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Research Article

Antifungal activity and chemical composition of Iranian medicinal herbs against fish pathogenic fungus, *Saprolegnia parasitica*

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Abstract

This study aimed to identify the chemical composition of essential oil of some Iranian medicinal plants and their antifungal activity against *Saprolegnia parasitica* in comparison to formalin as positive control under *in vitro* conditions. The essential oils of *Eryngium campestre, Pimpinella affinis, Mentha piperita, Achillea wilhelmsii* and *Cuminum cyminum* were analyzed for their activity by disk diffusion, Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) methods. Also, the oil constituents of investigated plants were analyzed by gas chromatography/mass spectrometry (GC/MS). The major constituent of the surveyed essential oils was bornyl acetate (17.9%) in *E. campestre*, Pregeijene (27.3%) in *P. affinis*, menthol (48.5%) in *M. piperita*, 1,8-cineol (25.2%) in *A. wilhelmsii*, and α-Pinene (29.1%) in *C. cyminum*. The MIC values for the surveyed essential oil were was 0.5 µg ml^{-1} for *C. cyminum* followed by *M piperita* and *E. campestre* both having 1 µg ml^{-1}, for *P. affinis* 2 µg ml^{-1} and was 4 µg/mL for *A. wilhelmsii*. The MFC for the mentioned essential oil were with 0.5 µg ml^{-1} again lowest for *C. cyminum*, followed by *M. piperita* and *E. campestre* with 2 µg ml^{-1}, for *P. affinis* MFC was 4 µg ml^{-1} while it was with 8 µg ml^{-1} highest for essential oils from *A. wilhelmsii*. The results indicate that the essential oils of *C. cyminun*, *E. campestre* and *M. piperita* could be potential candidates for new plant based antifungal components in aquaculture against *S. parasitica.*

Keywords: Iranian medicinal plants, Chemical composition, Antifungal activity, *Saprolegnia parasitica*

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Introduction

Saprolegnia parasitica is a pathogenic oomycete that can cause saprolegniasis. Freshwater fish, in particular salmon and trout species and their eggs are vulnerable to infection, which is characterized by cotton-like grayish mycelial growth on the surface of the fish (Ghiasi *et al.*, 2010). It is estimated that 10% of hatched salmon in aquaculture facilities die due to *S. parasitica* infection (Robertson *et al*., 2009). Besides losses in the salmonid industry due to infected eggs and fish (e.g. in Scotland, Scandinavia, Chile, Japan, Canada, Iran and the USA), estimated at tens of millions pound of fish (Woo and Bruno, 2011; Mohammadi *et al*., 2014; Noga, 2016), *Saprolegnia* spp. also cause the so called "winter kill" in channel catfish (Bly *et al*., 1992), estimated to cause world economic losses at around £25 million (Van West, 2006). Malachite green is the common effective drug to control saprolegniasis, but it was banned due to its potential carcinogenic, mutagenic and teratogenic effects (Meyer and Jorgenson, 1983). The currently mostly applied drugs and disinfectants are formaldehyde, peracetic acid and hydrogen peroxide and different commercial products with these substances as ingredients. However, it is supposed that formaldehyde as disinfectant will be prohibited soon (Van den Berg *et al*., 2013).

Antifungal resistance, increasing medication dosage and drug side effects have led to increased research in alternative treatments against *Saprolegnia* spp., such as medicinal herbs, in order to minimize the mentioned side effects and to reduce the price of antifungal components (Firouzbakhsh *et al*., 2014; Mohammadi *et al*., 2014).

