Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 890 - 898

Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

COMPARATIVE SCREENING OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SIX ETHNO-MEDICINALLY IMPORTANT PLANTS OF ASSAM

KANDARPA KR. SAIKIA*[1] Ph. D., VEDANT V BORAH[1] M. Sc., M C KALITA[1] Ph. D. AND MANGALA LAHKAR[2] M. D.

[1]Department of Biotechnology and Bioengineering, Institute of Science & Technology, Gauhati University, Guwahati, Assam, India - 781014.
[2]National Institute of Pharmaceutical Education & Resrarch, Guwahati, Assam, India - 781032

ABSTRACT

Emergence of drug resistance amongst clinical isolates of bacteria and fungi has led to the need of search for alternate drug candidates. Medicinal plants are a source of antimicrobial agents and some of which have been used for centuries by tribes in Assam. In this study, extracts of six medicinal plants namely *Cissus quadrangularis* L., *Moringa oleifera* L., *Ziziphus jujuba* M., *Cassia fistula* L., *Dillenia indica* L., and *Paederia foetida L.* were tested for activity against clinical isolates of bacteria and fungi. The preliminary phytochemical analysis of the extracts was tested for the presence of alkaloids, glycosides, steroids, terpenoids, carbohydrates, amino acids and saponins. The extracts were subjected to screening of *in vitro* antibacterial and antifungal activity against selected clinical isolates viz. *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Candida albicans* and *Candida trophicalis.* All the extracts exhibited inhibitory activity against the test pathogens. For positive control, a standard disc containing antibiotic drug Amoxicillin (10µg/disc), Kanamycin (30µg/disc) and Ketoconazole (50µg/disc) was used.

KEYWORDS : Antibacterial, Antifungal, Phytochemicals, Ayurveda

DR. KANDARPA KR. SAIKIA
Department of Biotechnology and Bioengineering, Institute of Science & Technology, Gauhati University, Guwahati, Assam, India - 781014.

INTRODUCTION

The North Eastern region of India is known for a rich heritage of traditional medical systems. Traditional knowledge of medicine developed by hundreds of tribal communities in NE India over centuries necessitates scientific validation. A large part of the NE India is botanically underexplored or even unexplored[1, 2]. The discovery, development and clinical use of antibiotics during the 19th century have substantially decreased public health hazards resulting from bacterial infections. However, there has been a parallel and alarming increase in bacterial resistance to existing chemotherapeutic agents as a result of their injudicious use [3]. In addition, antibiotics are occasionally associated with adverse effects to the host, including hypersensitivity, immune-suppression and allergic reactions [4]. These developments demand that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One possible strategy is the rational localization of bioactive products from folk medicines, with the hope that systematic screening of these will result in the discovery of novel effective compounds with potent and useful activities against clinically important bacterium. There is an ever-increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and, in most cases, easily available at affordable prices; they also have no side effects[5]. There are a limited number of antifungal drugs as compared to antibacterial drugs. Reports on increasing resistance to these antifungal worldwide necessitate the search of new drug candidates. Plants are reservoir of many known and unknown chemical compounds, some of these compounds are capable of restricting or retarding the growth of microorganism. Many plants are known to harbor active principle that confers the plants the ability to withstand microbial attack. These plants can be source for compounds that have pharmaceutical implication. We report here a systematic *in vitro* screening of antimicrobial effect of fruit and leaf extracts (aqueous, methanolic and hexane extracts) of six medicinal plants (*Cissus quadrangularis* L., *Moringa oleifera* L., *Ziziphus jujuba* M., *Cassia fistula* L., *Dillenia indica* L., and *Paederia foetida L.*) against clinical isolates of four bacterial species- *Staphylococcus aureus*, *Streptococcus pneumoniae, Escherichia coli*, *Klebsiella pneumoniae* and two fungi - *Candida albicans* and *Candida trophicalis*.

