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# Evaluation of the Biocidal Activity of Alkaloids, Saponins and Volatile Oil Extracted from *Nigella Sativa* Seeds against Miracidia and Cercariae of *Schistosoma mansoni*

[1]Khaled Abo-Zeid, [2,3]Mohamed Shohayeb
[1] *Department of parasitology, College of Medicine, Taif University, Saudi Arabia*
[2] *Pharmaceutical Biotechnology Unit, College of pharmacy Taif University, Saudi Arabia*
[3]*Department of Microbiology, Faculty of pharmacy Tanta University, Egypt*

***ABSTRACT:*** *Schistosomiasis is one of the most fatal diseases of humans. In Saudi Arabia, it is found in Jazan, Bishah, Aseer, Madina, Al-Bahah and Taif. In this study, the lethal properties of Nigella sativa alkaloids, saponins and volatile oil were tested in vitro against Schistosoma mansoni aquatic stages; miracidia and cercariae. The three bioactive constituents exerted a lethal effect on both miracidia and cercariae at concentrations below 1 ppm. Miracidia were more sensitive than cercariae to the lethal effect of three tested constituents. The volatile oil of N. sativa, was the most active constituent against both miracidia and cercariae. At 0.39 and 50ppm it killed 100% of the miracidia after 25 and 0.5 min and cercariae after 90 and 5 min respectively. Therefore it may be concluded that, the antimiracidial and anticercarial activity of sativa seeds could be attributed at least in part to its contents of volatile oil, saponins and alkaloids.Therefore, these three active constituents might be recommended for use in programs for controlling schistosomiasis.*

***KEYWORDS:*** *Schistosoma mansoni; Nigella sativa; alkaloids; volatile oil; saponins; cercariae; miracidia*

## I. INTRODUCTION

Schistosomiasis or bilharziasis is an ancient parasitic disease of man. Eggs of schistosoma have been recovered from Egyptian mummies several thousand years old [1]. Schistosomiasis is caused by a blood fluke of the genus Schistosoma, of which three species, namely *S. mansoni*, *S. haematobium* and *S. japonicum*, are the main causative agent of the disease in man [2]. Schistosomiasis is a fatal disease of humans which comes as the second parasitic disease after malaria in terms of overall morbidity and mortality. It is estimated that 200 million people are infected with schistosoma, of whom 20 million have severe disease [3]. Schistosomiasis is endemic in 54 countries in South America, Africa and Asia [4] and it is a major threat to public health in some Middle East countries like, Iraq, Sudan, Egypt, Yemen, and Saudi Arabia [1, 4-7] Both *S. mansoni*, *S. haematobium* are endemic in Saudi Arabia. According to the Ministry of Health, the prevalence of schistosomiasis in Saudi Arabia was 2.9/ 100,000 persons [8]. The highest prevalence was reported in Jazan, Bishah, Aseer, Al-Bahah and Taif. *S. mansoni* is more prevalent in Taif, Al- Bahah, Aseer, Bishah, Najran, Makkah Al- Mukarramah and Al-Medina [8] and it is presumably, transmitted by rodents, baboon monkeys and infected humans [9-11].

After the eggs of schistosoma parasite in faeces of hosts get into water, the ripe miracidia hatch out and invade the intermediate host freshwater snail where they form sporocysts. Cercariae are formed in sporocysts and emerge from the snails in water and search for humans or animals to penetrate their skin [12]. Therefore, to control schistosomiasis, the life cycle the life cycle of schistosoma should be interrupting for instance by killing cercaiae and miracidia [13-15]. The use of chemical compounds to control the aquatic snails, miracidia or cercariae, is not recommended because of their adverse effects on the environment [16]. Several plants that can decrease the shedding of cercariae and to kill both cercariae and miracidia have been reported. *Phytolacca dodecandra* (*Phytolaccaceae*) is considered as a natural efficient alternative to chemicals for controlling schistosomiasis [17,18] and it was environmentally acceptable [19]. Other examples include *Tetrapleura tetraptera*, which is used in South-west Nigeria under the name Aridan [20 ]; *Ambrosia maritima* L. (Damsisa) which is widely distributed throughout the Mediterranean region and was used to control of bilharziasis in Egypt [21,22] and *M. thonningii* which is highly active against both *S. mansoni* miracidia and cercariae [23]. The black seed, *Nigella sativa* L. is widely uses in folk medicine especially amongst Muslims as it was narrated that the prophet of Islam, Muhammad (peace be upon him), said " it is a cure from all ailments" [24]. The crude oil of *N. sativa* hindered the penetration of skin by cercariae [25], and its ingestion by infected albino mice lead to topographic changes in adult worms [26]. In vitro, crushed *N. sativa* seeds and crude extracts were found to be active against *S. mansoni* miracidia, cercariae, and the adult worms ([27]. In this study, we purified the alkaloids, saponins and volatile oil of *N. sativa* and evaluated their lethal effect on cercariae and miracidia.

