

PHARMACOGNOSTICAL STUDY OF AVICENNIA OFFICINALIS L.
GROWING IN EGYPT

PART III: TERPENOIDS, FLAVONOIDS AND IRIDOIDS.

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ABSTRACT

From hexane extract of Avicennia officinalis L. Lupeol, betulinic acid, ursolic acid and betulin were isolated. In addition, 4',5'-dihydroxy, 3',7'-dimethoxy flavone, and 8-O-acetyl harpagid iridoid were isolated from ethyl acetate extract of the plant. The identification of these compounds, was based on, physical, chemical and spectral studies including UV, IR, ¹H-NMR and mass analysis.

INTRODUCTION

Avicennia officinalis L. is a typical mangrove genus of the family Verbenaceae. Mangroves are wide spread in tropical and subtropical zones of sheltered coastlines. Literature survey on *Avicennia officinalis* L. revealed the presence of lapacol, betulinic and ursolic acids, taraxerol, triacontane, lupenone and lupeol 1-3. Beside, the iridoids: avicennoside, 7-cinnamoyl-8-epiloganic, geniposidic and 2-cinnamoyl-mussanosidic acids as well as velutin flavonoid were also isolated from the plant grown abroad 4,5.

Previously the authors described the isolation and identification of 7-methoxy, 2-hydroxy isoflavan,

isorhamnetin-3-O-glucosyl-5-O-rhannoside, hispidulin, isorhamnetin-3-O-glucopyranoside, and rutin from the plant grown in Egypt 6. The present investigation was planned to continue study of the chemical constituents of the leaves of *Avicennia officinalis* L. grown in Egypt.

EXPERIMENTAL

General Experimental Procedure:

Melting points were determined on Gallenkamp apparatus and are uncorrected. UV-spectrum were obtained in MeOH on Pye-Unicam SP 1750 spectrophotometer, IR spectra were determined on Perkin-Elmer 599 B spectrophotometer in KBr pellets. ¹H-NMR spectra were recorded in (CD₃OD) on Bruker spectrometer at 400 MHz,

Mass spectra were measured using a Varian MAT-112 S double-focusing spectrometer, operating at 70 ev. Silica gel 60 (0.040-0.063 mm) and G 60 & F 254 (E-Merck) were used for column & TLC chromatography.

Plant Material:

The plant used in this study was the overground parts of *A. officinalis* L. collected from Red Sea Coastal region around Marsaalam, Egypt. Identification of the plant was kindly confirmed by Dr. A.El-Gazzar, Associate Professor of Botany, Faculty of Science, Cairo University.

Extraction:

The air-dried powdered leaves of *A. officinalis* L. (500 g), were extracted with 70% ethanol on cold. The semisolid residue obtained after concentration was digested with warm distilled water, and successively extracted with hexane and ethyl acetate.

Column Chromatography of the Hexane Extract:

The hexane extract (10 g) was chromatographed over silica gel column (E-Merck, 300 g, 120x2.5 cm), eluted with pet. ether and then with mixtures of pet. ether with increasing amounts of ethyl acetate. Fractions collected (50 ml each) were monitored by TLC silica gel G, (pet. ether-ethyl acetate 9:1 v/v). The pet. ether eluate yielded compound 1, $R_f=0.96$. Further elution of the column with pet. ether-ethylacetate (9:1 v/v), yielded two crystalline components (2 and 3) $R_f=0.43$ and 0.32 respectively.

The pet. ether-ethyl acetate eluate (7:3 v/v) yielded compound (4), $R_f=0.13$.

Investigation of Ethyl Acetate Extract:

Column chromatographic fractionation of ethyl acetate extract (15 g) on silica gel column (E-Merck, 400 g, 120x3 cm) chromatography was performed eluting with chloroform and then with mixture of chloroform with increasing amounts of methanol.

The fractions collected (100 ml each) were screened by TLC on silica gel plates and chloroform-methanol (7:3 v/v) as solvent system. Two compounds 5 and 6 $R_f=0.32$ and 0.45 were isolated and identified.

Compound 1 (60 mg):

Colourless needle crystals, mp. 208-10°C (MeOH). It gave positive Liebermann-Burchard's test. IR showed bands at: 3300 (alc. OH), 2970, 2858 and 1640 cm^{-1} . An acetyl derivative of the compound was prepared using acetic anhydride and pyridine.

