



## **Saponins from the Pericarp of *Albizia lebbek* (Benth) L. and Their Biological Screening on Laboratory Animals**

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### **Authors' contributions**

This work was carried out in collaboration between both authors. Author NS designed the study, wrote the protocol, performed the spectroscopic analysis, wrote the manuscript and revised it's the final. Author BE carried out the biological experiment and shared in the final revision. Both authors read and approved the final manuscript.

### **Article Information**

DOI: 10.9734/JPRI/2021/v33i28B31553

#### Editor(s):

(1) Dr. Paola Angelini, University of Perugia, Italy.

#### Reviewers:

(1) Chaouche Tarik Mohammed, University Abu Bekr Belkaid, Algeria.

(2) Vishwanadham Yerragunta, Osmania University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/68285>

**Original Research Article**

**Received 06 March 2021**

**Accepted 11 May 2021**

**Published 14 May 2021**

### **ABSTRACT**

**Aim:** *Albizia lebbek* L. is widely used to treat many diseases. Saponins are one of the major constituents that are found in the tissues of the tree. The purpose of this study was aimed at the phytochemical and biological investigation of the saponins of the pericarp.

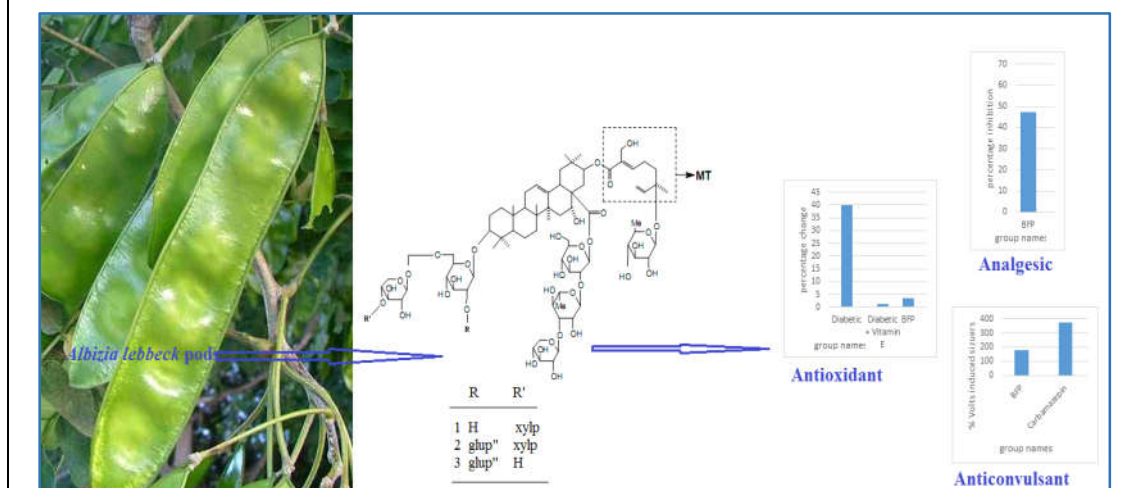
**Methodology:** 50% ethanol extract of the dried powdered pericarp of *A. lebbek* was fractionated with different organic solvents and examined for their oxidative stress radical, anticonvulsant and analgesic effects. The highly potent fraction was subjected to chromatographic and spectroscopic analysis of its saponins.

**Results:** The n-butanol fraction expressed the best results regarding the biological activities. Chromatographic and spectroscopic analysis of this fraction revealed the identification of three oleanane-type saponins.

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**Conclusion:** The present study constituted the first phytochemical analysis of the bioactive saponins content in the pericarp of *A. lebeck*.

### Graphical Abstract



**Keywords:** *Albizialebeck*; HPLC; LC-MS/MS; NMR; saponins.

## 1. INTRODUCTION

Genus *Albizia* family Fabaceae consist mainly of deciduous woody trees. Saponins in genus *Albizia* are triterpenoidal type [1-3], and are biologically active as antitumor, immunomodulatory, antimutagenic [4] and spermicidal [5]. *Albizia lebeck* L. is recognized as an ornamental plant in Egypt and a potent herbal remedy [6]. Its saponins are actively well known for their antipyretic, analgesic [7], nootropic and anxiolytic [8] activities.

In continuation to our studies on *A. lebeck* tree [9,10], the biological activity was performed on the 50% ethanol extract and its fractions of the pericarp. A detailed phytochemical investigation of the highly potent saponins in *n*-butanol fraction was presented here; structures were based mainly on NMR and LC-MSn (n=2).

