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# *In vitro* antioxidant activity of *Elaeocarpus tectorius* (Lour.) Poir – an Indian medicinal plant

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The study aimed to investigate bioactive phytochemicals and antioxidant activity of *Elaeocarpus tectorius* (Lour.) Poir leaf extract. The antioxidant properties were evaluated by four antioxidant methods, 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay, hydroxyl radical (HO%) scavenging assay, superoxide anion (O2%) radical scavenging assay, and nitric oxide (NO%) scavenging assay. The free radical scavenging activity of hydroethanolic extract of *E. tectorius* was found to be high for DPPH, nitric oxide, superoxide, and hydrogen peroxide ($H_2O_2$) radicals in a concentration-dependent manner. The content of total phenols, flavonoids, and tannins was also high in the hydroethanolic extract. The antioxidant capacity and total phenolic content were shown to be significantly related, indicating that phenolic chemicals are the primary contributors to plants' antioxidant capabilities. Based on the findings, it can be concluded that the hydroethanolic leaf extract of *E. tectorius* could serve as a potential source of antioxidants and can be explored as a therapeutic agent in free radical-induced diseases.

**Keywords**: Antioxidants, Bioactive phytochemical, *E. tectorius* (Lour.) Poir, Free radicals, Scavenging activity.
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## Introduction

Free radicals are produced continuously in the body as a result of normal physiological function and metabolism[1]. The products of normal cellular metabolism are reactive oxygen species (ROS) and reactive nitrogen species (RNS)[2]. ROS and RNS include radicals such as hydroxyl (OH•), superoxide (O2•), peroxyl (RO2•), alkoxyl (RO•), hydroperoxyl (HO2•), nitrogen dioxide (NO•), nitric acid (NO•), lipid peroxyl (LOO•) and non-radicals like hydrogen peroxide (H2O2), nitrogen dioxide (NO2•), hypochlorous acid (HOCl), ozone (O3), singlet oxygen, peroxynitrate (ONOO-), nitrous acid (HNO2), lipid peroxide (LOOH), dinitrogen trioxide (N2O3)[3]. The factors that increase the body's production of free radicals may be internal such as inflammation or external (pollution, UV exposure and cigarette smoke). Increased production of oxygen species beyond the antioxidant capacity of a biological system results in oxidative stress leading to the pathogenesis of various human diseases[4-6].

Antioxidants are macromolecules capable of trapping free radicals, offering resistance to oxidative stress by scavenging these free radicals, chelating ions of metals and inhibiting lipid peroxidation[7]. The source of antioxidants can be natural or man-made. Natural antioxidants are derived from plants and plant-based phytochemicals are quite effective when compared to synthetic source[8]. Some of the plant-produced antioxidants are carotenoids, flavonoids, tocopherols, beta carotene, lycopene, folic acid and so on[9].

Humans have used plants as a source of herbal medicine to cure and treat human ailments since ancient times. Natural phytochemicals in plants, such as alkaloids, flavonoids, phenols, and tannins are molecules of great potential to scavenge free radicals, thus preventing and inhibiting oxidative stress in various diseases such as cancer, atherosclerosis, and diabetes[10-13].

*E. tectorius* belongs to the family of Elaeocarpaceae, a traditional tree species which bears edible fruit and is eaten by tribal people of Western Ghats. Earlier research works demonstrated that members of the family Elaeocarpaceae possess a multitude of active molecules such as alkaloids, tannins, indolizilidine, flavonoids, and quercetin that confer desired pharmacological activities like anti-inflammatory, anti-microbial, anti-depressant, anti-epileptic, and anti-tumour properties[14-15].

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With this backdrop information, the present study was carried out to determine the phytochemical profile and antioxidant activity of *E.tectorius* leaf extract. This is the first report of the antioxidant potential of the leaves of the selected plant.

## Materials and Methods

**Plant material**

The fresh leaves of *E. tectorius* (Lour.) Poir were collected from Nilgiris District, Tamil Nadu, India, during the month of August 2019. The plant was identified and authenticated by G.V.S. Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agriculture University Campus, Coimbatore. (Ref No BSI/SC/5/23/05-06/Tech 304). A voucher specimen is available in the herbarium file of the Department of Biochemistry, PSG College of Arts and Science, Coimbatore.

