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Antibacterial activities of some Indian traditional plant extracts

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ABSTRACT

Objective: To evaluate the *in vitro* antibacterial activity of various solvent extracts of South Indian traditional medicinal plants *Ocimum sanctum*, *Ocimum gratissimum*, *Aegle marmelos*, and *Adhatoda vasica* leaves against clinical pathogens of human origin. **Methods:** The antimicrobial activity of different solvents crude extract of four medicinal plants used in traditional Indian medicine was tested by disc diffusion method against five bacterial pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi* and *klebsiella pneumoniae*.. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined for evaluating the potential plant extract. **Results:** The antibacterial results showed methanol extracts (0.4 g/ml) of *Ocimum gratissimum* and *Ocimum sanctum* showed maximum zone of inhibition (30 mm and 25.5 mm, respectively) against *Salmonella typhi*. MIC was tested at various concentrations from 0.625 mg/ml to 0.039 mg/ml for all the plant extracts. At the lowest concentration (0.039mg/ml) tested, methanol extracts of *Ocimum gratissimum* showed higher MIC against *Salmonella typhi* and *Salmonella paratyphi* where as the methanolic extracts of *Ocimum gratissimum* showed potent activity against *Staphylococcus aureus* at 0.078 mg/ml. Methanol extract (0.4 g/ml) of *Aegle marmelos* showed significant inhibitory activity of 22.5mm and MIC value of 0.156.mg/ml against *E. coli* strain. The *Klebsiella* spp was the most resistant strain of all and various concentrations *Adhatoda vasica* extract showed less activity against the tested pathogens. **Conclusions:** The present screening result demonstrated that the Indian traditional medicinal plants *Ocimum sanctum*, *Ocimum gratissimum*, *Aegle marmelos* methanol leaf extract has potent antibacterial activity and the studied plants may be new source for novel antibacterial compound discovery for treating drugs resistant human pathogens.

1. Introduction

Recently, the World Health Organization reports that at least 75 – 95% of the world populations of developing countries were chiefly rely on traditional medicines and major part of traditional therapies involves the use of plant extract products or their active constituents [1]. Traditional medicine usage is a common practice in developed and developing countries at the primary healthcare level [2]. Due to increased and indiscriminate use of antibiotics for treatment of humans and animals there develops the antibiotic resistance and multidrug resistance microorganisms like *Salmonella* spp. which has increased a great deal in developing countries [3]. It is estimated that nontyphoidal *Salmonella* cause between two hundred million and 1.3 billion cases of intestinal disease including 3 million of death each year worldwide [4]. The demand for more and more drugs from plant sources is continuously increasing which necessitates screening medicinal plants with promising biological activity [5]. Medicinal plants are gifts of nature to cure number of diseases among human beings and a large number of plants in different location around the world have been extracted, semi-purified to investigate individually their antimicrobial activity. [6].

The plant like *Ocimum sanctum*, *Ocimum gratissimum* has a versatile role to play in traditional medicines. These are popularly known as "Thulasi" also tulsi, tulasī, or Holy Basil is an aromatic plant in the family Lamiaceae. *Ocimum sanctum* is cultivated for its medicinal purpose as herbal

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tea in Ayurveda and religious purpose as performing worship with leaves across South Asia. It was well documented already on the bioactive compounds of *Ocimum sanctum* for medicinal aspects i.e. antimicrobial, adaptogenic, antidiabetic, hepatoprotective,anti-inflammatory, anti-carcinogenic, radioprotective, immunomodulatory, chemopreventive, cardio-protective, and safe guarding against possible deficiencies [7]. *Ocimum sanctum* extracts are effective against *Staphylococcus aureus, E. coli. P. aeruginosa , S. typhimurium* and used as better alternative in food preservation [8]. *Ocimum gratissimum* were cultivated in Indian houses, temples for pujas and traditional medicinal aspects. These plants were widely distributed in tropical and warm temperature regions in India and commonly known as "alfavaca". It is naturally used in the treatment of different diseases including upper respiratory tract infections, diarrhea, headache, conjunctivitis, skin diseases, pneumonia tooth and gum disorders[9].