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Pericarps of *A. lebeck* L. were harvested from trees grown in Agricultural Research Center (A.R.C), Egypt, during the year of 2019. Dr. Abd El Hafez, (A.R.C), verified the taxonomical identity. A plant sample (A-123) was kept at the Herbarium, Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

### 2.2 Techniques

A Finnigan LCQ Advantage ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), connected to the Agilent 1100 HPLC instrument via ESI interface for the LC-MS/MS-ESI analysis of saponins. HPLC conditions; column, Phenomenex – An Aqua C18 125 A, 150 x 3 mm; detector, UV at 250 nm; flow rate, 1.0 mL/min; injection volume, 20  $\mu$ L; mobile phase, acetonitrile/ water (50:50 v/v). ESI-MS conditions in the negative ion mode were: ions spray voltage, 4.5 kv; sheath gas, 60 arbitrary units; auxiliary gas, 15 units; capillary voltage, 5 V; capillary temperature, 320°C. The isolation width of precursor ions was 3.0 units. A Varian Unity Inova-600 (600 MHz for  $^1\text{H}$  and 150 MHz for  $^{13}\text{C}$ ) was used for NMR spectra in pyridine, with TMS (tetramethylsilane) as internal standard. IR spectra were taken out on a Shimadzu –IR-435 spectrophotometer. TLC, silica gel 60 F254 plates; elution system, *n*-butanol-acetic acid-water (4:1:5 v/v/v) (S1) (saponins) and toluene-ethyl acetate (5:5 v/v) (S2) (sapogenin), spots were detected by *p*-anisaldehyde and heated at 105°C.

### 2.3 Solvents

Acetonitrile (HPLC grade, Merck), deionized H<sub>2</sub>O was treated with pure aqua RC655. Solvents and chemical reagents were of analytical grade (BDH).

## 2.4 Standards and Reagents

Vitamin E, glacial acetic acid and carbamazepine were kindly supplied from the NRC, Giza, Egypt.

## 2.5 Extraction

50% ethanol was used to extract air-dried and defatted (using petroleum ether) powdered pericarp (700 g) of *Albizia lebbek* L. to give 71.5 g (10.1%) dry residue. A part of the residue was dissolved in water and portioned in succession with organic solvents. The dry yield was 15.7 g chloroform (CFP), 4.4 g ethyl acetate (EAFF) and 4.1 g butanol (BFP).

## 2.6 Sample for the Biology

The test samples (2 g, each) were dissolved separately in distilled water (20%, w/v) with tween 80 (few drops).

## 2.7 Animals

Male albino rats (130-150 g) of Sprague Dawley strain and albino mice (25-30 g) were employed in this study. They were fed a standard laboratory diet, and kept under standardized conditions with water given ad lib.

## 2.8 Preliminary Toxicity

LD<sub>50</sub> of 50% ethanol extract, CFP, EAFF and BFP were estimated based on OECD guidelines (2001) [11].

## 2.9 Antioxidant Activity

Glutathione in the blood was evaluated according to Beutler et al., (1963) [12]. Adult male albino rats (130-150 g) were split into seven groups (6 rats/group). Group 1 (control), while diabetes mellitus was induced in the remaining groups using a single intraperitoneal injection of 150 mg alloxan /kg b. wt, followed by fasting overnight [13]. Group 2 of diabetic rats were left untreated, group 3 received vitamin E (7.5 mg /kg b. wt.), and the groups (4-7) received orally 50% alcoholic extract, CFP, EAFF and BFP respectively, (100 mg / kg b. wt. each). The glutathione determination in blood was carried out after one week.

## 2.10 Analgesic Activity

The acetic acid-induced writhing test was used to assess the analgesic activity. Five groups (6

mice/group). Group 1 (control) received 1ml saline orally, the groups (2-5) of mice received 50% alcoholic extract, CFP, EAFF and BFP respectively, orally one hour later (100 mg/kg b. wt., each); 0.6% acetic acid was administered intraperitoneally (0.2 ml/mice) in the groups. Mice were then individually placed in a clear plastic observational chamber, and the number of writhes/30 min was counted for each mouse.

## 2.11 Anticonvulsant Activity

This activity was carried out, using Frequently Pulse Shock Current apparatus. Male albino mice (25-30 g) were split into six groups (6 mice/group). Group 1 (control) and was given 1ml saline orally, the groups (2-5) received the test samples. All doses were taken orally one hour before the experiment (100 mg/kg b. wt., each). Group 6 received carbamazepine. The stimulus duration was 0.2 second, the end-point was tonic hind limb extension, the maximum electric shock needed to emit a cry was determined.

