INNOVARE JOURNAL OF LIFE SCIENCE

Vol 5, Issue 3, 2017

ISSN - 2321-550X

Research Article

COMPARATIVE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF BENZENE EXTRACT OF THREE MEDICINAL PLANTS

SASIKALA RP, SANGEETHA K, MEENA KS*

Department of Chemistry, Bioinformatics Infrastructure Facility Centre of DBT Queen Marys College (Autonomous) Chennai, Tamil Nadu, India. Email: qmcbifc@gmail.com

Received: 01 March 2017, Revised and Accepted: 25 March 2017

ABSTRACT

Objective: This study was carried out with an objective to investigate the antibacterial and antifungal potential of three Indian medicinal plants.

Methods: Antimicrobial activity of benzene extracts of three plants namely *Abutilon indicum, Plectranthus amboinicus,* and *Aegle marmelos* were determined using agar disc diffusion method at different concentration from 5 to 30 µg/µl against two Gram-positive *Staphylococcus aureus, Enterococcus faecalis* and two fungal strains *Aspergillus niger, Aspergillus fumigatus* and compared with standard drugs norfloxacin and fluconazole, respectively.

Results: Benzene extract of fruits from *A. indicum* inhibited *S. aureus, E. faecalis* at 30 µg/µl, and leaves of *P. amboinicus* showed considerable inhibiting activity against the *A. niger, A. fumigatus* at 30 µg/µl.

Conclusion: Hence, these plants can be further used to determine the bioactive natural products that may provide as leads in the development of new drugs.

Keywords: *Abutilon indicum, Plectranthus amboinicus, Aegle marmelos*, Antimicrobial.

INTRODUCTION

The medicinal plants of the Indian traditional system of medicine possess various pharmacological properties and possess various active constituents such as carbohydrates, steroids, alkaloids, flavonoids, glycosides, starch, tannins, and phenolic compounds. *Abutilon indicum* belongs to the family of *Malvaceae*, is a medicinal plant, commonly known as Thuthi/Atibala, and is an erect, woody, shrubby plant widely distributed in the tropical countries [1-4]. *A. indicum* fruit is used to treat piles, gonorrhea, and cough. Fruit decoction mixed with ammonium chloride is given orally with water to treat hemorrhagic septicemia [5-8]. The main phytochemical constituents of the whole plant are carbohydrates, steroids, glycosides, flavonoids, tannins, and phenolic compounds [9]. However, few reports are available with respect to the pharmacological properties of the plant. So far, substantial studies on the antimicrobial activities of the various extracts of leaves, stem, and root have been performed. However, the fruit extract has not been explored for the antimicrobial activities [10-15].

Aegle marmelos belongs to the family of *Rutaceae* that has been widely used in native systems of Indian medicine due to its various medicinal properties [16]. Leaves are useful in jaundice and in the treatment of wounds. The extract of leaves is beneficial in the treatment of leukorrhea, conjunctivitis, and deafness. *A. marmelos* leaves contain phytoconstituents such as skimmianine, aegeline, lupeol, cineol, citral, citronella, cuminaldehyde, eugenol, and marmesinine [17]. Extensive studies on the antimicrobial activities of the various extracts of leaves, stem, and root of *A. marmelos* have been performed whereas the leaves of benzene extract have not been explored for the antimicrobial activities [18-22].

Plectranthus amboinicus belongs to the family of *Lamiaceae* which is grown as a household herb in Tamil Nadu. This herb is native to East Indies and is widely cultivated in Africa and almost all tropical countries and is distributed throughout India. Leaves roots and stems are used as carminative, digestive, stomachic, anthelmintic, expectorant, diuretic, otalgia, anorexia, diarrhea, and cholera, especially in children, convulsions epilepsy, chronic asthma, bronchitis, renal and vesical calculi, and malarial fever. Previous pharmacological studies showed that *P. amboinicus* possesses antiepileptic, antioxidant, anti-inflammatory, leishmanial, and antimicrobial properties of the different extracts of the leaves. Therefore, it is important to access the local *P. amboinicus* to screen the potential biological activity, especially antimicrobial properties and volatile components of the plant [23-28].

The survey of the literature reveals that the leaves of *P. amboinicus* are found to be used traditionally for antimicrobial activity. However, the antimicrobial activity of benzene extract leaves of *P. amboinicus* has not been investigated; hence the present study was undertaken to explore the antimicrobial activity of benzene extract.

Thus, this study was designed to explore the antimicrobial activity of benzene extract of three medicinal plants namely *A. indicum, A. marmelos,* and *P. amboinicus.*

METHODS

Plant material

Fruits of *A. indicum*, leaves of *A. marmelos, P. amboinicus* samples were collected from Chennai using clean polythene bag. The fruit samples were washed in tap water and shade dried for 20 days and authenticated and deposited at Queen Mary's College, Chennai.

