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***Research Article***
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# Bioactive Components and Effect Mechanism of Apilarnil

Apilarnilin Biyoaktif Bileşenleri ve Etki Mekanizması

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Anahtar Kelimeler:

Bal arısı, erkek arı larvası, streoid hormon, testosteron, yağ asidi.

## ABSTRACT

**Objective:** Apilarnil is a natural bee product produced from drone larvae. It has highly beneficial properties for both humans and animals. The chemical composition of drone larvae consists of mineral salts, vitamins, carbohydrates, lipids, and amino acids. These larvae affect the bio-stimulating hypothalamic–pituitary–adrenal axis, which has a direct impact on the essential measures of the genitalia function. The current study was designed to reveal a detailed physico-chemical characterization of apilarnil.

**Material and Methods:** In this study, a freshly harvested apilarnil sample from Turkey was examined based on the percentage of some physicochemical parameters (e.g. moisture, ash, total lipid, total protein, and cholesterol), the level of some hormones (estradiol, prolactin, progesterone, and testosterone), and the characterization of some fatty acids.

**Results:** According to the existing results, the percentage of total lipid, total protein, and cholesterol of apilarnil were defined as 5.68, 13.25, and 2.28, respectively. Furthermore, a high level of testosterone and progesterone was noted as 14.80±0.05 ng/g and 14.40±0.05 ng/g, and conjugated linoleic acid (52.62%) was defined as a marker of fatty acid by gas chromatography.

**Conclusion:** The evaluation of results show that apilarnil, thanks to its high-level bio-properties, could be considered as a remarkable natural source.

## ÖZ

**Amaç:** Apilarnil, erkek arı larvalarından üretilen doğal bir arı ürünüdür. Hem insan hem de hayvanlar için oldukça faydalı özelliklere sahiptir. Erkek arı larvalarının kimyasal bileşimi, mineral tuzlar, vitaminler, karbohidratlar, lipitler ve amino asitlerden meydana gelmektedir. Bu larvaların genital fonksiyonun temel ölçümleri üzerinde doğrudan etkisi olan biyo-uyarıcı hipotalamus-hipofiz-adrenal aks fonksiyonu üzerinde etkisi vardır. Mevcut çalışma, apilarnilin detaylı fizikokimyasal karakterizasyonunu ortaya çıkarmak ortaya çıkarmak adına tasarlanmıştır.

**Materyal ve Metot:** Bu çalışmada, Türkiye'den taze hasat edilmiş apilarnil örneği, bazı fizikokimyasal parametrelerin yüzdesi (nem, kül, toplam lipit, toplam protein ve kolesterol), bazı hormonların seviyesi (östradiol, prolaktin, progesteron ve testosteron) ve bazı yağ asitlerinin karakterizasyonu açısından incelenmiştir.

**Bulgular:** Mevcut sonuçlara göre; apilarnilin toplam lipit, toplam protein ve kolesterol yüzdesi sırasıyla; 5.68, 13.25 ve 2.28 olarak tanımlandı. Ayrıca, testosteron ve progesteronun seviyesi 14.80 ± 0.05 ng/g ve 14.40 ± 0.05 ng/g olarak yüksek düzeyde kaydedildi ve konjuge linoleik asit (% 52.62) gaz kromatografisi ile ana yağ asidi bileşeni olarak tanımlandı.

**Sonuç:** Değerlendirme sonuçları, apilarnilin, yüksek seviyeli biyolojik özelliklerinden dolayı kayda değer bir doğal kaynak olarak görülebileceği sonucunu ortaya koymuştur.

