

Original Article

Antioxidant and Larvicidal Activity of Areal Parts of *Scrophularia striata* against Malaria Vector *Anopheles stephensi*

Fatemeh Yousefbeyk¹, Hassan Vatandoost², Fereshteh Golfakhrabadi³, Zahra Mirzaee³,
Mohammad Reza Abai², Gholamreza Amin³, *Mahnaz Khanavi^{3,4}

¹Department of Pharmacognosy, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran

²Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

³Department of Pharmacognosy, Faculty of Pharmacy and Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁴Faculty of Land and Food Systems University of British Columbia, British Columbia, Canada

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Abstract

Background: *Scrophularia striata* is a perennial plant which is native in all parts of Iran, Turkey, and Azerbaijan. In this study, the total phenol content, antioxidant and larvicidal activities of total extract and different fractions of this plant were evaluated.

Methods: The aerial parts of *S. striata* were collected from Boli village, Illam Province, western Iran in Apr 2013. The total phenol content of total extract and different fractions were evaluated by Folin-Ciocalteu method. Moreover, antioxidant activity was tested by DPPH and FRAPS assays. Larvicidal activity was investigated according to standard method described by WHO.

Results: Ethyl acetate fraction (EF) had the highest content of total phenol (75.9±0.06mg Gallic acid equivalent/g dry extract). Furthermore, among the tested extract, methanol-water fraction (MWF), total methanol extract (TME) and water fraction (WF) showed the highest antioxidant activity in the DPPH assay (IC₅₀= 226.8, 283.66 and 299.4 µg.ml⁻¹, respectively). In FRAP assay MWF and WF and TME had the highest antioxidant activities (664.4±0.002, 565.3±0.003, 519.5±0.003mmol FeII/g dry extract, respectively). Ethyl acetate fraction had maximum larvicidal activity (LC₅₀ 49.1ppm) followed by TME (LC₅₀ 64.26ppm) and hexane fraction (HF) (LC₅₀ 89.69).

Conclusion: *Scrophularia striata* collected from west of Iran illustrated considerable antioxidant and larvicidal effects and further in vitro and in vivo experimental models for investigation would be required.

Keywords: *Scrophularia striata*, Antioxidant activity, Larvicidal activity, *Anopheles stephensi*

Introduction

According to the word malaria report, in 2014, 198 million cases of malaria occurred globally in 2013 and led to 584000 deaths (1-3). In southern parts of Iran including Sistan and Baluchistan, Hormozgan and some parts of Kerman provinces, this disease remains as a vital public health problem (4). Six *Anopheles* vectors exist in this area including *An. culicifacies*, *An. stephensi*, *An. dthali*, *An. fluviatilis*, *An. superpictus* and *An. pulcherrimus* (5). The present proliferation of malaria is due

basically to increasing resistance of mosquitoes to current insecticides (6).

Plant materials have been investigated for their susceptibility in controlling the malaria vector due to their ovicidal, larvicidal and adulticidal activities. The most susceptible stage to attack mosquitoes is larval stage as they are concentrated in smaller areas. Therefore, interrupting mosquito life cycle at larval stage is one of the important methods for controlling malaria transmission (7).

The genus *Scrophularia* (Scrophulariaceae) comprises about 300 known species. The various species of *Scrophularia* genus have been traditionally used in medical conditions including skin inflammatory disease like scabies, tumors, eczema (8), and psoriasis, inflammatory disorders fever, constipation, pharyngitis, neuritis, laryngitis affections (9). Among these species, *S. ningpoensis* Hemsl has been used for treatment of fever laryngitis, pharyngitis, neuritis and constipation swelling in China. Moreover, *S. grossheimi* and *S. nodosa* are used as diuretic agents (10). Recent studies reported antimalarial, antiprotozoal and antimycobacterial activities of *S. cryptophila* (11).

