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IN VITRO ANTISCHISTOSOMAL ACTIVITY OF *ALLIUM CEPA* L. (RED ONION) EXTRACTS AND IDENTIFICATION OF THE ESSENTIAL OIL COMPOSITION BY GC-MS

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ABSTRACT

Allium cepa L. (red onion) is one of the most famous vegetable crops grown in Egypt due to its medical and nutritional importance. *In vitro* antischistosomal bioassay of ethyl acetate (EtOAc) and butanolic (BuOH) fractions derived from methanolic (MeOH) extract of *A. cepa* as well as the essential oil of plant bulbs was carried out using ascending doses. The chemical constituents of essential oil were further investigated using GC-MS analysis. The results revealed that the MeOH extract, EtOAc fraction, BuOH fraction and essential oil have a significant effect on adult *Schistosoma mansoni* worms. The essential oil of *A. cepa* gave high worm mortality (%) at the concentration 500 µg/mL (75%), 250 µg/mL (50%) and 125 µg/mL (30%) death rate after 24 hours. GC-MS analysis of *A. cepa* essential oil exhibited different chemical volatile constituents such as organosulfur compounds, alcohols, acids, esters, furans, phenols, and aldehyde. 3, 5-Diethyl -1, 2, 4-trithiolane (10.17%), 1, 3, 5-trithiolane (7.80%), and 3-(2H-furanone, 2-hexyl-5-methyl) (7.74%) represented the highest contents percent in essential oil of *A. cepa* bulbs.
In conclusion, the bulbs of *A. cepa* exhibited antischistosomal activities and contain a variety of bioactive chemical constituents and can be considered as a natural antischistosomal agent.

Keywords: *Allium cepa* L., antischistosomal activity, GC-MS analysis

INTRODUCTION

Schistosomiasis or bilharzia is one of the most widespread parasitic diseases in the world that has been neglected by governments **(Mafud *et al.*, 2016)**. Schistosomiasis is a tropical disease spread in more poverty and poor living conditions areas **(WHO, 2010).** The World Health Organization (WHO) estimated that in the year 2015; approximately 240 million peoples around the world were infected and the mortality rate of people is 280000 annually **(Stein *et al.*, 2015).**Praziquantel (PZQ) is considered the drug of choice for schistosomiasis treatment (Hotez, 2009, Mantovani *et al.*, 2013). Although it has been documented that PZQ has least side effects, the control of *S.mansoni* using PZQ at a population level faces some limitations **(Metwalley, 2015).** Therefore, the scientific community are contineously searching for some alternative drugs by screening botanical and chemical compounds for their potential activity as antischistosomal agents. Many reports exhibited that the medicinal plants are seems to be the new sources of antischistosomal drugs **(Aline *et al.*, 2013).**
Allium (family Liliaceae) is the largest genus and the most important one in this family that include approximately 700 species. *Allium* genus is widely distributed in North Africa, Europe, Asia and America **(El-Wakil *et al.*, 2015).** *A. cepa* L. (red onion) is a vegetable plant which possesses a strong aromas and flavors and has made it as important food ingredients. Red onion bulbs and essential oil are important parts widely used in food processing **(Najjaa *et al.*, 2007; Che Othman *et al.*, 2011).** The bulbs of onion extracts were demonstrated several biological activities, such as antibacterial, antimutagenic, antitumor and antioxidants (**Ismail *et al.*, 2013; Ye *et al.*, 2013; Abdel-Gawad *et al.*, 2014a).**
The main purpose of this study is to evaluate the *in vitro* antischistosomal activity of essential oil, MeOH extract of *A. cepa* bulbs and its derived fractions. Also, investigation of the chemical constituents of *A. cepa* essential oil by GC-MS analysis.

MATERIAL AND METHODS

Plant materials

The fresh bulbs of *A. cepa* (red onion) were purchased from local market, Giza, Egypt in May 2015. The plant bulbs were kindly identified by Prof. Dr. Waffa Amer, Professor of plant taxonomy, Faculty of Science, Cairo University. The voucher sample was stored in Medicinal Chemistry Laboratory, Theodor Bilharz Research Institute. The fresh bulbs were cut into small piecies, milled with the electric mill and divided into parts. The first part was and kept for extraction of essential oil by hydrodistillation method and the other part was submitted to the extraction process.

Extraction and fractionation processes

One kilogram of freshly milled bulbs of *A. cepa* was extracted with MeOH. The methanolic extract was evaporated under vacuum to dryness using rotatory evaporator. The dried methanolic extract was defatted using petroleum ether then the defatted MeOH extract was successively fractionated by partition using EtOAc and BuOH. The two fractions were evaporated till dryness under reduced pressure. The dried extract and fractions were kept in dry vials for the antischistosomal test.

