Isolation, Characterization of Chemical Constituents and Validation of Herbal Potential of Folkloric Medicinal Plants *Pisonia grandis* R.Br. and *Andrographis stenophylla* C.B Clarke

By

G. Poongothai

IJSER

Supervisor

Dr. Shubashini K. Sripathi

Department of Chemistry

Thesis Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore - 641043

In Partial Fulfilment of the Requirements for the Degree of

Doctor of Philosophy in Chemistry

September 2014

DECLARATION

I declare that the thesis entitled "**Isolation, Characterization of Chemical Constituents and Validation of Herbal Potential of Folkloric Medicinal Plants *Pisonia grandis* R.Br. and *Andrographis stenophylla* C.B Clarke"** submitted by me for the Degree of **Doctor of Philosophy in Chemistry** is a record of original research work carried out by me during the period from October 2010 to September 2014 under the guidance of **Dr. Shubashini K. Sripathi,** Professor, Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, and it has not formed the basis for the award of any Degree/ Diploma/ Associateship/ Fellowship,Titles in this University or any other University or similar institutions of Higher Learning.

IJSER

G.Poongothai

Candidate

CONTENTS **Page No.**

IJSER

List of Figures

IJSER

IJSER

IJSER

IJSER

IJSER

IJSER

INTRODUCTION

"Medicinal herbs penetrate and get diffused into all limbs and joints of the sick like a sharp and strong moderator that destroys disease"
Rig Veda 10.97.12

The relationship between plants and mankind is an extended and uninterrupted one. The vital role of plants in the lives of humans is envisaged from the multi various products such as medicines, cosmetics, beverages, fuels, personal care products, furniture, textiles, tyres, and much more produced from plants. Plants are the only source of continuous supply of oxygen on earth. They provide oxygen for all organisms to survive. They control pollution by consuming carbon dioxide and prevent soil erosion and global warming. They are a source of rainfall. It is worthy of mention that plants make earth a heavenly abode by their greenery.

Medicinal plants represent the eternal kindness of almighty for the perpetuation of life in the universe. In terms of life forms, medicinal plants are equally distributed across habits, *viz,* trees, shrubs and herbs. Medicinal plants are used at various levels as home- made medicines; as medicine by the tribal shamans and by the practitioners of classical traditional systems of medicine such as Ayurveda, the Chinese medicine or the Japanese Kampo system **(Holley and Cherla,1998)**.The World Health Organization has defined medicinal plants as those which possess medicinal properties or those that synthesize metabolites to produce useful drugs **(WHO2008).**Medicinal plants are classified according to the part used, habit, habitat and therapeutic value besides the usual botanical classification. An estimated 13,000 species of medicinal plants have been in use to cure various ailments and diseases by various cultures of the world **(**www.agr.gc.ca**).**

There are around 2, 50,000 higher plant species on the earth and out of those 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value **(Joy *et al*., 2001)**. Most medicinal plants are collected from the wild. In the recent decades, industrialisation, urbanization, commercial exploitation, employing environment-unfriendly harvesting techniques and loss of growth habitats has led to the decrease in population of many important medicinal plant species.

IJSER © 202
http://www.ijser.org

In order to preserve plant wealth, suitable conservation methods have been recommended by global regulatory agencies. Apart from global agencies, local government organizations, non-government organizations have also initiated several schemes to encourage preservation and cultivation of medicinal plants among local people **(www.keralaagriculture.gov.in).**

Herbal medicine is also known as botanical medicine or plant medicine or phytomedicine. The World Health Organization has defined herbal medicine as herbal products in the medicines category in a national drug regulatory framework and may include “herbs”, “herbal materials”, “herbal preparations”, and “finished herbal products”/ “herbal medicinal products”. Medicines containing plant materials combined with chemically defined active substance including chemically defined isolated constituents of plants are not considered to be herbal medicines (**WHO 2007)**. 80 % of the world’s population depends on herbal medicine for their primary health care. *Rouvolfia serpentina, Andrographis paniculata, Withania somnifera, Allium sativum*, *Piper longum*, *Gymnema sylvestre*, *Tinospora cardifolia, Adathoda beddomei, Azardirachta indica, Ocimum teniflorum*are few medicinal plants whose market potential is very high due to their active ingredients.

IJSER

Traditional Herbal Medicine

Ancient wisdom has been the basis of modern medicine and will remain an important source of future medicine and therapeutics. The use of whole plantor various parts of the plant like leaves, stems, roots, bark, flowers, pods, seeds, etc., for treatment of various ailments in the ancient times is called traditional herbal medicine. All cultures have a history of herbal medicine. The history of the use of plants as a primary source of medicine can be traced back several millennia to the ancient written documents of early civilizations of India, China and the Middle East. Records from as early as 2700 BC from China, traced to the times of Emperor Shennung, indicate the usefulness of plants for treating disease, and the *Eberspapyrus*, written in 1550 BC includes many of the plants used in Egyptian medicine. Theophrastus, a Greek native (370-285 BC) began the scientific classification of plants, and *DioscoridesDeMateriaMedica* written by PedaniusDioscorides

IJSER © 202
http://www.ijser.org

(77 AD) documented the use of 600 medicinal plants. Ibn al-Baitar (1197-1248) listed over 1400 drugs and medicinal plants in his *Corpus of Simples* **(Reneela, 2010)**. The CharakSamhita written in India during 990BC has documented the method of manufacture of 340 herbal drugs and their indigenous uses for curing various ailments and diseases **(Chulet *et al.*, 2010).** Traditional systems of medicine continue to be widely practised on many dispensations. The rise in human population, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases has increased the emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments **(Joy *et al*., 2001**).

India is one of the twelve biodiversity centres of the world with the presence of over 45000 different plant species. There are 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species) that make uniqueness for the country. Out of 400 families of flowering plants in the world; at least 315 are present in India. Nearly three fourth of the drugs and perfumery products used in the world are available in the natural state in the country. Indian vedas describe the widespread use of plants in curing different disease. The *Rigveda* mentions the use of 67 plants for therapeutic applications; the *Yajurveda* enlist 81 plants and the *Atharvanaveda* enlist 290 plants for medicinal uses **(Joy *et al*., 2001**).

Ayurveda is an Indian traditional medicine system that has been known for nearly 5000 years and still practised widely in India. Ayurveda aims at maintenance of health of healthy individuals (*svasthasyasvaasthyarakshana*) and treatment of diseases of the diseased (*aaturasyavikaaraprasamana*). There are around 1990 plants employed in Ayurveda to treat various illnesses. Siddha medicine originated from South India especially from Tamilnadu, was developed by ancient sages called *Siddhars who* were imbued with divine power. The aim of siddha medicine is to make the body perfect, imperishable and to promote longevity. This is the first system to emphasize health as the perfect state of physical, mental, social, moral and

IJSER © 202
http://www.ijser.org

spiritual component of human beings. There are 600 plants employed in siddha medicine. The symbol of life's medicine is called Unani. It prescribes drugs, diet, drinks and other regiments including codes of conduct which are conducive to the maintenance and promotion of positive health, as well as the prevention and cure of disease. The ultimate aim of these scientific prescriptions is the creation of a healthy society. There are 700 plants used in Unani system of medicine**(**http://ism.kerala.gov.in/index.php/about-unani-and naturopathy.html**).**The Amchi system of medicine is known as "Tibetan Medicine" practised from east to west along the chain of the Himalayan Mountains including Himachal Pradesh and Sikkim, as well as the neighbouring states of Tibet, Nepal and Bhutan. Around 600 plants are used in this system of medicine to treat various ailments **(**http://www.lungtatravel.com/amchi-medicine.html**).** Plants that are used in the Indian traditional herbal medicine can provide biologically active molecules which are exemplary sources for development of novel drugs for various illnesses. The rich traditional knowledge on herbal medicine is considered as a wealth of India. Although the development in modern medicine is tremendous, people in rural and even urban cities of India depend on traditional herbal medicine for their healthy life because they believe and know that herbal medicines are non-toxic, have no side effects and are easily available at affordable price.

Medicinal plants contribute to global economy. Even during the recent economic recession, a robust growth of global economy was witnessed due to herbal trade. The world market for plant-derived chemicals – pharmaceuticals, fragrances, flavours, and colour ingredients alone exceeds several billion dollars per year. The demand for medicinal plant based raw material is growing at the rate of 15 to 25 % annually worldwide **(Vadera,2010)**. According to the World Health Organisation the international market of herbal products is around $6.2 billion, which is poised to grow to $5 trillion by the year 2050 **(Kumar and Janagam,2011)**. The International Council for Medicinal and Aromatic Plants estimates that the global demand for herbal products is currently growing at 8-10% annually. The global market for herbal

IJSER © 202
http://www.ijser.org

supplements and remedies is forecasted to reach $107 billion by the year 2017 **(**www.neutraceuticalsworld.com**).**Such a global growth is attributable to the increase in consumer awareness and demand for natural products and alternative medicines.

The Government of India has identified medicinal plants as one of the thrust areas of global trade. The Ministry of Environment and Forests, Government of India, has reported that there are over 8000 species of medicinal plants grown in the country. India is the second largest producer of castor seeds in the world. A large share of menthol and *psyllium* is exported to the world market by India. Indian medicinal plants are exported mainly to USA, Japan, Germany, France, UK, China, Hong Kong, UAE, Taiwan and Pakistan. According to a study on export of Indian medicinal plants, 880 medicinal plant species find place in All India Trade but India's share is only 0.5 % in global medicinal plant related export trade **(Kumar and Janagam,2011).**

IJSER

The global herbal market requires that a herbal medicine formulation should meet certain standards. A thorough documentation of traditional use of plants. Standardization based on chemical and activity profile. Safety and stability profile. Absence of pesticides and heavymetals in phyto medicines. Pharmacological studies in animals and in humans must support the herbal potential. Such scientifically generated data will project herbal medicine in a proper perspective and help in sustained global market **(Kamboj, 2000).** The Ministry of Health and Family Welfare of India has developed pharmacopoeia standards through pharmacopoeia committees. These standards are mandatory for drug testing under The Drugs and Cosmetic Act, 1940 and for examining samples of drugs available in the market for their safety and efficacy. If the quality, safety, and efficacy of medicinal plants, herbal medicines, herbal preparations, herbal extracts, and single medicines reach global standards certainly India will become the major contributor in the global market.

IJSER © 202
http://www.ijser.org

Need for Validation and Standardization

Quality, efficacy, safety and standardization are prime issues in medicinal plant research. The international regulatory authorities expect data generated on medicinal plants to meet the standards of GxPs (Good Practices), good agricultural practices (GAP), good laboratory practices (GLP), good clinical practices (GCP) and good manufacturing practices (GMP) (**Bhatt, 2010).** WHO has also issued guidelines for the assessment of medicinal plants, herbal medicines and simple herbal preparations on the basis of quality control such as identification, percentage of purity, water content, and assay of active ingredients, inorganic matters, heavy metal and pesticidesfor validation and standardization **(Bansal and Bansal, 2011)**

WHO monographs on medicinal plants contains two parts. The first part consists of pharmacopoeial summaries for quality assurance purposes including botanical features, distribution, identity tests, purity requirements, chemical assays and active or major chemical constituents. The second part consists of an extensive review of scientific research summaries on clinical applications of the plant material with detailed pharmacological information, contraindications, warnings, precautions, potential adverse reactions, and posology.

IJSER

Owing to the fact that the Indian subcontinent is a bountiful source of medicinal plants and owns a rich heritage of knowledge on medicinal plants, India’s prospects of achieving a significant share in global market is high. In order to materialize this there is an imperative need to validate and standardize herbal medicines. Indian governmental organizations namely “The Indian Council of Medical Research, The Central Council for Research in Homeopathy, Central Institute for Medicinal and Aromatic Plants, Directorate of Medicinal and Aromatic Plants Research, Indian Council of Agricultural Research, Centre for Medicinal Plants Research and National Medicinal Plants Board of Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy” provide assistance on the validation and standardization of plants by inflow of funds, trainings, workshops etc.

IJSER © 202
http://www.ijser.org

Medicinal Plants Selected for the Study

In the present research work two folkloric medicinal plants have been chosen for isolation and chemical characterisation of their phytoconstituents, which area of work, is one major stage towards chemical standardisation of the plants. This being the prime focus of the study; herbal standardisation strategies have also been applied to make it a wholesome record of validation of the folkloric use of the chosen plants. The plants chosen are:

Plant I: *Pisonia grandis* R.Br. of plant family Nyctaginaceae

Plant II: *Andrographis stenophylla* C.B Clarke of plant family Acanthaceae

Both plants have been extensively used by local people and tribal folk as anti-diabetic and anti-inflammatory agents.

Pisonia grandis* R.Br. (Figure 1)** is a flowering plant of the four O'clock family **Nyctaginaceae** and commonly called **lettucetree**. It is native to the Seychelles as well as other tropical areas of the world, especially the Indo-pacific islands. It is found to be widely distributed throughout India. The history of the plant dates back to the 18th century when it was discovered by Robert Brown from islands in the Gulf of Carpentaria **(Flinders,1814)**. The plant is fibrous and can store water allowing thus it to flower at any time of year independent of weather. It is known to be a salt resistant and drought tolerance plant **(**www.greenpatio.com**).**An interesting observation by bird watchers in the Seychelles reveals that birds flying away from the plant shed the seeds and propagate its growth, however due to the bulkiness of a sticky substance in the seeds **(Figure 2)** they lose their flight later and fall to the ground and hence the plant earns the name "Bird Eating Tree***."

IJSER

IJSER © 202
http://www.ijser.org

Figure 1 Photograph of the plant *Pisonia grandis* R.Br.

Figure 2 Sticky substance of the seed

Pisoniagrandis has been reported to have medicinal properties and is documented in NAPRALERTTM database. **Fresh leaves, moistened with Eau-de-Cologne, find use in reducing inflammation of a filarioid nature in the leg and other parts of the body. Chewing two leaves of the plant has been found to reduce sugar levels in the body and the roots are considered to be purgative (Anonymous 1969)**. South Indians cook and eat the young leaves as a salad and also use it for alleviating pain due to arthritis.

IJSER

General Information on *Pisonia grandis* R.Br.

Plant name	*Pisonia grandis* R.Br.	
Family	Nyctaginaceae	
Taxonomy Hierarachy Familia: Nyctaginaceae Genus: *Pisonia* Species: *Pisonia grandis*		
Synonyms	*Pisonia alba* Span; *Pisonia morindifolia* R.Br; *Pisonia sylvestris*Teijsm&Binn	
Vernacular names	Hindi	: Chinaisalit
	Tamil	: Lechai kottai, Maruval, Chandu
	Telugu	: Lanchamundaku
	Kannada	: Sulesoppu
	Gujaraty	: Velatisalet
Universal name	Lettuce tree; Cabbage tree; Bird eating tree	

IJSER © 202
http://www.ijser.org

Description	A tall attractive an evergreen tree of 9-12m high; Large Leaves 12-15 cm. pale greenish or yellowish; Smooth bark, brittle and soft nature; buttress-like roots; It produces flowers rarely; Fruits are narrow club-shaped;
Distribution	Seychelles; Indo-pacific islands; India, Indonesia, Malaysia, Sri Lanka, Australia, Madagascar
Biologically active constituents:	β- sitosterol, α-spinosterol, β- sitosterol glucoside, dulcitol, quercetin **(Natarajan *et al*., 1990)**
Folk use	Anti-diabetic; Anti-inflammatory
Parts used in medicinal preparation	Leaves and Roots
Pharmacological activities:	*The plant is reported to possess analgesic, antipyretic, diuretic, wound healing, anti-diabetic, free radical scavenging, anti-inflammatory, anti-arthritic, and antimicrobial, hepatoprotective activity, anxiolytic activity and antiplasmodial activities.*
Dosage and safety aspects extracts	Ethanolic extracts of ***Pisonia grandis*** are consideredsafe and non-toxic in nature. **(Anonymous, 1969).**

IJSER

***Andrographis stenophylla* C.B Clarke**

***Andrographis stenophylla* C.B Clarke (Figure 3 & 4)** is a medicinal plant of the **Acanthaceae** family. It is a rare and little-known endemic species of India. Tribes belonging to a local area named Marudhamalai call this plant “malaichiriyanagai” and claim that the leaves of this plant are potentially effective in the treatment of chronic fever, diabetes, wounds, ulcers, inflammations, cough, skin diseases and leprosy.

Figure 3 Photograph of the plant *Andrographis stenophylla* C.B Clarke

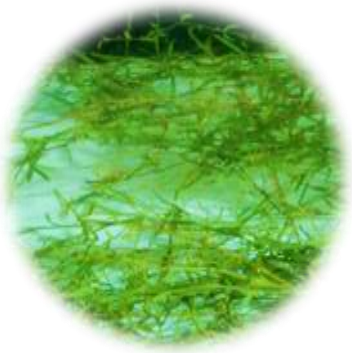


Figure 4 A close view of the plant

IJSER © 202
http://www.ijser.org

General Information on *Andrographis stenophylla* C.B Clarke

Plant name	*Andrographis stenophylla* C.B Clarke
Family	Acanthaceae
Taxonomy Hierarachy Familia: Acanthaceae	
Genus: *Andrographis* Species: *Andrographis stenophylla* C.B Clarke	
Vernacular names	Tamil : Malai chiriyanagai
Description	It is an erect glabrous perennial under shrub with very narrow leaves and stems from a stout root stock. Leaves are simple lanceolak, acute at both ends 2-3 cm long and 0.5 – 1.5 cm width, bitter in taste. Stems 4 angled; flowers distantly arranged 2-4 mm long;
Distribution	India (Andrapradesh, Kerala, Tamilnadu)
Biologically active constituents	Isosakuranetine, acacetine and andrographolide
Folk use	anti-diabetic, anti-inflammatory, and an antidote for snake bites
Parts used in medicinal preparation	Leaves; Whole plant.
Pharmacological activities	Plant *Andrographis stenophylla*is bestowed with anti-oxidant, anti-diabetic, anti-venom, anti-inflammatory, anti-microbial, anthelmintic, anti-histaminic and muscle relaxant activites
Dosage and safety aspects extracts	Extracts of *Andrographis stenophylla*are considered to be safe and non-toxic nature **(Gnasekaran*et al*., 2014)**

IJSER

IJSER © 202
http://www.ijser.org

Plants Chosen for the Study - The Rationale

There are only few reports on the chemical investigation of the leaves of the plant. As early as 1990, **Natarajan*et al.*** reported the presence of bio-active metabolites ***β-sitosterol, α-spinosterol, dulcitol, β-sitosterol glucoside, octacosanol and quercetin*** from the **leaves** of this plant. The first report of the isolation of highly valuable molecules ***pinitol*** **and** ***allantoin*** from the **leaves** of ***Pisonia grandis*** was published in 2011 as part of preliminary studies on this plant in our laboratory **(Shubashini *et al*., 2011a; Shubashini *et al*., 2011b).** Post this revelation, it was evident that publications on the medicinal potential of the leaves further mushroomed. There are not many reports on the chemical investigation of the stem and roots of the plant. Only one report on the hepato protective activity of root extract and one report on isolation of C-flavones from root extract exist. **Hence the plant *Pisonia grandis* was chosen in the present study for a thorough chemical investigation and validation as a safe medicinal plant for use in medicinal formulations.**

IJSER

Andrographis stenophylla **C.B Clarke** is a medicinal plant of the **Acanthaceae** family. It is a rare and little-known endemic species of India. Local tribes called this plant as “malai chiriyanagai”. ***Andrographis stenophylla*** is used in folk medicine as an anti-diabetic, anti-inflammatory agent, and as an antidote for snake bites. **Local tribes claim that the leaves ofthis plant are potentially effective in the treatment of chronic fever, diabetes, wounds, ulcers, inflammations, cough, skin diseases and leprosy.** There is only one report on the phytochemical investigation of leaves of the plant which describes the isolation of few flavones along with the terpene lactone andrographolide - a biomarker of the ***Andrographis*** genus **(Neelaveni and Gupta,2010).** There are no reports on scientific investigation of the stem, root, flower or pods of this plant. **Hence the aerial part of this plant has been chosen for a thorough chemical investigation and validation of its herbal potential.**

IJSER © 202
http://www.ijser.org

The main objectives

- To isolate and characterize the chemical constituents of two folkloric medicinal plants ***Pisonia grandis*** **R.Br. and** ***Andrographis stenophylla*** **C.B Clarke**

- To validate the herbal potential of the chosen plants by standardisation studies

IJSER

IJSER © 202
http://www.ijser.org

2. REVIEW OF LITERATURE

Review of literature pertaining to the study comprises,

- Earlier work on ***Pisonia grandis* R.Br**.
- Bio-pharma potential of **Pinitol**
- Bio-pharma potential **Allantoin**
- Earlier work on ***Andrographis stenophylla* C.B Clarke**
- Bio-pharma potentialof **Andrographolide**

2.1 Earlier Work on *Pisonia Grandis* R.Br.

The plant ***Pisonia grandis*** has been documented in many databases. **The Ethno pharmacopoeia of Rotuman** registered the use of leaves of the plant in Rotuman culture to control dysentery (**McClatchey,1996** and **Buenz *et al*. 2005)** identified *Pisonia grandis* R.Br. from the 17th century historic herbal text as a medicinal plant used by Rumphius and published those collections as **Ambonese Herbal: Volume I.** The ethnomedical and pharmacological uses of this plant has been documented in NAPRALERTM. Duke's phytochemical database documents it as a diuretic and purgative agent. It's use in the treatment of filariasis, inflammations, boils and edema has also been documented. **(Dr.Dukes Ethnobotany database 2014)**

IJSER

The plant has been extensively investigated for its pharmacological potential. However not much work on isolation of active principles/secondary metabolites has been done. Earlier reports of scientific investigations on this plant have been reviewed for the period from 1990 to till date.

2.1.1 Pharmacognostical Studies

Microscopical studies and physio-chemical analysis of leaves of *Pisonia grandis* have been carried by (**Jayakumari *et al.*,2011)** that could serve in the identification and preparation of a monograph on this medicinal plant to maintain the quality of the plant**.**

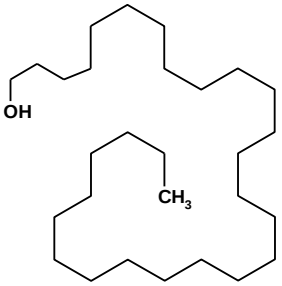
2.1.2 Metabolites from *Pisonia grandis* R.Br.

There are only few reports on the chemical investigation of the leaves of the plant. As early as 1990, **Natarajan *et al.*** reported the presence of bio-active metabolites ***β-sitosterol, α-spinosterol, dulcitol, β-sitosterol glucoside, octacosanol and quercetin*** from the **leaves** of this plant.

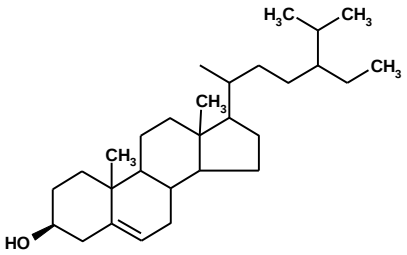
IJSER © 202
http://www.ijser.org

UV fluorescence microscopy of petiole of *Pisonia grandis* collected from botanical garden of Oxford University confirmed its similarity to the *Gramineae* and revealed the presence of Ferulic acid (**Hartley *et al*., 1981)**

The first report of chemical examination of *Pisonia grandis* reavealed the presence of octacosanol **(1)**, β-sitosterol **(2)**, α-spinosterol **(3)**, β-sitosterol glucoside **(4)**, dulcitol **(5)** and quercetin **(6) (Natarajan *et al*., 1990).**

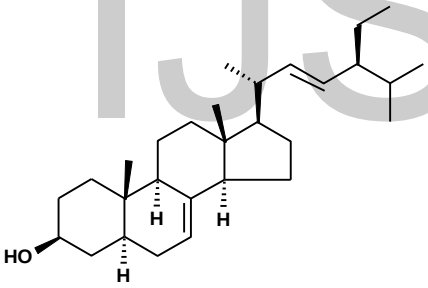


1

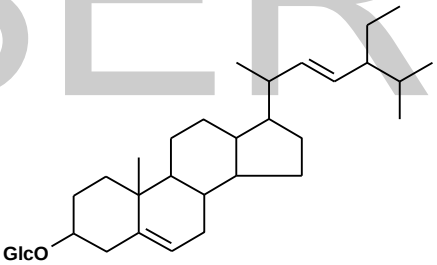


2

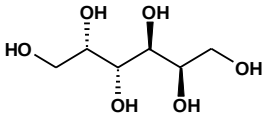
IJSER



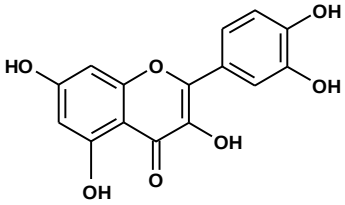
3



4



5

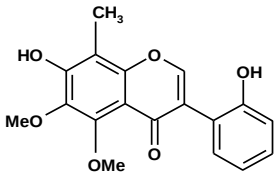


6

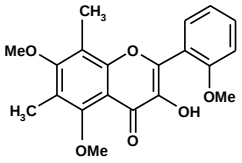
Five new C-methylated flavonoids (7,2'-Dihydroxy-5,6-dimethoxy-8-methylisoflavone (7); 6,2'-Dihydroxy-5,7-dimethoxy-8-methylisoflavone (8), 3-Hydroxy-5,7,2'-trimethoxy-6,8-dimethylflavone (9), 3,5,2'-Trihydroxy-7,3'-

IJSER © 202
http://www.ijser.org

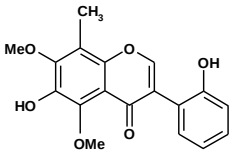
dimethoxy-6,8-dimethylflavone (10), 5,7,2'-Trihydroxy-,3'-methoxy-6,8-dimethylflavanone (11), together with seven known compounds (β -sitosterol (12), 6,8-dimethyli-sogenistein (13), leptorumol (14), 5,7,2'-trihydroxy-6-methoxy-8-methylisoflavone,pisonia-none(15), irilin A (16), 5,7,2'-trihydroxy-6,8-dimethylflavanone,2'-hydroxydemethoxymatteucinol (17) and 3-methoxy-4-hydroxybenzoic acid (18)have been isolated after chromatographic separation of the hexane and CH_2Cl_2 extracts of the roots of *Pisonia grandis* **(Sutthivaiyakit *et al*., 2013)**.



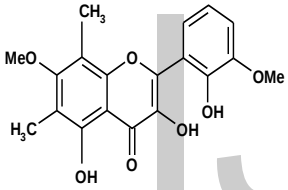
7



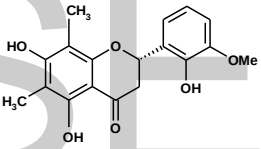
8



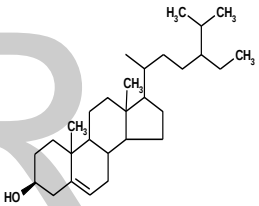
9



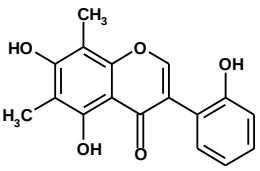
10



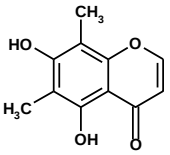
11



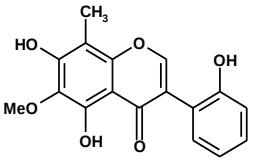
12



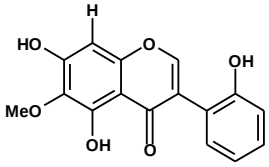
13



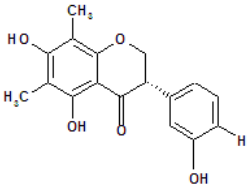
14



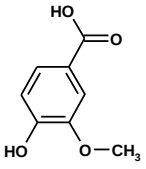
15



16



17



18

IJSER

IJSER © 202
http://www.ijser.org

2.1.3 Pharmacological Studies on *Pisonia grandis*

The plant *Pisonia grandis* is bestowed with immense pharmacological potential. Chewing two leaves of the plant has been found to reduce sugar levels in the body and the roots are considered to be purgative (**Anonymous, 1969**).

Analgesic Activity: The first scientific report on the medicinal potential of ***Pisonia grandis*** was published in **2002** which documented the analgesic potential of the leaves of *Pisonia grandis.* The methanol extract of leaves of ***Pisonia grandis*** showed significant analgesic activity in acetic acid induced writhing response method and in tail licks method **(Anbalagan *et al.,* 2002**).

Antipyretic Activity: Antipyretic effect of a poly-herbal formulation using *Pisonia grandis* was evaluated by **Elumalai *et al. (*2012).** The poly-herbal formulation containing *Pisonia grandis* showed significant reduction of yeast induced pyrexia in rats with respect to the control group.

Diuretic Activity: The chloroform and methanol extracts of the leaves of ***Pisoniagrandis*** were analysed for analgesic activity in dose response manner. The methanol extract possessed significant diuretic activity whereas the chloroform extract was completely devoid **(Anbalagan *et al.,* 2002**).

Anti-Inflammatory Activity: The plant *Pisonia grandis* is well known for its anti-inflammatory activity. It has been extensively used in Indian Traditional Medicine to treat inflammatory. **Fresh leaves, moistened with Eau-de-Cologne, find use in reducing inflammation of a filarioid nature in the leg and other parts of the body (Anonymous,1969)**. The first scientific report on anti-inflammatory activity of leaves of *Pisonia grandis* was published by **Anbalagan *et al.* (2002)** indicated that chloroform and methanol extracts of leaves of *Pisonia grandis* were exhibited graded dose response in both acute and chronic model.

Methanolic extract and flavonoid rich fraction separated from the leaves of *Pisonia grandis* were evaluated for anti-inflammatory activity by Carrageenan induced paw edema model. The flavonoid rich ethyl acetate fraction was showed maximum inhibition due to the presence of quercetin in

IJSER © 202
http://www.ijser.org

the leaves which strongly suggested that the defective effect inflammatory response by flavonoids **(Jayakumari *et al*.,2012)**. Ethanolic extracts of roots of *Pisonia grandis* showed significant reduction in paw edema compared to standard drug indomethacin (**Majumdar *et al*.**, **2012).**

Anti-Arthritic Activity: Anti-arthritic activity of ethanol extract of *Pisonia grandis* was evaluated by **Elumalai *et al. (*2012).** The observed anti-arthritic potential of the plant is due to the presences of phytoconstituents such as alkaloids, phenols and flavonoids.

Wound Healing Activity: Prabu*et al.* (2008) evaluated the wound-healing potential of methanolic extract of leaves of *Pisonia grandis* by incision and excision wound model. Ointments with 1% and 2% w/w extracts were capable of producing significant ($p<0.05$) wound healing activity in both models.

Anti-Diabetic Activity: The ethanolic extract of leaves of *Pisonia alba* Span was selected for administration in alloxan-induced diabetic rats. The extract reduced blood glucose levels of alloxan diabetic rats by elevating peripheral glucose utilization. Treatment with the ethanol extract of the plant restored the normal histological architecture of the liver, kidney and pancreas of alloxan-induced diabetic rats (**Sunil *et al.,*2009a).** **Y**et another report on the α-glucosidase inhibitory action and antidiabetic activity of *Pisonia grandis*revealed that the ethanolic extract of arial parts of *Pisonia grandis*showed intestinal α-glucosidase inhibitory activity. It also protects significantly from other metabolic aberrations caused by alloxan suggesting that ethanolic extract of aerial parts appears to be an attractive material for further studies leading to possible drug development for diabetes (**Sunil *et al.,*2009b).**

Free Radical Scavenging Activity: *Pisonia grandis* was found to contain very good anti-oxidant property. Methanolic extract of leaves of *Pisonia grandis* inhibited free radicals generated by DPPH, ABTS, lipid peroxides. **Subhasreeet *al.,*** (**2009)** found that the methanolic extractof leaves of *Pisonia grandis* has high potent in neutralizing ABTS cation radicals than the other radicals**.** The ethanolic extract of leaves of *Pisonia alba Span* showed dose dependent DPPH radical scavenging activity due to its ability for donating

IJSER © 202
http://www.ijser.org

hydrogen molecule. The extract also possesses antilipid peroxidant potential (**Sunil *et al.,*2009a).** The antioxidant and radical scavenging activity of methanolic extractof leaves of *Pisonia grandis* was established by **Jagadeesan *et al*., (2011).** Methanolic extract and it's fractionates (ethyl acetate and ethanol fractions) of leaves of *Pisonia grandis* were investigated by nitric oxide radical scavenging assay method and DPPH method. The ethanol fraction showed maximum scavenging of nitric oxide and DPPH radicals **(Jayakumari *et al.,* 2012).**

Anti-microbial Activity: The methanol extract of leaves of *Pisonia grandis* inhibited the growth of the gram-positive and gram-negative bacteria that aids in wound healing. The extract of 300 μg concentration showed inhibition equal to commercial antibiotics of 10 μg concentration (**Prabu *et al*., 2008).** Yet another evidence for antimicrobial activity of *Pisonia grandis* was reported by **Nivedhitha and Rani (2011)** against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus auresus*, *Aspergillus niger, Sacchraromyces cerevisiae*. Recently the aqueous extract of leaves of *Pisonia grandis* and its ethylacetate fractions were screened against *S. aureus, E. coli, K. pneumoniae* and *C. albicans* by agar disc diffusion and agar well diffusion methods. The ethyl acetate fraction depicted significant antimicrobial activity with 1.2 mg/ml as minimum inhibitory concentration **(Jayakumari *et al*.,2014).**

Hepatoprotective Activity: Hepatoprotective activity of 95% ethanolic extract of roots of *Pisonia grandis* R.Br. was studied against paracetamol induced hepatic injury in Wister rats. Histopathological observations revealed that pretreatment with the extract protected the animals from paracetamol induced liver damage **(Majumdar *et al.,* 2012)**. The ethanolic and aqueous extracts of leaves of *Pisonia grandis* screenedfor its hepatoprotective potential against liver injury induced by carbon tetrachloride, paracetamol or thioacetamide and chronic liver damage induced by carbon tetrachloride in rats. Pretreatment of animals with the extract reduced inflammation and degenerative changes. Histological examination of liver tissues supported the hapatoprotection by both the extracts and thus the ethanolic and aqueous extracts showed

IJSER © 202
http://www.ijser.org

significant hepatoprotective activity in carbon tetrachloride induced acute and chronic liver damage **(Thenmozhi *et al*.,2013).**

Anxiolytic Activity: The ethanolic extract of leaves of *Pisonia grandis* R.Br. possessed significant anxiolytic activity **(Rahman *et al.,* 2011).** This investigation was carried out to find alternate plant derived medications with anxiolytic effect.

In-vitro Antiplasmodial Activity: Antiplasmodial activity of leaf and bark extracts of *Pisonia grandis* was well established by **Sundaram *et al.,* (2012).**

Toxicity Study: The ethanol extract of leaves of *Pisonia alba* Span did not show any sign of drowsiness, restlessness, convulsions, piloerection and morality up to a dose of 4000 mg/kg **(Sunil *et al.,*2009a).** Non- toxic nature of the ethanol extract of leaves of ***Pisonia grandis*** was well established up to the dosage level of 2000 mg/kg body weights in rats **(Elumalai *et al.,* 2012).** Hence the extracts of ***Pisonia grandis*** was consider being safe and non-toxic nature.

"The literature review during the period from 1990 to till date revealed that the plant pisonia grandis possessed analgesic, antipyretic, diuretic, wound healing, anti-diabetic, free radical scavenging, anti-inflammatory, anti-arthritic, antimicrobial, hepatoprotective, anxiolytic activity and in-vitro antiplasmodial activity"

IJSER

2.2 BIO-PHARMA POTENTIAL OF PINITOL

Pinitol is the 3-O-methyl ether of D-chiroinositol, with both enantiomers occurring in various plant sources. The name pinitol derives from "pine", as it was first isolated from pine tree. As the demand for pinitol as a food supplement and as a pharmaceutical increased, any attempt to isolate it from natural sources including plants is considered highly worthy. Isolation of D-Pinitol from plants has been reviewed recently (**Poongothai *et al*., 2013)**. Number of scientific reports on the isolation of pinitol from plant families is illustrated in **Chart 1**

IJSER © 202
http://www.ijser.org

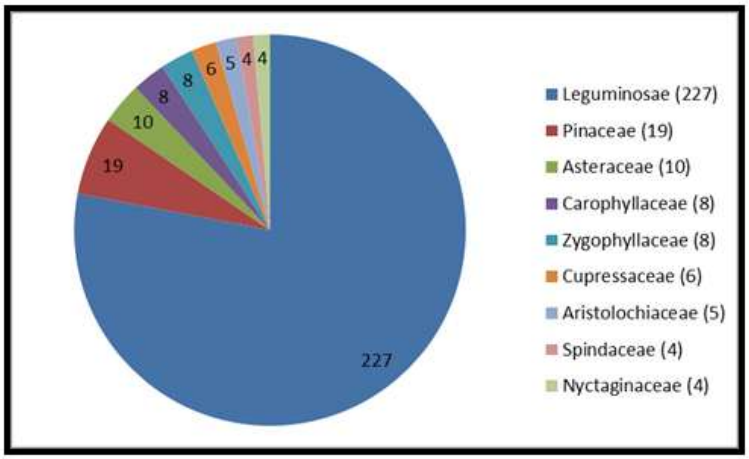


Chart 1 Number of Scientific Reports on isolation of D-Pinitol

Pinitol has immense pharmacological significance. It is bestowed with antidiabetic **(Ajuah *et al*., 2000)**, anti-inflammatory **(Singh*et al*., 2001)**, antioxidant **(Orthen *et al*., 1994)** and immunosuppressive potential **(Chauhan *et al*., 2011)** and is used in the treatment of hypertension, rheumatism, cardiovascular diseases, AIDS and neurological disorders **(Ostlund *et al*., 1996 and Kim *et al*., 2005)**. There is a growing interest in the use of D-pinitol as a food supplement because of its reported efficacy in lowering blood glucose levels with no side effects and nil toxicity **(Bates*et al*., 2000)** and thus D-pinitol has now become one of the better studied insulin mimickers in the food supplement industry. Report on pharmacological significance of pinitol is depicted in table1.

IJSER

2.2.1 Pharmacological Significance of D-Pinitol

Pinitol has immense pharmacological significance. As early as **1987**, **Narayanan *et al*.,** investigated the antihyperglycemic activity of pinitol from the leaves of *Bougainvillea spectabilis* which opened the door to the use pinitol as a food supplement in diabetes therapy. There are numerous reports on its pharmacological potential. It is proven to possess anti-hyperglycemic, anti-inflammatory, anti-obesity, anti-oxidant anti-hypertension activities and immunosuppressive potential. It also finds use in curing asthma, bone metabolic disorders and hepato toxicity.

A review of recent reports on the medicinal value of pinitol is presented.

IJSER © 202
http://www.ijser.org

Anti-diabetic Potential of Pinitol

D-pinitol is found to increase neural protection and memory ability in Wistar rat model of streptozotocin-induced diabetes by suppressing blood glucose and elevating insulin sensitivity (**Lee *et al.,* 2014)**. Oral ingestion of different doses of pinitol supplementation influences glucose tolerance, insulin sensitivity and plasma pinitol concentrations. Consumption of a nutritive beverage (Fruit Up) containing 2.5, 4.0 or 6.0 g of pinitol by thirty healthy subjects in two one-day trials revealed reduced serum glucose and insulin at 45 and 60 minutes at a dose of 6.0 g (**Mijares*et al.,* 2013**). A low dose of pinitol isolated from the stem bark of *Piliostigma thonningii showed significant anti-diabetic activity* compared to that of the therapeutic dose of the anti-diabetic drug glibenclamide **(Issac *et al.,* 2013).**

The pancreatic tissue protective nature of pinitol was analysed by its oral administration to streptozotocin-induced diabetic rats. Pinitol was found to enhance free radical-mediated alterations to near normalcy **(Sivakumar and Subramanian,2009a).** The oral administration of D-pinitol to diabetic rats also showed alterations in the activities of key metabolic enzymes involved in carbohydrate metabolism **(Sivakumar and Subramanian,2009b)**

Prolonged treatment of pinitol in Korean patients with type 2 *Diabetes mellitus* showed insulin- sensitizing effect without altering body weight and waist circumference of patients. This study reveals that pinitol can be effective as an oral agent in the treatment of type 2 diabetes and in the prevention of cardiovascular complications **(Kim *et al*., 2005).** Antihyperglycemic assay guided fractionation of roots of *Rhizophora apiculata and its* isolated constituent compounds revealed that the most active compound isolated from aqueous fraction was pinitol **(Lakshmi *et al*., 2006).** A study on the effect of D-pinitol, on the postprandial blood glucose response in type 2 diabetes patients revealed that ingestion of 1.2 g of pinitol one hour prior to consumption of rice controlled postprandial capillary blood glucose most effectively (**Kang *et.al*., 2006)**.

Hypoglycemic effect of pinitol isolated from soybeans has been reviewed (**Shin *et al.*,2002).** D-pinitol exerts insulin-like effect through post-

IJSER © 202
http://www.ijser.org

receptor pathway of insulin action affecting glucose uptake in hypoinsulinaemic STZ-diabetic mice **(Bates*et al.,* 2000).** A dose of 15 mg/kg promoted 21% decrease in plasma glucose in streptozotocin-treated rats (**Fonteles, 2000.).** Pinitol or combinations of pinitol with insulin in a synergistic amount has been proved to be effective for controlling insulin-dependent diabetes **(Weeks and Charles, 2000).** Oral administration of pinitol isolated from soybean is reported to improve insulin sensitivity (**Ajuah *et al.,*2000).**

Anti-inflammatory Activity of Pinitol

Pinitiol isolated from *Abies pindrow* leaves showed a significant anti-inflammatory effect in carrageenin-induced paw oedema in rats and it did not produce any behavioural change or mortality **(Singh *et al.,*2001).** When a combination of pinitol and glucosamine were administered, a synergistic anti-inflammatory effect against sub-acute inflammation was observed (**Kim*et al.,*2005**). The specific amount of glucosamine and pinitol needed to cure inflammatory diseases was determined by **Yun *et al.,*2007**. Pinitol suppresses inflammatory cellular response and inhibits cytokine secretion in LPS induced neutrophils **(Gautam*et al,*2008; Bhat *et al.,*2009**).

IJSER

Immunosuppressant and Neurological disorder suppressant Potential of Pinitol

The methods of using D-pinitol to promote a healthy nervous system and as a food supplement is well documented **(US Patent 20130123370 A1).** The immunosuppressant potential of the extracts of *Argyrolobium roseum* and its active constituent pinitol is well established **(Chauhan*et al.,* 2011).** D-Pinitol is suggested as a potent immunosupressor based on the results of well-established experimental models **(Chauhan *et al.,*2011).** The immunopharmacological functions of D-pinitol is documented **(Lee *et al.,*2007)**.

Biosynthesis of Pinitol

Pinitol biosynthesis in the angiosperm family proceeds *via* the intermediate formation of ononitol (1D-4-O-methyl myo-inositol) whereas in gymnosperms, it proceeds *via* the intermediate formation of sequoyitol (1D-5-

IJSER © 202
http://www.ijser.org

O-methyl myo-inosito). Since plants of the family Nyctaginaceae comprise of angiosperms, biosynthesis of pinitol may occur by the ononitol based pathway.

Pinitol biosynthesis pathway starts with the formation of myo-inositol-1-phosphate (myo-inositol 1- P) from glucose -6-phosphate (glucose 6- P) by inositol 1-P synthetase (INPSI). Then myo-inositol-1-phosphate dephosphorylated to myo-inositol by inositol 1- phosphatase (IMPI). Methylation of myo-inositol by inositol O-methyltransferase (IMTI) produces D-ononitol which is finally epimerased to d-pinitol by ononitol epimerase (OEPI). Epimerization of ononitol to pinitol is likely to proceed via a keto intermediate **(**www.pubchem.ncbi.nlm.nih.gov**).** Outline ofbiosynthetic pathway of pinitol was shown in **Figure 19.**

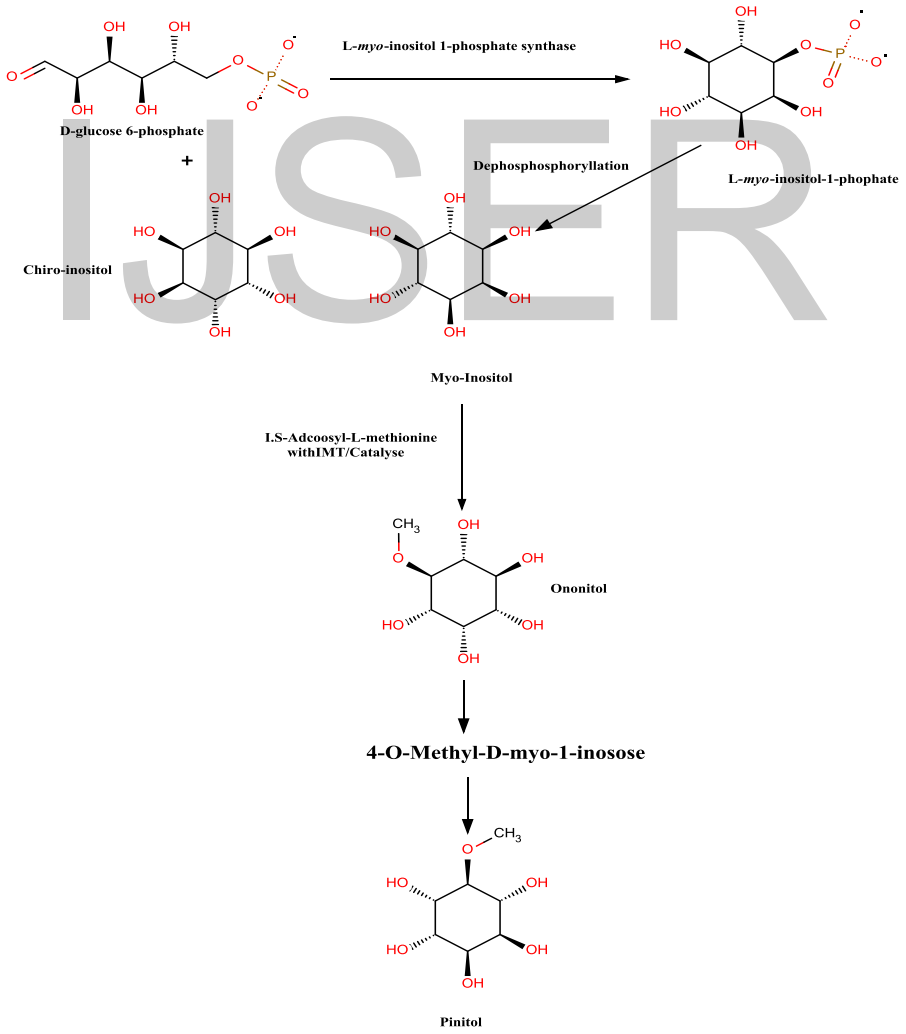


Figure 5 Biosynthetic pathway of pinitol

IJSER © 202
http://www.ijser.org

2.3 BIO-PHARMA POTENTIALOF ALLANTOIN

Allantoin is 2,5-dioxo-4-imidazolidinyl-urea and is known as a keratolytic molecule that removes warts, corns and hornylayer (hard layer) of the skin. It is a white, odourless, crystalline powder considered to be non-toxic, non-irritating and non-allergenic **(**www.balmtech.com**)**.The U.S. Food and Drug Administration (FDA) confirmed that allantoin is a safe and effective skin protectant in the recommended dosage range of 0.5 to 2.0% **(Federal Register)**. Allantoin is a compound that occurs naturally in wheat sprouts, tobacco seed, comfrey, and sugar beets. It is a 5-ureidohydantoin plays an essential role in the assimilation, metabolism, transport, and storage of nitrogen in numerous higher plants **(Wang *et al*., 2007).** Allantoin has been isolated from the plants of the family *Apiaceae, Theaceae, Rubiaceae, Dioscoreaceae, Fabaceae, Poaceae, Leguminosae, Lamiaceae, Boraginaceae,* Nyctaginaceae*, Annonaceae, Myristicaceae, Bignoniaceae, Bryaceae, Amaranthaceae, Selaginellaceae* and *Solanaceae.*

Allantoin is bestowed with lots of pharmacological significance. It is reported to be a free radical scavenger **(Guskov *et al*., 2002)** and wound healer **(Ranson 1984., Araújo *et al*., 2010)**. It reduces plasma glucose in streptozotocin-induced diabetic rats **(Shan Niu *et al*., 2010)**. Anti-inflammatory formulations **(Koho, 1984)**, antimicrobial dressings **(Ying Ko Sai, 1983)**, medicines that are used for treating gastroduodenal ulcer and chronic gastritis **(Dobrescu, 1998)** and ointments for treating plaque and psoriasis **(Pinheiro, 1997)** contain allantoin as one of the foremost ingredients.

Allantoin is used in a variety of skin care products **(Jewitt *et al.,* 2013)**, lip-care products**(www.ipr.net)**, hair care products **(Wu Ke, 2013)**, moisturizing cream **(Wang *et al.,*2013)**, sunburn lotions, diaper rash ointments and mouthwashes. Report on pharmacological significance of allantoin is presented in table2.

IJSER

IJSER © 202
http://www.ijser.org

2.3.1 Pharmacological activities of Allantoin

Anti-diabetic potential of Allantoin

The effect of allantoin on the plasma glucose of streptozotocin-induced diabetic rats (STZ-diabetic rats) exposed decrease in blood glucose level in the experimental animals in a dose-dependent manner (**Shan Niu *etal*.,2010)**. Allantoin is suggested to have increased the GLUT4 gene expression in muscle by increasing β-endorphin secretion from the adrenal gland in STZ-diabetic rats.

The study conducted by **Chen *et al.,*(2012**) on allantoin suggest that it can activate the imidazoline receptor I(2B)R to increase glucose uptake into cells I(2B)R is being proposed as a new target for diabetic therapy.

Anti- inflammatory effect and dermatological properties of Allantoin

In the study on correlation between anti-inflammatory activity and SPF of sunscreen lotions *in vivo*, significant correlation is reported. Phorbol myristate acetate on mice have been tested by **Celine *et al.,*(2012**) anti-inflammatory effect of allantoin and glycyrrhetinic acid-based emulsions as well as commercial sun product containing any one of these ingredients revealed significant results suggesting use of these chemicals in sunscreen products.

IJSER

The composition comprising a combination of methotrexate, bisabolol and allantoin is used to treat plaque psoriasis, atopic dermatitis and chronic eczema (**Alario *et al*., 2013**). A botanical basis soap of composition glycerol, 2,4,4'-trichloro-2'-hydroxyl di-Phether, Hamamelismollis ext., allantoin, 1-methyl-3-Ph propylamine, lanoline, anhydrous ethanol, and deionized water is capable of lubricating skin and softening hair, to shorten skin preservation (**Wang *et al*., 2013).**

The composition including propylene glycol, butanediol, betaine, sodium chloride, magnesium sulfate, methyl paraben, Propyl paraben, EDTA disodium salt, cetyl PEG/PPG-10/1 polydimethylsiloxane, simethicone, octanoic acid, triglycerides, shea butter oil, microcryst. wax, beeswax, polydimethylsiloxane, grape seed oil, sodium hyaluronate, essence, arbutin, adenosine, allantoin, iron oxide yellow, iron oxide red, black iron oxide, and

IJSER © 202
http://www.ijser.org

water, in refreshing cream can effectively isolate harmful substances in skin cream for makeup and in the air (**Jingyao, 2013**)

The combination of Allantoin along with other chemicals are used to treat acarid-caused acne, and has effect of inhibiting seborrhea of oily skin, relieving discomfort, whitening, removing wrinkles, antiaging and brightening skin, and also can be used for caring skin, moisturizing, and reducing formation of age pigment. The red nose caused by acarid, can be cured by using allantoin along with other chemicals by inhibiting excessive lipid secretion, repairing hair follicle and rugged tissue, reducing pigmentation and indentations, andpreventing acne formation (**Wu Ke, 2013**).

Endotoxin removal is efficiently achieved by the use of allantoin as a solid phase adsorbent which is effective than anion exchange, polymixin affinity and biological affinity methods for endotoxin clearance (**Vincent *et al*., 2013**).

A skin-caring cream comprising allantoin significantly improved skin laxity, aging, lines, dull skin and increased skin elasticity (**Zhang *et al*., 2013)**.

IJSER

Anti-bacterial Activity of Allantoin

The anti-bacterial, anti-viral, cytotoxicity and anti-microbial activities of allantoin along with the combination of some compounds are documented (**Berrin *et al*., 2011**).

Anti-ulcer Activity of Allantoin

Sixty-one alkaloids tested for anti-ulcer activity revealed fifty-five compounds to show significant antiulcer activity in ulcer induced animals (**Heloina *et al.,*2008**) .

Anti-hypertensiveActivity of Allantoin

Allantoin is recommended as an effective therapeutic agent for hypertension in the future. Administration of allantoin in rats decreased the blood pressure and in anesthetized rats inhibited cardiac contractility and heart rate. It is suggested for memory enhancement mediated by the PI3K – Akt-GSK-3β signal pathway and treating cognitive dysfunctions (**Chen *et al.,*2014**). Allantoin is also advocated for the cognitive dysfunctions observed in Alzheimer's disease **(Ahn *et al.,*2014).**

IJSER © 202
http://www.ijser.org

Wound healing Activity of Allantoin

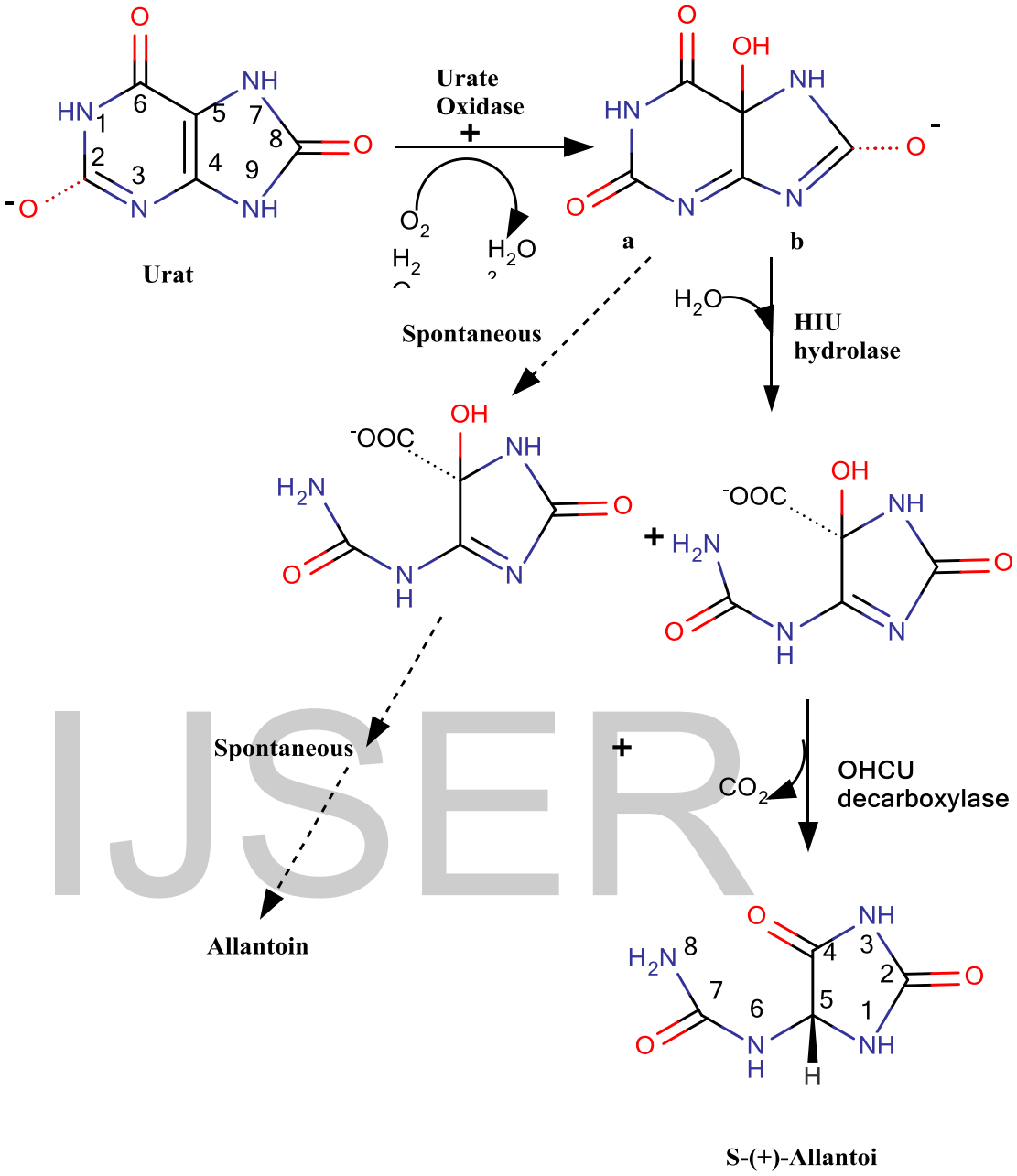
Comparison of the burn wound healing in rats by the application of *extra ctumcepae*, heparin and allantoin gel (CTBX) and silver sulfadiazine (SSD) cream revealed significant positive effect on wound healing by the application of CTBX (**Durmus *et al.,*2012**). Allantoin induces wound healing through the regulation of inflammatory response and stimulus to fibroblastic proliferation and extra cellular matrix synthesis. It is able to improve and fasten the reestablishment of the normal skin (**Lorena*et al.,*2010**).

Biosynthesis of Allantoin

Allantoin is nitrogen-rich compound derived from purine catabolism. A first step of purine catabolism occurs in the cytoplasm of infected nodule cell and leads to the production of oxopurines, such as hypoxanthine, xanthine and uric acid. Uric acid is then transferred to uninfected cells into the peroxisome and further metabolized to allantoin. The conversion of uric acid into allantoin is a single step process catalysed by urate oxidase. The urate oxidase reaction was determined by the conversion of urate to 5-hydroxyisourate (HIU), an unstable compound that decomposes spontaneously to 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU). Non-enzymatically decay of OHCU yield allantoin. The non-enzymatic decomposition of HIU generates a racemic mixture of allantoin (**Rossi, 2007).** Outline ofbiosynthetic pathway of allantoin was shown in **Figure 20.**

IJSER

IJSER © 202
http://www.ijser.org



IJSER

Fig.6 Biosynthetic Pathway of Allantoin

IJSER © 202
http://www.ijser.org

2.4 Earlier Work on *Andrographis stenophylla* C.B Clarke

Andrographis stenophyllais a medicinal plant belonging to the family **Acanthaceae***.* It is an erect shrub with very narrow leaves and stems from a stout rootstock; the corolla is pale with dark red stripes. In folk medicine the leaves are used for the treatment of diabetes and snake venom poisoning.

2.4.1 Pharmacognostical Studies

The physio-chemical and pharmacognostical properties of *Andrographis stenophylla* screened using light and confocal microscopy suggests use of physio-chemical, morphological and histological parameters as parameters to establish the authenticity of *Andrographis stenophylla* and that can possibly help to differentiate the drug from its other species (**Vijaya Bharathi *et al*.,2007)**.

Chemical constituents of the plants of the genus *Andrographis*

The genus *Andrographis* is rich in secondary metabolites particularly flavonoids. The following is a brief review of the scientific reports on isolation of flavonoidal compounds from the different species of *Andrographis.*

Andrographis paniculata

- Four flavonoids namely 7-*O*-methylwogonin, apigenin, onysilin and 3,4-dicaffeoylquinicwere isolated from *A.paniculata* (**Wen-Wan Chao et al., 2010)**
- Two new flavones, andropaniculosin and andropaniculoside and also 30 known compounds have been isolated from the plant *Andrographis paniculata (***Damu *et al*., 2008***)*
- Twelve flavonoids were isolated from the ethanol extract of ***Andrographis paniculata.*** Their structures were characterised by spectral analyses and chemical evidences **(Chen et al., 2006)**
- The roots and aerial parts of *Andrographis paniculata* yielded new flavones named 5-hydroxy- 7,2',6'-trimethoxyflavone and an unusual 23-carbon terpenoid, 14-deoxy-15-isopropylidene-11,12-didehydroandrographolide together with five known flavonoids and four known diterpenoids (**Rao *et al*., 2004**)

IJSER © 202
http://www.ijser.org

- **Rao *et al*.,(2004)** reported flavonoids and andrographolide diterpenoids, and other polyphenols from the whole plant of *Andrographis paniculata*

***Andrographis lineat*a**

- Three flavonoids, 5,7,2',3',4'-pentamethoxyflavone, 2'-hydroxy-2,4',6'-tri methoxychalcone and dihydroskullcap flavone together with 17,19,2'-trihydroxy-5, 8 H, 9 H,1' -labd-13-en-16,15-olactone , a known diterpenoid and six known flavonoids, 5-hydroxy-7,8-dimethoxyflavanone, 5-hydroxy-7,8,2',3',4'-pentamethoxyflavone, 5,2'-dihydroxy-7-methoxyflavanone , 5,2'-dihydroxy-7,8-dimethoxyflavone, 5,2'-dihydroxy-7-methoxyflavone and 5,2'-dihydroxy-7-methoxyflavone 2'-dglucopyranoside were isolated from the whole plant of *Andrographis lineat*a. The structures of these compounds were elucidated on the basis of spectral and chemical studies (**Hari Kishore *et al*., 2003).**

Andrographis affinis

- Three new 2'-oxygenated flavonoids, -5,7,2',3',4'-pentamethoxyflavanone , 5-hydroxy-7,8,2',5'-tetramethoxyflavone and echioidinin 2'-O-beta- glucopyranoside, together with four known flavonoids, 7-O-methyldihydrowogonin, 7-O-methylwogonin, skullcapflavone 2'-methyl ether, and skullcapflavone and two diterpenoids, andrograpanin and 14-deoxy-11,12-didehydroandrographolide, were isolated from the whole plant of *Andrographis affinis (***Reddy *et al*., 2003).**

Andrographis alata

- **Das *et al*.,(2006**), isolated, five acylated 5,7,2',6'-oxygenated flavone glycosides along with the known 5,2',6'-trihydroxy-7-methoxyflavone-2'-glucopyranoside from the whole plant of *Andrographis alata*. The structures of the compounds were established from 1D and 2D NMR spectral studies and chemical studies.
- **Damu et .al.,(1998),** isolated a new flavone glycoside, echioidinin 5-glucoside along with its known aglycone, echioidinin from the whole plant of *Andrographis alata*. Based on the spectral and chemical

IJSER

studies, the compounds structure was established as 5,2'-dihydroxy-7-methoxy favone 5-glucopyranoside.

Andrographis echioides

- Phytochemical investigation of the whole plants of *Andrographis echioides* afforded two new 2'-oxygenated flavonoids two new phenyl glycosides along with 37 known compounds. The structure of new compounds was elucidated byspectral analysis and chemical transformation studies (**Shen *et al*.,2013).**
- **Jayaprakasam *et al*.,(1999),** found a new flavanone, dihydroechioidinin together with four known flavones, echioidinin, echioidin, skullcap favone 2'-O-methyl ether and skullcap flavone 2'-O-glucoside. The structure of dihydroechioidinin was established as (2S)-5,2 '-dihydroxy-7-methoxy flavanone on the basis of spectral and chemical evidence.
- **Govindachari *et al*.,(1965),** isolated a new flavone glucoside echiodin from*Andrographis echioides and its structure was found to be* 5-hydroxy-2′-β-d-glucosidoxy-7-methoxyflavone (echioidinin-2′-β-d-glucoside).

IJSER

Andrographis viscosula

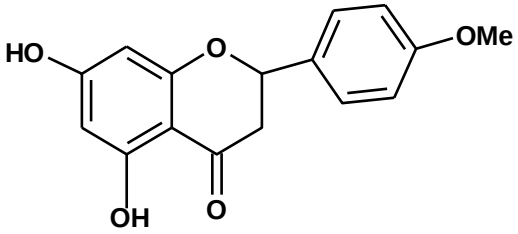
- Phytochemical investigation of the whole plant of *Andrographis viscosula* led to the isolation of three new 2'-oxygenated flavonoids, (2R)-5-hydroxy-7,2',3'-trimethoxyflavanone, 7,2',5'-trimethoxyflavone, 5,7,2',3'-tetramethoxyflavone and eight known flavones (**Rao *et al*.,2003).**
- **Rao *et al*., (2002)** isolated two new 2'-oxygenatedflavones, 5,7,2'-trimethoxyflavone and 5,7,2',4',6'-pentamethoxyflavone from the whole plant of *Andrographis viscosula.*

Chemical constituents of *Andrographis stenophylla*

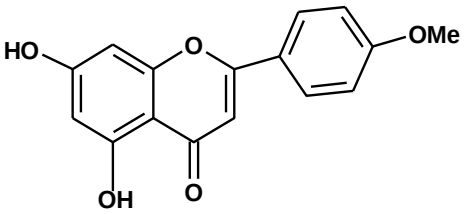
The phytochemical screening of the dichloroethane extract of the *Andrographis stenophylla* leaves showed the presence of a terpene and diterpenoid **(Parasuraman *et al*., 2010)**. Methanol extract of leaves of

IJSER © 202
http://www.ijser.org

Andrographis stenophylla was found to contain 2% oftotal phenolic compounds **(Neelaveni *et al*., 2010)**. Activity guided isolation of extracts of *Andrographis stenophylla*yielded secondary metabolites acacetine **(21)**, isosakuranetine **(22)** and andrographolide **(23)(Neelaveni *et al*, 2006)**.

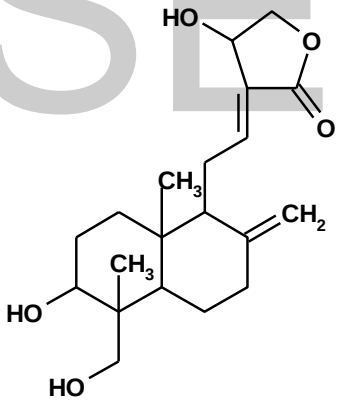


21



22

IJSER



23

Pharmacological Studies on *Andrographis stenophylla*

The dichloroethane extract of leaves of *Andrographis stenophylla* significantly reduced hypoglycaemia compared with the glucose treated group (**Parasuraman *et al*.,2010).** Various extracts of *Andrographis stenophylla tested for its* antioxidant potency, free radical scavenging activity and reductive ability revealed methanol extract to exhibit maximum antioxidant activity (**Neelaveni *et al*., 2010)**.

IJSER © 202
http://www.ijser.org

2.5 Bio-Pharma Potential of Andrographolide

Andrographolide is a natural di-terpenoid compound distributed in the *genera of Andrographis.Andrgraphis paniculata* **(Fujita *et al*., 1984)**, *Andrographis lineata* **(Hari *et al*., 2003)** and *Andrographis stenophylla* **(Neelaveni *et al*., 2010)** are sources of andrographolide and it is predominantly distributed in all the parts of *Andrgraphis paniculata* **(Koteswara *et al*., 2004, Mohmmed *et al*., 2013, Behera *et al*., 2010, Soumya *et al*., 2010, Priyanka et al., 2011, Bhan *et al*., 2006).**

Andrographolide exhibits immense biological activities. A literature review during the period from 1990 to till date revealed that the diterpenoid compound andrographolide is bestowed with anti-inflammatory, anti-diabetic, anti-oxidant anti-tumour, anti-ulcer and anti-arthritic activities. A report on neuroprotective effects on Parkinson disease by andrographolide is well established **(Zaijun *et al*., 2014).** It is a non-steroidal anti-inflammatory agent.

Andrographolide inhibits viral penetrations, synthesis of viral proteins and inhibits viral DNA polymerase There are more than 5000 consumer bands of andrographolide is currently available in the market which depicts the significance of the molecule **(www.pubchem.ncbi.nih.gov)**. Andrographolide, has traditionally been used for the treatment of colds, fever, laryngitis, and other infections with no or minimal side effects. Andrographolide treatment of mdx mice, an animal model for Duchenne muscular dystrophy (DMD), exhibited less severe muscular dystrophy than untreated dystrophic mice and was found to improve grafting efficiency upon intramuscular injection of dystrophin-positive satellite cells. These results suggest the prospects of andrographolide in improving the quality of life in individuals with DMD (**Cabrera, 2014**).

The protective effects of andrographolide on the development of autoimmune diabetes (NOD) was tested with mice. Oral supplementation of andrographolide significantly inhibited insulitis, delayed the onset, and suppressed the development of diabetes in 30-week-old NOD mice in a dose dependent manner (**Zhang,2013**).

IJSER

IJSER © 202
http://www.ijser.org

The anti-bacterial and anti-oxidant activity of andrographolide and echiodinin of *Andrographis paniculata* by broth micro-dilution method and DPPH assay, respectively, revealed andrographolide to be more effective against most of the strains tested including Mycobacterium smegmatis, showing broad spectrum of growth inhibition activity. Moderate anti-oxidant activity was noticed in the study and the results have been suggested to provide scientific rationale for the use of this plant in folkloric medicine **(Mohmmed,2013**).

Paracetamol overdose is often fatal due to progressive and irreversible hepatic necrosis. Engineered nanoparticles loaded with Andrographolide provides fast protection in Paracetamol induced acute liver failure due to the rapid regeneration of antioxidant capacity and hepatic GSH store (**Roy *et al*,2013**).

The study of effect of dietary andrographolide on growth, non-specific immune parameters and disease resistance against *Aeromonas hydrophila* infections in Indian major carp, Labeo rohita fingerlings revealed the fishes fed with formulated diet containing andrographolide to show significant stimulatory effect on non-specific immune parameters along with improved growth performance and increased disease resistance against A. Hydrophila infection in L. Rohita fingerlings (**Basha,2013).**

Andrographolide and its derivatives display anti-HIV activity *in vitro*. 3-nitrobenzylidene showed higher in vitro anti-HIV activity, whereas 2',6'-dichloro-nicotinoyl ester showed higher therapeutic index (**Uttekar *et al*.,2012).**A water-soluble polysaccharide (APP) isolated from *Andrographis paniculata* in synergism with andrographolide improved the metabolic abnormalities of diabetic mice and delayed the progression of diabetic renal complications suggesting its usefulness as a therapeutic agent for inhibiting the progression of diabetic nephropathy tested on renal complication in streptozotocin (STZ) induced diabetic mice. APP plus andrographolide increased the body weight and creatinine clearance rate (Ccr), and decreased the levels of serum creatinine, serum urea nitrogen, urinary albumin excretion

IJSER

IJSER © 202
http://www.ijser.org

(UAE), serum urea and blood glucose in diabetic rats, as well as the relative kidney weight (**Jie Xu,2012**).

Andrographolide extracted from the herb *Andrographis paniculata* exhibited concentration-dependent inhibition of human monocytic matrix metalloproteinases activation, induced by either tumor necrosis factor or lipopolysaccharide (LPS), in THP-1 cells suggesting new opportunities for the development of new anti-inflammatory and leukemic therapies **(Lee et al,2012**).

Andrographolide induces apoptosis of SiHa cells via suppression of HPV16 transcription activity, leading to decreased E6 oncoprotein and restored p53.These findings imply that the andrographolide may be an effective agent for cervical cancer prevention and treatment (**Fangkham,2012**). A series of andrographolide derivatives synthesized and evaluated for their anti-HIV activity in a cell-free virus infectivity assay using TZM-bl cell revealed andrographolide, 3-nitrobenzylidene derivative to show higher in vitro anti-HIV activity and 0-dichloro-nicotinoyl ester derivative to show higher Therapeutic Index suggesting andrographolide derivatives as promising candidates for prevention of HIV infection (**Uttekar,2012**).

IJSER

Acylated andrographolides synthesized through enzymatic acylation reactions using immobilized *Candida antarctica* lipase B (Novozym 435) as a biocatalyst revealed antibacterial activity against representative Gram-positive and Gram-negative bacteria with minimal inhibitory concentrations (MICs) as low as 4 microgram/mL **(Chen,2011).**

Inflammation and endothelial cell dysfunction are important initiating events in atherosclerosis. Tumor necrosis factor-R induces the expression of cell adhesion molecules and results in monocyte adherence and atheromatous plaque formation. Andrographolide a major bioactive diterpene lactone in Andrographis paniculata has anti-inflammatory activity. Heme oxygenase 1 plays a role in the inhibition of Tumor necrosis factor R (TNF-R) induced ICAM-1 expression by Andrographolide. The effect of Andrographolide on the IKK/NF-κB signaling pathway revealed Andrographolide to inhibit TNF-R-induced ICAM-1 mRNA and protein levels,

IJSER © 202
http://www.ijser.org

its expression on the cell surface, and subsequent adhesion of HL-60 cells to EA.hy926 cells suggesting Andrographolide to be a potent cardiovascular-protective agent (**Chao *et al*,2011**).

Andrographolide exhibits apoptosis of cancer cells at different concentrations portraying anticancer potential (**Jayakumar *et al*.,2010)**. Andrographolideshows potent immunomodulatory and anti-angiogenic activities in tumorous tissues (**Varma *et al*., 2011).** Andrographolide, neoandrographolide, isoandrographolide, andrograpanin, 14-deoxy- 11,12-didehydroandrographolide, 7-O-methylwogonin and skullcapflavone-I isolated from *Andrographis paniculata* exhibit anti-inflammatory/anti-allergic effects by modulating different inflammatory/allergic mediators and is suggested to provide useful phytomedical treatment against variety of inflammatory and allergic disorders (**Chandrasekaran,2011**).

Andrographolideanalogues, have been tested for their pharmacological potential. 14-deoxy-11,12-didehydroandrographolide(21) is immunostimulatory, anti-infective and anti-atherosclerotic;neoandrographolide is anti-inflammatory, anti-infective and anti-hepatotoxic; 14-deoxyandrographolide isimmunomodulatory and anti-atherosclerotic (**Chao *et al*.,2010)**.Oral administration of andrographolide at dosage of 50 mg/kg body weight of male mice was found to affect vascular reactivity and serum testosterone level in experimental animals in week4 suggesting the potential of andrographolide in enhancing sexual properties (**Sattayasai,2010**).

Andrographolide enhances 5-fluorouracil-induced apoptosis via caspase-8-dependent mitochondrial pathway involving p53 participation in hepatocellular carcinoma (SMMC-7721) cells suggesting its heady prospects in treating human carcinoma (**Yang,2009**).

Andrographolide possesses strong anti-inflammatory activity. The ability of andrographolide to inhibit the release of inflammatory cytokines in *in vitro* non-specific inflammation model reveals it to be anti-inflammatory drug that is active in vitro and in vivo, and affects both non-specific as well as antigen/antibody-dependent lung inflammation **(Abu-Ghefreh,2009).**

IJSER © 202
http://www.ijser.org

Invitroin vivo studies of the ethanolic extract of *Andrographispaniculata* and andrographolide reveal thepotential inhibitionof α-glucosidase and α-amylase enzymes (**Subramanian *et al*.,2008)**. The synthesis of andrographolide derivatives, 3,19-isopropylideneandrographolide, 14-acetyl-3,19-isopropylideneandrographolide and 14-acetylandrographolide , and their *in vitro* antitumour activity against breast cancer cell lines and colon cancer cell lines is reported(**Jada *et al*.,2007).** The antiangiogenic activity of *Andrographis paniculata* extract (APE) and its major component andrographolide (ANDLE) studied both *in vitro* and *in vivo* models revealed significantly inhibition of B16F-10 melanoma cell line induced capillary formation in C57BL/6 mice demonstrating the potent inhibition of tumour specific angiogenesis by APE and ANDLE **(Sheeja,2007**).

A series of analogues of andrographolide were found to be potent a-glucosidase inhibitors. Among them 23, 15-p-methoxylbenzylidene 14-deoxy-11,12- didehydroandrographolide showed comparatively higher activity with IC50 value was 16 lM **(Dai*et al*.,2006).**Urea adducts of andrographolide were isolated from human urine administrated with andrographolide(**Liang Cuia *et al*., 2008**). Larvicidal activity of andrographolide was reported by **Lingampally *et al*., (2012).**

IJSER

Review of literature pertaining to quantification of Andrographolide

A simple quantitative HPTLC method for determination of andrographolide in *Andrographis paniculata* at different stages of life cycle of crop from 30 days of plantation up to maturity of the crop was studied. Retention time for andrographolide was found to be 0.31. The average andrographolide content varied from 0.42% to 2.02% in the sample studied (**Sharma and Sharma,2013).** High performance liquid chromatographic (HPLC) and high-performance thin layer chromatographic (HPTLC) methods are established for quantitative determination of andrographolide **(Vijaykumar *et al*., 2007; Kumoro*a* and Hasan 2007; Senthil Kumaran *et al*.,2003).** The maximum andrographolide production by tissue culture was found to be 1.53 mg/g dry cell weight at the end of stationary phase during the growth curve.

IJSER © 202
http://www.ijser.org

The accumulation of andrographolide, was stimulated by the presence of yeast (**Gandi *et al*., (2012**).

Herbal powder and polyherbal formulation containing Andrographis peniculata were standardized and validated by high performance thin layer chromatographic method. Andrographolide in herbal powder and polyherbal formulations was identified and amount was estimated densitometrically by HPTLC (**jadhao , 2010)**

Biosynthesis of Andrographolide

Andrographolide, a diterpene lactone richly isolated from the species ***Andrographis paniculata*** was biosynthesised via mevalonate and non-mevalonate pathway. But the major biosynthetic pathway to this diterpenoid operates through non- mevalonate pathway which is known as deoxyxylulose (DXP) pathway **(Srivastava,2010**).

