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Article

Acaricidal potential of *Euphorbia seguieriana* and *E. helioscopia* (Euphorbiaceae) extracts against *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT
Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most dangerous agricultural pests worldwide. Since this mite has the potential to resist a wide variety of chemical acaricides, finding alternative natural sources for its management is suggested. This study estimated the lethal effect of methanol extracts obtained from two Euphorbiaceae plants against female adults after 24 hours using a spray tower. Probit analysis indicated that LC_{50} values (95% confidence intervals) of *Euphorbia helioscopia* and *E. seguieriana* were 4.54 (3.301–6.644) and 11.66 (8.498–21.043) mg/ml, respectively. Phytochemical analysis of both methanol extracts revealed that the total phenolic content, antioxidant rate, and total flavonoid capacity of *E. helioscopia* were significantly higher than in *E. seguieriana* ($P < 0.05$). In addition, HPLC analysis illustrated that rutin and quercetin content in *E. helioscopia* were 480 ± 0.01 and 2.73 ± 0.01 µg/ml, and in *E. seguieriana*, those were 230 ± 0.02 and 2.65 ± 0.01 µg/ml, respectively. According to the bioassay results, the methanol extract of *E. helioscopia* was 2.5 times more harmful than the methanol extract of *E. seguieriana*. Also, the metabolite contents with toxic activity in *E. helioscopia* were higher than in the other plant. Therefore, *E. helioscopia* may have the potential to act as a pesticide against *T. urticae*.

KEYWORDS: Acute toxicity, methanol extract, secondary metabolites, spurge, two-spotted spider mite.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most critical pests all around the world, which damages wide varieties of fruit, field, vegetable, and ornamental crops (Maciel *et al*. 2020). Since this polyphagous arthropod inserts its mouth parts into the aerial parts of the plants for sap sucking, either in between epidermal pavement cells or through a stomatal opening, the rate of photosynthesis decreases. It can severely affect plant growth and cause necrosis, deformation, and finally, death. Also, this feeding activity changes the capability of stomatal guard cells, resulting in decreased transpiration, and increased water stress (Deangelis *et al*. 1983; Bensoussan *et al*. 2016). Cosmetic damages are the other problems in which the quality of the crops

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attacked by the two-spotted spider mite declines, and the marketability of fruits, flowers, and vegetables reduces severely (Meck *et al*. 2012; Attia *et al*. 2013).

Control of *T. urticae* almost wholly depends on pesticides. There are many acaricides applied against this pest. However, due to the high resistant capacity to most chemicals, most mites can survive after spraying these pesticides (Maciel *et al*. 2020). The arthropod has a very short life cycle, high potential for fecundity, and ability to arrhenotokous reproduction besides inbreeding. So, it is clear why this Acari's selection for resistance is high-speed (Van Leeuwen *et al*. 2009). There are numerous reports explaining *T. urticae* resistance to wide varieties of acaricides with different modes of action (Cho *et al.* 1995; Stumpf and Nauen 2001; Lee *et al*. 2003; Van Leeuwen *et al*. 2010; Khajehali *et al*. 2011; Pavlidi *et al*. 2017; Wu *et al*. 2019; Alpkent *et al.* 2020; Erdem *et al.* 2020). There are several investigations to find an alternative source for control of this harmful arthropod, such as natural plant compounds (Çalmaşur *et al*. 2006; Dąbrowski and Seredyńska, 2007; El-Sharabasy 2010; Afify *et al*. 2011; Yanar *et al*. 2011; Erdogan *et al*. 2012; Pavela 2016; Abdelgaleil *et al*. 2019).

The plants' metabolites are relatively safe for humans, the environment, and non-target organisms. In addition, these ingredients possess various bioactivities such as toxicity, repellency, antifeedant effects, and oviposition deterrent activities toward insects and Acari pests (Afify *et al*. 2011; Tak and Isman 2017). One of the main groups of secondary metabolites in plants that comprises 5–10% of known secondary products is flavonoids. They work as enzyme inhibitors, antioxidants, and precursors to toxic substances. So, they have critical roles in plant biochemistry and physiology besides being involved in plant defense against organisms such as pathogens and insects (Upasani *et al*. 2003). Flavonoids are among those compounds with the ability to regulate insect oviposition and feeding (Mierziak *et al.* 2014). These compounds interrupt the biosynthesis of 20-hydroxyecdysone, the crucial enzyme in the development of the molting hormone, by adversely affecting insect ecdysone-20 monooxygenase (Smith 1985). Rutin and quercetin are two flavonoids that are phagostimulant to many polyphagous insects. However, insects' behavioral responses sometimes depend on their life stage and the phytochemical concentration they receive (Simmonds 2001). According to Diaz Napal and Palacios (2015), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae were deterred after feeding on leaves treated with 100 $\mu g/cm^2$ quercetin. Jadhav *et al*. (2012) showed that rutin caused a delay in the growth and development of *Helicoverpa armigera* (Hüb.) (Lepidoptera: Noctuidae) as well as *S. litura* (Fab.) larvae, interfering with their molting process.

