International Journal of Microbial Resource Technology

Available at http://ijmrt.inpressco.com

ISSN 2278 – 3822

Original Research Article

Antimicrobial potential of important medicinal plants of India

Poonam Khatri*, Pragati Jamdagni, Anil Sindhu and J.S. Rana

Department of Biotechnology
Deenbandhu Chhotu Ram University of Science and Technology
Murthal- 131 039, Sonipat , Haryana, India

*E-mail: poonamkhatri2@gmail.com

Accepted 10 Feb. 2016, Available online 1 March. 2016, **Vol.3, No. 1 (2016)**

Abstract

As a large part of population in the world is dependent on herbal medicines, especially in developing countries, study of medicinal plants becomes very crucial. Today in this modern era, multiple drug resistance has developed against many microbial infections because of arbitrary use of commercial synthetic drugs for the treatment of infectious diseases. This condition has forced researchers to find out new strategies to control microbial infections. The present study makes use of five medicinal plants, namely Acorus calamus, Chlorophytum borivilianum, Elettaria cardamomum, Nyctanthes arbortristis and Terminalia bellirica to determine their antibacterial potential in the terms of zone of inhibition of bacterial growth. Leaf and fruit extracts were prepared using water and organic solvents, such as methanol, chloroform, ethyl acetate and petroleum ether. The study, therefore, focuses on the antibacterial potential of five extracts from five plants against seven bacteria, viz. Escherichia coli, Salmonella typhi, Proteus mirabilis, Staphylococcus aureus, Acinetobacter baumanii, Pseudomonas aeruginosa and Streptococcus pyogenes. All the plant extracts have shown significant antibacterial activity against gram positive and gram negative bacteria, apart from E. cardamomum (Elaichi). Elaichi extracts showed no antibacterial activity against any of the tested bacteria. The largest zone of inhibition (22mm) was obtained with methanolic extract of T. bellirica and N. arbortristis against E. coli and S. typhi respectively. This study helps in identifying the plants with different antibacterial potential which could be further exploited for isolation and characterization of the novel phytochemicals for the treatment of infectious diseases esp. in the gleam of the emergence of drug resistant microorganisms and the demand to produce more effective antimicrobial agents.

Keywords*: Antimicrobial, Acorus, Nyctanthes, Elettaria, Terminalia, Chlorophytum*

1. Introduction

Since beginning of civilization, plants and their products are used as medicines. The medicinal use of plants has even been mentioned in 'Rigveda' between 4500- 1600B.C (Rastogi and Mehrotra, 2002). The interest in the study of medicinal plants as natural products is increasing day by day in diverse parts of the world (Gazzaneo *et al*., 2005). These plants contain active chemical compounds with high antioxidant properties, which help in prevention of various diseases (Hakkim *et al*., 2008). Generally, bacteria that are used as therapeutic or remedial agents have the genetic ability to transmit and develop resistance to drugs (Cohen, 1992) therefore it has now become crucial to find alternative treatments for bacterial infections (Joao *et al*., 2004). The antibiotic resistant pathogenic bacteria have been recognized as a serious problem to humans and animals by the World Organization for Animal Health (OIE), the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). Due to the high frequency of new resistant phenotypes, the only solution is the development of bacterial antibiotic resistance (OIE Terrestrial Manual, 2008). Montelli and Levy documented a high incidence of resistant microorganisms in clinical microbiology in Brazil from 1980 to 1990 (Montelli and Levy, 1991). The plant based medicines are more effective and cheaper

alternative as compared to synthesized compounds in the treatment of diseases (Rojas *et al*., 1992).

Acorus calamus (Family: Araceae) is very popular in Indian medicines for centuries. It is a perennial, smelly and semi- aquatic plant found in both temperate and sub temperate zones (Mehrotra e*t al*., 2003).The roots, rhizomes and essential oil obtained from these plant parts possess several important biological activities including antibacterial (McGraw *et al*., 2002; Phongpaichit *et al*., 2005), antifungal (Lee *et al*., 2004; Lee *et al*., 2005), immunosuppressive and anticellular (Mehrotra *et al*., 2003). Aromatic oils derived by alcoholic extraction of the rhizome are used in the oenological industries and pharmaceutical industries (Bertea *et al*., 2005). Earlier studies on chemical investigations have shown the presence of various compounds such as caryophyllene, asarone (alpha and beta), isoasarone, methyl isoeugenol and safrol in rhizome and roots (Namba, 1993; Wang *et al*., 1998).

