ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Preliminary phytochemical, FT- IR and antibacterial assessment of leaf of *Eugenia singampattiana* Bedd (Myrtaceae)

P.S.Tresina and V.R.Mohan*.

Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin-628008, Tamil Nadu, India

Manuscript Info

Manuscript History:

Received: 15 January 2014
Final Accepted: 23 February 2014
Published Online: March 2014

Key words:
E. singampattiana, Phytochemicals, FT-IR, Antibacterial activity

Corresponding Author

V.R.Mohan

Abstract

The present study is to investigate the phytochemical and antibacterial activity of different solvent extracts of leaf of *Eugenia singampattiana*. The preliminary phytochemical screening revealed that alkaloids, flavonoids, glycosides, terpenoids, steroids, saponins, phenols, tannins, coumarins, catechin and sugars were found to be present in the methanol and ethanol extracts of leaf of *E. singampattiana*. The FT-IR spectrum confirmed the presence of C=O, C-H, C=C, C-O and C=C groups. The antibacterial activity has been observed in the petroleum ether, methanol and ethanol extracts of leaf of *E. singampattiana* against the tested bacteria with varied activity. The methanol extract of leaf of *E. singampattiana* showed maximum zone of inhibition against *Staphylococcus aureus* (20 mm). It is hoped that, this study would direct to the establishment of some compounds that could be used to invent new and more potent antibacterial drugs of natural origin.

.

Introduction

Plants and their derivatives play key role in world health and have long been known to possess biological activity, 30% of all modern drugs are derived from plants (Riaz et al., 2010). According to WHO, about 80% of the world's population relies essentially on plants for primary health care (Mckay and Blumberg, 2007). Now-a-days, most of the research work is carried out for the development of drugs from medicinal plants for the treatment of microbial and non-microbial disease (Kawo et al., 2009). Traditional uses of medicinal plants have fewer side effects over allopathic medicine. Such promising fact leads to the development of herbal derived medicines all over the world (Pal and Shukla, 2003). Plants contain flavonoids, alkaloids, tannins, phenols etc., which have biological significance in terms of medicine development and extracts of aqueous, methanol and ethanol are good source of antiviral, antitumour and antibacterial agents (Cowan, 1999).

Eugenia singampattiana Bedd belong to the family Myrtaceae. It is commonly known as "Kattukorandi" by Kanikkar tribals of Agasthiarmalai, Biosphere Reserve, Western Ghats, Tamil Nadu, India. The paste prepared from the leaf of *E. singampattiana* is given to treat asthma and giddiness. Paste prepared from equal quantity of leaves and flowers is consumed by Kanikkar tribals to cure body pain and throat pain. Paste prepared from equal quantity of leaves, flowers and tender fruits are consumed by the Kanikkars to relief from leg sores and rheumatism. Paste prepared from equal quantity of stems, leaves and flowers is consumed with palm sugar to get relief from gastric complaints (Viswanathan et al., 2006). *E. singampattiana* leaf extracts were reported for the biological activities such as antitumor, antidiabetic, antihyperlipidaemic, hepatoprotective, antiinflammatory and *in vitro* antioxidant activity (Kala et al., 2011; 2012a; 2013a,b, Tresina et al., 2012).

The present study aimed at evaluating the phytochemical screening, FT-IR and *in vitro* antibacterial activity of *E. singampattiana* leaf against pathogenic bacteria.

Materials and Methods

Plant Material

The leaves of *Eugenia singampattiana* Bedd were freshly collected from well grown healthy plants inhabiting the natural forests of Karaiyar, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The plant was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of extracts for phytochemical screening and antimicrobial activity

Freshly collected leaf sample of *Eugenia singampattiana* were dried in shade, and then coarsely powdered separately in a Wiley mill. The coarse powder (100g) was extracted successively with petroleum ether, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through 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 (Saraf, 2010; Shajeela et al., 2012 and Murugan and Mohan, 2011). All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for antibacterial activity.

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between $4000 - 400\ cm^{-1}$ (Kareru et al., 2008).

