Citation/Atıf: Mokhtari MB, El Ouar I, Zeghina I,Tartouga MA, Ghorab A, Bahri L, Bensouici C. Palynological Analysis, Phenolic Components and Anti-Inflammatory Activity Of Some Bee Pollens Collected From The Northeast Region Of Algeria (Extended abstract in Turkish). U. Arı D. / U. Bee J. 2022, 22:45-58. DOI: 10.31467/uluaricilik.1073013

ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PALYNOLOGICAL ANALYSIS, PHENOLIC COMPONENTS AND ANTI-INFLAMMATORY ACTIVITY OF SOME BEE POLLENS COLLECTED FROM THE NORTHEAST REGION OF ALGERIA

Cezayir'in Kuzeydoğu Bölgesinden Toplanan Bazı Arı Polenlerinin Palinolojik Analizi, Fenolik Bileşenleri ve Anti-İnflamatuar Aktivitesi

Mohamed Badreddine MOKHTARI[1,2*], Ibtissem EL OUAR[1,3], Ibtissem ZEGHINA[1], Maya Abir TARTOUGA[1], Asma GHORAB [4,5], Laid BAHRI[1,2], Chawki BENSOUICI[6]

[1]Université Frères Mentouri Constantine 1, Faculté des Sciences de la Nature et de la Vie, Departement de Biologie Animale, Constantine 25000, ALGERIA, Corresponding author E-mail: mohamedbadreddine.mokhtari@umc.edu.dz, ORCID No: 0000-0003-3066-8346, E-mail: louar.ibtissem@umc.edu.dz, ORCID No: 0000-0002-0266-4965, E-mail: zeghina.ibtissem@umc.edu.dz, ORCID No: 0000-0002-9065-3421, E-mail: Tartouga.abirmaya@umc.edu.dz, ORCID No: 0000-0001-9692-5339.

[2]Université Frères Mentouri Constantine1, Institut des Sciences Vétérinaires, Laboratoire de Pharmacologie et Toxicologie, , Constantine 25000, ALGERIA, E-mail: bahri.laid@umc.edu.dz, ORCID No: 0000-0003-2611-5140.

[3]Centre de Recherche en Sciences Pharmaceutiques (CRSP), Constantine 25000, ALGERIA.

[4]Université A. Mira de Bejaia, Faculté des Sciences de la Nature et de la Vie, Laboratoire d'Ecologie et Environnement, Bejaia 06000, ALGERIA, E-mail: Asma.ghorab@univ-bejaia.dz, ORCID no: 0000-0001-6768-6316.

[5]Universida de de Vigo, Faculta de de Ciencias, Department of Vegetal Biology and Soil Sciences, 32004 Ourense, SPAIN.

[6]Centre de Recherche en Biotechnologie (CRBt), Constantine 25000, ALGERIA, E-mail: c.bensouici@crbt.dz, ORCID no: 0000-0003-4612-4642.

Geliş Tarihi / Received: 20.02.2022 Kabul Tarihi / Accepted:21.03.2022 DOI: 10.31467/uluaricilik.1073013

ABSTRACT

Bee pollen is multiplex blend of floral pollen and nectar agglutinated by bee salivary substances. It is famously known for being high in proteins, carbs, lipids, vitamins, and phenolic compounds, among other physiologically dynamic components. Its composition fluctuates incredibly agreeing to both botanical origins and edaphoclimatic conditions. In this work, the botanical origin, the phenolic components and the anti-inflammatory activity *in vivo* of eight bee pollens intended for human consumption were taken from distinctive apiaries in Algeria's northeast, were determined and compared. All samples were detected heterofloral based on the identification of forty pollen types belonging to 22 botanical families. Total phenolic contents varied between 752.94 ± 17.78 and 12247.06 ± 40.04 mg GAE/ 100g, while the total flavonoid contents ranged from 2680.55 ± 12.02 to 8506.94 ± 15.56 mg QE/ 100g, and the total flavonol contents were in the interval between 4978.87 ± 33.39 and 7903.75 ± 24.39 mg QE/ 100g. The obtained results showed that the bulk of the ethanolic extracts had a good anti-inflammatory activity. As a conclusion, all the aforementioned heterofloral bee pollen samples could significantly be a wealthy source of polyphenols with a potential anti-inflammatory activity.

Key words: Bee pollen, Palynological analysis, Phenolic contents, Anti-inflammatory activity

ÖZ

Arı poleni, çiçek poleni ve nektar ile arı tükürük maddelerinin karmaşık bir karışımıdır. Karbonhidratlar, proteinler, lipidler, vitaminler ve fenolik bileşikler gibi birçok biyolojik olarak aktif maddenin zengin bir kaynağı olarak ünlüdür. Bileşimi, botanik kökenlere ve edafoklimatik koşullara göre büyük ölçüde değişir. Bu çalışmada, Cezayir'in kuzeydoğusunda bulunan farklı arılıklardan toplanan insan tüketimine yönelik sekiz arı poleninin botanik orijini, toplam fenolik içerikleri, toplam flavonoid içerikleri, toplam flavonol içerikleri ve *in vivo* antiinflamatuar aktiviteleri belirlendi ve karşılaştırıldı. Tüm örnekler, 22 botanik familyaya ait kırk polen türünün tanımlanmasıyla heterofloral olarak saptandı. Toplam fenolik içerik 752,94 ± 17,78 ile 12247,06 ± 40,04 mg GAE/ 100 arasında değişirken, toplam flavonoid içeriği 2680,55 ± 12,02 ila 8506,94 ± 15,56 mg QE/ 100g arasında değişmekte ve toplam flavonol içeriği 4978,87 ± 33,39 ile arasında değişmektedir. 7903,75 ± 24,39 mg QE/ 100g. Sonuçlar etanolik ekstraktların büyük kısmının iyi bir anti-inflamatuar aktiviteye sahip olduğunu göstermektedir. Sonuç olarak, yukarıda bahsedilen tüm heterofloral arı poleni örnekleri, potansiyel bir anti-inflamatuar aktiviteye sahip zengin bir polifenol kaynağı olabilir.

Anahtar kelimeler: Arı poleni, Palinolojik analiz, Fenolik içerik, Antiinflamatuar aktivite

GENİŞLETİLMİŞ TÜRKÇE ÖZET

Çalışmanın amacı: Arı poleni nektarın arı tükürük maddeleriyle aglutine edilmiş multipleks karışımıdır. Proteinler, karbonhidratlar, lipidler, vitaminler ve fenolik bileşikler gibi etkileyici fizyolojik olarak dinamik bileşenlere sahiptir. Arı poleni, bağışıklık uyarıcı, antimikrobiyal, antienflamatuar, antioksidan ve antinosiseptif özelliklere sahip olduğundan, geleneksel Çin tıbbında genellikle apiterapötik bir çare olarak tavsiye edilir. Bununla birlikte, bileşimi, botanik kökenlere ve edafoklimatik koşullara göre inanılmaz derecede dalgalıdır. Buna göre bu çalışmada sekiz Cezayir arı poleninin in vivo botanik orijini, toplam fenolik içerikleri, toplam flavonoid içerikleri, toplam flavonol içerikleri ve antiinflamatuar aktiviteleri belirlenmiş ve karşılaştırılmıştır.