## 2.12 Phytochemical Work

BFP (2 g/200 ml methanol) was dissolved in 5 times of its volume acetone where a precipitate was formed, isolated by centrifuging and purified by repeated dissolution and precipitation to give 250 mg dry powder. A portion of the powder (200 mg) was dissolved in 50% aqueous acetonitrile (20 ml) and injected in preparative HPLC, at 25°C. Fractions relevant to each peak were collected and concentrated to afford compounds: **1**(40 mg), **2** (38 mg) and **3**(35 mg).

- 1: White powder; R<sub>f</sub> = 0.45; IR (KBr): 3300, 2930, 2830, 1718, 1700, 1550 cm<sup>-1</sup>.
- 2: White powder; R<sub>f</sub> = 0.38; IR (KBr): 3330, 2945, 2703, 1744, 1346, 1304 cm<sup>-1</sup>.
- 3: White powder; R<sub>f</sub> = 0.21; IR (KBr): 3348, 2900, 2803, 1775, 1712, 1500, 1413, 1336, 1302 cm<sup>-1</sup>.

NMR of compounds **1-3**(Tables 1 & 2).

Compounds **1-3** (8-10 mg, each) were separately refluxed with 2N HCl in 50% methanol (10 ml) for 2 h. The aglycone was extracted with chloroform and subjected to TLC (S1) and spectral analysis. The sugars were liberated after neutralization with Ag<sub>2</sub>CO<sub>3</sub> and identified by comparison with authentic using GC [14].

### 3. RESULTS AND DISCUSSION

#### 3.1 Biological Analysis

Toxicity study revealed that oral administration of the test samples in dose up to 1.3 g/ kg. Blood glutathione (Fig. 1) was restored in diabetic animals following oral administration of the test samples. The BFP showed a significant antioxidant activity (3.57%). It may be attributed to the presence of compounds can produce stable free radicals and thus inhibit the auto-oxidation of co-existing substances acting as biological antioxidants [14]. The 50% alcoholic extract induced significant analgesic effect (Fig. 2), as it increased numbers of abdominal constrictions of mice by 61.09% followed by the BFP that increased the constrictions by 47.28%.

Peripheral analgesic agents are known to specifically produce a constriction response of abdomen due to acetic acid. Prostaglandins are believed to underlie this response [15]. The 50% extract and BFP resulted in antinociceptive activity indicating that they may contain analgesic components which could be involved in modulation of the prostaglandin pathway.

50% alcoholic extract as well as the BFP and EAFP induced significant anticonvulsant effects (Fig. 3) through the increase of the % protection by: 241.66%,179.16% and 145.83, respectively. This activity may be attributed to fractions that resulted in an elevated level of serotonin and gamma-aminobutyric acid (GABA). In particular, these fractions exhibited a central nervous system depressant action [16].

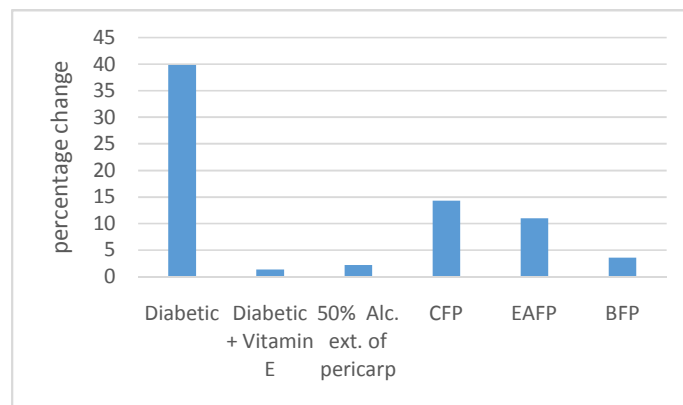


Fig. 1. Antioxidant activity of the test samples of *A. lebbeck* L. and vitamin E in male albino rats (n=6)

