International Journal of Secondary Metabolite
2022, Vol. 9, No. 2, 166–177
https://doi.org/10.21448/ijsm.1066884
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Comprehensive Study on BeeBread: Palynological Analysis, Chemical Composition, Antioxidant and Cytotoxic Activities

Gokhan Dervisoglu[1,*], **Duygu Nur Cobanoglu**[2], **Sedat Yelkovan**[3], **Davut Karahan**[3], **Yusuf Cakir**[4], **Serhat Kocyigit**[5]

[1]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Bingol University, Bingol, Türkiye
[2]Department of Crop and Animal Production, Vocational School of Food, Agriculture and Livestock, Beekeeping Program, Bingol University, Bingol, Türkiye
[3]Bee and Natural Products Research and Development Center, Bingol University, Bingol, Türkiye
[4]Department of Food Processing, Vocational School of Food, Agriculture and Livestock, Bingol University, Bingol, Türkiye
[5]Project Coordination Application and Research Center, Bingol University, Bingol, Türkiye

Abstract: Bee bread is a bee product obtained as a result of fermentation of pollen stored by honey bees in the cells of the honeycomb. Palynological analysis, chemical composition, antioxidant activity of bee bread and its cytotoxic effect against human lung carcinoma (A549), human prostate cancer (DU 145) and human neuroblastoma (SH-SY5Y) cell lines were investigated in this study. 25 plant taxa were identified with palynological analysis. Fatty acids, cyclic, aromatic, phenolic, terpenoid, diterpen and metallic complex structures were seen in GC-MS results. FTIR consequence were compatible with GC-MS results and the structure types of FTIR results were seen in the dominant compounds of GC-MS results. Radical scavenging activity (RSA) of bee bread showed inhibition variability between 20.15 ± 0.68% and 93.18 ± 0.44% depending on the concentration. In addition, the EC50 value was measured as 80.08 ± 0.10 mg/mL. Bee bread exhibited moderately cytotoxic effect at all concentrations (15.625 - 2000 µg/mL) against A549, DU 145, and SH-SY5Y cell lines. Bee bread can be used in medical fields because of it's antioxidant and anticancer properties.

ARTICLE HISTORY
Received: Feb 02, 2022
Revised: Mar. 28, 2022
Accepted: May 11, 2022

KEYWORDS
Bee bread,
Palynological analysis,
GC-MS,
FTIR,
Antioxidant,
Anticancer

1. INTRODUCTION

Honey bees collect pollen, nectar and water to meet their nutritional requirements (Gilliam, 1979). Pollen grains have the male reproductive cell of seed plants. It contains most of the nutrients necessary for the development of young worker bees and larvae (Liu *et al.*, 2015). The pollen pellets brought by the worker bees are filled into the honeycomb cells. The stored pollen grains are covered with honey and beeswax, undergoing a chemical change. Thereafter this chemical change, bee pollen turns into the bee bread (Gilliam, 1979). Bee bread is produced by

*CONTACT: Gokhan DERVISOGLU gdervisoglu@bingol.edu.tr, gokhandervisoglu@hotmail.com
Bingol University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Bingol, Türkiye

ISSN-e: 2148-6905 / **© IJSM 2022**

lactic acid fermentation of honey bee secretions, honey and bee pollen (Mărgăoan *et al.*, 2019). Adult honey bees and their larvae are fed with bee bread (Gilliam, 1979), which has beneficial effects on human health. Bee bread is among the functional foods because it contains bioactive compounds like omega fatty acids, essential amino acids, proteins, lipid, vitamin, mineral, and simple sugars (Bobiş *et al.*, 2010; Mărgăoan *et al.*, 2019).

The composition of bee bread varies according to the botanical origin of pollen, but basically, its structure consists of water, lipids, carbohydrates, proteins, vitamins B and C, reproductive hormones, nucleic acids, inorganic elements, acetylcholine, decanoic acid, pantothenic acid, gamma globulin, neopterin, and biopterin etc. (Giroud *et al.*, 2013; Sobral *et al.*, 2017).

Cancer is one of the deadliest diseases in the world, despite many developed methods and therapeutic drugs. However, it is difficult to cure because the immune system is damaged in the treatment of tumors. Consequently, there is a need to unearth new anti-tumor molecules that strengthen the immune system without harming the patient (Yang *et al.*, 2007). Lung cancer is the most occurring type of cancer that is diagnosed in males, and causes deaths. Moreover, it is the fourth most common cancer in women. Lung cancer also ranks second in cancer-related deaths in women. In this type of cancer, 13% (1.6 million) cases were seen in 2008. It has been reported that 18% (1.4 million) of deaths same year are caused by this disease (Jemal *et al.*, 2011). Thereafter lung cancer, prostate cancer is also the second most occurring cancer diagnosed in men. It ranks sixth in causes of death among men (Jemal *et al.*, 2011; Tuzcu *et al.*, 2017). In 2012, it was reported that 8.2 million died from cancer of 14.1 million new cases worldwide. By 2030, it is predicted that the number of new cases and deaths from cancer will increase approximately to double (Ferlay *et al.*, 2015; Tuzcu *et al.*, 2017). Neuroblastoma is also the most common tumor among children under 1 year old. Every year, 700 cases in the USA and Canada and 1,500 cases in Europe are reported. This is approximately twenty-eight percent of all cancers diagnosed in European and US babies (Heck *et al.*, 2009). Anticancer activity of honey and propolis has been extensively studied in some cancer cells (Barbarić *et al.*, 2011; Borges *et al.*, 2011; da Silva Frozza *et al.*, 2013; Markiewicz-Żukowska *et al.*, 2013). There are not many studies on the anticancer effects of bee bread (Sobral *et al.*, 2017).

