A novel β-lactam derivative, albactam from the flowers of *Albizia lebbeck* with platelets anti-aggregatory activity *in vitro*

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**Abstract:** A novel β-lactam derivative, albactam, was isolated from the alcoholic extract of the flowers of *Albizia lebbeck*. It showed a significant anti-aggregatory activity against adenosine diphosphate and arachidonic acid induced guinea-pigs' platelets aggregation *in vitro*. Six more known compounds were also isolated and fully characterized by measuring 1D and 2D NMR, two of them are the triterpenes β- amyrin and 11α, 12α-oxidotaraxerol, two ceramide derivatives and two flavonoids, kampferol 3-O-rutinoside and rutin.

**Keywords:** Albactam, *Albizia lebbeck*, flavonoids, platelet anti-aggregatory effect, triterpenes.

**INTRODUCTION**

The genus *Albizia* (Fabaceae) includes approximately 150 species; most of them are trees and shrubs inherent to tropical and subtropical regions of Asia and Africa. *Albizia lebbeck* is among the most important tree species that were imported many years ago from India and well adapted to the harsh environmental conditions of the central part of Saudi Arabia (Migahid, 1989).

In Indian traditional folk medicine *Albizia lebbeck* is used to treat several inflammatory illnesses such as asthma, arthritis and burns (Ayurvedic Pharmacopoeia of India, 2001). *A. lebbeck* inhibited the passive cutaneous anaphylaxis and mast cell degranulation in rat. Moreover, it could protect the sensitized guinea pig from antigen-induced anoxic convolution. Recently, it was found that the alcoholic extract of the plant has antihistaminic property, either direct antihistaminic effect or due to corticotrophic action as evidenced by increasing cortisol levels in plasma (Babu et al., 2009). It is also reported in Indian folk medicine that *A. lebbeck* has antiseptic, antisyndromic and anti-tubercular activities (Ayurvedic Pharmacopoeia of India, 2001). In addition, saponins isolated from *A. lebbeck* have been claimed to be useful in treatment of Alzheimer's and Parkinson's diseases (Sanjay, 2003). Moreover, in traditional Chinese medicine the flowers are used to treat anxiety, depression and insomnia (Kang et al., 2007). In previous study, we evaluate some of the biological activities of *A. lebbeck* flowers such as antipyretic, analgesic, estrogenic and anti-inflammatory activities of different fractions (Farag, 2013).

In the current study we report the isolation, characterization and investigation of the platelets anti-aggregatory activity *in vitro* of a novel four-membered β-lactam derivative, albactam, along with identification of six known metabolites.

The structures of known compounds were elucidated by comparing their chromatographic profile and NMR spectra with those of published data and were identified as β- Amyrin (Abbas et al., 2009; Tanaka and Matsumaga, 1989) 2; 11α, 12α-oxidotaraxerol (Ibrahim and Ali, 2007) 3; [(2S, 3S, 4R, 8E)-2-[(2’R)-hydroxyhexadecanoylaminol]-8-tetra-cosene-1, 3, 4-triol (Kang et al., 2007) 4; 1-O-β-D-glucopyranosyl- [(2S, 3S, 4R, 8E)-2-[(2’R)-hydroxyhexadecanoylaminol]-8-tetra-cosene-1, 3, 4-triol (Kang et al., 2007) 5; kaempferol-3-O-rutinoside (Song et al., 2007) 6 and rutin (Aderogba et al., 2006) 7. It is worthy to note that this is the first isolation of compound 3 from genus *Albizia*.

**MATERIALS AND METHODS**

**General experimental procedure**

Melting points were uncorrected and measured using Thermo system FP800 Metler apparatus. Ultraviolet absorption spectra were obtained in spectroscopic methanol on a Unicum Heyios UV -Visible Spectrophotometer. IR spectra (cm\(^{-1}\)) were recorded in KBr discs using Perkin-Elmer, FTIR, model 1600 spectrophotometer, USA. \(^{1}H\) and \(^{13}C\) NMR spectra were recorded on a Bruker AM 500 spectrometer (Germany) operating at 500 MHz (\(^{1}HNMR\)) and 125 MHz (\(^{13}CNMR\)) in spectroscopic grade CDCl\(_3\), CD\(_3\)OD, pyridine-\(_d_6\) or DMSO-\(_d_6\) (Central Lab., at the College of Pharmacy, KSU). Standard pulse sequences were used for generating 2D NMR spectra (COSY, HSQC and HMBC). The EIMS were obtained on a solid probe using Shimadzu QP-class-
500. HPLC (Agilent 1200) connected to mass detector (Agilent 6410 QQQ) was also utilized. Normal phase silica gel 60F254, 230-400 mesh, RP-18 (E. Merck, Germany) and Diaion (HP-20) were used for column chromatography. MPLC column, RP-18, Lobar 310-25 Lichroprep RP (40-53 µm) was also used.

