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Antioxidant Investigation of Dried Methanolic Extracts of *Gnaphalium polycaulon* Pers, An Indian Folkloric Ethnomedicinal Plant of the Nilgiri, Tamil Nadu, India

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Abstract

Objective: The present study is to evaluate the enzymic, non-enzymic and total antioxidant activity in *Gnaphalium polycaulon* by standard methods.

Methods: Each plants extract were subjected to Soxhlet extraction and then dried methanolic extracts (leaf, stem and flower) were carried out to assess the antioxidant activity for enzymic, non-enzymic and total antioxidants present in *Gnaphalium polycaulon* plant.

Results: Based on the results obtained in the present investigation, the antioxidant screening relieved that the dried methanolic leaf showed very significant antioxidant activity due to the presence of phytoconstituents in leaf than stem and flower extracts and thereby it confirmed the antioxidant activity in *Gnaphalium polycaulon*.

Conclusions: These present findings clearly concluded that polyphenolic components have antioxidant potentialin *Gnaphalium polycaulon* extracts. Therefore, further studies on the medicinal plants will be of great importance and widely used in number of pharmacological actions for the treatment of different diseases with high content of major phytoconstituents.

Keywords: Phytoconstituents; Antioxidant activity; Enzymic; Non-enzymic and total antioxidants; Correlation; *Gnaphalium polycaulon*

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Introduction

India is extremely rich in medicinal plants comprising about 8000 species and around 70% are found in the tropical areas mostly in the various forest types spread across the Western Ghats, Eastern Ghats, etc. [1]. Herbal medicines have continued to be a part of the medical field from the early Chinese Empires to modern physicians [2]. Medicinal and aromatic plants are used widely in the later years of the 21st century. It constitute the backbone of traditional medicine practices. The importance of traditional medicine (TM) as a source of primary healthcare was first officially recognized by the World Health Organization by Traditional Medicine Program. Researchers are increasingly turning their attention to herbal products, looking for new leads to investigate phytochemical investigation and to develop better drugs against Oxidative damage of cells. 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. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. Ethnobotany is the scientific study of relationship that exists between people and plants. It interface between indigenous people and their wild exploit of plants around them, which is a significant aspect of biological diversity conservation [3] Plants have always been the principal source of drugs for the prevention and treatment of disease and also for the production of some drugs currently used in modern medicine. Many reports stated that Asteraceae family is considered as abundant flowering plants with many therapeutic properties and reported as folkloric medicinal plants [4]. The genus, *Gnaphalium* was reported to

have antioxidant, antibacterial, antifungal, anti-complement, antitussive and expectorant, insect anti-feedant, cytotoxic, anti-inflammatory, anti-diabetic and anti-hypouricemic properties [5] due to the presence of flavonoids, phenolic glycosides, diterpenes, triterpenes, phytosterols and other major phytocompounds. The genus *Gnaphalium*, an herb distributed worldwide, comprises approximately 200 species of the Compositae (Asteraceae) family that belongs to the tribe Gnaphalieae. *G. polycaulon* is an annual widespread weed in tropical and subtropical Africa, Asia, Australia, and America [6]. The rich availability of *G. polycaulon* in the Western Ghats particularly in and around Kothagiri areas is due to the humid climatic conditions in hill stations. Based on these reports, *G. polycaulon* is a unique habitat of Western Ghats, The Nilgiris so this places were selected as the study area to carry out the present investigation Researchers on Biodiversity studies 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 biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous. Most of the modern researches on herbal medicine have hinged around traditional folklore medicine. The modern medicine has brought with it an array of drugs, none of which is non-toxic and quite safer for human consumption [7]. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the plant. So, this medicinal plant was validated with main objectives to screening and analyses antioxidant activity.

Materials and Methods

Collection and identification of plant samples

Following the collection, the plant samples were submitted to Botanical Survey of India (BSI), Coimbatore. They have been identified and confirmed as *Gnaphalium polycaulon* Pers. (*Gnaphalium indicum* Hook. f.) - Asteraceae family. Herbarium specimen has been authenticated and incorporated by the Madras Herbarium.

Chemicals required

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

Preparation and extraction of plant extracts

Following the confirmation, the standard protocols were followed to extract phytoconstituents from dried plant parts (leaves, stems and flowers), parallely with methanol as solvent. Resulting extracts were individually concentrated by applying vacuum to get dry powder using rotary evaporator and subjected to lyophilizaion. The obtained crude extracts were packed in air-tight plastic containers and stored in refrigerator at 4°C for further applications. The extracts were used for analysis the antioxidant activity in the present study.

Evaluation of *in vitro* antioxidant activity of *Gnaphalium polycaulon* extracts

Based on the phytoconstituents analysis, antioxidant activity was analysed, since there is no report available on *G. polycaulon* on these aspects in further studies.

Enzymic antioxidants

The enzymic antioxidants methods were followed to determine the antioxidant activity of dried *G. polycaulon* extracts. According to the previously reported standard protocols [7], all the assay methods such as Catalase (CAT), Peroxidase (POD), Glutathione S-Transferase (GST), Polyphenol Oxidase (PPO), Superoxide Dismutase (SOD), Glucose-6-phosphatedehydrogenase (G6PD) have been carried out in all extracts.

Non-enzymic antioxidants

According to the previously reported standard protocols [8], all non-enzymic antioxidants assays are Ascorbic acid, Total carotenoids and lycopene, Reduced glutathione, α-tocopherol, Total phenols, Total flavonols, Flavonoids have been carried out in all dried *Gnaphalium polycaulon* extracts.

Evaluation of free radical scavenging activity of *Gnaphalium polycaulon* extracts

The dried extracts of *Gnaphalium polycaulon* were analysed for their radical scavenging ability against a free radicals and oxidants using standard methods. The absorbance values were measured in a UV-Vis spectrophotometer and the percentage inhibition of free radical scavenging activity was calculated with standard formula as follows:

%inhibition of radical scavenging activity=A_{518}(control-A_{518}(sample)/A_{518} × 100

Where, A_{518} (control) is the absorbance of Control; A_{518} (sample) is the absorbance of free radical+sample extract/ standard. The graph against percentage inhibition versus concentration was plotted using Graph Pad Prism 5.0 program and its IC_{50} values (Concentration required for 50% inhibition of radicals) were calculated for all antioxidants assays in triplicates.

Dot-plot rapid screening assay

The rapid screening assay was performed by the method proposed by Soler-Rivas [9]. Aliquots of plant extracts were spotted carefully on TLC plates and dried for 3 min. The sheets bearing the dry spots were placed upside down for 10 secs in a 0.4 mM DPPH solution in methanol and the layer was dried. The stained silica layer revealed a purple background with yellow spots, which showed radical scavenging capacity. The ability of the plant extracts to scavenge the DPPH radical was tested in a rapid dot-plot screening and quantified using a spectrophotometer.