Phytochemical studies indicated the presence of saikosaponins, iridoids and phenylpropanoid glycosides such as angoroside A, angoroside C, angoroside D, acteoside and isoacteoside in *S. scorodonia* L. (8), iridoid glucosides including catalpol, 6-O-methylcatalpol and aucubin, phenylethanoid glycoside like angoroside C in *S. lepidota* (12), resin glycosides including cryptophilic acids A–C in *S. cryptophila* (11).

Many natural compounds have been reported for larvicidal activity. For example, flavonoids namely poncirin, rhoifolin, narigin and marmesin from *Poncirus trifoliata* (13), lupinfolin and rotenone and deguelin from *Derris trifoliata* (14), new iridoid glycosides like 6'-O-rhamnosylharpagide and 6-O-xylosylharpagide-B form *Ajuga remota* (15) and coumarins such as umbelliferone, herniarin, psoralen and xanthotoxin from *Cnidium monnieri* (16) and coumarin derivative pachyrrhizine from *Neorautanenia mitis* (17).

Scrophularia striata Boiss is a perennial plant grown in all parts of Iran as well as Turkey and Azerbaijan. These species have been used to cure different inflammatory diseases such as allergy, rheumatics and chronic inflammatory disorders in Iranian folk medicine (18). So far, phytochemical investigation revealed the presence of cinnamic acid,

flavonoids such as quercetine, isorhamnetin-3-O-rutinoside, and nepitrinandand, and one phenyl propanoid glycoside acteoside 1 in this plant (10). Studied showed the inhibitory effect of *S. striata* extract on matrix metalloproteinases and astrocyte cancer cell line (1321) (18, 19).

Different extracts from the aerial part of *S. canina* were evaluated for the insecticidal activity against larvae and adult females of *Culex pipiens molestus*. The most toxicity was revealed by the petroleum ether extract against second-instar larvae (48h, LC₅₀= 23.5 ppm) and by the hexane extract against fourth-instar larvae (48h, LC₅₀= 23.6ppm) (20). The leaves of *S. nodosa* showed larvicidal activity against *Cx. quinquefasciatus* (75.3% mortality) (21). The ethanol root extract of *S. lepidota* exhibited anti-protozoal activity (IC₅₀ 40.6µg.ml⁻¹) and plasmodial enoyl-ACP reductase (FabI) enzyme inhibitory activity (IC₅₀ 100µg.ml⁻¹), a key enzyme of fatty acid biosynthesis in *Plasmodium falciparum* (12).

In the present study, total extract and different fractions of *S. striata* were investigated for larvicidal properties against main malaria vector, *An. stephensi*. In addition, total phenol content and antioxidant activity (using DPPH and FRAP methods) of this plant were evaluated.

Materials and Methods

Plant material and extraction

The aerial parts of *S. striata* were collected from Boli village, Ilam Province, western Iran in Apr 2013. The voucher specimen was deposited in herbarium of faculty of Pharmacy, Tehran University of Medical Sciences (Herbarium number: 6748-TEH).

The aerial parts (500g) were aired dried in shade and fractionated with hexane, ethyl acetate, methanol, methanol-water (1:1) and water at room temperature, consequently. Total methanol extract was prepared from aerial

parts by percolation method. All the extract and fractions were dried using rotary evaporator to give 2.8g, 6.5g, 60g, 48.2g, 7.7g and 28.7g residues from hexane, ethyl acetate, methanol, methanol-water (1: 1), water fractions and total extract, respectively.

Antioxidant activity

DPPH radical-scavenging activity assay

The antioxidant activity of extracts was investigated by the DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging method according to an established protocol. Sample solutions (1ml) were prepared in methanol at different concentration. Then the solutions added to DPPH methanol solution (2ml, 40 $\mu\text{g}\cdot\text{ml}^{-1}$) and incubated at room temperature for 30 min. The absorbance was measured at 517nm. Vitamin E and butyl hydroxyanisole (BHA) were used as positive controls. IC_{50} values (indicate the concentration of the test samples providing 50% radical scavenging) were calculated from graph-plotted scavenging percentage against extract concentration (22).

