RESEARCH ARTICLE

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Investigation of Biological Properties of Two Pine Cone Molasses: A New Food Source from Turkey

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ABSTRACT: The study, aimed to determine the biological activities of molasses, in which the cones of pine species are used as raw material. The antioxidant activity (radical scavenging power) of two homemade natural molasses obtained from different regions was determined by DPPH and ABTS methods. Ethanol was preferred as a solvent in antioxidant measurements. Antimicrobial measurements of cone molasses samples were made according to the disc diffusion method. Gram (-) bacteria were used in the study. Three different extracts of molasses samples chloroform , ethanol, and methanol, were prepared for antimicrobial measurements. Cefadroxil (CFR 30) was administered as a comparative antibiotic. It was observed that the cone molasses samples had an antimicrobial effect. It was observed that the antibacterial effect changed in different extracts. High efficiency was obtained in the results of DPPH and ABTS radical scavenging activity determinations for cone molasses whose Brix values were measured. Today, free radicals play a role in the formation of many diseases, including cardiovascular diseases, cancer, and diabetes. The increase in the incidence of diseases leads people to seek new antioxidant sources. With this research for the first time, people will understand that pine cone molasses is a nutrient with antioxidant and antibacterial activity.

Keywords: Antioxidant activity, antibacterial activity, pine cone molasses, brix values.

INTRODUCTION

Inadequate and unbalanced food consumption can cause malnutrition, obesity, cardiovascular diseases, anemia, vitamin and mineral deficiencies, and growth and development retardation (Kapil and Bhavna, 2002). Food safety is defined as complying with the necessary rules and taking precautions during the production, processing, storage, transportation, and distribution stages of food to ensure healthy food production, and includes the concepts of healthy, beneficial, and healthy food (Giray and Soysal, 2007). Food safety problems are common in every society and foodborne pathogens have been recognized as an important public health problem in many countries for years (Pires et al., 2012; Xie et al., 2013). In recent years, the increase in the risk of infection caused by microorganisms resistant to antibiotics, the inadequacy of synthetic drugs against diseases, and the widespread observation of their side effects have increased the necessity of using natural products. Apart from this, the fact that many additives are added to foods for preservative purposes, especially those of synthetic nature, creates future concerns in consumers with allergic nature, encouraging organizations serving in this field to use natural preservatives (Sagdıç et al., 2003; Saridogan et al., 2021; Uysal et al., 2023). For this reason, it has been suggested to use medicinal plants as an alternative to drugs, and some traditional plants have also been used as antimicrobial agents and additives (Pires et al., 2012; Mohammed et al., 2023). The usage areas of antimicrobial secondary metabolites obtained from plants are quite wide, and they are used in many areas from preserving raw or processed foods to using them as raw materials for pharmaceuticals, from alternative medicine to natural therapies (Abu Zeid et al., 2010; Akgul et al., 2020). In addition, consumers' prejudices about synthetic additives increase their demands for safe and long shelf-life food products with minimal processing and cause the search for alternative methods for food preservation (Bagamboula et al., 2003). For this reason, intensive studies are still being carried out to identify new antimicrobial agents (Altuner and Canlı, 2012). Here is the some examples of medicinal plants that they used for the pharmacology. In a study it was investigated the protective effects of gintonin, a novel ginseng-derived lysophosphatidic acid receptor ligand, that improves brain functions and protects neurons from oxidative stress, on the developing cerebellum after prenatal and postnatal Pb exposure. The study revealed the ameliorating effects of gintonin against Pb, suggesting the potential use of gintonin as a preventive agent in Pb poisoning during pregnancy and lactation (Nam et al., 2020). Nguyen et al. evaluated ethanol and aqueous extracts of Adenosma bracteosum, used in traditional and modern medicine in Vietnam for curing hepatitis, for their alpha-glucosidase inhibitory activities and anti-hyperglycemic effects on glucose-loaded hyperglycemic and streptozotocin-induced diabetic mice (Nguyen et al., 2020). In another study the phenolic compounds in hydromethanolic extracts of *Salix alba* (L.) leaves and bark, as well as their antioxidant activity and cytotoxic potential examined. Leaf extract may be used as a potential new source of bioactive polyphenols with applications in cosmetics, and bark extracts can also be used but at lower concentrations (Piatczak et al., 2020). Alvarez-Collazo et al., studied whether the citrus flavanone hesperetin (HSP) has potentially beneficial effects on LQT3 syndromes (type 3 long QT syndromes), associated with arrhythmogenic gain

