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Fatty acids, bioactive substances, antioxidant and antimicrobial activity of *Ankyropetalum* spp., a novel source of nervonic acid

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SUMMARY: *Ankyropetalum* extracts were obtained by using two different extractors (Soxhlet and ultrasonic bath). The phenol, flavonoid, DPPH, FRAP, and antimicrobial activity properties of the extracts were investigated. In addition, the fatty acid composition was determined in GC-MS. High values were found in *A. reuteri* and *A. gypsophiloides* for total phenolic and flavonoid contents, respectively. DPPH and FRAP values were high in *A. arsusianum* and *A. gypsophiloides*, respectively. Better results were obtained by using methanol as the solvent and soxhlet as the extractor. The results showed that the extracts seem to be reasonably effective against test organisms including clinical isolates. The most promising results were obtained with all species USB extracts against *Candida parapsilosis*. It is notable that the levels of nervonic acid in *A. arsusianum* and *A. reuteri* reached 40%. Unlike other sources of nervonic acid in the world, the absence of erucic acid in plant oil increases the value of these plants.

KEYWORDS: Ankyropetalum*; Antimicrobial activity; Antioxidant activity; Fatty acid; Nervonic acid*

RESUMEN: ***Ácidos grasos, bioactivos y actividades antioxidante y antimicrobiana de Ankyropetalum spp., una nueva fuente de ácido nervónico.*** Los extractos de *Ankyropetalum* se obtuvieron usando dos medios de extracción diferentes (Soxhlet y baño ultrasónico). A estos extractos se les determinó el contenido fenólico, flavonoides, DPPH, FRAP, y la actividad antimicrobiana. Además, se determinó la composición de ácidos grasos mediante GC-MS. Se encontraron valores altos de contenido fenólico y flavonoide total en *A. reuteri* y *A. gypsophiloides*, respectivamente. Los valores de DPPH y FRAP fueron altos en *A. arsusianum* y *A. gypsophiloides*, respectivamente. Se obtuvieron mejores resultados utilizando metanol como disolvente y Soxhlet como extractor. Los resultados mostraron que los extractos parecen ser razonablemente efectivos contra los organismos ensayados, incluidos los aislados clínicos. Los resultados más prometedores se obtuvieron con todos los extractos USB de especies contra la *Cándida parapsilosis*. Es notable que los niveles de ácido nervónico en *A. arsusianum* y *A. reuteri* alcanzaron el 40%. A diferencia de otras fuentes de ácido nervónico en el mundo, la ausencia de ácido erúcico en el aceite vegetal aumenta el valor de estas plantas.

PALABRAS CLAVE: *Ácido graso; Ácido nervónico; Actividad antimicrobiana; Actividad antioxidante;* Ankyropetalum

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1. INTRODUCTION

Ankyropetalum Fenzl is a small genus which includes only three species in the world. It belongs to the Caryophyllaceae family with 32 genera and 494 species in Turkey (Davis, 1982). The species are *A. arsusianum* Kotschy ex Boiss., *A. reuteri* Boiss. and Hausskn. and *A. gypsophiloides* Fenzl (Barkoudah, 1962). The gene center of the genus is Turkey, and the taxa of this genus are spread mainly throughout South-Eastern Anatolia and partially in the Mediterranean region (Ozcelik and Muca, 2010). While *A. reuteri* is endemic and belongs to the EN category, the other species are rare and found in South-east Anatolia and within the borders of neighboring countries (Ekim *et al*., 2000; Korkmaz and Ozcelik, 2011). The *Ankyropetalum*, *Gypsophila* and *Saponaria* members are known as "Çöven Otu" in Turkey. It is generally difficult to distinguish the *Ankyropetalum* from the perennial *Gypsophila* species. *Ankyropetalum* and *Gypsophyla* are used for similar purposes. *A. gypsophiloides* has been used in the preparation of a local food and *A. reuteri* has been mixed with straw and used as animal feed (Ozcelik and Muca, 2010). They are also used for the production of tahini halvah, foam halvah, Turkish delight, herbed cheese, çöven bread, and the manufacturing of chemical cleaner, fire extinguisher manufacturing, liquor making, and soap making (Korkmaz and Ozcelik, 2011).

