Zag. J. Pharm. Sci., Vol. 3, No. 1, April 1994

PHYTOCHEMICAL INVESTIGATION OF OCHRADENUS BACCATUS DEL. GROWING IN EGYPT

Taha M. Sarg, Samia S. Hafez, Mahmoud M. Abd El-Aal and Amal A. Al-Gendy

Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt

ABSTRACT

Column chromatography of the USM yielded : long chain alcohol, lupeol, lanostane derivative and phytosterol mixture. GC-MS of the USM acetates showed the presence of 7-stigmastenol and 7-ergostenol. GC of the methylated fatty acids revealed the presence of 16 fatty acids. Chloroform extract yielded ß-sitosterol glucoside. The ethyl acetate extract afforded apigenin, kaempferol, quercetin, kaempferitin and rutin. The identification of these compounds was verified through spectral analysis and comparison with authenti samples.

INTRODUCTION

The genus Ochradenus (family Resedaceae) is represented in Egypt by one species only. This genus comprises six species distributed all over the world[1]. Many plants belonging to family Resedaceae exhibit anti-inflammatory activity [2] most probably is due to the fatty acids and steroidal contents [3,4]. It also exhibit antimicrobial activity [5] and as powdered hair dye [6].

In a previous phytochemical study of this plant we reported the isolation and identification of four different glucosinolates [7].

In the present work, we report the isolation and characterization of steroidal and flavonoidal contents of this plant.

EXPERIMENTAL

Plant material :

The wild flowering plant was collected in March (1992) from Eastern desert near Suez and from Sinai desert near to Mettla Valley. The identification was kindly verified by Prof. Dr. Nabil El-Hadidi, Professor of Taxonomy, Faculty of Science, Cairo University. A voucher specimen is deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University.

Methods and apparatus :

IR spectra were recorded on IR spectrometer, Perkin-Elmer 1430; Mass spectra were recorded on MS Hewlett-Packard 5988; UV spectra were recorded by Shimadzu UV 260. GC analysis using GC - MS HP 5988 under the following conditions:

Detector temp: 280°C, Injector temp: 250°C, Ion source temp: 200°C, Temp rate: 6°C/min, Column dimension: 12 m x 0.2 mm (capillary column), Carrier gas: Helium, Column: HP, 100% dimethyl poly syloxane.

Solvent systems :

I : Chloroform

II : Light petroleum - $CHCl_3$-MeOH (15:15:1)

III : Light petroleum - $CHCl_3$-glacial HAc (75:25:0.5)

IV : $CHCl_3$ - MeOH (9:1)

V : BuOH - HAc - H_2O (4:1:5)

VI : Benzene - EtOAc : Formic acid (5:4:0.5)

VII : EtOAc - HAc - Formic acid - H_2O (100:11:11:27)

Extraction and fractionation :

The air dried powdered plant (4 kg) was extracted on cold with ethanol (80%) until complete exhaustion (20 L). The concentrated total extract (150 g) was suspended in one litre of aqueous ethanol (1:1) and extracted with light petroleum chloroform and ethyl acetate to afford fractions 95, 4 and 5.5 g, respectively .

Investigation of the light petroleum extract :

A weight of 30 g of the light petroleum extract was subjected to saponification [8] to give 15 g USM and 3 g of free fatty acid contents.

Chromatography of the unsaponifiable matter :

About 10 g of the unsaponifiable matter was chromatographed on silica gel column (4x100 cm, 200 g) and gradiently eluted with light petroleum. The polarity was increased with benzene, chloroform and finally methanol. The eluted fractions (200 ml each) were monitored by TLC using solvent system I and anisaldehyde/sulphuric acid for visualization. Similar fractions were collected to give the following compounds :

Compound 1 :

Fractions 24-26 eluted by light petroleum-benzene (7:3), yielded (30 mg) white needles (chlorform - methanol) with R_f 0.7 (solvent system I) and m.p 85-87°C; IR: ν_{max} ($CHCl_3$), 3500-3250, 2990-2850, 1500, 1468, 1415-1380, 1240-1200, 1070, 940 and 860 cm^{-1}; MS : m/z (relative int.%) : 312 [M^+, 1.1), 297 (1.2), 284 (2), 269 (1.8), 256 (5.3), 241 (5), 227 (5.5), 213 (13.4), 199 (3.6), 185 (9), 171 (7.7), 157 (6), 143 (3.9), 129 (33), 115 (13.8), 101 (8.6), 87 (1.5), 73 (100), 60 (21.3), 43 (89) and 29 (24.5).

