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Research paper

Influence of extraction technique on nutrient content, antioxidant and antimicrobial activity of aqueous extracts of commercial apricot kernels

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Abstract

This paper presents the results of influence of extraction technique on phytochemical composition and biological activity of aqueous extracts of commercial apricot kernels. Three techniques were used for extraction: maceration, ultrasonic and Soxhlet extraction. The content of total phenols, flavonoids, bioelements, antioxidant and antimicrobial activity was analyzed in the extracts. Antioxidant activity was analyzed *in vitro* using DPPH and FRAP methods. Antimicrobial screening was performed by diffusion technique on reference strains from the ATCC collection. The content of total phenols and flavonoids is highest in extracts obtained by ultrasonic extraction and maceration. These techniques have proven to be the best for the extraction of macro and micronutrients. The aqueous extract obtained by maceration at 300 rpm for 3 hours showed a greater inhibitory effect against the tested microorganisms, compared to the extracts obtained by other techniques. The least efficient method of extracting bioactive components from apricot kernels is Soxhlet extraction, with the lowest dry extract yield of 5.5%.

Keywords: *Extraction; Apricot Kernels; Polyphenols; Antioxidants; Antimicrobial Activity; Nutrients.*

1. Introduction

Fruits and vegetables are known sources of many nutrients. They contain a large number of bioactive components necessary for the normal functioning of the human body and the treatment of many diseases (Yiğit et al. 2009). The use of natural remedies for the treatment of various diseases has a long history, starting with the Ayurvedic treatment and extending to the Chinese, European and other systems of traditional medicines (Raj et al. 2012). Apricot (*Prunus armeniaca* L.) is classified under the *Prunus* species of Rosaceae family of the Rosales group. Apricot has an important role in human diet, and can be used as fresh, dried or processed fruit (Ramadan et al. 2018). Due to the presence of cyanogenic glycosides (mainly amygdalin) in the apricot kernel, it is today alternatively used to treat various forms of cancer (Yan et al. 2006). In this paper, the influence of the extraction technique with the content of nutrients and phytochemicals, as well as the antioxidant and antimicrobial activity of apricot kernel extracts will be examined.

2. Material and methods

2.1. Chemicals

An apricot kernel sample was purchased at a local market and crushed using an electric grinder and as such used for extraction and other analyzes. Ultrapure water, prepared with a TKA Smart2Pure device, was used for the extraction process. The chemicals used in the experimental part are: methanol, diethyl ether, glacial acetic acid, hydrochloric acid, sodium carbonate, aluminum chloride, sodium nitrite, purchased from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO), 2,2`-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin & Ciocalteu's reagens, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Iron(II) sulphate heptahydrate and iron(III) chloride hexahydrate were purchased from Honeywell (Charlotte, North Carolina, USA). All reagents were used without further purification.

2.2. Preparation of extracts

Three methods were used for extraction: Soxhlet extraction, ultrasonic extraction and maceration. In all three cases, 20 grams of chopped apricot kernels were weighed and transferred to a flat-bottomed balloon or paper tube (in the case of Soxhlet extraction), and poured with 150 mL of ultrapure water. Ultrasonic extraction was performed in an Elmasonic S ultrasonic bath, without heating. Maceration was performed at room temperature with stirring at 300 rpm with Tehtnica Vibromix 40. After four hours of extraction, the extracts were filtered through filter paper. The obtained filtrates were stored in a dark and cool place before analyzing. Before determining the antioxidant activity, the content of total phenols and flavonoids, and bioelements, the extracts were centrifuged at 4000 rpm for five minutes, using a Tehtnica PLC-322 centrifuge.