The plant *Aegle marmelos* belong to family Rutaceae is commonly known as "Bael" in (Hindi), as "vilvam" in Tamil and leaves also auspiciously used in Indian temples for pujas. Also the leaves, stem, bark, fruits possess medicinal value and widely used in treating skin and eye diseases [10]. *A. marmelos* leaves extract exhibit broad spectrum antibacterial and antifungal activities [11]. *Aegle marmelos* solvent extract were reported to show potential antidiabetic in regeneration of ß-cells, antihyperlipidaemic, antioxidant, radio protective, hepatoprotective, antiarthritis activity and anti-inflammatory properties [12 - 18]. Because of its potent antibacterial activity, *Aegle marmelos* plants were used in folk-lore medicine for the production of bioactive compounds and for preliminary health care [19].

Adhatoda vasica (acanthaceae) known as chue mue, is a stout straggling prostrate shrubby plant with the compound leaves which gets sensitive on touching. The medicinal properties of *Adhatoda vasica* exert bacteriostatic and bactericidal effects on both gram positive and gram negative bacteria on animal models. These effects have been attributed to the peptides, alkaloids, and flavonols, which are major components in these plants [20- 21].

The usage of plant parts as traditional medicine is the most common practice in India, particularly as folk-lore medicines. Due to continuous usage of antibiotics against clinical pathogens, development of drug resistance is a major problem now-a-days. With this in view, the wild plant extracts of *Ocimum sanctum*, *Ocimum gratissimum*, *Aegle marmelos*, and *Adhatoda vasica* was tested for searching a potential source for new type of antibiotics for treating bacterial diseases. The folk-lore medicinal facts make the present work to investigate on the antibacterial activity of wild plant extracted compounds against clinical pathogenic isolates.

2. Materials and methods

2.1. Collection and identification of plant materials

The medicinal plants, *Ocimum sanctum*, *Ocimum gratissimum*, *Aegle marmelos*, and *Adhatoda vasica* were collected from Paallar river beds of Kanchipuram, Tamilnadu, India. The taxonomic position of the plants were identified and authenticated. Leaves from the plants were collected in a large quantity and washed with clear distilled water and dried in an oven at 60℃ for 5 mins for extraction purposes

2.2. Extraction of plant materials

250 gm of each plant leaves were taken for extraction procedure and grinded in a mortar and pestle separately under aseptic condition [22]. The solvents extraction was done by modified method of dissolving 5 g of dried plant powder in soxhlet apparatus with ethanol, methanol and acetone (200ml) separately for 24 hrs at 65℃. The extracts were concentrated to dryness in rotary pressure evaporator and stored at 40℃ for further antimicrobial study [23].

2.3. Preparation of test organisms

Staphylococcus aureus, *Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli* and *Klebsiella pneumoniae* were isolated from the clinical samples obtained from patients attending Government Hospital, Kanchipuram. Tamilnadu, India. The organisms were isolated in nutrient agar medium and selectively cultured at 37℃ for 24 hrs. The bacterial strains were identified by biochemical and standard antibiogram tests as per the directions from Bergy's manual for determinative bacteriology.

2.4. Antimicrobial assay

2.4.1. Antibacterial sensitivity testing using disc diffusion method

Circular disc of 6 mm diameter were made from the whatman no 1 filter paper. Discs were impregnated with equal volume (50 μl) of each plant extracts at four different concentrations (0.05 g/ml, 0.1g/ml, 0.2g/ml & 0.4g/ml). The discs were aseptically placed over plates of Muller Hinton agar (MHA ,Difco) seeded with each of test pathogens, and the inoculum was adjusted to 0.5 Mc Farland turbidometry [22]. The plates were incubated in an upright position at 37 ℃ for 24 hours and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 12 mm were considered as having low antibacterial activity. Diameters between 12 and 16 mm were considered moderately active, and these with >16mm were considered highly active [24]. The clinical strains were also tested for their sensitivity against the standard antibiotics, ciprofloxacin (5 mcg), nalidixic acid (10mcg), novobiocin (30 mcg) by the disk diffusion method.

