



***Elaeocarpus ganitrus*: A Phytochemical and Pharmacological Screening for Antioxidant Potential**

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Abstract

The primary objective of the research was to examine the antioxidant capacity of an ethanolic concentrate made from *Elaeocarpus ganitrus* leaves. The ABTS+ (2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical scavenging, hydroxyl radical scavenging, metal chelating, and overall antioxidant capability of an ethanolic extract of *Elaeocarpus ganitrus* leaves were all examined. The extract's maximal iron chelating activity (76.70%) and ABTS+ radical scavenging (55.77%) were both observed at 500 µg/ml. The extract's hydroxyl radical scavenging activity, however, was rather moderate (13.43%). At 500 µg/ml extract concentration, the total antioxidant capacity was 24.18 mg ascorbic acid equivalents. $R^2 = 0.8547$ indicated a positive association between total phenolic content and antioxidant capacity, while $R^2 = 0.8413$ indicated a negative correlation between total flavonoids and antioxidant capacity. The findings imply that the leaves' flavonoids and phenolics have strong antioxidant properties.

Keywords: *Elaeocarpus ganitrus*, antioxidant activity, DPPH assay, FRAP assay.

1. Introduction

In the face of unremitting advances in therapeutic interventional and surgical therapies for the treatment of atherosclerotic coronary disease the later remains the principal killer in the western and the developing world. [1] Dyslipidaemia and resultant atherosclerosis are believed to stem from the imbalance of the lipid metabolites in the affected organism. *Elaeocarpus ganitrus* Roxb. belongs to family Elaeocarpaceae is a large evergreen broad-leaved tree. In Hindi it is recognized as Rudraksha [2] is prevalent for its fascinating fruit stones and medicinal properties. The widespread investigation of literature exposed that E.



ganitrus Roxb. is an imperative basis of various pharmacologically and medicinally significant chemicals, such as indispensable triterpenes, tannins like geraniin and 3, 4, 5-trimethoxy geraniin, indolizidine alkaloids grandisines, rudrakine and flavnoids quercitin.

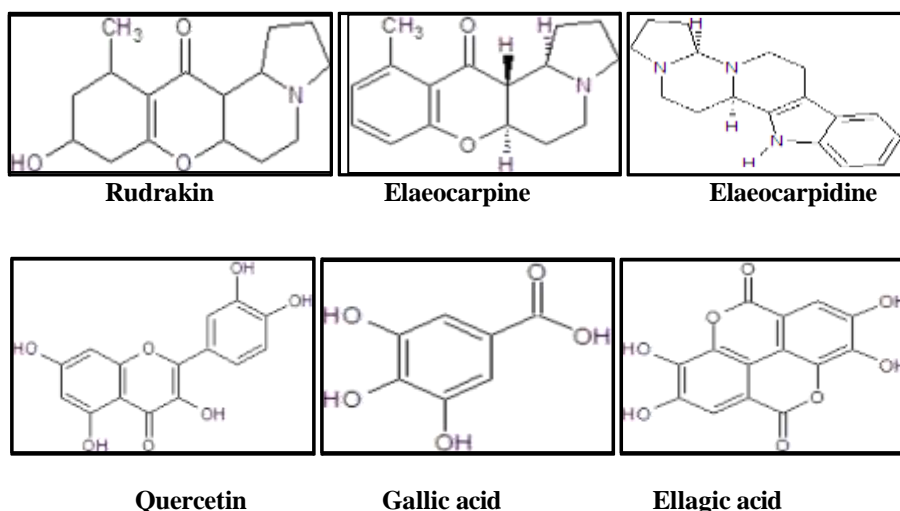
2. Materials and methods

a. Plant material

Elaeocarpus ganitrus were harvested in Botanical Garden, Oriental University, Indore, M.P., India. The collected plants were identified and authentication was done by Dr. S. N. Dwivedi, HOD, Dept. of Botany, Janta PG College, A.P.S. University, Rewa, M.P., India. Samples were washed with distilled water and dried in oven at 40 °C. After drying, a first grinding was performed using a copper household mortar, followed by an electrical grinder. The obtained powder was passed through a standard 125 µm sieve and only the fraction with particle size <125 µm was used. The powder was stored in airtight bags until use.

