Agriculture, Environment & Society (2022) Volume 2, Issue 1, P. 1-6

Agriculture, Environment & Society

Journal homepage: www.aes.uoz.ac.ir

Qualitative phytochemical screening and insecticidal effect of *Pimpinella stocksii* ethanolic extract on *Aphis gossypii*

Fatemeh Ordouni [a], Abbas Khani [*b], Najmeh Sahebzadeh [b], Ali Mirshekar [b]

[a] M.Sc. Graduate, Department of Plant Protection, Faculty of Agriculture, University of Zabol, Zabol, Iran
[b] Department of Plant Protection, Faculty of Agriculture, University of Zabol, Zabol, Iran

ARTICLE INFO

Article history:
Received: 31 January 2022
Accepted: 07 March 2022
Available online: 17 March 2022

Keywords:
Aphid
Bioassay
Contact toxicity
Phytochemical compounds
Pimpinella stocksii

ABSTRACT

In recent years, the use of herbal compounds to control pests has been proposed as one of the alternative sources to chemical control because of their selectivity, readily biodegradable, and low impacts on non-target organisms and the environment. In this research, qualitative photochemistry and the insecticidal effect of ethanol extract of aerial parts (without flowers) of *Pimpinella stocksii* Boiss. (Apiaceae) on one-day old adult aphids (*Aphis gossypii*) in a completely randomized design with four replications for each concentration, have been investigated under laboratory conditions (25 ± 1°C, 65 ± 5 % R. H, photoperiod 16:8 L: D). The results showed that the mortality of the tested insect increased significantly by increasing concentrations from 7 to 425 µg/cm^2. In the probit analysis, an ethanol extract of *Pimpinella stocksii* was found to be highly toxic to *Aphis gossypii* Glover, with a lethal concentration of 50 percent (LC_{50}), equal to 82.57 µg/cm^2. The mortality rate of one-day adult aphids treated with an ethanol extract of *P. stocksii* at concentrations ranging from 7 to 425 µg/cm^2 ranged from 25 to 80 percent. Preliminary phytochemical analysis showed the presence of various bioactive and insecticidal constituents in *P. stocksii*, like glycosides (anthraquinones), flavonoids, steroids, saponins, and triterpenoid compounds. The purpose of the study is to conduct preliminary and qualitative identification of the active components of the *Pimpinella stocksii* extract as well as to investigate its insecticidal activity.

Highlights

- Herbal compounds have recently been offered as an alternative to chemical pest control due to their selectivity, biodegradability, and little impact on non-target animals and the environment.
- An ethanol extract of *Pimpinella stocksii* was shown to be very poisonous to *Aphis gossypii* Glover, with an LC_{50} of 82.57 µg/cm^2.
- *P. stocksii* contains bioactive and insecticidal chemicals such as glycosides (anthraquinones), flavonoids, steroids, saponins, and triterpenoids.
- The study's goal is to identify the active components in *Pimpinella stocksii* extract and test its insecticidal activity.

1. Introduction

Aphids are among the economically important pests that are distributed worldwide and damage plants by feeding on plant sap. In addition to weakening and withering of the plant and resulting in young leaves being tangled, they cause the transmission of more than one hundred kinds of plant viruses. The cotton-melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a polyphagous species with a worldwide distribution and a variety of biotypes (Luo et al., 2016). With the increase in the number of people living on Earth, the need for foodstuffs has increased, and almost a third of crops are destroyed by damage caused by pests, so pest control is essential. Therefore, in order to control aphids, modern and effective methods like the use of herbal pesticides are emphasized. Herbal Insecticides have fewer adverse effects on the environment compared to conventional pesticides and are less toxic to humans and mammals. In

* Corresponding author.
E-mail address: abbkhani@uoz.ac.ir or abbkhani@yahoo.com (A. Khani)
http://dx.doi.org/10.22034/aes.2022.327310.1016

addition, due to their low durability and ease of use in nature, they can be an alternative to chemical pesticides to control pests such as aphids. In recent years, there has been a tendency to increase the efficiency of agricultural products. In this regard, indiscriminate pesticide use has resulted in serious issues such as direct toxicity on parasitoids, predators, pollinators, fish and humans, the development of pesticide resistance, pesticide residues in food products and negative environmental effects (Damalas and Koutroubas, 2016).

