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Original article

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *ARTEMISIA ANNUA* (L.) ESSENTIAL OIL AGAINST DIFFERENT FISH PATHOGENS

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Summary

Sakhaie, F., M. Adel, R. Safari, F. Firouzbakhsh, A. Nosrati Movafagh & T. Stadtlander, 2023. Chemical composition and antimicrobial activity of *Artemisia annua* (L.) essential oil against different fish pathogens. *Bulg. J. Vet. Med.* (online first).

In the present study, the composition of the essential oil and antimicrobial activity from aerial parts of *Artemisia annua* growing wild in north of Iran was investigated. The major ingredients of the essential oil of *A. annua* were camphor (29.2%), 1.8-cineole (13.3%), tetradecanol (6.16%), β-selinene (5.82%) and pinocarvone (3.86%). In the current study, antimicrobial activity of *A. annua* was tested against 4 fish pathogenic bacteria including: *Streptococcus iniae*, *Yersinia ruckeri*, *Aeromonas hydrophila* and *Lactococcus garvieae* and 3 fish pathogenic fungi namely *Saprolegnia* sp., *Fusarium solani* and *Aspergillus flavus*. Based on the results, *Y. ruckeri*, *A. hydrophila* and *Saprolegnia* sp. showed higher sensitivity to the essential oil of *A. annua* L. than to control antibiotic (ciprofloxacin, 0.3% w/v). Maximum antibacterial and antifungal activity was observed against *Y. ruckeri* (22.6±0.6 mm) and *Saprolegnia* sp. (18.7± 0.8 mm) respectively, while *S. iniae* (10.2±1.2 mm) and *A. flavus* (12.9± 0.82 mm) showed the least sensitivity. In addition, the minimum inhibitory concentration (MIC) test showed that concentrations of the essential oil within the range between 3.2 to 25 µg/L were able to inhibit the growth of the selected bacterial and fungal pathogens. According to the results, that the essential oil of *A. annua* could be a potential new and more effective antibacterial component for the aquaculture industry

Key words: antimicrobial activity, *Artemisia annua*, essential oil composition

INTRODUCTION

The genus *Artemisia* is an aromatic and medicinal plant belonging to the family *Asteraceae* and is widely distributed in Asia, Europe, and North America. It comprises 300 genera of which 37 species are endemic to Iran (Kazemi *et al.,* 2010; Sharopov *et al*., 2020). *Artemisia annua* occurs in different areas of Iran especially in Golestan, Mazandaran and Gilan provinces (Rasooli *et al*., 2003). It contains various secondary plant compounds with varying concentrations, e.g. coumarins, flavones and terpenes (Brown *et al*., 2003; Das *et al*., 2020). Pharmacological activity of this genus includes antifever, antimalaria, anticancer, antiviral, antifungal, antimicrobial (Juteau *et al.,* 2002) and antioxidant effects (Tajehmiri *et al.,* 2014). Traditionally, it has been used for treatment of fever, malaria, bacterial and parasitic infections in humans (Nigam *et al.,* 2019). The chemical composition of *A. annua* essential oil has been studied for different Iranian origins such as Gorgan (Verdian-rizi *et al.,* 2008), Gilan (Massiha *et al.,* 2013) and Azarbaijan (Mojarrab *et al.,* 2013) provinces, but there was no report on the composition of the essential oil of *A. annua* growing wild in Mazandaran province (northtern of Iran), which is an important geographical zone for medicinal plants in Iran. In several studies, antibacterial and antifungal activity of *A. annua* was reported on different strains. The composition of the essential oil of this species is highly dependable on the growing location (Risaliti *et al.,* 2020). In recent years, emergence of drug resistance against various pathogens and parasites turned out into a major problem for aquaculture (Love *et al.,* 2010; Aaen *et al.,* 2015; Watts *et al.,* 2017; Reverter *et al.,* 2020). Identification of the composition of the North Iranian *A. annua* essential oil and its major components and surveying its antimicrobial activity against typical fish pathogens could help develop new treatment and therapy options for important aquaculture species. Therefore, this study was conducted to test *A. annua* essential oil against some of the most important bacterial and fungal fish pathogens.

