Gas Chromatography-Mass Spectrometry and Fourier Transform Infrared Spectroscopy Characterisation of potential α-Glucosidase Inhibitors from *Terminalia Catappa* Leaf Extracts

Comment [md1]: Characterization

ABSTRACT

Aims: Diabetes mellitus is caused by insulin insufficiency or cellular glucose uptake complications, and conventional treatments often yield undesirable side effects. The aim of the current work was to evaluate the α-glucosidase inhibitory activities of *T. catappa*leaf extracts and use spectroscopic techniques to partially characterisepossible inhibitors.

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Study design: Phytochemical screening, enzyme inhibition assay and spectrometric and spectroscopic characterization of bioactive compounds from leaf extract and fractions of *T. catappa.*

Place and Duration of Study: The entire work was carried out at the Department of Applied Chemistry and Biochemistry, University for Development Studies within nine months.

Methodology: *T. catappa* leaf extract and fractions were qualitatively screened for phytochemicals and their biological activity assessed in α-glucosidase inhibition assay. The potential enzyme inhibitors were characterized by Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FT-IR) Spectroscopy.

Results: Phytochemical screening of the extract and the various solvent fractions revealed the presence of alkaloids, flavonoids, saponins, flavanol glycosides, phenolics, and terpenoids. The α-glucosidase inhibition potential of the crude leaf extract was concentration-dependent with a half-maximalinhibitory concentration (IC_{50}) of 337.5 µg/ml. Solvent fractions from the crude extract demonstrated α-glucosidase inhibitory activity with IC_{50} values ranging from 170.40 µg/ml for ethyl acetate (EtOAc) fraction to 71.90 µg/ml for n-hexane (n-Hex) fraction. Acarbose, the control drug, demonstrated significant α-glucosidase inhibition activity in a similar pattern with $IC_{50}$of 6.16 µg/ml. The enzyme inhibition kinetics parameters, specifically the Michaelis constant (K_M) and Maximum reaction velocity (Vmax) values, suggested that the inhibition of α-glucosidase by *T. catappa* extract followed a competitive mode.GC-MSand FT-IR analysis of the n-Hexanefraction identified the compounds Eugenol, Urs-12-en-24-oic acid, 3-oxo-, methyl ester (+)-, phytol, trans-isoeugenol, alpha-amyrin, and squalene as the potential antidiabetic agents that might be responsible for reducing postprandial blood glucose levels.

Conclusion: These findings show that *T. catappa* leaf extract contains α-glucosidase inhibitors and therefore serves as a valuable resource in the discovery of natural anti-diabetic agents.

Keywords: *Glucosidase inhibition, postprandial hyperglycemia, enzyme kinetics, phytochemical characterization, natural products*

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic condition resulting from the body's inadequate response to insulin, either due to insufficient production or the inability of target organs to respond effectively. The production of insulin by the pancreasregulates glucose and fat metabolism, and its dysfunction leads to elevated blood glucose levels. Over the past few decades, the global prevalence of DM has surged, with a growing number of cases, particularly among children and adolescents in low- and middle-income countries [1]. Chronic hyperglycemia is linked to several complications, including cardiovascular diseases, metabolic syndrome, and both micro- and macrovascular disorders [2].

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Conventional treatments for DM include insulin therapy and oral anti-diabetic medications. Commonly prescribed oral anti-diabetic agents include sulfonylureas (e.g., tolbutamide, glibenclamide), biguanides (e.g., metformin), and glycosidase inhibitors (e.g., voglibose, acarbose, miglitol) [3]. Sulfonylureas stimulate insulin release from pancreatic β-cells, while biguanidesare believed to

improve insulin sensitivity, reduce gluconeogenesis, and increase glucose uptake. Glycosidase inhibitors, such as acarbose, delay glucose absorption by inhibiting carbohydrate hydrolysing enzymes such as α-amylase and glucosidase, which mitigates post-prandial hyperglycemia (Horii et al., 1986; Mooradian & Thurman, 1999). There has been a growing interest in the discovery of novel α -glycosidase inhibitors. In spite of their efficacy, conventional α-glycosidase inhibitors often have undesirable side effects, such as gastrointestinal discomfort, necessitating the discovery of alternative therapies [4].

Medicinal plants have long been explored for their therapeutic properties, and over 400 plant species have been identified with anti-diabetic potential [5]. *Terminalia catappa* (Indian almond) has gained attention in the scientific community due to its anti-diabetic, antioxidant, and anti-inflammatory properties [6][7]. *T. catappa* has been reported to contain bioactive compounds such as tannins, flavonoids, and triterpenoids, which are thought to contribute to its anti-diabetic effects [8][9].

To the best of our knowledge, the α-glucosidase inhibitory potential of *T. catappa* extract is yet to be fully explored. Given the increasing interest in plant-derived bioactive compounds as safer alternatives to synthetic drugs, *T. catappa* could present a promising source for the discovery and development of new anti-diabetic therapies [10][11]. This study aims to evaluate the α-glucosidase inhibition activity of *T. catappa* leaf extracts and partially characterize potential bioactive compounds through spectroscopic and spectrometric techniques.

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2. MATERIALS AND METHODS

2.1 collection of samples

On January 31, 2021, mature *T. catappa* leaves were collected from Kakpagayili, a suburb of Tamale in the Northern Region of Ghana. Mr. GbalGordious of the Forestry Services Division, Tamale (F.S.D.) assisted in the identification of the plant sample.

