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Evaluation of the chemical composition and biological activities of medicinal plants *Alkanna tinctoria* and *Teucrium polium* from Turkey

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ABSTRACT

Since ancient times, natural products from various plants have been used for the treatment of different diseases. *Alkanna* and *Teucrium* species have been considered medicinal plants and are used popularly around the world. So, investigating the pharmaceutical properties of these plants has been the subject of interest for many researchers. In this work, total phenolic and flavonoid content, antioxydant, antimicrobial activity and *in vitro* enzyme inhibition properties of *Alkanna tinctoria* and *Teucrium polium* ethanol and methanol extracts were investigated. Additionally, GC-MS analyses of oil composition of the plant extracts were performed and 21 different fatty acids were identified in *A. tinctoria* and 18 in *T. polium.* Palmitic acid was the major component in *A. tinctoria* and saturated fatty acids constitutes a large part of the oil composistion. On the other hand, linoleic acid was the major fatty acid in *T. polium* and unsaturated fatty acids constitutes a large part of the oil composition. According to the results, methanol was more effective than the ethanol for the extraction of bioactive components. Both of the extracts showed a good antioxidant activity and the inhibition of bacteria and yeasts. Slight inhibitory activity of *A. tinctoria* and protective action of *T. polium* on enzyme with other results indicate that these plant extracts have potential to control microorganisms which can be promising for food industry.

Introduction

Medicinal plants are a natural and valuable resource that has been used for thousands of years to protect and improve human health (Pandey and Gupta, 2020). The therapeutic effects of plants are due to secondary metabolites with antioxidant properties such as vitamin C, vitamin E, carotenes and phenolic acids they synthesize (Kurt et al., 2018). High free radical concentration in cells and tissues caused by various factors such as UV and X-ray radiation, stress, processed foods, environmental conditions, smoking, alcohol and drugs cause oxidative stress (Yashin et al., 2017). Oxidative stress occurs with the continuous increase of free radicals in cells that cause the oxidation of protein molecules, lipids and DNA, and can lead to the formation of cancerous cells and different diseases (Farahmandfar et al., 2019). It has been reported that these harmful effects can be reduced by regular consumption of foods and beverages with antioxidant activity, as they are substances that can prevent or delay oxidation (Kurt et al., 2018). Therefore, in this study, the bioactivities of two different plants, which are known to have medicinal effects and are consumed in our country, were studied.

The Boraginaceae family is represented by approximately 130 genera and 2300 species, generally distributed in Europe (Mediterranean region) and Asia (Tufa et al., 2019). Anatolia is one of the most important centers of Boraginaceae, with a high endemism rate of up to 35% (Özcan, 2008). *Alkanna* is a genus of Boraginaceae and 37 of 42 taxa in Turkey are endemic and the rate of endemism is approximately 87% (Yıldırım & Şenol, 2014). *Alkanna tinctoria* (L.) naturally grows in the

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Mediterranean and Southern Anatolian regions of Turkey (Zannou and Koca, 2020). The plant is used to prevent and treat ulcers, wounds, fever, inflammation, aging and herpes. These beneficial effects are due to the phytochemical compounds it contains (Zannou and Koca, 2020). The genus *Teucrium*, which belongs to the Lamiaceae family, includes 300 species all over the world, 96% of which are in the Mediterranean basin (El Atki et al., 2019). There are 48 taxa of the genus represented by 36 species in Turkey and 17 of them are endemic. The endemism rate is approximately 35% (Aksoy et al., 2020). The plant *Teucrium polium* is known in the literature for its richness in bioactive compounds such as essential oils, flavonoids, tannins and phenolic acids (El Atki et al., 2019). Both plants used in traditional medicine exhibit important biological activities such as antimicrobial, antioxidant, anti-inflammatory and anticancer, and their pharmaceutical uses have been reported (Tufa et al., 2019; El Atki et al., 2020).

The aim of this study is to determine the fatty acid profile of plant extracts of *A. tinctoria* and *T. polium* by extracting them with ethanol and methanol solvents, as well as studying antioxidant, antimicrobial and enzyme activities as well as total phenolic and flavonoid content.

Materials and methods

Sample preparation

A. tinctoria and *T. polium* samples used in this study naturally grow in Nizip region of Gaziantep and Göksun region of Kahramanmaraş province, respectively. Both plant samples were collected from natural areas by the local people during the summer vegetation period of 2019.The plants brought to the laboratory were dried for about two weeks at room temperature in a dry environment. These samples were ground in a laboratory blender (Waring Commercial), powdered and stored in glass bottles protected from light and moisture for the use in further experiments.

Extraction method

The extraction was performed by slightly modifying the method described by Comlekcioglu and Kutlu (2021). All analyses were made from aerial parts of plants. Two different solvents were used for extraction, ethanol and methanol. Fifty ml of solvent was added to the 10 g of plant samples, and extraction was provided in the Ultrasonic Water Bath for an hour at room temperature. After ultrasonication, the plant extracts were filtered with filter paper. The filtrate was collected in another bottle and the plant sample remaining on the filter paper was extracted two more times in the same manner. All filtrates were combined in the same bottle and then centrifuged at 3500 rpm for 15 minutes. The solvent was then removed to the vacuum rotary evaporator and dry extract was obtained. These dried extracts were stored at -20 °C until analysis.

