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Phytochemical Contents and Antioxidant Activity *Paliurus spina-christi* Miller Leaf and Seed Extracts: PASS Predictions, *in silico* Studies on Xanthine Oxidase and Cytochrome P450 1A1

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Abstract

Paliurus spina-christi Miller (PSC) is a shrub plant that has significant biological activities. For this reason, the phytochemical and biological activities of the PSC leaves and seed extracts were investigated in our study. This study performs phytochemical analyses (total phenol and flavonoid content, LC-ESI-MS/MS, and GC-MS/MS) and bioactivity assays (antioxidant) for the PSC leaves and seeds. *In silico* study and PASS prediction of main compounds in LC-ESI-MS/MS and GC-MS/MS analysis were also investigated. The leaf extract showed a high total phenolic and flavonoid content. The hesperidin content (25.548 mg/g extract) was high in the LC-ESI-MS/MS and GC-MS/MS analyses. It was noted that the leaf extract's antioxidant activities were higher than standard. The molecular docking of hesperidin with xanthine oxidase and cytochrome P450 1A1 had high MolDock score (-179.68 and -149.156) and binding energy (-11.40 kcal/mol and -9.90 kcal/mol), respectively. This investigation pioneered using PSC leaf extracts as food supplements and medicine.

Key Words: *Paliurus spina-christi* Miller, Phytochemical content, Antioxidant activity, Molecular Docking, PASS prediction

1. Introduction

Different isoforms of cytochrome P450 enzymes (CYP) are essential in the metabolic process of xenobiotics and other substances (Jomova et al., 2023). CYP is an extrahepatic enzyme found in very low amounts in the lung and liver. Understanding the structural basis of CYP1A1 specificity is crucial for understanding the function and mechanism of enzymes and may also provide a basis for the sensitive development of drugs and inhibitors (Yan et al., 2016).

Lowering urate levels in the blood by promoting uric acid (UA) excretion or blocking uric acid synthesis is the most important therapeutic measure for gout patients (Hou et al., 2023). Xanthine oxidase (XO) is an enzyme that catalyzes hypoxanthine conversion to xanthine and, ultimately, to UA. XO is an important and

specific target for treating diseases related to hyperuricemia and gout (Singh et al., 2020).

Paliurus spina-christi Miller (PSC) is a shrub plant belonging to the *Rhamnaceae* family. It is a woody, perennial plant widespread in Turkey, southern Europe, the Balkans, and the Caucasus (Kaya & Arslan, 2021). PSC contains tannins, alkaloids, sterols, flavonoids, polyphenols, and natural-free fatty acids (Güner, 2005). The fruits of PSC are used in phytotherapy to prepare infusions that aid in removing uric acid and to prepare cosmetic formulations that address greasy skin because of its diuretic qualities. PSC, the species known in Artvin, is used by the public for medicinal purposes and as food (Eminağaoğlu, 2015; Erşen Bak & Çifci, 2020, 2022). PSC products are used as external wound healers for treating edema, antidiabetic, menstrual regulators, diuretics, and against infections (Harşıt, 2015). In addition, its active use in treating PSC urolithiasis has been reported in the literature (Bozyel & Merdamert-Bozyel, 2018). Due to the bioactivity mentioned above of PSC leaves and seeds, this study aimed to perform phytochemical analyses and bioactivity assays of PSC grown in Artvin-Ardanuç, which were conducted for the first time. In addition, PASS studies were performed using pharmacokinetic analyses.

2. Material and Methods

2.1. Plant Material and Extraction

PCS plant was harvested from the Ardanuç district of Artvin province, Turkey, and identified by Prof Dr. Özgür EMİNAĞAOĞLU, Department of Forest Botany, Faculty of Forestry, Department of Forest Engineering, Artvin Çoruh University. The seeds and leaves parts of the plant were separated and dried. The dried samples were ground into powder using a grinder. Methanol is mainly used to extract various polar compounds and is highly efficient. Therefore, methanol is often used to extract bioactive compounds (Y Başar et al., 2024). Hexane is a solvent widely used to extract products such as vegetable oils, fatty acids, fats, flavors, fragrances, color additives, or other bioactive ingredients (Cravotto et al., 2022). The seeds were extracted with hexane, and the leaves were extracted with methanol. The extracts obtained were stored under suitable conditions (+4 degrees) to analyze biological activity and phytochemical content.

2.2. LC-ESI-MS/MS and GC-MS/MS Analysis

We used LC-ESI-MS/MS (Agilent, 1200 Series Agillent Technologies 6460 TripleQuad HPLC-ESI-MS/MS) analysis to investigate the phenolic contents and quantities of the PSC methanol extract, as detailed in our already published study (Y Başar et al., 2024). Thirty-four phenolic standards were used for the investigation. The content analysis of the PSC hexane extract (seed) and methanol extract (leaf) was performed using a GC-MS/MS (Agilent 7000 A GC/MS Triple Quad with 7890 GC) instrument. The instrument conditions and method were described in detail in our previous study (Y Başar et al., 2024).

