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ARAŞTIRMA MAKALESI / RESEARCH ARTICLE

PHENOLIC COMPOSITION AND ANTIOXIDANT PROPERTIES OF *RANUNCULUS ARVENSIS* L. FLOWER POLLEN: IN VITRO AND SILICO INSIGHTS

Ranunculus arvensis L. Çiçek Polenlerinin Fenolik Bileşimi ve Antioksidan Özellikleri: in Vitro ve Siliko Görüşler

Aziza HUSEYNOVA[1], Elsever ASADOV[1], Houssem BOULEBD[2], Salah AKKAL[3], Leman NOVRUZOVA[1]

[1]Nakhchivan State University, Faculty of Medical, Nakhchivan, AZERBAIJAN, Corresponding author / Yazışma Yazarı E-mail: azizahuseynova@ndu.edu.az, ORCID No: 0000 0003 0943 5214, E-mail: asadoves1974@gmail.com, ORCID No: 0000 0003 2892 2974, E-mail: leman.novruzova.1990@gmail.com, ORCID No: 0009 0008 4269 3219

[2]Laboratory of Synthesis of Molecules with Biological Interest, Frères Mentouri Constantine 1 University, 25000 Constantine, ALGERIA, E-mail: boulebd.houssem@umc.edu.dz, ORCID No: 0000 0002 7727 8583

3Unit for the Valorization of Natural Resources, Faculty of Exact Sciences, Department of Chemistry, Bioactive Molecules and Physico-Chemical and Biological Analyzes, Mentouri University of Constantine 1, ALGERIA, E-mail: salah4dz@yahoo.fr, ORCID ID: 0000 0002 4999 5673

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ABSTRACT

***Ranunculus arvensis* L., although classified as a poisonous plant, holds significant value in medicine, food, and apiculture. Its pollen is actively collected by various bee species, including honeybees, and stored as a vital nutritional reserve for their larvae. This study investigates the polyphenol and flavonoid content, as well as the antioxidant properties, of *R. arvensis* flower pollen. The antioxidant activity of the pollen, sourced from the Nakhchivan Autonomous Republic, was measured as 179.102 ± 1.5919 *μ*mol $FeSO_4 \cdot 7H_2O$/g dw using the FRAP method and 0.137 ± 0.015 mg/mL using the DPPH method. The phenolic content of the methanolic extract was determined to be 17.952 ± 0.160 mg/g, while the flavonoid content was 5.660 ± 0.055 mg/g. Phenolic profiling via HPLC identified six key compounds: ferulic acid (521.163 *μ*g/g), caffeic acid (170.119 *μ*g/g), p-hydroxybenzoic acid (46.529 *μ*g/g), protocatechuic acid (22.377 *μ*g/g), chrysin (11.353 *μ*g/g), and pinocembrin (10.953 *μ*g/g). Quantum chemistry calculations revealed that ferulic acid and caffeic acid, the most abundant phenolic compounds, exhibited the most favorable profiles for antioxidant activity. These findings suggest that these two compounds are the primary contributors to the antioxidant potential of the pollen extract. Given the nutritional and pharmacological significance of bee products, continued investigation into the phytochemical composition of flower pollen is essential to better understand its functional properties and applications.**

Keywords: *Ranunculus arvensis,* Plant pollen, Polyphenol, Flavonoid, Antioxidant, Bee food

ÖZ

***Ranunculus arvensis* L., zehirli bir bitki olarak sınıflandırılmasına rağmen, tıp, gıda ve arıcılıkta önemli bir değere sahiptir. Poleni, bal arıları da dahil olmak üzere çeşitli arı türleri tarafından aktif olarak toplanır ve larvaları için hayati bir besin rezervi olarak depolanır. Bu çalışma, R. arvensis çiçek poleninin polifenol ve flavonoid içeriğinin yanı sıra antioksidan özelliklerini araştırmaktadır. Nahçıvan Özerk**

Cumhuriyeti'nden elde edilen polenin antioksidan aktivitesi FRAP yöntemi kullanılarak 179,102 ± 1,5919 µmol $FeSO_4$-$7H_2O$/g dw ve DPPH yöntemi kullanılarak 0,137 ± 0,015 mg/mL olarak ölçülmüştür. Metanolik ekstraktın fenolik içeriği 17.952 ± 0.160 mg/g, flavonoid içeriği ise 5.660 ± 0.055 mg/g olarak belirlenmiştir. HPLC yoluyla yapılan fenolik profilleme altı temel bileşiği tanımlamıştır: ferulik asit (521.163 µg/g), kafeik asit (170.119 µg/g), p-hidroksibenzoik asit (46.529 µg/g), protokateşuik asit (22.377 µg/g), krisin (11.353 µg/g) ve pinokembrin (10.953 µg/g). Kuantum kimyası hesaplamaları, en bol bulunan fenolik bileşikler olan ferulik asit ve kafeik asidin antioksidan aktivite için en uygun profilleri sergilediğini ortaya koymuştur. Bu bulgular, bu iki bileşiğin polen ekstraktının antioksidan potansiyeline birincil katkıda bulunan bileşikler olduğunu göstermektedir. Arı ürünlerinin besinsel ve farmakolojik önemi göz önüne alındığında, çiçek poleninin fitokimyasal bileşiminin sürekli araştırılması, fonksiyonel özelliklerinin daha iyi anlaşılması için gereklidir.