Plant phenolic compounds are plants' other defense mechanisms against arthropod pests ranging from simple phenol to polyphenols. They can block Gamma-Aminobutyric Acid (GABA) or the octopaminergic system and act as an efficient insecticide. In addition to their toxicity, phenols possess antifeedant, anti-ovipositional, and growth inhibitory effects (Pavela 2011). The existence of phenolic compounds in plants could decrease the fecundity of *T. urticae* and *Panonychus ulmi* (Koch) (Acari: Tetranychidae) (Dąbrowski and Bielak 1978). Also, an increase in total phenol content of host plants could suppress *T. urticae* infestation (Afifi *et al*. 2010).

Flavonoids and phenolic compounds are also considered potentially health-promoting substances due to having several physiological properties, including antithrombotic, antimicrobial, antiallergenic, cardioprotective, artherogenic, anti-inflammatory, vasodilatory, and antioxidant effects (Singh *et al*. 2014).

In this study, methanol extracts of *Euphorbia seguieriana* and *E. helioscopia* were examined to detect their antioxidant capacity, and phenolic and flavonoid contents. Both plants belong to Euphorbiaceae (Malpighiales), the spurge family, which comprises more than 300 genera and around 7500 species (Rampadarath *et al*. 2014). In this family, *Euphorbia* is a remarkable genus varying from annuals to trees, with more than 2000 known species containing toxic latex (Abbasi *et al.* 2013).

Numerous reports declare insecticidal and acaricidal effects of different species of this genus. Pinto *et al.* (2011) illustrated that all aqueous concentrations of *E. splendens* var. *hislopii* latex had lethal effects against eggs and larvae of *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae). Also, according to de Silva *et al.* (2008), the xylene-latex extract was extremely toxic to soft-bodied insects/ spiders. Devi *et al.* (2014) showed larval and pupal mortality of *H. armigera* after treatment by synthesized silver nanoparticles using leaf extract of *E. hirta*. After contact of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) with *E. hirta* leaves extracts using different solvents, Muthu *et al.* (2019) found that methanol extract was more effective with higher mortality. Moreover, the successful pesticidal activity of methanol extract of dried roots of *E. kansui* against *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), and *T. urticae* (Dang *et al.* 2010), Latex effect of *E. bupleuroides* on *Blattella germanica* L. (Dictyoptera: Blattidae) (Azoui *et al*. 2016), and effects of nine known diterpene polyesters extracted from *E. paralias* L. on *Biomphalaria alexandrina* (Ehrenberg) (Gastropoda: Planorbidae) (Abdelgaleil *et al*. 2002) were reported previously.

In the present study, in addition to the phytochemical analysis experiments including the determination of total phenolic quantity, antioxidant capacity, total flavonoid values, and measuring rutin and quercetin content, we evaluated *in vitro* acaricidal effect of methanol extracts of *E. seguieriana* and *E. helioscopia* aerial parts against adult females of *T. urticae*.

MATERIALS AND METHODS

Plant materials

Aerial parts of the two spurge plants, *Euphorbia seguieriana*, and *E. helioscopia*, were harvested during the flowering stage, respectively from the Gisoom region (Guilan province) and the Sarein region (Ardabil province), Iran, from April–May 2018. The geographic locations of both plants are shown in Table 1.

The plant materials were cleaned and washed under tap water. Then they were air dried in the shade at a temperature not higher than 28 °C. After that, they were ground to fine powder using an electric blender, stored in clean labelled paper pockets and kept at 4 °C for next experiments.

Table 1. Geographical locations of species *E. helioscopia* and *E. seguieriana*.

Species	Region and Province	Longitude (E)	Latitude (N)	Altitude (m)
E. helioscopia	Sarein region, Ardabil province	48° 07′ 52″	38° 15′ 03″	1663
E. seguieriana	Gisoom region, Guilan province	49° 04′ 50″	37° 06′ 77″	−17

Preparation of the plants' extracts

Fifty grams of each plant powder were added in 200 mL of methanol 95 % (Merck, Germany) in a conical flask with a plastic cap and then kept on a rotary shaker (TAT-Sheg) at 120 rpm for 72 h. After that, the mixtures were filtered through Whatman No. 1 filter papers, and the filtrates were concentrated by a rotary evaporator apparatus with a vacuum at the 35–40 °C temperature at 60 rpm. After evaporation of the solvent, the dry extracts were stored at 4 °C in airtight bottles for the subsequent experiments (Barbour *et al.* 2004).

Determination of total phenolic content

The total phenolic content of both extracts was estimated by the Folin-Ciocalteu reagent according to Basma *et al.* (2011). The test tubes containing 500 µL of 10% Folin-Ciocalteu's reagent, 500 µL of distilled water, and 200 µL of each plant extract were used for the assays. After 3–8 min. of incubation, 800 µL of 7.5% saturated aqueous sodium carbonate (Na_2CO_3) was added and mixed thoroughly. The mixtures were allowed to stand in dark conditions at room temperature for 30 min. Absorption was measured at 765 nm using a spectrophotometer. The total phenolic content was described as gallic acid equivalent per gram of dry weight (mg GAE/g) of extracts. The experiment was done in three replicates, and distilled water was used as a blank. Also, gallic acid was used to show a standard calibration curve.