Secondary compounds from some plants play an important role in natural defense and pest control. So the compounds derived from these plants can be a good alternative to synthetic insecticides in pest control programs. Moreover, these herbal compounds, compared to synthetic insecticides, have a lower degree of toxicity for mammals and non-target organisms, and they are also compatible with ecosystems and have less durability and stability in the environment (Geris et al., 2012; Lamarre et al., 2014).

All plants produce secondary metabolites, which are often specific to an individual genus or species and environmental conditions. A total of 74 plant species have been shown to hold secondary metabolites which have significant insecticidal properties and they are distributed in 33 families from which the major occurrence is found in the families Rutaceae (9.5%), Leguminosae (8.1%), Annonaceae (6.7%), Piperaceae (6.7%), Zingiberaceae (6.7%), Asteraceae (5.4%), and Verbenaceae (5.4%). The 51.5% remainder of the plant species are distributed into 26 families. The bioactive compounds reported here are diverse in structure and, according to their biosynthetic origins, are mainly distributed in terpenoids (30.2%), polyketides (including xanthones, quinones, and anthraquinones, 17.3%) and flavonoids (11.3%) classes (Geris et al., 2012).

The genus *Pimpinella* L., with about 170-180 species in the world, is one of the largest genera in the family Apiaceae (Umbelliferae). *Pimpinella stocksii* is a small, annual, herbaceous plant with white flowers and ovoid fruits that belongs to the Apiaceae family. Previous phytochemical studies of *Pimpinella* species have led to the isolation of various compounds like phenylpropanoids (Sajjadi et al., 2015; Reichling et al., 1991), sesquiterpenes, coumarins, and volatile oils (Sajjadi et al., 2015).

The main purpose of the study is to provide preliminary identification of the main active components of the *Pimpinella stocksii* extract that are responsible for its insecticidal activity against *Aphis gossypii* to support the usage of the plant as a safe insecticide. Based on our knowledge, no study has been reported previously relating to the activity of the tested plant extract against pest insects.

2. Materials and methods

2.1. Rearing insects in greenhouses

To create an initial population of A. gossypii Glover, leaves infected with aphids were collected from a greenhouse in the city of Hamoun, and they were transferred on the leaves of bush cucumbers, which were at five to six leaves stages; the pots containing cucumber plant were maintained in a greenhouse conditions (Temperature: 23-25 °C, Relative humidity: 70% and photoperiod 16:8 L: D). For conducting bioassay test, one-day old wingless adult aphids were applied (Tabacian et al., 2011).

2.2. Plant sample preparation and extraction

Pimpinella stocksii was collected in the city of Hamoun, Sistan and Baluchistan province (altitude 480 m, longitude 61° 14' East, latitude 30° 49' North) in October 2014. After recognition of the plant by the Research Center for Agriculture and Natural Resources of Sistan, it was transferred to the laboratory where aerial parts (without flowers) of the plant were held in a dark location at room temperature and dried under ventilation conditions, then held in paper bags in the refrigerator at 4 °C.

Extraction was conducted through a soaking plant on ethanol 96% (Noor Zakariayaye Razi Co., Iran) according to the method of Bahraminejad et al., 2008. The dried aerial parts of the plant were powdered by using electric mills. Then 50 grams of each powdered aerial part were mixed with 200 ml of ethanol solvent separately for 24 hours at room temperature on a shaker at 350 rpm. After each extraction, the considered extract was filtered using Whatman filter paper number one. Then the extract was stored in a dark glass jar in the refrigerator at 4 °C until the time of its usage.

In order to determine the concentration of the extract, the dry weight of the extract was measured in 1 mL of ethanol in three replicates. First the three empty watch glasses were weighed, then in each glass, 1 mL of extract was cast separately. The glasses were placed at 60 °C in the oven until their contents were completely dry. Watch glasses containing the dried extract were weighed and the difference between them and the empty watch glasses was determined, and then the amount of the dried extract in 1 mL of soluble was measured (Ghaemi et al., 2006). The yield of extraction was obtained at 4 mg/mL.

2.3. Bioassay test

This experiment was conducted in a completely randomized design with four replications for each concentration. Each tested unit consisted of a 6 cm petri dish (with an area of 28.26 cm^2). To study the effect of insecticide, different volumes of *P. stocksii* extract (0.05, 0.1, 0.3, 0.5, 1, 1.5, 2 and 3 ml) almost equal to the concentrations of 7.1, 14.2, 42.3, 70.8, 141.5, 212.3, 283.1 and 424.6 $\mu g/cm^2$ were gradually poured on the bottom of petri dishes by the sampler. In control, ethanol was poured by pipette on the bottom of the Petri Dish. The lid of the Petri Dish remained open for 30 minutes to evaporate the solvent, and then in each Petri Dish, 10 one-day old adults were placed, and the lid of the Petri Dish was closed (Rajashekar et al., 2010). The Petri dish was put in the incubator at 25±1°C with a relative humidity of 65 ± 5%, and a photoperiod of 16:8 L:D. Total mortality was determined based on the movement of the legs and antennae after 24 hours.

