Turkish Journal of Agriculture - Food Science and Technology, 6(10): 1501-1506, 2018

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X
www.agrifoodscience.com,
Turkish Science and Technology

An Investigate on Importance of the Major Components of Herb Essential Oils on Challenging with Fish Diseases

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ARTICLE INFO

Research Articles

Received 27 July 2018
Accepted 18 September 2018

Keywords:
Centauriumerythraea
Foeniculum vulgaris
Essential oil
Majorcomponents
Thymus vulgaris

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ABSTRACT

The presence of different phytochemical components of essential oil such as tannins, alkaloids, terpenoids and phenolic compounds has antibacterial, antifungal, and anti-inflammatory effects. The aim of this study is to investigate importance of the major components of three herb essential oils (*Thymus vulgaris* L., *Centauriumerythraea* Rafn. And *Foeniculumvulgare* Mill) on challenging with fish diseases. The components of essential oils provided from a commercial firm were made GC/MS analyzes. The major component of *T. vulgare*was carvacrol called as phenol, 2-methyl-5-(1-methylethyl) with 40%.The others were Linalool L (15.11%) and benzene, methyl(1-methylethyl)-(12.12%).The richest oil in *C. erythraea* was bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-(34.90%) called as alpha-pinene, followed by heptacosane (19.15%) and dotriacontane (17.72%), respectively.The evaluation of the essential oil of *F. vulgaris*presented benzene, 1-methoxy-4-(1-propenyl)- with 67.99%, followed by dl-Limonene (16.03%) and benzene, 1-methoxy-4-(2-propenyl)- (6.97%). The therapeutic effects of thyme are due to its high content of phenolic compounds, particularly carvacrol. The most important compounds of *F. vulgare* essential oil is anethole, fenchone, limonene that has antibacterial, antioxidant, antifungal and anticancer effects. The previous studies show that alpha pinene significantly inhibits many pathogenic Gram-negative bacteria. In aquaculture studies, *in vitro* and *in vivo* effects of the dominant compounds of essential oils in our study are consistent with the previous findings.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 6(10): 1501-1506, 2018

Balık Hastalıkları ile Mücadelede Bitki Esansiyel Yağlarının Major Komponentlerinin Önemi Üzerine bir Araştırma

MAKALE BİLGİSİ

Araştırma Makalesi

Geliş 27 Temmuz 2018
Kabul 18 Eylül 2018

Anahtar Kelimeler:
Centauriumerythraea
Foeniculumvulgare
Esansiyel yağ
Major bileşen
Thymus vulgaris

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ÖZ

Taninler, alkaloidler, terpenoidler ve fenolik bileşikler gibi esansiyel yağlardaki farklı fitokimyasalbileşenlerin mevcudiyeti anti-bakteriyel, anti-fungal ve anti-inflamatuar etkiler göstermektedir. Bu çalışmanın amacı üç bitkiye ait esansiyel yağın (*Thymus vulgaris* L., *Centaurium erythraea* Rafn. ve *Foeniculum vulgare* Mill) major bileşiklerinin balık hastalıkları ile mücadeledeki rolünü ortaya koymaktır. Ticari bir firmadan temin edilen esansiyel yağlara ait bileşenler GC/MS analizleriyle belirlendi. *T. vulgaris*'in en önemli bileşeni phenol, 2-methyl-5-(1-methylethyl) olarak adlandırılan karvakroldü (%40). Diğerleri ise linalool L (%15,11) ve benzene, methyl(1-methylethyl)-(%12,12) olarak belirlendi. *C. erythraea*'nın en zengin esansiyel yağı %34,90 ilebicyclo [3.1.1] hept-2-ene, 2,6,6-trimethyl-, (ñ)- diğer adıyla alfa-pinendi. Diğerleri sırasıyla heptacosane (%19,15) ve dotriacontane (%17,72) olarak bulundu. *F. vulgare* esansiyel yağı değerlendirildiğinde ise benzene, 1-methoxy-4-(1-propenyl)- (eş anlamlısı Cis-Anethol) %67,99 en önemli bileşen olarak bulundu, onu dl-Limonene (%16,03) ve benzene, 1-methoxy-4-(2-propenyl)- (%6,97) izledi. Kekiğin terapötik etkisi yüksek fenolik içeriğinden dolayıdır (özellikle karvakrol). *F. vulgare* esansyel yağındaki en önemli bileşenler antibakteriyel, antioksidan, antifungal ve antikanser etkileri olan anethole, fenchone ve limonene' dir. Önceki çalışmalar gösteriyor ki alfa-pinene birçok patojenik gram negatif bakteri üzerinde inhibe edici etkiler göstermektedir. Bizim araştırmamızda bahsi geçen esansiyel yağların dominant bileşenleri, balık yetiştiriciliği ile ilgili hem *in viv* hemde *in vitro* önceki bulgularla uyumluluk arz etmektedir.

