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Research Article

SCREENING FOR ANTIMICROBIAL ACTIVITY OF FRESH METHANOLIC EXTRACT OF *GNAPHALIUM POLYCAULON*, INDIAN FOLKLORIC MEDICINAL PLANT

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Abstract

Medicinal plants constitute an important natural wealth of a country and they play a significant role in providing primary health care services. Medicinal plants are important source for the therapeutic remedies of various ailments. The aim of the present study was designed to explore its antimicrobial analysis of the different fresh parts of *G. polycaulon* in methanolic solvents. The selected plant was collected and coarsely powdered for organic solvent extractionusing Soxhlet's method. Then all the extracts obtained were subjected for analysis of antimicrobial properties against bacteria and fungal cultures by standard procedure. The methanolic leaf extract showed high antimicrobial activity with MIC values at 125 µg/ml. In the current investigation, we have reported various isolated phytochemicals compounds of *G. polycaulon*-that might responsible for its antimicrobial effect against all the test bacteria and fungi.

Keywords: Medicinal plant, antibacterial, antifungal, MIC, bacteria, fungi.

INTRODUCTION

Medicinal plants constitute an important natural wealth of a country and they play a significant role in providing primary health care services to rural people as well as substantial amount of foreign exchange[1]. A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. A pharmaceutical drug is a drug that is produced in a laboratory to cure or help an illness. Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds that are for biological functions, including defence[2].

In 2001, researchers identified 122 compounds used in modern medicine which were derived from traditional plant sources; 80% of these have had a traditional use identical or related to the current use of the active elements of the plant. In a 2010 survey of 1000 plants, 356 had clinical trials published evaluating their "pharmacological activities and therapeutic applications" while 12% of the plants, although available in the Western market, had "no substantial studies" of their properties[3].

The use of herbs to treat disease is widespread in non-industrialized societies. The annual global export value of pharmaceutical plants in 2012 was over US$2.2 billion. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care. In fact, according to the World Health Organisation, approximately 25% of modern drugs used in the United States have been derived from plants[3].

All plants produce chemical compounds as part of their normal metabolic activities. Plants synthesize a bewildering variety of phytochemicals.The importance of plants has been realized and well do-

cumented by scholars since ancient period. Apart from the innumerable social benefits, much emphasis has been accorded to the plants of medicinal value. Majority of the population in developing countries depend on traditional system of medicine for their primary health care. Due to this increasing trend towards use of alternative system of medicine, natural medicinal plant resource in this world is under enormous pressure[1].

Gnaphalium polycaulon is a genus of flowering plants in the Asteraceae family of composite type[4], worldwide distribution are said to have anti-inflammatory[5], astringent, and antiseptic properties and are often prescribed as an herbal supplement for colds[4], flu, burns[1], wounds[6] and congestion.

Till now very few plants have been scientifically proved by different researchers for their medicinal potential but the therapeutic ability of number of plants are still unknown. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases[7]. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections[8].

Evidently, there are not sufficient scientific studies that confirm the antimicrobial properties of this plant. This study focused on this medicinal plant into screening its antimicrobial activity against selected microorganisms that cause most common cases of infectious diseases of tribal communities in South India and also.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves, stem and flower of *G. polycaulon* were collected from Kotagiri in Nilgiris District, Tamil Nadu. The plant parts were selected on the basis of the knowledge on their use in different medicine system of health care and identified as *Gnaphalium polycaulon* Pers. in the Botanical Survey of India, Coimbatore and herbarium specimen is authenticated and incorporated in the Madras Herbarium.

Preparation of extracts

The plant materials were washed, air dried and then coarsely powdered. Forty grams of the powdered leaf, stem and flower samples were extracted sequentially using Soxhlet's method for 72 h at a temperature not exceeding the boiling point of the solvent into 250ml of methanol for extract preparation[2]. Resulting extracts was concentrated in vacuum to dryness using a Rotary evaporator. Each powder was weighed and dissolved in the methanol solvents used for extraction separately and stored at 4 ºC. These extracts were subjected to screening antimicrobial study.

