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EFFICACY OF ANTI-BACTERIAL AND ANTI-FUNGAL ACTION ON THREE MEDICINAL PLANTS EXTRACT THE *Rosa gallica, Psidium guajava* AND *Vitis vinifera* AGAINST *Streptococcus mutans* AND *Candida albicans* -AN *In-vitro* STUDY

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: World health organization has been an initiative for the widespread practice of herbal medicine been practiced in developing countries and created a scientific basis to treat infection.
Aim: The aim of the study was to test the anti-bacterial and anti-fungal activity of three medical plant extract the *Rosa gallica, Psidium guajava* L and *Vitis vinifera* against the *streptococcus Mutans* and *Candida albicans* organisms.
Settings and Design: An in-vitro study was conducted collaborated with the department of microbiology and the Department of Public Health Dentistry in SRM Dental College and Hospital for a period of four months. The extract of medical plant of *Rosa gallica, Psidium guajava* L and *Vitis vinifera* was tested in different concentration to find out the effective anti-bacterial and anti-fungal action.
Methods and Materials: Extract obtained from the medicinal plant of *Rosa gallica, psidium guajava* L and *Vitis vinifera* was procured and powdered. The minimum inhibitory concentration of the obtained extracts was determined by using the tube dilution method and the bacterial strains of *Streptococcus mutans* and *Candida albicans* was cultured into the extract obtained at concentration of 1 mg/ml, 500 µg/ml, 250 µg/ml, and 100 µg/ml respectively.
Statistical Analysis Used: Descriptive statistics was done to analysis the bacterial count in different concentration.
Results: The extracts obtained from *Rosa gallica and psidium guajava* L had a better anti-bacterial property when compared to the other medicinal plants and the extracts obtained from *Rosa gallica* and *psidium guajava* L had a better anti-fungal property when compared to the other two medicinal plants.

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Conclusions: These plants extracts showed anti-bacterial property in previous studies to add on to the review of literature in our study it proved that *Rosa gallica and psidium guajava* L had a better anti-fungal activity when compared to another two medicinal plant extract. The clinical significance of the medicinal plant extract can be effectively used as anti-bacterial and anti-fungal agent as it was compared with the gold standard values of kanamycin and Ketoconazole.

Keywords: Anti-bacterial; anti-fungal; minimum inhibitory concentration and medicinal plant.

1. INTRODUCTION

Herbal medicine has been a recent trend to rise in the modern world due to increase in multidrug resistant caused by various antibiotics [1]. They are an increase in mortality and morbidity rate in immune-compromised patient due to higher occurrence of fungal and bacterial infection which has been an era to the modern world [2]. As plant extract are pure remedy obtained as serve as effective time-tested and cheaper alternative therapy than the medicine used to treat the infection caused by anti-bacterial and anti-fungal action [3]. People lived in ancient time believed that plant had a curative nature which treat the disease [4]. The people in recent times started to stress more on herbal medicine because it is cheaper and time-tested [5].World health organization has been an initiative for the widespread practice of herbal medicine been practiced in developing countries and created a scientific basis to treat infection [6]. According to the World Health Organization, 80 percent of people in African and Asian countries utilize herbal medicine for some part of primary health care, and they see herbal medicine as the best source of a wide range of pharmaceuticals.

"Among these herbs, rose has got many medicinal benefits. Rose is a woody perennial of Genus Rosa, within family Rosacae. Most are native to India, with small numbers native to Africa, Europe, and North America" [7]. "These are mostly known for their beauty and fragrance. The extracts in our study contains 80% essential fatty acids and antioxidants, thus helps to regenerate skin cells. Drinking rose water or tea removes inner body heat and brings down fever. It heals sunburn and wound" [8]. It also stimulates circulation in skin and reduces thread veins and broken capillaries. It acts as diuretic to flush toxins from body. It relieves bronchial and chest congestion. It also relieves from sore throat and running nose. It lessens cramping during menstruation. Local application of rose powder is helpful for expulsion of placenta [9]. "It acts as an antiseptic, antispasmodic, astringent, bactericidal (against typhoid, diarrhoea, cholera, food poisoning), antiviral (cold or influenza), antiphlogistic" [10].