Preparation of extract

The shade dried samples were crushed to get 200 g powder sample and successively extracted with 150 ml of benzene (60-75°C) in a Soxhlet extractor for 18-20 hrs. Furthermore, the extract was filtered and excess solvent was evaporated using a rotary evaporator.

Test microorganisms

Clinical isolates of bacterial (*Staphylococcus aureus*, *Enterococcus faecalis*) and fungal species (*Aspergillus niger, Aspergillus fumigatus*)

were procured from the Bacteriology Department, King Institute of Preventive Medicine and Research, Guindy, Chennai. The culture preparations for antibacterial assay were grown on nutrient broth at 37°C for 24 hrs in the test tube in an incubator. The turbidity was measured by adjusting to 0.5 McFarland standards (108 CFU/ml).

Agar well diffusion method

Antibacterial activity was tested in Muller Hinton agar plates in which wells of 6 mm were cut and swabbed with different cultures. Furthermore, the cut wells were filled with different concentration of extracts (5-30 µg/µl), and norfloxacin 10 µg/µl was used as positive control. Antifungal activity was tested at different concentration of extracts (5-30 µg/µl) and positive control (fluconazole 10 µg/µl). All the plates were kept in incubation at 37°C for 24 hrs.

Statistical analysis

The antimicrobial activities were analyzed using one-way analysis of variance between the benzene extract of three plants and standard drug by SPSS version 4. Significant differences were determined by Tukey's range test.

RESULTS AND DISCUSSION

The antimicrobial activity of all the three plant extracts was examined against Gram-positive and Gram-negative bacterial and fungal strains by measuring zone of inhibition. Antifungal activity of the plant extracts was studied on the growth of *A. niger* and *A. fumigatus* presented in Figs. 1 and 2. Minimal reduction in the growth of *A. niger* and *A. fumigatus* was observed with extracts of three medicinal plants when compared to the standard drug fluconazole. The comparative antifungal activities between the three plants revealed that benzene extract of *P. amboinicus* and *A. marmelos* were appreciable than *A. indicum*. However, when the concentration was increased to 30 µg/µl, all the three plants showed only slight difference in their activities against *A. niger*.

It is noteworthy that *A. marmelos* exhibited significant antifungal activity to *A. fumigatus* at 10 µg/µl; however, at the higher concentration of 30 µg/µl, *P. amboinicus* exhibited superior activity than the other two plant extracts. Furthermore, at 30 µg/µl concentration, the antifungal activity against *A. fumigatus* was comparable to that of fluconazole. Thus, the three plants extract showed better activity only when the concentrations were increased.

The antibacterial activity of the extracts was prepared from the dried leaves of *A. marmelos,* dried leaves of *P. amboinicus,* and dried fruits of *A. indicum*. Sweet was carried using agar well diffusion method against Gram-positive microorganisms. The antibacterial activities of the extracts investigated against Gram-positive bacteria are presented in Figs. 3 and 4. Of the three plants tested, *A. indicum* exhibited significant antibacterial activity to both *S. aureus* and *E. faecalis* at 10 µg/µl. Furthermore, the activity of the extract of *A. indicum* was comparable to the standard drug norfloxacin at 10 µg/µl.

The antimicrobial activities between the plant extracts and standard drugs showed significance differences with $p<0.05$-0.01 as tested by ANOVA with post Turkey's range test.

CONCLUSION

The study revealed that benzene extract of *P. amboinicus* exhibited promising antifungal activity, and *A. indicum* showed effective antibacterial activity to the tested organisms. Hence, it reconfirmed the potential of local plants for their usage in the treatment of bacterial diseases, and further studies are needed to determine the chemical

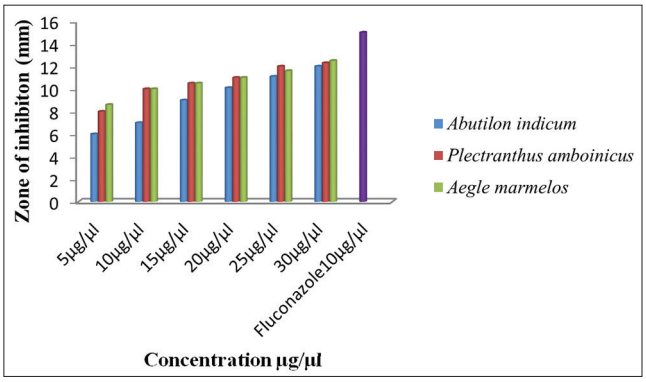

Fig. 1: Antifungal activities of the zone of inhibition against *Aspergillus niger* in mm

