Bull. Pharm. Sci., Assiut University, Vol. 29, Part 2, December 2006, pp. 371-387.

PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATIONS OF *FLACOURTIA CATAPHRACTA* ROXB. CULTIVATED IN EGYPT

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تمت تجزئة خلاصة الكحول الاثيلى (٧٠%) لكل من الاوراق الجافة وقلف الساق للنبات كل على حدة بواسطة الهكسان ، الكلوروفورم ثم خلات الإيثيل. وتم تركيز كل مستخلص وخضع للدراسة بواسطة كروماتوجرافيا الطبقة الرقيقة واتبع ذلك فصل وتنظيف والتعرف على مكونات كل مستخلص. وقد أسفرت الدراسة عن فصل أربعة عشر مركبا وتم التعرف عليها بدراسة خواصها الطبيعية والكيميائية والكروماتوجرافية وكذا بإستخدام طيف الأشعة البنفسجية وتحت الحمراء إضافة إلى الرنين النووى المغناطيسى بنوعيه البروتونى والكربونى ومقياس الكتلة وأيضا بمقارنة هذه النتائج بتلك المنشورة.

ومن مستخلص الهكسان للأوراق وقلف الساق تم فصل والتعرف على بيتا اميرين (١) ، ألفا اميرين (٢) وخليط من بيتاسيتوستيرول وستيجماستيرول (٣). أما من مستخلص الكلوروفورم لقلف الساق تم فصل ٢ أوكسو ١٨ بنزويل أوكسى ١٣ (١٦)، ١٤ رباعى هيدروكليرودا ٣ اين (٤) ، ٣ بيتا اسيتوكسى د:أ فريدوأوليانان ١٦ ، ٢٧ ألفا لاكتون (٥) ، ٤،٤' داى هيدوكسى شالكون (٦) ، أبيجينين (٧) ، وكامبيفيرول (٨). فى حين تم فصل بيتا سيتوستيرول ٣ أ جليكوزيد (٩) و ٥ أ حمض كافيول كوينيك (١٠) من مستخلص خلات الإيثيل للأوراق إضافة إلى فانيلين (١١) ، حمض بنزويك (١٢) ، حمض بروتوكاتشيوك (١٣) ، وفلاكورتين (١٤) من مستخلص خلات الإيثيل لقلف الساق.

وقد أجريت الدراسات البيولوجية لمختلف المستخلصات وكان من نتيجتها أن مستخلصات الهكسان وخلات الإيثيل والميثانول لنبات فلاكورتيا كاتفراكتا روكسب قليلة السمية ويمكن أن تستخدم كمضادات للإسهال والإلتهابات وكذا كمخفض للحرارة.

The concentrated 70% ethanolic extracts of the air-dried powdered leaves and stem bark of Flacourtia cataphracta were subjected separately to solvent fractionation by partitioning using

Received in 31/7/2006 & Accepted in 30/10/2006

n-hexane, chloroform and ethyl acetate respectively. Each concentrated fraction was subjected to TLC followed by isolation, purification and identification of the available constituents.

*Fourteen compounds were isolated and identified by different spectral tools (UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS) and comparison with corresponding literature data. β-amyrin (**1**), α-amyrin (**2**), and a mixture of β-sitosterol and stigmasterol (**3**) were isolated from n-hexane fraction of both leaves and stem bark. 2-Oxo-18-benzolyloxy-13(16), 14-tetrahydrocleroda-3-ene (**4**), 3-β-acetoxy-D:A friedo oleanan-27,16α-lactone (**5**), 4,4'-dihydroxychalcone (**6**), apigenin (**7**) and kampferol (**8**) were isolated from chloroform fraction of the stem bark. β-Sitosterol-3-O-β-D-glucoside (**9**), 5-O-caffeoylquinic acid (**10**) were isolated from the ethyl acetate fraction of the leaves in addition to vanillin (**11**), benzoic acid (**12**), protocatechuic acid (**13**) and flacourtin (**14**) from the ethyl acetate fraction of the stem bark. The different leaf extracts were subjected to biological study which revealed that n-hexane, ethyl acetate and methanol fractions of Flacourtia cataphracta Roxb. are safe to be used as antidiarrheal, anti-inflammatory and antipyretic drug.*

INTRODUCTION

Flacourtia cataphracta Roxb. is a shrub or erect low-branched tree,[1&2] indigenous to India,[1] commonly cultivated through out South East Asia[1,3&4] and is planted in Egypt and many tropical to subtropical parts of the world. It is known in Arabic as Talisfir or Zarnab.[1] It was reported that the leaves, stem bark and roots have been employed in Indian folk medicine as tonic, diaphoretic, stomachic, in treatment of diarrhea, and also to relieve bronchitis, cough, toothache and other ailments.[1-3]

Some members of the family Flacourtiaceae were reported to contain diversity of natural products as cyanogenic glycosides,[5&6] triterpenes,[7&8] sterols,[9&10] diterpenes,[10&11] phenolic compounds,[7&12] benzyl alcohol derivatives[7,13-15] and few alkaloids.[13,16&17] Few reports have been found concerning the chemistry of the genus *Flacourtia*, where flacourtin,[18] ramoutoside,[19] β-sitosterol glucoside,[19] caffeic acid,[20] ostruthin,[21] limonin,[21] jangomolide[21] and mortenone[22] were identified.