## INTRODUCTION

In the past decades, the least processed foods are in demand. Among them, apilarnil or bee drone larvae are one of the most intriguing and biologically active bee products, which includes a little amount of honey, propolis, bee bread and also royal jelly. The bee drone larvae are bioactive natural products. It owns a homogeneous texture and it is composed of milky substances. The components of apilarnil give its texture a yellowish-grey color and the acidic material inside the components brings out a slightly pungent odor and taste. Apilarnil is used in cosmetic and for the therapeutic purposes (Barnutiu, 2013; Speteanu et al., 1984; Stangaciu, 2002). The complicated composition supplied from the drone larvae and bee brood can be evaluated as a "luxury" food well-known valuable nutrition in terms of proteins and fatty acids. Essentially, apilarnil is produced from the special drone larvae comb cells that are 3rd or 7th days old. For storing and using of apilarnil, the four-step extraction procedures as trituration, homogenization, filtration, and lyophilization have to apply (Yücel et al., 2011).

The chemical composition and properties of apilarnil are similar to the royal jelly. The antiviral effect, the feature of immune system enhancer, and the anabolic stimulator are just a few of bio-properties of apilarnil. Moreover, the appetite, the body's energy, vitality, and regenerative power could be regulated with apilarnil (Iliesiu, 1987).

Apilarnil is a source of androgenic hormones thanks to mainly deriving from the male structure. This situation results in the stimulating of the spermatogenesis in men. Also, this positive result of apilarnil could be examined rather than drugs and chemicals to stimulate sexual development (Altan et al., 2013; Yücel et al., 2011). Not only the data, which has been reported on the subject of the increment of the hormone levels as an impact of the drone larvae in Eastern Europe and Asian folk medicine, demonstrates the androgen-like activity of apilarnil, but also previously described data is contributing to this characteristic feature of apilarnil in particular (Artık and Konar, 2015; Seres et al., 2014).

### The bioactive components of apilarnil

The main components of bee larvae are the amino acids (Topal et al., 2018). Apilarnil contains all of the essential amino acids. It is well-known that amino acids are important bio-marker for the body development of honeybees, too (Özbakır and Alisiroglu, 2019). However, alongside the amino acids; proteins, carbohydrates, fats, polyphenols, vitamins (A, $B_1$, $B_6$, PP and choline) and minerals (Ca, P, Na, Zn, Mn, Fe, Cu, and K) assigned increase the quality of apilarnil. Protein profile can be changed by the consumption of the pollen sources that are used by the bees. The carbohydrate compounds of apilarnil are known as glucose, fructose, and sucrose (Margaoan et al., 2017). Royal jelly has a high antioxidant level that strengthens the immune system as well as regenerates power. Apilarnil has even a higher antioxidant level than royal jelly. This property is based on the rich polyphenol content (Kolayli et al., 2016; Matsuka et al., 1973; Nagai et al., 2001). Apilarnil is rapidly destroyed by excessive heat. In the case of the lacking the refrigeration and lyophilization processes; it decomposes promptly (Barnutiu, 2013).

### The effect mechanism of apilarnil

Apilarnil is the first of all the natural products that is very rich in nutrients and sexual hormones. Apilarnil is very much in demand due to its preventive (prophylactic) property. It is widely used by many people in order to prevent potential diseases. A diseased individual requires to take more good nutrients, vitamins, enzymes, active pharmacological compounds etc. in their body than a healthy individual, because the body is usually lacking in these substances and bio-energy and therefore; cannot defend the body against the disease (Cosman et al., 1984; Iliesiu, 1991).

Until now; adverse, toxic or allergic reactions related to the use of apilarnil have not been reported. However, hence apilarnil contains small amounts of pollen, propolis and honey we can presume that its use may increase the problems that are similar to those associated with its components.

Apilarnil can set off possible allergy reactions to any of its components. Stomach algia, nausea-vomiting, diarrhea, toxicity, hyperandrogenism and hyper spermatogenesis may appear in some cases of overdose. Moreover, a vigorous antibacterial effect was determined against the gram positive bacteria (*Bacillus aureus*) (Eshraghi and Seifollahi, 2003).

