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Palynological Analyses of Malatya Propolis Samples

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ABSTRACT

Propolis is a sticky resinous mixture of plant secretions and bee wax. Propolis has used for ethnobotanical reasons from ancient cultures and nowadays the researchers focus on testing propolis extracts and raw forms for health problems. Knowledge about the quality of propolis samples is very important to solve various health problems. The quality of propolis samples highly associated with botanical origin. This preliminary study was conducted for determining the botanical origin of Malatya propolis samples in 2022. For palynological research, propolis samples were prepared to microscopic analyses. The dominant taxa was listed according to districts as; for Akcadag *Carduus* (9.2 %), *Astragalus* (7.4 %), *Onobrychis* (7.1 %), *Xanthium* (6.8 %), *Verbascum* (6.1 %), *Taraxacum* (5.3 %); for Battalgazi Poaceae (28.2 %), *Carduus* (7.7 %), *Astragalus* (5.9 %); for Darende *Onobrychis* (11.9 %), *Verbascum* (10.5 %), *Carduus* (8.7 %), Poaceae (5.8 %), for Hekimhan *Carduus* (9.3 %), Poaceae (8.9 %), *Astragalus* (6.8 %), *Verbascum* (6.1 %) *Xanthium* (5.4 %), for Puturge *Onobrychis* (14.1 %), *Astragalus* (8.8 %), Poaceae (8.7 %), *Carduus* (5.1 %), *Cistus* (5.1 %), for Yesilyurt Poaceae (10.7 %), *Astragalus* (6.1 %), *Carduus* (5.8 %), *Verbascum* (5.5 %). The aim of this study to determine the botanical origin of Malatya propolis for guiding beekeepers to place the beehives to the convenient areas.

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INTRODUCTION

Propolis is a natural resinous waxy bee product, obtain collecting from plants' (trees, flowers, buds and leaves exudates) secretions and mixed up bee wax by bees (Silva et al. 2012; Silva et al. 2013, Nada et al. 2022, Ali et al. 2023). Propolis gains strong and adhesive feature due to the modification of the resinous structure by bees (Silici 2019). The word of "Propolis" comes from Greek is "Pro" means "in defence of" and "Polis" means "city". Propolis uses by honeybees (*Apis mellifera* L.) for protecting the honeybees from microbial infections and diseases, protecting the hive from any external invaders and extreme environmental conditions, providing the hygienic situations in hives, making smaller the entrance pore of hives, repairing the hives and the usage of propolis in folk medicine dates ancient times (Vargas-Sánchez et al. 2016; Silici 2019). Besides propolis is used for food preservatives because of its natural protector features in food industry (Bahtiti 2010; Günhan et al. 2022).

Propolis colour varies reddish green to brown and has characteristically odour (Silici 2019). The components of propolis are resins (50 %); wax (30 %); volatile essential oils (10 %); pollen grains (5 %) consequently includes more than 100 compounds as fenolic acids and esters, flavonoids and terpenoids and this combines have antiviral, antifungal, anti-inflammatory, antibacterial, antiparasitic, immunomodulatory and hepatoprotective properties (Kujumgiev et al. 1999; Gao et al. 2014; Mahmoud and Rizk 2014; Chen et al. 2018; Lisbona-Gónzalez et al. 2021; Lopez-Válverde et al. 2021; Sahlan et al. 2021; Santos et al. 2021; Asma et al. 2022; Chavda et al. 2023). Propolis is effective in cleaning the cells of the honeycomb, the development of the egg laid by the queen bee in a sterile environment and the protection of the brood besides, bees working inside the hive, prevent various infections from entering the hive by brushing the worker bees coming from outside that infected various microorganisms during foraging, with propolis at the hive entrance hole (Kumova et al. 2002).

