Phytochemical Characterization of Bio-active Compounds in Hydroethanolic Extract of *Elaeocarpus ganitrus* leaves using HPLC, LC-MS, and HPTLC Analyses

Jyotsana Khushwaha[1,] Alpana Joshi[2,3*], Shiva Sharma[4], Subrata K. Das[4]

[1]Department of Biotechnology, Shobhit Institute of Engineering & Technology, (Deemed-to-be University), Meerut, 250110, India

[2]Department of Agriculture Technology & Agri-Informatics, Shobhit Institute of Engineering & Technology, (Deemed-to-be University), Meerut, 250110, India

[3]Department of Bioenvironmental Chemistry, College of Agriculture & Life Sciences, Jeonbuk National University, Jeonju, Republic of Korea

[4]Department of Biomedical Engineering., Shobhit Institute of Engineering & Technology, (Deemed-to-be University), Meerut, 250110, India

***Corresponding Email:-**alpana.joshi@shobhituniversity.ac.in; joshi.alpana@gmail.com

Abstract

Bioactive compounds have various applications in different industries, including food, pharmaceutical, and cosmetic industries, demonstrating the need to identify the best-standardized technique to screen the phytochemical profile of medicinal plants. This study aimed to characterize the bioactive compounds in the hydroethanolic extracts of *Elaeocarpus ganitrus* leaves using various analytical techniques: HPLC, LC-MS, and HPTLC. Air-dried leaves of *E. ganitrus* were extracted with 70% ethanol. The phytochemical composition of crude extracts was analyzed by the High performance liquid chromatography (HPLC) method, and a total of 93 compounds, including 46 flavonoids, 17 phenols, 14 polyphenols, 3 phenolic acid, 3 phenolic glycosides, 2 flavonoid glycosides, 2 glycosides, 2 phenylpropanoid glycoside, 1 hydroxycinnamic acid, 1 lignan, 1 tannin, and 1 terpene glycoside were detected and quantified. The Liquid chromatography mass spectrometry (LC-MS) analyses identified 11 major eleven compounds: quercetin (803.0215 µg/L), gallic acid (726.13 µg/L), ferulic acid (652.34 µg/L), chlorogenic acid (651.021µg/L), pinocembrin (264.11 µg/L), p-aminobenzoic acid (251.021 µg/L), epicatechin (246.02 µg/L), catechin (161.51 µg/L), caffeic acid (123.31 µg/L), syringaldehyde (116.31 µg/L), and naringenin (106.31 µg/L). The chemical fingerprinting was carried out by high performance thin layer chromatography (HPTLC), and HPTLC fingerprint qualitatively revealed a predominant amount of gallic acid (48.64 %), curcumin (15.21 %), caffeic acid (12.19 %) and cinnamic acid (6.50 %). A significant amount of bioactive constituents in a hydroethanolic extract of *E. ganitrus* leaves indicates the plant's therapeutic potential, including antioxidant, anti-inflammatory, antidiabetic, anticancer, neuroprotective, and cardio-protective activities.

Keywords: Bioactive compound, HPTLC, LC-MS, HPLC, *Elaeocarpus* ganitrus, Hydroethanolic extract.

1. Introduction

Medicinal plants constitute the basis of traditional and modern primary healthcare. Over 80% of the population, mainly of developing countries, depend on traditional and herbal medicine. In the past two decades, there has been substantial growth in the use of medicinal plants to pr event disease and promote health. The current pharmacopeia contains at least 25% plant-based medications; however, the medicinal plants must meet quality, safety, and efficacy standards for proper utilization. One of the most significant difficulties related to quality is that commercial medicinal plants are available in powdered form, making it challenging to identify specific plant parts or plant species (**Salmerón-Manzano *et al*., 2020**).

Elaeocarpus ganitrus (Rudraksha) belongs to the family Elaeocarpaceae and has been well-known from ancient times for its medicinal importance (**Kumari *et al*., 2018; Rai *et al*., 2018; Sharma *et al*., 2022; Joshi and Kushwaha, 2023**). These fruits are commonly found in India, specifically in the Himalayan and Gangetic plain regions, Nepal, Indonesia, and Java. The pharmacological action of *Elaeocarpus sp.* is due to the presence of bioactive phytochemicals and numerous studies revealed that petroleum ether, ethanol, and water extracts of *Elaeocarpus sp.* contain several alkaloids (elaeocarpidine, elaeocarpine, rudrakine), polyphenols (flavonoids, quercetin, tannin), phytosterols, fat, carbohydrates, proteins, gallic and ellagic acid (**Johns and Lamberton, 1973; Katavic *et al*., 2007; Sudrajat and Timotius, 2022**). The major identified biochemical compounds are isoelaeocarpine, epiisoelaeocarpiline, epielaeocarpiline, alloelaeocarpiline, and pseudo-epiiso-elaeocarpilline (**Johns *et al*., 1970; Katavic *et al*., 2006; Katavic *et al*., 2007; Ezeoke *et al*., 2018; Sudrajat and Timotius, 2022**).

E. ganitrus beads (Rudraksha) are known for their therapeutic potential against several disorders like stress, anxiety, insomnia, skin diseases, leprosy, hysteria, hyperglycemia, coma, leucorrhoea, infertility, asthma, hypertension, diabetes, arthritis, rheumatism, cardiovascular and liver diseases (**Rai *et al*., 2018; Sharma *et al*., 2022**). There are evidences in literature indicating their sedative, analgesic, anticonvulsant, anti-inflammatory, antioxidant, antiepileptic, hypnotic, antipyretic, antihypertensive, antidiabetic, antimicrobial, anxiolytic, anti-cancerous, anti-asthmatic, nephroprotective, immune-stimulator, and electromagnetic properties (**Ray *et al*., 1979; Fang *et al*., 1984; Ito *et al*., 2002; Katavic *et al*., 2006; Katavic *et al*., 2007; Meng *et al*., 2008; Shitamoto *et al*., 2010; Pan *et al*., 2012; Bordoloi *et al*., 2017; Liyanaarachchi *et al*., 2018; Kim *et al*., 2018; Ezeoke *et al*., 2018; Hong *et al*., 2019; Ogundele and Das, 2019; Turner *et al*., 2020; Ogundele *et al*., 2021; Kim *et al*., 2021; Banerjee *et al*., 2022; Joo *et al*., 2022**). A summary of phytochemical investigations on *Elaeocarpus* species is listed in **Table 1**.

The plant contains abundant bioactive compounds in different concentrations and polarity. A key challenge in screening plant phytochemical profiles is extraction and characterization methods. The combination of different analytical techniques, such as High-performance liquid chromatography (HPLC), Liquid chromatography-mass spectrometry (LC-MS), and high-performance thin-layer chromatography (HPTLC), can be applied to detect bioactive constituents in plant extracts. These analytical techniques are effective for ensuring the quality of raw plant material and can be used to analyze various plant extracts **(Nile and Park, 2014)**. The phytochemical profile of the ethanolic fraction of *E. floribundus* fruits displayed various biological activities, including antimicrobial **(Sircar *et al*., 2017)**. HPLC and GC-MS analyses was conducted out to examine the bioactive constituents present in the fruits of *E. oblongus, E. serratus,* and *E. tectorius* **(Muthuswamya and Senthamarai, 2014; Mundaragi *et al*., 2019; de Lima *et al*., 2019)**. LC-MS combines the separation abilities of liquid chromatography against a target compound. LC-MS profile of *E. grandiflorus* and *E. sphaericus* demonstrated the bioactive compounds significantly **(Primiani *et al*., 2021; Habibah *et al*., 2021)**.

However, the phytochemical profiling of *E. ganitrus* using HPLC, LC-MS, and HPTLC has not been reported. Hence, the present investigation aimed to conduct a qualitative and quantitative assessment of phytochemical constituents in the hydroethanolic extract of *E. ganitrus* leaves using three different analytical techniques: High-performance liquid chromatography (HPLC), Liquid chromatography-mass spectrometry (LC-MS), and high-performance thin layer chromatography (HPTLC).

