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Phillyrea latifolia L. :Biological Properties Screening of Different Extracts

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Abstract: *Phillyrea latifolia* L. is widely used as astringent, diuretic and hypoglycaemic in Mediterranean traditional medicine. This work focused on the biological properties (antioxidant and enzyme inhibitory) of *P. latifolia* L. leaves extracts, obtained by different solvents (ethyl acetate, methanol and aqueous). The amount of phenolics and flavonoids in *P. latifolia* L. extracts was also assessed by spectrophotometric methods. The methanol extract showed the highest total flavonoid content (68.07 mg RE g^{-1}). The ethyl acetate extract exhibited stronger DPPH radical scavenging activity (190.71 mg TE g^{-1}). The best CUPRAC activity was shown by the methanol extract (609.38 mg TE g^{-1}). The aqueous extract (14.83 mg EDTA g^{-1}) displayed the highest activity in metal chelating assay. Results showed that ethyl acetate extract indicated the highest activity in enzyme inhibition tests. Considering the obtained data, *P. latifolia* L. has potential to be used as sources of natural antioxidant and enzyme inhibitor

Phillyrea latifolia L: Farklı Ekstraktlarının Biyolojik Özelliklerinin İncelenmesi

Anahtar Kelimeler
Diyabet, *Phillyrea*, Polifenoller, Kolinesteraz, Tirozinaz

Öz: *Phillyrea latifolia* Akdeniz geleneksel tıbbında kanamayı durdurucu, diüretik ve hipoglisemik olarak yaygın şekilde kullanılmaktadır. Bu çalışma farklı çözücülerle (etil asetat, metanol ve su) elde edilen *P. latifolia* L. yapraklarının biyolojik özellikleri (antioksidan ve enzim inhibitör) üzerine odaklanmıştır. Ayrıca, *P. latifolia* L. ekstraktlarındaki fenoliklerin ve flavonoidlerin miktarı spektrofotometrik yöntemlerle değerlendirildi. Metanol ekstrak yüksek toplam flavonoid içerik gösterdi (68.07 mg RE g^{-1}). Etil asetat ekstraktı güçlü radikal süpürme aktivitesi sergiledi (190.71 mg TE g^{-1}). En iyi CUPRAC aktivitesi methanol ekstraktı tarafından gösterilmiştir (609.38 mg TE g^{-1}). Su ekstraktı (14.83 mg EDTA g^{-1}) metal şelatlama deneyinde yüksek aktivite sergiledi. Sonuçlar etil asetat ekstraktı enzim inhibitor testlerinde yüksek aktivite gösterdi. Elde edilen sonuçlar göz önüne alındığında, *P. latifolia* L. yaprakları umut verici bir doğal antioksidan kaynağıdır ve bulaşıcı olmayan hastalıkların tedavisi için sağlıklı yararlar sağlayabilir.

1. INTRODUCTION

In the past decades, the demand for natural products increased due to their potential health benefits against disease [1]. These therapeutic potentials of natural products are due to the presence of bioactive compounds especially phenolic compounds [2]. Many different studies have performed the biological activities including antioxidant and the inhibition of metabolic enzymes of natural products or bioactive compounds and their can reduce the risk of non-communicable including Alzheimer's and diabetes [3-5] Herein, natural products have been considered as a significant source for the development of new drugs [6,7].

Alzheimer's disease (AD) is neurodegenerative disorder, which is characterized by the progressive decline of memory and the progressive loss of cholinergic neurons [8,9]. However, there is no effective treatment for AD yet. The most prescribed drug for the symptomatic treatment of AD is cholinesterase inhibitors [10]. Diabetes mellitus (DM) is a major chronic non-communicable and metabolic disease with hyperglycaemia [11-13]. In 2017 there were just over 451 million were diabetes and this figure is expected to rise to 693 million by 2045 [14]. At present, the most know therapeutic approaches for treatment of DM is inhibition of the α-amylase and α-glucosidase enzymes that are involved in digestion of carbohydrates [15].