The following formula (Parthasarathy *et al*. 2009) was used for calculating the total content of phenolic compounds in the plant extract:

$$\text{Total phenolic content} = \text{GAE} \times \text{V/m}$$

where GAE is the gallic acid equivalence (mg/mL) or concentration of gallic acid established from the calibration curve; V is the extract volume (mL); and m is the weight (g) of the pure plant extract.

2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant capacity of both extracts was confirmed by using the DPPH assay, according to Blois (1985). In this experiment, different plant extract solutions, including 50, 100, 125, 400, 800, and 1000 µg extract/mL, were prepared, and 3 mL of 0.004% DPPH radical solution was added to each of them. After mixing by vortex, the mixtures were kept at room temperature under dark conditions for 30 min. The optical density (OD) was measured at 517 nm using a UV spectrophotometer. The experiment was prepared in three replicates. The solvent (methanol) was used as a blank, and Butylated hydroxytoluene (BHT) was applied as a positive control. Also, the baseline control involved methanol and DPPH solution.

By using the following equation (Basma *et al.* 2011), the DPPH radical concentration was estimated:

$$\text{Scavenging effect (\%)} = (A_0 - A_1) \times 100\%/A_0$$

where A_0 is the absorbance of the control reaction, and A_1 is the absorbance of the sample.

Determination of total flavonoid content

The aluminum chloride colorimetric method was used to measure the total flavonoid content of both *Euphorbia* extracts (Chang *et al.* 2002). Five hundred µL of each extract (1 mg/mL) was mixed with 1.5 mL methanol, 0.1 mL aluminum chloride, 0.1 mL potassium acetate (1 M), and 2.8 mL distilled water. After 30 min. of incubation at room temperature, the absorbance of the pinkish color mixture was evaluated at 415 nm by spectrophotometer. The reagent blank contained distilled water instead of the plant extract. The experiment was performed in four replications, and the standard curve was displayed by quercetin (Sigma) in concentrations between 250–1000 µg/mL.

The following formula (Parthasarathy *et al*. 2009) was used to calculate the total flavonoid content in both extracts:

$$\text{Total flavonoid content} = \text{CE}\times\text{V/m}$$

where CE is the quercetin equivalence (mg/mL) or concentration of quercetin solution established from the calibration curve; V is the extract volume (mL), and m is the weight (g) of the pure plant extract.

HPLC analysis of Rutin and Quercetin

High-Performance Liquid Chromatography technique (HPLC) was used to analyze rutin and quercetin content (Kreft *et al.* 2002). Twenty µl of each plant extract and prepared standards were injected into a Merck-Hitachi HPLC LaChrom instrument and analyzed using a reverse phase C-18 column with 25 cm length and 4.6 mm diameter. In this experiment, the mobile phase was 78.5: 21.5 of water: acetonitrile (isocratic elution) with a flow rate of 2 mL/min. To identify the compounds, two standards of rutin and quercetin (Sigma-Aldrich) were used. The peaks were recognized by comparing the standard compounds' retention time (RT) with that of different peaks obtained in HPLC analysis of extracts detecting at 256 and 255 nm for rutin and quercetin, respectively.

For developing the calibration curves of both chemicals, different dilution series of each standard stock (3.12, 6.25, 12.5, 25, 50, and 100 ppm) were prepared before injection into the HPLC instrument. According to the linear regressions of the standards of rutin (Y = 61075X + 124146; R^2 = 0.9987) (Fig. 1) and quercetin (Y = 133685X + 136275; R^2 = 0.9996) (Fig. 2), the quantification of quercein and rutin was determined.

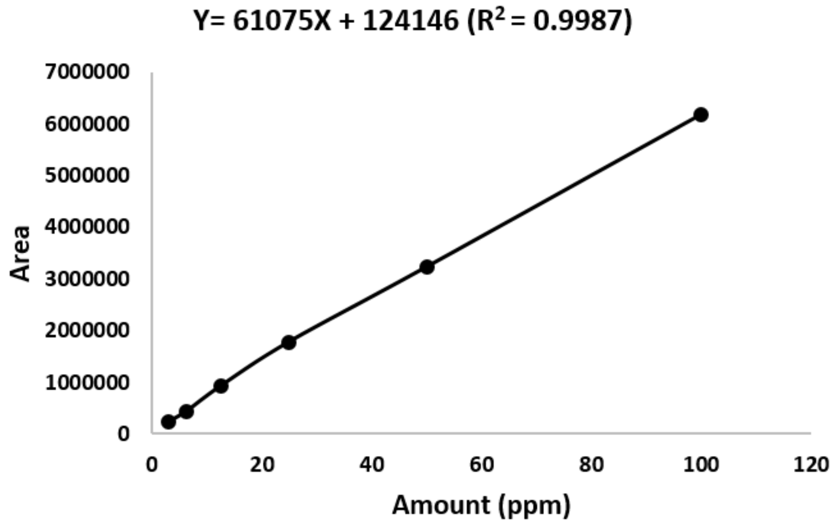

Figure 1. Rutin calibration curve.

