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Preliminary Phytochemical screening and Antimicrobial activityof fresh plant extract of Indian folk medicinal plant, *Gnaphalium polycaulon*

Shanmugapriya Kaminidevi[1]*, ThayumanavanThangavelu[1]*,Alamelu Lakshmanan[1], Divya Gunashekar[1], Hilda Thomas Chirayil[1], Jamuna Karanangattil Sukumaran[1], Srija Murugan[1]

***Corresponding author:**

Shanmugapriya Kaminidevi
ThayumanavanThangavelu

[1]School of Biotechnology,
Dr.G.R.Damodaran College of Science,
Coimbatore-641014, Tamil Nadu,
India.

Abstract

Nature has been a source of medicinal agents for thousands of years. Plants produce a diverse array of bioactive molecules, making them a rich source of diverse type of medicines. Thus, natural products with pharmacological or biological activities still play a very important role in medicine. The objective of the present study deals with the exploration of specific species of medicinal plants found in Western Ghats, India and was to investigate the presence of phytochemicals constituents and to determine antimicrobial analysis of the *Gnaphalium polycaulon* in hexane solvents.Fresh *G.polycaulon*was air dried and coarsely powdered. Then all the extracts obtained were subjected for phytochemical screening using standard procedure. Bacterial and fungal cultures were used for screening antimicrobial study by agar well diffusion method. It reported the presence of major phytochemicals in all solvent extracts. The phytochemical of the plant extract showed that the leaf and stem were rich in major phytochemicals such as alkaloids, flavonoids, tannins and saponins, than flower extract. The resource base of the traditional folk medical practices is more prevalent in rural and tribal villages of India.Our findings provided evidence that crude hexane extracts of selected plants contain medicinally important bioactive compounds and their use in the traditional folk medicines practices.

Keywords: phytochemicals, phenols, flavonoids, steroids, alkaloids, antimicrobial

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Introduction

Plants are a rich source of diverse type of medicines in different countries and produce a diverse array of bioactive molecules, the source of potential and powerful drugs [1]. Thus, natural products with pharmacological or biological activities still play a very important role in medicine [2]. Plant Extract has a potential application as natural medicine and to treat diseases as well as the microbiological safety of the human health [3].

Medicinal plants have a global distribution although they are most abundant in the tropics [4]. Medicinal plants are rich sources of antimicrobial agents [5]. Medicinal plants are the plants whose parts as extracts, infusions, and powders are used in the treatment of different diseases of humans, plants and animals [6]. Medicinal plants are sources of important therapeutic aids for alleviating human ailments [7]. Many medicinal plants traditionally used for thousands of years are present in a group of herbal preparation of the Indian traditional health care system [8].

Phytochemicals are natural and non-nutritive plant bioactive chemical compounds that have protective or disease preventive properties against external stress and pathogenic attack [5]. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine [9]. The plant-derived phytochemicals with therapeutic properties could be used as single therapeutic agent or as combined formulations in drug development [10, 11]. The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated [8]. The phytochemical investigation of a plant may involve extraction of plant materials, phytochemical screening, separation and isolation of the constituents, characterization of the isolated compounds [5].

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 [12]. Infectious diseases are the leading cause of death worldwide[13]. The potential for developing antimicrobials from higher plants appear rewarding as it might lead to the development of phytomedicine which would play a prominent role against microbes [14].The natural herbal products either as pure compounds or as standardized plant extracts provided unlimited opportunities for new drug leads because of the uncompared availability of diversities of chemical [9]. Therefore, researchers are increasingly turning their keen attention towards folk medicine from plants which leads into developing better natural drugs against microbial infections.

Asteraceae are popular garden plants due to their numerous and often brightly colored blossoms. *G. 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. The entire plant is harvested during flowering and is used to make herbal and homeopathic remedies [15]. Patients with rheumatism, diarrhea and an increase in urination, combined with sporadic upper jaw pain, may benefit from G. polycaulonplant [16].

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 [17]. World Health Organization (WHO) described plant as a plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1].Biodiversity studies revealed that the plant kingdom has not been exhausted based on the species of medicinal plants which are yet to be discovered. So, this medicinal plant was chosen for our present study with main objectives to screen the phytochemicals constituents and antimicrobial study.

