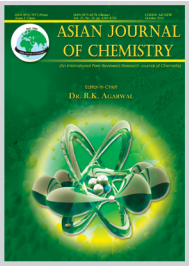
Asian Journal of Chemistry; Vol. 24, No. 8 (2012), 3548-3552

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Molluscicidal Activity and Screening of Forty Egyptian Medicinal Plants and Determination of the Active Fractions

KHALED A. SHAMS[1,*], LOBNA M. ABOU- SETTA[1], HANY M. RADWAN[1], NAGLAA M. NAZIF[1], RASMIA A. HASSAN[1] and ABDEL-MOHSEN M. SOLIMAN[2]

[1]Phytochemistry Department, National Research Centre, Dokki, Cairo-12311, Egypt
[2]Therapeutical Chemistry Department, National Research Centre, Dokki, Cairo-12311, Egypt

*Corresponding author: E-mail: khaledashams@hotmail.com

(*Received*: 30 July 2011; *Accepted*: 16 March 2012) AJC-11188

Schistosomiasis is considered as one of the most important trematode diseases of man. The most important goal of the present study is to use the natural plants as low cost and available sources for snail control. Screening of the molluscicidal activity for 40 Egyptian plants were carried out. Only 10 plants showed promising results, *Acacia nilotica, Lycopersicum esculentum, Ambrosia maritima, Zizyphus spina-christi, Coronopus squamatus, Diplotaxis acris, Spathodea campanulata, Eruca sativa, Cakile maritima*, *Nerium oleander* showed marked activity. The last 5 plants showed this activity for the first time.

Key Words: ***Acacia nilotica, Lycopersicum esculentum, Ambrosia maritima, Zizyphus spina-christi, Coronopus squamatus, Diplotaxis acris, Cakile maritima and Nerium oleander, Spathodea campanulata, Eruca sativa,*** **Molluscicidal activity.**

INTRODUCTION

A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but because such information may be of value in disclosing new sources of material. Schistosomiasis is a serious problem that infects millions of people in tropical and subtropical regions[1]. Though during last decades many investigators studied the chemical control of molluscan intermediate hosts of human schistosomiasis[2]. Therefore searching for new agents from natural products of different structures and mode of action is one of the important subjects to be considered in the hope to explore more effective control agents. Numerous investigations on naturally occurring molluscicides have been attributed to saponines[2-5], flavonoids[6,7], sesquiterpenes[8], diterpenes[9,10] and anacardic acid[11]. Also several plants showed to contain glucosinolate compounds, which found to be important to evaluate their biological activities against *Biomphalaria alexandrina* snails such as *Coronopus squamatus, Cakile maritime, Lepidium sativam, Eruca sativa* and *Diplotaxis acris*[12-15].

The present work aims to evaluate the biological activity of the alcoholic extracts of 40 Egyptian plants belonging to 25 families against *Biomphalaria alexandrina* snails, specific intermediate hosts of *Schistosoma mansoni* and the ultimate objective being to isolate and characterize the active constituents from the most promising species, therefore a phytochemical screening was carried out for the promising species to isolate the main classes of organic compounds in each plant. The different fractions of each promising plant were extracted and tested against *Biomphalaria alexandrina* to determine the active fraction responsible for molluscicidal activity.

EXPERIMENTAL

Nine of plant materials used in this study (Table-1), were purchased from local market. Whereas, *Senna alexandrina* Mill., *Lycopersicum esculentum* L., *Lepidium sativum*, *Capsicum annum* L. and *Morus alba* were collected from experimental station of medicinal plants, Pharmacognosy Dept., Faculty of Pharmacy, Cairo University at Giza. Also, *Zizyphus spina-christi* and *Leucaena glauca* were obtained from Orman Garden, Giza. *Coronopus squamatus* was collected from the farm of Saft Al-Laban, at Giza. Plant materials *Carpobortus edulis* N.E.Br., *Lotus hebranicus* Hochst. and *Lotus glinoides* Del were collected from Borg El-Arab near Alexandria during May 2009. *Mesembryanthemum forsskalei* Hochst., *Aerva javanica* (var. *bovi webb*), *Salsola vermiculata L., Forsskalei tenacissima* L. and *Zygophyllum coccineum* L. were collected from South Sinai beside Taba during June 2009. *Suaeda vermiculata* Forssk and *Chrozophera plicata* (vahl) were collected from El Arish, North Sinai during June 2009. *Ambrosia maritima* L. was collected from Nile region beside Faiyoum. Also, *Deplotaxis acris* plant was collected from

