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Research Article

SCREENING OF *POLYCARPAEA CORYMBOSA* Lam. (CARYOPHYLACEAE) FOR ITS *IN VITRO* ANTIOXIDANT ACTIVITY

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ABSTRACT

The methanolic extracts of *Polycarpaea corymbosa* Lam. root and aerial parts were evaluated for their *in vitro* antioxidant potential**.** The crude methanolic extracts exerted significant (P<0.005) antioxidant activity as evidenced by its Total Phenol content, Total Flavanoid content, 1,1-diphenyl-2- picrylhydrazyl radical (DPPH•), reducing power assay, ABTS•+ and metal chelating activities. The methanolic root extract was found to have significant radical scavenging activity as compared to the synthetic antioxidant BHT. From the results of the present investigation, it could be concluded that *Polycarpaea corymbosa* extracts can be explored as a potential source for the isolation of natural antioxidant agents. However, an appropriate solvent extraction system should be used to recover potent antioxidant components from *Polycarpaea corymbosa.*

Keywords: Antioxidant activity, methanolic extract of *P.corymbosa*, DPPH•, reducing power assay, ABTS•+, metal chelating

INTRODUCTION

Free radicals and its adverse effects were discovered in the last decade. These are dangerous substances produced in the body along with the toxins and wastes which are formed during the normal metabolic process of the body. The high levels of free radicals in living systems are able to oxidize biomolecules, leading to tissue damage, cell death or various diseases such as cancer, cardiovascular diseases, arteriosclerosis, neural disorders, skin irritations and inflammations [1,2]. It compounds can deactivate and scavenge free radicals. Antioxidants can inhibit the effect of oxidants by donating hydrogen atom or chelating metals [3,4,5]. Medicinal plants are the main source of natural antioxidants.

Polycarpaea corymbosa is an herb belongs to the family Caryophylaceae, a large group of medicinal plants and cosmopolitan in distribution. The whole plant of *Polycarpaea corymbosa* have been used in folk medicine especially in rural areas, for the treatment of various diseases like antidotes, jaundice, skin rashes and inflammations. Therefore the present study was carried out to asses the *in vitro* antioxidant activity in methanolic extract of root and aerial parts of *Polycarpaea corymbosa* Lam.

MATERIALS AND METHODS

Plant material

Polycarpaea corymbosa Lam. roots and their aerial parts were collected from Chennimalai, Erode district during November 2011. They were authenticated by Botanical Survey of India, Southern Circle, Coimbatore.

Preparation of the extract

Plant materials (aerial and root) were washed with distilled water and shade dried. The dried samples were manually ground to a fine powder. The coarsely powdered parts were exhaustively extracted with methanol for 8 h using Soxhlet apparatus. The filtrate was then evaporated to dryness under reduced pressure using rotary vaccum evaporator. The extracts were lyophilized until further use.

Chemicals

Sodium carbonate, Folin-Ciocalteu reagent, Potassium acetate, Aluminium chloride, Gallic acid, Rutin, Dimethyl Sulphoxide (DMSO), DPPH (1,1-diphenyl-2-picryl hydrazine), ABTS•+ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), Ascorbic acid, Potassium persulfate, Phosphate buffer, Potassium Ferricyanide, Trichloroacetic acid (TCA), Ferric chloride ($FeCl_3$), Ferrous sulphate (FeSO4), ferrozine, BHT (Butylated hydroxytoluene), EDTA (Ethylene diamine tetra aciticaacid), Trolex and all other chemicals were of analytical grade.

Total Phenolic Content

The total phenolic content of the extract was determined using the method of Macdonald *et al*. (2001)[6] with slight modifications. Absorbance values were measured at 765 nm and the standard curve was drawn after an incubation of 40 minutes in dark to determine the total phenolic content. All determinations were carried out in triplicate. The total phenolic content in the extract were presented as mg Gallic Acid Equivalents (GAE)/ g extract.

Determination of Total Flavonoid Content

Total Flavonoids of extracts were estimated as mg Rutin Equivalents (RE) /g extract, from the Rutin calibration curve. The reaction mixture was prepared by mixing 0.5 ml of extract solutions with 1.5ml of 95% ethanol followed by 0.1 ml (10 g/l) Aluminium chloride and 0.1 ml (98.5 g/l) of Potassium acetate. Each reaction flask was then immediately diluted with 2.8 ml of distilled water and mixed. The absorbance of reaction mixture was read at 415 nm [7].

DPPH• scavenging activity

DPPH (1,1-diphenyl-2-picryl hydrazine) free radical-scavenging capabilities of methanolic extracts were evaluated by the method of Blois (1958)[8]. Briefly, different concentrations (50, 100,150,200 and 250 mg/ml) of the extracts were pipetted out to the test tubes. 100µL of 0.2 mM alcoholic DPPH solution was added to the samples. These samples were vortexed, and incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank samples. Decreased absorbance of the sample indicates DPPH• free radical scavenging capability [9,10].

