Research Article **CODEN: IJPRNK** **ISSN: 2277-8713**
Mohan VR, IJPRBS, 2013; Volume 2(6): 140-155 **IJPRBS**

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

PHARMACOCHEMICAL CHARACTERISATION AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF LEAF OF *SESUVIUM PORTULACASTRUM*. L (AIZOACEAE)

M. PACKIA LINCY, K. PAULPRIYA, VR MOHAN
Ethnopharmocology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin-628008, Tamil Nadu, India.

Accepted Date: 01/11/2013; Published Date: 27/12/2013

Abstract: The pharmacochemical characterisation and *in vitro* antibacterial activity of the leaf of *Sesuvium portulacastrum*. L. were investigated. The total ash value of leaf of *S.portulacastrum* is 9.86%. The extractive value of water is more than the other solvents investigated. Preliminary phytochemical screening of leaf showed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, steroid, tannins, terpenoid, xanthoprotein, and sugar in the methanol and ethanol extracts. The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of leaf of *S.portulacastrum* were tested against *Bacillus thuringiensis*, *Bacillus subtilis, Streptococcus faecalis, Streptococcus pyogens, Staphylococcus aureus, Staphylococcus aureus* (Methicillin sensitive), *Enterococcus faecalis, Salmonella paratyphi* A and B, *Salmonella paratyphi, Proteus mirabilis, Proteus vulgaris, Escherichia coli, Escherrichia Escherichia coli (ESBL), Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas aeruginosa (ESBL) and Mycobacterium smegmatis,* by the agar disc diffusion method.

Keywords: *Sesuvium portulacastrum*, phytochemical, antibacterial, fluorescence, *B. subtilis*

PAPER-QR CODE

Corresponding Author: MR. MOHAN V. R.

Access Online On:

www.ijprbs.com

How to Cite This Article:

Mohan VR, IJPRBS, 2013; Volume 2(6): 140-155

INTRODUCTION

The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all countries have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants [1].

Plants have the major advantage of being effective and cheaper alternative source of drugs [2]. The local use of natural plants as primary health remedies, due to their pharmacological properties is quiet common in Asia, Latin America and Africa. Medicinal plants contain substances that can be used for therapeutic purposes or which are used as precursors for the synthesis of useful drugs [3,4]. Potential of higher plants as source of new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological and pharmacological screening is even smaller.

Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics.

More than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious disease. A vast knowledge of how to use the plants accumulated in areas where the use of plants is still of great importance [5]. The medicinal value of plant lies in some chemical substances present in them. The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds [6].

In India, many plants are widely used by all section of people either directly as folk remedies or indirectly in pharmaceuticals preparations of modern medicine. To determine the potential and promote the use of herbal medicine; it is essentially required to intensity the research to produce few antimicrobial agents from plants as on alternative to available antibiotics because they are effective against resistant pathogen of plants and animals to avoid to treat of post antibiotic era. Many pharmaceutical innovations are developed from a starting point of knowledge derived from the biological activities of natural organisms. Global level plants have been examined for their phytochemical properties, antimicrobial and pharmacological activity and they proved their fruitfulness [7,8,9,10].

Mangroves are a diverse group of trees that grow in intertidal tropical forests. In mangrove species, phenolics are abundant components, which prevent damage from herbivore [11,12] but they also exhibit a diversity of other biological activities of historic and potential importance to

humans [13]. Commercial use of mangroves as source of timber, fuel, has long been recognized in tropical coastal zones. Besides, mangrove also provided many non-timber products such as tannin, fish poison, medicine, food, fodder, etc [14] . They have been used as traditional medicine in South Asian Countries including India. Recently scientists are veering in search of effective remedies from mangroves for diseases such as diabetes, asthma, cancer, ulcer, wounds and AIDS [15,16,17]. Mangrove extracts have been used for diverse medicinal purposes and have a variety of antibacterial, antiherpetic and antihelminthic activities [18,19].