Nyctanthes arbortristis (Family: Oleaceae) also known as Harsingar or Night Jasmine. It is a small tree or wild hardy large shrub and decoction of its leaves are extensively used in Ayurvedic medicines for the treatment of arthritis, sciatica, fevers and as laxative (Prasad *et al*., 2014). Tranquilizing, purgative and antihistaminic activities were also shown by the leaves extract (Saxena *et al*., 2002). Roots are used for emaciation and stem bark of this plant is used for ulcer of palate, dysentery and also cure internal injuries (Ray *et al*., 1980). It has been stated that Nyctanthes possesses antipyretic, anti-inflammatory, analgesic, ulcerogenic, antiviral, antifungal, antileishmanial, antibacterial properties (Saxena *et al*., 1987; Puri *et al*., 1994; Khatune *et al*., 2001). Stem bark extract of Harsingar contain compounds like Nyctanthic acid, b-amyrin, Arbotristoside –A1, nyctoside-A2, oleonic acid (Balandrin *et al*., 1985).

Elettaria cardamomum (Zingiberaceae) commonly known as queen of spices (for the versatile use in cooking practices) is a perennial shrub with fleshy, thick and lateral roots which can grow to a height of eight feet (Kapoor, 2000). Cardamom oil can be used in food, liquor, perfumery while in field of medicine, it has proven as antiseptic, stimulant, stomachic, aromatic and anti- spasmodic. Cardamom is used in the preparation of 'Gahwa' a strong cardamom coffee concoction in most part of the world especially the Near East and Saudi Arabia (Korikontimath *et al*., 1999).It also works as a laxative and soothes dyspepsia and nausea even during pregnancy. Cardamom used as massage oil or diluted in the bath during aromatherapy, helps in the digestive system and also used as a general tonic (Nirmala *et al*., 2000).

Chlorophytum borivilianum (Liliaceae) is an endangered perennial herbaceous medicinal plant and commonly known as Safed Musli. It is an extensively grown species for commercial purposes. 17 out of 256 species of safed musli are known to occur in India. *Chlorophytum borivilianum* is used for oligospermia, male impotency treatment and for delaying the ageing process. It is also known to treat various gynecological disorders, arthritic conditions, diabetes mellitus and also for increasing lactation (Purohit *et al*., 2003). It also contains carbohydrates (41%), root fibres (4%), proteins (8-9%), saponins (2-17%). Saponins and alkaloids are the primary source of substantial medicinal properties (Pullaiah *et al*., 2002).

Terminalia bellirica belongs to the family Combretaceae and locally known as Bahera in India. *T.bellirica* has been used in the Ayurveda (an ancient medical science which was developed in India thousands of years ago). It is grown extensively upto 1200 meters in elevation throughout the Indian subcontinent, South East Asia and Sri Lanka (Amrithpal *et al*., 2011; Nadkarni *et al*., 2002). In India, Tribal people for treatment of fever, cough, diarrhea, oral thrush and skin diseases uses Bahera regularly as traditional medicine. Various chemical substances of gallic acid , ethle gallate, galloyl glucose and beta sitosterol, a new triterpene have been isolated from fruits of T.bellerica. Fruit extract of Bahera have shown fall in blood pressure of rats at a concentration of 70-mg/kg-body weight (Rastogi *et al*., 1999).

2. Materials and methods

2.1 Plants and microorganisms used

The medicinal plants used for the experiment were *Acorus calamus, Nyctanthes arbortristis, Elettaria cardamomum, Chlorophytum borivilianum, Terminalia bellirica*. The plants were collected from Ch. Devi Lal Herbal Nature Park - Chuharpur, Yamunanagar, Haryana and maintained in the University nursery.

The bacterial cultures used in this study were *Escherichia coli* (MTCC 448), *Staphylococcus aureus* (MTCC 3160), *Acinetobacter baumanii* (MTCC 1920), *Pseudomonas aeruginosa* (MTCC 647), *Salmonella typhi* (MTCC 733), *Proteus mirabilis* (MTCC 9493) and *Streptococcus pyogenes* (MTCC 442). All the pure cultures were procured in lyophilized form from Microbial Type Culture

Collection, IMTECH, Chandigarh.