DPPH radical scavenging activity

The scavenging ability of the natural antioxidants of the plant material towards the stable free radical DPPH assay was measured by the method of Mensor [10]. About 20 µl of various concentration of each plant extract was 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. Following incubation, the dis colouration of the purple colour and the absorbance values was measured at 518 nm using a UV-Vis spectrophotometer.

FRAP radical scavenging activity

The FRAP assay was carried out with a slight modified method, proposed by Benzie [11]. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM 2, 4, 6- tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. Different concentration (100-500 µg/ml) of plant extracts were allowed to react with 2850 ml of the FRAP solution for 30 min in the dark condition. Readings of the colored product were then taken at 593 nm using UV-Vis spectrophotometer. Results are expressed in mM ascorbic acid/g dry mass. Results are expressed in mM TE/g fresh mass. The final result was expressed as the concentration of antioxidant having a ferric reducing ability.

ABTS·$^+$radical scavenging activity

$ABTS^+$ or 2, 2'-azinobis (3-ethylbenzthiazoline)-6-sulphonic acid free radical cation decolourization assay of the antioxidant effect in the plant extracts was done according to the method of Shiwaiker [12]. The pre-formed radical monocation of $ABTS^+$ was generated by reacting $ABTS^+$ solution (7mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 15 h in dark at room temperature before use. The aliquot of 0.5 ml of each sample was added to 0.3 ml of $ABTS^+$ free radical cation solution and the final volume was made upto 1 ml with ethanol. The absorbance, monitored for 5 min was measured spectrophotometrically at 734 nm using UV-Vis spectrophotometer. Appropriate solvent blanks were run in each assay. The absorbance was read at 745 nm in a spectrophotometer.

ORAC radical scavenging activity

The ORAC (Oxygen radical absorbance capacity) free radical cation decolourization assay of the antioxidant effect in the plant extracts was performed by using the method of Prior [13]. In ORAC procedure, an automated plate reader with 96-well plates was used. Analyses were conducted in phosphate buffer pH 7.4 at 37°C. Peroxyl radical was generated using 2,2'-azobis (2-amidino-propane) dihydrochloride which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were excitation at 485 nm and emission at 520 nm. Results are expressed as mM Ascorbic acid/g dry weight.

Reducing power activity

The reducing power was quantified by the Fe^{3+}-Fe^{2+} transformation in the presence of the fractions according to the method of Oyaizu [14] Different concentrations of the plant extracts in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferric cyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%, 2.5 ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 ml) and the absorbance was measured at 700 nm using UV-Vis spectrophotometer. The reducing power of the extract was linearly proportional to the concentration of the sample. Higher absorbance of the reaction mixture indicated higher reductive potential.

Nitric oxide radical scavenging activity

The extent of inhibition of nitric oxide radical generation *in vitro* was followed as per the method reported by Makchuchit [15]. The reaction was initiated by adding 2.0 ml of 100 mM sodium nitroprusside, 0.5 ml of phosphate buffered saline (pH 7.4), 0.5 ml of plant extracts (100-500 µg/ml) and incubated at 25°C for 30 min. About 0.5 ml of Griess reagent was added and incubated for another 30 min. Control tubes were prepared without plant extracts. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiment was repeated in triplicate manner. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer.

Total Antioxidant Capacity (TAC) by phosphomolybdenum method

The total antioxidant capacity of the plant extracts was evaluated by the phosphomolybdenum method according to the procedure described by Prieto [16]. To 0.3 ml of plant extracts (100-500 µg/ml), 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added and mixed. The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature.

Hydrogen peroxide radical scavenging activity

The ability of the plant extracts to scavenge hydrogen peroxide was assessed according to Ruch [17]. Plant extracts were added to 0.6 ml H_2O_2 solution (40 mM H_2O_2 was prepared with phosphate buffer, 0.1 M, pH 7.4) and the total volume was made up to 3 ml and mixed properly. Then the absorbance was measured at 230 nm using a spectrophotometer (Hach DR 5000 UV Spectrophotometer). Phosphate buffer, without H_2O_2 was considered as blank solution.

Superoxide radical scavenging activity

The superoxide scavenging activity of the plant extracts was assessed according to Suprava [18]. Superoxide anions were generated in samples that contained in 3.0 ml, 0.02 ml of the plant extracts (100-500 µg/ml), 0.2 ml of EDTA (0.1 M containing 1.5 mg of NaCN), 0.1 ml of 1.5 mM Nitroblue tetrazolium [NBT]), 0.05 ml of 0.12 mM riboflavin and 2.64 ml of phosphate buffer (0.067 M, pH 7.6). DMSO was used as control. All the tubes were vortexed and the initial optical density was measured at 560 nm in a spectrophotometer. Then the tubes were illuminated using a fluorescent lamp for 30 min and absorbance was measured again at 560 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

Hydroxyl radical scavenging activity

The extent of hydroxyl radical scavenging was assayed according to Elizabeth [19]. The reaction mixture contained 0.1 ml of 2.8 mM deoxyribose, 0.1 ml of 0.1 mM $FeCl_3$, 0.1 ml of 0.1 mM EDTA, 0.1 ml of 1 mM H_2O_2, 0.1 ml of 0.1 mM ascorbate, 0.1 ml of KH_2PO_4-KOH buffer (20 mM, pH 7.4) and 20 µl of plant extracts (100-500 µg/ml) in a final volume of 1.0 ml. The mixture was incubated at 37°C for 1 h. Following incubation, 1.0 ml of thiobarbituric acid (TBA) was added and heated at 95°C for 20 min to observe colour development from yellow to orange. Then the content was cooled and TBARS formation was measured spectrophotometrically at 532 nm. Ascorbic acid was used as a positive control. Deoxyribose degradation was measured as thio barbituric acid-reactive species (TBARS).

Ferric Thiocyanate (FTC) method

The antioxidant potential of *G. polycaulon* extracts was determined according to the FTC method with slight modification of Lee [20]. The reaction mixtures contained 400 µl of different concentration of plant extracts, 200 µl of diluted linoleic acid (25 mg/ml in 99% ethanol) and 400 µl of 50 mM phosphate buffer (pH 7.4) was incubated for 15 min at 4°C. A 100 µl aliquot of the above was mixed with a reaction mixture containing 3 ml of 70% ethanol, 100 µl of ammonium thiocyanate (300 mg/ml in distilled water) and 100 µl of ferrous sulphate, which results in the development of red colour, which spectrophotometrically measured at 535 nm.

Assay of inhibition of free radicals using Thiobarbituric Acid (TBA) method

Assaying of inhibition of free radicals was done according to Kikuzaki and Ohkawa [20,21]. The sample preparation for this assay was similar to FTC method. To 2.0 ml of the sample solution, 1.0 ml of 20% aqueous trichloroacetic acid (TCA) and 2.0 ml of aqueous thiobarbituric acid (TBA) solution were added. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 min. Following cooling, centrifuged at 3000 rpm for 20 min and the absorbance was measured. On fifth day, prepared sample was spectrophotometrically analysed at 532 nm and recorded.