Gereç ve Yöntem: Cezayir'in kuzeydoğu bölgesindeki farklı arı kovanlarından insan tüketimine yönelik sekiz arı poleni örneği toplandı. Daha sonra, Louveaux ve diğerleri tarafından belirtildiği gibi slaytlarda (asetoliz olmadan hazırlanmış) 500'den fazla polen tanesinin değerlendirilmesiyle palinolojik tanımlama doğrulandı (1978) ve polen frekansları sınıflandırıldı (de França Alves ve de Assis Ribeiro DosSantos 2014). Toplam fenolik, flavonoid ve flavonol içerikleri üç yöntem kullanılarak belirlendi: sırasıyla Folin–Ciocalteu kolorimetrik yöntem (Singleton ve Rossi 1965) ve trikloroalüminyum kolorimetrik yöntemler (Topçu ve diğerleri 2007) ve (Kumaran ve Joel Karunakaran 2007). Olası antienflamatuar aktivite, sıçanlarda formalin testi kullanılarak in vivo olarak değerlendirildi (Arzi ve ark. 2015).

Bulgular: Mikroskobik analiz, tüm örneklerin heterofloral olduğunu ortaya koydu. 22 botanik familyaya ait toplam kırk polen tipi tespit edilmiş olup, örneklerin tamamında Cistus tipi mevcuttur. Toplam fenolik içerikler 752.94 ± 17.78 ve 12247.06 ± 40.04 mg GAE/ 100g arasında değişirken, toplam flavonoid içerikleri 2680.55 ± 12.02 ila 8506.94 ± 15.56 mg QE/ 100g arasında değişmektedir. Ayrıca, toplam flavonol içerikleri 4978.87 ± 33.39 ve 7903.75 ± 24.39 mg QE/ 100g aralığındaydı. Son olarak, numunelerimizin etanolik ekstraktlarının büyük kısmının iyi bir anti-inflamatuar aktiviteye sahip olduğu bulundu. Ek olarak, en iyi anti-enflamatuar aktivite, tüm değerlendirilen sürelerde, Diklofenak ile gözlemlenenden önemli ölçüde daha düşük şişme yüzdeleriyle ekstrak E'de gözlendi.

Sonuç: Bu çalışmada incelenen heterofloral arı poleni örnekleri, önemli bir anti-inflamatuar aktivite sergileyen zengin bir polifenol kaynağı olabilir. Bu nedenle, ilaç ve gıda endüstrilerinde kullanımı kesinlikle umut vericidir.

INTRODUCTION

In Algeria, beekeeping is a genealogical hone although its origin is lost in the mists of time. It has gained prominence over the last two decades and has become an indispensably portion for the sustainable economical advancement of agricultural and provincial exercises. The Algerian’s apiarian livestock has jumped from 360000 colonies in 2000 to over 1300000 colonies in 2014, thanks to the various agricultural and rural development plans

connected within the nation (Tamali and Özkırım 2019).

Bee pollen is one of the hive-derived products (together with honey, propolis, beeswax, royal jelly, beebread and bee venom) that is expecting more noteworthy popularity among beekeepers as a result of the growing request on it from agri-food, pharmaceutical and cosmetics industries around the world. Bee pollen is advertised as a nutrient-dense food with a wide range of medicinal and nutritional benefits, which is driving up demand (Nogueira et al., 2012). Commonly designated as "the life-giving dust", it is floral pollen gathered by foraging bees, mixed with nectar then agglutinated by bee salivary substances. This bee product is harvested in a form of pellets using a trap installed at the entrance of the beehives (Thakur and Nanda 2020).

Bee pollen is usually recommended as an apitherapeutic remedy in traditional Chinese medicine since it has immune-stimulating, antimicrobial, anti-inflammatory, antifungal, antioxidant, antinociceptive and antiviral properties (Komosinska-Vassev et al. 2015, Tutun et al. 2021).

It has the potential to provide vital nutrients like proteins, carbs, lipids and minerals. Furthermore, it contains some antioxidant vitamins, mainly vitamin C, E, all B-complex vitamins and provitamin A. Besides vitamins, it is considered as a rich source of polyphenols, mainly flavonoids (Khalifa et al. 2021). The chemical composition of this bee product fluctuates incredibly agreeing to plant origin and its nutrient status, geographical region, edaphoclimatic conditions, storage and degree of processing (Aylanc et al. 2021, Sattler et al. 2015)

In this context, the current work is devoted to determine the botanical origins, assess the phenolic components and evaluate the anti-inflammatory activities *in vivo* of eight bee pollens intended for human consumption with various botanical and geographical origins. As far as we are mindful, this could be the first ponder conducted on Algerian bee pollen aimed to compare and to look up for a possible relationship between the anti-inflammatory potential, the botanical origin, and the total phenolic, flavonoid and flavonol contents. In this regard, this study has the characteristic of being a preliminary study.

MATERIALS AND METHODS

Bee pollen samples

Eight bee pollen samples (A-H) intended for human consumption were collected by beekeepers, during 2019 flowering season, from different apiaries in Algeria's northeastern region: sample A (Constantine), sample B (Guelma), sample C (Boumerdès), sample D (Sétif), sample E (Jijel), sample F (Skikda), sample G (Khenchla) and sample H (Tizi Ouzou) (Figure 1). After the beekeepers had dried the bee pollen, the samples were delivered and kept in the dark at ambient temperature until their used.

