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Physicochemical characteristics of fresh bee pollen from different botanical origins

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Abstract

Bee pollen has a great importance in apiterapy through which it contributes to development complementary and alternative medical practices. Due to its specifficaly chemical characteristics during botanical sources, monofloral bee pollen represents a safe alternative in medical practice.
In this study were selected ten samples of fresh bee pollen collected from different apiaries situated in Romania and were determined botanical origin, physico chemical properties, nutritional value, and antibacterial activity. Physico-chemical characteristics of analised pollen were obtained as a result of the following laboratory parameters: moisture, free acidity, proteins, lipids, ash, total sugars and the amount of macro and micro elements by ICP OES. Pollen ethanolic extracts (PEE) were tested for antimicrobial activity against human pathogenic bacteria and fungal strains.
Bee pollen samples indicate large variations of the chemical composition due to the diferent botanical origin and great nutritional value. The highest variations are found among lipids and carbohydrates, ranged from 1.33 – 5.47% for lipids and 23.31 – 48.63% for carbohydrates. Prunus sp. pollen contained the highest amounts of Fe (150,9 ± 1,11 mg/Kg), while Mg level (1505 ± 1,43 mg/Kg) was highest in Brassica sp. pollen type. PEE possessed the best antimicrobial activity against Gram-positive bacteria. The obtained results support the use of bee pollen as ingredient in many supplements and medicines.

Key words: Fresh bee pollen, chemical composition, antimicrobial, botanical origin, monofloral.

1. Introduction

Fresh bee pollen represents the pollen produced in the anthers of flowers, collected by *Apis mellifera* honeybees worker from a variety of floral species, enriching with their own substances and obtaining the pellets with therapeutic and nutritional value (Mărgăoan & al. [1]). Bee pollen comes into attention of scientific community due to the healthy properties demonstrated from ancient times. Floral variety of a harvesting area supply pollen a complex chemical composition. This gives a great importance in terms of nutrition but a constant composition results from unifloral pollen (Bogdanov [2]) because only a monofloral pollen maintains chemical and senzorial characteristics as the plant source identify by colour, morphology, flavour, size (Almeida-Muradian & al. [3] Paola-Naranjo & al. [4]). Small differences among composition of bee pollen could results in gathering area or time in floral species, environmental conditions including soil type (Kai & al. [5] Xesús & al. [6], Morgano & al. [7]) but the major differences are mainly attributed to botanical origin (Araújo & al. [8]).

Valuable nutritional quality of pollen and "super food" name assigning, due to the content of essential substances such as carbohydrates, proteins, amino acids, lipids, vitamins, minerals (Chantarudee & al. [9]) determine nutrition and dietetics specialists to use in human nutrition as complex functional foods (Mateescu [10], Nogueira & al. [11]). Bee pollen also contains important bioactive elements such as polyphenols and flavonoids which consist in a rich source of antioxidants with the highest value of a foodstuff (Oltica & al. [12] Prelipcean [13]). Bee pollen extracts have also been reported to possess antimicrobial activity (Morais & al. [14]) and inhibit the growth of some Gram-positive, Gram-negative bacteria and yeasts, considered also a heath food with a wide range of biological activities (Pascoal & al. [15]).

Due to its nutritional and antibacterial properties, bee pollen is used as ingredient in many supplements (Glušac & al. [16] Solgajova & al. [17]), also is involved as adjuvant in many therapies (Sun & al. [18] Salles & al. [19] Wagenlehner & al. [20]).

2. Materials and Methods

Chemicals

All analytical reagents were purchased from Sigma Aldrich (Louis, MO, USA) and Fluka.

Samples collections

The pollen loads were collected with the use of pollen traps fixed on colony entrance from experimental apiaries situated in five counties (Figure 1), during the 2015 and 2016 floral seasons.

Figure 1: Geographic locations of experimental apiares

Pollen samples were collected at intervals of two days and packed in food grade polyethylene bags and stored in a − 4°C freezer until analyse. Bee pollen loads were hand-sorted by appearance (Kirk [21]) and classified according to Pantone Color Manager software for each sub-samples obtained.

Botanical Origin

Bee pollen was subjected to the unacetolised methodology (Barth & al. [22]) using two grams of each sub-samples. After frequency, pollen samples were classified according to their pollen grain percentages as monofloral or heterofloral batches. Bee pollen samples were classified as monofloral if a pollen source was presenting more than 90% (Freitas & al. [23]).

For microscopic identification of pollen grains was used an optical microscope with total magnification (40x). Reference collection slides from bee products chemistry laboratory of the Institute for Apicultural Research and Development and also pollen atlases (Bucher & al. [24] Ricciardelli [25]) were used for pollen recognition.

