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Antioxidant and anti-inflammatory activity of fruit, leaf and branch extracts of *Paliurus spina-christi* P. Mill.

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ABSTRACT: In this study, it is aimed to investigate antioxidant and anti-inflammatory activities of ethanol extracts and their *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the fruits, leaves, and branches of *Paliurus spina-christi* P. Mill. (Rhamnaceae). The antioxidant activity of the extracts was tested by DPPH and ABTS methods and the results were expressed as IC_{50} values. The total phenolic content was determined by Folin-Ciocalteu method and the results were expressed as mg of gallic acid equivalent per g dry extract. Anti-inflammatory activity of extracts were evaluated against 5-lipoxygenase enzyme. In particular, all extracts obtained from branches of *P. spina-christi*, except hexane extract, showed strong antioxidant activity against DPPH and ABTS radicals. Ethyl acetate and ethanol extracts of branches of *P. spina-christi* showed the highest antioxidant activity in DPPH and ABTS assays with IC_{50} values of 15.54 and 22.06 µg/ml, respectively. Also, the highest total phenol content was found in the ethyl acetate extract of branches of *P. spina-christi* (286.6 mg/g). Ethyl acetate and ethanol extracts of leaves of *P. spina-christi* with IC_{50} values of 66.28 and 76.75 µg/ml were found to have the best anti-inflammatory activity in the lipoxygenase (LOX) inhibition assay used to evaluate anti-inflammatory activities of extracts. These results demonstrate that ethanol and ethyl acetate extracts from branches and leaves of *Paliurus spina-christi* have significant antioxidant and anti-inflammatory activity. Also, these results confirm the folk use of the plant as an antiinflammatory medicine.

KEYWORDS: *Paliurus spina-christi* P. Mill.; Rhamnaceae; Antioxidant activity; Anti-inflammatory activity.

1. INTRODUCTION

The genus *Paliurus*, belongs to the Rhamnaceae family, is represented by one species in the flora of Turkey, *Paliurus spina-christi*. It is spiny shrub, growing two to four meters tall, with arching branches [1]. This species is known by local names such as "Karaçalı", "draga dikeni", "öküz gözü", "çaltı", "ilme", "çaltı dikeni", "sarı çalı", "sarı diken", "çalı bakıldağı", "çantı dikeni" [2-7] and is used as diuretic, antirheumatic, hypocholesterolemic, tonic, expectorant for children, anti-inflammatory, antitussive, tonic as well as in the treatment of cough, diarrhea, chronic obstructive pulmonary disease, abdominal pain, stomach pain, nausea, diabetes, nephralgia, kidney stones, sore throat, bronchitis, atherosclerosis, haemorrhoid, urinary tract infection in traditional Turkish herbal medicine [2-11]. It have also been reported in scientific studies that different extracts of this species have antimicrobial, hypolipidemic, antioxidant, and antigenotoxic activities [11-15]. The leaves, flowers, and fruits of *Paliurus spina-christi* contain flavonoids (quercetin hexoside, quercetin, naringenin-*C*-diglycoside, quercetin 3-*O*-rhamnoglucoside 7-*O*-rhamnoside, quercetin-3-*O*-triglycoside, quercetin-3-*O*-*a*-rhamnosyl(1→6)-*β*-galactoside, quercetin-3-*O*-*a*-rhamnosyl(1→6)-*β*-galactoside-7-O-α-rhamnoside, hyperoside, quercetin-3-*O*-[−*β*-xylosyl(1→2)-*a*-rhamnozil(1→6)]-*β*-glucoside, quercetin-3-rutinoside-7-rhamnoside, isoquercitrin, rutin, kaempferol-3-glycoside), tannins (epigallocatechol, gallocatechol, catechol, gallocatechin, gallocatechin, (+) and (−) catechin, epigallocatechin, catechin hydrate, proanthocyanidin B dimer), phenolic acids (5-caffeoyl quinic acid 5-p-coumaroylquinic acid), coumarins, amino acids, fatty acids, and alkaloids [9,11,12,15-18].

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There are only three studies on antioxidant activity of this species in the literature [9,13,14]. However, antioxidant activities of fruits, leaves and branches of *Paliurus spina-christi* were separately determined for first time in this study. In addition to, the antioxidant activities of the extracts obtained by using different solvents from the different parts of the plant were first evaluated comparatively in this study. Also, no literature could be found in respect of the anti-inflammatory activity of *Paliurus spina-christi*. The antioxidant and anti-inflammatory effects of various solvent extracts from different parts of the plant were therefore investigated.

