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

Venkatachalam Balamurugan[1,a], Manikandan Sridhivya[2,b], Ramachandran Dharani[1,c], Subramaniam Selvakumar[3,d], Krishnan Vasanth[1,e,*]

[1]*Department of Botany, Bharathiar University, Coimbatore – 641 046, Tamil Nadu, India*
[2]*Department of Biotechnology, Anna University, Regional Campus, Coimbatore – 641 046, Tamil Nadu, India*
[3]*Department of Biochemistry, Bharathiar University, Coimbatore – 641 046, Tamil Nadu, India*
[*]*Corresponding author*

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ABSTRACT

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^{2+} 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 IC_{50} 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.

[a] *vbalamurugan2@gmail.com* *https://orcid.org/0000-0002-3798-3632*
[b] *sridhivya13111994@gmail.com* *https://orcid.org/0000-0003-4900-577X*
[c] *sridharu101995@gmail.com* *https://orcid.org/0000-0002-3779-9130*
[d] *selvs20@yahoo.com* *https://orcid.org/0000-0001-9471-7632*
[e] *krishnanvasanth1975@buc.edu.in* *https://orcid.org/0000-0002-9091-1523*

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* Weibei, *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 hedyosmus* Zmarzty, *Elaeocarpus variablis* 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, bole 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

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.

$$\text{Extract \%} = \frac{\text{Extract + container (g)} - \text{Empty container (g)}}{\text{Sample weight (g)}} \times 100$$

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 m, 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 vortexes 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,

Tannins = Total phenolics - Non phenolics

Estimation of total flavonoids content

The flavonoids content was determined by the aluminumtrichloride 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 $NaNO_2$ was added to all the test tubes and incubated the whole mixture for 6 m at room

temperature. After incubation, 150 µL of 10% $AlCl_3$ 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 upto 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 colour 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 $FeCl_3$ 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 H_2SO_4 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-5MS (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 $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The inhibition curves were prepared and IC_{50} 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 m 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 $FeCl_3$ $6H_2O$ solution. The fresh working solution was prepared by mixing 25 Ml acetate buffer, 2.5 mLTPTZ solution, and 2.5 mL $FeCl_3$ $6H_2O$ 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 $FeCl_2$ (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 mg 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 alpha-amylase (0.5 mg/mL). The solution was incubated at 25ºC 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 tartarate, 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 calorimeter 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;

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

Determinationof Invitro 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 30ºC 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.

$$\% \text{ Inhibition} = \frac{\text{A410 control} - \text{A410 test}}{\text{A410 control}} \times 100$$

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 IC_{50} 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 isosaline 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 37^0C 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 ºC for 20 min and then heated to 51 ºC 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 and balanced salt solution (BSS) adjusted to contain 1.5 g Na_2CO_3, 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 37^0 C with a 5% CO_2 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 ofculture, the medium was removedby aspiration, and the cells were treatedwith different concentrations(0-50 µg/mL) of extracted compounds in 100 µL DMEM medium. Following 24 h of incubationat 37°C, to each welladded 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 factores. 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). Theywas 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 Alzheimer‟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 flavanoids 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*Blumeby analysis high nutritional value of pulp power, can be applied in diets in the form of dehydrated flour easily incorporated into food. The reported wild fruits of *Ziziphus mauritiana* Lam. and *Ziziphus nummularia* (Burm.f.) Wight & Arn. proximate investigation the rich quantity of crude fiber, vitamin-C, and minerals were observed in each species fruits (Sareen et al., 2020).

