



UNIVERSIDADE
LUSÓFONA

This report focuses on Centauri Cave Nymph honey, produced by Ahmet Eren Çakır by undisclosed formula, exploring its chemical composition and biological activity. Centauri Cave Nymph Honey is a unique type of cave honey extracted from an altitude of 2500 meters above sea level in a deep cave, with the assistance of professional speleologists. This exceptional honey is sourced from the Caucasus Mountains of Turkey. The bee type responsible for producing Centauri Cave Nymph Honey is *Apis mellifera* *Caucasica*, also known as Caucasian Honeybees. These bees are known for their resilience to cold temperatures and high altitudes. The bee colony is situated 50 kilometers away from the nearest human residences and is isolated from other bee colonies. This isolation helps to maintain the colony free from varroa mites, ensuring the purity and quality of the honey. The demand for Centauri Cave Nymph Honey is global, with customers from all around the world seeking this unique product. Due to its limited availability and the meticulous process of extraction, there is a waiting time of 3 to 6 months for customers to receive their orders. Annually, only 50 kilograms of this honey are available, and it is harvested during the best season to ensure its exceptional quality and taste.

A research study was conducted to scientifically validate the commercial sample of Centauri Cave Nymph Honey. This work examined several factors including the floral composition (mono/polifloral), altitude and cave depth in meters, visual color, and location. These parameters were carefully analyzed to ensure the authenticity and quality of the honey. By considering these aspects, Centauri ensures that their honey meets the highest standards and delivers a unique and exceptional medicinal product to their customers.

Physical-chemical parameters play a crucial role in assessing the quality of honey. The color of honey can range from white to dark brown, with lighter colors often having a milder flavor and darker honey having a more pronounced taste. Humidity is an important parameter affected by weather conditions and harvest, and it can impact other physicochemical parameters such as viscosity and crystallization, which in turn affect the overall quality of honey. Conductivity is a parameter related to the presence of minerals, proteins, and acids in honey. It is also indicative of the organic content, which is directly linked to the ash content. Conductivity serves as a useful parameter for nutritional assessment and can aid in the differentiation of honey from different botanical origins. International standards often recommend a maximum conductivity value of 0.8 mS/cm for nectar honey, which was in agreement with all the tested honey samples. Monitoring conductivity helps ensure the quality and authenticity of honey, as it provides insights into its mineral content and botanical origin. The ash content in honey is a quality criterion used to determine its botanical and geographical origin. It can provide insights into the floral sources and geographical locations from which the honey was derived. Additionally, the nutritional composition of honey, including its proline content, serves as an authenticity marker and can help detect adulteration attempts. Proteins in honey can be derived from nectar and introduced by bees during the honey-making process. The presence of specific enzymes in honey can also serve as a quality indicator, reflecting factors such as the freshness of the honey and its storage time. The



energy value and carbohydrate content of honey are important parameters for nutritional assessment, although they do not have specific limit regulations. These parameters provide information about the caloric content and carbohydrate composition of honey. Honey primarily consists of simple sugars or monosaccharides, with fructose and glucose being the predominant ones. Monitoring the energy value and carbohydrate content of honey allows for a better understanding of its nutritional profile and helps consumers make informed dietary choices. Monitoring these parameters can ensure the authenticity, quality, and nutritional integrity of honey products. These parameters provide valuable insights into the characteristics and quality of honey and are important considerations in the evaluation and assessment of honey samples.

In this study, the Centauri Honey was evaluated for its *in vitro* biological activities, including antioxidant anti-inflammatory and antimicrobial activities. For the anti-inflammatory activity, it was found that the honeys exhibited anti-inflammatory effects in an *in vitro* model using the RAW 264.7 cell line (cell line derived from murine macrophages). Specifically, the anti-inflammatory effect was assessed in Calu-3 lung cancer cells induced by lipopolysaccharide (LPS). After 24 hours of LPS induction, there was an increase in the expression of cytokines, specifically IL-6 and IL-1 β , at both the gene and protein levels. However, it was observed that honeys A, D, and E exerted a strong effect in reducing the levels of these cytokines in LPS-induced Calu-3 cells, indicating their potential anti-inflammatory properties. In the study evaluating the anti-inflammatory activity of honeys induced by lipopolysaccharide (LPS) in an *in vitro* model, the effects were also investigated in a normal colon cell line (FHC cell line). It was observed that the honeys exhibited a decrease in the level of LPS-induced cytokines in these cells, indicating their potential anti-inflammatory properties.