**Extraction of plant material**

The freshly collected leaves of *E. tectorius* were shade dried in the open air for three weeks. The leaves were further powdered with a suitable grinder. The dried powder was extracted with solvents of increasing polarity (petroleum ether, ethyl acetate, acetone, ethanol, hydro ethanol, aqueous). The sample powder (10 g) was extracted by stirring with 100 mL of the corresponding solvent at 25 °C for 24 h and filtered through Whatman No. 1 filter paper. The extract was then dried by rotary evaporation at 40 °C. The dried extracts were weighed to determine the percentage yield of the soluble constituents using the formula

$$\% \text{ Yield} = \left(\frac{(\text{Weight of dry extract}}{\text{Weight taken for extraction}}\right) \times 100$$

**Phytochemical screening**

The dried plant sample extracted with solvents of increasing order of polarity viz., petroleum ether, ethyl acetate, acetone, ethanol, hydro ethanol, and water were subjected to preliminary screening for the presence of active phytoconstituents using standard protocols[16-18].

**Determination of total phenolic content**

The total phenolic content in different solvent extracts of *E. tectorius* leaves was assessed by Folin Ciocalteau's method[19]. Exactly 1.0 mL of Folin Ciocalteau reagent was added to 1.0 mL of the sample. Further 1.0 mL of sodium carbonate (~35%) was added to the above mixture after 3 min and the final volume was made up to 10 mL with distilled water. After 90 minutes in the dark, the absorbance was measured in a Shimadzu spectrophotometer at 725 nm against a blank.

**Determination of total flavonoid content**

The total flavonoid content of selected plant extracts was evaluated by the method reported by Kumar with slight modification[20]. Exactly 0.5 mL of the sample was combined with 5% of 0.3 mL sodium nitrite. After 5 min, 10% of 0.3 mL aluminium chloride was added. Further, 2.0 mL of 1M sodium hydroxide was mixed after 6 min and the total volume was made up to 5.0 mL with distilled water.

**Determination of total tannin content**

The tannin content of various extracts of selected plant was evaluated by the method reported by Price and Butler *et al.*[21]. Exactly 1.0 mL of 1% ferric chloride and potassium ferric cyanide were added to 0.5 mL of the sample. Further, the final volume was made up to 10 mL with distilled water. The reaction mixture was kept at room temperature for 5 minutes before being measured in a Shimadzu spectrophotometer at 720 nm against a reagent blank.

**Determination of total alkaloid content**

About 5 g of sample was dissolved in 200 mL of 20% acetic acid in ethanol solution. This mixture was allowed to stand for 4 h which was then filtered. The filtrate collected was kept in a water bath and reduced such that the final volume was ¼ of the original volume of the filtrate. Few drops of concentrated ammonium hydroxide were added in a dropwise manner until precipitation. The solution was filtered and collected residue was weighed[22].

$$\% \text{ Alkaloid} = \left(\frac{(\text{Weight of the residue}}{\text{Weight of sample}}\right) \times 100$$

***In-vitro* free radical scavenging activity**

***DPPH radical scavenging activity***

DPPH radical scavenging assay is a widely used method to evaluate the free radical scavenging ability of natural compounds. This assay is based on the measurement of the scavenging ability of antioxidant substances towards the stable radical. The free radical scavenging activity of the extracts was examined *in-vitro* using DPPH radical as described by Shimada *et al.*[23] with slight modification. 1.0 mL of various concentrations of hydroethanolic extract (100 – 500 µg/mL) was mixed with 1.0 of 0.8 Mm

DPPH solution. The mixture was shaken vigorously and left to stand for 30 min and the absorbance was measured at 517 nm against a reagent blank. Ascorbic acid was used as standard. The inhibition percentage for scavenging DPPH radical was calculated according to the following equation

$$\% \text{ Decolorization} = \{1-(\text{ABS sample}/\text{ABS control})\} \times 100$$

***Nitric oxide radical scavenging assay***

Sodium nitroprusside generated from nitric oxide interacts with oxygen to produce nitrate ions which were measured by the Griess reaction. This assay was done by the procedure described by Green *et al.*[24]. The reaction mixture contained 3.0 mL of 10 mM sodium nitroprusside in phosphate-buffered saline (pH 7.4) and various concentration of (100 µg – 500 µg) hydroethanolic extract. The resulting solution was incubated for 60 min at 25 ºC. Added 5.0 mL of Griess reagent (1% sulphanilamide, 0.1% NEDD in 2% $H_3PO_4$) to the incubated sample and absorbance against the blank was calculated at 546 nm. The standard ascorbic acid was used for comparison. The free radical scavenging activity was determined by evaluating % inhibition as above.

