



Assessment of *Ocimum basilicum* Extracts on *Schistosoma mansoni* Infected *Biomphalaria alexandrina* Snails and Mice

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ABSTRACT

Schistosomiasis is one of the most prevalent diseases worldwide despite the conventional drug praziquantel and the molluscicides. The quest for a free-toxic clinical treatment and friendly-environmental and molluscicide has intensified. In this study, the compositions of African *Ocimum basilicum* plant extracts were prepared and analyzed. Then, their cytotoxicity, antioxidant, and anti-schistosomiasis impacts were studied. The constituents of *O. basilicum* essential oil and ethanol extract were analyzed by the HPLC and GS/MS techniques, respectively. DPPH and SRB assays were applied to test the antioxidant activity and cytotoxicity of the two extracts, respectively. Furthermore, the efficacy of both extracts on *Schistosoma mansoni*-infected *B. alexandrina* snails and mice was examined. The results demonstrated that the ethanol extract has fourteen compounds with different concentrations: Caffeic acid (553.46 mg) is the highest concentration, and carnosic acid (79.14 mg) is the lowest. However, the essential oil analysis revealed more components (n=25) with the highest content due to linalool (21.52%), and the lowest is phytol acetate (8%). Exposure of *B. alexandrina* snails to *O. basilicum* extracts (50 ppm, each) showed a significant ($P<0.001$) decrease in the infection rates (35% and 21%, respectively) and increase in the survival rates (95% and 96%, respectively) compared to the untreated, infected snails (85% and 95, respectively). The administration of mice with the mono *O. basilicum* extracts significantly reduced the worm burden, egg loads, and intact eggs in favor of the essential oil. Interestingly, our study demonstrated more fibro-cellular hepatic granulomas in the mice treated with the ethanol extract than in mice treated with the essential oil. In conclusion, our data is suggesting a promising anthelmintic and antioxidant herb. Further investigations are to be considered to examine the effectiveness of *O. basilicum* extracts in combination with praziquantel.

INTRODUCTION

Schistosoma mansoni is a neglected parasitic disease caused by the genus *Schistosoma*, resulting in a global socioeconomic affliction (McManus *et al.*, 2018). The WHO reported 250 million people infected with *Schistosoma spp.* and around 800 million people at risk of infection worldwide (WHO, 2020; Li *et al.*, 2021). The

life cycle of the parasite is complex and continuous between intermediate and definitive hosts. In freshwater, eggs hatch into miracidia that transform into cercariae in a specific snail host and depart from the snails to infect the definitive mammalian host (e.g., human) *via* skin. Through the host's bloodstream, the cercariae reach the intestinal mesentery, differentiate into male and female parasites that couple and mate to give rise to ova. Many intact eggs result in hepatic and intestinal pathological changes ending up with chronic granulomas, and the others depart the host through the feces (Olivier and Mao 1949; Cort *et al.*, 1954; Whitfield and Evans, 1983, Pearce and MacDonald, 2002)

Since the early eighties, praziquantel (PZQ) has been the only reliable treatment of schistosomiasis. It is affordable, safe, and highly efficient in treating trematodes and cestodes in humans and animals. Yet, there is an emergence of resistance to the PZQ drug and molluscicides in some areas (Ismail *et al.*, 1999; King *et al.*, 2000; Utzinger *et al.*, 2001; Jiwajinda *et al.*, 2002; Doenhoff *et al.*, 2008; Bergquist *et al.*, 2017). These challenges urged the researchers to study the botanical products potential effects on schistosomiasis as reported by WHO (2019) and Duarte Galhardo de Albuquerque *et al.* (2020).

The medicinal herbs have been utilized since 1993 in the control of *Schistosoma* intermediate host for being cheap and ecofriendly (Archiblad, 1933; Wager, 1936; Mozley, 1939; Lemma *et al.*, 1978; Hostettmann, 1984; Mølgaard *et al.*, 2001; Mossalem *et al.*, 2013; 2017; Simoben *et al.*, 2018). Many *in vitro* studies were conducted on *Schistosoma mansoni* to test the effect of some African plants on the growth of the parasite stages. At the same time, only a few *in vivo* assessments were considered to evaluate the efficacy of those plants (Duarte Galhardo de Albuquerque *et al.*, 2020). Many plants were screened for their efficacy against the adult worms (Yousif *et al.*, 2012), and only *Ocimum Americanum* extracts were tested for their anti-schistosomicidal activity in a murine model in Kenya (Waiganjo *et al.*, 2014). The current study aimed to evaluate the *anti-bilharzial* effects of the African *Ocimum basilicum* leaf extracts on infected snails and mice.

