Egypt. J. Chem. **Vol. 68**, No. 1 pp. 361-379 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Chemical Investigation, Antioxidant, Anti-inflammatory and Antidiabetic Activities of *Maclura spinosa* (Willd.) C.C.Berg: *In-vitro* and *In-vivo* Studies

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Abstract

The aim of this study was to investigate the chemical composition and different biological activities of the small twigs (aerial parts before flowering stage) of *Maclura spinosa* (Willd.) C.C.Berg commonly called "Paper cup flower" family Moraceae. Phytochemical investigation of the of *M. spinosa* total methanolic extract (MSTME) by different chromatographic and spectroscopic techniques led to the isolation and identification of ten known compounds: arachidic acid methyl ester **(1)**, lupeol acetate **(2)**, glutinol **(3)**, lupeol **(4)**, *β*-sitosterol and stigmasterol **(5)**, *β*-sitosterol-3-*O*-*β*-D-glucopyranoside **(6)**, apigenin **(7)**, 2,3-dihydrokaempferol **(8)**, orobol **(9)** and Kaempferol-7-*O*-*β*-D-glucopyranoside **(10)**. The gas chromatography-mass spectrometry (GC-MS) analysis of saponifiable and unsaponifiable matters led to the identification of eight and ten compounds, respectively. Moreover, total polyphenolic, total phenolic, and total flavonoid contents were determined by high-performance liquid chromatography (HPLC) analyses of MSTME. In the biological studies, the *in-vitro* antioxidant activity was determined by the DPPH method, *n*-butanol (MSBF), ethyl acetate (MSEF), and MSTME showed the highest activity at 91.5%, 90.8%, and 88%, respectively at concentration 1 mg/mL with comparison to ascorbic acid and quercetin as positive controls. Also, the anti-inflammatory activity was assessed on RAW 264.7 cells, the MSTME (81.5%) and MSEF (48.6%) exhibited the highest activity at a concentration of 10 µg/mL in comparison with standard quercetin. On the other hand, the *in-vivo* antidiabetic activity was evaluated in streptozotocin (STZ) induced diabetic rats, at a daily oral dose of (300 mg/kg, p.o.) for 15 days, the MSTME and DCM fraction (MSDF) showed a significant reduction in blood glucose levels, total cholesterol and serum triglycerides ($p < 0.01$). The findings suggest the potential of *M. spinosa* as a natural product of the development of herbal medicine for the treatment of diabetes and inflammatory diseases.

Keywords: *Maclura spinosa*, Moraceae, antioxidant, anti-inflammatory, antidiabetic, GC-MS.

1. Introduction

Moraceae is one of the widespread families of angiosperms, it contains 137 genera and 5047 species, and it includes trees, shrubs, and rarely herbs, the genus *Maclura*, a common genus in this family contains about 103 species, its synonyms include *Trophis aculeata* Roth, *Plecospermum spinosum* (Roxb. ex Wild.) trecul,*Cudrania spinosa* Hochr and *Trophis spinosa* Roxb. ex Wild. according to WFO. The native habitat of the species *M. spinosa* (Willd.) C.C.Berg. extends from northern India and China, passing through Malaysia and Indonesia to Australia, it is commonly called a "Paper cup flower" (in Tamil) [1].

Previous phytochemical studies of the genus *Maclura* have revealed the isolation and identification of different classes of compounds from the leaf, fruit, bark and root which include flavonoids [2], xanthones [3], chalcones [4], triterpenes [5], and sterols [6]. There are no previous phytochemical studies on *M. spinosa* except a preliminary phytochemical screening of the total extract of the leaves in India [1],there are no previous isolation or identification of any pure compounds from *M. spinosa*.

Worldwide, plants are important sources of medicines to develop newer drugs for treating several diseases [7]. various species of the genus *Maclura* were used in folk medicine for the treatment of different diseases such as; uterine hemorrhage and tooth pain [8], snake bites [9], eyes sores [10], insect repellents [11], and cholera [12]. Recent reported biological activities of different plant parts of the genus *Maclura* showed antibacterial [13], anti-oxidant [4], anti-inflammatory [14], anticancer [15] and antidiabetic activities [16-21], while previous studies about Indian *M. spinosa* leaves showed anti-oxidant [1], antibacterial [1], wound healing [22], neuroprotective, and fibrinolytic activities [23].

Free radicals can damage the cell membrane and DNA in the body, thus it's implicated in numerous pathological conditions, such as inflammation, metabolic disorders, cellular aging, and carcinogenesis [24]. Antioxidants can form a defense system to prevent free radicals from damaging the body. Many flavonoids are excellent free radical scavengers due to their strong abilities as hydrogen or electron donors [25]. DPPH is a stable free radical exhibiting violet colour in solution, which after

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Receive Date: 11 April 2024,Revise Date: 28 May 2024, Accept Date: 30 May 2024
DOI: 10.21608/ejchem.2024.282507.9583
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mixing with antioxidants, changes to a stable yellow colour, indicating antioxidant activity [26]. A previous anti-oxidant study on total methanolic extract of the leaves of *M. spinosa* growing in India was evaluated [1], however, in current study, the total methanolic extract and different fractions of *M. spinosa* small twigs were examined.

Inflammation is a normal protective response against infection by pathogens, exposure to bacterial endotoxin and chemical injury, it is required to maintain the homeostasis of the immune system, but uncontrolled or prolonged inflammation may be relate to various diseases such as rheumatoid arthritis, inflammatory bowel disease, atherosclerosis [27]. Inflammatory response and tissue damage are induced by inflammatory mediators generated through upregulation of a number of several inducible genes, including inducible nitric oxide (iNOS), cyclooxygenase (COX)-2, interleukin (IL)-6 and IL-8. Nitric oxide (NO) is a short-lived free radical, there are three isoforms of NO synthases (NOS) in tissues to generate it. The neural NOS and endothelial NOS isoforms are expressed in a select tissue. The third member is inducible NOS (iNOS), which has beneficial effects in response to inflammatory stimuli. Natural compounds in medical plants are always found to play an important role in inflammatory disease [28, 29]. Previous *in-vivo* anti-inflammatory study of the Indian *M. spinosa* leaves in India was evaluated on the total methanolic extract [1], but in this study the *in-vitro* anti-inflammatory activity of *M. spinosa* small twigs and different fractions using RAW 264.7 cell line was reported for the first time.

Diabetes mellitus (DM) is a chronic, and complex metabolic disorder with profound consequences. Its complications affect people in both developing and developed countries, leading to a major socioeconomic challenge [30]. It is estimated that 25% of the world population is affected by diabetes and its complications [31, 32]. There are several types of diabetes, which are type 1 DM and type 2 DM, type 1 DM is characterized by deficient insulin production due to pancreatic islet beta cell destruction. On the other hand, Type 2 DM, which is known as non-insulin-dependent diabetes, results from ineffective use of body insulin [33-35]. Despite the presence of several classes of oral hypoglycemic drugs that act through different mechanisms, it’s still far from perfect controlling of blood glucose levels in people, also these medications demonstrate prominent side effects. This represents the main motive for discovering alternative sources of antidiabetic agents [36]. For centuries, many natural products, particularly of plant origin have been considered a fundamental source of potent antidiabetic drugs in developing countries [32, 37], also, several studies have revealed the antidiabetic effect of the genus *Maclura* [17, 38-40], and no previous antidiabetic studies on *M. spinosa*, so, we investigated the *in-vivo* antidiabetic activity of *M. spinosa* (Willd.) C.C.Berg.

By reviewing the available current literature, nothing could be traced concerning phytochemical studies and few reports have been published about biological studies. This provoked us to carry out the antioxidant, anti-inflammatory and antidiabetic, activities of MSTME and its fractions, in addition to the isolation and identification of the major compounds from *M. spinosa* cultivated in Egypt for the first time.

2. Material and methods

2.1. Plant material

The small twigs (aerial parts before flowering stage) of *M. spinosa* (Willd.) C.C.Berg. were collected in October 2020 from Aswan Botanical Garden, Aswan, Egypt (24°05′37″N 32°53′13″E), as shown in figure S32, and kindly identified by Dr. Hafeez Rafael, director of Aswan Botanical Garden Herbarium. A specimen (voucher No M.S.1 2020) was deposited at a plant herbarium, Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

2.2. Materials and Equipment

^{1}H and ^{13}C-NMR spectral data were recorded on Brucker Ascend™ Aeon 400 MHz and Avance Neo console 600 MHz (Germany). ESI-MS positive ion acquisition mode with XEVO TQD mass spectrometer (U.S.A). EI-MS Thermo Scientific™ ISQ™ 70ev (Italy) was used for mass analysis. GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A), and the GC was equipped with an HP-5MS column (30 m×0.25 mm internal diameter and 0.25 µm film thickness), also, it was equipped with flame ionization detector (FID) using a Zebron ZB-FAME column (60 m×0.25 mm internal diameter×0.25 µm film thickness). Columns chromatography were made from silica gel for columns with mesh sizes (70-120) and (70-230) (Germany), Sephadex LH-20 gel (20-100 µm, Sigma-Aldrich), TLC Silica gel $G_{60}F_{254}$ MERK and Sigma-Aldrich (Germany). UV light lamb with two wavelengths (254 nm and 360 nm). STUART scientific Hot plate (England). Heidolph rotary evaporator. RADWAG Balance PS 360/C/1 (Poland). Ultrasonic cleaner (China). Microplate reader FluoStar Omega (Singapore). Spraying reagents like 10% *v/v* H_2SO_4 in MeOH and 5% *w/v* $ALCL_3$ in MeOH. All solvents were distilled before using *n*-hexane, DCM, EtOAc, *n*-but, and MeOH.

Streptozotocin (STZ) from Sigma-Aldrich Chemicals Co. (Germany), glibenclamide from (Sanofi Aventis Pharma (Pvt.) Ltd., Egypt). Sodium citrate buffer from Tribioscience (U.S.A). Dimethyl sulfoxide (DMSO) (SDS / France). Normal saline (Otsuka Pharma (Pvt.) Ltd., Egypt). GlucoDr™ Glucometer strips (Korea). Cholesterol-LQ kits from Spinreact (Spain). Triglycerides from Biomed diagnostics (Germany). 2,2-Diphenyl-1-picryl hydrazyl (DPPH) from Sigma-Aldrich Chemicals Co. (Germany), Ascorbic acid and quercetin from Sigma-Aldrich Chemicals Co, (Germany). RAW 264.7 mouse macrophage cell line was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Dulbecco’s modified Eagle medium (DMEM), 10% of heat-inactivated fetal bovine serum (FBS), 1% penicillin/ streptomycin (P/S) from Sigma-Aldrich Chemicals Co. (Germany). Infinite F50 microplate reader (TECAN, Switzerland).

