https://doi.org/10.31351/vol33iss2pp121-137

Alpha-Amylase and Glucoamylase Inhibitory Assessment of Methanol Extract of *Fagonia Indica* and its GC-MS analysis

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Received 26/2/2023, Accepted 9/5/2023, Published 27/6/2024

Abstract

Background

Plants have various biological activities due to the presence of bioactive compounds used in the treatment of human ailments.

Objective

The aim of the present study was to investigate the inhibition of enzymes alpha-amylase and glucoamylase by methanol extract of the plant *Fagonia indica* Burm.f. responsible for hypoglycemic activity.

Methods

This study was performed for phytochemical investigation of this plant using standard methods. Both qualitative and quantitative analysis of the methanol extract of the plant was done for the analysis and estimation of phytochemical constituents. Alpha-amylase and glucoamylase inhibition by this extract was assessed for the evaluation of hypoglycemic activity. Gas chromatographic-mass spectrometric (GC-MS) analysis of hypoglycemic methanol extract was done to identify the compounds responsible for this activity.

Results

Phytochemical analysis exhibited the presence of various phytoconstituents in methanol extract. The maximum %age of enzymes inhibition of this extract was 49.1 ± 2.4% (IC50 values of 104.2 ± 8.7 µg/ml) and 31.7 ± 2.9% (IC50 values of 273.7 ± 54.6 µg/ml) for alpha-amylase and glucoamylase enzymes respectively at 100 µg/ml concentration. The standard acarbose showed 68.91±3.0% (IC50=52.9±4.8 µg/ml) and 57.3±0.4% (IC50=70.2±3.4 µg/ml) inhibition of these enzymes respectively at the same concentration. GC-MS analysis identified 15 compounds in this extract.

Conclusion

This finding confirms the traditional use of this plant in managing diabetes. The compounds identified are pharmacologically significant.

Keywords: Hypoglycemic, *Fagonia indica,* phytochemical screening, acarbose, GC-M

Introduction

Diabetes mellitus (DM) has emerged as one of the most common problems, increasing the risk of cardiovascular disease and death [(1)]. It is characterized by an increase in blood glucose level and there are disturbances in the metabolism of fat, carbohydrate, and protein, which are secondary to insulin deficiency [(2)]. Internationally it is the biggest health problem of the twentieth century. If there is no response to the increasing epidemic of diabetes, the global figure will rise to 642 million by 2040[(3)]. During the last 10 years, the occurrence of DM has increased in low- and middle-income nations in comparison to high-income nations [(4)].

Postprandial hyperglycemia is the term for the high, unexpected increase in blood glucose levels which is caused by inability of cells to make or utilize insulin. The enzymes (alpha-amylase and alpha-glucosidase) are essential for the digestion of polysaccharides. A well-known pharmacological effect for reducing postprandial hyperglycemia is the

suppression of these enzymes. Natural products that may inhibit these enzymes are preferred over synthetic substances for decreasing postprandial glycemia due to less adverse effects [1].

A significant medicinal plant named *Fagonia indica* Burm.f. is distributed in tropical, subtropical, dry, and hot areas all over the world. *F. indica* (var. *indica*) is available westwards to tropical Africa (North and East) and in Pakistan [5]. It is used medicinally in the treatment of fever, asthma, dysentery, liver trouble, toothache, stomach troubles and skin diseases [6]. Traditionally, it is used therapeutically as a hypoglycemic agent [7]. The plant has a number of biological activities such as antibacterial, hepatoprotective, cytotoxicity and antioxidant activities [8-11].

Recently the techniques fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry (GC-MS) have been used to detect different functional groups and identify therapeutically biocompounds found in medicinal plants [12,13]. GC-MS is one of the best, fastest, and most accurate procedures for a variety of compound detection. A large number of natural compounds are present in the plants showing the inhibition of enzymes (alpha amylase and alpha glucosidase). The researchers have identified different therapeutic compounds through GC-MS [14,15].

No hypoglycemic activity involving alpha amylase and alpha glucosidase (glucoamylase) inhibitory models has yet been evaluated of methanol extract prepared from aerial parts of *Fagonia indica* (var. *indica*). Also, no analytical reports are present about GC-MS analysis of methanol extract of this plant indigenous to Pakistan. This investigation is useful for the characterization purpose of different phytocomponents. There is a need of the hour to characterize and identify the compounds present in this plant responsible for different biological activities. So, the aim of this investigation was to evaluate alpha amylase and glucoamylase inhibition of methanol extract of aerial parts of var. *indica* of the plant *Fagonia indica* for the assessment of hypoglycemic activity. It was followed by GC-MS analysis to detect and identify phytochemical compounds present in this extract which would be responsible for inhibiting these enzymes.

Materials and methods

Chemicals and solvents

Chemicals and solvents used during the study were of analytical grade. Chloroform, n-hexane, methanol, ethanol, ethyl acetate, dichloromethane, dimethyl sulfoxide, acetone, potassium acetate, aluminium nitrate, glacial acetic acid, concentrated sulfuric acid (H_2SO_4) of E. Merck, Darmstadt, Germany and Sigma Aldrich Chemical Co. USA were purchased. Sodium hydroxide, 3, 5-dinitro salicylic acid (DNS), sodium carbonate, potassium sodium tartrate, sodium chloride, potassium sodium tartarate, dimethyl sulfoxide (DMSO), the enzyme porcine pancreatic α-amylase and acetone powder of rat intestine for glucoamylase (Sigma Aldrich Chemical Co. USA) were used. Acarbose (Glucobay ®) was supplied by Bayer AG (Wuppertal, Germany).

Instruments

Rotary vacuum evaporator (Heidolph Laborota 4002, Germany), oven (Memert, UNB 500, Germany), freeze dryer (Vaco 2 Zirbus technology), GC-MS (5975C Agilent system), incubator (Memmert, W. Germany), vortex mixer (Biobase Meihua) and multichannel pipette (Dragon).

Plant material, its identification, and authentication

Aerial parts (stem, leaves, twigs, and fruit) of the plant *F. indica* were collected in July 2018 from District Khanewal, Pakistan. The plant was identified and authenticated by Prof. Dr. Zaheer-ud-Din Khan, a plant Taxonomist, The Department of Botany, GC University, Lahore, Pakistan. A voucher with the specimen (GC-Herb-Bot. 2967) was submitted to the same university.

Drying and pulverization

Aerial parts of the plant were washed, dried at room temperature in the shade, powdered and stored in airtight glass containers.

Preparation of plant extract

Powder of the aerial parts of plant *F. indica* was successively extracted with different organic solvents (n-Hexane, chloroform and methanol) in the order of increasing polarity. One kg of plant powder was macerated in a glass jar using a cold maceration method in 4 liter n-hexane solvent with frequent stirring for 3 days. Then it was first filtered with muslin cloth and after through Whatman No.1 filter paper. The residue was further re-extracted two times using n-hexane solvent till exhaustion and all the filtrates were pooled together. The resulting residue was air dried and further extracted with chloroform and it was followed by methanol similar to the method carried out for n-hexane extraction. The precipitate remaining in methanol extract was extracted twice with 30 ml of 80% ethanol to remove sugars in the precipitate. This process will not interfere with all the biological and chemical assays Finally the extracts (n-Hexane, chloroform and methanol) obtained after fractionation were concentrated at 40°C temperature and low pressure using a rotary vacuum evaporator (Heidolph, Laborota 4002, Germany). Methanol extract was packed in airtight glass containers and

stored in refrigerator below 10 ºC for biological and analytical study.

Preparation of stock solution of methanol extract/standard (acarbose)

Stock solutions of methanol extract/standard acarbose were prepared in methanol separately. Dilutions (20-100 µg/mL) of the extract and standard were prepared in the solvent methanol. In the preparation of 20 µg/mL solution of methanol extract/acarbose, 20 µL from the stock solution of methanol extract/standard was taken separately in the test tubes. Finally, the volume was made up to 1000 µL. Similarly, various dilutions i.e., 40-100 µg/mL of methanol extract/acarbose were prepared in the solvent methanol.

Phytochemical investigation

Qualitative analysis

It was carried out to analyze different phytochemical constituents present in the methanol extract following the modified standard procedures [16].

Quantitative analysis

Various extracts were subjected to quantitative analysis for the estimation of phytochemicals (tannins and glycosaponins) following the standard procedures.

Test for tannins

Each extract (2 g) was boiled with 5 ml (45% ethanol) for five minutes. The mixture was cooled and filtered. The filtrate (1 ml) was added to 3 drops of lead subacetate (CH_3COOPb) solution. The appearance of cream gelatinous precipitate showed the presence of tannins in solution [17].

