ORIGINAL ARTICLE

New acyclic secondary metabolites from the biologically active fraction of *Albizia lebbeck* flowers

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**Abstract** The total extract of *Albizia lebbeck* flowers was examined *in vivo* for its possible hepatoprotective activity in comparison with the standard drug silymarin at two doses. The higher dose expressed promising activity especially in reducing the levels of AST, ALT and bilirubin. Fractionation via liquid–liquid partition and reexamination of the fractions revealed that the *n*-butanol fraction was the best in improving liver biochemical parameters followed by the *n*-hexane fraction. However, serum lipid parameters were best improved with CHCl₃ fraction. The promising biological activity results initiated an intensive chromatographic purification of *A. lebbeck* flowers fractions. Two compounds were identified from natural source for the first time, the acyclic farnesyl sesquiterpene glycoside 1-O-[6-O-α-L-arabinopyranosyl-β-D-glucopyranoside]-2(E,6E)-farnesol (6) and the squalene derivative 2,3-dihydroxy-2,3-dihydrosqualene (9), in addition to eight compounds reported here for the first time from the genus *Albizia*; two benzyl glycosides, benzyl 1-O-β-D-glucopyranoside (1) and benzyl 6-O-α-L-arabinopyranosyl β-D-glucopyranoside (2); three acyclic monoterpene glycosides, linalyl β-D-glucopyranoside (3) and linalyl 6-O-α-L-arabinopyranosyl β-D-glucopyranoside (4); (2E)-3,7-dimethylocta-2,6-dienoate-6-O-α-L-arabinopyranosyl β-D-glucopyranoside (5), two oligoglycosides, *n*-hexyl-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside (creoside) (7) and *n*-octyl α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside (rhodiooctanoside) (8); and ethyl fructofuranoside (10). The structures of the isolated compounds were elucidated based on extensive...
1. Introduction

The genus *Albizia* (Fabaceae), embraces around 150 species, mostly trees and shrubs native to warm areas of Asia and Africa (Migahed, 1996). It is widely cultivated in tropical and subtropical regions as an ornamental plant. A perusal of the literature revealed that plants belonging to genus *Albizia* have great medicinal values. *A. anthelmintica* A. Brogne is used for the treatment of malaria and febrile convulsions (Carpiani et al., 1989; Johns et al., 1994), while the leaves, boiled with water, are given by traditional healers in Dar es Salaam, Tanzania, for the treatment of epilepsy (Moshi et al., 2005). On the other hand, the bark of *Albizia odoratissima* is used to treat cough, bronchitis, rheumatism and diabetes (Kumar et al., 2003; Varshney and Farooq, 1952). Recently, an antitumor triterpene saponin julibroside J28, isolated from the stem bark of *Albizia julibrissin* has displayed significant, *in vitro*, antitumor activity against PC-3M-1E8, Bel-7402 and HeLa cancer cell lines at 10 μM assayed by SRB method (Liang et al., 2005).

*Albizia lebbeck* L. is one of the most common species of *Albizia* worldwide, known by various names such as Indian sirs, flea tree, frywood, koko and Laback in Arabic. The tree was imported to Saudi Arabia from India, years ago, as an ornamental tree, well adapted to the hot environmental conditions of Najd state in the central region of Saudi Arabia (El Gamal et al., 2015).

It is usually flowering, between April and September, with cream-colored fragrant hermaphroditic flowers (Migahed, 1996). The plant is well-known, in traditional folk medicine, for the treatment of various ailments in several areas around the world. In Ayurveda, all parts of the tree including roots, leaves, bark and flowers are used to cure asthma and other inflammatory conditions such as, arthritis and burns (Ayurvedic Pharmacopoeia of India, 2001). In traditional Chinese medicine the flowers are commonly used to treat anxiety, depression and insomnia (Kang et al., 2007). The decoction of the flower in a dose of 50 mg/kg induces muscle relaxation and can protect the guinea pig against histamine-induced bronchospasm (Tripathi and Das, 1977).

In our previous research on *A. lebbeck*, we found that different fractions from the flowers of *A. lebbeck* possessed anti-inflammatory activities (Farag et al., 2013). Our earlier phytochemical study of the alcoholic extract of the flowers led to the isolation of taraxerol triterpenes, ceramide derivatives and two flavonoids, in addition to a novel β-lactam derivative, albacamt, which was also evaluated for platelet anti-aggregatory effect (El Gamal et al., 2015).

In view of the versatile biological activities of this plant and as a part of our continuing interest in identifying biologically active drug leads from natural sources, we conducted this research with the aim of discovering new compounds with hepatoprotective potential.

2. Materials and methods

2.1. General experimental procedures

1H and 13C NMR spectra were recorded at NMR Unit at the College of Pharmacy, Sattam Bin Abdulaziz University on a UltraShield Plus 500 MHz (Bruker) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the internal standard TMS or residual solvent peak, and the coupling constants (J) are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) were obtained using standard Bruker program. Jeol JMS-700 High Resolution Mass Spectrophotometer was used for accurate mass determination. Electron Impact mode with ionization energy of 70 eV was accustomed. Direct probe, temperature ramp setting was used with initial temperature 50 °C; increasing 32 °C per minute and final temperature 350 °C set up, and resolution was adjusted to 10k. Thin layer chromatography (TLC) was performed on pre-coated silica gel F254 plates (E. Merck, Darmstadt, Germany); detection was done at 254 nm and by spraying with p-anisaldehyde/ H2SO4 reagent followed by heating at 110 °C for 1–2 min. Centrifugal preparative thin layer chromatography (CPTLC) was performed on chromatotron (Harrison Research, Palo Alto, California, CA, USA). Plates coated with 2 mm of silica gel 60, 0.04–0.06 mm were used. All solvents used were of analytical grade. Silymarin was purchased from Sigma Aldrich (St. Louis, USA).

