THE FATTY ACID SCANNING IN COMMERCIAL BITTER GOURD AND MARIGOLD OILS AND ANTIBACTERIAL ACTIVITY

Elif Ayşe ERDOĞAN ELIUZ[1]
ORCID NO: 0000-0003-4317-3000
Erdal YABALAK[2]
ORCID NO: 0000-0002-4009-4174

[1]Mersin University, Vocational School of Technical Sciences, Department of Food Technology, Mersin, Turkey
[2]Mersin University, Faculty of Arts and Science, Department of Chemistry, Mersin, Turkey
[2]Mersin University, Department of Nanotechnology and Advanced Materials, Mersin, Turkey

ÖZET

Mikroorganizmaların antibiyotik direnci son iki yılda covid-19 pandemisi ile yeni bir boyut kazanmış olabilir. Covid-19 enfeksiyonu hem doğuştan gelen hem de adaptif antibakteriyel konak savunmasını bozduğundan, *Streptococcus pneumoniae*, *Staphylococcus aureus* gibi bakteriler, ikincil bakteriyel pnömonilere neden olmak için fiziksel ve immünolojik bir bariyerin bu geçici uzlaşmasını kullanır ve ciddi hastalıklara yol açabilir. Aynı zamanda, Covid ile ilgili olsun ya da olmasın tüm viral enfeksiyonların ölümcül doğası, bakteriyel süperenfeksiyonlarla ilişkilendirilmiştir.

Son yıllarda insanların doğal bitkilere olan ilgisi artmış ve başta bulaşıcı hastalıklar olmak üzere birçok hastalığa karşı tedavi amaçlı bitkisel yağlar kullanılmıştır. Bu nedenle piyasada satılan bitkisel yağlara büyük ilgi vardır. Ancak etiketlerinde içerikleri ile ilgili bilgiler yazılmamaktadır. Bu çalışmada, ticari olarak temin edilebilen iki bitkisel yağın içerik analizi ve antibakteriyel etkisi araştırılmıştır.

İnsanlarda cilt hastalıklarından yanıklara ve enfeksiyonlara kadar birçok hastalığın tedavisinde ticari olarak kullanılan acı kabak (*Momordica charantia* L.) ve kadife çiçeği (*Calendula officinalis* L.) tohum yağları birçok firma adı altında satılmaktadır. Bu bitkilerin yağ içerik analizleri bilimsel araştırmalarla detaylı olarak raporlanmış ve özellikle spor beslenmesinde ve obeziteye karşı tedavilerde kullanılan konjuge linolenik asit açısından zengin oldukları kanıtlanmıştır. Ticari olarak temin edilebilen bu yağların içeriği ilk kez bu çalışma ile kontrol edilmiştir. Bu çalışmada aynısefa ve kudrret narı olarak satılan iki firmanın ürün içeriği ve antimikrobiyal özellikleri araştırılmıştır.

Anahtar kelimeler: *Momordica charantia*, *Calendula officinalis*, antimikrobiyal aktivite, ticari yağlar

ABSTRACT

Antibiotic resistance of microorganisms may have gained a new dimension with the covid-19 pandemic in the last two years. Because infection by Covid-19 impairs both innate and adaptive antibacterial host defenses, bacteria such as *Streptococcus pneumoniae*, *Staphylococcus aureus* use this temporary compromise of a physical and immunological barrier to cause secondary bacterial pneumonia and can lead to serious illness. At the same time, the fatal nature of all viral infections, whether or not Covid-related, has been associated with bacterial superinfections.

In recent years, people's interest has increased in natural plants and they have used plant oils for treatment purposes against many diseases, especially infectious diseases. For this reason, there is a great interest in herbal oils sold in the market. However, information about their contents is not written on their labels. In this study, the content analysis and antibacterial effect of two commercially available vegetable oils were investigated.

Bitter gourd (*Momordica charantia* L.) and marigold (*Calendula officinalis* L.) seed oils, which are commercially used in the treatment of many diseases from skin diseases to burns and infections among humans, are sold under many company names. The oil content analyzes of these plants have been reported in detail with scientific research and it has been proven that they are rich in conjugated linolenic acid, which is used especially in sports nutrition and treatments against obesity. The content of these commercially available oils was controlled by this study for the first time. In this study, the product content and antimicrobial properties of two companies sold as bitter gourd and marigold oil were investigated.

Keywords: *Momordica charantia*, *Calendula officinalis*, antimicrobial activity, commercial oils

INTRODUCTION

Fats are one of the basic elements that must be taken into our body in the daily diet. They are powerful energy stores that provide the absorption of vitamins and minerals entering our body and maintaining body temperature. They may contain varying amounts of components such as mono, diacylglycerols, sterols, phosphatides, cerebrosides, color and fragrance substances (Willis et al., 2002). However, due to many reasons such as production conditions, improper consumption or chemical changes in the production stages, oils can also cause some diseases such as obesity and cancer in the human body (Erdoğan Eliuz, 2020).