2.2 Chemicals and solvents

Alpha-glucosidase EC: 3.2.1.20 (from Saccharomyces cerevisiae, 100U) and p-nitrophenyl-α-D-glucopyranoside-PNPG (1g) were procured from Sigma Aldrich, USA (CAS no.: 9001-42-7). Beijing Solarbio Science & Technology Co., Ltd. provided the phosphate buffer (Phosphate buffered saline, or PBS) pellets. Wagner's reagent was prepared by dissolving iodine and potassium iodide in water. Absolute hexane, 1-butanol, 99.5% anhydrous ethyl alcohol, sodium carbonate, and ethyl acetate, among others, were of analytical grade and used without further purification.

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2.3 Preparation of plant material and extraction

The leaves of *T. catappa* were plucked and cleaned with tap water to eliminate all undesired material and dust, then air dried under shade for 12 days at room temperature. The dried leaves were treated to size reduction to coarse powder by utilising dry grinder (Silver crest 2 in 1 easy clean SC-605 blender).

Finely ground leaf powder weighing 200 grams were soaked in 70% ethanol at a ratio of 1 part powder to 5 parts ethanol by weight. The mixture was left to soak for 5 days with intermittent stirring and filtered using Whatman's No. 1 filter paper to obtain the crude extract.

2.4 Phytochemical screening

The extract was screened for phytochemicals using standard protocols.

2.4.1 Test for Saponins

About 0.5 g of extract is dissolved in 10 ml of distilled water. This was vigorously shaken for 30 s. It was filtered into a conical flask, and the filtrate was warmed in a water bath for 15 min. It is again vigorously shaken when warm. The persistent frothing (foaming) appearance, when warm, indicates the presence of saponins[12].

2.4.2 Test for Alkaloids

To a 3 ml aqueous sample solution (filtrate), add 1% 1 ml HCl and Wagner's reagent, shake, and allow to settle. A reddish-brown precipitate shows a positive result for alkaloids [13].

2.4.3 Salkowski Test for Terpenoids

To 3.0 ml of ethanolic extract, add 1.0 ml of chloroform, and then 1.5 ml of concentrated H_2SO_4 along the sides of the tube. The reddish-brown at the interface is considered positive for terpenoid[13].

2.4.4 Test for Flavonoids

About 0.5 g of extract was dissolved in 10 ml of ethanol. It was filtered, and 2.0 ml of the filtrate was pipetted into a different flask. A few drops of 1.0 M sodium hydroxide were then added to the filtrate. A colour change was observed. Add a few drops of 1% aqueous hydrochloric acid. Another colour change was observed. The colour changes indicated the presence of flavonoids[12].

2.4.5 Test for Flavanol Glycosides

[The Magnesium and Hydrochloric Acid Reduction]
To the 5 ml of ethanolic filtrate, drop 1cm of magnesium ribbon into the solution, then add drops of conc. HCl. Pink or crimson colour indicates flavanol glycosides presence[13].

2.4.6 Test for tannins

To the 5 ml of extract, add a few drops of a neutral 5% ferric chloride solution. A dark green colour indicates the presence of tannins[12].

2.4.7 Test for Phenol

To 5 ml of ethanolic filtrate, add a few drops of ferric chloride solution. A bluish-black colour indicates phenol [14].

2.5 Acquisition of solvent fractions of crude extract

The crude extract was partitioned using solvents of varying polarities by adopting a modified version of the Kupchan and Tsou method[15]. Initially, 10 grams of crude extract were dissolved in 50 ml of distilled water. Subsequently, equal volumes (50 ml each) of n-hexane, ethyl acetate (EtOAc), and 1-butanol (1-BuOH) were sequentially added. The separating funnel was filled with the aqueous mixture initially, followed by the addition of n-hexane, which was then agitated and allowed to settle for fifteen minutes before decanting. This process was repeated for EtOAc and 1-BuOH sequentially.

2.6 Fourier Transform Infrared (FT-IR) Acquisition and Preprocessing

FT-IR spectrum of *T. catappa* leaf extract was obtained using a PerkinElmer FTIR spectrometer (Model: Spectrum Two) equipped with a $LiTaO_3$ detector with a resolution of 4 cm^{-1}at 24 scans. Spectra were scanned in the absorbance mode from 4000 to 400 cm^{-1}. FTIR analysis of the crude extract was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio. The spectral data were analyzed by Spectrum™ 10 software (version 10.03.09).
The protocol adopted was in accordance with the procedure reported by [16]. Briefly, a small amount of powdered leaf samples was made into pellets using KBr for FTIR analysis and a thin film was prepared by applying pressure. All the samples were analysed in triplicates with plain KBr pellets as blank. The spectral data were compared with a reference to identify the functional groups existing in the sample.

2.7 The gas chromatography-mass spectrometry analysis (GC-MS)

GC-MS is the best, fastest, and most accurate technique used for detecting various compounds like alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids with a small volume of plant extracts [17]. A Perkin Elmer GC Clarus 580 Gas Chromatograph interfaced to a Mass Spectrometer Perkin Elmer (Clarus SQ 8 S) fitted with a ZB-5HT MS (5% diphenyl/95% dimethyl polysiloxane) fused to a capillary column (30 × 0.25 µm ID × 0.25 µm DF) was used to analyse the samples. GC-MS detection was performed using an electron impact mode electron ionisation apparatus with an ionisation energy of 70 eV. A fixed flow rate of 1 ml/min was maintained using helium gas (99.999%) as the carrier gas, and an injection volume of 1 µl was utilised. The ion source was kept at 250 °C, while the injector was kept at 220 °C. The oven was set at 100 °C initially, and that temperature was held for two min. After that, the temperature increased by 10 °C/min until it reached 200 °C. The temperature was gradually adjusted by 5 °C/min until it reached 280 °C as the final oven temperature. Mass spectra were obtained at 70 eV with fragments ranging from 45 to 500 Da with a 0.2 s scan interval. The GC/MS running duration was 43 min overall, with a solvent delay of 0 to 3 min. Turbo-Mass was the mass detector used in this investigation, and Turbo-Mass ver-6.1.0 was the software used to manage the mass spectra and chromatograms.