2.2 Preparation of the Extract

The leaves and seeds were collected, weighed (2g each), washed and mashed in 10 ml of ethyl extract, chloroform, petroleum ether, methanol and aqueous separately. The mixtures were kept for 6hrs at room temperature and then filtered through sterile Whatman filter paper No. 1. The filtrates were centrifuged at 5000 rpm for 5 min and the supernatants were collected in the beaker. The solvents were evaporated to dryness and the residue was stored at 4^0 C in a refrigerator. The residue was dissolved in 2 ml DMSO at the time of antibacterial assays.

2.3 Culturing of Microorganisms

The bacterial strains were maintained on nutrient agar slants at 4^0 C. A loopful of each gram positive and gram negative bacterial strain was inoculated into 50 ml of sterile nutrient broth in 100 ml conical flask. The flasks were incubated on a rotary shaker for 24 hrs to activate the strain and Luria Bertani Agar medium was used as bacterial culture medium in the antibacterial assays.

2.4 Antibacterial Activity

The antibacterial activity of all the 5 medicinal plants from different organic and aqueous extracts (Chloroform, Methanol, Ethyl acetate, Petroleum ether and Aqueous) were evaluated by agar well diffusion method (Bauer *et al*., 1966).24 hr old broth cultures of the bacteria were used for the antibacterial assay. A sterile cotton swab was dipped into the bacterial suspension and then streaked evenly over the entire surface of sterile Luria Bertani Agar medium in order to get uniform inoculum. Wells were punched on the seeded plates by the use of sterile borer of 7 mm. The plates were allowed to dry for 2-3 mins. Ethyl acetate, chloroform, petroleum ether, methanol and aqueous extracts of 250 µl each were dispensed into each well using sterile micropipette. 2% tetracycline was used as positive control and DMSO was used as negative control. The plates were incubated overnight at 37^0C and antibacterial activity was determined by measuring the diameter of zone of inhibition (mm).

3. Results

Antibacterial potential of leaves and fruit (organic and aqueous) extracts were assessed in terms of zone of inhibition (mm) of bacterial growth. 250 microlitre of each extract was used for antimicrobial screening.

Fig 2 shows that *A.calamus* shows antibacterial activity against *E. coli and S. aureus* and was found to be resistant against *A. baumanii, P. aeruginosa, S.typhi, P. mirabilis* and *S. pyogenes*, by all the extracts. Chloroform and methanol extract showed maximum zone of inhibition against *E.coli* (21mm). Petroleum ether and Aqueous extract was found to give positive results with only *E.coli.*

N. arbortristis (Fig 3) displayed significant antibacterial activity against all Gram negative and Gram positive bacteria except *S. aureus* by water extract. The largest zone of inhibition recorded was 22mm with methanol extract against *S.typhi* and lowest zone was 14mm with Aqueous extract against *P. aeruginosa, P. mirabilis* and *S. pyogenes* and petroleum ether extract against *S.aureus* and *P.mirabilis.*

Fig 4 stated that *C.borivilianum* has also shown considerable antibacterial activity (21mm) against *S. typhi* and *S. aureus* by chloroform and ethyl acetate respectively. Aqueous and petroleum ether shows less effective results against all Gram positive and Gram negative bacteria. Chloroform and ethyl acetate extract showed almost similar results against all bacteria.

The largest zone of inhibition that is 22mm was obtained with methanol extract of *T. bellirica* (Figure-5). Methanol extract shows remarkable results in comparision to other extracts. *T. bellirica* was found to be resistant against *S. aureus, A. baumanii, S. typhi and S. pyogenes* by Aqueous extract. *E. cardamomum* shows no antibacterial activity against Gram negative and Gram positive bacteria.

4. Discussion

Plant essential extracts and oils have been used worldwide for thousands of years, in pharmaceuticals, food preservation, substitutive medicine and natural therapies. It is crucial to investigate those plants scientifically, which are medically important to improve the quality of healthcare. Plant extracts are promising sources of unique antimicrobial compounds against bacterial pathogens. The antibacterial activity of many plant extracts has already been reviewed and classified as weak, medium and strong (Zaika, 1998).