Bee Pollen Extract Preparation

For preparation of pollen ethanolic extracts (PEE) was weight 20g of bee pollen to 50 mL of 70% ethanolic solution and suspension was expose to ultrasonication. Alcoholic mixture was filtered using Whatman filter paper and stored at 4°C.

Physico-Chemical Determinations

Moisture: 3 g of sample of each study pollen was weight and heated in an electric oven at a temperature of 103 - 105°C to constant weight (Zenebon & al. [26]).

Titration method to fixed point was used for free acidity determination (Fuenmayor & al. [27]).

Ash content was determined through gravimetry after ignition the sample in an oven at 550°C±5°C until constant weight was obtained (Zenebon & al. [26]).

Mineral elements were quantified after etalon curves fitting and microwave mineralisation of the pollen with an ICP OES equipment (Morgano & al. [7]).

For total lipids determination, bee pollen pellets were extracted with petroleum ether in a Soxhlet automatic extractor (Somerville [28]).

Protein/nitrogen followed the Kjeldahl method. A standard solution of sulfuric acid was used for quantifying the nitrogen levels to protein and 6.25 conversion factor for calculate the crude protein content (Andrada & al. [29]).

For the carbohydrates determination, Elser method (N. Popescu & al. [30]) was applied. In order to avoid proteins interfering in chemical reaction, was carried out a deproteinisation of the sample.

Energy (kcal) = 4 × (g protein+ g carbohydrate) + 9 × (g lipid); was the equation used for estimated the total energy (Barros & al. [31]).

Antimicrobial Activity

Antimicrobial activity was tested using disc diffusion method against ATCC reference strains as follows: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212,.

Standard disks (Ø 10 mm) were impregnated with 20 µL PEE and placed on Muller-Hinton (MHA) agar plates and fungal strain on Sabouraud medium. The plates were inoculated with fresh culture suspension adjusted to McFarland standard 0.5 (equivalent to 1.5x108 UFC/mL). Chloramphenicol (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212) and Piperacillin (*Pseudomonas aeruginosa* ATCC 27853) were used as standard antimicrobial material against mention strains, while control was represented by ethanol 70%.

The antibacterial activity was evaluated by measuring the diameter of the inhibition areas. All tests were conducted in triplicate.

3. Results and Discussion

Microscopic identification demonstrated a large number of floral species which providing pollen, 20 taxa were identified. For determining the frequency of pollen samples, were considered significant only pollen types present with a higher frequency then three percent due to the fact that the presents of the minor pollen is attributed to surface contamination from other pollen pellets (Stimec & al. [32]). Botanical identification found that five samples were monofloral, two samples were heterofloral represented by eight botanical species and three were bifloral consists by *Aesculus*, *Tilia* and *Zea mays* pollen type (Figure 2). The *Asteraceae* family was dominant in this study with the higher number of species, present in samples two, three and six, followed by *Rosaceae* in samples eight and nine.

