EVALUATION OF PHYTOCHEMICAL ANALYSIS,ANTIOXIDANT,ANTIMICROBIAL ACTIVITY AND LEATHER APPLICATION OF THE SEA BLITE SUAEDA MARITIMA (L.)DUMORT [PDF]

Salomie Jennifer Samuel Johnson , Nithiya Rajaram

ABSTRACT

In this study an attempt was made to extract the phytochemials from the different parts of the plant using water, methanol, hexane and acetone. These extract were screened for its phytochemicals which showed the presence of alkaloids, glycosides, flavanoids, phenols, saponins, tannins, terpenoids, phlobatannins and steroids . The antioxidant study the leaf, stem and root part of plant in methanol, hexane, chloroform and distilled water extract showed maximum activity than the other extracts. In antimicrobial assay showed that the leaf extracts has maximum zone of inhibition against E.coli, bacillus, klebsilla, protease and pseudomonas. The pant parts were tested for its leather application in curing process. The result showed that salt less preservation technique using leaves of *Suaeda maritima* gave better results in leather preservation.

The phytochemical characterization and antibacterial activity of the leaf extracts of *Suaeda maritima* (L) Dumort was investigated using standard methods. Antimicrobial activity was studied using leaf, stem and root extracts, on the various test microorganisms, including multiple antibiotic resistant bacteria and phytopathogens. Antimicrobial activity of the extracts was determined by the Well Diffusion Method. The experimental results concluded that the hexane, methanol and water extracts of *S. maritima* leaves have greater potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms. The extractive value of water is more than in the solvents were investigated. The leaf extracts of *S.maritima* showed potent antibacterial activity. The present investigation revealed that the *S.maritima* leaves are potential good source of antibacterial agents. The phytochemical characterization study will be helpful to study the active principles using modern techniques in the later part of this work.

Keywords: Antimicrobial Activity, Multiple Antibiotic Resistant Bacteria, Well Diffusion Method, *Suaeda maritima*, Phytochemical screening.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

1. INTRODUCTION

1.1 PLANT DESCRIPTION

Suaeda maritima (L). is a coastal salt mash plant and it used coastal village peoples for traditional folk medicine method for varies disease no one evidence for scientific methods. In general salt tolerance plants have a more antioxidant constitute.

Suaeda maritima is a halophytic plant and its habitat is salt marsh. In order to adapt to saline or waterlogged conditions, plants have evolved mechanisms that include antioxidant protection. However, the combined effect of salinity and waterlogging on antioxidants in S. maritima is unknown. *Suaeda maritima* (L.) Dumort (Chenopodiaceae) is a mangrove herb, commonly known as Indian salt blite in English, found in salt marshy areas of India. The leaves of plant have been used as medicine for hepatitis traditionally. It is reported to possess antiviral, antibacterial activity and antioxidant activity etc. The present study was carried out to establish the pharmacognostical studies, physico-chemical parameters along with preliminary phytochemical screening of petroleum ether, chloroform, methanolic and aqueous extracts of *Suaeda maritima* (L.) Dumort.

Scientific Classification:-

- **Kingdom:** Plantae- Plants
- **Sub kingdom:** Trachiobionta-Vascular plants
- **Super division:** Spermatophyta- Seed plants
- **Division:** Magnoliophyta- Flowering plants
- **Class:** Magnoliopsida- Dicotyledons
- **Sub class:** Caryophyllidae
- **Order:** Caryophyllales
- **Family:** Chenopodiaceae- Goose foot family
- **Sub family:** Suaedoideae
- **Genus:** Suaeda Forssk.ex J.F.Gmel- Seep weed
- **Species:** Suaeda maritime (L.)Dumort- Herbaceous seep weed

Herbaceous sea-blite has a worldwide distribution, but in North America it is confined to the northeast coast. It is found in salt marshes and coastal beaches in New England. There are two varieties in New England, one of which is introduced, while the other is native and rare.

Plants of Suaeda are found in saline or alkaline wetlands or, occasionally, in upland habitats. Some species are cultivated and eaten as a vegetable; seeds of some have been ground and

eaten by native Americans, and some species are used as a source for red or black dye. The genus Suaeda includes widely distributed polymorphic species such as S. maritima, S. calceoliformis, and S. nigra. No infra specific taxa are recognized here. All three of these species show much variation in morphology and growth form characteristics, but no qualitative characters could be found during this review to reliably separate distinct taxa below the species level.

Future chromosomal and genetic studies may enable the recognition of distinct infra specific taxa or even species within these three polymorphic entities. Identification of Suaeda specimens is achieved most successfully when based upon material containing flowers (for ovary shape) and mature calyces (for lobe shape) containing seeds. Because of the succulent nature of most specimens, fresh material may appear quite different than dried material, especially in the accentuation of calyx features when dry. The raw or cooked young leaves has a pleasant salty flavour and mixed with other vegetables to reduce their saltiness. Traditionally, the leaf of suaeda maritima has been used as a medicine for Hepatitis and reported to have anti-bacterial activity and anti-oxidant property.

1.2PHYTO CHEMICALS

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome.

1.3ANTI MICROBIAL

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacteria and antifungals are used against fungi. They can also be classed according to their function. Antimicrobials that kill microbes are called *microbicidal*; those that merely inhibit their growth are called *microbiostatic*. Antiseptics help reduce infection during surgery. Disinfectants such as bleach are *nonselective antimicrobials*. They kill a wide range of microbes and are

valuable for cleaning inanimate surfaces to prevent the spread of illness, but they are generally not medicinal.

Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection. Wainwright.M (1989) More recently, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine. Kingston W (2008)

1.4ANTI OXIDANT

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such asthiols, ascorbic acid, or polyphenols. Sies, Helmut (1997)

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials with a limited number of antioxidants detected no benefit and even suggested that excess supplementation with certain putative antioxidants may be harmful. Jha, Prabhatet et al (1995) ; Baillie, J.K.et al(2009); Bjelakovic G et al (2007); Dabelstein et al(2007)

1.5LEATHER MAKING PROCESS

Leather manufacturing process is divided into three sub-processes: preparatory stages, tanning and crusting.

Preparatory stages may include:

- Preservation- the hide/skin is treated with a method which renders it temporarily unputrescible.
- Soaking - water for purposes of washing or rehydration is reintroduced.

- Liming - unwanted proteins and "opening up" is achieved.
- Unhairing - the majority of hair is removed.
- Fleshing - subcutaneous material is removed.
- Splitting - the hide/skin is cut into two or more horizontal layers.
- Reliming - the hide/skin is further treated to achieve more "opening up" or more protein removal.
- Deliming - liming and unhairing chemicals are removed from the pelt.
- Bating - proteolytic proteins are introduced to the skin to remove further proteins and to assist with softening of the pelt.
- Degreasing - natural fats/oils are stripped or as much as is possible from the hide/skin.
- Frizzing - physical removal of the fat layer inside the skin. Also similar to Slicking.
- Bleaching - chemical modification of dark pigments to yield a lighter coloured pelt.
- Pickling - lowering of the pH value to the acidic region. Must be done in the presence of salts. Pickling is normally done to help with the penetration of certain tanning agents, e.g., chromium (and other metals), aldehydic and some polymeric tanning agents
- Depickling - raising of the pH out of the acidic region to assist with penetration of certain tanning agents

Tanning

Tanning is the process that converts the protein of the raw hide or skin into a stable material which will not putrefy and is suitable for a wide variety of end applications. The principal difference between raw hides and tanned hides is that raw hides dry out to form a hard inflexible material that can putrefy when re-wetted (*wetted back*), while tanned material dries out to a flexible form that does not become putrid when wetted back. A large number of different tanning methods and materials can be used; the choice is ultimately dependent on the end application of the leather. The most commonly used tanning material is chromium, which leaves the leather, once tanned, a pale blue colour (due to the chromium), this product is commonly called “wet blue”.

The acidity of hides once they have finished pickling will typically be between pH of 2.8-3.2. At this point the hides are loaded in a drum and immersed in a float containing the tanning liquor. The hides are allowed to soak (while the drum slowly rotates about its axle) and the tanning liquor slowly penetrates through the full substance of the hide. Regular checks will be made to see the penetration by cutting the cross section of a hide and observing the degree

of penetration. Once an even degree of penetration is observed, the pH of the float is slowly raised in a process called basification. This basification process fixes the tanning material to the leather, and the more tanning material fixed, the higher the hydrothermal stability and increased shrinkage temperature resistance of the leather.

Crusting

Crusting is when the hide/skin is thinned, retanned and lubricated. Often a coloring operation is included in the crusting sub-process. The chemicals added during crusting have to be fixed in place. The culmination of the crusting sub-process is the drying and softening operations. Crusting may include the following operations:

- Wetting back - semi-processed leather is rehydrated.
- Sammying - 45-55% (m/m) water is squeezed out the leather.
- Splitting - the leather is split into one or more horizontal layers.
- Shaving - the leather is thinned using a machine which cuts leather fibres off.
- Neutralisation- the pH of the leather is adjusted to a value between 4.5 and 6.5.
- Retanning - additional tanning agents are added to impart properties.
- Dyeing - the leather is coloured.
- Fatliquoring - fats/oils and waxes are fixed to the leather fibres.
- Filling - heavy/dense chemicals that make the leather harder and heavier are added.
- Stuffing - fats/oils and waxes are added between the fibres.
- Stripping - superficially fixed tannins are removed.
- Whitening - the colour of the leather is lightened.
- Fixation - all unbound chemicals are chemically bonded/trapped or removed from the leather
- Setting - area, grain flatness are imparted and excess water removed.
- Drying - the leather is dried to various moisture levels (commonly 14-25%).
- Conditioning - water is added to the leather to a level of 18-28%.
- Softening - physical softening of the leather by separating the leather fibres.
- Buffing - abrasion of the surfaces of the leather to reduce nap or grain defects.

1.6 PRESERVATION OF SKIN AND CURING PROCESS

PRECURING PERIOD:

The precuring period is time between the actual slaughtering of animal and commencement of curing process . It is an important factor as the hides/skins become putrefied to some extent due to delay in curing. If the condition of the hide/skin is sufficiently fresh and without the onset of bacterial action, the aim of curing simply lies in quick dehydration of water from the material. It has been found that if the precuring period delay for *about 5 hours* is slight degenerative changes takes place in the cells lying around the sweat glands. After 11 hours, the remaining skin structure also gets affected leading to breakdown of the polypeptide into dipeptide level.

SALT CURING:

Salt is most common method for preservation. Sodium chloride is used amounting 30-40% of total flesh weight. Salt is not a real preservative, because it works on abstracting water from hide so that living condition for bacteria changes. Thus in short, the optimum growth in case of halophilic bacteria are.

- Moisture content -65%
- Temperature-30-45C
- pH -5.0-11.0
- salt content 5-15% for moderate and 15-30% for true halophiles
- Mainly, due to pollution related problems associated with the salt curing methods, many research groups have been actively involved in the development of alternative curing or preservation techniques. Some of the alternatives are described below.

ALTERNATIVES TO SALT CURING:

NEEM OIL:

The development of salt less preservation by the use of neem oil with alcohol has also been reported. The neem extracts were applied to both flesh and hair sides at a rate of about 1 percent on the green weight. After the treatment the experimental skins are allowed to dry in

the shade. The skins, by this method can be preserved for more than 6 months but the resultant leathers were of inferior quality.

In India, immersion of hides and skins for 4-8 h in a mixed solution of zinc chloride and sodium penta chloro phenate (pcp), at 0.15 and 0.16 percent respectively, prior to salt curing is found to preserve hides and skins for at least 7 d. But due to pollution concern the use of PCP is banned.

ANTIBIOTICS:

Use of antibiotics to control green hide biodegradation has been also reported, where *tetracycline, streptomycine* have been examined. The author has developed microbiological bioassays to determine the antibacterial activity of selected B-*lactams tetracyclines* and *aminoglycosides* against *Vibrioaliginolytious*, used as collagenolytic test

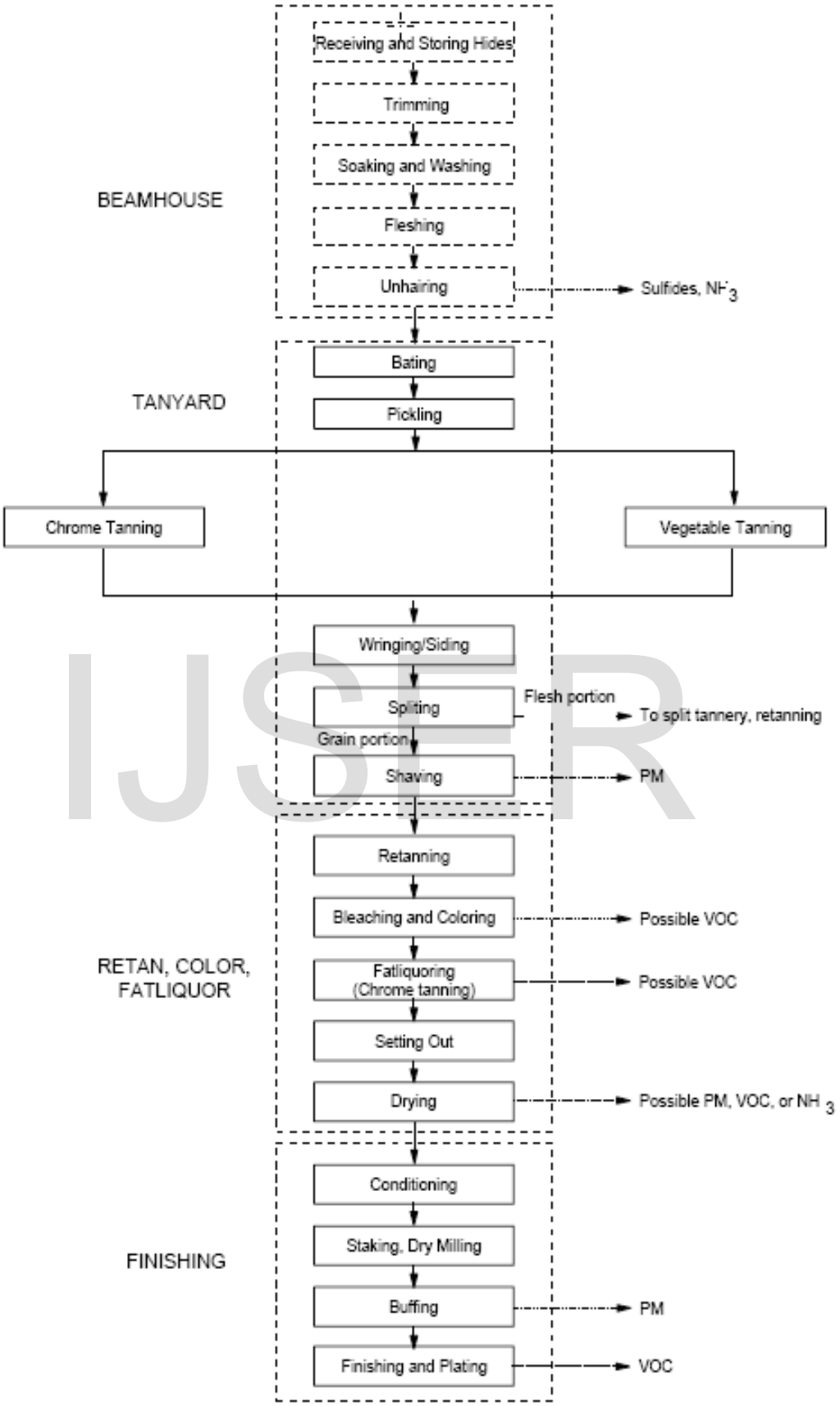


Fig. . leather making overall process

bacterium. Results indicated that the tetracycline type antibiotics are most effective at 1 percent, w/v, with *B-lactam* to a lesser extent.