Antimicrobial activity

One of the standard assay methods for testing antimicrobial activity is the Kirby-Bauer method, also referred to as the disc diffusion method. A Kirby-Bauer technique was used to screening the antimicrobial activity in different solvents of *G. polycaulon* plant parts[9]. The bacterial cultures of Gram positive (*Aeromonashydrophila, Escherichia coli* MTCC739, *Pseudomonas aeruginosa* MTCC424) and Gram negative (*Bacillus cereus* MTCC430, *Staphylococcus aureus* MTCC3381) bacteria; the fungal cultures of *Aspergillus fumigatus* MTCC343, *Aspergillus oryzae*, and *Candida albicans* MTCC227were used to test the antimicrobial activity.

Preparation of cultures:

To prepare the bacterial and fungal inoculums from each of the microorganisms, a loopful of each test organisms was taken and subsequently subcultured into separate test tubes containing nutrient agar broth. Then the tubes were subjected to incubation for 24 h at 37 ºC, the obtained broth with microorganisms was standardized to have a uniform population density of microorganisms in microbial culture laboratory.

Screening for antibacterial activity

The antibacterial activity of *G. polycaulon* was assayed by a modification of agar well diffusion method[10]. Different concentrations of the extracts were prepared by reconstituting with DMSO. The test organisms were maintained on agar slants were recovered for testing by inoculating into nutrient broth and incubated at 37 °C in a shaker at 180 rpm. The culture of each microorganism was inoculated in plates in nutrient agar and spread

evenly using sterile glass spreader. Test extracts were incorporated into the wells made by sterile 5 mm size borer in media and different concentration of methanolic extracts were added and water alone as a control. Plates were incubated at 37 ºC and after 24 h, the zone of inhibition of methanolic extract, standard control were measured using transparent ruler. Antibacterial screening was determined in triplicates manner.

Screening for antifungal activity

Antifungal activity of all various extracts was studied against two fungal strains by the agar well diffusion method[11]in triplicate manner. The fungal isolates were allowed to grow on a potato dextrose agar (PDA) at 25 ºC until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile distilled water. The fungal spore suspension was evenly spread on plate using sterile glass spreader. Wells were then bored into the agar media using sterile 5 mm cork borer and the wells filled with the solution of the extract and water alone as a control. The plates were allowed to stand on a laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25 ºC for 96 h and later observed for zones of inhibition of all extracts, standard control and measured using transparent ruler.

Minimum Inhibitory Concentration (MIC)

Organisms were subcultured on nutrient agar, followed by incubation for 24 h at 37 ºC. Inoculum was prepared by transferring several colonies of microorganisms to sterile nutrient broth[12]. The suspensions were mixed for 15 sec and incubated for 24 h at 37 ºC. Required volume of suspension culture was diluted to match the turbidity of 0.5 Mc Farland standards ($1.5x10^8$ CFU/mL). MIC was considered the lowest concentration of the sample that prevented visible growth. All samples were examined in triplicates manner. Samples were prepared in dimethyl sulphoxide at the concentration of 2 mg/ml. A series of 15 tubes were filled with 0.5 ml of sterilized nutrient broth. Sequentially, 2-14 test tubes received an additional 0.5 ml of the sample serially diluted to create a concentration sequence from 500-0.06 µg. The first tube served as the control. All the tubes received 0.5 ml of inoculum. The tubes were vortexed well and incubated for 24 h at 37 ºC. The resulting turbidity was observed, and after 24 h MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 600 nm.

RESULTS AND DISCUSSION

Medicinal plants products are responsible for several biological activities. In recent times, ethno medical and traditional pharmacological approaches are achieving great appreciation in modern medicine, because the search for new potential medicinal plants is often based on an ethnomedicinal origin[13]. Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites. Pharmaceutical and scientific communities have the attention of the medicinal plants to validate the claims of their biological activity.