No reports could be traced about *Flacourtia cataphracta* Roxb. cultivated in Egypt, so we decided to carry out phytochemical and biological studies of this plant in order to evaluate the therapeutic effects claimed by traditional medicine and also to provide a new source of natural biologically active compounds. In a previous publication, we reported the macro- and micromorphological characters of the

leaves, stem and stem bark of the titled plant.[23]

EXPERIMENTAL

General experimental procedures

1- Melting points are uncorrected and measured by Koffler hot stage microscope type (ESP, Boctius M).
2- UV spectra were measured in methanol and different ionizing and complexing agents using UVidec-320 spectrophotometer (JAS CO, Japan).
3- Schimadzu infra red-470 spectrophotometer (Japan) was used for measuring IR spectra as KBr discs.
4- ^{1}H-NMR and ^{13}C-NMR were recorded on JEOL TNM-LA 400 and 500 spectrophotometers using TMS as an internal standard.
5- Column chromatography was performed with silica gel 60 (E-Merck), Develosil Lop ODS (30-50 µ, Nomura Chemicals).
6- Silica gel 60 G F_{254} and precoated aluminium sheets to silica gel 60 G F_{254} (E-Merck) were used for TLC purposes.
7- The spots were visualized by UV lamp (254, 366 nm, VL, 6LC, Marinc Lavalec-Codex, France) and sprayed with 10% H_2SO_4, 5% $AlCl_3$ or $FeCl_3$.
8- Authentic samples were obtained from Department of Pharmacognosy, Faculty of Pharmacy, Assiut University.
9- Solvent systems:

I- n-Hexane – EtOAc (9 : 1).
II- $CHCl_3$ – MeOH (9.5 : 0.5).
III- n-Hexane – $CHCl_3$ – acetic acid (7.5 : 2.5 : 0.5).
IV- $CHCl_3$ – MeOH (9 : 1).
V- CHCl3 – MeOH (8 : 2).
VI- n-Butanol – acetic acid – water (4 : 1 : 2).

Plant material

Leaves and stem bark of *Flacourtia cataphracta* Roxb. were collected during flowering and fruiting stages in March-June 2001 from the Botanical Garden in Aswan. The plant identity was kindly confirmed by Prof. Dr. Naeem El-Keltawy, Professor of Horticulture, Faculty of Agriculture, Assiut University. A voucher specimen was deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Assiut University. The plant material was air-dried, powdered and kept for investigation.

Materials for biological study

1- Plant extracts for biological screening

i- The air-dried powdered leaves (100 g) was exhaustively extracted by percolation with methanol, the solvent was distilled under reduced pressure leaving 6.5 g residue, kept for antidiarrheal activity.
ii- The air-dried powdered leaves (200 g) was successively extracted by percolation with n-hexane, chloroform and ethyl acetate. The solvent in each case was evaporated under reduced pressure giving 4.5, 2.3 and 3.2 g respectively and kept for toxicological study, anti-

inflammatory and antipyretic activities.

2- Drugs and chemicals

Indomethacin (El-Nile Pharm. Co., Egypt), Brewer's dry yeast, castor oil, diphenoxylate (Kahira Pharm. and Chem. Ind. Co., Egypt), Normal saline 0.9% (El-Nasr Pharmaceutical and Chemical Co., Egypt).

3- Experimental animals

Adult male albino rats (each 100-120 g) were used. The animals were bred and housed under standardized environmental conditions in the pre-clinical animal house.

Extraction and isolation

2 Kg of the air-dried powdered leaves (Lvs) and ½ Kg stem bark (s.b.) were separately extracted (by maceration) with ethanol 70% at room temperature for 24 hours four successive times using 5L each (Lvs) and 2L each (stem bark) till exhaustion. The total ethanolic extract for each was separately concentrated under reduced pressure to give (130 g Lvs and 30 g s.b) residue, respectively and then separately subjected for successive solvent fractionation with n-hexane, chloroform and ethyl acetate, respectively. Each fraction was separately concentrated under reduced pressure to give the corresponding soluble, n-hexane (40 g Lvs and 8 g s.b.), chloroform (26 g Lvs and 5 g s.b.) and ethyl acetate (24 g Lvs and 12 g s.b.) fractions, respectively. Each of the obtained fractions was subjected to TLC study followed by chromatographic separation as follows:

1- Column chromatographic fractionation for 20 g of the n-hexane fraction of the leaves on silica gel (600 g, 150x5 cm) and elution with n-hexane and ethyl acetate with increasing polarity, gave three pure compounds (**1-3**) after rechromtographic and purification trials. TLC of the n-hexane fractions of s.b. (syst. I and II, spray with 10% H_2SO_4) against the isolated compounds from that of the leaves revealed the presence of compounds **1, 2** and **3** with the same R_f values and colour reactions.
2- Column chromatographic fractionation for 20 g of the chloroform fraction of the leaves on silica gel (600 g, 150x5 cm) and elution with gradient system of chloroform and methanol, gave five pure compounds (**4-8**) after rechromatographic and purification trials. TLC of the chloroform fraction of s.b. (syst. I and III spray with 10% H_2SO_4 and 5% $AlCl_3$) against the isolated compounds from that of the leaves, revealed the presence of compounds **4, 6, 7** and **8** with the same R_f values and colour reactions.
3- Column chromatographic fractionation for 20 g of the ethyl acetate fraction of the leaves on silica gel (600 g, 150x5 cm) and elution with gradient system of chloroform and methanol, gave

only two pure compounds (**9** and **10**) after rechromatographic and purification trials. While column chromatographic fractionation for 12 g of the ethyl acetate fraction of s.b. using silica gel (350 g, 120x4 cm) and elution with gradient system of chloroform and methanol afforded four pure compounds (**11-14**) after rechromatographic and purification trials.

