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CONSTITUENTS OF PLANTS GROWING IN QATAR XXIV PHYTOCHEMICAL INVESTIGATION OF *SALVIA AEGYPTIACA* L.

By

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مكونات نباتات دولة قطر : الجزء ٢٤

الفحص الكيميائي لنبات السالفيا (نعيم)

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أسفرت دراسة الفلافونيدات عن فصل والتعرف على المركبات الآتية : ليتيولين ، ليتيولين - ٧ - جليكوزيد ، أبيجنين ، أبيجنين - ٧ - جليكوزيد .

كما اسفرت دراسة الجزء الدهني للنبات عن فصل والتعرف على ألفا، بيتا - أميرين . وتم التعرف على الأحماض الدهنية .

Key Words: Salvia aegyptiaca, Triterpenes, Steroids, Fatty acids, Coumarin, Flavonoids.

ABSTRACT

The study of the flavonoids revealed the isolation and identification of luteolin, luteolin 7-*O*- glucoside, apigenin and apigenin 7-*O* glucoside; their identity was proved by m.p., TLC, UV, IR and MS. Moreover, a coumarin was isolated and identified as herniarin.

Investigation of the lipid fraction resulted in the isolation and identification of α and β amyrins. The fatty acids were identified as undecanoic, dodecanoic, tridecanoic, myristic, pentadecanoic, palmitic, heptadecanoic, stearic, oleic and linoleic acids.

INTRODUCTION

Salvia species, family Lamiaceae (Labiatea) are commonly used in folk medicine since a long time as a remedy for common cold, whooping cough, abdominal pains, in healing wounds [1], diarrhoae, gonorrhea, heamorrhoids and in eye diseases [2], antiseptic, cicatrizant, antispasmodic and stomachic [3].

Previous investigation showed that the herb contains pentacyclic triterpenes, diterpenes, coumarins, quinones, flavonoids [4], in addition to volatile oil, a mucilage [5], lupeol, sitosterol, lup-20 (29)-ene- 1β - 3β - diol, oleanolic acid and ursolic acid [2]. The roots contain two diterpene quinones (aegyptinones A and B) [6].

The present work deals with the chemical investigation of *Salvia aegyptiaca* L. growing in Qatar.

EXPERIMENTAL

Plant material

Salvia aegyptiaca L. was collected from Qatar in March. The plant was kindly identified by Prof. Dr. K.H. Batanouny. The whole plant was air dried and grounded into fine powder.

Apparatus and techniques

GLC was carried out according to the following conditions:

1- Terpenoids: Finn, S 1146, Column: 6 feet packed with OV-101, carrier gas, H_2 (40 ml/min), injector temperature 250°C with FIC detector.

2- Fatty acids: Methyl esters of fatty acids were analysed by coiled glass column (2.4 m. x 0.8 m.) coated with 10% PEGA; temp. programing 70-250°C, 8°C/min; chart speed 1 cm/min., carrier gas: N_2: 30 ml/min.

Thin layer chromatography was carried out using polyamide and developed with benzene: ethyl methyl ketone: methanol (4:3:3). Whatmann 3 MM paper chromatography was used for PPC. Detection was carried out in UV light at 366 nm before and after spraying with Naturstoff's reagent (β-aminodiethyl ester of diphenyl boric acid in methanol).

The UV and MS data were recorded on a Pye Unicam spectrophotometer model SP8-100 and Schimatzu Mass Spectrometer respectively.

Isolation and fractionation of the lipid constituents

About 300 gm of the dried powdered plant were extracted with petroleum ether (40-60°). The purified extract was evaporated and the residue (3.5 gm) was dissolved in boiling acetone (100 ml), cooled and the precipitated fatty alcohol was separated. The acetone soluble fraction (1.5 gm) was saponified and the unsaponifiable matter was chromatographed on Al_2O_3 column. Elution was affected with petroleum ether - benzene mixtures and collecting 100 ml fractions.

α and β- Amyrins

Fractions (77-103) eluted with pet. ether - benzene (80:20) gave after purification by PTLC (silica gel, toluene: acetone 9:1) followed by crystallization, a substance identical with authentic α amyrin (R_f and m.p. 180-183°C) (reported 186°C) [7].

MS showed a molecular ion peak at m/e 426 (C_{30} H_{50} O) and fragment ions at m/e 411, 399, 393, 299, 218 and 203. GLC showed that it was mixture of α- amyrin and β- amyrin in which α- amyrin constitutes the main component (77%).

Sterol fraction

Fractions (104-135) eluted with pet. ether : benzene (70 : 30) gave after purification by PTLC and crystallization from methanol, a steroid substance which melted at 134-136°C and was shown by GLC to be a mixture of β- sitosterol (81%), stigmasterol (18%) and campesterol (1%).