Metal chelation activity

The ability of chelating Fe^{2+} by the extracts was determined according to Dinis [22,23]. One ml of each extracts were individually incubated with 50 µl of 2 mM ferrous chloride and incubated at room temperature for 2 h. Following incubation, 200 µlferrozine (5 mM) was added and after 10 min, the green colour developed was recorded and measured spectrophotometrically at 562 nm and calculated using standard formula. Na_2EDTA served as standard. The percentage inhibition and IC_{50} values were calculated and graph was plotted.

Lipid Peroxidation (LPO) activity

The method of Dudonne [24] was carried out to measure the lipid peroxide formed in the plant samples. Egg homogenate (10% in 0.2 M PBS, 0.5 ml), 0.1 ml of plant extracts (100-500 µg/ml) and deionised water (0.85 ml) were mixed in a test tube. To this, $FeSO_4$ (0.07 M, 0.05 ml) was added and incubated at 37°C temperature for 30 min to induce lipid peroxidation. Thereafter, acetic acid (20%, 1.5 ml), thiobarbituric acid (TBA) (0.8% prepared in 1.1% sodium dodecyl sulphate, 1.5 ml) and TCA (20%, 0.05 ml) were added, vortexed and then heated in a boiling water bath for 60 min. Following cooling, butan-1-ol (5 ml) was added to each tube and centrifuged for 10 min at 3000 rpm. The absorbance of the organic upper layer was measured at 532 nm spectrophotometerically. The percentage inhibition and IC_{50} values were calculated and graph was plotted. The coefficient significance of phytoconstituents were correlated between all antioxidant assays of *G. polycaulon* using Graph Pad Prism 5.0 program. Based on the significant results of antioxidant activities, methanolic extract of dry leaf in *G. polycaulon* plant was observed to be more significant equal activities than other extracts.

Results

Enzymic antioxidants

The enzymic antioxidants of *G. polycaulon* reported that the methanolic extract of dried leaf exhibited higher amount of catalase of 0.74 U/mg, peroxidase of 0.827 and 0.938 U/mg, glutathione s-transferase of 0.71 and 0.70 U/mg, polyphenol oxidase of 1.73 U/mg, superoxide of 0.87 U/mg and glucose-6-phosphate dehydrogenase of 0.76 U/mg than other extracts **(Table 1).**

Non-enzymic antioxidants

The non-enzymic antioxidants were estimated in *G. polycaulon* extracts for the estimation of ascorbic acid (0.623), total carotenoids (0.642), lycopene (0.591), reduced glutathione (0.629), tocopherol (0.019), total phenols (0.78), total flavonols (0.79) and flavonoids (0.81 mg/g) was found that the methanolic extract of dried leaf exhibited maximum yield than other extracts **(Table 2).**

Evaluation of total free radical scavenging activity of *G. polycaulon*

Because of the complex nature of phytochemicals, the antioxidant activities of plant extracts must be evaluated by combining two or more different *in vitro* assays based on different features of the antioxidant effects, such as the ability to scavenge free radicals, or the metal ion chelation. The rapid screening of antioxidant was assayed by dot-blot antioxidant rapid Screening assay to visualize free radicals quantitatively in the tested samples and measured spectrophotometrically. Then it was detected in the TLC plate by the DPPH staining method. Among all plant extracts, stronger yellow spots with purple background effect were observed in methanolic extract of dried leaf of *Gnaphalium polycaulon* **(Figure 1)** than other extracts. However, initially faint spots appeared and several hours later, weak spots could be observed in loaded area, similar to the result of DPPH spectrophotometric assay. The results of DPPH radical scavenging activity of *G. polycaulon* exhibited very high percentage inhibition antioxidant activity of 73.2% at 100 µg/ml in methanolic extract of dried leaf extract than other extracts. Standard ascorbic acid

Table 1 *In vitro* enzymic antioxidant activity of methanolic extracts of dried *G. polycaulon*.

Enzymic antioxidant (U/mg)	OD (nm)		Dried *G. polycaulon* (µg/ml) Leaf	Stem	Flower
Catalase, CAT	240		0.74	0.67	0.65
Peroxidase, POD	430	30 sec	0.827	0.789	0.693
		60 sec	0.938	0.795	0.697
Glutathione S-Transferase, GST	340	15 sec	0.71	0.58	0.5
		30 sec	0.7	0.57	0.49
Polyphenol Oxidase, PPO	495		1.73	1.59	1.38
Superoxide Dismutase, SOD	543		0.87	0.81	0.71
Glucose-6-phosphate dehydrogenase, G6PD	343		0.76	0.73	0.72

All the values are means of three independent determinations, n=3, analyzed in triplicate.
Each value represents Mean ± SEM (n=3), one way ANOVA was analysed for mean values showed differ significantly from each other (p<0.005)

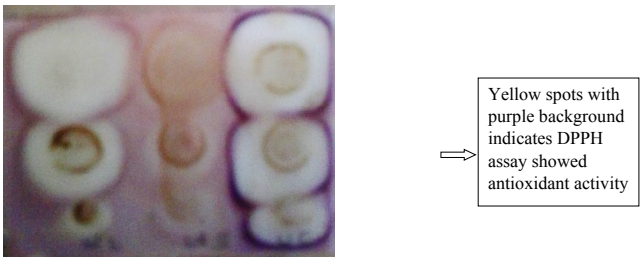

Figure 1 Dot-plot rapid Screening assay.

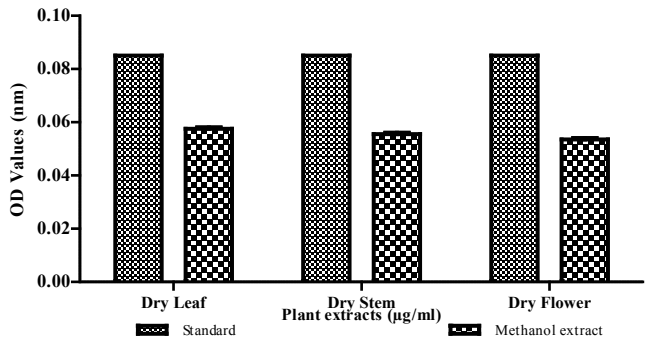

Figure 2 Estimation of Free radicals present in methanolic extracts of dried *G. polycaulon* Thiobarbituric acid (TBA) method.