Microorganisms

Bacterial strains of *Bacillus thuringiensis* (+), *Bacillus subtilis* (+),*Staphylococcus aureus* (+), *Staphylococcus aureus* (Methicillin sensitive) (+), *Enterococcus faecalis* (+), *Salmonella paratyphi* - A & B (-), *Salmonella paratyphi* (-), *Proteus mirabilis*(-), *Escherichia coli* (-), *Escherichia coli* (ESBL) (-), *Proteus vulgaris* (-), *Klebsiella pneumoniae* (-), *Pseudomonas aeruginosa* (-), *Pseudomonas aeruginosa* (ESBL) (-) and *Mycobacterium smegmatis* (+) bacterial strains were 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.

Antibacterial assay

Antimicrobial study was carried out by disc diffusion method (Gokhale, 2009) 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 tetracycline (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37ºC 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 phytochemical analysis of petroleum ether, methanol and ethanol extracts of *E. singampattiana* leaf were showed in Table 1. From the analysis, the presence of alkaloids, flavonoids, glycosides, terpenoids, steroids, saponins, phenols, tannins, coumarin, catechin and sugar were observed in methanol and ethanol extracts of *E. singampattiana* leaf.

From the FT-IR spectral data, presence of C=O, C-H, C=C, C-O, C-C were identified. The more intense bands occurring at 3279.33 cm^{-1}, 2917.82 cm^{-1}, 2849.45 cm^{-1}, 1727.24 cm^{-1} , 1685.51 cm^{-1}, 1313.94 cm^{-1} and 1443.94 cm^{-1} corresponds to OH, C-H (alkyl), C=O, C-O and C=C stretching vibrations respectively indicate the presence of polyhydroxy, aliphatic, carbonyl, alcohol, ether, ester and carboxylic acids (Table 2 Fig. 1).

The antibacterial activity of petroleum ether, methanol and ethanol extracts of E. singampattiana leaf was investigated against the selected pathogens by disc diffusion methods. All the examined plant extracts showed varying degree of antibacterial activities against the pathogens tested. The antibacterial activity of petroleum ether, methanol and ethanol extracts of *E. singampattiana* leaf against tested pathogens is depicted in Table 3. The petroleum ether extract of *E. singampattiana* leaf showed maximum zone of inhibition (17 mm) against *Proteus mirabilis*. The minimum inhibitory zone (6 mm) was exhibited against *Escherichia coli* and *Mycobacterium smegmatis*. Petroleum ether extract of leaf of *E. singampattiana* showed no zone of inhibition against *Bacillus*

thuringiensis, B. subtilis, Enterococcus faecalis, Salmonella paratyphi.A, Pseudomonas aeruginosa, Staphylococcus aureus (Methicillin sensitive), *Pseudomonas aeruginosa* (ESBL) and *Escherichia coli* (ESBL).

The methanol extract of *E. singampattiana* leaf showed maximum zone of inhibition against *Staphylococcus aureus* (20 mm); while minimum inhibitory zone was observed against *Pseudomonas aeruginosa* (6 mm). Methanol extract of *E. singampattiana* leaf showed no zone of inhibition against *Escherichia coli* (ESBL). The ethanol extract of *E. singampattiana* leaf showed maximum zone of inhibition against *Salmonella paratyphi* (18 mm); while minimum inhibitory zone was observed against *Mycobacterium smegmatis*.

Table 1 Phytochemical screening of powdered leaf of *E. singampattiana*

Tests	Benzene	Chloroform	Ethanol	Water
Alkaloid	+	-	+	+
Anthraquinone	-	+	-	-
Coumarin	-	-	+	+
Catechin	+	-	+	+
Glycoside	-	-	+	-
Flavonoid	-	-	+	+
Phenol	+	+	+	+
Quinone	+	-	-	-
Saponin	-	+	+	-
Steroid	-	-	+	-
Tannin	+	-	+	+
Terpenoid	+	+	+	-
Sugar	+	+	+	+
Xanthoprotein	+	+	+	+
Fixed Oil	-	-	+	+

Table 2 IR spectroscopic data of *E. singampattiana* stretching frequency (cm^{-1})

S.No.	Group	Stretching frequency (cm^{-1})
1	O-H	3407.87
2	C-H stretching (alkyl group)	2923.19
3	C=C	1448.02
4	C-H stretching (Phenyl group)	1235.09
5	C=O	1616.47
6	C-O	1384.19

Table 3 *In vitro* antibacterial activity of different extracts of *E. singampattiana* leaf