***Hydrogen peroxide scavenging assay***

The ability of plant extract to scavenge hydrogen peroxide was estimated according to the method reported by Sroka *et al.*[25] with minor modification. A solution of hydrogen peroxide (43 nM) was prepared in phosphate buffer (1M). Hydrogen peroxide solution (0.6 mL, 43 mM) was added to various concentrations of hydroethanolic extract (100-500 µg). The samples were incubated for 10 minutes and the absorbance was read at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating % inhibition as above.

***Superoxide anion radical scavenging assay***

The superoxide anion radical scavenging activity of *E. tectorius* leaf extract was assessed using the method described by Fontana *et al.*[26] with slight modification. To various concentration of the hydroethanolic extract (100 – 500 µg), 1.0 mL of phosphate buffer (0.1 M, pH 7.2), 1.0 mL of NADH (2 mM), 1.0 mL of NBT (0.5 mM) and 0.1 mL of PMS (0.03 mM) were added. After 5 min of incubation at room temperature, the absorbance was read at 562 nm against blank to determine the quantity of formazan generated. Ascorbic acid was used as standard. The % inhibition was determined as above.

## Results and Discussion

**Yield of sample**

The percentage yield for various extracts of *E. tectorius* is illustrated in Fig. 1. The variation in the extraction yield depends on the nature of the solvents and the chemical nature of the sample. Tripathy *et al.*[27] reported the percentage yield of different solvent extract of *Elaeocarpus Ganitrus (E. Ganitrus).*

**Preliminary phytochemical analysis**

The presence of several phytoconstituents such as flavonoids, tannins, alkaloids, protein, inulin, and steroids was identified in the preliminary phytochemical screening of different solvent extracts of *E. tectorius*; however, lignin, carbohydrates, and glycosides were absent (Table 1). Thenmozhi *et al.*[28] reported the presence of several phytochemicals in plant and fruit extract of *Elaeocarpus munronii* and *Elaeocarpus tuberculatus*. Similarly, Kharel *et al.*[29] revealed the presence of various phytochemicals in plant extract of *Elaeocarpus angustifolius*.

**Quantitative analysis of phytoconstituents**

Table 2 shows the concentrations of phenols, flavonoids, tannin, and alkaloids in several *E. tectorius* extracts. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Natural compounds such as phenolic acids and flavonoids, which are extremely fascinating for their antioxidant properties, are found in medicinal plants[30]. Quantitative analysis of phenolic and total flavonoid content in ethanolic extract *E. Ganitrus* was reported by Chandran *et al.*[31].

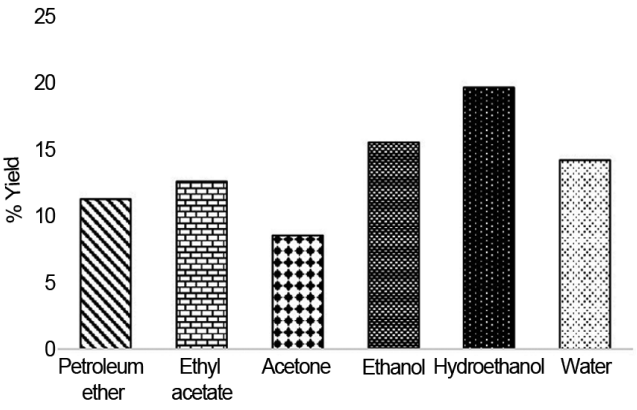


Fig. 1 — Per cent yield of extraction.