A number of mangroves and associates contain substances which show biological activities such as antiviral, antibacterial and antifungal properties [20,21,22]. The leaf extracts of *Bruguiera cylindrica* and bark of *Rhizophora mucronata* show antiviral activity against Newcastle disease, vaccinia and hepatitis B viruses. Mangroves are widely used by mangrove dwellers for bush medicine e.g. *Acanthus illicifolius* is used for skin disorders, boils and wounds [23]. Numerous medicines derived from mangroves (ashes or bark infusions) can be applied for skin disorders e.g. *Lumnitzera racemosa* and sores including leprosy.

Sesuvium portulacastrum(L.) (Seapurslane) is one of the fast growing, herbaceous, perennial, dichotomous, halophyte belonging to family Aizoaceae. In India it grows at the eastern and western coastal sides as a mangrove associate [24]. The plant has remarkable ability to survive under different stress conditions. Chanrasekaran *et al* [25] expressed the fatty acid methyl esters (FAME extract) from *S. portulacastrum* can be used in traditional medicine as a potential antimicrobial agent.

Perusal of literature survey reveals that, information on the pharmacochemical characterization and antibacterial studies on halophytes from Tuticorin coast, Tamil Nadu are totally lacking. With this background an attempt was made to evaluate the pharmacochemical characterization and *in vitro* antibacterial activity of leaf of *S. portulacastrum*.

MATERIALS AND METHODS

The leaf of *S. portulacastrum* was collected from Tuticorin coast, Gulf of Mannar, Tamil Nadu. 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 by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

(i) Determination of physicochemical parameters

Determination of physicochemical parameters, such as ash and extractive values were done following the methods of Kalpana devi *et al* [26] and Mohan *et al* [27] .The behaviour of the

powdered leaf with different chemical reagents was studied and the fluorescence character was observed under UV light [28]

(ii) Preparation of extracts for phytochemical screening and antimicrobial activity

Freshly collected leaf samples of *S. portulacastrum* were dried in shade, and then coarsely powdered separately in a willy mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures [29,30,31]. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for antibacterial activity.

(iii) Microorganisms

Bacterial strains of *Bacillus thuringiensis*(+), *Bacillus subtilis*(+), *Streptococcus faecalis*(+), *Staphylococcus aureus*(+), *Staphylococcus aureus* (Methicillin sensitive) (+),*Streptococcus pyogenes* (+), *Enterococcus faecalis*(+), *Salmonella paratyphi-A & B*(-), *Salmonella paratyphi*(-), *Proteus mirabilis*(-), *Escherichia coli*(-),*Escherichia coli* (ESBL) (-), *Proteus vulgaris*(-), *Klebsiella pneumoniae*(-), *Serratia marcescens*(-), *Pseudomonas aeruginosa*(-),*Pseudomonas aeruginosa* (ESBL) (-) and *Mycobacterium smegmatis(+) bacterial strains obtained from Department of Microbiology*, Bharathidasan University, Trichy, Tamil Nadu, India. The bacteria were incubated on a nutrient agar-slant (Stationary cultures) for 48h at 37ºC, followed by inoculation in Muller Hinton Agar (MHA) medium.

(iv) Antibacterial assay

Antimicrobial study was carried out by disc diffusion method [32] against the pathogens. A loopful of bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram Positive and Gram Negative bacteria. Respective solvents without plant extract served as negative control. Standard antibiotic of tetracycline (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37 ^{0}C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone and antibacterial activity against the pathogenic bacteria were recorded. The experiments were repeated in triplicate and the results were documented.

RESULTS

The results of the ash and extractive values of leaf of *S.portulacastrum* are depicted in Table-1. The total ash value of leaf is 9.86%. The extractive value of water is more than the other solvents investigated. The results of fluorescence analysis of leaf of *S.portulacastrum* are shown in Table-2. The powder of *S. portulacastrum* leaf fluoresced dark brown under day light and short UV light, deep blue under long UV light. The leaf of *S.portulacastrum* shows the characteristic fluorescent green colour treated with concentrated HCl, aqueous NaOH, Con HNO_3 and 50% H_2SO_4.

Petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S.portulacastrum* leaf were examined for their phytoconstituents and *in vitro* antibacterial activity against the human pathogenic bacteria and the results are given in the table 3 and 4. The results of the phytochemical screening revealed that alkaloids, coumarin, flavonoids, phenols, quinones, steroids, terpeniods, glycosides, xanthoprotein, sugar present in the five extracts tested. The *in vitro* antibacterial activity has been observed in the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S.portulacastrum* leaf against the gram positive and gram negative human pathogenic bacteria. All the extracts studied in the present investigation exhibited varying degree of inhibitory effect against all the tested human pathogenic bacteria. The maximum zone of inhibition was 25mm for *S. paratyphi* -B, and 19mm for *B. subtilis*, *P. aeruginosa* (ESBL) and *E.coli* (ESBL) in ethanol extract; 16mm *S. paratyphi-A* and *E.coli* and 15mm for *P. vulgaris* in methanol extract; 21mm for *E.coli* (ESBL) and 16mm for *S.aureus, S.paratyphi* and *S.aureus* (Methicillin sensitive) in ethyl acetate extract; 16mm and 14mm for *B.thuringiensis* and *E.coli* (ESBL) in benzene extract; 16mm for *K.pneumoniae* and 10mm for *S.paratyphi*-B and *E.coli* (ESBL) in petroleum ether respectively. The other pathogens showed the inhibition zone ranged between 9mm to 5mm. Antibacterial activity was comparable with that of positive control tetracycline (35mm to 18mm) against the organisms tested.

DISCUSSION

Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained [33,34,35]. Today with the present surge of interest in the phytotherapeutics, the availability of genuine plant material is become scarce. Since crude plant drugs from the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential part of its study. It becomes extremely important to make an effort towards standardization as the plant material as medicine.

Studies of physicochemical constant can serve as a valuable source of information and are usually used in judging the purity and quality of the drug. Extractive values are representing the presence of polar or nonpolar compounds. Ash values are indicating the purity of drug. The ash

value of any organic material is composed of their non volatile inorganic components. Control and incineration of crude drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose for evaluation of crude drugs. In certain drug, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted adulterants of drug, sometime posses a character that raises the ash value. A high ash value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for making [36].

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and solvent used. The use of a simple solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also gives the information regarding the quality of the drug. The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacological parameters and standards must be established [37]

Phytochemical screening is usually carried out to screen and to characterized the constituents available in a given plant sample. Generally in the phytochemical screening of any plant one normally identifies secondary metabolites that have accumulates to some extent at specific organ of the plant. These metabolites that are mainly used by the plant for protection against herbivores may have pharmacological activity when tested on animals [38]. Result of phytochemical screening of the leaf of *S.portulacastrum* of the various extracts showed the presence of alkaloids, steroids, coumarins, catachins, tannins, phenols, flavonoids, saponins, glycosides and xanthoprotein and sugar. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against prediction by many microorganisms, insects and other herbivores [39]. The present study carried out on the plant samples revealed the presence of medicinally active constituents. Analysis of plant extracts revealed the presence of coumarins, flavonoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antimicrobial property. These bioactive compounds are known to act by different mechanism and exert antimicrobial action.

Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is

probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [40]. Coumarins are also known act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity [41]. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [42]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes [43]

To understand the nature of the fluorescence emission from these crude preparations was compared. The comparative analysis clearly showed a correction between a compound present in it and their fluorescent behaviour under different conditions. The major bioactive compounds present in these crude preparations are the coumarin, flavones, tannins and alkaloids. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under UV light turns to bright yellow alkaline conditions. Similarly the phytosterols when treated with 50% H_2SO_4 show green fluorescence under UV light. Terpenoids especially sapogenins exhibit yellow green fluorescence under short UV light [44]. Quinine, aconitin, berberin and emetin show specific colour of fluorescence (Aconitin - light blue; berberin - light yellow; emetin - orange). Haydon [45] studied the photophysical characters of coumarins. Hydroxy methyl coumarin fluoresced in the 420 - 440nm when observed in different solvents with increasing polarity [46]. The fluorescence analysis of leaf of *S.portulacastrum* exhibited clear fluorescence behaviour at different radiations which can be taken as standard fluorescence pattern.