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of function mutations in the cardiac voltage-gated Na+ channel. Their conclusions were that HSP, despite its potential value for LQT3 treatment, is inadequate to treat some genetic variants (Alvarez-Collazo et al., 2020). There is a growing interest in finding and commercializing new compounds with antioxidant properties to prevent or protect against excessive oxidation. Antioxidants prevent DNA damage by acting as electron donors that neutralize Reactive Oxygen Species (ROS) and other free radicals (Willcox et al., 2004; El-Chaghaby et al., 2024). Plants contain many compounds with nutraceutical potential, including phenolic compounds, including a heterogeneous group of molecules with antioxidant potential, maintenance, and repair of DNA, inhibition of nitrosamine formation, cell differentiation, or deactivation of carcinogens (Yesil Celiktas et al., 2010; Mohammed et al., 2024). Considering the difficulty and high economic cost of synthesizing new molecules with antioxidant properties, extraction of such compounds from natural sources such as vegetables or fruits seems to be the most appropriate option (Charles, 2013).

It is a known fact that wild plants play an important role in human health due to their bioactive properties and nutritional content (Hendek, 2017). Although its commercial development is still limited, the bioactive compound content of extracts from pine tree fractions suggests that it may be an interesting and new ingredient that can be used in functional food development. There are few studies on the use of different forms of these fractions such as extracts and powders in foods. Although it is believed that the leaves, cones, and resins of plants belonging to the Pinaceae family have a healing effect on people, they are used in the treatment of various diseases. Pine seeds or nuts appear to have a positive effect on human health because of they contain much linoleic acid (Altaş, 2010).

Molasses is a food known as a sugary product called “bekmes” in Central Asia. It is a kind of thick consistency dessert obtained by cooking grape juice with white molasses soil (Köymen, 1982; Memiş and Ersoy, 2017; Genç, 1982). Although grapes are in the first place, molasses is also made with fruits such as apple, pomegranate, sugar beet, mulberry, plum, watermelon, carob, rose, and pear (Memiş and Ersoy, 2017). This study aimed to investigate the antioxidant and antibacterial activities of homemade samples taken from two different regions of pine cone molasses, which has been widely used in the treatment of various diseases in Turkey since ancient times.

MATERIALS AND METHODS

Natural homemade pine cone molasses obtained from the surrounding villages of Kahramanmaras province were used as material in the study. Molasses samples brought to the laboratory were stored in dark, opaque glass jars at 22 °C.

Brix (Water-soluble dry matter) determination

Abbe refractometer was used for Brix (water-soluble dry matter) analysis of molasses samples.

Measurements were performed at 20°C and results are expressed as °Brix. Before all measurements, the device was calibrated with ultrapure water (Cemeroglu, 1992).

Bacteria Strains

This study was carried out in Harran University SHMYO Biochemistry Research Laboratory between September and November 2021. In the study, three gram-negative strains were used to evaluate the antibacterial effect of pine cone extracts. These bacteria were *Escherichia coli* MTBB 100309, *Acinetobacterium boumannii* MTBB 120557, and *Pseudomonas aeuroginosa* MTBB 130203 strains.