MATERIALS AND METHODS

Plant material

Two kg of *A. annua* aerial parts were collected from Abbas Abad in Mazandaran province, north of Iran, (altitude: 132 m above sea level (asl); relative humidity: 75–82%, annual precipitation: 590–870 mm, temperature range: 3–34.6 ºC), in October 2023 and the voucher specimens were deposited in the herbarium of the Research Centre of Medicinal Plants at Mazandaran University of Medical Sciences, of Iran.

Essential oil preparation

The air-dried aerial parts of *A. annua* L. were dried at room temperature in a dark room, powdered and subjected to hydrodistillation using a clevenger-type apparatus for 4 h according to the method recommended by Boutabia *et al*. (2020). Anhydrous sodium sulfate was used to dehydrate the essential oil. The oil was stored (short time) at 4 ºC in dark bottle until use.

Gas chromatography/mass spectrometry (GC/MS) analysis

Analyses of the essential oil composition were performed using a Varian gas chromatograph 3600 with DB5 (methyl phenyl siloxane, 30 mm × 0.25 mm i.d.); the car-

rier gas was helium; split ratio 1:15 and flame ionisation detector. The initial temperature of the column was 60 °C (for 2 min) which was increased to 240 °C at 5 °C/min, the injector temperature was 250 °C and detector temperature of 260 °C. GC-MS was performed on a cross-linked 5% methyl phenylsiloxane (HP-5, 30 m × 0.25 mm i.d., 0.25 µm film thickness). Carrier gas was helium, split ratio 1:15 with a quadrupole mass spectrometer operating at 70 eV ionisation energy (Delazar *et al*., 2012). The retention indices for all components were calculated by using retention time 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 standards and also with those reported in the literatures (Charles *et al.,* 1991).

Microbial strains

In vitro antibacterial activities of *A. annua* essential oil were examined against 4 bacterial fish pathogens, including: *S. iniae* (LMG 14520), *Y. ruckeri* (KC291153), *A. hydrophila* (LMG 3770) and *L. garvieae*. These bacteria were obtained from the Persian Type Culture Collection, which were prepared from a lyophilized stock. Also, *in vitro* antifungal activity was determined on fish pathogenic fungi, including: *Saprolegnia* sp., *Fusarium solani* and *Aspergillus flavus*. Fungi strains were obtained from the Department of Aquatic Animal Health and Diseases, Research Organization of Caspian Sea, Iran.

Antimicrobial assasy

The disc diffusion method as described by Chebbac *et al*. (2023) was used to determine the growth inhibition effect of *A. annua* essential oil on selective pathogens. Bacterial suspensions with McFarland Standard 0.5 (equivalent to 1×10^7 cells/mL) were inoculated into Mueller-Hinton agar medium with the help of sterile cotton swabs. For fungal studies PDA (potato dextrose agar) the medium was dispensed in petri plates for different strains of fungi. Whatman No.1 filter paper discs with 4 mm diameter were impregnated with a defined concentration of test essential oil (0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8 and 16 µg mL^{-1}) while 4% DMSO and standard antibiotic disc (ciprofloxacin, 0.3% w/v) were applied as negative and positive controls, respectively. The impregnated discs along with the controls were kept on agar plates, previously seeded separately with either the test bacterial or fungal cultures. The bacterial plates were incubated for 24 h at 25 ^oC. The fungal plates (PDA) were incubated at 30 ^oC for 72-96 h to reveal any antimicrobial activity. The antibacterial activities were determined by measuring the diameter of the zone of inhibition in mm with all tests being performed in triplicate.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Essential oil of *A. annua* that showed antimicrobial activity was further tested for minimum inhibitory concentration (MIC). The MIC is defined as the lowest concentration of *A. annua* essential oil at which the respective pathogen does not demonstrate visible growth. MIC test for bacteria was carried out by using a broth microdilution method as described by Verdian-rizi *et al*. (2008). The MBC was defined as the lowest concentration of the essential oil at which incubated microorganisms are completely killed (Adel *et al*., 2016). Essential oil of *A. annua* was serially diluted two-fold using 100 µL of