2.4.2. Antibacterial activity by microdilution MIC assay methods

The minimum inhibitory concentration (MIC) was determined by comparing the various concentrations of plant extracts which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition [25]. The MIC had done by 96 well U bottom plates. The MIC plates were filled with Mueller Hinton Agar (MHA) and various concentrations

of plant extracts, antibiotics-ciprofloxacin or solvent control. Finally the MHB medium with overnight test organism (10-8cfu /ml-1) was equally distributed. All the samples were prepared in triplicates and incubated at 37 ℃for 24 hrs [26].

To determine the MBC, the treated broth culture from well which is not showing any visible growth in MIC assay was cultured on new sterile MHA plates. The least concentration (highest dilution) of the extract that inhibits colony formation on a solid agar medium after incubation at 37 ℃ for 24 hr was considered as MBC [22]

3. Results

The extracts of *Ocimum sanctum*, *Ocimum gratissimum*, *Aegle marmelos* and *Adhatoda vasica*, tested for antibacterial activity on five human pathogens were presented in Table 1. The zone of inhibition around the disc impregnated with plant extract over the lawn of bacterial culture plates determined the antibacterial activity as quantitatively. The result showed that the antibacterial activities of plant extract were increased with increasing concentration of crude extracts. Though the extracts showed prominent antibacterial activity against gram negative (*S. typhi*, *S. paratyphi*, *E. coli*) and gram positive (*S. aureus)* bacteria, only the *Klebsiella* sp appeared to be resistance with very less zone of inhibition. Among the tested plant extracts *Ocimum gratissimum* methonalic extract showed highest activity of 30 mm (0.4g/ml) inhibition zone against *S. typhi* and followed by methanol extract of *Ocimum sanctum* showed 25.5 mm (0.4g/ml) inhibition zone against *S. typhi* (Table.1). The *Ocimum gratissimum* methanol extract also showed maximum inhibition zone of 28 mm (0.4 g/ml) against *Salmonella paratyphi*.

On the other hand, the *E. coli* strain were mostly resistance to various solvents of *Adhatoda vasica* plant extract and moderately resistant to *Ocimum sanctum*, *Ocimum gratissimum*, while *Aegle marmelos* methanol extract demonstrated significant inhibitory activity of 22.5mm (0.4 g/ml) against *E. coli* strain tested. Also the *S. aureus* moderately sensitive to various solvent extracts of *Ocimum sanctum*, *A. marmelos*, *Adhatoda vasica* and showed maximum sensitivity to methanol extract of *Ocimum gratissimum* inhibition with 24 mm (0.4g/ml) zone of inhibition.

The *Klebsiella* sp was resistant to the different concentrations of ethanol, acetone plant extracts of *Ocimum sanctum*, *Aegle marmelos* and *Adhatoda vasica*, while *Ocimum gratissimum* methanol extract demonstrated a inhibition zone of 9.0 mm (0.4g/ml) only. The result showed that *S. typhi*, *S. paratyphi* and *S. aureus* were the most susceptible species to the different concentrations of methanol extract of *Ocimum gratissimum* and *E. coli* susceptible to the extracts of *Aegle marmelos*.

The MIC analysis of plant extracts showed the optimum bacteriostatic and bacteriocidal concentration for methanol crude extracts of the plants tested. The table 2 depicted the MIC and MBC of all plant extracts and the zone of inhibition results reflected in MIC. The MIC of all the plant extracts

Table 1

Antibacterial activity of different plant extract by disc diffusion method R= no zone of inhibition