Ancient Greeks, Egyptians and Romans used propolis as cosmetics, for doing mummies, wound antiception and healing tumors, then eventual years in Eastern Europe and Middle East propolis was started using as herbal medicine (Kuropatnicki et al. 2013). Nowadays the researches among propolis has intensified on human health. According to previous studies propolis has positive effects on diabetes (Zakerkish et al. 2019), gastrointestinal tract (Dallabona et al. 2020), rheumatoid arthritis (Farooqui and Farooqui 2014), tumors (Demir et al. 2016; Elumalai et al. 2022) and cardiovascular diseases (Hadi et al. 2017). In addition to these propolis prevent the chemical additives, that hazardous for people health, for protect the food in industry as a natural food preservative by its antimicrobial effect in solid and liquid aliments (Tosi et al. 2007; Yang et al. 2017). Antimicrobial effects of propolis had investigated on the food poisoning microorganisms like gram positive and negative bacteries (*Staphylococcus aureus, Bacillus cereus, Salmonella enteritidis, E. coli…* etc.), molds (*Candida albicans, Saccaromyces cerevisiae*… etc.), fungi (*Aspergillus niger, Fusarium solani*… etc.) (Tosi et al. 2007; Pobigea et al. 2019, Andre et al. 2022). In previous studies the alcohol extract of propolis were used as a natural food proservative (Tosi et al. 2007; Yang et al. 2017, Andre et al. 2022).

Propolis could be categorise according to geographical and botanical origin, climatic conditions, the genetic factors of bee, physical and chemical properties (Nada et al. 2022). The melissopalynological analyses of propolis provides determining the botanical origin. The pollen spectrum of propolis is a functional indicator for having a knowledge about the plants, foraging the bees (Silva et al. 2013) in other words the quality of propolis. Because the propolis biochemical content is directly related with plants that visited by bees. Either our country or around the world there are many melissopalynological researches on propolis (Park et al. 2002; Gencay Çelemli and Sorkun 2012; Popova et al. 2013; Bayram 2015; Kızılpınar et al. 2017; Tatlısulu and Özgör 2023). This preliminary study was carried out for contribute the former researches on this subject and lead beekeepers to choose valid areas to place their beehives.

MATERIALS AND METHODS

Study Area

The study conducted in 2022 and the propolis samples obtained from Malatya provinces; Akcadag, Battalgazi, Doganshehir, Hekimhan, Puturge, Yesilyurt (Figure 1). Malatya flora has 3 phytogeographic elements (Irano-Turanian (42.81 %), Mediterraneaen (7.82 %) and Euro-Siberian (3.84 %)) (Karakuş 2016). The families abundant

by species could be listed as Asteraceae, Fabaceae, Brassicaceae, Lamiaceae and Poaceae (Karakuş 2016). If focus on plant wealth of districts among local studies it could be seen the main families are identical only changes in order. Sürgü/Dogansehir has similarity with the general Malatya formation as Asteraceaea, Fabaceae, Brassicaceae, Lamiaceae, Poaceae (Tosyagülü Çelik and Kaya 2017), Flora of Beydağı consist of mainly Asteraceae, Fabaceae, Lamiaceae, Poaceae, Brassicaceae families (Yıldız et al. 2004), Inonu University Campus Area's largest families are listed as Fabaceae, Asteraceae and Poaceae (Mutlu and Karakuş 2015), Tohma Valley floristic records showed Asteraceae, Brassicaceae, Fabaceae, Poaceae, Lamiaceae taxa are the most abundant families (Karakuş and Mutlu 2017) and in Pütürge Fabaceae, Asteraceae, Brassicaceae families stands out with the rich number of studies (Altan 1984).