S.NO.	Name of the microorganisms	Name of the extract/ Zone of Inhibition (mm)			
		Petroleum ether	Methanol	Ethanol	AB
1	*Bacillus subtilis*	-	7	8	25
2	*Bacillus thuringiensis*	-	15	8	23
3	*Enterococcus faecalis*	-	10	15	24
4	*Escherrichia coli*	6	9	10	19
5	*Escherrichia coli* (ESBL)	-	-	8	21
6	*Klebsiella pneumoniae*	10	7	8	22
7	*Mycobacterium smegmatis*	6	15	6	15
8	*Proteus mirabilis*	17	9	9	17
9	*Proteus vulgaris*	7	9	12	22
10	*Pseudomonas aeruginosa*	-	6	8	17
11	*Psuedomonas aeruginosa* (ESBL)	-	9	10	24
12	*Salmonella paratyphi*	9	15	18	25
13	*Salmonella paratyphi-A*	-	11	17	25
14	*Salmonella paratyphi-B*	10	12	14	24
15	*Staphylococcus aureus*	7	20	15	26
16	*Staphylococcus aureus* (Methicillin sensitive)	-	12	13	23

Discussion

Phytochemicals are chemical compounds formed during the plant's normal metabolic processes. These chemicals are often referred to as 'secondary metabolites' of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids. In the present study, qualitative phytochemical investigation revealed that, the methanol and ethanol extracts contained some phytoconstituents. Alkaloid, coumarin, catechin, saponin, steroid, flavonoid, terpenoid, tannin and phenol are found in both the extracts.

Alkaloids possess antiinflammatory, antiasthmatic, and antianaphylatic properties with consequences of altered immunological status *in vivo*. Furthermore, alkaloid which is one of the largest phytochemical groups in plants has amazing effect on humans and this has led to the development of powerful painkiller medicines (Johnson et al., 2012). Flavonoids, the major group of phenolic compounds are reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids are able to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoids health promoting functions in organisms. They are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids on the other

hand, are potent water soluble antioxidants and few radical scavengers, which prevent oxidative cell damage and have strong anticancer activity (De Sousa et al., 2007). Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, antiinflammatory and anticancer activity (De Sousa et al., 2007, Jeeva and Johnson. 2012).