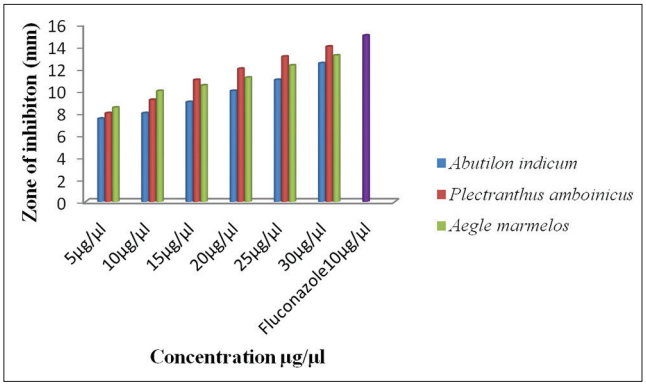

Fig. 2: Antifungal activities of the zone of inhibition against *Aspergillus fumigatus* in mm

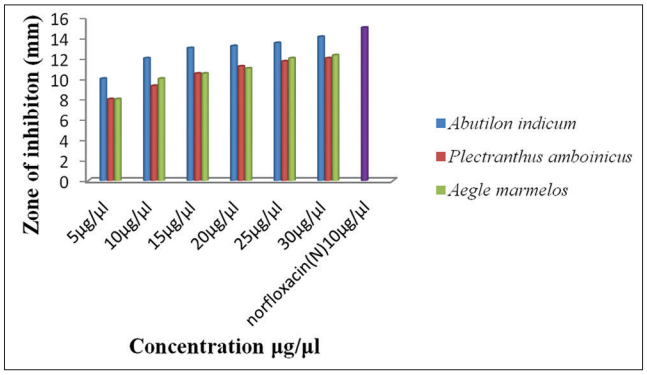

Fig. 3: Antibacterial activities of the zone of inhibition against *Staphylococcus aureus* in mm

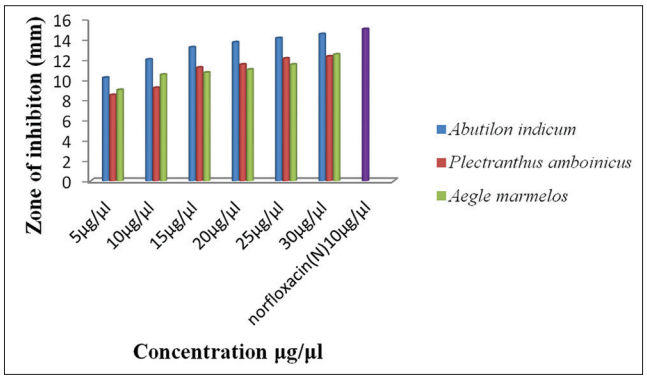

Fig. 4: Antibacterial activities of the zone of inhibition against *Enterococcus faecalis* in mm

identity of the bioactive compounds responsible for the observed antimicrobial activity.

ACKNOWLEDGMENT

This work was supported by Bioinformatics Infrastructure Facility Centre of Department of Biotechnology, Ministry of Science and Technology, Government of India Vide Grant No. BT/BI/25/068/2012/2015.