The extract of *Rosa gallica* (rose petal) belongs to the well-known rosoideae family is potential used as anti-inflammatory for skin. It plays an important role in chemotaxonomic marker and has an inhibitory action on digestive enzymes. The extract of *psidium guajava* has been used to treat various diseases which include cardiovascular disease, parasitic infection, cancer and diabetes mellitus. It has been proved to be effective in regulating the blood pressure level [11]. They are various studies published by other plant extract since these extracts have not been used to find out the anti-bacterial and anti-fungal property [12]. The extract of *Vitis vinifera* has been used to improve the level of proteomic and transcriptomic profile and also effective in suppressing the cells causing skin cancer.

2. MATERIALS AND METHODS

2.1 Collection of Plant Extract

Rosa gallica (Powder),*psidium guajava* L (dried fruit powder), *Vitis vinifera* (Fresh stem), were were dried ground into coarse powder and weighed into 5 gm, 10 gm, 50 gm powder. These were added to 10 ml of double deionised distilled water The experiments were carried out at Department of Microbiology, SRM Dental College, Chennai. The wild strains of *streptococcus mutans* and *candida albicans* obtained from the oral cavity were used. The quality control the organism and sterility control were carried out prior to the start of the study for satisfaction.

2.2 Preparation of Inoculum

100 g powder was soaked in 300 mL of ethanol in a soxhlet system to extract ethanolic extracts. For validation, the above steps were done three times. Ethanolic extracts were powdered and dissolved in DMSO solvents for antibacterial activity after being condensed with a rotary evaporator at 45ºC.

2.3 Well Diffusion Method

"Fresh bacterial culture of *Streptococcus mutans* MTTC 890 and fresh fungal culture *Candida albicans* MTTC 773 were used for the antimicrobial test. The colonies of the strains were inoculated to Muller Hinton broth for bacteria and Potato Dextrose Broth (PDB) for fungi and incubated at 37ºC for 24 h in orbit shaker at 200 rpm. Turbidity was adjusted with sterile broth to corresponds to the 0.5 McFarland standards before swabbing the microorganism;

standard inoculums of the microorganism was set at 1.5×10^6 colony counts and diluted to 1:100 and given suspension of turbidity equal to a McFarland standard 0.5. The turbidity was matched with a McFarland 0.5 mL of 1.175% w/v (0.048 M) $BaCl_2.H_2O$ to 99.5 mL of 1% w/v (0.36) sulphuric acid. The in vitro antimicrobial activities of test compounds were determined by the well diffusion method" as described by Perez et al. (1990). "Standard antibiotic kanamycin for *Streptococcus mutans* and fluconazole for *Candida albicans* was used as reference. Organisms (24 h old culture) were swabbed on the Mueller Hinton Agar (MHA) to bacteria and potato dextrose agar (PDA) for fungi plates with sterilized cotton swab sticks. Wells (9 mm diameter) were cut using a sterile cork borer. Ethanolic Stock solution Athimathuram powder, Soap nut coat, Gum Arabic and Guava leaf were dissolved in Dimethyl sulfoxide (DMSO). The stock solution was prepared with to 10 mg/ 2 mL of DMSO. Diluted measurements such as 50 µL, 100 µL, 200 µL (10 µL diluted sample contain 50 µg of the test compound) was dispensed into agar wells of culture inoculated plates (MHA) using sterilized micro tips. The plates were incubated at 37°C for overnight"[13]. The antibacterial activity was measured based on the zone of inhibition it was calculated using the following formula.

$$\text{Inhibition percentage (I \%)} = \frac{\text{Zone of Inhibition (mm)}}{\text{Diameter of Pertiplate (mm)}} \times 100$$

