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Chemical and pharmacological evaluation of the non-flowering aerial parts of *Acacia modesta* Wall. cultivated in Egypt

Eman Mohamed Salah[1*], Reham R. Ibrahim[2], Mariam H. Gonaid[1] and Hesham S. M. Soliman[2]

Abstract

Background: *Acacia modesta* Wall. (*A. modesta*), often recognized as Phulai, is belonging to family Fabaceae and sub-family Mimosaceae. *A. modesta* has many beneficial uses. Leaves, wood, flowers, and gum of *A. modesta* have been used frequently for multiple therapeutic purposes.

Results: The chemical investigation of butanol fraction of *A. modesta* non-flowering aerial parts yielded Vitexin-2″-*β*-*D*-glucopyranoside and Apigenin-6,8-di-*C*-*β*-*D*-glucopyranoside in a flavone mixture as well as (*β*-*D*-glucopyranosyl (1-3)-*β*-*D*-glucopyranosyl)-3-*β*-hydroxy-11-oxo-olean-12-en-28-oic acid) an oleanane-type triterpenoidal saponin. Metabolite profiling via ultra-performance liquid chromatography-electrospray ionization-mass spectrometry (UPLC-ESI-MS) of the ethyl acetate fraction resulted in recognizing of eighteen compounds tentatively compared with previously published data. Quantitative measurement of the overall value of flavonoids of *A. modesta* was found to be 2.824 μg/100 μg ± 0.01 calculated as quercetin. The acute toxicity study of the ethanol extract proved that the plant under investigation is safe and nontoxic to the male albino mice used. The anti-hyperglycemic activity of the ethanol extract performed on type 2 diabetic rats proved that the most potent dosage was 200 mg/kg b. wt. after 4 and 4 weeks of treatment respectively compared to metformin. Furthermore, evaluation of the hepato-protective activity of the ethanol extract of the plant under investigation showed that the most potent extract was with a dose level of 200 mg/kg b. wt. after 3 and 10 days of continuous treatment compared to silymarin.

Conclusion: It can be concluded that *A. modesta* Wall. cultivated in Egypt could be used as a promising anti-diabetic agent and a hepato-protective agent against hepatocellular damage induced by hepatotoxins.

Keywords: *Acacia*, Anti-hyperglycemic, Flavone, Hepato-protective, Saponin

Background

Traditional medicine is used globally and has significant economic benefits in both industrialized and developing economies using natural plants that are rich sources of active components, so knowledge about both the area's plant diversity and local people's medicinal uses is of utmost importance. Herbal plants play a key role in medicare and are therefore essential natural resources both for the traditional and innovative medicinal products [1].

Family Fabaceae is one of the fastest growing flowering plant families in the world, the third largest group of plants with 19,400 species, and has been categorized into 730 genera. Family Fabaceae plants supply a reliable and safe therapy for many diseases. Species of this family range from dwarf herbs to tropical rain forest massive trees [2]. Genus *Acacia* is a heterogeneous collection of ever more than 1000 species, most of which are trees, mainly found in Australia; others are found in South-East Asia [3]. *Acacia* species were first used in the beginnings of human civilization as traditional medicinal plants and have a really significant economic value [4]. Gum, leaves, flowers, and wood of *A. modesta* were used

* Correspondence: emanms4000@yahoo.com; eman.salah@fue.edu.eg
[1]Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt, Cairo, Egypt
Full list of author information is available at the end of the article

for various medical applications such as dysentery, leprosy, and cough [5]. Different parts of *A. modesta* have been previously investigated in several pharmacological activities such as antibacterial, antifungal, anti-hyperglycemic, analgesic, anti-inflammatory, anti-platelet aggregation, anti-termite, antioxidant, brine shrimp cytotoxicity, hemagglutination, insecticidal, phytotoxic, and spasmolytic activities. Reports on *A. modesta* aerial parts exposed the presence of flavonoids, alkaloids, terpenoids, and tannins [6]. Tracing the available current literature, there is scarce information on the chemical and pharmacological characters of *A. modesta* cultivated in Egypt. Therefore, the following work has been planned to examine the main active principles and to screen the biological activities to find out the potential benefits of the plant under investigation.

Methods

Collection of plant material

The non-flowering aerial parts of *A. modesta* have been collected in August 2015 from Giza zoo garden, then identified and authenticated by the taxonomist Dr. Threse Labib, specialist in the central gardening administration, Orman garden, Giza, Egypt.

Isolation and identification

Extraction of aerial parts of A. modesta

About 500 g of the air-dried powdered non-flowering aerial parts of *A. modesta* have been exhaustively extracted with 70% methanol, and filtered and then concentrated using rotary evaporator R-3 (Buchi, A.G., Switzerland). The crude extract (100 g) has been mixed with distilled water (500 ml) and then has been partitioned with the following solvents; n-hexane, methylene chloride, ethyl acetate, and n-butanol saturated with water several times and concentrated to give 3 g, 3 g, 8 g, and 17 g, respectively. TLC profile of the n-butanol fraction prompted to focus on its purification. The dried extract of the n-butanol fraction (17 g) was delivered over a silica gel (60) glass column and eluted with methylene chloride, and the polarity of the column was stepwise increased by gradient addition of methanol. Similar fractions of each 50 ml were collected together. Fractions eluted with solvent strength (methylene chloride: methanol 70:30) offered fraction 1 (1.08 g) which was purified using reversed-phase silica column and partitioned with water, then increasing polarity via addition of methanol gradually. Similar fractions (5 ml) each, eluted with (30% methanol), were concentrated to offer *compounds 1 and 2* (50 mg) (Supplementary file: Figures S1-S7). Similar fractions eluted from the main column using polarity (methylene chloride: methanol 60: 40) offered fraction 2 (1.37 g) which has been separated via a sephadex LH-20 glass column, using absolute methanol to offer *compound 3* (30 mg) (Supplementary file: Figures S8-S15).

Materials for pharmacological screening

Extract preparation

Non-flowering *A. modesta* aerial parts have been left to dry in the air, and grinded and then extracted with absolute ethanol for hepato-protective and anti-diabetic activities. The extracts were dried using rotary evaporator R-3 (Buchi, A.G., Switzerland).

Animals

Adult male albino *Sprague dawley* rats (130–150 g) have been used for the hepato-protective and anti-diabetic activities. Mice (25–30 g) have been used for the toxicological study. Both have been brought from the animal house colony belonging to the National Research Centre, Dokki, Giza, Egypt. Experiments and animal procedures have been carried out in compliance with the Ethics Committee of the National Research Centre following the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. After each experiment, animals would be sacrificed by cervical dislocation via light anesthesia with ether.

Quantitative estimation of flavonoid content

A spectrophotometric method using aluminum chloride was followed for total flavonoid content estimation based on the measurement of the intensity of the color developed when flavonoids complexed with aluminum chloride, at λ_{max} 415 nm using the standard quercetin (Sigma-Aldrich chemicals, Co., St. Louis, MO, USA). The assay was done in triplicate. The calibration curve was prepared using quercetin solution at a concentration of 5 to 100 μg/ml in methanol [7].

Metabolite profiling via UPLC-ESI-MS

The sample solution of the ethyl acetate fraction of *A. modesta* (100 μg/mL) non-flowering aerial parts was prepared, the chromatographic separation was conducted on an Acquity UPLC system (Waters) equipped with a reversed-phase BEH C18 column (50 × 2.1 mm, particle size 1.7 μm; Waters), and the analysis was carried out using a binary elution system. Mass spectra were detected between *m/z* 100–1000 in negative and positive ionization modes on a XEVO TQD triple quadrupole mass spectrometer (Waters Corporation, Milford, USA) [8]. Compounds were recognized tentatively by analyzing their mass data using the Maslynx 4.1 software and making a comparison between their retention time (RT) and mass spectrum with previously reported data.

