

A COMPARATIVE STUDY OF ANTIOXIDANT POTENTIAL OF *GNAPHALIUM POLYCAULON*, TRADITIONAL INDIAN FOLK MEDICINAL PLANT

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ABSTRACT

The current study was undertaken to examine the antioxidant value of *Gnaphalium polycaulon* in different solvents. The fresh plant parts were collected from Kodanadu, The Nilgiri District, South India. Plant materials are washed, air dried and coarsely powdered by soxhlet apparatus for organic solvent extraction methanol, ethanol, hexane and water at 4°C. Then all the extracts obtained were subjected for antioxidant analysis using enzymic, non-enzymic and total antioxidant assays. All the methanolic extracts exhibited antioxidant activity significantly. The order of antioxidant value in *G.polycaulon* showed that the leaf, stem and flower. we reported that our finding provided support that the crude solvent plant extracts contain medicinally important free radicals scavenging compounds due to the strongly presence of phytoconstituents. The study reveals that the consumption of the most valuable plant, *G.polycaulon* would exert several beneficial effects by virtue of their antioxidant activity in the traditional folk medicines for the treatment of different diseases.

Keywords: *Gnaphalium polycaulon*, medicinal plant, free radicals, antioxidant, DPPH*, ABTS*

1. INTRODUCTION

Aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties that can protect the human body against both cellular oxidation reactions and pathogens (Shanmugapriya and Thayumanavan, 2013). Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Bhore *et al.*, 2012). The use of plants for medicinal purposes and folk medical practices can be traced back to earlier civilization that is prevalent in rural and tribal villages (Badugu 2012). Traditional herbal medicine is an important component of primary health care system in developing countries for the revival of herbal plants (Shanmugapriya *et al.*, 2014). Medicinal plants have a global distribution although they are most abundant in the tropics. About 80% of the world population relies on herbal traditional medicine for their primary health care (Rubina and Qaiser, 2008).

Free radicals are highly reactive particles with an unpaired electron and are produced by radiation or as byproducts of metabolic processes (Shanmugapriya *et al.*, 2011). A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress (Chakraborty and Shah, 2011). Dietary antioxidants protect the body against free radicals (Sudha, 2011). Antioxidants are agents which scavenge the free radicals and prevent the damage

caused by reactive oxygen species (ROS), 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 DNA (Shanmugapriya *et al.*, 2012). Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones (Mishra and Satpal, 2011).

Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack (Chew *et al.*, 2011). Flavonoids are capable of treating certain physiological disorder and prevent oxidative cell damage (Okwu, 2004). In addition, vitamin C, vitamin E and carotenoids, polyphenols (a wide class of components including phenolic acids, catechins, flavonols and anthocyanins), have shown strong antioxidant capacity (Zhishen *et al.*, 1999). 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 persulphate and reduced to ABTS by the action of antioxidants available in the extracts (Mensor, 2001).

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 (Shih and Ming, 2006). The entire plant is harvested during flowering and is used to make herbal and homeopathic remedies (Bhupendra *et al.*, 2008). 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 (Uniyal and Shiva, 2005).

Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. Researchers revealed that the plant kingdom has not been exhausted based on the species of medicinal plants which are yet to be discovered. The investigations of natural antioxidants from medicinal plants are numerous. In current herbal drug scenario, plant derived antioxidants are gaining importance because of their potential health benefits, no toxicity and side effects over synthetic antioxidants (Jaina *et al.*, 2011). Plants may contain a wide variety of free radical scavenging molecules based drugs/formulations used for the prevention of complex diseases (Jinu *et al.*, 2014). So, this unique medicinal plant was chosen for our present study with main objectives to highly remarkable investigates antioxidant potential.

2. MATERIALS AND METHODS

2.1. 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.

2.2. Collection of plant material

The fresh leaves, stem and flower of *Gnaphalium polycaulon* plant were collected from Kodanadu near Kotagiri in The Nilgiri district, South India.

2.3. 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 72h at a temperature not exceeding the boiling point of the solvent into 250ml of methanol, 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 appropriate solvents used for extraction separately and stored at 4 °C for further use.

2.4. Antioxidant activity

2.4.1. Enzymic antioxidants

The enzymic antioxidants were analyzed in the fresh plant parts by standard methods. The assays were catalase (CAT) (Luck,1974), Peroxidase (POD) (Reddy *et al.*, 1995), Glutathione S-Transferase (GST) (Habig *et al.*, 1974) and Polyphenol Oxidase (PPO) (Esterbauer *et al.*,1977).

2.4.2. Non-enzymic antioxidants

The non-enzymic antioxidants were estimation out with standard methods such as Ascorbic acid (Sadasivam. and Manickam, 1997), Tocopherol (Sadasivam. and Manickam, 1997), Total Carotenoids and Lycopene (Zakaria *et al.*, 1979), and Reduced Glutathione (Boyne and Ellman, 1972).