A short term preservation technique for the cattle hides using a combination of sodium chloride and hydrolysed starch-poly acrylo nitrile graft co-polymers after washing with 4 percent acetic acid has been reported. The approach made is based on the principle that by regulating water activity to maintain micro bio-static conditions to produce a quality hide with minimum handling processing and storage. This method of short term preservation depends on control of gram-positive micrococci and bacilli and there was no sign of bacterial or mold growth after 11 wk of storage.

SODA ASH:

Another short term preservation of cattle hide using 20 percent, w/v, soda ash has also been reported. The raw cattle hides, thoroughly washed in cold water, are immersed in the solution for4, 6 or 8 h and then stacked flesh side up after drip draining for about 45 min and then covering samples with wrapping paper and preserved at ambient temperature of 25 C. The treatment may be used for preserving raw cattle hide for 8 days. sodium carbonate has been widely used as a salt additive for over 60 years, primarily with a view to prevention of stains due to calcium and magnesium salt impurities. Because of limited effectiveness, sodium carbonate against bacteria , particularly strains of halophilic bacteria, sodium carbonate(1%) has usually been used a salt additive in combination with naphthalene(1%) . (Kanagaraj.J 2001)

AIM AND OBJECTIVE

2. AIM AND OBJECTIVES

AIM

To evaluate phytochemical analysis, anti oxidant activity, anti microbial activity and preservation of goat skin of the sea blite *Suaeda maritima* (L) Dumort

OBJECTIVE

1, To screen the phytochemicals of *suaeda maritima*

2. To evaluate the anti oxidant activity of *suaeda maritima*

3. To evaluate the anti microbial activity of *suaeda maritima*

4. To preserve the skin sample in different concentrations of *suaeda maritima*

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

Anti oxidant activity of crude extracts were evaluated in anti oxidant activity, phyto chemical screening and DPPH radical scavenging activity. Anti microbial activity is determined using agar well diffusion method. With MIC activities against 10 gram positive and gram negative bacteria. The study reveals that presence of potential anti oxidants and anti microbial properties in plant extracts that could be exploited for pharmaceutical application.
(Rath. S.K, *et al.*(2009)

Many bioactive compounds are produced by the medicinal plants that have important pharmacological activities. The present study describes the chemical composition, antifungal, phytotoxic, brine shrimp cytotoxic, insecticidal and antibacterial activities of essential oils from Acacia modesta. The oils were extracted from the n-hexane fraction of the aerial parts of the plant and were analyzed by gas chromatograph and mass spectrometer (GC-MS). The results revealed 38 components from the oil of A. modesta. The oils exhibited moderate antifungal activity (40%) against Microsporum canis and low against Fusarium solani (25%). At the concentration of 1000 and 100 µg/ml, the oils showed moderate phytotoxic activity of 50 and 40%, respectively, against Lemna minor. The oils were highly cytotoxic at the concentration of 100 µg/ml killing all the shrimps in the experiment. At the concentration of 10 µg/ml only 2 and at concentration of 1 µg/ml, only 8 shrimps survived out of 30. The oils showed no antibacterial and insecticidal activity against the test organisms. (Bashir Ahmad *et al* (2012))

The antimicrobial properties of methanolic extracts of Aframomum melegueta seeds and rhizomes of Zingiber officinale were investigated on Helminthosporium solani, Aspergillus niger, Penicillium digitatum and Mucor piriformis isolated from tomato. This research was undertaken to control the growth of these rot fungi in vitro. Extracts at various concentrations ranging from 0-30% were separately added to PDA media. The plates were inoculated separately with the fungal isolates. Effects of these extracts on mycelial growth of the fungi were highly significant ($P < 0.05$) for all treatments. Z. officinale extract at 25% and A. melegueta at 30% concentration gave complete inhibition. Phytochemical analyses of extracts revealed the presence of tannins, phlobatannins, steroids, tarpenes, saponins, flavonoids and alkaloids. The presence of these compounds supports the use of the extracts as antimicrobial agents which can prolong the shelf–life of fresh tomato fruits.(Chiejina *et al* (2012))

Fatty acid methyl ester (FAME) extracts of four halophytic plants, *viz. Arthrocnemum indicum*, *Salicornia brachiata, Suaeda maritima* and *Suaeda monoica* belonging to the family Chenopodiaceae, were prepared and their composition was analyzed by GC-MS. The FAME extracts were also screened for antibacterial and antifungal activities. The GC-MS analysis revealed the presence of more saturated fatty acids than unsaturated fatty acids. Among the fatty acids analyzed, the relative percentage of lauric acid was high in *S. brachiata* (61.85%). The FAME extract of *S. brachiata* showed the highest antibacterial and antifungal activities among the extracts tested**.** Manivachagam Chandrasekaran *et al* (2008)

Suaeda maritima (L.) Dumort (Chenopodiaceae) is a mangrove herb, commonly known as Indian salt blite in English, found in salt marshy areas of India. The leaves of plant have been used as medicine for hepatitis traditionally. It is reported to possess antiviral, antibacterial activity and antioxidant activity etc. The present study was carried out to establish the pharmacognostical studies, physico-chemical parameters along with preliminary phytochemical screening of petroleum ether, chloroform, methanolic and aqueous extracts of Suaeda maritima (L.) Dumort. The macroscopical and microscopical characters were studied. The transverse section (T.S.) of root indicated the arrangement of various cells in cork, cortex, phelloderm and pith region. The histochemical colour reaction of T.S with different chemical reagents and preliminary phytochemical screening of various extracts revealed the presence of carbohydrate, alkaloids, glycosides, flavonoids sterols, phenolic and tannins compounds. The physico-chemical parameters such as total, acid insoluble, water insoluble and sulphated ash (2.7, 1.54, 2.17 and 3.6%w/w respectively), loss on drying (12.87 %w/w) extractive values and fluorescence analysis of extracts and powder treated with different chemical reagents were studied under ordinary light, short and long UV lights. The foaming and swelling index were also studied. These studies will be helpful in developing standards for quality, purity and sample identification of this plant (Sumitra Singh *et al* (2007))

Metabolites identified from mangrove plants are classified according to 'chemical classes', and some of their structures are illustrated. The article also presents some of the functions of the chemicals present and attempt to emphasize and create an awareness of the great of potential mangroves and mangal associates possess as a source of novel agrochemicals, compounds of medicinal value, and a new source of many already known biologically active compounds.(Bandaranayake.W.M *et al* (2009))

Natural products have been the single most productive source of leads for the development of drugs. Natural products from endophytic microbes have been showed many biological activities. The present study was carried out to find out the in vitro antibacterial activity of endophytic bacteria isolated from halophytic plants. A total of 14 endophytic bacterial strains were identified from the leaf tissue of 11 different halophytic plant species. Of them, 2 strains showed broad-spectrum of antibacterial activity against shrimp pathogens which were identified as Bacillus thuringiensis (FJ236808) and Bacillus pumilus (FJ236809) through 16S rDNA sequencing and deposited in the NCBI GenBank.(Ravikumar, Sundaram *et al* (2010))

Antioxidants are the chemical substances that reduce or prevent oxidation. The present study aimed to assess in vitro and ex vivo antioxidant activities of four acetonic extracts Tunisian halophytes (Suaeda fruticosa,Suaeda pruinosa,Suaeda mollis and Suaeda maritima). Various experimental models were used for characterization of antioxidant activities of shoot extracts. Eventually, the promising specie was subjected to phenolic identification using RP-HPLC. The analyzed shoot extracts exhibited that antioxidant activities varied considerably as function of species. The highest DPPH center dot scavenging ability was found in S. mollis with the lowest IC50 value (2.5 mu g/ml), followed by S. pruinosa, S. fruticosa and S. maritima. The same tendency was observed with ferric reducing power. Concerning beta-carotene bleaching assays and total antioxidant activity, results showed that S. fruticosa exhibited the highest antioxidant ability against the inhibition of beta-carotene bleaching, and a better total antioxidant capacity. Moreover antioxidant capacities using ORAC method and a cell based-assay showed that S. mollis. S. fruticosa, and S. pruinosa exhibit statistically similar antioxidant activity. These results suggested that Suaeda species showed a variability of their antioxidant activities.(Oueslati.S *et al* 2012)

Phenolics are secondary metabolites that play a role in the maintenance of the human body. The presence of phytoconstituents, such as phenols, flavonoids and tannin in plants, indicates

the possibility of antioxidant activity and this activity will helps in preventing a number of diseases through free radical scavenging activity. Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties. Many studies focused on the biological activities of phenolic compounds, which have potential antioxidants and free radical scavengers (Meenakshi, S., *et al* (2012))

Phenolic compounds are well known as antioxidant and scavenging agents free radicals associated with oxidative damage. Phenolic compounds have attracted much interest recently because *in vitro* studies suggest that they have a variety of beneficial biological properties like anti-inflammatory, antitumor and antimicrobial activities. (Turkoglu *et al., (*2007))

An analysis was made of the species used, parts of the plant employed, preparation methods, administration means, and the ailments treated in relation to pathological groups. A folk botanical survey was carried out. The informants reported data on 122 species, belonging to 49 botanical families, were claimed as medicinal. This work is focused on human medicinal plant uses, which represent 95% of the pharmaceutical uses. The most commonly represented families were Asteraceae (37.5%), Lamiaceae (20.8%), Rosaceae (18.7%), Fabaceae (16.7%) and Apiaceae (14.6%). Some of the uses were found to be new when compared with published literature on ethnomedicine of Iran. The folk knowledge about medicinal plant use is still alive in the studied region, and a number of scarcely reported plant uses has been detected, some of them with promising phytotherapeutical applications. The results of the study reveal that some of species play an important role in primary healthcare system of these tribal communities. Ghasemi Pirbalouti, A. *et al* (2013)

MATERIALS AND METHODS

4.MATERIALS AND METHODS

1. To screen the phytochemicals of *Suaeda maritima*

Materials required

- Methanol- 25 ml
- Hexane- 25 ml
- Chloroform-25 ml
- Distilled water-25 ml
- Whatmann filter paper No.42
- Wagner's reagent -1ml
- Glacial acetic acid -2ml
- Ferric chloride solution -1ml
- 20% sodium chloride -1ml
- 5% aqueous ferric chloride -1ml
- 10% alcoholic ferric chloride -1ml
- Chloroform -5ml
- Acetic anhydride -1ml
- Concentrated sulphuric acid -5ml
- Concentrated hydrochloric acid- 5ml
- Conical flask
- Test tubes

Collection of plant samples

The samples of *Suaeda maritima* were collected from Ennore, Chennai, TamilNadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles. The final uniform powder was used for the extraction of active constituents of the plant material.

Extraction- maceration method

The plant materials were air-dried at room temperature (26˚C) for 2 weeks, after which it was grinded to a uniform powder. The extracts were prepared by soaking 5 g each of the dry powdered plant materials in 25ml of methanol, hexane, chloroform and distilled water at room temperature. The extracts were kept in water bath for 8 hours and centrifuged at 2000

rpm for 20 minutes. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40˚C.

Procedure

Alkaloids

3-5 drops of wagners reagent is added to the extract. The reddish brown colour indicates the presence of alkaloids

Cardiac glycosides

2ml of glacial acetic acid is added to the extract, a drops of ferric chloride solution, 1ml of con.sulphuric acid is added. The brown ring at the interphase indicates the presence of cardiac glycosides.

Flavanoids

20% of NaCl is added to the extract. The yellow colour indicates the presence of flavanoids.

Phenols

5% aqueous ferric chloride is added to the extract. The deep blue or black colour indicates the presence of flavanoids.

Saponins

6ml of water is added to the extract and shaken vigorously. The foam produces indicates the presence of saponins.

Tannins

10% alcoholic ferric chloride is added to the extract. The blue or greenish colour indicates the presence of tannins.

Terpenoids

1 ml of chcl is added to the extract and few drops of con. Sulphuric acid is also added. The reddish brown precipitate indicates the presence of terpenoids.

Steroids

To the extract, a drops of chcl, acetic anhydride and con. Sulphuric acid is added. The dark pink or red colour indicates the presence of steroids.

2. To evaluate the anti oxidant activity of *suaeda maritima*

Materials required

- DPPH solution-100ml
- Hexane-50 ml
- Methanol-50 ml
- Chloroform -50 ml
- Distilled water-100 ml

Collection of plant samples

The samples of *Suaeda maritima* were collected from Ennore, Chennai, TamilNadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles. The final uniform powder was used for the extraction of active constituents of the plant material.

Extraction- maceration method

The plant materials were air-dried at room temperature (26˚C) for 2 weeks, after which it was grinded to a uniform powder. The extracts were prepared by soaking 5 g each of the dry powdered plant materials in 25ml of methanol, hexane, chloroform and distilled water at room temperature. The extracts were kept in water bath for 8 hours and centrifuged at 2000 rpm for 20 minutes. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40˚C.

Procedure

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay:

One ml of 0.135 mM DPPH prepared in methanol was mixed with 1.0 ml of aqueous extract ranging from 0.2-0.8 mg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the seedextract was calculated using this equation;

$$\text{DPPH scavenging activity}(\%) = \frac{(\text{Abs control} - \text{Abs sample}) \times 100}{(\text{Abs control})}$$

Where ,

Abs control is the absorbance of DPPH + methanol;

Abs sample is the absorbance of DPPH radical + sample

3. To evaluate the anti microbial activity of *suaeda maritima*

MATERIALS REQUIRED

- Distilled water-100 ml
- Methanol-50 ml
- Hexane-50 ml
- Chloroform- 50 ml
- Petriplates
- Nutrient broth

PROCEDURE

Collection of plant samples

The samples of *Suaeda maritima* were collected from Ennore, Chennai, TamilNadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles. The final uniform powder was used for the extraction of active constituents of the plant material.

Extraction- maceration method

The plant materials were air-dried at room temperature (26˚C) for 2 weeks, after which it was grinded to a uniform powder. The extracts were prepared by soaking 5 g each of the dry powdered plant materials in 25ml of methanol, hexane, chloroform and distilled water at room temperature. The extracts were kept in water bath for 8 hours and centrifuged at 2000 rpm for 20 minutes. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40˚C.

Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Agar well diffusion method

Petriplates containing 20ml Nutrient medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20µl of the plant extracts (namely aqueous, methanol and chloroform extracts) were added. The plates were then incubated at 37°C for 24 hours.
The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

4. **To preserve the skin sample in different concentrations of *suaeda maritima***

Collection of plant samples

The samples of *Suaeda maritima* were collected from Ennore, Chennai, TamilNadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles. The final uniform powder was used for the extraction of active constituents of the plant material.

Maceration

The plant materials were air-dried at room temperature (26˚C) for 2 weeks, after which it was grinded to a uniform powder. The extracts were prepared by soaking 5 g each of the dry powdered plant materials in 25ml of solvent at room temperature. The extracts were kept in water bath for 8 hours and centrifuged at 2000 rpm for 20 minutes. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40˚C.