Compound 2 :

Fractions 31-33 eluted by light petroleum-benzene (6:4), gave 650 mg of white needles crystals (chlorform - methanol), with m.p. 212-214°C; R_f 0.65 (solvent system I). IR: ν_{max} (KBr) 3581-3200, 2940, 2871, 1646, 1463, 1450, 1372, 1200, 1184, 1138, 1083 and 1047 cm^{-1} , MS : m/z (relative int.%) : 426 (M^+, 35), 408 (3.5), 393 (2.8), 257 (8), 218 (31), 207 (83), 203 (24), 189 (100), 175 (25.5), 149 (35), 147 (31), 109 (74), 105 (35) and 95 (78). Its acetyl derivative, needle crystals ; m.p. 215-217°C; R_f 0.95 (solvent system II); IR: ν_{max} : 2945, 2856, 1730, 1600, 1463, 1454, 1378 and 1247 cm^{-1}.

Compound 3 :

Fractions 39-42, eluted by light petroleum - benzene (6:4), to give white scales (35 mg , chlorform - methanol) with m.p. 154-157°C R_f 0.53 (solvent system I). IR : ν_{max} (KBr) : 3525-3250, 2960, 2870, 1640, 1520, 1465, 1450, 1379, 1125-1062, 750 and 640 cm^{-1} ; MS : m/z (relative int.%) : 440(M^+, 5.3), 425 (4.1), 328 (5.5), 313 (4.6), 285 (36.6), 273 (2.6), 245 (5.5), 227 (6.3), 203 (4.6), 189 (6.1), 175 (6.6), 147 (13.4), 123 (23.8), 121 (21.6), 109 (33), 107 (21), 105 (21), 55 (100).

Compound 4 :

Fractions 51-61 eluted by benzene, gave white needles (chlorform - methanol), (715 mg); m.p 133-135°C; R_f 0.45 (solvent system I). IR : ν_{max} (KBr) 3500-3250, 2956, 2870, 1635, 1463, 1450, 1380, 1328, 1082, 1060, 960 and 866 cm^{-1}; MS : m/z (relative int.%) : 414 (M^+, 17), 412 (M^+, 39), 396 (7), 381 (5), 303 (4), 300 (21), 271 (25), 255 (38), 229 (7.8), 213 (18), 185 (7), 173 (11),

145 (31), 133 (34), 107 (39), 95 (42) and 55 (100). Its acetyl derivative, colourless needle shape crystals (methanol), m.p 126-128°C, R_f 0.23 (system III (9), reactive TLC (10), and antimony trichloride reagent). IR: ν_{max} ($CHCl_3$) 3000, 2900, 1720, 1600, 1465, 1377, 1230-1200, 1040 and 925 cm^{-1}.

GC/MS of the acetyl derivative of USM revealed (in addition to previously isolated compounds) the presence of two more peaks, No. 28 (R_t 31.3 m); MS : m/z (relative int.%) : 442 (M^+; acetate, 42), 427 (12), 382 (46), 367 (37), 255 (17), 213 (30), 185 (23), 173 (33), 159 (56) and 147 (87), and peak No. 30 (R_t 32.5 m) with MS : m/z (relative int.%) : 396 (M^+ acetate, 91), 381 (42), 288 (21), 255 (29), 213 (34), 185 (15), 173 (22), 161 (36), 147 (100), 145 (36) and 133 (55).

Investigation of the fatty acids :

The methyl esters were analysed by GLC, the quantitative analysis was carried out by the peak area measurements and the results obtained are shown in table (1).

Investigation of the chloroform extract:

The chloroform extract (4 g) was chromatographed on silica gel column (2x100 cm, 120 g), in benzene. Elution was started with benzene and the polarity was increased by chloroform and finally with methanol. Fractions of 200 ml were collected, screened by TLC and similar fractions were collected and concentrated to afford compound **5**.