wadi- erabo, St. Cathrine. *Asteropterus leyseroides*, *Echium cericeum* and *Limoniastrum monopetalum* plants were collected from Mediterranean Coast near Marsa Mattrouh. Finally, cakile maritima, *Atriplex semibacata*, *Suaeda proinosa*, *Nerium oleander* and *Delonix elata* were collected from Borg El-Arab near Alex., Egypt during 2009-2010. All the plant samples were identified by Dr. M. El-Gebaly and Prof. Dr. S. El-Kawashty, Taxonomists, National Research Centre, Cairo, Egypt, to whom the authors are deeply indebted.

Materials for molluscicidal activity

Snails: The snails were collected from irrigation canals located in Giza Governorate. They were maintained as stock cultures in a well aeriated glass aquaria containing dechlorinated tap water and fed on fresh lettuce leaves daily at 25-27 ºC.

Methods: Samples of dried powdered plant materials (20 g). Each were extracted with boiling 70 % ethyl alcohol for few minutes and filtered while hot and the molluscicidal activity of these extracts against *Biomphalaria alexandrina* snails were carried out (Table-1).

Methods of screening: The phytochemical screening of the plants showed significant molluscicidal activity were carried out for the following constituents: alkaloids, flavonoids, saponins, sterols and/or triterpenes, glucosinolates, coumarins and tannins[16,17], (Table-2).

Extraction and fractionation of the promising plants: About 200 g of each dried plant materials were extracted separately with pet ether (br. 40-60 ºC) in a Soxhlet. The extract was evaporated and the residue was dissolved in boiling acetone, cooled and the precipitate formed was separated. The acetone soluble fraction was saponified (N/2 alc. KOH) and the unsaponifiable matter was isolated (Fr. A).

The defatted powdered plants were extracted separately with 70 % ethanol till exhaustion and divided into 2 portions. One portion was evaporated and dissolved in hot water, cooled, filtered and followed by successive extraction with chloroform (Fr. B), ethyl acetate (Fr. C) and finally with *n*-butanol[18]. The *n*-butanol fraction was evaporated, taken in methanol and a large volume of ether was added. Brown precipitate was separated (Fr. D) and the mother liquor (Fr. E) was evaporated.

The second portion of the ethyl alcohol extract was evaporated and dissolved in 100 mL 10 % HCl (in distilled water), filtered and extracted with chloroform, then the mother liquor rendered alkaline with ammonium hydroxide (30 %) till pH = 10 and then extracted with chloroform (Fr. F)[17,18].

Finally, about 100 g of each dried plant materials were extracted separately with pet ether and then with 70 % ethyl alcohol. In this technique the alcoholic extract containing the total glucosinolates is carried over an acidic alumina column (anionic). The total glucosinolates are eluted by potassium sulphate solution 2 %, the combined eluent is concentrated, desalted with hot methanol, dissolved in distilled water and freeze dried (Fr. G)[17].

The fractions of each plant were tested against *Biomphalaria alexandrina* snails to determine the active fractions, which are responsible for molluscicidal activity, (Table-3).