2.3. Total Phenol (TP) and Flavonoid (TF) Contents

The TP content (Folin-Ciocalteu Method) and the TF content (Aluminum Chloride Method) of methanol extract of the leaves and the hexane extract of the seeds of PCS was determined. Gallic acid (total phenol) and quercetin (total flavonoid) were used as standards (Golmakani et al., 2014).

2.4. Antioxidant Activity Assays

The antioxidant activity of the methanol extract of the leaves and the hexane extract of the seeds of PCS was tested with phosphomolybdenum reduction assay (PMRA)(Mohamed et al., 2007) and $DPPH^{\cdot}$ scavenging (Blois, 1958) activity. Also, the results were compared with standard ascorbic acid, recorded with $A_{0.5}$ (PMRA) and IC_{50} ($DPPH^{\cdot}$ scavenging), and expressed as µg/mL.

2.5. Statistical Analysis

ANOVA was used because the results of the three-way activity with standard deviation and the data obtained with the SPSS software showed a normal distribution. The data obtained were subjected to a multiple comparison test (Tukey HSD^{a,b}). The level of statistical significance was expressed as $p<0.05$.

2.6. Molecular Docking Studies

In the molecular docking studies, the molecular structures were drawn in ChemDraw ultra 18.0, the minimal energy was adjusted with Chem3D 18.0 programs, and the molecular structure was saved in mol2 format. XO [3NRZ], and CYP450 1A1 [4I8V] were selected by RSCB (Protein Data Bank). To determine the interaction of a molecule with enzymes, the active side Molegro Virtual Docker (MVD) and AutoDock Vina programs were used. All data were integrated to observe molecules' 2D and 3D interaction with enzymes' active sites using the Discovery Studio program (Yunus Başar et al., 2024; Yenigun et al., 2024).

2.7. PASS Prediction

The PASS analysis to determine the bioactivity spectra of compounds was performed via the PASS online web server (http://www.pharmaexpert.ru/passonline) (Lagunin et al., 2000). The PASS prediction compares the probability of being active (Pa) and probability of being inactive (Pi) of compounds based on their canonical smile. With an accuracy of 90%, this tool is intended to predict a wide range of biological activity.

3. Results and Discussion

3.1. LC-ESI-MS/MS and GC-MS/MS Analysis

Analysis of the phenolic content of the methanol extract of PSC leaves by LC-ESI-MS/MS. According to the analysis results, 15 phenolic compounds were detected. Accordingly, high amounts of hesperidin (25.548 mg/g extract) and rutin (9.687 mg/g extract) were determined (Table 1 and Figure 1).

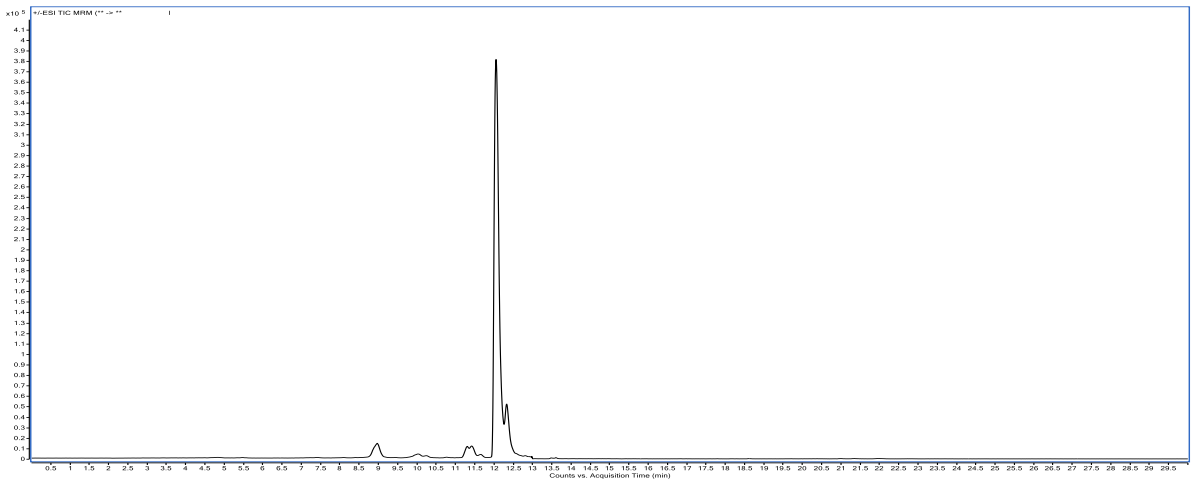

Figure 1. The LC-ESI-MS/MS chromatogram of the methanol extract of PSC lea

The fatty acid content of the hexane extract of the seeds and the methanol extract of the leaves of PSC was determined by GC-MS/MS. According to the analytical results, palmitic acid methyl ester (46.08%), linoleic acid methyl ester (29.93%), oleic acid methyl ester (15.51%), and stearic acid methyl ester (7.39%) were determined in the highest amounts in the PCS leaves. In comparison, palmitic acid methyl ester (36.32%), linoleic acid methyl ester (28.46%), oleic acid methyl ester (27.89%), and stearic acid methyl ester (7.34%) were determined in the highest amounts in PCS seeds (Figure 2 - Table 2).

