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Short Communication

PHYTOCHEMICAL INVESTIGATION OF FRUIT EXTRACT OF *ELAEOCARPUS GANITRUS*

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ABSTRACT

Objective: The present study aimed to investigate the qualitative and quantitative phytochemical analysis of ethanolic fruit extract of medicinally important plant of *Elaeocarpus ganitrus* for identification of major bioactive constituents.

Methods: Studies were carried out in terms of qualitative and quantitative estimation of phytoconstituents. The phenolic content was examined by using Folin-Ciocalteu assay. The total flavonoid was determined spectrophotometrically by using the aluminium chloride reagent.

Results: The yield extraction value in ethanolic fruit extracts was 28.65%. The preliminary phytochemical analysis results indicated the presence of alkaloids, glycosides, phenolic compounds, flavonoids, saponins, carbohydrates and fixed oils, but protein and amino acids were absent in this extract. The total phenolic content was 232.24 mg of gallic acid equivalent (GAE) per gram of ethanolic fruit extract of *E. ganitrus*. The total flavonoid concentration was 91.42 mg of quercetin equivalent (QE) per gram of ethanolic extract.

Conclusions: Phenolic and flavonoid compounds have several prevailing biological activity, which outlines the necessity of their determination. Results revealed the presence of different bioactive compounds which could be exploited for their potential applications for medicinal purposes.

Keywords: *Elaeocarpus ganitrus* fruit, Phytochemicals, Extracts, Phenolic compounds, Flavonoids.

Elaeocarpus ganitrus Roxb. (Syn. *E. sphaericus* Gaertn., *E. angustifolius* Blume) is an evergreen tree, ripe fruits which contain a hard and highly ornamental stony endocarp known as bead or nut and commonly termed as Rudraksha in India. It holds popular faith unbreakable by experiments that it has confirmed medicinal uses apart from its attractive stones [1, 2]. *Elaeocarpus* genus, has about 360 species, occurs during East Asia, Australia, Malaysia and the Pacific Islands. About 120 species belongs to this *Elaeocarpus* genus has been reported from different parts of Asia and out of this, 25 species occur in India alone [3]. Rudraksha beads have Ayurveda traits. Rudraksha beads used to alleviate different ailments like headache, fever, chicken pox, mental disorders, burn or pox marks and also to wound healing [4]. In most biological studies, *E. ganitrus* exhibited the wide range of pharmacological activities and identified as active against specific biological targets during large scale screening of multiple plant extracts. Literature reports are available on various pharmacological activities which include analgesic, anti-inflammatory [5], CNS activities, typical behavioral actions, sedative, tranquillizing, hypnosis potentiation, antiasthmatic, antidepressant, hydrocholeretic [6], antidiabetic [7], cardio stimulation, antihypertensive and anticonvulsant, etc [8]. Phytochemical constituents are non nutritive chemicals of plant; which are known to show protective or disease defensive properties against several ailments in man and therefore could explain the use traditional of medicinal plant for the treatment of some illnesses [9]. Several secondary metabolites such as phenolic compounds, alkaloids, terpenoids, steroids, saponins have complex structures and also have more restricted distribution than primary metabolites [10]. They are not crucial for the plant that contains them; at least their metabolic functions have not been discovered yet. Phenolic compounds are most active secondary metabolites present in the plant kingdom which has multiple biological effects including antioxidant, antimicrobial, anti-inflammatory, antidepressant, antiallergic and antidiabetic activities [11]. Phenolic compounds are which possess in common an aromatic ring posture one or more hydroxyl groups. There are about 8000 in nature occurring plant phenolics and about half of this number is flavonoids [12]. Flavonoids are necessary in human diets and also present in plants extracts that have been used for medical purpose [13]. Phenolic compounds are plant substance which possesses a broad spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as capability to change the gene expression [14]. Phenols and flavonoids are major secondary metabolites which are imperative source in plants for normal growth development and defense against infection and injury. Nowadays, isolation and identification of phytoconstituent of plant extract have always been a challenging task for research [15]. Secondary metabolites likes polyphenols or phenolics are that secondary plant metabolites which are ubiquitously nearby in plant products and plants. Many phenolic compounds have been shown to hold soaring levels of antioxidant activities [16, 17]. Usually the mechanisms of secondary phenolic metabolites intended for antioxidant activity are neutralizing lipid free radicals and preventing the disintegration of hydro peroxides into free radicals [18, 19].

Fig. 1: (A) Tree of *Elaeocarpus Ganitrus* (B) *Elaeocarpus Ganitrus* Fruit (C) Powder of *Elaeocarpus Ganitrus* fruits

Scientific advancement brought a positive approach for the systemic exploration of *E. ganitrus* for its medicinal properties and in last decades *E. ganitrus* has been reported to exhibit various pharmacological activities. However, most of these studies were carried out on the leaves and very few efforts have been made on the fruit extract and their fractions or pure isolated active compounds. The focus of this study was to determine the qualitative and quantitative determination of bioactive compounds i.e. Present in ethanolic fruit extract of *E. ganitrus*. Further research is going on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive principles with the help of advanced technologies to develop plant derived drugs.