On crystallization from methanol. It melted at 217-19°C and showed no depression in mp. on admixture with authentic lupeol acetate. The mobility of the parent compound and its acetate on a TLC plate of silica gel G in different solvent systems was the same as lupeol and its acetate respectively, thereby confirming the identity of the compound as lupeol.

Compound 2 (80 mg):

White plates, mp. 280-83°C (MeOH). It gave positive Liebermann-Burchard's and Salkowski's tests.

IR showed the following bands at: 3320 (alc. OH), 2950 (C-H stretch), 1690 (C=O), 1390 (C=C) and 1380 cm^{-1} . Ms showed $[M^+]$ at m/z 456 (30%), 438 $[M^+-H_2O]$, 411 $[M^+-$

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COOH], 393[M⁺-H₂O, COOH], 248(31.2), 220(20), 207(51.5) and 189(100).

Compound 3 (100 mg):

Fine needles mp. 276-79°C (MeOH). It gave positive Liebermann-Burchard's test and blue colour with H₂SO₄. IR: 3440(alc. OH), 1680(C=O), 2950, 1450 and 1360 cm⁻¹. MS showed [M⁺] at m/z 456(7.1%), 438[M⁺-H₂O], 411[M⁺-COOH], 393[M⁺-H₂O, COOH], 248(100) and 208(16.2).

Compound 4 (30 mg):

Needle crystals, mp. 220-22°C (MeOH), it gave positive Liebermann-Burchard's test. IR showed 3500(alc. OH), 1705, 1640, 1390, 1370, 1360 and 890 cm⁻¹. MS showed [M⁺] at m/z 442 other peaks at m/z 427(8%) [M⁺-CH₃] and 424(32) [M⁺-H₂O].

The above mentioned physical, chemical and spectral data of compound 4, are similar to those reported for triterpene alcohol betulin⁷.

Compound 5 (20 mg):

Yellow crystals, mp. 220-22°C (MeOH). It showed positive test for flavonoids¹⁰ and positive shinoda test. The IR spectrum displayed bands at 3410(OH), 3080(C-H), 1650, 1560 and 1170 cm⁻¹. The UV (MeOH) 250, 268, 350; +(NaOAc+H₃BO₃) 270, 347; +(AlCl₃) 275, 360, 390; +(AlCl₃/HCl) 270, 355; +(NaOMe) 260, 405; +(NaOAc) 260, 410 nm.

Compound 6 (100 mg):

Needle crystals, mp. 160-63°C (MeOH). It gave a violet-brown colour with 15% SbCl₃ in CHCl₃ and red

colour with anisaldehyde H₂SO₄. IR showed bands at: 3350, 2900, 1650, 1370, 1050 and 1010 cm⁻¹.

¹H-NMR (CD₃OD, 400 MHz) showed: δ 1.45(3H,s,CH₃); 2.01(3H,s,O-C-CH₃) 2.84(1H,s,H-9); 3.6(1H,d,J=4.4 Hz,H-6); 4.86(2H,d,J=4.4 Hz,H-7); 4.91(1H,d,J=6.4 Hz,H-4); 5.07(1H,s,H-1), 6.37(1H,d,J=6.4 Hz,H-3); Anomeric protons at δ 4.58(1H,d,J=7.3 Hz,H-1') and at 3.9(1H,d,J=12 Hz,H-6').

RESULTS AND DISCUSSION

The air-dried powdered leaves of *Avicennia officinalis* L. were extracted with ethanol on cold and the semisolid residue obtained after concentration was digested with warm distilled water and fractionated with hexane and ethyl acetate. Both the hexane and ethyl acetate extracts were separately fractionated over silica gel columns to give six pure compounds.

For compounds (1-4) were isolated from the hexane extract and two compounds (5-6) were isolated from the ethyl acetate extract.

Physical, chemical, IR spectra, acetate derivatives and co-chromatographic investigations of compounds 1 and 4 with authentic samples were found to be identical with those of lupeol and betulin respectively.

