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FULL LENGTH ARTICLE

Comparative GC-MS/MS studies on methanolic and acetone extract of leaves of *Emblica officinalis* Gaertn.

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Emblica officinalis Gaertn. is a plant species belonging to the family Euphorbiaceae. The main aim of this study is identification of bioactive phytochemicals present in methanolic and acetone extracts of leaves of *E. officinalis* by GC-MS/MS techniques. The shade dried plant material was powdered and extracted in methanol and acetone using soxhlet extraction method. Then the extracts were further subjected to Gas Chromatography Tandem – Mass spectrometry. GC-MS/MS analysis revealed the presence of 31 compounds in methanolic extract and 23 compounds in acetone extract. Some of the phytochemicals detected are Benzophenone, Coumarin, Tetra acetyl-d-xylonic nitrile, n-Hexadecanoic acid, squalene, Octadecanoic acid, 1,2,3-Benzenetriol and ethyl isoallocholate. Some of these compounds showanticancer, antioxidant and antiviral activities.

Keywords: *Emblica officinalis* Gaertn., Euphorbiaceae, Phytochemicals, GC-MS/MS analysis

INTRODUCTION

Pharmacognosy has always been a transient or multidisciplinary science. An alternative kind of medication needs to be introduced due to the emergence of superbugs in recent years as a result of the frequent and unprescribed use of antibiotics. A global concern has led researchers from all over the world to resort to herbal medicines (Rakib *et al.*, 2020). Phytochemicals found in therapeutic plants have long been used in phytomedicines. Medicinal herbs are essential for treating and preventing a wide range of illnesses. They also make up a significant portion of all current preventative methods. Numerous plant-based medications have demonstrated their possible safety and effectiveness (Sofowora *et al*., 2013). The contents of physiologically active chemicals are genetically specified and distinct to a given plant. However, other factors that may impact these substances include climate, illnesses and pests, developmental stage, ecology, and time of day the sample is collected (Alqethami *et al*., 2021).Plants have the capacity to create a wide range of compounds in the form of secondary metabolites that have a variety of biological properties and function as active medications against a variety of ailments (Gupta and Kumar, 2017; Rungsung *et al.*, 2015).

The plant species *Emblica officinalis* Gaertn. is a member of the Euphorbiaceae family. It is used as an aphrodisiac, antioxidant, chelating agent, and treatment for constipation, dental issues, diabetes, diarrhoea, diuretic, fever, gonorrhoea, hair growth,

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headaches, mouth ulcer irritation, respiratory issues, skin whitening, and other conditions. There is antineutrophilic action in plant leaves. It lowers blood levels of triglycerides, blood glucose, and blood cholesterol (Jacob *et al.*, 1988; Qureshi *et al.*, 2009). More specificity, ease of compound identification and data processing, and improved sensitivity can all be achieved with GC-MS/MS targeted analysis (Choudhury *et al*., 2022).

MATERIALS AND METHOD

E. officinalis used for the investigation was obtained from different regions of Thrissur and Malappuram district, Kerala, India. Fresh leaves of *E. officinalis* was washed under running tap water, shade dried for two weeks and powdered in electric blender. A 25 gm of sample of dried plant powder was extracted in 250 ml of methanol and acetone for 72 hrs in soxhlet solvent extractor. 64.7ºC is the boiling point of methanol and 56ºC is the boiling point of acetone. Obtained extract was evaporated to dryness and stored at 4ºC in an air tight container for further use. GC-MS/MS analysis was carried out in plant extracts (Duke and Wain, 1981).

GC-MS/MS analysis

GC-MS analysis of the leaf extract of *E. officinalis* was performed at the Mannuthy Veterinary College, Thrissur, Kerala by using the instrument Thermo fisher Scientific Triple Quadruple GC-MS/MS. The equipment has a DB-5MS Capillary Standard non-polar column with dimensions of Length - 30 m, Diameter - 0.25 m, Thickness - 0.25 m. The carrier gas used is Helium and the oven temperature was programmed as follows: Initial 110ºC Hold 2 min; 15ºC/min 150ºC Hold 1 min; 10ºC/min 250ºC/min Hold 5 min; total time : 20.67 min. The observed peaks which are segregated in a GC-MS/MS were determined by National Institute of Standards and Technology (NIST) Mass spectra databases. The components were determined by comparing them to those found in the NIST computer library, which is connected to the GC-MS/MS device. The outcomes acquired were then tabulated.