MATERIALS AND METHODS

1. *Ocimum basilicum* Plant

The fresh leaves of *O. basilicum* were collected from El- Orman Botanical Garden, Giza, Egypt, in June 2020. The plant was kindly identified by Mrs. Threase Labib, consultant of plant taxonomy at the Ministry of Agriculture; a voucher specimen (No. B6-2020) was kept at the Medicinal Chemistry Department, Theodor Bilharz Research Institute (Giza, Egypt).

1.1. Preparation of the Ethanol Extract and Essential Oil

Ethanol extract.

Dried powdered leaves of *O. basilicum* (200 g) were placed in a glass percolator with ethanol (1.2 L) and allowed to stand at room temperature, then

collected after 16 h. The extraction process was repeated four times. Finally, the combined extract was filtered, concentrated under vacuum using rotavapor at 40°C, and the weight of extract obtained was 16.46 g (Chatterjee *et al.*, 2011).

Essential Oil.

O. Ocimum basilicum fresh leaves (2 kg) were chopped into small pieces, and essential oil was extracted by hydro-distillation after 5h using an apparatus of Clevenger type. Then oil was dried over anhydrous sodium sulfate (Na₂SO₄), filtered, and stored in a sealed glass tube at 5°C for further analyses and biological activity tests (Saad *et al.*, 2017).

1.2. Phytochemical screening of the *O. basilicum*

According to the conventional reported methods, identifying the major phytoconstituents of ethanolic extract was carried out (Gerhardt *et al.*, 1983; Skaltsa *et al.*, 1986; Baritoux *et al.*, 1991; Grayer *et al.*, 2001; Bais *et al.*, 2002). The results were investigated by precipitation or change in color and exhibited glycosides, terpenoids, phenolics, and flavonoids. Qualitative and quantitative analysis of flavonoid and phenolic constituents of *O. basilicum* ethanolic extract carried out by HPLC method with UV- diode-array detector (Qureshi *et al.*, 2014). Analysis of the chemical compositions of the essential oil was performed by Gas Chromatography-Mass Spectrometry (GC/MS) following the procedures of (Adam, 1995).

1.3. Antioxidant DPPH Assay

Different concentrations of the extract were prepared in methanol and were added to 3 mL of 0.1 mM methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The tubes were shaken vigorously and allowed to stand for 30 min at room temperature in the dark. Changes in absorbance of samples were measured at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid, served as the standard free radical scavenging activity, was expressed as inhibition percentage and was calculated using the following formula:

$$\% \text{ inhibition} = ((A_0 - A_t)/A_0) \times 100$$

Where A₀ is the absorbance of the control, and A_t is the absorbance of test samples. All the tests were performed in triplicates, and the results are reported as IC₅₀, which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (Mansoori *et al.*, 2018).

1.4. Cytotoxic activity SRB assay

Evaluation of *in vitro* cytotoxic activity of *Ocimum basilicum* ethanol extract and essential oil against two human tumor cell lines, namely, hepatocellular carcinoma (HepG-2) and mammary gland breast cancer (MCF-7), was tested by sulforhodamine-B (SRB) assay at the National Cancer

Institute in Egypt, according to the method of **Skehan *et al.* (1990)**. The percentage of cell survival was calculated according to the following equation:

$$\% \text{ Survival fraction} = (\text{OD of treated cell/O.D. of control cells}) \times 100$$

According to the National Cancer Institute guideline, the extract with IC₅₀ values less than 20 µg/mL is considered active (**Houghton *et al.*, 2007; Refahy *et al.*, 2015**).

2. Experimental Design

2.1. Malacology Assays

B. alexandrina snails were infected with *S. mansoni* miracidia (8 miracidia/snail) and divided into three groups, each containing ten snails. A group of infected mice was treated with *O. basilicum* ethanol extract (50 ppm), another group was exposed to *O. basilicum* oil extract (50 ppm/snail), and a third one served as untreated infected controls. Three replicates were prepared for each group. The experimental snails were transferred to clean dechlorinated water (25 ± 1°C) and fed daily with oven-dried lettuce leaves throughout the pre-patent and patent periods. Dead snails were removed daily, and the surviving snails have been examined weekly for cercarial shedding 24 days post-miracidia exposure. The number of snails that prevailed at the first shedding and the number of infected snails were calculated (**Chernin and Dunavan 1962**).