Table 1. Literature survey on phytochemical profile for *Elaeocarpus* species

Name of the species	Phytochemicals	References
E. angustifolius	(±)−8,9-Dehydroelaeocarpine, (±)-Elaeocarpine trifluoroacetate, (±)− 9-Epielaeocarpine cis-N-oxide trifluoroacetate	Hong *et al.*, 2019
E. chinensis	Cucurbitacins D, Elaeocarpucins A-H	Pan *et al.*, 2012
E. dolichostylus	Cucurbitacin F	Fang *et al.*, 1984
E. flooribundus	Gallic acid, myricitrin, mearnsitrin, myricetin, and mearnsetin, Phytol, a-tocopherolquinone, Euphorbol, Phaeophytins	Ogundele and Das, 2019; Ogundele *et al.*, 2021, Banerjee *et al.*, 2022
E. fucoides	Elaeocarpine, Elaeocarpenine, Isoelaeocarpine,	Katavic *et al.*, 2007
E. ganitrus	Rudrakine	Ray *et al.*, 1979
E. grandis	(-)-Isoelaeocarpiline, Grandisine C, D, E, G,	Katavic *et al.*, 2006
E. hainanensis	Cucurbitacins D	Meng *et al.*, 2008
E. japonicus	Elaeocarpionoside	Shitamoto *et al.*, 2010
E. lanceofolius	Myricetin, 4′ -Methylmyricetin, Mearnsetin, Triacontanoic acid, Octatriacontan-1-ol, Dotriacontane	Ray *et al.*, 1979; Bordoloi *et al.*, 2017
E. mastersii	Cucurbitacins D, Cucurbitacin F, 4′-O-Methylellagic acid 3-(2″,3″-di-O-acetyl)-α-l-rhamnoside, 4,4′-O-Dimethylellagic acid 3-(2″,3″-di-O-acetyl)-α-l-rhamnoside	Ito *et al.*, 2002
E. reticulatus	Cucurbitacin-I, Proanthocyanidins anthocyanins,	Turner *et al.*, 2020
E. serratus	Dibutyl succinate, Phytosterol, Elastase, Hyaluronidase, Tyrosinase	Liyanaarachchi *et al.*, 2018

E. sylvestris	Geraniin, 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucose (PGG), elaeocarpusin,	Kim *et al.*, 2018; Kim *et al.*, 2021; Joo *et al.*, 2022
E. tectorius	Tectoricine, Tectoraline, Tectoramidines A, B	Ezeoke *et al.*, 2018

2. Material and Methods

2.1. Sample collection and hydroethanolic 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. Leaf samples were rinsed with double distilled water and air dried under shade conditions until all moisture content was gone. The plant samples (2 g) were ground into a fine powder using liquid nitrogen by mortar and pestle. The hydroethanolic extract was prepared by adding 70 % ethanol (10 ml) and incubated for 1 week at room temperature (**Figure 1**). Following centrifugation and filtration, extracts were lyophilized and stored at -80 ˚C.

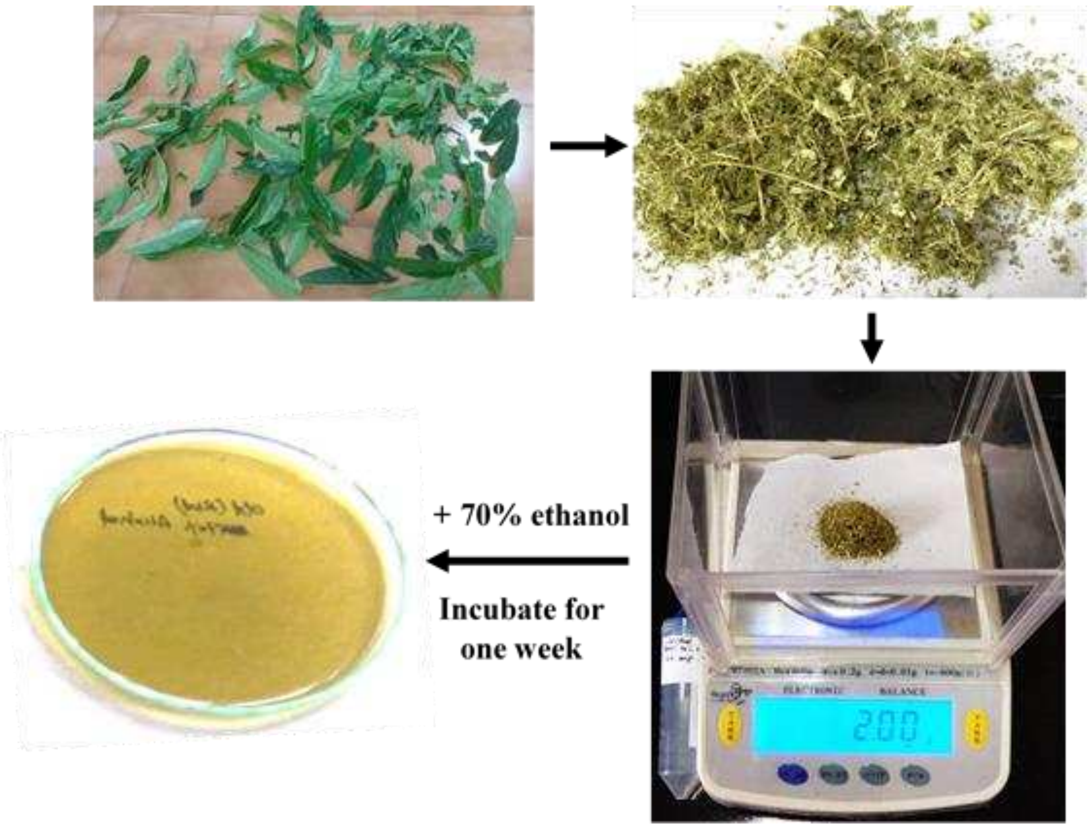


Figure 1. The process of hydroethanolic extract preparation from *E. ganitrus* leaves.

2.2. High-Performance Liquid Chromatography (HPLC) analysis

Reagents of analytical grade Toluene, Ethyl acetate, Formic acid, Gallic acid, Catechin, Caffeic acid, Berberine, Rutin, Cinnamic acid, and Curcumin were obtained (Sigma-Aldrich, India). Precoated TLC Aluminum sheets silica gel 60F254 (10 x 10 cm, 0.2 mm thick) were obtained from E. Merck Ltd, Mumbai. The extract was diluted in 50 % methanol (1 mg/ml) and subjected to HPLC analysis. Waters binary HPLC system (Waters Corporation, Milford, MA, USA), equipped with column oven, auto-sampler (Waters 2707), and photodiode array (PDA) detector (Waters 2998), was used for the analyses. A reversed-phase C18 analytical column (4.60×250 mm, 5 μm particle size; Sunfire, Waters, U.S.A.) was utilized at 30 ºC column temperature. Binary gradient was used with 0.1% HCOOH in water (A) and Acetonitrile (B), and a run time of 35 minutes at a flow rate of 1ml/min was used for the analysis. The injection volume was 20 μl (E. ganitrus leaf extracts) and 10 μl (standard mix) at different concentrations. The identities of constituents were also confirmed with a photodiode array (PDA) detector by comparison with ultraviolet (UV) spectra of standards in the wavelength at 280 nm and 325 nm.

2.3. LC-MS Analysis

The hydroethanolic extract of *E. ganitrus* leaves was used for LC-MS analysis **(Singh *et al.*, 2022)**. The LC/MS instrument is equipped with an Electron Spray Ionization (ESI) ion source operating in a positive and negative ion mode. The capillary temperature was kept at 280 °C, and the sample flow rate was 8 μL/min. A mass range was selected from 50-1000 Da with a scanning time of 0.2 s. The elution was carried out using 156 gradient elution of 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in 157 acetonitrile (solvent B) with a 400 μl/min flow rate. The solvent gradient program was started with 95-90 % of the mobile phase for 0-5 min, 90-80 % for 5-10 min, 80-60 % for 10-20 min, 60-40 % for 20-30 min, 40 % for 30-45 min, 40-95 % for 45-46 min, followed by 95 % for 46-50 min. Two microlitres of the test solution were used for screening, and the chromatograph was continuously tracked for 45 minutes.

2.4. HPTLC Analysis

Analysis was performed on a Camag HPTLC system equipped with a sample applicator ATS4, ADC2 development chamber, and TLC Scanner; TLC Visualizer and WinCats integration software were used. The standard solutions of gallic acid, catechin, caffeic acid, berberine, rutin, colchicine, cinnamic acid, and Curcumin were accurately weighed (10 mg), and the solution was made up to 10 ml with methanol (1 mg/ml). From the stock solution of the standards, 0.1 ml was pipetted out and further diluted up to 1 ml to obtain the final concentration of 100 µg/ml. For standard mixture preparation, different standards were mixed to get a 100 ppm final concentration for all standards in methanol. Hydroethanolic extract of *E. ganitrus* leaves, and the standards were spotted on a precoated TLC Aluminum sheets silica gel 60 F254 (20x10cm, 0.2mm thickness) as 8mm wide bandwidth by using automatic TLC applicator ATS 4,10mm from the bottom. The Mobile phase used was Toluene: Ethyl acetate: Formic acid (5:4:1v/v). The plates were saturated in ADC2 for 20 min. After development, the plates were dried in ADC2 and scanned at 254 nm, 366, and after derivatization at 540 nm using CAMAG Scanner. The plates were photographed at an optimized wavelength of 254 nm, 366 nm, and 540 nm.

