



Phenolic Content and Enzyme Inhibition Activities *Barbarea auriculata* var. *paludosa*, *B. integrifolia* and *B. plantaginea* (Brassicaceae)'s

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Authors' contributions

This work was carried out in collaboration among all authors. Author AK collected and identified the plants. Authors MB, SOS, NK, SK, SY, UO, RA designed the studies and performed the analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i930486

Editor(s):

(1) Dr. Vasudevan Mani, Qassim University, Saudi Arabia.

(2) Dr. Mohamed Fathy, Assiut University, Egypt.

Reviewers:

(1) Uzuazokaro M. Agatemor, Novena University, Nigeria.

(2) T. C. Venkateswarulu, Vignan's Foundation for Science, Technology & Research, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/57426>

Original Research Article

Received 07 April 2020

Accepted 12 June 2020

Published 20 June 2020

ABSTRACT

Barbarea genus has been presented about 12 species, 9 taxon are endemic, in Turkey. In this study, enzyme inhibition was carried out on methanolic extract, chloroform extract, ethyl acetate extract, and the remaining aqueous phases from the aerial parts of *B. auriculata* var *paludosa*, *B. integrifolia*, and *B. plantaginea* species and HPLC studies were carried on their methanolic extract in the present study for the first time. Phenolic compounds were determined using reverse phase-high performance liquid chromatography (RP-HPLC). *p*-OH benzoic acid, vanilic acid, syringaldehyde, coumaric acid, synapic acid and benzoic acid were detected as major phenolic compounds in the species. Assay of enzyme inhibition activities were done using

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spectrophotometric methods. Results of these studies reveal that the extracts from these species have moderate tyrosinase, AChE and BuChE inhibitory activity. In the biological activity studies, it was observed that *B. integrifolia* was the highest activity.

Keywords: *Barbarea*; *brassicaceae*; *enzyme inhibition*; *phytochemical*.

1. INTRODUCTION

Turkey is situated in the junction of three different phytogeographic regions (Mediterranean, Irano-Turanian, Euro-Siberian) [1]. Turkey being a leading country in terms of medicinal and endemic plants in the World, has a rich flora [2]. About 10000 flowering and fern plant species have inherently grown in Turkey and the rate of endemism is high (30% of them are endemic) [3-5].

Barbarea genus (Brassicaceae) has presented about 20 species in Europe and Asia, 12 species, 9 taxon are endemic, in Turkey. The leaves of *Barbarea* species have been used as food (for making salad), wound healing and diuretic [1].

Alzheimer's disease (AD) is a neurodegenerative ailment defined by memory defect. One of the most evident biochemical change for the ailment is a decrease acetylcholine levels in nerve cells [6]. Acetylcholinesterase (AChE) and butyryl-cholinesterase are two key enzymes hydrolysing acetylcholine [7]. Nowadays, synthetic cholinesterase inhibitors are one of the potent agent for treatment of AD. But, they have side effects such as hepatotoxicity and gastrointestinal problems [8]. So, researchers have been focused on cholinesterase inhibitors obtained from natural products.

Tyrosinase is well-known as a polyphenol oxidase enzyme, being charge of melanin biosynthesis [9]. Hyperpigmentation defined increasing of melanin production induces actinic damage, melasma, freckle and age-related stains [10]. The tyrosinase inhibitors are potential agents using to treat these hyperpigmentation diseases [11]. Moreover, Tyrosinase inhibitors have been evidenced to be used to cure neurotoxic diseases like Parkinson [12].

Phenolic compounds evidenced wide range of significant biological activities such as antioxidant, antiaging, antimicrobial, memory stimulating effect, hepatoprotective, anticholine-

sterase and antityrosinase. Some phenolic compounds including p-hydroxy benzoic acid, (2) vanillic acid, (3) syring aldehyde, (4) p-coumaric acid, (5) sinapic acid, (6) benzoic acid, (7) quercetin have anticholinesterase and antityrosinase activities [13,14].

The aim of the study was to investigate enzyme inhibition on methanolic extract, chloroform, ethyl acetate, and the remaining aqueous phases from the aerial parts of *B. auriculata* var *paludosa*, *B. integrifolia*, and *B. plantaginea* species and to screen phytochemical profile of methanolic extracts of the plants in terms of phenolic compounds (p-hydroxy benzoic acid, (2) vanillic acid, (3) syring aldehyde, (4) p-coumaric acid, (5) sinapic acid, (6) benzoic acid, (7) quercetin) using HPLC technique. It is first study about phytochemical and biological activity of these species.