2.4. Qualitative phytochemical screening

We characterized the different chemical groups with reference to the technical descriptions by Hossain et al., 2013 and Krisgnaveni et al., 2014.

2.5. Test's for steroids (Salkowski test)

The dry ethanol extract (4 mg) was taken in a test tube and dissolved with chloroform (2 ml), then an equal volume of concentrated sulphuric acid was added to the test tube slowly. The formation of red color in the upper layer (chloroform phase) and yellow with green fluorescence in the sulphuric acid layer (lower layer) shows the presence of steroids (Hossain et al., 2013).

2.6. Test's for tri terpenoids

The dry crude plant extract (4 mg) was mixed with chloroform (2 ml) and then acetic anhydride (1 ml) was added to it. Concentrated sulphuric acid (1 ml) was carefully added to the solution. The formation of a reddish violet color indicated positive results for the presence of triterpenoids (Hossain et al., 2013).

2.7. Test's for soponins (Foam test)

The stock solution (1 mL) was taken in a test tube and diluted with 20 mL of distilled water. It was shaken by hand for 15 min. A foam layer was obtained on the top of the test tube, which indicated the presence of saponins (Hossain et al., 2013).

2.8. Test's for flavonoids (Ferric Chloride Test)

Alcoholic solution of leaf extract (1 ml) evaporated and the dry crude plant extract (4 mg) was dissolved in distilled water and reacted with a few drop of freshly prepared 1% ferric chloride ($Fecl_3$) solution. Formation of black fish green color indicates the presence of the flavonoids (phenolic hydroxyl group) (Krisgnaveni et al., 2014).

2.9. Test's for glycosides (Anthraquinone test)

The powdered leaves are extracted with either ammonia or caustic soda. If the aqueous layer shows pink, it indicates the presence of glycosides (Krisgnaveni et al., 2014).

2.10. Test's for tannins (Ferric chloride test)

Three drops of diluted solution of FeCl3 was added to the extract, production of a blue (hydrolysable tannins) or greenish-black (condensed tannins) color that changes to olive green as more ferric chloride is added indicates the presence of tannins (Krisgnaveni et al., 2014).

2.11. Statistical Analysis

SPSS software version 21 was used to perform statistical analysis on the data. Normalization of raw data was evaluated by using the non-parametric One-Sample Kolmogorov-Smirnov Test. For grouping the average of significant data, Tukey's test was used. The effective concentration of 50% lethality was determined by linear probit analysis using SPSS software version 21.

3. Results and Discussion

3.1. Lethal effect of plant extract

The results of the contact toxicity test of ethanol extract on *P. stocksii* on adult *Aphis gossypii* showed that with increasing concentrations, the mortality rate of tested insects increased significantly ($F_{7,\ 31}$= 4.46, P= 0.002; Figure 1). The mortality rate of one-day adult aphids treated with ethanol extract of *P. stocksii* at concentrations ranging from 7.08 to 426.63 µg/cm^2 ranged from 25% to 80%. The calculated LC_{50} value for *A. gossypii* treated with the ethanol extract of *P. stocksii* after 24 hours was 82.6 µg/cm^2 (Figure 2, Table 1).

