

Antibacterial Activities of Some Medicinal Plants of the Western Region of India

Rathish NAIR, Sumitra V. CHANDA

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University,
Rajkot 360 005, Gujarat - INDIA

Received: 02.08.2007

Abstract: Ten medicinal plants, namely *Commiphora wightii*, *Hibiscus cannabinus*, *Anethum gravelons*, *Emblica officinalis*, *Ficus religiosa*, *Ficus racemosa*, *Ficus benghalensis*, *Ficus tisele*, *Mentha arvensis* and *Mimusops elengi*, were screened for potential antibacterial activity against medically important bacterial strains, namely *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Alcaligenes faecalis* and *Salmonella typhimurium*. The antibacterial activity was determined in aqueous and ethanol extracts using both agar disc diffusion and agar well diffusion methods. The ethanol extracts were more potent than aqueous extracts of all the plants studied. *Pseudomonas aeruginosa* and *Salmonella typhimurium* were the most resistant strains while the most susceptible bacterial strains were *Bacillus cereus* and *Proteus mirabilis*. *Emblica officinalis* showed strong activity against all the tested bacterial strains. Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address unmet therapeutic needs.

Key Words: *Commiphora wightii*, *Hibiscus cannabinus*, *Anethum gravelons*, *Emblica officinalis*, *Ficus* spp., *Mentha arvensis*, *Mimusops elengi*, antibacterial activity, aqueous extract, ethanol extract

Batı Hindistan Bölgesinin Bazı Tıbbi Bitkilerinin Antibakteriyel Aktiviteleri

Özet: On tıbbi bitkinin, *Commiphora wightii*, *Hibiscus cannabinus*, *Anethum gravelons*, *Emblica officinalis*, *Ficus religiosa*, *Ficus racemosa*, *Ficus benghalensis*, *Ficus tisele*, *Mentha arvensis* ve *Mimusops elengi* potansiyel antibakteriyel aktiviteleri *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Alcaligenes faecalis* ve *Salmonella typhimurium* bakterilerinde çalışılmıştır. Antibakteriyel aktivite, sıvı ve etanol özütü hem agar disk difüzyon hem de agar kuyu difüzyon yöntemi kullanılarak incelenmiştir. Bütün çalışılan bitkilerde etanol özütünün sıvı özütten daha etkili olduğu gözlenmiştir. *P. aeruginosa* ve *S. typhimurium* en dirençli türler olarak bulunurken *B. cereus* ve *P. mirabilis* türlerinin en hassas türler olduğu görülmüştür. *E. officinalis* denenen bütün türlere karşı çok yüksek aktivite göstermiştir. Bu bitki, biyoaktif doğal bitkileri ortaya çıkarmak için kullanılabilir, dolayısıyla henüz çaresi bulunmamış terapötik ihtiyaçlara cevap vermede ve yeni ilaç geliştirmede yardımcı olabilir.

Anahtar Sözcükler: *Commiphora wightii*, *Hibiscus cannabinus*, *Anethum gravelons*, *Emblica officinalis*, *Ficus* spp., *Mentha arvensis*, *Mimusops elengi*, antibakteriyel aktivite, sıvı özüt, etanol özüt

Introduction

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. The search for eternal health and longevity and for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, and minerals, etc. and the development of a variety of therapeutic agents. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green

medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects.

Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grow wild in different parts of our country. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times (1). India is rich in medicinal plant diversity. All known types of agro-climatic, ecologic and edaphic conditions are met within India. India is rich in all three levels of biodiversity, as species diversity, genetic diversity and habitat diversity (2).

Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extraction and can be followed by various organic extraction methods. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction (3).

Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs (4). Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms (5). Although some therapeutic benefits can be traced to specific plant compounds, many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value. Consequently, it is believed that the whole plant has more effective healing properties than its isolated constituents. Any part of the plant may contain active components (6). For example, the roots of the ginseng plant contain the saponins and essential oils, while eucalyptus leaves are harvested for essential oils and tannins.

Considering the aforesaid, it is assumed that the need of the hour is to search for new antimicrobials. With this in mind, in the present work, some selected plants are screened for their potential antimicrobial activity. A number of such studies have been done in various places of the world (7-10). There are several reports on the antimicrobial activity of different herbal extracts (11-15).