TABLE-1
MOLLUSCICIDAL ACTIVITY OF 70% ALCOHOLIC EXTRACTS OF THE PLANTS AGAINST *Biomphalaria alexandrina* SNAILS

No.	Family and species	Plant part	LC_{50} (ppm)	LC_{90} (ppm)
1	***Aizoaceae*** *Carpobortus edulis* N.E.Br.	Aerial part	101	143
2	*Mesembryanthemum forsskalei* Hochst	Aerial part	98	136
3	***Amaranthaceae*** *Aerva javanica* (var. *bovi webb*)	Aerial part	87	131
4	***Chenopodiaceae*** *Salsola vermiculata* L.	Aerial part	121	143
5	*Suaeda vermiculata* Forssk	Aerial part	107	125
6	*Suaeda pruinosa* lang.	Aerial part	118	133
7	*Atriplex semibaccata*	Aerial part	99	117
8	***Compositae*** *Ambrosia maritima* L.	Stem and Leaves	54	72
9	***Euphorbiaceae*** *Chrozophora plicata* (vahl)	Stem and Leaves	71	92
10	***Gramineae*** *Corn silk (Zea mays L.)*	Styles and Stigma	124	139
11	***Lauraceae*** *Cinnamoumum zelanicumnees*	Shoot bark	101	123
12	***Leguminoseae*** *Acacia nilotica*	Pods	41	63
13	*Senna alexandrina* Mill	Pods	146	163
14	*Lotus glinoides* Del	Aerial part	131	152
15	*Lotus hebranicus* Hochst	Aerial part	129	151
16	*Trigonella faenum groecum* L.	Seeds	113	129
17	*Leucaena glauca* (L.)	Aerial part	123	152
18	***Myrtaceae*** *Eugenia romatica* L.	Flower buds	141	162
19	***Rhamnaceae*** *Zizyphus spina-christi* L.	Leaves	51	81
20	***Solanaceae*** *Capsicum annuum* L.	Leaves and Shoots	119	141
21	*Capsicum minimum* Roxb	Ripe fruits	126	143
22	*Lycopersicum esculentum* L.	Leaves and Shoots	49	69
23	***Tiliaceae*** *Tilia europeae* L.		86	103
24	***Urticaceae*** *Forsskalei tenacissima* L.	Aerial part	72	91
25	***Zingiberaceae*** *Zingiber officinalis, Roseae*	Rhizome	106	127
26	***Zygophyllaceae*** *Zygophyllum coccineum* L.	Aerial part	79	94
27	***Brassicaceae*** *Coronopus squamatus*	Aerial part	38	61
28	*Diplotaxis acris*	Aerial part	43	68
29	*Cakile maritima* (scope)	Aerial part	55	84
30	*Lepidium sativam* (L.)	Aerial part and Seeds	49	67
31	*Eruca sativa*	Seeds	44	63
32	***Plumbaginaceae*** *Limoniastrum monopetalum*	Aerial part	78	115
33	***Caesalpinioideae*** *Delonix elata*	Aerial part & Flowers	88	144
34	***Apocynaceae*** *Nerium oleander*	Aerial part & Flowers	61	98
35	***Boraginaceae*** *Echium sericeum*	Aerial part	111	156
36	***Asteraceae*** *Asteropterus leyseroides*	Aerial part	103	129
37	***Myrtaceae*** *Syzygium cumini*	Leaves	88	124
38	***Moraceae*** *Mulberry* (*Morus alba*)	Leaves	71	104
39	***Bignoniaceae*** *Kigelia pinnata*	Fruits	111	145
40	*Spathodea campanulata*	Leaves	53	72

TABLE-2
PHYTOCHEMICAL SCREENING OF PLANTS HAVING SIGNIFICANT MOLLUSCICIDAL ACTIVITY

Family and name of plant	Alkaloids	Flavonoids	Saponins	Sterols and/or terpenes	Coumarins	Tannins	Glucosinolates
Leguminoseae *Acacia nilotica* L. (dried pods)	+	++	+	+	±	++	-
Compositae *Ambrosia maritima* L. (aerial parts)	±	-	+	+	++	+	-
Solanaceae *Lycopersicum esculentum* L. (dried leaves and shoots)	++	+	++	+	-	+	-
Rhamnaceae *Zizyphus spina-christi* L. (dried leaves	±	++	++	+	-	±	-
Brassicaceae *Coronopus squamatus* (aerial parts)	-	+	-	+	-	-	+
Diplotaxis acris (aerial parts)	-	+	-	+	+	±	+
Cakile maritima (scope) (aerial parts)	-	+	-	+	+	-	+
Lepidium sativum (L.)	+	+	±	+	+	-	+
Eruca sativa L. (Seeds)	±	+	-	+	-	-	+
Bigononiaceae *Spathodeae componulata* (Leaves)	-	+	++	-	-	+	-

