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RESEARCH PAPER

The moisture determination of bee pollen from Sivas Province in Anatolia and their antiproliferative activities in MCF-7 cell line

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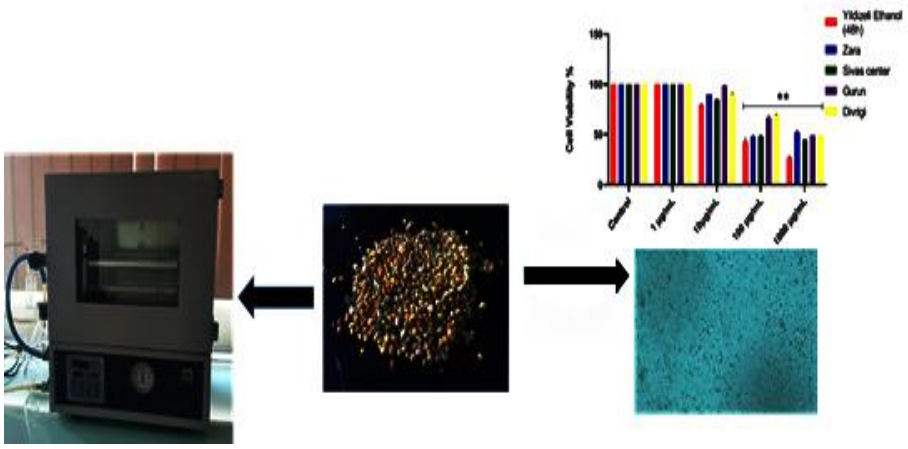
Highlights

- Pollen samples of Sivas province, which is one of the most important cities in the field of beekeeping in the center of Anatolia, were evaluated and compared.
- Moisture contents of pollen samples were evaluated.
- *In vitro* cytotoxic activities were evaluated in MCF-7 cell line by ethanol and water extraction of pollen samples.
- It was found that pollen samples showed moderate antiproliferative effect and their moisture content was within normal limits.

Graphical Abstract



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Abstract

Pollen, one of the bee products, is an important nutrient for bees. Pollen is mainly used for feeding adult, old and larval bees to meet their needs for protein, vitamins and minerals. Since the protein content of pollen meets the basic protein needs of bees, it should be brought into the hive in sufficient quantity and stored under suitable conditions. Likewise, bee pollen has nutritional and therapeutic properties for human health. Numerous studies have been conducted in different countries to evaluate the physicochemical and biological properties of bee pollen, which is widely used in apitherapy, pharmaceutical industry, food industry and cosmetic industry. The physical properties such as color, odor, and pH of pollen extracted from beehives may vary depending on the conditions, climate, and flower structure of each region. In addition, the pollen's constituents such as proteins, sugars, carbohydrates, fats, vitamins and minerals can change depending on the conditions in each region. In short, there are differences in the various physicochemical compositions of pollen. Moreover, it shows that the biological activity and functional effects of bee pollen in vitro and in vivo may vary according to the differences in chemical constituents. In this study, some properties of bee pollen were evaluated in Sivas province, central Anatolia, accompanied by scientific evidence showing the effectiveness of these traditional practices. The determination of moisture content of pollen samples from different places and their antiproliferative effect on MCF -7 cell line were compared. In general, it was found that the ethanol extracts of all pollen samples had cytotoxic activities on the MCF -7 cell line, while the moisture ratios were within standard ranges.

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Introduction

Apitherapy is the use of bee and bee products as a means of protecting health and supporting the treatment of some diseases (1, 2). Traditional and complementary medicine applications are very promising, especially in terms of preventive medicine, due to the positive effects of bee products on the immune system in general. With Anatolia in the top three places in the world in terms of quality beekeeping activities, it is very important to determine its potential in the field of Apitherapy (3, 4).