RESULT AND DISCUSSION

A total of 23 compounds were identified from the GC-MS/MS analysis of acetone extract and 31 compounds in the methanolic extract of *E. officinalis* leaves exhibiting various phytochemical activities. The chromatogram is presented in Fig. 1 and Fig. 2 while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW) and peak area concentration (%) are presented in Table 1 ans Table 2. The significant difference can be easily explained given that recovery of natural compounds depends on the type of solvent used, its polarity index (PI) and solubility of compounds in the extraction solvents (Siwe, 2019).

Among the identified phytocompounds Tetradecanoic acid, Squalene, 4-H-Pyran-4-one, 2,3-dihydro3,5-dihydroxy-6-methyl have the property of antioxidant, anticancer (Kala *et al*., 2011). 1,2,3-Benzenetriol

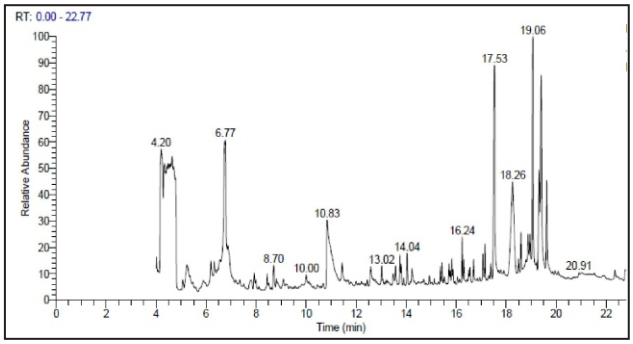

Fig. 1. GC-MS/MS chromatogram of acetone extract of leaves of *E. officinalis*

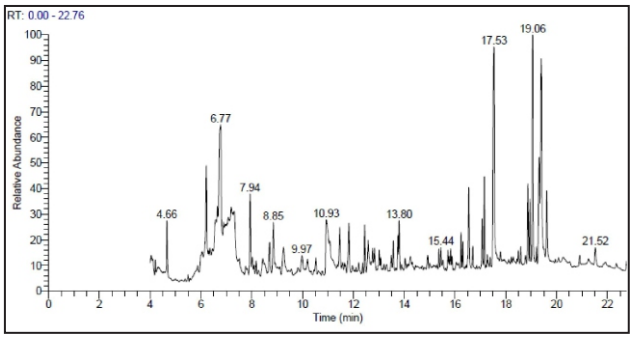

Fig. 2. GC-MS/MS chromatogram of methanolic extract of leaves of *E. officinalis*

Table 1. Bioactive compounds present in the acetone extract of leaves of *E. officinalis*

Sl. No.	Name of the compound	RT (min)	Molecular formula	MW g/mol	Peak Area %	Nature of the compound
1.	2-Pentanone, 4-hydroxy-4-methyl	4.32	$C_6H_{12}O_2$	116	6.28	Ketone
2.	Paromomycin	5.74	$C_{23}H_{45}N_5O_{14}$	615	0.03	Aminoglycoside antibiotic and an amino cyclitol glycoside
3.	2-H-Pyran-2,6-(3H)-dione	6.19	$C_5H_4O_3$	112	0.53	Alcohilic
4.	Glycerin	6.88	$C_3H_8O_3$	92	0.36	Aldoses/Aldehyde
5.	O-Butylisourea	6.32	$C_5H_{12}N_2O$	116	0.31	Urea derivatives
6.	4-H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	7.92	$C_6H_8O_4$	144	0.38	Flavonoid fraction
7.	1,4:3,6-Dianhydroadglucopyranose	8.80	$C_6H_8O_4$	144	0.24	Cyclic compound
8.	1,2,3-Benzenetriol	10.83	$C_6H_6O_3$	126	3.67	Phenol
9.	2-Cyclohexen-1-one, 4(3-hydro-butyl)-3,5,5-trimethyl	14.93	$C_{13}H_{22}O_2$	210	0.25	Cyclic ketone
10.	Tetradecanoic acid	15.36	$C_{14}H_{28}O_2$	228	0.30	Fatty acid
11.	3,6-Diisopropylpiperazin-2,5-dione	15.52	$C_6H_{10}N_2O_2$	198	0.12	Piperazine derivatives
12.	Benzene, 1,1´[1,2-ethanediylbis-(oxy)]bis	16.05	$C_{14}H_{14}O_2$	214	90.13	Dialkylbenzene
13.	2-Pentadecanone,6,10,14-trimethyl	16.31	$C_{18}H_{36}O$	268	0.39	Sesquiterpenoid
14.	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	16.70	$C_{20}H_{40}O$	296	0.44	Diterpene alcohol
15.	7,9-Diterbutyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	17.07	$C_{17}H_{24}O_3$	276	0.51	Steroid
16.	Hexadecanoic acid, methyl ester	17.15	$C_{17}H_{34}O_2$	270	0.58	Terpenoid
17.	Acridine, 9,10-dihydro-9,9-dimethyl	17.39	$C_{15}H_{15}N$	209	0.30	Heterocyclic compound
18.	Squalene	18.26	$C_{30}H_{50}$	410	5.04	Triterpene
19.	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl, 1-methylethyl-ester	18.59	$C_{13}H_{15}NO_3$	233	0.79	Carboxylic acid and its derivatives
20.	Phytol	19.06	$C_{20}H_{40}O$	296	7.06	Diterpene alcohol
21.	Octadecanoic acid	19.62	$C_{18}H_{36}O_2$	284	2.21	Fatty acid
22.	Ethyl isoallocholate	21.00	$C_{26}H_{44}O_5$	436	0.15	Steroid
23.	4,8,12,16-Tetramethylheptadecan-4-olide	22.36	$C_{21}H_{40}O_2$	324	0.29	Diterpenoid

