Radical Scavenging, Antioxidant and Antimicrobial Activity of *Paeonia peregrina* Mill., *Paeonia mascula* (L.) Mill. and *Paeonia officinalis (*L.)

Ajvazi, Majlinda Daci*+*

Faculty of Mathematical and Natural Sciences, Department of Chemistry, University of Prishtina, Prishtina, Republic of KOSOVO

Osmani, Ilirjana

Faculty of Medicine, Department of Pharmacy, University of Prishtina, Prishtina, Republic of KOSOVO

Gashi, Dafina

Faculty of Mathematical and Natural Sciences, Department of Chemistry, University of Prishtina, Prishtina, Republic of KOSOVO

Zeneli, Lulzim

University of Gjakova, Gjakova, Republic of KOSOVO

Krasniqi, Elez

Faculty of Mathematical and Natural Sciences, Department of Biology, University of Prishtina, Prishtina, Republic of KOSOVO

ABSTRACT: *This study aimed to investigate the potential of three Paeonia species, Paeonia peregrina Mill., Paeonia mascula (L.) Mill., and Paeonia officinalis L., as sources of natural antioxidants and antimicrobials. Different parts of the plant were extracted with solvents of varying polarities and the extracts were subjected to various tests to determine their total phenolic content, total flavonoid content, antiradical activity, potential antioxidant activity, ferric reducing antioxidant power, superoxide anion radical and hydrogen peroxide scavenging activity and microbial activity. Paeonia Officinalis was found to have the best antioxidant and antimicrobial activity. The results demonstrated that P. officinalis is a rich source of polyphenolic compounds with higher antioxidant and antimicrobial potential compared to P. peregrina and P. mascula. The ethyl acetate extract of P. officinalis showed the highest levels of phenolic content (62.45 mg GAE/g DW) and antioxidant potential as well as the strongest antimicrobial activity against various microbial strains. Ethyl acetate extract of P. officinalis effectively inhibited all of the examined bacteria and the fungus; however, it had a stronger impact on Gram-negative bacteria than it did on Gram-positive bacteria. The highest activity was observed against the Gram-negative pathogen P. aeruginosa with an inhibitory zone of 24 mm. Pearson correlation analysis revealed different correlations for the three Paeonia species in different extracts and plant parts. Overall, the study highlights the potential of P. officinalis as a source of natural antioxidants and antimicrobials.*

KEYWORDS: *Paeonia, Phenolics, Antioxidant activity, Superoxide, Antimicrobial activity, Correlations.*

* *To whom correspondence should be addressed.*
+ E-mail: majlinda.daci@uni-pr.edu
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INTRODUCTION

Smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents, and ozone are some of the external factors that help to promote the production of free radicals. It is paradoxical how these elements, essential to life (especially oxygen) have detrimental effects on the human body through these reactive species [1]. In every healthy human, there is a balance between the antioxidant defense system and Reactive Oxygen Species (ROS). However, this balance can be disrupted due to many factors, and excess of the ROS can induce oxidative damage to different biomolecules (protein, lipid, DNA, RNA, etc.) which play a crucial role in premature aging, prostaglandin-mediated processes degenerative diseases, cancer, etc. [2,3]. To prevent these oxidative damages caused by free radicals' different synthetic antioxidants were synthesized (BHT, BHA, etc.), however, toxicity and potential health hazards of these antioxidants came to focus, and every day more and more natural antioxidants (phenolics, flavonoids, tannins, anthocyanidins) were proved to be a safe alternative [4].

Antioxidants have been divided into two major classes, primary or chain-breaking antioxidants, and secondary or preventative antioxidants. Primary or chain-breaking antioxidants may occur naturally or they may be produced synthetically (BHT, BHA). These synthetic antioxidants are frequently used in the food industry [4]. However, use of the naturally occurring antioxidants has been promoted due to their potential health benefits. Plant-derived antioxidants, especially phenolics, have gained considerable importance based on their antioxidant and antimicrobial activities. Plant products are successful in drug discovery due to their chemical diversity and the possibility of creating different bioactive molecules [5].

The antioxidant activity of different phenolic compounds is mainly due to their redox properties, which play an important role in neutralizing free radicals, as singlet and triplet oxygen quenchers, metal chelators, or in decomposing peroxides [6]. For a preliminary screening of the antioxidant activity of plant extracts, several *in vitro* assays have been developed. These assays may be classified into Single Electron Transfer (SET) or Hydrogen Atom Transfer (HAT) methods. The former is based on the ability of antioxidants to scavenge radicals (radical cation decolorization assay ($ABTS^{\bullet+}$) and free radical scavenging activity ($DPPH^{\bullet}$)) or reduce the compounds present in the reaction mixture, e.g., Ferric Reducing Antioxidant Power (FRAP), total phenol content (by Folin-Ciocalteu)) by transferring one electron to them. The HAT method is based on inhibiting peroxyl-radicals (Oxygen Radical Absorbance Capacity (ORAC), Hydroxyl Radical Absorbance Capacity (HORAC), Hydroxyl Radical Scavenging Capacity (HOSC)), and, therefore, they are more relevant to the processes in the biological systems [7,8].

The human body uses an antioxidant defense mechanism to balance off excessive reactive oxygen species. This defense mechanism includes enzymatic (catalase, peroxidase, superoxide dismutase, and glutathione S-transferase) and non-enzymatic antioxidants (tocopherols, phenolic compounds, and ascorbic acid). The antioxidant capacity of food assessed in vitro has long been used to uncover health advantages from antioxidant activity at the cellular level.

Different epidemiological studies have shown that it is beneficial for health to consume plant foods rich in antioxidants because they regulate many degenerative processes and can effectively lower the occurrence of cancer and cardiovascular diseases [4,9-11].