Adult male Wistar albino rats weighing (180 ± 20 g) were utilized from the Department of Pharmacology, Faculty of Pharmacy, Al-Azhar University, Cairo, and were housed with standard food with free access to fresh water and 12/12 hrs. light/dark cycle in aplastic cages at 22-25 ºC. All animal procedures have been approved by the animal ethical committee (AZ-AS/PH-REC/20/2024) (Figure S31) [41, 42].

2.3. Extraction, fractionation, and isolation of pure compounds

About 5 Kg of dried milled twigs of *M. spinosa* were macerated with 90% MeOH/H_2O several times until complete exhaustion of the powder, the extract was filtered through Whatman filter paper then evaporated under reduced pressure using a rotary evaporator, dried in the open air to give semisolid paste (530 g). Approximately 500 g was dissolved in 500 mL distilled water and transferred to a separating funnel to successively partitioned with *n*-hex (5×500 mL), DCM (5×500 mL), EtOAc (5×500 mL) and *n*-but (5×500 mL) to give 23, 130, 30 and 40 g, respectively. While the aqueous portion was (250 g) after complete concentration.

n-Hex fraction (MSHF, 18 g) was oily, it was slurred by (15 g) silica and transferred to the top of the (5×120 cm) column backed with (550 g) silica slurred with *n*-hex, firstly the column was eluted by *n*-hex then by *n*-hex.- EtOAc at different concentrations until complete exhaustion of slurry, each 100 mL of eluents were collected and similar fractions were collected together after they compared with TLC precoated silica gel $G_{60}F_{254}$ to yield 9 sub-fractions labeled HF-1 to HF-9. Compound **1** (120 mg) was obtained from HF-1 after recrystallization with *n*-hex, compound **2** (8 mg), compound **3** (39 mg) and compound **4** (31 mg) were afforded from HF-2 after re-columns on silica gel with gradient elution of *n*-hex:EtOAc.

MSDF (30 g) was slurred and transferred to the top of the column (7.5×120 cm) filled with silica gel for column (900 g) slurred by DCM, the column was eluted by gradient elution of DCM-MeOH. Each 100 mL was collected separately in a flask and condensed under reduced pressure; similar sub-fractions were collected together after making TLC, and it yielded 15 sub-fractions labeled DF-1 to DF-15. Compound **5** (43 mg) was obtained from DF-10, while compound **6** (38 mg) was yielded from DF-13 after re-columned on silica gel and eluted by gradient elution of DCM:MeOH

MSEF (25 gm) was slurred by silica gel and transferred to the top of the column (7.5×100 cm) packed with (750 g) of silica gel slurred by DCM. The column was eluted by gradient elution of DCM-MeOH until exhaustion of the slurry. Fractions of 100 mL were collected in flasks and condensed under reduced pressure and similar fractions were gathered after they were monitored by TLC. The column gave 13 sub-fractions labeled EF-1 to EF-13. Three compounds **7** (9 mg), **8** (15 mg), and **9** (2 mg) were obtained from EF-6, while compound **10** (10 mg) was afforded from EF-9 after several re-columns on silica gel and Sephadex LH20 using gradient elution techniques.

2.4. Determination of total phenolic content

A gallic acid stock solution of 1 mg/mL in methanol was prepared and 8 serial dilutions in the concentrations of 25, 50, 100, 200, 400, 600, 800 and 1000 µg/mL. were prepared. The sample was prepared at the concentration of 9 mg/mL in methanol:distilled water, 1:1 *v/v*. The total phenolic content was determined using the Folin-Ciocalteu method [43]. Briefly, the procedure consisted of mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1:10) in a 96-well microplate. Then, 80 µL of 1M Na_2CO_3 was added and incubated at room temperature (25 °C) for 20 min in the dark. At the end of incubation time, the resulting blue complex color was measured at λ_{max} 630 nm using microplate reader FluoStar Omega. Data were represented as means ± SD.

2.5. Determination of total flavonoid content

A stock solution of standard rutin was prepared at 200 µg/mL in methanol, from which 5, 10, 20, 40, 80, 100, 120 and 160 µg/mL. dilutions were prepared. The sample was prepared at the concentration of 9 mg/mL in methanol:distilled water, 1:1 *v/v*. The total flavonoid content was determined using the aluminum chloride method with minor modifications [44].

Briefly, 15 µL of sample/standard was placed in a 96-well microplate, then, 175 µL of methanol was added followed by 30 µL of 1.25 % $AlCl_3$. Finally, 30 µL of 0.125 M sodium acetate ($C_2H_3NaO_2$) was added and incubated for 5 min. At the end of incubation time, the resulting yellow color was measured at λ_{max} 420 nm by using a microplate reader FluoStar Omega. Data are represented as means ± SD.

2.6. GC-MS analysis for determination of saponifiable and unsaponifiable matters

2.6.1. Sample derivatizations

The samples were subjected to saponification with KOH then extracted with heptane/diethyl ether and finally dried. 50 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added to the dried extract and incubated in a dry block heater at 70 °C for 30 min. [45, 46].

2.6.2. GC-MS of saponifiable matters

The GC-MS model (7890B) from Agilent Technologies was equipped with a flame ionization detector at Central Laboratories Network, National Research Centre, and Cairo, Egypt. Separation was achieved using a Zebron ZB-FAME column (60 m×0.25 mm internal diameter×0.25 µm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.8 mL/min at a split ratio 1:50 mode, injection volume of 1 µL and the following temperature program: 100 °C for 3 min; rising at 2.5 °C /min to 240 °C and held for 10 min. The injector and detector (FID) were held at 250 °C and 285 °C, respectively [46, 47].

2.6.3. GC-MS of unsaponifiable matters
The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with an HP-5MS column (30 m×0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using Hydrogen as the carrier gas at a flow rate of 1.0 mL/min at a split ratio of 10:1, injection volume of 1 µL, and the following temperature program: 240 °C; rising at 10 °C /min to 265 °C and held for 1 min; rising at 15 °C/min to 300 °C and held for 25 min. The injector and detector were held at 280 and 290°C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of *m/z* 50-550 and solvent delay of 3 min. The mass temperature was 230 °C and Quad 150 °C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

2.7. HPLC analysis for determination of polyphenol content
HPLC analysis was carried out on MSTME using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm×250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0-5 min (80% A); 5-12 min (60% A); 12-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µL for each of the sample solutions. The column temperature was maintained at 40 °C.

2.8. In-vitro biological studies
2.8.1. ***Antioxidant assay***
The concept is to measure the electron donation capacity of methanolic solutions of *M. spinosa* spectrometrically [25]. The bleaching of purple colored-methanolic solution of DPPH that was prepared at the time of the experiment was observed in MSHF, MSDF, MSEF, and MSTME at different methanolic concentrations (62.5, 125, 250, 500 and 1000 µg/mL). Ascorbic acid and quercetin were used as a control at the same concentrations. A constant 0.2 mL of 10^{-4} M of DPPH was mixed with 2 mL of each concentration from each fraction and kept in the dark for 30 min. then measured spectrometrically at λ_{max} 517 nm.

2.8.2. Determination of anti-inflammatory effect on RAW 264.7 cells
2.8.2.1. Cytotoxicity assay
2.8.2.1.1. Cell culture preparation
RAW 264.7 mouse macrophage cell line was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were cultured and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% of heat-inactivated fetal bovine serum (FBS) and 1% penicillin/ streptomycin (P/S) at 37 °C in a humidified atmosphere of 5% CO_2.

2.8.2.1.2. Determination of cytotoxicity
Cell viability was determined using a sulforhodamine (SRB) assay [48]. Briefly, aliquots of 100 µL cell suspension (5000 cells) were seeded in 96-well plates and incubated in complete media for 24 hrs. The cells were divided into control and treatment groups. The control group was exposed to varied concentrations of vehicle (DMSO), while the treatment groups were treated with another aliquot of 100 µL media containing different concentrations of plant extracts (1-10 µg/mL diluted in DMSO) for 48 hrs. After drug exposure, cells were fixed by replacing media with 150 µL of 10% trichloroacetic acid (TCA) and incubated at 4 °C for 1 hr. The trichloroacetic acid solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µL SRB solution (0.4% *w/v*) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µL of TRIS (10 mM) was added to dissolve the protein-bound SRB stain; the absorbance was measured at 540 nm [49].

2.8.2.2. Anti-inflammatory assay
After RAW264.7 Cells were seeded into a 96-well plate and incubated for 24 hrs. The inflammatory cascades were induced with 1 µg/mL of Lipopolysaccharides (LPS-group). The untreated cells will be replenished with fresh media (Control group); while the treated groups were divided into 5 groups as follows: group 1: treated with 1 µg/mL and 10 µg/mL of *n*-hex., group 2: treated with 1 µg/mL of DCM, group 3: treated with 1 µg/mL and 10 µg/mL of EtOAc, group 4: treated with 1 µg/mL and 10 µg/mL of MSTME, group 5: treated with quercetin as a positive control [50].
To measure nitric oxide (NO) secretion, equal volumes of the cell supernatant and Griess reagent were mixed for 10 min in the dark at room temperature. The absorbance was measured at 540 nm using an ELISA plate reader, which represents the nitrite concentration [51].