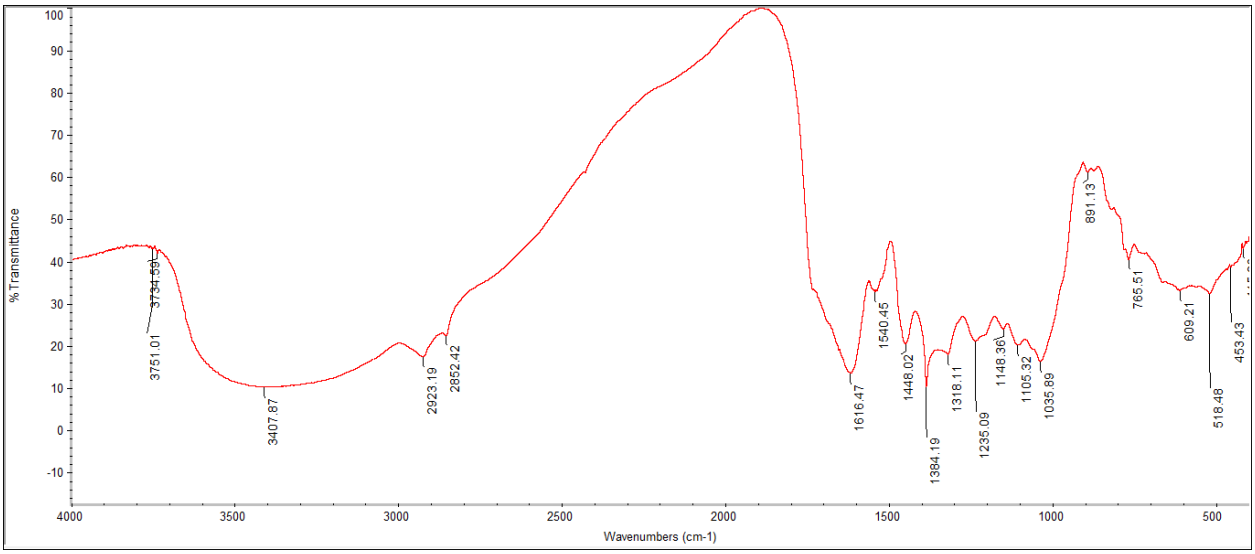

Figure 1: FT-IR Spectrum of leaf powder of *E. singampattiana*

Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and antiinflammatory activities (Arts et al., 2005, Mithraja et al., 2011). Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity (Kala et al., 2012a, Kala et al., 2012b). Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, and piles and as an antidote. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, antiinflammatory, antiulcer and antioxidant property for possible therapeutic applications. Tannins are known to possess general antimicrobial and antioxidant activities. Recent reports show that, tannins may have potential value as cytotoxic antineoplastic agents (Rievere et al., 2009). It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic (Mithraja et al., 2012). Coumarin has been used as anticoagulant drugs and to treat lymphedema (Online). Plant steroids are known to be important for their cardio tonic activities, posses insecticidal and antimicrobial properties (Jeeva et al., 2011). These observations cited on phytochemical compounds support the present findings on the usefulness of leaf of *E. singampattiana* in various medicaments. It suggests that *E. singampattiana* plant can be used as anti-microbial activity, antioxidant, antiallergic, anti-inflammatory, antidiabetic, anticarcinogenic, anticancer agents in the future.

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. From the spectral data, presence of C=O, C-H, C=C, C-O, C-C were identified. These bonding structures are responsible for the presence of alkyl group, methyl group, alcohol group, ethers, esters, carboxylic acid and anhydrides.

The more intense bands occurring at 3279.33 cm^{-1}, 2917.82 cm^{-1}, 2849.45 cm^{-1}, 1727.24 cm^{-1} , 1685.51 cm^{-1}, 1313.94 cm^{-1} and 1443.94 cm^{-1} corresponds to OH, C-H (alkyl), C=O, C-O and C=C stretching vibrations respectively indicate the presence of polyhydroxy, aliphatic, carbonyl, alcohol, ether, ester and carboxylic acids. Carboxylic acids present in the medicinal plant serves as main pharmaceutical product in curing ulcers, jaundice, headache, stomatitis, hemicranias, pain in liver, treatment of edema and rheumatic joint pains. Amines, amides and amino acids are the main groups of protein synthesis and herbs serves as herb oil and hair tonic.

Mohan et al. (2010) used chloroform, benzene and methanol as a solvent source for the extraction. Raja et al. (2011) used methanol and hexane as a solvent source. Anitha et al. (2012) used acetone, benzene and

petroleum ether as a solvent source. In the present study, petroleum ether, methanol and ethanol were used as solvent source for the extraction of metabolites. Of which, the ethanol extracted solvents showed high degree (16/16 pathogens) of antibacterial activity against the selected pathogens followed by methanol extracted solvents showed high degree (15/16 pathogens) of antibacterial activity. Since the polarity of methanol and ethanol are higher, most of the secondary metabolites of *E. singampattiana* leaves dissolved in methanol and ethanol extracts. Herbal drugs contain unique constituents which differs from one herb to another, hence the type and extent of their medicinal property also differs. Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts obtained using same herb with different solvents will be different (Roopashree et al., 2008). In the present study, *in vitro* antibacterial efficacy of *E. singampattiana* leaves of petroleum ether, methanol and ethanol extracts was quantitatively assessed on the basis of zone of inhibition. In the present investigation, *E. singampattiana* leaf exhibited varying degree if inhibitory effect against the selected bacterial pathogens. Eloff (1998) reported that methanol was the most effective solvent for plant extraction than hexane and water. The present study used petroleum ether, methanol and ethanol for extraction. The current investigation confirmed the Eloff's observations with maximum activity. It has been shown that, when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria (Shyamala Gowri et al., 2010). In the present study, petroleum ether, methanol and ethanol extracts of the selected plant also showed zone of inhibition against the human pathogens with varied diameter.

Enterococcus faecalis can cause endocarditis, bacteremia, urinary tract infections (UTI), meningitis, and other infections in humans as well as bladder, prostrate and epididymal infections; nervous system infections are less common (Anitha et al., 2012). The methanol and ethanol extracts of leaves of *E. singampattiana* exhibited antibacterial activity against the bacteria *E. faecalis* (10 mm and 15 mm respectively).

Staphylococcus aureus can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection. The methanol and ethanol extracts of leaves of *E. singampattiana* showed antibacterial activity against the bacteria *S. aureus* (20 mm and 15 mm).