Anahtar Kelimeler: *Ranunculus arvensis*, Bitki poleni, Polifenol, Flavonoid, Antioksidan, Arı besini

GENİŞLETİLMİŞ ÖZET

Amaç: *Ranunculus* L. cinsine ait bitkiler tıp, beslenme ve arıcılıkta çeşitli uygulamalarıyla bilinmektedir, ancak bu bitkiler aynı zamanda toksik özellikleri nedeniyle de sınıflandırılmaktadır (Al-Snafi, 2022). Zehirli olmasına rağmen hem bal arıları hem de diğer arı türleri *Ranunculus* bitkisinden polen toplar ve kovanlarında depolarlar. Ancak araştırmacılar, *Ranunculus* türlerinin polenlerinin düşük miktarda toksik madde protoanemioin içerdiğini ve bu nedenle arı larvaları üzerinde yıkıcı bir etkisinin olmadığını bulmuşlardır (Sedivy ve ark., 2012). Bu çalışmanın amacı, arılar tarafından çok sevilen *Ranunculus* cinsine ait *Ranunculus arvensis* L. poleninin arı larvalarının gelişimi açısından yararlı olan biyoaktif maddeleri ve antioksidan özelliklerini değerlendirmektir.

Gereç-Yöntem: *Ranunculus arvensis* L.'nin çiçekleri çiçeklenme evresinin başlangıcında toplanmış, polenler taç yapraklarından ayrılmış, kurutulmuş ve %98'lik metanol ile ekstrakte edilmiştir. Metanol ekstraktındaki (ME) toplam fenolik madde içeriği Folin-Ciocalteu reaktifi kullanılarak belirlenmiştir. Karışımın absorbansı Thermo Scientific Evolution TM 201 UV-VIS spektrofotometresi kullanılarak 760 nm'de ölçülmüşdür. Toplam fenolik içerik (TPC), gram kuru polen başına miligram gallik asit eşdeğeri (GAE) olarak ifade edilmiştir (Slinkard ve diğerleri, 1997). Toplam flavonoid içeriği (TFC), Fukumoto ve Mazza tarafından geliştirilen kolorimetrik yöntem kullanılarak 415 nm'de ölçülmüş. TFC, numunenin kurutulmuş ağırlığının (dw) gramı başına kuersetin eşdeğeri (QUE) miligramı olarak ifade edilmiştir (Fukumoto ve Mazza, 2000).

Polen özütünün toplam antioksidan kapasitesi Benzie ve Strain (1996) ve Pulido ve ark. tarafından belirlenmiştir. (2020) bitki özütlerinin antioksidan aktivitesinin belirlenmesi için uyarlanmış bir demir indirgeyici antioksidan gücü (FRAP) testi ile değerlendirilmişdir. Serbest radikal temizleme aktivitesi, Molyneux (2004) ve Erdogan ve ark. (2012) açıklanan yöntemle belirlenmiştir.

HPLC analizi, PDA dedektörü ve C18 kolonu ile donatılmış Shimadzu LC-20AT HPLC sistemi kullanılarak gerçekleştirilmişdir. Bu analizde 25 adet fenolik standart eş zamanlı olarak analiz edilmişdir. Polen ekstraktındaki polifenollerin moleküler geometrisi ve antioksidan mekanizmaları, yoğunluk fonksiyonel teorisi (DFT) hesaplamaları kullanılarak analiz edilmişdir. Bu hesaplamalar için, ampirik değişim korelasyonlarını içeren yüksek parametreli bir yöntem olan M06-2X fonksiyoneli ve 6-31+G(d,p) baz seti uygulanmışdır (Galano & Alvarez-Idaboy, 2014). Hidrojen atom transferi (HAT) için bağ ayrışma entalpisi (BDE) ve tek elektron transferi (SET) için iyonlaşma potansiyeli (IP) gibi antioksidan mekanizmalarla ilişkili termodinamik parametreler Boulebd ve arkadaşları (2022) ve Boulebd (2022) tarafından önerilen yöntemlere göre hesaplanmıştır.

Bulgular: Nahçıvan Özerk Cumhuriyeti'nde yaygın olarak yetişen *R.arvensis* L. bitkisinin poleninin polifenol içeriği ve antioksidan aktivitesi ilk kez araştırılmışdır. toplam fenolik içerik -17,952±0,160

mg GAE/g kuru ağırlık; toplam flavonoid içeriği 5.660±0.055mg QUE/g dw; toplam antioksidan kapasitesi: FRAP - 179,102±1,591 μmol FeSO4 ·7H2O/g dw; DPPH SC500'ün 137±0,015 mg/mL olduğu belirlenmişdir.

R. arvensis poleninin metanol ekstraktındaki fenolik bileşikler 25 standart kullanılarak analiz edilmiş ve bunlardan 6'sının miktarları tahmin edilmiştir: protokatekuik asit-22,377 μg/g, p-OH benzoik asit-46,529 μg/g, kafeik asit-170,119 μg/g, ferulik asit-512,163 μg/g, krizin-11,353 μg/g, pinocembrin-10,953 μg/g.

Hem HAT hem de SET mekanizmalarının bulgularına dayanarak, *R. arvensis* özütünde en aktif antioksidanların kafeik asit ve ferulik asit olduğu sonucuna varılabilir ve antioksidan aktiviteleri belirlenebilir.

Sonuç: Çiçek polenlerinden elde edilen arı poleninin sağlık açısından çok çeşitli faydaları olduğu biliniyor. Çalışmamızda, arı kolonilerinin gelişimi ve arı ürünlerinin kalitesinin artırılması amacıyla çiçek polenlerinin fenolik bileşenleri ve antioksidan aktivitesinin incelenmesi amaçlanmaktadır. *Ranunculus arvensis* çiçek poleninin biyokimyasal bileşiminde yararlı bileşenlerin incelenmesi literatüre ve araştırmacılara önemli katkı sağlaybileceyi düşünülmektedir.

INTRODUCTION

Beekeeping, which has an ancient history, has generated great interest in studying flowering plants' chemical composition, nutritional value and pharmacological benefits. Honeybees produce honey, bee pollen, bee bread, propolis, etc. from the nectar, pollen and resinous substances they collect from plants, and the chemical composition of these products largely depends on the plants used to collect them. Although the chemical composition of plants that are important for beekeeping has been widely studied, very few studies have been conducted analyzing the chemical composition of pollen. As you know, pollen is the main food source for bee colonies (Abd El-Wahab 2016).