Not only important as a decorating and beautiful plant but *Paeonia* species have also been used as medicinal plants [12]. For Example, Ottomans used them to treat internal diseases, pains, and epilepsy [13]. Some *Paeonia* species have been consumed as a tea, against constipation, epilepsy, and antitussive purposes [14]. In Chinese traditional medicine, *Paeonia* species have been used against atopic eczema as well as for anticoagulant, anti-inflammatory, antihyperglycemic, analgesic, anti-inflammatory, antispasmodic agent, and for sedative purposes [15]. In Persian traditional medicine, *P. officinalis* has been used in the treatment of epilepsy, nightmare, tremor, paralysis, and uterine complications [16]. Also, the roots of *P. officinalis* have been used in Unani and Ayurvedic medicine for years as an ingredient in many antioxidant preparations for treating disease states such as jaundice, dropsy, hepatitis, hepatomegaly, liver dysfunction, cirrhosis, and sluggish liver [17]. Also, *P. officinalis* has indicated strong protective effects against acute liver injury in rats [18].

For the isolation of plant antioxidant compounds, solvent extraction is the most frequently used technique. Antioxidant activity and the extract yields of the plant are

strongly dependent on the nature of extracting solvent. Since different antioxidant compounds with different chemical characteristics and polarities are present in plants, their solubility is different in a particular solvent. For the isolation of polyphenols from plants, frequently polar solvents are employed: aqueous mixtures containing ethanol, methanol, acetone, and/or ethyl acetate [19,20].

In this study, we have measured antioxidant and antimicrobial activity and the effects of solvents on the extraction of three *Paeonia* species extracts *P. peregrina*, *P. mascula,* and *P. officinalis*. Antioxidant activity was assessed by using different in vitro analytical methodologies such as 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging activity, 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity, total phenolic content by Folin-Ciocalteau's method, total flavonoid content by $AlCl_3$ colorimetric method, superoxide anion radical scavenging by riboflavin–NBT-illuminate system and hydrogen peroxide by 4-amino antipyrine colorimetric method. The search for new defensive compounds from plants is ongoing, given the availability of plentiful renewable resources that encompass a diverse range of natural products. Developing plant-derived chemicals is particularly significant, as they can aid in microbial control. Therefore, this study also aims to investigate the antimicrobial activities of three *Paeonia* species.

EXPERIMENTAL SECTION

Plant Material

The aerial components of *P. peregrina*, *P. mascula,* and *P. officinalis* were collected from an oak forest habitat in the hilly mountainous region of Pashtrik, located in the Republic of Kosovo. The plant specimen was identified and authenticated at the Herbarium of the Department of Biology, Faculty of Mathematical and Natural Sciences of the University of Prishtina, and voucher specimens were deposited (0000135/2019 (*P. peregrina*), 0000258/2019 (*P. mascula*) and 0000283/2019 (*P. officinalis*)).

The authentication process followed the IUCN criteria as outlined in the Red Book of Vascular Flora of the Republic of Kosovo (2013).

Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Germany): Folin – Ciocalteu reagent; Gallic acid; Na_2CO_3; $AlCl_3$; Catechin; NaOH; $NaNO_2$; Phosphate buffer (0.1M), pH=7.4; H_2O_2; Riboflavin; Nitroblue tetrazolium (NBT); EDTA; Acetate buffer; ABTS (2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid)); Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid); HCl; TPTZ (2,4,6-Tri(2-pyridyl)-*s*-triazine); $FeCl_3$; Acetic acid. All used reagents are from Sigma – Aldrich©, Germany.

Preparation of Extract

P. peregrina, *P. mascula,* and *P. officinalis* were cleaned, shade dried, divided separately into stems, leaves, sepals, and seeds, and then finely grounded. The powdered material was extracted by three different solvents hexane, ethyl acetate, and ethanol for 24 h in a magnetic stirrer at room temperature. The mixture was filtered through Whatman No. 1 filter paper and then extracts were evaporated using a rotary evaporator under reduced pressure to a crude extract. The extracts were stored at −18°C in a freezer until further analysis [20].

The yield percentage of the extracts were each determined using the formula (1).

$$\text{Extraction Yield (\%)} = (\text{Extract (mg)}/\text{dry weight (g)}) \times 100 \quad (1)$$

Total Phenol Content (TPC)

Total phenol content (TPC) in each extract was determined using the Folin-Ciocalteau's (FC) method described by *McDonald et al*. [21], with minor modifications. Diluted extract or gallic acid (1.6 mL) was added to 0.2 mL FC reagent and mixed thoroughly for 3 minutes. Sodium carbonate (0.2 mL, 10% w/v) was added to the mixture and the mixture was allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 760 nm using a UV-VIS spectrophotometer GENESYS 10S model (Thermo Scientific™). The calibration curve was established using gallic acid (0, 5, 10, 20, 30, 40, 50, 60 mg/mL). TPC was expressed as milligram gallic acid equivalent per gram of dry weight (mg GAE/g DW) using a regression equation ($y = 0.0005x + 0.1209$; $r^2=0.982$).

