A COMPARATIVE STUDY OF IN VITRO ANTIMICROBIAL ACTIVITY AND TLC STUDIES OF PETALS OF SELECTED INDIAN MEDICINAL PLANTS

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ABSTRACT

Objective: The present study was to evaluate the in vitro antibacterial activity, and thin-layer chromatography (TLC) studies from the petals of four different Indian medicinal plants (Punica granatum, Hibiscus rosa-sinensis, Cassia auriculata, and Moringa oleifera).

Methods: The phytochemical screening of the methanol extract of petals of four different Indian medicinal plants was performed using standard procedures. The antimicrobial activity was tested against various test organisms using the agar disc diffusion method.

Results: The preliminary phytochemical screening for petals of four different medicinal plants revealed the presence of flavonoids, alkaloids, tannins, and saponins. From the above study, the results indicated that the methanol extract of M. oleifera petals showed the highest antimicrobial activity against Staphylococcus aureus and Bacillus subtilis with zone of inhibition 17.93 and 23.40, respectively, at the concentration of 20 μl/ml and also showed the maximum inhibitory effect at the highest concentration (20 μl/ml) than the lowest concentration (5 μl/ml) against Gram-negative bacteria such as Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, and Gram-positive B. subtilis and S. aureus. TLC studies of methanol extracts of petals of Indian medicinal plants revealed the presence of different phytoconstituents as evidenced by separated compounds with different Rf values.

Conclusion: The results obtained in the present study indicate that the petals of four different Indian medicinal plants showed the highest antibacterial activity and can be used as an antibacterial agent against bacterial diseases.

Keywords: Phytochemicals, Antibacterial activity, Thin-layer chromatography.

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INTRODUCTION

Phytomedicines play a major role in human health-care system. Plants are also known to contain enormous biological active compounds such as flavonoids, glycosides, phenols, carotenoids, alkaloids, and terpenoids [1] which possess antibacterial properties. According to World Health Organization, 65-80% of the world populations rely on traditional medicine to treat various diseases [2]. In recent decades, many antimicrobial drugs have been discovered, developed, and widely used, but it was found that microorganisms build resistance to drugs [3,4]. Hence, development of new drugs without any side effects is the urgent need of the society. Plants have been a therapeutic source for a long time, and plant products played an essential role in ancient medicine. Plants are the cheapest and safest alternative sources of antimicrobials [5-7].

Hibiscus (Malvaceae) is a genus of herbs, shrubs, and trees. Its 250 species are widely distributed in tropical and subtropical regions of the world and are reported to possess various medicinal properties. Studies have shown that the plants of the Hibiscus genus have the potential to provide biologically active compounds that act as antioxidants and cardioprotective agents. Hence, Hibiscus genus may be a great natural source for the development of new drugs and may provide a cost-effective mean of treatment for cancer and other diseases in the developing world [8]. The ancient Indian medicinal literature reported that the flowers of Hibiscus rosa-sinensis have beneficial effects in heart diseases, mainly in myocardial ischemic disease and without producing any cytotoxic effects [9]. Recently, [10] suggested that H. rosa-sinensis had a protective role against age and scopolamine-induced amnesia, indicating its utility in the management of cognitive disorders.

Cassia auriculata commonly known as “avaram” and belonging to the family Caesalpinaceae. It is a common plant in Asia, profusely used in Ayurvedic medicine as a tonic, astringent, and as a remedy for diabetes, conjunctivitis, ulcers, leprosy, skin, and liver diseases [11]. There are many therapeutic uses, and medicinal properties are reported in different parts (leaves, stem, seeds, flowers, fruits, stem bark, etc.) of the plants. They are known for antihyperglycemic activity [12], hepatoprotective activity [13], antihelmintic activity [14], antimicrobial activity [15], and antioxidant activity [16]. The flowers of the plant are used in the preparation of tea, which is prescribed in diabetes. The seeds are used in diabetes, ophthalmia, and chylous urine [17]. The plant has been reported to antibacterial and microbicidal activity [18,19].