Preparation of Extract Containing Discs and Microorganism Cultures

The disk Diffusion Method (Bauer et al., 1966) was used to determine the antimicrobial activities of the samples. According to this method, 20 mL of the extracts obtained and the solvents used in the preparation of the extracts were impregnated on the commercially obtained empty sterile antibiotic discs with a diameter of 6 mm (Schleicher and Shüll No: 2668, Germany). Cefadroxil (CFR 30) antibiotic disc was also used as a control. Mueller Hinton Agar (Oxoid) was used as the medium to determine the antimicrobial activities of the extracts. Brain Heart Infusion Broth (Oxoid) was used to activate the bacterial cultures to be used in the experiments. Bacterial strains from stock cultures were inoculated into 4-5 ml of Brain Heart Infusion Broth (Oxoid) separately and incubated at 37 °C for 24-48 hours. At the end of the incubation period, after the bacterial suspensions were adjusted with sterile saline according to a 0.5 McFarland standard tube, 0.2 ml of the bacterial suspensions were distributed in 12 cm diameter Petri dishes containing 20 mL of medium (Mueller Hinton Agar). Then, discs impregnated with different extracts aseptically were placed into petri plates that were left to dry at room temperature for 5-15 minutes. In addition, only solvent-impregnated discs were used for control and Cefadroxil (CFR 30) antibiotic discs were used for comparison. Bacteria inoculated plates were incubated at 37 °C for 24-48 hours. At the end of the period, the inhibition zones formed around the discs were evaluated in mm. While measuring the diameters of the inhibition zones, the diameter of the discs was also included in the measurement, and the experiments were carried out in triplicate. As a result, the average of the inhibition zones of each extract and comparison antibiotic was taken into account.

Extraction of molasses samples

Sample groups were created for the extraction of molasses samples. 50 Ml of methanol-water (50:50 by volume) mixture was added to the weighed and 5 g samples. Afterward, the obtained solutions were homogenized with a vortex (Ika, Germany) for 30 minutes. The mouth was kept closed. The samples were kept in a centrifuge (Centurion Scientific K241, UK) at 5000 rpm for 15 min. The supernatant was taken by centrifugation. At the end of this period, the mixture was filtered into dark glass bottles with Whatman no.1 filter paper for analysis. The obtained extracts were stored at +4ºC to be used in the experiments.

Antioxidant Measurements

DPPH free radical scavenging activity was performed according to the Blois method (Blois 1958). A 0.1 mM DPPH solution was prepared in ethanol, and 1 mL of this solution was completed by taking 10, 20, and 40 mL of stock solutions of different concentrations to 3 mL with ethanol and added to the sample solution. These solutions were thoroughly vortexed and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm in a spectrophotometer. The lower absorbance of the reaction mixture indicates higher radical scavenging activity. The DPPH radical scavenging activity was calculated using the following equation.

DPPH Removal Effect (%)= (Absorbance of Control – Absorbance of Sample) / Absorbance of Control X 100

It was made by the color change method, which is an indication that the dark blue/green colored ABTS+ cation radical has lost its radical property as a result of the treatment with antioxidants (Pellegrini et al., 1999). ABTS+ cation radical was obtained by mixing the ABTS solution prepared with 2 mmol L^{-1} H_2O and 2.45 $mmoL^{-1}$ potassium persulfate ($K_2S_2O_8$) solution at a ratio of 1:2 and incubating for 14 hours in the dark and at room temperature. 10, 20, and 30 µL of stock solutions were taken on the extracts obtained from the plant, and phosphate buffer was added until the volume was 3 mL, and then 1 mL of ABTS+ solution was added to them and vortexed. Inhibition was calculated at 734 nm for each concentration. ABTS+ cation radical scavenging activity was calculated using the following equation.

ABTS+ Elimination Effect (%) = (Absorbance of Control – Absorbance of Sample) / Absorbance of Control X 100

Determination of Total Phenolic Substance

The total amount of phenolic substances was determined according to the Folin-Ciocalteu method. (Singleton and EnolViticult). Sample extracts (5 grams of sample: 50 ml of 80% ethanol mixed for 24 hours and filtered) 40 µL of the sample was mixed with 2400 µL of water. 200 µL of folin reagent was added to it, and 600 µL of 2% saturated sodium carbonate solution was added. Afterward, 760 µL of distilled water was added and kept in the dark for 2 hours, and the absorbance was measured at 765 nm. The results are given as gallic acid equivalents (mg Gallic acid equivalent/g sample).

Statistical analyzes

Statistical evaluations were made in the SPSS 22.0 package program. Mann Whitney U test and analysis of variance were used to compare samples with each other. The $p<0.05$ level was taken as the basis for statistical significance.