In this study, palynological analysis, chemical composition, and antioxidant activity of bee bread produced in Bingöl-Türkiye and its cytotoxic effect on some cancer cell lines were investigated. Studies haven't been conducted yet with bee bread on the cancer cell lines evaluated with this study. The aim of our study is to give an idea to the cancer research to be made with bee bread.

2. MATERIAL and METHODS

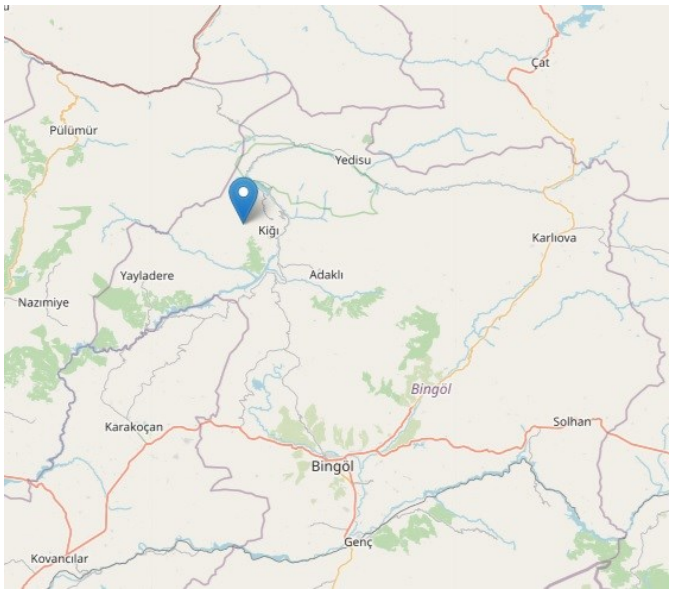
2.1. Bee Bread Sample

Bee bread was procured from beekeepers of the village Ölmez, in Kiğı Region, Bingöl Province, Türkiye (40° 17′ 21.9156"N - 39° 19′ 3.4032"E) in 2020 (Figure 1). The collected bee bread samples were stored at -20 °C until the bee bread extracts were prepared.

2.2. Chemicals and Reagents

Ethanol (~96% v/v) was procured from Alkomed Kimya Ltd. Sti., Türkiye. DMEM/F12 medium, fetal bovine serum (FBS) and penicillin-streptomycin solution were procured from Gibco Life Technologies, Paisley, UK. All other reagents and chemicals were purchased from Merck (Darmstadt, Germany).

Figure 1. A map giving the approximate location of procured bee bread sample.



2.3. Preparation of Bee Bread Extraction

Ethanolic extract of bee bread (EBB) was prepared at the rate of 10% (w/w) by using 96% ethanol (v/v), according to the method given by Markiewicz-Żukowska *et al.* (2013) with small modifications. Briefly, 10 g bee bread was immersed in a 90 g ethanol solution. The mixture was mixed for 24 hours with a magnetic stirrer at 25 °C and then was filtered by using Whatman no: 2 Cellulose Filter Paper (diameter: 125 mm). The filtrate was obtained and then evaporated at 40°C in a rotary evaporator (Rotavapor R-3, Buchi, Switzerland). The obtained residues were then stored in a refrigerator at -20°C for use in experimental analyses.

2.4. Palynological Analysis

Pollen analysis was performed with small modification by using the methods of Luz and Barth (2012). Bee bread pollen slides were prepared and investigated with Leica DM 2500 microscope. Minimum 500 pollen grains were counted on the slide. The pollen frequency percentages of the bee bread sample were indicated by using the methodology of Wróblewska *et al.* (2006). The pollen percentages were considered as <3% was sporadic group, 3-15% was minor group, 16-44% was secondary group, and ≥ 45% was dominant group.

2.5. Fourier-Transform Infrared Spectrometry (FTIR)

The properties of different chemical molecules and their organic bond structures in the EBB sample were determined via FTIR spectrometer. The analysis was carried out according to the study of ERGUN *et al.* (2017). FTIR spectrometer (Perkin-Elmer 100, Perkin-Elmer Inc., Norwalk, CT, USA) equipped with an attenuated total reflectance accessory (ATR; Perkin-Elmer) was used for acquiring spectra from EBB. The EBB sample was placed in the Diamond/ZnSe crystal cell. The sample was scanned with 4 cm^{-1} resolution for 5 scans in the wavenumber of 4000 – 650 cm^{-1}. EBB sample was read three times. For processing, the average spectrum within the sample was used. Spectrum 100 (version 6.3.5, 1999) and Spekwin32 (version 1.71.6.1, 2012) software were used for processing spectra of samples. The EBB data were statistically analyzed and compared utilizing Duncan multiple range test.

2.6. GC-MS Analysis

Chemical composition of EBB was determined with gas chromatography/mass spectrometry (GC-MS) analysis. The method was performed as described in previous published study of ÇAKIR *et al.* (2020).

2.7. Antioxidant Activity

The antioxidant activity of EBB was estimated with the $DPPH^+$ free radical scavenging assay, as described by Hatano *et al.* (1988); Kaya *et al.* (2018).