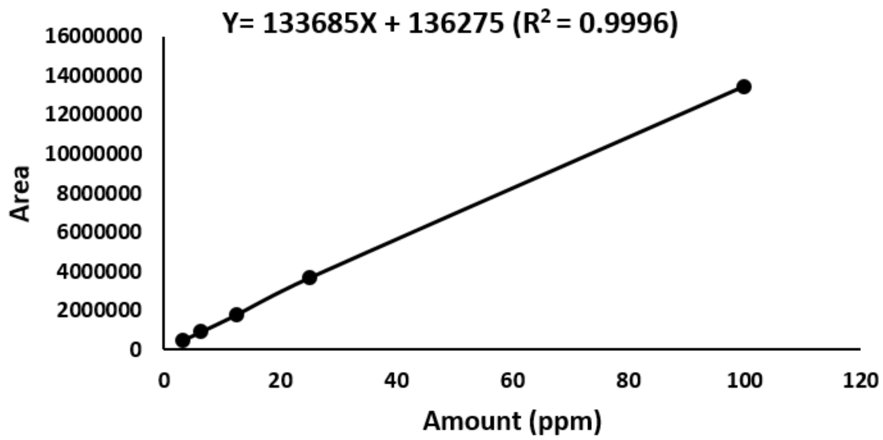

Figure 2. Quercetin calibration curve.

Biological materials

The first colony of *T. urticae* was obtained from a culture maintained in the Plant Protection Department, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran and was reared on the two-or three-week-old cowpea (*Vigna sinensis* L., var. Tarom) at 25 ± 1 °C and 60 ± 10% R.H., and 16: 8 (L: D). The cowpea plants were grown in pots filled with a mixture of soil, cocopeat, and perlite (1: 2: 3) and were kept at greenhouse environment with a temperature of 28 ± 1 °C, 40 ± 5% R.H., and 16: 8 (L: D). The plants were irrigated regularly and fed with 2 g/L of a complete fertilizer (HortiGrow-Hortiland Holland B.V.) every week. Several leaves with mites were cut out from infected cowpeas and placed on the young plants for mass rearing of the mites. The adult females were used for the bioassay experiment. In this case, several adults were put on clean leaves of the two-week-old cowpea plants by using a fine camel's hairbrush. After 24 hours, they were removed entirely, and the eggs were kept and reared until adulthood to produce the cohort individuals of the tests.

Acaricidal effects of the extracts on Tetranychus urticae

The acaricidal effects of *E. seguieriana* and *E. helioscopia* extracts were assayed on *T. urticae* adult females of the same age. Experimental units were the *V. sinensis* leaf discs held backward on wet cotton into 6 cm Petri dishes to keep the humidity. Twenty adult female individuals were put on the cowpea leaves using a fine camel's hairbrush. After the establishment of the mites on the leaves, five concentrations of each methanol extract could make mortality between 20 to 80 percent during pre-tests were prepared. Methanol extract concentrations related to *E. helioscopia* were 1, 1.68, 2.83, 4.76, and 8 mg extract/ml and to *E. seguieriana* were 2, 3.05, 4.68, 7.17, and 11 mg extract/ml.

The different concentrations of plant extracts were applied to the experimental unit by using a spray tower at 1 atm pressure (Salman *et al*. 2014) with 1.5 ml of each extract per plate, providing a uniform deposit of 2.0 ± 0.2 µl/cm^2 (Potter 1952). The experiments held at 25 ± 1 °C, 60 ± 10% R.H., and 16: 8 (L: D) were repeated three times on different days with 20 adult females per experimental unit, and the solvent (methanol) was used as control. After 24 hours, the experimental units were studied, and each individual could not move its appendages after stimulating with hairbrush was counted as dead.

Data analysis

In the toxicity test, concentration-mortality regression was evaluated using Probit analysis (Polo-Plus software; Robertson *et al.* 2007) to determine the lethal concentrations and slopes at 95% confidence intervals (CI). The comparison of means using Student's t-test was conducted to determine the differences between phytochemical traits of both plant extracts on the level of P < 0.05 (SAS Institute Inc. 2004).

RESULTS

Total phenolic contents

There was higher total phenolic content in *E. helioscopia* extract (52.82 ± 1.49 mg gallic acid/g F.W.) compared to *E. seguieriana* extract, and this difference was significant statistically (P < 0.05) (Table 2). The total phenolic content in *E. seguieriana* extract was 1.22 lower than the other extract.

Antioxidant activity

The methanol extract of *E. helioscopia* revealed a maximum DPPH scavenging activity of 51.07 ± 0.61% in comparison to *E. seguieriana* extract (P < 0.05) (Table 2). The latter species showed 1.06% activity lower than the first one.