Despite the current treatment strategy for abovementioned diseases is enzyme inhibitory (galanthamine for AD, acarbose and miglitol for DM, and kojic for skin disordes). it should be noted that the most commonly used drugs are limited by side effects (e.g., hepatotoxicity, gastrointestinal disturbances, diarrhea) [16-18]. Thus, screening phytochemical profile and pharmaceutical properties from plant for the discovery of new safe and effective drugs have gained considerable attentions.

Phillyrea latifolia L. is a Mediterranean maquis of the Oleaceae family [19]. This plant is used in Spanish folk medicine for the treat of ulcers, mouth inflammation, and as astringent, and diuretic agent [20]. Moreover, decoction and infusion of *P. latifolia* leaves are used for the treatment of kidney stones and as hypoglycaemic in Turkey [21]. Previous data on *P. latifolia* leaves have revealed the presence of iridoids, triterpenoid compounds, flavonoid glycosides, lignan, and phenylpropanoid [20,22,23]. Extracts and isolated compounds have indicated to possess anti-inflammatory and antibacterial activity [20,23,24].

Based on our literature search, there is very little knowledge on phytochemical profile and biological properties of P. latifolia leaves. In this regard, the present study has attempted to investigate the antioxidant capacity and enzyme inhibitory effect as well as total bioactive compounds of *P. latifolia* leaves. After a thorough review, this is the first report to on the antioxidant potential and enzyme inhibitory effect of *P. latifolia* leaves.

2. MATERIALS AND METHODS

2.1. Plant Materials

The leaves of *Phillyrea latifolia* L. were collected in 2018 at Silifke, Mersin, Turkey. Plant material was identified by Dr. botanist Evren Yıldıztugay from the Selcuk University. The leaves were dried at room temperature. Then, the dried leaves were grounded in a laboratory mill prior to extraction.

2.2. Preparation of Extraction

The dried materials (5 g) were macerated with ethyl acetate and methanol (100 mL) for 24 h at room temperature. Ethyl acetate and methanol extracts were evaporated by evaporation in vacuum at 40 °C. To aqueous extract, powdered material (5 g) was boiled with distilled water for 30 min. The aqueous extract was lyophilized. All samples were stored in a refrigerator for further analysis. The yield of the ethyl acetate, methanol, and aqueous extract was 7.75 %, 16.16%, and 30.37 %, respectively.

2.3. Total Polyphenols and Flavonoids

The phenols (TP) and flavonoids (TF) were evaluated by Folin- Ciocalteu and $AlCl_3$ methods, respectively [25]. The results were expressed as gallic acid equivalents for TP. The results were expressed as rutin equivalents for TF.

2.4. Biological Activities

The procedures of antioxidant capacity and enzyme inhibitory assays were given, described in our previously study [25].

2.4.1. *In vitro* antioxidant assays

Antioxidant capacity of *P. latifolia* leaves were assessed using different in vitro assays. The antioxidant results were expressed by using appropriate standard components: trolox for DPPH, ABTS, CUPRAC, FRAP, phosphomolybdenum; ethylenediaminetetraacetic acid for metal chelating.

2.4.2. *In vitro* enzyme inhibitory assays

The enzyme inhibitory potential of the extracts obtained from *P. latifolia* leaves were evaluated against cholinesterase, α-amylase, α-glucosidase and tyrosinase. The enzyme inhibition results were expressed by using appropriate standard components: galantamine for cholinesterase, kojic acid for tyrosinase, acarbose for amylase and glucosidase.

2.5. Statistical Analysis

One-way ANOVA and Tukey's post-hoc test with the confidence level of 95 % were done to assess the difference among samples for each evaluated biological activities. All statistical analysis were performed using R software v.3.6.2.