2.2. Plant materials

The flowers of *A. lebbeck* Linn. were collected in April, 2011, from Riyadh, kingdom of Saudi Arabia. The plant was identified by Dr. Mohammed Yusuf, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# 16182) has been deposited at the Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.3. Extraction, fractionation and isolation

The air-dried powdered flowers of *A. lebbeck* (1 kg) were extracted with 80% ethanol (3 × 4 L) at room temperature. The pooled extracts were concentrated under reduced pressure using rotatory evaporator to get a dark brownish residue.
Secondary metabolites from fraction of Albizia lebbeck flowers

(250 g), part of which (100 g) was suspended in methanol (400 mL) and filtered to remove the precipitated insoluble part. The dried methanol-soluble fraction (55 g) was dissolved in 40% ethanol and successively partitioned with n-hexane (300 mL x 4), chloroform (300 mL x 4), ethyl acetate (300 mL x 4) and n-butanol (300 mL x 4) to provide the corresponding fractions. The n-hexane fraction (12 g) was subjected to column chromatography on pre-packed silica gel column (35 mm i.d. x 350 mm) using n-hexane-ethyl acetate gradient; collected fractions were examined with TLC and similar fractions were pooled together. The fraction eluted with n-hexane/EtOAc (20/80) was further purified by subjecting to RP-18 column (MeOH–H2O, 65:35) to yield compound 18 column (MeOH–H2O, 65:35) to give compound 18.

The chloroform fraction (13 g) was applied on a silica gel column using CHCl3/MeOH gradient and collected fractions were grouped to obtain four main subfractions: A (3.7 g), B (3.4 g), C (2.6 g) and D (3.3 g). Fraction A was rechromatographed on a RP-18 column (MeOH–H2O, gradient) to afford compounds 1 (8 mg), 2 (4 mg) and 3 (1.6 mg). Fraction B was subjected to purification by chromatotron (8% MeOH/CHCl3), followed by RP-18 column chromatography (MeOH–H2O, 65:35) to yield compound 4 (23 mg). Fraction C was purified by a RP-18 column chromatography (MeOH–H2O, 65:35) to give compound 5 (8 mg), while compound 6 (19 mg) was obtained by RP-18 column (10% MeOH/H2O) of fraction D.

The n-butanol fraction (10 g) was chromatographed on Diaion HP-20 column, using MeOH/H2O gradient to afford two main subfractions (i and ii). Sub fraction i, eluted with 40% MeOH/H2O was further subjected to column chromatography (CHCl3/MeOH, gradient) to afford compounds 1-3 (using 9-15% MeOH/CHCl3 as eluents respectively). Sub fraction ii, eluted with 60% MeOH/H2O was further subjected to RP-18 column chromatography (100 g x 50 cm x 3 cm, MeOH/H2O gradient) to afford compounds 7 and 8 (eluted with 50% and 65% MeOH/H2O respectively).

2.4. Animals

Wistar male albino rats weighing 150–200 g, almost the same age (8–10 weeks), obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, were used. The animals were kept under constant conditions (12/12 h). They had ad libitum access to a dry commercial diet and free access to drinking water (Abdel-Kader et al., 2010). The procedures and experiments used in this research were approved by the Ethics Committee of the College of Pharmacy, King Saud University.

2.5. Hepatoprotective activity

The male albino rats were randomly assigned into four groups (5 animals each). Group IV was divided into eight sub groups of five animals each. Group I received only normal saline and served as control. Groups I-IV received single dose of CCl4 (1.25 mL/kg body weight). Group II received CCl4 treatment only. Group III administered silymarin at a dose of 10 mg/kg p.o. The sub groups of were treated with 200, 400 mg/kg of the total extract, 50 and 100 mg/kg of the different fractions. Treatment started 6 days prior to CCl4 and continued till day seven. After 24 h, following CCl4 administration in day 7, the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture and the serum was separated to evaluate the biochemical parameters (Abdel-kader and Alqasoumi, 2008).

2.6. Determination of AST, ALT, GGT, ALP, bilirubin, cholesterol, triglycerides, HDL, LDL and VLDL levels

Five biochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltranspeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin were estimated, as reflection of liver function, by reported method (Edwards and Bouchier, 1991). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE). TG (Foster and Dunn, 1973), TC (Zlatkis et al., 1953), HDL-C (Burstein et al., 1970), LDL-C and VLDL (Friedwald et al., 1972) were assessed using Roche Diagnostics Kits.

2.7. Determination of non-protein sulphydryl groups (NP-SH), malonaldehyde (MDA) and total protein (TP)

The livers were separately cooled in a beaker immersed in an ice bath. The tissues were homogenized in 0.02 M ethylenediaminetetraacetic acid (EDTA) in a Potter-Elvehjem type C homogenizer.

Homogenate equivalent of 100 mg tissues was used for the measurements. Non-protein sulphydryl groups (NP-SH) and MDA were quantified spectrophotometrically as previously described (Sedlak and Lindsay, 1968).

Total protein was determined according to the method of Lowry et al. (1951) Homogenates were treated with 0.7 mL of Lowry’s solution, mixed and incubated for 20 min in dark at room temperature. Diluted Folin’s reagent (1 mL) was then added and samples were incubated at room temperature in dark for 30 min. The absorbance of the resulted solutions was then measured at 750 nm (Lowry et al., 1951).

2.8. Statistical analyses

Results are expressed as Mean ± Standard Error (SE) of mean. Statistical analysis was achieved, using a one-way analysis of variance (ANOVA). When, the F-value was found statistically significant (p < 0.05), further comparisons among groups were made using Dunnett’s multiple comparisons test. All statistical analyses were performed using SPSS software version 17.0 (Released Aug. 23, 2008), Chicago, USA.

