Qatar Univ. Sci. J. (1994), 14 (C) : 143-146

STUDIES FOR DETERMINING ANTIMICROBIAL ACTIVITY OF *SOLENOSTEMMA ARGEL* (DEL) HAYNE. 2-EXTRACTION WITH CHLOROFORM/METHANOL IN DIFFERENT PROPORTIONS

By

FATEN K. ABD EL HADY*, A. G. HEGAZI**, NAGWA ATA** AND MONA L. ENBAAWY***
* Dept. of Natural Products, **Dept. of Parasitology & Animal Diseases, National Research Centre, Dokki, Giza
***Dept. of Microbiology, Faculty of Vet. Med. Cairo University, Egypt.

دراسات لقياس كفاءة نبات الحرجل كمضاد ميكروبي
٢ ـ الإستخلاص بنسب مختلفة من الكلوروفورم/ميثانول

فاتن كمال عبد الهادي و أحمد جعفر حجازي
نجوى سيد عطا و منى الإمبعاوي

أستخلص الجزء الهوائي لنبات الحرجل بإستخدام الكلوروفورم/ الميثانول بنسب مختلفة والذي أنتج أربعة مستخلصات تم دراستها فيتوكيميائيا وكروماتوجرافيا وكذلك التقدير الكيفي لمحتويات الفلافونات والصابونينات لكل مستخلص كما درس النشاط المضاد للميكروبات ممثلا في ثمانية أنواع من البكتريا وأربعة عشر فطراً .

وكان من الواضح التأثير الجلي للنشاط القوي للميكروب السبحي ، وفاعلية متوسطة للميكروب القولوني و عصويات الجمرة الخبيثة والميكروب العنقودي المكور الذهبي والكلبسيللا والإسيدوموناس ، بينما كان تأثير هذه المستخلصات ضعيفاً على الفطريات .

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

ABSTRACT

Aerial parts of *Solenostemma argel* plant were successively extracted with chloroform/methanol 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. A powerful effect was observed in case of *Streptococcus* spp. and moderate action against *E. coli. B. anthracis; S. aureus; Klebsiella pneumoniae* and *Proteus vulgaris*. There was no effect on *Micrococcus* and *Pseudomonas* while a weak fungicidal activity was observed.

INTRODUCTION

Medicinal plants are of world wide distribution. The use of such plants was directed to overcome the drug resistence especially against bacteria and avoid the side effects of synthetic drugs. Many authors studied the phytochemical analysis of such plants[1]. In a survey on some Egyptian plants about 60% of these plants have antimicrobial activity, whereas 15% exhibited a marked antifungal property[2].

Solenostemma argel (Del) Hayne is used in the folk medicine as an effective remedy for cough; infusion of leaves for gastrointestinal cramps, as laxative[3], stomachache; anticolic; for cold urinary tract; antisyphilitic if used for prolonged period of 40 to 80 days[4] and antiinflammatory[5].

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

MATERIAL AND METHODS

Solenostemma argel (family Asclepiadaceae) was collected from south Sinia, 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

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.* [6].

Chromatographic screening with TLC (Silica gel DF 254)

The probability of the presence of saponins, cardiac glycodsides was reported by Stahl[7] where differentiation between sitosterol, cardiac and steroidal glycosides was carried out on TLC by spraying with anisaldehyde reagent giving purple, blue and yellow colours, respectively. Also the differentiation between furostanol and spirostanol saponins is possible with Ehrlich reagent which gives red colour with furostanol only. The dominant kaempferol glucosides in *S. argel* were detected in comaprison with authentic samples [8].

Determination of total flavonoids

The standard calibration curve of flavonoids was done according to the procedure of Khalifa[9]. Kaempferol-3-glucoside[8], each weight was dissolved in methanolic AlCl3 (0.1 M) and measured at 400 nm. The curve obeys Beer's law from 20-340 mg. Spectrophotometric (UV spectra, Shimadzu-240) determination of total flavonoid content of the obtained fractions was done by dissolving 0.8 mg of each fraction in 5 ml AlCl3 (alch. solution) and measured at 400 nm. The flavonoid content was measured from standard calibration curve as kaempferol-3-glucoside.