Acute toxic activity

A preliminary experiment was done to determine the minimal dose that kills all animals (LD_{100}) and the minimal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between 2 doses; each dose was injected in a group of 10 animals by subcutaneous injection total of forty mice. The mice were then observed for 24 h, and symptoms of toxicity and mortality rates were recorded and LD_{50} was calculated [9].

Anti-hyperglycemic activity

Induction of hyperglycemia

Type 2 diabetes mellitus has been induced via alloxan injection [10]. Then, the measurement of the blood glucose levels was tested after 72 h to ensure hyperglycemia [11].

Hepato-protective activity

Induction of liver damage

Liver damage has been ensured by injection of toxic carbon tetrachloride (CCl_4) dissolved via liquid paraffin (5 ml/kg of 25%) via intra-peritoneal route, and blood samples were withdrawn for the biochemical study [12, 13]. Serum aspartate amino-transferase (AST), alanine amino-transferase (ALT) [14], and serum alkaline phosphatase (ALP) [15] were isolated and then analyzed. The data have been analyzed using Student's *t* test [16].

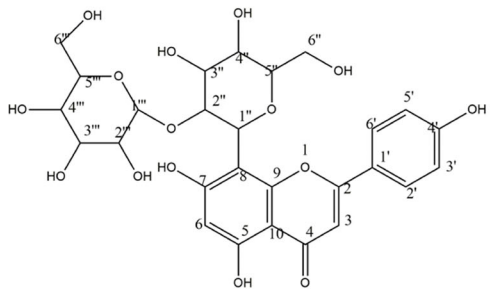
Results

Phytochemistry

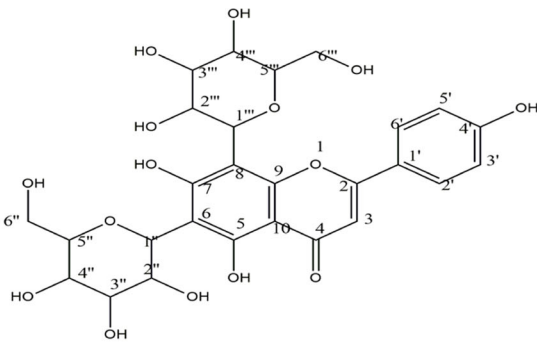
Phytochemical investigation of the butanol fraction of *A. modesta* non-flowering aerial parts has yielded three compounds. The chemical structures of the three compounds have been characterized using elemental analysis, ^{1}H &^{13}C NMR correlating with the existing literature data [17].

Vitexin-2″-*β*-glucopyranoside (compound 1)

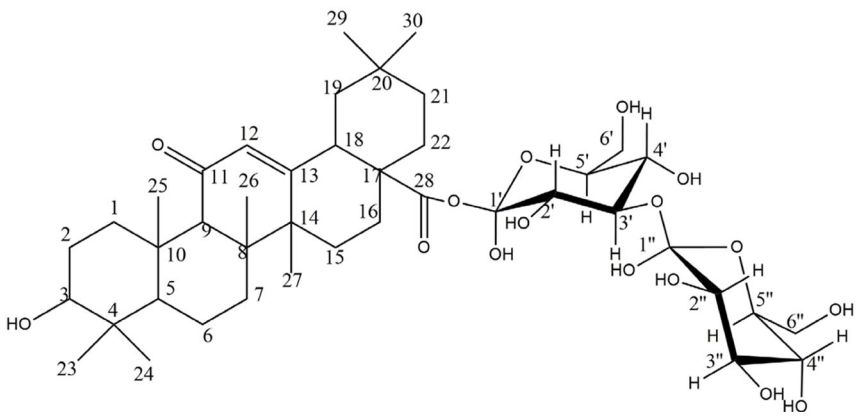
Brownish yellow amorphous powder, R_f: 0.53 (methylene chloride: methanol: distilled water 70:30:3). According to the chromatographic properties, this compound was expected to be an apigenin derivative [17] (Fig. 1).



Vitexin-2''- *β*-glucopyranoside compound 1



Apigenin-6,8-di-*C*-*β*-*D*-glucopyranoside (Vicenin II) compound 2



***β*-*D*-glucopyranosyl (1-3)-*β*-*D*-glucopyranosyl)-3-*β*-hydroxy-11-oxo-olean-12-en-28-oic acid compound 3**

Fig. 1 Isolated compounds of *Acacia modesta* Wall. non-flowering aerial parts. Figure 1 shows the three compounds isolated from the butanol fraction of *A. modesta* non-flowering aerial parts, compounds 1 and 2 were isolated in a flavone mixture, while compound 3 was isolated in a pure form

Apigenin-6,8-di-*C*-*β*-*D*-glucopyranoside (compound 2)

Yellow amorphous powder, R_f: 0.53 (methylene chloride: methanol: distilled water 70:30:3) (Fig. 1).

β-*D*-glucopyranosyl (1-3)-*β*-*D*-glucopyranosyl)-3-*β*-hydroxy-11-oxo-Olean-12-en-28-oic acid (compound 3)

Amorphous powder

It showed a positive test of Lieberman-Burchard test for terpenoid [18], R_f: 0.8 (methylene chloride: methanol: distilled water 70:30:3). The spectrum of ^{13}C NMR showed 30 carbon signals (Fig. 1).

Quantitative estimation of flavonoid content

The total flavonoid content of *A. modesta* Stock was found to be 2.824 μg/100 μg ± 0.01 calculated as quercetin.

UPLC-ESI-MS

Tentative identification of the ethyl acetate fraction of *A. modesta* non-flowering aerial part metabolites has led to the identification of eighteen compounds which were distributed into two major categories: flavonoids and phenolic acids. The compounds were analyzed depending on their molecular weight, mass fragmentation, and compared with previously revealed data to the extent of our knowledge. It is important to mention that this is the first study for evaluating any fraction of *A. modesta* non-flowering aerial parts via UPLC-ESI-MS analysis (Fig. 2).

Acute toxicity study (LD_{50})

It was found that the median lethal dose of ethanol extract of *A. modesta* non-flowering aerial parts (LD_{50}) is 7.1 g/kg b. wt., so it is possible to conclude that the LD_{50} of alcoholic extract of *A. modesta* is safe and nontoxic as LD_{50} greater than 50 mg/kg b. wt. is nontoxic [19].