Until the development of synthetic medicines from prehistoric times, plants were the basis of almost all medical treatments (Djeridane *et al*., 2006). Because of the less harmful effect than its synthetic counterparts, there is still interest in traditional herbal products today (Mocan *et al*., 2018). Herbal products contain biologically highly active compounds such as phenolic compounds, flavonoids, flavonols, and tocopherols, which play an important role in human nutrition and health (Mocan *et al*., 2018). It has been reported that there is an inverse relationship between the formation of human diseases and the consumption of various antioxidant plants (Dudonne *et al*., 2009). Therefore, research on the identification of antioxidative compounds is an important issue. Due to potential health risks and toxicity, there has also been an increased interest in the use of natural antioxidants in foodstuffs or medical materials in recent times (Djeridane *et al*., 2006). Fatty acids play an essential role in many body functions (Elias, 1983). Nervonic acid, one of the critical fatty acids, has been suggested to help maintain brain health, increase brain function, reduce fatigue, and accumulate less fat in the blood (Mohanty *et al*., 2013). There is a tendency for the use of nervonic acid to be added to foods for the treatment of neurological diseases such as Alzheimer's, multiple sclerosis, adrenoleuko-dystrophy and people with Zellweger's syndrome (Tang *et al*., 2013). It is also used as a medication in the symptomatic treatment of patients with schizophrenia, psychosis and attention deficit (Krishnan, 2009).

Since these species are endemic or rare and furthermore, they are not cultured, they are almost at the point of extinction. There are a limited number of studies on the taxonomy, ecology and economic importance of *Ankyropetalum*. *Ankyropetalum* is an economic plant with its commercial potential due to its addition to food. Despite its medicinal and economic value, there is no published biochemical or bioactivity study on this plant. Therefore, in this study, the total phenolic and flavonoid contents, antioxidant and antimicrobial activity of *A. arsusianum, A. reuteri* and *A. gypsophiloides* were investigated to find new potential sources of natural antioxidants. On the other hand, according to a GC-MS analysis, plant oils were found to be a novel source of nervonic acid.

2. MATERIALS AND METHODS

2.1. Plant materials

Three species of *Ankyropetalum* Fenzl were used in this study. The samples were collected from the natural habitats around Kahramanmaraş, Hatay and Gaziantep in the summer of 2015 (Table 1). The plants were identified according to the Flora of Turkey (Davis, 1982). The plants were kept in the herbarium of Kahramanmaras Sutcu Imam University [YZK-1123 (*A. reuteri*), YZK-1142 (*A. arsusianum*), YZK-1143 (*A. gypsophiloides*)].

2.2. Sample preparation and extraction

The plants were dried in the shade for about a week and then pulverized by grinding in a laboratory blender. Methanol and ethanol were used in two different extraction methods. Total phenolics, total flavonoids, antioxidant activity and antimicrobial activity were determined in these obtained extracts.

TABLE 1. Plant materials used in this study.

Species	Distribution	Altitude (m)	Location
A. arsusianum	narrow spread	10	Arsus/Hatay
A. gypsophiloides	narrow spread	700	Sahinbey/Gaziantep
A. reuteri	Endemic-narrow spread	565	Imali village-Turkoglu/ Kahramanmaras

2.2.1. Extraction method (SOX method)

The extraction was carried out using a Soxhlet apparatus at 60 °C for 6 hours with the addition of solvent (100 ml) to 10 g of plant material. After removal of the solvent in a vacuum rotary evaporator at 40 °C, the extract was kept at -20 °C for further analysis.

2.2.2. Extraction method (USB method)

Solvent (100 ml) was added to 10 g of plant and extraction was carried out in the Ultrasonic Water Bath for 1 hour at room temperature. The samples were centrifuged for 15 min at 3500 rpm. After centrifuging, the eluted liquid fraction was collected in another tube and the plant sample was extracted again as described above. The extracts were combined and the solvent was removed in a vacuum rotary evaporator at 40 °C and the extract was kept at -20 °C for further analysis.

2.3. Antioxidant assay

2.3.1. Determination of total phenolic and flavonoid content

Folin-Ciocalteau colorimetric method was used to determine the total phenolic contents of the fractions (Blainski *et al.*, 2013). Flavonoid compounds were extracted in the ultrasonic bath using 50 ml of 80% methanol:water (v/v) with 0.5 g of powdered plant samples for 20 min. Samples were centrifuged at 14000 rpm for 5 min. The total flavonoid content of the extracts was evaluated using spectrophotometry (Chang *et al.*, 2002). All extracts were tested in triplicate to confirm the reproducibility of the results.

2.3.2. DPPH and FRAP analysis

Scavenging free radical potentials were analyzed using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Brand-Williams *et al.*, 1995). Ascorbic acid was used as positive control. The antioxidant activity was expressed as IC_{50}, which denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The FRAP (The Ferric reducing antioxidant power) analysis was made according to Benzie and Strain (1996). Plant extracts (50 µl) were transferred to 2 ml eppendorf tubes and 600 µl FRAP agent were added. Absorbance was measured at 593 nm. The results were calculated as µmol ascorbic acid equivalent/g dry plant weight using the ascorbic acid (10-1000 $\mu mol \cdot l^{-1}$) calibration chart. Results were given in µmol/g dry plant weight. All extracts were tested in triplicate to confirm the reproducibility of the results.