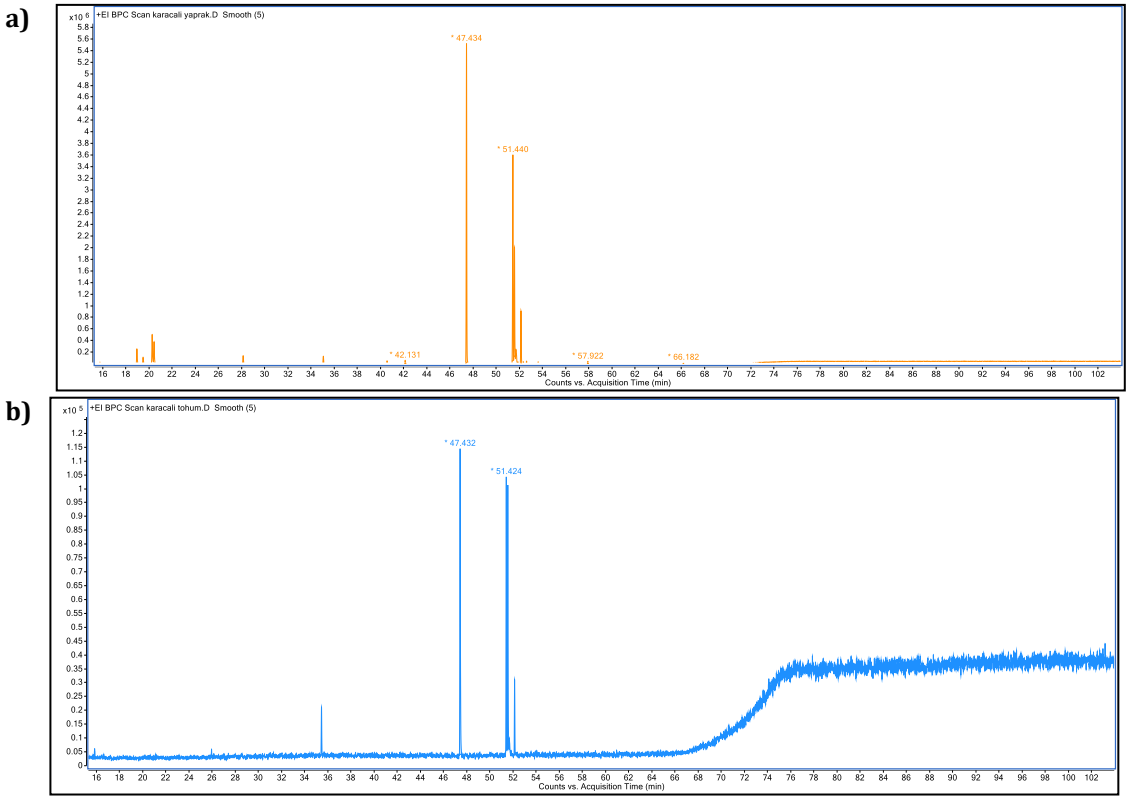

Figure 2. The GC-MS/MS chromatogram of the hexane extract of PSC leaf (a) and seed (b)

The phytochemical properties of this plant have been studied previously. The LC-ESI-MS/MS study of the fruits also showed that of the 22 phenolic components, rutin (233 µg/g) and malic acid (283 µg/g) had the highest values (Takım & Işık, 2020). In a similar study, the constituent analysis of the water extract of the fruits of *P. spina-Christi* using LC-ESI-MS/MS determined malic acid (17.54 ± 2.00 µg), quinic acid (382.78 ± 14.00 µg), hesperidin (47.44 ± 16.00 µg), rutin (98.75 ± 24.00 µg) and catechin (58.69 ± 13.00 µg) as the main constituentsTakım (2021).

3.2. TP and TF Contents and Antioxidant Activities

The TP and TF content in the methanol extract and hexane extract of PSC leaves was determined to be 16.98±1.40 mg GAE/g extract and 0.34±0.09 mg QE/g extract, respectively. In contrast, the TP and TF content in the methanol extract of PSC seeds was 1.10±0.11 mg GAE/g extract and 0.11±0.00 mg. It was determined as QE/g extract. From these results, the leaf extract's TP and TF content was higher than the seed extract's (Table 3).

In a study by Zengin, et al. (2023), it was found that the fruits of P. spina-Christi have a high TP content (75.91 ± 0.58 mg GAE/g) in the methanol extractZengin et al. (2023). The water, ethyl acetate, *n*-hexane, and dichloromethane extract were also investigated, and these extracts were observed to be lower than the methanol extract. Similarly, TF content ranged from 0.14±0.03 to 17.55±0.09 mg RE/g, and methanol extract had higher TF content than other extracts. It was found that the methanol extract of PSC fruit in our study had a TP content value of 16.98±1.40 mg GAE/g, which was lower than the value in the literature. This difference may be due to the region where it grows. Previous research has been done on this plant's phytochemical characteristics and found that the TF content of the fruit extract was 8.29±0.07 mg QE/g dry plant, while the TP content was 22.10±0.09 mg GAE/g dry plant (Takım &

Işık, 2020). The EA extract of *P. spina-Christi* branches had the most outstanding TP content (286.6 mg/g) in a different investigation, Şen (2018), but the other extracts varied from 2.44 to 216.2 mg GAE per g extract.