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 ± 0.04^{d}
Amino acid	3.55 ± 2.16^{b}
Carbohydrate	0.120 ± 0.03^{e}
Starch	0.015 ± 0.09^{e}
Fructose	0.028 ± 0.07^{e}
Vitamin-A	1.74 ± 0.08^{c}
Vitamin-B1	0.97 ± 0.04^{cd}
Vitamin-C	6.37 ± 0.07^{a}
Vitamin-E	0.50 ± 0.06^{d}
β-Carotene	1.38 ± 0.02^{c}

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

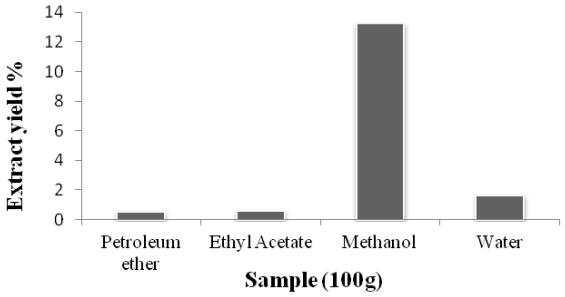

Figure 2. Estimation of Extract yield % of *Elaeocarpus variabilis* fruit

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*is 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 sounces 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 pracence of compounds 1,2,3-benzenetrio and lower number of compounds 1H-imidazole, 4, 5-dihydro-2-methyl-N, with presence of anti-oxidant related compound Dl-.alpha.-tocopherol reduced oxidative stress.The fruit extract compounds were responsible for antioxidant, anti-inflammation 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-á-Dglucopyranose (levoglucosan), 1,2,3-Benzenetriol, 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 alkane 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^{g}	$0.02\pm0.05^{g,f}$	0.21 ± 0.02^{g}	0.12 ± 0.02^{g}	0.01 ± 0.02^{c}
Ethyl acetate	0.20 ± 0.09^{b}	0.10 ± 0.01^{d}	17.70 ± 0.26^{e}	2.24 ± 0.06^{b}	0.04 ± 0.00^{ab}
Methanol	0.12 ± 0.05^{d}	0.14 ± 0.01^{c}	8.10 ± 0.10^{f}	1.42 ± 0.14^{e}	0.10 ± 0.00^{a}
Water	0.08 ± 0.004^{e}	0.11 ± 0.01^{d}	1.73 ± 0.15^{g}	1.34 ± 0.05^{e}	0.02 ± 0.00^{b}

GAE- Gallic acid equivalent, QE- Quinine 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. Phytocompounds 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	$C_4H_8N_2$	84	antifungal and anti-mycobacterium activities
2-[4-(4-methoxybenzyl) phenyl]propan-2-ol	4.281	12.092	$C_{17}H_{20}O_2$	256	No activity reported
1,2,3-benzenetriol	58.908	15.183	$C_6H_6O_3$	126	Antimicrobial, Anti-inflammatory, Antioxidant, Analgesic, Insecticide, Anticancer
3,7,11,15-tetramethyl-2-hexadecen-1-ol	6.970	16.259	$C_{20}H_{40}O$	296	Antimicrobial and Anti-inflammatory
1azabicyclo[2.2.2]oct-2-ene-3-carboxylic acid, methyl ester	2.012	16.534	$C_9H_{13}O_2N$	167	No activity reported
bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1s)	6.590	16.734	$C_{10}H_{16}O$	152	Immune enhancement and anti-microbial activity
1-(4-ethoxy-2-nitroanilino)-1-deoxy-.beta.-d-mannopyranose	2.018	17.509	$C_{14}H_{20}O_8N_2$	344344	No activity reported
n-hexadecanoic acid	7.300	18.135	$C_{16}H_{32}O_2$	256	antibacterial, antioxidant, insecticidal and cytotoxic activities
azetidine, 1-benzyl-3,3-dimethyl-2-phenyln	3.195	22.031	$C_{18}H_{21}N$	51	No activity reported
(2,3-diphenylcyclopropyl) methyl phenyl sulfoxide, transom	3.024	22.826	$C_{22}H_{20}OS$	332	No activity reported
Dl-.alpha.-tocopherol	3.744	28.589	$C_{29}H_{50}O_2$	430	antioxidant and inhibits angiogenesis

PA: % of Peak Area; RT: Retention Time (RT); MF: Molecular Formula (MF); MW: Molecular Weight (MW)