2.9. In-vivo biological studies
2.9.1. Acute toxicity study of MSTME
The acute toxicity study of crude methanolic extract of *M. spinosa* was determined before the starting of an anti-diabetic experiment to ensure its safety on adult male Wistar albino rats (180 ± 20 g) according to Dietrich Lorke's 1983 method. The experiment was divided into two phases, in the first phase 12 rats were randomizable and divided into 4 groups, one group was served as a control and received 1 mL of 0.04% DMSO in water. MSTME was given in a dose of (10, 100, and 1000 mg/kg b.w.) to each rat orally (one dose for each rat) to investigate the toxicity of the extract compared to the control group. There were no behavioral, physical, and pharmacological toxic effects within 24 hrs. after treatment. So, we started the second

phase by giving the plant extract at doses of (1600, 2900, and 5000 mg/kg b.w.), also, there were no toxicity signs recorded or death observed for 24 hrs. after treatment. All doses were prepared by dissolving the extract in 0.04% DMSO in water. This indicates the safety of MSTME at the dose of (5000 mg/kg b.w.) which is more than tenfold that will be used in the anti-diabetic experiment (300 mg/kg) according to OECD guidelines for screening of the antidiabetic activity [52].

2.9.2. Anti-diabetic activity

2.9.2.1. Induction of type 2 DM

Induction of diabetes was done by a single intraperitoneal dose of streptozotocin (STZ) 50 mg/kg which is freshly prepared by dissolving it in 0.1 mol/L cold sodium citrate buffer [53]. Rats were allowed free access to food and water for four days, and then blood sugar level was measured from the Retro-orbital vein with GlucoDr™ Glucometer strips using an SD glucometer (Korea). The rats with fasting blood glucose levels over 225 mg/dL were considered diabetic and selected for the study [42].

2.9.2.2. Experimental design

Rats were fasted overnight for 12 hrs. and randomly divided into six groups (n = 6). Group 1: normal control (non-diabetic) and received normal saline, group 2: STZ-induced diabetic control rats and received 1 mL of 0.04% DMSO in water, group 3: diabetic rats received glibenclamide 500 µg/kg (p.o.), group 4: diabetic rats received 300 mg/kg (p.o.) of DCM, group 5: diabetic rats received 300 mg/kg (p.o.) of EtOAc, group 6: diabetic rats received 300 mg/kg (p.o.) of MSTME. The extracts were given as a single oral dose dissolved in 1 mL of 0.04% DMSO in water. All groups received the treatment for 15 days using an intragastric tube, and blood glucose levels were measured on days 0, 10, and 15 [19, 42].

2.9.2.3. Estimation of serum lipid profile

On day 15, blood samples were collected from the Retro-orbital vein after 16 hrs. of fasting for biochemical parameters studies and animals were euthanized by cervical decapitation using the method described in Schedule 1 [54]. Collected blood was centrifuged at 1500 rpm for 10 min for serum separation. Total cholesterol (TC) and triglycerides (TGs) were determined in it according to manufacturer instructions.

2.10. Statistical analysis

All experimental values were expressed as mean ± SD and the statistical significance was analyzed by GraphPad Prism® software 8.0.2 (one-way ANOVA followed by Tukey's test for multiple comparisons). Differences between groups were considered significant at ($p < 0.05$).

3. Result and discussion

3.1. Identification of isolated compounds

Arachidic acid methyl ester (1)

White amorphous powder soluble in DCM but insoluble in MeOH; EI-MS showed $[M]^+$ at *m/z* 326.31 corresponding to molecular formula $C_{21}H_{42}O_2$, in addition to fragment peaks at *m/z* 298.28 (19%), 270.19 (44%), 199.09 (50%), 143.09 (85%), 86.73 (91%), 73.80 (100%) base peak,

^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 2.23 (2H, t, J = 7.6 Hz, H-2), 1.54 (2H, m, H-3), 1.18-1.22 (24H, br.s, H-4 to H-17), 0.81 (3H, t, J = 7 Hz, H-20), 3.59 (3H, s, H-1').^{13}C-NMR (150 MHz, $CDCl_3$): δc 174.28 (C-1), 34.09 (C-2), 24.95 (C-3), 29.15-29.7 (C-4 to C-17), 31.93 (C-18), 22.69 (C-19), 14.09 (C-20), 51.37 (C-1').

^{13}C-NMR spectrum (150 MHz, $CDCl_3$) revealed signals at δ_C 174.28, δ_C 51.37, and δ_C 14.09 ppm assigned to carbonyl, methyl ester, and terminal methyl groups respectively [55]. In addition, a group of signals from δ_C 22.69 to δ_C 34.09 ppm is assigned to the CH_2 cluster, which means the existence of long-chain fatty acid ester [55]. This was further approved from ^{1}H-NMR signals that showed at δ_H 3.59 (3H, s, H-1'), δ_H 2.23 (2H, t, J = 7.6 Hz, H-2) and δ_H 0.81 (3H, t, J = 7 Hz, H-20) characteristic for protons of methyl ester, downfield shifted CH_2 group neighbor to the carboxylic moiety in addition to a terminal methyl group [56]. Moreover, EI-MS showed $[M]^+$ at *m/z* 326.31 corresponding to molecular formula $C_{21}H_{42}O_2$ with fragment peaks at *m/z* 298.28 (19%), 270.19 (44%), 199.09 (50%), 143.09 (85%), 86.73 (91%) and base peak 73.80 (100%) [57]. Based on the above-mentioned data and by comparing with literature [55-57], compound **1** was identified as an arachidic acid methyl ester (Methyl eicosanoate), which is isolated for the first time from the genus *Maclura*.

Lupeol acetate (2)

White amorphous powder, soluble DCM but sparingly soluble in MeOH; ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 4.40 (1H, dd, J = 5.4, 10.8 Hz, H-3), 2.30 (1H, m, H-19), 0.78 (3H, s, H-23), 0.77 (3H, s, H-24), 0.96 (3H, s, H-25), 0.76 (3H, s, H-26), 0.72 (3H, s, H-27), 0.87 (3H, s, H-28), 4.49 (1H, br.s, H-29a), 4.61 (1H, br.s, H-29b), 1.61 (3H, s, H-30), 1.97 (3H, s, H-2'). ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C 38.39 (C-1), 23.72 (C-2), 80.98 (C-3), 37.8 (C-4), 55.38 (C-5), 18.21 (C-6), 34.21 (C-7), 40.85 (C-8), 50.35 (C-9), 37.09 (C-10), 20.95 (C-11), 25.1 (C-12), 38.04 (C-13), 42.83 (C-14), 27.44 (C-15), 35.58 (C-16), 43.0 (C-17), 48.01 (C-18), 48.29 (C-19), 150.97 (C-20), 29.84 (C-21), 40.01 (C-22), 27.96 (C-23), 15.98 (C-24), 16.19 (C-25), 16.51 (C-26), 14.51 (C-27), 18.01 (C-28), 109.37 (C-29), 19.3 (C-30), 171.03 (C-1'), 21.34 (C-2').

^{13}C-NMR spectrum (150 MHz, $CDCl_3$) of compound **2** indicated the presence of 32 carbon atoms which are similar to isolated compound **4** with two carbons at δ_C 171.03 and δ_C 21.34 with δ_H 1.97 (3H, s, H-2') which suggest the triterpene ester derivative [58], also, ^{1}H-NMR spectrum showed seven tertiary methyl groups at δ_H 0.72, 0.76, 0.77, 0.78, 0.87, 0.96, and 1.58 each (3H,s) corresponding to H-27, H-26, H-24, H-23, H-28, H-25, and H-30, which confirmed from ^{13}C-NMR signals at δ_C C-27 (14.51), C-26 (16.51), C-24 (15.98), C-23 (27.96), C-28 (18.01), C-25 (16.19), and C-30 (19.30) [59]. Moreover, a

characteristic pair of sp^2 hybridized carbon atoms were observed at δ_C 150.97 and 109.37 ppm for C-20 and C-29, respectively, that were approved from ^{1}H-NMR at δ_H 4.61, 4.49 (1H, br.s, H-29) which indicate the presence of terminal methylene moiety. Furthermore, the hydroxylated carbon atom was observed downfield shift than lupeol at δ_C 80.98 and δ_H 4.40 (1H, dd, J = 5.4, 10.8 Hz, H-3) for C-3 and H-3, respectively [60]. From the above-mentioned data and by comparison with the reported data [58, 59], compound **2** was assigned to be lupeol acetate, which was previously isolated from the genus *Maclura* [61].

Glutinol (3)

White crystalline needles, soluble in DCM and *n*-hex. but sparingly soluble in MeOH; ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 3.42 (1H, br.s, H-3), 5.56 (1H, d, J = 5.4 Hz, H-6), 1.07 (3H, s, H-23), 0.97 (3H, s, H-24), 0.78 (3H, s, H-25), 1.02 (3H, s, H-26), 0.93 (3H, s, H-27), 1.09 (3H, s, H-28), 0.88 (3H, s, H-29), 0.92 (3H, s, H-30).^{13}C-NMR (150 MHz, $CDCl_3$): δ_C 18.23 (C-1), 27.82 (C-2), 76.37 (C-3), 40.83 (C-4), 141.61 (C-5), 122.1 (C-6), 23.65 (C-7), 47.43 (C-8), 34.85 (C-9), 49.69 (C-10), 34.61 (C-11), 30.36 (C-12), 39.31 (C-13), 37.84 (C-14), 32.08 (C-15), 36.02 (C-16), 30.1 (C-17), 43.06 (C-18), 35.08 (C-19), 28.26 (C-20), 33.12 (C-21), 38.96 (C-22), 25.47 (C-23), 28.96 (C-24), 16.22 (C-25), 19.63 (C-26), 18.44 (C-27), 32.05 (C-28), 34.54 (C-29), 32.41 (C-30).

^{13}C-NMR spectrum (150 MHz, $CDCl_3$) of compound **3** indicated the presence of 30 signals including two olefinic carbon signals at δ_C 141.61 (C-5) and 122.1 (C- 6), also, one oxymethine carbon atom at δ_C 76.37 (C-3) which suggest its triterpenoid skeleton [62], in addition, ^{1}H-NMR spectrum approved the presence of eight tertiary methyl groups at δ_H 0.78, 0.88, 0.92, 0.93, 0.97, 1.02, 1.07 and 1.09 each (3H,s) corresponding to H-25, H- 29, H-30, H-27, H-24, H-26, H-23 and H-28, which confirmed from ^{13}C-NMR signals at δ_C C-25 (16.22), C-29 (34.54), C-30 (32.41), C-27 (18.44), C-24 (28.96), C-26 (19.63), C-23 (25.47) and C-28 (32.05), respectively. Moreover, the ^{13}C-NMR spectrum showed characteristic olefinic carbon signals at δ_C 141.61 (C-5) and δ_C 122.1 (C-6) which were approved from ^{1}H-NMR at δ_H 5.56, 5.55 (1H, br. d, J = 5.4 Hz, H-6) that indicate the presence of endocyclic double bond [63]. The hydroxylated carbon C-3 was observed at δ_C 76.37 corresponding to a carbinol proton with δ_H 3.42 (1H, br.s, H-3). From the above-mentioned data and by comparison with reported data [63, 64], compound **3** was identified as glutinol, and isolated for the first time from the genus *Maclura*.