2.5. Data analysis

The chemical structure for each compound identified in the hydroethanolic extract of *E. ganitrus* leaves using HPLC, LC-MS, and HPTLC was searched using online database software (www.chemspider.com).

3. Results & Discussion

3.1. Identification and quantification of marker compounds by HPLC

Phytochemical profiling of hydroethanolic extracts of *E. ganitrus* leaves was performed using the HPLC analysis. A binary gradient method for HPLC was developed and optimized. The hydroethanolic extract was analyzed along with the mixture of standard marker compounds. Altogether, 93 phenolic compounds in the leaves of *E. ganitrus* were identified and quantified, including 2 flavonoid glycosides, 46 flavonoids, 2 glycosides, 1 hydroxycinnamic acid, 1 lignan, 3 phenolic acid, 3 phenolic glycosides, 17 phenols, 2 phenylpropanoid glycoside, 14 polyphenols, 1 tannin, and 1 terpene glycoside using HPLC analysis **(Table 2)**. Each compound was identified and confirmed using its retention time (RT) and UV profile in a photodiode array (PDA) detector under similar conditions **(Figure 2 and Figure 3)**.

The flavonoids, including flavanones, flavanols, flavonols, flavones, and isoflavones, were the most abundant compounds annotated in the hydroethanolic fraction of *E. ganitrus* leaves (**Table 2**). Forty six flavonoids were reported including; 2-hydroxy-2-phenylacetic acid (72 µg/L) at RT 6.689, (+)-Gallocatechin 3-0-gallate (86 µg/L) at RT 7.736, Methylepigallocatechin 3-0 gallate (44 µg/L) at RT 8.264, Eriocitrin (35 µg/L) at RT 8.597, Naringin (18 µg/L) at RT 8.722, 8-prenylnaringenin (53 µg/L) at RT 8.854, Hesperidin (63 µg/L) at RT 8.895, Hesperetin 3'-0'glucuronide (57 µg/L) at RT 9.081, Apigenin 7-0-apiosyl-glucuoside (26 µg/L) at RT 9.123, Apigenin 7-0-glucuronide (44 µg/L) at RT 9.287, Apigenin 6,8-di-C-lucoside (34 µg/L) at RT 9.377, Chrysoeriol 7-0-glucoside (50 µg/L) at RT 9.514, Apigenin 6-C-glucoside(Isovitexin) (86 µg/L) at RT 9.676, Patuletin (59 µg/L) at RT 9.916, 3-0-glucosyl-(1->6)-[apiosyl (1->2]-glucoside (17 µg/L) at RT 10.12, Quercetin 3-0-xylosyl-rutinoside (71 µg/L) at RT 10.37, Myricetin 3-0-rutinoside (48 µg/L) at RT 10.48, Quercetin 3-0-glucosyl-xyloside (45 µg/L) at RT 10.51, Kaempferol 3,7-0-diglucoside (38 µg/L) at RT 10.56, Myricetin 3-0-glucoside (36 µg/L) at RT 10.74, Kaempferol 3-0-glucosyl-rhamnosyl-galactoside (71 µg/L) at RT 10.83, Kaempferol 3-0-(2"-rhamnosyl-galactoside)7-O-rhamnoside (69 µg/L) at RT 10.95, Rhamnoside (70 µg/L) at RT 11.07, Quercetin 3'-O-glucuronide (37 µg/L) at RT 11.09, Myricetin 3-O-rhamnoside (22 µg/L) at RT 11.27, Quercetin 3-O-arabinoside (78 µg/L) at RT 11.32, Isorhamnetin (25 µg/L) at RT 11.42, Dihydrochalcones (23 µg/L) at RT 11.52, 3-hydroxyphloretin 2'-O-xylosyl-glucoside (67 µg/L) at RT 11.64, 3-hydroxyphloretin 2'-O-glucoside (25 µg/L) at RT 11.71, Peonidin 3-O-diglucoside-5-O-glucoside (55 µg/L) at RT 12.13, Cyanidin 3-O-(6"-p-coumaroyl-glucoside) (14 µg/L) at RT 12.27, Delphinidin 3-O-glucosyl-glucoside (21 µg/L) at RT 12.49, Isopeonidin 3-O-arabinoside (85 µg/L) at RT 12.58, Cyanidin 3,5-O-diglucoside (56 µg/L) at RT 12.63, Pelargonidin 3-O-rutinoside (75 µg/L) at RT 12.77, 6"-O-malonylglycitin (45 µg/L) at RT 12.89, 5,6,7,3',4'-pentahydroxyisoflavone (81 µg/L) at RT 13.04, 6"-O-acetyldaidzin (10 µg/L) at RT 13.24, Violanone (50 µg/L) at RT 13.39,3'-hydroxydaidzein (55 µg/L) at RT 13.62, 6"-O-acetylglycitin (86 µg/L) at RT 13.79, 3'-hydroxygenistein (32 µg/L) at RT 13.91, Dihydrobiochanin A (14 µg/L) at RT 14.02, 2-dehydro-O-desmethylangolensin (82 µg/L) at RT 14.1, and 3',4',7-trihydroxyisoflavanone (32 µg/L) at RT 14.35. Various investigations reported the antiviral, anticancer, neuroprotective, and anti-inflammatory activities of flavonoids (**Muhammad *et al*., 2019; Yuan *et al*., 2021; Ortiz *et al*., 2022; Aboulaghras *et al*., 2022; Salehi *et al*., 2020; Ayvaz *et al*., 2022; Patel *et al*., 2023**).

The second abundant category was phenols, and 17 compounds were recognized including; Galloyl glucose (9 µg/L) at RT 3.749, Hydroxybenzoic acid (59 µg/L) at RT 3.905, 4-hydroxybenzoic acid 4-0 (44 µg/L) at RT 4.021, Hydroxycinnamic acids (79 µg/L) at RT 4.248, Cinnamic acid (73 µg/L) at RT 4.344, 3-p-coumaroylquinic acid (41 µg/L) at RT 4.552, M-coumaric acid (38 µg/L) at RT 4.63, 4-hydroxybenzoic acid 4-0 (18 µg/L) at RT 4.718, Caffeic acid (45 µg/L) at RT 4.993, Hydroxyphenylacetic acids (39 µg/L) at RT 6.454, 3-hydroxy-3(3-hydroxyphenyl) propionic acid (41 µg/L) at RT 7.588, 7-hydroxymatairesinol (15 µg/L) at RT

9.142, Anthocyanins (80 µg/L) at RT 12.01, Coumarin (75 µg/L) at RT 14.44, Salvianolic acid B (7 µg/L) at RT 14.78, Scopoletin (72 µg/L) at RT 14.82, and 4-vinylsyringol (53 µg/L) at RT 15.23. Phenols are known to exhibit various pharmacological activities such as., antioxidant, anti-inflammatory, antimicrobial, anti-adipogenic, antidiabetic anticancer, and neuroprotective (**Cardile *et al*., 2015; Li *et al*., 2020; Kowalska *et al*., 2021**).

