THE INHIBITORY EFFECT OF DOUM PALM (HYPHAENE THEBAICA L. MART.) LEAVES EXTRACT ON α-GLUCOSIDASE ACTIVITY

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__Background:__ Hyphaene thebaica L. Mart. (Doum-palm), rich in total phenolics content, is known for its medicinal value in the treatment of several health conditions, such as hypertension, and diabetes mellitus. __Aim of the Study:__ To investigate the hypoglycaemic activity of the dichloromethane, ethyl acetate, and aqueous fractions from doum palm leaves. Then, to characterize the metabolic profile of the most active fraction by LC-HR-MS/MS analysis to detect the responsible metabolites for this activity. __Material and Methods__: The present study examined the in vitro inhibitory effect of the extract fractions from Doum Palm Leaves at concentrations ranging from 7.81 to 1000.00 µg/ml on α-glucosidase activity, an enzyme responsible for carbohydrate-hydrolysis to monosaccharides and intestinal glucose absorption. Metabolic profiling for the dichloromethane fraction was obtained with LC-HR-MS/MS. __Results:__ The dichloromethane (DCM) fraction inhibited α-glucosidase activity in vitro with an IC_{50} of 52.40 µg/ml. Twenty-three compounds were identified in the DCM fraction by LC-HR-MS/MS analysis. Most of them were reported for their potential antidiabetic activity. Nevertheless, the III-DCM subfraction (IC_{50} 3.79 ± 0.17 µg/ml) and the IV-DCM subfraction (IC_{50} 5.13 ± 0.24 µg/ml) had the best inhibitory activity against α-glucosidase compared with acarbose (IC_{50} 2.33 ± 0.11 µg/ml). __Conclusions:__ The results support the use of these fractions obtained from Doum palm leaves to effectively inhibit a crucial enzyme linked to type 2 diabetes and suppress carbohydrate absorption from intestine, and thereby reducing the postprandial increase of blood glucose.

__Keywords:__ α -glucosidase, antidiabetic activity, doum, LC-HR-MS/MS, target metabolites

INTRODUCTION

Hyphaene thebaica L. Mart. is a dioecious palm belonging to the family Arecaceae that is characterized by its edible fruits. It is also known as doum-palm or doom-palm referring to the persistence of the tree under abnormal conditions (drought-tolerant) and its resistance to destruction by fire, as the word doum means permanence. Also, it is known as Gingerbread-tree. It is native to the northern half of Africa, Yemen, Saudi Arabia, and India. In Egypt, it is widely distributed in Sinai and along the Nile River especially in Upper Egypt [1-3]. Doum is an economically important product in Egypt a long time ago and it is extensively described in Pharaonic Egyptian tombs[4]. All parts of the tree (leaves, seeds, fruit, wood and roots) are applied for the manufacture of several traditional products[3,5] and are applied in folk medicine for the treatment of various disorders[3,6]. Phytochemical investigation of different parts of doum-palm indicated its high content of phenolics: flavonoids and phenolic acids (fruits[7-11] and leaves[12]), volatile oil [13], fatty acids[11], polysaccharides in particular mannan (seeds) [14,15] and estrone (seeds)[16].

Several biological studies were carried out to evaluate the biological activities of doum fruits. Doum fruits aqueous extracts have a hypotensive effect in normotensive and hypertensive anaesthetized dogs[17] and in

hypertensive rats [18], an antibacterial and a significant antifungal activities against a wide range of fungal and bacterial isolates[19-22], a nematicidal effect against root-knot nematode *Meloidogyne incognita* infecting sugar beet [23], a significant antioxidant activity[20,21, 24-29] and a significant hypocholesterolemic effect in normal diet albino rats[18,30,31] and a neuroprotective effect against Alzheimer's disease in an animal model[32]. Furthermore, nanoparticles of the extract of doum fruit showed anticancer activity against human prostate (PC-3), breast (MCF-7), and liver (HepG-2) cancer cell lines using MTT assay[12,33].

Type 2 diabetes mellitus is characterized by hyperglycemia, which may occur due to disorders in processing carbohydrates, proteins, and fats. Alpha-glucosidase is an imperative enzyme for the degradation of oligosaccharides into simple sugar. Thus, the inhibition of α-glucosidase would cause a decrease in the absorption of glucose and consequently inhibit the increase in postprandial blood glucose. The uses of medicinal plants are still continuing for the treatment of diabetes. The active ingredients present in medicinal plants can act on a variety of targets through a variety of mechanisms and have a great potential in the treatment of diabetes and its complications. The methanolic extract of doum-fruit exhibited *in vivo*[9, 34] and *in vitro* antidiabetic activity[35]. This antidiabetic activity is attributed either to its flavonoid [9, 36] or glycan content[37]. However, few studies were carried out to investigate the pharmacological activities of the leaves. Doum leaves are reported for their antioxidant activity that is attributed to their phenolic and flavonoid content[29, 38] and *in vitro* cytotoxic activity[39]. To the best of our knowledge, no reports for the hypoglycemic effect of doum leaves were recorded. In the light of these observations, the objective of this work is to evaluate the antidiabetic activity of dichloromethane, ethyl acetate and aqueous fractions of the doum leaves extract and to estimate the bioactive metabolites by LC-HR-MS/MS in the most active fraction.

