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Review Article

Bioactive potential of medlar (Mespilus germanica L.) leaves in terms of ABTS and DPPH antioxidant capacity assays

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Abstract

In strengthening the immune system, demand for natural products is increased rather than preferring food supplements and medicines. Instead of well-known, there are many hidden, valuable, naturally grown in own flora contents only appreciated by local people and utilized as traditional treatments. Medlar leaves are used for antioxidant, diuretic and diabetic properties as one this contents. For pointing the lights on it, antioxidant capacity of extractable, hydrolysable and bioaccessible extractions were evaluated in terms of $TEAC_{ABTS}$ and $TEAC_{DPPH}$ as a scope of this study. Samples were obtained from Yenicaga, Bolu from West Blacksea side of Turkey. Bioaccessible phenolics were determined as 37.41-43.84 μmol Trolox/g for $TEAC_{ABTS}$ and 130.26-145.79 μmol Trolox/g for $TEAC_{DPPH}$. %Bioaccessibility was % 31.43-39.78 over the extractable and hydrolysable fraction. This study was aimed be a clue for the bioactive potential of the medlar leaf and it should be revealed as bioactive contents with their provided effects for leaves on health in detail with further studies.

Keywords: medlar leaves, antioxidant capacity, ABTS, DPPH, %bioaccessibility

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Introduction

Bioactive potential of consumed foods is gained importance for determining their beneficials, improving diet quality for healthier life and preferring more effective foods for better nutrition. For this aim, the basis of the recent researches presents the evaluation of food and food components and determining their potential on health benefits. The cost valued raw materials with high bioactive potential are widespread cultivated and individuals could reach them easily. On the other hand, there are many fruit, vegetable and herbs that are naturally grown in own flora but not getting the deserved awareness and attention. These hidden contents are generally appreciated by local people and utilized as traditional treatments. Medlar is one these valuable sources and not recognized-well.

Medlar (*Mespilus germanica L.*), as a member of Rosacea family, is included in group of pome fruits. Although its homeland is South-West and South-East Europe, it is known that it was brought to Rome at BC 200 and to Greece at BC 700 (Phipps et al., 2003). In Turkey, it grows wild especially in the Marmara Black Sea and Aegean Regions (Yılmaz and Gercekcioglu, 2013). The flowering time of the tree is from May to June and fruits are harvested on September and October (Phipps et al., 2003). The fruits are known as good source of sugars, organic acids, vitamins, minerals (Haciseferogulları et al., 2005) and generally utilized as pickle, vinegar, jam and marmalade by the local people of presented areas. Bioactive potential of fruits is dedicated to phenolic compounds as *p*-coumaric, caffeic, ellagic, ferulic acids and pyrogallol (Voaides et al., 2021). Gulcin et al., (2021) evaluated the antioxidant properties of lyophilized fruits detailed. Voaides et al., (2021) handled medlar with comprehensive review study. They revealed its disregarded potential detailed not only in terms of fruits and leaves but also bud flowers and bark. Antioxidant and antimicrobial effects of medlar are emphasized by authors.

The plum-like fruit has thick, hard, oblong-shaped and hairy leaves with pointed-tips. The up-side of leaves is in dark green color while down-side is lighter green (Maral and Bostan, 2020). Instead of fruits, although not very well known, medlar leaves are picked and dried in autumn also brewed as tea or included in herb mixes because of their health benefits by local people. Voaides et al., (2021) indicated their diuretic, diabetic, hematopoietic effects on health. Alongside this usage, dried leaves are mixed with honey and fruits of resinous trees.

Antioxidants are gain importance with identification of the free radicals and their effect on human metabolism. They are significantly responsible from aging, chronic diseases, various health problems and disruption of metabolic balance. Therefore, consumption of antioxidant-containing foods became very crucial for human nutrition and wellness. In addition to revealing the chemical contents in detail, the bioactive potential determination is important point for understanding effects of these compounds. Antioxidant capacity (AC) assays are commonly used for interpretation of this relation. They are collected under two main headings as hydrogen atom transfer (HAT) and electron transfer (ET) methods (Prior et al., 2005). The most common ones are DPPH (2,2diphenyl-1-picrylhydrazyl), ABTS (2,20-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt), CUPRAC (Cupric Reducing Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power) and Total Phenolic Component analysis (Folin-Ciocalteu Method). these methods are used because of easily applicable and reproducible and provided comparison with the existing literature. Alongside the chosen methodology extraction procedure is more crucial for determining the potential. *In vitro* bioaccessible extraction is provides more realistic estimation by digestion enzymes with pH, temperature and duration

arrangement comparing to solutions that did not exist purely in metabolism.