	O.sanctum Ethanol extract (mg/mL)				*O.sanctum* Methanol extract (mg/mL)				*O. sanctum* Acetone extract (mg/mL)			
Bacterial species	50	100	200	400	50	100	200	400	50	100	200	400
S. aureus	8.2	9.4	10.2	11.4	12.0	13.5	14.0	14.5	8.0	9.0	10.0	10.5
E.coli	R	R	6.6	7.8	R	R	7.0	7.5	R	R	R	7.2
S.typhi	10.0	11.5	12.0	14.0	15.0	17.5	22.5	25.5	R	7.0	7.6	8.2
S. paratyphi	8.5	9.6	10.5	12.0	14.0	16.0	19.0	21.0	R	6.0	6.5	7.5
Klebsiella sp	R	R	R	7.0	R.0	7.0	7.0	8.0	R	R	R	7.2
	O. Gratissimum – Methonal extracts (mg/mL)				*O. Gratissimum* – Methonal extracts (mg/mL)				*O. Gratissimum* Acetone extract (mg/mL)			
Bacterial species	50	100	200	400	50	100	200	400	50	100	200	400
S. aureus	8.5	9.5	10.5	12.0	14.0	18.0	22	24.0	R	8.0	9.5	10.5
E.coli	R	R	6.5	7.0	7.0	8.0	8.5	9.0	R	R	6.6	7.2
S. typhi	12.2	14.0	15.5	16.7	19.0	24	28.0	30.0	9.0	10.5	12.2	14.1
S. paratyphi	10	11	12.3	14.5	16.0	22.0	25.0	28.0	7.6	8.6	9.2	10.2
Klebsiella sp	R	R	7	7.5	R	7.3	8.1	9.0	R	R	7.6	8.1
	A. Marmelos Ethanol extract (mg/mL)				*A. marmelos* extract (mg/mL)				*A. Marmelos* Acetone extract(mg/mL)			
Bacterial species	50	100	200	400	50	100	200	400	50	100	200	400
S. aureus	R	7.0	8.5	9.6	11.5	12.5	14	15	R	7.0	7.2	8.5
E.coli	R	8.0	9.0	10.5	16	17	17.5	22.5	R	7.0	8.0	9.0
S. typhi	R	6.9	8.5	10.2	12	14	17	18	R	R	8.0	8.6
S. paratyphi	R	7.0	7.6	8.2	9.0	10.5	11	11	R	R	7.0	7.0
Klebsiella sp	R	R	R	7	R	R	R	8.5	R	R	R	R
	A.vasica Ethanol extract (mg/mL)				*A. vasica* Methanol extract (mg/mL)				*A.vasica* Acetone extract(mg/mL)			
Bacterial species	50	100	200	400	50	100	200	400	50	100	200	400
S. aureus	R	R	R	7	R	R	R	7.2	R	R	R	6.9
E.coli	R	7.0	8.1	8.5	R	8.0	8.0	9.0	R	R	R	7.2
S. typhi	R	R	R	R	R	R	7.0	7.3	R	R	R	R
S. paratyphi	R	R	R	R	R	R	R	R	R	R	R	R
Klebsiella sp	R	R	R	R	R	R	R	R	R	R	R	R

was studied from the range of 0.625 to 0.039 mg/ml. The plant methanol extract showing more than 7 mm zone of inhibition was taken as observable MIC value (Table 2). The MIC Value of *Ocimum gratissimum* methanol extract for the bacterial strains *S. typhi* and *S. paratyphi* were 0.039.mg/ml (MIC=MBC). The *E. coli* appeared to be sensitive with a zone of inhibition of 22.5 mm and the MIC value of 0.156 mg/ml for *Aegle marmelos* respectively. The gram positive *Staphylococcus aureus* were the moderate sensitive of all against all the extracts tested, while the *Ocimum gratissimum* methanol crude extract shows maximum zone of inhibition of 24 mm and MIC of 0.078 mg/ml respectively. From MIC results of present study the methanol extracts of *Ocimum gratissimum* and *Aegle marmelos* showed prominent inhibitory action against all the pathogens tested, which indicates its possible application for antibacterial activity.

