Phytochemical Screening, Antioxidant, Antidiabetic and Anticancer Activities of *Elaeocarpus variabilis* Fruit

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**A R T I C L E   I N F O**

**Research Article**

Received : 13/11/2021
Accepted : 02/12/2021

**Keywords:**
*Elaeocarpus variabilis*
Fruit
Antioxidant
Antidiabetic
Anticancer

This study focused on the determination of secondary metabolites to an evaluation of the biological activity of *Elaeocarpus variabilis* Zmarzty fruit extract, an endemic medicinal plant. Their total Phenolic, tannin, flavonoids, alkaloids and saponins contents were quantified and their antioxidant activity, determined by DPPH, total antioxidant, ABTS, FRAP and Fe⁺ chelating activity. The ethyl acetate extract exhibited the highest radical scavenging activity. The most relevant contents in total phenolics (Gallic Acid Equivalent mg/g), tannin (Gallic Acid Equivalent mg/g), total flavonoids (Rutin equivalent mg/g), total alkaloids (Quinine equivalent mg/g), and total saponin (Diosgenin equivalent mg/g) were observed. Significant correlations were established between antioxidant activity and secondary metabolites. Though the *in vitro* anti-diabetic activity was carried out using α-amylase and α-glucosidase inhibition, this indicates that the absorption and digestion of carbohydrates would probably reduce the blood glucose levels and hence help the management of type-2 diabetes. The ethyl acetate extract exhibited the potential of anticancer activity on human HT-29 colon cancer cell line IC₅₀ values (30.0 ± 1.0) the valuable activity that compared to doxorubicin (17.0 ± 0.5). While this is the first study concerning extract from *E. variabilis*, our findings highlighted the richness in flavonoids of those extracts and their amazing potential as a source of antioxidant, anti-inflammatory, anti-diabetic, and anticancer agents.

**Introduction**

The *Elaeocarpus* are distributed worldwide, particularly in India, New Zealand, Australia, Malaysia, Fiji, Hawaii, China, Bangladesh, Nepal, and Bhutan (Tripathy et al., 2016). In India, almost 25 species are available (NayanTalukdar et al., 2016). Particularly in the Western Ghats, 12 species include 6 endemic species (Khan et al., 2003). Identified, around 8% of endemic species are confined to the Western Ghats, including four steno endemics namely, *Elaeocarpus blascoi* Weibel, *Elaeocarpus gaussenii* Weibel, *Elaeocarpus recurvatus* Corner, and *Elaeocarpus venustus* Bedd. According to a later revision of section in Southern India and Sri Lanka, 9 species were recognized, among them, three new species were included such as *Elaeocarpus hedyocosmus* Zmarzty, *Elaeocarpus variabilis* Zmarzty and *Elaeocarpus taprobanicus* Zmarzty (Zmarzty, 2001). The *E. variabilis* is commonly known as the South Indian Marble tree which is endemic to the Western Ghats and is found in moist deciduous and evergreen forests (Talukdar et al., 2016). Trees up to 20 m tall, bore buttressed, bark greyish-brown, blaze red. The Tribal of Nilgiri district todas, kurumbas, kothas, irulas, kattanaikes are broadly using these fruits for the therapeutic purpose the fruits were the very cheapest resource for the treatment of rheumatism and body pain, using this fruit for the treatment of leprosy, pneumonia, ulcers, piles, and dropsy (Jain, 1991). Leaves, seeds, and fruits have various medicinal properties and are used as an antipyretic agent to control fever, to treat malaria, diarrhoea, and typhoid (Bhattacharya et al., 1975; Hardainiyan et al., 2015; Lakshmi et al., 2016). These species have chemical constituents such as alkaloids, flavonoids, glycosides, tannins, triterpenes, fatty acids, ellagic acid derivatives and cytotoxic compounds. The previous studies also indicated that *Elaeocarpus* showed different therapeutic activities, such as anti-asthmatic, anxiolytic, antidepressant, antidiabetic and anti-
ulcerogenic also various other activities (Gaurav et al., 2014; Kumar et al., 2021). Therefore the present investigation was aimed to evaluate the nutritional profile, primary and secondary phytochemicals, antioxidant, anti-diabetic and anticancer potential of *E. variabilis* fruit extract as it is a locally available plant.

**Materials and Method**

**Collection and Identification of Plant Material**

The healthy fruits of *E. variabilis* (Figure 1) were collected from Munnar, Idukki District, Kerala. The plant specimen was identified (Ref. No. BSI/SRC/5/23/2017/Tech-3036) with the help of the Botanical Survey of India (BSI), Coimbatore.

![Figure 1. Habit of *Elaeocarpus variabilis* fruit](image)

**Nutritional Profiling**

*E. variabilis* fruits biochemical properties were analyzed by following methods, Total Carbohydrate (Hodge and Hofreiter, 1962), Starch (Hodge and Hofreiter, 1962), Fructose (Sadasivam and Manickam, 2004), Protein (Lowry et al., 1951), Total Free Amino acids (Moore and Stein, 1948), Vitamin-A (Neeld and Pearson, 1963), Vitamin-B1 (Okwu and Ndu, 2006), Vitamin-C (Benderitter, 1998), Vitamin-E (Arti and Archana, 2007) and β-Carotene (Kumari and Arti, 2014).