## II. MATERRIALS AND METHODS

**Extraction of *N. Sativa* with ethanol:** *N. sativa* seeds were purchased from the local market and were ground by an electric blender. The finely ground *N. sativa* seeds were extracted several times to exhaustion with 70% ethanol. Extracts were concentrated using a rotary vacuum evaporator.

**Separation of saponins** [28]
Equal volume of water was added to the alcoholic extract and the saponins were extracted several times with butanol. Butanol extracts were concentrated in a rotary evaporator at 60$^{o}$C and the thick extract was treated with chloroform-methanol (75:25 v/v). Chloroform/methanol soluble saponins were obtained by evaporating the solvents at 37$^{o}$C.

**Separation of alkaloids:**
The alcoholic extract was diluted, acidified with hydrochloric acid and extracted with chloroform, which was then discarded. The extract was alkalinized to pH9 with ammonium hydroxide and was extracted again with chloroform. The chloroform extract was evaporated at reduced pressure to obtain the crude alkaloids [29].

**Separation of the volatile oil:**
Volatile oil was separated by steam distillation of ground seeds suspended in distilled water.

**Preparation of S. mansoni miracidia:**
S. mansoni eggs were extracted and prepared from stools of patients, attending Edwani hospital in Taif who have received no treatment. Stools were emulsified in 10 volumes of 10% sodium chloride and the sediment was washed with cold saline and stored overnight in the refrigerator. The mixture was diluted by using tap water and exposed to bright light to allow the ova to hatch and to get the miracidia [18].

**Preparation of S. mansoni cercariae:**
Biomphalaria arabica snails previously collected from permanent fresh water ponds and wells in and around Taif were infected individually by putting each snail in a compartment of a haem-agglutination plate containing distilled water and 3-5 miracidia. The plates were maintained at 27-29$^{o}$C for a minimum of 5 hours. Infected snails were transferred to sandwich boxes containing de-chlorinated tap water. After 3 weeks of infection, snails were placed in a beaker containing 100 ml dechlorinated tap water, exposed to light from a 10 volt electric lamp for 4 hours to get the cercariae [30].

**Effect of purified constituents on miracidia:**
Tissue culture plates were used as test chambers to observe the viability and death of miracidia under a dissecting microscope [31]. Twenty miracidia were placed in 1 ml dechlorinated water in each well of the test chamber. Serial double concentrations of *N. sativa* extracted constituents were added to get a total of 2 ml in each experimental well. Three replicates were prepared for each tested concentration. Mortality of miracidia was recorded at different time intervals.

**Effect of purified constituents on cercariae:**
Series of 1 ml samples of water containing 20 freshly shed cercariae were mixed with 1 ml of double serial concentrations (0.39-50 ppm) of extracts. Three replicates were made for each tested concentration. Viability of the cercariae was monitored at different time intervals [18].

**Statistical analysis:** Data were analyzed by using SPSS statistical program [32]. The $LT_{50S}$ were calculated from the regression equation Y=a + bx, where (b) is the regression coefficient and (a) is the intercept of the extrapolated linear part of the sigmoid curve.

