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GC-MS ANALYSIS AND *IN-VITRO* ANTI-DIABETIC ACTIVITY OF BIOACTIVE FRACTIONS OF *FERONIA ELEPHANTUM* FRUIT

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Keywords:

GC-MS Analysis, *In-vitro* anti-diabetic, *Feronia elephantum* fruit, *α*-amylase, *α*-glucosidase

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ABSTRACT: The present study was carried out to characterize the bioactive phytoconstituents from the fractions of *F. elephantum* fruit and to evaluate their *in-vitro* anti-diabetic activity. Column chromatography of methanol extract of *F. elephantum* fruit yielded Hexane: Ethyl acetate (1:1 v/v) fraction (HEFE), Ethyl acetate fraction (EFE) and Ethyl acetate: Methanol (1:1 v/v) fraction (EMFE), which were subjected to GC-MS analysis. They were also tested for *in-vitro* α-amylase and *α*-glucosidase inhibitory potential. GC-MS analysis of EFE predominantly showed 2,5-Furandione, dihydro-3-methylene; n-Hexadecanoic acid; 5-Eicosene,(E)-; cis-13-Octadecenoic acid; and γ-Sitosterol; 2,5-Furandione, dihydro-3-methylene-; cis-Aconitic anhydride; Ethanol, 2,2'-[(1-methylethyl)imino]bis-; and Propanedioic acid, ethyl-, diethyl ester; were the major compounds in EMFE. HEFE showed 2,5-Furandione, dihydro-3-methylene (18.5%), Dodecanoic acid (4.48%), n-Hexadecanoic acid (15.18%) and cis-13-Octadecenoic acid (18.95%) which are biologically active. Moreover, the α-amylase IC_{50} values of HEFE, EFE, and EMFE were 68.77, 52.59, and 40.28 µg/mL, respectively, while that of acarbose was 41.99 µg/mL. And the α-glucosidase IC_{50} values of HEFE, EFE, and EMFE were 69.53, 35.08, and 42.49 µg/mL, respectively, which were comparable to that of acarbose (39.21 µg/ml). Findings of the present study clearly indicate that *F. elephantum* fruit possesses numerous bioactive components and potential *in-vitro* antidiabetic activity, thus justifies the use of this plant for different ailments by traditional medical practitioners.

INTRODUCTION: Plants produce an extensive range of bioactive phytochemical compounds with significant applications in different sectors. These compounds occur naturally in small quantities and are considered as secondary plant metabolites with pharmacological or toxicological properties in living organisms [1]. Among the secondary metabolites, polyphenolic compounds have a wide range of biological and physiological activities and serve as chemotaxonomic marker compounds [2].

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Feronia is a monotypic genus belonging to the family Rutaceae. *Feronia elephantum* correa (*Limonia acidissima* Linn, *Schinus limonia* Linnor *Feronia limonia*) is a moderate-sized tree whose parts such as fruits, leaves, root, bark, and gums have been used in traditional medicine for many ailments. The fruit (wood apple) contains flavonoids, saponins, glycosides, tannins, and some coumarins and tyramine derivatives [3]. Wood apple is a dry land fruit, which is a nutritious, rich in natural acids such as oxalic, tannic, mallic, and citric acid.

It is a source of calcium, phosphorus, iron and vitamins A, B and C. Seeds and fruits contained oil and protein; oil composed of palmitic, oleic, linoleic and linolenic acids besides traces of

palmitoleic and stearic acids; β-sitosterol, β-amyrin, lupeol and stigmasterol from unsaponifiable matter of seed oil. Fruit pulp has been reported for glycoside - 5,4-dihydroxy-3-(3-methyl-but-2-enyl) 3,5,6-trimethoxyflavone7 O-b-D-glucopyranoside [4]. According to Ayurveda, the fruits are used for heart diseases (cardiotonic), cough, vomiting, dysentery, removes biliousness, "tridosah", "vata", and blood impurities, thirst, fatigue, hiccough; tumors, asthma, leucorrhoea, ophthalmia. In Yunani, the fruits are cardiotonic, tonic to the lungs and the liver, diuretic, strengthening the gums; the juice is good for sore throat and stomatitis. The fruit pulp is also used by tribal of Rewa District of Madhya Pradesh against diabetes, boils and amoebiosis [5], and hence is regarded as one of the most valuable medicinal plants in India.

To the best of our knowledge, there have not been any earlier reports on fractions from the fruits of this plant. However, as part of our search for new natural products and bioactive compounds, an investigation of α-glucosidase and α-amylase inhibitory activity of crude methanolic and aqueous extracts of *F. elephantum* fruit was undertaken. We herein aim to examine the phytochemical constituents of different fractions of *F. elephantum* and their *in-vitro* antidiabetic activity using α-amylase and α-glucosidase inhibitory assay.

MATERIALS AND METHODS:

Chemicals and Reagents: P-Nitrophenyl-α-D-Glucopyranoside (pNPG), 3,5-DinitroSalicylicAcid (DNSA), α-amylase and α-glucosidase enzymes and acarbose, were purchased from M/S Sigma–Aldrich Chemicals Pvt., Limited, Bangalore. All other chemicals and reagents used were of high purity analytical grade.