Moringa oleifera (Moringaceae) is commonly known as a drumstick tree or horseradish tree. Traditionally, its roots are applied as plaster to reduce the swelling and rheumatism. Almost all parts of the plant are used culturally for its nutritional value, medicinal properties and for taste and flavor as a vegetable and seed [20]. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers, and immature pods act as cardiac and circulatory stimulants and possess antitumor, antipyretic, antiasthmatic, anti-inflammatory, antiulcer [21]. The whole M. oleifera plant is used in the treatment of psychosis, eye diseases, and fever, other important medicinal properties of the plant include antispasmodic [22], diuretic [23], antihypertensive [24], cholesterol lowering [25], antioxidant, antidiabetic, hepatoprotective [26], antibacterial, and antifungal activities [27].

The pomegranate (Punica granatum L., Punicaceae family) is a shrub, and its fruit is a rich source of bioactive phytochemicals such as tannins and other phenolics. Pomegranate fruit products have been...
used for centuries since ancient civilizations for medicinal purposes. In Ayurvedic and Siddha medicine, the pomegranate is considered “a pharmacy into itself” and is used as an antiparasitic agent [28] a “blood tonic” [29] and to heal diarrhea and ulcers. The potential therapeutic properties of pomegranate are wide ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation [30].

The present investigation is to study the in vitro antimicrobial activity and to assess the phytochemicals by thin-layer chromatography (TLC) of pets of four different medicinal plants.

METHODS

Plant material

The petals of four medicinal plants (H. rosa-sinensis, P. granatum, C. auriculata, and M. oleifera) were collected from the Government Siddha Medical College, Herbal garden, Tamil Nadu, India. The petals of medicinal plants were washed with distilled water and shade dried. The shade dried petals were powdered and stored in air tight containers for further studies.

Preparation of plant extract

About 20 g of the finely ground petals of four medicinal plants were soaked in 70% methanol at room temperature for 24 hrs. The extract was filtered using Whatman filter paper No. 1 and then concentrated in vacuum at 40°C-50°C (overnight) using a rotary evaporator. The residue was mixed with methanol and used for further studies.

Phytochemical screening

The extracts were subjected to phytochemical analysis to ascertain the presence metabolites such as alkaloids, tannins, flavonoids, terpenoids, and saponins using standard procedures [31].

TLC

TLC was performed using standard methods [32]. About 15 µl of extract was applied to the precoated aluminum silica gel 60 F, Merck F 254. Developing solvent system used was toluene, aceticone, and formic acid (6:6:1). All plates were visualized directly after drying and with the help of UV at 240 nm and 360 nm in UV TLC viewer. The Rf value of the different spots that were observed was calculated.

Bacterial cultures

Bacterial strains used were Staphylococcus aureus MTCC 29213, Bacillus subtilis MTCC441, Escherichia coli MTCC 25922, Pseudomonas aeruginosa MTCC 2488, and Proteus vulgaris MTCC 1771. All bacterial strains were obtained from microbial type culture collection and gene Bank, Institute of Microbial Technology, Chandigarh, India. All bacterial strains were stored and maintained at 4°C for further study.

Antibacterial activity assay

The antibacterial assay was performed by disc diffusion technique [33]. The disc diffusion technique is highly effective to determine the antibacterial activities for methanol extracts of 4 different medicinal plants. About 25 ml of Mueller-Hinton agar was poured into each Petri plate. Once the agar solidified, the bacteria were inoculated on the surface of the plates. The methanol extract impregnated discs (Whatman No. 1 filter paper) were prepared and air dried well. The test was conducted at four different concentrations of the crude extract (5, 10, 15, and 20 µl/ml) with 3 replicates. The loaded discs were placed on the surface of the medium and incubated at room temperature for 24 hrs. After 24 hrs incubation at 37°C, all plates were observed for zones of inhibition, and the diameter of these zones was measured in millimeters. All tests were performed in triplicate and the antibacterial activity was expressed as the mean ± standard deviation.