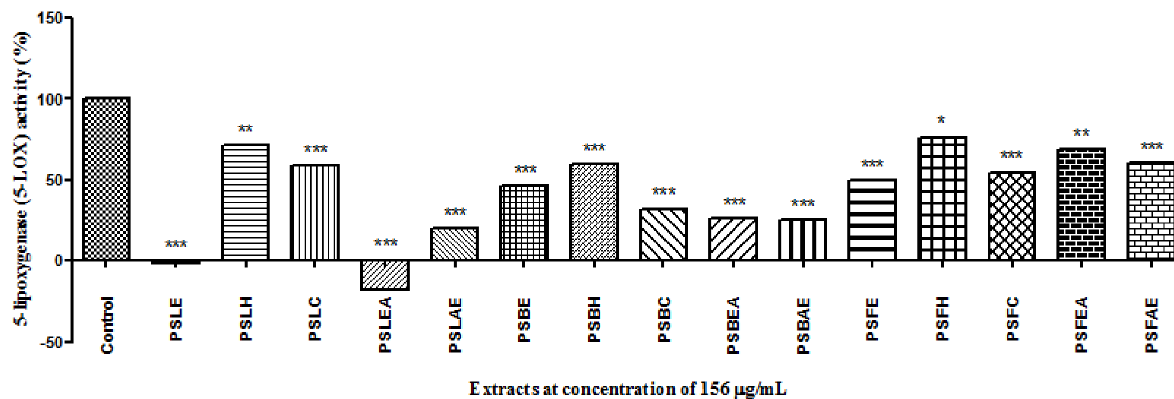
2. RESULTS

Almost all extracts except for the hexane extracts were found to have a significant antioxidant activity. PSBEA showed the highest antioxidant activity with IC_{50} value of 15.54 µg/ml, while PSFH showed the lowest antioxidant activity with IC_{50} value of 2174 µg/ml in DPPH assay. In the ABTS experiment, PSBE exhibited the highest antioxidant activity with IC_{50} value of 22.06 µg/ml, while PSFH exhibited the lowest antioxidant activity with IC_{50} value of 851.0 µg/ml. In both ABTS and DPPH assays, all extracts showed low antioxidant activity when compared to standards. Among all the tested extracts the highest amounts of total phenolic were found in the PSBEA (286.6 mg/g). The total phenol contents of other extracts ranged between 2.44 and 216.2 mg GAE per g extract (Table 1). PSLE and PSLEA inhibited lipoxygenase activity by 100% at a concentration of 156 µg/ml. PSLE and PSLEA showed strong anti-lipoxygenase activity with IC_{50} values of 76.75±1.99 and 66.28±0.73 µg/ml, respectively (IC_{50} for indomethacin: 18.05±0.95 µg/ml). Also, PSFH extract at a concentration of 156 µg/ml showed lowest anti-lipoxygenase activity with inhibition rate of 24.36% (Figure 1).

Table 3. Antioxidant activities and total phenolic contents of various extracts obtained from different parts of *Paliurus spina-christi.*

Extracts*/ Standards	Yield (%)	DPPH activity IC_{50} (µgml^{-1})	ABTS activity IC_{50} (µgml^{-1})	TPC (mg GAE/g extract)**
PSLE	7.03	50.88 ± 0.99^{b,c,d}	46.73 ± 0.72^{e}	106.9 ± 0.65^{g}
PSLH	2.44	1153 ± 35.36^{h}	313.4 ± 0.64^{h}	3.83 ± 0.42^{a}
PSLC	1.26	72.60 ± 1.81^{c,d,e}	46.21 ± 1.10^{e}	76.38 ± 0.68^{e}
PSLEA	0.49	20.19 ± 0.05^{b}	26.19 ± 0.79^{b,c}	210.0 ± 3.98^{j}
PSLAE	2.45	31.11 ± 0.01^{b,c}	41.83 ± 0.92^{d}	149.1 ± 3.66^{h}
PSBE	6.75	18.32 ± 0.11^{b}	22.06 ± 0.17^{b}	216.2 ± 2.92^{j}
PSBH	0.76	272.1 ± 0.42^{g}	92.55 ± 0.76^{f}	6.26 ± 0.16^{a}
PSBC	0.86	27.00 ± 0.65^{b,c}	27.18 ± 0.80^{c}	147.8 ± 0.80^{h}
PSBEA	1.30	15.54 ± 0.02^{b}	23.40 ± 0.47^{b,c}	286.6 ± 0.90^{k}
PSBAE	3.17	20.36 ± 0.04^{b}	27.66 ± 1.35^{c}	194.4 ± 2.28^{i}
PSFE	4.97	96.19 ± 0.98^{d,e}	47.76 ± 0.94^{e}	61.24 ± 2.28^{d}
PSFH	3.12	2174 ± 27.58^{i}	851.0 ± 1.27^{i}	2.44 ± 0.27^{a}
PSFC	0.48	98.21 ± 0.50^{e}	40.47 ± 1.05^{d}	85.13 ± 1.27^{f}
PSFEA	0.20	283.5 ± 2.05^{g}	108.8 ± 0.92^{g}	17.81 ± 0.27^{b}
PSFAE	1.37	189.6 ± 1.41^{f}	112.0 ± 2.55^{g}	36.49 ± 0.90^{c}
Ascorbic acid		2.5 ± 0.24^{a}		
Trolox			3.17 ± 0.03^{a}	

