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Research article

Determination of antioxidant, antimicrobial activities, total phenolic and flavonoid contents of *Allium rumelicum*, *Jurinea kilaea* and *Peucedanum obtusifolium*

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Abstract

A microwave-assisted extraction (MAE) process for polyphenols from *Allium rumelicum* Kocyigit & Ozhatay, *Jurinea kilaea* Azn. and *Peucedanum obtusifolium* Sibth. & Sm. was used. This research examined the methanolic extracts made from these three species' antioxidant, antimicrobial, total phenolic, and flavonoid contents. By using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method (DPPH), ABTS/Persulfate, and Cupric reducing antioxidant capacity (CUPRAC) methods, the total antioxidant activities and capacities were examined. Additionally, the Folin-Ciocalteu and $AlCl_3$/KAc techniques were used to calculate the total phenolic and flavonoid contents. To ascertain the antibacterial capabilities of plants, the disc diffusion method was applied. The *J. kilaea* showed the greatest total antioxidant capacity/activity levels when measured using the CUPRAC and ABTS/Persulfate techniques. *A. rumelicum* was found to have the highest quercetin concentration, while *P. obtusifolium* had the lowest. In *J. kilaea*, the gallic acid concentration was highest. The highest antimicrobial activity values were obtained in *P. obtusifolium*.

Keywords: *Antimicrobial activity; antioxidant activity; flavonoid; total phenolic; spectrophotometric method*

1. Introduction

People are exposed to various diseases throughout their lives and superior treatment methods are still being investigated by scientists to cure the corresponding diseases. Bioactive compounds, which are being used for healing, are mostly based on isolated natural compounds from plants. So far it is found through the research that the majority of isolated bioactive compounds consist of antioxidants. These antioxidants obtained from plants; either scavenge free oxygen radicals or prevent the formation of free radicals that cause various diseases and damage to the body (Ozturk et al., 2011). Antioxidants are important substances that can prevent and delay the oxidation that is likely to occur. Today, the interest in keeping reactive oxygen species away from living organisms and detecting or investigating antioxidants that can scavenge these species is increasing day by day and will continue to increase (Bener et al., 2018). Before the drug development in the pharmaceutical industry, many plants and plant ingredients were used instead of drugs to eliminate diseases. People are going to be treated by using plants or drugs obtained from plants in the treatment of various diseases (Ozturk et al., 2011; Karahan et al., 2023).

Allium rumelicum Kocyigit and Ozhatay (Amaryllidaceae) is an endemic plant and is also known as oak curry (Ozhatay et al., 2010). It grows only in Kırklareli Yıldız Mountains in Türkiye. When the medical benefits of *A. cepa* L., which is from its own family, are examined in the literature (Vierra et al., 2017), and it has been reported that it has an effect on the prevention of age-related changes in the vessels and the treatment of anorexia. It has been proven by experiments that it can inhibit platelet aggregation and thromboxane synthesis. Although it has an effect in the treatment of infections caused by

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bacteria such as dysentery, scars, wounds, ulcers, and keloids, it has been seen that its use is preferred in the treatment of asthma. It is used as adjuvant therapy in diabetes. The reports in the literature show that 50-100 mg of *A. cepa* reduces plasma fibrinogen and serum cholesterol levels. Clinical studies prove that *A. cepa* has anti-hyperglycemic activity. The use of 100 mg of the extract has been found to reduce glucose-induced hyperglycemia in men. In diabetes, it has been observed that the liquid taken by mouth (50 mg) reduces the level of glucose in the blood. It has been reported that *A. cepa* juice has antimicrobial activity against most bacteria. In addition, there are case reports that it is an effective method in the treatment of vitiligo and alopecia areata (Hannan et al., 2010; Onyeoziri et al., 2016; Sagar et al., 2020). In the literature, there are several studies on the antioxidant properties of *Allium* genus as well as their medicinal properties, but no study has been found on the endemic *A. rumelicum* plant (Sharma and Sharma, 1976; WHO, 1999). *J. kilaea* Azn. (Asteraceae) which is an interesting species of coastal dunes is a rare species on a national scale (Danin and Davis, 1975). Since it was first introduced to the scientific world with specimens collected in Kilyos, this species was named kilaea (Kilyos). Another name is Kilyos purple. The world distribution of *J. kilaea* is the dunes of Bulgaria. It is also found in Kırklareli in Türkiye and is an interesting species of coastal dunes. In the study conducted with *Jurinea consanguinea* DC., its anticholinesterase, antibacterial, and antioxidant properties were investigated (Ozturk et al., 2011). Phytochemical, total flavonoid and phenolic components, antioxidant properties of *Jurinea dolomiaea* Boiss. plant was determined. As a result of the studies, it has been revealed that the plant is very useful in terms of public health in line with the literature (Naseer et al., 2014). *P. obtusifolium* Sm. (Apiaceae) is a rare dune-growing species. It is known from Greece outside Türkiye (Chamberlain, 1972).