Determination of oil content and fatty acid composition of Plant extracts

Oil extraction was carried out in a FOSS Soxtec 2055 device using 3 g of plant sample and 100 ml of hexane. GC-MS analyses were performed with the Schimadzu GC 2025 system ®. The total of 0.1 g of plant extract was mixed with 1 ml of KOH solution prepared with 2 N methanol and then vortexed for 2 min. After 15 minutes, 10 ml of hexane was added and the mixture was stirred well. After centrifugation at 7000 rpm for 10 min, 1 microliter of the upper phase was injected into the GC-MS device (Comlekcioglu et al., 2019). The analysis of the fixed oils was made with GC-MS according to Comlekcioglu and Kutlu (2021). GC-MS analyses were performed with the Schimadzu GC 2025 system ®. A TRCN-100 (60m x 0.25 mm x 0.20 µm film thickness) SE-54 fused silica capillary column was used. Electron energy was 70 eV. The injection amount was 1 µl. The analysis of the samples was increased by 5 °C per minute after being kept at 80 °C for 2 minutes, and after reaching a temperature of 140 °C, it was kept at this temperature for 2 minutes. Following this process, it was kept for 5 more minutes at 240 °C with an increase of 3 °C per minute. The total analysis time was set as 61 minutes. The injection and detector temperatures were set at 240 and 250 °C, respectively. The fatty acids were expressed as percentage of the total fatty acids, calculated with peak areas.

Determination of total phenolic content

The total phenolic content of the samples was made according to the Folin-Coicalteau Reagent method by using the procedure of Obanda and Owuor (1997). Briefly, the stock solution of dried extracts (300 µg/mL) was dissolved into ddH2O. One ml of this stock extract solution was mixed with 5 ml of ddH2O, 500 µl of 2 N Folin-Ciocalteu's phenol reagent and 6 ml of 10.75% w/v anhydrous sodium carbonate (w/v). Blank was prepared by replacing the extract with ddH2O. Gallic acid (Sigma) was used as standard. The prepared solutions were read at 750 nm in a spectrophotometer (Perkin-Elmer Lambda EZ 150,

USA). Obtained absorbance values are given in terms of mg gallic acid equivalent (GAE) / g dry sample weight with the help of the calibration curve created with gallic acid solutions. All experiments were done in triplicate.

Determination of total flavonoid content

Total flavonoid content in plant extracts was determined spectrophotometrically according to Chang et al. (2002). The standard solution was calculated with different concentrations (25-200 µg/mL) of quercetin (Sigma) prepared according to the procedure mentioned above. Absorbance was read in a spectrophotometer at 415 nm. Obtained absorbance values were converted into µg quercetin equivalent / g dry sample weight. All experiments were done in triplicate.

Determination of DPPH radical-scavenging activity

Antioxidant capacity (reduction capacity of free radicals) was identified by modifying the DPPH method defined by Brand-Williams et al. (1995).

Dilution series of each plant crude extract were prepared with methanol at concentrations varying according to the plant. Dilution series for *A. tinctoria*/Ethanol, *A. tinctoria*/Methanol, *T. polium*/Ethanol and *T. polium*/Methanol were 1-2-3-4 mg/mL, 0.25-0.5-0.75-1mg/mL, 0.125-0.25-0.5-1 mg/mL and 0.125-0.25-0.5-1 mg/mL, respectively (Figure 1). Briefly, 50 µl of four different concentrations of each plant extract were mixed with 950 µl of methanolic DPPH solution. The mixture was left in the darkness for 15 min at 25 ºC and the absorbance was measured at 517 nm against a reagent blank (50 µl of methanol+950 µl of DPPH methanolic solution). All experiments were carried out in triplicate. Ascorbic acid was used as positive control. The results are shown as IC50, which is the concentration required to reduce 50% of DPPH free radicals.