Table 2. Bioactive compounds present in the methanolic extracts of leaves of *E. officinalis*

Sl. No.	Name of the compound	RT (min)	Molecular formula	MW g/mol	Peak Area %	Nature of the compound
1.	Paromomycin	5.87	$C_{23}H_{45}N_5O_{14}$	615	0.39	Aminoglycoside antibiotic and an amino cyclitol glycoside
2.	2-H-Pyran-2,6-(3H)-dione	6.21	$C_5H_4O_3$	112	2.88	Alcoholic
3.	Glycerin	6.45	$C_3H_8O_3$	92	0.07	Aldoses/Aldehyde
4.	Tetraacetyl-d-xylonic nitrile	7.76	$C_{14}H_{17}NO_9$	343	0.30	Aromatic nitro compound
5.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	7.94	$C_6H_8O_4$	144	2.49	Flavonoid
6.	5-Hydroxymethylfurfural	8.85	$C_6H_6O_3$	126	1.79	Heterocyclic compound
7.	2-Propen-1-ol, 3-phenyl	9.97	$C_9H_{10}O$	134	0.73	Cinnamyl alcohol
8.	1,2,3-Benzenetriol	1.93	$C_6H_6O_3$	126	4.32	Phenol
9.	Coumarin	11.82	$C_9H_6O_2$	146	1.64	Lactones
10.	Phenol, 2,4-bis(1,1-dimethylethyl)	12.44	$C_{17}H_{30}OSi$	206	1.09	Phenol
11.	Diethyl Phthalate	13.49	$C_{12}H_{14}O_4$	222	0.41	Phthalate ester
12.	Benzophenone	14.16	$C_{13}H_{10}O$	182	0.08	Aromatic ketone
13.	2-Cyclohexen-1-one, 4-(3-hydroxy-butyl)-3,5,-trimethyl	14.93	$C_{13}H_{22}O_2$	210	0.41	Ketones
14.	Tetradecanoic acid	15.36	$C_{14}H_{28}O_2$	228	0.43	Fatty acid
15.	3,6-Diisopropylpiperazin-2,5-dione	15.52	$C_{10}H_{18}N_2O_2$	198	0.30	Cyclic organic compound
16.	2-Pentadecanone, 6,10,14-trimethyl	16.31	$C_{18}H_{36}O$	268	0.49	Sesquiterpenoid
17.	Caffeine	16.54	$C_8H_{10}N_4O_2$	194	2.32	Alkaloid
18.	3,7,11,15-Tetramethyl-2-hexa-decen-l-ol	16.70	$C_{20}H_{40}O$	296	0.50	Diterpene alcohol
19.	Ethyl isoallocholate	16.85	$C_{26}H_{44}O_5$	436	0.06	Steroid
20.	7,9-Di-tert-butyl-1-oxaspiro(4,5)-deca-6,9-diene-2,8-dione	17.07	$C_{17}H_{24}O_3$	276	1.17	Steroid
21.	Hexadecanoic acid, methyl ester	17.15	$C_{17}H_{34}O_2$	270	1.75	Terpenoid
22.	Benzenepropanoic acid, 3,5-bis-(1,1-dimethylethyl)-4-hydroxy-, methyl ester	17.27	$C_{18}H_{28}O_3$	292	0.30	Phenol
23.	1-Hexadecen-3ol, 3,5,11,15-tetramethyl	17.39	$C_{20}H_{40}O$	296	0.17	Diterpene alcohol
24.	n-Hexadecanoic acid	17.53	$C_{16}H_{32}O_2$	256	9.11	Fatty acid
25.	1H-Naphtho[2,1b]pyran, 3-ethenyl dodecahydro-3,4a,7,7,10a pentamethyl-, [3R-(3à,4aá,6aà, 10aá,1bà)]-	18.22	$C_{20}H_{34}O$	290	0.13	Sesquiterpenoids
26.	Heptadecanoic acid	18.49	$C_{17}H_{34}O_2$	270	0.37	Fatty acid
27.	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	18.59	$C_{13}H_{15}NO_3$	233	0.39	Esters
28.	Phytol	19.06	$C_{20}H_{40}O$	296	6.77	Diterpenoid
29.	Methyl stearate	19.20	$C_{19}H_{38}O_2$	298	0.33	Fatty acid methyl ester
30.	Octadecanoid acid	19.61	$C_{18}H_{36}O_2$	284	1.86	Fatty acid
31.	4,8,12,16-Tetramethylhepatadecan-4-olide	22.35	$C_{21}H_{40}O_2$	324	0.15	Diterpenoid

