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Antimicrobial Properties and Characterization of Phytoconstituents of the Leaf Extracts of Some Medicinal Plants

Swati Bagde¹, Mayuri Khare¹, Rakesh Kumar Patidar¹, Vinod Singh^{*1}

1. Associate Professor, Department of Microbiology, Barkatullah University, Bhopal, Madhya Pradesh, India.
[E-mail: vsingh3@rediffmail.com, Tel: +91-9425712910]

Background: This study was designed to determine and compare the in-vitro antimicrobial effects of these plants phyto preparations. **Materials and Methods:** The leaves of *O. basilicum*, *C. citrates*, *C. roseus* and *A. paniculata* were collected from Bhopal (MP) India and leaves extract were examined to evaluate the phytoconstituents and antimicrobial activity by Agar well diffusion antimicrobial bioassay. Preliminary evaluation of the solvent fractions showed a broad spectrum of activity since the extracts inhibit the growth of both gram positive (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Enterococcus faecalis*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial isolates. **Results:** In the present study the plant parts contain levels of alkaloids, terpenoids, flavonoids and other phytochemical. In antimicrobial assay 100%, 75% and 25% methanolic extracts shows very potent antimicrobial agent on both gram positive *E. faecalis* (highest inhibition zone 3.6 mm) and gram negative bacterial isolates. The result from this study provides suitable support for the use of *A. paniculata* and *C. rosea* in the control and treatment of diabetes and use of *O. basilicum*, *C. citrates* for the control of infectious disease caused by both gram positive and gram negative bacteria. **Conclusion:** Among all the four investigated plants *C. citrates*, *C. roseus* and *A. paniculata* was found to be efficient against most of the investigated pathogens. This findings suggest that there is a potential in the discovery of novel antimicrobial agents from medicinal plants and further study should be made in order to identify the active phytochemical constituents and on toxicity of active plant principles to determine their safety use

Keyword: Antimicrobial Activity, Medicinal Plants, Phytoconstituents and Zone of Inhibition

1. Introduction

The use of traditional medicines holds a great promise as an easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. Medicinal plants and herbs are of great importance to the health of individual and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant

species has been studied for their application. However, in the recent past, increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers^[1].

The steadily increasing bacterial resistance to existing drugs is a serious problem in antimicrobial therapy and necessitates continuing

research into new classes of antimicrobials^[2,3]. One way to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents^[4]. It is anticipated that phytochemicals with adequate antimicrobial efficacy could be used for the treatment of bacterial infections^[5]. One of the great advantages of these medicinal plants is that they are easily available and have moderate side effects^[6]. There are several reports of antibiotic resistance of human pathogens to available antibiotics^[7-11].

Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human and plant pathogens and hence in the present investigation, leaves of *Ocimum basilicum*, *Cymbopogon citrates*, *Catharanthus roseus* and *Andrographis paniculata* was tested for its efficacy to inhibit against human pathogens. These plants are well known for its medicinal value as antiviral, antidiabetic, antioxidants and antimicrobial properties. In order to elucidate such a phenomenon, as well as seek highly effective plants, a number of plant extracts and isolated compounds have been tested

for their bioactivity by various in vitro model systems. Information on the biological functions and active constituents of each plant species may contribute to the improvement of food habits and public health in tropical countries. Therefore the main objectives of the present study were to determine and compare the in-vitro antimicrobial effects of these plants phyto preparations.

2. Materials and Methods

2.1 Bacterial Isolates and Sampling Procedure

Bacterial isolates were obtained from clinical samples belonging to different sources such as skin infection, throat sample and water samples. These samples were collected with sterile cotton swabs from the actual infection site aseptically into the sterile container containing normal saline. These swabs will capture the causative organism in most cases and the culture will allow the specific organism to be grown in the microbiology laboratory under certain conditions. Samples were taken to the laboratory in cold condition within 4 hr for further microbiological analysis.