REFERENCES

1. Ahmad J, Khan I. Antioxidant potential of *Abutilon indicum* (L.) Sw. J Plant Pathol Microbiol 2012;3:124.
2. Arora S, Singh DP, Saini A, Kumar A. Pharmacognostic investigation on roots and leaves extract of *Abutilon indicum* Linn. Int J Res Dev Pharm Life Sci 2013;2:567-73.
3. Rajurkar R, Jain R, Matake N, Aswar P, Khadbadi SS. Antiinflammatory action of *Abutilon indicum* (L.) Sweet leaves by HRBC membrane stablisation. Res J Pharm Technol 2009;2:415-6.
4. Razia M, Rajalakshmi BS, Lavanya K, Karthiga V, Bernala W, Deboral P. GCMS, FTIR and *in vitro* antibacterial activity of *Abutilon indicum*. Int J Biol Pharm Res 2012,4:3959-65.
5. Samy RP, Thwin MM, Gopalakrishnakone P, Ignacimuthu S. Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamil Nadu, India. J Ethnopharmacol 2008;115(2):302-12.
6. Ignacimuthu S, Ayyanar M, Sankarasivaraman K. Ethnobotanical study of medicinal plants used by Paliyar tribals in Theni district of Tamil Nadu, India. Fitoterapia 2008;79(7-8):562-8.
7. Ali ZA. Folk veterinary medicine in Moradabad district (Uttar Pradesh), India. Fitoterapia 1999;70(4):340-7.
8. Mohite MS, Shelar PA, Raje VN, Babar SJ, Sapkal RK. Review on pharmacological properties of *Abutilon indicum*. Asian J Pharm Res 2012;2(4):156-60.
9. Khadabadi SS, Bhajipale NS. A review on some important medicinal plants of *Abutilon* spp. Res J Pharm Biol Chem Sci 2010;1(4):718.
10. Tripathi P, Chauhan NS, Patel JR. Anti-inflammatory activity of *Abutilon indicum* extract. Nat Prod Res 2012;26(17):1659-61.
11. Darsini AI, Shamshad S. Antimicrobial activity and priliminary phytochemical screening of *Abutilon indicum*. Int J Pharm Biol Sci 2015;5(2):6-10.
12. Kaladhar DS, Saranya KS, Vadlapudi V, Yarla NS. Evaluation of anti-inflammatory and anti-proliferative activity of *Abutilon indicum* L. Plant ethanolic leaf extract on lung cancer cell line A549 for system network studies. J Cancer Sci Ther 2014;6:195-201.
13. Kousalya M, Kokila N, Sudha KM, Kalaiselvi B. *In vitro* anti inflammatory activity of *Abutilon indicum* root. Int J Front Sci Technol 2013;1(4):63-73.
14. Gnanavel V, Saral AM. Gc-Ms analysis of petroleum ether and ethanol leaf extracts from *Abrus precatorius* Linn. Int J Pharm Bio Sci 2013;4(3):37-44.
15. Golwala DK, Patel LD, Vaidya SK, Bothara SB, Mani M, Patel P. Anticonvulsant activity of *Abutilon indicum* Leaf. Int J Pharm Pharm Sci 2010;2(1):66-70.
16. Axay RP, Dipak G. *Aegle marmelos* (Linn): A therapeutic boon for human health. Int J Res Ayurveda Pharm 2012;3(2):159-63.
17. Balakumar S, Rajan S, Thirunalasundari T, Jeeva S. Antifungal activity of *Aegle marmelos* (L.) Correa (*Rutaceae*) leaf extract on dermatophytes. Asian Pac J Trop Biomed 2011;1(4):309-12.
18. Mohan V, Molly AG, Eldo AN. *Aegle marmelos* extract as a potent bactericide. Asian J Microbiol Biotechnol Environ Sci 2005;7(4):639-44.
19. Supriya D, Ashish S, Ankit S, Nirmala G, Agrawal RC. Evaluation of *in vitro* antibacterial potential of ripe fruits of *Aegle marmelos*. Int J Pharm Pharm Sci 2012;4(3):179-81.
20. Zaika LL. Spices and herbs: Their antimicrobial activity and its determination. J Food Saf 1988;9(2):97-118.
21. Suresh K, Senthilkumar PK, Karthikeyan B. Antimicrobial activity of *Aegle marmelos* against clinical pathogens. J Phytol 2009;1(5):323-7.
22. Yismaw G, Tessema B, Mulu A, Tiruneh M. The *in vitro* assessment of antibacterial effect of *papaya* seed extract against bacterial pathogens isolated from urine, wound and stool. Ethiop Med J 2008;46(1):71-7.
23. Bhatt P, Negi PS. Antioxidant and antibacterial activities in the leaf extracts of Indian borage (*Plectranthus amboinicus*). Food Nutr Sci 2012;3:146-52.
24. Gurgel AP, da Silva JG, Grangeiro AR, Oliveira DC, Lima CM, da Silva AC, *et al. In vivo* study of the anti-inflammatory and antitumor activities of leaves from *Plectranthus amboinicus* (Lour.) Spreng (*Lamiaceae*). J Ethnopharmacol 2009;125(2):361-3.
25. Lukhoba CW, Simmonds MS, Paton AJ. *Plectranthus*: A review of ethnobotanical uses. J Ethnopharmacol 2006;103(1):1-24.
26. Manjamalai A, Alexander T, Grace VM. Bioactive evaluation of the essential oil of *Plectranthus amboinicus* by GC-MS analysis and its role as a drug for microbial infections and inflammation. Int J Pharm Pharm Sci 2012;4(3):205-11.
27. Murthy PS, Ramalakshmi K, Srinivas P. Fungitoxic activity if Indian borage (*Plectranthus amboinicus*) volatiles. Food Chem 2009;114:1014-8.
28. Tiwari DK, Nagar H, Dwivedi G, Tripathi RK, Jena J. Evaluation of anti-anxiety activity of *Plectranthus amboinicus* (Lour.) On rats. Asian J Pharm Clin Res 2012;5(4):110-3.