In this content, this study is included bioactive potential determination of medlar leaves by ABTS and DPPH AC assays for attract notice and provide utilization.

2. Material and Methods

2.1. Material

Medlar leaves belong to *Mespilus germanica L.* species were obtained from Bolu province, Yenicaga district, Dere Village **(ML1)** and Oren Village **(ML2)** on October 2020. Samples obtained from Dere Village were codded as ML1, while Oren Village as ML2 in content of the study. After picking from tree, leaves were brought to laboratory inside of the plastic bag and kept in -18 °C till extraction for analysis.

2.2. Methods

2.2.1. Determination of antioxidant capacity

Medlar leaves were evaluated in terms of ABTS and DPPH antioxidant capacity (AC) assays as for three different bioactive extractions: extractable, hydrolysable and bioaccessible. Extractable phenolic fraction could ne considered as free-, while hydrolysable fraction as bounded-phenolic fractions. Bioaccessible fraction also considered as released potential of phenolics by digestion enzymes: pepsin and pancreatin (with bile salts) in terms of mimic *in vitro* digestion model.

Extraction

Free and bound phenolic fractions were obtained by the methodology of Vitali et al., (2009). 2.0 g of medlar leaves extracted for 2 h at 20°C by shaking water-bath (250 rpm) with 20 mL methanol/water /HCl (80:10:1 v/v). Then the mixture was centrifuged (Sigma centrifuge 3 K 30, Germany) at 3500 rpm for 10 min at 4°C. Supernatant was taken as free phenolic fraction (extractable phenolics), residue was extracted for 20 h at 85°C by shaking water-bath (250 rpm) with 20 mL H_2SO_4 / methanol (1:10 v/v). Then the mixture was centrifuged (Sigma centrifuge 3 K 30, Germany) at 3500 rpm for 10 min at 4°C. Supernatant was taken as bound phenolic fraction (hydrolysable phenolics). The obtained extracts were kept in in -18 °C till analyses.

Bioaccessible fractions were obtained by the methodology of Bouayed et al. (2012). 2.0 g of medlar leaves extracted for 2 h at 37°C by shaking water-bath (250 rpm) with pepsin enzyme (40 mg/mL; pH:2) as gastric conditions. The pancreatic enzyme (2 mg/mL) together with porcine bile (12 mg/mL) were added to mixture for intestinal conditions (pH:7.2) and extraction continued for 2 h at 37°C by shaking water-bath (250 rpm). Supernatant was taken as bioaccessible phenolic fraction. The obtained extracts were kept in in -18 °C till analysis.

ABTS antioxidant capacity assay

Determination of bioactive potential in terms of AC according to ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) assay produced by the methodology of Apak et al., (2008).

7mM ABTS solution (with distillated water) and 2.45 mM $K_2S_2O_8$ solution (with distillated water) were mixed and standed in dark for 12-16 h. The obtained stock ABTS solution was diluted by ethanol solution %1:10 (v /v). The obtained extracts were mixed with ABTS solution and kept in dark for 6 min. Spectrophotometric (Shimadzu UV 1208, Japan) values were determined at 734 nm over concentrated ethanol. Trolox equivalent calibration curve was obtained as $y = 3009.2x - 5.0458$ with $R^2 = 0.9983$. Triplicate results were given as mean±SD in terms of Trolox equivalent antioxidant capacity (TEAC) as μmol Trolox/g sample.

DPPH antioxidant capacity assay

Determination of bioactive potential in terms of AC according to DPPH (2.2diphenyl-1-picrylhydrazyl) assay produced by the methodology of Brand-Williams et al., (1995). The obtained extracts were mixed with 6 $\times 10^{-5}$ M DPPH solution (with methanol) and kept in dark for 30 min. Spectrophotometric (Shimadzu UV 1208, Japan) values were determined at 515 nm over concentrated methanol. Trolox equivalent calibration curve was obtained as y = 2527.1x + 0.4248 with R^2 = 0.9999. Triplicate results were given as mean±SD in terms of Trolox equivalent antioxidant capacity (TEAC) as μmol Trolox/g sample.

