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Research Article

Characterization of total phenolic content and antioxidant property in sweet cherry (*Prunus avium*) kernel extract and oil

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Abstract

During recent years, phenolic content and antioxidant activities of plant products gained lot of interest. Because they suppress the property of proliferation of free radical and protect the human body from various disorders. The cherry kernel, which is obtained from the processing-related waste from cherry fruit, is categorized as a non-consumable waste. In the present study kernel oil of sweet cherry (*Prunus avium*) was extracted by using a Soxhlet apparatus with n-hexane as a solvent and kernel methanolic extract was also obtained. By following the Folin-Ciocalteu method total phenolic content of the extracted oil and methanolic extract was determined. The obtained results showed that the percentage of total phenolic content in methanolic extract was 301.4% whereas 36.2% in the extracted oil. Antioxidant analysis was carried out by DPPH assay. The obtained results of study showed 83.52% inhibition of DPPH for methanol extract, while in extracted oil it was 98.75%. Studies suggest that daily intake of vegetables, and fruits have great importance because they are rich in bioactive substance especially anti-cancerous (breast cancer, colon cancer), antioxidant (free radical, ROS) and anti-inflammatory activity (insect bite, rheumatism, muscle swelling). It can be concluded that the solvent extract and the extracted oil from cherry kernel have relevant bioactive substances and can be used in pharmaceutical, cosmetics, and for curing disease.

Keywords: Antioxidant property; Kernel oil; Phenolic content; Sweet cherry

Introduction

Every year, large quantities of pits are generated during sweet cherry processing, without any substantial use. Cherries are tiny, ripe stone fruit from the genus *prunus* and the Rosaceae family. *Prunus avium* is the most favorite table fruit and it is cherished worldwide due to its tempting characteristics and sweetness **[1]**. Different cultivar of sweet cherry (*P. avium*) grows in European temperate forest. Till now, less than 100 sweet cherry cultivars are grown in the major production regions around the world. Consumption of sweet cherry can reduce the risk of oxidative stress, arthritis, and colon carcinomas etc. **[2]**. Cherry fruit contain low glycemic index, high level of water, low fats level, and calories without

cholesterol. Beside these aspects sweet cherry is often valued for its nutritional properties in addition to the health benefits, especially their bioactive compounds [3]. Quality and maturity of sweet cherry depends on anthocyanidin contents. Diverse phenolics and anthocyanins have been identified in sweet cherry, which contribute to total antioxidant activity. Furthermore, *P. avium* contain several dietary components like carbohydrates, vitamins, proteins, and minerals. It provides prevention from nutritional disorders like various illnesses and congestive heart failure [4, 5]. Cherry kernel oil contain various types of minerals and nutrients with a lots of health benefits. It also contains antioxidants as well as vitamins E [6]. Some health care benefits of cherry kernel include, reduce blood pressure, improve cardiovascular health, good for hairs, boost immune system, prevent various cancer [7]. Being a natural emollient, it improves dryness of skin and improves large pores. It is also helpful for the people with acne prone skin a prevent the skin from sun damaged [8]. In recent year, there is a lot of interest in finding phenolic compounds and antioxidants. Because they suppress the property of proliferation of free radical and protect the human body from disorders [9]. Generally, seeds are considered as waste product which has gained a lot of attention because of environmental concern associated with trash disposal. Additionally, it is widely known due to high value of bioactive compounds can be found in industrial waste such peel, seed, and pomace [10]. In this context, the objective of current work was to evaluate the phenolic content and antioxidant activity in sweet cherry (*Prunus avium*) kernel oil and methanolic extract.

Materials and Methods

Sample collection and storage

Sweet cherries (*P. avium*) were purchased from a local market of Ziarat district of Balochistan, province of Pakistan in July 2019, when farmers and consumers come to trade. At purchase time, information regarding the fruit origin was obtained. Fruit pulp and seeds were separated and then the outer shells of the seeds were removed. Seeds (kernels) were obtained from fruits by hand-processing, then they were grinded in electrical grinder to make powder, weight accurately in a digital balance and deposited in labeled polythene bag for further analysis.

Oil extraction

Thirty grams of seed powder was place in extraction thimble and was positioned into the Soxhlet extractor. The extraction was carried out with n-hexane at 69°C. After the extraction, oil was separated from the extraction solvent using rotary evaporator. The extracted oil samples were stored in the dark bottles at 4°C for further analysis.

