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, platellete nti-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 tothe 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 pigfrom antigen-induced anoxic convulsion. Recently, it was found that the alcoholic extract of the plant has antihistaminic property, eitherby 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, antidysentric 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 Matsunaga, 1989) 2; 11α, 12α-oxidotaraxerol (Ibrahim and Ali, 2007) 3; [(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol (Kang *et al*., 2007) 4; 1-*O*-β-D-glucopyranosyl- [(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol (Kang *et al*., 2007) 5; kaempferol-3-*O*-rutinoside (Song *et al*., 2007) 6 andrutin (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_3OD, pyridine-d_5 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-

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500. HPLC (Agilent 1200) connected to mass detector (Agilent 6410 QQQ) was also utilized. Normal phase silica gel $60F_{254}$, 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, 29 gm).10 Gm of fraction I was loaded onto the top of silica gel packed column (550gm silica gel, 150 x 4cm). Elution the column with *n*- hexane and n-hexane: acetone with increasing polarity was achieved. On the basis of TLC behavior, similar fractions were pooled together affording several subfractions in which two main subfractions A and B were subjected to repeated column chromatography. Subfraction A (46mg), was further purified on silica gel column (2.3gm silica gel, 20x2.5cm) eluted with *n*-hexane-acetone gradually yielded compound 2 (22mg) and compound 3 (15mg) as white fine crystals. Subfraction B (2.6gm), was rechromatographed over silica gel column (150gm silica gel, 100cm x 4cm) eluted with dichloromethane- methanol- water mixtures in a gradient system to give two subfractions C (27mg) and D (60mg). Repeated column chromatography over silica for fraction C using dichloromethane-methanol, gradient afforded 10mg of white amorphous powder, compound 4, while column chromatography of fraction D eluted by dichloromethane- methanol- water (85: 15: 1.5) followed by crystallization from methanol afforded 12mg of white powder of compound 5.

Fraction II (25gm) was applied to *Diaion* HP-20 column and eluted with water, water-methanol then acetone. The effluent, 250ml each, was concentrated to small volume, monitored by silica gel TLC using dichloromethane-methanol in a gradient system and similar fractions were pooled together to afford several fractions. Two fractions eluted by 40% methanol in water (E, 300mg) and 80% methanol in water (F, 3.4gm) were separately loaded onto the top of silica gel-packed columns using dichloromethane-methanol-water gradient. The effluent, 100 ml each, was concentrated and monitored by TLC in dichloromethane-methanol-water (70:30:3) and similar fractions were added together. Upon concentration, a subfraction from E 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 155-156 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^{o}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*