3. RESULTS AND DISCUSSION

Polyphenols are significant compounds found in plants and they possess potential health effects with high antioxidant properties [26]. The total phenols (TP) and total flavonoids (TF) were assessed by using colorimetric assays and the results are shown in Figure 1 and 2. The ethyl acetate extract exhibited the highest TP (111.37 mg GAE g^{-1}), followed by aqueous (106.71 mg GAE g^{-1}), and methanol (95.26 mg GAE g^{-1}). The TF of different extract was ranged from 35.13-68.07 mg RE g^{-1}.Flavonoids are important group of phenolic compounds and possess multiple health-promoting effects [27]. The results obtained in this work indicated that the phenolic and flavonoids contents of P. latifolia leaves are strongly affected by the different solvent employed. As can be seen on Figure 2, methanol is suitable solvent for extracting total flavonoid content from P. latifolia leaves. According to Ayrancı and Erkan [28], the methanol extract of *P. latifolia* fruit contained a high level TP (1652.9 mg GAE 100 g^{-1} fresh weight).

Many mechanisms have been suggested for the evaluation of antioxidant capacity in vitro. To this end several assays such as ABTS, DPPH, FRAP, CUPRAC, metal chelating and phosphomolybdenum are commonly employed to assess the antioxidant capacity of plants.

Table 1 shows the total antioxidant properties of tested extracts. In DPPH assay, the ethyl acetate and methanol extract (190.71 and 184.77 mg TE g^{-1}, respectively) exhibited high scavenging activity. Nevertheless, aqueous extract (158.04 mg TE g^{-1}) showed weak scavenging activity. Unlike the DPPH assay, the aqueous extract (347.40 mg TE g^{-1}) showed higher ABTS radical scavenging activity compared to the other extracts. In the previously study, the methanol extract of *P. latifolia* fruits showed DPPH (IC50: 69.4 µg ml^{-1}) and ABTS (1.8 mMTE g^{-1}) radical scavenging activity [28]. These values are not comparable to the our results because of values were given in various units.

In the CUPRAC and FRAP tests, all extracts displayed similar activity. In CUPRAC assay, reducing power activity of extracts was in the range from 575.59 to 609.38 mg TE g^{-1}. The FRAP activity of extracts was in the range from 356.72 to 399.09 mg TE g^{-1}. As for phosphomolybdenum assay, the highest total antioxidant activity was determined in ethyl acetate (5.62 mmol TE g^{-1}), followed by methanol (3.93 mmol TE g^{-1}) and aqueous (3.34 mmol TE g^{-1}). The methanol and aqueous extracts showed metal chelating activity, whereas, ethyl acetate extract did not show any metal chelating activity. To the best of our knowledge, study on antioxidant capacity of *P. latifolia* leaves has not presented in literature.

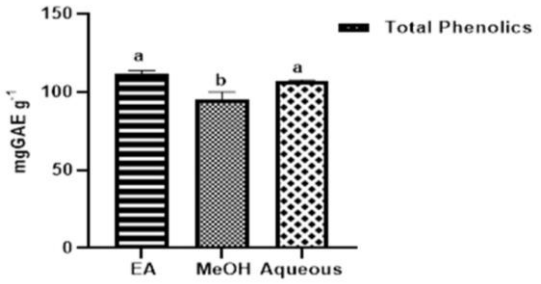

Figure 1. Total phenolic content of *P. latifolia*, GAE: gallic acid equivalents, For each solvent, values in the same column bearing different letters are significantly different at p <0.05

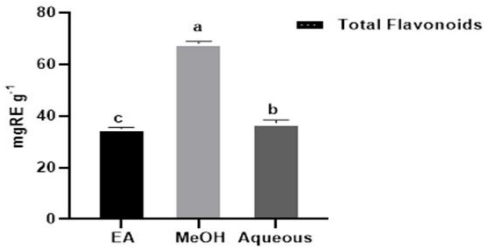

Figure 2. Total flavonoid content of *P. latifolia* RE: rutin equivalents, For each solvent, values in the same column bearing different letters are significantly different at p <0.05

