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Full Length Research Paper

Chemical constituents, larvicidal effects and antioxidant activity of petroleum ether extract from seeds of *Coriandrum sativum* L.

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The seeds of Korean *Coriandrum sativum* were extracted with petroleum ether and the chemical composition, larvicidal effects and antioxidant activity were studied. The analyses were conducted by gas chromatography and mass spectroscopy (GC-MS), which revealed the chemical composition of the petroleum ether extract of the seeds of *C. sativum*. Thirty seven components, representing 100% of the total extract were identified. The extract was dominated by linalool, a major component. The major components of petroleum ether extract are linalool (53.79%), 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)- (10.41%), hotrienol (7.82%), 3,7-Dimethylocta-1, 7-dien-3,6-diol (7.54%), 3,7-Octadiene-2,6-diol, 2,6-dimethyl- (5.87%), 7-Oxabicyclo[4.1.0]heptanes, 1-methyl-4-(2-methyloxiranyl)- (4.73), and 5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde (1.45%). The petroleum ether extract of the seeds had significant toxic effects against the larvae of *Aedes aegypti* with an LC_{50} value of 20.57 ppm and an LC_{90} value of 47.35 ppm. The petroleum ether extract from the *C. sativum* seeds was investigated for scavenging of the diphenylpicrylhydrazyl (DPPH) radical activity and the reducing power and the results demonstrate that the petroleum ether extract from the *C. sativum* seeds has potential as a natural antioxidant and thus inhibit unwanted oxidation process. The aforementioned data indicates that the major compounds may play an important role in the toxicity and also act as natural antioxidant.

Key words: *Aedes aegypti*, antioxidant activity, chemical composition, *Coriandrum sativum*, larvicidal activity.

INTRODUCTION

Blood feeding female mosquitoes are responsible for the intolerable biting nuisance and transmission of a large number of diseases, such as malaria, yellow fever, dengue, filariasis, chikengunya, and encephalitis. They cause serious health problems to humans and present obstacles to the socioeconomic development of developing countries, particularly in the tropical region (Senthilkumar et al., 2008). Synthetic insecticides create a number of ecological problems, such as the development of resistant insect strains, ecological imbalance and harm to mammals. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability (Prabakar and Jebanesan, 2004). *Aedes aegypti* is one of the mosquito species responsible for the transmission of both dengue fever and dengue hemorrhagic fever. The continuous application of the organophosphates such as temephos and fenthion and insect growth regulators such as diflubenzuron and methoprene, are generally used for the control of mosquito larvae (Yang et al., 2002). Although these organophosphates are effective, their continuous use has disrupted natural biological control systems and has led to outbreaks of insect species, which sometimes resulted in the widespread development of resistance, had undesirable effects on non-target organism and fostered environmental and human health concerns (Yang et al., 2002). These problems have highlighted the need for the development of new strategies for selective mosquito larval control. In the search for environmentally safe and relatively inexpensive methods to control mosquitoes, plant extracts have received much interest as potential bioactive agents against the mosquito larvae.

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Most mosquito control programs target the larval stage at their breeding sites with larvicides (Knio et al., 2008), since adulticides may reduce the adult population only temporarily (El Hag et al., 2001). Therefore, a more efficient approach to reduce the population of mosquitoes would be to target the larvae. In addition, discovery of the antioxidant activity has been reported in various essential oils which include rosemary, lavender (Lee and Shibamoto, 2002) and oregano (Rocha-Guzman et al., 2007). Consequently, antioxidant activity of essential oils has gained considerable attention among researchers. Since the benefits derived from essential oils have created renewed demand in their use by the common public, explorations of their antioxidant activities for their possible beneficial use in the prevention of oxidative damage is in order. Essential oils are natural volatile substances found in a variety of plants. It is well-known that plant-derived natural products are extensively used as biologically active compounds. Among them, essential oils were the first preservatives used by man, originally in their natural state within plant tissues and then as oils obtained by water distillation. Essential oils are composed of isoprenoid compounds, mainly mono- and sesquiterpenes are the carriers of the smell found in the aromatic plants (Cheng et al., 2003). During our search for new types of natural products possessing an immunotoxicity activity from wild and cultivated plants, we investigated the chemical constituents from the petroleum ether extract of Korean *Coriandrum sativum* seeds.