MATERIALS AND METHODS

Plant material

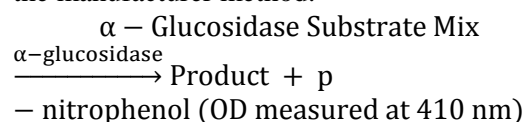Leaves of *H. thebaica L.* Mart. were collected during the flowering stage (April to July 2019) from Aswan, Egypt. The plants were identified and authenticated by Dr. Trease Labib (consultant of plant taxonomy at Ministry of Agriculture and director of El-Orman Garden, Giza, Egypt). Voucher (29469) specimen were kept in the herbarium, Aswan Botanical Garden, Aswan, Egypt.

Extraction and isolation

The air-dried powdered leaves of *H. thebaica L.* Mart. (3 kg) were exhaustively extracted with 70% ethanol by maceration at room temperature. The ethanolic extract was concentrated under reduced pressure to obtain a viscous residue (410 g). This residue was suspended in distilled water (2 L) and subjected to solvent fractionation using dichloromethane and ethyl acetate. Each fraction was separately concentrated to give 56.2 g, 18.9 g, respectively and the remaining aqueous fraction (315 g). Part of the aqueous fraction (200 g) was subjected to the Diaion HP-20 column chromatography and eluted with distilled water, distilled water: methanol 1:1, and methanol to give three main subfractions. The yield of water subfraction was 135.5 g, water: methanol 1:1 subfraction was 39.1 g, and methanol subfraction was 10.5 g. The dichloromethane fraction (50 g) was subjected to VLC using dichloromethane: methanol in a gradient elution manner, where four subfractions (I -IV) were obtained: subfraction I (100% DCM), subfraction II (DCM: MeOH 98: 2), subfraction III (DCM: MeOH 94: 6) and subfraction IV (DCM: MeOH 80: 20).

In-vitro α-Glucosidase Inhibitory Assay

Alpha-Glucosidase inhibitor Screening Kit (Colorimetric) (Catalog # K690-100) was purchased from Biovision. Acarbose was used as the positive control. The α-glucosidase inhibition assay depends on the ability of an active α-glucosidase to cleave a synthetic substrate, thus releasing a chromophore which is measured at optical density (OD) 410 nm. In the presence of an α-glucosidase-specific inhibitor, the enzymatic activity is greatly reduced, which is detected by a decrease in absorbance readings. An α-glucosidase inhibition assay was performed as described by the manufacturer method.

$$\alpha - \text{Glucosidase Substrate Mix} \xrightarrow{\alpha-\text{glucosidase}} \text{Product} + p - \text{nitrophenol (OD measured at 410 nm)}$$

Briefly, a mixture of 10 μL of each extract (or acarbose provided in the kit), and 10 μL of enzyme was preincubated at room temperature for 5 min before 20 μL of the preformed reaction mix substrate solution was added, and the reaction mixture was further incubated at room temperature for 15 min. The absorbance of the liberated *p*-nitrophenol was measured at 410 nm.

High Resolution Liquid Chromatography-Mass Spectrometry (LC-HR-MS) analysis

Samples were dissolved in water: acetonitrile 50: 50 containing 0.1% formic acid and analyzed by HR-LC-MS with an Agilent Technologies HPLC system equipped with an autosampler (G7129A), a quaternary pump (G7104C), and directly connected to a mass detector ESI-QTOF-MS\MS (6530, Agilent Technologies). Chromatographic separation was carried out at room temperature using a Zorbax RP-18 column (G7116A) (2.7 μ particle size, 150 × 3 mm i.d.). The mobile phase H_2O-CH_3CN (90:10, v/v) to (50:50, v/v), in 35.0 min, to (25:75, v/v) in 25 min. and to (0:100, v/v) in 80 min, linear gradient, and a flow rate of 0.4 mL/min. The injection volume was 4 μl. The chemical constituents were detected using ESI-QTOF-MS\MS (6530, Agilent Technologies) in both positive and negative ionization modes, The interface and MS parameters were as follows: The nebulization pressure was 40 psi; dry gas (N_2) flow rate (10.0 L/ min); dry gas temperature, 320 ℃; spray capillary voltage 4000 V; skimmer and fragmentation voltages were set at 65 and 130 V, respectively; ion transfer capillary exit, 100 V and the collision energy was 10 V; scan range in 100 to 2500 *m/z*. The tuning mixture of different organic compounds with different masses (Agilent Technologies) was used for constant MS calibration. From these MS spectra, the most abundant product ion was automatically isolated and further fragmented (with intensities > 10000) generating data-dependent MS/MS spectra.

Data analysis and evaluation

The data were acquired by Mass Hunter Workstation LC/MS Data Acquisition software (Agilent Technologies). Further, the data was analyzed using Agilent Mass Hunter qualitative analysis B.06. the compounds were detected according to the following parameters that were set to a peak filter of 1000 counts peak height,

ion species to "positive ions" with H^+, Na^+, K^+, and NH_4^+, "charge state" to 1, the mass to ± 5 ppm. The extracted ion chromatograms (EICs) were smoothed with a Gaussian function using 5 points function width and 5000 points Gaussian width. Further, the different compounds were characterized according to the local database embedded inside the software. The local database was collected from the available literature about the Doum palm identified compounds. The database contains 62 compounds. The detections were according to the following parameters: the mass deviation is ± 5 ppm, and the score was ≥75. The clustering analysis was performed using Mass Profiler Professional (MPP) 13.1.1 software packages. The distance was measured using Euclidean. The data for inhibitory activity of plant extracts for α-glucosidase enzyme are presented as mean ± standard deviation of the mean for n=3. The dose–response curve was obtained by plotting the percentage inhibition versus concentration. The concentration giving 50% inhibition (IC_{50}) was calculated by non-linear regression using Prism software (GraphPad Software, version 8.3.0 San Diego, CA, USA)