In the present study, *in vitro* antibacterial activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of leaf *S.portulacastrum* was quantitatively assessed on the basis of zone of inhibition. The present study indicates antibacterial property of the *S. portulacastrum* leaf against the bacterial strain of human pathogenic bacteria varies depends upon the solvent media used for extraction. The petroleum ether extract of *S. portulacastrum* leaf shows highest activity against bacterial pathogens such as *K. pneumoniae*, *S. marcescens*, *S.paratypi*-B and *E.coli* (ESBL). Benzene extract of leaf of *S.portulacastrum* exhibits highest activity against *B. thuringiensis*, *E.coli, E.coli* (ESBL). Ethyl acetate extract of leaf of *S. portulacastum* exhibits highest activity against *E.coli* (ESBL), *S.paratyphi*, *S. aureus and S. aureus* (Methicillin sensitive). Methanol extract of *S. portulacastrum* leaf exhibits highest antibacterial activity shows *S. Paratyphi A, E.coli* and *P. vulgaris* Ethanol extract shows highest activity against *S. paratyphi* –B, *S. paratyphi* and *Bacillus subtilis*. Among the solvents tested ethanol extract shows very good activity against *S.paratyphi* and *S. Paratyphi B.*

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *K. pneumoniae* is the most important member of the *Klebsiella* genus of Entero-bacteriaceae and it is emerging as an important cause of neonatal nosocomial infection [47]. *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients [48]. Infection caused by *Salmonella* is a serious public health problem in developing countries and represents a constant concern for the food industry [49]. *P. mirabilis* causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores [50,51]. The demonstration of activity against both gram-negative and gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against both clinical and laboratory isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms prevalent in hospital environments.

Qualitative phytochemical investigation revealed that the extracts contained some phytoconstituents. Saponins, tannins, alkaloids and flavonoids are present in the five different extracts. These bioactive components including thiocynate, nitrate, chloride and sulphates, beside other water soluble components which are naturally occurring in most plant materials, are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property to plants [52,53,2].

CONCLUSION

Extracts of *S. portulacastrum* (L) in this study demonstrated a broad-spectrum of antibacterial activity against both gram-positive and gram-negative bacteria. The antibacterial activity of the plant extract, possibly due to the identified phytoconstituents, further confirms its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections. Identification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view of formulating novel chemotherapeutic agents should be the future direction for investigation

Table 1 .Ash and extractive values of the powered leaf of *S portulacastrum*[a]

S.No	Types of ash	% of Ash
1	Total ash value of powder	9.86±0.04
2	Water soluble	3.80±0.03
3	Acid insoluble ash	1.34±0.02
4	Sulphated ash	10.08±0.08
Extractive values		
S.No	Name of the extract	Extractive Value (%)
1	Petroleum ether	5.66±0.03
2	Benzene	4.46±0.05
3	Chloroform	4.12±0.01
4	Acetone	6.88±0.07
5	Methanol	7.69±0.04
6	Ethanol	7.80±0.04
7	Water	8.12±0.10

[a] All values of mean of triplicate determinations

± Standard error.

Table 2. Fluorescence analysis of powdered leaf of *S.portulacastrum*

Treatement	Colour		
	Under Ordinary Light	Under UV light	
		365mm	245nm
Powder as such	Brown	Brown	Black
Powder + 1NAqueous NaoH	Fluorescent Green	Fluorescent Green	Black
Powder + 1NAlcoholic NaoH	Dark Brown	Dark Green	Violet
Powder + 1 N HCl	Brown	Fluorescent Green	Violet
Powder + Con. HCL	Fluorescent Green	Fluorescent Green	Dark Blue
Powder + Con.H_2So_4	Green	Dark Green	Violet
Powder+ 50% H_2So_4	Fluorescent Green	Fluorescent Green	Blue
Powder +Con. HNO_3	Red	Fluorescent Green	Black
Powder +40% NaoH + 10% Lead Acetate	Green	Black	Black
Powder + Acetic acid	Dark Brown	Fluorescent Green	Violet
Powder+ Ferric Chloride	Dark Brown	Fluorescent Green	Blue
Powder+ Chloroform	Green	Fluorescent Green	Blue
Powder + Benzene	Dark Brown	Black	Black
Powder + Petroleum ether	Brown	Dark Green	Black
Powder+ Methanol	Dark Green	Black	Violet
Powder+ Ethanol	Black	Fluorescent Green	Black
Powder+ acetone	Dark Brown	Dark Green	Black
Powder+ NH_3	Brownish Green	Fluorescent Green	Black
Powder+HNO_3+ NH_3	Red	Fluorescent Green	Violet
Powder+HNO_3+ 50% HNO_3	Black	Dark Green	Violet