2.3. Determination of total phenolic content (TPC)

Total phenolic compounds present in the aqueous extracts were quantified spectrophotometrically through the Folin-Ciocalteu test following the protocol (Singleton et al., 1999) with some modifications. 200 µL of extracts was mixed with 2.54 mL of 10% Folin-Ciocalteu reagent. After 5 min 420 µL of 10% sodium carbonate was added. 910 µL distilled water was added to each sample prior to measuring. The absorbance of the resulting blue-coloured solution was measured at 765 nm. Quantitative measurements were performed, based on a standard calibration curve of gallic acid ($y = 0{,}0042x + 0{,}0076$, $R^2 = 0{,}9998$). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gram of apricot kernels.

2.4. Determination of total flavonoid contents (TFC)

Total flavonoid content in the extracts was determined by the previously described method (Olajire and Azeez, 2011), with some modification. 1 mL of extract solution were mixed with 0.3 mL of 5% sodium nitrite. 0.3 mL of 10% aluminium chloride was added after 5 minutes. After 6 minutes incubation at room temperature, 1 mL of 1 M sodium hydroxide was added to the reaction mixture. Immediately the final volume was make upto 10 mL with distilled water. Absorbance of sample was measured against the blank at 510 nm using a spectrophotometer. The results were derived from the calibration curve ($y = 3{,}024x - 0{,}0034$; $R^2 = 0{,}9984$) of quercetin and expressed in quercetin equivalents (QE) per gram of apricot kernels.

2.5. Ferric-reducing antioxidant power (FRAP) Assay

The reducing powers of the extracts that reflected their antioxidant activity were determined following the protocol (Benzie and Strain, 1999). 3 mL of prepared FRAP reagent is mixed with 100 µL of diluted extracts. Absorbance at 593 nm is recorded after a 30 min incubation at 37 °C. The FRAP value was calculated from the calibration curve of iron (II) sulfate heptahydrate ($y = 0{,}001x + 0{,}0698$; $R^2 = 0{,}9997$) and expressed in mol per gram of apricot kernels.

2.6. DPPH radical scavenging activity

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method (Horozić et al., 2019). Lots of solutions were made in tubes by adding different volumes of extract supplemented with up to 2 mL of methanol. 0.5 mL of 0.5 mM DPPH solution were added and the samples were left to incubate for 30 minutes in a darkened room at a room temperature. The absorbance was measured at 517 nm with methanol as a blank sample. 0.5 mL of 0.5 mM DPPH dilution, diluted with 4 mL of methanol, was used as a control sample. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$$[(Ac - As) / Ac] \times 100$$

Where As is the absorbance of the solution containing the sample at 517 nm and Ac is the absorbance of the DPPH solution. The results are expressed as the IC_{50} value (mg/mL).

2.7. Determination of bioelement content in extracts and solid sample

3 grams of chopped apricot kernels were topped with aqua regia and incubated for 16 hours. After 16 hours, the mixture was heated to reflux for 2 hours. The content of bioelements was determined on ICP OPTIMA 2100 DV (Perkin Elmer). Aqueous extracts were taken directly without prior preparation.

2.8. *In vitro* antimicrobial activity

For antimicrobial screening, the prepared aqueous extracts were evaporated to remove water, and the dry residue was dissolved in dimethyl sulfoxide. Antibacterial activity were investigated by diffusion method on reference bacterial strains *E. coli*, *E. faecalis*, *S. aureus*, *B. subtilis*, *L. monocytogenes*, *S. enterica* and *P. aeruginosa*. Antifungal activity of the complexes was tested on *C. albicans*. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared. The strains were then placed on the surface of the nutrient substrate Mueller-Hinton agar, dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 50 and 100 µL of extract solutions in concentration of 100 mg/mL were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at 37°C/24 h. After the incubation period, the size of the inhibitory zone was measured.

3. Results and discussion

The lowest yield after evaporation of the extracts on rotavapor was obtained by Soxhlet extraction (5.5%), while the yield for the remaining two techniques was 27.85% (for maceration) and 31% for ultrasonic extraction. All extracts are light yellow.