Preparation of methanolic extract.

Cherry kernels weighed and soaked in the solvent 70% methanol and was store in airtight glass bottle for 12 days with occasional shaking. After 12 days the solution was stained with nylon cloth. The obtaining extract was stored in the bottle for further analysis.

Total phenolic content (TPC) Assay

The total phenolic content (TPC) of kernel oil methanolic extract was analyzed by applying the Folin-Ciocalteu method.

Procedure for determining total phenolic content (TPC)

Total phenolic content of cherry seed extract was assessed by using the procedure given by Pavlovic *et al.* [11]. In 5 mL extracted oil and 5 mL methanolic extract respectively, was added 2 mL distilled water, and 0.15 mL of 5% $NaNO_2$ solution were combined and incubated for 5 minutes. After that, 0.15mL of $AlCL_3$ 10% solution was added to mixture and then was incubated for 6 minutes before adding 4% NaOH solution. Volume of the reaction mixture was increased to 5mL by adding methanol and mixing thoroughly. After 15 minutes of incubation, the reaction mixture's absorbance was measured at 510nm. Dilution of gallic acids (10-200mg/L) were used as calibration standard. The total phenolic content (TPC)

was measured in mgs of Gallic Acid Equivalents (GAE) per gm of plant extract. Standard curve was constructed using a gallic acid standard solution. Total phenolic content (TPC) and absorbance of extract is presented in (Table 1).

Antioxidant assay by DPPH scavenging activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was employed to evaluate the antioxidant activity of each extract. The procedure of Yen and Chen [12] with slight modification was used to measure the DPPH radical scavenging activity. 1.0mL of freshly prepared 0.004% DPPH in methanol solution was added to 3mL of samples at different concentration, combined solution was placed in dark for about 30 minutes. Then the absorbance was measured at 517nm. An appreciative free radicle scavenging activity is indicated with a low absorbance. The percentage inhibition of the sample was calculated by using the following formula.

$$\% \text{ DPPH Inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of crude extract}}{\text{Absorbance of blank}} \text{X } 100$$

The antioxidant activity of butylated hydroxytoluene (BHT) and ascorbic acid, that serve as control (without plant extract) was also analyzed. All the tests were performed in triplicate.

Results and Discussion

Total phenolic content

Redox properties of phenolic content found in plant allow them to act as antioxidants [13]. Total phenolic content of oil extracted from the cherry (*P. avium*) kernel using the Folin-Ciocalteu assay. The concentration of total phenolic in methanolic extract was higher than those of extracted oil. Therefore, methanol solvent has the capacity to absorb phenol in comparison with hexane. The content varied between 301.4% in methanolic extract and 36.2% in extracted oil (Fig. 1). Phenolic content of kernel extract was similar to those obtained by Prvulovic *et al.* [14]. However, the values obtained for the kernel extract with methanol were higher than those reported by Afonso *et al.* [15].

The bioactivity of the methanolic extract is due to its higher phenolic content. Thus, this extract is expected to have good antioxidant properties. The reason might be due to various methods employ in sample preparation, extraction and could be due to different variety, mainly climatic condition.

Antioxidant assay

DPPH (2-2 diphenyl-1- picrylhydrazyl) scavenging activity is one of the prestigious methods for assessing the antioxidant activity. Because the free electron delocalized over the entire molecule DPPH is a stable free radical when H^+ is donated to the to the DPPH radical. Color of the solution changes violet to yellow. The DPPH radicals scavenging persistent by the antioxidants present in extract and oil determined is presented in the (Table 2 & Fig. 2) displays the absorbance and percentage inhibition of DPPH based on the results obtained. The sample with the larger proportion has a greater capacity for scavenging.

It was found that methanolic extract had an absorbance of 0.423 and a percentage inhibition of 83.52% which is found in agreement with the result where authors reported that the methanolic extract of cherry exhibit remarkable antioxidant activity (88.43%) [16]. Whereas the absorbance of oil was 0.032, with a percentage inhibition of 98.75. It was found that the extracted oil with n-Hexane exhibited remarkable activity, these data were in accordance with that reported by Kelebek & Selli [17]. The obtain result suggest that the extracted oil showed greater percentage of DPPH inhibition, then the methanolic extract. Consequently, it has been shown that kernel of *P. avium* can donate hydrogen ions which help to stabilize the free radicals. As a result, *P.*

avium seed oil is employed as an antioxidant source.