IJSER

IJSER © 202
http://www.ijser.org

3. MATERIALS AND METHODS

The present research work comprises isolation, characterization of chemical constituents and validation of herbal potential of folkloric medicinal plants ***Pisonia grandis* R.Br.** and ***Andrographis stenophylla* C.B Clarke**. The methodology adopted for the study is presented in the following sections

- Isolation and characterization of chemical constituents from the chosen plants ***Pisonia grandis*** and ***Andrographis stenophylla***
- Validation of the Herbal Potential of the Chosen Plants

The methodology for isolation and characterization of chemical constituents of the chosen plants involves:

- Systematic collection of stems, roots and leaves of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla***
- Sequential bulk extraction of the plant material
- Chromatographic isolation of chemical constituents of leaves stems and roots of *Pisonia grandis* and aerial parts of *Andrographis stenophylla*
- Characterization of isolated compounds by preliminary tests, physical data and spectral analysis

The methodology adopted for validation of herbal potential of chosen plants involves:

- ♣ Documentation of physical parameters of chosen plants
- ♣ NMR fingerprinting of extract concentrates of chosen plants
- ♣ Chemical standardization of extract concentrates of chosen plants with respect to the isolated biomarkers
- ♣ Biological standardization of extract concentrates and isolated compounds by anti-fungal, anti-oxidant, *in vitro* anti-cancer, *in vitro* wound healing, *in-vitro* anti-arthritic assays and *in vivo* wound healing studies

3.1.GENERAL

Chemical and Solvents Used

- All solvents used for extraction of plant material and isolation of constituents were purified by standard procedures **(Vogel, 2004)**.
- Chemicals and reagents used were of AR grade. Adsorbent for column chromatography: activated silica gel of 60-120 mesh (Acme brand)
- Adsorbent for TLC: silica gel (100 mesh) with 13 % $CaSO_4$.½ H_2O binder.

IJSER © 202
http://www.ijser.org

- Commercially available pre-coated aluminium sheets (TLC Silica gel 60 F_{254}) were also used for TLC.
- Visualisation of TLC spots: Sample spots in TLC were visualised under UV at 254 nm and 365 nm and in iodine vapours
- Spray reagent for TLC: ammoniacal silver nitrate, dimethylamino benzaldehyde, sulphuric acid (10%) and anisaldehyde reagent.
- Shift reagents used
- Sodium methoxide (Freshly cut metallic sodium dissolved in methanol)
- Anhydrous sodium acetate
- Boric acid
- Aluminium chloride (anhydrous aluminium chloride in methanol)

3.1.2. Instruments Used

- Toshiba electrical melting point equipment was used for determining melting point
- UV Double-beam spectrophotometer (Systronics 2202) was used recording UV spectra
- Infrared (IR) spectra were recorded on Shimadzu FT-IR (4000-400) spectrophotometer
- ^{1}H NMR, ^{13}C NMR, DEPT90, DEPT135, ^{1}H-^{1}H, COSY, HSQC and HMBC spectra were recorded on Burker Avance III 500 MHz Spectrophotometer. Deutriated solvents chloroform ($CDCl_3$), methanol (CD_3OD), dimethyl sulphoxide (DMSO-d6) and water (D_2O) were used for recording NMR spectra
- JEOL GCMATE II GC-MS instrument was employed for recording mass spectra
- GC-MS analysis was carried out in Hewlett–Packard 6890 gas chromatograph (Agilent Technologies, CA) connected to a HP5973 mass selective detector.
- HPLC analysis was done in SHIMADZU LC20AT instrument with CLASSVP software
- HPTLC analysis was done in CAMAG instrument with WINCATS software

IJSER © 202
http://www.ijser.org

3.2 Isolation and Characterization of Chemical Constituents from the Chosen plants *Pisonia grandis* and *Andrographis paniculata*

Plant Material

Two folkloric medicinal plants ***Pisonia grandis* R.Br.** of plant family ***Nyctaginaceae*** and ***Andrographis stenophylla* C.B Clarke** of plant family ***Acanthaceae*** were selected for the present investigation. The chosen plants collected from local areas (Saibaba colony and Marudhamalai) of Coimbatore were authenticated at the Institute of Forest Genetics & Tree Breeding (IFGTB) Coimbatore. Voucher specimens have been deposited in the herbarium of the Institute for further reference [F.No. 14932 for ***Pisonia grandis*** and Acc.No. 2413 for ***Andrographis stenophylla***]. The plant parts (leaves, stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** were pulverized into small pieces and air dried.

Bulk Extraction of Plant Material

Plant material for bulk extraction was collected from local area. Air-dried and pulverized parts of the chosen plant material (leaves, stem and roots of ***Pisonia grandis*** (1.5 kg each) and aerial parts of ***Andrographis stenophylla*** (1 Kg) were successively extracted with petroleum ether, ethanol and water. Two successive extractions of 3 hours duration each was carried out. Each extract was filtered; concentrated in vacuum and the residue weighed and stored.

IJSER

The extract concentrates have been designated as:

- PGLP, PGSP, PGRP (petroleum ether extract of leaves, stems and roots of ***Pisonia grandis*** respectively)
- DPGLE, DPGSE, DPGRE (dewaxed ethanol extract of leaves, stems and roots of ***Pisonia grandis*** respectively)
- PGLAq, PGSAq, PGRAq (aqueous extract of leaves, stems and roots of ***Pisonia grandis*** respectively)
- ASAP (petroleum ether extract of aerial parts of ***Andrographis stenophylla****)*
- DASAE (dewaxed ethanol extract of aerial parts of ***Andrographis stenophylla)***
- ASAAq (aqueous extract of aerial parts of ***Andrographis stenophylla***)

IJSER © 202
http://www.ijser.org

3.3 Column Chromatographic Isolation of Chemical Constituents

Isolation of chemical constituents from the various extract concentrates of chosen plants was mainly done by chromatographic methods.

Column Chromatographic Analysis

The extract concentrate of ***Pisonia grandis*** was dissolved in minimum volume of methanol and made into slurry with minimum amount of silica gel. The slurry was subjected to chromatographic separation over a column of silica gel (100 g) built in petroleum ether (PE) and eluted with increasing amounts of ethyl acetate (EA). Eluates of 100 ml were collected each time and the solvent was distilled off on a water bath. The homogeneity of the fractions was examined by TLC. The spots developed were visualized under UV light and then by exposure to iodine vapour. Similar fractions were combined and purified. Chart 2 shows the outline of the above procedure. A similar protocol as depicted in charts 3-5 was adopted for column chromatographic isolation of chemical constituents from various extract concentrates of both chosen plants. Chart 3 and 4 represent the column chromatography protocol adopted for isolation of chemical constituents of stems and roots of ***Pisonia grandis.*** Chart 5 represents the column chromatography protocol adopted for isolation of chemical constituents of aerial parts of ***Andrographis stenophylla.***

IJSER

IJSER © 202
http://www.ijser.org

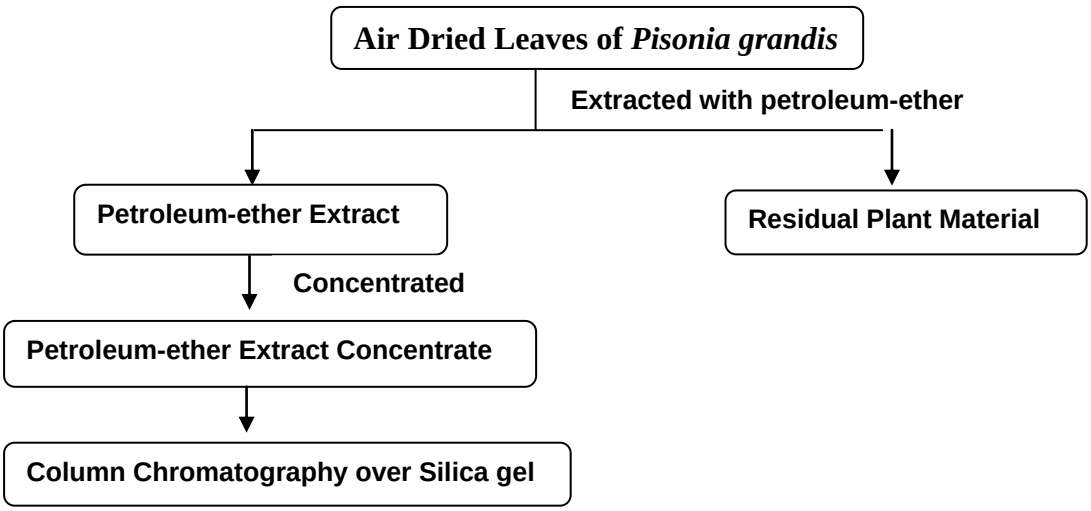
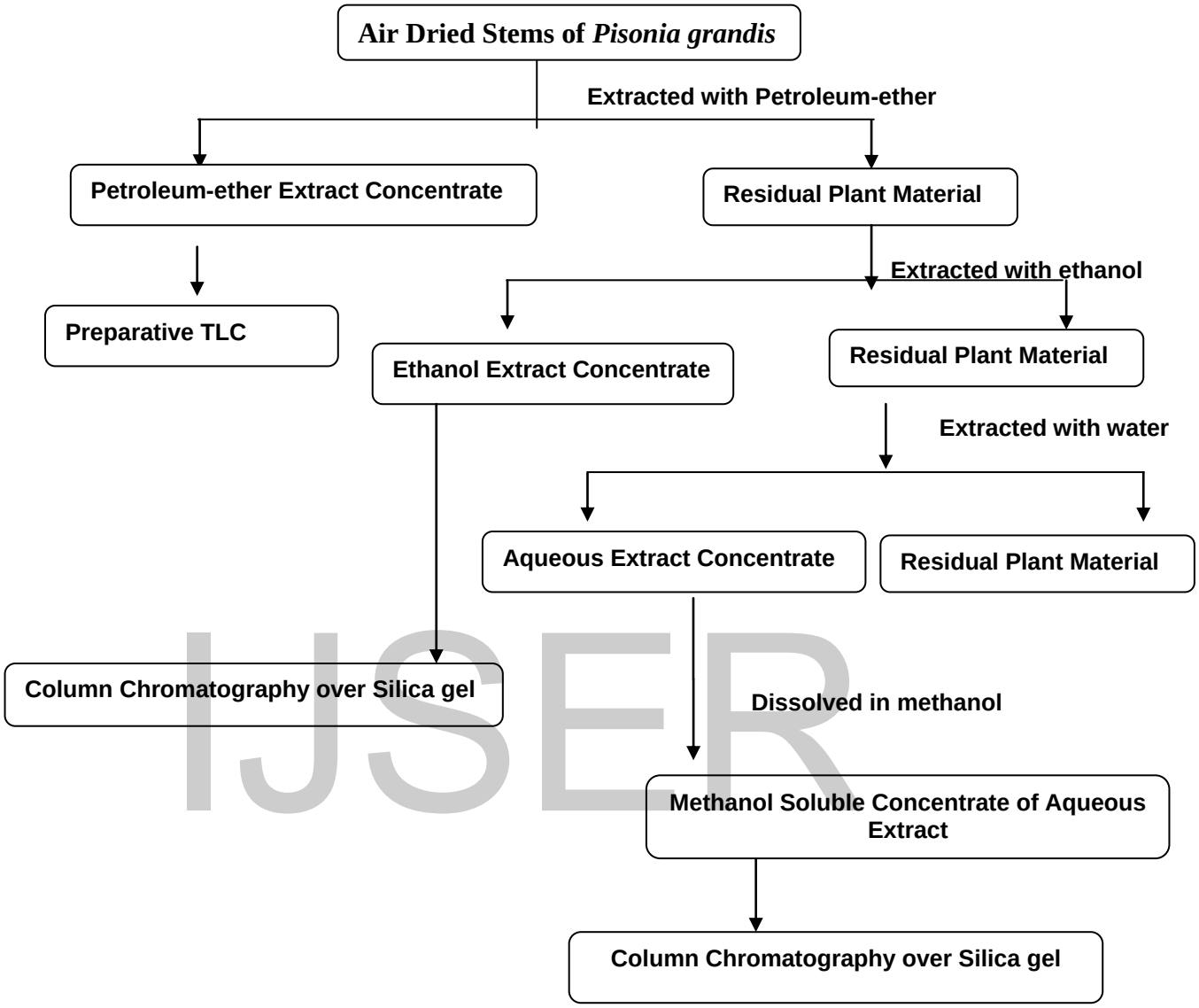


Chart 2 Outline of procedure adopted for isolating compounds from petroleum-ether extract concentrate of leaves of *Pisonia grandis*

IJSER

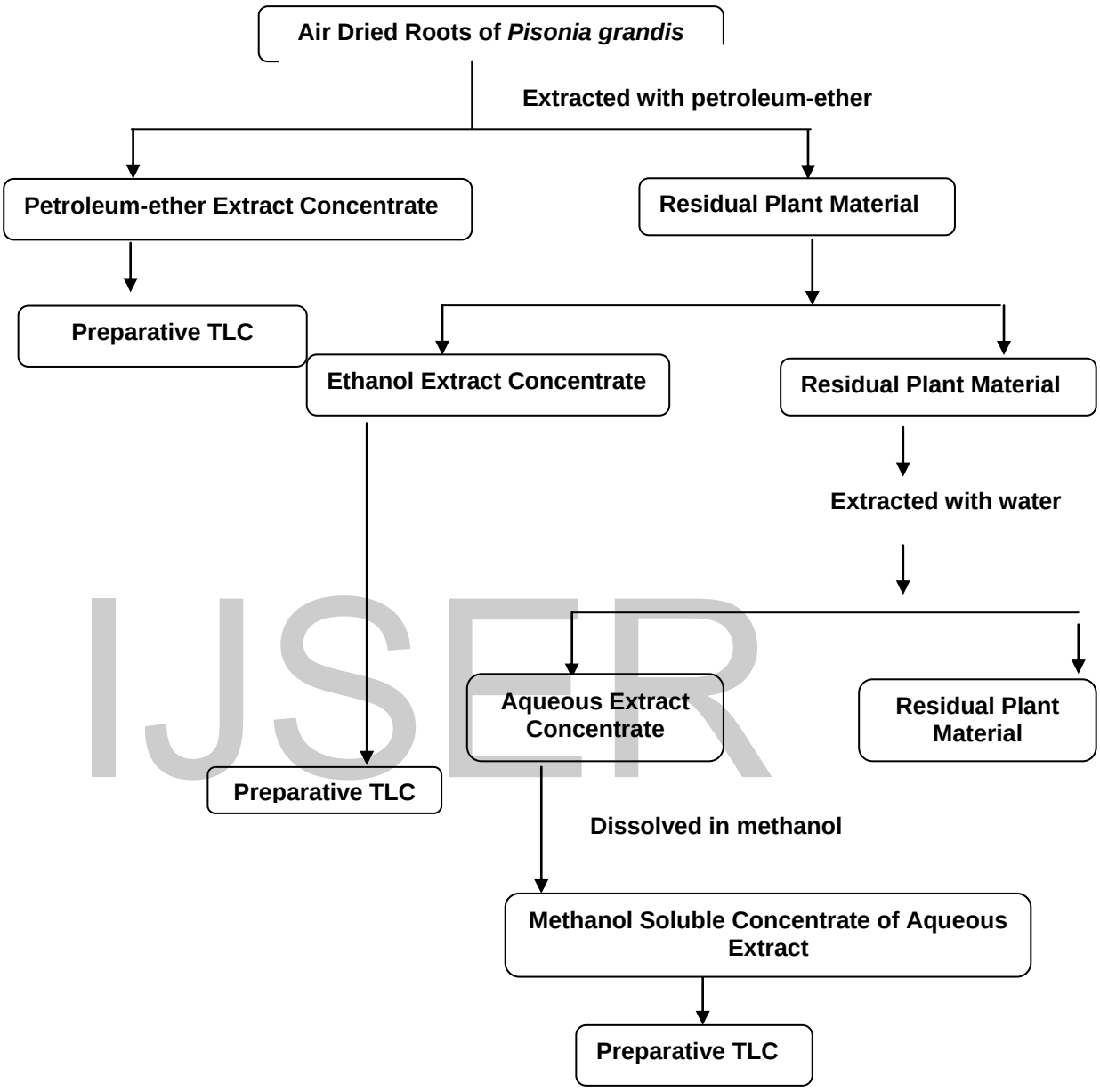
IJSER © 202
http://www.ijser.org



IJSER

Chart 3 Outline of procedure adopted for isolating compounds from extract concentrates of stems of *Pisonia grandis*

IJSER © 202
http://www.ijser.org



IJSER

Chart 4 Outline of procedure adopted for isolating compounds from extract concentrates of roots of *Pisonia grandis*

IJSER © 202
http://www.ijser.org

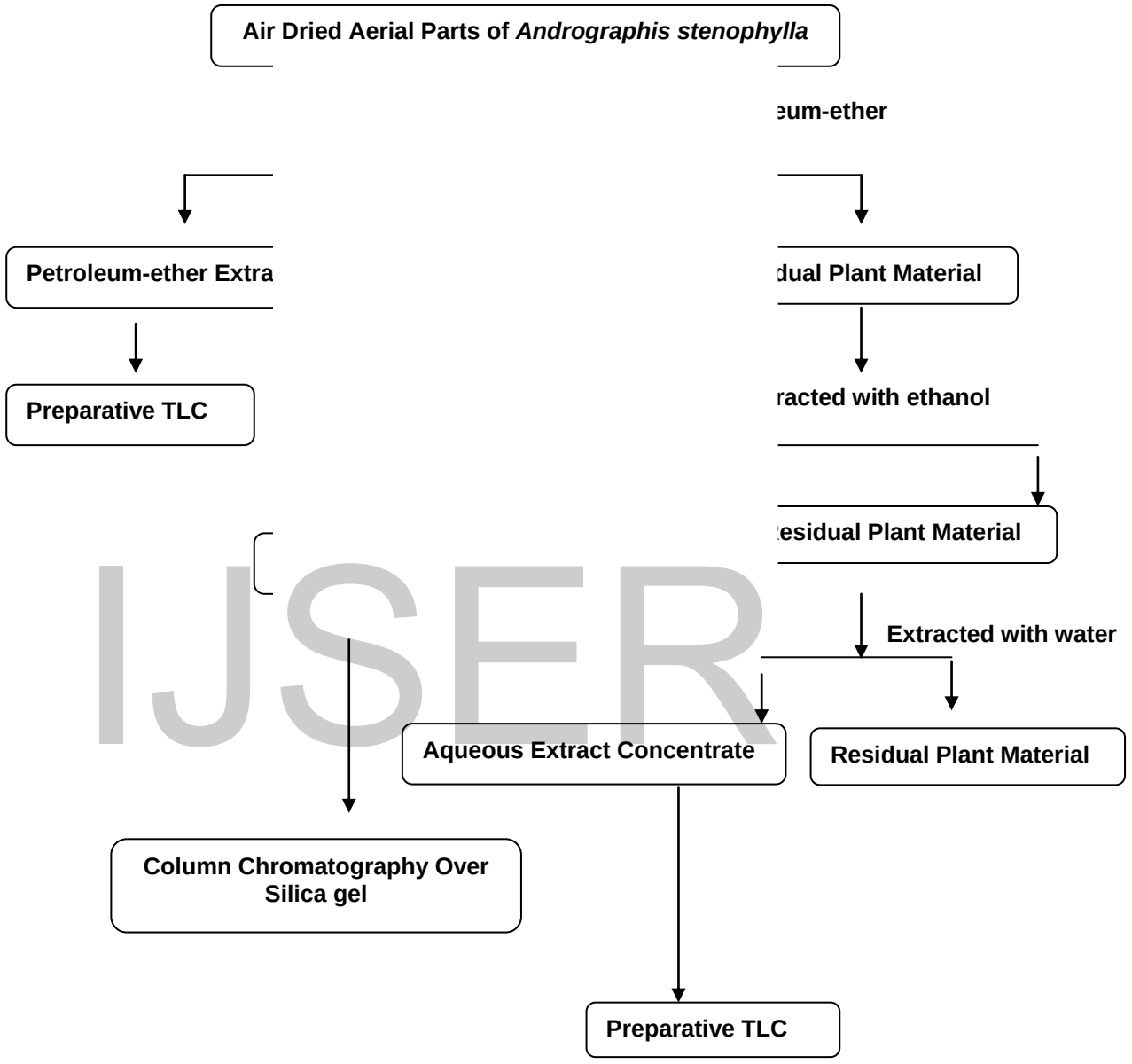


Chart 5 Outline of procedure adopted for Isolating compounds from extract concentrates of aerial parts of *Andrographis stenophylla*

IJSER © 202
http://www.ijser.org

3.4 Simple Method of Isolation of Constituents

A portion of the **dewaxed ethanol extract** of leaves, stem and roots of ***Pisonia grandis*** was also analysed for isolation of one or more of their constituents by a simple protocol adopting solvent fractionation and recrystallization. **Charts 6 and 7** represent the outline of the protocols adopted. **Chart 8** represents yet another simple method of isolation of constituents from the **dewaxed acetone extract** of the leaves of ***Pisonia grandis***. This work was taken up to analyse the feasibility of easy isolation of bio-active constituents from the extracts.

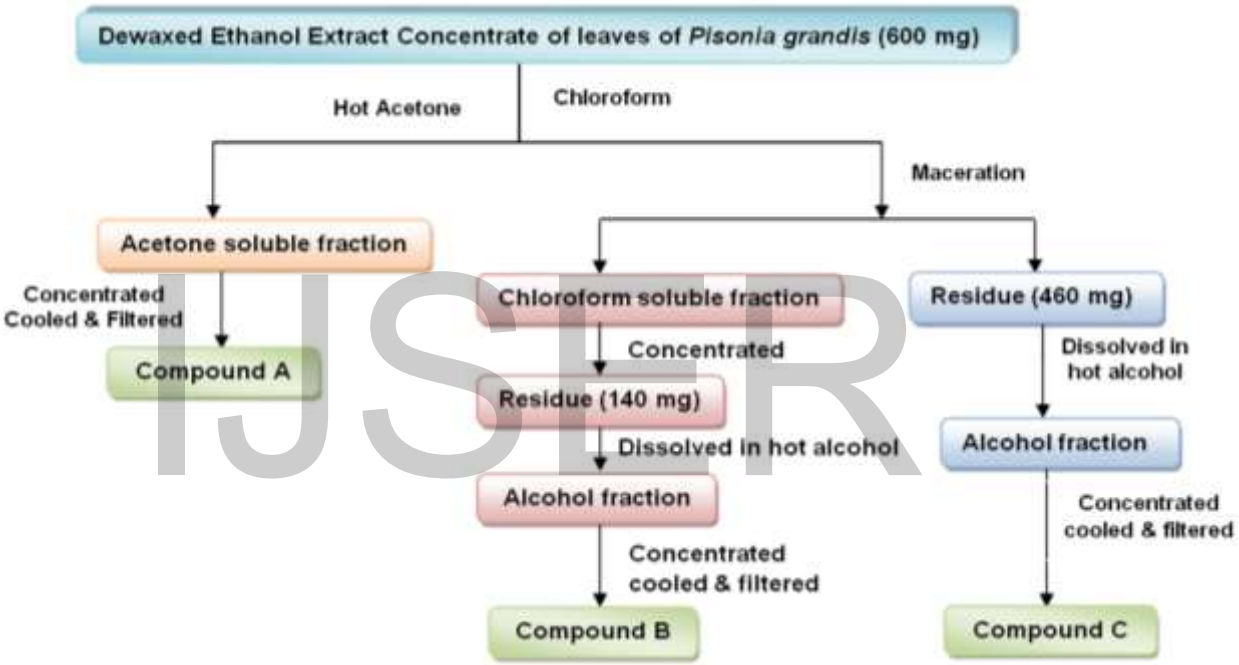


Chart 6

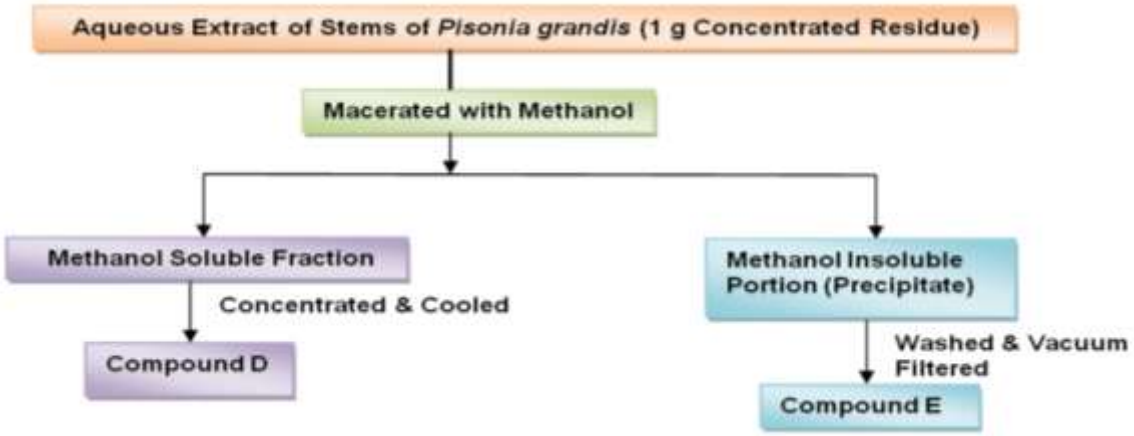


Chart 7

IJSER © 202
http://www.ijser.org

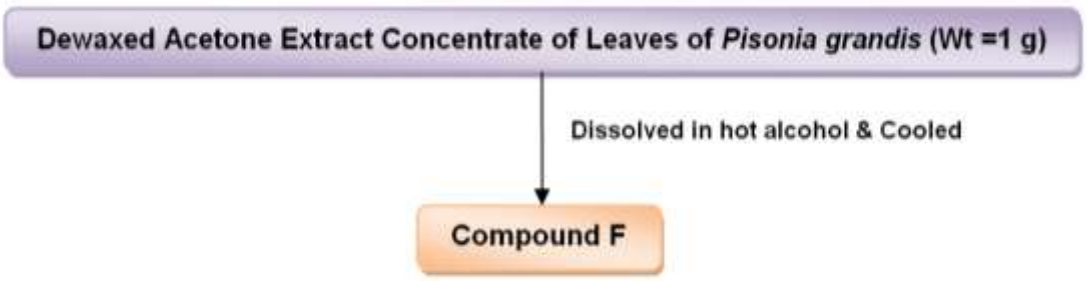


Chart 8

3.5 Characterization of Isolated Compounds

The isolated compounds were characterized by preliminary tests, physical data and spectral analysis. Preliminary colour tests were carried out for the isolated compounds by standard procedures**.** Melting points were determined in a digital melting point apparatus. Spectral techniques adopted for structure elucidation include UV, IR, 1D-NMR, 2D-NMR and Mass spectrometry. Wherever necessary, thermanalytical and X-ray diffraction techniques were utilised to augment the structural analysis of the compounds. Shift reagents were added to the methanol solution of the compound to study the shift in λ_{max} in UV spectrum and correlate the position of the hydroxyl groups of flavonoidal moieties if any.

IJSER

3.6 Validation of herbal potential of the chosen plants

In the present research work focus was also on standardization of the chosen folkloric plants and their extracts with a greater impetus on the chemical constituent characterisation and quantification of the extracts. This part of the research work will validate the herbal potential of the plant for use as a phytomedicine.

Herbal standardization was carried out by standard methods (**AOAC 2012**) as per protocol mentioned in The **Ayurvedic Pharmacopoeia of India (API) 2012**. The strategy adopted for the standardization is depicted in **Chart 9.**

IJSER © 202
http://www.ijser.org

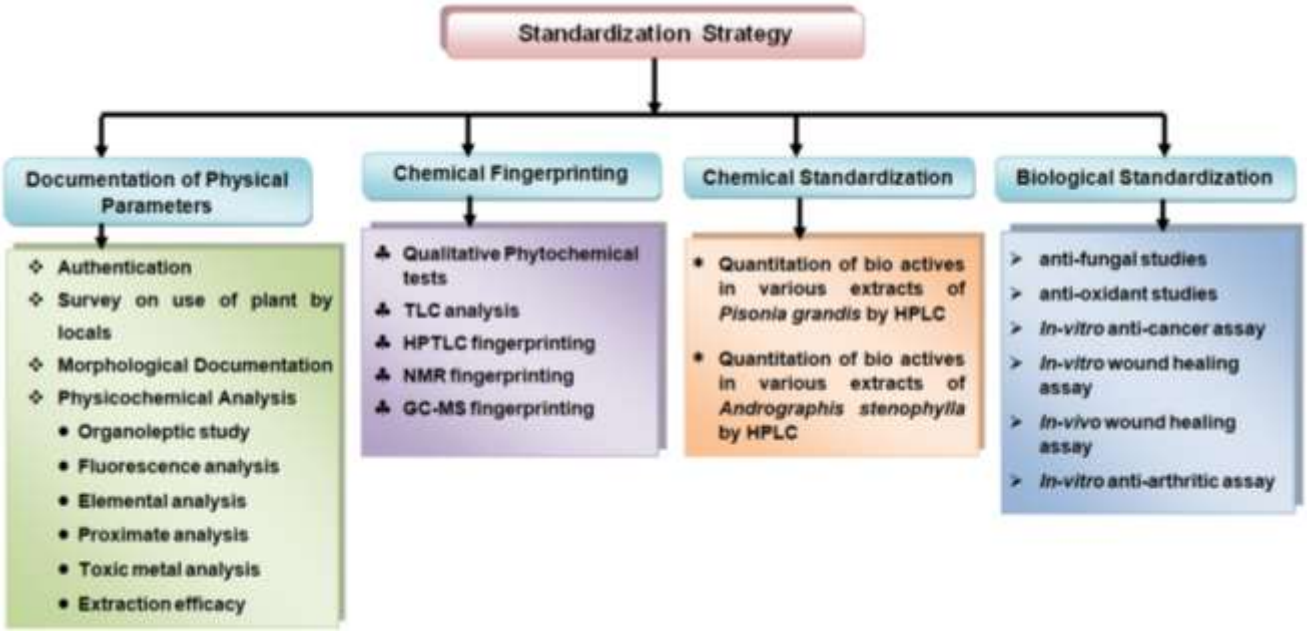


Chart 9

3.6.1 Documentation of Physical Parameters

A thorough documentation on scientifically generated data of traditional use of plants will project the herbals in a proper perspective and help in sustained global market.

IJSER

Rearing *Pisonia grandis* in Greenhouse

The plant *Pisonia grandis* was reared under greenhouse condition to compare its physiochemical data with that of the locally grown plant. This comparison was done since the plant ***Pisonia grandis*** (especially leaves) have been used by locals for internal consumption as an anti-diabetic agent and the safety of its internal use needs to be ascertained.

Greenhouse Conditions

The plant *Pisonia grandis* was grown in a greenhouse made of ultraviolet stabilized low density polyethylene sheets located at the Institute of Forest Genetics & Tree Breeding (IFGTB), Coimbatore during November 2011 to November 2012 under normal day-light conditions. Ten number of stem cuttings of *Pisonia grandis* were planted in plastic bags and kept in the greenhouse unit. Water was sprayed once in two days. A mechanical controller was used to control the spraying frequency. The growth of the plant

IJSER © 202
http://www.ijser.org

was monitored weekly. Four months later, two well grown plants were shifted to pots and further allowed to grow in the greenhouse condition. After a year of growth the leaves, stems and roots of the plant were harvested for physiochemical analysis.

Survey on use of the Chosen Plant by Local People

A survey on the folkloric use of the chosen plants ***Pisonia grandis*** and ***Andrographis stenophylla*** was carried out. Two local areas of Coimbatore namely Saibaba colony and Pothanur, where the plant ***Pisonia grandis*** was found to be grown widely along the road side, were chosen for the survey. A total of 64 houses were surveyed ***Pisonia grandis.*** The plant ***Andrographis stenophylla*** was indigenous to a local area called Marudhamalai. Twenty residents of that area were surveyed. The response from the local people was documented. Table 1 enlists the questions posed to the locals during the survey.

Table1 Survey Qusetion

IJSER

S.No.	Question
Q1	Do you know the name of the plant?
Q2	Are you growing this plant?
Q3	Do you use any of this plant parts (leaf, stem and root) for culinary purpose?
Q4	What recipe do you make with leaf of this plant?
Q5	Are you using it as a medicine?
Q6	For what ailments you have been using it?
Q7	How long have you been using it for medicinal use?
Q8	Do you make any decoction with leaves?
Q9	Do you use it for any other purposes apart from culinary and medicinal uses?

IJSER © 202
http://www.ijser.org

Morphological Documentation

The study of external features of the plant helps in selection of the correct species of the plant for formulation. Morphological data of both chosen plant has been documented in literature.(Parkinson1923)

Physicochemical Analysis

Organoleptic Study

Leaves, stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** were cleaned washed with water, air dried and pulverized. The colour, odour and taste of the selected plant parts was documented as per standard procedure **(Irfan et al., 2007).**

Fluorescence Analysis

Fluorescence study of the powders of plant material was carried out by standard protocol **(Nanna et al., 2013).** Around 0.5 g each of powdered leaves of ***Pisonia grandis*** was taken in 13 clean dry test tubes. Around 5 ml of the chosen solvents/regents (benzene, chloroform, acetone, ethylacetate, ethanol, methanol, iodine solution (5%), ferric chloride solution (5%), saturated picric acid solution, aqueous sodium hydroxide, alcoholic sodium hydroxide, conc. H_2SO_4, conc.HNO_3 and Conc.HCl and distilled water) was added one in each test tube. All the test tubes were shaken vigoursly and then allowed to stand for 25 mins and the contents filtered. The filtered solutions were observed under visible light and UV light and the fluorescence noted. The same was carried out for the pulverized stem and root material of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla.***

IJSER

Elemental Analysis

Elemental analysis of air-dried powdered parts of leaves, stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** was done. Each plant material was digested with a mixture of HNO_3 and $HClO_4$(5:2) and the solution made up to 50ml with HPLC grade water. The filtered solutions were analysed for their carbon, nitrogen, hydrogen, sulphur,

IJSER © 202
http://www.ijser.org

calcium, potassium, magnesium, sodium and zinc content in ICP-AES system. (Inductively Coupled Plasma - Atomic Emission Spectroscopy)

Proximate Analysis

Air-dried powdered parts of leaves, stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** were subjected to proximate analysis (**AOAC 2012**). Surface moisture, inherent moisture, ash, volatile matter, fixed carbon and gross calorific value (GCV) were determined as part of the analysis.

Determination of Surface Moisture

Air-dried and pulverized plant material (1g of 12.5 mm size) was taken in a petri dish and kept in a hot air oven for 1 hr. at 108°C. After an hour the petri dish was cooled in a desiccator and weighed. Percentage of surface moisture was calculated.

Percentage of Surface moisture= loss in weight / weight of the sample taken X100

Determination of Inherent Moisture

The residual plant material obtained after surface moisture analysis was finely powdered and taken in a petri dish. It was kept in a hot air oven for 1 hr. At 108°C,cooled in a desiccator and weighed. Percentage of inherent moisture was calculated.

IJSER

Percentage of Inherent moisture= loss in weight / weight of the sample taken X100

Determination of Ash Value

The air-dried plant material (2g) was taken in a pre-weighed silica crucible and heated at 450°C for about 1 hour in a muffle furnace and ignited to constant weight. After one hour the crucible was cooled in a desiccator and weighed. Percentage of ash was calculated.

Percentage of Ash = Residue weight / weight of sample X 100

Determination of Volatile Matter

Air-dried plant material was taken in a pre-weighed volatile matter crucible and heated at 900°C for about 7 minutes in a muffle furnace and ignited to constant weight. The crucible was cooled in desiccator and weighed. Percentage of volatile matter was calculated.

Percentage of Volatile Matter = Residue weight / weight of sample X 100

Determination of Gross Calorific Value

IJSER © 202
http://www.ijser.org

About 1 g of plant material was weighed in a crucible and placed inside the stainless-steel compartment of a bomb calorimeter. The decomposition vessel or bomb was filled with 30 bar of oxygen. The gross calorific value of the test substance was calculated from the temperature increase in the calorimeter. Water equivalent weight was calculated using benzoic acid.

$$\text{Gross Calorific Value} = \frac{(\text{Water Equivalent Weight} \times \text{Temp}^{\circ}\text{C}) - 31.1}{\text{Weight of the Sample}} \text{ Kcal/Kg}$$

Toxic Metal Analysis

The air-dried powdered plant material was digested with a mixture of HNO_3 and $HClO_4$ (5:2ml) and made up to 50ml using HPLC grade water. The filtered solutions were analysed is ICP-AES system to estimate the presence of lead, cadmium and arsenic.

Extraction Efficacy

Soaking, refluxing, and sonication methods were employed to find the extraction efficacy of solvent used for extracting the plant material.

IJSER

3.7 Chemical finger printing

A chemical finger printing has become one of the powerful tools of herbal research linking chemical constituents with biological potential of the plant **(Priya *et al*., 2012)**. Qualitative phytochemical tests, TLC analysis, HPTLC fingerprinting, NMR fingerprinting and GC-MS fingerprinting are effective tools for identifying biomarkers of plants. These techniques were adopted to document the chemical finger prints of the plant extracts.

Qualitative Phytochemical Tests

Qualitative phytochemical screening of the various extract concentrates of leaves, stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** was done as per standard protocol **(Harborne 2009**). The following tests were done with each of the extract concentrates and all the isolated compounds.

a) Test for alkaloids (Wagner's reagent, Mayer's reagent)

b) Test for steroids (Liberman-Burchard reagent, Salkowski reagent)

c) Test for flavonoids (Schinoda test, ferric chloride test)

IJSER © 202
http://www.ijser.org

d) Test for reducing sugar (Fehling's test)

e) Test for carbohydrates (Molish test)

f) Test for phenolic compounds (ferric chloride test)

g) Test for protein and amino acids (Ninhydrin test)

TLC Analysis

TLC analysis was done to analyse the homogeneity of the column eluates as well as the isolated compounds.

Preparation of TLC Plates

A known quantity of silica gel was (G) weighed and shaken well to a homogeneous suspension with distilled water. The suspension was poured onto a glass plate and dried in air overnight and then activated in the oven at 120^0C for two hours **(Harbone, 2009).**

Development of TLC plates

The plates were developed in the following solvent system

Pet-ether - ethyl acetate mixture (9:1 V/V) for pet-ether extracts
Chloroform: methanol mixture (7:3 V/V) for dewaxed ethanol extracts
Butanol: acetic acid: water mixture (5:0.5:4.5 V/V/V) for aqueous extracts
Chloroform: methanol: water mixture (6:3.5:0.5) for biomarker pinitol
Chloroform: methanol: watermixture (6:4:3 drops) for biomarker allantoin
Pet-ether - ethyl acetate mixture (10:0.25 V/V) for biomarker and ographolide

IJSER

HPTLC Fingerprinting

The extract concentrates of leaves, stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** were analysed by HPTLC to record the fingerprints of the extracts. The analysis was done Camag HPTLC system consisting of a Camag Linomat V semi automatic spotting device connected to a nitrogen tank and WinCATS 4 software (version 4.05, Camag)

Standard and Test solution preparation

Samples (10mg) and standard (1mg) were dissolved in 200 μl of dimethyl sulfoxide (DMSO).Each solution was centrifuged at 3000rpm for 2min and used for HPTLC analysis.

Sample application

IJSER © 202
http://www.ijser.org

The given standard solution and the sample were loaded as 6mm band length in a 3 x 10 Silica gel 60F_{254} TLC plate using Hamilton syringe.

Development

The sample loaded plate was kept in a twin trough developing chamber (after saturation with solvent vapour) in the optimised solvent system.

Photo-documentation

The developed plate was kept in a photo-documentation chamber and the images captured under visible light and UV light.

Derivatization

The developed plate was sprayed with a suitable spray reagent and dried at 100°C in a hot air oven and photo-documented in day light and UV 254nm mode in a photo-documentation chamber.

Scanning

Scanning of the developed TLC plate was done under white light. The peak display and peak densitogram details were noted.

IJSER

Optimized HPTLC protocol for extracts of Pisonia grandis and isolated biomarker pinitol

Mobile phase : chloroform-methanol-water (6: 3.5:0.5)

Spray reagent : Ammoniacal silver nitrate reagent

Detection : Yellowish brown coloured zones in day light.

Optimized HPTLC protocol for extracts of Andrographis stenophylla and isolated biomarker andrographis

Mobile phase : ethyl acetate: ethanol (10: 0.25)

Spray reagent : H_2SO_4(5 %)

Detection : Yellowish brown coloured zones in day light.

NMR Fingerprinting

^{1}H NMR and ^{13}C NMR spectra of pet-ether, dewaxed-ethanol and overall ethanol extract of leaves, stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** were recorded on BrukerAvance III 500MHz spectrophotometer using deutiriated dimethyl sulphoxide (DMSO-d6).

IJSER © 202
http://www.ijser.org

GC-MS Fingerprinting

GC-MS analysis was carried out in a Hewlett–Packard 6890 gas chromatograph (Agilent Technologies, CA) coupled to a HP5973 mass selective detector. Interpretation of mass spectrum was done with reference to National Institute of Standard and Technology (NIST) database. The relative percentage of the chemical constituents in crude extracts is expressed as percentage by peak area normalization.

Method Specification

- Column used: Agilent Ultra 2 fused silica capillary column (12 m length, 0.2 mm internal diameter)
- Carrier gas: Helium
- Flow rate: 1 ml/min
- Sample injection: Splitless mode
- Initial Temperature: 100 ^{o}C
- Final Temperature: 400 ^{o}C
- Concentration of the sample: 1 ppm
- Run time: 36 min

IJSER

3.8 Chemical Standardization

Chemical standardization by chromatographic techniques has become one of the powerful approaches for quantifying biomarkers of plants. High Performance Liquid Chromatography (HPLC) is a simple, quick, well resolved and reliable technique to quantity the biomarkers **(Parvin et *a*l., 2012).**

Quantification of Bio-actives in Extracts of the Chosen Plants by HPLC

Quantitation of biomarkers in the extracts of the chosen plants was carried out using a Shimadzu HPLC system. The extract concentrates of leaves, stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** and isolated compounds were analysed by HPLC.

IJSER © 202
http://www.ijser.org

Table 2 Method specification for HPLC

Method specification	Extracts of *Pisonia grandis* and its biomarkers		Extracts of *Andrographis stenophylla* and its biomarkers
Column	Amine column	C18 column	C18 column
Mobile phase	Acetonitrile : Water (70:30)	Acetonitrile: Phosphate buffer (20:80)	Acetonitrile : Water (30:70)
pH	-	3.5	-
Detector	RI (Refractive Index)	PDA – 200nm	PDA – Spectra max
Flow rate	1 ml/min		1.5 ml/min
Run time	20 minutes		

3.9 Biological standardization
Fractionation of Extract Concentrates

Each of the concentrated plant extracts was fractionated further. The fractionates and extract concentrates were analysed for their anti fungal potential to find the most active fraction.

The plant material (250g) was extracted with ethanol for 6 hours at reflux temperature. The extract was filtered and concentrated. A small portion of concentrated extract was set aside for testing its antifungal activity and the rest was macerated with equal volume of water and extracted with equal volume of chloroform. The liquid-liquid extraction (LLE) with chloroform was continued until the chloroform layer was colourless. The chloroform and aqueous layers were distilled under vacuum and a small portion of residues were stored for anti-fungal study. The remaining chloroform residue was dissolved in 10% aqueous ethanol for further extraction with petroleum ether. The LLE with petroleum ether was continued until the organic layer was colourless. The organic and aqueous layers were distilled under vacuum and the residue was stored for anti-fungal study. Chart 10 outlines the procedure adopted

IJSER

IJSER © 202
http://www.ijser.org

Assessment of *In-vitro* Anti-fungal Activity by Disk Diffusion Method

The concentrate extracts, extract fractionates of and isolated compounds in both plants were tested for their *in-vitro* anti-fungal activity by adopting standard protocol **(www.microlibrary.org)**

Method specification:

- Microorganisms: *Monoscus purpureus, Candida albicans and Aspergillus niger*
- Standard used: Clotrimazole
- Concentration of test solutions: standard disc of 10µg capacity
- sample disc of 100 µg capacity

Sample Designation

Leaf Extracts of ***Pisonia grandis***: PGLE, DPGLE, PGLP and the fractionates PGLC, PGLW, PGLW2, PGLP2

Stem Extracts of ***Pisonia grandis***: PGSE, DPGSE, PGSP and the fractionates PGSC, PGSW, PGSW2, and PGSP2

Root Extracts of ***Pisonia grandis***: PGRE, DPGRE, PGRP and the fractionates PGRC, PGRW, PGRW2, and PGRP2

Aerial parts of ***Andrographis stenophylla***: ASAE, DASAE, DASAP, and the fractionates ASAC, ASAW, ASAW2, and ASAP2

IJSER

Assessment of Anti-Oxidant Activity

DPPH Radical Scavenging Assay

The radical scavenging potential of extracts of ***Pisonia grandis*** and ***Andrographis stenophylla*** was assessed by standard method (**Nikhat *et al.* 2009)**.

- Standard used: ascorbic acid
- Concentration of test solutions:0.1mM solution of DPPH and varying concentration (10 to 60µg)of the extract
- Measured Wavelength: 517 nm
- The percentage of inhibition of the extract was calculated using the following equation:

IJSER © 202
http://www.ijser.org

- **DPPH Scavenged (%) = $A_{Control} - A_{Test} / A_{Control} \times 100$**

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts

Assessment of *In-vitro* Cytotoxicity study by Trypan Blue Exclusion Method

Short term *in-vitro* cytotoxicity study of extract concentrates of ***Pisonia grandis*** and ***Andrographis stenophylla*** was done using Dalton Lymphoma Ascites (DLA) cell line by trypan blue exclusion method. Percentage of dead cell was calculated using the formula:

$$\% \text{ Dead Cell} = \frac{\text{Number of Dead Cell}}{\text{Number of Viable Cell + Number of Dead Cell}} \times 100$$

Assessment of *In-vitro* Anti-arthritic Activity by Inhibition of Albumin Denaturation

Inhibition of albumin denaturation can be taken as a measure of *in-vitro* anti-arthritic activity **(Santhosh et al., 2013)**. The extract concentrates of Pisonia grandis have been assed for their anti-arthritic potential by a *In-vitro* method of inhibition of protein Denaturation which can be taken as a necessary in In-vitro anti-arthritic activity.

- Method name: Albumin denaturation assay
- Standard used: Diclofenac sodium
- Concentration of test solutions: extract at varying concentration (50 to 800µg)
- Control: double distilled water paper
- Measured wavelength: 660 nm
- **% of Inhibition was calculated using the formula**

% of Inhibition = 100 X [V_t/V_c -1]

V_t = absorbance of test sample; V_c= absorbance of control

IJSER © 202
http://www.ijser.org

Wound Healing Study

Assessment of *In-Vitro* Wound Healing Study by Angiogenic Model

The chick CAM (chorioallantoic membrane model) model was used as an *in vitro* model to assess angiogenic activity of extract concentrates of ***Pisonia grandis*** and ***Andrographis stenophylla***. **(Basanta *et al*., 2012).**

- ❖ Concentration of test solutions: 10mg of extract
- ❖ Control: Normal egg
- ❖ Blank: DMSO

Assessment of *In-vivo* Wound Healing Study by Incision Wound Model

In the present study incision wound healing activity of ointment prepared from the extract of ***Pisonia grandis*** was analysed by incision wound model **(Akkol *et al*., 2012)**.

IJSER

Preparation of Ointment

The simple ointment base used for this study was prepared by fusion method. Constituents of the base:

- Wool fat (0.5g),
- Hard paraffin (0.5g)
- Yellow soft paraffin (8.5g),
- Cetostearyl alcohol (0.5g).

The ingredient of the base were mixed and heated gently with stirring and then cooled. To 10 g of ointment base 1 g of dewaxed ethanol extract of leaves of *Pisonia grandis* was mixed and stirred well until a homogenous mass was obtained.

Incision Wound Healing Activity

Healthy male Albino Wistar rats (150 - 250 g) were used for the study. The animals were left for 3 days at room conditions for adaptation with the environment. The rats were anaesthetized with diethyl ether prior to and during creation of the wounds. The dorsal fur of the animals was shaved with an electrical clipper. A longitudinal paravertebral incision of 4cm long was made through the skin and cutaneous tissue on the back. After the incision,

IJSER © 202
http://www.ijser.org

the parted skin was sutured 1cm apart using a surgical thread and curved needle. The wounded animals were divided into 4 groups containing five animals each

- Control group (rats left untreated wound)
- Standard group (rats treated with providone iodine ointment)
- Extract group (rats treated with 10 % ointment)
- Vehicle group (rats treated with simple ointment base).

The ointment was applied once daily for ten days. When the wounds cured completely, the sutures were removed. Tensile strength was measured and histopathological parameters were assessed by standard procedure**.** Results are expressed statistically. (**Prabhu et al., 2008**)

IJSER

IJSER © 202
http://www.ijser.org

4. RESULTS AND DISCUSSION

The results pertaining to the present work titled **"Isolation, Characterization of Chemical Constituents and Validation of Herbal Potential of two Folkloric Medicinal Plants - *Pisonia grandis* R.Br. and *Andrographis stenophylla* C.B Clarke"** and the ensuing discussions are presented in this section.

4.1 Bulk Extraction of Plant Material

Air-dried and pulverized parts of the chosen plant material (leaves, stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla***) were successively extracted with petroleum ether, ethanol and water. The extract concentrates are designated as PGLP, PGSP, PGRP (petroleum ether extracts of leaves, stems and roots of ***Pisonia grandis*** respectively); DPGLE, DPGSE, DPGRE (dewaxed ethanol extracts of leaves, stems and roots of ***Pisonia grandis*** respectively); PGLAq, PGSAq, PGRAq (aqueous extracts of leaves, stems and roots of ***Pisonia grandis*** respectively); ASAP (petroleum ether extract of aerial parts of ***Andrographis stenophylla***); DASAE (dewaxed ethanol extract of aerial parts of ***Andrographis stenophylla***) and ASAAq (a aqueous extract of aerial parts of ***Andrographis stenophylla***). Table 3 lists the yield of the extracts.

IJSER

Table 3 Yield of the crude extracts

Plant parts used	Dry weight (Kg)	Yield (%)		
		Petroleum ether extract	Dewaxed ethanol extract	Aqueous extract
***Pisonia grandis* - Leaves**	0.75	3.47	17.26	20.11
***Pisonia grandis* - Stems**	0.75	0.27	4.68	12.5
***Pisonia grandis* - Roots**	0.75	0.53	5.36	13.67
***Andrographis stenophylla* - aerial parts**	0.75	1.87	10.19	9.85

IJSER © 202
http://www.ijser.org

The yield of extract is high with polar solvents alcohol and water. Leaves of *Pisonia grandis* are found to contain a higher percentage of constituents compared to its stem and roots. Both chosen plants are rich sources of polar constituents.

Preliminary Phytochemical Screening

The extract concentrates of ***Pisonia grandis*** and ***Andrographis stenophylla*** **were** subjected to preliminary phytochemical screening by carrying out suitable colour tests. The results are tabulated in Table 4

IJSER

IJSER © 202
http://www.ijser.org

Table 4 Phytochemical screening of extract concentrates of leaves stem and roots of *Pisonia grandis* and aerial parts of *Andrographis stenophylla*

	Leaves of *Pisonia grandis*			Stems of *Pisonia grandis*			Roots of *Pisonia grandis*			Aerial Parts of Andrographis stenophylla		
Tests For	**PGLP**	**DPGLE**	**PGLAq**	**PGSP**	**DPGSE**	**PGSAq**	**PGRP**	**DPGRE**	**PGRAq**	**ASAP**	**DASAE**	**ASAAq**
Alkaloids	-	-	-	-	+	+	-	+	+	-	+	+
Flavonoids	-	-	+	-	+	+	-	+	+	-	+	+
Phenols	-	+	+	-	+	+	-	+	-	-	+	+
Steroids	+	+	+	+	+	~ +	+	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-	-	+	-	+	+	-
Tanins	-	+	+	-	+	+	-	+	-	-	+	+
Carbohydrate	-	+	+	-	+	+	-	+	+	-	+	+
Saponins	-	+	+	-	+	+	+	~+	+	-	~+	~+
Aminoacids	-	+	+	-	+	+	-	+	~+	-	~+	~+
Lassaigne's test for Nitrogen	~+	+	+	-	+	+	-	+	+	-	~+	~+

+ = Positive to colour test; - = Negative to colour test; ~+ = May be positive

IJSER

IJSER © 202
http://www.ijser.org

4.2 Isolation and Characterization of Chemical Constituents from Chosen Plants *Pisonia grandis* R.Br. and *Andrographis stenophylla* C.B Clarke

Isolation of chemical constituents from the hitherto unexamined extracts of the chosen plants was achieved by chromatographic analysis. Main focus was on isolation of constituents from the polar extracts of the chosen plants. The separation and characterisation of isolated compounds is presented in the following pages.

Isolation of Constituents of *Pisonia grandis* R.Br.

Column chromatographic analysis of dewaxed ethanol extract concentrate of stems of Pisonia grandis gave 500 fractions. Further TLC analysis yielded 16 main fractions from which five compounds were isolated in the pure state

IJSER

- ♠ Fractions eluted with petroleum ether and ethyl acetate (20:80) yielded a dull white solid which on recrystallisation yielded a white solid designated as **PGS-1**
- ♠ Fractions eluted with ethyl acetate and ethanol (97:3) yielded a dark brown solid which on recrystallization yielded a pale brown solid designated as **PGS-2**
- ♠ Fractions eluted with ethyl acetate and ethanol (94:6) yielded a pale brown solid which on recrystallization gave a colourless solid designated as **PGS-3**
- ♠ Fractions eluted with ethyl acetate and ethanol (92:8) yielded a colourless solid designated as **PGS-4**
- ♠ Fractions eluted with ethyl acetate and ethanol (70:30) yielded a colourless solid designated as **PGS-5**

Column chromatographic analysis of methanol soluble portion of aqueous extract concentrate of stems of Pisonia grandis led to the isolation of three compounds.

IJSER © 202
http://www.ijser.org

- Fractions eluted with ethyl acetate and ethanol (85:15) yielded a brown solid which on recrystallization gave a colourless solid designated as **PGS-6**
- Fractions eluted with ethyl acetate and ethanol (80:20) yielded a brown solid which on recrystallization yielded a colourless solid designated as **PGS-7**
- Fractions eluted with ethyl acetate and ethanol (70:30) yielded a brown solid which on recrystallization gave a pale brown solid designated as **PGS-8**

The hitherto un investigated non-polar petroleum ether extract concentrates of leaves, stems and roots of Pisonia grandis were also analysed for phyto constituents.

The petroleum ether extracts concentrate of leaves of Pisonia grandis on column chromatographic analysis of yielded 250 fractions which upon TLC analysis yielded 16 major fractions.

IJSER

Fractions eluted with petroleum ether (100%) yielded a brown solid which on recrystallization gave a colourless solid designated as **PGL-1.** The other fractions did not yield sufficient quantity of compounds. These column fractions were analysed for their anti arthritic potential to find the most active fraction since non-polar constituents like palmitic acid have been reported to possess anti arthritic potential **(Aparna *et.al.,* 2012)**.

A preparative method of isolation of constituents from the petroleum ether extract concentrate of stems of *Pisonia grandis* was adopted to obtain a colourless solid designated as PGS -9

Further characterisation of the non-polar extracts of *Pisonia grandis* was done by GC-MS analysis and the results will be presented later.

4.3 Isolation of Constituents of *Pisonia grandis* by a Simple Protocol

A direct method of isolation of constituents of polar extract concentrates of leaves, stem and roots of Pisonia grandis was attempted in order to isolate one or more of their constituents by a simple protocol adopting solvent fractionation and recrystallization. Charts 7-9 depict the isolation protocol. The detail of constituents isolated directly from the concentrated extracts is listed below.

IJSER © 202
http://www.ijser.org

- The acetone soluble fraction of the ethanol extract of **leaves** of ***Pisonia grandis*** yielded a compound designated as **PGL-2** and its chloroform soluble fraction yielded a compound designated as **PGL-3 (chart 7)**
- The alcohol soluble fraction of the dewaxed ethanol extract of **leaves** of ***Pisonia grandis*** yielded a compound designated as **PGL-4 (chart 7)**
- The dewaxed acetone extract concentrate of leaves of ***Pisonia grandis*** on maceration with alcohol yielded a white solid designated as **PGL-5 (chart 7)**
- The methanol insoluble portion of aqueous extract concentrate of stems of *Pisonia grandis* on maceration with alcohol yielded mixture of two compounds as a pale white solid. Recrystallisation with water afforded a white solid designated as PGS-10. The filtrate on cooling yielded a white solid designated as PGS-11 **(chart 8)**
- The methanol soluble portion of aqueous extract of stems of ***Pisonia grandis*** on concentration and further purification yielded a solid designated as **PGS-12 (chart 8)**

IJSER

4.4 Isolation of Constituents from *Andrographis stenophylla*

The column chromatographic analysis of dewaxed ethanol extract concentrate of aerial parts of *Pisonia grandis* yielded 312 fractions which on TLC analysis yielded 16 main fractions. Further work up of the major fractions led to the isolation of five compounds in the pure form

* Fractions eluted with petroleum ether-ethyl acetate (70:30) yielded an yellow coloured solid designated as **ASA-1**
* Fractions eluted with petroleum ether-ethyl acetate (70:30) yielded a pale green solid designated as **ASA-2**
* Fractions eluted with petroleum ether-ethyl acetate (40:60) yielded a pale green coloured solid designated as **ASA-3**
* Fractions eluted with ethyl acetate-ethanol (70:30) yielded a pale yellowish green coloured solid designated as **ASA-4**

IJSER © 202
http://www.ijser.org

* Fractions eluted with ethyl acetate-ethanol (40:60) yielded a pale green coloured solid which on re-crystallization gave a colourless solid designated as **ASA-5**

4.5 Isolation of Constituents of *Andrographis stenophylla* by a Simple Protocol

The methanol insoluble portion of aqueous extract concentrate of aerial parts of ***Andrographis stenophylla*** on alcohol purification yielded a compound designated as **ASA-6**

4.6 Characterization of Isolated Compounds

All the isolated compounds were characterized by preliminary tests, physical data and spectral analysis. Spectral techniques used for structure elucidation include UV, IR, 1D-NMR, 2D-NMR and mass spectrometry techniques. Wherever necessary, thermanalytical and X-ray diffraction techniques were utilised to augment the structural analysis of the compounds.

IJSER

4.3.1. Characterization of compounds isolated from dewaxed ethanol extract concentrate of stems of *Pisonia grandis*

Characterization of PGS-1

Compound **PGS-1** was obtained by elution with petroleum ether and ethyl acetate (20:80) Yield: 115 mg. Melting point: 268-274°C. TLC R_f =0.56 (chloroform: methanol: water (9:1:3 drops). It gave positive Libermann-Buchard test

Spectral data of **PGS-1**:

IR (KBr) $\gamma_{max}cm^{-1}$: 3370, 2948, 2868, 1641, 1450, 1372, 10261, 1022 **(Figure 7)**

1H NMR (500MHz, DMSO, δ ppm):0.65-0.67(3H), 0.76-0.79(3H), 0.81-0.80(3H), 0.83-0.84(3H), 0.90-0.91(3H), 0.96(3H), 4.21-4.23(H), 5.04(2H), 5.13-5.18(2H), 5.33(H) **(Figure 8)**

^{13}C NMR (125 MHz, $CDCl_3$, δ): **Table 5 & Figure 9; DEPT 135 NMR spectra (figure 10)**

EI-MS: m/z = 412[M^+] calculated for $C_{29}H_{48}O$ **(Figure 11)**

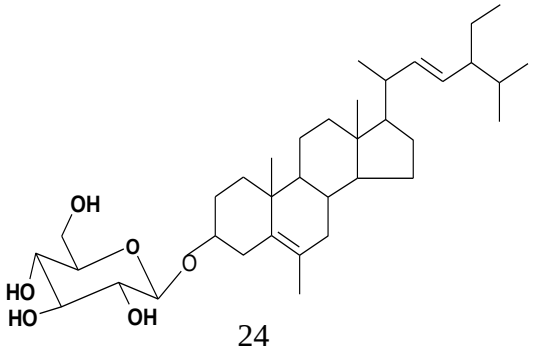
Compound **PGS-1** was inferred as a steroid moiety from the Libermann-Burchard test. The IR spectrum revealed a characteristic

IJSER © 202
http://www.ijser.org

absorption due to hydroxyl group at 3370 cm^{-1}. Strong absorption bands at 3370 cm^{-1} and 1021 cm^{-1} are characteristic of a glycoside. The absorption at 2948 cm^{-1} and 2868 cm^{-1} indicate the largely aliphatic nature of the molecule. The molecular mass of **PGS-1** was found to be 412 from mass spectrum indicating a molecular formula of $C_{29}H_{48}O$. The EI mass spectrum gave a fragment at *m/z* 396 due to the loss of glucose noveity from the molecular ion peak.

The ^{1}H NMR spectrum **(Figure 8)** indicated two tertiary methyl groups at δ 0.65-0.67 and δ 0.96; three secondary methyl groups at δ 0.90-0.91; δ 0.83-0.84 and δ 0.81-0.80 and one primary methyl group at δ 0.79. The olefinic protons exhibited signals at δ 5.33; δ 5.13 -5.18 and δ 5.00-5.06. The anomeric proton appeared at δ 4.21-4.23.

^{13}C NMR spectrum **(Figure 9)** of **PGS-1** showed the existence of 35 carbons in the molecule. The signal at δ 101.3 due to one anomeric carbon indicated the presence of one monosaccharide unit in the compound. The four methine carbons at δ 70.5, δ 73.9, δ 76.7 and δ 77.2 and methylene carbon at δ 61.7 were assigned to C-2', C-3', C-4', C-5' and C-6' of a β-D-glucopyranoside moiety. Further analysis of **^{13}C NMR** data and its comparison with that reported in literature **(Ahmad *et.al.,* 2012)** led to the characterisation of **PGS-1** as stigmasterol glucoside (24).



24

IJSER

IJSER © 202
http://www.ijser.org

Table 5 ^{13}C NMR chemical shifts δ of PGS-1

Chemical Shift δ (ppm)					
Carbon number	PGS-1	Literature Data Ahmad *et al.*(2012)	Carbon number	PGS-1	Literature Data Ahmad *et al.*(2012)
C-1	38.5	38.3	C-19	19.3	19.0
C-2	34.3	33.3	C-20	34.3	35.3
C-3	76.7	76.9	C-21	19.4	18.8
C-4	37.0	36.8	C-22	138.0	137.9
C-5	142.0	140.4	C-23	129.7	128.8
C-6	124.6	121.0	C-24	32.2	31.2
C-7	31.7	31.3	C-25	34.3	31.2
C-8	32.5	31.4	C-26	19.4	19.3
C-9	49.4	49.6	C-27	18.8	18.9
C-10	37.0	36.1	C-28	23.3	23.8
C-11	22.0	22.6	C-29	15.8	11.6
C-12	41.0	41.7	1’	101.3	100.7
C-13	42.5	41.8	2’	70.5	70.1
C-14	55.8	56.2	3’	77.2	76.6
C-15	24.8	24.7	4’	73.9	73.4
C-16	29.5	29.2	5’	76.7	76.6
C-17	56.0	56.1	6’	61.7	61.1
C-18	12.2	11.8			

IJSER

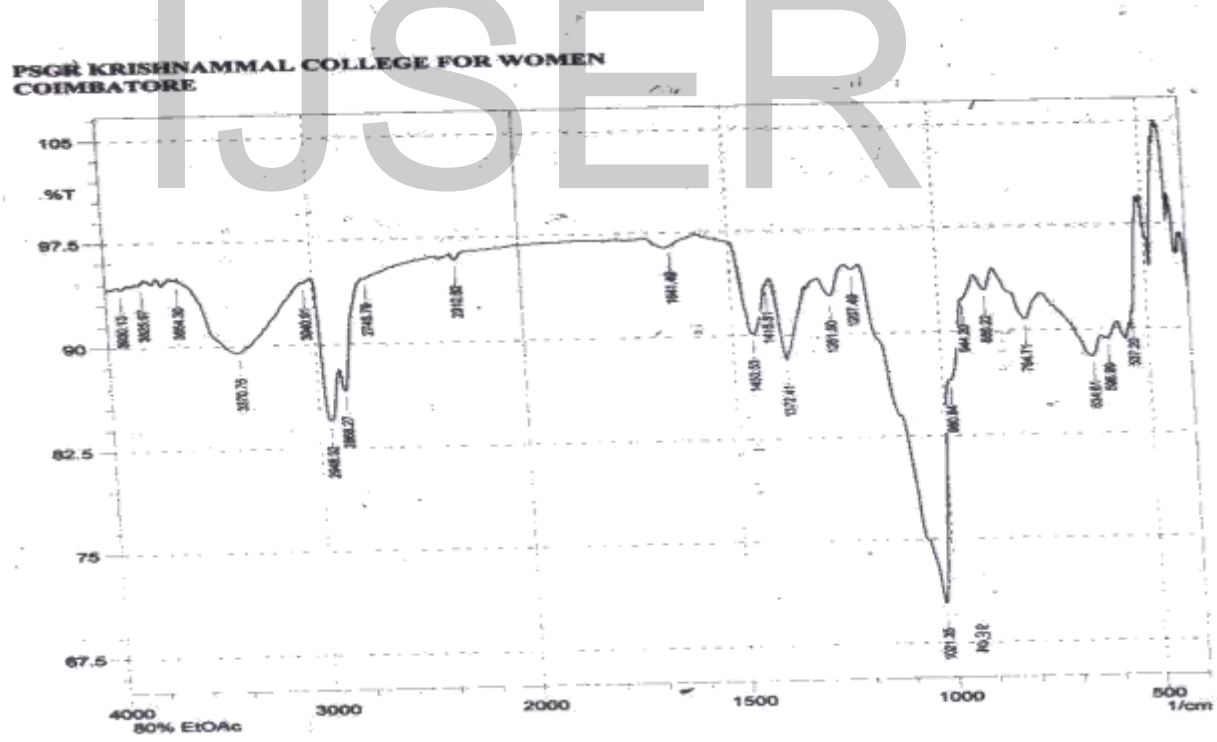


Figure 7
IR spectrum of PGS-1

IJSER © 202
http://www.ijser.org

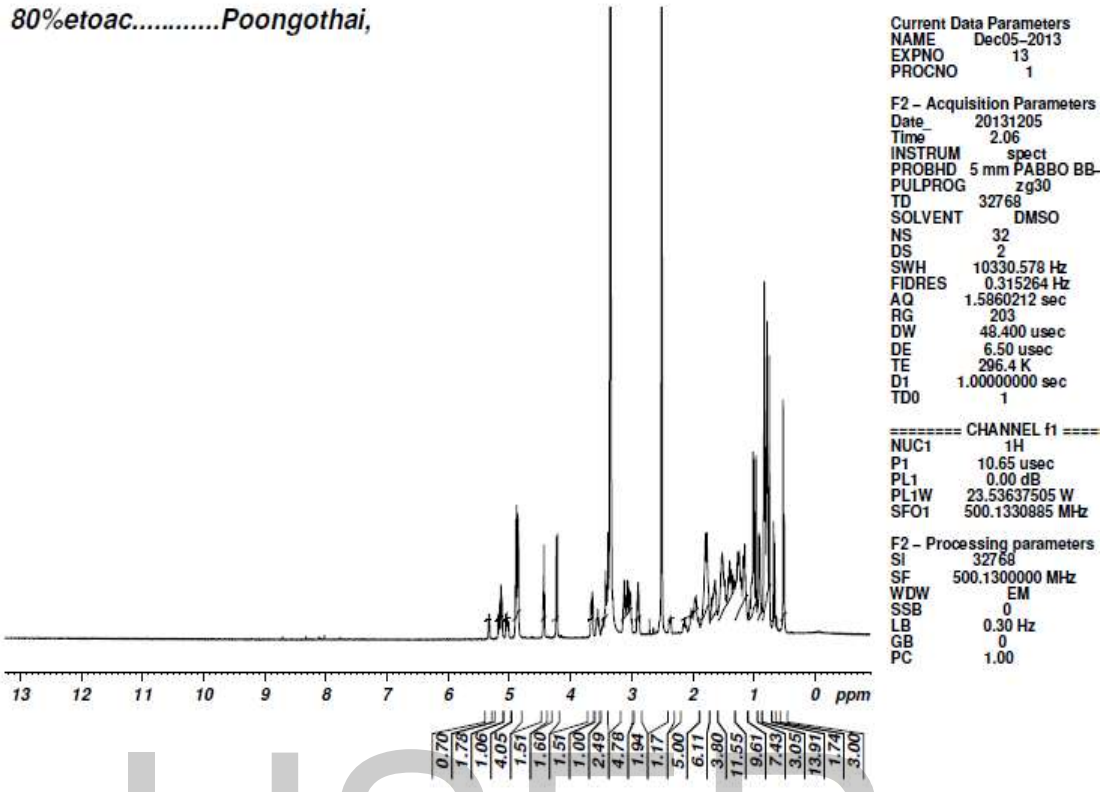


Figure 8
^{1}HNMR spectrum of PGS-1

IJSER

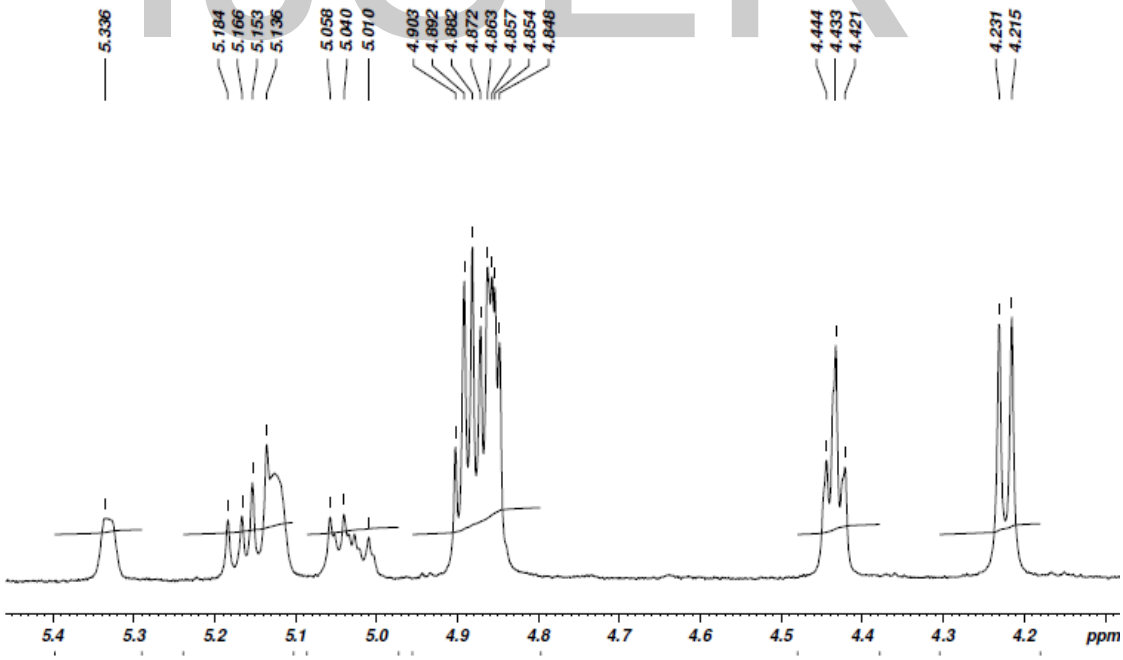


Figure 8a
Expansion of ^{1}HNMR spectrum of PGS-1

IJSER © 202
http://www.ijser.org

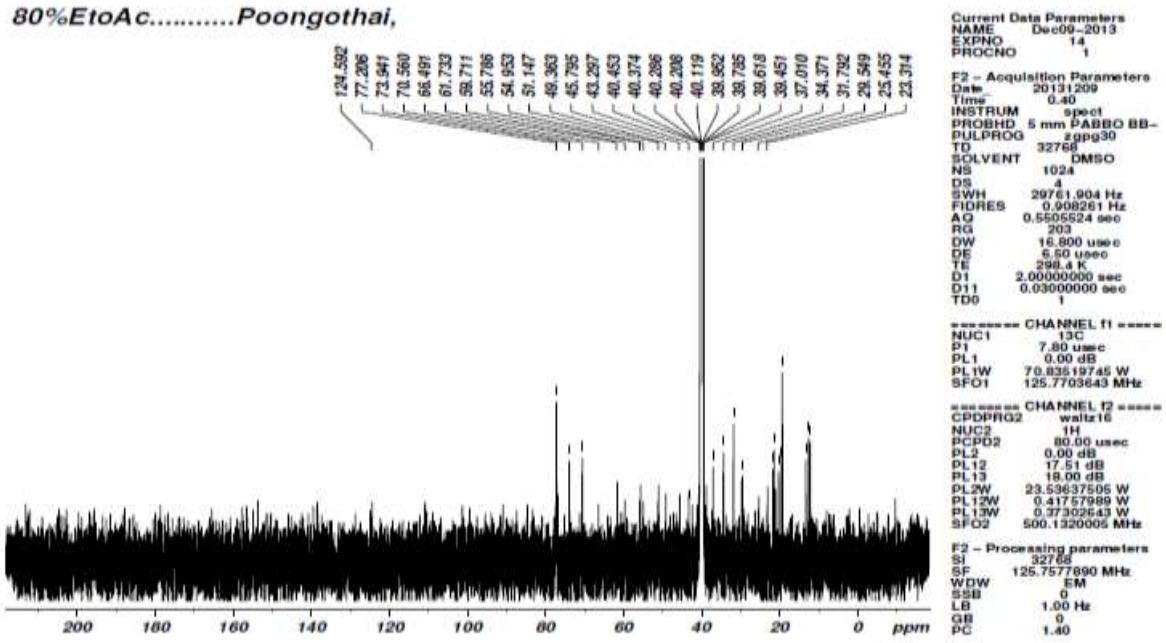


Figure 9
^{13}CNMR spectrum of PGS-1

IJSER

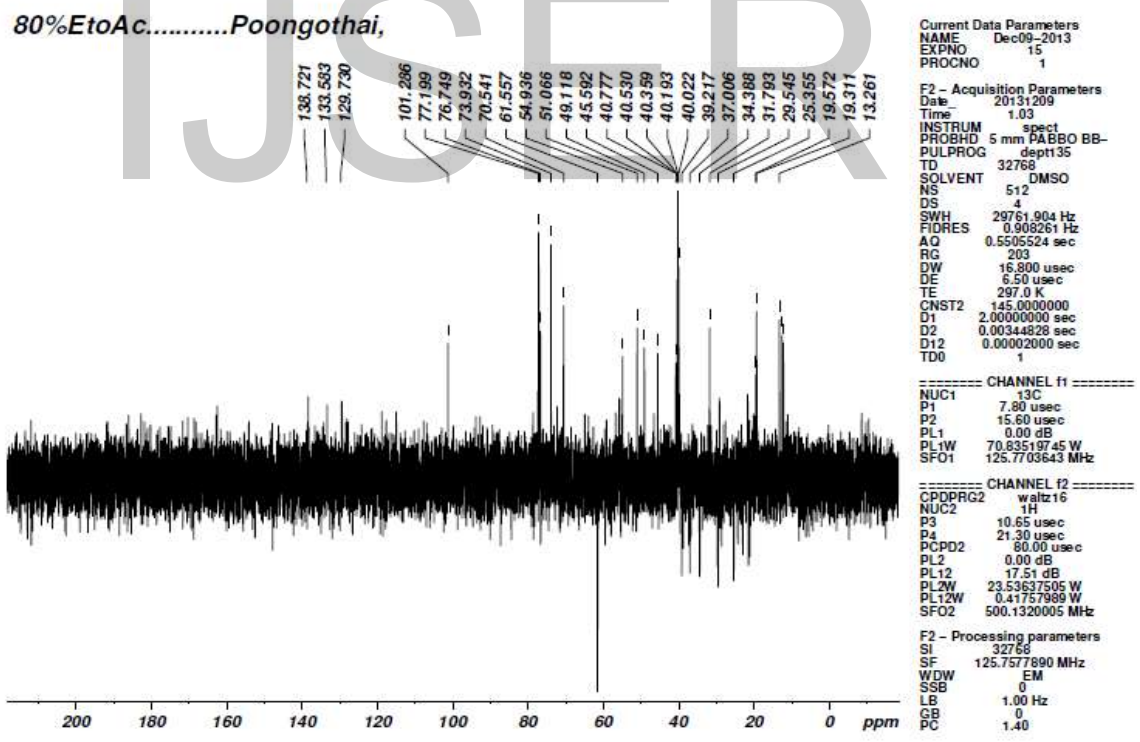


Figure 10
DEPT 135 NMR spectrum of PGS-1

IJSER © 202
http://www.ijser.org

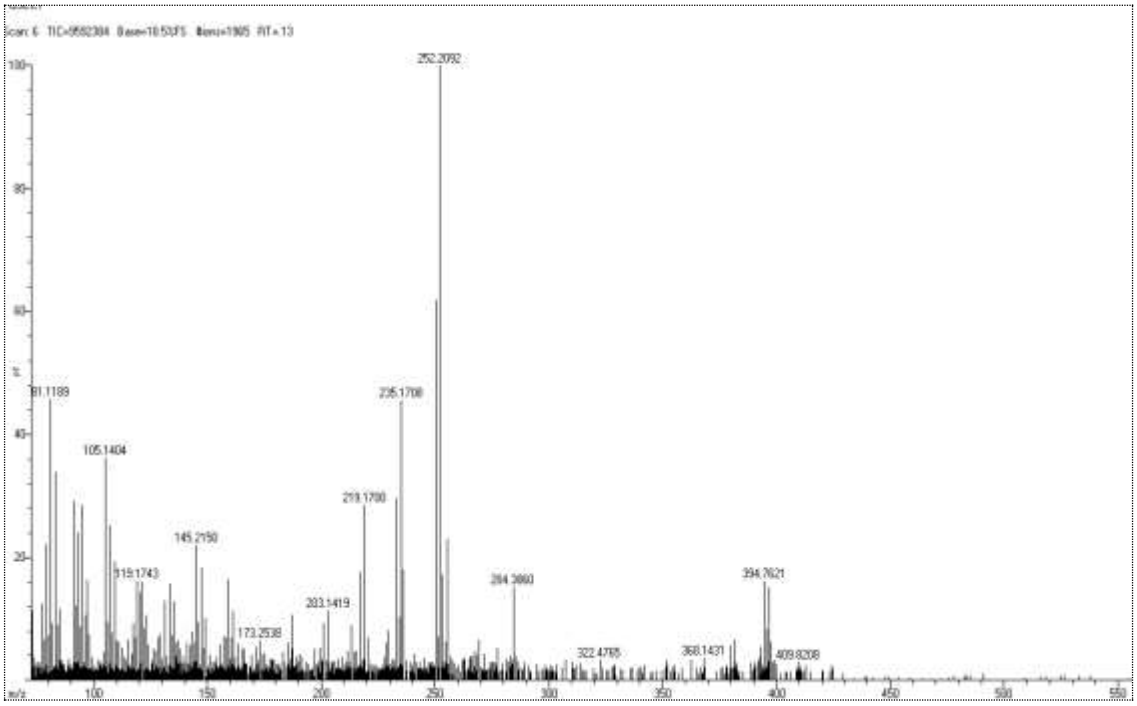


Figure 11
Mass spectrum of PGS-1

IJSER

4.3.2 Characterization of PGS-2

PGS-2 was isolated as a pale brown solid**.** Yield: 158 mg. Melting Point: 222-228°C.