**Minerals Analysis**

The *E. variabilis* fruit, mineral analysis was done by the ICP-MS instrument of (NeX Ion 300 X, Perkin Elmer, USA). The dried fruits sample was ground using mortar and pestle. From taking 0.5 g of sample was digested using 10 mL tri-acid (9:2:1, Nitric: Sulfuric: Perchloric acid) and the sample was made up to 10 mL, using deionized water, filtered using Whatman No-1 filter paper, then filtered in a syringe filter with a pore size of 0.22 μm and directly analyzed. The elements such as Be, Na, Mg, Al, K, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Mo, Cd, and Pb were analyzed and expressed in μg/L and mg/g.

**Extract Preparations**

The *E. variabilis* dried fruit extract was extracted by the soxhlet method with different solvents like petroleum ether, ethyl acetate and methanol. Finally, the water extract was used the maceration technique. Each extract was dried in a hot air oven at below 40°C overnight and collected, then stored at -4°C until further analysis.

**Extract Recovery Percentage**

From the extract, a known volume was taken, dried in an oven at an incubator temperature of 40°C. (Until sample gets a constant weight) and the recovery percent was calculated as follows.

\[
\text{Extract %} = \frac{\text{Extract + container (g) – Empty container (g)}}{\text{Sample weight (g)}} \times 100
\]

**Secondary Metabolites**

**Determination of total phenols content**

The total phenol content was determined by the method Makkar, (2003). In this method, 100 μL of fruit extracts were taken into a series of test tubes and made up to 1 mL with distilled water. A test tube with 1 mL of distilled water served as the blank. Then, 500 μL of folin-ciocalteau phenol reagent (1N) was added to all the test tubes including the blank. After 5 min, 2.5 mL of sodium carbonate solution (20%) was added to all the test tubes. The test tubes were vortexed well to mix the contents and incubated in dark for 40 min. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation, the absorbance was read at 725 nm against the reagent blank. Gallic acid standard was also prepared and the results were expressed as Gallic acid equivalents (GAE). The analyses were performed in triplicates.

**Determination of tannin content**

The tannin content of the sample was determined according to the method Makkar, (2003). 100 μL of each fruit sample were incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 μL of distilled water taken in a 2 mL Eppendorf tube for 4 h at 4°C. After incubation, the Eppendorf tubes were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant contains only the non-tannin phenolics since the tannins would have been precipitated along with PVPP. The supernatant was collected and the non-tannin phenolics were determined by the same method described for the quantification. The 100 μL of plant extracts were taken into a series of test tubes and made up to 1 mL with distilled water. A test tube with 1 mL of distilled water served as the blank. Then, 500 μL of folin-ciocalteau phenol reagent (1N) was added to all the test tubes including the blank. After 5 min, 2.5 mL of sodium carbonate solution (20%) was added to all the test tubes. The test tubes were vortexed well to mix the contents and incubated in dark for 40 min. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation, the absorbance was read at 725 nm against the reagent blank. The analyses were also performed in triplicates and the results were expressed in Gallic acid equivalents. From these two results, the tannin content of the plant samples was calculated as follows,

\[
\text{Tannins} = \text{Total phenolics} - \text{Non phenolics}
\]

**Estimation of total flavonoids content**

The flavonoids content was determined by the aluminumchloride method Zhishen et al., (1999). A volume of 200 μL of fruit extract was taken in different test tubes and 2 mL of distilled water was added to each test tube. A tube containing 2.5 mL of distilled water served as a blank. To this 150 μL of 5% of NaNO₂ was added to all the test tubes and incubated the whole mixture for 6 h at room
temperature. After incubation, 150 μL of 10% AlCl3 was added to all the test tubes including the blank. Then, the test tubes were incubated for 6 min at room temperature. Then 2 mL of 4% NaOH was added and made up to 5 mL using distilled water. The contents in all the test tubes were vortex well and allowed to stand for 15 min at room temperature. The development of pink color due to the presence of flavonoids was read spectrophotometrically against prepared reagent blank at 510 nm. Samples were analysed in triplicates and the amounts of flavonoids were expressed in rutin equivalents.

**Determination of total alkaloid content**

The total alkaloid content of the sample was measured using the 1, 10-phenanthroline method described by Singh et al., (2004). The reaction mixture contained 0.2 mL fruit extract, 1 mL of 0.025 M FeCl3 in 0.5 M HCl and 1 mL of 0.05 M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 min in a hot water bath with a maintained temperature of 70 ± 2°C. The absorbance of the red-colored complex was measured at 510 nm against the reagent blank. Alkaloid contents were estimated and it was calculated with the help of a standard curve of quinine (0.1 mg/mL, 10 mg dissolved in 10 mL ethanol and diluted to 100 mL with distilled water). The values were expressed as mg/g of dry weight.

