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Research Article

INVESTIGATION OF ANTIOXIDANT ACTIVITY IN DIFFERENT SOLVENTS OF *GNAPHALIUM POLYCAULON*

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ABSTRACT

Objective: The objectives of the present study were to investigate antioxidant activity of the *Gnaphalium polycaulon* in different solvents.

Methods: Fresh *Gnaphalium polycaulon* was collected from Kodanadu, The Nilgiri District. Plant materials are washed, air dried and coarsely powdered by soxhlet apparatus for organic solvent extraction with hexane and ethanol at 4°C. Then all the extracts obtained were subjected for antioxidant analysis using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3- ethylbenzothiazoline- 6-sulphonic acid) assay (ABTS).

Results: The antioxidant analysis of *G.polycaulon* showed that the leaf and stem were rich in antioxidant than flower.

Conclusion: Our findings provided evidence that crude solvent extracts contain medicinally important free radicals compounds for the treatment of different diseases like cancer in the traditional folk medicines.

Keywords: Free radicals, antioxidant, DPPH, ABTS, *G. polycaulon*

INTRODUCTION

India is a varietal emporium of medicinal plants among the richest countries in the world in regard to genetic resources of medicinal plants. Natural plant products with pharmacological and biological activities play a very important role in medicine [1]. The use of local plants in folk medical practices has a long history. The resource base of the traditional medical practices is prevalent in rural and tribal villages of India and abroad. Medicinal plants are used to maintain and promote healthy life, prevent disease and cure ailments. About 80 % of the world population gets relies on herbal traditional medicine for their primary health care [2].

Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine [3]. Traditional herbal medicine is an important component of primary health care system in developing countries [4]. Medicinal plants and their extracts are used in traditional treatments of various diseases [5]. Aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules [6].

Plants are the important source for free radical scavenging molecules. Intake of natural antioxidant has been associated with reduced risk of cancer, cardiovascular diseases, diabetes and other diseases associated ageing [7]. Free radical oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders. Free radicals are highly reactive particles with an unpaired electron and are produced by radiation or as byproducts of metabolic processes that lead to disintegration of cell membranes and cell compounds [8]. A serious imbalance between the production of free radicals and antioxidant defense system is responsible for oxidative stress.

Oxidative stress is related to the aging process and some chronic diseases such as cancer, cardiovascular diseases and diabetes. Dietary antioxidants protect the body against free radicals [9]. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS), and reactive nitrogen species (RNS). Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates, and DNA. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases [10].

Antioxidant activity has been proposed to play vital role in various pharmacological activities such as anti-aging, anti-inflammatory, and anti-atherosclerosis activities. Several synthetic antioxidants are available, but are quite unsafe and their toxicity is of great concern. Natural products with antioxidant activity may be used for human consumption because of their safety [7]. Antioxidant is one of the most essential ingredients of today's menu/therapy because the antioxidative system protects the animal against reactive oxygen species that induced oxidative damage. Various synthetic antioxidants are on the use, but they are suspected to be carcinogenic. Natural antioxidants, therefore, have gained importance [10].

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. Chromatophore ABTS+ was formed by the reaction between ABTS and potassium per sulphate and reduced to ABTS by the action of antioxidants available in the extracts [10]. Flavonoids and some other phenolic compounds of plant origin have been reported as scavengers of free radicals. Hence, nowadays search for natural antioxidant source is gaining much importance [11]. Reactive oxygen species are produced as a natural byproduct/ intermediates in biological processes in body by the normal oxygen metabolism [12].

G. polycaulon is a genus of flowering plants in the Asteraceae family of compositae type, worldwide distribution and is mostly found in temperate and subtropical regions of the world. The entire plant is harvested during flowering and is used to make herbal and homeopathic remedies [13]. The homeopathic remedy has no known side effects. 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 [14]. Patients with rheumatism, diarrhea and an increase in urination, combined with sporadic upper jaw pain, may benefit from *G. polycaulon* [15].