Collection of Plant and Preparation of Fractions: Ripe fruits of *F. elephantum* were collected (voucher no. 1328) and processed as described in our previous work [6]. *F. elephantum* fruits powder was extracted with methanol by soxhlation, filtered, and evaporated under reduced pressure for viscous extract using Rotavapor (Buchi R-200). It was fractionated using column chromatography on silica gel with n-hexane yielding insoluble fraction, which was further fractionated with a mixture of an equal ratio of n-hexane and Ethyl acetate (1:1) yielding a soluble fraction and an insoluble fraction. The insoluble fraction of n-hexane and Ethyl acetate was then fractionated using ethyl acetate, yielding the ethyl acetate soluble fraction and insoluble fraction, which was then fractionated using methanol. All the fractions were concentrated by rotary vacuum evaporator and labeled as follows; Hexane: Ethyl acetate (1:1 v/v) fraction of *F. elephantum* as HEFE, Ethyl acetate fraction of *F. elephantum* as EFE and Ethyl acetate: Methanol (1:1 v/v) fraction of *F. elephantum* as EMFE.

Phytochemical Analysis of Bioactive Fractions using Gas Chromatography-Mass Spectrometry (GC-MS): GC-MS analysis of the fractions of *F. elephantum viz*., HEFE, EFE, and EMFE was carried out using GC (Agilent 7890A) with DB 5 Ms Column (30m L × 0.25mm ID × 0.25um film thickness). Helium (99.9995%) was used as carrier gas (flow rate 1 mL/min), and an injection volume of 1 µL was employed in a splitless mode. The injection temperature was 250 °C, and the auxiliary temp was 290 °C. Mass Spectrophotometer (5975C MSD) with Electron Impact Ionization and Quadrupole Mass Analyzer was used with the Scan Mass range of 30m/z to 700m/z. The MS source temperature was 250 °C, and the MS quad temperature was 180 °C in **Table 1**.

Sample Preparation: Given a sample made up to 2 mL with the respective solvent. It again diluted with 20 µL in 980 µL of solvent and injected 1uL into the GCMS instrument.

TABLE 1: TEMPERATURE RAMP

	Rate °C/min	Temp. (°C)	Hold time (min)	Run time (min)
Initial		50	1	1
Ramp 1	10	280	5	29

The phytoconstituents of HEFE, EFE, and EMFE were identified by comparison of mass spectra with the national libraries (NIST - 11). The molecular formula, molecular weight, and structure of the identified compounds were ascertained.

***In-vitro* α-Amylase Inhibitory Activity:** The α-amylase inhibitory potential of all fractions was evaluated using 3, 5-dinitrosalicylic acid (DNSA) which is based on the spectrophotometric method using acarbose as standard reference 6. Stock

solutions (500 µg /mL in distilled water) of HEFE, EFE, EMFE, and positive control, acarbose were prepared.500 µL of different concentrations (10, 20, 40, 80 and 160 µg/mL) of each sample were added to a 500 µL solution of α-amylase (0.5 mg/mL in 0.02 M,pH 6.9 sodium phosphate buffer) and was incubated for 10 min. Then add 500 µL of starch solution 1% (w/v) and incubate for 10 min at 25 °C.

The coloring reagent, DNSA (1 mL)was added, and heat the reaction mixture in a boiling water bath for 5 min, cool to room temperature. Then dilute it with 10 mL of distilled water and measure the absorbance at 540 nm using a UV-VIS spectro-photometer (ELICO SL159). A blank solution was prepared by substituting the α-amylase enzyme solution with 500 µl of sodium phosphate buffer. The tests were repeated thrice with the same protocol.

***In-vitro* α-glucosidase Inhibitory Activity:** The study was performed using α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (pNPG) as per previously reported model.6Each of 100 µLof HEFE, EFE, EMFE and positive control, acarbose at different doses (10, 20, 40, 80 and 160 µg/mL) was added to 50 µL of α-glucosidase (1 U/mL) prepared in 0.1 M phosphate buffer (pH 6.9). Then, add 250 µL of 0.1 M phosphate buffer. The mixture was incubated at 37 °C for 20 min. Then, 10 µl of 10 mM pNPG (in 0.1 M phosphate buffer, pH 6.9) was added and incubated at 37 °C for 30 min. The reactions were stopped by adding 650 µl of 1 M sodium carbonate, and the absorbance was measured at 405 nm in triplicate against the blank solution with 100% enzyme activity

Method for Calculation of α-amylase and α-Glucosidase Inhibitory Concentration (IC_{50}): The percentage of enzyme inhibition was calculated using the formula:

$$\% \text{ inhibition} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control (blank with 100% enzyme activity), and A_{sample} is the absorbance of the sample.

The concentration of the fraction required to inhibit 50% of α-amylase and α-glucosidase activity under the assay conditions is defined as the IC_{50} value. IC_{50} was calculated by using the percentage inhibition at five different concentrations of the fractions by plotting percentage inhibition against the concentrations. The IC_{50} value was calculated by using Linear Regression analysis.