$$\%\ AA = \frac{\left[A_{control} - A_{sample}\right]}{A_{control}} \cdot 100$$

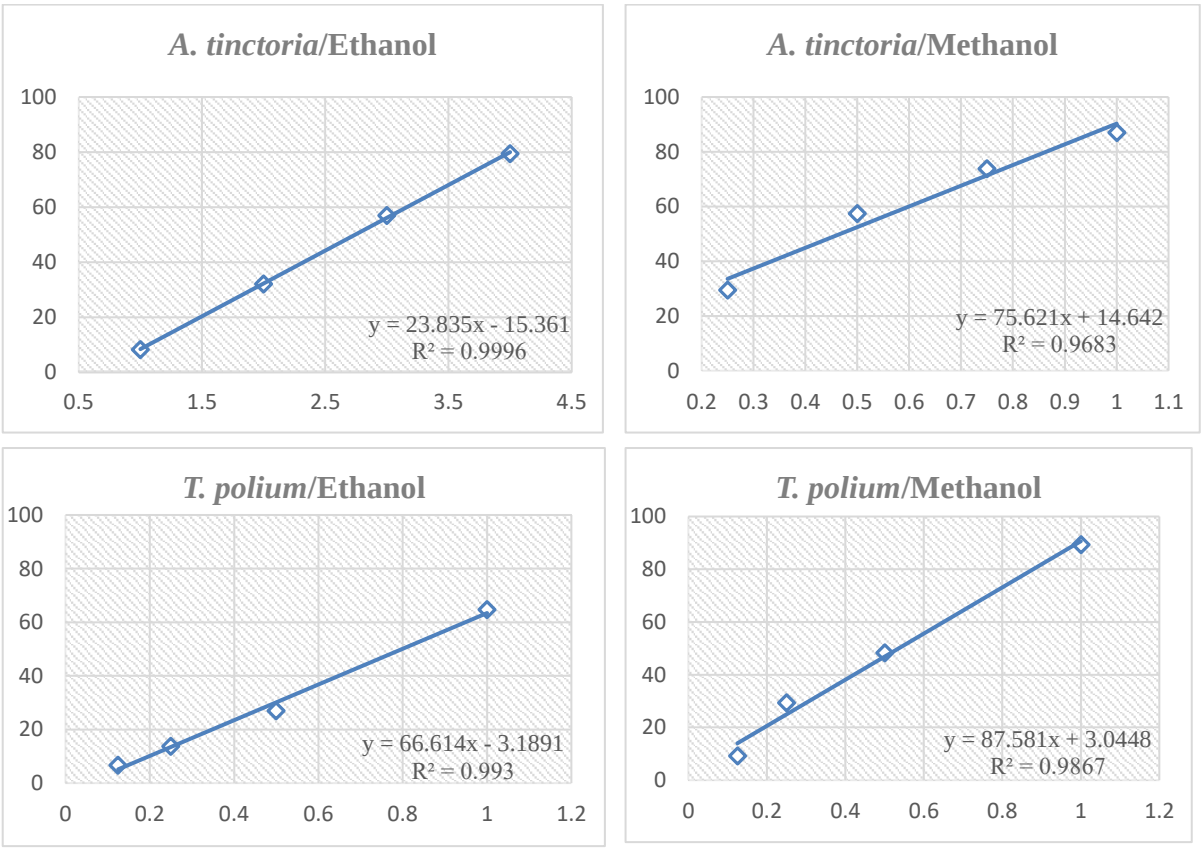

Figure 1. Calibration grafics prepared with percent inhibitions of plant extracts

Determination of FRAP activity

FRAP method was done according to Benzie and Strain (1996). Absorbance was measured at 593 nm. Results were calculated as μmol ascorbic acid equivalent/g dry plant weight using ascorbic acid (100–1000 μmol/L) calibration graph. Results are given as μmol/g dry plant weight.

Antimicrobial activity assay

Antimicrobial activities of the *A. tinctoria* and *T. polium* extracts were investigated by well diffusion assay. The extracts were tested against *Bacillus subtilis* (ATCC 6633), *Sarcina lutea* (ATCC 9341NA), *Staphylococcus aureus* (ATCC 29213), *Serratia marcessens, Escherichia coli* (ATCC 309628), and clinical isolates *Enterococcus faecium, Acinetobacter baumannii, Klebsiella pneumoniae, Candida parapsilosis and Candida albicans.* The cultures were maintained in Nutrient broth (Oxoid) and Sabouraud dextrose broth (Oxoid). Mueller Hinton plates were inoculated with a standardized suspension of McFarland 0.5 turbidity giving $1.5x10^8$ cfu/mL of bacterial strains and also Sabouraud dextrose agar was inoculated with yeast strains (Collins et al., 1989). Fifty μL of *A. tinctoria* and *T. polium* extracts (16 mg/mL), dissolved in DMSO (10%), were loaded into the wells (6 mm) aseptically after inoculation of the test microorganisms with a sterile cotton swab. After the incubation period (37 ºC for 24 h and 48 h), the diameter of inhibition zones was measured. DMSO (10%) was used as solvent control. The extracts producing an inhibition zone were tested for MIC determination in micro-well plates (Collins et al., 1989). The extracts were dissolved in Mueller Hinton Broth and serially diluted in microwell plates. Then inoculation was completed with 10 μL of the microorganism from overnight culture (McFarland 0.5 turbidity). After incubation period, the plates were checked in a microplate reader (SPECTRAmax 384 PLUS) for the lowest concentration showing no growth which was considered as MIC.

Determination of in vitro enzyme inhibiton activity

Enzyme inhibition activity of *A. tinctoria* and *T. polium* extracts was tested on alpha-amylase enzyme with optimum temperature and pH as 40 °C and 7.6, respectively. Plant extracts were dissolved (16mg/mL) in 100 mM phosphate buffer at pH 7.6 and centrifuged for 3 min at 4000 rpm. The enzyme inhibition activity of extracts was analysed by mixing and preincubating the extract (20μL) with enzyme at 40 °C for 0, 15 and 30 min. The remaining enzyme activity was tested by addition of 400 μL of soluble starch (1%) (Merck). After incubating the final mixture for 15 min at 40 °C, the reaction was ended by addition of 3,5-dinitrosalicylic acid reagent (500 μL) and absorbance (550 nm) was measured (Bernfeld, 1955) in a spectrophotometer (Perkin Elmer Lambda EZ 150). The enzymatic activity without preincubating the enzyme and substrate mixture in the absence of plant extracts was taken as 100%. The results were expressed as mean values of triplicates.