2.4. Antimicrobial assay

2.4.1. Microorganisms and culturing

The bacteria and yeast were obtained from the culture collection of the Biotechnology Laboratory in Kahramanmaras Sutcu Imam University. *Bacillus subtilis* ATCC 6633, *Enterobacter cloacae* ATCC 13047D, *Escherichia coli* ATCC 39628, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538P, *Sarcina lutea* ATCC 9341NA, *Klebsiella pneumonia, Candida albicans, Candida parapsilosis, Candida glabrata* and *Saccharomyces cerevisiae* were handled as test organisms. They were maintained and activated on Sabouraud Dextrose and Nutrient Broth/Agar (Oxoid).

2.4.2. Antimicrobial activity assay

The antimicrobial activity of the ethanol and methanol extracts of *A. arsusianum, A. gypsophiloides* and *A. reuteri* were determined by the well diffusion method (Collins *et al.*, 1989). Mueller Hinton Agar (Difco) and Sabouraud Dextrose Agar (SDA) (Oxoid) plates were inoculated with a standardized inoculum, giving 1.5×10^8 bacteria and 2.1×10^3cfu ml^{-1} yeast (Collins *et al.*, 1989). The wells (6 mm) were prepared with a cork borer and filled with 50 µl of extract (20 mg·ml) dissolved in dimethyl

sulfoxide (DMSO). The plates inoculated with bacteria and yeast were then incubated at 37 and 30 °C for 24 and 48 hours, respectively. The inhibition zones produced were measured and the presence of antimicrobial substances was evaluated after an incubation period.

2.4.3. MIC determination

Minimum inhibition concentrations (MIC) of the extracts were determined according to the micro dilution method (Collins *et al*., 1989) in culture broth media. The extracts presented an inhibition zone in the well-diffusion method dissolved in DMSO and mixed with the Mueller Hinton and Sabouraud dextrose broth in a designed volume. Later, a dilution series was accomplished in micro well plates. As a control, culture medium and DMSO were set as growth control as well as test dilution for sterility control. After inoculation of the test well, 5 µl of organisms were changed, and the plates were incubated for 24/48 hours. The results were stated as $mg \cdot ml^{-1}$.

2.5. Determination of fatty acid content

The fatty acid content was analysed in a Shimadzu 2025 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and a column TR-CN100, 60 m × 0.25 mm × 0.20 mm. He was used as carrier gas at a flow rate of 1.5 $ml \cdot min^{-1}$. Initial column oven temperature was 80 °C for 2 min, then elevated to 140 °C at a rate of 5 $°C \cdot min^{-1}$ (maintained for 2 min at 140 °C), and then elevated to 240 °C at 3 $°C \cdot min^{-1}$ (maintained for 5 min at 240 °C). The detector and injection temperatures were programmed to 250 and 240 °C, respectively. Peak areas were used to calculate the relative percentage of the fatty acids as total fatty acid.

2.6. Statistical analysis

The statistical analysis appropriate for the entirely randomized (3x2x2) x3 factorial design with three replicates was performed. The hypotheses for the means of the primary and interaction effects were tested using the analysis of variance (ANOVA). Since factors of "solvent" and "extractor" have two levels, the direct use of F test is adequate for the related comparisons. If the results of the F test were significant, the means were determined to be statistically different. In addition, for the factors with more than two levels, comparisons of means were made by Tukey's test at the 0.05 significance level (Efe *et al*., 2000).

3. RESULTS

3.1. Antioxidant assay

Oxidative stress is thought to cause the development and progress of diseases as well as ageing. Many phenolic compounds with biologically essential effects are considered to be the most abundant antioxidants in foods (Mocan *et al*., 2018; Abeywickrama *et al*., 2016; Locatelli *et al*., 2017). Since different radicals and oxidants have different mechanisms of antioxidant response, there is no single way to measure antioxidant capacity (Isık *et al.,* 2015). Therefore, in this study, different solvents and extractors were applied to compare the total phenolic and flavonoid contents of *Ankyropetalum* species by using Folin-Ciocâlteu and $AlCl_3$ assays and the results are given in Table 2.

When means of the species and two-way interactions of species x extractor and species x solvent were examined, the highest total of phenolic substances was observed for *A. reuteri* extract (52.75, 48.04, 48.51 mg $GAE \cdot g^{-1}$, respectively). Two-way interactions of solvent x indicated the statistical superiority of methanolic extracts over ethanolic extracts in all types. On the other hand, interactions of the x extractor showed that soxhlet was statistically superior to USB in all types (Table 3).