2.4.3. Free radical scavenging activity

2.4.3.1. 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.*, 2001. Each plant extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH• in methanol, without the plant extracts, served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer. The radical scavenging activity was calculated as follows:

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

2.4.3.2. 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.*, 2006. ABTS radical cations (ABTS••) were produced by reacting ABTS solution (7mM) with 2.45mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the three different extracts were added to 0.3ml of ABTS•• solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm in a spectrophotometer and the percent inhibition was calculated using the formula

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

3. RESULTS AND DISCUSSION

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 disease.

3.1. Antioxidant Activity

The enzymic antioxidants in the fresh plant parts were analyzed by the assay of catalase (CAT), peroxidase (POD), glutathione S-transferase (GST) and polyphenol oxidase (PPO). The results are tabulated in Table-1. In compared with all extracts, the values of methanolic leaf extract of each assay were found to be CAT (0.71), POD at 30 sec and 60 sec (0.803 and 0.935), GST at 15 sec and 30 sec (0.69 and 0.68) and PPO (1.60) are expressed in U/mg protein. All assays reported that the fresh methanolic leaf extracts of *G.polycaulon* showed high enzymic antioxidants than other extracts.

3.2. Non-enzymic Antioxidant

The non-enzymic antioxidants were ascorbic acid, α -tocopherol, total carotenoids, lycopene, and reduced glutathione. The results were reported and tabulated (Table-2). In compared with all extracts, the values of methanolic leaf extract of each assay were found to be Ascorbic acid (0.614), Total Carotenoids and Lycopene at 450 and 503 nm (0.631 and 0.582), Reduced Glutathione at 412 nm (0.621) and Tocopherol (0.017) in mg/g. The fresh methanolic leaf extracts of *G.polycaulon* showed high enzyme antioxidants than other extracts.

3.3. 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 (Shanmugapriya *et al.*, 2014). 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 (Mensor *et al.*, 2001). The results of the DPPH radical scavenging activity of *G.polycaulon* showed that the fresh methanolic extract possesses very high percentage antioxidant activity of 84.5% at a concentration of 500 $\mu\text{g/ml}$ than stem and flower in others solvents. The results shows that *G.polycaulon* plant extracts have hydrogen donors that scavenge the free radical DPPH•, with high Antioxidant activity in methanolic

fresh leaf extract that was observed to be higher than standard (Table-3 and Figure-1).

The result of DPPH• scavenging activity assay in this study indicates that the plant was potently active (Yogesh *et al.*, 2011). The ability of this plant extract to scavenge DPPH• could also reflect its ability to inhibit the formation of ABTS•+ (Stephanie *et al.*, 2009). 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 (Tyagi *et al.*, 2010). 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 power of the extract.

3.4. ABTS•+ radical scavenging activity

ABTS•+ radical, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals. The ABTS•+ radical cation scavenging activity of the plant extracts were lesser in percentage when compared with that of DPPH• (Kriengsak *et al.*, 2006). Higher concentrations of the extracts were more effective in quenching free radicals in the system (Stephanie *et al.*, 2009). The highest percentage of antioxidant activity was 82.0% in 500 $\mu\text{g/ml}$ concentration of methanolic extract (Table-4 and Figure-2). Among all the extracts, methanolic leaf extracts showed better results, fast and effective scavengers of the ABTS•+ radicals. Higher concentrations of the extracts were more effective in quenching free radicals in the system.

4. CONCLUSION

Antioxidant activity of medicinal plants plays a significantly part in postnatal recovery. Herbal medicines are not only providing traditional medicine but also promising for highly efficient novel bioactive molecules. The results revealed the presence of phytoconstituents in the plants showed that *G.polycaulon* plant is good source of antioxidant. The free scavenging activities of the *G.polycaulon* plants extracts are more effective, safe and non toxicity than synthetic antioxidant effects. Many medicinal plants lie unexplored or remain under explored. This finding supports the efficiency of selected plant as antioxidant additives or as nutritional supplements for human health in traditional medicine.

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Table 1. Enzymic antioxidant activity of fresh samples of *G.polycaulon*.

Enzymic antioxidant (U/mg protein)	OD (nm)	OD Value (nm) of fresh samples of <i>G.polycaulon</i>											
		Fresh leaf				Fresh stem				Fresh flower			
		M	E	H	W	M	E	H	W	M	E	H	W
CAT	240	0.67	0.65	0.70	0.64	0.60	0.58	0.61	0.62	0.60	0.51	0.56	
	30 sec	0.792	0.787	0.799	0.781	0.756	0.721	0.772	0.685	0.601	0.628	0.673	
	60 sec	0.889	0.853	0.922	0.801	0.786	0.691	0.790	0.699	0.678	0.656	0.687	
PPO	15 sec	0.53	0.49	0.61	0.57	0.51	0.47	0.50	0.49	0.32	0.34	0.44	
	30 sec	0.51	0.48	0.60	0.56	0.49	0.42	0.48	0.51	0.48	0.43	0.49	
PPO	495	1.54	1.52	1.56	1.56	1.41	1.39	1.42	1.36	1.33	1.42	1.33	

Table 2. Non-Enzymic antioxidant activity of fresh samples of *G. polycaulon*.