Apilarnil's main prosperity is to support the fight of the progression of the diseases and even to cure them. The diseases that apilarnil provides benefits are;

anorexia(lack of appetite with various causes), hypoproteinemia (lack of proteins in the blood which may lead to many kinds of metabolic, nervous system, endocrine system diseases), metabolic diseases (diabetes, obesity, gout), diminution of the muscular body weight especially in males, asthenia of all levels, chronic fatigue syndrome, physical exhaustion, convalescence, premature ageing, depression in old people, nervous system diseases (weak memory, mental disorders, neuro-psychomotricity diseases in children, insomnia), respiratory apparatus diseases, dermal (skin) insufficiencies, diseases from the genital areas related to the lack of hormones, vitamins and minerals which are necessary for the proper development and functioning of these organs, sexual impotence (low spermatogenesis, erectile dysfunction, short duration of sexual act in men), endocrine system diseases (weak hypophysis and adrenal glands, premenstrual syndrome) and immune system diseases (weak immune system, flu, infections) (Iliesiu, 1987; Stangaciu, 2002).

Several factors can diminish the effectiveness of apilarnil. In the case of diathesis; the body becomes unable to digest or absorb apilarnil, hence it is weak to process the components of apilarnil. The alternate factor is related to the genetic problems. The "target" organs (like the endocrine glands in this case), have major structural or genetic problems; thus they cannot use the active compounds of apilarnil properly. A final conclusion here is that; like all other bee products. Apilarnil cannot cure every disease nor every problem in the body in the case of a diathesis or a genetic problem (Stangaciu, 2002).

The aim of this study was to establish some bioactive components and chemical composition (moisture, ash, total lipid, fatty acids, total protein contents and hormone analyses) of apilarnil.

## MATERIAL and METHODS

### Apilarnil (bee drone larvae) preparation

Apilarnil freshly harvested from opened or unsealed cells from the bee hives. All the cells were filled with clean water and then the larvae were shaken out (Schmidt and Buchmann, 1992). Since larvae defecated just before pupation; larvae was washed in clean water before further processing. Pupae had clean, empty intestines. Apilarnil was packed and the samples transferred to the laboratory. The samples were triturated, homogenized, filtrated and finally lyophilized using CHRIST Alpha 1-4 LD plus (Germany). The lyophilized samples were stored at -20 ºC until analyzed.

### Analyses

Moisture, total lipids, crude protein contents of the samples were determined using the AOAC method (Helrich, 1990)**.** Ash contents were defined by placing the sample inside the incineration oven at 550 ºC for 6h, until a white powder was obtained. The crucible was weighted at the beginning and at the end. The difference was expressed in the percentage alteration of the ash content from the beginning to the end. The Kjeldahl method with distillation parameter optimization (Digester K-424, Distiller KjelFlex K-360 and titrator Schott Titro Line) was applied to evaluate the total protein contents of the sample. Fatty acid methyl esters (FAMEs) was prepared according to ISO 12966-4 (Anonymous, 2015), Supelco 37 component FAME mix was used in the internal standard.

Agilent 5975 C FID gas chromatograph equipped with a Supelco DB-5 capillary column (60 m × 0.25 mm ID, 0.2 µm HP-88) was used to analyzing the fatty acid compounds. 1.0 mL helium at a minute was used as a carrier gas. The injector temperature was programmed to remain at 240 °C. First, the heating degree of the oven was adjusted at 80 °C for 4 minutes, and then a gradual increase was applied to 220 °C at a rate of 3.0 °C per minute. An Agilent 5975 C gas chromatography equipped with a flame ionization detector (FID) was utilized to obtain the percentage of the fatty acids. The results were given according to the current device's library (Wiley and Nist) and previously the methodology described by Adams (2007).