In addition to the value given to honey as a bee product in Anatolian ethnopharmacology, many scientific studies are carried out on bee products by many scientists today (5-7). In recent years, scientists have contributed to the development of evidence-based Apitherapy with their efforts to develop new innovative products and scientific studies by using bee products (8). When look at the traditional medicine practices of different cultures in history, it is seen that apitherapy practices date back to ancient times. Today, Apitherapy, one of the oldest known treatment methods with a history of 7 thousand years, is being researched with modern scientific methods with its new products and applications. It is beneficial for all humanity to prove these applications, which are mostly based on experience in the past, with current scientific methods (8-10).

In the cultures of China, Egypt and Mesopotamia, bee pollen has been described as a "miracle remedy for rejuvenation and treatment". In the documents of ancient Roman and Greek civilizations, bee pollen is defined as "life-giving dust". It is recorded that Hippocrates, who is accepted as the founder of modern medicine, used bee pollen for treatment purposes (11). In terms of content, bee pollen consists of a large number of biologically active components such as carbohydrates, protein compounds, various vitamins, 28 different minerals such as iron, calcium, magnesium, sodium, potassium, phosphorus, sulfur, some essential fatty acids, phenolic, flavonoid, anthocyanin compounds (11-13).

It is thought that bee pollen provides protection against damage caused by free oxygen radicals due to its rich phenolic content, and this antioxidant effect is the basis of various biological effects claimed for bee pollen (14). Bee pollen demonstrated that it has some important biological activities like antioxidant, anti-inflammatory, anti-allergic, antimicrobial, neurotonic, antidiabetic, aphrodisiac, antiulcer, antihypertensive, antihyperlipidemia, antihepatotoxic, antimutagenic, wound healing, cell repair enhancer, immune booster, slowing down the aging effects, tissue-repairing inflamed tissues in various animal experimental researches (11, 15-19). In this study, it was aimed to determine the humidity of bee pollen collected from Sivas region, where beekeeping is common in Anatolia, and to evaluate the in vitro antiproliferative activity of water and ethyl alcohol extracts of these pollens on the MCF-7 cell line.

Material and Method

Collection of bee pollen samples

In the province of Sivas, bee pollen samples were taken from beekeepers engaged in beekeeping in the 2021 harvest period in Yıldızeli, Zara, Gürün, Divriği, and Merkez districts. The samples were taken to the appropriate storage environment and brought to the laboratory for analysis.

Determination of moisture

Moisture determination processes were carried out in homogeneously mixed pollen samples in accordance with the Turkish Standards Institute 6318 (TS 6318) protocol. The closed weighting container containing the test sample was placed in the vacuum oven and the lid was removed and left in the oven. The test samples were kept in an oven at 80°C for 4 hours at a pressure of less than 13.5 kPa (135 mbar). During drying, a slow stream of air was introduced through the oven drying device. The vacuum pump was turned off after 4 hours and the air passed through the drying system was allowed to enter the oven slowly until atmospheric pressure was achieved. The weigh bucket lid was closed before it was removed from the oven. The closed weighing cup was placed in the desiccator. It was cooled to room temperature and weighed with an accuracy of 0.0002 g.

Extraction

Each pollen sample was weighed and taken into a flask, and ethyl alcohol and water solvents were added according to the ratio of 1/10 (w/v) and extracted separately by maceration method at room temperature. With occasional shaking, maceration was ensured. After 24 hours, the macerates were filtered and the ethanol extract was recovered from its solvents using a vacuum at low temperature (40°C) in a rotary evaporator, and the % yield was calculated. The water extracts were placed in the lyophilizer in order to remove the water completely. After a period of four days, the remaining extract in the lyophilizer was taken into a dark-colored glass container and the % yield was calculated. All extracts were stored in a refrigerator at -20°C until used in experimental studies.