**Determination of total saponin content**

The total saponin content of the sample was determined by Makkar et al., (2007). The 0.2 mL of extract was taken to which 0.25 mL vanillin reagent (8% vanillin in ethanol) and 2.5 mL of 72% aqueous H2SO4 were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 min. After that tubes were cooled in ice for 4 min and then allowed to cool to room temperature. Subsequently, the absorbance was measured in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

**GC-MS analysis**

The ethyl acetate extract used GC-MS analyzed for identified volatile compounds presence of extract. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-SMS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL min. The injector temperature was set at 260°C during the chromatographic run. The 1 μL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300 °C at the rate of 10°C min and 300°C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments ranged from 50 to 600 Da. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

**In vitro Antioxidant Activities**

**Determination of DPPH radicals scavenging activity**

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl test according to the method described by Braca et al., (2001). The fruit extract 0.1 mL was added to 3 mL of 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated from [(A0−A1)/A0] ×100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard. The inhibition curves were prepared and IC50 values were obtained.

**Determination of total antioxidant activity**

The antioxidant power of the extracts has been assayed with the phosphomolybdenum reduction assay by Prieto et al., (1999). It was based on the reduction of the extract and subsequent formation of a complex (green color) at acidic pH, 0.3 mL of extract (50, 100, 150 μg/mL) were combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min at 695 nm using spectrophotometer against blank.

**Determination of ABTS+• radical scavenging activity**

Free radical scavenging activity ABTS assay was determined followed by Arnao et al., (2001), method with some modifications. The stock solutions included 7.4 mM ABTS+• solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS+• solution with 60 mL methanol to obtain an absorbance of 1.170.02 units at 734 nm using the spectrophotometer. The Fresh ABTS+• solution were prepared for each assay. The 0.2 mL of leaves extract was allowed to react with 2850 μL of the ABTS+• solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The standard curve was linear between 25 and 600 mM Trolox. Results are expressed in mM Trolox equivalents (TE)/g fresh mass.

**Determination of FRAP assay**

The FRAP assay was done according to Benzie and Strain, (1996), with some modifications, stock solutions included 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM FeCl3 6H2O solution. The fresh working solution was prepared by mixing 25 Ml acetate buffer, 2.5 mM TPTZ solution, and 2.5 mL FeCl3 6H2O solution and then warmed at 37°C before use. Leaves extracts (150 ml) were allowed to react with 2850 μL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The result was expressed in mM TE/g fresh mass.

**Determination of Fe2+ chelating assay**

The Fe2+ chelating assay was performed based on the method as described by Dinis et al., (1994). The chelating activity of fruit extracts for ferrous ions Fe2+ was measured according to the method previously described. Briefly, 0.5 mL of extract in deionized water at different concentrations 1.6 mL of deionized water and 0.05 mL of FeCl2 (2 mM) were added. After 30 sec, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe2+-Ferrozine complex was measured at 562 nm. EDTA was used as the standard metal chelating agent and the results were expressed as μg EDTA equivalents/g extract.
In vitro Anti-diabetic Activity

Determination of α-amylase inhibition assay

The α-amylase inhibition activity of the according to the method described by Worthington, (1993). The 100-500 μL of the extract was taken into different test tubes. Made up to the volume to 0.5 mL with phosphate buffer of pH 6.9. A blank was measured by taking 1 mL of phosphate buffer and the control was measured by taking 0.5 mL of phosphate buffer. The solution was then treated with 0.5 mL of α-amylase solution (0.5 mg/mL). The solution was incubated at 25°C for 10 min. Added 0.5 mL of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubated at 25°C for 10 min. The reaction was stopped by adding 1.0 mL of DNSA solution (1 g of DNSA, 30 g of sodiumpotassium tartrate, and 20 mL of 2 N sodium hydroxide was added and made up to a final volume of 100 mL with distilled water and the reaction mixture was kept in boiling water bath for 5 min, cooled to room temperature. The solution was mixed with 8 mL distilled water. Read the absorbance of the solution in a colorimeter at 570 nm against the blank solution. The amount of maltose produced is calculated using standard maltose curve, and Enzyme activity is calculated by using the formula:

\[
\text{% Inhibition} = \frac{\text{Abs (control)} - \text{Abs (extract)}}{\text{Abs (control)}} \times 100
\]

Determination of In vitro α-glucosidase inhibition assay

The α-glucosidase inhibition activity of the according to the method described by Apostolidis et al., (2007). The α-glucosidase was dissolved in 100 mM phosphate buffer at pH 6.8 was used as an enzyme source; 10 mM paranitrophenyl-α-D-glucopyranoside (PNPG) was used as substrate. The different concentration of fruit extract was mixed with 320 μL of 100 mM phosphate buffer (pH 6.8) and 50 μL of 10 mM PNPG in the buffer and then it was incubated at 30°C for 5 min. After the incubation, 20 μL of the buffer containing 0.5 mg/mL of the enzyme was added and further incubated at 30°C for 5 min. Finally, 3.0 mL of 50 mM sodium hydroxide was added to the mixture and the absorbance (A) was measured at 410 nm on a spectrophotometer. The enzyme without plant extract was used as a control.

\[
\text{% Inhibition} = \frac{A_{410 \text{ control}} - A_{410 \text{ test}}}{A_{410 \text{ control}}} \times 100
\]

Acarbose was taken as a reference standard with the same concentration as that of the sample. The percentage inhibition vs. concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC₅₀ value.