b. Preparation of sample extracts for biochemical assays

The seeds were coarsely powdered and extracted with 70% ethanol for 48 hrs, by soxhlet extraction method. Then ethanol was filtered and then separated under reduced pressure to obtain solid mass in a rotary evaporator and this was stored in a desiccator. Ethanolic extract of *E. ganitrus* seed contains indolizidine types of alkaloids, flavanoids, tannins, carbohydrate and proteins. 15 Some dominant phytoconstituents in ethanolic extract of *E. ganitrus* which possess hypolipidaemic and antioxidant activity are depicted as:



Phenolic compounds were extracted using a stirred conventional solvent extraction method. Briefly, fifteen grams of powder were mixed with 300 mL of 80% (v/v) aqueous methanol in a

flask and the mixture was kept in a thermostatic water bath at 40 °C, with shaking at a speed of 500 rpm for 12 hours. After this period, the residue was recovered and re-extracted with 150 mL of the same solvent for another 12 hours cycle to complete a total of 24 hours of extraction process. The extracts were combined and concentrated in a rotary evaporator (Singla Scientific, India) until the complete elimination of methanol. Afterwards, the aqueous extract was lyophilized (24 hours) and kept in hermetic amber flasks at -22 °C. Before each trial, the lyophilized extracts were dissolved in distilled water.

c. Determination of total phenolic content

The total phenolic content (TPC) of the Leaf extracts, was determined using the Folin–Ciocalteu assay as reported by Blainski A, Lopes GC and De Mello JCP [13], with slight modifications. Briefly, 500 µL of extract were mixed with 1500 µL of a 10-fold diluted Folin–Ciocalteu reagent. Then, 1500 µL of a sodium carbonate solution (7.5%, w/v) were added. The mixture was incubated in the dark for 90 min with intermittent shaking. Finally, the absorbances of the reaction mixtures were measured at 725 nm (1 cm optical path) against a blank using a UV-Vis spectrophotometer (Shimadzu™ UVmini-1240 Model Spectrophotometer). The phenolic content was calculated as gallic acid equivalents GAE/g based on standard curve of gallic acid. The TPC was expressed as mg of gallic acid equivalents (GAE) per gram of the plant material. The experiment was carried out five times.

d. Determination of total flavonoid content

The content of total flavonoids (TF) was estimated by the AlCl₃ method [14]. Briefly, 1.5 mL of extract were added to 1.5 mL of 2% (w/v) methanolic AlCl₃ incubated for 1 hour at room temperature. The absorbance was then read at 420 nm (1 cm optical path). The TF was expressed as mg of Quercetin Equivalent (QE) per gram of plant material. The experiment was carried out five times.

e. Determination of reducing power

The reducing power of extracts was determined according to the method of Yen GC and Duh PD [16]. A volume (1 mL) of aqueous extracts was mixed with 2.5 mL of phosphate buffer (0.2 M; pH 6.6) and 2.5 mL of potassium ferricyanide (1%, w/v). The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (TCA) (10%, w/v) was added to the mixture, and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (1%, w/v). The reducing power was expressed as the absorbance of this mixture measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

f. Determination of DPPH radical scavenging activity

The electron donation ability of the obtained aqueous extracts was measured by bleaching of the purple-colored solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and was performed



according to the method of [17]. A DPPH[•] solution in methanol (6×10^{-5} M) was prepared, and 3.9 mL of this solution were mixed with 0.1 mL of extract and the mixture was incubated in the dark at room temperature for 30 min, and then the decrease in absorbance at 515 nm was recorded. The antioxidant capacity was expressed as a percentage of inhibition of DPPH[•] radical (% inhibition of DPPH radical) calculated according to the following equation (Equation 2).

$$\% \text{ inhibition of DPPH radical} = \frac{\text{AC (DPPH)} - \text{AA (sample)}}{\text{AC (DPPH)}} \times 100 \quad (2)$$

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Where, AC (DPPH) was the absorbance of the control (without extract) after 30 min and AA (sample) was the absorbance of extract after 30 min. IC₅₀ was calculated as the concentration of extracts causing a 50% inhibition of DPPH[•] radical.

g. Determination of ABTS^{•+} radical scavenging activity

The antioxidant activity of the aqueous extracts was assessed by ABTS^{•+} assay [18], which is based on the ability of antioxidants to interact with the ABTS^{•+} radical, decreasing its absorbance at 734 nm. Briefly, a radical solution (7 mM ABTS and 2.45 mM potassium persulfate) was prepared in absolute ethanol and left in the dark at room-temperature for 16 hours before using it in the assay. This solution was then diluted with ethanol to get an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. For the antioxidant activity analysis, 990 μ l of the diluted radical solution were mixed with 10 μ l of the extract at different concentrations and the absorbance was read at 734 nm against ethanol. Antioxidant activity (AOX) was calculated as the percent inhibition of absorbance at 734 nm (Equation 3):

$$\% \text{ inhibition of ABTS}^{\bullet+} \text{ radical} = \frac{\text{Abs control} - \text{Abs (sample)}}{\text{Abs control}} \times 100$$

Where, Abs control was the absorbance value of diluted ABTS^{•+} solution without extract and Abs (sample) was the absorbance of the sample extract.