The antioxidant and antimicrobial properties of *P. japonicum* Thunb., were investigated. (Kim et al., 2018). The antioxidant properties of *P. pastinacifolium* Boiss. & Hohen were analyzed. (Movehedian et al., 2016). The antioxidant and antimicrobial properties of four different species of *Peucedanum, (P. officinale* L., Besser, *P. longifolium* Waldst. & Kit., *P. aegopodioides* (Boiss.) Vandas, *P.m. alsaticum* Poir.), have been studied (Matejic et al., 2013).

Microwave-assisted extraction (MAE) is a green technique that provides speed of extraction and less solvent consumption (Bagade and Patil, 2021). MAE is a non-ionizing wave at frequencies ranging from 300 MHz up to 300 GHz (Lasunon and Sengkhamparn, 2022).There are a lot of current studies regarding the MAE technique in literature. (Destandau and Michel, 2022; Solaberrieta et al., 2022; Georgiopoulou et al., 2023; Tomasi et al., 2023; Tran et al., 2023; Zayed et al., 2023).

The main target of this study was to analyze the total antioxidant and antimicrobial properties, total phenolic and total flavonoid contents of *A. rumelicum, J. kilaea* and *P. obtusifolium*. To the best of our knowledge, no previous literature study reported antioxidant, antimicrobial activities, and total contents of phenolic and flavonoid for *A. rumelicum, P. obtusifolium,* and *J. kilaea*.

2. Materials and methods

2.1. Preparation of plant extracts and sampling area

A. rumelicum Kocyigit and Ozhatay was collected from around Demirköy, Dupnisa cave, Kırklareli in Türkiye (NAKU 000004). *P. obtusifolium* Sibth. & Sm. was collected from Gökçetepe coastline, Keşan, Edirne in Türkiye (NAKU 000005). *J. kilaea* Azn. was collected from Kastro dunes, Saray, Tekirdağ in Türkiye (NAKU 000006). (Kocyigit et al., 2010). Examined specimens were cited in the appendix. Microwave extraction was carried out in Teflon (PTFE) containers using a fiber optic temperature control system and a closed oven system (The ETHOS-One (Milestone, Shelton, CT). After adding 15 mL of solvent (80% methanol-water (v/v) by weighing 0.1 g from the homogenized dry raw plant sample, it was placed in Teflon extraction vessels and placed in the device. During the extraction process, 500 W of power was applied and the working temperature of 80 ° C was reached in 3 minutes (1st stage). The temperature was kept constant at 80 ° C within the following 3 minutes (2nd stage) and the extraction process was completed in 11 minutes by allowing it to cool in the last 5 minutes (3rd stage). All plant extracts obtained were filtered with a membrane filter that has a pore size of 0.45 µm.

A. rumelicum was collected from Dupnisa cave, Demirköy of Kırklareli. *P. obtusifolium* was collected from, Gökçetepe coastline, Keşan of Edirne and *J. kilaea* was collected from Kastro dunes, Saray of Tekirdağ. The identifications of all plants were made by Prof. Dr. E. Cabi.

2.2. DPPH method

Since DPPH is a stable free radical at room temperature, it causes ethanol to turn violet when added to it. During the interaction with an antioxidant molecule, it is decreased, resulting in colorless ethanol solutions. The use of DPPH offers a simple and quick method to assess antioxidants. 2 mL of a 1 mM DPPH which is prepared in ethanol solution and 2 mL ethanol were added to 0.2 mL of sample solutions of diluted plant extracts (1:5 v/v). The reproducibility is checked by repeating the experiments three times. The absorbance readings were obtained at 517 nm after waiting 30 minutes, and the following formula was used to translate these into the percentage of antioxidant activity (AA):

$$\text{Inhibition \%} = (A_0\text{-}A_S)/A_0 \times 100$$

Where A_0 denotes the control solution's absorption and A_S is the absorption of the solution which to be extracted and tested. Inhibition % values were calculated for all plant extracts by using the formula (Huang et al., 2005). The experiments were repeated three times.