Biological screening

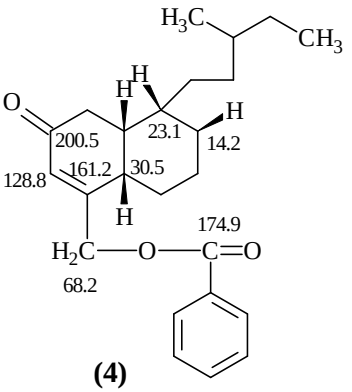
a) Determination of LD_{50} of the previously prepared extracts of the leaves according to the reported method,[24] using male albino rats. LD_{50} (g/kg) for n-hexane and $CHCl_3$ were eight while for EtOAc eleven and for MeOH ten.
b) Antidiarrheal activity: Adopting castor oil induced diarrhea method,[25] the activity of the methanolic extract of the leaves was measured in doses of (100, 200 and 400 mg/kg) suspeneded in 2% v/v aqueous Tween 80, orally), against diphenoxylate (5 mg/kg, orally) as standard antidiarrheal drug using five groups (x 6 animal).
c) Antiinflammatory activity: Adopting paw-oedema method,[26] where oedema was induced by s.c. injection of 20% w/v yeast aqueous suspension in normal saline in the left hind paw under the sub-planter region. The activities of the different extracts of the leaves were measured in doses of (200 and 400 mg/kg suspended in 2.5% Tween 80 in normal saline, orally) against indomethacin (8 g/kg) as standard anti-inflammatory using 10 groups (x 6 animal). The thickness of the paw of the tested animals was measured in mm using Varinier Caliber after 1, 2, 3 and 4 hours following the administration of the tested extracts.
d) Antipyretic activity: Using Brewer's yeast method,[27] where pyrexia was induced by s.c. injection of 20% w/v yeast aqueous suspension in normal saline in the left hand paw of the tested rats. The activities of the different extracts of the leaves were measured in doses of (200 and 400 mg/kg suspended in 2.5% Tween 80 in saline, orally) against indomethacine (8 mg/kg, orally) as standard antipyretic using 10 groups (x 6 animal). The rectal temperature of each rat was recorded at 1, 2, 3 and 4 hours after administration of the tested extracts.

Compound 1

White fine needles (ethanol), (30 mg), m.p 200-202°, R_f= 0.43 (sys. I). IR ν cm^{-1} (KBr): 3450, 2940, 1380 and 1037.

Compound 2

White needles (acetone), (50 mg), m.p 184-186°, R_f= 0.4 (sys. I). IR ν cm^{-1} (KBr): 3450, 2940, 1385 and 1037.



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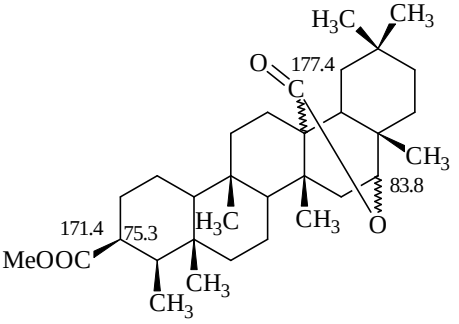

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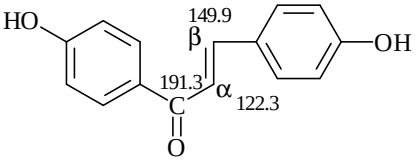

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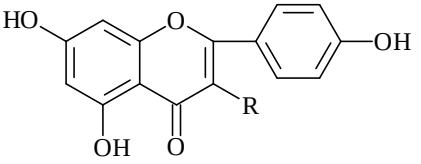
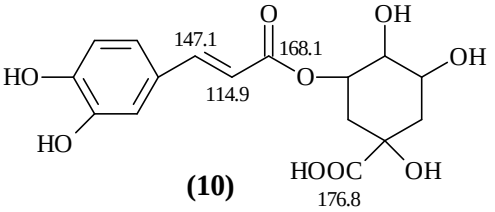


R = H **(7)**
R = OH **(8)**



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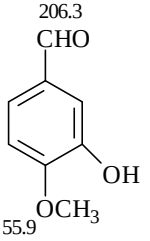

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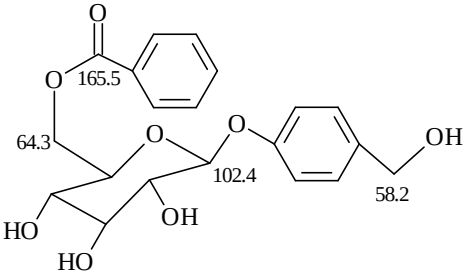

(14)

Compound 3

White needles (methanol), (200 mg), m.p 134-136°, R_f= 0.79 (sys. II).

Compound 4

Dark yellow oil, (40 mg), R_f= 0.71 (sys. II). IR ν cm^{-1} (KBr): 2945, 1765, 1646, 1520-1420, 1245 and 1056. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 0.89 (3H, t, J= 7.5 Hz, Me-15), 0.90 (3H, d, J= 7.5 Hz, Me-16), 0.92 (3H, d, J= 7.5 Hz, Me-17), 0.93 (3H, br.s, Me-20), 1.26 (3H, s, Me-19), 1.30-1.69 (m, CH_2 groups), 1.89 (1H, br.s, H-10), 3.16 (2H, t, J= 5.7 Hz, H-1), 5.90 (1H, br.s, H-3), 6.90 (2H, ABq, J= 8.80 Hz, H-18), 7.46 (2H, t, J= 7.5 Hz, H-3',5'), 7.52 (1H, dd, J= 7.5, 1.3 Hz, H-4') and 8.08 (2H, dt, J= 7.5, 1.3 Hz, H-2',6'). ^{13}C-NMR ($CDCl_3$, 125 MHz), δ: 11.0 (Me-15), 14.2 (Me-17), 20.9 (Me-16), 23.1 (Me-20), 23.9 (C-6, C-12), 26.7 (C-14), 28.9 (C-7), 29.7 (C-8, C-11), 30.5 (Me-19), 31.9 (C-31), 36.0 (C-1), 38.9 (C-6, C-9), 47.3 (C-10), 68.2 (C-18), 127.0 (C-3',5'), 128.8 (C-3), 130.2 (C-2',6'), 132.0 (C-1'), 136.0 (C-4'), 161.2 (C-4), 174.9 (O-C=O) and 200.5 (C-2). EIMS, no molecular ion peak, fragments at m/z; 325 (10%), 220 (2%), 135 (3%), 105 (10%), 85 (20%), 77 (31%) and 43 (39.86%).