+ Present, - Absent, ± Traces

TABLE-3
MOLLUSCICIDAL ACTIVITY OF DIFFERENT FRACTIONS FROM THE PROMISING PLANTS

No.	Family and species	LC_{50} (ppm)	LC_{90} (ppm)
1	*Acacia nilotica* L.		
	Fraction A	107	131
	B	91	113
	C	61	77
	D	31	42
	E	47	62
	F	131	149
	G	124	152
2	*Ambrosia maritima* L.		
	Fraction A	99	116
	B	104	122
	C	98	125
	D	52	88
	E	59	81
	F	115	132
	G	103	114
3	*Lycopersicum esculentum*		
	L.	132	157
	Fraction A	72	99
	B	46	58
	C	53	79
	D	67	99
	E	141	167
	F	79	94
	G		
4	*Zizyphus spina-christi* L.		
	Fraction A	112	135
	B	106	134
	C	94	119
	D	71	105
	E	84	112
	F	136	157
	G	107	143
5	*Coronopus squamatus*		
	Fraction A	87	101
	B	72	99
	C	69	82
	D	108	139
	E	97	122
	F	105	117
	G	33	42
6	*Diplotaxis acris*		
	Fraction A	107	139
	B	75	103
	C	109	146
	D	111	155
	E	91	98
	F	68	82
	G	35	52
7	*Cakile maritima* (scope)		
	Fraction A	99	138
	B	94	107
	C	79	91
	D	104	126
	E	83	94
	F	58	91
	G	46	71
8	*Nerium oleander*		
	Fraction A	72	89
	B	94	111
	C	61	74
	D	135	161
	E	101	132
	F	74	90
	G	48	67
9	*Spathodea campanulata*		
	Fraction A	89	117
	B	111	142
	C	81	96
	D	69	94
	E	57	83
	F	68	94
	G	144	161
10	*Eruca sativa*		
	Fraction A	114	132
	B	88	101
	C	71	101
	D	110	146
	E	102	151
	F	91	114
	G	60	88

Fraction A: unsaponifiable matter; B: Chloroform fraction; C: Ethyl acetate fraction; D: Brown ppt. from butanol; E: Mother liquor from butanol; F: Alkaloid fraction; G: Glucosinolate fraction

Detection: (I) Detection of sterols and terpenes in (Fr. A) isolated from the 10 promising plants were proved by using TLC (silica gel, toluene-acetone 9:1) and sprayed with 5 % sulphuric acid[19].

(II) Detection of coumarins in (Fr. B) isolated from *Acacia nilotica* L., *Lepidium sativum*, *Diplotaxis acris*, *Eruca sativa* and *Ambrosia maritima* L. were proved by using TLC (cellulose, benzene-acetic acid-water 6:7:3 and sprayed with 5 % sodium hydroxide solution)[18].

(III) Detection of flavonoids in (Fr. C) isolated from *Acacia nilotica* L., *Lycopersicum esculentum* L., *Coronopus squamatus*, *Cakile maritima*, *Diplotaxis acris*, *Lepidium sativum*, *Eruca sativa, Spathodea campanulata* and *Zizyphus spina-christi* L. were proved by using PC (3 mm, 15 % acetic acid) and sprayed with (NA)[18].

(IV) Detection of alkaloids in (Fr. F) isolated from *Acacia nilotica, Lycopersicum esculentum, Ambrosia maritima, Lepidium sativum, Eruca sativa* and *Zizyphus spina-christi* were proved by using TLC (silica gel, chloroform-methanol-ammonium hydroxide 85:14.5: 0.5)[20].

(V) Detection of glucosinolates in (Fr. G) isolated from *Coronopus squamatus, Diplotaxis acris, Cakile maritima, Lepidium sativum* and *Eruca sativa* were proved by using PC (butanol- acetic acid-water 12:3:5, which give brown spot after spraying with $AgNO_3$ reagent)[17].

