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A PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF ARTEMISIA ARGENTEA L'HER GROWING IN EGYPT.

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The Phytochemical examination of Artemisia argentea L'Her. established the Pharmacopoeial constants and the percentage of extractives of the different organs. Such examination proved the presence of volatile oil, flavonoid aglycones and glycosides, sesquiterpene lactones, unsaturated sterols and or/ triterpenes, carbohydrates, besides traces of alkaloids or nitrogenous bases in the examined organs.

Artemisia argentea L'Her. is an ornamental shrub of the family Compositae [1] The flowering plant is used in folklore medicine as a decoction for the treatment of coughs and colds and in diabetes [2].

The genus Artemisia to which the plant belongs, includes about 350 species widely distributed all over the world [3]. These species are well known for their valuable medicinal constituents [4].

EXPERIMENTAL

Meterial :

The matrial used in this investigation consisted of the leaves, stems, flowers and flowering tops of the plant cultivated in the Experimental Station of Medicinal Plants of the Faculty of Pharmacy, Assiut University and in other gardens in Assiut, and it was identified by Prof. Dr. Fouad Yehia Amine, Prof. of Floriculture and Ornamental Horticulture, Faculty of Agriculture, Assiut University.

1- Preliminary Phytochemical Screening :

The powdered samples of the leaves, stems, flowers and flowering tops were separately subjected to the various chemical tests proposed by Wall et al [5]. Results obtained revealed that the examined organs of Artemisia argentea L'Her, contain volatile oil, carbohydrates and/or glycosides, tannins, sesquiterpene lactones, unsaturated sterols and/or triterpenes, flavonoid aglycones and glycosides and alkaloids or nitrogenous bases.

2- Proximate analysis :

A proximate analysis of each of the dried powdered orangs was carried out according to the monograph of the Egyptian Pharmacopoeia (E.P. 1972) . The results (which are the average of three determination), are given in Table 1.

TABLE 1. Proximate analysis of the different organs of Artemisia argentea 1'Her growing in Egypt.

Standard	*Organ*			
	Leaves	*Stems*	*Flowers*	*Fl. tops*
Moisture	5.32	5.45	6.63	6.4o
Ash	9.82	4.27	8.16	10.62
Acid insol. ash	2.82	0.92	1.96	1.08
Crude fibres	7.32	13.33	9.85	9.40
EXTRACTIVES:				
Pet. ether $^{60-80}$	6.40	3.96	4.27	8.93
Ether	5.90	1.94	8.35	5.43
Chloroform	4.43	2.89	2.48	4.17
Alcohol (95%)	17.76	12.55	15.39	14.20
% YIELD OF VOL.OIL :				
Fresh	1.2	0.8	2.5	1.5
Air-dried.	1.7	1.0	3.7	2.1

The aqueous alcoholic extracts were condensed and the residue was extracted with chloroform.

The chloroform extract was investigated for its content of sesquiterpene lactones using TLC chromatography.

Adsorbents :

Silica gel H type "Merck"

Solvents :

a- Chloroform : Benzene (3 : 2).

b- Chloroform : Ethyl acetate (9 : 1).

c- Chloroform : Petroleum ether : Ethyl acetate (2 : 2 : 1)

Spray reagent :

50% sulphuric acid, then heat at 70°C for 10 min. The solvent, chloroform : benzene (3 : 2), proved to be the most suitable and the chromatogram was visualized in UV light. The results are compiled in Table 3.

TABLE 3 : Thin layer chromatographic investigation of the sesquiterpene lactone fraction .

Spot No.	R_f	*SA*	*UV*
1	0.95	rr	y
2	0.80	g	O
3	0.65	p	O
4	0.58	p	O
5	0.52	y	y
6	0.40	p	gr
7	0.20	p	y
8	0.15	p	O
9	0.10	p	O

SA : colour with H_2SO_4; UV colour in UV light after spray with acid; rr; rose red; y :yellow ; g : green ; O : orange ; p : pink ; gr : grey .

Spot No. 3 with an R_f. value of 0.65 has been found to show a typical behaviour like an authentic sample of arborescine especially in giving a deep pink colour with sulphuric acid and the orange colour aquired in UV light. The other spots seems to be new sesquiterpene lactones .

3-Chromatographic investigation of the crude extracts:

A- The crude extracts which gave strong positive tests for flavonoids viz., petroleum ether and alcohol extracts of all the examined organs were subjected to a preliminary chromatographic study.

Adsorbents :

1- For aglycones : a- Whatman No. 1 paper .
b- Silica gel G. "Merck"

Solvents :

a- Acetic acid 15%
b- Chloroform-benzene (4 : 1)
c- Chloroform .