Interpretation of mass-spectrum GC-MS was conducted using the database of the National Institute of Standards and Technology (NIST), which has more than 62,000 patterns.
Generally, 55 µl of each standard solution (50 µM), or 4 µl from the plant leaf extract, were poured into a vial (with insert) and spin-dried at 39°C. Samples were derivatised (automatically with a preparative robot) with 20 µl methoxyamine (20 mg ml^{-1} in pyridine; 90 min at 37°C) and 30 µl ***N***-methyl-***N***-(trimethylsilyl) -trifluoroacetamide (MSTFA) for 30 min at 37°C. Before injection, 5 µl of alkane mix (14 alkanes from C_9 to C_{36}, 3 µg $µl^{-1}$, Connecticut n-Hydrocarbon Mix, Supelco) were added in each sample to compute the retention index. Analyses were performed by injecting 1 µl in splitless mode at 230°C (injector temperature) in a TG-5 SILMS column (30 m × 0.25 mm × 0.25 µm; Thermo Scientific) set in a Trace 1300 Series GC (Thermo Scientific)[18].
Specific to the sample under study, the extract was homogenised by mixing 15 ml of n-hexane: acetone (ratio 4:1) with 5 g of the semi-solid (paste-like) sample, then shaken for 5 hours. After extracting the precipitate for 24 hours, filter paper was used to separate the liquid from the solid.

2.8 Enzyme Inhibition Assay

The procedure for enzyme inhibition followed those reported by[19]with little modifications. Breifly,a mixture containing 20 µl α- glucosidase enzyme (1.0 unit/ml), 250 µl 0.1 M phosphate buffer (pH 6.9), and 100 µl plant extract (or fractions) at various concentrations were incubated at 37°C for 15 mins. After pre-incubation, an enzymatic reaction was initiated by adding 40 µl 5 mM PNPG solution in 250 µl buffer and the reaction mixture was further incubated for another 20 mins at 37°C. 100 µl 0.1N (0.05 M) sodium carbonate solution was then added to the reaction mixture to terminate it. Absorbance readings were recorded at 405 nm. The reaction systems without plant extracts were used as control, and the system without α-glucosidase was blank to correct the background absorbance (Bhatia et al., 2019). The inhibitory activity of sample on α-glucosidase was then calculated from Eq. 1.

$$\%\ inhibition = \frac{controlabsorbance - sampleabsorbance}{controlabsorbance} \times 100 \text{-------- Eq. 1}$$

2.9 Alpha-glucosidase Inhibition Kinetics study

The enzyme kinetic study was in accordance with Bhatia and co. (2019), with some modifications. Briefly, 20 µl enzyme (α-glucosidase (1.0 unit/ml)) in 250 µl 0.1 M phosphate buffer (pH 6.9) was incubated for 15 mins at 37°C. The reaction was initiated with 40 µl of PNPG at varied concentrations, and the absorbance (of p-nitrophenol) was recorded at a 15 s interval for 120 s. This was done without an inhibitor to determine the rate of enzyme inhibition. In a separate experiment, 20 µl of the enzyme (α-glucosidase (1.0 unit/ml)), 250 µl of 0.1 M phosphate buffer (pH 6.9), and 100 µl of plant extract at 400 µg/ml and 200 µg/ml were incubated for 15 min at 37°C. The reactions were then initiated with 40 µl of PNPG at varied concentrations, and absorbance was recorded at 15 s interval for 120 s.

2.10 Analysis of data

The data for the α-glucosidase inhibitory activity of the various plant extracts are presented as mean ± standard error. The data was further analysed using one-way analysis of variances (ANOVA) and Tukey's test for multiple comparison tests assuming variances are equal using GraphPad Prism 9.3.1 (47) for Windows OS. ($p < 0.05$ is considered statistically significant). Enzyme kinetics parameters as well as IC_{50} values were all calculated using GraphPad Prism 9.3.1 (47).

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical screening of crude extract of *T. catappa*

Phytochemical screening of the crude extract revealed the presence of key phytochemicals, as presented in Table 1. The synergistic effects of alkaloids, phenolics, and terpenoids have been

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associated with anti-diabetic activity, consistent with findings by [20], [21] and [22]. Studies link the alpha-glucosidase inhibition by plant extracts to alkaloids [23]and tannins [24]. *Caulerpin*, an alkaloid, has shown significant inhibition of the human Protein Tyrosine Phosphatase 1B enzyme (hPTP1B) with an IC_{50} of 3.77 µM, potentially aiding in the management of type 2 diabetes and obesity [25]. The saponin*dammarane*, exhibits hypoglycemic, antimicrobial, and hypolipidemicactivities [26]. Flavonoids are reported to exhibit therapeutic properties including anti-inflammatory, anti-hypertension, and anti-diabetic [20]. The anti-diabetic property demonstrated by *T. catappa* extract may result from these phytochemicals.

