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Research Article

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Assessment of antioxidant activity, total phenolic content of some medicinal plants used by the tribes in Wayanad, kerala

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ABSTRACT

The tribal medicines play vital role in curing human diseases in Wayanad district Kerala. In our continuous effort 500 plants are already been reported. The objective of the present study is to examine the antioxidant activity of methanolic and aqueous extracts of leaves of Embelia tsjeriam-cottam(Roem.&Schult.)DC., Gomphostemma heyneanum Benth.var. heyneanum, Hackeria subpeltata (Willd.)Kunth and Nothapodytes nimmoniana (Graham)Mabb. which are extensively used by the tribes for curing cancer, arthritis, piles, dysentery, diarrhoea and skin problems. The DPPH radical scavenging activity, phosphomelybdenum assay and Fe (3) to Fe (2) reducing activity were employed in the present study to determine the antioxidant activity of these plants. The DPPH radical scavenging activity for the methanolic leaf extract of, Hackeria subpeltata (Willd.)Kunth is significant when compared to the commonly used BHA followed by 99.24 % at 517nm (Methanolic extract of Gomphostemma heyneanum Benth.var. heyneanum), 93.90 % at 517nm (Methanolic extract of Embelia tsjeriam-cottam(Roem.&Schult.)DC. The maximum value for phenol estimation, reducing power and antioxidant capacity by phosphomolybdenum were shown by the methanolic leaf extract of Hackeria subpeltata (Willd.) which forms a good basis to propose the plant material for further phytochemical and pharmacological analysis.

Key Words: Antioxidants, Reducing power, Medicinal plants. DPPH, Tribal medicines.

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological process. However the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with ageing [1-3]. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [4]. There is some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanesole (BHA), commonly used in processed foods. However, it has been suggested that these compounds have side effects [5]. Now a days natural antioxidants have become one of the major areas of scientific research [6-7].Therefore the importance of searching for an attractive exploiting natural antioxidants especially of plant origin, has increased greatly in recent years. In plants the antioxidant effect is mainly due to phenolic components such as flavonoides, phenolic acids, and phenolic diterpenes [[8-9].

The antioxidant activity of various plant parts can be measured using numerous assays. Due to complex composition of different plant products, more than one method is recommended for the evaluation of antioxidant activity [10].

The methods commonly used include DPPH assay [11], total antioxidant capacity by phosphomolybdenum method [12]. Fe (III) to Fe (II) - reducing activity [13] and total phenol estimation [14]

This study is part of our work related to the documentation of tribal medicines and therapeutic uses of plants used by the kurichia, kuruma, kattunaika, adiya and the paniya tribes of Wayanad district Kerala. The objective of the present study is to examine the antioxidant activity of methanolic and water extracts of leaves of *Embelia tsjeriam-cottam* (Roem. & Schult.)DC., *Gomphostemma heyneanum* Benth.*var. heyneanum, Hackeria subpeltata* (Willd.)Kunth and *Nothapodytes nimmoniana* (Graham) Mabb.which are extensively used by the tribes for curing cancer, arthritis, piles, and dysentery, diarrhoea and skin problems.

EXPERIMENTAL SECTION

Chemicals

0.004% DPPH in ethanol, 0.6M H_2SO_4,28mM ammonium molybdate, ascorbic acid, phosphate buffer, 1%K3 Fe $(CN)_6$ potassium hexacynoferrate, trichloroacetic acid,0.1%$Fecl_3$, 20% $Na2CO_3$, folin ciocalteau reagent, standard Gallic acid,butylated hydroxyanesole.(All chemicals used were analytical grade and obtained from sigma –aldrich and Merck.

Plant Material

Fresh leaves of *Embelia tsjeriam-cottam* (Roem. & Schult.)DC., *Gomphostemma heyneanum* Benth.var. *heyneanum, Hackeria subpeltata* (Willd.) Kunth and *Nothapodytes nimmoniana* (Graham) Mabb.were collected from the forests of Wayanad district, Kerala. Plant specimens were deposited in Mysore university environmental science department. The tribal medical practitioners use the fresh leaves of these plants for treating various diseases.

Sample preparation

Aqueous and methanol extracts were prepared by homogenizing the fresh samples of leaves with a mortar and pestle in the respective solvents to a concentration of 0.01g /ml.The extracts were centrifuged at 5000 g for 10 min.and the supernatant were used for the *invitro* antioxidant activity assay.