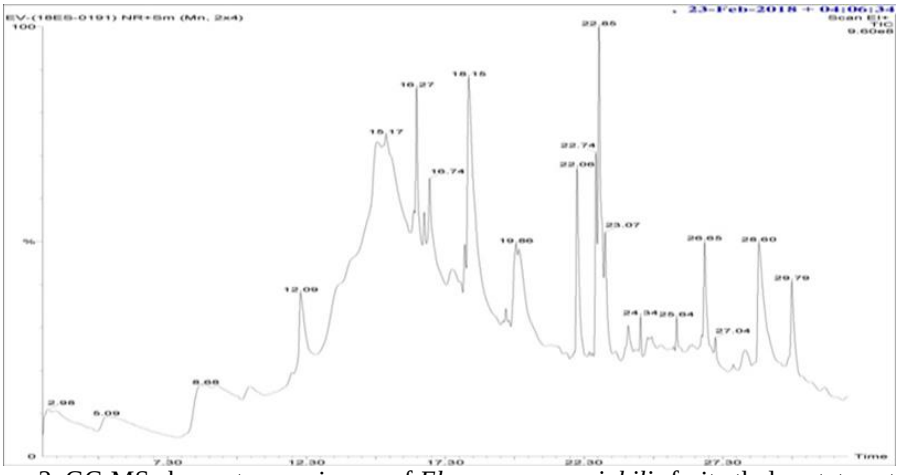

Figure 3. GC-MS chromatogram image of *Elaeocarpus variabilis* fruit ethyl acetate extract

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

Solvent	DPPH % Inhibition	Total Antioxidant (VC-Equivalent)	ABTS % Inhibition	FRAP (TE)	Fe_2+Chelating (EDTA- Equivalent)
Petroleum ether	13.33±1.70^{d}	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^{d}	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).

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* fruit 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 *Rubusniveus* and *E. oblongus* exhibited impressive DPPH radical scavenging of fruit (Shamna 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-glucosidase. 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%, and 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).

Talukdar et al. (2016) state that *in vitro* anti-diabetic activity of *E. ganitrus* bark extract has an inhibitory effect on the α-amylase enzyme which may be used to control high blood glucose levels. These findings justify that the

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; Selamoğlu 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- inflammation 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. reticulates* extract was treated with pancreatic cells line (HPDE and BXPC-3) in 24 hours treatments after was IC_{50} 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

	% inhibition µg/mL	
Solvent	α-Amylase	α-Glucosidase
Petroleum ether	75.31 ± 0.65^{c}	91.74 ± 0.29^{b}
Ethyl acetate	78.83 ± 0.49^{ab}	95.39 ± 0.13^{a}
Methanol	64.26 ± 1.74^{d}	85.49 ± 0.50^{c}
Water	81.59 ± 0.39^{a}	57.02 ± 2.76^{d}
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

	% Inhibition µg/mL	
Solvent	HRBC	Albumin Denaturation
Petroleum ether	3.49 ± 3.02^{d}	13.953 ± 2.32^{c}
Ethyl acetate	20.61 ± 2.71^{a}	59.60 ± 10.70^{a}
Methanol	14.81 ± 2.39^{b}	31.00 ± 40.00^{b}
Water	11.72 ± 1.48^{c}	11.85 ± 1.34^{d}
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 ($P<0.05$).