extract was significantly different (p<0.005) but all extracts were significantly different from standard (p>0.005). All fresh and dried extracts showed steady increase in percentage inhibition of the DPPH radicals with concentration. The percentage inhibition of antioxidant values was determined in all extracts **(Table 3a)**. Among all the extracts, methanolic extract of dried leaf exhibited more potential ABTS⁺antioxidants. The percentage inhibition of antioxidant activity was 66.9% at 100 µg/ml concentration with IC_{50} of 0.131 ± 0.002 mg/ml in methanolic extract of dried leaf than other extracts **(Table 3a)**. The FRAP radical scavenging activity exhibited very high antioxidant percentage activity of 71.2% at 100 µg/ml in methanolic extract of dried leaf than other extracts with an IC_{50}, 71.2 ± 0.009 mg/ml. Standard ascorbic acid extract were significantly different (p<0.005) All dried extracts showed steady increase in percentage inhibition of the DPPH radicals with concentration and dependent. The percentage inhibition of antioxidant values was tabulated in **Table 3a**. The methanolic extract of dried leaf exhibited very high significant ORAC activity than other extracts and found to be 41%. The IC_{50} was calculated and found to be high antioxidant potential when compared to the standard and other extracts. It showed more significant than standard values **(Table 3b)**. Reducing power of methanolic extract of dried leaf was significantly higher than that other extracts. All dried extracts were found to be significantly different from ascorbic acid (p<0.005) **(Table 3b).** However, methanolic leaf extracts showed better activity than the ascorbic acid at 100 µg/mL. The methanolic extract of dried leaf exhibited a maximum nitric oxide radical scavenging activity of 45.9% at 100 µg/ml with IC_{50} of 200 µg/ml **(Table 3b)** in methanolic extract of dried leaf had higher scavenging activity than standard. All plant extracts showed consistent increase in scavenging nitric oxide radicals with increase in concentration. The different plant extracts were significantly different with each other as well as from standard ascorbic acid (p>0.005). The results of phosphomolybdenum assay were measured and found to be 45.7% at 100 µg/ml in the methanolic extract of dried leaf than other extracts **(Table 3c)**. The percentage scavenging of hydrogen peroxide in all extracts were determined and found to be 36% at 100 µg/ml in the methanolic extract of dried leaf than other extracts with IC_{50} of 200 µg/ml **(Table 3c).** The maximum superoxide radical scavenging activity was found to be 61.6% at 100 µg/ml with IC_{50} of 100 µg/ml in methanolic dried leaf extract **(Table 3c)**. There was no significant difference in superoxide radical scavenging activity of extracts and standard ascorbic acid (p>0.005) by statistical analysis. The Hydroxyl radical scavenging activity exhibited more activity in methanolic extract of dried leaf than other extracts and found to be 39.8% with IC_{50} of 300 µg/ml **(Table 3d)**. All plant extracts had been oxidized at 40-45°C for seven days. Initially, the absorbance of all extracts were measured and exhibited as the lowest values from 0.026. After seven days storage, all samples exhibited good effect in inhibiting linoleic acid oxidation compared to standard **(Table 3e)**. Antioxidant activity of four different extracts were measured to inhibit lipid peroxidation by FTC and TBA methods. In general, the antioxidant capacity of all extracts by TBA method is higher than that of FTC method. The methanolic extract of dried leaf revealed high antioxidant capacity than others extracts. The TBA analysis of the methanolic extract of dried leaf at seventh day

Table 2 *In vitro* non-enzymic antioxidant activity of methanolic extracts of dried *G. polycaulon*.

Non-Enzymic antioxidant (mg/g)	OD (nm)	Dried *G. polycaulon* (µg/ml)		
		Leaf	Stem	Flower
Ascorbic acid	540	0.623	0.619	0.593
Total Carotenoids	450	0. 642	0.633	0.579
Lycopene	503	0.591	0.586	0.562
Reduced Glutathione	412	0.629	0.617	0.615
Tocopherol	460	0.019	0.015	0.012
Total phenols	725	0.78	0.54	0.4
Total flavonols	440	0.79	0.57	0.51
Flavonoids	510	0.81	0.63	0.58

All the values are means of three independent determinations, n=3, analyzed in triplicate.
Each value represents Mean ± SEM (n=3), one way ANOVA was analysed for mean values with differ significantly from each other ($p<0.005$).

storage exhibited high antioxidant capacity than other extracts and found to be 0.57% in methanolic extract of dried leaf **(Figure 2)**. The methanolic extract of dried leaf at 100 µg/ml exhibited higher metal chelating effects of 33.8% than other extracts with IC_{50} of 300 µg/ml. The good chelating effects of *G. polycaulon* extracts on ferrous ions increased with increasing concentrations **(Table 3d)**. The Lipid peroxidation assay exhibited high inhibiting activity in the methanolic extract of dried leaf at 100 µg/ml (46.2%) with IC_{50} of 300 µg/ml than other extracts **(Table 3d).** Methanolic extract of driedleaf of *G. polycaulon* was found to have maximum antioxidant activity with significant IC_{50} values, followed by other extracts. The correlation of coefficient significance among major phytoconstituents with antioxidant assays of fresh and dried *G. polycaulon* plant was analysed **(Tables 4a-4c)**. The correlation results clearly stated that methanolic extract of driedleaf of *G. polycaulon* statistically exhibited more significant results in total phenol, tannin, saponin, alkaloids and flavonoids contents with maximum antioxidant potential than other extracts. Based on the significant results in phytoconstituents analysis, antimicrobial and antioxidant activities, methanolic extract of dried leaf and aqueous extract of fresh leaf in *G. polycaulon* plant was found to be more significant equal activities than other extracts. So, both extracts were considered and parallely compared in further analysis.

Discussion

During the last two decades, there has been in search for new plant derived drugs containing medically useful alkaloids, polyphenolics, steroids and terpenoids derivatives, which contributes to the antioxidant property. Many research studies have demonstrated that medicinal plants contain various phytocomponents with antioxidant activities that are responsible for their beneficial health effects. Antioxidants played an effective defense role in inhibiting and scavenging free radicals with synthetic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) [25]. It providing supported to defense mechanism of the body against risk of free radicals mediated deadly diseases [26]. Therefore, the discovery of natural antioxidants is currently a foremost theme of intensive investigation. It is believed that folk remedies are major sources of new phytoconstituents for antimicrobial and antioxidant treatments. Antioxidant assay was analysed and evaluated as high antioxidant activity in *G. polycaulon* extracts.

Enzymic antioxidants

The dried extracts of *G. polycaulon* have exhibited interesting phytoconstituents that leads to good antioxidant by enzymic, non-enzymic and total free scavenging assays. The enzymic antioxidants were analyzed and reported as methanolic extract of dried leaf have significant antioxidant activity than other solvents. Hydrogen peroxide is generated by the dismutation of superoxide radical by superoxide dismutase. SOD generates H_2O_2 as a more toxic product to the cells and required catalase or peroxidases to scavenge free radicals. An increase in catalase or peroxidase is essential for beneficial antioxidant effect from increase superoxide dismutase activity. Glucose-6-phospate-dehydrogenase enzyme is to maintain GSH in reduced state [27]. In addition to vitamin C, vitamin E and carotenoids, polyphenols exhibited strong antioxidant capacity [28].