3. Results

Phytochemical study of A. lebbeck flowers resulted in the isolation of ten compounds, benzyl 1-O-β-D-glucopyranoside (1), benzyl6-O-α-L-arabinopyranosyl β-D-glucopyranoside (2), linalyl β-D-glucopyranoside (3), linanal 6-O-α-L-arabinopyranosyl-β-D-glucopyranoside (4), (2E)-3,7-dimethyl-2,6-dienoate-6-O-α-L-arabinopyranosyl-β-D-glucopyranoside (5), 1-O-[6-O-
3.1. The physical and spectral data of compounds 1–10

3.1.1. Benzyl-1-O-β-D-glucopyranoside (1)

C_{13}H_{18}O_{6}, white solid; 1H NMR (500 MHz, MeOD): δ_H 7.43 (br d, H-2/6), 7.33 (br t, H-3/5), 7.28 (m, H-4), 4.93 (dd, J = 11.9 Hz, H-7α), 4.70 (d, J = 11.9 Hz, H-7β), 4.34 (dd, J = 7.5 Hz, H-1'), 3.20 (m, H-2'), 3.35 (m, H-3'), 3.36 (m, H-4'), 3.17 (m, H-5'), 3.80 (dd, J = 11.8, 2.5 Hz, H-6'-a), 3.65 (dd, J = 11.8, 5.6 Hz, H-6'-b); 13C NMR (125 MHz, MeOD): δ_C 139.1 (C-1), 129.4 (C-2/6), 129.4 (C-3/5), 128.8 (C-4), 71.8 (C-7), 103.5 (C-1'), 75.1 (C-2'), 78.0 (C-3'), 71.8 (C-4'), 78.1 (C-5'), 62.8 (C-6'); HREIMS m/z 270.2780 [M^+].

3.1.2. Benzyl-6-O-α-L-arabinopyranosyl β-D-glucopyranoside (2)

C_{13}H_{26}O_{10}, white solid; 1H NMR (500 MHz, MeOD): δ_H 7.43 (br d, H-2/6), 7.33 (br t, H-3/5), 7.28 (m, H-4), 4.93 (d, J = 11.9 Hz, H-7α), 4.70 (d, J = 11.9 Hz, H-7β), 4.35 (d, J = 8.0 Hz, H-1'), 3.22 (t, J = 8.1 Hz, H-2'), 3.42 (d, J = 2.3 Hz, H-3'), 3.43 (m, H-4'), 3.44 (m, H-5'), 4.33 (dd, J = 11.0, 5.7 Hz, H-6'-a), 3.75 (dd, J = 11.0, 5.6 Hz, H-6'-b), 4.34 (d, J = 7.0 Hz, H-1''), 3.59 (dd, J = 9.0, 7.1 Hz, H-2''), 3.64 (dd, J = 9.1, 3.0 Hz, H-3''), 3.95 (m, H-4''), 3.89 (dd, J = 13.0, 2.2 Hz, H-5''a), 3.65 (br d, J = 12.4 Hz, H-5''b); 13C NMR (125 MHz, MeOD): δ_C 139.1 (C-1), 129.4 (C-2/6), 129.3 (C-3/5), 128.9 (C-4), 72.0 (C-7), 103.4 (C-1'), 75.1 (C-2'), 77.9 (C-3'), 71.6 (C-4'), 76.9 (C-5'), 69.6 (C-6'), 105.3 (C-1''), 72.4 (C-2''), 74.2 (C-3''), 69.6 (C-4''), 66.9 (C-5''); HREIMS m/z 402.1526 [M^+].

3.1.3. Linalyl-β-D-glucopyranoside (3)

C_{10}H_{16}O_{5}, white viscous liquid; 1H NMR (500 MHz, MeOD): δ_H 5.17 (d, J = 11.3 Hz, H-1-a), 5.22 (d, J = 18.0 Hz, H-1-b), 6.07 (dd, J = 17.7, 11.0 Hz, H-2), 1.64 (m, H-4), 2.04 (m, H-5), 5.11 (br t, J = 7.0 Hz, H-6), 1.68 (s, H-8), 1.61 (s, H-9), 1.35 (s, H-10), 4.33 (d, J = 7.4 Hz, H-1'), 3.18 (m, H-2'), 3.34 (m, H-3'), 3.36 (m, H-4'), 3.16 (m, H-5'), 3.80 (dd, J = 11.9, 2.3 Hz, H-6'-a), 3.65 (dd, J = 11.9, 5.5 Hz, H-6'-b); 13C NMR (125 MHz, MeOD): δ_C 115.1 (C-1), 144.5 (C-2), 81.5 (C-3), 41.6 (C-4), 23.5 (C-5), 125.8 (C-6), 32.2 (C-7), 23.7 (C-8), 26.0 (C-9), 17.9 (C-10), 99.2 (C-1'), 75.1 (C-2'), 78.2 (C-3'), 71.7 (C-4'), 77.6 (C-5'), 62.9 (C-6'); HREIMS m/z 316.1886 [M^+].

3.1.4. Linalyl-6-O-α-L-arabinopyranosyl-β-D-glucopyranoside (4)

C_{10}H_{20}O_{10}, white solid; 1H NMR (500 MHz, MeOD): δ_H 5.23 (d, J = 11.3 Hz, H-1-a), 5.17 (d, J = 11.3 Hz, H-1-b), 6.09 (dd, J = 17.7, 11.0 Hz, H-2), 1.56 (m, H-4a), 1.62 (m, H-4b), 2.07 (m, H-5a), 2.04 (m, H-5b), 5.11 (br t, J = 7.0 Hz, H-6), 1.68 (s, H-8), 1.61 (s, H-9), 1.35 (s, H-10), 4.35 (d, J = 7.9 Hz, H-1'), 3.20 (t, J = 8.0 Hz, H-2'), 3.40 (m, H-3'), 3.43 (m, H-4'), 3.43 (m, H-5'), 4.33 (dd, J = 11.0, 5.7 Hz, H-6'-a), 3.75 (dd, J = 11.0, 5.5 Hz, H-6'-b), 4.33 (d, J = 7.0 Hz, H-1''), 3.60 (dd, J = 9.0, 7.0 Hz, H-2''), 3.63 (dd, J = 9.1, 3.0 Hz, H-3''), 3.93 (m, H-4''), 3.88 (dd, J = 13.0, 2.2 Hz, H-5''a), 3.66 (br d, J = 12.4 Hz, H-5''b); 13C NMR (125 MHz, MeOD): δ_C Table 2; HREIMS m/z 448.2308.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Chemical structures of the isolated compounds from *A. lebbeck* flowers.
Secondary metabolites from fraction of Albizia lebbeck flowers