Table 1. The LC-ESI-MS/MS compounds of the methanol extract of PSC leaf

No	Compound name	RT (min.)	PSC Leaf (mg/g extract)
1	Epigallocatechin	7.626	0.025
2	Chlorogenic acid	8.946	0.187
3	Vanillic acid	11.370	0.555
4	Caffeic acid	10.058	0.017
5	Hydroxybenzaldeyde	10.431	0.014
6	Vanillin	10.856	0.007
7	Rutin	12.078	9.687
8	*trans*-Ferulic acid	12.618	0.124
9	*o*-Coumaric acid	11.484	0.045
10	Taxifolin	11.517	0.043
11	Salicylic acid	12.367	0.207
12	Isoquercitrin	12.116	0.777
13	Hesperidin	12.078	25.548
14	Morin	12.483	0.026
15	*trans*-Cinnamic acid	13.721	0.017

RT: Retention Time, PSC: *Paliurus spina-christi*

Table 2. The GC-MS/MS fatty acids of the methanol extract of PSC leaf and seed

No	Compound Name	RT (min.)	PSC Seed (%)	PSC Leaf (%)
1	Myristic acid, methyl ester	42.13	-	0.51
2	Palmitic acid, methyl ester	47.43	36.32	46.08
3	Linoleic acid, methyl ester	51.44	28.46	29.93
4	Oleic acid, methyl ester	51.58	27.89	15.51
5	Stearic acid, methyl ester	52.14	7.34	7.39
6	Heneicosanoic acid, methyl ester	57.92	-	0.44
7	Behenic acid, methyl ester	66.18	-	0.14
Total			100	100

RT: Retention time, PSC: *Paliurus spina-christi*

For DPPH· scavenging activity, the IC_{50} value of methanol extracts of PSC leaves and seeds were found to be 15.11±0.85 µg/mL, and 2.20±0.12 µg/mL, respectively, while the IC_{50} value of ascorbic acid was determined to be 42.15±1.35 µg/mL (Table 3). Based on these results, it was found that the DPPH· scavenging activities of the PSC leaves and seed extracts were higher than that of the standard, and the seed extract was higher than all others.

In the study by Zengin et al. (2023), methanol extract showed the most potent antioxidant effect among the different extracts of *P. spina christi* fruit in terms of DPPH· filtering (245.59±4.46 mg TE/g) and FRAP reducing power (292.94±6.60 mg TE/g). This study also found that although the W extract demonstrated high, it showed lower antioxidant activity than the methanol extract. Using prior findings, Şen [23] evaluated the antioxidant activity of P. spina-christi fruit, leaf, and branch extracts. It was found that all extracts except the H extract of the twigs exhibited high antioxidant activity, with the ethyl acetate extract showing an IC_{50} value of 15.54 µg/mL in DPPH· analysis. Another study investigated the DPPH· scavenging activity of methanol extracts from leaves and fruits. The IC_{50} value was determined to be 53.41±1.24 µg/mL for the extract from the leaves and was lower than that of ascorbic acid (86.06±1.92 µg/mL) (Grande et al., 2024).

In reducing power capacity, the $A_{0.5}$ value of PSC leaf methanol extract was 54.60±0.20 µg/mL, and the $A_{0.5}$ value of ascorbic acid was recorded as 87.24±2.44 µg/mL (Table 3). According to these results, it was found that the reducing power capacity of PSC leaf extract was higher than the standard. The extract from the seeds showed no signs of action.

Table 3. Antioxidant activity, TP, and TF contents of PSC extracts

Sample/ Standard	Yield, %	TP content, mg GAE/g	TF content, mg QE/g	Antioxidant activity	
				PMRA, $A_{0.5}$: µg/mL	DPPH· scavenging, IC_{50}: µg/mL
PSC Leaf	3.84	16.98±1.40	0.34±0.09	54.60±0.20^{a}	15.11±0.85^{b}
PSC Seed	4.98	1.10±0.11	0.11±0.00	-	2.20±0.12^{a}
Ascorbic acid	-	-	-	87.24±2.44^{b}	42.15±1.35^{c}

PSC: *Paliurus spina-christi,* PMRA: Phosphomolybdenum reducing assay, $p<0.05$

3.3. Molecular Docking Studies

Hesperidin molecule interacted with CYP1A1 by three CHBs (GLY459), two carbon-HBs (GLY318, GLY459), two pi-sulfur (CYS457), one pi-pi t-shaped (PHE450), three alkyls (LEU496, MET121, ILE458), and three pi-alkyl (VAL382, LEU496, ALA463) (Figure 3 - Table 4). Palmitic acid molecules interacted with CYP1A1 by three CHBs (TRP131, ARG135, MET121), one carbon HB (ARG455), and fourteen alkyls (ALA317, VAL322, CYS457, ALA463, VAL197 ILE198, ILE201, ILE462, ILE458, MET121, ILE458) (Figure 3 - Table 5).