Table 1. Phytochemical constituents of leaf extract of *O. basilicum*, *C. citrates*, *C. roseus* and *A. paniculata*

Phytochemical constituent	Methanolic extract			
	<i>O. basilicum</i>	<i>C. citrates</i>	<i>C. roseus</i>	<i>A. paniculata</i>
Terpenoids	+	+	+	+
Alkaloids	-	-	+	+
Coumarins	+	+	+	+
Flavonoids	-	-	-	+
Tannins	+	+	+	-
Saponins	-	+	-	+

Negative (-) absent, Positive (+) present

2.2 Bacterial Isolation and Identification

The samples were inoculated on nutrient agar and MacConkey agar media plate and incubated at 37°C for 24 hr. The colonies of isolated organism have been sub culture on nutrient agar plate and

pure culture were obtained in various selective media such as Mannitol Salt agar, Blood agar and Chocolate agar and further identified by biochemical tests for confirmation of bacteria. For the confirmation of the isolated bacteria and

their species specific biochemical tests such as IMViC test, catalase test, coagulase test, urease test, oxidase test, bile solubility test, Deoxyribonuclease (DNase) test and carbohydrate fermentation test were performed. *S. aureus*, was identified by the positive catalase, from IMViC-MR positive and acid-gas production by carbohydrate fermentation test and other biochemical characters. *S. pneumoniae*, was isolated on Streptococcus selection agar and identified positive for IMViC-VP and bile

solubility test. *Enterococcus faecalis* isolated on Brain Heart infusion agar and Blood agar, showed positive results of Methyl red and nitrate reduction.

Escherichia coli, isolated on Nutrient agar media and identified positive for IMViC-Indole, MR, catalase and carbohydrate fermentation. *P. aeruginosa*, isolated on Pseudomonas agar base and identified positive for catalase, oxidase and citrate utilization shown in Figure 1.

Table 2. Results of the antimicrobial activity of the selected plants

Test organisms	A	B	C	D
<i>S. aureus</i>	+	+	+	+
<i>S. pneumoniae</i>	+	-	-	-
<i>E. faecalis</i>	-	+	+	+
<i>E. coli</i>	-	+	+	-
<i>P. aeruginosa</i>	-	-	-	+

(A) *Ocimum basilicum*, (B) *Cymbopogon citrates*, (C) *Catharanthus roseus* and (D) *Andrographis paniculata*

Negative (-) absent, Positive (+) present

2.3 Collection of Plant Material

(A) *Ocimum basilicum*, (B) *Cymbopogon citrates*, (C) *Catharanthus roseus* and (D) *Andrographis paniculata* plants are known for their medicinal importance. These plants were collected from different localities in Bhopal (MP), India. Dirt was removed from the plant parts by rinsing in clean water. The leaves were air-dried at room temperature for 2 weeks. All dried material were chopped into small fragments and reduced into fine powder with mortar and pestle, which can pass through 0.5 mm pore size sieve.

2.4 Preparation of Solvent Extracts

The methanol extracts were prepared by soaking each of the dry powdered plant materials in methanol at room temperature for 48 h. This step was repeated several times till the extraction was complete. The total extracted volume of each plant subjected to filtration after 48 h using Whatman No.1 filter paper and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set

at 40° C. All the dried samples were subjected to antimicrobial activity assay and phytochemical analysis.

3. Phytochemical Analysis

Phytochemicals screening of active plant extracts was done by following the standard method of Khandelwal^[12] for the qualitative analysis of various phytochemicals studies such as terpenoids, alkaloids, coumarins, flavonoids, tannins and saponins.

3.1 Terpenoids Determination

A portion of 0.5 gm of each of the extract was taken, 2 ml of chloroform and few drops of conc. H₂SO₄ were added. A reddish brown colouration of the interface indicates the presence of terpenoids.

3.2 Alkaloids Determination

a. Mayer's test: Few drops of Mayer's reagent (Prepared by dissolving 1.36 gm HgCl₃ in 60 ml double distilled water and 5 gm KI in 20 ml

double distilled water) added in 2-3 ml test sample, creamy precipitate observed indicates presence of alkaloids.

b. Wagner's test: Few drops of Wagner's reagent (Prepared by dissolving 1.27 gm of

Iodine and 2 gm KI in 100 ml double distilled water) added in 2-3 ml test sample, reddish brown color observed indicates presence of alkaloids.