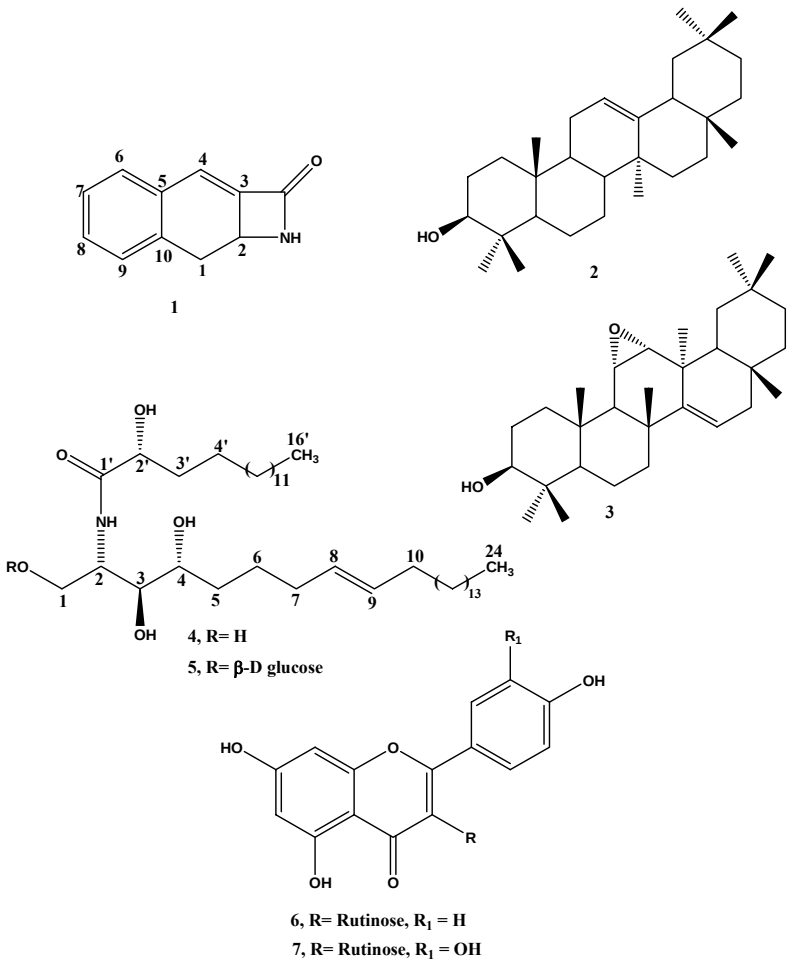

Fig. 1: Structures of compounds **1-7**

$C_{11}H_9ON$; Pale yellow fine needles. M.P: 142-143°C.UV λ_{max} (MeOH): 224, 273, 280, 289 (nm). IR (KBr): ν(cm^{-1})=3408 (N-H), 3010 (=C-H), 1680 (C=O), 1605, 1504 (C=C). ^{1}H NMR (500MHz, CD_3OD), (δppm): 7.60 (d, *J*= 8.0Hz, 1H, H-9), 7.34 (d, *J*=6.5Hz, 1H, H-6), 7.19 (s, 1H, H-4), 7.09 (t, *J*=7.0Hz, 1H, H-8), 7.02 (t, *J*=7.5Hz, 1H, H-7), 3.77 (q, *J*=9.5, 3.5Hz, 1H, H-2),), 3.14 (q, *J*=15.5, 9.5 Hz, 1H, H-1a), 3.49 (dd, *J*=15.5, 3.5Hz, 1H, H-1b). ^{13}C NMR (125 MHz, CD_3OD), δ: 28.4 (CH_2, 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 $[M-C_2HNO]^+$, 105 $[M- C_3HNO]^+$, 91 [tropolium ion]$^+$, 79 [benzonium ion]$^+$.

2: β- Amyrin

$C_{29}H_{48}O$; White crystalline needles from acetone; M.P:193°-195 °C. IR (KBr):ν (cm^{-1}) =3460 (O-H), 3030 (vinyl C-H), 1625 (C=C),1065 (C-O). ^{1}H NMR (500 MHz, $CDCl_3$): (δppm): 5.12 (t, *J*=8.5 Hz, 1H, H-12), 3.15 (dd, *J*=11.5, 5.0 Hz, 1H, H-3), 1.93 (t, *J*=5.0 Hz, 2H, H-16), 1.90 (t, *J*=3.5 Hz, 1H, H-18), 1.80 (m, 1H, H-11), 1.70 (t, *J*=5.0 Hz,2H, H-15), 1.60 (d, *J*=3.5 Hz, 2H, H-19), 1.54 (m, 2H, H-2), 1.45 (t, *J*=4.0 Hz, 1H, H-9), 1.41 (t, *J*=4.0 Hz, 2H, H-1), 1.39 (t, *J*=5.0 Hz, 2H, H-22), 1.33 (m, 2H, H-6), 1.32 (m, 2H, H-21), 1.29 (t, *J*=5.5 Hz, 2H, 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, $CDCl_3$), δ: 38.8 (CH2, C-1), 27.3(CH_2, C-2), 79.0 (CH, C-3), 39.8 (C, C-4), 55.2 (CH, C-5), 18.4 (CH_2, C-6), 32.7 (CH_2, C-7), 38.6 (C, C-8), 47.7 (CH, C-9), 37.2 (C, C-10), 23.5 (CH_2, C-11), 121.8 (CH, C-12), 145.2 (C, C-13), 41.7 (C, C-14), 26.2 (CH_2, C-15), 26.9 (CH_2, C-16), 32.5 (C, C-17), 47.3 (CH, C-18), 46.9 (CH_2, C-19), 31.1 (C, C-20), 34.8 (CH_2, C-21), 36.9 (CH_2, C-22), 28.1 (CH_3, C-23), 15.2 (CH_3, C-24), 15.1 (CH_3, C-25), 16.8 (CH_3, C-26), 25.9 (CH_3, C-27), 28.4 (CH_3, C-28), 33.3 (CH_3, C-29), 23.7 (CH_3, C-30).EIMS (m/z): 498 $[M+TMSi]^+$, 483 $[M-CH_3]$, 408 [M-TMSiOH], 393 [M-TMSiOH and CH_3], 279 [M-C*[a]DE rings],218 [M-ABC rings], 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 β-Amyrin (2) (Abbas *et al*., 2009; Tanaka and Matsunaga, 1989).