Results

Results on fatty acid composition

Studies on lipids and their structural elements, fatty acids, are one of the most interesting topics in biology and nutrition (German and Dillard, 2004). The research of lipid profiles is of great importance, because of their protective or therapeutic effects, such as providing information about the specific roles of lipid molecules in health and diseases, and the ability of lipid-related interventions to improve human health (Mohanty et al., 2013; Johnson and Stolzing, 2019). As a result of the fatty acid analysis of the plant extracts, 21 and 18 fatty acids were found in *A. tinctoria* and *T. polium*, respectively (Table 1 and Figure 2). The predominance of saturated fatty acids in the composition of *A. tinctoria* oil is also seen in the main components (myristic, palmitic, stearic acids). Oleic and gamma linolenic acids, which are the main components of *T. polium*, are also high in *A. tinctoria*. Unlike *A. tinctoria*, the major fatty acid of *T. polium* is linoleic acid (31.67%), which makes up one-third of the total oil. The consumption of certain amounts of linoleic acid is necessary for the presumed benefit of linoleic acid in heart diseases and in preventing the symptoms of essential fatty acid deficiency. Various health organizations, such as the American Heart Association and WHO, recommend consuming linoleic acid up to 10% energy level for health for linoleic acid intake (Jandacek, 2017). Gamma-linolenic acid (GLA), another essential fatty acid required by our body, is the precursor of prostaglandins and it has anti-inflammatory and antitumoral effects (Goffman and Galletti, 2001). The absence of GLA in diets causes serious health problems such as atopic eczema, diabetic neuropathy, rheumatoid arthritis, fertility, cardiovascular disorders and autoimmune diseases (Özcan, 2008). It is stated that dietary supplementation with monounsaturated fatty acids oleic and palmitoleic and PUFA treatment can prolong life (Johnson and Stolzing, 2019).

Table 1. Fatty acid compositions of *A. tinctoria* and *T. polium* (%)

		A. tinctoria	***T. polium***
Carbon Numbers	**Fatty Acids**	**Amount (%)**	
C4:0	Butyric acid	1.93 ± 0.02	-
C6:0	Caproic Acid	2.11 ± 0.02	-
C8:0	Caprylic Acid	1.76 ± 0.01	-
C10:0	Capric Acid	3.21 ± 0.01	-
C12:0	Lauric Acid	3.85 ± 0.02	-
C14:0	**Myristic Acid**	**9.54** ± 0.02	0.37 ± 0.01
C15:0	Pentadecanoic Acid	0.89 ± 0.01	-
C16:0	**Palmitic Acid**	**29.17** ± 0.12	6.67 ± 0.16
C18:0	**Stearic Acid**	**9.14 ± 0.08**	0.73 ± 0.01
C20:0	Arachidic Acid	-	0.81 ± 0.03
C21:0	Heneicosanoic Acid	0.34 ± 0.02	0.29 ± 0.01
C22:0	Behenic Acid	5.31 ± 0.14	1.78 ± 0.11
C23:0	Tricosanoic Acid	0.45 ± 0.02	0.48 ± 0.01
C24:0	Lignoceric Acid	3.05 ± 0.05	4.88 ± 0.14
C14:1	Myristoleic Acid Ω5	0.51 ± 0.02	-
C16:1	Palmiteloic Acid Ω7	0.66 ± 0.02	0.13 ± 0.00
C17:1	Heptadecanoic Acid	0.45 ± 0.01	2.46 ± 0.015
C18:1	**Oleic Acid Ω9**	**13.22** ± 0.16	**18.71** ± 0.18
C18:1	Elaidic Acid Ω9	-	0.48 ± 0.00
C20:1	Cis-11-Eicosenoic Acid Ω9	0.74 ± 0.02	-
C24:1	Nervonic Acid Ω9	-	0.94 ± 0.016
C18:2	**Linoleic Acid Ω6**	5.01 ± 0.15	**31.67** ± 0.32
C18:2	Linolelaidic Acid Ω6	-	7.37 ± 0.15
C18:3	Alfa-Linolenic Acid Ω3	-	0.84 ± 0.08
C18:3	**Gamma-Linolenic Acid Ω6**	**8.1** ± 0.13	**16.70** ± 0.23
C22:6	Cis-4,7,10,13,16,19-Docosahexaenoic Ω3	0.57 ± 0.01	4.71 ± 0.04

Since the fatty acid formation of *T. polium* contains these three important fatty acids (oleic, linoleic and gamma-linolenic acids) as the major component, it is a potentially beneficial plant due to its therapeutic effects. In addition to being important structural components of cell membranes, some saturated fatty acids are also necessary for the construction of various structural elements in cells and tissues, the production of signaling compounds, hormone production, and energy-storage (German and Dillard, 2004; Mohanty et al., 2013). Saturated fatty acids that play an important role in these processes are known as palmitic acid, myristic acid, stearic and lauric acid (Mohanty et al., 2013). The *A. tinctoria* plant contains these fatty acids, mainly palmitic acid. However, the general opinion is that excess fat intake (especially saturated fat) increases the possibility of getting different diseases such as obesity, diabetes, cholesterol, heart diseases.