In current herbal drug scenario, plant derived antioxidants are of gaining importance because of their potential health benefits, no toxicity and side effects over synthetic antioxidants like butyl hydroxy anisole and butyl hydroxy toluene respectively. Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites [3]. Epidemiological and *invitro* studies suggested that plants are major constituents in antioxidant based drugs/formulations used for the prevention of complex diseases [10]. The plant kingdom has not been exhausted based on the species of medicinal plants which are yet to be discovered. Based on this, the investigations on biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are abundant. So, this medicinal plant was

chosen for our present study with main objectives to screening the phytochemicals constituents that involved in antioxidant activity.

MATERIALS AND METHODS

Chemicals required

All chemicals used for this study were high quality analytical grade reagents. The methanol solvent was 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

Fresh *G. polycaulon* plants were collected form Kodanadu, near Kotagiri in Nilgiri District, Tamil Nadu, India, them, fresh leaf, stem and flower have been separately collected and sun dried for one day and powdered. Then the samples were separately used for the extraction process.

Extraction of plant material

About 40 g of the each powdered samples were individually subjected for methanolic extraction process by using Soxhlet's extractor for 72 h at a temperature not exceeding the boiling point of the solvent. Resulting extracts was filtered using Whatman filter paper (No.1) and concentrated in vacuum to dryness using a Rotary evaporator and stored at 4^{o}C.

Antioxidant activity / Free radical scavenging activity

DPPH scavenging assay

The scavenging ability of the natural antioxidants of the plant extracts towards the stable free radical DPPH were measured by the method of Mensor *et al.* [16]. Each plant extracts (20 µl) were added to 0.5 ml of methanolic solution of DPPH and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 min. Methanol served as the blank and DPPH in methanol, without the plant extracts, served as the positive control. After 30 min of incubation, the discolouration of the purple colour was measured at 518 nm in a spectrophotometer. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = 100 - \frac{A_{518}\ (\text{Sample}) - A_{518}\ (\text{Blank})}{A_{518}\ (\text{Blank})} \times 100$$

ABTS scavenging assay

The antioxidant effect of the plant extracts were studied using ABTS (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method of Shirwaikar *et al.* [17]. ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium per sulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 h before use. Aliquots (0.5 ml) of the three different extracts were added to 0.3 ml of ABTS solution and the final volume was made up to 1 ml with ethanol. The absorbance was read at 745 nm in a spectrophotometer and the percent inhibition was calculated using the formula

$$\text{Scavenging activity \%} = 100 - \frac{A_{745}\ (\text{Sample}) - A_{745}\ (\text{Blank})}{A_{745}\ (\text{Blank})} \times 100$$

RESULTS AND DISCUSSIONS

Plants and herbs contain more number of secondary metabolites as they are responsible for several biological activities in human beings and animals. It has been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases.

Antioxidant activity

DPPH scavenging activity

The antioxidant activity of different plant extracts was determined using methanol solution of DPPH reagent. DPPH is a very stable free radical. The effect of an antioxidant on DPPH radical scavenging is due to their hydrogen donating ability or radical scavenging activity [9]. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenylpicryl hydrazine with the loss of its violet color [10].

Table 1: DPPH scavenging activity of dry samples of *G. polycaulon*

Plant samples	Concentration (µg/ml)	Percentage of Inhibition (%) DPPH (2, 2-diphenyl-2-picryl hydrazyl hydrate) assay		Standard Ascorbic acid
		Hexane	Ethanol	
Stem	100	61.7±0.03	66.0±0.01	88.30±0.01
	200	64.0±0.01	68.9±0.03	76.15±0.01
	300	66.0±0.02	71.8±0.02	67.03±0.02
	400	69.0±0.01	74.5±0.01	53.98±0.03
	500	70.5±0.01	77.8±0.01	45.11±0.01
Leaf	100	64.7±0.01	65.9±0.03	88.30±0.01
	200	68.4±0.02	67.8±0.01	76.15±0.02
	300	73.6±0.04	71.1±0.01	67.03±0.01
	400	76.2±0.03	73.3±0.02	53.98±0.01
	500	82.6±0.01	79.1±0.03	45.11±0.01
Flower	100	61.1±0.02	63.1±0.01	88.30±0.01
	200	64.7±0.03	66.5±0.01	76.15±0.01
	300	68.0±0.01	70.3±0.02	67.03±0.02
	400	70.3±0.01	72.4±0.03	53.98±0.02
	500	73.8±0.02	76.9±0.02	45.11±0.03

All values are expressed as mean ± SD for three determinations

The results of the DPPH radical scavenging activity of dry leaves sample of *G. polycaulon* shows that it possesses very high percentage antioxidant activity of 82.6 %, and 79.1 % at a concentration of 500 µg/ml of Hexane, and ethanolic extract.

