ORIGINAL ARTICLE

Antibacterial activity of plants used in Indian herbal medicine

Pavithra P. S., Janani V. S., Charumathi K. H., Indumathy R., Sirisha Potala, Rama S. Verma
Department of Biotechnology, Indian Institute of Technology Madras, Chennai - 600 036, Tamil Nadu, India

Delonix elata, *Enicostemma axillare, Merremia tridentata, Mollugo cerviana* and *Solanum incanum* are medicinal plants used in traditional Indian medicine for the treatment of various ailments. These plants were selected to evaluate their potential antibacterial activity. To determine antibacterial activity and phytochemicals in the crude extracts of five medicinal plants used in traditional Indian medicine for the treatment of various ailments like rheumatism, piles fever, skin diseases and snake bite. The antibacterial activity of organic solvent extracts of these plants were determined by disc diffusion and broth dilution techniques against gram-positive bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*) and gram-negative bacterial strains (*Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*). Results revealed that the chloroform and methanol extracts of *D. elata* and methanol extracts of *M. cerviana* exhibited significant antibacterial activity against gram-positive and gram-negative strains with minimum bactericidal concentration (MBC) ranging from 1.5 to 100 mg/ml. Methanol extracts of *M. tridentata* exhibited activity only against gram-positive bacterial strains with MBC ranging from 12.5 to 100 mg/ml. Extracts of *E. axillare* and *S. incanum* showed activity only against *B. subtilis* and were not bactericidal at 100 mg/ml. The most susceptible organism to the organic extracts from all the studied plants was *B. subtilis* and the most resistant organism was *P. aeruginosa*. The presence of phytochemicals such as alkaloids, tannins, triterpenoids, steroids and glycosides in the extracts of these plants supports their traditional uses as medicinal plants for the treatment of various ailments. The present study reveals potential use of these plants for developing new antibacterial compounds against pathogenic microorganisms.

Key words: Antibacterial, *Enicostemma axillare, Merremia tridentata, Mollugo cerviana, Solanum incanum*

INTRODUCTION

About 80,000 species of plants are utilized for treating various diseases in different systems of Indian medicine. Since 1990s there has been a growing shift in interest towards plants as significant sources for new pharmaceuticals. Many pharmaceutical companies show interest in plant-derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects. As per the World Health Organization (WHO) report, 80% of the world population, presently use herbal medicine for some aspect of primary health care.[1] Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.[2,3] With the advancement of modern medicinal technology, it is now easier to identify specific botanical constituents and assess their potential antimicrobial activity. Many herbs contain dozens of active constituents that combine to give the plant its therapeutic value. In the present study, five plants viz., *Delonix elata, Merremia tridentata, Mollugo cerviana, Enicostemma axillare* and *Solanum incanum* were selected based upon their traditional medicinal uses in the treatment of various ailments. This paper reports the antibacterial activity and phytochemicals present in the organic extracts of the above medicinal plants.

D. elata (L.) Gamble (Fabaceae) has been used in traditional Indian medicine for the treatment of rheumatism, stomach disorders,[4] and its leaves are used in the treatment of bronchitis and pneumonia in infants.[5] Leaf extracts of *D. elata* are reported for strong anti-inflammatory activity.[6] *M. cerviana* (L.) Ser. (Molluginaceae) has been widely used as a pot herb, enhances eyesight, reduces body odour, acts as a good antiseptic and is used in the treatment of cough.[4] *E. axillare* (Lam.) A. Raynal (Gentianaceae) is used as laxative and in the treatment of rheumatism. The plant has bitter glycosides and ophelic acid.[7] *M. cerviana* and *E. axillare* have been documented for anti-inflammatory activity.[8] *M. tridentata* (L.) Hallier f. (Convolvulaceae) is used in traditional Indian medicine as a tonic, laxative and astringent.[9] The anti-hypertensive activity[10] and wound healing properties[11] of this plant have been reported. *S. incanum* (L.) (Solanaceae) is used in the treatment of cough, cold and as expectorant.[4]