In fact, it is important to note that the most of foods containing saturated fatty acids without side effects are also nutritionally adequate, as they provide valuable nutrients at the same time. Excessive intake of these fats in the diet should be avoided (German and Dillard, 2004). Although lipids have been traditionally viewed as harmful and simply associated with age-related diseases, numerous studies have shown that lipid metabolism strongly regulates aging and lifespan. The importance of lipids in life span depends on the appropriate interventions regarding different dietary, genetic, pharmacological and surgical lipids (Johnson and Stolzing, 2019).

In the literature, no fatty acid analysis was found from the aerial parts of *T. polium* and *A. tinctoria*. However, Hachicha et al. (2009) stated the main fatty acids in the seeds of *T. polium* as linoleic, linolenic and palmitic acids. In this study, unlike the seed, oleic acid was found to be one of the major components instead of palmitic acid in the aeral parts.

The fatty acid composition stated by Özcan (2008) in the seeds of *A. tinctoria* is very different from the results of this study. SFA:MUFA:PUFA ratio indicated as 9.60:23.66:65.86 in seeds whereas in this study, it was obtained as 70.75:15.58:13.68 in the aeral part of the plant (Figure 3).

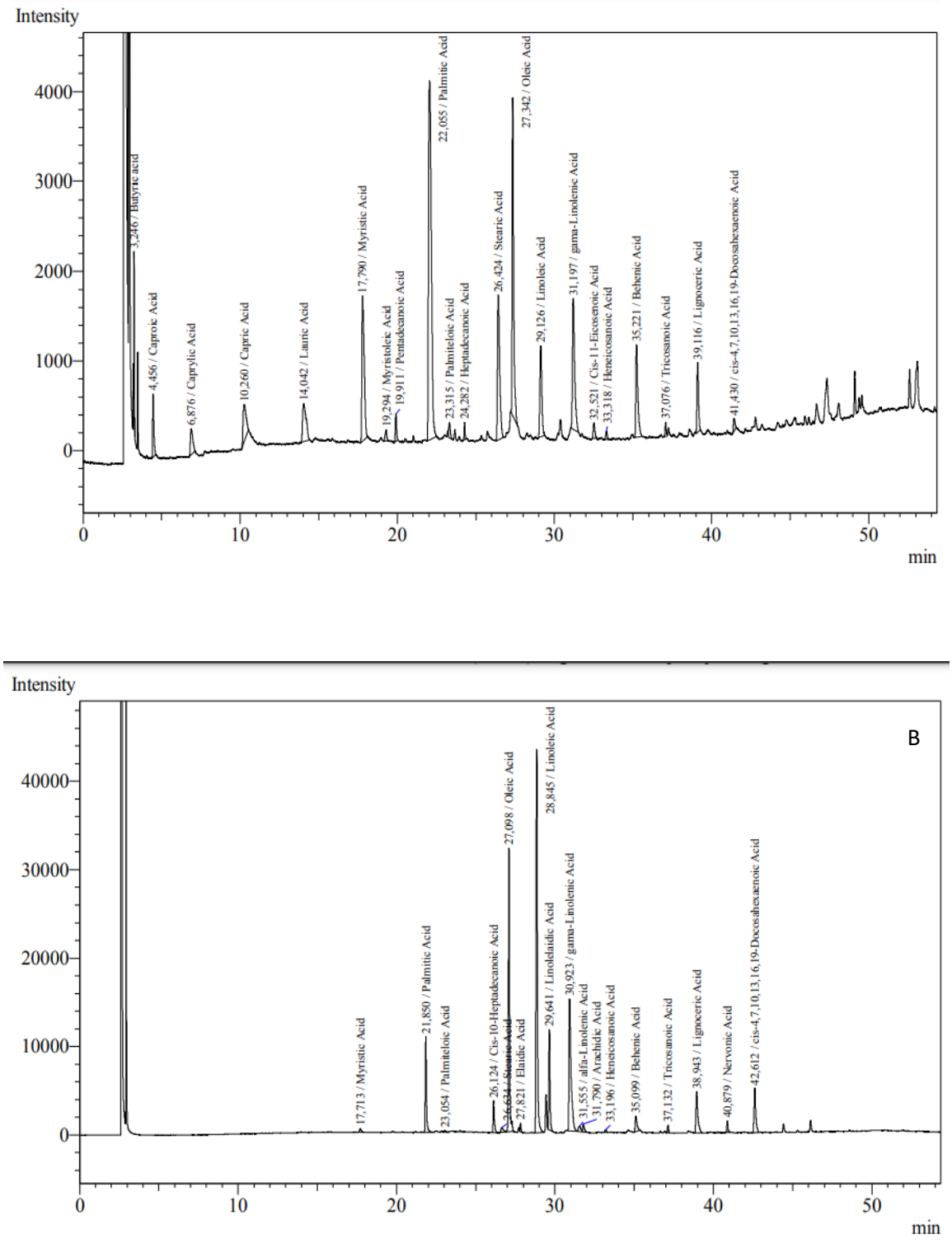

Figure 2. GC-MS chromatograms obtained from plant extracts of *A. tinctoria* (A) and *T. polium* (B)