Coriander (*C. sativum* L.) is an annual herbaceous plant originally from the Mediterranean and Middle Eastern regions, cultivated for its culinary, aromatic and medicinal uses (Diederichsen, 1996). This plant is widely distributed and mainly cultivated for its seeds, which are used for different purposes, such as food, drugs, cosmetics and perfumery. Coriander fruit is widely studied for its chemical constituents and it possesses an essential oil content of up to 1%, where linalool is the main component. The essential oil and various extracts from coriander have been shown to possess antibacterial, antioxidant, antidiabetic, anticancerous and antimutagenic activities (Msaada et al., 2007). The composition of the volatile oil isolated from coriander seeds has been reported from different geographical regions (Zoubiri and Baaliouamer, 2010).

The aim of this study was to investigate the chemical composition, larvicidal properties, and antioxidant activity of the petroleum ether extract of coriander seeds grown in the Korean region. To the best of our knowledge, this is the first report on the chemical constituents, larvicidal properties, and antioxidant activity from petroleum ether extracts of coriander seeds grown in the Korean region.

MATERIALS AND METHODS

Plant material

The *C. sativum* seeds were procured from Danong Company, Namyanju-si, Gyeonggi-do, South Korea in October 2010. A voucher specimen was deposited in the Department of Applied Life Science, Konkuk University, Seoul, South Korea.

Petroleum ether extract

The crushed seeds of *C. sativum* (200 g) were immersed in petroleum ether (500 ml, 35 to 60°C) overnight at room temperature, and then the supernatant was concentrated under vacuum to yield 0.5 g of the extract, of which a small sample was dissolved in hexane (spectroscopic grade), after which the sample was filtered for the GC-MS analysis.

GC-MS analysis of petroleum ether extract

Samples of petroleum ether extract were diluted in hexane (spectroscopic grade) and analyzed in a Finnigan focus GC/Finnigan focus DSQ MS system (Thermo Co., Germany) apparatus equipped with Vesteckrtx-50 capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The temperature program used for the analysis was as follows: The temperature of the injector was set at 300°C. The initial temperature was set at 80°C and held for 5 min, set at 5.0°C/min to reach 280°C, and held for 10 min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. One microliter of the sample (diluted 1:10 with hexane) was injected with a split ratio of 1:100. The percent composition of the petroleum ether extract was calculated by comparing the areas of the GC peaks. The temperature of the ion source and of the injector was set at 200 and 210°C, respectively. The interface was kept at 280°C and the mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The sector mass analyzer was set to scan from 50 to 500 amu every 0.5 s. The different components of the petroleum ether extract were identified by comparing the mass spectra of each peak with those of authentic samples found in a library of mass spectra (The Wiley Registry of Mass Spectral Data, 7th ed.).

Larvicidal activity

The F^{21} laboratory strain of *Aedes aegypti* was obtained in 2008 from the National Institute of Health, Seoul, South Korea. Adult female mosquitoes were maintained on a 10% sucrose solution and anaesthetized mice were used for blood feeding the mosquitoes. Larvae were reared in plastic trays and fed a diet of chicken chow and yeast (8:2). Mosquitoes were maintained at 27 ± 2°C, 70 ± 5% relative humidity, and a photoperiod of 16L:8D. The larvicidal activity was analyzed according to the standard procedures recommended by the World Health Organization (WHO, 1997). The petroleum ether extract was dissolved in acetone at a concentration of 1.0 mg/ml and different concentrations were prepared (0, 6.25, 12.5, 25 and 50 ppm) using distilled water. Twenty larvae at the fourth stage were used in the larvicidal assay and five replicates were maintained for each concentration. The larval mortality was calculated after 24 h of exposure. The lethal concentrations LC_{50} and LC_{90} were calculated.