Table 1. Phytochemical screening for the 70% ethanol crude extract of *T. Catappa*

Phytochemical	Observation
Alkaloids	+
Flavonoids	+
Flavonol Glycosides	+
Phenolics	+
Saponins	+
Tannins	+
Terpenoids	+

Detected (+)

3.2Phytochemical screening of solvent fractions of *T. catappa*leaf extract

Table 2 presents results for phytochemical screening of solvent fractions obtained from *T. catappa* leaf extract. Phenolics and flavonoids were absent in the aqueous fraction, likely influenced by factors such as geographical location, harvest time, and plant age. However, hydrophilic substances like saponins and flavanol glycosides were detected in aqueous extracts. This corroborates with report by[26]. Ethyl acetate fraction contained flavonoids and total phenolics, while 1-butanol fraction showed phenolic presence due to its polarity. Saponins, typically undetected by lipophilic solvents, were found. The 70% EtOH leaf extracts and fractions of *T. catappa* exhibited significant biological activity, including antidiabetic effects attributed to flavanol glycosides, terpenoids, and phenolics. Other studies confirmed that phenolics show strong inhibitory activity against L-tyrosine[27], and α-glucosidase, [28].

Table 2: Phytochemical screening of the fractions of the 70% ethanol *T. catappa* crude extract

Solvent	Phytochemical

Fraction	Alkaloids	Flavonoids	Flavanol Glycosides	Phenolics	Saponins	Tannins	Terpenoids
Aqueous	+	–	+	–	+	+	+
1-BuOH	+	+	+	+	–	–	+
EtOAc	+	+	+	+	–	+	+
n-Hexane	–	–	+	+	–	–	+

Detected (+), Not detected (-)

3.3Alpha-glucosidase inhibition activity of *T. catappa* crude leaf extract and fractions

Table 3 displays the α-glucosidase inhibitory activity of *T. catappa* leaf extract compared to acarbose, a known enzyme inhibitor used in diabetes management. The crude extract exhibited concentration-dependent inhibition, reaching a maximum of 51.58±1.05% at 400 µg/ml, while acarbose reached 96.18±0.44 % at the same concentration. Acarbose was found to be 500 times more potent (IC_{50} = 6.16 µg/ml) than the crude extract (IC_{50} = 337.5 µg/ml), suggesting possible presence of antagonistic compounds potentially masking active principles in the crude extract. This assertion is supported by the apparent increase in alpha-glucosidase inhibition activity as observed from the IC_{50} values of the solvent fractions.

Significantly, the selection of solventgreatly influences inhibition percentages, typically showing higher inhibitory effects with extreme solvent polarities, such as polar-aqueous and non-polar n-hexane[26].

The data further demonstrates clear dose-response relationships, indicating that higher concentrations generally lead to greater inhibition, aiding in the determination of optimal dosages for α-glucosidase inhibitors. Comparing IC_{50} values indicates differing potencies among solvents, with n-hexane demonstrating the highest potency and ethyl acetate exhibiting the lowest.

Table 2. Alpha-glucosidase inhibition activity of *T. catappa* extracts and the control

	% Inhibition						
Sample/ Conc.	12.50 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	400 (µg/ml)	IC_{50}(µg/ml)
Crude	16.54±0.25	25.95±4.97	30.28±2.39	38.93±0.44	42.75±0.88	51.58±1.05	337.5***
Acarbose	63.87±3.85	72.01±0.51	76.84±1.11	91.09±0.25	92.11±0.25	96.18±0.44	6.16*
Aqua frac.	11.63±8.39	34.88±1.70	44.19±6.98	51.16±8.06	65.12±8.06	79.07±1.66	90.59**
BuOH frac.	12.24±2.56	14.29±1.14	38.78±1.43	46.94±4.42	51.02±2.69	53.06±0.66	109.60**
EtOAc frac.	10.20±1.41	14.29±0.14	20.41±1.12	29.18±3.22	46.73±8.55	46.94±0.68	170.40***

n-Hex	20.41±4.4	22.41±0.2	36.73±5.4	44.90±3.5	69.39±9.3	73.47±2.0	71.90*
frac.	9	3	0	3	5	4	

*Values are mean ± standard error, measured in triplicates. Values with different * are significantly different (p<0.05) from each other.*

3.4Alpha-glucosidase Inhibition Kinetics study

The mechanism of inhibition was determined by comparing through statistical analysis the K_M and V_{max} of 1 U/ml of enzyme reacting with different amount of substrate under the experimental conditions of the assay, with those of 1 U/ml of enzyme reacting with the same amount of substrate, under the same conditions, adding the extract at 400 µg/ml and 200 µg/ml.

The enzyme kinetics parameters were obtained from Michaelis-Menten plot and displayed by the Linewaver-Burk plot. The results for kinetic parameters are presented in Table 4. Michaelis-Menten plot is displayed in Figure 1. There was an observed increase in the values of the Michaelis constant K_M at different inhibitor concentrations while maximum velocity (Vmax) values remained relatively unchanged as the extract was added at 200 and 400 µg/ml. This is an indication of competitive inhibition of the enzyme.

Table 4. Parameters of α-glucosidase inhibition kinetics in the presence and absence of inhibitor

	Vmax (mM/min)	**K_M (mM)$^{-1}$**
No inhibitor	204.10±10.80	0.115±0.027
+ 200 µg/ml Inhibitor	217.30±15.40	0.206±0.049
+ 400 µg/ml Inhibitor	222.10±11.60	0.3069±0.047

Values are mean ± standard error, measured in triplicates

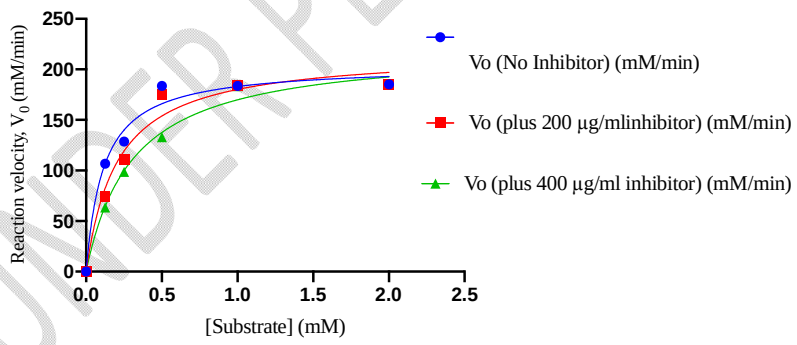


Fig.1. Michaelis-Menten plot of α-glucosidase reaction in the presence and/or absence of inhibitor

