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Original Article

Biochemical Investigation of Different Extracts and Larvicidal Activity of *Tagetes minuta* L. on *Anopheles stephensi* Larvae

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Abstract

Tagetes minuta L. is considered as an annual plant growing in the temperate zones of South America. It has been used as carminative, anti-inflammatory, anti-abortion, vermifuge, bronchodilator and hypotensive. In the current study the locally cultured plant of *Tagetes minuta* L. was studied for phytochemical and larvicidal properties. The phytochemical analysis of aerial parts of the plant exhibited the presence of saponins, terpenoids, tannins, alkaloids and flavonoids. The efficacy of methanolic extract of the plant was tested against malaria vector, *Anopheles stephensi*. The probit analysis from the concentration and mortality exhibited the LC_{50} and LC_{90} values of 2.5 mg/l and 11.0 mg/l, respectively. Also the efficacies of the ethyl acetate, chloroform, methanolic and aqueous extracts of the plant were tested on *Anopheles stephensi* with the LC_{50} concentration of the methanolic extract. The results showed that the chloroform extract had the highest efficacy.

Keywords: Tagetes minuta L.; Larvicidal activity; *Anopheles stephensi*.
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1. Introduction

Tagetes species are growing worldwide, and *Tagetes minuta* L. from Asteraceae family is endemic of South America. The solvent or oil extracts of the genus *Tagetes* have been the subject of many studies [1-4]. *Tagetes minuta* L. is rich in flavonoids [3, 5], and also contains tannins [6, 7] and terpenoids [4], and the major category of phytochemicals of interest, terpenoids, flavonoids, alkaloids, polyacetylenes and fatty acids, have been the subjects of studies by different groups [8].

Tagetes minuta L. is used in folk medicine, which is approved for treating gastrointestinal diseases [9], however, some studies have shown other effects including antimicrobial and larvicidal activities by the plants of this species [3]. It is known as suico, chinchilla or zuico in South America, and it has been used in the folk medicine as antimicrobial, antihelminthic and antispasmodic remedy [10]. Antibacterial activity of its flavonoids has

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been reported against microorganisms like *Proteus vulgaris* [11]. Enzyme inhibition, antioxidant and cytotoxic effects have also been reported for flavonoids [12]. Here, we report the chemical composition and larvicidal activity of different extracts of *Tagetes minuta* L.

2. Materials and methods

2.1. Plant materials

Tagetes minuta L. was collected from Karaj, Tehran province, Iran during the flowering stage. Voucher specimen was deposited at the Faculty of Agriculture, Tehran University, Iran, with the herbarium number of 6540 TEH.

2.2. Preparation of extracts

Dried and finely powdered aerial parts of *Tagetes minuta* L. (1000 g) were extracted with ethanol (3.5 l) at room temperature for two weeks. After removal of the solvent in vacuum at 50 ºC, the residue (30 g, 3% w/w) was stored at 4 ºC in sealed vials until usage. Three fractions of this extract were taken up with 600 ml of the following solvents at the room temperature: chloroform, ethyl acetate, and water with the yield of 9%, 35%, and 26% w/w, respectively.

2.3. Biological study

Different extracts of *Tagetes minuta* were evaluated against the late third and the early fourth instar larvae of *Anopheles stephensi*. The mosquitos were collected from malarious areas of Iran, Sistan and Baluchistan province and then were maintained at the School of Public Health and Institute of Health Research. The larvae were exposed to different concentrations of the *Tagetes minuta* extracts which were prepared in methanol. The minimum concentration was 0.625 mg/l and the maximum concentration was 1mg/l, to gain the appropriate mortality. Mortality was determined after a 24 h exposure period. All the tests were conduced at 25 ºC in the laboratory condition. For each concentration, at least 2 replicates of 25 individuals were used. The mortality data were subjected to probit analysis using Finney studies [13]. From the regression line between logarithmic dose and probit mortality all the parameters including LC_{50} and 95% confidence interval, LC_{90} and 95% confidence interval were determined.