Mueller-Hinton broth (Difco Laboratories, Detroit, MI, USA) in order to determine the minimum concentration that can be used to inhibit the growth of the specific pathogen. Fifty µL of overnight inoculum were then added into each tube containing different concentrations of essential oil and incubated at 37 ^{o}C for 24 h.

Determination of antifungal activity

In vitro antifungal activity was determined against *Saprolegnia* sp., *F. solani* and *A. flavus*. Fungi species were cultured on Sabouraud's dextrose agar (SDA) and incubated at 37 ^{o}C for 48 h (Liu *et al.,* 2001). Several colonies of each fungal species were collected in 2 mL sterile PBS to prepare a suspension. The suspension was adjusted to 70% transmittance by a spectrophotometer at 530 nm. This should result in a suspension containing about 1×10^7 cfu per mL. MIC was carried out according to Pirbalouti *et al*. (2009), in brief, a serial dilution of essential oil of *A. annua* in dimethylsulfoxide (DMSO) was prepared in SDA tubes. The solvent (4% DMSO) was also used as a negative control. A tube was considered as positive control (formalin 10 µL mL^{-1}) without *A. annua* essential oils and solvents. Twenty mL of standardised suspension of different fungal species were inoculated into each tube (0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40 µg/mL). The tubes were incubated at 30 ^{o}C for 24 h to 5 days. The lowest *A. annua* essential oils dosage at which the respective tubes showed no visible growth (e.g. were clear) was defined as the minimal inhibitory concentration (MIC). For the determination of MFC (minimum fungicidal concentration), a portion of liquid (10 µL) from each tube that was clear was placed on SDA for further incubation at 37 °C for 24 h to 5 days (Lu *et al.,* 2000). The lowest dosage that yielded no growth after this sub-culturing was defined as the MFC, with 3 replicates for each experiment.

RESULTS

The essential oil yields of *A. annua* collected in the Mazandaran region was 0.58%. The major composition of its essential oil were camphor (29.2%), 1.8-cineol*e* (13.3%), tetradecanol (6.16%), β-selinene (5.82%) and Pinocarvone (3.86%) (Table 1).

The *in vitro* antimicrobial activity of *A. annua* essential oil against selective pathogens are shown in Table 2 and 3. The maximum antibacterial activity was observed against *Y. ruckeri* with an average of 22.6±0.6 mm diameter of inhibition zone. This was followed by *A. hydrophila* (20.0±1.2 mm), *L. garvieae* (12.7±0.3 mm) and *S. iniae* (10.2±0.3 mm). The minimum antibacterial activity was observed against *S. iniae* (Table 2). Also, the highest antifungal activity of *A. annua* essential oil was observed against *Saprolegnia* sp. with 18.7 ± 0.8 mm diameter of inhibition zones, followed by *F. solani* (13.8±0.4 mm) and the minimum antifungal activity was observed against *A. flavus* (12.9 ± 0.3 mm) (Table 3).

