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PHYTOCHEMICAL INVESTIGATION OF AMARANTHUS CHLOROSTACHYS GROWING IN EGYPT

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ABSTRACT

Chromatographic study of the petroleum ether extract afforded : lupeol, lupeol acetate , α - spinasterol, long chain ester and long chain ketonic ester. The chloroformic extract gave α-spinasterol glucoside and β-sitosterol glucoside. From the ethyl acetate extract quercetin and rutin were isolated. In addition, two triterpenoidal saponin glycosides, choline and basic nitrogenous substance were isolated from the aqueous mother liquor. GC of the methylated fatty acids revealed the presence of 22 fatty acids.

INTRODUCTION

Amaranthus chlorostachys Willd. (family Amaranthaceae) is an annual herb, and one of 12 species of the genus Amaranthus which are wildly growing in Egypt [1]. The genus Amaranthus comprises plants that are known to have different economic values as well as different folkloric medicinal uses [2-4]. They are reported to be used as an internal remedy to improve vision, to strengthen the liver, to control haemorrhage, dressing boils and itches in the form of ointment. Moreover, they are reported to be used as diuretic, antiscorbutic, anthelmintic, anti-diarrhaea, antirheumatic, galactogugue and for the treatment of gonorrhea and bronchitis. Although numerous Amaranthus species have been studied,[5-8] nothing was traced in the available literature concerning the phytochemical constituents of Amaranthus chlorostachys. In continuation of our research for potentially active drugs from the Egyptian plants, this rearaused the interest of the authors to investigate this plant aiming to isolate and identify its chemical constituents, and to test promising biologically active components.

EXPERIMENTAL

Plant Material :

The whole flowering plant was collected in July 1988 from a region 20 km west of Zagazig. The plant was air dried and grounded. The authenticity of the plant was kindly verified by Prof Dr. Loutfy Boulos, National Research Center, Cairo, Egypt. A voucher specimen is deposited at the Department of Pharmacognosy, Faculty of Pharmacy , University of Zagazig, Egypt .

Methods and Apparatus :

All solvents were analytical grade; melting points were determined on Buchi B 5/2 and were uncorrected ; IR were measured on a Beckmann IR - 4220 and the UV on Shimadzu UV 260. The mass spectra were determined on LKB - 9000. ^{1}Hnmr were recorded on 400 MHz using a bruker WM 400. Solvent systems for TLC:

system I : pet.ether : ethyl acetate (6 : 1)
" II : pet.ether : choroform : methanol , (4 : 3 : 1)
" III: ethyl acetate : methanol : water (15 : 2 : 2)
" IV: chloroform : methanol (8 : 2)
" V : ethyl acetate : ethanol (3 : 1)

Spray reagent ; anisaldehyde and sulphuric acid.

Extraction and Fractionation :

The powdered plant (5 kg) was extracted with ethanol (95 % , 30 L) till complete exhaustion.The total extract was concentrated to give 360 g, diluted with H_2O (2 L) and partitioned into light petroleum (92 g), chloroform (16 g), ethyl acetate (6 g), and water soluble fraction (245 g).

Chromatography of the Light Petroleum Extract :

About 28 g was chromatographed on silica gel column (700 g, 3. 5 x 120 cm) eluted with light petroleum and polarity was increased with ethyl acetate to yield several fractions , examined by tlc and pooled to yield the following compounds :

Compound 1 :

Fractions 1- 5 yielded 100 mg with R_f 0. 94(system I) ; m.p. 82 - 83° C; IR (KBr) : ν 2900 - 2830, 1700, 1480, 1420, 1300 cm^{-1}; MS :m/z(% rel. int): 452 ($M^{+\cdot}$ 6), 424 (54), 396(28),381 (6), 368(12),325(5) 185 (10),129(34),73 (64) and 57 (100).

Compound 2 :

Fractions 18 - 22 on concentration yielded white needle crystals (200mg) with R_f 0.82 (system 1), m.p 216 - 217° C; IR(KBr) :ν 2930 - 2890 , 1700 , 1450, 1360 , 1280, 1110 cm^{-1} and

gave positive Liebermann's and Salkowskis tests. Hydrolysis of this compound (100 mg, H_2SO_4 7%) yielded deacetylated product (60 mg) having m. p. 214 - 216° C.