Commiphora wightii (Arn) Bhandari belongs to the family Burseraceae. It is found in Bihar, Mysore, Deccan, Khandesh, Rajputana desert, Sindh, Baluchistan, Arabia and Gujarat. It contains gum, resin and essential oil containing mycerene. It is used as astringent, antiseptic, antispasmodic, diaphoretic, aphrodisiac, emmenagogue, antirheumatic, antihyperlipidemic, demulcent, aperient, carminative, alterative, maturant, resolvent, and expectorant. It is also used for muscular rheumatism, lung complaints, dyspepsia, piles, skin and nervous diseases, scrofulous affections, and urinary disorders. It is

locally applied as a paste in hemorrhoids, incipient abscesses and ulcers.

Hibiscus cannabinus L. belongs to the family Malvaceae. It is distributed throughout India. It is an annual or perennial herb reaching up to 4 m in height. The inflorescence is solitary or raceme. The flowers are axillary. The seeds, leaves and flowers are medicinally useful. The seeds are used as appetizer, aphrodisiac, and flattening and also in external application in pains and bruises. The leaves are purgative, and used in dysentery and diseases of blood, bile and throat. An infusion is administered in cough.

Anethum graveolens L. belongs to the family Umbelliferae (Apiaceae). It is an annual erect glabrous herb. The leaves are alternate, 2-3 pinnately compound. The flowers are complete and yellow. It contains volatile oil and fixed oil, anethine, phellandrene, and d-limonene. The seed is hot, bitter, stomachic, antipyretic, carminative, and anthelmintic, and used in ulcers, abdominal pain, eye diseases, and urinary pains. The fruit is hot, bitter, carminative, antidysenteric, stomachic, alexeteric, diuretic, laxative, emmenagogue, maturant vulnerary, and relieves griping pains. It is also used in piles and mental disorders. Fruits are used in hyperglycemia and blood pressure regulation. Dried and ripe fruits are used in pediatric complaints, such as flatulence and disordered digestion.

Embllica officinalis Gaertn belongs to the family Euphorbiaceae. It is found throughout India, Pakistan, Bangladesh, China, Sri Lanka and the Malayan Peninsula. It is a deciduous tree 8-15 m tall. The leaves are alternate, subsessile, narrowly linear-lanceolate or linear-oblong, light-green, imbricate, base truncate to cordate, apex apiculate; stipules ovate, finely acute. The flowers are greenish to creamy-yellow, unisexual, actinomorphic, trimerous. The male flowers are numerous; pedicle short, slender, staminate. The female flowers are few, subsessile, pistillate, hypogenous. The leaves, bark, fruit, root bark, etc are used to cure many diseases. The plant is known for digestion power and improving liver function and is liver-protective. It has anti-viral, anti-bacterial, anti-cancer, anti-allergy, and anti-mutagenic properties. It is rich in phenols, flavonoids and tannins.

Ficus religiosa L., *Ficus benghalensis* L., *Ficus tisel* Roxb. and *Ficus racemosa* L. belong to the family Moraceae. They are large deciduous trees, distributed

throughout India; wild as well as cultivated. Various parts of the plant like bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important. The bark contains tannin, rubber and wax. The plant parts are used in diseases of blood, vagina, uterus and leukorrhea, burning sensation, gonorrhoea, diarrhoea, dysentery, hemorrhoids, gastrohelcosis. The bark is used in inflammations, swelling of neck, gonorrhoea, scabies, mouth wash for toothache and for strengthening gums, and steeped freshly burnt bark has been said to cure cases of obstinate hiccup. The latex is used in inflammations and hemorrhages.

Mentha arvensis L. belongs to the family Labiateae. It is an erect branched perennial herb with running rootstocks and rigid branching stem up to 75 cm tall. The plant is used to treat liver and spleen diseases, asthma and jaundice. The oil yield is 5% by distillation of leaves, which contain 40-50% menthol. The oil is antiseptic, carminative, refrigerant, stimulant and diuretic. Menthol is used in medicine for stomach disorders and in ointments for headache. The infusion of leaves is used in indigestion and rheumatic pains.