2.3. Aluminum chloride/potassium acetate spectrophotometric method

The $AlCl_3$/KAc method essentially uses the color of the Al(III)-complexes to measure the flavonoid compounds. Naturally, the $AlCl_3$/KAc approach may also measure a small number of polyphenolic compounds that do not belong to the flavonoid class but do possess the Al(III)-chelating 1-hydroxyanthraquinone or o-dihydroxycatechol moieties. Separate mixtures of 1.5 mL of 95% C_2H_5OH, 0.1 mL of 1 M CH_3COOK, 0.1 mL of 10% $AlCl_3$, and (3.3-x) mL of distilled water was made with the analyte mixture (x mL) (Woisky and Salatino, 1998; Chang et al., 2002). After keeping the samples at room temperature for 30 minutes, the absorbance measurement is conducted at 427 nm. The experiments were

repeated three times.

2.4. CUPRAC assay of total antioxidant capacity

Bis(2,9-dimethyl-1,10-phenanthroline) copper(II), the chromogenic oxidizing reagent used in the CUPRAC assay, is straightforward, versatile in application to both hydrophilic and lipophilic antioxidants, stable, and inexpensive. The CUPRAC method has been effectively used to assess the antioxidant capabilities of human serum and food plants. The reaction between chromogenic oxidizing reagent from the CUPRAC method, which is cupric neocuproine, and n-electron-reductant antioxidants (AOX) undergoes as:

n $Cu(Nc)_2^{2+}$ +n-electron reductant antioxidants (AOX)→ n $Cu(Nc)_2^{+}$ +n-electron oxidized product+n H^+

x mL of the sample was mixed with the following ingredients: i) 1 mL of $CuCl_2$ solution, ii) 1 mL of neocuproine alcoholic solution iii) 1 mL of ammonium acetate solution iv) (1.1-x) mL of distilled water. The volume of the total mixture added up to 4.1 mL. The incubation period was 30 mins, after which the absorbance measurements were conducted at 450 nm against the reagent blank (Apak et al., 2004). The repetition of experiments is also checked three times.

2.5. ABTS/Persulphate methods

Even in TAC measurement via decolorization of the $ABTS^+$ cation generated by persulfate oxidation; various results are achieved as a result of different modifications. The ABTS assay has been criticized for being overly dependent on the chromogenic radical-generation approach. 7.0 mM ABTS radical reagent was prepared in water and $K_2S_2O_8$ was added to this solution. The mixture's final persulfate content is 2.45 mM. For 12 to 16 hours, the ABTS-radical cation solution was stored at room temperature and in the dark. An ABTS-radical cation solution that was blue-green in color was diluted with 96% ethanol at a ratio of 1:10 before being combined with x mL of plant extract. The absorbance was measured at the end of the sixth minute (λ =734 nm) (Celik et al., 2010). All experiments were repeated three times.

2.6. Folin ciocalteu method

The Folin Ciocalteu assay is an electron transfer-based reaction that assesses an antioxidant's capacity for reductive activity. It has been frequently used to assess the total polyphenol content of plant-derived foods and biological samples in nutritional and therapeutic studies. Lowry A solution: it was prepared by dissolving 2.0 g of Na_2CO_3 in 100 mL of 0.1 M NaOH solution. Lowry B solution: it was prepared by dissolving 0.5 M $CuSO_4$ in 1% sodium potassium tartrate ($NaKC_4H_4O_6$) solution. Lowry C solution: it was prepared by adding 1 mL of Lowry B solution to 50 mL of Lowry A solution and diluting the Folin reagent with distilled water at a ratio of 1:3 (v/v). After adding x mL of sample solution + (1-x) mL of H_2O + 2.5 mL of Lowry C solution, the resulting solution mixture is left for 10 minutes. Then, 0.25 mL of Folin reagent is added to this solution and solutions are prepared with a total volume of 3.75 mL. After the prepared solutions were kept at room conditions for 40 minutes, their absorbance against the blind solution was measured at a wavelength of 750 nm (Prior et al., 2005). All experiments were repeated three times.

2.7. Bacterial strains

A. rumelicum, J. kilaea, P. obtusifolium extracted by 80% methanol were analyzed against a panel of bacteria including, *Listeria monocytogenes* ATCC 7644, *Salmonella enteritidis*, ATCC 13076, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 2592. All the aforementioned strains were acquired from the American Type Culture Collection as living cultures (ATCC). For stock cultures, suspensions were adjusted to 0.5 McFarland standard turbidity (equivalent to 10^7-10^8 cfu/mL for bacteria, depending on species) after growing them in Nutrient Broth (Merck, Germany) at 37°C for 24 hours.