Lupeol (4)

White crystalline needles, soluble in DCM and *n*-hex. but sparingly soluble in MeOH; ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 2.31 (1H, m, H-2), 3.12 (1H, dd, J = 4.8, 10.4 Hz, H-3), 1.85 (1H, m, H-19), 0.89 (3H, s, H-23), 0.69 (3H, s, H-24), 0.76 (3H, s, H-25), 0.96 (3H, s, H-26), 0.87 (3H, s, H-27), 0.72 (3H, s, H-28), 4.62 (1H, br.s, H-29a), 4.50 (1H, br.s, H-29b), 1.61 (3H, s, H-30).^{13}C-NMR (150 MHz, $CDCl_3$): δ_C 38.72 (C-1), 25.14 (C-2), 79.04 (C-3), 38.87 (C-4), 55.31 (C-5), 18.33 (C-6), 34.29 (C-7), 40.84 (C-8), 50.44 (C-9), 37.17 (C-10), 20.94 (C-11), 27.41 (C-12), 38.06 (C-13), 42.84 (C-14), 27.46 (C-15), 35.59 (C-16), 43.01 (C-17), 48.31 (C-18), 48.0 (C-19), 150.98 (C-20), 29.86 (C-21), 40.02 (C-22), 28.01 (C-23), 15.4 (C-24), 16.14 (C-25), 15.99 (C-26), 14.56 (C-27), 18.02 (C-28), 109.35 (C-29), 19.32 (C-30).

^{13}C-NMR spectrum (150 MHz, $CDCl_3$) of compound **4** indicated the presence of 30 carbon signals as seven methyl, eleven methylene, six methine, and six quaternary carbons, which suggests lupane triterpenoid skeleton [65], also ^{1}H-NMR spectrum approved the presence of seven tertiary methyl groups at δ_H 0.69, 0.72, 0.76, 0.87, 0.89, 0.96 and 1.61 each (3H, s) corresponding to H-24, H-28, H-25, H-27, H-23, H-26 and H-30 which confirmed from ^{13}C-NMR signals at δ_C C-24 (15.4), C-28 (18.02), C-25 (16.14), C-27 (14.56), C-23 (28.01), C-26 (15.99) and C-30 (19.32) [66]. In addition, the ^{13}C-NMR spectrum showed two characteristic signals at δ_C 150.98 (C-20) and δ_C 109.32 (C-29) which were approved from ^{1}H-NMR at δ_H 4.62 and δ_H 4.50 (1H, br.s, H-29) representing the exocyclic double bond [67]. Moreover, there is δ_C 79.04 which indicates the presence of hydroxyl group at carbon C-3 that can be confirmed by δ_H 3.12 (1H, dd, J = 4.8, 10.4 Hz, H-3) [65]. From the above-mentioned data and by comparison with the reported data [68, 69], compound **4** was identified as lupeol, which was previously isolated from the genus *Maclura* [70].

β-Sitosterol and stigmasterol (5)

White crystalline needles, soluble in DCM but sparingly soluble in MeOH; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 3.54 (1H, m, H-3), 2.27 (2H, m, H-4), 5.37 (1H, m, H-6), 0.70 (3H, s, H-18), 1.02 (3H, s, H-19), 5.17 (1H, m, H-22b), 5.03 (1H, m, H-23b). ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 37.26 (C-1), 31.66 (C-2), 71.81 (C-3), 42.31 (C-4), 140.76 (C-5), 121.72 (C-6), 31.91 (C-7), 31.91 (C-8), 50.15 (C-9), 36.51 (C-10), 21.09 (C-11), 39.69/39.78b (C-12), 42.22 (C-13), 56.77/56.87b (C-14), 24.31/24.37b (C-15), 28.25 (C-16), 56.06/55.96b (C-17), 11.86 (C-18), 19.40 (C-19), 36.15/40.50b (C-20), 18.79/21.09b (C-21), 33.95/138.32b (C-22), 26.09/129.28b (C-23), 45.84/51.24b (C-24), 29.16/31.91b (C-25), 19.81/18.99b (C-26), 19.04/21.22b (C-27), 23.07/25.41b (C-28), 12.26 (C-29).

^{13}C-NMR spectrum (100 MHz, $CDCl_3$) of compound **5** indicated the presence of 29 carbon atoms which suggests its steroidal skeleton [71], which accounted for six methyls, nine methylenes, eleven methanes, and three quaternary carbons. Two pairs of sp^2 hybridized carbon atoms were observed at δ_C 140.76 and δ_C 121.72 ppm with δ_H 5.37 (1H, m, H-6) indicating the presence of a double bond in the B-ring. also, shown at δ_C 138.32 and δ_C 129.28 ppm with δ_H 5.17 (1H, m, H-22) and δ_H 5.03 (1H, m, H-23) indicated the presence of another double bond in the chain. In addition to, there is δ_C 71.81 with δ_H 3.54 (1H, m, H-3) which indicates the presence of a hydroxyl group at carbon C-3 [72]. From the above-mentioned physical, chemical, and spectral data and by comparison with reported data and authentication with co-chromatography [68]. Compound **5** was

identified as a mixture of *β*-sitosterol (a) and stigmasterol (b), which was previously reported in the genus for *β*-sitosterol (a) [6], but stigmasterol (b) is reported here for the first time from the genus *Maclura*.

***β*-Sitosterol-3-*O*-*β*-D-glucopyranoside (6)**

White crystalline flakes, insoluble in *n*-hex. and DCM, sparingly soluble in MeOH, but soluble in hot MeOH and mixture of DCM and MeOH (1:1); ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 3.43 (1H, m, H-3), 5.31 (1H, m, H-6), 0.64 (3H, s, H-18), 0.95 (3H, s, H-19), 0.89 (3H, d, J = 6.4 Hz, H-21), 0.82 (3H, d, J = 6.8 Hz, H-26), 0.79 (3H, d, J = 6.8 Hz, H-27), 0.81 (3H, t, J = 7.2 Hz, H-29); glucose; 4.22 (1H, d, J = 7.6 Hz, H-1'), 2.86-3.66 (5H, m, H-2' to H-6'). ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 36.86 (C-1), 28.73 (C-2), 76.98 (C-3), 38.34 (C-4), 140.47 (C-5), 121.19 (C-6), 31.44 (C-7), 31.40 (C-8), 49.64 (C-9), 36.23 (C-10), 20.63 (C-11), 41.87 (C-12), 41.76 (C-13), 55.47 (C-14), 22.63 (C-15), 25.47 (C-16), 56.21 (C-17), 11.79 (C-18), 19.11 (C-19), 35.52 (C-20), 18.63 (C-21), 33.38 (C-22), 23.89 (C-23), 45.18 (C-24), 27.82 (C-25), 11.68 (C-26), 18.95 (C-27), 29.28 (C-28), 19.72 (C-29); glucose; 100.85 (C-1'), 73.48 (C-2'), 76.78 (C-3'), 70.09 (C-4'), 76.74 (C-5'), 61.10 (C-6').

^{13}C-NMR spectrum (100 MHz, DMSO-d_6) of compound **6** indicated the presence of 35 carbon atoms from which there were 6 signals attributed to sugar moiety at δ_C 73.48, 76.78, 70.09, 76.74, 61.10 and anomeric carbon at δ_C 100.35 with the *β*-configuration of its proton δ_H 4.22 (1H, d, J = 7.6 Hz, H-1'), while the other carbon signals (29 carbon atoms) are similar to the previously isolated compound **5**; six methyls, nine methylenes, eleven methanes, and three quaternary carbons, that suggest the glycosidic structure of *β*-sitosterol [73]. Moreover, the ^{13}C-NMR spectrum showed two sp^2 hybridized carbon atoms at δ_C 140.47 and δ_C 121.19 with δ_H 5.31 (1H, m, H-6) that indicate the presence of double bond between C-5 and C-6 in B-ring [72]. Furthermore, investigation of the ^{1}H-NMR spectrum showed six methyl groups at δ_H 0.64 (H-18), 0.79 (H-27), 0.81 (H-29), 0.82 (H-26), 0.89 (H-21) and 0.95 (H-19) each (3H, s). From the above-mentioned physical, chemical, and spectral data and by comparison with reported data and authentication with co-chromatography [73], compound **6** was identified as *β*-sitosterol-3-*O*-*β*-D-glucopyranoside, and isolated for the first time from the genus *Maclura*.

Apigenin (7)

Yellow amorphous powder, insoluble in DCM, sparingly soluble in MeOH and soluble in DMSO; ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 6.78 (1H, s, H-3), 12.96 (1H, s, H-5), 6.19 (1H, d, J = 1.6 Hz, H-6), 6.48 (1H, d, J = 1.6, H-8), 7.92 (2H, d, J = 8.8 Hz, H-2', H-6'), 6.93 (2H, d, J = 8.8Hz, H-3', H-5'). ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 163.75 (C-2), 102.85 (C-3), 181.76 (C-4), 161.17 (C-5), 98.85 (C-6), 164.16 (C-7), 93.97 (C-8), 157.32 (C-9), 103.70 (C-10), 121.19 (C-1'), 128.48 (C-2'), 115.96 (C-3'), 161.46 (C-4'), 115.96 (C-5'), 128.48 (C-6').