2.2. Animals, infection, and regimens

Six to seven-week-old male, Swiss albino mice (20 ± 2 g) and *S. mansoni* cercariae were purchased from Schistosomiasis Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI) in Giza, Egypt. Eight-week after subcutaneous infection (**Muchirah *et al.*, 2012**) with a 100 cercariae/mouse, the animals were divided randomly into three groups (n=5). One group received a 200 mg/kg *Ocimum basilicum* ethanol extract, and another group received 100 µL/kg essential oil for five consecutive days each. A third group served as untreated infected control mice. In parallel, the fourth group with normal mice was maintained under the same laboratory and nutrition conditions. Two weeks post-treatment (10 weeks post-infection), mice were euthanized by decapitation and perfused with saline solution followed by formalin solution (10 minutes, each). Livers and small intestines were collected and processed for the parasitological and histopathological studies. The experiment was repeated three times.

2.4. Parasitological Assays

Egg developmental stages (Oogram)

The percentages of immature, mature, and dead eggs from the small intestinal wall of infected mice were computed from a hundred eggs per intestinal segment. Immature eggs were characterized by partially developed embryos with clear

transparent parts within the eggs shell. The mature ones contain fully developed miracidium. Dead eggs exhibited dark retraction and irregular outlines of dead seeds. Three segments per animal were examined (**Pellegrino and Jane Faria 1965**).

Tissue eggs load and worm burden

The number of eggs per gram tissue (liver and intestine) of infected mice was determined. The mean number of worms/mouse was determined for each experimental group (**Ebeid et al., 2005**).

2.5. Histopathological Parameters

Livers were harvested from the mice, fixed in 10% buffered formalin, and processed to paraffin blocks. Sections (4 µm thick) were cut every 250 µm to avoid measuring the same granuloma. Five liver sections were prepared from each animal and stained with hematoxylin and eosin and Masson trichrome stains. An ocular micrometer was utilized to measure the non-contiguous granulomas; each contained a single intact or degenerated egg. The mean diameter of each granuloma was calculated by measuring 2 diameters of the lesion at right angles to each other (**Ebeid et al., 2005**). Granuloma structural configurations, including cellular components and associated hepatic histopathological changes, were recorded.

3. Statistical analysis

Data were expressed as means ± SD or percentage and analyzed using the statistical package SPSS (version 7.5 Windows). Comparisons between the groups were made using Chi-square test for the qualitative variables and the *t*-student test for the quantitative variables. The probability value less than 0.05 was considered statistically significant.

RESULTS

HPLC fingerprint chromatography was applied to compare the retention time of the extract constituents and standard solutions. The chromatography detected fourteen significant peaks to which the retention time of the standards matched. The identified components are listed in the Table (1), showing that caffeic acid accounted for the highest content, whereas carnosic acid accounted for the most negligible content. On the other hand, the analysis of the essential oil obtained by GC/MS demonstrated twenty-five compounds. The greatest concentration was due to the linalool (21.52%), and the least in concentration was due to phytol acetate (0.08%) depicted in Figure (1) and Table (2). The DPPH assay recorded the radical solid scavenging effect of the ethanol extract at 27.13 µg/mL; SC₅₀ value.

The effect of the plant extracts on snails

The exposure of the snails to a mono-treatment of *O. basilicum* ethanol extract and essential oil resulted in a highly significant ($p < 0.0001$) increase (95 and

96%, respectively) of the survival rates and a significant ($p < 0.001$) decrease (35% and 21%, respectively) of the infection rates compared to the normal controls (85% and 95%, respectively) as shown in Table (3).

The effect *O. Basilicum* extracts on the stages of the *Schistosoma* parasite

The mice treated with either ethanol extract or oil showed a significant reduction ($p < 0.0001$) of the intestinal and hepatic egg loads and worm counts compared to the controls (22061.33 ± 451.95 and 21458.3 ± 1011.99 ; respectively). However, the hepatic egg counts in of mice treated with the 200mg/kg ethanol extract (12558.25 ± 1033.37) were significantly ($p < 0.01$) more than their counterparts (13870.00 ± 730.36) of mice treated with 100 μ l/kg essential oil as depicted in the table (4).