2.8. Disc diffusion assay

Using the agar disc diffusion method, the antimicrobial properties of *A. rumelicum, J. kilaea,* and *P. obtusifolium* were examined (NCCLS, 1997). A suspension of the tested microorganism (0.1 mL, 10^8 cells per ml) was put on the solid medium plates. Filter paper discs (6 mm in diameter) that had been impregnated with 20 µl of the extracts were placed on the inoculation plates. Methanol served as the negative control and tetracycline the positive control. All petri dishes were incubated for 24 hours at 37°C after 2 hours at 4°C, except for *L. monocytogenes*, which was incubated for 48 hours. The inhibitory zones' sizes were measured in millimeters. Three times each was done for every experiment.

3. Results and discussion

Traditional extraction techniques have advanced in recent years to become environmentally friendly techniques like ultrasound-assisted extraction and microwave-assisted extraction (MAE). Important benefits of MAE include its compatibility with automation, simplicity of working with multiple samples, low solvent requirement, and extremely quick processing time (Bener, 2022).

There is no study on antioxidant, antimicrobial activities, phenolic and flavonoid contents of *A. rumelicum* M. Kocyigit & Ozhatay, *J. kilaea* Azn., *P. obtusifolium* Sibth. & Sm., in literature but there are studies on species from their own family.

A. schoenoprasum and *A. ursinum* were tested for their antioxidant activity using a variety of techniques, including the DPPH radical-scavenging ability, ferric reducing antioxidant power (FRAP) assay, and ABTS radical scavenging tests. The same research was conducted by Parvu et al. (2014) using the trolox equivalent antioxidant capacity (TEAC) test and the DPPH bleaching method. The TEAC method revealed that the leaf extract's antioxidant activity was higher than that of the DPPH method (Dikdik et al., 2021). Nencini et al. (2011) found gallic acid concentrations analyzed by the FRAP method *of A. neapolitanum* Cyr., *A. roseum* L., *A. subhirsutum* L., *A. sativum* L. All gallic acid concentrations in the samples were higher than *A. rumelicum*. Santas et al. (2010) investigated antioxidant activities and total phenolic contents of *A. cepa* extracts by TEAC and Folin methods and trolox and rutin concentrations were higher than *A. rumelicum*. Hisamoto et al. (2003) analyzed antioxidant compounds of *P. japonicum*'s leaves. The results of DPPH radical scavenging activity were mostly higher than present study. The level of inhibition of DPPH free radicals is measured by DPPH free radical scavenging activity. Regarding

the antioxidant activity, *P. Japonicum* in ethyl acetate fraction exhibited the highest antioxidant activity (Kim et al., 2018). Tepe et al. (2011) determined DPPH free radical scavenging activity of *P. longifolium* and *P. palimbioides* and analysis results were lower than *P. obtusifolium*. Ozturk et al. (2011) analyzed *J. consanguinea DC.* Total phenolic and flavonoid content results according to quercetin were found higher than *J. kilaea*. Ayad et al. (2017) investigated the antioxidant and antimicrobial activity of *J. humilis* DC. Gallic acid and rutin values are higher than *J. kilaea*.

The spectrums of *A. rumelicum, P. obtusifolium and J. kilaea* were measured by diluting 80% methanol. The photos of plants, given in Fig. 1 and Fig. 2-4, show absorption spectrums of plant extracts.

Fig. 1. Dried *Allium rumelicum, Peucedanum obtusifolium* and *Jurinea kilaea* samples.