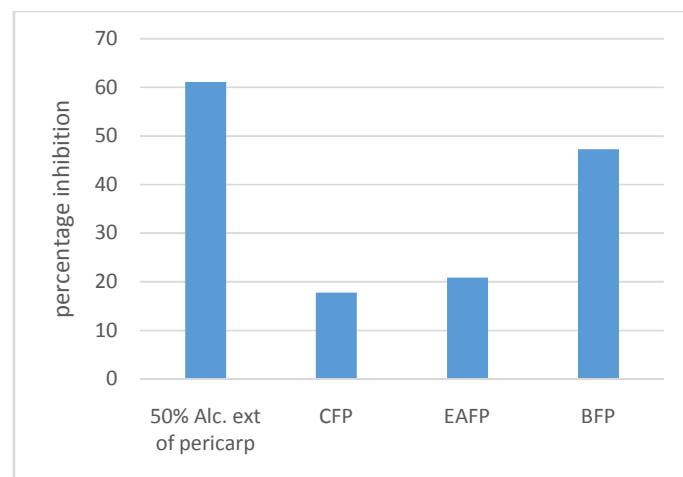
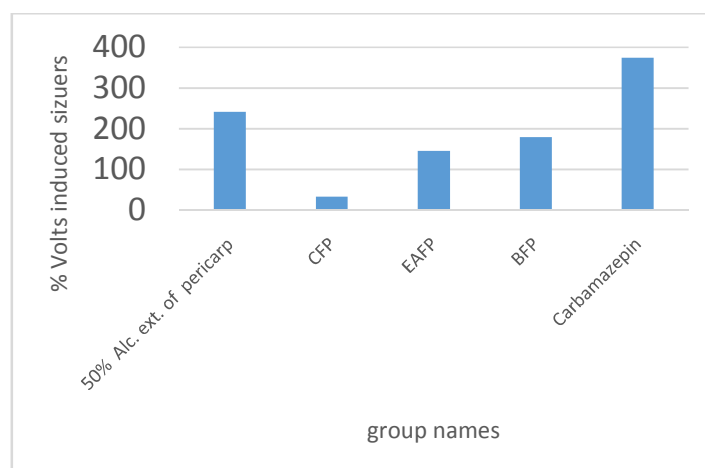


Fig. 2. Effect of the test samples of *Albizzia lebbeck* L. and glacial acetic acid on numbers of abdominal constrictions in mice (n=6)



**Fig. 3. Anticonvulsant activity of the test samples of the pericarp of *A. lebbeck* L. and carbamazepine in mice (n=6)**

### 3.2 Phytochemical Investigation

The significant results of the BFP encouraged the authors for its phytochemical investigation. Previous work discussed saponins isolated from different tissues of *A. lebbeck* of echinocystic acid [3], acacic acid lactone [1] and oleanolic acid [2] aglycones. In the present study, the saponins were isolated by preparative HPLC. Their acid hydrolysis gave oneaglycone; TLC (S2) showed one violet spot at  $R_f = 0.48$  and its EIMS gave  $M^+$  at 470 ( $C_{30}H_{46}O_4$ ), and fragments at  $m/z$  452 ( $-H_2O$ ), 263 and 244.  $^1H$ NMR data (Table 1) were identical to that of acacic acid lactone [17].

Gas chromatography of the sugars alongside with authenticals revealed the glucose, rhamnose, arabinose, xylose and quinovose.

LC-ESI-MS/MS (Fig. 4) of the saponin mixture showed (MS2,  $[M-H]^-$ ) at  $m/z$  469 due to the ( $-$  oligosaccharides) and ions at  $m/z$  243 and at  $m/z$  206.7 relevant to the aglycone. LC-ESI-MS of peak 1 ( $R_t = 5.03$ ) displayed a quasi-molecular ion peak at  $m/z$  1661.72  $[M - H]^-$ , consistent with a molecular composition of  $C_{79}H_{122}O_{37}$ . The fragments: A at  $m/z$   $[M-H-328]^-$  ( $-MT$  linked to methyl pentose), B at  $m/z$  1530  $[M-H-132]^-$  ( $-$  terminal pentose), C at  $m/z$  1398  $[M-H-264]^-$  ( $-$  two pentoses one of them was terminal) and D at 1235  $[M-H-426]^-$  ( $-$  an oligosaccharide "a"). The fragments:  $m/z$  1090  $[1222.4-132]^-$ ,  $m/z$  958  $[1222.4-132-132]^-$ ,  $m/z$  796  $[1222.4-132-132-162]^-$  (successive loss of two pentoses and one hexose of "a"). E at  $m/z$  1384  $[M-H-278]^-$  ( $-$  pentose attached to a methyl hexose) while, F