Extraction of essential oil from *A. cepa* (red onion) bulbs

Fresh bulbs (2.5 kg) of *A. cepa* were submitted to hydrodistillation process using a Clevenger-type apparatus. The plant sample was immersed in distilled water (2.5 L) in round flask. The extraction step was executed for 7 h until complete plant exhaustion. The distillation process was started after 40 min of heating. The condensation of oil drops was obtained with continuous chilled water (10 °C). The experiments were repeated three successive times. The resulted essential oils were kept in tightly closed vials and preserved at 4 °C in a refrigerator to evaluate its antischistosomal activity and characterize of its chemical composition by GC-MS.

In vitro antischistosomal bioassay screening

Schistosoma mansoni worms were colected from the Schistosoma Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The antischistosomal assay was carried out using the method described by **Metwalley, (2015).** *S. mansoni* worms were washed several times in sterile RPMI-1640 media (Cutilab, São Paulo, Brazil) buffered to pH 7.5, with HEPES 20 mM and completed with streptomycin (100 mg/mL), 10% fetal calf serum (Gibco, UK) and penicillin (100 U/mL). In 35 mm diameter (35 × 10 mm)

polystyrene petri dish, 10 adult *S. mansoni* worms were cultured in 10 mL sterile RPMI-1640 media with descending concentrations of plant extracts and oil(500, 250 and 125 µg/mL) then incubated in a humid 5 % CO_2 shaking incubator **(**SSI10R Large Refrigerated Incubator Shaker, Germany**)** at 37 °C for 24 hrs. In parallel, the adult worms were inserted in cultured media (RPMI-1640) containing 10% DMSO as solvent control. Worms exhibited no signs of motility for one minute, in addition to those showing deformities such as twisting, blackening, contracting and shrinking were considered dead.The efficacy of plant extracts on Schistosoma worms (viability, mortality and shrinking) was recorded using a stereomicroscope at different time intervals (1 h, 3 and 24 hrs) of incubation.

GC-MS analysis

The essential oil of *A. cepa* was performed using GC-MS instrument (Agilent Technologies, Palo Alto, CA). 9 µL of essential oil were diluted with 991 µL of EtOAc for GC. 0.5 µL of the sample solution were injected into the gas chromatograph model (6890N Network GC system) coupled with a mass spectrometer (MS) model 5973 Network Mass Selective Detector (Agilent Technologies). A capillary column HP-5MS with 0.25 mm internal diameter, 0.25 µm film thickness, and 30 m length was used. Helium gas was used as a carrier at a flow rate of 1.2 mL/min (linear velocity: 33 cm/s). The injected sample was subjected into a split-splitless injector (split ratio 50:1) at 250 °C, the program of oven temperature was the following: 45 °C for 5 min.; an increase of 7 °C/min. up to 100 °C, held for 15 minutes, from 100 °C to 150 °C with an increment of 5 °C per minute, held for 20 minutes, from 150 °C to 200 °C with an increment of 15 °C per minute, held for 5 minutes. The MSD transfer line was set at a temperature of 250 °C; MSD temperature quadrupole was 150 °C and ionization temperature was 230 °C. Mass spectra were acquired at energy 70 eV and the scan acquisition was performed in the range between 35 and 300 *m/z*.The characterization process of the essential oil chemical composition was determined by matching their mass spectra with database of NIST 02 and WILEY 275 libraries**.**

RESULTS AND DISCUSSION

***In vitro* antischistosomal activity**

Schistosomiasis is one of the most predominant parasitic infection diseases worldwide. The results in Table 1 exhibited that the essential oil of *A. cepa* bulbs showed high antischistosomal activity (25 % - 75 %) and shrinking rate (50% - 70%) at concentration 500µg/mL at time interval 1hr to 24 hrs (Hassan *et al.,* 2016). Also, BuOH fraction derived from MeOH extract showed more potent antischistosomal activity (25 % - 50 %) and the highest shrinking rate (25 % - 75 %) at concentration 500 µg/mL at time interval 1hr to 24hrs. Praziquantel (PZQ) exhibited the highest antischistosomal activity (50 % - 100 %) and high shrinking rate (50% - 100%) at concentration 125 µg/mL at 1hr to 24 hrs time interval.