2.8. Cell Culture

Human prostate cancer DU 145 (ATCC: HTB-81), human neuroblastoma SH-SY5Y (ATCC: CRL-2266) and human lung carcinoma A549 (ATCC: CCL-185) cell lines were used in this study. The related cells were cultured in a Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 medium (DMEM/F12) supplemented with 12% fetal bovine serum and 0.5% penicillin-streptomycin antibiotic solution. The cells were grown in an incubator at 37 °C with 5% CO_2 and subcultured every two or three days.

2.9. Cell Viability Assay

WST-1 cell proliferation and cytotoxicity assay kit (AR1159, Boster, China) was used to analyze the cytotoxic activity of EBB on the A549, DU 145, and SH-SY5Y tumor cell lines. The tumor cells were grown in *T25* flasks. The cells were harvested and then counted using a Thoma hemocytometer. 1×10^4 cells/well in a 100 µL medium were seeded in a 96-well plate. After 24 h, the cells were treated with different concentrations of EBB (15.625–2000 µg/mL). After 48 h, 5 µl of WST-1 reagent was added to each well and approximately 4 h later of incubation, the absorbance was measured at 450 nm using a microplate reader (SpectraMax Plus 384 Molecular Devices, USA).

2.10. Statistical Analysis

All measurements were repeated three times, and GraphPad Prism 5.01 software was applied for statistical analysis. Comparable datasets were assessed and the analyses were conducted by using two-tailed Student's t-test ($p<0.05$ was considered significant).

3. RESULTS and DISCUSSION

Plant source analysis of bee bread is quite limited. As a result of palynological analysis of bee bread, the pollen grains of 25 different taxa belonging to 13 different families were identified (Table 1) and belonged to Asteraceae, Caryophyllaceae, Caprifoliaceae, Convolvulaceae, Eleagnaceae, Fabaceae, Hypericaceae, Lamiaceae, Malvaceae, Onagraceae, Plumbaginaceae, Rosaceae, Scrophulariaceae. Pollen grains of Convolvulus sp. (33%) and Pyrus sp. (24%) were counted secondary. The pollen grains of 4 taxa belonging to Fabaceae, Lamiaceae and Plumbaginaceae family were determined as minor.

The results of the current study showed that Convolvulaceae, Rosaceae, Fabaceae, Asteraceae, Lamiaceae are the most preferred families by the honey bees. Behçet and Yapar (2019) has also reported that the top 5 families, which are Asteraceae, Lamiaceae, Fabaceae, Rosaceae, Apiaceae, are important for beekeeping in Bingöl Matan Mountains. Wróblewska *et al.* (2006) conducted a pollen analysis in 10 bee bread samples from North East Poland. They found the pollens of the genus Anthriscus, Brassicaceae, Centaurea cyanus and Trifolium repens in the highest frequency. In current study, dominant plant taxa were not found, but similar to this study, the frequency of Convolvulus pollen was found in range of 25-50% (Wróblewska *et al.*, 2006). In the Brazilian bee bread samples, 32 pollen types belonging to 27 genus and 22 families were identified. As a result of microscopic analysis, the most common pollen types belonged to Asteraceae, Mimosaceae, Euphorbiaceae, Lythraceae, Moraceae, Poaceae, Rubiaceae, Sapindaceae, and Tiliaceae families (Luz & Barth, 2012). Kaplan *et al.* (2019) conducted a study on five bee bread samples from Asteraceae, Fabaceae and Brassicaceae families at minor and rare levels. Mayda *et al.* (2020) found that Asteraceae, Fabaceae, Plantaginaceae and Rosaceae were common families in bee bread samples from Türkiye.

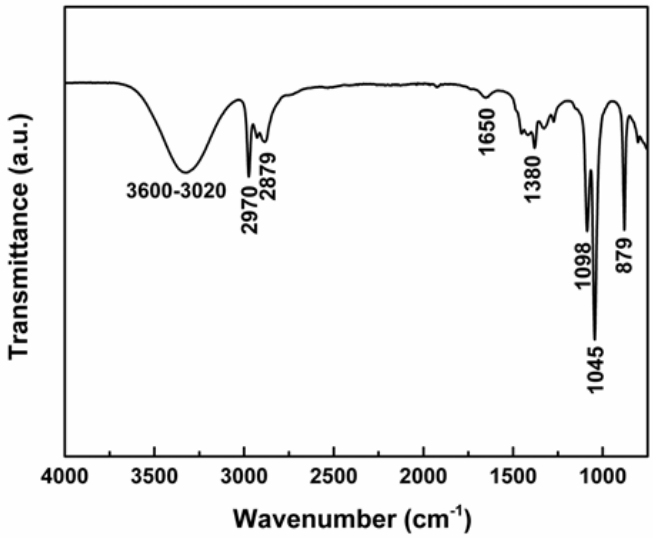
In our study Asteraceae, Fabaceae, Convolvulaceae and Rosaceae families were the most common.

Table 1. Frequency of pollen taxa in bee bread.