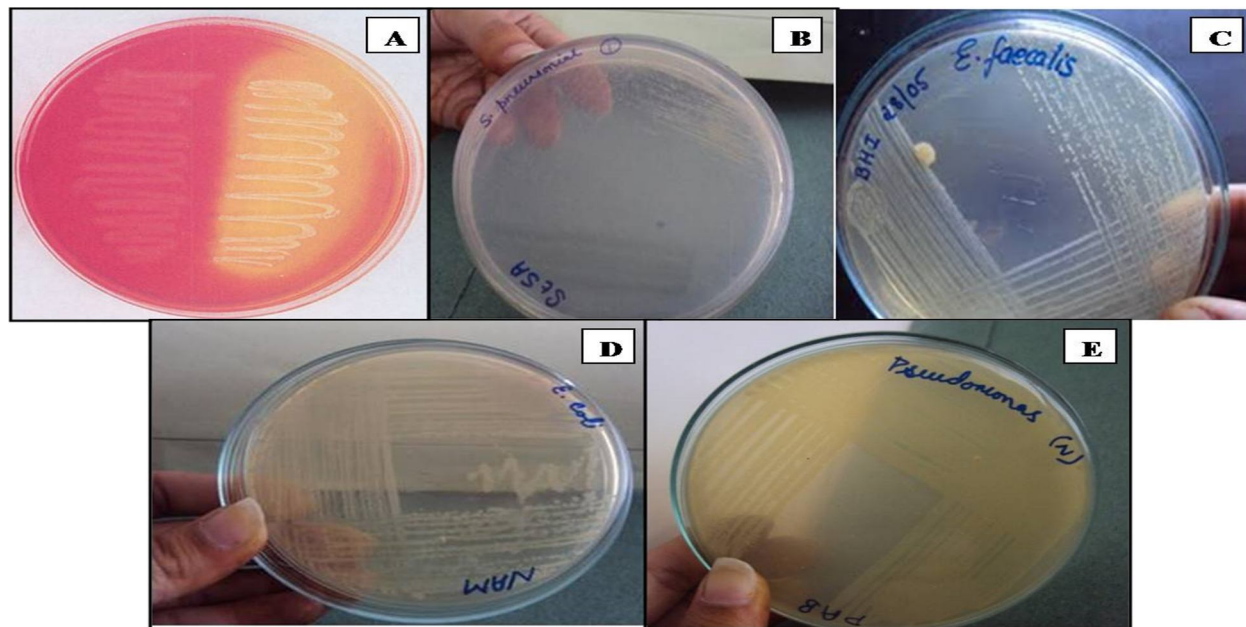


Figure 1. Pure Bacterial cultures of Gram Positive Bacteria; (A) *S. aureus* on Mannitol Salt agar, (B) *S. pneumoniae* on Streptococcus Selection agar, (C) *E. faecalis* on Brain Heart Infusion agar and Gram Negative Bacteria; (D) *E. coli* on Nutrient agar media, (E) *P. aeruginosa* on Pseudomonas Agar Base.

3.3 Coumarins Determination

a. Aromatic odor: Coumarin given aromatic odor.

b. Filter Paper Test: Test samples taken in test tube and covered with filter paper soaked in dilute NaOH and kept in hot water bath, after some time filter paper gives yellowish green fluorescence.

3.4 Flavonoids Determination

A portion of each extract solution was taken in a test tube and 5 ml of dilute ammonia solution and 1 ml conc. H_2SO_4 were added. A yellow color appears that disappear on standing indicating the presence of flavonoids.

A portion of each extract solution was taken in a test tube and 10 ml of ethyl acetate was added and heated over the steam bath for 3 minutes. Mixture was filtrated, on 4 ml filtrate 1 ml of dilute ammonium solution were added. A yellow coloration indicates the presence of flavonoids.

3.5 Tannins Determination

A portion of 0.5 gm extract was boiled in 10 ml of water in attest tube and then filtered. Few drops of 0.1% ferric chloride were added. Brownish green or a blue-black color develops confirms presence of tannins.

3.6 Saponins Determination

Shake the plant extract vigorously with water, persistent foam observed, indicates the presence of saponins.

3.7 Glycoside Determination

A portion of 0.5 gm extract was diluted in 5 ml of distilled water and 2 ml of glacial acetic acid and 1% of ferric chloride solution were added with few drops of conc. H₂SO₄. A violet ring appear below the brown ring or a greenish ring form just above the brown ring and gradually spread throughout this layer which confirms the presence of glycoside.