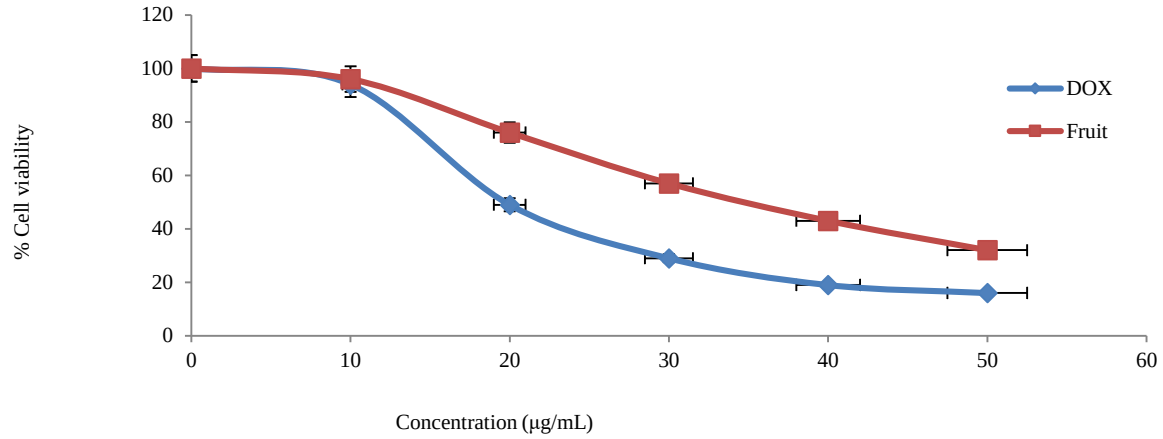

Figure 4. Anticancer activity of HT-29 colon cancer cell line used for various concentrations of *Elaeocarpus variabilis* fruit ethyl acetate extract was triplicate determined after 24 h of treatment compared to DOX standard, the IC_{50} values of fruit extract 30.0 ± 1.0 and DOX 17.0 ± 0.5 respectively.

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_{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_{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.

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References

Apostolidis E, Kwon YI, Shetty K. 2007. 'Inhibitory potential of herb, fruit and fungal-enrichd cheese against key enzymes linked to type 2 diabetes and hypertension. Innovative Food Science and Emerging Technologies, 8(1): 46-54.

Aragaw HS, Nohr D,Callo-Concha D. 2021.Nutritional potential of underutilized edible plant species in coffee agroforestry systems of Yayu, southwestern Ethiopia. Agroforest Syst, 95: 1047–1059.

Arnao MB, Cano A, Acosta M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food chemistry, 73(2): 239-244.

Arti N, Archana A. 2007. Lab Manual in Biochemistry, Immmunology and Biotechnology'. Tata MC Graw-Hill Publishing Company Limited, 1: 84-85.

Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry, 239(1): 70-76.

Benderitter M. 1998. Studies by Electron Paramagnetic Resonance of the Importance of Iron in the Hydroxyl Scavenging Properties of Ascorbic acid in plasma: Effects of Iron Chelators. Fundamental and Clinic Pharmacol, 12(5): 510-516.

Bharti A. 2013. Determination of Quercetin in extract of *Elaeocarpus ganitrus*Roxb seeds by using HPTLC method. International Reseach Journal of Pharmacy, 4(3): 186-188.

Bhatt BD,Dahal P. 2019. Antioxidant and Antimicrobial Efficacy of Various Solvent Extracts of Seed of Rudrakshya (*Elaeocarpus ganitrus*) from Ilam District of Nepal. Journal of Nepal Chemical Society, 40: 11-18.

Bhattacharya SK, Debnath PK, Pandey VB, Sanyal AK. 1975. Pharmacological in vestigation on *Elaeocarpus ganitrus.* Planta Medica,28(06): 174-177.

Braca A, Tommasi ND, Bari LD, Pizza C, Politi, Morelli I. 2001. Antioxidant principles from *Bauhuniaterapotensis*. Journal of Natural Products, 64(7): 892-895.

Carmichael J, DeGra WG, Gazdar AF, Minna JD, Mitchell JB. 1987. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Researh*, 47: 936- 942.

Cespedes CL, Eihafidi M, Pavon N, Alarcon J. 2010. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis*(Elaeocarpaceae), Maqui. Food Chemisty, 107(2): 820–829.

Chen YH, Yang CY. 2020. Ultrasound-Assisted Extraction of Bioactive Compounds and Antioxidant Capacity for the Valorization of *Elaeocarpus serratus* L. Leaves. Processes,8(10): 1218.

Chopra RN. 1933. Indigenous Drugs of India. The Art Press. Calcutta, 58-76.

Chowdhury A, Azam S, Jainul MA, Faruq KO, Islam A. 2014. Antibacterial activities and *in vitro* anti-inflammatory (membrane stability) properties of methanolic extracts of *Gardenia coronaria* leaves. International Journal of Microbiology, 1-5.