The antifungal activity of some medicinal plants on some fish and shrimp fungal pathogens such as *Candida albicans*, *Aphanomyces invadans*, *Saprolegnia australis*, *S. parasitica* and *Fusarium solani* were surveyed in several studies (Muniruzzaman and Chowdhury, 2006; Pirbalouti *et al*., 2009; Caruana *et al*., 2012; Mousavi *et al*., 2012; Sharif Rohani *et al*., 2013). Some researchers have confirmed antifungal effects of *Origanum onites*, *Thymbra spicata* (Gormez and Diler, 2012), *Thymus daenensis*, *T. khuzestanicum*, *Tanacetum parthenium*, *Mentha longifolia* (Pirbalouti *et al*., 2009), *Zataria multiflora*, *Eucalyptus camaldolensis* (Khosravi *et al*., 2012) and *Citrullus colocynthis* (Azizi *et al*., 2012) on *S. parasitica* under *in vitro* conditions. *Terminalia catappa* extract showed highest antimicrobial effect of MIC and MBC, respectively 25 and 12.5 mg mL^{-1} (Kanchan, et al., 2018).

E. campestre as native plant in Mazandaran province is an edible flowering plant belonging to the family Apiaceae (Thiem *et al*., 2010). Essential oil of *E. campestre* includes phenylpropanoids, eugenol, methylisoeugenol and benzaldehyde with antibacterial, antifungal and antioxidant activity (Thiem *et al*., 2010). Species of the genus *Eryngium* have been used as a diuretic and against

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pertussis, urinary infections and renal calculus in traditional medicine (Thiem *et al*., 2010).

Pimpinella affinis is another member of the family *Apiaceae*. This biennial herb grows up to 110 cm and is native in the center and North of Iran (Gulcin *et al*., 2003). This herb is useful as carminative agent, appetizer, diuretic, antispasmodic drug, antimicrobial, sedative and lactation medication in traditional medicine. It has also been shown to possess antioxidant and antifungal properties (Tabanca *et al*., 2007).

Mentha piperita (peppermint) is a perennial herb of the family Lamiaceae. This species is originally native in the Mediterranean region but it has been commercially cultivated in temperate locations such as India, North America, China and Iran (Iscan *et al*., 2002). It is mainly used for its antispasmodic, anti-inflammatory, antiemetic, carminative, anticancer, antibacterial and anti-fungal properties (Talpur, 2014). The primary chemical compounds of *M. pulegium*, another of the 20 *Mentha* species occurring worldwide, have been identified as piperitenone, piperitone and □-terpineol (Mahboubi and Haghi, 2008).

Achillea wilhelmsii is a flowering plant in the family Asteraceae. Up to date 85 species of the genus have been identified and 7 species are exclusively native to Iran (Javidnia *et al*., 2004). Flowers of the closely related *A. millefoium* contain chamazulene which has anti-inflammatory and anti-allergic effects. Besides these two compounds, *A. wilhelmsii* also contained high amounts of carvacrol, linalool, 1,8-cineol, E-nerolidol and borneol (Javidnia *et al*., 2004). Tips of the flowered branches of this plant have flavonoids and sesquiterpenes that have noticeable antifungal effects on fungal pathogens such as *Candida albicans* (Amjad *et al*., 2012).

Cuminum cyminum (cumin) is a flowering plant in the family Apiaceae. This aromatic species is native to many regions of Iran including Kerman, Semnan, Yazd and Mazandaran provinces. It is used as food additive and spice. Moreover, cumin is utilized in modern and traditional medicine as carminative and antimicrobial agent (Kedia *et al*., 2014). It was also used to treat indigestion as appetizer and in digestive problems. Antifungal effects of *C. cyminum* on some fungal pathogens such as *Candida* species (*C. albicans*, *C. parapsilosis*, *C. glabrata* and *C. krusei*) and *Saccharomyces cerevisae* have been proven (Hajlaoui *et al*., 2010).

The main objective of the current study was to identify the chemical compositions and investigate the *in vitro* antifungal activity of essential oils of these Iranian medicinal plants on *S. parasitica*.