Determination of the saponin content

The quantitative determination of saponin content was carried out using the cholesterol complex method[10]. One gram of each fraction was dissolved in a suitable volume of 70% aqueous ethanol. The free saponins were obtained by decomposing the cholesterol complex with pyridine.

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.* [11].

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[12,13]. 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 inoculated 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 o f the disk[13].

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).

Table 1
Phytochemical screening of *S. argel* fractions.

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

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

TLC chromatographic screening (Table 2) revealed the probable presence of cardiac glycosides (positive Libermann Burchardt and Killer Killiani tests), blue colour with anisaldehyde reagent[7] and negative colour with Kedde reagent[14]. Also the probable existance of steroidal saponins of furostanol type [(positive to saponins and steroidal test[6], and yellow colour with anisaldehyde reagent and the red colour with Ehrlich[7] was noticed. The results obtained from spectrophotometric determinattion of the flavonoid and saponin contents are shown in Table 3. The flavonoid content of the four fractions was 0, 19.0, 3.6 and 7.4% in fractions number 1,2,3 and 4, respectively while the saponin content was 1, 0, 0.05 and 0.9% in the same fractions.

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

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	Other
1	I	0.84, 0.7, 0.5, 0.48, 0.4, 0.37 ++ ++ +++ +++ +++ ++	0.8, 0.6 ++ +++	The same -	-	-
	II	-	-	-	-	-
2	I	-	-	-	-	-
	II	-	-	-	0.87, +++	0.55 ++
3	I	-	-	-	-	-
	II	-	-	-	0.55 ++	0.28 ++
4	I	-	-	-	-	-
	II	-	-	-	0.87, 0.55, 0.28 + ++ ++	

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 EtOH
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	0.0	0.035	1.000
2	19.0	0.000	0.000
3	3.6	0.005	0.050
4	7.4	0.026	0.900

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

The results obtained (Table 4) revealed that the four fractions extracted from *S. argel* by chloroform/methanol in different proportions has antimicrobial activity in variable degrees. The most powerful effect was observed against *Streptococcus* spp and moderate actions against *E. coli, B. anthracis, S. aureus, Klebsilla pneumoniae* and *Proteus vulgaris*. There was no effect against *Micrococcus* and *Pseudomonas aeruginosa*. 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. Though Tharib *et al.*[15] isolated 4 components from stems of the desert shrub (*S. argel*), yet there was only one fraction (from the saponifiable fraction) which showed antimicrobial activity against both Gram positive and Gram negative bacteria.

Table 4
Antimicrobial activity of *S. argel* fractions.

Fraction No.	Flavones	Saponins	Inhibition zone of microorganisms measured by mm I	II	III	IV	V	VI	VII	VIII
1	0.0	1.00	8	0	17	17	11	10	0	10
2	19.0	0.00	7	0	25	0	0	0	0	0
3	3.6	0.05	7	0	24	5	11	5	0	6
4	7.4	0.90	0	0	20	5	5	0	0	6

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

From Table 5 it was clear that fraction No. 3 showed no antifungal activity while fraction 2 showed activity only against *Penicillum*. Fraction 1 was effective against *Can* spp and *Rhodotorula*. On the other hand fraction 4 was effective against *Cr. neoformans*. These 4 fractions possessed a weak effect on 14 fungal species. These results were comparable with the previous findings of Ross *et al* [2] who found that alcoholic extracts of *P. harmala* and *Solenostemma argel* have a marked antifungal activity.