15 genera and 57 species represent the family of Ranunculaceae Adans. in the Nakhchivan Autonomous Republic. These species are considered useful plants in medicine, food and beekeeping, but are also known to be poisonous plants. *Ranunculus arvensis* L., belonging to the genus *Ranunculus* L. section, is widespread in the middle mountain zone of the autonomous republic. It is an annual mesophytic plant with a height of 10-40 cm, a branching stem, yellow flowers and abundant pollen. According to its geographical type, it is considered a Mediterranean-Iranian-Turanian plant. The plant blooms in May-June (Talibov & Ibrahimov 2008).

Plants belonging to the genus *Ranunculus* L. are considered to be useful for medicine, food and beekeeping, but they are also known to be poisonous plants (Al-Snafi 2022). All bee genera and also honeybees collect the pollen of plants belonging to the *Ranunculus* L. family, which are toxic, and store them in the hives to feed their larvae. The toxic effect of these plant pollens on the development of bee larvae has long been a matter of debate. However, it is clear from some studies that protoanemoin, the substance that causes plant toxicity, is found in large amounts in the buds of the plant and in small amounts in the pollen. It was also established that the amount of protoanemoin in bee pollen was drastically reduced as protoanemoin was detoxified and turned into harmless substances as a result of enzymatic processes. For this reason, the pollen of plants of the *Ranunculus L*. genus contained in bee pollen was found to have no adverse effect on the bee larvae development (Sedivy et al, 2012). Although the plant belongs to the group of poisonous plants, it is also widely used in pharmacology because it is non-toxic after drying (Kurkin 2004).

In terms of chemical composition, the plant is rich in proteins, amino acids, carbohydrates, glycosides, saponins, polyphenols, and flavonoids (Boroomand et al. 2018). This plant also contains ranunculus glycoside, the enzymatic breakdown of which produces a toxic substance called protoanemonin. For this reason, the plant is considered poisonous. However, since the dried form or infusion of the plant does not contain protoanemonin, it does not have a toxic effect (An et al. 2018; Jürgens & Dötterl 2004). The flavonoid-rich plant *R. arvensis* has strong antioxidant and antimicrobial effects (Hachelaf et al. 2015; Al-Snafi 2022).

This study aimed to evaluate the phenolic compounds and antioxidant properties of *Ranunculus arvensis* L. pollen, which serves as an important food source for bees in the Nakhchivan Autonomous Republic.

MATERIALS AND METHODS

Plant Materials

Ranunculus arvensis L. (Order: Ranunculales/Family: Ranunculaceae) was collected from the Anagut village in the Ordubad district of the Nakhchivan Autonomous Republic for research purposes. Anagut is located at an altitude of 38°58'53" N. E. 45°57'51" N. U. At the end of May, During the period of full flowering of the species *R. arvensis*, parts of flowers were collected, the stamens were separated and dried in a shaded and ventilated place.

Pollen Extraction

A methanolic extract of dried flower pollen was prepared. One g of pollen was weighed, 50 mL of 98% methanol was added to it and extracted on a magnetic shaker for 24 h. The mixture was filtered through the Whatman No. 4 filter paper and then through the Whatman No. 1 filter paper. The extract was stored in a deep freezer (-18°C). In the phytochemical analysis of pollen extract of *R.arvensis,* total phenolic content, total flavonoid content, and antioxidant activity were examined.

Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent was used to measure the total phenol concentration in the methanol extract (ME). 400 µL of 0.5 N Folin-Ciocalteu reagent was mixed with 20 µL of the prepared methanol extract (*R.arvensis*). 680 µL of distilled water was added to dilute the mixture. Folin-Ciocalteu reagent is a strong oxidizing agent that reacts with the extract's phenolic compounds to form a blue complex. The mixture was incubated for 3-4 min. to allow the initial reactions between the reagent and phenolic compounds in the extract to occur. Then 400 µL of Na_2CO_3 (10%) was added and kept at room temperature for 2 h. The absorbance of the mixture was measured at 760 nm using a Thermo Scientific Evolution TM 201 UV-VIS spectrophotometer. TPC was expressed in milligrams of gallic acid equivalents (GAE) per gram of dry pollen (Slinkard et al. 1997).

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was determined using the colorimetric assay developed by Fukumoto and Mazza. This assay added 50 µL of a 10% $Al(NO_3)_3$ solution and 50 µL of a 1.0 M NH_4CH_3COO solution to 25 µL of the pollen extract. The aluminum ions (Al^{3+}) are responsible for forming complexes with the flavonoid molecules in the extract. The mixture was incubated at room temperature for 45 min., after which the absorbance was read at 415 nm. Quercetin standards were utilized to express TFC as milligrams of quercetin (QUE) per gram of dried weight (dw) of the sample (Fukumoto & Mazza 2000).

Total Antioxidant Capacity

The total antioxidant capacity of the pollen extract was measured using the ferric-reducing antioxidant power assay (FRAP) method. The method was developed by Benzie and Strain (1996) and adapted by Pulido et al, (2020) for the determination of the antioxidant activity of plant extracts. The FRAP reagent was prepared by mixing 2.5 mL of 10 mM TPTZ, 2.5 mL of 20 mM $FeCl_3$, and 25 mL of 300 mM acetate buffer at pH 3.6. Three milliliters of FRAP reagent and 100 µL of the sample were mixed and incubated for 4 min at 37°C. During the incubation period, the Fe^{+3} ions in the ferryl tripyridyltriazine reagent are reduced to Fe^{+2} ions, and the color of the reagent turns dark blue. The absorbance was then read at 595 nm. The FRAP results were reported as micromoles of $FeSO_4 \cdot 7H_2O$ gram of dry weight.

DPPH (2,2- diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

Free radical scavenging activity was measured using the method described by Molyneux (2004) and Erdogan et al. (2012). Briefly, 750 µL extract of the sample was mixed with 750 µL of DPPH radical solution. This mixture was then kept in the dark for 45 min at 25°C, absorbance being read at 517 nm. The result was calculated as SC_{50}, with lower SC_{50} values indicating higher radical scavenging activity.

