Plant Archives Vol. 23, No. 2, 2023 pp.389-396 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2023.v23.no2.066

PHYTOCHEMICAL SCREENING AND COMPARATIVE ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS ISOLATED FROM *E. GANITRUS* LEAVES

Jyotsana Khushwaha[1] and Alpana Joshi[2]*
[1]Department of Biotechnology, Shobhit Institute of Engineering and Technology, (Deemed to be University), Meerut, 250110, India
[2]Department of Agriculture Technology and Agri–Informatics, Shobhit Institute of Engineering & Technology, (Deemed to be University), Meerut, 250110, India
*Corresponding Author Email: alpana.joshi@shobhituniversity.ac.in; joshi.alpana@gmail.com
(Date of Receiving : 04-08-2023; Date of Acceptance : 30-09-2023)

ABSTRACT

Elaeocarpus ganitrus (Rudraksha) contains many bioactive compounds with potent antioxidant and pharmacological benefits. This study designed to identify the bioactive compounds in the three extracts prepared from *E. ganitrus* leaves using UV–Vis and FTIR spectroscopy and evaluate their antioxidant potential. The preliminary phytochemical screening showed that all three extracts are rich in alkaloids, flavonoids, phenolics, tannins, and saponins. The UV–Vis analysis displayed two distinct peaks at 212.6 nm and 275 nmin the absorption range of 0.9 – 1.0 with hydroalcoholic extract (EGHA), 0.4 – 0.5 with ethanolic extract (EGE), and 0.3 – 0.4 with water extract (EGW), respectively. The FT–IR spectrum showed the presence of alkene, amine and fluoro compound, alkyl aryl ether, alkane, ester, δ–lactone, cyclopentanone, amine salt, alcohol, and carboxylic acid. All three extracts of *E. ganitrus* leaves have been subjected to the assessment of antioxidant properties by using 1, 1–diphenyl–2–picryl–hydrazyl (DPPH) and Nitric oxide (NO) scavenging radical scavenging assays. The results revealed that hydroalcoholic extract (EGHA) displayed better antioxidant potential as compared to ethanolic extract (EGE) and water extract (EGW) in all tested concentrations. This study demonstrates the significance of the FTIR and UV–Vis spectrum of *E. ganitrus*, which can be exploited to isolate significant phytoconstituents. Furthermore, biologically active isolates can be analysed to investigate their diverse biological activities depending on their therapeutic applications in the pharmaceutical industries.
***Keywords*:** Antioxidant, *Elaeocarpus ganitrus*, FT–IT, Phytochemicals, Rudraksha, UV–Vis spectroscopy

Introduction

Elaeocarpus ganitrus (Rudraksha) is a large, evergreen, big–leavedtree in tropicalandsubtropical regions. Most *Elaeocarpus* species are evergreen trees or shrubs, although a few species can occur as epiphytes or lianes, and some are briefly deciduous (Coode, 2010; Phoon, 2015). *Elaeocarpus* has small (less than 1 cm in diameter) to big (4–7.5 x 3–5 cm), typically blue drupes. However, other species (*E. holopetalus, E. ruminatus*, and *E. grandiflorus*) have brown, black, or red fruits. The *Elaeocarpus* tree is famous for its spiritual or aesthetic values and is known as Rudraksha in India. The International Union for Conservation of Nature and Natural Resources (IUCN) has enlisted 38 species of *Elaeocarpus* under diverse conditions, of which three species were procured to be critically endangered, four species as endangered, twenty as vulnerable, two as near threatened, eight as conservation dependent and one species as data deficient. In India, the majority of them were confined to the northeast (Assam, Arunachal Pradesh, Sikkim, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura) and southern parts of India (Maynard, 2004; Crayn *et al*., 2006; Baba, 2013; Kushwaha and Joshi, 2023).

The ethnobotanical research conducted on *Elaeocarpus* has predominantly concentrated on its biological functioning and medicinal properties. Rudraksha fruit (beads) have been used for thousands of years to cure various conditions, including stress, anxiety, analgesia, asthma, depression, hypertension, and epilepsy. Additionally, this plant has a range of bioactivities, such as antioxidant, anti–inflammatory, antibacterial, and antidiabetic activities (Dixit *et al*., 2018). The phytochemical examination of *Elaeocarpus* species revealed the presence of a variety of desirable bioactive compounds, including indolizilidine alkaloids, triterpenes, tannins such as geraniin, 3,4,5–trimethoxygeraniin, grandisines, rudrakine and flavonoids such as quercetin (Nain *et al*., 2012). The qualitative and quantitative phytochemical examination of the ethanolic fruit extract of the medicinally important plant, *E. ganitrus* revealed the presence of varied bioactive constituents such as alkaloids, glycosides, phenolic compounds, steroids, flavonoids, saponins, carbohydrates, and fixed oils (Hardainiyan *et al*., 2015). Phytochemical investigations of *E. Ganitrus* leaves bark and fruit displayed the presence of active principles such as alkaloids, tannins, and flavonoids. Further, phytochemical screening of *E. ganitrus* seed revealed the presence of phytosterols, fats, alkaloids,

flavonoids, carbohydrates, proteins and tannins, triterpenoids, glycosides, and cardiac glycosides with potent anticonvulsant activity (Sakat *et al*., 2009). Similarly, the fruit and seed parts of *E. Dentatus* supported the presence of fatty acids such as palmitic, oleic, linoleic acid, and hexadecenoic acid (Morice, 2001). Likewise, the methanolic and aqueous extracts of *E. Recurvatus* leaves demonstrated the presence of various phytochemical constituents such as alkaloids, phenolics, flavonoids, and tannins (Lakshmi *et al*., 2016). The fruit pulp of *E. angustifolius*, when investigated for its phytochemical profile, demonstrated the presence of various phytochemicals such as glycosides, flavonoids, saponins, alkaloids, steroids, tannins, and phenolic compounds(Jawlaand Rai, 2016). Phytochemical screening of different extracts of *E. Tuberculatus* leaves and stem revealed the presence of various phytochemical constituents. Similarly, *E. Tuberculatus* leaves and fruit, along with their seeds, recorded the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, phenols, terpenoids, steroids, triterpenoids, coumarin, saponins, quinine, glycosides, gum, starch, and fixed oil (Anusuya *et al*., 2018; Rajeswari *et al*., 2018).

