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SCREENING OF PHYTOCONSTITUENTS ACTIVITY AND ANTIMICROBIAL EFFICACY OF DIFFERENT EXTRACTS OF GNAPHALIUM POLYCAULON

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ABSTRACT

The present study comprises the efficiency of phytochemical and antimicrobial activity of aqueous, ethanol and hexane extracts of leaf, stem and flower of *Gnaphalium polycaulon.* The qualitative phytochemical investigation was carry out by identifying the presence of major phytochemicals using standard procedure. The selected plant used in traditional Indian medicine was examined against human pathogenic Gram positive and Gram negative bacteria and fungus using the agar well diffusion method against various diseases causing drug resistant microorganisms. Based on the results obtained, the phytochemical screening relieved that the leaf and stem were rich in alkaloids, flavonoids, tannins, carbohydrates, proteins, tannins, Alkaloids, amino acids, fatty acids, glycosides, steroids and saponins, than flower extract and confirmed the presence of all Phytoconstituents in selected plant. The antimicrobial activity of various extracts of *G. polycaulon* showed varied levels of antimicrobial activity against the studied bacterial and fungal pathogens. Result findings provided evidence that crude organic solvent extracts of selected plants contain medicinally important phytochemical compounds and their use in the traditional herbal medicines. Therefore, further studies on this medicinal plant will be widely used in number of pharmacological actions for the treatment of different diseases.

KEYWORDS: Phytochemical, qualitatively analysis, antimicrobial, bacteria, fungus, *G. polycaulon.*

INTRODUCTION

Medicinal plants have been used for centuries as remedy for human diseases because they contain the compounds of therapeutic values [1]. This are of prime importance to the health of individuals and communities and the medicinal values of these economically important plant species is due to the presence of certain secondary metabolites. Aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules. Medicinal plants have a global distribution although they are most abundant in the tropics[2]. Basic phytoinvestigations of the extracts for their major phytocompounds is vital as the active principles of many drugs are the secondary metabolites found in plants [3]. Medicinal plants and their extracts are used in traditional treatments of various diseases [4].

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants [5]. India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants [6]. About three quarters of the World's population relies on plants and its extracts for health care. 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 [7].

From the traditional era, edible plants have been used for treatment of many chronic and fatal diseases. According to the World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. In recent times, natural resources has grown to interest due to the increasing and alarming levels of side effects exhibited by the chemically synthesized drugs [8]. Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs [9].

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 [6]. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides [10]. The presence of diverse phytochemicals suggested that the secondary metabolites vary widely which probably is responsible in conferring a wide spectrum of biological activities. The successive extraction

using solvents of varying polarities can maximize the exploitation of diverse bioactive compounds [3].

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [11]. Plant synthesizes natural bioactive/phytochemical (lipid, protein, starch, sugars, phenol etc.) for the normal growth and development itself [12]. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. Phytochemicals act in numerous ways to assist the body in combating diseases and health problems. The medicinal value of the title plant can be correlated due to the presence of various bioactive chemical constituents [5]. Over 50% of the modern clinical drugs are of natural origin and natural products play an important role in drug development program of the pharmaceutical industry [13].

In recent years, secondary metabolites (phytochemicals) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal plants. The phytochemicals with adequate anti-infective efficacy will be used for the treatment of various infections caused by pathogens. Therefore, there is a need to develop the efficient, safe and inexpensive drugs from plant source are of great importance. Some traditionally useful plants have been shown to exhibit fungi-toxic property. Control measures by using plant extracts are cost effective and non-toxic methods. Efforts have been devoted to search for antifungal material from natural resources by many researchers [10].

Infectious diseases caused by bacteria and fungi affect millions of people worldwide and continue the major cause of death in tropical countries like India and China. The improved hygiene and development of new antimicrobial leads a remarkable progress in prevention, control and eradication of infectious diseases. However, 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 [14]. The active ingredients in medicinal plants are defined as chemical compounds that act directly or indirectly prevent or treat disease. In the recent years, antimicrobial resistance has become a major global problem [15]. 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 [16]. One of the measures to combat this problem is to have continuous investigations into new, safe and effective antimicrobials as alternative agents to substitute the present less effective ones. Plants have been traditionally proved to be a rich

source of novel drug compounds, and the herbal mixtures have made large contributions to human health and well-being [17]. Plant based antimicrobials will help to overcome the resistance problems as well as it will be more reliable than the synthetic products [14].