Non-Enzymic antioxidant (mg/g)	OD (nm)	OD Value (nm) of fresh samples of <i>G. polycaulon</i>											
		Fresh leaf				Fresh stem				Fresh flower			
		M	E	H	W	M	E	H	W	M	E	H	W
Ascorbic acid	540	0.614	0.587	0.563	0.605	0.612	0.594	0.566	0.600	0.581	0.578	0.546	0.531
Total	450	0.	0.	0.	0.	0.629	0.581	0.572	0.617	0.574	0.566	0.552	0.572
Carotenoids and Lycopene	503	631	598	584	613								
Reduced Glutathione	412	0.582	0.537	0.528	0.576	0.580	0.543	0.530	0.578	0.558	0.521	0.519	0.542
Tocopherol	460, 520	0.621	0.605	0.591	0.611	0.615	0.590	0.592	0.607	0.611	0.596	0.584	0.605
		0.017	0.011	0.008	0.013	0.015	0.011	0.009	0.012	0.011	0.008	0.005	0.010

Table 3. DPPH• scavenging activity of fresh samples of *G. polycaulon*.

Plant samples	Concentration (µg/ml)	Percentage of Inhibition (%)				Standard Ascorbic acid
		DPPH assay				
		M	H	W	P.E	
Fresh Stem	100	67.9	66.2	64	66.7	88.30
	200	71	69.6	66.4	70.2	76.15
	300	78.6	72.3	70.1	76.4	67.03
	400	84	78.1	73.2	79.2	53.98
	500	87	82.1	77.5	82.1	45.11
Fresh Leaf	100	71.9	64.1	69.6	71.2	88.30
	200	75.7	67.3	72.7	73.3	76.15
	300	78.4	72.4	75.7	77.2	67.03
	400	82.3	79	78.8	80.7	53.98
	500	84.5	83.5	82.3	83.9	45.11
Fresh Flower	100	61.5	60.1	60	60.3	88.30
	200	68.7	65.2	64.2	64.4	76.15
	300	70.2	67.5	65.6	69.3	67.03
	400	72.7	71.8	71	72.4	53.98
	500	78	73.8	72.3	74.7	45.11

Table 4. ABTS^{•+} radical scavenging activity of fresh samples of *G. polycaulon*.

Plant samples	Concentration (µg/ml)	Percentage of Inhibition (%)				Standard Ascorbic acid
		ABTS assay				
		Methanol	Hexane	Water	Ethanol	
Fresh Stem	100	64.5	63.9	63.3	63.7	82.50
	200	67.5	67.3	66.8	66	71.03
	300	70.9	69.2	70.1	67.8	60.88
	400	73.9	71.4	71.3	69.2	51.08
	500	78	74.9	75.3	70.9	42.79
Fresh Leaf	100	65.4	61.4	65.1	64.8	82.50
	200	67.7	65.5	65.5	67.1	71.03
	300	71.9	68.7	70.6	69.6	60.88
	400	78.2	72.4	72.7	71.7	51.08
	500	82	77.6	74.8	73.7	42.79
Fresh Flower	100	60.3	59.6	58.8	56.1	82.50
	200	64.2	63	62.6	58.5	71.03
	300	65.7	65.1	63.5	61.6	60.88
	400	71	69.5	66.1	64	51.08
	500	72.5	72.1	68.2	66.4	42.79

Fig. 1 DPPH• scavenging activity of fresh samples of *G. polycaulon*

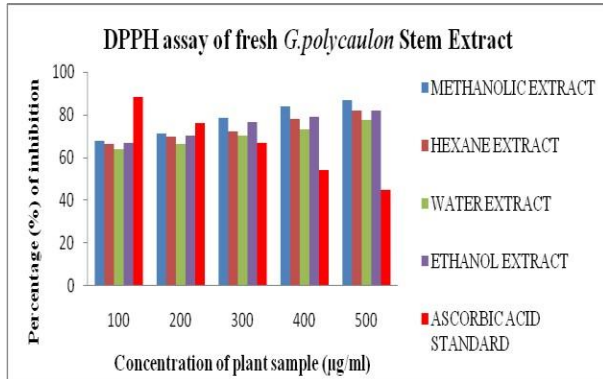


Fig. 2 ABTS^{•+} scavenging activity of fresh stem, leaf and flower samples of *G. polycaulon*.