**Plant material**
The air-dried flowers of *A. lebbeck* were collected from Riyadh district, Saudi Arabia in spring 2008. The identity of the plant was kindly authenticated by Professor Dr. Ahmad Alfarhan, Department of Botany, College of Science, King Saud University. A voucher specimen was placed at the Pharmacognosy Department, College of pharmacy, King Saud University.

**Extraction and isolation**
The air-dried flowers were crushed to a coarse powder. A sample of 700gm was soaked in 70% ethanol for 3 days with occasional shaking at room temperature. This process was repeated four times until complete exhaustion. The alcoholic extract was then concentrated to dryness at 40°C using a rotary vacuum evaporator. The crude dried alcoholic extract (95gm) was then liquefied in water-alcohol mixture (20: 80) and subjected to successive solvent fractionation with ethyl acetate in a separating funnel to give two parts; ethyl acetate soluble part (I, 11gm) and ethyl acetate insoluble part (II, 29gm). 10 Gm of fraction I was loaded onto the top of silica gel packed column (550gm silica gel, 150 x 4cm). Elution with dichloromethane-methanol-water (70:30:3) and similar fractions were added together. Upon concentration, a subfraction from I afforded grayish white amorphous powder of 1(15mg). Subfraction 129-141 from fraction F was separated on MPLC (RP-18) column using water-methanol gradient to give yellow needle crystals of 6 (45mg), While subfractions 155-165 eluted with dichloromethane-methanol-water (65:35:3.5) afforded yellow amorphous powder of 7(30mg).

**The anti-aggregatory activity**
Platelet-rich plasma (PRP) was obtained from guinea-pigs and prepared for aggregation studies (Mahato et al., 1983). Albino guinea-pigs (350-450g) were anaesthetized with diethyl ether. Nine mls of blood were collected using cardiac puncture into 12ml plastic centrifuge tubes each containing 1ml of 3.6-3.8% aqueous sodium tricitrate solution. Blood was mixed gently and centrifuged at 1000 rpm for 10min. The platelet-rich plasma (PRP) was aspirated and distributed in 1ml plastic cuvettes. An aliquot of the PRP was centrifuged at 14330rpm for 20 min to precipitate all platelets to get platelet-poor plasma (PPP).

Each cuvette containing PRP was inserted into a chronologaggregometer that was calibrated such that light transmission through PRP was zero and through PPP was 100%. Each cuvette was heated (37°C) with stirring (1000 rpm) for 2min. Then different doses of compound 1 in volumes of (5-20µl) were added to the PRP and their ability to aggregate the platelets was assessed. Thereafter the ability of compound 1 to inhibit chemically-induced aggregation was examined. For this purpose, aggregation was induced by adenosine diphosphate (ADP) (10µM) and arachidonic acid (0.5-1µM) and each one was added to the aliquots of PRP. The concentration of the agonist that produced just irreversible aggregation was selected. PRP was then treated with various concentrations of compound 1 for 2-5 min. Then the aggregating agent was added and allowed to react with the platelets for 4-5 min. The percentage change induced by the treatment on the agonist -induced aggregation was evaluated (El-Tahir, 2007).

**RESULTS**
Seven compounds were isolated from the alcoholic extract of *A. Lebbeck*. The structure of compounds 1-7 are presented in fig. 1 and their structures were assigned by different spectroscopic methods. The anti-aggregatory activity of compound 1 was studied.