RESULTS AND DISCUSSION:

Characterization of the Phytochemical Compounds of HEFE, EFE and EMFE using GC-MS: Three fractions were separated from methanol extract of *F. elephantum* fruit *viz*., HEFE, EFE, and EMFE. GC-MS analysis of HEFE, EFE, and EMFE revealed the presence of various complex compounds. GC-MS analysis of EFE shown 2,5-Furandione, dihydro-3-methylene (44.78%), n-Hexadecanoic acid (6.62%), 5-Eicosene, (E)- (4.04%), cis-13-Octadecenoic acid (6.08%) and γ-Sitosterol (2.99) as prominent compounds as presented in **Fig. 1** along with other phytoconstituents, as reported in **Table 2**.

2,5-Furandione, dihydro-3-methylene- (68.47%), cis-Aconitic anhydride (5.19%), Ethanol, 2,2'-[(1-methylethyl) imino]bis- (6.27%) and Propanedioic acid, ethyl-, diethyl ester (7.11%) are the major compounds with higher peak areas in EMFE as seen in **Fig. 2**, listed in **Table 3**.

GC-MS profiling of HEFE shown different compounds as presented in **Table 4**, out of which the prominent constituents with predominant peak area, as shown in **Fig. 3** are 2,5-Furandione, dihydro-3-methylene (18.5%), Dodecanoic acid (4.48%), n-Hexadecanoic acid (15.18%) and cis-13-Octadecenoic acid (18.95%) which are biologically active. Other important bioactive compounds present in the fractions of *F. elephantum* fruit are L-Glutamic acid, dimethyl ester; E-15-Hepta-decenal; Phenol, 2, 4-bis (1, 1-dimethylethyl; cis-Linaloloxide; citric acid; 6-Isopropenyl-4, 8a-dimethyl-1,2,3,5,6,7,8,8a-octa-hydro-naphthalen-2-ol; 9,12-Octadecadienoic acid (Z,Z)-; Dichloro-acetic acid, heptadecyl ester *etc*., which have been reported for anti-oxidant, antiinflammatory, hypo-cholesterolemic, anti-diabetic, anti-cancer, anti-microbial, antitubercular, antibacterial, antifungal activities which are represented in respective tables. These bioactive compounds are reported for the first time in the fractions of *F. elephantum* fruit through the present study.