Preservation of raw skins

Raw goat skins

↓

Cut into two halves and completely washed

(to remove blood and dirt)

↓

Inclined bench for 30 minutes

↓

10% salt applied for 30 minutes

↓

(salt + crushed leaves, stem and root for different concentrations)

↓

Kept for conditioning

i. Protein estimation-Lowry's method

Materials required:

Alkaline copper sulphate reagent

The reagent contains 250ml solution A, 0.5ml solution B and 1ml of solution C

- Solution A: 2% sodium carbonate solution in 0.1N sodium hydroxide
- Solution B: 0.5% copper sulphate solution
- Solution C: 1% sodium potassium tartarate

Folin's reagent

10 ml of Folin liocalteus phenol is diluted in 20 ml distilled water.

Stock solution

100 mg of BSA in 100 ml of distilled water.

Working standard

10ml stock solution in 100 ml distilled water.

Procedure

5g of sample is suspended in 10ml and kept incubated for 20 minutes. 0.5 ml sample is taken in test tube. 4.5 ml copper sulphate solution,2ml distilled water and 0.5ml folin's reagent were added. The solution is kept incubation for 20 minutes. The optical density were observed at 630 nm.

ii. Hydroxy proline test

Materials required

A stock solution

10 mg hydroxyl proline in 100 ml 0.001N HCl

Standard solution

Diluting stock with water to obtain 2 to 10 µg in 2 ml.

Chloramine-T solution

0.05M solution was prepared finally by dissolving 1.141g of chloramine-T in 20 ml of water. 30 ml of methyl cellosolve (methoxy ethanol) and 50 ml of buffer were added.

Buffer solution

50g of citric acid monohydrate,12 ml of glacial acetic acid, 120 gram of sodium acetate trihydrate and 34 gram of sodium hydroxide were made up to a final volume of 1 litre in distilled water. The pH was carefully adjusted to 6.0 an the buffer was stored in refrigerator.

Perchloric acid solution

3.15M solution obtained by diluting 27ml of 70 % perchloric acid to 100ml.

PDAB solution

20% of solution was prepared shortly before the use of adding cellosolve to 20 gram of PDAB to final volume of 100 ml. This was warmed at 60 degree Celsius to facilitate solubilisation.

Procedure

- 10 mg lyophilized sample was hydrolysed with 5ml of 6N HCl at 110 degree Celsius for 20 minutes in sealed tubes. After hydrolysis the samples were evaporated to dryness and made up to a known volume of water.
- 2ml portion containing 2.10 µg hydroxylproline were placed in test tube and series of standard were prepared containing 0.10 µg hydroxylproline in a total volume of 2ml. Hydroxyproline oxidation was initiated by adding 1ml perchloric acid and allowed to stand for 5 min

- 1ml of PDAB solution was added and the mixture is shaken well. The tubes were placed at 60ºc water bath for 20 minutes and then cooled in a tap water for 5 minutes. The colour developed was read at 537 nm.
- 750µl of collagen sample and 750µl of 6N HCl for hydrolysis were taken in a hydrolysis tube and the tube was sealed. The sealed tube was kept in an oven (110ºc) over night.
- Next day the top of the hydrolysis tube was broken and the content were taken in a water bath (80ºc) and left out for 3 to 4 hours for evaporation.
- Standard stock solutions at different concentrations were added corresponds to different concentrations of hydroxylproline.
- The sample after evaporation in a china dish was made upto 5 ml from which 2 ml of the solution is estimated for hydroxyl proline.
- Chloramines-T was prepared after adding sample and water in test tubes. 7ml of chloramines-T was added to all samples and standards as well as blank which contains 2ml of water. This was left for 20 minutes
- The PDAB was prepared and warmed in water bath at 60ºc for solubilisation
- After 20 minutes, addition of chloramine-T, 1 ml perchloric acid was added and left for few minutes and PDAB was added and kept in water bath for colour to develop and the readings were taken at 557 nm.

iii.Moisture content

Materials required

- China dish
- Skin samples
- Hot air oven

Procedure

5 gram of different day samples were taken and weighed. China dish were taken for different samples and kept it on an oven for 3 hours. After 3 hours the crucible were taken and weighed. The moisture content of the skin is calculated by the following formula

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

iv.Microbial count

Materials method

- Animal skin-3kg

- Sterile water-100ml
- Arbitral shaker
- Test tubes
- Petriplates
- Nutrient agar-1000ml

Procedure

Preserved skin pieces weighing 5g were taken and soaked in 50 ml sterile water. The skin extract was prepared by shaking in an arbitral shaker at 200 rpm for 30 minutes. 1ml of liquid in which the skin pieces had been soaked was taken in 9 ml of sterile water and shaken well to get uniform suspension of bacteria. A volume of 0.1 ml of resulting diluted solution was taken in sterile petriplates and molten nutrient agar at 40ºc was poured and shaken gently to obtain uniform distribution of the bacteria. The plates were incubated at 37ºc for 48 hours. The no. of colonies in the agar medium was counted and the results were tabulated.

v.Leather processing

Material required

- Ammonium chloride-100 g
- Alcoholic bate-100 g
- Sodium bicarbonate- 100 g
- Sulphuric acid- 50 ml
- BCS-50 g
- Sodium formate-50 ml
- Acetic acid-50 ml

Solutions required

Solution A

- Water 100%
- Sx20 2%

Solution B

- SXDI5%
- FB6 5%
- PF18 5%

Solution C

- Sx25 4%
- LP16 4%

- GL 2%
- BLSF0 1%
- EXP 3%
- EAI 2%

Procedure

1. The preserved skin is introduced into soaking. After soaking the hair of the skin is removed by unhairing process.
2. Then the skin is washed with water and 20 ml of the OE solution is poured into the drum and washed twice.
3. Then 100% water of the skin of the skin weight and 2% ammonium chloride is added and allowed to 45 minutes
4. After 30 minutes alcoholic bate 1% to drum and test the skin for tanning process.
5. The water is drained and 100 % water to the skin is added to the drum.
6. 10% of the common salt is added and the run is allowed to run for 1 hour.0.5% formic acid in 5% of water for 2 feeds at 5 minutes interval is added.
7. After 30 minutes, 1% of sulphuric acid is added and 10% of water for 3 feeds at 10 minutes interval is added.The pH value is checked after the pickling process.
8. The skin is incubated for 24 hours. The next day, the drum is allowed to run for 10 minutes.
9. 4% chrome is added by draining 50% of water from the drum.After 30 minutes 4% of chrome is added again and the skin is checked for tanning by cutting the skin.
10. 1.5% sodium formate is added for the basification process.
11. 1% of sodium carbonate is diluted in 10% water and separated into 3 parts and added into the drum by 3 feeds in the interval of 10 minutes.
12. After 30 minutes of the final feed, the pH is tested.
13. Then the dextran fungicide liquid is added and the drum is allowed to run for 5 minutes and washed and packed in a clean cover.
14. The skin is incubated for 12 hours and shaved to remove the unwanted flesh in the skin.
15. 200% of water to the final weight of the skin is added and 0.2% of acetic acid is added by 3 feeds for 10 minutes.

16. Then 5% of BCS is added is allowed for 90 minutes. 200 % of water is added and allowed for 20 minutes. 1% of sodium formate is added and allowed for 30 minutes. 0.5% of sodium bicarbonate is prepared and given for 3 feeds for 10 minutes.
17. After 30 minutes solution A is added and allowed for 20 minutes.
18. Then solution B is added and the drum is allowed for 20 minutes.
19. The dye is added and solution C is added and allowed to run in the drum for 1 hour.
20. 3% of formic acid is added for 2 feeds for 20 minutes and soaked by water and packed.

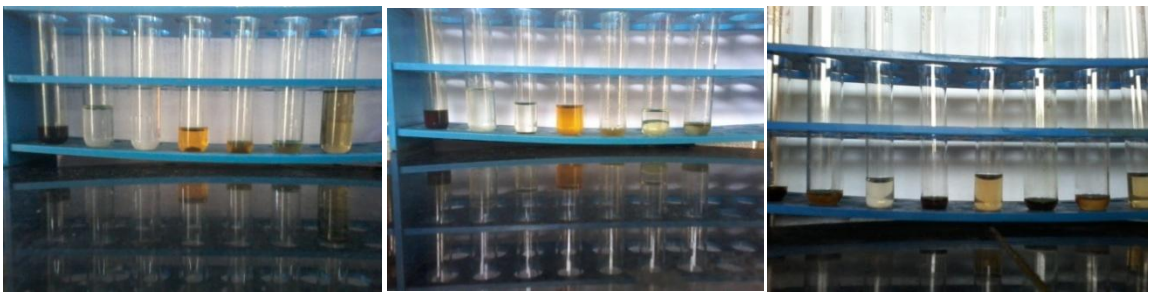
RESULT

5. RESULT

OBJECTIVE 1: TO SCREEN THE PHYTOCHEMICALS OF *Suaeda maritima*

TABLE 1 PHYTOCHEMICAL ANALYSIS

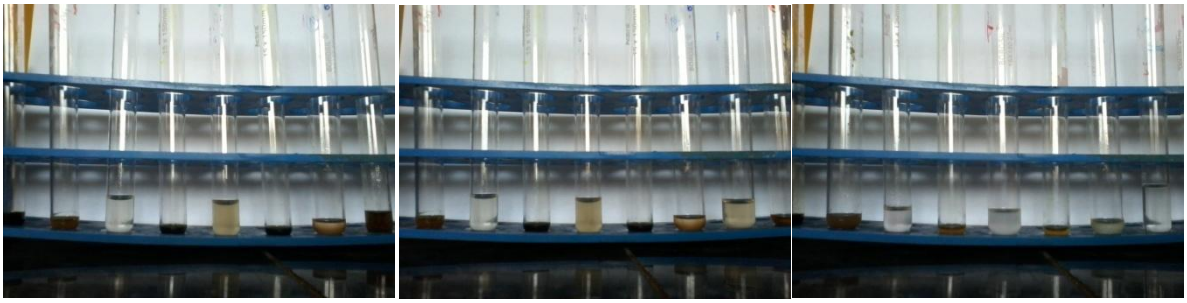
TEST	METHANOL			CHOLROFORM			HEXANE			DISTILLED WATER		
	LEAF	STEM	ROOT	LEAF	STEM	ROOT	LEAF	STEM	ROOT	LEAF	STEM	ROOT
ALKALOIDS	+	+	+	+	+	+	+	+	+	+	+	+
CARDIAC GLYCOSIDES	+	+	-	+	-	+	-	+	-	-	-	-
FLAVONOIDS	+	+	+	+	+	-	+	+	+	-	-	-
PHENOLS	+	-	-	-	-	-	+	-	+	+	-	-
TANNINS	-	-	-	-	+	+	+	-	+	-	-	-
TERPENOIDS	-	-	+	-	-	-	-	-	-	-	-	-
STEROIDS	-	-	+	-	-	-	-	+	-	-	-	-



A B C D E F G A B C D E F G A B C D E F G

(a) (b) (c)

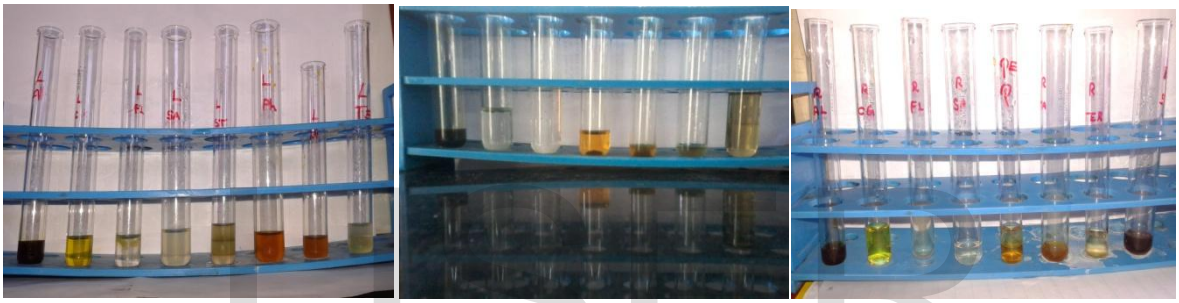
Fig 1.1 shows the methanol extract of phyto chemical analysis of (a) leaf, (b) stem, (c) root of *suaeda maritima ;* A.Alkaloids ,B.Cardiac glycosides, C.Flavanoids, D. Phenols, E.Tannins, F.Terpenoids, G.Steroids



A B C D E F G A B C D E F G A B C D E F G

(a) (b) (c)

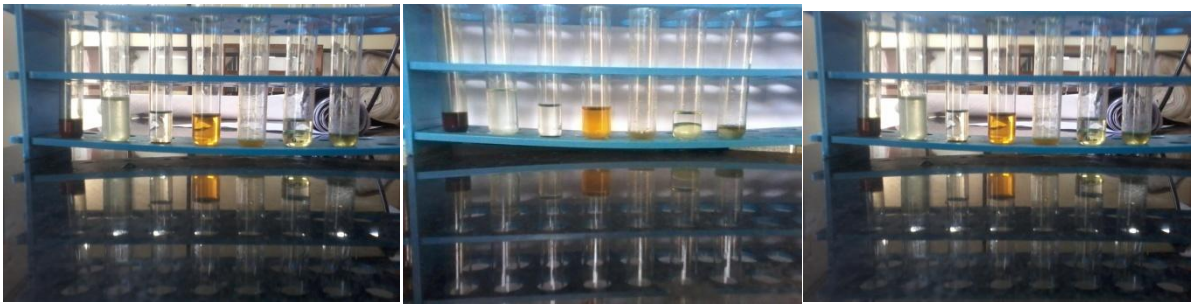
Fig 1.2 shows the chloroform extract of phyto chemical analysis of (a) leaf ,(b) stem, (c) root of *suaeda maritima* . A.Alkaloids ,B.Cardiac glycosides, C.Flavanoids, D. Phenols, E.Tannins, F.Terpenoids, G.Steroids



A B C D E F G A B C D E F G A B C D E F G

(a) (b) (c)

Fig 1.3 shows the hexane extract of phyto chemical analysis of (a) leaf ,(b) stem, (c) root of *suaeda maritima* . A.Alkaloids ,B.Cardiac glycosides, C.Flavanoids, D. Phenols, E.Tannins, F.Terpenoids, G.Steroids



A B C D E F G A B C D E F G A B C D E F G

(a) (b) (c)

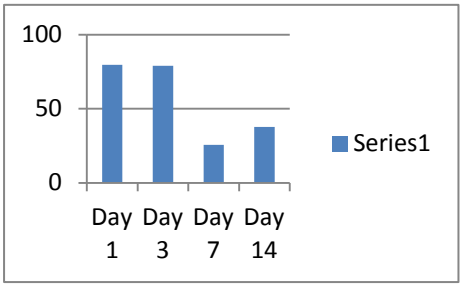
Fig 1.4 shows the distilled water extract of phyto chemical analysis of (a) leaf ,(b) stem, (c) root of *suaeda maritima* . A.Alkaloids ,B.Cardiac glycosides, C.Flavanoids, D. Phenols, E.Tannins, F.Terpenoids, G.Steroids

