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PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON TWO ACALYPHA SPECIES

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ABSTRACT

The investigation and evaluation of the tannin contents of Acalypha wilkesiana Mull. Arg. and Acalypha marginata Mort. not. (Poir) K. Spring, were carried out. The study revealed the presence of high contents of the condensed and hydrolysable tannins in the leaves of the two species. In addition one flavonoidal compound was isolated from the leaves of the two species and identified as liguiritigenin. Chromatographic study of the unsaponifiable fraction of the petroleum ether extract of the leaves of the two species revealed the presence of ß-sitosterol and α-amyrine. The antimicrobial and antifungal activities were carried out for the different extracts of the two Acalypha species.

INTRODUCTION

Acalypha species (Family Euophorbiaceae) have been widely used in folk medicine as diuretic, expectorant and as a mouth wash for sore gums[1,2].

Chemical studies of the two species revealed the presence of alkaloids, cyanogenetic glycosides, essential oil, resin and tannin[1-4]. Despite the presence of high percentage of tannin in Acalypha species, nothing was found in the literatures about their qualitative and quantitative analysis. In a previous publication, it was found that the two species grown in Egypt contain alkaloids, glycosides and/or carbohydrates, flavonoids, saponins and sterols and/or triterpenes[5].

In the present work, a comprehensive investigation of the flavonoid, tannin and lipid contents of the two species is presented. Biological studies of the extracts were also performed.

EXPERIMENTAL

Plant material :

Samples of stems and leaves of Acalypha witkesiana Mull. Arg. and Acalypha marginata Mort. not. (poir) K. spring[6] were collected in June 1991 from the plants cultivated in El-Orman Garden, Giza, Egypt. The plants were kindly authenticated by Dr. N. El-Hadidi, Professor of Plant Taxonomy, Faculty of Science, Cairo University. Identity was also confirmed by comparison with Herbarium specimens kept at El-Orman Garden in Giza. Voucher specimens are kept in the Pharmacognosy Department Herbarium, Faculty of Pharmacy, Cairo University. Each organ of the two species was separately air-dried in the shade and powdered.

A- Study of the tannin content :

1- Extraction and investigation of tannin content :

Three quantities each of twenty grams of powdered leaves and stems of the two species under investigation were separately extracted by cold water, boiling water and 40% acetone. Each extract was separately subjected to the colour and precipitation tests for tannins [7,9].

2-Thermographical identification of tannin using TAS technique[10] :

Two grams of each powdered drug were extracted with 100 ml of boiling water and warmed in water bath for 30 minutes. After rapid cooling, the solution was filtered and evaporated to dryness under reduced pressure. Tannin extract (1-6 mg) were inserted in the TAS-patron and submitted to a linear temperature gradient from 50-300°C and the product was condensed on a silica gel G thin-layer plate. One uL of authentic phenols were spotted on TLC plate. The chromatogram was developed with benzene: ethyl acetate: formic acid (30:19:1 v/v/v), dried for 5 minutes then visualized in UV at 254 nm.

3- Determination of the tannin content :

The quantitation of the tannin content was done by adopting Hide-powder method [11] . The results are listed in Table 1 .

4- Evaluation of tannin using titrimetric method [12,13] :

Quantitation was carried out based on precipitation of the tannin content separately from each extract by standard solution of copper acetate. The excess copper acetate was determined iodometrically [12,13] . The results are recorded in Table 1.

B- Extraction and isolation of the flavonoid content :

About 500 g of the air dried powdered plant were exhaustively extracted by successive percolation using petroleum ether and methanol. The methanolic extract was concentrated under reduced pressure and the residue was extracted with boiling water. The aqueous filtrate was extracted with ethyl acetate. The ethyl acetate extract was concentrated to dryness under reduced pressure. Paper chromatography screening of the ethyl acetate extract using water: acetic acid (85:15) as developing solvent system, showed the presence of three spots visualised by UV at 365 nm, NH_3-UV 365 nm and $ALCl_3$-UV 365 nm. One flavonoidal compound was isolated using preparative paper chromatography. Water: acetic acid (85:15) was used as a developing solvent system. The eluted bands were separately concentrated under reduced pressure and the residue was monitored by paper chromatography using the above mentioned solvent system.