3.1.5. (2E)-3,7-dimethylocta-2,6-dienoate-6-O-\textit{z}-arabinopyranosyl-\textit{z}-o-glucopyranoside (5)

C_{19}H_{36}O_{10}; colorless amorphous powder; $^1$H NMR Table 1; $^{13}$C NMR Table 2; HREIMS m/z 426.2101 [M$^+$.]

3.1.6. 1-O-[6-O-\textit{z}-arabinopyranosyl \textit{z}-o-glucopyranoside] – (2E,6-E-)–farnesol (6)

C_{34}H_{46}O_{16} viscous liquid; $^1$H NMR Table 1; $^{13}$C NMR Table 2; HREIMS m/z 516.2934 [M$^+$.]

3.1.7. Hexyl-\textit{z}-arabinopyranosyl-(1 $\rightarrow$ 6)-\textit{z}-o-glucopyranoside (Creoside IV) (7)

C_{13}H_{24}O_{10}; colorless amorphous powder; $^1$H NMR Table 1; $^{13}$C NMR Table 2; HREIMS m/z 396.1995 [M$^+$.]

3.1.8. Octyl-\textit{z}-arabinopyranosyl-(1 $\rightarrow$ 6)-\textit{z}-o-glucopyranoside (rhodiooactanoside) (8)

C_{16}H_{30}O_{10}; white amorphous powder; $^1$H NMR Table 1; $^{13}$C NMR Table 2; HREIMS m/z 424.2308 [M$^+$.]

3.1.9. 2,3-Dihydroxy-2,3-dihydroxyalcanes (9)

C_{38}H_{56}O_{12}; oily; $^1$H NMR (500 MHz, MeOD); $^1$H NMR Table 1; $^{13}$C NMR Table 2; HREIMS m/z 694.3798 [M$^+$.]

Table 2

$^{13}$C NMR data of compounds 1–8 (125 MHz, MeOD).

| 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|-----|-----|-----|-----|-----|-----|-----|-----|
| 139.1 | 139.1 | 115.1 | 115.1 | 166.5 | 66.7 | 71.1 | 71.1 |
| 129.4 | 129.4 | 144.5 | 144.3 | 115.9 | 121.6 | 30.8 | 30.8 |
| 129.3 | 129.3 | 81.5 | 81.5 | 164.5 | 142.4 | 26.8 | 27.1 |
| 128.8 | 128.9 | 41.6 | 41.7 | 42.0 | 40.8 | 32.9 | 30.6 |
| 129.3 | 129.3 | 23.5 | 23.8 | 27.1 | 27.5 | 23.7 | 30.4 |
| 129.4 | 129.4 | 125.8 | 125.8 | 124.1 | 125.2 | 14.4 | 33.0 |
| 71.8 | 72.0 | 132.2 | 132.2 | 133.6 | 136.3 | – | 23.7 |
| 83.5 | – | 26.0 | 26.0 | 29.9 | 40.9 | – | 14.5 |
| 129.1 | – | 17.9 | 17.9 | 19.3 | 125.5 | – | – |
| 129.1 | – | 132.1 | – | – | – | – | – |
| 129.1 | – | 26.1 | – | – | – | – | – |
| 129.1 | – | 19.7 | – | – | – | – | – |
| 129.1 | – | 16.3 | – | – | – | – | – |
| 129.1 | – | 16.8 | – | – | – | – | – |
| 103.3 | 103.4 | 99.2 | 99.3 | 95.2 | 103.0 | 104.4 | 104.4 |
| 75.1 | 75.1 | 75.1 | 75.1 | 77.9 | 75.0 | 75.2 | 75.2 |
| 78.0 | 77.9 | 78.2 | 77.6 | 77.9 | 78.1 | 78.1 | 78.1 |
| 71.8 | 71.6 | 71.7 | 71.7 | 73.9 | 71.5 | 71.7 | 71.7 |
| 78.1 | 76.9 | 76.6 | 76.4 | 71.2 | 76.8 | 76.9 | 76.8 |
| 62.8 | 69.6 | 62.9 | 69.4 | 69.2 | 69.5 | 69.6 | 69.5 |
| 105.3 | – | 104.9 | 104.8 | 105.1 | 105.2 | 105.2 | 105.2 |
| 72.4 | – | 72.5 | 72.4 | 72.4 | 72.5 | 72.5 | 72.5 |
| 74.2 | – | 74.2 | 74.2 | 74.2 | 74.4 | 74.4 | 74.4 |
| 69.6 | – | 69.4 | 69.6 | 69.4 | 69.5 | 69.6 | 69.6 |
| 66.9 | – | 66.4 | 66.8 | 66.8 | 66.8 | 66.8 | 66.8 |

Table 1

$^1$H NMR data of compounds 5–8 (500 MHz, MeOD).