Determination of total flavonoid content

The amount of this ingredient in *E. helioscopia* extract was 1.2 times higher compared with the other plant (P < 0.05). In fact, the total flavonoid content in *E. helioscopia* and *E. seguieriana* extract was 11.75 ± 0.82 and 9.71 ± 0.13 mg quercetin/g F.W., respectively (Table 2).

HPLC analysis of Rutin and Quercetin

According to the results obtained from HPLC graphs, the retention time of rutin in *E. helioscopia* and *E. seguieriana* was found to be 27.693 and 27.891, respectively (Fig. 3a, b). Also, the retention time of quercetin in those species was respectively 34.947 and 34.960 (Fig. 4a, b). Rutin content in *E. helioscopia* and *E. seguieriana* was 840 ± 0.01 and 230 ± 0.02 µg/mL, respectively (Fig. 3c). On the other hand, quercetin content in *E. helioscopia* was 2.73 ± 0.01 µg/mL and 2.65 ± 0.01 µg/mL in

E. seguieriana (Fig. 4c).

Table 2. Metabolites content in aerial parts of *E. helioscopia* and *E. seguieriana*.

Phytochemicals	Methanol extracts of Euphorbiaceae species	
	E. helioscopia	***E. seguieriana***
phenolics content (mg gallic acid/g F.W.)	52.85 ± 1.49a	43.10 ± 1.73b
Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay (%)	51.07 ± 0.61a	47.87 ± 0.83b
Total flavonoid content (mg quercitin/g F.W.)	11.75 ± 0.82a	9.71 ± 0.13b

[*] Means followed by the different letters show statistically significant differences within rows (Student's t-test; $P < 0.05$).

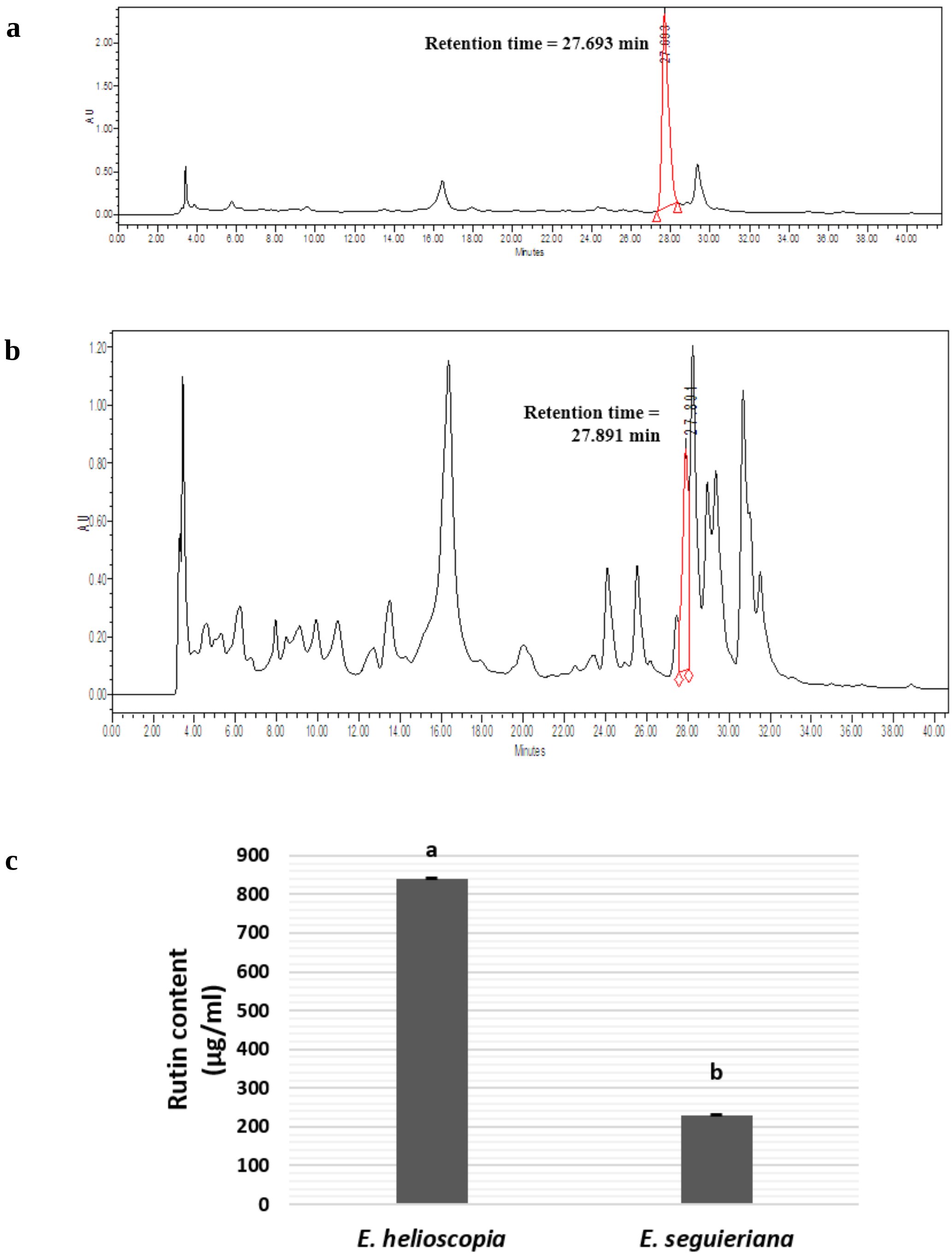

Figure 3. HPLC chromatograms of *Euphorbia helioscopia* (a) and *E. seguieriana* (b) methanol extracts used for measuring rutin content (c) in both plant species (the data is based on only one replicate measurement).