Address for correspondence: Dr. Rama. S. Verma, Department of Biotechnology, Indian Institute of Technology Madras, Chennai - 600 036, Tamil Nadu, India. E-mail: vermars@iitm.ac.in
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The aqueous and methanol extracts of the leaves of *S. incanum* were studied for their antibacterial effect against *E. coli* strains and results indicated that the extracts were bacteriostatic at higher concentrations.[12] The fruits extracts of *S. incanum* exhibited strong anti-fungal activity against five opportunistic human fungal pathogens.[13] The plants selected for the study are known for therapeutic uses as carminative, stomachic, antiseptic, laxative and anti-inflammatory properties in the treatment of various ailments in traditional Indian medicine. The lack of scientific data regarding the presence of antibacterial activity of these medicinal plants led us to investigate the antibacterial activity and phytochemicals present in the organic extracts of these plants that may provide scientific justification to the traditional uses in treating various ailments.

MATERIALS AND METHODS

Plant Material

The plants used for the study were *D. elata* (Fabaceae), *E. axillare* (Gentianaceae), *M. tridentata* (Convolvulaceae), *M. cerviana* (Molluginaceae) and *S. incanum* (Solanaceae). Plants were procured from Tamil Nadu Medicinal Plant Farms and Herbal Medicine Corporation Ltd (TAMPCOL), Chennai, India. Parts of the plants used in the study and their ethno botanical uses are mentioned in Table 1.

Preparation of Extracts

A 30 g of air-dried plant powder (leaves, stem, and fruits) was soaked in 300 ml of organic solvents, viz., methanol, hexane and chloroform separately for 24 h in a round bottomed flask at room temperature. Extracts were filtered through the Whatman filter paper No.1. The filtrate was allowed to dry at room temperature and hexane, methanol and chloroform extracts were obtained. Condensed extracts were weighed and stored in air-tight containers at 4°C till further investigation.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening was performed to identify phytochemicals in the methanol, chloroform and hexane extracts of plants parts (leaves, stem, aerial parts and fruits) used in this study. There are several sophisticated techniques, e.g. thin layer chromatography, ultra violet and infrared spectroscopy, nuclear magnetic resonance and high-performance liquid chromatography for identification of various groups of phytochemical compounds in plant extracts; however, in the present work, the phytochemicals were detected by colour tests. A 200 mg extracts were dissolved in 20 ml of its mother solvents. These extracts were subjected to preliminary phytochemical tests as described earlier.[14] Briefly, following tests has been performed for identifying the class of compounds.

Test for alkaloids

Of each extract 2 ml was acidified with a few drops of dilute hydrochloric acid and then 1 ml of Dragendorff reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

Test for tannins

To 2 ml of each extract, a few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

Test for saponins

To 1 ml of each extract taken in a measuring jar, 9 ml of distilled water was added and shaken vigorously for 15 s and extracts were allowed to stand for 10 min. Formation of stable foam (1 cm) indicates the presence of saponins.

Test for steroids

Chloroform 10 ml was added to 2 ml of all the three plant extracts. To these extracts, 1 ml of acetic anhydride was added: then, 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Colour formation at the junction is noted. The appearance of blue-green colour indicates the presence of steroids.

Test for triterpenoids

The test for triterpenoids is same as that for steroids. The

Table 1: Ethno botanical information of traditionally used Indian medicinal plant species selected for antibacterial activity

Plant	Family	Common name	Part used	Action/therapeutic uses
Delonix elata	Fabaceae	Vedanarayanan	Leaves	Carminative, fever, malaria,[32] rheumatism, stomachic, flatulence, paralysis[4]
Enicostemma axillare	Gentianaceae	Vallari, Vellarugu	Aerial parts	Stomachic, bitter tonic, laxative, carminative,[35] bug bites, swellings, depurative, dropsy,[32] locally applied in snake bite, fever[34]
Merremia tridentata	Convolvulaceae	Thirupal Pullu, Mudhiyaar Koondal	Stem	Astringent, calefacient, laxative, anodyne, hemiplegia, hemorrhoids, uropathy, mouth wash, piles, inflammation,[29] fever, leprosy[4]
Mollugo cerviana	Molluginaceae	Parpadagam	Aerial parts	Blood purifier, fever, post partum discharges,[34] antiseptic, stomachic, febrifuge, gout and in rheumatic complaints[35]
Solanum incanum	Solanaceae	Kandan Kathri	Fruits	Cough, cold, flatulence, as expectorant, urination[4]

appearance of red, pink or violet colour at the junction indicates the presence of triterpenoids.

