Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 3
ISSN :2321–3647(online)

Screening for Antimicrobial efficacy of phytochemicals extracted from two medicinally important plants of Cucurbitaceae

Renu Sarin*[1] Sangeeta Samariya[2]
1.Laboratory of Bioactive compounds from Plant Tissue cultures,Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India,
2 Senior research fellow, Laboratory of Bioactive compounds from Plant Tissue cultures, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

ABSTRACT

Medicinal plants are being popular these days to cure diseases due to rapid increase in the rate of infectious diseases day by day and their less side effects over synthetic antibiotics. In the present study two medicinally important plants of family cucurbitaceae vis. *Luffacylindrica*Linn. and*Citrulluscolocynthis*Linn. Leaf, stem and calli phytochemical extracts viz. alkaloids, flavonoids and sterols were evaluated against some pathogenic strains of bacteria and fungi viz. *Staphylococcus epidermis* (MTCC 3615), *Micromonaspora* (MTCC 3296), *Fusariumculmorum*(MTCC349), *Alternariasolani* (MTCC 2101), *Penicilliumchrysogenum* (MTCC 161). The antibacterial and antifungal activities were performed by Disc diffusion method. The on the basis of inhibition zone(IZ) and minimum inhibitory concentration (MIC).Among all the extracts sterol extracts of both the plant species showed highest antimicrobial activity. Results obtained in the present study indicate *Luffa cylindrical* Linn. and*Citrulluscolocynthis*Linn. Leaf, stem and calli phytochemical extracts viz. alkaloids, flavonoids and sterols possesses antimicrobial properties that can be exploited for future natural plant based antimicrobial agents.

Keywords: *Luffacylindrica*, *Citrulluscolocynthis*, alkaloids, flavonoids, sterolsantimicrobial activity, Minimum inhibitory concentration

*Corresponding Author Email:renusarin@sify.com
Received 07 March 2014, Accepted14March2014

Please cite this article in press as: Sarin R.*et al.*,Screening for Antimicrobial efficacy of phytochemicals extracted from two medicinally important plants of Cucurbitaceae. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Plants have been playing an important role in alleviating human sufferings by containing herbal medicines in the primary health care systems of rural and remote hilly areas. Plants produce a diverse range of bioactive molecules making them as a rich source of different types of medicines .[1]Plants are important therapeutic aids for alleviating various ailment of human beings. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. [2]*i.e*. any part of the plant may contain active components. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Plants are source of many chemical therapeutic agents such as gum, resin, latex, oils and dyes. Infectious diseases are leading cause of death world-wide.

Now a days multiple drug resistance microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. [3] Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs.[4] Due to alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. . Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Essential oils, the vast reservoir of secondary metabolites produced by aromatic and officinal plants are of specific interest due to potent biological activity.[5,6]They are complex mixture of monoterpenes and sesquiterpenes which are hydrocarbons with the general formula (C5H8) n. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity.[7] Therefore, researchers are increasingly turning their attention to the field of medicines and looking for new leads to develop better drug against microbial infections. It has been reported that plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases.[8]

Green plants represent a reservoir of effective chemotherapeutic agent and can provide valuable sources of natural pesticides. Biopesticides have been suggested as an effective substitute for chemicals. Reports are available on the use of several plant byproducts which posses antimicrobial properties, on several pathogenic bacteria and fungi. Antimicrobial activity has been reported in many plants by various workers viz.,[9,10,11,12,13,14,15,16,17]

Luffacylindrica Linn. And *Citrulluscolocynthis* Linn. are two common medicinal plants belonging to the family Cucurbitaceae. The phytochemical investigation of these two plants have revealed the presence alkaloids, flavonoids andsterols. The purpose of this study was to screen the antimicrobial activity of these phytochemical extracts of *Luffacylindrica* Linn. And *Citrulluscolocynthis* Linn. that could be useful for the development of new tools as antimicrobial agents for the control of infectious diseases.

MATERIALS AND METHODS

The plant material was collected from University of Rajasthan campus, Jaipur. The plant was identified and voucher specimen was deposited to the Herbarium, Department of Botany, University of Rajasthan, Jaipur. The callus cultures of both the plants were raised on Murashige and Skoog's .[18]medium using seeds, leaves and nodal segments as explants. The various plant parts (leaves and stem) as well as calli of *Luffacylindrica* Linn. And *Citrulluscolocynthis* Linn. were separately dried, powdered and extracted for various secondary metabolites viz. alkaloids, flavonoids and sterols using various standard techniques of extraction.