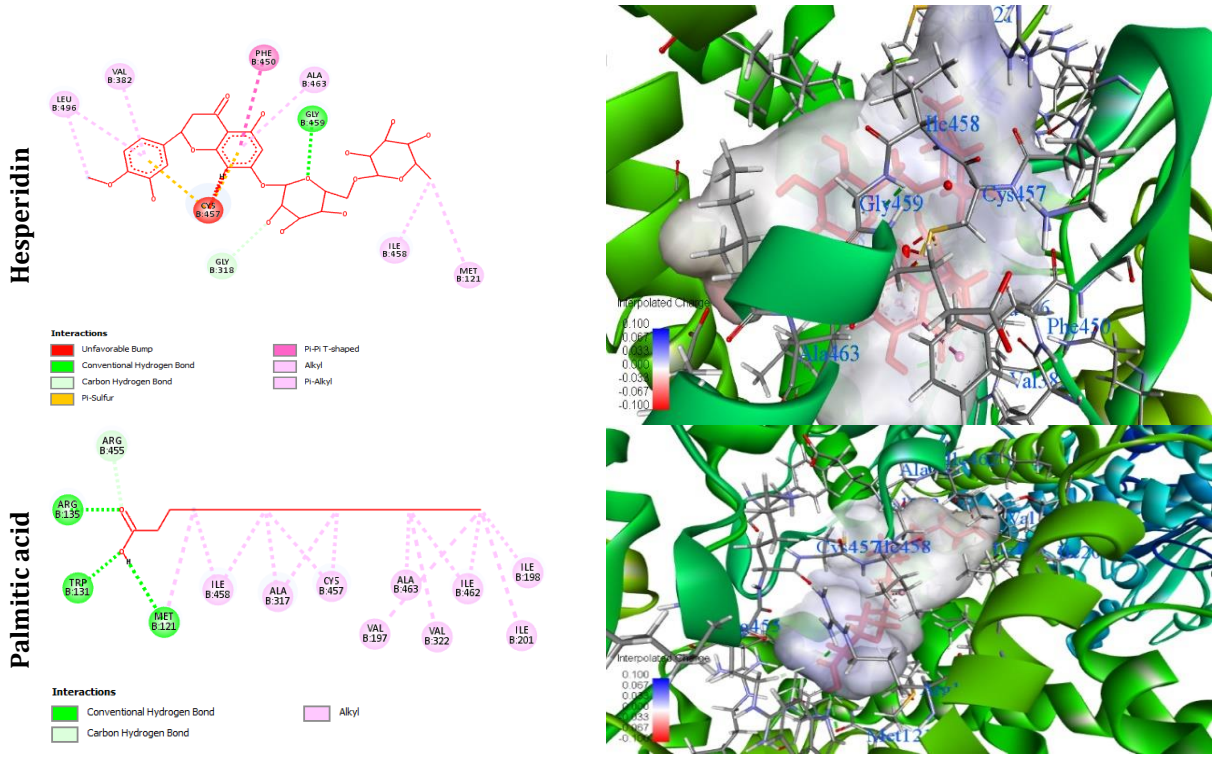

Figure 3. Hesperidin, and palmitic acid interaction with CYP1A1 2D images, and 3D interpolated load vie

Table 4. Interaction types, categories, and distances of molecular insertion of the hesperidin with cytochrome P450 1A1

No	Name	Distance	Category	Type	From Chemistry	To Chemistry
1	B:GLY459:HN - :[001:O4	3.01625	HB	CHB	H-Donor	H-Acceptor
2	:[001:H7 - :[001:O6	2.75856	HB	CHB	H-Donor	H-Acceptor
3	:[001:H34 - :[001:O15	1.95969	HB	CHB	H-Donor	H-Acceptor
4	B:GLY318:HA2 - :[001:O13	2.27638	HB	Carbon HB	H-Donor	H-Acceptor
5	B:GLY459:HA2 - :[001:O4	2.14485	HB	Carbon HB	H-Donor	H-Acceptor
6	B:CYS457:SG - :[001	5.57562	Other	Pi-Sulphur	Sulfur	Pi-Orbitals
7	B:CYS457:SG - :[001	3.7062	Other	Pi-Sulphur	Sulfur	Pi-Orbitals
8	B:PHE450 - :[001	4.67065	H	Pi-Pi T-shaped	Pi-Orbitals	Pi-Orbitals
9	:[001:C12 - B:LEU496	4.77858	H	Alkyl	Alkyl	Alkyl
10	:[001:C28 - B:MET121	5.27025	H	Alkyl	Alkyl	Alkyl
11	:[001:C28 - B:ILE458	4.41797	H	Alkyl	Alkyl	Alkyl
12	:[001 - B:VAL382	4.57024	H	Pi-Alkyl	Pi-Orbitals	Alkyl
13	:[001 - B:LEU496	5.4774	H	Pi-Alkyl	Pi-Orbitals	Alkyl
14	:[001 - B:ALA463	4.0757	H	Pi-Alkyl	Pi-Orbitals	Alkyl

HB: Hydrogen Bond, **H:** Hydrophobic, **CHB:** Conventional Hydrogen Bond, **Carbon HB:** Carbon Hydrogen Bond