Table 1: Total phenolic content of *P. avium* seed extract

S. No.	Sample	TPC/mg	Absorbance
1	Methanolic extract	301.4	1.605
2	Extracted oil	36.2	0.279

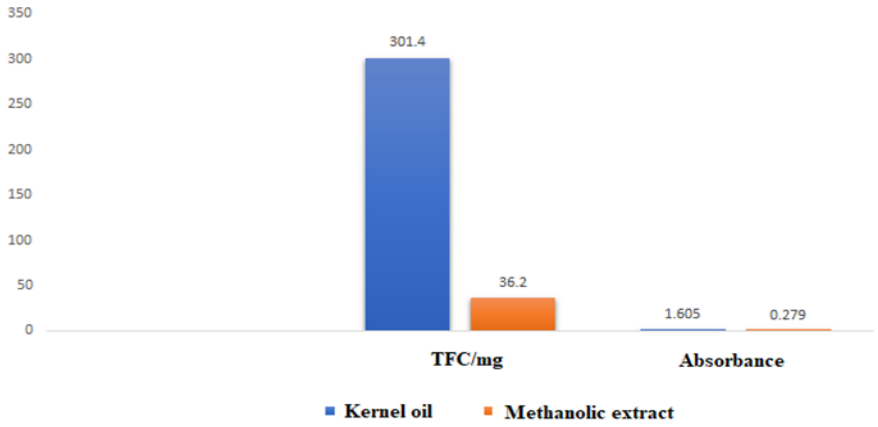

Figure 1: Total phenolic content in *P. avium* Kernel oil and methanolic extract

Table 2: Absorbance and percentage inhibition of DPPH of kernel oil and extract

S. No.	Sample	Blank Abs.	Sample Abs.	% Inhibition of DPPH
1	Methanolic extract	2.567	0.423	83.52
2	Extracted oil	2.567	0.032	98.75

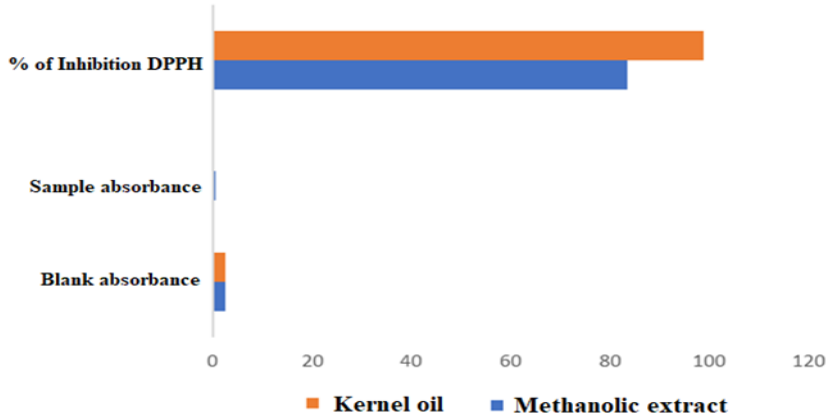

Figure 2: Absorbance and percentage inhibition of DPPH *P. avium* Kernel oil and methanolic extract

Conclusion

Annually, millions of pounds of fruit seeds are disposed of, as a result of fruit processing and domestic intake. This is not only wasting potentially valuable agriculture resources, but it also exacerbates an already major disposal issue. Sweet cherries are grown on a commercial scale, hold a high nutritional and medicinal significance. Cherry berries consist of high content of anthocyanin (red pigment) that decrease pain, inflammation, and help with cardiac disease and diabetes. This study

demonstrates the bioactive qualities of sweet cherry by-products, particularly their richness in phenolics and antioxidants capabilities. However, to investigate the impact of antioxidant preservatives for the food industry, nutraceutical and pharmaceutical formulations on human health, further research is needed and should be done on these by-products.

Authors' contributions

Conceived and designed the experiments: I Saeed, A Sajjad & I Haq Performed the experiments: I Saeed, A Masood & K Baloch, Analyzed the data: A Sajjad & A Masood, Contributed reagents/ materials/ analysis tools: A Hussain & A Masood, Wrote the paper: I Saeed & A Sajjad.

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