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STUDIES FOR DETERMINING ANTIMICROBIAL ACTIVITY OF *SOLENOSTEMMA ARGEL* (DEL) HAYNE. 1-EXTRACTION WITH METHANOL/WATER IN DIFFERENT PROPORTIONS

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دراسات لقياس كفاءة نبات الحرجل كمضاد ميكروبي

١ - الاستخلاص بنسب مختلفة من الميثانول/والماء

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أستخلص الجزء الهوائي لنبات الحرجل بإستخدام الميثانول/ والماء بنسبة مختلفة والذي أنتج أربعة مستخلصات حيث فحصت فيتوكميائياً وكروماتوجرافيا وكذلك التقدير الكيفي لمحتويات الفلافونات والصابونينات لكل مستخلص كما درس النشاط المضاد للميكروبات ممثلا في ثمانية أنواع من البكتريا وأربعة عشر فطراً .

وقد كان واضحاً الأثر الفعال القوي للمستخلصات على الميكروب السبحي ومتوسط التأثير على الميكروب القولوني وميكروب الجمرة الخبيثة والميكروب المكور العنقودي الذهبي والكلبسيللا والبروتيس .

كما أسفر نشاط المستخلص رقم ١ كمضاد فطري ضد الأسبريجيلس نيجر (١٩مم) والميوكر (١٢مم) بينما أثر المستخلص رقم ٢ فكان أوضح تأثير له ضد الأسبريجيلس نيجر وكاند والكريزوسبورم والكاندا أليكانز وجنس الكنديدا والرودوتيللا بدرجات ١٠ و١٠ و١٨ و٥ و٦ و٩ مم على التوالي . ولكن المستخلص رقم ٣ أظهر نشاط على الأسبريجلس نيجر والكنديدا والروديتيللا (٥ مم) لكل من الفطريات الثلاث . وعلى الجانب الآخر فإن المستخلص رقم ٤ كان ذو نشاط فعال على السبريجليس بارازيتيكس (٣٦ مم) والأسبؤيجيلس كاند (١٥ مم) والكريزوسبورم والكربتوكوكس نيوفورمانز ٥ و٦ مم على التوالي .

Key Words: Solenostemma argel, Asclepiadiaceae, Flavones, Saponins, Antimicrobial.

ABSTRACT

Aerial parts of *Solenostemma argel* plant were successively extracted with methanol/water in different proportions (4 fractions). The phytochemical and chromatographic screening as well as quantitative determination of the flavonoid and saponin contents were carried out to each fraction. The antimicrobial activity of the four fractions against eight bacteria: *Staphylococcus aureus*; *Micrococcus*; *Streptococcus* spp; *Bacillus anthracis*; *E. coli; Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; and *Proteus vulgaris* and 14 fungi: *Fusarium; Aspergillus parasiticus*; *A. flavus*; *A. niger; A. candidus; A. glaucus; Penicillum; Chrisosporium; Cr. neoformans; Candida* spp; *C. albicans; Can.* spp 20; *Mucor* and *Rhodotorula* were studied. It was clear that the most powerful effect was observed in case of *Streptococcus* spp.; moderate action against *E. coli. B. anthracis; S. aureus; Klebsiella pneumoniae* and *Proteus vulgaris*. The fungicidal activity of fraction No. 1 showed antifungal activity to *A. niger; Mucor* while fraction 2 showed the activity against *A. niger; A. candidus chrisosporium, Cand. albicans, Cand.* spp 20 and *Rhodotorula*. But fraction 3 had an effect on *A. niger, Cand.* spp 20 and *Rhodotorula*. On the other hand, fraction 4 was highly effective to *A. parasiticus* and *A. candidus*. It was clear that 4 fractions gave different degrees of antifungal activity to the examined 14 fungal species.