Specifically, honeys A, D, and E showed the best effect in reducing the levels of cytokines in the LPS-induced FHC cells. This suggests that these honeys have the ability to modulate the inflammatory response and potentially provide therapeutic benefits in inflammatory conditions associated with the colon. In the study investigating the morphological changes after honey treatment in the FHC cell line (normal colon cell line), it was observed that no significant changes were observed in the normal FHC cells when treated with honey at 1% and 5% concentrations. The lack of morphological changes suggests that the honey treatment at these concentrations did not induce any noticeable alterations in the cellular structure or morphology of the normal FHC cells. This finding is important as it indicates that the honey treatment did not have any adverse effects on the normal colon cells under the conditions tested.

In conclusion, in the evaluation of Centauri Honey, various physical-chemical and quality parameters were assessed across all honey samples. These parameters included color, moisture content, conductivity, pH, and acidity tests. By evaluating these physical-chemical and quality parameters, a comprehensive understanding of the Centauri Honey samples can be obtained. These analyses contribute to ensuring the authenticity, purity, and overall quality of the honey.

During the evaluation of Centauri Honey, several bioactivity tests were conducted. These findings provide insights into the potential health benefits and safety profile of the honey. Moreover, the anti-

inflammatory activity of Centauri Cave honey was investigated, particularly in an in vitro model using the FHC cell line (normal colon cell line) and LPS (lipopolysaccharide) induction. The results demonstrated that Centauri Cave honey exhibited a highly effective anti-inflammatory potential. When the FHC cell line was treated with LPS, which typically induces the release of inflammatory cytokines, the honey was able to decrease the levels of these cytokines. This indicates that Centauri Cave honey has the ability to mitigate the inflammatory response triggered by LPS. Among the different honey samples tested, honeys A, D, and E showed the most pronounced anti-inflammatory effects. These honeys demonstrated a more significant decrease in the levels of cytokines induced by LPS, suggesting their higher potency in suppressing inflammation. Based on these findings, Centauri Cave honey holds great potential as an agent to enhance the immune response by reducing inflammation. Its anti-inflammatory properties make it a promising candidate for further research and potential therapeutic applications.

Commercial sample E of Centauri Cave honey was found to be the most bioactive among the samples tested, exhibiting the most relevant physical-chemical parameters. This indicates its potential as a highly valuable and beneficial honey variant. Furthermore, future studies will explore the potential health benefits of consuming Centauri Cave honey, particularly in relation to its protective effects against colon cancer and others such as prostate cancer. Ongoing studies are currently being conducted to further investigate these potential protective properties.

In addition, future in vivo studies are planned to delve deeper into the health benefits of Centauri Cave honey and uncover additional advantages it may offer. These studies aim to provide a more comprehensive understanding of the potential health-promoting properties of this honey. The ongoing and future research endeavors highlight the importance of studying the consumption of Centauri Cave honey and its potential impact on human health. By expanding our knowledge in this area, we may uncover further health benefits and applications for this unique honey variant.

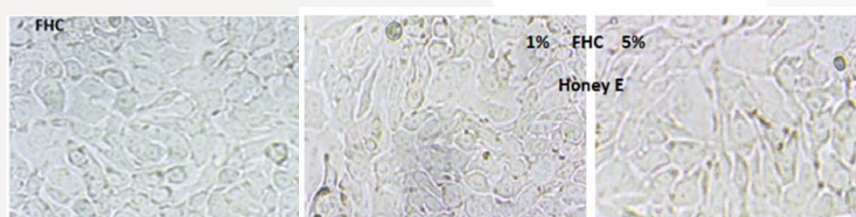


Figure 1: Morphological changes after honey treatment in FHC (normal colon cell line) were not observed at 1 and 5% concentration (Cell morphology before and after honey stimulation: Magnification x40).