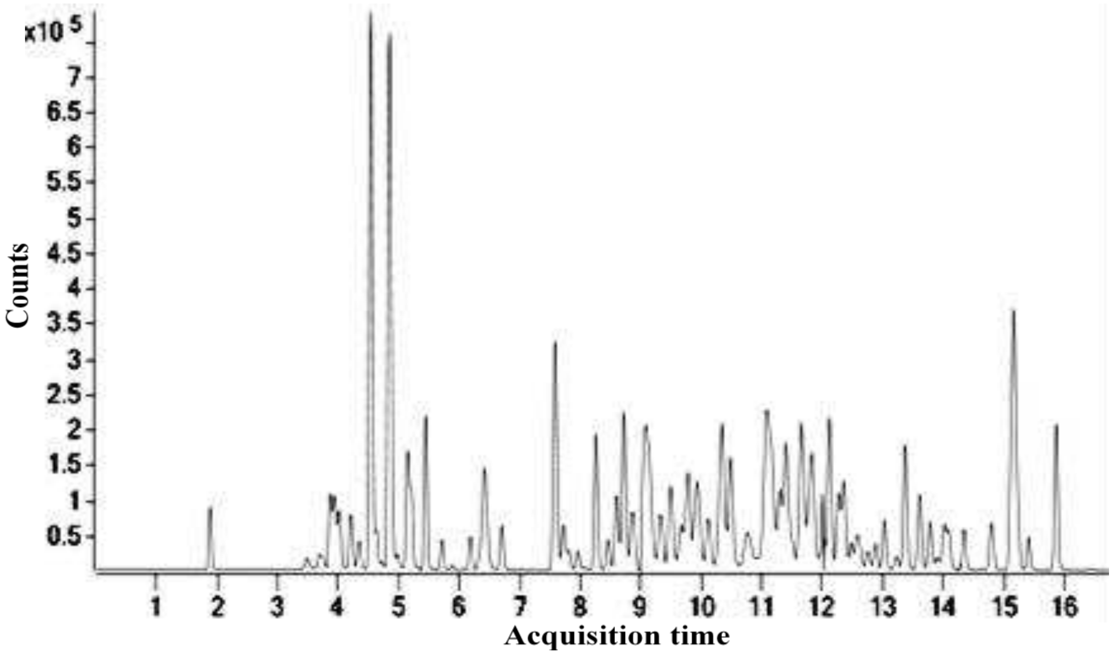


Figure 2. HPLC chromatogram of identified phytochemical constituents' profile hydroethanolic extract of *E. ganitrus* leaves

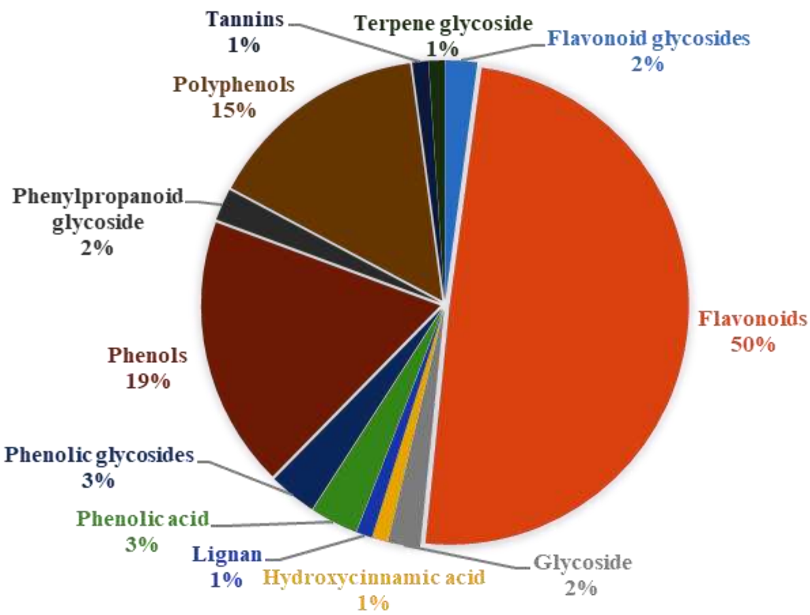


Figure 3. The phytochemical composition identified in HPLC analysis in a hydroethanolic fraction of *E. ganitrus* leaves.

The third abundant category of phytochemicals was polyphenols. The present study detected 14 polyphenols, including 1-sinapoyl-2-feruloylgentiobiose (22 µg/L) at RT 6.434, (+)-Catechin 3-0-gallate (72 µg/L) at RT 7.609, (-)-Epigallocatechin (65 µg/L) at RT 7.815, Procyanidin trimer C1 (79 µg/L) at RT 7.961, (-)-Epicatechin 4"-0-(4 µg/L) at RT 8.263, Procyanidin dimer B1 (76 µg/L) at RT 8.473, 3,4-dihydroxyphenylacetic acid (60 µg/L) at RT 8.895, Todolactol A (54 µg/L) at RT 9.083, Stilbenes (41 µg/L) at RT 9.151, Phloridzin (33 µg/L) at RT 11.81, Alkylmethoxyphenols (56 µg/L) at RT 15.13, Hydroxybenzoketones (65 µg/L) at RT 15.42, 2,3-dihydroxy-1-guaiacylpropanone (10 µg/L) at RT 15.88, and 2-hydroxy-4-methoxyacetophenone 5-sulfate (21 µg/L) at RT 16.48. Polyphenols are secondary metabolites that exhibit multiple pharmacological activities: anti-infectious, anti-inflammatory, cardio-protective, antimicrobial, antiviral, antimutagenic, antihyperglycemic, and anti-allergic **(Rue *et al.*, 2018)**. The major bioactive compounds identified by HPLC analysis were displayed with their classification and pharmacological activities **(Table 4)**.

Table 2. The phytochemical profile of the *E. ganitrus* hydroethanolic extract identified using HPLC

Compound	RT	Response	Concentration	Category
Galloyl glucose	3.749	3940	9 µg/L	Phenols
Hydroxybenzoic acid	3.905	547355	59 µg/L	Phenols
4-hydroxybenzoic acid 4-0	4.021	244884	44 µg/L	Phenols
Gallic acid	4.196	25297	63 µg/L	Phenolic acid
Paeoniflorin	4.211	132476	38 µg/L	Glycoside
Hydroxycinnamic acids	4.248	36410	79 µg/L	Phenols
Cinnamic acid	4.344	80249	73 µg/L	Phenols
3-p-coumaroylquinic acid	4.552	224901	41 µg/L	Phenols
M-coumaric acid	4.63	88404	38 µg/L	Phenols
4-hydroxybenzoic acid 4-0	4.718	14194	18 µg/L	Phenols
Caffeoyl glucose	4.848	2004689	54 µg/L	Phenylpropanoid glycoside
Caffeic acid	4.993	32645	45 µg/L	Phenols
3-feruloylquinic acid	5.151	562991	10 µg/L	Phenolic acid
Ferulic acid 4-0-glucoside	5.212	233991	73 µg/L	Phenolic glycosides

Isoferulic acid	5.443	474103	10 µg/L	Hydroxycinnamic acid
P-coumaric acid 4-0 glucoside	5.716	134202	26 µg/L	Phenolic Glycosides
Sinapic acid	6.191	147932	76 µg/L	Phenolic Acid
Verbascoside	6.347	44183	8 µg/L	Phenylpropanoid glycoside
1-sinapoyl-2-feruloylgentiobiose	6.434	167708	22 µg/L	Polyphenols
Hydroxyphenylacetic acids	6.454	185413	39 µg/L	Phenols
2-hydroxy-2-phenylacetic acid	6.689	2078	72 µg/L	Flavonoids
3-hydroxy-3(3-hydroxyphenyl) propionic acid	7.588	344193	41 µg/L	Phenols
(+)-Catechin 3-0-gallate	7.609	140339	72 µg/L	Polyphenols
(+)-Gallocatechin 3-0-gallate	7.736	154358	86 µg/L	Flavonoids
(-)-Epigallocatechin	7.815	105280	65 µg/L	Polyphenols
Procyanidin trimer C1	7.961	57531	79 µg/L	Polyphenols
Cinnamtannin A2	8.082	11012	86 µg/L	Tannins
(-)-Epicatechin 4"-0-	8.263	261452	4 µg/L	Polyphenols
Hydroxytyrosol 4-O-glucoside	8.264	124797	34 µg/L	Phenolic glycosides
Methylepigallocatechin 3-0 gallate	8.264	231580	44 µg/L	Flavonoids
Procyanidin dimer B1	8.473	112349	76 µg/L	Polyphenols
Eriocitrin	8.597	38440	35 µg/L	Flavonoids
Naringin	8.722	199063	18 µg/L	Flavonoids
Demethyloleuropein	8.729	206757	21 µg/L	Terpene glycoside
8-prenylnaringenin	8.854	194299	53 µg/L	Flavonoids
3,4-dihydroxyphenylacetic acid	8.895	63118	60 µg/L	Polyphenols
Hesperidin	8.895	116122	63 µg/L	Flavonoids
Hesperetin 3'-0'glucuronide	9.081	295808	57 µg/L	Flavonoids
Todolactol A	9.083	267752	54 µg/L	Polyphenols
Apigenin 7-0-apiosyl-glucuoside	9.123	276639	26 µg/L	Flavonoids
7-hydroxymatairesinol	9.142	9091	15 µg/L	Phenols
Stilbenes	9.151	141612	41 µg/L	Polyphenols