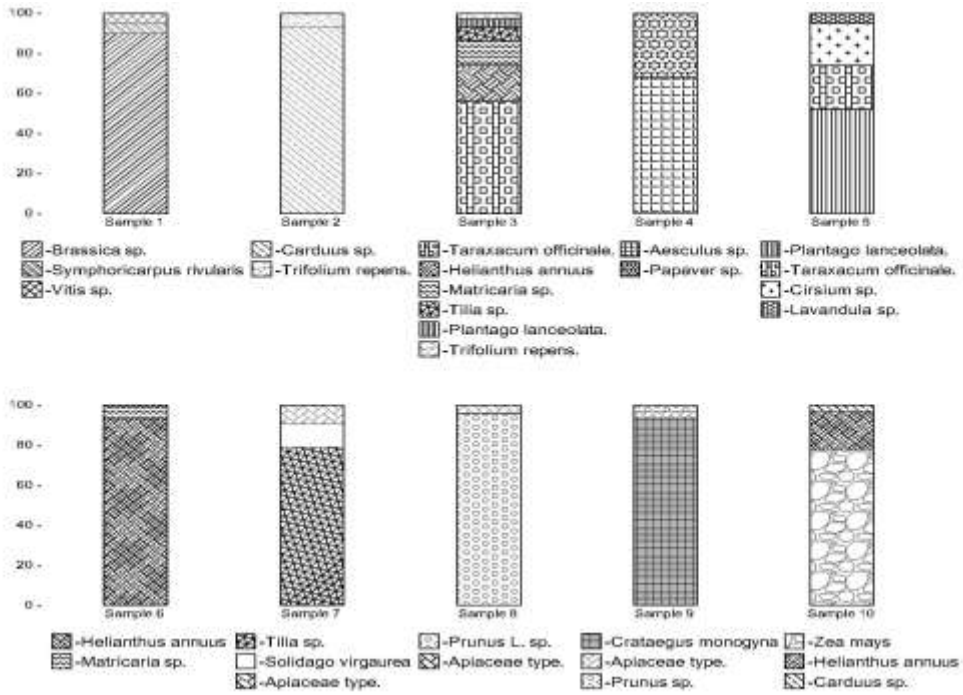

Figure 2: Botanical sources of bee pollen samples

Colour identification of bee pollen pellets showed an evident variability by botanical origin which could be an useful tool for macroscopic identification associated with collection time and floral composition of the area. We find a variety of colors (Table 1) but yellow color is the most prevalent. Pollen type collected by the bees colonies has colours from light yellow (102 C) to purple (5115 C) and dark red (1685 C) colour. Most of the samples presented an uniform colour throughout all pellets, different shades of colour were found in some samples, while the other presented two or more groups of colours on the same pellets. That was a mixture pollen types confirmed after botanical identification.

Table 1: Floral composition, year of collection, frequency, sample classification, colour of pollen pellets

Sample	Dominant botanical family identified	The main pollen type	Year of collection	Frequency (%)	Color	Sample classification
S1	*Brassicaceae*	*Brassica sp.*	2015	91	Yellow 102 C	monofloral
S2	*Asteraceae*	*Carduus sp.*	2015	93	Purple 5115 C	monofloral
S3	*Asteraceae*	*Taraxacum officinale*	2015	56	Orange 130 C	heterofloral
S4	*Fagaceae*	*Aesculus sp.*	2015	68	Red 1685 C	bifloral
S5	*Plantaginaceae*	*Plantago lanceolata*	2015	52	Light cream 601 C	hetrofloral
S6	*Asteraceae*	*Helianthus annuus*	2016	91	Orange 130 C	monofloral
S7	*Malvaceae*	*Tilia sp.*	2016	79	Yellow 108 C	bifloral
S8	*Rosaceae*	*Prunus L. sp.*	2016	94	Green 584 C	monofloral
S9	*Rosaceae*	*Crataegus monogyna*	2016	94	Yellow-green 585 C	monofloral
S10	*Poaceae*	*Zea mays*	2016	78	Dark yellow 115 C	bifloral

Bee pollen samples were analised from physicochemical characteristics and nutritional point of view and obtained results were compared to the same pollen type from the other area or countries. Chemical composition results indicate significant variations of analyzed pollen types (Table 2).

Table 2: Chemical composition and energetic value of pollen species collected by honeybees, from corbicular loads

Sample	Dominant botanical source	Moisture (g/100g)	Free acidity (mEq/Kg)	Ash* (g/100g)	Fat/lipids* (g/100g)	Protein* (g/100g)	Carbohydrates* (g/100g)	Energy (Kcal/100g)
S1	Rape	16.92 ± 1.34	146 ± 0.05	2.66 ± 0.40	5.47 ± 0.73	20.41 ± 1.89	42.57 ± 2.03	301.15 ± 4.08
S2	Thistle	22.77 ± 1.99	143 ± 0.03	1.66 ± 0.25	3.21 ± 0.81	15.54 ± 1.27	41.15 ± 3.23	255.65 ± 3.56
S3	Dandelion	20.13 ± 1.75	128 ± 0.01	1.38 ± 0.31	2.07 ± 0.55	13.76 ± 0.99	43.55 ± 2.98	247.87 ± 2.72
S4	Chestnut	24.94 ± 2.00	288 ± 0.07	2.04 ± 0.23	1.33 ± 0.37	20.61 ± 1.35	45.30 ± 3.02	275.61 ± 2.86
S5	Plantain	18.68 ± 1.55	134 ± 0.05	1.80 ± 0.10	3.16 ± 0.46	14.83 ± 1.74	48.63 ± 2.76	282.28 ± 3.33
S6	Sunflower	26.36 ± 1.49	195 ± 0.04	1.34 ± 0.12	5.20 ± 0.77	13.16 ± 0.76	35.81 ± 1.85	242.68 ± 2.56
S7	Lime	21.96 ± 1.32	168 ± 0.01	2.62 ± 0.25	2.30 ± 0.42	16.31 ± 0.88	32.23 ± 1.74	214.86 ± 3.78
S8	Fruit trees	23.36 ± 1.53	268 ± 0.03	2.81 ± 0.22	3.26 ± 0.51	24.14 ± 1.14	43.27 ± 2.01	298.98 ± 4.11
S9	Hawthorn	31.08 ± 2.16	294 ± 0.05	2.09 ± 0.17	2.80 ± 0.46	22.06 ± 2.01	41.89 ± 3.11	281.0 ± 3.07
S10	Maize	21.77 ± 1.09	221 ± 0.05	1.55 ± 0.21	1.71 ± 0.26	14.08 ± 1.53	23.31 ± 1.95	164.95 ± 2.99