Mimusops elengi Linn. belongs to the family Sapotaceae. It is an evergreen tree, 5-8 m tall. It is cultivated throughout as an ornamental tree. The bark is used as a gargle for odontopathy, ulitis, and ulemorrhagia. The fruit is used as astringent, coolant, anthelmintic. The tender stems are used as tooth brushes, and in cystorrhoea, diarrhoea and dysentery. The flowers' lotion is used for wounds and ulcers. The seeds are used in constipation.

Materials and Methods

Plant Collection

Fresh plant or plant parts were collected randomly from the semi-arid region of Rajkot Gujarat, India in August 2003. The taxonomic identities of these plants were determined by Dr. P. S. Nagar, Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The ethnobotanical information of the screened plants is given in Table 1. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Crude Extraction

Aqueous extraction

Ten grams of dried powder was extracted in distilled water for 6 h at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This process was repeated twice and after 6 h, the supernatant was concentrated to make the final volume one-fourth of the original volume (16). It was then autoclaved at 121 °C and 15 lbs pressure and then stored at 4 °C.

Solvent extraction

Ten g of dried powder was extracted with 100 ml of ethanol kept on a rotary shaker at 190-220 rpm for 24 h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (16). It was stored at 4 °C in airtight bottles for further studies.

Table 1. Ethnobotanical information of some medicinal plant species of Western India selected for antibacterial activity.

Plant species	Family	Common name	Part used
<i>Commiphora wightii</i> (Arn) Bhandari	Burseraceae	Guggad	Stem
<i>Hibiscus cannabinus</i> L.	Malvaceae	Sheria	Stem
<i>Anethum graveolens</i> L.	Apiaceae	Suva bhaji	Whole plant
<i>Emblica officinalis</i> Gaertn	Euphorbiaceae	Amla	Leaf
<i>Ficus religiosa</i> L.	Moraceae	Pipado	Bark
<i>Ficus benghalensis</i> L.	Moraceae	Vad	Bark
<i>Ficus tesila</i> Roxb	Moraceae	Pipat	Bark
<i>Ficus racemosa</i> L.	Moraceae	Umaro	Bark
<i>Mentha arvensis</i> L.	Labiataeae	Pudina	Whole plant
<i>Mimusops elengi</i> Linn.	Sapotaceae	Borseli	Leaf

Bacterial Strains

The microbial strains are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The studied bacterial strains were *Pseudomonas aeruginosa* ATCC27853, *Proteus mirabilis* NCIM2241, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC11778, *Alcaligenes faecalis* ATCC8750 and *Salmonella typhimurium* ATCC23564.

Antibacterial Activity

The antibacterial activity of different plant species was evaluated by agar disc diffusion method (17,18) for aqueous extract and agar well diffusion (19,20) for solvent extract using Mueller Hinton agar No. 2 medium for the assay. The microorganism was activated by inoculating a loopful of the strain in the nutrient broth (30 ml) and incubated on a rotary shaker. Then 0.2 ml of inoculum (inoculum size was 10^8 cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the Petri plate (Hi-media). For agar disc diffusion method, the test compound (0.1 ml) was introduced on the disc (0.7 cm) (Hi-Media) and then allowed to dry. Then the disc was impregnated on the seeded agar plate. For agar well diffusion method, a well was made in the seeded plates with the help of a cup-borer (0.85 cm). The test compound was introduced into the well and the plates were incubated at 37 °C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter is shown in the graph. The experiment was done three times and the mean values are presented.

Results and Discussion

The antibacterial activity of both aqueous and ethanolic extracts of the different plants against *P. aeruginosa* is shown in Figure 1. Of aqueous extracts, only that of *E. officinalis* showed maximum activity against *P. aeruginosa*; none of the others showed any activity. Ethanolic extract of 5 of the 10 plants, *E. officinalis*, *F. benghalensis*, *F. tisela*, *F. racemosa* and *C. wightii*, showed considerable antibacterial activity. Maximum activity was shown by *E. officinalis*, followed by *F. benghalenses*, *F. racemosa*, and *F. tisela*. Minimum antibacterial activity was shown by *C. wightii*. Other plants demonstrated no activity against *P. aeruginosa*.