Compound 3 :

Fractions 36 - 40 yielded white crystals (110 mg) with R_f 0. 65 (solvent system 1) , m.p. 107- 108° C ; IR(KBr) :ν 2900 - 2890 , 1740 , 1700 , 1450 , 1340, 1150 cm^{-1} ; MS : m/z (% rel. int.) 452 (M^+,2) , 393 (0.5) , 362 (1.25), 124 (2.5), 110 (4) , 96 (14) , 82(12), 68 (6) and 59 (100) .

Compound 4 :

Fractions 47- 51 yielded (150 mg) of white needle crystals with R_f 0 . 52 (system 1) ; m.p. 214 - 215° C; IR (KBr) : ν 3360 , 2900 , 2850 , 1450 , 1370 , 1180 cm^{-1} . The acetate derivative was also prepared (acetic anhydride + pyridine) with m. p. 217 - 218° C.

Compound 5 :

Fractions 62 - 66 gave white crystals (350 mg) with R_f 0.43 (system 1); m. p. 168 - 169° C; IR (KBr) : ν 3450 , 2900 , 1660, 1455, 1370 , 1150 cm^{-1}; MS: m/z (%rel. int.) 412(M^+ ,41), 397 (13) , 369 (18), 314 (2) , 271 (100), 255 (43) and Hnm R ; (δ, C D Cl_3) 0.55 (s , H - 1 , H - 19) , 0.81(t , H - 28) , 0.86 (d,H- 29, H- 26) , 1.03 (d , H - 21) , 5. 04 (dd , H - 23) , 5.15 (dd, H - 22) , 5.16 (br.d, H - 7) and 3.6 (m , H - 3).

Preparation of the Fatty Acids :

A fraction of the petroleum ether extract (15 g) was subjected to saponification [9]. The resulted fatty acids were methylated [10] and the resulting methyl esters were analysed by GLC with the following operating conditions:

detector (FID) temp : 300° C,
column temp : 195 ° C.
hydrogen flow rate:300 ml / min.
column length : 6 Feet
chart speed : 1 cm / min,
carrier gas : hydrogen
column package:20% DEGS
sample size 7 M of 10% solution of methyl esters and ether

Quantitative analysis was carried out by the peak area measurment and the results obtained are shown in Table (1).

Chromatography of the Chloroform Extract :

About 15 g of the chloroform extract was chromatographed on silica gel column (450 g , 3 x 120 cm) , eluted with light petroleum and increased with ethyl acetate and methanol to yield several fractions.

Compound 6 :

Fractions 67 - 71 yielded (350 mg) white needle crystals with R_f 0.45 (system II) m.p. 282 - 286° C ; positive Liebermann's , Salkowski's and Molish's tests; IR (KBr) : ν 3410 - 3300, 1640 , 1165 , 1110, 1025 cm^{-1}; MS: m/z (% rel. int.) 574 (M^+ , 45) , 556 (6) , 476 (4) ,

463 (6) , 440 (10), 412 (12) , 395 (8) , 273 (10) , 271 (24) 246 (6) , 87 (100) and 55 (92). Acid hydrolysis (100 mg) using 7% H_2SO_4 yielded an aglycone (60 mg) with m.p 168 - 169°C, R_f 0.43 (system I) and a sugar part chromatographed against authentic sugars .

Compound 7 :

Fractions 72 - 77 yielded (250 mg) white needle crystals with R_f 0.42 (system II), m.p. 287 - 289° C ; gave positive Liebermann's ,Salkowski's and Molish's tests; IR (KBr) : ν 3550 - 3200 (br) , 2930 - 2860 , 1640 , 1460 , 1160 , 1070 , 1020 cm^{-1}. Acid hydrolysis (50 mg) using 7 % H_2SO_4 yielded an aglycone with m. p. 135 ° C ; MS :m/z (% rel. int.) 414 (M^+, 15) , 396 (2) , 369 (12) , 301 (4) , and 55 (100) with a sugar part co-chromatographed against authentic sugars.