* parameters expressed on dry basis; Mean ± standard deviation

Regarding moisture content, our data recorded values between 16.92 and 31.08% with the higer content in *Crataegus sp.* sample. This probably occurred because the high relative humidity of the air in harvest time, an influenced factor in fresh bee pollen mentioned in previous studies (Morgano & al. [33]). Our results are comparable with those obtained in Transylvania for fresh bee pollen (Mărgăoan & al. [34]) and corresponded in interval proposed for bee pollen standardisation (Campos & al. [35]).

Free acidity ranged from 128 meq/Kg in sample dominated by *Taraxacum* pollen type and 294 meq/Kg in that dominated by *Crataegus monogyna* pollen type. This results confirmed the acidic character of the bee pollen, found also in brazilian multifloral bee pollen (Martins & al. [36]) with minimum value of 105.3 meq/Kg and maximum of 609.9 meq/Kg and colombian bee pollen ranged from 155 to 402 meq/kg with 256 (±67) meq/kg as mean value (Fuenmayor & al. [27]).

The highest variations are found among lipids and carbohydrates ranged from 1.33 – 5.47% for lipids and 23.31 – 48.63% for carbohydrates. The highest value for carbohydrate content was found for the pollen dominated by *Plantago sp.* and the lowest for *Zea mays* pollen, a similar range was also obtained in western France when was used the same method for carbohydrates determination (Odoux & al. [37]).

The results obtained for ash and total protein content were similar to those described in brazilian bee pollen with a minimum content of ash and proteins of 1.9 and 15%, respectively (Solange & al. [38]). Some authors confirmed low protein content of some taxa investigated also in our study, for instance, *Helianthus sp.* (Nicolson & al. [39]) and *Zea Mays* (Odoux & al. [37]).

Mineral composition results are reported in Table 3.

Table 3: Mineral elements content in pollen (mg/kg)

Sample	Potassium	Magnesium	Calcium	Iron	Zinc
S1	4099 ± 3.44	1505 ± 1.43	2437 ± 1.78	58.03 ± 0.27	28.78 ± 0.65
S2	2296 ± 2.81	474.3 ± 0.98	1282 ± 0.95	27.45 ± 0.61	59.57 ± 1.15
S3	1980 ± 0.99	286.1 ± 0.77	1373 ± 0.64	24.20 ± 0.36	23.31 ± 0.33
S4	4284 ± 3.24	412.2 ± 2.02	294.1 ± 1.09	37.11 ± 0.42	34.41 ± 0.47
S5	3212 ± 2.80	398.1 ± 2.00	1275 ± 1.75	29.34 ± 0.57	20.21 ± 0.67
S6	2245 ± 3.03	315.4 ± 1.64	1294 ± 1.63	22.98 ± 0.52	26.76 ± 0.99
S7	2866 ± 1.75	1277 ± 0.87	4335 ± 2.06	27.94 ± 0.39	57.08 ± 0.75
S8	4073 ± 3.21	666.7 ± 1.05	1155 ± 1.34	150.9 ± 1.11	32.92 ± 0.62
S9	3405 ± 2.66	672.2 ± 1.34	1231 ± 1.77	40.19 ± 0.89	28.41 ± 0.40
S10	2628 ± 1.86	647.9 ± 0.96	414.8 ± 0.89	21.73 ± 0.23	38.66 ± 0.53

Mean ± standard deviation

Our data were comparable with those obtained (Morgano & al. [7]) with the same method and equipment but we detected low levels of all elements in relation to values found by (Taha [40]) with atomic absorption. We could arrange the values found for mineral elements in the following decreasing order: potassium, calcium, magnesium, iron and zinc. Our Ca and Mg levels have wider ranges (414-2437 and 315-1505 mg/kg), that are in accordance with other work with the following results: 999-2820 and 571-1405 μg/g, respectively (Kai & al. [5]), for *Brassica, Helianthus and Zea Mays* pollen. Prunus sp. pollen contained the highest amounts of Fe, followed by *Brassica*, *Crataegus* and *Carduus sp.* which had higher contents of Zn. The calcium content of Ethiopian *Zea mays* pollen investigated by Admassu was higher than potassium content (Admassu & al. [41]) a different situation comparative with previous scientific basis.