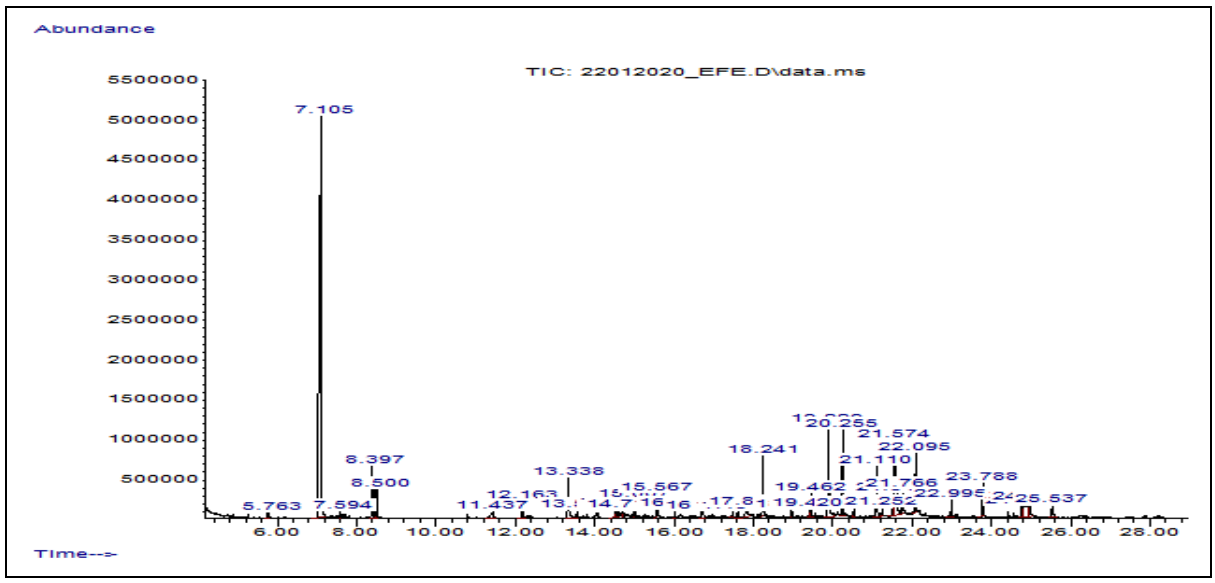

FIG. 1: GCMS CHROMATOGRAM OF EFE

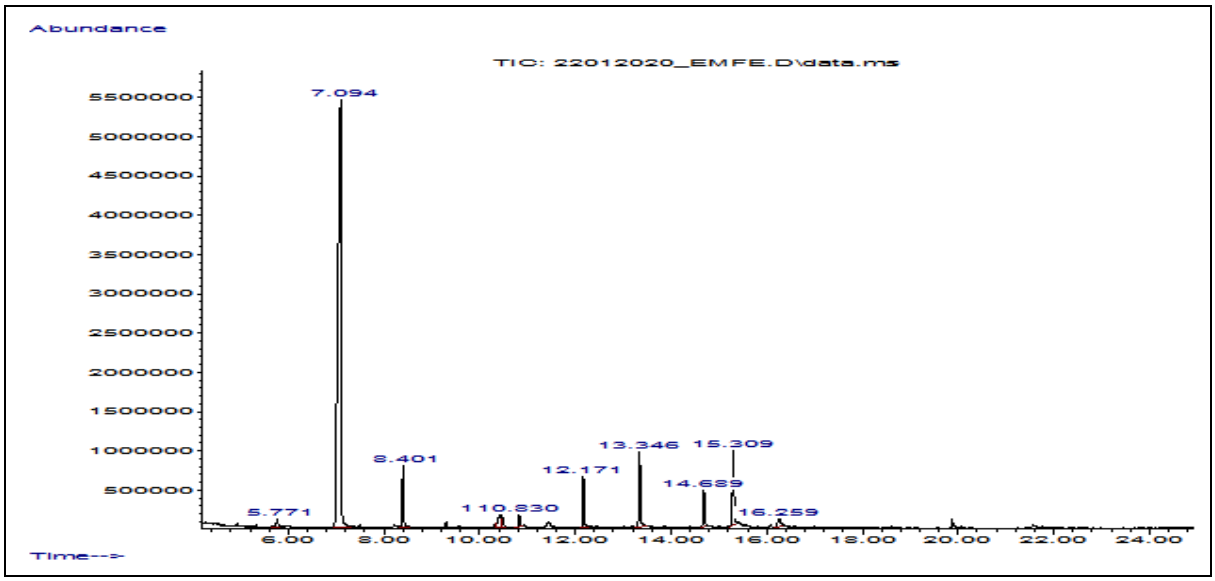

FIG. 2: GCMS CHROMATOGRAM OF EMFE

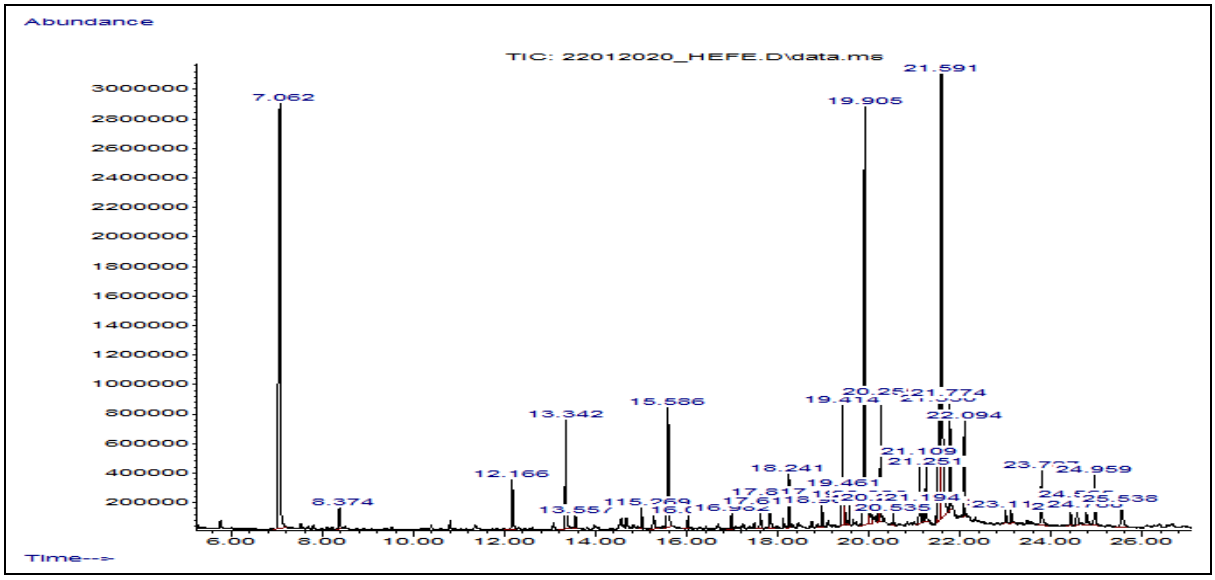

FIG. 3: GCMS CHROMATOGRAM OF HEFE

TABLE 2: PHYTOCONSTITUENTS IDENTIFIED FROM EFE BY GC-MS ANALYSIS AND THEIR REPORTED BIOLOGICAL ACTIVITIES

RT (min)	% Area	Compound Name	CAS #	MF and MW (g/mol)	Reported Biological Activities
5.762	0.283	Maleic anhydride	000108-31-6	$C_4H_2O_3$ 98.06	Antitumor, immunostimulator antiviral,[7] antifungal [8]
*7.105, 8.398	44.78, 3.24	2,5-Furandione, dihydro-3-methylene	002170-03-8	$C_5H_4O_3$ 112.08	Antitubercular, antibacterial, antifungal, antileprotic, anticancer [9]
7.595	0.26	Allyl(ethoxy)dimethylsilane	018269-47-1	$C_7H_{16}OSi$ 144.29	Not reported
8.500	1.51	2-Pyrrolidinone, 1-methyl-	000872-50-4	C_5H_9NO 99.13	Not reported
11.435	0.99	Butanedioic acid, methylene-	000097-65-4	$C_5H_6O_4$ 130.09	Not Reported
12.163	1.11	2-Furanmethanol	000098-00-0	$C_5H_6O_2$ 98.10	Not Reported
13.337	2.46	L-Glutamic acid, dimethyl ester	006525-53-7	$C_7H_{13}NO_4$ 175.18	Antidiabetic [10]
13.558	0.37	Dichloroacetic acid, tridecyl ester	1000280-48-3	$C_{15}H_{28}Cl_2O_2$ 311.30	Not Reported
14.517	0.31	Benzoic acid,4-hydroxy-	000099-96-7	$C_7H_6O_3$ 138.12	Antibacterial, antifungal, antialgal, antimutagenic, antisickling, estrogenic, antiatherogenic, antiplatelet, hypoglycemic, anti-inflammatory, antioxidant [11]
14.558	0.49	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	000719-22-2	$C_{14}H_{20}O_2$ 220.30	Not reported
14.714	0.40	Ala-Gly, N-trimethylsilyl-, trimethylsilyl ester	1000333-69-9	$C_8H_{18}N_2O_3Si$ 218.32	Not reported
15.007	0.80	Phenol, 2,4-bis(1,1-dimethylethyl)	000096-76-4	$C_{14}H_{22}O$ 206.32	anti-pathogenic agent (drug resistant infections),[12]Antioxidant, Anti-Inflammatory, Anticancer, Insecticidal and Nematicidal [13]
15.568	1.28	Dodecanoic acid	000143-07-7	$C_{12}H_{24}O_2$ 200.32	Hypolipidemic, Antimicrobial, In cardiovascular disorders, Antihypertensive, prostatic hyperplasia and colon cancer prevention, antioxidant, Anticancer [14]
*16.024, 18.242 & 25.538	0.43, 2.78 and 1.09	1-Nonadecene	018435-45-5	$C_{19}H_{38}$ 266.50	Antifungal, Antioxidant, antitubercular, anticancer [15]
16.714	0.79	Disilane, ethylpentamethyl-	015063-64-6	$C_7H_{20}Si_2$ 160.40	Not reported