Although the use of PZQ is considered as a drug of choice for treatment of schistosomiasis, there are significant limitations associated with its use. The most important one is, there are strains drug-resistant of the parasite that unable to weakness the oxidative stress directly in the tissue, but only it can decrease the activity of host's antioxidant system **(Benkeblia *et al.*, 2005; Obonyo *et al.*, 2015).** Therefore the new studies are focused on the parasite antioxidant pathway; since the parasite is subjected to a high oxidative stress mainly because of host's immune response **(Benkeblia *et al.*, 2005; Muema *et al.*, 2015).** Also, red onion may be expected as a natural antischistosomal drug due to the reactive oxygen species contribute to a large variety of diseases including schistosomiasis (Rizk *et al.,* 2006). Thus, the plant under investigation was selected on the basis of its antioxidant potential **(Abdel-Gawad *et al.*, 2014b).**

This study is matched with other previous studies which showed that the extracts of the stem and root of *Abrus precatorius* have a high activity against *schistosomules* **(Molgaard *et al.*, 2001).** Another study was reported that *Zingiber officinale* has antischistosomal properties against *S. Mansoni* **(Sanderson *et al.* 2002).** Also, *Allium sativum* showed antischistosomal activity against *S.mansoni* **(Mohamed *et al.*, 2005).**

GC-MS analysis of the essential oil of *A. cepa* (red onion)

According to the high antischistosomal activity of *A. Cepa* essential oil of, this oil was subjected to GC-MS analysis in order to identify the chemical composition of this oil and investigate the relationships between antischistosomal properties of this oil and its chemical composition. The tentative identification of these phytochemicals was done by comparing their mass spectra with the WILEY 275 and NIST 02 libraries. The investigation of essential oil of *A. cepa* led to characterized 50 chemical compounds representing about 96.75 % of the total essential oil content including organosulfur compounds (49.47 %) and other chemical constituents such as alcohols, acids, esters, furans, phenols, and hydrocarbons represent 47.28 % of total as shown in Figure 1 and Table 2. The major chemical components were identified as 3, 5-diethyl -1, 2, 4-trithiolane (10.17 %), 1, 3, 5-trithiolane (7.80 %), 3-(2H-furanone, 2-hexyl-5-methyl) (7.74 %), dodecane (6.77 %), 4 -dibutylaminobut-2-yn-1-ol (5.94 %), 3(2H)-furanone, 5-methyl-2-octyl (5.27 %). These results are in agreement with previous studies reported by **Colina-Coca *et al*. (2013), Mnayer *et al.* (2014)** and **El-Wakil *et al.* (2015).** This suggests that the *in vitro* antischistosomicidal activity of the essential oil of red onion may be attributed to the sulfur and phenolic compounds which have antioxidant properties and may exert important protective effects against oxidative stress that occur during *S. mansoni* infection.