ABTS•+ radical scavenging assay

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity assay was carried out using procedures described by Re *et al.* (1999)[11]. ABTS•+ radical cations are produced by reacting ABTS 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (7 mM) and potassium persulfate (2.45 mM) and incubating the mixture at room temperature in the dark for 16 hour. The solution thus obtained was further diluted with 89% ethanol to give an absorbance of 0.700 at 734 nm.20µL of the test sample were added to 2 ml of ABTS and the absorbance was recorded at 734 nm after 30 minutes of incubation [12]. Trolox was used as reference standard. The percent inhibition was calculated from the following equation:

$$\text{Percentage of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Reducing power

Methanol extracts were determined for their reducing power by modifying the method of Yildrin *et al*. (2001)[13]. Reaction mixtures were prepared by adding 1 ml of phosphate buffer (200m M, pH 6.6), 1 ml Potassium Ferricyanide (1%) and varying concentrations of extracts (300-700µg). After, the reaction mixtures were incubated at 50°C in water bath for 20 min, it was allowed to cool at room temperature (28°C), and 1 ml of 10% TCA (Trichloroacetic acid) were added to each reaction mixture, and then centrifuged at 3000 rpm for 10 mins. The supernatant (2 ml) was separated in the test tube and added with 2 ml of distilled water and 0.5 ml $FeCl_3$ (1.0%), and allowed to react at room temperature and the absorbance was measured at 700 nm. Ascorbic acid was used as a standard.

Ferrous ion-chelating ability

The ferrous ion-chelating (FIC) assay reported by Singh and Rajini (2004)[14] was adopted. 2 mM FeSO4 (100µl) was mixed with different concentrations of extracts (1000, 2000, 3000, 4000 and 5000 µl), followed by 5mM ferrozine (500 µl). Absorbance was measured at 562 nm after 10 min. The ability of extracts to chelate ferrous ions was calculated as follows:

$$\text{Percentage of inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Statistical analysis

Statistical analysis of the data was performed by analysis of variance (ANOVA), using DMRT software. Statistically significance difference was denoted by probability value of $P < 0.05$. All the data were presented as Mean ± Standard Deviation (SD) for triplicates determinations.

RESULTS AND DISCUSSION

Total Phenol and Flavonoid Content

Phenolic compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups [15]. The phenolic compounds may contribute directly to antioxidative action [16]. The concentration of phenolics in the extracts expressed as Gallic acid equivalents (GAE) is shown in Table 1. Methanol extract of the roots (5.33 ± 0.38 gm of GAE/gm extract) had a higher phenolic content than the aerial part (1.60 ± 0.22 gm of GAE/gm extract).

Flavonoids are natural phenolic compounds and well known antioxidants. In various studies, antioxidant activity of the plant extracts was found to be fairly high which are rich in flavonoids [17]. The concentration of flavonoid in the extracts was expressed as Rutin Equivalents per mg of the extract, as shown in Table 1. The most flavonoid rich extract was found to be Methanol extract of the aerial part (1.52 ± 0.414 gm of RE/gm extract), than root (1.26±0.518 gm of RE/gm extract).

DPPH· assay

DPPH radical is a stable organic free radical with adsorption maxima at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow [18]. DPPH radical scavenging activity of the extracts is concentration dependent and a lower IC_{50} value reflects better protective action. The results revealed that the methanolic root parts of *P.corymbosa* . The root extract exhibited higher DPPH free radical scavenging ability (IC_{50} 136.92 mg/ml) followed by the aerial parts (IC_{50} 444.94 mg/ml), when compared to the standard BHA (Fig 1).

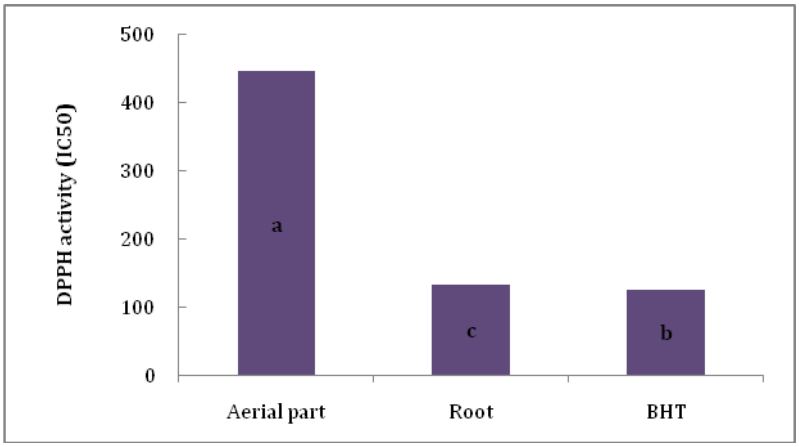

Figure 1: DPPH activity of *P.corymbosa* compared to BHT standard

Table 1: Total Phenolic Content, Total Flavonoid Contents and $ABTS^{\bullet+}$ of *P.corymbosa* Root and Aerial extracts