The medicinal plants like *A. calamus, T. bellirica, N. arbortritis, C. borivilianum and E. cardamomum* are

being used traditionally for the treatment of arthritis, sciatica, fevers, cough, oral thrush, diarrhea, skin diseases, antiseptics expectorant and stomatitis. The antibacterial activity has been accredited to the presence of some active compounds in the extracts.

The antibacterial activity by Gram positive or Gram negative bacteria, it would generally be expected that a much greater number would be active against Gram positive than Gram negative bacteria (McCutcheon et al. 1992). Whereas, in our study both Gram negative and Gram positive bacteria are found to be active against organic and aqueous extracts. The differences in the antibacterial effects of plant extracts may be due to the differences in their phyto-chemical compositions (Nair et al. 2005).

Resistant strains have been developed due to intensive use of antibiotics, which create a problem in treatment of infectious diseases (Sydney *et al*., 1980). Furthermore antibiotics contain lots of side effects (Cunha, 2001). Therefore, there are some advantages of using antimicrobial compounds of medicinal plants with less side effects, less expensive, better patient tolerance and acceptance due to long history of use and also being renewable source of nature (Vermani and Garg, 2002). Hence, these findings support the traditional knowledge of local users and it's a primary, scientific, authentication for the use of these plants for antibacterial activity to promote proper conservation and viable use of such plant resources.

Combining the traditional knowledge with scientific findings should augment awareness of local community. In conclusion, the present study has shown the traditional usage of the studied plants and also suggests that some of the plant extracts consists of compounds with antimicrobial properties. Furthermore, this antibacterial study of the plant extracts demonstrated that folk or traditional medicine can be as valuable as modern medicine against pathogenic microorganisms. The tremendous use of traditional medicine suggests that they are more economic and safe alternative to treat infectious disease.

Acknowledgements

The author PJ is thankful to Assured Opportunity for Research Careers (AORC), Department of Science and Technology (DST), Ministry of Science and Technology, New Delhi (INDIA) for awarding INSPIRE fellowship.

References

1. Balandrin, M.F., Kjocke, A.J. and Wurtele. (1985). Natural plant chemicals: sources of industrial and medicinal materials. Science., 228: 1154-1160.
2. Bauer, A.W., Kirby, W.M.M. and Sherris, J.C. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
3. Bertea, C.M., Azzolin, C.M.M., Bossi, S., Doglia, G. and Maffei, M.E. (2005). Identification of an EcoR1 restriction site for rapid and precise determination of Basarone-free Acorus calamuscrtotypes. Phytochemistry., 66: 507-514.
4. Cohen, M.L. (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. Science., 257: 1050-1055.
5. Cunha, B.A. (2001). Antibiotics side effects. Med. Clin. North Am., 85: 149-185.
6. GazzaneoIr, De lucena, P.R.F and Paulino de Albu, Q.U. (2005). In vitro antioxidant activities of ocimum species: ocimum basilicum and ocimum sanctum. J. Ethnobiol. Ethnomed., Pp1- 9.
7. Hakkim, F.L., Arivazhagan, G. and Boopathy, R. (2008). Antioxidant property of selected Ocimum species and their secondary metabolite content. Journal of Medicinal Plants Research., Vol. 2(9), pp. 250-257.
8. João, P. C., Tome´, J., Neves, M. G.P.M.S., Tome´, A.C., Cavaleiro, J. A. S., Soncin, M., Magaraggia, M., Ferro, S. and Jori, G. (2004). Synthesis and Antibacterial Activity of New Poly-S - lysine- Porphyrin Conjugates. J. Med. Chem., 47: 6649- 6652.
9. Kapoor LD.(Hand book of ayurvedic medicinal plants). CRC Press, Boca Raton, FL.2000.
10. Khatune, N.A., Mosaddik, M.A. and Haque, M.E. (2001). Antibacterial activity and cytotoxicity of Nyctanthes arbortristis flowers. Fitoterapia., 72: 412-414.
11. Korikontimath, V.S., Mulge, R. and Zachariah, J.T. (1999). Variations in essential oil constituents in high yielding selections of cardamom. J. Plantation Craps., 27: 230-232.
12. Lee, H.S. (2005). Pesticidal constituents derived from Piperaceae fruits. Agric Chem Biotechnol., 48: 65-74.
13. Lee, J.Y., Lee, J.Y., Yun, B.S. and Hwang, B.K. (2004). Antifungal activity

