MELLIFERA SCIENTIFIC NOTE

A New Record for Propolis Substitute: Pruning Sealer

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A B S T R A C T

Propolis is produced by honeybees from various secretions of the plants and is combined with digestive enzymes and wax. Factors such as plant content, climate, and location of propolis affect propolis content and its physical and chemical properties. Although honeybees are very selective when collecting propolis, some studies have shown that bees carry asphalt, paint and similar substances to the hive according to the location of the beehive. In this study, it has been observed that the propolis taken from on orchard in Ankara was light brown and red. After this observation, possible source of the propolis was investigated. As a result of the analysis, a total of 28 compounds were observed in propolis and four of them (decanol, homofuraneol, tetradecane, 2-phenylethanol) were derived from the pruning sealer that was applied to the trees located around the beehive.

Keywords: Propolis, Pruning sealer, Chemical analysis

Introduction

Propolis is collected by honeybees from different parts of specific plant species [1, 2]. Leaf or flower buds, leaf glands, exudates, wounds in the bark or stems of plants and exudates produced in response to microbial infection or insect attack are some of the different plant parts [3].The resinous material collected by mandibles of bees and transferred to their hind leg 'pollen baskets' where they form it into a mass that typically appears as smooth, shiny, semi-transparent droplets [3].

The resinous material is not ingested by bees, and just mixed with beeswax in the hive [4].The propolis introduced into the hive is used in many functions such as cleaning the honeycomb eyes before laying the eggs, narrowing the entrance hole of the bucket to prevent cold weather, repairing the structural defects in the hive and preventing bacterial growth [5, 6]. Propolis has a complex chemical composition that includes polyphenols, terpenoids, steroids, and amino acids which are related to the plant source that honeybees prefer to collect propolis, because it has been proved that bees do not change its chemical composition [7]. Factors such as region, plant source and climate affect the chemical structure of propolis. It has been found that this chemical structure provides antibacterial, antiviral, antifungal, antitumoral, immunostimulator and local anesthetic effects. And it can be used in the treatment of many diseases [8].

Honeybees are very selective in the material they collect to produce propolis [3]. *Acer* spp., *Salix* spp., *Aesculus hippocastanum*., *Pinus* spp., *Castanea sativa* spp., *Eucalyptus* spp. and *Tilia* spp.

are some of the major plants bees prefer as a source of propolis [8]. But some researchers and apiarists reported that honey bees would likely collect other substances such as paints, mineral oils or asphaltic tar to make bee glue-like substance (propolis) when vegetation was scarce in an area [9-11].There are observations about researchers of honey bees collecting matter from asphalt [11, 12].

The aim of our study is to determine that the honey bees collect pruning sealer as a source of propolis or not.

Materials and Methods

Propolis and pruning sealer sampling

The samples were collected by a beekeeper from the hives which were located in an orchard, in Ankara,Turkey. The sample divided into three pieces according to its coloration. A commercial pruning sealer was bought to analyze.

Extraction and sample derivatization

According to the coloration on the propolis sample, only propolis (1), mixed with propolis (2) and red (3) areas were extracted and separated. In addition, the pruning sealer was also extracted. Extraction were done according to the Popova et al. (2007) [13]. Materials were grated and a sample of 1 gram was dissolved in 70% ethanol and mixed in an ultrasound bath. After extraction the sample was filtered and the filtrate diluted to 100 ml with70% ethanol (this process was done three times).

GC–MS analysis

A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS5973, Hewlett-Packard) was used for the analysis of samples. Experimental conditions of GC-MS system were as follows: DB 5MS column (30 m×0.25 mm and 0.25 μm of film thickness) was used and flow rate of mobile phase (He) was set at 0.7 ml/min. In the gas chromatography part, temperature was kept for 1 min at 50 °C and then increased to 150 °C with 10 °C/min heating ramp. After this period, temperature was kept at 150 °C for 2 min. Finally, temperature was increased to 280 with 20 °C/min heating ramp and then kept at 280 °C for 30 min.

Identification and Quantification

The compounds were identified using the GC-MS response data (i.e. key ion fragmentograms and mass spectra).