Regarding flavonoid content, *A. gypsophiloides* shows superiority over other species. As in the total phenolic content, flavonoid content was also found to be poorer than *A. arsusianum* in comparison to the other species. Soxhlet and methanol were found to be more effective in eliciting the flavonoid contents in the species. Other researchers working with different species of Caryophyllaceae have also reported that methanolic extracts have higher phenolic and flavonoid contents than other solvents (Nikolova *et al*., 2011; Chima *et al*., 2014).

The lower the IC_{50} in DPPH analysis, the better the free radicals can be scavenged and thus impair the free radical chain reaction (Lim *et al*., 2007). Antioxidant activities were determined by DPPH and FRAP tests in this study and the results are presented in Table 1. The extracts of *A. arsusianum* and *A. gypsophiloides* were more active against DPPH free radical than *A. reuteri*.

TABLE 2. Total phenolic, total flavonoid, FRAP and DPPH results of *Ankyropetalum* species (The results were the mean of three replicates)

		Soxhlet		Ultrasonic Bath (USB)		
Method	**Species**	**Ethanol***	**Methanol***	**Ethanol***	**Methanol***	**Species Mean****
Total Phenolic Content (mg GAE·g^{-1})	***A. arsusianum***	30.94± 1.64^{d}	42.67± 2.02 ^{bc}	11.16± 1.13 ^{e}	41.94± 1.10 ^{bc}	31.68^{c}
	A. gypsophiloides	40.53± 0.78 ^{bc}	46.17 ± 1.92 ^{b}	13.27± 0.50 ^{e}	38.54± 0.21 ^{c}	34.62^{b}
	A. reuteri	43.33± 0.25 ^{bc}	52.75 ± 0.58 ^{a}	15.81± 0.29 ^{e}	44.27± 2.17 ^{bc}	39.04^{a}
		Ethanol**	**Methanol****	**Ethanol****	**Methanol****	**Species Mean****
Total Flavonoid Content (mg QE·g^{-1})	***A. arsusianum***	0.9 ± 0,03 ^{f}	1.10 ± 0.06 ^{ef}	0.66 ± 0.03 ^{g}	1.04 ± 0.04 ^{ef}	0.93^{c}
	A. gypsophiloides	2.97 ± 0.13 ^{b}	3.56 ± 0.01 ^{a}	1.21 ± 0.02 ^{de}	2.24 ± 0.03 ^{c}	2.50^{a}
	A. reuteri	1.21 ± 0.01 ^{e}	1.29 ± 0.05 ^{de}	0.62 ± 0.02 ^{g}	1.47 ± 0.07 ^{d}	1.15^{b}
		Ethanol**	**Methanol****	**Ethanol****	**Methanol****	**Species Mean****
DPPH (mg dw·g^{-1})	***A. arsusianum***	1. 70 ± 0.00 ^{c}	1.47 ± 0.02 ^{ab}	1.91 ± 0.00 ^{e}	1.41 ± 0.03 ^{a}	1.62^{b}
	A. gypsophiloides	1.72 ± 0.01 ^{cd}	1.43 ± 0.01 ^{a}	1.70 ± 0.01 ^{c}	1.42 ± 0.02 ^{a}	1.58^{a}
	A. reuteri	1.90 ± 0.05 ^{e}	1.53 ± 0.01 ^{b}	1.81 ± 0.01 ^{d}	1.51 ± 0.02 ^{b}	1.65^{b}
		Ethanol **	**Methanol ****	**Ethanol ****	**Methanol ****	**Species Mean****
FRAP (µg AAE·g^{-1})	***A. arsusianum***	36.65 ± 0.47 ^{bc}	42.06 ± 0.35 ^{a}	15.61 ± 0.30 ^{e}	33.73 ± 0.55 ^{c}	32.34^{a}
	A.gypsophiloides	39.08 ± 0.52 ^{ab}	41.57 ± 0.34 ^{a}	14.86 ± 0.15 ^{e}	36.35 ± 0.32 ^{c}	32.64^{a}
	A. reuteri	29.86 ± 0.32 ^{d}	35.26 ± 0.71 ^{c}	10.48 ± 0.28 ^{f}	35.34 ± 0.58 ^{c}	27.74^{b}

* $P < 0.05$, ** $P < 0.01$

Methanolic extracts were more active than ethanolic extracts; although the extractors did not significantly affect DPPH activity. FRAP results were also found to be similar to DPPH. *A. reuteri* also showed lower activity than the other two species. Methanolic extracts were more active in all three species. However, unlike DPPH, it was statistically significant that the extracts obtained from the soxhlet were more active than USB extracts.