Anti-inflammatory Activity

Determination of Membrane Stabilization Method

The human red blood cell (HRBC) membrane stabilization is based on the method as described by Gandhisan et al., (1991). The blood was collected from a healthy human volunteer who had not taken any NSAIDS for 2 weeks before the experiment and mixed with an equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) and centrifuged at 3000 rpm. The packed cells were washed with isosalone and a 10% suspension was made. The extracts were prepared (100 μg/mL) using distilled water and to each concentration 1 mL of phosphate buffer, 2 mL hyposaline, and 0.5 mL of HRBC suspension were added. It was incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. The diclofenac (100 and 200 μg/mL) was used as reference standard and a control was prepared by omitting the extracts. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

\[
\text{Percentage stabilization} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100
\]

Inhibition of albumin denaturation activity

The anti-inflammatory activity was studied by using the inhibition of albumin denaturation technique according to this method Sakat et al., (2010), with followed minor modifications. The reaction mixture consisted of test extracts and a 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using a small amount of 1N HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min, after cooling the samples the turbidity was measured at 660 nm The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as:

\[
\text{Percentage inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100
\]

Anticancer Activity

Cell culture condition

The Human colon cancer cell HT-29 was procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were maintained in McCoy’s medium supplemented with FCS and 2 mM L-glutamine balanced salt solution (BSS) adjusted to contain 1.5 g Na₂CO₃, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g L-glucose, 10 mM (4-2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100 μg) were adjusted to 1 mL. The cells were maintained at 37°C with a 5% CO₂ atmosphere.

MTT assay

The MTT assay was determined by Carmichael et al. (1987) method. HT-29 cell lines were seeded in 96-well microtiter plates (2000 cells/well) ina DMEM medium. After one day of culture, the medium was removed by aspiration, and the cells were treated with different concentrations (0-50 μg/mL) of extracted compounds in 100 μL DMEM medium. Following 24 h of incubation at 37°C, to each wellladded 100 μL (2 mg/mL) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma, USA) and incubated for another 3 h at 37°C. Finally, to all the wells added 100 μL of DMSO to lyse the cells and the absorbance of the cell lysates was measured at 540 nm using a microplate reader (Dynatech MR 5000, USA). Similarly, the control wells contained DMSO in the culture medium and the blank contained only DMEM medium.
Statistical Analysis
The analysis of variance one-way ANOVA test was carried out for statistical analysis using SPSS 20 software followed by Duncan’s multiple range test. The significance level was fixed at P<0.05.

Result and Discussion
Nutritional Factors
The screening of nutritional profile such as total protein, amino acid, carbohydrate, starch, fructose, vitamin-A, vitamin-B1, vitamin-C, vitamin-E, β-carotene and elements were analyzed, among, the maximum amount of vitamin-A, vitamin-C, Amino acid and β-Carotene contents were found to be maximum content present in E. variabilis fruit when compared to others nutrient factors. This results was in (Table 1 and Table 2). Generally, the Vitamin–A, Vitamin-C and β-Carotene have been showed antioxidant properties.

The early look at of exact resources of protein and minerals by using leafy vegetables like Amaranthus graecizans L., Portulaca oleracea L. and Solanum nigrum L. (Aragaw et al., 2021). The wild fruits had rich sources of minerals, vitamins and fiber (Rajapaksha, 1998). They were reported to another species observed, carotenoids and vitamin-C the associated with antioxidant properties. Carotenoids are one of the pro-vitamin-A that can avoid the development of chronic diseases (Singh et al. 2015), whereas fruit is rich in vitamin-C for the prevention of cardiovascular diseases and obesity (Gonzalez-Molina et al., 2010; Ramful et al., 2011). E. serratus fruits are used in dysentery, as well as diarrhoea (Chopra, 1993). E. oblongus influence is used as a complement in affected growth retardation, reproductive abnormalities, or reduced RBC synthesis. Vitamin-A prevents night blindness and regular performance of the body epithelia. Vitamin-K is vital for blood clotting and Vitamin-D acting bones, kidneys, intestinal mucosa, avoid Osteomalacia, guard against Alzheimers’s and Parkinson’s illness (Meenakshi et al., 2012). Prihantini and Tachibana (2017) reported the β-carotene bleaching assay in E. sylvestris which is typically used to determine the ability of antioxidants. Reported that the estimated fats and water-soluble vitamins, phenolics, and flavonoids contents in the fruit pulp of E. oblongus (Raghunathan, 2017). Jawla and Rai (2016) analyzed the elemental composition in the fruit pulp of E. angustifolius Blumenbey analysis high nutritional value of fruit by ICP-MS.

Extract recovery
The successive solvent extraction of E. variabilis fruit extract was obtained and the recovery percentage was calculatedshowed (Figure 2) estimation of extract yield percentage in the different solvents used, the methanol extract showed maximum recovery percentage in fruit. Whereas, the lowest amount of yield percentage was found in petroleum ether, ethyl acetate, and water.