RESULTS

Brix is a unit of measurement used to calculate the amount of solute in a solution based on the principle of refraction of light in different media. The pine molasses samples used in the research were produced by the traditional production method by boiling in open boilers and removing the water. According to the results of the analysis, Brix values were found to be 67.36% and 64.28% in N1 and N2, respectively (Table 1).

Table 1. *Comparison of Brix values in Cone molasses*

	Briks %
Cone molasses (N1)	% 67.36± 0.14
Cone molasses (N2)	% 64.28± 0.21

Antioxidant Activity

The reducing capacity of DPPH radicals was determined by the reduction in absorbance at 517 nm as a result of the induction of antioxidants. The maximum absorbance of a stable DPPH radical in ethanol was recorded as 517 nm. They make the antioxidant molecules an inactive radicals by donating a hydrogen proton to the DPPH radical. It makes the antioxidant molecules inactive radical by donating a hydrogen proton to the DPPH radical.

The DPPH radical scavenging activity of cone molasses was compared with the radical scavenging activity of the standards (Trolox, BHT, BHA). At the same concentration, the 1st sample (1N) pineapple molasses and the scavenging effect of the standards on DPPH radical decreased in the order Trolox (89%) gt; N1 (76) gt; BHA (75%) gt; BHT (70%) (Figure-1) . In Figure-2, it is seen that the scavenging effect of the 2nd sample (2N) pineapple molasses and standards on the DPPH radical at the same concentration decreased in the order Trolox (89%) gt; N2 (83) gt; BHA (75%) gt; BHT (70%). These results show the conclusion that pine cone molasses has a strong effect on free radical scavenging.

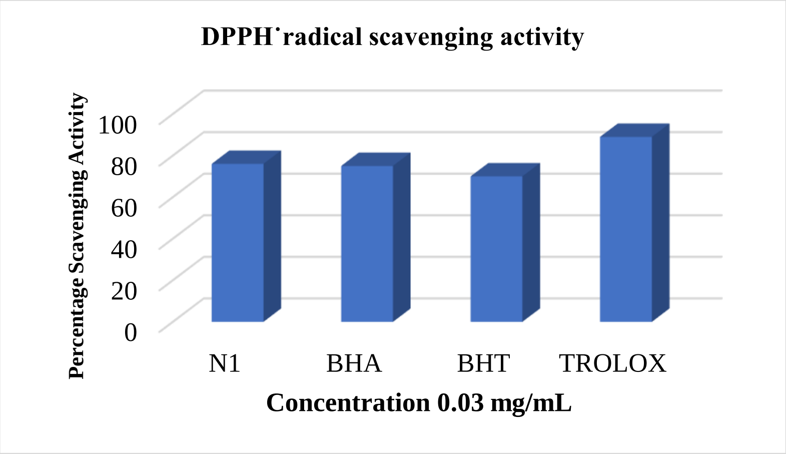

Figure 1. *Comparison of DPPH• radical scavenging activities of ethanol extract of pine cone molasses (N1) with standard antioxidants BHA, BHT, and Trolox*

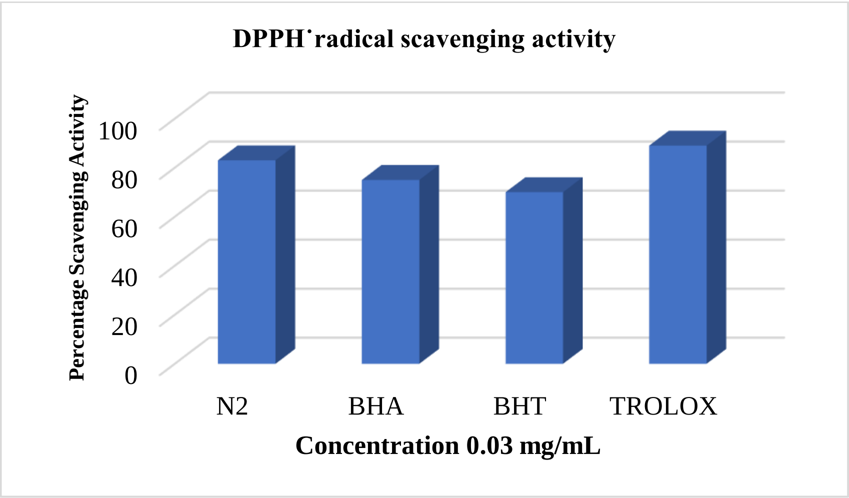

Figure 2. *Comparison of DPPH• radical scavenging activities of pine cone molasses (N2) ethanol extract with standard antioxidants BHA, BHT, and Trolox.*