Quantitative estimation

Estimation of tannins was carried out by Follin-Ciocalteu (FC) method. Various concentrations i.e., 10 ppm, 25 ppm, 50 ppm, 100 ppm and 150 ppm were prepared from 1000 ppm of standard tannic acid (TA). The calibration curve was obtained from these dilutions. To 1 ml of FC reagent, 25 ml of each concentration of dilution of TA was added. After five min, 20 ml of sodium carbonate (25%) was added to each test tube. The absorbance was taken after 20 min at 700 nm using a UV spectrophotometer (UV-1800, Shimadzu, Japan). A standard graph between absorbance and various concentrations was plotted. Tannins that were extracted from the sample by extracting with aqueous acetone (70%) were filtered through filter paper and was dilute with distilled water (DW) in a ratio (1:1000 ml). The method was revised with the sample by replacing TA and the absorbance was taken [17].

Total glycosaponins

Total glycosaponins in the plant extracts were estimated as followed by the method of Hussain [18]. Each extract (1 g) was refluxed in methanol (50 ml) for half an hour. The method was repeated; the extracts were mixed and dried using a rotary vacuum evaporator until the final volume (10 ml) of the extracts was obtained. Each of the extracts was added drop wise to a 50 mL tarred beaker containing acetone. Afterwards, there was desiccation of precipitates using an oven at 100 ºC. The precipitates were cooled and weighed to calculate the content of glycosaponins as follows:

$$\textbf{Total glycosaponins}\ (\%) = \frac{\text{weight of precipitate (g)}}{\text{total weight of the extract (g)}} \times 100$$

Antidiabetic activity (Anti-postprandial hyperglycemic studies)

Alpha amylase inhibition assay

In vitro alpha amylase enzyme inhibition of the methanol extract was determined by following the procedure with minor modifications [19] with some modifications. The sample solutions in various concentrations (20 to 100 µg/mL) were prepared in methanol. Methanol extract and standard acarbose at the concentration (20 - 100 µg/ml) with sodium phosphate (Na_2PO_4) buffer (500 µl, 0.006 molar NaCl, 0.02M, pH 6.9) and 0.5 mg/mL of alpha-amylase was put separately in an incubator at 25 °C for ten minutes. Control was conducted in a similar way, replacing with a similar volume of 0.02 M sodium phosphate and methanol extract/standard. Then all the solutions were incubated with starch solution (1%) in Na_2PO_4 buffers. The reaction mixture was then stopped by mixing 1 mL of Dinitrosalicylic acid (DNS), a coloring reagent. Then the solution mixtures were incubated for 5 minutes in a boiling water bath at 85°C and later cooled at room temperature. UV absorbance was noted at a wavelength (540 nm) by using a spectrophotometer (UV-1800 Shimadzu Corporation, Tokyo, Japan). Positive control used during the assay was a reference of alpha amylase inhibitor (acarbose). Alpha amylase inhibition by methanol extract and the standard acarbose was estimated as follows:

$$\%\ \boldsymbol{Alpha-amylase\ inhibition} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{(\text{Absorbance of control})}\right] \times 100$$

Alpha-amylase inhibition exhibited by methanol extract against α-amylase was shown as a percentage of enzyme inhibition. The IC_{50} (the concentration of methanol extract/standard, which is required to show 50% enzyme inhibition) was calculated by using Microsoft Excel from the linear regression line of concentration versus %age of inhibition.

Alpha glucosidase (glucoamylase) inhibition assay

It was measured by following the Nisar method [20] with some modifications. The glucoamylase reaction mixture contained starch, varying concentrations (20 µg/ml to 100 µg/ml) of methanol extract (500 µl) of aerial parts of the plant, a solution of a rat intestinal alpha-glucosidase (20 µl) in phosphate buffer (0.1 M, 100 M) at pH 7.0 and a solution of 1% starch (500 µl) as a substrate. Control test tubes had starch as substrate and enzyme glucoamylase. Test tubes without the enzyme, acarbose and methanol extract served as blanks. A reference drug (acarbose) with various concentrations (20 to 100 µg/ml) was used as a standard inhibitor of glucoamylase. The reaction mixture was then incubated at 37 °C temperature for half an hour using a water bath. The reaction mixture was terminated by boiling it for two minutes. The absorbance of resulting glucose was taken at a wavelength of 540 nm using a UV spectrophotometer which is directly proportional to the activity of enzyme. IC_{50} was calculated from the plots of %age inhibition.

Glucoamylase inhibition by methanol extract and the standard acarbose were estimated as follows:

$$\textbf{Inhibition of glucoamylase} = \left[\frac{\text{Absorbance of control without sample} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100$$

All the assays were performed in triplicates.

Gas chromatography-mass spectroscopic analysis

The methanol extract of the plant aerial parts was subjected to GC-MS analysis, which is helpful to identify various bioactive natural chemical compounds. This was done using the GC-MS5975C Agilent system which was coupled with a mass spectrometer, MS model (7890A). It worked with the conditions mentioned as: capillary column (30 m Length and 50 µm internal diameters). An injection in the volume of I µl of helium as carrier gas having a flow rate (0.7 ml/min) was used. The column temperature was maintained at 60 °C - 310 °C with 10 °C/minute. Detector and injector temperature was set at 250 °C and an injection volume (4 µl) was inserted in GC-MS. The spectra were taken with a scan range (50-650 m/z) with 70 eV energy of ionization. The software used for the analysis was Mass Hunter with NIST (National Institute of Standard Technology) library.

Identification of compounds

GC-MS was conducted by interpretation of mass spectrum using the data (NIST) which had more than 62000 patterns [21]. Identification of chemical compounds was done by comparing GC-MS spectrum with the spectrum of known (reference) compounds. The mass spectra and retention times of unknown compounds were compared with the spectra of reference compounds in a database system of GC-MS. In this way the chemical compounds with chemical structures and molecular weight present in the methanol extract of aerial parts of *F. indica* were identified.

Statistical analysis

All the performances were done in triplicate and the findings obtained were presented as mean ± SD. The analysis was done using one-way analysis of variance (ANOVA) which was followed by Tukey's Multiple Comparison as post hoc tests (GraphPad Prism version 6.0). A difference ($P < 0.05$) between methanol extract of the plant and the standard acarbose was considered as significant statistically.

Results and Discussion

Phytochemical profile

Phytochemicals i.e., flavonoids, tannins, phenols, glycosides, saponins, terpenoids, alkaloids, glycosides, and coumarins were present in methanol extract of the plant while anthraquinones were not found in this extract. Total tannins and glycosaponins present in various extracts of the plant which may be responsible for antidiabetic activity are given in Table 1. A Literature survey showed that aqueous and ethanolic extracts of *F. indica* had amino acids, flavonoids saponins and terpenoid glycosides [22]. Similarly, var. schewenfurthii of *F. indica* had terpenoids, flavonoids, glycosides, alkaloids, saponins, tannins, sterols, and coumarins [11,23]. The analysis of plant extracts quantitatively showed different quantities of phytochemicals with reference to the solvents used. n- Hexane and chloroform extracts had a higher content of glycosaponins as compared to methanol extract. This was due to the greater solubility of glycosaponins in this solvent. A Maximum amount of total tannins was seen in the methanol extract.

Table 1. Quantification of tannins and glycosaponins present in various extracts of aerial parts of *F. indica.*

Phytochemicals	n-Hexane extract (mg/g ± SD, % content)	Chloroform extract (mg/g ± SD, % content)	Methanol extract (mg/g ± SD, % content)	Aqueous extract (mg/g ± SD, % content)
Total tannins	31.55 ± 0.11, 3.15	43.14 ± 0.40, 4.31	60.43 ± 0.45, 6.04	10.44 ± 0.15, 1.04
Total glycosaponins	38.23 ± 2.34, 3.82	64.32 ± 2.13, 6.43	32.72 ± 1.98, 3.27	13.61 ± 2.02, 1.36

mg/g = milli gram per gram, SD = standard deviation

Alpha-amylase and glucoamylase inhibitory activity

Various concentrations i.e., 20-100 µg/ml of methanol extract of *F. indica* were subjected to hypoglycemic activity using both alpha-amylase and glucoamylase

inhibitory assays. The plant showed potent inhibition of both enzymes (alpha-amylase and glucoamylase). The inhibition of enzymes by methanol extract increased as the concentration was increased. The maximum %age of enzyme inhibition shown by methanol extract at the maximum concentration (100 µg/ml) was 49.1±2.4% (IC_{50}=104.2±8.7 µg/ml) and 31.7±2.9% (IC_{50}=273.7±54.6 µg/ml) inhibition of alpha-amylase and glucoamylase enzymes respectively. Methanol extract had a greater enzyme inhibitory tendency for alpha-amylase in comparison with glucoamylase which is supported by the previous literature as a number of plants showed more inhibition of alpha-amylase as compared to alpha-glucosidase [(1)]. The standard acarbose showed 68.9±3.09% (IC_{50}=52.9±4.8 µg/ml) and 57.3±0.4% (IC_{50}=70.2±3.4 µg/ml) inhibition of alpha-amylase and glucoamylase respectively at the same concentration. The %age inhibition of both enzymes by the methanol extract of *F. indica* and the standard acarbose is given in Figure 1.