Anti-hyperglycemic activity

Results revealed that the ethanol extract of *A. modesta* showed a potent anti-diabetic activity at the two tested doses. From the examination of the two dose levels at 100 mg/kg b. wt. and at 200 mg/kg b. wt., respectively, of

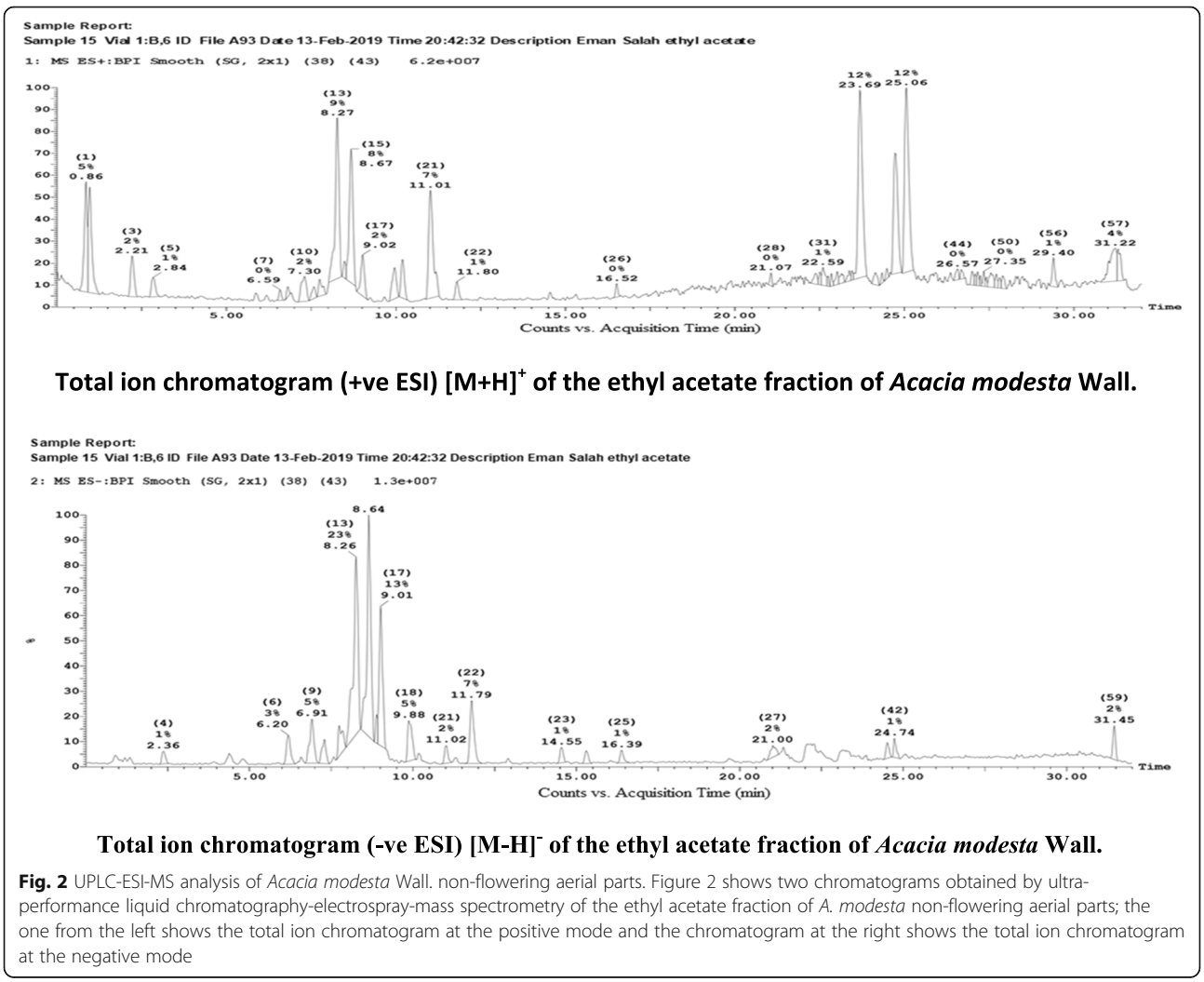


Fig. 2 UPLC-ESI-MS analysis of *Acacia modesta* Wall. non-flowering aerial parts. Figure 2 shows two chromatograms obtained by ultra-performance liquid chromatography-electrospray-mass spectrometry of the ethyl acetate fraction of *A. modesta* non-flowering aerial parts; the one from the left shows the total ion chromatogram at the positive mode and the chromatogram at the right shows the total ion chromatogram at the negative mode

the ethanol extract of *A. modesta* non-flowering aerial parts cultivated in Egypt in AITD (Alloxan-induced type 2 diabetic rats) after 4 and 8 weeks of continuous treatment against standard anti-diabetic drug metformin with dose 100 mg/kg b. wt.: results showed a substantial decrease in blood glucose levels from 259.8 mg/dl in non-treated AITD to 209.1 mg/dl to 186.8 mg/dl in AITD treated with the two tested doses, respectively, compared with metformin (143.6 mg/dl). Furthermore, after 8 weeks of continuous treatment blood glucose levels was lowered from 268.4 mg/dl in non-treated AITD to 153.6 mg/dl to 139.9 mg/dl in AITD treated with the two tested doses, respectively, compared with metformin (85.4 mg/dl) (Supplementary file: Table S1) (Fig. 3).

Hepato-protective activity

From the examination of the two doses 100 and 200 mg/kg b. wt., respectively, of the ethanol extract of *A. modesta* non-flowering aerial parts cultivated in Egypt in comparison with silymarin as a standard hepato-protective drug, blood samples were collected at zero-time, 1 week before CCl_4 injection, 3 days, and then 10 days after CCl_4 injection. Results showed a substantial increase in serum levels of ALP, ALT, and AST in non-treated animals, i.e., control (3 days and then 10 days after CCl_4 injection). On the other side, the pretreated animals with the two tested doses respectively revealed a great decrease in the previously mentioned enzymes. Serum ALP, ALT, and AST levels in rats treated with the dose of 200 mg/kg b. wt. were (24.2, 21.2 KAU), (61.3, 51.9 u/l), and (65.9, 53.9 u/l) after 3 days and 10 days, respectively. This significant reduction could be comparable to that of silymarin in which the serum AST, ALT, and ALP levels were (48.2, 39.2 u/l) and (63.8, 39.1 u/l) and (16.8, 7.9 KAU) after 3 days and 10 days, respectively (Supplementary file: Table S2-S4) (Figs. 4, 5, and 6).

Discussion

Genus *Acacia* belongs to the family Fabaceae. *Acacia* species were being used in the beginnings of civilization as traditional medicinal herbs which are of considerable medicinal and economic value. Therefore, investigation of non-flowering aerial parts of *A. modesta* cultivated in Egypt has led to the following findings: The chemical investigation of butanol fraction of *A. modesta* non-flowering aerial parts yielded three compounds. According to the chromatographic properties, compound 1 was expected to be apigenin derivative [17], confirmed by ^{1}H NMR spectrum and ^{13}C NMR spectrum showing typical signals of the apigenin aglycone moieties giving the confirmation of vitexin-2′′-*β*-glucopyranoside that has been separated for the first time from genus *Acacia.* In the same spectra of ^{13}C-NMR, another compound having signal strength close to 1:2 of the first compound, the aglycone moiety of the second compound seems to be