^{13}C-NMR spectrum (100 MHz, DMSO-d_6) of compound **7** indicated the presence of 13 peaks corresponding to 15 carbon atoms, together with its physical characteristics suggesting its flavonoid skeleton [74]. The carbonyl atom was observed downfield at δ_C 181.76 that suggest the presence of hydroxyl group at C-5, also, there is a peak at δ_C 164.16 which indicate the presence of another hydroxyl group in ring A (C-7) that can be approved from ^{1}H-NMR with two doublet peaks at δ_H 6.19 (1H, d, J = 1.6 Hz, H-6) and 6.48 (1H, d, J = 1.6 Hz, H-8) for C-6 and C-8, respectively. Moreover, ^{1}H-NMR spectrum showed two sets of more downfield doublet peaks at δ_H 7.92 (2H, d, J = 8.8 Hz, H-2', H-6') and 6.93 (2H, d, J = 8.8 Hz, H-3', H-5') with δ_C 128.48 and 115.96 for C2′, C6′ and C3′, C8′, respectively [75]. From the above-mentioned physical, chemical, and spectral data and by comparison with reported data [76], compound **7** was identified as apigenin, which was previously reported from the genus *Maclura* [6].

2,3-Dihydrokaempferol (8)

Yellowish white powder, soluble in MeOH; ^{1}H-NMR (400 MHz, CD_3OD): δ_H 4.99 (1H, d, J = 11.6 Hz, H-2), 4.55 (1H, d, J = 11.6 Hz, H-3), 5.89 (1H, br.s, H-6), 5.94 (1H, d, J = 2 Hz, H-8), 7.37 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.85 (2H, d, J = 8.4 Hz, H-3', H-5'). APT NMR (100 MHz, CD_3OD): δ_C 84.94 (C-2), 73.61 (C-3), 198.47 (C-4), 165.27 (C-5), 97.32 (C-6), 168.71 (C-7), 96.28 (C-8), 164.52 (C-9), 101.83 (C-10), 129.27 (C-1'), 130.36 (C-2'), 116.13 (C-3'), 159.19 (C-4'), 116.13 (C-5'), 130.36 (C-6').

APT NMR spectrum (100 MHz, CD_3OD) of compound **8** indicated the presence of 15 carbon atoms, also from its physical characters, it showed a yellow spot with ammonia vapor and 5% $ALCL_3$ which suggests its flavonoid skeleton [74]. Carbonyl group was observed at δ_C 198.47 which is highly desheilded owing to molecular hydrogen bonding between the two hydroxyl groups in C-3 and C-5. In addition, the coupling constant of C-2 and C-3 protons (11.6 Hz) is typical for 2,3-diaxial protons, also, the presence of two carbon signals at δ_C 84.94 and δ_C 73.61 with δ_H 4.99 (1H, d, J = 11.6 Hz, H-2) and 4.55 (1H, d, J = 11.6 Hz, H-3) suggest the 2,3-trans dihydroflavonol nucleus [74]. Moreover, the presence of δ_H 5.89 (1H, br.s, H-6) and 5.94 (1H, d, J = 2 Hz, H-8) indicate that C-6 and C-8 are free from any substitution. Furthermore, there are two highly intense peaks at δ_C 116.13 and 130.36 with δ_H 6.85 (1H, d, J = 8.4 Hz, H-3', H-5') and 7.37 (1H, d, J = 8.4 Hz, H-2', H-6') which represent the (C-3′, C-5′) and (C-2′, C-6′), respectively [77]. From the above mentioned physical, chemical, and spectral data and by comparison with reported data [25, 78], compound **8** was identified as 2,3-dihydrokaempferol, which was previously isolated from the genus *Maclura* [79].

Orobol (9)

Colourless amorphous powder, soluble in MeOH and DMSO; ^{1}H-NMR (400 MHz, CD_3OD): δ_H 7.94 (1H, s, H-2), 6.12 (1H, d, J = 2.4 Hz, H-6), 6.23 (1H, d, J = 2 Hz, H-8), 6.91 (1H, d, J = 2 Hz, H-2'), 6.71 (1H, d, J = 8 Hz, H-5'), 6.75 (1H, dd, J = 2, 8 Hz, H-6'). ^{13}C-NMR (100 MHz, CD_3OD): δ_C 154.83 (C-2), 124.85 (C-3), 182.26 (C-4), 159.71 (C-5), 100.15 (C-6), 166.07

(C-7), 94.79 (C-8), 163.88 (C-9), 106.25 (C-10), 123.82 (C-1'), 117.44 (C-2'), 146.83 (C-3'), 146.25 (C-4'), 116.33 (C-5'), 121.68 (C-6').

^{13}C-NMR spectrum (100 MHz, CD_3OD) of compound **9** indicated the presence of 15 carbon atoms also from its physical characters, it showed a yellow spot with ammonia vapor and florescent colour under UV. with 5% $AlCl_3$. ^{1}H-NMR and ^{13}C-NMR spectra showed a single peak at δ_H 7.94 (1H, s, H-2) and δ_C 154.83 ppm, which suggests the iso-flavonoid nucleus [74]. Carbonyl group was observed downfield at δ_C 182.26 ppm owing to the molecular hydrogen bonding formation with the hydroxyl group at C-5, moreover, there were two doublet signals at δ_H 6.12 (1H, d, J = 2.4 Hz, H-6) and 6.23 (1H, d, J = 2 Hz, H-8) with δ_C 100.15 and δ_C 94.79 ppm corresponding to C-6 and C-8 protons. Furthermore, the substitution pattern of protons in ring B showed three peaks at δ_H 6.91 (1H, d, J = 2 Hz, H-2'), δ_H 6.71 (1H, d, J = 2 Hz, H-5') and δ_H 6.75 (1H, dd, J = 2, 8 Hz, H-6') that revealed the ortho di hydroxyl substitution in C-3′ and C-4′ [75]. From the above-mentioned physical, chemical, and spectral data and by comparison with reported data [80], compound **9** was identified as orobol, and was previously reported from the genus *Maclura* [2, 81].

Kaempferol-7-O-β-D-glucopyranoside (10)

Yellow amorphous solid, insoluble in DCM, soluble in MeOH and DMSO; ^{1}H-NMR (400 MHz, CD_3OD): δ_H 6.36 (1H, d, J = 2 Hz, H-6), 6.72 (1H, d, J = 2 Hz, H-8), 8.02 (2H, d, J = 8.8 Hz, H-2', H-6'), 6.81 (2H, d, J = 8.8 Hz, H-3', H-5'); glucose; 4.96 (1H, d, J = 7.2 Hz, H-1''), 3.28-3.85 (5H, m). APT NMR (100 MHz, CD_3OD): δ_C 147.43 (C-2), 136.20 (C-3), 176.19 (C-4), 163.08 (C-5), 100.25 (C-6), 159.36 (C-7), 98.84 (C-8), 156.37 (C-9), 104.91 (C-10), 122.17 (C-1'), 129.49 (C-2'), 114.94 (C-3'), 160.79 (C-4'), 114.94 (C-5'), 129.49 (C-6'); glucose; 94.17 (C-1''), 73.35 (C-2''), 76.46 (C-3''), 69.88 (C-4''), 76.97 (C-5''), 61.06 (C-6'').

APT NMR spectrum (100 MHz, CD_3OD) of compound **10** indicates the presence of 19 signals corresponding to 21 carbon atoms. APT NMR with ^{1}H-NMR spectra revealed a signal at δ_C 94.17 ppm with δ_H 4.96 (1H d, J = 7.2 Hz, H-1'') which corresponded to the anomeric carbon and its proton, in addition to, five sugar signals were appeared from 61.07 to 77.64 ppm with δ_H 3.28 to 3.85 (5H, m), together with the physical characters, this indicated the presence of flavonoid glycoside skeleton [75]. Moreover, there were two downfield aromatic signals at δ_C 100.25 and 98.84 ppm with δ_H 6.36 (1H, d, J = 2 Hz, H-6) and 6.72 (1H, d, J = 2 Hz, H-8) corresponding to C-6 and C-8, on the other hand, there was upfield signal at δ_C 159.36 ppm corresponding to C-7, which indicate the attachment of glucose moiety to hydroxyl group of C-7, furthermore, it showed two intense signals at δ_C 129.49 and 114.94 ppm with δ_H 8.02 (2H, d, J = 8.8 Hz, H-2', H-6') and 6.81 (2H, d, J = 8.8 Hz, H-3', H-5') corresponding to para-substituted ring B [74]. From the above-mentioned physical, chemical, and spectral data and by comparison with reported data [82], compound **10** was identified as kaempferol-7-*O*-*β*-D-glucopyranoside, and was previously isolated from the genus *Maclura* [83].

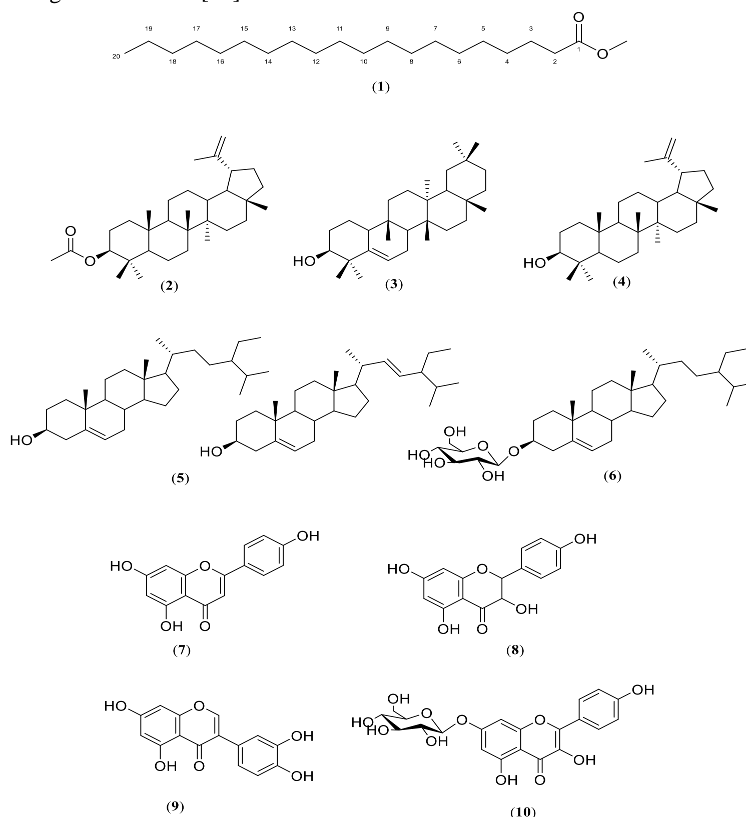

Fig. 1: Chemical structure of isolated compounds from *M. spinosa*

Table 1: Total phenolic content in MSTME

Sample	Average reading at 630 nm	Curve equation (µg GA/mg sample) or (µg GA/mL sample solution)	Total phenolics content (µg GA/mg extract)	SD
MSTME	0.9638	372.56	41.39	1.99