Steroid hormones of lyophilized apilarnil (estradiol, progesterone, prolactin and testosterone) were analyzed for this purpose. Lyophilized apilarnil samples were dissolved in 0.9% NaCl and centrifuged at 4000xg for 10 min and supernatant was used for hormone tests. Steroid hormones of estradiol, progesterone, prolactin, and testosterone of the samples were measured by a Beckman Coulter© Analyzer, by Access© Immunoassay system Kits, for Estradiol REF 33540, for Progesterone REF33550, for Prolactin REF33530 and for Testosterone REF332609 were used.

Total cholesterol of the sample was determined according to a modified version of spectrometric method of Franey and Amador (1968). Lyophilized apilarnil samples were dissolved in 0.9% NaCl. The

reaction mixed was contained 1 mL sample, 4 mL $FeCl_3$ (140 mg $FeCl_3.6H_2O$ was dissolved in 100 mL glacial acetic acid) and incubated 30 min, then centrifuged at 2500 rpm at 10 min, and supernatant was used. 2 mL supernatant, 2 mL acetic acid and 2 mL $H_2SO_4$ was mixed and incubated 30 min and absorbance was read at 560 nm against reagents blank. For calibration curve, standard cholesterol solutions were prepared with glacial acetic acid, and the results were expressed mg/dL.

All results were expressed as mean±standard deviation by using Microsoft Office Excel 2013 software. For standard deviations, the results of some bioactive components of apilarnil were performed three times.

## RESULTS and DISCUSSION

The data of some physicochemical analysis of the lyophilized apilarnil sample were given in Table 1. Percentage of the moisture and the ash content were 73.00±1.20, 1.04±0.02, respectively. Total lipid content was determined 5.68±0.50% whereas total protein and cholesterol level were 13.25±0.50%, 2.28±0.20%. Steroid hormones results were expressed as ng per g lyophilized apilarnil sample. In the current study, testosterone had the highest (14.80±0.05 ng/g) followed by progesterone as 14.4 ±0.05 ng/g, while PRL (prolactin) had the lowest (1.2±0.01 ng/g) followed by $E_2$ (estradiol) as 4.12±0.02 ng/g.

The fatty acid composition of apilarnil was given in Table 2. According to the result, conjugated linoleic acid (CLA) (52.695%) and palmitic acid (PA) (27.19%) were found higher than the other fatty acids of apilarnil. Oleic acid, gamma linoleic acid, myristic acid, 10-heptadecenoic acid, 11-eicosenoic acid, 11, 14, 17-eicosatrienoic acid were evaluated as moderate and low values.

**Table 1.** The results of some bioactive components of Apilarnil
***Tablo 1.*** *Apilarnil'in bazı biyoaktif bileşenlerinin sonuçları*

| Apilarnil (lyophilized) | | | | |
|---|---|---|---|---|
| **Moisture%** | **Ash%** | **Total lipid%** | **Total protein%** | **Cholesterol (%)** |
| 73.00±1.20 | 1.04±0.02 | 5.68±0.50 | 13.25±0.50 | 2.28±0.20 |
| **Hormones (ng/g lyophilized)** | | | | |
| | **$E_2$ (estradiol)** | **PRL (prolactin)** | **Progesterone** | **Testosterone** |
| | 4.12±0.02 | 1.20±0.01 | 14.40±0.05 | 14.80±0.05 |

**Table 2.** The fatty acid compositions of Apilarnil
***Tablo 2.*** *Apilarnil'in yağ asidi bileşimleri*

| | Fatty Acids | Value (%) |
|---|---|---|
| **1** | Myristic acid (C14:0) | 1.69 |
| **2** | Palmitic acid (C16:0) | 27.10 |
| **3** | 10-Heptadecenoic acid (C17:1n7) | 1.41 |
| **4** | Oleic acid (C18:1n9c) | 12.40 |
| **5** | Conjugated linoleic acid (C18:2n6c) | 52.62 |
| **6** | Gamma linoleic acid (C18:3n6c) | 2.54 |
| **7** | 11-Eicosenoic acid (C20:1n9c) | 1.11 |
| **8** | 11,14,17-Eicosatrienoic acid (C20:3n3c) | 0.97 |
| | **Total** | **99.57** |