Compound 5

White plates (methanol), (30 mg), m.p 322-324°, R_f= 0.87 (sys. IV). ^{1}H-NMR ($CDCl_3$, 500 MHz), δ: 0.80 (3H, d, J= 7.1 Hz, Me-23), 0.89 (3H, s, Me-25), 0.90 (3H, s, Me-30), 0.91 (3H, s, Me-24), 0.99 (3H, s, Me-29), 1.19 (3H, s, Me-26), 1.20 (3H, s, Me-28), 1.5-2 (m, -CH_2), 2.04 (3H, s, Me-acetoxy), 3.97 (1H, br.t, H-16) and 4.6 (1H, dt, J= 5.7, 2.8, 2.8 Hz, H-3). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 11.30 (Me-23), 15.80 (Me-24), 16.50 (C-7), 18.20 (Me-25), 18.30 (C-12), 20.70 (Me-26), 21.60 (Me-ester), 21.70 (C-1), 23.70 (Me-28), 28.30 (C-20), 30.41 (C-22), 30.90 (Me-29), 32.70 (C-2, 19), 36.70 (C-17), 36.80 (C-11, 21), 36.90 (Me-30), 37.60 (C-9), 39.30 (C-18), 39.4 (C-4, 14), 39.6 (C-15), 41.0 (C-6), 49.90 (C-4), 51.80 (C-13), 57.80 (C-8), 60.0 (C-10), 75.30 (C-3), 83.80 (C-16), 171.40 (C=O ester) and 177.40 (C-27). EIMS, no molecular ion peak, fragments at m/z (rel. int.): 439 (25%), 438 (69%), 423 (75%), 371 (18%), 370 (28%), 369 (16%) and 355 (11%).

Compound 6

Yellow amorphous powder (methanol), (30 mg), R_f= 0.65 (sys. IV). UV (λ_{max}, nm MeOH): 225, 346; +NaOMe: 248, 322 (sh), 317; +$AlCl_3$: 225, 346; +$AlCl_3$/HCl: 225, 346; +NaOAc: 240 (sh), 354; +NaOAc/H_3BO_3: 225, 245. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.83 (4H, d, J= 8.5 Hz, H-2',6', H-3',5'), 6.97 (4H, d, J= 8.5 Hz, H2,6, H3,5), 7.30 (1H, d, J= 15.3 Hz, H-α), 7.70 (1H, d, J= 15.3, H-β) and 10.20 (2H, s, OH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 112.70 (C-3,5), 118.20 (C-3',5'), 122.30 (C-α), 126.80 (C-1), 127.40 (C-1'), 128.60 (C-2,6), 131.50 (C-2',6'), 149.90 (C-β), 153.90 (C-4), 166.90 (C-4') and 191.30 (C=O). EIMS at m/z (rel. int.): 240 (0.6%),

239 (1.3%), 185.2 (40%), 148.2 (60%), 93 (100%) and 43 (30%).

Compound 7

Yellow amorphous powder (methanol), (20 mg), R_f= 0.53 (sys. IV). UV (λ_{max}, nm MeOH): 268, 338; +NaOMe: 274, 382; +$AlCl_3$: 278, 354, 384; +$AlCl_3$/HCl: 278, 354, 384; +NaOAc: 276, 338; +NaOAc/H_3BO_3: 268, 338. ^{1}H-NMR (CD_3OD, 400 MHz) δ: 6.21 (1H, d, J= 3.16 Hz, H-6), 6.46 (1H, d, J= 3.16 Hz, H-8), 6.59 (1H, s, H-3), 6.92 (2H, d, J= 8.7 Hz, H-3',5') and 7.85 (2H, d, J= 8.7 Hz, H-2',6').

Compound 8

Yellow amorphous powder (methanol), (30 mg), R_f= 0.50 (sys. IV). UV (λ_{max}, nm, MeOH): 270, 374; +NaOMe: 288, 442; +$AlCl_3$: 276, 434, 358; +$AlCl_3$/HCl: 274, 434, 358; +NaOAc: 276, 378; +NaOAc/H_3BO_3: 270, 374. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 6.17 (1H, br.s., H-6), 6.39 (1H, br.s., H-8), 6.87 (2H, d, J= 8.5 Hz, H-3',5'), 7.51 (2H, d, J= 8.5 Hz, H-2',6') and 12.48 (1H, s, 5-OH). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 93.30 (C-8), 98.20 (C-6), 103.0 (C-10), 115.60 (C-3',5'), 122.0 (C-1'), 129.40 (C-2',6'), 135.7 (C-3), 146.70 (C-2), 156.10 (C-9), 160.0 (C-4'), 160.70 (C-5), 164.0 (C-7) and 175.80 (C-4).