Table 1. Nutritional Factors in Elaeocarpus variabilis fruit

| Primary metabolism (mg/g) |  |
|---------------------------|--|
| Protein                   | 0.60 ± 0.04d |
| Amino acid                | 3.55 ± 2.16b |
| Carbohydrate              | 0.12± 0.03c |
| Starch                    | 0.015 ± 0.09e |
| Fructose                  | 0.028 ± 0.07e |
| Vitamin-A                 | 1.74 ± 0.08c |
| Vitamin-B1                | 0.97 ± 0.04cd |
| Vitamin-C                 | 6.37 ± 0.07a |
| Vitamin-E                 | 0.50 ± 0.06d |
| β-Carotene                | 1.38 ± 0.02c |

Values are presented as means ± SEM of triplicate determinations and expressed per g of fruit sample extracts. Means significantly different between the extract (P<0.05).

Table 2. Analysis of Minerals content in Elaeocarpus variabilis fruit by ICP-MS

| Element Name | Symbol | Conc. (mg/100g) |
|--------------|--------|----------------|
| Beryllium    | Be     | 0.009          |
| Sodium       | Na     | 42.015         |
| Magnesium    | Mg     | 43.628         |
| Aluminium    | Al     | 34.977         |
| Potassium    | K      | 2.464          |
| Chromium     | Cr     | 0.002          |
| Manganese    | Mn     | 0.003          |
| Iron         | Fe     | 0.062          |
| Nickel       | Ni     | 0.002          |
| Copper       | Cu     | 0.003          |
| Zinc         | Zn     | 0.049          |
| Arsenic      | As     | 0.009          |
| Selenium     | Se     | 0.002          |
| Molybdenum   | Mo     | 0.007          |
| Cadmium      | Cd     | 0.000          |
| Lead         | Pb     | 0.002          |

Figure 2. Estimation of Extract yield % of Elaeocarpus variabilis fruit

Quantification of Secondary Metabolites
The amounts of secondary metabolites by different solvent extracts, such as petroleum ether, ethyl acetate, methanol, and water of E. variabilis were analyzed and represented in Table 3. The maximum amount of total phenolics, alkaloids, and flavonoids were found to be in the ethyl acetate extract when compared to another solvent extract. The earlier reported for E. serratus was observed, the highest amount of total phenolic in methanolic extract by fruit (Swapana et al., 2012). Flavonoids are ubiquitous in photosynthesizing cells and therefore occur widely in the plant kingdom (Havsteen, 1983). The highly rich flavonoid content of ethanolic extract in E. ganitrus fruit is the most important pigment for flower coloration producing yellow,
red and blue pigmentation (Singh et al., 2010). The similarly reported was good flavonoids in *E. ganitrus* ethanolic leaves extract that equivalents to rutin (Sathish Kumar et al., 2010). These findings recommend 85% of the antioxidant capacity of *E. ganitrus* by flavonoid components (Bharti, 2013). The organic solvents had been a significant presence of higher levels of total phenolic, flavonoid, and Proanthocyanidins (Vuong et al., 2018). Similarly seed was showed high level of Phenolic and flavonoid (Bhatt and Dahal, 2019). In the earlier report for *E. ganitrus* leaves extract several isomeric alkaloids had been isolated (Kumar et al., 2021). *E. serratus* leaves had been good sources of phenolic the significant for antioxidant properties (Chen and Yang, 2020).

**GC-MS Analysis**

The GC-MS analysis was

Inpresen’s study, was analyzed in ethyl acetate extract observed seven active compounds by *E. variabilis* fruits. The analyzed active principles with their retention time, molecular formula, molecular weight, peak area percentage are presented in Table 4, and the chromatogram was represented in Figure 3. The identified with a highest praceence of compounds 1,2,3-benzenetriol and lower number of compounds 1H-imidazole, 4, 5-dihydro-2-methyl-N, with presence of anti-oxidant related compound Di-.alpha.-tocopherol reduced oxidative stress. The fruit extract compounds were responsible for antioxidant, anti-inflammatory and cytotoxic activities. The bioactive compounds present in the ethanolic leaf *E. serratus* extract had been diagnosed using GC-MS analysis in thirty compounds were detected. The most obtainable compounds are 1,6-Anhydro-4-Dglucopyranose (levoglucosan), 1,2,3-Benzenetrio, levoglucosenone, phenol. The 1,2,3-benzenetriol can be obtained by microbial degradation of tannins, which are complex combinations of glucose obtained from oak bark and gallnuts (Vijayan, 2017). GC-MS analysis was *E. serratus* seed ethanol extract identified 30 various group compounds like alcohol, alkane, ester, aldehyde, amide, and ketone in this family mostly were found alkaline and alcohol groups (Geetha et al., 2013). The reported *E. tuberculatus* leaf extract the presence of α-amyrin it has chemically similar to steroids like compound that prevention of many diseases particularly oxidative and inflammatory stress (Jayashree et al., 2019).