The compound showed pale yellow powder (10 mg), with R_f 0.49 in water: acetic acid (85:15); m.p. 208°C; UV (using methanol and shift reagents) λ_{max} (MeOH): 276, 365 (sh); NaOMe: 328, 420 (sh); $ALCl_{,3}$: 296, 362 (sh); $ALCl_3$/HCl: 279, 290; Sod. acetate: 254, 288, 334; Sod./ acetate/Boric acid: 277, 312 nm. The mass spectra showed m/z 256 (M^+), 213, 184, 163, 137, 120, 109, 93 and 55.

C- Study of the lipid content :

Preparation of the unsaponifiable matter of the petroleum ether extracts of the two species were performed[14] . Column chromtography gave two compounds, with R_f 0.81 and 0.56 in Bezene-ethyl aetate (86-14) identified against authentic samples.

D- Antimicrobial activity :

The extracted phenolic compounds were dissolved in DMSO (for flavonoids containing extracts) and in water (for those containing tannins) at a concentration 5 mg/ml. The dissolved extracts were screened for their antimicrobial activity in vitro against the following micro-organisms: E. coli ATCC 10536; Pseudomonas aeruginosa CNCM A 21; Serratia marcescens (Gram-negative bacteria); Staphylococcus aureus ATCC 9141 (Gram positive bacteria) and Mycobacterium semegma CIP 7326 (Acid fast bacteria). The antifungal activity was determined against Candida albicans; Candid para-cursi; Candida tropicalis; Torulopsis glabrata; Geotricum candidum (yeasts); Aspergillus niger; Aspergillus nodulans and Penicillium glabrium (Mycelium fungi). Applying the disc[15] technique using sterile discs of Whatman filter paper (5 mm diameter) with a final charge 250 mg/disc. The discs were placed onto the surface of inoculated agar plates. The plates were incubated at 37°C (24-48 hours for bacteria) and 25°C (24-72 hours in case of fungi). After incubation, the inhibition zones were recorded in mm diameter, less than 5 mm indicates no effect. A disc impregenated with 50 uL of DMSO was used as control for each microorganism.

RESULTS AND DISCUSSION

The qualitative investigation of the aqueous extracts of the leaves and stems of Acalypha wilkesiana and A. marginata proved the pressence of hydrolysable and condensed tannins. These results were confirmed by thermographic study of the aqueous extracts of the two species. This

technique showed the presence of several polyphenolic constituents, such as pyrogallic acid and catechol. This indicated that the tannins present are of the condensed and hydrolysable types.

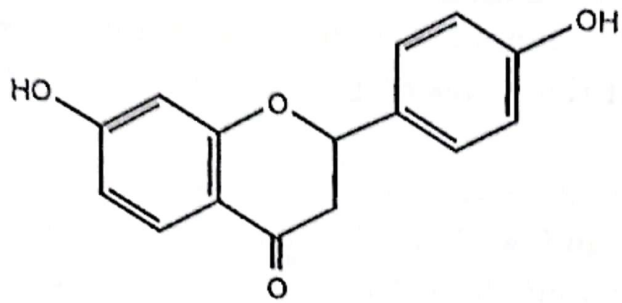
In the present study, two different methods were used to evaluate the tannin content of the two species. The hide powder method showed that the leaves of A. wilkesiana contain 32.5% of tannin, while that of A. marginata contain 30.6% tannin. On the other hand, the copper acetate method revealed that the leaves of A. wilkesiana contain the highest percentage of tannin (39.5%, 37.6% and 35.1%) using 40% acetone, hot water and cold water as extracting solvents, respectively. In A. marginata the percentages of tannin found were 37.0%, 35.8% and 33.0% using the previously mentioned solvents for extraction. In conclusion, these results showed the presence of high tannin content in the leaves of A. wilkesiana and A. marginata.