DISCUSSION

The essential oil yields of *A. annua* collected in the Mazandaran region was 0.58%. The major composition of its essential oil were camphor (29.2%), 1.8-cineole (13.3%), tetradecanol (6.16%), β-selinene (5.82%) and pinocarvone (3.86%). The main components of the species of the Gilan province were 1,8-cineole (11.40 %), linalool (8.01%), spathulenol (4.97%) and α-pinenes (3.67%) (Kazemi

Table 1. Chemical composition (%) of the essential oils of *Artemisia annua* L. aerial parts

No	Name of compounds	RI	Percentage
1	Acoradiene	1459	0.24
2	Tricylene	914	0.09
3	α–cadinol	1683	1.06
4	α–pinene	939	2.06
5	Camphor	1143	29.2
6	α–thujene	919	0.28
7	Camphene	953	3.67
8	1,8-cineole	1033	13.27
9	Trans pinocarveol	1139	0.1
10	Myrtenol	1194	1.26
11	Artemisia alcohol	1053	1.17
12	Trans-caryophyllene	1481	0.16
13	Tetradecanol	1729	6.16
14	Borneol	1165	2.43
15	Cis-sabinene hydrate	1054	0.32
16	Terpinene-4-ol	1176	1.9
17	α-terpinolene	1181	1.14
18	γ-cadinene	1508	0.76
19	Spathulenol	1562	1.48
20	Artemisia ketone	1057	2.6
21	Myrtenol	1198	1.52
22	Pinocarvone	1163	3.86
23	Verbenone	1205	0.13
24	Isocedrol	1635	0.87
25	Apiol	1734	1.36
26	Germacrene B	1492	3.38
27	Cederannon	1642	3.12
28	Elemol	1651	0.17
29	γ-eudesmol	1658	1.06
30	β-selinene	1468	5.82
Total			93.07

et al., 2010). Camphor (48.0%), 1,8-cineole (9.39%), camphene (6.98%) and spathulenol (4.89%) were the main compounds identified in *A. annua* collected from Tehran province, Centre of Iran as reported by Verdian *et al.* (2008). In another study for *A. annua* essential oils collected in Azarbaijan province (Mojarrab *et al*., 2013), the major components of the oil were α–pinene (10.7%), Nonadecane (10.0%), 6,10,14-trimethyl-2-pentadecanone (9.4%), spathulenol (7.8%) and Z-verbenol (5.8%). In Sharopov *et al.* (2020) survey, camphor (32.5%), 1,8-cineole (17.8%), camphene (8.4%), and α-pinene (7.3%) were the major components of the essential oil obtained from the aerial parts of *A. annua*, growing wild in Tajikistan.

In the present study, Camphor was considered as an important constituent of *A. annua* of the Mazandaran area, this result was similar to Verdian *et al.* (2008) and Massiha *et al*. (2013) studies. Tetradecanol, Apiol, Acoradiene and Isocedrol have been identified only in essential oil of *A. annua* collected in Mazandaran province. This fact could conduce us to the identification of different chemotypes and also the effect of climatic conditions

Table 2. Antibacterial activity of essential oil of *Artemisia annua* L.

Bacterial pathogens	Test sample	Zone of inhibition (mm) positive control (ciprofloxacin)	Negative control	MBC (µg/mL)	MIC (µg/mL)
S. iniae	10.2±0.3[b]	17.1±0.4[a]	–	24.8	12.4
Y. ruckeri	22.6±0.6[a]	18.2±0.7[b]	–	3.2	1.6
A. hydrophila	20.0±1.2[a]	18.3±0.8[b]	–	>6.4	3.2
L. garvieae	12.7±0.3[a]	18.6±0.4[a]	–	12.8	6.4

*Values in the same column with different superscripts show significant difference (P<0.05). MBC = minimum bactericidal concentration, MIC = minimum inhibitory concentration.

Table 3. Antifungal activitiy of the essential oils of *Artemisia annua* L. against selective fungi

Fungal pathogens	Test sample	Zone of inhibition (mm) positive control (ketoconazole)	Negative control	MIC (ppm)	MFC (ppm)
Saprolegnia sp.	18.7± 0.8[a]	15.3± 0.7[b]	–	3.6	7.2
F. solani	13.8± 0.4[a]	14.8± 0.6[a]	–	15.6	31.2
A. flavus	12.9± 0.3[a]	14.7± 0.6[a]	–	6.2	12.4

*Values in the same column with different superscripts show significant difference (P<0.05). MIC = minimum inhibitory concentration, MFC = minimum fungicidal concentration.

on constitutes of herbal plants. These variations may be attributed to variations in their agroclimatic and geographical conditions, environmental and seasonal conditions, plant strain, age of plants, time of harvest, methods of drying and oil extracting and genetic differences (Javidnia *et al*., 2004; Esmaeili *et al*., 2006).