### Table 3. Quantification of secondary metabolites various solvent extract from *Elaeocarpus variabilis* fruit mg/g

| Solvent            | Total Phenols (GAE) | Tannin (GAE) | Total Flavonoids (RE) | Total Alkaloids (QE) | Total Saponin (DE) |
|--------------------|---------------------|--------------|-----------------------|----------------------|-------------------|
| Petroleum ether    | 0.05±0.02           | 0.02±0.05    | 0.21±0.02             | 0.12±0.02            | 0.01±0.02         |
| Ethyl acetate      | 0.20±0.09           | 0.10±0.01    | 17.70±0.26            | 2.24±0.06            | 0.04±0.00         |
| Methanol           | 0.12±0.05           | 0.14±0.01    | 8.10±0.10             | 1.42±0.14            | 0.10±0.00         |
| Water              | 0.08±0.004          | 0.11±0.01    | 1.73±0.15             | 1.34±0.05            | 0.02±0.00         |

GAE: Gallic acid equivalent, QE: Quinone equivalent, RE: Rutin equivalent, DE: Diosgenin equivalent. Values are presented as means ± SEM of triplicate determinations and expressed per g of fruit extracts. Means significantly different between the extract (P<0.05).

### Table 4. Phytochemicals identified in the ethyl acetate extract of *Elaeocarpus variabilis* fruit by GC-MS

| Compound Name                          | PA     | RT    | MF    | MW    | Biological Activities                                                  |
|----------------------------------------|--------|-------|-------|-------|------------------------------------------------------------------------|
| 1H-imidazole, 4,5-dihydro-2-methyl-n    | 1.958  | 8.651 | C₆H₄N₂| 84    | antifungal and anti-mycobacterium activities                            |
| 2-[4-(4-methoxybenzyl) phenyl]propan-2-ol| 4.281  | 12.092| C₆H₆O₂| 256   | No activity reported                                                   |
| 1,2,3-benzenetriol                     | 58.908 | 15.183| C₆H₆O₃| 126   | Antimicrobial, Anti-inflammatory, Antioxidant, Analgesic, Insecticide,  |
|                                        |        |       |       |       | Anticancer                                                             |
| 3,7,11,15-tetramethyl-2-hexadecen-1-ol | 6.970  | 16.259| C₆H₄O  | 296   | Antimicrobial and Anti-inflammatory                                      |
| Tazabicyclo[2.2.2]oct-2-ene-3-carboxylic acid, methyl ester | 2.012  | 16.534| C₄H₁₃O₂N | 167   | No activity reported                                                   |
| bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl- , (1s) | 6.590  | 16.734| C₆H₁₀O  | 152   | Immune enhancement and anti-microbial activity                          |
| 1-(4-ethoxy-2-nitroanilino)-1-deoxy-β-d-mannopyranose | 2.018  | 17.509| C₆H₆O₈N₂| 3443  | 44 No activity reported                                               |
| n-hexadecanoic acid                    | 7.300  | 18.135| C₁₈H₃₅O₂| 256   | antibacterial, antioxidant, insecticidal and cytotoxic activities       |
| azetidine, 1-benzyl-3,3-dimethyl-2-phenyl | 3.195  | 22.031| C₆H₁₁N  | 51    | No activity reported                                                   |
| (2,3-diphenylclopopyrrol) methyl phenyl sulfoxide, transom | 3.024  | 22.826| C₂₅H₂₀OS| 332   |                                                                 |
| Di-.alpha.-tocopherol                   | 3.744  | 28.589| C₂₄H₄₀O₂| 430   | antioxidant and inhibits angiogenesis                                   |

PA: % of Peak Area; RT: Retention Time (RT); MF: Molecular Formula (MF); MW: Molecular Weight (MW)
Antioxidant Activity
The antioxidant activity of all solvent extracts was evaluated in different ways, due to their complex nature of phytochemical. The identified various antioxidant compounds like 1,2,3-benzenetriol, n-hexadecanoic acid, and α-tocopherol have been found in this fruit extract. In the present study DPPH, Power reducing assay, and phosphomolydenum assays were analyzed. In Table 5, showed that antioxidant activity of a fruit solvent extract, the highest scavenging activity was obtained in ethyl acetate extract DPPH 83.57% total antioxidant 0.06 μg/mL, ABTS 78.76% and FRAP 0.04 μg/mL which metal chelating activity 0.06 μg/mL in water extract if compared to other solvents extract. The antioxidant capacity was observed in E. tectorius leaf extract to discover the ability of free radical scavenging properties do due to the presence of phenolic acids and vitamins (Manoharan et al., 2019). Many of them phenolics contain have been high levels of antioxidant activities (Razali et al., 2012). Polyphenolic contents of all the sample extracts appear to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reactions (Yu, 2001). The Rubus niveus and E. oblongus exhibited impressive DPPH radical scavenging of fruit (Sharma et al., 2011). The highest DPPH radical scavenging activity was observed in E. recurvatus stem (Lakshmi et al., 2014). The decolouration of ABTS in the present study reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactive this radical caution the E. ganitrus leaf extract was observed strongest chelating activities (Sathish Kumar et al., 2010). Similarly ABTS+ colour reduction activity in leaf extract of E. sphaericus (Sharma et al., 2015).