Contrary to this, plants used for medicinal purpose have been found to have little or no side effects [18]. 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 [19]. 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 [20]. Plant extract has a potential application as natural medicine and to treat diseases as well as the microbiological safety of the human health [21].

Gnaphalium polycaulon is a genus of flowering plants in the Asteraceae family of composite 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 [22]. 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, laryngitis, and congestion [23]. Patients with rheumatism, diarrhea and an increase in urination, combined with sporadic upper jaw pain, may benefit from *G. polycaulon* plant [24]. The investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous. So, this medicinal plant was chosen for our present study with main objectives to screen the phytochemicals constituents and antimicrobial activity.

MATERIALS AND METHODS

The methodology adopted for the study entitled "Screening of Phytoconstituents activity and antimicrobial efficacy of different extracts of *Gnaphalium polycaulon*" is given below:

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 HiMedia, India. 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 Kodanadu, near 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.

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's extractor for 72 h at a temperature not exceeding the boiling point of the solvent into 250 ml of ethanol, hexane 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: Hexane, ethanol and water separately and stored at 4˚C.

Qualitative Analysis of Phytochemical

Standard phytochemical screening tests of plant extracts were carried out for various plant constituents. The tests are carbohydrates (Molisch's test, Fehling test, Keller Killiani test), tests for proteins and amino acids (Millon's test, Ninhydrin test, Biuret test), glycosides (Legal test, Keller-killiani test), anthraquinone glycoside (Borntrager's test, Modified Borntrager's test), sterols and triterpenoids (Liberman Buchardt test, Salkowski reaction), Saponins (Froth test, Haemolytic Zone), Coumarins (test with Ammonia, test with Hydroxylamine hydrochloride), flavonoids (Shinoda test, Fluorescence test, Alkaline Reagent test, Lead acetate test), tannins(test with gelatin, Ferric chloride test, Lead acetate test, Pot. Dichromate test), phenolic compounds (test with $FeCl_3$, test with Folin ciocalteu reagent), alkaloids (Dragendroff's test, Hager's test, Mayer's test, Wagner's test), test for Anthocyanins, test for Leucoanthocyanins and Detection of Fatty acids. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthroquinones using standard procedures [25, 26].

ANTIMICROBIAL ACTIVITY

Test Organisms

The following cultures of Gram-negative bacteria (*Salmonella typhimurium, Yersinia enterocolitica, Flavobacterium* sp.) and Gram-positive bacteria *(Listeria monocytogenes*) were collected and were cultured in nutrient agar and allowed to grow at 37 °C in microbial

culture laboratory. The fungal cultures of *Aspergillus flavus* and *Pencillium notatum* were used for screening antifungal study. The fungal isolates were allowed to grow on a potato dextrose agar (PDA) until they sporulated [27].

Screening for Antibacterial Activity

The antibacterial activity was assayed by a slight modification of agar well diffusion method [28,29]. 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 extract (ethanol, hexane and water) 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 [28, 29]. 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 DISCUSSIONS

Plants and herbs contain more number of secondary metabolites and are responsible for various biologically important activities in human and animals as well. Plants are recognized for their ability to produce a wealth of secondary metabolites, extensively used for centuries to treat a variety of disease.

Phytochemicals Analysis

Different photochemical have been found to possess a wide range of activities, which may help in protection against chronic diseases [5]. Preliminary phytochemical screening of the

plant showed the presence of carbohydrate, alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannin, saponin, glycosides, coumarine, phenol, resin, amino acids and the absence of acidic compounds, anthraquinone, phlobatinin and gum/mucilage in dry samples. The qualitative phytoconstitutent analysis of *G. polycaulon* was evaluated and tabulated (Table1).

Table1. Qualitative analysis in dry samples of *G. polycaulon*

S. No	Phytochemical constituents	Dry leaves			Dry stem			Dry flower		
		Ethanol	Hexane	Water	Ethanol	Hexane	Water	Ethanol	Hexane	Water
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	+	+	+	+	+	+	+	+	+
18.	Anthocyanins	-	-	-	-	-	-	-	-	-
19.	Leucoanthocyanins	-	-	-	-	-	-	-	-	-
20.	Fatty acids	+	+	+	+	+	+	+	+	+

Phenolics are one of the major largest and most ubiquitous groups of plant metabolites that can be found ubiquitously in certain plants [18], which are considered as bioactive and non-nutritional compounds, due to their antioxidant properties, against free radicals effects that exhibit various significant biological activities [8]. Phenolic content of plant extracts possess antimicrobial activity and highly oxidized phenols are inhibitorier because of phenolic toxicity to microorganisms [1]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [16].

Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganism *in vitro* [16]. They are also effective antioxidant and

exhibit stronger anticancer activities. Flavonoids are capable of treating certain physiological disorder and diseases [30]. The biological functions of flavonoids, on the other hand, include protection against allergies, inflammation, free radicals scavenging, hepatotoxins, platelets aggregation, microorganisms, ulcers, hepatoxins and tumors [31]. Tannins are known to possess general antimicrobial and antioxidant activities [32]. A recent report shows that tannins may have potential value as cytotoxic and antineoplastic agents [33].

Saponins have been implicated as bioactive antibacterial agents of plants, which are a glycoside, have the property of precipitating and coagulating red blood cells, which are occurring widely in plants [16]. Saponins protect against hypercholesterolemia and antibiotic properties [5]. Other compounds like saponins also have antifungal properties. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [34]. Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids [16].

Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and antimicrobial properties. They are also used in nutritional preparation, herbal medicine and cosmetics. The efficiency of plant derived antimicrobials is needed to be determined completely [18]. Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones [16]. Steroids and saponins were responsible for central nervous system activities [5].

Antimicrobial Activity

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 [1]. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes [35]. In this study, antibacterial activity of different extracts of *G. polycaulon* was evaluated. The extracts were screened against Gram positive (*S. typhimurium, Y. enterocolitica* and *Flavobacterium* sp.) and Gram negative (*Listeria monocytogenes*) 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 is 23 mm respectively. The zone of inhibition of all various fresh and dry extracts of *G. polycaulon* was measured and tabulated (Table 2).

Fungi can cause damage to the structures, decoration of buildings and are also responsible for their indoor air quality [36]. The antifungal activity of all extracts of *G. polycaulon* parts was evaluated using agar well diffusion method. Antifungal activity of *G. polycaulon* was tested against *A. flavus* and *P. notatum*. The extract exhibited significant activity against all the tested fungi compared with the standard drug, Nystatin (10µg/disc). The maximum inhibition shown in dry leaf extracts is 18 mm respectively. 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 the 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 [37].

Table 2. Antimicrobial Activity of Dry Samples of *G. Polycaulon*

Microorganisms	Solvents	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**
S. typhimurium	Ethanol	20	12	10	17	10	8	23	8	8	18
	Hexane	23	8	8	12	10	6	18	12	10	
	Water	12	6	6	10	12	12	7	7	7	
Y. enterocolitica	Ethanol	7	7	7	10	13	15	8	12	11	24
	Hexane	8	14	15	12	13	13	12	14	19	
	Water	9	9	9	12	13	16	8	10	12	
Flavobacterium sp.	Ethanol	10	11	16	11	12	12	13	14	14	20
	Hexane	14	15	16	8	9	10	8	19	20	
	Water	12	12	12	8	10	12	8	9	10	
L. monocytogenes	Ethanol	9	12	17	12	15	16	10	10	22	32
	Hexane	13	15	19	11	11	16	13	13	16	
	Water	12	15	16	6	8	12	6	18	18	
Fungus											**Nystatin**
A. flavus	Ethanol	9	12	14	13	14	15	8	9	12	08
	Hexane	15	16	18	10	12	14	9	10	12	
	Water	12	13	14	8	9	14	8	9	10	
P. notatum	Ethanol	13	14	13	12	9	17	15	14	16	12
	Hexane	12	17	9	13	8	18	13	15	9	
	Water	16	19	11	11	11	12	12	8	9	

The reason for the difference sensitivity between the Gram positive and Gram negative bacteria could be described to the morphological differences between these microorganisms, Gram negative pathogens having an outer phospholipid 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 peptidoglycan layer, which is not an effective permeability barrier [1].

CONCLUSION

The results in my present study was concluded that *G. polycaulon* plant has acts as rich source of phytochemical due to the presence of medicinally important major phytoconstituents. *in vitro* evaluations of this plant for antimicrobial property help in the identification of therapeutically potent 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 bioactive compounds reported in various extracts evidences of their medicinal activities provide ample proof to the therapeutic and pharmacological potential of *G. polycaulon* which needs to be further explored and some pharmacological activities has to be performed and validated so as to use it as a potential force in the field of healthcare against many diseases to isolate, identity, characterize and elucidate the structure of the bioactive components.

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