RESULTS AND DISCUSSION

### Preparation of doum leaves extracts

The total ethanolic extract of doum leaves was fractionated as described in the experimental part to give dichloromethane fraction (DCM fraction), ethyl acetate fraction and the remaining aqueous fraction. DCM fraction was further subjected to silica gel column chromatography to give four main subfractions (I - IV DCM). On additions the remaining aqueous fraction was applied to Diaion HP-20 to give three subfractions: methanol subfraction, water: methanol 1:1 subfraction, and water subfraction. All mentioned fractions were tested for their α-glucosidase inhibitory effect as presented in the following text.

Alpha-glucosidase inhibition of *Hyphaene thebaica* L. Mart.

In the preliminary screening with the fractions, the dichloromethane fraction exerted the chief inhibiting effect on α-glucosidase activity with IC_{50} = 52.40 (Figure 1). The methanol subfraction, the water: methanol 1:1 subfraction and the ethyl acetate fraction

exerted less inhibiting effect on α-glucosidase activity with IC_{50}, 91.60, 102.90, and 265.20 respectively, while the water subfraction did not exert inhibiting effect on α-glucosidase activity. Furthermore, the optimal concentrations of the four subfractions (I -IV) obtained from the dichloromethane fraction

required for the 50% inhibition (IC_{50}) against α-glucosidase using acarbose as a positive control were most prominent in subfraction III (93.4% of inhibition) with IC_{50}= 3.79, while acarbose % of inhibition on α-glucosidase activity was 92.7% with IC_{50}= 2.33 (Table 1).
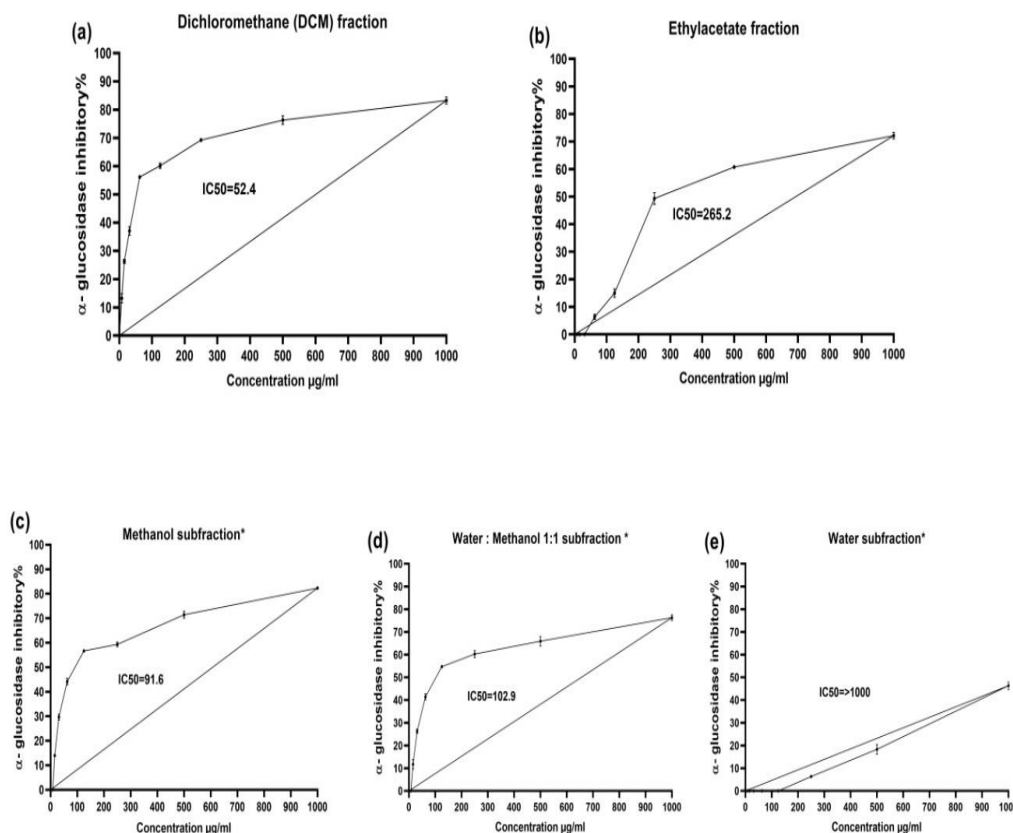
Fig. 1: α-glucosidase inhibitory activity of Doum Palm (*Hyphaene thebaica* L.) Leaves Extracts (IC_{50} μg/ml). Dose‑dependent inhibitory activity of (a): Dichloromethane (DCM) fraction, (b): Ethylacetate fraction, (c): Methanol subfraction*, (d): Water : Methanol 1:1 subfraction *, (e): Water subfraction* (* represents subfractions of the aqueous fraction by Diaion HP-20 chromatography). α-glucosidase inhibitory % values are the mean ± S.D for (n = 3). Data were analyzed using nonlinear regression analysis using GraphPad Prism software.