According to the findings, methanol extract exhibited hypoglycemic activity by the inhibition of alpha-amylase and alpha-glucosidase. The inhibition of enzymes leads to less absorption of glucose in the body. It has been established that inhibiting these enzymes (alpha-amylase and alpha-glucosidase is the best method for preventing postprandial hyperglycemia. So, the inhibition of these enzymes may be one of the challenges in controlling diabetes mellitus [(24)]. Acarbose (a synthetic drug) has the ability to inhibit the enzymes. So, it acts as a standard drug for both assays. A number of known medicinal plants have alpha amylase and alpha-glucosidase inhibitory properties. Plants exhibit biological activities as a result of the phytochemicals present. Phytoconstituents present in this plant justify its use in the treatment of various diseases. Secondary metabolites like alkaloids, saponins, tannins and flavonoids in the plant are responsible for anti-diabetic and free radical scavenging activities [(25)]. Most natural plants have the ability to reduce glucose absorption in the blood. It is because of the presence of flavonoids, tannins, and terpenoids in the plants [(26)]. According to the results of multiple analysis, flavonoids are found to show active alpha-amylase inhibitory action. This was verified by a prior study that revealed that alpha-amylase enzyme might be inhibited by flavonoids present in plant extracts. The inhibition of enzymes alpha-amylase and glucoamylase has been shown by tannins. The inhibition of alpha amylase by tannins was due to the binding abilities of proteins and carbohydrates. A prior study on alpha-glucosidase inhibition by flavonoids and tannins [(27,28)] confirmed that alpha-glucosidase enzyme inhibition was caused by flavonoids, tannins, and reducing sugar. The antidiabetic potential in the plant *Melia azedarach* was mostly because of the presence of terpenoids and tannins. Glycosaponins are reported to show hypoglycemic, cholesterol-lowering, and anti-cancer activities [(29,30,31)]. It is shown from the present studies that the secondary metabolites present in the plant inhibited the enzymes alpha-amylase and glucoamylase in an effective way.

The whole plant of *F. indica* (var. *Schweinfurthii*) *was* evaluated for potential hypoglycemic activity [(32)] but there is no report of alpha-amylase and alpha-glucosidase (glucoamylase) inhibitory evaluation of methanol extract of aerial parts of *F. indica* (var. *indica*).

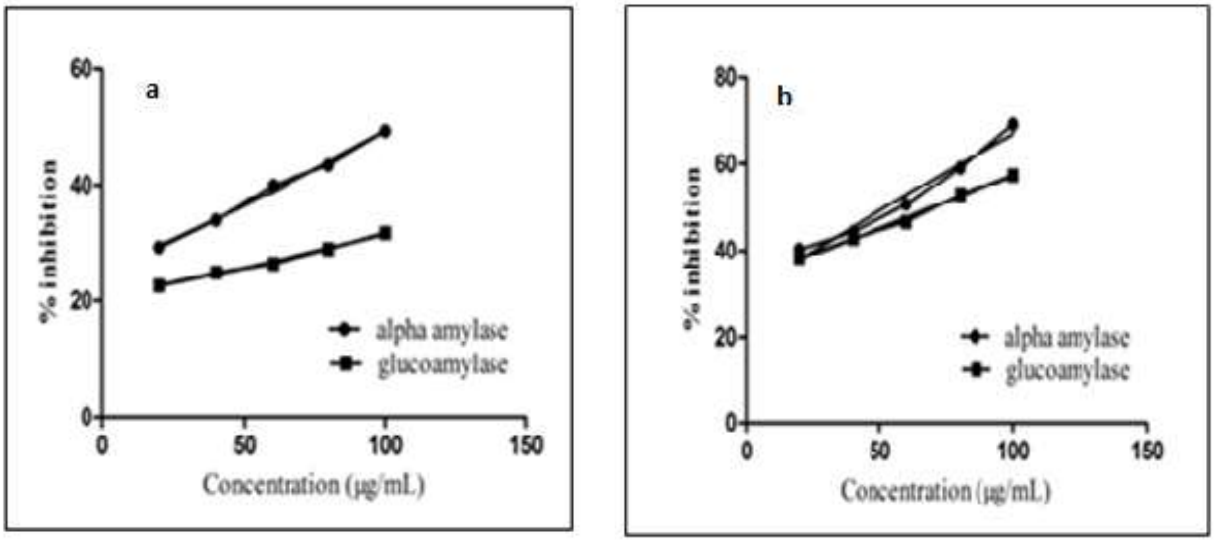
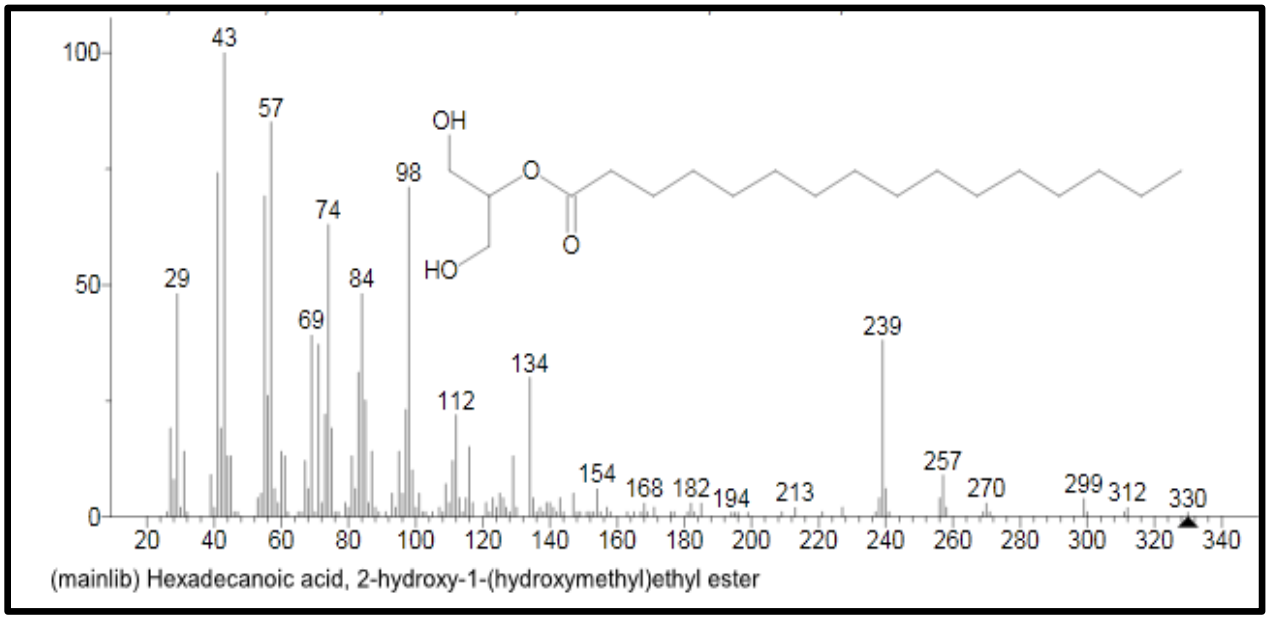


Figure 1. Comparison of the enzymes (α-amylase and glucoamylase) inhibition. a: % alpha-amylase and glucoamylase inhibition by various concentrations of *F. indica*. b: % alpha-amylase and glucoamylase inhibition by various concentrations of standard acarbose. Statistically significant difference* *p*<0.001when the enzyme inhibition of both enzymes by methanol extract was compared with the standard acarbose.**

By carefully examining the spectrum pattern and comparing it to the GC-MS NIST database library, various compounds were identified in the methanol extract of the plant. GC-MS analysis identified 15 compounds having therapeutic importance and contributing to the medicinal properties of the plant (Figure 2). The names of chemical compounds with molecular formulas and molecular weights are given in Table 2. In this study, fatty esters are among the non-polar molecules discovered. Plant esters of hexadecatrienoic acid, palmitic acid, and octadecatrienoic acid are the major compounds found in this plant.