Compound 5 :

Fractions 64-73 eluted by chloroform-methanol (98:2), gave white needles (methanol), with m.p. 281-283°C, R_f 0.53 (System IV). It gave positive Molisch's test; IR: ν_{max} (KBr) 3500-3200, 2922, 2852, 1646, 1463, 1450, 1377, 1278, 1118-1071 and 719 cm^{-1}. Acid hydrolysis with 5% H_2SO_4 yielded and aglycone having the same R_f and m.p (138-142°C) of ß-sitosterol and glucose as the sugar part (R_f 0.25, System V).

Chromatographic investigation of the ethyl acetate extract :

About 4.5 g of ethyl acetate extract was chromatographed on silica gel column (2.5 x 120 cm, 130 g). Elution was started with benzene and the polarity was increased by ethyl acetate to methanol. Fractions each of 250 ml were collected, concentrated and examined by TLC, similar fractions were combined and the following compounds were isolated.

Compounds 6,7 and 8 :

Fractions 6-12 eluted by benzene - ethyl acetate (1:1), (0.9 g); preparative TLC (System VI) yielded three compounds with R_f values of 0.86, 0.79 and 0.68.

Compound 6 : 150 mg, yellow sandy crystals (methanol), m.p. 350-352°C, R_f 0.86 (System VI).

Compound 7 : 70 mg, yellow needle, (methanol), m.p 279-281°C, R_f 0.79 (System VI).

Compound 8 : 150 mg yellow needles (methanol), m.p 316-318°C, R_f 0.68 (System VI).

Isolations of compounds 9 and 10 :

Fractions 26-35 eluted by ethyl acetate-methanol (7:3)to yield (2.5 g), two major spots R_f 0.54 and 0.47 (system VII). By PTLC compounds **9** and **10** were isolated.

Compound 9 : Whitish yellow rosetts (methanol), 180 mg, m.p. 200-203°C, R_f 0.54 (System VII), gave positive Molisch's test.

Compound 10 : Yellow sandy crystals (methanol), 200 mg, m.p 190-192°C, R_f

Table (1) : Results of GLC analysis of fatty acids methyl esters.

Retention time	Area %	No. of carbon & double bond	Systemic name	Trivial name
1.4	1.35	6 : 0	hexanoic	caproic
1.81	0.14	7 : 0	heptanoic	enanthic
2.43	4.38	8 : 0	octanoic	caprylic
4.46	5.24	10 : 0	decanoic	capric
5.81	4.84	11 : 0	hendecanoic	-
7.35	7.85	12 : 0	dodecanoic	lauric
8.85	1.22	13 : 0	tridecanoic	-
10.6	10.45	14 : 0	tetradecanoic	myristic
13.8	14.26	16 : 0	hexadecanoic	plamitic
15.11	1.41	17 : 0	heptadecanoic	margaric
16.77	8.37	18 : 0	octadecanoic	stearic
17.92	12.37	18 : 1	cis-9-octa- decanoic	oleic
18.69	11.95	18 : 2	9,12, octadecadienoic	linoleic
19.58	9.85	18 : 3		linolenic
20.65	1.68	21 : 0	heneicosanoic	-
21.81	0.54	22 : 0	docasanoic	behenic

0.47 (System VII) and gave positive Molisch's test.

The UV and MS analysis of the isolated compounds are presented in table (2).

Acid hydrolysis of compound 9 and 10:

About 30 mg, of compound **9** in 7% H_2SO_4 yielded an aglycone with m.p 2.79-281°C and UV spectral analysis similar to those of compounds **7**. The sugar part was screened by TLC alongside reference sugar samples and sprayed with aniline phthalate, gave a spot with R_f 0.43 (System V) identical with rhamnose.

Acid hydrolysis of compound **10**, yielded an aglycone with m.p 316-318°C, UV spectra were similar to those of compound **8** and sugar parts with R_f 0.43 and 0.24 (System V) were identical with those of rhamnose and glucose.

DISCUSSION

The aqueous ethanolic extract of Ochradenus baccatus Del. was partitioned into light petroleum, chloroform and ethyl acetate fractions.

The USM yielded lupeol **2**, phytosterol **4** Identification of these compounds were established by their m.p., ir, ms as well as by direct comparison with authentic samples (m.p, m.m.p., co - tlc) and preparation of their acetyl derivatives (mp, ir). The light petroleum extract was chromatographed to yield compounds **1** and **3**.