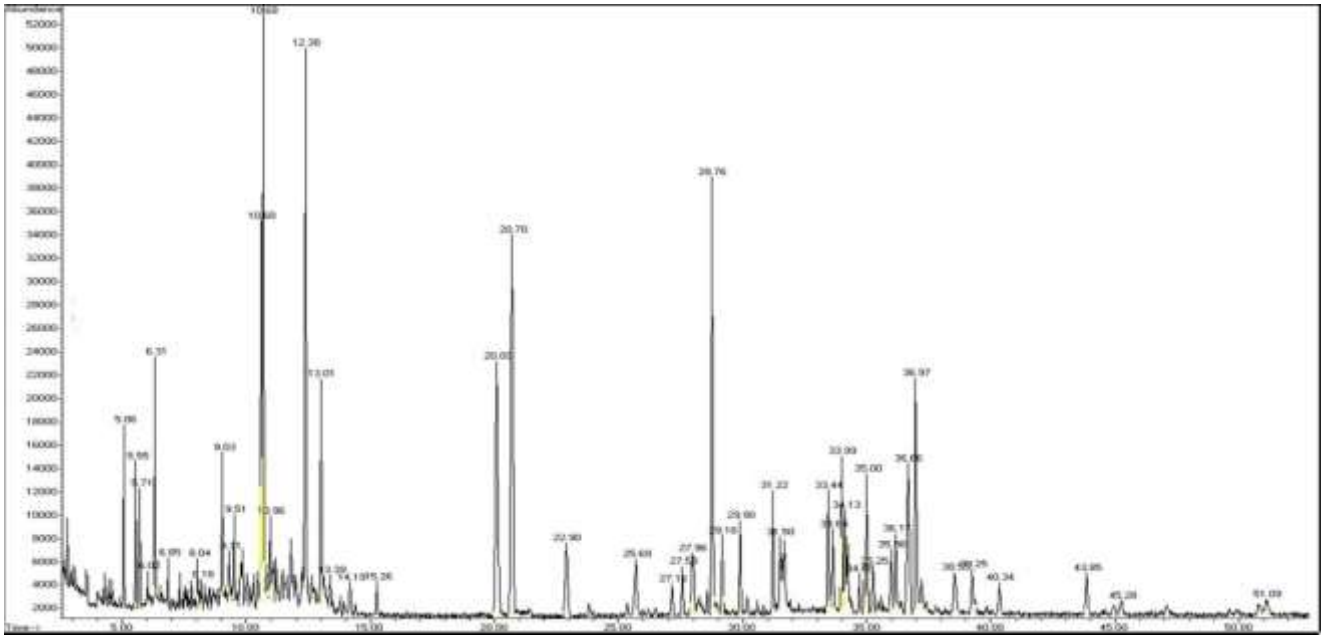

Figure 1 GC-MS chromatogram of the essential oil of *A. cepa* (red onion) bulbs**.**

Table 1 Antischistosomal activity of *A. cepa* (red onion) essential oil, MeOH extracts EtOAc fraction and BuOH fraction

Sample	Mortality %									Viability %									Shrinking %								
	500 µg/mL			250 µg/mL			125 µg/mL			500 µg/mL			250 µg/mL			125 µg/mL			500 µg/mL			250 µg/mL			125 µg/mL		
	1 *hr*	3 *hrs*	24 *hrs*	1 *hr*	3 *hrs*	24 *hrs*	1 *hr*	3 *hrs*	24 *hrs*	1 *hr*	3 *hrs*	24 *hrs*	1 *hr*	3 *hrs*	24 *hrs*	1 *hr*	3 *hrs*	24 *hrs*	1 *hr*	3 *hrs*	24 *hrs*	1 *hr*	3 *hrs*	24 *hrs*	1*hr*	3 *hrs*	24 *hrs*
Essential Oil	25	50	75	0	40	50	0	25	30	75	50	25	100	60	50	100	100	50	50	50	70	0	20	40	0	0	40
MeOH extract	0	25	50	0	0	25	0	0	25	100	75	50	100	100	75	100	100	75	0	35	50	0	0	25	0	0	25
EtOAc fraction	0	25	50	0	0	25	0	0	25	100	75	50	100	100	75	100	100	75	0	35	50	0	0	25	0	0	25
BuOH fraction	25	25	50	0	25	50	0	0	50	75	75	50	100	75	50	100	100	50	25	50	75	0	25	50	0	0	50
PZQ	75	100	100	75	100	100	50	75	100	25	0	0	25	0	0	50	25	0	75	100	100	75	100	100	50	75	100

Negative control showed 0% mortality, 100% viability and 0% shrinking

Table 2 Chemical composition of the essential oil of *A. cepa* (red onion) blubs.