% Bioaccessibility

From the obtained results of extractable, hydrolysable and bioaccessible extractions, %bioaccessibility was calculated according to Anson et al., (2009, Eq:1). This value was considered as potential of bioaccessibility over free and bounded phenolics.

$$B\% = \frac{B}{E+H} \times 100 \qquad \text{Eq(1)}$$

**B%: %bioaccessibility; E: Result belong to extractable phenolics for related assay; H: Result belong to hydrolysable phenolics for related assay, B: Result belong to bioaccessible phenolics for related assay*

2.2.2. Statistical Evaluation

Obtained results from AC assays were evaluated by JMP IN 7.0.0 (Statistical Discovery from SAS 2005. Institute Inc.). The mean values obtained with standard deviations and the statistical difference between the two samples in terms of extraction were determined by LSD (Least Significant Difference) test.

3. Results and Discussion

AC results of two medlar leaf samples as extractable, hydrolysable, bioaccessible phenolic fractions are given in Table 1. Samples were obtained from two close villages as Dere Village and Oren Village in Yenicaga district of Bolu (Turkey). Instead of samples-based differences, hydrolysable fraction was showed higher potential than extractable and bioaccessible fractions. Higher temperature (85 °C) with longer extraction duration (20 h) thought to be reason of releasing more of phenolics. For evaluating of bioactive potential, evaluation and prediction the process in metabolism will be more realistic with *in vitro* bioaccessible extractions of digestive enzymes. Bioaccessible phenolics were determined as 37.41-43.84 μmol Trolox/g for $TEAC_{ABTS}$ and 130.26-145.79 μmol Trolox/g for $TEAC_{DPPH}$. %Bioaccessibility was % 31.43-39.78 over the extractable and hydrolysable fraction. As geographical and growing conditions are difference caused parameters on bioactivity, structure and releasability of compounds are effective on obtained results. In terms of AC assays, higher results were obtained with DPPH, and similar potential pattern was given by ABTS as well. In this sense, for potential determination ABTS could be preferred as to shorter waiting time (6 min) of the relevant reagent relaxions in the methodology in order to analyze more samples in the same time.

Conclusion

Recently, individuals are intended to change habits in natural and traditional. West Blacksea, as obtained area of samples, because forestry landscape and traditional knowledge from elders, people are utilized from many naturally grown species. Most of the information are originated from colloquial knowledge. %Bioaccessibility was % 31.43-39.78 over the extractable and hydrolysable fraction in terms of obtained $TEAC_{ABTS}$ and $TEAC_{DPPH}$ results. Studies on this subject will be provided increase the awareness and consume of medlar leaves and other contents.

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Tables

Table 1. Antioxidant capacity results of medlar leaves in terms of different phenolic fractions

Phenolic Fraction	ABTS Assay		DPPH Assay	
	ML1*	ML2	ML1	ML2
Extractable Phenolics as Free (μmol Trolox/g)	47.97 ± 0.12^{b**}	54.83 ±0.57^{a}	241.24 ± 0.52^{b}	246.07 ± 0.44^{a}
Hydrolysable Phenolics as Bounded (μmol Trolox/g)	71.05 ± 0.20^{a}	64.67 ± 0.69^{b}	138.03 ± 1.37^{a}	120.48 ± 0.41^{b}
Total Phenolics Extractable + Hydrolysable	119.02 ± 0.23^{a}	119.50 ± 0.59^{a}	379.27 ± 1.37^{a}	366.55 ± 0.82^{b}
Bioaccessible Phenolic fraction (μmol Trolox/g)	37.41 ± 0.12^{b}	43.84 ± 0.27^{a}	130.26 ± 0.90^{b}	145.79 ± 0.41^{a}
%Bioaccessibility	31.43 ± 0.04^{b}	36.69 ± 0.07^{a}	34.34 ± 0.23^{b}	39.78 ± 0.19^{a}

ML1: Medlar leaf sample that obtained from Dere Village, Yenicaga, Bolu; **ML1: Medlar Leaf sample that obtained from Oren Village, Yenicaga, Bolu*

*** Different letters (a-b) represent statistical significancy ($p<0.05$) in terms of sample for same phenolic extraction*