* Abbreviations: PSLE, PSLH, PSLC, PSLEA, PSLAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the leaves of *Paliurus spina-christi*, respectively. PSBE, PSBH, PSBC, PSBEA, PSBAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the branches of *Paliurus spina-christi*, respectively. PSFE, PSFH, PSFC, PSFEA, PSFAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the fruits of *Paliurus spina-christi*, respectively. ** Results were expressed as gallic acid equivalent (GAE). Each value in the table is represented as mean ± SD (n = 3). Different letter superscripts in the same column indicate significant differences ($P < 0.05$).



*p<0.05, **p<0.01, ***p<0.001 versus control.

Figure 1. Anti-inflammatory activity of *Paliurus spina-christi* extracts**.**

3. DISCUSSION

In the present study, the antioxidant and anti-inflammatory activities of ethanol extracts and their *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the fruits, leaves, and branches of *Paliurus spina-christi* were evaluated. PSLEA, PSBE, PSBEA, PSBAE against DPPH radical and PSLEA, PSBE, PSBC, PSBAE against ABTS radical showed significant antioxidant activity.

Only three studies have been found in the literature on the antioxidant activity of *Paliurus spina-christi*. In one of these studies, Jukic et al. (2012) reported methanol, chloroform, ethyl acetate extracts of *Paliurus spina-christi* flos have antioxidant activity against DPPH radical with IC_{50} values of 1.75, 4.15 and 4.35 mg/ml, respectively. Also, total phenol contents of methanol, chloroform, ethyl acetate extracts of *Paliurus spina-christi* were found to be 64.85, 10.27, 5.99 mg/g, respectively [13]. In another study, it was demonstrated that methanol extracts of flower and branches with leaf of *Paliurus spina-christii* have antioxidant activity against DPPH radical with IC_{50} values of 0.211 and 0.302 mg/ml, respectively. In the same study, it was reported that total phenol contents of methanol extracts of flower and branches with leaf of plant was 91.20 and 75.50 mg/g, respectively [14]. Kayalar et al. (2016) have showed that methanol extract of *Paliurus spina-christi* fruits has DPPH radical scavenging activity with IC_{50} value of 0.062 mg/ml. Also, total phenolic content of methanol extract of the plant was found to be 109.54 mg/g [9]. When we compared these results with our current study in terms of ethanol or methanol extracts of the plant, generally there were better results in this study, except for the studies of Kayalar et al (2016). However, the antioxidant activities and total phenol contents of the extracts obtained from the leaves and branches of the plant were better than those obtained in these studies. In particular, ethanol and ethyl acetate extracts obtained from branches and leaves of the plant have been found to have significant antioxidant activity and high amount of phenolic contents. Phenolic compounds present in plants are known to be powerful antioxidants [19]. Also, in the phytochemical studies on different parts of the plant, it have been reported that this species contained phenolic compounds such as flavonoids and tannins intensively. Therefore, the high antioxidant activity of these extracts might be resulting from the high total phenolic contents of them.

No studies on the anti-inflammatory activity of the plant have been reported in the literature. However, some parts of the plant have been used by the people for anti-inflammatory purposes. In our current study, it was found that especially the PSLE and PSLEA had significant anti-inflammatory activity. These results confirm ethno-botanical use of the plant. Also, flavonoids are known as compounds with an important anti-inflammatory activity [20]. Therefore, the reason for the good anti-inflammatory activity of PSLE and PSLEA may be due to the presence of high phenolic compounds.

4. CONCLUSION

These findings suggest that ethanol and ethyl acetate extracts from leaves and branches of *Paliurus spina-christii* are good sources of antioxidant and anti-inflammatory agents. Also, these results support traditional use of the plant for anti-inflammatory purposes.