Table 3: Preliminary phytochemical screening of leaf of *S.portulacastrum*

Bioactive components	Nature of extract				
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
Alkaloids	+	+	+	+	+
Anthroquinones	-	-	-	-	-
Catechin	-	-	-	-	-
Coumanin	+	+	+	+	+
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	+
Quinones	+	+	+	+	+
Saponins	+	+	+	-	-
Steroids	+	+	+	+	+
Tannins	+	+	+	-	-
Terpenoids	+	+	+	+	+
Glycosides	+	+	+	+	+
Xanthoprotein	+	+	+	+	+
Sugar	+	+	+	+	+
Fixed oil	-	-	-	-	-

Table 4: Antibacteial activity of different extracts of leaf of *S. portulacastrum*

Microorganisms	Name of the extract/ Zone of Inhibition (mm)					
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	AB
1.*Bacillus thuringiensis*	7	16	10	11	8	25
2.*Bacillus subtilis*	-	8	9	13	19	20
3.*Streptococcus faecalis*	8	7	10	10	12	26
4.Staphylococcus aureus	8	6	16	7	11	25
5. *Streptococcus pyogens*	6	7	7	9	10	21
6.*Enterococcus faecalis*	6	10	8	10	5	21
7.*Salmonella paratyphi-A*	-	8	7	16	13	35
8. *Salmonella paratyphi-B*	10	9	11	10	25	18
9. *Salmonella paratyphi*	5	11	16	11	21	19
10.*Proteus mirabilis*	5	9	8	4	10	18
11.*Escherichia coli*	6	14	9	16	10	20
12. *Proteus vulgaris*	6	6	12	15	11	22
13.*Klepsiella pneumonia*	16	7	15	10	12	21
14.*Pseudomonas aeruginosa*	-	6	8	7	-	21
15.*Serratia marcescens*	10	9	8	11	10	21
16.*Mycobacterium smegmatis*	-	7	9	7	-	25
17.*Staphylococcus aureus* (Methicillin sensitive)	8	6	16	7	11	25
18.*Psuedomonas aeruginosa* (ESBL)	9	8	9	11	19	20
19. *Escherichia coli* (ESBL)	10	14	21	10	19	19

REFERENCES

1. Newman DJ, Cragg GM and Snader KM: The influence of natural products upon drug discovery. Natural Products Research 2000; 17: 215-234.
2. Pretorius CJ and Watt E: Purification and identification of active components of *Carpobrotus edulis* L. Journal of Ethnopharmarcology 2001; 76: 87-91.
3. Bibitha B, Jisha VK, Salitha CV, Mohan S and Valsa AK: Antibacterial activity of different plant extracts. Short communication. Indian Journal of Microbiology 2002; 42: 361–363.
4. Sofowora A. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd (Pub.), Ibadan. 1993.
5. Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS and Maiga A: An ethnobotanical survey of herbal drugs of Gourma district, Mali, Pharmaceutical Biology 1999; 37: 80–91.
6. Edeoga HO, Okwu DE and Mbaebie BO: Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4: 685-688
7. Chendurpandy P, Mohan VR and Kalidass C: Antimicrobial activity of ethnomedicinal plants from Kanyakumari District, Western Ghats, Tamil Nadu, Journal of Economic Taxonomic Botany 2010; 34: 380-387.
8. Chendurpandy P, Mohan VR and Kalidass C: Screening of ethnomedicinal plants for antimicrobial activity. Journal of Economic Taxonomic Botany 2010; 34: 663-669.
9. Kala SMJ, Tresina Soris P and Mohan VR: Antitumor activity of *Eugenia floccosa* Bedd and *Eugenia singampattiana* Bedd leaves against Dalton ascites lymphoma in Swiss albino rats. International Journal of Pharmtech Research 2011; 3: 1796-1800.
10. Feller IC, Whigham DF, O'Neill JP and McKee KL: Effects of nutrient enrichment on within-stand cycling in a mangrove forest. Ecology 1999; 80: 2193–2205.
11. Hernes PJ, Benner R, Cowie L, Goni MA, Bergamaschi BA and Hedges JI: Tannin diagenesis in mangrove leaves from a tropical estuary: A novel molecular approach. *Geochim.Cosmochim. Acta* 2001; 65: 3109–3122.
12. Mainoya J, Mesaki S and Banyikwa FF: The distribution and socio-economic aspects of mangrove forests in Tanzania. In *Man in the Mangroves: the Socio-Economic Situation of Human Settlements in Mangrove Forests*; Kunstadter, P.E., Bird, C.F., Sabhasri, S., Eds.; United Nations University: Tokyo, Japan, 1986, 87–95.
13. Bandarnayake WM: Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology Management, 2002; 10: 421-452.
14. Premananthan M, Arakaki R, Izumi H, Kathiresan K and Nakano M &Yamamoto N: Antiviral properties of a mangrove plant, *Rhizophora apiculata* Blume, against human immunodeficiency virus, antiviral Research 1999; 44: 113-122.
15. Babu BH, Shylesh BS and Padikkala L: Antioxidant and hepatoprotective effect of *Acanthus ilicifolius*. Fitoterapia 2001; 72: 272-277.

