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Research Article

ANTIOXIDANT POTENTIAL, TOTAL PHENOLIC AND TOTAL FLAVONOIDS CONTENT OF VARIOUS EXTRACTS FROM WHOLE PLANT OF *POLYCARPAEA CORYMBOSA* LAM.

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ABSTRACT

Objective: The objective of the study was to evaluate antioxidant potential, total phenolic and total flavonoids content of various extracts of *P.corymbosa.*

Methods: The antioxidant activity of various extracts of *P.corymbosa* were analysed by using DPPH assay, superoxide radical scavenging assay and total antioxidant activity method.

Results: The ethanolic extract of *P.corymbosa* had showed significant radical scavenging activity. Similar result was not observed in other two extracts. The higher content phenolic and flavonoids were found in ethanolic extract of *P.corymbosa* in comparison with other two extracts.

Conclusion: The results obtained from this study indicate that *P.corymbosa* is a potential source of antioxidants and thus could prevent many radical diseases.

Keywords: P.corymbosa, antioxidant, phenols, flavonoids.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals [1,2] like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10000 oxidative hits per second [3]. Various reactive oxygen species (ROSs) are formed in the living organism in different ways i.e. normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages and peroxisomes. These appear to be the endogenous source of oxidants. Exogenous sources of free radical include tobacco smoking, ionizing radiation, certain pollutants, organic solvents and pesticides [4].

All these free radicals are capable of reacting with membrane lipids, nucleic acids, proteins, enzymes and other micro molecules resulting in cellular damage [5]. Free radicals are involved in the development of degenerative diseases [6]. They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, and in the process of aging [7]. To protect these free radical induced damage, antioxidants are the most popular agents that interactively and synergistically neutralize free radicals. Hence, there has been an increased interest in the food industry as well as in preventive medicine in the development of ''Natural antioxidants'' from plant materials. Therefore, the plants with antioxidant properties are becoming more and more popular all over the world [3].

Polycarpaea corymbosa Lam. is a herb of annual or perennial, small shrubs with taproots slender to stout, stems erect, branched, terete, leaves opposite, sometimes appearing whorled belonging to the family Caryophyllaceae. Flavonoids and phenolic compounds widely distributed in plants have been reported to exert multiple biological effects, including antioxidant, anti-inflammatory, anti carcinogenic, etc. Leaves, flower heads of *P.corymbosa* are used in reducing fever; anti-inflammatory and as a poultice for boils and other swellings; antidote for snakebite, leaves were reported to posses potent antioxidant property and are used for treatments of jaundice, demulcent and astringent in Indian folk medicine**.** The whole parts of *P.corymbosa* are used in Indian traditional medicinal system in inflammatory swellings and in treatment of ulcer, jaundice [8], liver diseases [9]. Antimicrobial activity reported different extracts of *P.corymbosa* against human pathogens [10].

To the best of our knowledge, no reports are available on the antioxidant potential of various extracts of whole plant of *P.corymbosa.* In this paper, we investigated the antioxidant activity of various extracts that were derived from *P.corymbosa.* Furthermore, the total phenolic and total flavonoid content of all extract were also determined.

MATERIALS AND METHODS

Chemicals

Chemical reagents nitroblue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Germany, Catechol (Loba Chemie, Mumbai), sodium carbonate, sodium phosphate, EDTA and Ammonium molybdate (S.D-fine chemicals, Mum- bai).

Plant Material

Whole plant of *P.corymbosa* were collected from –thirunelveli Dt-, Tamil Nadu, India and plant authentication were done by the Botanical survey of India -. The whole plant of *P.corymbosa* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of the extracts

The dried powder was extracted sequentially by hot continuous percolation method using Soxhlet apparatus [11], using different polarities of solvents like petroleum ether, ethyl acetate and Ethanol. The dried powder was packed in Soxhlet apparatus and successively extracted with petroleum ether, ethyl acetate, ethanol extraction. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

DPPH radical scavenging activity [12]

A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

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

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide anion radical scavenging activity [13]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Total antioxidant activity (Phosphomolybdic acid method)[14]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

Determination of total phenolic content [15]

A 1.0ml aliquot of sample was added to 1.5ml of deionized water and 0.5 ml of Folins phenol reagent, and the contents were mixed thoroughly. After 1min, 1.0ml of 20% sodium carbonate was added, and the mixture was again mixed thoroughly. After 30min of incubation at 37°C, the absorbance was measured at 650nm in a spectrophotometer.