Massiha *et al*. (2013) examined the antibacterial activity of essential oil of *A. annua* against 7 pathogenic bacteria and reported highest antibacterial activity on *Escherichia coli.* In another study, no antibacterial activity of *A. annua* was observed against various bacterial and fungal species (Beiki & Alizadeh, 2006), that was different from our results.

The dissimilarity in antimicrobial activity may be due to the high variation in the chemical compounds that cause antibacterial and anti-fungal effects. In the current investigation, the results of MIC determination showed that a minimum concentration of essential oil of *A. annua* ranging between 3.2 to 25 µg/L was able to inhibit the growth of the bacterial and fungal pathogens. In a study published by Fabien *et al.* (2002), essential oil of *A. annua* inhibited the growth of gram positive bacteria *Enterococcus hirae* and the MIC values obtained were in the same ranges as in the current work. In a similar study, *A. annua* essential oil showed maximum activity against *Staphylococcus aureus* and *Salmonella enterica* with inhibitory, 16.5 and 15.5 mm (Tajehmiri *et al*., 2014). Very similar to our finding, antifungal activity of *A. annua* was observed against *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis*, *Helminthosporium satium*, *Fusarium graminearum*, *Gerlachia nialis* and *Phytophthora capsici* (Lu *et al.,* 2000). Earlier found and reported antifungal and

antibacterial activity of *A. annua* essential oil was suggested due to flavonoids and phenolic compounds such as isoalantolactone (Tan *et al*., 1998), which could also contribute to an antimicrobial effect.

CONCLUSION

In conclusion, this research demonstrated that *Artemisia annua* essential oil holds a certain potential to form new plant-based drugs for treatment in aquaculture against important pathogens such as *Y. ruckeri*, *A. hydrophila* and *Saprolegnia* sp. However, the high variation in the composition of *A. annua* essential oil presents a certain challenge, as their activitiy and efficacy against different fish pathogens likely depends on exact composition and concentrations. Since this composition depends on a variety of factors, as mentioned earlier, further research is necessary to determine and monitor quality parameters and efficacy of *A. annua* essential oil throughout different seasons, climatic conditions and collected from different regions. Another important aspect in utilization of wild harvested plants is the danger of a potential depletion of wild stocks and thus the necessity to culture *A. annua*. Cultured plants might show different compounds and chemical composition compared to wild harvested plants (Briskin, 2000; Canter *et al*., 2005) but present the benefit of being more stable and reliable in their chemical composition when cultured and harvested under equal conditions.

Further studies are also needed to purify, fractional and characterize various antimicrobial compounds from the essential oil of *A. annua* in different conditions of Iran.

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REFERENCES

Aaen, S. M., K. O. Helgesen, M. J. Bakke, K. Kaur & T. E. Horsberg, 2015. Drug resistance in sea lice: A Threat to salmonid aquaculture. *Trends in Parasitology,* **31**, 72–81.

Adel, M., M. Dadar, M. J. Zorriehzahra, R. Elahi & T. Stadtlander, 2020. Antifungal activity and chemical composition of Iranian medicinal herbs against fish pathogenic fungus, *Saprolegnia parasitica. Iranian Journal of Fisheries Sciences,* **19**, 3239–3254.

Adel, M., R. Safari, A. H. Ghitanchi & M. J. Zorriehzahra, 2016. Chemical composition and in vitro antimicrobial activity of some Iranian medical herbs against *Yersinia ruckeri. Iranian Journal of Fisheries Sciences,* **15**, 108–123.

