ORIGINAL ARTICLE

Antimicrobial activity of methanolic extracts of indigenous traditional Indian folk Medicinal Plant, *Gnaphalium polycaulon*

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Background and Aim: *Gnaphalium polycaulon* (L.) Pers. (*Asteraceae*) plant, locally known as Nerabu chedi, collected from Nilgiri District, Tamil Nadu was subjected to antimicrobial screening and minimum inhibitory concentration of methanolic extracts of leaf, stem, and flower. **Methodology:** The selected plant used in traditional Indian medicine was examined for antimicrobial activity and minimum inhibitory concentration against human pathogenic bacteria and fungus using the agar well diffusion method. The antilog of the corresponding value of concentration was taken as the minimum inhibitory concentration value. **Statistical Analysis:** All the values of the results of the assay were expressed as means of triplicates, mean ± standard deviation. **Results:** The antimicrobial activity of methanolic leaf extracts of *G. polycaulon* showed a high level of antimicrobial activity against the studied bacterial and fungal pathogens. **Conclusion:** Based on the results obtained, the medicinal value of this plant could be attributed to the presence of secondary metabolites in the traditional herbal medicines. Therefore, this antimicrobial activity shows a source for traditional use of the plant as a local health remedy to the indigenous communities of Tamil Nadu. Further studies on knowledge of the medicinal plant used medicinally by indigenous people could lead to further research and new drug discovery for the treatment of different diseases.

Key words: Antimicrobial activity, folk medicine, fungus, *Gnaphalium*, Gram-negative bacteria, Gram-positive bacteria, minimum inhibitory concentration

INTRODUCTION

Aromatic and medicinal plants are known to produce certain bioactive molecules that can react with other organisms in the environment to inhibit bacterial or fungal growth (antimicrobial activity). Many plants showed antioxidant and antimicrobial properties that can protect the human body against both cellular oxidation reactions and pathogen.[1] India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plant.[2] Medicinal plants have a global distribution although they are most abundant in the tropics.[3] Medicinal plants and their extracts are used in traditional treatments of various diseases.[4] Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drug.[5]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.150921

Medicinal plants have their intrinsic ability to resist pathogenic microorganisms, and this has led the researchers to investigate their mechanisms of action and isolation of active compounds. This has enabled exploitation of medicinal plants for the treatment of microbial infections of both plants and humans by developing new antimicrobial agents. This novel search entails extensive research, and it is, therefore, imperative to follow standard methods to authenticate claims of antimicrobial action.[6]

Herbal medicine is the use of medicinal plants for the prevention and treatment of diseases, it ranges from traditional and popular medicines of every country to the use of standardized herbal extracts. Herbal knowledge from local indigenous communities has long been the basis for investigating the further potential of plants as therapeutic agents.[7]

The past, present, and future of medicinal plants was analyzed in both as potential antimicrobial crude drugs as well as a source for natural compounds that

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Received: 28-06-2014; **Accepted:** 10-09-2014

act as new antiinfection agents.[8] Many diseases caused by pathogenic microbes have been successfully treated to this day with a variety of available antibacterial drugs.[7] The primary benefit of the utilization of plant derived medicine is that they are relatively safer than synthetic alternatives, offering very good therapeutic benefit and affordable treatment.[9] Secondary metabolite is crucial for plant defenses as an antioxidant or antimicrobial agent that has enabled plants to survive.[10] The detection plays a strategic role in the phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological effects.[11]

Microorganisms are closely associated with the health and welfare of human beings, some microorganisms are beneficial, and others are detrimental.[12] Throughout the history of mankind, many infectious diseases have been known to be treated with herbal remedies.[13] The natural herbal products either as pure compounds or as standardized plant extracts provided unlimited opportunities for new drug leads to a never ending and urgent need to discover new antimicrobial compounds with different chemical structure and new mechanisms of action for re-emerging and new infectious diseases.[14] Therefore, researchers are increasingly turning their keen attention towards folk medicine from plants that leads into developing better natural drugs against microbial infections.[15]