Total Flavonoid Content (TFC)

Total flavonoid content was determined by aluminum chloride colorimetric method [22], with minor modifications. The diluted extract was added to 0.15 mL of a $NaNO_2$ (5%) solution, after 6 min 0.15 mL of aluminum chloride hexahydrate (10%) solution was added

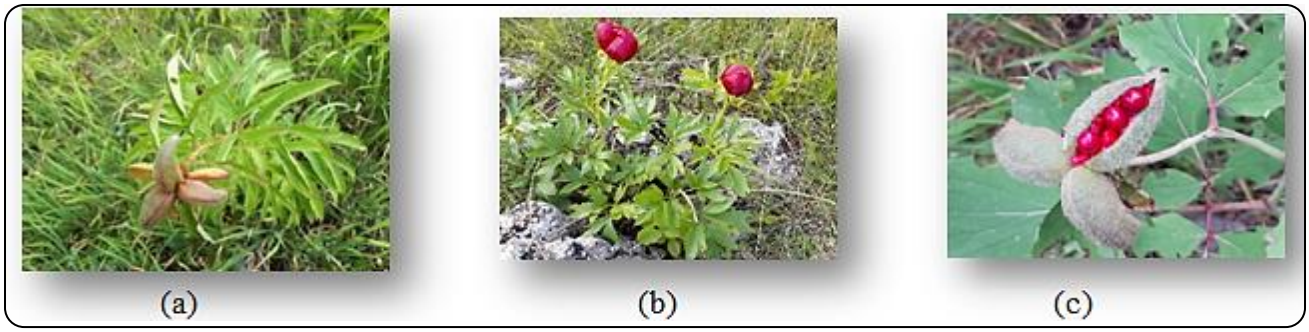

Fig. 1: Three Paeonia species were collected in Pashtrik, Republic of Kosovo: (a) P. peregrina, (b) P. mascula, and (c) P. officinalis.

and the mixture was allowed to stand for another 6 min and 2 mL of 4% NaOH was added, and the total volume was made up to 5 mL with distilled water. The solution was well mixed and was allowed to stand for 15 min. The absorbance was measured immediately against the blank at 510 nm in comparison with the standards prepared similarly with known catechin concentrations and results were expressed in catechin equivalent per gram of dry weight (mg CE/g DW) using a regression equation ($y = 5.1534x + 0.0796$; R^2=0.994).

Determination of Antioxidant Activity (AA)

DPPH Free Radical Scavenging Activity

The principle of this antioxidant assay is the capability of DPPH, a stable free radical, to diminish the color of the DPPH solution in the presence of antioxidants. The radical scavenging activity of extracts was measured by the DPPH method [23], with minor modifications. DPPH solution (0.1 mM) was prepared in methanol and 0.5 mL of this solution was added to 1.5 mL of extract solution in methanol at different concentrations (0, 10, 20, 30, 40, 50, 60 µg/mL). Solutions were vortexed thoroughly and incubated in dark. After 30 minutes the absorbance was measured at 517 nm against blank samples. Lower absorbance of the reaction mixture shows higher DPPH free radical scavenging activity. The percentage of radical inhibition was calculated by the Eq. (2).

$$\% \text{ Scavenging [DPPH]} = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

A_0 - absorbance of the control and A_1- absorbance in the presence of the sample.

ABTS Radical Cation Decolorization Assay

ABTS radical scavenging activity was determined according to *Re et al*. [24], in which ABTS is oxidized with potassium persulfate. The working solution was prepared by mixing 7.4 mM $ABTS^{\bullet+}$ solution and 2.6 mM potassium persulfate solution in equal quantities and allowing them to react for 12 h at room temperature in the dark. Before usage, the $ABTS^{\bullet+}$ solution was diluted with sodium phosphate buffer (0.1 M, pH 7.4). In 3 mL of extract solution in methanol, 1 mL of $ABTS^{+}$ solution was added. After 30 min, the absorbance was measured at 734 nm. The percentage of radical inhibition was calculated by the Eq. (3).

$$\% \text{ Scavenging [ABTS]} = [(A_0 - A_1)/A_0] \times 100 \quad (3)$$

A_0 - absorbance of the control and A_1 - absorbance in the presence of the sample.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP method was determined according to Benzie and Strain [25]. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous, colored form (Fe^{2+}-TPTZ) in the presence of antioxidants. The Frap reagent contained 1 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine), 1 mL 20 mM $FeCl_3$ diluted in 10 mL 300 mM acetate buffer, pH 3.6. This reagent was prepared daily and warmed to 37ºC. The measurement was performed by mixing 200 µL of the extract with 1.8 mL of Frap reagent, incubating for 10 minutes at 37 ºC and finally measuring the absorbance at 593 nm, with a blank (1.8 mL Frap reagent, + 200 µL distilled water). FRAP was expressed as a milligram of Trolox equivalent per gram of dry weight (mg TE/g DW) using a regression equation:

$R^2 = y = 0.0025x + 0.1209$; 0.768

Hydrogen Peroxide Scavenging Activity (H_2O_2)

The scavenging activity of hydrogen peroxide (H_2O_2) assay was performed according to the method of *Ruch et al*. [26]. with minor modifications. Hydrogen

peroxide solution (40 mmol/L) was prepared in a phosphate buffer (50 mmol/L, pH 7.4) (4), and the concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. W=30%, þ= 1.45g/cm^3, c_2=40mM, v_2=100ml

$$c1 = \frac{\text{þ x w 100}}{\text{M}} V1 = \frac{\text{c2 x V2}}{\text{c1}} \quad (4)$$

The diluted extract was added to hydrogen peroxide solution and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenged hydrogen peroxide was calculated using Eq. (5).

$$\% \text{ Scavenging } [H_2O_2] = [(A_0 - A_1)/A_0] \times 100 \quad (5)$$

A_0 - absorbance of the control and A_1 - absorbance in the presence of the sample.

Superoxide Anion Radical Scavenging Activity (Sox)

The superoxide radical (Sox) is a destructive free radical. The scavenging activity of hydrogen peroxide superoxide anion radicals was estimated according to *Zhishen et al*. [27], with minor modifications. 1 mL of extract was added to the reaction mixture that contained 1 mL of each riboflavin (1.33×10^{-5} M), NBT (8.15×10^{-8} M), and methionine (4.46×10^{-5} M). Solutions were prepared using 0.05 M phosphate buffer (pH 7.8). The reaction mixture was illuminated at room temperature for 30 min. As a blank, the un-illuminated reaction mixture was used. The absorbance of the reaction mixture was measured at 560 nm. The percentage of scavenged superoxide anion was calculated using Eq. (6).

$$\% \text{ Scavenging} = [(A_0 - A_1)/A_0] \times 100 \quad (6)$$

A_0 - absorbance of the control and A_1 - absorbance in the presence of the sample.