%	Pollen taxa
45-100 dominant	none
16-44 secondary	*Convolvulus sp., Pyrus sp.,*
3-15 minor	Lamiaceae, *Astragalus sp., Trifolium sp., Acantholimon Type I.*
<3 sporadic	Asteraceae, *Cichorium sp.,* Caryophyllaceae, Fabaceae, *Astragalus gummifer, Verbascum sp., Hedysarum sp., Lotus sp., Melilotus sp., Trifolium pratense, Eleagnus sp., Lamium sp., Malvaceae, Epilobium sp., Acantholimon TypeII,* Rosaceae, *Fragaria sp., Scabiosa sp., Hypericum sp.,*

The FTIR graph of bee bread extraction is given in Figure 2. According to the FTIR results, the wavenumber had the peak values of 3600-3020 cm^{-1} had the properties of O-H stretching vibration (Kuptsov & Zhizhin, 1998). 2970 cm^{-1} and 2879 cm^{-1} showed the properties of CH_3 and CH_2 asymmetric stretching vibration, respectively (Kostova, 2006). Carboxylate anion (COO-) was seen at the peak value of 1650 cm^{-1} (Kuptsov & Zhizhin, 1998). O-H bending in –COOH or/and CH_3 bending was/were seen at 1380 cm^{-1} (Kostova, 2006). Asymmetric vibration of ester link (C-O-C), C-O stretch in CH_3-COO-R, and predominantly C-C stretch corresponded to the peak value of 1098, 1045, and 879 cm^{-1}, respectively (Kostova, 2006; Kuptsov & Zhizhin, 1998).

Figure 2. FTIR graph as wave number versus transmittance of bee bread extract.



GC-MS graph of the EBB was given in Figure 3 and compound names, properties and quantities obtained from GC-MS results were given in Table 2. Figure 3 and Table 2 showed that compounds of different properties determined in the EBB. According to the Table 2, the compounds identified as Hexadecanoic acid, Hexadecanoic acid ethyl ester, Octadecanoic acid, Octadecanoic acid ethyl ester, 9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z), Methyl-3-(3,5-Ditertbutyl-4-Hydroxyphenyl) Propionate, Cholest-5-En-3-Ol(3Beta), Ruthenium organometallic ($C_{14}H_{21}BO_3RuSeSi$), and Bornyl ester of 3-isopropylidene cyclopentane carboxylic acid were seen over area of 1%. The structure types as fatty acids, cyclic, aromatic, phenolic, terpenoid, diterpen and metallic complex structures were seen in GC-MS results.

Figure 3. GC-MS graph as time versus abundance of bee bread extract.

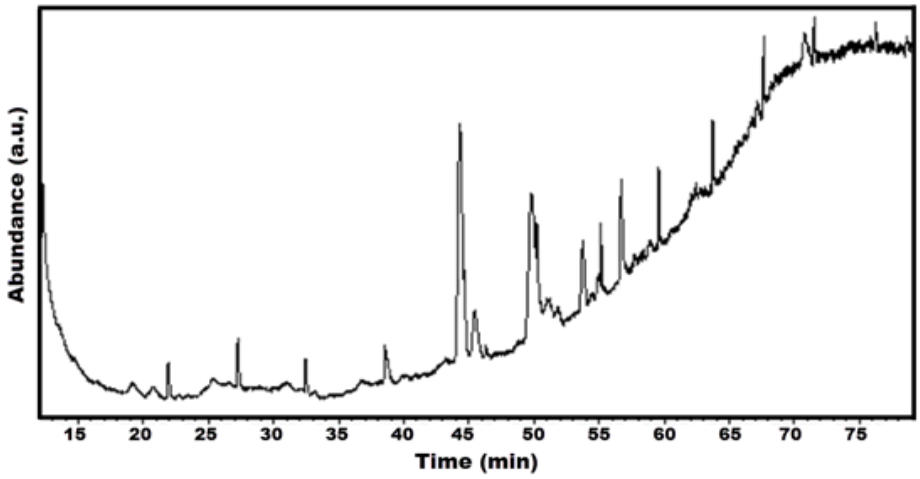


Table 2. The data about compound names, properties and quantities obtained from GC-MS results.

RT	Area%	Library/ID	Structure Type	Ref#	CAS#	Qual
44.290	9.13	Palmitic acid (Hexadecanoic acid)	Fatty acid	12	000112-39-0	99
45.469	2.19	Hexadecanoic acid, ethyl ester	Fatty acid	696	000628-97-7	89
49.777	6.30	Stearic acid (Octadecanoic acid)	Fatty acid	16	000112-61-8	97
51.168	1.75	Octadecanoic acid, ethyl ester	Fatty acid	151552	000111-61-5	94
51.803	0.65	Methyl linoleate	Fatty acid	133505	000112-63-0	90
53.743	1.87	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	Fatty acid	22	000301-00-8	96
54.944	0.50	4-Pentyl-1-(4-Propylcyclohexyl)-1-Cyclohexene	Cyclic	133389	108067-17-0	92
55.139	0.75	Benzo[G]Quinoline-2-Carboxylic-Acid	Aromatic	522489	071295-05-1	80
56.684	2.21	Methyl-3-(3,5-Ditertbutyl-4-Hydroxyphenyl)-Propionate	Phenolic	469837	000000-00-0	95
62.692	0.95	4-(4-Ethylcyclohexyl)-1-Pentyl-1-Cyclohexene	Cyclic	59858	301643-32-3	90
63.121	0.63	1,5-Dimethyl-2-Vinylcyclohexanecarboxylic-Acid	Cyclic	286470	106542-17-0	86
64.203	0.20	1-Allyl-3-Methylindole-2-Carbaldehyde	Terpenoid	55113	000000-00-0	90
65.456	1.52	Cholest-5-En-3-Ol(3.Beta.) /Cholesterol	Terpenoid	57079	000057-88-5	90
70.411	0.58	1,5-Dimethyl-2-Vinylcyclohexanecarboxylic-Acid	Cyclic	286470	106542-17-0	83
71.504	3.12	Ruthenium organometallic ($C_{14}H_{21}BO_3RuSeSi$)	Metallic Complex	2028	118772-38-6	90
73.552	3.03	Ruthenium organometallic ($C_{14}H_{21}BO_3RuSeSi$)	Metallic Complex	2028	118772-38-6	92
73.650	0.32	(7R,14S,1E,3E,8E,11E)-Cembra-1,3,8,11-Tetraene-7,14-Diol	Diterpen	287197	000000-00-0	83
75.023	1.21	Bornyl-Ester-Of-3-Isopropylidene-Cyclopentanecarboxylic-Acid	Cyclic	287113	000000-00-0	86