4. Antimicrobial activity

The antimicrobial assay was performed by Agar well diffusion method^[13, 14] for solvent extract. Petri plates were prepared with 20 mL of sterile brain heart infusion agar (BHI) (Himedia, Mumbai). The test cultures (100 µL of suspension containing 10⁸ CFU/mL bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. For agar well diffusion method, a well was prepared in the plates with the help of a cork borer (6.0 mm). The active extract fractions were serially diluted in the respective solvent used for its extraction. The active extract fractions were diluted and used at concentrations of 0%, 25%, 50%, 75% and 100%. Normal saline was used as the negative control while Ampicillin of 30 µg/ml was used as the positive control. Into the well, 100 µl of the test compound was introduced. The plates were incubated overnight at 37°C. The positive antimicrobial growth was determined by measuring the diameter of the zone of inhibition after 24h in which square radius of the clear zone around each well (y²) were measured and divided over the square well radii (x²) to obtain absolute unit (AU) for the inhibition zone^[15]. Minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited growth of the microorganism.

5. Results

5.1 Phytochemical analyses

The phytochemical screening of the selected plants showed the presence of terpenoids and coumarins in all the four plants. Alkaloids were present in *C. roseus* and *A. paniculata*. Flavonoids were absent in all plants except for *A. paniculata*. Tannins were present in all the plants

except *A. paniculata*. Saponins were present in *C. citrates* and *A. paniculata* as presented in Table 1.

5.2 Glycoside determination

O. basilicum, *C. citrates* and *C. roseus* methanolic extract showed the presence of glycoside whereas *A. paniculata* showed negative result.

5.3 Antimicrobial assay

The antimicrobial efficacy of *O. basilicum*, *C. citrates*, *C. roseus* and *A. paniculata* extracts of 100%, 75% and 50% Methanol, plants against bacterial isolates showed varied level of inhibition. Almost all the selected plants showed antimicrobial activity against the investigated five human pathogens, the presence and absence of antimicrobial activity is summarized in the Table 2. The zone of inhibition ranged from 0.6 mm to 3.6 mm. The highest inhibition zone 3.6 mm (100 %) was formed by the extract of *A. paniculata* against *E. faecalis* at the highest concentration, followed by 3 mm (75%), 1.3 mm (50% and 25%) whereas, 3.3 mm (100%), 3 mm (75%) and 1.3 mm (50% and 25%) zone recorded in *S. aureus*. *C. roseus* plant extract showed 2.5 mm (100%), 1.8 mm (75%) and 0.8 mm (50%) inhibition zone against *E. faecalis* followed by 2.6 mm (100%) and 1.3 mm (50% and 25%) inhibition zone against *S. aureus*, 1.3 mm (100% and 50%) and 0.6 mm (25%) inhibition zone against *E. coli*. *C. citrates* plant extract showed 3.3 mm (100%), 1.6 (75% and 50%) and 1.3 mm (25%) inhibition zone against *S. aureus* followed by 3 mm (100%), 1.6 mm (75%) and 0.6 mm (50%) inhibition zone against *E. faecalis*. The positive control (Ampicillin) exhibited far stronger antimicrobial activity with inhibition diameters 10.0 mm while no zone was observed by negative control. The rest of the zones of inhibition (mm) are given in the Table 3. None of the investigated plants showed activity against *P. aeruginosa*. All plant extracts showed antimicrobial activity against *S. aureus* (Figure 2). There was no significant difference in the activity of the various extracts suggesting that, maybe none of the extraction modes used affected the bioactive components of the plant extracts or that

none of the extraction modes adequately extracted the bioactive components from the raw plant materials.

Table 3. Inhibition of bacterial isolates exposed to selected plant extracts using agar well diffusion technique at different concentration dose