Biodiversity studies still reveal that the plant kingdom has not been exhausted based on the species of medicinal plants that are yet to be discovered. Based on history, plants have been found to be active against a wide variety of microorganisms.[16] According to World Health Organization, 65–80% of the world populations rely on traditional medicine to treat various diseases.[17] About three-quarters of World's population relies on plants and its extracts for health care.[18] Traditional herbal and folk medicine practices are based on the use of plants and plant extracts. Standardization and phytochemical investigation of the extract plays a very important role in determining the active constituents and relative purity.[19] In the recent years, multiple drug resistance has been developed in human pathogens due to the indiscriminate use of commonly available antibiotics in the treatment of infectious diseases.[20] The increased prevalence of antibiotic-resistant bacteria due to the extensive use of antibiotics has rendered the current antimicrobial agents inefficient to control several bacterial diseases.[21]

Plant based antimicrobials will help to overcome the resistance problems as well as it will be more reliable than the synthetic products.[20] A recent ethno-botanical survey of traditional and folk medicine in India has revealed that most of these plants are still in use by the local tribal people, from ancient time.[22] Plant extract has a potential application as natural medicine and to treat diseases as well as the microbiological safety of the human health.[23]

Asteraceae are popular garden plants due to their numerous and often brightly colored blossoms. *Gnaphalium polycaulon* is a genus of flowering plants in the *Asteraceae* family of compositae type, worldwide distribution and is mostly found in temperate regions, although some are found on tropical mountains or in the subtropical regions of the world. *Gnaphalium* plants can survive in − 10°F (−23.3°C). The entire plant is harvested during flowering and is used to make herbal and homeopathic remedies.[24] Species in this genus are said to have anti-inflammatory, astringent, and antiseptic properties and are often prescribed as an herbal supplement for colds, flu, pneumonia, tonsillitis, larygitis, and congestion.[25] Practitioners prescribe the herb for respiratory, digestive, and musculoskeletal conditions as well as an aid to quit smoking. The homeopathic remedy has no known side-effects. This species are said to have anti-inflammatory, astringent, and antiseptic properties and are often prescribed as an herbal supplement for colds, flu, pneumonia, tonsillitis, laryngitis, and congestion. It is a popular treatment for respiratory problems and neuritis among tribe. Patients with rheumatism, diarrhea and an increase in urination, combined with sporadic upper jaw pain, may also benefit from *G. polycaulon* plant.[26] The investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous.

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.[27] Hence, in tune with this effort, the objective for the present study is to screening the antimicrobial activity of the medicinal plant in order to understand the nature of the principle component responsible for its medicinal property.

METHODOLOGY

Chemicals Required

All chemicals used for this study were high quality analytical grade reagents. The solvents such as ethanol, water and hexane were purchased from S.D. Fine Chemicals Pvt. Ltd., Sigma chemicals, Lobe chemicals, Merck Chemical Supplies, Nice Chemicals and Hi media. All other chemicals used for the study were obtained commercially and were of analytical grade.

Collection of Plant Material

The fresh leaves, stem and flower of *G. polycaulon* were collected from Nedugula (Latitude - 11.41871 and Longitutte - 76.87816), Kotagiri in Nilgiri 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 *G. polycaulon* Pers. (=*Gnaphalium indicum* Hook.f.) - *Asteraceae* family and the herbarium specimen is authenticated and incorporated in the Madras Herbarium with accession number of 175619–175620.

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 methanol for extract preparation. 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 °C. 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, 1996, also referred to as the disc diffusion method. A selective culture media were prepared in the antimicrobial assay container and subsequently streaked uniformly with the selected test microorganisms.

Test Organisms

A Kirby-Bauer technique was used to screen the antimicrobial activity for the methaolic leaf, stem and flower extract of *G. polycaulon*. The bacterial cultures of Gram-positive (*Aeromonas hydrophila, Escherichia coli* MTCC739, *Flavobacterium* sp., *Pseudomonas aeruginosa* MTCC424, *Salmonella typhimurium* and *Yersinia enterocolitica*) and Gram-negative (*Bacillus cereus* MTCC430, *Listeria monocytogenes, Staphylococcus aureus* MTCC3381) bacteria; the fungal cultures of *Aspergillus flavus, Aspergillus fumigatus* MTCC343, *Aspergillus oryzae, Candida albicans* MTCC227 and *Pencillium notatum* were used to test the antimicrobial activity.