RP-HPLC-PDA (Reversed-Phase High-Performance Liquid Chromatography) Analysis and Determination of Phenolic Derivatives

The HPLC analyses were conducted on a Shimadzu liquid Corporation LC 20AT HPLC system equipped with a PDA detector, C18 column (250 mm × 4.6 mm, 5 µm; GL Sciences). The elution was performed using mobile phase A (10% Acetonitrile-ultra pure water solution), and mobile ase B (2% acetic acid in water). The flow rate was 1 ml/min and the injection volume was 20 µL. The detection was performed at 250, 280, 320, and 360 nm, at a column temperature of 30ºC. The gradient program changed according to the following conditions: 95% A and 5% B as initial

conditions,15% A and 85% B for 8 min, 21% A and 79% B for 10 min, 52% A and 48% B for 20 min, 67% A and 33% B for 35 min, 90% A and 10% B for 50.5% A and 95% B for 50.1 and finally 5% A and 95% B for 60 min. Before all the prepared samples were applied to the device, they were injected and filtered through 0.45 *μ*m membranes (Kolayli et al, 2024; Zehra et al. 2015).

Standard Phenolics

25 phenolic standards including gallic acid, protocatechuic acid, p-OH benzoic acid, m-OH benzoic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, epicatechin, rutin, myricetin, quercetin, apigenin, resveratrol, daidzein, t-cinnamic acid, hesperetin, luteolin, rhamnetin, chrysin, pinocembrin, CAPE, curcumin and ellagic acid were analyzed simultaneously.

DFT calculations

The molecular geometry and antioxidant mechanisms of the polyphenols of the pollen extract were investigated using density functional theory (DFT) calculations. The M06-2X functional, which is highly parameterized and includes empirical exchange-correlation, was used for all calculations along with the 6-31+G(d,p) basis set. M06-2X is one of the most accurate methods for thermodynamic calculations of radical reactions (Galano & Alvarez-Idaboy, 2014). The effects of ethanol as a solvent were simulated using Truhlar's SMD solvation model. The thermodynamic descriptors of the investigated antioxidant mechanisms, including bond dissociation enthalpy (BDE) related to the hydrogen atom transfer (HAT) mechanism and ionization potential (IP) related to the single electron transfer (SET) mechanism, were calculated as follows (Boulebd et al. 2022.; Boulebd 2022).

$$BDE = H(HZ{-}N^{\cdot}) + H(H^{\cdot}) - H(HZ)$$

$$IP = H(HZ^{+\cdot}) + H(e^{-}) - H(HZ)$$

Where, $H(HZ)$, $H(HZ{-}N^{\cdot})$, $H(HZ^{+\cdot})$, $H(e^{-})$, and $H(H^{+})$ are enthalpies of the neutral molecule, radical, radical cation, electron, and proton, respectively. All calculations were performed using Gaussian09 software (Frisch et al. 2009). The analysis and visualization of the results were conducted using Multiwfn and VMD software (Lu & Chen 2012; Humphrey et al. 1996).

Statistical Analysis

Three replicate experiments were conducted, and the resulting data were analyzed on SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Tukey's test were used to compare TPC, TFC, FRAP, and DPPH parameters among the different species.

RESULTS

We studied the content of polyphenols and antioxidant activity of the pollen of the *R.arvensis* plant, common in the Nakhchivan Autonomous Republic, for the first time. Since methanol is the best solvent for most polyphenol derivatives, 98% methanol was used to prepare the extract. Table 1 displays the results of the quantitative analysis of total phenol and flavonoid concentrations and antioxidant activity of the *R. arvensis* pollen.

Table 1. Total phenolic content, total flavonoid content, and antioxidant capacity of the *R.arvensis* pollen

	***R.arvensis* flower pollen extract (Mean ±st)**
Total phenolic content (mg GAE/g dw)	17.952±0.160
Total flavonoid content (mg QUE/g dw)	5.660±0.055
Total antioxidant capacity (FRAP) (*μ*mol $FeSO_4 \cdot 7H_2O$/g dw)	179.102±1.591
DPPH SC_{50} (mg/mL)	0.137±0.015

Phenolic compounds in the methanolic extract of *R.arvensis* pollen were analyzed using 25 standards. Of these, the amount of 6 substances was rated quite highly. The results of the RP-HPLC-PDA analysis are presented in Table 2.

Table 2. Phenolic composition of the *R.arvensis* pollen based on spectrophotometry and high-performance liquid chromatography

Phenolic acids (*µ*g/g)		Flavonoids (*µ*g/g)	
Gallic Acid	nd*	Resveratrol	nd
Protocatechuic Acid	22.377	Daidzein	nd
Chlorogenic Acid	nd	Luteolin	nd
p-OH Benzoic Acid	46.529	Quercetin	nd
t-Cinnamic Acid	nd	Epicatechin	nd
Caffeic Acid	170.119	Apigenin	nd
Syringic Acid	nd	Hesperidin	nd
m-OH Benzoic Acid	nd	Rhamnetin	nd
p-Coumaric Acid	nd	Chrysin	11.353
Ellagic Acid	nd	Pinocembrin	10.953
Ferulic Acid	512.163	CAPE	nd
		Curcumin	nd
		Rutin	nd
		Myricetin	nd

nd*: Not detected*

According to the HPLC chromatogram, the amount of pinocembrin was lower (10.953 *µ*g/g), while the amount of ferulic acid was higher (521.163 *µ*g/g). The antioxidant function of each of the resulting phenol derivatives has been studied since they are substances widely used in the food, pharmaceutical, and cosmetic industries.

In silico studies

The main phenols found in the *R.arvensis* extract, which are protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, chrysin, and pinocembrin, were individually investigated for their antioxidant capacity using DFT calculations.