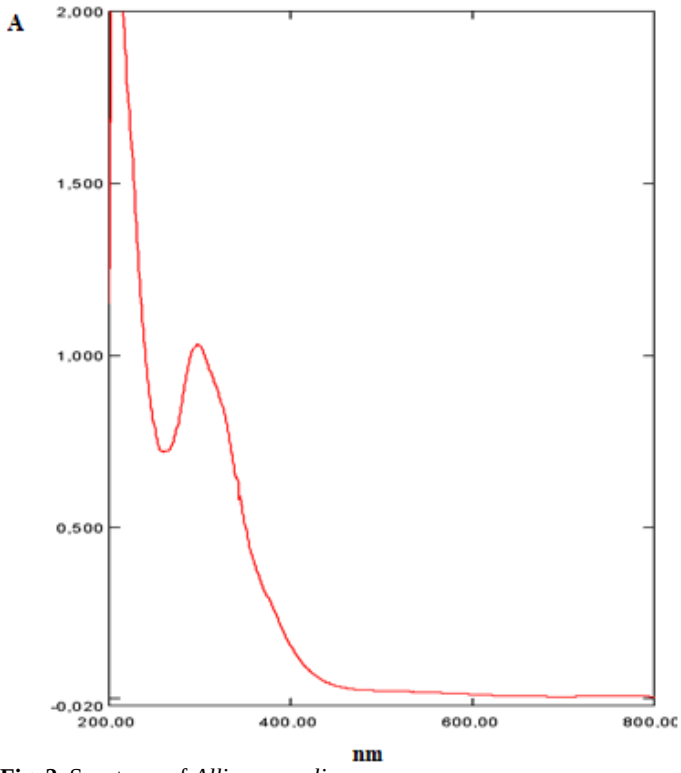

Fig. 2. Spectrum of *Allium rumelicum*.

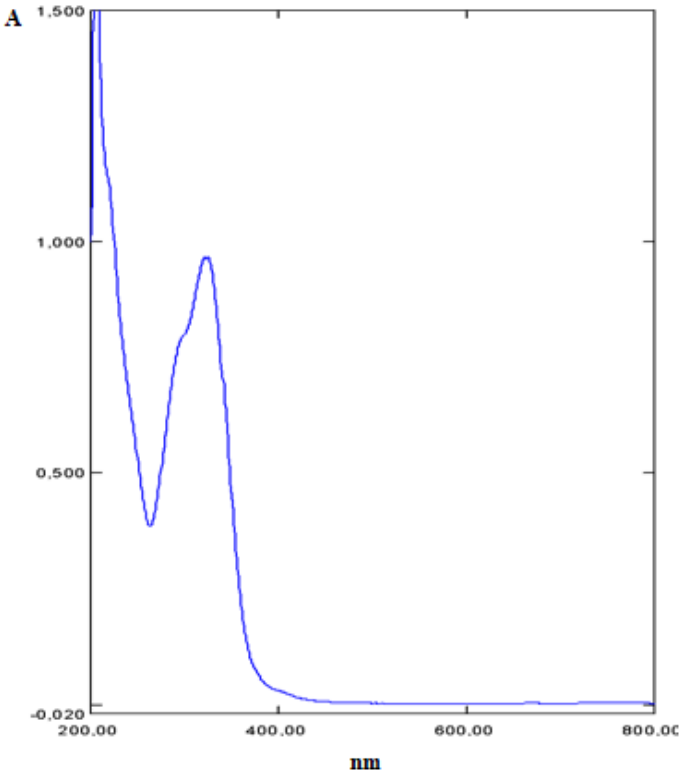

Fig. 3. Spectrum of *Peucedenum obtisifolium*.

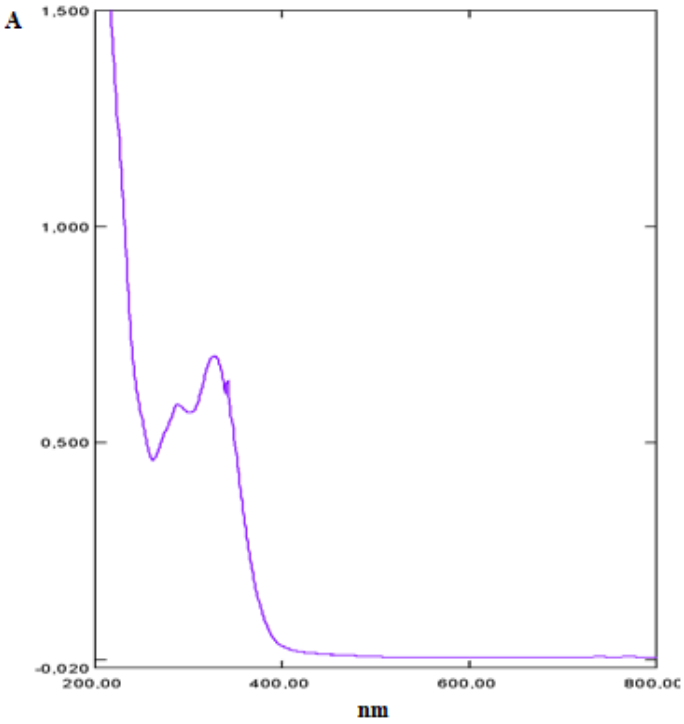

Fig. 4. Spectrum of *Jurinea kilaea*.