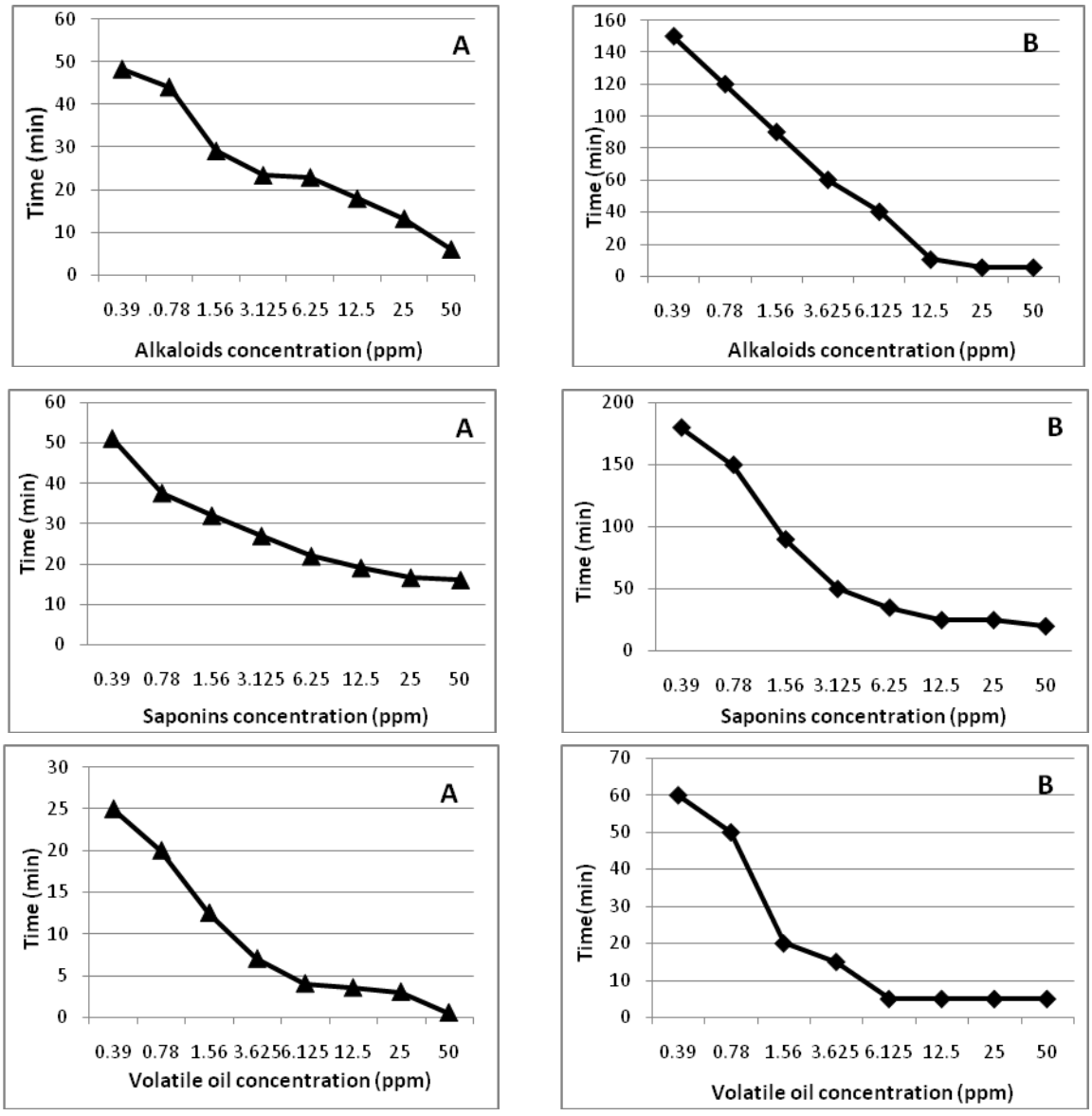


Fig 1: Lethal time for killing 50% (A) and 100% (B) of *Schistosoma mansoni* miracidiaat different concentrations of alkaloids, saponins and volatile oil of *Nigella sativa*.