Antimicrobial Activity

The agar well diffusion method was used to assess the extracts' antibacterial activity [28,29]. Molten agar (15 mL, at 45 °C) was placed into sterile Petri dishes (90 mm), and then 50 µL of each tested bacteria's five-hour-old culture and 100 µL of *C. albicans* fungus five-hour-old culture were distributed uniformly over the surface of the agar plates. A sterile cork borer was used to puncture 5 mm wells into the agar after the plates had been aseptically dried. Each extract was diluted to a final concentration of 1 mg/mL in dimethyl sulfoxide (DMSO-water, 1-9; v/v), then filtered through 0.22 µm pore-size black polycarbonate filters (Millipore). The plates were incubated at the proper temperature for 24 hours for bacterial strains and 48 hours for *C. albicans*. The diameter of the inhibitory zone formed around the wells was measured in millimeters. The measures were done at the National Institute of Public Health.

Table 1: %Yields of hexane, ethyl acetate, and ethanol extract of P. officinalis, P. mascula, and P. peregrina.

	Yields %		
Extracts	*P. officinalis*	*P. mascula*	*P. peregrine*
Hexane	1.12	0.98	0.89
Ethyl acetate	0.85	0.74	0.66
Ethanol	2.95	2.71	2.70

Statistical Analysis

The data obtained from the experiment were analyzed using an SPSS one-way analysis of variance (ANOVA) to determine if there were significant differences between the groups. To further investigate any significant differences, we conducted Duncan's multiple-range test with a significance level of $P<0.05$. All reported values are expressed as means of triplicate determinations ± standard deviation to provide a measure of the variability in our data. In addition, we conducted Pearson's correlation test to determine the linear correlations among variables. This allowed us to examine any potential relationship between variables and assess their strength and direction. Overall, the statistical analyses performed in this study provide a robust and reliable basis for interpreting our results and drawing valid conclusions from our data.

RESULTS AND DISCUSSION

Our results showed that ethanol extract yielded the highest amount, whereas ethyl acetate extract yielded the lowest amount. The variability in extract yields could be attributed to the similarity in polarity between the solvent and the plant material being extracted [20] (Table 1).

Total phenol and flavonoid content

In this study, we extracted different parts (stem, leaf, sepal, and seed) of three *Paeonia* species: *P. officinalis, P. mascula, and P. peregrina* using solvents of different polarities (hexane, ethyl acetate, and ethanol) to measure the total phenolics and total antioxidant potential.

Table 2: Summarized mean results for total phenol content (TPC), total flavonoid content (TFC), antiradical activity (DPPH) and (ABTS), ferric reducing antioxidant power assay (FRAP), hydrogen peroxide scavenging activity (H_2O_2), superoxide scavenging activity (Sox) in all three Paeonia species P. peregrina, P. mascula and P. officinalis.

Plant sample	TPC (mg GAE/g DW)	TFC (mg CE/g DW)	Sox (%)	H_2O_2 (%)	FRAP (mg TE/g DW)	ABTS %	DPPH %
P. officinalis	24.94± 12.72	76.12±5.99	49.99±16.06	50.47±6.74	287.46±9.21	86.20±11.32	87.99±15.17
P. mascula	20.33± 7.70	77.81±12.33	51.99±13.79	46.79±7.16	251.94±13.43	83.28±15.69	90.77± 4.89
P. peregrina	18.29± 4.93	65.99±19.44	55.93±9.72	52.77±5.52	284.79±17.14	85.98± 11.43	89.92± 6.75

Table 3: Total phenol and flavonoid content and antioxidant activity of hexane, ethanol, and ethyl acetate extracts of P. officinalis.

Solvent	TPC (mg GAE/g DW)	TF (mg CE/g DW)	Sox (%)	H_2O_2 (%)	FRAP (mg TE/g DW)	ABTS %	DPPH %
Hexane	26.52±15.69	79.22±5.60	69.68±3.73	57.96±4.80	170.52±37.04	72.61± 2.52	80.56± 26.63
Ethanol	20.45±4.69	74.72±6.93	38.63±8.70	43.62±8.27	380.95±40.26	91.29±9.34	92.13±1.43
Ethyl acetate	28.80±17.53	73.84±8.09	41.43±8.82	49.84±5.46	309.00±51.38	94.27±3.88	93.23±2.44

Table 4: Total phenol and flavonoid content and antioxidant activity of hexane, ethanol and ethyl acetate extracts of P. peregrina.

Solvent	TPC (mg GAE/g DW)	TF (mg CE/g DW)	Sox (%)	H_2O_2 (%)	FRAP (mg TE/g DW)	ABTS %	DPPH %
Hexane	18.01± 6.08	59.12±20.70	62.59±10.27	56.5±4.84	130.18±37.09	74.97± 12.94	87.37± 6.86
Ethanol	17.01± 4.93	68.66±18.06	52.75±8.92	50.36±5.37	363.19±20.25	94.38±2.51	89.23±8.33
Ethyl acetate	19.54± 3.60	72.25±21.96	51.61±7.11	51.28±5.92	369.33±45.51	90.61±4.72	92.18±5.82

Total phenolic content (Table 2), was determined using the Folin–Ciocalteu method, with the highest values observed for *P. officinalis* at 24.94 mg GAE/g DW, followed by *P. mascula* at 20.33 mg GAE/g DW and *P. peregrina* at 18.29 mg GAE/g DW. Although our results were lower than those reported by *Dienaitė et al*. [30], (43.5 mg GAE/g DW), and *Surveswaran et al*. [31], (41.47 mg GAE/g DW), they were higher to those reported by different authors [32] (14.1 mg GAE/g DW). However, direct comparison with other studies is difficult due to differences in plant families, parts used, extraction techniques, solvents, and other factors.