Dinis TCP, Madeira VMC, Almeida LM. 1994. Action of Phenolic Derivatives (Acetaminophen, Salicylate and 5-Aminosalicylate) as Inhibitors of Membrane Lipid Per oxidation and as Peroxyl Radical Scavengers. Biophysics Journal, 315(1): 161-169.

El Omari N, Sayah K, Fettach S, El Blidi O, Bouyahya A, Faouzi MEA, Barkiyou M. 2019. Evaluation of *in vitro* antioxidant and antidiabetic activities of *Aristolochia longa* extracts. Evidence-Based Complementary and Alternative Medicine, 1-9.

Gandhisan R, Thamaraichelvan A, Baburaj. 1991. Antiinflammatory action of *Lannea coromandelica* Hrbc membrane stabilization. Fitotherapia, 62(1): 81- 83.

Gaurav K, Karthik L, Rao KVB. 2014. A Review on Medicinal Properties of *Elaeocarpus ganitrus* Roxb.ex G. Don (Elaeocarpaceae). Research Journal of Pharmacy and technology, 7(10): 1184-1186.

Geetha DH, Rajeswari M,Indiramuthu J. 2013. Chemical profiling of *Elaeocarpus serratus* L. by GC-MS. Asian Pacific Journal of Tropical Biomedicine, 3(12): 985-987.

Gonzalez-Molina E, Domínguez-Perles R, Moreno DA, García-Viguera C. 2010. Natural bioactive compounds of *Citrus limon* for food and health. Journal of Pharmaceutical and Biomedical Analysis, 51(2): 327-345.

Hardainiyan S, Nandy BC, Saxena R. 2015. Phytochemical investigation of fruit extract of *Elaeocarpus ganitrus*. International Journal of Pharmacy and Pharmaceutical Sciences, 7(6): 415-418.

Havsteen B. 1983. Flavonoids, a class of natural products of high pharmacological potency. Biochemical pharmacology, 32(7): 1141-1148.

Hendrickson BA, Gokhale R, Cho JH. 2002. Clinical aspects and pathophysiology of inflammatory bowel disease. Clinical Microbiology Reviews, 15(1): 79-94.

Hodge JE,Hofreiter BT. 1962. Determination of reducing sugar and carbohydrates. In: methods in carbohydrate chemistry, whistler, RL, ML wolfram, JN Be-Miller and F Shafizadeh (Eds.). Academic Press Inc, New York USA, pp: 380-394.

Jain SK. 1991. Dictionary of Indian folk medicine and ethnobotany. New Delhi: Deep publication,53: 75 -82.

Jawla S, Rai DV. 2016. Pharmacognostic Studies on Rudraksh (*Elaeocarpus angustifolius* Blume) Fruit. Advances in Biological Research, 10(6): 382-387.

Jayashree I, Geetha DH, Rajeswari M. 2019. GC-MS Analysis of Ethanolic Extract of Leaf of *Elaeocarpus tuberculatus*Roxb. International Journal of Pharmacognosy and Phytochemical Research, 11: 299-303.

Joshi S, Gupta P, Kumar N, Rai N, Gautam P, Thapliyal A. 2012. A comprehensive report on therapeutic potential of *Elaeocarpus ganitrus* Roxb.(Rudraksha). Environment Conservation Journal, 13(3): 147-150.

Khan ML, Bhuyan P, Tripathi RS. 2003. Regeneration of Rudraksh (*Elaeocarpus ganitrus* Roxb.) – a threatened tree species: germination strategies. International Journal of Ecology and Environmental Science, 29: 255–260.

Korkmaz N, Dayangac A, Sevindik M. 2021. Antioxidant, antimicrobial and antiproliferative activities of *Galium aparine*. Journal of Faculty of Pharmacy of Ankara University, 45(3): 554-564.

Krishnan V, Subramaniam S, Chia–Chuan C, Venkatachalam B, Thomas Cheeran A, Chi-Ying FH. 2020. Anticancer Activity of *Leonurus sibiricus* L.: Possible Involvement of Intrinsic Apoptotic Pathway. Nutrition and Cancer, 1-12.

