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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi. 2024; 10 (2): 115-120 Araştırma Makalesi/Research Article

Pistacia vera Yumuşak Kabuğundan Elde Edilen Hidrosolün Uçucu Profili ve Balık Yemi Endüstrisindeki Potansiyel Kullanımı

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Öz

Çalışmanın amacı, Antep fıstığı üretiminin bir yan ürünü olan Pistacia vera'nın atılan yumuşak kabuklarından elde edilen hidrosolün etkinliğini değerlendirmektir. Çalışma, hidrosolde bulunan uçucu bileşenlerin nitel yapısını incelemeyi ve balık yeminde katkı maddesi olarak potansiyelini değerlendirmeyi amaçlamaktadır. Bu hedefe ulaşmak için 1-kilogram yumuşak Pistacia vera kabuğunu çıkarmak için bir hidrodistilasyon prosedürü kullanılmıştır. Hidrosol, kilogram başına 170 mililitrelik bir performans seviyesine ulaşmıştır. Ürün, %93,37'sini oluşturan 16 bileşeni tanımlayan gaz kromatografisi-kütle spektrometrisi kullanılarak analiz edilmiştir. Ürünün ana bileşeni, ürünün %44,60'ını oluşturan Limonene-4-ol olarak tanımlanmıştır. Ayrıca, ürün önemli miktarda Benzenemethanol, α.α.,4-trimethyl, α-terpineol, Bornyl asetat, Karvon ve Okaliptol (1,8 cineol) içeriyordu. P. vera softshell'de bulunan biyoaktif kimyasallar üzerine yapılan önceki araştırmalar, ondan üretilen hidrosolün balık yemi katkı maddesi olarak kullanılabileceğini öne sürüyor. Bu çoğunlukla antibakteriyel ve antioksidan etkilerinden kaynaklanmaktadır.

Volatile Profile of Hydrosol Extracted from *Pistacia vera* Soft Bark and of Its Potential Use in Fish Feed Industry

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Abstract

The study aimed to evaluate the effectiveness of hydrosol obtained from discarded soft shells of *Pistacia vera*, a byproduct of Pistachio production. The study sought to examine the qualitative makeup of the volatile components found in the hydrosol and assess its potential as an additive in fish feed. A hydrodistillation procedure was used to extract 1 kilogram of soft Pistacia vera peel to accomplish this goal. The hydrosol achieved a performance level of 170 milliliters per kilogram. The product was analyzed using gas chromatography-mass spectrometry, which identified 16 components comprising 93.37%. The product's main component was identified as Limonene-4-ol, making up 44.60% of the product. Furthermore, the product contained substantial amounts of Benzenemethanol, α. α.,4-trimethyl, α-terpineol, Bornyl acetate, Carvone, and Eucalyptol (1,8 cineol). Previous research on the bioactive chemicals found in *P. vera* softshell suggests that the hydrosol generated from it could be used as a fish feed additive. This is mostly due to its antibacterial and antioxidant effects.

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INTRODUCTION

Pistacia vera is a fruit that belongs to the Anacardiaceae family and is native to Central Asia and the Middle East. People extensively consume Pistachios since they have a high economic worth, are flavorful, contain valuable nutrients, and offer health advantages (Dreher, 2012). Turkey is the world's second-largest pistachio producer, following the United States (FAO, 2020). Turkey's pistachio production has shown remarkable growth over the past 50 years, increasing from 15 tons per year in 1970 to a production capacity of 296 thousand tons per year in 2020. According to TUIK (2020), Şanlıurfa, Gaziantep, Siirt, Adıyaman, and Kahramanmaraş are the leading cities in terms of production. The pistachio business has flourished in the provinces of Gaziantep and Şanlıurfa, contingent upon the circumstances.

Long and red types dominate pistachio production in Turkey (Tekin, 2017). As these cultivars reach maturity, their outer shell color changes to a red tint, and they are commonly used in the production of baklava. The baklava and desert industries collect the pistachio approximately 30-40 days prior to its complete maturation. Each day, our nation's distinct peanut harvest flourishes, resulting in the generation of a substantial quantity of peanut shells throughout the process of peanut processing (Barreca et al., 2016). The outer shell of the nut constitutes 10-18% of its total composition (Demiral et al., 2008; Martorana et al., 2013). According to data from 2020, the yearly production of soft outer bark in Turkey amounts to around 30-55 thousand tons. However, a significant portion of this waste stays unused resulting in environmental problems (Abolhasani et al., 2018).