Non-enzymic antioxidants

The non-enzymic antioxidants were estimated in different dried plant parts of *G. polycaulon* extracts and reported for ascorbic acid, total carotenoids, lycopene, reduced glutathione, tocopherol, total phenols, total flavonols and flavonoids in methanolic extract of dried leaf as high antioxidant activity than other extracts. Medicinal plants exhibited free radical scavenging molecules such as vitamins, reduced glutathione, tocopherol, lycopene, phenolic acids, flavonoids, and alkaloids which are rich in antioxidant activity. Specifically, non-enzymic antioxidants are essential for redox buffering [29]. Ascorbic acid is a natural water soluble antioxidant defense that protects cells against lipid peroxidation [30] and acts as strongest reductants and primary defense against radicals due to increasing utilization in the trapping of free radicals. The previous studies reported that lycopene acts as effective dietary antioxidant and free radicals. Reduced Glutathione is a major endogenous antioxidant defense substances and also acts as essential factor for glutathione peroxidase. Carotenoids are particularly efficient scavengers of singlet oxygen but it can also trap peroxyl radicals at low oxygen pressure with efficiency and also played a role in preventing *in vivo* lipid peroxidation [31]. Total phenolic act as reducing agents, hydrogen donors, singlet oxygen quenchers, and a metal chelating potential. Flavonoids exhibited structural diversity, biological and ecological significance, and health-promoting, anti-cancer properties [32] and responsible for antioxidant production.

Total free radical scavenging assays

Antioxidant activities (Dot-plot, DPPH, FRAP, ABTS, ORAC, reducing power, nitric oxide radical, phosphomolybdenum method, hydrogen peroxide, superoxide radical, hydroxyl radical, ferric thiocyanate method, thiobarbituric acid method, metal chelation and lipid peroxidation assays) were measured in all dried *G. polycaulon* extracts. The Dot-plot rapid screening assay is based on the inhibition of the accumulation of oxidized products by scavenging

Table 3a Total radical Scavenging activity of different extracts of *G. polycaulon*.

Assay	Conc. (µg/ml)	Dried Methanolic Samples						Standard	
		Leaf		Stem		Flower			
		%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM
DPPH	100	73.2	0.663 ± 0.001	69.1	0.763 ± 0.001	63.4	0.904 ± 0.004	45.11	1.358 ± 0.001
	200	76.6	0.578 ± 0.001	72.2	0.686 ± 0.001	69.1	0.764 ± 0.002	53.98	1.138 ± 0.001
	300	82.7	0.427 ± 0.001	79.4	0.507 ± 0.001	71.3	0.71 ± 0.004	67.03	0.815 ± 0.002
	400	85.6	0.355 ± 0.001	84.9	0.371 ± 0.001	72.9	0.674 ± 0.002	76.15	0.590 ± 0.001
	500	88.3	0.29 ± 0.001	88.1	0.294 ± 0.001	78.7	0.526 ± 0.003	88.31	0.289 ± 0.001
ABTS	100	66.9	0.924 ± 0.001	66.8	0.932 ± 0.002	63.7	0.897 ± 0.001	42.79	1.598 ± 0.001
	200	69.4	0.854 ± 0.002	69	0.870 ± 0.003	69.1	0.764 ± 0.002	51.08	1.366 ± 0.001
	300	73.1	0.750 ± 0.004	72.9	0.761 ± 0.001	69.5	0.754 ± 0.001	60.88	1.093 ± 0.001
	400	79.5	0.572 ± 0.001	75.1	0.699 ± 0.001	71.9	0.694 ± 0.003	71.03	0.809 ± 0.001
	500	83.9	0.449 ± 0.001	78.5	0.604 ± 0.002	79.3	0.510 ± 0.002	82.52	0.488 ± 0.001
FRAP	100	71.2	0.734 ± 0.002	70.6	0.724 ± 0.001	66.8	0.802 ± 0.005	37.34	0.802 ± 0.005
	200	74.9	0.643 ± 0.001	72.6	0.673 ± 0.001	69.6	0.735 ± 0.003	48.17	0.735 ± 0.003
	300	79.3	0.530 ± 0.004	76	0.590 ± 0.002	70.6	0.710 ± 0.001	53.11	0.710 ± 0.001
	400	80.6	0.495 ± 0.002	79.3	0.509 ± 0.003	73.2	0.647 ± 0.003	64.22	0.647 ± 0.003
	500	84.1	0.407 ± 0.002	82.4	0.432 ± 0.002	75.5	0.593 ± 0.003	76.36	0.593 ± 0.003

the free radical. When flavonoids were analyzed, fast-reacted and strong intensities of white-yellow spots were appeared When a DPPH solution is mixed with plant extract that can donate a hydrogen atom to the reduced form, diphenylpicryl hydrazine with the loss of its violet color The degree of reduction in absorbance measurement is indicative of free radical scavenging of extract. The scavenging effect elicited by the ethanolic extract of leaves of *G. uniflorum* was concentration-dependent in DPPH test the amount of antioxidant was necessary to decrease 50% of initial concentration. The reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue colour is related to the amount of antioxidant reductants in the samples. The reductive ability of plant samples suggested that the extracts were able to donate electron to free radicals in biological systems, for stable and unreactiveradicals. A decrease of the ABTS·+ concentration is linearly dependent on the antioxidant concentration Higher concentrations of the extracts were more effective in quenching free radicals in the system. Trolox, BHT, rutin, ascorbic acid and gallic acid can be used as a positive control. The essential oil from *G. affine* was observed to possess strong radical scavenging activity against 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonate with the IC_{50} being 0.32 ± 0.89 µg/mL. The ORAC assay is based on the scavenging of peroxyl radicals generated that prevent degradation of the fluorescein probe and prevent the loss of fluorescence of the probeby spontaneous decomposition of 2, 2'- azo-bis, 2-amidinopropanedihydrochloride (AAPH), was estimated and Similar results was reported. Increasing absorbance indicated an increase in the reductive ability. The reducing capacity of plant extract served as a significant indicator of its potential electron donating and antioxidant activity that inhibition of lipid oxidation and peroxidation supported the present study and are significant to superoxide dismutase and catalase activities. Excess production of NO is associated with several diseases. It reacts with O_2 to produce stable product nitrate and nitrite through intermediates NO_2, N_2O_4 and N_3O_4 using Griess reagent. The methanolic extract of dried leaf of *G. polycaulon* exhibits significant antioxidant activity which compete with oxygen to react with nitric oxide and inhibited the generation of nitrite. The antioxidant capacity measured is based on the reduction of (IV) to (V) by the sample analyte and subsequent formation of green formation / Mo (V) compounds. It might be attributed to the presence of phytochemicals such as flavonoids and biflavones. Hydrogen peroxide is decomposing to yield highly reactive hydroxyl radical. Hydrogen peroxide is not very reactive, but it

Table 3b Total Radical Scavenging activity of different extracts of *G. polycaulon*.