## III. RESULTS

**Effect of *N. sativa* saponins, alkaloids and volatile oil on miracidia of *S. mansoni***

The lethal times for different concentration of *N. sativa* saponins, alkaloids and volatile oil which caused 50% and 100% mortalities of *S. mansoni* miracidia were characterized by a sigmoid-shape curves and there was a steady decrease in the lethal time as the concentration increases. The $LT_{50}$ for 50 ppm and 0.39 ppm of alkaloids were 6min and 48min respectively (Fig. 1). The corresponding $LT_{100S}$ for the same concentrations were 5min and 150min respectively. The $LT_{100}$ decreased steadily by increasing the concentration of alkaloids between 0.39 and 12.5 ppm and then no much change in lethal times were observed between 12.5 and 50 ppm (Fig. 1).The $LT_{50S}$ and $LT_{100S}$ of *S. mansoni* miracidia at different concentrations (0.39 ppm and 50 pp) of *N. sativa* saponins ranged between 51 min and 16 min. On the other hand, the $LT_{100S}$ ranged between 180 and 25 min when miracidia were exposed to the same concentrations (Fig. 1).The $LT_{50S}$ and $LT_{100S}$ of miracidia at different concentrations of *N. sativa* volatile oil decreased steadily between 0.39 and 6.125 ppm and then little changes in lethal times were observed between 6.125 and 50 ppm

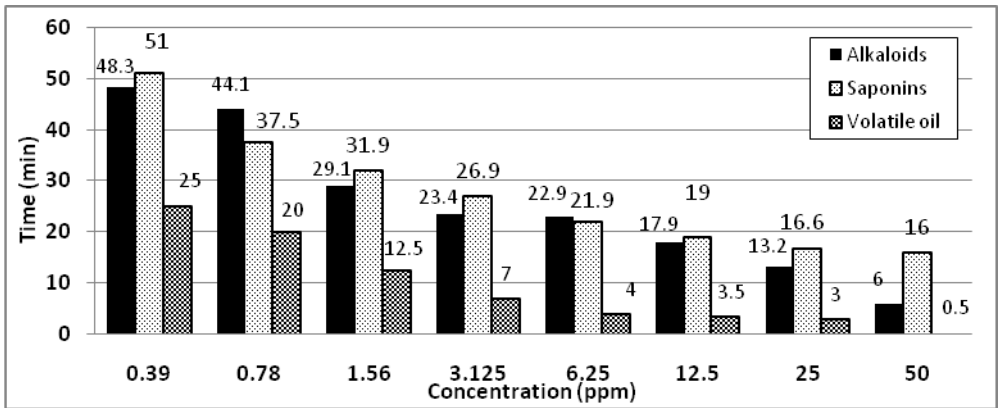


Fig 2: Comparison between the $LT_{50s}$ of different concentrations of saponins, alkaloids and volatile oil of *Nigella sativa* on miracidia.