The phenolic compounds and flavonoids obtained from plants were shown to have abundant antioxidant activity in food products (Van Acker *et al*., 1996). In general, extracts with high radical scavenging activity had a high phenolic content. However, there was no significant relationship between total phenolic and flavonoid contents and antioxidant activity in this study. Phenolic content was highest in *A. reuterii*, whereas antioxidant activity was found to be lower than the other two species in both DPPH and FRAP tests. A similar situation was seen in total flavonoid content. According to the results from the DPPH and FRAP tests, *A. arsusianum* and *A. gypsophiloides* species had high activity; while *A. gypsophiloides* alone had superior flavonoid content. This lack of relationship is also present in different studies. For example, Arslan *et al.,* (2013) studied three species of *Gypsophila* (*G. arrostii*, *G. pilulifera*, *G. simonii*) from the same family and obtained the highest total phenolic content in *G. simonii* (15.15 mg·g^{-1}). According to the results from the ABTS and DPPH analyses, *G. pilulifera* had a stronger antioxidant activity compared to the other two species. A similar result was reported by Stankovic *et al.,* (2015). According to their studies, while the phenolic contents in *Hordeum hystrix* and *Puccinella limosa* were found to be low, these plants were shown to have the highest antioxidant activity. On the other hand, the total phenolic content, IC_{50} value and FRAP value of *G. pilulifera* extracts were 6.5 mg·g^{-1}, 4.56 mg·ml^{-1} and 23.5 µg·g^{-1}, respectively (Yazici and Ozmen, 2017). These values were considerably lower than the values obtained in this study.

There may be several reasons why *A. reuterii* had lower antioxidant potency than the other two species despite its high phenol content and relatively high flavonoid content. The reaction of DPPH or FRAP may have been reversed with some phenols (Percentage of disappearance in antioxidant activity), or the reaction between DPPH or FRAP and substrate molecules may have been slow (Lim *et al*., 2007; Huang *et al*., 2000).

TABLE 3. Two-way interactions of species x extractor and species x solvent

		Species x extractor		Species x solvent	
Method	**Species**	**Species x SOX****	**Species x USB****	**Species x Ethanol Mean****	**Species x Methanol Mean****
Total Phenolic Content (mg GAE·g^{-1})	***A. arsusianum***	36.81^{c}	26.55^{d}	21.05^{d}	42.305^{b}
	A.gypsophiloides	43.35^{b}	25.91^{de}	26.90^{c}	42.353^{b}
	A. reuteri	48.04^{a}	30.0^{4d}	29.57^{c}	48.513^{a}
		Species x SOX**	**Species x USB****	**Species x Ethanol Mean****	**Species x Methanol Mean****
Total Flavonoid Content (mg QE·g^{-1})	***A. arsusianum***	1.01^{d}	0.85^{e}	0.78^{e}	1.07^{d}
	A. gypsophiloides	3.27^{a}	1.723^{b}	2.10^{b}	2.90^{a}
	A. reuteri	1.25^{c}	1.05^{d}	0.91^{de}	1.38^{c}
		Species x SOX**	**Species x USB****	**Species x Ethanol Mean***	**Species x Methanol Mean***
DPPH (mg dw·g^{-1})	***A. arsusianum***	1.59^{ab}	1.66^{c}	1.81^{c}	1.44^{a}
	A.gypsophiloides	1.58^{ab}	1.56^{a}	1.71^{b}	1.43^{a}
	A. reuteri	1.72^{c}	1.66^{c}	1.86^{c}	1.52^{a}
		Species x SOX**	**Species x USB****	**Species x Ethanol Mean****	**Species x Methanol Mean****
FRAP (µg AAE·g^{-1})	***A. arsusianum***	39.35^{a}	25.33^{c}	26.13^{c}	38.55^{a}
	A.gypsophiloides	40.32^{a}	24.95^{c}	26.97^{c}	38.31^{a}
	A. reuteri	32.56^{b}	22.91^{d}	20.17^{d}	35.30^{b}