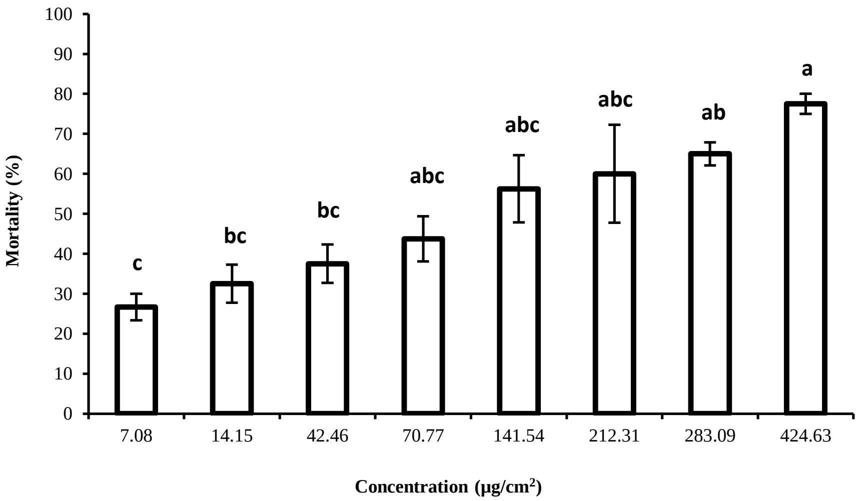

Figure 1. Mortality percentage of *Aphis gossypii* exposed to *Pimpinella stocksii* ethanol extract for 24 h. Means with the different letters are significantly different (p< 0.05) (Tukey post-hoc test after analysis of variance).

Table 1. The Contact toxicity of the *Pimpinella stocksii* ethanol extract on *Aphis gossypii* Glover after 24 hours

No of insects	95% CL	χ^2 (df)	Probability	LC_{50} (μg/cm^2)	Slope ± SE
390	(52.98-126.66)	2.73 (6)	0.84	82.57	0.72 ± 0.13

CL: Confidence limit, χ^2: Chi-square test, df: degrees of freedom and SE: standard error

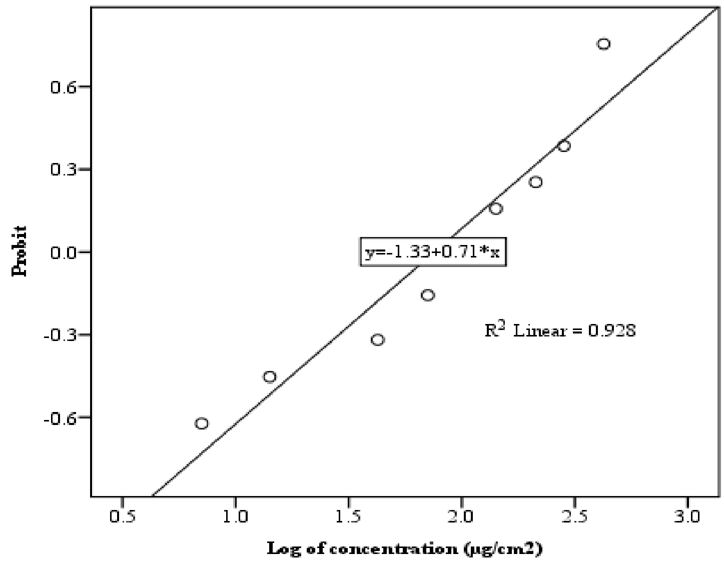

Figure 2. Mortality probit line and 95% confidence limits for *Pimpinella stocksii* on adult *Aphis gossypii*.

Studies indicated that no report suggesting the contact toxicity of ethanol extracts of *P. stocksii* on insects has been disclosed yet. However, the insecticidal activities of other *Pimpinella* species have been reported in several studies (Knio et al., 2007; Tunç and Şahinkaya, 1998). Tunç and Şahinkaya found that anise essential oils (*Pimpinella anisium* L.) were found to be effective fumigants against the cotton aphid (*Aphis gossypii* Glover) and *Tetranychus cinnabarinus* Boisd.

3.2. Qualitative phytochemical screening of plant extract

Preliminary phytochemical analysis showed the presence of various bioactive and insecticidal constituents in *P. stocksii*, like glycosides (anthraquinones), flavonoids, steroids, saponins, and triterpenoid compounds. But tannin (hydrolysable and condensed tannins) was not detected.

It has also been suggested that plant secondary metabolites may be involved in plant defense against insect pests. Similar to our results, phenylpropanoids (Reichling et al., 1991; Tabanca et al., 2005), flavonoids (Özbek et al., 2015), terpenes (Burkhardt et al., 1986), triterpene saponins and steroids (Özbek et al., 2015) were reported form other plants of genus *Pimpinella* in the previous phytochemical studies.

The insecticidal activities of some of these compounds were reported by Reichling *et al.*, 1991 and Tabanca *et al.*, 2005. Both the extracts and essential oils of *Pimpinella* are known to have a high content of pseudoisoeugenol type phenylpropanoids, which is unique to the genus (Sajjadi et al., 2015; Reichling et al., 1991; Knio et al., 2007; Tabanca et al., 2005). In fumigation tests, Anetol phenylpropanoid, the most important component of anise, was very efficient in controlling *Aedes aegypti* and *Culex pipiens* L. (Knio et al., 2007).