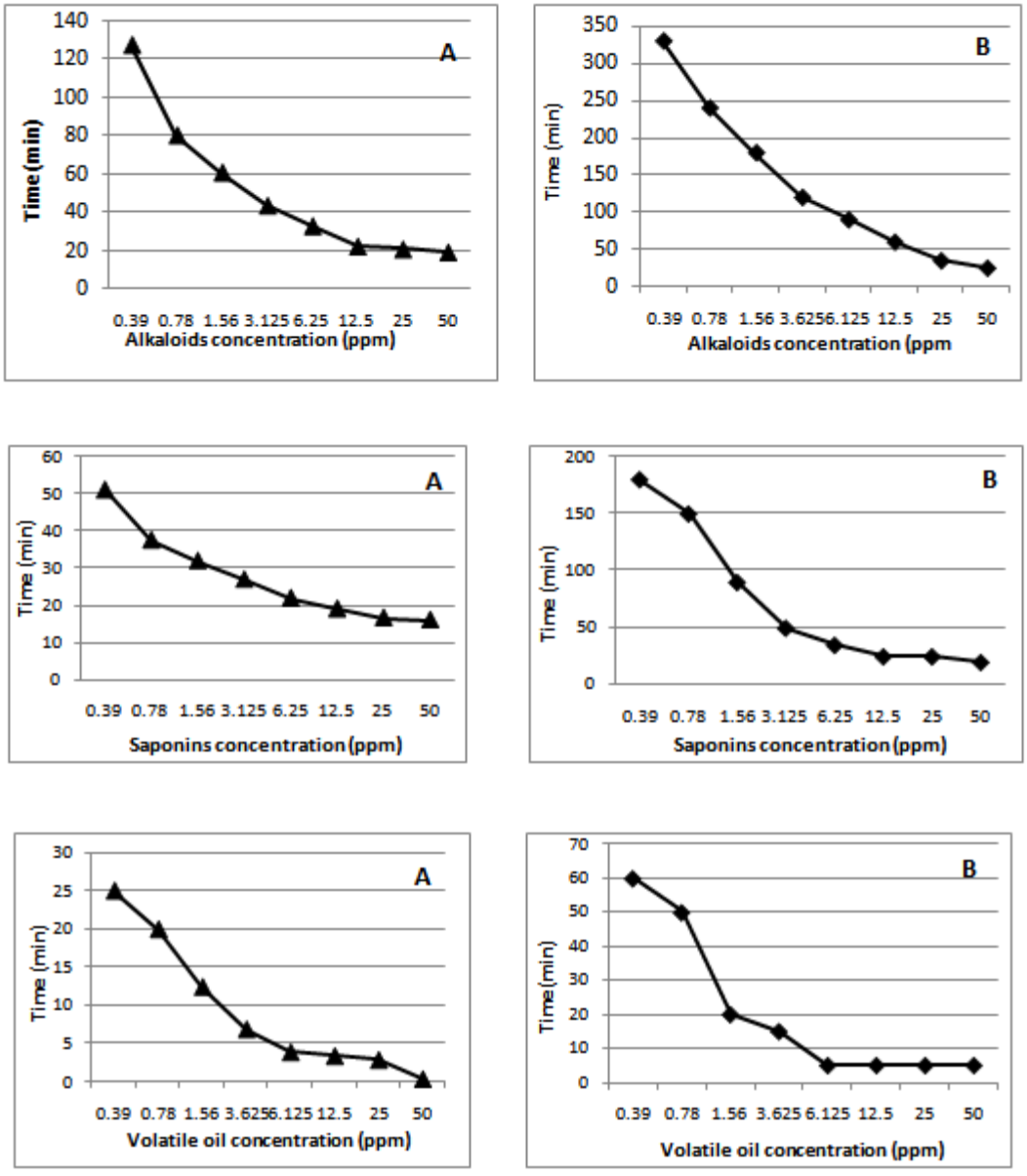


Fig 3: Lethal time for killing 50% (A) and 100% (B) of *Schistosoma mansoni* cercariae at different concentrations of alkaloids, saponins and volatile oil of *Nigella sativa.*

(Fig.1). The lethal times of 50% of miracidia was 0.5 minutes at 50 ppm and 25 min at 0.39 ppm. To attain 100%mortality of the miracidia, 5min and 60 min were required at 6.125 ppm and 0.39 ppm respectively (Fig 1).

The activities of the three tested constituents are compared in figure 2. While the lethal times of different concentrations of both saponins and alkaloids were comparable, the lethal times of volatile oil were significantly (P <0.001) less than those of the other two tested active constituents (Fig 2).

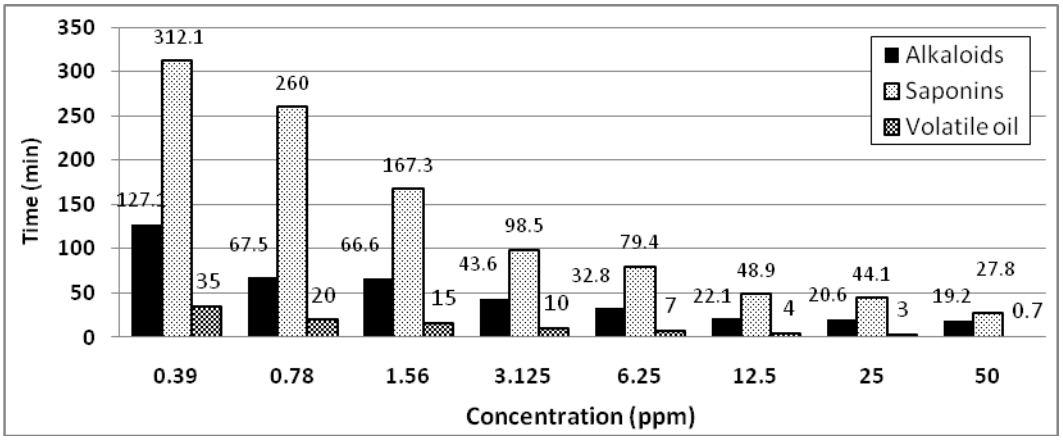


Fig 4: Comparison between the $LT_{50s}$ of different concentrations of saponins, alkaloids and volatile oil of *Nigella sativa* on cercariae.