OBJECTIVE 2: TO EVALUATE ANTI OXIDANT ACTIVITY OF *Suaeda maritima*

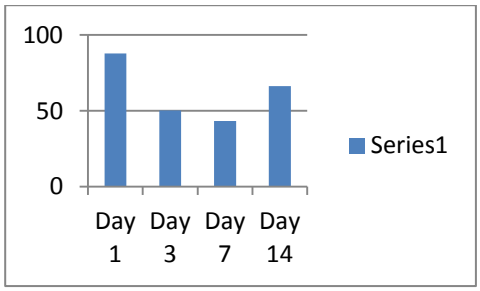
TABLE 2 ANTIOXIDANT ACTIVITY

S.NO	EXTRACT	LEAF	STEM	ROOT
1	METHANOL	79.6	87.7	69.1
2	CHLOROFORM	79.0	50.1	67.4
3	HEXANE	25.5	43.2	5.1
4	DISTILLED WATER	37.7	66.1	69.7

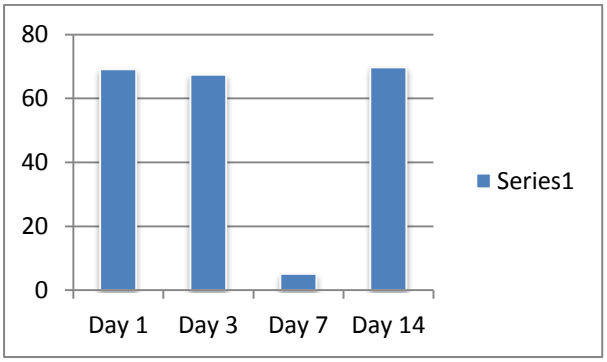
Graph 2.a Antioxidant in leaf Extract(%activity)



Graph 2.b Antioxidant in stem extract (%activity)



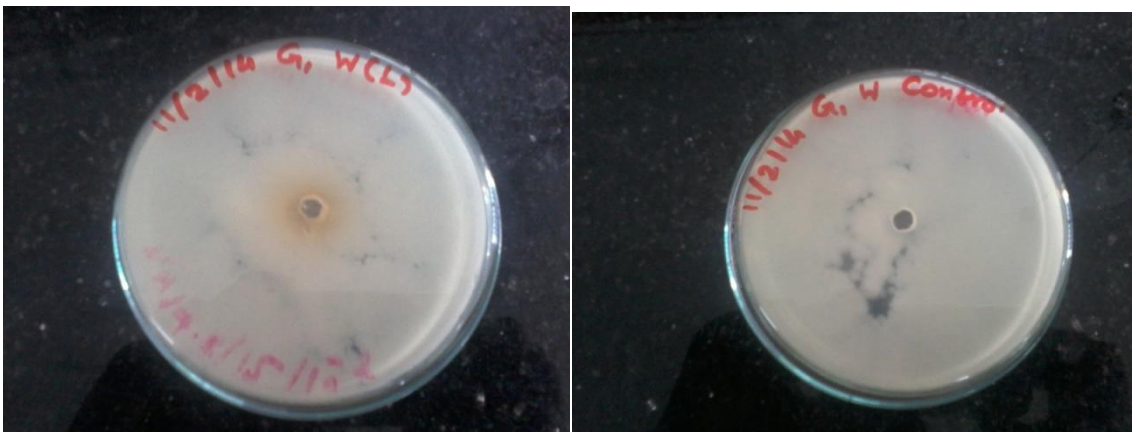
Graph 2.c Antioxidant in root extract(%activity)



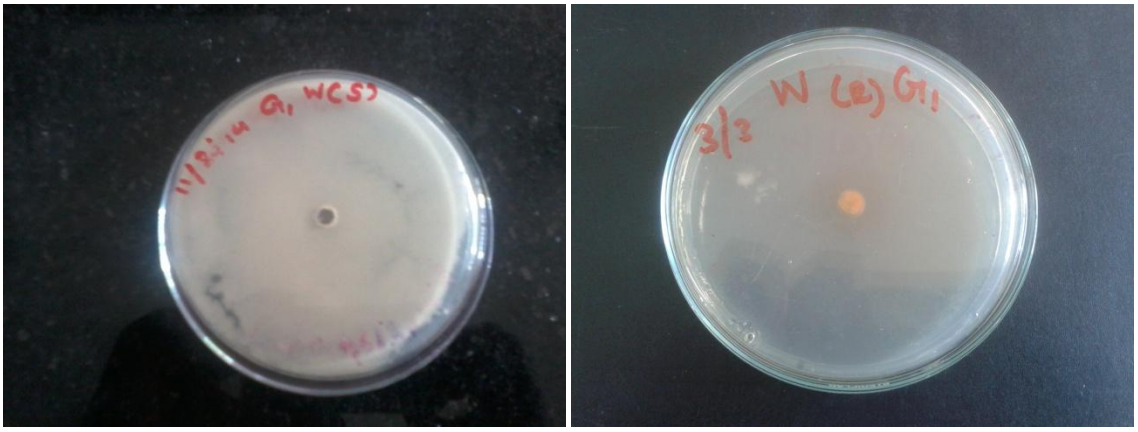
OBJECTIVE 3: TO EVALUATE ANTI MICROBIAL ACTIVITY OF *Suaeda maritima*

TABLE 3 ANTI-MICROBIAL ACTIVITY -ZONE OF DIFFUSION IN mm

BACTERIA	METHANOL				CHOLROFORM				HEXANE				DISTILLED WATER			
	CONTROL	LEAF	STEM	ROOT	CONTROL	LEAF	STEM	ROOT	CONTROL	LEAF	STEM	ROOT	CONTROL	LEAF	STEM	ROOT
E.COLI	nil	1.4	1.2	0.8	nil	1.0	1.6	nil	nil	1.5	1.2	2.0	nil	nil	nil	Nil
BACILLUS	nil	nil	1.2	nil	Nil	nil	nil	nil	nil	0.4	1.5	nil	nil	2.2	1.9	2.5
KLEBSILLA	nil	1.3	1.5	1.0	Nil	1.2	1.1	nil	nil	1.4	1.2	nil	nil	1.0	8.0	3.5
PROTEASE	1.1	1.5	1.8	1.2	2.7	2.2	2.2	2.3	1.0	1.5	2.3	1.0	2.0	2.3	1.7	2.9
PSEUDOMONAS	nil	1.2	2.0	1.3	Nil	1.2	1.4	2.0	nil	0.8	nil	nil	1.8	2.0	nil	1.6

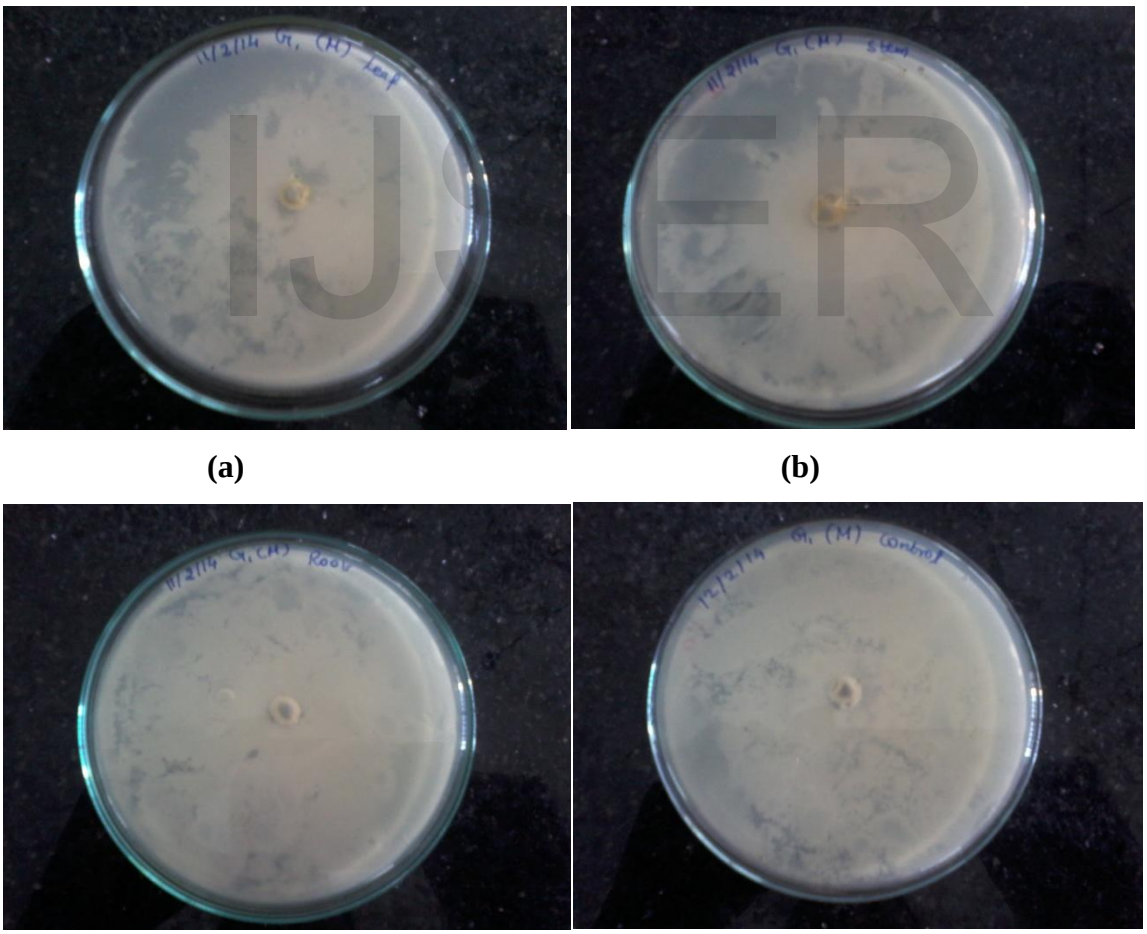


(a) **(b)**



(c) **(d)**

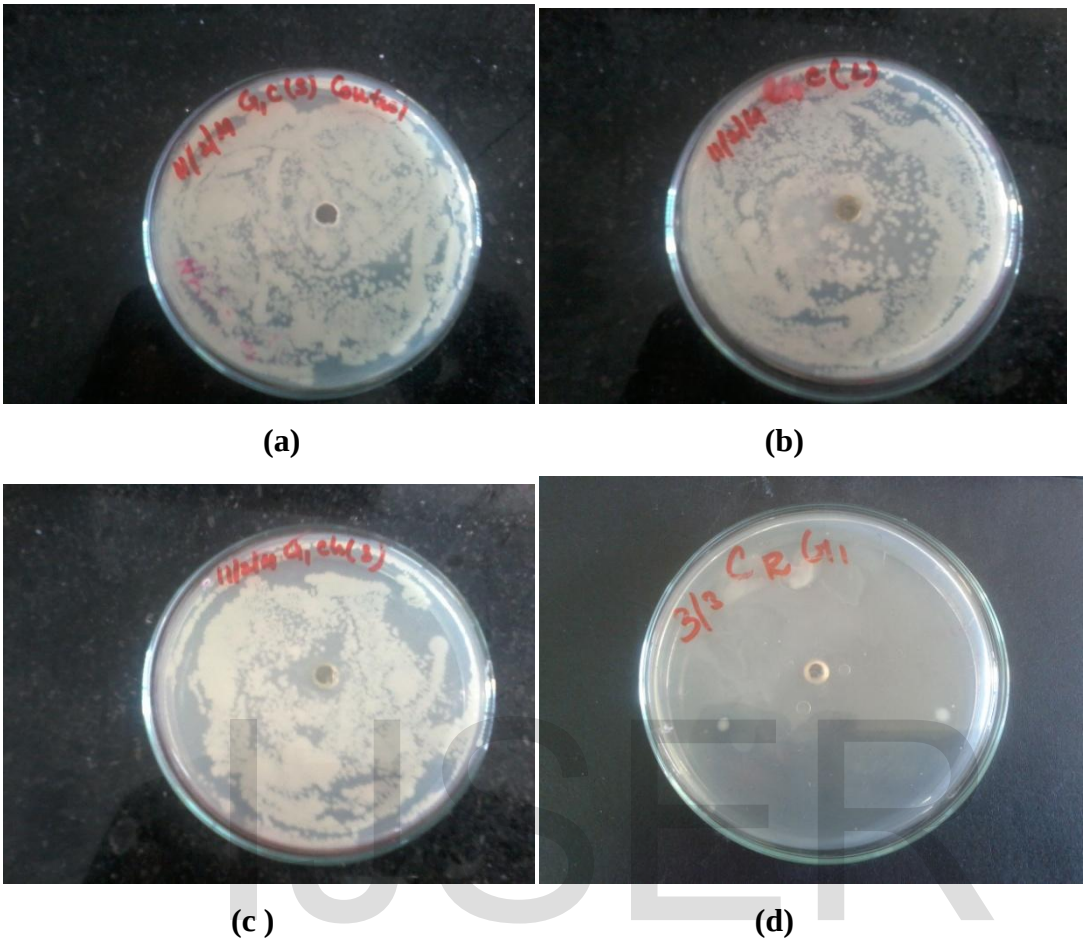
Fig 3.1.1 shows the Anti microbial activity of the E.coli in distilled water extract of *Suaeda maritima* ; (a) leaf, (b) control, (c) stem, (d) root



(a) **(b)**

(c) **(d)**

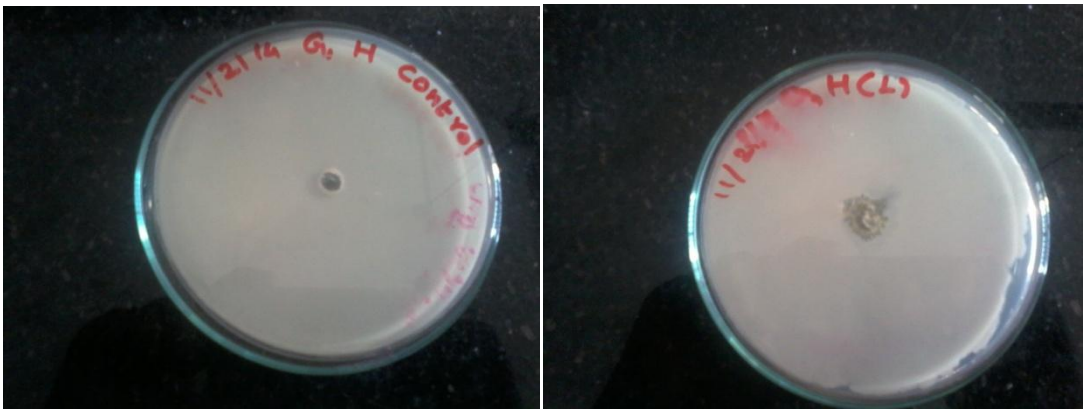
Fig 3.1.2 shows the Anti microbial activity of the E.coli in methanol extract of *Suaeda maritima* ; (a) leaf, (b) stem, (c) root, (d) control



(a) **(b)**

(c) **(d)**

Fig 3.1.3 shows the Anti microbial activity of the E.coli in chloroform extract of *Suaeda maritima* ; (a) control, (b) leaf, (c) stem, (d) root