3.1. Total phenolic and flavonoids content

Table 1 shows the results of the content of total phenols and flavonoids in the extracts obtained by different extraction techniques. The lowest content of total phenols and flavonoids has the extract obtained by Soxhlet extraction (**U-1**) while the content of these groups of compounds in extracts obtained by ultrasonic extraction (**U-2**) and maceration (**U-3**) is significantly higher but with very close values. This indicates almost equal extraction ability of the above techniques to extract bioactive components from chopped apricot kernels.

Table 1: Content of Total Phenols and Flavonoids in Apricot Kernel Extracts

Extract	TPC [mg GAE/g]	TFC [mg QE/g]
U-1	1.29	0.184
U-2	3.11	0.490
U-3	2.84	0.484

Phytochemical composition and in vitro antioxidant activity have been studied in the core of apricot cultivars grown in northern areas of Pakistan. The total phenol content obtained through this study ranges from 209.4 ± 0.91 to 10.60±0.20 mg GAE/100 g DW, with an average value of 59.41±0.54 mg GAE/100 g (Chen et al. 2020). However, the flavonoid content is lower, probably due to the use of water as the extraction solvent, since flavonoids are most easily isolated by pure organic solvents (acetonitrile, methanol, ethanol, acetone, ethyl acetate) or by a mixture of organic solvents with a lower water content (aqueous methanol) (Stalikas 2007, Xu and Chang 2007). Considering that Soxhlet extraction was carried out at a temperature higher than 70 °C for 4 hours, its isolation efficiency is lower due to the possibility of decomposition of certain phenolic compounds that are subject to oxidation and/or hydrolysis (Havlikova and Mikova 1985).

3.2. Antioxidant activity

The results of antioxidant capacity obtained by FRAP and DPPH method are shown in Table 2. Extracts U-2 and U-3, obtained by ultrasonic extraction and maceration, have the highest antioxidant capacity. The above data correspond to the content of total phenols which are responsible for the antioxidant activity of the samples. Vitamin C has shown extremely higher antioxidant capacity, compared to aqueous extracts (IC_{50} = 0.03 mg/m.

Table 2: Results of Antioxidant Capacity and Reduction Ability of Extracts Obtained by DPPH and FRAP Method

Extract	FRAP value [µmol/g]	IC_{50} value [mg/mL]
U-1	7.62	77.2
U-2	21.32	42.2
U-3	17.71	55.9
Vitamin C	14 250	0.03

Differences in the published values of antioxidant capacity obtained by the DPPH method for apricot kernel extracts may be the result of the geographical origin of the sample, the method of its cultivation, storage and the method of extract preparation. In this regard, it was observed that the extracts prepared for our study generally showed better antioxidant capacity compared to other studies where the extracts were prepared differently (with different extraction techniques and/or solvents).

3.3. Bioelements content

Table 3 shows the results of the content of bioelements in the extracts and the parent sample. Alkaline and alkaline earth elements (potassium, calcium, magnesium and sodium) are the most common bioelements in the sample of apricot kernels and prepared extracts. In a paper published by Tušimová et al. the content of alkaline and alkaline earth elements in apricot seeds was reported, with a slightly higher content of calcium and sodium compared to the sample of seeds used in this study. The magnesium content is relatively similar to our results while the potassium content is significantly higher in the apricot kernels used in our analysis. Gezer et al. they worked on the physico-chemical analysis of five types of apricot kernels. Compared to our results, they reported higher content of iron, zinc, calcium, sodium and potassium, while the content of magnesium was lower. The content of copper and nickel is approximately equal to the results we obtained (Gezer et al. 2011).