FTIR results showed properties of O-H stretching, CH_3 and CH_2 stretching vibration, carboxylate anion peak, O-H bending in –COOH, CH_3 bending, ester link (C-O-C), C-O stretch in CH_3-COO-R, and predominantly C-C stretching according to literature data. C-C, CH_2 and CH_3 structure types, which have the longest peak value, were generally seen in all of the compounds. Especially, -COOH structure types were seen in fatty acids and carboxylic acid compounds. C-O-C and CH3-COO-R structure types were also seen in the compounds with ester; the compounds of bornyl ester of 3-isopropylidene cyclopentane carboxylic acid, Methyl-3-(3,5-Ditertbutyl-4-Hydroxyphenyl) Propionate and (7R, 14S, 1E, 3E, 8E,1 1E)-Cembra-1,3,8,11-Tetraene-7,14-Diol. As it was seen in Figure 2, FTIR results were compatible with GC-MS results and the structure types of FTIR results were seen in the dominant compounds of GC-MS results. The GC-MS results showed EBB had different properties such as antibacterial, antioxidant, anticancer activities etc., when the structures were based on the GC-MS results. In the literature data, complex structures containing ruthenium exhibited anticancer activity due to rapid ligand exchange, biological stability, and different and high oxidation states thanks to ruthenium (Reedijk, 2008; Syamdidi & Irianto, 2016). Cholest-5-En-3-Ol (3Beta) compound had antioxidant activity (Greenland & Bowden, 1994; Khan *et al.*, 2019). (7R, 14S, 1E, 3E, 8E,1 1E)-Cembra-1,3,8,11-Tetraene-7,14-Diol molecule named as cytotoxic diterpene was detected showing antitumor effect (Chandrasekaran *et al.*, 2008). Fatty acids, their methyl and ethyl ester compounds showed antibacterial, antioxidant and anticancer activities (Akbari *et al.*, 2019; Ghosh & Indra, 2014; Pinto *et al.*, 2017). It was seen that the structures with bornyl ester, cyclopentanecarboxylic acid and cyclohexanecarboxylic acid showed anti-imflammatory and antimicrobial activities (Bakour *et al.*, 2019; Etehadpour & Tavassolian, 2019; Soumya *et al.*, 2014). Bee bread can be used as a healthy food and a supplement for medicine (Magalhães *et al.*, 2008; Mărgăoan *et al.*, 2019). Bee bread has a unique chemical composition providing antioxidant, antimicrobial and cytotoxic effects (Magalhães *et al.*, 2008; Mărgăoan *et al.*, 2019; Sobral *et al.*, 2017).

There are several methods to evaluate the antioxidant activity of bee bread in the literature (Sreeramulu *et al.*, 2013). DPPH free radical-scavenging activity was chosen for this study to evaluate the antioxidant property at different concentrations of EBB. DPPH radical scavenging activities of bee bread and its EC50 values are given in Table 3. The scavenging activity (RSA) of EBB showed inhibition variability between 20.15 ± 0.68% and 93.18 ± 0.44% depending on the concentration. The 200 mg/mL sample presented the best RSA, while sample 25 mg/mL showed the worst performance. In addition, the EC50 value of bee bread sample was measured as 80.08 ± 0.10 mg/mL.

Table 3. DPPH radical scavenging activities (RSA) and EC50 value of bee bread extracts at different concentrations.

EBB	Concentrations				EC50 (mg/mL)
	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL	
RSA(%)	20.15 ± 0.68	48.20 ± 0.44	76.93 ± 0.34	93.18 ± 0.44	80.08 ± 0.10

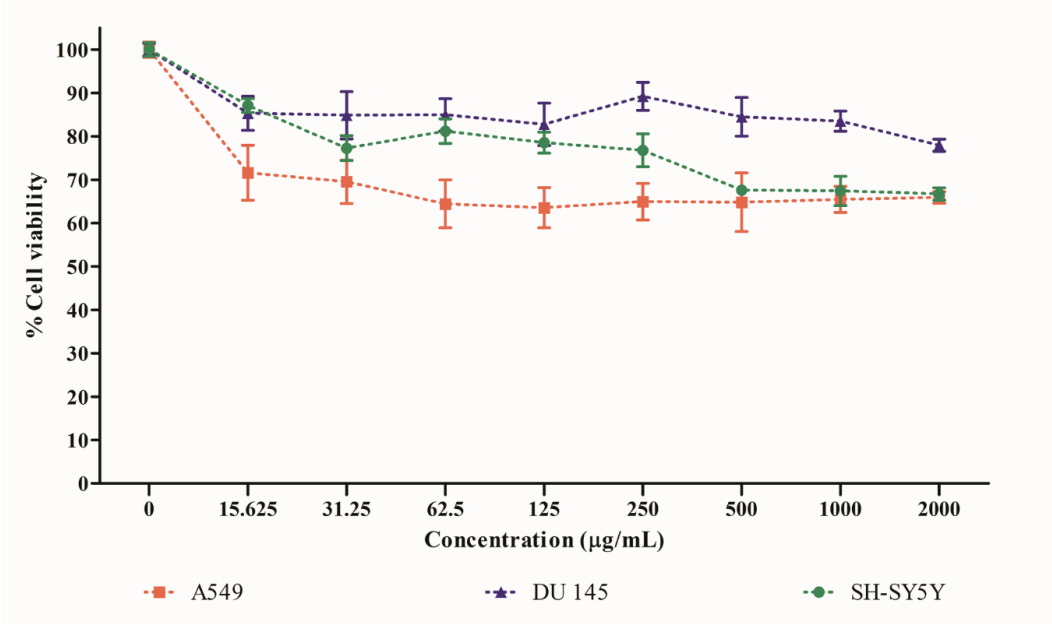
All data were expressed as mean ± SD of triplicate.