In contrast, total flavonoid content showed lower variation compared to total phenolic content, with the highest values observed for *P. mascula* at 77.81 mg CE/g DW, followed by *P. officinalis* at 76.12 mg CE/g DW, and *P. peregrina* at 65.99 mg CE/g DW. These results were higher than those reported by *Tusevski et al*. [33], (58.97 mg CE/g DW) but much lower than those reported by other studies [37-40] (382 mg CE/g DW).

The results presented in Tables 3, 4, and 5, suggest that the choice of solvent for extraction can have a significant impact on the total phenolic content of plant extracts. The moderate polarity of phenolic compounds means that they tend to accumulate in the solvents of medium polarity such as ethyl acetate [34-36]. This is consistent with our finding (Table 2) that the ethyl acetate extract of the *P. officinalis* had the highest total phenol content (62.45 mg GAE/g DW) while according to Table 3 and Table 4, hexane extract had the highest total phenol content in *P. peregrina* (27.32 mg GAE/g DW) and in *P. mascula* (47.21 mg GAE/g DW), respectively.

The total flavonoid content did not show significant differences among the different solvents used for extraction, (Tables 3, 4, 5 and Figs. 2, 3, 4). The highest flavonoid content was in hexane extract in *P. mascula* (83.80 mg CE/g DW) and *P. officinalis* (79.22 mg CE/g DW). However, total flavonoid content was higher in the ethyl acetate extract for *P. peregrina* (72.25 mg CE/g DW) which is similar to other authors [36,37].

Overall, these results suggest that although the choice of solvent may have an impact on the total phenolic content of plant extracts, it may not have that impact on the total flavonoid content. However, the specific pattern of solvent extraction may vary depending on the plant species, as seen from the above results.

Based on the results from Tabels 6, 7, and 8, it appears that the distribution of phenols and flavonoids varies among different plant parts of the same plant species [41]. Specifically, in *P. mascula*, the stem has the highest total phenol content (47.21 mg GAE/g DW) as well as the highest total flavonoid content (80.43 mg CE/g DW).

Table 5: Total phenol and flavonoid content and antioxidant activity of hexane, ethanol and ethyl acetate extracts of P. mascula.

Solvent	TPC (mg GAE/g DW)	TF (mg CE/g DW)	Sox (%)	H_2O_2 (%)	FRAP (mg TE/g DW)	ABTS %	DPPH %
Hexane	22.66±13.41	83.80±12.65	64.14±7.20	53.32±4.33	126.78±57.59	63.73± 7.52	90.23± 6.08
Ethanol	18.13± 1.90	73.89±10.25	46.24±11.39	39.74±4.34	286.07±67.84	92.86±5.10	90.99±3.59
Ethyl acetate	22.48±7.87	73.76±13.42	47.13±15.11	47.19±5.08	345.61±68.69	94.55±5.32	90.80±6.34

Table 6: Total phenol and flavonoid content and antioxidant activity in plant parts (stem, sepal, leaves, seed) of P. officinalis.

Plant part	TPC (mg GAE/g DW)	TF (mg CE/g DW)	Sox (%)	H_2O_2 (%)	FRAP (mg TE/g DW)	ABTS %	DPPH %
Stem	24.06±12.34	75.09±10.34	47.87±17.47	54.86±7.61	299.81±75.57	87.33± 12.67	92.91± 2.41
Sepal	29.15±19.72	79.33±4.56	54.25±13.63	43.62±10.05	294.48±130.14	86.50±11.96	76.46±28.25
Leaves	21.71±8.82	76.61±5.04	52.16±16.91	53.89±3.90	295.28±108.53	84.21±10.99	94.26±2.92
Seed	27.02±14.26	74.17±6.78	47.76±18.49	50.13±6.33	250.07±87.24	86.06±12.20	92.18±2.50

Table 7: Total phenol and flavonoid content and antioxidant activity in plant parts (stem, sepal, leaves, seed) of P. peregrina.

Plant part	TPC (mg GAE/g DW)	TF (mg CE/g DW)	Sox (%)	H_2O_2 (%)	FRAP (mg TE/g DW)	ABTS %	DPPH %
Stem	18.67±6.38	94.86±8.57	50.80±6.27	56.46±2.54	277.21±99.96	79.23± 14.90	94.28± 2.59
Sepal	16.70±4.08	65.34±3.13	53.05±7.46	54.00±7.67	278.55±123.38	93.26±4.35	92.78±4.947
Leaves	18.95±4.95	50.10±12.41	63.59±6.61	47.65±4.10	289.59±108.58	88.50±8.20	82.40±5.03
Seed	18.72±4.60	51.79±2.78	56.33±13.46	52.77±3.88	274.65±152.16	82.41±13.20	89.31±7.84

Table 8: Total phenolics and antioxidant activity in plant parts (stem, sepal, leaves, seed) of P. mascula.