Matairesinol	9.181	119015	47 µg/L	Lignan
Apigenin 7-0-glucuronide	9.287	26576	44 µg/L	Flavonoids
Apigenin 6,8-di-C-lucoside	9.377	264250	34 µg/L	Flavonoids
Chrysoeriol 7-0-glucoside	9.514	436876	50 µg/L	Flavonoids
Apigenin 6-C-glucoside(Isovitexin)	9.676	104039	86 µg/L	Flavonoids
Neodiosmin	9.716	127475	60 µg/L	Flavonoid glycosides
Patuletin	9.916	64119	59 µg/L	Flavonoids
3-0-glucosyl-(1->6)-[apiosyl (1->2]-glucoside	10.12	215634	17 µg/L	Flavonoids
Quercetin 3-0-xylosyl-rutinoside	10.37	855408	71 µg/L	Flavonoids
Myricetin 3-0-rutinoside	10.48	20422	48 µg/L	Flavonoids
Quercetin 3-0-glucosyl-xyloside	10.51	452348	45 µg/L	Flavonoids
Kaempferol 3,7-0-diglucoside	10.56	3406	38 µg/L	Flavonoids
Myricetin 3-0-glucoside	10.74	29613	36 µg/L	Flavonoids
Kaempferol 3-0-glucosyl-rhamnosyl-galactoside	10.83	36162	71 µg/L	Flavonoids
Kaempferol 3-0-(2''-rhamnosyl-galactoside)7-O-rhamnoside	10.95	4300	69 µg/L	Flavonoids
Rhamnoside	11.07	216751	70 µg/L	Flavonoids
Quercetin 3'-O-glucuronide	11.09	567900	37 µg/L	Flavonoids
Myricetin 3-O-rhamnoside	11.27	189474	22 µg/L	Flavonoids
Quercetin 3-O-arabinoside	11.32	326216	78 µg/L	Flavonoids
Isorhamnetin	11.42	305651	25 µg/L	Flavonoids
Dihydrochalcones	11.52	13954	23 µg/L	Flavonoids
3-hydroxyphloretin 2'-O-xylosyl-glucoside	11.64	56793	67 µg/L	Flavonoids
3-hydroxyphloretin 2'-O-glucoside	11.71	279595	25 µg/L	Flavonoids
Phloridzin	11.81	278976	33 µg/L	Polyphenols
Anthocyanins	12.01	165071	80 µg/L	Phenols
Peonidin 3-O-diglucoside-5-O-glucoside	12.13	825636	55 µg/L	Flavonoids

Cyanidin 3-O-(6"-p-coumaroyl-glucoside)	12.27	593935	14 µg/L	Flavonoids
Delphinidin 3-O-glucoside	12.36	158245	55 µg/L	Flavonoid glycosides
Delphinidin 3-O-glucosyl-glucoside	12.49	81014	21 µg/L	Flavonoids
Isopeonidin 3-O-arabinoside	12.58	112968	85 µg/L	Flavonoids
Cyanidin 3,5-O-diglucoside	12.63	88400	56 µg/L	Flavonoids
Pelargonidin 3-O-rutinoside	12.77	91061	75 µg/L	Flavonoids
6"-O-malonylglycitin	12.89	84742	45 µg/L	Flavonoids
5,6,7,3',4'-pentahydroxyisoflavone	13.04	239694	81 µg/L	Flavonoids
6"-O-acetyldaidzin	13.24	64871	10 µg/L	Flavonoids
Violanone	13.39	492839	50 µg/L	Flavonoids
3'-hydroxydaidzein	13.62	228757	55 µg/L	Flavonoids
6"-O-acetylglycitin	13.79	226902	86 µg/L	Flavonoids
3'-hydroxygenistein	13.91	45909	32 µg/L	Flavonoids
Dihydrobiochanin A	14.02	194915	14 µg/L	Flavonoids
2-dehydro-O-desmethylangolensin	14.1	197210	82 µg/L	Flavonoids
3',4',7-trihydroxyisoflavanone	14.35	125408	32 µg/L	Flavonoids
Coumarin	14.44	560	75 µg/L	Phenols
Esculin	14.64	5603	87 µg/L	Glucoside
Salvianolic acid B	14.78	61866	7 µg/L	Phenols
Scopoletin	14.82	142959	72 µg/L	Phenols
Alkylmethoxyphenols	15.13	533112	56 µg/L	Polyphenols
4-vinylsyringol	15.23	91460	53 µg/L	Phenols
Hydroxybenzoketones	15.42	138903	65 µg/L	Polyphenols
2,3-dihydroxy-1-guaiacylpropanone	15.88	458347	10 µg/L	Polyphenols
2-hydroxy-4-methoxy acetophenone 5-sulfate	16.48	3595	21 µg/L	Polyphenols

3.2. Identification and quantification of marker compounds by LC-MS

Detailed phytochemical profiling of hydroethanolic extract was performed using LC-MS analysis. LC-MS analysis showed a total of 22 phytochemicals, including 11 flavonoids, 2 polyphenols, 2 hydroxybenzoic acids, 4 hydroxycinnamic acid, 1 phenolic acid, and 2 phenolic aldehydes in a hydroethanolic fraction of *E. ganitrus* leaves, and the chromatogram was displayed in **Figure 4**. LC-MS data of the identified compounds with their retention time, responses (frequency), and concentration was provided in **Table 3**. The major eleven identified compounds were quercetin (803.0215 µg/L) at RT 7.319, Gallic acid (726.13 µg/L) at RT 6.223, Ferullic acid (652.34 µg/L) at RT 7.672, Chlorogenic acid (651.021 µg/L) at RT 8.812, Pinocembrin (264.11 µg/L) at RT 13.387, p-aminobenzoic acid (251.021 µg/L) at RT 1.678, Epicatechin (246.02 µg/L) at RT 1.336, Catechin (161.51 µg/L) at RT 1.336, Caffeic acid (123.31 µg/L) at RT 9.555, Syringaldehyde (116.31 µg/L) at RT 7.696, and Naringenin (106.31 µg/L) at RT 8.697 **(Figure 4 and 5)**.

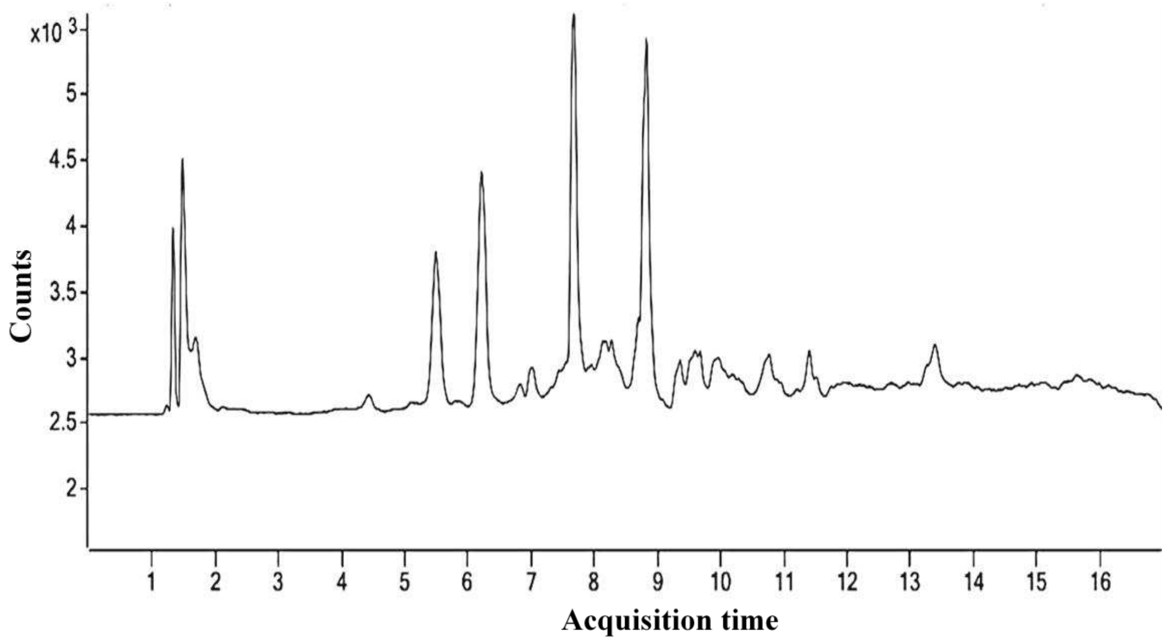


Figure 4. LC-MS chromatogram of identified phytochemical constituents' profile hydrolethanolic extract of *E. ganitrus* leaves.