ANTIMICROBIAL SCREENING

Test microorganisms

Antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive bacteria- *Staphylococcus epidermis* (MTCC 3615) and *Micromonaspora* (MTCC 3296), and fungi – *Fusariumculmorum* (MTCC349), *Alternariasolani* (MTCC 2101), *Penicilliumchrysogenum* (MTCC 161). All the microbial strains (bacteria and fungi) of human pathogens used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India. Pure cultures of all experimental bacteria and fungi were maintained on nutrient agar and Potato Dextrose Agar (PDA) (Hi-media) respectively in Institute of Applied Sciences and Biotechnology (ChemindBiosolutions Laboratory), Jaipur. Each bacterial and fungal culture was further maintained on the same medium after every 48 hours of transferring and stored at 4^{o}C before use in experiments.

ANTIMICROBIAL ACTIVITY

Disc Diffusion Method

Antimicrobial assay of the crude extracts was performed against pathogenic strains by disc diffusion method.[19]The nutrient agar plates and potato dextrose agar plates were seeded with suspension (106cfu/ml) of the bacterial and fungal strains vice-versa. The empty sterilized Whatmann No. 1 filter paper disc (6mm) were impregnated with 1 mg/ml of extracts dried and

placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (Pre diffusion time). The standard discs (6mm) impregnated with antibiotics ampicillin and fluconazols (1.0mg/disc) were used as positive control. The plates were incubated at 37ºC for 24 hr and 25ºC for 48 hr for bacteria and fungi, respectively and observed for zone of inhibition. The diameter of the inhibition zone (mm) was measured. The experiment was done in triplicate and the mean values(±SD) calculated for conclusion.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method.[20]For broth dilution, 1 ml of standardized suspension of a strain (106cfu/ml) was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at37ºC for 24h (for bacterial strains) and 25ºC for 48h (for fungal strains) and observed for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

RESULTS AND DISCUSSION

World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs. No doubt, some studies have identified and isolated the main active ingredients in the plants responsible for this antimicrobial activity. However, the study on medicinal plants will allow for the demonstration of their physiological activity and also catalyze many pharmacological studies that will lead to the development of more toxicity and high sensitivity especially towards the emerging microbial agents.[21] There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action dueto an alarming increase in the incidence of new and reemerging infectious diseases and development of resistance to the antibiotics in current clinical use.[22] Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments.[23,24,25]

In the present investigation, antimicrobial efficacy of the crude extracts of alkaloids, flavonoids and sterols from plant parts (stem and leaf) and calli cultures of *Luffacylindrica* and *Citrulluscolocynthis* were quantitatively assessed on the basis of inhibition zone and Minimum Inhibitory Concentration. The various extracts of*Luffacylindrica* and

*Citrulluscolocynthis*exhibited varying degree of inhibitory effect against all tested pathogenic strains. The results obtained are as following :

Luffa cylindrical:

Among all three extracts (alkaloids, flavonoids and sterols) from plant parts (stem and leaf) and calli cultures of *Luffacylindrica* sterol extract of stem showed maximum antimicrobial activity than other extracts. The stem sterol extract showed highest zone of inhibition (IZ) and activity index (AI) against *Staphylococus epidermis* (MTCC 3615) (30 mm; 1.363mm respectively). The alkaloid and flavonoid extracts also found to effective against pathogenic strains. In alkaloid extract highest zone of inhibition and activity index was to be found against *Micromonaspora* (MTCC 3296) (13mm; 0.625mm respectively) in calli extract whereas in flavonoid extract highest zone of inhibition and activity index was found in calli extract against*Micromonaspora* (MTCC 3296) (26mm; 1.083 respectively). Alkaloid extract of leaf and stem showed very low activity against tested pathogens.

Citrulluscolocynthis:

Among all three extracts (alkaloids, flavonoids and sterols), sterol extract of stem of *Citrulluscolocynthis* showed more antimicrobial activity than other extracts. The stem sterol extract showed highest zone of inhibition (IZ) and activity index (AI) against *Staphylo cocus epidermis* (MTCC 3615) (33 mm; 1.5mm; respectively). The alkaloid and flavonoid extracts also found to effective against pathogenic strains. In alkaloid extract hightest zone of inhibition was found in stem extract against *Staphylo cocus epidermis* (MTCC 3615) (20 mm; 1.272mm; respectively). and in flavonoid extract hightest zone of inhibition was found in leaf extract against *Micromonaspora* (MTCC 3296) (25mm; 1.041 respectively Alkaloid extract of leaf and calli showed very low activity against tested pathogens. *Fusariumculmorum* (MTCC 349), *Alternariasolani*, (MTCC 2101), *Penicillium chrysogenum* (MTCC 161).

CONCLUSION

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.[26] Crude extracts of alkaloids, flavonoids and sterols from plant parts (stem and leaf) and calli cultures of *Luffacylindrica* and *Citrulluscolocynthis* in the present investigation revealed broad spectrum of activity against a range of microorganisms which explain the basis for its use in traditional medicines The result

shows that maximum antimicrobial activity was shown by sterol extract of stem in both the plants. This

explains the basis for the use of these two plants in traditional medicines.