Plant part	TPC (mg GAE/g DW)	TF (mg CE/g DW)	Sox (%)	H_2O_2 (%)	FRAP (mg TE/g DW)	ABTS %	DPPH %
Stem	22.43±14.96	80.43±9.70	50.71±16.50	46.86±9.36	204.27±127.60	85.26± 14.15	89.32± 4.86
Sepal	21.58±2.59	75.68±9.27	50.62±11.58	47.56±7.15	236.23±79.30	81.64±18.22	92.22±3.28
Leaves	17.57±1.40	78.10±14.09	63.99±6.09	47.18±7.85	286.92±138.72	80.29±19.58	88.58±6.88
Seed	22.88±8.75	75.49±17.22	44.78±13.31	46.00±6.19	278.91±104.33	84.18±13.08	93.04±3.61

Similarly, in *P. peregrina*, the stem has the highest total phenol content (27.32 mg GAE/g DW) as well as the highest total flavonoid content (94.86 mg CE/g DW). However, in *P. officinalis*, sepal has the highest phenol content (62.45 mg GAE/g DW) as well as the highest total flavonoid content (85.4 mg CE/g DW). These results suggest that the distribution of phenols and flavonoids in plants can be complex and may vary depending on the plant species and the specific plant part being studied.

Antioxidant activities

Ferric reducing antioxidant power (FRAP)

Based on the results from Table 2, it appears that the Ferric Reducing Antioxidant Power (FRAP) of the examined plant species varies, with *P. officinalis* having the highest FRAP content (434.57 mg TE/g DW), followed by *P. peregrina* (434.21 mg TE/g DW) and *P. mascula* (434.21 mg TE/g DW). The highest FRAP content was observed in the ethyl acetate extract of *P. peregrina* (369.33 mg TE/g DW) and *P. mascula* (345.61 mg TE /g DW), while the highest FRAP content in *P. officinalis* was observed in ethanol extract (380.95 mg TE /g DW).

Additionally, the FRAP content also varies among different plant parts. In *P. officinalis*, the stem has the highest FRAP content (299.81 mg TE /g DW), while in *P. peregrina (*289.59 mg TE /g DW) and *P. mascula* (286.92 mg TE /g DW) the leaves have the highest FRAP content (tables 6, 7, 8; Figs. 3, 4, 5).

These results suggest that different plant parts may contain varying levels of antioxidants, and the choice solvent and the extraction method may also affect the FRAP content and their potential health benefits.

DPPH radical-scavenging activity

Tables 2, 3, 4 present the results of the DPPH radical scavenging activity for different solvent extracts and plant parts, in all three *Paeonia* species. The results show that all three plant species exhibited varying levels of DPPH radical

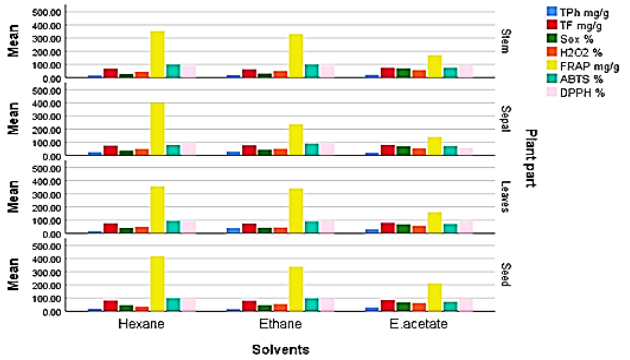

Fig. 2: Mean results for Total Phenol Content (TPC), Total Flavonoid Content (TFC) and antioxidant activity in different solvents and plant parts in P. officinalis.

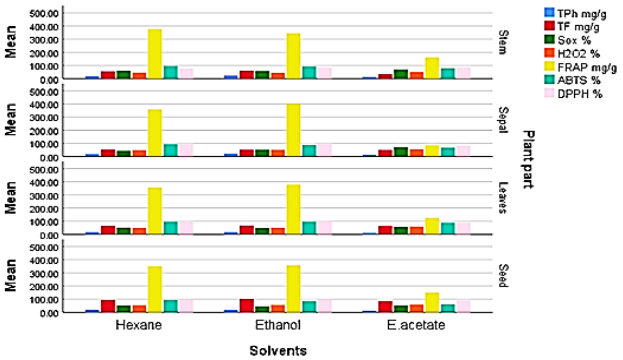

Fig. 3: Mean results for total phenols (TPC), total flavonoids (TFC) and antioxidant activity in different solvents and plant parts in P. peregrina.

scavenging activity, with the highest activity observed in *P. peregrina* (52.77%), followed by *P. officinalis* (50.47%) and *P. mascula* (46.79%). The ethyl acetate extracts of all three *Paeonia* species had the highest percentage of DPPH radical scavenging activity, 93.23% for *P. officinalis*, 92.18% for *P. peregrina*, and 90.8% for *P. mascula.*

The highest DPPH radical scavenging activity in *P. officinalis* was observed in leaves (94.26%), in *P. peregrina* was in stem (94.28%), and in *P. mascula* was in seeds (93.04%), (tables 6, 7, 8 and Figs. 3, 4, 5).

ABTS radical-scavenging activity

Varying levels of ABTS radical scavenging activity, according to Table 2, were found for all three *Paeonia* species, with *P. officinalis* having the highest activity (86.2%), followed by *P. peregrina* (85.98%) and *P. mascula* (83.28%).

The ethyl acetate extracts of the *P. officinalis* and *P. peregrina* had the highest percentage of ABTS radical scavenging activity at 94%, while the lowest was in hexane extracts. However, in *P. peregrina*, the highest percentage of ABTS radical scavenging activity was observed in ethanol extract (94.38 %), and the lowest was in the hexane extract (74.97%).

Table 7 shows that in *P. peregrina*, *the highest* ABTS radical scavenging activity was observed in sepal (93.26%), while in *P. officinalis* and *P. mascula*, the highest activity was observed in the stem 87.33% and 85.26%, respectively (tables 6, 8 and Figs. 3, 4, 5).