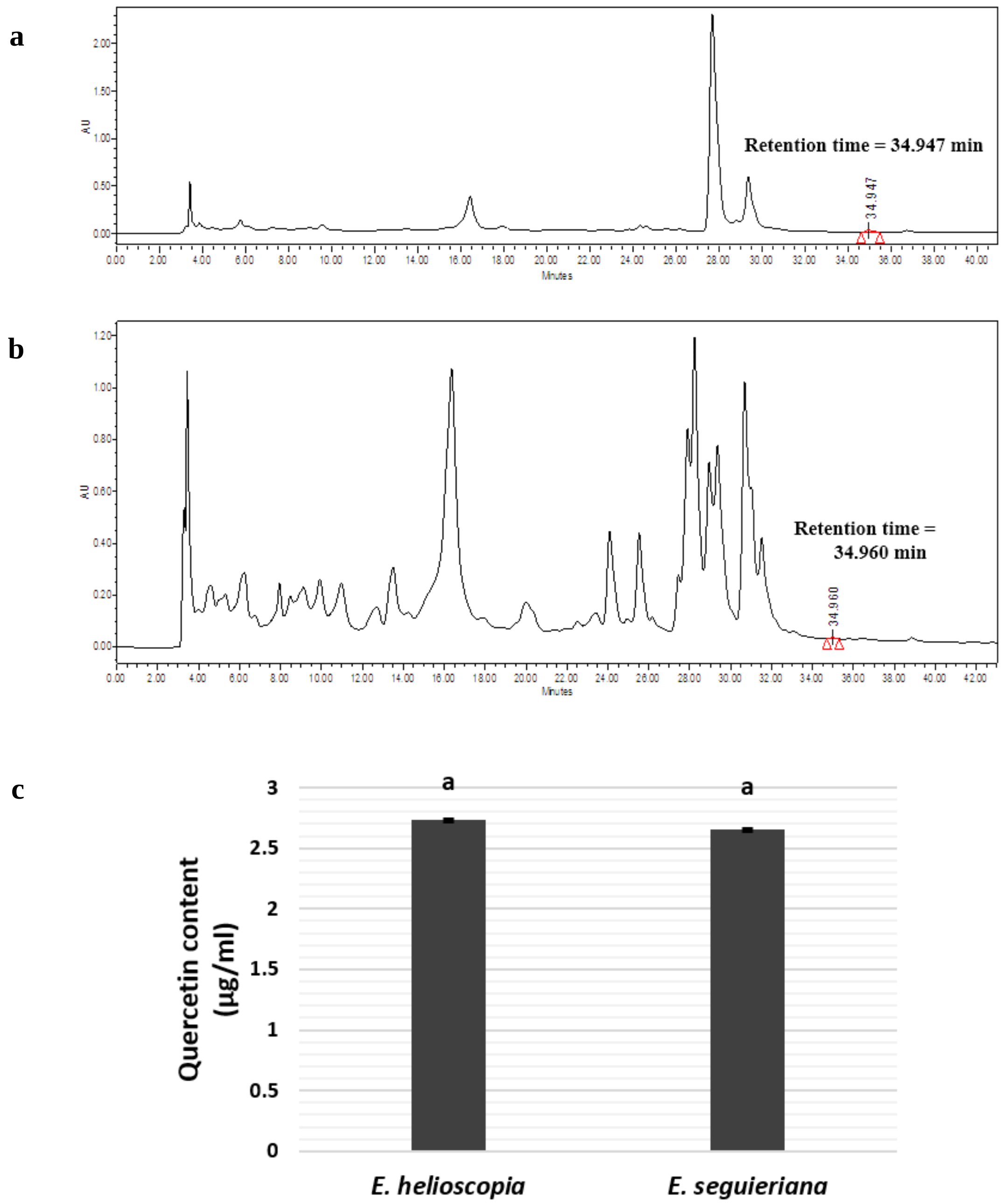

Figure 4. HPLC chromatograms of *Euphorbia helioscopia* (a) and *E. seguieriana* (b) methanol extracts used for measuring quercetin content (c) in both plant species (the data is based on only one replicate measurement).

Table 3. Lethal concentrations (mg extract/ml) of *E. helioscopia* and *E. seguieriana* methanol extracts against *T. urticae* adult females after 24 hours.