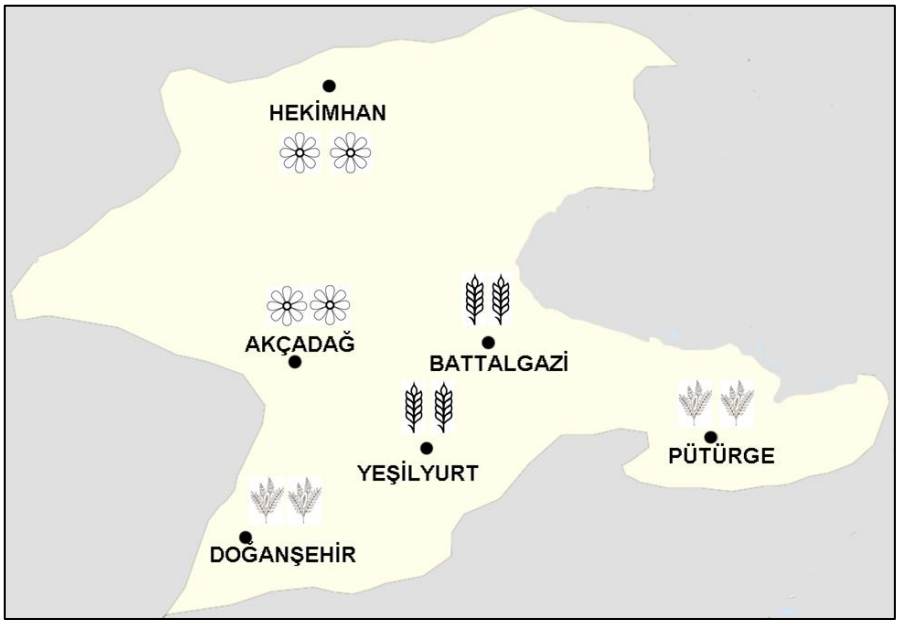

Figure 1. *The study area stated districts*

Palynological Method

The modified Barth (1998) method was applied while preparing the propolis samples. Accordingly, 0.5 g of propolis sample was weighed, kept in EtOH (Absolute > 99.9%) (13 ml) for 1 night, the samples were centrifuged (NÜVE, NF 200), the precipitate was again treated with EtOH and centrifuged. Afterwards, the supernatant was discarded, the precipitate was kept in a 10% KOH (85%, pellet) solution in a water bath for 2 minutes and vortexed for 5 minutes. Then the acetolysis mixture was applied, centrifuged, washed with distilled water, and stained with glycerine-gelatin after centrifugation again.

The samples were examined by counting 1000 pollen grains under the microscope (Euromax-Bioblue Lab.) and pollen percentages were given. Reference pollen samples and pollen descriptions from to Erdtman (1952; 1969), Wodehouse (1965), Aytuğ (1967), Charpin et al., (1974), Faegri and Iversen (1975) were used as identification of pollen grains in propolis samples.

RESULTS AND DISCUSSION

In this study 46 different taxa, belongs to 31 families, was determined and according to provinces 42 taxa in Akcadag (A), 45 taxa in Battalgazi (B), Dogansehir (D) and Yesilyurt (Y), 43 taxa in Hekimhan (H), 39 taxa in Puturge (P) were defined (Table 1). The value of taxa calculated over 5 % accepted as dominant. The dominant taxa determined as; for Akcadag *Carduus* (9.2 %), *Astragalus* (7.4 %), *Onobrychis* (7.1 %), *Xanthium* (6.8 %), *Verbascum* (6.1 %), *Taraxacum* (5.3 %); for Battalgazi Poaceae (28.2 %), *Carduus* (7.7 %), *Astragalus* (5.9 %); for Darende *Onobrychis* (11.9 %), *Verbascum* (10.5 %), *Carduus* (8.7 %), Poaceae (5.8 %), for Hekimhan

Carduus (9.3 %), Poaceae (8.9 %), *Astragalus* (6.8 %), *Verbascum* (6.1 %) *Xanthium* (5.4 %), for Puturge *Onobrychis* (14.1 %), *Astragalus* (8.8 %), Poaceae (8.7 %), *Carduus* (5.1 %), *Cistus* (5.1 %), for Yesilyurt Poaceae (10.7 %), *Astragalus* (6.1 %), *Carduus* (5.8 %), *Verbascum* (5.5 %) (Figure 1, 2).