Hydrogen peroxide scavenging activity

According to the results from Table 2, the highest percentage of hydrogen peroxide scavenging activity

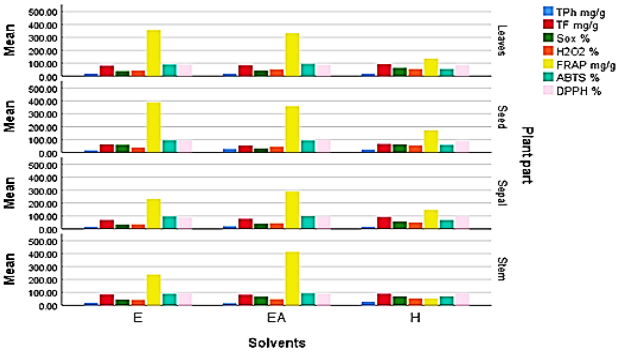

Fig. 4: Mean results for total phenols (TPC), total flavonoids (TFC) and antioxidant activity in different solvents and plant parts in Paeonia Mascula.

was observed in *P. peregrina* (52.77%), followed by *P. officinalis* (50.47%) and *P. mascula* (46.79%).

In all three *Paeonia* species, the highest percentage of hydrogen peroxide scavenging activity was observed in hexane extracts (Table 3, 4, 5), being 57.96% in *P. officinalis*, 56.5% in *P. peregrina*, and 53.32% in *P. mascula.*

Additionally, the percentage of hydrogen peroxide scavenging activity also varies among different plant parts. In *P. peregrina* and *P. officinalis* the stem has the highest percentage of hydrogen peroxide scavenging activity of 56.46% and 54.86% respectively, while in *P. mascula* the sepal has the highest percentage of hydrogen peroxide scavenging activity of 47.56% (Tables 6, 7, 8 and Figs. 3, 4, 5).

Superoxide anion radical scavenging activity

The highest percentage of superoxide anion radical scavenging activity, according to Table 2, was observed for *P. peregrina* (55.93%), followed by *P. mascula* (51.99%) and *P. officinalis* (49.99%).

Table 9: Antimicrobial activity of P. officinalis extract of hexane, ethyl acetate, and ethanol. The diameters of inhibition zones were reported in millimeters (mm).

Microorganism Extract	L. monocytogenes	S. aureus	P. aeruginosa	E. coli	C. albicans
Hexane	10	-	12	10	-
Ethyl acetate	18	18	24	21	18
Etanol	-	-	22	17	22

In all three *Paeonia* species, hexane extracts had the highest percentage of superoxide anion radical scavenging activity compared to ethyl acetate and ethanol extracts (Table 3, 4, 5), being 69.68% for *P. officinalis*, 64.14% for *P. mascula*, and 62.59% for *P. peregrina*. From the analysis of the superoxide anion radical scavenging activity in different plant parts, according to Tables 7 and 8, the highest percentage was found in leaves for *P. peregrina* (63.59%) and for *P. mascula* (63.99%), while in *P. officinalis* (Table 6) highest percentage of superoxide anion radical scavenging activity was found in sepal (54.25%), Figs. 2, 3, 4.

Antimicrobial activity

The hexane, ethyl acetate, and ethanol extracts of *P. Officinalis, P. Mascula,* and *P. Peregrina* were examined for their antibacterial activity against five pathogenic microorganisms: two Gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus*; two Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli,* and a fungus *Candida albicans*. Human invasive illnesses, particularly infections of the central nervous system, are caused by *L. monocytogenes* [42]. In humans, *S. aureus* is a very adaptable pathogen that can cause a variety of syndromes, including systemic and deep-seated infections, and toxemic syndromes [43]. The most common cause of morbidity and mortality in people with cystic fibrosis is caused by *P. aeruginosa* [44].

E. coli in addition to playing a significant role in the normal intestinal microbiota of humans and other mammals comprises also of numerous pathotypes that are responsible for a wide range of disorders [45]. The most prevalent human fungus, *C. albicans*, is an opportunistic pathogen that poses a hazard to immunologically weakened and immunocompromised individuals and can result in serious, perhaps fatal bloodstream infections in susceptible patients [46].

Table 9 presents the results of the antimicrobial activity of *P. Officinalis* extracts, which were compared to *P. Mascula*, and *P. Peregrina* extracts that showed very low or no activity against the five tested microbial strains. The study examined the antimicrobial activity of three different *P. Officinalis* extracts, namely hexane, ethyl acetate, and ethanol extracts, against various microbial strains and the fungus *C. albicans*. The hexane extract of *P. Officinalis* demonstrated moderate inhibitory activity [47] against Gram-positive pathogen *L. monocytogenes* and two Gram-negative pathogens *P. aeruginosa* and *E. coli*. However, it had no effect on the fungus *C. albicans*. On the other hand, the ethyl acetate extract of *P. Officinalis* was found to be effective against all tested bacteria and the fungus *C. albicans,* with a stronger impact on Gram-negative bacteria than Gram-positive bacteria. The highest activity was observed against the Gram-negative pathogen *P. aeruginosa* with an inhibitory zone of 24 mm. Finally, the ethanol extract of *P. Officinalis* was only effective against Gram-negative bacteria and the fungus *C. albicans,* with an inhibitory zone of 22 mm observed for both *P. aeruginosa* and *C. albicans*.

Overall, the study suggests that *P. Officinalis* extracts have varying degrees of antimicrobial activity, with ethyl acetate extract being the most effective against all tested microorganisms.

Statistical analysis

This study also investigated the correlations between the phenolic levels and antioxidant activity of extracts of the aerial portions of three different *Paeonia* species, namely *P. Officinalis, P. Mascula,* and *P. Peregrina.* The aim was to determine the influence of phytochemical elements on antioxidant capacity.