DPPH radical scavenging activity

The antioxidant activity of the petroleum ether extract of the seeds of *C. sativum* was based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, and was determined by the method of Katerere and Eloff, 2005. The different concentrations (0, 100, 200, 300, 400 and 500 µg) of the tested

samples (0.02 ml; extracts and BHT) were taken in different test tubes with 4 ml of a 0.006% MeOH solution of DPPH. Water (0.02 ml) in place of the extract was used as control. Absorbance at 517 nm was determined after 30 min incubation at 37°C. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula: % Radical scavenging activity = $[(A_0 – A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

Reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (Konig et al., 2004). Different extracts of concentration (100, 200, 300, 400 and 500 µg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. All analysis were run in triplicate and averaged.

Statistical analysis

The data for the larvicidal activity were collected from five replicates for each concentration. The lethal concentrations LC_{50} and LC_{90}, the 95% confidence intervals, and the upper and lower confidence levels were calculated using probit analysis (SAS 9.2 version software). For the antioxidant activity, all the analysis were run in triplicate and averaged.

RESULTS

The petroleum ether extract of *C. sativum* seeds was analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty seven components, representing 100% of the total extract were identified. The extract was dominated by linalool, a major component. The major components of petroleum ether extract are linalool (53.79%), 2,6-octadien-1-ol, 3,7-dimethyl-, acetate, (E)- (10.41%), hotrienol (7.82%), 3,7-dimethylocta-1, 7-dien-3,6-diol (7.54%), 3,7-octadiene-2,6-diol, 2,6-dimethyl- (5.87%), 7-oxabicyclo[4.1.0]heptanes, 1-methyl-4-(2-methyloxiranyl)- (4.73), and 5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde (1.45%). Table 1 summarizes the current composition on the analysis of the petroleum ether extract from seeds of *C. sativum*. This is the first report on the chemical constituents from the petroleum ether extract of the seeds of *C. sativum*. The larvicidal effects of the petroleum ether extract from seeds of coriander were studied. The petroleum ether extract had significant toxic effects against the larvae of *A. aegypti* with an IC_{50} value of 20.57 ppm and an IC_{90} value of 47.35 ppm. The petroleum ether extract from the seeds of *C. sativum* exhibited a concentration-dependent immunotoxicity activity. At the concentration of 6.25 ppm, the mortality rate was 10.0% and reached the maximum of 98.0% mortality at the concentration of 50.0 ppm (Table 2). The control substance caused no mortality for the larvae. Also, phellandrene (≥ 95.0%), geranial (≥ 95.0%) and citronellol (≥ 95.0%) were tested against the F^{21} laboratory strain of *A. aegypti*. The current petroleum ether extract from seeds of coriander was tested for the first time against *A. aegypti*.

The free radical scavenging activity of the petroleum ether extract from the seeds of coriander was tested through DPPH method (Katerere and Eloff, 2005) and the results were compared with BHT. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The petroleum ether extract was able to reduce the stable radical DPPH to the yellow colored DPPH. The petroleum ether extract exhibited a concentration dependent antiradical activity by inhibiting DPPH radical (Figure 1). The IC_{50} value of the petroleum ether extract was 192.56 µg/ml. Of the different concentrations tested, 500 µg/ml exhibited the highest radical scavenging activity of 86.43%. The DPPH activity of BHT showed higher degree of free radical-scavenging activity than that of the extract at very low concentration points. The DPPH activity of BHT exhibited 92.04% at 50 µg/ml concentration with an IC_{50} value of 27.16 µg/ml (data not shown).

Antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power (Tanaka et al., 1988). As shown in Figure 2, the reducing power of the petroleum ether extracts from seeds of coriander increased with increase in the concentration from 100 to 500 µg. The activity of BHT had higher activity than the test extracts (data not shown).