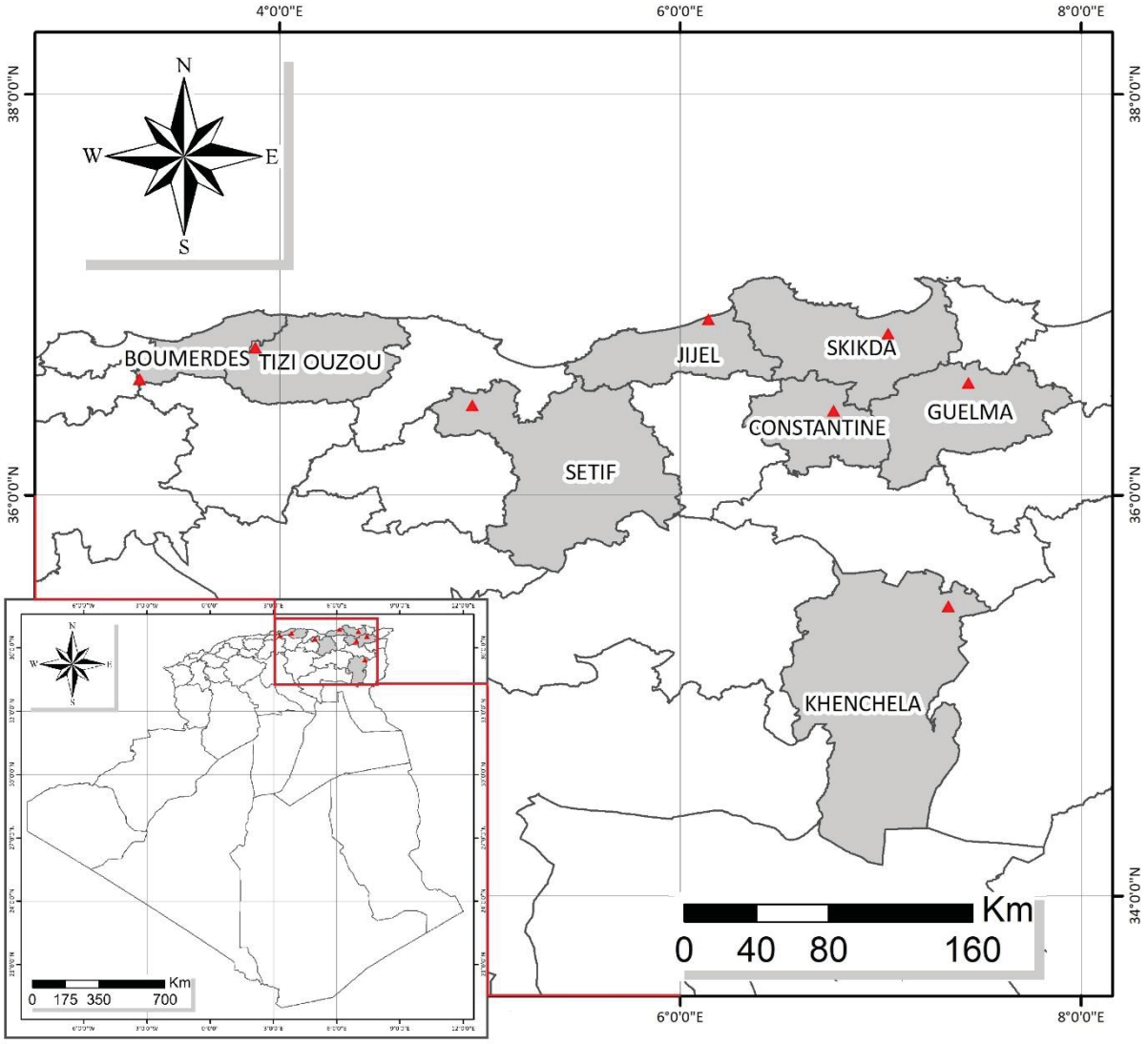
Palynological analysis

A ten-gram sample of each specimen was mixed with 100 milliliters of warm water. Two hours later, the pollen was totally macerated with a glass stick. The supernatant was drawn off after centrifugation (10 min, 2500 r/min). Water: glycerin solution (50 mL), at ratio 1:1 (v/v), was added to the pollen residue. After 2 hours and 24 hours, another centrifugation was done following the same conditions. Finally, the slides were prepared using the glycerol jelly method (Riding 2021) with the final pollen residue. During the slides mounting process, basic fuchsine was used as dye to stain pollen grains. Palynological identification of bee pollen samples was ascertained by examining over 500 pollen grains in slides prepared without acetolysis as outlined by Louveaux et al. (1978) with some modifications.

The pollen grains observation was carried out using an optical microscope, BIOSTAR B4 SP Microscope (Exacta Optech, München, Germany), at 400X and a picture analysis system EOS 450 D (CANON Inc., Japan). The authors' reference collection was used to identify pollen types, as well as specialized atlases and literature when necessary.

When the pollen grains were identified as a belonging to a particular genus and a specific species, their scientific names; consisting of the genus and the species, were applied. However, when the pollen grains had a similar morphology for some species and genus or even botanical families, the nomenclature pollen type was used. Therefore, a pollen type can be defined as a group of plants with similar pollen grains under a light microscope.

Figure 1: Apiaries geopositioning in the study area (Source: Arc Gis 10.7).



The frequency of occurrence (FO) was assigned to the types in each sample based on the percentage of each pollen type's occurrence in the sample set: De França Alves and de Assis Ribeiro DosSantos (2014) defined Rare (less than 10 percent), Less Frequent (10–20 percent), Frequent (21–50 percent), and Very Frequent (more than 50 percent).

Extracts preparation

The extracts were prepared by macerating 300 g of the powdered bee pollen samples with Ethanol (96%) at a ratio of 1:2 (w/v) for 5 days at room temperature, with solvent renovation in the third day and every 24 hours thereafter. The ethanolic extracts of bee pollen were obtained by mixing, filtering, and then concentrating the products of all extractions for each sample in a rotavap at 40 °C.

Phenolic components

Total phenolic content (TPC)

The total phenolics contents of the eight (8) extracts were assessed spectrophotometrically using the Folin–Ciocalteu method (Singleton and Rossi 1965). In every well of the microtiter plate, 100 µL of 1/10 (v/v) diluted Folin–Ciocalteu reagent and 7.5µL of sodium carbonate (7.5%) were added to

20 µL extract. After incubating the microtiter plate for 2 hours in the dark, the absorbances of each mixture were measured at 765 nm using a 96-well microtiter plate reader (Perkin Elmer, Enspire). The results were expressed as milligram gallic acid equivalents per 100 gram of bee pollen extract (mg GAE/100 g).

Total flavonoid content (TFC)

The total flavonoids contents were determined by the trichloroaluminum colorimetric method (Topçu et al. 2007). In every well, 130 µL of methanol, 10 µL of aluminum nitrate (10%) and 10 µL of aqueous potassium acetate (1 M) were added to 20 µL extract. After a 40-minutes incubation period, the absorbances were read at 415 nm using the microtiter platereader. Results were expressed as milligram quercetin equivalent per 100 gram of bee pollen extract (mg QE/100 g).

Total flavonols content (TFIC)

The total flavonols contents were carried out using the trichloroaluminum colorimetric method with slight modifications (Kumaran and Joel Karunakaran 2007). In every well, 50 µL of each extract were placed then 50 µL of aluminum nitrate (2%) and 150 µL of aqueous sodium acetate (5%) were added. After a 2-hours incubation period, the absorbances were recorded at 440 nm using the microtiter platereader. Results were expressed as milligram equivalent per 100 gram of bee pollen extract (mg QE/100 g).

Animals

Fifty male Wistar rats weighing 250–300 g, aged 3–4 months, were used for *in vivo* anti- inflammatory activity. The animals were produced and raised at the laboratory animal facility of the "Department of Animal Biology, Faculty of Natural and Life Sciences, Frères Mentouri Constantine 1 University, Algeria". The rats were kept in groups of five per cage, for acclimatization for seven days before the start of the experiment, under standard laboratory condition (temperature 22 ± 2 °C, photoperiodic cycle (light/darkness) of 12h and relative humidity 50 ± 5%) and unrestricted access to food and water *ad libitum*. This protocol was used in accordance with the Laboratory Animals Care and Use guidelines and approved under the PRFU project (D01N01UN250120210003) by the Ethical Committee of the DGRSDT at the Algerian Ministry of Higher Education and Scientific Research.

***In vivo* Anti-Inflammatory Activity**

Formalin-induced paw edema test

The formalin-induced inflammation test was carried out based on the method of Arzi et al. (2015) with slight modifications. The Wistar rats were randomly allocated into ten homogeneous groups (n=5). After a 12-hours fast period, the rats of each group received the tested samples intraperitoneally. Group 1 received normal saline solution (5 mL/kg) and treated as negative control. Group 2 was administered by an anti-inflammatory drug (Diclofenac) in a dose of 20 mg/kg of Body Weight (BW) and considered as standard. Group 3 to 10 were given different bee pollen extracts in a dose of 200 mg/kg of BW. Thirty minutes later, acute paw edema was induced on the right hind paw by a subplantar injection of 1% formalin (100 µL). Paw volume was measured before and after formalin injection at 0, 30, 60, 120, 180 and 240 minutes, using a water displacement plethysmometer (Fereidoni et al. 2000). The results (% swelling) were expressed as the proportional rise in paw volume before formalin injection. The following formula was used to calculate it:

$$\%\ swelling = \left[\frac{V_t - V_i}{V_i}\right] \times 100$$

Where V_i is the paw volume before formalin injection and V_t is the paw volume after formalin injection at different time points.