In Lineweaver-Burk plot, the common intersection at the y-intercept is a further illustration of a competitive mechanism of inhibition. As the inhibitor concentration increased, the slope (K_M/V_{max}) in the re-arranged Michaelis-Menten Equation,Eq. 2, increased, while the y-intercept ($1/V_{max}$) remained unchanged.

$$\frac{1}{V_0} = \frac{K_M}{Vmax[S]} + \frac{1}{Vmax} \quad \text{-------- Eq. 2}$$

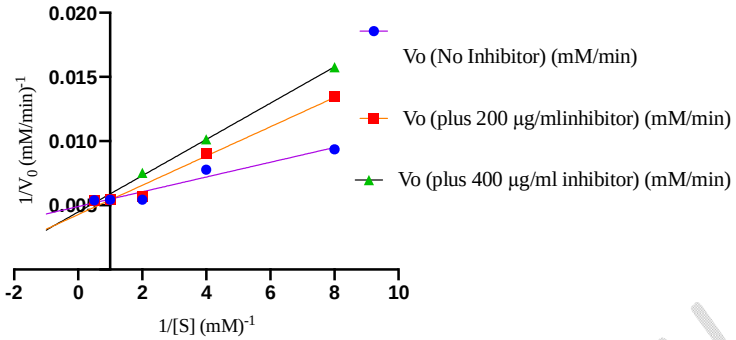


Fig.2. Lineweaver-Burk plot of α-glucosidase reaction in the presence and/or absence of the inhibitor

3.5GC-MS analysis of *T. catappa* leaf extract

The analysis adhered to standard procedures[29] [17] using PerkinElmer GC Clarus 580 Gas Chromatograph connected to a Mass Spectrometer PerkinElmer (Clarus SQ 8 S) equipped with ZB-5HT MS, for identifying phytoconstituents in the n-hexane fraction of *T. catappa* leaf. By comparing peak retention time, area, height, and mass spectral patterns to known compounds in the NIST library, 15 peaks (Figure 3) were identified in the GC-MS chromatogram. Thirty-one compounds (Figure 4) were suggested with their retention times (RT), molecular formulas (MF), molecular weights (MW), and concentrations (peak area %) detailed in Table 5.

Table 5. Suggested phytoconstituents in the GC-MS chromatogram of*T. catappa* leaf extract

#	RT	Area%	MF	MW	Other Name	IUPAC Name
1	6.840	2.007	$C_{10}H_{12}O_2$	164.20	Eugenol*	4-allyl-2-methoxyphenol
2	12.378	1.172	$C_{10}H_{12}O_2$	164.20	Trans-isoeugenol	(E)-2-methoxy-4-(prop-1-en-1-yl) phenol
			$C_{10}H_{18}$	138.25	Cyclopentane,1,2-dimethyl-3-(1-methylethenyl)-	1,2-dimethyl-3-(prop-1-en-2-yl) cyclopentane
			$C_{13}H_{24}O_2$	212.33	6-octen-1-ol,3,7-dimethyl-, propanoate	3,7-dimethyloct-6-en-1-yl propionate
3	25.524	26.429	$C_{14}H_{30}O_2$	230.39	1,14-tetradecanediol	Tetradecane-1,14-diol
			$C_{30}H_{50}$	410.72	Squalene*	(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene
			$C_{19}H_{40}$	268.52	Octadecane, 2-methyl-	2-methyloctadecane
4	26.459	8.063	$C_{21}H_{44}$	296.57	Eicosane, 2-methyl-*	2-methylicosane
			$C_{27}H_{56}$	380.73	Heptacosane	Heptacosane
5	27.248	1.332	$C_{25}H_{42}$	342.60	2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosane pentaene	(6E,10E,14E,18E)-2,6,10,14,18-pentamethylicosa-2,6,10,14,18-pentaene
6	27.761	1.264	$C_{19}H_{39}Br$	347.42	Nonadecane, 1-bromo-	1-bromononadecane
			$C_{20}H_{41}Br$	361.44	1-bromoeicosane	1-bromoicosane
			$C_{22}H_{45}Br$	389.50	1-bromodocosane	1-bromodocosane
7	28.109	1.281	$C_{12}H_{38}O_5Si_6$	430.94	Hexasiloxane,1,1,3,3	1,1,3,3,5,5,7,7,9,9,11,11-

