

PHYTOCHEMICAL STUDY OF ARTEMISIA ARGENTEA L'HER

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From the alcoholic extract of the defatted powdered aerial parts of the flowering plant, six flavonoid glycosides have been isolated together with large amounts of sucrose and sodium chloride. Four glycosides were hydrolysed and their aglycones were identified as 3,6,7-trimethoxy-4',5'-dihydroxyflavone (penduletin); kaempferol-6-methyl ether; 5,7-dihydroxy-4'-5'-dimethoxyflavone (diosmetin) and kaempferol-6,7-dimethylether. The other two glycosides were minors and unidentified, but spectroscopic data are given. The sugar parts of the identified four glycosides were, glucosyl, arabinosyl, glucosyl and rhamnosyl respectively as identified by paper chromatography and TLC against authentic carbohydrates.

Artemisia argentea L'Her. is an ornamental perennial shrub of the family Compositae¹. The flowering plant is used in folklore medicine, as a decoction, for the treatment of coughs and colds and in diabetes².

In previous publications³⁻⁵, we reported the botanical study of the different organs, and a study of the analysis of its volatile oil. In a separate publication⁶, we will present a study of the sesquiterpene lactones and steroidal content.

The present work, will report the isolation of six flavonoid glycosides from the alcoholic extract of the aerial parts. These glycosides were studied by spectroscopic methods.

EXPERIMENTAL

Material

The aerial parts of the flowering *Artemisia argentea* L'Her., were used including the stems, leaves, and the flower heads. The

samples were collected from the plants cultivated in the Experimental Station of Medicinal Plants, Pharmacognosy Dept., Faculty of Pharmacy, Assiut University. The material was indentified after Prof. Dr. F. Y. Amin, Prof. of Floriculture and Ornamental Plants, Faculty of Agriculture, Assiut University.

Authentic samples of diosmetin-7-glucoside and kaempferol-6,7-dimethylether-3-rhamonsyl have been provided by Prof. Dr. V. Herout, Institute of Organic Chemistry, Czechoslovakian Akademy of Science, to whom the authors are grateful.

Melting points were measured with Electrothermal Melting point Apparatus in a capillary tube and un-corrected; IR spectra were measured with a Pye-Unicam SP-1025 spectrometer using KBr discs; UV spectra were measured on a Pye-Unicam SP-1850 spectrometer (220-460 nm), using Methyl alcohol of El-Nasr Co. as the solvent.

Extraction Procedure:

The defatted ground aerial parts of the title plant (1.8 Kg.) were extracted in a soxhlet apparatus with ethanol till exhaustion. The ethanolic extract was condensed under reduced pressure to give a syrupy residue (132 g.). This residue was mixed with 150 g. cellulose powder and placed on the top of a column packed with 850 g. cellulose. Elution was started with chloroform and increasing proportions of methyl alcohol 10, 20, 40, 50, 70 and 80%, then elution with alcohol and alcohol-water (1:1). Fractions eluted with methanol-chloroform 40, 50 and 70% were found to contain the flavonoid glycosides, but it was contaminated with quantities of phenolic acids, and therefore purification was necessary.

Purification of the glycosides:

This was accomplished by application of the glycoside solution in methanol-water (1:1), as a band on sheets of Whatmenn No. 3 papers and developed in 25% acetic acid solution overnight (about 16 hours). After drying the papers in air, to have been visulaized in UV light, and the glycoside bands have been marked and cut into small pieces about 1 cm², then placed in a flask and refluxed with methyl alcohol-water (1:1) to recover the glycosides. The solution was filtered off and condensed to precipitate the glycosides as amorphous yellow

powder after recrystallization several times from dilute alcohol.

Melting points as well as the chromatographic characters of the isolated glycosides will be summarized in Table 1.

Table 1. Chromatographic data of the isolated glycosides and its melting points

Compound	R_f 1	R_f 2	mp. °C
I	0.91	0.85	180
II	0.88	0.81	233
III	0.80	0.72	250
IV	0.66	0.65	210
V	0.21	0.20	300
VI	0.01	0.05	276

R_f 1 : 25% acetic acid, Whatman NO. 1

R_f 2 : Chloroform : ethanol : water (75 : 22 : 3) , Whatman NO. 1.

Spray reagent : 5% Aluminium chloride in Methanol.

Hydrolysis of the glycosides⁷ : The isolated glycosides were hydrolysed separately, by mixing few mgm. of each with 10% aqueous sulphuric acid (5 ml.) using a minimum of methanol to effect complete solution. The solution was heated on a water bath for 45 min., after cooling, the precipitated aglycones were filtered off, washed with water and the mother liquors were shaken with ethyl acetate thoroughly to extract the last traces of the aglycones in the hydrolysate solutions. The aglycones were collected and recrystallized from methanol-ether mixture. Evaporation of the aqueous layers yielded the corresponding sugar of each glycoside.

The UV analysis of the aglycones and the shifts obtained with the different reagents, will be summarized in Table 2.

Table 2. Ultra Violet spectral analysis of the aglycones.

Compound	A	B	C	D	E	F
I	270,340,	265,280, 303,369, 398.	265,283, 303,360, 404.	255,272, 302,350, 388.	272,294, 384,394,	272,340,
II	253,270, 300,320, 366,	253,272, 305,350, 420.	254,270, 300,347, 420.	280,323, 410,	260,274, 300,387,	268,300, 319.367,
III	252,278, 291,344,	268,272, 300,362, 390,	265,275, 295,350, 384.	272,304, 386,	272,320, 368,	354,270, 348.
IV	252,270, 369.	265,300, 360,429.	244,265, 304,362, 428.	244,268, 420.	258,266, 330,386, 420	256,272. 374.
V	276,338,	258,276, 340,384,	260,276, 338,400,	266,284, 406.	260,278, 334.	260,280, 334
VI	270,338,	278,296, 342,384,	278,296, 342,384,	266,274, 404.	272,344,	368,336.