The molecular geometries of the compounds were optimized using the M06-2X/6-31+G(d,p) level of theory, with ethanol (EtOH) as the solvent to simulate the environment of the in vitro experiments. The most stable molecular geometries are presented in **Figure 1**. Except for pinocembrin, all compounds exhibit a planar geometry, reflecting significant electron delocalization throughout their molecular structures. Pinocembrin, which differs from the others by the saturation of the pyran ring, displays a non-planar geometry. In this case, the benzene moiety is inclined at an angle of 87° from the molecular plane. The distribution of HOMO and LUMO orbitals, also shown in **Figure 1**, further highlights the extensive electron delocalization in these compounds. Analysis of the HOMO energy levels reveals that caffeic acid and ferulic acid have the highest values (-7.21 to -7.26 eV compared to -7.63 to -7.96 eV for other compounds), indicating superior electron-donating capacities. Additionally, the HOMO-LUMO energy gap analysis demonstrates that caffeic acid and ferulic acid exhibit the smallest gaps (6.12–6.16 eV versus 6.38–7.49 eV for other derivatives), suggesting their higher chemical reactivity relative to the other compounds.

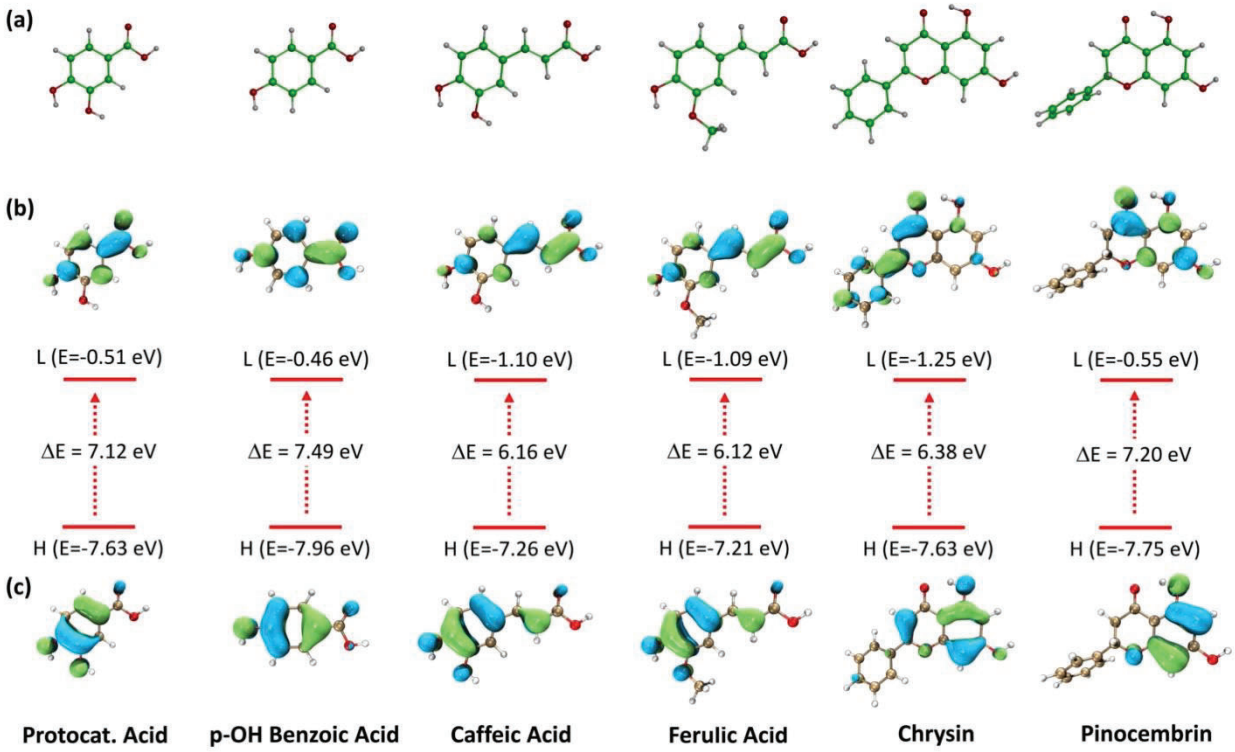

Figure 1. Molecular geometry (a), LUMO (b), and HOMO (c) of the main polyphenols of *R.arvensis* extract obtained at M06-2X/6-31+G(d,p) level in EtOH.

The two main mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET), which govern the experimental assays, were investigated (Lü et al. 2010; Spiegel 2022). In the HAT mechanism, the antioxidant transfers a hydrogen atom to the DPPH radical, transforming it into a more stable radical species (Boulebd, 2023). This mechanism is characterized by the bond dissociation enthalpy (BDE) of the active OH bond. For the SET mechanism, the antioxidant donates an electron to the Fe ion or a free radical, neutralizing it (Boulebd 2024). This mechanism is characterized by the ionization potential (IP) of the antioxidant. The BDE and IP values calculated for the HAT and SET mechanisms, respectively, of the compounds studied are shown in Figure 2. Regarding the HAT mechanism, the lowest BDE value was obtained for the 4-OH group of caffeic acid (79.9 kcal/mol), followed by protocatechuic acid and ferulic acid, which showed approximately the same values of 83.3 and 83.4 kcal/mol, respectively. These results indicate that caffeic acid, protocatechuic acid, and ferulic acid are the most active compounds in the HAT mechanism. On the other hand, the analysis of IP values also shows the lowest values for caffeic acid and ferulic acid (112.4 and 111.7 kcal/mol, respectively), indicating that these molecules may also exhibit the highest electron-donating capacity compared to the other phenolic derivatives. Based on the findings from both the HAT and SET mechanisms, we can conclude that caffeic acid and ferulic acid are the most active antioxidants in the *R.arvensis* extract and determine their antioxidant activity.