ACKNOWLEDGEMENTS

The authors are thankful to UGC, New Delhi for financial support by granting S.R.F to one of the authors, the Head of Botany Department, University of Rajasthan, Jaipur and Institute of Applied Sciences and Biotechnology (ChemindBiosolutions Laboratory), Jaipur for providing all necessary facilities for the present work. They are also thankful to Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India for providing tested microorganisms for antimicrobial activity.

REFERENCES

1. Stuffness M, Douros J. Current status of the NCl plant and animal product program. J. Nat. Prod.1982; 45**:** 1-14
2. Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm. Biol.2001;39 :8-17.
3. Bhaskarwar B, Itankar P, Fulke, A. Roumanian Biotechnological Letters. 2008; 13 :3873-3877.
4. Silver, LL. Discovery and development of new antibiotics: the problem of antibiotic resistance. Antimicrob. Agents Chemother. 1993**;** 37**:**377-383.
5. Pattnaik S, Subramanyam VR, Bapaji M, Kole CR. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios.1997; 89: 39-46.
6. Durate MCT, Leme EE, Delarmelin, C, Soares AA, Figuerira GM, Sartoratto A. Activity of essential oils from Brazilian medicinal plants on *Escherchia coli*. J. Ethanopharm. 2007; 111: 197- 201.
7. Heinrich M, Gibbons S. Ethanopharmacology in drug discovery: An analysis of its role and potential contribution. J. Ethanopharm.2001; 53: 425-432.
8. Cragg GM, Boyd MR, Khanna R, Kneller R, Mays TD, Mazan, KD, Newman,D J, Sausville EA. Pure Appl. Chem. 1999; 71: 1619-1633.
9. Sarin R. Useful metabolites from plant tissue cultures, Biotechnology. 2005; 4:79-83.
10. Bansal S, Malwal M, Sarin R. Antibacterial efficacy of some plants used in folkloric medicines in arid zone. J.Pharm. Res. 2010; 3 :2640-2642.

11. Chahal JK, Sarin R, Malwal M.. Efficacy of *Clerodendruminerme* L. (Garden quinine) against some human pathogenic strains Intl. J. Pharma and Biosciences. 2010; 1: 219-223.
12. Seth, R, Sarin R. Analysis of the phytochemical content and antimicrobial activity of *J. gossypifolia* L. Arch Appl. Sci. Res.2010; 2: 285-291.
13. Malwal M, SarinRAntimicrobial efficacy of *Murrayakoenigii* (Linn.)Spreng root extract. Indian J. of Nat. Prod. Res. .2011**;** 2 : 48-51.
14. Vyas A, Sarin R. Analysis of the phytochemical content and antimicrobial activity of *Nyctanthes arbor-tristis*. Int. J. Pharm. Bio. Sci. .2013; 4 :201-206.
15. Vyas A, Sarin R. Efficacy of leaf extracts of *Cassia angustifolia*(Linn.) for antimicrobial activity and phytochemical analysis Int. J Pharma Bio. Sci**.** 2013.;4: 713 -718.
16. Samariya K, Sarin R. Screening for Phytochemical content and Antimicrobial activity of *Cyperusrotundus*Linn. World Journal of Pharmacy and Pharmaceutical Science 2013; 2 : 2956-2968
17. Agarwal,M, Sarin R. Screening of antimicrobial potential of flavonoids extracted from *Erythrinaindica.*Intl. J. Pharma Res. &Review. 2014.3: 21-27.
18. Murashige T and Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures.Physiol. Plant. 1962;15:473–497
19. Gould JC, Bowie JH. The determination of bacterial sensitivity to antibiotics. Edinb. Med. J. 1952; 59: 178-199.
20. Basri DF, Fan, SH. The potential of aqueous and acetone extract galls of *Quercusinfectoria*as antibacterial agents. Indian J Pharmacol. 2005; 37: 26-29.
21. Fabricant, DS.,Famsworth NR. The value of plant used in traditional medicine for drug discovery. Environmental Health Perspectives Supplements. 2001; 109: 69-75.
22. Bauer, J, Rojas R, Bustamante B.. Antimicrobial activity of selected Peruvian medicinal plants. J. Ethnopharmacol. 2003**;** 88: 199- 20
23. Diallo D, Hvee B, Mahmoud MA, Betge G, Paulsen BS. Maiga A.An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharm Biol. 1999**;** 37: 80-91.
24. Rojas .J., Ocho J., Ocampo, SA, Munoz, J.F. 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.
25. Erdogrul OT. Antibacterial activities of some plant extracts used in folk medicine. *Pharm Biol*. 2002; 40: 269-273.

26. Iwu MW, Duncan AR, Okunj, CO.. New antimicrobials of plant origin. In: *J.Janick(ed.)*, Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. 1999; 457-462.