Test for cardiac glycosides

To 1 ml of each extract, a few drops of glacial acetic acid and ferric chloride, and 3-4 drops of concentrated sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides.

Microorganisms

The antibacterial activity of the extracts was tested individually on gram-positive and gram-negative bacterial strains. All bacterial strains were obtained from National Collection of Industrial Micro organisms (NCIM), Pune, India. The gram-positive bacterial strains used were *Bacillus subtilis* (NCIM 2718) and *Staphylococcus aureus* (ATCC 25923) and gram-negative bacterial strains used were *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063) and *Pseudomonas aeruginosa* (ATCC 27853). Bacterial strains were maintained on nutrient agar at 4°C and sub-cultured every month in our laboratory.

Antibacterial Activity

Agar disc diffusion assay

The antibacterial activity of the extracts was determined by the disc diffusion method.[15] Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth (O.D. 600=0.08) to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Five filter paper discs (Whatman No. 1, 6 mm diameter) were placed on the inoculated agar surface. A 20 µl of the extracts (100 mg/ml) were loaded on to the filter paper discs and were allowed to dry completely. Standard antibiotics ampicillin (10 µg), gentamicin (10 µg) and 20 µl of DMSO were placed as controls. Plates were incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicate.

Determination of minimum inhibitory concentration

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution in the Muller-Hinton broth to obtain concentrations from 100 mg/ml to 0.19 mg/ml. Standard antibiotics ampicillin, gentamicin and DMSO were placed as controls. A 10 µl of 10^7 (CFU) bacterial cultures were added to the tubes and were incubated at 37°C for 18 h. MIC was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration.[15]

Determination of minimum bactericidal concentration

A minimum bactericidal concentration (MBC) is the lowest concentration of an antibiotic required to kill a microorganism. The MBC was determined by sub-culturing 10 µl of the test dilutions from MIC tubes on to fresh Mueller-Hinton agar plates. Plates were incubated for 18-24 h. The highest dilution that yielded no single bacterial colony on the plates was recorded as MBC.

MIC index

The MIC index (MBC/MIC) was calculated for each extract and standard control drug to determine whether an extract is bactericidal (MBC/MIC <4) or bacteriostatic (MBC/MIC >4) on growth of bacterial organisms.[16,17] Also, the range of MIC index values greater than 4 and less than 32 are considered as bacteriostatic.[18]

RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed the presence of alkaloids, saponins, tannins, steroids, glycosides and triterpenoids [Table 2] in methanol, chloroform and hexane extracts, respectively. The observed antibacterial activity is attributed to the presence of bioactive compounds in the extracts of plants tested. The presence of these bioactive compounds in crude extracts is known to confer antibacterial activity against disease-causing microorganisms[19] and offer protection to plants themselves against pathogenic microbial infections.[20] The antibacterial activity of the five plants species was assayed by the agar disc diffusion method against five bacterial strains. All the five plants exhibited antibacterial activity against one or more test organisms. Methanol extracts from all the five plants were found to have highest antibacterial activity, whereas the hexane and chloroform extracts were less effective in inhibiting bacterial growth. Among the five bacterial strains tested for antibacterial activity, *B. subtilis* was most susceptible with inhibition zones ranging from 8.66±0.57 to 12±1 mm and *P. aeruginosa* was least susceptible organism to the plant extracts. The resistance conferred by *P. aeruginosa* against many antibiotics and non-antibiotic antimicrobial agents may be due to the permeability barrier exhibited by their outer membranes.[21] Further, the chloroform and methanol extracts of *D. elata* was found to be most effective among five tested plant species in inhibiting the bacterial growth with zones sizes in the range of 7±0-12±1 mm. Our results also support the previous finding of antibacterial activity of *D. Elata*'s methanol extract against *E. coli*, *B. subtilis* and *P. aeruginosa*.[22] Chloroform and methanol extracts of *D. elata* inhibited gram-positive strains *B. subtilis,*