Amin, G., 1991. Traditional Medicinal Plants of Iran. 1th edn, Ministry of Health, Treatment and Medical Education Press, Tehran, Iran.

Beiki, F. & A. Alizadeh, 2006. Antimicrobial activity of some herbal essential oils and plant extracts on the causal agents of bacterial leaf streak in wheat and barley. *Journal of Agriculture and Natural Resources Sciences,* **13**, 1–9.

Boutabia, L., S. Telailia, F. Guenadil & A. Chefrour, 2020. Chemical composition and antibacterial activity of essential oils from *Mentha pulegium* L. and *Mentha suaveolens* Ehrh. growing in North-East of Algeria. *Analele Universitatii din Oradea,* **2**,143–148.

Briskin, D. P., 2000. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiology,* **124**, 507–514.

Brown, G. D., G. Y. Liang & L. Sy, 2003. Terpenoids from the seeds of *Artemisia annua*. *Phytochemistry,* **64**, 303–323.

Canter, P. H., H. Thomas & E. Ernst, 2005. Bringing medicinal plants into cultivation: Opportunities and challenges for biotechnology. *TRENDS in Biotechnnology,* **23**, 180–185.

Charles, D. J., E. Cebert & J. E. Simon, 1991. Charecterization of the essential oil of *Artemisia annua* L. and constituents of its essential oil. *Journal of Essential Oil Research,* **3**, 33–39.

Chebbac, K., Z. Benziane Ouaritini, A. El Moussaoui, M. Chalkha, S. Lafraxo, Y.A. Bin Jardan & R. Guemmouh, 2023. Antimicrobial and antioxidant properties of chemically analyzed essential oil of *Artemisia annua* L. (Asteraceae) native to Mediterranean area. *Life,* **13**, 807.

Das, S., B. Vörös-Horváth, T. Bencsik, G. Micalizzi, L. Mondello, G. Horváth & A. Széchenyi, 2020. Antimicrobial activity of different Artemisia essential oil formulations. *Molecules,* **25**, 2390.

Delazar, A., M.-R. Delnavazi, N. Yassa, S. Parkhideh, N. Delazar, L. Nahar & S. D. Sarker, 2012. Essential oil composition and isolation of freeradical-scavenging phenolic glycosides from the aerial parts of *Ajuga chamaepitys* growing in Iran. *Revista Brasileira de Farmacognosia,* **22**, 299–305.

Esmaeili, A., F. Nematollahi, A.H. Rustaiyan, N. Moazzemi, Sh. Masoudi & Sh. Bamasian, 2006. Volatile constituents of *Achillea pachycephala*, *A. oxyodonta* and *A. biebersteinii* from Iran. *Flavour and Fragrance Journal,* **21**, 250–253.

Fabien, J., V. Masottia & J. Marie Bessie, 2002. Antibacterial and antioxidant activities of Artemisia annua essential oil. *Fitoterapia,* **73**, 532–535.

Javidnia, K., R. Miri & H. Sadegh Pour, 2004. Composition of the volatile oil of *Achillea wilhelmsii* C. Koch from Iran. *Daru,* **12**, 63–66.

Juteau, F., V. Masotti, J. M. Bessière, M. Dherbomez & J. Viano, 2002. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. *Fitoterapia,* **73**, 532–535.

Kazemi, M., M. R. Zand, K. Roshanaei, M. Mehrzad & A. Rustaiyan, 2010. Composition of the volatile oils of *Artemisia armenica* lam. and *Artemisia splendens* willd from Iran. *Journal of Essential Oil Research,* **22**, 126–128.

Liu, C. H., W. X. Zou, H. Lu & R. X. Tan, 2001. Antifungal activity of *Artemisia annua* endophyte cultures against phytopathogenic fungi. *Journal of Biotechnology*, **88**, 277–282.