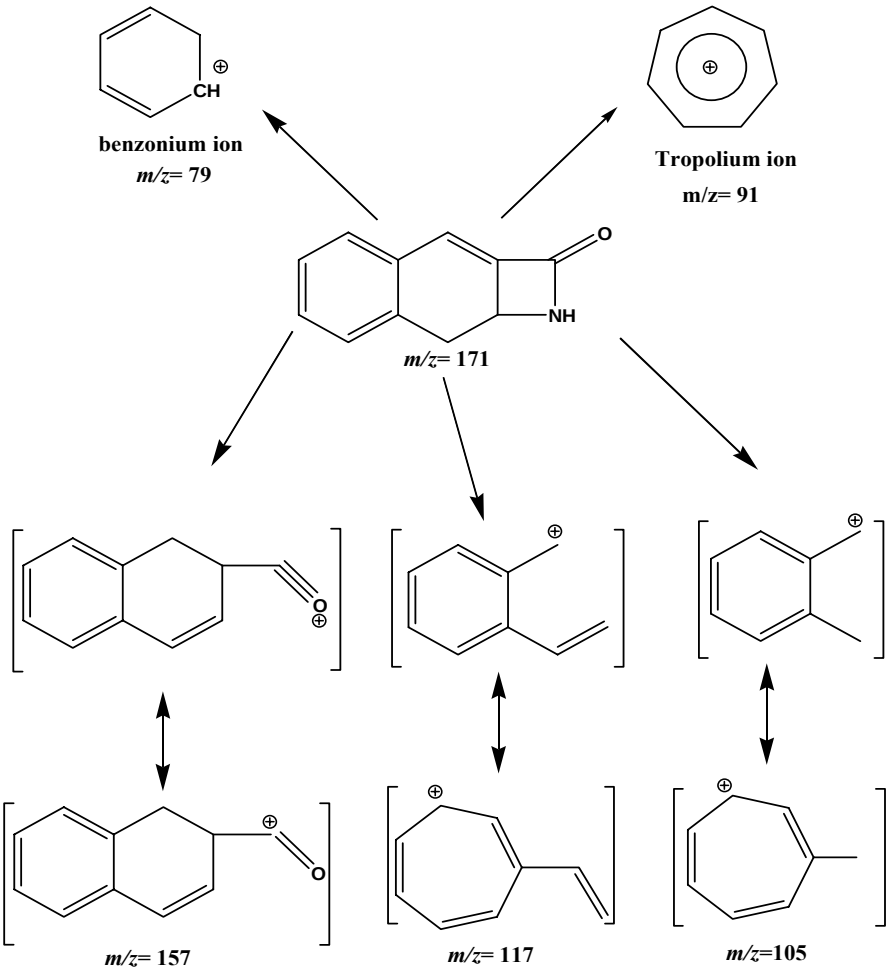

Fig. 3: Proposed fragmentation pattern of compound 1

3: 11α, 12α-oxidotaraxerol

$C_{30}H_{48}O_2$; White crystalline needles; M.P:425-427^{0}C.IR (KBr)*v* (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* = 5.5Hz, 1H, H-12), 2.11 (t, *J*=, 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*=4.0Hz, 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*=2.0Hz, 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 (CH2, C-6), 33.2 (CH2, 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_2O], 404 [M-2H_2O], 299 [RDA]. These data are in good agreement with that reported for 11α, 12α-oxidotaraxerol (3) (Ibrahim and Ali, 2007).