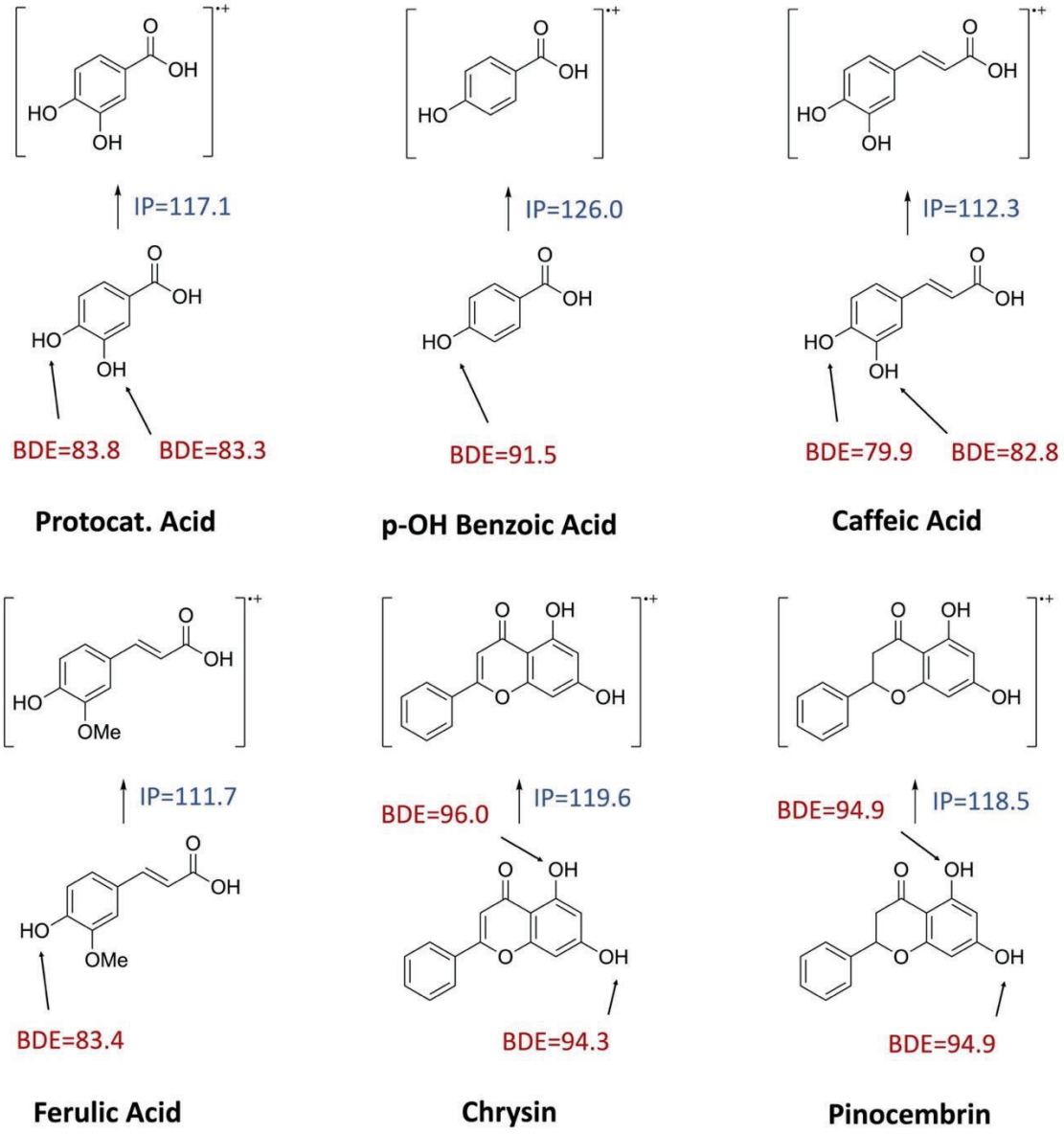

Figure 2. Computed BDE and IP kcal/mol of the main polyphenols and antioxidant mechanisms (d) of the main compounds of the *R.arvensis* extract obtained at M06-2X/6-31+G(d,p) level in EtOH.

DISCUSSIONS

There are no studies on phytochemical analysis of *R.arvensis* pollen extract in scientific databases. In this study, the phytochemical composition of *R.arvensis* pollen was compared with the composition of flower pollens of other plants that are food sources for bees. Such comparisons help to better understand the biologically active components of different types of pollens for the development of bee colonies and the benefits of bee products.

Kostic et al. (2021) analyzed the phytochemical composition of pollen collected by honey bees from the artichoke (*Cynara scolymus*) plant in the Belgrade region. In this study, phenolic compounds and their amounts in the pollen were studied. According to the results, TPC in Artichoke pollen was

5.3 mg/g GAE DW, TFC 0.81 mg/g QE DW. HPLC DAD MS/MS analysis identified 10 phenolic compounds. Among phenolic compounds, the highest amount was found in Isorhamnetin 3-O-glucoside (49,171 mg/kg), which accounts for more than 70% of the total phenol content identified (Kostic et al. 2021). Based on the results obtained in *R.arvensis* pollen, it was found that the amounts of TPC and TFC were 3-5 times higher than those of artichoke pollen. The highest amount of phenolic compounds identified was also in ferulic acid, which accounts for more than 60% of the total phenolic compounds.

In a study conducted by De-Melo et al., (2018) the phenol and flavonoid contents and antioxidant properties of 8 monofloral bee pollen samples collected from the state of Brasilia, with more than 90% pollen from the same plant, were studied. As a result, TPC was obtained in various amounts, depending on the plant species, ranging from 5.6 to 29.7 mg GAE/g, and flavonoid content was obtained in amounts ranging from 0.3 to 19.0 mg GAE/g. Antioxidant evaluation according to the DPPH method revealed 10.3 to 110.8 µmol TE/g. Based on the results obtained from HPLC-MS analysis, quercetin, kaempferol 3-O-glycosides, and isorhamnetin flavonoid glycosides predominated (De-Melo et al. 2018).