3. Results and discussion

a. Total phenolic, total flavonoid contents

Table 1 shows the total phenolic, total flavonoid and anthocyanin contents of Leaf *Elaeocarpus ganitrus* extracts. The phenolic compound content of Ethanolic concentrate of leaves of *Elaeocarpus ganitrus* ranged from 19.85 to GAE/g of DM. The rate of flavonoids of *Elaeocarpus ganitrus* leaves was 9.69 mg of EQ mg/g of DM.



Table 1: Total phenolic content (TPC), total flavonoids (TF) of leaves extracts

	TPC	TF
	(EAG mg/g DM)	(EQ mg/g DM)
	Mean ± SD	Mean ± SD
Leaves Extracts	19.85 ± 1.38 ^a	9.69 ± 0.09 ^a

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Table 2: C50 (µg/mL) values of both leaves extracts on FRAP assay and scavenging activity against the ABTS*,+ radical and DPPH*, expressed as mean ± SD (n = 9)

	IC50 (µg/ml)		
	FRAP	ABTS* ⁺	DPPH*
Leaves Extract	50.12 ± 6.86 ^b	287.56 ± 4.98 ^b	346.97 ± 22.07 ^b

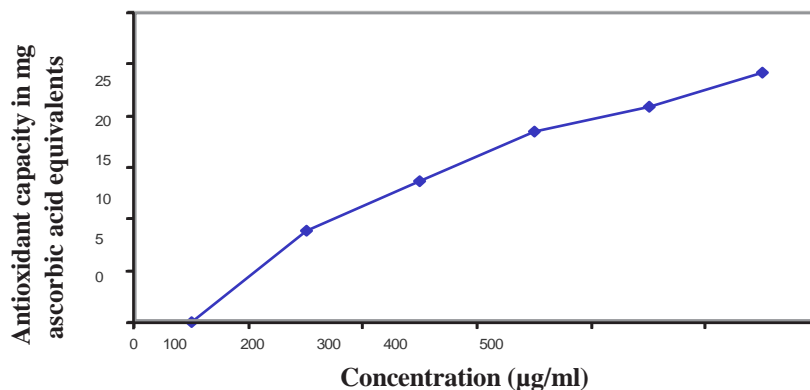
It depend on several intrinsic (genetic) and extrinsic Agro-ecological factors in the plant development area [27]. Exposure to light and preservation technique of the plants may also affect the flavonoid content [28], furthermore, the temperature influences the extraction of a given compound [29].

Total antioxidant activity

DPPH * radical-scavenging activity

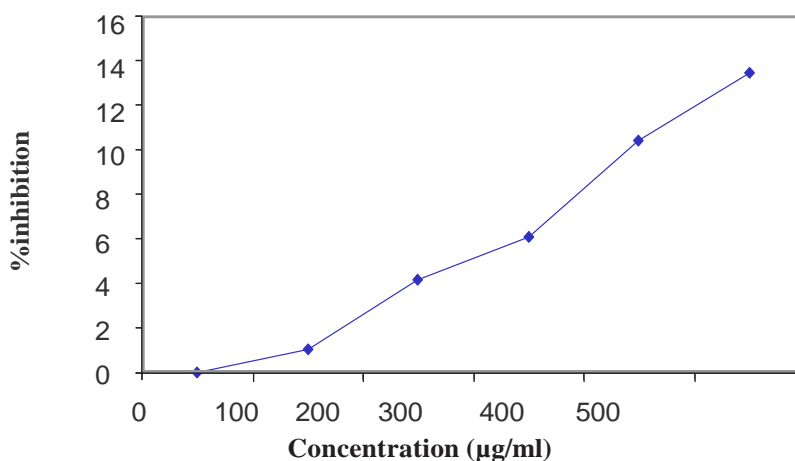
Many studies illustrate the importance given to natural antioxidants in the fields of food processing and medical industry and to their protective roles against the oxygen reactive species and the correlation between bioactive compounds of plant materials and their antioxidant capacity [34, 35]. The results of this study showed that the inhibition concentration (IC50) of DPPH* scavenging activity of Ethanolic concentrate of leaves of *Elaeocarpus ganitrus* were ranging from 346.9 with significant difference (p < 0.05) (Table 2). The leaf extracts showed a better DPPH* scavenging activity compared to that of the twig extracts. Ghazi F, Rahmat A, Yassin Z, Ramli NS and Buslima NA [19] reported that leaves extracts of *F. carica* harvested in India presented percentages of DPPH* scavenging activity ranging from 59.42 ± 1.53% to 63.29 ± 2.51%. The results of the present investigation are in accordance with several previous studies that showed significant correlations (p < 0.05) between the inhibitory power of the radical DPPH* and both total polyphenol (R = 0.71) and flavonoid (R = 0.76) contents [14, 36]. The DPPH*scavenging activity of plant extracts has been attributed to the presence of phenolic compounds which easily yield protons for reduction [37–39] while the anti-radical power is influenced by several factors: the extraction method, the nature and concentration solvent, the temperature and the time of extraction [40, 41].