Sample	Extraction yield	TPC(gm of GAE/gm extract)	TFC(gm of RE/gm extract)	$ABTS^{\bullet+}$ (µ molar Trolox equivalent/ g sample extract)
Aerial part	6.2	1.6±0.22	1.52±0.414	1447.9±175.7
Root	14.6	5.33±0.38	1.26±0.518	4586.6±300.9

$ABTS^{\bullet+}$ radical scavenging activity

$ABTS^{\bullet+}$ assay is an excellent tool to determine the antioxidant activity of hydrogen donating and chain breaking antioxidants. Though all the samples exhibited strong $ABTS^{\bullet+}$ radical scavenging activity, the root extract exhibited higher TAA (4586.6 µmol TE/g methanol extract) followed by aerial extract (1447.9µmol TE/g methanol extract). Pietta *et al.* (1998)[19] evaluated the total antioxidant potential of commonly used medicinal plants and concluded that the phenolic compounds play a vital role in scavenging of $ABTS^{\bullet+}$. The methanol extract of *P.corymbosa* aerial part indicates that they could terminate the oxidation process by converting free radicals to the stable form.

Reducing power

The antioxidant activity has been reported to be concomitant with reducing power [20]. In the assay, the presence of reductants in the antioxidant sample causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous ion.[21] (Gulcin, 2006), so the reducing power of the sample could be monitored by measuring the formation of Perl's Prussian blue at 700 nm [22]. There were no much significant difference in reducing power values among parts of methanol extract. In the present study, the aerial parts of the extract showed significant reducing power ($P<0.05$) than the root (Fig 2). It is suggested that methanol soluble compounds of root might contribute to its effect.

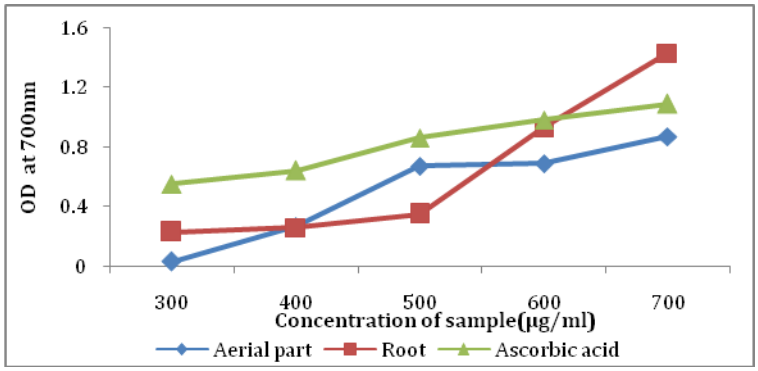

Figure 2. Reducing ability of methanolic root and aerial extract of *P.corymbosa*

Ferrous ion-chelating ability

Presence of transition metal ions in a biological system could catalyse the Haber–Weiss and Fenton-type reactions, resulting in generation of hydroxyl radicals (OH•). However, these transition metal ions could form chelates with the antioxidants, which result in the suppression of OH• generation, and inhibit ion of peroxidation processes of biological molecules. In this assay, the presence of chelating agents in the extracts of *P.corymbosa* disrupts the ferrozine (Fe^{2+}) complex formation, thus decreasing the red colour. The metal ion scavenging effects of aerial and root extracts were 35.94 µM/100 g and 22.84 µM/100 g respectively. It is reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [23]. The data presented in this study indicated that *P.corymbosa* ability for iron binding could reduce the generation of hydroxyl radicals.

Table 2:Effect of Methanolic extract of *P.corymbosa.* on Ferrous Iron Chelating method

S. No	Concentration (µg/ml)	% of activity		
		Aerial part	Root	Standard EDTA
1	1000	29.02±0.22 [a]	14.86±0.11 [a]	56.28±0.19 [a]
2	2000	29.79±0.05 [b]	17.41±0.14 [b]	71.55±0.33 [b]
3	3000	30.91±0.16 [c]	21.04±0.45 [c]	83.46±0.20 [c]
4	4000	31.78±0.12 [d]	22.45±0.09 [d]	93.19±0.21 [d]
5	5000	35.94±0.08 [e]	22.84±0.12 [e]	96.69±0.15 [e]

Each value in the table was obtained by calculating the average of triplicates ±SD.
Mean values followed by different superscript in a column are significantly different (P<0.05).

CONCLUSION

The crude methanol extract and solvent fractions of *P.corymbosa* have indicated strong antioxidant activities at least, *in vitro.* The plant contained phenolic compounds which can serve as a natural source of antioxidants. The study highlights the significance of the free radical scavenging capacity and the potentials of *P.corymbosa* as a source of therapeutic agents. Furthermore, the *in vivo* evaluations as well as the isolation and spectral characterization of the radical scavenging components, were under progress.

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