Phytochemical Screening and GC-MS Analysis of Ethanol Extract of Polyherbal Drug

Revathi, G[1]., Elavarasi, S[2]. and Saravanan, K[1]

Abstract

The present study focused on a traditionally used polyherbal drug (mixture of *Andrographis paniculata*, *Andrographis alata*, *Adhatoda zeylanica*, *Gymnema sylvestre*, *Syzygium cumini* and *Justicia glabra*) by tribal healers of Kolli hills, and given to diabetic patient to treat diabetes mellitus. The polyherbal drug was extracted using ethanol and the preliminary phytochemical screening of the extract revealed the presence of bioactive compounds such as alkaloids, protein, carbohydrate, saponin, phenols, sterols, and fatty acid. And the extract was subjected to GC- MS analysis, the results revealed the presence of 12 bioactive compounds, and these compounds are recommended to treat antidiabetic activity.

Index Terms—: Polyherbal drug, phytochemical screening, GC-MS study, Antidiabetic activity.

1. INTRODUCTION

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Cragg and David, 2001) i.e. any part of the plant may contain active components. Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with less side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use (Sofowora, 1993). The medicinal properties of some plants have been documented by some researchers (Ayitey *et al*., 1977; Gill, 1992; Banso and Adeyemo, 2007). Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova *et al*., 2005). Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs (Mandal *et al*., 2007).

[1]*PG & Research Dept. of Zoology, Nehru Memorial College (Autonomous), Puthanampatti-621 007, India.*
[2]*PG & Research Dept. of Zoology, Holy Cross College (Autonomous), Trichy, India.*

Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity (Misra, 2009). Knowledge of the chemical constituents of plants is desirable because such information will be value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many researchers (Mojab *et al.,* 2003; Parekh and Chanda, 2007; Parekh and Chanda, 2008). A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (Hertog *et al*., 1993). It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana and Shekhawat, 2010). Phytochemical screening of plants has revealed the presence of numerous chemicals including alkaloids, flavonoids, tannins, steroids, glycosides and saponins. Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Cown, 1999, Nishaa *et al*., 2013). Polyherbal plants (mixture of *Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini* and *Justicia glabra*) by tribal healers of Kolli hills, Namakkal district, Tamilnadu.

The polyherbal plants possessed antimicrobial activity, anti-inflammatory activity, antifungal activity and antidiabetic activity. The reports on the biological activity of polyherbal plants in this composition in the literature are rare. This forms the basis to reveal the biological significant

compound present in polyherbal plants. So that polyherbal mixture can be used as a therapeutic sources. GC-MS is one of the best techniques to identify the bioactive constituents of long chain branched alkaloids, protein, carbohydrate, saponin, phenols, sterols, and fatty acid. To explore the medicinal importance of the polyherbal plants were screened primarily for the phytochemicals present in it and was analyzed using GC-MS.

2. MATERIALS AND METHODS

Preparation of extracts

The process of extraction was followed by the method of Swami Handa *et al* (2008). The Polyherbal drug was prepared by mixing equal quantity of whole plant *of A. paniculata*, *A. alata, G. sylvestre* and *J. glabra,* leaves of *A. zeylanica,* and bark of *S. cumini* powder. Then it was extracted by cold extraction method using ethanol. They were concentrated to a dry mass by vacuum evaporator and stored separately in desiccator until use.

Preliminary phytochemical screening

Different extracts of polyherbal mixer were subjected to screen the preliminary phytochemicals such as alkaloids, flavonoids, glycosides, phenolic compounds, saponins, terpenoids, steroids, tannins, fatty acids, protein and carbohydrate according to the standard methods (Kokate, 1994; Harborne, 1973; Rajpal, 2002; Raman, 2006).

Test for flavonoids: One ml of extract was taken in a test tube and a few drops of dilute sodium hydroxide were added. An intensive yellow colour is produced and become colourless on addition of few drops of dilute HCl which indicates the presence of flavonoids.

Test for alkaloids: Extract (0.5g) was diluted to 10 ml with acid alcohol then boiled and filtered. Two ml of dilute ammonia was added to 5 ml of the filtrate. Then 5 ml of chloroform was added to this content and shaken gently. The chloroform layer was extracted with 10 ml of acetic acid which was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream with Mayer's reagent or reddish brown precipitate with Draggendorff's reagent indicates the presence of alkaloids.