S. aureus, gram-negative strains *K. pneumoniae* and *E. coli* with MIC ranging from 0.78 to 3.125 mg/ml and 12.5 to 25 mg/ml, respectively. However, the extracts did not exert any inhibitory activity on *P. aeruginosa*, contrary to earlier studies[22] where they reported the inhibitory activity of methanol extract of *D. elata*.

M. cerviana had broad, but relatively weak activity in this study. Methanol extracts inhibited the growth of *B. subtilis, S. aureus, K. pneumoniae* and *E. coli* with zones of 7.33±0.57 to 11±1, while hexane and chloroform extracts were ineffective against these bacterial strains. The methanol extract of *M. tridentata* was found to have strong antibacterial activity only against gram-positive strains *B. subtilis* and *S. aureus* with inhibition zones of 11.33±0.57 to 12±0. Hexane, chloroform and methanol extracts of *E. axillare* and *S. incanum* were only active against gram-positive strain *B. subtilis* with zones of 8.66±0.57 to 11.3±0.57. The inhibition zones for the standard antibiotics gentamicin and ampicillin against all bacterial strains ranged from 13 to 25 mm and 18 to 37 mm, respectively.

Extracts of *D. elata, M. cerviana* and *M. tridentata* exhibited bactericidal activity with MBC ranging from 1.5 mg/ml to 100 mg/ml against bacterial strains, whereas the extracts of *S. incanum* and *E. axillare* were not bactericidal at the tested range. The minimum inhibitory and bactericidal concentrations of the extracts are shown in Tables 3 and 4, respectively. Based upon the MIC and MBC values, the MIC index is calculated for the plant extracts. These data elucidate the observed antibacterial activity of the extract as bactericidal or bacteriostatic. The methanol extract of *D. elata* was found to be bacteriostatic against *S. aureus, K. pneumoniae* and methanol extract of *M. tridentata* was found to be bacteriostatic against gram-positive species *B. subtilis*. The MIC index values of all the other plant extracts are tabulated in Table 5.

In general, the methanol extract of the tested plants was most effective in inhibiting the bacterial growth suggesting that polar solvent methanol was most successful in extracting secondary metabolites responsible for the antibacterial property than chloroform and hexane solvents.

Table 2: Preliminary phytochemical analysis of the plant extracts

Plants	Tannins			Saponins			Triterpenoids/steroids			Cardiac glycosides			Alkaloids		
	M*	C*	H*	M	C	H	M	C	H	M	C	H	M	C	H
Delonix elata	+*	+	+	-*	-	-	+/+	+/+	+/-	+	+	+	+	+	-
Mollugo cerviana	-	+	+	+	-	-	+/-	-/-	-/-	-	+	+	-	+	+
Merremia tridentate	-	+	+	+	-	-	+/-	-/-	+/-	+	+	+	+	+	+
Enicostemma axillare	+	+	+	-	-	-	-/-	-/-	-/-	+	-	+	-	+	-
Solanum incanum	+	+	-	+	-	-	-/-	-/-	-/-	-	-	-	-	-	-

*(+) and (–) indicate the presence and the absence of the phytochemicals, respectively; M: methanol, C: chloroform, H: hexane

Table 3: Minimum inhibitory concentration of the extracts (mg/ml)

Plant/extract		Bacterial organisms				
		B. subtilis	*Staph. aureus*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
Delonix elata	M*	12.5	12.5	12.5	25.0	- *
	C*	0.78	3.125	3.125	3.125	-
	H*	25.0	-	-	-	-
Mollugo cerviana	M	25.0	100.0	50.0	50.0	-
	C	25.0	-	-	-	-
	H	12.5	-	-	-	-
Merremia tridentata	M	6.25	3.125	-	-	-
	C	12.5	-	-	-	-
	H	6.25	-	-	-	-
Enicostemma axillare	M	12.5	-	-	-	-
	C	12.5	-	-	-	-
	H	100.0	-	-	-	-
Solanum incanum	M	100.0	-	-	-	-
	C	100.0	-	-	-	-
	H	-	-	-	-	-
Ampicillin (μg/ml)		-	10	-	10	-
Gentamicin (μg/ml)		10	10	10	10	10