According to the Pearson correlation analysis presented in Fig. 5, in the case of *P. officinalis*, there is a highly significant strong positive correlation between FRAP-ABTS (R^2=0.777**) with p=0.001, TFC-Sox (R^2=0.679**) with p=0.001. Additionally, there is a highly significant strong negative correlation between Sox-ABTS (R^2=-0.833**) with p=0.001. When analyzing the correlations

Fig. 5: The relationship of (a) positive correlation between ABTS (%) and FRAP (mg TE/g DW) (R^2=0.777**, p<0.001); (b) negative correlation between ABTS (%) and Sox (%) (R^2=-0.833**, p<0.001) in P. officinalis.

Fig. 6: The relationship of (a) positive correlation between ABTS (%) and FRAP (mg TE/g DW) (r=0.762**, p<0.001); (b) negative correlation between H_2O_2 (%) and ABTS (%) (r=-0.639**, p<0.001) in P. Mascula.

in different solvents, the hexane extracts showed a highly significant strong positive correlation between TPC-DPPH (R^2=0.772**) with p=0.025.

Finally, when looking at the correlations in different plant parts, the leaves of *P. officinalis* showed a highly significant very strong positive correlation between FRAP-ABTS (R^2=0.980**) with p=0.001.

Overall, the study highlights the strong relationship between phenolic levels and antioxidant activity of *P. officinalis* extracts, with the FRAP-ABTS and TFC-Sox assays being the most indicative of antioxidant activity. The results also suggest that different solvents and plant parts may have an impact on the antioxidant activity of the extracts and that the hexane extract is rich in phenolic compounds and possesses significant antioxidant activity. Also, results suggest that the leaves of *P. officinalis* contain a high level of phenolic compounds and have potent antioxidant activity.

The Pearson correlation analysis presented in (Fig. 6) pertains to the correlations between the phenolic levels and antioxidant activity of extracts of the aerial portions of *P. mascula.* The data from Pearson correlation shows that in *P. mascula* there is a highly significant medium-strong positive correlation between FRAP-ABTS (R^2=0.762**) with p=0.001, which indicates that the phenolic content of the extract is positively associated with its antioxidant activity. Additionally, there is a highly significant medium-strong negative correlation between ABTS-H_2O_2 (R^2=-0.639**) with p=0.001. This suggests that the presence of phenolic compounds in the extract may reduce the effectiveness of the ABTS-H_2O_2 assay in measuring antioxidant activity.

When analyzing the correlations in different plant parts, the leaves of *P. mascula* showed a highly significant very strong positive correlation between TFC-Sox (R^2=-0.947**) with p=0.004 and between FRAP-ABTS: (R^2=-0.959**) with p=0.003.

On the other hand, data from Pearson correlation in different solvents shows that there is no significant correlation between parameters in hexane and ethyl acetate extracts. However, in ethanol extract, there is a highly

Fig. 7: The relationship between: (a) positive correlation between TPC (mg GAE/g DW) and FRAP (mg TE/g DW) (r=0.650**, p<0.001); (b) negative correlation between Sox (%) and FRAP (mg TE/g DW) (r=-0.608** p<0.002) in P. Peregrina.

significant very strong negative correlation between TPC-ABTS (R^2=-0.986**) with p=0.001.

Overall, this information provides insights into the correlation between phenolic levels and antioxidant activity of *P. mascula* extracts, with leaves being a particularly rich source of phenolic compounds and antioxidant activity. The results also suggest that different solvents may extract different phytochemical components, which may influence the antioxidant activity of the extracts.

The data from Pearson correlation analysis of the phenolic levels and antioxidant activity of extracts from the aerial parts of *P. Peregrina* (Fig. 7), revealed a highly significant medium-strong positive correlation between TPC-FRAP (r=0.650**) with p=0.001 and ABTS-FRAP (r=0.757**) with p=0.001, which indicates that the phenolic content of the extract is positively associated with its antioxidant activity. Additionally, there is a highly significant medium-strong negative correlation between Sox-FRAP (r=-0.608**) with p=0.002.

When analyzing the correlation in different plant parts, the leaves of *P. Peregrina* showed that there is a significant very strong positive correlation between TPC-ABTS (r=0.849*) with p=0.03 and DPPH-ABTS (r=0.916*) with p=0.01. On the other hand, data from Pearson correlation in different solvents shows that the highest correlation was observed in the ethanol extract, with a highly significant medium-strong negative correlation between H_2O_2-Sox (r=-0.874**) with p=0.005.

CONCLUSIONS

Overall, the study found that among the three studied *Paeonia* species, *P. officinalis* is a rich source of polyphenolic compounds with higher antioxidant and antimicrobial potential compared to *P. peregrina* and *P. mascula*. The choice of solvent for extraction was found to have a significant impact on the total phenolic content of plant extracts but may not have a significant impact on the total flavonoid content. Our results also suggest that different plant parts may contain varying levels of antioxidants. Therefore, it is important to carefully consider the choice of solvent for extraction when analyzing the phytochemical content of plant extracts. Antimicrobial analysis showed that *P. mascula* and *P. peregrina* showed very low or no activity at all, while *P. officinalis* extracts showed varying degrees of antimicrobial activity against five tested microbial strains. Ethyl acetate extract effectively inhibited all of the examined bacteria and the fungus; however, it had a stronger impact on Gram-negative bacteria than it did on Gram-positive bacteria. This study also established correlations between the phytochemical content and biological activities of the different extracts, which could be useful in predicting the activity of these extracts in various applications. Data from Pearson correlation showed different correlations for *P. officinalis*, *P. peregrina*, and *P. mascula* in different extracts and in different plant parts. There is a strong correlation between phenolic levels and antioxidant activity of the extracts, with different correlations observed in different species, plant parts, and solvents. These results indicate that the antioxidant activity of these plants is largely due to the presence of phenolic compounds. These findings suggest that *Paeonia* species have the potential as natural sources of antioxidants and antimicrobial agents, and could have valuable applications in the pharmaceutical and food industries.

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