Plant material (fruit) was collected from the herbal garden of Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India in the month of April 2013. The plant was authentified taxonomically and confirmed by the department of Botany, Rajasthan University, Jaipur. A voucher specimen *Elaeocarpus ganitrus* (RUBL-211325) has been deposited in the same department's herbarium. The fruits of *Elaeocarpus ganitrus* were dried under shade at room temperature for 20 days. The dried fruits of the plant were crushed into powdered with the help of mechanical grinder and sieved to give particle size 50 to 150 mm. The dried fruit powder of *Elaeocarpus ganitrus* was filled in the thimble and extracted successively with 90% ethanol in the Soxhlet apparatus for about 48 hours [20]. The extract was concentrated and solvent removed by vacuum evaporator at 40 ˚C under reduced pressure and extract were transferred to glass vials and kept at 4 ˚C before use. Yield of the extract obtained was calculated by formula as mentioned below:

$$\text{Extractive yield value} = \frac{\text{weight of concentrated extract}}{\text{weight of dried plant power}} \times 100$$

The fruit extracts of *E. ganitrus* was subjected to preliminary qualitative phytochemical screening for the detection of plant constituents. Various colour reactions were used to identify the nature of the components. The crude fruit extract was tested for the presence or absence of several phytoconstituents such as carbohydrate, alkaloids, the phenolic compound, tannins, glycoside, flavonoids, saponins and fixed oils. All phytochemical screening of extract was carried out as per the procedure mentioned in the standard book of "Pharmacognosy" [21].

The amount of total phenolic content in the fruit extracts of *E. ganitrus* was determined by the spectrophotometric methods of Jan *et al.,* (2013) with slight modification [22]. An aliquot of extract or standard gallic acid was used in this analysis. The reaction mixture was prepared into a 25 ml of the volumetric flask. 1 ml of diluted extract in ethanol (100µg/ml) was mixed with 9 ml of distilled water and 1 ml of Folin-Ciocalteu phenol reagent was also added to the mixture and shaken. The solution was allowed to stand at 25˚C for 5-8 min before adding 10 ml of 7% sodium carbonate solution which was made in distilled water. The test sample was diluted to 25 ml with distilled water and mixed thoroughly. The reaction mixture was allowed to stand for 2 h at room temperature. The absorbance was determined by U. V. Spectrophotometer at 765 nm. A calibration curve was plotted by using different concentration of standard Gallic acid. Total phenolic contents were calculated as mg of Gallic acid equivalent (GAE) per gram of the dried sample (mg/g) (table 3).

Total flavonoid content was estimated according to the method published earlier by Jasuja *et al.,* 2013, with slight modification [15]. In a test tube, plant extract (0.5 ml) was taken from pre prepared stock solution of extract was mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and diluted with 2.8 ml distilled water. Then the mixtures were tolerable to stand for incubation at room temperature for 30 min and absorbance of the reaction mixtures were measured at 510 nm against the prepared blank solution using spectrophotometer. Blank solution was prepared by addition of all the other reagents except the plant extracts. Total flavonoids content was expressed in mg of quercetin equivalent (QE) per gram of the dry plant extract (mg/g) (table 3).