Test for tannins: About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration which indicates the presence of tannins.

Test for saponins: Extract (0.5g) was taken in a test tube and 5 ml of distilled water added. This mixture was shaken vigorously and observed for a stable persistent froth. That froth was mixed with 3 drops of olive oil and shaken vigorously. The formation of an emulsion indicates the presence of saponins.

Test for glycosides: The extract was hydrolysed by hydrochloric acid for few hours on a water bath. Then 1 ml of pyrimidine and a few drops of sodium nitroprusside solutions were added to this hydrolysate. Then it was made alkaline by adding sodium hydroxide solution. Appearance of pink to red colour showed the presence of glycosides.

Detection of phenols: Presence of phenols was detected by ferric chloride test. Extract was treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for steroids: One ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. Formation of yellow with green fluorescence colour indicates the presence of steroids.

Test for triterpenoids: Ten milligram of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for emodins: Two ml of 10% NH_4OH and 3ml of benzene was added to the extract. Appearance of red colour indicates the emodins.

Test for Coumarins: 3ml of 10% NAOH was added to 2ml of extract. Formation of yellow color indicates the presence of coumarins.

Test for fatty acids: 0.5 ml of extract was mixed with 5 ml of ether. These extract was allowed it for evaporation, on filter paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids (Ayoola *et al*., 2008).

Test for proteins: The extract was treated with few drops of concentrate nitric acid. Formation of yellow colour indicates the presence of proteins.

Test for carbohydrates: Extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to test for the presence of carbohydrates. Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

GC-MS analysis

Presence of individual compounds in the study plant extracts were analyzed using GC-MS/MS of Thermo Fisher make, ITQ900 model. One micro

litre of the sample was run in a DB-1 fused silica capillary column with helium (1ml/min) as carrier gas, 250°C injector temperature, 280°C ion-source temperature and isothermal temperature 110°C (2 min), with an increase of 10°C/min to 200°C then 5°C/min to 280°C and 9 min to 280°C. The mass spectrum interpretation was performed using the library of National Institute Standard and Technology (NIST) and the compounds were identified.

3. RESULTS AND DISCUSSION

The phytochemical substances such as alkaloids, protein, carbohydrate, saponin, phenols, sterols and fatty acid were present in the ethanol extract of polyherbal drug.

Table. 1: Preliminary phytochemical screening of Ethanol extracts of Polyherbal plants

S. No.	Name of the compound	Presence
1	Flavonoids	**(+)**
2	Alkaloids	**(+)**
3	Tannin	**(-)**
4	Protein	**(-)**
5	Carbohydrate	**(-)**
6	Saponin	**(-)**
7	Glycosides	**(-)**
8	Phenols	**(+)**
10	Sterols	**(-)**
11	Triterpenoids	**(-)**
12	Coumarins	**(-)**
13	Emodins	**(-)**
14	Fatty acid	**(-)**

(+)Present (-) Absent

GC-MS analyses

The results pertaining to GC-MS analysis led to the identification of number compounds from the GC fractionations of the ethanol extract of polyherbal drug. The identified compounds and their molecular formula and peak area are presented in table 2, and peaks and their RT are shown in GC-MS chromatogram (Figure 1).

Table 2: Identified compounds from Ethanol extract of polyherbal formulation by GC-MS.

S. No.	Compound name	Molecular formula	M.W	R.T
1	Tetradecanoic acid, 12-methyl-, methylester	$C_{16}H_{32}O_2$	256	12.19
2	Phytol	$C_{20}H_{40}O$	296	13.53
3	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	28	14.27
4	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	21.02
5	Squalene	$C_{30}H_{50}$	410	22.54
6	Methoxyacetic acid, 4-tetradecyl ester	$C_{17}H_{34}O_3$	286	23.64
7	Vitamin E	$C_{29}H_{50}O_2$	430	26.75
8	Vitamin A aldehyde	$C_{20}H_{28}O$	284	28.38
9	Lupeol	$C_{30}H_{50}O$	426	30.65
10	Acetic acid, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester.	$C_{17}H_{26}O_3$	278	31.27
11	Thunbergol	$C_{20}H_{34}O$	290	34.21
12	Cedran-diol,8s,14-	$C_{15}H_{26}O_2$	238	34.47