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	0
A. Flavus	0	0	0	0
A. niger	0	0	0	0
A. Candidus	0	0	0	0
A. glaucus	0	0	0	0
Penicillum	0	6	0	0
Chrisosporium	0	0	0	0
Cryptococcus	0	0	0	5
Candida spp.	0	0	0	0
Cand. albicans	0	0	0	0
Cand spp. 20	6	0	0	0
Mucor	0	0	0	0
Rhodotorula	9	0	0	0

*Inhibition zone of microorganism measured by mm

From these data it could be concluded that the different fractions of *Solensotemma argel* extracted by chloroform/methanol possess an antimicrobial activity to some Gram positive and Gram negative bacteria in a variable manner and a weak fungicidal activity.

REFERENCES

[1] **Korsun, V. P., 1983.** The uses of propolis in treating trophic ulcer. Vestnik Dermatololii ivenerologii, ii, 46-48.

[2] **Ross, S. A., S. E. Megalla, D. W. Bishay** and **A. H. Awaad, 1980**. Studies for determining antibiotic substancces in some Egyptian plants. 1- Screening for antimicrobial activity. Fitoterapia, 51(6): 303-308.

[3] **Filipescu, G. G., A. Gerorgeto, E. Grizorescu, E. Streil** and **T. Gabriela, 1985.** Pharmocognostic study of some *Cassia* L and *Solenostemma* L. I-Preliminary morphoanatomiccal and chemical study of *Cassia alata* L. *C. dentolis* L., C.- mimosolides L-C, Angustifolial and *Solenostemma argeria* L. An Stiint Univ. M. I. Cuza "Iasia, Sect. 2a: 31: 53-56.

[4] **Boulos, L., 1983.** Medicinal plant of North Africa. Reference publication, Inc., Michigan, U.S.A.

[5] **Jabeen, F. S. M., Tharib** and **G. B. A. Veitch, 1984.** An investigation of the antiinflammatory activity of *Solenostemma oleifolium.* Fitoteropia, 55(3): 186-189.

[6] **El Gamal, M. H. A., F. K. Abd El Hady** and **H. S. M. Soliman, 1990.** Constituents of local plants XXX. Phytochemical screening of some selected local saponin bearing plants, Bull. NRC 15(4): 215-220.

[7] **Stahl, E., 1969.** Thin Layer Chromatography, Berlin, Heidelberg, New York.

[8] **Abd El Hady, F. K.** and **S. A. Ouf, 1993.** Fungi toxic of material from *Solenostemma argel* (Del) Hayne on some shoot surface fungi. Zentralblatt für Mikrobiologie 148: 598-607.

[9] **Khalifa, T. I., F. J. Muhtadi** and **M. M. A. Hassan, 1983.** Analytical profiles of drug substances. Academic press subsidiary of Horcourt Brace, Jovanocish Publicher, New York, USA. Vol 12: p. 630.

[10] **Wulff, G., 1968.** Neuere entewicklungen auf dem saponingebiet. Deutche Apotheker Zeitung, 108(23): 797.

[11] **Cruickshank, R., J. P. Duguid** and **R. H. Swain, 1970.** In medical microbiology, 11th Ed. E. and S. livingstone Ltd., UK.

[12] **Bauer, A. W., D. M, Perry** and **W. M. Kirby, 1959.** Single disk antibiotic sensitivity testing of Staphylococci: An analysis of technique and results. Arch Intern Med. 104: 208-216.

[13] **Hegazi, A. G., F. K. Abd El Hady, M. L., El Enbaawy** and **N. Ata, 1993**. Studies for determinining antimicrobial activity of *Solenstemma argel* (Del) Hayne. 3-Extraction with petroleum ether and ether. Ist Int. Conf. in Chemistry and its Applications. Qatar, Dec. 1993., p. 190.

[14] **Balbaa, S. I., S. H. Hilal** and **A. Y. Zaki, 1976.** Medicinal plant constituents, p. 305-329. General Organisattion of University Books, Egypt.

[15] **Tharib, S. M., S. El-Migirab** and **G. B. Veitch, 1986.** A preliminary investgation of the potential antimicrobial activity of *Solenostemma argel.* Int. J. Crude Drug Res., 24(2): 101-104.