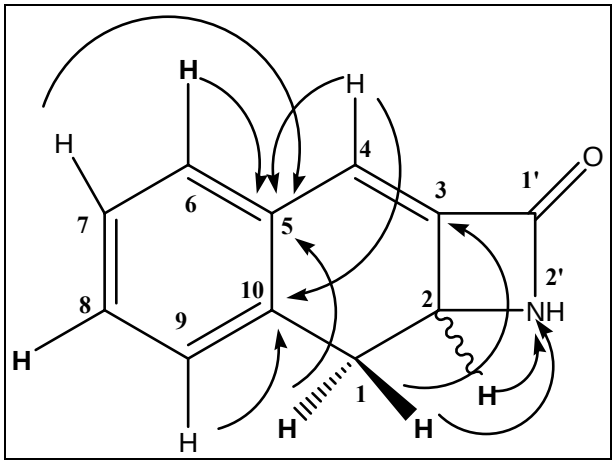

Fig. 3: HMBC correlations of compound 1

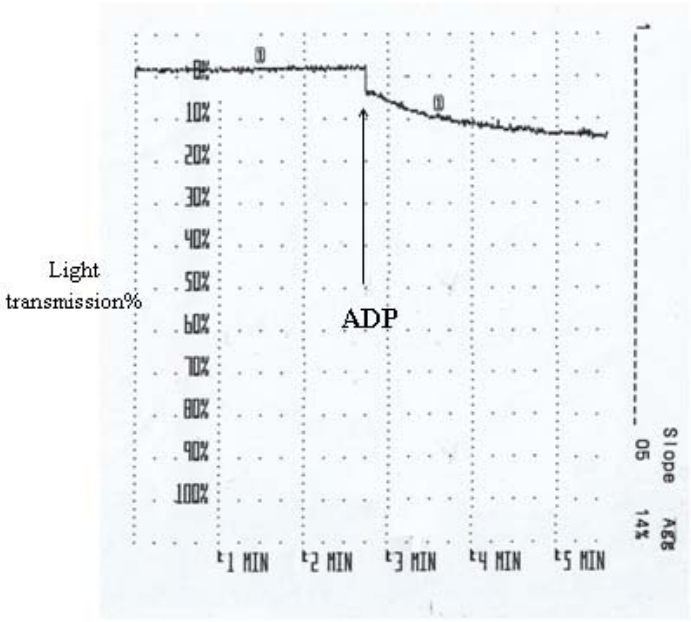

Fig. 4A: Platelete anti-agreggatory effect of ADP on PRB.

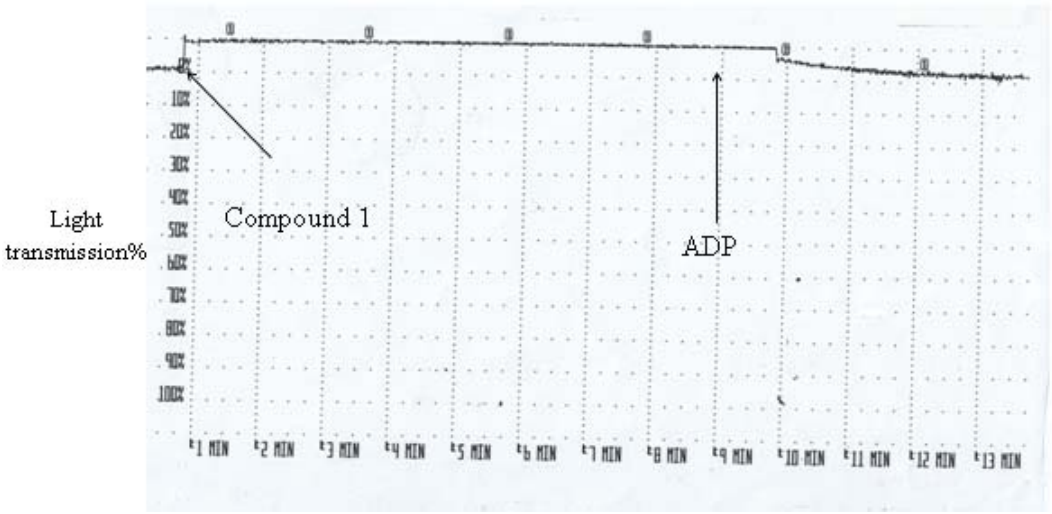

Fig. 4B: Platelete anti-agreggatory effect of compound 1and ADP on PRB.