| 5   | 6   | 7     | 8     |
|-----|-----|-------|-------|
| 4.21 (dd, $J = 11.8$, 7.5 Hz) | 4.20 (dd, $J = 12.4$, 7.6 Hz) | 3.83 (m) |
| 4.36 (dd, $J = 9.3$, 6.5 Ha) | 4.35 (dd, $J = 12.4$, 6.9 Hz) | – |
| 5.41 (t, $J = 6.5$ Hz) | 1.61 (m) | 1.61 (m) | – |
| 2.08 (m) | 1.37 (m) | 1.22–1.47 (m) | – |
| 2.16 (m) | 1.34 (m) | – | – |
| 5.14 (m) | 0.91 (t, $J = 6.8$ Hz) | – | – |
| 1.99 (m) | – | – | 0.90 (t, $J = 6.9$ Hz) |
| 2.10 (m) | – | – | – |
| 5.11 (m) | – | – | – |
| 1.69 (s) | – | – | – |
| 1.62 (s) | – | – | – |
| 1.63 (s) | – | – | – |
| 1.72 (s) | – | – | – |
| 4.32 (d, $J = 7.9$ Hz) | 4.26 (d, $J = 8.0$ Hz) | 4.22 (d, $J = 8.0$ Hz) | – |
| 3.23 (t, $J = 8.2$ Hz) | 3.18 (dd, $J = 8.2$, 8.9 Hz), 3.19 (m) | – | – |
| 3.40 (d, $J = 2.3$ Hz) | 3.34 (m) | 3.32 (m) | – |
| 3.42 (m) | 3.34 (m) | 3.32 (m) | – |
| 3.43 (m) | 3.42 (m) | 3.40 (m) | – |
| 4.11 (dd, $J = 11.5$, 1.9 Hz) | 4.09 (brd, $J = 10.0$ Hz) | 4.09 (brd, $J = 11.4$ Hz) | – |
| 3.76 (d, $J = 11.1$, 5.8 Hz) | 3.73 (m) | 3.77 (d, $J = 6.6$ Hz) | – |
| 4.33 (d, $J = 6.8$ Hz) | 4.31 (d, $J = 6.5$ Hz) | 4.31 (d, $J = 6.7$ Hz) | – |
| 3.60 (dd, $J = 9.3$, 7.0 Hz) | 3.59 (m) | 3.62 (m) | – |
| 3.65 (d, $J = 9.3$, 3.1) | 3.53 (m) | 3.53 (m) | – |
| 3.87 (d, $J = 8.2$, 8.7 Hz) | 3.80 (m) | 3.83 (m) | – |
| 3.90 (dd, $J = 12.6$, 2.1 Hz) | 3.85 (m) | 3.83 (m) | – |
| 3.66 (brd, $J = 12.4$ Hz) | 3.52 (m) | – | – |
3.1.10. 4-Ethyl fructofuranoside (10)

C₆H₁₄O₅; viscous liquid; ¹H NMR (500 MHz, MeOD): δ_H 3.90 (m, H-1), 3.70 (dd, J = 2.8, 3.0 Hz, H-2), 4.04 (d, J = 2.8 Hz, H-3), 3.66, 3.90 (dd 2.5, 12.0 CH₂-5), 3.56, 3.77 (m, CH₂-6), 3.57 (m, J = 7.4 Hz CH₂-1), 1.20 (t, J = 7.5 Hz CH₃-2'), 5.15 ¹³C NMR (125 MHz, MeOD); δ_C 84.1 (C-1), 78.6 (C-2), 83.0 (C-3), 109.0 (C-4), 61.5 (C-5), 62.7 (C-6), 57.8 (C-1'), 16.1 (C-2'); HREIMS m/z 208.0947 [M⁺].

4. Discussion

The administration of CCl₄ causes damage of the hepatocytes which is reflected by increase in the biochemical parameter levels such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin levels such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin (Table 3). Liquid–Liquid fractionation of the total extracts revealed that the alcoholic extract at two doses revealed that pretreatment of rats with 400 mg/kg provided protection comparable to that of silymarin. The levels of AST, ALT, GGT and bilirubin were reduced by 52.01%, 33.67%, 29.67% and 48.05% respectively. All results were highly significant (p < 0.001) (Table 3). Liquid–Liquid fractionation of the total extracts with n-hexane, CHCl₃ and n-butanol and evaluation of the hepatoprotective potential of the fractions at 50 and 100 mg/kg revealed that the n-butanol fraction is the most active in reduction of the levels of AST (36.54%), ALT (48.56%), MDA (1.82 ± 0.20) and restoring the levels of NP-SH (4.50 ± 0.19) (p < 0.001 and p < 0.01) (Tables 3 and 5). In the second place in improving these parameters comes the n-hexane fraction (Tables 3 and 5). However, the CHCl₃ fraction was superior in the improvement of blood lipid picture (Table 4). Cholesterol, triglycerides, HDL, LDL and VLDL were improved significantly (p < 0.001) following the treatment of the rats with 100 mg/kg.

Phytochemical analysis of the n-hexane, chloroform and n-butanol fractions obtained from the flowers of A. lebbeck led to the isolation of two benzyl glycosides, benzyl 1-O-β-D-glucopyranoside (1) (De Rosa et al., 1996; Wen et al., 2012) and benzyl 6-O-β-D-arabinopyranosyl β-D-glucopyranoside (2) (Chassagne et al., 1996); three acyclic monoterpenes glycosides, linalyl β-D-glucopyranoside (3) (Moon et al., 1994), linalyl 6-O-β-D-arabinopyranosyl-β-D-glucopyranoside (4) (Chassagne et al., 1996; Pabst et al., 1991) and (2E)-3,7-dimethyl-2,6-dienoate-6-O-β-D-arabinopyranosyl-β-D-glucopyranoside (5) (Yang et al., 2013), an acyclic sesquiterpene glycoside 1-O-[6-O-β-D-arabinopyranosyl-β-D-glucopyranosyl]-[2(E,6E)-farnesol (6) (Magid et al., 2005, 2008) in addition to creoside IV (7) (Nakamura et al., 2008), octyl β-D-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside (rhodioactonane) (8) (Yoshikawa et al., 1996), the squalene derivative, 2,3-dihydroxy-2,3-dihydroxysqualene (9) (Brown and Martens, 1977) and ethyl fructofuranoside (10) (Liu et al., 2012; Lu et al., 2013) which were identified using 1D and 2D-NMR and HREIMS and in correlation with known compounds. The compounds 6 and 9 are reported here for the first time from natural sources. On the other hand and from the chemotaxonomic point of view, the sugar part of the isolated bioside glycosides consists of l-α-arabinopyranosyl(1 → 6)-β-D-glucopyranoside which might be considered as chemotaxonomic marker for A. lebbeck.