The pistachio is encased in a soft outer bark, which is then surrounded by a tough shell and a yellow-red meaty layer (Ayatollahi et al., 2021). The pistachio develops within and expands to occupy the entire crust, eventually causing the tough crust to fracture. The pistachio's inherent state of being open is a distinctive attribute (Grace et al., 2016). In this process, the pistachio's fragile outer shell generally remains undamaged, providing protection against insects and infections, with few exceptions. Immediate processing of freshly harvested peanuts is necessary to initiate natural chemical processes. This directly impacts the quality of the product's sales, as freezing the granules in the eagerly awaited container might result in stains on the firm outer layer that covers the juicy fruit. Hence, the soft barks derived from processed pistachio are recognized as the most abundant residual product of the post-harvest pistachio manufacturing sector.

Occasionally, by-products in the vegetable food sector might serve as appealing reservoirs of natural antioxidants and bioactive substances. These by-products can be utilized by the food and feed industries to inhibit lipid oxidation or mitigate the consequences of radical oxygen degradation (Balasundram et al., 2006). The plant's soft and hard shells are by-products of the agricultural production. They typically have elevated levels of antioxidant phenolic chemicals as a result of the protective outer shell that envelops the fruit or dish. (Grace et al., 2014). Previous studies have presented bioactive molecules with antioxidant properties in the outer shells of peanuts, beans, strawberries, rice, almonds, and other foodstuffs. (Yen & Duh, 1993; Duh et al., 1997; Watanabe et al., 1997; Ramarathnam et al., 1989; Takeoka & Dao, 2003).

While distilled bioactive products such as pistachio soft bark provide substantial value and advantages, their storage can frequently result in considerable environmental degradation. Consequently, we can categorize it as a formidable waste. These wastes frequently contain bioactive compounds that exhibit antimicrobial properties, rendering them resistant to microbial degradation and unsuitable for composting. When they come into contact with soil and/or water, they might also disturb the microbiome of their habitat. Animal feed rations cannot contain these wastes or can only contain them in minimal amounts because they are high in aromatic volatile compounds. They release their aroma on the consumed animal flesh and dairy. Concurrently, the elevated moisture in these waste materials frequently renders them challenging to incinerate, resulting in an unfavorable carbon footprint and exacerbating climate change. Consequently, disposing of products containing high levels of moisture and bioactive substances, such as peanut peel, directly into natural resources is both a significant environmental catastrophe and a substantial economic detriment.

Studying the biologically active components of *Pistacia vera* L. soft barks is essential since these discoveries can increase the worth of this leftover material in many sectors, such as the herbal medicine industry. The current study characterized the volatile components present in the hydrosol extracted from the pistachio soft bark and emphasized the potential advantages of utilizing these identified volatiles in aquafeeds.

MATERIAL AND METHOD

The economic manufacturer in Siirt provides the necessary pistachios for the task. We conveyed the recently gathered pistachios to the laboratory, doing the examination in containers filled with ambient air to avoid compression and distortion. We utilized a plastic knife to delicately extract the shells from the pistachios before the hydrosol removal procedure, ensuring they remained intact and uncrushed.

Extraction of hydrosol from the soft barks of pistachios. We employed microwave solvent-free extraction equipment (Milestone ETHOS X, Italy) to extract hydrosol from the soft outer barks of pistachios. The system treated 1 kilogram of soft outer bark by introducing 300 mL of deionized water into its reactor. We conducted a 30-minute. operation of the system, applying a power of 800 W, in order to produce hydrosol. At the conclusion of the process, we successfully recovered 170 mL of hydrosol from the outer shell of 1 kg of soft pistachio. Following the processing, we promptly transferred the resultant product to a 250 mL amber container in order to avoid oxidation. The bottle was then sealed with nitrogen gas, and the product was stored for a short duration to facilitate the analysis of its volatile component (Kesbiç, 2019b).

The composition of the hydrosol's volatile compounds was analyzed using pistachio soft shells. The volatile component of the hydrosol was identified through qualitative analysis utilizing a gas chromatography-mass spectrometer (GC-MS) manufactured by Shimadzu (model GCMS-QP 2010 ULTRA, Japan). The material was combined with n-Hexan in a 1:9 (v:v) ratio using a tube mixer (Velp, Classic Vector Mixer, Italy) for analysis. For the investigation, the device utilized RTX-5MS gate columns of the brand (30 m in length, 0.25 mm in diameter, and 0.25 µm in particle size) along with helium as the carrier gas. The column oven temperature was adjusted to 40 °C, the interface temperature was set to 250 °C, and the ion source temperature was set to 200 °C. The volume of the injection was 1 microliter, and the split method with a ratio of 1/5 was employed. During the analysis, we executed a furnace program with the following steps: The temperature was initially set at 40°C and lasted for 3 minutes. The temperature was then increased at a rate of 4°C per minute until it reached 240°C, where it stayed for 10 minutes. After that, the temperature was further increased from 240°C to 260°C. Finally, the temperature was maintained at 260°C for 10 minutes. The entire program lasted for a total of 78 minutes. The peaks with the highest intensity on the chromatogram, obtained after injecting the product extracted from hydrosol into the device, were compared to those in the W9N11 library. The similarity rates of 85% or higher were observed, and the retention times were documented (Kesbiç, 2019a).