Assay	Conc. (µg/ml)	Dried Methanolic Samples						Standard	
		Leaf		Stem		Flower			
		%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM
ORAC	100	41	1.431 ± 0.001	40.9	1.453 ± 0.001	38	1.445 ± 0.001	20.15	0.901 ± 0.001
	200	47.3	1.279 ± 0.001	45.8	1.332 ± 0.001	44.1	1.304 ± 0.002	31.74	0.770 ± 0.001
	300	53.6	1.127 ± 0.001	49.6	1.239 ± 0.001	47.9	1.215 ± 0.001	59.52	0.457 ± 0.001
	400	60.7	0.953 ± 0.001	54.3	1.124 ± 0.002	53	1.096 ± 0.002	68.14	0.359 ± 0.001
	500	63.6	0.883 ± 0.001	59.6	0.994 ± 0.001	57.4	0.993 ± 0.001	72.46	0.311 ± 0.001
Reducing Power	100	42.3	0.562 ± 0.001	39.2	0.562 ± 0.001	37.5	0.571 ± 0.001	56.59	0.202 ± 0.001
	200	49.4	0.493 ± 0.001	45.5	0.503 ± 0.001	43.9	0.513 ± 0.001	65.89	0.214 ± 0.001
	300	57.6	0.413 ± 0.001	54.2	0.423 ± 0.001	53.7	0.423 ± 0.001	78.29	0.230 ± 0.001
	400	62.3	0.367 ± 0.001	58.4	0.384 ± 0.001	57.7	0.386 ± 0.001	82.17	0.235 ± 0.001
	500	71	0.282 ± 0.001	67.4	0.301 ± 0.001	63.4	0.334 ± 0.001	95.35	0.252 ± 0.001
Nitric Acid	100	45.9	0.802 ± 0.001	44.3	0.817 ± 0.001	42	0.843 ± 0.001	66.77	0.767 ± 0.001
	200	51.1	0.725 ± 0.001	49.2	0.746 ± 0.001	47.6	0.763 ± 0.001	69.08	0.714 ± 0.001
	300	55.9	0.654 ± 0.001	54.9	0.662 ± 0.001	53	0.683 ± 0.001	72.6	0.633 ± 0.001
	400	58.6	0.615 ± 0.002	57.9	0.618 ± 0.001	56.4	0.633 ± 0.001	76.39	0.545 ± 0.001
	500	63.8	0.537 ± 0.001	61.7	0.562 ± 0.001	60.8	0.571 ± 0.001	80.37	0.453 ± 0.001

All the values are means of three independent determinations, n=3, analyzed in triplicate.
Each value represents Mean ± SEM (n=3), one way ANOVA was analysed for mean values with differ significantly from each other ($p<0.005$).

Table 3c Total Radical Scavenging activity of different extracts of *G. polycaulon.*

Assay	Conc. (µg/ml)	Dried Methanolic Samples						Standard	
		Leaf		Stem		Flower			
		%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM
Phosphomolydoenum	100	45.7	0.897 ± 0.001	44.2	0.817 ± 0.001	41.4	0.893 ± 0.001	31.8	1.063 ± 0.001
	200	48.5	0.852 ± 0.001	48.3	0.758 ± 0.001	46.5	0.816 ± 0.002	47.26	0.822 ± 0.001
	300	54.8	0.747 ± 0.001	53.2	0.686 ± 0.001	52.5	0.724 ± 0.002	59.88	0.625 ± 0.001
	400	57.9	0.695 ± 0.001	56.8	0.632 ± 0.001	55.8	0.673 ± 0.001	68.35	0.493 ± 0.001
	500	62.9	0.613 ± 0.001	60.9	0.572 ± 0.001	60.2	0.607 ± 0.001	79.15	0.325 ± 0.001
Hydrogen peroxide	100	36	1.578 ± 0.001	33.6	1.656 ± 0.001	32.4	1.603 ± 0.001	22.51	0.732 ± 0.001
	200	42.1	1.427 ± 0.001	40.2	1.493 ± 0.001	39.2	1.443 ± 0.001	28.55	0.675 ± 0.001
	300	50.2	1.227 ± 0.001	48.5	1.284 ± 0.001	46.7	1.263 ± 0.001	38.04	0.585 ± 0.001
	400	55.1	1.106 ± 0.001	53	1.173±0.001	52.3	1.132 ± 0.001	47.39	0.497 ± 0.001
	500	60.9	0.963 ± 0.001	58.2	1.044 ± 0.001	57.3	1.013 ± 0.001	54.94	0.426 ± 0.002
Superoxide	100	61.6	0.983±0.001	60.5	0.962 ± 0.001	60.3	0.943 ± 0.001	36.09	1.225 ± 0.002
	200	67.4	0.836 ± 0.001	66.2	0.824 ± 0.001	65.2	0.825 ± 0.001	52.87	0.902 ± 0.001
	300	70.3	0.762 ± 0.001	69.3	0.747 ± 0.001	67.8	0.764 ± 0.001	67.97	0.612 ± 0.001
	400	79.8	0.518 ± 0.001	77.7	0.542 ± 0.001	76.3	0.562 ± 0.001	76.38	0.453 ± 0.001
	500	86.1	0.355 ± 0.001	84.3	0.382 ± 0.001	83.9	0.383 ± 0.001	84.64	0.293 ± 0.001

Table 3d Total radical Scavenging activity of different extracts of *G. polycaulon.*