It is noteworthy that linalyl β-D-glucopyranoside (3) and linalyl 6-O-β-D-arabinopyranosyl-β-D-glucopyranoside (4) were reported as aroma precursors from Jasminum sambac and passion fruit (Passiflora edulis), respectively (Chassagne et al., 1996; Moon et al., 1994). On the other hand, ethyl fructofuranoside (10) is formed as a by-product by the Wickhamomyces anomala, a yeast strain capable of selective utilization of fructooligosaccharides (FOSs) syrup (Lu et al., 2013).

Compound 6 was obtained as a viscous liquid. HREIMS of compound 6 showed a molecular ion peak at m/z 516.2934 [M⁺] suggesting the molecular formula C₃₆H₅₄O₁₀ and 5 degrees of unsaturation. Careful examination of NMR spectra for compound 6 revealed the presence of a farnesyl type sesquiterpene glycoside. Its ¹³C NMR and DEPT spectrum (Table 1) exhibited 26 carbons including four olefinic methyls, seven methylenes, twelve methines nine of which are oxygenated and three quaternary carbons. The aglycone part was proved to be a farnesyl type sesquiterpene by four olefinic methyls observed in the NMR spectra, at δ_C 121.6, 121.6, 120.5 and 120.5 respectively. Long range coupling was observed between the olefinic protons at δ_H 5.11 ppm (H-10, m) and Me-12 and Me-13; and 5.14 (H-6, m) and Me 14; and 5.41 (H-2, t) with Me-15. The presence of oxymethylene at C-1 was clarified by one pair of two coupled oxygenated protons at δ_H 4.21 ppm (H-1a, dd, J = 11.8, 7.6 Hz), 4.36 ppm (H-1b, dd, J = 9.3, 6.5 Hz), both showing cross peak correlations with the olefinic proton at δ_H 5.41 (H-2, t, J = 6.45 Hz).

Moreover, NMR showed the presence of four upfield methylene protons at δ_H 2.08 (H-4, m), 2.16 (H-5, m), 1.99 (H-8, m) and 2.10 ppm (H-9, m). The above mentioned spectral data together with 15 carbon atoms ascribed by ¹³C NMR and DEPT experiments as 4 methyls, 5 methylenes, three olefinic methines at 16.8 ppm and those for the two methylene at C-4 and C-8 appeared at δ_C 40.8 and 40.9 ppm, respectively closely similar for those reported for (2E,6E)-farnesol (Kasai et al., 1986) while the C-12 resonated at δ_H 20.1 ppm. The chemical structure of the sugar part of the molecule was confirmed to be a bioside of β-D-glucose and z-1-arabinose based on the data obtained from 1D (¹H, ¹³C, and DEPT) experiments and 2D (COSY, HSQC and HMBC). The presence of two anomic protons at δ_H 4.32 and 4.33 ppm with large coupling constants J = 7.9 and 6.8 correlates directly through one bond length in HSQC to their carbons at δ_C 103.1 and 105.1 ppm indicating the presence of β-D-glucose and z-1-arabinose respectively. Additionally two oxymethylene protons appeared at δ_H 3.76

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Table 3 Effect of *A. lebbeck* total extract (Total) and fractions on serum marker enzymes of control and experimental rats.

| Treatment (n = 5) | Biochemical parameters | AST (units/l) % Change | ALT (units/l) % Change | GGT (units/l) % Change | ALP (units/l) % Change | Bilirubin (mg/dl) % Change |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|---------------------------|
| Normal            |                        | 106.01 ± 5.44          | 29.48 ± 1.50           | 3.55 ± 0.27            | 283.00 ± 14.36         | 0.53 ± 0.02                |
| CCl4              |                        | 297.66 ± 10.01***,a    | 192.5 ± 5.70***,a      | 10.90 ± 0.41***,a      | 503.66 ± 14.84***,a    | 2.22 ± 0.16***,a           |
| Silymarin 200 mg  |                        | 119.83 ± 5.68***,b     | 100.68 ± 4.27***,b     | 6.28 ± 0.29***,b       | 372.00 ± 13.03***,b    | 1.08 ± 0.04***,b           |
| Silymarin 400 mg  |                        | 163.16 ± 5.05***,b     | 139.50 ± 3.76***,b     | 8.31 ± 0.30***,b       | 452.50 ± 5.97**,b      | 1.33 ± 0.04***,b           |
| Normal            |                        | 142.83 ± 4.72***,b     | 127.66 ± 3.48***,b     | 7.66 ± 0.22**          | 408.50 ± 7.57**        | 1.15 ± 0.04**              |
| Normal            |                        | 106.85 ± 4.18          | 30.95 ± 1.20           | 4.96 ± 0.19            | 337.66 ± 6.11          | 0.54 ± 0.02                |
| CCl4              |                        | 331.83 ± 9.78***,a     | 288.83 ± 11.01***,a    | 13.40 ± 0.56***,a      | 598.16 ± 8.97***,a     | 2.33 ± 0.09***,a           |
| Silymarin 200 mg  |                        | 149.66 ± 8.04***,b     | 108.63 ± 7.47***,b     | 10.40 ± 0.36**         | 420.50 ± 6.23**        | 1.03 ± 0.05**              |
| Silymarin 400 mg  |                        | 284.66 ± 10.48**       | 240.00 ± 8.61**        | 11.93 ± 0.29**         | 564.33 ± 14.72**       | 2.05 ± 0.13**              |
| n-Hexane fraction 50 mg |                | 212.50 ± 9.37***,b     | 183.83 ± 8.04***,b     | 7.40 ± 0.57**          | 531.33 ± 22.83**       | 1.95 ± 0.10**              |
| n-Hexane fraction 100 mg |               | 101.33 ± 3.08          | 27.36 ± 1.51           | 4.73 ± 0.35            | 326.50 ± 10.55         | 0.53 ± 0.01                |
| Normal            |                        | 148.83 ± 6.97***,b     | 108.51 ± 5.03***,b     | 6.01 ± 0.18**          | 401.50 ± 9.29**        | 0.98 ± 0.04**              |
| CHCl3 fraction 50 mg |                       | 300.83 ± 9.18***,b     | 280.50 ± 7.21**        | 12.31 ± 0.49**         | 596.66 ± 12.58**       | 1.98 ± 0.04**              |
| CHCl3 fraction 100 mg |                      | 273.66 ± 8.89***,b     | 237.16 ± 13.47***,b    | 8.40 ± 0.39**          | 510.33 ± 7.13**        | 1.53 ± 0.07**              |
| Normal            |                        | 109.25 ± 4.25          | 28.40 ± 1.66           | 5.21 ± 0.21            | 312.16 ± 10.36         | 0.54 ± 0.01                |
| CCl4              |                        | 304.66 ± 7.21***,b     | 282.50 ± 8.98***,b     | 11.83 ± 0.60***,a      | 586.83 ± 10.36***,a    | 2.75 ± 0.05***,a           |
| Silymarin 200 mg  |                        | 137.83 ± 3.93***,b     | 107.16 ± 5.16***,b     | 6.53 ± 0.23**          | 401.33 ± 9.69**        | 1.50 ± 0.05**              |
| n-Butanol fraction 50 mg |                   | 223.33 ± 8.94***,b     | 224.66 ± 16.43***,b    | 9.96 ± 0.29**          | 577.50 ± 13.48**       | 2.52 ± 0.08**              |
| n-Butanol fraction 100 mg |                   | 193.33 ± 6.43***,b     | 145.33 ± 4.55***,b     | 8.86 ± 0.45**          | 532.00 ± 11.98***,b    | 2.15 ± 0.09**              |