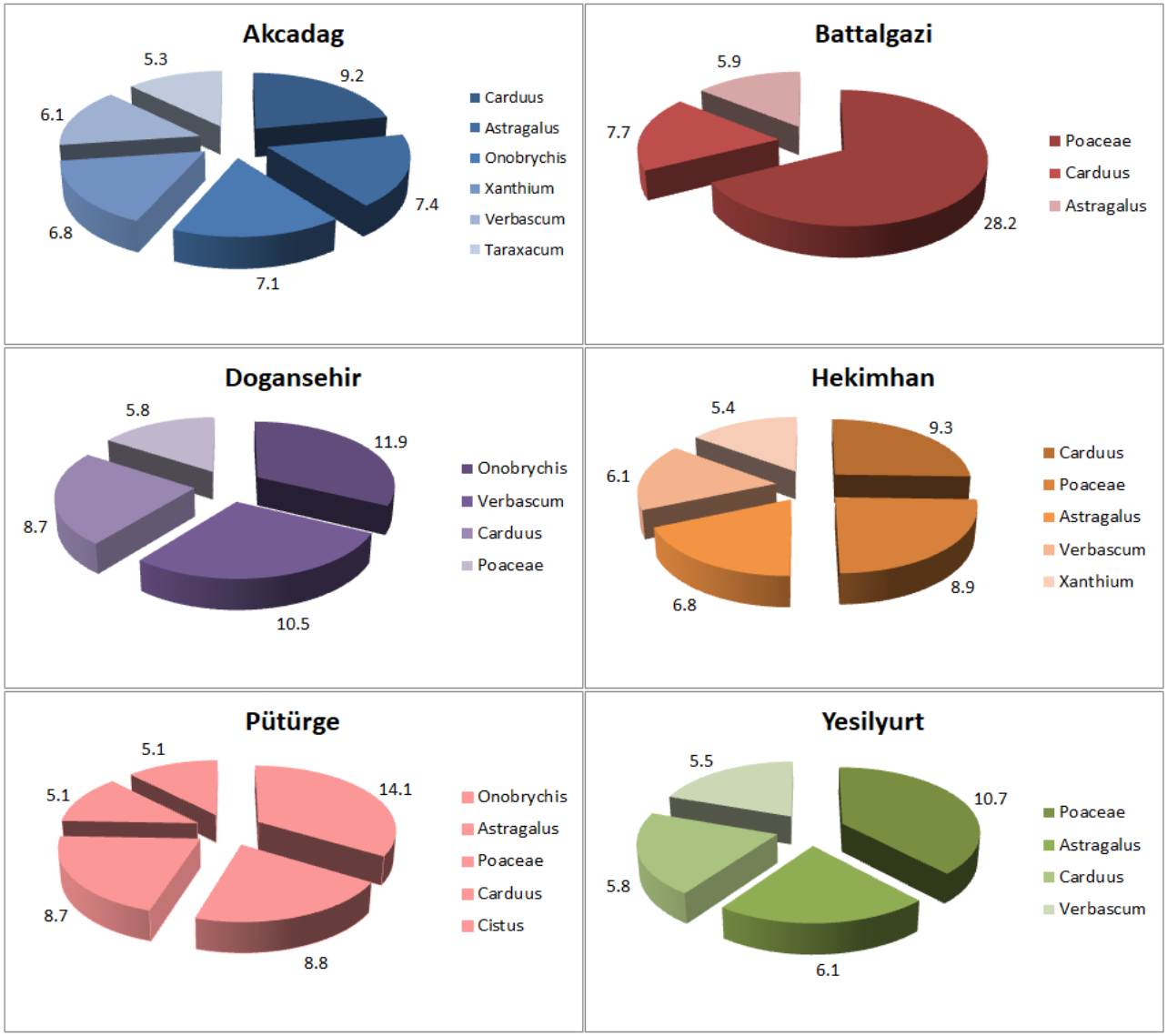

Figure 2. *Dominant pollen taxa percent values of propolis samples according to provinces*