Determination of total flavonoids content [16]

A 0.5 ml of aliquot of sample was added with 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Inhibition of DPPH radical

The DPPH assay constitutes a quick and low cost method has frequently been used for the evaluation of the antioxidative potential of various natural producats [17]. The percentage of DPPH radical scavenging activity of petroleum ether extract of *P.corymbosa* was presented in Table 1. The DPPH radical scavenging activity of the petroleum ether extract was increases with increasing concentration, 47.53% DPPH radical scavenging. Nevertheless, it was 70.65% in the presence of 1000µg/ml Rutin. The IC_{50} values of the petroleum ether extract of *P.corymbosa* and Rutin were recorded at 1250µg/ml and 270µg/ml respectively.

Table 1: DPPH radical scavenging activity of petroleum ether extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	12.64 ± 0.03	28.39 ± 0.25
2	250	14.43 ± 0.09	48.98 ± 0.46
3	500	25.45 ± 0.30	65.18 ± 0.13
4	1000	47.53 ± 0.02	70.65 ± 0.20
		IC_{50} = 1250µg/ml	**IC_{50} = 270µg/ml**

* Values are expressed as mean ± SEM of 3 observations.

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *P.corymbosa* was presented in Table 2. The DPPH radical scavenging activity of the ethyl acetate extract was increases with increasing concentration, only 53.45% DPPH radical scavenging. Nevertheless, it was 70.65% in the presence of 1000µg/ml Rutin. The IC_{50} values of the ethyl acetate extract of *P.corymbosa* and Rutin were recorded at 875µg/ml and 270µg/ml respectively.

Table 2: DPPH radical scavenging activity of ethyl acetate extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	14.61 ± 0.04	28.39 ± 0.25
2	250	19.58 ± 0.06	48.98 ± 0.46
3	500	25.97 ± 0.27	65.18 ± 0.13
4	1000	53.45 ± 0.14	70.65 ± 0.20
		IC_{50} = 875µg/ml	**IC_{50} = 270µg/ml**

* Values are expressed as mean ± SEM of 3 observations.

The percentage of DPPH radical scavenging activity of ethanolic extract of *P.corymbosa* was presented in Table 3. The DPPH radical scavenging activity of the ethyl acetate extract was increases with increasing concentration, only 72.02% DPPH radical scavenging. Nevertheless, it was 70.65% in the presence of 1000µg/ml Rutin. The IC_{50} values of the ethanolic extract of *P.corymbosa* Rutin were recorded at 225µg/ml and 270µg/ml respectively.

Table 3: DPPH radical scavenging activity of ethanolic extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethanolic extract)	Standard (Rutin)
1	125	38.65 ± 0.16	28.39 ± 0.25
2	250	56.51 ± 0.03	48.98 ± 0.46
3	500	59.76 ± 0.18	65.18 ± 0.13
4	1000	72.02 ± 0.43	70.65 ± 0.20
		IC_{50} = 225µg/ml	**IC_{50} = 270µg/ml**

* Values are expressed as mean ± SEM of 3 observations.

On the DPPH radical, ethanolic extract of *P.corymbosa* had significant radical scavenging effect with increasing concentration in the range of 125-1000µg/ml when compared with that of Rutin (standard), the scavenging activity of other two extracts were little lower. An IC_{50} value of ethanolic extract of *P.corymbosa* and Rutin was recorded as 225µg/ml and 270µg/ml respectively.