Antibiotic resistance of microorganisms may have gained a new dimension with the covid-19 pandemic in the last two years. Because infection by Covid-19 impairs both innate and adaptive antibacterial host defenses, bacteria such as *Streptococcus pneumoniae*, *Staphylococcus aureus* infect people faster. At the same time, the fatal nature of all viral infections, whether or not Covid-related, has been associated with bacterial superinfections (Clancy et al., 2021). Therefore, people's interest has increased in natural plants and they have used plant oils for treatment purposes against many diseases, especially infectious diseases. Among them, Bitter Gourd and Marigold plants, which we use in screening, are used in the treatment of many diseases such as infection, pain and burns among people (Şekeroğlu and Gezici, 2020).

Calendula officinalis is a member of the Asteraceae family and is cultivated especially in parks and gardens due to its attractive appearance. In our country, it is known with names such as "*aynısefa, altıncık, kadifeçiçeği, tıbbi nergiz*". Calendula seed is a plant rich in fatty acids and has chemical components such as triterpene saponosides, triterpene esters, phenolic compounds, carotenoids and volatile compounds (Çeçen 2009). *Momordica charantia* L., known as bitter gourd, karela, balsam pear or balsam apple, bitter gourd, is a tropical fruit belonging to the Cucurbitaceae family. In Turkey, it is known with names such as "*kudret narı, acı kabak, acı kavun, acı hıyar, acı dülek, balsam armudu*". It is included in the Momordica genus fruit group and has a thin body and a delicate structure. It is an annual, fruit 10-20 cm long, rough, ivy structure, yellow or red in summer blooming plant. Its homeland is India, however, it is produced in many parts of the world. In Turkey, it is grown in the Marmara and Aegean Regions. Bitter melon seeds are very rich in oil and protein content (Akihisa et al., 2007; Şavşatli et al., 2018).

In our study, content analysis and antimicrobial activity of commercial bitter gourd and marigold oils were performed.

MATERIALS AND METHODS

Preparation of the cold-pressed oil and analysis

Calendula officinalis (Lokman Sema Sultan) and *Momordica charantia* (Karden) were purchased from the herbal market, Mersin/Turkey. Gas chromatography (GC) was used to identify the fatty acid compositions of bitter gourd and marigold oil. Plant fatty acids were subjected to the preparation of methyl esters before GC analysis. Therefore, 6 mL of 0.5 N methanolic NaOH was added to 0.5 g of plant fatty acid, and the mixture was heated in a reflux water bath for 5-10 minutes. At the end of the period, the BF3-methanol complex was added, followed by the addition of 5 mL of heptane. At the end of the formed phase separation, the upper phase containing Na_2SO_4 containing fatty acids was taken to ideal sample apparatuses and prepared for GC. The GC-FID analysis was carried out on an Agilent gas chromatograph equipped with a flame ionization detector (FID). The separation was achieved using an HP Innowax capillary column (30 m x 0.25 mm x 0.25 μm film thickness) and helium as carrier gas. The temperature of column was 40 ^{o}C with 5 min initial hold, and to 140 °C at 10 °C/min, and then to 250 °C after 10 min; injection mode 1 μL, 280 ^{o}C, split 100:1. The results were compared with Fatty Acid Methyl Esters (FAME) standard (Sigma-Aldrich-supelco) (Erdoğan Eliuz et al., 2021).

Antimicrobial analysis

The antimicrobial properties of the oils were determined. For antimicrobial property: well dilution techniques were used to determine the antimicrobial activities of the oils. The standard types of microorganisms to be examined were: *Klebsiella pneumoniae*, *Escherichia coli* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 25853), *Staphylococcus aureus* (ATCC 25813), *Bacillus subtilis* (ATCC 6633), *Candida parapsilosis* (ATCC 22019), *Candida glabrata* (ATCC 4322). These microorganisms are incubated one day in Nutrient Broth (bacteria) and Sabouraud dextrose broth (yeasts) medium. Bacteria (~10^4 CFU) and fungal (~10^5 CFU) density are adjusted according to McFarland. Growing cultures are then seeded on Mueller Hinton agar plates by the spread method. The 6 mm wells are drilled in the middle of the Petri dishes. Oils (15μL) were added to the wells. At the end of the 24-hour incubation, the zones formed are measured.