* p < 0.05.
** p < 0.01.
*** p < 0.001.

a Compared to normal saline group.
b Compared to Carbon tetrachloride group.
Table 4 Effect of *A. lebbeck* fractions on serum lipid metabolism and serum lipoproteins of control and experimental rats.

| Treatment (n = 5) | Cholesterol (mg/dl) | % Change | Triglycerides (mg/dl) | % Change | HDL (mg/dl) | % Change | LDL (mg/dl) | % Change | VLDL (mg/dl) | % Change |
|------------------|---------------------|----------|-----------------------|----------|-------------|----------|-------------|----------|-------------|----------|
| Normal           | 105.16 ± 4.52       |          | 60.83 ± 2.70          |          | 56.06 ± 2.69|          | 36.92 ± 4.76|          | 12.17 ± 0.54|          |
| CCl₄             | 175.66 ± 3.42***,a  |          | 150.33 ± 3.94***,a    |          | 25.78 ± 1.78|          | 119.81 ± 4.83|          | 30.06 ± 0.78***,a|
| Silymarin        | 152.33 ± 5.60**     | 13.28    | 127.83 ± 3.41**      | 14.96    | 38.33 ± 2.40** | 48.68   | 87.83 ± 7.20** | 26.69 | 25.66 ± 0.68** | 14.64 |
| n-Hexane fraction 50 mg | 160.50 ± 6.30**b | –        | 141.00 ± 3.56**b      | –        | 33.38 ± 1.80**b | 29.48   | 98.91 ± 6.81**b | 17.44 | 28.20 ± 0.71**b | –        |
| n-Hexane fraction 100 mg | 155.00 ± 7.15**b | 11.76    | 131.83 ± 4.07**b      | 12.31    | 35.35 ± 2.75**b | 37.12   | 93.38 ± 8.98**b | 21.86 | 26.36 ± 0.81**b | 12.31 |
| Normal           | 103.96 ± 4.93       |          | 68.40 ± 2.04          |          | 56.21 ± 2.08 |          | 34.07 ± 5.48  |          | 13.68 ± 0.40  |          |
| CCl₄             | 189.66 ± 6.76***,a  |          | 170.66 ± 6.11***,a    |          | 26.66 ± 1.02  |          | 128.96 ± 6.20***,a|          | 34.03 ± 1.22***,a|
| Silymarin        | 148.66 ± 6.72**     | 21.61    | 123.66 ± 4.42**      | 27.54    | 42.28 ± 2.05** | 58.58   | 83.41 ± 6.81** | 35.32 | 24.73 ± 0.88** | 27.33 |
| CHCl₃ fraction 50 mg | 165.16 ± 4.47**b | 12.91    | 146.16 ± 4.00**       | 14.36    | 40.51 ± 2.64** | 51.95   | 100.00 ± 4.73** | 22.46 | 29.23 ± 0.80** | 14.11 |
| CHCl₃ fraction 100 mg | 144.33 ± 4.12**b | 23.90    | 117.66 ± 4.91**      | 31.05    | 42.28 ± 1.68** | 58.58   | 78.51 ± 4.58** | 39.12 | 23.53 ± 0.98** | 30.86 |
| Normal           | 100.63 ± 4.32       |          | 70.13 ± 3.61          |          | 58.61 ± 2.79 |          | 28.00 ± 5.32  |          | 14.02 ± 0.72  |          |
| CCl₄             | 196.83 ± 7.42***,a  |          | 172.83 ± 4.32***,a    |          | 23.10 ± 1.44***,a|          | 139.16 ± 6.74***,a|          | 34.56 ± 0.86***,a|
| Silymarin        | 148.66 ± 6.72**     | 24.47    | 123.66 ± 4.42**      | 28.45    | 42.28 ± 2.05** | 83.03   | 83.41 ± 6.81** | 40.06 | 24.73 ± 0.88** | 28.44 |
| n-Butanol fraction 50 mg | 203.00 ± 10.27b | –        | 159.33 ± 3.73**       | 7.81     | 34.03 ± 1.69** | 47.31   | 137.10 ± 10.82** | –     | 31.86 ± 0.74** | 7.81 |
| n-Butanol fraction 100 mg | 188.50 ± 5.07b | –        | 142.50 ± 3.25**       | 17.55    | 41.50 ± 1.80** | 79.65   | 118.49 ± 4.67** | 14.85 | 28.50 ± 0.64** | 17.53 |

All values represent mean ± SEM. ANOVA, followed by Dunnett’s multiple comparison test.