Table 1 — Preliminary phytochemical screening of various extracts of *E. tectorius*

| Test | Petroleum ether | Ethyl acetate | Acetone | Ethanol | Hydroethanol | Water |
|---|---|---|---|---|---|---|
| Flavonoids | +++ | ++ | - | - | +++ | + |
| Amino acids | + | + | + | + | ++ | + |
| Alkaloids | + | - | - | + | +++ | + |
| Carbohydrates | - | ++ | ++ | - | +++ | + |
| Lignin | - | - | ++ | +++ | +++ | ++ |
| Protein | + | ++ | + | + | + | + |
| Glycoside | - | - | - | - | +++ | + |
| Inulin | + | +++ | + | + | + | ++ |
| Tannin | + | ++ | ++ | +++ | +++ | +++ |
| Steroids | +++ | ++ | ++ | +++ | +++ | +++ |

Table 2 — Quantitative analysis of phytoconstituents

| Extracts | Flavonoids (mg/g) | Phenols (mg/g) | Tannins (mg/g) | Alkaloids (mg/g) |
|---|---|---|---|---|
| Petroleum ether | 2. | 3.7 | 4.4 | 5.2 |
| Ethyl acetate | 3.1 | 3.7 | 6.5 | - |
| Acetone | 2.8 | 5.0 | 6.1 | - |
| Ethanol | 2.7 | 5.1 | 5.2 | 6.1 |
| Hydro ethanol | 2.7 | 4.3 | 6.8 | 17.4 |
| Water | 3.3 | 3.1 | 5.3 | 12.2 |

**_In-vitro_ free radical scavenging activity**

***DPPH radical scavenging activity***

The 2,2- diphenylpicrylhydrazyl (DPPH) assay is commonly used in plant biochemistry for testing the free radical scavenging activity of plant constituents. The violet colour of the DPPH radical was reduced to yellow coloured Diphenylpicrylhydrazine radical which was measured colourimetrically at 517 nm. The hydroethanolic leaf extract of the plant showed a better antioxidant potential when compared to standard ascorbic acid. There was a dose-dependent increase in the percentage antioxidant activity for all concentrations tested. The % inhibition at various concentrations (100-500 µg/mL) of leaf extract as well as standard ascorbic acid (100-500 µg/mL) was calculated (Fig. 2a). The IC50 values are calculated from the graph and were found to be 202.17 µg/mL (standard) and 186.35 µg/mL (sample). The capture of more free radicals formed by DPPH by ethanolic plant extract resulted in the increase in IC50 value and decrease in absorbance. The DPPH results are consistent with the phenol concentrations found for each sample. Phenolics are potent antioxidants that can slow or stop oxidative processes by chelating transition metals that are involved in the initiation of free radical reactions[32]. As a result, we can deduce that these polyphenols are responsible for the antioxidant activity shown in this study.

***Nitric oxide radical scavenging assay***

Nitric oxide (NO) is involved in the regulation of various physiological processes. It is a diffusible free radical generated by endothelial cells, macrophages, neurons etc, which acts as an effector molecule. Incubation of sodium nitroprusside ion PBS at 25 °C for 2 h resulted in the production of nitrate which was reduced by the hydroethanolic plant extract of *E. tectorius*. The percentage inhibition at various concentrations (100-500 µg/mL) of leaf extract as well as standard Ascorbic acid (100-500 µg/mL) was calculated and is shown in Fig. 2b. The IC50 values were calculated from the graph and were found to be 210.75 µg/mL for ascorbic acid and 249.66 µg/mL for leaf extract. It is likely that the observed NO scavenging activity is due to flavonoids and phenolic compounds[33], which have been shown to compete with oxygen to scavenge NO and inhibit the generation of nitrites.

***Hydrogen peroxide scavenging assay***

$H_2O_2$ is an essential chemical messenger that plays an important role in transmitting signals by oxidizing specific proteins at sites. $H_2O_2$ itself is not very reactive, but it can be toxic to the cell as it may give rise to hydroxyl radicals. The scavenging ability of the hydroethanolic extract of *E. tectorius* on hydrogen peroxide is shown in Fig. 2c and compared with ascorbic acid as standard. The percentage inhibition at various concentrations (100-500 µg/mL) of plant extracts as well as standard ascorbic acid (100-500 µg/mL) was calculated and is shown in Fig. 2c. The IC50 values for the extract and that of the standard were found to be 162.21 µg/mL and 179.24 µg/ mL respectively. *E. tectorius* extract caused a strong dose-dependent inhibition of hydrogen peroxide. The hydroethanolic extract showed good scavenging ability compared to the standard compound. The presence of phenolic groups