a



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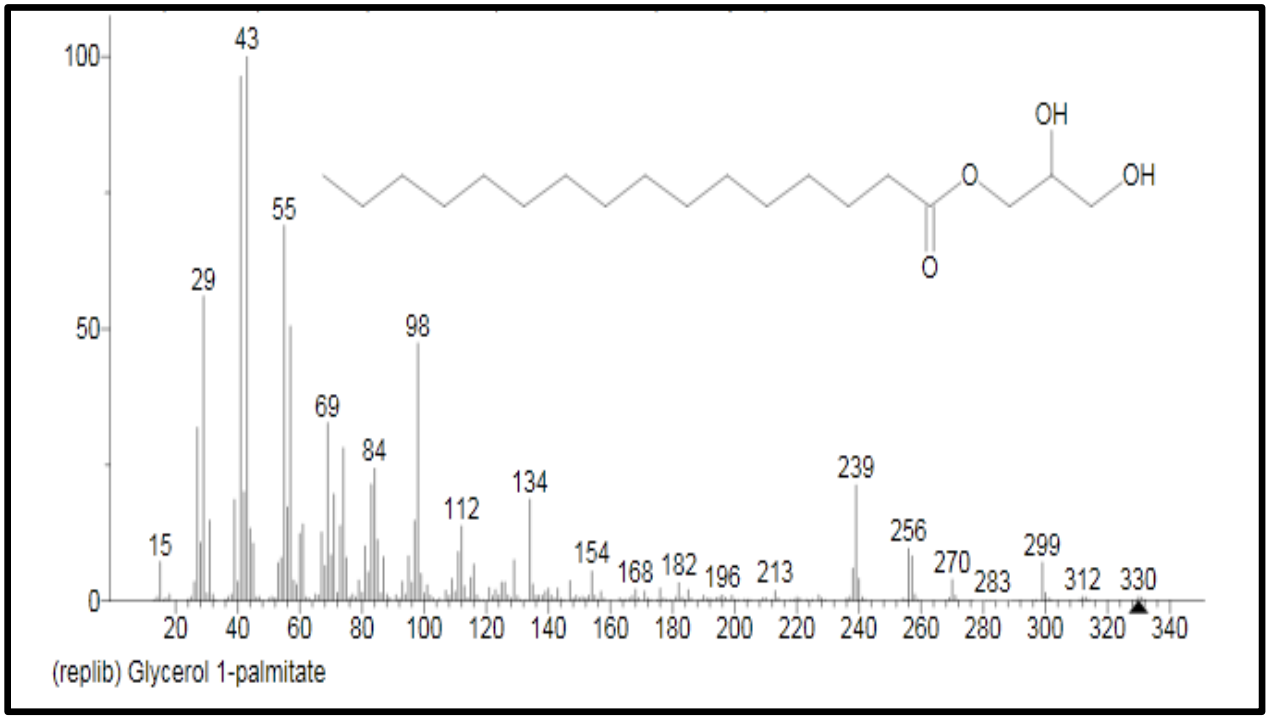
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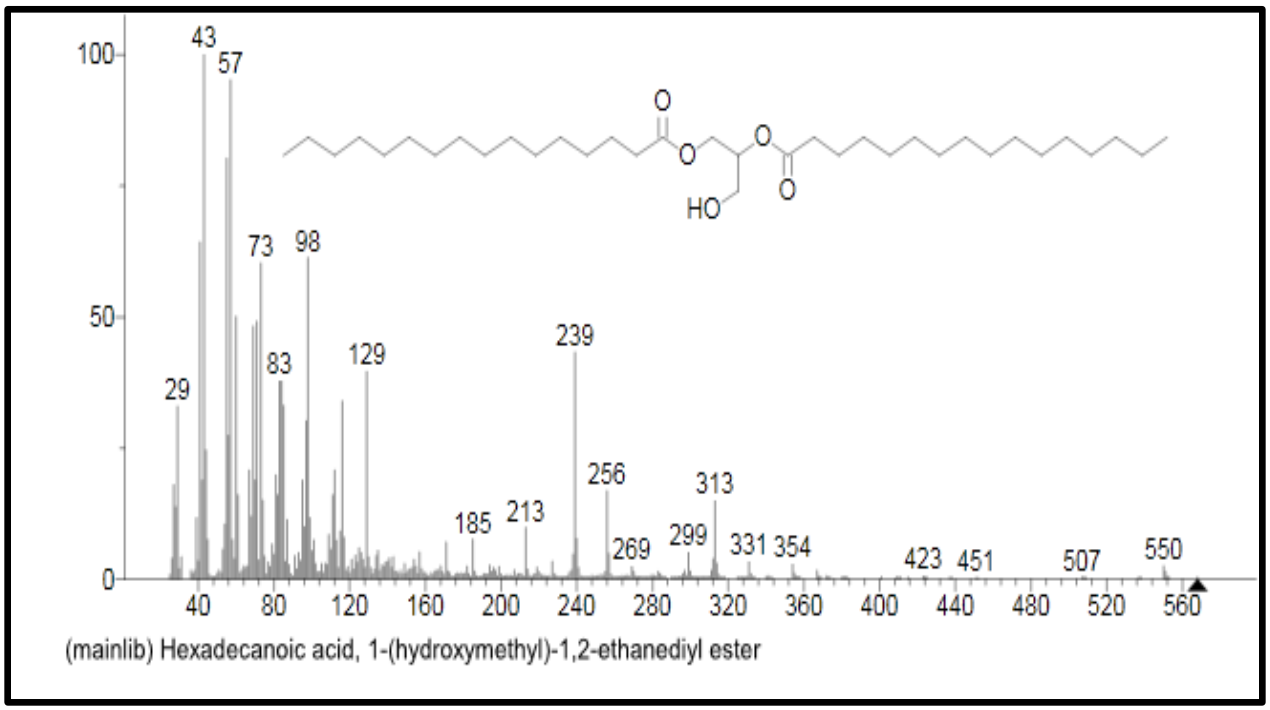
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(mainlib) Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester

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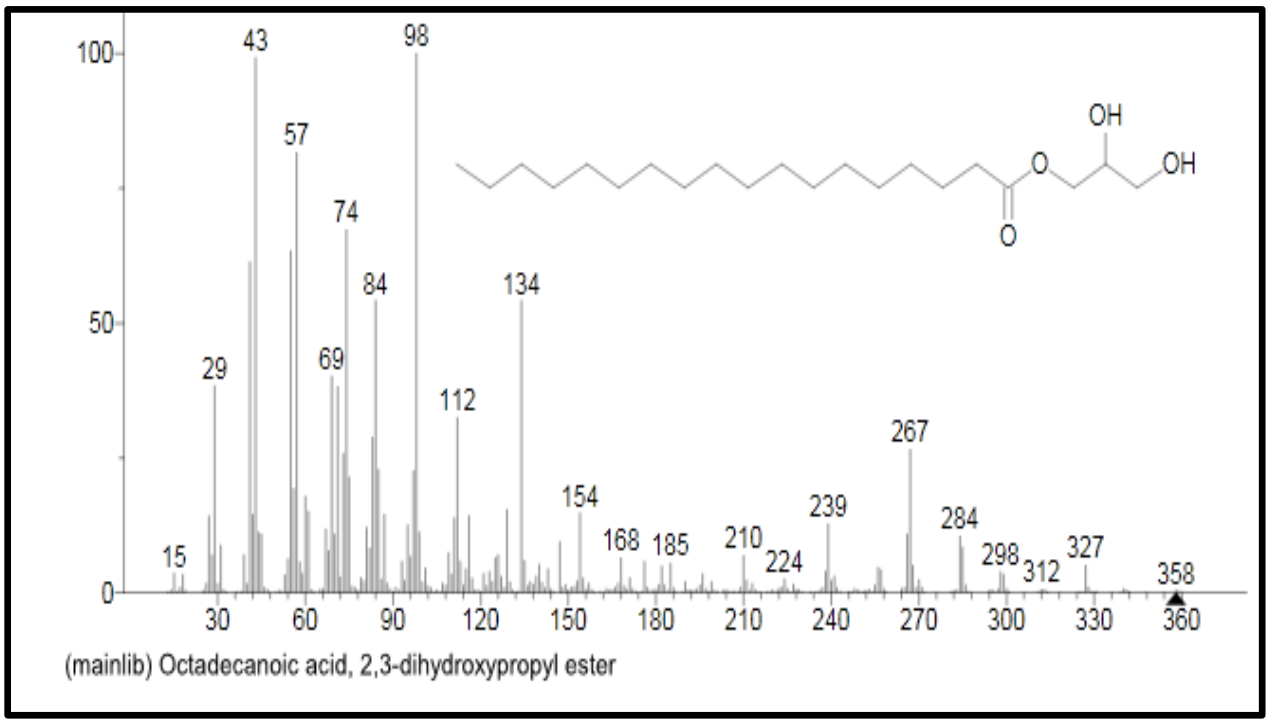
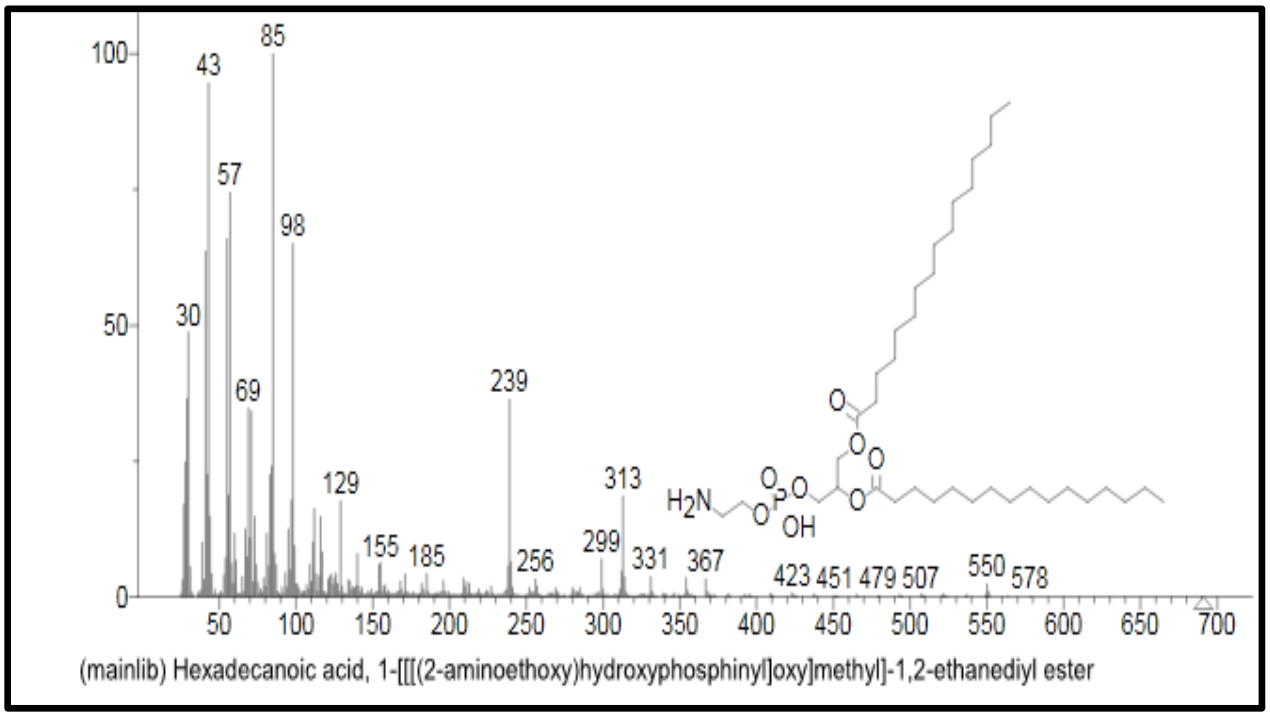