The blue-green ABTS radical cation scavenging activity of the cone molasses extract was measured relative to the radical scavenging activity of the standard antioxidants BHA, BHT, and Trolox. In this method, antioxidants oxidize the ABTS+ dark-colored cation radical, resulting in a reduction of dark color. The color change that occurs in this reaction is used as a parameter for the measurement of antioxidant potential (Gulcin 2012). The scavenging effect on ABTS+ radical appears to decrease in the order of BHA (100%) > BHT (99%) > N2 (91%) > N1 (88) > Trolox (63%) (Figure 3, Figure 4).

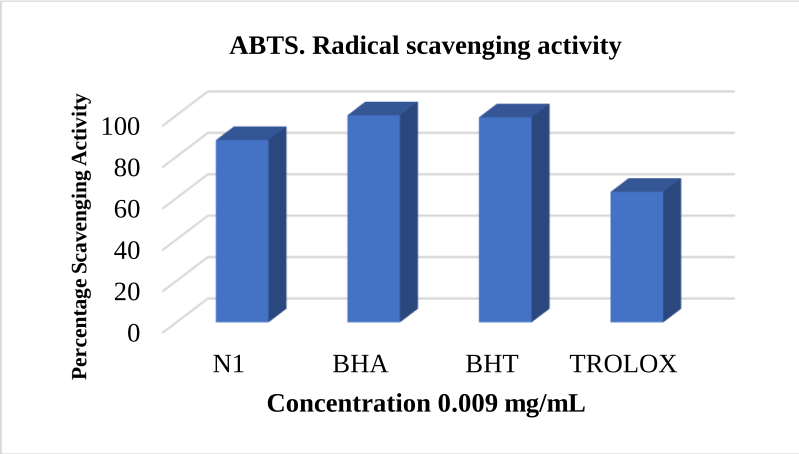

Figure 3. *Comparison of ABTS radical scavenging activities of pine cone molasses (N1) ethanol extract with standard antioxidants BHA, BHT, and Trolox*

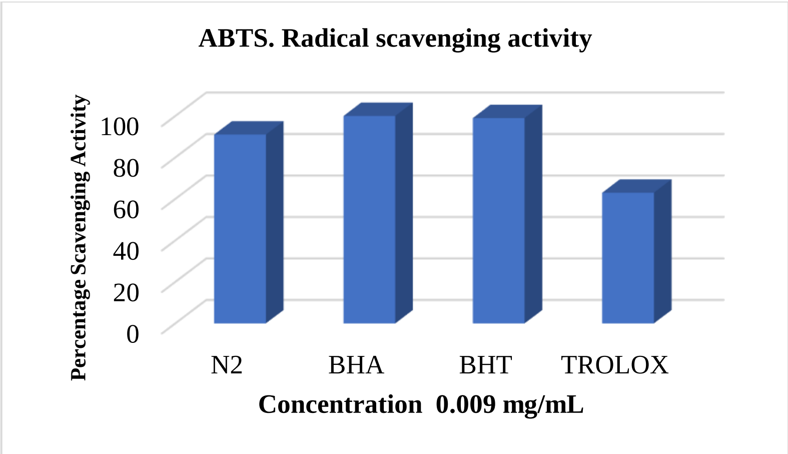

Figure 4. *Comparison of ABTS radical scavenging activities of pine cone molasses (N2) ethanol extract with standard antioxidants BHA, BHT, and Trolox*

Antibacterial Activity Results

Inhibitory effect of ethanol, methanol, and chloroform extracts of N1 and N2 from cone molasses samples against some bacterial species (3 Gram-negative bacteria) Table 1. has also been given.