					,5,5,7,7,9,9,11,11-dodecamethyl-	dodecamethylhexasiloxane
8	28.421	1.095	$C_{14}H_{22}O_2$	222.32	2-(2,6,6-trimethylcyclohex-1-enyl) cyclopropane carboxylic acid, methyl ester	Methyl 2-(2,6,6-trimethylcyclohex-1-en-1-yl)cyclopropanecarboxylate
			$C_{13}H_{19}BrO_2$	287.19	Methyl 3-bromo-1-adamantaneacetate	Methyl 2-(3-bromoadamantan-1-yl) acetate
			$C_{24}H_{46}O_4Si$	426.71	1-monolinoleoylglycerol trimethylsilyl ether*	(9Z,12Z)-3-hydroxy-2-((trimethylsilyl)oxy) propyl octadeca-9,12-dienoate
9	28.641	1.507	$C_{12}H_{38}O_5Si_6$	430.94	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane
			$C_{14}H_{24}O_3Si_2$	296.51	2',6'-dihydroxyacetophenone, bis (trimethylsilyl) ether*	1-(2,6-bis((trimethylsilyl)oxy) phenyl)ethanone
			$C_{14}H_{24}O_3Si_2$	296.51	2,5-dihydroxyacetophenone, bis(trimethylsilyl) ether	1-(2,5-bis((trimethylsilyl)oxy)phenyl) ethanone
10	29.099	7.709	$C_{29}H_{60}$	408.79	2-methyloctacosane	2-methyloctacosane
			$C_{20}H_{42}$	282.55	Nonadecane, 2-methyl-	2-methylnonadecane
11	29.851	3.365	$C_{29}H_{50}O_2$	430.71	dl-alpha-tocopherol/ Vitamin E*	(R)-2,5,7,8-tetramethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman-6-ol
12	34.435	1.799	$C_{31}H_{48}O_3$	468.71	Urs-12-en-24-oic acid, 3-oxo-, methyl ester (+)-*	(6aR,6bS,8aR,11R,12S,12aR,14aR,14bR)-methyl 4,6a,6b,8a,11,12,14b-heptamethyl-3-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicene-4-carboxylate
13	35.113	1.235	$C_{30}H_{50}O$	426.72	Alpha-amyrin*	(3S,4aR,6aR,6bS,8aR,11R,12S,12aR,14bR)-4,4,6a,6b,8a,11,12,14b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-icosahydropicen-3-ol
14	35.443	1.389	$C_{20}H_{36}O_4$	340.50	Oxalic acid, allyl pentadecyl ester	allyl pentadecyl oxalate
			$C_{23}H_{42}O_4$	382.58	Oxalic acid, allyl octadecyl ester	allyl octadecyl oxalate
			$C_{16}H_{30}$	222.41	Cyclohexane,1,1'-(2-methyl-1-3-propanedilyl) bis-	(2-methylpropane-1,3-diyl) dicyclohexane
15	39.771	2.031	$C_{20}H_{40}O_2$	312.53	Ethanol, 2-(9-	(Z)-2-(octadec-9-en-1-yloxy)

		octadecenyloxy)-(Z)-	ethanol
$C_{20}H_{40}O$	296.53	3,7,11,15-tetramethyl-2-hexadecen-1-ol	(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol
$C_{17}H_{32}O_2$	268.43	Z-8-methyl-9-tetradecen-1-ol acetate	(Z)-8-methyltetradec-9-en-1-yl acetate

** Represent compounds that were repeated in spectra.*

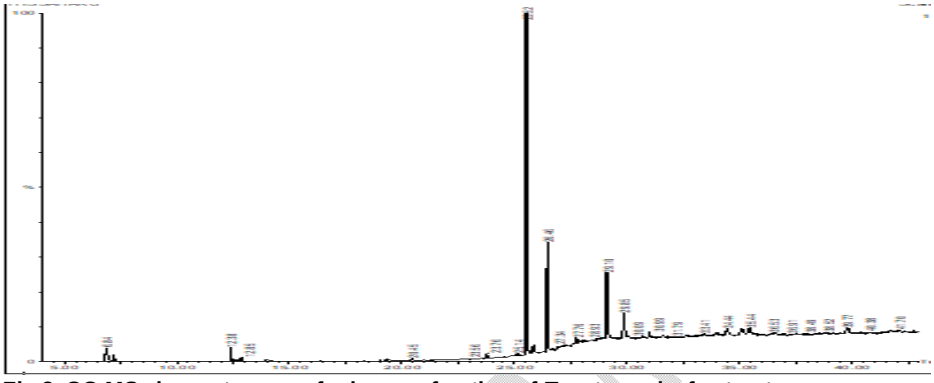
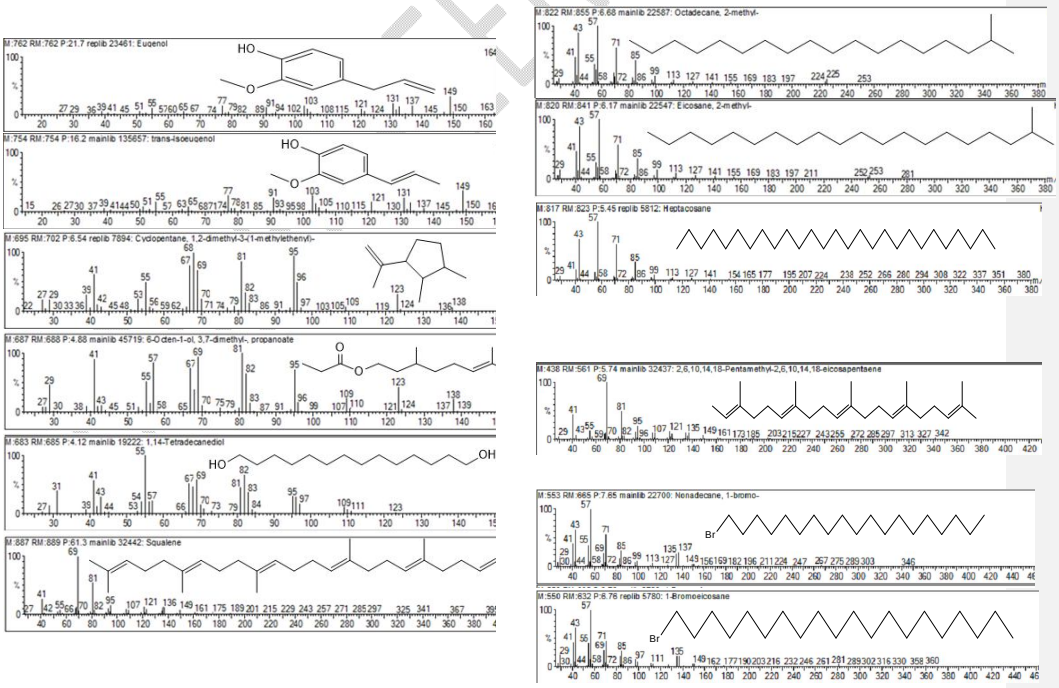