In vitro Anti-diabetic activity
Table 6 showed that the inhibition of α-glucosidase and α-amylase were estimated by using different solvents of E. variabilis fruit. As the results showed in table 5, which had the fruit, therefore the highest percentage of inhibition was present 95.39% in ethyl acetate extract then followed by petroleum ether 91.74%, methanol 85.49%, and water 57.02%, when compared to Acarbose standard was 94.51% inhibited on α-glucoaldase. In the analysis of α-amylase inhibitory activity the highest inhibition was present in water extract 81.59%, then followed by ethyl acetate 78.83%, petroleum ether 75.31%, methanol 64.26% when compared to acarbose 71.68% standard was inhibited the alpha-amylase. Hence, the greater values than the standard values are directly indicated the highest inhibition of α-amylase and α-glucosidase. The digestive enzyme (α-amylase) is liable for hydrolyzing nutritional starch, which breaks down into glucose before absorption. Inhibition of α-amylase can lead to a reduction in post prandial hyperglycemia in diabetic situations (Lonkisch et al., 1998; Roux et al., 2001). α-glucosidase is a membrane-bound enzyme located in the epithelium of the small gut, catalyzing the cleavage of disaccharides to form glucose. Inhibitors can retard the uptake of nutritional carbohydrates and suppress post-prandial hyperglycemia. Therefore, inhibition of α-glucosidase will be one of the most effective approaches to control diabetes (Sheikh et al., 2008). Elaeocarpus genus is used as folk medicines for diabetics and hypertensive disease (Okselni et al., 2019).

Figure 3. GC-MS chromatogram image of Elaeocarpus variabilis fruit ethyl acetate extract

Table 5. Estimation of in vitro antioxidant activities various solvent extract from Elaeocarpus variabilis fruit μg/mL

| Solvent       | DPPH % Inhibition (VC-Equivalent) | Total Antioxidant (VC-Equivalent) | ABTS % Inhibition | FRAP (TE) | Fe⁺Chelating (EDTA-Equivalent) |
|---------------|-----------------------------------|-----------------------------------|-------------------|-----------|-------------------------------|
| Petroleum ether | 13.3±1.70 ab                      | 0.03±3.61 bc                      | 6.74±4.39 d       | 0.03±0.01 ab | 0.01±0.01 cd                  |
| Ethyl acetate | 83.57±2.52 a                       | 0.06±0.01 a                       | 78.76±3.01 a      | 0.04±0.01 a  | 0.01±0.01 cd                  |
| Methanol      | 57.24±0.35 b                      | 0.04±5.51 b                       | 54.08±14.24 b     | 0.03±0.01 ab | 0.02±0.01 b                   |
| Water         | 37.05±1.89 c                      | 0.02±3.06 d                       | 31.9±2.39 c       | 0.02±0.01 c  | 0.06±0.01 a                   |
| Standard      | Vitamin-C (96.35±1.43)             | ---                               | Vitamin-C         | (93.94±0.95) | ---                           |

TE= Trolox equivalent, EDTA- ethylenediaminetetraacetic acid equivalent, VC-Vitamin-C equivalent. Values are presented as means ± SEM of triplicate determinations and expressed per mL of fruit extracts. Means significantly different between the extract (P<0.05).
bark of *E. ganitrus* may be used as a potent anti-diabetic agent. Prihantini et al. (2014) similarly that α-glucosidase inhibitory activity in *E. sylvestris* obtained had the highest activity against α-glucosidase. Phenolic and flavonoids, compounds have been inhibiting carbohydrate hydrolyzing enzymes because of the capability to bind to proteins (Shobana et al., 2009). These compounds can also inhibit glucosidase have fewer side effects and are less expensive if compared to synthetic compounds. Moreover, prevent the malfunction of pancreatic β-cell due to oxidative stress and reduced type 2 diabetes (El omari et al., 2019).

**In vitro Anti-Inflammatory Activity**

The result showed in Table 7 protein denaturations were done by *E. variabilis* fruit different solvent extracts the highest albumin denaturation activity in ethyl acetate extract 59.60% and minimum activity in water extracts 11.85%. The estimation of HRBC membrane stabilization was done by fruit extract these results expressed was aspirin used as standard and it should be obtained as 43.09% stabilization. In a fruit ethyl acetate extract of 20.61% was higher activity than remaining solvents followed by methanol 14.81%, water 11.72% finally petroleum ether extract 3.49%. The present study was documented some anti-inflammatory compounds through GC-MS analysis, in this fruit extract showed good activities.

The many phenolic acids, anthocyanins, and flavonoids inhibitory activities on nitric oxide implicated in the physiological and pathological process as chronic inflammation (Matsuda et al., 2000; Mohammed et al., 2020; Selamoglu et al., 2021; Sevindik, 2021). The findings shows that the anthocyanins, flavonoids, and phenolic acids may be responsible for the anti-inflammatory activity of this fruit (Cespedes et al., 2010). The anti-inflammatory drug makes half of the analgesics, producing pain by reducing inflammation at a particular body part of an organism. The HRBC membrane stabilization has been used as a technique to study the in vitro anti-inflammatory interest due to the fact the erythrocyte membrane is analogous to the lysosomal membrane and stabilization means that the extract may well stabilize. Stabilization of lysosomal is essential in limiting the informative response. The lysosomal enzymes released during inflammation produce various disorders. The inhibited these lysosomal enzymes are stabilizing the lysosomal membrane (Chowdhury et al., 2014). Inflammation is a complex pathophysiological process mediated through a variety of signaling molecules produced by way of leukocytes, macrophages, and mast cells in addition to by way of the activation of complement factors, which bring about edema formation because of extravasations of fluid and proteins and accumulation of leukocytes on the inflammatory site (Hendrickson et al., 2002).

