Research Article

Determination of the Chemical Composition, Antioxidant, and Enzyme Inhibitory Activity of *Onosma mollis* DC

**Arzuhan Sihoglu Tepe**

*Kilis 7 Aralik University, Vocational School of Health Sciences, Department of Pharmacy Services, Kilis 79000, Turkey*

Correspondence should be addressed to Arzuhan Sihoglu Tepe; arzuhan.tepe@kilis.edu.tr

Received 21 June 2021; Accepted 2 August 2021; Published 7 August 2021

Academic Editor: Claudia Crestini

Copyright © 2021 Arzuhan Sihoglu Tepe. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Onosma* species have long been used traditionally for respiratory tract infections, abdominal pain, wound treatment, burns, and constipation. This study aims to investigate the chemical composition and *in vitro* antioxidant and enzyme inhibitory activities of ethyl acetate (EtOAc), methanol (MeOH), and water extracts of *Onosma mollis* DC. MeOH extract was richer in both phenolics and flavonoids than other extracts (44.06 mg GAEs/g and 41.57 mg QEs/g, respectively). The findings obtained from the results of the chromatographic analysis also supported the results of the spectrophotometric analysis. The MeOH extract was the richest in terms of most of the phytochemicals screened. Apigenin 7-glucoside, luteolin 7-glucoside, rosmarinic acid, vanillic acid, and pinosylvin were over 1000.0 μg/g in MeOH extract. The extract in question showed the highest activity in phosphomolybdenum, DPPH, and ABTS radical scavenging and CUPRAC and FRAP reducing power activity assays (2.01, 3.33, 2.30, 1.48, and 0.79 mg/ml, respectively). The water extract presented the highest activity in the ferrous ion chelating assay (1.01 mg/ml). While EtOAc extract showed high activity in acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glucosidase inhibitory activity tests (1.11, 1.49, and 1.07 mg/ml, respectively), MeOH extract showed significant efficacy in tyrosinase and α-amylase inhibitory activity assays (2.94 and 2.08 mg/ml, respectively). There was a high correlation between the total phenolics/flavonoids of the extracts and their antioxidant activities (correlation coefficients were over 0.9). In addition, the phytochemicals mentioned above were found to contribute significantly to the antioxidant activity. It was concluded that a more detailed analysis should be done to determine the compounds responsible for the enzyme inhibitory activities of the extracts.

### 1. Introduction

Plants and plant-based nutrients are an indispensable part of the human diet. Many researchers agree that, in addition to fruits and vegetables, many medicinal and aromatic plants that we consume in our daily lives serve as an additional source of natural antioxidants [1–4]. Studies have shown that plant-derived flavonoids, some alkaloids, phenolic compounds, some diterpenoids, and sulfur compounds have a high degree of antioxidant activity [4–6]. These compounds help protect our body from the harmful effects of these compounds by eliminating oxidant substances that occur due to both normal metabolic reactions and some environmental conditions (gamma, UV, and X-ray radiation, etc.). Therefore, plants continue to be of great interest as additional sources of antioxidant compounds [4, 5, 7–10].

Alzheimer’s disease (AD) is a critical neurodegenerative disorder affecting older people [11]. Various strategies have been developed to treat this health problem. Some of these strategies are targeting amyloid-beta (Aβ) aggregation and modulating enzymes responsible for metabolizing the amyloid precursor protein (APP) or tau phosphorylation [12]. The first pharmacological treatment method approved by the US Food and Drug Administration (FDA) in AD treatment is the use of cholinesterase (ChE) inhibitors. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) hydrolyze acetylcholine (ACh), impairing signal transduction. This situation is considered among the fundamental reasons for the progression of AD [13]. Some ChE inhibitors such as galantamine, rivastigmine, donepezil, or tacrine have been shown to reduce symptoms in AD patients. However, these agents exhibit some unwanted side
effects that have limited their use [14–16]. Therefore, researchers are screening plants for the discovery of new and alternative CH inhibitors.

Melanin is a heteropolymer synthesized by the catalytic activity of tyrosine in melanosomes. Researchers have reported that metal ions TRP-1 and TRP-2 play a critical role in melanin synthesis [17–20]. However, the most crucial task in the melanogenesis process is fulfilled by tyrosinase. The use of tyrosinase inhibitors to treat hyperpigmentation disorders due to excessive melanin synthesis is considered a critical treatment approach. Tyrosinase inhibitors are also frequently used in skin whitening applications and are extremely important for the cosmetic industry. Over the last decades, researchers have been conducting intensive research on plants to discover new and effective tyrosinase inhibitors [21–28].