3.1. CUPRAC and ABTS/Persulphate method

Maximum absorption is provided at 450nm by the reduction of bis(2,9-dimethyl-1,10-phenanthroline: neocuproine)Cu(II) chelate cation in the presence of antioxidants to the cuprous neocuproine chelate [Cu(I)–Nc], which is known as the CUPRAC method (Apak et al., 2004). The CUPRAC values of trolox (TR), flavon (FV), gallic acid (GA), rutin (RT), and quercetin (QR) were higher than ABTS/Persulphate values. Total antioxidant activity contents of the plant extracts were found through the equation $y = 1.60.10^{4}x$

- 0.002 (R^2 = 0.999) which are calculated according to the reference (TR), y = 5.10^4x + 0.02 (R^2 = 0.999) which are calculated according to the reference (GA), y = 8.10^4x + 0.01 (R^2 = 0.999) which are calculated according to the reference (QR), y = 5.10^4x + 0.026 (R^2 = 0.993) which are calculated according to the reference (RT), y = $0.6.10^4$x + 0.006 (R^2 = 0.998) which are calculated according to the reference (FV) by CUPRAC method. Total antioxidant activity contents of the plant extracts were found through the equation y = $2.65.10^4$x + 0.018 (R^2 = 0.999) which are calculated according to

the reference (TR), y = 1.10^5x – 0.08(R^2 = 0.999) which are calculated according to the reference (GA), y = 1.10^5x + 0.02 (R^2 = 0.999) which are calculated according to the reference (QR), y = 8.10^4x + 0.03 (R^2 = 0.999) which are calculated according to the reference (RT), y = $0.14.10^4$x + 0.027 (R^2 = 0.998) which are calculated according to the reference (FV) by ABTS/Persulphate method.

Solvent polarity is important for antioxidants because it causes showing some variant of antioxidants. Table 1-2 are given the results analyzed by CUPRAC, ABTS/persulfate methods. The values of CUPRAC and ABTS Persulfate of trolox (TR), gallic acid (GA), quercetin (QR), rutin (RT), flavon (FV) were investigated in the CUPRAC assay results. Total antioxidant contents of *A. rumelicum*, *P. obtusifolium* and *J. kilaea* were found within the 0.022±0.08-0.294±0.07; 0.001±0.02-0.020±0.02; 0.041±0.04-0.543±0.02 mmol g^{-1} range by CUPRAC Method, respectively. Total antioxidant contents of *A. rumelicum*, *P. obtusifolium* and *J. kilaea* were found within the 0.016±0.04-1.23±0.05; 0.010±0.03-0.756±0.02; 0.020±0.03-1.59±0.04 mmol g^{-1} range by ABTS/Persulphate Method, respectively. The order of the CUPRAC values was determined to be FV>TR>RT=GA>QR for *A. rumelicum*, *P. obtusifolium* and *J. kilaea*. The order of the ABTS/Persulphate values was determined to be FV>TR>RT>GA>QR for *A. rumelicum*, *P. obtusifolium* and *J. kilaea* (Table 1-2). The highest antioxidant content was obtained in flavon for CUPRAC and ABTS/Persulphate Method. MeOH 80% was chosen due to well-known electron transfer in existence of ionizing solvents. In addition, MeOH is determined to be the best ionization-supporting alcohol.

3.2. DPPH method

The DPPH method aims to measure the scavenging effects of antioxidants on a stable free radical (DPPH radical). It is reduced to hydrazine when hydrogen interacts with donors. The maximum absorption of deep violet-colored DPPH radical measures at 517 nm. (Prior et al., 2005).

DPPH method is a valid, inexpensive, rapid, accurate, easy, and economical method for measuring the activity of antioxidants in plant samples (Sagar and Pareek, 2011).

The approach is distinctive in that it uses a sample reaction with DPPH in methanol to make it easier to extract antioxidant chemicals from the sample. The DPPH method has the advantage of allowing DPPH to react with the entire sample and, given enough time, allowing DPPH to react slowly. This phenomenon occurs even with weak antioxidants. The DPPH method can be used to investigate both hydrophilic and lipophilic antioxidants in aqueous and nonpolar organic solvents. (Prior et al., 2005).

The experimental results of antioxidant activity as determined by the DPPH technique are displayed in Fig. 5. The experimental findings were presented using IC50 values (concentration needed to inhibit 50% of the oxidative process). The investigated samples' maximal inhibition percentages had similar values, ranging from 51.0% to 64.3%. The highest value of inhibition % was found in *P. obtusifolium.* Inhibition % of *J. kilaea* was found as the lowest value. The order of inhibition % was determined to be *P. obtusifolium*>*A. rumelicum*>*J. kilaea.* % Inhibition values analyzed by the DPPH Method are given in Fig.5.