All values ranked it among the powerful antioxidant foods (Li *et al.*, 2014). This high antioxidant property of EBB can be probably due to the functional compounds in its content such as phenolic compound (Methyl-3-(3,5-Ditertbutyl-4-Hydroxyphenyl)-Propionate), aromatic group (Benzo[G]Quinoline-2-Carboxylic-Acid), and terpenoid (Cholest-5-En-3-Ol(3Beta)) (Borawska *et al.*, 2014; Greenland & Bowden, 1994; Khan *et al.*, 2019).

Bee bread has also cytotoxic activity on tumor cells besides antioxidant effects but there are a few researches on cytotoxic effect on tumor cells of bee bread in the literature. In this study,

we examined the cytotoxic effects of EBB in concentrations of 15.625-2000 µg/mL on human lung adenocarcinoma (A549), human prostate cancer (DU 145), and human neuroblastoma (SH-SY5Y) cell lines and we found that EBB exhibited moderately cytotoxic effect on all of 3 tumor cell lines (A549, DU 145, and SH-SY5H) at all concentrations with 24h incubation and decreased cell viabilities to 63.55, 77.94, and 66.71% respectively. Figure 4 indicates the results of cell viability expressed as a percentage of the control after a 24-hour incubation using an EBB concentration range of 15.625 - 2000 µg/mL.

Figure 4. Viabilities of A549, DU 145, and SH-SY5Y cancer cells (% of the control) after incubation with bee bread extract. Values are presented as mean ± SEM and are statistically significant at $p<0.05$.



EBB exhibited moderate cytotoxic activity at all concentrations against A549, DU 145 and SH-SY5Y cancer cells. It showed the maximum cytotoxic effect at 125 µg/mL concentration with 63.55 ± 8.03% viability on A549 cell line, at 2000 µg/mL concentration with 77.94 ± 2.41% viability on DU 145 cell line, and at 2000 µg/mL concentration with 66.71 ± 2.48% viability on SH-SY5Y cell line. These values were statistically significant compared to the control. Markiewicz-Żukowska *et al.* (2013) studied cytotoxic effect of 3 different ethanolic EBBs in concentrations of 10–100 µg/mL on U87MG cell lines and they reported that, after incubation of 24 h, while one of the 3 different EBBs showed a moderately cytotoxic effect, the other two did not show any cytotoxic effect. Borawska *et al.* (2014) examined the effects of EBB in concentration of 50 µg/mL on astrocytoma (DASC), human glioblastoma multiforme (U87MG), and normal human astroglia (SVGp12) cell lines. They demonstrated that, in a 24 h incubation, while EBB moderately inhibits the growth of U87MG and SVGp12 cells, but not DASC. Sobral *et al.* (2017) studied the effects of bee bread against some human tumor cells. Bee bread extracts collected from northeastern Portugal were tested on breast adenocarcinoma, hepatocellular carcinoma, cervical carcinoma and non-tumor liver cells, and also against non-small cell lung cancer. The extracted bee bread showed normal levels of antitumor activity; but, extractions did not cause toxicity in normal cells (Sobral *et al.*, 2017). The other studies conducted on different cell lines confirmed the antitumor activity of bee bread.

Based on all of this data, it can be said that the EBB had moderately cytotoxic effects on cancer cells, and this data matched up with our cell viability test. Cytotoxic property of EBB can be probably due to the presence of another functional compound in its content such as Ruthenium organometallic ($C_{14}H_{21}BO_3RuSeSi$) groups, (7R,14S,1E,3E,8E,11E)-Cembra-

1,3,8,11-Tetraene-7,14-Diol molecule named as cytotoxic diterpene, fatty acids, fatty acid methyl esters, and fatty acid ethyl ester compounds (Akbari *et al.*, 2019; Chandrasekaran *et al.*, 2008; Ghosh & Indra, 2014; Pinto *et al.*, 2017; Reedijk, 2008; Syamdidi & Irianto, 2016). In addition, more studies are needed to clarify the anticancer mechanisms of bee bread.

4. CONCLUSION

The composition of bee bread varies depending on the botanical origin. There are endemic plants in Bingöl-Türkiye. Bee bread is less studied than pollen and the existence of a region-specific flora reveals the importance of the studies in this region. Bee bread is anti-cancer, protects the nervous system and is effective against viruses. Cancer has many treatment methods; despite therapeutic and diagnostic drugs, it is one of the deadliest diseases in the world. The main reason for this is the damage of the immune system in the treatment of tumors. Therefore, there is a need to reveal new anti-tumor molecules that strengthen the immune system without harming the person. The ingredients in bee bread support the immune system. Also, bee bread has cytotoxic activity on tumor cells beside of antioxidant and antimicrobial effects but there are a few researches on cytotoxic effect on tumor cells of bee bread in the literature. In our study EBB exhibited moderate cytotoxic activity at all concentrations against A549, DU 145, and SH-SY5Y cancer cells. We think that our study will give an idea about cancer research and drug development studies.