Measurement of LC_{50} of the extracts and the isolated fractions: Stock solutions (100 ppm) of extract or isolated fractions were prepared separately by dissolving 100 mg in 1 mL ethanol and then diluted with dechlorinated water (1 L). Series of dilution that would permit the computation of LC_{50} - LC_{90} values were used.

10 matured snails (8-10 mm in diameter) were immersed in suitable beaker containing 100 mL of the tested concentration. Three replicates were employed in each concentration beside a control groups containing methanol and water only. The exposure period was 24 h. The snails were then washed with dechlorinated water and transferred to a new beaker for a recovery period of another 24 h. The molluscicidal activity of the studied extracts were done according to standard procedure[21] and the effectiveness of the extracts was expressed in terms of LC_{50} and LC_{90} *via* statistical analysis[22].

RESULTS AND DISCUSSION

The biological activity of alcoholic extracts of 40 Egyptian plants belonging to 25 families were carried out against *Biomphalaria alexandrina snails*. Data listed (Table-1) revealed that only *Acacia nilotica, Lycopersicum esculentum, Ambrosia maritima, Zizyphus spina-christi, Coronopus squamatus, Diplotaxis acris, Cakile maritima, Lepidium sativum, Eruca sativa* and *Spathodea campanulata* showed significant molluscicidal activity (LC_{50} = 41, 49, 54, 51, 38, 43, 55, 49, 44 and 53 respectively), while the other plants gave low molluscicidal activity. The different fractions of each of the promising plants were extracted and tested against *Biomphalaria alexandrina snails*. The data listed (Table-3) revealed that fraction D and E isolated from *Acacia nilotica* showed significant molluscicidal activity (LC_{50} = 31, 47 respectively), while fractions B and C showed moderate activity and the other fractions showed the lowest activity. It is obvious that fraction D and E displayed moderate toxicity followed by fraction B and C, while the other fractions showed less toxicity by 3-4 folds.

From the phytochemical screening of the plant (Table-2), the significant activity of fractions D and E may be attributed to the presence of tannins and/or saponins present in the plant. This result was in agreement with those obtained by Hussein Ayoub[23].

On the other hand, the data listed in (Table-3) revealed that fraction C isolated from *Lycopersicum esculentum* possessed the highest activity against snails (LC_{50} = 46 ppm) followed by fractions D and E, while the other fractions showed low activity. The significant activity of the plant may be attributed to the presence of flavonoids in fraction C or to the presence of saponin and/or tannins in fractions D and E.

Also, from the data listed in (Table-3), it is clear that fractions D and E isolated from *Zizyphus spina-christi* possessed moderate activity against snails and the other fractions showed low activity. This moderate activity may be attributed to the presence of saponins in the plant and this activity is demonstrated in this genus for the first time.

Data listed in (Table-3) revealed that fractions D and E isolated from *Ambrosia maritima* possessed moderate activity against snails and the other fractions showed low activity that may be attributed to the presence of saponins and/or tannins present in the plant[24].

The data listed in (Table-3) also revealed that the fraction G isolated from *Coronopus squamatus* showed significant activity against snails (LC_{50} = 33 ppm) and the other fractions showed low activities. The significant activity of the plant may be attributed to the presence of glucosinolate in fraction G.

Data listed in (Table-3) revealed that fraction G isolated from *Diplotaxis acris* showed strong activity (LC_{50} = 35 ppm) and the other fractions showed between low and moderate activities. The significant activity of fraction G may be attributed to the presence of glucosinolates.

Also, the G fraction of *Cakile maritima* showed high activity against snails due to the presence of glucosinolate compounds, while as fraction F of the plant showed moderate activity that may be attributed to the presence of alkaloidal compounds.

On the other hand, the data listed in (Table-3) revealed that fraction G isolated from *Nerium oleander* possessed the highest activity against snails (LC_{50} = 48 ppm) followed by fractions C, A and F, while the other fractions showed low activity. The significant activity of the plant may be attributed to the presence of glucosinolate compounds in fraction G, while the moderate activity may be attributed to the presence of flavonoidal and/or alkaloidal compounds in fractions C and F.