Fabaceae family is represented most numerous genera, by 7 taxa, in the study. Total provinces percentage of this family has highest value (21.86 %) (Table 2). Asteraceae family is the secondary taxon either by member genera (*Carduus, Xanthium,Taraxacum, Artemisia*) and sum of pollen percentages (17.34 %) (Table 2). Poaceae family was able to increase over 10 % with merely 2 taxa member (Table 2).

There are some previous palynological studies on propolis. In a study involving 30 propolis samples collected from Kemaliye-Erzincan region 32 different plant families were identified. Similar to our study, it was emphasized that Asteraceae and Fabaceae families were dominant taxa (Gencay and Sorkun 2006). 28 propolis samples were used in the study conducted in Brazil, and the dominant pollen types were determined as *Mimosa verrucosa*, *Borreria*, *Acacia*, Asterarceae, *Eucalyptus, Cecropia* and Solanaceae (Freitas et al. 2011). In the study analysis on 8 propolis samples in Mexico, 42 pollen types were defined, 6 samples were found to be bifloral and 2 samples were multiforal; *Mimosa distachya* var. *laxifora* and *Prosopis velutina* are characteristic pollen types (Vargas-

Sánchez et al. 2016). In the palynological study on Turkey and Serbia propolis, 60 samples, 48 from Turkey and 12 from Serbia, were used and dominant pollen taxa were determined as Fabaceae, Lamiaceae, Rosaceae, *Castanea sativa* Mill., *Lotus corniculatus* L., *Salix* spp. (Guzelmeric et al. 2018). According to Kekecoglu et al. (2021) *Castanea sativa, Lathyrus laxiflorus* and *Geranium* pollen grains were found important values in Yiğilca Region propolis samples.