Enzyme inhibitors have long been an attractive strategy for the treatment of non-communicable including Alzheimer's and diabetes. The effect of *P. latifolia* on cholinesterase, α-amylase, α-glucosidase and tyrosinase are displayed in Table 2. The results concluded that all extracts of *P. latifolia* possess anti-cholinesterase, anti-diabetic and anti-tyrosinase activities. In tyrosinase assay, the methanol extract (231.70 mg KAE g^{-1}) showed the highest inhibitory activity, followed by ethyl acetate (214.61 mg KAE g^{-1}) and aqueous (26.18 mg KAE g^{-1}). Tyrosinase plays a crucial role in the biosynthesis of melanin [29]. Nevertheless, an abnormal high level of tyrosinase is associated with several skin diseases such as albinism and skin hyperpigmentation [30]. Hence, the inhibition of tyrosinase may be helpful in cosmetic and pharmaceutical applications.

The ethyl acetate extract exhibited the highest inhibitory effect for all tested enzymes (except tyrosinase). However, aqueous extract exhibited very low enzyme inhibitory effect. For example, the activity of methanol extract was about 9 times of aqueous extract in tyrosinase assay. In the light of the results obtained, the enzyme inhibitory effect of studied extracts was related to total phenolic content. This result is in agreement with previous studies [31,32]. No study on the cholinesterase, α-amylase, α-glucosidase and tyrosinase inhibitory effect of *P. latifolia* leaves has been carried out.

Table 1. Total antioxidant capacity of *P. latifolia*

Assays	EA	MeOH	Aqueous
DPPH assay (mgTE g^{-1}extract)	190.71±8.73*[a]	184.77±10.03[a]	158.04±5.29[b]
ABTS assay (mgTE g^{-1}extract)	256.77±52.95[b]	278.64±24.21[ab]	347.40±20.98[a]
CUPRAC assay (mgTE g^{-1}extract)	599.41±21.36[a]	609.38±4.67[a]	575.59±19.97[a]
FRAP assay (mgTE/g^{-1}extract)	356.72±5.65[a]	357.05±5.55[a]	399.09±6.65[a]
Phosphomolybdenum (mmolTE g^{-1}extract)	5.62±0.29[a]	3.93±0.24[b]	3.34±0.06[c]
Metal chelating activity (mgEDTAE g^{-1}extract)	na	11.55±1.01[b]	14.83±0.98[a]

*Values expressed are means ± S.D., TE: Trolox equivalent; EDTAE: EDTA equivalent, na: not activity; For each solvent, values in the same column bearing different letters are significantly different at p <0.05.

Table 2. Enzyme inhibitory effect of *P. latifolia*

Assays	EA	MeOH	Aqueous
Acetylcholinesterase (mgGALAE g $^{-1}$extract)	4.01±0.07^{*a}	3.49±0.09^{b}	1.51±0.24^{c}
Butyrylcholinesterase (mgGALAE g $^{-1}$extract)	3.95±0.22^{a}	2.68±0.18^{b}	0.71±0.19^{c}
α-Amylase (mmolACE g^{-1}extract)	0.43±0.04^{a}	0.35±0.02^{b}	0.09±0.01^{c}
α-Glucosidase (mmolACE g^{-1}extract)	1.73±0.08^{a}	1.71±0.01^{a}	0.68±0.09^{b}
Tyrosinase (mgKAE g^{-1}extract)	214.61±0.72^{b}	231.70±1.74^{a}	26.18±2.96^{c}

*Values expressed are means ±SD, GALAE: galanthamine equivalets; ACE: acarbose equivalents, KAE: kojic acid equivalents. For each solvent, values in the same column bearing different letters are
significantly different at p <0.05.

4. CONCLUSION

In brief, the biological properties (antioxidant capacity and enzyme inhibitory effect) of *P. latifolia* leaves were assessed in vitro. This is first report on the study of antioxidant and enzyme inhibitory effect of *P. latifolia* leaves. The results showed that extracts of *P. latifolia* leaves have potent antioxidant and enzyme inhibitory effect. Thus, *P. latifolia* could be used natural antioxidant and enzyme inhibitory. Further studies especially identification of the compounds responsible for biological activity is also important..

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