* $P < 0.05$, ** $P < 0.01$, SOX: Soxhlet, USB: Ultrasonic bath

3.2. Antimicrobial assay

The inhibitory activity of plant extracts was assayed against seven bacteria and four yeasts. The results showed that both solvent extracts of *A. arsusianum, A. gypsophiloides* and *A. reuteri* had substantial inhibitory activity against all the Gram-positive bacteria tested (Table 4). As *E. coli,* a member of gram-negative bacteria, was inhibited with all extracts, *Enterobacter cloaca* was the only one inhibited with the *A. reuteri* extract. However, *K. pneumonia* was not affected by any of the extracts. According to the general opinion, Gram-negative bacteria are already more resistant than Gram-positive organisms (Stickler and King, 1992). Among the 4 yeast strains, *Candida parapsilosis* was inhibited by three plant extracts, while *C. albicans* was inhibited only by *A. gypsophiloides* with the extract. On the contrary, *C. glabrata* and *Saccharomyces cerevisiae* were not affected. It could be an essential property having an inhibitory and non-inhibitory activity against pathogenic and non-pathogenic strains, respectively, for food and pharmaceuticals. Concerning extraction method, although neither soxhlet and USB nor methanol and ethanol were found superior to each other, the most promising results were obtained from the activity of *A.arsusianum, A.gypsophiloides* and *A.reuteri* USB extracts against *C. parapsilosis* (MIC: 0.781mg·ml^{-1}).

Around the world, numerous plants have been screened by many researchers with different methods against different microorganisms. Here in this study, the extract from *A. arsusianum*, *A. gypsophiloides* and *A. reuteri* obtained with different methods and solvents were tested against common microorganisms. As a result, these extracts seem to be reasonably effective against test organisms, including clinical isolates.

3.3. Fatty acid content

The oil content of *Ankyropetalum* plant extracts was 5.79% for *A. arsusianum*, 6.86% for *A. gypsophiloides* and 5.77% for *A. reuteri*. As a result of the fatty acid analysis of plant extracts, 18, 21 and 26 fatty acids were found in *A. arsusianum*, *A. reuteri* and *A. gypsophiloides*, respectively (Table 5). The major components were nervonic acid (23.66%, 39.76% and 42.88%), butyric acid (10.64%, 19.42% and 21.59%), palmitic, oleic and linoleic acids. Butyric acid was the major SFA in *A. arsusianum* and *A. reuteri* (19.42% and 21.59%, respectively), while it was palmitic acid (13.10%) in

TABLE 4. The antimicrobial activity of *Ankyropetalum* spp. against test microorganisms. (The results were the mean of three replicates)

			A. arsusianum		***A. gypsophiloides***		***A. reuteri***		
			Inhibition Zone (mm)	**MIC (mg·ml^{-1})**	**Inhibition Zone (mm)**	**MIC (mg·ml^{-1})**	**Inhibition Zone (mm)**	**MIC (mg·ml^{-1})**	**Gnc**
B. subtilis	SOX	Ethanol	9±1.52	6.25	11±0.57	6.25	10±1.54	25	21
		Methanol	12±0.57	6.25	8±0.54	12.5	8±1.52	12.5	
	USB	Ethanol	12±0.57	12.5	12±1.54	12.5	12±0.54	12.5	
		Methanol	14±1.54	12.5	14±0.52	12.5	12±0.57	12.5	
*E. cloaca**	SOX	Ethanol	-	NT	-	NT	7±1.00	50	16
		Methanol	-	NT	-	NT	9±1.52	50	
	USB	Ethanol	-	NT	-	NT	8±0.57	25	
		Methanol	-	NT	-	NT	9±0.52	25	
E. coli	SOX	Ethanol	10±2.00	25	10±1.15	12.5	11±2.00	25	24
		Methanol	10±1.15	6.25	8±1.15	12.5	10±1.00	50	
	USB	Ethanol	8±1.00	12.5	11±1.52	25	10±1.15	12.5	
		Methanol	12±1.15	12.5	10±1.57	25	12±1.15	25	
*E. faecalis**	SOX	Ethanol	11±0.00	12.5	11±1.52	12.5	12±1.52	25	26
		Methanol	12±1.52	12.5	8±1.00	25	10±1.52	12.5	
	USB	Ethanol	12±0.54	12.5	10±1.15	6.25	12±1.52	12.5	
		Methanol	12±0.54	12.5	11±1.15	6.25	11±1.52	12.5	
*S. aureus**	SOX	Ethanol	12±1.52	12.5	9±1.15	6.25	12±1.54	12.5	25
		Methanol	9±1.72	6.25	8±1.52	25	8±1.52	12.5	
	USB	Ethanol	10±1.15	12.5	9±1.73	12.5	10±1.00	25	
		Methanol	11±2.00	25	8±0.00	25	8±1.00	25	
S. lutea	SOX	Ethanol	14±1.52	25	13±0.00	25	13±1.52	12.5	28
		Methanol	11±1.52	6.25	12±..54	25	9±1.57	12.5	
	USB	Ethanol	10±1.00	12.5	13±1.73	12.5	10±1.00	12.5	
		Methanol	10±1.00	12.5	14±1.52	3.125	9±1.52	>50	
									Nys
*C. albicans**	SOX	Ethanol	-	NT	8±1.15	25	-	NT	18
		Methanol	-	NT	8±1.54	25	-	NT	
	USB	Ethanol	-	NT	8±1.52	25	-	NT	
		Methanol	-	NT	8±1.52	50	-	NT	
*C. parapsilosis**	SOX	Ethanol	11±1.00	6.25	9±0.00	3.125	11±1.52	12.5	12
		Methanol	10±1.00	3.125	12±0.52	0.781	8±1.54	25	
	USB	Ethanol	11±1.15	1.562	12±0.52	0.781	12±1.54	0.781	
		Methanol	12±1.15	0.781	11±0.57	0.781	9±0.00	3.125	