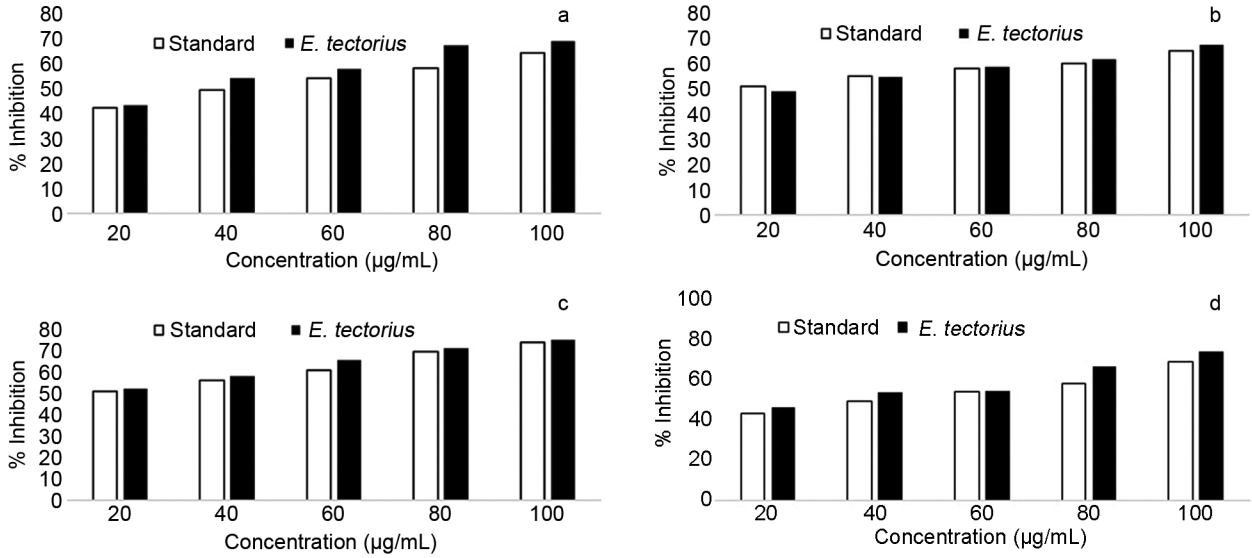


Fig. 2 — a) DPPH radical scavenging activity of hydroethanolic extract of *E. tectorius*. b) Nitric oxide scavenging activity of hydroethanolic extract of *E. tectorius*. c) Hydrogen peroxide scavenging activity of hydroethanolic extract of *E. tectorius*. d) Superoxide scavenging activity of hydroethanolic extract of *E. tectorius*.

in the ethanolic extract of *E. tectorius* may have contributed to the ability of the extract to scavenge hydrogen peroxide and neutralise it into $H_2O$[34].

***Superoxide anion scavenging assay***

Superoxide anion is a weak oxidant produced during various biological reactions is highly toxic. Superoxide anion radical is known as an initial radical and plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide or singlet oxygen. Superoxide is generated *in-vivo* by several oxidative enzymes, including xanthine oxidase. Superoxide anion radical scavenging activity of hydroethanolic extract of *E. tectorius* is shown in Fig. 2d. The EC50 value of ascorbic acid was found to be 180.25 µg/mL. The scavenging of superoxide anion radicals of the extracts was found to be 205.11 µg/mL. A decrease in absorbance indicated the antioxidant activity of the plant extract due to the presence of flavonoids[35] which may have led to the inactivation or consumption of superoxide anion radicals produced in the reaction mixture.

## Conclusion

Antioxidant properties of plants have become a topic of great interest because of their potential use as a natural additive to replace synthetic ones. Based on the results obtained in the present study, it is evident that the ethanolic extract of *E. tectorius* contains a considerable amount of flavonoid, alkaloids, and phenolic compounds that exhibit high antioxidant and free radical scavenging activities. Phytochemical research on *Elaeocarpus* species has also revealed that the species contain biologically active phytoconstituents. Pharmacological research characterized biological effectiveness using crude extracts and specific phytoconstituents. Thus, present data suggest that ethanolic extract of *E. tectorius* can be used as a good source of natural antioxidants for health benefits. Further isolation of bioactive compounds is required for identifying the unknown compounds to establish their pharmacological properties.

## Conflict of interest

The authors declare that they have no conflicts of interest.

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