Table 2

The minimum inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC)

Plants/Bacterium	*Ocimum sanctum*	*Ocimum gratissimum*	*A. Marmelos*	*A.vasica*	+control Cf
S.a	0.156 mg/mL	0.078 mg/mL	0.312 mg/mL	0.625 mg/mL	0.001mg/mL
E.c	0.625 mg/mL	0.0625 mg/mL	0.156 mg/mL	0.625 mg/mL	0.002mg/mL
S.t	0.156 mg/mL	0.039 mg/mL	0.625mg/mL	0.625 mg/mL	0.004mg/mL
S.pt	0.156 mg/mL	0.039 mg/mL	0.625 mg/mL	–	0.001mg/mL
K.n	–	–	–	–	0.002mg/mL

S.a : *Staphylococcus aureus*, E.c : *Escherichia coli*, S.t : *Salmonella typhi*, S.pt : *Salmonella paratyphi*, K.n : *Klebsiella* pneumonia , –: Not Determined, +ve control cf : ciprofloxin.

4. Discussion

The Medicinal plants have been main source for drugs over many centuries in many countries, in both developed and developing world. Traditional medicines products are not officially recognized in many countries, and the European union presently developing regulatory laws for quality traditional medicines [1]. It is estimated that at least 25% of all modern medicines are derived either directly or indirectly from medicinal plants. Traditional medicines play important role in world health treating millions of people [1]. The medicinal property of herbs is due to the presence of different complex chemical substance as secondary metabolites, which are exclusively accumulated in different parts of the plants [22]. The tropical, subtropical regions of India are rich with various kinds of herbs/plant species with good medicinal properties. The plant extracts contain wide range of secondary metabolites such as flavonoides, , terpenoids, tannins, glycosides and alkaloids. These natural metabolites are important as potential antimicrobial crude drug and source for natural compounds as new anti-infection agents. [27]. The occurrence of bacterial diseases is becoming common in south Asia particularly in India, because of development of antibacterial drug resistant pathogens. To resolve the problem and to detect alternative chemotherapeutic agents, the search for novel forms from newer sources is global challenges [22].

Our present investigation for the newer antibacterial bioactive compounds targetted on the unexplored folk medicinal plants, being used for centuries in treating local population. The plant extracts are considered as best source of bioactive compounds particularly for traditional healers as they contain components of therapeutic values. The bioactive compounds have been detected for either bacteriostatic or bacteriocidal property and have very minimum or no toxicity to host. In this study, three different polarity plant extracts , *Ocimum sanctum*, *Ocimum gratissimum*, *Aegle marmelos*, *Adhatoda vasica* have been tested for antimicrobial activity on five different human clinical pathogens viz. *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli* and *Klebsiella pneumoniae*. In an earlier study Bishnu *et al* [28] observed that *Ocimum sanctum* and other natural plants extracts inhibit the growth of similar clinical pathogens. Among the three different extracts *Ocimum gratissimum* methanol extract showed highest antibacterial activity with a MIC of 0.039 mg/ml against *Salmonella typhi* , *Salmonella paratyphi* and MIC of 0.078 mg/ml active against *Staphylococcus aureus*. Similarly Adebolu *et al* [29] reported the steam distillation extracts of *Ocimum gratissimum* were highly effective against *Salmonella typhi*, *Staphylococcus aureus* and *E. coli* strains.

Apart from *Salmonella typhi* infection, *Salmonella paratyphi A* and *B* also widely persist in Indian population. The present study reported, methanol extracts of *Ocimum gratissimum* showed considerable inhibitory activity against both enteric isolates of *Salmonella typhi* , *Salmonella paratyphi* and reports of such similar work on enteric *Salmonella paratyphi* from scientific group is very minimal. The methanol extract of *Aegle marmelos* showed potent antimicrobial activity against clinical pathogenic *E. coli*; while suresh *et al* [30] showed that *Aegle marmelos* leaf extracts having reasonable antibacterial activity against *E. coli*. In fact the results of different extracts of *Adhatoda vasica* shows less antibacterial activity against the clinical pathogenic isolates. On the other hand the *Klebsiella* spp was the most resistant of all the tested plant extracts and it is suggested that this bacterial strains may possess resistant mechanism and concentration of compound used may be lesser to inactivate the bacterial activity.