Statistical analysis

The *in vivo* experiment was carried out in quintuplicate and the data were recorded as means ± standard deviation. The one-Factor ANOVA test was used to determine whether there were significant differences between pollen samples, with the Tukey test serving as post-hoc test. Moreover, the principal components analysis (PCA) was used to check the relationships between pollen types, phenolic components and anti-inflammatory activity. All statistical analyses were performed using IBM SPSS Statistics 23.0 software (IBM® SPSS Inc.). For all analyses, the level of significance was set at 5% ($p < 0.05$).

RESULTS

Pollen analysis revealed that all samples were heterofloral. A total of 40 pollen types belonging to 22 botanical families were identified in eight pollen

samples (Table 1). The most diverse families were *Asteraceae* (8 types), *Brassicaceae* (4 types), *Cistaceae*, *Fabaceae*, and *Fagaceae* (3 types) respectively. The other families were represented by one or two types only.

Cistus type was the most represented, found in all samples. *Bellis*, *Boraginaceae*, *Bryonia*, *Buxus*, *Centaurea*, *Cerinthe major,* *Convolvulus*, *Cucurbitaceae*, *Euphorbiaceae*, *Fraxinus*, *Galactites*, *Helianthemum*, *Juglans*, *Myrtus communis*, *Oxalis*, *Poaceae*, *Quercus suber*, *Raphanus*, *Rhamnus*, *Sonchus*, *Verbenaceae*, *Vicia* and *Vitex* pollen types were in turn observed in only a single sample.

For sample A, eight different pollen types were identified, of which *Brassicaceae* and *Cistus* types were the two most frequent pollen types. Twelve pollen types were recorded in sample B without any frequent pollen types. The *Pistacia lentiscus* was the most frequent pollen type among 10 different pollen types recorded in sample C. In sample D, the two most frequent pollen types were *Cistus* and *Quercus*, out of a total of 9 pollen types. Thirteen different pollen types were found in sample E where *Quercus* type was the most frequent type. For sample F, *Brassica* was the most repeated type out of a total of 14 pollen types. The recurrent pollen types in sample G were *Brassica* and *Pistacia lentiscus* types from 7 pollen types. Finally, ten different pollen types were found in sample H, among which three pollen types were frequent: *Bryonia*, *Cistus* and *Quercus ilex*.

Table 1: Frequency of occurrence data recorded in the eight bee pollen samples

Family	Pollen Type	Samples (%)							
		A	B	C	D	E	F	G	H
Anacardiaceae	***Pistacia lentiscus*** type	-	R. 9.12%	F. 45.70%	-	-	L.F. 14.97%	F. 43.04%	L.F. 11.46%
Asteraceae	***Anthemis*** type	-	-	R. 0.17%	-	-	R. 0.66%	R. 0.72%	-
	Bellis type	-	-	-	-	-	-	-	R. 0.83%
	Carduus type	-	-	R. 0.33%	-	R. 0.18%	R. 0.49%	-	-
	Centaurea type	-	-	-	-	L.F. 19.85%	-	-	-
	Cichorium type	-	-	-	-	-	R. 0.33%	R. 0.36%	R. 0.66%
	Galactites type	L.F. 10.07%	-	-	-	-	-	-	-
	Picris type	-	R. 5.34%	-	R. 0.64%	R. 0.72%	-	-	-
	Sonchus type	R. 0.72%	-	-	-	-	-	-	-
Boraginaceae	***Boraginaceae*** type	-	-	-	-	-	-	-	R. 2.49%
	Cerinthe major type	-	-	L.F. 19.25%	-	-	-	-	-
Brassicaceae	***Brassica*** type	-	-	R. 4.51%	R. 5.14%	-	F. 30.60%	F. 41.96%	L.F. 13.95%
	Brassicaceae type	F. 39.76%	L.F. 10.53%	-	L.F. 12.05%	-	R. 3.95%	-	-
	Other Brassicaceae type	-	-	-	-	R. 3.43%	-	L.F. 12.11%	R. 3.99%
	Raphanus type	-	-	-	-	-	R. 3.29%	-	-
Buxaceae	***Buxus*** type	-	R. 7.07%	-	-	-	-	-	-
Cistaceae	***Cistus*** type	F. 21.40%	L.F. 16.20%	R. 1.64%	F. 24.30%	R. 3.97%	R. 9.55%	R. 0.36%	F. 20.10%
	Halimium type	-	R. 9.90%	-	-	R. 3.43%	R. 2.96%	-	-

	Helianthemum type	-	R. 5.97%	-	-	-	-	-	-
Convolvulaceae	***Convolvulus*** type	-	-	-	-	-	-	-	R. 5.81%
Cucurbitaceae	***Bryonia*** type	-	-	-	-	-	-	-	F. 20.61%
	Cucurbitaceae type	-	-	-	-	R. 3.79%	-	-	-
Ericaceae	***Erica arborea*** type	-	-	R. 6.03%	-	-	R. 4.28%	-	-
Euphorbiaceae	***Euphorbiaceae*** type	-	-	-	-	L.F. 19.68	-	-	-
Fabaceae	***Fabaceae*** type	-	L.F. 18.88%	R. 8.49%	L.F. 13.18%	-	-	R. 1.45%	-
	Lotus type	-	R. 5.19%	-	-	-	-	-	-
	Vicia type	-	-	-	-	R. 7.04%	-	-	-
Fagaceae	***Quercus ilex*** type	R. 1.08%	-	-	R. 8.84%	-	R. 5.26%	-	F. 20.10%
	Quercus suber type	-	R. 2.99%	-	-	-	-	-	-
	Quercus type	L.F. 10.07%	R. 4.25%	-	F. 20.74%	F. 22.92%	L.F. 16.43%	-	-
Juglandaceae	***Juglans*** type	-	-	-	R. 7.88%	-	-	-	-
Lamiaceae	***Vitex*** type	R. 5.57%	-	-	-	-	-	-	-
Myrtaceae	***Myrtus communis*** type	-	-	R. 2.78%	-	-	-	-	-
Oleaceae	***Fraxinus*** type	-	-	-	-	R. 2.17%	-	-	-
Oxalidaceae	***Oxalis*** type	-	-	-	R. 7.23%	-	-	-	-
Papaveraceae	***Papaver*** type	L.F. 11.33%	-	-	-	R. 2.53%	-	-	-
Poaceae	***Poaceae*** type	-	-	-	-	-	R. 3.78%	-	-
Rhamnaceae	***Rhamnus*** type	-	R. 4.56%	-	-	-	-	-	-
Rosaceae	***Rosaceae*** type	-	-	L.F. 11.10%	-	-	R. 3.45%	-	-
Verbenaceae	***Verbenaceae*** type	-	-	-	-	L.F. 10.29%	-	-	-
Number of pollen types		8	12	10	9	13	14	7	10

* F.: Frequent, L.F.: Less Frequent, R.: Rare

The results of phenolic components of the eight ethanolic extracts indicated significant differences in their total contents. Whereby, high total phenolics contents were recorded in the extract D followed by the extract G then the extracts C and E (12247.06 ± 40.04, 9050.98 ± 17.93, 7854.90 ± 33.05, and 7541.27 ± 48.71 mg GAE/100 g; respectively) with insignificant difference in the two last ones (Table 2), while the lowest content was obtained in the extract A (752.94 ± 17.78 mg GAE/100 g). For flavonoids, the extract D provides the highest content (8506.94 ± 15.56 mg QE/100 g), followed by the extract G (6076.39 ± 20.01 mg QE/100 g), whereas low values were registered in extracts A and B (2694.44 ± 22.85 and 2680.55 ± 12.02 mg QE/100 g; consecutively). Flavonols contents values ranged from 4978.87 ± 33.39 mg QE/100 g (extract B) to 7903.75 ± 24.39 mg QE/100 g (extract D).