Table 1: Maximum inhibition of α-glucosidase enzyme and IC_{50} values of isolated DCM subfractions (I-IV DCM subfractions) and the positive control acarbose

Fractions	Maximum inhibition (%)	$IC_{50} \pm SD$ ug/ml
I DCM	87.4	20.06 ± 0.92
II DCM	81.0	42.86 ± 1.97
III DCM	93.4	3.79 ± 0.17
IV DCM	88.5	5.13 ± 0.24
Acarbose	92.7	2.33 ± 0.11

Values are expressed as the means ± SD of the mean of three replicate samples. IC_{50} values were calculated by linear regression analysis using Prism software (GraphPad Software, San Diego, CA, USA).

Hyphaene thebaica L. Mart. metabolic profile obtained with LC-HR-MS/MS

The mass spectrometric total ion chromatogram (TIC) was obtained with reversed-phase (RP)-QTOF-MS/MS and interpreted to acquire the extracted ion chromatograms (EICs), which enabled the extraction of the retention time (RT)/mass for each feature. The RT of features was plotted on the x-axis in minutes while the masses were plotted on the y-axis in *dalton* units (Figure 2). The positive metabolic profile was represented in light blue squares. Further, the negative metabolic profile of *H. thebaica L. Mart. H.*

thebaica L. Mart. Mart. was represented in grey circles. The positive metabolic profile consisted of 1236 features, and the negative one was 1024 features. This might be due to a large number of compounds that are ionized in positive mode like amino acids, vitamins, sterols, and flavonoids. The latter is ionized in both modes[41]. The dendrogram clustering of the negative and positive metabolic profile were revealed that the two clouds complete each other. Thus, we could consider the data as the almost the complete metabolic profile of *H. thebaica* L.
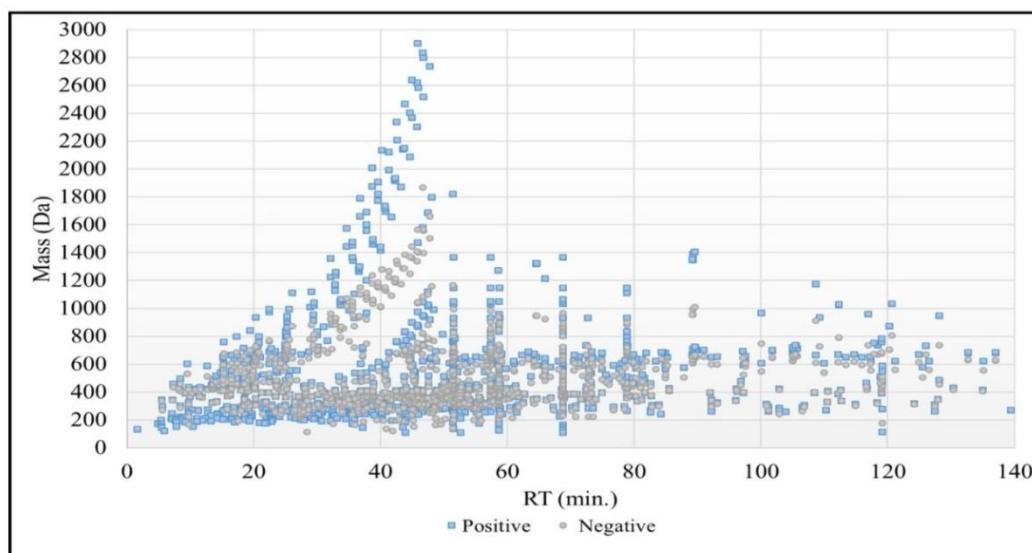
Fig. 2: Retention time/mass plot of *Hyphaene thebaica* L. Mart. metabolic profile. The compounds identified in the positive ionization mode were represented as light blue colored squares. The compounds identified in negative ionization mode were represented as grey-colored circles.
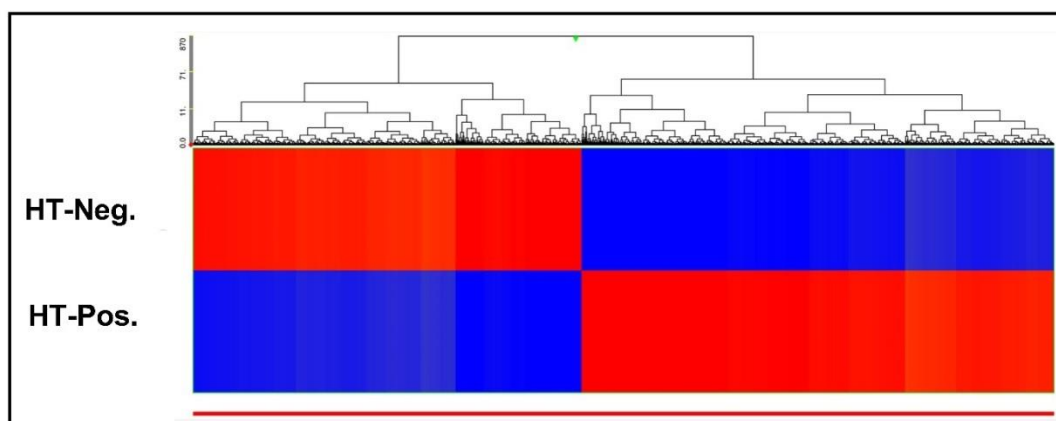

Fig. 3: The dendrogram showed the hierarchical clustering results of *Hyphaene thebaica* L. Mart. negative (HT-Neg.) and positive (HT-Pos.) metabolic profile. Also, the heat map of both was plotted. The blue color represents the lowest intensity and the red color represents the highest intensity.