Materials and methods

Plant materials and extraction of the essential oils

The aerial parts of *E. campestre*, *P. affinis*, *M. piperita*, *A. wilhelmsii* and *C. cyminum* were collected between May and September 2013 from their natural habitats of Iran. Identifications were confirmed according to standard

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methods by Shahrekord University botany section (Table 1). The sampled plant parts were air dried at room temperature for one week before they were ground and 200 g each subjected to hydro-distillation for 4 h with a Clevenger-type apparatus to extract the essential oils according to the European Pharmacopoeia method and stored in tightly closed and dark vials at 4 °C for GC-MS analysis (Mohammadi *et al*., 2014).

Table 1: Geographical and environmental conditions of used medicinal herbs.

Plant	Region	Altitude (m)	Latitude	Longitude
Eryngium campestre	Mazandaran province	132	36°(N)	36°4’(E)
Pimpinella affinis	Mazandaran province	132	36°(N)	36°4’(E)
Cumminum cyminum	Khorasan province	1444	36°20’(N)	59°35’(E)
Achillea wilhelmsii	Chaharmahalvabakhtiary province	2080	32°39’(N)	51°43’(E)
Mentha piperita	Mazandaran province	1230	31°41’(N)	53°49’(E)

Gas Chromatography–Mass Spectroscopy analyses

Essential oil compositions were analyzed by using gas chromatograph-mass spectrometry (GC-MS, Agilent 5975 GC-MSD system). The following conditions were set in order to acquire data: initial temperature 50 °C; program rate 3 °C min^{-1}; final temperature 300 °C and injector temperature 290 °C. The carrier gas was helium and the split ratio was 0.8 ml min^{-1}. For GC–MS detection, an electron ionization system with an ionization energy of 70 eV was used (Sarac *et al*., 2009). The retention indices for all the components were calculated by using the retention times of n-alkenes (C8-C25) that were injected after the essential oil under the same condition. The components were identified by comparing retention indices (RRI, DB-5) with those of the standards and also with those reported in the literature.

Strain tested

The *Saprolegnia parasitica* strain, which was used for the antifungal assays, was isolated from *Oncorhynchus mykiss* eggs in Mazandaran province during autumn and winter 2013. It was cultured on glucose-yeast extract (GY) agar medium which consisted of 0.5 g of yeast extract, 10 g of glucose and 20 g of agar in 1000 ml of distilled water. To inhibit bacterial growth, 250 μg ml^{-1} each of penicillin and streptomycin, were added (Gormez and Diler, 2012). The purified strain was kept at 18 °C for 7 days and transferred to fresh GY agar at regular intervals (Gormez and Diler, 2012).

Antifungal assays

The disc diffusion method as described by Gormez and Diler (2012) was used to determine the growth inhibition zone diameters of the extracted essential oils

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on *S. parasitica*. At first, the essential oils were diluted in 4% dimethyl sulfoxide (DMSO), then, the autoclaved and sterile discs (6 mm diameter) were impregnated with 50 µl of the diluted oil with concentrations of 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8 and 16 µg ml^{-1} and placed onto the agar which was inoculated by a 4 mm diameter agar plug punched from previously cultivated *S. parasitica* agar plates (Gormez and Diler, 2012). In this study, 4% DMSO was used as negative control and 10 µl of formalin were prepared as positive control (Gormez and Diler, 2012). The impregnated discs along with the controls were kept on GY agar plates and incubated at 18°C for 72 h. After this period, diameters of the zone of inhibition were measured in mm (all tests were performed in triplicate).

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Serial dilution assays were used for determination of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). First, 50µ of essential oils plus DMSO from 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8 and 16 µg ml^{-1} concentratins were poured into tubes containing 2 ml GY-broth, then, inocula (agar plugs as used for the disc diffusion assay) from 72 h old *S. parasitica* cultures were dispensed and finally incubated at 18 °C for 96 h. As negative control served 4% DMSO while formalin was used as positive control. After incubation the MIC was determined by the lowest concentration of the essential oil at which the fungus does not demonstrate visible growth (GY-broth was clear). MIC and higher concentrations were re-inoculated to each agar plate and incubated for 72 h at 18 °C. The MFC was defined as the lowest concentration of the essential oil at which 99.9% of the incubated fungi are completely killed (Sharif Rohani *et al*., 2013).