TLC R_f = 0.62 (chloroform: methanol: water 6:4:3 drops).

Spectral data:

IR (KBr) $\gamma_{max} cm^{-1}$: 3435, 3339, 1778, 1706, 1655, 1600, 1524, 1427, 1393, 1326, 1272, 1183, 1058, 1015, 806 **(Figure 12)**

1H NMR (500MHz, DMSO, δ ppm):δ 5.23 - 5.25 (d, 1H) , 5.89 (s, 2H) , 6.88-6.89 (d, 1H) , 8.06 (s ,1H) and 10.54 (br. s ,1H). **(Figure 13)**

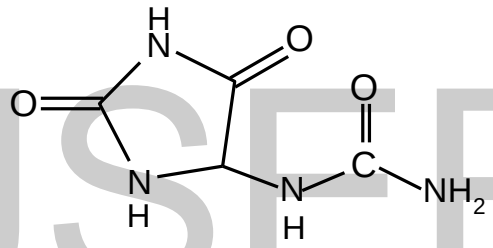
^{13}C NMR (125 MHz, $CDCl_3$, δ): δ 62.9, 157.2, 157.8 and 174.0. **(Figure 14)**

EI-MS: m/z = 158 $[M^+]$ calculated for $C_4H_6N_4O_3$ **(Figure 15)**

The compound **PGS-2** did not show good solubility in organic solvents but was soluble in water and DMSO thus indicating the high polar nature of the compound. It was not positive to the colour tests meant for the common class of natural products. It gave a positive Lassaigne's test indicating the presence of nitrogen. Enhancement of yellow colouration with dimethyl amino benzaldehyde indicated a ureide kind of moiety. The IR spectrum **(Figure 12)**

IJSER © 202
http://www.ijser.org

revealed characteristic absorptions due to carbonyl group at 1778 cm^{-1} and –NH group at 3435 cm^{-1}. The ^{1}H NMR spectrum of **PGS-2** clearly indicated five distinct signals in five different environments **(Figure 13)**.The compound also showed distinct signals in the **^{13}C** NMR spectrum **(Figure 14)**. On the basis of above evidences the compound was identified as the keratolytic molecule allantoin. **Table 6** gives the comparison of ^{1}H and **^{13}C**NMR spectral data of **PGS-2** with that of allantoin reported in literature **(Rashed *et.al.,* 2004)**. A mixed melting point of **PGS-2** with authentic sample of allantoin was recorded and it was not depressed. Further confirmation of the structure of **PGS-2** was done by reversed phase HPLC analysis of this compound on C18 column using PDA detector. The HPLC chromatograms (**Figure 16)** of **PGS-2** and an authentic sample of allantoin exhibited peaks at same retention time (R_t=5.5).



20

Table 6: Comparison of ^{1}H NMR and ^{13}C NMR spectral data of PGS-2 with that of authentic allantoin

Position of Proton	Chemical Shift δ (ppm)				
	PGS-3	Literature Data	Carbon Position	PGS-3	Literature Data
H-1	10.54	10.50	C2	157.3	157.2
H-3	6.89-6.87	6.9	C4	62.9	62.9
H-4	8.06	8.1	C5	174.0	174.0
H-6	5.23-5.25	5.3	C7	157.9	157.8
H-8	5.78	5.8			

IJSER © 202
http://www.ijser.org

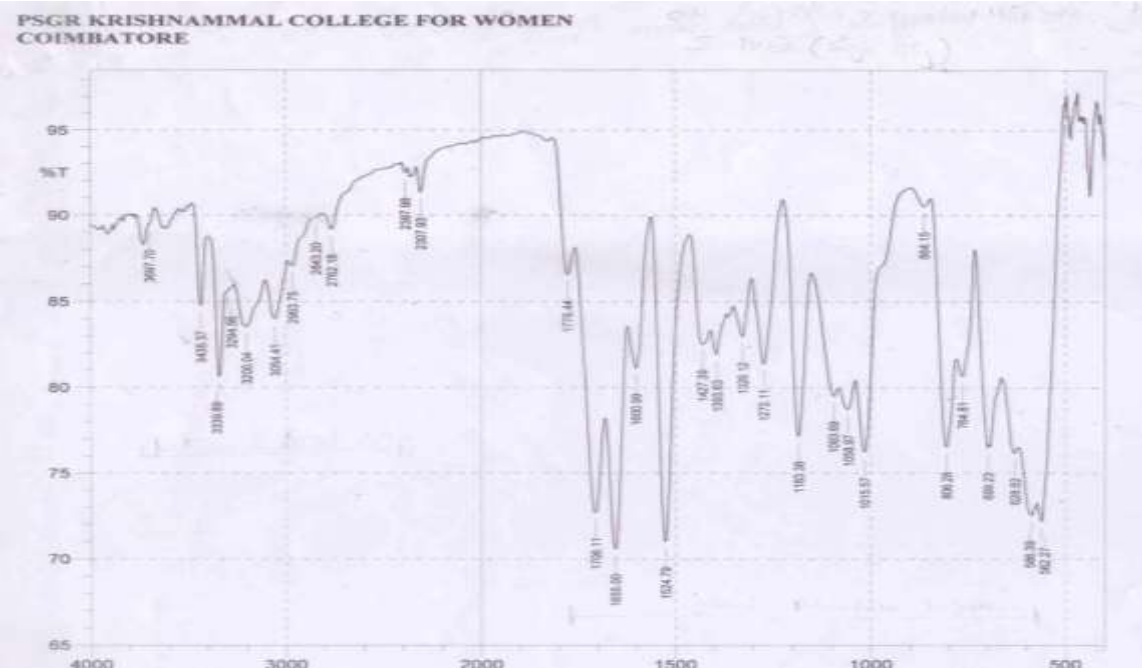


Figure 12
IR spectrum of PGS-2

IJSER

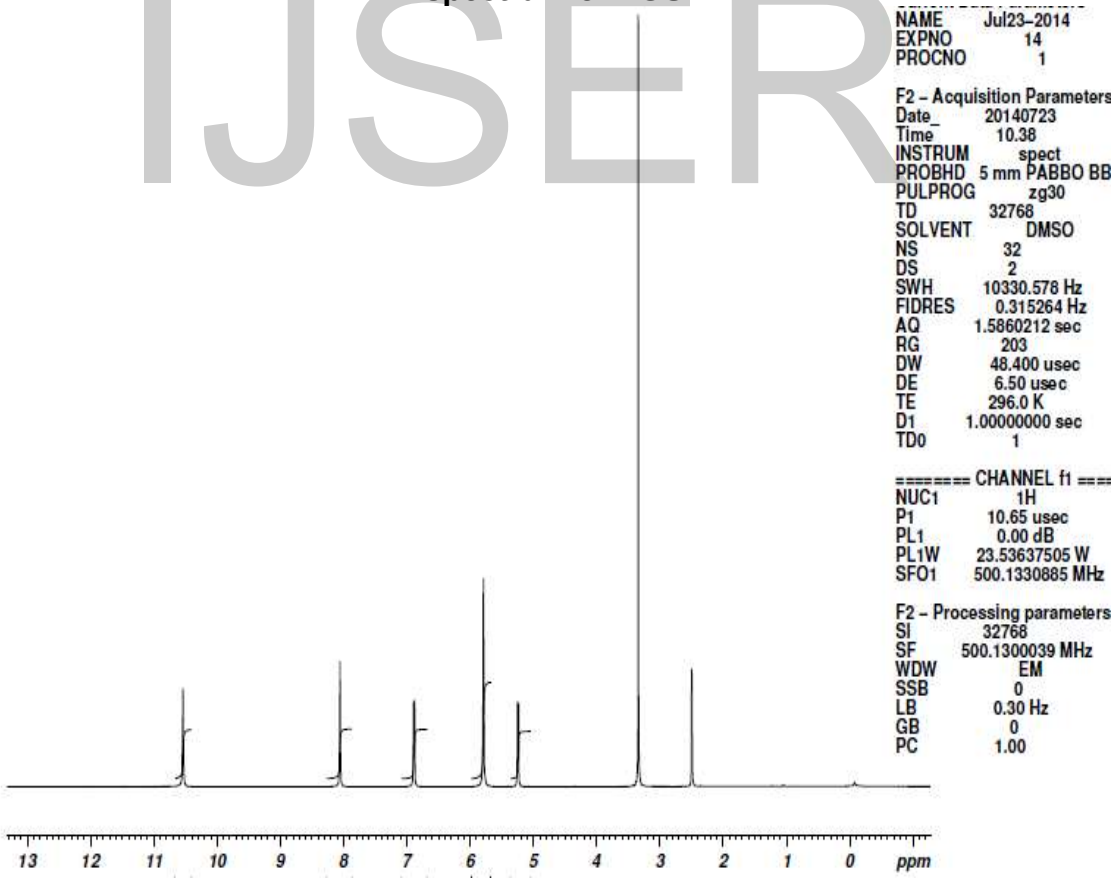


Figure 13
^{1}H NMR spectrum of PGS-2

IJSER © 202
http://www.ijser.org

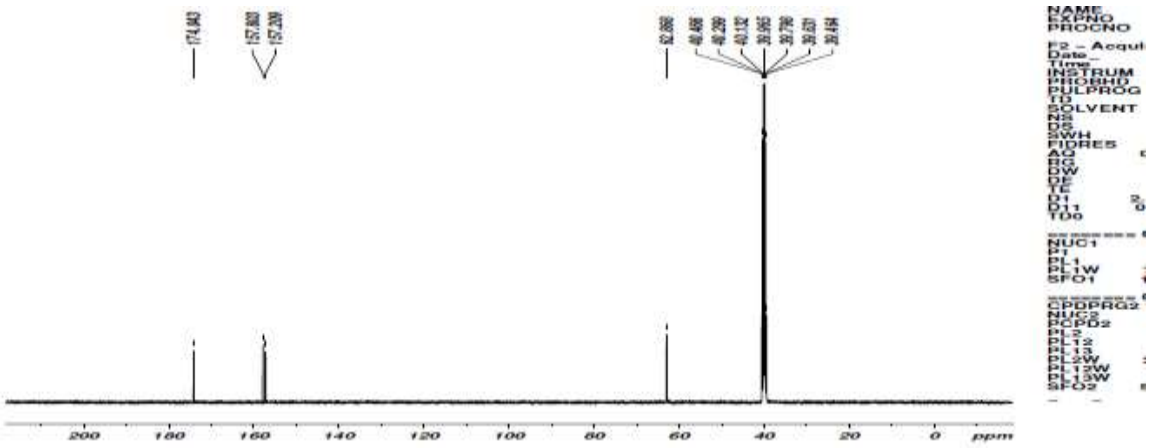
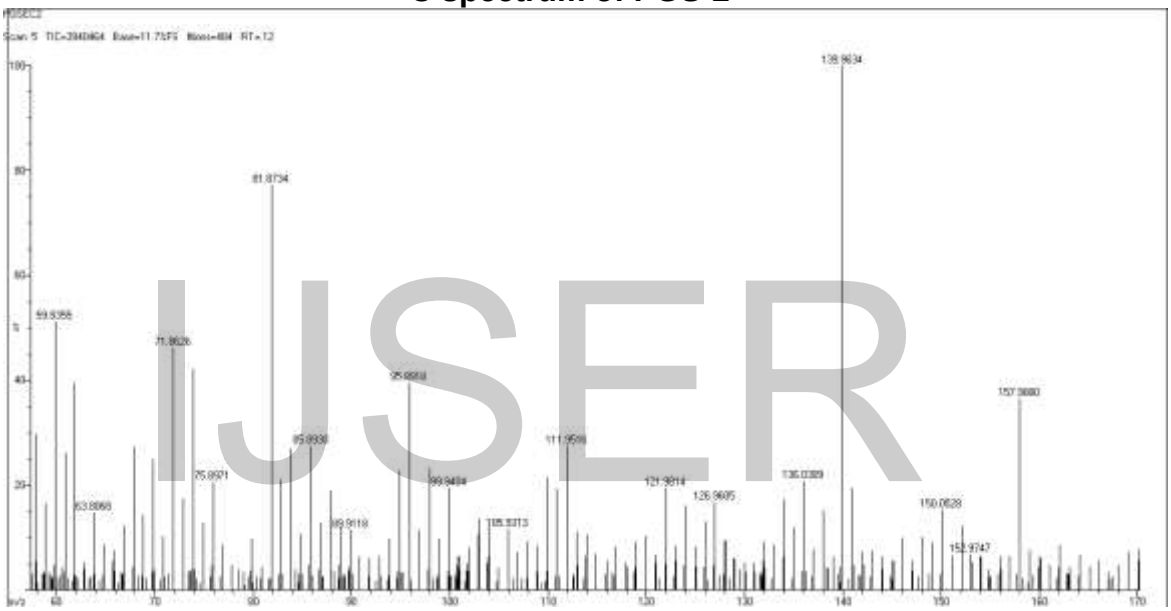


Figure 14
^{13}C spectrum of PGS-2



IJSER

Figure 15
Mass spectrum of PGS-2

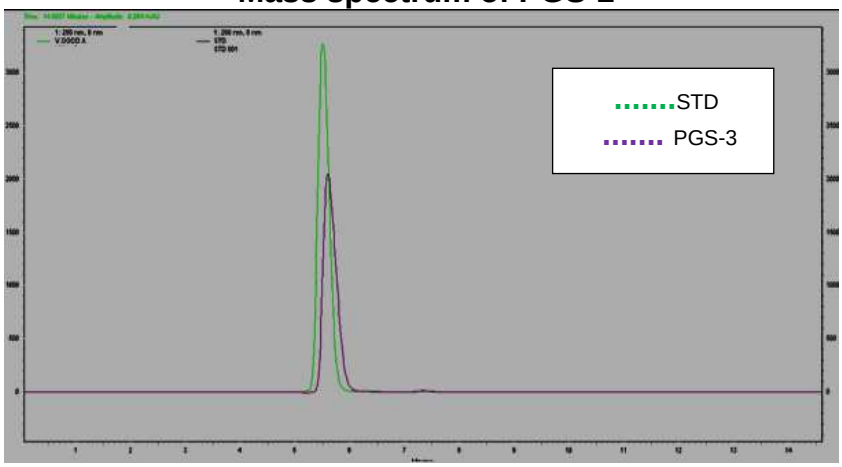


Figure 16
Comparison of HPLC chromatogram of PGS-2 with standard allantoin

IJSER © 202
http://www.ijser.org

4.3.3 Characterization of PGS-3

Compound **PGS-3** was obtained by column chromatographic analysis of the dewaxed ethanol extract concentrate of ***Pisonia grandis*** on elution with ethyl acetate and ethanol (92:8) as a white solid (yield:184 mg). Melting point: 192-197°C. TLC R_f of 0.47 (chloroform: methanol: water 6:3.5:0.5). The compound did not show good solubility in organic solvents but was highly soluble in water and DMSO thus indicating the high polar nature of the compound. It was not positive to the colour tests meant for common class of natural products.

Spectral data:

IR (KBr) γ_{max} cm^{-1}: 3394, 3301, 2305, 1709, 1651, 1522, 1441, 1265, 1190, 1113, 1063, 1012, 801,668, 577.

The IR spectrum of **PGS-3 (Figure 17)** possessed signals in the same regions expressed by previously isolated compounds **PGS-2 (allantoin) and PGS-4 (pinitol)).** A **c**omparison of IR spectral data of all the three compounds indicated that the compound **PGS-3** contains functional groups of allantoin and pinitol **(Figure 17a).**

IJSER

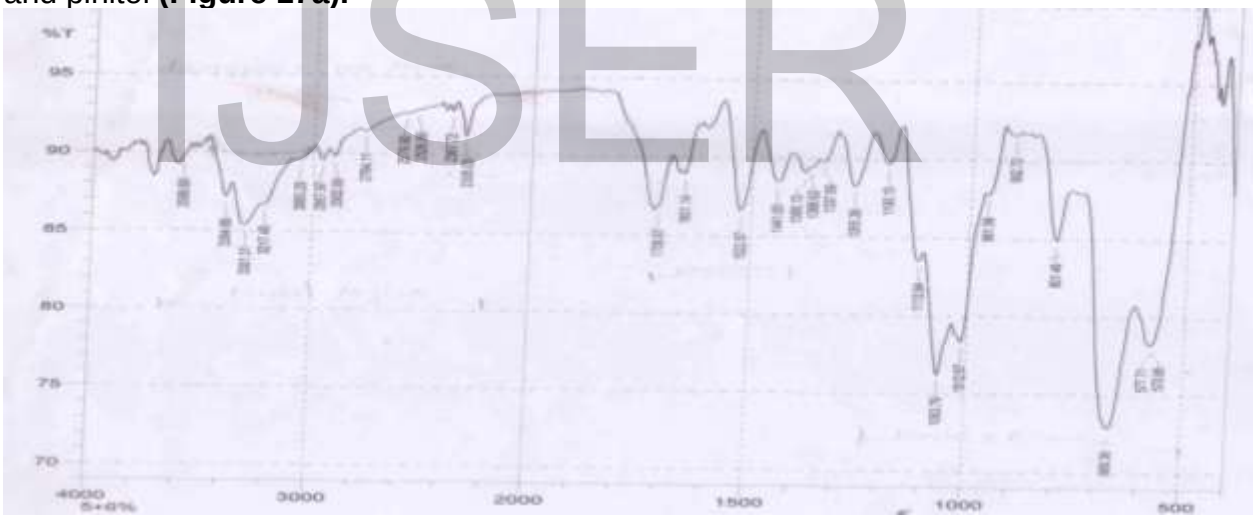
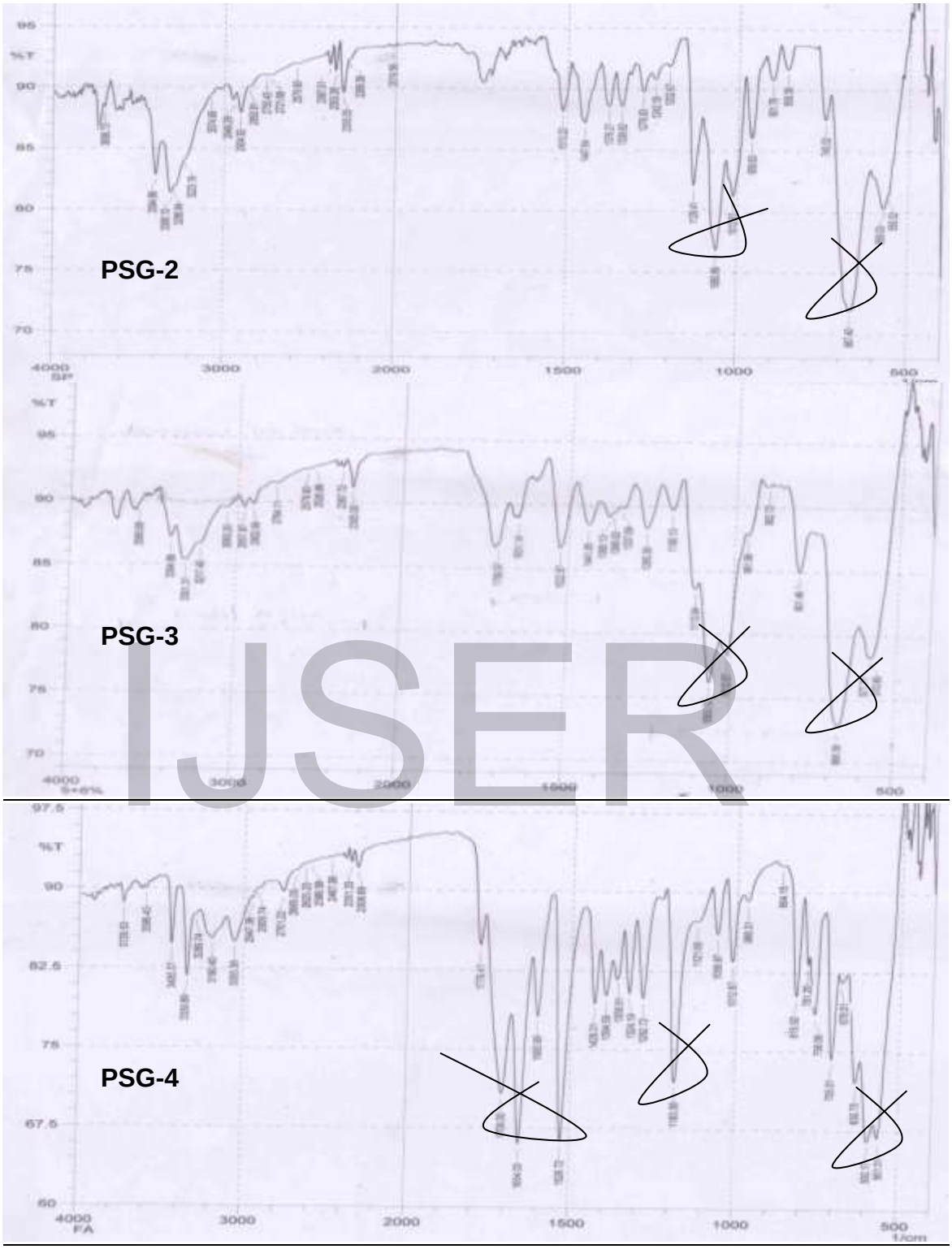


Figure 17

IR spectrum of PGS-3

IJSER © 202
http://www.ijser.org



IJSER

Figure 17a

Comparison of IR spectra of PGS-3 with PGS-2 (allantoin) and PGS-4 (pinitol)

IJSER © 202
http://www.ijser.org

^{1}H NMR (500MHz, DMSO, **δ** ppm) spectral data of **PGS-3,** allantoin and pinitol is presented in table

^{13}C NMR (125 MHz, $CDCl_3$, **δ**): Table -- is the comparison table of ^{13}C NMR spectral data of **PGS-3 with that of PGS-2 and PGS-4.**

The ^{1}H NMR **(Figure 18)**, ^{13}C NMR (**Figure 19)** and two dimensional NMR spectrum of **PGS-3** clearly indicated that it constitutes non-coupling partners of allantoin and pinitol. The only difference was in its IR spectrum. The prominent peaks due to allantoin at 1654, 1526, 1183, 592 and 561 cm^{-1}) were slightly reduced in intensity in the IR spectrum of **PGS-3** which is an evidence of formation of addition compound of allantoin. There are reports in literature on complex compounds of allantoin and pinitol with small molecules (**Hirohide *et.al.,* 1977) which suggest the diminution of the IR absorption peaks due to the imide carbonyls at 1780 and 1720** cm^{-1} **as an evidence of the formation of the complexes.** The same characteristic of IR peaks was seen in the IR spectrum of **PGS-3** in which absorptions due to cyclic imide got diminished. The relative shift of prominent peaks to lower frequency also suggests that the **PGS-3** may be bonded intermolecularly with weak interactions between allantoin and pinitol moiety.

IJSER

The compound pinitol exerts acute and chronic insulin like antihyperglycemic effect in STZ diabetic mice **(Bates *et.al.,* 2000).** Soybean derived pinitol was found to be an effective oral agent in the treatment of type-II diabetes **(Kim *et.al.,* 2005).** Similarly allantoin was found to decrease plasma glucose in streptozotocin induced diabetic rats **(Niu *et.al.,* 2010)**.However the study also reported that allantoin gets degraded in the gastrointestinal tract and may be lost after oral administration. A combination of allantoin and pinitol may be of much pharmacological significance especially in diabetes treatment. This is the first report of the isolation of such an addition complex of allantoin and pinitol.

IJSER © 202
http://www.ijser.org

Table 7 Comparison of ^{1}H NMR chemical shift δ of PGS-3 with that of PGS-2 and PGS-4

Proton Position	^{1}H NMR Chemical Shift δ (ppm)		
	PGS-2	PGS-3	PGS-4
H-1	10.53	10.53	-
H-3	6.89-6.87	6.90 – 6.88	-
H-4	8.06	8.05	-
H-6	5.25-5.23	5.26 – 5.24	-
H-8	5.78	5.79	-
H-1		2.99 – 3.03	2.99 -3.02
H-2		3.49 - 3.53	3.49 – 3.52
H-3		3.64	3.62
H-4		3.45	3.44
H-5		3.64	3.66
H-6		3.35	3.32
Methoxy proton		3.44	4.42
C2-OH		4.47 – 4.46	4.46 – 4.45
C3-OH		4.63 - 4.62	4.62 - 4.61
C4-OH		4.34 – 4.33	4.34 – 4.33
C5-OH		4.72- 4.71	4.71 – 4.70
C6-OH		4.51 – 4.50	4.51 - 4.50

IJSER

Table 8 Comparison of ^{13}C NMR chemical shift δ of PGS-3 with that of PGS-2 and PGS-4

Chemical Shift δ (ppm)			
Carbon Position	PGS-2	PGS-3	PGS-4
C2	157.3	157.2	
C4	62.9	62.9	
C5	174.0	174.0	
C7	157.8	157.8	
C1 Methoxy		60.1	60.1
C1		84.3	84.2
C2		70.5	70.5
C3		72.9	72.8
C4		71.4	71.3
C5		72.4	72.4
C6		73.1	73.0

IJSER © 202
http://www.ijser.org

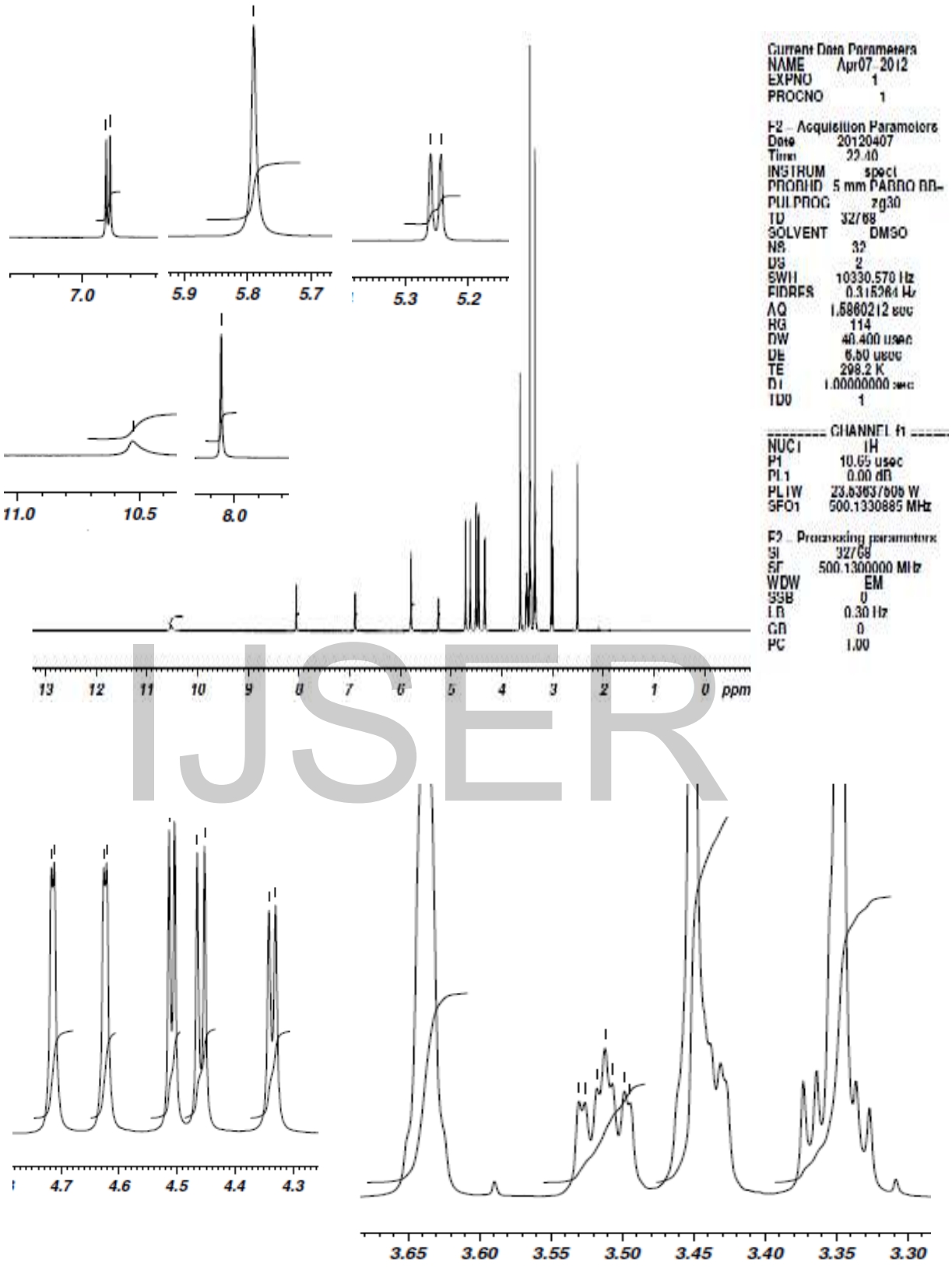


Figure 18
^{1}H spectrum of PGS-3

IJSER © 202
http://www.ijser.org

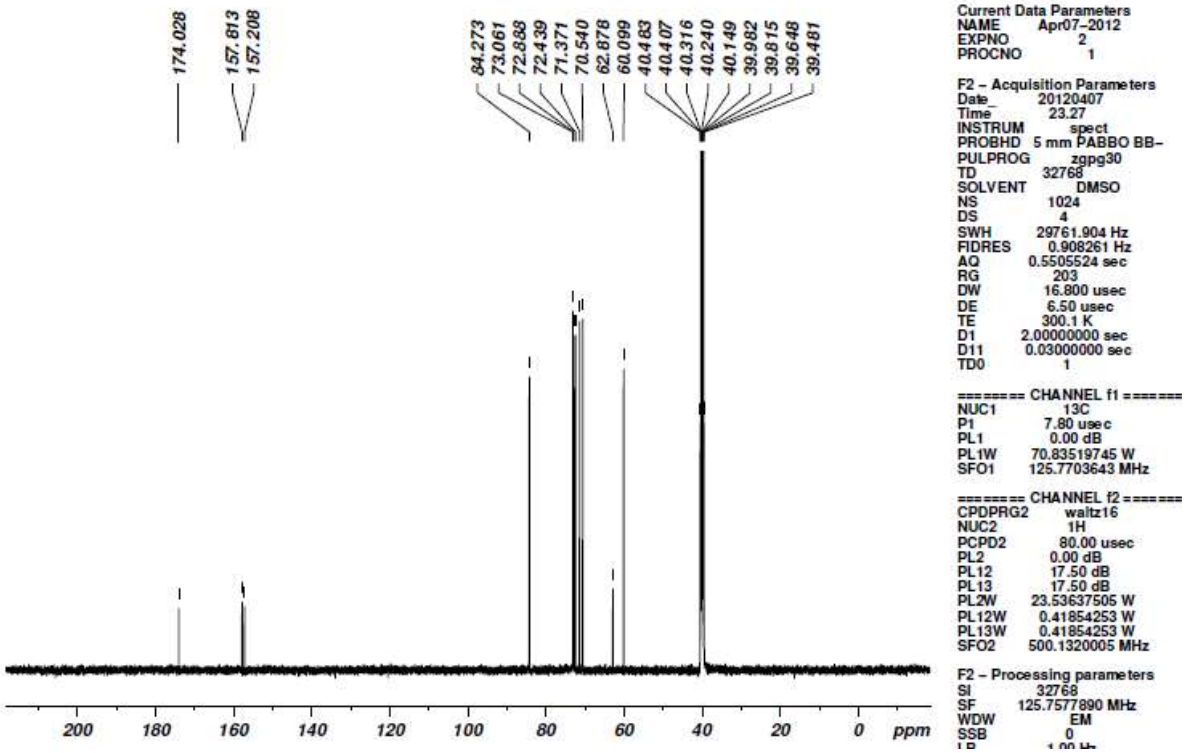


Figure 19
^{13}C spectrum of PGS-3

IJSER

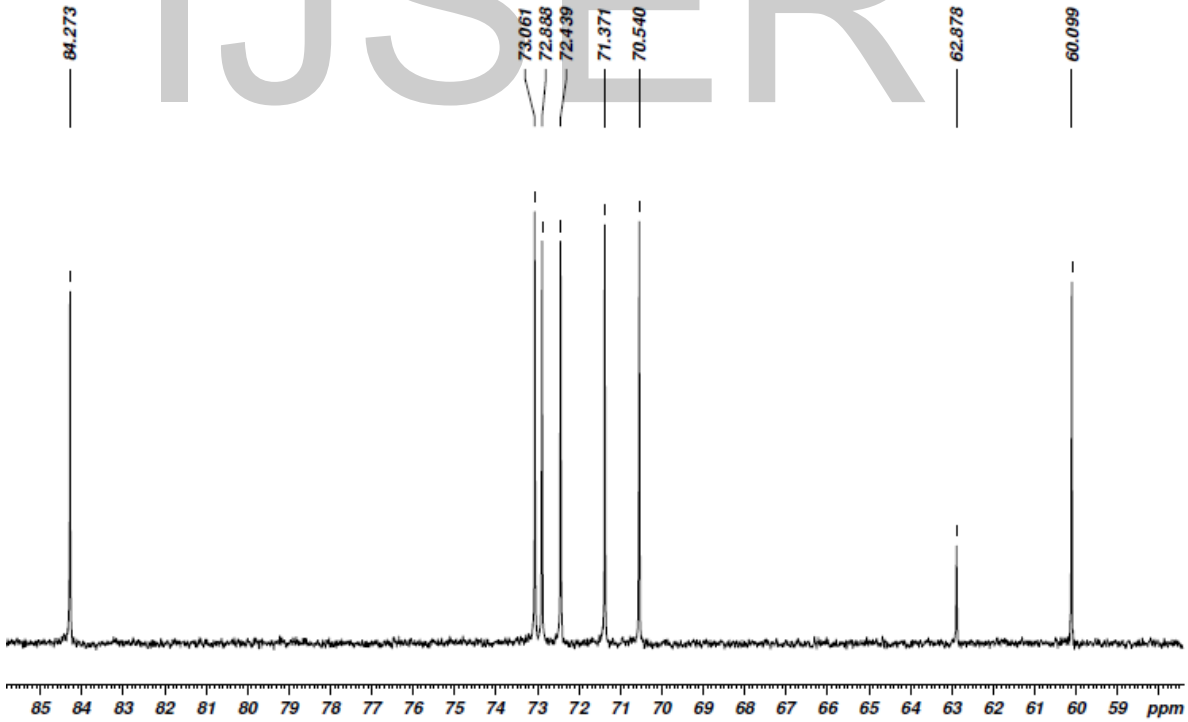


Figure 20
Expansion of 13C spectrum of PGS-3

IJSER © 202
http://www.ijser.org

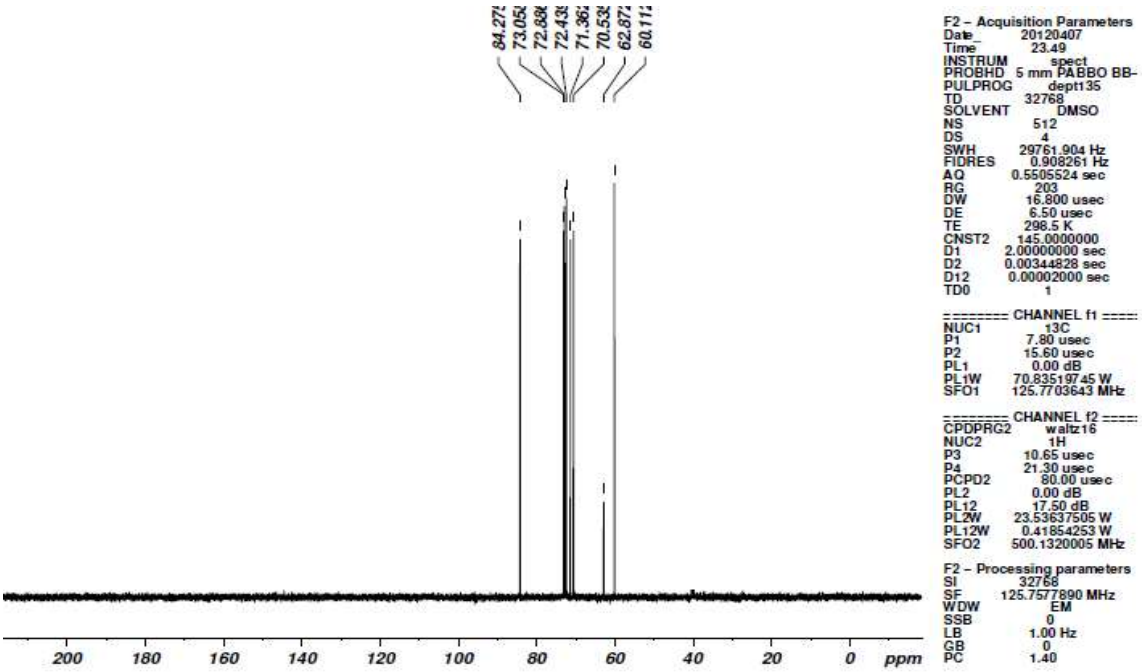
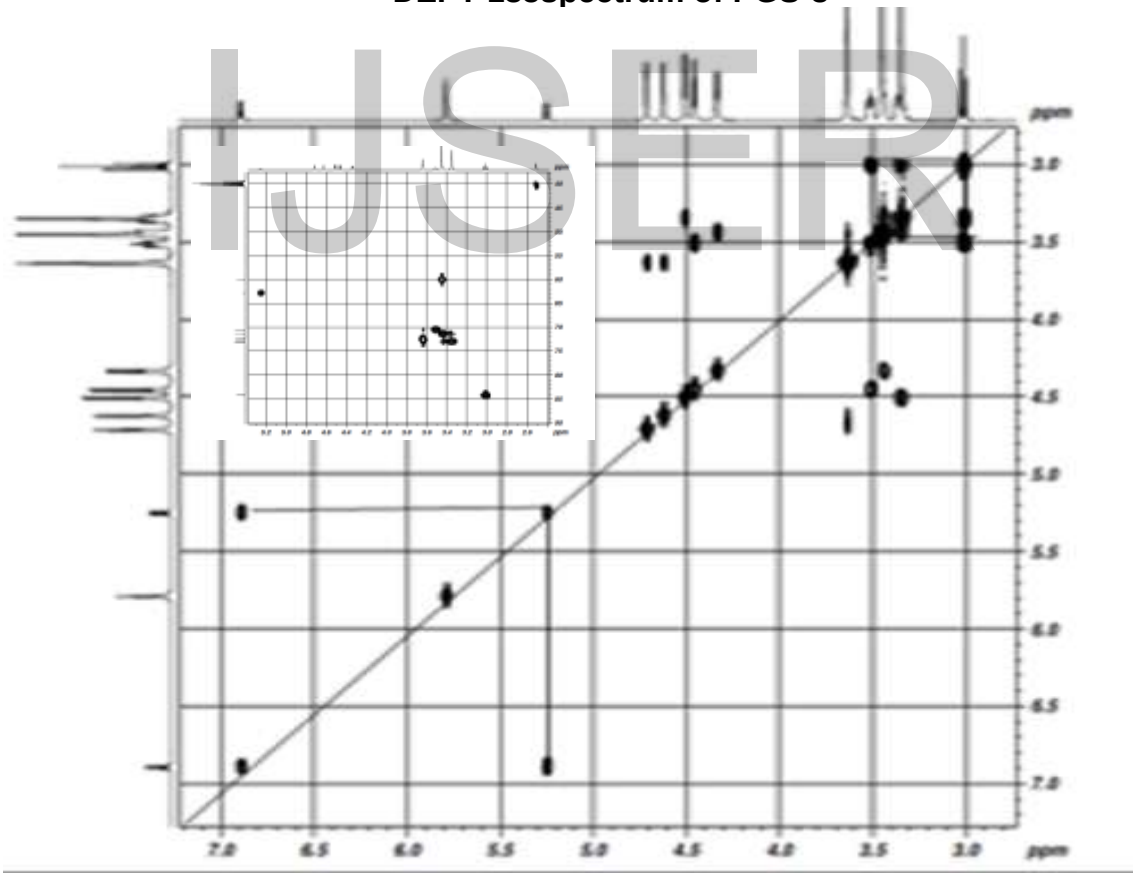


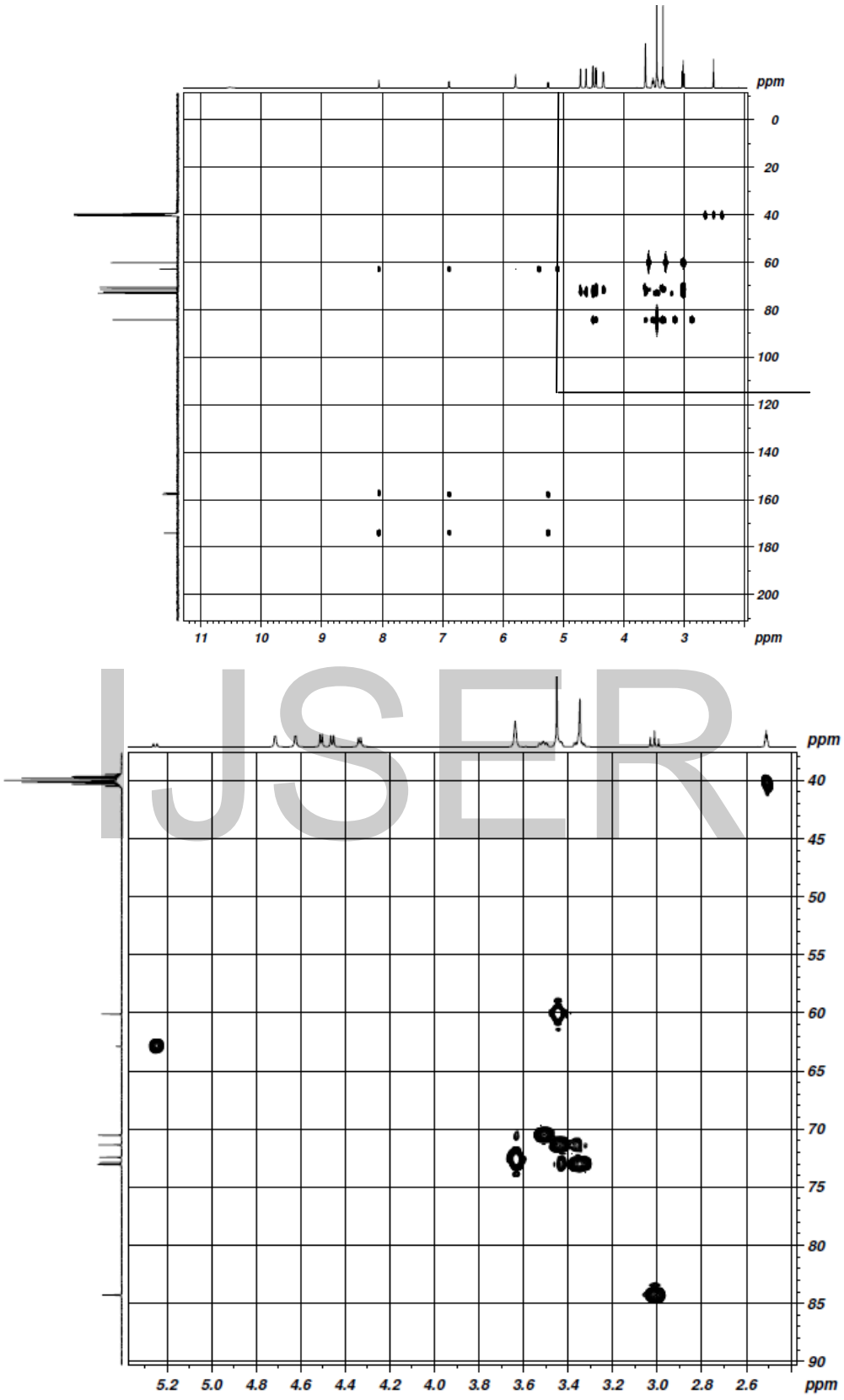
Figure 21
DEPT 135spectrum of PGS-3



IJSER

Figure 22
COSY spectrum of PGS-3

IJSER © 202
http://www.ijser.org



IJSER

Figure 23
HSQC spectrum of PGS-3

IJSER © 202
http://www.ijser.org

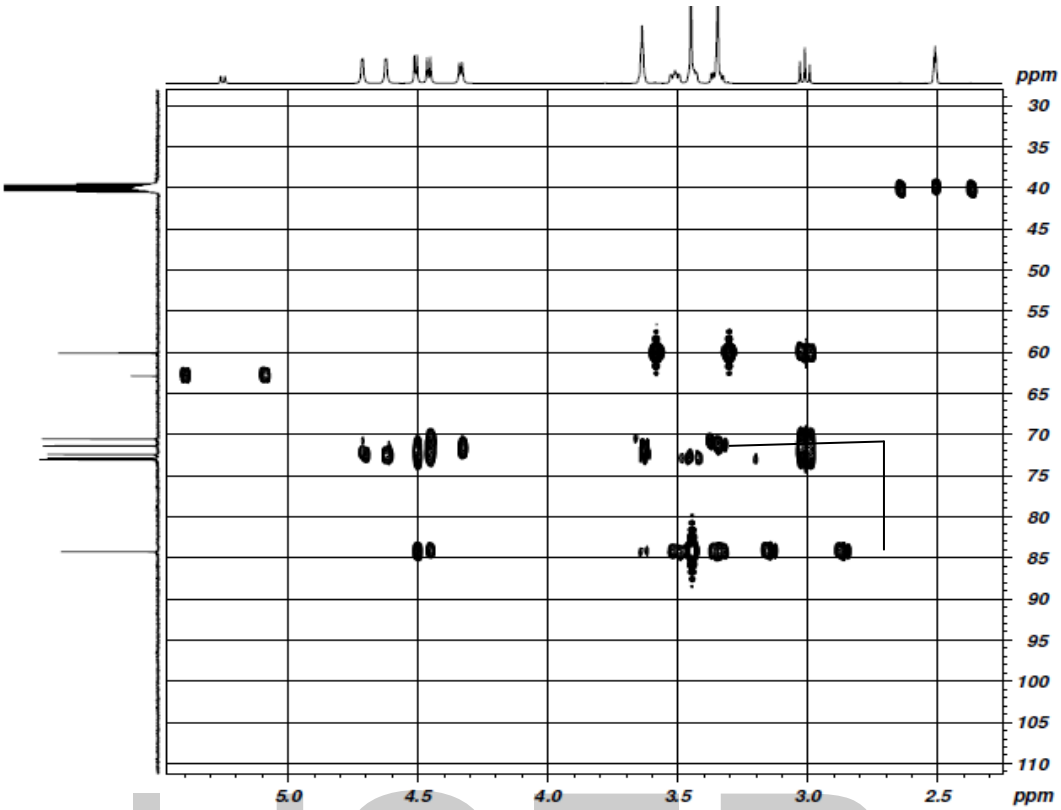
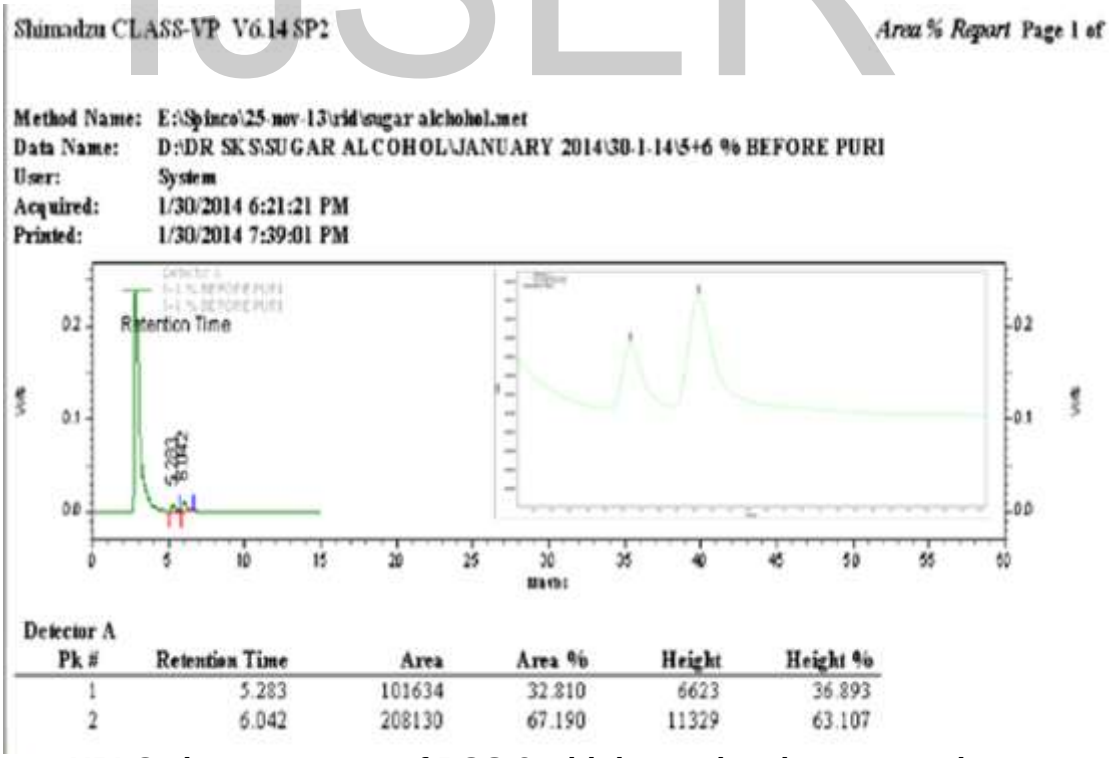


Figure 24
Expansion of HMBC spectrum of PGS-3

IJSER



HPLC chromatogram of PGS-3 with inset showing two peaks

IJSER © 202
http://www.ijser.org

4.3.4 Characterization of PGS-4

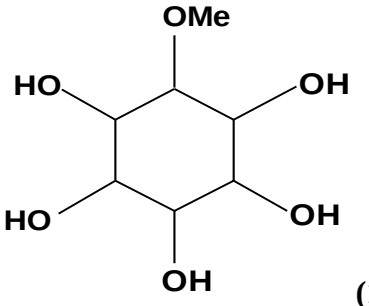
Compound **PGS-4** was obtained by elution with ethyl acetate-ethanol (88:12) as a white solid. Yield: 1.06 g. Melting point: 184-191°C. TLC R_f = 0.50 (chloroform: methanol: water (6:3.5:0.5)

Spectral data of **PGS-4**:

IR (KBr) γ_{max} cm^{-1}: 3393, 3304, 2901, 1448, 1123, 1064, 668 **(Figure 26)**
1H NMR (500MHz, DMSO, δ ppm):3.49,2.99-3.02,3.32-3.35,3.37,3.50-3.52,3.63,4.33-4.34,4.45-4.47,4.50-4.5,4.62,4.71**(Figure 27)**

^{13}C NMR (125 MHz, $CDCl_3$, δ): 60.1, 84.2, 73.0, 71.3, 72.4, 70.5, 72.9 **(Figure 28)**
EI-MS: m/z = 194 $[M^+]$ calculated for $C_7H_{14}O_6$ **(Figure 29)**

Compound **PGS-4** did not show good solubility in organic solvents but it was soluble in water and DMSO thus indicating the high polar nature of the compound. It was not positive to the colour tests meant for the common class of natural products. The IR spectrum **(Figure 16)** revealed characteristic absorptions due to -OH group at 3304 cm^{-1}. The ^1H NMR spectrum clearly indicated six methine protons, one methoxy proton and five heteroatom protons.**(Figure 17)**.The ^{13}C NMR spectrum revealed seven carbons out which 6 carbons were methine carbons and one was a methoxy carbon **(Figure 18)**. The molecular mass of **PGS-5** was found to be 194 from mass spectrum **(Figure 19)** indicating a molecular formula of $C_7H_{14}O_6$. Table 9 represents the comparison of the assigned ^1H and **^{13}C**NMR spectral data of **PGS-4** with that of pinitol reported in literature **(Rashed *et.al.,* 2004)**.Further confirmation of **PGS-4** was done by reversed phase HPLC analysis of this compound on an amine column using RI detector. The HPLC chromatograms of **PGS-4** and authentic sample (**Figure 20)** exhibited peaks at the same retention time (R_t=6.2). On the basis of above evidences **PGS-4** was confirmed as the insulinomimetic molecule pinitol (19).



(19)

IJSER © 202
http://www.ijser.org

Table 9 **H NMR and ^{13}C NMR data of PGS-4**

Chemical Shift δ (ppm)			
Proton Position	**PGS-4**	**Carbon Position**	**PGS-4**
H-1	2.99 -3.02	C1 Methoxy	60.1
H-2	3.49 – 3.52	C1	84.2
H-3	3.62	C2	70.5
H-4	3.44	C3	72.8
H-5	3.66	C4	71.3
H-6	3.32	C5	72.4
Methoxy proton	4.42	C6	73.0
C2-OH	4.46 – 4.45	-	-
C3-OH	4.62 - 4.61	-	-
C4-OH	4.34 – 4.33	-	-
C5-OH	4.71 – 4.70	-	-
C6-OH	4.51 - 4.50	-	-

IJSER

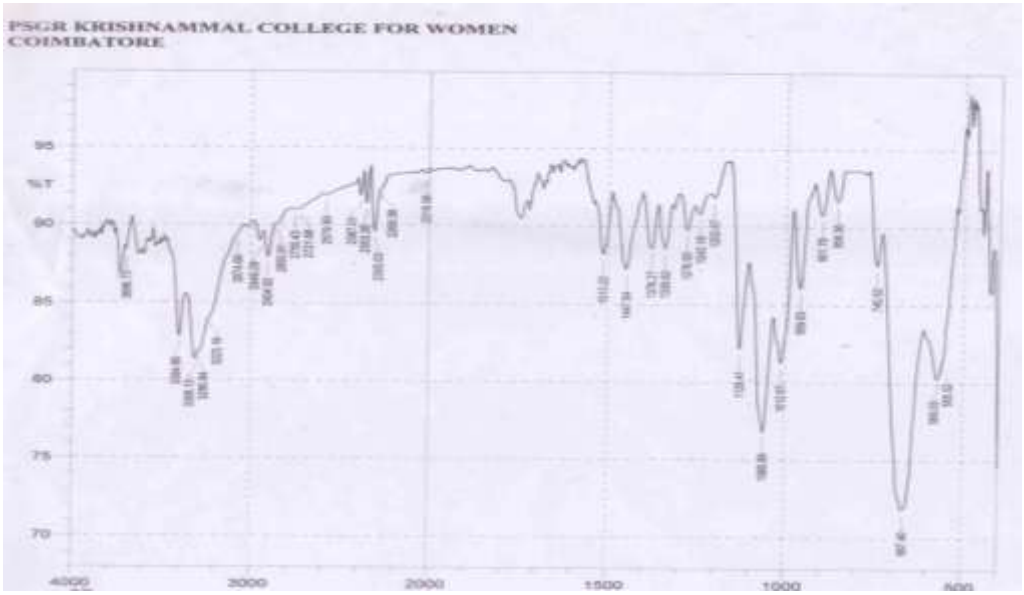


Figure 26
IR spectrum of PGS-4

IJSER © 202
http://www.ijser.org

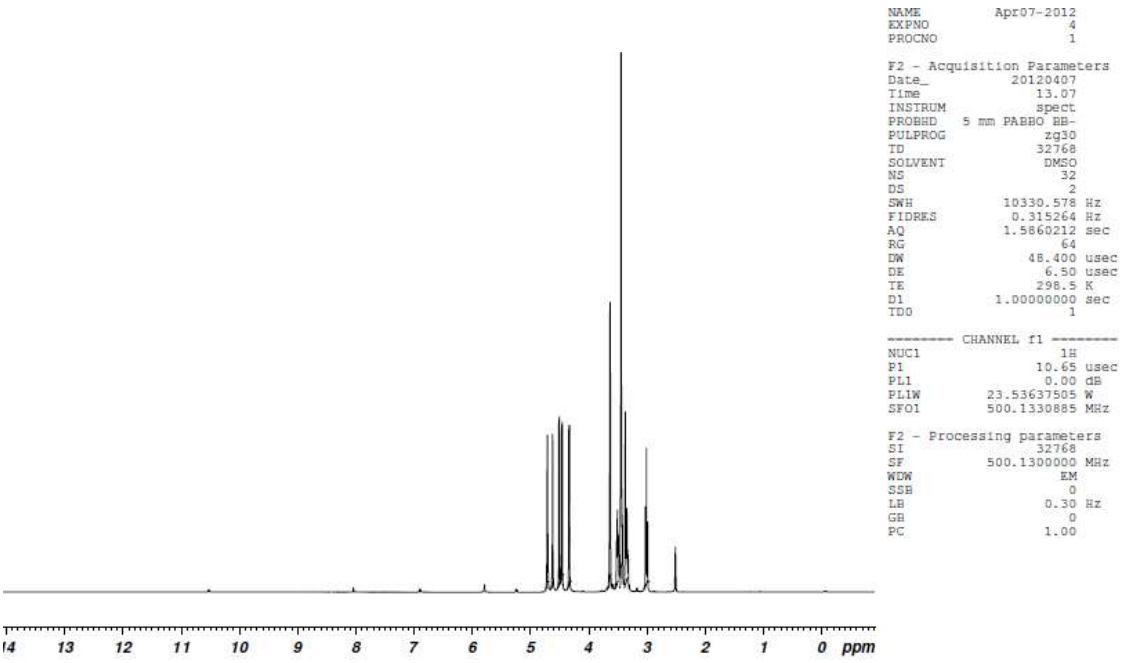


Figure 27
^{1}H spectrum of PGS-4

IJSER

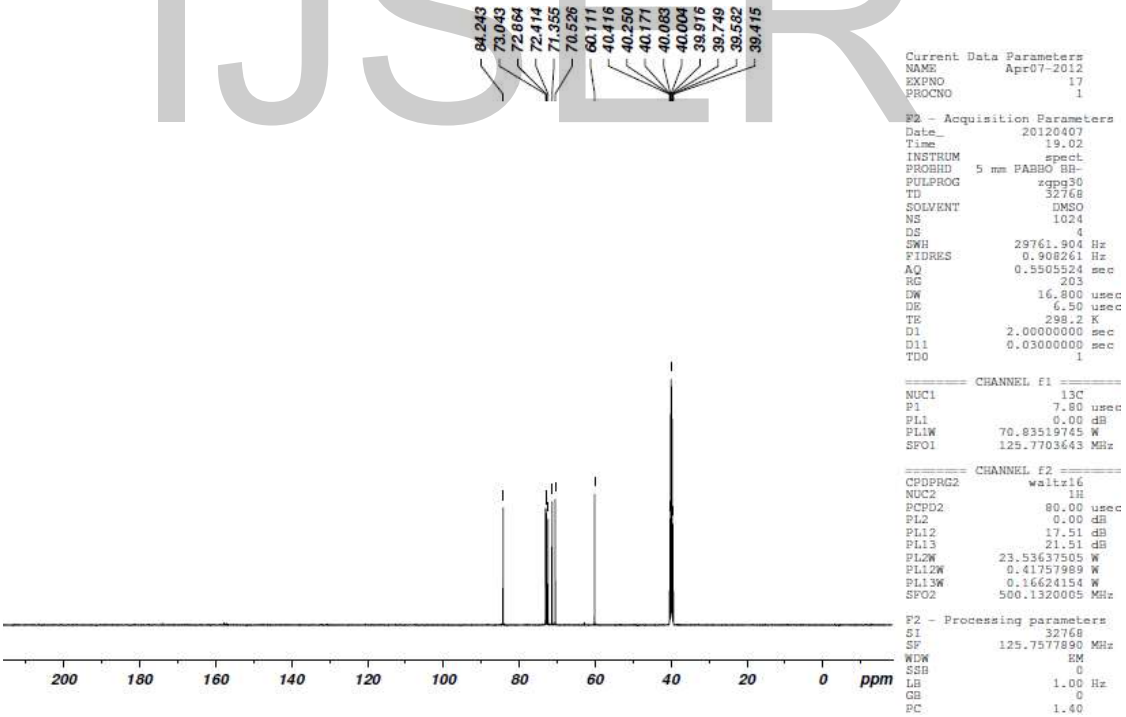


Figure 28
^{13}C spectrum of PGS-4

IJSER © 202
http://www.ijser.org

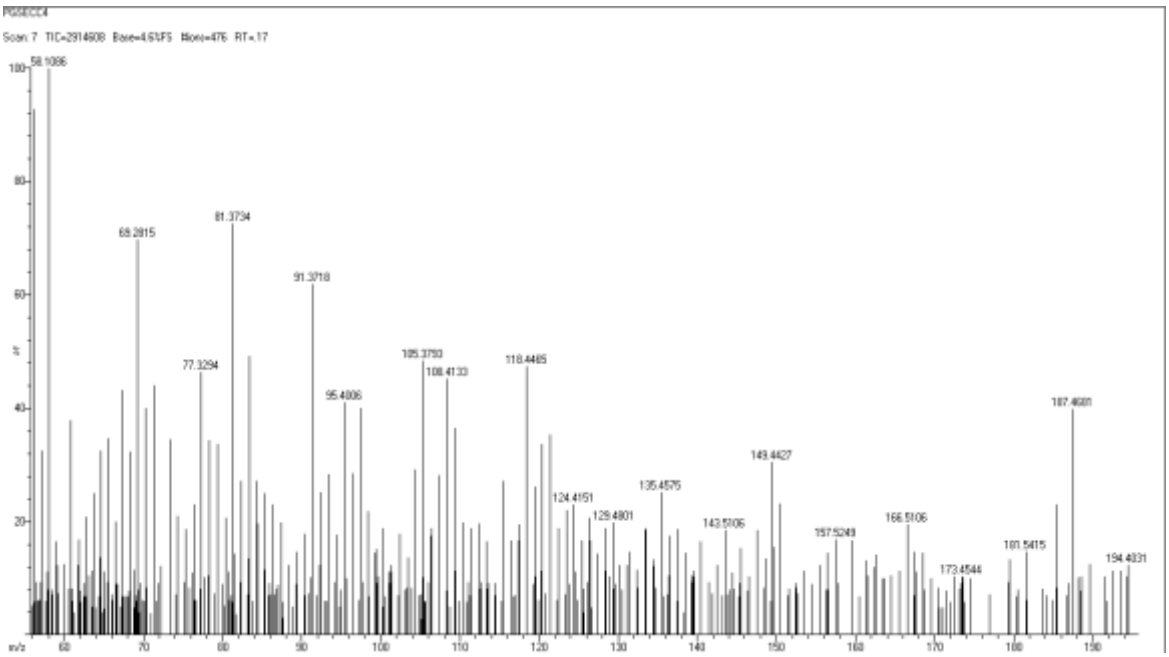


Figure 29
Mass spectrum of PGS-4

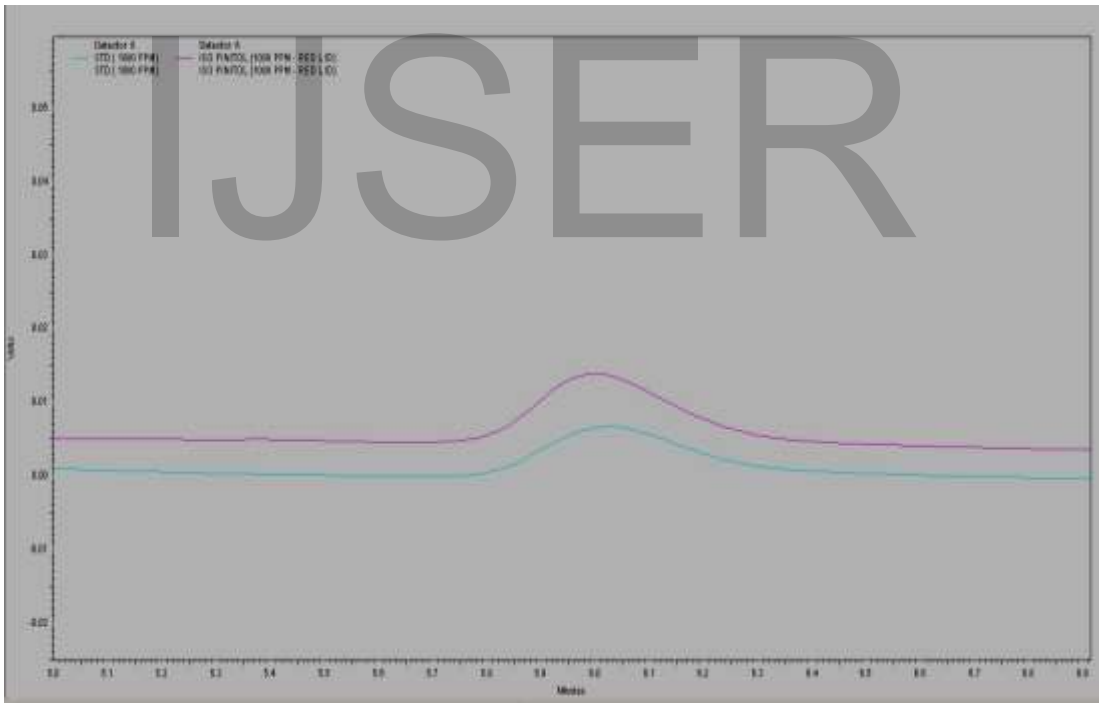


Figure 30
HPLC chromatogram of PGS-4 and standard pinitol

IJSER

IJSER © 202
http://www.ijser.org

4.3.5 Characterization of PGS-5

Compound **PGS-5** was obtained by elution with ethyl acetate-ethanol (70:30) as a white solid. Yield: 123mg. Melting point: > 240^{o}C .It appeared as a white patch in TLC. The compound answered colour tests meant for carbohydrates; pale violet ring in Molisch's test and reddish brown precipitate in Fehling's test.**The IR spectrum showed peaks due hydroxyl group and the C-O-C linkage of sugars at 3422 cm^{-1} and 1261.**

The proton NMR spectrum (Figure 32) indicated the carbohydrate nature of the molecule.

IJSER

Figure 31
IR spectrum of PGS-5

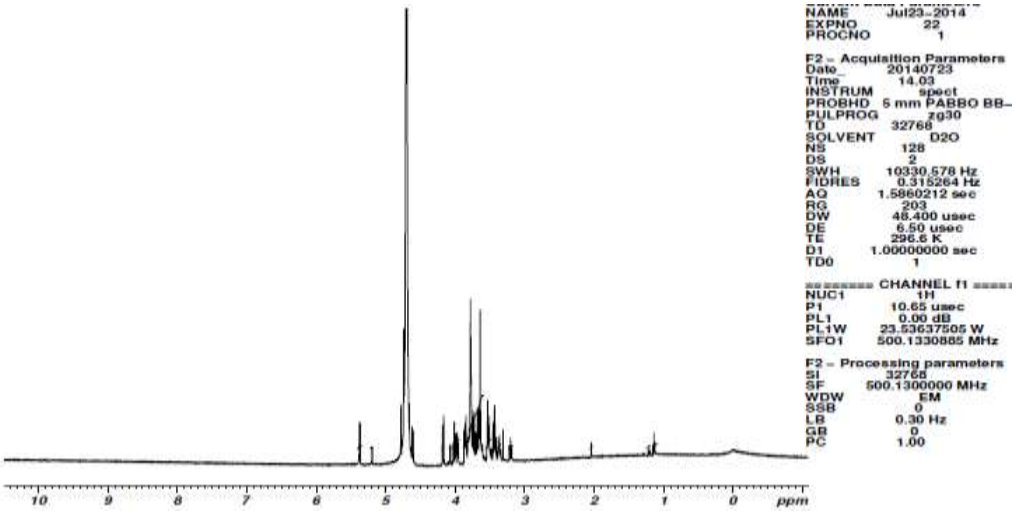


Figure 32 ^{1}H spectrum of PGS-5

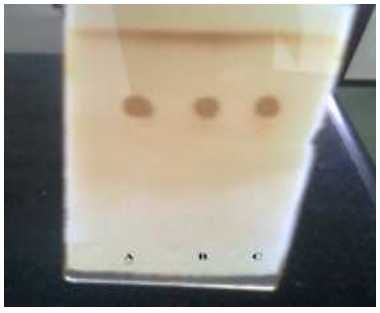
IJSER © 202
http://www.ijser.org

4.5 Characterization of compounds isolated from the methanol soluble fraction of aqueous extract of stem of PG

4.5.1 Characterization of PGS-6

Column chromatographic analysis of methanol soluble portion of aqueous extract concentrate of stems of ***Pisonia grandis*** led to the isolation of brown solid designated as **PGS-6.** Yield: 200 mg. Melting Point: 220-226˚C. TLC R_f = 0.62 (chloroform: methanol: water 6:4:3 drops **Figure 33**). The compound **PGS-6** did not show good solubility in organic solvents like chloroform, acetone etc. But it was soluble in water and DMSO thus indicating the high polar nature of the compound. It was not positive to the colour tests meant for the common class of natural products. It gave a positive Lessaigne's test indicating the presence of free nitrogen. Enhancement of yellow colouration with dimethyl amino benzaldehyde indicated a ureide kind of moiety. IR spectrum **(Figure 34) PGS-6** gave a characteristic absorption due to carbonyl group (1778 cm^{-1}), and –NH groups at (3435 cm^{-1}). IR spectrum of **PGS-6** was found to be identical with that of PGS-3 and identified to be the ureide molecule allantoin. A Co TLC of PGS7 done with PGS3 and standard allantoin ascertained the identify of PGS3 as allantoin. The mixed melting point was undipped.

IJSER



A = Standard allantoin
B = PGS-2
C= PGS-6

Figure 33
Thin layer chromatogram of PGS-6, PGS-2 and standard allantoin

IJSER © 202
http://www.ijser.org

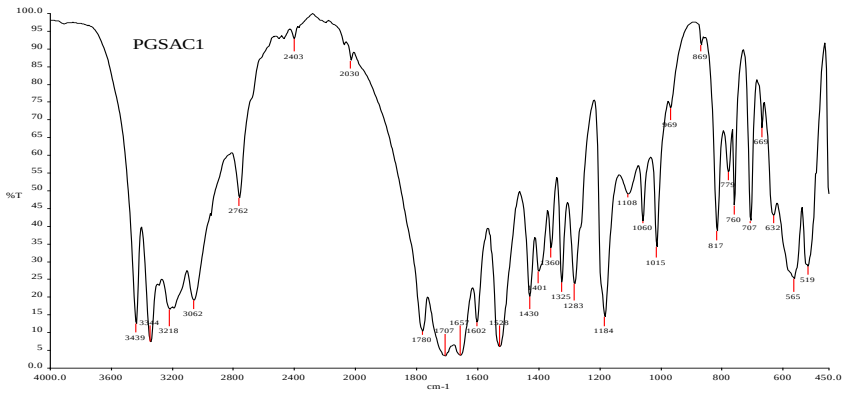


Figure 34

IR spectrum of PGS-6

4.5.2 Characterization of PGS-7

Compound **PGS-7** obtained by elution with ethyl acetate and ethanol (80:20) as a brown solid (Yield: 315 mg). Melting point: >250^{o}C. TLC of this compound showed a white streak. The compound was freely soluble in water. It was not positive to the colour tests meant for the common class of natural products.

The IR Figure 35 and NMR spectrum Figure 36 of this compound indicated the compound to be hydrogen bonded entity of the degradation product of allantoin namely allantoic acid

IJSER

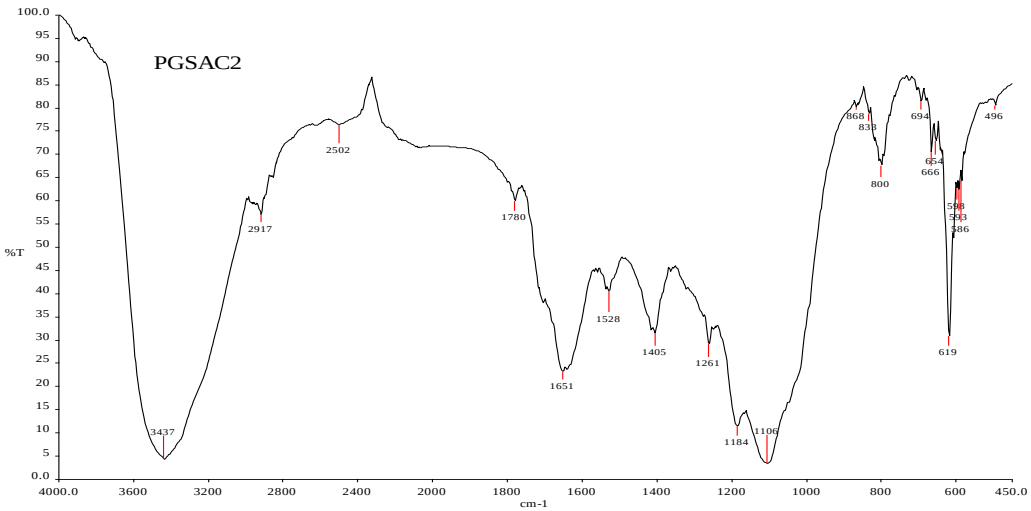


Figure 35

IR spectrum of PGS-7

IJSER © 202
http://www.ijser.org

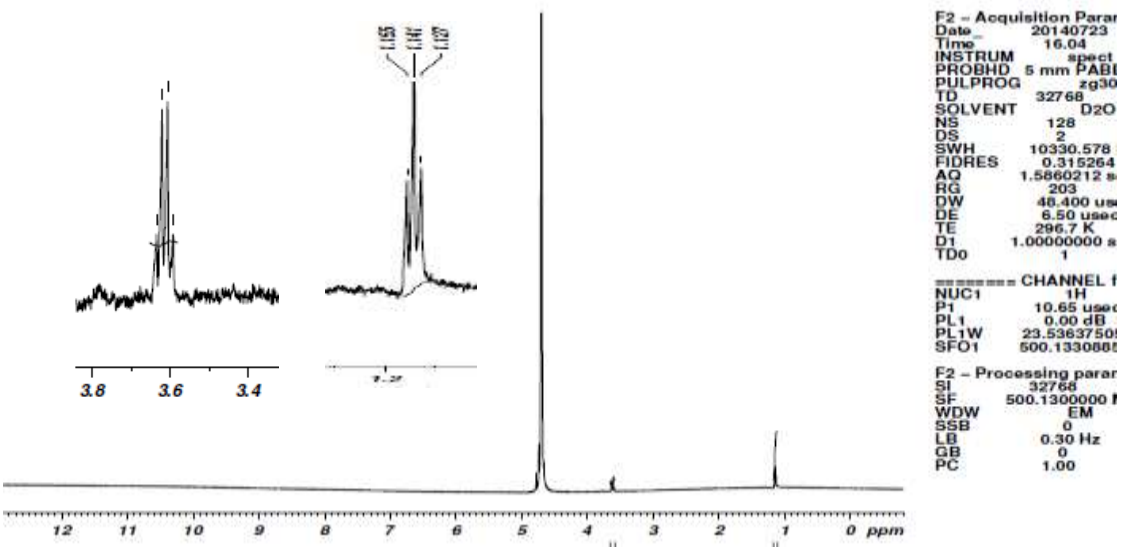
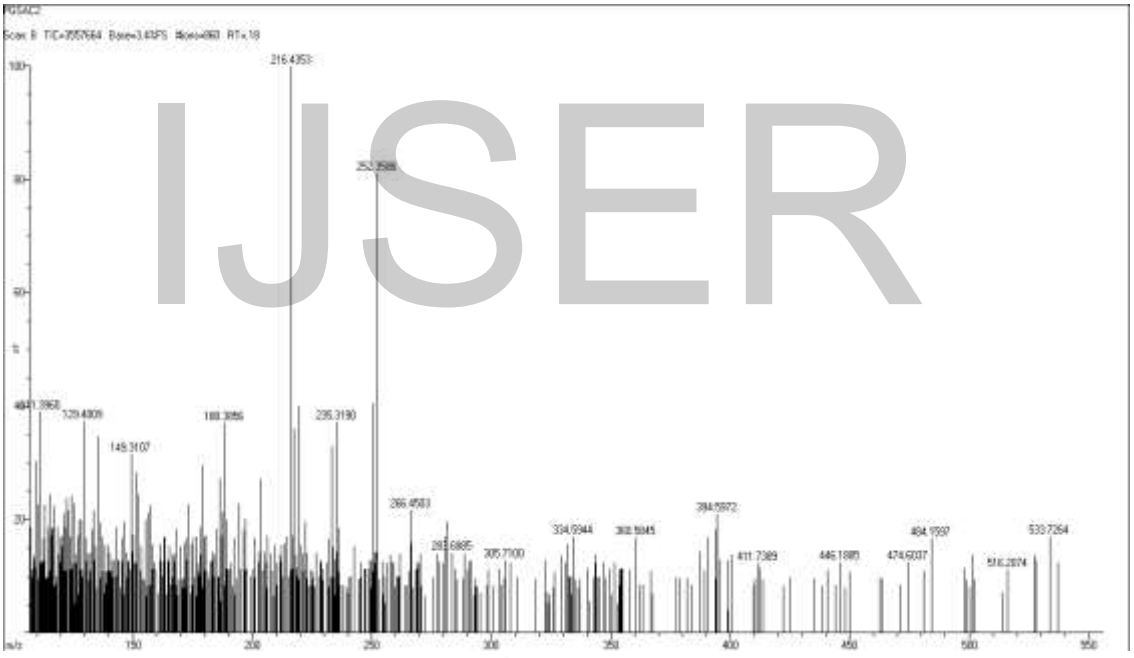


Figure 36
^{1}H NMR spectrum of PGS-7



IJSER

Figure 37
Mass spectrum of PGS-7

IJSER © 202
http://www.ijser.org

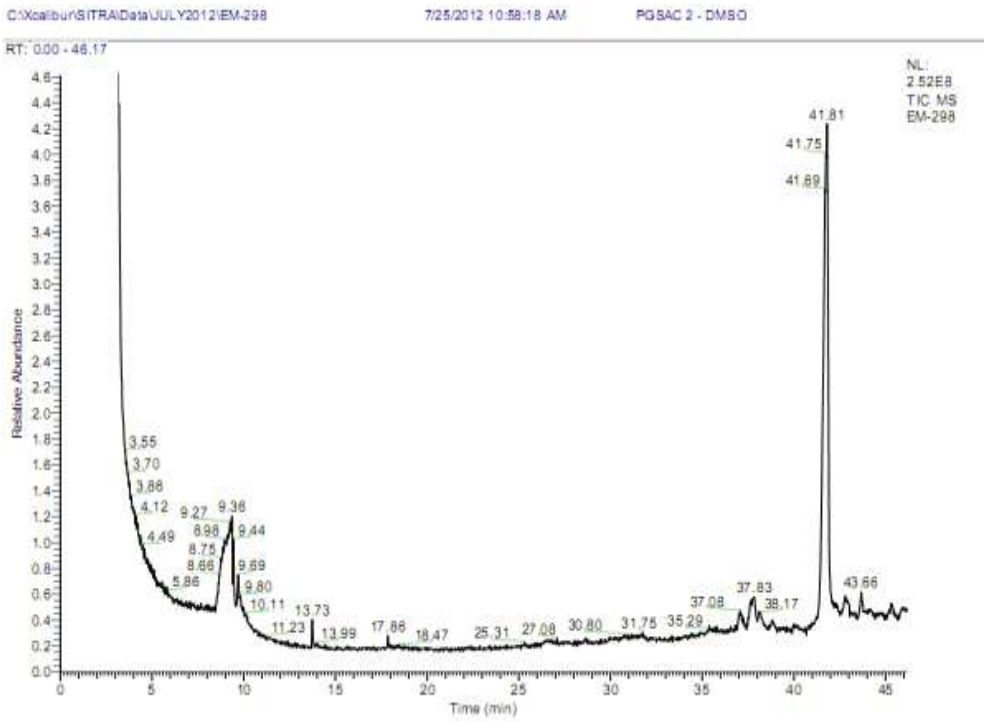


Figure 38

Gas chromatogram of PGS-7

IJSER

4.5.3 Characterization of PGS-8

Column chromatographic analysis of methanol soluble portion of aqueous extract concentrate of stems of ***Pisonia grandis*** by elution with ethyl acetate and ethanol (70:30) led to the isolation of brown solid designated as **PGS-8.** Yield: 80 mg. Melting Point: >250^{o}C. TLC: no characteristic spot. The compound **PGS-8** did not show solubility in organic solvents but it was soluble in water and DMSO.