Preparation of the Inoculum

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 the nutrient agar broth. Then, the tubes were subjected to incubation for 24 h at 37°C, the obtained broth with microorganisms was standardized to have an 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.[28,29] Different concentrations of the extracts were prepared by reconstituting with dimethyl sulphoxide (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°C and after 24 h, the zone of inhibition of methanolic extract, standard control were measured using transparent ruler. Antibacterial screening was done in triplicates.

Screening for Antifungal Activity

Antifungal activity of all various extracts was studied against two fungal strains by the agar well diffusion method.[28,29] The fungal isolates were allowed to grow on a potato dextrose agar at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile distilled water. The fungal spores 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°C for 96 h and later observed for zones of inhibition of methanolic extract, standard control and measured using transparent ruler. Antifungal screening was done in triplicates.

Minimum Inhibitory Concentration

Preparation of Inoculum

Organisms were subcultured on nutrient agar, followed by incubation for 24 h at 37°C. Inoculum was prepared by transferring several colonies of microorganisms to sterile nutrient broth.[30] The suspensions were mixed for 15 s and incubated for 24 h at 37°C. Required volume of suspension culture was diluted to match the turbidity of 0.5 Mc Farland standard (1.5×10^8 CFU/mL). Minimum inhibitory concentration (MIC) was considered the lowest concentration of the sample that prevented visible growth. All samples were examined in triplicates manner.

Preparation of Sample

Samples were prepared in DMSO at the concentration of 2 mg/ml.

Broth Dilution Assay

A series of 15 tubes were filled with 0.5 ml of sterilized nutrient broth. Sequentially, test tubes 2–14 received an additional 0.5 ml of the sample serially diluted to create a concentration sequence from 500 to 0.06 µg. The first tube served as a control. All the tubes received 0.5 ml of inoculum. The tubes were vortexed well and incubated for 24 h at 37°C. 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

Plants are recognized for their ability to produce a wealth of secondary metabolites, extensively used for traditional medicine for centuries to treat a variety of disease. 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 ethanomedicinal origin.[31]

Secondary metabolites in plant products are responsible for several biological activities in living systems. Antimicrobial properties of several plant extracts have been attributed due to the secondary metabolites.[32] Pharmaceutical and scientific communities have recently received the attention of the medicinal plants, and various publications have documented the therapeutic worth of natural compounds to validate the claims of their biological activity.[6]

In this study, antibacterial activity of methanolic leaf, stem and flower extracts of *G. polycaulon* were evaluated. The extracts were screened against Gram-positive and Gram-negative bacteria. 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, but the maximum inhibition shown in dry leaf extracts. The zone of inhibition of all various dry extracts of *G. polycaulon* was measured and tabulated [Table 1].

Fungi can cause damage to the structures, decoration of buildings and are also responsible for their indoor air quality.[33] The antifungal activity of all extracts of *G. polycaulon* parts was evaluated using agar well diffusion method. The extract exhibited high significant activity in dry 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 8 to 18 mm. In conclusion, the results showed that all various extract of *G. polycaulon* is a broad spectrum agent which can be used against both Gram-positive and Gram-negative bacteria and also fungi.[34]

The reason for the difference sensitivity between the Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms, Gram-negative pathogens having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da. The Gram-positive bacteria should be more susceptible having only an outer peptidoglycone layer, which is not an effective permeability barrier.[31]

Minimum Inhibitory Concentrations of crude methanolic extracts was determined. The results are showing high

Table 1: Antimicrobial activity of methanolic extracts of *Gnaphalium polycaulon*