In the anti-inflammatory activity, after formalin injection into the hind paw, the paw edema in the normal saline group; increased along with the time course and the peak edema, was registered after 240 min in which the mean of swelling percentage was 63.99 ± 2.98 % (Table 3). Our results showed

that the extract E had the best anti-inflammatory activity with the least significant swelling percentage mean in all assessed time. Followed by extract F which had also strongly and significantly activity, compared to the control group, inhibited the formalin induced paw edema, with comparable effect to the reference drug (diclofenac at 20 mg/kg BW). Furthermore, extracts A, D, G, and H exhibited mild anti-inflammatory effect, with significant statistical difference when compared to the normal saline group. However, extracts B and C did not affect the paw edema with non-significant difference compared to the control group.

Table 2: Phenolic components contents in the pollen types ethanolic extracts

Extracts	TPC (mg GAE/100 g)	TFC (mg QE /100 g)	TFIC (mg QE /100 g)
A	752.94 ± 17.78 ^{e}	2694.44 ± 22.85 ^{e}	5058.68 ± 12.97 ^{d}
B	5913.72 ± 19.13 ^{d}	2680.55 ± 12.02 ^{e}	4978.87 ± 33.39 ^{d}
C	7854.90 ± 33.05 ^{c}	5201.39 ± 37.87 ^{c}	6950.70 ± 31.72 ^{a,b}
D	12247.06 ± 40.04 ^{a}	8506.94 ± 15.56 ^{a}	7903.75 ± 24.39 ^{a}
E	7541.27 ± 48.71 ^{c}	4847.22 ± 36.10 ^{c,d}	7406.10 ± 17.13 ^{a}
F	6482.35 ± 15.5 ^{d}	4965.28 ± 27.43 ^{c}	5744.13 ± 50.46 ^{c,d}
G	9050.98 ± 17.93 ^{b}	6076.39 ± 20.01 ^{b}	6208.92 ± 43.97 ^{b,c}
H	6178.43 ± 34.45 ^{d}	4347.22 ± 27.43 ^{d}	7485.91 ± 54.82 ^{a}

*The outcomes were presented as Means ± SD of three measurements. Analysis of variance (One-Factor ANOVA and Tukey tests) revealed statistical difference ($P < 0.05$). Different superscripts (a, b, c…) for the values in the same columns are statistically different.

The obtained data on the main pollen types, the total bioactive components and the swelling percentages were ordinated with PCA analysis. In total, 48 standardized variables were introduced to create covariance matrix. Whereby, Varimex method was used as a factor analysis rotation technique and the number of extracted factors was fixed at two principal components (PCs). The PCA indicated that the two PCs accounted for 71.16% of the total variance. The first principal component (PC1) represented 40.42% and had the highest positive correlation coefficients with the swilling percentages (t+30: 0.975; t+120: 0.972; t+60: 0.964; t+180: 0.960 and t+240: 0.947). The PC2 (30.74% of the variance) had the main correlation coefficients with the total bioactive components (TPC: 0.985; TFC: 0.908 and TFIC: 0.689). The variables with high correlation coefficients appeared together in the biplot (Figure 2). Focusing on the PC1, the PCA demonstrated four bee pollen types; *Centaurea*, *Cucurbitaceae*, *Euphorbiaceae* and *Fraxinus*, with negative correlation coefficient (-0.796) with the swilling percentages which means that these pollen types were characterized by potent anti-inflammatory activity. Regarding PC2, it can be concluded that *Oxalis* and *Juglans* types are positively correlated (0.575) with the total bioactive components (TPC, TFC, and TFIC) and that their presence matches up with high total bioactive components. Whereas, the three pollen types; *Galactites*, *Sonchus* and *Vitex* had a negative relationship (-0.777) with the total bioactive components.

Table 3: Effects of bee pollen and diclofenac on formalin-induced paw edema in rats.

Extracts and standards	T+30 (%)	T+60 (%)	T+120 (%)	T+180 (%)	T+240 (%)
A	19.80 ± 1.29 ^{d,e}	26.42 ± 2.89 ^{b,c}	32.49 ± 2.57 ^{c}	32.86 ± 2.60 ^{c,d}	42.38 ± 2.06 ^{c}
B	30.84 ± 2.31 ^{a}	40.01 ± 1.74 ^{a}	44.44 ± 2.55 ^{b}	55.59 ± 2.96 ^{a}	61.08 ± 1.90 ^{a}
C	29.75 ± 2.25 ^{a,b}	42.40 ± 2.51 ^{a}	53.49 ± 2.76 ^{a}	55.68 ± 3.75 ^{a}	63.71 ± 3.28 ^{a}
D	15.12 ± 1.78 ^{e,f}	23.87 ± 1.93 ^{b,c}	35.14 ± 1.51 ^{c}	38.10 ± 1.67 ^{b}	49.53 ± 1.03 ^{b}
E	3.89 ± 0.66 ^{h}	3.67 ± 0.66 ^{e}	4.22 ± 1.27 ^{f}	7.71 ± 1.46 ^{h}	10.17 ± 2.31 ^{h}
F	12.68 ± 2.74 ^{f,g}	21.97 ± 1.77 ^{c}	25.54 ± 2.47 ^{d}	28.04 ± 1.89 ^{d}	31.95 ± 2.14 ^{d}
G	18.73 ± 2.09 ^{e,}	26.75 ± 1.52 ^{b}	30.18 ± 2.07 ^{c,d}	37.70 ± 2.55 ^{b,c}	43.71 ± 1.90 ^{c}
H	23.04 ± 2.90 ^{c,d}	24.29 ± 2.48 ^{b,c}	34.97 ± 2.92 ^{c}	39.02 ± 2.21 ^{b}	49.73 ± 2.60 ^{b}
Control	26.61 ± 2.90 $^{b, c}$	37.99 ± 2.37 ^{a}	57.81 ± 2.57 ^{a}	59.74 ± 1.17 ^{a}	63.99 ± 2.98 ^{a}
Diclofenac	8.74 ± 1.17 ^{g}	12.54 ± 2.98 ^{d}	11.53 ± 2.63 ^{e}	16.50 ± 2.73 ^{e}	22.49 ± 1.94 ^{e}

* The swelling percentages values mean ± SD (n = 5) were checked by One-Factor ANOVA test followed by multiple comparison test Turkey ($p < 0.05$). Outcomes with distinct superscript letters are statistically different.

DISCUSSION

The botanical identification of our samples confirmed the richness of the northeastern region of Algeria in important species resulting from the particular climate (Mediterranean climate for both tell and steppe regions), orography and human impact. Most of the found pollen types were from spontaneous species known by their high melliferous potential (Ghorabet al. 2021b, Saadia TamaliandÖzkırım 2019, Zerrouk et al. 2014). Therefrom, this floral diversity represents a great feature that favours the sustainable development of beekeeping activities in Algeria.