Target analysis of *Hyphaene thebaica* L. Mart.

The antidiabetic activity of the *H. thebaica L. Mart.* metabolic profile was investigated using the target analysis to detect the responsible compounds for this activity. The identification was performed using the finding compounds with the target MS/MS function in Agilent software. The software suggests the most important MS/MS fragments and compared them with the literature. An exemplary extracted ion chromatogram, MS/MS spectra, and MS/MS spectra predicted by the software according to the molecular formula of apigenin and tricin were identified in the negative and positive metabolic profiles of *H. thebaica* L. *Mart.*, respectively, which were shown in Figure 4.

In the negative mode the following organic acids: protocatechuic acid, chlorogenic acid, 5-*O*-caffeoyl shikimic acid, cinnamic acid, ferulic acid, coumaric acid, and 3-hydroxy tyrosol were identified in the DCM fraction. In the positive mode, ferulic acid and vanillic acid were identified.
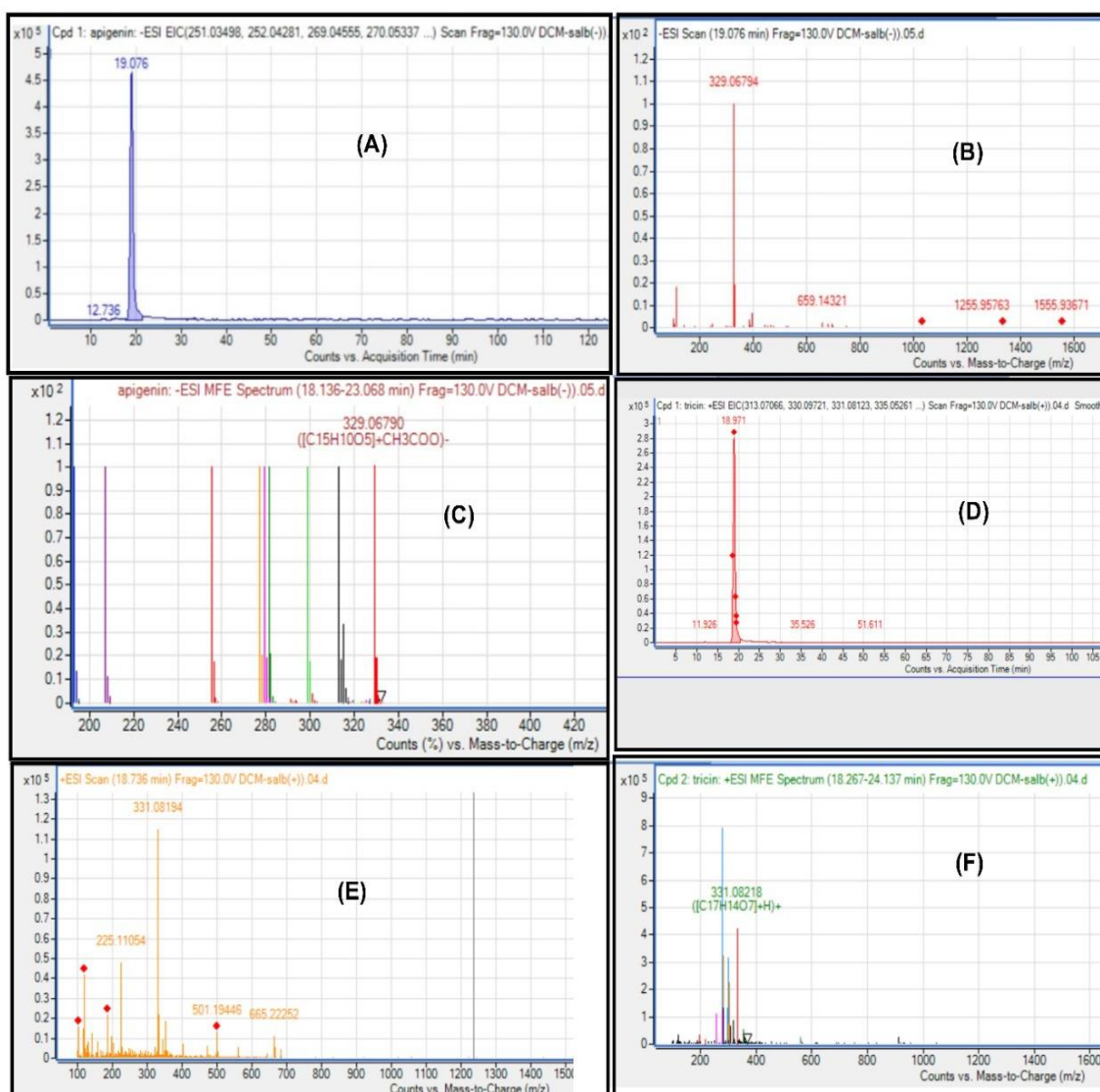
Fig. 4: (**A**), Extracted ion chromatogram of apigenin in the negative metabolic profile of *Hyphaene thebaica* L.; (B), MS/MS spectra of apigenin; (C), MFE MS/MS spectra of apigenin the red color represents the MS selected and the blue color represent the MS/MS precursor ion which was predicated according to its molecular formula by the software package; (D), Extracted ion chromatogram of tricin in the negative metabolic profile of *Hyphaene thebaica* L.; E)-MS/MS spectra of tricin; (F), MFE MS/MS spectra of tricin the red color represents the MS selected and the blue color represent the MS/MS precursor ion which was predicated according to its molecular formula by the software package

Table 2: The list of compounds identified in the *H. thebaica* L. dichloromethane extract metabolic profile in the negative mode. The calculated mass in the database (DB) and the extract (HT) were listed beside the deviation between them (Δppm). Also, the retention time (RT) was listed. The references for the most abundant fragment (MS/MS Ref.), and the isolated compounds from doum-palm (REF. C.) were listed.