RESULTS and DISCUSSION

The components of fatty acid of *C. officinalis* and *M. charantia* with their retention time (Rt) and relative abundance (%) were listed in Table 1. The *C. officinalis* was abundant in methyl linoleate (53.34%), cis-9-oleic acid methylester (32.65%), palmitic acid (6.04%), stearic acid (4.29%), methyl-cis-11-eicosanoate (1.64%), while the major FAs in *M. charantia* oil were methyl linoleate (57.24%), cis-9-oleic acid methylester (27.24%), palmitic acid (6.51%), stearic acid (3.84%), ligniseric acid (1.32%), decosadienoic acid (1.11%).

The results of the chemical composition of *C. officinalis* in this report compared with previous reports and it was found that they are essentially different. Linoleic (78.82%) and oleic acids (10.67%) were the main fatty acids of *C. officinalis* extracted in laboratory conditions (Erdoğan Eliuz 2020). Ashwlayan et al., (2018) reported that oleic acid was in the range of 68–73% and linoleic acid of 9.6-11.8% in *C. officinalis*. By contrast, both fatty acids were not detected in commercial *C. officinalis* in this work.

For *M. charantia* oil, in Zubair's study (2018), stearic acid and eleostearic acid were the most abundant fatty acids making up 37.60% and 39.16%, respectively. Palmitic acid (2.36%), oleic acid (8.71%), and linoleic acid 0.67% were the smaller amount in the oil. In another study, Ghaffar et al. (2017) showed that stearic acid (66.57%) and oleic acid (9.76%) were abundant in *M. charantia* oil. In our study, we did not detect oleic acid, eleostearic acid, and linoleic in commercial *M. charantia*.

Table 1. Chemical composition of *C. officinalis and M. charantia*

Fatty acid	***C. officinalis***		***M. charantia***	
	Rt	Ra	Rt	Ra
Miristic acid	19.746	**0.157**	-	-
Palmitic acid	23.118	**6.04**	23.053	**6.51**
Palmitoleic acid	24.148	**0.145**	-	-
Margaric acid	24.672	**0.070**	-	-
Stearic acid	26.336	**4.29**	26.193	**3.84**
Cis-9-oleic acid methylester	27.295	**32.65**	27.158	**27.24**
Methyl linoleate	28.714	**53.34**	28.561	**57.24**
Arachidic acid	-	-	29.134	**0.21**
N6 gama linolenic acid	29.536	**0.09**	-	-
Methyl-cis-11-eicosanoate	30.078	**1.64**	30.047	**0.36**
Behenic acid	31.945	**0.59**	31.907	**0.85**
N3 cis-11,14,17-eicotrienoic acid	32.870	**0.13**	-	-
Decosadienoic acid	-	-	34.449	**1.11**
Ligniseric acid	34.665	**0.24**	34.635	**1.32**

The antimicrobial activity of *C. officinalis* and *M. charantia* against *K. pneumoniae*, *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *C. parapsilosis*, *C. glabrata* were shown in Table 2. No antimicrobial activity was determined for both oils (IZ=0).

Table 2. IZ (mm) of *C. officinalis and M. charantia* against the pathogens

	IZ (mm)		
	C. officinalis	***M. charantia***	**Antibiotic**
K. pneumoniae	0	0	10.7±0.11
E. coli	0	0	7.7±0.2
E. faecalis	0	0	8.1±0.1
P. aeruginosa	0	0	12.1±0.01
S. aureus	0	0	9.0±0.01
B. subtilis	0	0	13.7±0.01
C. albicans	0	0	7.9±0.01
C. parapsilosis	0	0	9.2±0.02
C. glabrata	0	0	11±0.01

As positive control, ampicillin for bacteria and fluconazole for yeast (16 $\mu g.ml^{-1}$).

Erdoğan Eliuz (2020) showed that oil of *C. officinalis* was strong natural agent against many pathogens such *as E. coli, B. subtilis, S. aureus, E. fecalis, C. albicans,* and *C. parapsilosis*. The bioactive components such as proteins, seed oil, tannins of *M. charantia* showed antimicrobial activity against many pathogens including *Helicobacter pylori* (Gupta et al., 2011; Villarreal-La Torre et al., 2020).

CONCLUSION

In the study, the content analyzes of commercial *C. officinalis* and *M. charantia* fatty acids were similar to each other in terms of basic substances. Cis-9-oleic acid methyl ester and methyl linoleate were detected at high rates in both plants. The fatty acids of both plants were not active against bacteria, which may be due to their essential components. As a result, although oleic acid and linoleic acid are natural antimicrobial agents, it has been shown that Cis-9-oleic acid methyl ester and methyl linoleate fatty acids should not effective against microorganisms.

As a result;

* Cis-9-oleic acid methyl ester and methyl linoleate are esterification products. The esterification reaction may occur due to storage or early harvesting. It affects the quality.

* The contents of commercial oils should be written on their labels for food safety

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