Compound 9

White granular powder (methanol), (500 mg), R_f= 0.37 (sys. IV). ^{1}H-NMR (C_5D_5N, 400 MHz) δ: 0.63 (3H, s, Me-18), 0.83, 0.85 and 0.90 (9H, m, Me-26, 27, 29), 0.89 (3H, s, Me-19), 0.97 (3H, d, J= 5.9 Hz, Me-21), 1.60-2.74 (m, CH_2 & CH), 3.94-4.60 (m, sugar protons), 3.99 (1H, m, H-3α) and 5.33 (1H, br.s., H-6). ^{13}C-NMR (C_5D_5N, 400 MHz) δ: 12.0 (Me-18), 12.1 (Me-29), 19.0 (Me-21), 19.2 (Me-26), 19.4 (Me-19), 20.0 (Me-27), 21.3 (C-11), 23.4 (C-28), 24.5 (C-15), 26.3 (C-23), 28.5 (C-16), 29.4 (C-2), 30.2 (C-25), 32.0 (C-8), 32.2 (C-7), 34.2 (C-22), 36.4 (C-20), 37.5 (C-18), 39.3 (C-4), 36.90 (C-10), 36.92 (C-12), 42.5 (C-13), 46.0 (C-24), 50.3 (C-9), 56.2 (C-17), 56.8 (C-14), 62.8 (C-6'), 71.6 (C-4'), 75.3 (C-2'), 78.4 (C-5'), 78.1 (C-3'), 78.6 (C-4), 102.3 (C-1'), 121.9 (C-6) and 140.8 (C-5). Acid hydrolysis: 5 mg of **9** in 5 ml MeOH and 5 ml N/2 methanolic sulphuric acid were refluxed for 3 hours, then the aglycone was extracted with $CHCl_3$ and purified and the produced sugar was identified by silica gel PC and system VI.

Compound 10

Yellowish brown amorphous powder (methanol), (50 mg), R_f= 0.3 (sys. V). UV (λ_{max}, nm, MeOH): 330. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.94-2.16 (4H, m, H-2,6), 3.67 (1H, dd, J= 8.5, 3.1 Hz, H-4), 4.11 (1H, m, H-3), 5.36 (1H, m, H-5), 6.27 (1H, d, J= 15.8 Hz, H-8'), 6.76 (1H, d, J= 8.2 Hz, H-5'), 6.94 (1H, dd, J= 8.2, 1.9 Hz, H-6'), 7.03 (1H, d, J= 1.9 Hz, H-2') and 7.54 (1H, d, J= 15.8 Hz, H-7'). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 38.5 (C-2), 39.9 (C-6), 72.3 (C-3), 72.4 (C-5), 74.3 (C-4), 77.7 (C-1), 114.9 (C-8'), 115.6 (C-2'), 116.5 (C-

5'), 123.0 (C-6'), 127.7 (C-1'), 146.8 (C-4'), 147.1 (C-7'), 149.6 (C-3'), 168.1 (C-9') and 176.8 (C-7). EIMS at m/z (rel. int.): M^+ 354.7 (15%), 191 (5%) and 163 (18%).

Compound 11

Pale yellow amorphous powder with fragrant odour (chloroform), (15 mg), R_f= 0.6 (sys. II). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 55.9 (OCH_3), 114.9 (C-5), 117.9 (C-2), 124.0 (C-6), 130.4 (C-1), 140.9 (C-3), 152.9 (C-4) and 206.3 (CHO).

Compound 12

White needles (methanol), (40 mg), m.p 122°, R_f= 0.5 (sys. II). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.48 (2H, t, J= 7.5 Hz, H-3,5), 7.62 (1H, dt, J= 7.5, 1.5 Hz, H-4) and 8.15 (2H, dd, J= 7.5, 1.5 Hz, H-2,6). ^{13}C-NMR ($CDCl_3$, 125 MHz), δ: 128.4 (C-3,5), 129.3 (C-1), 130.0 (C-2,6), 133.8 (C-4) and 172.4 (C=O). EIMS m/z (rel. int.) M^+ at 122 (92%), 105 (100%), 77 (82%) and 51 (50%).

Compound 13

Yellowish brown powder (methanol), (30 mg), R_f= 0.4 (sys. V). IR ν cm^{-1} (KBr): 3450, 1633, 620 and 473. ^{1}H-NMR (CD_3OD, 400 MHz) δ: 6.54 (1H, dd, J= 2.9, 8.5 Hz, H-6), 6.6 (1H, d, J= 8.5 Hz, H-5) and 6.75 (1H, d, J= 2.9 Hz, H-2). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 116.5 (C-5), 117.4 (C-2), 130.3 (C-6), 130.6 (C-1), 149.7 (C-3), 151.9 (C-4) and 170.1 (C=O).

Compound 14

White needles (acetone), (60 mg), m.p 212°, R_f= 0.3 (sys. V). UV (λ_{max}, nm, MeOH): 228 and 285. IR ν cm^{-1} (KBr): 3475, 2940, 1747, 1717, 1616, 1646, 1385 and 1038. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.98 (1H, br.s, phenolic OH), 7.96 (2H, dd, J= 7.8, 2.5 Hz, H-2,6), 7.69 (1H, t, J= 7.5, H-4), 7.55 (2H, t, J= 7.5 Hz, H-3,5), 6.88 (1H, d, J= 8.7 Hz, H-5"), 6.76 (1H, s, H-2"), 6.33 (1H, dd, J= 8.7, 2.6 Hz, H-6"), 4.61 (1H, d, J= 7.0 Hz, H-1'), 4.54 (1H, d, J= 15.1 Hz, H-7"a), 4.36 (1H, d, J= 15.1 Hz, H-7"b), 4.29, 4.36, 3.26 (m, sugar protons) and 4.39 (1H, br.s., benzoylic OH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 129.7 (C-1), 129.2 (C-2,6), 128.8 (C-3,5), 133.2 (C-4), 165.5 (C-7), 64.3 (C-6'), 71.1 (C-4'), 73.4 (C-2'), 73.8 (C-5'), 76.3 (C-3'), 102.4 (C-1'), 147.1 (C-1"), 133.4 (C-2"), 152.7 (C-3"), 116.9 (C-4"), 113.0 (C-5"), 113.8 (C-6") and 58.2 (C-7"). EIMS m/z (rel. int.), no molecular ion peak, 267 (2.2%), 140.1 (28.6%), 122.1 (100%), 105.1 (82%) and 77.1 (40.6%).