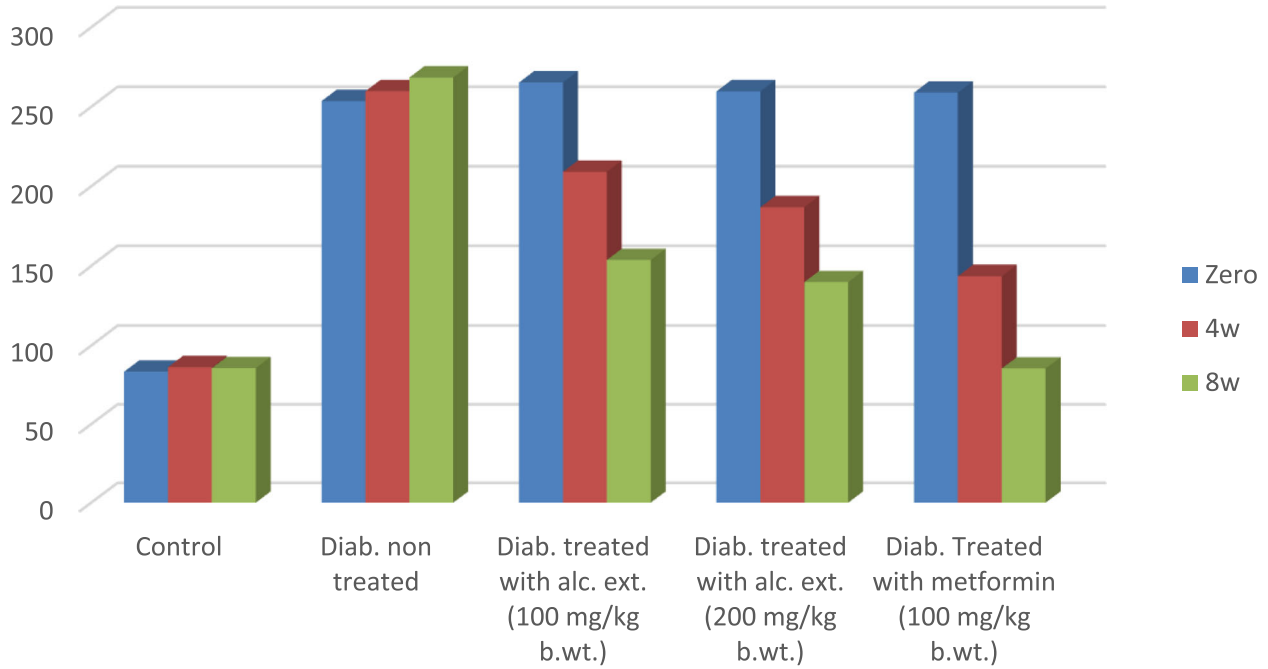


Fig. 3 Effect of the alcoholic extracts of *Acacia modesta* Wall. non-flowering aerial parts and metformin on blood glucose level in male albino rats. Figure 3 presents blood glucose level in male albino rats after treatment with two doses of *A. modesta* non-flowering aerial parts (100 and 200 mg/kg b. wt.) for 4 weeks and 8 weeks against standard drug metformin with the dose level of 100 mg/kg b. wt

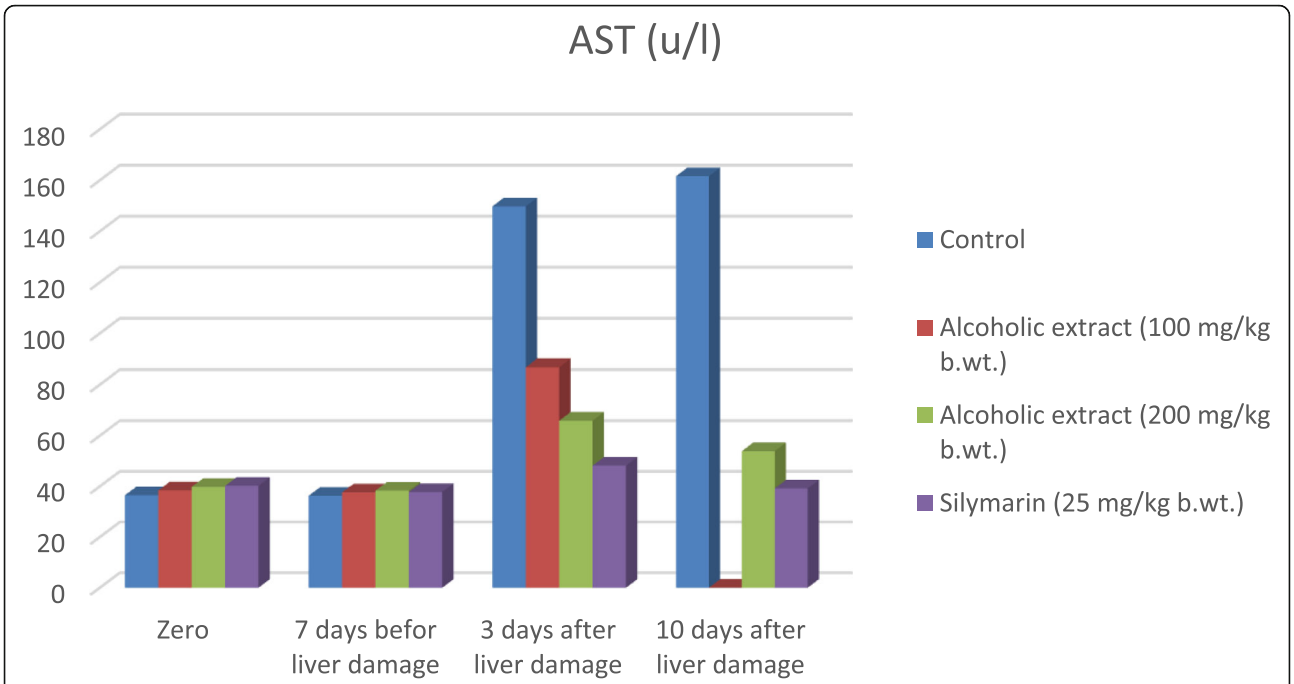


Fig. 4 Effect of alcoholic extract of *Acacia modesta* Wall. non-flowering aerial parts and silymarin on serum aspartate amino-transferase AST level in liver damaged rats. Figure 4 presents serum aspartate amino-transferase AST level in the liver-damaged rats with the collection of blood samples at zero time, 7 days before liver damage with continuous administration of the two doses of *A. modesta* non-flowering aerial parts (100 and 200 mg/kg b. wt.) and then 3 days and 10 days after induction of liver damage with continuous administration of the two doses of *A. modesta* non-flowering aerial parts (100 and 200 mg/kg b. wt.) showing a great reduction of serum levels of AST after treatment with the two doses of *A. modesta* against standard drug silymarin with the dose level of 25 mg/kg b. wt