Also, from the data listed in (Table-3), it is clear that fractions E and F isolated from *Spathodea campanulata* showed moderate activity against snails while, other fractions showed low activities. This moderate activity may be attributed to the presence of tannins and/or alkaloidal compounds in fractions E and F.

Finally, from the data listed before, it is clear that fractions G and C isolated from *Eruca sativa* possessed moderate activity

against the snails. The moderate activity of the plant may be attributed to the presence of glucosinolates and/or flavonoides in fractions G and C. All of these fractions will be subjected to further investigations to identify their constituents.

REFERENCES

1. M. Andrew and K. Hosttmann. *Phytochemistry*, **24**, 639 (1985).
2. A. Lemma, *Ethiopian Med. J*., **3**, 84 (1965).
3. K. Hostettmann, H. Kizu and T. Tomimori, *Planta Med.*, **44**, 34 (1982).
4. H.W. Liu and K. Nakanishi, *Tetrahedron*, **38**, 513 (1982).
5. K. Hostettmann, *Helv. Chim. Acta*, **63**, 606 (1980).
6. F.R. Medina and L.S. Ritchie, *Econ. Bot.*, **34**, 368 (1980).
7. S. Dossaji and I. Kubo, *Phytochemistry*, **19**, 482 (1980).
8. D.S.J. Santos Filho, W.S. Vichnewski, M.S. Bulhoes and H. de Freitas Leitao Filho, *Rev. Fac. Farm. Odontol. Ribeirao Preto (Univ. Sao Paulo),* **17**, 43 (1980).
9. K. Nakanishi and I. Ubo, *Israel J. Chem.*, **16**, 28 (1977).
10. T.C.B. Tomassini and M.E.O. Matos, *Phytochemistry*, **18**, 663 (1979).
11. J.T. Sullivan, C.S. Richards, H.A. Lloyd and G. Krishna, *Planta Med.*, **44**, 175 (1982).
12. M.A. Wael, Ph. D. Thesis, Chemical and Biological Studies on *Diplotaxis acris* and *Cronopus squamatus* Growing in Egypt, Chemistry Department, Faculty of Science, Ain Shams Univeristy, Egypt (2010).
13. H.M. Radwan, K.A. Shams, W.A. Tawfik and A.M. Soliman, *Res. J. Med. Med. Sci.*, **3**, 182 (2008).
14. M.N. Naglaa, A.H. Amira, A.T. Wafaa and A.H. Rasmia, *Asian J. Chem.*, **22**, 2407 (2010).
15. H.M. Radwan, M.M. El-Missiry, W.M. Al-Said, A.S. Ismail, K.A. Abdel-Shafeek and M.M. Seif El-Nasr, *Res. J. Med. Medical Sci.*, **2**, 127 (2007).
16. A.M. Rizk, *Fitotrapia*, **52**, 35 (1982).
17. A. Bran Hanely, K. Heany Robert and G. Fewwick, *J. Sci. Food Agri.*, **34**, 869 (1983).
18. J.B. Harborne, Phytochemical Methods, Chapman and Hall, London, New York, edn. 2 (1984).
19. H.M. Radwan, M.M. El-Missiry and M.M. Seif El-Nasr, *Bull. Fac. Pharma. Cairo Univ.*, 35, (1977).
20. R.T. Morsy, B. Sc. Thesis, Chemical Studies on Certain Plants of Family Convolvulaceae, Chemistry Department, Faculty of Science, Cairo University, Cairo, Egypt, p. 59 (1999).
21. WHO, Molluscicidal Screening and Evaluation, *Bull. W.H.O*., **33**, 567 (1965).
22. J.T. Litchfeild and E. Wilcoxon, *J. Pharmaco. Exper. Therap.*, **69**, 99 (1996). Cf. M.T. Omar and A.M. Soliman, *Al-Azhar Bull. Sci.*, **7**, 111.
23. S.M. Hussein Ayoub, *Planta Med.*, **46**, 181 (1982).
24. K. Hostettmann and D. Schaufelberger, *Planta Med.*, **48**, 105 (1983).