Table 5. Interaction types, categories, and distances of molecular insertion of the palmitic acid with cytochrome P450 1A1

No	Name	Distance	Category	Type	From Chemistry	To Chemistry
1	B:TRP131:HE1 - :[001:O1	1.8161	HB	CHB	H-Donor	H-Acceptor
2	B:ARG135:HH11 - :[001:O2	1.91015	HB	CHB	H-Donor	H-Acceptor
3	:[001:H32 - B:MET121:O	2.74877	HB	CHB	H-Donor	H-Acceptor
4	B:ARG455:HD2 - :[001:O2	2.41378	HB	Carbon HB	H-Donor	H-Acceptor
5	B:ALA317 - :[001	4.38453	H	Alkyl	Alkyl	Alkyl
6	B:ALA317 - :[001	3.46189	H	Alkyl	Alkyl	Alkyl
7	B:VAL322 - :[001	4.59032	H	Alkyl	Alkyl	Alkyl
8	B:CYS457 - :[001	4.70082	H	Alkyl	Alkyl	Alkyl
9	B:CYS457 - :[001	5.28121	H	Alkyl	Alkyl	Alkyl
10	B:ALA463 - :[001	4.57441	H	Alkyl	Alkyl	Alkyl
11	:[001:C1 - B:VAL197	5.31291	H	Alkyl	Alkyl	Alkyl
12	:[001:C1 - B:ILE198	4.0271	H	Alkyl	Alkyl	Alkyl
13	:[001:C1 - B:ILE201	4.62305	H	Alkyl	Alkyl	Alkyl
14	:[001:C1 - B:ILE462	4.15308	H	Alkyl	Alkyl	Alkyl
15	:[001 - B:ILE462	4.80541	H	Alkyl	Alkyl	Alkyl
16	:[001 - B:ILE458	4.84342	H	Alkyl	Alkyl	Alkyl
17	:[001 - B:MET121	5.44933	H	Alkyl	Alkyl	Alkyl
18	:[001 - B:ILE458	3.79587	H	Alkyl	Alkyl	Alkyl

HB: Hydrogen Bond, **H:** Hydrophobic, **CHB:** Conventional Hydrogen Bond, **Carbon HB:** Carbon Hydrogen Bond

Hesperidin molecules interacted with XO by eight CHBs (LYS792, ARG790, PRO753, GLY755, LYS754, GLY820, GLU756), eight carbon HBs (CYS593, PRO597, LYS792, ARG793, PRO753, LYS754), two pi-sigma (ILE596, LYS754), two alkyls (CYS593, ARG37), and three pi-alkyl (YS792, VAL591, ILE596) (Figure 4 - Table 6). Palmitic acid molecules interacted with XO by two CHBs (ARG37, GLY35), nine alkyls (LYS754, VAL591, PRO597, ILE752, ILE596), and pi-alkyls (PHE763) (Figure 4 – Table 7).