2. MATERIALS AND METHODS

2.1 Chemicals and Instrumentation

The following chemicals and reagents were: chloroform, ethyl acetate, methanol, ethanol, galanthamine, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), α -Kojic acid, tyrosinase, hexane, vanillin, formic acid, p-OH benzoic acid, vanilic acid, syringaldehyde, coumaric acid, sinapic acid, quercetin, benzoic acid (Sigma-Aldrich); acetic acid and acetonitrile (Merck). BMG Labtech Spectrostar Nano spectrophotometer has been used to measure the absorbance in biological activity studies.

2.2 Plant Material

B. auriculata var. *paludosa*, *B. plantaginea* and *B. integrifolia* were collected from The mount of Pöske in Erzincan (2100 m), Çimen village of Kelkit district (Gümüşhane, 1750 m) and Uzunkol village of Kelkit (Gümüşhane, 1900 m), respectively and identified by Ali Kandemir. The herbarium materials have been stored at the Herbarium Faculty of Science, Erzincan Binali Yıldırım University (Kandemir 10863, 10864, 10865).

2.3 Preparation of Extracts of the *Barbarea* Species

Dried and powdered of aerial parts of the species (min. 250 g) were extracted with 1000 mL of methanol at room temperature and filtered. The filtrates were concentrated under pressure and the methanol extracts were obtained. The methanol extracts were dissolved in water:methanol (9:1) and partitioned with chloroform and ethyl acetate, respectively. Then the solvents were concentrated at low pressure to give the chloroform, ethyl acetate and remaining aqueous extracts. The extracts were further used for HPLC analysis and biological activity studies.

2.4 Determination of Enzyme Inhibition Activity

2.4.1 Cholinesterase inhibition

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitions were determined to assess cholinesterase inhibitory activity in the study. The assay was carried on by a modified colorimetric Ellman method with a 96-well microplate by ELISA microplate reader [15]. Acetylthiocholine iodide and butyrylthiocholine iodide were employed as substrates. (DTNB) was used as reagent. The samples were dissolved in sodium phosphate buffer (pH 8) range of concentration of 25-1000 µg/mL. Next, 130 µL of sodium phosphate buffer, 10 µL of the tested sample and 20 µL of the enzyme were added and incubated for 15 min at 25°C. Then, 20 µL of DTNB and 20 µL of substrat were put in all wells. Absorbance was recorded at 412 nm spectrophotometrically. The percentage of AChE and BChE inhibition values were calculated as shown the following formula and compared with galantamine used as reference. The results were given as IC₅₀ levels.

$$\% \text{ Inhibition} = 100 - [(A1 / A2) \times 100]$$

A1 = Absorbance of the sample solutions at 412 nm

A2 = Average absorbance of the control solutions at 412 nm.

2.4.2 Tyrosinase inhibition

A modified dopachrome method was used to assess the tyrosinase inhibitory activity [16]. L-DOPA and various concentrations of α -kojic acid solutions was used as a substrat and as

reference. The analysis was performed with a 96-well microplate by ELISA microplate reader. The samples were prepared a concentration range of 25-1000 µg/mL in DMSO of phosphate buffer (pH 6.8). 120 µL of 0.2 M phosphate buffer (pH 6.8) and 40 µL tyrosinase solution for A wells; 160 µL of 0.2 M phosphate buffer (pH 6.8) for B wells; 80 µL of 0.2 M phosphate buffer (pH 6.8), 40 µL tyrosinase solution and 40 µL sample solution for C wells; 120 µL of 0.2 M phosphate buffer (pH 7.0) and 40 µL sample solution for D wells were added and incubated for 10 min at 23°C. Then, 2.5 mM L-DOPA solution (40 µL) was added to the wells. After the incubation for 10 min at 23°C, the absorbance was measured at 490 nm. The percentage of tyrosinase inhibition was calculated by the following formula and the results were given as IC₅₀ levels.