Table 1. *Pollen taxa percents of different provinces'propolis samples*

TAXA	A %	TAXA	B %	TAXA	D %	TAXA	H %	TAXA	P %	TAXA	Y %
Carduus	9.2	Poaceae	28.2	*Onobrychis*	11.9	*Carduus*	9.3	*Onobrychis*	14.1	Poaceae	10.7
Astragalus	7.4	*Carduus*	7.7	*Verbascum*	10.5	Poaceae	8.9	*Astragalus*	8.8	*Astragalus*	6.1
Onobrychis	7.1	*Astragalus*	5.9	*Carduus*	8.7	*Astragalus*	6.8	Poaceae	8.7	*Carduus*	5.8
Xanthium	6.8	Apiaceae	4.6	Poaceae	5.8	*Verbascum*	6.1	*Carduus*	5.1	*Verbascum*	5.5
Verbascum	6.1	*Verbascum*	4.4	*Hedysarum*	4.9	*Xanthium*	5.4	*Cistus*	5.1	*Xanthium*	4.9
Taraxacum	5.3	Cheno./Amar.	3.2	*Astragalus*	4.8	*Juglans*	4.4	*Verbascum*	4.7	*Onobrychis*	4.2
Dianthus	4.4	*Xanthium*	2.9	*Plantago*	4.2	*Taraxacum*	4.3	Apiaceae	3.6	*Taraxacum*	4.1
Cheno./Amar.	4.3	*Quercus*	2.8	Brassicaceae	3.6	*Berberis*	3.9	*Berberis*	3.4	*Hedysarum*	3.9
Hedysarum	3.7	*Taraxacum*	2.6	*Taraxacum*	3.4	Apiaceae	3.6	*Xanthium*	2.9	Apiaceae	3.1
Echium	3.6	*Dianthus*	2.3	Apiaceae	3.3	*Echium*	2.8	*Taraxacum*	2.7	Brassicaceae	2.8
Apiaceae	3.4	Brassicaceae	2.1	*Cistus*	3.3	*Pinus*	2.7	*Echium*	2.6	*Dianthus*	2.8
Quercus	3.1	*Artemisia*	1.9	*Quercus*	3.2	*Onobrychis*	2.6	*Hedysarum*	2.6	Cheno./Amar.	2.7
Plantago	2.6	*Zea mays*	1.9	*Artemisia*	2.9	*Quercus*	2.5	*Quercus*	2.4	*Plantago*	2.6
Poaceae	2.6	Cupressaceae/ Taxaceae	1.8	*Trifolium*	1.9	*Dianthus*	2.4	Oleaceae	2.3	*Juglans*	2.6
Vicia	2.1	Lamiaceae	1.8	*Salix*	1.9	Cheno./Amar.	2.3	*Juglans*	2.3	Oleaceae	2.5
Lamiaceae	1.9	*Onobrychis*	1.7	Oleaceae	1.7	*Cistus*	2.3	*Vicia*	2.2	*Cistus*	2.3
Cupressaceae/ Taxacaea	1.8	*Robinia pseudoacacia*	1.6	*Xanthium*	1.6	*Plantago*	2.2	Brassicaceae	1.9	*Berberis*	2.2
Urticaceae	1.8	*Salix*	1.4	*Dianthus*	1.6	*Artemisia*	2.1	*Dianthus*	1.8	*Pinus*	2.1
Pinus	1.6	*Platanus*	1.3	*Vicia*	1.6	Urticaceae	2.1	*Artemisia*	1.7	*Quercus*	2.1
Geranium	1.6	*Hedysarum*	1.3	Rosaceae	1.5	*Robinia pseudoacacia*	1.8	*Convolvulus*	1.7	*Trifolium*	1.9
Brassicaceae	1.4	*Trifolium*	1.3	*Medicago*	1.4	*Hedysarum*	1.7	Cheno./Amar.	1.6	Lamiaceae	1.9
Cistus	1.4	*Convolvulus*	1.3	Lamiaceae	1.3	*Vicia*	1.7	Lamiaceae	1.5	*Artemisia*	1.8
Oleaceae	1.3	*Plantago*	1.2	Urticaceae	1.3	Brassicaceae	1.6	Cupressaceae/ Taxaceae	1.4	*Convolvulus*	1.8
Berberis	1.3	*Geranium*	1.2	Cheno./Amar.	1.2	*Salix*	1.6	*Trifolium*	1.4	*Echium*	1.7
Rumex	1.3	*Vicia*	1.2	*Juglans*	1.2	Oleaceae	1.4	*Medicago*	1.3	*Geranium*	1.7
Malvaceae	1.3	Oleaceae	1.1	*Echium*	1.1	Cupressaceae/ Taxaceae	1.3	Rosaceae	1.3	*Salix*	1.6
Salix	1.2	Rosaceae	1.1	*Robinia pseudoacacia*	1.0	*Rosaceae*	1.3	*Zea mays*	1.3	*Medicago*	1.4
Anchusa	1.1	*Cistus*	1.0	*Platanus*	0.9	Malvaceae	1.2	*Pinus*	1.2	*Vicia*	1.4
Artemisia	1.0	Campanulaceae	0.9	*Berberis*	0.8	*Anchusa*	1.1	*Salix*	1.2	Cupressaceae/ Taxaceae	1.3
Trifolium	1.0	*Anchusa*	0.9	*Rumex*	0.8	*Trifolium*	1.0	*Plantago*	1.1	*R.pseudoacacia*	1.3
Medicago	0.9	*Juglans*	0.8	*Anchusa*	0.8	*Papaver*	0.9	*Platanus*	1.0	*Anchusa*	1.3
Thalictrum	0.9	*Papaver*	0.8	*Pinus*	0.7	*Platanus*	0.8	*Robinia pseudoacacia*	0.9	*Platanus*	1.2
Papaver	0.8	Urticaceae	0.8	Campanulaceae	0.7	*Rumex*	0.8	Urticaceae	0.9	*Zea mays*	1.2
Platanus	0.7	*Berberis*	0.7	*Papaver*	0.7	*Populus*	0.8	Campanulaceae	0.8	Rosaceae	1.0
Campanulaceae	0.7	*Echium*	0.6	Cupressaceae/ Taxaceae	0.6	*Zea mays*	0.8	*Anchusa*	0.8	Urticaceae	0.9
Ligustrum	0.7	*Abies*	0.6	*Convolvulus*	0.6	*Ligustrum*	0.8	*Papaver*	0.7	Campanulaceae	0.6
Robinia pseudoacacia	0.6	*Populus*	0.6	*Zea mays*	0.6	Lamiaceae	0.7	*Ligustrum*	0.6	Malvaceae	0.6
Rosaceae	0.6	*Pinus*	0.5	*Populus*	0.5	*Thalictrum*	0.6	*Rumex*	0.4	*Papaver*	0.5
Juglans	0.5	*Rumex*	0.5	*Acer*	0.4	Campanulaceae	0.5	*Abies*	0	*Rumex*	0.5