Statistical analysis

The data were subjected to statistical analysis using the SPSS (software version no. 18). One-way analysis of variance (ANOVA) along with Duncan's post-hoc method was carried out to examine differences between the essential oils. A p-value of less than 0.05 was statistically considered significant ($p<0.05$).

Results

Chemical composition of essential oils

The chemical composition of the examined essential oils is presented in Tables 2-6. A total of 20 different components were identified in the essential oil of *E. campestre* with the most important constituent being bornyl acetate (17.9%) (Table 2). The GC-MS analysis of *P. affinis* essential oil revealed 19 different compounds with pregeijene as the main component (Table 3). The yield of the essential oil of *C. cyminum* is summarized in Table 4 and based on the GC–MS analysis, 32 components were identified. The most significant compound of *C. cyminum* was α-Pinene, which made up 29.1% of the total essential oil. Besides that, other important compounds were

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limonene (21.5%), 1,8-Cineole (17.9%) and linalool (10.4%). Essential oils from aerial parts of *A. wilhelmsii*, contained in total 33 different identified chemical compounds (Table 5) with 1,8-cineol as the dominant component. For *M. piperita* menthol was the predominant compound (48.5%, Table 6) followed by neomenthyl acetate (15.1%) and menthofuran (11.2%).

Table 2: Essential oil chemical composition of *Eryngium campestre*

Constituents	Retention index (RI)	Percentage
β-Ylangene	1420	0.9
Bornyl acetate	1274	17.9
Terpinen-4-ol	1164	8.7
Camphene	952	5.2
β-Pinene	978	1.3
Myrcene	991	0.2
Terpinolene	1082	0.2
γ-Terpinene	1051	0.2
α-Terpineol	1172	0.9
2,3,6-Trimethylbenzaldehyde	1293	1.4
α-Copaene	1378	0.9
β-Elemene	1389	1.2
α-Gurjunene	1412	0.1
Bicyclogermacrene	1492	1.8
Carotol	1594	0.4
γ-Muurolene	1474	1.1
trans-Pinocarveol	1128	0.3
Linalool	1085	0.1
Limonene	1025	11.6
neo-3-Thujanol	1148	0.05

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Table 3: Chemical components of the essential oils distilled from *Pimpinella affinis*

Constituents	RI	Percentage
Cis-β - Ocimene	1038	1.9
α-Pinene	939	0.9
Trans-b-Ocimene	1042	3.96
Linalool	1082	0.2
3-Octanore	986	0.3
Myrcene	991	0.6
Geijerene	1145	15.7
Decanal	1192	3.6
β-Cubebene	1382	0.5
Terpiene	1018	0.4
Delta elemene	1342	0.4
Limonene	1031	11.28
Valencene	1490	1.3
Methyl cinnamate	1342	3.18
Germacrene D	1482	12.8
Trans-dictamnol	1425	1.8
Longipinanole	1565	0.3
Pregeijene	1285	27.3
Methyleeugenol	1403	1.9

Table 4: Chemical composition of *Cuminum cyminum* essential oil

Constituents	RI	Percentage
Isobutyl isoutyrate	892	0.8
α-Thujene	922	0.3
α-Pinene	931	29.1
Sabinene	971	0.6
Myrcene	981	0.2
δ-3-Carene	998	0.2
ρ-Cymene	1013	0.3
Limonene	1025	21.5
1,8-Cineole	1028	17.9
(E)-Ocimene	1038	0.1
γ-Terpinene	1051	0.6