$$\% \text{ inhibition} = [[(A-B)-(C-D)] / (A-B)] \times 100$$

2.5 HPLC Analysis of Phenolic Compounds and Benzoic Acid in the Species

Vanillic acid, *p*-hydroxybenzoic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, benzoic acid and quercetin were analysed using HPLC technique. a reverse phase column (150 × 4.6 mm i.d, 5 µm) (Waters Spherisorb, Milfort, MA, USA), on a gradient program with the assistance of a two-solvents system [A: 100% methanol; B: 2% acetic acid in water (pH 2.8)], and a constant solvent flow rate set to 1.5 mL min⁻¹ on a HPLC system (Shimadzu Corporation, LC 20 AT, Kyoto, Japan) (Table 1) were used for HPLC analysis. The injection volume was 20 µL. Signals were identified using DAD detection at a column temperature of 25°C. HPLC analyses were performed modified methods in our previous study [17,18].

3. RESULTS

3.1 The Results of Enzyme Inhibition Studies

The results from enzyme inhibition studies of *B. auriculata* var *paludosa*, *B. integrifolia*, and *B. plantaginea* have been included in Table 1.

3.2 The Results of HPLC Analysis of Phenolic Compounds and Benzoic acid in the Species

The results from HPLC analysis methanolic extracts of *B. auriculata* var. *paludosa*, *B.*

integrifolia, and *B. plantaginea* have been showed in Table 2. The chromatograms of phenolic standards and methanolic extract of the species are displayed in Figs. 1-4.

4. DISCUSSION

Previous studies show that some *Barbarea* species and their isolated compounds have important biological effects for human health. It

was reported that (R) -5-phenyl-2-oxazolidinone called barbarin was obtained from *B. orthoceras* and barbarin has inhibitory effect on the tyrosinase enzyme as strong as kojic acid [19]. Some studies showed that *B. orthoceras* has anti-inflammatory effect by inhibiting NO synthesis in various cell types and may have a preventive effect in neurodegenerative diseases such as NO-related Parkinson and Alzheimer's disease [20].

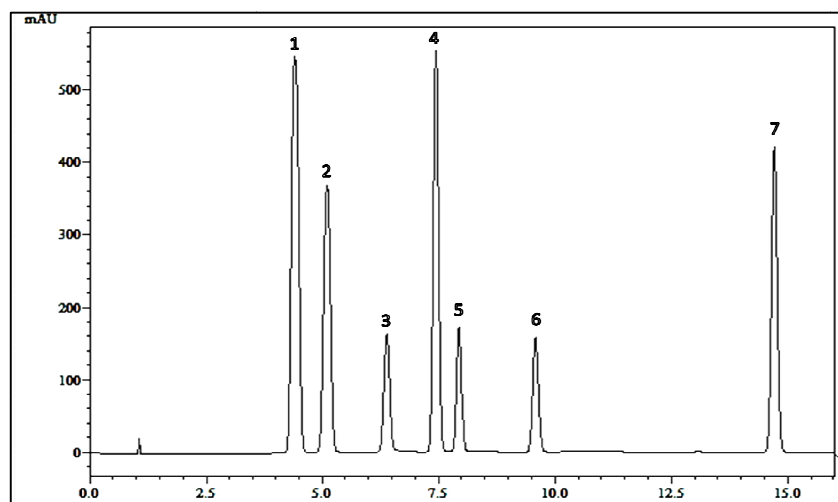


Fig. 1. HPLC chromatogram of phenolic standards

Peak identification: (1) *p*-hydroxy benzoic acid, (2) vanillic acid, (3) syring aldehyde, (4) *p*-coumaric acid, (5) sinapic acid, (6) benzoic acid, (7) quercetin

Table 1. Acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase inhibitor activities

Samples		AChE Inhibitory Activity IC ₅₀ değeri (µg/mL)	BuChE Inhibitory Activity IC ₅₀ değeri (µg/mL)	Tyrosinase Inhibitory Activity IC ₅₀ değeri (µg/mL)
<i>B. auriculata</i>	Methanolic extract	150.95±2.65	679.04±9.36	502.5824±3.68
	Etoc extract	203.176±6.02	158.8892±4.31	582.6123±3.26
	Aqueous extract	152.1257±2.81	161.9107±2.03	587.1096±0.32
	Chloroform extract	269.9255±0.86	165.9243±1.61	859.456±1.67
<i>B. integrifolia</i>	Methanolic extract	206.9±4.48	247.68±3.89	413.8071±1.13
	Etoc extract	202.7765±1.23	147.0933±1.79	623.563±0.95
	Aqueous extract	189.5929±2.18	69.17957±2.25	480.9188±1.02
	Chloroform extract	193.4644±1.25	133.8981±1.79	960.9375±2.03
<i>B. plantaginea</i>	Methanolic extract	243.26±4.18	308.14±5.21	>1000
	Etoc extract	379.94±1.38	229.7585±1.02	>1000
	Aqueous extract	202.0273±2.08	243.0346±1.15	964.5861±2.32
	Chloroform extract	320.4593±1.25	200.0563±1.16	>1000
	α -Kojic acid			3.4819±1.19
	Galanthamine	1.4454±0.06	7.1154±0.21	