Flavonoids and other phenolic compounds have extensive distribution in plants, and various biological activities of these compounds, including insecticidal (Céspedes et al., 2014; Ghaly et al., 2014; Upasani et al., 2003), antioxidant, anti-microbial, and anti-inflammatory types (Jamshidi et al., 2010), have been reported in many surveys.

It has been proven that the flavonoids isolated from the methanolic extracts of the leaves of *Kalanchoe beharensis* and *K. longiflora* family Crassulaceae, has a high degree of inhibitory activity on pupal formation and adult emergence of cotton leaf worm, *Spodoptera littoralis* (Ghaly et al., 2014). The isolated flavonoids from aqueous leaf extract of *Ricinus communis* L. (Euphorbiaceae), showed potential insecticidal, ovicidal and oviposition deterrent activities against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) (Upasani et al., 2003). Céspedes *et al.*, 2014, found that extracts from *Calceolaria* that contained secondary metabolites, including flavonoids, revealed insecticidal properties against *Spodoptera frugiperda* and *Drosophila melanogaster*. Flavonoids in particular have also been found to affect insect ecdysone-20 monoxygenase, which is responsible for the biosynthesis of 20- hydroxyecdysone (Mitchell et al., 1993).

Herbal essences may impact the inhibition of the acetylcholinesterase enzyme and inhibit the aforementioned enzyme (Savelev et al., 2003). Ethanolic extract from the fruits of *Pimpinella anisoides*, an aromatic plant, exhibited activity against acetylcholinesterase, with IC_{50} values of 227.5 μg/ml (Menichini et al., 2009).

4. Conclusion

Due to the toxicity of *Pimpinella stocksii* and its low risk to humans and other mammals, it seems that the extract from this plant could become a proper and viable alternative to conventional chemical control strategies. However, further studies need to be conducted in order to evaluate the safety of this plant before its practical use in insect pest control. Therefore, we can be hopeful that further studies will reveal, in the future by the extraction of active ingredients of *Pimpinella stocksii*, and the possibility of applying an insecticide in integrated pest management to reduce pesticide usage will be generated. These compounds can be applied along with pesticides in integrated pest management. It is also possible to open up a new horizon for producing effective and low-risk pesticides.

Acknowledgments

This study is a part of MSc program at the University of Zabol, Iran. The corresponding author gratefully acknowledges the financial support from the University of Zabol (Grant No 9618-63).

References

Bahraminejad, S., Asestorfer, R.E., Riley, I.T., Schalta, C.J., 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the schools of oats (*Avena sativa* L.). Journal of Phytopathology, 156, 1-7.

Burkhardt, G., Reichling, J., Martin, R., Becker, H., 1986. Terpene hydrocarbons in *Pimpinella anisum* L. Pharmaceutisch Weekblad, 8 (3), 190-193.

Céspedes, C.L., Salazar, J.R., Ariza-Castolo, A., Yamaguchi, L., Ávila, J.G., Aqueveque, P., Kubo, I., Alarcón, J., 2014. Biopesticides from plants: *Calceolaria integrifolia*. Environmental Research, 132, 391-406.

Damalas, C.A., Koutroubas, S.D., 2016. Farmers' exposure to pesticides: Toxicity types and ways of prevention. Toxics 4 (1), 1.

Geris, R., Ribeiro, P.R., Brandão, M.S., Silva, H.H.G., Silva, I.G., 2012. Bioactive natural products as potential candidates to control *Aedes aegypti*, the vector of dengue. In: Rahman A Ed. Studies in natural products chemistry, Vol. 37, Elsevier, Amsterdam, 277-376.

Ghaemi, A., Soleimani jahi, H., Farshbaf moghadam, M., Yazdani, N., Zaki dizaji, H., 2006. Assess the antiviral of the extract of milk thistle flower head growing body control virus Herpes simplex human type 1. Hakim Research Journal, (4), 59-64. (In Persian).

Ghaly, N.S., Mina, S.A., Abdel-Aziz, N.F., Sammour, E.A., 2014. Insecticidal activity of the main flavonoids from the leaves of *Kalanchoe beharensis* and *Kalanchoe longiflora*. Journal of Natural Products, 7, 196-202.

Hossain, M.A., AL-Raqmi, K.A., AL-Mijizy, Z.H., Weli, A.M., Al-Riyami, Q., 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pacific Journal of Tropical Biomedicine, 3(9), 705-10.