* p < 0.05.
** p < 0.01.
*** p < 0.001.

a – as compared to normal saline group.
b – as compared to Carbon tetrachloride group.
Secondary metabolites from fraction of Albizia lebbeck flowers

Table 5  Effect of A. lebbeck fractions on NP-SH, MDA and TP in rat liver.

| Treatment                  | MDA (nmol/g) | NP-SH (nmol/g) | Total protein (g/l) |
|----------------------------|--------------|----------------|--------------------|
| Normal                     | 0.46 ± 0.04  | 6.01 ± 0.37    | 98.61 ± 6.74       |
| CCl₄                       | 6.37 ± 0.46**| 3.37 ± 0.28**  | 25.04 ± 3.41**     |
| Silymarin                  | 1.89 ± 0.20**| 5.84 ± 0.29**  | 59.16 ± 4.50**     |
| n-Hexane fraction 50 mg    | 5.31 ± 0.42b | 3.92 ± 0.17b   | 34.28 ± 2.95b      |
| n-Hexane fraction 100 mg   | 2.98 ± 0.25**| 4.61 ± 0.28**  | 51.20 ± 5.56**     |
| Normal                     | 0.46 ± 0.03  | 7.19 ± 0.31    | 131.20 ± 9.47      |
| CCl₄                       | 7.64 ± 0.35**| 4.35 ± 0.28**  | 30.20 ± 2.63**     |
| Silymarin                  | 2.02 ± 0.21**| 6.25 ± 0.22**  | 63.53 ± 4.63**     |
| CHCl₃ fraction 50 mg       | 5.09 ± 0.42**| 3.26 ± 0.16**  | 47.98 ± 3.32**     |
| CHCl₃ fraction 100 mg      | 2.87 ± 0.20**| 3.35 ± 0.21**  | 72.85 ± 5.80**     |
| Normal                     | 0.52 ± 0.04  | 7.20 ± 0.38    | 128.44 ± 9.21      |
| CCl₄                       | 5.66 ± 0.54**| 3.61 ± 0.13**  | 38.54 ± 2.82**     |
| Silymarin                  | 1.46 ± 0.19**| 4.99 ± 0.25**  | 94.98 ± 11.06**    |
| n-Butanol fraction 50 mg   | 4.46 ± 0.29**| 4.30 ± 0.32**  | 59.24 ± 4.83**     |
| n-Butanol fraction 100 mg  | 1.82 ± 0.20**| 4.50 ± 0.30**  | 82.19 ± 7.19**     |

All values represent mean ± SEM. ANOVA, followed by Dunnett’s multiple comparison test.

- $p < 0.05$
- $**p < 0.01$
- $***p < 0.001$

- $\alpha$ – as compared to Normal saline group.
- $\beta$ – as compared to Carbon tetrachloride group.

(\(dd, J = 11.1, 5.8\) Hz), \(4.11 (dd, J = 11.5, 1.9 \) Hz) and \(\delta_H 3.90\) (\(dd, J = 12.6, 2.1 \) Hz), \(3.66 (brd, J = 12.4 \) Hz) assigned for Glc-6 and Ara-5. The remaining protons and carbons data for \(\beta\)-d-glucopyranose and \(\alpha\)-l-arabinofuranose were matched with those reported (Nakamura et al., 2007) and with the glycosides isolated from the currently studied plant (compounds 5-8) (Nakamura et al., 2008; Yang et al., 2013; Yoshikawa et al., 1996). The linkage between the two sugar moieties was found to be (1→6) by clear downfield shift of C-6 of \(\beta\)-d-glucose (\(\delta_C 69.5 \) ppm) and significant three bond correlations, observed in HMBC spectrum, from the protons at \(\delta_H 3.76, 4.11 \) ppm (H-6’a and H-6’b) and the anomeric carbon of arabinose resonating at \(\delta_C 105.1 \) ppm. On the other hand, the linkage, between the farnesyl and the sugar moiety, was proved to be at C-1 of the aglycone by a significant downfield shift of C-1 of the farnesol (\(\delta_C 66.7 \) ppm) as well as the three bond correlation, observed in HMBC experiment, between H-1 (\(\delta_H 4.21, 4.36 \) ppm) and the \(\beta\)-d-glucose anomeric carbon at \(\delta_C 103.0 \) ppm and the above aforementioned data prove compound 6 to be 1-O-[6-O-\(\alpha\)-l-arabinopyranosyl \(\beta\)-d-glucopyranoside]-[2,6,6E]-farnesol. It is worth to note that crenulatoside derivatives (A-G) have been isolated before from *Guioa crenulata* (Magid et al., 2005).

Based on collective data, compound 9 (2,3-dihydroxy-2,3-dihydroxyisouquinoline), is identified here from natural source for the first time. However, it was previously synthesized (Brown and Martens, 1977) and also used as precursor in the synthesis of squalene oxides (D’Accolti et al., 2005).

This study proved that *A. lebbeck* is a rich source of terpenoid compounds, where we isolate mono, sesqui and triterpenes derivatives. Moreover the sugar part of the isolated biosides (compounds 4-8) consists of \(\alpha\)-l-arabinopyranosyl (1→6)\(\beta\)-d-glucopyranoside and this finding is considered as a chemotaxonomic marker for genus *Albizia*.

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