Assay	Conc. (µg/ml)	Dried Methanolic Samples						Standard	
		Leaf		Stem		Flower			
		%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM	%	Mean ± SEM
Hydroxyl	100	39.8	1.478 ± 0.001	38.1	1.463 ± 0.001	37.6	1.345 ± 0.001	21.15	1.533 ± 0.001
	200	47.4	1.292 ± 0.001	45.6	1.286 ± 0.001	42.2	1.246 ± 0.001	31.96	1.323 ± 0.001
	300	53.8	1.135 ± 0.001	51.5	1.146 ± 0.001	47.9	1.123 ± 0.001	53.55	0.903 ± 0.001
	400	64.3	0.876 ± 0.001	62.9	0.877 ± 0.001	60.2	0.857 ± 0.001	62.31	0.733 ± 0.001
	500	72.1	0.684 ± 0.001	71	0.686 ± 0.001	68.2	0.685 ± 0.001	70.53	0.573 ± 0.001
Metal Chelation	100	33.8	1.665 ± 0.001	32.9	1.653 ± 0.001	30.4	1.704 ± 0.001	28.96	1.383 ± 0.001
	200	42	1.457 ± 0.001	39.7	1.486 ± 0.001	38.9	1.495 ± 0.001	37.71	1.213 ± 0.001
	300	51.2	1.227 ± 0.001	50.6	1.217 ± 0.001	47.9	1.275 ± 0.001	48.48	1.003 ± 0.001
	400	62.4	0.944 ± 0.001	58.5	1.022 ± 0.001	57.7	1.035 ± 0.001	61.33	0.753 ± 0.001
	500	71.6	0.715 ± 0.002	67.8	0.794 ± 0.001	66.8	0.813 ± 0.001	71.09	0.563 ± 0.001
Lipid Peroxidation	100	46.2	1.185 ± 0.001	44.6	1.255 ± 0.001	39.7	1.384 ± 0.002	38.04	0.585 ± 0.001
	200	54.9	0.995 ± 0.001	52.1	1.085 ± 0.002	50.6	1.134 ± 0.001	47.39	0.497 ± 0.001
	300	66.1	0.747 ± 0.001	64.4	0.807 ± 0.001	63.6	0.835 ± 0.001	54.94	0.426 ± 0.002
	400	70.2	0.657 ± 0.001	66.4	0.762 ± 0.001	65.5	0.792 ± 0.001	62.49	0.354 ± 0.002
	500	76.2	0.524 ± 0.001	75.9	0.546 ± 0.001	74.5	0.585 ± 0.001	76.61	0.221 ± 0.001

All the values are means of three independent determinations, n=3, analyzed in triplicate.

Each value represents Mean ± SEM (n=3), one way ANOVA was analysed for mean values with differ significantly from each other ($p<0.005$).

Table 3e Estimation of Free radicals present in methanolic extracts of dried *G. polycaulon* by Ferric thiocyanate (FTC) method.

Free radicals	Days	Dried Samples			Standard
		Leaf	Stem	Flower	
Ferric thiocyanate (FTC) method	1	0.026	0.024	0.021	0.041
	2	0.032	0.031	0.03	0.043
	3	0.049	0.047	0.045	0.053
	4	0.053	0.051	0.05	0.064
	5	0.059	0.056	0.055	0.07
	6	0.049	0.047	0.046	0.074
	7	0.057	0.055	0.053	0.084

Table 4a Coefficient of correlation between antioxidant assays of methanolic extracts of *G. polycaulon.*

MDL*	DPPH	FRAP	ABTS	ORAC	RP	NO	PM	HP	SOD	HO	METAL	LPO	TPC	TTC	TAC	TFC	TSC
DDPH	1																
FRAP	0.9855	1															
ABTS	0.9311	0.9164	1														
ORAC	0.9845	0.9647	0.9577	1													
RP	0.9769	0.9905	0.9516	0.9703	1												
NO	0.9753	0.9941	0.9429	0.9694	0.9979	1											
PM	0.9819	0.9795	0.966	0.9677	0.9916	0.9824	1										
HP	0.9933	0.9918	0.9553	0.9869	0.9944	0.9926	0.9916	1									
SOD	0.9128	0.9096	0.9912	0.954	0.9527	0.9438	0.9454	0.9457	1								
HO	0.9545	0.9496	0.9917	0.9996	0.9788	0.9719	0.9743	0.9774	0.9928	1							
METAL	0.9684	0.962	0.9893	0.9844	0.9858	0.9787	0.9848	0.9866	0.9848	0.9984	1						
LPO	0.9948	0.9948	0.9094	0.9757	0.9791	0.9831	0.9729	0.9911	0.8991	0.9436	0.9575	1					
TOC	0.9581	0.9784	0.9585	0.9677	0.9938	0.995	0.9743	0.9842	0.9673	0.984	0.9853	0.9642	1				
TTC	0.9474	0.9064	0.9606	0.9844	0.9258	0.9193	0.9324	0.9493	0.9557	0.9687	0.9684	0.9246	0.9275	1			
TAC	0.9801	0.9574	0.9218	0.945	0.9546	0.9424	0.9813	0.9691	0.8813	0.9259	0.9452	0.9634	0.9193	0.9012	1		
TFC	0.9954	0.969	0.9213	0.9885	0.9579	0.9576	0.9638	0.9828	0.9047	0.9463	0.9593	0.987	0.9413	0.9616	0.9669	1	
TSC	0.9572	0.949	0.9934	0.9653	0.9795	0.9662	0.9887	0.9766	0.9784	0.9907	0.9941	0.9405	0.9719	0.951	0.9545	0.9422	1

Table 4b Coefficient of correlation between antioxidant assays of methanolic extracts of *G. polycaulon*.

MDS*	DPPH	FRAP	ABTS	ORAC	RP	NO	PM	HP	SOD	HO	METAL	LPO	TPC	TTC	TAC	TFC	TSC
DPPH	1																
FRAP	0.9859	1															
ABTS	0.9825	0.9934	1														
ORAC	0.9634	0.9909	0.9872	1													
RP	0.9672	0.9861	0.9971	0.9888	1												
NO	0.9748	0.9737	0.9869	0.9771	0.9873	1											
PM	0.9835	0.9917	0.996	0.9918	0.994	0.9946	1										
HP	0.9794	0.9753	0.9875	0.9757	0.9861	0.9997	0.9947	1									
SOD	0.9468	0.9829	0.9627	0.9888	0.9598	0.9398	0.9677	0.9394	1								
HO	0.9667	0.9917	0.9748	0.9924	0.9693	0.957	0.9802	0.9576	0.9974	1							
METAL	0.9873	0.9971	0.9988	0.9906	0.9941	0.9868	0.9976	0.9878	0.9713	0.983	1						
LPO	0.9533	0.9569	0.9832	0.959	0.9881	0.9884	0.9804	0.9871	0.9093	0.9266	0.9758	1					
TPC	0.9677	0.9813	0.9675	0.9878	0.9629	0.9696	0.9825	0.97	0.9847	0.9919	0.9775	0.93	1				
TTC	0.9393	0.9785	0.9544	0.9736	0.9474	0.914	9505	0.9149	0.993	0.9887	0.962	0.8897	0.9624	1			
TAC	0.9648	0.9946	0.9882	0.9901	0.9851	0.9575	0.9814	0.9578	0.9856	0.9883	0.9897	0.949	0.9677	0.9868	1		
TFC	0.9385	0.9694	0.9793	0.9904	0.9898	0.9809	0.9852	0.9769	0.9623	0.9664	0.9781	0.9741	0.9677	0.9381	0.971	1	
TSC	0.9599	0.9905	0.9743	0.9806	0.9666	0.9361	0.9673	0.9376	0.9889	0.9897	0.979	0.9198	0.9648	0.996	0.9959	0.9498	1

Table 4c Coefficient of correlation between antioxidant assays of methanolic extracts of *G. polycaulon*.