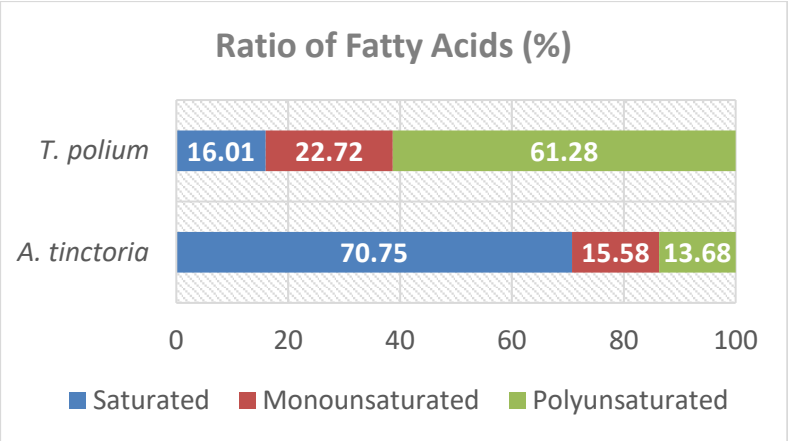

Figure 3. Fatty acids ratio (%) of *A. tinctoria* (A) and *T. polium* (B)

Results of phenolic content and antioxidant activity

Oxidative stress causes many ailments, such as Alzheimer's, Parkinson's, aging and cancer, but antioxidants neutralize free radicals. Since antioxidant-rich plants, which are the main source of nutrients are commercially important, the determination of antioxidants is one of the main research topics (Uttara et al., 2009). Phenols and flavonoids, which are the main sources of antioxidants, are the most common secondary components in plants (Pandey and Rizvi, 2009). However, a single method is not sufficient to evaluate the antioxidant capacity of foods, since the response of antioxidants to different radicals and oxidants is also through different mechanisms (Comlekcioglu and Kutlu, 2021). Therefore, in this study, Folin-Ciocâlteu and AlCl3 analyses were applied by preparing extracts with two different solvents, in order to compare the phenol and flavonoid contents of plants. DPPH and FRAP analyses were used to determine the antioxidant activity and the results are given in Table 2.

Table 2. Total phenolic and flavonoid content and antioxidant activity values

	Alkanna tinctoria		*Teucrium polium*	
	Ethanol	**Methanol**	**Ethanol**	**Methanol**
Phenol (mg GAE/g)	70.12 ± 0.21	81.92 ± 0.55	39.32 ± 0.76	74.68 ± 0.36
Flavonoid (mg QE/g)	9.71 ± 0.20	10.53 ± 0.05	8.31 ± 0.21	8.19 ± 0.11
FRAP (mmol AAE/g)	20.34 ± 0.36	20.67 ± 0.25	11.51 ± 0.10	12.72 ± 0.51
IC_{50} Value (%DPPH) (mg/mL)	2.09 ± 0.15	0.65 ± 0.33	0.75 ± 0.11	0.57 ± 0.21

According to the measurement results, it was observed that the total phenolic content and DPPH results in both plants were higher in methanolic extracts. In terms of these properties, methanol was found to be more effective in revealing bioactive components and antioxidant power compared to ethanol. Since there is an inverse ratio between the antioxidant activity value and the IC_{50} value calculated using the DPPH analysis, it is desirable for this value to be low. The IC_{50} values in the extracts vary between 0.57-2.09 mg/mL. Especially those plant extracts with values below 1.0 mg/ml have very high antioxidant power. It is known that phenolic compounds and flavonoids obtained from plants have abundant antioxidant activity in food products. In general, extracts with high radical scavenging activity have a high phenolic content (Comlekcioglu and Kutlu, 2021). In this study, both total phenolic and flavonoid contents and antioxydant power were found to be high in *A. tinctoria*. However, in *T. polium*, where the phenolic content is twice as high as in methanolic extract, the antioxidant power is similar in terms of IC_{50} and FRAP values of both extracts. Zannou and Koca (2020) investigated the antioxidant power in ethanolic and methanolic root extracts of *A. tinctoria*. They have determined the phenolic content, flavonoid content and FRAP value as 49.71 and 33.12 mg/g; 1.93 and 2.14 mg/g; 96.11 and 140.81 mmol/g, respectively. Compared to Zannou and Koca (2020), phenol and flavonoid values were higher in this study, but FRAP values were lower. In addition, Zannou and Koca (2020) found methanolic extract more effective in flavonoid and FRAP, but ethanolic extract in phenolic content. Guemmaz et al. (2020) found the phenolic content of

the aerial parts of *A. tinctoria* between 144.50-189.66 mg/g and the flavonoid content between 1.98-26.80 mg/g. These values are considerably higher than the amounts obtained in this study. On the other hand, El Atki et al. (2019) determined the antioxidant activities, total phenolic and total flavonoid contents of methanol, ethanol, water and ethyl acetate extracts of *T. polium* and found the bioactivity values of the methanolic extract significantly higher than in all other extracts. They found DPPH, FRAP, phenolic and flavonoid contents values in the methanolic extract, as 0.41, 0.21, 95.53 and 101.9 mg/g, respectively. These values are higher than those obtained in this study. El Atki et al. (2020) found quite different results in another study in which they studied two subspecies of *T. polium* (*T. polium* subsp. *polium* and *T. polium* subsp. *aureum*). In their study, they stated DPPH and FRAP values as 7.2 and 3.5 mg/g for a *T. polium* subsp. *aureum* and as 3.7 and 2.31 mg/g for *T. polium* subsp. *Polium*. As a result, many factors, such as location, climatic conditions, solvent used in extraction and genetics, cause differences in the chemical contents of plants.