Fig.3. GC-MS chromatogram of n-hexane fraction of *T. catappa* leaf extract



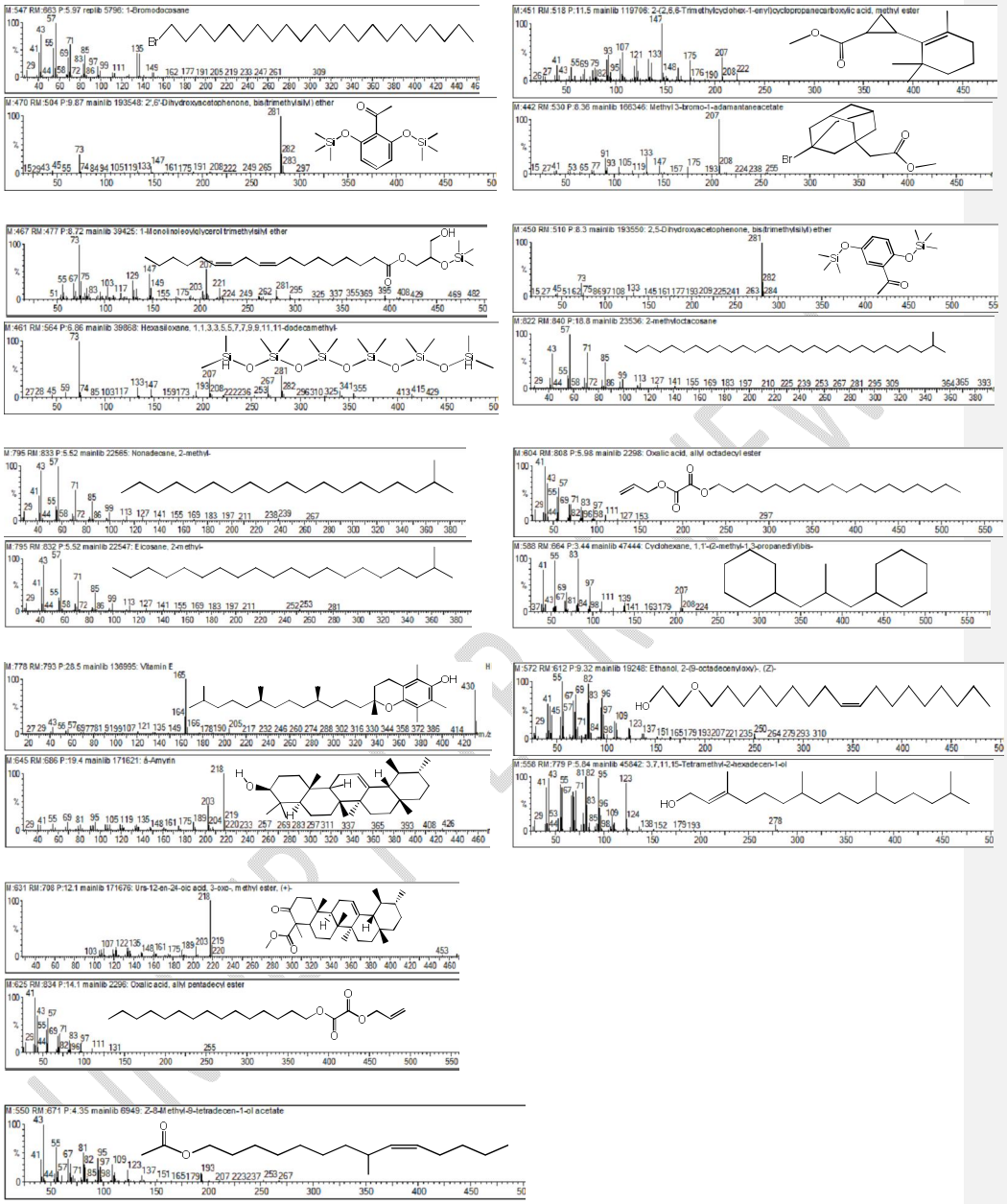


Fig. 4. Mass spectra of identified compounds from n-hexane fraction of *T. catappa leaf* extract

The antidiabetic activities of some of these suggested compounds have fully been documented. For instance, Urs-12-en-24-oic acid, 3-oxo-, methyl ester (+)-, is a member of the terpenoid class and is known to possess biological properties including anti-inflammatory, antioxidantand antidiabetic effects[30]. According to Al-Qahtani et al., 2023, Urs-12-en-24-oic acid, 3-oxo-, methyl ester (+)-binds more affinitively than acarbose to α-glucosidase and α-amylase. The molecule binds by preventing hydrophobic interactions and hydrogen bonding, with a value of -9.8 kcal/mol. [31] reported that the phenolic monoterpenoid eugenol possesses antibacterial, anti-inflammatory, analgesic, neuroprotective, anti-diabetic, and anti-tumour properties. The WHO also asserts the compound is safe for use and does not cause mutations [31].[32]reported the anti-

hyperglycemic potentials of α-amyrin in mice. [33]reported that squalene has a broad antidiabetic effect capable of modulating glucose at the pancreatic tissue level and in peripheral tissues such as the liver, muscles, and adipose. Thus, the alpha-glucosidase inhibition activity demonstrated by the *T. catappa* extracts might be due to the presence of thesecompounds. Results from qualitative phytochemical screening also support the suggestion that these compounds are possibly present in *T. catappa* extracts.