MATERIALS AND METHODS

i.Collection of plant material

The plants were collected from nearby areas of Guwahati city in Assam, India. The leaves and fruits were rinsed with distilled water and dried with paper towel; shade dried and powdered using electric grinder. The fine plant powder was transferred into sterile, air-tight container and stored for future use.

ii. Preparation of Extracts

Each plant powder was extracted by using different solvents, according to the protocol described earlier[6] wherein we have introduced a slight variation. Here, 10 grams powder of the plant leaves and fruits was extracted with 200 ml of 70% methanol with continuous stirring for 30 minutes, centrifuged at 10000 rpm for 15 minutes and filtered through What mann No.1 into a 250 ml round-bottom flask. Methanol was removed under reduced pressure, dried under nitrogen atmosphere and freeze dried using a lyophilizer at -92°C to obtain the extract. For aqueous extracts, 10 grams of plant leaf powder was extracted with 150 ml of distilled water, with continuous heating at 40°C and stirring for 30 minutes on a mechanical shaker. The resulting slurry was centrifuged at 10000 rpm for 15 minutes, filtered under vacuum (using the Buchner funnel) and freeze dried to obtain the aqueous extract.

iii. Phytochemical analysis

The various plant parts (root, stem bark and leaves) were screened for the presence of metabolites for which pulverized samples were used. Standard techniques of Brain and Turner, 1975[7] were employed in the phytochemical screening.

iv. Test organisms

The clinical isolates bacteria and fungi were obtained from Department of Microbiology, Gauhati Medical College and Hospital, Assam, India. Identification and maintenance of cultures were performed by using classical diagnostic microbiology procedures[8]. The tested strains were cultured in Mueller Hinton Agar and Nutrient broth at 37°C for 16-18 hours and stored at 4°C. Two gram positive (*Staphylococcus aureus, Streptococcus pneumoniae*) and two gram negative *(Escherichia coli, Klebsiella pneumoniae*) bacteria and two fungi (*Candida albicans, Candida trophicalis*) were used for the study.

v. Antibacterial screening

a. Disk diffusion method

Antibacterial activity of the plant extracts prepared was assayed separately using disc diffusion method [9]. Petri dishes containing 25ml of Mueller Hinton Agar medium were plated with 16-18 hour old culture of a selected bacterial strain. Sterile filter paper discs (6 mm in diameter) containing a plant solvent residue dissolved in DMSO, were placed on the surface of the medium. DMSO and distilled water alone served as negative controls. A standard disc containing chloramphenicol, ciprofloxacin antibiotic drug (10µg/disc) was used as a positive control. Incubation was done for 16-18 hours at 37°C. The assessment of antibacterial activity was based on the measurement of the diameter of inhibition zone formed around the disc. Sterilized antibiotic discs (6 mm) were used as per published literature [10].

b. Agar well diffusion method

Bacterial strains grown on Nutrient agar at 37°C for 18 hours were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (10^8CFU/ml). The suspension was used to inoculate 90 mm diameter Petri plates. Wells with diameter 6 mm were punched in the agar and filled with 3 ml of 2 g/ml extracts. The dissolution of the organic extracts (Methanolic) was facilitated with the addition of 1% (v/v) DMSO and that of the aqueous extracts with the addition of sterile distilled water, neither of which affected the growth of microorganisms as shown by our control experiments.

vi. Antifungal Assay

Antifungal assay was assessed by "Poisoned food technique" [11, 12]. 20 ml of Saubourad dextrose agar was poured in sterile Petri dishes and 2 mg/ml of each extract was added and allowed to solidify. After the medium solidified, inoculums was placed in the centre of each assay plate which was incubated at 25±2°C and on the 7th day the radial growth of the inoculums in each plate was observed.

vii. Determination of Minimal Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined using the tube dilution method. A set of seven capped small test tubes were used for each extract against each organism. Nutrient broth was prepared and 1 ml of it was transferred into each of the test tubes. 1 ml of the solution of the extract with the highest concentration (50 mg/ml) that inhibited the growth of the organism was transferred into the first test tube and then diluted serially by a factor of two into the seven test tubes. A loop-full from the culture, which showed zone of inhibition was introduced into each of the test tubes, capped and left at room temperature for 72 h. The tubes were then examined for the presence or absence of growth of the microorganisms which was visualized by the level of turbidity of the solution in the test tube. The test tube containing the solution of lowest concentration of extract that produced a clear solution was taken and recorded as the MIC of the crude extract.