Final Remarks:

This report focuses on Centauri Cave Nymph honey, produced by Ahmet Eren Çakır by undisclosed formula, exploring its chemical composition and biological activity. To scientifically validate the commercial sample of Centauri Cave Nymph Honey, a research study was conducted. This work examined several factors including the floral composition (mono/polifloral), altitude and cave depth in meters, visual color, and location (A to E honey samples). These parameters were carefully analyzed to ensure the authenticity and quality of the honey. By considering these aspects, Centauri ensures that their honey meets the highest standards and delivers a unique and exceptional medicinal product to their customers.

In the evaluation of Centauri Honey, various physical-chemical and quality parameters were assessed across all honey samples. These parameters included color, moisture content, conductivity, pH, and acidity tests. By evaluating these physical-chemical and quality parameters, a comprehensive understanding of the Centauri Honey samples can be obtained. These analyses contribute to ensuring the authenticity, purity, and overall quality of the honey.

During the evaluation of Centauri Honey, several in vitro bioactivity tests were conducted. These tests included antioxidant, antimicrobial, and cytotoxicity assessments. These findings provide insights into the potential health benefits and safety profile of the honey. Moreover, the anti-inflammatory activity of Centauri Cave honey samples was investigated, particularly in an in vitro model using the FHC cell line (normal colon cell line) and LPS (lipopolysaccharide) induction. The results demonstrated that Centauri Cave honey exhibited a highly effective anti-inflammatory potential. When the FHC cell line was treated with LPS, which typically induces the release of inflammatory cytokines, the honey was able to decrease the levels of these cytokines. This indicates that Centauri Cave honey has the ability to mitigate the inflammatory response triggered by LPS. Among the different honey samples tested, honeys A, D, and E (the commercial sample) showed the most pronounced anti-inflammatory effects. These honeys demonstrated a more significant decrease in the levels of cytokines induced by LPS, suggesting their higher potency in suppressing inflammation. Based on these findings, Centauri Cave honey holds great potential as an agent to enhance the immune response by reducing inflammation. Its anti-inflammatory properties make it a promising candidate for further research and potential therapeutic applications. Furthermore, the morphological changes after honey treatment in the FHC cell line (normal colon cell line), was observed and no significant changes were observed when treated with honey at 1% and 5% concentrations. The lack of morphological changes suggests that the honey treatment at these concentrations did not induce any noticeable alterations in the cellular structure or morphology of the normal FHC cells. This finding is important as it indicates that the honey treatment did not have any adverse effects on the normal colon cells under the conditions tested.

Commercial sample E of Centauri Cave honey was found to be the most bioactive among the samples tested, exhibiting the most relevant physical-chemical parameters. This indicates its potential as a highly valuable and beneficial honey variant. Furthermore, future studies will explore the potential health benefits of consuming Centauri Cave honey, particularly in relation to its protective effects against colon cancer or others such as prostate cancers. Ongoing studies are currently being conducted to further investigate these potential protective properties.

In addition, future in vivo studies are planned to delve deeper into the health benefits of Centauri Cave honey and uncover additional advantages it may offer. These studies aim to provide a more comprehensive understanding of the potential health-promoting properties of this honey and contribute to its potential use as a medicinal honey. The ongoing and future research endeavors highlight the importance of studying the consumption of Centauri Cave honey and its potential impact on human health. By expanding our knowledge in this area, we may uncover further health benefits and applications for this unique honey variant.

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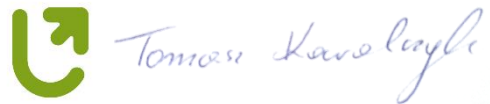
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