Zea mays	0.5	Malvaceae	0.5	Malvaceae	0.4	*Medicago*	0.4	*Rubia*	0	*Ligustrum*	0.5
Abies	0.4	*Ligustrum*	0.4	*Ligustrum*	0.3	*Abies*	0.3	*Acer*	0	*Populus*	0.4
Rubia	0	*Medicago*	0.2	*Abies*	0.2	*Acer*	0.2	*Populus*	0	*Abies*	0.2
Acer	0	*Rubia*	0.2	*Rubia*	0.1	*Rubia*	0	*Thalictrum*	0	*Acer*	0.2
Populus	0	*Thalictrum*	0.2	*Thalictrum*	0.1	*Geranium*	0	*Geranium*	0	*Rubia*	0.1
Convolvulus	0	*Acer*	0	*Geranium*	0	*Convolvulus*	0	Malvaceae	0	*Thalictrum*	0

Onobrychis (Fabaceae) pollen grains were found most represented taxa in Dogansehir and Puturge. The genus is widespread in Malatya and has 13 taxa including 4 endemic (Karakuş 2016; Tosyagülü Çelik and Kaya 2017). Fabaceae members also dominant in Brazilian propolis samples (Avelino and Santos 2018). *Carduus* (Asteraceae) has 4 taxa in Malatya and *C. nutans* subsp. *nutans* were recorded Akcadag and Hekimhan (Karakuş 2016). This taxon is dominant in Akcadag and Hekimhan in our study supportively. *Carduus* species have been reported important beekeeping plants for our country (Sıralı and Deveci 2002). Poaceae family has a great number of members in Turkey. The distrubition area of Poaceae is quite wide and this takson is also cultivated intensely. In Malatya 124 species and in Battalgazi 47 species of Poaceae were recorded (Karakuş 2016; Mutlu and Karakuş 2015). The pollen grains of this family were determined dominantly in Battalgazi and Yesilyurt propolis samples. When the results, which are generally accepted as dominant, are examined, it is seen that they are compatible with the flora of the region. *Astragalus* pollen grains are also dominant in whole sampling areas except only Dogansehir with 4.8%. The genus *Astragalus* is rife and represented with 74 species in Malatya (Karakuş 2016).