In a phytochemical investigation, *E. floribundus* leaves and fruits divulged the presence of various secondary metabolites such as alkaloids, phytosterol, saponins, tannin, phenol, fixed oil and terpenoids (Deivasigamani and Devi, 2018). Several bioactive components like flavonoids, glycosides, steroids, tannins, and terpenoids with potent antibacterial effects were determined from the aqueous leaves extract of *E. variabilis* (Sharvani *et al*., 2015). Phytochemical components such as cardiac glycosides, anthraquinone glycosides, steroids, terpenoids, quinone and phenols were reported from the fruit parts of *E. Floribundus* (Sircar *et al*., 2017). Chemical investigation of *E. Serratus* leaves revealed the presence of major bioactive constituents such as myricitrin, mearnsetin 3–O–β–D–glucopyranoside, mearnsitrin, and tamarixetin 3–O–α–L–rhamnopyranoside (Jayasinghe *et al*., 2012). Earlier studies suggested that the leaves extract of *E. ganitrus* manifested the presence of significant compounds like quercetin, gallic, and ellagic acids (Singh *et al*., 2013). The ethanolic bark extract of *E. ganitrus* contains several important bioactive metabolites (Talukdar *et al.,* 2017). Correspondingly, the methanolic leaves extract of *E. sphaericus*also displayed the presence of various bioactive principles (Kumar *et al*., 2017). The ethanolic leaves extract of *E. blascoi* confirmed the presence of sixty–three compounds (Vijayan *et al*., 2017). Thirty different phytochemical compounds were determined from the ethanolic leaves extracts of *E. Serrates* revealed the presence of thirty different bioactive principle compounds, each belonging to various functional groups like alcohol, alkane, esters, aldehyde, amide, acids, and ketone (Geetha *et al*., 2013). *E. oblongus*leavesdemonstrates the presence of a flavonoid derivative, mearnesetin, and myricetin, and *E. floribundas* leaves exhibited the presence of vitamin C, myricetin, myricitrin, mearnsetin, and ellagic acid (Ragunathan *et al*., 2014).

Several studies on *Elaeocarpus* species were conducted to isolate, identify, and characterize bioactive compounds by using various techniques such as Gas chromatography–mass spectrometry (GC–MS), Fourier transform infrared (FT–IR), and Ultraviolet–visible spectroscopy (UV–Vis) (Geetha *et al*., 2013; Ragunathan *et al*., 2014; Vijayan *et al*., 2017; Tripathi *et al*., 2021; Singh *et al*., 2022).The seeds and leaves of *Elaeocarpus* species also possess antioxidant, anti–inflammatory, anti asthmatic, and antimicrobial activities. Several studies evaluate the antioxidant properties of *E.ganitrus* and related species of genus *Elaeocarpus*. The ethanolic extract of leaves was analysed and their total antioxidant efficiency and ABTS+ (2, 2–azinobis–(3–ethylbenzothiazoline–6–sulphonate) radical and DPPH (1, 1–diphenyl–2–picryl–hydrazyl) scavenging activities (Singh *et al*., 2013; Talukdar *et al.,* 2017). The leaves have possessed antioxidant activity may be due to the presence of flavonoids and biflavones. Acetone (50%) extract of *E. reticulatus* fruit has potent antioxidant properties. Acetone extract of this fruit contains high levels of total phenolic content (104 mg GAE/g) and flavonoid (155 mg RUE/g). The free radical scavenging efficiency of *E. recurvatus* bark extract was reported (Kumar *et al*., 2007; Lakshmi*et al*., 2016; Vuong *et al*., 2018; Sharma *et al*., 2021).

Elaeocarpus species is known for its extensive therapeutic potential.The phytochemical screening and the evaluation of core bioactive compounds in various fractions isolated from different plant parts (majorly fruits) were determined in several studies. However, phytochemical screening of different fractions isolated from *E. ganitrus* leaves and comparative assessment of all extracts were scarcely studied. Hence, the present study aimed to compare the phytochemical composition and antioxidantpotential of *E. Ganitrus* leaves using spectrophotometers based assays.

Material and Methods

Sample collection and Extract Preparation

Fresh leaves samples of *E. ganitrus* were harvested at the Shobhit Institute of Engineering & Technology (Deemed to be University), Modipuram, Meerut, India, with the coordinates of the sites, Latitude 29.071274º and Longitude 77.711929º. The samples were washed with double distilled water and air dried under shade conditions until all moisture content was gone. The plant samples were ground into a fine powder using liquid nitrogen by mortar and pestle. The final dried sample was stored under vacuum for further experiments. The hydroalcoholic (EGHA) and ethanolic (EGE) extracts were prepared by mixing 20 ml of 70 % ethanol and 100 % ethanol with 2 g of powdered plant samples and incubated for 72 h at 4 °C. The samples were centrifuged at 4000 rpm for 15 minutes. The supernatant was filtered with Whatman No.1 filter paper, transferred into pre–weighed glass containers, and lyophilized for further analysis. Water extract (EGW) was prepared by mixing the fine ground powder with 2 parts of water and incubated at room temperature for 2–3 days. The sample was centrifuged (4000 rpm, 15 min), filtered, and lyophilized.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was performed with all three extracts using the following standard procedures for identifying various phytoconstituents.

Test for Alkaloids

Each extract was mixed with 5 mL of hydrochloric acid (HCL) and filtered. Each filtrate was tested with the following reagents:

Mayer's Test: A few drops of Mayer's reagent were added to 2 mL of the extract in a test tube. The formation of a

green colour or a white precipitate indicates the presence of alkaloids.

Wagner Test: A few drops of Wagner's reagent (a solution of iodine in potassium iodide) were mixed with 2 mL of the extract in a test tube. The formation of a reddish–brown precipitate indicates the presence of alkaloids.

Test for Tannins and Phenolic Compounds

Ferric Chloride Test: The extract was treated with a few drops of ferric chloride solution, and the formation of a bluish–black colour indicates the presence of phenols and tannins.

Gelatin Test: The extract was treated with 1% gelatin solution containing 10% sodium chloride. The formation of a precipitate indicates the presence of tannins and phenolic compounds.

Iodine Test:Small quantities of different extracts were treated with diluted iodine solution separately. The appearance of a transient red colour indicates the presence of tannins and phenolic compounds.