Fatty acids

About 3.5 gm of the pet. ether extract was saponified using N/2 alc. KOH. The fatty acids (0.62 gm) were methylated (MeOH, 4-5% dry HCl) and then subjected to GLC analysis.

The fatty acids were identified as octanoic (11.7%), undecanoic (4.73%), dodecanoic (4.93%) tridecanoic (9.57%), myristic (7.62%) pentadecanoic (8.48%), palmitic (22.11%), heptadecanoic (9.39%), stearic (7.31%), oleic (4.41%), linoleic (6.20%) and arachidic acids.

Isolation and identification of herniarin

Fractions (51-70) eluted with pet. ether - benzene (90:10) were purified by PTLC (silica gel G, toluene: ethyl acetate : formic acid 5 : 4 : 1). A coumarine substance identical with authentic herniarin (R_f) was obtained. UV shows maximum absorption at 252, 320 nm. MS shows a molecular ion peak at m/e 176 ($C_{10}H_8O_3$) and fragment ions at m/e 148, 133, 105, and 77 which are in agreement with the fragmentation pattern of herniarin [8].

Extraction and fractionation of the flavonoids

About 300 gm of the defatted powdered plant were extracted with 70% ethanol. The solvent free extract was taken with hot water (500 ml), filtered and extracted with chloroform (3x200 ml) followed by ethyl acetate (3x200).

The chloroform fraction (0.8 gm) was subjected to column chromatographic fractionation using polyamide and eluting with benzene: ethyl methyl ketone: methanol (4:3:3). The flavonoidal fractions were subjected for further purification on sephadex LH-20 column using 90% methanol as eluent.

The ethyl acetate fraction 0.5 gm was subjected to preparative paper chromatography (PLC) using n-butanol: acetic acid: water (3:1:1) followed by sephadex LH-20 column using 90% methanol.

Apigenin

Fractions (26-36, polyamide column) of the chloroform extract gave after further purification and crystallization from methanol a crystalline substance m.p. 345-346°C both alone and when admixed with authentic apigenin, Its identity as apigenin was confirmed by UV (Table 1) and MS (m/e 270, $C_{15}H_{10}O_5$) and fragment ions at 242, 153, 124, and 118)[9].

Luteolin

Fractions (46-52, polyamide column) of the chloroform extract gave after crystallization form methanol, yellow crystals which melted at 326-329°C, Its identity as luteolin was confirmed by UV (Table 1) and MS (m/e 286 $C_{15}H_{10}O_6$); and fragment ions at m/e 258, 153, 134 and 124 [9, 10].

Apigenin 7-O glucoside

Fractions (29-35) eluted with 90% methanol (Sephadex LH-20 column) from the ethyl acetate extract were shown by TLC to contain a single flavonoidal component corresponding to that of apigenin - 7-O glucoside. Acid hydrolysis gave apigenin identified by PC, TLC, m.m.p., UV (Table 1) and glucose (PC) [10].

Luteolin - 7-O glucoside

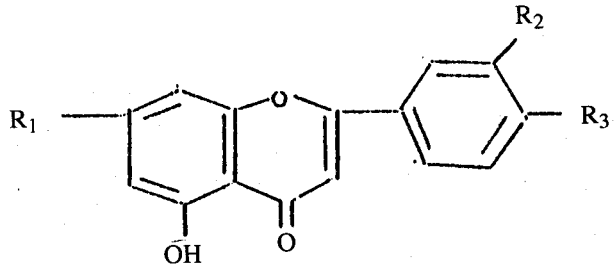
Fractions (33-39) eluted with 90% methanol (Sephadex LH-20 column) of the ethyl acetate extract were shown by TLC to contain a single flavonoidal component corresponding to that of luteoline - 7-O glucoside. Acid hydrolysis gave luteolin identified by TLC, PC, UV (Table 1) and glucose (PC) [10].

Table 1
Ultraviolet spectral data of the isolated compounds and their aglycones

	MeOH	NaOMe	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+H_3Bo_3
Apigenin	267 291 sh 330	272 320 sh 329	272 298 337 375	272 299 342 379	274 303 378	268 303 340
Luteolin	251 264 290 345	265 327 400	259 272 425	259 272 395 sh 356 380	252 326 383	259 369 420 sh

Table 1 Contd.

	MeOH	NaOMe	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+H_3Bo_3
Apigenin 7-O	267	245sh	275	275	252 sh	265
glucoside	334	270	300	295	265	340
		300	345	335	352	
		385	387	380	385	
Luteolin 7-O	252	265	273	273	260	262
glucoside	270	280 sh	295	294	367	300
	345	299 sh	329	360	402	370
		390	428	386		



	R_1	R_2	R_3
Apigenin	-OH	-H	-OH
Luteolin	-OH	-OH	-OH
Apigenin-7.O glucoside	glucose	-H	-OH
Luteolin-7.O glucoside	glucose	-OH	-OH

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