Compound **1** was identified as long chain alcohol through its IR analysis (peaks at 3500-3250 cm-1 characteristic for -OH group). The MS showed successive loss of 14 units for CH_2. It was given a molecular formula $C_{21}H_{44}O$.

Compound **3** was identified as lanosta - 7,9 (11), 24-triene - 3B, 21-diol

Table (2) : *Ultraviolet* and mass spectral data of compounds 6-10

Compound		UV						MS
		MeOH	NaOMe	$AlCl_3$	$AlCl_3$/ HCl	NaOAc	NaOAc/ H_3BO_3	m/z (rel. int. %)
6	Band II	267	275	277,301 (sh)	277,299	275,302 (sh)	267,300 (sh)	270 (M^+,100), 257(10), 242 (11), 153(24), 152(12), 124(13), 121 (171), 118 (11), 112 (4).
	Band I	336	324,392	348,380	340,375	350	340	
7	Band II	265	274	268,303 (sh)	269,303 (sh)	271	265	286 (M^+,4), 257(4), 242 (10), 185(14), 153(53), 152(15), 136 (16), 124(18), 123(18), 118(11), 107(38).
	Band I	366	350,442	347,425	350,423	345,435	340,430	
8	Band II	255,270 (sh),300 (sh)	272	270,304 (sh)	265,302 (sh)	270	261	302 (M^+,8), 153(14), 152(12), 137 (16), 124(24), 123(23), 118(14).
	Band I	372	445	350,435	359,420	345,440	425	
9	Band II	267	275,322 (sh)	270,300 (sh)	267,300 (sh)	267,325 (sh)	267,322 (sh)	578 (M^+,10), 557(62), 388(100), 303(16), 286(27), 258(10), 242 (14), 152(0.2), 124(11).
	Band I	340	385	346	343	345	342	
10	Band II	258	277	268,295 (sh)	268,295 (sh)	265	258	610 (M^+,0.02), 575(10), 557(28), 388(37),302(0.09), 301(0.07), 287 (4), 153(0.4), 123(5).
	Band I	355	414	358,397	391	359	376	

through IR (band for OH at 3525-3250 and 1125-1062 cm^{-1} for C-O). The mass spectrum gave an evidence that compound **3** is lanostane derivative with 13,14-dimethyl groups with the characteristic fragments 440 (M^+), 425 ($M^+ - CH_3$), 328 (M^+ - 112), 313 (M^+ - 127) and given the molecular formula $C_{30}H_{48}O_2$.

GC- MS of the acetylated USM revealed the presence of 7-stigmastenol and 7-ergostenol by direct comparison with published data (11). Fatty acids methyl esters were analysed by GC and results revealed the presence of 16 fatty acids the main components were lauric (7.8), palmitic (14.2), stearic (8.4), oleic (12.4) and linoleic (11.95).

Column chromatography of the chloroform extract yielded ß-sitosterol glucoside. Its structure was proved by IR, acid hydrolysis and comparing the resultant aglycone and sugar part with authentic ß-sitosterol and glucose respectively.

Chromatographic fractionation of the ethyl acetate extract on silica gel column yielded five compounds. The colour reactions with NH_4OH as well as $FeCl_3$ and conc. H_2SO_4 indicated the flavonoidal nature of these compounds. Compounds **6,7** and **8** gave negative Molisch's test indicating their aglycone nature.

Compound **6** was proved to be apigenin from the MS and UV analysis (Table 2). The UV analysis showed a flavone nature (MeOH) and a bathochromic shift by addition NaOMe (56 nm, B and I) without decrease in intensity this indicated 4-OH, also $AlCl_3/HCl_3$ gave bathochromic shift, band I (44 nm) for free 5-OH. Free 7-OH was proved by addition of NaOAc; MS showed M^+ at m/z 270 (100) for $C_{15}H_{10}O_5$ and fragments at m/z 242, 153 and 121 proved the presence of a flavone with one OH at ring B.