In the study carried out by Sadeq et al., (2021) the phytochemical composition, antioxidant and antibacterial properties of *Micromeria fruticosa*, *Achilleafra grantissima*, *Phoenix dactylifera* plant pollens distributed in the Palestinian Territory were studied. Among the samples, high results were observed in the *Micromeria fruticosa* plant pollen according to all indicators. *Micromeria fruticosa* pollen extract contained TPC 56.78 ± 0.49 mg GAE /g, TFC 2.48 ± 0.05 and 8.03 ± 0.01 mg QE/g, and IC50 values of 0.047 and 0.039 mg/mL were found in DPPH and FRAP assays, respectively (Sadeq et al. 2021). The study of plant pollen, which is a food source for honey bees, makes a great contribution to determining both the development of bee colonies and the nutritional and pharmacological value of bee products. *Micromeria fruticosa* (*Lamiaceae* L) is one of the nectar- and pollen-producing plants for honey bees (Albaba 2015).

These analyses show that *R.arvensis* pollen, a species of *Ranunculus* L., which is widespread in forests and meadows, is a source of phenolic and flavonoids for honey bees.

Quantum chemical calculations using the DFT method at the M06-2X/6-31+G(d,p) level revealed significant differences in the reactivity of the extract's primary polyphenols. Caffeic acid and ferulic acid emerged as the most reactive compounds, following both hydrogen atom transfer (HAT) and single electron transfer (SET) pathways. These results are consistent with the potent and well-documented antioxidant activity of these compounds, making *Ranunculus arvensis* L. flower pollen a rich source of antioxidants.

Ranunculus arvensis L., which has a mass flowering period in April-May in many regions of the Nakhchivan Autonomous Republic, has been observed to be used as a food source by honey bees during the active beekeeping season. X et al. Y et al. etc. studies indicate that monofloral bee pollen, which constitutes 90-100% of *R.arvensis* plant pollen, is obtained. This shows that *R. arvensis* is a plant loved by bees and its pollen is used as food. In this study, the TPC, TFC and antioxidant content of *R.arvensis* flower pollen is studied to understand the value of this plant for the development of bee colonies. *R.arvensis*, which has a higher TPC, TFC and antioxidant value than most flower pollens, is antimicrobial for bees. It has anti-inflammatory and antioxidant effects. This also means the healthy development of bee colonies.

We have observed the use of Ranunculus arvensis L., which has a mass flowering period in April-May in many regions of the Nakhchivan Autonomous Republic, as a food source by honey bees during the active beekeeping season. The lack of literature data on the biochemical analysis of Ranunculus arvensis flower pollen is a factor that emphasizes the originality and importance of this study. Thus, the fact that these analyses were performed by us for the first time not only presents a new approach scientifically, but also creates a basic database for other studies to be conducted in this field in the future. Such studies are also of particular importance in terms of their contribution to the development of plant pollens and the field of beekeeping. The results of biochemical analyses of *Ranunculus arvensis* flower pollen show that, unlike other plant pollens loved by bees, it has rich phenolic components. Phenolic components play an important role in the healthy development of bee colonies and in leading a healthy lifestyle in general. Thanks to the antioxidant properties of phenols, they strengthen the immune system of bees and increase their resistance to diseases. The result confirms the idea

that pollen plays an important role in the development of beekeeping. This also supports the development of the beekeeping industry and has a positive impact on its productivity.

This topic opens up an interesting area for further research into apitherapy and the role of bees in the ecosystem. A more extensive biochemical analysis of the relationship between bee health and plant pollen composition may offer new approaches to protecting bees in agriculture and natural ecosystems.

Conclusion: The present study highlights the significance of *Ranunculus arvensis* as a valuable pollen source for honey bees during the mass flowering period in April-May within the Nakhchivan Autonomous Republic. Comprehensive phytochemical, antioxidant, and HPLC analyses revealed that ferulic acid and caffeic acid are abundant phenolic components in the pollen. DFT calculations further identified these compounds as key contributors to the antioxidant activity of the extract. The bioactive-rich pollen of *R. Arvensis* underscores its potential to support the healthy development of bee colonies. Furthermore, the transfer of diverse phenolic compounds from pollen to bee-derived products enhances their nutritional and medicinal value, providing a strong foundation for future applications in apiculture and human health.

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REFERENCES

Abd El-Wahab, T.E., Ghania, A.M.M. and Zidan, E.W. Assessment a new pollen supplement diet for honey bee colonies and their effects on some biological activities./ Journal of Agricultural Technology, 2016; Vol. 12(1):55-62

Albaba, İ. A List of Important Honeybee Nectariferous and Polleniferous Plant Species in the West Bank Governorates, Palestine / Journal of Agricultural Science and Technology A. 2015; 5(2), 114-121 doi: 10.17265/2161-6256/2015.02.005

Al-Snafi, A. E. Pharmacological and toxicological effects of the Ranunculus species (Ranunculus arvensis and Ranunculus sceleratus) grown in Iraq. *Int. J. Biol. Pharm. Sci. Arch*, 2022; 3, 64-72. Article DOI: https://doi.org/10.53771/ijbpsa.2022.3.1.0032

An, İ., Ucmak, D., Esen, M., & Gevher, O. D. Phytocontact dermatitis due to Ranunculus arvensis: Report of three cases. *North. Clin. Istanb*, 2019, *6(1)*, 81-84. doi: 10.14744/nci.2018.93695

Benzie, I. F., & Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical biochemistry*, 1996, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Boroomand, N., Sadat-Hosseini, M., Moghbeli, M., & Farajpour, M. Phytochemical components, total phenol and mineral contents and antioxidant activity of six major medicinal plants from Rayen, Iran. *Natural product research*, 2018, *32*(5), 564-567. http://dx.doi.org/10.1080/14786419.2017.1315579

Boulebd, H. Insights on the antiradical capacity and mechanism of phytocannabinoids: H-abstraction and electron transfer processes in physiological media and the influence of the acid-base equilibrium. *Phytochemistry***,** 2023; *208*, 113608. https://doi.org/10.1016/j.phytochem.2023.113608

Boulebd, H. Is cannabidiolic acid an overlooked natural antioxidant? Insights from quantum chemistry calculations. *New Journal of*

Chemistry, 2022; *46* (1), 162-168. DOI: 10.1039/D1NJ04771J.

