



Research Article

EVALUATION OF PHYTOCHEMICAL PROPERTIES AND ANTI OXIDANT POTENTIAL OF *PRIVA CORDIFOLIA*

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ABSTRACT

Priva cordifolia (Verbenaceae) is broadly dispersed all through India in states like Andhra Pradesh, Gujarat, Karnataka, Kerala and Tamil Nadu. The species of genus *Priva* are known for their rich composition of Phenolic compounds. Therefore, phytochemical properties and antioxidant potential of *Priva cordifolia* were evaluated in the present study. The ethanol extract of *P.cordifolia* [EEPC] was obtained by Soxhletion, with ethanol and was further processed for phytochemical screening, Total Phenol Content, Total Flavonoid Content and various *in vitro* antioxidant assays. The phytochemicals present in EEPC were Carbohydrates, Proteins, Steroids, Phenolic compounds, Flavonoids and Triterpenoids. The Total Phenolic and Flavonoid contents of EEPC were found to be 98.62 mg Gallic acid equivalents (GAE) per gram of dry extract and 49.76 mg Quercetin equivalents (QE) per gram of dry extract, respectively. In DPPH radical scavenging activity, EEPC exerted an inhibition of 71% and that of ascorbic acid was 79% at 500µg/ml. In the FRAP assay, the absorbance of EEPC clearly increased, due to the formation of the Fe²⁺-TPTZ complex with increasing concentration. Hydroxyl radical scavenging activity of the EEPC was 64% while ascorbic acid reached 73% at 500µg/ml. Superoxide radical scavenging activity of the EEPC was 79% while ascorbic acid reached 84% at 1000 µg/ml. Nitric oxide radical scavenging activity of the EEPC reached 54% while ascorbic acid reached 70% at 500µg/ml. The present study suggests that this plant could be of great importance for the treatment of free radical related diseases.

Key words: *Priva cordifolia*, phytochemical screening, Phenolic compounds, antioxidant activity.

INTRODUCTION

Medicinal plants aids as the repository of potent useful chemical compounds which could serve as bioactive leads and clues for modern drug design¹. The most crucial of these bioactive constituents of plants are alkaloids, terpenoids, flavonoids, phenolic compounds and tannins². Antioxidant compounds have a highly important role in the well being of human health. Many scientific proofs have shown that antioxidant compounds can cut down the risk of chronic diseases such as cancer and coronary heart disease. In addition, antioxidants also inhibits or delays the oxidation of oxidized substrates in chain reactions, hence these compounds are very important for disease prevention³⁻⁹. The major characteristic of antioxidant compounds is its ability to capture free radicals¹⁰. Free radicals can oxidize nucleic acids, proteins, fats, and even DNA as well as initiate the onset of degenerative diseases¹¹. Antioxidant compounds obtained from plants, including vitamin C, vitamin E, carotene, phenol groups especially polyphenols, and flavonoids, are known to potentially diminish the risk of degenerative diseases^{10,12}. The living system has different antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which play an important role in scavenging the free radicals and protecting cell membrane from injury¹³. A large number of medicinal plants have been studied and proved to possess antioxidant property¹⁴. Hence the objective of the study was to evaluate phytochemicals present in *Priva cordifolia* belonging to

the family Verbenaceae which is known to possess a wide range of therapeutic activities.

The plant *P.cordifolia* is commonly known as *Heart-Leaf Velvet Bur*. The local name of *P.cordifolia* in Telugu is Magalingaku and in Tamil it is called Ottu urinji. *P.cordifolia* is an erect, perennial herb, growing up to 1 m height. The plant is branched with notably elongated fruiting branches. Leaves are ovate, ovate-triangular to elliptic, 3-6 cm in length, 2-5 cm wide, toothed, blunt, nearly heart-shaped at the base with hooked hairs. Leaf stalks are 1-2 cm long. Stem is quadrangular, velvety with hooked hairs, particularly at nodes. Flowers are white, slaver form, rarely violetish, about 1 cm across, nearly stalkless. Spike-like flower-racemes are 10-20 cm long. Sepal-tube is cylindrical, clearly toothed, 6-8 mm long, 2-2.5 mm broad. Flowers are 8-12 mm in length and are 2-lipped with 5, unequal petals. Fruit is 4-5 mm in length, broadly obcordate, enclosed within inflated and persistent calyx¹⁵.