**Anticancer Activity**

The plant extracts have a complex mixture of secondary metabolites proposed a model mechanism of actions that induced breaking of DNA the result initiation of apoptosis endonuclease activation and also enhanced chemotherapeutic drugs improved apoptosis was related with caspase activation (Singh et al., 2015; Korkmaz et al., 2021; Mohammed et al., 2021). The *E. reticulat* extract was treated with pancreatic cells line (HPDE and BXPC-3) in 24 hours treatments after was IC₅₀ values measured for HPDE 68 µg/mL, and BXPC-3 22 µg/mL there for significantly reduced (Turner et al., 2020). The screening of cytotoxic activity of crude extracts of leaves and bark of *E. floribundus* leaves demonstrated potent activity against human T4 lymphoblastoid and cervical cancer cells (Utami et al., 2013). The strong cytotoxic activities were against the proliferation for four human cancer cells A375, L292, HeLa, and THP-1(Lu et al., 2010). *E. mastersil* was shown significant cytotoxic activity against the human oral epidermoid carcinoma cell line. Phytochemical analysis revealed that the presence of ellagic acid and curarbitacin of the bark has shown a cytotoxicity effect against tumor cells (Joshi et al., 2012). The β-carotene and lutein were used antitumorigenic activities for numerous concentrations on HSC-T6 and Huh-7 cell viability test (Krishnan et al., 2020).

### Table 6. Estimation of in vitro anti-diabetic activities various solvent extract from *Elaeocarpus variabilis* fruit

| Solvent         | α-Amylase (%) | α-Glucosidase (%) |
|-----------------|---------------|-------------------|
| Petroleum ether | 75.3 ± 0.65    | 91.74 ± 0.29      |
| Ethyl acetate   | 78.83 ± 0.49   | 95.39 ± 0.13      |
| Methanol        | 64.26 ± 1.74   | 85.49 ± 0.50      |
| Water           | 81.59 ± 0.39   | 57.02 ± 2.76      |
| Acarbose        | 71.68 ± 0.23   | 94.51 ± 0.11      |

Values are presented as means ± SEM of triplicate determinations and expressed per mL of fruit extracts. Means significantly different between the extract (P<0.05).

### Table 7. Estimation of in vitro anti-inflammatory activities various solvent extract from *Elaeocarpus variabilis* fruit

| Solvent         | HRBC Inhibition (%) | Albumin Denaturation (%) |
|-----------------|---------------------|--------------------------|
| Petroleum ether | 3.49 ± 3.02         | 13.95±3.23              |
| Ethyl acetate   | 20.61 ± 2.71        | 59.60±10.70             |
| Methanol        | 14.81 ± 2.39        | 31.00±40.00             |
| Water           | 11.72 ± 1.48        | 11.85±1.34              |
| Aspirin         | 43.09 ± 2.56        | 93.35 ± 1.25            |

Values are presented as means ± SEM of triplicate determinations and expressed per mL of fruit extracts. Means significantly different between the extract (P<0.05).
In the evaluation of the GC-MS study, n-hexadecanoic acid analysed was responsible for cytotoxic activity extracted by ethyl acetate. The cytotoxicity effects on the cell response of the HT-29 cell line by using the MTT assay shows Figure 4. used ethyl acetate extract different concentrations 10, 20, 30, 40, and 50 µg/mL against selected HT-29 human colon cancer cells. The experimental results of the extract can inhibit cell proliferation in dose-dependent IC\textsubscript{50} values that compared to the ethyl acetate extract against colon cancer cells were calculated and found to be ethyl acetate 30.0 ± 1.0 if compared to doxorubicin (DOX) standard 17.0 ± 0.5 respectively. The results were observed IC\textsubscript{50} values of a fruit extract against HT-29 cancer cells were significantly inhibited the cell proliferation in vitro conditions.

Conclusion

The present study investigated the phytochemicals, antioxidant, anti-inflammatory, anti-diabetic and anticancer properties of various solvent fruit extracts. It can be inferred from the results of the study, which, among ethyl acetate extract exhibited good antioxidant and anti-inflammatory potentials. This additional investigation of anti-diabetic properties to inhibition of α-glucosidase and α-amylase activities is very important that could be developed into a drug for the management of diabetic Mellitus. In furthermore in vitro study was used HT-29 human colon cancer cell line to better activity for fruit extract. The results concluded that the E. variabilis fruit has been valuable nutritional properties for human health.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgement

We kindly acknowledge the Botanical Survey of India, Southern region of Coimbatore, Tamil Nadu, for identifying and authenticated the plant specimen. We thank Bharathiar University, Coimbatore, 641 046 for the financial support and access the lab facility.

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