Plant extracts	N[a]	LC_{50} (95% confidence intervals)	Slope ± SE	X^2 (df)	Heterogeneity
E. helioscopia	300	4.54 (3.301–6.644)	1.517 ± 0.298	4.989 (13)	0.384
E. seguieriana	320	11.66 (8.498–21.043)	1.898 ± 0.458	5.921 (13)	0.455

X^2 is significant ($p < 0.05$); [a] Number of subjects.

Acaricidal effects of the extracts on Tetranychus urticae

Probit analysis of the 24-hour exposure of *T. urticae* to methanolic extracts of *E. helioscopia* and *E. seguieriana* is shown in Table 2. LC_{50} of *E. helioscopia* extract against *T. urticae* showed 4.54

mg/ml. However, the concentration of *E. seguieriana* extract causing 50% mortality in the two-spotted spider mites was estimated at 11.66 mg/ml (Table 3). Thus, methanol extract of *E. helioscopia* was 2.5 times more toxic than the other one against *T. urticae* female adults.

DISCUSSION

The results indicated that *E. helioscopia* methanol extract had a greater acaricidal effect against the two-spotted spider mites. Zhou and Zhao (2018) determined that extracts from *E. helioscopia* roots at a concentration of 200 mg/mL after a slide dip method had 49.4% killing action against *Tetranychus cinnabarinus* Boisduval (Acari: Tetranychidae). This plant species was also shown to have wide variety of pesticidal activities like insecticidal, antibacterial, antiviral, nematicidal, antifungal, and molluscicidal effects (Singh *et al*. 2018). Nisa and Buhroo (2021) found that ethanolic and aqueous extracts of *E. helioscopia* at 5.0% concentration had a maximum mortality of 85.83 and 72.50%, as well as the maximum repellent activity of 86.67% and 80.00% among six plants collected from Kashmir valley assayed against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) after 96 h. On the other hand, according to Yousuf *et al*. (2021), *E. helioscopia* was the least repellent against *Macrosiphum rosae* (L.) (Hemiptera: Aphididae), even when its ethanolic extracts were used against this sucking pest. Possessing cytotoxicity effects, the water and chloroform extracts of *E. helioscopia* leaves at concentrations of 300 ppm showed respectively, a maximum mortality rate of 10.34 and 14.67% against *Cimex lectularius* L. (Hemiptera: Cimicidae) during 48 h (Haleem *et al.* 2016). Despite the pest-killing potential, this extract is less toxic than the chemical insecticides. For example, Shehzadi *et al.* (2015) evaluated that after 168 h of exposure, the LC_{50} value of chlorpyrifos in a lethal assay against second instar larvae of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) was 0.60 ppm, while *E. helioscopia* extract showed 8.18 times less toxicity against this pest.

There are wide varieties of secondary metabolites in different species of Euphorbiaceae plants. In this study, methanolic extracts of *E. helioscopia* and *E. seguieriana* aerial parts possessed phenols, antioxidants, and flavonoids. However, *E. helioscopia* was evaluated as richer than the second species, *E. seguieriana*, regarding all these ingredients. Previous phytochemical studies revealed the presence of such products in *Euphorbia* species (Shi *et al*. 2008; Noori *et al.* 2009; Zeghad *et al.* 2016; Mahomoodally *et al*. 2020), and different authors illustrated differences in the content of these chemicals in different species as well as in each aerial part of *Euphorbia* plants. Until now, a total of 173 terpenoids, as well as phenolics, tannins, flavonoids, phenylpropanoids, steroids, lipids, and volatile oils have been isolated and identified from *E. helioscopia*. Among them, diterpenoids and flavonoids are considered as the most important and plentiful bioactive ingredients (Yang *et al.* 2021), as Hua *et al.* (2017) found that macrocyclic diterpenoids isolated from *E. helioscopia* indicated cytotoxicity and an antifeedant activity against the herbivorous insect, *H. armigera*, with EC_{50} values ranging from 2.05 to 4.34 μg/cm^2. In addition, thirteen compounds from *E. helioscopia*, including nine undescribed diterpenoids with anti-inflammatory effects, were displayed by Zhang *et al.* (2022). *Euphorbia helioscopia* has also been widely used for decades to treat various diseases, including phlegm and cough, edema, dysentery, scab, malaria, osteomyelitis, tuberculous fistula, and cancer with diverse biological properties, such as antiproliferative, lipid-lowering, wound-healing, insecticidal, and antibacterial activities (Yang *et al*. 2021). Through the screening of 57 extracts of 54 plant species from 30 families, Nikolova *et al.* (2011) found that the methanol extract of *E. helioscopia* was among the first five extracts that exhibited the most potent antioxidant activity. Also, Maoulainine *et al*. (2012) evaluated that the total phenolic and flavonoid contents and antioxidant activity in flowers of *E. helioscopia* were significantly higher than these metabolites in stem and leave extracts ($P \leq 0.05$).

