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Determination the Fatty Acid Composition of the *Rumex patientia* L. Leaves and in vitro Antimicrobial Activity of their Different Extracts

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Abstract: *Rumex patientia* L. is from Polygonaceae family and there are 25 species of it in Turkey. *Rumex patientia* L. has antifebrile, laxative, diuretic, and pain killer properties. In this study, the extraction of the *Rumex patientia* L. leaf were done and determination the fat content and fatty acid composition by using GC device and to determinate in vitro antimicrobial activity of their different extracts were aimed. On the purpose of detection of antimicrobial activity; *Rumex patientia* L. leaf was extracted with ethanol, methanol, acetone, petroleum ether and water. *Rumex patientia* L. leaves was tested their different extracts using *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeroginosa, Pasteurella multocida, Yersinia enterocolitica, Klebsiella pneumoniae, Staphylococcus aureus* bacteria and *Candida albicans* yeast by agar-well diffusion technique. As a result of the study, 26 fatty acid components were detected on *Rumex patientia* L. leaf. In addition, among obtained extracts the highest antimicrobial activity was formed by methyl alcohol extract on *K. pneumoniae* bacteria (32mm).

Rumex patientia L. Yapraklarının Yağ Asitleri Kompozisyonunun ve Farklı Ekstraktlarının in vitro Antimikrobiyal Aktivitesinin Belirlenmesi

Anahtar Kelimeler
Antimikrobiyal,
Yağ asit kompozisyonu,
Rumex patientia L.

Özet: *Rumex patientia* L. (efelek) Polygonaceae familyasından olup, Türkiye'de 25 türü bulunmaktadır. *Rumex patientia* L. ateş düşürücü, kabızlığı giderici, idrar söktürücü, yara iyileştirici ve ağrı kesici özelliklere sahiptir. Yapılan çalışmada, *Rumex patientia* L. yaprağının ekstraksiyonu yapılarak yağ oranı ve yağ asidi kompozisyonu GC cihazı kullanılarak belirlenmesi ve farklı ekstraklarının in vitro antimikrobiyal aktivitesinin tespit edilmesi amaçlanmıştır. Antimikrobiyal aktivite tayini için; *Rumex patientia L.* (efelek) yaprağının etanol, metanol, aseton, petrol eteri ve su ile ekstraktı çıkarılmıştır. *Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas aeroginosa, Pasteurella multocida, Yersinia enterocolitica, Klebsiella pneumoniae, Staphylococcus aureus* bakterileri ve *Candida albicans* mayası kullanılarak agar kuyucuk difüzyon tekniği ile *Rumex patientia* L. (efelek) yaprağının farklı ekstraktları test edilmiştir. Çalışma sonucunda, *Rumex patientia* L. yaprağında toplam 26 adet yağ asit bileşenleri tespit edilmiştir. Ayrıca elde edilen ekstraktlardan antimikrobiyal olarak en yüksek aktivitenin; *K. pneumoniae* (32mm) bakterisi üzerine metil alkol ekstraktı tarafından oluşturulduğu görülmüştür.

1. Introduction

Rumex patient L. is from Polygonaceae family and there are 25 species of it in Turkey. It is a plant that grows in Anatolia and has green leaves between 25 and 50 cm [1, 2]. The most common species are *R. patientia* L., *R. crispus* L., *R. acetosa* L. *R. caucasicus* RECH., and *R. alpinus* L. [3].

Rumex species belonging to the Poligonaceae family are known to produce numerous biologically important secondary metabolites such as

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anthraquinones, naphthalenes, stilenoids, steroids, flavonoid glycosides, leucoanthocyanidins and phenolic acids [4]. Besides, one of the main component of *R. patientia* plant is catechin [5]. Several studies on catechins have shown that they have anti-diabetic potentials [6-8]. *R. patientia* L. is also known to have anti-oxidant properties by easing lipid peroxidation caused by various free radicals [9]. It has been reported that *R. patientia* can reduce abnormal changes in blood glucose levels when used in the subchronic treatment of diabetic rats and partly due to decreased lipid peroxidation in liver tissue may enhance lipid profile related HDL- and LDL-cholesterol [10]. The chemical components of *R. patientia* were investigated and the nepodine and 1,3,5-trihydroxy-7-methylanthraquinone compounds were isolated from this plant for the first time. The leaves and roots of plants are used in the treatment of various disorders such as infections, diarrhea, constipation, mild diabetes, edema, heptatitis antihypertensive, diuretic and analgesic and also in conventional medicine [11]. Because of the medicinal properties of the genus Rumex has been the center of the interest for many researchers. In several studies, the extracts of these plants and the compounds isolated from them have various pharmacological activities in vitro and in vivo, including anti-inflammatory, antioxidant, antitumor, antibacterial, antiviral and antifungal properties have shown [12]. *R. patientia* subsp. which is used to treat inflammatory disorders, pains, fever and infections in the Pamir Mountains in North-East Afghanistan has a weak inhibitory effect on *S. aureus*, *E. coli, B. subtilis* and *P. aeruginosa* was indicated [13].