Antiproliferative activity assay

The ethanol and water extracts of pollen samples were administered to MCF-7 cell line and their cytotoxic activity was analyzed using the MTT method (20, 21). The cell lines were grown in DMEM containing 1% penicillin-streptomycin and 10% FBS. The cell line was incubated at 37 °C, 95% moisture and 5% CO_2. Cells ($1x10^5$/well) were then sowed into 96 well plates after 24 hours of growth and extracts were applied to the cells at different doses ranging from 1-1000 µg/mL. Cells were incubated with these drugs for 48 hours. After incubation of cells, 10 µL of MTT was added to each well. After the addition of MTT, incubation was done at 37 °C for 3 hours. MTT was aspirated from the cells. Then, 100 µL of dimethyl sulfoxide (DMSO) was attached to each well and incubated for 15 minutes. In order to determine the cytotoxic activity of the cells, absorbance values were read with a microplate reader at 570 nm. Data were analyzed using the GraphPad Prism method. 100 µg/mL extracts were added to the cells. The morphology of the cells after the applied dose was examined on the device at 20X magnification (ZEISS Axio Vert.A1).

Statistical analysis

Data were analyzed at 95% confidence level and a p value less than 0.05 was considered significant. All the experiments were repeated as triplicate. The statistical significance levels of the doses are given on the *$p<0.05$, ** $p<0.01$).

Results

The shape and color of pollen varies depending on the plant variety. Even different plant species belonging to the same family can have different pollen shape and color from each other. The diameter of the pollen pellets collected by the bee varies between 1 mm and 4 mm (13). The foreign matter content should be at most 2.5% by mass. Although the chemical structure of the pollen varies according to the plant species, the moisture content of the dried pollen should not be more than 10-12% by mass. The moisture content of the pollen varies according to the pollen type (13, 22). Moisture content also correlates with atmospheric conditions when pollen is collected (13, 23). The desired drying condition for pollen prepared for marketing is a moisture content of 2.5-7%. The water content of the pollen determines the shelf life as well as the microbiological and organoleptic quality and causes changes in the biological activity values of the pollen (13, 24-26). High water capacity in pollen increases the number of microorganisms. In this case, with the increase of microorganisms, it is observed that pollen spoils, changes in taste and smell. At the same time, with the increasing microbial load, the accumulation of unwanted toxin compounds in the pollen chemical composition and the breakdown of macromolecules such as oil and protein may ocur with increased enzyme activity.

In this study, the moisture content of pollen samples collected from different localities in Sivas province were investigated and it was determined that they were within the value ranges specified in the TSE TS 10255 communiqué (Table 1). The highest moisture content was obtained from the pollen taken from the Gurun locality of Sivas province with a value of 11.85%. The lowest moisture content was observed in the pollen samples collected from Sivas central localities with a value of 6.43%.

Table 1. Moisture contents of pollen samples collected from different localities in Sivas.

	Yildızeli	Zara	Gurun	Divriği	Sivas central localities
Total moisture (%)	7.86	11.12	11.85	7.26	6.43

The yields of pollen extracts prepared with different solvents were found as 16.6% in ethyl alcohol extract and 5.05% in water extract. It was observed that both ethyl alcohol, and water isolates of pollen samples showed moderate antiproliferative activity in MCF-7 cell line (IC50 < 100 µg/mL). It was determined that ethyl alcohol isolates of the pollen samples showed IC50 values in the range of 35.0-176.8 µg/mL and water isolates in the range of 94.6 -286.4 µg/mL. It was determined that ethyl alcohol isolates of pollen samples generally showed higher activity than water isolates (Table 2, Figures 1 and 2). In some studies in the literature, it has been shown that ethanol extracts of pollen obtained from different bee breeds can reduce the cell viability value up to 20% on the MCF-7 cell line (27). In another study conducted in Indonesia, water extracts of bee pollen and propolis samples were morphologically shown to have an in vitro cytotoxic effect on the MCF-7 cell line (28). In another study conducted with Malaysian pollens in 2016, the IC50 values in the MCF-7 cell line were found to be 15 mg/mL, while it was determined that the IC50 values decreased by 50-80% depending on the dose when the pollen was used together with cisplatin chemotherapeutic drug (29).