RESULTS

The investigation yielded a hydrosol quantity of 170 mL per kilogram of peanut peel. 93.37% of the volatile component profile of the hydrosol generated was analyzed using qualitative methods. Approximately 44% of the identifiable substance is composed of Limonen-4-ol, as indicated in Table 1.

Table 1. The volatile component profile of *Pistacia vera* soft bark hydrosol**.**

No.	Component Name	Retention Time (min)	Percentage (%)
1	Isoamyl acetate	6.996	1.20
2	Limonene	12.565	1.43
3	Eucalyptol (1,8-cineole)	12.646	4.75
4	Linalool	15.477	1.01
5	Ho-trienol	15.638	1.24
6	Carveol	16.824	2.52
7	Limonene-4-ol	18.420	44.60
8	Benzenemethanol, α,α,4-trimethyl-	18.798	7.83
9	α-Terpineol	18.995	7.74
10	Carvone	19.438	5.52
11	Verbenol	19.617	1.42
12	L-Piperitone	21.275	2.10
13	Phenyl acetate	21.406	2.50
14	Bornyl acetate	22.415	6.21
15	β-Pinene	22.926	1.06
16	Carvacrol	23.232	2.24

DISCUSSION

The pistachio soft bark exhibits similarities to pine resin due to its fluid and methanolic extracts, which contain a high concentration of volatile compounds (Arjeh et al., 2020). Additionally, it shares olfactory characteristics with pine due to the presence of similar aromatic components (Sonmezdag et al., 2017). The hydrosol derived from the shells utilized in the study has a comparable abundance of volatile components and encompasses compounds belonging to the pine group. Research has demonstrated that substances derived from *P. vera* barks have antioxidant and antibacterial properties, mostly attributed to bioactive chemicals within them (Tomaino et al., 2010; Smeriglio et al., 2017). A study investigating the protective effects of *P. vera* extract against lipid oxidation found that the extract can effectively inhibit the degradation of soybean fat heated to 60 °C. This extract can be a substitute for the synthetic antioxidant BHA, which has issues with residual substances. The previous study published by Goli et al. (2005) reported that peanut shell extracts have a high concentration of phenolic compounds with strong antioxidant properties. These compounds have the potential to be used as a replacement for synthetic antioxidants such as BHA and BHT. Scientists have discovered that peanut peel's liquid, unprocessed, and refined extracts hinder the growth of gram-positive bacteria (specifically Bacillus cereus) at concentrations of 1.0 and 0.5 mg/mL, respectively. Additionally, these extracts can prevent mutations caused by 2-nitroflorenine, a mutagenic substance found in the same product (Rajaei et al., 2010). Nevertheless, adequate data regarding the phytochemical substances found in the outer barks is lacking. Research has examined *Pistachia atlantica*, a species of interest, to evaluate the antioxidant activity and funding profile of its outer crust, hard crust, and nucleic tissues. Hatamniaa et al. (2014) presented that the outer bark extracts have a higher concentration of total phenolic and flavonoid chemicals and a greater antioxidant capacity than the hard shell and nucleus tissues. According to previous research, these chemicals have been reported to have the potential to provide cost-effective health benefits.