Compound name	Mass (Calc) (DB)	Mass (HT)	Δppm	RT	MS/MS Fragments	MS/MS Ref.	Ref. C.
Protocatechuic acid	154.0266	154.026	3.83	3.34	———	———	12
Chlorogenic acid	354.0951	354.0968	-4.72	5.46	———	———	11
5-*O*-caffeoyl shikimic acid	336.0845	336.0859	-4.23	5.46	———	———	11
Catechin	290.079	290.0798	-2.69	5.46	———	———	12
Luteolin 7-*O*-β-rutinoside	594.1585	594.1607	-3.62	7.81	———	———	13
Coumaric acid	164.0473	164.0469	2.50	7.92	163.04; 119.9	40	12
3-Hydroxy Tyrosol	154.063	154.0631	-0.39	8.04	———	———	13
Quercetin-3-*O*-β-rhamnoside	448.1006	448.1016	-2.14	8.63	———	———	13
Cinnamic Acid	148.0524	148.0526	-1.28	12.50	147; 145.05; 102.95	40	12
Oleuropein	540.1843	540.1826	3.17	12.74	———	———	12
Rhamnetin	316.0583	316.0575	2.44	15.67	———	———	12
Apigenin	270.0528	270.0538	-3.81	19.08	269.0; 225.0; 201.12	41	13
Tricin	330.074	330.075	-2.91	19.08	331.07; 330.07; 329.07; 328.22; 327.22	40	13
Chrysin	254.0579	254.0585	-2.40	19.31	183; 181; 141	40	36
Naringenin	272.0685	272.069	-1.95	19.31	271.06; 259.0; 112.9	42	12
Chrysoeriol	300.0634	300.064	-1.97	19.31	283.15; 183	40	7-9, 40
Hesperetin	302.079	302.0791	-0.26	27.29	301; 283	40	12
Linolenic acid	278.2246	278.2253	-2.59	51.48	209.0; 277.2	40	11
Linoleic acid	280.2402	280.2411	-3.18	58.53	279.2; 235.9	40	11
Palmitic acid	256.2402	256.2398	1.64	68.56	225.23; 195; 145.05	40	11
Oleic acid	282.2559	282.2568	-3.33	72.61	281.25; 282.25	40	11

Further, flavonoids aglycone and glycosides were identified in the negative ionization mode in the DCM metabolic profile of the doum palm including rhamnetin, apigenin, tricin, chrysoeriol, chrysin, naringenin, luteolin-7-*O*-β-rutinoside, quercetin-3-*O*-β-rhamnoside, and hesperetin. furthermore, anthocyanin as catechin and catechol members oleuropein (secoiridoid glycoside) were identified. Besides, long-chain fatty acids such as linoleic acid, and oleic acid were identified in both negative and positive modes. Further, palmitic acid and linolenic acid were identified in the negative, and their masses were detected also in the positive,

however, at different RT which might be due to the presence of their isomers or derivatives.

The presence of the compounds in the positive and negative ionization modes confirmed the identification of the compounds. The calculated mass, the measured mass, and the deviation between them (Δppm) were listed in Tables (2&3). The RT and the most important MS/MS fragments and the corresponding references were listed in the Tables (2&3). The software suggested the different fragments for the major identified compounds their intensities were less than 10000, which also matched the references which were shown in Figure 5.

Table 3: the list of compounds identified in the *H. thebaica* L. dichloromethane extract metabolic profile in the positive ionization mode. The calculated mass in the database (DB) and the extract (HT) were listed beside the deviation between them Δppm. Also, the retention time (RT) was listed. The references for the most abundant fragment (MS/MS Ref.), and the isolated compounds from doum-palm (Ref. C.) were listed.

Compound name	Mass (DB)	Mass (HT)	Δ ppm	RT	MS/MS Fragments	MS/MS Ref.	Ref. C.
Ferulic acid	194.0579	194.0589	-4.90	8.62	177.05; 194.08	40	12
Tricin[*]	330.074	330.0749	-2.67	18.92	331.08; 120.01; 196.58; 239.95; 297.76	43	13
Linolenic acid derivative or isomer[*]	278.2246	278.2246	-0.04	39.4	277.22; 119.06; 257.1	40	11
Linoleic acid[*]	280.2402	280.2411	-3.10	58.68	281.25; 263.24; 245.23; 197.16	40	11
Oleic acid[*]	282.2559	282.2565	-2.02	72.85	265.25; 282.28	43	11
Palmitic acid derivate or isomer[*]	256.2402	256.2407	-1.99	103.89	257.25; 256.26; 239.24	43	11
Vanillic acid	168.0423	168.0419	-2.14	66.76	164.05	43	12