**Structure elucidation**

1: 2a,3-dihydronaphtho[2,3-b]azet-2(1H)-one Albactam
C_{11}H_{9}ON; Pale yellow fine needles. M.P: 142-143 °C. UV \( \lambda_{\text{max}} \) (MeOH): 224, 273, 280, 289 (nm). IR (KBr): \( \nu \) (cm\(^{-1}\)) = 3408 (N-H), 3010 (=C-H), 1680 (C=O), 1605, 1504 (C=C). \(^1\)H NMR (500MHz, CD3OD), \( (\delta \text{ppm}) \): 7.60 (d, \( J = 8.0 \text{Hz}, 1\text{H}, \text{H-9} \)), 7.34 (d, \( J =6.5 \text{Hz}, 1\text{H}, \text{H-6} \)), 7.19 (s, 1H, H-4), 7.09 (t, \( J =7.0 \text{Hz}, 1\text{H}, \text{H-8} \)), 7.02 (t, \( J =7.5 \text{Hz}, 1\text{H}, \text{H-7} \)), 3.77 (q, \( J =9.5, 3.5 \text{Hz}, 1\text{H}, \text{H-2} \)), 3.14 (q, \( J =15.5, 9.5 \text{Hz}, 1\text{H}, \text{H-1a} \)), 3.49 (dd, \( J =15.5, 3.5 \text{Hz}, 1\text{H}, \text{H-1b} \)). \(^{13}\)C NMR (125 MHz, CD3OD), \( \delta \): 28.4 (CH2, C-1), 56.7 (CH, C-2), 109.6 (C, C-3), 125.2 (CH, C-4), 128.5 (C, C-5), 112.5 (CH, C-6), 120.1 (CH, C-7), 122.8 (CH, C-8), 119.4 (CH, C-9), 138.4 (C, C-10), 174.5 (CH, C-2'). ESI-Ion(m/z): 193 [M+Na]+, 171 [presence of one N-atom, M]+, 157 [M-NH]+, 117 [M-C3HNO]+, 105 [M-C3HNO]+, 91 [tropolium ion]+, 79 [benzonium ion]+.

2: \( \beta \)-Amyrin

C_{29}H_{48}O; White crystalline needles from acetone; M.P: 193-195 °C. IR (KBr): \( \nu \) (cm\(^{-1}\)) = 3460 (O-H), 3030 (vinyl C-H), 1625 (C=C), 1065 (C-O). \(^1\)H NMR (500MHz, CDCl3), \( (\delta \text{ppm}) \): 5.12 (t, \( J =8.5 \text{Hz}, 1\text{H}, \text{H-12} \)), 3.15 (dd, \( J =11.5, 5.0 \text{Hz}, 1\text{H}, \text{H-3} \)), 1.93 (t, \( J =5.0 \text{Hz}, 2\text{H}, \text{H-16} \)), 1.90 (t, \( J =3.5 \text{Hz}, 1\text{H}, \text{H-18} \)), 1.80 (m, 1H, H-11), 1.70 (t, \( J =5.0 \text{Hz},2\text{H}, \text{H-15} \)), 1.60 (d, \( J =3.5 \text{Hz}, 2\text{H}, \text{H-19} \)), 1.54 (m, 2H, H-2), 1.45 (t, \( J =4.0 \text{Hz}, 1\text{H}, \text{H-9} \)), 1.41 (t, \( J =4.0 \text{Hz}, 2\text{H}, \text{H-1} \)), 1.39 (t, \( J =5.0 \text{Hz}, 2\text{H}, \text{H-22} \)), 1.33 (m, 2H, H-6), 1.32 (m, 2H, H-21), 1.29 (t, \( J =5.5 \text{Hz}, 2\text{H}, \text{H-7} \)), 1.27 (m, 1H, H-5), 1.07 (s, 3H, Me-27), 0.93 (s, 3H, Me-28), 0.90 (s, 3H, Me-26), 0.87 (s, 3H, Me-24), 0.80 (s, 3H, Me-29), 0.80 (s, 3H, Me-30), 0.76 (s, 3H, Me-23), 0.72 (s, 3H, H-25); \(^{13}\)C NMR (125 MHz, CDCl 3), \( \delta \): 38.8 (CH2, C-1), 27.3(CH2, C-2), 79.0 (CH, C-3), 39.8 (C, C-4), 55.2 (CH, C-5), 18.4 (CH2, C-6), 32.7 (CH2, C-7), 38.6 (C, C-8), 47.7 (CH, C-9), 37.2 (C, C-10), 23.5 (CH2, C-11), 121.8 (CH, C-12), 145.2 (C, C-13), 41.7 (C, C-14), 26.2 (CH2, C-15), 26.9 (CH2, C-16), 32.5 (C, C-17), 47.3 (CH, C-18), 46.9 (CH2, C-19), 31.1 (C, C-20), 34.8 (CH2, C-21), 36.9 (CH2, C-22), 28.1 (CH3, C-23), 15.2 (CH3, C-24), 15.1 (CH3, C-25), 16.8 (CH3, C-26), 25.9 (CH3, C-27), 28.4 (CH3, C-28), 33.3 (CH3, C-29), 23.7 (CH3, C-30). EIMS (m/z): 498 [M+TMSi]+, 483 [M-CH3], 408 [M-TMSiOH], 393 [M-TMSiOH and CH3], 279 [M-C*DE rings], 218 [M-TMSiOH and CH3], 205 [M-ABC rings and C-11 moiety], 203 [M-ABC* rings and C-28 moiety]. These data are in good agreement with that reported for \( \beta \)-Amyrin (2) (Abbas et al., 2009; Tanaka and Matsunaga, 1989).