Fatty acid, a straight hydrocarbon chain carrying a carboxyl group in its structure, is the most important element of the oil. They are classified as saturated and unsaturated fatty acids. Saturated fatty acids are less reactive than unsaturated fatty acids since they don't contain double bonds in their chain and no other functional group than the carboxyl group. This reactivity varies according to the number of double bonds in the fatty acid [14].

A great majority of unsaturated fats are of vegetable origin and are in liquid form. These oils contain essential fatty acids which are necessary for the body. The aim of this study is to determine the fatty acid composition of *Rumex patientia* L. leaf and to compare the antimicrobial effect of different extracts. It is thought that since there is not enough study about the fatty acid composition of *Rumex patientia* L. leaf this study will shed light on the literature.

2. Material and Method

2.1. Material

Rumex patientia L. plant used in the experiments was obtained from Kars province and the leaves of the plant were dried and ground for use in the analysis.

2.1.1. Microorganisms

The standard strains which are *Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pasteurella multocida* (ATCC 12945), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 25213), *Yersinia enterocolitica* (ATCC 9610) and *Candida albicans* (ATCC 10231) were used to determine the antimicrobial effects of *Rumex patientia* L. leaf extract.

2.2. Method

2.2.1. Determination of fat content and fatty acid compounds

The crude oil, total fat in the leaves was determined by Soxhelet device (Foss Soxtec 2055). Fatty acid analysis was carried out by Flame Ionizer Detector (FID), Shimadzu Gas Chromatography (Model 2025) after the fatty acids were methylated.

Approximately 2-4 grams of homogeneously milled leaf samples were passed through a 1 mm micropellet and placed in soxhelet cartridges. It was then dried at 105 ° C for 1 hour in an oven, cooled in a desiccator, and 80-100 ml of hexane were added to the weighed extraction vessels. Samples were analyzed by inserting them into the Soxhelet device. After approximately 1.5 hours of analysis, the oil accumulated in containers was dried for 30 minutes in the oven and then cooled in the desiccator and weighed. Finally, % of fat was calculated from the weight difference (1).

$$\% \text{ fat} = (\text{tare} + \text{sample}) - (\text{tare}) * 100/\text{sample amount(gr)} \quad (1)$$

0.1 g of oil were taken into a 15 ml capped plastic centrifuge tube and 10 ml of n-hexane was added. Cover closed and then the tube vigorously shaken and 1 ml of 2N methanolic KOH solution was added. The cover was closed and again vigorously stirred and esterification occurred. Until the upper phase was clarified the sample were left for 1-2 hours and 1 microliter was taken from the supernatant by centrifugation. After the fatty acids were methylated, injection was applied to the Shimadzu Gas Chromatography (Model 2025) with Flame Ionizer Detector (FID). Supelco 37 Component Mix certified STD from Supelco was used in the analysis process. TR-CN100 column which is Teknocroma brand was used. The length is 60m, the film thickness is 0.25 micron and the inner diameter is 0.20 mm. The column oven was heated at 80°C for 2 minutes. Then, after reaching to 140°C by increasing 5°C per minute, it was kept at this temperature for 2 minutes. This was followed by increasing of 3°C /min to reach 240 °C and it was kept at this temperature for 5 minutes.

Total analysis time was 61 minutes. The injector temperature was 240°C and the detector temperature was 250°C. Helium was used as the carrier gas and the flow rate was set to 30 ml/min. The gas flows used were determined as H_2 = 40 ml/ min and dry air = 400 ml/min.

2.2.2. Preparation of *Rumex patientia* L. leaves extracts

Rumex patientia L. leaves were finely pulverized, the sample and distilled water, ethyl alcohol, methyl alcohol, acetone and petroleum ether was kept at 40°C for 48 hours in the rinsing water bath by maceration method for making plant: solvent ratio 1:20. After that, the solution was filtered through the Whatman type filter paper and the extracts were prepared by extracting the solvents at 50°C

2.2.3. Preparation of microorganism cultures and agar well diffusion Technique

Bacteria strains taken from stock cultures were suspended in 5 ml broth separately an incubated in incubator for 2-5 hours [15]. At the end of this period, after the bacterial suspension was adjusted to 10^8 cfu/ml for bacteria and 10^6 cfu/ml for yeasts, 100 µL of them was inoculated to petri dishes. A sterile swab was inoculated by drawing it in three different directions across the petri dish at frequent intervals. All petri dishes were then allowed to dry at room temperature for 5-15 minutes.