(a) **(b)**

(c) **(d)**

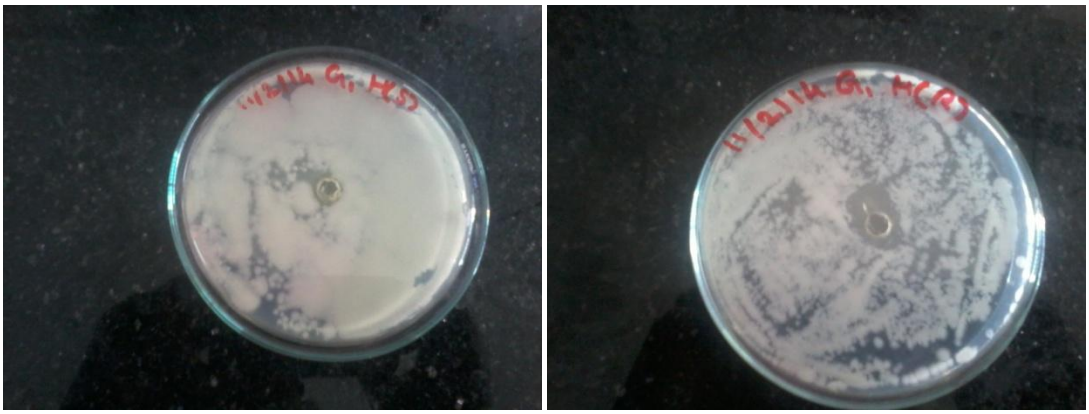
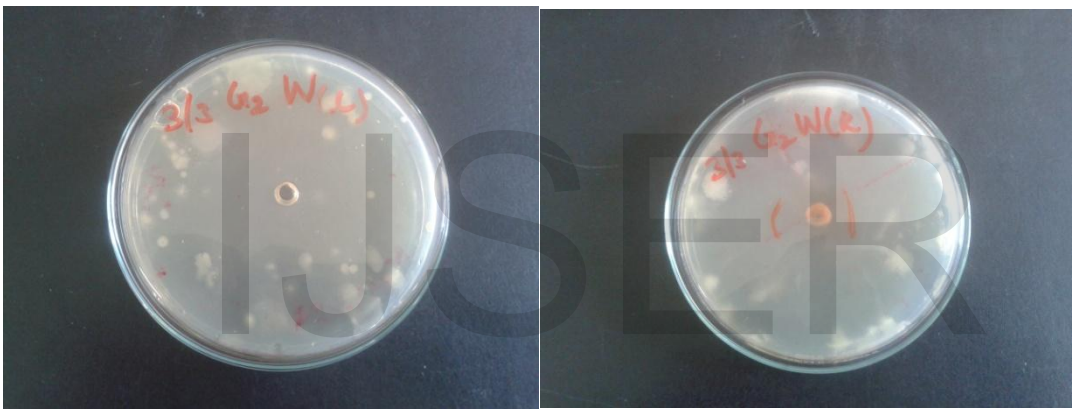
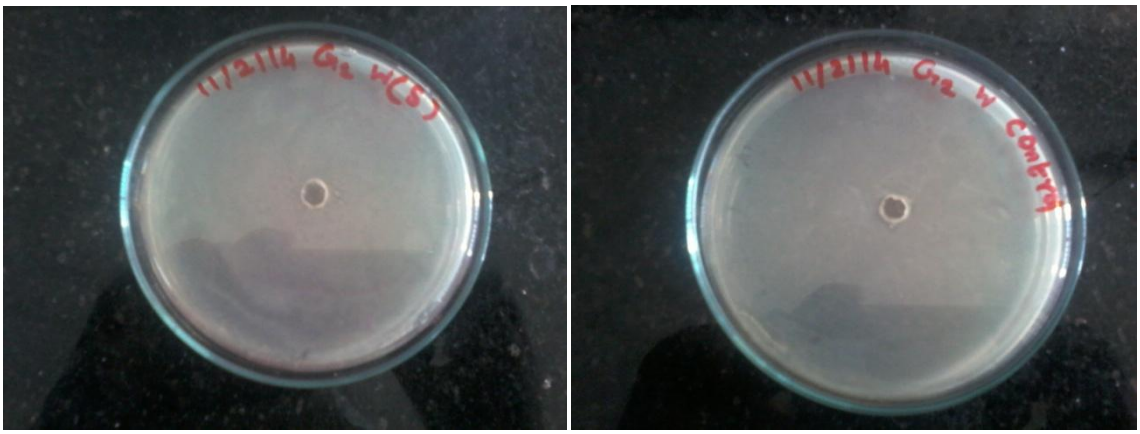


Fig 3.1.4 shows the Anti microbial activity of the E.coli in hexane extract of *Suaeda maritima* ; (a) control, (b) leaf, (c) stem, (d) root

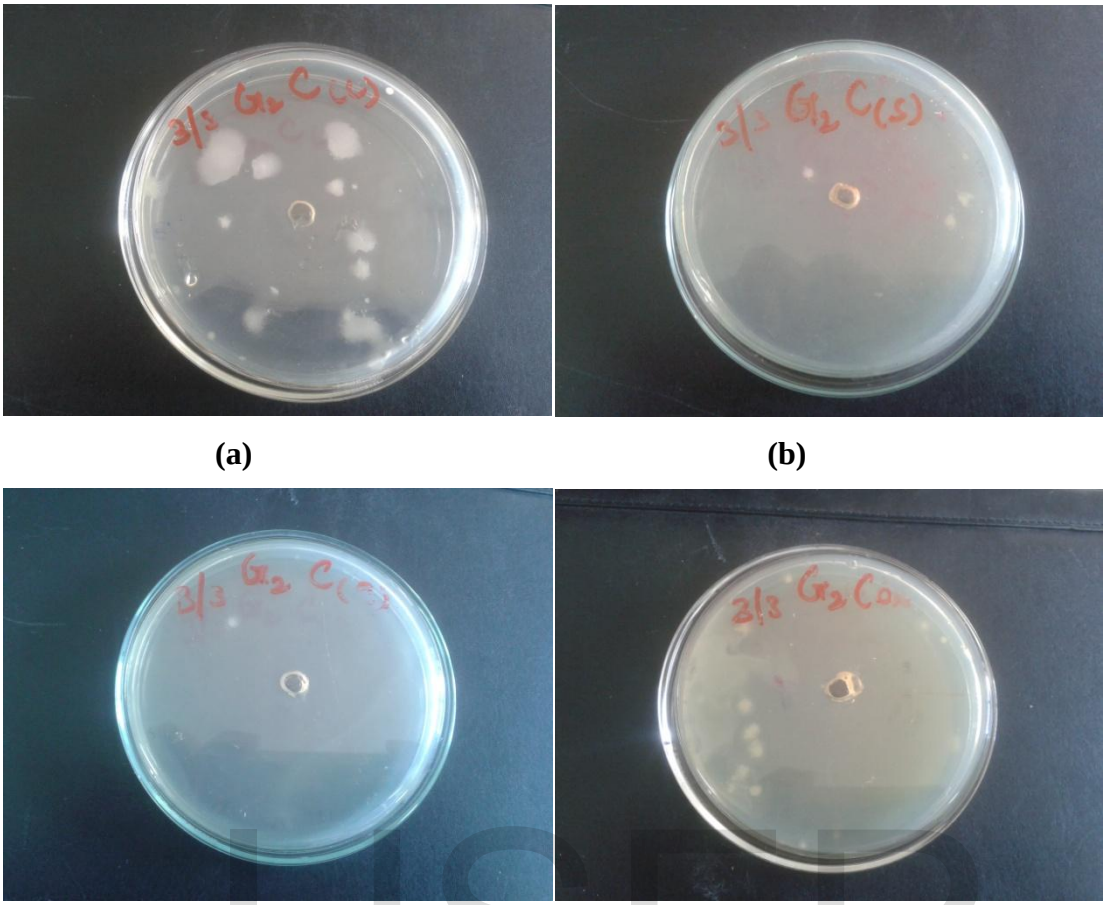


(a) **(b)**



(c) **(d)**

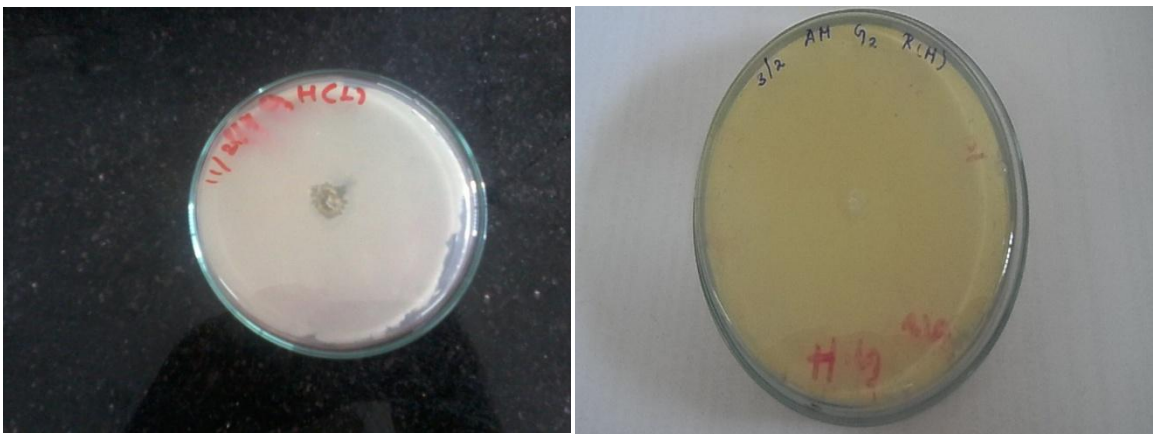
Fig 3.2.1shows the Anti microbial activity of the Bacillus in distilled water of *Suaeda maritima* ; (a) leaf, (b) root, (c) stem, (d) control



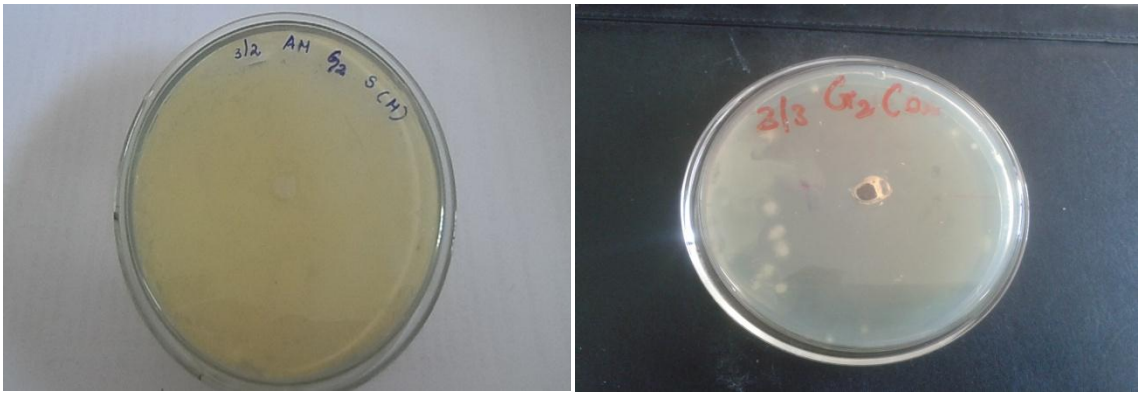
(a) **(b)**

(c) **(d)**

Fig 3.2.2shows the Anti microbial activity of the Bacillus in chloroform extract of *Suaeda maritima* ; (a) leaf, (b) stem, (c) root, (d) control



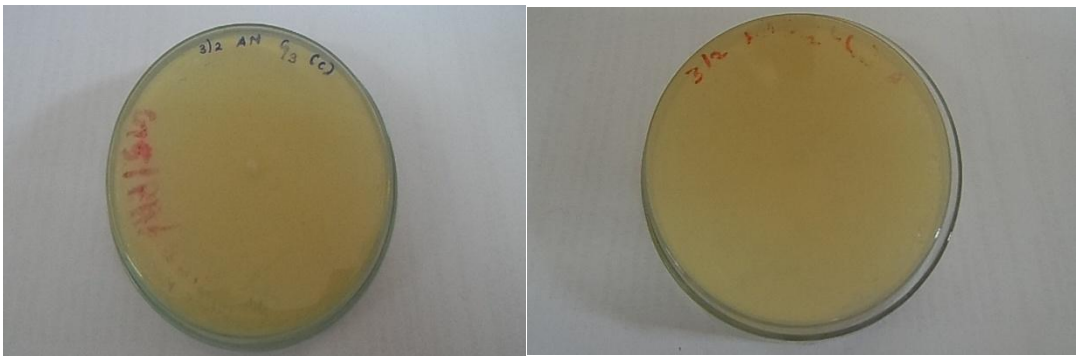
(a) **(b)**



(c) **(d)**

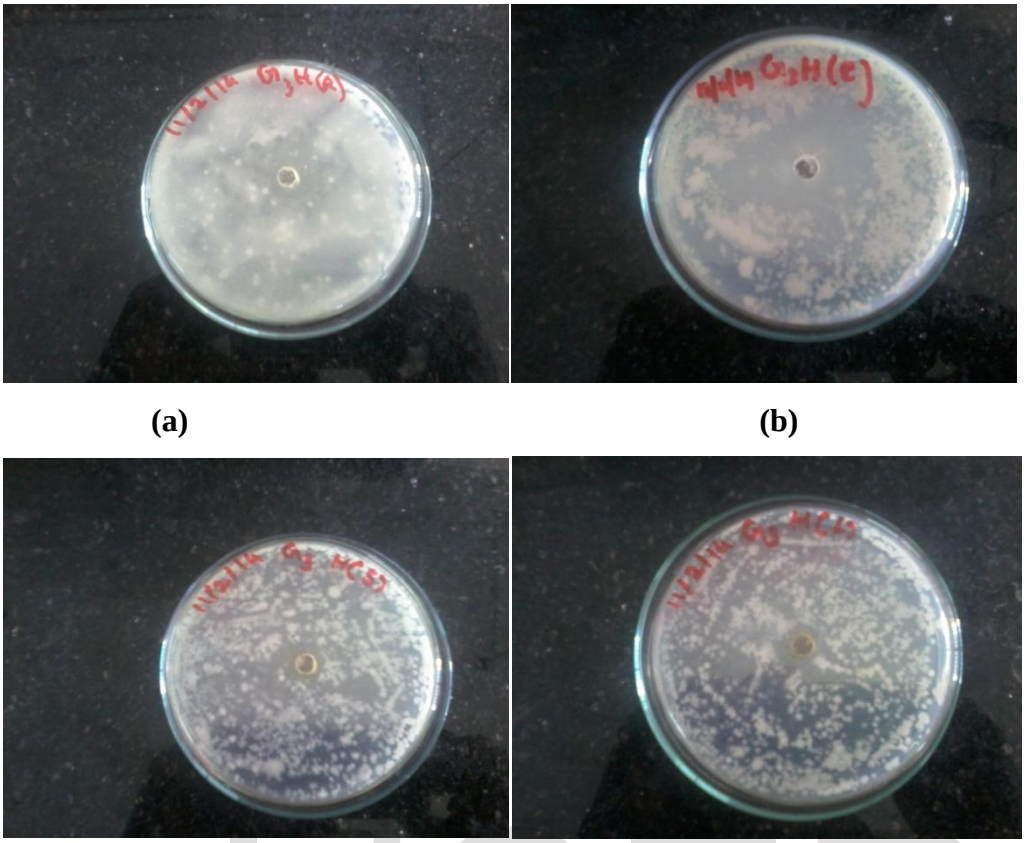
Fig 3.2.3shows the Anti microbial activity of the Bacillus in hexane extract of *Suaeda maritima* ; (a) leaf, (b) root, (c) stem, (d) control