*M: methanol, C: chloroform, H: hexane; (-): no activity

Table 4: Minimum bactericidal concentration of extracts (mg/ml)

Plant/Extract		Bacterial organisms				
		B. subtilis	***Staph. aureus***	***K. pneumoniae***	***E. coli***	***P. aeruginosa***
Delonix elata	M*	25.0	100.0	100.0	50.0	-*
	C*	1.5	6.25	12.5	12.5	-
	H*	25.0	-	-	-	-
Mollugo cerviana	M	50.0	-	100.0	100.0	-
	C	50.0	-	-	-	-
	H	25.0	-	-	-	-
Merremia tridentata	M	100.0	12.5	-	-	-
	C	50.0	-	-	-	-
	H	-	-	-	-	-
Enicostemma axillare	M	-	-	-	-	-
	C	-	-	-	-	-
	H	-	-	-	-	-
Solanum incanum	M	-	-	-	-	-
	C	-	-	-	-	-
	H	-	-	-	-	-
Ampicillin (μg/ml)		-	10	-	10	-
Gentamicin (μg/ml)		10	10	10	10	10

*M: methanol, C: chloroform, H: hexane; (-): no activity

Table 5: Antibacterial activity of the extracts on bacterial strains in terms of MIC index [=MBC/MIC]

Plant/Extract		MIC index			
		Bactericidal activity on specific strains, MIC index ≤4		**Bacteriostatic activity on specific strain, MIC index >4 and <32**	
Delonix elata	M*	*B. subtilis, E. coli*	2	*S. Aureus; K. pneumoniae*	8
	C*	*B. subtilis* *S. aureus* *E. coli, K. pneumoniae*	1.9 2 4	-	-
	H*	*B. subtilis*	1	-	-
Mollugo cerviana	M	*B. subtilis, E. coli, K. pneumoniae*	2	-	-
	C	*B. subtilis*	2	-	-
	H	*B. subtilis*	2		
Merremia tridentate	M	*S. aureus*	4	*B. subtilis*	16
	C	*B. subtilis*	4	-	-
Ampicillin		*S. aureus, E. coli*	1	-	-
Gentamicin		*B. subtilis, S. aureus, E. coli, K. pneumoniae*	1	-	-

*M: methanol, C: chloroform, H: hexane; (-): no activity

Bioactive secondary metabolites have been utilized as natural medicines and plants containing those compounds have been used as medicinal plants and are prescribed in many recipes as forms of crude drugs.[23,24] In the present study, the extracts of *D. elata* revealed the presence of steroids and triterpenoids. Triterpenoids are well known to have anti-inflammatory activity.[25] This plant is used in traditional Indian medicine as an anti-inflammatory agent and as a traditional remedy for rheumatism.[4] Phytochemical analysis revealed the presence of tannins in the extracts of *M. tridentata*. Tannins are known for their astringent property, antimicrobial activity,[26] anti-inflammatory[27] and anti-diarrhoeal properties.[28] *M. tridentata* plant is used as an astringent, anodyne,[29] for treatment of rheumatism, piles and urinary disorders in traditional medicine.[9] Also, the extracts of *E. axiallare* revealed the presence of tannins. This plant is applied locally in snake bite, bug bites, treatment of rheumatism and swellings.[30]

The extracts of *S. incanum* and *M. cerviana* showed the presence of tannins and saponins. Saponins are known for their medicinal properties as a natural blood cleanser, expectorant and antibiotics.[31] The plant *S. incanum* is used in the treatment of stomach disorders, cold and as expectorant[4] and *M. cerviana* is used to treat fever and to purify blood.[32] Alkaloids are known to have significant

physiological activities by acting mainly on the central nervous system.[33] Alkaloids and glycosides were found in the extracts of *D. elata, M. cerviana, E. axillare* and *M. tridentata.*