Table 2. IC50 levels in MCF-7 cell line of bee pollen extracts from Sivas.

	IC50 (µg/mL) against MCF-7 cell line ±SD				
	Yildizeli	**Zara**	**Gurun**	**Divrigi**	**Sivas central localities**
Ethanolic ekstract	35.0±5.2	88.9±8.9	176.8±12.5	135.6±15.9	55.5±7.1
Water extract	94.6±10.3	119.9±10.2	141.6±12.9	286.4±20.4	98.5±7.6

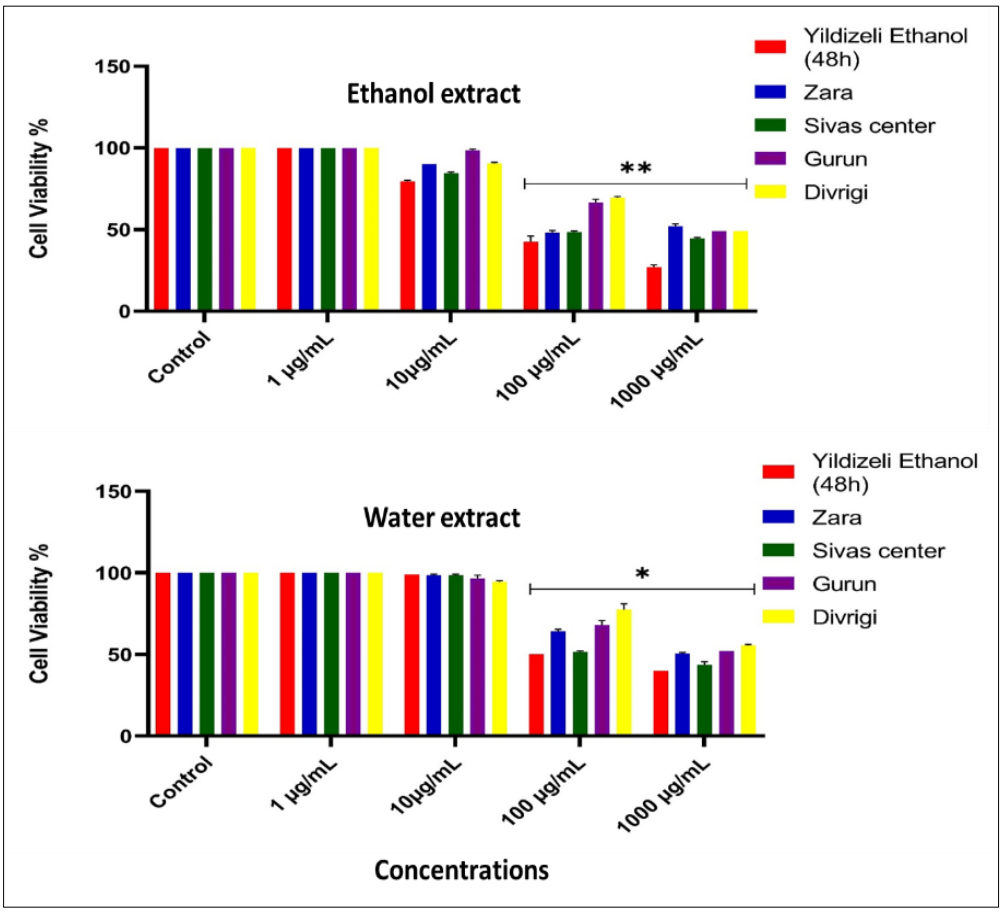

Figure 1. Determination of the cytotoxic activity of pollen extracts in MCF-7 cell line. Activities of pollen samples after 48 hour incubation at concentrations ranging from 1-1000 µg/mL. (*p<0.05, ** p<0.01).