The histopathological changes of the definitive host

Compared to the control, a significant ($p < 0.0001$) diminution in the hepatic granuloma size (35.37%), numbers (16.2 ± 1.21 vs. 10.47 ± 1.45), and cellular type (78 vs. 62) was due to the treatment with 100 μ L/kg essential oil. Yet, the percentages of hepatic cellular granulomas (62%) and degenerated eggs (15%) were significantly greater ($p < 0.0001$ and $p < 0.001$, respectively) in the mice treated with a 100 μ L/kg essential oil than their counterparts of the mice treated with 200 mg/kg ethanol extract (55% and 12%, respectively) as shown in Table (5). The liver tissue sections of untreated-infected mice showed various lobular, portal, cellular, and fibro-cellular granulomas with intact eggs. The lobular granulomas were infiltrated with Kupffer cells, monocytes, and mild fibrosis. Meanwhile, the portal granulomas had an influx of inflammatory cells with fibrous tissue disposition. The highest percentage of fibro cellular granulomas was detected in the liver tissues of the mice treated with 200 mg/kg ethanol extract. However, the hepatocytes were ameliorated in the groups treated with both extracts (Figure 2).

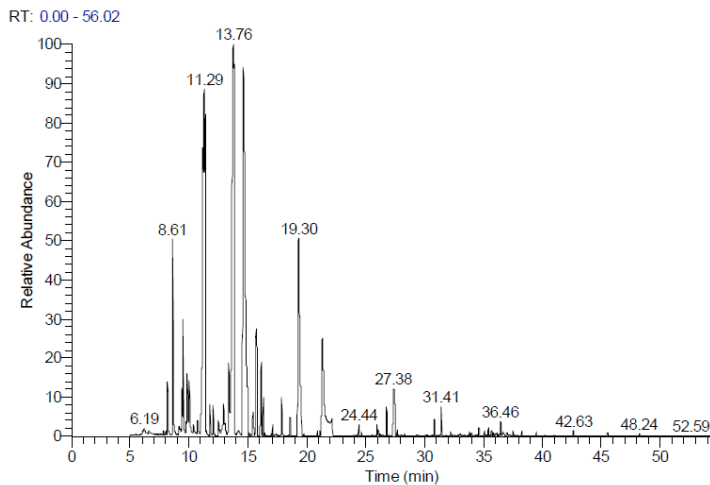


Figure 1: The chemical constituents of *O. basilicum* essential oil using HPLC techniques

Table (1): The constituents of *O. basilicum* ethanolic extracts by reverse phase HPLC with diode-array detection

No.	Retention time (min)	Components	Concentration mg/100 mL extract)
1	6.11	Catechin	451.71
2	11.4	Rutin	179.14
3	17.44	Caffeic acid	553.46
4	25.99	Dihydrokaempferol-3-O-glucoside	91.87
5	27.15	Luteolin acetyl-glucuronide	86.02
6	29.27	Luteolin 5-O-glucoside	222.01
7	30.04	Ferulic acid,	182.25
8	34.56	Rosmarinic acid	506.70
9	35.14	Caffeoyl -3-O-rutiniside	146.18
10	38.49	Apigenin,	99.76
11	45.32	Carnosic acid	79.14
12	50.16	Chlorogenic acid	237.01
13	51.41	Acacetin	312.05
14	53.20	Ursolic acid	97.18

Table (2): The chemical constituents identified from *O. basilicum* essential oil by GC-MS analysis.

No.	Retention time (min)	components	Peak area %	Molecular Weight
1	8.18	α -Pinene	0.80	136
2	8.61	Camphene	3.06	136
3	9.17	Benzaldehyde	0.21	106
4	9.47	β -Pinene	1.78	136
5	10.02	α - Myrcene	0.74	136
6	10.37	1-Phellandrene	0.20	136
7	11.29	1,8- Cineole	16.68	154
8	11.77	Carvone	5.51	150
9	12.02	γ - Terpinene	0.43	136
10	12.48	Linalool oxide	0.41	170
11	13.76	Linalool	21.52	154
12	14.17	P-mentha-1(7),8-dien-2-ol	0.13	152
13	14.60	Camphor	17.02	152
14	15.71	p-Cymene	5.35	134
15	16.14	Geraniol formate	2.14	182
16	16.31	Myrtenol	0.53	152
17	17.10	Nerol	0.14	154
18	17.86	Isopulegol	0.72	154
19	18.58	Bornyl acetate	0.26	196
20	19.28	Germacrene	7.37	204
21	24.43	Methyl-3-phenyl-2-propenoate	0.19	162
22	26.76	Cubenol	0.48	222
23	27.38	α - Cadinol	2.09	222
24	34.61	Delta-3-Carene	0.11	136
25	38.26	Phytol acetate	0.08	338