DOI: https://doi.org/10.24925/turjaf.v6i10.1501-1506.2151

Introduction

In recent years, development of aquaculture industry has largely increased. However, the diseases may occur under the influence of various factors such as biotic and abiotic effects (particularly disease-causing agents and dense fish stock etc.) in freshwater and marine aquaculture. To prevent problems with disease, antibiotics are frequently used for many years. However, because of the antibiotic resistance and residue problem, etc. scientists focused to use products of natural herbs as alternative to antibiotics that specially the essential oils of these natural herbs that have powerful health benefits.

The volatile and aromatic compounds derived from plants are called essential oils, which has the medical value. The presence of different phytochemical components of essential oil such as tannins, alkaloids, terpenoids and phenolic compounds has anti-bacterial, anti-fungal, and anti-inflammatory effects (Hill, 1952 Yağız et al., 2017).

Thyme, which is one of the highest medical values, belongs to Lamiaceae family. Additionally, it is known as common thyme and it is indigenous to Mediterranean region, to the North Africa and several parts of Asia along with carefully discriminating in areas of the entire world. The pharmacological activities of *Thymus vulgaris* both of plant extracts and essential oil with the last decades studies were reported (Zheng et al., 2009; Lambert et al., 2011). The therapeutic effects of thyme are due to its high content of phenolic compounds, particularly carvacrol and thymol. Many *in vivo* studies have reported that oral administration of combination of carvacrol and thymol on *Ictaluruspunctatus* (Zheng et al., 2009) and *Oncorhynchusmykiss* (Ahmadifar et al., 2011; Küçükgül et al., 2013) improved growth performance, disease resistance and/or immunity.

*Centauriumerythraea*Rafn. commonly known as centaury is an annual or biennial medicinal plant belonging to the Gentianaceae family. The species inhabits dry grassland, scrub, and mountain habitats (Flora EuropaeaDatabase, 2002) *C. erythraea* is one of medicinal plants, which appears many countries of Europe, Mediterranean regions, America and Azores (Flora Europaea database 2002). In Turkey, it shows distribution Marmara, Black Sea, Aegean, Central and Eastern Anatolia, Mediterranean and South-East Anatolia regions (Davis, 1988). Previous studies investigated the antibacterial effect of *C. erythraea* essential oil showed that phytochemical components have alkaloids, coumarins, phenolic acids, secoiridoids, and xanthone (Valentão et al., 2003). In other *in vivo* study evaluated the effect of dietary centaury oil on growth performance, some environmental stress parameters and antioxidant activity in juvenile rainbow trout, positive effects upon oxidant status and oxidative stress index were observed (Çilingir et al., 2017).

Fennel (*Foeniculumvulgare*Mill) is one of the world's most important medical plants has different pharmacological properties such as anti-inflammatory, anti-oxidant, anti-bacterial, anti-stress, cytotoxicity, etc. Fennel sp. is used in many forms (essential oil, extract and hydrosol) on the treatment of bacterial, fungal and viral infectious diseases. In aquaculture sector, in addition to the treatment of disease, *F. vulgare* has been used to accelerate development and increase product quality (Muanda et al., 2011). There are more than 30 types of terpene compounds in the essential oil of fennel, the most important of them are 50 to 80% trans-anethole, 8% fenshon and limonene 5% (SalehiSurmaghi, 2006). The main part of *F. vulgare*essential oil is anethole, which has beneficial effects on growth, survival, body composition and hematological parameters. The main active compound (anethole) has been proposed as active agent on antioxidant, antibacterial activity, antifungal and anticancer effects (Mohamad et al., 2011; Dadalioglu and Evrendilek 2004; Mimica-Dukic et al., 2003).

The essential oil compositions of the three most studied medicinal herbs were determined by this research. The major components of them were determined by GC-MS analyzes and compared with the literature before.

Material and Methods

Essential Oils

Essential oils of three herbs, thyme (*Thymus vulgare*), fennel (*Foeniculumvulgare*) and centaury (*Centauriumerythraea)* were provided from a commercial firm, Elazığ, Turkey.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The components of essential oils were made by GC MS analyzes (Marmara Research Center Food Institute, Turkey). GC-MS analyses were carried out on a Shimadzu GC-9A gas chromatograph equipped with Thermon-600 T (30 m × 0.25 mm ID × 0.25-µm film). The scan mode was used for the total data. The structure of each compound was described using mass spectra with the Xcalibur program (Wiley 7). The retention times (RT) of the compounds detected and the RT value of volatile oil compounds were compared and the indices calculated (Bagamboula, 2004).