Even if *Asteraceae* family was the most diverse (8 types), it was not the most frequent plant family (represents just 5.25% of identified pollen types). The most abundant families in our samples were *Brassicaceae* (its 4 pollen types constituted 23.15% of identified pollen types), followed by *Anacardiaceae* (represented by its unique type *Pistacia lentiscus* with 15.53% of identified pollen types) then *Cistaceae* (14.99%) and *Fagaceae* (14.10%). Excluding *Brassicaceae* family, all the cited families are well known as good polliniferous species. However, most species of *Brassicaceae* and *Asteraceae* families were considered as nectariferous. *Fabaceae* family members (6.68% of identified pollen types) were known as good nectar and pollen producers (Ghorabet al. 2021a).

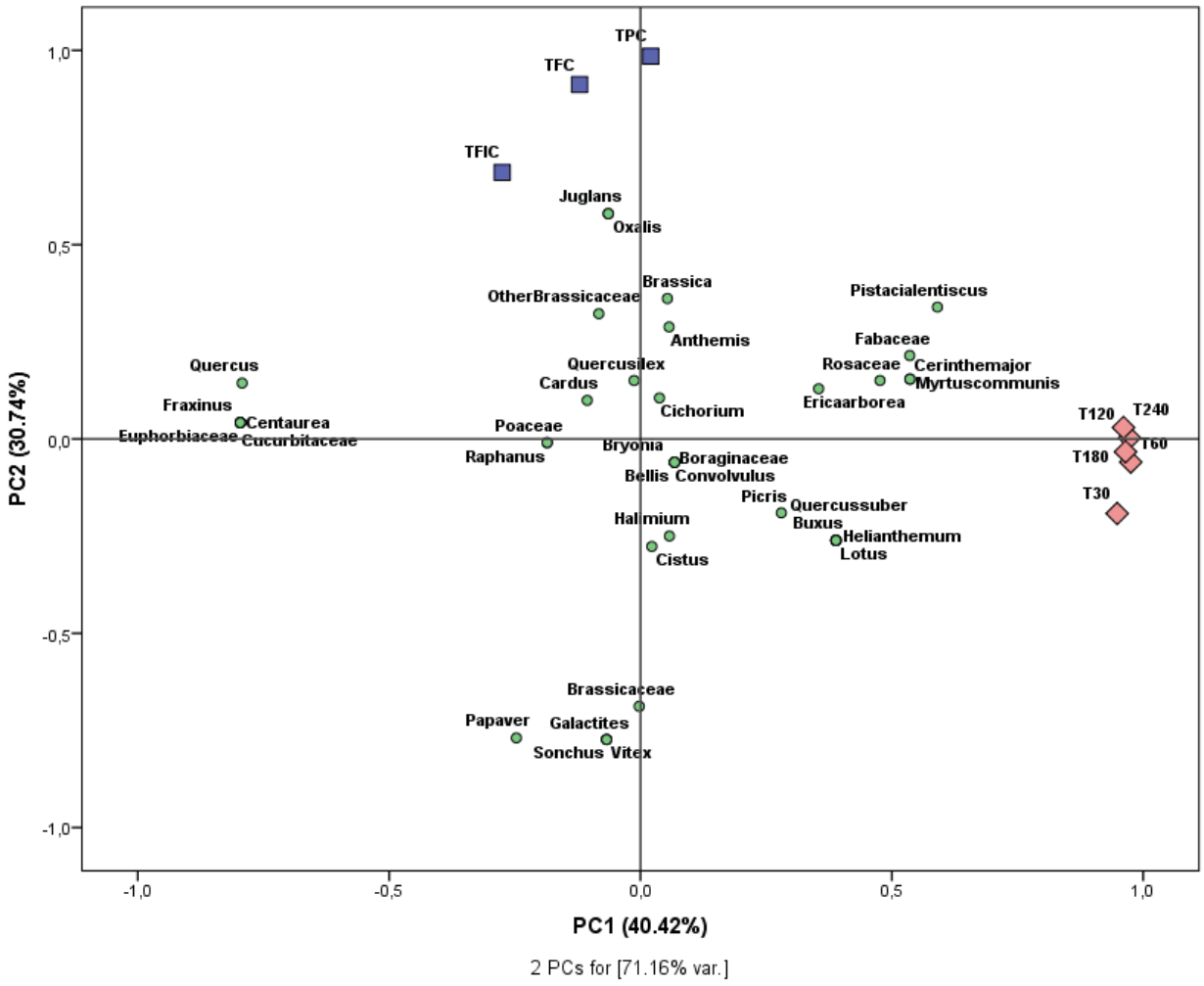


Figure 2: Loading biplot of variables (Pollen types, phenolic components and swilling percentages) included in the Principal Component Analysis (PCA)

Numerous studies in many countries have been conducted on bee pollen total bioactive components. Concerning our bee pollens, the TPCs were ranging from 752.94 to 12247.06 mg GAE/100 g, TFC ranging from 2680.55 to 8506.94 mg QE/100 g and TFIC ranging from 4978.87 to 7903.75 mg QE/100 g. Although different extraction and assay methods were used, these results were tad higher than those reported by (Asmae et al. 2021, Eraslan et al. 2009, Fatrcová-Šramková et al. 2013, LeBlanc et al. 2009, Yildiz et al. 2013). Our results can also be comparable with those obtained by (ŞahinandKarkar 2019, Žilić et al. 2014). Nevertheless, the present findings were moderately lower than those found on Turkish bee pollen (Gercek et al. 2021).

This variation is common and may be ascribed to variations in geographical origin and edaphoclimatic condition (Araújo et al. 2017, Nogueira et al. 2012). However, the most influencing factor remains botanical origin (Bogdanov 2004, Daoud et al. 2019, Estevinho et al. 2012).

The anti-inflammatory effect of our ethanolic bee pollen extracts was investigated by a method of formalin-induced paw edema in rats. This method is commonly used as a model for anti-nociceptive and anti-inflammatory activities assessment which mainly results from a neurogenic inflammation mediated by neuropeptides such as substance P (Damas et al. 1999).

The diclofenac was chosen as an anti-inflammatory drug reference. It is a proven nonsteroidal anti-inflammatory drug (NSAID) with antipyretic, anti-inflammatory and analgesic properties. Diclofenac exerts its action via cyclooxygenases (COX-1 and 2) inhibition with relative equipotency. However, extensive research shows that the mechanisms of action of diclofenac goes beyond COX inhibition and confirms that it can inhibit substance P, inhibit lipoxygenase enzymes, influence the release and uptake of arachidonic acid, activate the nitric oxide–cGMP antinociceptive pathway and alter the interleukin-6 production (Gan 2010).

The bulk of the tested bee pollen ethanolic extracts suppressed paw edema. Homogeneous effect was found in an ethanolic bee pollen extract from *Cistus sp.* of Spanish (Maruyama et al. 2010), hydroethanolic extracts of bee pollen from *M.fasculata* (Lopes et al. 2019) and from *S.aff.postica* (Lopes et al. 2020) at comparable doses showed high anti-inflammatory activity in rat carrageenan-induced paw edema models. These results confirm the hypothesis that bee pollen extract may act by COX-2 inhibition and also probably acts by NO release inhibition and as H1 histamine receptor antagonist (Lopes et al. 2019).