Figure 2 (a). GC-MS spectra with structural representation of compounds (a-d) identified in *F. indica*.

e



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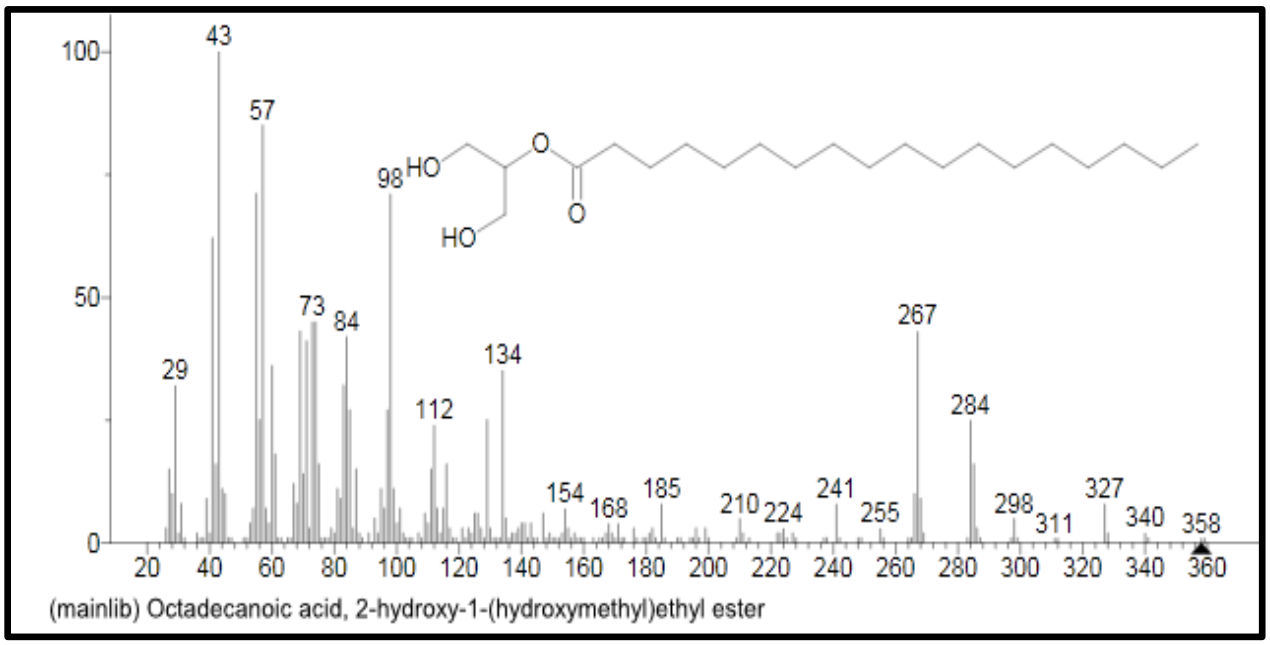
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(mainlib) Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