Fig. 1: Structures of compounds 1-7
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3: 11α, 12α-oxidotaraxerol

C_{30}H_{48}O_{2}; White crystalline needles; M.P:425-427°C. IR (KBr) ν (cm\(^{-1}\)) =3510 (O-H), 3030 (vinyl C-H), 1630 (C=C), 1150 (C-O). \(^1\)H NMR (500 MHz, CDCl\(_3\)); (δppm):5.57 (dd, \(J=8.5, 3.5\), 1H, H-15), 3.26 (dd, \(J=11.5, 5.5\) Hz, 1H, H-3), 3.14 (t, \(J=10.5, 5.5\) Hz, 1H, H-11), 2.82 (d, \(J=5.5\)Hz, 1H, H-12), 2.11 (t, \(J=7\), 1H, H-19b), 2.0 (d, \(J=3.0\) Hz, 1H, H-1a), 1.90 (t, 1H, H-22b), 1.72 (d, \(J=5.0\) Hz, 1H, H-9), 1.70 (m, 1H, H-1b), 1.69 (m, 2H, H-2), 1.37 (d, \(J=4.0\)Hz, 1H, H-19a), 1.33 (m, 2H, H-7), 1.28 (m, 2H, H-6), 1.25 (d, \(J=3.0\) Hz, 2H, H-21), 1.23 (m, 1H, H-22a), 1.21 (s, 1H, H-18), 1.18 (d, \(J=3.5\), 1H, H-16), 1.10 (s, 3H, H-26), 1.10 (s, 3H, H-27), 1.02 (s, 3H, -29H), 1.02 (s, 3H, H-30), 0.99 (s, 3H, H-23), 0.89 (s, 3H, H-25), 0.85 (d, \(J=2.0\)Hz, 3H, H-24), 0.85 (s, 3H, H-28), 0.76 (d, \(J=2.0\) Hz, 1H, H-1). \(^13\)C NMR (125MHz, CDCl\(_3\)); δ: 38.3 (CH\(_2\), C-1), 26.9 (CH\(_2\), C-2), 78.9 (CH, C-3), 38.7 (C, C-4), 54.6 (CH, C-5), 18.9 (CH\(_2\), C-6), 33.2 (CH\(_2\), C-7), 38.9 (CH, C-8), 51.9 (CH, C-9), 37.5 (C, C-10), 53.7 (CH, C-11), 58.3 (CH, C-12), 36.6 (C, C-13), 157.2 (C, C-14), 118.9 (CH, C-15), 35.3 (CH\(_2\), C-16), 35.4 (C, C-17), 48.1 (CH, C-18), 40.4 (CH\(_2\), C-19), 28.7 (CH\(_2\), C-20), 36.6 (CH\(_2\), C-21), 38.2 (CH\(_2\), C-22), 27.9 (CH\(_3\), C-23), 16.9 (CH\(_3\), C-24), 15.4 (CH\(_3\), C-25), 27.0 (CH\(_3\), C-26), 30.2 (CH\(_3\), C-27), 29.9 (CH\(_3\), C-28), 33.6 (CH\(_3\), C-29), 19.5 (CH\(_3\), C-30). EIMS (m/z): 440 [M]\(^+\), 422 [M-H\(_2\)O], 404 [M-H\(_2\)O], 299 [RDA]. These data are in good agreement with that reported for 11α, 12α-oxidotaraxerol (3) (Ibrahim and Ali, 2007).