2.4 Minimum Inhibitory Concentration (MIC)

Ethanolic extracts was chosen to assay for MIC. MIC was determined by modification of the Wariso and Ebong technique (1996). Muller Hinton broth was prepared and sterilized using autoclave. Hundred µL of the prepared broth was dispensed in to the well numbered 1-12 using sterile micropipette. A stock solution containing mg/ml of the extract was prepared. Then 100 µL of the solution was dispensed into the tubes numbered 1. Subsequently, from well 1, serial dilution was carried out and 100 µL from tube 1 was transferred up to well number 10 and 100 µL from the well 10 was discarded. Well 11, and 12 was viability of the organism control. An overnight culture (inoculums) of each of the test isolates was prepared in sterile nutrient broth. 100 µL of the inoculums was transferred into each tube from well 1 to 12. The final concentration of the ethanol extract in each of the plate well numbered 1-10 after dilution 500 µg/mL 250 µg/mL; 125 µg/mL; 62.5 µg/mL; µg/mL 31.25 µg/mL; 15.625 µg/mL; 7.812 µg/mL; 3.90 µg/mL; 1.953 µg/mL and 0.976 µg/mLwere incubated at 37°C for 24 h and examined for growth. After that, each well was filled with 20 µl produced Blue tetrazolium (5 mg/mL) and incubated for 30 minutes. After incubation, the presence of blue color indicates that the organisms are alive, whereas the presence of yellow color or the absence of blue color indicates that bacteria and fungus are completely inhibited.

3. RESULTS

Table 1 showed that three samples of different medicinal plant extract were used with the positive control as kanamycin (10 µg/ disc) and negative control as Dimethyl Sulfoxide and the concentration which were tested are 1000 µg, 500 µg and 250 µg and the target organism was *streptococcus mutans*.

Table 2 showed that four samples of different medicinal plant extract were used with the positive control as Ketoconazole (19 µg/ disc) and negative control as Dimethyl Sulfoxide and the concentration which were tested are 1000 µg, 500 µg and 250 µg/disc and the target organism was *Candida albicans*.

Figs. 1,2,3 showed the anti-bacterial activity of the three medicinal plant extract the *Rosa gallica*, *psidium guajava* L and *Vitis vinifera* against *streptococcus mutans* clockwise from the top to bottom in different concentration were tested from 1000 µg, 500 µg and 250 µg/disc and solvent of Dimethyl sulfoxide were used with kanamycin (10 µg/ disc) as control.

Figs. 4,5,6 showed the anti-fungal activity of the three medicinal plant extract the *Rosa gallica*, *psidium guajava* L and *Vitis vinifera* against *candida albicans* clockwise from the top to bottom in different concentration were tested from 1000 µg, 500 µg and 250 µg/disc and solvent of Dimethyl sulfoxide were used with Ketoconazole (19 µg/ disc) as control.

Table 1. zone of inhibition seen in different concentration by inhibiting the micro-organism – *streptococcus mutans*

S.No	Microorganism	Zone of Inhibition in mm			
		1000 µg	500µg	250µg	kanamycin (10µg/ disc)
	Streptococcus mutans				
1.	Rose petal	18mm	16mm	10mm	24 mm
2.	Guava leaf	16mm	13mm	10 mm	22 mm
3.	Grape seed	18mm	15mm	10mm	19mm

Table 2. Zone of inhibition seen in different concentration by inhibiting the micro-organism- *candida albicans*

S.No	Microorganisms	Zone of Inhibition (in mm)			
		1000 µg	500µg	250µg	Ketoconazole(19ug/ml)
	Candida albicans				
1.	Rose petal	26 mm	24mm	22mm	19mm
2.	Guava leaf	22mm	19mm	17mm	15mm
3.	Grape seed	15	12	9	14mm

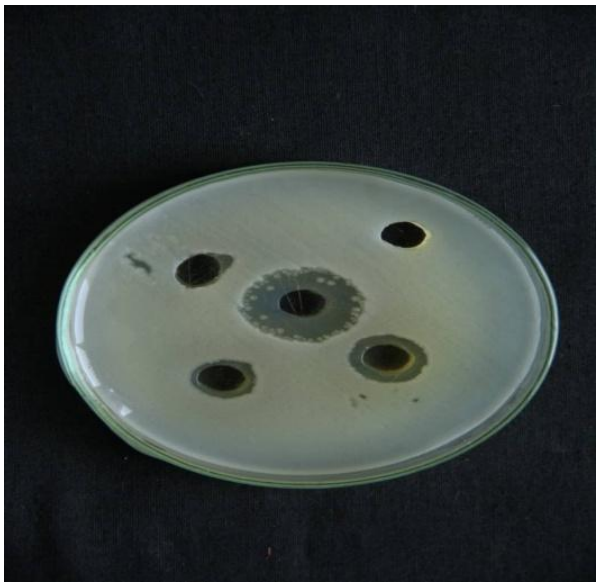

Fig. 1. Depicts zone of inhibition of Kanamycin and rose petal extract at different concentration in Muller Hinton agar swabbed with *Streptococcus mutans*. Zone of inhibition of Kanamycin is more than the sample