Table 3: Content of Bioelements in Extracts and Parent Sample (Mg/Kg)

Bioelement	U-1	U-2	U-3	Apricot kernels
Iron	2,43	7,88	8,19	24,30
Copper	3,39	7,52	7,89	15,66
Zinc	2,85	17,82	23,05	38,23
Nickel	0,64	0,98	1,08	1,10
Selenium	0,48	0,89	0,87	1,36
Calcium	215,1	406,57	399,07	1270,0
Magnesium	710,4	1652,62	1383	1992,0
Sodium	116,1	126,52	132,37	424,33
Potassium	4548,75	8479,28	8311,75	8556,66

Figure 1 shows a graph of the extraction efficiency of micro and macroelements using the extraction techniques used. The efficiency of extraction of bioelements from apricot kernels varies depending on the technique used. As in the case of extraction of organic bioactive components, the most efficient techniques for the extraction of bioelements are ultrasonic extraction and maceration. Soxhlet extraction proved to be the least efficient method for mineral extraction, with extraction efficiencies ranging from 7.45% (for zinc) to 58.18% (for nickel). Almost equal efficiency of the extraction techniques used was found only in the case of sodium (from 27.36% for Soxhlet extraction to 31.19% for maceration), while ultrasonic extraction and maceration were significantly more efficient for the extraction of other bioelements. Bioelements such as iron, copper, selenium, calcium and potassium are extracted by maceration and ultrasonic extraction

with approximately equal efficiency. However, maceration at 300 rpm proved to be slightly more efficient for zinc, nickel and magnesium extraction, compared to ultrasound-assisted extraction.

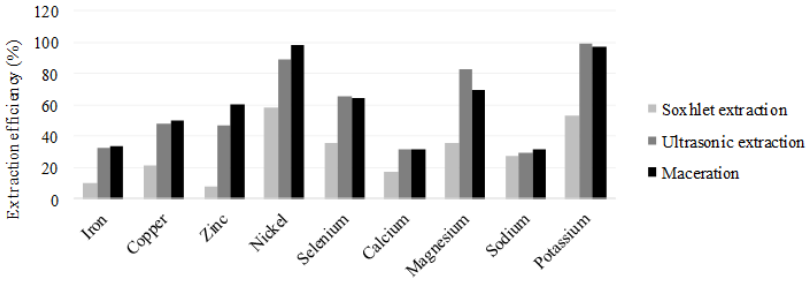

Fig. 1: Efficiency of Bioelement Extraction by Different Extraction Techniques.

3.4. Antimicrobial activity

The results of the antimicrobial activity of aqueous extracts of apricot kernels are shown in Table 4. In the tests in which an extract volume of 50 μL was used, no inhibition zone was recorded. Zones of growth inhibition of the tested microorganisms were recorded for a series of tests in which an extract volume of 100 μL was used. The extracts showed the greatest antibacterial activity against *S. enterica* and *E coli*. The complete absence of antimicrobial activity was noted in the case of *E. faecalis* and *P. aeruginosa*. Maceration and ultrasonic extraction have proven to be the most effective for the extraction of bioactive components from apricot kernels, which have a certain antimicrobial action. The weakest antimicrobial activity was recorded in the aqueous extract obtained by Soxhlet extraction, and the best in the extract obtained by maceration at 300 rpm. Ciprofloxacin (in the case of bacterial strains) and nystatin (in the case of *C. albicans*) were used as controls and showed stronger antimicrobial activity at much lower concentrations compared to extracts, with zones of inhibition greater than 20 mm.

Table 4: Results of Antimicrobial Activity of Extracts

Microorganism	ATCC	Inhibition zone [mm]		
		U-1	U-2	U-3
Staphylococcus aureus	25923	11	-	10
Enterococcus faecalis	51299	-	-	-
Escherichia coli	25922	-	12	12
Bacillus subtilis	6633	-	12	-
Salmonella enterica	13076	10	10	14
Listeria monocytogenes	19118	-	-	11
Pseudomonas aeruginosa	27853	-	-	-
Candida albicans	2091	-	-	10

4. Conclusion

In order to achieve the most efficient extraction and avoid organic solvents, a comparison of extraction techniques for the preparation of aqueous extracts of apricot kernels was performed. For the extraction of organic bioactive components from apricot kernels, ultrasonic extraction and maceration had the most efficient results, while a significantly lower content of bioactive components was recorded in the extract obtained by Soxhlet extraction.

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