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**Fig. 3: HMBC correlations of compound 1**
Fig. 4A: Platelet anti-aggregatory effect of ADP on PRB.

Fig. 4B: Platelet anti-aggregatory effect of compound 1 and ADP on PRB.

Fig. 4C: Platelet anti-aggregatory effect of arachidonic on PRB.
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**Fig. 4D**: Platelet anti-aggregatory effect of compound 1 and arachidonic on PRB.

4: [(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol

C_{40}H_{79}O_{5}; White fine needles from alcohol; M.P: 141-142°C; IR (KBr) ν(cm⁻¹) = 3200-3500 (-OH & –NH), 3100 (olefinic C–H), 1650 (C=O). 1H NMR (500MHz, Pyr-d5): (δ ppm): 8.57 (d, J=9.0Hz, 1H, NH), 5.52 (dt, J=15.5, 5.5Hz, 1H, H-8), 5.11 (m, 1H, H-2), 4.62 (dd, J=3.5, 7.5Hz, 1H, H-2'), 4.5 (dd, J=10.5, 4.5Hz, 1H, H-1a), 4.4 (dd, J=10.5, 4.5Hz, 1H, H-1b), 4.33 (m, 1H, H-3a), 4.29 (m, 1H, H-4), 2.23 (m, 1H, H-3'), 1.97 (m, 2H, H-5), 1.95 (m, 2H, H-7), 2.05 (m, 2H, H-10), 1.93 (m, 2H, H-22), 1.79 (m, 2H, H-6), 1.71 (m, 2H, H-4'), 1.43 (m, 2H, H-23), 1.29-1.40 (m, 22H, H-11-21), 1.29-1.40 (m, 18H, H-5'-13'), 1.27 (m, 2H, H-15'), 1.23 (m, 2H, H-14'), 0.85 (t, J=7.0Hz, 3H, Me-24), 0.85 (t, J=7.0Hz, 3H, Me-16'); 13C NMR (125MHz, Pyr-d5), δ: 62.5 (CH2, C-1), 53.5 (CH, C-2), 77.2 (CH, C-3), 73.5 (CH, C-4), 34.6 (CH2, C-5), 27.1 (CH2, C-6), 32.6 (CH2, C-7), 130.7 (CH, C-8), 130.8 (CH, C-9), 33.6 (CH2, C-10), 30.1-30.8 (CH2, C-11-21), 33.8 (CH2, C-22), 23.4 (CH2, C-23), 14.7 (CH3, C-24), 175.8 (C, C-1'), 73.0 (CH, C-2'), 36.2 (CH2, C-3'), 26.3 (CH3, C-4'), 30.1-30.8 (CH2, C-5'-13'), 33.8 (CH2, C-14'), 23.3 (CH2, C-15'), 14.7 (CH3, C-16'); FABMS m/z 654 [M+H]+; EIMS m/z 575 [M-C_{16}H_{31}O_{2}]+, 356 [M-H_{2}O-C_{20}H_{39}]+, 388 [M-2H_{2}O-C_{20}H_{38}]+. These data are in good agreement with that reported for [(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol, 4 (Kang et al., 2007).

5: 1-O-β-D-glucopyranosyl- [(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol

C_{46}H_{89}O_{10}N; White fine needles from alcohol; M.P: 207-208°C; IR (almost the same as 4). 1H NMR (500MHz, CD3OD), δ (ppm): 5.32 (m, 1H, H-8), 5.32 (m, 1H, H-9), 4.75 (d, J=8.5Hz, 1H, H-1''), 4.20 (m, 1H, H-2'), 3.91 (m, 1H, H-5''), 3.78 (dd, J=10.5, 4.5Hz, 1H, H-1''), 3.77 (s, 2H, H-6''), 3.50 (dd, J=10.5, 4.5Hz, 1H, H-1''), 3.49 (m, 1H, H-2''), 3.45 (m, 1H, H-2''), 3.28 (m, 1H, H-3''), 3.20 (m, 1H, H-4''), 3.17 (m, 1H, H-3), 3.15 (m, 1H, H-4), 2.05 (m, 2H, H-10), 1.97 (m, 2H, H-7), 1.64 (m, 2H, H-3'), 1.61 (m, 2H, H-22), 1.52 (m, 2H, H-23), 1.32 (m, 2H, H-15'), 1.29 (m, 2H, H-4'), 1.23 (m, 2H, H-6), 1.21-1.40 (m, 22H, H-11-21), 1.21-1.40 (m, 18H, H-5'-13'), 1.21 (m, 2H, H-1'), 1.19 (m, 2H, H-5), 0.80 (t, J=6.5Hz, 3H, Me-24), 0.80 (t, J=7.0Hz, 3H, Me-16'); 13C NMR (125MHz, CD3OD), δ: 71.7 (CH2, C-1), 51.7 (CH, C-2), 75.6 (CH, C-3), 70.9 (CH, C-4), 30.7 (CH2, C-5), 23.7 (CH3, C-6), 32.8 (CH2, C-7), 131.1 (CH, C-8), 131.6 (CH, C-9), 33.1 (CH2, C-10), 30.5-32.8 (CH2, C-11-21), 33.7 (CH3, C-22), 23.7 (CH2, C-23), 14.5 (CH3, C-24), 174.7 (C, C-1''), 72.9 (CH, C-2''), 35.7 (CH2, C-3'), 27.2 (CH2, C-4'), 30.5-32.8 (CH2, C-5'-13'), 30.4 (CH2, C-14'), 26.1 (CH2, C-15'), 14.5 (CH3, C-16'), 104.7 (CH, C-1''), 75.1 (CH, C-2''), 77.9 (CH, C-3''), 71.6 (CH, C-4''), 78.0 (CH, C-5''), 62.7 (CH3, C-6'); FABMS m/z 816 [M+H]+; EIMS m/z 635 [M-glucose]+. These data are in good agreement with that reported for 1-O-β-D-glucopyranosyl- [(2S, 3S, 4R, 8E)-2-[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol

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[(2'R)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol 5 (Kang et al., 2007).

6: kaempferol-3-O-rutinoside

C_{27}H_{30}O_{15}; Yellowish amorphous powder.UV \( \lambda_{\text{max}} \) (CD2OD): 355, 270 (nm). IR (KBr), \( \nu \) (cm\(^{-1}\)) = 3380 (OH), 1150 (C-O), 3050, 1460, 1600 (aromatic \( =C-H \)), 1580 \( \times \) (C=C (stretching)) and medium absorption at 1605 cm\(^{-1}\) and 1504 cm\(^{-1}\) for C=C (stretching).

The ESI-Ion Trap mass spectrum revealed a pseudo-molecular ion peak [M + Na] at \( m/z\) 1939 in addition to fragments at \( m/z\) 157 [M-NH]\(^+\), 117 [M-C\(_2\)HNO]\(^+\), 105 [M-C\(_3\)HNO]\(^+\), 91 [tropolium ion]\(^+\) and 79 [characteristic benzonion ion]\(^+\). The odd molecular weight (m/z 2171) indicated the presence of one N-atom, corresponding to molecular formula C\(_{11}\)H\(_9\)NO. The proposed fragmentation pattern of compound 1 is shown in fig. 2.

7: Rutin

C\(_{27}\)H\(_{30}\)O\(_{15}\); Yellowish amorphous powder.M.P: 241-242°C. UV \( \lambda_{\text{max}} \) (CD2OD): 380, 275 (nm).IR (KBr): \( \nu \) (cm\(^{-1}\)) = 3380 (OH), 1605, 1504 (aromatic rings), 1160 (C=O). ESI-Ion (m/z): 593[M-1]\(^+\), 578 [M-CH\(_2\)H\(_2\)O]\(^+\).\(^1\)H NMR (500MHz, DMSO-d\(_6\)), (\( \delta \) ppm): 7.70 (d, \( J=1.5 \text{ Hz} \), H-2'), 7.60 (d, \( J=8.5 \text{ Hz} \), 1H, H-2'), 6.90 (d, \( J=8.5 \text{ Hz} \), 2H, H-3',5'), 3.40 (m, 1H, H-4'), 3.39 (m, 1H, H-5'), 3.40 H-6'), 3.49 (m, 1H, H-3'), 3.49 (m, 1H, H-5'''), 3.48 (m, 1H, H-3''), 3.40 (m, 1H, H-4''), 3.40 (m, 1H, H-4''), 3.40 (m, 1H, H-4''). These data are in good agreement with that reported for kaempferol-3-O-rutinoside 6 (Song et al., 2007).
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$^{13}$C NMR (table 1) revealed an amide carbonyl signal at $\delta_c 174.5$, six aromatic carbons at $\delta_c 112.5$, $\delta_c 119.4$, $\delta_c 120.1$, $\delta_c 122.8$, $\delta_c 128.5$ and $\delta_c 138.4$. Two olefinic carbons appeared at $\delta_c 109.6$ ($s$) and $\delta_c 125.2$ ($d$). Dept 135° experiment represented six methine proton and one methylene protons. HSQC experiment confirmed the assignment of each proton to its corresponding carbon.