Clearly, antioxidant potency is highly related to the existence of phenolic and flavonoid metabolites in the plants. Total phenolic and flavonoids have chelating proprieties and work as antioxidant agents against free radicals (Maoulainine *et al*. 2012). Several factors can affect polyphenols levels. Plant species or varieties, different parts of plants, and different life cycles of plants as genetic factors, and climatic conditions and different kinds of stresses as environmental factors may determine the number of polyphenols in the plant (Woodhead 1981; Conor *et al.* 2002; Fujita *et al.* 2002; Lin *et al*. 2003; Silva *et al*. 2007; Bystricka *et al.* 2010; Stine *et al.* 2011; Chrysargyris *et al*. 2020).

In this study, we only used methanol extracts of both *Euphorbia* species. However, using different solvents, we may extract the phytochemical components in different quantities. The polarity of solvents determines the recovery of natural metabolites from herbals; thus, the choice of extraction solvent is an essential step for the assessing the activity of the extracts. It was revealed that extracts prepared with methanol solvent had higher antioxidant properties than other organic solvents used (Felhi *et al*. 2017). Maoulainine *et al*. (2012) indicated that methanolic extracts showed higher DPPH inhibition percentages than ethanol extracts. Moreover, phenolic contents of methanolic extractions of flowers, leaves, and stem were 51.49, 21.27, and 13.54 mg GAE/g dry weight, respectively. These values in ethanol extractions were respectively 1.3, 1.4, and 3.1 times less than in methanol extractions. Similarly, methanol extracts of different aerial parts of *E. helioscopia* showed about 1.2 times higher flavonoid contents than extractions with ethanol. As well, Deveci *et al*. (2018) reported that the water extract of *E. helioscopia* with the highest phenolic (161.20 ± 0.98 µg pyrocatechol equivalents/mg) and flavonoid (11.22 ± 0.05 µg quercetin equivalents/mg) contents had the highest antioxidant activity followed by the methanol extract. In another study, Rauf *et al.* (2012) revealed that DPPH radical scavenging activities of *E. helioscopia* methanol extract had the highest activity (80.88%). Hence, the antioxidant potency decreases in the order of ethyl acetate, ethanol, chloroform, and hexane (Rauf *et al.* 2012).

According to the present data, *E. helioscopia* showed better acaricidal activity against *T. urticae* compared to *E. seguieriana*. In addition, all critical secondary metabolites studied here, which were extracted by methanol solvent and participated in pesticidal activities, were higher than the other species. Thus, *E. helioscopia* may be promising as a new plant extract pesticide. However, there should be further studies focused on bioactivity-guided isolation and characterization of each active constituent involved in the mites' mortality.

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اثر کنه‌کشی عصاره‌های *Euphorbia seguieriana* و *E. helioscopia* (Euphorbiaceae) روی *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

کنهٔ *Tetranychus urticae* Koch (Acari: Tetranychidae) از جمله خطرناک‌ترین آفات کشاورزی در سراسر دنیا است. از آن جایی که این کنه توان مقاومت به طیف گسترده‌ای از کنه‌کش‌های شیمیایی را دارد، یافتن منابع طبیعی جایگزین به منظور مدیریت آن پیشنهاد می‌شود. در این بررسی، اثر کشندگی عصاره‌های متانولی به دست آمده از دو گیاه Euphorbiaceae بر کنهٔ بالغ ماده پس از ۲۴ ساعت با استفاده از برج پاشش ارزیابی شد. بر اساس آنالیز پروبیت، مقادیر LC_{50} (حدود اطمینان ۹۵ درصد) *Euphorbia helioscopia* و *E. seguieriana* به ترتیب، ۴/۵۴ (۳/۳۰۱– ۶/٦٤٤) و ۱۱/٦٦ (۸/٤۹۸ – ۲۱/۰٤۳) میلی‌گرم/میلی‌لیتر به دست آمد. بر اساس تجزیه و تحلیل فیتوشیمیایی هر دو عصاره متانولی، میزان فنل کل، فعالیت آنتی‌اکسیدانی و محتوای فلاونوئیدی کل در *E. helioscopia* به طور معنی‌داری بیشتر از *E. seguieriana* گزارش شد ($P < 0.05$). افزون بر این، آنالیز HPLC نشان داد که میزان روتین و کوئرستین به ترتیب در *E. helioscopia* ۴۸۰±۰/۰۱ و ۲/۷۳±۰/۰۱ میکروگرم/میلی‌لیتر و در *E. seguieriana* برابر با ۲۳۰±۰/۰۲ و ۲/٦۵±۰/۰۱ میکروگرم/میلی‌لیتر بود. بر اساس نتایج حاصل از زیست‌سنجی، عصاره متانولی *E. helioscopia* ۲/۵ برابر زیان‌بارتر از عصاره متانولی *E. seguieriana* برآورد شد. همچنین، محتوای متابولیتی با فعالیت سمی در *E. helioscopia* بیشتر از عصارهٔ گیاه دیگر ارزیابی شد. بدین ترتیب، *E. helioscopia* این قابلیت را دارد که به عنوان آفت‌کش علیه *T. urticae* مورد توجه قرار گیرد.

واژگان کلیدی: سمیت حاد، عصارهٔ متانولی، متابولیت‌های ثانویه، فرفیون، کنهٔ تارتن دولکه‌ای.

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