M. spinosa total methanolic extract, MSTME

Table 2: Gallic acid standard absorbance

Concentration (µg/mL)	Absorbance
25	0.044
50	0.107
100	0.231
200	0.493
400	0.986
600	1.584
800	2.144
1000	2.671

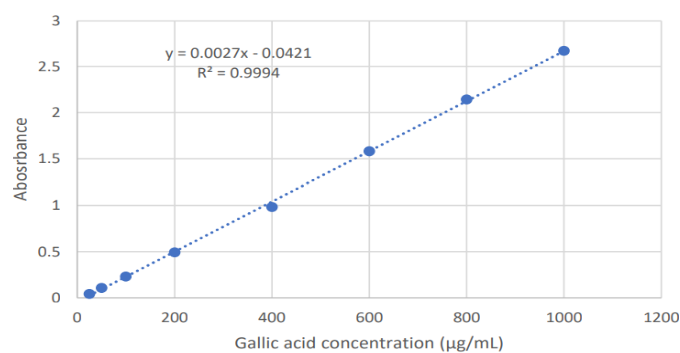

Fig. 2: Gallic acid standard calibration curve

Table 3: Total flavonoid content in MSTME

Sample name	Average reading at 420 nm	Total flavonoids content (µg rutin eq/mg sample)	SD
MSTME	0.175	1.932	0.09

M. spinosa total methanolic extract, MSTME

Table 4: Rutin standard absorbance

Concentration (µg/mL)	Absorbance
5	0.023
10	0.025
20	0.038
40	0.054
80	0.095
100	0.108
120	0.13
160	0.167
200	0.202

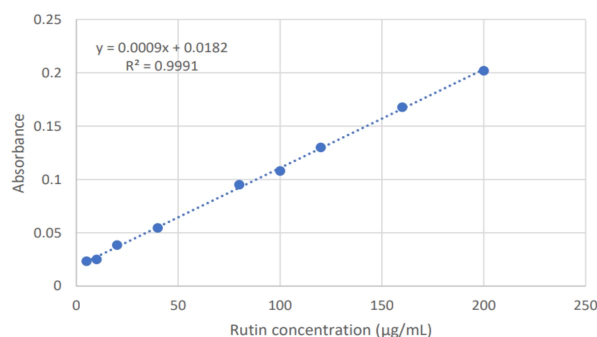

Fig. 3: Rutin standard calibration curv

3.4. GC-MS analysis of saponifiable and unsaponifiable matters of M. spinosa

3.4.1. GC-MS analysis of saponifiable matters

Eight compounds were identified as four saturated (62.22%) and four unsaturated (37.76%) fatty acids. The four saturated fatty acids were identified as palmitic acid (54.17%), stearic acid (6.04%), margaric acid (1.45%) and myristic acid (0.55%), while the four unsaturated fatty acids were determined as linolenic acid (14.15%), linoleic acid (13.17%), oleic acid (8.74%) and palmitoleic acid (1.70%). It was obvious that unsaturated fatty acids were more abundant than saturated fatty acids. For the unsaturated fatty acids, linolenic acid was the major acid followed by linoleic acid, while stearic acid and palmitic acid constitute the main saturated fatty acids, as shown in Table 5 and Fig. S24. The previous study revealed the identification of thirty-five compounds from the leaves of this plant growing in India [1]. However, this study showed high unsaturated fatty

acid content which induced us to study the possible anti-inflammatory activity of the plant fractions to determine the most active one.

3.4.2. GC-MS analysis *of unsaponifiable matter*

Ten compounds were identified as six triterpenes (93.74%), two sterols (3.81%), one sesquiterpene (1.36%), and one diterpene (1.06%). The six triterpenes were identified as lupeol (70.37%), *β*-amyrin (13.04%), lupeol acetate (5.04%), *α*-amyrin (3.32%), 23-(phenylsulfanyl)lanosta-8,24-dien-3-ol) (1.21%), and 9,19-cyclolanost-23-ene-3,25-diol (3*β*,23*E*)- (0.73%). The two sterols were determined as *γ*-sitosterol (2.75%) and stigmasterol (1.06%). Moreover, there was one sesquiterpene and one diterpene compound identified as *β*-chamigrene (1.36%) and 3,3,3',3',5,5,5',5'-octamethyl-bi-1-cyclohexen-1-yl (1.06%), respectively (Table 6 and Fig. S25). From these ten identified compounds there were three major pure compounds were first isolated and identified from this species namely (lupeol, lupeol acetate and stigmasterol), which have previously antidiabetic and anti-inflammatory activities [87, 88].

Table 5: List of identified compounds by GC/MS analysis of saponifiable matters of *M. spinosa*

Peak no.	Compounds	MF	Saturation degree	MW	Rt (min)	Area %	RA %
1	Myristic acid	$C_{14}H_{28}O_2$	C14:0	228.37	22.81	1.02	0.552%
2	Palmitic acid	$C_{16}H_{32}O_2$	C16:0	256.42	28.876	100	54.179%
3	Palmitoleic acid	$C_{16}H_{30}O_2$	C16:1	254.41	29.937	3.14	1.701%
4	Margaric acid	$C_{17}H_{34}O_2$	C17:0	270.50	31.581	2.69	1.457%
5	Stearic acid	$C_{18}H_{36}O_2$	C18:0	284.50	34.388	11.15	6.041%
6	Oleic acid	$C_{18}H_{34}O_2$	C18:1	282.50	35.216	16.14	8.744%
7	Linoleic acid	$C_{18}H_{32}O_2$	C18:2	280.40	36.943	24.31	13.171%
8	Linolenic acid	$C_{18}H_{30}O_2$	C18:3	278.40	39.062	26.12	14.151%

MF, Molecular formula; MW, Molecular weight (g/mol); RT, Retention time; RA, Relative area

Table 6: List of compounds identified by GC/MS analysis of unsaponifiable matters of *M. spinosa*

Peak no.	Compounds	MF	MW	Rt (min)	Area %	RA %
1	Stigmasterol	$C_{29}H_{48}O$	412.7	6.405	1.51	1.062%
2	*γ*-Sitosterol	$C_{29}H_{50}O$	414.7	6.803	3.91	2.751%
3	*β*-Amyrin	$C_{30}H_{50}O$	426.7	7.022	18.54	13.047%
4	Lupeol	$C_{30}H_{50}O$	426.7	7.563	100	70.372%
5	*α*-Amyrin	$C_{30}H_{50}O$	426.7	7.77	4.73	3.328%
6	*β*-Chamigrene	$C_{15}H_{24}$	204.35	7.883	1.94	1.365%
7	3,3,3',3',5,5,5',5'-octamethyl-bi-1-cyclohexen-1-yl	$C_{20}H_{34}$	274.5	7.978	1.52	1.069%
8	Lupeol acetate	$C_{32}H_{52}O_2$	468.8	8.109	7.17	5.045%
9	9,19-Cyclolanost-23-ene-3,25-diol, (3*β*,23*E*)-	$C_{30}H_{50}O_2$	442.7	8.494	1.05	0.738%
10	23-(Phenylsulfanyl) lanosta-8,24-dien-3-Ol	$C_{36}H_{54}OS$	534.9	8.714	1.73	1.217%

MF, Molecular formula; MW, Molecular weight (g/mol); RT, Retention time; RA, Relative area

3.5. Determination of polyphenol by HPLC

Fifteen compounds were identified from MSTME as eight phenolic acid derivatives (81.2%) and seven flavonoids (18.8%). Flavonoids can be classified as two flavanones (8.18%) (naringenin and hesperetin), one flavonoid glycoside (4.88%) (rutin), two flavonol (2.76%) (kaempferol and quercetin), one flavone (2.16%) (apigenin), and one flavanol (0.83) (catechin). The most abundant phenolic acid derivatives were chlorogenic acid (41.81%), gallic acid (16.26%), and caffeic acid (11.41%) as listed in Table 7 and shown in Figure S26. These results reveled that the plant was rich with flavonoids and phenolic components which agreed with the previously published literatures about genus *Maclura* [89]. Apigenin and kaempferol were isolated as major components from EtOAc fraction in agreement with the HPLC analysis, in addition to, the isolation of dihydrokaempferol and orobol flavonoids.

Table 7: List of compounds identified by HPLC analysis for MSTME

No.	Compound name	MF	R_t (min)	Conc. (µg/g)	Area %	RA%
1	Gallic acid	$C_7H_6O_5$	3.44	1991.63	4.89	16.267%
2	Chlorogenic acid	$C_{16}H_{18}O_9$	4.27	7451.22	12.57	41.816%
3	Catechin	$C_{15}H_{14}O_6$	4.68	281.58	0.25	0.831%
4	Caffeic acid	$C_9H_8O_4$	6.06	4791.15	3.43	11.410%
5	Syringic acid	$C_9H_{10}O_5$	6.58	496.13	1.52	5.056%
6	Rutin	$C_{27}H_{30}O_{16}$	7.93	734.91	1.46	4.8856%
7	Ellagic acid	$C_{14}H_6O_8$	8.89	1276.09	0.46	1.530%
8	Coumaric acid	$C_9H_6O_2$	9.28	53.69	0.36	1.197%
9	Ferulic acid	$C_{10}H_{10}O_4$	10.04	254.53	0.86	2.860%
10	Naringenin	$C_{15}H_{12}O_5$	10.44	941.48	1.83	6.087%
11	Quercetin	$C_{15}H_{10}O_7$	12.66	18.46	0.03	0.099%
12	Cinnamic acid	$C_9H_8O_2$	14.00	28.36	0.32	1.064%
13	Apigenin	$C_{15}H_{10}O_5$	14.49	225.07	0.65	2.162%
14	Kaempferol	$C_{15}H_{10}O_6$	15.04	437.85	0.80	2.661%
15	Hesperetin	$C_{16}H_{14}O_6$	15.85	152.96	0.63	2.095%

MF, Molecular formula; RT, Retention time; RA, Relative area

3.6. In-vitro biological studies

3.6.1. Antioxidant activity

The result showed that MSBF exhibited the highest antioxidant activity at 91.56 ± 0.39 followed by MSEF at 90.87 ± 0.03, while MSHF showed the lowest activity in comparison to quercetin and ascorbic acid at the same concentrations as shown in Table 8 and Fig. 4. MSDF, MSEF, and MSTME exhibited almost the same effect at a concentration of 250, 500 and 1000 µg/mL, which indicates the high phenolic and flavonoid content that was confirmed from HPLC and total phenolics and flavonoids analysis.