3. Results

Chemical composition of different extracts of the aerial parts of *Tagetes minuta* L. were determined semiquantitively by phytochemical studies. As shown in Table 1, flavonoids, saponins, tannins and alkaloids were present in the methanolic and aqueous extracts, while terpenoids, alkaloids and flavonoids were present in the non polar organic extracts. There were no acetogenin in the extracts (Table 1).

The result of the bioassay tests of the methanolic extract of *Tagetes minuta* on the *Anopheles stephensi* larvae are presented in Table 2. The extract was larvicid with a concentration as low as 0.625 ppm and its LC_{50} and LC_{90} values were about 2.5 and 10.97 ppm, respectively (Table 2).

Other extracts of *Tagetes minuta* L., were also tested at the 2.5 ppm for their larvicidal activity against *Anopheles stephensi*. As shown in Table 3, the chloroform extract

Table 1. Components of different extracts of *Tagetes minuta* L..

Type of Extract	Flavonoid	Saponin	Tannin	Alkaloid	Terpenoid	Acetogenin
Methanolic	+++	++	+	+	-	-
Aqueous	++	+	+	+	-	-
Ethylacetate	+	-	-	+	+	-
Chloroform	+	-	-	+	++	-

Table 2. Larvicidal effect of different concentrations of *Tagetes minuta* L. methanolic extract on *Anopheles stephensi* larvae.

Concentrations	Total tested	Total dead	Mortality rate (%)
Control	73	3	4
0.625	73	10	14
1.25	75	19	25
2.5	75	35	47
5	74	48	65
10	73	69	95

Note: The larvae were exposed to different concentrations of the *Tagetes minuta* methanolic extract. Mortality was determined after a 24 h exposure period. All the tests were conduced at 25 ºC in the laboratory condition. For each concentration, at least 2 replicates of 25 individuals were used.

showed the highest activity against the larvae than the other extracts.

4. Discussion

Many biological effects have been attributed to *Tagetes minuta* L., including antimicrobial and larvicidal activities [3, 10, 11]. The present study was performed to find out if it has larvicidal effect against *Anopheles stephensi* larvae. The results showed that different extracts of the aerial parts of the plant have a good effect against the larvae of *Anopheles stephensi*.

The chloroform extract of *Tagetes minuta* L. showed a good larvicidal activity possibly because of its terpenoids, whereas the methanolic extract contains Aglycon flavonoids and saponins which may be the effective components that have larvicidal effect.

Perich et al. also [14] previously reported that the fraction 1 of the *Tagetes minuta* L. showed an LC_{50} value of 0.16 mg/l (Fiducial Limit, 0.14-0.19) and an LC_{90} of 0.46 mg/l (Fiducial Limits, 0.37-0.63) against larvae of *Anopheles stephensi*. In the fraction 2 they found higher concentrations to cause LC_{50} and LC_{90} [14]. In another study, they also evaluated extractions of different parts of *Tagetes minuta* L. against 3rd instar larvae of *Anopheles stephensi*, and found the LC_{50} values between 2.35 and 18.88 mg/l which are higher than the values found in the present study. These figures were based on the solvent used for extraction as well as different parts of plants [15]. There were some reports about ocimenone derivatives and whole essential oil of *Tagetes minuta* L. that showed some larvicidal activity [16, 17].

In conclusion, the extracts from this plant may be useful for development of new biorational insecticide, however, further investigations are needed to identify the effective components and their mechanisms of actions of this species.

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Table 3. Comparison of larvicidal effect of different extracts of *Tagetes minuta* L. on *Anopheles stephensi* larvae at the LC_{50} level of the methanolic extract.

Extract type	Total tested	Total dead	Mortality (%)
Control	50	2	4 ±3
Chloroform	50	32	64 ±7
Ethyl acetate	50	23	46 ±7
Methanol	50	28	56 ±7
Aqueous	50	0	0

Note: The larvae were exposed to a 2.5 ppm concentration of the *Tagetes minuta* extracts which were prepared in methanol. Mortality was determined after a 24 h exposure period. All the tests were conduced at 25 ºC in the laboratory condition.

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