Test for Flavonoids

NaOH and Acid Test: 1 mL of each extract was treated with dilute sodium hydroxide (NaOH) drops. The formation of an intense yellow colour that turns colourless upon mixing with a few drops of dilute HCl indicates the presence of flavonoids.

Lead Acetate Test: Each extract was treated with lead acetate solution, and the formation of a yellow colour precipitate after a few minutes indicates the presence of flavonoids.

Test for Saponins

Foam test: Extract was diluted with distilled water up to 20 ml and was agitated for 10–15 min. Formation of the foam layer indicated the presence of saponins.

UV–VIS Spectrum Analysis

The phytochemicals screening of different plant extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) of *E. ganitrus* were conducted using UV–VIS spectrum analysis. Each extracted fraction was diluted to 1:10 (w/v) using the same solvent. The aliquot of the diluted sample was scanned using a UV–visible spectrophotometer at a wavelength range from200–600 nm (Shimadzu UV–1800, Kyoto, Japan), and the specific peaks of each extract were recorded.

Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Fourier Transform Infrared Spectrophotometer (FT–IR) is a powerful tool for identifying different functional groups present in compounds. The infrared absorption spectrum can be used as a molecular fingerprint to determine a molecule's chemical bonds. The dried extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) of *E. ganitrus* were used for FTIR analysis. A thin pellet was prepared using 10 mg of dried extract powder and 100 mg of Potassium bromide (KBr) pellet. The thin pressed pellet was loaded in an FTIR spectroscope (Agilent), and infrared spectra were recorded between 4000–650 cm^{-1}.

Total Antioxidant Capacity

The phosphomolybdate method evaluated the total antioxidant capacity of three different extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) of *E. ganitrus*. The test samples (0.3 ml) were mixed with 3 ml of the reagent solution (0.6 M Sulfuric acid, 28 mM Sodium phosphate, and 4 mM Ammonium molybdate). The reaction mixture tubes were incubated at 95 ºC for 90 min, and absorbance was recorded using a UV–VIS spectrophotometer at the wavelength of 695 nm (Shimadzu UV–1800, Kyoto, Japan)against the control. The total antioxidant capacity of all extracts under study was expressed as mg Ascorbic acid gram equivalents.

DPPH Radical Scavenging Activity

An antioxidant capacity to scavenge free radicals is measured using the DPPH (2,2–Diphenyl–1–picrylhydrazyl) Radical–Scavenging analysis. Different concentrations of *E. ganitrus* leaves extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) were evaluated for their antioxidant potential. One ml of DPPH solution dissolved in extracts at various concentrations (0, 25, 50, 75, 100, 500, 750, 1000 µg/ml) and incubated for 30 minsin dark condition at room temperature. This method is based on the reduction of DPPH (deep violet colour) in the presence of a hydrogen–donating antioxidant compound, and DPPH turns yellow when neutralized. The absorbance of each sample was recorded at 517 nm using a UV–VIS spectrophotometer (Shimadzu UV–1800, Kyoto, Japan). L–ascorbic acid was used as an antioxidant standard, and the IC 50 value of the sample was calculated based on the absorbance.

The percentage DPPH radical scavenging activity of each extract was calculated using the formula:

$$\%\ \text{DPPH radical scavenging activity} = \frac{(\text{absorbance of the control} - \text{absorbance of the control})}{\text{absorbance of the control}} \times 100$$

Nitric Oxide (NO) Radical Scavenging Assay

The nitric oxide scavenging activity of the *E. ganitrus* leaves extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) was measured at different concentrations (50, 100, 200, 400, 800, 1000 µg/mL). Sodium nitroprusside 100 µl (10 mM) was prepared in saline phosphate buffer and was added to 100 µl of each extract. Then 1 mL of Griess reagent (prepared by mixing an equal amount of 1% sulphanilamide in 2.5 % phosphoric acid and 0.1% naphthyl ethylene diaminedihydrochloride (NED) in water) was added to reaction mixtures, and incubated for 3 h. The absorbance of solutions was recorded at 540 nm wavelength. The percentage nitrite radical scavenging activity of all the extracts under study wasmeasured using the following formula:

$$\text{Nitric oxide radical scavenging} = \frac{(\text{absorbance of the control sample} - \text{absorbance of samples or standards})}{\text{absorbance of the control sample}} \times 100$$

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± SEM. A one–way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t–test was used to calculate statistical

differences. Statistical significance was considered at a 5% or 1% level of significance (*p< 0.05 or **p<0.01).

Results and Discussion

Preliminary Phytochemical Screening of *E. ganitrus* leaves extracts

Freshly prepared extracts were subjected to preliminary phytochemical screening to detect the presence of alkaloids, tannins, phenolics, flavonoids, and saponins. The phytochemical investigation revealed that the extracts are enriched with vital phytochemicals, including alkaloids, phenolic, tannins, flavonoid and saponins. Hydroalcoholic and ethanolic extracts demonstrated similar phytochemical profiles. The negative result was reported in the iodine test for tannins and phenolic compounds for all extracts under study (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW). Water extract showed a negative result in the Mayer test, FeCl3 test, iodine test, lead acetate test, and foam test for alkaloids, phenolic, flavonoids, and saponins, respectively (Table 1).

Table 1: Preliminary screening of phytochemical constituents in various extracts prepared from *E. ganitrus* leaves samples

Phytochemical test		Hydroalcoholic (EGHA)	Ethanolic (EGE)	Water (EGW)
Alkaloids	Mayer test	+	+	–
	Wagner test	+	+	+
Tannins and Phenolic	$FeCl_3$ test	+	+	+
	Iodine test	–	–	–
	Gelatin test	+	+	–
Flavonoids	NaOH and acid test	+	+	+
	Lead acetate test	+	+	–
Saponins	Foam test	–	–	–
+ indicates the presence, and – indicates the absence of phytochemicals in respective extracts.				

Characterization of *E. ganitrus* leaves extracts using UV–VIS Spectrophotometer

Phytochemicals exhibit a broad spectrum of pharmacological properties, including antioxidant, and anti–microbial. All three extracts of *E. ganitrus* (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW)were scanned using a UV–VIS spectrophotometer in the wavelength range from 200 – 600 nm, and the characteristic peaks were documented (Figure 1). Two distinct peaks at 212.6 nm and 275 nm were observed in all leaves extracts of *E. ganitrus* spectrum at same concentration. EGHA spectra profile showed the peaks at 212.6 nm and 275 nm in the absorption range of 0.9–1.0. Similarly, two peaks at 212.6 nm and 275 nm were observed in the absorption range of 0.4–0.5 with EGE and 0.3–0.4 with EGW. This result indicates that EGHA produced superior and distinct peaks at the same concentration as EGE and EGW.