RESULTS AND DISCUSSION

The ethanolic extracts of the leaves and stem bark were separately concentrated and partionated between n-hexane, $CHCl_3$ and EtOAc. Column chromatography of the n-hexane fraction of the leaves provided compounds **1-3**. Compounds **1** and **2** were identified as β-amyrin and α-amyrin by comparison with authentic samples (mmp, IR, co-chromatography), while compound **3** was

proved to be a mixture of β-sitosterol and stigmasterol (where TLC of acetylated product on a wedged $AgNO_3$ impregnated silica gel (system III), showed two spots with the same R_f values).

Column fractionation of $CHCl_3$ fraction of the leaves provided compounds **4-8**.

^{1}H-NMR spectrum of compound **4** exhibited typical signals for bicyclic clerodane diterpene skeletone;[10&28] signals at δ 3.16 and 1.89 are suggested for H-1 and H-10 protons in 2-oxo-clerodanes,[10&28] in addition to five methyl resonances at δ 0.89-1.26 for two tertiary, two secondary and a primary one. ^{13}C-NMR spectrum displayed signals at δ 14.2, 23.1 and 30.5 which are characteristic for Me-17, Me-20 and Me-19 respectively of clerodane diterpene.[10&28] It also revealed a downfield signal at δ 200.5 assigned for C-2 with oxo-substitution[29&30] which was confirmed by IR band at 1646 cm^{-1}. The presence of benzoyl moiety was observed from ^{1}H-NMR and MS spectra where the former showed aromatic signals at δ 7.46 (2H, t, J= 7.5 Hz), 7.52 (1H, dd, J= 7.5, 1.3 Hz) and 8.08 (2H, dd, J= 7.5, 1.3 H) for monosubstituted benzene ring which was confirmed by four signals at δ 130.2, 127, 132 and 136 for C-2',6', C-3',5', C-1' and C-4' in ^{13}C-NMR spectrum; in addition to the appearance of mass fragments at m/z 105 for C_7H_5O. A mass fragment at m/z 85 indicated side chain of C_6H_{13} (fission at C-9/C-11 for clerodane),[10,28&31] in addition to another fragment at m/z 325, indicated that the benzoyl moiety could be attached to the bicyclic skeleton not to the side chain. The ester function detected from IR spectrum (band at ν 1765 cm^{-1}) was confirmed by a signal at δ 174.9 in ^{13}C-NMR. The benzoyl ester could be attached to C-18, depending upon its chemical shift in ^{1}H- and ^{13}C-NMR spectra (δ 6.90 and 68.2 respectively). Comparison of the obtained data with those previously published for a large group of related clerodane derivatives,[10,28&30] led to the assignment of its structure as 2-oxo-18-benozyloxy, 13(16), 14 tetra-hydrocleroda-3-ene.

Compound **5** gave positive colour test for triterpene lactones and/or esters.[32] Its ^{1}H-NMR spectrum revealed the presence of seven methyl groups at δ 0.89-1.2 in accordance with those of friedelane skeleton, which are common in Flacourtiaceae.[9&33] In addition to a signal at δ 4.6 (1H, dt, J= 5.7, 2.8, 2.8 Hz) assigned for H-3 oxomethine proton and the small coupling constant between H-3, H-4 and H-2 protons, indicated β-configuration of C-3 substituent.[34] In addition to a broad triplet at δ 3.97 for H-16[34] and a signal at δ 2.04 for Me-CO while ^{13}C-NMR and DEPT experiment showed signals for 32 carbon atoms, representing 8 methyl, 2 carbonyl, 10 methylene, 4 methine, 2 oxo-methine and 6 quaternary carbons. The spectrum also displayed signals attributed to two carbinols at δ 75.3 and 83.8 for C-3 and C-16 and

carbonyls, an ester one at δ 171.4 and a lactone one at δ 177.4, respectively. The MS data failed to show the molecular ion peak, but showed characteristic fragments at m/z 439 (M-ester) and other fragments diagnostic for friedo-oleanane triterpenes.[34] The previously reported data for some lactonized friedo-oleananes from other flacouriaceous plants revealed that the lactone at C-27 was joined to C-15α, but according to our data the lactone is attached to C-16α, consequently **5** could be identified as 3β-acetoxy-D:A friedo-oleanan-27,16α-lactone.[34]

Compound **6** gave positive $FeCl_3$ test, indicating its phenolic nature. Its UV spectral data suggested its chalcone nature;[35] a bathochromic shift upon addition of NaOMe and NaOAc indicated the presence of free 4-OH at ring B and free 4'-OH at ring A respectively, while absence of any shift with $AlCl_3$ indicated the absence of 6'-OH. ^{1}H-NMR spectrum displayed eight aromatic protons, attributed to two units of 1,4-disubstituted benzene rings, represented by two doublets with ortho coupling at δ 6.83 and 6.97 respectively. The two proton doublets at δ 7.3 and 7.7 with J= 15.3 Hz, indicated a *trans*-configuration of the ethylenic protons, characteristic for H-α and H-β of chalcones.[35] The signals at δ 10.2 for non hydrogen bonded OH, confirmed the absence of 6'-OH. The presence of one hydroxyl group in each ring was confirmed by EIMS and fragmentation pattern, where it showed M^+ at m/z 240 and characteristic fragments at m/z 148, 120 and 93. The ^{13}C-NMR spectrum showed eleven signals attributed to fifteen carbon atoms, with a downfield signal at δ 191.3 for C=O of chalcone skeleton, in addition to signals at δ 122.3 and 149.9 for C-α and C-β position.[36] It could be concluded that **6** is 4,4'-dihydroxy chalcone where its ^{1}H-NMR and MS spectral data are in accordance with those previously published.[37]

Compounds **7** and **8** gave positive colour reactions for flavonoidal aglycone.[38] The UV spectral data in methanol for **7** indicated its flavone nature while **8** was flavonol one. They were identified as apigenin and kaempferol by direct comparison of their spectral data[35&38] with literature data and co-chromatography with authentic samples.