Chromotagraphy of the Ethyl Acetate Extract :

About 5 g of the ethyl acetate extract was chromatographed on silica gel column (150 g, 2.5x 120 cm) eluted with ethyl acetate and increased with methanol to yield several fractions monitored by TLC and the pooled fractions yielded the following compounds :

Compound 8 :

Fractions 7 - 12 yielded (15 mg) yellow sandy crystals with R_f 0. 85 (system III) m.p. 316 - 318° C; UV(Me OH): λ 258 and 370nm displaced by addition of NaOMe,$AlCl_3$ / HCL and NaOAc / H_3BO_3 .

Compound 9 :

Fractions 41 - 46 yielded (100 mg) minute yellow crystals with R_f 0.25 (system III) m.p. 188 - 190° C; UV(MeOH) : λ 259 and 359 nm displaced by addition of NaO Me, Na OAc / H_3BO_3 and Al CL_3 / HCL ; IR (KBr) :ν 3350 , 2900 , 1650 , 1600 , 1350 , 1290 cm^{-1}. Acid hydrolysis (60 mg) using 7% H_2SO_4 yielded aglycone with m. p. 316 - 317° C and a sugar part chromatographed against authentic sugars.

Chromatography of the Aqueous Mother Liquor :

About 15 g of the aqueous mother liquor residue was chromatographed on silica gel column (450 g, 3 x 120 cm) eluted with ethyl acetate increased with ethanol to yield several fractions examined by TLC and the pooled fractions yielded the following compounds :

Compound 10 and 11 :

Fractions 23 - 29 and 40 - 45 yielded two compounds with R_f 0.34 and 0.28 (system IV), both gave persistant froth on shaking with water and failed to be crystallized. Hydrolysis (10 mg separately) using 10 % HCL , gave two aglycones. Both white needle crystals , mp. 168 - 169° C, same R_f 0.43 (system 1). They were found to be the same through co - tlc; IR (KBr) : ν 3450 , 2900 , 1660 cm^{-1}, MS :m/z (% rel. int) 412 (M^+, 20) 314 (24) , 271 (36) , 246 (10) and 69 (100) ; PMR (δ, $CDCl_3$): 0.55 (s) , 0.81 (t) , 0. 86 (d) 1.03 (d) , 5.04 (dd) , 5.15 (dd) , 5.16 (br.d) , 3.6 (m). The mp., the R_f, the IR , MS and PMR spectra of the

aglycones were found to be identical with those of compound 5 previously isolated from light petroleum extract .The glycone part was chromatographed against authentic sugars.

Isolation of a Basic Nitrogenous Compound :

About 18 g of the aqueous mother liquor residue was chromatographed on alumina column (500 g , 3 x 120 cm), eluted with ethyl acetate and polarity was increased with ethanol .The pooled fractions was examind by TLC (system V) and Dragendorff's reagent as visulizing reagent.

Isolation of Compound 12 :

Fractions 12 -16 yielded a viscous residue (R_f 0.5 system V),with saturated aqueous solution of ammonium reineckate , it gave needle crystals with m.p .271 - 272° C . It also gave positive tests with Mayer's, Wagner's and Dragendorff's reagents for alkaloids. IR (KBr): ν 3425 - 3350 , 3300 - 3280 , 2950 , 2100 -2020,1600 -1550,1450,1100 cm^{-1}.

Results and Discussion

The aqueous ethanolic extract of Amaranthus clorostachys was partitioned into : light petroleum , chloroform and ethyl acetate fractions. Chromatography on silica gel column yielded five crystalline substances : lupeol acetate, lupeol, α-spinasterol (compounds 2 , 4 , 5 respectively). Identification of these compounds was established through their mmp, Co -TLC,IR, MS and PMR and comparison with authentic samples. In addition , a crystalline long chain ester (compound 1) was isolated. This compound was identified through IR peaks characteristic for the presence of an ester at 1700 cm^{-1} and 1300 cm^{-1}, also the MS spectrum showed a molecular ion peak at 452 with a base peak at 57 (100) suggest that the compound may be a long chain ester having the molecular formula ($C_{30}H_{60}O_2$) and structural formula $CH_3 - (CH_2)_{26} - O - CH_2 - CH_3$. Compound 3 was identifed as along chain ketonic ester through the presence of two peaks at 1740 and 1700 cm^{-1} for C = O group and two peaks at 2900 and 2890 cm^{-1}. MS spectrum showed a molecular ion peak at 452 (M^+) with the loss of (CH_3COO) to give peak at 393 and subsequent loss of 14 or 28 mass units for CH_2 - or $CH_2 - CH_2$ to give peaks at 362 , 124, 110 , 96 , 82 and base peak at 59 (100) for (CH_3COO).

