Research Article

Minimum Inhibitory Concentration (MIC) of antimicrobial activity in various dried extracts of *Gnaphalium polycaulon*, Indian folkloric medicinal plant

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ABSTRACT

Objective: Medicinal plants have been identified and used throughout human history. The aim of the current study was designed to determine MIC values for its antimicrobial analysis of the different dried parts of *G. polycaulon* in all solvents. **Methods**: The selected plant was collected and coarsely powdered for organic solvent extraction using Soxhlet's method. Then all the extracts obtained were subjected for analysis of antimicrobial properties against bacteria and fungal cultures by standard procedure. **Results**: All extracts showed MIC values at 125 µg/ml, determined high antimicrobial activity. **Conclusions**: In the current investigation, we have reported major secondary metabolites of *G. polycaulon* that might responsible for its antimicrobial effect against all the test bacteria and fungi.

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

INTRODUCTION

Medicinal plants play a significant role in providing primary health care services. Medicinal plants have been identified and used throughout human history[1] (Borkataky et al., 2013). Medicinal and aromatic plants are used widely in the later years of the 21st century. It constitute the backbone of traditional medicine practices. The importance of traditional medicine (TM) as a source of primary healthcare was first officially recognized by the World Health Organization (1991) by Traditional Medicine Program. Researchers are increasingly turning their attention to herbal products, looking for new leads to investigate phytochemical investigation and to develop better drugs against Oxidative damage of cells [2] (Mowobi et al., 2016).

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world [3] (Saiba et al., 2013). Ethnobotany is the scientific study of relationship that exists between people and plants. It interface between indigenous people and their wild exploit of plants around them, which is a significant aspect of biological diversity conservation[4] (Shanmugapriya and Thayumanavan, 2016).

The study of traditional human uses of plants, is recognized as an effective way to discover future medicines [5] (Sofowora, 1993). 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 medicinal plant (Shanmugapriya and Thayumanavan, 2015).

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 Organization, approximately 25% of modern drugs used in the United States have been derived from plants [7] (Sadasivam and Manickam, 1996).

Plants have always been the principal source of drugs for the prevention and treatment of disease and also for the production of some drugs currently used in modern medicine. Many reports stated that Asteraceae family is

considered as abundant flowering plants with many therapeutic properties and reported as folkloric medicinal plants [8] (Li et al., 2015).

Gnaphalium polycaulon is a genus of flowering plants in the Asteraceae family of composite type, worldwide distribution are said to have anti-inflammatory [9] (Shanmugapriya et al., 2014), antioxidant, astringent, and antiseptic properties and are often prescribed as an herbal supplement for colds [10] (Abid, and Qaiser, 2008), flu, burns [11] (Acharya, 2011), wounds [12] (Uniyal and Shiva, 2005) and congestion. There is a urgent need to discover new antimicrobial compounds for new and reemerging infectious diseases [13] (Bisht and Purohit, 2010). Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs as well as viral and microbial infections [14] (Ibarra et al., 2001). 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 by its MIC values that cause most common cases of infectious diseases of tribal communities in South India.

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 and dried. 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 250 ml of Aqueous, ethanol and hexane for extract preparation [15] (Harborne, 1998). Resulting extracts was concentrated in vacuum to dryness using a Rotary evaporator. Each powder was weighed and dissolved in the respective solvents used for extraction separately and stored at 4 ºC. These extracts were subjected to screening the MIC values for its 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 [16] [Shanmugapriya *et al.,* 2011]. The bacterial cultures of Gram positive (*Listeria monocytogenes*) and Gram negative (*Flavobacterium* sp., *Salmonella typhimurium*, *Yersinia enterocolitica*) bacteria; the fungal cultures of *Aspergillus flavus,* and *Pencillium notatum* were used to test the MIC values for its 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 sub-cultured 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.

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 [17] [Upadhay *et al.,* 1996]. The suspensions were mixed for 15 seconds and incubated for 24h at 37 ºC. Required volume of suspension culture was diluted to match the turbidity of 0.5 Mc Farland standards (1.5×10^8 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.

RESULT AND DISCUSSION

Medicinal plants have several biological activities. In recent times, ethno medical and traditional pharmacological approaches are achieving great appreciation in modern medicine, based on an ethnomedicinal origin [5] [Sofowora, 1993].

Antimicrobial properties of several plant extracts have been recognized mainly due to the secondary metabolites. Pharmaceutical and scientific communities have the attention of the medicinal plants to validate the claims of their biological activity.

Antimicrobial activity in all solvents of leaf, stem and flower extracts of *G. polycaulon* were evaluated against Gram positive and Gram negative bacteria, and fungi for determined MIC values. Results were compared with the standard drugs such as gentamycin and Nystatin (10 µg/disc) for bacterial and fungal cultures. Minimum Inhibitory Concentrations (MIC) of crude plant extracts 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 extracts (leaf, stem and flower) of *G. polycaulon* tested in present study had specific potential antimicrobial activity against the reference (standard) strains. The MIC values were evaluated and showed no growth at 125 µg/ml in all leaf extracts tabulated (Table 1).

Table 1: Minimum Inhibitory Concentration (MIC) of Antimicrobial activity in various extracts of dried *G. polycaulon*

Extracts	Plant parts	Antibacterial activity (Zone of inhibition, mm)				Antifungal activity (Zone of inhibition, mm)	
		Flavobacterium sp.	*Salmonella typhimurium*	*Yersinia enterocolitica*	*Listeria monocytogenes*	*Aspergillus flavus*	*Pencillium notatum*
Aqueous extract	Fresh leaf	125	125	125	125	125	125
	Fresh stem	-	125	-	-	125	125
	Fresh flower	-	125	-	-	125	125
	Dried leaf	125	125	125	125	125	125
	Dried stem	125	125	125	125	125	125
	Dried flower	-	125	-	125	125	125
Ethanolic extract	Fresh leaf	125	125	125	125	125	125
	Fresh stem	-	125	-	-	125	125
	Fresh flower	-	125	-	-	125	125
	Dried leaf	125	125	125	125	125	125
	Dried stem	125	125	125	125	125	125
	Dried flower	-	125	-	125	125	125
Hexane extract	Fresh leaf	125	125	125	125	125	125
	Fresh stem	-	125	-	-	125	125
	Fresh flower	-	125	-	-	125	125
	Dried leaf	125	125	125	125	125	125
	Dried stem	125	125	125	125	125	125
	Dried flower	-	125	-	125	125	125
Standard		**20**	**18**	**20**	**32**	**8**	**12**

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. The results strongly supported that the medicinal use of *G. polycaulon* plant in traditional medicine that can be used as antimicrobial agents in the search for new drugs [4] [Shanmugapriya et al., 2016]. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs as well as viral and microbial infections was documented [18] (Zheng et al., 2013). 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. Major phytochemicals present in *G. polycaulon* plant was significant source for various biological 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}. All leaf extract of *G. polycaulon* recorded significant MIC values for its 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.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Biotechnology, Dr. G.R. Damodaran College of Science, Coimbatore, Tamil Nadu, India for providing necessary facilities to carry out this work

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