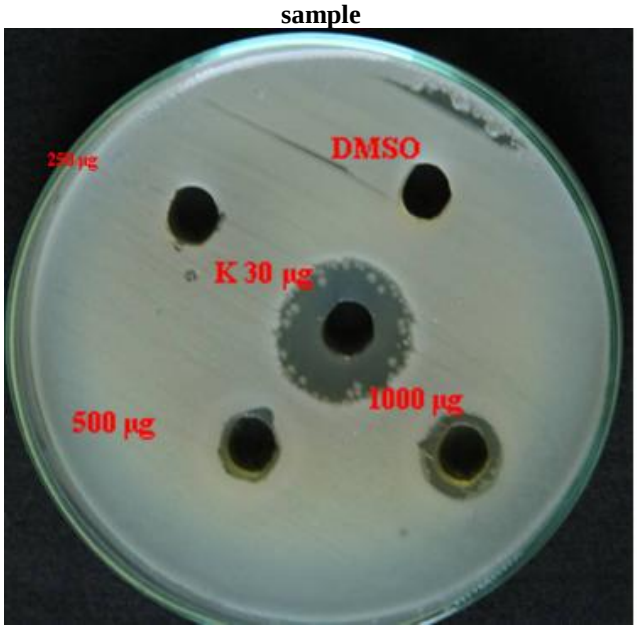

Fig. 2. Anti-bacterial activity of extract of *psidium guajava* L against steptococcusmutansclockwise from top: 1000, 500 and 250 µg/disc and solvent (Dimethyl sulfoxide) control center: kanamycin (10 µg/ disc) control

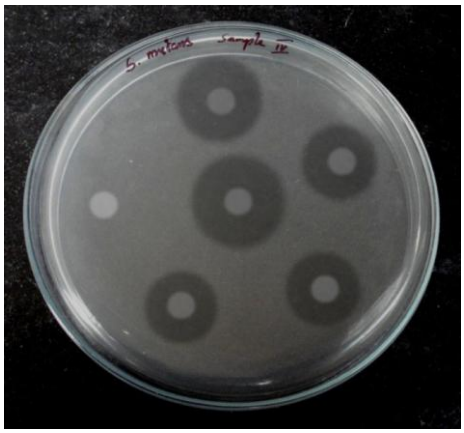

Fig. 3. Anti-bacterial activity of extract of *Vitis vinifera* againststeptococcusmutansclockwise from top: 1000, 500 and 250 µg/disc and solvent (Dimethyl sulfoxide) control center: kanamycin (10 µg/ disc) control

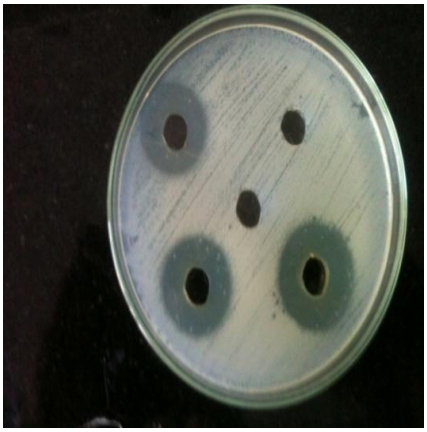

Fig. 4. Depicts zone of inhibition of Ketoconazole and Rose petal extract at different concentration in Potato Dextrose agar swabbed with *Candida albicans*. Zone of inhibition of the sample was than Ketoconazole