of B-asarone from rhizomes of Acorusgramineus. J Agric Food Chem., 52: 776-780.
14. McCutcheon, A.R., Ellis, S.M., Hancock, R.E.W. and Towers, G.H.N. (1992). Antibiotic screening of medicinal plants of the British Columbian native peoples. J. Ethnopharmacol., 37:213-223.
15. McGaw, L.J., Jager, A.K. and Van Staden, J. (2002). Isolation of B-asarone, an antibacterial and anthelmintic compound, from Acorus calamus in South Africa. South African J Bot., 68: 31-35.
16. Mehrotra, S., Mishra, K.P., Maurya, R., Srimal, R.C., Yadav, V.S., Pandey, R. and Singh, V.K. (2003). Anticellular and Immunosuppressive Properties of Ethanolic extract of Acoruscalamus rhizome. International Immunopharmacol., 3: 53-61.
17. Montelli, A.C. and Levy, C.E. (1991). Sistema COBA - Aspectosrelativosaos dados dos laboratórios de referência. Rev. Microbiol., 22: 197-205.
18. Nadkarni, K.M. (2002). Indian MeteriaMedica, Published by RamdasBhatkal for Popular rakashan Pvt.Ltd.Mumbai., 01: 202- 1205.
19. Nair, R.T., Kalariya and Chanda, S. (2005). Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol., 29: 41-47.
20. Namba, T. (1993). The encyclopedia of Wakan-Yuku (tranditional Sino Japanese Medicines, with color pictures). Hoikusha. Osaka Japan., 1- 606.
21. Nirmala, M.A. (2000). Studies on the volatile of cardamom (Elettaria cardamomum) J.FoodScience Technology., 37: 406-408.
22. OIE Terrestrial Manual (2008). Chapter 1.1.6. - Laboratory methodologies for bacterial antimicrobial susceptibility testing: 56-65.
23. Phongpaichit, S., Pujenjob, N., Rukachaisirikul, V. and Ongsakul, M. (2005). Antimicrobial activities of the crude methanol extract of Acorus calamus Linn. M J Sci Technol., 27: 517-523.
24. Prasad, M.P. and Shekhar, S. (2014). *In-vitro* phytochemical and antimicrobial activity of *Nyctanthes arbortristis linn* against human pathogens. *Int. J. Pure App. Biosci.*, 2(1), 1-5.
25. publications, Jodhpur. pp 43.
26. Pullaiah, T. (2002). Medicinal Plants of India, Regency Publications, New Delhi, pp 62.
27. Puri, A., Saxena, R., Saxena, R.P., Saxena, K.C., Srivastava, V. and Tandon, J.S. (1994). Immunostimulant activity of Nyctanthes arbor-tristis L. J Ethanopharmacol., 42: 31-37.
28. Purohit, S.S. and Prajapati, N.D. (2003). Agro's Colour Atlas of Medicinal Plants, Agrobios.
29. Rastogi, P. and Mehrotra, B.N. (1999). Compendium of Indian Medicinal Plants, Drug research perspective, CDRI Lucknow and NISCOM, New Delhi., 2: 1-859.
30. Rastogi, R.P. and Mehrotra, B.N., (2002). Glossary of Indian Medicinal Plants. National Institute of Science Communication, New Delhi, India.
31. Ray, P. and Gupta, H.N. (1980). Medicinal plants and plant products and their uses. In Ray P, Gupta, HN, editors. CharakSamitha. p.71.
32. Rojas, A.L., Hernandez, M.R. and Mata, R. (1992). Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J. Ethnopharmacol., 35: 127- 149.
33. Saroya, A.S. (2011). Herbalism phytochemistry and Ethanopharmacology, Science Publishers. 357-361.
34. Saxena, R.S., Gupta, B. and Lata, S. (2002). Transquilizing, antihistaminic, and purgative activity of Nyctanthes arbor-tristis leaf extract. J Ethanopharmacol., 81: 321-325.
35. Saxena, R.S., Gupta, B., Saxena, K.K., Srivastava, V.K. and Prasad, D.N. (1987). Analgesic,antipyretic and ulcerogenic activity of Nyctanthes arbor –tristis leaf extract. J Ethanopharmacol., 19: 93-2000.
36. Sydney, S., Lacy, R.W. and Bakhtiar, M. (1980). In : The Betalactam antibiotics Penicillin and Cephalosporin in perspective, Hodder and stongton, London. p. 224.
37. Vermani, K. and Garg, S. (2002). Herbal medicine for sexually transmitted diseases and AIDS. J. Ethnopharmacol., 80: 49-66.
38. Wang, H.Z., Cheng, Y.G. and Fan, C.S. (1998). Review of studies on chemical constituents and pharmacology of genus Acorus. Acta Bot Yunnanica., 5: 96-100.