Salmonella are potential enteric pathogens and a leading cause of bacterial foodborne illness. In addition, *Salmonella* species have been implicated in a spectrum of other diseases, including enteric or typhoid fever (primarily *Salmonella typhi* and *Salmonella paratyphi*), bacteremia, endovascular infections, faecal infections (eg, osteomyelitis), and enterocolitis (typically *Salmonella typhimurium*, *Salmonella enteritidis*, and *Salmonella heidelberg*). The petroleum ether, methanol and ethanol extracts of leaf of *E. singampattiana* exhibited antibacterial activity against *Salmonella paratyphi* B (10 mm, 12 mm and 14 mm respectively) and *Salmonella paratyphi* (9 mm, 15 mm and 18 mm respectively).

The results of the present study suggest that, the methanol and ethanol extracts of *E. singampattiana* leaf can be used to treat urinary tract infections, skin infections and food borne illness. Based on the present study on phytochemical and antibacterial activities, it is concluded that, the leaf of *E. singampattiana* contains various bioactive compounds with high degree of antibacterial activity against various pathogens. It is hoped that, this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Further study will emphasize the isolation and characterization of active principles responsible for bioefficacy and bioactivity.

Acknowledgement

The authors are thankful to University Grants Commission – New Delhi, for their financial support (Ref. No: 39-429/2010(SR) dated 7th JAN 2011).

References

Anitha, V.T., Antonisamy, J. M. and Jeeva, S. (2012). Antibacterial studies on *Hemigraphis colorata* (Blume) H.G. Hallier and *Elephantopus scaber* L. Asian Pac. J. Trop. Med., 5: 52-57.

Arts, I.C. and Hollman, P.C. (2005). Polyphenols are disease risk in epidemiological studies. Amer. J. Clin. Nut., 81: 317-325.