RESULTS

On complete extraction from the plant samples - *Cissus quadrangularis* L., *Moringa oleifera* L., *Ziziphus jujuba* M., *Cassia fistula* L., *Dillenia indica* L., and *Paederia foetida* L., the percentage yield of each extract obtained was found to be 2.35 %, 2.0 %, 4.0 %, 4.34 %, 2.5 %, 2.3 %, 15.5 % (w/w), respectively. The results of the phytochemical analysis are presented in Table 1. The antimicrobial properties of the medicinal plants assessed showed higher inhibition with that the methanolic extracts of the plant samples have higher inhibition as compared to hexane and aqueous extracts for both bacteria and fungi. The MIC was interpreted as the lowest concentration of the extracts that did not permit any visible growth when compared with that of the control. *C. quadrangularis* L. aqueous and methanol extract showed highest inhibition against *K. pneumoniae* of 15mm each (Fig. 1), while *M. oleifera* L. showed zones of 15mm against *E. coli* and 12mm against *K. pneumoniae* and *C. albicans* (Fig. 2). *Z. jujuba* M. showed the lowest inhibition of 15mm, 12mm and 10mm against *E. coli*, *K. pneumoniae* and *C. tropicalis,* and *C. albicans* respectively (Fig. 3). Hexane extract of *C. fistula* L. showed 12.3 mm and 12.2 mm zone against *K. pneumoniae* and *S. aureus* (Fig. 4). *D. indica* L. hexane extract showed a zone of 15mm against *C. tropicalis* while methanol extract showed a 14mm zone against both *E. coli* and *C. tropicalis*, however, aqueous extract showed no inhibition at all (Fig. 5). Methanol extract of *P. foetida* L. showed the highest inhibition amongst all other plants with 19mm, 17mm and 15mm zones against *E. coli*, *S. pneumoniae* and *K. pneumoniae* respectively. Table 3 gives a detailed report on the MIC values in mg/ml of all the active plant extracts.

Table 1
Result of phytochemical screening of plant extracts

Plants	Alkaloids	Glycosides	Steroids	Terpenoids	Carbohydrates	Amino acids	Saponins
C.quadrangularis L.	-	-	+	+	+	-	-
M.oleifera L.	-	+	-	+	+	+	-
Z. jujuba M.	+	+	-	+	-	-	-
C.fistula L.	+	-	-	+	-	-	-
D.indica L.	-	+	+	+	+	-	+
P.foetida L.	+	+	-	+	+	+	+

(+) Presence of respective constituents, (-) Absence of respective constituents

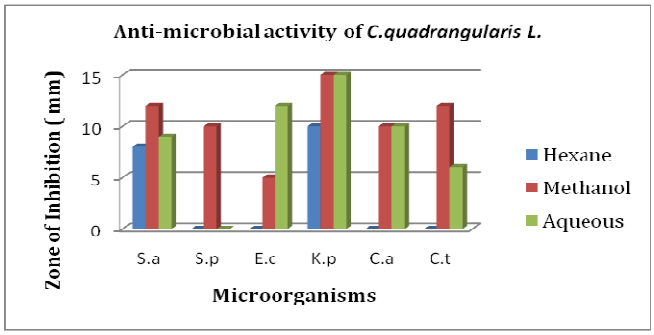

Figure 1
Graph representing anti-microbial activity of Cissus quadrangularis L. where S.a = Staphylococcus aureus, S.p = Streptococcus pneumoniae, E.c = Escherichia coli, K.p = Klebsiella pneumoniae, C.a = Candida albicans and C.t = Candida trophicalis

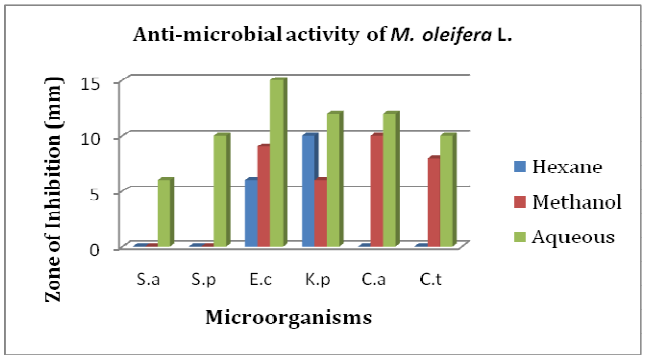

Figure 2

Graph representing anti-microbial activity of Moringa oleifera L. where S.a = Staphylococcus aureus, S.p = Streptococcus pneumoniae, E.c = Escherichia coli, K.p = Klebsiella pneumoniae, C.a = Candida albicans and C.t = Candida trophicalis