70.5 % and 77.8 % of antioxidant activity was observed at a concentration of 500 µg/ml of Hexane, and ethanolic extract of dry stem of *G. polycaulon*. In the dry flower sample of concentration 500 µg/ml of Hexane, and ethanolic extract, an antioxidant activity of 73.8 %, and 76.9 % was observed and tabulated in Table 1.

The results showed that *G. polycaulon* extracts have hydrogen donors that scavenge the free radical DPPH, with high Antioxidant activity about 82.6 % at 500 µg/ml in Hexane extract of dry leaves that was observed to be higher than standard Ascorbic acid.The *in vitro* antioxidant assay of the plant extract reveals significant antioxidant potential compared with standard.

The result of DPPH scavenging activity assay in this study indicates that the plant was potently active [8]. The ability of this plant extract to scavenge DPPH could also reflect its ability to inhibit the

formation of ABTS+. The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in visible region [11]. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the color changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract [18].

ABTS radical scavenging activity

ABTS radical, a protonated radical has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals [9]. The ABTS radical cation scavenging activity of the plant extracts were lesser in percentage when compared with that of DPPH [11].

Among all the extracts, hexane extracts gave better results. The highest percentage of antioxidant activity was 77.10 % in 500 µg/ml concentration of hexane extract than 76.3 % in 500 µg/ml of ethanolic extract in dry leaves of *G. polycaulon*. In Fresh stem, hexane and ethanolic extract showed 71.90 % and 72.70 % antioxidant activity at a concentration of 500 µg/ml (Table 2). The dry flower of hexane and ethanolic extract showed antioxidant activity of 71.7 % and 69.2 % at a concentration of 500 µg/ml respectively. Higher concentrations of the extracts were more effective in quenching free radicals in the system [8].

Table 2: ABTS radical scavenging activity of dry samples of *G.polycaulon*

Plant samples	Concentration (µg/ml)	Percentage of Inhibition (%)		Standard Ascorbic acid
		ABTS (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) assay		
		Hexane	Ethanol	
Stem	100	63.0±0.02	63.0±0.01	82.50±0.01
	200	65.4±0.01	64.4±0.02	71.03±0.01
	300	67.7±0.03	68.2±0.04	60.88±0.02
	400	68.8±0.01	69.8±0.03	51.08±0.02
	500	71.9±0.02	72.7±0.01	42.79±0.03
Leaf	100	63.9±0.02	67.6±0.02	82.50±0.01
	200	68.4±0.01	70.0±0.01	71.03±0.02
	300	71.3±0.01	71.0±0.03	60.88±0.02
	400	74.9±0.01	74.0±0.02	51.08±0.01
	500	77.1±0.01	76.3±0.01	42.79±0.03
Flower	100	61.1±0.03	61.6±0.01	82.50±0.02
	200	63.9±0.04	64.3±0.02	71.03±0.01
	300	65.8±0.02	66.1±0.01	60.88±0.01
	400	68.5±0.01	67.6±0.02	51.08±0.03
	500	71.7±0.01	69.2±0.02	42.79±0.02

All values are expressed as mean ± SD for three determinations

CONCLUSION

The present study showed the antioxidant properties of medicinal plants playing part significantly in postnatal recovery. Herbal medicines are not only providing traditional and ethnic medicine but also promising for highly efficient novel bioactive molecules. The results revealed the presence of medicinally important phytoconstituents in the plants showed that *G. polycaulon* plant is good source of antioxidant. Many plants with strong therapeutic, medicinal, aromatic and aesthetic effect lie unexplored or remain under explored.

These findings help to identify the active components responsible for the development of drugs for therapeutic uses as traditional medicine and also have a potential application as natural medicine. Further studies are on going to isolate, identity, characterize and elucidate the structure of the bioactive components.

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