Microorganisms	% Conc. (µg/ml)	Zone of inhibition (mm)											
		A		B		C		D		PC		NC	
		Y	AU	Y	AU	Y	AU	Y	AU	Y	AU	Y	AU
<i>S. aureus</i>	100	12	2	20	3.3	16	2.6	20	3.3	60	10	-	-
	75	9	1.5	10	1.6	8	1.3	18	3	NT	NT	NT	NT
	50	8	1.3	10	1.6	8	1.3	8	1.3	NT	NT	NT	NT
	25	4	0.6	8	1.3	6	1	8	1.3	NT	NT	NT	NT
<i>S. pneumonia</i>	100	15	2.5	-	-	-	-	-	-	53	8.6	-	-
	75	10	1.6	-	-	-	-	-	-	NT	NT	NT	NT
	50	-	-	-	-	-	-	-	-	NT	NT	NT	NT
	25	-	-	-	-	-	-	-	-	NT	NT	NT	NT
<i>E. faecalis</i>	100	-	-	18	3	15	2.5	22	3.6	50	8.3	-	-
	75	-	-	10	1.6	11	1.8	18	3	NT	NT	NT	NT
	50	-	-	4	0.6	5	0.8	8	1.3	NT	NT	NT	NT
	25	-	-	-	-	-	-	8	1.3	NT	NT	NT	NT
<i>E. coli</i>	100	-	-	10	1.6	8	1.3	-	-	60	10	-	-
	75	-	-	8	1.3	8	1.3	-	-	NT	NT	NT	NT
	50	-	-	-	-	-	-	-	-	NT	NT	NT	NT
	25	-	-	6	1	4	0.6	-	-	NT	NT	NT	NT
<i>P. aeruginosa</i>	100	-	-	-	-	-	-	-	-	48	8	-	-
	75	-	-	-	-	-	-	-	-	NT	NT	NT	NT

Y: diameter of inhibition zone (mm); AU=y/x µl of plant extract where x: Diameter of well (6mm), NT: Not tested, -: Absent

A: *Ocimum basilicum*, B: *Cymbopogon citrates*, C: *Catharanthus roseus*, D: *Andrographis paniculata*, PC: Positive control (Ampicillin), NC: Negative control (Normal saline)

Among all the five investigated plants *C. citrates*, *C. roseus* and *A. paniculata* was found to be efficient against most of the investigated pathogens.

6. Discussion

Medicinal plants are used in traditional medicine for several purposes. The secondary metabolites produced by plants constitute a source of bioactive substances and nowadays scientific interest has increased due to the search for new drugs of plant origin^[16,17].

Considering the evolution of resistance genes to antibiotics of microbial origin and non-antibiotic Chemicals^[18], plant materials have become the subject of public attention and therefore the pharmaceutical industry is moving away from drug discovery or screening towards compounds isolated by medicinal plants.

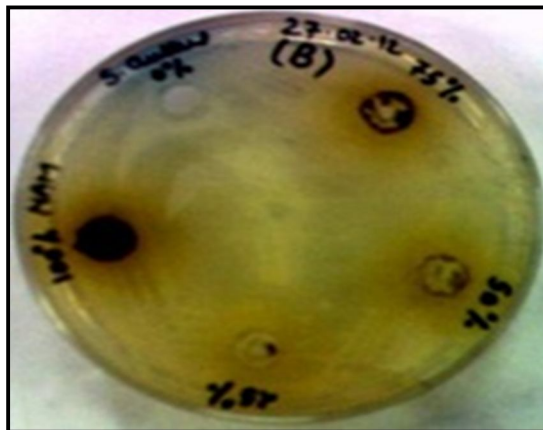


Figure 2. Anti-microbial activity of selected plant extracts against *S. aureus* by the well diffusion method.

Standardization of herbal drugs is most desirable part of plant based drugs designing that includes macroscopic, microscopic and phytochemical studies of investigated plant parts. Macroscopic characters involve size, arrangement, venation, texture, surface characters, markings and hardness of the plant materials. The microscopical studies (anatomical and histochemical) are often necessary to establish the botanical identity of commercial samples of medicinal plants, timbers, fibers etc. and may play an important part in checking adulteration and substitution.

The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavonoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpenes, etc., are effective antimicrobial substances against a wide range of microorganisms. It can be seen from the above results that the leaf extract contains alkaloids and other phytochemicals. These compounds could be used as substitutes for synthetic antibiotics for the treatment of chronic

kidney infection, bacterial endocarditis and carrier conditions of typhoid^[19].

The in vitro antimicrobial activity of *O. basilicum*, *C. citrates*, *C. roseus* and *A. paniculata* against Gram positive *S. aureus* and *E. faecalis* and efficient result was observed against *S. aureus*. Very less or no activity was observed against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). This can be explained because the outer membrane of Gram-negative bacteria is known to present a barrier to the penetration of numerous antibiotic molecules and the periplasmic space contains enzymes which are able of breaking down foreign molecules introduced from outside.