Spectral data

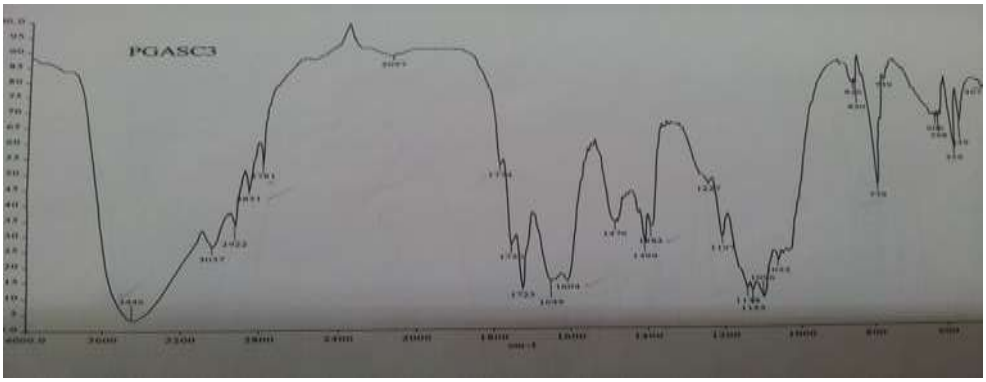


Figure 39 IR Spectrum of PGS-8

IJSER © 202
http://www.ijser.org

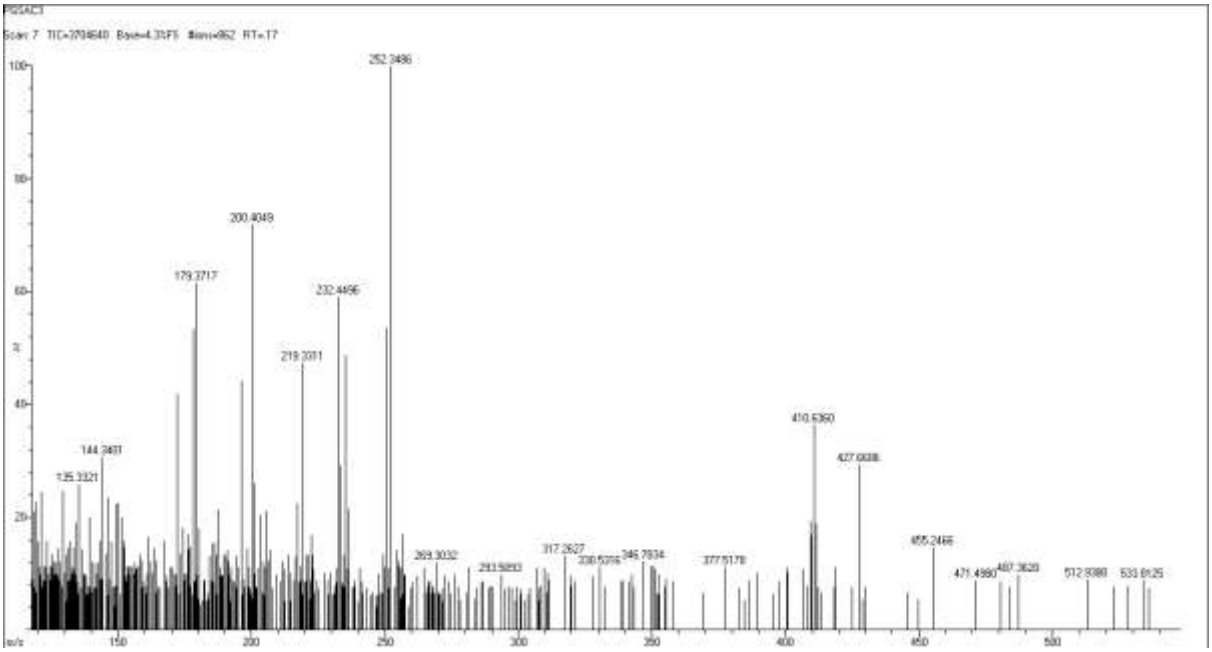


Figure 40
Mass spectrum of PGS-8

IJSER

IJSER © 202
http://www.ijser.org

Compounds PGS-6, PGS-7 and PGS-8 have been isolated from the methanol soluble fraction of the aqueous extract of stems of *Pisonia grandis*. PGS-6 has been characterised as allantoin. This molecule has been reported to degrade in aqueous conditions to form allantoic acid. Allantoin also form polymeric entities by intermolecular hydrogen bonding. Hence compounds PGS-7 and PGS-8 may be the degradation products of allantoin. Since compound PGS-8 did not reveal any prominent signals in the NMR spectra its part characterisation could only be made. Further characterisation will be made by recording a nitrogen NMR spectrum in future studies.

4.5.4 Characterization of PGS-9

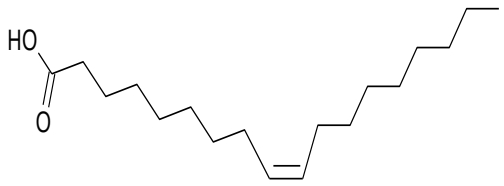
PGS-9 was isolated by preparative TLC analysis of petroleum ether extract concentrate of stems of ***Pisonia grandis*** as a pale greenish yellow solid. Yield: 42 mg. It was low melting and freely soluble in chloroform.

Spectral data:

IR (KBr) γ_{max} cm^{-1}: 3425, 2924, 2854, 2677, 2376, 1712, 1458, 1280, 1172, 1111, 1041 941,871, 725,478 **(Figure 41)**

IJSER

GC-MS chromatogram of **PGS-9** showed an intense peak of 100% abundance at retention time 20.23 minutes. It corresponded to mass fragmentation peaks at m/e 41, 55,69,83,97110,125,138,151,222,264 and m/e 282. Based on the GC-MS data and compound PGS-10 was identified as 9-Octadecenoic acid **(25).**



25

IJSER © 202
http://www.ijser.org

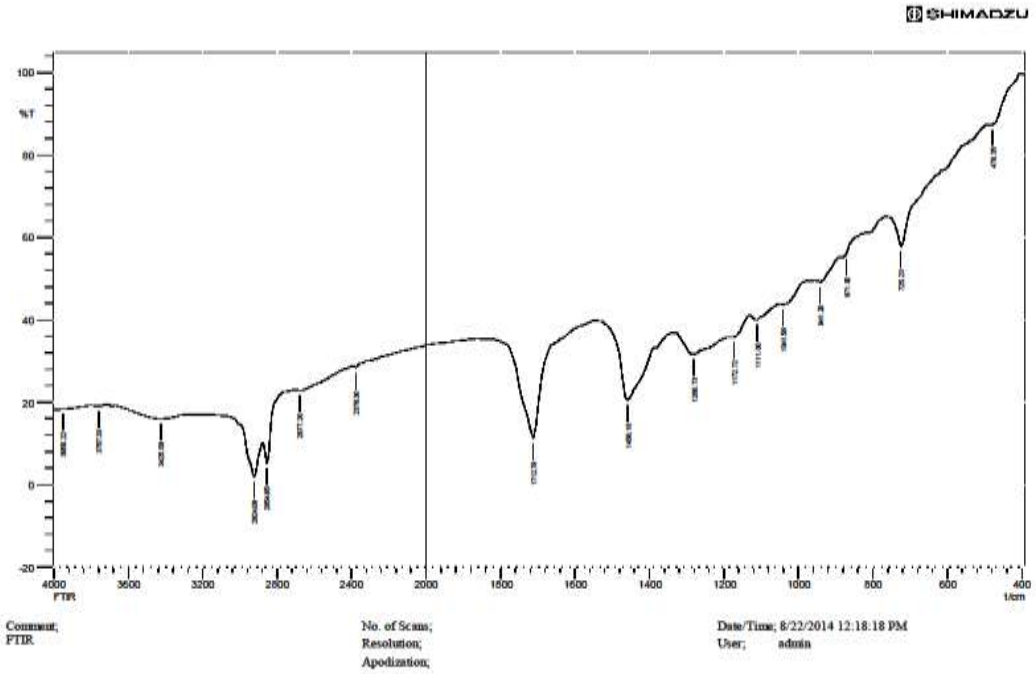


Figure 41
Figre 83

IJSER

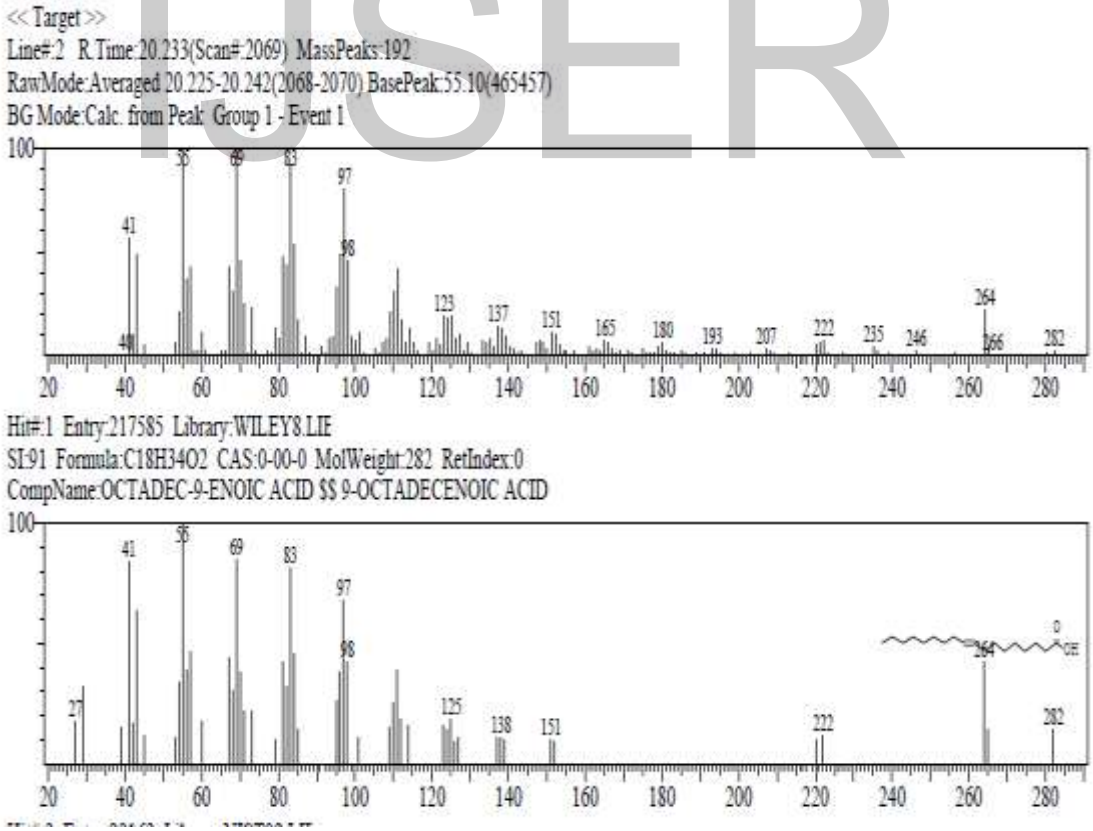


Figure 42

Comparison of mass spectrum of PGS-9 and 9-octadecenoic acid

IJSER © 202
http://www.ijser.org

4.5.5 Characterization of PGS-10

The methanol insoluble portion of aqueous extract concentrate of stems of ***Pisonia grandis*** on maceration with alcohol yielded a brown solid designated as **PGS-10.**

Yield:400 mg. Melting Point: Decomposition with formation of residue at 168-171˚C. A positive Fehling's test indicated sugar moiety. Brown colouration with ammoniacal silver nitrate indicated the presence of sugar alcohol moiety.

Spectral data

IR (KBr) γ_{max} cm^{-1}: 3393, 2924, 2155, 1617, 1413, 1324, 1243, 1148, 1078, 1023, 832, 763, 694, 616, 526.

^{1}H NMR and ^{13}C NMR : Figure 43, 44 and 45 represent the NMR spectra of **PGS 10**

The proton NMR spectrum of this compound revealed the presence of signals due to sugar alcohols and and anomeric protons. The prominent signals in the proton NMR spectrum were seen at δ3.7 may **due to a sugar alcohol moiety and the glycoside moiety.** Since the sugar alcohol pinitol has been elaborated by *Pisonia grandis* as revealed by the present study, it is most probable that the sugar alcohol constituting compound **PGS 10 is pinitol. A thorough analysis of the NMR spectra indicated that this compound may be glycosides of sugar alcohol.**

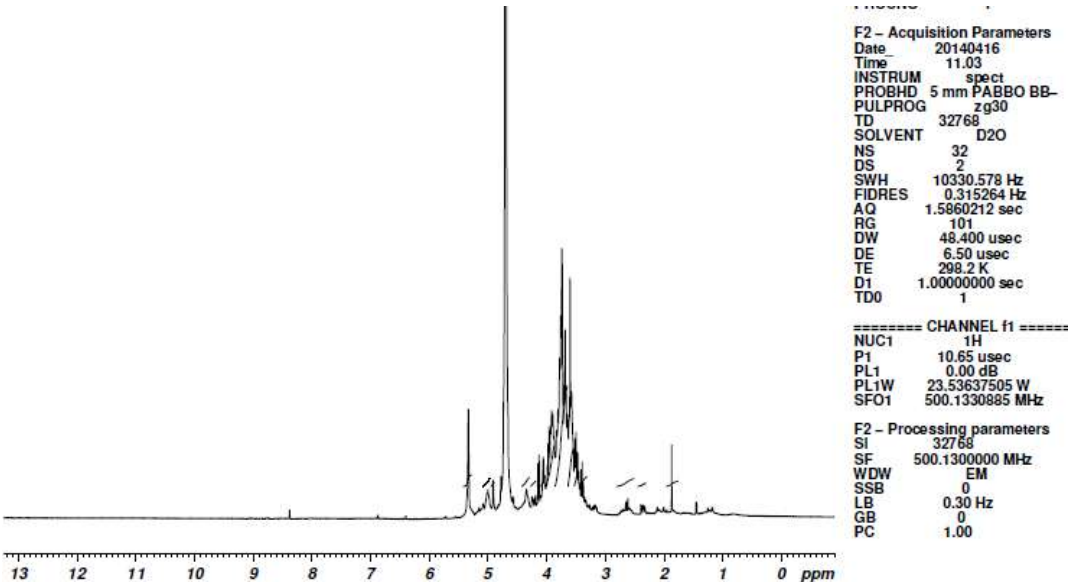


Figure 43
^{1}H NMR spectrum of PGS-10

IJSER © 202
http://www.ijser.org

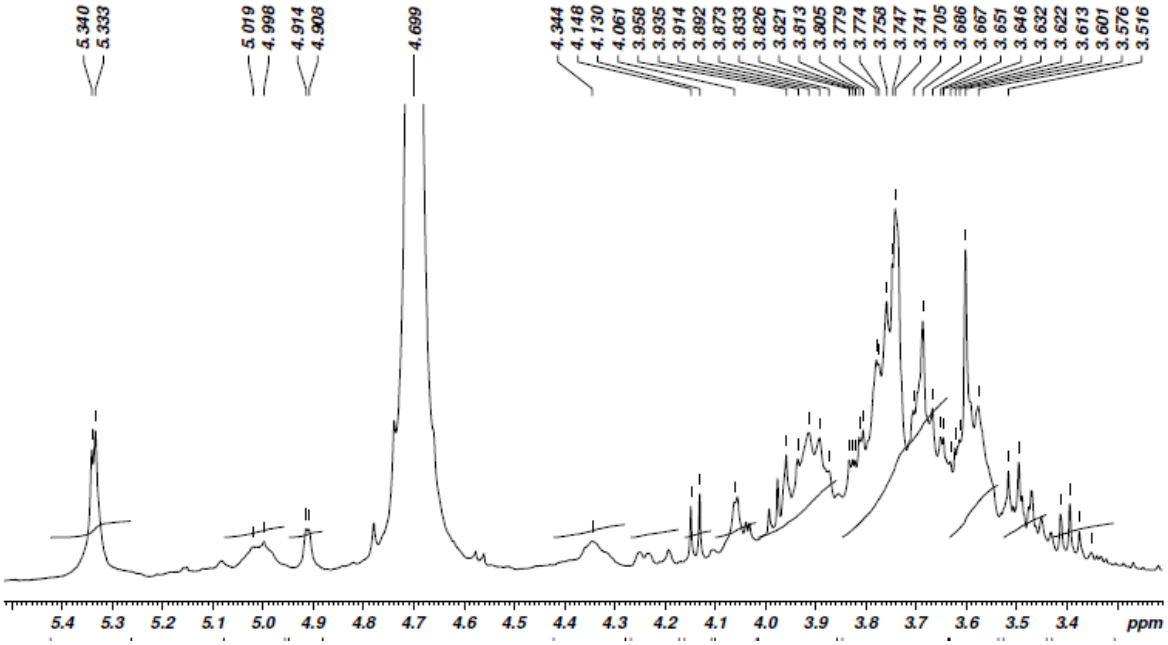


Figure 44
Expanded scale of ^{13}H NMR spectrum of PGS-10

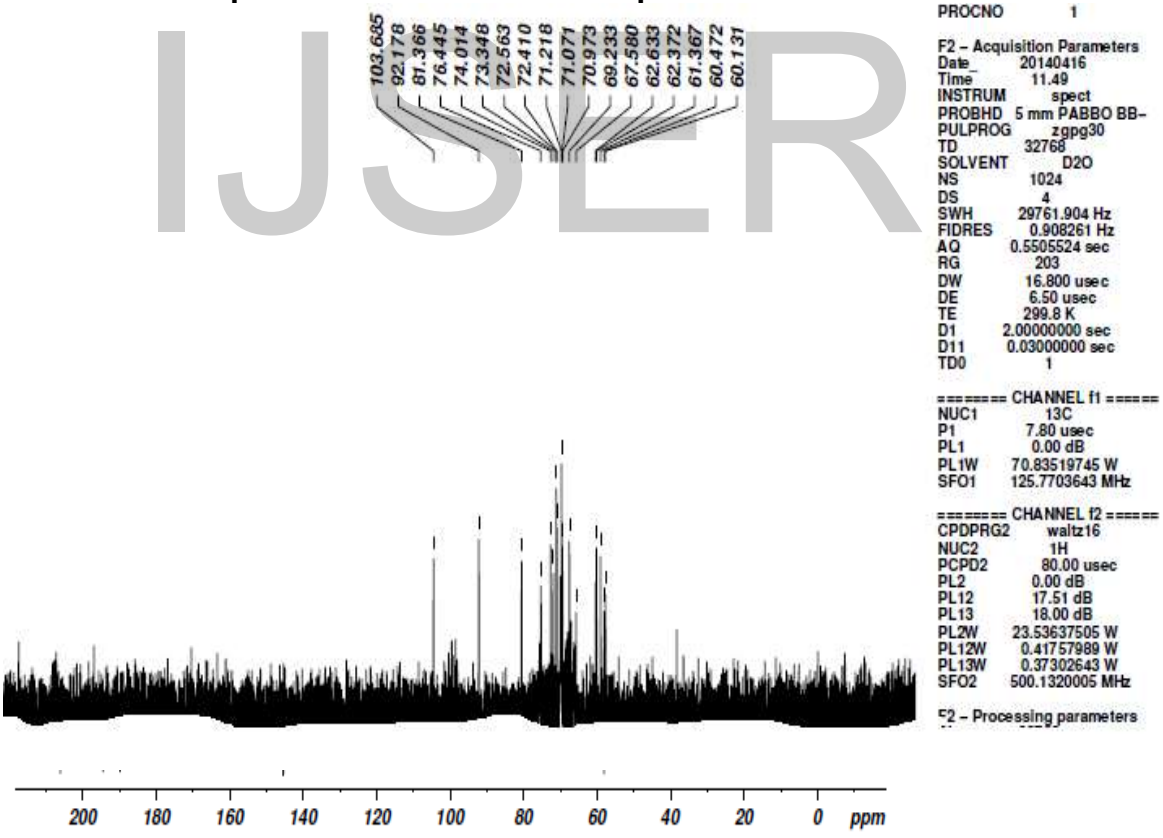


Figure 45
^{13}C NMR spectrum of PGS-10

IJSER

IJSER © 202
http://www.ijser.org

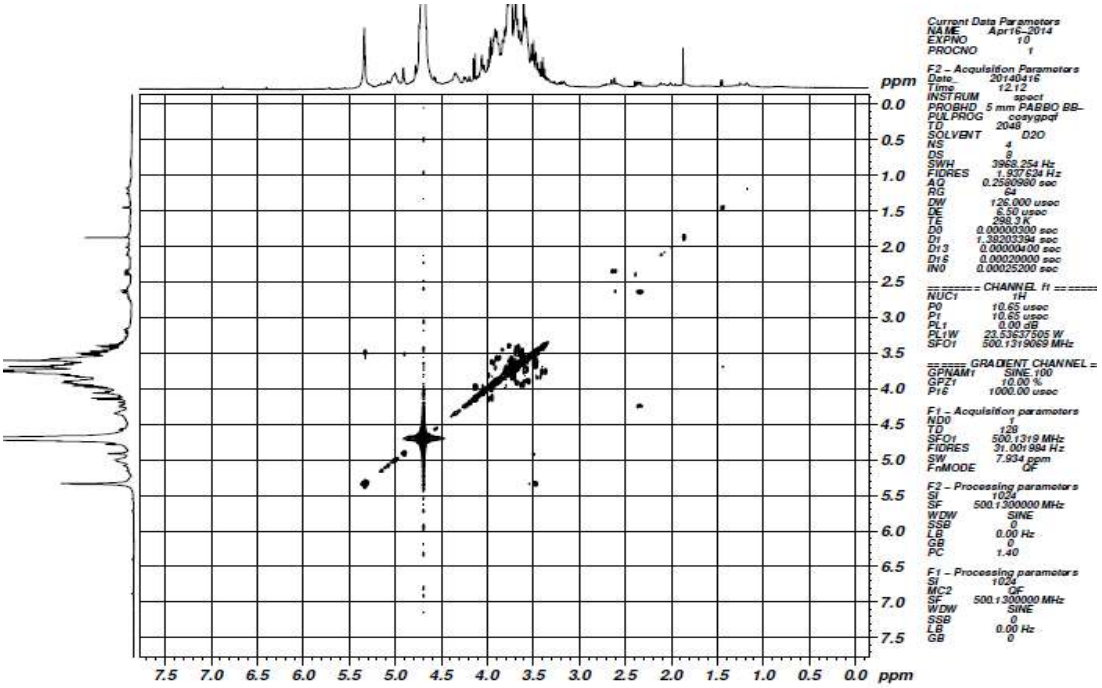


Figure 46
COSY spectrum of PGS-10

IJSER

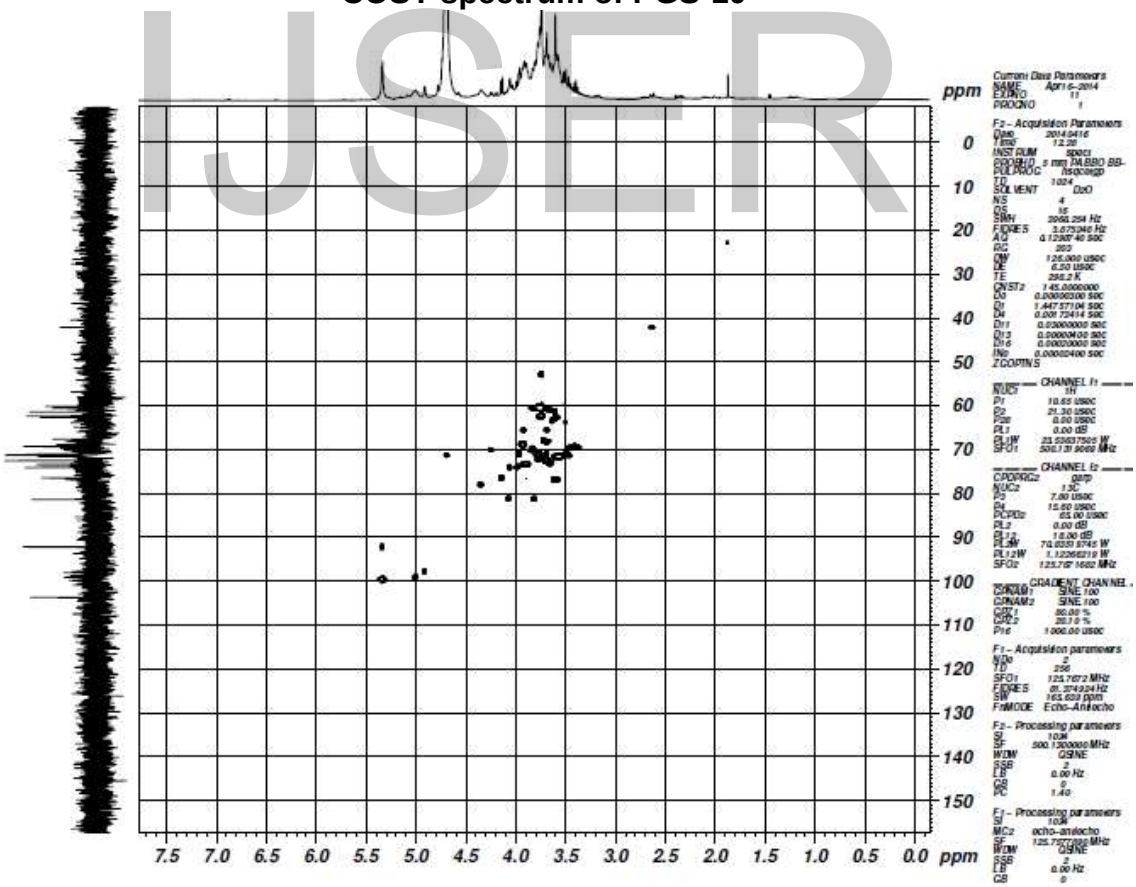


Figure 47
HSQC spectrum of PGS-10

IJSER © 202
http://www.ijser.org

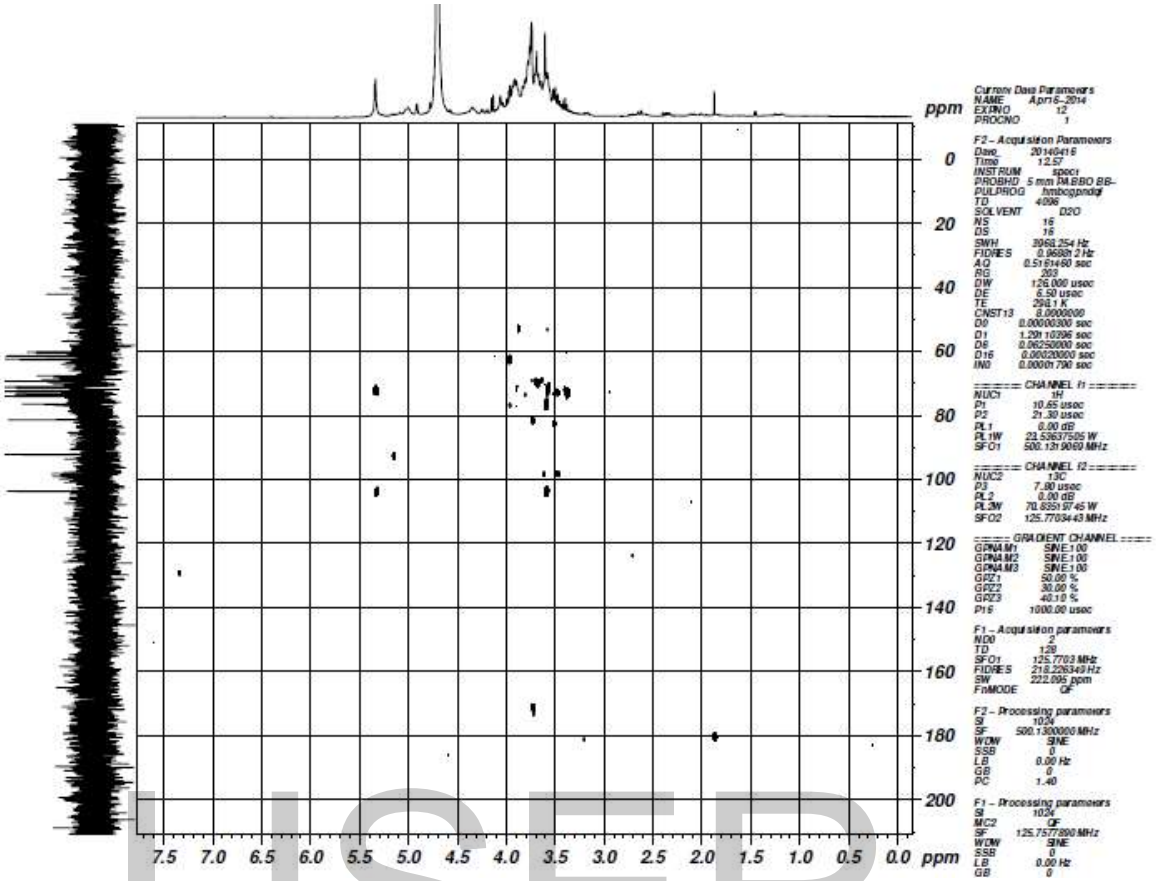


Figure 48
HMBC spectrum of PGS-10

IJSER

4.5.6 Characterization of PGS-11

The methanol insoluble portion of aqueous extract concentrate of stems of ***Pisonia grandis*** on maceration with alcohol yielded a white precipitate designated as **PGS-11. It was freely soluble in water.** Yield: 88 mg. The compound did not melt upto 250 ˚C. No characteristic spot was observed in TLC. Compound **PGS-11** gave an orange red flame on heating over a flame. It was positive to test for oxalates. Based on these observations **PGS-11** and from its IR spectrum (Figure 49) has been identified as an oxalate salt.

IJSER © 202
http://www.ijser.org

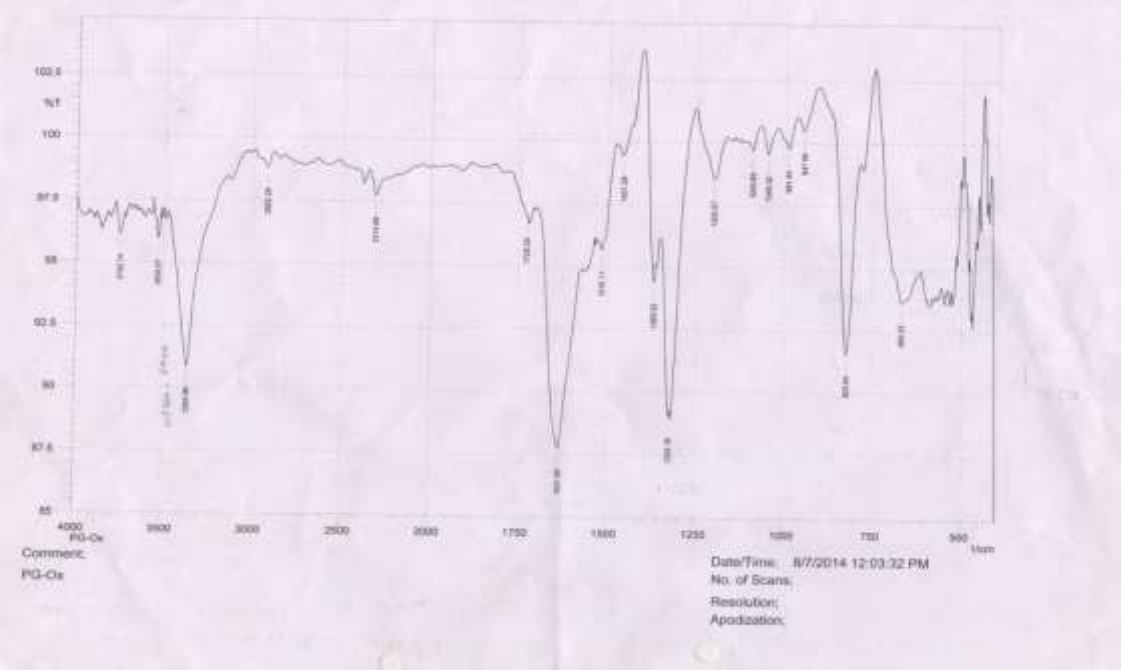


Figure 49

IR spectrum of PGS-11

IJSER

4.5.6 Characterization of PGS-12

The methanol soluble portion of aqueous extract concentrate of stems of *Pisonia grandis* on maceration with alcohol yielded a pale white solid designated as **PGS-12.**

Yield : 43 mg. Melting point: >250^{o}C. TLC: no characteristic spot.

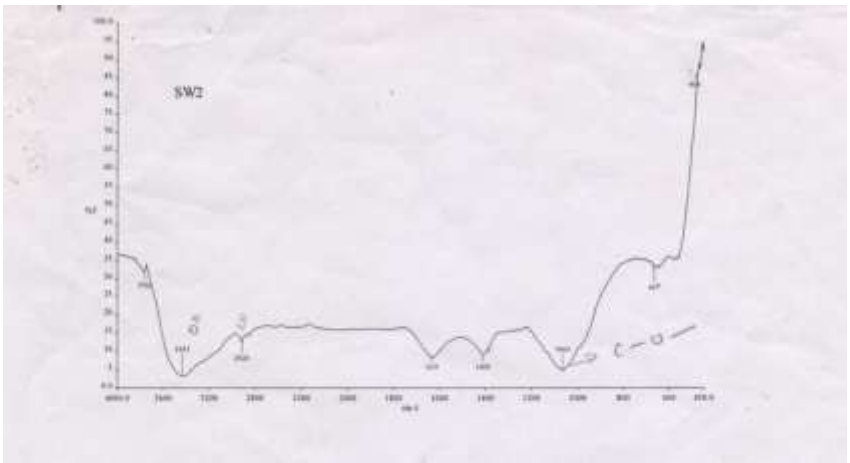


Figure 50

IR spectrum of PGS-12

IJSER © 202
http://www.ijser.org

Characterization of PGL-1

The compound **PGL-1** was obtained by column chromatographic analysis of the petroleum ether extract concentrate of leaves of ***Pisonia grandis*** on elution with 100 % petroleum ether. Melting point: 142-146°C. TLC R_f =0.56 (petroleum ether: ethyl acetate 8:2 v/v).

Compound **PGL-1** was inferred as steroid moiety from the test.

Spectral data

IR (KBr) γ_{max} cm^{-1}: 3376, 2940, 1643, 1458,1382, 888

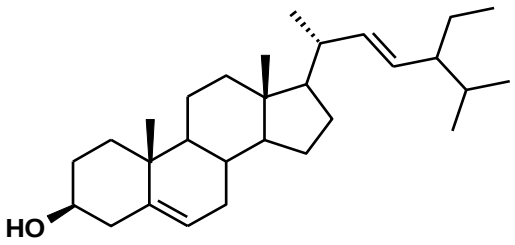
^{1}H NMR (500MHz, $CDCl_3$, δ ppm): 0.79-0.82 (3H), 0.84-0.88 (3H), 1.01(3H), 1.25 (3H), 5.0 (1H), 5.18 (1H) (Figure 51)

^{13}C NMR (125 MHz, $CDCl_3$, δ) : **Table 10** (Figure 52)

The IR spectrum of PGL-1 revealed a characteristic absorption due to hydroxy group at 3376 cm^{-1}. The ^{1}H NMR spectrum of **PGL-1** indicated the presence of six methyl groups that appeared at δ 0.79-0.82, 0.84-0.88, 1.01 and δ 1.25. The signals which appeared as a doublet of doublet at δ 5.18 – 5.14 (1H) and δ 5.01 were attributed to two olefinic protons. The broad singlet at δ 5.34 and was assigned to a third olefinic proton.

IJSER

The ^{13}C NMR spectrum of **PGL-1** indicated twenty nine carbon signals. Olefinic protons were seen at δ 139.7(C-5), 117.5(C-6), 138.2(C-22) and 129.5(C-23). A hydroxyl bearing carbon was seen at 71.1 (C-3). The presence of a steroid skeleton was confirmed by the ^{13}C NMR signals at δ 129.5 and 138.2 characteristic of Δ^{22} sterol and at δ139.7 and 117.5 characteristic of Δ^5 sterol. The above spectral evidences indicated that compound **PGL-1** to be stigmasterol. A comparision of the ^{13}C NMR spectra data **PGL-1** with that reported in the literature was made (**Table 10**)



26

IJSER © 202
http://www.ijser.org

Table 10 Comparison of ^{13}C NMR chemical shifts of PGL-1 and Stigmasterol

Chemical Shift δ (ppm)					
Carbon number	PGL-1	Stigmasterol Alma *et al.*(1996)	Carbon number	PGL-1	Stigmasterol Alma *et al.*(1996)
C-1	37.2	37.0	C-16	28.5	28.5
C-2	31.5	31.6	C-17	55.9	55.8
C-3	71.1	71.7	C-18	12.1	11.6
C-4	40.8	41.5	C-19	19.0	19.2
C-5	139.7	140.2	C-20	40.3	40.1
C-6	117.5	121.6	C-21	21.1	20.4
C-7	31.4	31.7	C-22	138.2	138
C-8	31.9	31.9	C-23	129.5	129.2
C-9	49.9	50.1	C-24	51.2	51.0
C-10	37.1	36.7	C-25	31.9	32.0
C-11	21.5	20.8	C-26	21.1	19.0
C-12	39.5	39.5	C-27	21.4	21.2
C-13	43.2	42.5	C-28	25.4	25.4
C-14	55.1	56.6	C-29	12.1	12.0
C-15	25.4	24.0			

IJSER

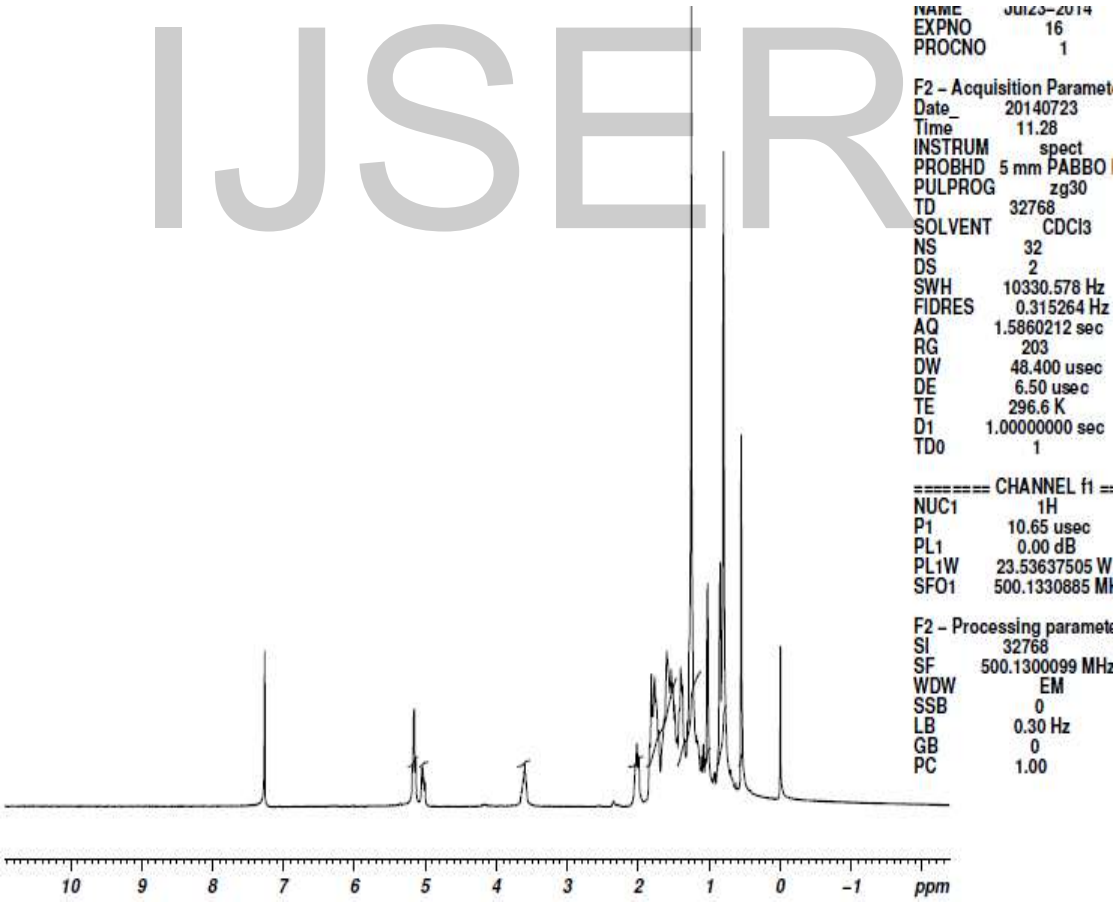


Figure 51
^{1}H NMR spectrum of PGL-1

IJSER © 202
http://www.ijser.org

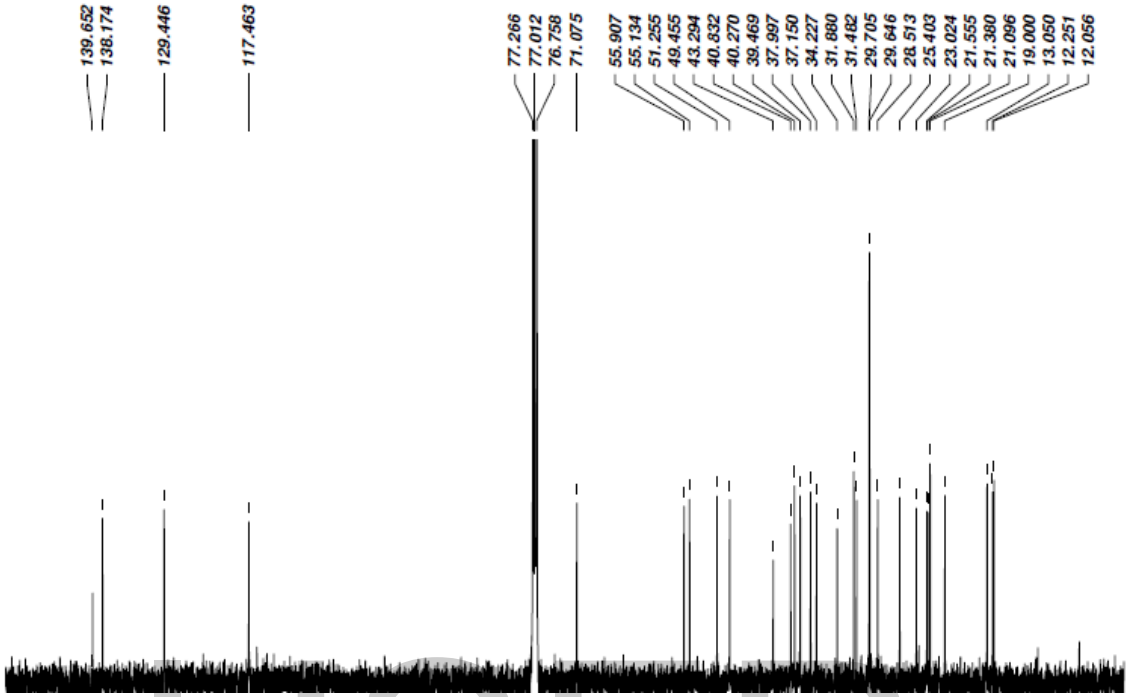


Figure 52
^{13}C NMR spectrum of PGL-1

IJSER

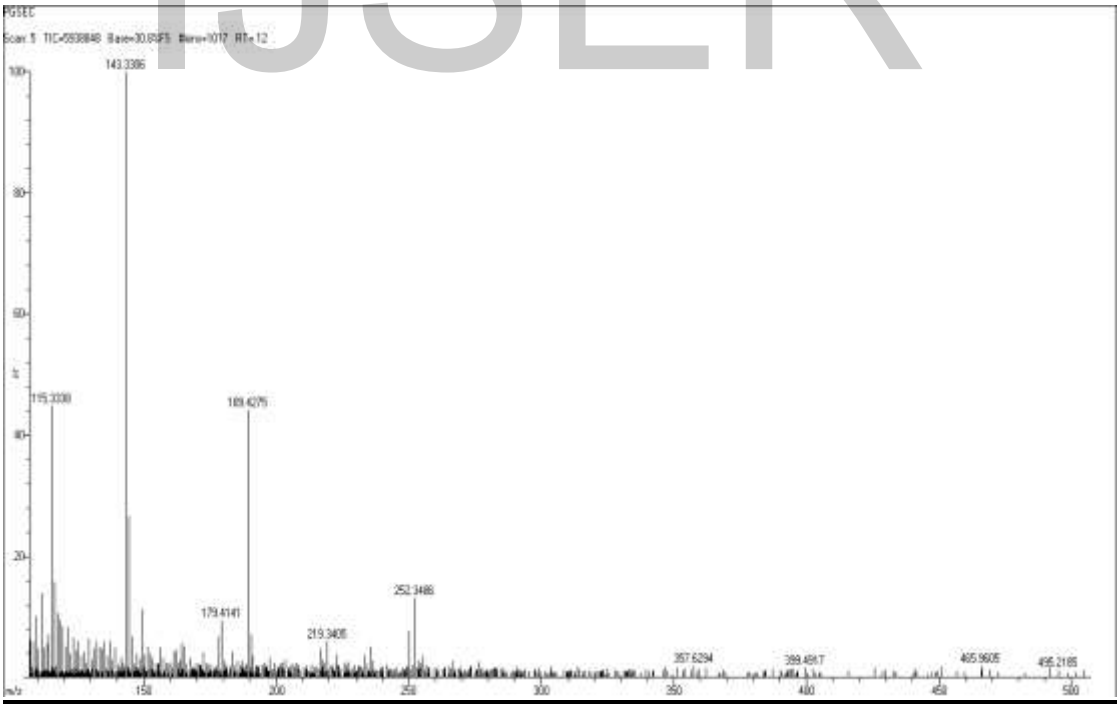


Figure 53
Mass spectrum of PGL-1

IJSER © 202
http://www.ijser.org

Characterisation of constituents of *Pisonia grandis* isolated by simple protocol

Characterization of PGL-2

The acetone soluble fraction of dewaxed ethanol extract on concentration and cooling yielded a pale white solid designated as **PGL-2**. Yield: 19.6 mg. Melting Point: 182 to186°C . TLC R_f = 0.50 (chloroform: methanol: water (6:3.5:0.5). A Co-TLC (Fig.54) of **PGL-2** with pinitol isolated from the stems **(PGS-4)** and an authentic sample of pinitol revealed that the compounds are identical. The mixed melting point was undipped.

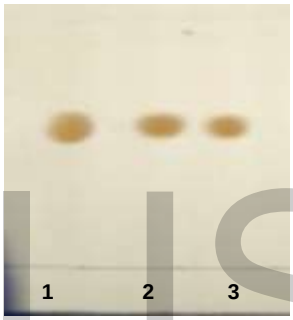


Figure 54

1 =Standard Pinitol
2 =PGL-2
3= PGS-4

IJSER

Thin layer chromatogram of PGL-2, PGS-4 and standard pinitol

Characterization of PGL-3

The chloroform soluble fraction of the dewaxed ethanol extract of leaves of ***Pisonia grandis*** yielded pale white solid. Yield: 7.2 mg. Melting Point: 86 – 89 °C.

Spectral Data

IR (KBr) γ_{max} cm^{-1}: 3282, 2956, 2916, 2848, 2312, 1466, 1377, 1212, 1061,724,679 (Figure 55).

^{1}H NMR (500MHz, $CDCl_3$, δ ppm): (Figure 56)

^{13}C NMR (125 MHz, $CDCl_3$, δ) : (Figure 57)

Analysis of ^{13}C **NMR** data and its comparison with reported data **(Table 11)** led to the characterisation of **PGL-3** as octacosanol

IJSER © 202
http://www.ijser.org

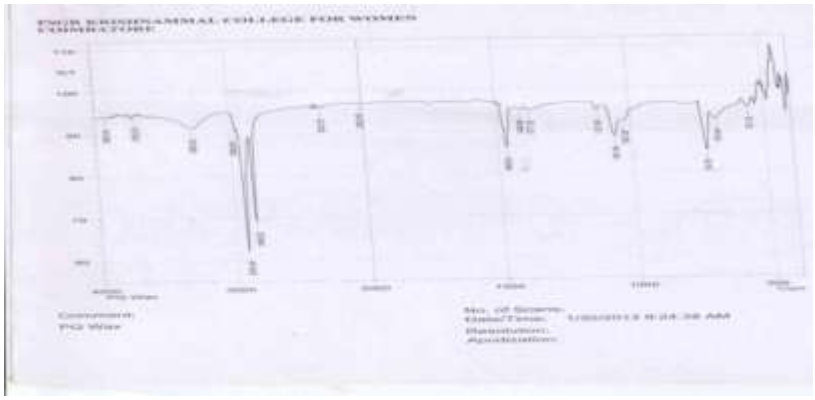


Figure 55
IR spectrum of PGL-3

Table 11 ^{13}C NMR chemical shifts δ of PGL-3

Chemical Shift δ (ppm)	
PGL-3	**Octacosanol (SDBS Database)**
17.86	14.2
26.53	22.7
29.63	25.75
33.21	29.37
33.34	29.47
33.50	29.63
33.54	29.71
35.79	31.74
36.39	32.84
66.31	63.13

IJSER

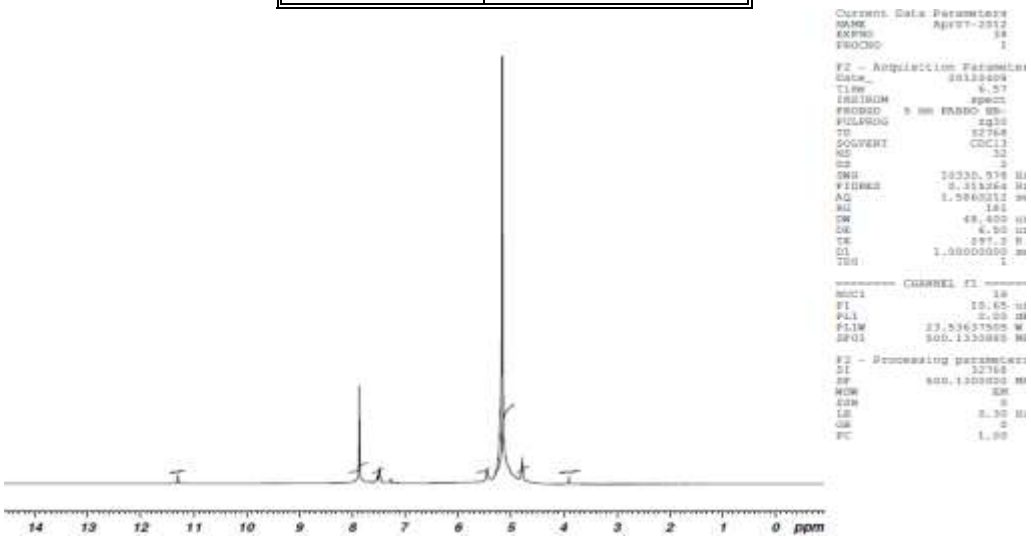


Figure 56
^{1}H NMR of PGL-3

IJSER © 202
http://www.ijser.org

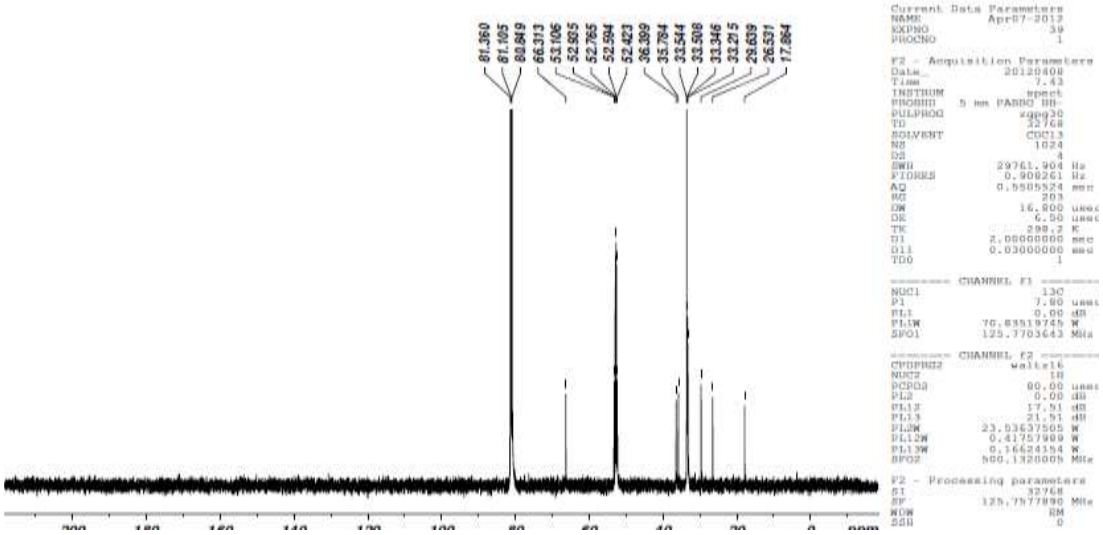


Figure 57
^{13}C NMR of PGL-3

Characterization of PGL-4

Compound **PGL-4 was isolated as** a pale white compound (Chart 7). Yield =32 mg. Melting Point: 224-230˚C. TLC R_f = 0.62 (chloroform: methanol: water 6:4:3 drops. It gave a positive Lessaigne's test indicating the presence of nitrogen. Enhancement of yellow colouration with dimethyl amino benzaldehyde indicated a ureide kind of moiety. Further confirmation of **PGL-4** was done by reversed phase HPLC analysis of this compound on C18 column using PDA detector (Figure 58 and 59). The HPLC chromatograms of **PGL-4** and an authentic sample of allantoin exhibited peaks at same retention time.

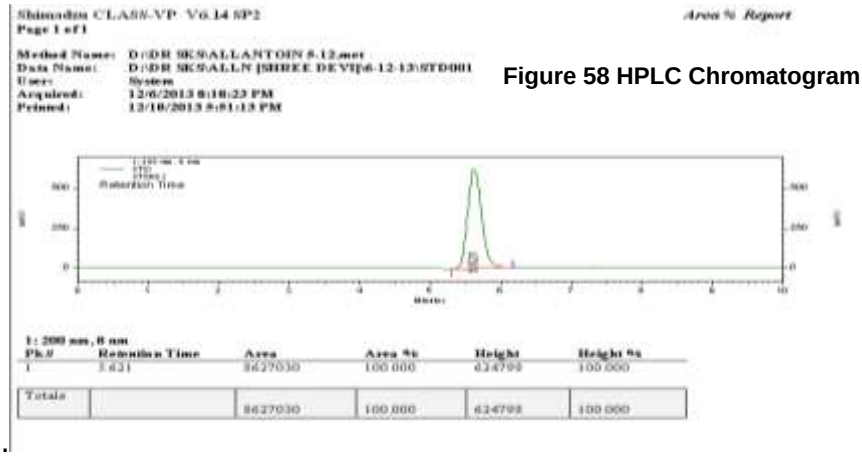


Figure 58 HPLC Chromatogram of Standard allantoin

IJSER © 202
http://www.ijser.org

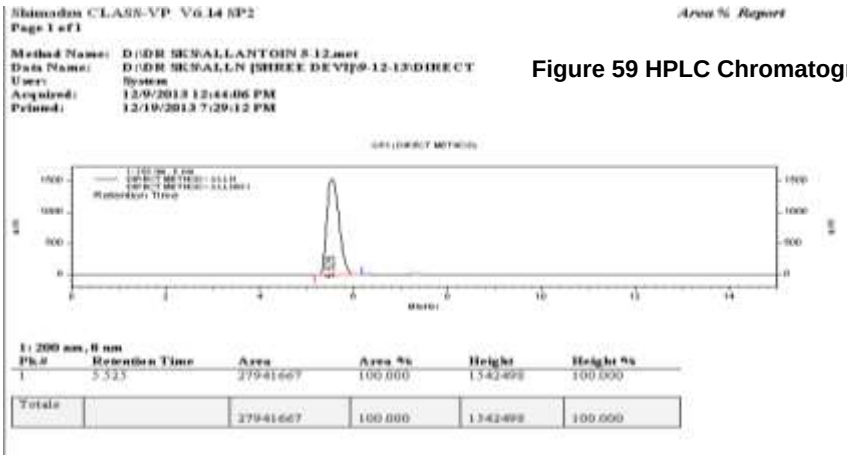


Figure 59 HPLC Chromatogram of PGL-4

Characterization of Compounds Isolated from *Andrographis stenophylla*

Column chromatographic analysis of dewaxed ethanol extract of concentrate of aerial parts of *Andrographis stenophylla* led to the isolation of five compounds designated as **ASA-1, ASA-2, ASA-3, ASA-4 and ASA-5**.

Characterization of ASA-1

Compound **ASA-1** was obtained by column chromatographic analysis of the dewaxed ethanol extract of aerial parts of *Andrographis stenophylla* by elution with petroleum ether and ethyl acetate (70:30). Yield: 11 g. Melting Point: 176-182^{o}C. The compound gave a dark grey colour with alcoholic $FeCl_3$ solution. The compound **ASA-1** responded to Shinoda's test (Mg/HCl) giving a pale pink colour indicating the presence of flavones with chelated hydroxyl group.

Spectral data

1H NMR (500MHz, DMSO, δ ppm): Table 12

^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): : Table 13

EI-MS (m/z): 298 $[M]^+$

The UV absorption band at 272 & 352 cm-1 indicate a flavone moiety. No shift in λ_{max} was observed with sodium acetate shift reagent. Hence the 7-position of flavone ring must be a substituted hydroxyl group.

The IR spectrum showed absorption bands for a hydroxyl group (3946 cm^{-1}) and conjugated carbonyl group (1655 cm^{-1}). The mass spectrum showed the highest mass peak at m/z 283 consistent with molecular formula

IJSER © 202
http://www.ijser.org

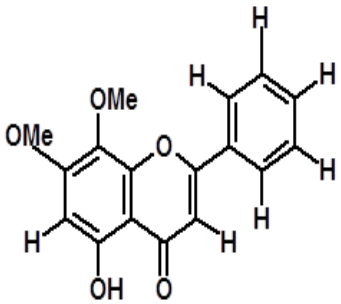
$C_{17}H_{16}O_4$ and associated with ten degrees of unsaturation. The ^1H NMR spectrum showed downfield resonance at δ 12.6 attributed to a chelated hydroxyl proton. The appearance of two singlet's at δ 3.92 and 3.85 indicated the presence of two methoxyl groups. Further, a group of 5 signals were observed in the down field region. The group of the signals at δ 8.07-8.08 integrating to 2 protons and at δ 7.61-7.62 integrating to 3 protons suggested a substituted C ring of flavone moiety.

The ^{13}C NMR spectrum (Figure 61) showed fifteen signals. The signals at δ182.8 implies characteristic absorption of carbonyl carbon at C-4. The signal at δ 163.9 was indicative of C-2 carbon of flavonoid. The other characteristic signals were observed at δ149 and δ104.6 and attributed to C9 and C10 carbons. 105.4, 157.1, 159.1, signals were assigned for C-3, C-5, C-7 of the flavonoid moiety.

The 1H-1HCOSY spectrum (Figure 62) showed the expected correlations between proton signals. The HSQC spectrum (Figure 63) established the connectivity between the carbons and respective protons. The HMBC spectrum (Figure 64) showed the 3-bond correlations.

IJSER

The mass spectrum gave the molecular ion peak at 298.3 associated with the molecular formula $C_{17}H_{14}O_5$. Based up on the above evidences and comparing the spectral data with literature, the compound ASA-1 was identified as 5-hydroxy-7,8-dimethoxy-flavone (27).



27

IJSER © 202
http://www.ijser.org

Table 12 ^{1}H NMR chemical shifts of ASA-1

Chemical shift (signal position δ)	Multiplicity	Proton Number
3.85	Singlet	3H
3.92	Singlet	3H
6.60	Singlet	1H
7.02	Singlet	1H
7.61-7.62	Multiplet	3H
8.07-8.08	Doublet of doublet	2H
12.63	Singlet	1H

Table 13 Assignment of ^{1}H and ^{13}C NMR signals

Carbon	^{1}H NMR (δ)	C^{13} NMR (δ)
C-2	-	163.9
C-3	7.02	105.4
C-4	-	182.8
C-5 (OH)	12.63	157.1
C-6	6.60	96.6
C-7 (OMe)	3.92	159.1
C-8 (OMe)	3.84	129.7
C-9	-	149.3
C-10	-	104.6
C-1’	-	131.2
C-2’,6’	8.07-8.08	126.8
C-3’,4’,5’	7.61-7.62	129.0

IJSER

IJSER © 202
http://www.ijser.org

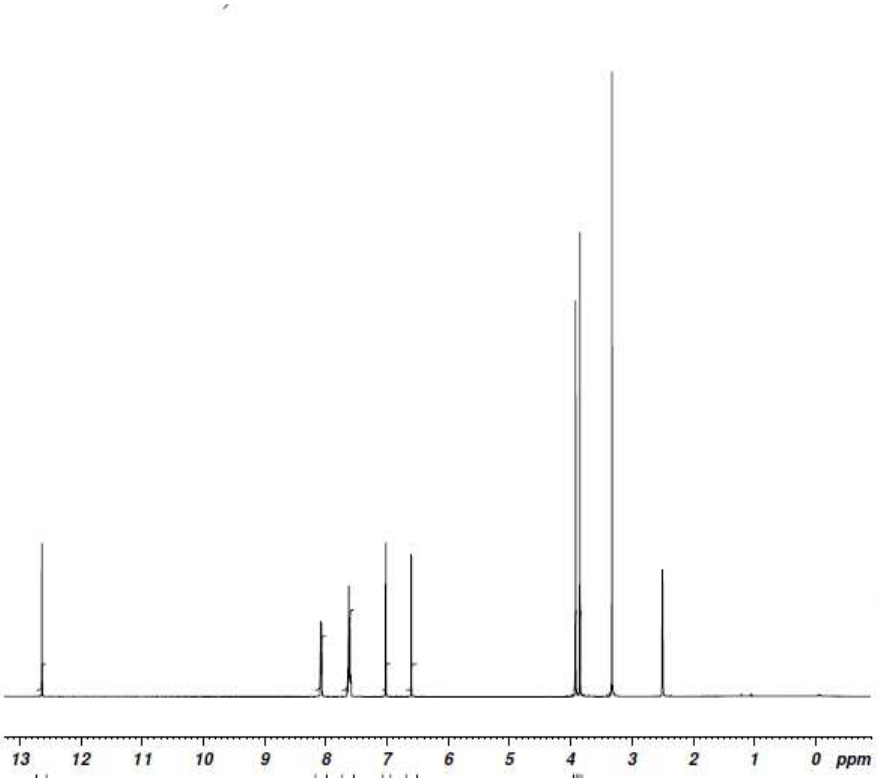


Figure 60

^{1}H NMR spectrum of ASA-1

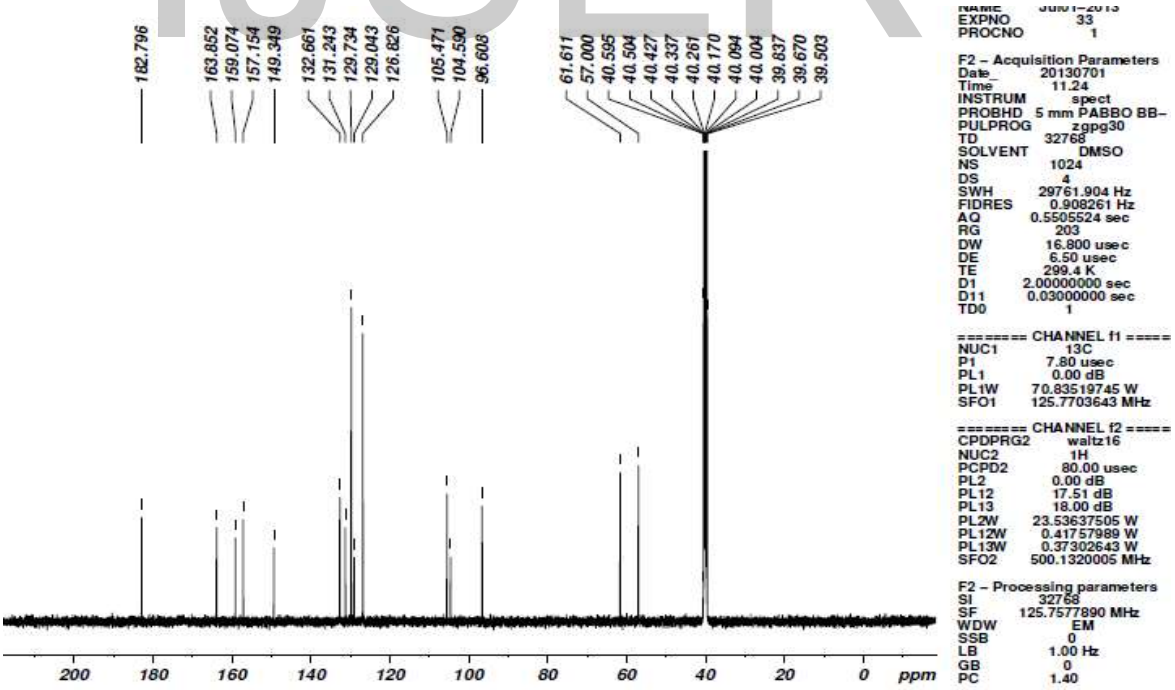


Figure 61

^{13}C NMR spectrum of ASA-1

IJSER

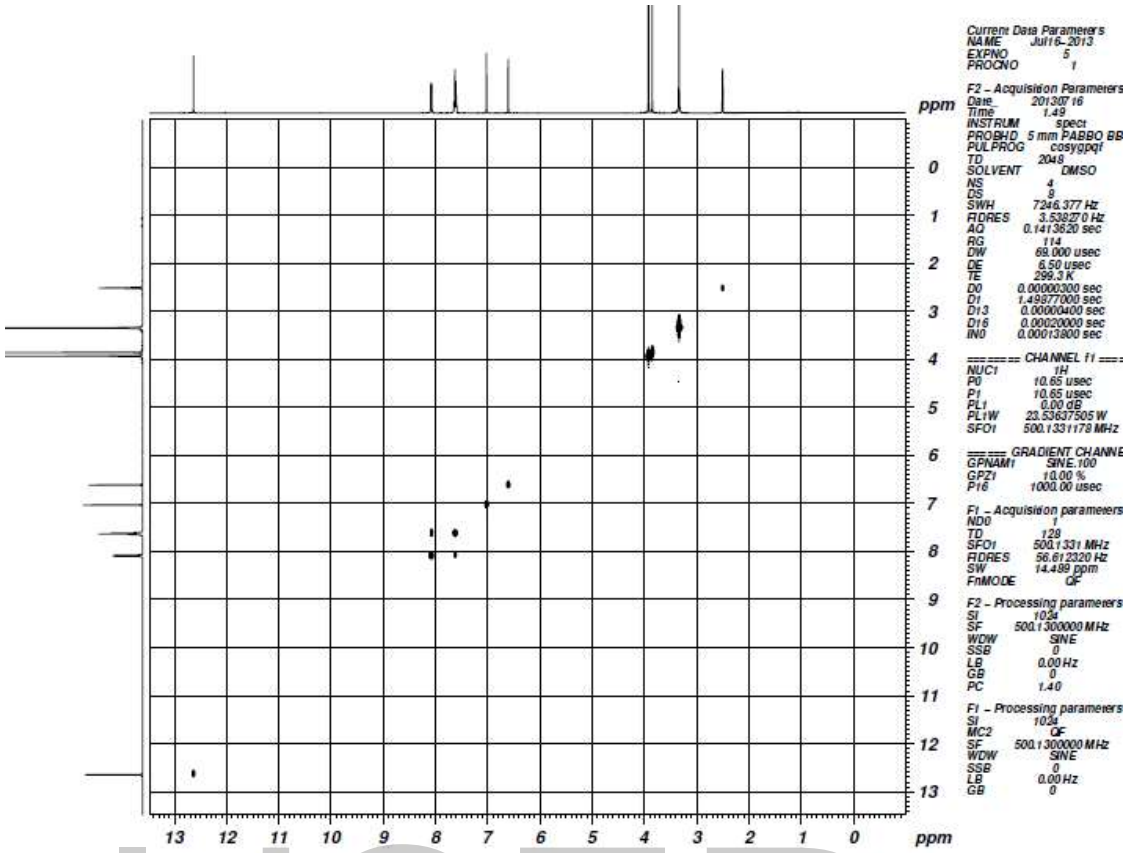


Figure 62

^{1}H-^{1}H COSY spectrum of ASA-1

IJSER

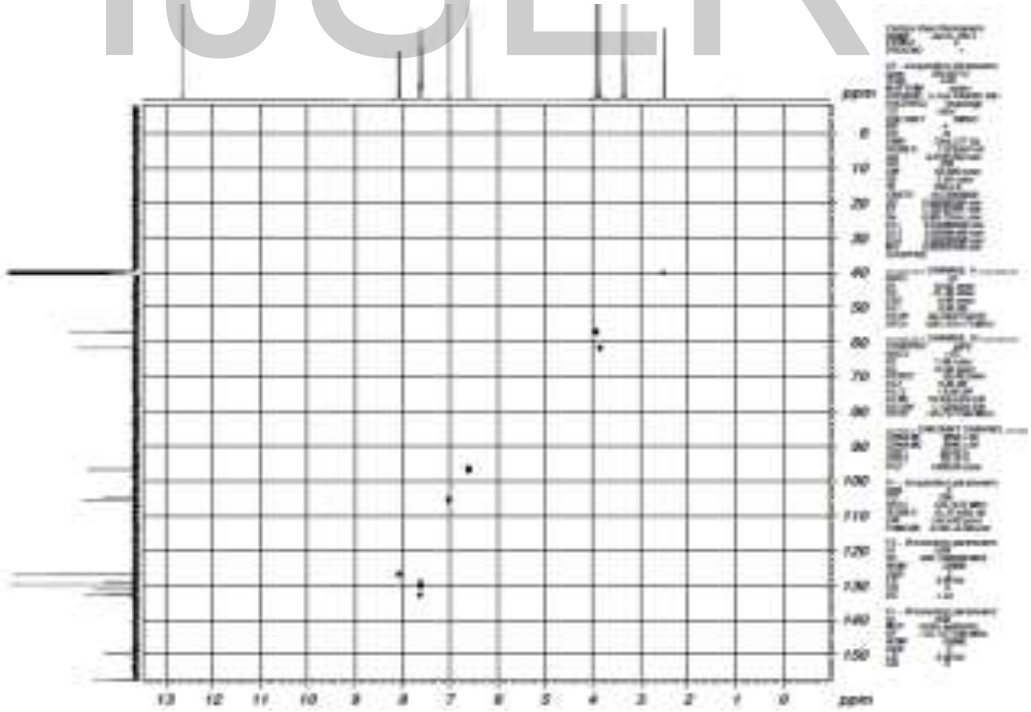


Figure 63

HSQC (^{1}H -^{13}C) spectrum of ASA-1

IJSER © 202
http://www.ijser.org

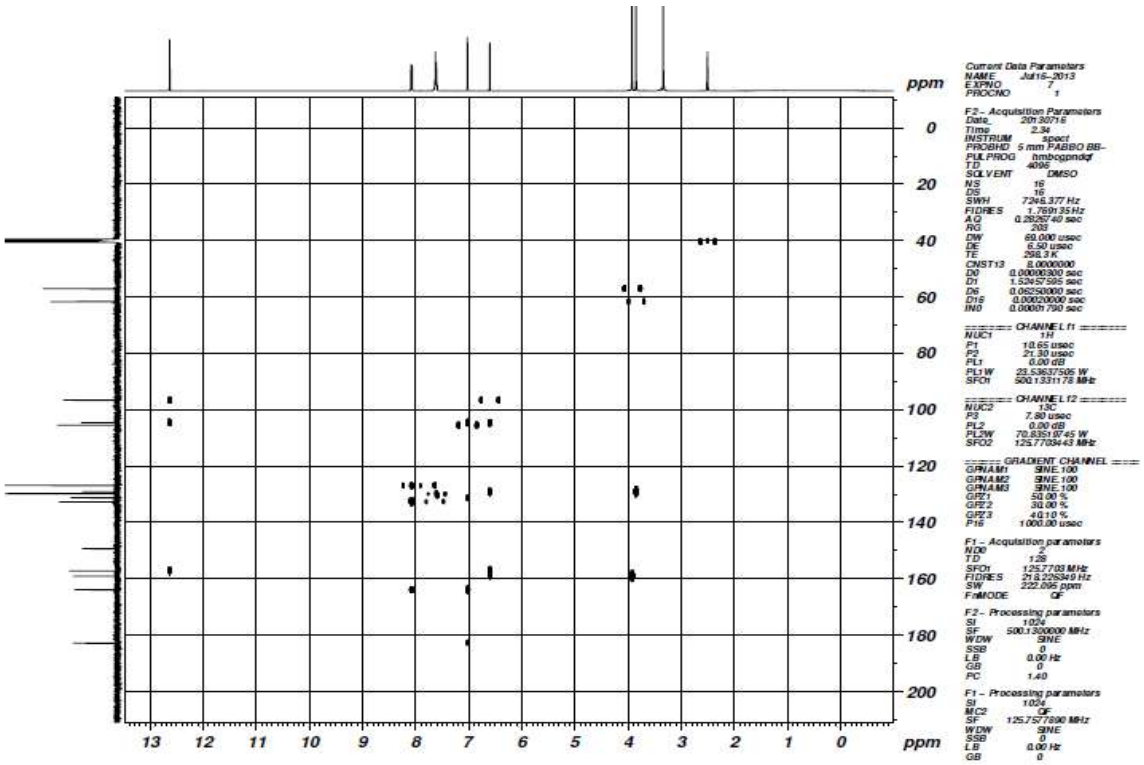


Figure 64

HMBC spectrum of ASA-1

IJSER

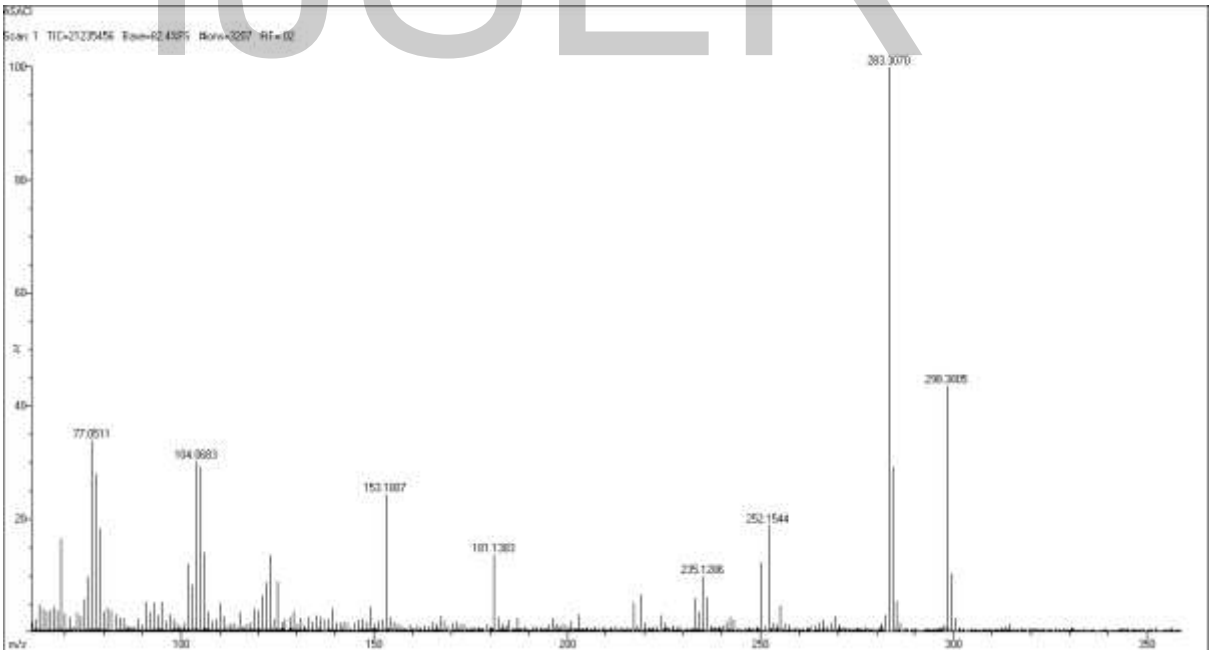


Figure 65

Mass spectrum of ASA-1

IJSER © 202
http://www.ijser.org

Characterization of ASA-2

Compound ASA-2 was obtained by column chromatographic analysis of the dewaxed ethanol extract of aerial parts of *Andrographis stenophylla* by elution with petroleum ether and ethyl acetate (70:30). Yield: 20 g. Melting Point: 238-242^{o}C. The compound gave a dark grey colour with alcoholic $FeCl_3$ solution. It gave a pale pink colour in Shinoda test indicating a flavone.