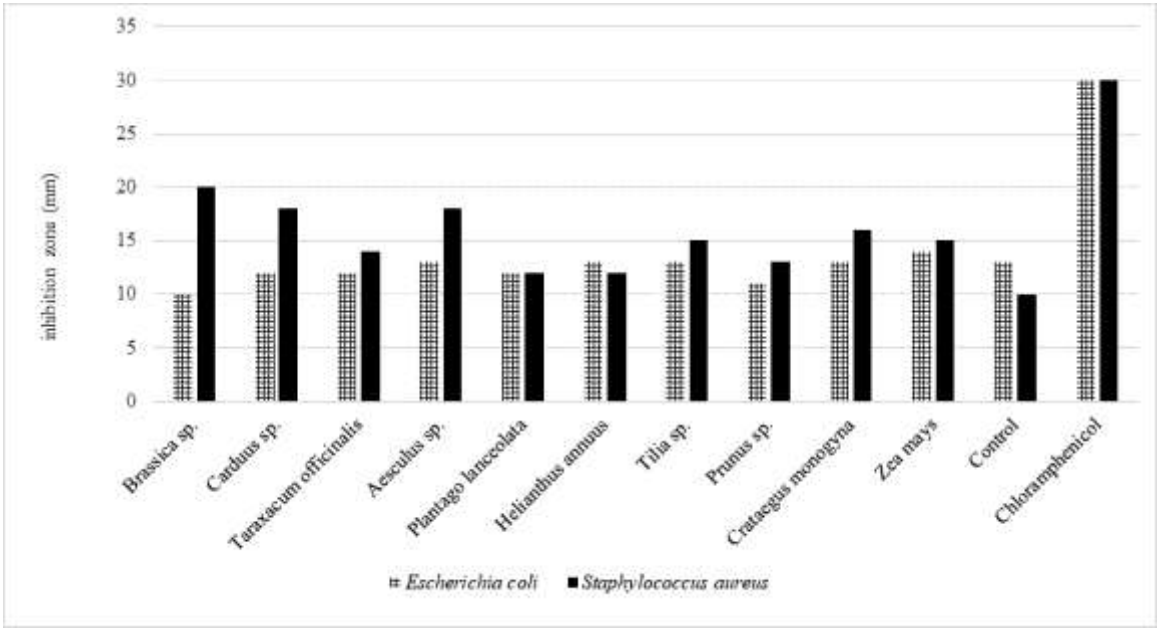

Figure 3a. Antibacterial activity of PEE against *E. coli* and *S. aureus*

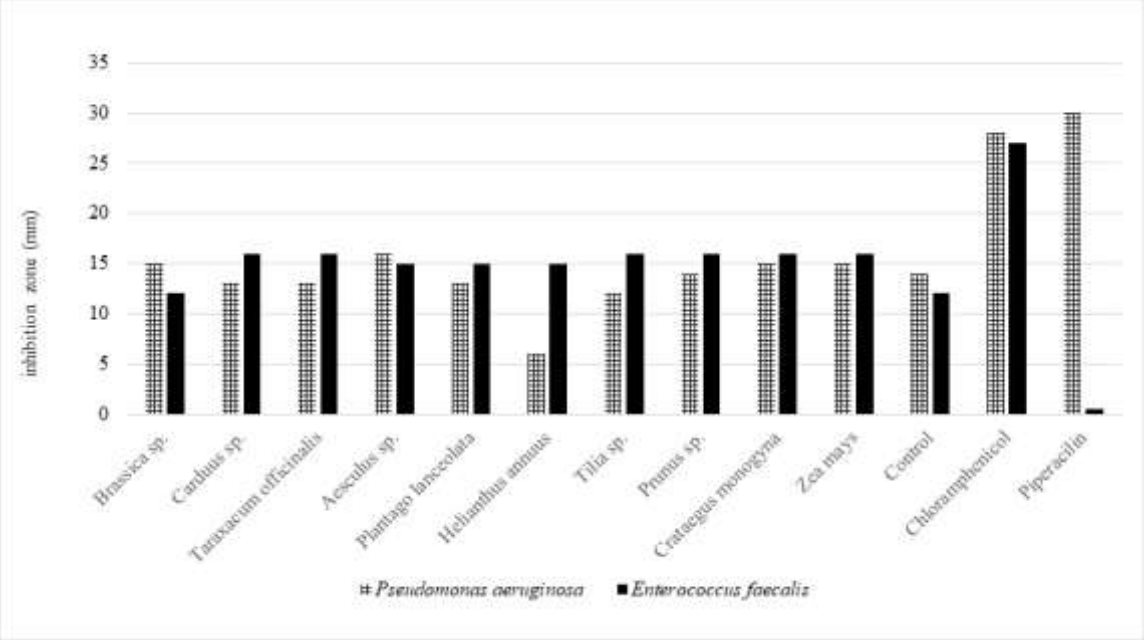

Figure 3b. Antibacterial activity of PEE against *P.aeruginosa* and *E. faecalis*