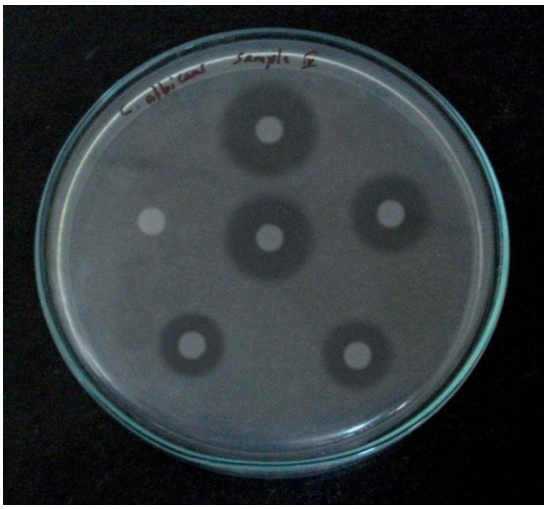

Fig. 5. Anti-fungal activity of extract of *psidium guajava* L against *candida albicans* clockwise from top: 1000, 500 and 250µg/disc and solvent (Dimethyl sulfoxide) control center: Ketoconazole (19ug/ml) control

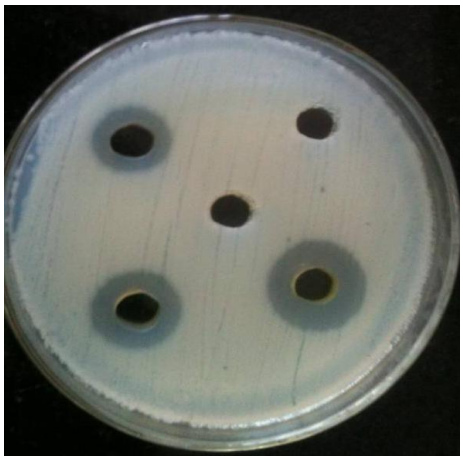

Fig. 6. Anti-fungal activity of extract of *Vitis vinifera* against *candida albicans* clockwise from top: 1000, 500 and 250 µg/disc and solvent (Dimethyl sulfoxide) control center: Ketoconazole(19 ug/ml) control

4. DISCUSSION

Here the extracts of *Rosa gallica*, *psidium guajava* L and *Vitis vinifera* are herbal medicine used in our study since large to moderate numbers of trees are found throughout the world, chiefly in light rainfall and deciduous forest areas [14]. The contraindication of the extracts present in our studies includes systemic intake in pregnancy, weak digestion, dehydration and heat stroke condition [15]. Gnan et al have reported "a complete inhibition of growth of Staphylococcus aureus; aqueous guava leaf extract Vieira et al, 42 reported the microbiocidal effect of guava sprout extract (acetone). Abdelrahimet al, 29 also reported a complete inhibition of Bacillus subtilis, Staphylococcus aureus with extract of the guava leaf". Takte et al states that "Methanolic extract of Rosa damascenashowed Zone of inhibition of 8.3, 14.9, 21 mm at 20, 40, 80mg respectively against *Candida albicans* to that of Fluconazole, control 15 mm [16]. This shows Methanolic extract of Rosa damascena has more Antifungal activity against *Candida albicans*".

The minimum inhibitory concentration of the plant extracts was determined using dilution method as it has been reported in the studies to be more sensitive and is able to distinguish between anti-fungal and anti-bacterial effects and is used for quantitative determination.

In our present study MIC aqueous solution was determined and the test tube showed that the zone of inhibition was matched with the gold standard values of kanamycin when incubated in higher concentration, as the concentration became low the values gradually decreased and did not match the gold standard values of kanamycin and showed the anti-bacterial property for the *streptococcus mutans*. Whereas, in antifungal property the extracts of plants when incubated in high concentration matched to the gold standard value of ketoconozole and higher values of extract were found to exist equal effect in the concentration of 250 ug when compared to the amphotericin B value and as the concentration were tested in higher concentration it found that it exert effect more than the gold standard value of Ketoconazole tested in *candida albicans* organisms. As the available data suggest that the extracts used in our study found other effect, but in our study it proves its effectiveness as anti-fungal agent which adds on more credit to our study.

The limitation of our study is they have taken concentration from 1000µg/ml and gradually decrease the concentration and has been tested, so further studies can be carried out with a higher concentration of more than 1000 µg/ml to prove its anti-bacterial and anti-fungal effect of the plant extract we have used in our study.