Further investigation is required for the isolation of the active principle which could serve as a broad-spectrum antimicrobial agent for treating bacterial infections.

7. Conclusion

The phytochemicals that have efficient antimicrobial activity could be screened, isolated and used as substitute for antibiotics. In the present study almost all the plants showed antimicrobial activity but *C. citrates*, *C. roseus* and *A. paniculata* were found to be more effective against most of the investigated pathogens.

In contrast to chemical drugs, herbs have sometimes been claimed to be non-toxic, because of their natural origin and long-term use as folk medicines. However, problems may arise due to intrinsic toxicity, adulteration, substitution, contamination, misidentification, drug-herb interactions and lack of standardization^[20]. This unfavourable fact urges the study of medicinal plants and plant derived compounds used in medicine and food industry.

8. Acknowledgment

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9. References

- Zhang X. Traditional medicine and WHO, World health, the magazine of World Health Organization 49th year 1976; No. 2:4-5.
- Essawi T, Srour M. Screening of some Palestinian medicinal plants for Antimicrobial activity. J Ethnopharmacol 2000; 70:343-349.
- Woodford N. Novel agents for the treatment of resistant Gram positive infections. Expert Opin Investig Drugs 2003; 12:117.
- Shah PM. The need for new therapeutic agents: What is in the pipeline? Clinical Microbiology and infection 2005; 11:36-42.
- Balandrin MF, Kjocke AJ, Wurtele. Natural plant chemicals sources of industrial and mechanical materials. Science 1985; 228:1154-1160.
- Mehta KC. Indian herbal drugs in the treatment of diabetics. Current medical practice 1982; 26:305-308.
- Mitsuyama J, Hiruma R, Yamaguchi A, Sawai T. Identification of porins in outer membrane of *Proteus*, *Morganella* and *Providencia* spp. and their role in outer membrane permeation of β -lactams. Antimicrob Agents Chemother 1987; 31:379-384.
- Gutmann L, Billot-Klein D, Williamson R, Goldstein FW, Mounier J, et al. Antimicrob Agents Chemothe 1988; 32:195-201.
- Mathias AJ, Somashekar RK, Sumithra S, Subramanya S. Anti-microbe Agents. Indian J Microbiol 2000; 40:183-190.
- Ganguly R, Mishra P, Sharma A. Indian J Microbio 2001; 41:211-213.
- Martino PD, Gagniere H, Berry H, Bret L. Antibiotic resistance and virulence properties of *Pseudomonas aeruginosa* strains from mechanically ventilated patients with pneumonia in intensive care units: Comparison with imipenem-resistant extra-respiratory tract isolates from uninfected patients. Microbes and infection 2002; 4: 613-620.
- Chapter in book: Khandelwal K.R. Textbook of Practical pharmacognosy. 7th ed. Pune: Nirali publication; 2000. pp. 149-189.
- Perez C, Paul M, Bazerque P. An antibiotic assay by the agar well diffusion method. Acta Biol Med Exp 1990; 15:113-115.
- Nair R, Chanda S. Antimicrobial activity of *Punica granatum* indifferent solvents. Indian J Pharm Sci 2005; 67:239-243.
- Tagg JR and Mcgiven AR. Assay system for bacteriocins. Appl Microbiol 1971; 21(5):943-944.
- Basile A, Sorbo S, Giordano S, Ricciardi L, Ferrara S, Montesano D, et al. Antimicrobial and allelopathic activity of extract from *Castanea sativa* leaves. Fitoterapia 2000; 71 (5):110-116.
- Paiva SR, Figueiredo MR, Aragao TV, Kaplan MAC. Antimicrobial activity in vitro of plumbagin isolated from *Plumbago* species. Mem. Inst. Oswaldo Cruz 2003; 98: 959-961.
- Kohler T, Pechere JC, Ple siat P. Bacterial antibiotic efflux systems of medical importance. Cell Mol Life Sci 1999; 56:771-778.
- John A, Steena RA, Mary SR. Phytochemical and Antimicrobial screening of seven apocynaceae species against human pathogens. Int. J Pharm and Pharm Sci 2011; 3 (5):278-281.
- Zhou S, Koh HL, Gao Y, Gong ZY, Lee E JD. Herbal bioactivation: the good, the bad and the ugly. Life Sci 2004; 74:935-968.