At the end of the period, 50 µL of the different *R. patienta* leaf extracts (distilled water, ethyl alcohol, methyl alcohol, acetone and petroleum ether) was transferred to the agar wells having 5 mm diameter. Plates in which bacteria were inoculated were incubated at 36 °C for 24 hours. After 24 and 48 hours, the diameters of the inhibition zones formed around the wells were measured. The antimicrobial activity experiments against all test microorganisms were done with three repetitions.

For control purposes; water, ethanol, methanol, acetone and petroleum ether, which are our solvents, were inoculated on the same medium to see if there was inhibition.

3. Results

3.1. Results of fatty acid components

The fat rate of *R. patientia* L. leaf was found as 2.3%. 26 fatty acid components were totally detected on *R. patientia* L. leaf. These are shown in Table 1.

As shown in Table 1, arachidic acid (%43.217) was found the highest one among the fatty acids determined by gas chromatography. Other fatty acids, 13.564% of linoleic acid, 11.620% of alpha-linolenic acid, 11.283% of Palmitic acid, 7.590% of Butyric acid and 4,297% of Elaidic acid were determined.

Table 1. Gas chromatographic analysis results of total fatty acids in *Rumex patientia* L.

Common and Systematic Name	Fatty acid percentages (%)
Butyric acid	**7.590**
Capric Acid	0.072
Tridecanoic Acid	0.185
Myristic Acid	0.305
Pentadecanoic Acid	0.236
Palmitic Acid	**11.283**
Palmiteloic Acid	0.662
Heptadecanoic Acid	0.142
Cis-10-Heptadecanoic Acid	0.549
Stearic Acid	1.193
Elaidic Acid	**4.297**
Oleic Acid	0.265
Linoleic Acid	**13.564**
gama-Linolenic Acid	0.364
alfa-Linolenic Acid	**11.620**
Arachidic Acid	**43.217**
Heneicosanoic Acid	0.129
Cis-11,14-Eicosadienoic Acid	0.327
Cis-8,11,14-Eicosatrienoic Acid	0.315
Behenic Acid	0.129
Erucic Acid	0.330
cis-13.16-Docosadienoic Acid	0.283
cis-5.8.11.14.17-Eicosapentaenoic Acid (EPA)	0.405
Lignoceric Acid	1.015
Nervonic Acid	0.498
cis-4,7,10,13,16,19-Docosahexaenoic Acid (DHA)	1.024

The saturated fatty acids were found most in the fatty acids composition of *R. patientia* L. leaves. As shown in Figure 1, among these saturated fatty acids, arachidic acid was detected in highest level. Arachidic acid is a saturated fatty acid with twenty-carbon, used as meaning of eicosanoic acid. They are found in adipocytes and milk fat of some animals and also breast milk fat in small quantities.

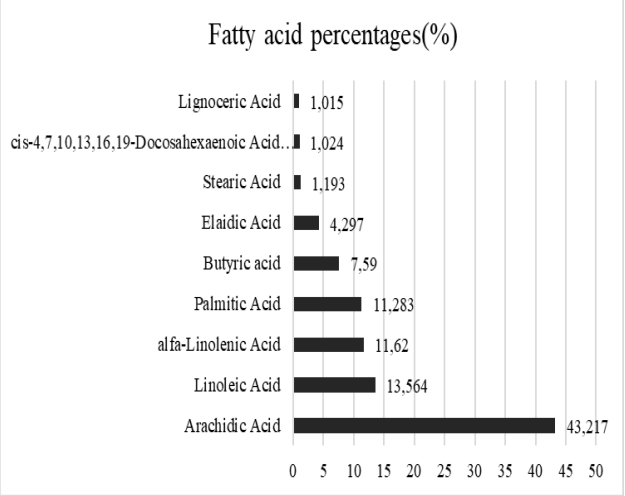

Figure 1. Most common fatty acid varities in *Rumex patientia L.*

3.2. Results of antimicrobial activity

The results of the study are given in Table 2. As seen in table, it has been observed that different extracts prepared from *R. patientia* L. leaf have high antimicrobial effect against test bacteria and yeasts. There is no difference between zone diameters after 24 and 48 hours.

Table 2. The inhibition diameters on the test bacteria and yeasts made by different extracts prepared from *R. patientia* L. leaf (mm).