(Synonym: Pyrogallol) is known for its fungicidal and fungistatic properties (Shukla *et al*., 1999). It is a polyphenol compound and an effective antimicrobial agent and its toxicity is attributed to the three hydroxyl groups present in its structure (Kocacaliskan *et al*., 2006; Cowan, 1999). It has also shown antitumor, antiviral, antibacterial, cardioprotective, prooxidant and anti-mutagenic activities (Rice *et al*., 1996; Chen *et al*., 1998). 2-Pentadecanone-6,10,14-trimethyl is a terpene ketone compound and has antimicrobial, antiosteoporotic properties (Govindaraj and Rajangam, 2017). 3,7,11,15-Tetramethyl-2-hexadecen-1-ol is a terpenol compound and has shown antioxidant antimicrobial activities, analgesic, anti-inflammatory, antipyretic, anticancer and antidiuretic bioactivity (Tsunoda, 1965; Dahpour *et al*., 2012). Hexadecanoic acid, methyl ester is a fatty acid ester and it possesses antioxidant and antifungal activities (Kosasih *et al*., 2023). Squalene (triterpene) is a phenolic compound and it possesses anti-microbial activity, chemo preventive activity against colon carcinogenesis (Rao *et al*., 1998).

Squalene is also reported to have anticancer, antitumour, chemopreventive, gastropreventive and hepatoprotective effects, pesticide, anti-tumor and sunscreen properties(Sunitha *et al.*, 2001; Ukiva *et al.*, 2002; Katerere *et al*., 2003). Phytol exhibits various pharmacological properties including toxicity and cytotoxicity, and exerts antitumor activity. It modulates pro-carcinogens as well as produced genotoxicity and death in breast cancer cells. It has also demonstrated DNA damage repair capabilities in mouse lymphocytes (deAlencar *et al*., 2019). It also has an antinociceptive effect. It may be associated with the antioxidant activity of phytol as demonstrated by *in vitro* methods used (Santos *et al.*, 2013). Additionally, phytol increases the activity of natural killer cells, which identify and eliminate cancer cells, and stimulates macrophage roles in immunity (Jeong, 2018). Phytol exhibits anti-angiogenic properties by inducing apoptosis in cancer cells, such as lung adenocarcinoma cells (Venugopala *et al*., 2013). Octadecanoic acid has an anticancer, antimicrobial and antioxidant activity. Ethyl isoallocholate possesses Antibacterial, Antioxidant, Anti-tumour, Cancer preventive, Chemo preventive and Pesticide activity. A natural product derived from plants, coumarin (2H-1-benzopyran-2-one) has a number of pharmacological benefits, including anti-inflammatory, anticoagulant, antiviral, antibacterial, antifungal, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, antihyperglycemic, antioxidant, and neuroprotective effects (Sakthivel *et al*., 2018). As a natural compound, phenol, 2,4-bis(1,1-dimethylethyl) has been reported to have many functions for medicine, food and agriculture. In medicine, it has antioxidant, anticancer, antifungal, antibacterial properties (Gupta and Kumar, 2017; Rice *et al*., 1996; Rao *et al.*, 1998).

CONCLUSION

The present study was focused on identification of various bioactive compounds from leaves of *Emblica officinalis* in acetone and methanol extracts by GC-MS/MS analysis. Among the identified compounds 1,2,3 Benzenetriol, Tetradecanoic acid, Squalene, Phytol and Phenol, 2,4-bis (1,1-dimethylethyl) have the role in antioxidant, anticancer, antifungal and antimicrobial properties. This study may provide future prospects for isolation, biological and medicinal characterization of some more compounds from the plant.

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DISCLAIMER

The author(s) declare no conflict of interest in the work.

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