Column chromatography fraction-ation of EtOAc fraction of the leaves provided compounds **9** and **10**.

Compound **9** gave positive colour reaction for sterols and positive Molish's test indicating its glycosidic nature. The IR spectrum showed broad band at 3450 cm^{-1} for hydroxyl groups and at 2960 cm^{-1} for C-H stretching. The ^{1}H-NMR spectrum showed signal at δ 5.33 for Δ^{5-6}. It also showed a multiplet at δ 3.99 attributed to H-3α. Two tertiary methyl groups (singlets) and four secondary ones are observed while the sugar protons appeared at δ 4.07-4.56 and the signal of the anomeric proton was hidden under the solvent signals. ^{13}C-NMR spectra of compound **9** showed signals for 35

carbon atoms. Six of them are methyl groups at δ range from δ 12.0-20.0 and an olefinic carbons at δ 140.8 and 121.9, in addition to the characteristic signals for B-glucose carbons. The other ^{13}C-NMR data were in a good agreement with those published for β-sitosterol-3-O-D-glucoside. Acid hydrolysis of **9** gave glucose as a sugar moiety and β-sitosterol as the aglycone which were identified by co-chromatography with authentic samples. From the aforementioned data, compound **9** is identified as β-sitosterol-3-O-D-glucopyranoside by comparison of IR, ^{1}H- and ^{13}C-NMR spectral data with the literature data,[39] direct comparison with authentic sample and acid hydrolysis followed by co-TLC for each of the algycone and sugar part with authentic samples.

^{1}H-NMR spectrum of compound **10** revealed the presence of three aromatic protons characteristic of 1,3,4-trisubstituted benzene ring at δ 7.03 (1H, d, J= 1.9 Hz), 6.94 (1H, dd, J= 8.2, 1.9 Hz) and 6.76 (1H, d, J= 8.2 Hz) for H-2', H-6' and H-5' respectively, in addition to two doublets at 7.54 and 6.27 with J= 15.8 Hz for two *trans*-olefinic protons, characteristic for caffeoyl derivatives.[40] Other signals at δ 5.39, 4.11, 3.67 and 1.94-2.16, attributed to quinic acid protons[41] were also observed. The ^{13}C-NMR spectral data displayed two characteristic signals for carbonyl carbons at C-9' and C-7 of the acidic moieties at δ 168.1 and 176.8, respectively, in addition to other characteristic signals. Comparison of the obtained spectral data with those previously published[40&41] leads to identification of **10** as 5-O-caffeoylquinic acid (chlorogenic acid).

Column chromatographic fractionation of the EtOAc fraction of s.b, provided four compounds (**11-14**).

Compound **11** gave positive $FeCl_3$ (T.S) test, indicating its phenolic nature, and it has a fragrant odour. Its ^{13}C-NMR spectrum exhibited six aromatic carbon signals, methoxyl signal at δ 55.9 and a signal at δ 206 indicating the presence of aromatic aldehydic group.[42] So it was identified as 3-hydroxy-4-methoxy benzaldehyde (vanillin) and confirmed by direct comparison with authentic sample (co-TLC).

^{1}H-NMR spectrum of compound **12**, showed monosubstituted benzene ring pattern and the ^{13}C-NMR showed five signals displayed for 7 carbons characteristic for benzoic acid,[42] which was confirmed by molecular ion peak at m/z 122 in EIMS and other characteristic fragments at m/z 105 and 77. These data leads to identification of **12** as benzoic acid which was confirmed by co-TLC with authentic.

Compound **13** gave positive $FeCl_3$ T.S, indicating its phenolic nature. Its ^{1}H-NMr spectrum revealed the presence of 1,3,4-trisubstituted benzene ring while ^{13}C-NMR displayed seven carbons, one of them at δ 170.1 characteristic for carboxylic carbonyl group. By comparing the available data was those previously published,[43]

compound **13** was identified as 3,4-dihydroxy benzoic acid (proto-catechuic acid).

^{1}H-NMR spectrum of compound **14** revealed the presence of eight aromatic protons, five of them have characteristic pattern for monosubstituted benzene ring (signals at δ 7.96, 7.69 and 7.55 respectively) and three other aromatic protons at δ 6.88, 6.78 and 6.33 for 1,3,5-trisubstituted benzene. Furthermore, a phenolic and alcoholic protons were observed at δ 6.8 and 4.93, beside sugar protons and anomeric proton appeared at δ 4.7 (d, J= 7.0 Hz) indicating its β-configuration.[43] EIMS showed a fragment at m/z 267 suggesting a benzoylated glucose moiety[44] with other fragments at m/z 122, 105 and 77. This was confirmed by acid hydrolysis which gave benzoic acid (co-TLC) and glucose (PC, sys. VI). The obtained ^{1}H-NMR and EIMS data are in accordance with those previously published for the phenolic glucoside ester flacourtin.[45] The ^{13}C-NMR confirmed the structure by appearance of 20 signals, seven of them are at δ 129.7, 129.2, 128.8, 133.2 and 165.5 (benzoic acid moiety), six for glucose (δ 102.4, 76.3 and 64.3) and seven for benzoyl alcohol. From the previous data compound **14** was identified as 3''-hydoxy-4''-hydroxy methyl phenyl-6'-O-benzoyl-β-D-glucopyranoside; flacourtin. To the best of our knowledge, this is the first compilation of the ^{13}C-NMR spectra of this compound.

Toxicological study, revealed that the n-hexane (a), $CHCl_3$ (b), EtOAc (c) and methanol (d) extracts of the leaves were safe to be used internally.