Figure 3 shows that bee pollen extracts action differentially against microbial strains growth being influenced by the type of PEE used. Pollen ethanolic extracts (PEE) possessed the best activity against Gram-positive bacteria (*Staphylococcus aureus ATCC 29213*, *Enterococcus faecalis ATCC 29212*). Qualitative antimicrobial screening revealed that *Brassica sp.* pollen extract exhibited the highest value of zone of inhibition against *Staphylococcus aureus ATCC 29213* (20 ±1 mm) compared with control (10 ± 0.4 mm). Gram positive and fungal microorganisms were also inhibited by *Carduus sp., Aesculus sp.* and *Helianthus annuus* pollen extracts. These results might have been due to high lipids content with some exceptions. Regarding Gram-negative bacteria, pollen extracts showed low to moderate antibacterial activity which can be explained by the cell membrane structure of this class of bacteria (Daoud & al. [42]).

5. Conclusion

Microscopic examination allowed us to identified the botanical sources of bee pollen and we found that half of the samples have a frequency up to 90% of one dominant floral source.

Physico chemical properties of Romanian fresh bee pollen samples show a great variation among plant sources. *Prunus L. sp.*, *Aesculus sp.* and *Brassica sp.* pollen are important sources of protein, *Plantago lanceolata* contains the greatest macronutrient (carbohydrates) amount and the highest total lipids content was found in *Brassica sp*. pollen. The important source of mineral elements content of fresh bee pollen gives a high nutritive value of the product, important in human food.

Bee pollen ethanolic extracts possess different antimicrobial properties against various pathogenic species of microorganisms in relation with botanical origins of pollen. The most sensitive bacteria was *Staphylococcus aureus* ATCC 29213 to rape pollen extract. Our results demonstrated that bee pollen extracts exhibited a stronger antimicrobial activity against Gram-positive bacteria compared with Gram-negative bacteria species.

These findings might be used to improve the scientific basis for monofloral bee pollen characterstics and assesment of this natural product of beehive.