DISCUSSION

In general, plant essential oils have been recognized as an important natural source for insecticides (Gbolade et al., 2000; Adebayo et al., 1999). The differences in the toxicity of essential oils against different mosquito species are well known (Sukumar et al., 1991) and are due to qualitative and quantitative variations of the components. Recently, the clinical use of essential oils has expanded worldwide also including therapy against various kinds of inflammatory diseases, such as allergy, immunotoxicity, rheumatism and arthritis. These activities have mainly been recognized through clinical experience, but there have been relatively little scientific studies on biological actions of these natural essential oil extracts. For instance, Chung et al. (2011) reported that *Allium victorialis* L. var. platyphyllum growing in the South Korea contained allyl methyl disulfide (24.36%), dimethyl trisulfide (11.78%), allyl cis-1-propenyl disulfide (9.17%), allyl methyl trisulfide (4.13%) and dipropyl trisulfide (7.22%) as major components and the essential oils from the *A. victorialis* L. var. platyphyllum showed good immunotoxicity activity. Park et al. (2010) described

Table 1. Chemical constituents from the petroleum ether extract of *Coriandrum sativum* seeds.

S/No	RT	Components	Peak area (%)
1	3.81	Permetrinic acid methylamide	0.07
2	4.12	1,5,9,13-Tetrathia-3, 11-cyclohexadecaediol	0.07
3	4.52	Dichloromethyl ethyl sulfone	0.10
4	4.72	3,5-Cyclohexadiene-1,2-dione, 5-(hydroxymethyl)-3-methoxy-	0.05
5	4.95	1,6-Octadien-3-ol, 3,7-dimethyl	0.04
6	5.18	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	0.06
7	5.27	Pyrrolidine, 1-(1,6-dioxooctadecyl)-	0.03
8	5.43	Octanal	0.05
9	5.53	5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	1.45
10	5.71	3-Carene	0.97
11	5.98	Linalool oxide	1.16
12	6.17	Linalool	53.79
13	6.53	Ethyl linalool	0.03
14	6.67	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-, [1R-(1a,2a,5a)]-	0.14
15	6.89	1,6-Octadiene, 3-ethoxy- 3,7-dimethyl-	0.63
16	7.04	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	0.17
17	7.17	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	5.87
18	7.39	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	0.35
19	7.52	Geraniol	1.00
20	7.63	1,2-15,16-Diepoxyhexadecane	0.45
21	7.99	3,7-Dimethylocta-1, 7-dien-3,6-diol	7.54
22	8.19	Myrtenyl acetate	0.73
23	8.28	Geranyl vinyl ether	0.25
24	8.50	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	10.41
25	8.77	Hotrienol	7.82
26	8.97	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	0.23
27	9.23	7-Oxabicyclo[4.1.0]heptanes, 1-methyl-4-(2-methyloxiranyl)-	4.73
28	9.56	Cholestan-3-ol, 2-methylene-, (3a,5a)-	0.06
29	10.07	2-Octen-1-ol, 3,7-dimethyl-, isobutyrate, (Z)-	0.12
30	10.18	Cis-ρ-Mentha-2,8-dien-1-ol	0.45
31	11.16	2,6,10-Dodecatrienal	0.49
32	12.00	2,2-Dideutero octadecanal	0.10
33	12.51	Tetradecanoic acid	0.09
34	14.69	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methy]cyclopropyl]methyl]-methyl ester	0.04
35	15.37	1,2,3,5,6,7-Hexahydro-inden-4-one	0.11
36	15.67	5-Hydroxy-1,3,4-trimethoxy-7-methyl-6-propargynaphthalene	0.29
37	18.28	9-Octadecenoic acid (Z)-, methyl ester	0.06

composition and immunotoxicity activity of the major essential oil of *Angelica purpuraefolia* Chung against *A. aegypti*. Chung et al. (2011) reported the composition and immunotoxicity activity of the essential oil from leaves and stems of the Korean *C. sativum*.

The free radical-scavenging activity of the petroleum ether extract was tested through di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) method (Katerere and Eloff, 2005). DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic $DPPH^{-}$ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The petroleum ether extract was able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. It has been found that

Table 2. Larvicidal activity of the petroleum ether extract from seeds of *Coriander sativum* against *Aedes aegypti.*

Concentration (ppm)	Percentage of mortality ± SE	LC_{50} (ppm)	LC_{90} (ppm)	95% confidence interval for LC_{50}		95% confidence interval for LC_{90}	
				LCL[a]	UCL[b]	LCL[a]	UCL[b]
6.25	10.0 ± 2.2	20.57	47.35	18.80	22.61	42.87	62.34
12.50	22.0 ± 1.8						
18.75	36.0 ± 2.4						
25.0	54.0 ± 2.6						
50.0	98.0 ± 1.6						

[a]Lower confidence level; [b]Upper confidence level.