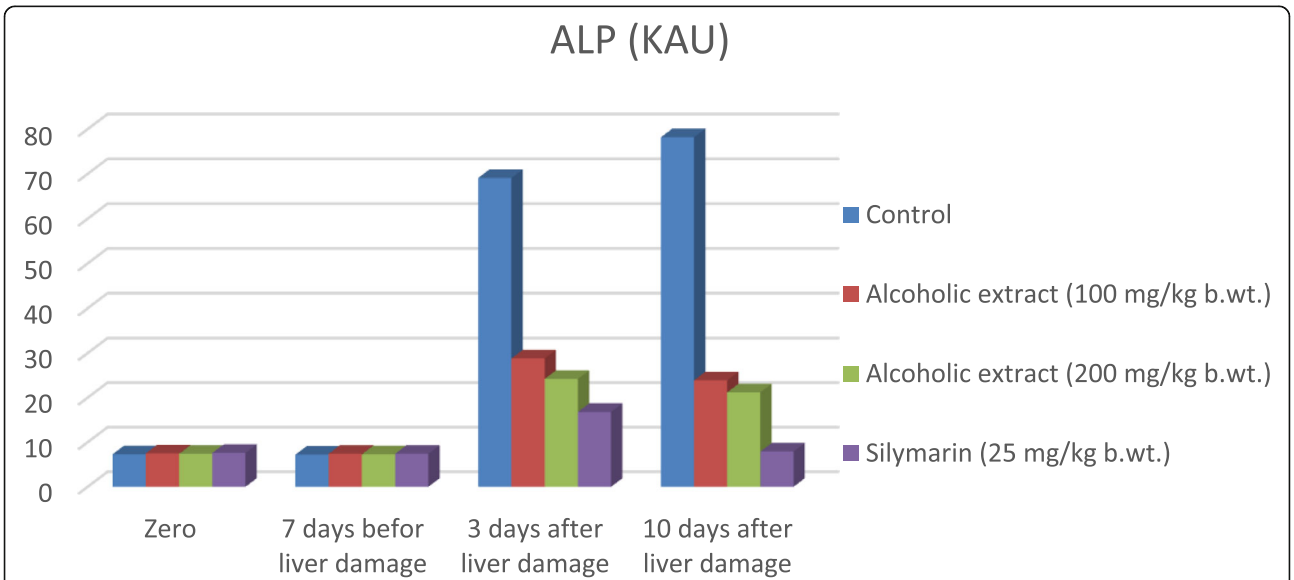


Fig. 5 Effect of alcoholic extract of *Acacia modesta* Wall. non-flowering aerial parts and silymarin on serum alkaline phosphatase enzymes ALP level in liver-damaged rats. Figure 5 presents serum alkaline phosphatase enzymes ALP level in liver-damaged rats with the collection of blood samples at zero time, 7 days before liver damage with continuous administration of the two doses of *A. modesta* non-flowering aerial parts (100 and 200 mg/kg b. wt.) and then 3 days and 10 days after induction of liver damage with continuous administration of the two doses of *A. modesta* non-flowering aerial parts (100 and 200 mg/kg b. wt.) showing a great reduction of serum levels of ALP after treatment with the two doses of *A. modesta* against standard drug silymarin with the dose level of 25 mg/kg b. wt

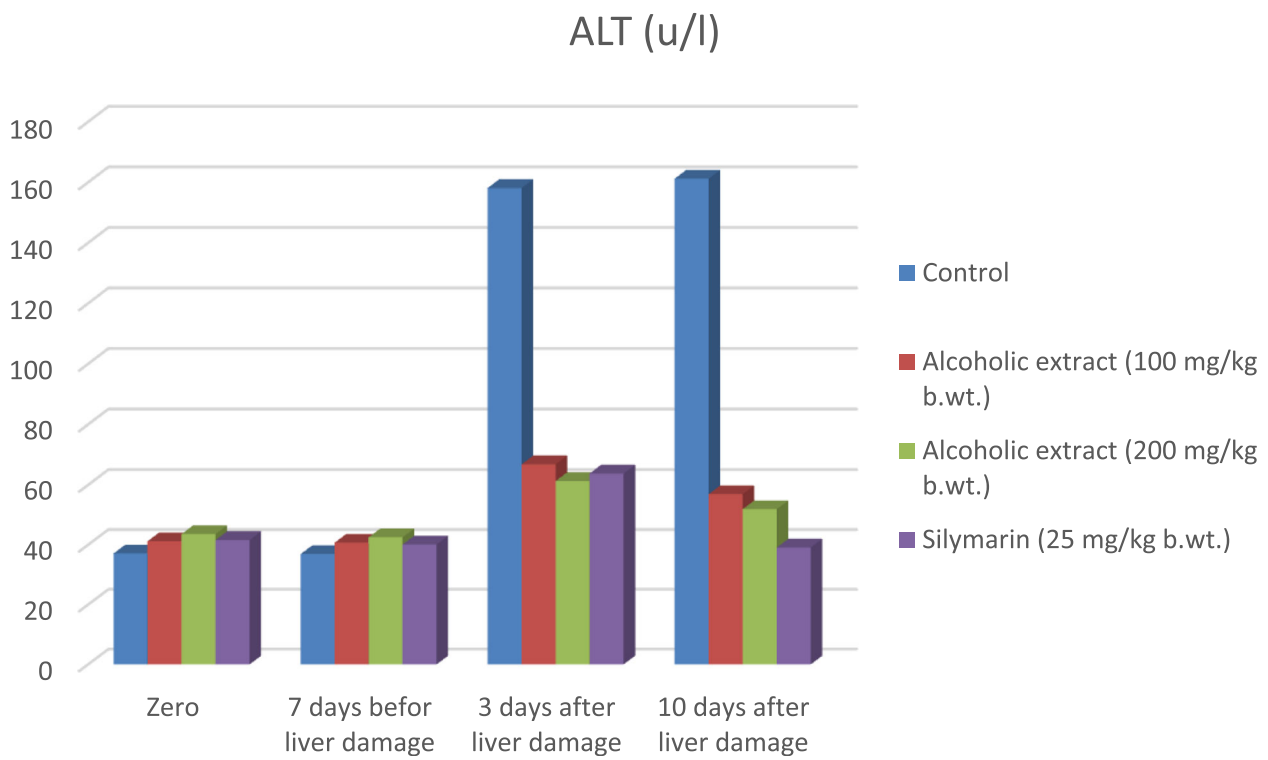


Fig. 6 Effect of alcoholic extract of *Acacia modesta* Wall. non-flowering aerial parts and silymarin on serum alanine amino-transferase ALT level in liver-damaged rats. Figure 6 presents serum alanine amino-transferase ALT level in liver-damaged rats with the collection of blood samples at zero time, 7 days before liver damage with continuous administration of the two doses of *A. modesta* non-flowering aerial parts (100 and 200 mg/kg b. wt.) and then 3 days and 10 days after induction of liver damage with continuous administration of the two doses of *A. modesta* non-flowering aerial parts (100 and 200 mg/kg b. wt.) showing a great reduction of serum levels of ALT after treatment with the two doses of *A. modesta* against standard drug silymarin with the dose level of 25 mg/kg b. wt

identical to the first one except in carbon number 6 of the aglycone. Comparing the chemical shift of the carbon spectra, compound 2 was confirmed to be apigenin-6,8-di-*C*-*β*-*D*-glucopyranoside (vicenin II), that was separated for the first time from genus *Acacia*. According to the chromatographic properties, ^{1}H and ^{13}C NMR spectrum of compound 3 revealed 30 carbon signals of the aglycone that have been sorted into nine methylenes, nine quaternary carbons, seven methyls, and six methines by DEPT experiments. This data suggested that it is a triterpene of an oleanane-type, the carbonyl function appears at δ 200.1 corresponding to ketone function at C-11. The previous data in addition to the other carbons in the spectra with the 2D-NMR experiments (H-H COSY, HSQC, DEPT, HMBC) came in complete accordance with the previously published data of *β*-*D*-glucopyranosyl (1-3)-*β*-*D*-glucopyranosyl)-3-*β*-hydroxy-11-oxo-olean-12-en-28-oic acid which was separated for the first time from family Fabaceae.

Eighteen compounds were tentatively identified using (UPLC-ESI-MS) analysis of the ethyl acetate fraction compared with previously published references presented in Table 1 and are discussed in the following section:

Compound 1 RT 0.86; it has been tentatively proposed as a caffeic acid ester derivative with $[M + H]^+$ of *m/z* 295 amu, because of showing similar peaks. The MS fragmentation shows the characteristic fragment ion of caffeic acid at *m/z* 136 (Supplementary file: Figure S16). *Compound 2 RT 6.91*; the molecular formula of this compound was found to be $C_{21}H_{20}O_{11}$ (orientin). With the characteristic base peak at *m/z* 327 (Supplementary file: Figure S17). *Compound 3 RT 7.75*; it has been tentatively identified as coumaroyl diferuoyl spermidine, with the molecular formula of $C_{36}H_{41}N_3O_8$, with the deprotonated ion $[M - H]^-$ at *m/z* 642.2 (Supplementary file: Figures S18-S19). *Compound 4 RT 8.27*; with the precursor ion peak of $[M - H]^-$ at *m/z* 626.2. It can be tentatively supposed to be quercetin dihexose, with the characteristic aglycone at *m/z* 301.1, known for quercetin $[(M - H)\text{-}2Hexose]^-$ (Supplementary file: Figure S20). *Compound 5 RT 8.48*; could be tentatively assigned as apigenin-*O*-pentosyl hexoside with the molecular formula $C_{26}H_{28}O_{14}$. The precursor ion peak $[M + H]^+$ at *m/z* 565.2 and the $[M + Na]^+$ ion at *m/z* 587 (Supplementary file: Figure S21). *Compound 6 RT 8.67*; it has been tentatively identified as apiin with the molecular