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Table 4 Continued:		
Terpinolene	1082	0.3
Linalool	1089	10.4
α-Campholenal	1122	0.03
trans-Pinocarveole	1130	0.07
δ-Terpineole	1154	0.09
Terpinene-4-ol	1169	0.5
α-Terpineole	1180	3.17
trans-Carveole	1213	0.4
cis-Carveole	1217	0.07
Geraniol	1242	1.1
Linalyl acetate	1248	4.8
Methyl geranate	1310	0.2
α-Terpinyl acetate	1342	1.3
Neryl acetate	1351	0.09
Methyl eugenol	1369	1.6
β-Caryophyllene	1430	0.2
α-Humulene	1463	0.2
Spathulenol	1562	0.07
Caryophylleneb epoxide	1586	0.1
Acetocyclohexanedione (2)	1704	0.4

Table 5: Essential oil compounds in the aerial parts of *Achillea wilhelmsii*

Constituents	RI	Percentage
Sabinene	976	3.2
α-Pinene	939	2.06
Terpineneol	1185	2.2
Camphene	953	0.87
1,8-cineol	1033	25.2
trans pinocarveol	1139	0.1
Myrtenol	1194	0.8
Artemisia alcohol	1083	4.3
trans-Linalool oxide	1076	0.2
Camphor	1143	18.9
Borneol	1165	5.7

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Table 5 Continued:		
Cis-sabinene hydrate	1064	0.18
Terpinene-4-ol	1176	1.9
Bornyl acetate	1289	1.08
α-Terpinolene	1201	1.84
γ—cadinene	1508	0.76
Isospathulenol	1592	2.45
Fargano	1209	1.75
para-Cymen-8-ol	1180	1.2
Verbenone	1205	0.06
Isopentylisovalerate	1113	0.07
Pinocarveone	1161	1.1
Linalool	1098	6.7
Caryophyllene oxide	1577	2.9
Thymol	1288	0.5
α-Campholenal	1123	0.23
Cuminyl aldehyde	1235	0.8
Dihydrocarvone	1239	4.6
ρ-Cymene	1027	2.3
b-Selinene	1418	0.5
Isobornyl n-butanoate	1472	1.2
Pentyl benzoate	1475	0.1

Table 6: Chemical components of the essential oils of *Mentha piperita*

Constituents	**RI**	**Percentage**
α-Pinene	939	0.31
Sabinene	975	0.26
β-pinene	979	0.58
1,8 Cineole	1031	6.69
Cis-Sabinene hydrate	1152	2.56
Menthone	998	0.23
Menthofuran	1164	7.2
Neomenthol	1165	2.38
Menthol	1171	48.52

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Table 6 continued:		
Neomenthyl acetate	1295	12.13
Menthyl acetate	1051	0.52
Eucarvone	1343	0.61
β-Bourbonene	1089	10.12
(z)-Caryophyllene	1408	2.09
E-*β*-farnesene	1456	0.36
Germacrene D	1485	1.1
Caryophyllene oxide	1575	0.16
Linalool	1087	0.36
DL-Carvone	1253	2.83
Piperitone	1227	0.39

Antifungal activity

According to the results, essential oil of *M. piperita, E. campestre* and *C. cyminum* had significantly larger inhibition zones than formalin (p<0.05). In contrast, no significant difference was observed between inhibition zones of *P. affinis* and *A. wilhelmsii* to formalin (p>0.05). No inhibition of *S. parasitica* growth was observed by DMSO. The highest activity against *S. parasitica* was observed for *C. cyminum* with a zone of inhibition of 27.4±2.1 mm and MIC and MFC of 0.5 μg ml^{-1} each. With a zone of inhibition of 24.8±1.5 mm and 2 μg ml^{-1} each and a zone of inhibition of 22.6±1.2 mm and 1 μg ml^{-1} for MIC and 2 μg ml^{-1} for MFC. *E. campestre* and *M. piperita*, respectively, were slightly less active than *C. cyminum* but significantly more active than formalin. *P. affinis* and *A. wilhelmsii* both showed significantly smaller zones of inhibition in comparison to formalin (15.7±0.8 and 13.2±0.5, respectively), their MIC were 2 and 4 μg ml^{-1}, respectively and their MFC were 4 and 8 μg ml^{-1}, respectively (Table 7).