In mouse formalin-induced paw edema model, Choi (2007) noted that the ethanolic extract of pine (*Pinus densiflora*) bee pollen has demonstrated at the same dose (200mg/kg BW) a strong anti-inflammatory activity significantly better than that of the used anti-inflammatory drug reference (indomethacin at 10 mg/kg BW). This finding corroborates with the result observed with extract E.

The results of the PCA showed that there is no correlation between the total phenolic, flavonoid and flavonol contents of the samples with the anti-inflammatory activity (0.057, -0.042 and -0.167 respectively). Therein, the registered variation of the anti-inflammatory activity in our results between the eight bee pollen extracts could be explained by the different botanical origins of our bee pollen which certainly implies a variation in their secondary metabolites composition. The high anti-inflammatory activity registered with extract E compared to remaining extracts could be attributed to its possible richness in flavonoids from the subgroup of flavonols with potent anti-inflammatory effect like quercetin and kaempherol, likewise their glycosides especially rutin (Panche et al. 2016; Rzepecka-Stojko et al. 2015). Therefore, additional research is required to identify the phenolic profile of each extract and thus clarify the possible mechanism.

This finding gives us more information and details about the relation between pollen types and phenolic components and anti-inflammatory activity of a bee pollen ethanolic extract. However, these results remain preliminary, whereby future investigations are needed to affirm them.

Several studies must be launched inside and outside Algeria on monofloral bee pollens to pinpoint the biological characteristics and the chemical composition of each pollen type, for better comprehension of their anti/pro-inflammatory activity mechanisms.

Conclusion

All bee pollen samples, intended for human consumption, collected from the northeast of Algeria, an area known for its flora diversity, are heterofloral. The ethanolic extracts of the studied samples are rich in total phenolic, flavonoid and flavonol contents. Most extracts exhibited a good anti-inflammatory activity. In this fact, Algerian bee pollen can be an important candidate which opens up new possibilities for developing many food supplements and pharmaceutical products. It is therefore advisable to give more attention and support more research on this bee hive product.

Author contributions: Conceptualization: MOKHTARI M.B., EL OUAR I. and GHORAB A., Methodology: MOKHTARI M.B. and EL OUAR I.Sample collection: MOKHTARI M.B., ZEGHINA I. and TARTOUGA M.A., Palynological Identification: MOKHTARI M.B., GHORAB A., ZEGHINA I. and TARTOUGA M.A, Phenolic components dosages: MOKHTARI M.B and BENSOUICI C., Anti-inflammatory activity: MOKHTARI M.B. and BAHRI L., Statistical analysis: MOKHTARI M.B., Writing original draft: MOKHTARI M.B, Writing -review and editing: ZEGHINA I., GHORAB A., EL OUAR I., TARTOUGA M.A. and BENSOUICI C.

Conflicf of Interest: There are no conflicting interests in the realization of the present work.

Ethical issue: Not applicable.

Funding: The authors are thankful to DGRSDT and MESRS for financial support.

Declaration of Interest:

The authors declare that they have no conflicts of interest, financial or otherwise. The authors alone are responsible for the content and writing of the paper.

Acknowledgments: The authors are very grateful to Dr Maria Carmen Seijo Coello & Dr Rifka Nakib for their generous support. They would like also to express their sincere appreciation for all the staff of the Faculty of Life and Natural Sciences (UFMC1), of the CRBt and CRSP.

REFERENCES

Araújo, J., Chambó, E., Costa, M., Cavalcante da Silva, S., Lopes de Carvalho, C., M. Estevinho, L. (2017). Chemical Composition and Biological Activities of Mono- and Heterofloral Bee Pollen of Different Geographical Origins. International Journal of Molecular Sciences, 18(5), 921. https://doi.org/10.3390/ijms18050921.

Arzi, A., Olapour, S., Yaghooti, H., Sistani Karampour, N. (2015). Effect of Royal Jelly on Formalin Induced-Inflammation in Rat Hind Paw. Jundishapur Journal of Natural Pharmaceutical Products, 10(1), 8–11. https://doi.org/10.17795/jjnpp-22466.

Asmae, EG., Nawal, EM., Bakour, M., Lyoussi, B. (2021). Moroccan Monofloral Bee Pollen: Botanical Origin, Physicochemical Characterization, and Antioxidant Activities. Journal of Food Quality, 2021. https://doi.org/10.1155/2021/8877266.

Aylanc, V., Tomás, A., Russo-Almeida, P., Falcão, S. I., Vilas-Boas, M. (2021). Assessment of bioactive compounds under simulated gastrointestinal digestion of bee pollen and bee bread: Bioaccessibility and antioxidant activity. Antioxidants, 10(5). https://doi.org/10.3390/antiox10050651.

Bogdanov, S. (2004). Quality and Standards of Pollen and Beeswax. APIACTA, 38, 334–341.

Choi, EM. (2007). Antinociceptive and Antiinflammatory Activities of Pine (*Pinus densiflora*) Pollen Extract. Phytotherapy Research, 21, 471–475. https://doi.org/10.1002/ptr.

Damas, J., Liégeois, J.-F. (1999). The inflammatory reaction induced by formalin in the rat paw. In Naunyn-Schmiedeberg's Arch Pharmacol, 359 (3): 220-7.

Daoud, A., Malika, D., Bakari, S., Hfaiedh, N., Mnafgui, K., Kadri, A., Gharsallah, N. (2019). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. Arabian Journal of Chemistry, 12(8), 3075–3086. https://doi.org/10.1016/j.arabjc.2015.07.014

de França Alves, R., de Assis Ribeiro dos Santos, F. (2014). Plant sources for bee pollen load production in Sergipe, northeast Brazil. Palynology, 38(1), 90–100. https://doi.org/10.1080/01916122.2013.846280.

Eraslan, G., Kanbur, M., Silici, S., Cem Liman, B., Altinordulu, Ş., Soyer Sarica, Z. (2009). Evaluation of protective effect of bee pollen against propoxur toxicity in rat. Ecotoxicology and Environmental Safety, 72(3), 931–937. https://doi.org/10.1016/j.ecoenv.2008.06.008.

Estevinho, LM., Rodrigues, S., Pereira, AP., Feás, X. (2012). Portuguese bee pollen: Palynological study, nutritional and microbiological evaluation. International Journal of Food Science and Technology, 47(2), 429–435. https://doi.org/10.1111/j.1365-2621.2011.02859.x.

Fatrcová-Šramková, K., Nôžková, J., Kačániová, M., Máriássyová, M., Rovná, K., Stričík, M. (2013). Antioxidant and antimicrobial properties of monofloral bee pollen. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes, 48(2), 133–138. https://doi.org/10.1080/03601234.2013.727664

Fereidoni, M., Ahmadiani, A., Semnanian, S., Javan, M. (2000). An accurate and simple method for measurement of paw edema. Journal of Pharmacological and Toxicological Methods, 43(1), 11–14. https://doi.org/10.1016/S1056-8719(00)00089-7.

Gan, TJ. (2010). Diclofenac: An update on its mechanism of action and safety profile. In Current Medical Research and Opinion 26(7)1715–1731. Informa Healthcare. https://doi.org/10.1185/03007995.2010.486301.

Gercek, YC., Celik, S., Bayram, S. (2021). Screening of Plant Pollen Sources, Polyphenolic Compounds, Fatty Acids and Antioxidant/Antimicrobial Activity from Bee Pollen. Molecules, 27(1), 117. https://doi.org/10.3390/molecules27010117.