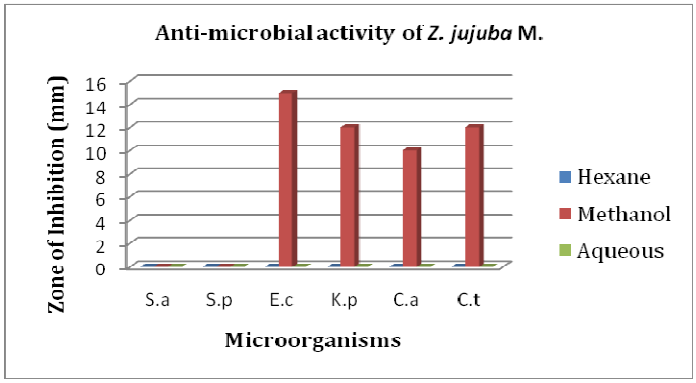

Figure 3

Graph representing anti-microbial activity of Ziziphus jujuba M. where S.a = Staphylococcus aureus, S.p = Streptococcus pneumoniae, E.c = Escherichia coli, K.p = Klebsiella pneumoniae, C.a = Candida albicans and C.t = Candida trophicalis

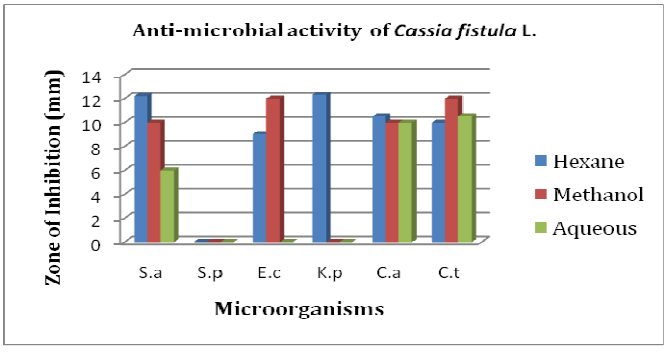

Figure 4

Graph representing anti-microbial activity of Cassia fistula L. where S.a = Staphylococcus aureus, S.p = Streptococcus pneumoniae, E.c = Escherichia coli, K.p = Klebsiella pneumoniae, C.a = Candida albicans and C.t = Candida trophicalis

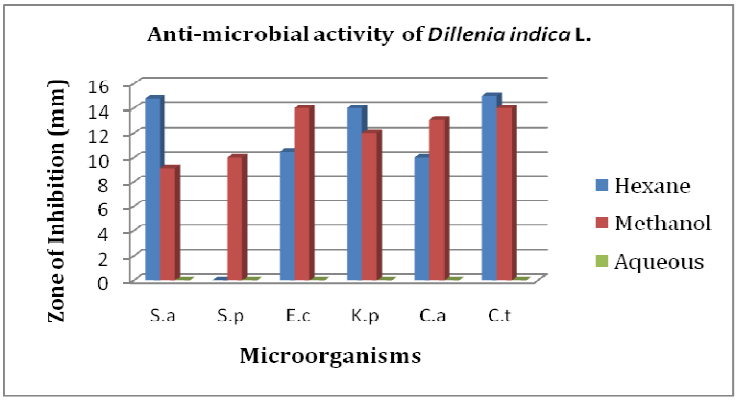

Figure 5

Graph representing anti-microbial activity of Dillenia indica L. where S.a = Staphylococcus aureus, S.p = Streptococcus pneumoniae, E.c = Escherichia coli, K.p = Klebsiella pneumoniae, C.a = Candida albicans and C.t = Candida trophicalis

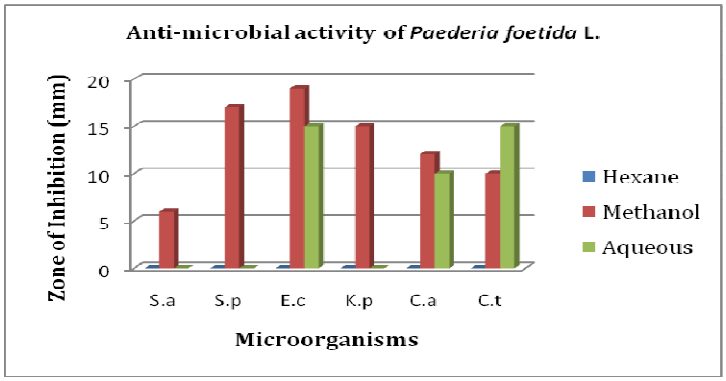

Figure 6

Graph representing anti-microbial activity of Paederia foetida L.where S.a = Staphylococcus aureus, S.p = Streptococcus pneumoniae, E.c = Escherichia coli, K.p = Klebsiella pneumoniae, C.a = Candida albicans and C.t = Candida trophicalis