39. Zaika, L.L. (1988). Spices and herbs: their antimicrobial activity and its determination. J. Food Saf., 23: 97-118.

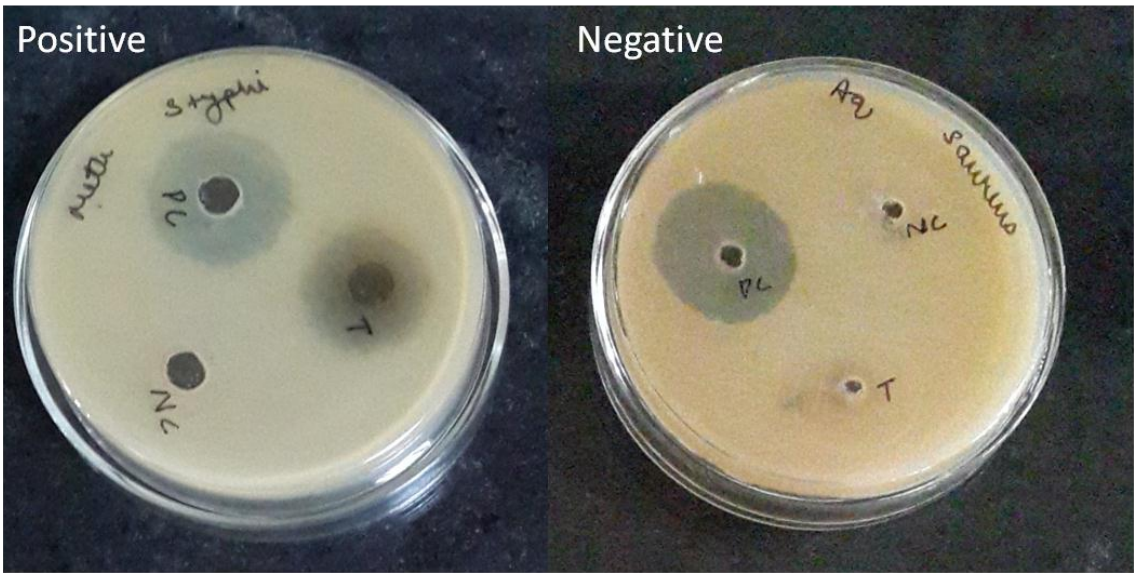

Fig. 1: Representative results for positive and negative antibacterial activity

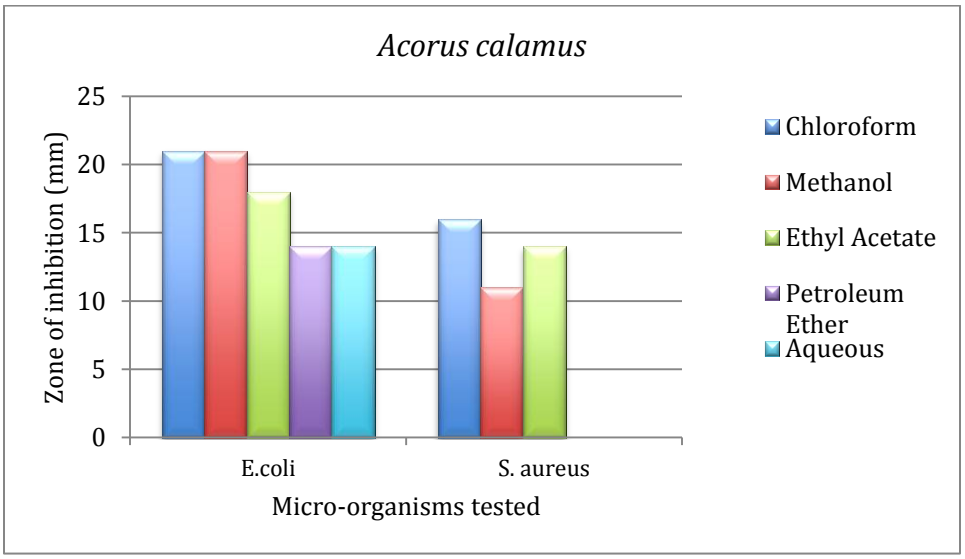

Fig. 2: Graphical representation of antibacterial activity of *Acorus calamus* (Leaves Extract).