**Effect of *N. sativa* alkaloids, saponins and volatile oil on cercariae of *S. mansoni*:** The lethal effects of *N. sativa* alkaloids, saponins and volatile oil against S. mansoni cercariae are presented in figures 3. While, the $LT_{50S}$ of S. mansoni cercariae at different concentrations of *N. sativa* alkaloids ranged between 19 min and 127 min at 50 and 0.39 ppm respectively, the $LT_{100S}$ ranged between 25 and 330 min respectively (Fig 3). The $LT_{50S}$ of cercariae by *N. sativa* saponins ranged between 312 min and 27 min at 0.39 and 25 ppm respectively. Both concentrations killed 100% of cercariae after 500 min and 50 min respectively (Fig 3). Volatile oil of *N. sativa* was highly active against cercariae. At 0.39 ppm and 50 ppm it killed 50% of the cercariae after 35 min and 0.7 min respectively and killed 100% of cercariae after 90 min and 5 min respectively (Fig 3).

Figure 4 compares between the lethal effect of 50% of cercariae at different concentrations of alkaloids, saponins, and volatile oil of *N. sativa* on cercariae. The volatile oil was significantly more active than alkaloids and saponins particularly at higher concentrations (p<0.001). Alkaloids were also significantly (P <0.05) more active than saponins (Fig 4).

The comparison between the susceptibility of miracidia and cercariae to different concentrations of alkaloids, saponins, and volatile oil of *N. sativa* is shown in Figure 5. The miracidia were more sensitive than cercariae at all the tested constituents. The higher susceptibility of miracidia was significantly more obvious in case of saponins (P <0.001) followed by alkaloids and volatile oil (Fig. 5).

## IV. DISCUSSION

Schistosomiasis is the second most important parasitic disease after malaria in terms of overall morbidity and mortality. In this study three constituents of *N. sativa*, namely alkaloids, saponins and volatile oil, were tested for their lethal effect on miracidia and cercariae of *S. mansoni*. All the tested constituents were lethal to both miracidia and cercariae. The biocidal activity of alkaloids and saponins of other plants against cercariae and miracidia has been also reported. For instance alkaloids of *Teclea nobilis* and *Jatropha elliptica*and saponins of *Phytolacca dodecandra* were previously found to be lethal to both cercariae and miracidia [33-35]. In a previous study, crude extracts and crushed seeds of *N. sativa* were found to be lethal to cercariae and miracidia [27]. Therefore, data obtained in this study suggest that this activity could be attributed at least in part to volatile oil, saponins and alkaloids of *N. sativa*.