(a) **(b)**



(c) **(d)**

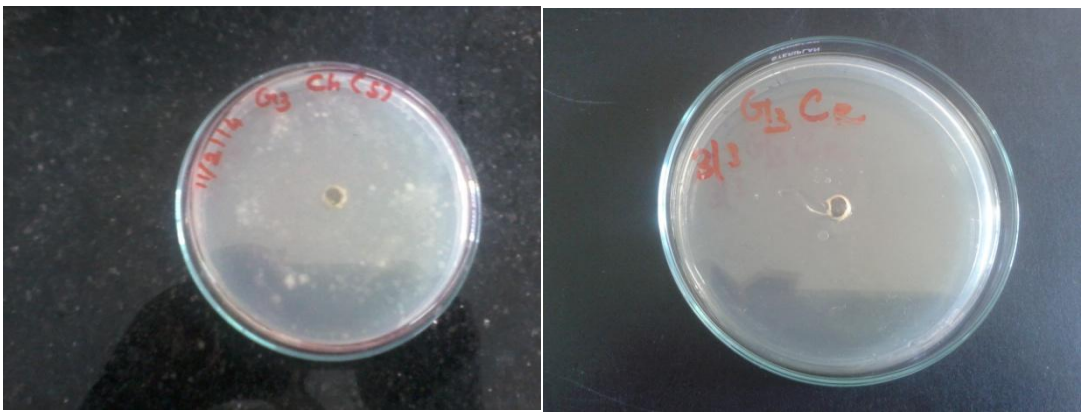
Fig 3.3.1shows the Anti microbial activity of the Klebsilla in distilled water extract of *Suaeda maritima* ; (a) leaf, (b) root, (c) stem, (d) control



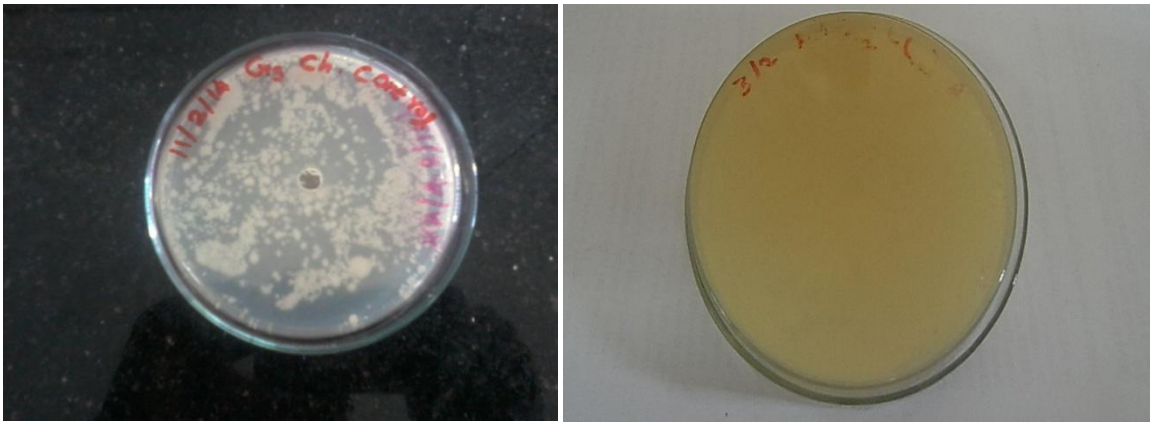
(a) **(b)**

(c) **(d)**

FIG 3.3.2 shows the Anti microbial activity of the Klebsilla in hexane extract of *Suaeda maritima* ; (a) leaf, (b) root, (c) stem, (d) control



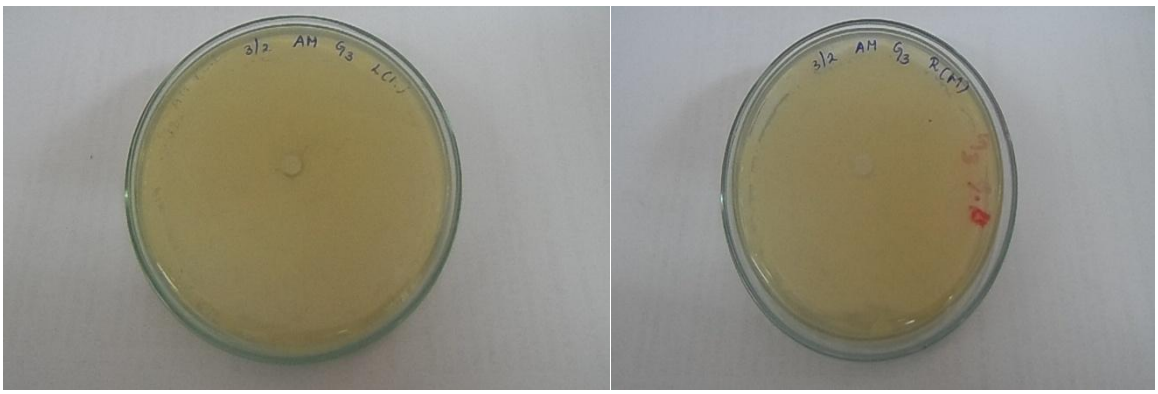
(a) **(b)**



(c) **(d)**

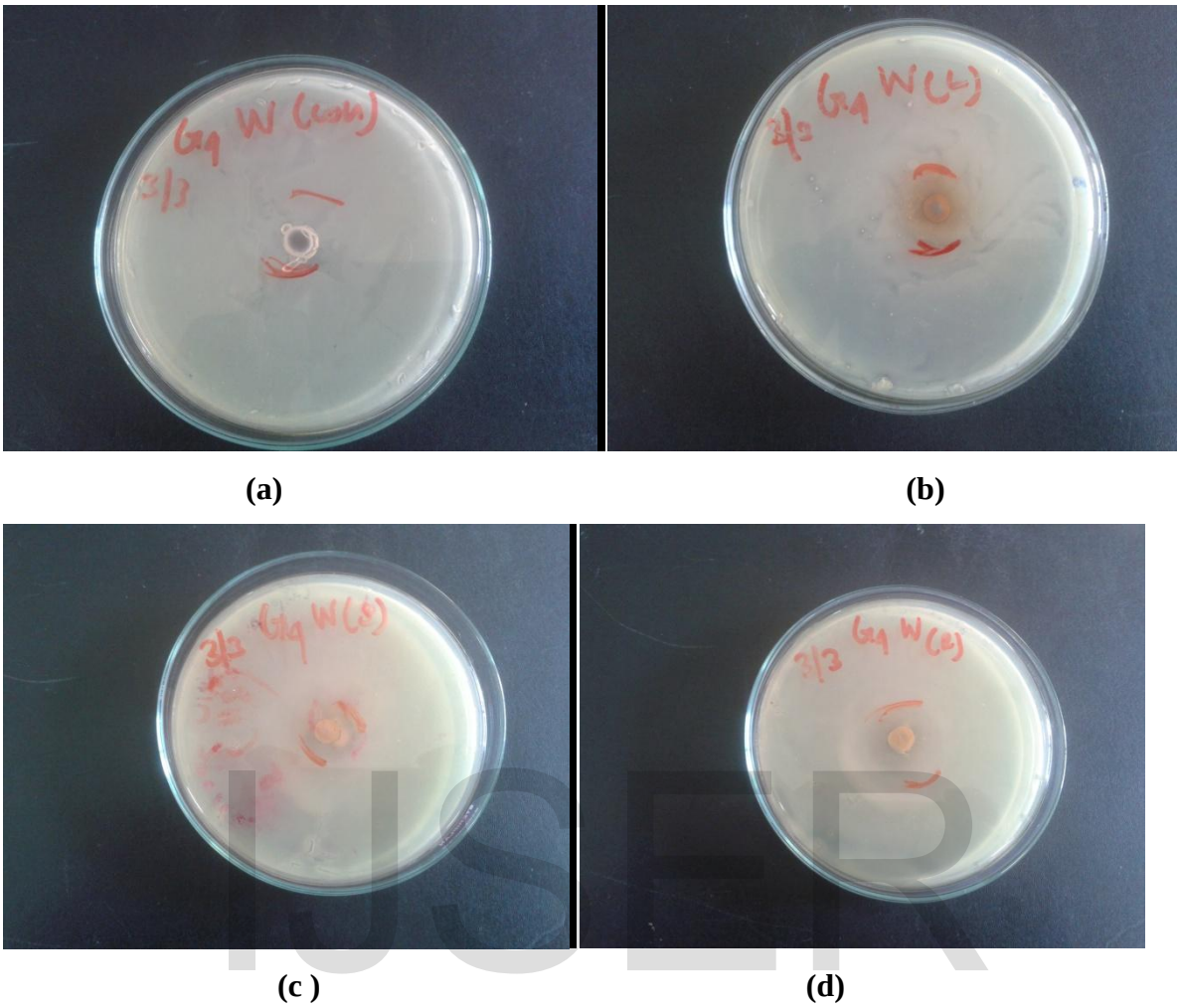
FIG 3.3.3 shows the Anti microbial activity of the Klebsilla in chloroform extract of *Suaeda maritima* ; (a) stem, (b) root, (c) control, (d) leaf

(a) **(b)**



(c) **(d)**

FIG 3.3.4 shows the Anti microbial activity of the Klebsilla in methanolic extract of *Suaeda maritima* ; (a) control, (b) stem, (c) leaf, (d) root

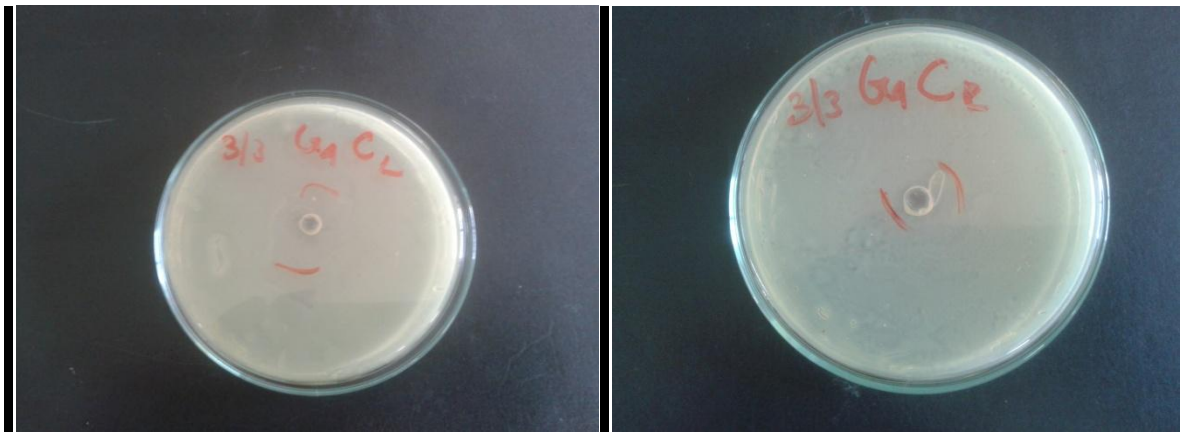


(a) **(b)**

(c) **(d)**

FIG 3.4.1 shows the Anti microbial activity of the Protease in distilled water extract of *Suaeda maritima* ; (a) control, (b) leaf, (c) stem, (d) root

(a) **(b)**



(c) **(d)**

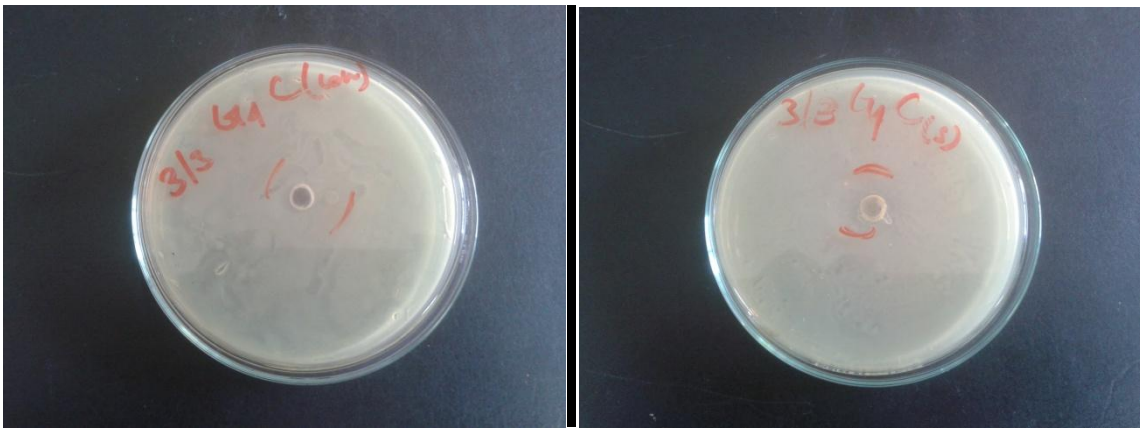
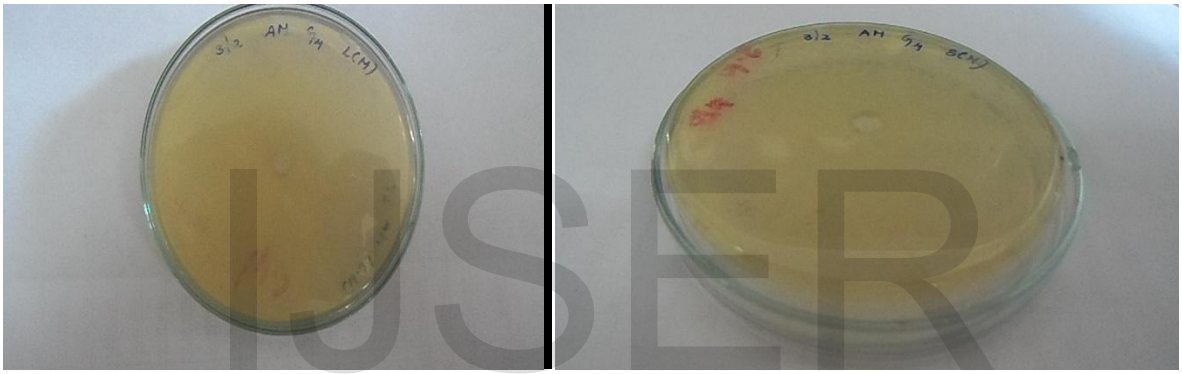
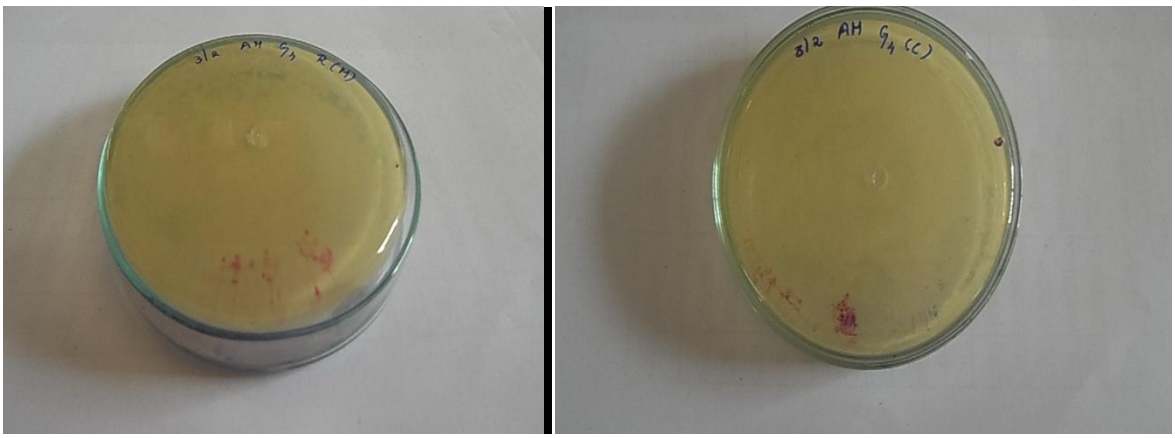