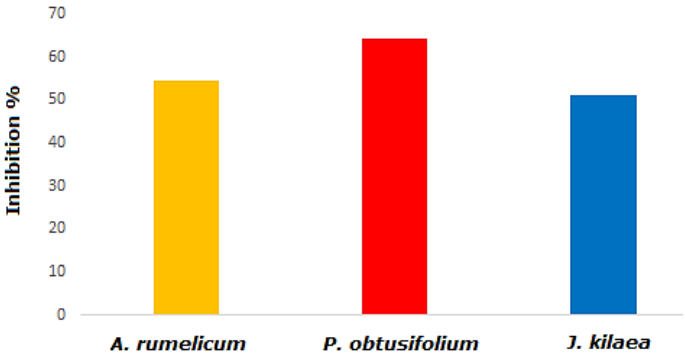

Fig. 5. % Inhibition values analyzed by DPPH Method.

3.3. Total phenol and total flavonoid contents of extracts

The ability of flavonoids to reduce alpha-tocopherol radicals, bind metal catalysts, activate antioxidant enzymes, transfer electrons to free radicals, and block oxidases are all factors that contribute to their protective effects in biological systems (Heim et al., 2002). Most of the plant phenolic chemicals, and flavonoids are individuated with a benzo-pyrone

Table 1
Total antioxidant contents of *Allium rumelicum*, *Peucedanum obtusifolium* and *Jurinea kilaea* analyzed by CUPRAC method.

Plant name	Trolox (mmol g^{-1})	Gallic acid (mmol g^{-1})	Quercetin (mmol g^{-1})	Rutin (mmol g^{-1})	Flavon (mmol g^{-1})
A. rumelicum	0.103±0.02	0.033±0.01	0.022±0.08	0.033±0.05	0.294±0.07
P. obtusifolium	0.007±0.07	0.002±0.03	0.001±0.02	0.002±0.01	0.020±0.02
J. kilaea	0.190±0.05	0.061±0.02	0.041±0.04	0.061±0.04	0.543±0.02
Mean ± SD (n=3)					

Table 2
Total antioxidant contents of *Allium rumelicum*, *Peucedanum obtusifolium* and *Jurinea kilaea* analyzed by ABTS/Persulphate method.

Plant name	Trolox (mmol g^{-1})	Gallic acid (mmol g^{-1})	Quercetin (mmol g^{-1})	Rutin (mmol g^{-1})	Flavon (mmol g^{-1})
A. rumelicum	0.065±0.01	0.017±0.07	0.016±0.04	0.021±0.01	1.23±0.05
P. obtusifolium	0.040±0.02	0.011±0.06	0.010±0.03	0.013±0.02	0.756±0.02
J. kilaea	0.084±0.04	0.022±0.05	0.020±0.03	0.027±0.01	1.59±0.04
Mean ± SD (n=3)					

structure. In both fruits and vegetables, it is common. $AlCl_3$/KAc was used to assess the total flavonoid content. Quercetin served as a benchmark. Total flavonoid contents were calculated through the equation $y = 2.31.10^4x + 0.018$ ($R^2 = 0.999$). Total flavonoid contents within the extracts were found within the 0.017-0.0032 mmol g^{-1} range. The order of the total flavonoid values was determined to be *A. rumelicum>J. kilaea>P. obtusifolium*.

Total phenolic contents were analyzed by the Folin-Ciocalteu method by using gallic acid as a standard phenolic compound. The equation $y = 6.10.10^3 x + 0.011$ ($R^2 = 0.999$) supplied a linear calibration curve for gallic acid. The results of total phenolic and flavonoid contents are given in Table 3. Table 3 shows that the total phenol content of plant extracts fell between the ranges of 0.277 to 0.099 mmol g^{-1}. As was already demonstrated, it has been discovered to be normal to see the methanol extracts with the largest phenolic concentration and the best polarity to extract phenol components. Therefore, it is not surprising to see that methanol extracts have the highest antioxidant activity, which is quite similar to the antioxidant activity of gallic acid. The order of the total phenolic values was determined to be *J. kilaea>A. rumelicum>P. obtusifolium.* Table 3 shows the total phenolic and flavonoid contents of the studied species.

Table 3
Total phenolic and flavonoid contents *of Allium rumelicum, Peucedanum obtusifolium* and *Jurinea kilaea*.