Jamshidi, M., Ahmadi, H.R., Rezazadeh, S.h., Fathi, F., Mazanderani, M., 2010. Study on phenolicd and anioxidant activity of some selected plant of Mazandaran province. Journal of Medicinal Plants, 9, 177-183. (In Persian).

Knio, K.M., Usta, J., Dagher, S., Zournajian, H., Kreydiyyeh, S., 2007. Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, *Ochlerotatus caspius*. Bioresource Technology, 99 (4), 763-768.

Krisgnaveni, G., Sailaja, O., Mounika, K., 2014. Identification and estimation of phytochemicals from the plant *Pedicularis bicornuta* leaf extract by uv-spectrophotometry. Research Desk, 3(1), 410-418.

Lamarre, G.P.A., Mendoza, I., Fine, P.V.A., Baraloto, C., 2014. Leaf synchrony and insect herbivory among tropical tree habitat specialists. Plant Ecology, 215, 209–220.

Luo, J.Y., Zhang, S., Wang, L., Lv, L.M., Wang, C.Y., Li, C.H., Zhu, X.Z., Zhou, Z.G., Cui, J.J., 2016. The Distribution and host shifts of cotton-melon aphids in northern China. Plos One, 11(3), e0152103.

Menichini, F., Tundis, R., Loizzo, M.R., Bonesi, M., Marrelli, M., Statti, G.A., Menichini, F., Conforti, F., 2009. Acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from *Pimpinella anisoides* V Brig. (Apiaceae). Fitoterapia, 80, 297-300.

Mitchell, J.M., Keogh, D.P., Crooks, J.R., Smith, S.L., 1993. Effects of plant flavonoids and other allelochemicals on insect cytochrome P-450 dependent steroid hydroxylase activity. Insect Biochemistry and Molecular Biology, 65, 65–71.

Özbek, H., Güvenalp, Z., Kuruüzüm-Uz, A., Kazaz, C., Demirezer, L.Ö., 2015. Trinorguaian and germacradiene type sesquiterpenes along with flavonoids from the herbs of *Pimpinella cappadocica* Boiss. & Bal. Phytochemistry Letters, 11, 74-79.

Özbek, H., Güvenalp, Z., Kuruüzüm-Uz, A., Kazaz, C., Demirezer, L.Ö., 2016. β-Hydroxydihydrochalcone and flavonoid glycosides along with triterpene saponin and sesquiterpene from the herbs of *Pimpinella rhodantha* Boiss. Natural Product Research, 30(7), 750-754.

Rajashekar, Y., Gunasekaran, N., Shivanandappa, T., 2010. Insecticidal activity of the root extract of *Decalepis hamiltonii* against stored-product insect pests and its application in grain protection. Journal of Food Science and Technology, 47(3), 310-314.

Reichling, J., Merkel, B., Hofmeister, P., 1991. Studies on the biological activities of rare phenylpropanoids of the genus *Pimpinella*. Journal of Natural Products, 54, 1416-1418.

Sajjadi, S.E., Ghanadian, M., Ahmadi, S.h., Shafiyan, S., 2015. Isolation and identification of a new phenylpropanoid derivative from the fruits of *Pimpinella Haussknechtii* and GC-MS analysis of the essential oil. Iranian Journal of Pharmaceutical Research, 14(4), 1233-1239.

Savelev, S., Okello, E., Perry, N.S.L., Wilkins, R.M., Perry, E.K., 2003. Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. Pharmacology Biochemistry and Behavior, 75, 661-668.

Tabacian, H., Ravan, S., Bandani, A., 2011. Susceptibilities of two populations of *Aphis gossypii* Glover to selected insecticides. African Journal of Biotechnology, 10(4), 670-674.

Tabanca, N., Bedir, E., Ferreira, D., Slade, D., Wedge, D.E., Jacob, M.R., Khan, I.S., Kırımer, N., Baser, K.H.C., Khan, I.A., 2005. Bioactive constituents from Turkish *Pimpinella* species. Chemistry and Biodiversity, 2(2), 221-232.

Tunç, I., Şahinkaya, Ş., 1998. Sensitivity of two greenhouse pests to vapours of essential oils. Entomologia Experimentalis et Applicata, 86(2), 183-187.

Upasani, S.M., Kotkar, H.M., Mendki, P.S., Maheshwari, V.L., 2003. Partial characterization and insecticidal properties of *Ricinus communis* L foliage flavonoids. Pest Management Science, 59(12), 1349-54.