ion at  $m/z$  1222.54  $[M-H-440]^-$  ( $-$  oligosaccharide "b"). LC-ESI-MS of peak 2 ( $R_t = 6.00$ ) displayed an ion peak at  $m/z$  1824.6 calculated for  $C_{85}H_{132}O_{42}$  and is more than 1 with 162 mu (terminal hexose). Fragments: B at  $m/z$  1692.58, C at  $m/z$  1560 and D at  $m/z$  1236.61  $[M - H - 588]^-$  ( $-$  oligosaccharide "a"), A at  $m/z$  1496, E at  $m/z$  1546, F at  $m/z$  1384, G at  $m/z$  1662.60  $[M - H - 162]^-$ . Peak 3 ( $R_t = 7.37$ ) has an ion peak at  $m/z$  1691.50 calculated for  $C_{80}H_{124}O_{38}$  and a fragment at  $m/z$  1560.55 ( $-$  terminal pentose at C-3). Structure elucidation of the isolated compounds was achieved by extensive spectroscopic mass and 1D- and 2D-NMR. The coupling constants indicated  $\beta$ -d-configuration for glup, xylp and quinp and  $\alpha$ -l-configuration for rhap and arap [18]. D-configuration for glup, xylp and quip and L-configuration for rhap and arap were assumed, according to those present in plant glycosides [19]. The sugars were identified as three pentoses: two  $\beta$ -xylopyranosyl (xylp, xylp') and one  $\alpha$ -arabinopyranosyl (arap); two deoxy sugars:  $\alpha$ -rhamnopyranosyl (rhap) and  $\beta$ -quinovopyranosyl (quinp); two  $\beta$ -glucopyranosyl units (glup, glup').  $^1H$ -NMR spectrum (Table 1) of compound 1 showed seven anomeric proton signals at  $\delta_H$  5.83(s, H-rhap-1), 5.52(d, J = 7.7 Hz, H- glup'-1), 5.42(d, J = 7.9 Hz, H-arap-1), 4.91(d, J = 7 Hz, H-xylp'-1), 4.87(d, J = 7.6 Hz, H-quip-1), 4.83(d, J = 6.6 Hz, H-glup-1), 4.72(d, J = 6.8 Hz, H-xylp-1), two methyl signals of the two deoxysugars at  $\delta_H$  1.09(d, J = 6.1 Hz), and 1.47(d, J = 6.2 Hz). The anomeric protons are correlated in the HSQC spectrum with  $^{13}C$ -NMR signals at:  $\delta_C$  104.6 (arap-1), 102.0 (glup-1), 101.0 (xylp'-1), 100.3 (xylp-1), 99.5 (rhap-1), 97.7

(quip-1), 92.2 (glup'-1) and the methyls of the two deoxy sugars showed signals at  $\delta_C$  18.0 (rhap-6) and 17.5 (quip-6) [17].

$^{13}\text{C}$ -NMR spectrum (Tables 1&2) of the aglycone showed signals at  $\delta_C$  166.8 (carbonyl ester),  $\delta_C$  142.4, 115.0 (a monosubstituted C=C),  $\delta_C$  130.2, 142.0 (a trisubstituted C=C) which corresponded to the monoterpene moiety (MT) and  $\delta_C$  123.5, 141.4 (trisubstituted C=C) of the oleanane (C-12). NMR spectra in Tables 1 and 2 and results of COSY, TOCSY, HSQC, and HMBC NMR proved a  $3\beta$ ,  $16\alpha$ ,  $21\beta$ -trihydroxyolean-12-en-28-oic acid aglycone [20]. The interglycosidic linkages were figured out through the long range HMBC correlations. The downfield and upfield shifts at  $\delta_C$  89.5 (C-3) and  $\delta_C$  171.4 (C-28) showed HMBC correlation to the sugar positions H-glup-1 and H-glup'-1 at  $\delta_H$  4.83(d, J =6.6 Hz) and  $\delta_H$  5.52( d, J =7.7Hz), respectively; also the shift at  $\delta_C$  79.4 (MT-C-6) and the anomeric protons of quip at  $\delta_H$  4.87(d, J=7.6 Hz) confirmed the glycosidic linkage of the suggested tridesmosidic glycoside (17) with an ester link at C-21 that appeared as downfield shift at  $\delta_C$  78.7 and proved by HMBC correlations to MT-C-1 at  $\delta_C$  166.8 [21].  $^{13}\text{C}$ NMR chemical shifts suggested the connections C-arap-1, C-arap-2, C-xylp-1, C-xylp'-1, to other sugars at  $\delta_C$  104.6, 81.4, 100.3, 101.0; the connection of C-rhap-4 at  $\delta_C$  81.4 and C-glup-6 and C-glup'-2, at  $\delta_C$  69.2 and 81.5, respectively, indicated their attachments [19]. HMBC spectrum identified the H and C signals correlation of the oligosaccharides. H-arap-1 / C- glup-6 at  $\delta_H$  (d, 5.42, J =7.9 Hz) /  $\delta_C$  69.2, H-

xylp-1/ C-arap-2 at  $\delta_H$  4.72(d, J= 6.8 Hz)/  $\delta_C$  81.4 forming: xylose (1→2) arabinose (1→6) glucose of the oligosaccharide "a" [21]. H-rhap-1 / C- glup'-2 at  $\delta$  5.83s /  $\delta_C$  81.5, H-xylp'-1 / C-rhap-4 at  $\delta_H$  4.91(d, J=7Hz) /  $\delta_C$  84.4 forming: xylose (1→4) rhamnose (1→2) glucose of the oligosaccharide "b" [22]. Compound 1 is a jilibrosides analogue [4, 23] the similarity lies in the aglycone structure with one MT group (with a monosaccharide at C-6) attached to the C-21, and differs in the oligosaccharides at the positions C-3[xylose (1→4) rhamnose (1→2) glucose] and C-28 [xylose (1→4) rhamnose (1→2) glucose] of the triterpene.