Determination of minimum inhibitory concentration (MIC)

The effect of MIC of the methanol extracts was carried out using the method of [34]. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism. To 0.5 ml of bacterial cultures, varying concentrations of the extracts (5, 10, 15, and 20 µl/ml) were added to test tubes. The culture tubes were then incubated at 37°C for 24 hrs. After incubation, the tubes were then examined for microbial growth by observing for turbidity, and OD was measured spectrophotometrically at 580 nm.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of petals of four different medicinal plants (H. rosa-sinensis, P. granatum, C. auriculata, and M. oleifera) revealed the presence of flavonoids, alkaloid, saponins, and tannins (Table 1).

TLC studies of petals of four different Indian medicinal plants

TLC studies of methanol extract of petals of four medicinal plants were carried out using mobile phase toluene:acetone:formic acid (6:6:1). The spots were visualized under 240 nm and 360 nm (Figs. 1-4).

Antibacterial activity

The antibacterial potential of petals of four medicinal plants was compared according to their zone of inhibition against several pathogenic organisms by disc diffusion method (Table 2). M. oleifera showed the highest antimicrobial activity against S. aureus and B. subtilis with zone of inhibition 17.93 and 23.40, respectively, at the concentration of 20 µl/ml. Among the four medicinal plants, the methanol extract of M. oleifera petals showed the maximum inhibitory activity at the highest concentration (20 µl/ml) than the lowest concentration (5 µl/ml) against Gram-negative bacteria such as E. coli, P. vulgaris, P. aeruginosa, and Gram-positive B. subtilis and S. aureus (Table 3). In the present investigation, it shows that the methanol extracts of petals of four medicinal plants can inhibit the growth of pathogenic organisms.

Table 1: Phytochemical screening of petals of four Indian medicinal plants

| Phytochemical constituent | Hibiscus rosa-sinensis | Punica granatum | Cassia auriculata | Moringa oleifera |
|---------------------------|------------------------|----------------|-----------------|-----------------|
| Flavonoid                 | +                      | +              | +               | +               |
| Alkaloid                  | +                      | +              | +               | +               |
| Saponin                   | +                      | +              | +               | +               |
| Tannins                   | +                      | +              | +               | +               |

Hibiscus rosa-sinensis: H. rosa-sinensis, Punica granatum: P. granatum, Cassia auriculata: C. auriculata, Moringa oleifera: M. oleifera
The minimal inhibitory concentration was determined by measuring the turbidity of the bacterial culture that is the mean±SD of three replicates.

DISCUSSION

According to the present study, the preliminary phytochemical screening of methanol extract of petals of four different medicinal plants (H. rosa-sinensis, P. graminum, C. auriculata, and M. oleifera) showed the presence of flavonoids, alkaloids, saponins and tannins. Phytoconstituents have been found to inhibit bacteria [35]. The four medicinal plants screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health [36].

The methanol extract of petals of different medicinal plants showed varying degree of antibacterial activities against the test organisms (Table 2). Among the four medicinal plants, the methanol extract of M. oleifera at the concentration of 20 µl/ml showed maximum zone of inhibition percentage of 23.4 mm against B. subtilis. This is followed by P. graminum, S. aureus, and E. coli, with Zone of inhibition [in mm diameter]a of 17.93, 15.43, 12.40, and 11.43 mm zone of inhibition against S. aureus, E. coli, P. vulgaris, and P. aeruginosa, respectively. The MIC of the methanol extract for different organisms ranged between 5-20 µl/ml. TLC results showed the presence of phytoconstituents and it is active to the present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by the previous literature [39]. It supports the earlier investigation that the phytoconstituents isolated from the flower of M. oleifera possess remarkable toxic activity against bacteria and may assume pharmacological importance [38]. The traditional method of treating a bacterial infection, decoction of the plant parts, or boiling the plant in water is employed, whereas according to the present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by the previous literature [39].