The plant has claims to treat wounds¹⁶, ulcer and as anti-fertility drug¹⁷ and in the treatment for diarrhoea. There is also report on its use to treat migraine¹⁸. Leaf powder is used to drive away bugs and also acts as mosquito repellent and leaf paste is used to treat eczema¹⁹. These claims seem to be mostly confined to tribal medicinal practices and scientific evidence in well-established systems is lacking. But other species of *Priva* like *Priva mexicana* was proved to contain high levels of total phenolics (53.4 mg g⁻¹ dt), flavonoids and Total Antioxidant Capacity was 3.93 ± 0.04

mg AAE mL⁻¹ 20. Therefore, the present study was aimed to evaluate phytochemical properties, Total Phenolic content, total flavonoid content and antioxidant potential of *P.cordifolia*.

MATERIALS AND METHODS

Fresh leaves of *Priva cordifolia* were collected from Talakona forest, Chittoor District, Andhra Pradesh, in the month of December. The taxonomical identification and authentication of the plant was done by Dr. K. Madhava Chetty, Assistant Professor, S.V. University, Tirupati, Andhra Pradesh. The leaves were collected and dried under shade at room temperature for 5 days. Then the leaves were ground into coarse powder and passed through mesh size no. 50. The powdered sample of leaf was stored in a closed container free from pollution and environmental contaminants²¹. A voucher specimen has been kept in the herbarium (PC/SNS/SVCP/2017/12) of the Department of Pharmacognosy, Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh (India).

Preparation of extract

50 gm of powdered sample was evenly packed in Soxhlet apparatus and extraction was carried out with 90% Ethanol. The solvent was evaporated at low temperature. The residual ethanol extract of *Priva cordifolia* (EEPC) was used for phytochemical screening²¹.

Preliminary Phytochemical screening

This involves screening and analysis of EEPC for different phytochemical compounds. The Phytochemical screening was done as per WHO guidelines²²⁻²⁵. The results are shown in Table 1.

Estimation of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method²⁶. Samples (100µL) were mixed vigorously with 2 ml of 2% Sodium Carbonate (Na₂CO₃). After 2 min. 100 µL of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of Gallic acid equivalents per 100 gram of dry weight of the plant samples.

Estimation of Flavonoids

The flavonoids content was assessed according to Eom *et al.*²⁷. An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1ml of 10% aluminium chloride and 0.1ml of potassium acetate (1M). 4.3ml of 80% methanol was added to this mixture to make it 5 mL volume. Then this mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The optical density value was used to calculate the total flavonoid content of the sample.

In vitro Antioxidant Activity

DPPH radical scavenging activity

The DPPH radical is a stable free radical and is mostly used to calculate the radical scavenging activity of antioxidant component. This method is depended on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the production of the non radical form DPPH-H. The free radical scavenging activity of EEPC was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the reported method²⁸. In short, 0.1mm solution of DPPH in

methanol was prepared, and 1mL of the prepared solution was added to 3 ml of the solution of extract in methanol at different concentration (125,250,500 & 1000 µg/mL). The absorbance was measured using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation) at 517 nm. Ascorbic acid was used as the reference standard. A lower absorbance value of reaction mixture suggests higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A₀ is the absorbance of the control and A₁ is the absorbance in presence of the extract and reference. All the tests were performed in triplicates and the results were averaged.