The methanol extract (d) of the leaves showed a significant and dose dependent anti-diarrheal activity (Fig. 1) nearly similar to reference drug diphenoxylate (5 mg/kg). The different extracts a, b and c exhibited a significant anti-inflammatory activity on yeast-induced oedema in a dose dependent way (200 and 400 mg/kg) ranged from intermediate potency in non polar extracts (a and b) to high potency with polar fractions (c and d) compared with indomethacin as a reference compound (Fig 2). While extracts (b and c) showed a well-marked antipyretic activity at different doses (200 and 400 mg/kg) for yeast induced fever compared with reference indoemthainc, extracts (a and d) showed no significant effect (Fig. 3).

In conclusion, it is the first report for isolation and identification of such constituents from the studied plant, the first report for isolation of compounds **4, 5, 7, 8** and **13** from the genus Flacourtia and the first report for isolation of compounds **6, 10, 11** and **12** from the family.

Since the preliminary phytochemical screening of the tested extractives and isolation of their contents, showed the presence of sterols and/or triterpenes and diversity of phenolic compounds, we can speculate that these constituents might be responsible for the observed

pharmacological effects, even if other studies are needed, the obtained result seems to support the use of the plant in phytotherapy.

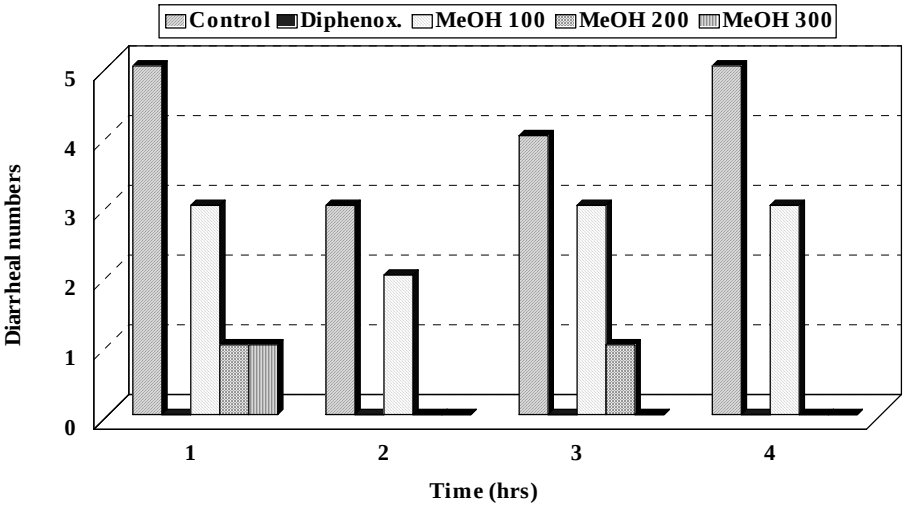

Fig. 1: The anti-diarrheal activity of *Flacourtia cataphracta* Roxb. leaves.

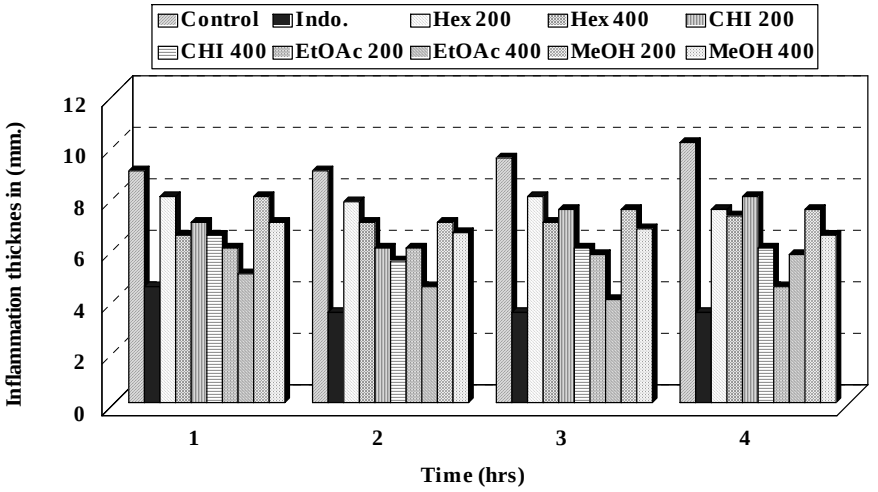

Fig. 2: The anti-inflammatory activity of *Flacourtia cataphracta* Roxb. leaves.

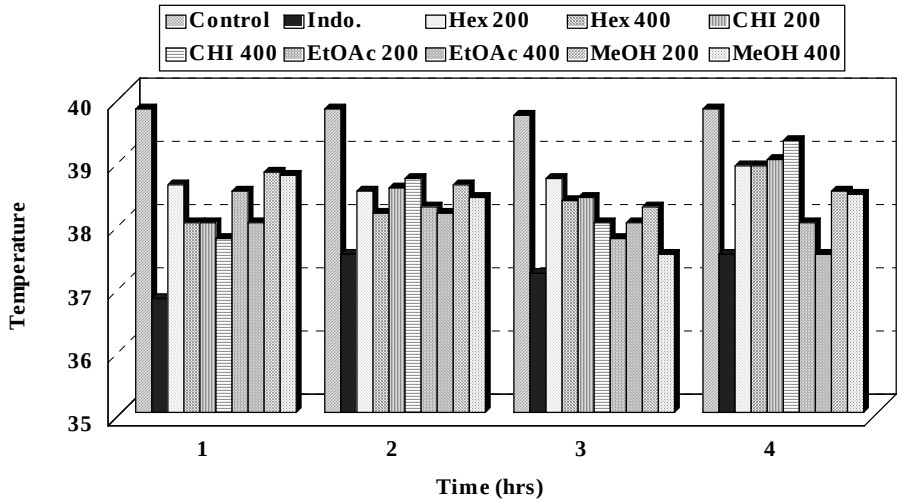

Fig. 3: The anti-pyretic activity of *Flacourtia cataphracta* Roxb. leaves.

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