The main flavonoid aglycone present in the petroleum ether extract and which has been crystallized on ccoling of the extract was filtered off, washed with ether and chromatographed on Whatmann No. 1 paper using acetic acid 15% as the developing solvent . It was also chromatographed on TLC using Chloroform-benzene (4:1) as the developing solvent. The R_f. value of this compound was 0.82 which is co-incident with the R_f. value of an authentic sample of Artemetin (Artemisetin), a pentamethoxyflavonoid isolated from some artemisia species [6] , and from Kuhnia eupatorioides L. var. pyramidalis [7] .The identification of the characters of artemetin was confirmed by spectroscopic analysis as well as by the mixture mp. with no depression. Another minor flavonoid aglycone with R_f. 0.80 was also detected with the mentioned solvent system on TLC.

2- For glycosides :

Adsorbents :

a- Whatman No. 1 paper .
b- Silica gel G. "Merck"

Solvents :

a- Acetic acid 25%
b- Ethyl-methyl ketone : Ethyl acetate : Formic acid: Water (3 : 5 : 1 : 1)
c- Ethyl acetate : Formic acid : Water (10 : 2 : 3).

d- Benzene : Pyridine : Formic acid (36 : 9 : 5)

The flavone glycosides present in the alcoholic extract , were successfully separated with developing solvent of benzene : formic acid : pyridine (36 : 5 : 9), which is the best solvent on TLC. The spots were visualized in UV light before and after spraying with 0.1 M alcoholic Aluminium chloride solution. Results are compiled in Table 2 .

TABLE 2 . Thin layer chromatography of the glycosidal fraction using Benzene: pyridine: formic acid (36:9:5)

Spot No.	R_f	*DL*	UV_1	*AC*	UV_2
1	0.95	gy	p	y	y
2	0.90	py	p	y	y
3	0.78	1y	p	y	p
4	0.75	gy	p	y	gy
5	0.70	1y	p	y	p
6	0.65	py	p	y	p
7	0.62	py	p	y	p
8	0.50	1y	p	y	p
9	0.20	1y	p	y	y
10	0.15	1y	y	y	y
11	0.08	y	y	y	y

DL : day light; UV_1, UV light before spray; AC: aluminium chloride; UV_2: UV after spray ; gy : grey yellow ; p : purple: y : yellow ; 1y : lemon yellow ; py : pale yellow .
spots No. 3 and 6 has been found to have the same R_f. values of authentic samples of diosmetin and kaempferol-6,7-dimethyl ether glycosides respectively.

4- Examination of the sesquiterpene lactone fraction :

The sesquiterpene lactone containing fraction was prepared from the defatted powdered plant organs, by extraction with chloroform. The extract was purified by evaporation of the solvent to dryness and the residue left was treated with boiling alcohol, then boiling water added. The aqueous alcohol extract was filtered from the insoluble material and the process repeated twice .

Conclusion :

A thorough phytochemical screening of the different extractives of each organ of the plant revealed the presence of steroids, and/or triterpenes, volatile oil and a flavonoid aglycone (artemetin) which has been compared with an authentic specimen, in the petroleum ether extract together with a minor flavonoid aglycone.

The chloroform extracts were found to contain mainly sesquiterpene lactones and arborescine has been detected by comparison with an authentic sample, in addition to a small amount of the flavonoid aglycone artemetin.

The alcoholic extract, was tested and tannins, free sugars and flavone glycosides especially diosmetin and kaempferol-6,7-dimethoxyflavone glycosides has been detected on chromatographic studies and comparison with authentic specimens.

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دراسة كيميائية لنبات الشيبة الذى ينمو فى مصر

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اشتملت الدراسة الكيميائية على مايلى :

١ـ عينت بعض الثوابت الدستورية لاجزاء النباتات المختلفة (الرماد ، الكلى والغير قابل للذوبان فى الحمض ونسبة الرطوبة والخلاصات الناتجة بالمذيبات المختلفة .

٢ـ اسفر البحث الكيميائى الاولى عن وجود مواد فلافونية جليكوزيدية وحرة وكومارات راتينية واستيرولات غير مشبعه وسيسكيتربينات لاكتونية وقواعد نيتروجينية .

٣ـ تم فصل الفلافونيدات الجليكوزيدية والحرة بواسطة كروماتوجرافيا الطبقة الرقيقة وقد امكن فصل مادة الارتيميتين من خلاصة البترول الاثيرى

٤ـ تم فصل السيسكيتربينات اللاكتونية بواسطة كروماتوجرافيا الطبقة الرقيقة وامكن التعرف على الاربوريسين .