[*] Compounds identified in the positive and negative ionization modes
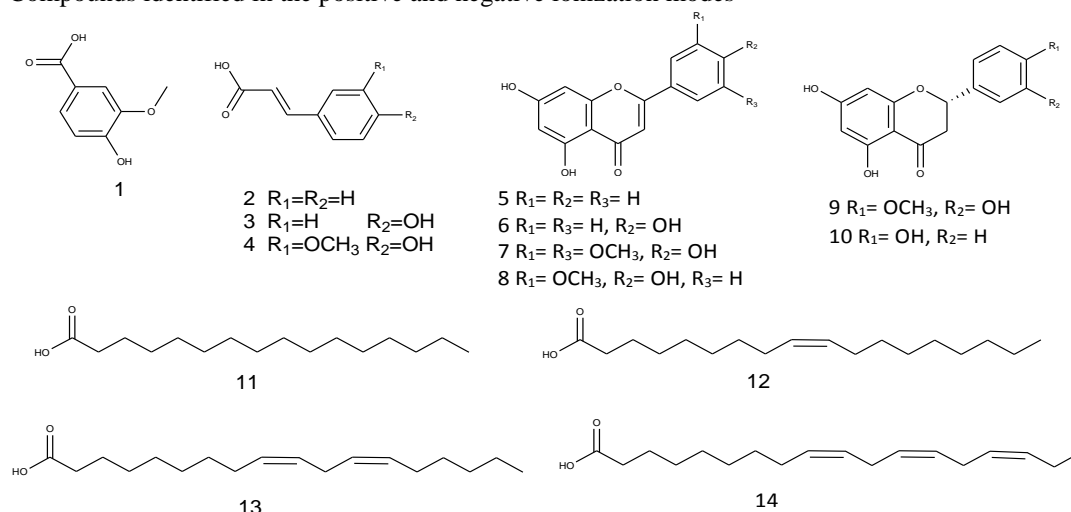
Fig. 5: Chemical structures of major compounds identified in the *H. thebaica* L. dichloromethane extract by LC-HR-MS/MS, Vanillic (1), cinnamic (2), coumaric (3), ferulic (4) acids, chrysin (5), apigenin (6), tricin (7), chrysoeriol (8), hesperetin (9), naringenin (10), palmitic (11), oleic (12), linoleic (13), linolenic (14) acids.

The tricin flavonoids show a peak at 589.2464 which indicated the presence of tricin derivatives. This confirms the identification of tricin inside the extract[42, 43].

The peak of chrysin was detected at *m/z* 299.06 (M+HCOO)⁻, and the characteristic MS/MS fragments were compared with the mass bank database, which was listed in Table 1. The same fragment (M+HCOO)⁻ was detected for cinnamic acid, apigenin, chrysin, and palmitic acid is due to the usage of formic acid.

The protocatechuic acid, luteolin-7-*O*-rutinoside, 3-hydroxy tyrosol, Quercetin-3-*O*-β-rhamnoside, oleuropein, rhamnetin, catechin, chlorogenic acid, 5-*O*-caffeoyl shikimic acid did not have MS/MS fragments, however, the high-resolution mass detection, as well as, they have been identified before in doum palm extracts suggested their identification (Tables 2,3).

Many researchers reported the potential antidiabetic activity of natural products with less side effects as polyphenols (Curcumin, resveratrol, carotenoid, flavonoids and berberine phenolic acids, lignans, anthocyans and stilbenes), coixol, andrographolide, polypeptide p, charantin, and different plants extract as *Tinospora cordifolia*, *Annona squamosa*, *Aloe vera*, cinnamon and Nigella. Polyphenols can modulate carbohydrate metabolism, decrease hyperglycemia and control insulin resistance, increase lipid metabolism, optimize oxidative stress and inflammatory processes, inhibit α-glucosidase and α-amylase in the digestive tract. Also, polyphenols may modulate glucose uptake and expression of glucose transporters; stimulate insulin secretion and pancreatic β-cell proliferation[44-47]. Although pancreatic α-amylase is an important enzyme that breaks down dietary long chain carbohydrates, still α-glucosidase is a concluding enzyme that acts on shorter starch chains and disaccharides to produce glucose. Therefore, inhibiting α-glucosidase can suppress carbohydrate digestion and reduce blood glucose levels in patients with type-2 diabetes. A limited number of studies evaluated doum palms and focused mainly on the fruit extract [48]. The current study highlighted the α-glucosidase inhibitory activity of *H. thebaica* L leaves extract as a proposed mechanism for the evident hypoglycemic effect. The activity of these fractions can be attributed to their phytoconstituents, such as flavonoids and,