CONCLUSION

From our study, it clearly indicates that the methanol extract of petals of four different medicinal plants is rich in phytochemicals which have potent antibacterial activity against pathogenic organisms. The result

Table 2: Antibacterial activity of methanol extract of petals of four different Indian medicinal plants

| Medicinal plants | Methanol extract (µl/ml) | Zone of inhibition [in mm diameter]a |
|------------------|--------------------------|-----------------------------------|
| Punica graminum   | 5 | 9.4±0.65 | 6.3±0.60 | - | 0.3 ±0.06 | 7.4±0.6 |
|                  | 10| 11.3±0.0.83 | 7.5±0.40 | 9.1±0.45 | 6.3±0.60 | 9.3±0.77 |
|                  | 15| 14.06±0.60 | 7.4±0.92 | 10.9±0.45 | 9.0±0.40 | 11.3±0.70 |
|                  | 20| 16.1±0.60 | 8.3±0.90 | 13.0±0.60 | 11.0±0.40 | 14.0±0.45 |
| Hibiscus rosa-sinensis | 5 | 9.0±0.45 | 10.3±0.70 | 7.4±0.65 | 11.3±0.77 | 9.3±0.70 |
|                  | 10| 11.0±0.40 | 12.4±0.66 | 11.0±0.40 | 15.1±0.45 | 11.4±0.65 |
|                  | 15| 12.9±0.45 | 14.3±0.76 | 11.9±0.55 | 17.1±0.41 | 13.3±0.76 |
|                  | 20| 14.4±0.61 | 17.0±0.40 | 14±0.4 | 18.9±0.45 | 16.2±0.43 |
| Moringa oleifera  | 5 | 11.3±0.67 | 7.4±0.60 | 11.4±0.79 | 7.0±0.40 | 6.3±0.0.6 |
|                  | 10| 13.4±0.73 | 11.5±0.72 | 16.1±0.45 | 8.4±0.6 | 7.3±0.75 |
|                  | 15| 15.3±0.75 | 13.9±0.45 | 19.3±0.81 | 10.4±0.60 | 9.3±0.81 |
|                  | 20| 17.9±0.60 | 15.4±0.60 | 23.4±0.79 | 12±0.79 | 11.4±0.81 |
| Cassia auriculata | 5 | 10.3±0.75 | 11.3±0.75 | 9.4±0.65 | 12.2±0.91 | 8.3±0.70 |
|                  | 10| 12.9±0.45 | 14.0±0.40 | 12.0±0.4 | 14.3±0.60 | 10.3±0.77 |
|                  | 15| 15.1±0.47 | 16.0±0.60 | 13.9±0.45 | 14.5±0.79 | 12.3±0.81 |
|                  | 20| 16.8±0.41 | 18.0±0.55 | 1.6±0.50 | 19.0±0.40 | 14.4±0.65 |

Table 3: Minimum inhibitory concentration of methanol extract of petals of four different Indian medicinal plants

| Medicinal plants | Methanol extract (µl/ml) | Zone of inhibition [in mm diameter]a |
|------------------|--------------------------|-----------------------------------|
| Punica graminum   | 5 | 0.652±0.010 | 0.527±0.009 | 0.572±0.009 | 0.576±0.011 | 0.653±0.009 |
|                  | 10| 0.557±0.011 | 0.447±0.008 | 0.490±0.006 | 0.471±0.011 | 0.538±0.009 |
|                  | 15| 0.463±0.010 | 0.336±0.008 | 0.355±0.010 | 0.352±0.011 | 0.456±0.009 |
|                  | 20| 0.373±0.011 | 0.242±0.009 | 0.242±0.011 | 0.275±0.005 | 0.340±0.011 |
| Hibiscus rosa-sinensis | 5 | 0.655±0.012 | 0.715±0.008 | 0.628±0.008 | 0.759±0.011 | 0.666±0.011 |
|                  | 10| 0.510±0.011 | 0.621±0.010 | 0.521±0.010 | 0.533±0.009 | 0.567±0.007 |
|                  | 15| 0.443±0.008 | 0.491±0.011 | 0.434±0.009 | 0.421±0.010 | 0.448±0.007 |
|                  | 20| 0.315±0.015 | 0.385±0.005 | 0.343±0.009 | 0.362±0.010 | 0.354±0.008 |
| Moringa oleifera  | 5 | 0.642±0.010 | 0.666±0.012 | 0.759±0.011 | 0.699±0.011 | 0.513±0.009 |
|                  | 10| 0.56±0.006 | 0.502±0.010 | 0.652±0.010 | 0.495±0.011 | 0.426±0.008 |
|                  | 15| 0.523±0.010 | 0.416±0.009 | 0.526±0.011 | 0.403±0.010 | 0.305±0.011 |
|                  | 20| 0.472±0.010 | 0.313±0.010 | 0.432±0.010 | 0.272±0.010 | 0.222±0.010 |
| Cassia auriculata | 5 | 0.312±0.010 | 0.180±0.011 | 0.206±0.010 | 0.168±0.012 | 0.162±0.010 |
|                  | 10| 0.38±0.008 | 0.262±0.010 | 0.357±0.010 | 0.257±0.011 | 0.353±0.001 |
|                  | 15| 0.46±0.008 | 0.491±0.01 | 0.505±0.008 | 0.57±0.011 | 0.424±0.008 |
|                  | 20| 0.23±0.009 | 0.25±0.011 | 0.27±0.008 | 0.37±0.008 | 0.230±0.009 |