3.6FT-IR analysis of crude leaf extract of *T. Catappa*

Fourier Transform Infrared (FT-IR) spectroscopy is commonly used in the analysis of plant extracts for the presence of phytochemicals. The FT-IR spectra provide valuable information about functional groups present in molecules and this aids in the identification of various phytochemicals such as flavonoids, alkaloids, phenolics, glycosides and terpenoids [34] [27].

The FT-IR spectrum of *T. catappa* leaf extract is presented in Figure 5.Flavonoids, phenolics and terpenoids may typically exhibit absorption bands in the range of 3000-3500 cm^{-1} due to the presence of hydroxyl (OH) or amino (NH) groups in the molecule[35] [36]. The peak at 3404.89 cm^{-1} could correspond to these groups.

Flavonoids and phenolics also exhibit absorption bands in the region of 1650-1760 cm^{-1} due to the carbonyl group (C=O) present in the molecule. The peak at 1735.67 cm^{-1} may be attributed to the presence of these phytochemicals.

Absorption bands in the region of 1050-1300 cm^{-1} are characteristic of saponins due to the presence of O-H (hydroxyl) and C-O (alcohol) groups. The peaks at 1306.82 cm^{-1}, 1242.69 cm^{-1}, and 1164.65 cm^{-1} might be indicative of these functionalities.

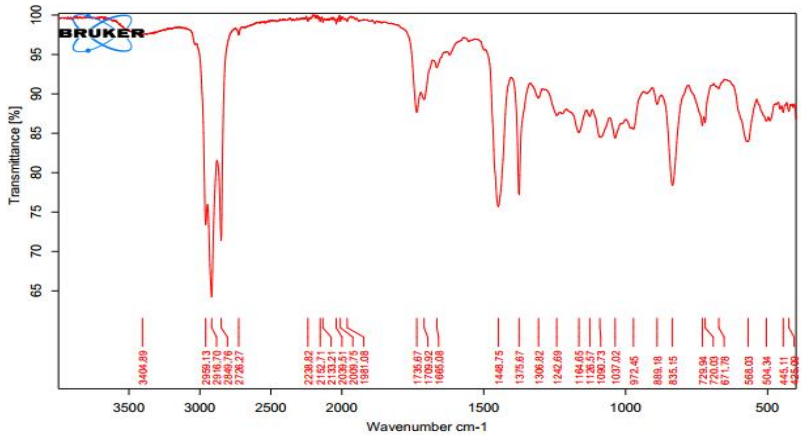


Figure 5: FT-IR spectrum of *T. Catappa* leaf extract

Tannins and phenolics often display absorption bands in the region of 1600-1700 cm^{-1} due to C=C stretching vibrations. The peak at 1665.08 cm^{-1} may also be relevant here. Additionally, they may exhibit absorption bands in the region of 1300-1500 cm^{-1} due to C-O stretching vibrations. The peak at 1448.75 cm^{-1} might indicate the presence of this functional group.

Terpenoids often show absorption bands in the region of 1450-1700 cm^{-1} due to the presence of C=C bonds. Peaks at 1448.75 cm^{-1} and 1665.08 cm^{-1} could be indicative of terpenoids.

Peaks in the region of 1000-1200 cm^{-1} are often associated with C-O-C stretching vibrations, indicative of ether bonds. These bonds are common in compounds like flavonol glycosides. The peaks at 1164.65 cm^{-1} and 1242.69 cm^{-1} might suggest the presence of such phytochemicals.

Absorption bands around 2800-3000 cm^{-1} usually correspond to hydrocarbon groups such as CH_3 and CH_2. These groups are common in terpenoids, fatty acids, and other lipid-like compounds. The peaks at 2959.13 cm^{-1}, 2916.70 cm^{-1}, 2849.76 cm^{-1}, and 2726.27 cm^{-1} could be indicative of the presence of terpenoids.

Absorption bands around 1400-1600 cm^{-1} often indicate the presence of aromatic rings with C=C bonds. These bonds are characteristic of flavonoids, phenolics, and terpenoids. The peaks at 1665.08 cm^{-1} and 1448.75 cm^{-1} might also suggest the presence of aromatic rings that are common with these phytochemicals.

The FT-IR spectra in general corroborates results from the phytochemical screening indicating that the leaf extract of *T. catappa* is rich in bioactive phytochemicals.

4. CONCLUSION

The hydroalcoholic leaf extract and solvent fractions of *T. catappa* were evaluated for α-glucosidase inhibitory activity and the bioactive constituents partially characterised by GC-MS and FT-IR. The leaf extract was found to be rich in phytoconstituents and also competitively inhibited the activity of α-glucosidase in a concentration-dependent manner. Eugenol, Urs-12-en-24-oic acid, 3-oxo-, methyl ester (+)-, phytol, trans-isoeugenol, alpha-amyrin, and squalene/ supraene were suspected to be the active α-glucosidase inhibitors from *T. catappa* extract. These findings underscore the promising role of *T. catappa* leaf extract as a source of bioactive compounds with potential applications in glycaemic control and the treatment of related metabolic disorders.

On the basis of these findings, it is recommended to further isolate and completely characterize potential α-glucosidase inhibitors from *T. catappa* leaf extract. Conducting an *insilico* investigation using molecular docking techniques to predict the binding modes and affinities of active isolated compound(s) with the target enzyme is desirable.

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