Ghorab, A., Mesbah, M., Nakib, R., Kabli, N., Bekdouche, F., Seijo, MC. (2021a). Input to the knowledge of the melliferous plants diversity in Babors Kabylia's region (North-East of Algeria). Livestock Research for Rural Development, 33. www.lrrd.org/lrrd33/10/33119asma.html.

Ghorab, A., Rodríguez-Flores, M. S., Nakib, R., Escuredo, O., Haderbache, L., Bekdouche, F., & Seijo, M. C. (2021b). Sensorial, melissopalynological and physico-chemical characteristics of honey from Babors Kabylia's region (Algeria). Foods, 10(2). https://doi.org/10.3390/foods10020225.

Khalifa, SAM., Elashal, MH., Yosri, N., Du, M., Musharraf, SG., Nahar, L., Sarker, SD., Guo, Z., Cao, W., Zou, X., Abd El-Wahed, AA., Xiao, J., Omar, HA., Hegazy, MEF., El-Seedi, HR. (2021). Bee pollen: Current status and therapeutic potential. In Nutrients (Vol. 13, Issue 6). MDPI AG. https://doi.org/10.3390/nu13061876.

Komosinska-Vassev, K., Olczyk, P., Ka, J., Mencner, L., Olczyk, K. (2015). Bee Pollen: Chemical Composition and Therapeutic Application. 2015. https://doi.org/http://dx.doi.org/10.1155/2015/297425.

Kumaran, A., oel Karunakaran, R. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT - Food Science and Technology, 40(2), 344–352. https://doi.org/10.1016/j.lwt.2005.09.011.

LeBlanc, BW., Davis, OK., Boue, S., DeLucca, A., Deeby, T. (2009). Antioxidant activity of Sonoran Desert bee pollen. Food Chemistry, 115(4), 1299–1305. https://doi.org/10.1016/j.foodchem.2009.01.055.

Lopes, AJO., Vasconcelos, CC., Garcia, JBS., Dória Pinheiro, MS., Pereira, FAN., Camelo, DD. S., de Morais, SV., Freitas, JRB., da Rocha, CQ., de Sousa Ribeiro, MN., do Socorro de Sousa Cartágenes, M. (2020). Anti-inflammatory and antioxidant activity of pollen extract collected by *Scaptotrigona affinis postica*: *In silico*, *in vitro*, and *in vivo* studies. Antioxidants, 9(2), 1–16. https://doi.org/10.3390/antiox9020103.

Lopes, AJO., Vasconcelos, CC., Pereira, FAN., Silva, RHM., Queiroz, PFDSQ., Fernandes, CV., Garcia, JBS., Ramos, RM., da Rocha, CQ., Lima, STDJRM., Cartágenes, MDSDS., de Sousa Ribeiro, MN. (2019). Anti-Inflammatory and antinociceptive activity of pollen extract collected by stingless bee *Melipona fasciculata*. International Journal of Molecular Sciences, 20(18). https://doi.org/10.3390/ijms20184512.

Louveaux, J., Maurizio, A., Vorwohl, G. (1978). Methods of Melissopalynology. Bee World, 59(4), 139–157. https://doi.org/10.1080/0005772X.1978.11097714.

Maruyama, H., Sakamoto, T., Araki, Y., Hara, H. (2010). Anti-inflammatory effect of bee pollen ethanol extract from *Cistus sp.* of Spanish on carrageenan-induced rat hind paw edema. In BMC Complementary and Alternative Medicine (Vol. 10). http://www.biomedcentral.com/1472-6882/10/30.

Nogueira, C., Iglesias, A., Feás, X., Estevinho, LM. (2012). Commercial bee pollen with different geographical origins: A comprehensive approach. International Journal of Molecular Sciences, 13(9), 11173–11187. https://doi.org/10.3390/ijms130911173.

Panche, AN., Diwan, AD., & Chandra, SR. (2016). Flavonoids: An overview. In Journal of Nutritional Science (Vol. 5). Cambridge University Press. https://doi.org/10.1017/jns.2016.41.

Riding, JB. (2021). A guide to preparation protocols in palynology. Palynology, 45(S1), 1–110.

https://doi.org/10.1080/01916122.2021.1878305.

Rzepecka-Stojko, A., Stojko, J., Kurek-Górecka, A., Górecki, M., Kabała-Dzik, A., Kubina, R., Moździerz, A., Buszman, E., Iriti, M. (2015). Polyphenols from Bee Pollen: Structure, absorption, metabolism and biological activity. In Molecules (Vol. 20, Issue 12, pp. 21732–21749). MDPI AG. https://doi.org/10.3390/molecules201219800.

Saadia Tamali, H., Özkırım, A. (2019). Beekeeping Activities in Turkey and Algeria. In Mellifera (Vol. 19, Issue 1).

Şahin, S., Karkar, B. (2019). The antioxidant properties of the chestnut bee pollen extract and its preventive action against oxidatively induced damage in DNA bases. Journal of Food Biochemistry, 43(7), 1–8. https://doi.org/10.1111/jfbc.12888.

Sattler, JAG., de Melo, ILP., Granato, D., Araújo, E., da Silva de Freitas, A., Barth, OM., Sattler, A., de Almeida-Muradian, LB. (2015). Impact of origin on bioactive compounds and nutritional composition of bee pollen from southern Brazil: A screening study. Food Research International, 77, 82–91. https://doi.org/10.1016/j.foodres.2015.09.013

Singleton, VL., Rossi, JA. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, 16(3), 144 LP – 158.

Thakur, M., Nanda, V. (2020). Composition and functionality of bee pollen: A review. Trends in Food Science and Technology, 98, 82–106. https://doi.org/10.1016/j.tifs.2020.02.001.

Topçu, G., Ay, M., Bilici, A., Sarikürkcü, C., Öztürk, M., Ulubelen, A. (2007). A new flavone from antioxidant extracts of *Pistacia terebinthus*. Food Chemistry, 103(3), 816–822. https://doi.org/10.1016/j.foodchem.2006.09.028.

Tutun, H., Kaya, MM., Usluer, MS., Kahraman, HA. (2021). Bee pollen: Its antioxidant activity. Uludağ Arıcılık Dergisi, 21(1), 8–20. https://doi.org/10.31467/uluaricilik.896045.

Yildiz, O., Can, Z., Saral, Ö., Yuluğ, E., Öztürk, F., Aliyazicioğlu, R., Canpolat, S., Kolayli, S. (2013). Hepatoprotective potential of chestnut bee pollen on carbon tetrachloride-induced hepatic damages in rats. Evidence-Based Complementary and Alternative Medicine, 2013. https://doi.org/10.1155/2013/461478.

Zerrouk, S., Seijo, MC., Boughediri, L., Escuredo, O., Rodríguez-Flores, MS. (2014). Palynological characterisation of Algerian honeys according to their geographical and botanical origin. Grana, 53(2), 147–158. https://doi.org/10.1080/00173134.2014.897751.

Žilić, S., Vančetović, J., Janković, M., Maksimović, V. (2014). Chemical composition, bioactive compounds, antioxidant capacity and stability of floral maize (*Zea mays L.*) pollen. Journal of Functional Foods, 10, 65–74. https://doi.org/10.1016/j.jff.2014.05.007.