Inhibition of Superoxide anion radical scavenging activity

Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to tissue damage and various diseases [18]. The percentage of superoxide anion scavenging activity of petroleum ether extract of *P.corymbosa* was presented in Table 4. A maximum scavenging activity of petroleum ether extract and Quercetin at 1000 µg/ml was found to be 39.14% and 89.28% respectively. IC_{50} value of petroleum ether extract on superoxide radical scavenging activity was found to be 1365 µg/ml, whereas the IC_{50} value of Quercetin was found to be 145µg/ml.

Table 4: Superoxide anion radical scavenging activity of Petroleum ether extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)* Sample (Pet. ether extract)	Standard (Quercetin)
1	125	13.26 ± 0.48	49.44 ± 0.48
2	250	25.17 ± 0.42	61.88 ± 0.49
3	500	32.06 ± 0.52	78.39 ± 0.18
4	1000	39.14 ± 0.43	89.28 ± 0.09
		IC_{50} = 1365 µg/ml	**IC_{50} = 145 µg/ml**

* Values are expressed as mean ± SEM of 3 observations.

The percentage of superoxide anion scavenging activity of ethyl acetate extract of *P.corymbosa* was presented in Table 5. A maximum scavenging activity of ethyl acetate extract and Quercetin at 1000 µg/ml was found to be 49.42% and 89.28% respectively. IC_{50} value of ethyl acetate extract on superoxide radical scavenging activity was found to be 1005 µg/ml, whereas the IC_{50} value of Quercetin was found to be 145µg/ml.

Table 5: Superoxide anion radical scavenging activity of Ethyl acetate extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)* Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	21.87 ± 0.33	49.44 ± 0.48
2	250	33.84 ± 0.58	61.88 ± 0.49
3	500	40.67 ± 0.22	78.39 ± 0.18
4	1000	49.42 ± 0.15	89.28 ± 0.09
		IC_{50} = 1005 µg/ml	**IC_{50} = 145 µg/ml**

* Values are expressed as mean ± SEM of 3 observations.

The percentage of superoxide anion scavenging activity of ethanolic extract of *P.corymbosa* was presented in Table 6. A maximum scavenging activity of ethanolic extract and Quercetin at 1000 µg/ml was found to be 49.42% and 89.28% respectively. IC_{50} value of ethyl acetate extract on superoxide radical scavenging activity was found to be 110µg/ml, whereas the IC_{50} value of Quercetin was found to be 145µg/ml.

Table 6: Superoxide anion radical scavenging activity of ethanolic extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)* Sample (Ethanolic extract)	Standard (Quercetin)
1	125	51.11 ± 0.50	49.44 ± 0.48
2	250	68.22 ± 0.11	61.88 ± 0.49
3	500	81.10 ± 0.31	78.39 ± 0.18
4	1000	84.41 ± 0.03	89.28 ± 0.09
		IC_{50} =110 µg/ml	**IC_{50} = 145 µg/ml**

* Values are expressed as mean ± SEM of 3 observations.

On the superoxide anion radical scavenging activity, the ethanolic extract of *P.corymbosa* had significant when compared to standard Quercetin. Similar result was not obtained into other two extracts. IC_{50} value of ethanolic extract of *P.corymbosa* and Quercetin was found to be 85µg/ml and 145µg/ml respectively.

Determination of Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *P.corymbosa* was depicted in Table 7. The petroleum ether extract of *P.corymbosa* exhibited a maximum total antioxidant activity of 39.41% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. IC_{50}values of the petroleum ether extract of *P.corymbosa* and ascorbate were found to be 1320µg/ml and 410µg/ml respectively.

Table 7: Total antioxidant activity of Petroleum ether extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)* Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	18.26±0.09	26.87 ± 0.08
2	250	24.39±0.19	30.30 ± 0.05
3	500	33.42±0.27	60.64 ± 0.02
4	1000	39.41±0.14	55.23 ± 0.01
		IC_{50} = 1320µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements.