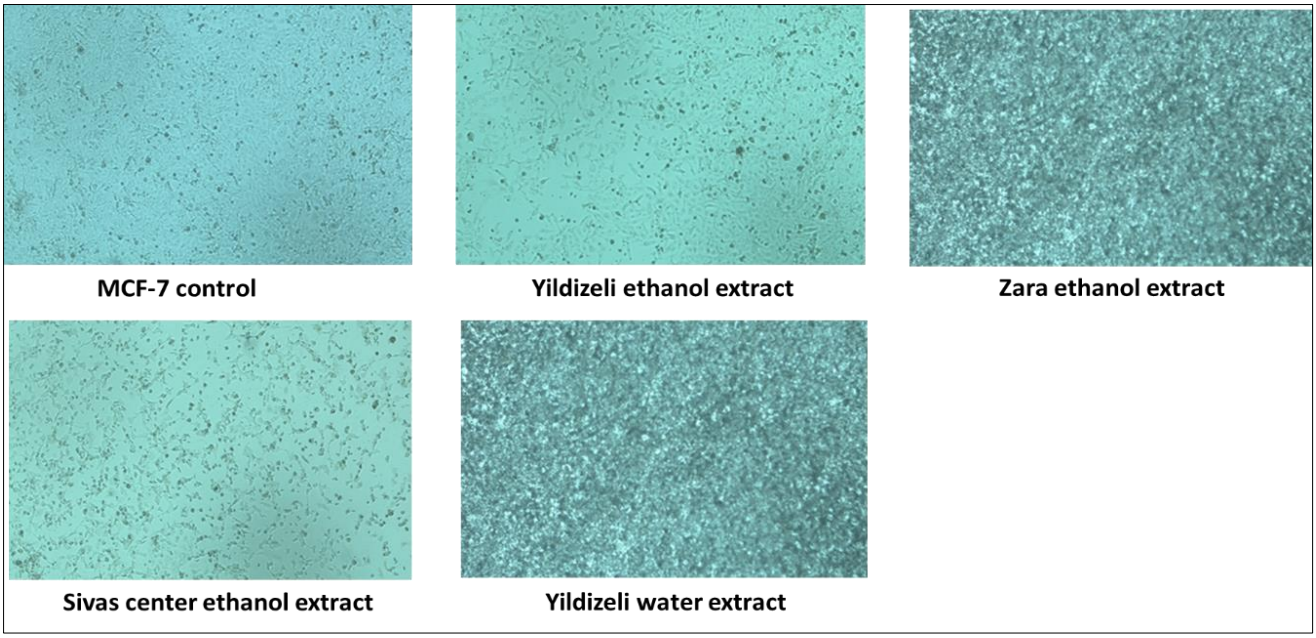

Figure 2. Morphological changes of cells after 48 hours of incubation with 100 µg/mL dose of pollen samples.

Discussion

The great duties of honey bee breeds in maintaining the ecological balance, the use of bee products as a food source, their benefits in maintaining health and their therapeutic qualities, as well as their effects in reducing the side effects of diseases are known from various sources in the ancient history of mankind. Every day, new studies are carried out on the benefits of bees and bee products and important information is brought to the literature. The biological activity values coming from bee pollen content properties such as proteins, minerals, carbohydrates, fats, vitamins and secondary metabolites contained in bee pollen are highly shown in various i*n vitro* and *in vivo* studies, and with these properties the bee pollen has an important place in apitherapy.

Conclusion

In order to determine the benefits of bee pollen, which has an important place in apitherapy and ethnopharmacology, on human health, it is necessary to reveal all its biological and chemical properties first, and it will provide great convenience in the use of pollen for therapy. In this study, moisture determination of pollen samples taken from some localities of Sivas province and evaluation of in vitro cytotoxic activities on MCF-7 cell line were made. With the study carried out, it is thought that the pollen samples, which were extracted with ethyl alcohol and water under laboratory conditions, are promising for the preparation of new and effective supplement products in terms of pharmacognosy. It is very important to reveal the pollen fingerprint of all beekeeping regions in the world geography and to determine their various therapeutic qualities. This research will guide the development of new therapeutic products and methods in the fields of cosmetics, food supplements and health, which can be done in the future.

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