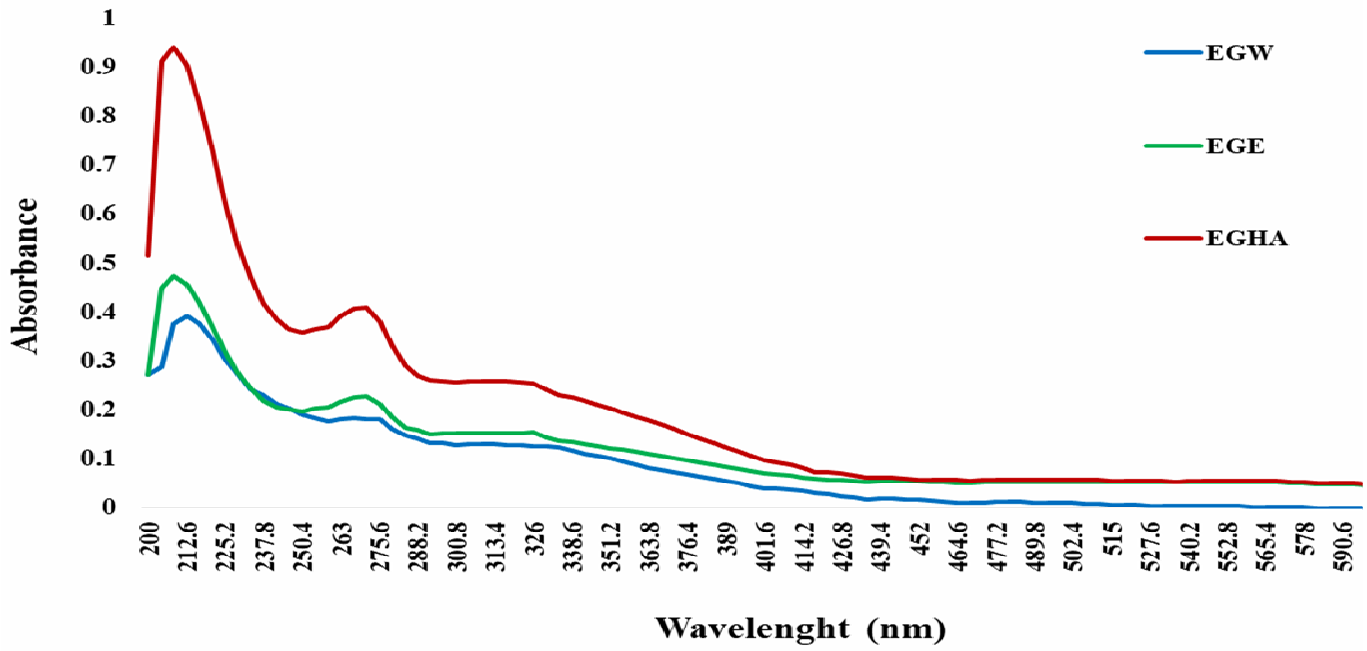

Fig. 1 : The UV–VIS absorptionspectra of *E. ganitrus*leaves extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW)

Characterization of *E. ganitrus* leaves extracts using FT–IR Analysis

The functional group of active components in the hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) extracts is based on band values identified in the FT–IR spectrum (Table 1).The IR spectra of EGHA showed the presence of a band at 950 cm^{-1}, indicating the presence of an alkene (C=C) functional group in the band range of 1000–

650 cm^{-1}. Two potential bands were observed in the 1400–1000 cm^{-1} range at 1180 cm^{-1} and 1290 cm^{-1}, representing Amine and fluoro compound (C–N/F) and alkyl aryl ether (C–O). All three bands identified in the wavenumber range of 1600–1300 cm^{-1},including bands at 1420 cm^{-1} and 1450 cm^{-1}, indicate an alkane (C–H) functional group, and the band at 1500 cm^{-1} showed the presence of alkene (C=C) functional group. One band identified at 1850 cm^{-1} representsthe ester, δ–lactone, and cyclopentanone (C=O) functional group. Three potential bands were detected in the wave number range of 4000–2500 cm^{-1} at 2830 cm^{-1} and 2960 cm^{-1}, indicating the presence of alkane, amine salt and alcohol, and carboxylic acid (C–H, N–H, and O–H), and one band at 3710 cm^{-1} indicating the presence of alcohol (O–H) functional groups (Figure 2).

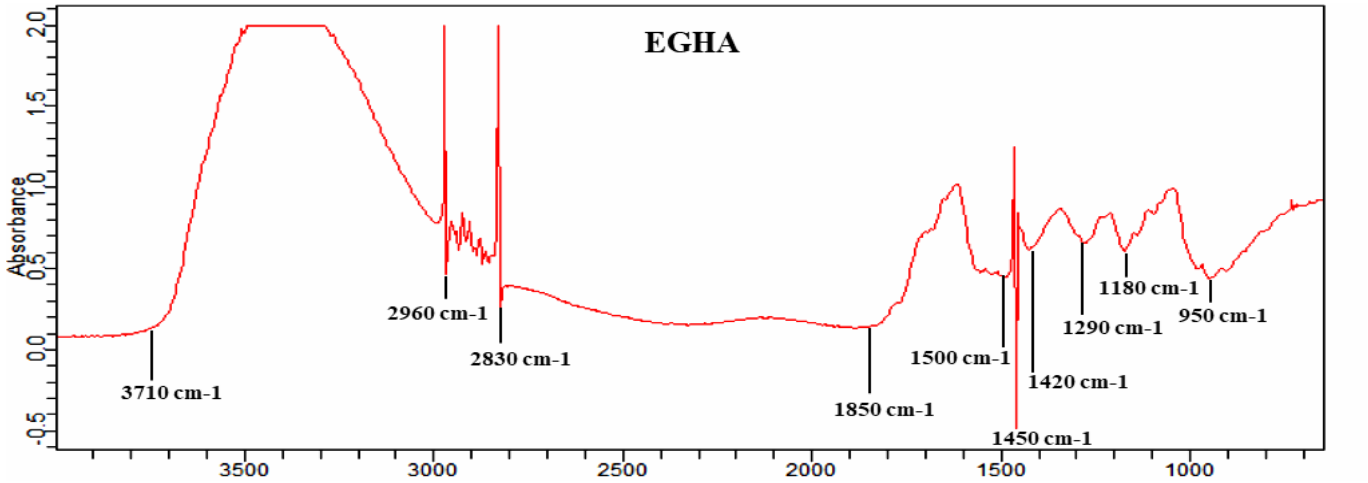

Fig. 2 : FT–IR spectrum analysis of hydroalcoholic extract (EGHA) of *E. ganitrus* and its probable functional groups with wave numbers