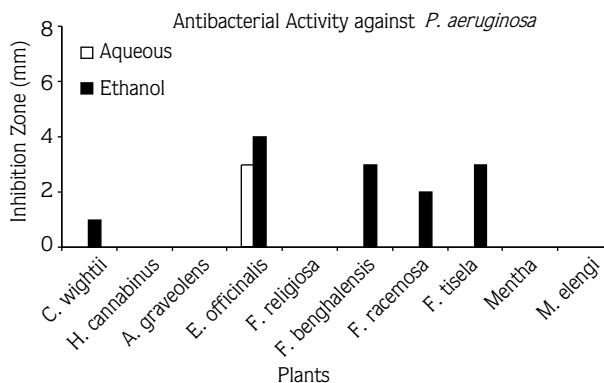


Figure 1. Antibacterial activity of some medicinal plant extracts (aqueous and ethanol) against *P. aeruginosa*.

The antibacterial activity of the 10 plants against *P. mirabilis* is shown in Figure 2. The ethanolic extracts of all the plants showed antibacterial activity while the aqueous extracts of only 2-3 plants showed antibacterial activity. The antibacterial activity of ethanolic extract was more than that of aqueous extract. Maximum antibacterial activity was shown by *E. officinalis* followed by *M. elengi*, while minimum activity was shown by *F. religiosa* and *H. cannabinus*.

The antibacterial activity of both aqueous and ethanolic extracts of the 10 plants against *S. aureus* is shown in Figure 3. Of the 10 plants, 7 showed antibacterial activity against this bacterial strain. Maximum activity of ethanolic extract was shown by *E. officinalis*, followed by *F. religiosa*, *M. elengi*, *A. graveolens*, *F. racemosa*, *F. tisela* and *Mentha* sp. Aqueous extract of only 5 of the 10 plants showed antibacterial activity against *S. aureus*; maximum activity was shown by *M. elengi*, followed by *E. officinalis*, *F. racemosa*, *F. tisela* and *C. wightii* extracts.

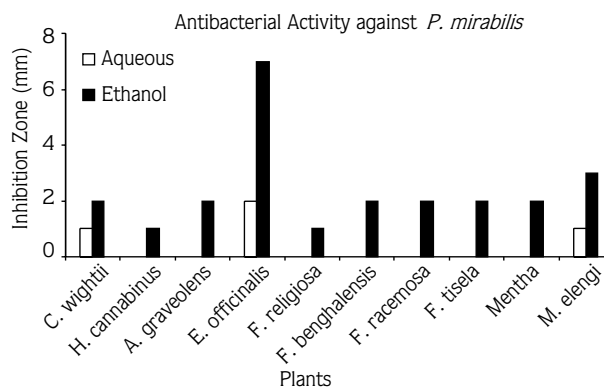


Figure 2. Antibacterial activity of some medicinal plant extracts (aqueous and ethanol) against *P. mirabilis*.

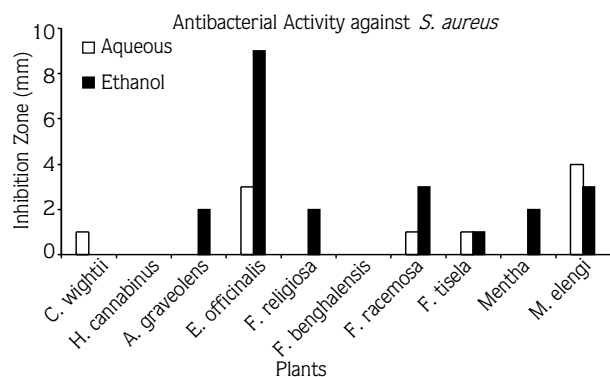


Figure 3. Antibacterial activity of some medicinal plant extracts (aqueous and ethanol) against *S. aureus*.

Antibacterial activity of both aqueous and ethanolic extracts of the 10 plants against *B. cereus* is shown in Figure 4. Ethanolic extract of all the plants showed considerable antibacterial activity against this bacterial strain. Maximum activity of ethanolic extract was shown by *E. officinalis*, *F. religiosa*, and *F. racemosa*. Except for *C. wightii*, ethanolic extract of all other plants showed antibacterial activity to a certain extent. Of the 10 plants, aqueous extract of 7 showed antibacterial activity against *B. cereus*. Maximum activity of aqueous extract was shown by *E. officinalis*.