Ferric reducing antioxidant potential (FRAP scavenging) assay

The FRAP assay was done by using the method described (23, 24). The FRAP reagent contained 5 ml of a (10 $\text{mmol}\cdot\text{l}^{-1}$) TPTZ (2, 4, 6-tripyridyl- s- triazine) solution in 40 $\text{mmol}\cdot\text{l}^{-1}$ HCl plus 5ml of (20 $\text{mmol}\cdot\text{l}^{-1}$) FeCl_3 and 50ml of (0.3 $\text{mmol}\cdot\text{l}^{-1}$) acetate buffer, pH 3.6 was prepared freshly. Aliquots of each extract (50 μl) were mixed with FRAP reagent (1.5ml). The mixtures were incubated at 37 °C, for 10min. Finally, the absorbance of each sample was measured at 593nm. For construction of calibration curve, five concentrations of $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ (125, 250, 500, 750, 1000 $\text{mmol}\cdot\text{l}^{-1}$) were prepared to used. The antioxidant activities were calculated as the concentration of antioxidants having a reducing ability equivalent for 1 $\text{mmol}\cdot\text{l}^{-1}$ FeSO_4 (22, 25).

Determination of total phenolic contents

Total phenolic contents of extracts were determined by Folin-Ciocalteu method (26). Folin-Ciocalteu (Merk) reagent diluted to tenfold with distilled water. Then 5ml of this solution was added to 1ml of each extract (1 $\text{mg}\cdot\text{ml}^{-1}$) and allowed to stand at room temperature for 10min. A 4ml sodium bicarbonate solution (75 $\text{g}\cdot\text{l}^{-1}$) was added to the mixture. After 30min at room temperature, absorbance was measured at 765nm using a UV spectrophotometer (Pharmacia Biotech). Total phenolic contents were quantified by calibration curve obtained by measuring the absorbance of a known concentration of Gallic acid (GA) standard (20–200 $\text{mg}\cdot\text{l}^{-1}$). The concentrations are expressed as milligrams of Gallic acid equivalents (GA) per gr dry extract (22, 27).

Bioassays and larval mortality

Fourth instar larvae of *An. stephensi* Bandar-Abbas strain was exposed to test concentrations of 20, 40, 80, 160 and 320ppm of each extract (solvent: Ethanol) for 24h according to standard method described by WHO (1981).

Briefly, 1ml of appropriate dilution of each extract with 224ml of water and 25 larvae in 25ml water mixed and total volume was 250 ml (2). For control, only 1ml of ethanol with 224ml of water and 25 larvae in 25ml water mixed and total volume was 250ml. The experiment was repeated four times on different days. The percentage of mortality was reported from the average for the four replicates after 24h exposure period. From the regression line between logarithmic dose and probit mortality, the LC_{50} was measured (28, 29).

The investigation of larvicidal activity has been carried out in the insectarium of Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Results

Ethyl acetate fraction (EF) had the highest content of total phenol (75.9±0.06mg Gallic acid equivalent/g dry extract) (Table 1). Among the tested extract, methanol-water fraction (MWF), total methanol extract (TME) and water fraction (WF) showed highest antioxidant activity in the DPPH assay (IC₅₀= 226.8, 283.66 and 299.4µg.ml⁻¹, respectively). In FRAP assay MWF and WF and TME had the highest anti

oxidant activities (664.4±0.002, 565.3±0.003, 519.5±0.003mmol FeII/g dry extract, respectively).

The total extract and fractions of *S. striata* were effective against *An. stephensi* with LC₅₀ ranged between 49.15 to 1265.96ppm. Ethyl acetate fraction had maximum larvicidal activity (LC₅₀ 49.15ppm) followed by TME (LC₅₀ 64.2623ppm) and HF (LC₅₀ 89.69) (Table 2).