Spectral data

IR (KBr) γ_{max} cm^{-1}: 3516, 2922, 1739, 1610, 1151

1H NMR (500MHz, DMSO, δ ppm): **Table 14**

^{13}C NMR (125 MHz, $CDCl_3$, δ) : **Table 15**

EI-MS (m/z): 284 $[M]^+$

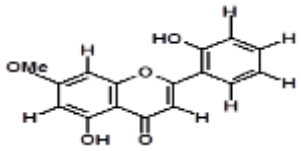
The UV absorption band at 305 and 356 nm indicating the flavonoid nature of the molecule. No shift in λ_{max} was observed with sodium acetate shift reagent. Hence the 7- position of flavone ring must be a substituted hydroxyl group. The IR spectrum showed absorption band for a hydroxyl group (3946 cm^{-1}), CH-group (2922 cm^{-1}) and conjugated carbonyl group (1610 cm^{-1}). The mass spectrum showed the highest mass peak at m/z 243. The ^1H NMR spectrum showed downfield resonances at δ 12.88 attributable to a chelated hydroxyl proton and at δ 10.9 due to an aromatic hydroxyl proton. Presence of a singlet at δ3.87 indicates the presence of one methoxyl group. Further a group of seven signals was observed red in the downfield region.

The ^{13}C NMR spectrum (Figure 67) revealed sixteen carbons. The signal at δ182.6 is characteristic of a flavones carbonyl carbon at C-4. The signal at δ162.1 was indicative of C-2 carbon of flavonoid. The other characteristic signals were observed at δ158 and δ106.6 and attributed to C9 and C10 carbons. Signals at δ110.4, 161.1, 166 and δ93 were assigned for C-3, C-5, C-7, C-8 of the flavonoid moiety.

The COSY spectrum (Figure 68) showed the expected correlations between proton signals. The HSQC spectrum (Figure 69) established the connectivity between the carbons and respective protons. The HMBC spectrum (Figure 70) established the 3-bond correlations. The mass

IJSER © 202
http://www.ijser.org

spectrum gave the molecular ion peak at 284.3. Based up on the above spectral details and comparing the spectral data with literature, the compound ASA-2 was identified as 5-,2'-dihydroxy -7- methoxy-flavone (28).



28

Table 14 ^{1}H NMR chemical shift

Chemical shift (δ)	Multiplicity	Proton Number
3.87	Singlet	3H
6.38-6.39	Doublet	1H
6.77	Doublet	1H
7.00-7.03	Multiplet	1H
7.06-7.08	Doublet	1H
7.13	Singlet	1H
7.40-7.44	Multiplet	1H
7.92-7.94	Doublet of doublet	1H
10.87	Singlet	1H
12.88	Singlet	1H

IJSER

Table 15 Assignment of ^{1}H and ^{13}C NMR signals

Carbon	^{1}H NMR (δ)	C^{13} NMR (δ)
C-2	-	162.1
C-3	7.13	109.7
C-4	-	182.6
C-5 (OH)	12.88	161.1
C-6	6.38-6.39	98.8
C-7 (OMe)	3.87	165.7
C-8 (OMe)	6.77	93.1
C-9	-	157.9
C-10	-	105.2
C-1'	-	117.5
C-2',	10.87	157.3
C-3'	7.06-7.08	117.6
C-4'	7.00-7.03	120.0
C-5'	7.40-7.44	133.4
C-6'	7.92-7.94	129.0

IJSER © 202
http://www.ijser.org

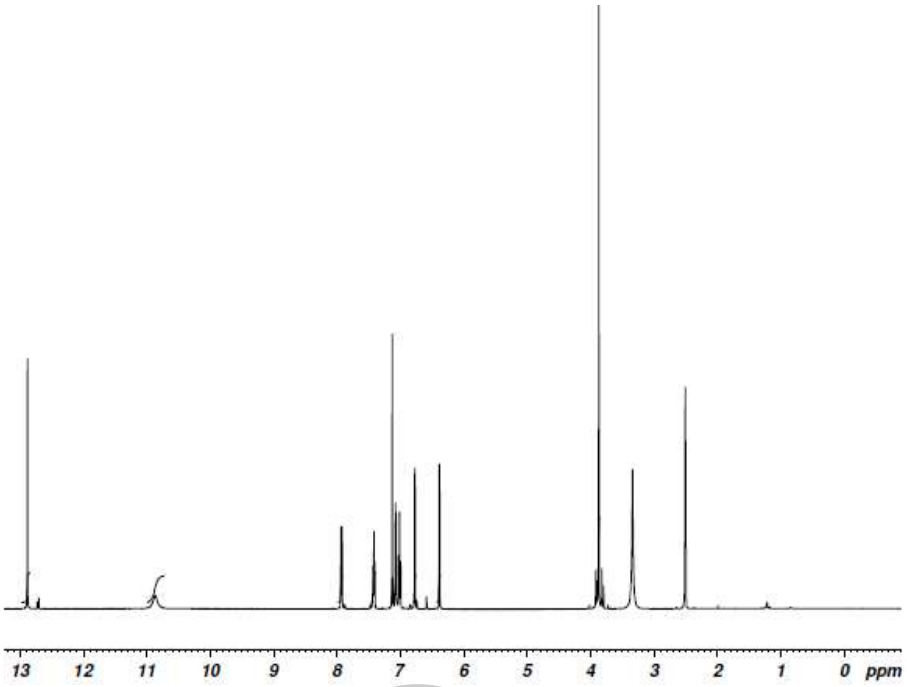


Figure 66

^{1}H NMR spectrum of ASA-2

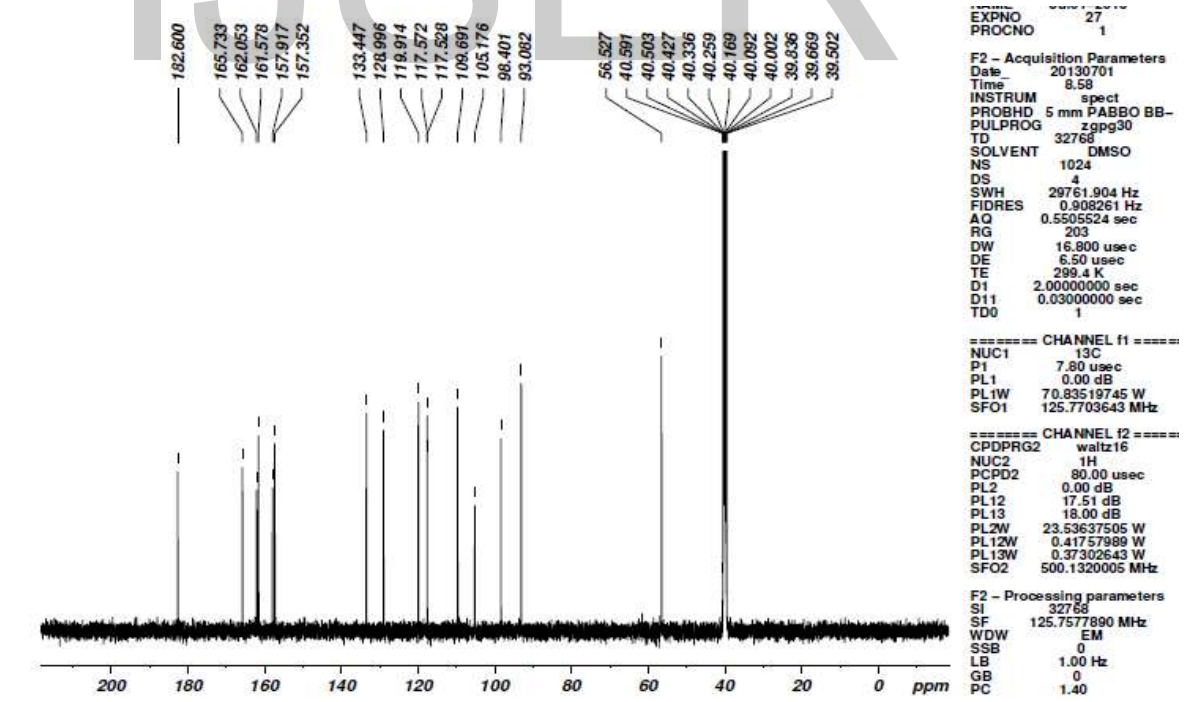
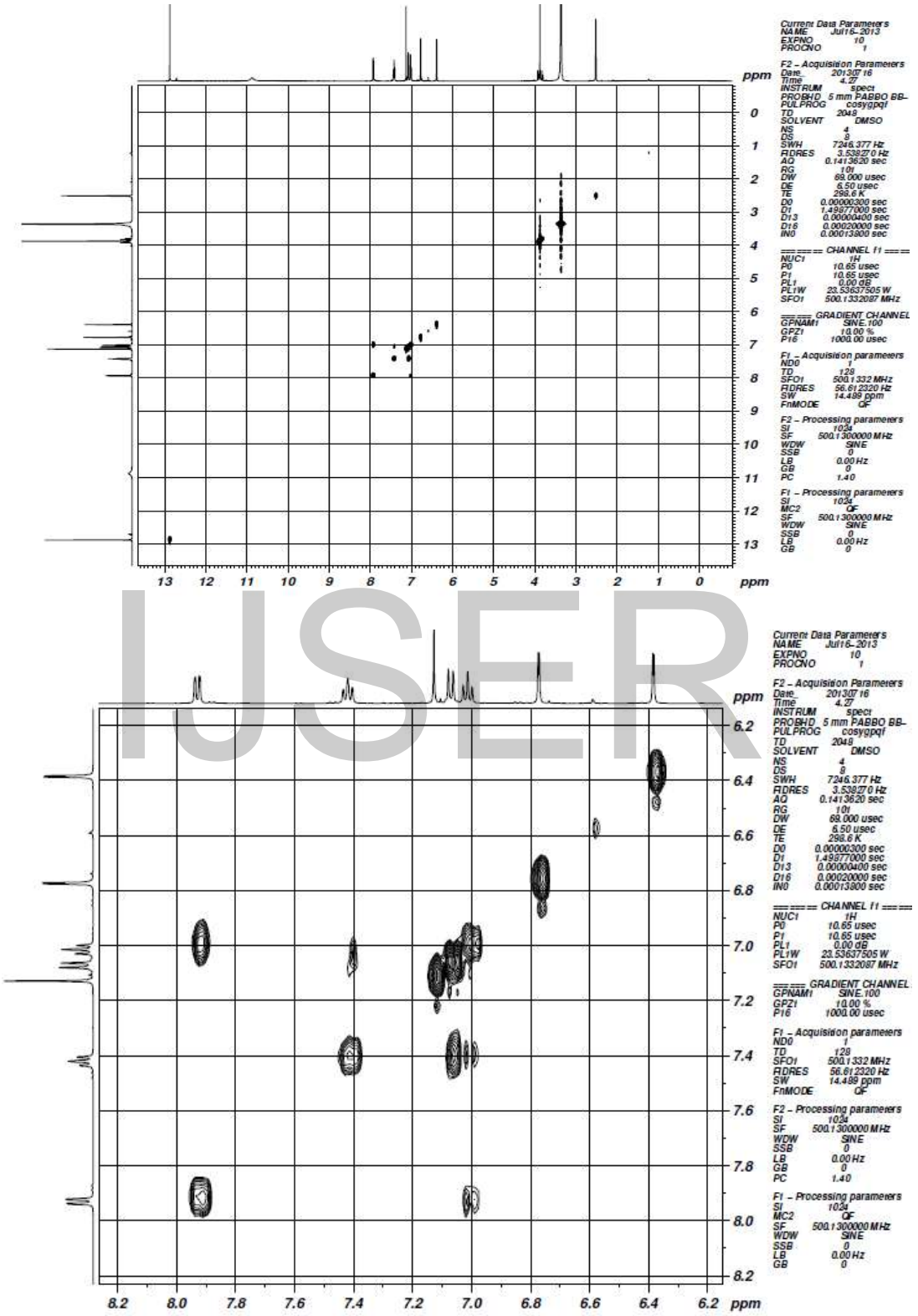


Figure 67

^{13}C NMR spectrum of ASA-2

IJSER © 202
http://www.ijser.org



IJSER

Figure 68

^{1}H-^{1}H COSY spectrum of ASA-2

IJSER © 202
http://www.ijser.org

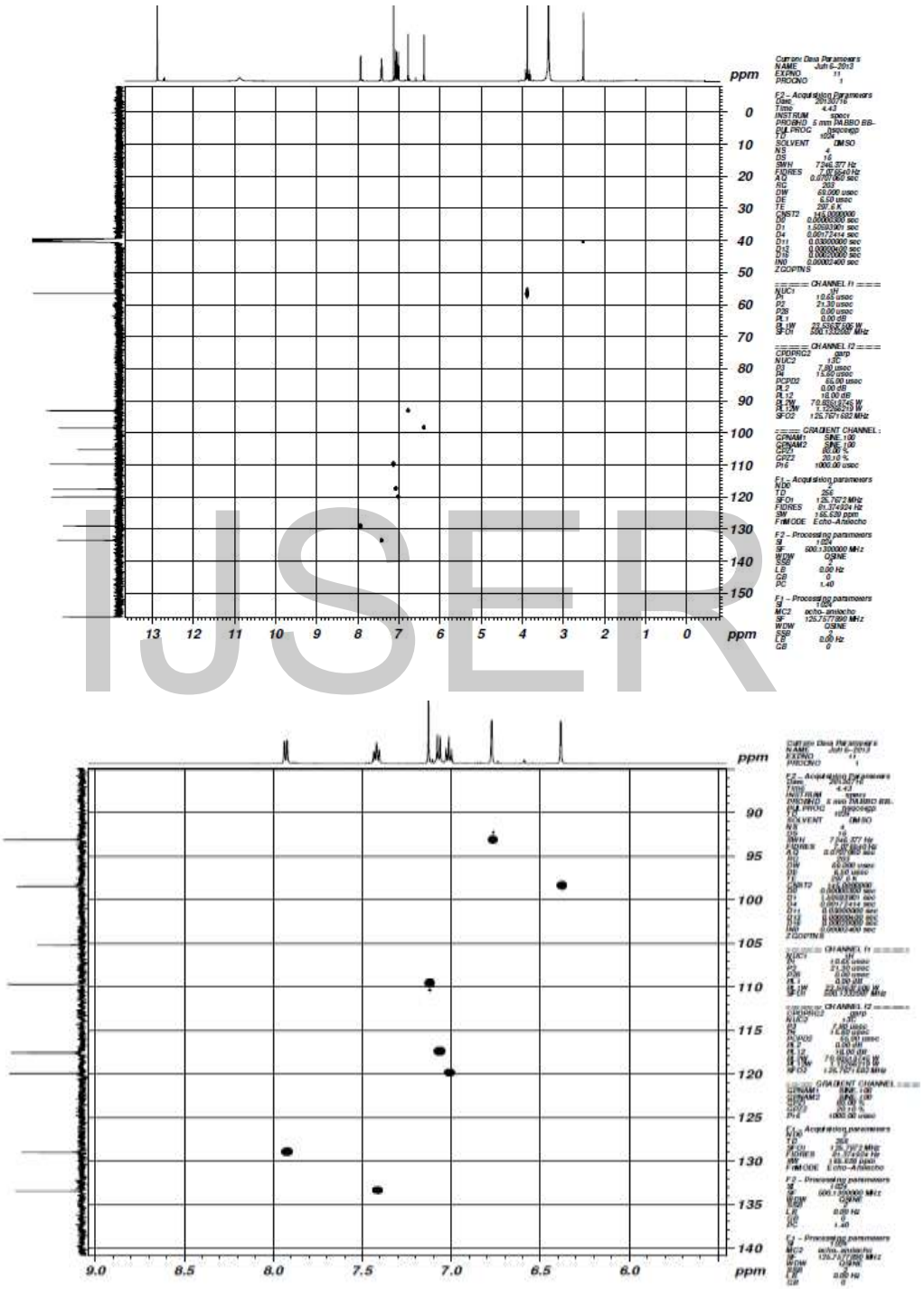


Figure 69

HSQC spectrum of ASA-2

IJSER © 202
http://www.ijser.org

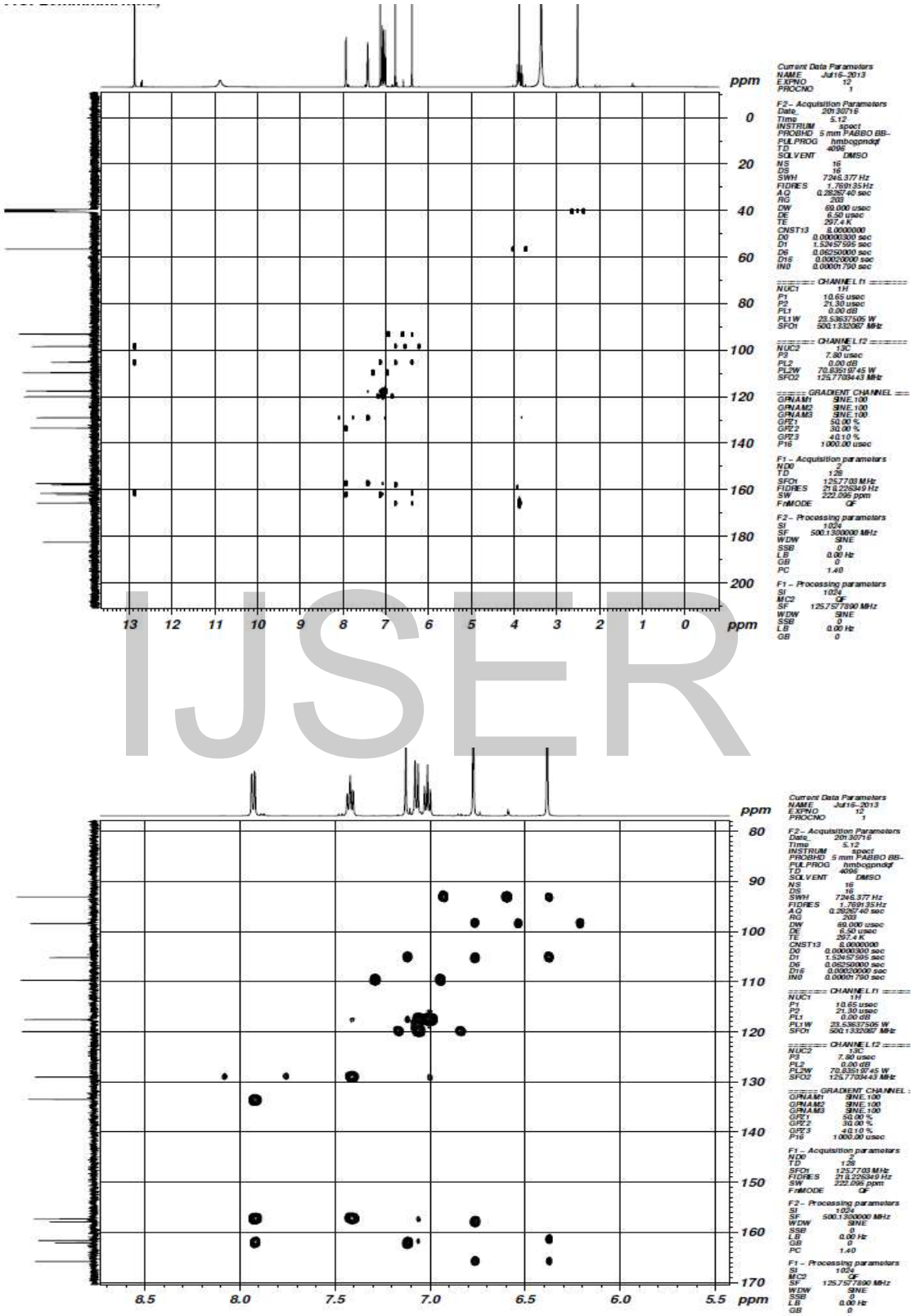


Figure 70

HMBC spectrum of ASA-2

IJSER © 202
http://www.ijser.org

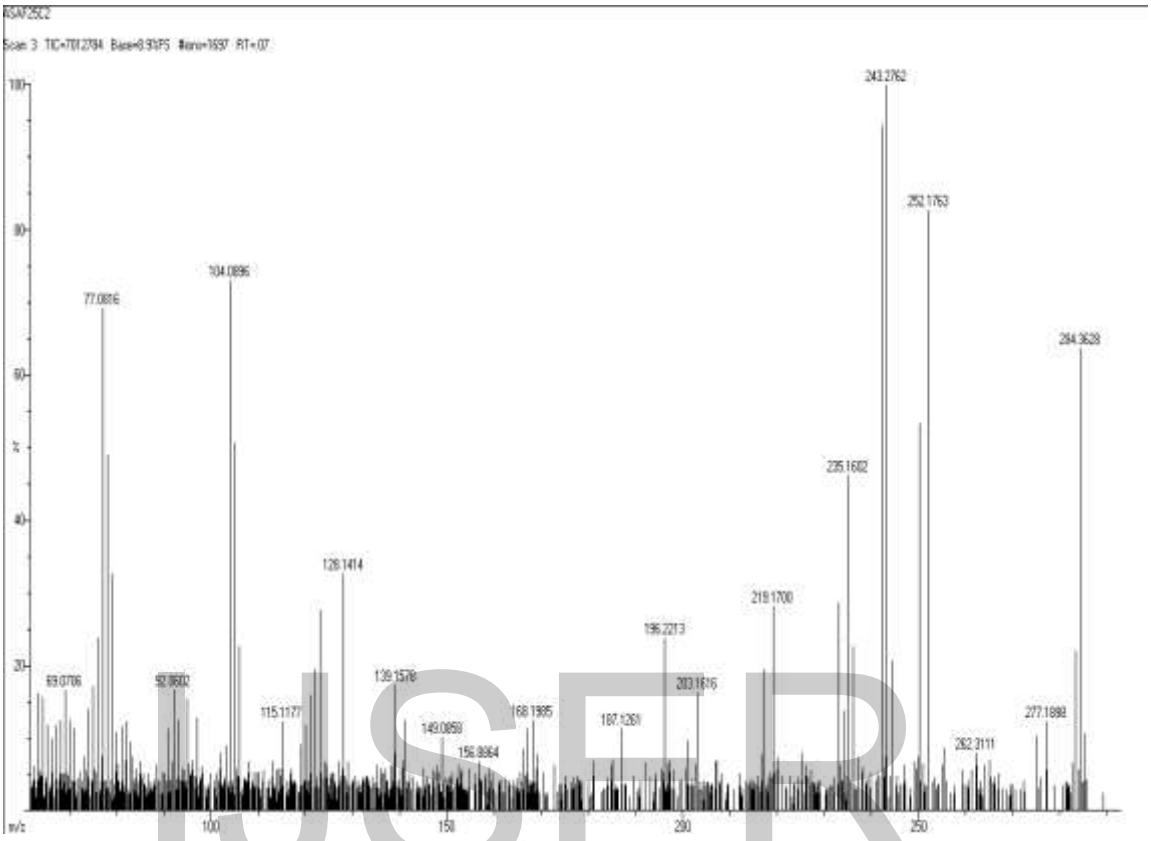


Figure 71
Mass spectrum of ASA-2

IJSER

Characterization of ASA-3

Column chromatographic analysis of dewaxed ethanol extract of aerial parts of *Andrographis stenophylla* by elution with ethanol extract petroleum ether and ethyl acetate (40:60) yielded pale green colour solid. Yield: 52 g. Melting Point: 216-222^{o}C. It was freely soluble in alcohol. TLC (R_F) =0.65 (ethyl acetate: ethanol 10:0.25).

Spectral Data:

UV :

The UV absorption bands at 227.2 and 241.6 nm indicates lactone nature of the compound. The finger print regions in the IR spectrum of **ASA-3** matched with that of the terpenoidal lactone andrographolide (Table 16). Further confirmation was done by reversed phase HPLC analysis of this compound on C18 column using PDA detector. The HPLC chromatograms of

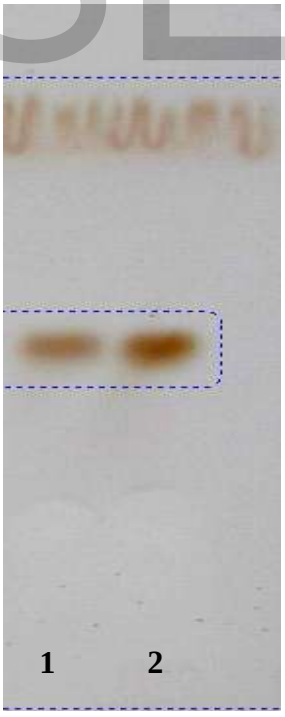
IJSER © 202
http://www.ijser.org

ASA-3 and an authentic sample of andrographolide exhibited peaks at same retention time 5.2 minutes

Table 16 Comparison of IR peaks with authentic sample

Andrographolide (Authentic sample γ_{max} cm^{-1})	ASA-3 γ_{max} cm^{-1}
3407	3391
3316	3308
2925	2926
2868	2852
2348	2375
1724	1724
1674	1667
1459	1455
1366	1362
1294	1302
1220	1209
1136	1120
1032	1025
980	981

IJSER



1 =Standard Andrographolide
2 = ASA-3

Figure 72

IJSER © 202
http://www.ijser.org

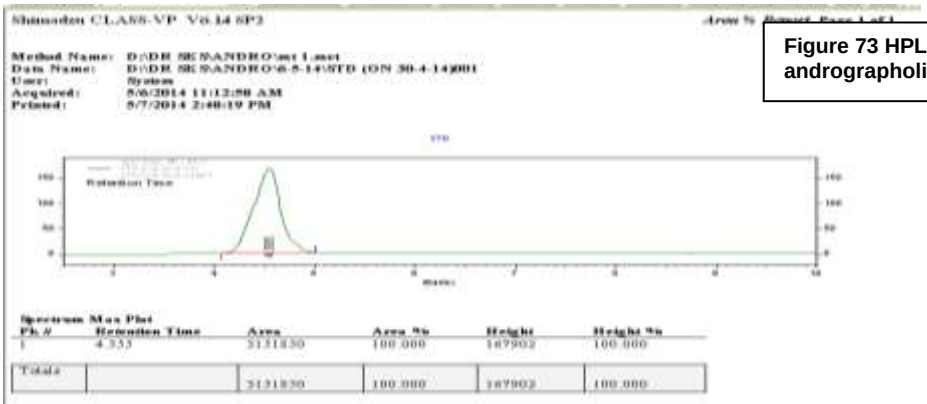


Figure 73 HPLC chromatogram of standard andrographolide

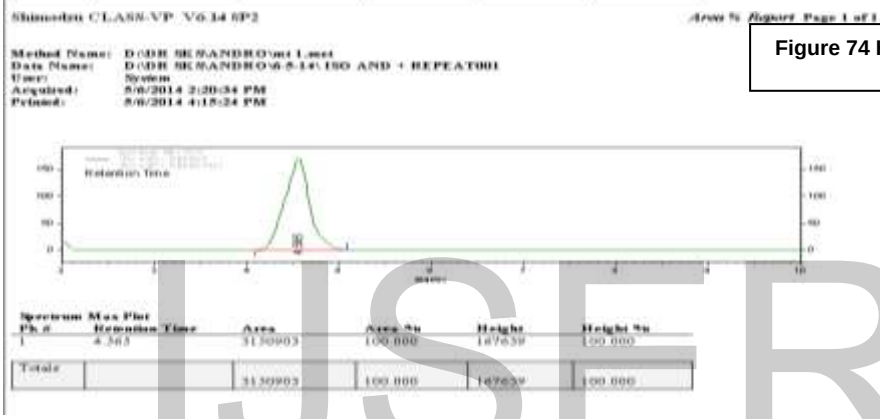


Figure 74 HPLC chromatogram of ASA-3

IJSER

Characterization of ASA-4

Column chromatographic analysis of dewaxed ethanol extract of aerial parts of *Andrographis stenophylla* by elution with ethanol extract ethyl acetate and ethanol (70:30) yielded pale green colour solid. Yield: 115 g. Melting Point: >240^{o}C. The compound ASA-4 responded to Shinoda's test and Molisch test indicating the presence of a flavonoidal glycoside.

Spectral data

UV λ_{max} 219.2, 267.2 & 320.0 cm^{-1}

1H NMR (500MHz, DMSO, δ ppm): 2.0 (3H,S); 3.20-3.25 (1H, m); 3.34 (S); 3.67-3.70 (1H, m); 3.87 (3H,S); 4.07-4.11(1H,m); 4.28 -4.31 (1H d/d); 5.17-5.18 (1H,d); 5.2 (1H,wd); 5.35 (1H,d); 5.4 (1H,d); 6.4 (1H, d); 6.7 (1H d); 7.06 (1H,S); 7.22 -7.25 (1H,t); 7.33-7.35 (1H,d); 7.57 -7.60 (1H,t); 7.92 -7.94 (1H, d); 12.89 (1H,) (Figure 75)

^{13}C NMR (125 MHz, $CDCl_3$, δ) Table 17

IJSER © 202
http://www.ijser.org

No shift in λ_{max} was observed with sodium acetate shift reagent indicating a protected 7 position of flavones. The IR spectrum showed the absorptions expected of a flavone. The ^{1}H NMR spectrum showed downfield resonance at δ 12.88 attributed to a chelated hydroxyl proton. Presence of a broad singlet at δ 3.87 indicates the presence of one methoxyl group. Presence of a three proton singlet at δ 2.0 is attributed to an acetyl group. The upfield signals observed with respect to protons at δ 7.25, 7.35, 7.60, 7.93 and its multiplicity indicate that the C 2' proton is protected. The meta coupling resonance at δ 6.40, and δ6.75 indicated a mono substituted **A** ring of flavones.

Table 17 Comparison of ^{13}C NMR Data of ASA-4 with Literature data

Chemical shift (δ ppm)		
Carbon number	**ASA-4**	**Literature data (Biswanath *et.al.*,2006)**
C-2	**158.0**	**157.6**
C-3	**120.0**	**113.9**
C-4	**182.5**	**183.3**
C-4a	**105.2**	**107.9**
C-5	**161.6**	**162.2**
C-6	**98.4**	**98.8**
C-7	**165.7**	**166.6**
C-8	**93.1**	**93.2**
C-8a	**155.1**	**157.3**
C-1'	**116.2**	**112.2**
C-2'	**161.5**	**157.6**
C-3'	**122.0**	**107.9**
C-4'	**133.4**	**133.0**
C-5'	**110.9**	**111.1**
C-6'	**158.0**	**157.3**
C-1"	**100.6**	**102.3**

IJSER

IJSER © 202
http://www.ijser.org

C-2”	**73.7**	**74.5**
C-3"	**76.9**	**77.9**
C-4”	**70.1**	**71.2**
C-5”	**74.2**	**75.1**
C-6”	**63.7**	**64.2**
OMe-7	**56.4**	**56.3**
-OCOMe-2”	**21.1**	**20.7**
-OCOMe-2”	**170.6**	**171.1**

The ^{13}C NMR spectrum (Figure 76) further confirms the linkage of sugar moiety. The signal at δ 101.6 due to one anomeric carbon indicated the presence of a single monosaccharide unit in the compound. The methoxy group and the acetyl group resonated at δ56.0 and 21.1 in the carbon NMR. The COSY spectrum clearly showed the correlation between anomeric proton with a downfield proton indicating the presence of acetoxy group at 2'' position. The mass spectrum of ASA-4 gave the molecular ion peak at 504. Based on the above evidences the structure of ASA-4 is proposed as 2-5–dihydroxy-7-methoxy-2'-glycoflavone.

IJSER

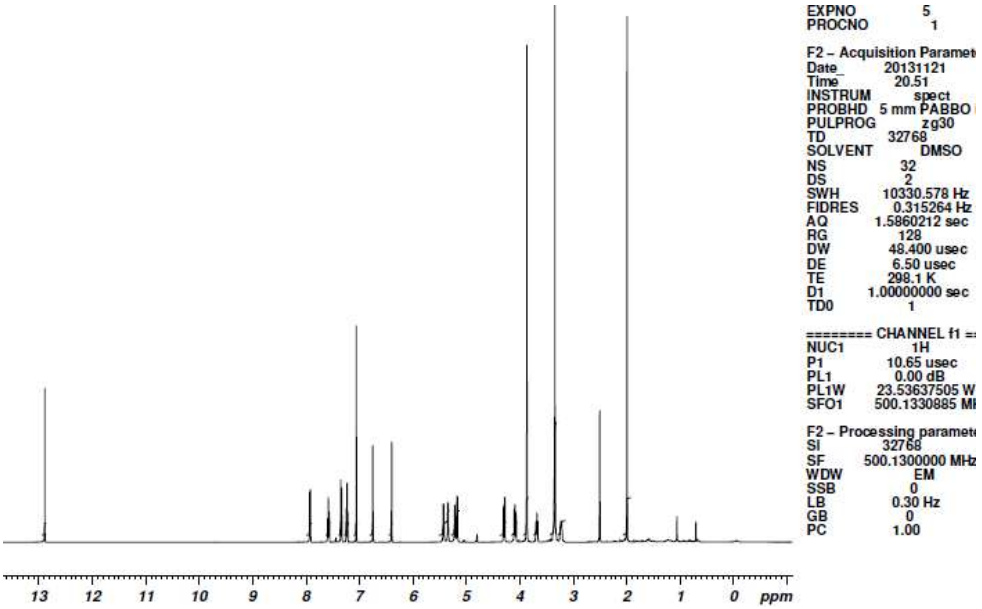


Figure 75

^{1}H NMR spectrum of ASA-4

IJSER © 202
http://www.ijser.org

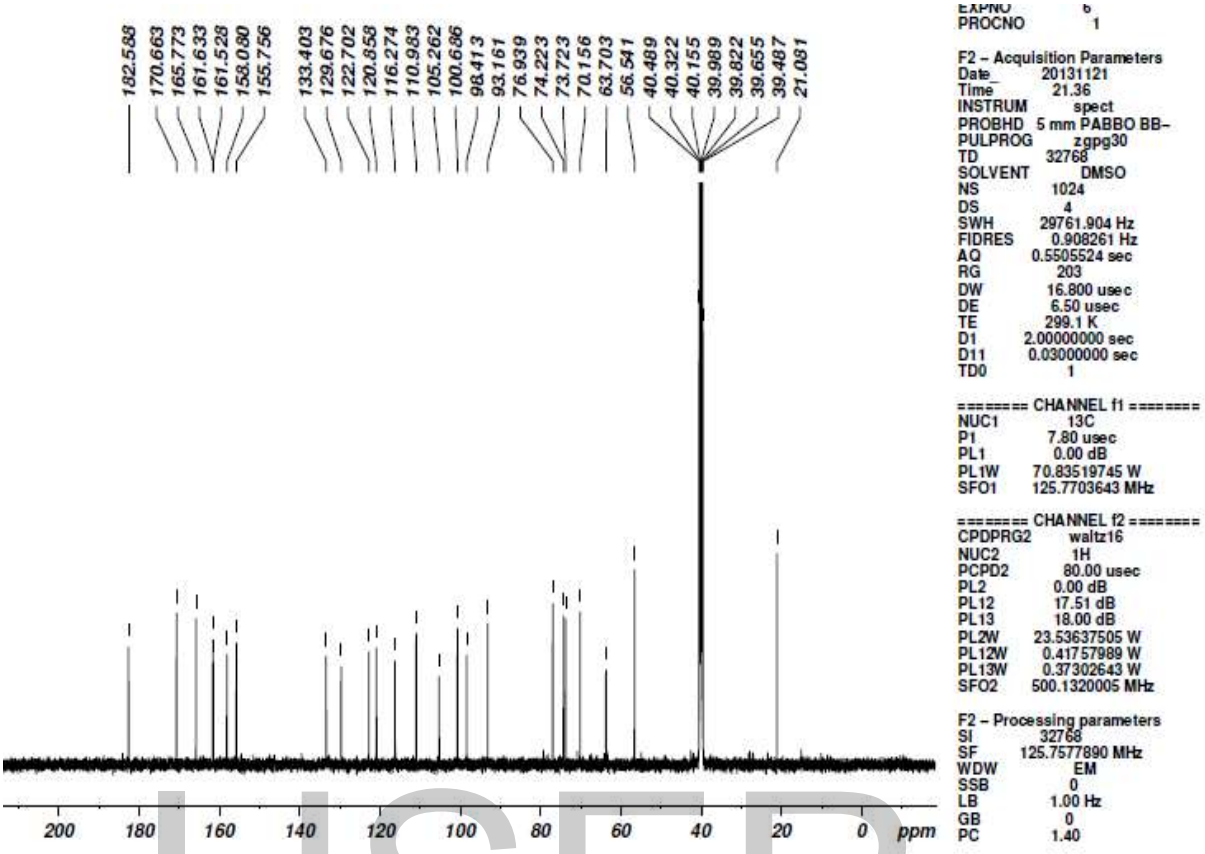


Figure 76

^{13}C NMR spectrum of ASA-4

IJSER

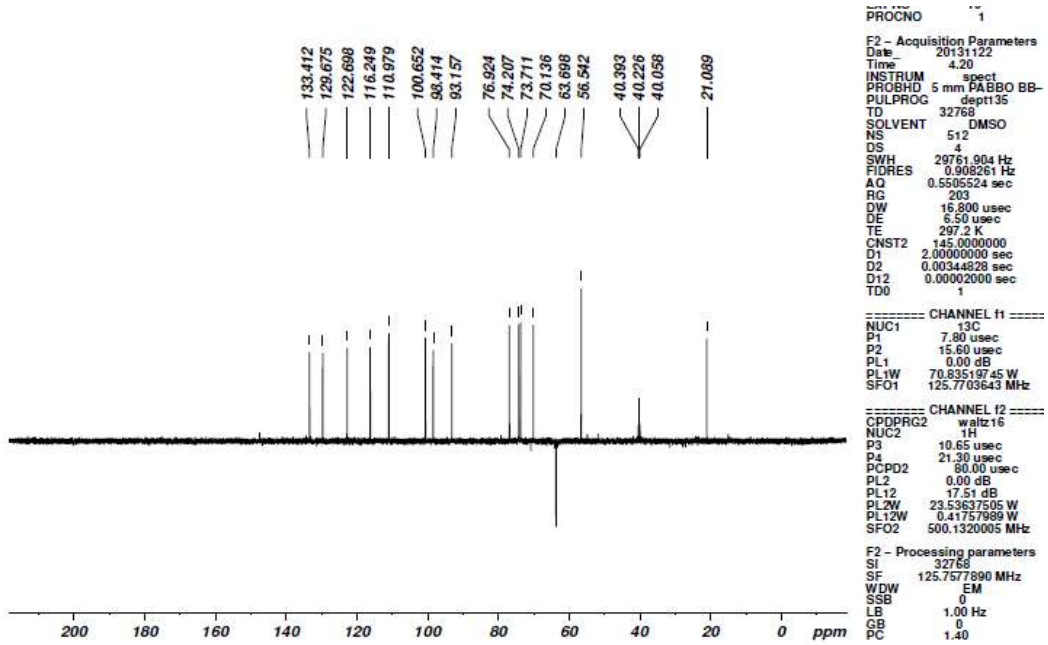
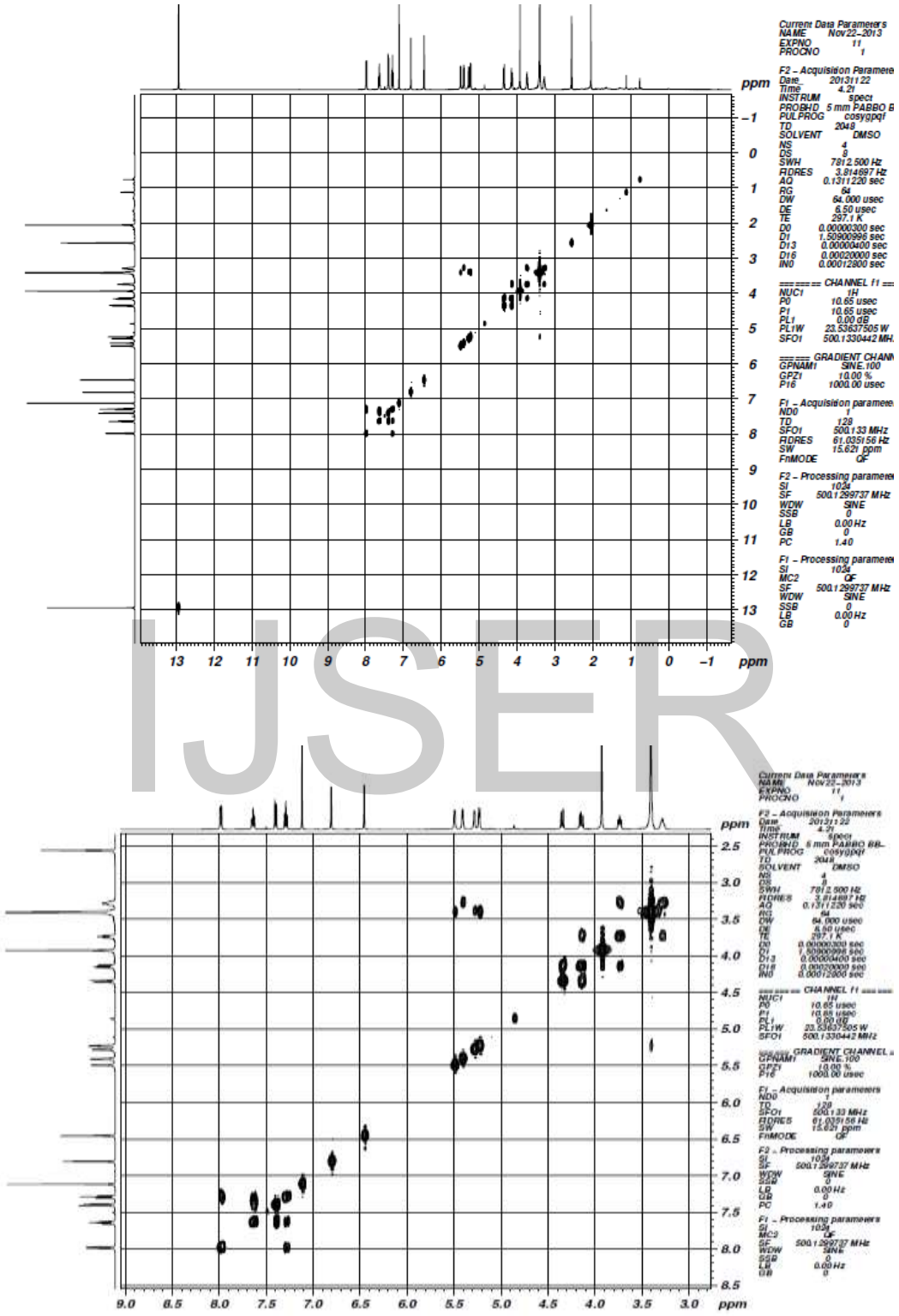


Figure 77

DEPT 135 spectrum of ASA-4

IJSER © 202
http://www.ijser.org



IJSER

Figure 78

COSY spectrum of ASA-4

IJSER © 202
http://www.ijser.org

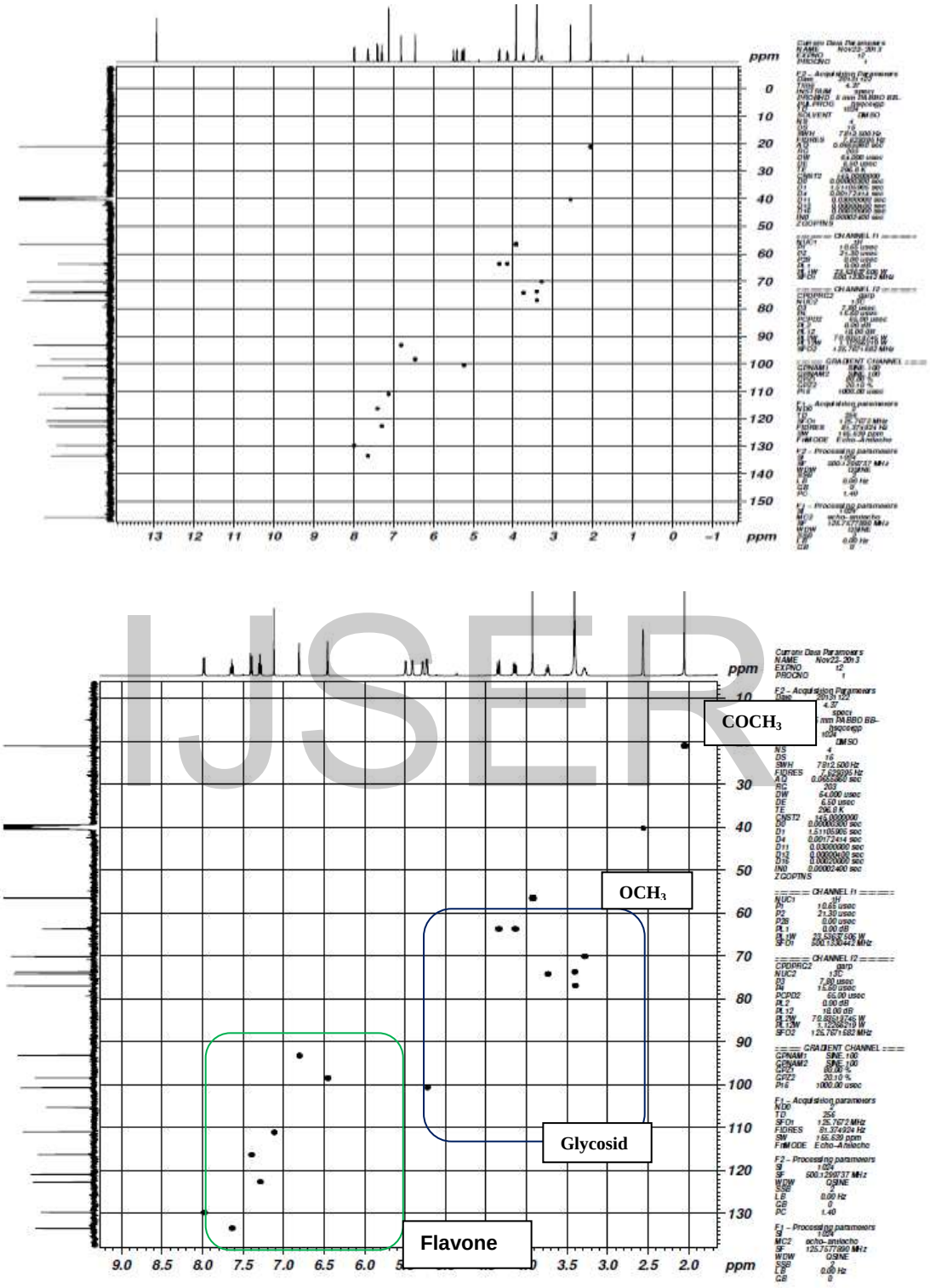


Figure 79

HSQC spectrum of ASA-4

IJSER © 202
http://www.ijser.org

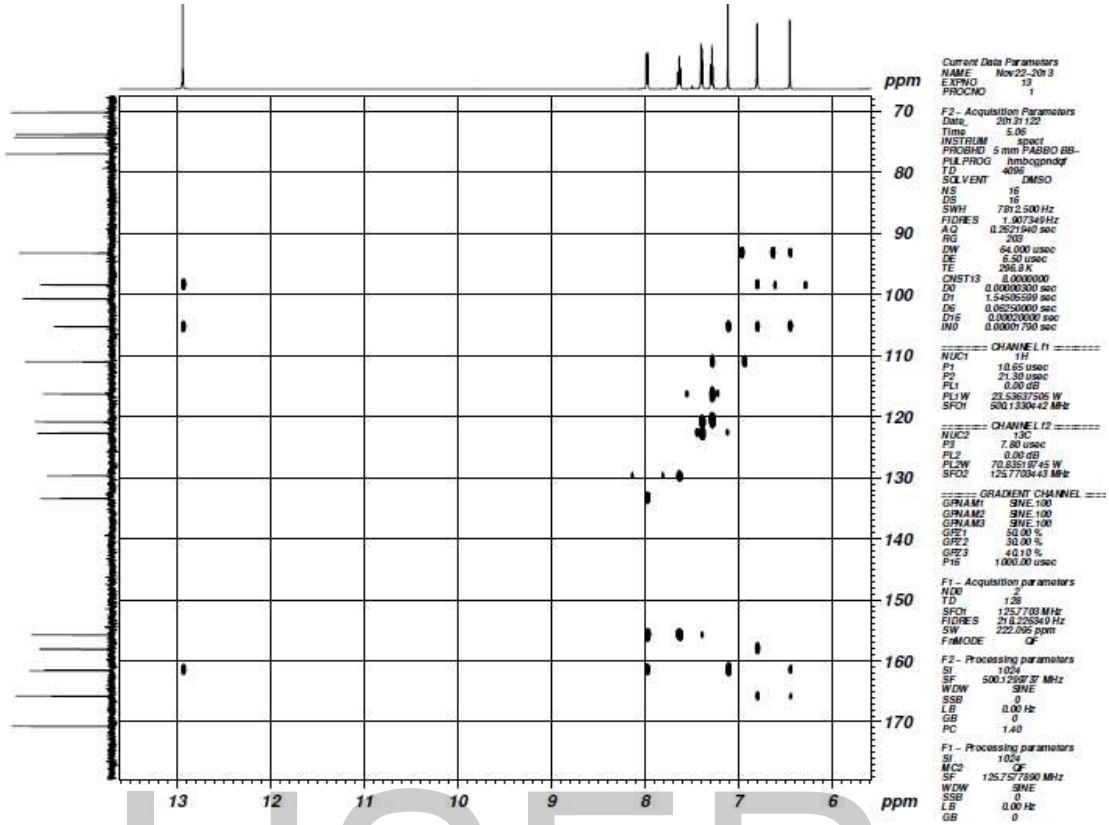


Figure 80

HMBC spectrum of ASA-4

IJSER

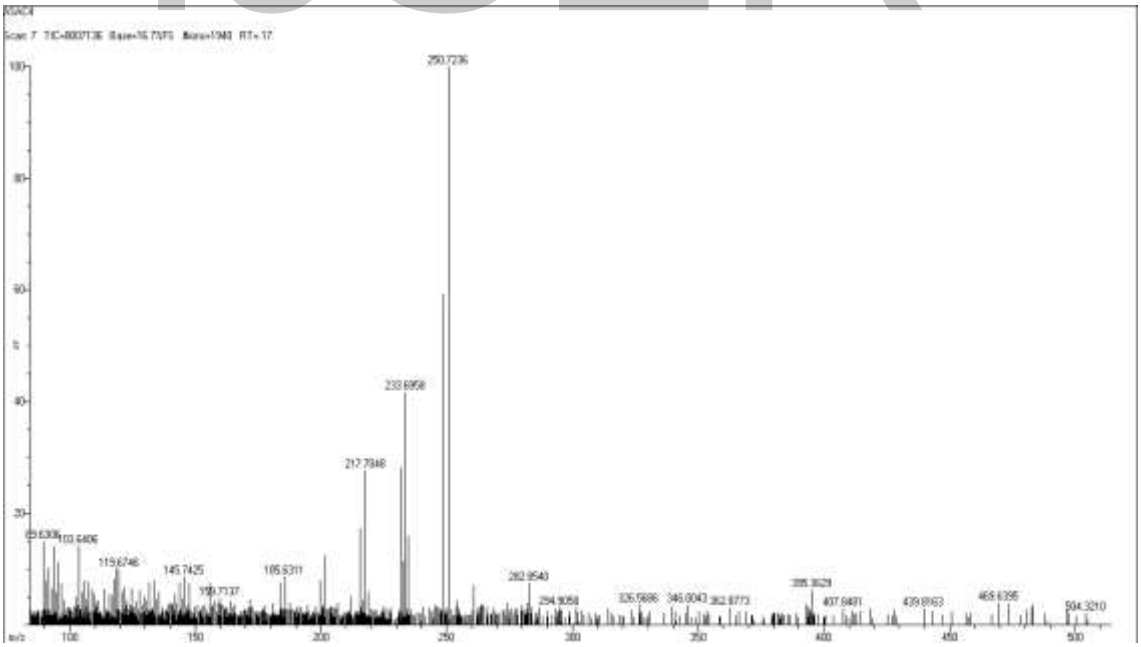


Figure 81

Mass spectrum of ASA-4

IJSER © 202
http://www.ijser.org

Characterization of ASA-5

Column chromatographic analysis of dewaxed ethanol extract of aerial parts of *Andrographis stenophylla* by elution with ethanol extract ethyl acetate and ethanol (40:60) yielded pale green colour solid. Yield: 105 g. Melting Point: 218-220^{o}C. The compound was highly soluble in water and DMSO indicating its polar nature.. The compound ASA-4 responded to Shinoda and Molisch test indicating the presences of flavonanoidal glycoside.

Spectral data

UV: Absorption band fall into the flavone class of compounds (227.2, 264.2 & 312 .0) No shift in λ_{max} was observed with sodium acetate shift reagent indicated 7th position as protected. The IR spectrum showed absorption band due to the presence of OH, -CH, -C-O-C-, -C=O functional groups.

1H NMR (500MHz, DMSO, δ ppm): 3.24-3.28 (1H, q); 3.37-3.39 (t); 3.45 -3.48 (t); 3.52- 3.56 (1H,S); 4.07 (S); 4.28 -4.31 (1H d/d); 5.17-5.18 (1H,d); 5.2 (1H,wd); 5.35 (1H,d); 5.4 (1H,d); 6.5 (1H, S); 6.8 (1H S); 7.15 (1H,S); 7.29 -7.26 (1H,t); 7.43-7.42 (1H,d); 7.64 -7.61 (1H,t); 7.99 -7.9 (71H, d); 12.89 (1H,) (Figure 82)

^{13}C NMR (125 MHz, $CDCl_3$, δ) Table 18

The ^{1}H NMR spectrum showed a downfield resonance at δ 12.94 attributed to a chelated hydroxyl proton. Presence of a broad singlet at δ 3.92 indicated the presence of one methoxyl group. The slightly downfield signals observed with respect to the C ring protons suggest substitution at C 2' position.

The ^{13}C NMR spectrum (Figure 83) further confirms the linkage of sugar moiety. The signal at δ100.7 due to the anomeric carbon indicated the presence of a monosaccharide unit in the compound. A methoxy group was clearly observed at δ 56.5 in ^{13}C spectrum. Based on the above evidences the The structure of ASA-4 was concluded as 5 –hydroxy-7-methoxy-2'-glucoflavone.

IJSER © 202
http://www.ijser.org

Table 18 ^{13}C NMR chemical shift of ASA-5

Carbon Position	Chemical shift (δ)	Carbon Position	Chemical shift (δ)
C-2	161.6	C-1’	120.5
C-3	110.9	C-2’,	156.0
C-4	182.6	C-3’	117
C-5	161.5	C-4’	122.5
C-6	98.4	C-5’	133.5
C-7	165.8	C-6’	129.6
C-8	93.1	C-1’’	100.7
C-9	158.1	C-2’’	73.8
C-10	105.2	C-3’’	70.0
		C-4’’	77.2
		C-5’’	77.7
		C-6’’	61.0

IJSER

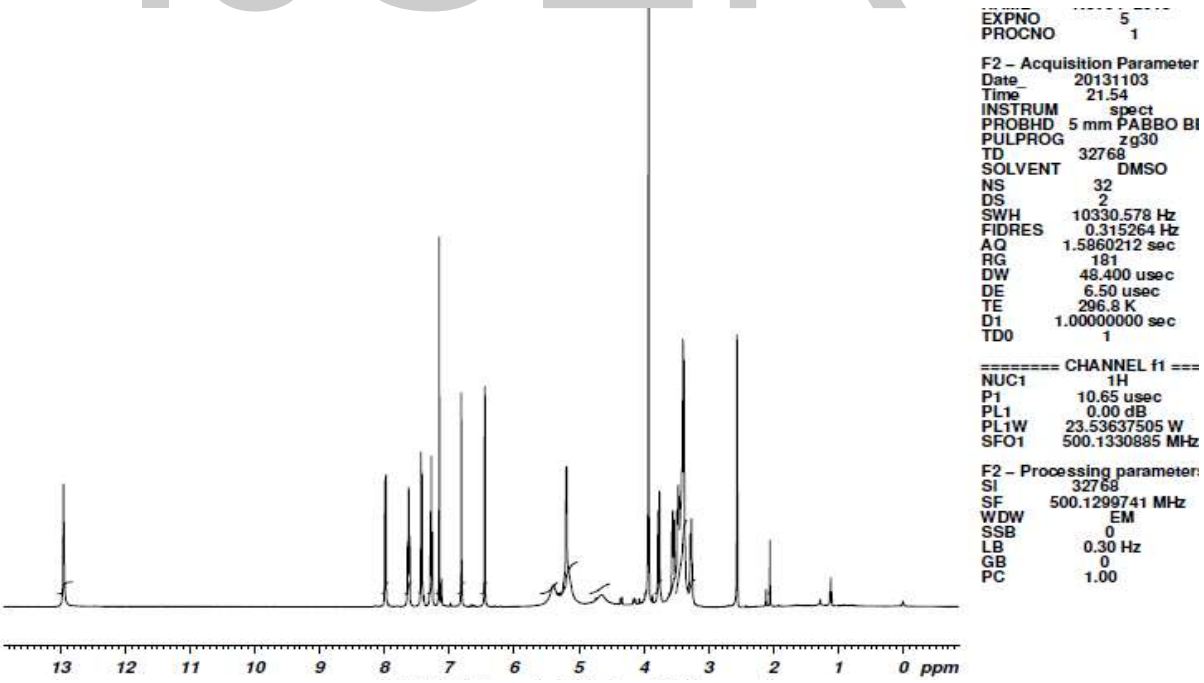


Figure 82

^{1}HNMR spectrum of ASA-5

IJSER © 202
http://www.ijser.org

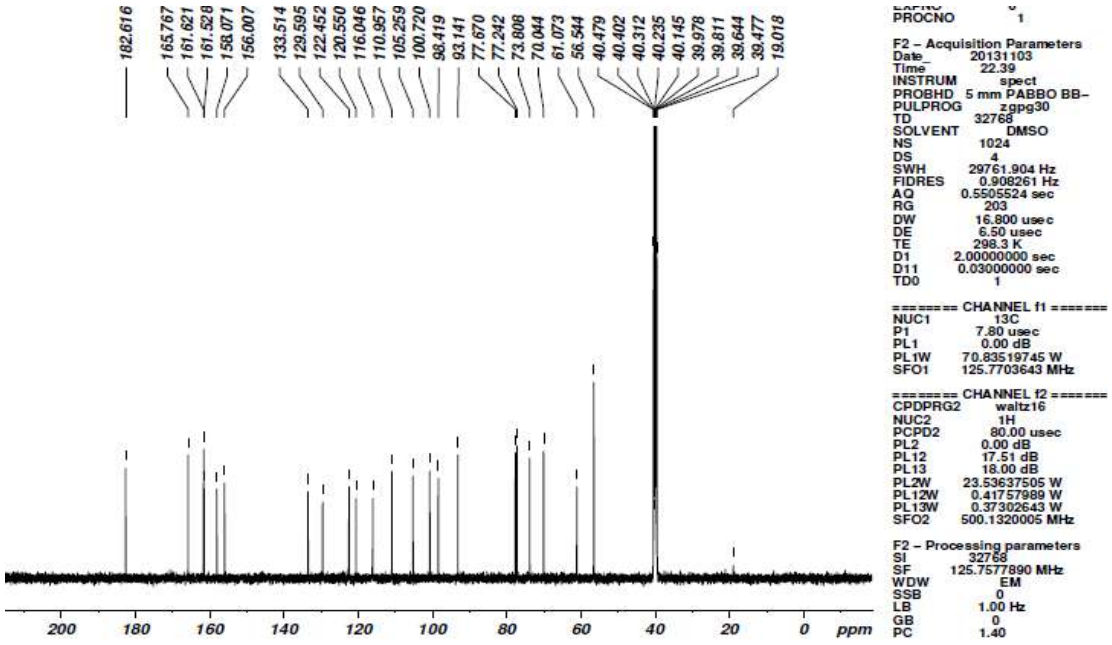


Figure 83

^{13}CNMR spectrum of ASA-5

IJSER

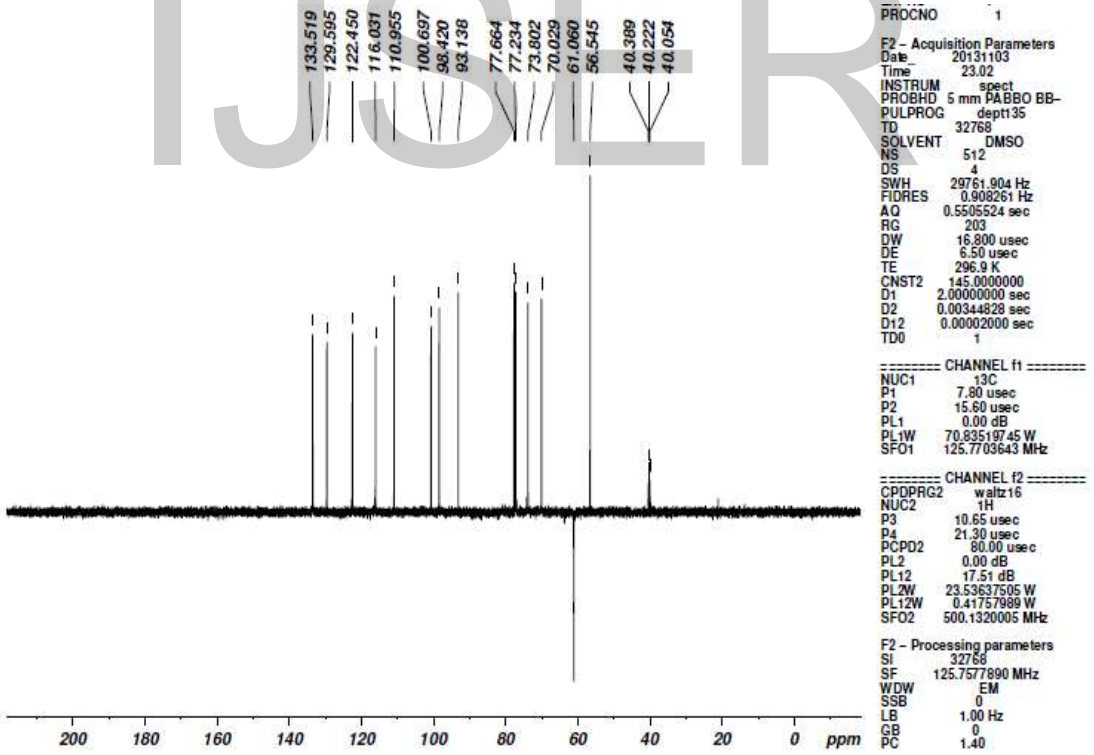


Figure 84

DEPT 135 spectrum of ASA-5

IJSER © 202
http://www.ijser.org

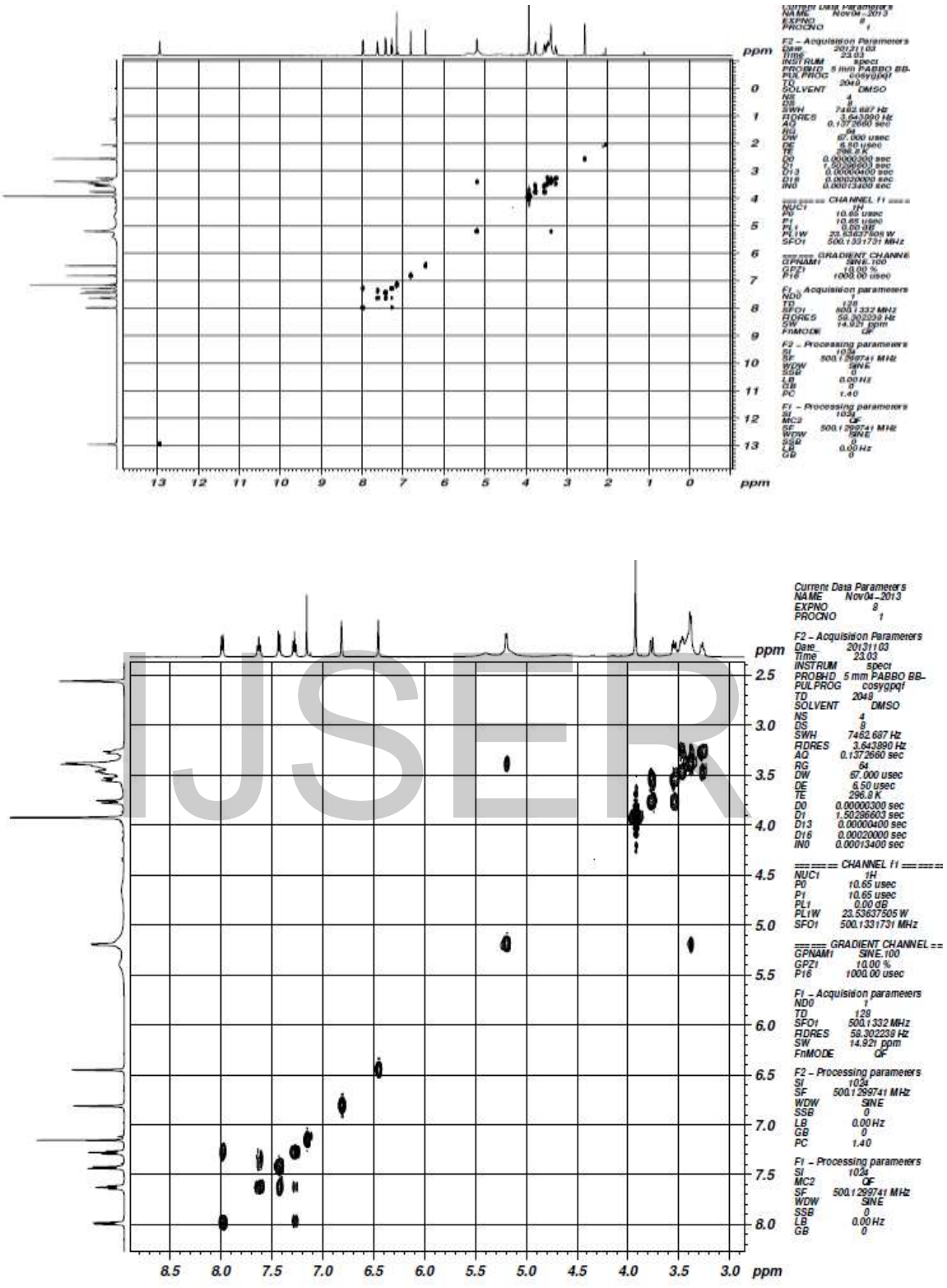


Figure 85

COSY spectrum of ASA-5

IJSER © 202
http://www.ijser.org

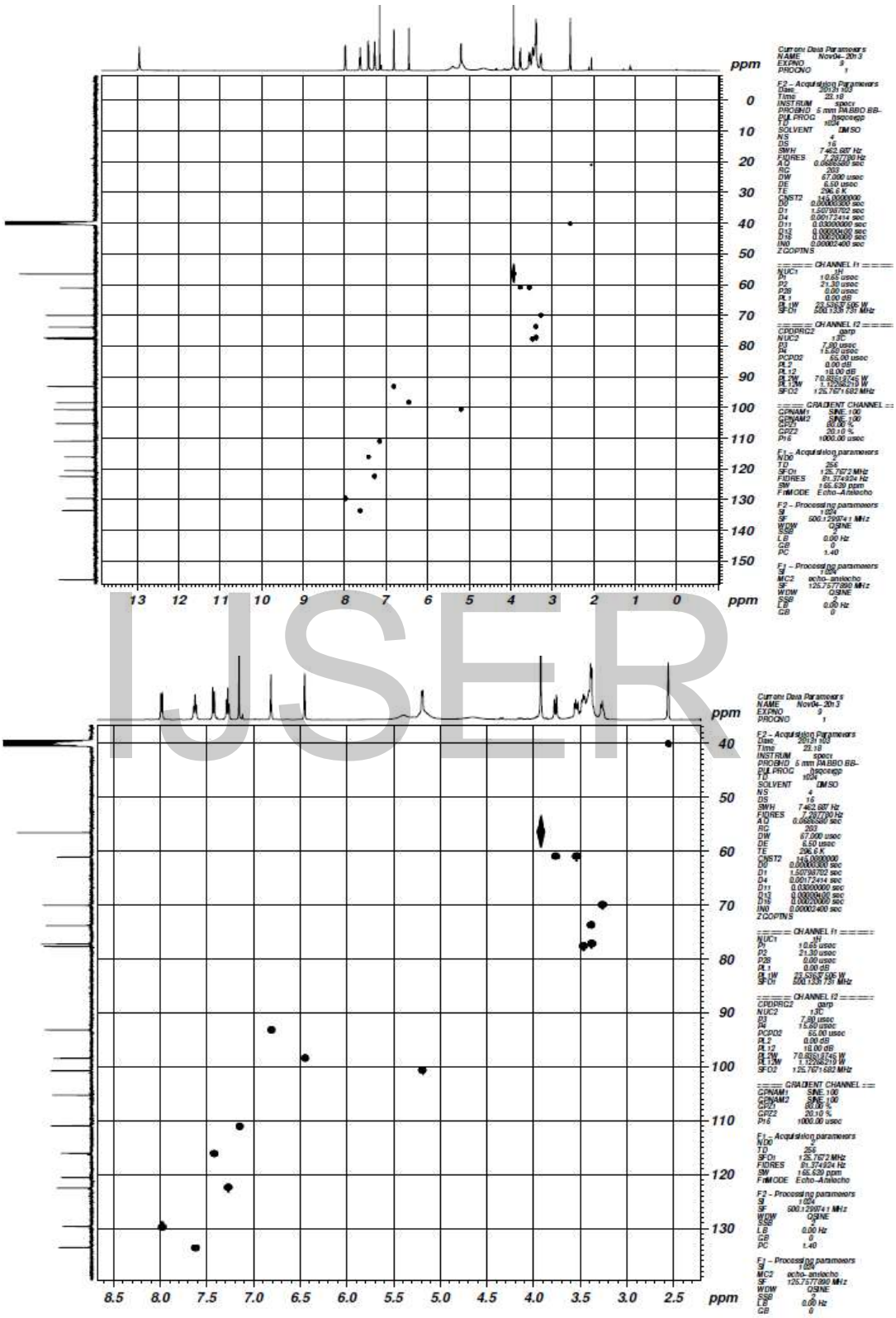


Figure 86

HSQC spectrum of ASA-5

IJSER © 202
http://www.ijser.org

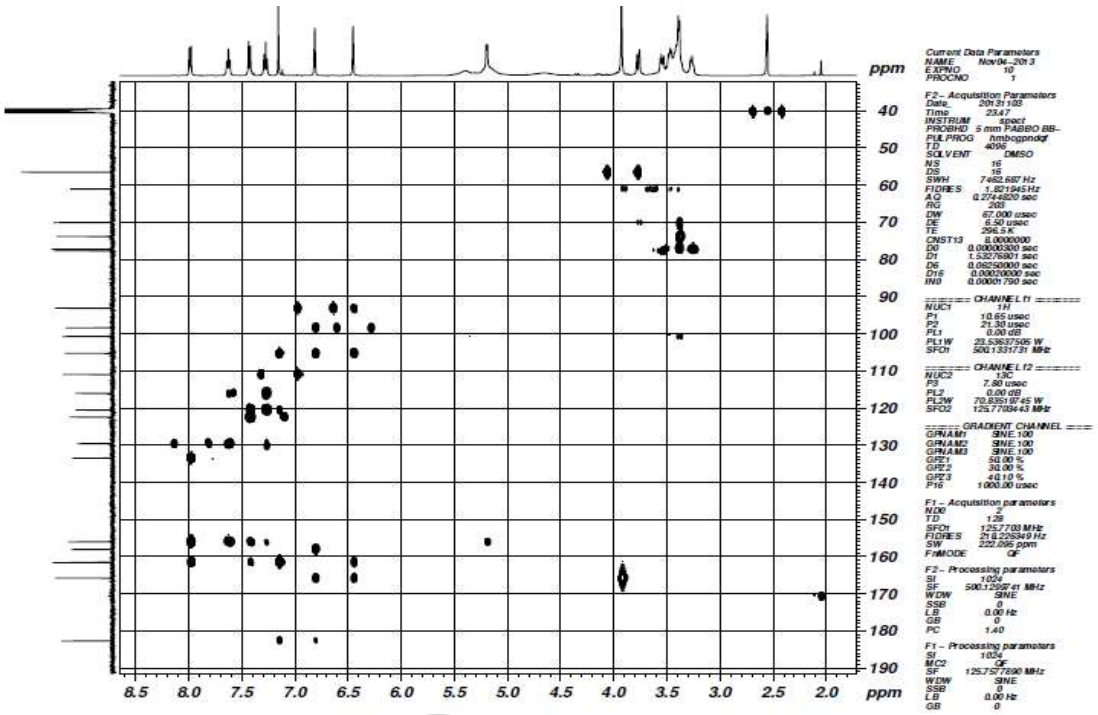


Figure 87

HMBC spectrum of ASA-5

IJSER

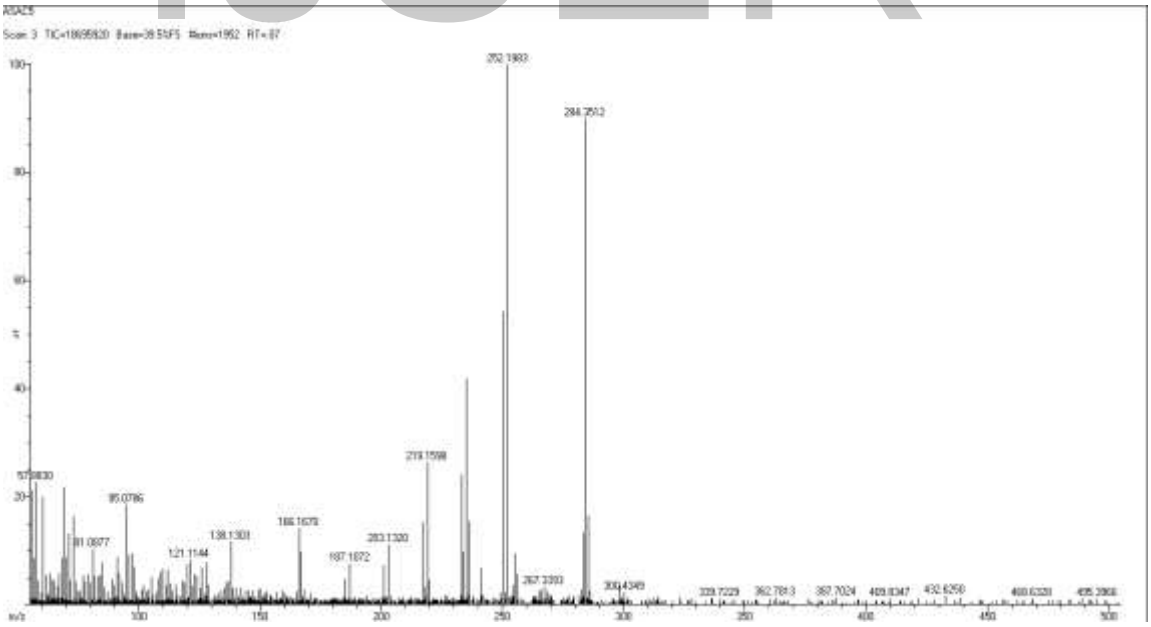


Figure 88

Mass spectrum of ASA-5

IJSER © 202
http://www.ijser.org

Validation of the Herbal Potential of the Chosen Plants

Quality, efficacy, safety and standardization are prime issues in the medicinal plant research. Hence, the present research work was also focussed on standardization of the chosen folkloric plants and their extracts. The result of the standardisation studies is presented in this section.