Table 1 : Functional groups and possible compounds identified in the *E. ganitrus* leaves extracts using FT–IR Spectroscopy

Band range (cm^{-1})	**Band position** (cm^{-1})	**Band assignments**	**Possible compounds**
1000–650 cm^{-1}	950	C=C	alkene
1400–1000 cm^{-1}	1140, 1180	C–N/F	Amine and fluoro compound
	1260, 1290	C–O	alkyl aryl ether
1600–1300 cm^{-1}	1420, 1450	C–H	alkane
	1500	C=C	alkene
2000–1650 cm^{-1}	1820, 1850	C=O	ester, δ–lactone, and cyclopentanone
4000–2500 cm^{-1}	2830, 2960	C–H, N–H, and O–H	alkane, amine salt and alcohol, and carboxylic acid
	3710	O–H	alcohol

The IR spectra of EGE indicated the presence of a band at 950 cm^{-1}, signifying the presence of an alkene (C=C) functional group in the wavenumber range of 1000–650 cm^{-1}. Two significant bands were observed in the 1400–1000 cm^{-1} range at 1140 cm^{-1} and 1290 cm^{-1}, indicating the presence of Amine and fluoro compound (C–N/F) and alkyl aryl ether (C–O) functional groups. Three potential bands identified in the wavenumber range of 1600–1300 cm^{-1}, including bands at 1420 cm^{-1} and 1450 cm^{-1}, indicate an alkane (C–H) functional group, and the band at 1500 cm^{-1} showed the presence of alkene (C=C) functional group. One band was identified at 1850 cm–1, indicating the presence of ester, δ–lactone, and cyclopentanone (C=O) functional group. Three bands were identified in the wavenumber range of 4000–2500 cm^{-1}at 2830 cm^{-1} and 2960 cm^{-1}, demonstrating the presence of alkane, amine salt and alcohol, and carboxylic acid (C–H, N–H, and O–H), and one band detected at 3710 cm^{-1}indicating the presence of alcohol (O–H) functional groups (Figure 3)

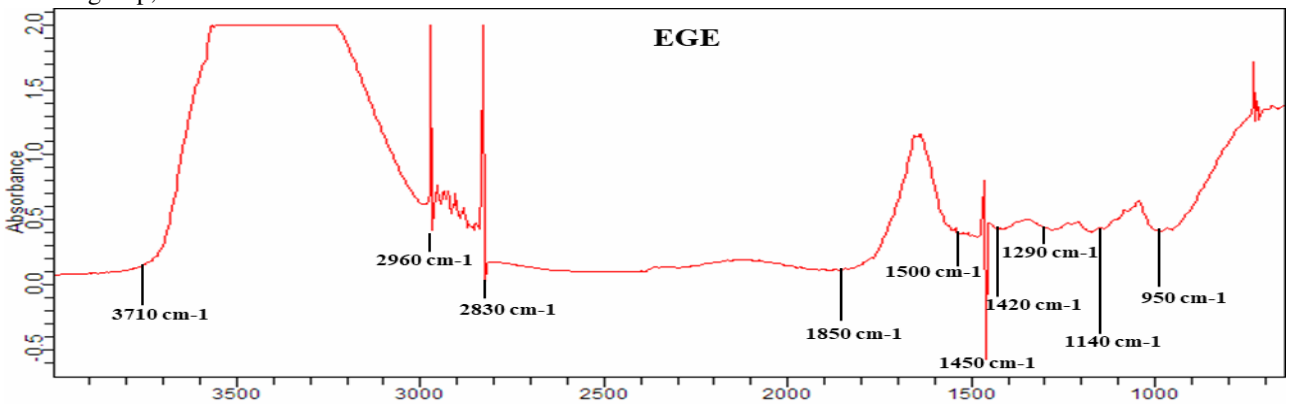

Fig. 3: FT–IR spectrum analysis of ethanolic extract (EGE) of *E. ganitrus* and its probable functional groups with wave numbers

The IR spectra of EGW showed a band at 950 cm^{-1}, indicating the presence of an alkene (C=C) functional group in the wavenumber range of 1000–650 cm^{-1}. Two potential bands were observed in the 1400–1000 cm^{-1} range at 1140 cm^{-1} and 1260 cm^{-1}, suggesting the presence of Amine and fluoro compound (C–N/F) and alkyl aryl ether (C–O) functional groups. Three bands were detected in the band range of 1600–1300 cm^{-1}, including bands at 1420 cm^{-1} and 1450 cm^{-1}, indicate an alkane (C–H) functional group, and the band at 1500 cm^{-1} showed the presence of alkene (C=C) functional group. One band identified at 1820 cm^{-1} indicates the ester, δ–lactone, and cyclopentanone (C=O) functional group. All three potential bands were detected in the wave number range of 4000–2500 cm^{-1} at 2830 cm^{-1} and 2960 cm^{-1}, indicating the presence of alkane, amine salt and alcohol, and carboxylic acid (C–H, N–H, and O–H), and one band at 3710 cm^{-1} indicating the presence of alcohol (O–H) functional groups (Figure4).