Acknowledgments

The authors are thankful to beekeepers who helped with the supply of bee bread, from the village of Ölmez, Kiğı Region, Bingöl Province, Türkiye.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship contribution statement

Gokhan Dervisoglu: Performed the experimental part and statistical analysis, wrote and edited the original draft. **Duygu Nur Cobanoglu** and **Serhat Kocyigit**: did the experimental part, helped write the draft. **Sedat Yelkovan** and **Davut Karahan**: helped to carry out the experimental part and to write the draft. **Yusuf Cakir**: helped carry out the experimental part.

Orcid

Gokhan Dervisoglu https://orcid.org/0000-0001-7195-2031
Duygu Nur Cobanoglu https://orcid.org/0000-0002-8583-8114
Sedat Yelkovan https://orcid.org/0000-0002-7209-6350
Davut Karahan https://orcid.org/0000-0003-4571-1095
Yusuf Cakir https://orcid.org/0000-0002-3789-3039
Serhat Kocyigit https://orcid.org/0000-0003-0172-6180

REFERENCES

Akbari, S., Abdurahman, N.H., Yunus, R.M., Alara, O.R., & Abayomi, O.O. (2019). Extraction, characterization and antioxidant activity of fenugreek (Trigonella-foenum graecum) seed oil. *Materials Science for Energy Technologies*, *2*(2), 349-355.

Bakour, M., Fernandes, Â., Barros, L., Sokovic, M., & Ferreira, I.C. (2019). Bee bread as a functional product: Chemical composition and bioactive properties. *LWT*, *109*, 276-282.

Barbarić, M., Mišković, K., Bojić, M., Lončar, M.B., Smolčić-Bubalo, A., Debeljak, Ž., & Medić-Šarić, M. (2011). Chemical composition of the ethanolic propolis extracts and its effect on HeLa cells. *Journal of Ethnopharmacology*, *135*(3), 772-778.

Behçet, L., & Yapar, Y. (2019). Important plants at the Matan Mountain (Bingöl/Turkey) flora with regard to beekeeping. *Biological Diversity and Conservation*, *12*(1), 149-159.

Bobiş, O., Mărghitaş, L.A., Dezmirean, D., Morar, O., Bonta, V., & Chirilă, F. (2010). Quality parameters and nutritional value of different commercial bee products. *Bulletin UASVM Animal Science and Biotechnologies*, *67*, 1-2.

Borawska, M.H., Markiewicz-Żukowska, R., Naliwajko, S.K., Moskwa, J., Bartosiuk, E., Socha, K., . . . Mariak, Z. (2014). The interaction of bee products with temozolomide in human diffuse astrocytoma, glioblastoma multiforme and astroglia cell lines. *Nutrition and cancer*, *66*(7), 1247-1256.

Borges, K.S., Brassesco, M.S., Scrideli, C.A., Soares, A.E.E., & Tone, L.G. (2011). Antiproliferative effects of Tubi-bee propolis in glioblastoma cell lines. *Genetics and Molecular Biology*, *34*, 310-314.

Chandrasekaran, M., Kannathasan, K., & Venkatesalu, V. (2008). Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae. *Zeitschrift für Naturforschung C*, *63*(5-6), 331-336.

Çakır, Y., Çobanoğlu, D.N., Dervişoğlu, G., Koçyiğit, S., Karahan, D., & Yelkovan, S. (2020). Determination Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey. *Mellifera*, *20*(1), 41-60.

da Silva Frozza, C.O., Garcia, C.S.C., Gambato, G., de Souza, M.D.O., Salvador, M., Moura, S., . . . Borsuk, S. (2013). Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. *Food and Chemical Toxicology*, *52*, 137-142.

Ergun, M., Süslüoğlu, Z., & Bengü, A.Ş. (2017). Studying Structural Differentiation of Plant Parts of Sideritis pisidica Boiss. & Heldr. Using FTIR Spectroscopy. *Türk Tarım ve Doğa Bilimleri Dergisi*, *4*(4), 461-467.

Etehadpour, M., & Tavassolian, I. (2019). Ecological factors regulate essential oil yield, percent and compositions of endemic yarrow (Achillea eriophora DC.) in Southeast Iran. *International Journal of Horticultural Science and Technology*, *6*(2), 201-215.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., . . . Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer*, *136*(5), E359-E386.

Ghosh, K., & Indra, N. (2014). Phytochemistry, in vitro free radical scavenging, chelating and toxicity of Centela asiatica L.(Apiaceae) ethanolic leaf extract. *International Journal of Pharmaceutical Sciences Review and Research*, *29*, 328-334.

Gilliam, M. (1979). Microbiology of pollen and bee bread: the yeasts. *Apidologie*, *10*(1), 43-53.

Giroud, B., Vauchez, A., Vulliet, E., Wiest, L., & Buleté, A. (2013). Trace level determination of pyrethroid and neonicotinoid insecticides in beebread using acetonitrile-based extraction followed by analysis with ultra-high-performance liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1316*, 53-61.

Greenland, G.J., & Bowden, B.F. (1994). Cembranoid diterpenes related to sarcophytol A from the soft coral Sarcophyton trocheliophorum (Alcyonacea). *Australian Journal of Chemistry*, *47*(11), 2013-2021.

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chemical and pharmaceutical bulletin*, *36*(6), 2090-2097.