Table 2

Zones of inhibition (mm) of the Standard drugs

Microorganisms /Standard drugs	S.a	S.p	E.c	K.p	C.a	C.t
Amoxicillin	22.71	20.3	17.45	16	-	-
Kanamycin	26.61	19.45	22.43	17	-	-
Ketoconazole	-	-	-	-	22.53	19.67

Table 3
Minimum Inhibitory Concentration (MIC) of Plant extracts in mg/ml

Plant Extracts/ Microorganisms	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Escherichia coli*	*Klebsiella pneumoniae*	*Candida albicans*	*Candida tropicalis*
C. quadrangularis L.	5.0	2.5	2.5	0.625	2.5	1.25
M. oleifera L.*	2.5	2.5	1.25	1.25	2.5	1.25
Z. jujuba M.	-	-	1.25	2.5	2.5	1.25
C. fistula L.	2.5	-	0.625	-	1.5	1.25
D. indica L.	5.0	2.5	1.25	2.5	0.625	0.32
P. foetida L.	5.0	0.625	0.32	1.25	1.25	2.5

**** - Aqueous extract***

DISCUSSION

The emergence of multiple drug resistance in human and animal pathogenic bacteria and fungi as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis. The easy availability of antimicrobial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents. The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella 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[13]. *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients[14]. Different solvents have various degrees of solubility for different phytoconstituents[15]. *In vitro* evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new bio-molecules of plant origin. The extracts demonstrated antimicrobial activity against both the test bacteria and fungi. In the present study, methanolic extract of plants showed a wide range of anti microbial activity against all microorganisms. For instance, methanolic extract of *C. quadrangularis L.* and *D. indica L.* showed activity against both bacteria and fungi. Both Gram negative bacterial and fungal strains are sensitive towards methanolic extract of *Z. jujuba* M. and *P. foetida*. Aqueous extract of *M. oleifera L.* showed activity against *S. aureus, E. coli, K. pneumoniae* and fungal strains. Methanolic extract of *I. staphylina R*. showed activity against both bacteria and fungi. Aqueous extract of D*. indica L., Z. jujuba* and Hexane extract of *P. foetida* L. did not show any activity against bacteria.

In Ayurveda, these plants have shown to have certain medicinal properties. In S*iddha* medicine, *Cissus quadrangularis* it is considered a tonic and analgesic, and is believed to help heal broken bones, thus its name *asthisamharaka* (that which prevents the destruction of bones). It is said to have antibacterial, antifungal, antioxidant, anthelmintic, antihemorrhoidal and analgesic activities. It contains a rich source of carotenoids, triterpenoids and ascorbic acid. In a study in Cameroon, high doses of the extracts have been shown to have beneficial effects against obesity and associated oxidative stress[16]. The immature green pods of *Moringa*

oleifera L., called “drumstick” are used as a sexual virility drug for treating erectile dysfunction in men and also in women for prolonging sexual activity[17]. The fruits of *Ziziphus jujuba* M. are used in Chinese and Korean traditional medicine, where they are believed to alleviate stress, and traditionally for antifungal, antibacterial, antiulcer, anti-inflammatory, sedative, antispastic, antifertility/contraception, hypotensive and Antinephritic, cardiotonic, antioxidant, immunostimulant, and wound healing properties[18, 19]. In Ayurvedic medicine, *Cassia fistula or* golden shower tree is known as *aragvadha*, meaning "disease killer". The root is considered a very strong purgative, and self-medication or any use without medical supervision is strongly advised against in Ayurvedic texts[20]. The juice of *D. indica* leaves; bark and fruits are mixed and given orally (5-15ml, two to five times daily) in the treatment of cancer and diarrhea. The fruit juice of this plant has anti-leukemic effect, cardiotonic effect[21]. *Paederia foetida* is known for the strong, sulphurous odour exuded when its leaves or stems are crushed or bruised. This is because the oil responsible for the smell, and found primarily within the leaves, contains sulphur compounds, including largely dimethyl disulphide[22]. These plants should be further analyzed, screened for metabolites and assessed of the individual component’s medicinal properties and the toxicity studies. Ayurveda practitioners can employ these plants as medicine and with further evaluation of these medicinal plants in the laboratory and practical use, there will be more data and evidence about their potential use.