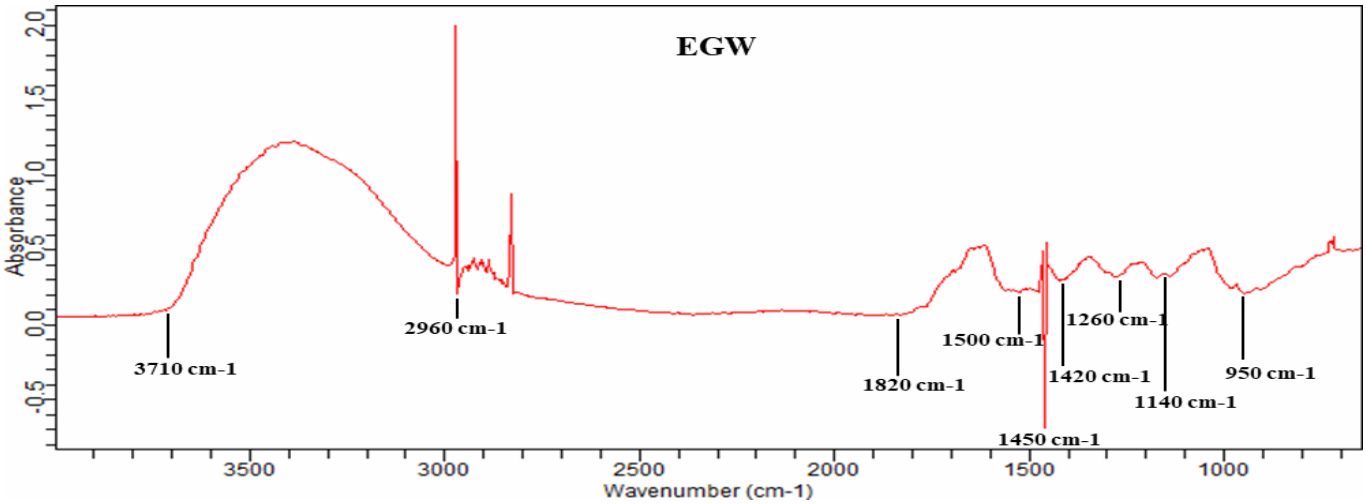

Fig. 4: FT–IR spectrum analysis of water extract (EGW) of *E. ganitrus* and its probable functional groups with wave numbers

Among all the extracts tested, significant bands were identified in the hydroalcoholic, ethanolic, and water extracts of *E. ganitrus* leaves, indicating the presence of C=C, C–N/F, C–O, C–H, C=C, C=O, N–H, and O–H functional groups. The identified functional groups play a vital role in plants' antioxidant and anti–inflammatory activities.

Total Antioxidant Capacity

The total antioxidant capacity estimates both water–soluble and fat–soluble antioxidants and involves the reduction of phosphomolybdate by the extracts following the formation of the phosphomolybdenum complex (Singh *et al.*, 2022). In the present study, the antioxidant potential of three different plant extracts (Hydroalcoholic (EGHA), Ethanolic (EGE), and Water (EGW) prepared from *E. ganitrus* was estimated and expressed as microgram equivalents of Ascorbic acid per gram of extract (µgAAE/g). The highest antioxidant capacity was observed in EGHA (218.26 ± 1.07µgAAE/g),followed by EGE (209.04 ± 1.18 µgAAE/g) and EGW (199.21 ± 1.01 µgAAE/g).

Antioxidant Analysis using DPPH radical scavenging assay

The DPPH radical has been used widely to assess the antioxidant potential of plant extracts. In the present study, the antioxidant potential of all three extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) of *E. ganitrus* leaves was evaluated at varied concentrations ranging from 0–1000µg/mL.The DPPH activity of EGHA, EGE, and EGW revealed a dose–dependent response at all tested concentrations with IC_{50} values of 440± 0.45 µg /mL, 600± 0.52 µg /mL, and 680 ± 0.44 µg /mL, respectively. The percent of DPPH radical scavenging activity at the highest tested concentration (1000 µg/mL) was recorded at 85% in EGHA, 77 % in EGE, and 62% in EGW. The antioxidant capacity of the extract was compared with ascorbic acid (IC50 value: 95.12± 0.43 µg /mL) as the standard antioxidant (Figure 5). The highest antioxidant activities of EGHA as compared to EGE and EGW might be due to the presence of high phenolic content of the plant.

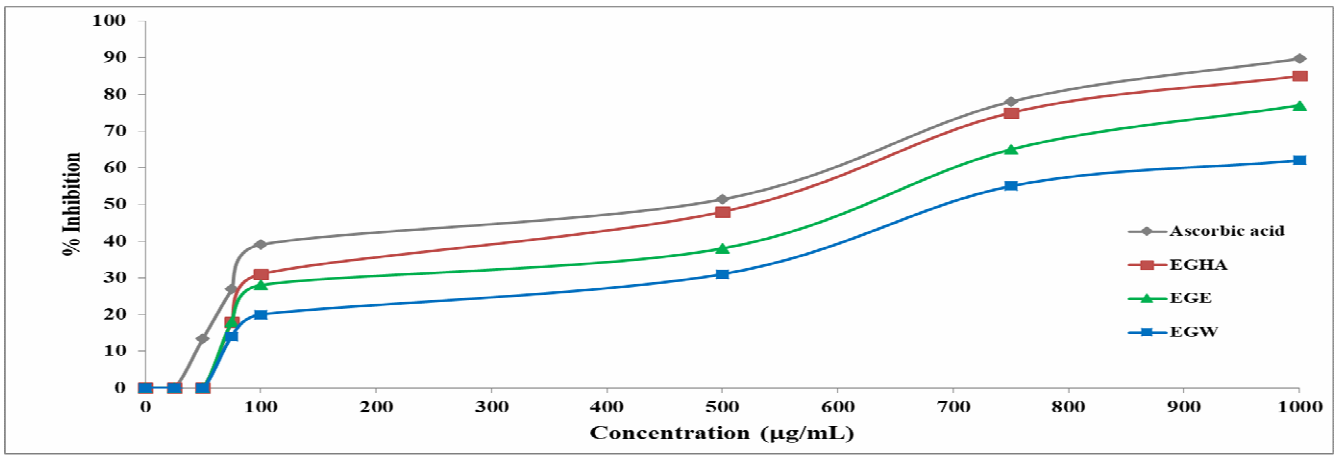

Fig. 5 : The DPPH scavenging activity of (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) isolated from *E. ganitrus* leaves. All experiments were performed in triplicate for all tested concentrations