DPPH assay

Ability of extracts to scavenge DPPH free radical was determined by [11]. An aliquot of the sample is taken and made up to 5 ml with alcohol and then mixed well with 1.0 ml of 0.004% DPPH solution (in ethanol, prepared fresh just before the assay). The tubes are incubated in dark for 30 min and absorbance read at 517 nm.

DPPH radical scavenging capability (%) was calculated using the formula

$$\frac{\text{(ABS of control-ABS of sample) x 100}}{\text{(ABS of control)}}$$

Total Antioxidant Capacity by Phosphomolybdenum method.

An aliquot of each sample is made up to 3 ml with ethanol and combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium sulphate and 4 mM ammonium molybdate). Ethanol (0.1 ml) is used, in place of sample solution, for the blank. The tubes are capped and incubated in a boiling water bath at 95°C for 90 min. Later the samples cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank and using ascorbic acid as standard. Antioxidant capacity expressed as ascorbic acid equivalents (µmol/g).

Fe (III) to Fe (II) - reducing activity

Fe (III) reducing activity was measured by the method of [13]. An aliquot of each extract dissolved in water, was mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% aqueous potassium hexacyanoferrate solution. After 30 min incubation at 50°C, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged for 10 min. 2.5 ml of aliquot of the upper layer was mixed with 2.5 ml of water and 0.5 ml of 0.1% aqueous ferric chloride. The absorbance was measured at 700 nm. Fe (III) reducing activity was determined as ascorbic acid equivalents (mmol ascorbic acid/g extract).

Total phenol Estimation:

The total phenol content is estimated by the method of [14]. An aliquot of each extract dissolved in water, was mixed with 250ml of Folin-Ciocaltean reagent (Undiluted) and allowed for 1 min; later 750µg of 20% sodium bicarbonate was added and incubated for 2 hours. The absorbance was measured at 760nm.Antioxidant capacity is expressed as gallic acid equivalents. (µg Gallic acid /gm fresh weight).

RESULTS AND DISCUSSION

In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effect [15-16]. This concern has resulted in the investigation of the effectiveness of the naturally occurring compounds with antioxidant properties [17-19]. In the present investigation the methanolic and water extracts of leaves of *Embelia tsjeriam-cottam*(Roem.&Schult.) DC., *Gomphostemma heyneanum* Benth.*var. heyneanum, Hackeria subpeltata* (Willd.) Kunth and *Nothapodytes nimmoniana* (Graham) Mabb. were subjected to screening for their possible antioxidant activity.

The radical scavenging activities of the extracts were determined by using DPPH a stable free radical with radical scavenging activity 175 at 517nm. 1, 1-diphenyl-2-picrylhydrazyl is a nitrogen-centered free radical, color of which changes from violet to yellow on reduction by H or e donation. Substances able to perform this reaction are antioxidants and therefore radical scavengers Ascorbic acid, BHA, BHT, gallic acid are references.The decrease in absorption is taken as the measure of the extend of radical scavenging. The free radical scavenging activity of extracts by DPPH assay are shown in Table 1. Here the DPPH radical scavenging activity 107.05% at 517nm for the methanolic leaf extract of, *Hackeria subpeltata* (Willd.)Kunth is significant when compared to the commonly used BHA (175 % at 517 nm.) followed by 99.24 % at 517nm (Methanolic extract of *Gomphostemma heyneanum* Benth.*var. heyneanum),* 93.90 % at 517nm (Methanolic extract of *Embelia tsjeriam-cottam* (Roem. &Schult.)DC.

Total quantitative determination of antioxidant capacity of the sample extracts were evaluated by phosphomolybdenum method [12]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The measurement of absorbance of each extracts at 695nm showed significant values for methanolic extract of *Hackeria subpeltata* (Willd.)Kunth (743.6 µg ascorbic acid equivalents/gm fresh leaf weight) followed by methanolic extract of *Embelia tsjeriam-cottam* (Roem. &Schult.)DC. (404.8 µg ascorbic acid equivalents/gm fresh leaf weight) and methanolic extract of *Gomphostemma heyneanum* Benth.*var. heyneanum* (365.6 ascorbic acid equivalents/gm fresh leaf weight).Table 2.

Table 1: DPPH Radical scavenging Capacity (%) of plant extracts.