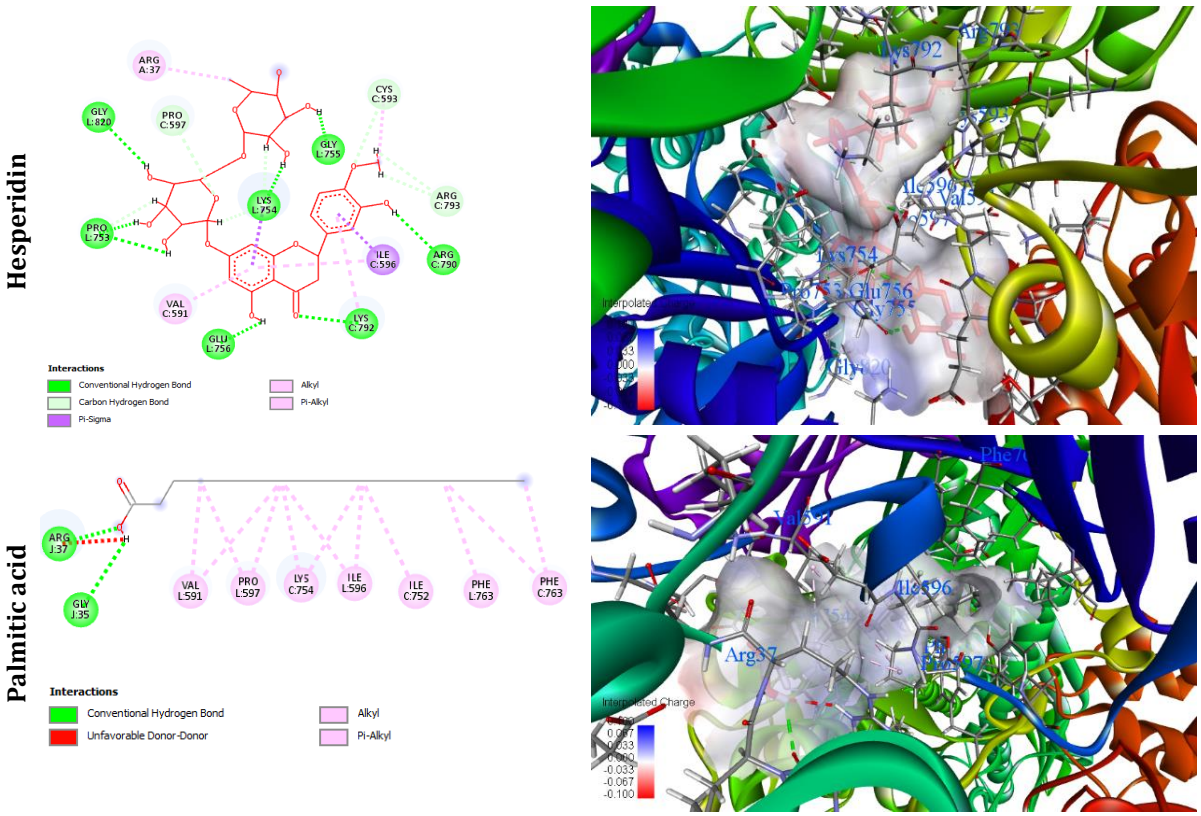

Figure 4. Hesperidin and palmitic acid, interaction with XO 2D images, and 3D interpolated load view

Table 6. Interaction types, categories, and distances of molecular insertion of the hesperidin with XO

No	Name	Distance	Category	Type	From Chemistry	To Chemistry
1	C:LYS792:HZ1 - :[001:O15	2.44191	HB	CHB	H-Donor	H-Acceptor
2	:[001:H7 - C:ARG790:O	2.07364	HB	CHB	H-Donor	H-Acceptor
3	:[001:H26 - L:PRO753:O	2.14762	HB	CHB	H-Donor	H-Acceptor
4	:[001:H27 - L:GLY755:O	2.38624	HB	CHB	H-Donor	H-Acceptor
5	:[001:H28 - L:LYS754:O	2.37468	HB	CHB	H-Donor	H-Acceptor
6	:[001:H32 - L:GLY820:O	2.62496	HB	CHB	H-Donor	H-Acceptor
7	:[001:H33 - L:PRO753:O	2.03668	HB	CHB	H-Donor	H-Acceptor
8	:[001:H34 - L:GLU756:OE2	2.27772	HB	CHB	H-Donor	H-Acceptor
9	C:CYS593:HA - :[001:O3	2.86225	HB	Carbon HB	H-Donor	H-Acceptor
10	C:PRO597:HD2 - :[001:O4	2.30554	HB	Carbon HB	H-Donor	H-Acceptor
11	C:LYS792:HE2 - :[001:O15	1.71557	HB	Carbon HB	H-Donor	H-Acceptor
12	:[001:H8 - C:ARG793:O	2.66738	HB	Carbon HB	H-Donor	H-Acceptor
13	:[001:H10 - C:ARG793:O	3.05984	HB	Carbon HB	H-Donor	H-Acceptor
14	:[001:H15 - L:PRO753:O	1.75075	HB	Carbon HB	H-Donor	H-Acceptor
15	:[001:H17 - L:LYS754:O	2.20118	HB	Carbon HB	H-Donor	H-Acceptor
16	:[001:H22 - L:LYS754:O	2.89313	HB	Carbon HB	H-Donor	H-Acceptor
17	C:ILE596:HD13 - :[001	2.3307	H	Pi-Sigma	C-H	Pi-Orbitals
18	L:LYS754:HD2 - :[001	2.47469	H	Pi-Sigma	C-H	Pi-Orbitals
19	:[001:C12 - C:CYS593	3.85548	H	Alkyl	Alkyl	Alkyl
20	:[001:C28 - A:ARG37	4.75728	H	Alkyl	Alkyl	Alkyl
21	:[001 - C:LYS792	4.18674	H	Pi-Alkyl	Pi-Orbitals	Alkyl
22	:[001 - C:VAL591	3.97314	H	Pi-Alkyl	Pi-Orbitals	Alkyl
23	:[001 - C:ILE596	5.11882	H	Pi-Alkyl	Pi-Orbitals	Alkyl

HB: Hydrogen Bond, **H:** Hydrophobic, **CHB:** Conventional Hydrogen Bond, **Carbon HB:** Carbon Hydrogen Bond

Table 7. Interaction types, categories, and distances of molecular insertion of the palmitic acid with xanthine oxidase

No	Name	Distance	Category	Type	From Chemistry	To Chemistry
1	J:ARG37:HN - :[011:O1	1.7627	HB	CHB	H-Donor	H-Acceptor
2	:[011:H32 - J:GLY35:O	2.5911	HB	CHB	H-Donor	H-Acceptor
3	C:LYS754 - :[011	5.42612	H	Alkyl	Alkyl	Alkyl
4	C:LYS754 - :[011	4.61331	H	Alkyl	Alkyl	Alkyl
5	L:VAL591 - :[011	4.77883	H	Alkyl	Alkyl	Alkyl
6	L:VAL591 - :[011	4.00137	H	Alkyl	Alkyl	Alkyl
7	L:PRO597 - :[011	4.08831	H	Alkyl	Alkyl	Alkyl
8	L:PRO597 - :[011	4.89531	H	Alkyl	Alkyl	Alkyl
9	:[011 - C:ILE752	4.97429	H	Alkyl	Alkyl	Alkyl
10	:[011 - L:ILE596	4.86617	H	Alkyl	Alkyl	Alkyl
11	:[011 - L:ILE596	4.97151	H	Alkyl	Alkyl	Alkyl
12	C:PHE763 - :[011:C1	4.63377	H	Pi-Alkyl	Pi-Orbitals	Alkyl
13	C:PHE763 - :[011	5.41644	H	Pi-Alkyl	Pi-Orbitals	Alkyl
14	L:PHE763 - :[011	4.9976	H	Pi-Alkyl	Pi-Orbitals	Alkyl