Results of antimicrobial activity

The antimicrobial activity and MIC values of the *A. tinctoria* and *T. polium* extracts obtained by two different solvents are presented in Table 3. The *A. tinctoria* and *T. polium* extracts produced an antimicrobial effect against both test bacteria and yeasts. Only *A. baumannii* and *K. pneumoniae* were not inhibited by *A. tinctoria* extracts unlike Khan et al. (2015) results. But other test microorganisms were effected in varying proportions. Antimicrobial activity properties of roots were also reported along with aerial part of *A. tinctoria* (Alwahibi and Parween, 2017; Aljanaby, 2018).

The results showed that *T. polium* did not effect only *E. faecium* and *C. parapsilosis*. Generally, the extracts of *T. polium* had a potential to inhibit the growth of all microorganisms tested (Table 3). The results obtained in this study are in agreement with the results of other researchers (Sarac and Ugur, 2007; Darabpour et al., 2010; Shabba et al., 2014). Antimicrobial activity of essential oil of *T. polium* against various microorganisms was also documented (Belmekki et al, 2013; Lograda et al., 2014; El Atki et al., 2019; Benali et al.,2021). The largest inhibition zone and the lowest MIC value was observed against *S. marcessens* with *A. tinct*oria extracts. *T. polium* produced a promising inhibition against *A. baumannii* and *K. pnemonia* too. Similar result was also reported by Shahba et al., (2014).

Table 3. Antimicrobial activity and MIC values of *A. tinctoria* and *T. polium* extracts against some microorganisms

	Inhibition Zone (mm) and MIC values				Antibiotic	
	A. tinctoria		*T. polium*			
Microorganisms	EtOH/**MIC**	MeOH/**MIC**	EtOH/**MIC**	MeOH/**MIC**	Cxm	Nys
B. subtilis ATCC 6633	12±0.50 /**2**	18±1.46 /**4**	16±3,10 /**4**	14± 2.70 /**2**	-	NT
S. lutea ATCC 9341NA	14±1.30 /**4**	-	14±2,13 /**4**	14±2.10 /**4**	15	NT
S. aureus ATCC 29213	14±1.50 /**4**	12±1.69 /**4**	12±0.58 /**4**	14±1.36 /**4**	27	NT
*S. marcessens**	25±3.91 /**2**	25±5.56 /**2**	11±0.74 /**4**	13±1.52 /**4**	30	NT
E. coli ATCC 13846	12±0.96 /**4**	17±2.2 /**4**	15±3.21 /**4**	12±1.41 /**4**	12	NT
*E. faecium**	14±0.75 /**4**	16±0.89 /**4**	-	-	25	NT
*A. baumannii**	-	-	14±1.23/**4**	15±1.15 /**4**	-	NT
*K. pneumonia**	-	-	11±2.05 /**4**	11±1.68 /**4**	17	NT
*C.albicans**	11±0.23 /**4**	11±0.82 /**4**	10±1.27/**4**	9±1.89 /**4**	NT	16
*C.parapsilosis**	14±3.97 /**4**	9±0.32 /**8**	-	-	NT	16

EtOH: Ethanol Extract, MeOH: Methanol Extract; Mic: Minimal inhibitory concentration (mg/mL); Cxm: Cefuroxime sodium (30µg)-Oxoid; Nys: Nystatine 100U; NT: Not Tested; *: Clinic Isolate

Results of In-vitro enzymeiInhibiton activity

The ethanol extract of *A. tinctoria* showed a slightly increasing inhibition over time (Figure 4). The enzyme lost its original activity by 2.1% and 5.2%, within 15 and 30 min, respectively. However, methanol extract did not inhibit amylase enzyme more than 0.7% up to 30 min. On the other hand, methanol extract of *T. polium* produced a slight enzyme inhibition about 4.6% up to 30 min. However, ethanol extract of *T. polium* did not inhibit amylase. On the contrary, it showed a protective action on the enzyme (Figure 5).