Antibacterial activity of both aqueous and ethanolic extracts of the 10 plants against *A. faecalis* is shown in Figure 5. Aqueous extract of only 2 plants (*M. elengi* and *E. officinalis*) showed antibacterial activity against this bacterial strain. *M. elengi* showed more activity than *E. officinalis*. Aqueous extract of other plants had no activity against *A. faecalis*. Except for *C. wightii* and *H. cannabinus*, ethanolic extracts of all other plants showed antibacterial

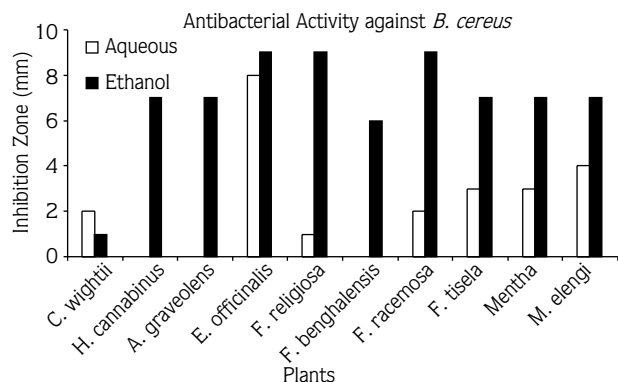


Figure 4. Antibacterial activity of some medicinal plant extracts (aqueous and ethanol) against *B. cereus*.

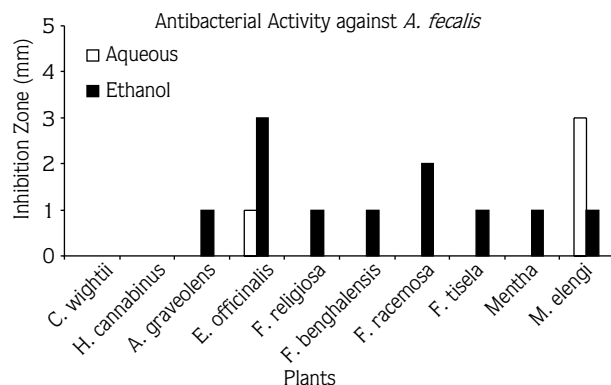


Figure 5. Antibacterial activity of some medicinal plant extracts (aqueous and ethanol) against *A. faecalis*.

activity; maximum activity was again shown by *E. officinalis*.

Antibacterial activity of both aqueous and ethanolic extracts of the 10 plants against *S. typhimurium* is shown in Figure 6. Aqueous extract of one plant, i.e. *E. officinalis*, showed antibacterial activity against this bacterial strain while ethanolic extracts of 7 plants showed antibacterial activity against *S. typhimurium*. Maximum antibacterial activity was shown by *E. officinalis*.

All plants showed greater inhibitory activity in the ethanolic when compared to aqueous extract, with the exception of the aqueous extract of *M. elengi*, which showed better activity than the ethanolic extract against *A. faecalis*. Aqueous plant extract of *H. cannabinus* and *F. benghalensis* had no antibacterial activity against any of the six bacterial strains investigated. Of the investigated 6 bacterial strains, *P. aeruginosa* and *S. typhimurium* were the most resistant strains. The most susceptible bacterial strains were *B. cereus* and *P. mirabilis*. Gram-negative

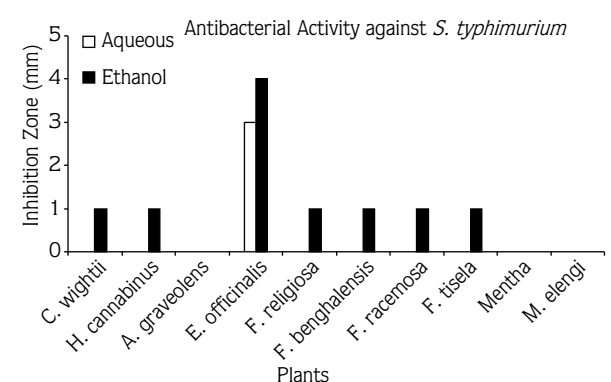


Figure 6. Antibacterial activity of some medicinal plant extracts (aqueous and ethanol) against *S. typhimurium*.

bacterial strains were more resistant than Gram-positive bacterial strains (21,22). Based on our results, it is concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. *E. officinalis* showed stronger activity than the other plants against all the tested bacterial strains, as reported earlier (23). Therefore, *E. officinalis* can be selected for further analysis. It can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address unmet therapeutic needs. Such screening of various natural organic compounds and identification of

active agents is the need of the hour because successful prediction of lead molecule and drug-like properties at the onset of drug discovery will pay off later in drug development.