17.480	0.36	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	000088-58-4	$C_{14}H_{22}O_2$ 222.32	Not reported
17.616	0.36	2-Chloro-5,6-dihydro-4H-benzothiazol-7-one	330203-55-9	C_7H_6ClNOS 187.65	Not reported
17.813	1.16	Tetradecanoic acid	000544-63-8	$C_{14}H_{28}O_2$ 228.37	Larvicidal and repellent, antitumor activity, Antibacterial [16]
18.966	0.34	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	000084-69-5	$C_{16}H_{22}O_4$ 278.34	Anticancer, antimicrobial, antiarthritic [17]
19.412	0.37	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.α,4a.α,7.β,7.a.β,7.b.α)]-	006750-60-3	$C_{15}H_{24}O$ 220.35	Not Reported
19.463	1.17	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	082304-66-3	$C_{17}H_{24}O_3$ 276.37	Not Reported
19.888	6.62	n-Hexadecanoic acid	000057-10-3	$C_{16}H_{32}O_2$ 256.42	Antiinflammatory, hypocholesterolemic antispasmodic, anticancer and antiviral, nematicide,

					pesticide, hemolytic,[18] 5-Alpha reductase inhibitor, potent mosquito larvicide [19]
20.255	4.04	5-Eicosene,(E)-	074685-30-6	$C_{20}H_{40}$ 280.53	Antitumor, antifungal, cytotoxic, Antibacterial [20]
20.534	0.38	Octadecanal	000638-66-4	$C_{18}H_{36}O$ 268.47	Not Reported
21.109	2.61	Cyclohexadecane	000295-65-8	$C_{16}H_{32}$ 224.42	Antioxidant, antibacterial, antifungal [21]
21.252	0.42	trans-13-Octadecenoic acid, methyl ester	1000333-61-3	$C_{19}H_{36}O_2$ 296.00	Anti-cancer, Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene-D4, hypocholesterolemic,5-alpha reductase inhibitor, anemiagenic [19]
21.517	1.04	9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	$C_{18}H_{32}O_2$ 280.40	Antiarthritic, Anti-inflammatory [22]
21.575	6.08	cis-13-Octadecenoic acid	013126-39-1	$C_{18}H_{34}O_2$ 282.46	Not reported
21.765	1.27	Octadecanoic acid	000057-11-4	$C_{18}H_{36}O_2$ 284.00	Antimicrobial [19]
22.095	3.04	E-15-Heptadecenal	1000130-97-9	$C_{17}H_{32}O$ 252.40	Not reported.
22.993	0.88	1-Octadecene	000112-88-9	$C_{18}H_{36}$ 252.47	Antibacterial, antioxidant, anticancer [15]
23.789	1.58	1-Heneicosanol	015594-90-8	$C_{21}H_{44}O$ 312.57	Antifungal [23]
24.769	0.76	Hexadecanoic acid,2,3-dihydroxypropyl ester	000542-44-9	$C_{19}H_{38}O_4$ 330.50	Antimicrobial [16]
24.861	2.99	γ-Sitosterol	000083-47-6	$C_{29}H_{50}O$ 414.70	Anticancerous, hepatoprotective, antihyperglycemic, antidiabetic [22]
24.959	1.14	1,2-Benzenedicarboxylic acid, diisooctyl ester	027554-26-3	$C_{24}H_{38}O_4$ 390.55	Antimicrobial, antifungal [24]