5. MATERIALS AND METHODS

5.1. Plant material

Plant samples were collected from the Çatalca district of İstanbul province of Turkey in 2009 and identified by Dr. Ahmet Doğan, a botanist of the Faculty of Pharmacy, University of Marmara. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE No: 19205).

5.2. Extraction

About 15 g of each dried and ground the fruits (PSFE), leaves (PSLE), and branches of *Paliurus spina-christi* (PSBE) were extracted with 6×200 ml EtOH, using an ultrasonic bath. After filtration and evaporation, the ethanol extracts was dissolved in 30 ml 60% aqueous ethanol, and subjected to solvent-solvent partition between *n*-hexane (5×50 ml), chloroform (3×50 ml), and ethyl acetate extract (2x50 ml). The *n*-hexane, chloroform, ethyl acetate extract and aqueous ethanol extracts of *Paliurus spina-christi* fruits obtained by this method were coded as PSFH, PSFC, PSFEA and PSFAE, respectively. The *n*-hexane, chloroform, ethyl acetate extract and aqueous ethanol extracts of *Paliurus spina-christi* leaves obtained by this method were coded as PSLH, PSLC, PSLEA and PSLAE, respectively. The *n*-hexane, chloroform, ethyl acetate extract and aqueous ethanol extracts of *Paliurus spina-christi* branches obtained by this method were coded as PSBH, PSBC, PSBEA and PSBAE, respectively. Extraction yields have been summarized in Table 1. All extracts were stored under refrigeration for further analysis.

5.3. Antioxidant activity

5.3.1. DPPH radical scavenging activity

Free radical scavenging capacity of *Paliurus spina-christi* extracts were evaluated according to the previously reported procedure using the stable DPPH [21]. Briefly, 10 µl of extracts in DMSO at different concentrations (250-0.048 µg/ml) were added to 190 µl methanol solution of DPPH (0.1 mM) in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 517 nm. The percent radical scavenging activity of extracts and standard against DPPH were calculated according to the following:

DPPH radical-scavenging activity (%) = $[(A_0–A_1)/A_0]×100$

where A_0 is the absorbance of the control (containing all reagents except the test compounds), and A_1 is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Ascorbic acid was used as positive control.

5.3.2. ABTS radical-scavenging activity

Free radical scavenging capacity of *Paliurus spina-christi* extracts were evaluated according to the previously reported procedure [21]. ABTS radical cations were prepared by mixing equal volume of ABTS (7 mM in H_2O) and potassium persulfate (4.9 mM in H_2O), allowing them to react for 12-16 h at room temperature in the dark. Then, ABTS radical solution was diluted with 96% ethanol to an absorbance of about 0.7 at 734 nm. 10 µl of extracts in DMSO at different concentrations (250-0.048µg/mL) were added to 190 µl of ABTS radical solution in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 734 nm. The percent radical scavenging activity of extracts and standard against ABTS were calculated according to the following:

ABTS radical-scavenging activity (%) = $[(A_0–A_1)/A_0]×100$

where A_0 is the absorbance of the control (containing all reagents except the test compounds), and A_1 is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Trolox was used as positive control.

5.4. Determination of Total Phenolic Contents (TPC)

Total phenolic contents of *Paliurus spina-christi* extracts were measured using Folin–Ciocalteau reagent [22]. The assay was adapted to the 96 well microplate format. 10 µl of extracts in various concentrations (151.52-18.94 µg/ml) were mixed with 20 µl Folin-Ciocalteu reagent (Sigma), 200 µl of H_2O, and 100 µl of 15% Na_2CO_3, and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid was used as a standard and the total phenolics were expressed as mg GAE/g extract.

5.5. *In vitro* anti-inflammatory activity

The anti-inflammatory activity was evaluated according to the method described by Phosrithong et al [23]. An aliquot of 500 µl at different concentrations of *Paliurus spina-christi* extracts was added to 250 µl of 0.1 M borate buffer pH 9.0, followed by addition of 250 µl of type V soybean lipoxygenase solution in buffer (20.000 U/ml). After the mixture was incubated at 25 °C for 5 min, 1000 µl of 0.6 mM linoleic acid solution was added, mixed well and the change in absorbance at 234 nm was recorded for 6 min. Indomethacin was used as a reference standard. The percent inhibition was calculated from the following equation:

$$\% \text{ inhibition} = [(A_{control} - A_{sample}) / Ac_{ontrol}] \times 100$$

A dose-response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum anti-inflammatory activity. All tests and analyses were performed in triplicates.

5.6. Statistical analysis

The data were given as means±standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests using GraphPad Prism 5. Differences between means at $p<0.05$, $p<0.01$, $p<0.001$ levels were considered significant.

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