Documentation of Physical Parameters

Authentication of Chosen Plants: The first stage in assuring the quality, efficacy and safety of medicinal plant is identification of the plant species. The chosen plants collected from local areas of Coimbatore were authenticated at the Institute of Forest Genetics & Tree Breeding (IFGTB). Voucher specimens have been deposited in the Herbarium of the Institute [F.No. 14932 for ***Pisonia grandis***and Acc.No. 2413 for ***Andrographis stenophylla***].

Greenhouse Plant: The plant *Pisonia grandis*was also reared under greenhouse condition to compare physiochemical data of the greenhouse grown plant and the locally grown plant. This comparison was done since the plant ***Pisonia grandis*** (especially leaves) has been used by locals for internal consumption as an anti-diabetic agent. To ascertain the safety of its internal use a comparative of phytochemical analysis of the locally available plant and the greenhouse grown plant was undertaken.

IJSER

For the plants grown under green house conditions, the height, colour and size of the leaves were continuously monitored. Every month the plant was grew by 4.5”. At the initial stage only 6 to 9 numbers of leaves were observed in the plants. Size of the matured leaves of ***Pisonia grandis***was 15” with dark green colour. After a year of growth, the height of the plant was found to be around 50 to 54”. The leaves, stems and roots of well grown plant were harvested and subjected to physiochemical analysis. Figure 89 are photographic pictures of greenhouse grown ***Pisonia grandis***.

IJSER © 202
http://www.ijser.org

Stems of *Pisonia grandis* planted on 16-11-2011

30 days later

90 days later

120 days later

Plant in Pot

one year later

IJSER © 202
http://www.ijser.org

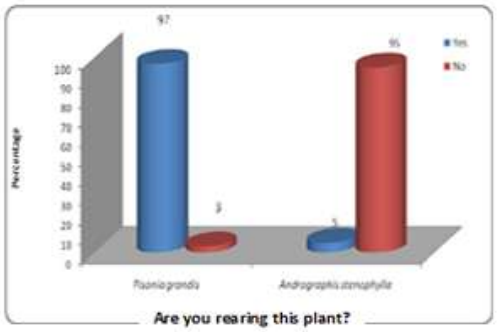
Survey on Use of Plant by Local People: A survey was conducted on the folkloric use of the chosen plants ***Pisonia grandis*** and ***Andrographis stenophylla***. The survey led to the documentation of the folkloric use of the plants. It was found that the plants chosen for the present investigation have been extensively used by locals and tribals as anti-diabetic and anti-inflammatory agent. Only leaves of the chosen plants were found to be largely used. The survey results expressed as percentage of overall response by the local people is presented in Table 19 below.

Table 19 Survey Questions

S.No.	Survey Question		Response (%)	
			Pisonia grandis	*Andrographis stenophylla*
1	**Are you rearing this plant?**	**Yes**	97	5
		No	3	95
		<10 years	66	25
		11-20 years	31	20
		>20 years	3	55
2	**Do you use any of this plant parts (leaf, stem and root) for culinarypurpose?**	**Yes**	86 (leaf)	0
		No	14	100
3	**What recipe do you make with leaf of this plant?**	**Fried Curry**	58	NA
		Kootu	11	
		Vadai	2	
		Dosai	4	
		Sambar	11	
		Salad	2	
		All of the above	12	
4	**Are you using it as a medicine?**	**Yes**	84	100
		No	16	-
5	**Which part of the plant do you use for medication?**	**Yes(Leaf)**	88	100
6	**For what ailment you have been using it?**	**Joint pain**	20	20
		Hip pain	19	-
		Leg pain	17	-
		Stroke	9	-
		Blood Pressure	15	-
		Diabetes	17	10
		Body pain	3	-
		Skin disease		15
		Anti-venom		40
		Wound healing		15
7	**How long have you been using it for medicinal use?**	**<10 years**	87	20
		11 to 20 years	9	15
		>20	4	65
		No	16	-
8	**Do you make any decoction with leaves?**	**Yes**	8	15
		No	92	85
9	**Do you use it for any other purposes apart from culinary and medicinal uses?**	**Ornamental**	19	-
		Shade	9	-
		Dust control	8	-
		Fresh air	3	-
		All of the above	9	-

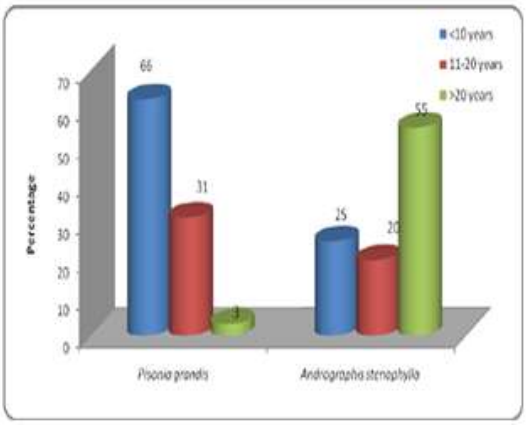
IJSER

IJSER © 202
http://www.ijser.org

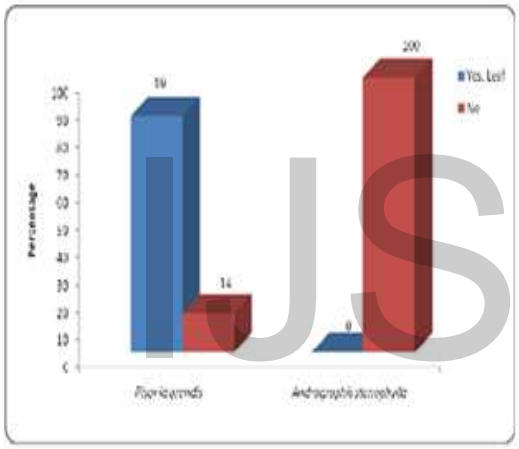


Are you rearing this plant?

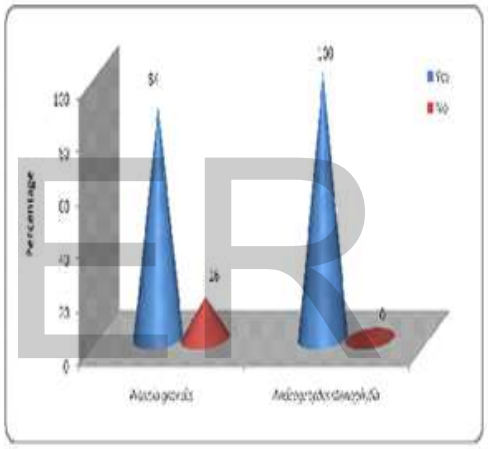
- The survey revealed that the majority of the local people were rearing this plant.
- Andrographis stenophyll is an endemic plant found growing in the mountains of Marudhamalai



- The histogram reveals the long time acquaintance of the locals with the plants



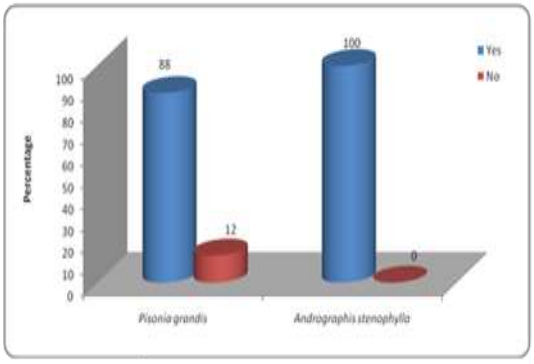
Do you use any of the plant parts (leaf, stem and roots) for culinary purpose?

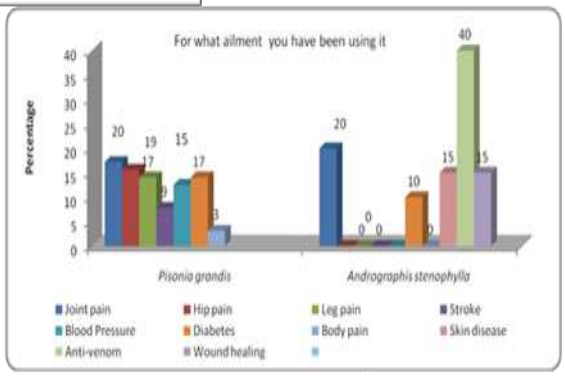


- The leaves of PG have been largely used for culinary purpose

Are you using it as a medicine?

Which part of this plant do you use for medication?

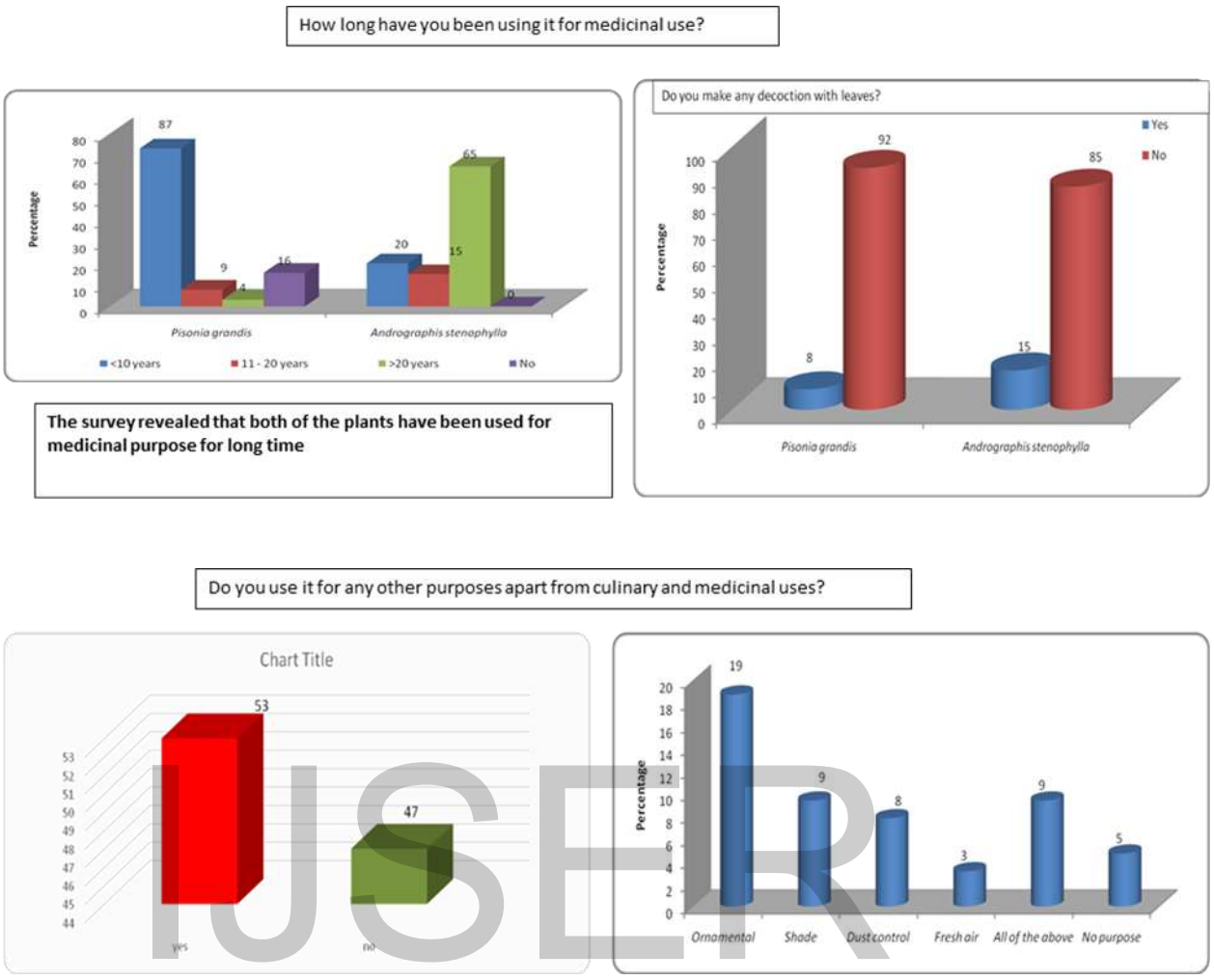




Leaves of PG have been largely used by locals to cure impair due to arthritis, as an anti-oxidant and for reducing blood pressure.

Figure 90 Histogram of survey result

IJSER © 202
http://www.ijser.org



IJSER

Figure 90a Histogram of survey result

The results of the survey revealed that,

1. *Pisonia grandis* is found to be reared by locals whereas *Andrographis stenophylla*being an endemic plant is not.
2. Both the plants are widely used by locals for curingailments related to diabetes and arthritis
3. *Pisonia grandis*is also being used for culinary purposes.
4. *Pisonia grandis* was found to be reared by the locals as an ornamental plant and as a shade.

The folkloric use of both plants has been ascertained by the survey. It also establishes the safe use of the leaves of Pisonia grandis as an internal medicament since it has been used for culinary and internal medicinal purposes since long time.

IJSER © 202
http://www.ijser.org

Morphological Documentation: Morphological data of the plant ***Pisonia grandis***has been documented by many botanists. In a book titled 'A forest flora of the Andaman Islands' written by Parkinson, *Pisonia grandis* was mentioned as an evergreen tree with leaves 6-10 " long and linear, club shaped fruit ½ " long with 5 corners, each of which has a line of recurred prickles **(Parkinson, 1923)**. 'The flora of British India' has documented the plant as a tree with large leaves (6-10"), that may be long-ovate or oblong, acute or acuminate, stout branches; flowers in dense corymbose terminal cymes; narrow fruits (1/2-3/4"long) which are club-shaped, 5-angled angler with one row of princkles **(Hooker *et al*., 1973).** In 'Flora of China' the plant has been reported to have the following features, tree of 14(-30) m with a trunk of 30-50(-70)cm in diameter; Bark as white-grey with conspicuous furrows and large leaf-scars; puberulous to near glabrous. Petiole 1-8 cm long; leaf blade elliptic, oblong, or ovate, (7–)10–20(–30) × (4–)8–15(–20) cm, papery or membranous, puberulous or glabrescent, lateral veins 8–10 pairs, base rounded or slightly cordate, mostly oblique, margin entire, apex acute to acuminate. Cymes terminal, 1–4 × 3–5 cm; peduncle ca. 1.5 cm, with light brown hairs. Flowers bisexual. Pedicel 1– 1.5 mm, apex with 2–4 oblong bracteoles. Perianth tube funnelform, ca. 4 mm, 5-lobed, with 5 rows of black glands Stamens 6–10, exserted. Stigma fimbriate, included. Fruit clavate, ca. 1.2 cm × 2.5 mm, 5-ribbed, with sessile glands, without persistent perianth, rib with a row of viscid prickles, hairy between the ribs. Seed 9–10 × 1.5–2 mm. Flower in summer, fruit in late summer–autumn **(Shu, 2003).**

IJSER

Morphological data of ***Andrographis stenophylla*** has been documented recently by Botanical Survey of India which reports the features of the plant as follows. It is a straggling herbs, up to 1 m high; rootstock very thick. Stems 4-angled, reddish at base, swollen at nodes, glabrous. Leaves narrowly oblanceolate at base of plant, linear towards apex, attenuate at base, revolute at margins, acute or obtuse at apex, 1.5–5 × 0.2–0.5 cm; midrib conspicuous below, lateral veins 3 or 4 pairs, inconspicuous, glabrous; petioles 1–2 mm long, glabrous, at times puberulous. Inflorescences axillary,

IJSER © 202
http://www.ijser.org

racemose, 4–14 cm long, simple or at times branched; axis 4-angled, glabrous; flowers distantly arranged (internodes 0.5–1.5 cm long), twisted in right angle to each other, one-sided; pedicels slender, 2–4 mm long, puberulous to glabrous **(Gnanasekaran and Murthy, 2014).**

Physicochemical Analysis

Organoleptic Study: It is a sensory evaluation technique to analyse colour, odour and taste of the chosen plants to satisfy consumer's expectation. Ayurvedic medicines without proper sensory analysis have a higher risk of market failure. Organoleptic characteristics of leaves stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** revealed that the chosen plants parts are suitable for use as an Ayurvedic medicine **(Table 20).**

Table 20 Organoleptic characteristic of leaves, stems and roots of *Pisonia grandis* and aerial parts of *Andrographis stenophylla*

IJSER

Plant Part	Color	Odor	Taste
Leaf Powder of PG	Green	Medicine like	Taste less
Stem Powder of PG	Pale yellowish green	Tree odor	Bitter
Root powder of PG	Pale orangish yellow	Odorless	Taste less
Aerial part powder of AS	Dark green	Medicine like	Highly bitter

Fluorescence Analysis: The dried powdered parts of leaves, stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** displayed fluorescence with chemical reagents under day light and UV light indicating that the selected plant parts are sources of natural metabolites, since many natural products produce fluorescence under UV light.Figure 91 is the photographic image of fluorescence exhibited by the plant parts with various reagents. Table 3 lists the fluorescence characteristic of the plants.

IJSER © 202
http://www.ijser.org

IJSER

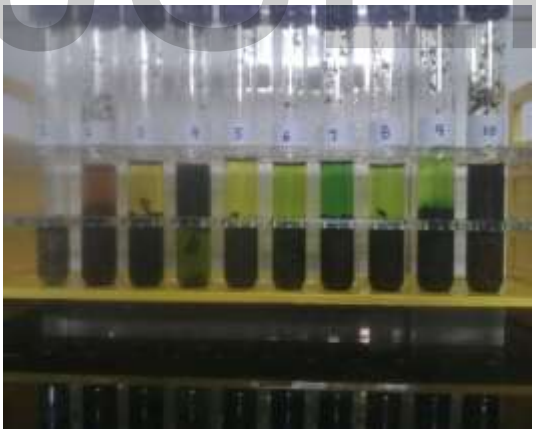


Figure 91

Photographic image of plant parts with reagents

IJSER © 202
http://www.ijser.org

Table 21 Fluorescence characteristic of leaves, stems and roots of *Pisonia grandis* and aerial parts of *Andrographis stenophylla*

Reagents	*Pisoniagrandis*						Andrographis stenophylla	
	Leaf Powder		Stem Powder		Root Powder		Aerial Parts	
	White Light	UV	White Light	UV	White Light	UV	White Light	UV
As such Powder	Green	Green	Yellow	Yellow	Orange	Orange	Green	Green
Distilled water	Orangish brown	Orangish brown	Pale yellowish orange	Pale green	Pale orange	Pale green	Brownish Green	Dark
Benzene	Green	Yellowish red	Pale Yellow	Green	Pale Yellow	Green	Green	Yellowish orange
Chloroform	Green	Red	Pale Yellow	Green	Pale Yellow	Green	Dark Green	Red
Ethyl acetate	Dark green	Dark red	Yellow	Yellow	Yellow	Yellow	Dark Green	Greenish red
Acetone	Green	Reddish yellow	Yellow	Yellowish green	Yellow	Yellowish green	Dark Green	Greenish red
Ethanol	Dark green	Greenish red	Yellow	Green	Yellow	Green	Dark Green	Reddish green
Methanol	Dark green	Greenish red	Yellowish green	Green	Yellowish green	Green	Dark Green	Yellow
Liq.Ammonia	Pale orangish green	Blackish green	Orangish brown	Dark green	Orangish brown	Dark green	Yellowish orange	Dark
Glacial acetic acid	Pale green	Yellowish blue	Ivory	Greenish Yellow	Sandal yellow	Greenish Yellow	Blackish green	Dark
5% Iodine	Reddish brown	Dark	Reddish brown	Reddish brown	Reddish brown	Reddish brown	Reddish brown	Dark
5 % Ferric Chloride	Dark orange	Dark	Orangish Yellow	Dark	Orangish Yellow	Dark	Orange	Dark brown
Saturated Picric acid solution	Green	Dark	Dark Yellow	Dark	Dark Yellow	Dark	Yellow	Pale green
Aqueous Sodium hydroxide	Orange	Dark	Dark orange	Greenish orange	Dark orange	Greenish orange	Orangish brown	Dark
Alcoholic Sodium hydroxide	Dark green	Purple	Yellow	Green	Yellow	Green	Yellowish green	Yellowish green
50% H_2SO_4	Dark red	Dark	Dark orange	Dark red	Dark orange	Dark red	Dark	Dark
50% HNO_3	Orange	Dark	Orange	Dark	Orange	Dark	Green	Dark green
Conc.HCl	Dark Organge	Dark coloration	Orange	Reddish orange	Orange	Reddish orange	Orange	Dark orange

IJSER

Elemental Analysis: Elemental analysis of each of the chosen parts indicated that the chosen plants are good sources of micro and macro nutrients. Table 22 gives the results of the elemental analysis. Green house grown plant ***Pisonia grandis*** showed distinguished change in elements when compared with the locally grown plant. The comparison revealed that the element content of leaves of greenhouse plant showed significant change in nutrients, whereas not much of a change was observed with respect to stems and roots. The content of zinc, sodium and magnesium increased in leaves of givengreenhouse growing plant but the content of carbon, hydrogen, nitrogen, sulphur, calcium and potassium decreased which may due to change in growth conditions and efficiency of mineral uptake from the soil. The content of carbon, hydrogen, nitrogen and sulphur were highest in leaves of ***Pisonia grandis*** which suggests that leaves are good source of organic compounds compared to stem and roots. Leaves of ***Pisonia grandis*** are reported to be a rich source of calcium potassium and sodium. Presence of potassium in the plant increases the disease resistance and intake of water efficiently. Potassium is vital for its diuretic nature in humans. Sodium plays significant role in the transport of metabolites. The ratio of potassium/sodium prevents hypertension and arteriosclerosis. Calcium in plants counteracts the effect of alkali salts and organic acids within the plants. It is considered an important element because of its role in bones, teeth, muscular system and heart functions in humans. Presence of magnesium was seen almost equally in leaves, stem and roots of ***Pisonia grandis.*** *The*aerial parts of ***Andrographis stenophylla*** is a rich source of zinc. It regulates the consumption of sugars and transformation of carbohydrates. Zinc is used to prevent many childhood diseases and zinc deficiency is considered as significant health problem in developing countries, and 20% of the world's population could be at risk of zinc deficiency **(Bhowmik and Datta, 2012)**. The results of the elemental analysis reveal that the chosen plants are good sources of minerals.

IJSER

IJSER © 202
http://www.ijser.org

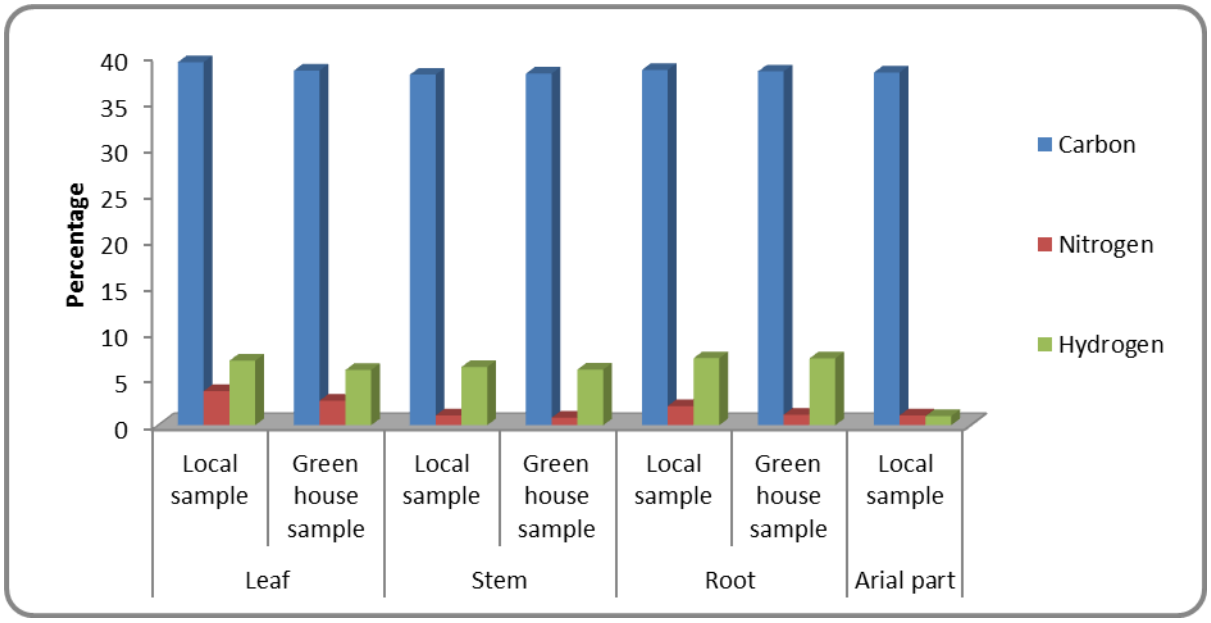
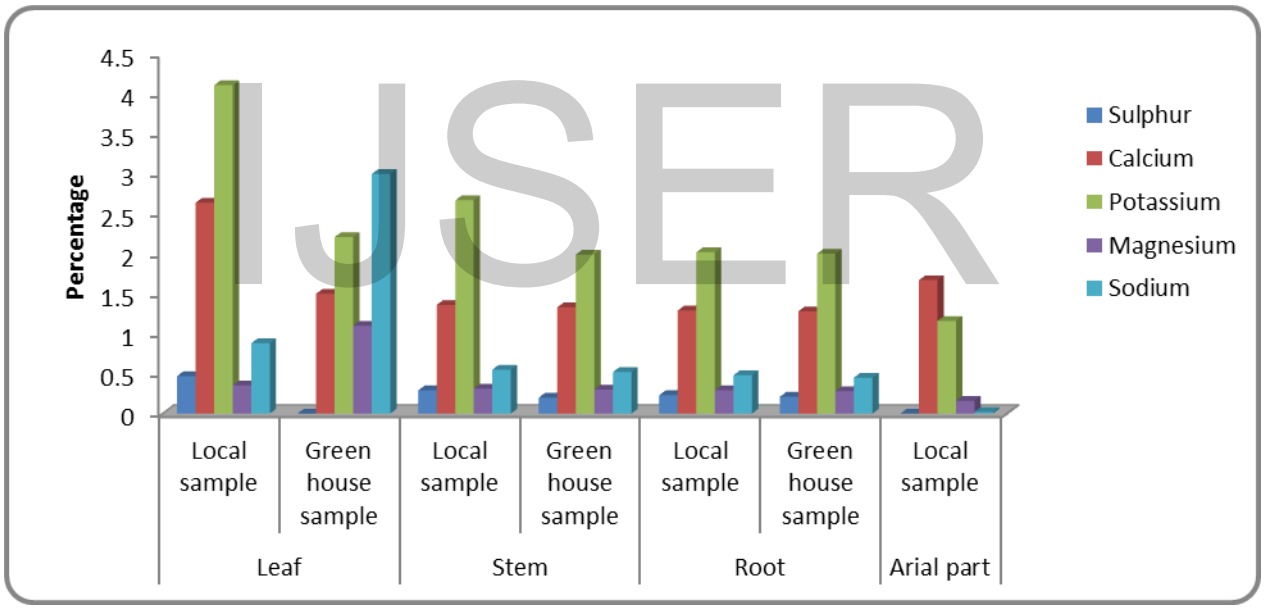


Figure 92 Comparison of carbon, nitrogen and hydrogen content in PG & AS



IJSER

Figure 93 Comparison of sulphur, calcium, potassium, magnesium and sodium content in *Pisonia grandis&Andrographis stenophylla*

IJSER © 202
http://www.ijser.org

Table 22 Comparison of results of elemental analysis of greenhouse reared and locally grown *Pisonia grandis* and aerial parts of *Andrographis stenophylla*

Elements	*Pisoniagrandis*						Andrographis stenophylla
	Leaf (%)		Stem (%)		Root (%)		Arial Parts (%)
	LS	GHS	LS	GHS	LS	GHS	LS
Carbon	39.36	38.45	38.01	38.12	38.52	38.36	38.25
Nitrogen	3.69	2.66	1.06	0.82	2.08	1.14	1.07
Hydrogen	6.99	5.99	6.31	6.04	7.26	7.24	0.99
Sulphur	0.47	ND	0.29	0.20	0.23	0.21	ND
Calcium	2.64	1.50	1.36	1.33	1.29	1.28	1.67
Potassium	4.11	2.21	2.67	1.99	2.02	2.00	1.16
Magnesium	0.35	1.10	0.31	0.30	0.29	0.28	0.16
Sodium	0.88	3.00	0.55	0.52	0.48	0.45	0.02
Zinc (ppm)	31.58	33.90	24.56	24.21	19.26	20.02	40.67

LS = Local sample; GHS= Greenhouse sample

IJSER

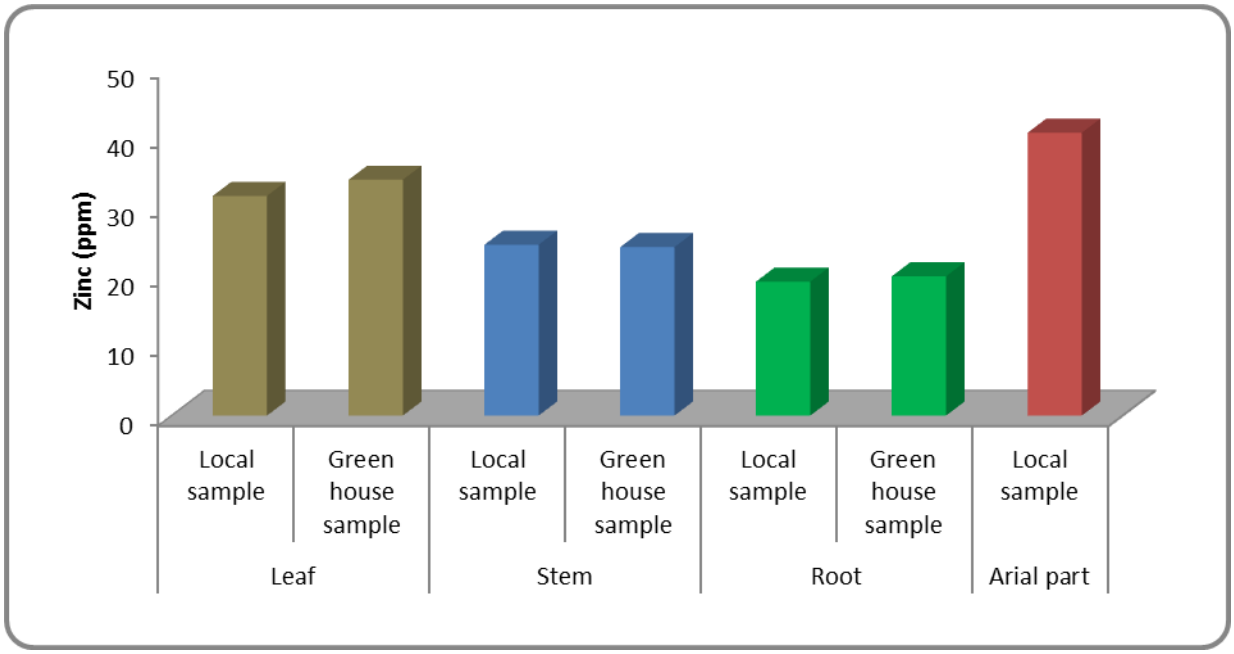


Figure 94 Comparison of zinc content in *Pisonia grandis&Andrographis stenophylla*

IJSER © 202
http://www.ijser.org

Proximate Analysis: The quality of a plant can be assed sampleon the basis of moisture, ash, volatile matter and fibre content, extractive value and calorific value of the plants. Dried powdered parts of leaves, stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** were subjected to proximate analysis. The results indicated that the chosen plant parts are a good source of energy, minerals and fiber.

Green house grown plant parts were found to possess significantly greater moisture content than the commercial samples which may due to the humidity in the green house environment. Surface moisture content of *Pisonia grandis* is in the order leaf>root>stem whereas inherent moisture content of the plant is in the order stem> leaf >root. Measurement of ash values gives an idea about minerals contained in the plantsand these minerals may be the cause of pharmacological effect. Ash content of greenhouse reared plant parts is significantly higher than the locally collected plants. Specifically the leaves of ***Pisonia grandis*** were found to possess higher minerals than parts stems and roots. An aerial part of Andrographis stenophylla wasfound topossess 9 % mineral content.The higher extractive value of aqueous extract of leaves, stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** indicated higher assimilation efficacy on intake. Leaves, stems and roots of green house plant were not significantly possessing alcohol extractive values than the commercial samples but they significantly differ in mean

IJSER

IJSER © 202
http://www.ijser.org

Water extractive value for greenhouse leaf showed significant value. Similarly fiber content of the greenhouse reared plant parts is higher than the commercial samples. Higher the calorific value, higher will be energy and protein content. A plant food that provides more than 12% of their calorific value from proteins and thus it was found to be good source of protein **(Aberoumand, 2011).** In this perspective, data obtained from the analysis revealed that commercial plant parts and greenhouse plant parts are not significant but they were found to possess significant mean value. The root of commercial sample of ***Pisonia grandis*** was found to have highest energy as well as protein content followed by leaf and stem. Aerial parts of ***Andrographis stenophylla***isalso a good source of protein. These results concluded that ***Pisonia grandis*** and ***Andrographis stenophylla*** are good source of minerals, fiber and proteins.

Table 23Proximate analysis of aerial parts of *Andrographis stenophylla*

Tests	Aerial Parts (%)
Surface Moisture	8.0
Inherent Moisture	6.3
Ash	9.3
Volatile Matter	76.5
Alcohol Extractive	13.6
Water Extractive	16.2
Crude Fiber	10.98
GCV Kcal/kg	3831

IJSER

Toxic Metal Analysis: Medicinal herbs can be easily contaminated with heavy metals from the environment. Rainfall, atmospheric dust, plant protective agents and fertilizers are the other sources for heavy metal contamination **(Flamini *et al*., 2007).** Heavy metal analysis of leaves stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** revealed that the metal contaminations in both of plants was within the WHO permissible limits and the chosen medicinal plant parts are non-toxic **(Table 24).**

IJSER © 202
http://www.ijser.org

Table 24Comparison on toxic metal content of greenhouse reared and commercial reared plant *Pisonia grandis* and aerial parts of *Andrographis stenophylla*

Toxic Metals	*Pisonia grandis*						*Andrographis stenophylla*
	Leaf		Stem		Root		Aerial Parts
	Local sample	Green house sample	Local sample	Green house sample	Local sample	Green house sample	Local sample
Lead (Pb)	3.3	2.72	0.42	BDL	BDL	ND	1.45
Cadmium (Cd)	ND	0.02	0.01	0.01	0.01	0.01	0.28
Arsenic (As)	ND	BDL	0.09	0.08	0.09	ND	BDL

WHO Permissible Level (ppm) Pb= 10; Cd=0.3, As = 1;
BDL= Below detection limit; ND = not detected

Extraction Efficacy: Extraction of leaves stems and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** was carried outin two solvents namely ethanol and water by four different methods *viz* soaking, refluxing, sonicating (Homogenizing) and ultrasonication irradiation.

IJSER

Yield of the crude ethanolic extracts is given in**Table 25**. The reflux method gave higher yield of extract compared to other methods.

Extraction Method	Pisonia grandis (%)			Andrographis stenophylla (%)
	Leaves	Stems	Roots	Aerial Parts
Soaking	4	1	3	2
Sonication (Homogenizer)	5	4	5	4
Ultrasonic Irradiation	5	4	5	4
Reflux	5.9	5	6	8.8

Extraction with water by reflux method gave higher yield particularly with leaves of ***Pisonia grandis*** and aerial parts of***Andrographis stenophylla.*** The stems and roots of ***Pisonia grandis*** gave higher yield by sonication method. In a homogenizer generally high intensity and high frequency sound waves are used to interact with plant material which helps to raise permeability of cell walls and

IJSER © 202
http://www.ijser.org

generate cavitation to maximize the yield of extract **(Arulpriya and Lalitha, 2013).**Yield of the crude aqueous extracts is given in table 26.

Table 26Yield of extracts obtained by different extraction methods

Extraction Method	Yield of Extracts (%)			
	Pisonia grandis			*Andrographis stenophylla*
	Leaves	Stems	Roots	Aerial Parts
Soaking	19	9	13	8
Sonication (Homogenizer)	19	11	16	9.5
Ultrasonic Irradiation	20	10	14	9.5
Reflux	29	10	14.5	11

Chemical Fingerprinting

Qualitative Phytochemical Tests

Phytochemical screening of the various extract concentrates of leaves stem and roots of ***Pisonia grandis*** and aerial parts of ***Andrographis stenophylla*** revealed the presence of phenols, flavonoids, alkaloids, terpenoids, sterols, tannins, carbohydrates, and proteins. The results have been summarized in **Table 4** of this thesis.

IJSER

TLC Analysis

An optimized condition for TLC analysis was achieved for the various extracts with solvent mixtures.

- Pet-ether - ethyl acetate mixture (9:1 V/V) for pet-ether extracts
- Chloroform: methanol mixture (7:3 V/V) for dewaxed ethanol extracts
- Butanol: acetic acid: water (5:0.5:4.5 V/V/V) for aqueous extracts

HPTLC Fingerprinting

HPTLC finger print analysis was carried out to find a way to cluster, discriminate and identify the plant material according to their fingerprints. HPTLC is an imaging process that can be influenced by various factors. It can be stored as images and provide objective source to compare and to identify the markers **(Ambuhl, 2011)**. In the present investigation HPTLC finger prints of ***Pisonia grandis*** and ***Andrographis stenophylla*** have been developed. An optimized condition for the extracts of ***Pisonia grandis*** with respect to active constituent was achieved with $CHCl_3$: MeOH: H_2O as mobile phase and ammoniacal silver nitrate as a detecting

IJSER © 202
http://www.ijser.org

agent. The HPTLC finger print analysis indicated an orangish brown band at R_f = 0.59 assigned to that biomarker compound pinitol found to present in the ethanol extract concentrates, dewaxed ethanol extract concentrates and aqueous extract concentrates of leaves, stems and roots of ***Pisonia grandis.*** **Table 27** summarizes the HPTLC data of various extracts of leaves, stems and roots of ***Pisonia grandis***. Quantitation of the marker compound presented in these extracts was also achieved using standard. The method was found to be linear with respect to standard and the regression coefficient was found to be 0.99. Data analysis indicated that pinitol as a major constituent in the extracts of ***Pisonia grandis*** and relative percentage of pinitol in those extracts was found to be high in DPGRE and PGSAq followed by DPGSE, DPGLE. **Table 28** showed the relative percentage of pinitol in PGLE, DPGLE, PGLAQ, PGSE, DPGSE, PGSAQ, PGRE, DPGRE and PGRAQ.

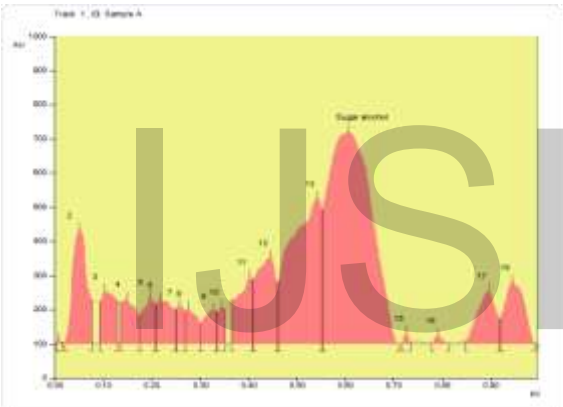


Fig.95. Densitogram of A (DPGSE)

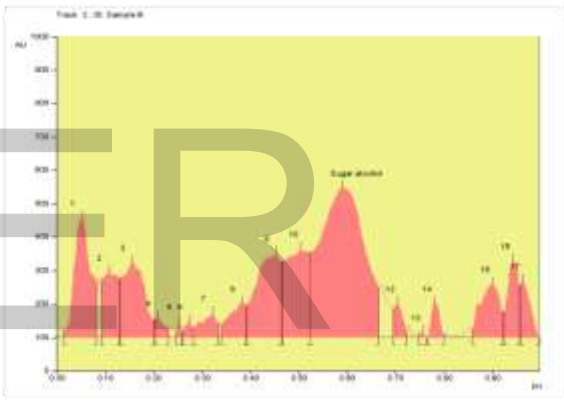


Fig.96.Densitogram of B (PGRAQ)

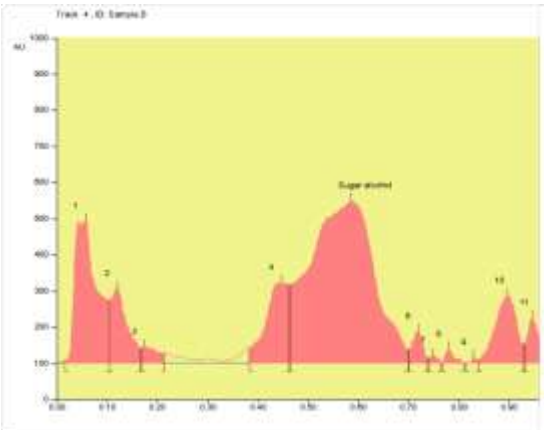


Fig.97. Densitogram of D (PGSAQ)

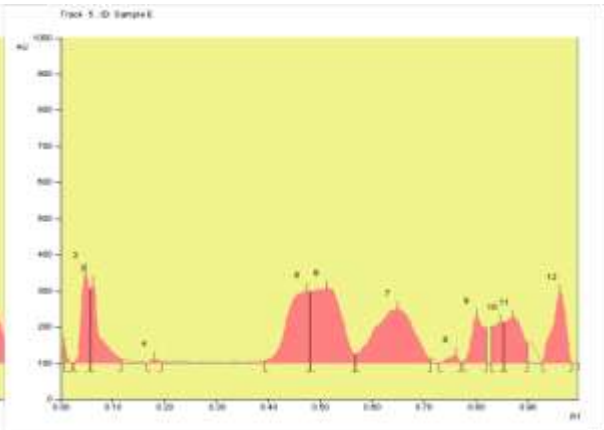
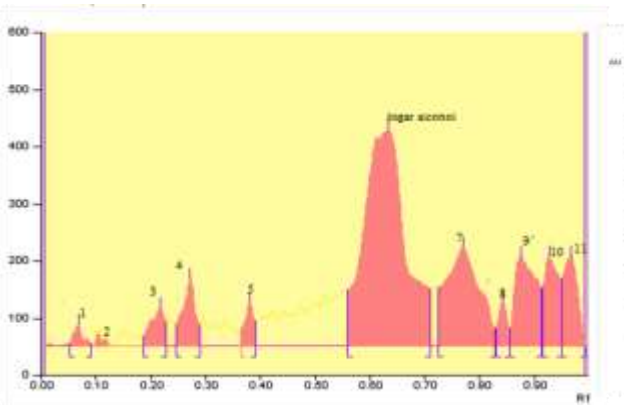


Fig.98.Densitogram of E (PGLAQ)

IJSER

IJSER © 202
http://www.ijser.org



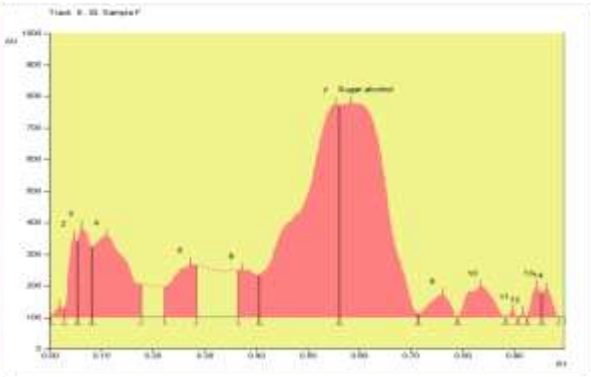
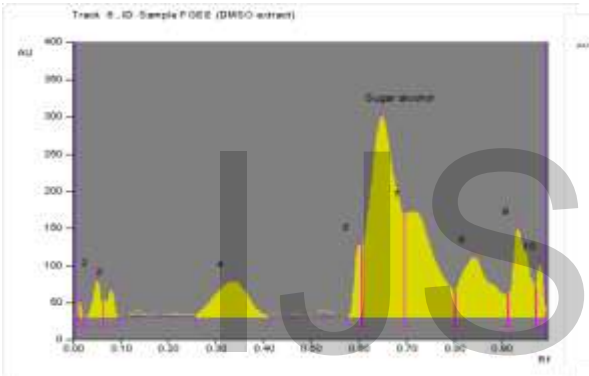


Fig.99. Densitogram of F DPGRE **Fig.100.Densitogram of H (DPGLE)**



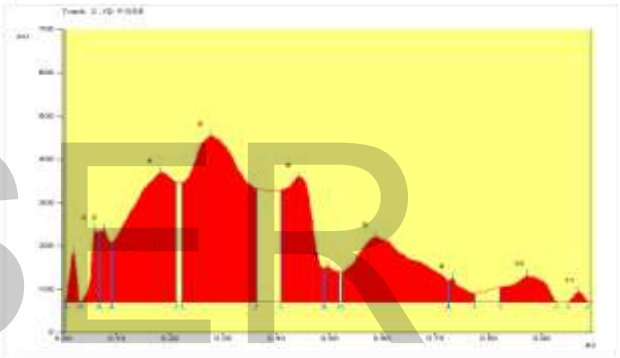
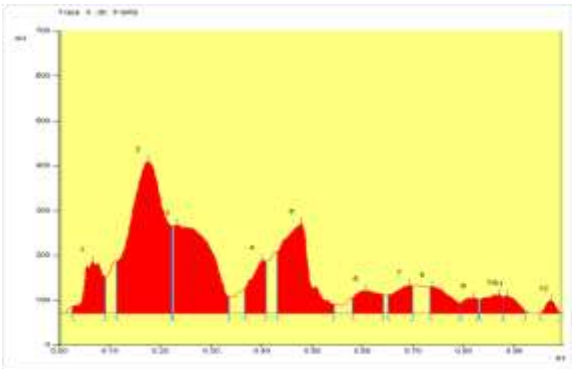


Fig.101 Densitogram of G (PGLE) **Fig.102 Densitogram of (PGSE)**



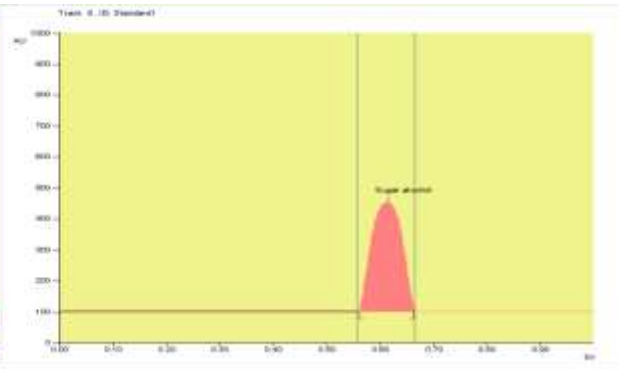


Fig.103 Densitogram of G (PGRE) **Fig.104Densitogram of Standard@ 10 µg**

IJSER

IJSER © 202
http://www.ijser.org

Table 27 HPTLC data of various extracts of leaves, stems and roots of *Pisonia grandis*

Track	Sample Code	Peak	R_f	Height	Area	Assigned substance
Sample A	DPGSE	1	0.01	11.9	80.1	Unknown
Sample A	DPGSE	2	0.05	332.2	8963.4	Unknown
Sample A	DPGSE	3	0.10	153.3	4451.4	Unknown
Sample A	DPGSE	4	0.15	130.7	3484.2	Unknown
Sample A	DPGSE	5	0.20	137.4	3056.4	Unknown
Sample A	DPGSE	6	0.22	127.8	3844.7	Unknown
Sample A	DPGSE	7	0.26	107.9	1661.2	Unknown
Sample A	DPGSE	8	0.28	102.7	2150.9	Unknown
Sample A	DPGSE	9	0.30	96.9	2297.3	Unknown
Sample A	DPGSE	10	0.34	102.7	1428.2	Unknown
Sample A	DPGSE	11	0.40	195.0	5613.9	Unknown
Sample A	DPGSE	12	0.45	252.7	9055.9	Unknown
Sample A	DPGSE	13	0.54	425.4	24300.0	Unknown
Sample A	**DPGSE**	**14**	**0.60**	**619.5**	**51758.8**	**Sugar alcohol 1**
Sample A	DPGSE	15	0.73	31.3	239.7	Unknown
Sample A	DPGSE	16	0.79	23.7	320.0	Unknown
Sample A	DPGSE	17	0.90	148.9	5072.5	Unknown
Sample A	DPGSE	18	0.94	171.6	6081.8	Unknown
Sample B	PGRAQ	1	0.05	356.5	11008.2	Unknown
Sample B	PGRAQ	2	0.11	193.5	5673.4	Unknown
Sample B	PGRAQ	3	0.16	224.4	9175.6	Unknown
Sample B	PGRAQ	4	0.21	58.8	1013.7	Unknown
Sample B	PGRAQ	5	0.25	46.7	331.4	Unknown
Sample B	PGRAQ	6	0.27	48.0	631.9	Unknown
Sample B	PGRAQ	7	0.32	71.5	2024.4	Unknown
Sample B	PGRAQ	8	0.38	101.7	3178.6	Unknown
Sample B	PGRAQ	9	0.45	251.4	11388.9	Unknown
Sample B	PGRAQ	10	0.50	165.6	11543.7	Unknown
Sample B	**PGRAQ**	**11**	**0.59**	**446.3**	**37391.5**	**Sugar alcohol 1**
Sample B	PGRAQ	12	0.70	100.1	1647.6	Unknown
Sample B	PGRAQ	13	0.76	16.1	136.7	Unknown
Sample B	PGRAQ	14	0.78	101.9	1523.9	Unknown
Sample B	PGRAQ	15	0.90	140.7	5027.0	Unknown
Sample B	PGRAQ	16	0.94	197.7	3840.5	Unknown
Sample B	PGRAQ	17	0.96	132.7	1908.8	Unknown
Sample C	PGSAQ	1	0.06	394.0	16256.0	Unknown
Sample C	PGSAQ	2	0.12	206.0	6333.4	Unknown
Sample C	PGSAQ	3	0.17	45.4	1395.0	Unknown
Sample C	PGSAQ	4	0.45	221.9	9277.9	Unknown
Sample C	PGSAQ	**5**	**0.59**	**449.0**	**53654.2**	**Sugar alcohol 1**
Sample C	PGSAQ	6	0.72	87.9	1764.9	Unknown
Sample C	PGSAQ	7	0.75	20.6	304.9	Unknown
Sample C	PGSAQ	8	0.78	37.6	594.0	Unknown
Sample C	PGSAQ	9	0.83	15.6	132.4	Unknown
Sample C	PGSAQ	10	0.90	187.2	7414.3	Unknown
Sample C	PGSAQ	11	0.95	125.4	2734.4	Unknown
Sample D	PGLAQ	1	0.05	256.3	3494.3	Unknown
Sample D	PGLAQ	2	0.06	220.6	3551.0	Unknown
Sample D	PGLAQ	3	0.18	10.9	158.6	Unknown
Sample D	PGLAQ	4	0.47	199.1	7831.0	Unknown
Sample D	PGLAQ	5	0.51	207.8	10801.7	Unknown
Sample D	**PGLAQ**	**6**	**0.61**	**151.1**	**10485.5**	**Sugar alcohol 1**

IJSER

Sample D	PGLAQ	7	0.76	22.6	390.2	Unknown
Sample D	PGLAQ	8	0.80	130.3	2843.1	Unknown
Sample D	PGLAQ	9	0.85	113.4	2152.7	Unknown
Sample D	PGLAQ	10	0.87	125.8	3864.2	Unknown
Sample D	PGLAQ	11	0.96	195.9	4494.7	Unknown
Sample E	DPGRE	1	0.02	27.7	310.9	Unknown
Sample E	DPGRE	2	0.05	246.7	3325.9	Unknown
Sample E	DPGRE	3	0.06	278.9	5783.1	Unknown
Sample E	DPGRE	4	0.11	250.5	14687.1	Unknown
Sample E	DPGRE	5	0.27	163.1	6919.3	Unknown
Sample E	DPGRE	6	0.37	146.2	4763.4	Unknown
Sample E	DPGRE	7	0.55	671.1	47462.6	Unknown
Sample E	**DPGRE**	**8**	**0.59**	**676.2**	**52698.7**	**Sugar alcohol 1**
Sample E	DPGRE	9	0.76	72.1	2514.4	Unknown
Sample E	DPGRE	10	0.83	97.7	4283.2	Unknown
Sample E	DPGRE	11	0.90	22.7	1807.2	Unknown
Sample E	DPGRE	12	0.92	14.6	108.5	Unknown
Sample E	DPGRE	13	0.94	95.6	1518.5	Unknown
Sample E	DPGRE	14	0.96	90.2	1427.4	Unknown
Sample F	DPGLE	1	0.07	36.7	546.7	Unknown
Sample F	DPGLE	2	0.14	29.2	423.4	Unknown
Sample F	DPGLE	3	0.22	64.9	1568.9	Unknown
Sample F	DPGLE	4	0.28	112.3	2488.4	Unknown
Sample F	DPGLE	5	0.40	80.1	1204.6	Unknown
Sample F	**DPGLE**	**6**	**0.60**	**496.9**	**44332.0**	**Sugar alcohol 1**
Sample F	DPGLE	7	0.76	180.7	9744.7	Unknown
Sample F	DPGLE	8	0.83	81.9	1098.4	Unknown
Sample F	DPGLE	9	0.89	145.6	5586.6	Unknown
Sample F	DPGLE	10	0.93	157.2	3892.6	Unknown
Sample F	DPGLE	11	0.98	157.2	3657.1	Unknown
Sample G	PGLE	1	0.01	20.7	110.9	Unknown
Sample G	PGLE	2	0.05	49.6	669.4	Unknown
Sample G	PGLE	3	0.08	37.4	528.6	Unknown
Sample G	PGLE	4	0.34	47.1	3061.8	Unknown
Sample G	PGLE	5	0.60	96.9	1303.2	Unknown
Sample G	**PGLE**	**6**	**0.65**	**270.0**	**13543.0**	**Sugar alcohol 1**
Sample G	PGLE	7	0.71	141.3	7969.5	Unknown
Sample G	PGLE	8	0.84	80.2	4683.5	Unknown
Sample G	PGLE	9	0.93	118.7	3379.9	Unknown
Sample G	PGLE	10	0.98	70.6	799.2	Unknown
Sample H	PGSE	1	0.59	119.1	4816.1	Unknown
Sample H	PGSE	1	0.02	114.0	1236.1	Unknown
Sample H	PGSE	2	0.06	168.8	2094.9	Unknown
Sample H	PGSE	3	0.08	168.2	2945.1	Unknown
Sample H	PGSE	4	0.18	299.4	23324.6	Unknown
Sample H	PGSE	5	0.28	384.0	36921.0	Unknown
Sample H	PGSE	6	0.45	289.6	14572.7	Unknown
Sample H	PGSE	7	0.50	81.0	1746.0	Unknown

IJSER

IJSER © 202
http://www.ijser.org

Sample H	PGSE	8	0.59	150.4	16778.0	Sugar alcohol 1
Sample H	PGSE	9	0.73	55.5	1347.5	Unknown
Sample H	PGSE	10	0.87	61.3	3642.2	Unknown
Sample H	PGSE	11	0.97	23.0	379.4	Unknown
Sample I	PGRE	1	0.07	115.1	2832.4	Unknown
Sample I	PGRE	2	0.18	338.7	21188.6	Unknown
Sample I	PGRE	3	0.23	196.7	13212.0	Unknown
Sample I	PGRE	4	0.40	118.6	3191.9	Unknown
Sample I	PGRE	5	0.48	199.4	10215.1	Unknown
Sample I	PGRE	6	0.61	50.8	2285.6	Sugar alcohol 1
Sample I	PGRE	7	0.69	62.9	2064.5	Unknown
Sample I	PGRE	8	0.74	59.6	2123.4	Unknown
Sample I	PGRE	9	0.82	34.4	901.8	Unknown
Sample I	PGRE	10	0.87	41.9	1410.7	Unknown
Sample I	PGRE	11	0.89	39.2	932.0	Unknown
Sample I	PGRE	12	0.97	28.4	495.1	Unknown
STD 1	**-**	**1**	**0.59**	**606.6**	**41998.8**	**Sugar alcohol 1**
STD 2	**-**	**1**	**0.60**	**660.9**	**79338.3**	**Sugar alcohol 2**
STD 3	**-**	**1**	**0.59**	**669.3**	**115996**	**Sugar alcohol 3**
STD 4	**-**	**1**	**0.59**	**698.6**	**157995**	**Sugar alcohol 4**
STD 5	**-**	**1**	**0.59**	**688.5**	**179994**	**Sugar alcohol 5**

IJSER

IJSER © 202
http://www.ijser.org

Table 28 Relative quantity of Pinitol in ethanol and aqueous extracts of leaves, stems and roots of *Pisonia grandis*

Sample Code	Relative Quantity of Pinitol (μg) in 100 μg Extract
PGLE	16.56
DPGLE	25.5
PGLAq	0.92
PGSE	0.63
DPGSE	29.95
PGSAq	31.35
PGRE	0.38
DPGRE	31.28
PGRAq	20.12

IJSER

HPTLC finger printing of *Andrographis stenophylla*

The HPTLC finger printing of various extracts of ***Andrographis stenophylla*** was carried out under optimized condition. Optimized condition for extracts of ***Andrographis stenophylla*** with respect to active constituent was achieved with $CHCl_3$:MeOH as mobile phase and 5% H_2SO_4 as a detecting agent. The HPTLC finger print indicated an orangish brown band at R_f =0.69 assigned to the biomarker compound of ***Andrographis stenophylla*** found to be present in extracts DASAE, ASAAQ and ASAE***.*** Table 12 summarize the HPTLC data of various extract concentrates of aerial parts of ***Andrographis stenophylla***. Relative quantitation of marker compound presented in those extracts was also achieved using standard ***Andrographis stenophylla***. The method was found to be linear with respect to standard. Relative quantities of andrographolide present in the extracts of ***Andrographis stenophylla***is given in **Table 29**.

IJSER © 202
http://www.ijser.org

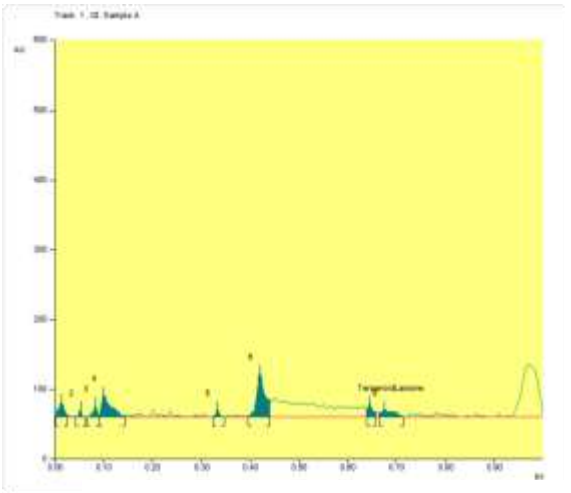


Fig.105. Densitogram of ASAP

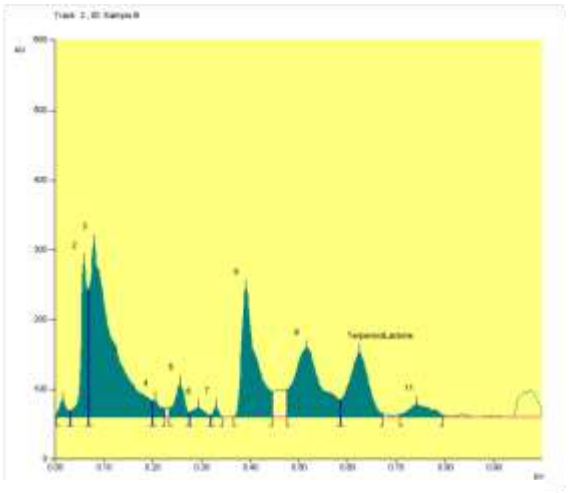


Fig.106. Densitogram of DASAE

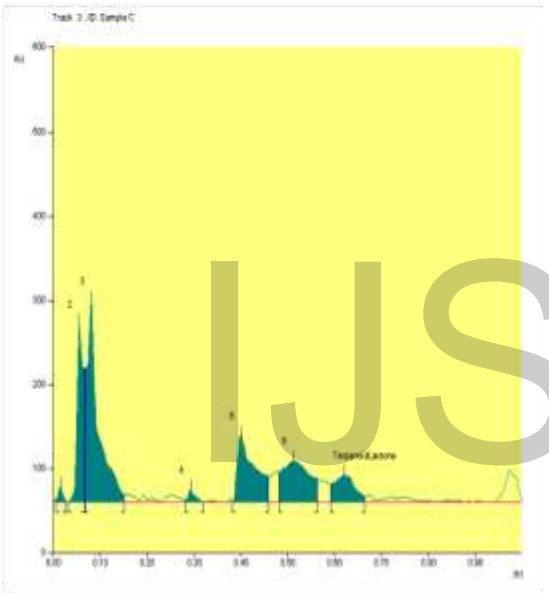


Fig.107. Densitogram of ASAQ

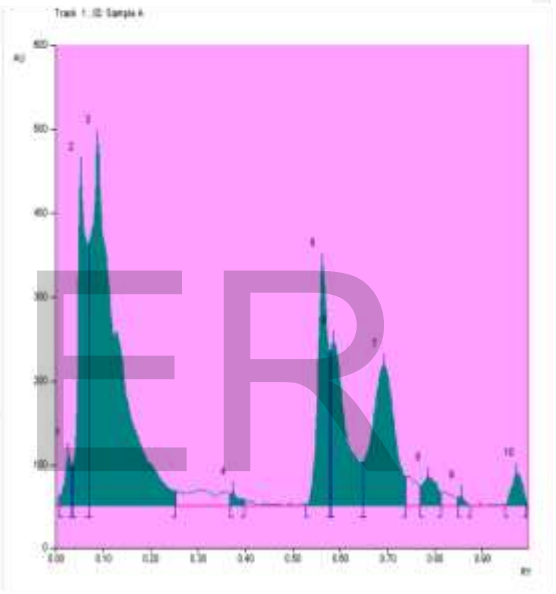


Fig.108. Densitogram of ASAE

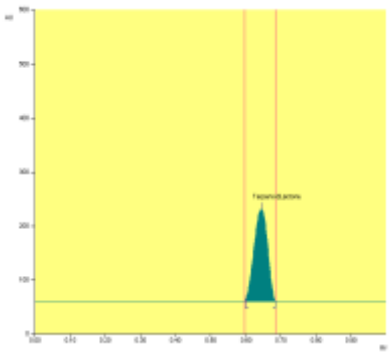


Fig.109. Densitogram of Standard (2.5 μg)

IJSER

IJSER © 202
http://www.ijser.org

Table 29 HPTLC data of various extract concentrates of aerial parts of *Andrographis stenophylla*.

Track	Sample Code	Peak	R_f	Height	Area	Assigned substance
Sample A	ASAP	1	0.01	13.4	106.2	Unknown
Sample A	ASAP	2	0.05	10.9	53.4	Unknown
Sample A	ASAP	3	0.08	16.7	125.8	Unknown
Sample A	ASAP	4	0.10	32.2	576.3	Unknown
Sample A	ASAP	5	0.33	11.1	75.8	Unknown
Sample A	ASAP	6	0.42	63.3	993.4	Unknown
Sample A	**ASAP**	**7**	**0.65**	**18.7**	**187.5**	**Terpenoid Lactone 1**
Sample A	ASAP	8	0.68	10.7	254.5	Unknown
Sample B	DASAE	1	0.02	16.8	126.9	Unknown
Sample B	DASAE	2	0.06	216.4	2455.3	Unknown
Sample B	DASAE	3	0.08	243.5	10097.0	Unknown
Sample B	DASAE	4	0.20	22.0	229.7	Unknown
Sample B	DASAE	5	0.26	45.4	786.8	Unknown
Sample B	DASAE	6	0.29	12.3	259.3	Unknown
Sample B	DASAE	7	0.33	14.4	135.9	Unknown
Sample B	DASAE	8	0.39	184.9	4947.7	Unknown
Sample B	DASAE	9	0.52	98.9	5033.1	Unknown
Sample B	**DASAE**	**10**	**0.64**	**93.2**	**3322.8**	**Terpenoid Lactone 1**
Sample B	DASAE	11	0.74	18.3	780.4	Unknown
Sample C	ASAAQ	1	0.01	16.0	127.9	Unknown
Sample C	ASAAQ	2	0.06	211.4	2514.0	Unknown
Sample C	ASAAQ	3	0.08	239.3	5490.5	Unknown
Sample C	ASAAQ	4	0.29	15.2	194.8	Unknown
Sample C	ASAAQ	5	0.40	79.4	2681.8	Unknown
Sample C	ASAAQ	6	0.51	48.7	2595.4	Unknown
Sample C	ASAAQ	7	0.65	31.6	1306.6	Terpenoid Lactone 1
Sample D	ASAE	1	0.03	62.5	798.0	Unknown
Sample D	**ASAE**	**2**	**0.05**	**402.9**	**6837.7**	**Terpenoid Lactone 1**
Sample D	ASAE	3	0.09	435.4	22044.0	Unknown
Sample D	ASAE	4	0.37	15.8	265.6	Unknown
Sample D	ASAE	5	0.56	288.1	5625.3	Terpenoid Lactone 2
Sample D	ASAE	6	0.59	195.8	5988.2	Unknown

IJSER

IJSER © 202
http://www.ijser.org

Sample D	ASAE	7	0.69	168.0	7347.1	Terpenoid Lactone 3
Sample D	ASAE	8	0.79	34.0	985.4	Unknown
Sample D	ASAE	9	0.86	11.6	134.9	Unknown
Sample D	ASAE	10	0.97	39.0	315.9	Unknown
STD	**standard**	**1**	**0.65**	**187.8**	**7567.3**	**standard**
STD	**standard**	**2**	**0.64**	**309.3**	**14157.5**	**standard**
STD	**standard**	**3**	**0.65**	**403.9**	**22572.1**	**standard**

Table 30Relative quantity of andrographolide in extracts of *Andrographis stenophylla*

Sample Code	Relative quantity of Andrographolide (μg/100 μg of extract)
DASAE	3.96
ASAAQ	0.13
ASAE	6.22

IJSER

NMR Fingerprinting of Extracts

Fingerprinting by NMR is a quick, expedient and effective tool to identify molecule by means of clusters of information that are obtained from the spectrum. Many scientists have reported the versatility of NMR instrument in metabolite analysis. It isreported that NMR can be used as non-destructive analysis to record the spectra of cell suspensions, tissues, whole plant, plant extracts and purified metabolites can be recorded **(Ratcliffe *et al*., 2001)**.NMR fingerprinting of various extract concentrates of leaves, stems and roots of ***Pisonia grandis*** and aerial part extract concentrates of ***Andrographis stenophylla*** was done using 500 MHz NMR. The following figures 110-113 represent the NMR spectra of the various extracts.

IJSER © 202
http://www.ijser.org

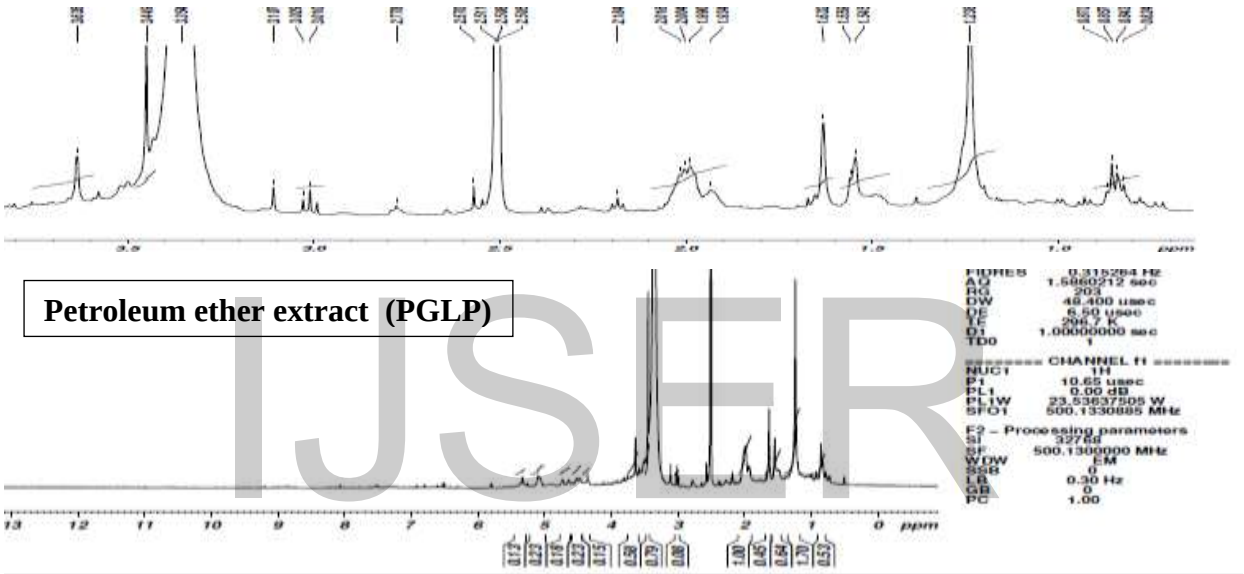


Figure 110.H[1]NMR Spectra of petroleum ether extract of leaves of PG

IJSER

IJSER © 202
http://www.ijser.org

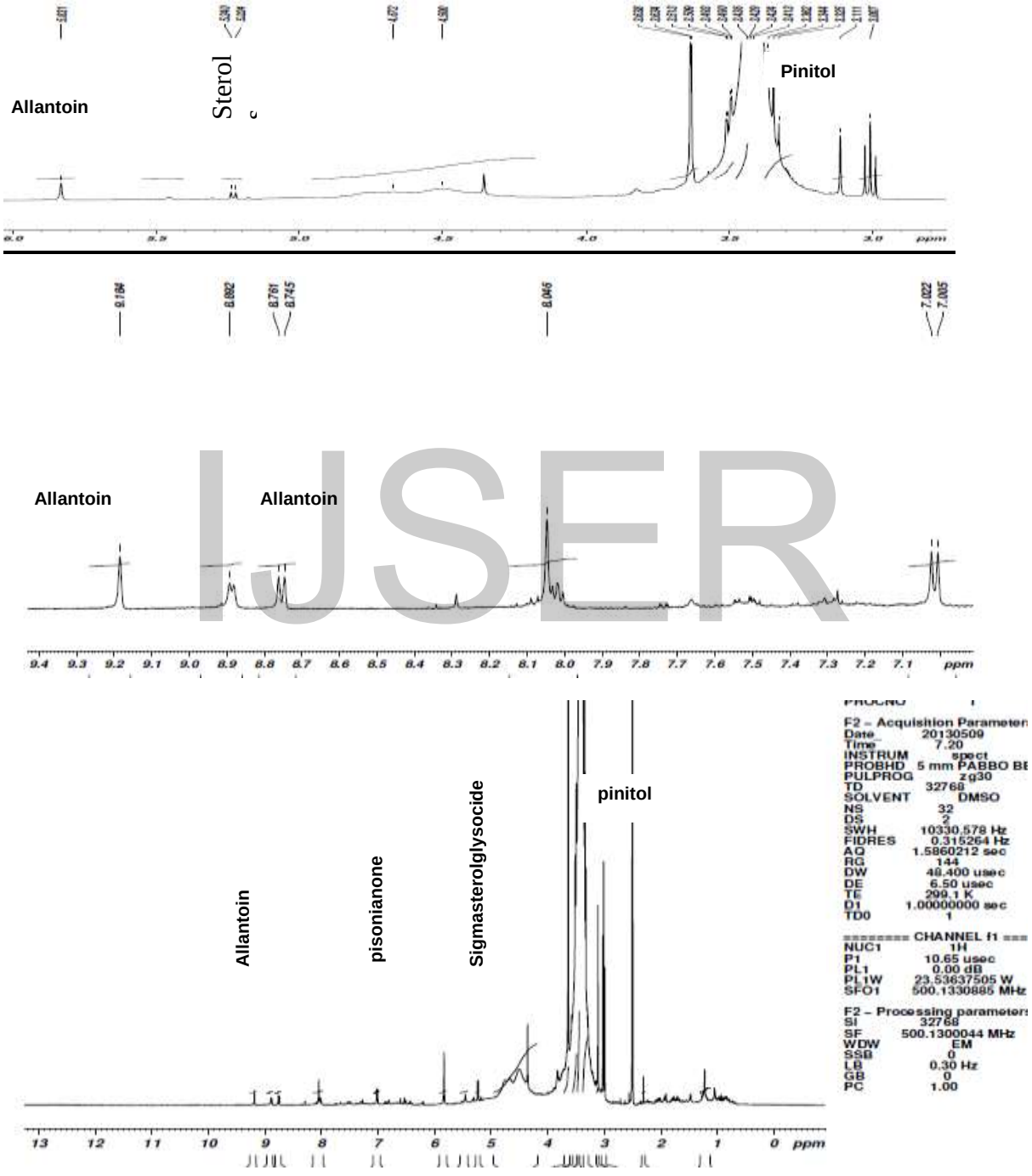


Figure 11 ^{1}H NMR Spectra of Dewaxed ethanol extract

IJSER

IJSER © 202
http://www.ijser.org

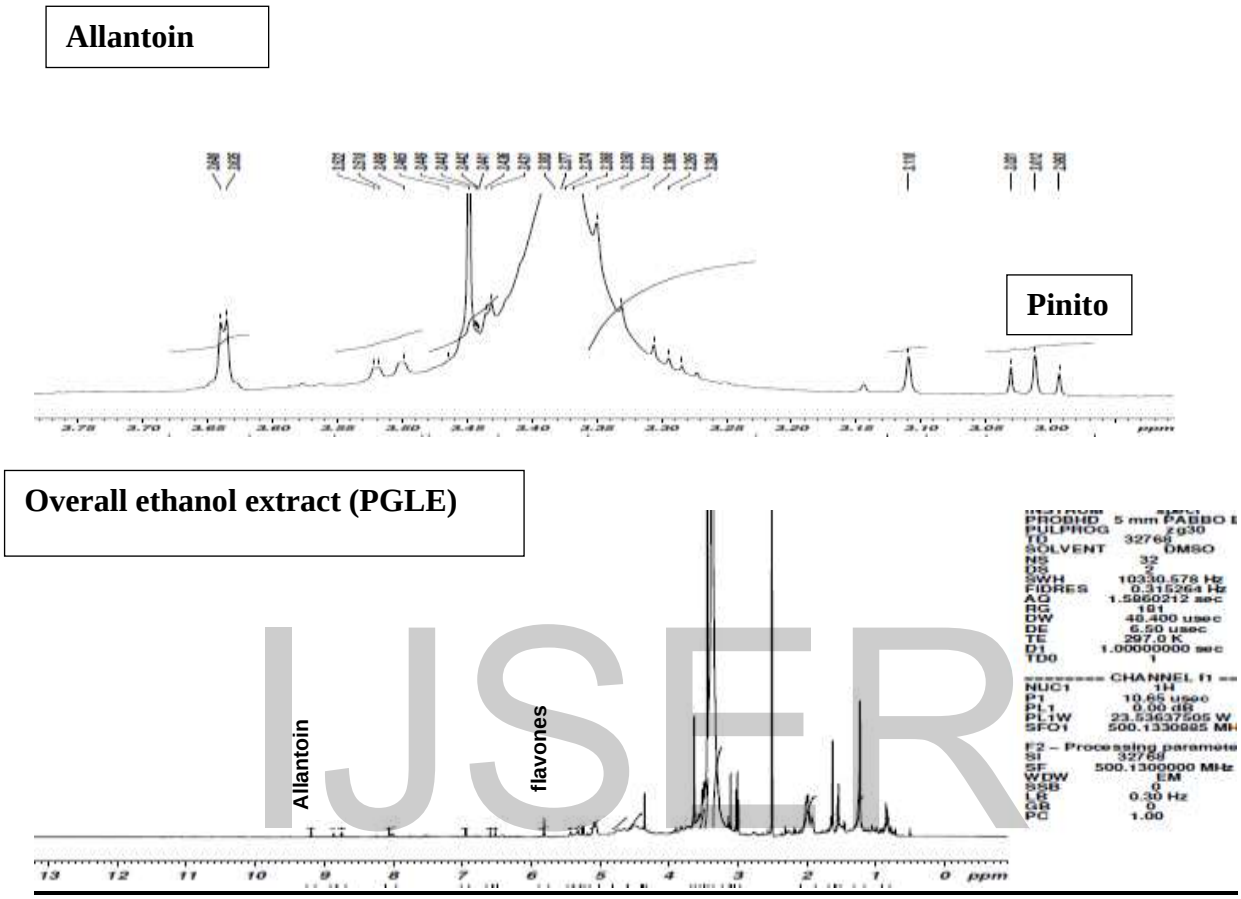


Figure 112
H[1]NMR Spectra of Leaf Extracts of PG

IJSER © 202
http://www.ijser.org

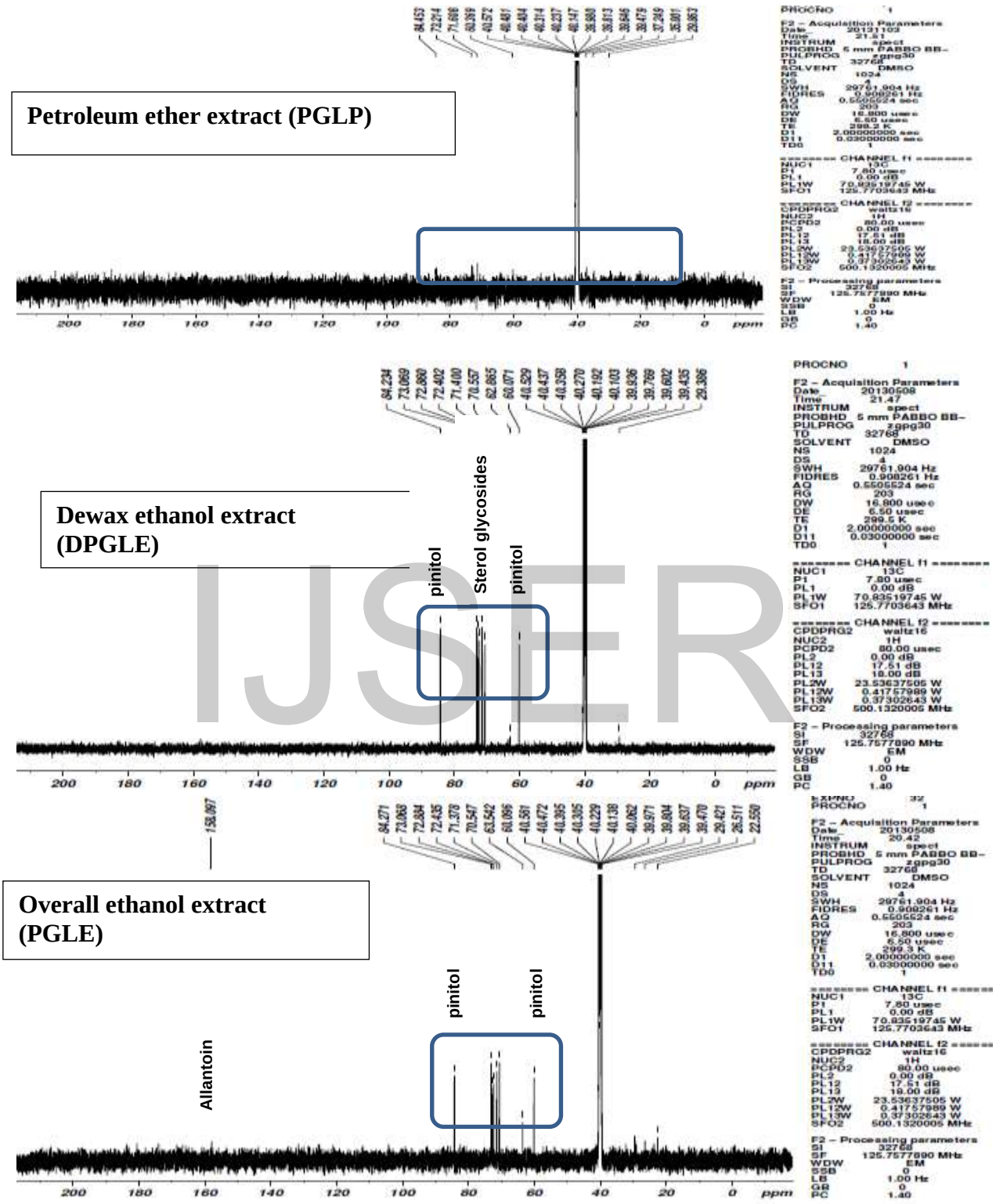


Figure 113

C^{13} NMR Spectra of Leaf Extracts of PG

IJSER © 202
http://www.ijser.org

H^1 NMR spectrum of polar extract concentrates of leaves stems and roots of ***Pisonia grandis*** (PGLE, DPLE, PGSE, DPGSE, PGRE, and DPGRE) uniformly depicted a cluster of signals at δ3 to 4 ppm. The spectrum of non-polar extract concentrate (PGLP, PGSP and PGRP) showed few peaks at 0.5 ppm to 2 ppm. These peaks may be due to aliphatic alicyclic or β- substituted aliphatic compounds respectively as confirmed by the standard chart of **Silverstein *et al*., (2005).** Overall ethanol extract is the additive spectrum of pet-ether and dewaxed-ethanol extract.The spectrum of bio-marker compound pinitol exhibited characteristic peaks at 3 to 4 ppm and 4 to 5 ppm. The presence of these peaks in the extracts was ascertained in the 'HNMR spectrum.

