The Pharma Innovation

ISSN: 2277- 7695
CODEN Code: PIHNBQ
ZDB-Number: 2663038-2
IC Journal No: 7725

Received: 03-04-2013
Accepted: 05-06-2013

Vol. 2 No. 5 2013
Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION - JOURNAL

# Antioxidant Activities of *Polycarpaea corymbosa* Lam. (Caryophyllaceae) Using Various *In vitro* Assay Models

Sindhu S [1*] and S Manorama [2]

1. Sindhu S, PG and Research Dept. of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-29, Tamil Nadu, India.
   [E-mail: ssindhu389@gmail.com; Tel: +91-9976498001]
2. S Manorama, PG and Research Dept. of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-29, Tamil Nadu, India.
   [E-mail: drsmanorama@gmail.com; Tel: +91-9976498001]

Natural products have the potential to be developed into new drugs for the treatment of various diseases. The aim of the present study was to screen the antioxidant activities of medicinal plants indigenous to Tamil Nadu, India. The antioxidant potential of both extracts of roots and stems were evaluated using different antioxidant tests, namely total antioxidant (ABTS), DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and metal chelating activities. The *Polycarpaea corymbosa* Lam. extracts had higher antioxidant activities which were 94.56% (DPPH), 65.39 mol $l^{-1}$ (FRAP), and 5332.5 (ABTS) and 82.51% (DPPH), 48.28 mol $l^{-1}$ (FRAP), and 5224.5 (ABTS) in aerial and root respectively. The study suggests that *Polycarpaea corymbosa* extracts exhibit great potential for antioxidant activity and may be useful for their nutritional and medicinal functions.

*Keyword:* *Polycarpaea corymbosa,* DPPH, ABTS, Metal Chelating Assay, Total Phenol And Flavonoid Content.

## 1. Introduction

Virtually all the medicinal plants available in the world have great potential sources for discovery as well as production of new drugs beneficial to mankind. Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries[1]. Natural antioxidants exhibit a wide range of pharmacological activities, and have been shown to have anticancer, anti-inflammatory and anti-aging properties[2]. Many works have been expanded to evaluate and discover new antioxidant, antimicrobial ingredients from different kinds of natural sources like soil, microorganisms, animals and plants. Different types of folk medicine or herbal medicine are among the most important resources. In the recent years, natural antioxidants, particularly those present in fruits and vegetables have gained increasing interests among consumers and the scientific community. There has been worldwide trend towards the use and ingestion of natural antioxidants such as phenolic acids, flavanoids and tannins, which possesses more potent antioxidant activity then dietary plants due to their phytochemical constituents[3,4].

*Polycarpaea corymbosa*, commonly known as Old's man cap is a herb species in the family are

mostly found as weed on open, often moist, sandy soils, less often in grassy places on mountains and slopes about 1200 m sea level. Flavanoids and phenolic compounds widely distributed in plants have been reported to exert multiple biological effects, including antioxidant, anti-inflammatory, anticarcinogenic etc. Traditionally, the whole plant is taken orally for inflammation, ulcer and jaundice[5]. Apart from this the plant possesses antioxidant property and antimicrobial activity, used in reducing fever and antidote for snake bite.

This study was designed for the evaluation of possible beneficial antioxidative potency of the *Polycarpaea corymbosa* extracts by employing different methods and techniques.

## 2. Materials and Methods

### 2.1 Plant material

*Polycarpaea corymbosa* L. roots and their aerial parts were collected from Chennimalai, Erode district during November 2012. They were authenticated by Botanical Survey of India, Southern Circle, and Coimbatore.

### 2.2 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 petroleum ether, chloroform, acetone and methanol for 8 h using Soxhlet apparatus. The filtrate was then evaporated to dryness under reduced pressure using rotary vacuum evaporator. The extracts were lyophilized until further use.

### 2.3 Chemicals

Dimethyl Sulphoxide (DMSO), Folin-Ciocalteu reagent, Sodium bicarbonate($Na_2Co_3$), Gallic acid, Aluminium chloride, Potassium acetate, Rutin, DPPH (1,1-diphenyl-2-picryl hydrazine), BHT (Butylated hydroxytoluene), Potassium persulfate, $ABTS^{\bullet+}$ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), Trolox, Ferrous sulphate ($FeSO_4$), ferrozine, EDTA (Ethylene diamine tetra acetic acid), and all other chemicals were of analytical grade.

### 2.4 Determination of 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.

### 2.5 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].

### 2.6 $DPPH^{\bullet}$ 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 $DPPH^{\bullet}$ free radical scavenging capability [9,10].