FIG 3.4.2shows the Anti microbial activity of the Protease in chloroform extract of *Suaeda maritima* ; (a) leaf, (b) root, (c) control, (d)stem



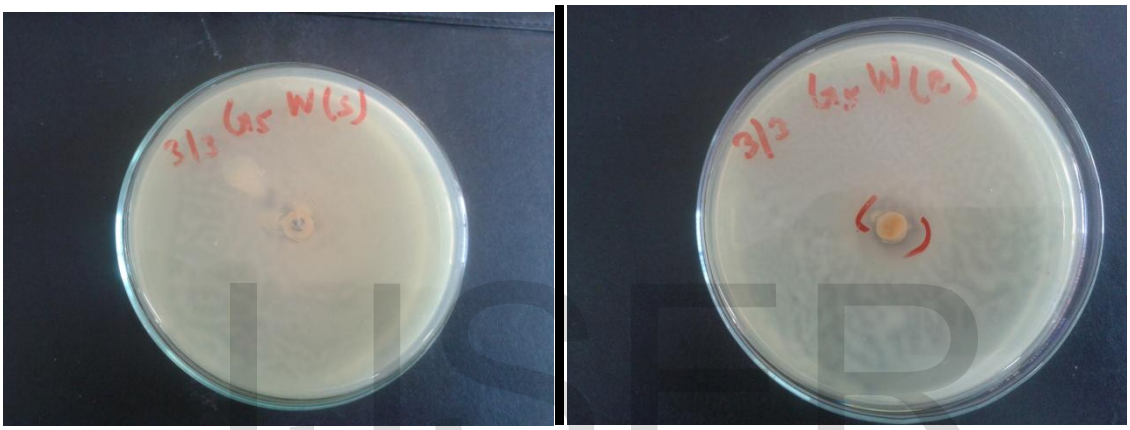
(a) **(b)**



(c) **(d)**

FIG 3.4.3shows the Anti microbial activity of the Protease in methanolic extract of *Suaeda maritima* ; (a) leaf, (b) stem, (c) root, (d)control

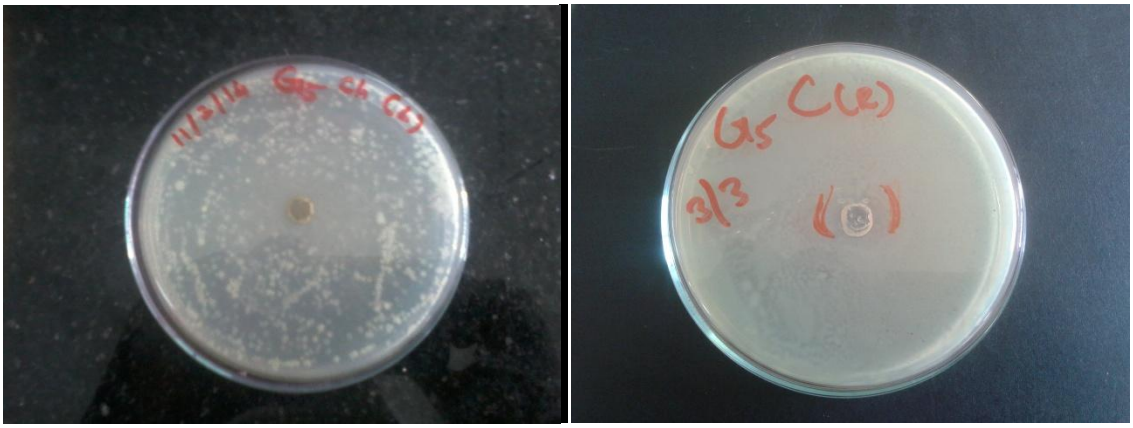
(a) **(b)**



(c) **(d)**

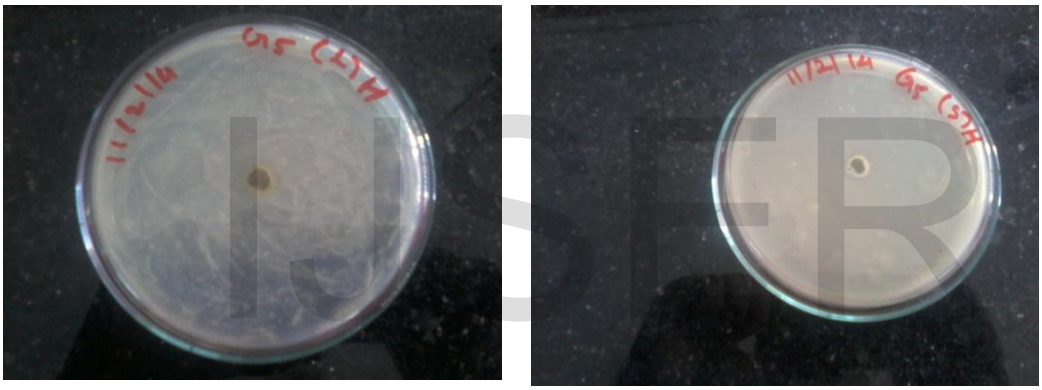
FIG 3.5.1shows the Anti microbial activity of the Pseudomonas in distilled water extract of *Suaeda maritima* ; (a) control, (b) leaf, (c) stem, (d)root

(a) **(b)**

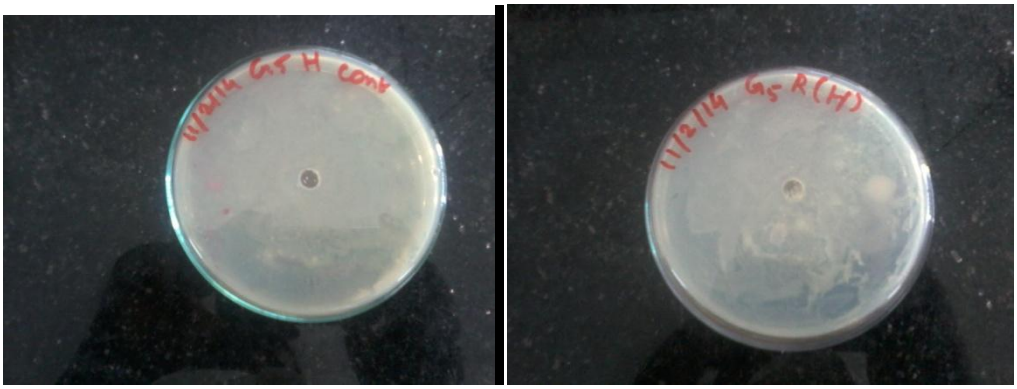


(c) **(d)**

FIG 3.5.2shows the Anti microbial activity of the Pseudomonas in chloroform extract of *Suaeda maritima* ; (a) control, (b) stem, (c) leaf, (d)root



(a) **(b)**



(c) **(d)**

FIG 3.5.3shows the Anti microbial activity of the Pseudomonas in hexane extract of *Suaeda maritima* ; (a) leaf, (b) stem, (c) control, (d)root

OBJECTIVE 4. TO PRESERVE THE SKIN USING DIFFERENT CONCENTRATIONS OF SUAEDA MARITIMA

TABLE.4. PRESERVATION OF SKIN

S.NO	Days	Control	Experiment
1	1st	no hair slip, putrefaction and smell	no hair slip, putrefaction and smell
2	3rd	no hair slip, putrefaction and smell	no hair slip, putrefaction and smell
3	7th	no hair slip, putrefaction and smell	no hair slip, putrefaction and smell
4	14th	no hair slip, putrefaction and smell	no hair slip, putrefaction and smell

Fig 4.1 skin preservation by curing method of *suaeda maritima*

1. 15%Salt,15%leaf **2. 15%salt,10%leaf**

3. 15%salt, 5%leaf

4. 40% salt

5. 10% salt, 15% leaf

6.10%salt, 10%leaf

7. 10% salt, 5% leaf

8.5% salt, 15% salt

9. 5% salt, 5% leaf

10. 15%salt, 15% stem

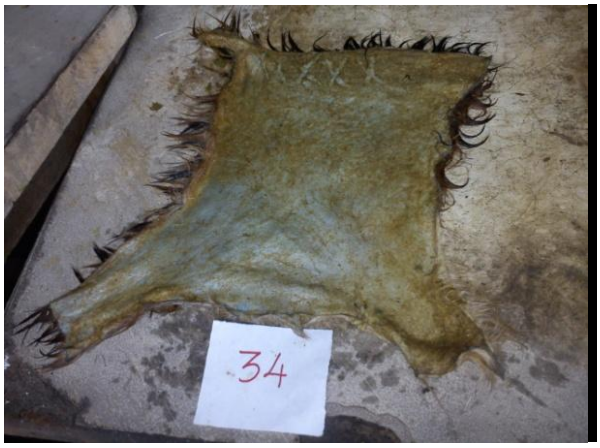
11. 15%salt , 10%stem

12. 15% salt, 5% stem

13. 10% salt , 15% stem

14. 10%salt, 10% stem

15. 10% salt , 5% stem



16. 5% salt, 15% stem

17. 5% salt , 10% stem

18. 5% salt, 5% stem

TABLE 4.1 PROTEIN ESTIMATION (LOWRY'S METHOD)

SAMPLE	DAY 1 (µg/ml)	DAY 3 (µg/ml)	DAY 7 (µg/ml)	DAY 14 (µg/ml)
23(CONTROL)	0.00	0.00	0.00	0.00
20	11.41	11.41	55.89	11.41
21	91.25	114.06	13.69	17.11
22	11.41	11.41	141.44	38.78
24	57.03	22.81	9.13	9.13
25	68.44	45.63	25.09	114.06
26	91.25	193.91	61.59	11.41

27	57.03	45.63	34.22	71.86
28	57.03	307.97	150.56	5.7
29	79.84	250.94	1.14	19.39
30	22.81	216.72	58.17	21.67
31	11.41	68.44	77.56	36.5
32	79.84	296.57	163.11	54.75
33	45.63	182.5	17.11	45.63
34	946.73	193.91	57.03	20.53
35	159.69	273.75	7.30	94.67
36	91.25	581.73	46.77	42.2
37	45.63	273.75	101.52	77.56
38	125.47	342.19	70.72	69.58

Graph 4.1 Protein estimation of skin by lowry's method

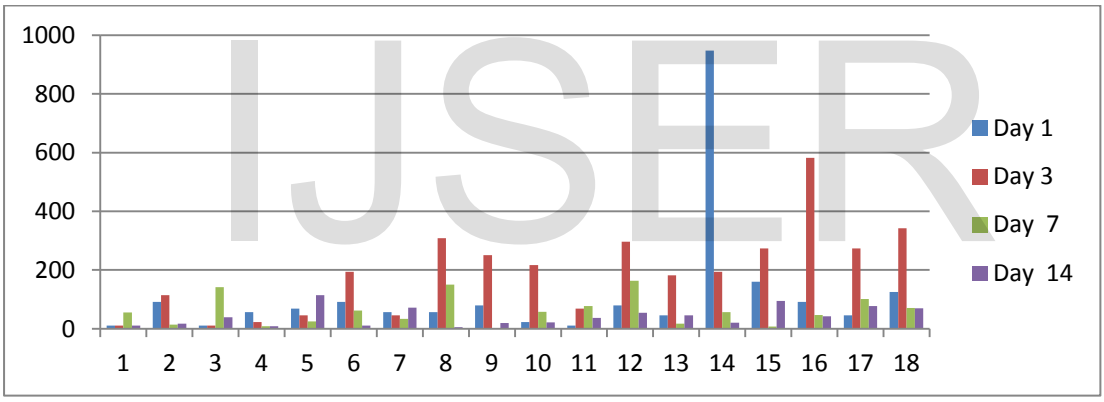


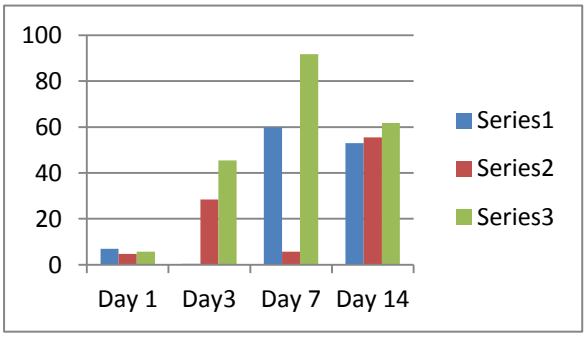
TABLE 4.2 HYDROXY PROLINE TEST

SAMPLE	**DAY 1 (µg/ml)**	**DAY 3 (µg/ml)**	**DAY 7 (µg/ml)**	**DAY 14 (µg/ml)**
23(CONTROL)	0.00	0.00	0.00	0.00
20	6.92	0.45	59.83	52.95
21	4.70	28.50	5.75	55.54
22	5.67	45.48	91.68	61.76

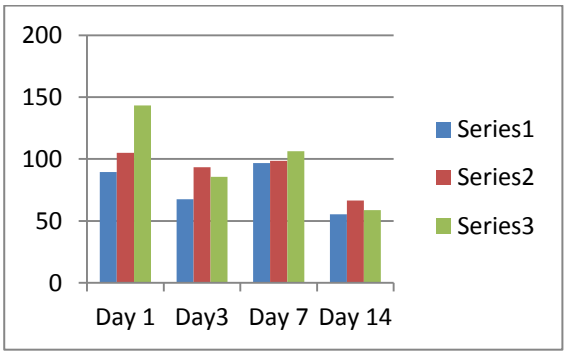
24	3.74	9.52	38.46	67.63
25	8.44	29.66	15.96	55.17
26	45.98	21.51	62.60	72.82
27	9.44	41.8	79.86	65.12
28	6.24	56.88	82.69	59.27
29	9.29	1.23	87.54	63.54
30	130.3	22.18	88.50	67.22
31	98.73	101.7	93.06	79.55
32	104.8	92.22	95.11	80.35
33	89.49	67.61	96.77	55.31
34	104.9	93.30	98.6	66.52
35	143.3	85.69	106.4	58.76
36	167.5	81.48	96.20	76.66
37	101.4	93.59	82.14	58.88
38	83.7	98.80	69.10	47.86