Love, D. C., S. Rodman, R. A. Neff & K. E. Nachman, 2010. Veterinary drug residues in seafood inspected by the European Union, United States, Canada, and Japan from 2000 to 2009. *Environmental Science and Technology,* **45**, 7232–7240.

Lu, H., W. Xin Zou, J. Cai Meng, J. Hu & R. Xiang Tan, 2000. New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*. *Plant Science,* **151**, 67–73.

Massiha, A., M. M. Khoshkholgh-Pahlaviani, Kh. Issazadeh, S. Bidarigh & S. Zarrabi, 2013. Antibacterial activity of essential oils and plant extracts of Artemisia (*Artemisia annua* L.) *in vitro*. *Zahedan Journal of Research in Medical Sciences,* **15**, 14–18.

Mojarrab, M., A. Delazar, S. Esnaashari & F. Heshmati Afshar, 2013. Chemical composition and general toxicity of essential oils extracted from the aerial parts of *Artemisia armeniaca* Lam. and *A. incana* (L.) Druce growing in Iran. *Research in Pharmaceutical Sciences,* **8**, 65–69.

Nigam, M., M. Atanassova, A. P. Mishra, R. Pezzani, H. P. Devkota, S. Plygun & J. Sharifi-Rad, 2019. Bioactive compounds and health benefits of Artemisia species. *Natural product Communications*, **14**, 1934578X19850354.

Pirbalouti, A. G., M. Taheri, M. Raisee, H. R. Bahrami & R. Abdizadeh, 2009. In vitro antifungal activity of plant extracts on *Saprolegnia parasitica* from cutaneous lesions of rainbow trout (*Oncorhynchus mykiss*) eggs. *Journal of Food Agriculture and Environment*, **7**, 94–96.

Rasooli, I., M. B. Rezaee, M. L. Moosavi & K. Jaimand, 2003. Microbial sensitivity to and chemical properties of the essential oil of *Artemisia annua* L. *Journal of Essential Oil Research,* **15**, 59–62.

Reverter, M., S. Sarter, D. Caruso, J. C. Avarre, M. Combe, E. Pepey, L. Pouyaud, S. Vega-Heredía, H. de Verdal & R. E. Gozlan, 2020. Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nature Communications,* **11**, 1870.

Risaliti, L., G. Pini, R. Ascrizzi, R. Donato, C. Sacco, M. C. Bergonzi & A. R. Bilia, 2020. *Artemisia annua* essential oil extraction, characterization, and incorporation in nanoliposomes, smart drug delivery systems against *Candida species. Journal of Drug Delivery Science and Technology,* **59**, 101849.

Sharopov, F. S., A. Salimov, S. Numonov, A. Safomuddin, M. Bakri, T. Salimov & M. Habasi, 2020. Chemical composition, antioxidant, and antimicrobial activities of the essential oils from *Artemisia annua* L. growing wild in Tajikistan. *Natural Product Communications,* **15**, 1934578X2092 7814.

Tajehmiri, A., F. Issapour, M. Nasiri Moslem, M. Tavakoli Lakeh & M. Hassani Kolavani, 2014. In vitro Antimicrobial activity of *Artemisia annua* leaf extracts against pathogenic bacteria. *Advanced Studies in Biology,* **6**, 93–97.

Tan, R., H. Tang, J. Hu & B. Shuai, 1998. Lignans and sesquiterperpene lactones from *Artemisia sieversiana* and *Inula recemosa. Phytochemistry,* **49**, 157–161.

Verdian-rizi, M. R., E. Sadat-Ebrahimi, A. Hadjiakhoondi, M. R. Fazeli & M. Pirali Hamedani, 2008. Chemical composition and antimicrobial activity of *Artemisia annua* L. essential oil from Iran. *Journal of Medicinal Plants,* **4**, 58–62.

Watts, J. E. M., H. J. Schreier, L. Lanska & M. S. Hale, 2017. The rising tide of antimicrobial resistance in aquaculture: Sources, sinks and solutions. *Marine Drugs,* **15**, 158.

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