The antibacterial activities of the extracts tested at a concentration of 100 mg/ml (2 mg/disc) were found to be less effective than standard antibiotics gentamicin (10 μg) and ampicillin (10 μg). These extracts may provide activity comparable to the standards if tested at higher doses since similar results have been reported for other plant extracts.[34] The bacterial strains *B. subtilis, K. pneumoniae* and *P. aeruginosa* were resistant to ampicillin (10 μg). However, all the bacterial strains were susceptible to gentamicin. The plant extracts exhibited antibacterial activity against the bacterial strains *B. subtilis* and *K. pneumoniae* that were resistant to ampicillin. Interestingly, our study revealed antibacterial potency of *D. elata, M. cerviana,* and *M. tridentata* with MIC values over the range of 0.78-100 mg/ml and MBC values from 1.5 to 100 mg/ml. However, these crude plant extracts may not have enough pure compounds to exert their activity at the dose levels tested. Extracts of *E. axillare* and *S. incanum* were ineffective in exhibiting the antibacterial property which may be due to the absence of antibacterial constituents or the concentration of extract studied was not sufficient to exhibit antibacterial activity.

CONCLUSIONS

The present study reports for the first time the antibacterial activity exhibited by extracts of *D. elata, M. cerviana* and *M. tridentata* used in traditional Indian medicine for the treatment of various ailments. MIC, MBC activities and MIC index of the plant extracts have been determined to give an idea about its antibacterial potency and antibacterial action. Two other plants studied viz., *S. incanum* and *E. axillare* were less effective in inhibiting the bacterial growth at the tested concentrations. These active plant extracts may be further subjected to biological and pharmacological investigations for isolation of antibacterial and therapeutic compounds. The results of phytochemical analysis and antibacterial activity studies of these plants extracts confirm their therapeutic usage as depicted in the literature.