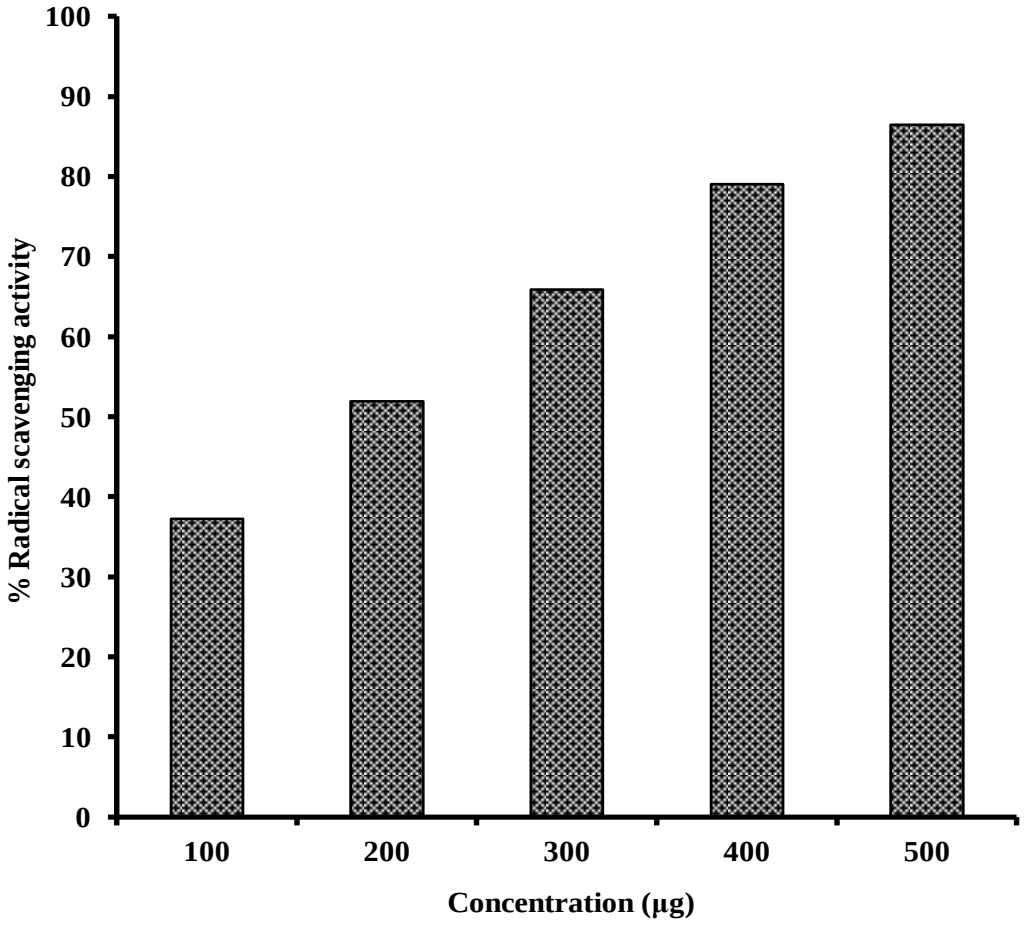

Figure 1. Antioxidant activity of the petroleum ether extract of *Coriandrum sativum* seeds at different concentrations levels as measured by DPPH radical scavenging activity.

cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g., hydroquinone, pyrogallol, gallic acid), and aromatic amines (e.g., *p*-phenylene diamine, *p*-aminophenol), reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Blois, 1958). In the present study, petroleum ether extract from *C. sativum* seeds also showed a remarkable antioxidant activity, one of the possible mechanisms is polyphenolic-associated compounds (formation of non-extractable complex between high molecular weight phenolics and polysaccharides). Those kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing

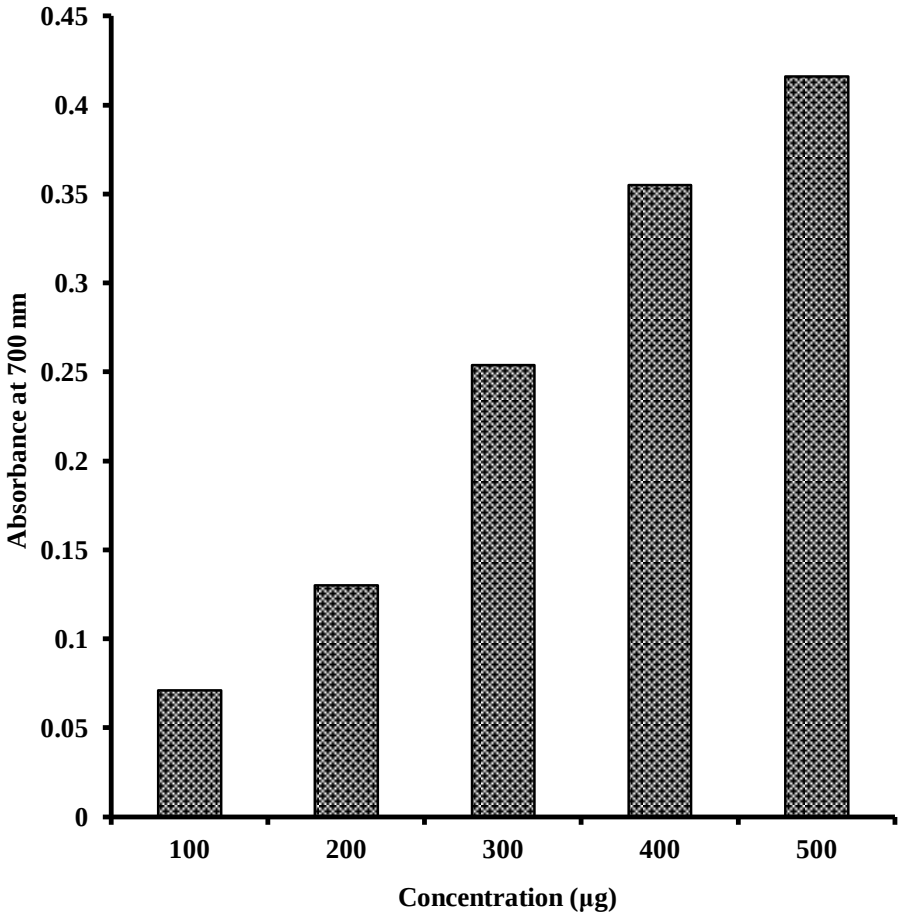

Figure 2. Reducing power of the petroleum ether extract of *Coriandrum sativum* seeds at different concentrations levels.

free radicals, quenching singlet and triple oxygen or decomposing peroxide. The extract exhibited a concentration-dependent antiradical activity by inhibiting DPPH radical, the activity increased with increasing concentration. The petroleum ether extract concentration of 500 µg exhibited good free radical scavenging activities, that is, 86.43%. The higher concentration of essential oil from thyme species exhibited highest antioxidant activity (Anahi Dandlen et al., 2010). Nagella et al. (2011) also reported that higher concentration of oil exhibited highest antiradical activity from leaves of *Apium graveolens*.

Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. Okuda et al. (1983) reported that the reducing power of tannins from medicinal plants prevent injury by inhibiting formation of lipid peroxides. In the present study, the petroleum ether extract exhibited concentration dependent activity, that is, the reducing power of the extract increased with increase in the concentration. The activity of BHT was higher than the test sample at all concentration points. This is in line with the observations of several other workers wherein the reducing power of BHA (Otkay et al., 2003), BHT, and α-tocopherol (Chung et al., 2002) was higher than the food samples. In the present study, though the petroleum ether extract exhibited a moderate reducing power, they did have an activity that reveals that the extract from coriander seeds are electron donors and can react with free radicals and convert them to stable products thus terminating the free radical chain reactions.

Conclusion

In conclusion**,** the findings of the present study indicate that the petroleum ether extract from the seeds of *C. sativum* could be used as a potential natural larvicidal agent and also as antioxidant.

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