These results agreed with the traditional use of the plant and previously reported antioxidant activity of *M. spinosa* (*Plecospermum spinosum*) that was determined by DPPH and ABTS radical scavenging assay on total methanolic extract [1]. In this study, MSTME and different fractions were evaluated to determine the most active fraction. The EtOAc fraction showed high antioxidant activity due to flavonoids isolated (apigenin, dihydrokaempferol, orobol and kaempferol glucopyranoside) from this fraction which were previously reported for their antioxidant activity [90-92]. From these results, *M. spinosa* can be used as a natural source of low-cost antioxidants [93].

Table 8: Antioxidant activity of MSTME and different fractions

Fractions	Concentration (µg/mL)				
	62.5	125	250	500	1000
	Inhibition %				
Ascorbic acid	89.17 ± 0.04%	93.72 ± 0.25%	96.25 ± 0.12%	96.74 ± 0.03%	96.76 ± 0.05%
Quercetin	88.99 ± 0.20%	92.62 ± 0.39%	93.17 ± 0.06%	93.79 ± 0.06%	94.12 ± 0.20%
MSTME	32.12 ± 0.15%	57.94 ± 0.24%	87.93 ± 0.04%	87.96 ± 0.05%	88.01 ± 0.06%
MSHF	0.01 ± 0.03%	0.06 ± 0.00%	03.43 ± 0.44%	4.84 ± 3.6%	7.18 ± 0.55%
MSDF	31.19 ± 0.24%	53.64 ± 0.20%	85.56 ± 0.31%	85.24 ± 0.24%	85.18 ± 0.12%
MSEF	28.73 ± 0.53%	52.25 ± 1.96%	90.11 ± 0.54%	90.75 ± 0.04%	90.87 ± 0.03%
MSBF	10.59 ± 0.91%	19.24 ± 1.17%	35.73 ± 0.79%	61.09 ± 0.73%	91.56 ± 0.39%

n-Hexane fraction, MSHF; DCM fraction, MSDF; EtOAc fraction, MSEF; *n*-but. Fraction, MSBF; *M. spinosa* total methanolic extract, MSTME

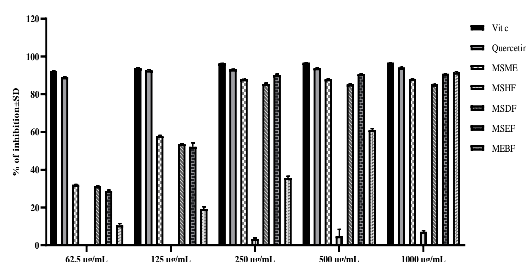

Fig. 4: Antioxidant activity of *n*-hex. (MSHF), DCM (MSDF), EtOAc (MSEF) and *n*-but (MSBF) fractions in addition to *M. spinosa* total methanolic extract (MSTME) against standard

3.6.2. Anti-inflammatory effect on RAW 264.7 mouse macrophage cells

3.6.2.1. Cytotoxicity study

The toxic effect of MSHF, MSDF, MSEF, and MSTME was determined at two concentrations (1 and 10 μg/mL) on RAW 264.7 cells before the anti-inflammatory study to avoid it, as shown in Table 9 and Fig. 5, S27, S28. The percentages of cell viabilities ranged from (90.90 ± 4.49%) to (101.10 ± 0.46%) after 48 h. of treatment except in MSDF at a concentration of 10 μg/mL that showed a significant reduction in cell viability (39.53 ± 2.15%); therefore, all concentrations are convenient except 10 μg/mL of MSDF for studying the possible anti-inflammatory effect of these fractions in RAW 264.7 cells

Table 9: Effect of different plant fractions on RAW 264.7 macrophage cells viability

No.	Fraction	Cell viability%	
		1 μg/mL	10 μg/mL
1	Quercetin	100.43 ± 0.41%	99.13 ± 2.53%
2	MSHF	98.31 ± 0.51%	100.36 ± 0.42%
3	MSDF	99.29 ± 1.52%	39.53 ± 2.15%
4	MSEF	95.71 ± 0.21%	101.10 ± 0.46%
5	MSTME	100.33 ± 0.53%	90.90 ± 4.49%

n-Hexane fraction, MSHF; DCM fraction, MSDF; EtOAc fraction, MSEF; *M. spinosa* total methanolic extract, MSTME

3.6.2.2. Anti-inflammatory study

Nitric oxide (NO) is an important messenger molecule that has critical functions in vascular regulation, host immune defense and neuronal signal transduction [94], it is produced during conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS) in the presence of molecular oxygen and NADPH [95], its concentration reflects the degree of inflammation, thus providing a measure of the inflammatory process [96]. To determine the effects of MSHF. MSDF, MSEF and MSTME on NO production, the cells were treated with different concentrations of extract (1 and 10 μg/ml), followed by stimulation with LPS (100 ng/ml) for 24 h. The release of NO was significantly increased in the LPS-treated group, while the treatment with MSDF, MSEF and MSTME significantly decreased NO in a concentration-dependent manner. The percent of NO inhibition of MSDF, MSEF and MSTME was greater than the quercetin (positive control) in all concentrations (1 and 10 μg/mL). On the other hand, MSHF didn't exhibit any remarkable activity in comparison to the control group as shown in Table 10 and Fig 6, S29, S30. Moreover, the test was not accomplished on a concentration 10 μg/mL of MSDF because of the toxic effect on RAW 264.7 cells.

This promising anti-inflammatory effect of *M. spinosa* fractions may be at least in part, to the synergistic effect of its high sterols, triterpenes, flavonoid and phenolic contents. It was noted that the anti-inflammatory effects induced by plant sterols have been well demonstrated in many *in-vitro* and *in-vivo* studies [97]. The MSDF showed the highest activity at concentration (10 μg/mL), the activity could be attributed to the presence of sterols (β-sitosterol, stigmasterol and β-sitosterol glucopyranoside) which were previously reported for their anti-inflammatory effect [98-100], also, apigenin and kaempferol glucopyranoside were isolated from MSEF, which previously displayed a significant anti-inflammatory activity against LPS-induced human lung A549 cells through the inhibition of iNOS, COX-2, expression of pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, and TNF-α) [101, 102]. Hence, *M. spinosa* is considered a valuable source of potent anti-inflammatory activity with lower side effects.

Table 10: Anti-inflammatory activity of MSTME and different fractions

No.	Fraction	Nitric oxide inhibition %	
		1 μg/mL	10 μg/mL
1	MSHF	1.85 ± 1.30%	11.23 ± 2.18%
2	MSDF	23.17 ± 1.91%	None
3	MSEF	12.60 ± 1.73%	48.46 ± 0.86%
4	MSTME	19.74 ± 1.46%	81.50 ± 0.11%
5	Quercetin	11.56 ± 1.42%	43.90 ± 1.05%

n-Hexane fraction, MSHF; DCM fraction, MSDF; EtOAc fraction, MSEF; *M. spinosa* total methanolic extract, MSTME

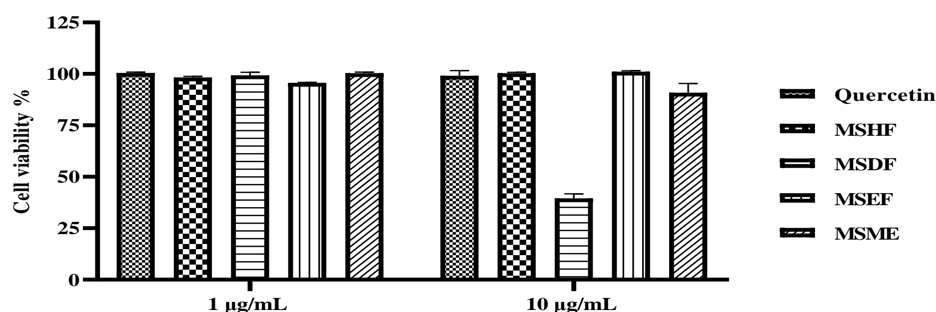

Fig. 5: Cytotoxicity assay of *n*-hex. (MSHF), DCM (MSDF), EtOAc (MSEF) fractions in addition to *M. spinosa* total methanolic extract (MSTME) on RAW 264.7 cells

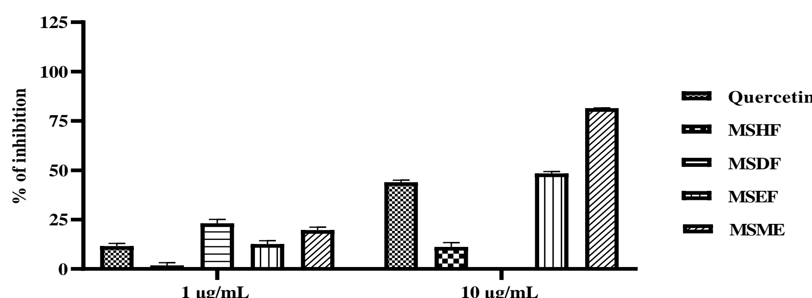

Fig. 6: Anti-inflammatory activity of *n*-hex (MSHF), DCM (MSDF), EtOAc (MSEF) fractions in addition to *M. spinosa* total methanolic extract (MSTME) against quercetin on RAW 264.7 cells

3.7. In-vivo biological studies

3.7.1. Acute toxicity study of MSTME

MSTME was tested before the anti-diabetic experiment to ensure its safety. The result of the acute toxicity study showed that there are no significant changes in the behavior of the animals as observed by lack of convulsions, respiratory distress, changes to reflex activity or mortality up to 5000 mg/kg b.w. after oral administration of MSTME during the first 24 hrs. Therefore, it was considered a safe herbal drug due to its being more than ten times the dose that will be used (300 mg/kg) in antidiabetic experiment.