Results illustrated that ethanolic fruit extracts of *E. ganitrus* were subjected to routine qualitative and quantitative phytochemical analysis to identify the nature of bioactive compounds present in it. The extractive value of fruit extract was found 28.65 % (table 1). The qualitative phytochemical screening of fruit extracts of *E. ganitrus* revealed the presence of alkaloids, flavonoids, steroids, glycosides, terpenoids, saponins, carbohydrates and fixed oils. Protein and Amino acids were absent in the fruit extract (table 2). *E. ganitrus* plant extracts and their active ingredients such as alkaloids, flavonoids, tannins and phenols were used to serve as antioxidants, antibacterial, antifungal, analgesic and anti-inflammatory, CNS activities, typical behavioral action, sedative, tranquillizing, hypnosis potentiation, antidepressant, antiasthmatic, hydrocholeretic, antidiabetic, cardiostimulation, antihypertensive and anticonvulsant, etc [5-8, 10]. Recently, Singh *et al.,* (2013) reported that ethanolic extract of *E. ganitrus* fruits exhibited significant anti anxiety activity. An anxiolytic constituent of quercetin derivative was isolated from ethanol extract of *E. ganitrus* beads and it was also used as the marker to standardize the plant material [3]. This research work provides a scientific validation for utilization of ecofriendly fruit of medicinal plant *E. ganitrus,* in having the potential to be a good drug. Several constituents such as (+)-elaeocarpiline, isoelaeocarpiline, (-)-epielaeocarpiline, (+)-epi-isoelaeocarpiline, (+)-epialloelaeocarpiline, (-)-alloelaeocarpiline, elaeocarpidine, and pseudoepi-isoelaeocarpiline having a dihydro-γ-pyrone chromophore in their molecules have also been reported by Ray et al., (1979) [23]. The study of Kumar *et al.,* (2008) revealed the presence of phenolic and flavonoids in the leaves of *E. ganitrus* which provide substantial antioxidant activity [24]. Earlier, study suggested that Leaves extract of *E. ganitrus* contain quercetin, gallic and ellagic acids [25]. Singh *et al.,* (2013) also reported a non aromatic indolizidine alkaloid such as rudrakine which was isolated from the leaves of *E. ganitrus* [3]. Furthermore, Sakat *et al.,* (2009) were also studies on phytochemical analysis of bioactive compounds by using aqueous seed extract of *E. ganitrus* and evaluated for antihypertensive activity in renal artery occluded hypertensive rats [8]. Similarly, Bharti, (2013) revealed that Kaempferol and Quercetin were found to dominate in seeds of *E. ganitrus* The content of C-glycosides derivatives of apigenin and quercetin in all seed samples was in a standard quantity than flavonol type compounds. On the contrary, in sample C-glycosides derivatives of apigenin and luteolin were dominant [26]. Phenolic acids have repeatedly been concerned as natural antioxidants in fruits, vegetables and several plants. Phenolic compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma and flavour and also in providing health beneficial effects [27, 28]. Total phenolic concentration of extracts was determined by Folin-Ciocalteu (F-C) assay. Gallic acid used as a standard phenolic compound. The F-C assay for total phenolic content is a simple, fast, and the standard method that can be useful in characterizing and standardizing botanical samples. F-C method is based on oxidation of phenolics by a molybdotungstate in F-C reagent to give up a coloured product with λmax 765 nm [29]. The total phenolic content of the test extract was calculated using the standard curve equation of gallic acid: $y = 0.091x+0.065$; $R^2 = 0.945$; Where y is absorbance at 765 nm and x is total phenolic content in the ethanolic fruit extract of *E. ganitrus* expressed as gallic acid equivalent in mg/g. Total phenolic content was 232.24 mg GAE/g of extract (table 3). The amount of total flavonoid was determined with aluminum chloride reagent. Quercetin was used as a standard flavonoid compound. Total flavonoid contents were calculated using the standard curve equation of quercetin $y = 0.017x-0.030$; $R^2 = 0.982$; Where y is absorbance at 510 nm and x is total flavonoid content in the fruit extracts of *E. ganitrus* expressed as quercetin equivalents (QE) per gram of the plant extract. The total flavonoid content was 91.42 mg QE/g of extract (table 3). The results showed that the family of Elaeocarpaceae plant i.e. *E. ganitrus* is the richest source of phenolic compounds. There is an encouraging connection between phenolic content and free-radical scavenging activity. Phenolic acids have been implicated as natural antioxidants in fruits, vegetables and several plants [30. 31]. Flavonoids are essential in human diet and are present in plant extracts that have been used for medicinal purpose [32]. Further studies are needed with fruit extract of *E. ganitrus* to identify the unknown flavonoids and phenolic acids in the analysed ethanolic extracts by isolating, characterizing and elucidating the structure of the bioactive compounds of plant in order to prepare natural pharmaceutical products with a of high medicinal value.

Table 1: The percentage yield of crude extract of *E. ganitrus* prepared by soxhlet extraction techniques

Solvent	Weight of fruit powder	Volume of solvent	Weight of extract	% of yield extraction
90% Ethanol	40 gm	200 ml	11.46 gm	28.65%

Table 2: Qualitative screening of the phytochemical composition of ethanolic extract of *Elaeocarpus ganitrus*

Chemical constituents	Observation of plant extract
Alkaloids	+ve
Flavonoid	+ve
Saponins	+ve
Phenol	+ve
Carbohydrates	+ve
Glycosides	+ve
Protein & Amino Acid	-ve
Steroids & Terpenoids	+ve
Fixed Oils and fat test	+ve

(+ve means presence of compounds &-ve means absences of compound in the extract)

Table 3: Quantitative estimation of total Phenolic content and total Flavonoid contents in the ethanolic extract of *E. ganitrus*

S. No.	Plant material	Total phenolic contents (mg/gm)	Total flavonoids contents (mg/gm)
1.	*E. ganitrus* fruit extract	232.24±0.31	91.42±0.44

CONCLUSION

Present study revealed that *E. ganitrus* fruit extract was rich in phenolic constituents. The objective of this study was to provide information about the significant amount of phenolic compounds and flavonoids in ethanolic fruit extract of *E. ganitrus*. Further studies are going on concerning this plant in order to isolate, identify, characterize and elucidate the structure of bioactive compounds by various techniques such as high performance liquid chromatography (HPLC), Fourier transform infrared (FTIR) spectroscopy and Nuclear magnetic resonance (NMR) etc. There is no doubt that this plant is a reservoir of potentially useful bioactive compounds which serve as drugs, provide newer leads and clues for modern drug design by synthesis. Due to its many medicinal properties, there is enormous scope for further research on *E. ganitrus* and further clinical and pharmacological can be conducted to investigate the unexploited potential of this plant.

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CONFLICT OF INTERESTS

Declared None

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