INTRODUCTION

Solenostemma argel (Del) Hayne is used in the folk medicine as an effective remedy for cough; infusion of leaves for gastrointestinal cramps, as laxative[1]; stomachache; anticolic; for cold urinary tract; antisyphilitic if used for prolonged period of 40 to 80 days[2] and antiinflammatory [3]. Many authors studied the phytochemical analysis of such plants[4]. In a survey on Egyptian plants about 60% of these plants have antimicrobial activity, whereas 15% exhibited a marked antifungal property[5]. Hegazi et al.[6,7] found that *S. argel* has antimicrobial effect to some bacteria and fungi. In another study Hegazi et al.[8] reported the antiviral activity of *S. argel* to Newcastle disease virus.

The aim of the present investigation is to determine the possible antimicrobial activity of *Solenostemma argel* successively extracted with methanol/water in different proportions.

MATERIAL AND METHODS

Solenostemma argel (family Asclepiadaceae) was collected from south Sinai, Egypt in June 1991. The plant was kindly identified by Dr. M. El Gebaly, National Research Centre. Aerial parts of *S. argel* plant were successively extracted with methanol/water in different proportions [methanol, methanol/water (30 and 60), and water].

Phytochemical screening for alkaloids, flavonoids, unsaturated sterols and/ or triterpenoids, saponins, coumarins, cardiac glycosides, anthraquinones and tannins were done according to El Gamal *et al.*,[9]. TLC chromatographic screening (Silica gel DF 254) of the steroidal saponin, cardiac and flavonoidal glycosides was carried out according to Stahl[10].

The quantitative determination and standard calibration curve of flavonoids were done according to the procedure of Khalifa[11]. Kaempferol-3-glucoside[12] for the standard curve was dissolved in methanolic AlCl3 (0.1 M) and measured at 400 nm. The curve obeys Beer's law from 20-340 mg (Fig. 1).

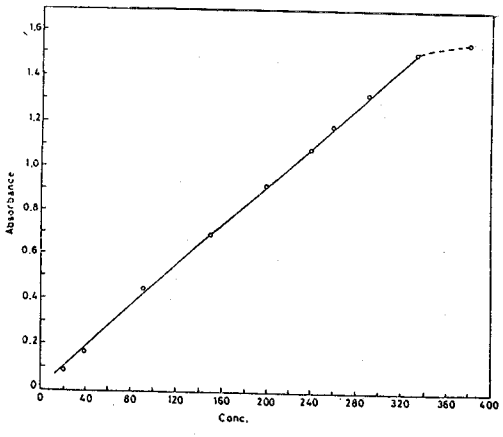

Fig. 1. Standard calibration curve of 3-kaempferol glucoside (µgs/5ml) with 0.1 M alcoholic $AlCl_3$ reagent.

Spectrophotometric (UV spectra, Shimadzu-240) determination of total flavonoid content of the obtained fractions were done by dissolving 0.8 mg of each fraction in AlCl3 (alch. solution) and measured at 400 nm.

The quanititative determination of saponin content was done with the cholesterol complex method[13]. The complex was precipitated with cholesterol (7% alc. solution). The dry weight of the complex was decomposed with pyridine to give the final weight of the free saponin.

The antimicrobial activity of the fractions was tested on 8 bacterial (*S. aureus; Micrococcus*; *Streptococcus* spp.; *B. anthracis; E. coli; Klebsiella* spp.; *Pseudomonas* spp.; and *Proteus* spp) and 14 fungi (*Fusarium*; *A. parasiticus*; *A. flavus; A. niger*; *A. candidus; A. glaucus; Penicillum; Chrisosporium; Cr. neoformans; Candida* spp.; *Cand. albicans; Cand.* spp 20; *Mucor* and *Rhodotorula*). Bacteria and fungi were isolated and identified according to the procedure of Cruickshunk et al[14].