Plant name	Gallic acid (mmol g^{-1})	Quercetin (mmol g^{-1})
A. rumelicum	0.179±0.02	0.017±0.01
P. obtusifolium	0.099±0.04	0.0032±0.01
J. kilaea	0.277±0.07	0.013±0.05
Mean ± SD (n=3)		

3.4. Antimicrobial activities

In this study, the antimicrobial effects of *A. rumelicum, J. kilaea, and P. obtusifolium* extracts at 80% methanol were evaluated against *Enterococcus faecalis* ATCC51299, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC6538, *Listeria monocytogenes* DSM12464, and *Salmonella* Enteritidis ATCC Alcoholic extracts exhibit greater antibacterial action than aqueous extracts, according to the literature (Al-Hashimi, 2012). Thus, 80% methanol was used for the plants studied. The antibacterial activities of the methanol extracts of the species examined in this article were compared with tetracycline (reference antibiotic), which was used as a positive control reported in Table 4. 80 % methanol extracts of *A. rumelicum* and *P. obtusifolium* showed antimicrobial activity against *S. aureus* ATCC6538. In addition, *P. obtusifolium* indicated antimicrobial activity against *E. faecalis* ATCC51299. The highest antimicrobial activity value was obtained in *P. obtusifolium* against *E. coli* ATCC 25922. The plants studied showed no effect on *Listeria monocytogenes* DSM12464. 80 % methanol extracts of *A. rumelicum, J. kilaea,* and *P. obtusifolium* had inhibition zones with a diameter ranging from 6.46-7.23 mm. *J. kilaea* showed antimicrobial activity against *Salmonella enteritidis* ATCC 13076.

Many studies have shown the influence of phenolic compounds and organosulfur compounds in *Allium spp*. as important inhibitors of pathogenic bacteria growth (Skerget et al., 2009; Santas et al., 2010; Xiao, 2017). In parallel with the results of present study (Sagar et al., 2020) found that *Allium cepa* L. (Cultivar Phursungi Local) inhibited the growth of *S. aureus* NCDC 109 up to 7.0 ± 0.0 mm. Viera et al. (2017) also reported that no antibacterial (*Salmonella* Enteritidis clinical isolate, *Listeria monocytogenes* ATCC 7644, *S. aureus* ATCC 25923) activity was found for the extracts of red onion skin (*Allium cepa* L.). Contrary to this study Ye et al., (2013) showed that the essential oil of *A. cepa* showed antibacterial activity against *E. coli* ATCC 25922 (13.4 ± 0.9). Different results have been obtained in the literature due to the presence of antimicrobial components, environmental conditions, plant species, and type of solvent (Sivropoulou et al., 1995; Cushnie and Lamb, 2005). Ozturk (2011) found that the methanol extract of *J. consanguinea* had antimicrobial activity to *S. aureus* with a 10 mm zone diameter. Vellutini et al., (2005) observed the antimicrobial activity of *Peucedanum paniculatum* leaf and root oils against *S. aureus, Serratia marcescens, Micrococcus luteus, Bacillus subtilis, Enterobacter cloacae,* and *E. coli*, similar to the results of present study.

Table 4
Antimicrobial activity results of *Allium rumelicum, Peucedanum obtusifolium* and *Jurinea kilaea* analyzed by disc diffusion methods (mm).

Microorganisms	*A. rumelicum* (mm)	*J. kilaea* (mm)	*P. obtusifolium* (mm)	Positive Control
Enterococcus faecalis **ATCC51299**	-	-	6.50±1.42	27±1.35
Escherichia coli **ATCC 25922**	-	-	7.23±1.27	30±1.74
Staphylococcus aureus **ATCC6538**	6.46±1.32	-	6.87±1.18	32±1.41
Listeria monocytogenes **DSM12464**	-	-	-	25±1.22
Salmonella Enteritidis **ATCC 13076**	-	6.50±1.25	-	20±1.16

Inhibition zone is the mean of three replicates including the disc diameter (6 mm); (-): no activity; negative control (Methanol) had no inhibitory effects on pathogenic bacteria tested, Positive control: Tetracycline (30 µg).
Mean ± SD (n=3)

4. Conclusion

For the analysis of antioxidants, which are important for human health and foodstuffs, antioxidant activity/capacity properties were determined by CUPRAC, ABTS/Persulfate, and DPPH methods, which were made with simple, highly reproducible, low reaction steps, inexpensive and non-specific devices. Folin Ciocalteu and $AlCl_3$/KAc methods were used for the analysis of components for total phenolic and flavonoid. Antimicrobial activity analyzes were also analyzed by the disk diffusion method. The 80 % methanolic extracts of *A. rumelicum, J. kilaea* and *P. obtusifolium* had no antimicrobial activity against *S. aureus* ATCC6538 but *A. rumelicum, P. obtusifolium* and *J. kilaea* were most effective against bacterial strains tested.

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