5. CONCLUSION

These plants extracts showed anti-bacterial property in previous studies to add on to the review of literature in our study it proved that *Rosa gallica* and *psidium guajava* L had a better anti-fungal activity when compared to another two medicinal plant extract. The clinical significance of the medicinal plant extract can be effectively used as anti-bacterial and anti-fungal agent as it was compared with the gold standard values of kanamycin and Ketoconazole.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al-Bari MA, Sayeed MA, Rahman MS, Mossadik MA. Characterization and antimicrobial activities of a phenolic acid derivative produced by Streptomycesangla-deshiensis, a novel species collected in Bangladesh. Res J Med Med Sci 2006;1:77-81.
2. Barani K, Manipal S, Prabu D, Ahmed A, Adusumilli P, Jeevika C. Anti-fungal activity of Morindacitrifolia (noni) extracts against *Candida albicans*: An in vitro study. Indian Journal of Dental Research. 2014;25(2):188.
3. Boskabady MH, Shafei MN, Saberi Z, Amini S. Pharmacological effects of Rosa damascena. Iranian journal of basic medical sciences. 2011 Jul;14(4):295.
4. Dilhuydy JM. Patients′ attraction to complementary and alternative medicine (CAM): A reality which physicians can neither ignore nor deny. Bull Cancer 2003;90:623-8
5. Díaz-de-Cerio E, Verardo V, Gómez-Caravaca A, Fernández-Gutiérrez A, Segura-Carretero A. Health effects of *Psidium guajava* L. Leaves: An overview of the last decade. International journal of molecular sciences. 2017;18(4):897.
6. El Astal ZY, Ashour AE, Kerrit AA. Antimicrobial activity of some medicinal plant extracts in Palestine. Pak. J. Med. Sci. 2005;21(2):187-93.
7. Kumarasamy B, Manipal S, Prabu Duraisamy AA, Mohanaganesh SP, Jeevika C, Role of aqueous extract of Morindacitrifolia (Indian noni) ripe fruits in inhibiting dental caries-causing *Streptococcus mutans* and Streptococcus mitis. Journal of Dentistry (Tehran, Iran). 2014;11(6):703
8. Lee MH, Nam TG, Lee I, Shin EJ, Han AR, Lee P, Lee SY, Lim TG. Skin anti-inflammatory activity of rose petal extract (*Rosa gallica*) through reduction of MAPK signaling pathway. Food Science & Nutrition. 2018 Nov;6(8):2560-7.
9. Ochir S, Nishizawa M, Park BJ, Ishii K, Kanazawa T, Funaki M, Yamagishi T. Inhibitory effects of *Rosa gallica* on the digestive enzymes. Journal of natural medicines. 2010 Jul 1;64(3):275-80.
10. Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complement Altern Med 2006;6:2
11. Rekha, V., Jayamathi, R., Vijayalakshmi, D., Prabu, N.K. and Sunayana Manipal, K., 2014. Anti cariogenic effect of Terminalia chebula. Journal of clinical and diagnostic research: JCDR, 8(8), p.ZC51.
12. Sarangowa O, Kanazawa T, Nishizawa M, Myoda T, Bai C, Yamagishi T. Flavonol glycosides in the petal of Rosa species as chemotaxonomic markers. Phytochemistry. 2014 Nov 1;107:61-8.
13. Swetha R, Shobana R, Sunayana M, Prabu D, Raj Mohan and Naveen Raj. Antibacterial and Antifungal Efficacy of Rose Petal (*Rosa Indica*) Extract on Oral Microbes - An In Vitro Study. Research & Reviews: Journal of Dental Sciences. 2019;7(2).
14. Shireen F, Manipal S, Prabu D. Anti-fungal activity of Aloe vera: In vitro study. SRM Journal of Research in Dental Sciences. 2015;6(2):92.
15. Van der Watt E, Pretorius JC. Purification and identification of active antibacterial component in Carpobrotus edulis L. J Ethnopharmacol. 2001;76:87-91
16. Vijaya K, Ananthan S. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. J Altern Complement Med. 1997;3:13-20.