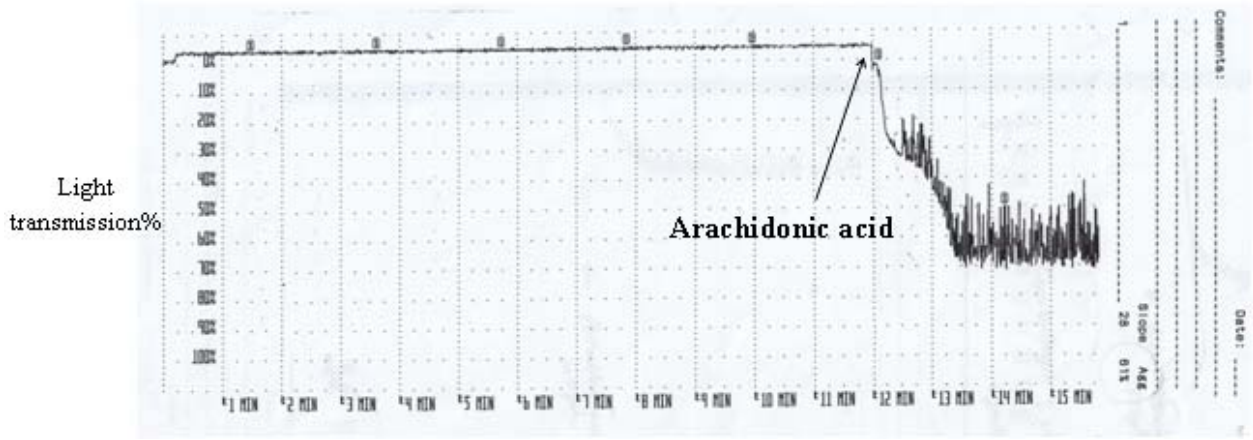

Fig. 4C: Platelete anti-agreggatory effect of arachidonic on PRB.

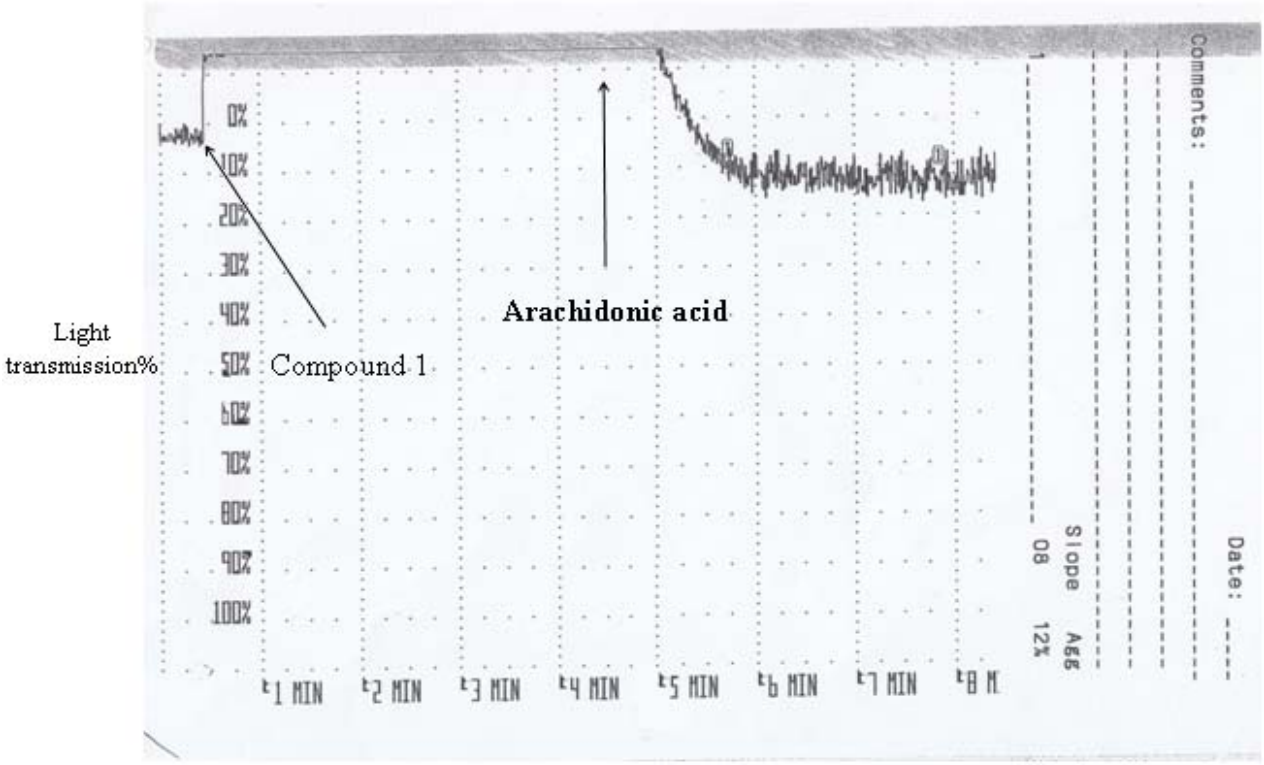

Fig. 4D: Platelete anti-agreggatory effect of compound 1and arachidonic on PRB.