According to our findings, various extracts of N1 and N2 species prepared with different solvents have a CFR 30 (Cephadroxil) anti-bacterial activity of comparative antibiotic against *Escherichia coli* MTBB 100309, *Acinetobacterium boumannii* MTBB 120557, and *Pseudomonas aeuroginosa* MTBB 130203 against Gram (-) bacteria used in the research. found to have. Values of chloroform, ethyl alcohol, and methyl alcohol solvents are given below (Table 2).

Table 2. *Antibacterial (inhibition zones in mm) results of N1 and N2 standard comparison antibiotics**

Bacteria tested	N1			N2			Comparative antibiotic CFR 30 (30 µg/disk)
	TCM	EtOH	MeOH	TCM	EtOH	MeOH	
Escherichia coli MTBB 100309	12	6	6	-	13	-	25
Pseudomonos aeuroginosa MTBB 130203	11	8	7	9	-	6	27
Acinetobacterium boumannii MTBB 120557	9	-	-	-	-	-	40

(*) The numbers indicate the diameters of the zones of inhibition. Each disc was 6mm in diameter and 20 µl of extract was impregnated. The results are the average of three experiments. tcm: Chloroform extract, EtOH: Ethyl alcohol extract, MeOH: Methanol extract, CFR 30: Cefadroxil, (-): No inhibition.

It was observed that N1 chloroform extract was most effective against *Escherichia coli* MTBB 100309 with a 12 mm inhibition zone, while ethanol and methanol extracts were less effective against the same bacteria compared to the comparison antibiotic with 6 mm inhibition zones. N1 chloroform extract appears to have a high degree of antibacterial effect with an inhibition zone of 11 mm compared to the comparison antibiotic against *Pseudomonas aeuroginosa* MTBB 130203. It was observed that ethanol extract with an inhibition zone of 8 mm and methanol extract with an inhibition zone of 7 mm followed this against the same bacterial species. Chloroform extract of N1 created a 9 mm (table 1.) inhibition zone against Acinetobacterium boumannii MTBB 120557. It was understood that ethanol and methanol extracts of N1 had no antibacterial effect against *Acinetobacterium boumannii* MTBB 120557.

The ethanol extract of the N2 pine cone molasses sample showed inhibitory activity against *Escherichia coli* MTBB 100309 by showing 13 mm zone formation. It can be said that chloroform and methanol extracts prepared from N2 showed moderate antibacterial activity (inhibition zones of 9 mm and 6 mm, respectively) against *Pseudomonas aeuroginosa* MTBB 130203. It was observed that N2 had no antibacterial effect against *Acinetobacterium boumannii* MTBB 120557 in Chloroform ethanol and methanol extract samples.

Total Phenolic Substance Results

In our study, the phenolic content of pine molasses samples was found to be between 890,00 µg GA/g (N1) and 1160,00 µgGA/g (N2), and the differences between them were evaluated as statistically significant ($p<0.05$).

In this study, the biological and some bioactive qualities of pine molasses were obtained by using pine cones traditionally produced in Kahramanmaras city from Turkey. The pine molasses samples in the research were boiled in open boilers with the traditional production method and the water was removed. Since pine molasses is produced on a domestic scale and with local techniques, it has been determined that the data obtained on the investigated qualities are in a wide range.

Brix values of the analyzed pine cone molasses species were found as 67.36 and 64.28.

DISCUSSION

As a result of this reaction, low absorbance is obtained. This interaction is visually noticeable as the color changes from purple to yellow. Therefore, DPPH is often used as a substrate to evaluate the antioxidant activity of antioxidant molecules (Duh et al., 1999; Chang et al., 2002). DPPH is a stable free radical and takes an electron or a hydrogen radical to become a stable diamagnetic molecule.

According to the Turkish Food Codex Grape Molasses Communiqué and TS 3792 Grape Molasses Standard, °Brix must be at least 68% in liquid grape molasses. (Turkish Food Codex 2019). In our study, it is seen that the values of N1 and N2 cone molasses samples are outside the limits. In a study of 15 pinecone molasses samples, Brix values were found between 64.63% and 78.68%. Brix values vary according to the type of fruit used in making molasses. It is reported that the Brix value should be 72% in mulberry molasses according to the TS12001 standard, and 70% according to the TS 13717 standard in carob molasses. No molasses communiqué has been published by the Turkish Food Codex for pine cone molasses.