Table 1 Identification of the metabolites of the ethyl acetate fraction of *Acacia modesta* Wall. non-flowering aerial parts

No.	RT	Name	M − H	M + H	M + Na	M + K	Mol. formula	Fragments	Reference
1	0.86	Caffeic acid ester derivative		295			---------	136	[20]
2	6.91	Orientin	448.2				$C_{21}H_{20}O_{11}$	**327.1**	[21–23]
3	7.75	Coumaroyl diferuoyl spremidine	642.2				$C_{36}H_{41}N_3O_8$	147.1, 307.1, **327.1**	[24]
4	8.27	Quercetin dihexose	626.2				$C_{27}H_{30}O_{17}$	**301.1**	[24, 25]
5	8.48	Apigenin-*O*-pentosyl hexoside		565.2	587.2		$C_{26}H_{28}O_{14}$	**273.2**	[26, 27]
6	8.67	Apiin		565.2	588.2		$C_{26}H_{28}O_4$	**433.1**	[28, 29]
7	8.89	Myricetin-rhamnose malic acid	579.3				$C_{24}H_{23}O_{16}$	**316.1**, 463.1	[29]
8	9.02	Granatin B		951.3			$C_{41}H_{28}O_{27}$	**303.1**, 465.1	[30, 31]
9	9.88	Catechin trigallate	745.3				$C_{36}H_{15}O_{18}$	**447.1**, 593.2	[32]
10	10.20	Ellagic acid derivative		799.3			---------	**271.1**, 331.1, 395.2	[30]
11	11.80	Kaempferol hexose glucuronide	623.3		647.1		$C_{27}H_{28}O_{17}$	**287.1**	[24]
12	22.59	Tricaffeoyl-quinic acid		677.5			$C_{34}H_{30}O_{15}$	**351.2**, 515.3	[33]
13	22.84	Pentagalloyl hexoside		993.2			$C_{41}H_{32}O_{26}$	**496.4**	[34]
14	23.46	Quercetin hexose glucuronide		641.2			$C_{27}H_{28}O_{18}$	**304.3**,	[24]
15	23.69	Digalloyl hexose			507. 7	524.7	$C_{20}H_{20}O_{14}$	313.4, **331.3**	[35]
16	24.35	Quercetin-tri-*O*-hexoside				827.7	$C_{33}H_{39}O_{21}$	**303.1**, 624.3	[20]
17	24.49	Galloyl-valoneic acid bilactone		623.2			---------	469.4	[29, 30]
18	27.56	7-*O*-Methyl- delphinidin-3-*O*-(2″galloyl)-hexoside		631.1			$C_{29}H_{26}O_{16}$	**153.1**, 235	[29]

M + *H*$^+$ protonated molecular ion; *M* − *H*− deprotonated molecular ion; *m/z* mass to charge; *M* + *K* protonated/deprotonated, K$^+$ adduct; *M* + *Na* protonated/deprotonated, Na$^+$ adduct; *Mol. formula* molecular formula; *RT* retention time

formula $C_{26}H_{28}O_4$ and $[M + H]^+$ peak at *m/z* 565.2 with a molecular weight 564 with the characteristic base peak ion at *m/z* 433 due to the loss of a pentose unit $[(M + H)^+ - 132]^+$ and the $[M + Na]^+$ ion at *m/z* 588.2 (Supplementary file: Figure S22). *Compound 7 RT 8.89*; it has been tentatively identified as myricetin-rhamnose malic acid was detected in −ve ESI mode showing a precursor ion peak at *m/z* 579 having the molecular formula $C_{27}H_{30}O_{13}$. The base peak ion at *m/z* 463.1 appeared due to the removal of malic acid $[(M - H) - 116]^-$ at *m/z* 579 to give a myricetin-rhamnose moiety. A characteristic fragment of the aglycone myricetin appeared at m/z 316, also the $[(M - H) + K]^-$ ion at *m/z* 609. (Supplementary file: Figure S23). *Compound 8 RT 9.02*; exhibited a precursor ion at *m/z* 951.3 which is assigned for granatin B; (Galloyl-hexahydroxydiphenoyl-di-hexahydroxydiphenoyl-hexoside), hexa-hydroxy-diphenoyl (dehydro-ellagitannins of type III-tannins) (Supplementary file: Figure S24). *Compound 9 RT 9.88*; it is tentatively assigned as catechin trigallate with the precursor ion peak $[M - H]^-$ at *m/z* 745.3, showing a minor production $[(M - H) - 152]^-$ at *m/z* 593.2 due to removal of a gallic acid unit (Supplementary file: Figure S25). *Compound 10 RT 10.20* is tentatively assigned as ellagic acid derivative showing a precursor ion at *m/z* 799.3 and the base peak ion at *m/z* 271.1 (Supplementary file: Figure S26). *Compound 11 RT 11.80*; with the molecular formula $C_{27}H_{28}O_{17}$ and molecular weight 624, it can be recognized as kaempferol hexose glucuronide with a precursor ion peak $[M - H]^-$ at *m/z* 623.3 and the base peak ion fragment [(M + H)-hexose-Glucuronide]$^+$at *m/z* 287.1 (Supplementary file: Figure S27-S28). *Compound 12 RT 22.59*; it has been tentatively identified as tricaffeoyl-quinic acid with the base peak ion at *m/z* 515 (Supplementary file: Figure S29). *Compound 13 RT 22.84*; it has been assigned as pentagalloyl hexoside as a hydrolysable tannin with $[M + H]^+$ ion peak at *m/z* 993 and the base peak ion at *m/z* 496.4 (Supplementary file: Figure S30). Compound 14 RT 23.46; with the molecular formula of $C_{27}H_{28}O_{18}$ and molecular weight 640 and the precursor ion peak $[M + H]^+$ at *m/z* 641.2, it can be recognized tentatively as quercetin hexose glucuronide (Supplementary file: Figure S31). *Compound 15 RT 23.69*; it has been tentatively identified as an isomer of digalloyl hexose with the ion peak of $[M + Na]^+$ at *m/z* 507.7 and the $[M + K]^+$ ion at *m/z* 524.7 (Supplementary file: Figure S32). *Compound 16 RT 24.35*; it has been assigned as quercetin-tri-*O*-hexoside with the precursor ion peak $[M + K]^+$ at *m/z* 827.7 and the base peak ion at *m/z* 303.1 corresponding to the aglycone quercetin (Supplementary file: Figure S33). *Compound 17 RT 24.49* exhibited a precursor ion peak at m/z 623.2 and also an observance of galloyl moiety removal $[(M - H) - 152]^-$ *m/z* 469.4. Thus, this compound was tentatively identified as galloyl-valoneic acid bilactone (Supplementary file: Figure S34). *Compound 18 RT 27.56* has been assigned as 7-*O*-methyl-delphinidin-3-*O*-(2′′galloyl)-hexoside with the precursor

fragment at *m/z* 631.1 and characteristic ions of *m/z* 153 [(M + H)-delphinidin-hexose]$^+$ due to loss of delphinidin and hexose moieties (Supplementary file: Figure S35).