In this study, antibacterial activity in methanolic solvents of leaf, stem and flower extracts of *G. polycaulon* were evaluated against Gram positive and Gram negative bacteria, and fungi. Results were compared with the standard drugs such as gentamycin for bacterial cultures. The zone of inhibition was seen in all extract against all cultures. The MIC values were evaluated and showed no growth at 125 µg/ml in methanolic leaf extracts. The zone of inhibition of all various fresh extracts of *G. polycaulon* was measured and tabulated in Table 1.

Fungi can cause damage to the structures, decoration of buildings and are also responsible for their indoor air quality[14]. The antifungal activity of all extracts of *G. polycaulon* parts was evaluated using agar well diffusion method. The extract exhibited high significant activity in methanolic leaf extracts against all the tested fungi compared with the standard drug, Nystatin (10 µg/disc). All extracts showed good activity against the fungal isolates with zones of inhibition ranging from 1 to 4 mm. In conclusion, the results showed that the all various fresh extract of *G. polycaulon* is a broad spectrum agent which can be used against both Gram positive and Gram negative bacteria and also fungi.

Table 1 Antibacterial activity of methanolic extracts of fresh *G. polycaulon*

Extracts	Plant parts	Concentration(µg/ml)	Antibacterial activity (Zone of inhibition,mm)					Antifungal activity (Zone of inhibition,mm)		
			Ah	*Ec*	*Pa*	*Bc*	*Sa*	*Afu*	*Aor*	*Can*
Methanolic extract	Fresh leaf	50	2	2	2	2	2	2	2	2
		100	2	2	2	2	3	3	3	3
		150	3	5	5	5	6	3	3	4
		MIC	125	125	125	125	62.5	125	125	125
	Fresh stem	50	1	1	2	2	2	1	2	2
		100	1	2	2	2	3	2	2	3
		150	4	4	2	4	3	3	2	3
		MIC	-	125	-	-	125	125	125	125
	Fresh flower	50	1	1	2	2	2	1	1	1
		100	1	2	2	2	3	1	2	2
		150	3	3	3	3	3	2	1	2
		MIC	-	125	-	-	125	125	125	125
	Standard	**100**	**18**	**17**	**19**	**28**	**28**	**8**	**8**	**14**

Ah: Aeromonashydrophila; Ec: Escherichia coli; Pa: Pseudomonas aeruginosa; Bc: Bacillus cereus;

Sa: Staphylococcus aureus; Afu: Aspergillus fumigatus; Aor: Aspergillus oryzae; Can: Candida albicans;

Minimum Inhibitory Concentrations (MIC) of crude methanolicextracts was determined. The results showed high resistant antimicrobial activity. MICs of active extracts ranged from 500-0.06 µg/mL against test bacterial and fungal cultures were tabulated in Table 1.All of methanolicextracts (leaf, stem and flower) of *G. polycaulon* tested in present study had specific potential antimicrobial activity against the reference (standard) strains. Our results strongly support the medicinal use of this plant in traditional medicine that can be used as antimicrobial agents in the search for new drugs[15]. With the emergence of multiple strains of antibiotic resistance microorganism, great interest has been generated in search for potential compounds from plants for therapeutic, medicinal, aromatic and aesthetic uses.

Conclusion

In conclusion,many medicinal plant lie unexplored or remain under explored. The results reported the major phytochemicals showed that *G. polycaulon* plant is good source of phytochemical constituents for antimicrobial activity. It demonstrated broad spectrum activity against all bacteria and fungi tested with inhibition zones in the range of 1-6mm. The MIC values were found to range from 0.6 to 125 µgml^{-1}. The methanolic leaf extract of *G. polycaulon* recorded significant antimicrobial activity against all the test bacteria and fungi. The results strongly supported that the efficiency of crude solvent plant extracts contain medicinally important bioactive compounds due to the strongly presence of plant secondary metabolites which can be used to fight against resistant bacteria and fungi for the treatment of different diseases in traditional medicine.