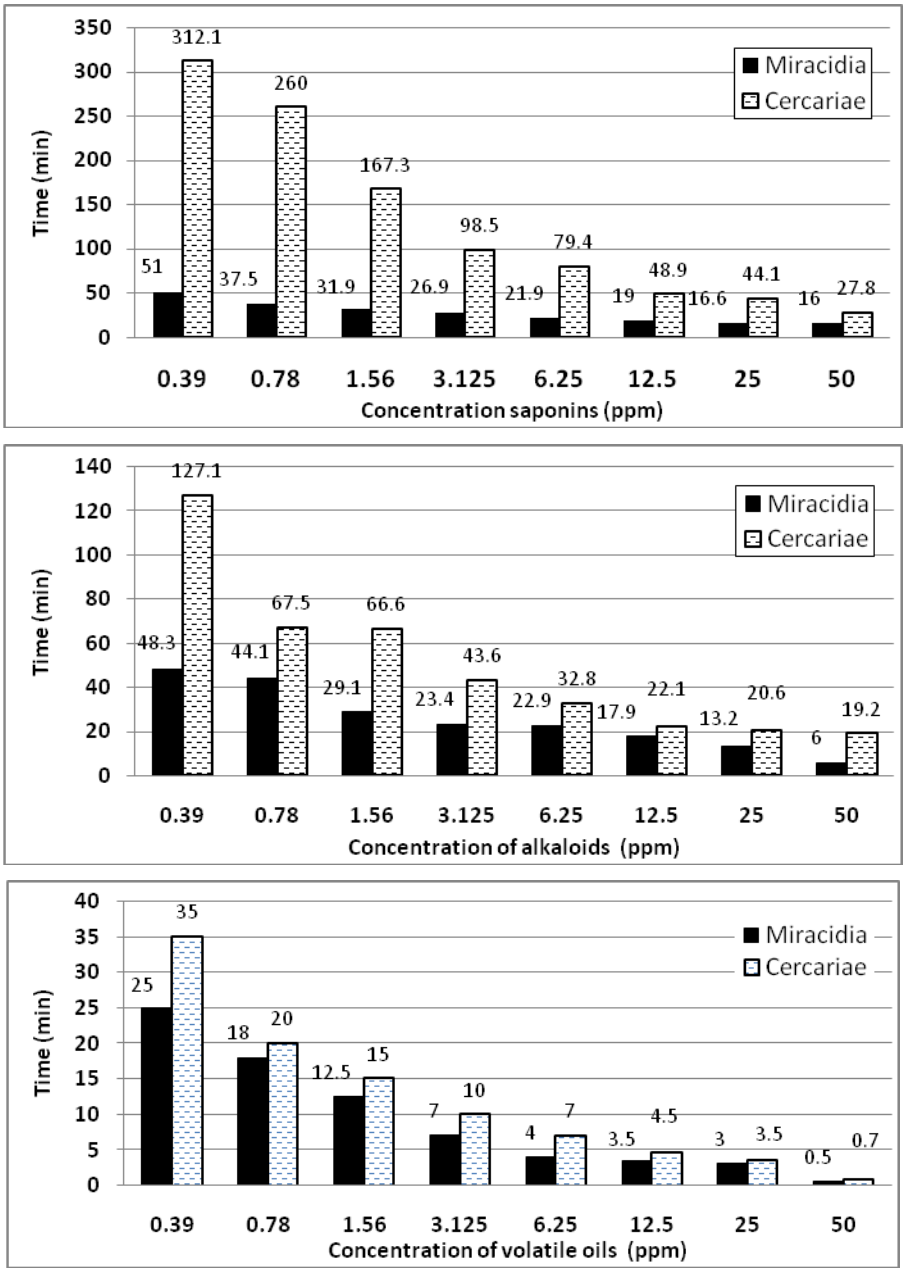


Fig. 5: Comparison between the $LT_{50S}$ of miracidia and cercariae at different concentrations of saponins, alkaloids and volatile oil of *Nigella sativa*.

In this study the volatile oil was significantly more active than alkaloids and saponins, particularly at higher concentrations, against both miracidia and cercariae ($p<0.001$) and alkaloids were significantly more active than saponins against cercariae ($p<0.001$). Miracidia were found to be more sensitive than cercariae to the lethal effect of the tested constituents of black seeds. This higher susceptibility of miracidia was significant to both alkaloids and saponins ($p<0.001$ and $<0.05$, respectively). The crushed *N. sativa* seeds were previously found to be more active against miracidia than cercariae [27], which is in agreement with the data of the activity of the tested active constituents evaluated in this study. Higher susceptibility of miracidia was also observed in for extracts of berries of *Phytolaccadodecandra* [35] and latex of *Euphorbia milli* [36]. However, this is not always the case as cercariae are more commonly sensitive to other medicinal plant extracts. For instance, while alkaloids of the rhizome of *Jatropha elliptica* were highly lethal to cercariae ($LT_{100}$ of 4 ppm after 30 min), they were ineffective against miracidia [33]. Also, *Iris pseudacorus*, [37], *Allium sativum* [38], mirazid resin of *Commiphora molmol* [39] and *Plectranthus tenuiflorus* [40] had lesser activities against miracidia compared to cercariae.

Miracidia after infecting snails, they form sporocysts, which produce thousands of cercariae [41]. Therefore, the observed higher activity of the active constituents of *N. sativa* against miracidia is advantageous since killing one miracidium prevents the formation of thousands of cercariae.

In this study, the constituents of *N. sativa* killed cercariae at concentrations below 1 ppm, as did, other potent medicinal plants like *Origanum compactum* [42], *Iris pseudacorus* [7], *Iris germanica* [43] and *Lagenaria breviflora* [44]. Therefore, the three tested active constituents of *N. sativa* could be categorized as potent cercaricides and miracicides.

## V. CONCLUSION

Volatile oil, alkaloids and saponins of *N. sativa* seeds are potent larvicides both against miracidia and cercariae, the aquatic stages of *S. mansoni* and consequently, they could be used in programmes to control and eliminate the parasite.

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