M.W- Molecular weight, RT- Retention time

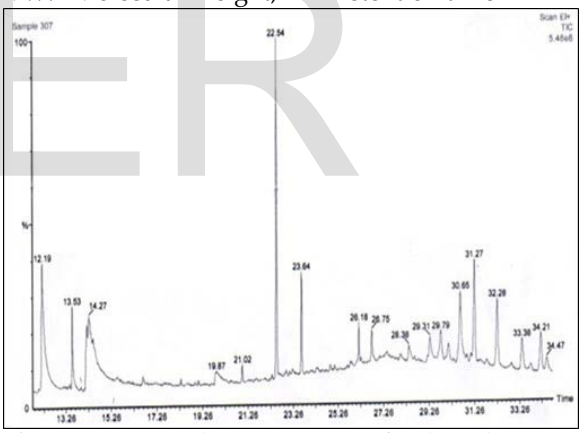

Fig 1: GC-MS Chromatogram of Ethanol extract of polyherbal formulation

Totally 12 compounds were identified in the spectrum profile of GC-MS of ethanol extract of polyherbal drug. The prevailing compounds were Tetradecanoic acid, Phytol, 9, 12- Octadeconoic acid (Z,Z)-, Geraanyl isovalerate, Squalene, Methoxycetic acid,4-teradecyl ester, Vitamin E, Vitamin A aldehyde, Lupeol, Acetic acid, 3-hydroxy-7-isopropenyl-,4a- dimethyl-2,3,4,4a,5,6,7,8- octahydronaphthalen- 2-yl ester, Thunbergol, Cedran-diol, 8s, 14-, the spectrum profile of GC-MS confirmed the presence of twelve major components with retention time of 12.19, 13.53, 14.27, 21.02, 22.54, 23.64, 26.75, 28.38, 30.65,

31.27, 34.21 and 34.47, respectively. The fragmentations of the components are illustrated in GC-MS chromatogram (Figure 1).

Arising from their biodiversity and perhaps the rich complement of phytochemicals and secondary metabolites, plants have from antiquity been used as sources of medicament against various ailments (Atangwho, 2009). A wide variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Several phytochemicals have been found to possess a variety of medicinal properties, which may help in protection against chronic diseases and metabolic diseases. Many traditional plants remedies are known in folk medicine and used for treatment and management of diabetes mellitus (Aktar and Ali, 1984), and some have been validated by scientific studies to actually exert biological action against diabetes or its complications. For example, phytochemicals such as glycosides, flavonoids, tannins and alkaloids have hypoglycaemic activity (Cherian and Augusti, 1995; Elavarasi, 2015; Revathi *et al*., 2015). Rupasinghe *et al.,* 2003) have reported that saponins possess hypocholesterolemic and antidiabetic properties. The terpenoids have also been shown to decrease blood sugar level in animal studies (Luo *et al*., 1999). Over 150 plant extracts and some of their active principles, including coumarins, flavonoids, terpenoids, and most of other secondary plant metabolites, including arginine and glutamic acid and flavonoids are known for the treatment of diabetes (Erenmemisoglu *et al*., 1995). Several flavonoids, glycosides, terpenoids and other phenolic compounds have been shown to possess antidiabetic properties (Chandramohan, 2005).

The phytochemical substances such as alkaloids, protein, carbohydrate, saponin, phenols, sterols and fatty acid were present in the ethanol extract of polyherbal drug which included a mixture of whole plant powder of *Andrographis paniculata, Andrographis alata, Justicia glabra, Gymnema sylvestre,* leaf powder of *Adhatoda zeylanica* and bark powder of *Syzygium cumini*.. Polyherbal drug can able to reduce blood glucose level due to the presence of above chemical fractions. Even though, this is only a preliminary study of the occurrence of certain chemicals of polyherbal drug isolation and characterization of chemical constituents would provide a good concrete base of all the phytochemicals functions.

CONCLUSION

Totally 12 compounds were identified in the GC-MS spectrum. Most of them can be possessed antidiabetic and antioxidant activities due to the presence of steroids, flavonoids, alkaloids, coumarin and tannin.

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