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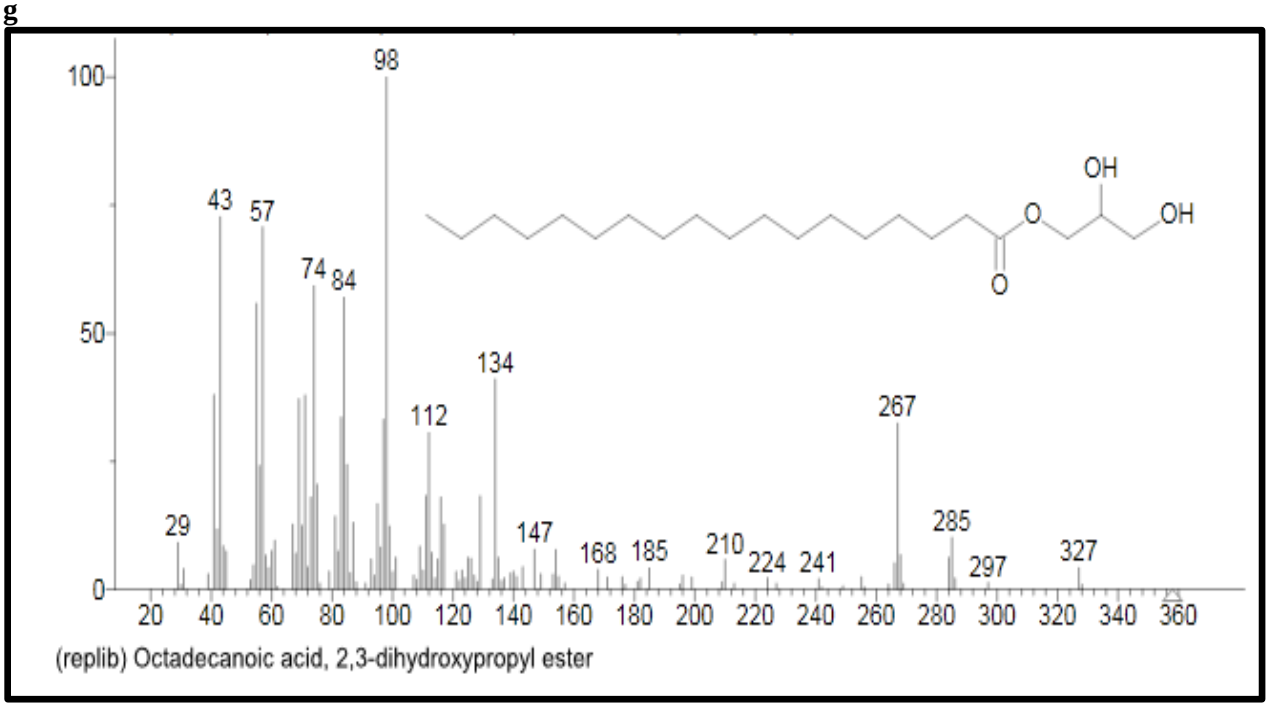
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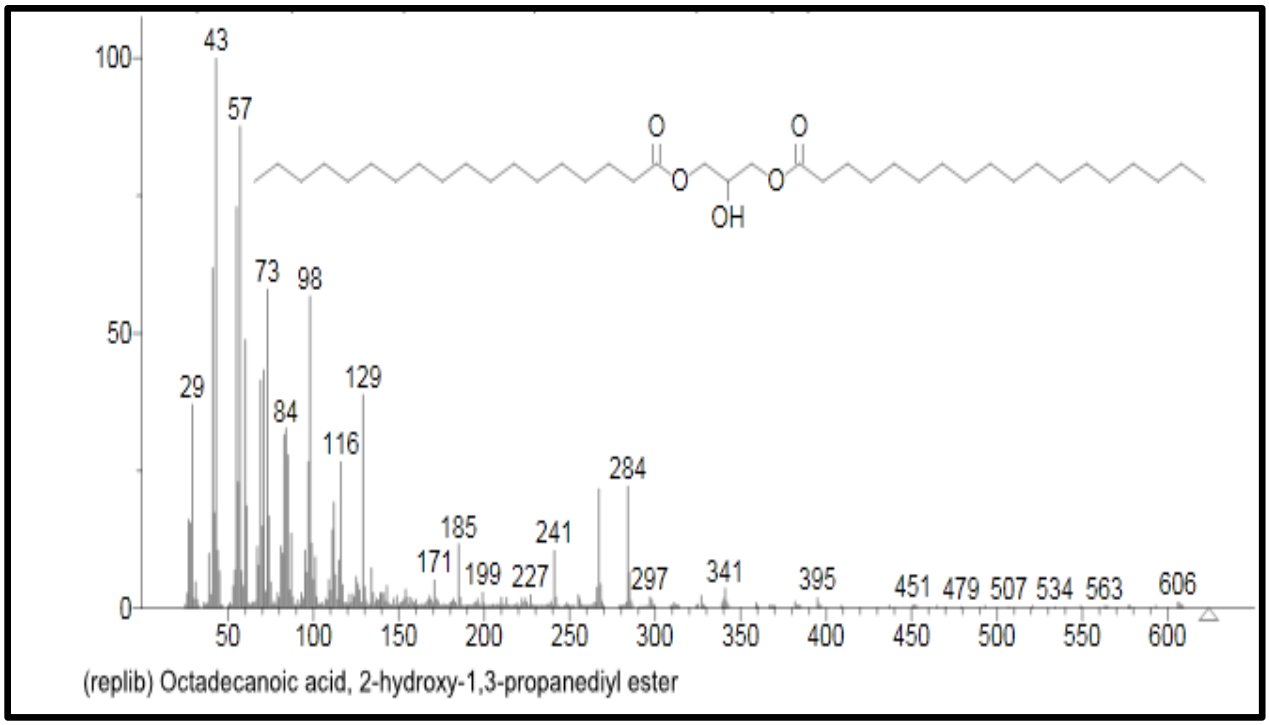
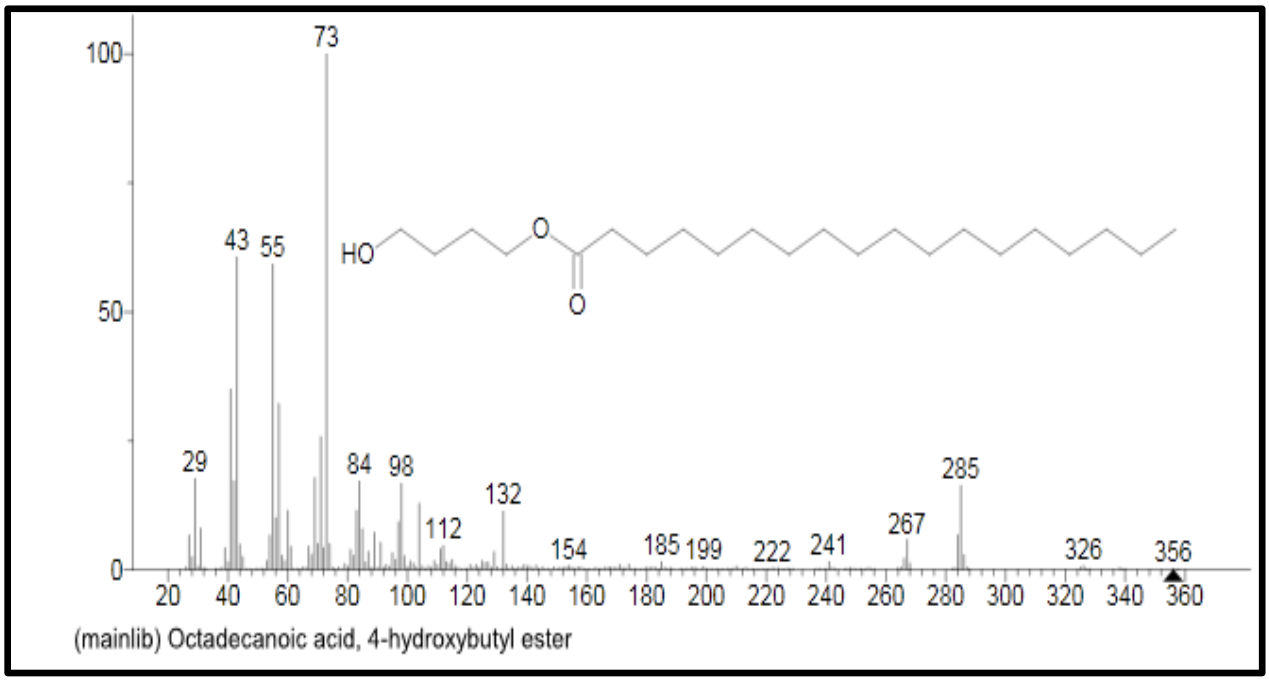


Figure 2 (b). GC-MS spectra with structural representation of compounds (e-h) identified in *F. indica.*

I



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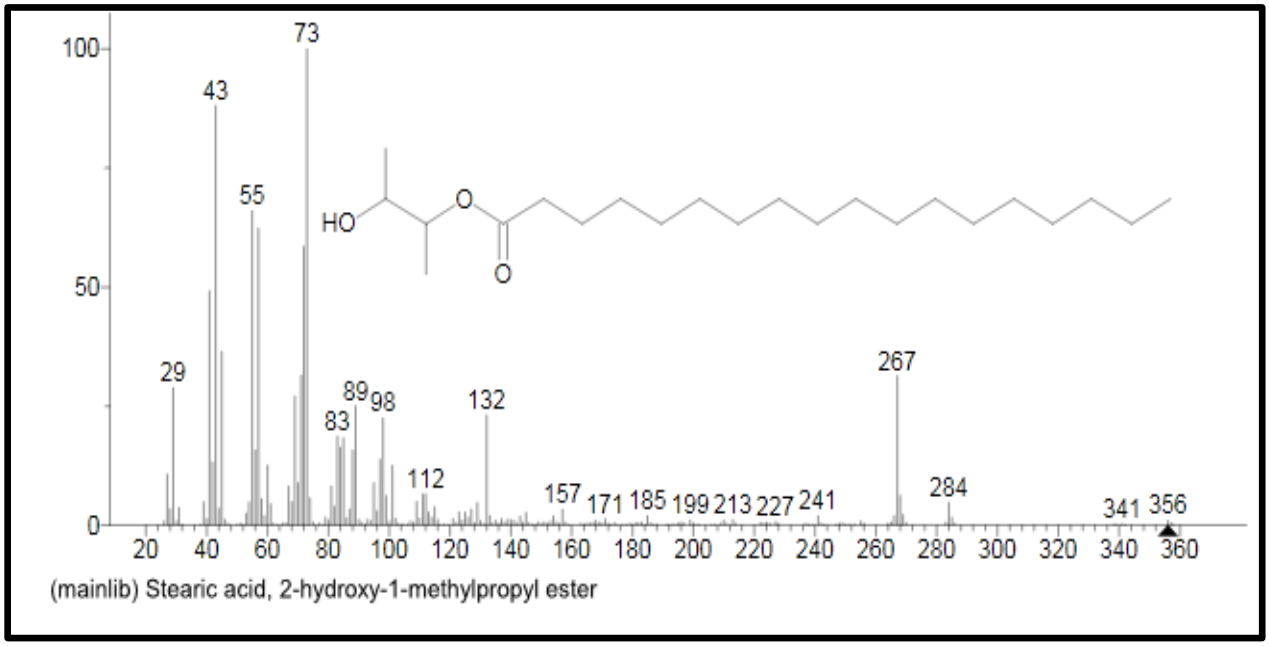
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(mainlib) Stearic acid, 2-hydroxy-1-methylpropyl ester