Ferric reducing antioxidant power [FRAP] assay

The capability to reduce ferric ions was measured using the method described by Benzie and Strain²⁹. The FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripiryridyl triazine) TPTZ solution and 20.0 mM FeCl₃.6H₂O solution in a ratio of 10:1:1 in volume. Different concentrations of the samples (100,200,300,400 and 500 µg/ml) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of Ferrous Sulphate (FeSO₄) were used for calibration. The antioxidant potential based on the capacity to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mmol FeSO₄ equivalents per gram of sample.

Hydroxyl radical scavenging activity

The scavenging ability for hydroxyl radical was established based on the modified method of Halliwell *et al* (1987)³⁰. Stock solutions of Ferric Chloride (FeCl₃) (10mM), EDTA (1mM), Hydrogen Peroxide (H₂O₂) (10mM), Ascorbic Acid (1mM) and Deoxyribose (10 mM) were prepared in distilled deionised water. The assay was performed by adding 0.1mL EDTA, 0.01mL of FeCl₃, 0.1mL H₂O₂, 0.36mL of deoxyribose, 1.0mL to the extract of different concentration (125, 250, 500 & 1000 µg/mL) dissolved in distilled water, 0.33mL of phosphate buffer (50mM, pH 7.9), 0.1mL of ascorbic acid in sequence. The mixture was later incubated at 37°C for 1 hour. 1.0 mL portion of the incubated mixture was mixed with 1.0 mL of 10% Trichloro acetic acid (TCA) and 1.0 mL of 0.5% Thiobarbituric acid (TBA) (in 0.025M NaOH containing 0.025% BHA) for the formation of the pink chromogen which was measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation and is calculated using the following equation

$$\text{Hydroxyl radical scavenging activity (\% inhibition)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A₀ is the absorbance of the control and A₁ is the absorbance in presence of the extract and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was determined with reference to Srinivasan *et al*³¹. The superoxide anion radicals were produced in 3.0 ml of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of Nitroblue tetrazolium (NBT) (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 mL extract of different concentrations (125,250,500 & 1000 µg/ml) and 0.5 mL Tris – HCl buffer (16 mM, PH 8.0). The reaction was started by adding 0.5 mL Phenazine methosulfate (PMS) solution (0.12 mM) to the mixture and incubated at 25°C for 5 min and then the absorbance

was measured at 560 nm against a blank sample and reference standard used was ascorbic acid. The percentage inhibition was calculated by using the following equation

$$\text{Superoxide radical scavenging activity (\% inhibition)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A₀ is the absorbance of the control and A₁ is the absorbance in presence of the extract and reference. All the tests were performed in triplicates and the results were averaged.

Nitric oxide radical scavenging

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside in aqueous solution, at physiological pH spontaneously generate nitric oxide, which interact with oxygen to produce nitrite ions that can be estimated by Griess reagent. Scavenger of nitric oxide competes with oxygen resulting in decreased production of nitric oxide. The absorbance of chromophore generated during diazotization of nitrite with sulphanilamide following coupling with naphthyl ethylene diamine dihydrochloride was read at 540 nm and compared to the absorbance of standard. The decrease in absorbance of Griess reagent at its absorption maximum of 540 nm is proportional to the concentration of free radical scavenger added to the Griess reagent solution³². Ascorbic acid was used as a standard solution. NO radical scavenging activity was calculated by using following equation:

$$\text{NO Scavenged (\% inhibition)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where, A₀ is the absorbance of the control and A₁ is the absorbance in presence of the extract and reference. All the tests were performed in triplicates and the results were averaged.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening

The Phytochemical study (Table 1) shows ethanol extract of *Priva cordifolia* leaves contains Carbohydrates, Proteins, Steroids, Phenolic compounds, Flavonoids and Triterpenoids.

Table 1. Phytochemical screening of EEPC

Test	Phytochemicals present in EEPC
Alkaloids	-
Glycosides	-
Carbohydrates	+
Steroids	+
Triterpenoids	+
Fixed oils & fats	-
Tannins	-
Proteins & amino acids	+
Gums & mucilage	-
Flavonoids	+
Saponins	-
Phenols & Phenolic compounds	+

Key: "+" - Present; "-" - Absent.