Treatments	Sample	DPPH % over control
1	WGH	60.57 ± 1.23
2	WNN	10.8 ± 1.4
3	WEJC	76.00 ± 2.14
4	WHP	53.52 ± .27
5	MEGH	99.24 ± 1.5
6	MENN	49.52 ± 2.35
7	MEEJC	93.90 ± .54
8	MEHP	107.05 ± .27
9	BHA(control)	175 ± 1.25

Table 2: Antioxidant capacity of plant extracts by phosphomolybdenum method.

Treatment	Sample	PM(µM)Ascorbic acid equivalents/gm fresh leaf weight.
1	WGH	265.6 ± 29.06
2	WNN	221.5 ± 34.72
3	WEJC	286.9 ± 15.69
4	WHP	293.5 ± 56.75
5	MEGH	365.6 ± 42.80
6	MENN	177.1 ± 21.18
7	MEEJC	404.8 ± 38.6
8	MEHP	743.6 ± 73.9

Reduction is an important indicator of e^- donating activity, which is an important mechanism of phenolic antioxidant action.When substances exhibiting high reducing tendencies donate electrons which can react with free radicals converting them to more stable products in the process radical chain reactions could be terminated. In Fe (III) to Fe (II) - reducing activity experiments the increased absorbance values denotes the increased reducing ability of plant extracts by converting Fe^{3+} to Fe^{2+} .The water extract of *Embelia tsjeriam-cottam* (Roem. & Schult.)DC. Shows high reducing power, 40.04mM ascorbic acid equivalents/gm fresh leaf weight at 700nm followed by methanolic extract of *Hackeria subpeltata* (Willd.)Kunth, 26.12 mM ascorbic acid equivalents/gm fresh leaf weight and water extract of *Gomphostemma heyneanum* Benth.*var. heyneanum,* 25.42 mM ascorbic acid equivalents/gm fresh leaf weight **(**Table 3).

Table 3: Fe (III) to Fe (II) - reducing activity of plant extracts.

Treatment	Sample	Fe(3)-Fe(2) Reducing activity(mM Ascorbic acid equivalents/gm fresh leaf weight.
1	WGH	25.42± 1.41
2	WNN	2.06± .43
3	WEJC	40.04±1.2
4	WHP	19.29± .95
5	MEGH	25.2± 4.2
6	MENN	3.65± 1.75
7	MEEJC	20.35± 1.6
8	MEHP	26.12± 5.5

Phenolic moieties present in the molecular structure of natural antioxidants often help in enhancing their antioxidant activity [20-21]. The phenol content of plant extracts were measured based on the method of Singleton et al., 1999 at 760nm and tabulated the results in Table 4. An increase in the absorbance of the reaction mixture which indicates the increased antioxidant activity.The methanolic extract of *Hackeria subpeltata* (Willd.)Kunth has the maximum value (23.02mg /gm fresh leaf) followed by water extract of *Embelia tsjeriam-cottam* (Roem. & Schult.)DC (16.78 mg /gm fresh leaf), methanolic extract of *Embelia tsjeriam-cottam* (Roem. & Schult.) (13.10 mg /gm fresh leaf).

Table 4: Total phenole content of plant extracts.

Treatment	**Sample**	**Total phenols (mg/gm fresh leaf weight**
1	WGH	11.91 ±.13
2	WNN	10.49 ±.45
3	WEJC	16.78 ±.44
4	WHP	12.25 ±.12
5	MEGH	12.38 ±.27
6	MENN	8.43 ±.14
7	MEEJC	13.10 ±.30
8	MEHP	23.02 ±.60

(WGH, WNN, WEJC, WHP**:** Water extracts of *Gomphostemma heyneanum* Benth.*var. heyneanum, Nothapodytes nimmoniana* (Graham) Mabb *Embelia tsjeriam-cottam*(Roem.&Schult.)DC., and *Hackeria subpeltata* (Willd.)Kunth MEGH, MENN, MEEJC, MEHP: Methanolic extracts of *Gomphostemma heyneanum* Benth.*var. heyneanum, Nothapodytes nimmoniana* (Graham) Mabb *Embelia tsjeriam-cottam*(Roem.&Schult.)DC., and *Hackeria subpeltata* (Willd.)Kunth)

The present study result highlights the potential of traditional plants as part of tribal medicines in therapeutic uses. The results form a good basis for the selection of plants for further investigations in the preparation of medicines for various ailments.

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