4: [(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-hydroxyhexadecanoy-lamino]-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)*v*(cm^{-1}) =3200-3500 (-OH & –NH), 3100 (olefenic C-H), 1650 (C=O).^{1}H NMR (500MHz, *Pyr-d*$_5$): (δppm): 8.57 (d, *J*=9.0Hz, 1H, NH), 5.52 (dt, *J*=15.5, 5.5Hz, 1H, H-8), 5.52 (dt, 15,5, 5.5Hz, 1H, H-9), 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-3), 4,29 (m, 1H, H-4),2.23 (m, 1H, H-3'a), 2.21 (m, 1H, H-3'b), 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'); ^{13}C NMR (125MHz, *pyr-d*$_5$), δ: 62.5 (CH_2, C-1), 53.5 (CH, C-2), 77.2 (CH, C-3), 73.5 (CH, C-4), 34.6 (CH_2, C-5), 27.1 (CH_2, C-6), 32.6 (CH_2, C-7), 130.7 (CH, C-8), 130.8 (CH, C-9), 33.6 (CH_2, C-10), 30.1-30.8 (CH_2, C-11-21), 33.8 (CH_2, C-22), 23.4 (CH_2, C-23), 14.7 (CH_3, C-24), 175.8 (C, C-1'), 73, 0 (CH, C-2'), 36.2 (CH_2, C-3'), 26,3 (CH_2, C-4'), 30.1- 30.8 (CH_2, C-5'-13'), 33,8 (CH_2, C-14'), 23.3 (CH_2, C-15'), 14.7 (CH_3, C-16'); FABMS m/z 654 $[M+H]^+$; EIMS *m/z*398 $[M-C_{16}H_{31}O_2]^+$, 356 $[M-H_2O-C_{20}H_{39}]^+$, 388 $[M-2H_2O-C_{20}H_{39}]^+$. These data are in good agreement with that reported for [(2*S*, 3*S*, 4*R*, 8*E*)-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).^{1}H NMR (500MHz, CD_3OD), (δppm):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-1a), 3.77 (s, 2H,H-6"), 3.50 (dd, *J*=10.5, 4.5Hz, 1H, H-1b), 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 (t, 18H, H-5'-13'), 1.21 (m, 2H, H-14'), 1.19 (m, 2H, H-5), 0.80 (t, *J*=6.5Hz, 3H, Me-24), 0.80 (t, *J*=7.0Hz, 3H, Me-16'). ^{13}C NMR (125MHz, CD_3OD), δ: 71.7 (CH_2, C-1), 51.7 (CH, C-2), 75.6 (CH, C-3), 70.9 (CH, C-4), 30.7 (CH_2, C-5), 23.7 (CH_2, C-6), 32.8 (CH_2, C-7), 131 (CH, C-8), 131.6 (CH, C-9), 33.1 (CH_2, C-10), 30.5-32.8 (CH_2, C-11-21), 33.7 (CH_2, C-22), 23.7 (CH_2, C-23), 14.5 (CH_3, C-24), 174.7 (C, C-1'), 72.9 (CH, C-2'), 35.7 (CH_2, C-3'), 27.2 (CH_2, C-4'), 30.5-32.8 (CH_2, C-5'-13'), 30.4 (CH_2, C-14'), 26.1 (CH_2, C-15'), 14.5 (CH_3, 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 (CH_2, C-6").FABMS (m/z):816$[M+H]^+$; EIMS*m/z* 635 $[M-glucose]^+$.These data are in good agreement with that reported for1-*O*-β-D-glucopyranosyl- [(2*S*, 3*S*, 4*R*, 8*E*)-2-