Total Phenol Content and Total Flavonoid Content in EEPC

The Total Phenolic and Flavonoid contents of ethanol extract of *Priva cordifolia* leaves were found to be 98.62 mg Gallic acid equivalents (GAE) per gram of dry extract and 49.76 mg Quercetin equivalents (QE) per gram of dry extract, respectively.

In vitro Antioxidant Activity

DPPH Radical Scavenging Activity

The reactivity of EEPC was analyzed with DPPH, a stable free radical. As DPPH takes up one electron in the presence of a free radical scavenger, the absorption reduces and the resulting discoloration is stoichiometrically related to the number of electrons gained³³. The DPPH radical scavenging (%) activity is shown in the Fig 1, EEPC exhibited an inhibition of 71% and that of ascorbic acid was 79% at 500µg/ml and the IC₅₀ of the extract was 222.79µg/ml, while that of ascorbic acid was 202.72µg/ml.

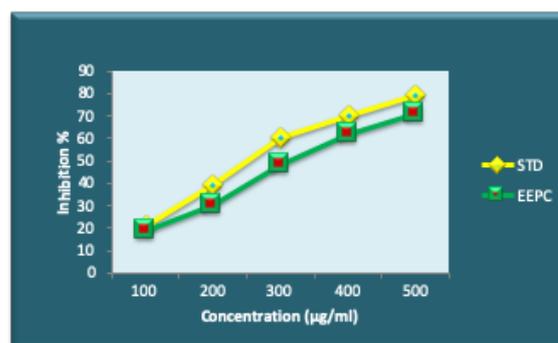


Fig 1. DPPH radical scavenging activity of EEPC

Ferric reducing antioxidant power [FRAP] assay

Frap assay measures the reducing capacity of an antioxidant reacting with a ferric tripyridyl triazine [Fe³⁺-TPTZ] complex and generating a coloured ferrous tripyridyl triazine [Fe²⁺-TPTZ]. In general, the reducing properties are correlated with the presence of compounds which exhibit their action by breaking the free radical chain by donating a hydrogen atom³⁴. Frap assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction³⁵. In the present study, the trend for ferric ion reducing activities of EEPC and Ascorbic acid are shown in Fig 2. The absorbance of EEPC clearly increased, due to the formation of the Fe²⁺-TPTZ complex with increasing concentration.

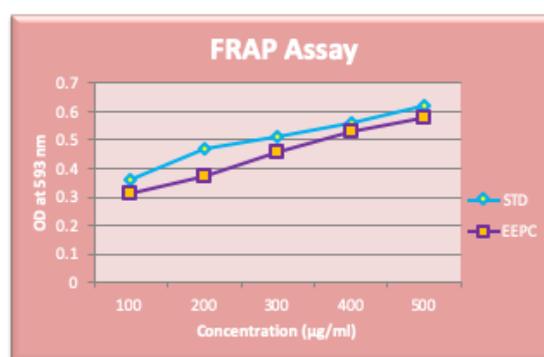


Fig 2. FRAP Assay of EEPC

Hydroxyl radical scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, generally by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H₂O₂ can probably react with Fe²⁺, and possibly Cu²⁺ to form hydroxyl radical and this may be the cause of many of its toxic effects³⁶. Hydrogen peroxide scavenging activity of the extract is presented in Fig. 3; the extract exhibited a concentration dependent scavenging. At a concentration of 500µg/ml, the Hydroxyl radical scavenging

activity of the EEPC was 64% while at the same concentration, the ascorbic acid reached 73%. The IC₅₀ value of the extract was 184.9µg/ml, whereas the standard exerted an IC₅₀ of 112.5µg/ml³⁷.