MDF*	DPPH	FRAP	ABTS	ORAC	RP	NO	PM	HP	SOD	HO	METAL	LPO	TPC	TTC	TAC	TFC	TSC
DDPH	1																
FRAP	0.9637	1															
ABTS	0.9691	0.9373	1														
ORAC	0.9606	0.9931	0.9078	1													
RP	0.9286	0.9438	0.8389	0.9744	1												
NO	0.9553	0.9695	0.8781	0.9906	0.9947	1											
PM	0.9476	0.9631	0.8682	0.9868	0.9977	0.9992	1										
HP	0.9392	0.969	0.8619	0.9908	0.995	0.9981	0.9983	1									
SOD	0.9217	0.9788	0.9315	0.9601	0.9015	0.9249	0.9219	0.9301	1								
HO	0.889	0.9665	0.8807	0.9583	0.9172	0.9299	0.9307	0.9417	0.9909	1							
METAL	0.9454	0.986	0.8985	0.9941	0.9777	0.9986	0.9866	0.9905	0.9709	0.9768	1						
LPO	0.9429	0.9204	0.8423	0.9545	0.9877	0.9849	0.9857	0.9761	0.8572	0.8612	0.9476	1					
TOC	0.9382	0.9642	0.8761	0.9828	0.9912	0.9903	0.9934	0.9926	0.9455	0.9573	0.9935	0.967	1				
TTC	0.9009	0.9645	0.874	0.9671	0.9484	0.9529	0.9561	0.963	0.9799	0.9943	0.9877	0.8989	0.9787	1			
TAC	0.9486	0.9736	0.8743	0.9934	0.9935	0.9991	0.9985	0.9995	0.9333	0.941	0.991	0.9775	0.918	0.9613	1		
TFC	0.9518	0.986	0.9003	0.9959	0.9821	0.9912	0.9907	0.9934	0.9651	0.9696	0.9995	0.9565	0.9945	0.9826	0.9943	1	
TSC	0.9345	0.9866	0.8914	0.9924	0.9701	0.9804	0.98	0.9864	0.9755	0.9833	0.9989	0.9341	0.9886	0.9905	0.9864	0.9973	1

*Different extracts

Antioxidant assays

DPP: DPPH radical Scavenging activity, FRAP: FRAP radical Scavenging activity, ABTS: ABTS radical Scavenging activity, ORAC: ORAC radical Scavenging activity, RP: Reducing Power Assay, NO: Nitric Acid Radical Scavenging Activity, PM: Phosphomolydoenum Assay, HP: Hydrogen Peroxide Radical Scavenging activity, SOD :Superoxide Radical Scavenging activity, HO: Hydroxyl Radical Scavenging activity, METAL : Metal Chelation activity, LPO :Lipid Peroxidation activity; TPC:Total Phenol Content, TTC :Total Tannin Content, TAC :Total Alkaloid Content, TFC :Total flavonoid Content, TSC :Total Saponin Content

can sometimes be toxic to cell because it can give rise to hydroxyl radical in the cells. Thus, the removal of H_2O_2 is very important for antioxidant defense in cell systems. Superoxide anions served as precursors of singlet oxygen and hydroxyl radicals. The plant extracts decreased the mean rate of absorption by inhibiting NBT reduction by the superoxide anion radicals through metabolic processes. SOD enzymes present in aerobic and anaerobic organisms catalyzed the breakdown of superoxide radical. The hydroxyl radical scavenging activity is measured as percentage of inhibition of hydroxyl radicals generated in the Fentons reaction mixture by studying the composition between deoxyribose and the plant extract for hydroxyl radical generated. Plant extracts and the standard inhibited the production of hydroxyl radicals. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The Ferric thiocyanate method measured the amount of peroxide value in the beginning of the lipid peroxidation, where ferric ion was formed upon reaction of peroxide with ferrous chloride. The ferric ion reacted with

ammonium thiocyanate to formed ferric thiocyanate, a red-coloured substance. The darker colour will gives the absorbance higher The same samples as prepared for the FTC method were used in TBA test. FTC is used to measure the production of peroxide compound at the initial stage of oxidation while TBA test is used to measure the secondary product of oxidation such as aldehyde and ketone. Furthermore, the secondary product is much more stable for a period of time. It turned into alcohol and acid, which cannot be detected by a spectrophotometer. According to the results, the methanolic extract of dried leaf of *G. polycaulon* reported high ferrous ion-chelating activity through scavenging or chelating processes. Ferrozine combines with ferrous ions forming a red coloured complex. It was reported that the chelating agents which form σ bond with a metal, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion. In addition, metal chelating ability of the plant extract demonstrated that they reduce concentration of the catalyzing transition metal involved in the peroxidation of lipids. The high lipid peroxidation scavenging effects was measured in methanolic extract of dried leaf due to high contents of phenolic compounds and other radical scavengers present in the plant extracts which can terminate the peroxidation chain reaction easily and quench reactive oxygen species or nitrogen species, thereby inhibiting the oxidation of lipid and other biological molecules. In addition, it was reported that phenolic substances were associated with antioxidant activity played important role in stabilizing lipid peroxidation. Plant parts having better protection against free radical induced lipid peroxidation that can be used as anti-lipid peroxidation and anti-mutagenic substances. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. The correlation results stated that the total phenol content is more significant to antioxidant activity also. A positive correlation was found between phenolics, alkaloids, tannins, saponins, and flavonoid contents and antioxidant properties of the *G. polycaulon* extracts. These findings indicated that polyphenolic components may have antioxidant potential, as reported in other medicinal plants among major phytoconstituents. Based on the findings of the present investigation, standardization of antioxidant activity clearly reported that the methanolic extract of dried leaf of *G. polycaulon* exhibited highly significant values than other extracts. Most of the ethnobotanical studies confirmed that leaves are the major portion of the plant used for the treatment of various diseases due to their easily accessible, active in photosynthesis and production of various valuable metabolites, which has been reflected in the present investigation too.

Conclusion

According to the knowledge of tribals, *G. polycaulon* is having variety of medicinal properties. The results also clearly revealed the presence of medicinally important phytoconstituents in the plants studied and showed that *G. polycaulon* plant is good source of antioxidant due to its phytochemicals. Only very few reports available on this plant and this report is the first report from India. This plant is said to be location specific and present throughout the year. These findings stated that *G. polycaulon* extracts has a potential application as natural medicine and to treat diseases as well as the microbiological safety of the human health. Further studies are ongoing to isolate, identity, characterize and elucidate the structure of the bioactive components. Such kind of plants should be given with lot of importance and their bioactive properties should be studied in an elaborative manner and should be documented. Specifically, preservation of medicinal plants is urgent need to protect biological diversity for the betterment of future generation.

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