*Clinical isolate, NT: Not tested, -: No inhibition zone, MIC: Minimum inhibition concentration, Gnc: Gentamicin, Nys: Nystatine, *SOX: Soxhlet, USB: Ultrasonic bath

TABLE 5. Fatty acid compositions (%) of the plant extract of *Ankyropetalum* species (The results were the mean of three replicates)

	Number of Carbon Atoms	Fatty acids	*A. arsusianum* %	*A. gypsophiloides* %	*A. reuteri* %
1	C4:0	Butyric acid	**19.42 ± 0.03**	**10.64 ± 0.03**	**21.59 ± 0.04**
2	C6:0	Caproic Acid	0.14 ± 0.02	0.05 ± 0.01	0.13 ± 0.00
3	C8:0	Caprylic Acid	0.21 ± 0.00	0.09 ± 0.00	0.14 ± 0.00
4	C10:0	Capric Acid	-	0.04 ± 0.00	0.13 ± 0.00
5	C12:0	Lauric Acid	0.46 ± 0.00	0.45 ± 0.02	0.76 ± 0.01
6	C13:0	Tridecanoic Acid	-	0.159 ± 0.01	-
7	C14:0	Myristic Acid	0.91 ± 0.00	1.25 ± 0.01	0.92 ± 0.01
8	C15:0	Pentadecanoic Acid	-	-	0.21 ± 0.01
9	C16:0	Palmitic Acid	**9.11 ± 0.01**	**13.10 ± 0.03**	**6.95 ± 0.03**
10	C17:0	Heptadecanoic Acid	-	0.15 ± 0.00	-
11	C18:0	Stearic Acid	1.87 ± 0.02	3.13 ± 0.02	1.50 ± 0.01
12	C20:0	Arachidic Acid	2.29 ± 0.02	6.71 ± 0.02	2.58 ± 0.01
13	C22:0	Behenic Acid	-	0.49 ± 0.01	0.36 ± 0.00
14	C23:0	Tricosanoic Acid	-	0.22 ± 0.00	-
15	C24:0	Lignoceric Acid	-	0.37 ± 0.00	0.28 ± 0.00
16	C15:1	*Cis*-10-Pentadecanoic Acid	-	0.18 ± 0.00	-
17	C16:1	Palmitoleic Acid	-	0.37 ± 0.00	-
18	C17:1	*Cis*-10-Heptadecanoic Acid	2.23 ± 0.01	1.86 ± 0.02	2.48 ± 0.01
19	C18:1	Oleic Acid **Ω9**	**5.92 ± 0.02**	**13.88 ± 0.03**	**9.97 ± 0.03**
20	C20:1	*Cis*-11-Eicosenoic Acid **Ω9**	0.48 ± 0.00	0.38 ± 0.01	1.06 ± 0.01
21	C24:1	Nervonic Acid **Ω9**	**42.88 ± 0.03**	**23.66 ± 0.04**	**39.76 ± 0.04**
22	C18:2	Linoleic Acid **Ω6**	**6.09 ± 0.01**	**17.50 ± 0.03**	**3.31 ± 0.02**
23	C18:3	Gamma-Linolenic Acid **Ω6**	1.66 ± 0.01	1.34 ± 0.01	1.84 ± 0.01
24	C18:3	Alfa-Linolenic Acid **Ω3**	0.39 ± 0.00	0.60 ± 0.0	-
25	C20:4	Arachidonic Acid **Ω6**	1.43 ± 0.01	1.15 ± 0.02	2.66 ± 0.01
26	C20:5	*Cis*-5.8.11.14.17-Eicosapentaenoic **Ω3**	1.01 ± 0.01	0.60 ± 0.00	1.22 ± 0.01
27	C22:6	*Cis*-4,7,10,13,16,19-Docosahexaenoic **Ω3**	3.50 ± 0.02	1.62 ± 0.01	2.15 ± 0.01
		SFA (Saturated Fatty Acid)	34.41	36.85	35.52
		MUFA (Monounsaturated Fatty Acid)	51.51	40.33	53.27
		PUFA (Polyunsaturated Fatty Acid)	14.08	22.81	11.18
		Total	**100.00**	**99.99**	**99.24**