Nitric Oxide (NO) Radical Scavenging Assay

Nitric radical scavenging assay was conducted using all three extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) of *E. ganitrus* leaves at different concentrations (50, 100, 200, 400, 800, and 1000 µg/mL). The dose–dependent activity of all three extracts was noted with an increase in concentration and the maximum free radical scavenging activity was observed in EGHA, with 89 % inhibition at 1000 µg/mL followed by 79 % inhibition at 800 µg/mL, 61 % inhibition at 400 µg/mL, 45 % inhibition at 200 µg/mL, 28 % inhibition at 100 µg/mL, and 19 % inhibition at 50 µg/mL. A significant difference in NO scavenging activity in EGHA was found at 200 µg/mL, 400 µg/mL, 800 µg/mL, and 1000 µg/mL. The maximum NO scavenging activity was noted in EGE at the highest tested concentrations (78 % inhibition), followed by 61 % inhibition at 800 µg/mL, 42 % inhibition at 400 µg/mL, 35 % inhibition at 200 µg/mL, 22 % inhibition at 100 µg/mL, and 13 % inhibition at 50 µg/mL. A significant difference in NO scavenging activity was noted at 400 µg/mL, 800 µg/mL, and 1000 µg/mL. Similarly, the dose–dependent response was observed in EGW with 59 % inhibition at the highest tested concentration (1000 µg/mL), followed by 44 % inhibition at 800 µg/mL, 37 % inhibition at 400 µg/mL, 33 % inhibition at 200 µg/mL, 17 % inhibition at 100 µg/mL, and 8 % inhibition at 50 µg/mL. A significant difference in NO scavenging activity was observed at 800 µg/mL and 1000 µg/mL. The percentage of free radical NO scavenging activity was plotted against the concentration of the extracts (Figure 6).

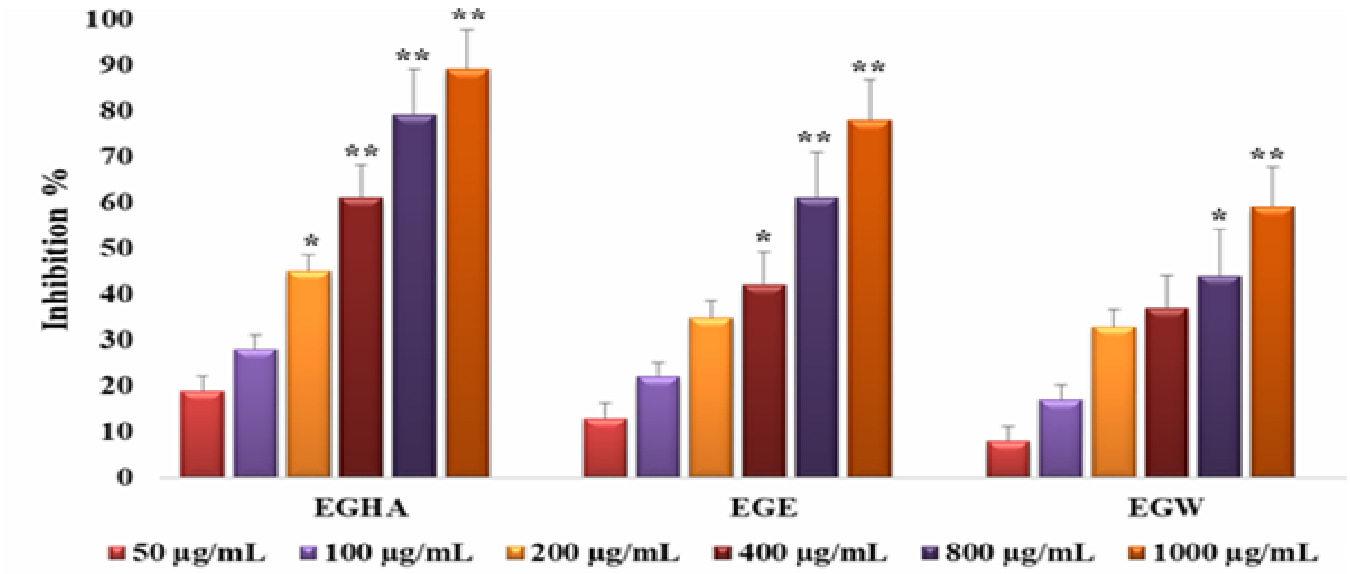

Fig. 6 : The Nitric Oxide (NO) Radical Scavenging activity of three extracts (Hydroalcoholic (EGHA), Ethanolic (EGE), and Water (EGW) isolated from *E. ganitrus* leaves. All experiments were performed in triplicate for all tested concentrations varied from 50 µg/mL to 1000 µg/mL

Conclusion

Medicinal plants are abundant with bioactive phytochemicals with significant pharmacological properties and can be used as a therapeutic alternative. This study reported the phytochemical screening and antioxidant activities of three different extracts (hydroalcoholic (EGHA), ethanolic (EGE), and water (EGW) of *E. ganitrus* leaves. The preliminary phytochemical screening indicated the presence of crucial phytochemicals, including alkaloids, phenolics, tannins, flavonoid, and saponins in all extracts. UV-Vis and FT–IR analyses of the plant extracts also indicated the presence of functional groups, suggesting an antioxidant nature of *E. ganitrus*. The present study determined the comparative antioxidant potential of *E. ganitrus* leaves extracts usingDPPH Radical–Scavenging and Nitric Oxide Radical Scavenging Assay, and the highest antioxidant activities might be due to the presence of significant amount of phytochemicals. Further study is required to identify, isolate, and evaluate the bioactive components of these plant extracts to elucidate the mechanism of action and possible key compounds for developing novel antioxidant agents.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding Statement

This research received no specific grant from any funding agency.

Acknowledgment

The authors acknowledge all the faculty members of the Department of Biotechnology, and Department of Agriculture Technology & Agri–Informatics, Shobhit Institute of Engineering & Technology, (Deemed to be University), Meerut, 250110, India for their support and motivation.

References

Anusuya, D.R., Arumugam, S., Thenmozhi, K. and Veena, B. (2018). Phytochemical and in vitro antioxidant of an endemic medicinal plant species, *Elaeocarpus munronii* (wt.) mast. and *Elaeocarpus tuberculatus* Roxb. (Elaeocarpaceae). *Journal of Pharmacognosy and Phytochemistry,* 7(6): 159–164.

Baba, Y. (2013). Evolution, systematics and taxonomy of *Elaeocarpus* (Elaeocarpaceae) in Australasia. Ph.D. thesis, James Cook University, Queensland.

Coode, M.J.E. (2010). *Elaeocarpus* for Flora Malesiana: new taxa and understanding in the Ganitrus group. *Kew Bulletin*, 65(3): 355–399.