16. Itigowa M, Ito C, Tan HT, Okuda M, Tokuda H, Nishino H and Furukawa H: Cancer chemopreventive activity of naphthoquinones andtheir analogs from *Avicennia* plants.Cancer Lett 2001; 174: 135-139.
17. Banerjee D, Chakrabarti S, Hazra A, Banerjee S, Ray J and Mukherjee B: Antioxidant activity and total phenolics of some mangroves in Sundarbans. African Journal of. Biotechnology 2008; 7**:** 805–810.
18. Rahim AA, Rocca E, Steinmetz J, Jain Kassim M, Sani Ibrahim M and Osman H: Antioxidant activities of mangrove *Rhizophora apiculata* bark extracts. Food Chemistry 2008; 107: 200–207.
19. Bandaranayake W: Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management 2002; 10: 421–52.
20. Chandrasekaran M, Kannathasan K, Venkatesalu V and Prabhakar K: Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant *Staphylococcus aureus*. World Journal of Microbiology Biotechnology 2009; 2: 155–60.
21. Bandaranayake WM: Survey of mangrove plants from Northern Australia for phytochemical constituents and uv-absorping compounds. Current Topics Phytochemistry 1995; 14: 60–72.
22. Bandaranayake WM: Economic, traditional and medicinal uses of mangroves. Townsville: Australian Institute of Marine Science; 1999. AIMS Report No.28.
23. Lokhande VH, Srivastava S, Patade VY, Dwivedi S, Tripathi RD and Nikam TD: Investigation of arsenic accumulation and tolerance potential of *Sesuvium portulacastrum* (L.) Chemosphere 2011; 4: 529-34.
24. Chandrasekaran M, Senthilkumar A and Venkatesalu V: Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. European Review for Medical and Pharmcological Science 2011; 7: 775-80.
25. Kalpanadevi V, Murugan M, Maruthupandian A, Murugan P and Mohan VR: Pharmacochemical characterization and antibacterial activity of *Pleiospermium alatum* (Wall ex Wight & Arn.) Swingle (Rutaceae) and *Balanites aegyptiaca* (L) Del. (Balanitaceae). Journal of Economic Taxonomic Botany 2011; 35: 609-617.
26. Mohan VR, Chenthurpandy P and Kalidass C: Pharmacognostic and phytochemical investigation of *Elephantopus scaber* L.(Asteraceae). Journal of Pharmaceutical Science and Technology 2010; 2 : 191-197.
27. Tresina Soris P, Esakkiraja N, Maruthupandian A and Mohan VR: Pharmaco-chemical characterization of *Phyllanthus reticulatus* Poir, *Phyllanthus urinaria* L. and *Leptadenia reticulata* (Retz.) Wight & Arn. Journal of Economic Taxonomic Botany 2012; 36**:** 324-329.
28. Gowari SS and Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* L. (Myrtaceae) leaves extracts. International Journal Pharmtech Research 2010; 2: 1569-1573.
29. Saraf A: Phytochemical and antibacterial studies of medicinal plant *Costus specious* Koer. E Journal of Chemistry 2010; 7: S405-S413.