Inflammation is a defensive mechanism that safeguards the overall well-being of the organism by countering physical, chemical, and viral threats. Reactive oxygen (RO) is a natural byproduct due to regular oxidative cell action. While the detrimental impacts of reactive oxygen species (ROS) are acknowledged, ongoing study reveals their potential for regulating and maintaining normal biological processes in living organisms. Environmental stress greatly enhances the production of RO, leading to detrimental effects on the cellular structure. Inducible nitrogen oxide synthase (iNOS) generates nitric oxide (NO), a highly reactive form of nitrogen (RNS), mainly observed in macrophages following stimulation by lipopolysaccharides. The body prevents the accumulation of reactive radicals by utilizing oxygen phytochemicals such as carotenoids, vitamins, and polyphenols found in ordinary foods. These phytochemicals help regulate the activity of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione systems, thereby maintaining a balanced metabolism. (Lei et al., 2016; Scalbert et al., 2005). Previous research has elucidated that pistachio peels are a valuable reservoir of antioxidants. Behgar et al. (2011) suggest that peanuts could serve as a viable substitute for synthetic antioxidants due to their high-quality natural antioxidant properties. Therefore, it is thought that hydrosol extracted from pistachio soft bark will have an effect on ROS, and by consuming both environmentally friendly and nutritionally beneficial food, we can assess these waste products and generate advantages for the relevant industries (Garavand et al., 2016). Agricultural waste is the undesirable waste produced due to agricultural activity (Ramirez-Garcia et al., 2019). In emerging nations globally, the rise in agricultural output leads to a corresponding increase in agricultural by-products and waste. It is important to promptly retrieve agricultural waste, store it to prevent decay, or prepare it for processing to produce the desired end result (Obi et al., 2016). Taghizadeb-Alisaraeia et al. (2017) classified the waste products of pistachio into three categories: soft bark, hard shell, and plant leaves. Reassessing agricultural waste will decrease environmental contamination and yield economic advantages (Shurson 2020).

Feed is stored in industrial fish aquaculture. Alterations occur during the process due to raw materials, including the oxidation of lipids in the feed, an increase in microbial load, and a loss in nutritional values (Hardy & Lee, 2010). The quick degradation of fish feeds is mainly caused by fish oil, which serves as the main raw ingredient (Bell & Koppe, 2010). Scientists have investigated different oil sources that could replace fish oil and use fish harvests for industrial purposes. Nevertheless, scientific study suggests that it is impossible to entirely remove fish oils from aquaculture's species diet, as some species can replace them with alternative oils (Demir et al., 2014; Kesbiç et al., 2023). Due to its high content of polyunsaturated fatty acids, fish oil is prone to oxidation and can lead to deterioration in the feed (Turchini et al., 2010). The feed is stored under non-aseptic storage conditions. Consequently, due to the susceptibility of several bacteria to contamination during the manufacture and storage of feeds, the quantity of grains in the feed also rises during storage. We incorporate numerous feed additives into the meal as a preventive measure against such disruptions (Yitbarek & Tamir, 2014). The initial breeding phases in the past relied on natural feed sources like little fish and silk insects for nourishment. However, nowadays, we utilize industrial fish feed. Industrial feed is a blend of organic and inorganic ingredients that supply the essential nutrients and energy needed for the growth and development of animals (Erteken & Hasimolu, 2005). The substitution of natural feed with concentrated feed resulted in the use of synthetic protection additives. In the past decade, a range of artificial preservatives, including antibiotics, have been employed to maintain the quality of animal feed. However, in recent times, certain governments have implemented limitations and even prohibited using these additives (OJEU, 2003). In aquaculture, feed additives containing antibiotics and other chemicals have been utilized to improve the fish's growth performance and disease resistance. Nevertheless, critics contend that using antibiotics results in drug resistance in the natural microbiota and the accumulation of residual antibiotics in the body. As stated in the Official Journal of the European Union, the European Parliament and the Council have imposed limitations on antibiotics and synthetic growth accelerators to regulate supplements used in animal feed. This action was taken in response to the issues arising from synthetic products. Consequently, a novel practice has arisen in the development of aquatic goods, which entails incorporating non-synthetic additives into the feed, mirroring practices seen in other sectors of natural product production. Scientists have discovered and reported that natural extracts derived from plants have advantageous benefits, including the ability to improve appetite, alleviate stress, enhance the immune system, diminish pigmentation and egg loss in fish, and improve the survival rate of larvae. The main benefit of herbal supplements is their inherent natural state, often enabling direct consumption. Consequently, synthetic chemicals are considered less human and environmentally friendly than these products (Li et al., 2014).

CONCLUSIONS

In conclusion, the hydrosol extracted from the soft shell of P. vera contains a high concentration of bioactive compounds such as Limonene-4-ol. The volatile components of the product mostly consist of molecules with antioxidant and antibacterial properties. This indicates that the product has the potential to function as a sustainable feed additive generated from plants for the fish feed industry**.**

a) Authors' contributions

1. OSK: Designed the study and interpreted the data.
1. OSK: Conducted laboratory work.
1. OSK: Conducted laboratory work and prepared the article.

b) Conflict of interest

The authors declare that they have no conflict of interest.

c) Statement on Animal Welfare

This research does not contain any studies subject to ethical approval.

d) Declaration of Human Rights

This study is within the scope of human rights.

e) Acknowledgements

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