Kumari N, Atri NS. 2014. Nutritional and Nutraceutical Potential of Wild Edible Macrolepiotoid Mushroom of North India. International Journal of Pharmacy and Pharamaceutical Science, 6(2): 200-204.

Kumar A, Kumar M, Verma RK, Kumar A, Punar S, Ram L, Maheshwari RK. 2021. A Comprehensive Review on Phytochemical, Pharmacological, Dielectric and Therapeutic Attributes of Multifarious Rudraksha (*Elaeocarpus Ganitrus* Roxb.). Applied Sciences, 9(1): 97-109.

Lakshmi MA, Srinivas K, Rani SU,Praneetha V. 2016. Evaluation of *in vitro* anti inflammatory activity of *Elaeocarpus ganitrus* of bark extract by hrbc membrane stabilization. International Journal of Pharmaceutical Chemical and Biological Sciences,6(4).

Lakshmi SR, Kumar TS, Rao MV. 2014. Phytochemical Analysis, Histochemical localization and Antioxidant Activity of Hoya wightii ssp. palniensis and *Elaeocarpus recurvatus*. American Journal of Phytomedicine and Clinical Therapeutics, 2(3): 57-366.

Lonkisch M, Layer P, Rizza RA, Magno EP. 1998. Acute post prandial gastrointestinal and metabolic effects of wheat amylase inhibitor (WAI) in normal, obsess and diabetic humans. Pancreas,17(2): 176-181.

Lowry OH, Rosebrough NJ, Farr AL, anandall RJ. 1951. Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193: 265-275.

Lu B, Liu L, Zhen X, Wu X, Zhang Y. 2010. Anti-tumor activity of triterpenoid-rich extract from bamboo shavings (Caulis bambusae in Taeniam). African Journal of Biotechnology, 9(38): 6430-6436.

Makkar HP, Siddhuraju P, Becker K. 2007. Plant secondary metabolites, Totowa: Humana Press Inc, p93-100.

Makkar HPS. 2003. Quantification of Tannins in Tree and Shrub Foliage, Makkar, H.P.S., (ed.). Kluwer Academic Press. New York,pp. 102.

Manoharan AL, Thamburaj S, Muniyandi K, Jagadeesan G, Sathyanarayanan S, Nataraj G,Thangaraj P. 2019. Antioxidant and antimicrobial investigations of *Elaeocarpus tectorius* (Lour.) Poir. fruits against urinary tract infection pathogens. Biocatalysis and Agricultural Biotechnology, 20: 101260.

Matsuda H, Kagerura T, Toguchida I, Ueda H, Morikawa T, Yoshikawa M. 2000. Inhibitory effects of sesquiterpenes from bay leaf on nitric oxide production in lipopolysaccharide-activated macrophages: Structure requirement and role of heat shock proteininduction. Life Science, 66(22): 2151-2157.

Meenakshi VK, Gomathi S, Senthamarai S, Paripooranaselvi M, Chamundeswari KP. 2012. Analysis of vitamins by HPLC and phenolic-compounds, flavonoids by HPTLC in *Microcosmus-exasperatus*. European Journal of Zoological Research, 1(4): 105-110.

Mohammed FS, Günal S, Pehlivan M, Doğan M, Sevindik M, Akgül H. 2020. Phenolic content, antioxidant and antimicrobial potential of endemic *Ferulago platycarpa*. Gazi University Journal of Science, 33(4): 670-677.

Mohammed FS, Pehlivan M, SevindikE, Akgul H, Sevindik M, Bozgeyik I, Yumrutas O. 2021. Pharmacological properties of edible *Asparagus acutifolius* and *Asparagus officinalis* collected from North Iraq and Turkey (Hatay). Acta Alimentaria, 50(1): 136-143.

Moore S, Stein WH. 1948. In methods in enzymol. Academic Press. New York, 3, 468.

Nayan talukdar N, Dutta AM,Karabi D. 2016. Anti-Oxidant, Antimicrobial and Inhibitory effect on α-Amylase of Ethyl acetate extracts from the bark of *Elaeocarpus ganitrus.*Indo American Journal of Pharmaceutical Research, 6: 6926-6931.