C^{13}NMR is preferred tool for metabolite fingerprinting because after all, the carbon of skeleton of rings and chains are central to the organic chemistry. The C^{13}spectrum of polar extract concentrates of leaves stems and roots of ***Pisonia grandis*** (PGLE, DPLE, PGSE, DPGSE, PGRE, and DPGRE)uniformly depicted the cluster of signal at 60 to 90 ppm which corresponds to –CH-O; $-CH_2$-O; -O-CH_3 groups. The spectrum of non-polar extract concentrate (PGLP, PGSP and PGRP) showed few peaks at 0 to 40 ppm which corresponds to -CH-N; $-CH_2$-N; $-CH_3$-N; -CH-CO; $-CH_2$-CO; $-CH_3$-CO;$-CH_3$-C=C; $-CH_3$-C; $-CH_2$ respectively peaks at 120 to 140 ppm corresponds to aromatic -CH and alkene –CH; a peaks at 120 to 140 ppm corresponds to conjugated alkene respectively as confirmed by the standard chart of **Silverstein *et al*., (2005).** The spectrum of bio-marker compound pinitol exhibited five peaks at 70 to 75 ppm; and a peak at 60 and 85 ppm. Presence of these respective peaks in the extracts such as PGLE, DPLE, PGSE, DPGSE, PGRE, and DPGRE confirms the presence pinitol like compound and considered it as fingerprint region of the plant ***Pisonia grandis***.

IJSER

IJSER © 202
http://www.ijser.org

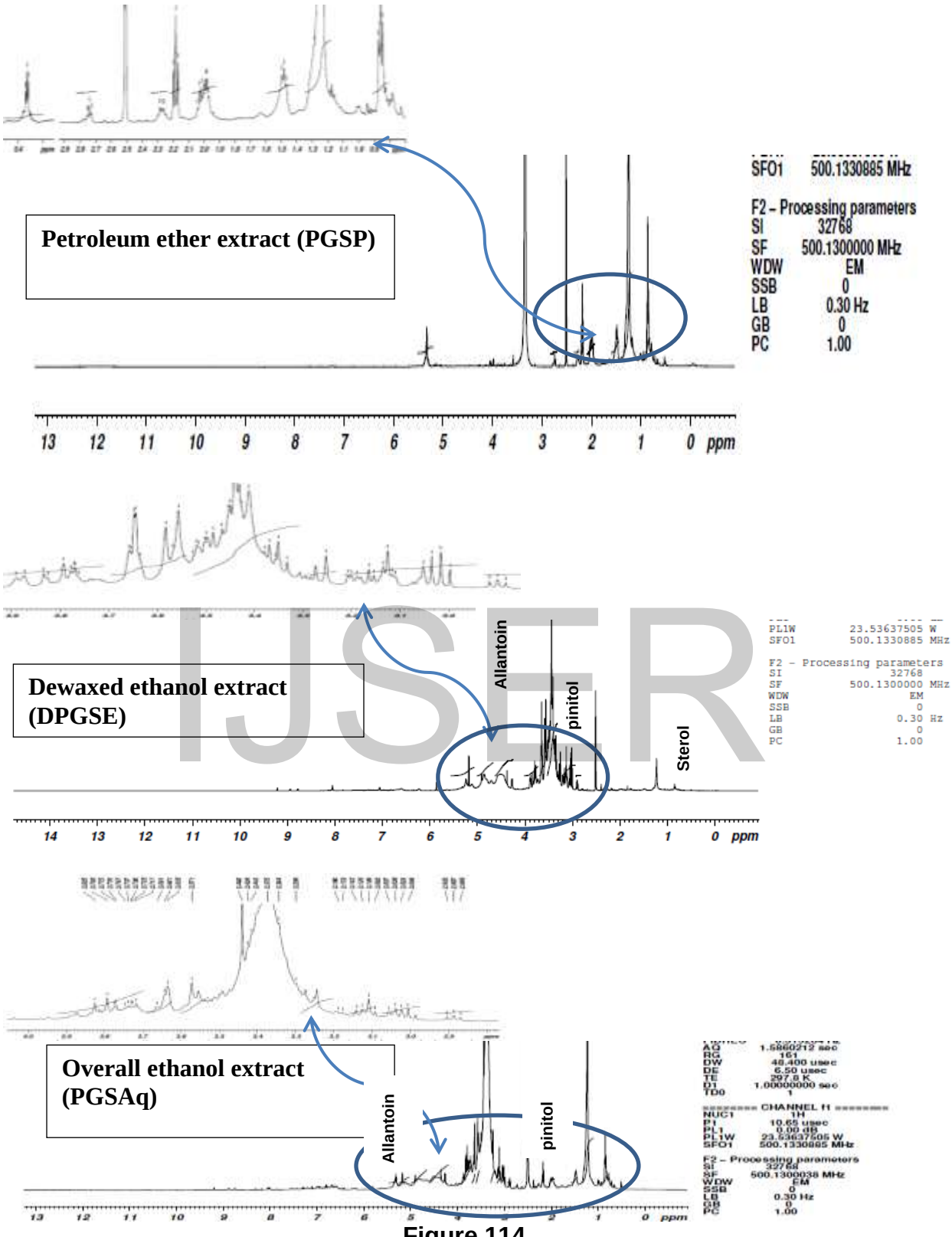


Figure 114
H[1]NMR Spectra of Stem Extracts of PG

IJSER

IJSER © 202
http://www.ijser.org

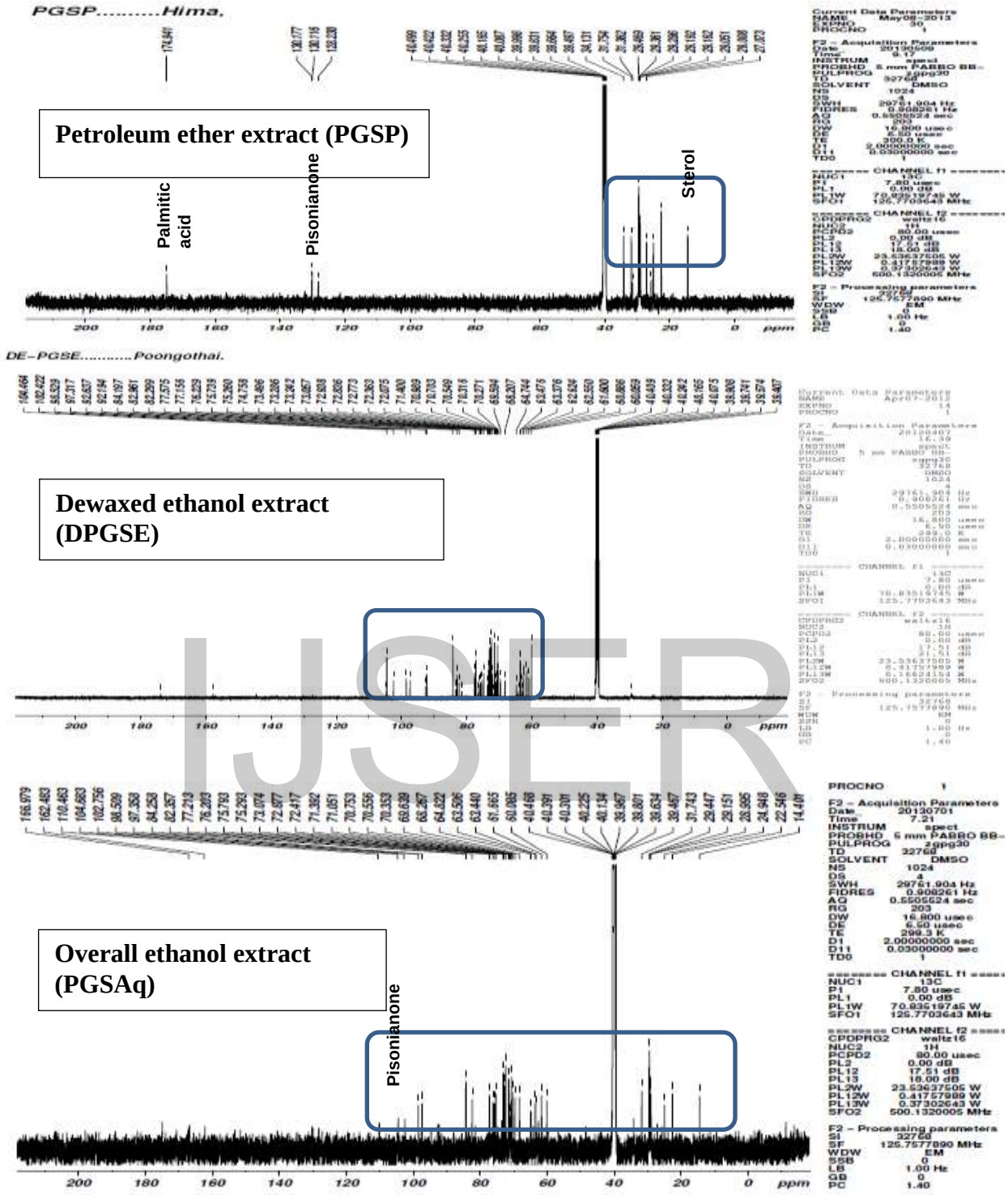


Figure 115 C^{13}NMR Spectra of Stem Extracts of PG

IJSER

IJSER © 202
http://www.ijser.org

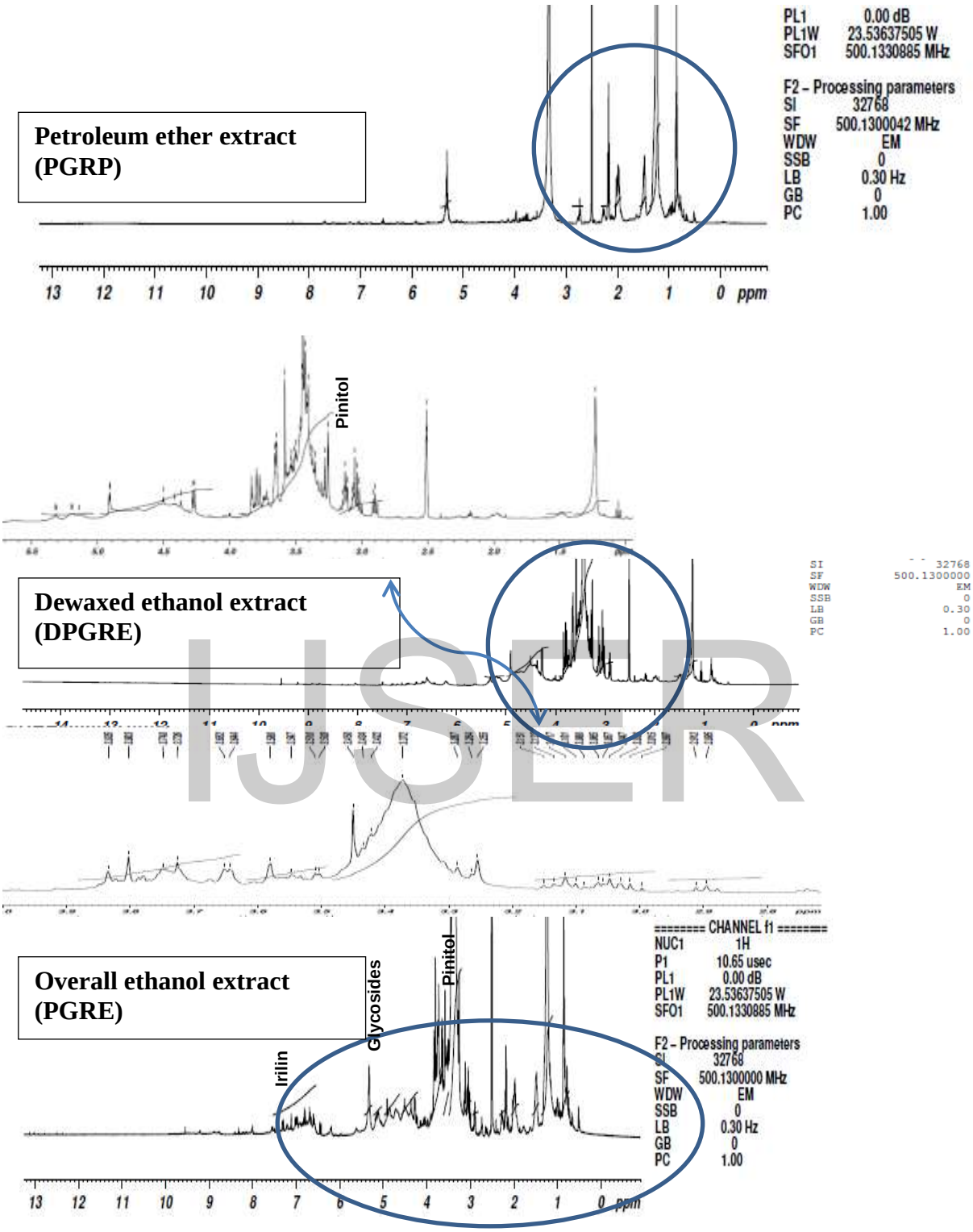


Figure 116 H[1]NMR Spectrum of Root Extracts of PG

IJSER

IJSER © 202
http://www.ijser.org

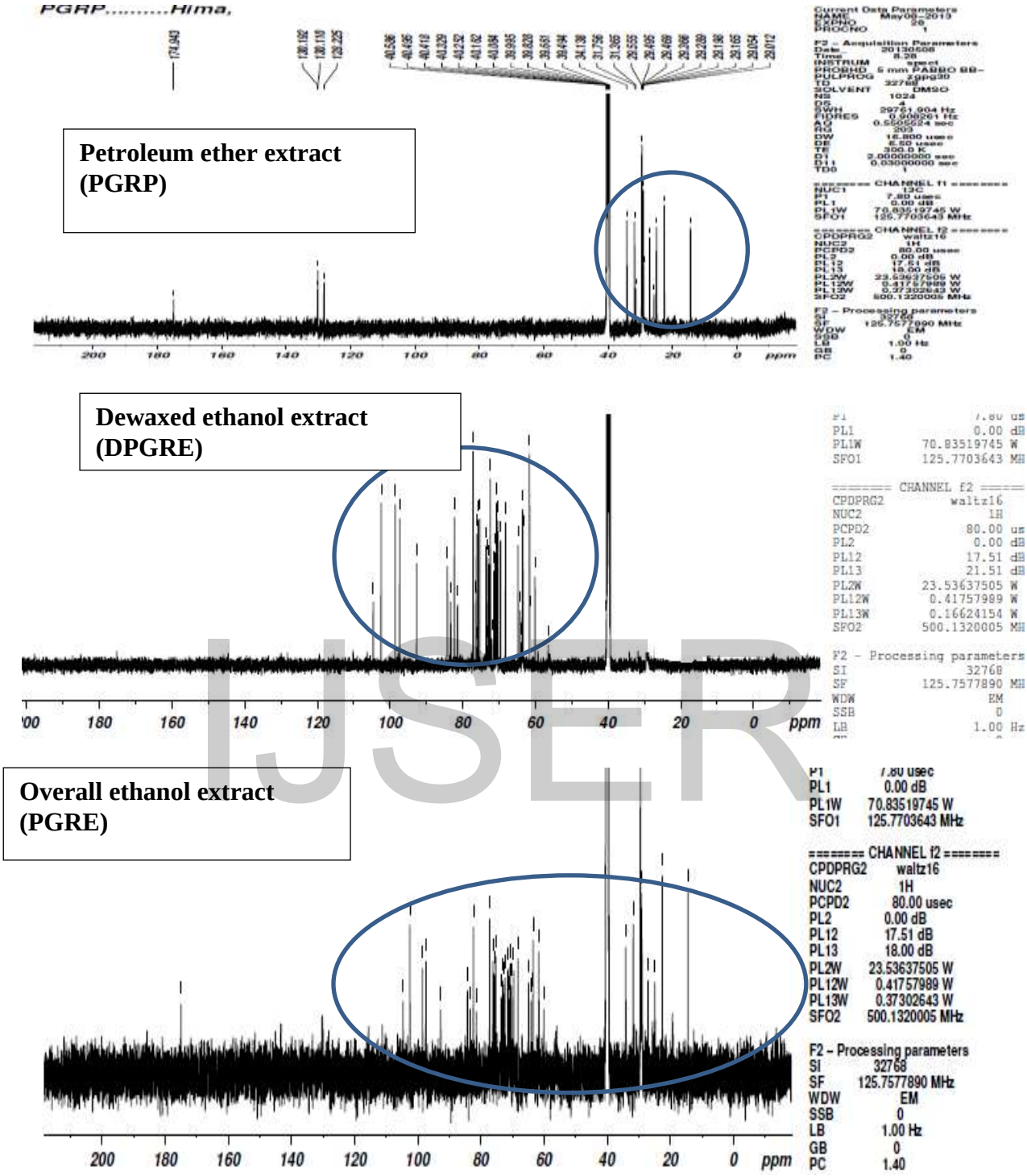


Figure 117
C^{13}NMR Spectrum of Root Extracts of PG

IJSER

IJSER © 202
http://www.ijser.org

The H^1 NMR spectrum of polar extract concentrates of aerial parts of ***Andrographis stenophylla*** **(ASAE and DASAE)** depicted the cluster of signal at 0 to 8 ppm thatfalls at the aliphatic and aromatic region. The spectrum of non-polar extract concentrate (ASAP) showed strong peaks at 0 ppm to 3 ppm. These peaks may be for aliphatic alicyclic, β- substituted aliphatic, α-substituted aliphatic or alkyne group of compounds respectively as confirmed by the standard chart of **Silverstein *et al* (2005).** Overall ethanol extract is the additive spectrum of pet-ether and dewaxed-ethanol extract which showed peaks at 0.5 ppm to 10 ppm which indicated the presence of both aliphatic, aromatic, heteroaromatic groups of compounds that might be responsible for the bio-potential of the plant. The spectrum of bio-marker compound andrographolide exhibited characteristic peaks at 0.74;1.20; and 4.0 to 5.5 ppm. The presence of these peaks in the extracts was observed and assigned those regions as finger print region of H^1 NMR spectrum of the plant ***Andrographis stenophylla.***

The C^{13}NMR is preferred tool for metabolite fingerprinting because after all, the carbon of skeleton of rings and chains are central to the organic chemistry. The C^{13}spectrum of polar extract concentrates of aerial parts of ***Andrographis stenophylla*** **(ASAE and DASAE)**uniformly depicted the cluster of signal at 20 to 80 and 120-180 ppm which corresponds to acyclic carbons, alkyne carbons and hetero-aromatic carbons. The spectrum of non-polar extract concentrate (ASAP) showed few peaks mainly at 0 to 40 ppm which corresponds to -CH-N;-CH_2-N; -CH_3-N; -CH-CO; -CH_2-CO; -CH_3-CO;-CH_3-C=C; -CH_3-C; C-CH_2-C carbons respectively peaks at 120 to 140 ppm corresponds to aromatic -CH and alkene –CH as confirmed by the standard chart of **Silverstein *et al* (2005).** The spectrum of bio-marker compound andrographolide exhibited cluster of peaks at 10 to 50 ppm; and few peaks at 60 to 85 ppm was considered as fingerprint region of andrographolide. Presence of these respective peaks in the ASAE confirms the presence andrographolide like compound and considered the region as fingerprint region of the plant ***Andrographis stenophylla***. Figures 114-117 show the NMR spectrum of extracts.

IJSER

IJSER © 202
http://www.ijser.org

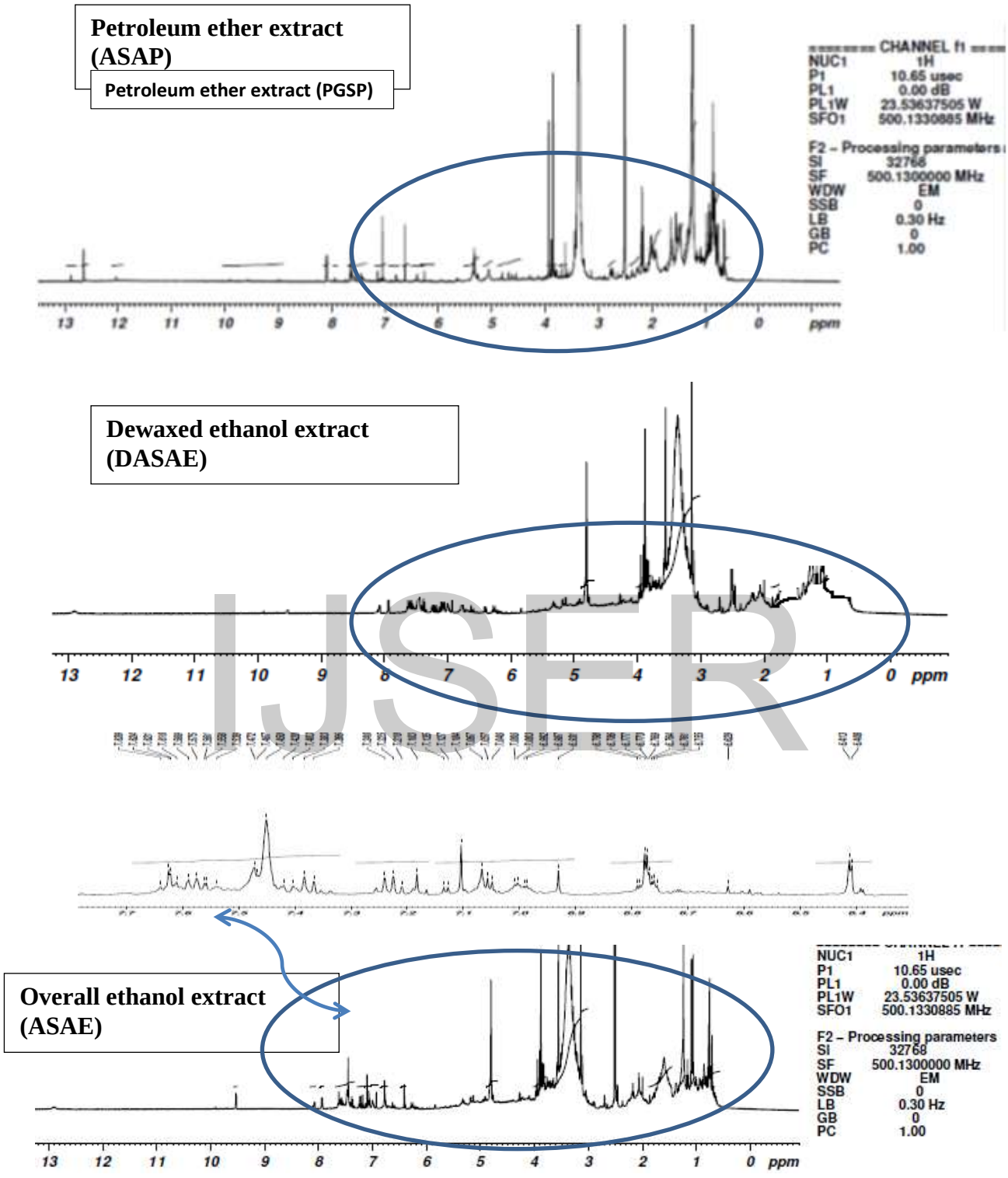


Figure 118
H[1]NMR Spectra of Aerial Parts Extract of AS

IJSER © 202
http://www.ijser.org

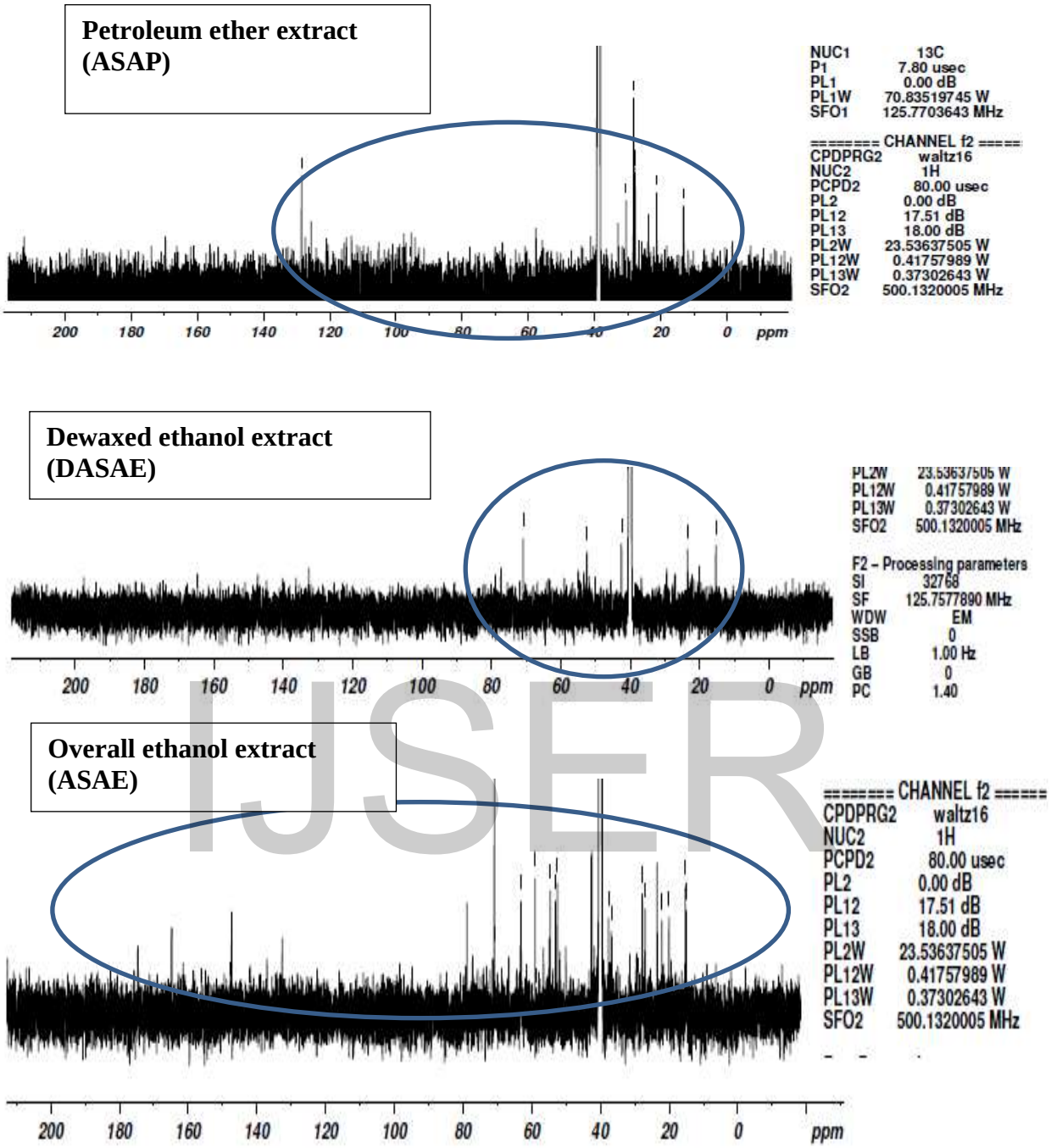


Figure 119
C^{13}NMR Spectra of Root Extracts of AS

IJSER

GC-MS Fingerprinting

GC-MS analysis of non-polar extract concentrates of chosen Plants

GC-MS analysis is the first step towards exploring the phytoconstituents present in medicinal plants. The present investigation was designed to explore the secondary metabolites present in the PGLP, PGSP, PGRP and ASAP using gas chromatography-mass spectrometry (GC-MS). The results are summarized for

IJSER © 202
http://www.ijser.org

chromatographic peaks exhibiting more than 1% peak area in the chromatogram**(Table 31-33).**GC-MS chromatograms of all the extracts are shown in the **figure120-122. Mass spectrum of major metabolites identified in PGSP, PGRP and PGLP are depicted in figures 123-127.**

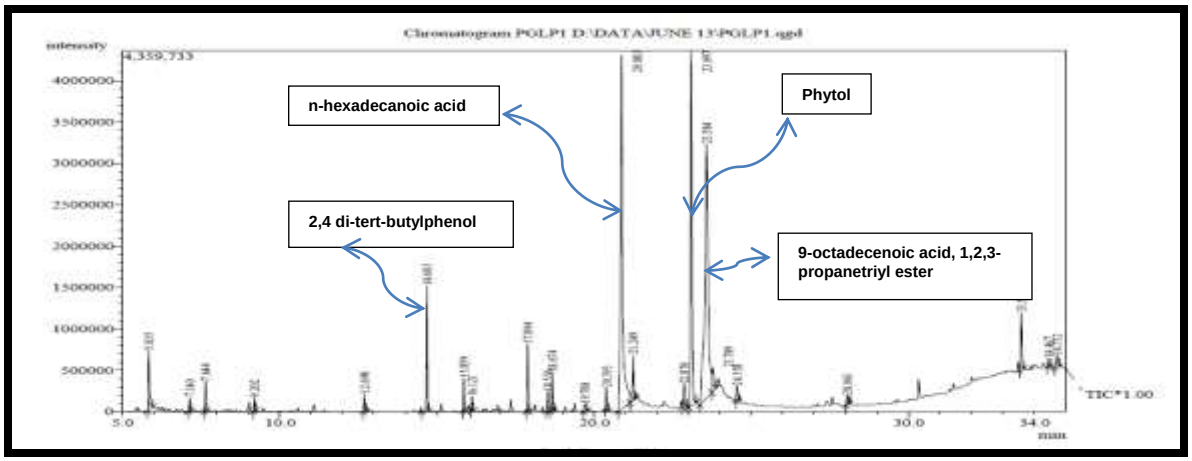


Figure 120
GC-MS chromatogram of PGLP

Table 31 Metabolites identified in PGLP by GC-MS

RT	Area %	Molecular Weight	Molecular Formula	Name of the Compound
5.83	2.64	99	C_5H_9NO	Methyl pyrrolidin
7.64	1.02	138	$C_9H_{14}O$	α- isophorone (Isoacetophorone)
14.68	3.76	206	$C_{14}H_{22}O$	2,4 di-tert-butylphenol
18.67	1.44	266	$C_{19}H_{38}$	1-nonadecene
20.39	0.98	270	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester (n-hexadecanoic acid methyl ester)
20.88	26.22	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid (Palmitic acid)
21.24	2.18	354	$C_{24}H_{50}O$	Lignoceric alcohol (1-tetracosanol)
22.87	1.19	296	$C_{19}H_{36}O_2$	9-octadecenoic acid, methyl ester
23.09	18.36	296	$C_{20}H_{40}O$	Phytol
23.58	29.35	884	$C_{57}H_{10}4O_6$	2,3-bis-(9E)-9-octadecenoyloxy]propyl(9E)-9-octadecenoate (9-octadecenoic acid, 1,2,3-propanetriyl ester)
23.78	1.55	340	$C_{20}H_{36}O_4$	2-Ethylhexyl maleate
33.59	2.45	410	$C_{30}H_{50}$	trans-Squalene (2,6,10,14,18,22tetracosahexaene,2,6,10,15,19,23-hexamethyl)

IJSER

IJSER © 202
http://www.ijser.org

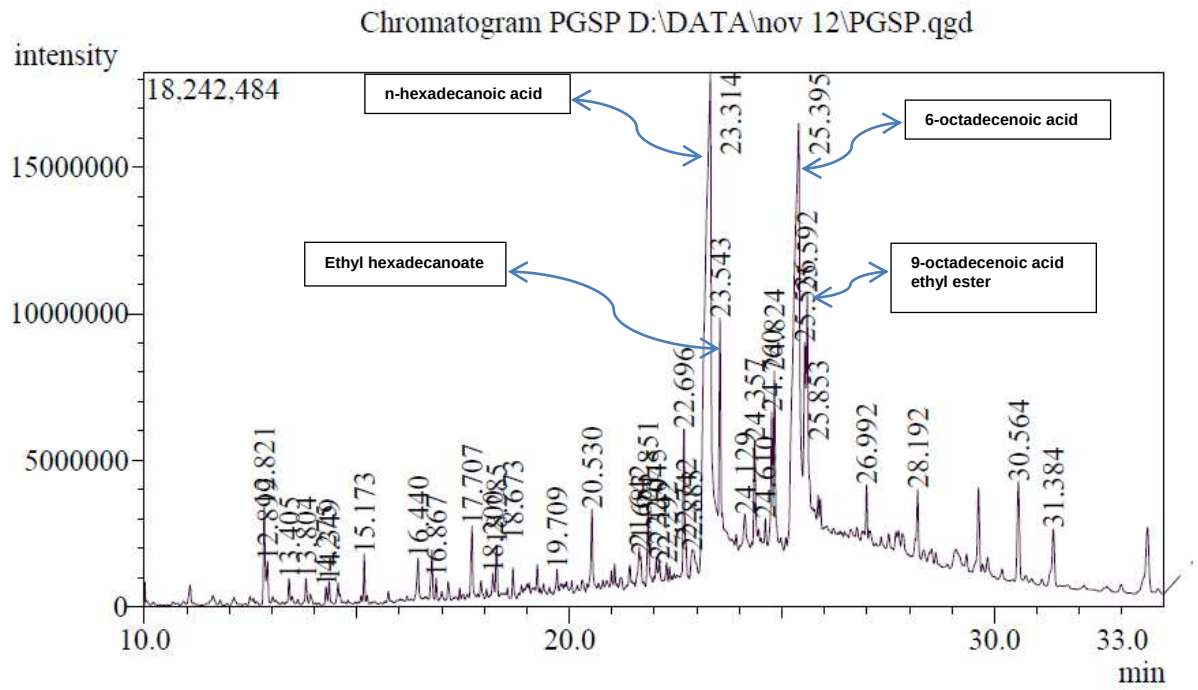


Figure 121
GC-MS chromatogram of PGSP

IJSER

Table 32 Metabolites identified in PGSP by GC-MS

RT	Area %	Molecular Weight	Molecular Formula	Name of the Compound
23.31	30.60	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid (Palmitic acid)
25.39	25.88	282	$C_{18}H_{34}O_2$	6-octadecenoic acid (Petroselenic acid)
25.59	5.11	310	$C_{20}H_{38}O_2$	9-octadecenoic acid ethyl ester (Ethyl oleate)
25.53	4.11	322	$C_{21}H_{38}O_2$	n-prpopyl-9,12-octadecadienoate
23.54	3.78	284	$C_{18}H_{36}O_2$	Ethyl hexadecanoate (Palmitic acid ethyl ester)
24.82	2.84	296	$C_{19}H_{36}O_2$	9-octadecenoic acid methyl ester (Methyl elaidate)
24.76	2.30	294	$C_{19}H_{34}O_2$	Methyl-10-trans,12-cis-octadecadienoate
22.69	2.28	270	$C_{17}H_{34}O_2$	Methyl hexadecanoate (Palmitic acid methyl ester)
30.56	2.27	279	$C_{16}H_{22}O_4$	Mono-(2-ethylhexyl)phthalate
25.85	1.84	312	$C_{20}H_{40}O_2$	Methyl-17-methyl-octadecanoate
24.35	1.71	270	$C_{17}H_{34}O_2$	Heptadecanoic acid (Margaric acid)
21.85	1.46	242	$C_{15}H_{30}O_2$	Pentadecanoic acid
17.70	1.23	200	$C_{12}H_{24}O_2$	Dodecanoic acid (Lauric acid)
20.53	1.07	228	$C_{14}H_{28}O_2$	Tetradecanoic acid (Myristic acid)

IJSER © 202
http://www.ijser.org

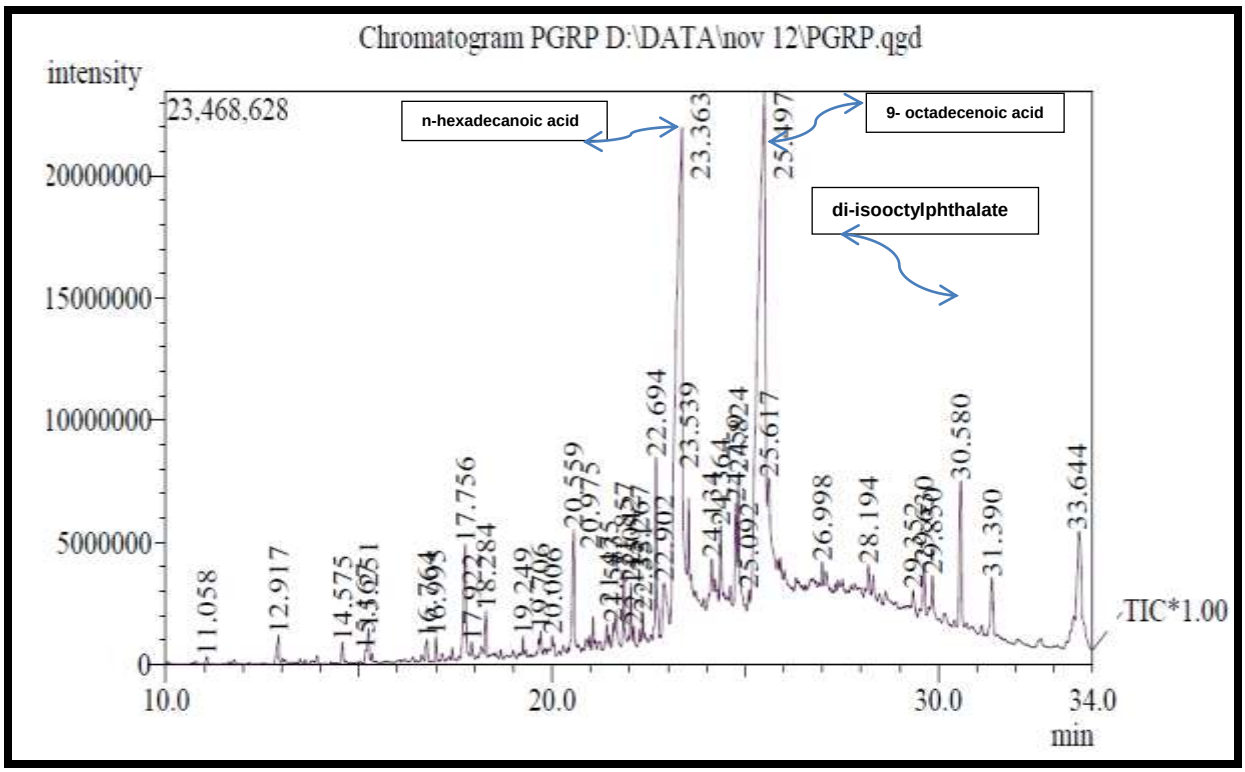


Figure 122
GC-MS chromatogram of PGRP

Table 33 Metabolites identified in PGRP by GC-MS

RT	Area %	Molecular Weight	Molecular Formula	Name of the Compound
25.49	35.50	282	$C_{18}H_{34}O_2$	9- octadecenoic acid (Oleic acid)
23.36	30.30	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid (Palmitic acid)
30.58	3.38	390	$C_{24}H_{38}O_4$	di-isooctylphthalate
33.64	3.35	404	$C_{25}H_{40}O_4$	Oxalic acid-hexadecyl 2-methylphenyl ester
17.75	2.65	200	$C_{12}H_{24}O_2$	Dodecanoic acid (Lauric acid)
22.69	2.53	270	$C_{17}H_{34}O_2$	Methyl hexadecanoate (Palmitic acid methyl ester)
20.55	2.03	228	$C_{14}H_{28}O_2$	Tetradecanoic acid (Myristic acid)
24.82	1.47	296	$C_{19}H_{36}O_2$	9-octadecenoic acid methyl ester (Methyl elaidate)
24.75	1.31	294	$C_{19}H_{34}O_2$	Methyl-10-trans,12-cis octadecadienoate (Linoleic acid methyl ester)
31.39	1.25	506	$C_{36}H_{74}$	Hexatriacontane
24.36	1.24	270	$C_{17}H_{34}O_2$	Heptadecanoic acid (Margaric acid)
21.85	1.12	242	$C_{15}H_{30}O_2$	Pentadecanoic acid
23.53	1.10	284	$C_{18}H_{36}O_2$	Ethyl hexadecanoate (Palmitic acid ethyl ester)
22.04	0.95	278	$C_{16}H_{22}O_2$	di-isobutyl phthalate

IJSER

IJSER © 202
http://www.ijser.org

Table 34 Major Metabolites identified in PGSP, PGRP and PGLP by GC-MS

Sample Code	R_t	Area(%)	MW	MF	Name of the Compound
PGSP	23.31	30.60	256	$C_{16}H_{32}O_2$	*n*-hexadecanoic acid (palmitic acid)
	25.39	25.88	282	$C_{18}H_{34}O_2$	6-octadecenoic acid (petroselenic acid)
PGRP	25.49	35.50	282	$C_{18}H_{34}O_2$	9-octadecenoic acid (oleic acid)
	23.36	30.30	256	$C_{16}H_{32}O_2$	*n*-Hexadecanoic acid (palmitic acid)
PGLP	23.58	29.35	884	$C_{57}H_{10}4O_6$	2,3-bis[(9E)-9-octadecenoyloxy]propyl (9E)-9-octadecenoate (9-octadecenoic acid, 1,2,3-propanetriyl ester)
	20.88	26.22	256	$C_{16}H_{32}O_2$	*n*-hexadecanoic acid (palmitic acid)
	23.09	18.36	296	$C_{20}H_{40}O$	Phytol

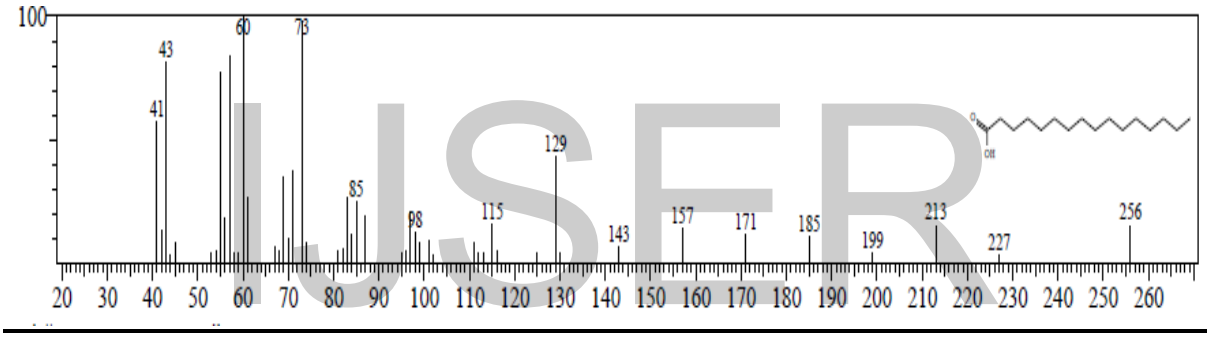


Figure 123 Mass spectrum of *n*-hexadecanoic acid (palmitic acid)

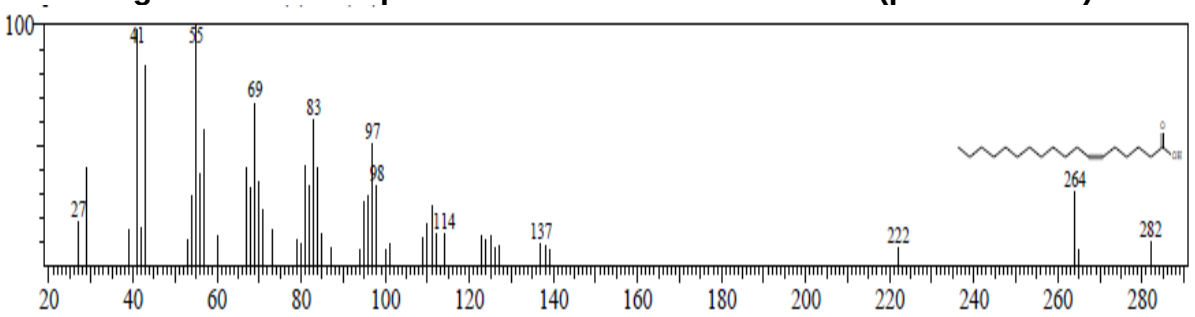


Figure 124 Mass spectrum of 6-octadecenoic acid (petroselenic acid)

IJSER

IJSER © 202
http://www.ijser.org

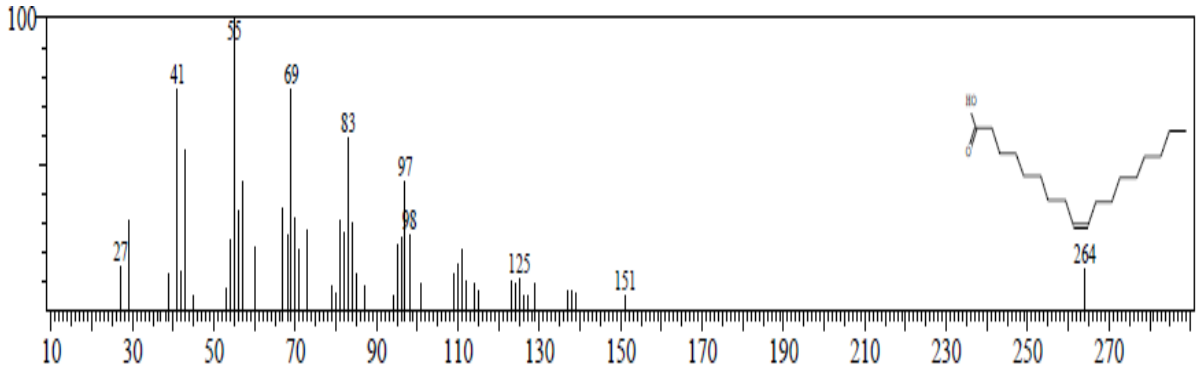


Figure 125 Mass spectrum of 9-octadecenoic acid (oleic acid))

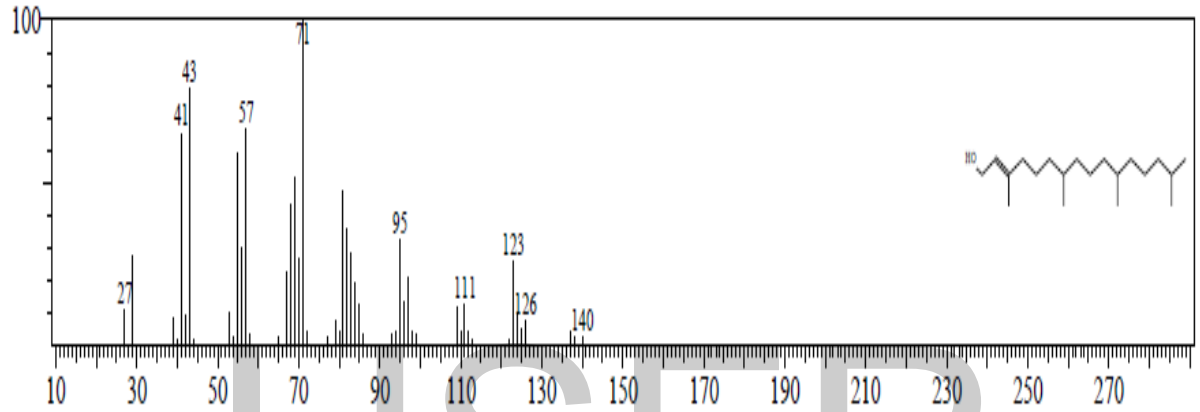


Figure 126 Mass spectrum of Phytol

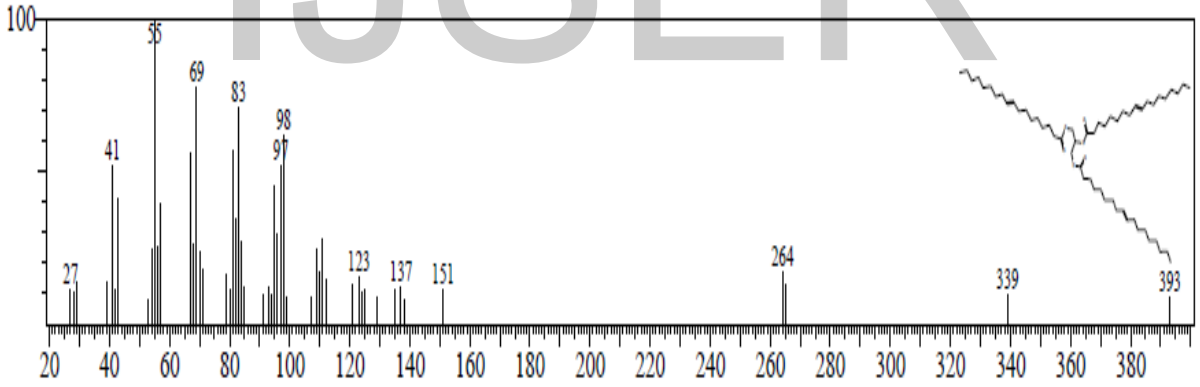


Figure 127 Mass spectrum 9-octadecenoic acid, 1, 2, 3- propanetriyl ester

Metabolite analysis of the petroleum ether extract concentrates of leaves, stems and roots of ***Pisonia grandis*** revealed that n-hexadecanoic acid (palmitic acid) is the most prevalent phytoconstituent in all the three extracts. Peak area percentage of palmitic acid in the chromatograms of PGSP, PGRP and PGLP are 30.60 %; 30.30 %and 26.22 %.Palmitic acid has been reported to possess anti-inflammatory activity**(Aparna *et al*., 2012)** and it finds use in the Ayurvedic system of medicine as a component of medicated oil used to treat rheumatism. This fact validates the traditional use of ***Pisonia grandis*** as an anti-arthritic agent.

IJSER © 202
http://www.ijser.org

GC-MS analysis of **Andrographis stenophylla** was designed to explore the secondary the metabolites present in its extracts. The results are summarized for chromatographic peaks exhibiting more than 1% peak area in the chromatogram **(Table 35).**GC-MS chromatograms of ASAP is shown in the **figures 128.** The most prevailing phytoconstituents present in the extract are chondrillasterol, stigmasterol, tetrapentacontane, n-hexadecanoic acid and dotriacontane. Mass spectrum of major metabolites identified in ASAP are shown in figures 129-132.

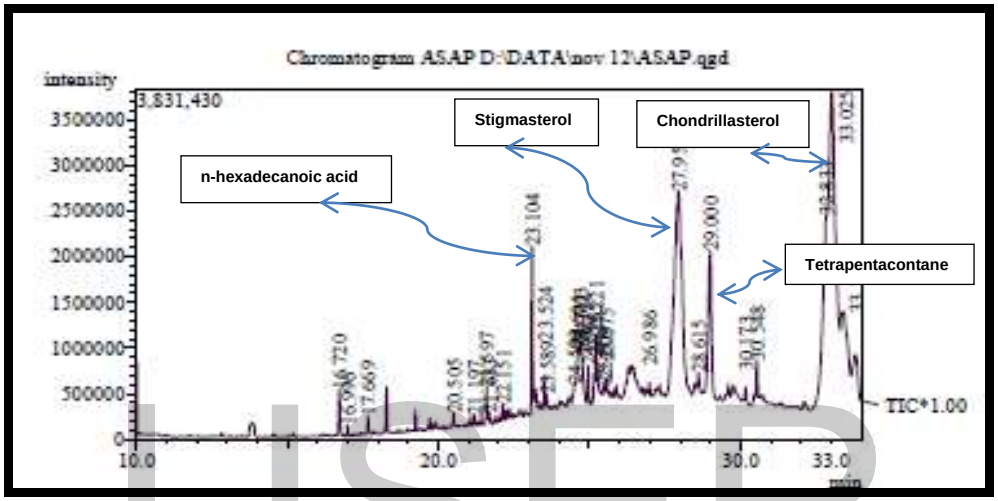


Figure 128

GC-MS chromatogram of ASAP

IJSER

Table 35 Metabolites identified in ASAP by GC-MS

RT	Area %	Molecular Weight	Molecular Formula	Name of the Compound
33.02	30.58	412	$C_{29}H_{48}O$	Chondrillasterol
27.95	24.49	412	$C_{29}H_{48}O$	Stigmasterol
32.83	13.35	412	$C_{29}H_{48}O$	Stigmasta-7,25-dien-3ol
29.00	7.44	758	$C_{54}H_{110}$	Tetrapentacontane
33.36	6.97	414	$C_{29}H_{50}O$	Stigmast-5-en-3ol(3-beta)
23.10	3.32	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid (Palmitic acid)
24.70	2.18	450	$C_{32}H_{66}$	Dotriacontane
25.22	1.91	282	$C_{18}H_{34}O_2$	9-octadecenoic acid
24.74	1.58	268	$C_{19}H_{40}$	Nonadecane
24.60	1.10	228	$C_{15}H_{32}O$	n-Pentadecanol
30.54	0.82	279	$C_{16}H_{22}O_4$	Mono(2-ethylhexyl)phthalate

IJSER © 202
http://www.ijser.org

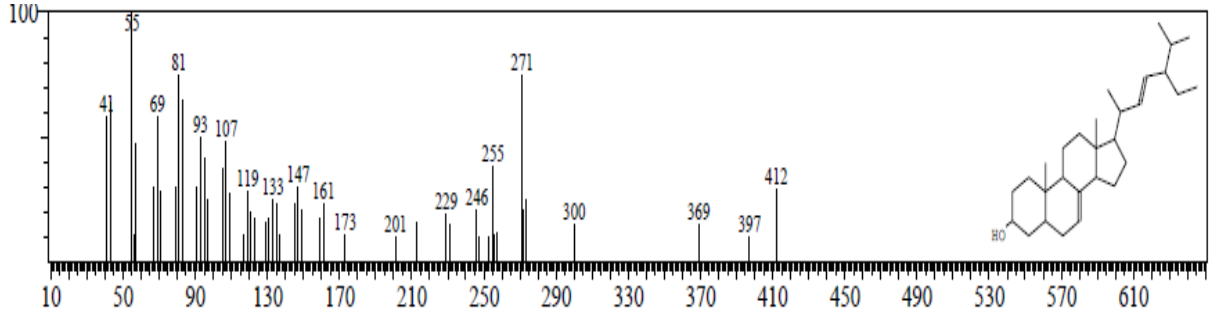


Figure 129 Mass spectrum of Chondrillasterol

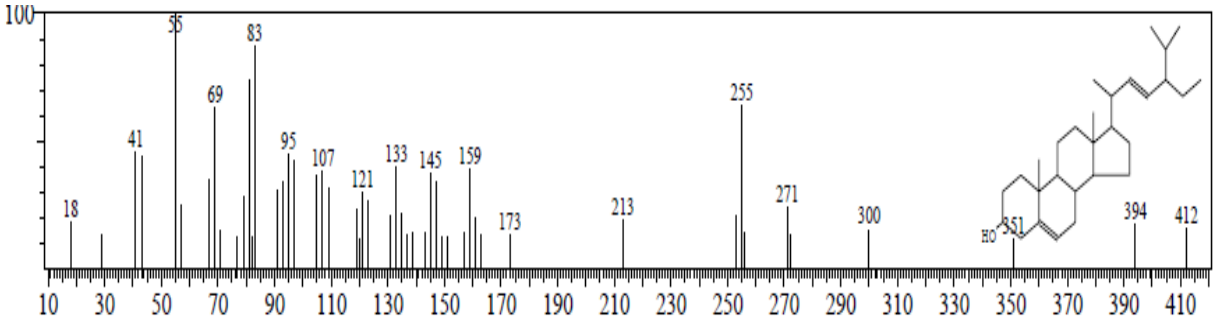


Figure 130 Mass spectrum of Stigmasterol

IJSER

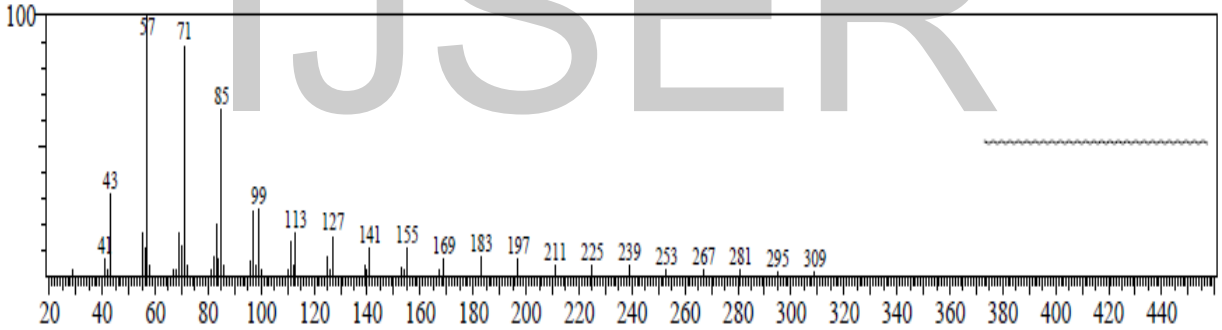


Figure 131 Mass spectrum of Tetrapentacontane

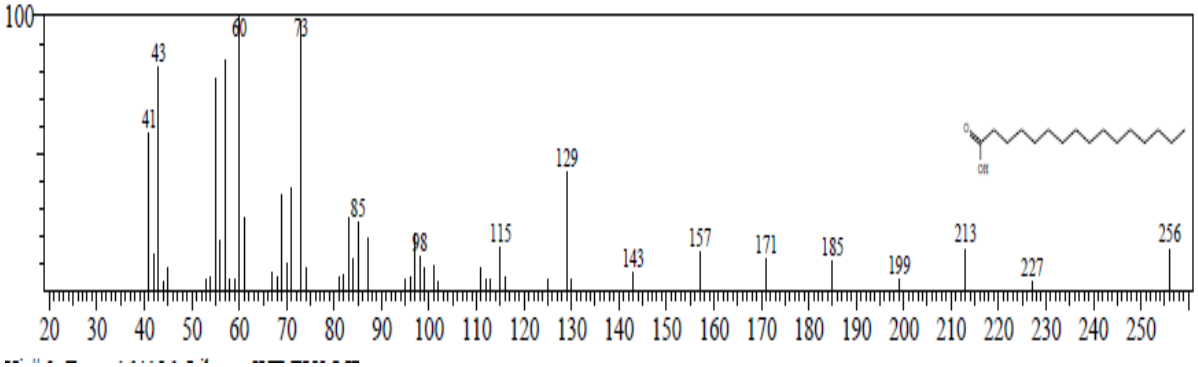


Figure 132 Mass spectrum of n-hexadecanoic acid

IJSER © 202
http://www.ijser.org

Among the identified phytochemicals oleic acid, pentadecanoic acid, margaric acid and myristic acid, lauric acid, phytol and palmitic acid are pharmacologically active compounds. Oleic acid is found to have anti-inflammatory, anticancer, antialopecic, antiandrogenicproperties. Pentadecanoic acid, margaric acid and myristic acid are good antioxidants. Lauric acid is used for treating viral infections including swine flu and avian flu. It is also used for preventing the transmission of HIV from mothers to children. Antimicrobial potential was well established by lauric acid. Phytol and myristic acid are cancer preventers**(www.ars-grin.gov/duke)**. Stigmasterol was found to inhibit the growth of the tumour. It is used as precursor in manufacturing of synthetic progesterone (**pbs.org 2007, lipidlibrary2010)**. Existence of these molecules validates the traditional usage of the plant ***Pisonia grandis*** and ***Andrographis stenophylla*** by tribals in the preparation of several folk medicines

Chemical Standardization

Quantitation of Bio-actives in various extracts of *Pisonia grandis* by HPLC

IJSER

HPLC is a powerful tool in analytical chemistry because of its high accuracy and precision. In HPLC, a liquid or a solid sample dissolved in suitable solvent is carried into a column by a liquid mobile phase. Selection of column and mobile phase determines the efficacy of separations in HPLC. A reversed-phase high performance liquid chromatography (RP-HPLC) is the most common system in HPLC where the stationary phase is non-polar and the mobile phase is polar. RP-HPLC method with RI detection has been developed for the determination of pinitol. In the current investigation Luna 5μ amine column (100°A); acetonitrile : water (30:70); refractive index detector was used for a satisfactory separation. Linearity of the method was proved by analysing various concentrations of the standard. Figure 133-138 depicts the HPLC profile of standards and samples. Table 36 illustrate the percentage concentration of pinitol in extract concentrates of leaves stems and roots of ***Pisonia grandis.***Figure140 is the histogram representing the pinitol content in the extracts. Quantity of pinitol in extract concentrates of leaves stems and roots of ***Pisonia grandis*** is in the order of DPGSE>DPGRE>PGSE>PGLE>PGRE>DPGLE>PGSAQ>PGRAQ.

IJSER © 202
http://www.ijser.org

Table 36 Quantity of Pinitol in various extracts of *Pisonia grandis*

	Sample Detail	R_t	Area	Quantity of Pinitol (µg/ 100 µg of extract)
STD	Standard @ 100 µg	6.2	687529	-
STD	Standard @ 200 µg	6.17	1355058	-
STD	Standard @ 400 µg	6.17	3128388	-
STD	Standard @ 600 µg	6.15	4447700	-
PGLE	Over all leaf extract	6.3	237613	14.35
D.PGLE	De-wax leaf extract	6.25	91741	8.02
PGLAQ	Aqueous extract of leaf	ND	ND	ND
PGSE	Over all stem extract	6.12	272712	15.87
D.PGSE	De-wax stem extract	6.25	381894	20.60
PGSAQ	Aqueous extract of stem	6.24	71689	7.15
PGRE	Over all root extract	6.14	146953	10.41
D.PGRE	De-wax root extract	6.23	322749	18.04
PGRAQ	Aqueous extract of root	6.26	51430	6.27

IJSER

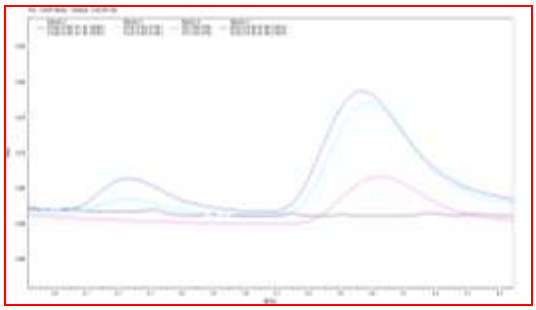


Fig 133. Chromatogram of extract of leaves of PG

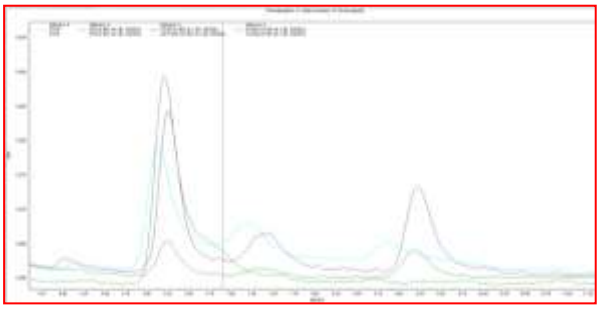


Fig.134. Chromatogram of extract of stems of PG

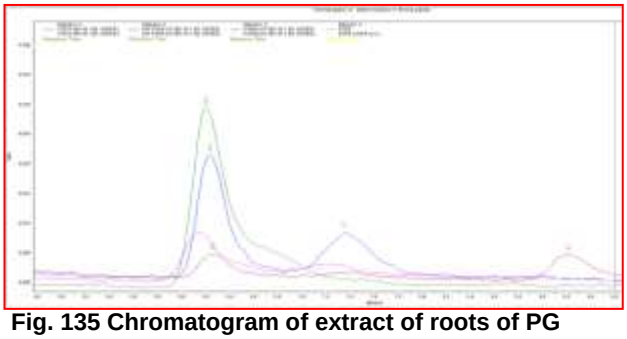


Fig. 135 Chromatogram of extract of roots of PG

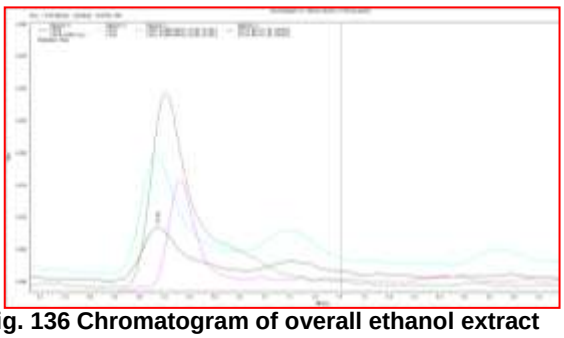
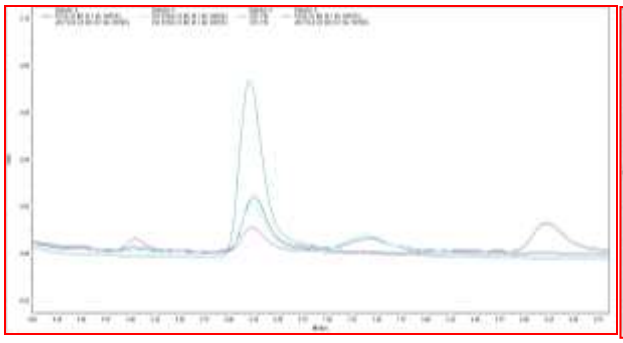


Fig. 136 Chromatogram of overall ethanol extract

IJSER © 202
http://www.ijser.org

of PG



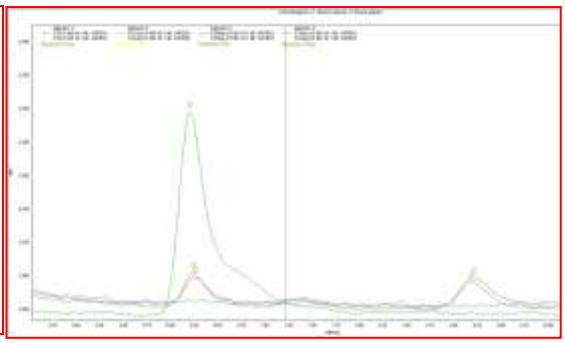
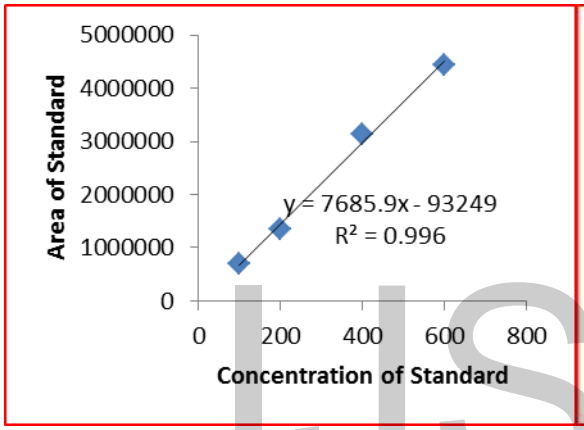
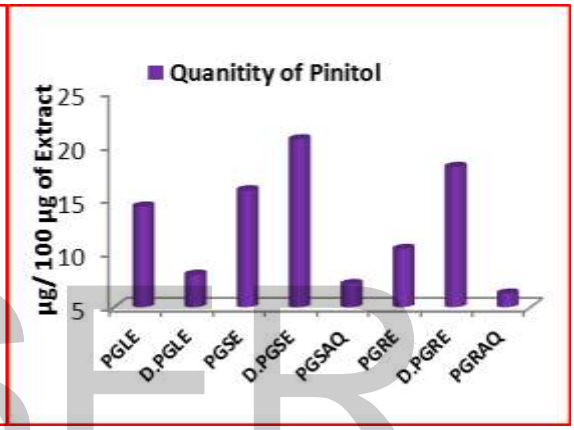


Fig.137 Chromatogram of dewaxed ethanol extract concentrates leaves stems and roots of PG

Fig.138 Chromatogram of aqueous extract concentrates of leaves stems and roots of PG





IJSER

Fig.139 Linearity graph for standard

Fig.140 Quantity of pinitol in extracts

Data analysis depicted that out of the extracts analysed the quantity of pinitol was more in DPGSE and thus found to be a good source of pinitol. Quantity of pinitol in overall ethanol extract concentrates of leaves stems and roots of ***pisonia grandis*** were found to be in the order of PGSE>PGLE>PGRE. Quantity of pinitol in sequential extracts was found to be DPGSE>DPGRE>DPGLE>PGSAQ>PGRAQ. The result of this study indicated that stems of ***Pisonia grandis***are the good source of pinitol.

Pinitol has immense pharmacological significance. It is bestowed with anti-diabetic **(Ajuah *et al*., 2000)**, anti-inflammatory **(Singh *et al*., 2001)**, antioxidant **(Orthen *et al*., 1994)** and immunosuppressive potential **(Chauhan *et al*., 2011)** and is used in the treatment of hypertension, rheumatism, cardiovascular diseases, AIDS and neurological disorders **(Ostlund *et al*., 1996, and Kim *et al*., 2005)** .Existence of pinitol in ***Pisonia grandis*** validates the traditional usage of this plant for diabetic.

Quantitation of Allantoin by HPLC

IJSER © 202
http://www.ijser.org

A reverse phase HPLC method with PDA detection has been developed for the determination of allantoin. In the current investigation ODS C18 column; acetonitrile: phosphate buffer (20:80) and photodiode array detector (PDA) was used for a satisfactory separation. Linearity of the method was proved by analysing various concentrations of the standard. Figures141-146depicts the HPLC profile of extracts. Table 37 illustrate the percentage concentration of allantoin in extract concentrates of leaves stems and roots of ***Pisonia grandis.*** Data analysis depicted that out of the extracts analysed the quantity of allantoin was more in DPGSE and thus found to be a good source of allantoin. Quantity of allantoin in overall ethanol extract concentrates of leaves stems and roots of ***pisonia grandis*** were found to be in the order of PGSE>PGLE>PGRE. Quantity of allantoin in sequential extracts was found to be DPGSE>DPGLE >PGSAQ> DPGRE >PGRAQ. The result of this study indicated that stems of ***Pisonia grandis*** are the good source of allantoin.

Table 37 Quantity of Allantoin in various extracts of *Pisonia grandis*

Sample Code	R_t	Area/20 µg	Quantity of Allantion (20 µg) of extract	Quantity of Allantion (100 µg) of extract
STD(Standard 10 µg)		Area: 35966869		
PGLE	5.6	15158584	4.210	21.05
DPGLE	5.6	18645823	5.180	25.90
DDPGLE	5.6	14736604	4.100	20.50
PGSE	5.6	13282518	3.690	18.45
DPGSE	5.6	19177817	5.330	26.62
PGSAQ	5.6	8004746	2.220	11.01
PGRE	5.8	2331842	0.650	3.250
DPGRE	5.6	1328641	0.370	1.850
PGRAQ	5.6	1498967	0.420	2.100

IJSER

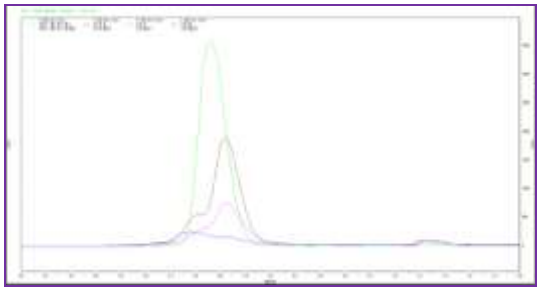


Fig.141 Chromatogram of extract concentrates of leaves of PG

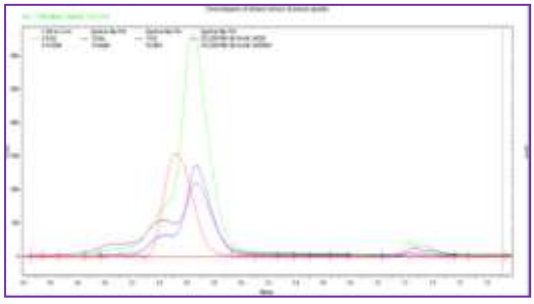


Fig.142. Chromatogram of extract concentrates of stems of

IJSER © 202
http://www.ijser.org

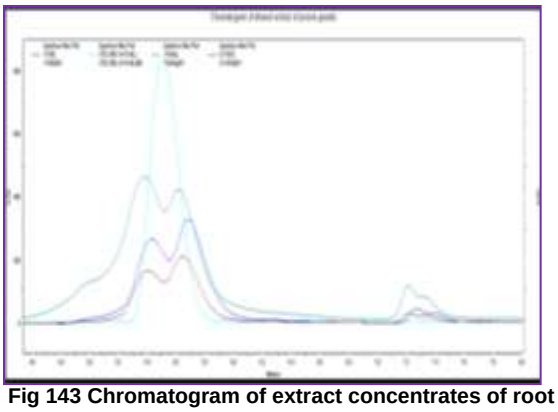
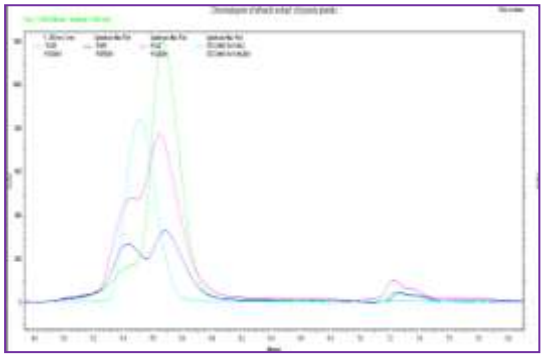


Fig 143 Chromatogram of extract concentrates of root of PG



PG Fig 144 Chromatogram of ethanol extract concentratesof PG

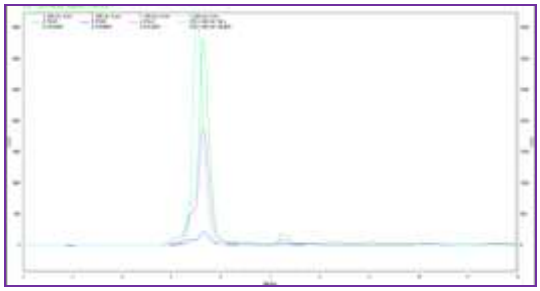


Fig 145 Chromatogram of dewaxed ethanol extract of PG

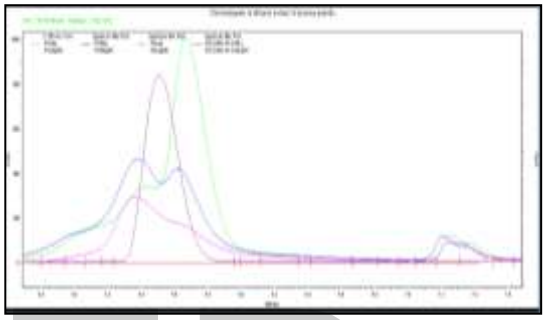


Fig 146 Chromatogram of aqueous extract of PG

IJSER

Allantoin is bestowed with immense pharmacologicalsignificance. The U.S. Food and Drug Administration (FDA) confirmed that allantoin is a safe and effective skin protectant in the recommended dosage range of 0.5 to 2.0% **(Federal Register)**.reported to be a free radical scavenger **(Guskov *et al*., 2002)** and wound healer **(Ranson 1984., and Araújo *et al*., 2010)**. It reduces plasma glucose in streptozotocin-induced diabetic rats **(Shan Niu *et al*., 2010)**. Anti-inflammatory formulations **(Koho, 1984)**, antimicrobial dressings **(Sai,1983)**, medicines that are used for treating gastroduodenal ulcer and chronic gastritis **(Dobrescu, 1998)** and ointments for treating plaque and psoriasis **(Pinheiro, 1997)**contain allantoin as one of the foremost ingredients. Existence of allantoin in ***Pisonia grandis*** validates the traditional usage of this plant by tribals.