RT = Retention Time, MF = Molecular formula and MW=Molecular Weight. *Same compound but appeared at two different RT (min) showing two distinct peaks on the spectrum and with different % compositions

TABLE 3: PHYTOCOMPONENTS IDENTIFIED FROM EMFE BY GC-MS ANALYSIS AND THEIR REPORTED BIOLOGICAL ACTIVITIES

RT (min)	% Area	Compound Name	CAS #	MF and MW (g/mol)	Reported Biological Activities
5.772	0.89	4-Methylthieno[2,3-b]pyridine	013362-81-7	C_8H_7NS 149.21	Antitumor [25]
7.095	68.47	2,5-Furandione, dihydro-3-methylene-	002170-03-8	$C_5H_4O_3$, 112.08	Antitubercular, antibacterial, antifungal, antileprotic [9]
8.401	5.19	cis-Aconitic anhydride	006318-55-4	$C_6H_4O_5$, 156.09	Not Reported
10.422	2.05	Furan	000110-00-9	C_4H_4O, 68.07	Its Derivatives are used.
10.473	1.12	4-Methyl itaconate	007338-27-4	$C_6H_8O_4$, 144.12	Anticancer, Antiinflammatory, antioxidant [26]
10.830	1.05	2-(Bromomethyl)acrylic acid	072707-66-5	$C_4H_5BrO_2$, 164.99	Not reported
12.170	3.77	cis-Linaloloxide	1000121-97-4	$C_{10}H_{18}O_2$, 170.24	Nematicide
13.347	6.27	Ethanol,2,2'-[(1-methylethyl)imino]bis-	000121-93-7	$C_7H_{17}NO_2$, 147.21	Not reported
14.691	2.90	Ethanamine, N-methyl-N-nitroso-	010595-95-6	$C_3H_8N_2O$, 88.1084	Not reported
15.310	7.11	Propanedioic acid, ethyl-, diethylester	000133-13-1	$C_9H_{16}O_4$, 188.22	Antiinflammatory [27]
16.259	1.18	Citric Acid	000077-92-9	$C_6H_8O_7$, 192.12	Antioxidant