Higher moisture content is one of the prominent parameters in larvae and pupa processes. It directly causes the growth and development of them. For this reason, moisture content could be considered as one of the criteria in estimating the larvae quality (Rahmathulla et al., 2006). Our finding of the moisture degree is the similar (72%) to Bărnuțiu et al., (2013). Apilarnil had more water content in the compression with the royal jelly. Wytrychowski et al., (2013) specified the average value of water content of 500 royal jelly samples as 65.3%, whereas Kolayli et al. (2016) found 66.8% for 18 samples in average.

Besides the moisture content in this study, the ash content is the residue remaining all the moisture has been removed at high temperature that it can also be an indirect way to measure of the total amount of minerals present within larvae. The ash content of apilarnil (1.04%) was similar to Bărnuțiu et al., (2013) (1%) but lower than Stangaciu, (2002) (2%) findings.

According to Bayne et al., (1975), protein and lipid contents make a significant contribution to energy metabolism during the early stages of larval development. After this claim, a positive response could be exhibited that apilarnil was very rich in protein and lipid contents. Furthermore, total cholesterol and cholesterol percentage were found for comparing with other chemical components in directly apilarnil sample. For this reality, total cholesterol and cholesterol percentage were determined as 114.28±2.02 mg/dL and 2.28%±0.20, respectively.

In the literature, especially steroid hormones which are E2 (estradiol), PRL (prolactin), progesterone, testosterone have been mentioned that a significant effect on the regulating of the growth, development, and proliferation in vital larvae (Nazari and Ghomi, 2010). In the current study these hormones were quantified as a quality marker. Paludo et al., (2018) showed that bee pupation required ecdysteroid hormones to developing, allowing successful pupation.

Other parameter was the fatty acid composition of apilarnil. Dietary conjugated linoleic acid (CLA) known as a possible agent of the effect of anti-carcinogenic and anti-atherogenic was precursor with its high value in the current study. Besides these features, CLA has also been seen as a potent immune modulating activator and a reducing factor of body fat mass (Aydin, 2005; Blankson et al., 2000). Kelly, (2001) mentioned that serum lipid degrees in humans were affected by CLA due to the decreasing of the total, LDL, and HDL cholesterol, but the increasing of Lp(a). According to the study result about rodent models of cancer, CLA directly showed anticancer activity owing to a decrease in the number of tumor burden and metastasis in experimental models of transplanted tumors (Thompson et al., 1997). Although there is no effect on tumor mass, the low-fat diet shows a protective act solely in the tumorigenesis process. Moreover, palmitic acid (PA) which is taken in the diet also influences the carcinogenesis process. Hence, the regulation of tumor growth may be related to PA (Mancini et al., 2015).

All these results enucleate the impact of apilarnil's prosperities on the body that is essential for not only the diseases but also for the overall balance of the body. Their activities include supplying a high anabolic activity level acting as a stabilizer to the blood cholesterol level and enhancing the immune system in general.

In conclusion, apilarnil which stimulates growth and sexual development thanks to its androgenic hormones is a member of natural bee-products. Also, it is suggested as a natural anabolism stimulator in males by the reason of its impact on the increment of the muscular bodyweight. Besides *in-vitro* examination, the further *in-vivo* studies are also needed to evaluate the potency of the androgenic and anabolic effect of apilarnil. There were many limitations in our study. First, there were no comparing studies. For this reason, the discussion did not improve. The second was sample limitation. While we had one sample, it assumed as a fixed nearly same properties in all apilarnil samples. Therefore, carrying out such studies with bioactive components and effect mechanism of apilarnil will be more beneficial to see the results clearly. However, the use of apilarnil for therapeutically purposes should be actualized only after a quality control.

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