Compound **7** was identified as kaempferol by its MS analysis, M^+ at m/z 286 for $C_{15}H_{10}O_6$, other fragments at m/z 153 and 136. The UV analysis (Table 2) proved the presence of a flavone skeleton (MeOH) also, exhibited a bathochromic shift for band II by addition NaOAc (+ 6 nm) for free 7-OH NaOMe caused bathochromic shift, band I (76 nm) for free 4-OH

Compound **8** identified as quercetin, by UV and mass spectral analysis (table 2). The UV indicated the presence of flavonol skeleton (MeOH), NaOMe caused bathochromic shift for band I (73 nm) for ortho- dihydroxy-system. This was confirmed by bathochromic shift with $NaOAc/H_3BO_3$, band I (53 nm), free 5-OH was proved by $AlCl_3/HCl$ shift.

The mass showed M^+ at m/z 302 and other fragment at m/z 152 for two hydroxyls at ring A. Compound 9 and 10 were identified as kaempfretrin and rutin respectively through MS, UV acid hydrolysis and comparison of the aglycones and sugar parts with authentics.

REFERENCES

(1) Abdallah, M. (1967). "The Resedaceae, A Taxonomical Revision of the Family" Laboratory of plant taxonomy and plant geography. Agricultural University, Wageningen, The Netherland, 12, 50.

(2) Pierre, M., Fr Demande. F.R., 2, 581, 310 **(1986).**.

(3) Susplugas, Taillade, C.; Susplugas, P. and Michel, F., Pharm. Acta. Helv. 63 (2), 59-63 **(1988).**

(4) Pierre, M. and Lucas, T. Fr Demand F.R.m 2, 649, 322 **(1991).**

(5) Melkumyam, J. and Nauk, A., Biol.SSR, 16 (9), 83-8 Russian **(1963).** throuth C. A. 60 : 9601a (1964)

(6) Rosenbaum, G.; Catteret, J.; Grollier, J. and Francois, C., Fr. Appl. 81(3), 946, 23 **(1981).**

(7) **Sarg, T.M.; Hafez, S.S.; El-Ayouty, M. and Al-Gendy, A.A.,** Acta Pharm. Hung. Under Publication **(1994).**

(8) **El-Said, F. and Amer, M.,** "Oil, Fats, Waxes and Surfactants" Anglo - Egyptian Book, Cairo, p.130 **(1965)**

(9) **Ikan, R.,** "Natural Products", Academic Press Inc., London, New York, and San Francisco **(1976).**

(10) **Stock, R. and Rice, C.,** "Chromatographic Methods" , 2nd Ed., Science Paper Backs, Chapman and Hall, London, p. 111 **(1976).**

(11) **Sihua, X.; Patterson, W. and Schmid, K.,** Phytochemistry, 25, (8) 1883 **(1986).**

دراسة المحتويات الكيميائية لنبات اوكرادينس بكاتس دل (جرثى) الذى ينمو فى مصر

طه مصطفى سرج - سامية صلاح حافظ - محمود محمد عبدالعال وامل امين الجندى

قسم العقاقير - كلية الصيدلة - جامعة الزقازيق - مصر

فى هذا البحث تم استخلاص النبات بواسطة الكحول المخفف وتجزئة الخلاصة الى الاثير البترولى وخلاصة الكلوروفورم وخلاصة خلات الإثيل ولقد تم تحليل هذه الخلاصات باستخدام كروماتوجرافيا العمود كلا على حده وقد تم فصل والتعرف على ستة مركبات من خلاصة الاثير البترولى وهى كحول طويل السلسلة ، اللوبيول ، احد مشتقات اللانوستان ، الفيتوستيرول ، ٧-ارجوستينول و ٧-ستجماستينول. ومن خلاصة الكلوروفورم أمكن فصل بيتا سيتوستيرول جلوكوزيد .

ومن خلاصة خلات الايثيل امكن فصل خمسة فلافونيدات وهى الابيجينين ، الكامفيرول ، الكوارستين ، الكامفورترين والروتين ، كما تم التعرف على ١٦ من الاحماض الدهنية الموجودة فى النبات .

وقد تم تحديد التركيب الكيميائى للمركبات باستخدام التحاليل الطيفية المختلفة متمثلة فى التحليل الطيفى بالاشعة الحمراء ، طيف الكتلة وايضا باستخدام جهاز كروماتوجرافيا الغار وكروماتوجرافيا الغاز المتصلة بمطياف الكتلة.