[(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 λ_{max} (CD_3OD): 355, 270 (nm). IR (KBr), ν (cm^{-1}) = 3380 (OH), 1605, 1501 (aromatic rings), 1160 (C=O). ESI-Ion (m/z):593$[M-1]^+$, 578 $[M-CH_3]^+$, 560 $[M-CH_3-H_2O]^+$.^{1}H NMR (500MHz, DMSO-d_6), (δppm):8.00 (d, *J*=8.0, 8.5Hz, 2H, H-2',6'), 6.90 (d, *J*=8.5Hz, 2H, H-3',5'), 6.42 (d, *J*=1.5Hz, 1H, H-8), 6.22 (d, *J*=1.5Hz, 1H, H-6), 5.30 (d, J=7.0Hz, 1H, H-1''), 4.39 (d, *J*=3.5Hz, 1H, H-1'''), 3.90 (m, 1H, H-2''), 3.70 (m, 1H, H-2'''), 3.50 (m, 2H, H-6''), 3.49 (m, 1H, H-5'''), 3.49 (m, 1H, H-3''), 3.48 (m, 1H, H-3'''), 3.40 (m, 1H, H-4'''), 3.40 (m, 1H, H-4''), 3.39 (m, 1H, H-5''), 1.10 (d, *J*=6.5Hz, 3H, Me-6'''). ^{13}C NMR (125MHz, DMSO-d_6),δ:156.4 (C, C-2), 133.2 (C, C-3), 177.3 (C, C-4), 161.1 (C, C-5), 98.7 (CH, C-6), 164.2 (C, C-7), 93.7 (CH, C-8), 156.8 (C, C-9), 103.9 (C, C-10), 120.8 (C, C-1'), 130.9 (CH, C-2'), 115.1 (CH, C-3'), 159.8 (C, C-4'), 115.1 (CH, C-5'), 130.9 (CH, C-6'), 101.3 (CH, C-1''), 74.1 (CH, C-2''), 76.3 (CH, C-3''), 69.9 (CH, C-4''), 75.7 (CH, C-5''), 66.8 (CH_2, C-6''), 100.7 (CH, C-1'''), 70.3 (CH, C-2'''), 70.5 (CH, C-3'''), 71.8 (CH, C-4'''), 68.2 (CH, C-5'''), 17.9 (CH3, C-6''').These data are in good agreement with that reported forkaempferol-3-*O*-rutinoside 6 (Song *et al*., 2007).

7: Rutin

$C_{27}H_{30}O_{16}$; Yellowish amorphous powder.M.P: 241-242^{0}C.UV λ_{max}(CD_3OD):380, 275 (nm).IR(KBr):ν (cm^{-1}) =3400 (OH), 1150 (C-O), 3050 , 1460, 1600 (aromatic =C-H), 1660 (C=O).MS (m/z): 609 $[M-1]^+$.^{1}H NMR (500MHz, CD_3OD), (δppm): 7.70 (d, *J*=1.5 Hz, 1H, H-2'), 7.60 (dd, *J*=8.50Hz, 1H, H-6'), 6.9 (d, *J*=8.5Hz, 1H, H-5'), 6.40 (d, *J*=1.2Hz, 1H, H-8), 6.21 (d, *J*=1.2Hz, 1H, H-6), 5.30 (d, *J*=7.0Hz, 1H, H-1''), 4.60 (d, *J*=1.50Hz, 1H, H-1'''), 3.90 (m, 1H, H-2''), 3.70 (m, 1H, H-2'''), 3.50 (m, 2H, 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''), 3.39 (m, 1H, H-5''), 3.40 (m, 1H, H-4'''), 1.2 (d, *J*=6.5Hz, 3H, H-6'''). ^{13}C NMR (125 MHz, CD_3OD),δ: 158.5 (C, C2), 135.6 (C, C-3), 179.4 (C, C-4), 159.2 (C, C-5), 99.9 (CH, C-6), 166.1 (C, C-7), 94.8 (CH, C-8), 162.9 (C, C-9), 105.6 (C, C-10), 123.1 (C, C-1'), 116.0 (CH, C-2'), 145.8 (C, C-3'), 149.8 (C, C-4'), 117.6 (CH, C-5'), 123.5 (CH, C-6'), 102.4 (CH, C-1''), 73.9 (CH, C-2''), 77.1 (CH, C-3''), 69.7 (CH, C-4''), 75.7 (CH, C-5''), 68.5 (CH_2, C-6''), 104.7 (CH, C-1'''), 71.4 (CH, C-2'''), 72.2 (CH, C-3'''), 73.9 (CH, C-4'''), 78.1 (CH, C-5'''), 17.9 (CH_3, C-6''').These data are matched with those reported forrutin **7** (Aderogba *et al*., 2006)**.**