Table 2. Phenolic and benzoic acid composition of the methanolic extracts of *Barbarea* species

	<i>p</i> -OH Benzoic Acid	Vanillic Acid	Syring aldehyde	<i>p</i> -coumaric Acid	Sinapic Acid	Benzoic Acid	Quercetin
	mg/g						
<i>B. integrifolia</i>	12.535	9.245	19.543	76.180	97.261	90.589	-
<i>B. auriculata</i> var. <i>paluosa</i>	-	66.445	60.553	22.342	170.443	48.754	-
<i>B. plantaginea</i>	-	-	-	-	-	16.784	-

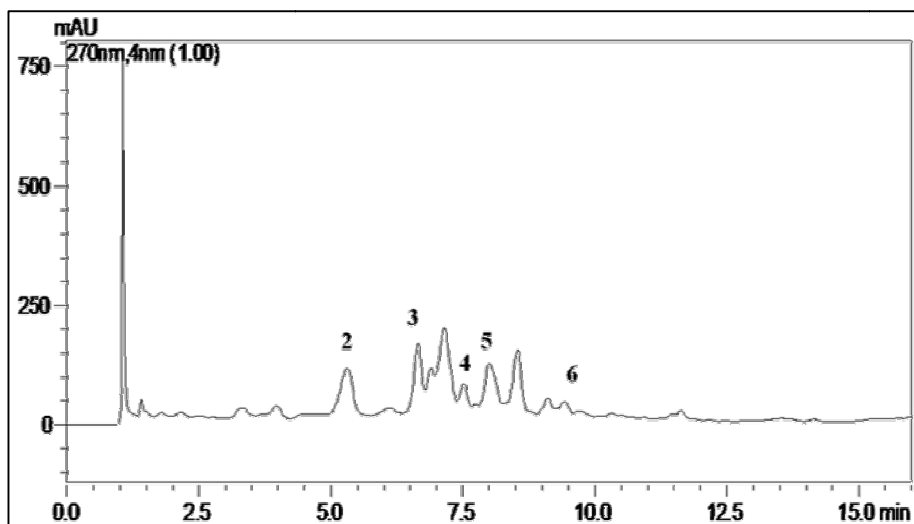


Fig. 2. HPLC chromatogram of *B. auriculata* var. *paluosa* methanolic extract

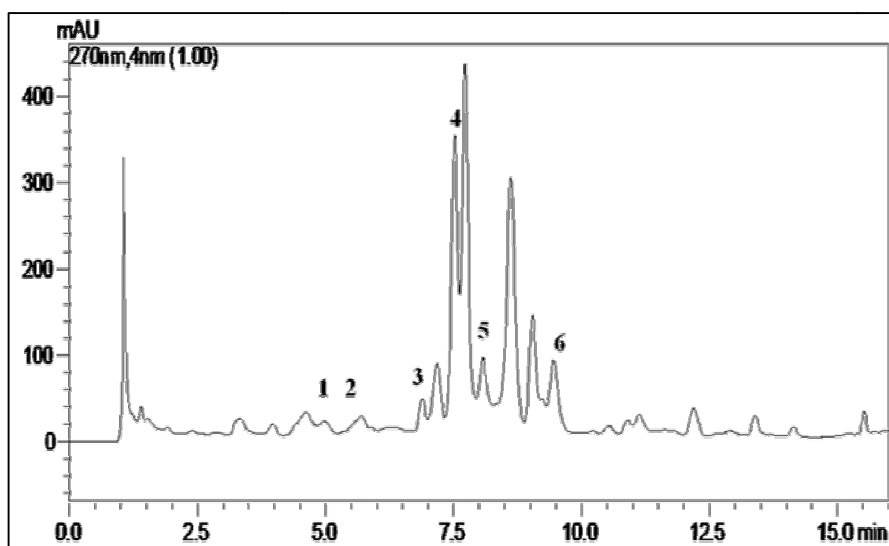


Fig. 3. HPLC chromatogram of *B. integrifolia* methanolic extract

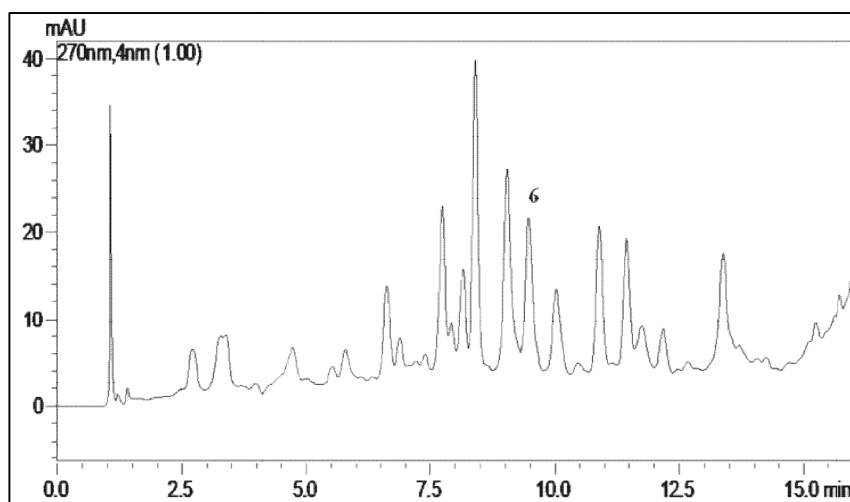


Fig. 4. HPLC chromatogram of *B. plantaginea* methanolic extract

Phenolic compounds have an inhibitory effect on cholinesterase enzymes so they can be protective against neurodegenerative diseases [21-27]. Based on this, tyrosinase and cholinesterase inhibitor effects of three *Barbarea* species and their phenolic profiles were examined in study.

α -kojic acid was used as positive control to evaluate the tyrosinase inhibitory activity of extracts from species. IC_{50} values of α -kojic acid and extracts are shown in Table 1. According to the results, *B. plantaginea* has weak inhibitory effect on tyrosinase enzyme. Also, the study have showed that *B. integrifolia* and *B. auriculata* var. *paludosa* have moderate tyrosinase inhibitory effect.

The effect of the species on acetyl and butyrylcholinesterase enzymes was investigated by comparison with the galantamine standard. IC_{50} values of galantamine and extracts as shown in Table 2. According to the results of acetylcholinesterase enzyme inhibition, the highest inhibitory effect was observed on aqueous extract of *B. auriculata*. the highest butyrylcholinesterase enzyme inhibition was evidenced aqueous extract of *B. integrifolia*.