3.8. Antidiabetic effect of MSTME and different fractions

The diabetic control rats showed a highly significant ($p < 0.001$) elevation in blood glucose level from 99.33 ± 6.65 to 440.0 ± 43.51 mg/dL compared to the normal rats. Oral administration of different plant fractions in addition to the crude methanolic extract (300 mg/kg) leads to a significant ($p < 0.01$) reduction in blood glucose levels in MSDF and MSTME groups in comparison to the diabetic control rats. MSDF was the most considerable fraction which decreased the blood glucose level from 372.3 ± 57.36 to 325.7 ± 11.68 mg/dL, followed by the MSTME which also decreased the blood glucose level from 436.66 ± .22 to 346.0 ± 21.0 mg/dL, this effect was evident till the 10th day. Moreover, the hypoglycemic effect was increased from the 10th day onwards till the 15th day in MSDF only while MSTME was still significant, the MSDF showed a high significant ($p < 0.001$) reduction in blood glucose level from 372.3 ± 57.36 to 223.3 ± 81.93 mg/dL, while, the MSTME still show only a significant ($p < 0.05$) reduction in blood glucose level and decrease it from 436.66 ± .22 to 305.0 ± 21.51 mg/dL in comparison to the diabetic control rats. On the other hand, the MSEF fraction didn't exhibit any significant changes in blood glucose level on the 10th and 15th days as shown in Figure 7. The diabetic group treated with glibenclamide (500 µg/kg, p.o.) showed a highly significant ($p < 0.001$) reduction in blood glucose level, the mean blood glucose level was decreased from 377.3 ± 51.5 to 223.7 ± 67.88 mg/dL till the 10th day and from 377.3 ± 51.5 to 97.0 ± 3.91 mg/dL at the end of the experiment as listed in Table 11.The increase in serum glucose level in STZ-diabetic rats could be due to a concomitant decrease in the serum insulin levels because of pancreatic β-cells necrosis mediated by STZ, which enhances ATP dephosphorylation and results in the consequent generation of superoxide, hydrogen peroxide and hydroxyl radicals. In the present study, the treatment of diabetic rats with MSDF fraction which contains the isolated sterols (β-sitosterol and β-sitosterol glucopyranoside) prevents the development of diabetes. The possible reasons may be due to the increase of insulin secretion through either antioxidant activity or the regeneration of β-cells [103], also, the activity of MSTME could be attributed to the synergistic effect of flavonoids, phenolics, triterpenes and sterols that was identified by HPLC and GC/MS analysis [104-106]. These results provoke us to use *M. spinosa* as a new candidate for development of antidiabetic drug.

Table 11: Effect of MSTME and different fractions on blood glucose level in STZ-induced diabetic rats compared with standard and negative control groups

No.	Tested samples	Blood glucose level (mg/dL ± S.D)/day		
		0 day	10 days	15 days
1	Normal rats	99.33 ± 6.65	98.33 ± 9.07	89.00 ± 13.11
2	Diabetic rats	440.00 ± 43.51	409.70 ± 31.79	390.30 ± 45.61
3	Glibenclamide	377.30 ± 51.5	223.70 ± 67.88***	97.00 ± 3.91***
4	MSDF	372.30 ± 57.36	325.70 ± 11.68**	223.30 ± 81.93***
5	MSEF	394.30 ± 38.81	359.30 ± 11.59	321.00 ± 27.87
6	MSTME	436.66 ± 36.22	346.00 ± 21.00*	305.00 ± 21.51*

Values were expressed as mean ± S.D; $n = 6$, Significant difference (*$P < 0.05$, **$P < 0.01$ *and* ***$P < 0.001$). All doses were 300 mg/kg except glibenclamide 500 µg/kg; DCM fraction, MSDF; EtOAc fraction, MSEF; *M. spinosa* total methanolic extract, MSTME

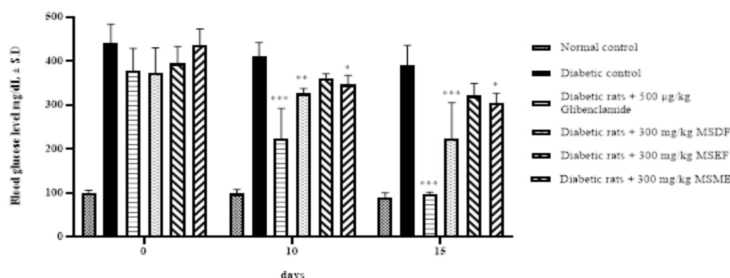

Fig. 7: Antidiabetic effect of daily oral dose 300 mg/kg of DCM (MSDF) and EtOAc (MAEF) fractions in addition to *M. spinosa* total methanolic extract (MSTME) on blood glucose level in STZ-induced diabetic rats. Columns represent mean ± SD. A significant difference (*p <0.05, **p <0.01, ***p <0.001) when compared to diabetic control (one way ANOVA followed by Tukey's test for multiple comparisons)

3.9. Effects of MSTME and different fractions on lipid profile in STZ-induced diabetic rats

Injection of STZ resulted in a significant ($p < 0.01$) elevation in total cholesterol, and triglycerides ($p < 0.001$) level in diabetic control rats compared to normal animals. Administration of MSTME for 15 days showed a significant ($p < 0.05$) reduction in total cholesterol level from 97.33 ± 3.78 to 71 ± 9.29 mg/dL and triglycerides level from 132.33 ± 6.65 to 95 ± 19.69 mg/dL in comparison to STZ-induced diabetic control rats, but the MSDF exhibits a significant ($p < 0.05$) reduction on total cholesterol level only, resulting in decrease the level from 97.33 ± 3.78 to 75.00 ± 16.82 mg/dL as shown in figure 8 and 9. On the other hand MSEF didn't have any effect on both as listed in Table 12.

Table 12: Effect of the MSTME and different fractions on lipid profile in STZ-induced diabetic rats compared with standard and negative control groups

Parameters	Blood lipids level (mg/dL ± S.D)/ on 15th day					
	Normal rats	Diabetic rats	Glibenclamide	MSDF	MSEF	MSTME
Cholesterol	89 ± 7.00	97.33 ± 3.78	82 ± 5.29	75.00 ± 16.82**	83.66 ± 9.29	71 ± 9.29***
Triglycerides	84 ± 13.45	132.33 ± 6.65	96.66 ± 5.85	123 ± 22.06	140.33 ± 4.16	95 ± 19.69**

Values are expressed as mean ± SD; $n = 6$, Significant difference (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). All doses were 300 mg/kg except glibenclamide 500 µg/kg; DCM fraction, MSDF, EtOAc fraction, MSEF; *M. spinosa* total methanolic extract, MSTME

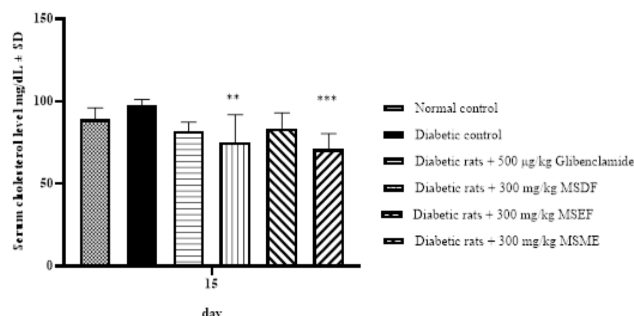

Fig. 8: Effect of DCM (MSDF) and EtOAc (MSEF) fractions in addition to *M. spinosa* total methanolic extract (MSTME) on serum cholesterol level in STZ-induced diabetic rats. Columns represent mean ± SD. A significant difference (*p <0.05, **p <0.01, ***p <0.001) when compared to diabetic control (one-way ANOVA followed by Tukey's test for multiple comparisons)

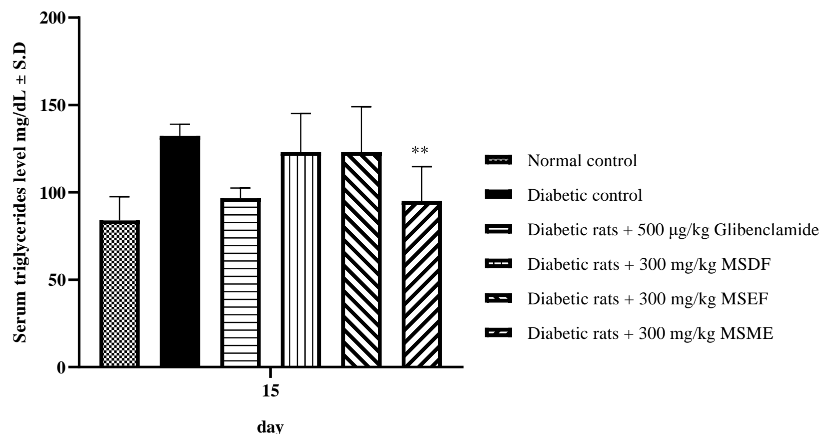

Fig 9: Effect of DCM (MSDF) and EtOAc (MSEF) fractions in addition to *M. spinosa* total methanolic extract (MSTME) on serum triglycerides level in STZ-induced diabetic rats. Columns represent mean ± SD. A significant difference (*p <0.05, **p <0.01, ***p <0.001) when compared to diabetic control (one-way ANOVA followed by Tukey's test for multiple comparisons)

4. Conclusion

The phytochemical study of *M. spinosa* (Willd.) C.C.Berg twigs (aerial parts before flowering stage) afforded ten known compounds that were identified and classified as one fatty acid ester, three triterpenes, two sterols and four flavonoids. Moreover, GC-MS and HPLC analysis led to the identification of nineteen and fifteen compounds, respectively. The biological studies were determined on the *M. spinosa* total methanolic extract and its different fractions, which exhibited potent anti-inflammatory and moderate antioxidant activities in addition to mild antidiabetic effect. Consequently, *M. spinosa* could be considered a promising plant for the development of herbal medicine for the treatment of inflammatory disease and diabetes disorders. The future of work encompasses the isolation and identification of polar fractions, in addition to carrying out the biological study, molecular docking and dynamics of the isolated compounds from the species *M. spinosa.*

5. Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

6. Acknowledgment

I would like to express my hearty appreciation to Dr. Hafeez Rafael for the identification of the plant; Dr. Mohamed E. Abouelela for NMR analysis and providing STZ for antidiabetic study, Dr. Emad H.M. Hassanein for providing a lot of advice in biological studies. Author.

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