Table 7: Antifungal activity of essential oils from Iranian medicinal herbs on *Saprolegnia parasitica*

Plant	Zone of inhibition (mm)	MIC (μg ml^{-1})	MFC (μg ml^{-1})
Eryngium campestre	24.8±1.5^{a}	1	2
Pimpinella affinis	15.7±0.8^{c}	2	4
Cuminum cyminum	27.4±2.1^{a}	0.5	0.5
Achillea wilhelmsii	13.2±0.5^{c}	4	8
Mentha piperita	22.6±1.2^{ab}	1	2
Formalin	18.3 ± 0.3^{b}	1	2

* Values in the same column with different superscripts show significant difference (p<0.05).

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Discussion

The control of fungi of the genus *Saprolegnia* has long been a major objective in aquaculture. For decades, hatchery personnel have been depending either on laborious manual labor by hand sorting dead eggs or on chemicals to control disease outbreaks on fish eggs. Although it is necessary to control these outbreaks, it is also important to find an environmentally friendly and hazard free alternative disease control method to reduce the use of potentially hazardous and chemicals as a matter of safety for consumers and environment (Ghiasi *et al*., 2010). Failure in disease treatment, antifungal side effects and increasing drug resistance have led researchers to consider herbal extracts and essential oil effects on aquatic diseases because of their effectiveness and low side effects (Firouzbakhsh *et al*., 2014). Several studies were done in Iran on medicinal plant effects as antifungal agents against *S. parasitica* (Pirbalouti *et al*., 2009; Azizi *et al*., 2012; Mousavi *et al*., 2012) and some herbal plants such as *Zataria multiflora* and *Eucalyptus camaldolensis* were introduced as alternative for malachite green and other chemical drugs (Khosravi *et al*., 2012).

In the present study, essential oil acquired from *C. cyminum* showed the highest inhibitory activity on *S. parasitica* with MIC and MFC values of 0.5 µg ml^{-1} each. Effectiveness of *C. cyminum* on other fungal pathogens such as *C. Albicans* (Naeini *et al*., 2009), *Aspergillus clavatus*, *Cladosporium musae*, *Fusarium oxysporum*, *Paecilomyces carneus*, *Trichoderma hamatum*, *T. viride* and *Ulocladium chartarum* have been approved as well (El-Said and Goder, 2014). Moreover, Hajlaoui *et al*. (2010) assessments of *C. cyminum* antifungal activity on different *Candida* species have been successful and *Candida glabrata* was the most sensitive fungus with an inhibitory zone 22.7±0.58 mm in diameter. High antifungal activity of *C. cyminum* is due to large amounts of the highly volatile components in the cumin oil (Romagnoli *et al*., 2010).

Analyses of essential oil from *E. campestre* aerial parts in the current study, revealed high concentrations of α -Pinene, Limonene and 1,8-cineole. The antifungal activity of this species might be due to the mentioned components (Thiem *et al*., 2010). The MIC value of *E. campestre* essential oil on *S. parasitica* in this survey was 2 µg/mL, while in comparison the effectiveness of a similar species, *E. tricuspidatum*, against *C. albicans* was with a MIC of 4.6 µg ml^{-1} lower (Merghache *et al*., 2014). In another study, MIC concentrations of *E. campestre* extract on *Candida glabrata*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans* were determined as 50-100, 100 and 50-200 µg ml^{-1}, respectively (Thiem *et al*., 2010). The observed variance in MIC values from genus *Eryngium* is probably related to different chemical compositions of the essential oil and might correlate with species, geographical range, plant age, seasonal patterns, desiccation and extraction methods, genetic polymorphisms and differences

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between studied fungi strains (Pirbalouti *et al*., 2009).