Results and Discussion

The basic chemical content of propolis is composed of flovanoids, phenolics and various aromatic components. As a result of the analyzes, more than 300 compounds were obtained from the samples of propolis with different plant origin. Among these compounds flavonoids have a large place. In addition, caffeic acid and esters, terpenes

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and aromatic esters are also present naturally in propolis [6].

According to the results of the GC–MS analysis, the four extracts from the sample contain about 98 compounds. Although the sample of propolis mixed with the pruning sealer was separated according to the color differences, the origin of some compounds could not be determined, 10 compounds were common in almost all samples (Table 1). Three compounds (Benzoic acid, Homofuronol; 2-ethyl-4-hydroxy-5-methyl-3(2H) furanone, n-Tetracosane) were found in all samples. Two compounds (cis-6-Nonenal, n-Tetradecane) were found in pruning sample and red area (3). As a result of GC-MS analysis, it was determined that the products of decanol, Homofuraneol, Tetradecane, 2-phenylethanol were caused by pruning sealer.

Decanol, which has a straight oil chain alcohol structure, is used in the construction of plasticizers, lubricants, surfactants and solvents. This material was obtained from both the pruning sealer and the red zone after GC-MS analysis. Although it has serious irritative effects in contact eyes skin and skin, it is insoluble in water and harmful in the environment [14].

Homofuraneol is a compound commonly used as a thickener and flavoring agent. It is used to give a caramel-like aroma in foodstuffs. No toxicity has been reported to date this substance is thought to be used as a thickener in the puruning sealer [14].

N-Tetracosane, a long-chain alkane, is a volatile component naturally occurring in the plant's structure and is involved in plant metabolism. Tetradecane is an acrylic alkans, it is used in the production of many chemicals with its lubricating properties, and it can cause irritations by contact with eyes and skin [14].

2-phenylethanol is an ethanol derivative. Through its antimicrobial, antiseptic and disinfectant properties, it is used as a preservative in the pharmaceutical and perfumery industries [14].

Owing to the results we can say that environmental conditions such as the location of apiaries or the applications around the beehives effect directly the quality of bee products and unfortunately limited their usages.

Table 1: Common compounds in all samples.

Compounds	Pruning sealer	Propolis (1)	Mixed area (2)	Red area (3)
1-Decanol	0.2	0.65	-	0.91
Benzoic acid	0.03	4.55	0.06	2.26
cis-6-Nonenal	2.39	-	-	4.04
Homofuronol; 2-ethyl-4-hydroxy-5-methyl-3(2H)furanone	4.81	1.02	0.84	0.35
Myristic acid	0.13	-	1.32	2.27
n-Tetracosane	2.44	0.5	7.2	1.32
n-Tetradecane	4.62	-	-	1.50
Phenethyl alcohol	-	0.02	0.14	0.37
Stearic acid	0.69	-	5.4	5.95
Piperonal	0.07	7.77	-	0.20

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Propolis Yapımında Kullanılan Kaynaklar Için Yeni Bir Kayıt: Aşı Macunu

Öz: Propolis, arılar tarafından, bitkilerin çeşitli salgılarından üretilen, enzimleri ve mum ile karıştırdıkları bir üründür. Bitki içeriği, iklim ve lokasyon gibi etkenler propolisin içeriğini, fiziksel ve kimyasal özelliklerini etkilemektedir. Balarıları propolis toplarken çok seçici olmalarına rağmen, bazı çalışmalar göstermiştir ki arılığın yerine bağlı olarak arılar kovana asfalt, boya ve benzeri maddeleri taşıyabilmektedirler. Bu çalışmada, Ankara'da buluna bir meyve bahçesinden toplanılan propolis örneğinin açık kahverengi ve kırmızı renklerde olduğu gözlemlenmiştir. Bu gözlemden sonra propolisin olası kaynağı incelenmiştir. Analiz sonucunda propolis örneğinde 28 bileşik tanımlanmıştır ve bunlardan dördünün (dekanol, homofuraneol, tetradecane, 2-phenylethanol), arılığın etrafındaki ağaçlara uygulanan aşı macunundan kaynaklandığı tespit edilmiştir.

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