Test microorganism	İnhibition zones (mm) Extracts				
	Ethyl Alcohol	Methyl Alcohol	Acetone	Petroleum Ether	Distilled Water
Bacillus cereus	R	R	R	12	R
Bacillus subtilis	30	20	20	20	R
Escherichia coli	10	8	10	14	R
Klebsiella pneumoniae	24	32	26	28	R
Pasteurella multocida	16	16	20	16	R
Pseudomonas aeruginosa	20	24	30	22	R
Staphylococcus aureus	24	20	20	18	8
Yersinia enterocolitica	18	16	20	22	R
Candida albicans	10	R	R	10	R

* R: Resistant

Among the extracts obtained, the highest activity belonged to methyl alcohol extract which formed it on *K. pneumoniae* (32mm). Water extract had no effect on any bacteria other than *S. aureus* (8 mm). According to the results obtained, it was determined that the extracts had low antimicrobial activity. It was found that the extract of ethyl alcohol and methyl alcohol had effect on *B. subtilis* (30 mm) and *K. pneumoniae* (32 mm), respectively and also the extract of acetone was the most effective on *P. aeroginosa* (30 mm).

4. Discussion and Conclusion

In several studies, the antimicrobial properties of the genus *Rumex* were determined [16]. The methanol and ether extracts of the different parts of *R. vesicarius* (collected in different vegetative stages) were found to be effective on *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. pneumoniae* and *S. pyogenes*. [17]. Methanol extract of *R. nepalensis* leaves have been reported to have moderate antimicrobial activity against *Bacillus cereus, B. subtilis, E. coli* and *P. aeruginosa* [18]. It was indicated that the water extract of *R. nepalensis* leaves produced the highest inhibition diameter against *E. coli* [19]. Benzene and extracts prepared from the roots of the same plant showed significant antibacterial activity against *S. aureus, S. mutans, E. coli* and *P. aeruginosa* [20]. In another antimicrobial study on the genus *Rumex*, ether extract of *Rumex crispus L*. leaves and seeds and and ethanol extract of the leaves have been shown to exhibit antimicrobial activity on the *Staphylococcus aureus* and *Bacillus subtilis* however none of the water extracts showed antimicrobial activity [21]. Orbán-Gyapai et al. [22] reported that hexane, chloroform and water extracts obtained from *R. patienta* leaves did not have any antibacterial activity. Our study supports these findings; among *R. patienta L*. extracts obtained from different solvent extracts, water extract almost did not show the antimicrobial activity and the highest antimicrobial activity was detected in alcohol and acetone extracts.

Omarova et al. [23] declared that in the leaves and roots of hybrid Rumex K-1 produced by *Rumex patientia L*. and *Rumex tianschanicus A.* hybridization and used as a feed additive has oleic acid, linolenic acid and palmitic acid and also the ratio of unsaturated fat was higher.

In our study, it has been seen that the saturated fat content ratio of total fatty acids in *R. patientia L.* leaves is higher than the unsaturated fat content ratio.

As seen in Table 1, the alpha-linolenic acid, linoleic, eicosapentaenoic and docosahexaenoic acids were found in *R. patientia L.* leaves, which are the most important polyunsaturated fatty acids. Since they aren't synthesized in the body, they must be taken through nutrients and are therefore called as essential fatty acids. They must exist at certain levels in fats and various fat products [14]. Omega fatty acids consisting of Omega 3 (alpha-linoleic acid), Omega 6 (linoleic acid) and Omega 9 (oleic acid) have many features such as brain development, prevention of coronary heart diseases, strengthening the immune system. Alpha-linolenic acid also participate in synthesizing of EPA and DHA [24]. Linoleic and alpha-linolenic acid exist abundantly in plant and fish fats. The essential fatty acids are used in the formation of hormones like prostaglandins and these compounds have involved in inflammation-related reactions [25]. In our study, the ratio of linoleic and alpha-linolenic acid in *R. patientia L.* leaves was determined as 25.184% (Figure 1).

It is clear that patience dock which uses for making soup, sarma and roast and consumed willingly in Kars province of Turkey in daily life, is rich in these essential fatty acids of the fish and this therefore will contribute to the healthy nutrition of people living in this shoreless city. Furthermore, these essential fatty acids found in *R. patientia L*. plants also vary according to soil, climate and applied planting conditions. The ratio of higher quality unsaturated fatty acids can be increased by means of making investigations on the location of the plant, and thus can be made much more beneficial to human health.

Since comprehensive datas about the fatty acid composition of *R. patientia* in Turkey were not observed in the literature, our study as a first one will shed light on the studies in terms of elaboration the fatty acid profile of *R. patientia*.

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