Table 3: Effect of the 4-week exposure of 50 ppm *O. basilicum* extracts on *B. alexandrina* snails' infection and survival rates

Infected snails	% Infection rate	% Survival rate
Untreated Control	95%	80%
Treated with ethanol extract	35%***	95***
Treated with essential oil	21%***	96***

*** ($P < 0.001$) highly significant

Table 4: Effect of the *O. basilicum* extracts on *S. mansoni* worm burden, ova development, and egg count in the intestines and livers of the experimental mice, 10 weeks post-infection.

Groups	Ova count/g tissue		% Ova developmental stages			Mean worm burden \pm SD in Liver and Porto-mesenteric			
	Intestine	Liver	Dead	Mature	Immature	Couples	Female	Male	Total
Infected Control	22061.33 \pm 451.95	21458.3 \pm 1011.99	05.67 \pm 1.53	46.67 \pm 2.08	47.67 \pm 0.58	12.33 \pm 1.53	0.67 \pm 1.15	2.67 \pm 0.58	28.00 \pm 2.65
200 mg/kg ethanol extract	14280.00 \pm 1546.91** *	12558.25 \pm 1033.37***	14.75 \pm 3.21***	45.5 \pm 7.41	39.75 \pm 7.41	7.50 \pm 1.29***	0.75 \pm 0.96	2.0 \pm 0.82	17.75 \pm 1.89***
100 μL/kg essential oil	12853.33 \pm 772.05***	13870 \pm 730.36***	17.00 \pm 4.36***	46.67 \pm 7.23	35.33 \pm 4.73***	7.00 \pm 1.00***	0.00	1.67 \pm 0.58	15.67 \pm 1.53***