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REFERENCES

1. Acharya, S., 2011. Presage biology: lesson from nature in weather forecasting. Ind. JTrad. Knowl, 10(1), 114-124.
2. Harborne J.B. Phytochemical Methods: A guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK, 2ndedition, (1998).
3. Shanmugapriya, K., Thayumanavan, Tha., 2016. Phytochemical screening and antimicrobial potential on ethanolic extract of *Gnaphalium polycaulon* Pers., ethnomedicinal plant used by Niligiri tribe from Western Ghats, Tamil Nadu, India, Medicinal Plants – Int. J.Phytomed. Rel. Ind. 8 (2), 168-171.

4. Abid, R., Qaiser, M., 2008. Cypsela morphology of *gnaphalium*L and its allied genera (gnaphalieae-asteraceae) from Pakistan.Pak. J. Bot. 40(1), 25-31.
5. Shanmugapriya, K., Thayumanavan, Tha.,Alamelu, L., Divya, G., Hilda, T.C., Jamuna K.S., Srija, M, 2014. Preliminary phytochemical screening and antimicrobial activity of fresh plant extract of Indian folk medicinal plant, *Gnaphalium polycaulon.* Int. J.Phytomed.6(1), 82-86.
6. Uniyal, B., Shiva, V., 2005. Traditional knowledge on medicinal plants among rural women of the Garhwal Himalaya. Uttaranchal. Ind. JTrad. Knowl. 4(3), 259-266.
7. Bisht, V.K., Purohit, V., 2010. Medicinal and Aromatic Plants Diversity of Asteraceae in Uttarakhand. Nat. Sci. 2, 121-128.
8. Krishnaveni, T., Valliappan,R.,Selvaraju,R., Nagendra, P.P.,2016. Antioxidant activity and preliminary phytochemical analsysis of *Loranthuselasticus*of *loranthaceae.* Int. J. Pharm.Drug Anal. 4(10), 480 – 479.
9. Shanmugapriya, K., Saravana, P. S., Harsha, P., Peer, M. S., Williams, B., 2011. A comparative study of antimicrobial potential and phytochemical analysis of *Artocarpusheterophyllus* and *Manilkarazapota* seed extracts. J. Pharm. Res. 4(8), 2587-2589.
10. Perez, C., Pauli, M., Bazevque, P., 1990. An antibiotic assay by the agar well diffusion method. ActaBiologiaeet Medicine Experimentalis. 15,113-115.
11. Verma, R.K., Chaurasia, L., Kumar, M., 2011. Antifungal activity of essential oils against selected building fungi. Ind. J Nat. Prod. Res. 2(4), 448-451.
12. Upadhyay, O.P., Singh, R.M.,Dutta, K., 1996. Studies on antidiabetic medicinal plants used in Indian folklore. Aryavaidyan. 9 (3), 159-167.
13. Sofowora, A., 1993. Screening plants for Bioactive Agents. In: medicinal plants and traditional medicinal in Africa. 2nd *Eds*. Spectrum Books Ltd, Pp. 134-256. Sunshine house, Ibadan, Nigeria.
14. Shanmugapriya, K., Saravana, P. S., Harsha, P., Peer, M. S., Williams, B., 2012. Antioxidant potential of pepper (*Piper nigrum* L.) leaves and its antimicrobial potential against some pathogenic microbes. Ind. J. Nat. Prod. Res. 3(4), 570-577.
15. Anshika, S., Kuldeep, S., Suresh, K. N., Vivek, D., Vidyakant, K., 2016. Antiulcer activity of methanolic root extract of *Baliospermummontanum*in ethanol and indomethacin induced ulcer models. Int. J. Pharm.Drug Anal. 4(12), 522 – 532.