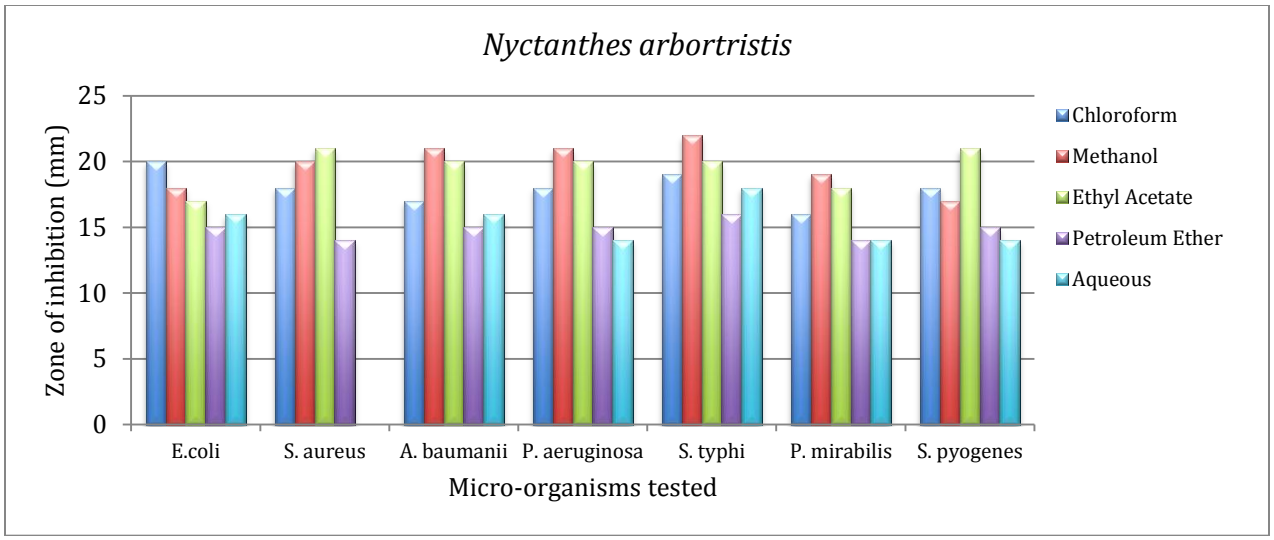

Fig. 3: Graphical representation of antibacterial activity of *Nyctanthes arbortristis* (Leaves Extract).