### 2.7 $ABTS^{\bullet+}$ 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]. $ABTS^{\bullet+}$ 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 $ABTS^{\bullet+}$ 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:

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

### 2.8 Ferrous ion-chelating Ability

The ferrous ion-chelating (FIC) assay reported by Singh and Rajini (2004)[13] was adopted. 2 mM $FeSO_4$ (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:

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

### 2.9. 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.

## 3. Result and Discussion

### 3.1 Total phenol

The acetone aerial fraction showed the highest amount of total phenolic compounds 5.33±0.38 gm of GAE/gm extract while that of acetone root fraction it was found to be 4.65±0.78 gm of GAE/gm extract. The total phenolic contents of petroleum ether, chloroform, acetone and methanol are shown in the table 1. These compounds exhibit antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals[14]. Phenolic compounds are known to inhibit various types of oxidizing enzymes. These potential mechanisms make the diverse group of phenolic compounds an interesting target in the search for beneficial phytochemicals[15]. Phenols are one of the major groups of nonessential inhibition of atherosclerosis that have been associated with the inhibition of atherosclerosis and cancer, as well as for age-related degenerative brain disorders[16].

### 3.2 Total Flavonoid

The total flavonoid was determined based on detection of colored flavonoids-aluminum complex. Aluminum chloride formed acid stable compound at keto or hydroxyl groups of the flavonoids which lead to formation of the colored flavonoids - aluminium complexes[17]. Rutin was used as a standard ($y = 0.0097x + 0.0127$, $r2 = 0.9995$) and the total flavonoid content of *P. corymbosa* extract was expressed in microgram of Rutin equivalents per gram of extract (lg RE/g extract). The total flavonoid content of *Polycarpaea corymbosa* extracts was varied considerably from 1.26 to 4.50 gm of RE/gm extract. The data presented in Table 1 indicates that the highest flavonoid content of 4.50 gm of RE/gm extract was observed in the acetone extract of root and the lowest content was observed in the methanol extract of the root (1.26 gm of RE/gm extract).

### 3.3 DPPH

The effect of *Polycarpaea corymbosa* extract and standard BHT on DPPH radical was compared and shown in figure1. The scavenging effect increases with the concentration of standard and sample at 50 µg /ml concentration of petroleum ether, chloroform, acetone and methanol extract of aerial and root of *Polycarpaea corymbosa* plant more scavenging activity on DPPH. Antioxidant activity of an ethanolic extract of *PC* has previously been reported by Anagha *et al.*[18]. The activity was expressed as the concentration of sample necessary to give a 50% reduction in the sample absorbance ($IC_{50}$).

The DPPH tests provide information on the reactivity of test compounds with a stable free radical. The efficacies of antioxidants are often

associated with their ability to scavenge stable free radicals[19]. It has been shown that the scavenging affects on the DPPH radical increases sharply with standards to a certain extent[20].

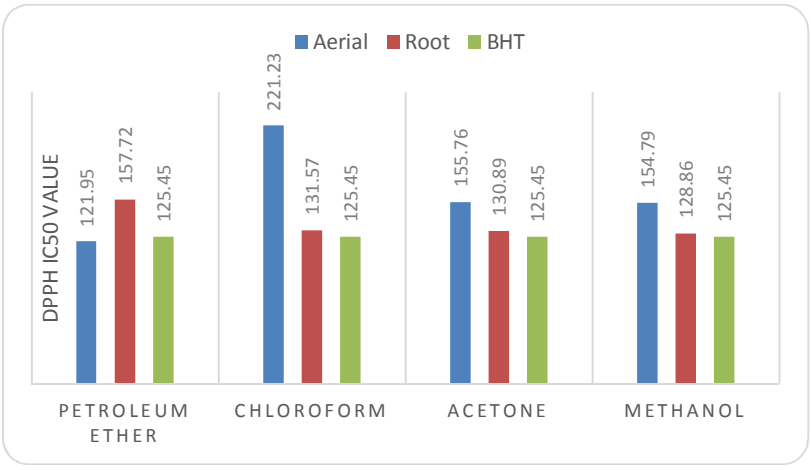


**Fig 1:** Free radical scavenging activity of *Polycarpaea corymbosa* crude extract by DPPH Method

### 3.4 ABTS$^{\bullet+}$

The ABTS$^{\bullet+}$ radical cation scavenging activity was found to be increased in a dose dependent manner from 4509.0 (chloroform) – 5332.5 (Acetone) and 3152.2 (Petroleum ether) – 5224.5 (Chloroform) % in aerial and root respectively at a concentration of 20 µmolTE/g sample extract (table 1). ABT's radical scavenging activity is relatively is recent one, which involves a more drastic radical, chemically produced is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids. The extracts showed potent antioxidant activity in ABT's method which is comparable to the standard Trolox. The ABTS$^{\bullet+}$ radical scavenging activities its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain breaking reaction. The limitation of this method is the fact that the ABTS reagent, in combination with flavonoids, develops strong antioxidative complexes leading to the determination of overestimated values of the antioxidative potential[21].