The locations of the amide group as well as the olefinic double bond were unambiguously determined by careful assignment of HMBC correlations as shown in (fig. 3). Two and three bond correlations were observed from H-1 assignment of HMBC correlations as shown in (fig. 3). The presence of lactam ring was confirmed double bond were unambiguously determined by careful assignment of HMBC correlations as shown in (fig. 3). The presence of lactam ring was confirmed by HMBC correlations from H$_{1a}$ at $\delta 3.14$ ($q$, $J=15.5$, 9.5 Hz) and H$_{1b}$ at $\delta 3.49$ (dd, $J=15$, 3.5 Hz) to the carbonyl carbon at $\delta 174.5$. The lack of HMBC between the olefinic proton at C4 and the carbonyl carbon confirmed our assumption.

Based on the above data the structure of compound 1 identified as 2a,3-dihydonaphtho[2,3-b]azet-2(1H)-one and was named as albactam as it is the first time to isolate it from natural source during this study.

It worth to note that the tetrahydro derivative of albactam (2a,3,8a-tetrahydonaphtho[2,3-b]azet-2(1H)-one) had been synthesized and commercially available. (CAS Registry Number 903639-29-2) 2014 available at http://www.sigmaaldrich.com/catalog/product/aldrich/t172170?lang=en&region=EG).

Several amide-containing compounds were previously isolated from leguminosae plants as in case of isolation of three spermidine alkaloids from the leaves of Caesalpiniadigyna (Mahato et al., 1983). One of these alkaloids, caesalpinine A, has five-membered β-lactam ring which supported the possible biosynthesis of the four-membered lactamI, most probably through condensation of decarboxylated L-phenylalanine and malonyl-S CoA.

Platelet aggregation activity
Compound (1) (albactam) inhibit both adenosine diphosphate (ADP) and arachidonic acid platelets induced aggregation in a dose dependant manneras shown in fig. 4 (4a-4d). Figures. 4a and 4b showed the reduction in the percentage of light transmission, which indicating the antiaggregatory effect, from 14% (ADP alone, control) to 8% (after addition Compound 1) indicating that compound 1 in a dose of 1mg/ml PRB inhibited ADP-induced aggregation by 43%. On the other hand the percentage decrease of light transmission was shifted from 61% to 12% (figs. 4C & D) upon addition of arachidonic acid at a dose of 1mg/ml PRB indicating that the percentage of antiaggregatory effect of compound 1 is equal to 80% using arachidonic acid as a reference standard. Therefore compound 1 was proved to have anti-aggregatory activity against adenosine diphosphate (ADP) and arachidonic acid-induced guinea-pigs' platelets aggregation in vitro at doses 208 µg/ml and 172 µg/ml respectively (El-Tahir, Williams, 1980).

The inherent property of 1 to suppress platelets aggregation points to its potential as a prophylactic against thrombin formation in cases associated with platelets hyper-aggregation (El-Tahir, 2007).

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CONCLUSION
The alcoholic extract of A. lebbeck flowers afforded a novel β- lactam derivative designated as albactam, which shows a significant anti-aggregatory activity. Six more known compounds were also isolated and fully characterized by extensive spectroscopic techniques measurement including 1D and 2D NMR. The isolated compounds were identified as β- amyrin and 11α, 12α- oxidotaraxerol, two ceramide derivatives (aglycone and its glucoside), kampferol 3- O- rutinoside and rutin.

REFERENCES