The minimal inhibitory concentration was determined by measuring the turbidity of the bacterial culture that is the mean±SD of three replicates.
of the study supports the traditional application of the plants and suggests that the methanol extract of four medicinal plants possess phytocompounds and can be used as antibacterial agents in novel drugs for the treatment of bacterial diseases. The use of these plants in traditional medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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REFERENCES
1. Alabe PI, Irobi ON. Antibacterial activities of crude extract of Acalypha wilkesiana from Manna Nigeria. J Ethnopharmacol 1993;39:235-6.
2. Gurinder JK, Daljit SA. Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. BMC Complement Alter Med 2009;9:30.
3. Franklin TJ, Snow CA. Biochemistry of Antimicrobial Action. 4th ed. New York: International Book Distributors; 1987. p. 867-8.
4. Prescott L, Harley J, Klein DA. Microbiology. 5th ed. London: McGraw-Hill; 2002. p. 820-950.
5. van der Watt E, Pretorius JC. Purification and identification of active antibacterial components in Carroporus edulis L. J Ethnopharmacol 2001;76(1):87-91.
6. Sharif MD, Banik GR. Status and utilization of medicinal plants in Rangamati of Bangladesh. Res J Agric Biol Sci 2006;2(6):268-73.
7. Doughhari JH, El-mahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of Carica papaya L. Arch Microbiol Res 2007;1(3):37-41.
8. Maganha EG, Da costa HR, Pegas HO, De Paula RA, Saffi J. Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. Food Chem 2009;118(1):1-10.
9. Gauthaman KK, Saleem MT, Thansidas PT, Prabhu VV, Krishnamoorthy KK, Devaraj NS, et al. Cardioprotective effect of the Hibiscus rosa sinensis flowers in an oxidative stress model of myocardial ischemic reperfusion injury in rat. BMC Complement Altern Med 2006;6:32.
10. Nade VS, Kanhere SV, Kawale LA, Yadav AV. Cognitive enhancing and antioxidant activity of ethyl acetate soluble fraction of the methanol extract of Hibiscus rosa sinensis in scopalamine-induced amnesia. Indian J Pharmacol 2011;43(2):137-42.
11. Kirthikar KR, Basu BD. Indian Medicinal Plants. 2nd ed., Vol. III. Dehradun: International Book Distributors; 1987. p. 867-8.
12. Gupta S, Sharma SB, Prabhu KM, Bansal SK. Antibacterial activity of Carica papaya L. Int J Pharm Pharm Sci 2014;6(9):52-4.
13. Das S, Sarma G, Barman S. Hepatoprotective activity of aqueous extract of fruit pulp of Cassia auriculata and antioxidant activity of ethyl acetate soluble fraction of the methanol extract of Hibiscus rosa sinensis in scopalamine-induced amnesia. Indian J Pharmacol 2011;43(2):137-42.
14. Deore SL, Khadabadi SS, Kamdi KS, Ingle VP, Kawalkar NG, Sawarkar PS, et al. In vitro anthelmintic activity of Cassia tora. J Ethnopharmacol 2009;1(2):177-9.
15. Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of Cassia fistula L.: An ethnomedicinal plant. J Ethnopharmacol 2007;112(3):590-4.
16. Kaur G, Alam MS, Jabbar Z, Javed K, Athar M. Evaluation of its safety evaluation. Indian J Biochem Biophys 2009;46(3):340-8.