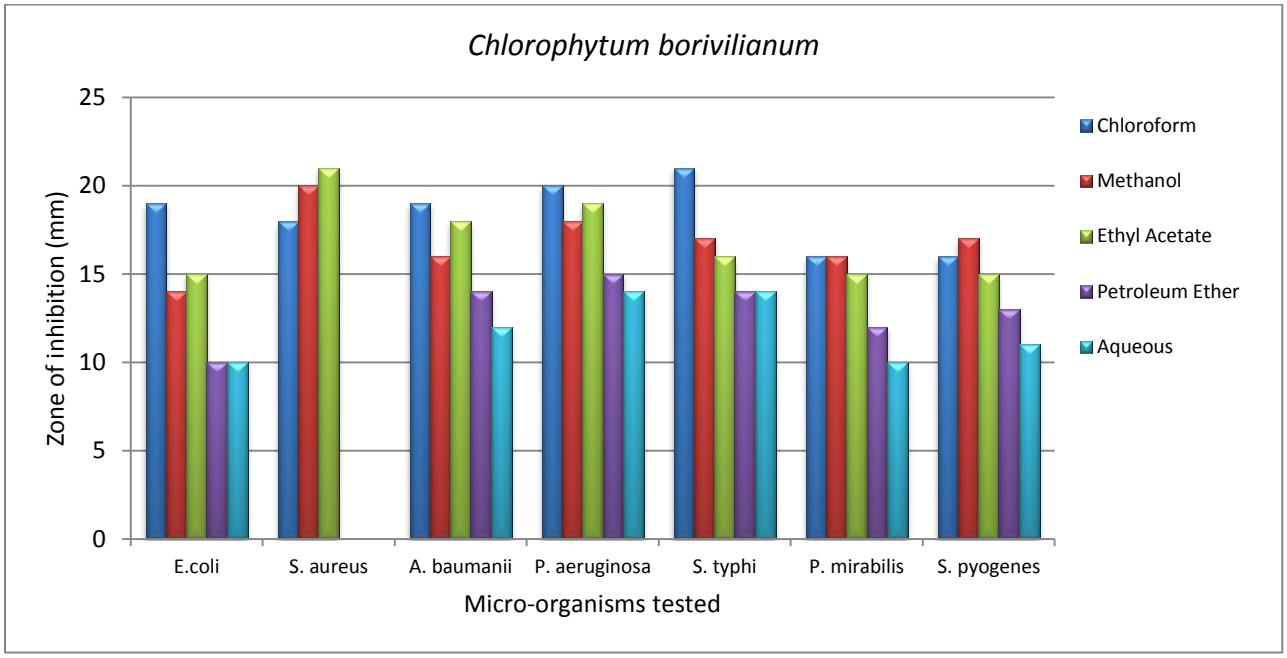

Fig. 4: Graphical representation of antibacterial activity of *Chlorophytum borivilianum* (Leaves Extract).

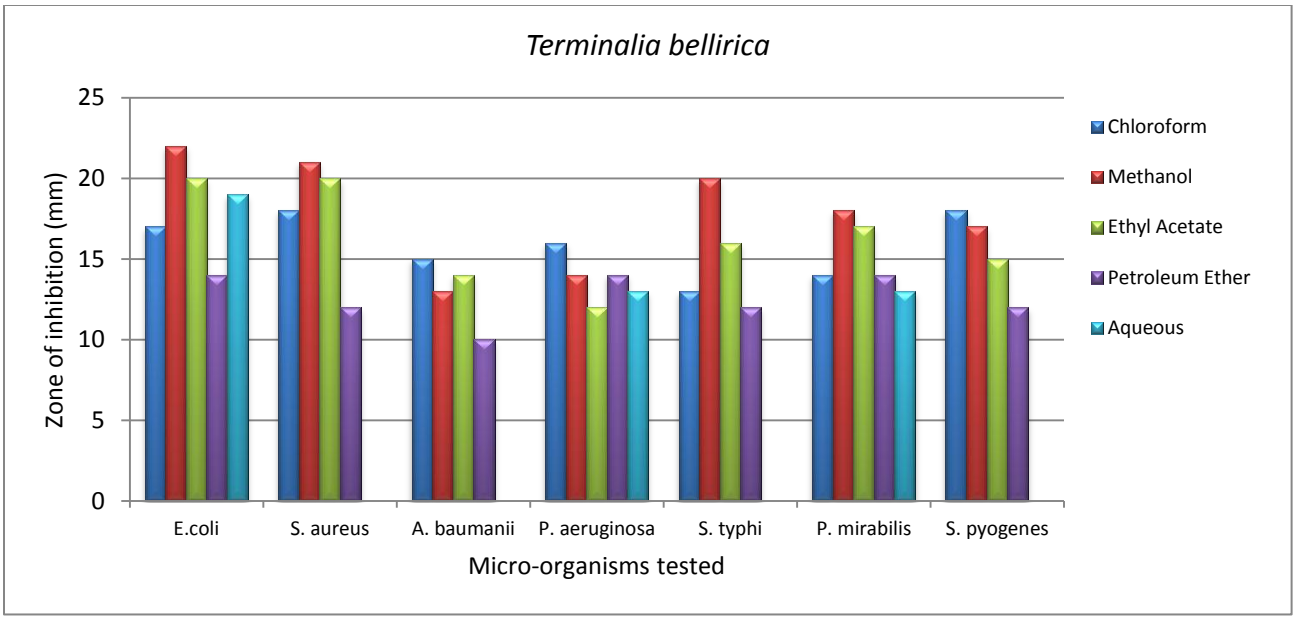

Fig. 5: Graphical representation of antibacterial activity of *Terminalia bellirica* (Fruit Extract).