A : Spectra measured in Methanol.

B : Spectra in Aluminium chloride solution.

C : Spectra in Aluminium chloride and Hydrochloric acid.

D : Spectra in Sodium Methoxide.

E : Spectra in Sodium acetate

F : Spectra in Sodium acetate and Boric acid.

N.B. UV spectra, measured in standard cuvettes of 1 cm.

bath length. The spectra with Sodium methoxide and Sodium acetate , were measured immediately after adding the reagent and then after 10 min., where in all cases no change occurred

The infra-red spectra of the aglycones, were measured in KBr discs and the spectra of all compounds indicated the presence of free hydroxyl groups due to the presence of hydroxyl absorption bands in the range $3560-3450\text{ cm}^{-1}$; also the absorption bands of the benzpyrone fragments of the flavonoid nucleus at $1650-1635\text{ cm}^{-1}$, were very diagnostic.

From the spectroscopic analysis, chromatographic characters, melting points, comparison with the available authentic samples and the data available in the literature,⁸⁻¹¹ the aglycones isolated from the four glycosides 1-IV, have been identified as 3,6,7-trimethoxy-4,5'-dihydroxyflavone; kaempferol-6-methylether; 5,7-dimethoxy-4,5'-flavone-dimethylether and kaempferol-6,7-dimethylether respectively.

Investigation Of The Carbohydrates In The Hydrolysates:

Chromatographic investigation of the carbohydrate moiety in the hydrolysates was performed using paper as well as TLC chromatography. The results of this investigation are given in Table 3.

Table 3- Chromatographic identification of the suger moiety of the hydrolysates.

REFERENCE	PC	TLC	I	II	III	IV	V	VI
Glucose	0.25	0.42	+	-	+	-	+	+
Rhamnose	0.36	0.63	-	-	-	+	-	-
Xylose	0.31	0.52	-	-	-	-	-	-
Arabinose	0.28	0.43	-	+	-	-	-	-
Galactose	0.24	0.40	-	-	-	-	-	-

PC: R_f values of sugars on Whatman No. 3 paper and n-butanol: acetic acid: water (120:30:50 v/v/v) as the developing solvent.

TLC: R_f Values on silica gel GF₂₅₄ plates impregnated with 0.2M sodium acetate¹².

Spray Reagent:

Aniline phthalate and subsequent heating at 110°C for 10 minutes.

The collective results of the obtained glycosides and their hydrolysis products are given in Table 4

Table 4- Yield of glycosides and characterization of their aglycones and sugar moieties.

GLYCOSIDES	YIELD	AGLYCONE	MP, °C	SUGAR
I	86 mg.	3,6,7-trimethoxy-4,5-dihydroxyflavone	218	
II	60 mg.	kaempferol-6-methyl ether.	290	glucose arabinose
III	114 mg.	5,7dihydroxy-4',5'-dimethoxyflavone.	258	glucose
IV	65 mg	kaempferol-6,7-dimethylether.	243	rhamnose
V	22 mg	unknown	268	glucose
VI	18 mg	unknown	294	glucose

CONCLUSION

Artemisia argentea L'Her., a fragrant ornamental plant, which is known in folklore medicine and has not been studied phytochemically elsewhere. In this publication we report the isolation of 4 flavonoid glycosides which have been identified as: 3,6,7-trimethoxy-4-glucosyl-5-hydroxyflavone, 6-methoxy-3-arabinosyl-5,7,4'-trihydroxyflavone; 5-hydroxy-7-glucosyl-4'5'-dimethoxyflavone and 6,7-dimethoxy-3-rhmnosyl-5,4'-dihydroxyflavone. In addition there are two other minor glycosides which has not been identified, although their spectral data have been given. Especially glycoside VI., is very unstable towards acids and turns black and its aglycone also turns black few hours after its separation.

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دراسة كيميائية لنبات الشيبة "أرتيميزيا أرجنتيا" المنزوع في مصر

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قسم العقاقير — كلية الصيدلة — جامعة أسسوط

قام الباحثان بتحضير خلاصة الكحول لسيقان وأوراق ونورات الشيبة المجففة بمعد
استخلاصها بالبتروال الاثيمري .

وقد تمكن الباحثان من فصل مجموعة من جلوكوزيدات الفلافونز تم تحليلها طيفيا بواسطة

الأشعة فوق البنفسجية والمكروماتوجرافيا .

كذلك تم دراسة السكاكر الناتجة من عملية الحلماة وتم التعرف على أربعة من هذه

الجلوكوزيدات وهي ٣ ٤ ٦ ٧ — تراي ميثوكسي — ٤ — جلوكوزيل — ٥ — هيدروكسي

فلافون و ٦ — ميثوكسي — ٣ — أرابينوزيل — ٥ ٦ ٧ — ٤ — تراي هيدروكسي فلافون

و ٥ — هيدروكسي — ٧ — جلوكوزيل — ٤ ٥ ٦ — داي ميثوكسي فلافون و ٦ ٥ ٧ — داي

ميثوكسي — ٣ — رامنوزيل — ٥ — ٤ — داي هيدروكسي فلافون بينما الاخرين مازالا تحت

الدراسة وقد أمكن التعرف على اجزائها السكرية وبعض ثوابتها .