Neeld JB, Pearson WN. 1963. Macro and Micro Methods for the Determination of Serum Vitamin A using Trifluoroacetic acid. The Journal of nutrition, 79(4): 454-462.

Okselni T, Santoni A, Dharma A,Efdi M. 2019. Biological activity of methanol extract of *Elaeocarpus mastersii* king: antioxidant, antibacterial, and α-glucosidase inhibitor. Rasayan Journal of Chemistry, 12(1): 146-151.

Okwu DE, Ndu CU. 2006. Evaluation of the phytonutrient, mineral and vitamin content of some varieties of yam (*Discorea*spp). International Journal of Molecular Medicine and Advance Science, 2(2): 199-203.

Prieto P, Pineda M, Aguilar M. 1999. Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application of vitamin-E. Analytical Biochemistry, 269(2): 337-341.

Prihantini AI, Tachibana S. 2017. Antioxidant compounds produced by Pseudocercospora sp. ESL 02, an endophytic fungus isolated from *Elaeocarpus sylvestris*. Asian Pacific Journal of Tropical Biomedicine, 7(2): 110-115.

Prihantini AI, Tachibana S, Itoh K. 2014. Evaluation of antioxidant and α-glucosidase inhibitory activities of some subtropical plants. Pakistan journal of biological sciences, 17(10): 1106-1114.

Raghunathan M. 2017. Assessment of Vitamins, Flavonoids And Phenolic-compounds by Hplc in the fruit Of *Elaeocarpus Oblongus.* European Journal of Pharmaceutical and Medical Research,4: 235-241.

Rajapaksha U. 1998. Traditional Food Plants in Sri Lanka. Hector Kobbekaduwa Agrarian Research and Training Institute. Sri Lanka, 231.

Ramful D, Tarnus E, Aruoma O I, Bourdon E,Bahorun T. 2011. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. Food Research International, 44(7): 2088-2099.

Razali N, Mat-Junit S, Abdul-Muthalib AF, Subramaniam S, Abdul-Aziz A. 2012. Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of *Tamarindus indica* L. Food Chemistry, 131(2): 441-448.

Roux GF, Perrier J, Forest E, MourenGM, Puigserver A, Santimone M. 2001. The human pancreatic alpha-amylase isoforms: isolation, structural studies and kinetics of inhibition by Acarbose.Asian Journal of Pharmaceutical Research,1388(1): 10-20.

Sadasivam S, Manickam A. 2004. Biochemical Methods, 2nd edition, New Age International (P) Ltd, Publishers, New Delhi.

Sakat S, Juvekar AR,Gambhire MN. 2010. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharmacy and Pharmaceutical Sciences, 2: 146-155.

Sareen A, Gupta RC, Bansal G, Singh V. 2020. Comparison of key mineral elements in wild edible fruits of *Ziziphus mauritiana* and *Ziziphus nummularia* using atomic absorption spectrophotometer (AAS) and flame photometer. International Journal of Fruit Science, 20(sup2): S987-S994.

Sathish Kumar T, Shanmugam S, Palvannan T, Bharathi Kumar VM. 2010. Evaluation of antioxidant properties of *Elaeocarpus ganitrus* Roxb leaves. Iranian Journal of Pharmaceutical Research, 3: 211-215.

Selamoglu Z, Sevindik M, Bal C, Ozaltun B, Sen İ, Pasdaran A. 2020. Antioxidant, antimicrobial and DNA protection activities of phenolic content of *Tricholoma virgatum* (Fr.) P.Kumm. Biointerface Research in Applied Chemistry, 10(3): 5500-5506

Sevindik M. 2021. Phenolic content, antioxidant and antimicrobial potential of *Melanoleucamelaleuca* edible mushroom. Journal of Animal & Plant Sciences, 31(3): 824-830

Shamna R, Sasikumar JM,Sugathakumar E. 2011. Antioxidant Capacity of *Rubus niveus*Thunb. and *Elaeocarpus oblongus* Gaestn Fruits. Global science book, 5: 96-101.