Identified compounds belonged to various classes, including flavonoids, polyphenols, hydroxybenzoic acid, hydroxycinnamic acid, phenolic acid, and phenolic aldehyde. The major bioactive compounds identified by LC-MS analysis were presented along with their classification and pharmacological activities **(Table 4)**. The bioactive compounds have diverse therapeutic potential which includes anti-inflammatory, antioxidant, antifungal, anticancer, antidiabetic, anti-adipogenic, cardio-protective and neuroprotective activities (**Laborda *et al*., 2018; Imran *et al*., 2019; Zhang *et al*., 2019; Musial *et al*., 2020; Gong *et al*., 2020; Pimpley *et al*., 2020; Yi *et al*., 2021; Mirzaei *et al*., 2021; Dong *et al*., 2022; Dicks *et al*., 2022; Bai *et al*., 2022; Jiang *et al*., 2022; Wu *et al*., 2022; De Luca *et al*., 2022**).

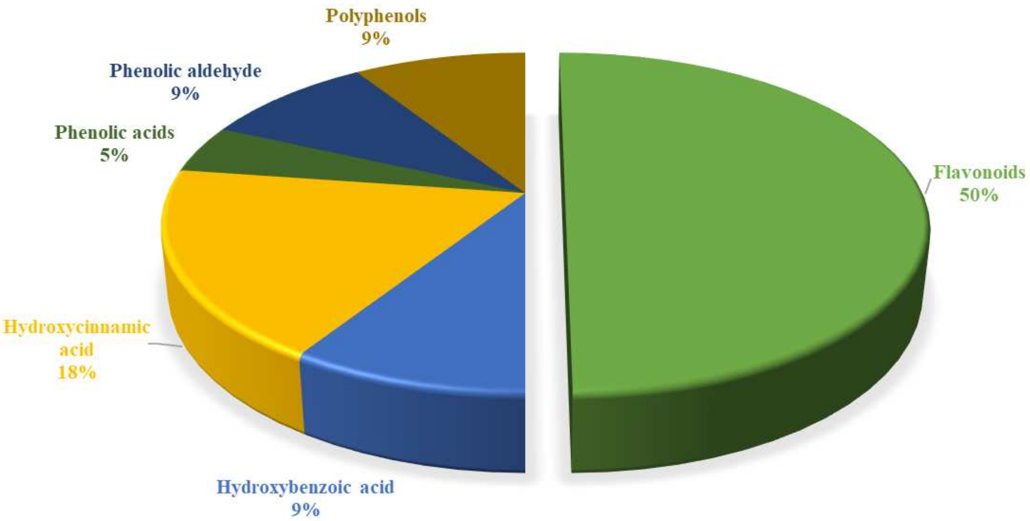


Figure 5. The phytochemical composition identified in LC-MS analysis in a hydroethanolic fraction of *E. ganitrus* leaves.

Table 1: Quantification of phytochemicals in hydroethanolic extract of *E. ganitrus* leaves through LC-MS

Compound	**RT**	**Response**	**Concentration**	**Category**
Epicatechin	1.336	4762	246.02 µg/l	Flavonoids
Catechin	1.336	2852	161.51 µg/l	Flavonoids
P-Aminobenzoic acid	1.678	5716	251.021 µg/l	Phenolic acids
Hesperidin	2.461	29	1.021 µg/l	Flavonoids
Gallic acid	6.223	14924	726.13 µg/l	Hydroxybenzoic acid
Syringic acid	7.014	128	2.261 µg/l	Hydroxybenzoic acid
Quercetin	7.319	17295	803.0215 µg/l	Flavonoids

Coniferaldehyde	7.58	143	2.2614 µg/l	Phenolic aldehyde
Cyanidin 3-glucoside	7.582	362	3.0215 µg/l	Flavonoids
Apigenin	7.654	73	2.0541 µg/l	Flavonoids
Ferullic acid	7.672	10776	652.34 µg/l	Hydroxycinnamic acid
Syringaldehyde	7.696	2113	116.31 µg/l	Phenolic aldehyde
Ellagic acid	8.013	25	1.0215 µg/l	Polyphenols
Naringenin	8.697	1832	106.31 µg/l	Flavonoids
Chlorogenic acid	8.812	12167	651.021 µg/l	Polyphenols
Luteolin	9.337	67	6.02 µg/l	Flavonoids
p-Coumaric acid	9.364	545	3.051 µg/l	Hydroxycinnamic acid
Taxifolin	9.552	13	1.001 µg/l	Flavonoids
Caffeic acid	9.555	1662	123.31 µg/l	Hydroxycinnamic acid
Kaempeferol	10.04	51	2.021 µg/l	Flavonoids
Sinapic Acid	10.877	921	3.021 µg/l	Hydroxycinnamic acid
Pinocembrin	13.387	3925	264.11 µg/l	Flavonoids

HPTLC

The HPTLC analysis of the hydroethanolic extract of *E. ganitrus* leaves showed the presence of various phytoconstituents in different concentrations, such as Gallic acid (48.64 %), Curcumin (15.21 %), Caffeic acid (12.19%) and Cinnamic acid (6.50%) (**Figure 6**). The developed HPTLC method will assist in the standardization of *E. ganitrus* extract using biologically active chemical markers. Several pharmacological activities of identified phytochemicals exhibit antioxidant, anti-inflammatory, antifungal, and antibacterial activities due to the presence of bioactive phytochemicals such as phenolic acid, phenols, hydroxycinnamic acid, and flavonoids **(Zhang *et al*., 2019; Mirzaei *et al*., 2021; Fu *et al*., 2021; Bai *et al*., 2022; Jiang *et al*., 2022)**. The major bioactive compounds identified by HPTLC analysis were displayed with their classification and pharmacological activities in **Table 4**.