The fatty acids were prepared and esterified and their methyl esters analysed by GC which revealed the presence of 22 fatty acids. The main

components are palmitic acid (36.75%), linoleic acid (11.37 %) and linolenic acid (6.3%) .

Column chromatography of the chloroformic extract yielded two steroidal glucosides , α - spinasterol glucoside and β - sitosterol glucoside. The structure of which was proved by IR , MS and PMR as well as comparison with authentic samples.

Chromatographic fractionation of the ethyl acetate extract on silica gel column yielded two flavonoidal compounds; quercetin and rutin. Identity was confirmed by UV , IR spectral analysis and comparison with authentic samples and reported data (11).

The aqueous mother liquor was dried and chromatographed on silica gel column which led to the isolation of two saponin glycosides.

Although insufficient pure materials were available to fully characterize these two compounds (10 & 11) in their glycosidic forms , acid hydrolysis (10 % HCL) afforded α – spinasterol as aglycone for both glycosides and the sugar parts were found to be only glucose units in the two compounds.

Also the quaternary base, choline was separated and identified as reinekate .The identity was confirmed by IR and comparison with authentic sample through the mp and CO - TLC.

This is the first report about the constituents of this plant which is the second of the genus.

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Table 1 : GLC Analysis of the Methyl Esters of the Fatty Acids

Peak No.	Carbon No. & Double Bonds	%	Fatty Acid
1-	C_4	0.37	Butyric
2-	C_6	0.39	Caproic
3-	-	2.36	Unknown
4-	C_8	1.83	Caprylic
5-	-	2.0	Unknown
6-	$C_{10\ iso}$	1.74	Isocapric
7-	C_{10}	0.84	Capric
8-	-	0.17	Hendecanoic
9-	C_{12}	0.61	Lauric
10-	C_{12-1}	0.9	Lauroleic
11-	C_{14}	3.14	Myristic
12-	$C_{14:1}$	5.7	Myristoleic
13-	C_{15}	6.2	Pentadecanoic
14-	-	1.31	Unknown
15-	$C_{16\text{-}iso}$	1.57	Isopalmitic
16-	C_{16}	36.75	Palmitic
17-	$C_{16:1}$	3.5	Palmitoleic
18-	C_{18}	1.57	Stearic
19-	$C_{18:1}$	5.51	Oleic
20-	$C_{18:2}$	11.37	Linoleic
21-	$C_{18:3}$	6.3	Linolenic
22-	-	5.2	Unknown

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المحتـــــويات الكيميائيـــة لنبـــات امارانثوس كلوروستاكس (الرُعـــاف) الذي ينمو فى مصر

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يشمل هذا البحث دراسة كيميائية لنبات أمارانثوس كلوروستاكس (الرُعاف) الذي ينمو في مصر ونتيجة لهذه الدراسة أمكن فصل ما يلي :

اللوبيول وخلات اللوبيول وألفا سبيناستيرول ومادتين إسترأليفاتي وكذلك تم التعرف علي الأحماض الدهنية من خلاصة الأثير البترولي كما تم فصل الفاسبينا ستيرول جلوكوزيد وبيتاسيتو ستيرول جلوكوزيد من خلاصة الكلوروفورم وفصل الكويرسيتين والروتين من خلاصه خلات الأثيل. وكذلك تم فصل أثنين من الجلوكزيدات الصابونية من باقي الخلاصة المائية وأثبت أن الجزء الصابوني الحر للمركبين هو الفا سبينا ستيرول بالاضافة الي فصل مادة قاعدية هي الكولين وقد تم التعرف علي التركيب الكيميائي لهذه المواد بدراسة خواصها الطبيعية والكيميائية وأجراء الدراسات الطيفية اللازمة (أشعة تحت حمراء وفوق بنفسجية ومطياف الكتلة والرنين النووي المغناطيسي) وكذلك مقارنتها بعينات أصلية نقية من هذه المواد المعروفة .