The antimicrobial activity of each fraction as well as of the solvent were determined against 8 bacteria and 14 fungi using paper disk plate methods[6,15]. Whatman No. 1 filter paper disks (10 mm) were saturated with the tested materials, then placed on the agar plate surface which previously inoculated with bacteria (enriched on nutrient broth for 24 hours) and fungi (enriched on Czapek dextrose agar for 48 hours) for one hour at 37°C. The plates were reincubated at 37°C for further 24 (bacteria) and 48 (fungi) hours. The disks which had been previously incubated on the agar plate were observed concerning the zone of growth inhibition adjacent to those disks containing the tested materials to which the bacterium is sensitive. The development of a zone of growth inhibition of any size around a disk indicated that the organism was susceptible to the examined material. Resistant bacteria grow right up to the margin of the disk. The minimal inhibitory concentration of each tested compound was determined by using a constant amount (60 mg) of the components.

RESULTS AND DISCUSSION

Aerial parts of *S. argel* plant were successively extracted with methanol/water in different proportions which produced 4 fractions. The phytochemical screening revealed the presence of flavonoids (aglycones and glycosides), unsaturated sterols and/or triterpenoids, saponins and tannins (Table 1).

TLC chromatographic screening revealed the probable presence of furostanol steroids, saponins, cardiac glycosides and kaempferol glucosides (Table 2). The flavonoid content of the four fractions was 1.4, 3.0, 2.4 and 2.4% in fractions number 1,2,3 and 4 respectively while the saponin content was 2.2, 3.7, 4.7 and 3.68% in the same fractions (Table 3).

Table 1
Phytochemical screening of *S. argel* fractions

Fraction No.	Alkaloids 1,2,3	4	Leuc.	Flavonoids Agl.	Gly.	Unsat. st. LB	H_2SO_4	Saponins	Cardiacglycosides LB	KK	Ked	Anth F	C	Coumarins	Tannins
1	-	-	-	+	+++	++	++	+++	+	-	-	-	-	-	+
2	-	-	-	++	++	++	++	+++	+	+	-	-	-	-	++
3	-	-	-	++	++	++	++	+++	+	+	-	-	-	-	++
4	-	-	+	+++	+++	++	++	+++	++	++	-	-	-	-	++

Leuc. = Leucoanthocyanidines Agl. = Aglycones Glyc. = Glycosides
LB. = Liebermann Burchardt test KK. = Keller Kiliani test
Anth. = Anthraquinones F = Free C = Combined

Table 2
Chromatographic screening of *S. argel* fractionated with methanol/water

Fraction No.	Eluent system	Anisaldehyde reagent (Steroid, spiro or furostenol) R_f (yellow)	Cardiac glycosides R_f (pink)	Ehrlich reagent Steroids (furostanol) Rf (pink)	10% H_2SO_4 Kaempferol glucosides	Others
1	I	-	-	-	-	-
	II	-	-	-	0.45 0.2 0.1 + ++ +	
2	I	0.84, 0.7 ++ ++	0.81 ++	The same	-	-
	II	-	-	-	-	-
3	I	0.84, 0.7 ++ ++	0.81 ++	The same	-	-
	II	-	-	-	0.45 0.31 0.2 + + +++	
4	I	0.84 0.7 0.54 0.5 0.4 + + + ++ +	0.81 +	The same	- 0.45 +	- 0.2 +++
	II	-	-	-		

Eluent I = ($CHCl_3$: MeOH: H_2O, lower layer) 8: 2.5: 1
II = ($CHCl_3$: MeOH : H_2O : CH_3COOH) 15: 8: 1: 1
Anisaldehyde reagent: (0.5 ml anisald. + 9 ml EtOH + 0.5 ml H_2SO_4 + 0.1 ml CH_3COOH)
Ehrlich reagent: 1 g *p*-dimethylaminobenzaldehyde + 50 ml 36% HCl + 50 ml ÈtOH
Colour intensity: +++ = Major ++ = Moderate + = Traces