Boulebd, H. Mechanistic Insights into the Antioxidant and Pro-oxidant Activities of Bromophenols from Marine Algae: A DFT Investigation. *The Journal of Organic Chemistry*, 2024; *89* (11), 8168-8177. https://doi.org/10.1021/acs.joc.4c00851.

Boulebd, H.; Pereira, D. M.; Amine Khodja, I.; Hoa, N. T.; Mechler, A.; Vo, Q. V. Assessment of the free radical scavenging potential of cannabidiol under physiological conditions: Theoretical and experimental investigations. *Journal of Molecular Liquids*, 2022; *346*, 118277. DOI: 10.1016/j.molliq.2021.118277.

De-Melo AAM., Estevinho LM., Moreira MM., Delerue-Matos C., Freitas AS., Barth OM., Almeida-Muradian LB. Phenolic profile by HPLC-MS, biological potential, and nutritional value of a promising food: Monofloral bee pollen/ J Food Biochem.; 2018; 1-21 https://doi.org/10.1111/jfbc.12536

Erdogan, T. F., Kivcak, B., Onur, M. A., & Braca, A. Chemical constituents and cytotoxic activity of Ranunculus pedatus subsp. pedatus. *Chemistry of Natural Compounds*, 2012; *48*, 486-488. DOI: 10.1007/s10600-012-0284-3.

Frisch M. J.; Trucks G. W.; Schlegel H. B.; Scuseria G. E.; M. A. Robb; J. R. Cheeseman; G. Scalmani et al. *Gaussian 09, Gaussian, Inc., Wallingford CT,* 2009.

Fukumoto, L. R., & Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of agricultural and food chemistry*, 2000; *48*(8), 3597-3604. https://doi.org/10.1021/jf000220w.

Galano, A.; Alvarez-Idaboy, J. R. Kinetics of radical-molecule reactions in aqueous solution: A benchmark study of the performance of density functional methods. *Journal of Computational Chemistry*, 2014; *35* (28), 2019-2026. DOI: 10.1002/jcc.23715.

Hachelaf, A., Touil, A., Zellagui, A., & Rhouati, S. Antioxidant and antibacterial activities of essential oil extracted from Ranunculus arvensis L. *Der Pharma Chem*, 2015, 7(9):170-173.

Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual molecular dynamics. *Journal of Molecular Graphics*, 1996; *14* (1), 33-38. https://doi.org/10.1016/0263-7855(96)00018-5.

Jürgens, A., & Dötterl, S. Chemical composition of anther volatiles in Ranunculaceae: genera-specific profiles in Anemone, Aquilegia, Caltha, Pulsatilla, Ranunculus, and Trollius species. *American Journal of Botany*, 2004; *91*(12), 1969-1980. DOI: 10.3732/ajb.91.12.1969.

Kolaylı, S., Asadov, E., Huseynova, A., Rahimova, S., & Kara, Y. Phenolic composition and antioxidant properties of black mulberry (Morus nigra l.) fruits and leaves. *Journal of Wildlife and Biodiversity*, 2024; 8(2), 355-364. DOI: https://doi.org/10.5281/zenodo.11078022.

Kostic, A., Milincic, D.D., Nedic, N., Gašic, U.M., Trifunovic, B., Vojt, D. et al. Phytochemical Profile and Antioxidant Properties of Bee-Collected Artichoke (*Cynara scolymus*) Pollen. Antioxidants- 2021; 10(7), 1091. https://doi.org/10.3390/antiox10071091.

Kurkin, V. Pharmacognosy. Textbook for students of pharmaceutical universities (faculties). Samara: OFORT LLC, *State Educational Institution of Higher Professional Education* "Sam GMU", 2004; 1180 p.

Lu, T.; Chen, F. Multiwfn: A multifunctional wavefunction analyzer. *Journal of Computational Chemistry*, 2012; *33* (5), 580-592. DOI: 10.1002/jcc.22885.

Lü, J.-M.; Lin, P. H.; Yao, Q.; Chen, C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *Journal of Cellular and Molecular Medicine*, 2010; 14 (4), 840-860. DOI: 10.1111/j.1582-4934.2009.00897.x

Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. sci. technol*, 2004; *26*(2), 211-219.

Pulido, R., Bravo, L., & Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of agricultural and food*

chemistry, 2000; *48*(8), 3396-3402. DOI: 10.1021/jf9913458.

Sadeq, O.; Mechchate, H.; Es-safi, I.; Bouhrim, M.; Jawhari, F.z.; Ouassou, H.; Kharchoufa, L. et al. Phytochemical Screening, Antioxidant and Antibacterial Activities of Pollen Extracts from Micromeria fruticosa, Achillea fragrantissima, and Phoenix dactylifera. Plants, 2021; 10, 676. https://doi.org/ 10.3390/plants10040676.

Sedivy, C., Piskorski, R., Müller, A., & Dorn, S. Too low to kill: concentration of the secondary metabolite ranunculin in buttercup pollen does not affect bee larval survival. *Journal of chemical ecology*, 2012. *38*, 996-1002. DOI 10.1007/s10886-012-0153-3

Slinkard, K., & Singleton, V. L. Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture*, 1997; *28*(1), 49-55. DOI:10.5344/ajev.1977.28.1.49.

Spiegel, M. Current Trends in Computational Quantum Chemistry Studies on Antioxidant Radical Scavenging Activity. *Journal of Chemical Information and Modeling***,** 2022; *62* (11), 2639-2658. https://doi.org/10.1021/acs.jcim.2c00104.

Talibov, T. H., & Ibrahimov, A. S. Taxonomic spectre of the flora of Nakhchivan Autonomous Republic. *Nakhchivan: Ajamy*, 2008, *364*.

Zehra, C., Yildiz, O., Şahin, H., Asadov, A., & Kolayli, S. Phenolic profile and antioxidant potential of propolis from Azerbaijan. *Mellifera*, 2015, *15*(1), 16-28.