Eventually, the ethanol extract of *A. modesta* Stocks of the two tested doses of 100 mg/kg b. wt. and 200 mg/kg b. wt. decreased the blood sugar level of AITD after 4 weeks by 21.1% and 28%, respectively, when compared to the reference drug metformin (44.5%). Also, the ethanol extract of *A. modesta* Stocks decreased the blood sugar level of AITD after 8 weeks by 42.1% and 46.1% in the two tested doses, respectively, when compared to the reference drug metformin (67%). It can be concluded that the most potent extracts were *A. modesta* (200 mg/kg b. wt.) followed by *A. modesta* (100 mg/kg b. wt.) after 4 weeks and 8 weeks with a potency 62.9% and 68.8% and a potency 47.6% and 62.8%, respectively, compared to metformin (100 mg/kg b. wt.) which is considered 100% potent. It is important to mention that levels of blood glucose were tested in normal and AITD, given ethanol and ethanol: water (1:1) leaf extracts of *A. modesta* cultivated in India, at two dose levels of 100 and 300 mg/kg/day [36]. Nevertheless, this is the first report for the anti-diabetic activity of the plant cultivated in Egypt. Regarding the hepato-protective activity, a remarkable reduction in the levels of serum enzymes was observed. After treatment with the two doses of 100 mg/kg b. wt. and 200 mg/kg b. wt., respectively; the percent of reduction were found to be 42.1%, 56% for AST; 57.7%, 61.2% for ALT; and 58.3%, 65% for ALP compared with the reference drug silymarin 67.8%, 59.6%, and 75.7% for AST, ALT, and ALP, respectively, after 3 days. While after 10 days, percent of reduction was found to be (53.7%, 66% for AST and 64.7%, 67.8% for ALT and 69.4%, 73% for ALP compared with the reference drug silymarin 75.7%, 75.7%, and 90% for AST, ALT, and ALP after treatment with the two tested doses, respectively. It could be concluded that the most potent extract was that of dose level 200 mg/kg b. wt. with percent of potency (82.6%, 102.7%, and 85.9%) for AST, ALT, and ALP, respectively, after 3 days of treatment. While the percent of potency was found to be (88%, 89.6%, and 81.1%) for AST, ALT, and ALP, respectively, after 10 days of treatment compared to the standard drug silymarin (which is considered 100% potent). It is important to mention that the hepato-protective activity of the stem bark of *A. modesta* was previously investigated using 80% methanolic extract of the Pakistanian cultivated species crude extract [37]. This is the first report for the hepato-protective activity of *A. modesta* cultivated in Egypt.

Conclusion

Acacia modesta Wall. cultivated in Egypt revealed the presence of a variety of phytochemical constituents and showed low toxicity profiles with high safety margins and valuable hypoglycemic, hepato-protective activities. This superior activity would be attributed to their high contents of phenolic components and flavonoids. Further investigation is recommended on the total extracts and individual components. Clinical trials should be performed in order to support the above investigation and to facilitate their pharmaceutical formulation.

Supplementary Information

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Additional file 1: Figure S1. ^{1}H-NMR spectral data of the flavone mixture (400 MHz, CD3OD). **Figure S2.** ^{13}C-NMR total carbons spectral data of the flavone mixture (100 MHz, CD3OD). **Figure S3.** ^{13}C-NMR total carbons spectral data of the flavone mixture (100 MHz, CD3OD). **Figure S4.** HMBC-DEPT 90 spectral data of the flavone mixture (CD3OD). F**igure S5.** HMBC-DEPT 135 spectral data of the flavone mixture (CD3OD). **Figure S6.** HSQC-DEPT 90 spectral data of the flavone mixture (CD3OD). **Figure S7.** HSQC-DEPT 135 spectral data of the flavone mixture (CD3OD). **Figure S8.** ^{1}H-NMR spectral data of compound three (400 MHz, CD3OD). **Figure S9.** ^{13}C-NMR DEPT 90 spectral data of compound three (100 MHz, CD3OD). **Figure S10.** ^{13}C-NMR DEPT 135 spectral data of compound three (100 MHz, CD3OD). **Figure S11.** HMBC-DEPT 90 spectral data of compound three (CD3OD). **Figure S12.** HMBC-DEPT 135 spectral data of compound three (CD3OD). **Figure S13.** HSQC-DEPT 90 spectral data of compound three (CD3OD). **Figure S14.** HSQC-DEPT 135 spectral data of compound three (CD3OD). **Figure S15.** H-H Cosy spectral data of compound three (CD3OD)

Abbreviations

ALP: Alkaline phosphatase; ALT: Alanine amino-transferase; AST: Aspartate amino-transferase; UPLC-ESI-MS: Ultra-performance liquid chromatography-electrospray ionization - mass spectrometry; +ve ESI: Positive mode of electrospray ionization; *A. modesta*: *Acacia modesta*; AITD: Alloxan-induced type 2 diabetic rats; b. wt.: Body weight; BEH C-18: Ethylene-bridged hybrid carbon 18 reversed-phase; CCl_4: Carbon tetrachloride; DEPT: Distortion-less enhancement by polarization transfer; –ve ESI: Negative mode of electrospray ionization; ext.: Extract; H-H cosy: Proton-proton correlation spectroscopy; HMBC: Hetero-nuclear multiple quantum coherence; HSQC: Hetero-nuclear single quantum coherence; LD_{50}: Median lethal dose; R_f: Retention factor; RP: Reversed-phase; RT: Retention time; TLC: Thin layer chromatography

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Authors' contributions

EMS is a research scholar who carried out the practical work, data collection, data interpretation, and analysis and was a major contributor in writing the manuscript. MHG is a professor in Pharmacognosy who designed the work and helped in data interpretation and analysis and critical revision of the article. RRI is a lecturer in Pharmacognosy who helped in practical work and data collection. HSMS is a professor in Pharmacognosy who revised the article and gave the final approval of the manuscript to be published. The authors read and approved the final manuscript.

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Data and materials are available upon request.

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Experiments and animal procedures have been carried out in compliance with the Ethics Committee of the National Research Centre following the recommendations of the National Institutes of Health Guide for care and use of laboratory animals and approved by the Ethical Committee of Faculty of Pharmacy, Helwan University, Ain-Helwan, Cairo, Egypt, of the protocol numbered (008A-16).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt, Cairo, Egypt. [2]Faculty of Pharmacy, Pharmacognosy Department, Helwan University, P.O. Box 11795, Ain-Helwan, Cairo, Egypt.