Compound 2 (Table 1) is an analogue to 1 with the difference of the presence of the terminal glup" in the oligosaccharide "a" at C-3.  $^1\text{H}$ - NMR showed its anomeric proton at  $\delta_H$  5.38 (d, J=7.8Hz) which correlated in the HSQC spectrum with  $^{13}\text{C}$ -NMR spectrum of C-1 at  $\delta_C$  101.7. The HMBC suggested the attachment between H-glup"-1 / C-glup- 2 at  $\delta_H$  5.38 /  $\delta_C$  83.4.

NMR spectrum of 3 (Tables 1 & 2) showed a structure close to 1 and 2; their C-arap-2 showed more downfield shifts ( $\delta_C$  81.4 and 81.1, respectively) for the sugar substitutions than in 3 which appeared at  $\delta_C$ 72.6 indicating its terminal position. The structure of "a" fragment is superimposable to that of the oligosaccharide chain attached to C-1 of kinmoonosides isolated from *Acacia concinna* [20].

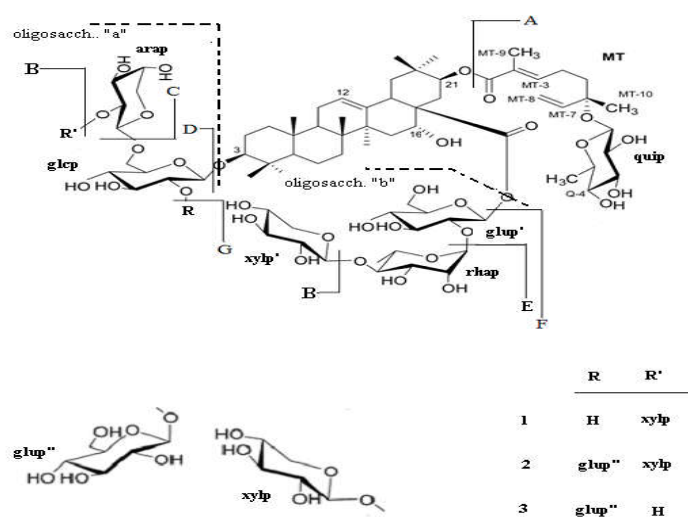


Fig. 4. Fragmentations of the saponins

Table 1. NMR data of the aglycone

Position	1 <sup>b</sup>		2 <sup>b</sup>		3 <sup>b</sup>	
	$\delta C$	$\delta H(J Hz)$	$\delta C$	$\delta H(J Hz)$	$\delta C$	$\delta H(J Hz)$
CH <sub>2</sub> 1	38.2	1.55m	39.0	1.53m	38.1	1.56m
CH <sub>2</sub> 2	27.0	1.79m	27.1	1.78m	27.0	1.70m
CH 3	89.5	3.35m	89.0	3.30m	89.2	3.33m
C 4	41.6	—	41.5	—	41.8	—
CH 5	56.1	0.80d (10.0)	56.4	0.87d (10.0)	56.5	0.87d (10.0)
CH <sub>2</sub> 6	18.0	1.50m	18.8	1.50m	18.6	1.50m
CH <sub>2</sub> 7	33.3	1.39m	33.7	1.37m	33.0	1.39m
C 8	40.1	—	41.4	—	41.0	—
CH 9	47.2	1.65m	47.8	1.66m	48.4	1.66m
C 10	36.0	—	36.4	—	36.5	—
CH <sub>2</sub> 11	23.4	2.21m	23.0	2.18m	23.8	2.20m
CH 12	123.5	5.57m	123.0	5.54m	123.5	5.54m
C 13	141.4	—	141.3	—	141.3	—
C 14	41.6	—	41.4	—	41.5	—
CH <sub>2</sub> 15	35.1	1.50 m	34.2	1.52m	34.1	1.50m
CH 16	73.4	5.07brS	73.4	5.10 brS	73.0	5.12 brS
C 17	51.3	—	51.0	—	51.0	—
CH 18	40.6	2.98m	41.0	2.94m	41.2	2.96m
CH <sub>2</sub> 19	47.8	2.58dd (12,11) 1.75(11,5.4)	48.3	2.57dd (12.4,11) 1.85 (12, 5.5)	48.5	2.59dd (12,10.5) 1.80 (12.5,5.0)
C 20	35.6	—	35.7	—	35.2	—
CH 21	78.7	5.41dd (10.4,5.4)	78.4	5.43dd(11.0, 5.0)	78.4	5.40dd (10.8,5.0)
CH <sub>2</sub> 22	35.3	2.07m,2.64 (10.4,5.0)	35.5	2.10m2.64(11.0,4.8)	35.0	2.102.68(10.5,4.8)
Me 23	28.0	1.16 s	28.2	1.14 s	28.2	1.12 s
Me 24	16.5	0.91s	17.1	0.91 s	16.8	0.91 s
Me 25	16.0	0.74s	16.2	0.79 s	16.2	0.75 s
Me 26	17.0	0.92 s	17.5	0.92s	17.1	0.92 s
Me 27	27.7	1.65 s	27.8	1.68 s	27.7	1.68 s
C 28	171.4	—	171.0	—	171.6	—
Me 29	28.9	0.94 s	28.6	0.94 s	29.0	0.94 s
Me 30	18.8	1.03s	18.4	1.02s	18.0	1.03s