17. Kirthikar KR, Basu BD. Medicinal Plants of India. Vol. I. New Delhi: CSIR; 1965. p. 518-20.
18. Samy RP, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharmacol 2000;69(1):63-71.
19. Parkas SK. Effects of Herbal Extracts Towards Microbiidal Activity Against Pathogenic Escherichia coli in Poultry. Int J Poult Sci 2006;5(3):259-61.
20. Singh K, Tafida GM. Antibacterial activity of Moringa oleifera (LAM) leaves extract against some selected bacteria. Int J Pharm Pharm Sci 2014;6(9):52-4.
21. Pal SK, Mukherjee PK, Saha BP. Studies on the antibacterial activity of Moringa oleifera leaf extract on gastric ulcer models in rats. Phytother Res 1995;9(6):463-5.
22. Cáceres A, Saravia A, Rizzo S, Zabala L, De Leon E, Navé F. Pharmacologic properties of Moringa oleifera. 2: Screening...
for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol 1992;36(3):233-7.
23. Morton JF. The horse radish tree: M. Pterigosperma (Moringaceae). A boon to arid lands. Econ Bot 1991;45(3):318-33.
24. Dahot MU. Vitamin contents of flowers and seeds of Moringa oleifera. Pak J Biochem 1988;21:1-24.
25. Mehta K, Balaraman R, Amin AH, Bafna PA, Gulati OD. Effect of fruits of Moringa oleifera on the lipid profile of normal and hypercholesterolaemic rabbits. J Ethnopharmacol 2003;86(2-3):191-5.
26. Ruckmani K, Kavimani S, Anandan R, Jaykar B. Effect of Moringa oleifera Lam on paracetamol – Induced hepatotoxicity. Indian J Pharm Sci 1998;60(1):33-5.
27. Nickon F, Saad ZA, Rehman MH, Haque ME. In vitro antimicrobial activity of the compound isolated from chloroform extract of Moringa oleifera Lam. Pak J Biol Sci 2003;6(22):1888-90.
28. Naqvi SA, Khan MS, Vohora SB. Antibacterial, antifungal, and anthelmintic investigations of Indian medicinal plants. Fitoterapia 1991;62(3):221-8.
29. Lad V, Frawley D. The Yoga of Herbs. Santa Fe, NM: Lotus Press; 1986. p. 135-6.
30. Cáceres A, Girón LM, Alvarado SR, Torres MF. Screening of antimicrobial activity of plants popularly used in Guatemala for the treatment of dermatomucosal diseases. J Ethnopharmacol 1987;20(3):223-37.
31. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hill; 1998. p. 279.
32. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol 1988;23(2-3):127-49.
33. Akinpelu DA, Kolawole DO. Phytochemical and antimicrobial activity of leaf extract of Piliostigma thonningii (Schum.). Sci Focus J 2004;7:64-70.
34. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. New Delhi, India: Springer Pvt. Ltd.; 1998.
35. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12(4):564-82.
36. Swathi S. Phytochemical screening and TLC studies of Moringa oleifera: Their antibacterial and antioxidant activities. Int J Curr Pharm Res 2016;8(1):46-9.
37. de Boer HJ, Kool A, Broberg A, Mziray WR, Hedberg I, Levenfors JJ. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. J Ethnopharmacol 2005;96(3):461-9.
38. Singh DV, Gupta MM, Kumar TR, Saikia D, Kharuna SP. Antibacterial principles from the bark of Terminalia arjuna. Curr Sci 2008;94(1):27-9.
39. Nair R, Kalariya T, Sumitra C. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol Sci 2005;29:41-7.