Results

Essential oil compositions of the herbs were viewed by chromatogram method (Figure 1).

The major component was phenol, 2-methyl-5-(1-methylethyl)- (or synonymous carvacrol) with 40% in composition of *T. vulgare* essential oil belonging toLamiaceae. The others were Linalool L (15.11%) and benzene, methyl (1-methylethyl)- (12.12%) (or synonymous cymene) (Table 1).

The chemical compositions of *C. erythraea*essential oil from this study are listed in Table 2. Bicyclo [3.1.1] hept-2-ene, 2,6,6-trimethyl-, (ñ)- (or synonmyeα-Pienene) in *C. erythraea essential oil* was the richest (34.90%). Other major compounds in the oil were Heptacosane (19.15%) and Dotriacontane (17.72%), respectively.

The major component in essential oil of *F. vulgaris* was benzene, 1-methoxy-4-(1-propenyl)- (or synonym Cis-Anethol) with 67.99%. Fennel essential oil also contains other components such as dl-Limonene (16.03%), Benzene, 1-methoxy-4-(2-propenyl)- (6.97%) (in Table 3).

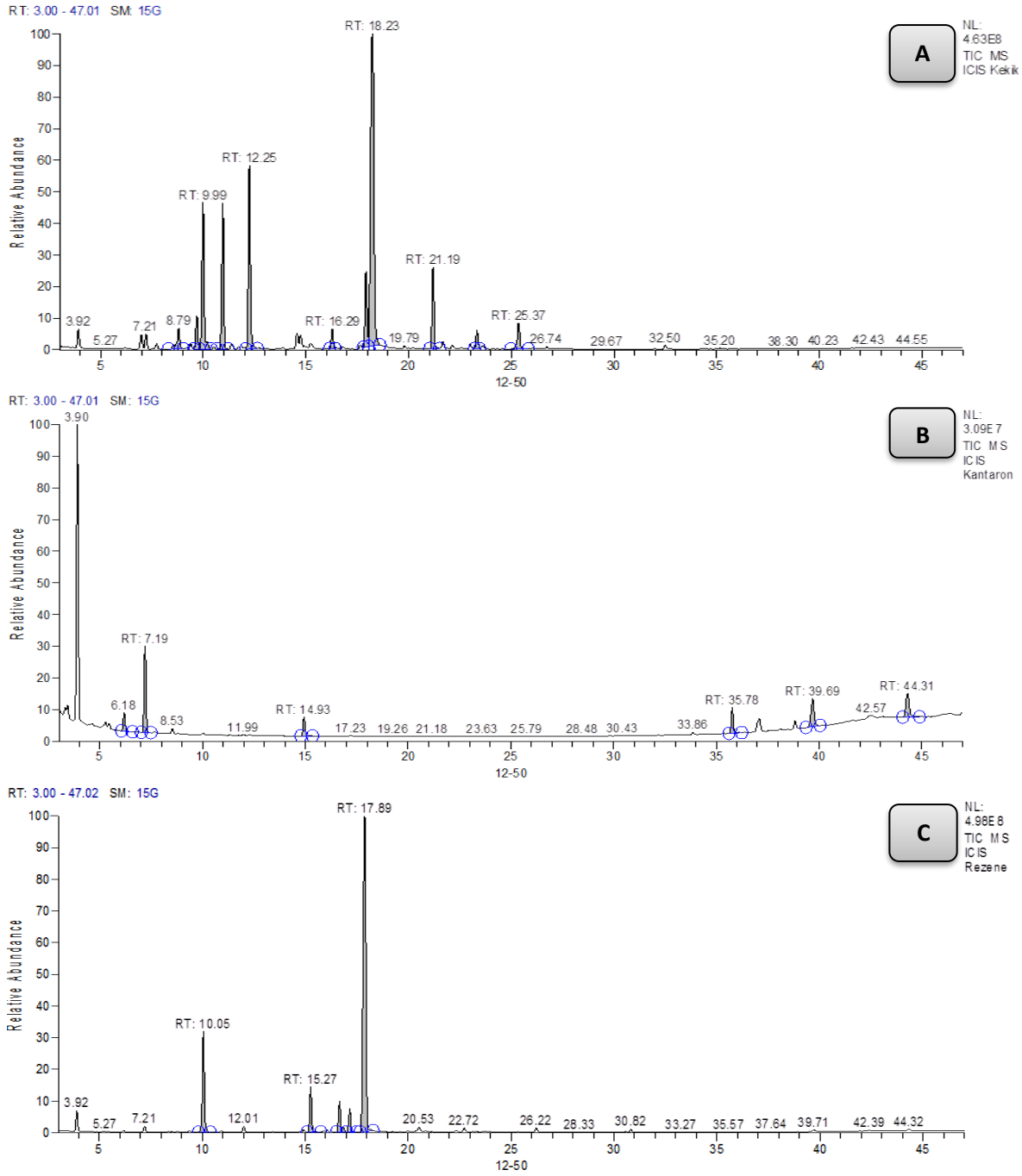

Figure 1 Essential oil compositions of the herbs chromatogram images (A: *T. vulgaris*; B: *C. erythraea*; C: *F. Vulgaris*)

Table 1 Chemical compositions of *Thymus vulgaris* essential oil

RT	T. vulgaris *(Thyme)*					
	PA	A	Compound Name (CA)	CAS	SI	RSI
8.79	285961843	2.22	á-Myrcene	123-35-3	935	941
9.68	311712797	2.42	1,3-Cyclohexadiene, 1 methyl-4-(1-methylethyl)-	99-86-5	929	938
9.99	1558067595	12.12	Benzene, methyl(1-methylethyl)- (CAS)	25155-15-1	937	940
10.94	1403300636	10.92	ç-Terpinene	99-85-4	935	938
12.25	1942135393	15.11	Linalool L	78-70-6	950	950
16.29	184987199	1.44	Carvacrol Methyl Ether	6379-73-3	949	951
17.93	755823234	5.88	Phenol, 5-methyl-2-(1-methylethyl)- (CAS)	89-83-8	933	937
18.23	5148364354	40.05	Phenol, 2-methyl-5-(1-methylethyl)- (CAS)	499-75-2	932	934
21.19	782585735	6.09	Caryophyllene	87-44-5	946	947
23.33	175887713	1.37	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	495-61-4	912	922
25.37	305952384	2.38	(-)-Caryophyllene oxide	1139-30-6	941	943

RT: Retention Time, PA: Peak Area, A: Area (%), CAS: CAS Registry Number