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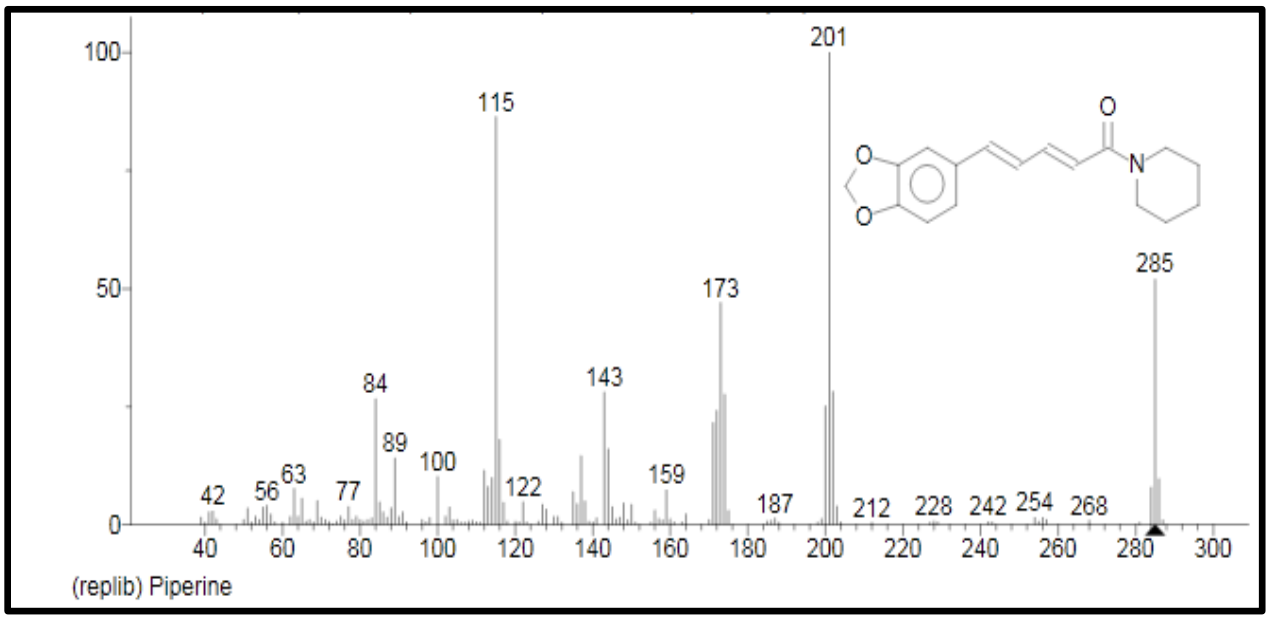
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(replib) Piperine

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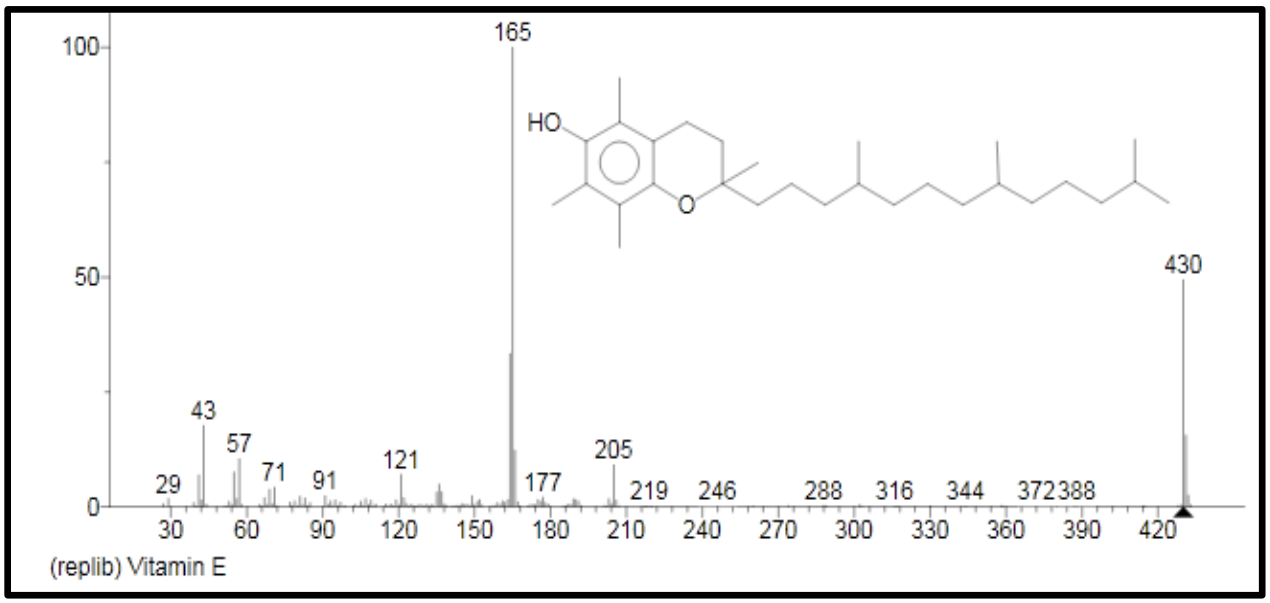
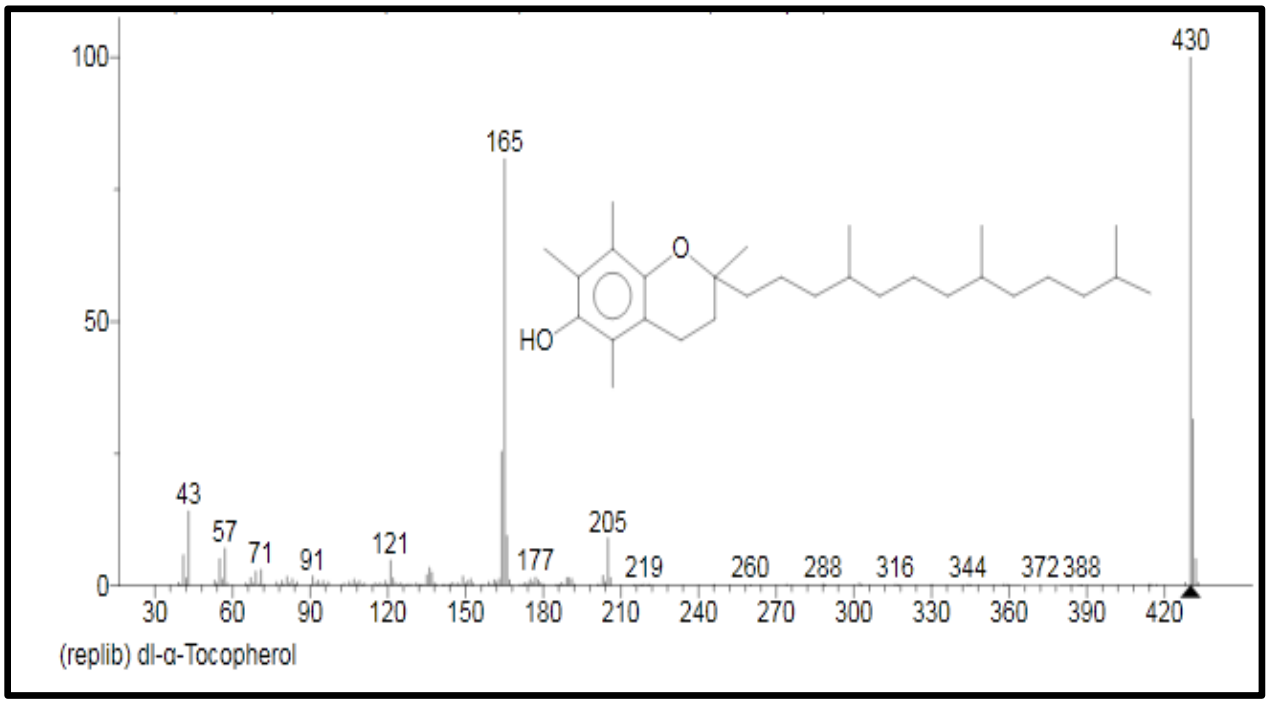


Figure 2 (c). GC-MS spectra with structural representation of compounds (i-l) identified in F. indica

m



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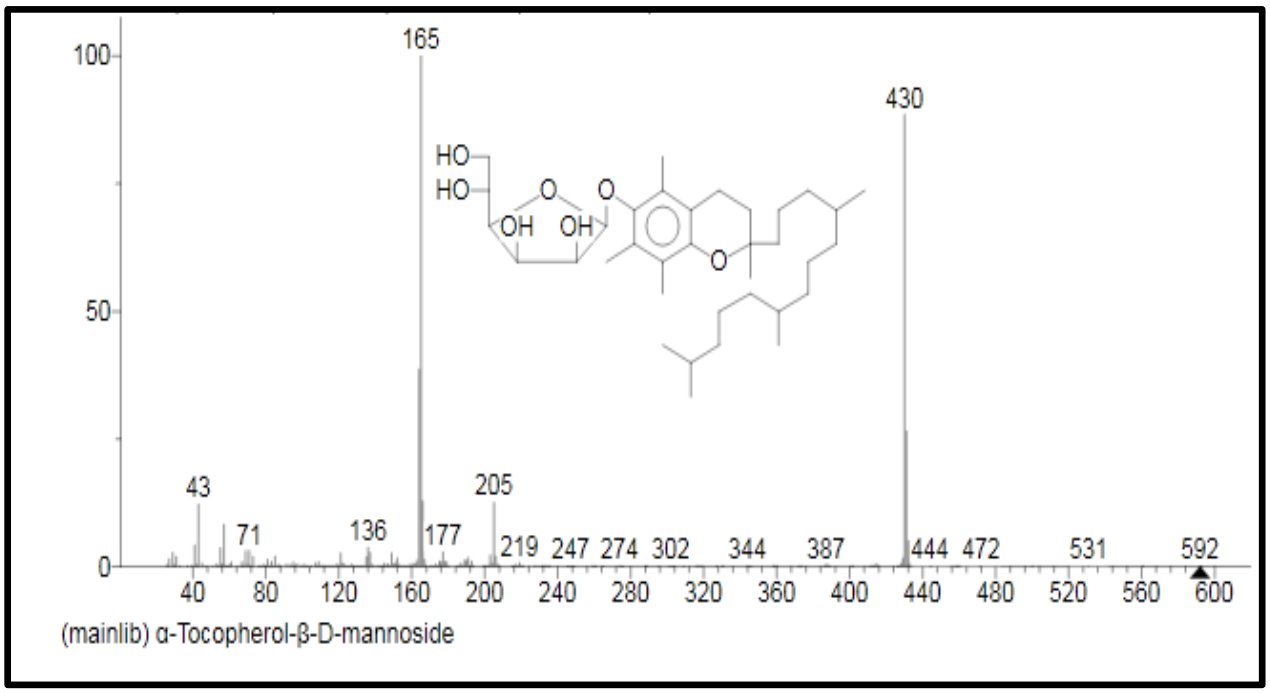