Materials and Methods

Chemicals required

All chemicals used for this study were high quality analytical grade reagents. The solvents such as ethanol, methanol, petroleum ether 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* plant were collected from Kodanadu near Kotagiri in Nilgiri district, Tamil Nadu, India and identified in Botanical Survey of India, Southern Regional Centre, T.N.A.U. Campus, Coimbatore, Tamil Nadu, India.

Extraction of Plant Material

The plant materials were washed, air dried and coarsely powdered. Forty grams of the powdered sample was extracted sequentially by using Soxhlet extractor for 72h at a temperature not exceeding the boiling point of the solvent into 250ml of methanol, ethanol and water for extract preparation. Resulting extracts was filtered using Whatman filter paper (No.1) and concentrated in vacuum to dryness using a Rotary evaporator. Powder was weighed and dissolved in the solvents used for extraction: methanol, ethanol and water separately and stored at 4˚C for further use.

Phytochemical Analysis

The preliminary screening of phytochemicals of plant extracts for the presence or absence of secondary metabolites such as carbohydrates (Molisch's test and Benedict's test), protein (Biuret test), aminoacids (Ninhydrin test), alkaloids (Wagner and Dragendorff's tests), flavonoids, tannins, saponins, cardiac glycosides (Keller-Killiani test),terpenoids(Salkowski test), steroids (Liberman-Burchard Test), gum(Molisch's test), anthraquinone (Borntrager's test) and phlobatinin by standard methods[18-20].

Antimicrobial activity

Test organisms

The following cultures of Gram negative bacteria (*Salmonella typhimurium, Yersinia enterocolitica, Flavobacterium*sp.) and Gram-positive bacteria (*Listeria monocytogenes*) were cultured in nutrient agar and allowed to grow at 37 C in microbial culture laboratory. The fungal cultures of *Aspergillusflavus* and *Pencilliumnotatum* were used for screening antifungal study. The fungal isolates were allowed to grow on a potato dextrose agar (PDA) until they sporulated [20].

Screening for antibacterial activity

The antibacterial activity was assayed by a modification of agar well diffusion method [11]. 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 180rpm. 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 5mm size borer in media and different concentration of Hexane extract were added and water alone as a control. Plates were incubated at 37 C and zone of inhibition was observed after 24 h.

Screening for antifungal activity

Antifungal activity of all various extracts was studied against two fungal strains by the agar well diffusion method [11]. The fungal isolates were allowed to grow on a potato dextrose agar (PDA) 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.

Results and Discussion

Plants are used medicinally in different countries as the rich source of potential and powerful drugs.

Phytochemicals analysis

A recent ethno-botanical survey of traditional and folk medicine in India has revealed that most of these plants were still in use from ancient time by the local tribal people [21]. Preliminary phytochemical screening showed the presence of carbohydrate, alkaloids, flavonoids, tannin, saponin, glycosides, phenol, amino acids and the absence of acidic compounds, Steroids, Phlobatinin, Coumarine, Anthraquinone, Resin and gum/mucilage in fresh leaves, stem and flower samples of hexane extract. Whereas the phytochemicals such as terpenoids, cardiac glycosides was present in the fresh leaves of hexane extract and absent in fresh stem and flower. The qualitative phytochemical analysis in hexane extract of fresh samples of *G.polycaulon* was tabulated (Table 1).

A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index [10]. Much of the protective effect of fruits and vegetables has been attributed by phytochemicals [11]. Different phytochemicals have been found to possess a wide range of activities that may help in protection against chronic diseases [22]. The phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects [5].

A large number of phytochemicals belonging to several chemical classes have inhibitory effects on all types of microorganisms *in vitro* [23]. Phenolic compounds, flavanoids and tannins were the major group of compounds acting as primary antioxidants or free radical scavengers [24]. Tannins were known to possess general antimicrobial and antioxidant activities [25]. Saponin was a mild detergent used as hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory, antibacterial and antifungal properties[26]. Flavonoids are capable of treating certain physiological disorder and diseases [27]. Plant steroids possess cardiotonic activity, insecticidal and antimicrobial properties in nutrition, herbal medicine and cosmetics [12].