*** $P < 0.0001$

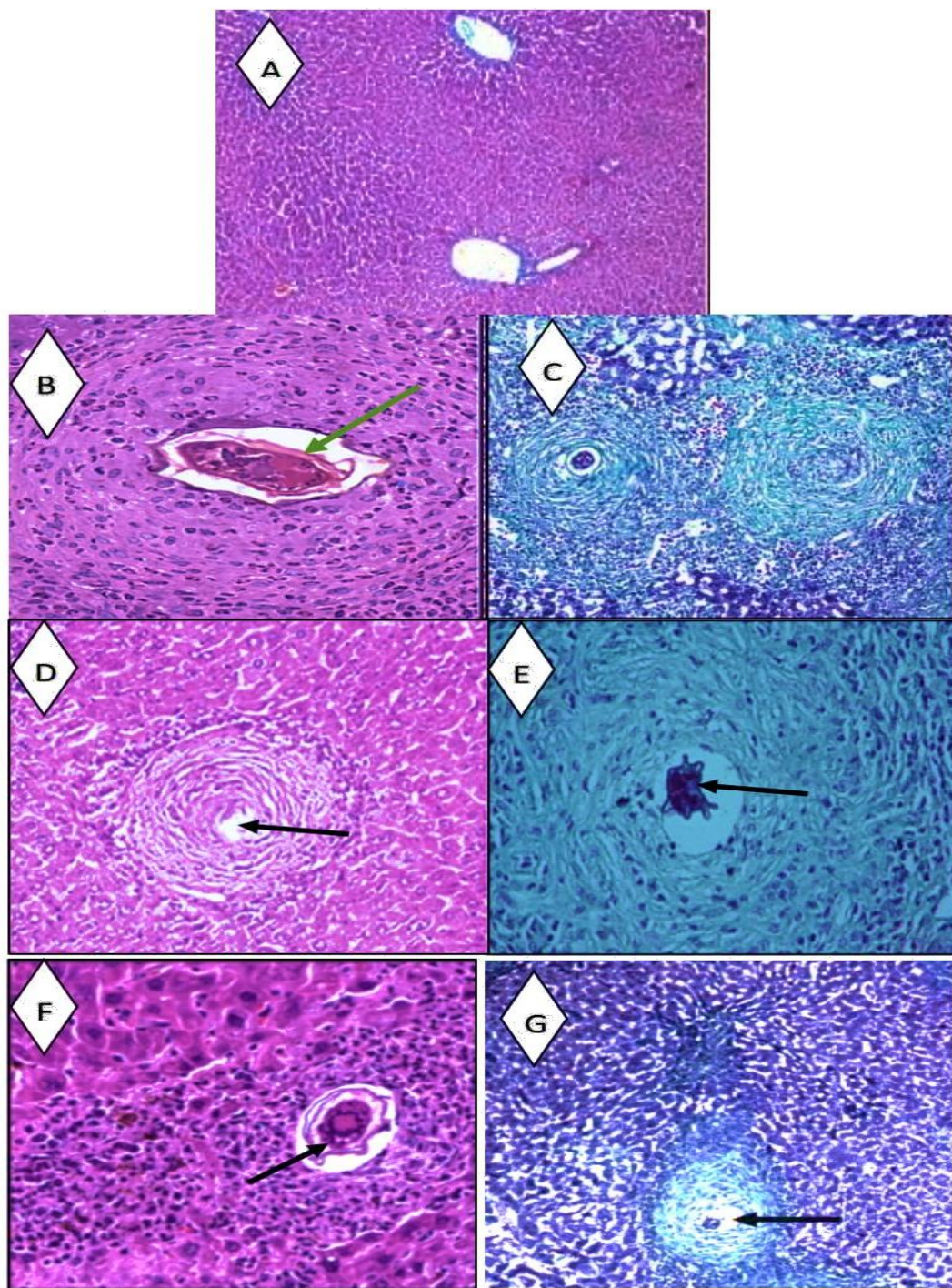


Figure 2: (A) Healthy hepatic tissue with intact hepatocytes in normal mice, H and E, x10.

(B and C) Hydropic hepatocytes, an influx of monocytes, eosinophils, and fibrocytes surrounding intact *Schistosoma* eggs (green arrow) in the infected mice (H and E, x20; MT, x10, respectively). (D and E) Ameliorated hepatocytes and well-defined granuloma with a mild number of monocytes and an increase of fibrocytes surrounding degenerated eggs (black arrow) in the liver sections of mice treated with 200 mg/kg ethanol extract. H and E, x20;

MT, x40, respectively. (F and E) intact hepatic architectures with a considerable number of lymphocytes, eosinophils (H and E, x20), and fibrocytes (MT x10) in mice treated with 100µl/kg essential oil, respectively.

DISCUSSION

Schistosomiasis is reported as the most second prevalent disease in the world after malaria (WHO 2021). The transmission of *Schistosoma spp.* is controlled by molluscicides, and the treatment is managed by the commercial drug praziquantel. Recently, several cases of resistance to the therapeutic agents (Ismail *et al.* 1999, Gryseels *et al.* 2002, Doenhoff *et al.* 2008, Melman *et al.* 2009, Zhang and Coultas, 2013, Mossalem *et al.* 2014) and molluscicides were reported (Duarte Galhardo de Albuquerque *et al.*, 2020).

Over the past years (2013, 2014, 2017), Mossalem and colleagues worked on the folkloric herbal extracts to control schistosomiasis transmission via disturbing their specific snails, the intermediate host. In this study, the demonstrated antioxidant constituents of *O. basilicum* ethanol extract and essential oil are verified by matching them with many references (Gerhardt *et al.* 1983, Skaltsa *et al.*, 1986, Baritoux *et al.*, 1991, Grayer *et al.*, 2001, Bais *et al.*, 2002). We used the lowest dose of either plant extract, which is 50ppm, because all the tested concentrations had harmless impacts on the infected snails for the fact of being antioxidants, not molluscicides. It was believed that a pesticide manipulated *Biomphalaria alexandrina* snails ameliorated when treated with *O. Ocimum* oil extract (Mossalem and Ibrahim, 2019). Hence, we designed this research work to assess and compare the impacts of *Ocimum basilicum* ethanol and oil extracts on both *Schistosoma mansoni* intermediate and definitive hosts.

Our data showed that the treatment protocols significantly increased the survival rates and decreased the infection rates of *S. mansoni*-infected *B. alexandrina* snails, which agrees with other findings (Sestili *et al.* 2018, Mossalem and Ibrahim, 2019). *O. basilicum* extracts may have their protective influences through the active antioxidant components such as polyphenolic and flavonoids (Mossalem and Ibrahim, 2019).