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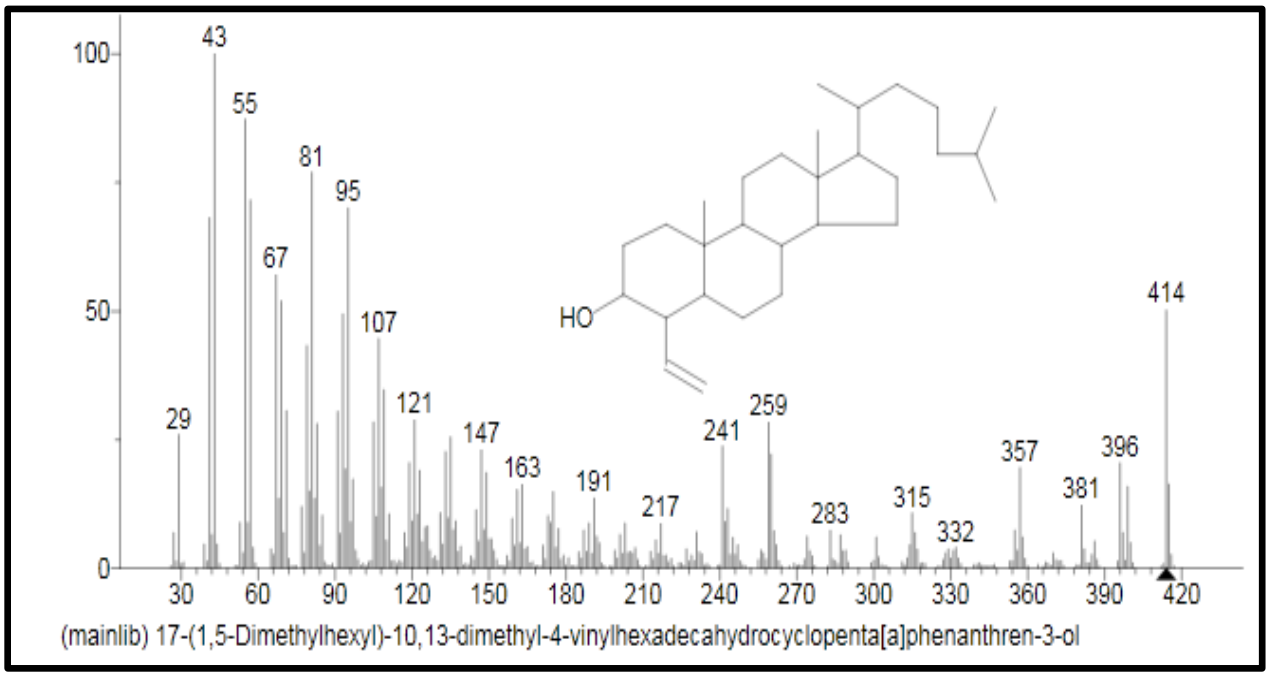

Figure 2 (d). GC-MS spectra with structural representation of compounds (m-o) identified in *F. indica.*

Table 2. Chemical Compounds identified in the methanol extract of *F. indica* by GC-MS analysis technique.

S. No.	RT (Minute)	Name of the chemical compound	Molecular formula	Molecular weight
1.	17.876	A) Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330
		B) Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330
		C) Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568
		D) Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358
		E) Hexadecanoic acid, 1-[[[(2-aminoethoxy) hydroxyphosphinyl] oxy] methyl]-1,2-ethanediyl ester	$C_{37}H_{74}NO_8P$	691
2.	20.915	F) Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	358
		G) Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358
		H) Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	624
		I) Octadecanoic acid, 4-hydroxybutyl ester	$C_{22}H_{44}O_3$	356
		J) Stearic acid, 2-hydroxy-1-methylpropyl ester	$C_{22}H_{44}O_3$	356
3.	23.156	K) Piperine	$C_{17}H_{19}NO_3$	285
4.	25.135	L) Vitamin E	$C_{29}H_{50}O_2$	430
		M) dl-α-Tocopherol	$C_{29}H_{50}O_2$	430
		N) α-Tocopherol-β-D-mannoside	$C_{35}H_{60}O_7$	592
5.	26.579	O)17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	$C_{29}H_{50}O$	414

RT = Retention time

This investigation is supported by GC-MS analysis of methanol extract having hypoglycemic activity. It is one of the most popular methods to identify phytocomponents. A crucial tool for analyzing chemical substances is gas chromatography in conjunction with mass spectroscopy. It is a qualitative data source about the chemical constituents and the substances are characterized using their mass spectra. The plant has a variety of pharmacological effects, including antioxidant, antimicrobial, antidiabetic, anti-inflammatory, analgesic, hepatoprotective, and anticancer due to the presence of various compounds

in the plant. It was discovered that some of the identified compounds in the plant have antidiabetic and antioxidant activities. Among 15 compounds, most of the compounds have the potential of lowering blood glucose levels. The hypoglycemic compounds identified in the methanol extract of *F. indica* included hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester and hexadecanoic acid, 1-[[[(2-aminoethoxy) hydroxyphosphinyl] oxy] methyl]-1,2-ethanediyl ester are antioxidants and have antidiabetic activities by their antioxidant nature. DL -α-Tocopherol, α-Tocopherol-β-D-mannoside have been reported in the literature to be effective in the alleviation of diabetes by their antioxidant activities [21,32,33]. Similarly, the compounds Octadecanoic acid, 2,3-dihydroxypropyl ester, Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Octadecanoic acid, 2,3-dihydroxypropyl ester, Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester, Octadecanoic acid, 4-hydroxybutyl ester identified in the plant are antidiabetic compounds**.** Vitamin E has an important role in slowing down diabetic complications [34,35,36]. The compounds identified in the plant have antidiabetic activity by inhibiting alpha-amylase and glucoamylases.

Major chemical compounds identified in the literature by HPLC analysis of hypoglycemic methanol extract of *F. indica* (var. *Schweinfurthii*) included chromatotropic acid, ferulic acid, vit C, caffeic acid, vanilic acid, cinnamic acid, gallic acid, benzoic acid, syringic acid and quercetin [32]. The above investigational study showed for the first time the hypoglycemic nature of the phytochemical compounds that are present in the methanol extract of *F. indica.* This quality shows the natural usage of aerial parts of *F. indica* for hypoglycemic activity. Also, it is the first report on GC-MS analysis of methanol extract of *F. indica* to the best of my knowledge. Thus, this investigation may be useful in discovering new drugs from the methanol extract of *F. indica*.

Conclusion

It is concluded from this study that hypoglycemic chemical compounds identified by GC-MS in the methanol extract of *F. indica* inhibit the enzymes alpha-amylase and glucoamylase. It is suggested that these compounds may also show an effect that is synergistic toward antidiabetic properties. This finding supports the traditional use of this plant in diabetes management. Further studies are required to isolate and characterize these compounds from the methanol extract of the plant. Also, *in vivo,* studies are required to assess and evaluate the antidiabetic potential of this extract.

Acknowledgement

The author is very thankful to the Director, Drug Testing Laboratory, Lahore for GC-MS analysis of the samples.

Conflict of Interest

The authors have declared no conflict of interest.

Funding

The study received no external funding.

Author Contribution

Dr. Atiq designed and supervised the study. He revised critically and finalized the manuscript for submission. Dr. Sana Ullah interpreted the study and conducted the experiments and statistically analyzed the data. Dr. Fatima wrote the first version of the manuscript. Also helped in the GC-MS analysis of the sample. The authors read and approved the final manuscript

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