Peak no	t_R	% of total	MF	MW	Name
1	4.28	0.23	$C_6H_{14}S_2$	150	Dipropyl- disulfide
2	4.38	0.07	$C_3H_6N_4O_2$	130	3,3- bis(carbamino)diaziridine
3	4.47	0.14	C_6H_8S	112	2,5-dimethyl- thiophene
4	4.93	0.11	$C_9H_{21}NO_2$	175	N,N-dimethylformamide-dipropylacetal
5	5.06	1.40	C_6H_8S	112	3,4 –dimethyl-thiophene
6	5.55	1.43	$C_4H_{10}S_2$	122	Disulfide- methyl –propyl
7	5.71	1.10	$C_4H_8S_2$	120	Disulfide- methyl-1- propenyl
8	6.02	0.23	$C_{15}H_{32}O$	228	3,7,11-trimethyl-3- dodecanol
9	6.31	2.15	$C_2H_6S_3$	126	Dimethyl- trisulfide
10	6.54	0.11	$C_{10}H_{20}$	140	1-methyl-2-propyl- cyclohexan
11	6.85	0.28	$C_{10}H_{22}$	142	Decane
12	7.31	0.29	$C_{11}H_{24}$	156	Decane-4-methyl
13	9.02	1.14	$C_{11}H_{24}$	156	Undecane
14	9.30	0.89	$C_4H_5ClN_2S$	148	2-ethyl-5-chloro-1,3,4-triazole
15	9.51	1.06	$C_9H_6F_3NO_2$	217	4,4,4-trifloro-1-(3-pyridinyl)- 1,3-butadienone
16	10.60	5.94	$C_{12}H_{23}NO$	197	4-dibutylaminobut-2-yn-1-ol
17	10.68	7.80	$C_3H_6S_3$	138	1,3,5-trithiolane
18	10.96	0.84	$C_{17}H_{37}$	240	2,6,10-trimethyl –tetradecane
19	11.80	0.67	$C_{20}H_{40}O$	296	1-ethenyloxy –octadecane
20	12.38	6.77	$C_{12}H_{26}$	170	Dodecane
21	13.00	2.97	$C_{13}H_{28}$	184	2,6-dimethyl –undecane
22	13.13	0.48	$C_2H_6S_4$	158	Dimethyl –tetrasulfide
23	13.38	0.40	$C_{14}H_{30}O$	214	2-hexyl-octanol
24	14.16	0.72	$C_5H_{10}S_3$	166	4,6-dimethyl-1,2,3-trithiolane
25	15.25	0.84	$C_5H_{12}S_2$	136	2,2-bis(methylthiol)-propane
26	20.72	10.17	$C_6H_{12}S_3$	180	3,5diethyl -1,2,4-trithiolane
27	25.71	2.96	$C_6H_3ClN_2OS$	186	4-chloro-benzo(1,2,5trithiazol-5ol)
28	26.19	0.32	$C_{16}H_{34}O$	242	2-hexadecanol
29	27.57	1.14	$C_5H_{12}S_2$	136	2,2-bis(methyl thio)-propane
30	27.95	2.93	$C_6H_{11}NO_2S$	161	1-nitro-2(2-propenyl thio)-propane
31	28.57	0.40	C_6H_8S	112	2,4-dimethyl –thiophene
32	28.76	7.74	$C_{11}H_{18}O_2$	183	3(2H-furanone,2-hexyl-5-methyl)
33	29.89	2.45	$C_8H_{18}S_3$	210	1,1-thiobis(3-methylthiol)- propane
34	31.21	1.87	$C_{13}H_{26}O$	198	2-tridecanone
35	32.58	0.61	$C_3H_6O_2S_2$	138	1,1dioxide -1,2-dithiolane
36	33.43	2.83	$C_{12}H_{23}NO_2$	213	N,(1-Cyclohexylrthyl)-2-methoxy- propanamide
37	33.64	1.50	$C_{12}H_{26}O_2$	202	4,5-decanediol-6-ethyl
38	33.99	3.06	$C_6H_{12}S_3$	180	3,5-diethyl -1,2,4-tritholan
39	34.70	0.89	$C_{21}H_{40}O_2$	324	Cyclohpetane carboxylic acid- pentadecyl ester
40	35.00	2.53	$C_9H_{18}S_3$	222	2,2,4,4,6,6-hexamethyl -1,3,5-trithiolane
41	35.26	1.18	$C_6H_{12}S_3$	180	Trans-3,5-diethyl-1,2,4-trithiolane
42	35.99	1.56	$C_8H_{11}NO_4S_2$	249	3-(methylsulfonyl amino)-thiphene-2-carboxyli acid
43	36.17	2.02	$C_6H_{13}ClOSi$	164	trans-2-chlorovinyl (cimethylethoxysilane)
44	36.96	5.27	$C_{13}H_{22}O_2$	210	3(2H)-furanone,5-methyl-2-octyl
45	37.21	0.83	$C_{10}H_{18}OS_2$	218	1,5-Dithiaspiro (5,6) dodecan-7-ol
46	39.24	1.72	$C_5H_{12}S_2$	136	2,2-bis(methylthiol) propane
47	43.85	2.32	$C_8H_{16}O_2$	144	2,2,4,6-tetramethyl-trans-1,3-dioxane
48	44.96	0.67	$C_{11}H_9NO_2$	187	2-quinolinecarboxylic acid, methyl ester
49	45.27	0.97	$C_{10}H_{22}O$	158	3-methyl ,3-nonanol
50	50.80	0.75	$C_6H_{12}S_3$	180	3,5-diethyl -1,2,4-trithiolane
Total %		**96.75**			

(t_R) Retention time, (MF) Molecular formula, (MW) Molecular weight.

CONCLUSION

The present study demonstrated that *A. cepa* essential oil and BuOH fraction showed antischistosomal activities in a time and dose-dependent manner. The higher antischistosomal activity of *A. cepa* essential oil was related to its chemical composition such as sulfur compounds, alcohols, acids, esters, and hydrocarbons. Therefore, it was suggested that *A. cepa* may be used as a natural and safe therapeutic agent for human parasitic infectious diseases.

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