Micro organisms	Zone of inhibition in mm									Standard
	Dry leaf (µg/ml)			Dry stem (µg/ml)			Dry flower (µg/ml)			
	50	100	150	50	100	150	50	100	150	
Bacteria										Gentamycin
Aeromonas hydrophila	10	10	11	8	9	10	8	8	11	18
Escherichia coli	11	11	13	9	11	11	9	9	10	17
Flavobacterium sp.	11	11	12	10	12	12	11	11	17	20
Pseudomonas aeruginosa	12	11	12	11	14	12	11	12	15	19
Salmonella typhimurium	21	12	10	16	15	15	28	17	12	18
Yersinia enterocolitica	8	9	10	10	10	12	8	10	8	24
Bacillus cereus	12	13	15	4	9	12	6	11	14	28
Listeria monocytogenes	13	18	18	5	8	14	7	13	15	32
Staphylococcus aureus	11	13	15	5	7	13	6	11	13	28
Fungus										Nystatin
Aspergillus flavus	10	12	16	6	8	10	7	12	15	08
Aspergillus fumigatus	10	11	14	5	6	7	6	11	10	08
Aspergillus oryzae	11	11	12	6	7	10	7	11	12	08
Candida albicans	14	13	15	8	9	11	8	15	11	14
Penicillium notatum	17	11	15	14	15	12	11	12	11	12

resistant activity in the order of test bacterial cultures such as *S. aureus*, *A. hydrophila*, *E. coli*, *Flavobacterium* sp., *P. aeruginosa*, *S. tryphimurium*, *Y. enterocolitica*, *B. cereus* and *L. monocytogenes* and the fungal cultures are *C. albicans*, *P. notatum A. flavus*, *A. fumigates* and *A. oryzae*. MICs of active extracts ranged from 500 to 0.06 µg/mL against test bacterial and fungal cultures were tabulated in Table 2.

In conclusion, all of methanolic extracts (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. Until date, many plants have been claimed to pose beneficial health effects such as antioxidant and antimicrobial properties.[35] With the emergence of multiple strains of antibiotic resistance microorganism, great interest has been generated in the search for potential compounds from plants for therapeutic, medicinal, aromatic and esthetic uses.

CONCLUSION

The results obtained in this plant concluded that the antimicrobial property plays an important role in the identification of therapeutically potent bioactive compounds. This exploration on plant-derived antimicrobials was carried out to determine the identification of antimicrobial compounds within this plant and also to determine their full spectrum of efficacy. Many plants with strong therapeutic, medicinal, aromatic and aesthetic effect lie unexplored or remain under explored. The extract showed antibacterial activity against some types of microorganisms upon which the extract was employed. Its antimicrobial property against certain microbes and presence of secondary metabolites presents a potential for treating various infectious disease. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation. The bioactive compounds reported in various extracts evidences of their medicinal activities of *G. polycaulon* which needs to be further explored and some pharmacological active constituents has to be performed and validated so as to use it as a potential force in the field of healthcare for the treatment of many diseases to determine, isolate, identity, characterize and elucidate the structure of the specific bioactive components responsible for such activity.

Table 2: Minimum inhibitory concentration of methanolic extracts of *Gnaphalium polycaulon*

Micro organisms	Minimum inhibitory concentration (µg/ml)		
	Dry leaf	Dry stem	Dry flower
Bacteria			
Aeromonas hydrophila	125	125	-
Escherichia coli	125	125	125
Flavobacterium sp.	125	-	-
Pseudomonas aeruginosa	125	125	125
Salmonella typhimurium	125	125	125
Yersinia enterocolitica	125	125	-
Bacillus cereus	125	125	125
Listeria monocytogenes	125	125	125
Staphylococcus aureus	62.5	125	-
Fungus			
Aspergillus flavus	125	125	125
Aspergillus fumigatus	125	125	125
Aspergillus oryzae	125	125	125
Candida albicans	125	125	125
Penicillium notatum	125	125	125

ACKNOWLEDGMENTS

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

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How to cite this article: Kaminidevi S, Thangavelu T, Udayabhanu J, Thangavel SM. Antimicrobial activity of methanolic extracts of indigenous traditional Indian folk Medicinal Plant, *Gnaphalium polycaulon.* Int J Green Pharm 2015;9:39-44.

Source of Support: Nil, **Conflict of Interest:** None declared.