RT = Retention Time, MF = Molecular formula and MW = Molecular Weight

TABLE 4: PHYTOCOMPONENTS IDENTIFIED FROM HEFE BY GC-MS ANALYSIS AND THEIR REPORTED BIOLOGICAL ACTIVITIES

RT (min)	% Area	Compound Name	CAS #	MF and MW (g/mol)	Reported Biological Activities
7.061	18.52	2,5-Furandione, dihydro-3-methylene-	002170-03	$C_5H_4O_3$ 112.08	Antitubercular, antibacterial, antifungal, antileprotic [9]
8.374	0.63	cis-Aconitic anhydride	006318-55-4	$C_6H_4O_5$ 156.09	Not reported
12.167	1.65	3-Pyridinecarboxylic acid, 1,6-dihydro-6-oxo	005006-66-6	$C_6H_5NO_3$ 139.11	Cardiotonic [28]
13.343	3.55	Ethanol,2,2'-[(1-methylethyl)imino]bis-	000121-93-7	$C_7H_{17}NO_2$ 147.21	Not reported
13.558	0.37	1-Tetradecene	001120-36-1	$C_{14}H_{28}$ 196.37	Not reported
15.007	0.55	Phenol,2,4-bis(1,1-dimethylethyl)	000096-76-4	$C_{14}H_{22}O$ 206.32	Antimicrobial, antifungal, antioxidant,[12]Antibacterial [13]
15.269	0.72	Malonic acid, butyl 2-hexyl ester	1000349-32-0	$C_{13}H_{24}O_4$ 244.32	Not reported
15.585	4.48	Dodecanoic acid	000143-07-7	$C_{12}H_{24}O_2$ 200.31	Hypolipidemic, Antimicrobial, In cardiovascular disorders, antihypertensive prostatic hyperplasia and colon cancer-preventive, antioxidant, Anticancer [14]
16.024	0.36	Tridecylpentafluoropropionate	1000351-80-2	$C_{16}H_{27}F$ 346.38	Not reported
16.983	0.46	2-Naphthalenemethanol, decahydro-.α,α.,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]-	000473-15-4	$C_{15}H_{26}O$ 222.37	Not reported
17.612	0.62	Neoisolongifolene, 8,9-dehydro-	067517-14-0	$C_{15}H_{22}$ 202.33	Not reported
17.816	1.06	Tetradecanoic acid	000544-63-8	$C_{14}H_{28}O_2$ 228.37	Larvicidal and repellent, antitumor activity, Antibacterial [16]
18.242	1.43	1-Nonadecene	018435-45-5	$C_{19}H_{38}$ 266.50	Antituberculosis, anticancer, antioxidant, antimicrobial [15]
18.966	0.61	Phthalic acid, isobutyl octyl ester	1000309-04-5	$C_{20}H_{30}O_4$ 334.40	Antimicrobial.
19.415	3.43	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1000189-10-2	$C_{15}H_{24}O$ 220.35	Not reported
19.459	0.98	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	082304-66-3	$C_{17}H_{24}O_3$ 276.40	Not reported
19.568	0.62	Octadecanoic acid	000057-11-4	$C_{18}H_{36}O_2$ 284.48	Antimicrobial [19]
19.905	15.18	n-Hexadecanoic acid	000057-10-3	$C_{16}H_{32}O_2$ 256.42	Antiinflammatory antispasmodic,[18] anticancer and antiviral, hypocholesterolemicnematicide, pesticide, antiandrogenicflavor, hemolytic, 5-Alpha reductase inhibitor,[19] potent mosquito larvicide
20.031	0.83	2-Methyl-5-(1-adamantyl)pentan-2-o	095477-25-1	$C_{16}H_{28}Oc$ 236.39	Not reported
20.208	0.53	2-Naphthalenemethanol, decahydro-α.,α.,4a-trimethyl-8-methylene-[2R-(2.α.4a.α.,8a.β)]-	000473-15-4	$C_{15}H_{26}O$ 222.37	Not reported
20.255	3.20	5-Eicosene,(E)-	074685-30-6	$C_{20}H_{40}$ 280.53	Antibacterial Antitumor, antifungal, cytotoxic [20]
20.534	0.40	1,19-Eicosadiene	014811-95-1	$C_{20}H_{38}$ 278.50	Not reported
21.109	1.84	n-Nonadecanol-1	001454-84-8	$C_{19}H_{40}O$ 284.50	Not reported
21.194	0.47	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	000112-63-0	$C_{19}H_{34}O_2$ 294.00	Anti-cancer, Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, hypocholesterolemic

					[22]
21.252	1.40	11-Octadecenoic acid, methyl ester	052380-33-3	$C_{19}H_{36}O_2$ 296.5	Not reported
21.531	4.00	9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	$C_{18}H_{32}O_2$ 280.4	antiarthritic, Anti-inflammatory, cancer preventive, hypocholesterolemic [22]
21.592	18.95	cis-13-Octadecenoic acid	013126-39-1	$C_{18}H_{34}O_2$ 282.4614	Not reported
21.776	3.16	Octadecanoic acid	000057-11-4	$C_{18}H_{36}O_2$ 284.48	Antimicrobial [22]
22.095	2.32	Dichloroacetic acid, heptadecyl ester	1000282-98-2	$C_{19}H_{36}Cl_2O_2$367.40	Not reported
22.997	0.48	Octadecyltrifluoroacetate	079392-43-1	$C_{20}H_{37}F_3O_2$ 366.50	Not reported
23.116	0.43	1-Phenyl-3,6-diazahomoadamantan-9-one hydrazone	1000216-29-0	$C_{15}H_{20}N_4$ 256.35	Not reported
23.786	1.96	Pentadecyltrifluoroacetate	1000351-74-4	$C_{17}H_{31}F_3O_2$ 324.40	Not reported
24.425	0.39	Naphthalene, 1,2,3,4-tetrahydro-1-methoxy-	001008-18-0	$C_{11}H_{14}O$ 162.23	Not Reported.
24.565	0.78	Benzene,1,1'-(2-butene-1,4-diyl)bis-	013657-49-3	$C_{16}H_{16}$ 208.30	Not reported
24.769	0.71	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	023470-00-0	$C_{19}H_{38}O_4$ 330.30	antioxidant, antiinflammatory, anthelmintic [27]
24.959	1.65	Mono(2-ethylhexyl) phthalate	004376-20-9	$C_{16}H_{22}O_4$ 278.34	Not reported
25.538	1.29	9-Hexacosene	071502-22-2	$C_{26}H_{52}$ 364.70	Not reported

RT = Retention Time, MF = Molecular formula and MW = Molecular Weight

***In-vitro* α-amylase Inhibitory Activity of HEFE, EFE and EMFE:** Three fractions *viz*., HEFE, EFE, and EMFE were screened at different doses *for in-vitro* α-amylase inhibitory potential, which might be deduced to perceive their antidiabetic potential. The results showed a concentration-dependent rise in the percentage inhibitory activity against the α-amylase enzyme, as represented in **Table 5**. HEFE, EFE, and EMFE at the maximum tested concentration, 160 µg/mL showed a percentage inhibition of 90.21, 91.65 and 97.53 respectively, while acarbose showed 95.06, which infers the inhibitory potential of fractions is comparable to that of standard reference, acarbose.