Table 1. Antioxidant activity and total phenolic content of total methanol extract and different fractions from aerial parts of *Scrophularia striata*

	DPPH (IC ₅₀ : µg.ml ⁻¹)	FRAP (mmol FeII/g dry extract)	Total phenol contents (mg GAE/g dry extract)
TME	283.7	519.5±0.003	64.8±0.03
HF	>1000	248.8±0.035	- ^a
EF	505.2	444.28±0.002	75.9±0.06
MF	511.3	513.7±0.026	41.7±0.04
MWF	226.8	664.4±0.002	56.2±0.02
WF	299.4	565.3±0.003	33±0.06

Key to extracts employed: TME: Total methanol extract, HF: hexane fraction, EF: ethyl acetate fraction, MF: methanol fraction, MWF: methanol-water fraction, WF: water fraction. ^a not detected.

Table 2. Probit regression line parameters of total extract and different fractions of *Scrophularia striata* against *Anopheles stephensi*

	Intercept	Slope ± SE	LC ₅₀ (ppm) , 95%C.I.	LC ₉₀ (ppm) , 95%C.I.	λ ² table (df)	P-value
TME	-4.5092	2.4941±0.537	64.2623 (27.2325-131.1766)	209.8017 (109.8552-2827.5650)	24.405*	0.001
HF	-2.4518	1.2556±0.144	89.6911 (72.3973-112.2429)	940.7311 (577.0739-1990.1654)	13.345*	0.001
EF	-2.7150	1.6051±0.156	49.1497 (40.1770-58.6299)	309.0209 (231.3864-462.1751)	13.345*	0.001
MF	-4.6954	1.9658±0.199	244.6640 (203.2745-310.4233)	1097.8066 (747.3601-1921.5333)	13.345	0.001
MWF	-6.5143	2.0977±0.424	1265.9601 (607.7026-2645.3743)	5161.5548 (2523.4135-75009.0794)	13.345	0.001
WF	-7.9868	2.6359±0.195	1071.3972 (949.6278-1205.7662)	3282.1074 (2756.2862-4091.2804)	13.345	0.001

Key to extracts employed: TME: Total methanol extract, HF: hexane fraction, EF: ethyl acetate fraction, MF: methanol fraction, MWF: methanol water fraction, WF: water fraction, LC₅₀±95% C.L= lethal dose cause 50% mortality, 95% confidence interval, LC₉₀±95% C.I= lethal dose cause 90% mortality, 95% confidence interval, (df)= degree of freedom, P= P-value

Discussion

Scrophularia striata is a perennial plant which is native in all parts of Iran as well as Turkey and Azerbaijan. In this study total methanol extract and different fractions from aerial parts of *S. striata* were investigated for antioxidant and larvicidal activities. According to our results, EF and TME had the highest amount of total phenolic compounds. *Scrophularia striata*, as highest amount of total phenolic compounds, was reported in ethanol 70% and ethyl acetate extracts (79.7 and 65.5mg Gallic acid equivalent/g dry extract, respectively). Moreover, water extract of this plant was reported to have better radical scavenging activity (IC_{50} 195 μ g.ml⁻¹) (30).

Furthermore, evaluation of larvicidal activity of *S. striata* revealed that LC_{50} value of total extract and fractions ranged between 49.15 to 1265.96ppm, and the EF had maximum larvicidal activity (LC_{50} 49.15ppm). Essential oils and extracts of different plant species have been investigated for larvicidal activity. For instance, the essential oil prepared from seeds of *Heracleum persicum* had larvicidal effect with LC_{50} value of 104.80ppm. The essential oil of *Cupressus arizonica* revealed significant larvicidal activity against *An. stephensi* with LC_{50} 79.30ppm (31). Moreover, for *Coriandrum sativum* LC_{50} value of 120.95ppm and for *Cymbopogon olivieri* 321.90ppm were reported (5), which their activities were low when compared with EF and TME of *S. striata*. The LC_{50} of essential oils from *Tagetes minuta* and *Foeniculum vulgare* were 1.05 and 20.10ppm, respectively (5), these plants were more effective than *S. striata*.