Cowan, M.M. (1999). Plant products as microbial agents. Clin. Micro. Rev., 12: 564-582.
De Sousa RR, Queiroz KC, Souza AC, Gurgueira SA, Augusto AC, Miranda MA, *et al*. (2007). Phosphoprotein levels, MAPK activities and NFkappaB expression are effective by fisetin. J Enzyme Inhib Med Chem., 22: 439-444.
Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol., 60: 1-8.
Gokhale, S. B. (2009). Pharmacognosy 29th ed. Nirali Prakashan.
Jeeva, S. and Johnson, M (2012). Antibacterial and phytochemical studies on methanolic extracts of *Begonia floccifera* Bedd flower. Asian Pac. J. Trop. Biomed., 2: S151-S154.
Jeeva, S., Johnson, M., Aparna, J.S. and Irudayaraj, V (2011). Premilary phytochemical and antibacterial studies on flower of selected medicinal plants. Int. J. Med. Arom. Plants. 1: 107-114.
Johnson, M., Aparna, J.S., Jeeva, S., Sukumaran, S. and Anantham, B. (2012). Preliminary phytochemical studies on the methanolic flower extracts of some selected medicinal plants from India. Asian Pac. J. Trop. Biomed., 1(S1): S79-S82
Kala, S.M.J., Tresina, P.S. and Mohan, V.R. (2011). Antitumour activity of *Eugenia flocossa* Bedd and *Eugenia singampattiana* Bedd leaves against Dalton ascites lymphoma in Swiss albino rats. Int. J. PharmTech Res., 3: 1796-1800.
Kala, S.M.J., Tresina, P.S. and Mohan, V.R. (2012a). Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia singampattiana* Bedd leaves in alloxan induced diabetic rats. Int. J. Pharm. Pharmaceut. Sci,. 4: 412-416.
Kala, S.M.J., Tresina, P.S. and Mohan, V.R. (2012b). Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia floccosa* Bedd leaves in alloxan induced diabetic rats. J. Bas. Clin. Pharm., 3: 235-240.
Kala, S.M.J., Tresina, P.S. and Mohan, V.R. (2013a). Evaluation of anti-inflammatory activity of *Eugenia singampattiana* Bedd leaf. Int. J. Adv. Res., 6:248-251.
Kala, S.M.J., Tresina, P.S. and Mohan, V.R. (2013b). Hepatoprotective Effect of *Eugenia singampattiana* Bedd Leaf Extract on Carbon Tetrachloride Induced Jaundice. Int. J. Pharmaceu. Sci. Rev. Res., 21: 41-45.
Kareru, P.G., Keriko, J.M., Gachanja, A.N. and Kenji, G.M. (2008). Direct detection of triterpenoid saponins in medicinal plants. Afr. J. Tradit. Complement. Altern. Med., 5: 56–60.
Kawo, A.M., Mustapha, A., Abdullahi, B.A., Rogo, L.D., Gaiya, Z.A. and Kumurya, A.S. (2009). Phytochemical properties and antibacterial activities of the leaf and latex extracts of *Calotropis procera*. AITF. Bajopas., 2: 34-40.
Mckay, D.L. and Blumberg, J.B. (2007). A review of the bioactivity of South African herbal teas: Roobos (*A. linearis*) and honey comb (*C. intermedia*). Phytother. Res., 21: 1-16.
Mithraja, M.J, Johnson, M., Mahesh, M., Miller Paul, Z. and Jeeva, S. (2012). Interspecific variation studies on the Phyto-constituents of Christella and Adiantum using phytochemical methods. Asian Pac. J. Trop. Biomed., 2: S40-S45
Mithraja, M.J., Johnson, M., Mahesh, M., Paul, Z.M. and Jeeva, S (2011). Phytochemical studies on *Azolla pinnata* R.Br., *Marsilea minuta* L and *Salvinia molesta* Mitch. Asian Pac. J. Trop. Biomed., 1: S26-S29.
Mohan, V.R., Chendurpandy, P. and Kalidass, C. (2010). Pharmacognostic and phytochemical investigation of *Elephantopus scaber* L. (Asteraceae). J. Pharm. Sci. Tech., 2: 191-197.
Murugan, M. and Mohan, V.R. (2011). Evaluation of phytochemical analysis and antibacterial activity of *Bauhinia purpurea* L . and *Hiptage bengalensis* (L.) Kurz. J. Appl. Pharmaceut. Sci., 1: 157-160.
Pal, S. K. and Shukla, Y. (2003). Current status and the future. Asian. Pac. J. Can. Prev., 4: 281-288.
Raja, R.D.A., Jeeva, S., Prakash, J.W. Antonisamy,J. M. and Irudayaraj, V. (2011). Antibacterial activity of selected ethnomedicinal plants from South India. Asian Pac. J.Trop. Med., 4: 375-378.
Riaz, A., Khan, R.A., Ahmed, S. and Afroz, S. (2010). Assessment of acute toxicity and reproduction capability of herbal combination. Pak. J. Pharmaceu. Sci., 23: 291-294.
Rievere, C., Van Nguyen, J.H., Pieters L., Degaegher, B., Heyder, Y.V. and Minh, C.V. (2009). Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. Phytochem. 70: 86-91.
Roopashree, T.S., Dang, R., Rani, R.H.S. and Narendra, C. 2008. Antibacterial activity of antiploriatic herbs: *Cassia tora, Momordica charantia* and *Calendula officinalis*. Int. J. App. Res. Nat. Prod., 1: 20-28.
Saraf, A. (2010). Phytochemical and antibacterial studies of medicinal plant *Costus specious* Koer. E. J. Chem., 7: S405-S413.
Scalbert, A., Manach, C., Morand, C., Remesy, C. and Jimenez, L. (2005). Dietary polyphenols and the prevention of diseases. Crit. Rev.Food Sci. Nutr., 45: 287-306.
Shajeela, P.S., Kalpanadevi, V. and Mohan, V.R. (2012). Potential antidiabetic, hyperlipidaemic and antioxidant effects of *Nymphaea pubescens* extract in alloxan induced diabetic rats. J. Appl. Pharmaceut. Sci., 2: 83-88.

Shyamala Gowri, S. and Vasantha, K. (2010). Phytochemical screening and antibacterial activity of S*yzygium cumini* L. (Myrtaceae) leaves extracts. Int. J. PharmTech Res., 2: 1569-1573.
Tresina, P.S., Kala, S.M.J. and Mohan, V.R. (2012). HPTLC finger print analysis of phytocompounds and *in vitro* antioxidant activity of *Eugenia singampattiana* Bedd. J. App. Pharmaceut. Sci., 2: 112-124.
Viswanathan, M.B., Harrison Prem Kumar, E. and Ramesh, N. (2006). Ethnobotany of Kanis. Bishen Singh Mahendra Pal Singh. Dehra Dun. Pp: 87-88.