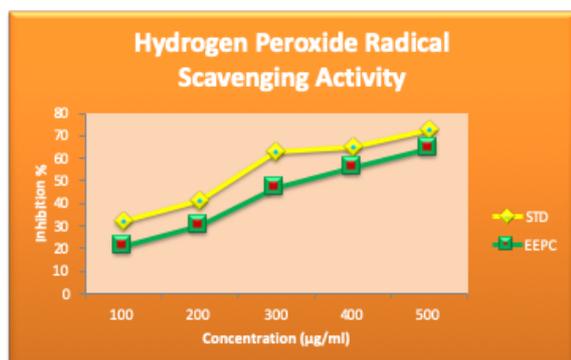


Fig 3. Hydroxyl radical scavenging activity of EEPC

Superoxide radical scavenging activity

Though superoxide anion is a weak oxidant, it produces powerful and dangerous hydroxyl radicals as well as singlet oxygen, which contribute to oxidative stress³⁸. Number of biological reactions gives rise to superoxide anions which are intensely toxic species. At a concentration of 1000 µg/ml, the superoxide radical scavenging activity of the EEPC was 79% while at the same concentration, ascorbic acid reached 84%, which was significantly comparable (Figure 4). The IC₅₀ values were found to be 70.6 µg/mL and 40.8 µg/mL respectively for EEPC and ascorbic acid. The results clearly indicate that EEPC have a noticeable effect as scavenging superoxide radical.

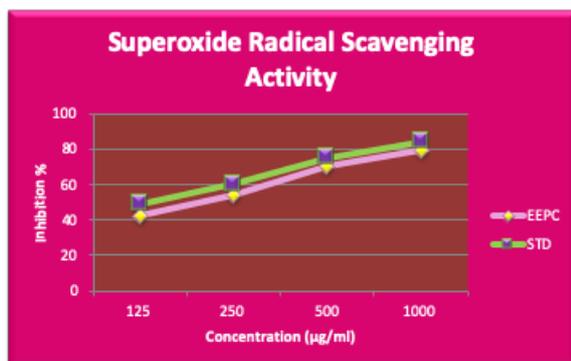


Fig 4. Superoxide radical scavenging activity of EEPC

Nitric oxide radical scavenging assay

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities³⁹. Suppression of released NO may be partially attributed to direct NO scavenging, as the EEPC decreased the amount of nitrite generated from the decomposition of Sodium Nitroprusside *in vitro*. The scavenging of NO by the extract was increased in concentration dependent manner. Fig 5 illustrates a significant reduction in the NO radical due to the scavenging ability of extract and ascorbic acid. At a concentration of 500µg/ml, the Nitric oxide radical scavenging activity of the EEPC reached 54% while at the same concentration, the ascorbic

acid reached 70%. The IC₅₀ values were found to be 191.11µg/ml and 195.79µg/ml for EEPC and ascorbic acid respectively.

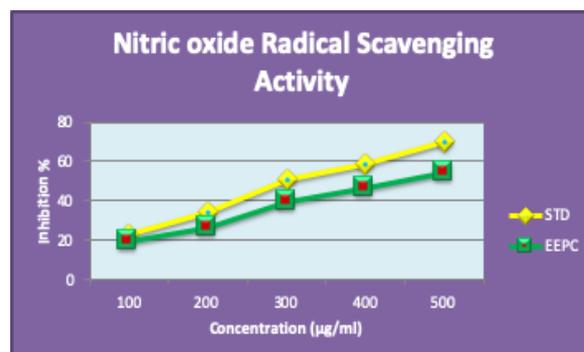


Fig 5. Nitric oxide radical scavenging activity of EEPC

CONCLUSION

The results obtained in the present study indicate that *Priva cordifolia* extract exhibits free radical scavenging potential. The overall antioxidant activity of *P. cordifolia* extract might be due to its flavonoid, polyphenolic and other phytochemicals constituents. The findings of the present study suggested that *P. cordifolia* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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