Corresponding author:

Sumitra V. CHANDA
 Phytochemical, Pharmacological and
 Microbiological Laboratory,
 Department of Biosciences,
 Saurashtra University,
 Rajkot 360 005, Gujarat - INDIA
 E-mail: sumitrachanda@yahoo.com

References

- Bhattacharjee SK. Handbook of Medicinal Plants. Pointer Pub, Jaipur-03, India; 1998; pp. 1-6.
- Zafar M, Iqbal A, Faiz M et al. Indian medicinal plants: a potential source for anticandidal drugs. *J Ethnopharmacol* 37: 237-242, 1999.
- Vilegs JH, DeMarchi E, Lancas FM. Extraction of low polarity compounds from *Milania glomerata* leaves. *J Phytochem Anal* 8: 266-270, 1997.
- Cheesbrough M. Medical Laboratory Manual for Tropical Countries, Butterworth, Oxford, pp. 260, 2000.
- Gordon MC, David JN. Natural product drug discovery in the next millennium. *Pharm Biol* 39: 8-17, 2001.
- Nair R, Chanda S. Antibacterial activity of some medicinal plants of Saurashtra region. *J Tissue Res* 4: 117-120, 2004.
- Martinez MJ, Betancourt J, Alonso-Gonzalez N et al. Screening of some medicinal plants for antimicrobial activity. *J Ethnopharmacol* 52: 171-174, 1996.
- Kumarsamy Y, Cox PJ, Jaspars M et al. Screening seeds of Scottish plants for antibacterial activity. *J Ethnopharmacol* 83: 73-77, 2002.
- Parekh J, Chanda S. Screening of aqueous and alcoholic extracts of some Indian medicinal plants for antibacterial activity. *Ind J Pharma Sci* 68: 835-838, 2006.
- Sudharameshwari K, Radhika J. Antibacterial screening of *Aegle marmelos*, *Lawsonia inermis* and *Albizia libbeck*. *Afr J Trad Comp Alt Med* 4: 199-204, 2007.
- Samy PR, Ignacimuthu S. Antibacterial effects of the bark of *Terminalia arjuna*: justification of folklore beliefs. *Pharm Biol* 39: 417-420, 2001.
- Shahidi Bonjar GH. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Bordetella bronchiseptica*. *Asian J Plant Sci* 3: 82-86, 2004.
- Albinu I, Adenipekun T, Adelowotan T et al. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. *Afr J Trad Comp Alt Med* 4: 185-190, 2007.
- Parekh J, Chanda S. In vitro antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz flower (Lythraceae). *Braz J Micro* 38: 204-207, 2007.
- Parekh J, Chanda S. In vitro antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. *Afr J Biotech* 6: 766-770, 2007.
- Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk J Biol* 29: 203-210, 2005.
- Bauer AW, Kirby WMM, Sherris JC et al. Antibiotic susceptibility testing by a standardized single disk method. *Ame J Clin Pathol* 45: 493-496, 1966.
- Vaghasiya Y, Nair R, Chanda S. Investigation of some *Piper* species for antibacterial and anti-inflammatory property. *Int J Pharma* (In Press), 2007.
- Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion method. *Acta Bio Med Exp* 15: 113-115, 1990.
- Parekh J, Chanda S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol* 31: 53-58, 2007.
- Nair R, Chanda S. In vitro antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically important pathogenic microbial strains. *Braz J Microbiol* 38: 452-458, 2007.
- Yaghoubi SMJ, Ghorbani GR, Soleimani Zad S. Antimicrobial activity of Iranian propolis and its chemical composition. *DARU* 15: 45-48, 2007.
- Nair R, Chanda S. Assessment of antibacterial activity of *Emblca officinalis* Gaertn leaf extract. *J Cell Tissue Res* 6: 715-717, 2006.