In this study, *E. campestre* and *M. piperita* have the same MFC values against *S. parasitica.* Antifungal activity of *M. piperita* on *Aspergillus niger* and *C. albicans* have been successfully proved in a study by Erturk (2006), their zones of inhibition were 22 and 25 mm, respectively. Mousavi *et al*. (2012) reported a new combination of four essential oils including *M. piperita*, *E. globulus*, *S. officinalis* and *T. vulgaris* which can be used as a good antifungal therapeutic agent in order to control *S. parasitica* and *F. solani* outbreaks in rainbow trout, *Oncorhynchus mykiss* and hatcheries. Results of Hu *et al.* (2013) showed that traditional Chinese methanolic extract including *Cnidium monnieri*, *Magnolia officinalis*, and *Aucklandia lappa* at a concentration of 62.5 mg ml^{-1} have inhibitory activity on *Saprolegnia* sp. and *Achlya klebsiana*. In our study the MIC value for *M. piperita* essential oil was 1 µg ml^{-1} against *S. parasitica*, much lower than the values of 125 and 250 mg ml^{-1} for *Mentha haplocalyx* against *Saprolegnia* sp. and *Achlya klebsiana*, respectively (Hu *et al*., 2013). Also, in a study by Pirbalouti *et al*. (2009), an ethanolic extract of *Mentha longifolia* showed good inhibitory effects against *S. parasitica* isolated from rainbow trout eggs. Antifungal activity of *M. piperita* is mainly considered to be related to the compounds pulegone, isomenthone, carvone, piperitone and dehydrocarvone (Tassou *et al*., 2010).

In the current study, *P. affinis* was more effective against *S. parasitica* than essential oil from *A. wilhelmsii*. Extracts of *Pimpinella anisum* were assessed on 90 different fungi species and it had acceptable effects on *Chaetomium oblatum, Drechslera erythrospila, D. euphorbiae, Epicoccum purpurascens, Fusarium sulphureum, Gibberella tricincta, Scopulariopsis brevicaulis, Setosphaeria rostrata* and *Stemphylium solani* (El-Said and Goder, 2014). In another study, the highest antifungal activity of *P. anisum* extracts on dermatophytic and saprophytic fungi such as *A. niger, C. albicans, Trichophyton mentagrophytes* and *Microsporum canis* was observed to be at a concentration of 16 mg ml^{-1} (Yazdani *et al*., 2009).

In this study, among all surveyed medicinal plants, the essential oil of *A. wilhelmsii* had the lowest effects on *S. parasitica* and MIC value was 4 µg ml^{-1}. This result was similar to results of Firouzbakhsh *et al*. (2014) who showed that *A. millefolium* had lower effects on *S. parasitica* compared to *Artemisia annua* and the control group (formalin) and MIC value was determined to be >2048 µg mL^{-1}. On the other hand, the aerial parts of *A. clavennae, A. holosericea, A. lingulata* and *A. millefolium* had a good antifungal activity against *A. niger* and *C. albicans* (Stojanovic *et al*., 2005). The suggested antifungal activity of the essential oil of *A. Wilhelmsii* is supposed to be related to flavonoid and phenolic compounds such as 1,8-cineole and α-Pinene (Jose Abad *et al*., 2007). However, the content of α-Pinene was found to be

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rather low in *A. wilhelmsii* in this study (Table 5).

In conclusion, this research approved good antifungal activities of *C. cyminun*, *E. campestre* and *M. piperita* essential oils against *S. parasitica* under *in vitro* conditions. Further studies, primarily *in vivo* and if also successful, *in situ*, are necessary in order to determine the effective dosage, mode of application and its duration, the active ingredients, biosafety and ecotoxicity of these medicinal plants prior to an introduction as new antifungal remedies against *S. parasitica*. Also application against other critical oomycetes such as *S. australis*, *S. salmonis*, *A. astaci*, *A. invadans* and even terrestrial ones like *Phytophthora* spp. should be elucidated further. Also, the unknown and unwelcome effects of these essential oils on fish and aquatic microorganism such as planktons should be studied.

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