Heck, J.E., Ritz, B., Hung, R.J., Hashibe, M., & Boffetta, P. (2009). The epidemiology of neuroblastoma: a review. *Paediatric and perinatal epidemiology*, *23*(2), 125-143.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: a cancer journal for clinicians*, *61*(2), 69-90.

Kaplan, M., Karaoğlu, Ö., & Silici, S. (2019). An Evaluation on Bee Bread: Chemical and Palynological Analysis. *Mellifera*, *19*(1), 21-29.

Kaya, B., Darendelioğlu, E., Dervişoğlu, G., & Tartik, M. (2018). Determination of comparative biological activities of silver nanoparticles formed by biological synthesis using Achillea Vermicularis. *Pak. J. Bot*, *50*(4), 1423-1432.

Khan, F., Abdullah Asuhaimi, F., Jalal, T.K., Roheem, F.O., Natto, H.A., Johan, M.F., . . . Abdul Wahab, R. (2019). Hystrix Brachyura Bezoar Characterization, Antioxidant Activity Screening, and Anticancer Activity on Melanoma Cells (A375): A Preliminary Study. *Antioxidants*, *8*(2), 39.

Kostova, I. (2006). Ruthenium complexes as anticancer agents. *Current medicinal chemistry*, *13*(9), 1085-1107.

Kuptsov, A., & Zhizhin, G.N. (1998). *Handbook of Fourier transform Raman and infrared spectra of polymers*. Elsevier.

Li, X., Wang, Z.G., Chen, H.H., & Liu, S.G. (2014). The antioxidant methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate. *Acta Crystallogr C Struct Chem*, *70*(Pt 11), 1050-1053. https://doi.org/10.1107/s2053229614021445

Liu, X.D., Zhang, F.B., Zhou, B., Shan, H., & Chen, P.Y. (2015). Effect of sonication on different quality parameters of Pinus massoniana pollen. *Ultrasonics sonochemistry*, *22*, 174-181.

Luz, C.F.P.D., & Barth, O.M. (2012). Pollen analysis of honey and beebread derived from Brazilian mangroves. *Brazilian Journal of Botany*, *35*(1), 79-85.

Magalhães, L.M., Segundo, M.A., Reis, S., & Lima, J.L. (2008). Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica chimica acta*, *613*(1), 1-19.

Mărgăoan, R., Stranț, M., Varadi, A., Topal, E., Yücel, B., Cornea-Cipcigan, M., . . . Vodnar, D.C. (2019). Bee collected pollen and bee bread: Bioactive constituents and health benefits. *Antioxidants*, *8*(12), 568.

Markiewicz-Żukowska, R., Naliwajko, S.K., Bartosiuk, E., Moskwa, J., Isidorov, V., Soroczyńska, J., & Borawska, M.H. (2013). Chemical composition and antioxidant activity of beebread, and its influence on the glioblastoma cell line (U87MG). *Journal of Apicultural Science*, *57*(2), 147-157. https://doi.org/doi:10.2478/jas-2013-0025

Mayda, N., Özkök, A., Bayram, N.E., Gerçek, Y.C., & Sorkun, K. (2020). Bee bread and bee pollen of different plant sources: Determination of phenolic content, antioxidant activity, fatty acid and element profiles. *Journal of Food Measurement and Characterization*, *14*(4), 1795-1809.

Pinto, M.E., Araujo, S.G., Morais, M.I., Sá, N.P., Lima, C.M., Rosa, C.A., . . . Lima, L.A. (2017). Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. *Anais da Academia Brasileira de Ciências*, *89*, 1671-1681.

Reedijk, B.J. (2008). Metal-ligand exchange kinetics in platinum and ruthenium complexes. *Platinum Metals Review*, *52*(1), 2-11.

Sobral, F., Calhelha, R.C., Barros, L., Dueñas, M., Tomás, A., Santos-Buelga, C., . . . Ferreira, I.C. (2017). Flavonoid composition and antitumor activity of bee bread collected in northeast Portugal. *Molecules*, *22*(2), 248.

Soumya, V., Muzib, Y.I., Venkatesh, P., & Hariprasath, K. (2014). GC-MS analysis of Cocus nucifera flower extract and its effects on heterogeneous symptoms of polycystic ovarian disease in female Wistar rats. *Chinese journal of natural medicines*, *12*(9), 677-684.

Sreeramulu, D., Reddy, C., Chauhan, A., Balakrishna, N., & Raghunath, M. (2013). Natural antioxidant activity of commonly consumed plant foods in India: effect of domestic processing. *Oxidative Medicine and Cellular Longevity*, *2013*.

Syamdidi, H.E.I., & Irianto, G. (2016). Agar-abundant Marine Carbohydrate from Seaweeds in Indonesia: Production, Bioactivity, and Utilization. In *Marine Glycobiology* (pp. 275-282). CRC Press.

Tuzcu, Z., Koclar, G., Agca, C.A., Aykutoglu, G., Dervisoglu, G., Tartik, M., . . . Sahin, K. (2017). Antioxidant, antimicrobial and anticancer effects of different extracts from wild edible plant Eremurus spectabilis leaves and roots. *International journal of clinical and experimental medicine*, *10*(3), 4787-4797.

Wróblewska, A., Warakomska, Z., & Koter, M. (2006). Pollen analysis of bee products from the north-eastern Poland. *Journal of Apicultural Science*, *50*(1), 71-83.

Yang, X., Guo, D., Zhang, J., & Wu, M. (2007). Characterization and anti-tumor activity of pollen polysaccharide. *International immunopharmacology*, *7*(3), 401-408.