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References
1. Ayyanar M, Silambarasan R (2015) An ethnobotanical study of medicinal plants in Palamalai region of eastern Ghats. India. J Ethnopharmacol 172:162–178
2. Ahmad F, Anwar F, Hira S (2016) Review on medicinal importance of Fabaceae family. Pharmacol Online 3:151–156
3. Or K, Ward D (2003) Three-way interactions between *Acacia*, large mammalian herbivores and bruchid beetles. Afr J Ecol 41:257–265
4. Subhan N (2016) Phytochemical and pharmacological investigations of Australian *Acacia*: an ethnomedicine-guided bioprospective approach. Charles Sturt University, Dhaka, Bangladesh, p 317 Available from: https://researchoutput.csu.edu.au/en/publications/phytochemical-and-pharmacological-investigations-of-australian-ac-3
5. Bukhari IA, Khan RA, Gilani AH, Sagheer A, Arshad SS (2010) Analgesic, anti-inflammatory and anti-platelet activities of the methanolic extract of *Acacia modesta* leaves. Inflammopharmacology 18:187–196
6. Sarwar W (2016) Pharmacological and phytochemical studies on *Acacia modesta* wall; a review. JPHYTO 5:160–166
7. Kalita P, Tapan B, Pal T, Kalita R (2013) Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. JDDT 3:33–37. https://doi.org/10.22270/jddt.v3i4.546 Available from: http://jddtonline.info/index.php/jddt/article/view/546
8. Osama S, El Sherei M, Al-Mahdy DA, Bishr M, Salama O (2019) Effect of salicylic acid foliar spraying on growth parameters, γ-pyrones, phenolic content and radical scavenging activity of drought stressed *Ammi visnaga* L. plant. Ind Crop Prod 134:1–10. https://doi.org/10.1016/j.indcrop.2019.03.035
9. Kärber G (1931) Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Archiv f Exper Pathol u Pharmakol 162:480–483. https://doi.org/10.1007/BF01863914
10. Eliasson SG, Samet TM (1969) Alloxan induced neuropathies lipid changes in nerve and root fragments. Life Sci 8:493–498
11. Trinder P (1969) Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem:6–24 Available from: https://journals.sagepub.com/doi/abs/10.1177/000456326900600108
12. Klassen CD, Plaa GL (1969) Comparison of the biochemical alteration elicted in liver of rats treated with carbon tetrachloride, chloroform 1,1,2-trichloroethane and 1,1,1-trichloroethane. Biochem Pharmacol 18:2019–2022
13. El-Askary HI, El-Olemy MM, Salama MM, Amer MH (2013) Development and validation of a high-performance liquid chromatography method for standardization of the bioactive ethyl acetate fraction of *Alstonia scholaris* (Linn.) R. Br. Growing in Egypt. Z Naturforsch C J Biosci 68:376–383
14. Thewfweld W (1974) The enzymatic methods for determination of serum AST and ALT. Deutsch Med 99:343–347
15. Kind PR, King EG (1954) A colorimetric method for the determination of serum alkaline phosphatase. J Clin Pathol 7:322
16. Snedecor GW, Cochran WG (1971) Méthodes Statistiques, original title: statistical methods, 6th edn. Iowa State University Press, Ames, p 649
17. Mabry TJ, Markham KR, Thomas MB (1970) The systematic identification of flavonoids. Springer- Verlag, New York, p 354
18. Xiong Q, Wilson WK, Pang J (2007) The Liebermann-Burchard reaction: sulfonation, desaturation, and rearrangement of cholesterol in acid. Lipids 42:87–96
19. Buck WB, Osweiler GD, Van Gelder GA (1976) Iodine in clinical and diagnostic veterinary toxicology, 2nd edn. Kendall/Hunt, Dubuque, pp 69–75
20. Francescato LN, Debenedetti SL, Schwanz TG, Bassani VL, Henriques AT (2013) Identification of phenolic compounds in *Equisetum giganteum* by LC–ESI-MS/MS and a new approach to total flavonoid quantification. Talanta 105:192–203
21. Krasteva I, Nikolov S (2008) Flavonoids in *Astragalus corniculatus*. Quim Nova 31:59–60
22. Pereira OR, Silva AMS, Domingues MRM, Cardoso SM (2012) Identification of phenolic constituents of *Cytisus multiflorus*. Food Chem 131:652–659
23. Brito A, Ramirez JE, Areche C, Sepúlveda B, Simirgiotis MJ (2014) HPLC-UV-MS profiles of phenolic compounds and antioxidant activity of fruits from three citrus species consumed in northern Chile. Molecules 19:17400–17421
24. Hanhineva K, Rogachev I, Kokko H, Mintz-Oron S, Venger I, Kärenlampi S, Aharoni A (2008) Non-targeted analysis of spatial metabolite composition in strawberry (*Fragaria* × *ananassa*) flowers. Phytochemistry 69:2463–2481
25. Truchado P, Vit P, Heard TA, Tomás-Barberán FA, Ferreres F (2015) Determination of interglycosidic linkages in *O*-glycosyl flavones by high-performance liquid chromatography/photodiode-array detection coupled to electrospray ionization ion trap mass spectrometry. Its application to *Tetragonula carbonaria* honey from Australia. Rapid Commun Mass Spectrom 29:948–954
26. Wojakowska A, Muth D, Narozna D, Madrzak C, Stobiecki M, Kachlicki P (2013) Changes of phenolic secondary metabolite profiles in the reaction of narrow leaf lupin (*Lupinus angustifolius*) plants to infections with *Colletotrichum lupini* fungus or treatment with its toxin. Metabolomics 9:575–589
27. Wojakowska A, Piasecka A, García-López PM, Zamora-Natera F, Krajewski P, Marczak L, Kachlicki P, Stobiecki M (2013) Structural analysis and profiling of phenolic secondary metabolites of Mexican lupine species using LC–MS techniques. Phytochemistry 92:71–86
28. Blunder M, Orthaber A, Bauer R, Bucar F, Kunert O (2017) Efficient identification of flavones, flavanones and their glycosides in routine analysis via off-line combination of sensitive NMR and HPLC experiments. Food Chem 218:600–609
29. Abu-Reidah IM, Ali-Shtayeh MS, Jamous RM, Arráez-Román D, Segura-Carretero A (2015) HPLC-DAD-ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (sumac) fruits. Food Chem 166:179–191
30. Fischer UA, Carle R, Kammerer DR (2011) Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MS^n. Food Chem 127:807–821
31. Mena P, Calani L, Dall'Asta C, Galaverna G, García-Viguera C, Bruni R, Crozier A, Del-Rio D (2012) Rapid and comprehensive evaluation of (poly)-phenolic compounds in pomegranate (*Punica granatum* L.) juice by UHPLC-MS^n. Molecules 17:14821–14840
32. Maldini M, Montoro P, Hamed AI, Mahalel UA, Oleszek W, Stochmal A, Piacente S (2011) Strong antioxidant phenolics from *Acacia nilotica*: profiling by ESI-MS and qualitative-quantitative determination by LC-ESI-MS. J Pharm Biomed Anal 56:228–239
33. Llorent-Martínez EJ, Zengin G, Fernández-de Córdova ML, Bender O, Atalay A, Ceylan R, Mollica A, Mocan A, Uysal S, Guler GO, Aktumsek A (2017) Traditionally used lathyrus species: phytochemical composition, antioxidant activity, enzyme inhibitory properties, cytotoxic effects, and in silico studies of *Lathyrus czeczottianus* and *Lathyrus nissolia*. Front Pharmacol 8:1–20
34. Sobeh M, El-Hawary E, Peixoto H, Labib RM, Handoussa H, Swilam N, El-Khatib AA, Sharapov F, Mohamed T, Krstin S, Linscheid MW, Singab AN, Wink M, Ayoub N (2016) Identification of phenolic secondary metabolites from *Schotia brachypetala* Sond. (Fabaceae) and demonstration of their antioxidant activities in *Caenorhabditis elegans*. Peer J 4:e2404
35. Lee JH, Johnson JV, Talcott ST (2005) Identification of ellagic acid conjugates and other polyphenolics in muscadine grapes by HPLC-ESI-MS. J Agric Food Chem 53:6003–6010
36. Jawla S, Kumar Y, Khan MSY (2011) Antimicrobial and anti-hyperglycemic activities of *Acacia modesta* leaves. Pharmacologyonline 2:331–347 Available from: https://pharmacologyonline.silae.it/files/archives/2011/vol2/036.kumar.pdf
37. Rahaman M, Chaudhry M (2015) Evaluation of antioxidant and hepato-protective effect of *Acacia modesta* wall. Against paracetamol induced hepatotoxicity. BJPR 5:336–343

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