References

1. R. MĂRGĂOAN, L. MĂRGHITAȘ, D. DEZMIREAN, C. MIHAI, O. BOBIȘ, Bee Collected Pollen – General Aspects and Chemical Composition, Bulletin USAMV Animal Science and Biotechnologies, 67 (1-2), 254, 259, (2010).
2. Ș. BOGDANOV. Pollen: Nutrition, Functional Properties, Health: A Review, http://www.bee-hexagon.net, (2014).
3. L.B. ALMEIDA-MURADIAN, L.C. PAMPLONA, S. COIMBRA, O.M. BARTH, Chemical composition and botanical evaluation of dried bee pollen pellets, Journal of Food Composition and Analysis 18 (1), 105, 111, (2005).
4. R.D.D PAOLA-NARANJO, J. SÁNCHEZ-SÁNCHEZ, A.M. GONZÁLEZ-PARAMÁS, J.C. RIVAS-GONZALO, Liquid chromatographic−mass spectrometric analysis of anthocyanin composition of dark blue bee pollen from *Echium plantagineum*, Journal Chromatography, 1054, 205, 210, (2004).
5. Y. KAI, W. DAN, Y. XINGQIAN, L. DONGHONG, C. JIANCHU, S. PEILONG, Characterization of Chemical Composition of Bee Pollen in China, Journal Agric. Food Chem., 61 (3), 708, 718, (2013).
6. F. XESÚS, M.V.T. PILAR, L. ESTEVINHO, J.A. SEIJAS, A. IGLESIAS, Organic Bee Pollen: Botanical Origin, Nutritional Value, Bioactive Compounds, Antioxidant Activity and Microbiological Quality, Molecules 17 (7), 8359, 8377, (2012).
7. M.A. MORGANO, M.C.T. MARTINS, L.C. RABONATO, R.F. MILANI, K. YOTSUYANAGIA, D.B.R. AMAYA, A Comprehensive Investigation of the Mineral Composition of Brazilian Bee Pollen: Geographic and Seasonal Variations and Contribution to Human Diet, Journal Braz. Chem. Soc., 23 (4), 727, 736 (2012**)**.
8. S.J. ARAÚJO, E.D. CHAMBÓ, M.A. COSTA, S.M.P. CAVALCANTE DA SILVA, C.A. LOPES DE CARVALHO, L.M. ESTEVINHO, Chemical Composition and Biological Activities of Mono- and Heterofloral Bee Pollen of Different Geographical Origins, Int. J. Mol. Sci. 18 (5), 921, (2017).
9. A. CHANTARUDEE, P. PHUWAPRAISIRISAN, K. KIMURA, M. OKUYAMA, H. MORI, A. KIMURA, C. CHANCHAO, Chemical constituents and free radical scavenging activity of corn pollen collected from *Apis mellifera* hives compared to floral corn pollen at Nan, Thailand, BMC Complementary and Alternative Medicine, 12 (45), 1472, 6882, (2012**)**.
10. C. MATEESCU, Apitherapy or how could we use hive products, Fiat Lux Publishing, Bucharest, 2008, pp 5-9.
11. C. NOGUEIRA, A. IGLESIAS, F. XESÚS, L.A.M. ESTEVINHO, Commercial Bee Pollen with Different Geographical Origins: A Comprehensive Approach, International Journal of Molecular Sciences, 13(9), 11173, 11187, (2012).
12. C. OLTICA; L.A. MĂRGHITAS, D.S. DEZMIREAN, Total Polyphenols, Flavonoids and Radical Scavenging Activity of Bee pollen and Beebread Collected from Transylvania Area, Bulletin of University of Agricultural Sciences and Veterinary Medicine, Animal Science and Biotechnologies, 62, 149, 152, (2006).
13. A. PRELIPCEAN, The Dynamics of Total Polyphenols, Flavonoids and Antioxidant Activity of Bee pollen Collected from Moldavia Area, Romania, Agronomical Research in Moldavia, 45 (1), 81, 92, (2012).
14. M. MORAIS, L. MOREIRA, X. FEAS, L.M. ESTEVINHO, Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity, Food and Chemical Toxicology, 49 (5), 1096, 110, (2011).
15. A. PASCOAL, S. RODRIGUES, A. TEIXEIRA, X. FEAS, L.M. ESTEVINHO, Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory, Food and Chemical Toxicology 63, 233, 239, (2014).
16. J.R. GLUŠAC, M.J. STIJEPIC, S.D. MILANOVIC, D.M.D. MILOŠEVIC, Physicochemical properties of honeybee pollen enriched acidophilus milk and probiotic yoghurt, Biblid. 46, 45, 54, (2015).
17. M. SOLGAJOVA, J. NOZKOVA, M. KADAKOVA, Quality of durable cookies enriched with rape bee pollen, Journal of Central European Agriculture, 15 (1), 24, 38, (2014).
18. L. SUN, Y. GUO, Y. ZHANG, Y. ZHUANG, Antioxidant and Anti-tyrosinase Activities of Phenolic Extracts from Rape Bee Pollen and Inhibitory Melanogenesis by cAMP/MITF/TYR Pathway in B16 Mouse Melanoma Cells, Front. Pharmacol., 8:104, (2017).
19. J. SALLES, N. CARDINAULT, V. PATRAC, A. BERRY, C. GIRAUDET, M.L. COLLIN, A. CHANET, C. TAGLIAFERRI, P. DENIS, C. POUYET, Y. BOIRIE, S. WALRAND, Bee Pollen Improves Muscle Protein and Energy Metabolism in Malnourished Old Rats through Interfering with the Mtor Signaling Pathway and Mitochondrial Activity, Nutrients, 6 (12), 5500, 5516 (2014).
20. F.M.E WAGENLEHNER, H. SCHNEIDER, M. LUDWIG, J. SCHNITKER, E. BRAHLER, W. WEINER, A Pollen Extract (Cernilton) in Patients with Inflammatory Chronic Prostatitis–Chronic Pelvic Pain