Table 2 Chemical compositions of *Centaurium erythraea* essential oil

RT	Centauriumerythraea (Centaury)					
	PA	A	Compound Name (CA)	CAS	SI	RSI
6.18	11767021	7.58	Nonane (CAS	111-84-2	906	925
7.19	54154612	34.90	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (ñ)-	2437-95-8	939	951
14.93	13896077	8.96	Dodecane (CAS)	112-40-3	880	911
35.78	18121940	11.68	2-(7-Hydroxymethyl-3,11-Dımethyl-Dodeca-2,6,10-Trıenyl)	NA	728	769
39.69	29717648	19.15	Heptacosane (CAS)	593-49-7	776	808
44.31	27492892	17.72	Dotriacontane (CAS)	544-85-4	724	737

RT: Retention Time, PA: Peak Area, A: Area (%), CAS: CAS Registry Number

Table 3 Chemical compositions of *Foeniculum vulgare* essential oil

RT	*Foeniculumvulgare* (Fennel)					
	PA	A	Compound Name (CA)	CAS	SI	RSI
10.05	1113831854	16.03	dl-Limonene	138-86-3	925	926
15.27	484400216	6.97	Benzene, 1-methoxy-4-(2-propenyl)- (CAS)	140-67-0	946	953
16.67	372032722	5.37	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (S)-	2244-16-8	937	953
17.16	254578110	3.66	Benzaldehyde, 4-methoxy- (CAS)	123-11-5	949	950
17.89	4725541671	67.99	Benzene, 1-methoxy-4-(1-propenyl)- (CAS)	104-46-1	943	943

RT: Retention Time, PA: Peak Area, A: Area (%), CAS: CAS Registry Number

Discussion

Herbs have been used for many years in the treatment of diseases because of low side effect compared to chemical drugs and being good alternative (Kooti et al., 2014; Beyrami-Miavagi et al., 2014). Medical herbs has many beneficial effects such as anti-oxidant, anti-bacterial, anti-fungal and anti-cancer (Duško et al., 2006; Kaur et al., 2009; Faudale et al., 2008; Baranauskiene et al, 2003; Bogucka-Kocka et al., 2008). Recently, the use of herbals has become widespread to treat fish diseases (Rattanachaikusopon and Phumkhachorn, 2009; Seden et al., 2009; Maack and Segner, 2003; Küçükgül et al., 2013). The most studied plants in the treatment of fish diseases were thyme, centaury and fennel in previous studies (Küçükgül et al., 2013; Viuda-Martos et al., 2011; Seden et al., 2009). As a result, the objective of the present study was to evaluate effects of the major components on the essential oils of these three herbs.