The results of the present study along with early reports concluded that the methanol extracts of traditional wild plant *Ocimum gratissimum*, *Ocimum sanctum* has potent antibacterial activity against the clinical human pathogens isolates particularly pathogenic *Salmonella typhi* and *salmonella paratyphi*. The traditionally used

*Aegle marmelos*leaves methanol extracts possess effective antibacterial activity against *E. coli*. The present investigation data on antibacterial potency of wild *Ocimum sanctum*, *Ocimum gratissimum*, *Aegle marmelos*helps to design further study for synthesis of novel antibiotics.

References

[1] Molly Meri Robinson Classifications, Terminology and Standards, WHO, Geneva :Xiaorui Zhang Traditional Medicines, WHO. 2011. traditional medicines: global situation, issues and challenges. 3rd Edition.

[2] Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. *J Ethnopharmacology* 2000 **;70** : 343-349.

[3] Gordana Mijovic , Bogdanka Andric , Dragica Terzic , Milena Lopicic , Brankica Dupanovic. Antibiotic Susceptibility Of *Salmonella* Spp.: A Comparison Of Two Surveys With A 5 Years Interval. *Journal of IMAB – Annual Proceeding (Scientific Papers)* 2012; **18**:1. 216 -219 .

[4] Goburn B, Grassl GA, Finlay BB. *Salmonella*, the host and disease: A brief review. Immunol. *Cell. Biol.* 2007; **(85)**: 112-118.

[5] Sumathi P , Parvathi A. Antimicrobial activity of some traditional medicinal plants. *Journal of Medicinal Plants Research* 2010; **4**(4): 316-321.

[6] Vadlapudi Varahalarao, Chandrashekar N K, In Vitro Bioactivity Of Indian Medicinal Plant Calotropis Procera (Ait.). Journal of Global Pharma Technology 2010; 2(2): 43-45.

[7] Ekta Singh, Sheel Sharma, Jaya Dwivedi, Swapnil Sharma. Diversified Potentials Of *Ocimum sanctum* Linn (Tulsi): An Exhaustive Survey. *J. Nat. Prod. Plant Resour*., 2012; **2** (1):39-48.

[8] Poonam Mishra, Saniav Mishra . Study of antibacterial activity of *Ocimum sanctum* extract against gram positive and gram negative bacteria. *American journal of food technology* 2011; **6**(4): 336-341.

[9] Okigbo RN, Ogbonnanya OU . Antifungal effects of two tropical plants extracts *Ocimum gratissimum* and Afromaomum melegueta on post harvest yam Discorea spp rot. *Afr J Biotechnol* 2006; **5** (9): 727-731.

[10]Kingston C , Jeeva S , Jeeva G M , Kiruba S, Mishra B P , Kannan D . Indigenous Knowledge Of Using Medicinal Plants In Treating Skin Diseases In Kanyakumari District , Southern India. *Indian journal of traditional knowledge* 2009; **8**(2):196-200.

[11]Rajeshwari Sivaraj, Balakrishnan A, Thenmozhi M, Venckatesh R. Antimicrobial activity of *Aegle marmelos*, Ruta graveolens, Opuntia dellini, Euphorbia royleana and Euphorbia antiquorum. *Journal of Pharmacy Research* 2011; **4**: 1507-1508.

[12]Gopalsamy Rajiv Gandhi, Savarimuthu Ignacimuthu , Michael Gabriel Paulraj . Hypoglycemic and ß-cells regenerative effects of *Aegle marmelos* (L.) Corr. Bark extract in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology* 2012; **50**: 1667-1674.

[13]Vijaya C, Ramanathan M, Suresh B. Lipid lowering activity of ethanolic extracts of leaves of *Aegle marmelos* (linn.) in hyperlipidaemic models of wistar albino rats. *Indian journal of experimental biology* 2009 ; **47** (3): 182-185.