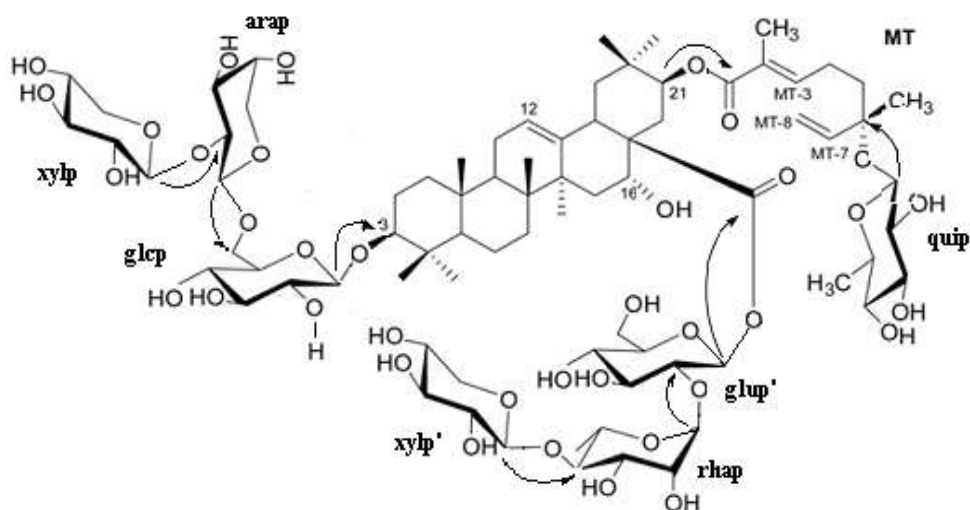


Fig. 5. Important HMBC correlations of Compound 2



Table 2. NMR of MT and sugars of compounds 1-3<sup>a,b</sup>

	1		2		3	
	$\delta C$	$\delta H(J Hz)$	$\delta C$	$\delta H(J Hz)$	$\delta C$	$\delta H (J Hz)$
MT-	166.8	-	166.6	-	165.5	-
-2	130.2	-	131.2	-	130.1	-
-3	142.0	7.00t (7.3)	141.8	7.03t (7.3)	144.2	7.00t (7.3)
-4	22.8	2.30m	22.2	2.31m	22.5	2.32m
-5	40.8	1.70m	40.1	1.74m	40.8	1.71m
-6	79.4	-	82.1	-	81.3	-
-7	142.4	6.66dd (17.7, 11.0)	141.8	6.67dd (17.7, 11.0)	142.3	6.65dd (17.7, 11.0)
-8	115.0	5.12 dd (17.7, 0.8)	114.4	5.10dd (17.7, 1.1)	114.7	5.07dd (17.7, 1.1)
-9	12.0	4.34s	13.5	4.25s	12.4	4.44s
-10	22.4	1.36 s	23.1	1.35s	23.4	1.33 s
quip	97.7	4.87d(7.6)	98.0	4.87d(7.8)	97.5	4.86d(8.0)
-2	75.2	3.63 dd(7.8, 8.2)	75.4	3.64 dd(7.6, 8.1)	76.6	3.60 dd(7.8, 8.1)
-3	75.0	4.28m	75.2	4.18m	76.4	4.23m
-4	74.0	5.25m	73.5	5.22m	78.1	5.22m
-5	69.4	3.64m	70.1	3.44m	69.7	3.63m
-6	17.5	1.09d(6.1)	17.2	1.12d (6.0)	17.2	1.09d (6.2)
glup	102.0	4.83d (6.6)	103.2	4.80d (6.6)	106.5	4.76d (6.6)
-2	78.8	4.02dd(7,8.5)	83.4	4.02dd(7,8.5)	82.2	4.06(7.1,8.7)
-3	78.0	3.78m	78.1	3.55m	78.3	3.65m
-4	72.0	3.82m	72.4	3.42m	72.4	3.42m
-5	78.2	3.98m	78.4	3.94m	79.0	3.92m
-6	69.2	4.23m,4.52m	71.2	4.23m,4.53m	68.2	4.23m,4.55m
glup <sup>n</sup>	-	-	101.7	5.38d(7.8)	102.3	5.46d(7.8)
-2	-	-	79.7	3.90m	80.1	3.79m
-3	-	-	77.5	3.85m	77.3	3.58m
-4	-	-	71.5	4.02m	71.4	4.02m
-5	-	-	79.2	3.93m	78.8	3.93m
-6	-	-	61.4	4.01m,4.2m	62.8	4.05m,4.2m
xylp	100.3	4.72d(6.8)	100.2	4.82d(6.8)	-	-
-2	74.7	4.02m	75.8	4.00m	-	-
-3	78.8	4.08m	77.5	4.08m	-	-
-4	70.0	4.02m	70.8	4.14m	-	-
-5	66.0	3.57dd(10.4,11.7)/4.65m	66.2	3.32dd(10.0,10.8)/4.61m	-	-
arap	104.6	5.42d(7.9)	104.3	5.41d (7.9)	105	5.42d (7.9)
-2	81.4	4.40	80.1	4.43	72.6	4.43
-3	72.1	4.35	72.4	4.31	73.1	4.32
-4	70.4	4.02	70.3	4.12	70.0	4.15
-5	65.4	3.56, 4.65m	64.8	3.53, 4.65m	65.0	3.52, 4.61m
glup <sup>o</sup>	92.2	5.52d (7.7)	93.4	5.50d(7.7)	94.8	5.51d(7.7)