ABTS*+ assay

The cation radical of 2,2'-azino bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) is stable in its free form. It is formed by oxidation of the colorless ABTS*+ with potassium persulfate [18]. The results (Table 2) indicated that the IC₅₀ (µg/mL) values of the ethanolic concentrate of leaves of *Elaeocarpus ganitrus* on scavenging activity against the ABTS*+ radical varied significantly ($p < 0.05$) from 287.56 µg/mL. In contrast, the leaf extracts showed a better ABTS*+ scavenging activity. Several studies have shown that the ABTS*+ scavenging activity is strongly influenced by the extraction solvent, which could modify the quantity and quality of the extracted antioxidants [42]. Manian R, Anusuya N, Siddhuraju P and Manian S [43] reported that phenolic compounds with high molecular weights have more ability to capture free ABTS*+ radicals.

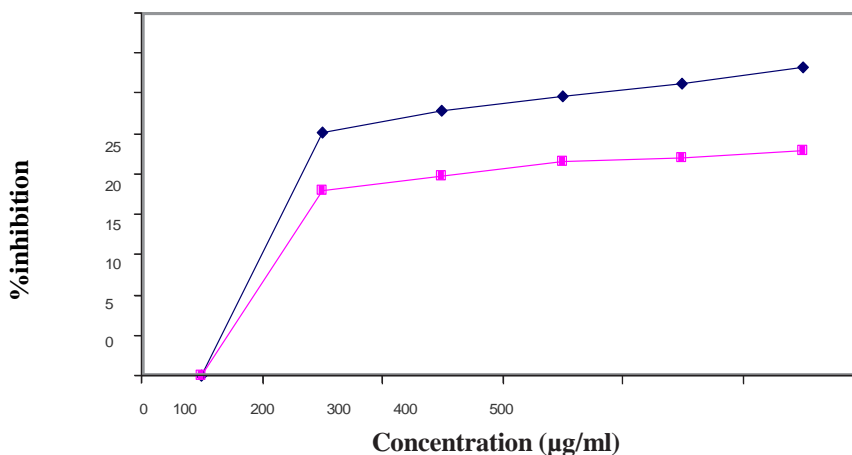


FRAP (Ferric reducing/antioxidant power) assay

This test is used to determine the ability of the extracts to give electrons [44], or to give a



hydrogen atom, which allows them to act on the peroxidation of lipids as primary and secondary antioxidant [45]. It is based on the ability of extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The reduction of ferric ions is accompanied by a color change from yellow to green. The intensity of the color depends on the reduction potential of the compounds present in the reaction medium. The results given in Table 2 show that the IC_{50} ($\mu g/mL$) values on FRAP assay varied significantly ($p < 0.05$) from 50.12 for Leaf extracts, respectively.



4. Conclusion

The results of this study showed that leaves of *Elaeocarpus ganitrus* is a promising antioxidant has therapeutic potentials on Swiss albinos mice. Clarification of mechanisms generating these activities needs more investigations. These results suggest 85% of the antioxidant capacity of *E. ganitrus* is due to the contribution of phenolics and flavonoid components. In addition, the antioxidant activity may be due to enzymatic and other non-enzymatic antioxidants, which needs further analysis. Thus, it will be a wide-open spectrum of biological and clinical studies.

5. Acknowledgments

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6. Conflicts of interest

No known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.



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