[14]Vanitha Reddy P, Sahana N, Asna Urooj . Antioxidant activity of *Aegle marmelos* and Pisidium Guajava leaves. *Int.J. Med.Arom.Plants* 2012; **2** (1): 155-160.

[15]Manjeshwar Shrinath Baliga, Harshith P, Bhat, Manisha Maria Pereira, Nishan Mathias, Ponemone Venkatesh. Radioprotective Effects of *Aegle marmelos* (L.) Correa (Bael): A Concise Review. *The Journal of Alternative and Complementary Medicine* 2010; **16**(10): 1109-1116.

[16]Sumitha P, Thirunalasundari T. Hepatoprotective Activity of *Aegle marmelos* in CCl4 Induced Toxicity – An In-vivo Study. *Journal of Phytology* 2011; **3**(9): 05-09

[17]Benni JM, Jayanthi MK, Suresha RN. Evaluation of the anti-inflammatory activity of *Aegle marmelos* (Bilwa) root. *Indian J Pharmacol* 2011; **43**: 393-397.

[18]Trivedi HP, Pathak NL, Gavaniya, MG, Patel AK, Trivedi HD, Panchal NM. *International Journal of Pharmaceutical Research and Development* 2011; **3**: 38-45.

[19]Saradha JK, SubbaRao B. Antibacterial Activity of Extracts from *Aegle marmelos* against Standard Pathogenic Bacterial Strains. *International Journal of PharmTech Research* 2010; **2**(3): 1824-1826.

[20]Umamaheswari M, Chaterjee TK. In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. African Journal of Traditional, *Complementary and Alternative Medicines* 2007; **5**: 61-73.

[21]Vinothapooshan G, Sundar K. Wound Healing Effect Of Various Extracts Of *Adhatoda vasica*. *International Journal of Pharma and Bio Sciences* 2010; **1**(4):530-536.

[22]Haniyeh Koochak, Seyyed Mansour Seyyednejad, Hussein Motamedi. Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran). A*sian Pacific Journal of Tropical Medicine* 2010; **3**(3)180-184.

[23]Nwinyi Obinna C, Chinedu Nwodo S, Ajani Olayinka O, Ikpo Chinwe O, Ogunniran Kehinde O. Antibacterial effects of extracts of *Ocimum gratissimum* and piper guineense on Escherichia oli and *Staphylococcus aureus*. *African Journal of Food Science* 2009; **3**(1): 022-025.

[24]Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G. Antimicrobial Activity of Some of The South-Indian Spices Against Serotypes of *Escherichia Coli*, *Salmonella*, Listeria Monocytogenes and Aeromonas Hydrophila. *Brazilian Journal of Microbiology* 2006; **37**:153-158.

[25]Agatemor C . Antimcrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Elec J Environ Agric food chem* 2009; **8**(3): 195-200.

[26]Shahbudin Saad , Muhammad Taher , Deny Susanti , Haitham Qaralleh , Nurul Afifah Binti AbdulRahim. Antimicrobial activity of mangrove plant (Lumnitzera littorea) . *Asian Pacific Journal of Tropical Medicine* 2011; **4**(7) 523-525.

[27]Dwivedi SC, Dudey R, Richa tyagi, Meeta Masand, Uma Advani. Medicinal Bioactives as antimicrobial agents : an overview. *International journal of pharmaceutical research and development* 2011: **3**(7) 24-30.

[28]Bishnu J, Govind Prasad S, Buddha BB, Megh RB, Dinita S, Krishna S, et al. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem). *Journal of Microbiology and Antimicrobials* 2011; **3**(1): 1-7.

[29]Adebolu TT, Oladimeji SA. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in Southwestern Nigeria. *African Journal of Biotechnology* 2005; **4**(7): 682-684.

[30]Suresh K, Senthilkumar PK, Karthikeyan B. Antimicrobial activity of *Aegle marmelos* against clinical pathogens. *Journal of Phytology* 2009; **1**(5): 323-327.