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

| Crude extract | TPC (gm of GAE/gm extract) | | TFC (gm of RE/gm extract) | | ABTS (µ molar Trolox equivalent/ g sample extract) | |
|---|---|---|---|---|---|---|
| | Aerial | Root | Aerial | Root | Aerial | Root |
| Petroleum ether | 1.00±0.07 | 0.54±0.09 | 1.52±2.2 | 2.55±2.3 | 4623.7±5.8 | 3152.2±5.8 |
| Chloroform | 0.87±0.19 | 1.02±0.30 | 2.28±0.2 | 4.06±2.33 | 4509.0±5.8 | 5224.5±44.1 |
| Acetone | 4.30±0.24 | 4.65±0.78 | 2.8±2.62 | 4.50±3.1 | 5332.5±5.8 | 4964.6±32.5 |
| Methanol | 1.60±0.22 | 5.33±0.38 | 1.5±0.41 | 1.26±0.51 | 5116.5±5.8 | 4046.6±71.1 |

### 3.5 Ferrousion ($Fe^{2+}$) Chelating activity

The results of antioxidant activity of the different extracts of *Polycarpaea corymbosa* based on metal chelating activity are given in fig 2. The percentage of metal chelating activity was determined to be sample concentration dependent and it was increasing with the increase in concentration of extract from 500 to 2500 µg/ml. The percentage of inhibition of the metal chelating was varying from 8.28% (in 500 µg/ml of acetone extract) to 65.39% (in 2500 µg /ml of chloroform extract). The activity was expressed as the concentration of sample necessary to give a 50% reduction in the sample absorbance ($IC_{50}$).

The values obtained were compared with the standard EDTA. The aerial and root extracts highest metal chelating activity was observed in chloroform extract (65.39 µM/100 g and 48.28 µM/100 g respectively). Presence of transition metal ions in a biological system could catalyze 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.

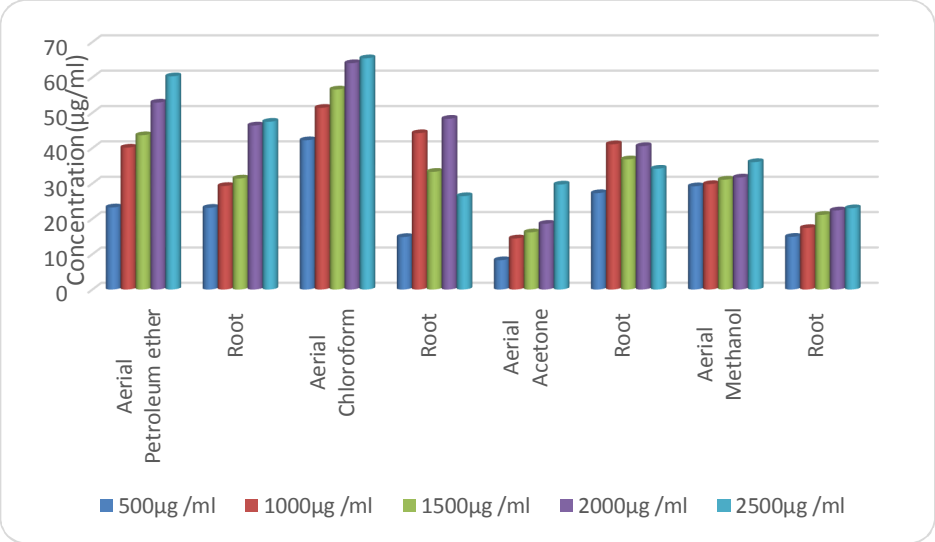


**Fig. 2**: Free radical scavenging activity of *Polycarpaea corymbosa* crude extract by Ferrous ion ($Fe^{2+}$) Chelating activity

## 4. Conclusion

Flavonoid play vital role in scavenging the free radicals and these are the phytoconstituents to be focused on for investigation of many biological activities. Due to the highly presence of phenolic content in *Polycarpaea corymbosa*, it has more potent for antioxidant activity. Antioxidant properties can be used as easily accessible source of natural antioxidants and as a possible food supplement and also in pharmaceutical industry. The work further reveals that the *Polycarpaea corymbosa* could be an interesting source of antioxidants of potential use in different fields, namely food, cosmetics, and pharmaceuticals. A detailed chemical investigation of these extract is underway to identify the compounds responsible for the antioxidant activity.