Table 3
The flavonoid and saponin content of *S. argel* fractions

Fraction No.	Flavonoids %*	Weight of cholesterol complex	** % of free saponins
1	1.4	0.970	2.20
2	3.0	0.250	3.70
3	2.4	0.200	4.70
4	2.4	0.110	3.68

* = Calculated as kaempferol-3-glucoside.
** = Some saponin complexes are easily formed but decomposed with difficulty (Wulff 1968)

The obtained results (Table 4) revealed that the four different fractions have antimicrobial activity in variable degrees. The most powerful effect was observed in case of Streptococcus spp. and a moderate action against *E. coli*, *B. anthracis*, *S. aureus*, *Klebsilla pneumoniae* and *Proteus vulgaris*. There was no effect against *Micrococcus* and *Pseudomonas aeruginosa*. These results may be attributed to the effect of different constituents of these fractions as well as the type of solvent used for fractionation. Tharib *et al*[16] who isolated 4 components from stems of the desert shrub *S. argel* and they found that there was only one fraction (from the saponifiable fraction) showed antimicrobial activity against both Gram positive and Gram negative bacteria. Hegazi *et al*[6] found that the four fractions extracted with chloroform/methanol in different proportions from *S. argel* showed antimicrobial activity.

Table 4
Antimicrobial activity of *S. argel* fractions

Fraction No.	Flavone	Saponin	Inhibition zone of microorganisms measured by mm							
			I	II	III	IV	V	VI	VII	VIII
1	1.4	2.20	6	0	25	0	5	0	13	6
2	3.0	3.70	6	6	0	5	0	0	0	0
3	2.4	4.70	0	0	17	5	6	5	10	6
4	2.4	3.68	6	18	15	15	12	6	0	12

I = *S. aureus.* II = *Micrococcus* III = *Streptococcus* spp. IV = *B. anthracis*
V = *E. coli* VI = *Klebseilla pneumoniae* VII = *Pseudomonas aeruginosa*
VIII = *Proteus vulgaris*

From Table 5, it was clear that fraction No. 1 showed antifungal activity against *A. niger* (19 mm); *Mucor* (12 mm) while fraction 2 showed activity against *A. niger; A. candidus chrisoporium, Cand. albicans, Cand.* spp 20 and *Rhodotorula* in an inhibition zone 10, 10, 18, 5,6 and 9 mm respectively. Fraction 3 is active on *A. niger, Cand.* spp 20 and *Rhodotorula* with inhibition zone (5 mm) in the 3 fungi. On the other hand fraction 4 was highly effective to *A. parasiticus* (36 mm), and *A. candidus* (15 mm) while *Chrisosporium* and *Cr. neoformans* were 6 and 5 mm respectively. The four fractions gave different degrees of antifungal activity to examined 14 fungal species. The results were in agreement with the findings by Ross *et al*[5] who found that the alcoholic extracts of *P. harmala* and *Solenostemma argel* possessed a marked antifungal activity. Also Hegazi *et al*[6] showed a week antifungal activity of the four fractions obtained by extraction of *S. argel* with chloroform/methanol in different proportions.

Table 5
Fungicidal activity of *S. argel* fractions

Fungi	Fraction No.1*	Fraction No.2*	Fraction No.3*	Fraction No.4*
Fusarium	0	0	0	0
Aspergillus	0	0	0	36
A. Flavus	0	0	0	0
A. niger	19	10	5	0
A. Candidus	0	10	0	15
A. glaucus	0	0	0	0
Penicillum	0	0	0	0
Chrisosporium	0	18	0	6
Cryptococcus	0	0	0	5
Candida spp.	0	0	0	0
Cand. albicans	0	5	0	0
Cand spp. 20	0	6	5	0
Mucor	12	0	0	0
Rhodotorula	0	9	5	0

*Inhibition zone of microorganism measured by mm

From these data it could be concluded that the different fractions of *Solenostemma argel* extracted by methanol/water possess an antimicrobial and fungicidal activities in a variable manner.

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