In the literatures, it was found that the phenolic compounds in *Barbarea* species are as chlorogenic acid, 3,5-dicaffeilkinic acid and 1,5-dicaffeilkinic acid [28]. *p*-OH benzoic acid, vanilic acid, syringaldehyde, coumaric acid, synapic acid and benzoic acid were found as phenolic compounds in the *Barbarea* species by our HPLC analysis. While only benzoic acid is

detected in *B. plantaginea*; It was determined that *B. integrifolia* contains *p*-OH benzoic acid, vanilic acid, syringaldehyde, coumaric acid, synapic acid and benzoic acid. Also, the presence of vanilic acid, syringaldehyde, coumaric acid, synapic acid and benzoic acid in *B. auriculata* var. *paludosa* was proved.

5. CONCLUSION

Phytochemical and biological activity studies were carried out on three *Barbarea* species for the first time. The datas obtained from the studies will contribute to literatures regarding these species. In addition, the results of biological activity studies have shown that the species have a moderate inhibitory effect on tyrosinase, AChE and BuChE enzymes. Of them, *B. integrifolia* has the highest BuChE inhibition. It can be related to its rich phenolic contents. The data obtained are important in terms of light on the further studies about the species, as well as showing that the species may be potential sources in the development of drugs of natural origin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

Merve Badem, Sıla Özlem Şener and Nuriye Korkmaz would like to acknowledge the

scholarship by the Turkish Scientific and Technical Research Council.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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