HB: Hydrogen Bond, **H:** Hydrophobic, **CHB:** Conventional Hydrogen Bond

Hesperidin and palmitic acid interaction with CYP1A1 was saved as a MolDock score of -149.156 and -107.23, respectively. The binding energies of -9.90 kcal/mol and -3.90 kcal/mol, respectively. Hesperidin and palmitic acid interaction with XO was saved as a MolDock score of -179.68 and -118.53, respectively. The binding energies are -11.40 kcal/mol and -4.30 kcal/mol. Molecular docking of hesperidin with XO and CYP1A1 was observed to have a high MolDock score and binding energy. According to these results, hesperidin could inhibit these enzymes.

3.4. PASS Prediction

Several biological activities describing compounds were predicted using PASS. The results showing the probability of activity (Pa) and the probability of inactivity (Pi) were summarized in Table 8. In PASS analysis, it is more likely to find activity experimentally when Pa>0.7. Accordingly, it has high α-glucosidase inhibitor, anticarcinogenic, lipid peroxidase inhibitor, free radical scavenger, antioxidant, antifungal, toxic (vascular), toxic, toxic (gastrointestinal), and inflammation effects for rutin and hesperidin; antimutagenic, eye irritation (inactive), ulcer (aphthous), gastrointestinal bleeding, and toxic (vascular) effects for palmitic acid.

4. Conclusion

This study used leaf and seed parts of the PSC plant, which belongs to the Rhamnaceae family. The leaf parts were extracted with methanol and the seed parts with hexane, and both parts' phytochemical and biological activities were investigated. The leaf part's total phenol and flavonoid contents are higher than the seed part. According to LC-ESI-MS/MS and GC-MS/MS analyses, it was observed that hesperidin and palmitic acid components were present in high amounts. According to the antioxidant capacity and urease inhibition applied to the leaf and seed extracts, it was found that the leaf extract had a higher effect than the standard and seed extracts. However, it was noted that the seed extract did not affect PMRA capacity. Molecular docking of the hesperidin molecule, found in high amounts in LC-ESI-MS/MS analysis, was executed with XO and CYP1A1 enzymes, and its interaction with XO was higher than its interaction with CYP1A1. The prospective use of PSC as an alternative source of bioactive chemicals for developing pharmaceutical drugs has a scientific foundation thanks to this investigation. However, further *in vitro*, *in vivo*, and clinical

research is required. The species' toxicity profile and bioavailability also need to be determined.

Table 8. The PASS prediction activity of major compounds of LC-ESI-MS/MS and GC-MS/MS

No	Activity	Hesperidin		Palmitic acid	
		Pa	Pi	Pa	Pi
1	Alpha glucosidase inhibitor	0.852	0.001	-	-
2	Anti-inflammatory, intestinal	-	-	0.727	0.002
3	Anti-inflammatory	0.691	0.017	0.515	0.052
4	Antiviral (Influenza)	-	-	0.565	0.016
5	Antimutagenic	-	-	0.783	0.004
6	Anticarcinogenic	0.982	0.001	0.359	0.039
7	Urease inhibitor	-	-	0.665	0.003
8	Antibacterial	0.650	0.006	-	-
9	Antipruritic, allergic	-	-	0.630	0.008
10	Anti-infective	0.632	0.011	0.655	0.009
11	Eye irritation, inactive	-	-	0.805	0.004
12	Reductant	0.498	0.025	0.690	0.006
13	Lipid peroxidase inhibitor	0.991	0.001	0.401	0.035
14	DNA ligase (ATP) inhibitor	0.461	0.009	0.406	0.015
15	Free radical scavenger	0.989	0.001	0.315	0.027
16	Antioxidant	0.846	0.003	-	-
17	Antifungal	0.803	0.005	0.407	0.048
18	Antiulcerative	0.710	0.005	0.525	0.019
19	Ulcer, aphthous	-	-	0.867	0.006
20	Ulcer, gastric	-	-	0.663	0.005
21	Gastrointestinal haemorrhage	-	-	0.861	0.004
22	Toxic, vascular	0.789	0.020	0.800	0.017
23	Toxic	0.860	0.017	0.559	0.069
24	Toxic, gastrointestinal	0.723	0.041	0.689	0.047
25	Gastrointestinal disturbance	-	-	0.713	0.011
26	Inflammation	0.946	0.005	0.682	0.026

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Author Contribution

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Conflicts of Interest

There is no conflict of interest, according to all of the writers.

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