The food preservation function of propolis has investigated on many aliments. Propolis extends the shelf life of nutriments and relays chemical protectors naturally. El-Deeb (2017) approved discriminated percents of propolis water extract in raw milk for natural microbial safety and according to results 20 % propolis water extract was the convenient proportion for raw milk. In another dairy product, yoghurt microbial quality was analysed by using encapsulated propolis and concluded propolis extend the shelf life of yoghurt (Günhan et al. 2022). The use of propolis alcohol extracts as preservatives in liquid food products has become widespread. Mandarin, apple, orange, white grape juices protection of fungal and bacterial infection by propolis extracts were determined (Koc et al. 2007, Silici and Kevser 2014, Luis-Villaroya et al. 2015, Yang et al. 2017). Not only the liquid foods but also solid foods' spoilage can be prevented by addition of propolis. To Bahtiti's research (2010); mashed potatoes treated with different ppm proportions of propolis and considered that propolis prohibited the microbial growth and prolong the shelf life of mashed potatoes. Propolis extracts are preferable to chemical preservatives for meat products also for all fresh beef, livestock products (Tosi et al. 2007; Natsir et al. 2017, Andre et al. 2022).

Table 2. *The family names of determined plant taxa in propolis samples and their total percentages*

FAMILY	TAXON	%	Total %
Fabaceae	*Onobrychis*	6.93	
	Astragalus	6.63	
	Hedysarum	3.04	
	Vicia	1.7	21.86
	Trifolium	1.43	
	Robinia pseudoacacia	1.2	
	Medicago	0.93	
Asteraceae	*Carduus*	7.63	
	Xanthium	4.08	17.34
	Taraxacum	3.73	
	Artemisia	1.9	
Boraginaceae	*Echium*	2.08	3.08
	Anchusa	1	
Poaceae	Poaceae	10.82	11.87
	Zea mays	1.05	
Oleaceae	Oleaceae	1.72	2.27
	Ligustrum	0.55	
Pinaceae	*Pinus*	1.47	1.75
	Abies	0.28	

Salicaceae	*Salix*	1.48	1.86
	Populus	0.38	
Apiaceae	Apiaceae	3.6	3.6
Berberidaceae	*Berberis*	2.05	2.05
Brassicaceae	Brassicaceae	2.23	2.23
Campanulaceae	Campanulaceae	0.7	0.7
Caryophyllaceae	*Dianthus*	2.55	2.55
Chenopodiaceae/Amaranthaceae	Chenopodiaceae/Amaranthaceae	2.55	2.55
Cistaceae	*Cistus*	2.57	2.57
Convolvulaceae	*Convolvulus*	0.9	0.9
Cupressaceae/Taxaceae	Cupressaceae/Taxaceae	1.37	1.37
Fagaceae	*Quercus*	2.68	2.68
Geraniaceae	*Geranium*	0.75	0.75
Juglandaceae	*Juglans*	1.97	1.97
Lamiaceae	Lamiaceae	1.52	1.52
Malvaceae	Malvaceae	0.67	0.67
Papaveraceae	*Papaver*	0.72	0.72
Plantaginaceae	*Plantago*	2.32	2.32
Platanaceae	*Platanus*	0.98	0.98
Polygonaceae	*Rumex*	0.72	0.72
Ranunculaceae	*Thalictrum*	0.3	0.3
Rosaceae	Rosaceae	1.13	1.13
Rubiaceae	*Rubia*	0.07	0.07
Sapindaceae	*Acer*	0.13	0.13
Scrophulariaceae	*Verbascum*	6.22	6.22
Urticaceae	Urticaceae	1.28	1.28

CONCLUSION

This study is conducted in Malatya city through 2022 for revealling the origin of Malatya propolis samples. It is important to determine pollen content of propolis by the reason of determining the quality of propolis. The biochemical functions of propolis are directly associated with the plants that forage by the bees therefore this preliminary study will lead for beekeeping activities.

ETHICAL STATEMENT

During the writing process of the study titled "Palynological Analyses of Malatya Propolis Samples", scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. Our study was presented as an oral presentation in "1st International Conference on Frontiers in Academic Research on 18-21 February in 2023 at Konya/Turkey" and published in the abstract book. Since this research is based on document analysis and descriptive analysis, there is no obligation for an ethics committee decision.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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