Investigation of the methanolic extract of Acalypha wilkesiana Mull. Arg. and A. marginata Mort. not. (poir) K. Spring exhibited the presence of three flavonoidal compounds. One of them gave negative test for glycosides. The yield of the other two compounds was to low to be identified. The identification of this compound was based on UV and Mass spectral data. The compound gave light blue colour under UV changed to yellow with NH_3 and with $ALCl_3$.

The UV spectral data showed the absence of strong peak above 300 nm. This indicated the possible presence of dihydroflavone skeleton. The NaOMe revealed the presence of few-OH gps, due to the absence of decomposition of the compound on standing.

The bathochromic shift with NaOMe in Band I indicated the presence of free-OH gp at 4'-position, while the Na acetate confirmed the presence of free-OH gp at 7-position. The $ALCl_3$ and $ALCl_3$/HCl indicated the absence of orthodihydroxy pattern and free-OH gp at 3 or 5 positions. By comparison the UV data was found to be indentical with published data for

Liquirtigenin[16] . The Mass spectral data showed fragment at m/z 137 corresponding to $C_9H_5O_3$, m/z 120 for C_8H_8O, m/z 109 and m/z 93. This showed further confirmation of the isolated compound to be liquirtigenin.



Liquirtigenin

The chromatographic study of the obtained unsaponifiable matters resulted in the isolation of ß-sitosterol and α-amyrin. The identification of these two compounds were performed by co-chromatography and mixed m.p. compared with authentic samples.

Concerning the antimicrobial activity, all aqueous extracts (containing tannin) showed no antimicrobial activity except two of them having moderate antibacterial activity (Table 2). Candida tropicalis was not affected by all of these tested extracts. On the other hand, ethyl acetate extracts (containing flavonoids) showed a prefrential antibacterial activity and a weak or moderate antifungal effect especially those isolated from the leaves of A. wilkesiana.

In addition; the stem of A. marginata. extract has only antibacterial activity and that of A. wilkesiana, showed antibacterial and some antifungal action. This may be attributed to some constituents other than the flavonoids which present in A. wilkesiana and absent in A. marginata.

Table (1): Percentage of tannin contents of the leaves and stems of Acalypha wilkesiana and A. marginata.

Method	Tannin percentage			
	A. w.		A. m.	
	leaf	stem	leaf	stem
Hide Powder	32.5	5.3	30.6	4.7
Titrimetric:				
Cold H_2O	35.0	6.1	33.0	5.4
Hot H_2O	37.6	6.5	35.8	5.8
40% acetone	39.5	6.9	37.0	6.1

Table (2): Antimicrobial activity of extracts of A. wilkesiana and A. marginata.

Micro-organism	Tannin		Flavonoids			
	A. m. leaf	A. w. leaf	A. m. leaf	A. m. stem	A. w. leaf	A. w. stem
Staph. aureus ATCC 9141.	18	16	15	-	30	15
E. coli ATCC 1536	16	20	-	28	24	13
P. aeruginosa CNCM A 21	-	-	-	25	20	15
S. marcescens	-	-	-	-	24	14
M. semegma CIP 7326	-	-	22	32	35	25
C. albicans	-	-	-	-	30	22
C. para-crusi	-	-	-	-	25	23
T. glabrata	-	-	-	-	15	-
G. candidum	-	-	-	-	15	-
A. niger	-	-	-	-	22	-
A. nodulans	-	-	-	-	23	-
P. glabrium	-	-	-	-	15	-

A.m. = Acalypha marginata. - = no effect.
A.w. = Acalypha wilkesiana.

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دراسة كيميائية وبيولوجية لنوعين من جنس الاكاليفا

جمال مهران - ابراهيم عطيه - محمود كحيل

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