The IC_{50} values of HEFE, EFE, and EMFE were 68.77, 52.59, and 40.28 µg/mL, respectively, while that of acarbose was 41.99 µg/mL. Since α-amylase plays a vital role in starch absorption in human beings and animals, the presence of such inhibitors in plant extracts or foodstuffs may be responsible for impaired starch digestion and thus anti-hyperglycemic effect [29].

***In-vitro* α-glucosidase Inhibitory Activity of HEFE, EFE and EMFE:** The obtained results showed a dose-dependent escalation in the percentage inhibition of α-glucosidase enzyme as depicted in **Table 5**. HEFE, EFE, and EMFE at the highest tested concentration, 160 µg/mL showed a percentage inhibition of 89.34, 93.70 and 94.38, respectively, which were comparable to that of standard drug acarbose with 90.65. The IC_{50} values of HEFE, EFE, and EMFE were 69.53, 35.08 and 42.49 µg/mL, respectively, which were comparable to that of acarbose (39.21 µg/ml), however, there was a significant difference between the IC_{50} of acarbose and HEFE. The α-glucosidase inhibitory effect exhibited by all the fractions indicates their potential effectiveness at managing Diabetes Mellitus, possibly by reducing postprandial glycemic levels and the total range of postprandial glucose levels [30].

HEFE, EFE, and EMFE efficiently inhibited α-amylase and α-glucosidase enzymes *in-vitro*, which might be due to the presence of bioactive phytoconstituents like L-Glutamic acid dimethyl ester11, Benzoic acid 4-hydroxy12, Dodecanoic acid, 132,5-Furandione, dihydro-3-methylene, n-Hexadecanoic acid, 5-Eicosene,(E)-, γ-Sitosterol, 9,12-Octadecadienoic acid (Z,Z)-,methyl ester that were earlier reported for antidiabetic activity.

These results indicate that *F. elephantum* fruit could be used to reduce post-prandial blood glucose levels and may be of worth as novel therapeutic agents in the treatment of Diabetes Mellitus.

TABLE 5: α-AMYLASE AND α-GLUCOSIDASE INHIBITION ASSAY OF HEFE, EFE AND EMFE

S. no.	Sample	Concentration (µg/mL)	% inhibition of α-amylase activity	IC_{50} (µg/mL)	% inhibition of α-glucosidase activity	IC_{50} (µg/mL)
1	HEFE	10	8.10 ± 0.84	68.77	10.32 ± 1.15	69.53
		20	15.73 ± 1.09		18.28 ± 1.84	
		40	28.03 ± 2.11		29.09 ± 2.30	
		80	58.18 ± 2.82		60.04 ± 1.95	
		160	81.21 ± 2.56		83.34 ± 2.71	
2	EFE	10	14.08± 1.06	52.60	16.35 ± 1.21	35.08
		20	25.84 ± 1.57		30.28 ± 2.07	
		40	46.57 ± 2.32		57.92 ± 1.14	
		80	72.68 ± 2.15		74.11 ± 1.45	
		160	91.65 ± 2.63		90.65 ± 3.58	
3	EMFE	10	18. 28± 1.34	40.29	17.16 ± 0.90	42.49
		20	34. 05 ± 1.98		30.13 ± 1.96	
		40	59.57 ± 2.20		55.18 ± 2.14	
		80	73.19 ± 2.36		76.29 ± 2.57	
		160	95.06 ± 2.59		93.70 ± 2.32	
4	Acarbose	10	16.10 ± 1.01	41.99	18.07 ±1.03	39.21
		20	31.18 ± 1.54		32.09 ±1.51	
		40	56.12 ± 2.03		57.23±1.67	
		80	80.12 ± 2.38		79.16 ±1.43	
		160	97.53 ± 2.75		94.38 ±1.89	

All determinations were carried out in the triplicate manner, and values are expressed as the mean ± SEM

CONCLUSION: Findings of the present study clearly indicate that *F. elephantum* fruit possesses considerable inhibitory activity against α-amylases and α-glucosidases, with remarkable activity in EFE and EMFE. This observed antidiabetic activity of EFE, EMFE, and HEFE might be attributed to the bioactive compounds like L-Glutamic acid dimethyl ester, Benzoic acid 4-hydroxy, Dodecanoic acid, 2,5-Furandione,dihydro-3-methylene, n-Hexadecanoic acid, 5-Eicosene,(E)-, γ-Sitosterol, 9,12-Octadecadienoic acid (Z,Z)-,methyl ester which are identified as prominent compounds by GC-MS analysis.

This present work discloses the goodness of *F. elephantum* with numerous bioactive components, potential *in-vitro* antidiabetic activity, *and* other earlier reported biological activities justifies the use of this plant for different ailments by traditional medical practitioners. However, isolation of specific phytoconstituents and studying their biological activity will certainly give productive results.

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