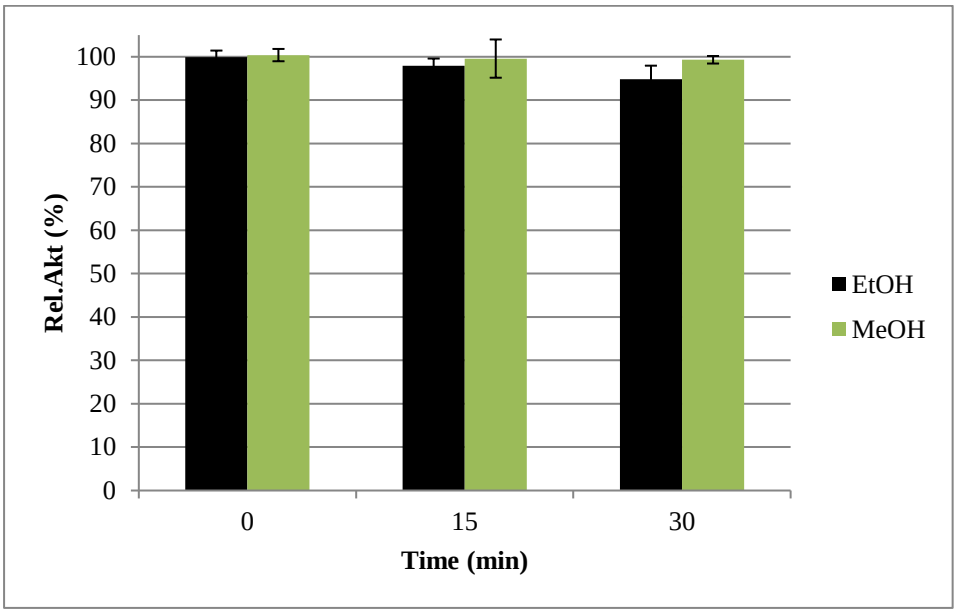

Figure 4. Inhibitory activity of *A. tinctoria* on alpha amylase enzyme (EtOH: Ethanol extract; MeOH: Methanol extract)

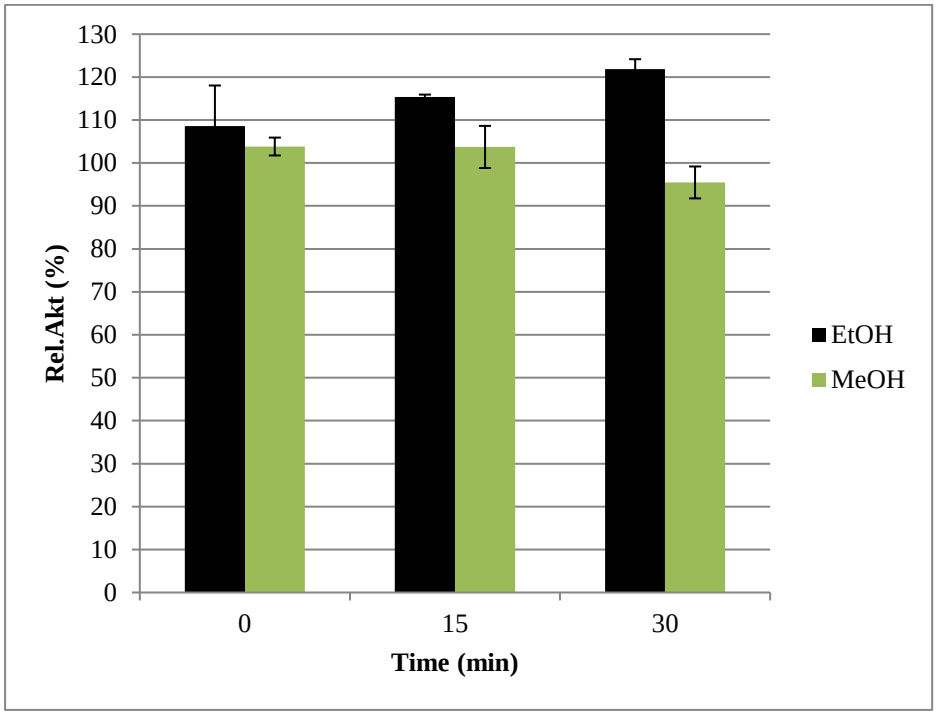

Figure 5. Inhibitory activity of *T. polium* on alpha amylase enzyme (EtOH: Ethanol extract; MeOH: Methanol extract)

Conclusions

This study was carried out on two plants, which were collected by local people, in order to benefit from their medicinal benefits from their natural environments. It has been determined that both plants contain significant amounts of flavonoid and phenolic compounds and have high antioxidant power. Since the polarity values are different, the variety and amount of solute in solvents can be different. It has been determined that methanol is more effective than ethanol in extracting bioactive substances from plants. Fats, which are a high energy source and are found in basic foods, are very important for human health. One of the interesting results of this study is that *A. tinctoria* has a high ratio of saturated fatty acids. On the other hand, omega 6 and 9 fatty acids, which are the main fatty acids in the 84% unsaturated fatty acid of *T. polium*, combined with the high bioactive content, make the plant a healthy alternative. In this study, the results showed that the extracts of A. tinctoria and T. polium possess antimicrobial properties and the compatibility of digestive enzyme amylase could have a potential natural product that could be used as a controlling agent for microorganisms and an additive material for food preservation.

Author Contributions: This work was carried out in collaboration of three authors. Authors NC and AA designed the study, provided the plant material, wrote the protocol and interpreted the data. Authors RC and AK conducted the laboratory work, gathered the initial data and performed preliminary data analysis. The authors NC and AA managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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