Syndrome: A Multicentre, Randomised, Prospective, Double-Blind, Placebo-Controlled Phase 3 Study, European Urology, 56, 544, 551, (2009).
21. W.D. KIRK, Recording the colours of pollen loads, Bee World 75 (4), 169, 180, (1994).
22. O.M. BARTH, A.S. FREITAS, E.S. OLIVEIRA, R.A. SILVA, F.M. MAESTER, R.R.S. ANDRELLA, G.M.B.Q. CARDOSO, Evaluation of the botanical origin of commercial dry bee pollen load batches using pollen analysis: a proposal for technical standardization, Annals of the Brazilian Academy of Sciences 82 (4), 893, 902 (2010).
23. A.S. FREITAS, V.A.S. ARRUDA, L.B. ALMEIDA-MURADIAN, O.M. BARTH, The Botanical Profiles of Dried Bee Pollen Loads Collected by *Apis mellifera* (*Linnaeus*) in Brazil, Sociobiology 60 (1), 56, 64, (2013).
24. E. BUCHER, V. KOFLER, G. VORWOHL, E. ZIEGER, Lo spettro pollinico dei mieli dell'Alto Adige, *Agenzia Provinciale per la Protezione Dell'Ambiente e la Tutela del Lavoro*, Bolzano, 17, 37, (2004).
25. G.D'ALBORE RICCIARDELLI, Textbook of Melissopalynology, Apimondia Publishing House, Bucharest, pp 18-33, (1997).
26. O. ZENEBON, N. TIGLEA, S.P. PASCUET, *Métodos Físico-Químicos Para Análise de Alimentos*, Ed. Digital, Brazil, 2008, pp 1018-1020.
27. C.B. FUENMAYOR, C.D. ZULUAGA, C.M. DÍAZ, M.C. QUICAZÁN, M. COSIO, Evaluation of the physicochemical and functional properties of Colombian bee pollen, The Journal MVZ Córdoba, 19 (1), 4003, 4014, (2014).
28. D.C. SOMERVILLE, Lipid content of honey bee-collected pollen from south-east Australia, *Australian Journal of Experimental Agriculture*, 45 (2006) 1659–1661.
29. A.C. ANDRADA, M.C. TELLERÍA, Pollen collected by honey bees (*Apis mellifera L.)* from south of Caldén district (Argentina): botanical origin and protein content, Grana, 44, 115, 122, (2005).
30. N. POPESCU, S. MEICA, Bee products and their chemical analysis, Diacon Coresi Publishing, Bucharest, 1997, pp 57-62.
31. L. BARROS, S.A. HELENO, A.M. CARVALHO, I.C.F.R. FERREIRA, *Lamiaceae* often used in Portuguese folk medicine as a source of powerful antioxidants: Vitamins and phenolics, LWT-Food Sci. Technol., 43 (3), 544, 550, (2010).
32. J. Stimec, S.C.D. Dupree, J.H. McAndrews, Honey Bee, *Apis mellifera*, pollen foraging in southern Ontario, Canadian Field-Naturalist, 111 1 (3), 454, 456, (1997).
33. M.A. Morgano, R.F. Milani, C.T.M. Márcia, D.B. Rodriguez-Amaya, Determination of water content in Brazilian honeybee-collected pollen by Karl Fischer titration, Food Control, 22, 1604, 1608, (2011).
34. R. Mărgăoan, L.A. Mărghitaş, D.S. Dezmirean, O. Bobiş, C.M. Mihai, Physical-Chemical Composition of Fresh Bee Pollen from Transylvania, Bulletin UASVM Animal Science and Biotechnologies, 69 (1-2), 351, 355, (2012).
35. M.G.R. Campos, S. Bogdanov, L.B. Almeida-Muradian, T. Szczesna, Y. Mancebo, Pollen composition and standardisation of analytical methods, Journal of Apicultural Research and Bee World, 47 (2), 156, 163, (2008).
36. M. Martins, M. Morgano, E. Vicente, S. Baggio, D. Rodriguez-Amaya, Physicochemical composition of Bee Pollen from Eleven Brazilian States, Journal of Apicultural Science, 55 (2), 107, 116, (2011).
37. J.F. Odoux, D. Feuillet, P. Aupinel, Y. Loublier, J.N. Tasei, Territorial biodiversity and consequences on physico-chemical characteristics of pollen collected by honey bee colonies, Apidologie, 43 (5), 561, 575, (2012).
38. T.C. SOLANGE, G.B. MOURAO, S.M. ALENCAR, M. L. MASSON, Chemical composition and free radical scavenging activity of *Apis mellifera* bee pollen from Southern Brazil, Brazilian Journal Food Technol., 12 (3), 220, 229, (2009).
39. S.W. NICOLSON, H. HUMAN, Chemical composition of the 'low quality' pollen of sunflower (*Helianthus annuus, Asteraceae*), Apidologie, 44 (2), 144, 152, (2013).
40. E.K.A. TAHA, Chemical Composition and Amounts of Mineral Elements in Honeybee-Collected Pollen in Relation to Botanical Origin, Journal of Apicultural Science, 59 (1), 75, 81, (2015).
41. A. ADMASSU, K. ENSERMU, S. TESHOME, G. PETER, B. LULSEGDE, M.G. CAMPOS, Proximate composition and antioxidant power of bee collected pollen from moist Afromontan forests in southwest Ethiopia, Agricultural Science Research Journal 7 (3), 83, 95, (2017).
42. A. DAOUD, D. MALIKA, S. BAKARI, N. HFAIEDH, K. MNAFGUI, Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars, Arabian Journal of Chemistry, (in press).