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REFERENCES

1. Sujatha S. Complementary and alternative therapies in palliative care: A transition from modern medicine to traditional medicine in India. J Cancer Pain Symptom Palliation 2005;1:25-9..
2. Colombo ML, Bosisio E. Pharmacological activities of *Chelidomium majus L* (Paveraceae). Pharmacol Res 1996;33:127-34.
3. Iwu MM, Duncan AR, Okunji CO. New Antimicrobials of Plant Origin. In: Janick J,editor. Perspectives on new crops and new uses. Alexandria: Ashs Press; 1999. p. 457-62.
4. Thirugnanam S. Mooligai Maruthuvam. (Tamil) Trichy: Selvi Publishers; 2003. p. 33, 117, 131,139,147.
5. Compendium of Medicinal Plants. Delhi: National Institute of Industrial Research (NIIR) publication; 2005. p. 91.
6. Sethuraman MG, Sulochana N. The anti-inflammatory activity of *Delonix elata*. Curr Sci 1986;55:343.
7. Jayvir A, Minoo P, Gauri B, Raipal K. Nature heals - A Glossary of Selected Indigenous Medicinal Plants of India. Ahamdabad: Sristi Innovations; 1997. p. 25, 27.
8. Sadique J, Chandra T, Thenmozhi V, Elango V. The anti-inflammatory activity of *Enicostemma littorale* and *Mollugo cerviana*. Biochem Med Metab Biol 1987;37:167-76.
9. Krithikar KR, Basu BD. Indian Medicinal Plants In: Blatter E, Caius JF, Bhaskar KS, editors. Vol 1-5. Bishen Singh, Mahendra Pal Singh. Dehradun: 1991. p. 1734.
10. Klaus H, Nyman U, Smitt UW, Adsersen A, Gudiksen L, Sreedharan R, Pushpangadan P. *In vitro* screening of traditional medicines for anti-hypertensive effect based on inhibition of the angiotensin converting enzyme (ACE). J Ethnopharmacol 1995;48:43-51.
11. Hatapakki BC, Hukkeri VI, Patil DN, Chavan MJ. Wound healing activity of aerial parts of *Merremia tridentate*. Indian Drugs 2004;41:532-35.
12. Britto SJ, Senthilkumar S. Antibacterial activity of *Solanum incanum* L leaf extracts. Asian J Microbiol Biotechnol Environ Exp Sci 2001;3:65-6.
13. Mohammed-Al Fatimi, Martina W, Gudrun S, Hansen UL. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J Ethnopharmacol 2007;3:657-66.
14. Harborne JB. Phytochemical methods. London: Chapman and Hall Ltd; 1996. p. 52-105.
15. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol 1988;23:127-49.
16. Kone WM, Kamanzi AK, Terreaux C, Hostettmann K, Traore D, Dosso M. Traditional medicine in north Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. J Ethnopharmacol 2004;93:43-9.
17. Chattopadhyay RR, Bhattacharyya SK, Medda C, Chanda S, Datta S, Pal NK. Antibacterial activity of black myrobalan (Fruit of Terminalia chebula Retz.) against uropathogen *Escherichia coli*. Phcog Mag 2007;11:212-5.
18. Cutler NRC, Sramek, John JS, Prem KN. Pharmacodynamics and drug development: Perspectives in clinical pharmacology. New York: John Wiley and Sons; 1994. p. 318.
19. Farnsworth AC. The role of ethnopharmacology drug development from plants. England Ciba: John Wiley and Sons; 1982. p. 2-10.
20. De N, Ifeoma E. Antimicrobial effects of components of the bark extract of neem *Azadirachta indica* A. Juss Technol Dev 2002;8:23-8.
21. Lino A, Degracious O. The *in vitro* antibacterial activity of *Annona senegalensis, Securidacca longipendiculata* and *Steganotaenia araliaceae*-Ugandan medicinal plants. Afr Health Sci 2006;6:31-5.
22. Srinivasan K, Abdul Nazar Dheen M, Perumal G, Mohanasundari C, Natrajan D. Screening of methanolic leaf extracts of some medicinal plants against pathogenic bacteria. Adv Plant Sci 2005;18:605-7.
23. Zuin VG, Vilegas JH. Pesticide residue in medicinal plants and phytomedicines. Phytother Res 2002;14:73-88.
24. Rios JL, Recio MC. Medicinal plant and antimicrobial activity. J Ethnopharmacol 2005;100:80-4.

25. Rios JL, Recio MC, Manez S, Giner RM. Natural triterpenoids as anti- inflammatory agents. In: Atta-ur- Rahman, editor. Studies in Natural Products Chemistry. Elsevier; 2000. p. 93-143.
26. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
27. Hu Fenglina, Lu Ruilia, Huang Baoa, Ming Liangb. Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. Fitoterapia 2004;75:14-23.
28. Palombo EA. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: Modes of action and effects on intestinal function. Phytother Res 2006;20:717-24.
29. Warrier PK, Longman Staff, Nambiar VP, Ramankutty C, Vasudevan NR. Indian Medicinal Plants: A Compendium of 500 Species. India: Orient Blackswan; 1996. p. 22.
30. Johnson T. CRC Ethnobotany Desk Reference. United Kingdom: CRC Press; 1999. p. 264, 302.
31. Kalanithi N, Lester P. Micronutrients and Health: Molecular Biological Mechanisms. The American Oil Chemists Society; 2001. p. 136.
32. Chhabra SC, Uiso FC, Mshiu EN. Phytochemical screening of Tanzanian medicinal plants. I. J Ethnopharmacol 1984;11:157-79.
33. Chhabra SC, Uiso FC, Mshiu EN. Phytochemical Screening of Tanzanian Medicinal Plants. J of Ethnopharmacol 1984;11:157-79.
34. Parekh J, Chanda S. *In vitro* Antimicrobial activity and phytochemical analysis of some Indian Medicinal Plants. Turk J Bio 2007;31:53-8.
35. Nadkarni KM. Dr. K.M. Nadkarni's Indian Materia Medica: With Ayurvedic, Unani-tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home Remedies, and Appendices and Indexes. Mumbai: Popular Prakashan; 1954. p. 485, 804.

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