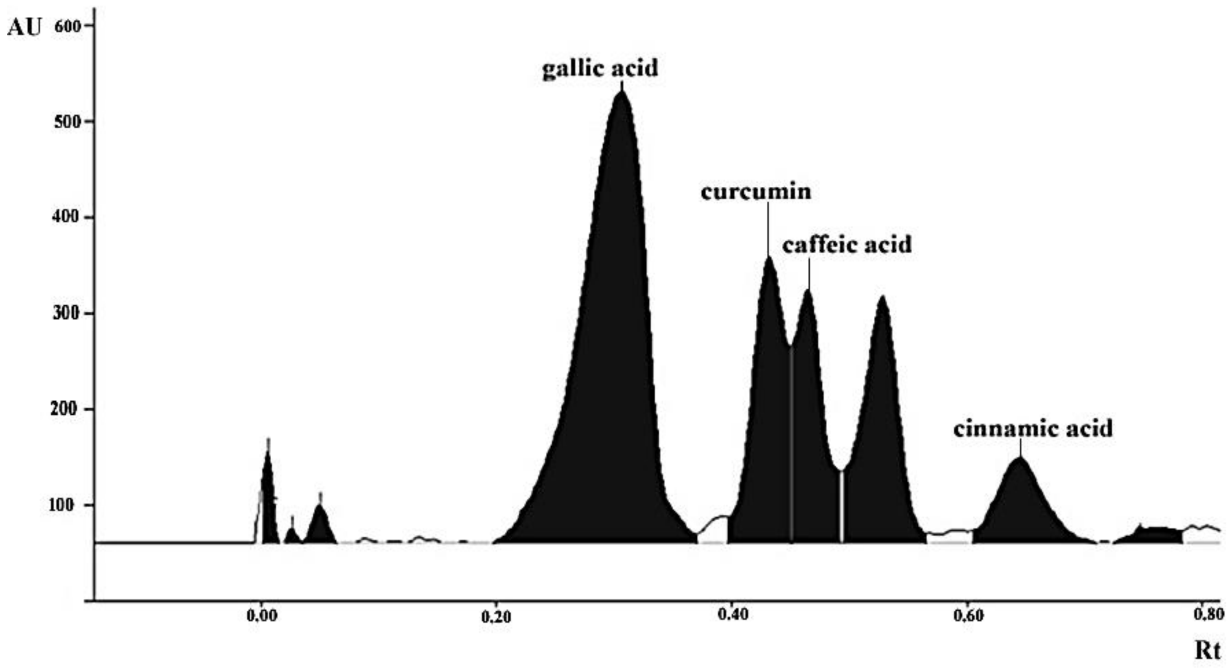
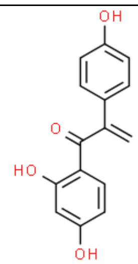
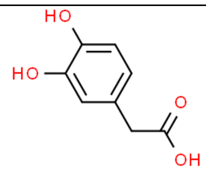
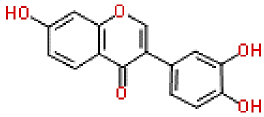
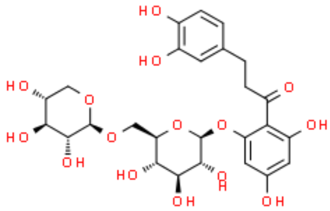
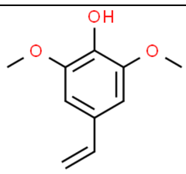
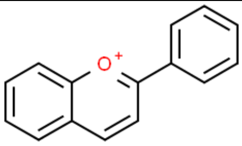
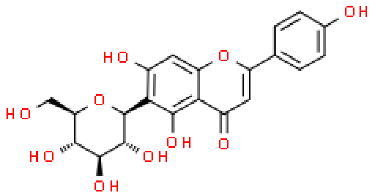
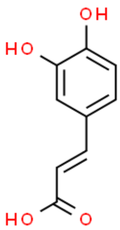
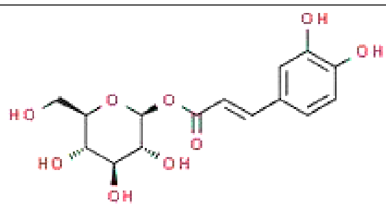
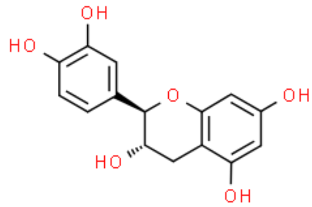
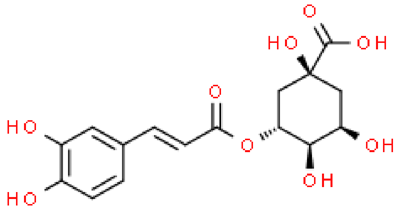
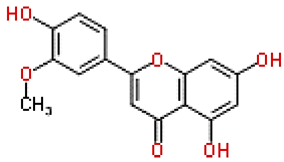
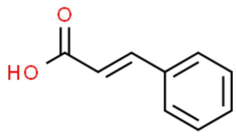
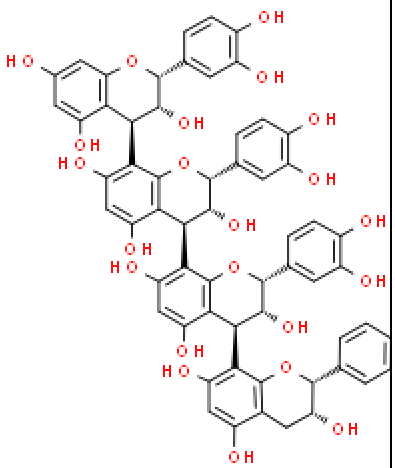
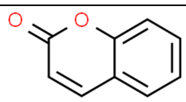


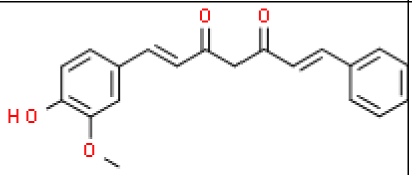
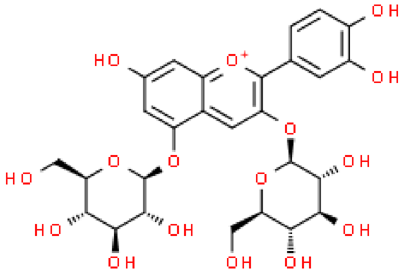
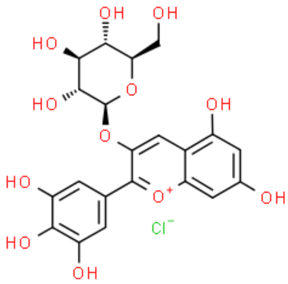
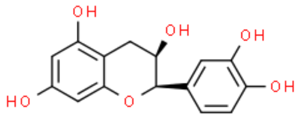
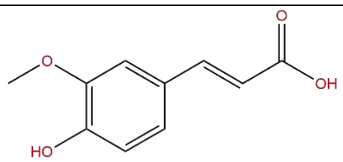
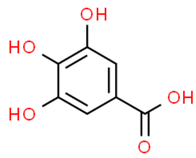
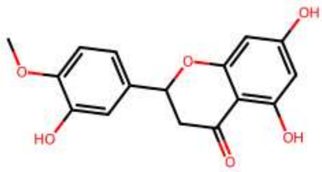
Figure 6. HPTLC chromatogram of identified phytochemical constituents' profile hydroethanolic extract of *E. ganitrus* leaves.

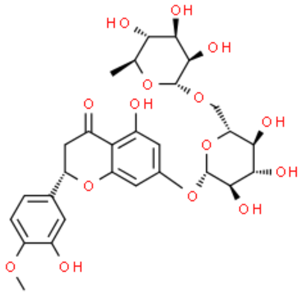
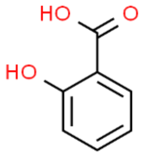
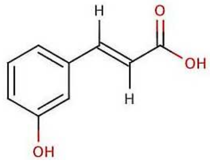
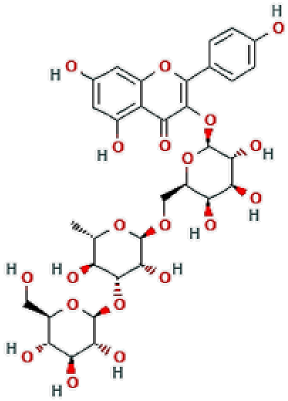
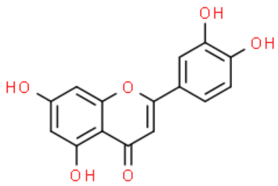
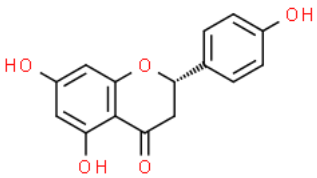
Table 4: Pharmacological properties of major bioactive compounds identified in LC-MS, HPLC, and HPTLC analyses

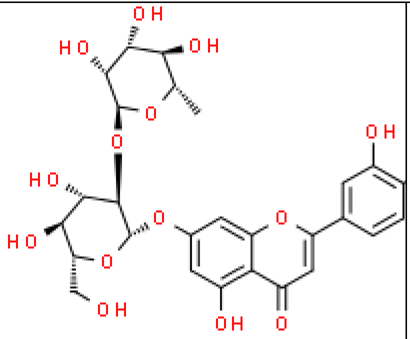
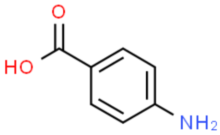
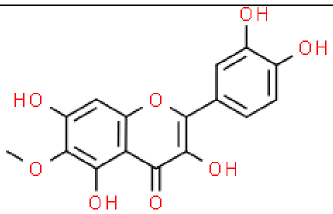
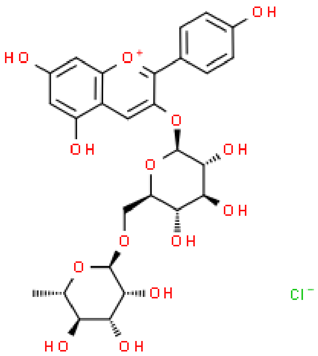
Compound name	Pharmacological properties	Chemical Structure	References
2-dehydro-O-desmethylangolensin	antioxidant		Ali *et al*., 2021