The methanol extract of *T. minuta* had better LC_{50} value (2.5ppm) followed by methanol and aqueous extract of *Nelumbo nucifera* (8.89 and 11.82ppm, respectively) and methanol extract of *Cassia fistula* (17.97ppm) when compared with the extract and fractions of *S. striata* (32-34).

Different extracts of fruits and leaves of *Centratherum anthelminticum* were tested for larvicidal activity against *An. stephensi* and the petroleum ether extract of fruits (LC_{50} 162.60) were more toxic than that of leaf extract (LC_{50} 522.94) (7). Petroleum ether extract from leaves of *Artemisia annua* had larvicidal activity with LC_{50} value of 263 ppm (7). The leaf and seed methanol extracts of *Clitoria ternatea* showed dose-dependent larvicidal activity against *An. stephensi* with LC_{50} values of 555.6 and 116.8ppm, respectively (35). The leaves petroleum ether extract of *Gymnema sylvestre* exhibited the highest mortality in the concentration of 1000ppm against the larvae of *An. subpictus* (LC_{50} 166.28 ppm) (36). Moreover, the leave methanol extract of *Calotropis gigantea* showed larvicidal activity (LC_{50} 121.69ppm) (37). Larvicidal activity of acetone extract of *Millingtonia hortensis* (LC_{50} 223.9ppm) and ethanol extract was from peels of *Citrus sinensis* (LC_{50} 291.69ppm) (35, 38).

Extracts of *A. annua*, *C. anthelminticum* (7), *Citrus sinensis*, *C. ternatea* (35), *C. gigantea* (37), *G. sylvestre* (36) and *M. hortensis* (38) were less active than EF and TME of *S. striata*.

Conclusion

EF and TME of *S. striata* possesses better larvicidal activity against *An. stephensi* than other fractions. Moreover, antioxidant activity of MWF, WF, and TME were higher than other fractions in both DPPH and FRAP assays. Finally, complete phytochemical investigation is suggested to reveal most effective compound in this native species.

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The authors declare that there is no conflict of interest.

References

- Olepu S, Suryadevara PK, Rivas K, Yokoyama K, Verlinde CL, Chakrabarti D, Van Voorhis WC, Gelb MH (2008) 2-Oxo-tetrahydro-1, 8-naphthyridines as selective inhibitors of malarial protein farnesyltransferase and as anti-malarials. *Bioorg Med Chem Lett*. 18(2): 494–497.
- Ghanbarzadeh S, Ghasemi S, Shayanfar A, Ebrahimi-Najafabadi H (2015) 2D-QSAR study of some 2,5-diaminobenzophenone farnesyltransferase inhibitors by different chemometric methods. *EXCLI*. 14: 484–495.
- World malaria report (2014) WHO Press GMP, World Health Organization. Geneva, Switzerland. World malaria report, WHO Press, Global Malaria Program, World Health Organization. Available at: http://www.who.int/malaria/publications/world_malaria_report_2014/en/.
- Hanafi-Bojd AA, Azari-Hamidian S, Vatan-doost H, Zabihollah C (2011) Spatio-temporal distribution of malaria vectors (Diptera: Culicidae) across different climatic zones of Iran. *Asian Pac J Trop Dis*. 4(6): 498–504.
- Mahnaz K, Alireza F, Hassan V, Mahdi S, Reza AM, Abbas H (2012) Larvicidal activity of essential oil and methanol extract of *Nepeta menthoides* against malaria vector *Anopheles stephensi*. *Asian Pac J Trop Dis*. 5(12): 962–965.
- Zoubiri S, Baaliouamer A (2014) Potentiality of plants as source of insecticide principles. *J Saudi Chem Soc*. 18(6): 925–938.
- Srivastava A, Bartarya R, Tonk S, Srivastava S, Kumari KM (2008) Larvicidal activity of an indigenous plant, *Centratherrum anthelminticum*. *J Environ Biol*. 29(5): 669–672.
- Díaz AMa, Abad Ma J, Fernández L, Silván AM, De Santos J, Bermejo P (2004) Phenylpropanoid glycosides from *Scrophularia scorodonia*: in vitro anti-inflammatory activity. *Life Sci*. 74(20): 2515–2526.
- Salavati P, Ramezani M, Monsef-Esfahani HR, Hajiagha R, Parsa M, Tavajohi S, Seyed Nasser Ostad (2013) Neuroprotective effect of total and sequential extract of *Scrophularia striata* Boiss. in rat cerebellar granule neurons following glutamate-induced neurotoxicity: an in-vitro study. *Iran J Pharm Res*. 12(2): 389–394.
- Monsef-Esfahani HR, Hajiaghae R, Shahverdi AR, Khorramizadeh MR, Amini M (2010) Flavonoids, cinnamic acid and phenyl propanoid from aerial parts of *Scrophularia striata*. *Pharm Biol*. 48(3): 333–336.
- Tasdemir D, Brun R, Franzblau SG, Sezgin Y, Çalı I (2008) Evaluation of anti-protozoal and antimycobacterial activities of the resin glycosides and the other metabolites of *Scrophularia cryptophila*. *Phytomedicine*. 15(3): 209–215.
- Tasdemir D, Güner ND, Perozzo R, Brun R, Dönmez AA, Calı I, Rüedi P (2005) Anti-protozoal and plasmodial FabI enzyme inhibiting metabolites of *Scrophularia lepidota* roots. *Phytochemistry*. 66(3): 355–362.
- Rajkumar S, Jebanesan A (2008) Bioactivity of flavonoid compounds from *Poncirus trifoliata* L. (Family: Rutaceae) against the dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). *Parasitol Res*. 104(1): 19–25.
- Yenesew A, Twinomuhwezi H, Kabaru JM,