Graph.4.2a hydroxyl proline leaf extract

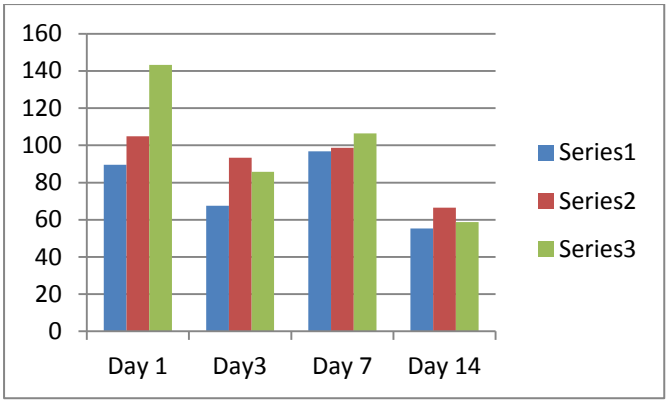
15% salt content



10% salt content

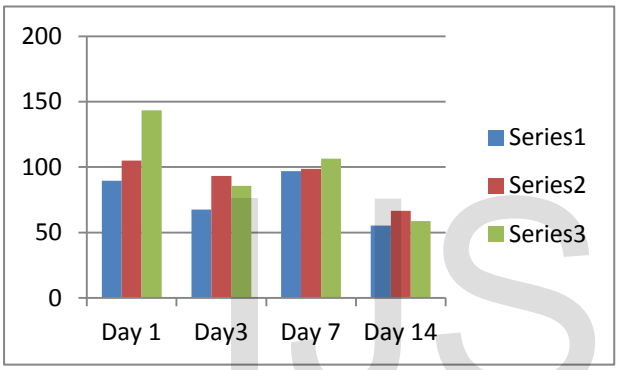


5% salt

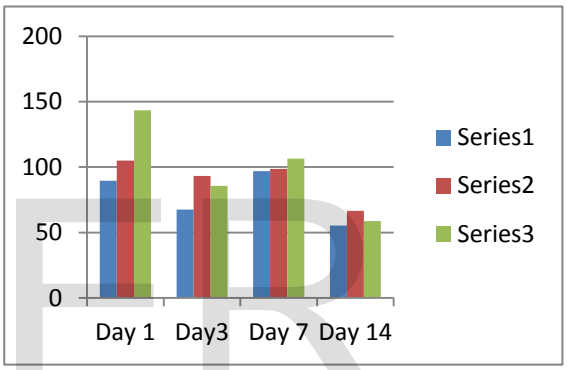


Graph.4.2b hydroxyproline stem extract

15% salt content



10% salt content



5% salt content

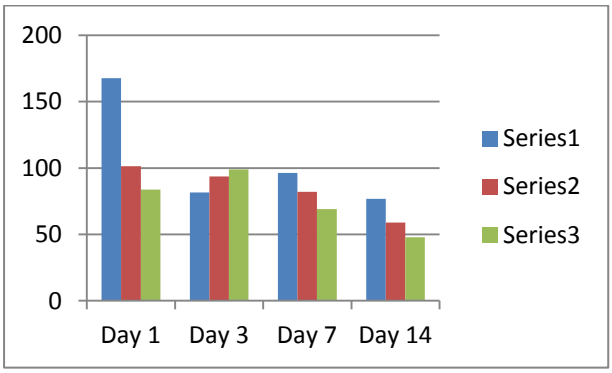


TABLE 4.3 MOISTURE CONTENT

S.NO	Day 1 (mg/ml)	Day 3 (mg/ml)	Day 7 (mg/ml)	Day 14 (mg/ml)
23(control)	9.40	5.94	2.75	6.00
20	8.35	2.53	2.88	4.95

21	11.61	4.95	6.02	7.85
22	5.39	2.50	3.53	8.91
24	9.40	1.82	4.53	1.09
25	13.99	7.71	7.65	6.05
26	10.99	3.75	3.87	2.25
27	0.69	2.64	6.53	1.40
28	16.47	4.80	1.54	3.74
29	4.46	3.83	1.33	3.18
30	10.48	3.37	3.69	2.25
31	6.45	5.92	1.79	1.41
32	7.29	6.19	6.79	0.97
33	6.14	0.13	1.62	5.85
34	8.45	3.24	1.98	2.00
35	6.02	4.17	2.38	5.65
36	4.46	10.77	3.71	3.11
37	9.36	1.48	2.09	2.32
38	3.74	6.12	0.71	5.72

GRAPH 4.3 MOISTURE CONTENT

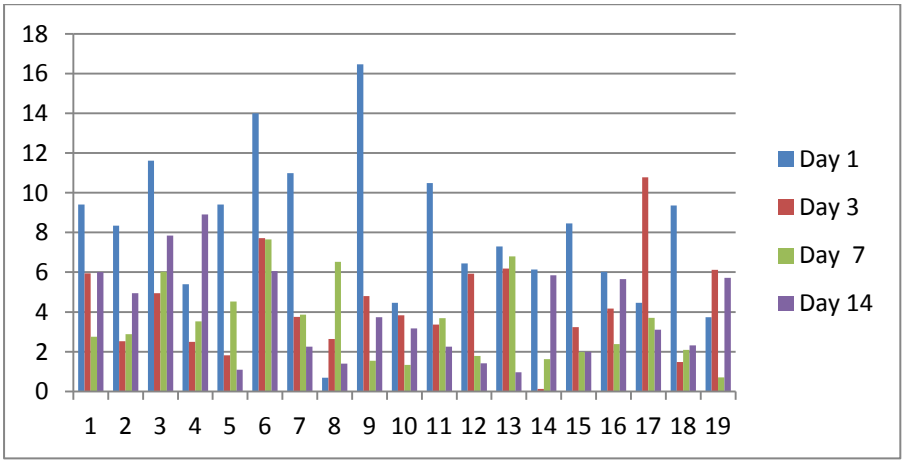


TABLE 4.4 MICROBIAL COUNT

S.NO	Day 1			Day 3			Day 7			Day 14		
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}

23(control)	72	24	4	40	12	0	40	8	0	32	16	40
20	68	44	0	40	24	4	4	16	32	24	0	0
21	24	8	0	80	40	8	108	72	8	0	4	0
22	28	16	8	12	8	0	20	8	0	40	32	24
24	44	16	4	28	24	4	112	60	12	40	12	12
25	32	8	4	28	20	4	104	24	24	40	32	20
26	28	20	12	20	16	4	96	20	32	48	40	40
27	60	32	16	68	16	12	60	20	4	40	40	16
28	108	36	40	40	28	4	84	16	12	20	0	16
29	120	96	16	56	40	36	112	12	28	32	20	20
30	68	28	0	80	16	4	120	76	28	40	20	16
31	120	28	8	68	24	4	100	16	20	56	40	32
32	120	32	28	56	76	16	120	108	36	40	60	32
33	120	104	8	60	16	0	120	104	32	120	100	80
34	36	24	12	120	48	16	120	108	84	52	24	20
35	116	64	24	36	28	24	40	60	16	52	48	40
36	76	16	0	24	12	0	120	88	20	88	80	68
37	20	4	0	48	0	0	120	52	24	120	120	24
38	120	84	36	56	0	0	72	28	16	120	120	120

GRAPH 4.4 MICROBIAL COUNT

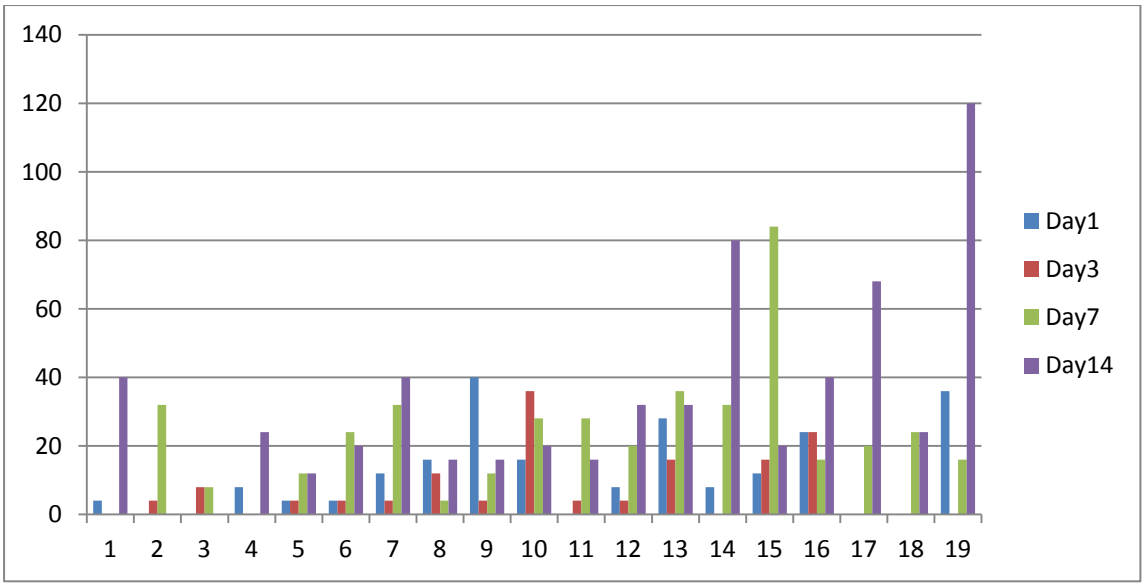
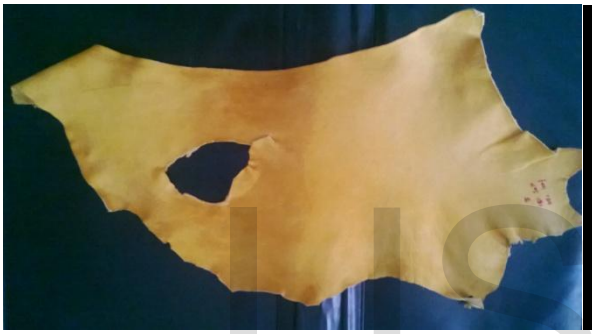


Fig. 4.2finished leather process by the preservation of *suaeda maritima*

1. 15% SALT ,15% LEAF

2. 15% SALT, 10% LEAF

3. 15% SALT, 5% LEAF



4. 40% SALT

5. 10% SALT, 15% LEAF

6. 10% SALT, 10% LEAF

7. 10% SALT, 5% LEAF

8. 5% SALT, 15% LEAF


9. 5% SALT, 10% LEAF **10. 5% SALT, 5% LEAF**


11. 15% SALT, 15% STEM **12. 15% SALT, 10% STEM**


13. 15% SALT, 5% STEM **14. 10% SALT, 15% STEM**


15. 10% SALT, 10% STEM **16. 10% SALT, 5% STEM**

17. 5% SALT, 15% STEM

18. 5% SALT, 5% STEM

DISCUSSION

6. DISCUSSION

In this study, it was found that Alkaloids, cardiac glycosides, flavonoids, phenols, tannins, terpenoids, sterols and saponins were present in the different extract obtained by maceration process using methanol, chloroform, hexane and distilled water of leaf, stem and roots of *Suaeda maritima*.

Studies on antibacterial activity found that *Protease sps.* was inhibited by all the extract of all the plant parts of *Suaeda maritima.* The chloroform extract of stem of *s. maritima* showed maximum zone of clearance of 1.6 cm against *E. Coli*. The distilled water extract of root showed maximum zone of clearance of 2.5 cm against *Bacillus sp.* Distilled water extract of stem showed maximum zone of clearance against *Klebsilla sps.*of 8.0 cm. The methanolic stem extract, chloroform extract of root and distilled water extract of leaf showed maximum zone of clearance against *Pseudomonas sps.*by well diffusion method.

From the observation it is evident that methanolic extract is showing the maximum antioxidant activity in leaf – 79.6, stem – 87.7 and root – 69.1. Than the extract from chloroform, hexane and distilled water from leaf, stem and root parts of *Suaeda maritima* in this present study by*in vivo* antioxidant activity by DPPH radical cation scavenging activities.

The *Suaeda maritima* was tested for its leather application in curing process minimising the salt content. The skins were grouped with salt and plant extract of varying concentration were applied and compared with the control (40% Salt alone).

It was observed for 14days for hair slip, smell and putrefaction and there was no hair slip, no smell and no putrefaction on these days. The samples were collected on the 1, 3, 7 and the 14 day and tested for its moisture content, hydroxyl proline, protein content and microbial load. There was moisture content was normal compared to the control. Only little protein and hydroxyproline were detected in the samples.

The 14days preserved skin was processed into leather and tested for its physical properties and it was even compared with the conventional method.

REFERENCES

7. REFERENCES

1. Baillie, J.K.; Thompson, A.A.R.; Irving, J.B.; Bates, M.G.D.; Sutherland, A.I.; MacNee, W.; Maxwell, S.R.J.; Webb, D.J. (2009). "Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized placebo-controlled trial". *Oxford Journals QJM* **102** (5): 341–8.

2. Bandaranayake.W.M. et al (2009) "Mangrove derived fungal endophytes – a chemical and biological perception" *Fungal Diversity* 61(1);1-27

3. Bashir Ahmad, Ibrar Khan, Shumaila Bashir and Sadiq Azam et al (2012), "Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal and antibacterial activities of the essential oils of Acacia modesta" ,*Journal of Medicinal Plants Research* ;6(31),4653-4659

4. Bjelakovic G; Nikolova, D; Gluud, LL; Simonetti, RG; Gluud, C (2007). "Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis". *PubMed JAMA* **297** (8): 842–57.

5. Chiejina, Nneka V. and Ukeh, Jude A Antimicrobial Properties and Phytochemical (2012) "Analysis of Methanolic Extracts of Aframomum Melegueta and Zingiber Officinale on Fungal Diseases of Tomato Fruit". *Journal of Natural Sciences Research*; 2(6): 64-68

6. Ghasemi Pirbalouti, A., Momeni, M. and Bahmani, M. (2013) "Ethno botanical study of medicinal plants used by kurd tribe in dehloran and abdanan districts, ilam province, Iran" *African journal of traditional,complementary and alternative medicine* 10(2):368-385

7. Jha, Prabhat; Marcus Flather, Eva Lonn, Michael Farkouh, and Salim Yusuf (1995). "The Antioxidant Vitamins and Cardiovascular Disease: A Critical Review of Epidemiologic and Clinical Trial Data". *Annals of Internal Medicine* **123**(11): 860–872.

8. Kanagaraj J, Chandrababu NK (2001) "Alternatives to salt curing techniques", *Journal of science and industrial research*, 61(1) 339-348

9. Kingston W (June 2008). "Irish contributions to the origins of antibiotics". *Irish journal of medical science* **177** (2): 87–92.

10. Manivachagam Chandrasekaran, Krishnan Kannathasan, Venugopalan Venkatesalu (2008)"Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae" *PubMed* 63(5-6):331-6.

11. Meenakshi, S., Umayaparvath, S., Arumugam, M. and Balasubramanian, T. (2012). "In vitro antioxidant properties and FTIR analysis of two seeweeds of Gulf of Mannar." *Asian Pac. J. Trop. Biomed*., 2(10):66 – 70

12. Oueslati.S , Trabelsi.N , Boulaaba.M , Legault.J , Abdelly.C ,Ksouri.R (2008) "Utilization of Halophyte Species as New Sources of Bioactive Substances" *European Journalism Centre* 22(1), 41 -44

13. Rath S.K, Mohapatra.N, Dubey.D, Panda.S.K, Thatoi.H.N and Dutta.S.K.(2009) "Antimicrobial activity of Diospyros melanoxylon bark from Similipal Biosphere Reserve, Orissa, India". *African Journal of Biotechnology*,8 (9),1924-1928

14. Ravikumar, Sundaram; Inbaneson, Samuel Jacob; Sengottuvel, Ramasamy; Ramu, Andy et al (2010) "Assessment of endophytic bacterial diversity among mangrove plants and their antibacterial activity against bacterial pathogens" *Annals of Biological Research*, 1(4) ;240-247

15. Sumitra Singh, Rajinder Mann and Surendra Kr. Sharma et al (2007) "Anti hyperlipidemic activity of sueada maritiima (L) Dumortier stem in riton induced rats ayurveda pharm" *International journal of research in ayurveda and pharmacy*4(2); 1-5

16. Turkoglu ,Jose and Radhamary, 2012 "Identification and determination of antioxidant constituents of bioluminescent mushroom" *Asian Pac. J. Trop Biomed.,* 2(4)386 - S391.

17. Wainwright.M (1989). "Moulds in ancient and more recent medicine". *Mycologist* **3** (1): 21–23.