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

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**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-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 hepatoprotective 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 hepatoprotective 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 medicine 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...
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 $\lambda_{\text{max}}$ 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 $\mu$g/ml in methanol [7].

Metabolite profiling via UPLC-ESI-MS
The sample solution of the ethyl acetate fraction of A. modesta (100 $\mu$g/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 $\times$ 2.1 mm, particle size 1.7$\mu$m; 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 (methylen chloride: methanol: distilled water 70:30:3). According to the chromatographic properties, this compound was expected to be an apigenin derivative [17] (Fig. 1).
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,
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 hepatoprotective drug, blood samples were collected at zero-time, 1 week before CCl4 injection, 3 days, and then 10 days after CCl4 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 CCl4 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 1H NMR spectrum and 13C 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 13C-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. 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.
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-\(\beta\)-D-glucopyranoside (vicenin II), that was separated for the first time from genus Acacia. According to the chromatographic properties, 1H and 13C 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 \(\delta\) 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 \(\beta\)-D-glucopyranosyl (1-3)-\(\beta\)-D-glucopyranosyl-3-\(\beta\)-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\(_{3}\)O\(_{8}\), 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)-2Hexose]**\(^-\) (Supplementary file: Figure S20). **Compound 5 RT 8.48**; could be tentatively assigned as apigenin-O-pentosyl hexoside with the molecular formula C\(_{29}\)H\(_{23}\)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
| No. | Compound | RT  | Name                          | M – H | M + H | M + Na | M + K | Mol. formula | Fragments | Reference |
|-----|----------|-----|-------------------------------|-------|-------|--------|-------|-------------|-----------|-----------|
| 1   | Caffeic acid ester derivative | 0.86 |  | 295 | ------- | 136 | [20] |
| 2   | Orientin | 6.91 |  | 448.2 | C₃₀H₂₂O₁₁ | 327.1 | [21–23] |
| 3   | Coumaroyl diferuoyl spermidine | 7.75 |  | 642.2 | C₂₉H₄₈N₂O₆ | 147.1, 307.1, 327.1 | [24] |
| 4   | Quercetin dihexose | 8.27 |  | 626.2 | C₁₃H₁₂O₁₇ | 301.1 | [24, 25] |
| 5   | Apigenin-O-pentosyl hexoside | 8.48 |  | 565.2 | C₂₆H₂₈O₄ | 273.2 | [26, 27] |
| 6   | Apiin | 8.67 |  | 565.2 | C₂₆H₂₈O₄ | 433.1 | [28, 29] |
| 7   | Myricetin-rhamnose malic acid | 8.89 |  | 579.3 | C₂₅H₂₄O₁₆ | 316.1, 463.1 | [29] |
| 8   | Granatin B | 9.02 |  | 951.3 | C₃₉H₂₈O₂₇ | 303.1, 465.1 | [30, 31] |
| 9   | Catechin trigallocate | 9.88 |  | 745.3 | C₃₀H₂₀O₁₇ | 447.1, 593.2 | [32] |
| 10  | Ellagic acid derivative | 10.20 |  | 799.3 | ------- | 271.1, 331.1, 395.2 | [30] |
| 11  | Kaempferol hexose glucuronide | 11.80 |  | 623.3 | C₁₇H₁₆O₁₅ | 287.1 | [24] |
| 12  | Tricaffeoyl-quinic acid | 12.59 |  | 677.5 | C₂₆H₂₈O₁₄ | 351.2, 515.3 | [33] |
| 13  | Pantagalloy hexoside | 12.84 |  | 952.3 | C₄₀H₃₂O₂₆ | 496.4 | [34] |
| 14  | Quercetin hexose glucuronide | 13.46 |  | 641.2 | C₁₇H₁₆O₁₄ | 304.3 | [24] |
| 15  | Digalloyl hexose | 13.69 |  | 507.7 | C₂₀H₂₀O₁₄ | 313.4, 331.3 | [35] |
| 16  | Quercetin-tri-hexoside | 14.35 |  | 627.7 | C₃₃H₃₀O₁₃ | 303.1, 624.3 | [20] |
| 17  | Galloyl-valonic acid bilactone | 14.49 |  | 623.2 | C₂₀H₂₀O₁₆ | 469.4 | [29, 30] |
| 18  | 7-O-Methyl-delphinidin-3-O-(2′galloyl)-hexoside | 27.56 |  | 631.1 | C₃₁H₂₆O₁₆ | 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

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

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formula C₂₆H₂₄O₄ 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 C₂₇H₃₀O₁₃. 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 triggallocate with the precursor ion peak [M – H]⁻ at m/z 745.3, showing a minor production [(M – H) – 152]⁻ at m/z 593.2 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 C₂₇H₂₈O₁₇ 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 tricaffeyl-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 pantagalloy 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 C₂₈H₂₈O₁₆ 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-valonec 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 andAITD, 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 Pakistani 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**

The online version contains supplementary material available at https://doi.org/10.1186/s43094-020-00134-x.

**Additional file 1:** Figure S1. 1H-NMR spectral data of the flavone mixture (400 MHz, CD3OD). Figure S2. 13C-NMR total carbons spectral data of the flavone mixture (100 MHz, CD3OD). Figure S3. 13C-NMR total carbons spectral data of the flavone mixture (100 MHz, CD3OD). Figure S4. HMBC-DEPT 90 spectral data of the flavone mixture (CD3OD). Figure 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. 1H-NMR spectral data of compound three (400 MHz, CD3OD). Figure S9. 13C-NMR DEPT 90 spectral data of compound three (100 MHz, CD3OD). Figure S10. 13C-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; CCl₄: 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; LD₅₀: Median lethal dose; Rf: Retention factor; RP: Reversed-phase; RT: Retention time; TLC: Thin layer chromatography

**Acknowledgements**

The authors are thankful to prof. Amany Sleem professor of Pharmacology, Department of Pharmacology, National Research Centre, Dokky, Giza, Egypt, who carried out the experimental activities in the pharmacology section.

**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. RR is a lecturer in Pharmacognosy who helped in practical work and data collection. HSM 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.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Availability of data and materials**

Data and materials are available upon request.
Ethics approval and consent to participate
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 number (008A-16).

Consent for publication
Not applicable.

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

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Received: 2 May 2020 Accepted: 6 August 2020
Published online: 14 December 2020

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