Quantitation of Andrographolide in *Andrographis stenophylla*

A reverse phase HPLC method with PDA detection has been developed for the determination of andrographolide. In the current investigation ODS C18 column;

IJSER © 202
http://www.ijser.org

acetonitrile: water (30:70) and photodiode array detector (PDA) was used for a satisfactory separation. Linearity of the method was proved by analysing various concentrations of the standard. Figure 147 depicts the HPLC profile of standard and samples. Table 38illustrate the percentage concentration of andrographolide in extract concentrates of aerial parts of ***Andrographis stenophylla.*** Figure 148 is the calibration curve drawn for proving the linearity of the method. Data analysis depicted that out of the extracts analysed the quantity of andrographolide was more in DASAE and thus found to be a good source of andrographolide. The result of this study indicated that ethanol extract concentrates and dewaxed extract concentrates of ***Andrographis stenophylla*** are the good source of andrographolide.

Table 38 Quantity of Andrographolide in various extracts of *Andrographis stenophylla*

Sample Code	Sample Detail	R_t	Area	Quantity of Andrographolide (µg/ 100 µg of extract)
STD	Standard @ 10 µg	4.76	306603	-
STD	Standard @ 20 µg	4.70	647106	-
STD	Standard @ 30 µg	4.56	948902	-
STD	Standard @ 40 µg	5.05	1380218	-
STD	Standard @ 50 µg	4.80	1673846	-
ASAE	Over all extract of aerial parts	4.68	1048289	31.64
DASAE	De-waxed extract of aerial parts	4.61	1314569	39.32
ASAAq	Aqueous extract of aerial parts	4.82	342497	11.29

IJSER

IJSER © 202
http://www.ijser.org

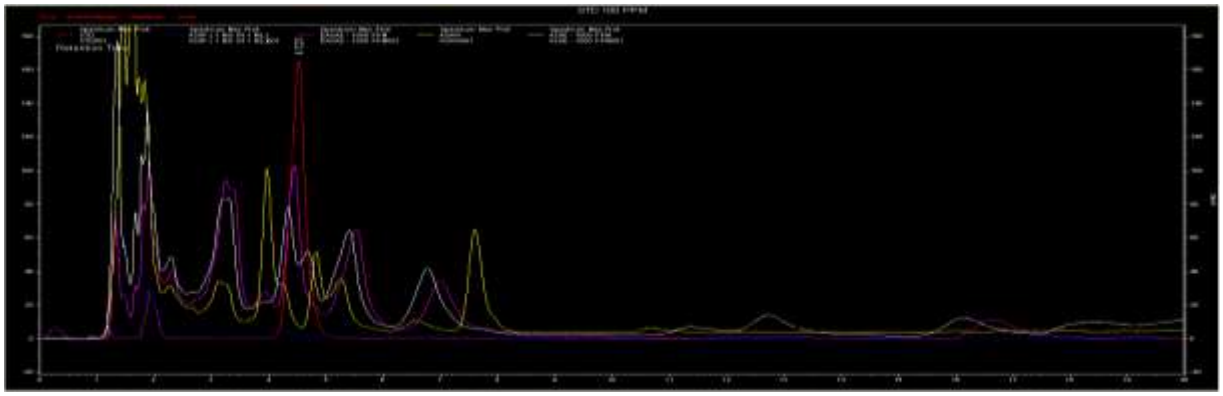


Fig. 147 HPLC chromatogram of extract concentrates of AS

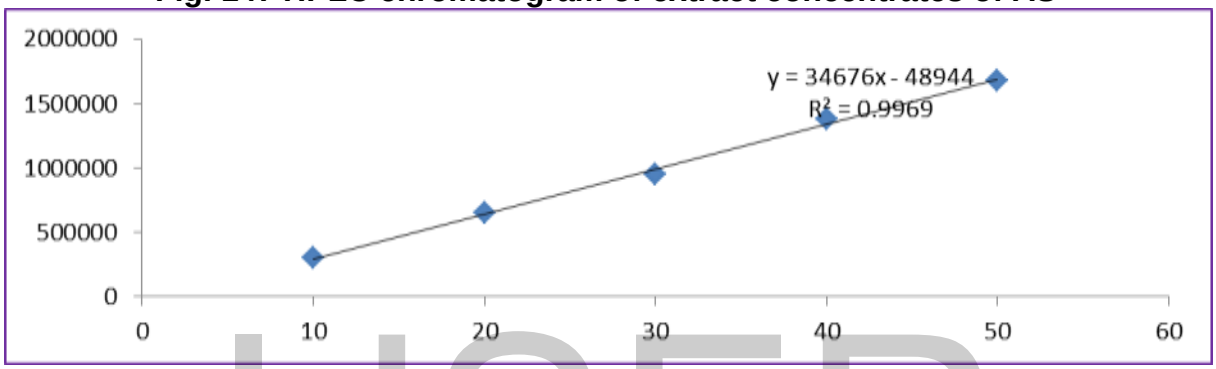


Figure 148 Linearity graph for standard

IJSER

Andrographolide exhibits various biological activities. It is bestowed with anti-inflammatory (**Woan-Ruoh *et al*., 2012**), anti-diabetic (**Zhang *et al*., 2013**), anti-oxidant (**Soumya *et al*., 2010**) anti-tumour (**Das*et al*., 2012**), anti-ulcer (**Saranya *et al*., 2011**) and anti-arthritic activities (**Parichatikanond *et al*., 2010**). A report on neuroprotective effects on Parkinson disease by andrographolide was well established **(Zaijuin *et al*., 2014).**Existence of andrographolide in ***Andrographis stenophylla*** validates the traditional usage of this plant by tribals.

Biological Standardization

The various extract concentrate of the plants ***Pisonia grandis*** and ***Andrographis stenophylla*** were standardized by biological activity studies. The biological activity studies may be considered as biological fingerprints of the extracts of these plants.

Activity studies carried out for the extracts of ***Pisonia grandis***

- Anti-fungal study
- *in-vitro*anti-oxidant activity
- *in-vitro* cytotoxicity study
- *in-vitro* wound healing study
- *in-vitro* anti-arthritic study

IJSER © 202
http://www.ijser.org

- *in-vivo* wound healing study

Activity studies carried out for the extracts of ***Andrographis stenophylla***

- Anti-fungal study
- *in-vitro*anti-oxidant activity
- *in-vitro* cytotoxicity study
- *in-vitro* wound healing study
- *in-vitro* anti-arthritic study

Anti-fungal assay of extract concentrates and their solvent fractionates

The extract concentrates and their fractionates, combined column fractions and isolated compounds of both plants were screened for their antifungal activity against microbes ***Aspergillus niger, Candida albicans*** and ***Monoscus purpureus*** by disk diffusion method**. Table 39** gives the zone of inhibition values for the extracts concentrate.

Table 39 Anti-fungal assessment of extract concentrates

Sample Code	Zone of Inhibition (mm)		
	Aspergillus niger	***Candida albicans***	***Monoscus purpureus***
Standard*	15	13	16
PGLE	09	07	10
PGLP	10	08	10
DPGLE	07	10	**15**
PGLAq	**11**	**12**	10
PGSE	**16**	**14**	**14**
PGSP	**12**	**12**	09
DPGSE	08	09	09
PGSAq	10	08	08
PGRE	**14**	**12**	**15**
PGRP	**12**	**11**	10
DPGRE	10	10	08
PGRAq	09	10	08
ASAE	09	08	08
ASAP	09	10	10
DASAE	10	**12**	**12**
ASAAq	**12**	08	10

*Standard = Clotrimazole (10µg/ Disk); Extracts = 100µg/ Disk

The graph represent below (Figure 149) depicts the variation in anti-fungal potential of the various extracts

IJSER © 202
http://www.ijser.org

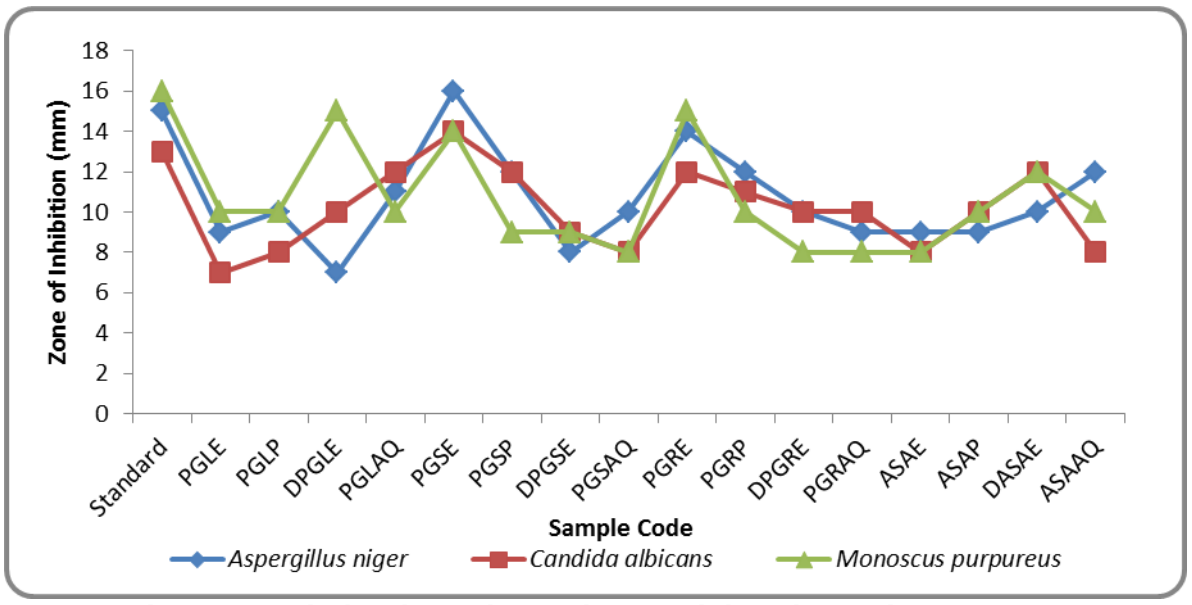


Fig. 149 Variation in antifungal potential of the various extract

Table 40 Anti-fungal assessment of extract fractionates of leaf, stem and roots of *Pisonia grandis*

Sample code	Zone of Inhibition (mm)								
	Aspergillusniger			***Candida albicans***			***Monoscuspurpureus***		
	Leaf	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**
Standard*	**11**			**12**			**15**		
PGLE, PGSE, PGRE	09	**16**	**14**	07	**14**	**12**	**25**	**14**	**15**
PGLC, PGSC, PGRC	10	**14**	13	10	10	**13**	**20**	11	12
PGLW1, PGSW1, PGRW1	12	**13**	11	12	**14**	09	**15**	10	10
PGLW2, PGSW2, PGRW2	08	**12**	10	**14**	06	09	**20**	11	09
PGLP,PGSP,PGRP	12	**14**	09	**06**	**11**	**12**	**12**	10	09

*Standard = Clotrimazole (10µg/ Disk); Extracts = 100µg/ Disk

IJSER

IJSER © 202
http://www.ijser.org

Table 41 Anti-fungal assessment of extract fractionates of aerial parts of *Andrographis stenophylla*

Sample code	Zone of Inhibition(mm)		
	Aspergillu sniger	***Candida albicans***	***Monoscuspu rpureus***
Standard*	**13**	**10**	**11**
ASAE	09	08	08
ASAC	09	08	**10**
$ASAW_1$	08	07	08
$ASAW_2$	**12**	08	**09**
ASAP	10	**09**	**09**

*Standard = Clotrimazole (10µg/ Disk); Extracts = 100µg/ Disk

Tables 40 & 41 represent the zone of inhibition values for the various solvent fractionates of the concentrated extract of the chosen plants respectively. The stems of *Pisonia grandis* are found to exhibit better anti-fungal potential than the leaves and roots of *Pisonia grandis*.

Antifungal Assay of Combined Column Fractions and Isolated Compounds

The column eluates of dewaxed ethanol extract concentrate of stems of *Pisonia grandis,* and the column elutes of methanol soluble portion of aqueous extract concentrate of stems of *Pisonia grandis* and that of dewaxed ethanol extract concentrate of aerial parts of *Andrographis stenophylla* were screened for anti-fungal assay against ***Aspergillus niger, Candida albicans*** and ***Monoscus purpureus along with respective isolated compounds.*** The resultsare tabulated (table 42)

The results show that the column eluates PGSEF1 to F5 exhibit notable activity against Candidaalbicans comparable to that of standard clotrimazole. Fraction PGSEF6 and the respective compound PGS-2 isolated from it express antifungal potential against all the three microbes and comparable to that of standard.

The column extracts PGSEF6 to F14 express notable activity against ***Aspergillusniger***comparable with that of standard. Allantoin isolated from PGSEF7 and pinitol isolated from PGSEF9 are found to be the bioactive constituents of the fractions. Table below gives the zone of inhibition data for the anti-fungal assay **of column elutes of dewaxed ethanol extract of stems of *Pisonia grandis***.

IJSER

IJSER © 202
http://www.ijser.org

Table 42 Anti-fungal assessment of column eluates of dewaxed ethanol extract of stems of *Pisonia grandis* (DPGSE)

Sample code	Zone of Inhibition (mm)		
	Aspergillusniger	***Candida albicans***	***Monoscuspurpureus***
Standard	**14**	**15**	**12**
DPGSE	**08**	**09**	**09**
PGSEF1	09	**12**	09
PGSEF2	09	**12**	09
PGSEF3	08	**13**	08
PGSEF4	09	**12**	09
PGS-1 Isolated gel	08	09	07
PGSEF5	09	**11**	09
PGSEF6	**11**	**11**	**11**
PGS-2 (Stigmasterol glucoside)	10	09	10
PGSEF7	09	10	09
PGS-3 (Allantoin)	**11**	08	07
PGSEF8	09	10	09
PGS-4 (Complex)	08	09	07
PGSEF09	**11**	07	07
PGS-5 (Pinitol)	10	09	07
PGSEF10	**12**	07	07
PGSEF11	10	08	06
PGS-6 (sugar like)	09	09	06
PGSEF12	10	07	07
PGSEF13	**11**	09	08
PGSEF14	10	09	07
PGSEF15	08	09	06
PGSEF16	10	10	09

*Standard = Clotrimazole (10µg/ Disk); Extracts = 100µg/ Disk

IJSER

Table 43gives the zone of inhibition details for the anti-fungal assay of column eluates of methanol soluble portion of aqueous extract of stems of *Pisonia grandis*

Sample code	***Zone of Inhibition (mm)***		
	Aspergillusniger	***Candida albicans***	***Monoscuspurpureus***
Standard	***14***	***15***	***12***
PGSAq	**10**	**09**	**10**
PGSAF1	10	08	**11**
PGSAF2	08	**11**	**15**
PGSAF3	09	**12**	**14**
PGSAF4	09	10	**11**

IJSER © 202
http://www.ijser.org

PGSAF5	09	08	**10**
PGSAF6	10	07	**11**
PGSAF7	10	06	09
PGS-7	10	07	07
PGSAF8	**11**	07	10
PGSAF9	10	07	06
PGS-8	09	06	07
PGSAF10	08	10	10
PGS-9	09	07	08
PGSAF11	10	07	07
PGSAF12	**12**	07	09
PGSAF13	**11**	06	09
PGSAF14	**11**	06	**11**

*Standard = Clotrimazole (10µg/ Disk); Extracts = 100µg/ Disk

Column eluates of dewaxed ethanol extract concentrate of aerial parts of *Andrographis stenophylla* and the isolated compounds showed good inhibition against ***Monoscuspurpureus.***ASEF69 and ASEF153 showed inhibition against ***Candida albicans.*** ASEF7 and its isolate **ASC-1** both showed good activity against ***Aspergillus niger***. It is evident that the presence of isolated compound **ASC-1 characterized as 5-hydroxy-7,8-dimethoxy flavone** in the fractionASEF7 may be the probable cause for its activity. Zone of inhibition of all fractions and the isolated compound is tabulated in table 44.

IJSER

Table 44Anti-fungal assessment of column eluates of dewaxed ethanol extract of aerial parts of *Andrographis stenophylla*

SampleCode	*Zone of Inhibition (mm)*		
	Aspergillusniger	***Candida albicans***	***Monoscuspurpureus***
Standard	***14***	***15***	***12***
ASEF3	07	10	**15**
ASEF5	07	10	**17**
ASEF7	**10**	07	10
ASC-1 5-hydroxy-7,8 dimethoxy flavone	**10**	10	**14**
ASEF12	08	07	**13**
ASEF30	08	08	**11**
ASC-2 5,2 'dihydroxy-7methoxy flavone	08	09	**19**
ASEF69	09	**12**	**15**
ASEF96	07	09	**12**
ASEF133	07	07	**11**
ASEF153	10	**13**	**16**
ASEF199	08	07	**14**

IJSER © 202
http://www.ijser.org

ASC-3 Andrographolide	07	09	**14**
ASC-4 Acetylated-5-hydroxy,7-methoxy-2'-glycoflavone	07	07	**15**
ASEF225	06	08	**12**
ASEF279	07	07	09
ASC-5 5-hydroxy,7-methoxy-2'-glycoflavone	09	07	**15**
ASEF342	09	07	**13**

*Standard = Clotrimazole (10µg/ Disk); Extracts = 100µg/ Disk

***in-vitro*antioxidant activity**

DPPH Radical Scavenging Assay

There are a numbers of reports on the anti-oxidant potential of the leaves of ***Pisonia grandis***. This study was aimed at comparing the antioxidant potential of leaves of ***Pisonia grandis***withthat of its stems and roots by **DPPH Radical Scavenging Assay.** The anti-oxidant potential of ***Andrographis stenophylla*** was analysed by standard method (**Nikhat *et al.,* 2009)**. Percentage inhibition increase as the concentration increase. At a concentration of 50 **µg/mL**the dewaxed stem ethanol concentrateDPSE snowed highest activity(48.91). The same was the trend in the activity of PGRP. At 90 **µg/mL**the DPGSE showed 80 % inhibition. The pet ether extracts showed very low anti-oxidant potential also the aqueous extract concentrate. In the case of DASAE the ethanol extract shows the highest activity at 90

Table 45 Percentage Inhibition of DPPH radical by extracts of *Pisonia grandis*

Drug (µg/mL)	% Inhibition in scavenging DPPH radical											
	PGLE	**PGSE**	**PGRE**	**PGLP**	**PGSP**	**PGRP**	**DPGLE**	**DPGSE**	**DPGRE**	**PGLAQ**	**PGSAQ**	**PGRAQ**
10	1.46	3.14	1.24	2.18	4.02	3.14	4.12	9.36	3.56	1.11	Negative	1.15
30	9.42	7.46	4.68	7.46	16.01	12.43	16.63	25.64	12.32	5.85	2.65	5.32
50	19.19	17.43	7.02	10.43	24.01	26.38	32.06	48.91	27.4	13.19	3.56	12.43
70	25.92	24.23	10.23	15.42	32.98	42.32	48.42	63.02	42.74	20.96	5.87	15.98
90	34.89	31.96	15.68	21.09	42.67	61.27	66.25	80.14	58.95	24.38	8.76	22.98
IC_{50} value	126	134	8109	218	106	78	70	54	78	168	9690	188

Radical 2,2-diphenyl-1-picrylhydrazine = DPPH

IJSER © 202
http://www.ijser.org

Table 47Percentage Inhibition of DPPH radical by extracts of *Andrographis stenophylla*

Drug (µg/mL)	% Inhibition in scavenging DPPH radical			
	ASAE	ASAP	DASAE	ASAAq
10	6.52	Negative	5.97	Negative
30	18.56	Negative	19.00	3.72
50	35.42	1.11	38.32	6.89
70	47.81	2.34	51.94	11.43
90	64.25	5.87	66.23	14.87
IC_{50} value	70	6980	68	270

Radical 2,2-diphenyl-1-picrylhydrazine = DPPH

*In-vitro*Cytotoxicity Assay

*in-vitro*cytotoxicity of extract concentrates of ***Pisonia grandis*** and ***Andrographis stenophylla*** was analysedby the tryphan blue exclusion method using cell DLA cell linesand the results are presented here. Table 48 and 49 depicted that the potential of percentage of cell death was dose dependent against DLA cell lines. The IC_{50}values are presented the in table 48 and 49. Out of the extracts screened the stem ethanol extract of ***Pisonia grandis*****(PGSE)**showed better activity than the other extracts.

IJSER

Table 48 -*In vitro* cytotoxicity assay of various extract concentrates of *Andrographis stenophylla* using DLA cell lines

Drug (µg/ml)	Percentage of Cell Death											
	Leaf				Stem				Root			
	PGLE	PGLP	DPGLE	PGLAq	PGSE	PGSP	DPGSE	PGSAq	PGRE	PGRP	D.PGRE	PGRAQ
10	0	0	0	0	0	0	0	0	0	0	0	0
20	0	2	0	0	2	0	0	0	6	0	2	0
50	4	8	3	3	7	5	5	2	13	4	6	0
100	10	16	10	9	17	10	11	8	19	12	12	4
200	23	24	18	18	28	15	23	15	26	20	27	10
IC_{50} value	434	393.28	567.45	572.36	357.14	627.50	448.25	684.70	384.6	488.25	382.15	1105.22

IJSER © 202
http://www.ijser.org

Table 49*In vitro* cytotoxicity assay of various extract concentrates of *Andrographis stenophylla* using DLA cell lines

Concentration of the Drug (µg/mL)	Percentage of Cell Death			
	ASAE	ASAP	DASAE	ASAAq
10	0	0	2	0
20	6	5	6	0
50	9	10	10	4
100	18	15	17	10
200	34	33	35	20
IC_{50} value	294.1	302.18	285.30	515.62

Wound Healing assay by *in-vitro* Wound Healing by Angiogenic Model

Angiogenesis is the complex physiological process required for healing of wounds and for restoring blood flow to injured tissue. Wherein new capillaries form fromvarious pre-existing vascular network.The chorioallantoic membrane (CAM) is a vascular extra embryonic membrane found in eggs of birds and is formed on the fourth day of incubation. Extracts and natural substanceshave been tested for their in *in-vitro*wound healing potential by the CAM model**(Baruaet *al*., 2009)**. The percentage of blood vessel formation, taken as an indication of the extent of angiogenesis; increase in blood vessel formation indicates stimulation of angiogenesis by the test substance/extracts while decrease implies inhibition of angiogenesis (anti angiogenetic effect). The higher the percentage, the higher the extent of angiogenesis and this implies higher wound healing potential. Inhibition of angiogenesis has been proposed to be indicative of inhibition of tumour growth**(Jena *et al*.,2012).**

Evaluation of angiogenic potential of extract concentrates of ***Pisonia grandis*** and ***Andrographis stenophylla*** was carried out by the chick CAM model. The extracts show varying degrees of blood vessel formation. The results are presented in table 50 . Among the extract concentrates of ***Pisonia grandis***, PGLE, PGLP, PGSAq, PGRP and PGRAq showed increase in blood vessel formation which indicates the*in vitro* wound healing potential of these extracts. **It is also worthy of mention that the leaf petroleum ether extract of *P.grandis*shows slightly higher percentage of blood vessel formation (50% increase) than the leaf ethanol extract (44%) whereas the dewaxed ethanol extract of leaves of *P.grandis*express an equivalent decrease (44%). It may be proposed that the non-polar constituents of the leaf petroleum ether extract of *P.grandis* might play a greater role in stimulating angiogenesis than the constituents of the dewaxed leaf ethanol extract; being identified to contain the bio actives**

IJSER © 202
http://www.ijser.org

allantoin and pinitol (Shubashini *et al*., 2011a and Shubashini *et al*., 2011b).It is also of significance to note that extract PGSAq and PGRAq expressed an increase of94 % and 72 % respectively.It has been reported that saponins may promote angiogenesis **(Shanshan*et al*., 2004) and hence the angiogenesis stimulating effect of these aqueous extracts may be attributed to the presence of saponins.**

The extract concentrates of ***Andrographis stenophylla*** (ASAE and DASAE) showed moderate blood vessel formation in the chick CAM model.

Table 50 depicted the percentage blood vessel formation by various extract concentrates of *Pisonia grandis* and *Andrographis stenophylla*

Sample Code	Blood Vessel Formation
PGLE	44 % Increase
PGLP	50 % Increase
DPGLE	44 % Decrease
PGLAQ	No Blood Vessel Formation
PGSE	No Blood Vessel Formation
PGSP	No Blood Vessel Formation
DPGSE	77.7 % Decrease
PGSAQ	94 % Increase
PGRE	16 % Decrease
PGRP	58 % Increase
DPGRE	No Blood Vessel Formation
PGRAQ	72 % Increase
ASAE	12 % Increase
ASAP	50 % Decrease
DASAE	16 % Increase
ASAAQ	No Blood Vessel Formation
DMSO	No Blood Vessel Formation

IJSER

IJSER © 202
http://www.ijser.org

Figure 150 Chick CAM after the treatment of extracts of PG and AS

IJSER © 202
http://www.ijser.org

*In-vitro*anti-arthritic assay

Assessment of anti-arthritic potential of various extracts of ***Pisonia grandis*** and ***Andrographis stenophylla*** was carried out by *in-vitro* studies. Denaturation of proteins is one of the main causes of inflammatory and rheumatoid arthritis.Inhibition of protein denaturation can be taken as a measure of*In-vitro* anti-arthritic activity. The results show that inhibition of protein denaturation was achieved in a dose dependent response and the non-polar extracts of the both plants ASAP and PGLP exhibited maximum inhibition **(90.79±0.043; 90.51±0.050)**at 50 μg/ml and their effects were comparable with standard drug Diclofenac sodium. GC-MSanalysis of ASAP and PGLP revealed the presence of palmitic acid. Palmitic acid has been reported to possess anti-inflammatory activity **(Aparna*et al*., 2012)** and it finds use in the Ayurvedic system of medicine as a component of medicated oil used to treat rheumatism. This fact validates the traditional use of ***Pisonia grandis***and ***Andrographis stenophylla***as ananti-arthritic agent. The results of present study support that both plants extract may be good sources of novel anti-arthritic drug formulations.

IJSER

Table 51 Percentage inhibition of protein denaturation of various extracts of *Pisonia grandis* and *Andrographis stenophylla*

Drug (µg/mL)	% Inhibition							
	PGLP	**PGLE**	**PGSP**	**PGSE**	**PGRP**	**PGRE**	**ASAP**	**STD***
50	90.79± 0.043	85.89± 0.075	63.49± 0.043	35.19± 0.043	49.07± 0.043	4.08±0 .115	90.51± 0.050	91.22± 0.075
100	90.75± 0.048	88.07± 0.083	61.70± 0.048	39.82± 0.048	43.52± 0.048	19.88± 0.083	90.01± 0.055	90.64± 0.083
200	88.97± 0.597	85.42± 0.103	59.57± 0.103	28.37± 0.060	39.23± 0.060	30.58± 0.060	87.95± 0.066	88.97± 0.597
400	87.39± 0.068	86.29± 0.118	51.26± 0.068	7.60±0 .068	40.46± 0.651	30.14± 0.118	86.88± 0.119	87.27± 0.068
800	73.21± 0.151	70.59± 0.151	22.95± 0.756	7.94±0 .151	36.56± 0.151	43.54± 0.151	72.66± 0.135	73.04± 0.262

*Diclofenac sodium

IJSER © 202
http://www.ijser.org

Anti-arthritic Assessment ofColumn Eluatesof Petroleum EtherExtract Concentrate of *Pisonia grandis* andIsolated compounds

Column chromatographic analysis of **PGLP** on elution with 100% petroleum ether andmixture of petroleum ether and ethyl acetateyielded **16** main fractions. Assessment of anti-arthritic potential of all the fractions and isolated compound revealed that Fractions I, II, IV, VII, XII and PGLPC1 possesseddose-dependent response to anti-arthritic activity. Out of the fractions screened, Fractions I,II ,VII and XII showed significant activity even at lowconcentration. Fraction **IV** andthe isolated molecule PGLC1showed significantactivity however the fraction containing isolated compound showed 97 % inhibition at 600**µg/mL** concentration whereas the isolated compound showed only 51 % inhibition at 600 **µg/mL** concentration.So it is clearly indicated that the presence of other molecules in the fraction**IV**may bethe reason for thehighest percentage of activity. The commercially available anti-arthritic drug palmitic acid showed 69% inhibitionat 600 **µg/mL.**This study revealed potentially active fractions of ***Pisonia grandis***by which novel drug formulation for arthritis can be achieved.

IJSER

IJSER © 202
http://www.ijser.org

Table 52 Percentage inhibition of protein denaturation of column fractions of PGLP

Drug (µg/mL)	% Inhibition									
	Frn I	FrnII	Frn IV	Frn VI	Frn VII	Frn X	Frn XII	Frn XVI	PGLPC1	Palmitic acid
100	35.27± 0.008	17.16± 0.231	-37.04± 0.040	2.92±0.209	12.54± 0.042	-30.13±0.057	10.33± 0.088	-24.85± 0.143	-2.26±0.034	10.55±0.174
200	44.15± 0.083	36.05± 0.137	-62.75± 0.073	14.74± 0.067	26.22± 0.056	-13.65±0.125	27.72± 0.014	36.11± 0.084	14.87±0.135	33.79±0.076
400	55.58± 0.138	55.44± 0.150	49.09± 0.075	39.03± 0.142	39.48± 0.062	-25.84±0.095	42.32± 0.071	13.27± 0.070	34.87±0.120	57.90±0.115
600	60.98± 0.119	64.53± 0.127	97.03± 0.177	65.72± 0.132	71.11± 0.050	-13.11±0.057	55.53± 0.112	21.44± 0.095	51.21±0.046	69.85±0.185
800	85.11± 0.037	71.63± 0.092	83.76± 0.118	73.37± 0.015	77.21± 0.070	6.19±0.077	64.86± 0.110	-35.08± 0.078	66.43±0.025	75.95±0.067

In-vivo Wound Healing

The wound healing efficacy of the ointment was analysed by incision wound model based wound healing study. The ointment formulated with the leaf extract gave promising wound healing results comparable to standard ointment. The wound healing efficacy of this product would be tested by excision wound model in future.

IJSER

Tensile strength

S. No	Group	Tensile Strength (maximum load) (kg f)
1	Control	2.21 ± 0.42
2	Standard	4.20 ± 1.04^{ns}
3	10% w/w ointment	$7.57 \pm 1.75^{**}$

Values are mean ± SD of two samples in each group
** - $p<0.01$ significant ns – Not significant

IJSER © 202
http://www.ijser.org

5. Summary and Conclusion

The present research work is on phytochemical investigation and validation of herbal potential of two folkloric medicinal plants ***Pisonia grandis*** **R.Br. of plant family *Nyctaginaceae* and *Andrographis stenophylla* C.B Clarke of plant family *Acanthaceae.* The prime focus of the study is the isolation and chemical characterisation of their phytoconstituents, which area of work is one major stage towards chemical standardisation of the plants. Herbal standardisation strategies have also been applied to make it a wholesome record of validation of the folkloric use of the chosen plants.**

This research study is presented in five chapters.

Chapter I comprises of a brief introduction to the study and a mention of its prime focus.
The main objectives of this research work are:

- To isolate and characterize the chemical constituents of two folkloric medicinal plants ***Pisonia grandis*** **R.Br. and *Andrographis stenophylla* C.B Clarke**
- To validate the herbal potential of the chosen plants by standardisation studies

IJSER

Review of literature pertaining to the research study is presented in Chapter II.

It covers recent reports on:

- ♣ Earlier work on the chosen plants ***Pisonia grandis*** and ***Andrographis stenophylla***
- ♣ Earlier work on the bio-pharma potential of the bio-actives of the chosen plants

The methodology adopted for the research work comprising of two major stages is presented in Chapter III.

The outline of the strategy adopted for the research work is presented in the following sections:

- Isolation and characterization of chemical constituents from the chosen plants ***Pisonia grandis*** *and* ***Andrographis stenophylla***
- Validation of the Herbal Potential of the chosen plants

IJSER © 202
http://www.ijser.org

The results pertaining to the research work and the ensuing discussions are presented in Chapter IV.

Two folkloric plants have been extensively investigated in this research study. The constituents of their non-polar and polar extracts have been revealed. Both plants have been validated for their herbal potential by exhaustive standardisation studies.

The Highlights of the results and their significance:

As an outcome of the work on *isolation and characterization of chemical constituents from the chosen medicinal plants*, potentially active constituents elaborated by both plants have been identified in their extracts.

- Five compounds have been characterised from the dewaxed ethanol extract concentrate of stems of Pisonia grandis.
- Three compounds have been characterised from the methanol soluble portion of aqueous extract concentrate of stems of Pisonia grandis
- Five compounds have been characterised from the dewaxed ethanol extract concentrate of aerial parts of Andrographis stenophylla
- The non-polar extracts of both plants are found to contain palmitic acid with a larger percentage of it found in extracts of Pisonia grandis. Oleic acid and phytol are the other major constituents of Pisonia grandis

IJSER

The bio active molecules allantoin and pinitol identified to be present in the stem and roots of *Pisonia grandis* R.Br. are also significant constituents of its leaves also as revealed by a preliminary lead from our laboratory.

These bio actives could be isolated directly from the concentrated extract. Stem extract has a higher percentage of allantoin (5.3%) and pinitol (6%) compared to leaves and roots. This quantification is based on the direct isolation protocol adopted in the study without resorting to column chromatography. This has been ascertained by analytical quantification studies also.

Both allantoin and pinitol isolated *from Pisonia grandis* possess immense pharmacological potential as seen from the large number of scientific reports on their bio pharma potential. Hence ***extracts of Pisonia grandis of leaves, stems and roots of Pisonia grandis are of pharmacological significance***

IJSER © 202
http://www.ijser.org

owing to the presence of medicinally valuable allantoin and pinitol. A molecular entity comprising of a combination of allantoin and pinitol has also been isolated from the leaf, stem and roots of this plant in the present study. This revelation is of significance since such an entity has not been reported in literature though combination compounds of allantoin with various small molecules and of pinitol with small molecules have been earlier reported.

Allantoin and pinitol have proven anti diabetic potential however it was found that allantoin gets degraded in the gastrointestinal tract and may be lost after oral administration. A combination compound with allantoin and pinitol may be of much pharmacological significance especially in diabetes treatment. This is the first report of the isolation of such an addition complex of allantoin and pinitol. Ascertaining the bio potential of these two compounds in the combined form especially the anti diabetic potential in future work may lend lead to a valuable anti diabetic herbal formaulation with the active extracts of *Pisonia grandis.*

IJSER

The plant ***Andrographis stenophylla*** **C.B Clarke** is a medicinal plant of the ***Acanthaceae*** family. It is a rare and little known endemic species of India. Chemical investigation of polar extracts of this plant revealed the presence of flavonoidal constituents. **Andrographolide**, a terpenoidal lactone has been also isolated as a major constituent of these extracts. This molecule is a bio marker of the genus ***Andrographis.***

Extracts of Andrographis stenophylla possessing this molecule are also **are of pharmacological significance.**

In the present research work focus was on standardization of the chosen folkloric plants and their extracts to validate the herbal potential of the plant for use as a Phytomedicine.

- As part of the standardisation studies a survey was conducted on the folkloric use of the chosen plants. The survey led to the documentation of the folkloric use of the plants. It was found that the plants chosen for the present investigation have been extensively used by locals and tribals as anti-diabetic and anti-inflammatory agent. Mostly leaves of the chosen

IJSER © 202
http://www.ijser.org

plants have been more prioritized among the people to treat various ailments.

- Morphological data of both plants has been documented in literature and this has mention in the present work since documentation of morphological data of medicinal plants is an essential part of the validation and this will help in selection of the correct species of the plant for formulation.
- Physicochemical characteristics of both plants have been recorded and the results reveal that both plants are suitable for use as an Ayurvedic medicine.
- Heavy metal analysis of leaves stem and roots of *Pisonia grandis* and aerial parts of *Andrographis stenophylla* revealed that the metal contaminations are within the WHO permissible limits and thus the extracts of the chosen medicinal plant parts are safe for use
- Chemical fingerprinting of the plants was done by phytochemical analysis HPTLC, HPLC, NMR and GC-MS finger printing of extracts and quantification of bio actives.
- The NMR finger prints of the polar extracts of ***Pisonia grandis*** showed that the dewaxed ethanol extracts possess fewer constituents than its non polar pet-ether extracts. The NMR resonances expressed by the polar extracts correlate with the active constituents isolated from the same by direct fractionation and by column chromatography and the NMR finger prints reveal these constituents as the major constituents.
- The chemical shift ranges exhibited by the extracts in ^{1}H NMR and ^{13} CNMR represent the metabolite finger prints of the polar extracts
- GC-MS analysis of pet-ether concentrates of leaves, stems and roots of *Pisonia grandis* (PGSP, PGRP, and PGLP) revealed that the most prevailing phytoconstituents are palmitic acid, oleic acid and phytol. The GC-MS chromatograms represent the fingerprints.
- The GC-MS analysis of pet-ether extract of ***Andrographis stenophylla*** (ASAP) revealed the presence of chondrillasterol, stigmasterol, palmitic acid, oleic acid, dotriacontane, nonadecane and n-pentadecanol. The GC-MS chromatograms represent the fingerprints.

IJSER © 202
http://www.ijser.org

- The bioactives isolated from both plants were quantified in the extracts by HPLC method. This led to a revelation on the relative percentage of the bio actives in the various extracts. The relevance of this study lies in the validation of the extracts for its bioactive content which is of principal significance in the development of any herbal formulation.
- Stem and roots of *Pisonia grandis* contain a higher percentage of pinitol whereas leaves and stems possess a higher percentage of allantoin.
- The ethanol extracts of *Andrographis stenophylla* (ASAE and dASAE) have a higher percentage of the biomarker molecule andrographolide **(32 µg and 39 µg per 100 µg of extract respectively)** compared to the non-polar extract **(ASAP)**
- **Biological Standardization:** The various extract concentrate of the plants ***Pisonia grandis*** and ***Andrographis stenophylla*** were standardized by biological activity studies. The biological activity studies may be considered as biological fingerprints of the extracts of these plants.
- The efficacy of the isolated constituents also has been tested and compared with that of the extract concentrates of ***Pisonia grandis*** and ***Andrographis stenophylla***.
- At a concentration of 50 µg/mL the dewaxed stem ethanol concentrate DPSE showed highest DPPH Radical Scavenging potential compared to the other extracts. At 90 µg/mL DPGSE showed 80 % inhibition.
- In the analysis of the anti angiogenic potential of the extracts it is worthy of mention that the leaf petroleum ether extract of *P.grandis* shows slightly higher percentage of blood vessel formation (50% increase) than the leaf ethanol extract (44%) whereas the dewaxed ethanol extract of leaves of *P.grandis* express an equivalent decrease (44%). It may be proposed that the non-polar constituents of the leaf petroleum ether extract of *P.grandis* might play a greater role in stimulating angiogenesis than the constituents of the dewaxed leaf ethano l extract; being identified to contain the bio actives allantoin and pinitol
- The biological standardisation study forms part of the validation of the herbal potential of the chosen plants.

IJSER

IJSER © 202
http://www.ijser.org

Conclusion

In the present research work, two folkloric medicinal plants have been investigated extensively for their chemical constituents. Medically valuable molecules have been identified and characterized from the non-polar and polar extracts of leaves, stem and roots of Pisonia grandis and aerial parts of Andrographis stenophylla. All feasible herbal standardisation strategies have been applied to make it a wholesome record of validation of the folkloric use of both the plants. This work may lend a major lead to the development of herbal formulations particularly anti-diabetic, wound healing and anti-arthritic formulations.

IJSER

IJSER © 202
http://www.ijser.org

References

Aberoumand, A. 2011, Determination and Comparison of Potential NutritiveValues and Mineral Elements of Three Important Food Edible Plants from Southern Part of Iran ,*Croatian Journal of Food Technology, Biotechnology and Nutrition* 6 (3-4), 148-151.

Abu-Ghefreh, A., Canatan, H., Ezeamuzie,I.C. 2009, In vitro and in vivo anti-inflammatory effects of andrographolide, *International Immunopharmacology* , 9 ,313–318.

Ahmad, Z., Zamhuri, K. F., Yaacob, A., Siong, C. H., Malarvili S., Ismail, A. and Hakim, M. N. 2012, In Vitro Anti-diabetic Activities and Chemical Analysis of Polypeptide-k and Oil Isolated from Seeds of Momordicacharantia (Bitter Gourd),*Molecules*, 17, 9631-9640.

Ajuah, d., Christiansen, nM., Horowitz, F.J., Klein,S., Hellerstein,K,M. and Richard E. Ostlundjr, 2000, Effect of pinitol treatment on insulin action in subjects with insulin resistance, *Diabetes care*, 23: 1000 – 1005**.**

Akkol, K.E., Süntar, I., Orhana,I. E, Keles, H., Kanc, A., Çoksar, G. 2011, Assessment of dermal wound healing and in vitro antioxidant properties of Avenasativa L. *Journal of Cereal Science*, 53, 285-290.

Akowuah,G.A., Zhari, I., Mariam, A., 2008, Analysis of urinary andrographolides and antioxidant status after oraladministration of Andrographispaniculata leaf extract in rats, *Food and Chemical Toxicology*, 46, 3616–3620.

Alario, J. D. and Pereira, J.R.C. 2013, Topical pharmaceutical composition comprising a combination of methotrexate, _-bisabolol and allantoin for the treatment of psoriasis, atopic dermatitis or chronic eczema, *PCT Int. Appl.*, WO 2013155584 A1

Ambuhl, R. 2011, Pattern Recognition in HPTLC-Fingerprints of Medicinal Plants. Master Thesis. Institute of Pharmaceutical Biology, Department of Pharmaceutical Science, University of Basel, Switzerland.

Anbalagan, N., Rajinikanth, K. N., Gnanasam, S. Kishore., Leonard, J. Thomas., Balakrishna, K., Ramachandran, S. and Sridhar, S. K. 2002, Analgesic, Anti-inflammatory and Diuretic Activities of *Pisonia grandis*, *Natural product sciences*, 8(3),97-99.

Anonymous.1969.The Wealth of India – An Encyclopedia of India's Rawmaterial ResourcesEdn 1, VIII (Ph-Re).

Aparna ,V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. andHaridas, M. 2012, Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment.*ChemBiol Drug Des,* 80(3), 434-9.

Araújo, L.U., Grabe-Guimarães, A., Vanessa Carla Furtado Mosqueira, Carneiro, C.M. and Silva-Barcellos, N.M. 2010.Profile of wound healing process induced by allantoin, *ActaCirúrgicaBrasileira*, 25 (5):460.

Arulpriya, P. and Lalitha, P. 2013, Evaluation of Different Extraction Methods for Optimization of Extraction of Aerial Roots of Rhaphidophoraaurea Entwined over Two Diverse Host Trees, *International Journal of Chem Tech Research*, 5(5), 2173-2176.

Arun Bhatt. 2010, Ayurvedic Herbal Industry: QUEST for Global Acceptance.

Bansal, Y., and Bansal, G. 2011, Analytical methods for standardization of Aeglemarmelos: A review,*Journal of Pharmaceutical Education and Research,*2(2).

IJSER

IJSER © 202
http://www.ijser.org

Barua , C.C., Talukdar, A., Begum, S.A., Sarma , D. K., Pathak , D.C., Barua, A. G. and Bora,R.S. 2009, Wound healing activity of ethanolic extract of leaves of *Alternantherabrasiliana*Kuntz using in vivo and in vitro model. Indian Journal of Experimental Biology, 47,1001-1005.

Bates, S.H., Jones, R.B. and Bailey ,C.J. 2000, Insulin-like effect of pinitol,*British Journal of Pharmacology*, 130 (8): 1944-1948.

Behera, P.R., Nayak,P., Barik, D.P., Rautray, T.R., Thirunavoukkarasu, M.,Chand, P.K., 2010, ED-XRF spectrometricanalysisofcomparativeelementalcomposition of in vivo and in vitro roots of Andrographis paniculata (Burm.f.) Wall.exNees—a multi-medicinalherb, *Applied RadiationandIsotopes,*68, 2229–2236

Berrin, O., Murat, K. and Ilkay,**O.** 2011, Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids, *Pharmaceutical Biology,* 49(4): 396-402.

Bhan, M.K., Dhar, A.K., Khan,S., Lattoo, S.K., Gupta, K.K. and Choudhary, D.K. 2006, Screening and optimization of Andrographis paniculata (Burm.f.) Nees for total andrographolide content, yield and its components, *ScientiaHorticulturae*, 107,386–391.

Bhaskar Reddy, M. V., Kishore, P. H., Rao, C. V., Gunasekar, D., Caux, C. and Bodo, B. 2003, New 2'-oxygenated flavonoids from Andrographis affinis. *Journal of Natural Products*, *66*(2), 295–7.

Bhat, K. A., Shah,B, A., Gupta, K, K., Pandey, A., SarangBani and Subhash, T.C. 2009, Semi-synthetic analogs of pinitol as potential inhibitors of TNF-α cytokine expression in human neutrophils,*Bioorganic& Medicinal Chemistry Letters*, 19(7): 1939-1943.

Bhowmik, S. and Datta, K.B. 2012**,** Elemental analysis of some ethnomedicinaly important hydrophytes and marsh plants of India used in traditional medicine, Asian Pacific Journal of Tropical Biomedicine, S1227-S1231.

Buenz, E.J., Johnson, H.E., Beekman, E.M. , Motley, T.J. and Bauer, B.A. 2005, BioprospectingRumphius's*Ambonese Herbal*: Volume I, *Journal of Ethnopharmacology*, 96, 57–70.

Cabrera, D., Gutiérrez, J., Cabello-Verrugio, C., Morales, G.M., Mezzano, S., Fadic, R., Casar, C.J., Hancke, L.J. and Brandan, E. 2014, Andrographolide attenuates skeletal muscle dystrophy in mdx mice and increases efficiency of cell therapy by reducing fibrosis, *Skeletal Muscle*, 4:6.

Celine,C., Catherine,C., Eva, P. and Laurence, **C.** 2012, Influence of certain ingredients on the SPF determined, *Archives of dermatological research,* 304(10)**:** 817-21.

Chandrasekaran, C.V., Thiyagarajan, P.,Deepak, H.B., AmitAgarwal. 2011,In vitro modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of Andrographis paniculata (King of bitters) extract, *International Immunopharmacology*,11,79–84.

Chao,C.Y., Lii, K.C., Tsai,I.T., Li, C.C., Liu, L.K., Tsai, W.C. and Chen,W.H., 2011, Andrographolide Inhibits ICAM-1 Expression and NF-KB Activation in TNF-α-Treated EA.hy926 Cells, Journal of Agricultural and Food chemistry, 59, 5263–5271. Andrographolide exhibits apoptosis of cancer cells at different concentrations portraying anticancer potential (**Jayakumar*et al*.,2010)**.

Chauhan, P.S., Gupta, K.K. and SarangBani, 2011, The immunosuppressive effects of *Agyrolobiumroseum* and pinitol in experimental animals, *International Immunopharmacology*, 11: 286-291.

Chen, M. F.,Yang, T. T.,Yeh, L. R.,Chung, H. H.,Wen, Y. J.,Lee, W.J. and Cheng, J. T. 2012,Activation of imidazoline I-2B receptors by allantoin toincrease glucose uptake into cells,*Hormone and metabolic research,* 44(4): 268-72.

IJSER © 202
http://www.ijser.org

Chulet, R., Joseph, L., George, M. and Pradhan, P. 2010. Pharmacognostic standardization and phytochemical screening of albizzialebbeck, J. Chem. Pharm. Res., 2(1), 432-443.

Dai, G. F., Xu, H. W., Wang, J. F., Liu, F. W. and Liu, H. M. 2006, Studies on the novel a-glucosidase inhibitory activity and structure–activity relationships for andrographolide analogues, *Bioorganic & Medicinal Chemistry Letters*, 16, 2710–2713.

Damu, A.G., Jayaprakasam, B., Rao, K. V. and Gunasekar, D. 1998, A flavone glycoside from Andrographis alata. *Phytochemistry*, *49*(6), 1811–1813.

Das, B., Ramu, R., KoteswaraRao, Y., Ravinder Reddy, M., Harish,H.,Saidi Reddy, V., Ramakrishna, K.V.S., 2006, Acylated 5,7,2',6'-oxygenated flavone glycosidesfromAndrographisalata, *Phytochemistry*, 67, 978–983.

Das, P., Kumar, D., Roy, R., Chowdhury, C. and Chatterjee, **M.** 2012, Andrographolide Analogue Induces Apoptosis and Autophagy Mediated Cell Death in U937 Cells, *European Journal of cancer 48*, suppl. 5, S25–S288.

Dobrescu, D. 1998. Medicine for treating gastroduodenal ulcer and chronic gastritis.RO 113805.

D-pinitol to diabetic rats also showed alterations in the activities of key metabolic enzymes involved in carbohydrate metabolism **(Sivakumar and Elumalai, A., Eswaraiah, M.C., Sindhura, S., Rajendra, D., Manikanta, K.V.C. and Rajkumar, C.H.** 2012, Acute toxicity studies and antipyretic activity of a poly herbal formulation, *International Journal of Biological & Pharmaceutical Research,* 3(1): 130-132.

Fangkham, S., Ekalaksananan ,T.,Aromdee, C.,Seubsasana, S.,Kongyingyoes ,B., Patarapadungkit ,N., Pientong, C.,2012, The effect of andrographolide on Human papillomavirus type16 (HPV16) positive cervical cancer cells (SiHa), 15th ICID Abstracts / International Journal of Infectious Diseases 16S e2–e157 Final Abstract Number: 40.030

Federal Register, 43(151): 34632-34.

Flamini, G., Luigi Cion, P., Morelli, I. and Bader, A. 2007, Essential oil of the aerial parts of three Salvia species from Jordan: Salvia Lanigera, S. spinosa and S.syriaca. *Food chem*., 100, 732-735.

Flinders, M. 1814, 153-7.

Fonteles, M. C., Almeida, M. Q. and Larner, J. 2000, Antihyperglycemic effects of 3-O-Methyl-D-Chiro-inositol and D-Chiro-inositol associated with manganese in streptozotocin diabetic rats, *Hormone and Metabolic Research***,** 32(4):129-132.

Fujita, T., Fujitani, R., Takeda, Y., Takaishi, Y., Yamada, T., Kido, M. and Miura, I.1984, On the diterpenoids of Andrographis paniculata X-ray crystallographic analysis of andrographolide and structure determination of new minor diterpenoids. *Chem. Pharm. Bull*, 32, 2117– 2125.

Gautam, S., Seok,A.K., Bokyung, S. and Bharat, A. 2008, Pinitol targets nuclear factor-_ B activation pathway leading to inhibition of gene products associated with proliferation, apoptosis, invasion, and angiogenesis, *Molecular Cancer Therapeutics* , 7(6): 1604-1614.

Gnanasekaran, G. and Murthy, G.V.S. 2010, Taxonomy, lectotypification and distribution of Andrographis stenophylla (Acanthaceae), a little known endemic species from southern India, *Phytotaxa* 156 (3),175–181.

Gnanasekaran, G. and Murthy, G.V.S. 2010, Taxonomy, lectotypification and distribution of Andrographis stenophylla (Acanthaceae), a little known endemic species from southern India,*Phytotaxa* 156 (3),175–181.

Gnanasekaran,G., and Murthy,G.V.S. 2014,Taxonomy, lectotypification and distribution of Andrographis stenophylla (Acanthaceae), a little known endemic species from southern India.*Phytotaxa*156 (3), 175–181

IJSER © 202
http://www.ijser.org

Gupta, S.K., Amit Gupta, A.K. Gupta, Vedpal, D.P. 2013**,** In vitro anti-arthritic activity of ethanolic extract of CallicarpaMarophylla flower. *International Research Journal of Pharmacy*, 4(3):160-162.

Guskov, E.P., Kletskii, M.E., Kornienko, I.V., Olekhnovich, L.P., Chistyakov,V.A., Shkurat, T.P., Prokof'ev, V.N. and Yu. A. Zhdanov,2002. Allantoin as a Free-Radical Scavenger,*Doklady Biochemistry and Biophysics*,383:105–107.

Harborne, J.B., 2009,Phytochemical methods- a guide to modern techniques of plant analysis, Chapman and Hall, London.

Hari Kishore, P**., VijayaBhaskar Reddy, M., Kesava Reddy, M., Gunasekar,D., CristelleCaux, Bernard Bodo.** 2003, Flavonoids from Andrographis lineata, *Phytochemistry*, 63, 457–461.

Hartley, D. Roy.and Harris, J. Pilip. 1981, Phenolic Constituents of the Cell Walls of Dicotyledons, *BiochemicalSystematlcs and Ecology*, 9(2/3), 189-203.

Heloina de Sousa Falcão, Jacqueline AlvesLeite , José Maria Barbosa-Filho , PetrônioFilgueiras de Athayde-Filho , Maria Célia de Oliveira Chaves , Marcelo DantasMoura , Anderson Luiz Ferreira, Ana Beatriz Albino de Almeida, Alba Regina Monteiro Souza-Brito,Margareth de FátimaFormigaMeloDiniz and Leônia Maria Batista,Gastric and Duodenal Antiulcer Activity of Alkaloids: A Review,2008, *Molecules, 13,* 3198-3223.

Holley, J. and Cherla, **K.** 1998, The medicinal plants sector in India: A review. International Development Research Centre.

Hypoglycemic effect of pinitol isolated from soybeans has been reviewed (**Shin *et al.*,2002)**

in refreshing cream can effectively isolate harmful substances in skin cream for makeup and in the air (**Jingyao, 2013**)

IrfanHashmi, 2007, Sensory Evaluation Techniques, 18th Annual IAOM Conference (MEA District) Muscat-Oman.

Jada, S. R., Subur, G. S., Matthews, C., Hamzah, A. S., Lajis, N. H., Saad, M. S. and Stanslas, J. *2007. Semisynthesis and in vitro anticancer activities of andrographolide analogues.Phytochemistry, 68(6), 904–12.*

Jadhao, M. 2010. Estimation of Andrographolide in Herbal Powder and PolyherbalAsava by HPTLC, *International Journal of Pharma and Bio Sciences1*(4), 242–245.

Jagadeesan, P., Arun Prasad, D., Pandikumar, P. and Ignacimuthu, S. 2011. Antioxidant and free radical scavenging activities of common wild greens from Tiruvallur District of Tamilnadu, India*, Indian Journal of Natural Products and Resources*.,2(2), 156 -163.

Jayakumari , S., Ravichandiran ,V. and NageeswaraRao. 2014, Antimicrobial Activity of Pisonia Grandis R . Br leaf extract and its fraction, *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(2), 2290-2302.

Jayakumari, S., Arthanareswaran, A., Vijayalakshmi, A., Velraj, M. and Ravichandran, V. 2012**,** Free radical scavenging activity of *Pisonia grandis* R.Br leaves*. Indian J Pharm Edu Res* 2012; 46: 37-40.

Jayakumari, S., MalarkodiVelraj, Vijayalakshmi, A . and Arthanarieswaran,P. 2011, Pharmacognostical studies on the leaves of *Pisonia grandis* R. Br, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(2), 193.

Jayaprakasam, B., Damu, A. G., Gunasekar, D., Blond, A. and Bodo, B. 1999, Dihydroechioidinin , a flavanone from Andrographis echioides, *52*, 935–937.

IJSER © 202
http://www.ijser.org

Jena, B.K., Ratha,B., Kar,S., Mohanta,S., Tripathy, M. and Nayak, A.M. 2012, Wound healing potential of ziziphusxylopyruswilld. (rhamnaceae) stem bark ethanol extract using in vitro and in vivo model, *Journal of Drug Delivery & Therapeutics,* 2(6), 41-46.

Jewitt, Douglas; Cheng, Lixiu; Cai, Jingyao. 2012**,** Aloe gel skin care product and preparation method there of. Faming ZhuanliShenqing, CN 102525848.

JieXu, Zhanting Li , Min Cao, Han Zhang, Jifeng Sun, Jie Zhao, QiangZhouc,**ZhifenWua,, Lianjia Yang**, 2012, Synergetic effect of Andrographis paniculata polysaccharide on diabetic nephropathy with andrographolide,International*Journal of Biological Macromolecules* 51,738– 742.

Joy, P. P., Thomas, J., Mathew, S. and Skaria, B. P. 2001, Medicinal Plants, *Tropical Horticulture Vol. 2*. (eds. Bose, T.K., Kabir, J.,Das, P. and Joy, P.P.). NayaProkash, Calcutta, 449-632.

Kamboj, V.P., 2000, Herbal medicine,*Current science*, 78(1).

Kang, M.J., Kim J.I., Yoon S. Y., Kim J. C. and Cha I.J. 2006, Pinitol from Soybeans Reduces Postprandial Blood Glucose in Patients with Type 2 Diabetes Mellitus, *Journal of Medicinal Food*, 9(2): 182-186.

Kim, J.L., Kim, J.C, Kand , M.J., Lee, M.S., Kim, J.J. and Cha, I.J. 2005, Effects of pinitol isolated from soybeans on glycaemic control and cardiovascular risk factors in Korean patients with type II diabetes mellitus: a randomised controlled study, *European Journal of Clinical Nutrient*, 56: 456-458.

Kim, K., Park, J. and Rhee, S. 2007. Structural and functional basis for (S)-allantoin formation in the ureide pathway.*The Journal of Biological Chemistry*, *282*(32), 23457–64.

KokaiTokkyoKoho. 1984. Topical anti-inflammatory formulations containing Aloe extracts. JP 59013716.

KoteswaraRao,Y., Vimalamma,G., VenkataRao,C., Yew-Min Tzeng, 2004, Flavonoids and andrographolides from Andrographis paniculata, *Phytochemistry* 65 , 2317–2321.

Kumoro, A. C. and Hasan, M. 2007, Supercritical Carbon Dioxide Extraction of Andrographolide from Andro- graphis paniculata: Effect of the Solvent Flow Rate, Pressure, and Temperature. Chin. J. Chem. Eng. 15, 877–883.

KusunurAhamedBasha,A.K., Raman, P.R K. Pani Prasad, KundanKumar,EzhilNilavan, Saurav Kumar,2013,Effect of dietary supplemented andrographolide on growth,non-specific immune parameters and resistance against Aeromonashydrophila in Labeorohita (Hamilton), *Fish & Shellfish Immunology* xxx ,1-9.

Lakshmi, V., Gupta, P., Tiwari, P. and Srivastava, A. K. 2006.Antihyperglycemic activity of Rhizophora apiculata Bl. in rats.*Natural Product Research*, *20*(14), 1295–1299.

Lee Jun Sik, Lee Chang-Min, Jeong Young-Il, Jung In Duk, Kim Bo-Hye, SeongEun-Young, Kim Jong-Il, Choi Il-Whan, Chung Hae Young and Park Yeong-Min, 2007, D-pinitol regulates Th1/Th2 balance via suppressing Th2 immune response in ovalbumin-induced asthma,*FEBS Letters,*581(1): 57-64.

Lee, B. H., Lee, C.C.andWu ,S.C. 2014, Ice plant (Mesembryanthemumcrystallinum) improves hyperglycaemia and memory impairments in a Wistar rat model of streptozotocin-induced diabetes, J Sci Food Agric, 94(11), 2266-73.

IJSER

IJSER © 202
http://www.ijser.org

Lorena UlhoaAraujo, Andrea Grabe-Guimaraes, Vanessa Carla Furtado Mosqueira, Claudia Martins Carneiro, Neila Marcia Silva-Barcellos, 2010, Profile of wound healing process induced by allantoin, *Actacirurgicabrasileira/ SociedadeBrasileiraparaDesenvolvimento PesquisaemCirurgia,*25 (5): 460-6

Majumdar, A., Sahu, M., Saraf, S., Verma, S. and Yadav, R. 2012, Evaluation of hepatoprotective & anti-inflammatory activity of roots of *Pisonia grandis*R.Br *, Inventi Rapid: PlantaActiva.*

Mei-Fen Chen, Jo-Ting Tsai, Li-Jen Chen, Tung-Pi Wu, Jia-Jang Yang, Li-Te Yin, Yu-lin Yang, Tai-An Chiang, Han-Lin Lu and Ming-Chang Wu1, 2014, Antihypertensive Action of Allantoin in Animals, *BioMed Research*, ID 690135.

Mijares, A.H., Banuls, C., Peris, J.E., Monzo,N., Jover,A., Bellod, L., Victor, M.V. and Rocha,**M.** 2013, A single acute dose of pinitol from a naturally-occurring food ingredient decreases hyperglycaemia and circulating insulin levels in healthy subjects, *Journal of Food chemistry.*

MohmmedArifullah, NimaDanduNamsa, ManabendraMandal, Kishore Kumar Chiruvella, Paritala Vikrama1, Ghanta Rama Gopal4. Evaluation of anti-bacterial and anti-oxidant potential of andrographolide and echiodinin isolated from callus culture of Andrographis paniculata Nees. Asian Pac J Trop Biomed 2013; 3(8), 604-610.

Nanna, S.R., Banala, M., Pamulaparthi, A., Kurra, A. and Kagithoju, S., 2012, Evaluation of Phytochemicals and fluorescent analysis of seed and leaf extracts of Cajanuscajan L, Int. J.Pharm. Sci.Rev.Res. 22(1), 11-18.

Natarajan, R.K., Ragothaman, P., Veluchamy, G. and Balakrishna, K. 1990, Chemical examination of Pisonia grandis*. Bull Med-Ethno Bot Res*,11: 110-111.

Neelaveni, T. and Gupta, J.K. 2010. Activity-Guided Isolation of Antioxidant Compounds from Andrographis stenophylla Leaf, *E-Journal of Chemistry*, 7(2), 363-368**.**

Nikhat,F., Satynarayana, D. and Subhramanyam E. V. S. 2009,Isolation, Characterization and Screening of Antioxidant Activity of the Roots of Syzygiumcuminii (L) Skeel, *Asian J. Research Chem. 2(2).*

Niu, S.C., Chen, W., Wu, H. T., Cheng, C.K., Wen, J.Y., Lin, C.K. and Cheng, T.J. 2010, Decrease of plasma glucose by allantoin, an Active principle of Yam (Dioscorea spp.), in Streptozotocin- induced diabetic rats, *J.Agric.FoodChem*, 58, 12031-12035.

Nivedhitha, R. and JaqulineChinna Rani, I. 2011. "A study on the antimicrobial activity of *Pisonia grandis* and *Pithecellobiumdulce* on selected microflora". *National Congress on Bioscience Research Proceedings 56*.

on wound healing by the application of CTBX (Durmus et al., 2012).

Orthen, B., Popp, M., and Smirnoff N. 1994, Hydroxyl radical scavenging properties of cyclitols**,** , Proceedings of the Royal Society of Edinburgh Section B: Biology, 102: 269-272.

Ostlund, R. and Sherman, W. 1996**,**Pinitol and derivatives thereof for the treatment of metabolic disorders,U.S. Patent US 5882896, 1996.

Parasuraman ,S., Sankar, N., Chandrasekar ,T., Murugesh ,K.,, Neelaveni, T. 2010, Phytochemical analysis and oral hypoglycemic activity of Leaf extract of leaves of *andrographis stenophylla* c.b. clarke(acanthaceae), *International Journal of Applied Biology and Pharmaceutical Technology, Volume I(2)*442.

Parkinson,**C.E.** 1923, A forest flora of the Andaman Islands, Published by Government Central Press. P.222

IJSER © 202
http://www.ijser.org

Pekthong, D., Blanchard, N., Abadie,C., Bonet,A., Heyd, B., Mantion, G., Berthelot, Richert, A.L., Martin, H., 2009, Effects of ***Andrographispaniculata***extract and Andrographolide on hepaticcytochrome P450 mRNA expression and monooxygenase activities after ***in vivo*** administration to rats and ***in vitro*** in rat and human hepatocyte cultures, *Chemico-Biological Interactions*, 179, 247–255.

Pinheiro, N.C. 1997, Comparative effects of calcipotriol ointment and 5% coal tar 2% allantoin 0.5% hydrocortisone cream in treating plaque psoriasis, *British Journal of Clinical Practice*, 51(1):16-19.

Pinitol or combinations of pinitol with insulin in a synergistic amount has been proved to be effective for controlling insulin-dependent diabetes

Poongothai, G. and Shubashini K. Sripathi, 2013,Quantitative Analysis of Allantoin in Leaves, Stem And Roots Of *PisoniaGrandis*R.Br. By RP-HPLC, *International Journal of Current Research,* 5(08), 2105-2108.

Prabu D., Nappinnai M., Ponnudurai K. and Prabhu K. 2008. Evaluation of Wound-Healing Potential of *Pisonia grandis* R.Br*:* A Preclinical Study in Wistar Rats, *The International Journal of Lower Extremity Wounds*, 7(1): 21-27.

Prabu D., Nappinnai M., Ponnudurai K. and Prabhu K. 2008. Evaluation of Wound-Healing Potential of *Pisonia grandis* R.Br*:* A Preclinical Study in Wistar Rats, *The International Journal of Lower Extremity Wounds*, 7(1): 21-27**.**

PriyankaTrivedi, Rashmi Singh, and Karuna Shanker,2011, Micellar Extraction and Cloud-Point preconcentration of LabdaneDiterpenoidsfrom Andrographis Paniculata, *Journal of Liquid Chromatography & Related Technologies*, 34:2085–2102.

Rahman, A.H., Elumalai, M., Eswaraiah,C. and Bardalai, D. 2011, Evaluation of anxiolytic activity of ethanolic extract of Pisonia grandis R. Br leaves in mice. *J Chem Pharm Res*; 3(5):646-652.

Ramesh Kumar,M. and Janagam, D., 2011, Export and import pattern of medicinal plants in India. *Indian Journal of Science and Technology*, 4 (3).

Ranson, Michele. (Fr.).1984. Allantoin and DNA combination for wound healing. EP 109873.

Rao, Y. K., Harikishore, P., Rao, C. V., Gunasekar, D., Blond, A., and Bodo, B. 2002.Flavones from Andrographis viscosula.*Phytochemistry*, *61*(8), 927–929.

Rashed, N.A., Afifi, U.F., Shaedah.M.andTaha, O.M. 2004, "Investigation of the active constituents of *portulacaoleraceae* L. (*Portulacaceae*) growing in jordan", *Pakistan Journal of Pharmaceutical Sciences*, 17(1):37-45.

Reneela, **P**. 2010. Phytochemical Investigation of two Medicinal Plants Endemic to Western Ghats, India. Ph.DThesis submitted to Avinashilingam University for Women.

Roy, P., Das ,S., Auddy , G. R., Saha , A. and Mukherjee, A. 2013,Engineered Andrographolide Nanoparticles Mitigate Paracetamol Hepatotoxicity in Mice, *Pharm Res*, 30:1252–1262, DOI 10.1007/s11095-012-0964-5.

Sattayasai ,J., Srisuwan , S., Arkaravichien ,T. and Aromdee,**C.** 2010, Effects of andrographolide on sexual functions, vascular reactivity and serum testosterone level in rodents,*Food and Chemical Toxicology*, 48, 1934–1938.

Shan Niu, C., Chen, W., Tsung Wu, H., Chun Cheng, K., Ya-Ju Wen, Kao-Chang Iin, and Tang Cheng, J. 2010. Decrease of Plasma Glucose by Allantoin, an Active Principle of Yam (Dioscorea spp.), in Streptozotocin-Induced Diabetic Rats. J. Agric. Food Chem., 58: 12031–12035.

IJSER © 202
http://www.ijser.org

Sharma, M., and Sharma, R. G. 2013, Identification, Purification and Quantification of Andrographolide from Andrographis Paniculata (Burm . F .)NeesBy HPTLC at different Stages of Life Cycle of Crop, *3*(1), 23–32.

Sheeja, K., Guruvayoorappan, C. and Kuttan , G. 2007,Antiangiogenic activity of Andrographis paniculata extract and andrographolide, *International Immunopharmacology*, 7, 211–221.

Shubashini K. Sripathi, Poongothai G., and Lalitha , P. 2011a, Anti-diabetic agent Pinitol from the leaves of *Pisonia grandis* (R.Br.), J.Natural Remedies, , 11 (1), 39-43.

Shubashini K. Sripathi., Poongothai ,G., and Lalitha, P. 2011b, Allantoin from the leaves of Pisonia grandis R.Br, Int. J. of Pharm. & Life Sci.2(6), , 815-817 815.

Silverstein, M.R., Webster, X. F. and Kiemle, J.D. .2005, Spectrometric Identification of Organic Compounds.John Wiley & Sons, 7th edition p.142.

Singh, R. K., Pandey, B. L., Tripathi, M. and Pandey, V. B.2001, Anti-inflammatory effect of (+)-pinitol.*Fitoterapia*, 72(2): 168-170.

Singh, R. K., Pandey, B. L., Tripathi, M. and Pandey, V. B., 2001, Anti-inflammatory effect of (+)-pinitol.*Fitoterapia*, 72(2): 168-170.

Singh, V. and Vadera, S. 2010, Export Potential of Herbal and Medicinal Plants in India, www.smeworld.org

Sir J.D. Hooker; C.B, K.C.S.I. 1973, The flora of British India..Vol IV: 711

Sivakumar, S. and Subramanian, S. 2009, Pancreatic tissue protective nature of D-Pinitol studied in streptozotocin-mediated oxidative stress in experimental diabetic rats. European Journal of Pharmacology (2009), 622(1-3), 65-70.

Soumya V.,Venkatesh,P. and Shrishaillapa,B.2010, A novel microwave-assisted extraction for the isolation ofandrographolide from Andrographispaniculata and its invitroantioxidant activity, *Natural Product Research,* 24(16), 1560–1567.

Srivastava, N. Akhila, **A.** 2014, Biosynthesis of andrographolide in Andrographis paniculata, *Phytochemistry*, 71,1298–1304.

Subhasree, B., Baskar, R., Laxmi Keerthana, R., Lijina Susan, R. and Rajasekaran, P, 2009,Evaluation of antioxidant potential in selected green leafy vegetables, *Food Chemistry*, 115(4),1213-1220.

Subramanian, R., Asmawi, M.Z. and Sadikun, A. 2008, In vitro alpha glucosidase and alpha amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide.ActaBiochem Pol, 55:391-398.

Sundaram, U. and Gurumoorthi, P. 2012. Validation Of HPTLC Method For Quantitative Estimation Of L-Dopa, *3*(4), 300–304.

Sunil, C., Latha, G., Mohanraj,P., Kaliyamoorthy, K. andAgastian.P. 2009. "*4* α-Glucosidase inhibitory and antidiabetic activities of ethanolic extract of *Pisonia alba*Span. Leaves."*International journal of Integrative Biology*, 6(1):41 -45.b

Sunil, C., Latha, G., Suja, S.R., Shine, V.J., Shyamal ,S., Anuja, G.I., Sini, S., Rajasekharan, S., Ajastian, P., Ignacimuthu, S. and Kaliselvan,V. 2009. "Effect of ethanolic extract of *Pisonia alba* Span. Leaves on blood glucose levels and Histological changes in tissues of Alloxan-induced diabetic rats." *International journal of Applied Research in natural products*. 2(2), 4-11.a

IJSER © 202
http://www.ijser.org

Sutthivaiyakit, S., Seeka, C., Wetprasit, N. and Sutthivaiyakit, P. 2013, C-Methylated flavonoids from Pisonia grandis roots, *Phytochemistry Letters*, xxx (xxx–xxx), article in press.

The Hindu India's National News Paper. 2013. Chennai Edition, Jan 14,

Thenmozhi, S., Kameshwaran, S., Subasini ,U., Sathyamurthy, D. and Dhanalakshmi, M. 2013**.** Hepatoprotective Constituents from the leaves of *Pisonia grandis* R.Br. *Pharmacologia*, 383-390**.**

US Patent 20130123370 A1

Uttekar, M. M., Das, T., Pawar, R. S., Bhandari, B., Menon, V., Nutan, S.K.G. and Bhat, S. V. 2012, Anti-HIV activity of semisynthetic derivatives of andrographolide and computational study of HIV-1 gp120 protein binding. *European Journal of Medicinal Chemistry*, *56*, 368–74. doi:10.1016/j.ejmech.2012.07.030

VijayaBharathi , R. and Vamsadhara, C., 2007, Pharmacognostical Evaluation of Andrographis stenophylla C. B. Clarke, *Natural Product Sciences*,13(3) : 241-246

Vijaykumar, K., Murthy, P.B.S., Kannababu, S., Syamasundar, B., Subbaraju, G.V., 2007. Int. J. Appl. Sci. Eng. 5, 27–39.

Vincent, V., Jang, C.T., Jing, R.C. and Pete,**G.** 2013, Allantoin as a solid phase adsorbent for removing endotoxins, *Journal of Chromatography A,*1310: 15-20.

Vogel, 2004, Qualitative Analysis.

Wang P., Kong C.H., Hu F. and Xu X.H. 2007, Allantoin involved in species interactions with rice and other organisms in paddy soil. Plant Soil, 296:43–51.

Wang, X. and Liu, S.2013, Preparation and application of head skin-preservation liquid used in neurological surgery, *Faming ZhuanliShenqing*, CN 102988236.

Wen-wan chao, Yueh-hsiungkuo,and Bi-fonglin, 2010, Anti-inflammatory Activity of New Compounds from Andrographis paniculata by NF-KB Transactivation Inhibition,Journal of Agricultural and Food chemistry,58, 2505–2512.

WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues.2007

Will McClatchey. 1996, Theethnopharmacopocia of rotuna, Journal of Ethnopharmacology, 50, 147-156.

Woan-Ruoh Lee, Chi-Li Chung, Che-Jen Hsiao, Yung-Chen Chouc , Po-Jen Hsueh, Po-ChihYang,Jing-Shiun Jan, Yu-Wen Cheng, George Hsiao, 2012, Suppression of matrix metalloproteinase-9 expression by andrographolide in human monocytic THP-1 cells via inhibition of NF-ᴷB activation, *Phytomedicine* ,19 , 270– 277.

Wu, Ke Hair ironing and dyeing lotion Faming ZhuanliShenqing 2013, CN 103284911

Xianguotengshu, 2003, PISONIA Linnaeus, Sp. Pl. 2: 1026. 1753. *Flora of China* 5: 430-431. 2003.

Yang,L., Wu,D., Luo,K., Wu,S., Wu,P., Zhejiang. 2009, Andrographolide enhances 5-fluorouracil-induced apoptosis via caspase-8-dependent mitochondrial pathway involving p53 participation in hepatocellular carcinoma (SMMC-7721) cells, *Cancer Letters*, 276, 180–188.

Ying KoSai. 1983. Antimicrobial dressing. AU 527826.

Young, Je Ahn, Se Jin Park, Hyun Woo, HyungEun Lee, Hyun Ji Kim, Guyoung, Kwon, QingtaoGao, DaeSik Jang and Jong HoonRyu, 2014, Effects of allantoin on cognitive function and hippocampal neurogenesis, *Food and Chemical Toxicology,* 64, 210–216.

Yun Y.C., Choi C.M., Jeon Y.J. and Shin Y.C. 2007, Composition for preventing and treating inflammatory disease by using synergistical anti-inflammatory effects of glucosamine and pinitol,KR2007002401.

IJSER

IJSER © 202
http://www.ijser.org

Zaijun Zhang, Daoxu Lai, LiangWang, Pei Yu, Longjun Zhu , BaojianGuo, LipengXu,Libing Zhou, Yewei Sun, Simon Ming Yuen Lee, YuqiangWang, 2014,Neuroprotective effects of the andrographolide analogue AL-1 in the MPP+/MPTP-induced Parkinson's disease model in vitro and in mice, *Pharmacology, Biochemistry and Behavior,* 122, 191–202.

Zhang and Jingyao. 2013, Refreshing cream used for UV light or radiation protective agent, *FamingZhuanliShenqing*, CN 103340812.

Zhi-Gang Chen, Quan Zhu, Min-HuaZong, Zhen-XinGu, Yong-Bin Han,2011, Enzymatic synthesis and antibacterial activity of andrographolide derivatives, *Process Biochemistry* 46 , 1649–1653.

Dr.Dukes Ethno botany Data Base 2014

www.ars-grin.gov/duke

www.microlibrary.org

www.neutraceuticalsworld.com

www.pubchem.ncbi.nih.gov

http://ism.kerala.gov.in/index.php/about-unani-and-naturopathy.html

http://www.lungtatravel.com/amchi-medicine.html

www.agr.gc.ca

www.balmtech.com

www.greenpatio.com

www.ipr.net

www.keralaagriculture.gov.in

www.pubchem.ncbi.nlm.nih.gov

IJSER

IJSER © 202
http://www.ijser.org