Diabetes is a severe disease that affects a large number of people worldwide [29, 30]. It is evaluated in two categories, Type 1 or Type 2. The leading cause of type 2 diabetes is the imbalance between the transfer of blood sugar to tissues and insulin secretion. Postprandial hyperglycemia is one of the most important reasons for Type 2 diabetes to develop [31]. In the treatment of Type 2, the plasma glucose level is tried to be kept under control. One of the ways to achieve this is to stimulate insulin secretion through medical intervention or diet [30, 32, 33]. Another solution is to slow down the digestion of starch and lower the glucose concentration, which is the end product of digestion. Thus, the amount of glucose absorbed from the small intestine is reduced [33]. This strategy is more practical and economical than controlling insulin secretion [30]. Digestive enzymes such as α-amylase and α-glucosidase must be inhibited to limit glucose production [34–36]. In addition to the biological activities documented above, the researchers also analyze the plants for their antidiabetic activity.

Onosma, one of the important genera of Boraginaceae, consists of approximately 150 species distributed worldwide. Literature records show that only 10% of these species are reported in terms of their chemical constituents and biological activity potential. These reports indicate that Onosma species are rich in shikonins and onosmins. These species have long been used traditionally for respiratory tract infections, abdominal pain, wound treatment, burns, and constipation. Onosma species are also used to color foods, medicinal preparations, and wool because of the dye contained in the root [37]. According to literature data, it is known that some Onosma species (such as O. siehoana, O. bourgaei, O. brachytricha, O. hispidum, and O. heterophyllum) have antioxidant, anti-inflammatory, antiinociceptive, enzyme inhibitory, hypoglycemic, antimicrobial, etc. activities [38–42]. There is also a report in the literature on the antioxidant activity of methanol (MeOH) extract from the aerial parts of O. mollis [43]. However, in the report in question, ethyl acetate (EtOAc) and water extracts, which include nonpolar compounds, were not evaluated, and the enzyme inhibitory activity of O. mollis was not included.

This study aimed to determine the chemical composition, antioxidant, and enzyme inhibitor activities of ethyl acetate (EtOAc), methanol (MeOH), and water extracts obtained from the aerial parts of Onosma mollis DC.

2. Experimental

2.1. Plant Material and Extract Preparation. Aerial parts of O. mollis were collected from Yazihan–Hekimhan highway, Yazihan, Malatya, Turkey (1140m., 38° 41’ 58”N38° 06’ 54”E). Dr. Olçay Ceylan (MuglaSitkiKocman University) performed the identification of the species.

Aerial parts of the plants were used as the study material to obtain solvent extracts. Details of the extraction procedure can be found in the supplementary file.

2.2. Determination of the Phenolic Compositions of the Extracts. Details of the spectrophotometric and chromatographic methods are given in the Supplementary file [44, 45].

2.3. Biological Activity. The antioxidant [45–49] and enzyme inhibitory activity [41] tests are given in the Supplementary file.

2.4. Statistical Analysis. The details of the statistical analysis are presented in the Supplementary file.

3. Results

3.1. Chemical Composition of the Extracts. Table 1 shows the yield of extracts from O. mollis. The yield of the water extract was found to be higher than the others (14.9%). The yields of MeOH and EtOAc extracts were 4.58 and 3.91%, respectively.

The phytochemical composition data of the extracts obtained by spectrophotometric method are also given in Table 1. The total amount of phenolic compounds of the extracts was found to be higher than the total amount of flavonoid compounds. The total amounts of phenolic and flavonoid compounds of the MeOH extract were 44.06 mg GAEs/g and 41.57 mg QEs/g, respectively. The EtOAc extract was extremely poor in these compounds (20.43 mg GAEs/g and 1.69 mg QEs/g, respectively).

The results of the chromatographic analysis are presented in Table 2. Apigenin 7-glucoside, luteolin 7-glucoside, rosmarinic acid, vanillic acid, and pinoresinol were found in MeOH extract at a concentration above 1000 μg/g. In addition, the MeOH extract was found to contain significant amounts of apigenin (679.43 μg/g), luteolin (581.55 μg/g), and 4-hydroxybenzoic acid (553.68 μg/g). On the other hand, vanillic acid and pinoresinol were higher concentrations in water extract than other phytochemicals (896.81 and 841.68 μg/g, respectively). Compared to the others, the EtOAc extract was the poorest in terms of phytochemicals screened. Apigenin 7-glucoside in this extract stood out with its concentration (752.01 μg/g) than other phytochemicals.

3.2. Antioxidant Activity Potential of the Extracts. Table 3 shows the antioxidant activities of the extracts. MeOH
Table 1: Total flavonoid and phenolic contents of *O. mollis* extracts.

| Assays | EtOAc | MeOH | Water |
|--------|-------|------|-------|
| Yield (%) | 3.91 | 4.58 | 14.09 |
| Total flavonoids (mg QEs/g extracts) | 1.69 ± 0.35<sup>c</sup> | 41.57 ± 0.60<sup>a</sup> | 13.44 ± 0.76<sup>b</sup> |
| Total phenolics (mg GAES/g extracts) | 20.43 ± 0.46<sup>b</sup> | 44.06 ± 3.48<sup>a</sup> | 21.80 ± 3.54<sup>b</sup> |

QEs: Quercetine equivalent. GAEs: Gallic acid equivalent. The different superscripts within the same row are different statistically at a 5% significance level.