IR spectrum of 2 revealed the presence of alc. OH and C=O functional groups. MS spectral analysis revealed [M⁺] at m/z 456 other characteristic fragments at m/z 248, 220, 219, 218 and base peak at m/z 189 which are characteristic for lupan series of triter-

penes ⁷. From the a formentioned spectral analysis in addition to physical and chromatographic characters, compound 2 was found to be betulinic acid.

The IR spectrum of compound 3 is similar to that of 2. MS spectral analysis showed $[M^+]$ at m/z 456. other characteristic peaks at m/z 235, 303, 191, 189, 133 and a base peak at 248 which are characteristic for ursane series of triterpenes.

From the above physical, chemical and spectral studies of compound 3 in addition to co-chromatography with authentic sample, compound 3 was found to be identical with those reported for ursolic acid ⁷.

By chromatographing the acetate extract over silica gel column. two compounds were isolated. Compound 5 was proven to be flavone in nature, brown spot under UV and λ_{max} at 250, and 350 nm. Compound 6 gave a violet-brown spot with $SbCl_3$, red colour with anisaldehyde H_2SO_4 and positive Trim-Hill reagent ⁸. The spectral analysis showed a bathochromic shifts (+55 nm) in (NaOMe) and (+40 nm) in ($AlCl_3$) confirming the presence of OH groups at 4' and 5 positions respectively.

The presence of free OH-group at C-5 was confirmed by giving a deep yellow fluorescence with boric and citric acids ⁹ and green colour with $FeCl_3$ ^{10,11}.

Comparing these data with those reported for 4',5-dihydroxy, 3',7-dimethoxy flavone (velutin), it was superimposable ^{2,12}.

Preliminary chemical tests confirmed that compound 6 is an iridoid glycoside. It exhibits a green-brown

reaction with vanillin reagent. Its IR spectrum showed a band at 1650 cm^{-1} attributable to the conjugated iridoid system ¹³.

The ¹H-NMR spectrum confirm its iridoid structure and of harpagid type, which showed a signal at 1.45 ppm integrating for protons due to methyl group and at 2.01 ppm for acetyl group. The signal at 3.6 ppm, corresponding to one proton at H-6. If C-6 is substituted with acetyl group it will be downfield ¹⁴, also H-9 at 2.84 ppm, which agrees with that reported for 8-acetyl iridoids ¹⁴.

The anomeric protons at 3.9 and 4.58 ppm with coupling constant 12 and 7.88 Hz for H-6' and H-1' respectively, the H-6 and H-7 are coupled with each other at 3.6 and 4.86 with coupling constant around 4.4 Hz, this confirmed the presence of OH group at C-6.

The previous physical, chemical and spectral studies proved that compound 6 has one methyl and one acetyl located at C-8, and two OH groups at C-5 and C-6.

So that compound 6 was found to be identical with those reported for 8-acetyl harpagid ¹⁵.

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ACKNOWLEDGEMENT

The authors express their thanks and appreciation to Prof. Dr. H.Itokawa, Tokyo College of Pharmacy, Tokyo, Japan, for the spectral analysis of the isolated compounds.

دراسة عقاقيره لنبات الافسينا اوفسينال ل

الذى ينمو فى مصر

الجزء الثالث: التربينات، الفلافونيدات الايريديديات

عفاف محمد عبد الباقي - مقبول أحمد مقبول - داود ونيس بشاى

وسمير أنيس روس

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تم استخلاص الاوراق بالكحول الايثيلى وبعد تركيز الخلاصه تم الاستخلاص المتتابع مستخدما الهكسان وخلات الايثيل.

من خلاصة الهكسان تم فصل ليوبيلول، حمض يورسولك، حمض بتيولينك وبتيولين وذلك باستخدام عمود من هلام السليكا.

كما تم فصل فلافونيد حر ٤، ٥ ثنائى هيدروكسى ٣، ٧ ثنائى ميثوكسى فلافون "فليوتين" وكذلك ايريديويد ٨- استيل هيرباجيد.

وقد تم التعرف على هذه المركبات باستخدام الطرق الكيمياء الحديده مثل الاشعه فوق البنفسجيه ودون الحمراء والرنين النووى المغناطيسى ومطياف الكتله كما تم تحضير بعض خلات هذه المركبات.