CONCLUSION

This comparative study provides the basic information required for the development of drugs and medicine from plants that are available in abundance in this region. Although the nature and number of active components involved in each extract have not been evaluated yet, these findings are promising and further studies about the toxicity of plant extracts and the isolation of active compounds are important to propose.

ACKNOWLEDGEMENT

We are grateful to the Department of Biotechnology, Government of India for funding this research (Grant no. BT/213/NE/TBP/2011).

REFERENCES

1. Jamir NS, Ethnobotanical studies among Naga tribes in Nagaland: Biodiversity North east India perspective (ed., B Karbuli, D. Syiem, and H. Kayang) 128-40, (1999).
2. Sharma HK, Chhangte L, Dolui AK, Traditional medicinal plants in Mizoram, India. Fitoterapia, 72: 146-61, (2001).
3. Service RF, Antibiotics that Resist Resistance, Scien, 270:724-727 (1995).
4. Ahmad I, Mehmood Z and Mohammad F, Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol, 62: 183-193 (1998).
5. Pandey P, Mehta A and Hajra S, Evaluation of Antimicrobial Activity of Ruta graveolens Stem extracts by Disc Diffusion Method, Journal of Phytology, 3(3): 92-95 (2011).
6. Ljubuncic P, Song H, Cogan U, Azaizeh H and Bomzon H, The effects of aqueous extractsprepared from the leaves of *Pistacia lentiscus*in experimental liver

disease, Journal of Ethnopharmacology, 100: 198-204 (2005).
7. Brain KR and Turner TD, The practical evaluation of phytopharmaceuticals, Wrightscientechnica, Bristol, pp. 7-9 & 81-85 (1975).
8. Finegold SM, Martin WJ, Bailey and Scott's Diagnostic Microbiology 6th ed. St. Louis. The C.V. Mosby Company. 1982.
9. Bauer AW, Kirby WMM, Sherris JC and Turck M, Antibiotic susceptibility testing by a standardized single disk method, Amer. I. C/in. Pathol, 45:493-96 (1966).
10. Collins CM and Lyne PM, Microbiologicial Methods Buttermorths & Co (publishers) Ltd. London, pp. 450, (1987).
11. Grover RK and Moore JD, Toximetric studies of fungicides against brown rot organism *Sclerotina fruticola, Phytopathology,* 52:876-880, (1962).
12. Thippeswamy S, Praveen P, Mohana DC and Manjunath K, Antimicrobial evaluation and phytochemical analysis of a known medicinal plant *Samanea saman* (Jacq.) Merr. against some human and plant pathogenic bacteria and fungi, International journal of Pharma and Biosciences, 2(2): 443-52, (2011).
13. Gupta P, Murali P, Murali MV, Faridi MMA, Kaul PB, Ramachandran VC, Talwar V, Clinical profile of *Klebsiella septicaemia* in neonates, Ind J Paediatr, 60: 565-72, (1993).
14. Black JG, Microbiology: Principles and application. New York, Prentice Hall, pp. 260, (1996).
15. Marjorie MC, Plant products as antimicrobial agents, Clinical Microbiology Reviews, 12(4): 564-582, (1999).
16. Mishra G, Srivastava S and Nagori BP, Pharmacological and Therapeutic Activity of *Cissus quadrangularis*: An Overview, International Journal of PharmTech Research, Vol.2, No.2, pp 1298-310, (2010).
17. Oluduro OA, Idowu TO, Aderiye BI, Famurewa O and Omoboye OO, Evaluation of Antibacterial Potential of Crude Extract of *Moringa oleifera* seed on Orthopaedics Wound Isolates and Characterization of Phenylmethanamine and Benzyl Isothiocyanate Derivatives, *Research Journal of Medicinal Plant,* 6: 383-394, (2012).
18. Fahey JW, *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties, Part 1, Trees Life J. Vol. 1, (2005).
19. Posmontier B, The medicinal qualities of *Moringa oleifera*, Holistic Nursing Pract, 25: 80-87, (2011).
20. Jayasuriya AMH, Longest legume inflorescence in Sri Lanka, *Ceylon Journal of Science* (*Bio. Sci.*), 41 (1): 79-82, (2012).
21. Sharma HK, Chhangte L, Dolui AK, Traditional medicinal plants in Mizoram, India. Fitoterapia, 72, 146-61, (2001).
22. Wong KC, and Tan GL, Steam volatile constituents of the aerial parts of *paederia foetida* L., Flavour and Fragrance Journal, Vol 9 Issue 1, p25–28, (2006).