## 5. References

1. S. Mathew, and T.E. Abraham TE. In vitro antioxidant activity and scavenging effects of Cinnamomum vernum leaf extract assayed by different methodologies. Food Chem Toxicol, , 44, 2006, pp198-206.
2. M.E. Abalaka, A. Mann, and S.O. Adeyemo. Studies on in vitro antioxidant and free radical scavenging potential and phytochemical screening of leaves of Zizipus mauritiana L. and Zizipus spinachrish L. compared with ascorbic acid. J Med Genet Genomics, 3, 2011, pp28-34.
3. K. Madhava chetty, K. Sivaji , and K. Tulasi Rao. "Polycarpaea corymbosa L." Flowering Plants of Chittoor District, Andhra Pradesh, India. 2008.
4. S. McDonald, P.D. Prenzler, M. Autolovich, and K. Robards. Phenolic content and antioxidant activity of olive oil extracts. Food Chem., 73, 2001, pp73-84.
5. G. Miliauskas, P.R. Venskutonis, and T.A. Van Beek. Screening of radical scavenging activity of some medicinal plants and aromatic plant extract. Food Chem., 85, 2004, pp231-237.
6. M. S. Blois. Antioxidant determinations by the use of a stable free radical. Nature,1958, 26.
7. I. Gulcin, Beydemir , H.A. Alici, M. Elmasta, and M. E. Buyukokurolu. In vitro antioxidant properties of morphine. Pharmacol. Res., 49, 2004a, pp 59- 66.

8. Gulcin,V. Mshvildadze, A. Gepdiremen, and R. Elias. Antioxidant activity of saponins isolated from ivy: a-Hederin, hederasaponin-C, hederacolchiside-E and hederacolchiside. F. Planta Med., 70, 2004b, pp 561- 563.
9. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice- Evans. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Me., 26, 1999, pp 1231 – 1237.
10. B. Auddy, M. Ferreira , F. Blasina, L. Lafon , F. Arredondo, F. Dajas, P.C. Tripathi, T. Seal and B. Mukherjee. Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. J. Ethnopharmacol, 84, 2003,pp 131.
11. N. Singh and P.S. Rajini. Free radical scavenging activity of an aqueous extract of potato peel. Food Chemistry, 85, 2004, pp 611–616.
12. F.O. Jimoh, A.A. Adedapo, A.A Aliero, and A.J Afolayan. Polyphenolic contents and biology activities of Rumex ecklonianus. Pharm. Biol., 46(5), 2008, pp333-340.
13. D.A. Akinpelu, O.A. Aiyegoro, and A.I. Okoh. The in vitro antioxidant property of methanolic extract of Afzelia africana (Smith.). J .Med. Plants Res., 4(19), 2010, pp2021-2027.
14. L. M. Cheung, P. C. K. Cheung , and V. E. C. Ooi. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem., 81, 2003, pp249-255.
15. C. C. Chang, M. H. Yang, H. M. Wen and J. C. Chern. Estimation of total flavonoid contents in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10(3), 2002, pp 178-182.
16. Anagha A Rajopadhye and Anuradha S Upadhye. Determination of phenolic content and in vitro antioxidant potential of ethanol extract of seven sources of Ayurvedic drug'pittapapda'. Indian journal of Natural products and resources, 4(1), 2012, pp 81-87.
17. A.V. Krishnaraju, C.V. Rao, T.V.N. Rao, K.N. Reddy, and G. Trimurtular. In vitro and in vivo antioxidant activity of Aphanamixis polystachya bark. Am j infe Dis, 5, 2009, pp60-67.
18. G. Motalleb, P. Hanachi, S.H. Kua, O. Fauzia and R. Asmah. Evaluation of phenolic content and total antioxidant activity in Berberis vulgaris fruit extracts . J Biol Sci, 5, 2005, pp648-453.
19. M. Arts, G. Haenen, H. Voss, and A. Bast. Antioxidant capacity of reaction products limits the applicability of the Trolox Equivalent Antioxidant Capacity (TEAC) assay. Food Chem. Toxicol. 42, 2004, pp 45-49.
20. B. Halliwell. Antioxidants in human health and disease. Annu. Rev. Nutr. 16, 1996, pp 33-50.
21. J.M. Matés, C. Pérez-Gómez., and I. Núñez de Castro. Antioxidant enzymes and human diseases. Clin. Biochem. 3,1999, pp 595-603.