Crayn, D.M., Rossetto, M. and Maynard, D.J. (2006). Molecular phylogeny and dating reveals an Oligo–Miocene radiation of dry–adapted shrubs (former Tremandraceae) from rainforest tree progenitors (Elaeocarpaceae) in Australia. *American Journal of Botany,* 93(9): 1328–1342.

Deivasigamani, R. and Devi, M.S. (2018). Phytochemical analysis of *Elaeocarpus floribundus* Blume. *World Journal of Pharmacy and Pharmaceutical Sciences,* 7(10): 1452–1457.

Dixit, P.K., Dixit, S., Bhardwaj, M., Chauhan, B., Nagarajan, K. And Sahoo, J. (2018). A review on traditional and ethnomedicinal uses of *Elaeocarpus ganitrus* (Rudraksha). *International Journal of Pharmaceutical Sciences Review and Research*, 53(2): 1–7.

Geetha, D.H., Rajeswari, M. and Jayashree, I. (2013). Chemical profiling of *Elaeocarpus serratus* L. by GC–MS. *Asian Pacific Journal of Tropical Biomedicine,* 3(12): 985–987.

Hardainiyan, S., Nandy, B.C. and Kumar, K. (2015). *Elaeocarpus ganitrus* (Rudraksha): a reservoir plant with their pharmacological effects. *International Journal of Pharmaceutical Sciences and Research,* 34(1): 55–64.

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

Jayasinghe, L., Amarasinghe, N.R., Arundathie, B.G., Rupasinghe, G.K., Jayatilake, N.H. and Fujimoto, Y. (2012). Antioxidant flavonol glycosides from *Elaeocarpus serratus* and *Filicium decipiens*. *Natural Product Research*, 26(8): 717–721.

Kumar, D., Sanghi, A., Chandra, R., Arora, R. and Kumar, A. (2017). Membrane Stabilizing and antioxidant activities of extracts from leaves of *Elaeocarpus sphaericus. International Journal of Chem. Tech Research*, 10(6): 668–673.

Kumar, S.T., Shanmugam, S., Palvannan, T. and Bharathi, V.M.K. (2007). Evaluation of antioxidant properties of *Elaeocarpus ganitrus*, *Iranian Journal of Pharmaceutical Research*, 7(3): 211–215.

Kushwaha, J. and Joshi, A. (2023). In silico characterization and phylogenetic analysis of *Elaeocarpus ganitrus* based on ITS2 barcode sequence. *International Journal of Biotech Trends and Technology*, 13: 26–37.

Lakshmi, M.A., Srinivas, K., Rani, S.U. and 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): 395–399.

Maynard, D. (2004). A molecular phylogeny for Australian *Elaeocarpus* (Elaeocarpaceae) and the affinities of a putative new taxon.Honours thesis. University of New South Wales, New South Wales.

Morice, I.M. (2001). Fruit–coat and seed fats of Rhopalostylis, *Elaeocarpus* and *Nestegis* species. *Phytochemistry*, 14(3): 765–767.

Nain, J., Garg, K. And Dhahiya, S. (2012). Analgesic and anti–inflammatory activity of *Elaeocarpus sphaericus* leaf extract. *International Journal of Pharmacy Pharmaceutical Science,* 4(1): 379–381.

Phoon, S.N. (2015). Systematics and biogeography of *Elaeocarpus* (Elaeocarpaceae)", Ph.D. thesis, James Cook University.

Ragunathan, M. and Senthamarai, R.M. (2014). Pharmacognostical studies on the fruit of *Elaeocarpus oblongus* Gaertn. *Pharmacognacy Journal*, 6(3): 72–78.

Rajeswari, M. (2018). GC–MS analysis of bioactive compounds in the plant of methanolic leaf extract of *Elaeocarpus tuberculatus* roxb. *International Journal of Scientific Research,* 8(1): 13–18.

Sakat, S.S., Wankhede, S.S., Juvekar, A.R., Mali, V.R. and Bodhankar, S.L. (2009). Antihypertensive effect of aqueous extract of *Elaeocarpus ganitrus* Roxb. fruits in renal artery occluded hypertensive rats. *International Journal of Pharm Tech Research,* 1(3): 779–782.

Sharma, S., Rai, D.V. and Rastogi, M. (2021). Compositional characteristics of rudraksha (*Elaeocarpus ganitrus* Roxb. *Plant Archives*, 21(1): 627–63.

Sharvani, K.A., Jagadeesh, D., Chandrakanth, R., Sumana, K., Seema, M. and Devaki, N.S. (2015). Antimicrobial assay of *Elaeocarpus* species of Western Ghats of Karnataka. *Asian J Pharm Anal Med Chem,* 3(1): 14–19.

Singh, B., Ishar, M.P.S. and Sharma, A. (2013). Estimation of quercetin, an anxiolytic constituent, in *Elaeocarpus ganitrus*. *J. Pharmacogn Phytochem,* 1(6): 117–121.

Singh, P.K., Singh, J., Medhi, T. and Kumar, A. (2022). Phytochemical Screening, Quantification, FT–IR Analysis, and In Silico Characterization of Potential Bio–active Compounds Identified in HR–LC/MS Analysis of the Polyherbal Formulation from Northeast India. *ACS omega*,7(37): 33067–33078.

Sircar, B., Mandal, M., Mondal, M.A. and Mandal, S. (2017). Biosynthesis of *Elaeocarpus floribundus* mediated silver nanoparticles with broad antibacterial spectrum. *Acta Scientific Pharmaceutical Sciences*, 1(2): 59–67.

Talukdar, N., Dutta, A.M., Chakraborty, R. And Das, K. (2017). Screening of phytochemicals, antioxidant and inhibitory effect on alpha–amylase by ethanolic extract of *Elaeocarpus ganitrus* (bark), *International Journal of Pharmaceutical Sciences and Research*, 8(12): 5270–5275.

Tripathy, S., Mishra, A. and Mishra, A.K. (2021). UV–VIS spectroscopy and GC–MS analysis of *Elaeocarpus ganitrus* (Rudraksha) seeds. *International Journal of Botany Studies,* 6(2): 417–420.

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, Q.V., Ngoc Thuy Pham, H., Vu,H.T., Dang, T.T., Van Ngo, T. and Chalmers, A.C. (2018). Fruit characteristics, phytochemical and antioxidant properties of blueberry ash (*Elaeocarpus reticulatus*). *Heliyon*, 4(10): e00834.