phenolic compounds. The dichloromethane fraction explicated a notable inhibition potential (83.25%) against the α-glucosidase enzyme, while the other fractions were found to be less effective against the α-glucosidase enzyme. Furthermore, the dichloromethane fraction was further fractionated successively and searching published data for the potential antidiabetic activity of the identified compounds in the dichloromethane fraction, protocatechuic acid had insulin-like activities[49,50]. The methanol leaf extracts from *Cecropia obtusifolia* and *C peltata* had significant hypoglycemic effect and was correlated with the chlorogenic acid contents in both species[51], in addition, molecular docking findings indicated that chlorogenic acid inhibited α-amylase and α-glucosidase[52]. Many *in vitro* studies and animal models, cinnamic acid and its derivatives as coumaric and ferulic acids act on different mechanism of actions, including stimulation of insulin secretion, improvement of pancreatic β-cell functionality, inhibition of hepatic gluconeogenesis, enhanced glucose uptake, increased insulin signaling pathway, delay of carbohydrate digestion and glucose absorption, and inhibition of protein glycation and insulin fibrillation [53-55]. Tyrosol treatment significantly reduced plasma glucose and significantly increased plasma insulin and high-density lipoprotein cholesterol in STZ-induced diabetic rats [56]. Vanillic acid might ameliorate insulin resistance via improving hepatic insulin signaling and alleviating inflammation pathways [57]. Apigenin and naringenin significantly decreased the levels of blood glucose and down-regulating oxidative stress and inflammation [58]. Identified metabolites from *Cosmos caudatus* leaf extracts were correlated with total phenolic content and α-glucosidase inhibitory activity resulted in quercetin-3-*O*-rhamnoside were identified as one of the major bioactive metabolites [59]. The administration of hesperetin attenuates the hyperglycemia and dyslipidemia through ameliorating antioxidant competence in STZ-induced experimental rats [60]. Citrus flavonoids, including hesperetin and naringin have antidiabetic potential. These flavonoids regulated biomarkers of glycemic control, lipid profiles, renal function, hepatic enzymes, and antioxidant enzymes, and modulated signaling pathways related to glucose uptake and insulin sensitivity that are involved in the pathogenesis of diabetes and its related complications[61].

Oleuropein is a promising compound for diabetes and diabetes complications management and can be used as a nutraceutical to fight against these diseases[62]. A recent analysis revealed that higher linoleic acid biomarker is associated with dose-dependent decreases in the incidence of type 2 diabetes, better glycemic control and/or insulin sensitivity [63]. Oleic acid compared with palmitic acid on insulin resistance and type 2 diabetes, including its anti-inflammatory actions, and its capacity to inhibit endoplasmic reticulum stress, prevent attenuation of the insulin signaling pathway, and improve β-cell survival [64].

Conclusion

In the current study, dichloromethane fraction and its subfractions III, IV obtained from doum palm (*H. thebaica* L.) leaves efficiently and effectively inhibited the primary carbohydrate-hydrolyzing enzyme (α-glucosidase) linked to type 2 diabetes. A limitation of this study was to focus on studying the α-glucosidase, however, further different mechanisms caused by the bioactive components which can lead to hypoglycemic activity of these subfractions will be investigated. Moreover, the toxicological consequences of utilizing these fractions and the resulting isolated chemicals should be thoroughly investigated in addition to quantitative study using HPLC analysis will be conducted.

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نشرة العلوم الصيدليــــة
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التأثير المثبط لمستخلص اوراق نخلة الدوم (هيفان ثيبايكا مارت.) على نشاط ألفا جلوكوزيداز

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الخلفية: هيفان ثيبايكا مارت. (نخلة الدوم) ، الغني بالمحتوى الفينولي ، معروف بقيمته الطبية في علاج العديد من الامراض ، مثل ارتفاع ضغط الدم ، وداء السكري.

الهدف من الدراسة: التحقق من فعالية خافض سكر الدم للمستخلصات المختلفة (ثنائي كلورو ميثان ، أسيتات الإيثيل ، و المائي) من أوراق نخيل الدوم. ثم بعد ذلك ، تحديد المركبات للجزء الأكثر نشاطًا عن طريق التحليل الطيفي للكتلة اللوني السائل عالي الدقة و المسئولة عن هذا النشاط.

المواد والطرق: فحصت الدراسة الحالية التأثير المثبط في المختبر لمستخلصات أوراق النخيل الدوم (هيفان ثيبايكا مارت) بتركيزات تتراوح من ٧,٨١ إلى ١٠٠٠,٠٠ ميكروجرام / مل على نشاط ألفا جلوكوزيداز ، وهو إنزيم مسئول عن تحلل الكربوهيدرات. للسكريات الأحادية وامتصاص الجلوكوز المعوي. تم التعرف على المركبات الكيميائية لمستخلص ثنائي كلورو ميثان باستخدام التحليل الطيفي للكتلة اللوني السائل عالي الدقة.

النتائج: أدى جزء ثنائي كلورو ميثان (DCM) إلى تثبيط نشاط ألفا جلوكوزيداز في المختبر (التركيز المثبط لنصف الحد الأقصى ٥٢,٤٠ ميكروجرام / مل)، تم تحديد ثلاثة وعشرين مركبًا في جزء DCM عن طريق التحليل الطيفي للكتلة اللوني السائل عالي الدقة. و معظمهم لهم نشاط محتمل في علاج مرض السكري. وكذلك ، فإن الجزء الثالث من DCM (التركيز المثبط لنصف الحد الأقصى ٠,١٧ ± ٣,٧٩ ميكروجرام / مل) والجزء الرابع من DCM (التركيز المثبط لنصف الحد الأقصى ٠,٢٤ ± ٥,١٣ ميكروجرام / مل) كان لهما أفضل نشاط مثبط ضد ألفا جلوكوزيداز مقارنة مع أكاربوز (التركيز المثبط لنصف الحد الأقصى ٠,١١ ± ٢,٣٣ ميكروجرام / مل).

الاستنتاجات: النتائج تدعم استخدام هذه الأجزاء التي تم الحصول عليها من أوراق نخيل الدوم (هيفان ثيبايكا مارت.) لتثبيط بشكل فعال إنزيم مهم مرتبط بمرض السكري من النوع ٢ ومنع امتصاص الكربوهيدرات من الأمعاء ، وبالتالي تقليل الزيادة بعد الأكل في نسبة الجلوكوز في الدم.