Antimicrobial activity

The antibacterial and antifungal activity of hexane solvent in leaf, stem and flower extracts of *G.polycaulon* were screened against bacterial cultures of Gram-negative bacteria (*S. typhimurium, Y. enterocolitica* and *Flavobacterium* sp.) and Gram-positive bacteria (*L. monocytogenes*) and fungal cultures such as *A. flavus* and *P. notatum* by agar well diffusion method. Results were compared with the standard drugs such as gentamycin for bacterial cultures. The zone of inhibition was seen in hexane extract against all cultures but the maximum inhibition was found to be 23 mm, 19mm and 20mm at 50µg/ml respectively. Gentamycin have a universal activity against the entire test organism, with zones of inhibition ranging from 18to 32 mm respectively. The extract exhibited significant activity against all the tested fungi compared with the standard drug, Nystatin (10µg/disc) ranging from 8 to 12 mm respectively. The maximum inhibition showed in fresh leaf extract at 50 µg/ml was about 14 mm, whereas fresh stem and flower was found to be 15mm, 9mm at 150µg/ml respectively. All extracts showed good activity against the fungal isolates with zones of inhibition ranging from 8 to 18 mm. The zone of inhibition of fresh hexane extracts of *G. polycaulon* was measured and tabulated (Table 2).

Table 1: Qualitative analysis of hexane extract in fresh samples of *G.polycaulon*

Sl.No	Phytochemicals	Fresh leaves	Fresh stem	Fresh flower
		Hexane	Hexane	Hexane
1.	Carbohydrates	+	+	+
2.	Alkaloids	+	+	+
3.	Flavonoids	+	+	+
4.	Terpenoids	+	-	-
5.	Cardiac glycosides	+	-	-
6.	Steroids	-	-	-
7.	Tannin	+	+	+
8.	Saponin	+	+	+
9.	Phlobatinin	-	-	-
10.	Gum/mucilage	-	-	-
11.	Glycosides	+	+	+
12.	Coumarine	-	-	-
13.	Acidic compounds	-	-	-
14.	Phenol	+	+	+
15.	Resin	-	-	-
16.	Anthraquinone	-	-	-
17.	Aminoacids	+	+	+

Table 2: Antimicrobial activity of hexane extract in fresh samples of *G.polycaulon*

Micro Organisms	Zone of inhibition in mm									Standard
	Fresh leaf (µg/ml)			Fresh stem (µg/ml)			Fresh flower (µg/ml)			
	50	100	150	50	100	150	50	100	150	
	BACTERIA									Gentamycin
Salmonella typhimurium (Gram -ve)	18	12	8	18	13	10	13	12	12	18
Yersinia enterocolitica (Gram -ve)	14	13	10	10	9	9	16	15	15	24
Flavobacterium sp. (Gram -ve)	23	19	10	8	19	8	20	19	8	20
Listeria monocytogenes (Gram +ve)	15	10	6	18	18	16	16	12	6	32
	FUNGUS									Nystatin
Aspergillusflavus	14	15	16	12	14	15	6	8	9	08
PenicilliumNotatum	10	12	14	8	13	14	13	15	16	12

The result was suggested that antibacterial property helps the bacteria to interact strongly with the treatment many bacterial diseases [14]. Fungi can cause damage to the structures, decoration of buildings and also responsible for their indoor air quality [28]. Since time immemorial, plants based drugs have been used for various ailments ranging from common cold to cancer [29].In conclusion, the results showed that the hexane extract of *G.polycaulon* was a broad spectrum agent against both Gram-positive and Gram-negative bacteria and fungi.

Conclusion

Plants contain thousands of phytoconstituents and were valuable sources of new biologically active molecules possess antimicrobial property. Many reports were available regarding phytochemical, anti-bacterial and anti-fungal properties of Medicinal plants. At present, scientists are investigating for medicinal plant as natural products of antimicrobial properties. The present study reveals that the selected plant, *G.polycaulon* serves as a source of phytochemicals and antimicrobials in food and pharmaceuticals industries. Many medicinal plants contain secondary metabolites that possess strong therapeutic, medicinal, aromatic and aesthetic effect lie unexplored or remain under explored. These report help to identify the active components responsible for the development of drugs with good antibacterial properties for therapeutic uses as traditional medicine.

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