30. Shajeela PS, Kalpanadevi V and Mohan VR: Potential antidiabetic, hyperlipidaemic and antioxidant effects of *Nymphaea pubescens* extract in alloxan induced diabetic rats, Journal of Applied Pharmaceutical Science 2012; 2: 83-88.
31. Murugan M and Mohan VR: Evaluation of phytochemical analysis and antibacterial activity of *Bauhinia purpurea* L . and *Hiptage bengalensis* (L.) Kurz. Journal of Applied Pharmaceutical Science 2011; 1: 157-160.
32. Gokhale S B, Pharmacognosy 29th ed. Nirali Prakashan; 2009.
33. Padmavathi D, Sussheela L and Bharathi RV: Pharmacognostical evaluation of *Barringhonia acutangula* leaf. International Journal Ayurveda Research 2011; 2: 37-41.
34. Soni H, Nayak C, Patel SS, Mishra K, Singh RV and Singhai AK: Pharmacognostic studies of the leaves of *Tinospora cordifolia*. IJPI'S J Pharm Herb Formul 2011; 1: 3.
35. Janandhanan UK, Varadharajan V and Krishnamurthy V: Physicochemical evaluation, Phytochemical screening and chromatographic fingerprint profile of *Aegle marmelos* (L.) leaf extracts, World Journal Pharmaceutical Research 2012; 1: 813-837.
36. Mahendra SK and Nityanand PV: Pharmacognostical and physio-chemocal standardization of leaves of *Wrightia tinctoria* R.Br. International Journal Pharm Research Devolpment 2009; 8: 1-10.
37. Ambi AA, Abdurrahman EM, Sule MI, Pateh UU, Abdurrahman VR and Ibrahim NDG: Phytochemical screening and histological studies on the seeds of *Colocynthis eitrullus* in Albino rats. Nigerean Journal of Pharmaceutical Science 2007; 6: 7-13.
38. Bonjar GHS, Nik AK and Aghighi S: Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions. Iranian Journal of Biological Science 2004; 4: 405-412.
39. Shimada T: Salivary proteins as a defence against dietary tannins. Journal of Chemical Ecology 2006; 32: 1149-1163.
40. Marjorie C: Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999; 12: 564-582.
41. Hoult, JRS, and Paya M: Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. General Pharmacology. 1996; 27: 713–722.
42. Zablotowicz RM, Hoagland RE and Wagner SC: Effect of saponins on the growth and activity of rhizosphere bacteria. Advances in Experimental Medicine and Biology 1996; 405: 83-95.
43. Epand RF: Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. Biochimica et Biophysica Acta 2007; 2500–2509.
44. Horborne JB: Phytochemical methods. Chapman & Hall, New York.1976: 1- 288.
45. Haydon SC: Spectroscopic letters 1975; 8: 815
46. Chaltopudhyay N, Mallick A and Sengupt S: Photophysical studies of hydroxyl methyl coumarin: A new fluorescent chemosensor for Zinc and Nickel ions in water, Journal of Photochemistry and Photobiology 2006; 177: 55-60.

47. Gupta P, Murali P, Murali MV, Faridi MMA, Kaul PB, Ramachandran VC and Talwar V: Clinical profile of *Klebsiella septicaemia* in neonates. Indian Journal of Paediatrics 1993; 60: 565-572.
48. Black JG: Microbiology: Principles and Application. Prentice Hall, New York. 1996 p. 260.
49. Mastroeni P: Immunity to systemic *Salmonella* infections. Current Molecular Medicine 2002; 2: 393-406.
50. Cheesbrough M: Medical Laboratory Manual for Tropical Countries. Microbiology, Linacre House, Jordan Hill Oxford. 2000 p.260.
51. Parekh J and Chanda S: *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. African Journal of Microbiology Research 2007; 1: 92-99.
52. Lutterodt GD, Ismail A, Basheer RH and Baharudin HM: Antimicrobial effects of *Psidium guajava* extracts as one mechanism of its antidiarrhoeal action. Malaysian Journal of Medicinal Science 1999; 6: 17-20.
53. El astal ZY, Aera A and Aam A: Antimicrobial activity of some medicinal plant extracts in Palestine. Pakistan Journal of Medicinal Science 2005; 21:187.