The percentage of total antioxidant activity of ethyl acetate extract of *P.corymbosa* was presented in Table 8. The ethyl acetate extract of *P.corymbosa* exhibited a maximum total antioxidant activity of 45.49% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the ethyl acetate extract of *P.corymbosa* and ascorbate were found to be 1090µg/ml and 410µg/ml respectively.

Table 8: Total antioxidant activity of Ethyl acetate extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)* Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	13.36±0.16	26.87 ± 0.08
2	250	17.71±0.50	30.30 ± 0.05
3	500	40.12±0.43	60.64 ± 0.02
4	1000	45.49±0.16	55.23 ± 0.01
		IC_{50} = 1090µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements.

The percentage of total antioxidant activity of ethanolic extract of *P.corymbosa* presented in Table 9. The ethanolic extract of *P.corymbosa* exhibited a maximum total antioxidant activity of 69.65% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the ethanolic extract of *P.corymbosa* and ascorbate were found to be 255µg/ml and 410µg/ml respectively.

Table 9: Total antioxidant activity of ethanolic extract of *P.corymbosa*

S.No	Concentration (µg/ml)	% of activity(±SEM)* Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	39.20±0.02	26.87 ± 0.08
2	250	49.73±0.29	30.30 ± 0.05
3	500	61.29±0.70	60.64 ± 0.02
4	1000	69.65±0.05	55.23 ± 0.01
		IC_{50} = 255µg/ml	**IC_{50} = 410 µg/ml**

*Data presented as the mean ± SEM for three measurements.

Based on the above data result clearly indicated the ethanolic extract of *P.corymbosa* had more effective antioxidant activity when compared to standard ascorbate. Similar results were not revealed in other two extracts. IC_{50} values of the ethanolic extract of *P.corymbosa* and Ascorbate were found to be 255µg/ml and 410µg/ml respectively.

Total phenolic content

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [19]. The phenolic compounds may contribute directly to antioxidative action [20]. The content of phenolic compounds (mg/g) in various extracts of *P.corymbosa* was presented in Table 10. The ethanolic extract of *P.corymbosa* was found higher content of phenolic components than that extracts. These results suggest that the high levels of antioxidant activity were due to the presence of phenolic components. From all these observations it can be concluded that the plant extracts with high level of polyphenolic compounds show good antioxidant activity *in vitro* systems.

Table 10: The total Phenolic content of various extracts of *P.corymbosa*

S.No	Extracts	Total phenol content (mg/g of
1.	Petroleum ether extract of *P.corymbosa*	1.94 ± 0.22
2.	Ethyl acetate extract of *P.corymbosa*	2.04 ± 0.73
3.	Ethanolic extract of *P.corymbosa*	4.60 ± 0.05

*All values are expressed as mean ± SEM for three determinations

Total flavonoids content

The total amount of flavonoids content of various extract of *P.corymbosa* was summarized in Table 11. Flavonoids present in food of plant origin are also potential antioxidants [21,22]. The higher content of flavonoids was found in ethanolic extract of *P.corymbosa* than that of other extracts.

Table 11: The total flavonoids content of various extracts of *P.corymbosa*

S.No	Extracts	Total flavonoids content of Catechol (mg/g) (±SEM)*
1.	Petroleum ether extract of *P.corymbosa*	0.97 ± 0.06
2.	Ethyl acetate extract of *P.corymbosa*	1.70 ± 0.09
3.	Ethanolic extract of *P.corymbosa*	3.63 ± 0.93

*All values are expressed as mean ± SEM for three determinations

CONCLUSION

In the present investigation, we observed that ethanolic extract of whole plant of *P.corymbosa* contained higher levels of total phenolic and flavonoid compounds and was capable of inhibiting, quenching free radicals to terminate the radical chain reaction, and acting as a reducing agent. Significant antioxidant activity of ethanolic extract of *P.corymbosa* provides a scientific validation for this plant as an accessible source of natural antioxidants with consequent health benefits. Further work on isolation and identification of active compounds and their efficacy needs to be done.

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