A. gypsophiloides. There is a need for saturated fats for energy, hormone production, cellular membranes and organs. Butyric acid reduces virulence (a disease-causing effect) and it is used both in hygiene measures and in protection measures (Van Immerseel *et al*., 2005). In addition, since butyric acid esters have pleasant odors or flavors, they are often used as food and perfume additives. Some saturated fatty acids are also necessary for important signalling and stabilization processes in the body. Saturated fatty acids that play an important role in these processes are known as palmitic acid, myristic acid and lauric acid (Mohanty *et al*., 2013). *Ankyropetalum*, which contains all three fatty acids, contains palmitic acid predominantly.

Parameters associated with significant risk factors for cardiovascular disease have been associated with dietary habits. Olive oil rich in MUFA is one of the main components of the Mediterranean diet (Teres *et al*., 2008). On the other hand, the literature is increasingly showing the benefits of PUFAs for alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorders, diabetes and other diseases (Finley and Shadidi, 2001). It has been observed that MUFAs contribute to the majority of the unsaturated fatty acid content in *Ankyropetalum* species. In all three species, the major MUFA is nervonic acid and the major PUFA is linoleic acid. It is known that some unsaturated fatty acids, such as nervonic acid, linoleic acid and *cis*-11 eicosenoic acid are suitable for human nutrition (Carvalho *et al*., 2006). The studied species contain all three fatty acids, two of which are dominant.

Interestingly, the amount of nervonic acid, especially in *A. arsusianum* plant oil, was higher than *Tropaeolum speciosum* (NA: 42.5%, EA: 17.3%) (Carlson *et al.*, 1993), *Lunaria annua* (NA: 30%, EA: 45%) (Guo *et al*., 2009); *Lunaria biennis* L. (NA: 36-48%, EA: 14-25%) (Katavic *et al*., 2012); transgenic *Brassica napus* (NA: 30%, EA: 20%) (Napier and Graham, 2010); *Cardamine graeca* L. (NA: 9-10%, EA: 43-54%) (Katavic *et al*., 2012); *Acer truncatum* (NA: 5.8%, EA: 17.2%) (Wang *et al*., 2006) seed oils, which are known as nervonic acid sources. However, as you can see, these plants contain erucic acid. Nervonic acid is abundant in the white matter of the brain and the peripheral nervous tissue. Nervonic acid, which plays a role in nerve cell myelin biosynthesis, is one of the major fatty acids that make up about 40% of the total fatty acids in the brain sphingolipids (Sandhir *et al.*, 1998; Taylor, 2010).

Interest in dietary therapy with nervonic acid-containing oils and fats has increased with the suggestion of Sargent *et al.,* (1994) that dietary nervonic acid may support the normal synthesis and function of myelin in brain and nerve tissues. This recommendation encouraged the development of refined, nervonic acid-enriched vegetable oil to make experiments on humans and animals. Nervonic acid can be evaluated as a bioactive lipid supplement for the promotion of human and animal health, but it has been reported that nervonic acid-rich vegetable oil with the minimal amount of erucic acid has to be developed to be able to carry out these applications (Guo *et al.,* 2009). In this context, *Ankyropetalum* oil which does not contain erucic acid at all and which contains nervonic acid at a satisfactory level will be preferred among other sources of nervonic acid.

4. CONCLUSIONS

Ankyropetalum is consumed locally as food and food additives. However, this plant has not been studied thoroughly enough. For this reason, the bioactive properties of *Ankyropetalum* have been examined in this study. The results show that *Ankyropetalum* is rich in phenols and flavonoids, as well as antioxidants and antimicrobials. The effects of extraction methods were evaluated and soxhlet extraction was found to be more effective in total phenolic content and FRAP. Methanol was superior to ethanol as solvent in extraction methods. With high MUFA and PUFA contents, *Ankyropetalum* oils can be evaluated in various fields as a valuable raw material that can be used in the pharmaceutical, cosmetic, perfume, and food and medicine industry. *Ankyropetalum* oil is rich in nervonic acid, so it has great potential for the symptomatic treatment of many neurodegenerative diseases such as multiple sclerosis, schizophrenia and Parkinson's disease. Further work on the isolation and identification of bioactive compounds will be beneficial for a better and specifically directed application.

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