- Akala HM, Kiremire BT, Heydenreich M, Martin G. Peter, Fredrick L. Eyase, Norman C. Waters, Douglas S. Walsh (2009) Antiplasmodial and larvicidal flavonoids from *Derris trifoliata*. Bull Chem Soc Ethiop. 23(3): 409–414.
15. Manguro LOA, Lemmen P, Hao P (2011) Iridoid Glycosides from Underground Parts of *Ajuga remota*. Rec Nat Prod. 5(3): 147–157.
 16. Wang Z, Kim JR, Wang M, Shu S, Ahn YJ (2012) Larvicidal activity of *Cnidium monnieri* fruit coumarins and structurally related compounds against insecticide susceptible and insecticide-resistant *Culex pipiens pallens* and *Aedes aegypti*. Pest Manag Sci. 68(7): 1041–1047.
 17. Joseph C, Ndoile M, Malima R, Nkonya M (2004) Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpan from *Neorautanenia mitis*. Trans R Soc Trop Med Hyg. 98(8): 451–455.
 18. Azadmehr A, Afshari A, Baradaran B, Hajiaghachee R, Rezazadeh S, Monsef-Esfahani H (2009) Suppression of nitric oxide production in activated murine peritoneal macrophages in vitro and ex vivo by *Scrophularia striata* ethanolic extract. J ethnopharmacol. 124(1): 166–169.
 19. Rezaie-Tavirani M, Mortazavi SA, Barzegar M, Moghadamnia SH, Rezaee MB (2010) Study of anti cancer property of *Scrophularia striata* extract on the human astrocytoma cell line (1321). Iranian J Pharm Res. 9(4): 403.
 20. Germinara GS, Frontera AM, De Cristofaro A, Rotundo G (2011) Insecticidal activity of different extracts from *Scrophularia canina* L. against *Culex pipiens molestus* Forskal (Diptera, Culicidae). J Environ Sci Health, Part B. 46(6): 473–479.
 21. Pavela R (2009) Larvicidal effects of some Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitol Res. 105(3): 887.
 22. Yousfbeyk F, Esmaili T, Pashna Z, Hozori Z, Ghohari A, Ostad SN, GhR Amin (2014a) Antioxidant Activity, Total Phenol and Total Anthocyanin Contents of *Cornus sanguinea* L subsp *australis*. (CA Mey.) Jáv. J Med Plant. 1(49): 69–74.
 23. Moradi-Afrapoli F, Asghari B, Saeidnia S, Ajani Y, Mirjani M, Malmir M, Dolatabadi Bazaz R, Hadjiakhoondi A, Salehi P, Hamburger M, Yassa N (2012) In vitro α -glucosidase inhibitory activity of phenolic constituents from aerial parts of *Polygonum hyrcanicum*. Daru. 20(1): 37.
 24. Benzie IF, Strain J (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem. 239(1): 70–76.
 25. Yousefbeyk F, Gohari AR, Hashemighaderijani Z, Ostad SN, Sourmaghi MHS, Amini M, Golfakhrabadi F, Jamalifar H, Amin G (2014b) Bioactive Terpenoids and Flavonoids from *Daucus littoralis* Smith subsp. *Hyrcanicus* Rech. f, an Endemic Species of Iran. Daru. 22(1): 12.
 26. Miliuskas G, Venskutonis P, Van Beek T (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 85(2): 231–237.
 27. Golfakhrabadi F, Ardekani MRS, Saeidnia S, Yousefbeyk F, Jamalifar H, Ramezani N, Akbarzadeh T, Khanavi M (2016) Phytochemical analysis, antimicrobial, antioxidant activities and total phenols of *Ferulago carduchorum* in two vegetative stages (flower and fruit). Pak J Pharm Sci. 29(2): 623–628.
 28. Dharmagadda V, Naik S, Mittal P, Vasu-