-2	81.5	3.80m	79.1	3.83m	81.2	3.81m
-3	78.4	3.96m	78.2	3.92m	78.0	3.94m
-4	70.4	4.28m	71.0	4.28m	70.4	4.29m
-5	78.5	3.60m	78.2	3.50m	78.2	3.64m
-6	68.2	4.00m/4.19m	68.1	4.00m/4.18m	68.4	4.00m/4.19m
rhap	99.5	5.83br s	99.5	5.80 br s	99.3	5.80 br s
-2	71.4	5.28br s	72.2	5.29 br s	71.1	5.26 br s
-3	73.4	4.62 dd (3.9,9.2)	73.5	4.72 dd(3.4,8.9)	73.0	4.69 dd(3.5,9.0)
-4	81.4	4.69 dd(8.6, 9.4)	81.9	4.64 dd(8.3,9.2)	82.4	4.68 dd(8.7, 9.6)
-5	67.5	4.41m	69.2	4.51m	69.2	4.56 m
-6	18.0	1.47d(6.2)	18.4	1.54 d(6.2)	17.5	1.46d(6.2)
xylp <sup>r</sup>	101.0	4.91d(7)	102.3	4.95d(7)	104.1	4.86d(7)
-2	76.1	4.42m	76.0	4.42m	77.4	4.40
-3	79.2	4.41m	79.0	4.41m	78.2	4.41m
-4	70.2	4.42m	70.8	4.41m	70.1	4.41m
-5	66.3	3.52dd(8.9,10.0), 4.65m	66.0	3.52dd(8.9,10.0), 4.65m	67.6	3.51dd(8.9,10.0), 4.61m

<sup>a,b</sup>Assignments of the sugars based on HSQC-TOCSY and <sup>13</sup>C NMR.



#### 4. CONCLUSION

This study constitutes an investigation of the saponins in the pericarp of *A. lebbeck*. They proved to have a valuable antioxidant, analgesic and anticonvulsant activities; through examination of the *n*-butanol fraction, containing them and having a complex structure of an acacic acid aglycone with oligosaccharide substitutions at C-3 and C-28 and by acyl groups at C-21. They are different from the Albiziahexosides previously isolated from leaves and the by-product of its degradation reaction albiatrioside A [2], Albiziasaponins isolated from the bark [1] or echinocystic acid glycosides isolated from the seeds [3] of *A. lebbeck*. To our knowledge, this is the first investigation of saponins identification from the pericarp of *A. lebbeck*.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

The animal study was approved by the Research Ethics Committee, Faculty of Pharmacy, King Abdulaziz University, approval number PH-1442-74.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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