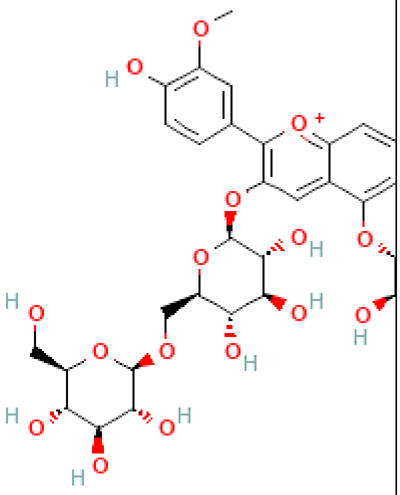
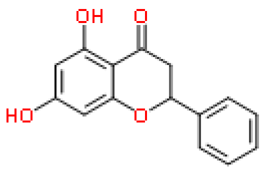
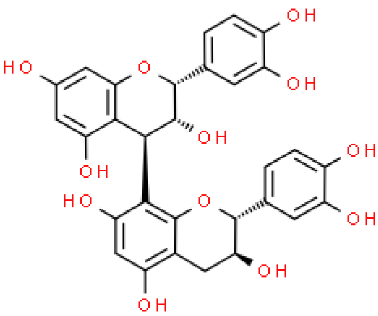
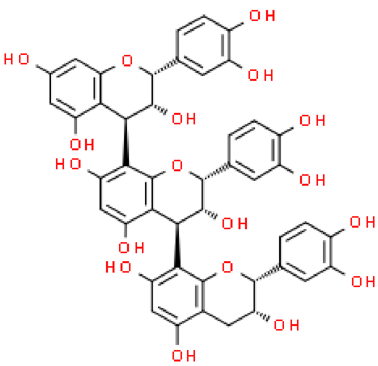
3,4-dihydroxyphenylacetic acid (DOPAC)	antioxidant, anti-inflammatory		Liu *et al.*, 2017
Daidzein (3'-hydroxydaidzein)	antioxidant		Zhang *et al.*, 2 023
3-hydroxyphloretin 2'-O-xylosyl-glucoside	antioxidant		Zhu *et al.*, 2022
Canolol (4-vinylsyringol)	antimutagens and anticarcinogens, antioxidant		Kraljić *et al.*, 2015
Anthocyanins (flavylium)	anti-inflammatory, antioxidant, antidiabetic, cardio protective, neuroprotective		Salehi *et al.*, 2020; Ayvaz *et al.*, 2022
Apigenin 6-C-glucoside(Isovitexin)	Antidiabetic		Abdulai *et al.*, 2021
Caffeic acid	anticancer and neuroprotective		Zhang *et al.*, 2019; Mirzaei *et al.*, 2021
Caffeoyl glucose	Antidiabetic, antioxidant		Alcázar Magaña *et al.*, 2021

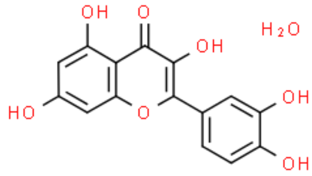
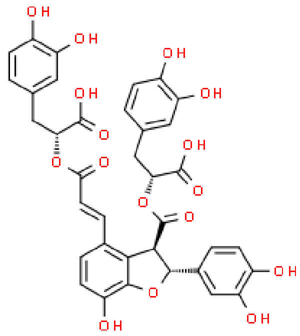
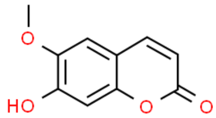
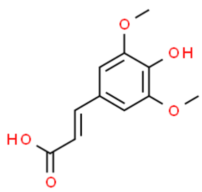
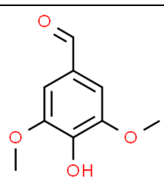
Catechin	anticancer and anti-inflammatory		Musial *et al*., 2020
Chlorogenic acid	antidiabetic, anti-adipogenic, neuroprotective		Pimpley *et al*., 2020
Chrysoeriol 7-0-glucoside	anticancer, antidiabetic, anti-diabetic, cardio-protective, neuroprotective		Aboulaghras *et al*., 2022
Cinnamic acid	Antioxidant, antimicrobial, antidiabetic, anti-inflammatory, anticancer, neuroprotective		Kowalska *et al*., 2021
Cinnamtannin A2	antioxidant, anti-inflammatory		Li *et al*., 2020
Coumarin	anticancer, anti-inflammatory, anticonvulsant,		Srikrishna *et al*., 2018

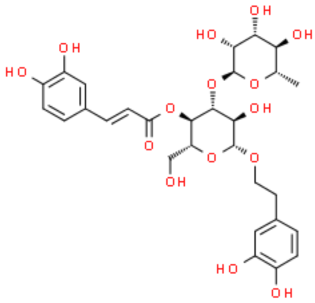
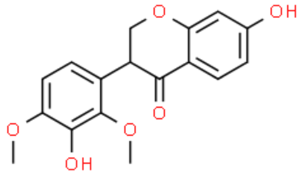
	antimicrobial, antioxidant		
Curcumin	anticancer, anti-inflammatory, antioxidant, neuroprotective		Fu *et al.*, 2021
Cyanidin 3,5-O-diglucoside	antioxidant, antidiabetic, antiaging, cardio-protective		Olivas-Aguirre *et al.*, 2016
(Myrtillin) delphinidin 3-O-glucoside	antioxidant, anti-inflammatory, anticancer		Sharma *et al.*, 2021
Epicatechin	Cardio-protective		Dicks *et al.*, 2022
Ferullic acid	neuroprotective		Dong *et al.*, 2022
Gallic acid	antioxidant, antimicrobial, anticancer		Bai *et al.*, 2022; Jiang *et al.*, 2022
Hesperetin 3'-0'glucuronide	antiviral, anticancer, neuroprotective, anti-inflammatory		Muhammad *et al.*, 2019

Hesperidin	anti-inflammatory, neuroprotective		Ortiz *et al.*, 2022
(Salicylic acid) hydroxybenzoic acid	antioxidant, antimicrobial		Kalinowska *et al.*, 2021
Hydroxycinnamic acids	antioxidant, anti-adipogenic		Cardile *et al.*, 2015
kaempferol 3-0-glucosyl-rhamnosyl-galactoside	antioxidant, neuroprotective		Yuan *et al.*, 2021
Luteolin	anticancer and neuroprotective		Imran *et al* 2019; De Luca *et al.*, 2022
Naringenin	anti-inflammatory activity		Wang *et al.*, 2020

Neodiosmin	anticancer		Zheng *et al.*, 2020
P-Aminobenzoic acid	antifungal		Laborda *et al.*, 2018
Patuletin	anti-inflammatory, antinociceptive, antioxidant, antiplatelet, antiproliferative, hepatoprotective		Patel *et al.*, 2023
Pelargonidin 3-O-rutinoside	antidiabetic		Xu *et al.*, 2018

Peonidin 3-O-diglucoside-5-O-glucoside	anti-inflammatory, antioxidant,		Sari *et al.*, 2019
Pinocembrin	neuroprotective		Gong *et al.*, 2020
procyanidin dimer B1	anti-infectious, anti-inflammatory, cardio protective, antimicrobial, antiviral, antimutagenic, antihyperglycemic, anti-allergic		Rue *et al.*, 2018
Procyanidin trimer C1	anti-infectious, anti-inflammatory, cardio protective, antimicrobial, antiviral, antimutagenic, antihyperglycemic, anti-allergic		Rue *et al.*, 2018

Quercetin	anti-inflammatory		Yi *et al.*, 2021
Salvianolic acid B	anti-cancer, antifibrosis, anti-diabetic		Ma *et al.*, 2019
Scopoletin	Antioxidant, antimicrobial, anticarcinogenic, anti-metabolic disorder, neuroprotective		Antika *et al.*, 2022
Sinapic acid	antioxidant, anti-inflammatory, anticancer, hepatoprotective, cardioprotective, renoprotective, neuroprotective, antidiabetic, anxiolytic and antibacterial		Pandi and Kalappan, 2021
Syringaldehyde	antioxidant, anti-inflammatory, and antidiabetes		Wu *et al.*, 2022

Verbascoside	neuroprotective		Zhao *et al*., 2023
Violanone	antimicrobial, antifungal		Deesamer *et al*., 2007

Conclusion

The phytochemical profile of *E. ganitrus* leaf extract was characterized using HPLC, LC-MS, and HPTLC analyses. The hydroethanolic fraction of *E. ganitrus* leaf was found to contain valuable metabolites: phenolic acid, polyphenols, flavonoids, phenols, phenolic glycosides, flavonoid glycosides, terpene glycoside, phenylpropanoid glycoside, hydroxycinnamic acid, hydroxybenzoic acid, phenolic aldehyde, lignin, and tannins. The phytochemicals could be employed as potential biochemical markers because different phytochemicals were detected in three studied (HPLC, LC-MS, and HPTLC) analytical techniques. Previous research has shown that *Elaeocarpus* species contain beneficial bioactive compounds in significant amounts, which have a wide range of applications in the pharmaceutical, food, and cosmetic industries. Moreover, only a limited number of researches are available on the phytochemical profiling of *E. ganitrus*. Hence, extensive investigations on phytochemical analysis and pharmacological activities of different fractions of leaf, fruit (bead), and pulp of *E. ganitrus* would be of much interest using a combination of modern analytical techniques or assays. Further research is required to isolate and characterize individual bioactive compounds and to validate their therapeutic potential.

Conflicts of Interest

The authors declare no conflict of interest.

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