DISCUSSION

Phytochemical investigation of the flowers of *A. lebbeck* resulted in the isolation of a novel β-lactam, albactam 1, along with six known compounds named as β- Amyrin 2, 11α, 12α-oxidotaraxerol 3, 2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-hydroxyhexadecanoylamino]-8-tetra-cosene-1, 3, 4-triol 4, 1-O-β-D-glucopyranosyl- [(2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-hydroxyhexadecanoylamino]-8-tetra-cosene-1,3,4-triol 5, kaempferol-3-O-rutinoside 6, Rutin 7 which were identifed by a comparison of their data (physical and spectral) with those reported in the literature as well as direct comparison with authentic materials whenever avilable.

The UV spectrum of 1 showed absorption maxima at λ_{max} at 224, 273, 280 and 289 nm suggesting an aromatic ring with extended conjugation. The IR spectrum (KBr) showed typical absorption band at 3408cm^{-1} suggesting the presence of N-H or O-H group (s) and a strong absorption of C=O at 1680cm^{-1} that supported the presence of an amide group. An aromatic ring was indicated by a weak absorption at 3010cm^{-1} indicated =C-H (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*193 in addition to fragments at *m/z*157 $[M- NH]^+$, 117 $[M-C_2HNO]^+$, 105 $[M-C_3HNO]^+$,91 [tropolium ion]$^+$and 79 [characteristic benzonium ion ring]$^+$. The odd molecular weight (*m/z*171) indicated the presence of one N-atom, corresponding to molecular formula $C_{11}H_9NO$. The proposed fragmentation pattern of compound 1 is shown in fig. 2

^{1}H NMR indicated the presence of four aromatic protons at δ_H 7.02 (*t, J*=7.5 Hz, H-7), δ_H 7.09 (*t, J*=7Hz, H-8),δ_H 7.34 (*d, J*=6.5Hz, H-6) andδ_H 7.60 (*d, J*=8 Hz, H-9) suggesting *O*-disubstituted benzene. In addition, an isolatedolefinic proton singlet at δ_H7.19 (1H, *s*) was assigned to H-4 and three aliphatic protons appeared atδ_H3.14 (*q, J*=15.5, 9.5 Hz), δ_H 3.49 (*dd, J*=15, 3.5 Hz) assigned for H-1a and H-1b and δ_H 3.77 (*q, J*=9.5, 3.5 Hz) assigned for H-2.

Table 1: ^{1}H and ^{13}C NMR assignment of compound 1 (500 MHz, CD_3OD)

Position	δ_H (Multiplicity, *J* in Hz)	δ_C
1a 1b	3.14 (*q*, 15.5, 9.5) 3.49 (*dd*, 15.5, 3.5)	28.4
2	3.77 (*q*, 9.5, 3.5)	56.7
3	-	109.6
4	7.19 (*s*)	125.2
5	-	128.5
6	7.34 (*d*, 6.5)	112.5
7	7.02 (*t*, 7.5)	120.1
8	7.09 (*t*, 7)	122.8
9	7.60 (*d*,8)	119.4
10	-	138.4
2'	-	174.5

^{13}C NMR (table 1) revealed an amide carbonyl signal at δ_C174.5, six aromatic carbons at δ_C112.5, δ_C119.4,δ_C 120.1,δ_C 122.8, δ_C128.5 and δ_C138.4. Two olefenic carbons appeared at δ_C 109.6 (*s*) and δ_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 carful assignment of HMBC correlations as shown in (fig. 3). Two and three bond correlations were observed from H-1 to C-2', C-3, C-5; from H-2 to C-2 and from H-4 to C-5, C-10 (fig. 3). The presence of lactam ring was confirmed by HMBC correlations from H_{1a} at δ 3.14 (*q*, *J*=15.5, 9.5 Hz) and H_{1b} at δ3.49 (*dd*, *J*=15, 3.5 Hz) to the carbonyl carbon at d 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-dihydronaphtho[2,3-*b*]azet-2(1*H*)-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,3,8,8a-tetrahydronaphtho[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 lactam**1**, 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 antiagregatory 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.

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