- devan P (2005) Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. *Bioresour Technol.* 96(11): 1235–1240.
29. Golfakhrabadi F, Khanavi M, Ostad SN, Saeidnia S, Vatandoost H, Abai MR, Hafizi M, Yousefbeyk F, Rad YR, Baghenegadian A, Ardekani MR (2014) Biological Activities and Composition of *Ferulago carduchorum* Essential Oil. *J Arthropod Borne Dis.* 9(1): 104–115.
30. Mahboubi M, Kazempour N, Boland Nazar AR (2013) Total phenolic, total flavonoids, antioxidant and antimicrobial activities of *Scrophularia striata* Boiss Extracts. *Jundishapur J Nat Pharm Prod.* 8(1): 15–19.
31. Sedaghat MM, Dehkordi AS, Khanavi M, Abai MR, Mohtarami F, Vatandoost H (2011) Chemical composition and larvicidal activity of essential oil of *Cupressus arizonica* EL Greene against malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Pharmacogn Res.* 3(2): 135.
32. Hadjiakhoondi A, Vatandoost H, Khanavi M, Abaee MR, Karami M (2005) Biochemical investigation of different extracts and larvicidal activity of *Tagetes minuta* L. on *Anopheles stephensi* larvae. *Iranian J Pharm Sci.* 1(2): 81–84.
33. Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C (2011) Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. *Parasitol Res.* 108(3): 693–702.
34. Govindarajan M, Jebanesan A, Pushpanathan T (2008) Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitol Res.* 102(2): 289–292.
35. Murugan K, Kumar PM, Kovendan K, Amerasan D, Subramaniam J, Hwang JS (2012) Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res.* 111(4): 1757–1769.
36. Khanna VG, Kannabiran K, Rajakumar G, Rahuman AA, Santhoshkumar T (2011) Biolarvicidal compound gym-nemagenol isolated from leaf extract of miracle fruit plant, *Gymnema sylvestre* (Retz) Schult against malaria and filariasis vectors. *Parasitol Res.* 109(5): 1373–1386.
37. Kovendan K, Murugan K, Kumar KP, Panneerselvam C, Kumar PM, Amerasan D, Subramaniam J, Vincent S (2012) Mosquitocidal properties of *Calotropis gigantea* (Family: Asclepiadaceae) leaf extract and bacterial insecticide, *Bacillus thuringiensis*, against the mosquito vectors. *Parasitol Res.* 111(2): 531–544.
38. Kaushik R, Saini P (2008) Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. *J Vector Dis.* 45(1): 66.