Sharma A, Swati J,Navin K. 2015. Antioxidant and antibacterial properties of leaves of *Elaeocarpus sphaericus* Roxb. And *Pinus wallichiana*from Uttarakhand region of India.International Journal of Green Pharmacy, 9(4): 246-250.

Sheikh JH, Iyo Tsujiyama MT, Ashabul I, Rajat SB. 2008. Hitoshi, A. Total Phenolic Content, Anti-oxidative, anti-Amylase, Anti- Glucosidase and Anti-Histamine release activities of Bangladeshi fruits.Food Science and Technology Research,14(3): 261-268.

Shobana S, Sreerama YN, Malleshi NG. 2009. Composition and enzyme inhibitory properties of fnger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of α-glucosidase and pancreatic amylase. Food Chemistry, 115(4): 1268–1273.

Singh B, Chopra A,Ishar MPS, Sharma A, Raj T. 2010. Pharmacognostic and antifungal investigations of *Elaeocarpus ganitrus* (Rudrakasha). Indian Journal of Pharmaceutical Sciences 72(2): 261-265.

Singh DK, Srivastva B,Sahu A. 2004. Spectrophotometric determination of *Rauwolfia* alkaloids, estimation of reserpine in pharmaceutical. Analytical sciences, 20(3): 571-573.

Singh TD, Meitei HT, Sharma AL, Robinson A, Singh LS, Singh TR. 2015. Anticancer properties and enhancement of therapeutic potential of cisplatin by leaf extract of *Zanthoxylum armatum* DC. Biological research,48(1): 1-9.

Swapana N, Jotinkumar T, Devi CB, Singh MS, Singh SB, Singh CB. 2012. Total Phenolic, Total Flavonoid Contents And Antioxidant Activity of A Fe w Indigenous Fruits Grown In Manipur. The bioscan, 7: 73-76.

Talukdar M, Duarah A, Talukdar S, Buragohain M, Debnath R, Yadav A, Jha DK , Bora TC, 2016. Bioprospecting *Micromonospora* from Kaziranga National Park of India and their *anti-infective* potential. World Journal of Microbiology and Biotechnology, 28(8): 2703–2712.

Tripathy S, Anil M, Sudhansu RS. 2016. Phytochemical screening and thin layer chromatographic studies of *Elaeocarpus ganitrus* Seed, the magical electromagnetic bead (Rudraksha). International Journal of Pharmacy and Biological Sciences, 6(3): P16-24.

Turner A, Bond DR, Vuong QV, Chalmers A, Beckett EL, Weidenhofer J, Scarlett CJ. 2020. *Elaeocarpus reticulatus* fruit extracts reduce viability and induce apoptosis in pancreatic cancer cells *in vitro*. Molecular biology reports, 47(3): 2073-2084.

Utami R, Nurhasniza K, MohdAspollah S, Mawardi R, Ahmad Bustaman A,Dachriyanus H. 2013. Phenolic contents, antioxidant and cytotoxic activities of *Elaeocarpus floribundus* Blume. Pakistan Journal of Pharmaceutical Sciences, 26(2): 245-250.

Vijayan A. 2017. Phytochemical Analysis of *Elaeocarpus blascoi* Weibel using Gas Chromatography – Mass Spectroscopy. Journal of Natural Products and Resources, 3(2): 125–129.

Vuong QV, Pham HNT, Vu HT, Dang TT, Van Ngo T, Chalmers AC. 2018. Fruit characteristics, phytochemical and antioxidant properties of blueberry ash (*Elaeocarpus reticulatus*). Heliyon, 4(10): e00834.

Worthington V. 1993. Alpha amylase, in: Worthington (Ed.), Worthington Enzyme Manual Freehold', Worthington Biochemical Corporation. NJ, pp.36-41.

Yu L. 2001. Free radical scavenging properties of conjugated linoleic acids. Journal of Agricultural and Food Chemistry, 49(7): 3452-3456.

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemisry, 64(4): 555-559.

Zmarzty S. 2001. '*Elaeocarpus* in Southern India and Sri Lanka', Kew Bulletin,56: 405-447.