The results obtained in terms of antioxidant activity and total phenolic content reveal that the bioactive quality of the cone molasses samples (N1, N2) is high. In this study, which was conducted for the first time, the total phenolic content of pine molasses samples was found to be between 890.00 µg GA/g (N1) and 1160.00 µgGA/g (N2), and the differences between them were evaluated as statistically significant ($p<0.05$). When the literature studies are examined, different types of molasses have been studied and different values have been obtained. In a study conducted on grape molasses, the phenolic content was found to be 329.40 mgGAE/100g (Dağ and Tarakçı, 2016). In a different study, the total phenolic content of grape molasses varied between 24.18±0.17 and 25.81±0.38 (mg / L) (Ozcan, 2015). Studies of phenolic content in fruits such as mulberry, apricot, carob, and fruits are recorded in the literature (Aliyazıcıoğlu et al., 2009; Türkben et al., 2015). In our study, similar results were obtained in terms of antioxidant activity . Antioxidant activities were found to be (N1-N2) 88 and 91, respectively, in the analyzes performed by the ABTS method. In the analyzes performed with the DPPH method, the antioxidant activity (N1-N2) values of 76 and 83 were obtained, respectively. It was observed that there was no significant difference between the samples in terms of the radical scavenging effect (P<0.05). ABTS and DPPH radical scavenging activities were found to be 98% and 82%, respectively, in the antioxidant activity study with pomegranate molasses (Aliyazıcıoglu et al., 2009). In the same study, while the ABTS scavenging activity of black mulberry molasses was 94.5%, DPPH activity was evaluated as 59.76%. In the archive studies, we can see only one study evaluating the antioxidant activity of pine cone molasses. According to the results of this study, it is reported that the antioxidant capacity varies between 44.06% and 89.77%.

According to our findings, various extracts of N1 and N2 species prepared with different solvents were found to have different levels of antibacterial activity against *Escherichia coli, Acinetobacterium boumannii*, *Pseudomonas aeuroginosa*. No antimicrobial effect was found in all solutions of pinecone molasses prepared in different extracts. It has been observed that the antibacterial effect is against some strains depending on the type of solution. As no effect was observed in some samples makes the fact that pine cone molasses has an antimicrobial effect is debatable. Phenolic compounds increase the permeability of intracellular vital structures or disrupt the enzyme systems of microorganisms by stimulating the phospholipid layer in the cell membrane of microorganisms (Halender, 1998). It is thought that the antimicrobial effect detected against some bacterial species may be due to the phenolic compounds in the structure of pine cone molasses.

In our research, the most antimicrobial activity was obtained against *Escherichia coli* bacteria. In the study of Jayaprakasha et al. with grape molasses, the highest antibacterial activity was obtained against *Escherichia coli*. In the antibacterial studies with molasses varieties, the inhibition zone range was measured as 6-30.67 (Baydar et al., 2006). In our study, the lowest zone was measured as 6 mm against *Escherichia coli* in the ethanol extract; the highest zone of 13 mm was also observed in the ethanol extract against *Escherichia coli*. It is seen that the inhibition zone intervals vary in different studies (Rhodes, 2005). The value of the inhibition zone range varies depending on the molasses sample extract and bacteria type. In our study, the highest zone of pine cone molasses was measured in the ethanol extract, while in another study, the highest zone value was obtained in the methanol extract.

CONCLUSION

Nowadays, people are moving away from synthetic products containing chemicals and turning to natural products. However, there is an increase in the use of plants with medicinal importance. Many studies have shown that these plants have been used in every aspect of our lives from past to present. Due to increasing needs, the collection, drying, storage and use of these plants from nature should be done in a controlled manner. When the literature studies were examined, no study was found that investigated the bioactive properties of pine cone molasses. With this study, the protection and sustainability of pine forests and ensuring that people stay healthy today when diseases are increasingly common. In addition, it is aimed to raise awareness of pine cone molasses, which is a natural food among people. We believe that the information obtained on the biological properties of pine cone molasses through this research, which was carried out for the first time, will shed light on future in vivo and in vitro studies.

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None

CONFLICT OF INTEREST

There is no conflict of interest between the authors

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