The mice infected and treated with *O. basilicum* essential oil had the female worm count vanished (zero) alongside a significant reduction of ova disposition in intestinal tissues. These results may indicate a metabolic action mode on the development or fecundity of female worms, which is supported by observations reported from an *in vitro* study used *O. basilicum* ethanol and hexane extracts on earthworms (Clement Osei Akoto *et al.*, 2020). Therefore, we suggest further morphological studies and protein analysis on the *Schistosoma* adult worms to validate this hypothesis.

The higher number of fibro cellular hepatic granulomas in the group treated with ethanol extract (200mg/kg) may indicate an acceleration towards the chronic

inflammation in favor of Th2 response. Unlikely, the major cellular granulomas in the hepatic tissues of mice treated with the oil extract retained the Th1 response of the acute phase. These data may explain the high degeneration rate of eggs in the hepatic granulomas of the mice treated with 100µl /kg oil extract (Xu *et al.* 2010).

In conclusion, the biochemical assays, malacology assessments, and *in vivo* model indicated a potential therapeutic effect of *O. basilicum* ethanol and oil extracts. Further studies are needed to consider combining *O. basilicum* extracts with the other medicinal herbs tested for their actions on schistosomiasis *mansoni* to develop an efficient treatment and reduce or eradicate the transmission.

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CONCLUSION

O. basilicum was considered a prolific source for several biologically active metabolites. The plant's ethanol extract and essential oil showed appreciable anti-schistosomiasis activities in the intermediate and definitive hosts.

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الملخص العربي

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يعد داء البلهارسيا أحد أكثر الأمراض انتشاراً في جميع أنحاء العالم على الرغم من عقر البرازيكونتيل التقليدي ومبيدات الرخويات. تم تكثيف البحث عن علاج سريري خالٍ من الأعراض الجانبية ومبيدات للرخويات صديقة للبيئة. في هذه الدراسة تم تحضير وتحليل تركيبات مستخلصات نبات الريحان الأفريقي ودراسة سميته على الخلايا ومحتوياته لمضادات الأكسدة ومضادات البلهارسيا. تم تحليل مكونات زيت نبات الريحان الأفريقي الأساسي ومستخلص الإيثانول بواسطة تقنيات "HPLC و GS/MS"، على التوالي. تم تطبيق قياسات "DPPH و SRB" لاختبار نشاط مضادات الأكسدة والسمية الخلوية للمستخلصين، على التوالي. علاوة على ذلك، تم فحص فعالية كلا المستخلصين على القواقع والفئران المصابة بالبلهارسيا. أظهرت النتائج أن مستخلص الإيثانول يحتوي على أربعة عشر مركباً بتركيزات مختلفة: حمض الكافيك (٤٦.٥٣ مجم) هو أعلى تركيز، وحمض الكرونوسيك (١٤.٧٩ مجم) هو الأقل. ومع ذلك، أظهر تحليل الزيت العطري المزيد من المكونات (ن = ٢٥) مع أعلى محتوى بسبب اللينالول (٢١.٥٢٪)، وأقلها هو أسيتات فيتول (٨٪). أظهر تعرض حلزون "B. alexandrina" لمستخلص نبات الريحان الأفريقي (٥٠ جزء في المليون، لكل حلزون) انخفاضاً معنوياً ($P < 0.001$) في معدلات الإصابة (٣٥٪ و ٢١٪، على التوالي) وزيادة في معدلات البقاء على قيد الحياة (٩٥٪ و ٩٦٪، على التوالي) بالمقارنة مع القواقع المصابة غير المعالجة (٨٥٪ و ٩٥، على التوالي). أدت معاملة الفئران بمستخلصات نبات الريحان الأفريقي الأحادية إلى تقليل عبء الدودة، وأحمال البيض، والبيض السليم لصالح الزيت العطري. ومن المثير للاهتمام، أن دراستنا أظهرت المزيد من الأورام الحبيبية الكبدية الليفية الخلوية في الفئران المعالجة بمستخلص الإيثانول مقارنة بالفئران المعالجة بالزيت العطري لنبات الريحان الأفريقي. في الختام، تشير بياناتنا إلى عشب واعد طارد للديدان ومضاد للأكسدة. مزيد من التحقيقات للنظر في فعالية مستخلصات نبات الريحان الأفريقي بالاشتراك مع البرازيكونتيل.