Many studies reveal that the main constituents of thyme include thymol, carvacrol and flavonoids. Martinez et al. (2006) reported that the active components of thyme essential oil were carvacrol, thymol and p-cymene. Our results on the major component of *F. vulgaris* confirm the findings of Martinez et al. (2006). In contrast to our results, Aziziet al. (2012) showed that thymol (62.5%) was the dominant compound. Some researches emphasize the existence of differences in the chemical composition among the extracted oils of different species or varieties (Martins et al., 2003; Gobbo-Neto and Lopez, 2007). Zheng et al. (2009) carvacrol and thymol are the two main active components of oregano essential oil added to the diets of channel catfish (*I. punctatus*) and evaluated the antibacterial effects of fish to *Aeromonashydrophila* infection. These researchers found that carvacrol and thymol reduced fish mortality. In other study, diets supplemented with *Origaniumvulgare* improve performance of Nile tilapia fingerlings (Seden et al., 2009). In aquaculture, *in vivo* and *in vitro* studies result that of both carvacrol and thymol has beneficial effect to treat of fish diseases (Küçükgül et al., 2003).

Fennel (*F. vulgare*) essential oil, one of the other important medical herbs, contains more than thirty types of terpenes or terpenoids compounds, of which anethole, fenchone, limonene are the most important ones (Albert-Puleo 1980). In one study, it was reported that the essential oil content of fennel was trans-anethole as the main component and varied between 81.63% and 87.85% (Telci et al., 2009). Tognolini et al. (2007) who they observed the phenylpropenesestragol and trans-anethole as the major constituents of *F. vulgare* reported similar results. These results confirm our findings on the major component of fennel (anethole 67.99%). The differences in the makeup of the essential oils from the same plant species depending on the phonological state and origin of the fennel, also the method of extraction and geographical origin (Diaaz-Maroto et al., 2006). Many scientists have focused on the therapeutic usage of the fennel due to anethole, which has important role beneficial activity such as antibacterial, antifungal, antioxidant, etc. (Küçükgül et al., 2013; Dadalioglu and Evrendilek 2004; Mimica-Dukic et al., 2003).Küçükgül et al (2013) studied the therapeutic effects of thyme and fennel essential oils on rainbow trout (*O. mykiss*)*Yersinia ruckeri*-infected. According to these results, the authors proposed that essential oils used at the appropriate dose could be natural defenders against the infections of fish.

Previous phytochemical investigations on *C. erythraea* show that it includes alkaloids, coumarins, phenolic acids, secoiridoids, triterpenes and xanthone derivatives (Phytochemical and Ethnobotanical Database, 2002). Kumarasamy et al. (2003) isolated the most abundant monoterpene in GC-MS analyses of *C. erythraea*oil. Our results show that alpha-pienene (34.90%) is the main component of *C. erythraea*essential oil.Many studies have conducted to reveal the activities such asanti-bacterial, anti-fungal and anti-oxidant of this plant thatis traditional medicinal specie(Laranjinha et al., 1995; Valentão et al., 2003; Dzotamet al., 2016). In a study, antibacterial activity of *C. erytraea* on gram positive bacteria (*Staphylococcusaureus* and *Listeria*

monocytogenes) was examined, and this finding revealed that it have indicated the sensitivity of Gram-positive bacteria (Dzotamet al., 2016). Some authors have attributed the antimicrobial activity of some essential oils to monoterpenessuch as α-pinene (Aligiannis et al., 2001; Leite et al., 2007). Davidson and Naidu (2000) identified a group of terpenes (borneol, camphore, 1, 8 cineole, α-pinene, camphene, verbenone and bornyl acetate) in rosemary, which are responsible for its antimicrobial activity. It is reasonable to assume that*C. erythraea*essential oil has potential usage as antimicrobials.

Conclusion

Composition and quantities of essential oils are depend on the type of plant, the part of the plant from which it is obtained, production seclusion, the age and the geographical structure of the region where it is cultivated (Baydar, 2005; Angioni et al., 2006; Masotti et al., 2002). The results of the research on the antibacterial, antifungal, antiviral and antioxidative effects of the major compounds of plant essential oils are generally positive and compatible, particulary *in vivo* and *in vitro* treatments of fish diseases. Therefore, plant essential oils can be used in the treatment of various diseases also may play an important role as an alternative to antibiotics.

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