Table 2: Concentration (µg/g extract) of selected phenolic compounds in *O. mollis* extracts.

| Compound                              | EtOAc | MeOH | Water |
|---------------------------------------|-------|------|-------|
| Apigenin 7-glucoside                  | 752.01 ± 2.56<sup>b</sup> | 22320.63 ± 302.76<sup>a</sup> | 27.96 ± 0.71<sup>b</sup> |
| Luteolin 7-glucoside                  | 253.14 ± 1.09<sup>b</sup> | 15684.92 ± 77.23<sup>b</sup> | 243.7 ± 5.56<sup>b</sup> |
| Rosmarinic acid                       | 232.48 ± 9.98<sup>b</sup> | 13552.66 ± 144.22<sup>a</sup> | 16.18 ± 4.86<sup>b</sup> |
| Vanillic acid                         | 48.31 ± 3.41<sup>c</sup> | 1623.24 ± 79.44<sup>a</sup> | 896.81 ± 46.15<sup>b</sup> |
| Pinoresinol                           | 109.14 ± 2.69<sup>b</sup> | 1292.32 ± 36.50<sup>a</sup> | 841.68 ± 44.49<sup>b</sup> |
| Apigenin                              | 123.25 ± 19.23<sup>b</sup> | 679.43 ± 16.50<sup>a</sup> | 22.52 ± 2.36<sup>c</sup> |
| Luteolin                              | 101.72 ± 5.28<sup>b</sup> | 581.55 ± 0.38<sup>a</sup> | 459.34 ± 5.47<sup>b</sup> |
| 4-hydroxybenzoic acid                 | 27.18 ± 2.47<sup>b</sup> | 1623.24 ± 79.44<sup>a</sup> | 33.04 ± 0.59<sup>b</sup> |
| Ferulic acid                          | 12.1 ± 0.14<sup>c</sup> | 581.55 ± 0.38<sup>a</sup> | 34.15 ± 1.17<sup>b</sup> |
| Rosmarinic acid                       | 232.48 ± 9.98<sup>b</sup> | 13552.66 ± 144.22<sup>a</sup> | 16.18 ± 4.86<sup>b</sup> |
| Caffeic acid                          | 1.53 ± 0.07<sup>b</sup> | 171.48 ± 1.65<sup>a</sup> | 0.66 ± 0.09<sup>b</sup> |
| Hyperoside                            | 2.25 ± 0.12<sup>b</sup> | 22320.63 ± 302.76<sup>a</sup> | 27.96 ± 0.71<sup>b</sup> |
| Vanillin                              | nd    | 152.12 ± 0.95<sup>b</sup> | Nd |
| 2,5-Dihydroxybenzoic acid             | nd    | 129.41 ± 0.79<sup>a</sup> | 3.97 ± 0.33<sup>a</sup> |
| Syringic acid                         | 6.59 ± 0.69<sup>b</sup> | 115.61 ± 1.41<sup>a</sup> | 115.54 ± 6.99<sup>a</sup> |
| Hesperidin                            | 9.75 ± 0.02<sup>b</sup> | 90.93 ± 0.49<sup>a</sup> | Nd |
| p-Coumaric acid                       | 1.98 ± 0.12<sup>c</sup> | 68.44 ± 0.41<sup>a</sup> | 24.86 ± 0.04<sup>b</sup> |
| Protocatechuic acid                   | 3.01 ± 0.08<sup>b</sup> | 65.74 ± 1.01<sup>a</sup> | 69.40 ± 20.5<sup>a</sup> |
| Sinapic acid                          | nd    | 37.84 ± 1.99<sup>a</sup> | Nd |
| 3-Hydroxybenzoic acid                 | nd    | 14.6 ± 0.95<sup>a</sup> | 2.40 ± 0.20<sup>b</sup> |
| Gallic acid                           | nd    | 4.21 ± 0.02<sup>b</sup> | Nd |
| 3,4-Dihydroxyphenylacetic acid        | nd    | 6.80 ± 0.69<sup>b</sup> | Nd |
| Quercetin                             | 2.98 ± 0.14<sup>b</sup> | Nd | Nd |
| (+)-Catechin                          | nd    | Nd | Nd |
| Pyrocatechol                          | nd    | Nd | Nd |
| (-)-Epicatechin                      | nd    | Nd | Nd |
| Verbascoside                          | nd    | Nd | Nd |
| Taxifolin                             | nd    | Nd | Nd |
| 2-Hydroxycinnamic acid                | nd    | Nd | Nd |
| Eriodictyol                           | nd    | Nd | Nd |
| Kaempferol                            | nd    | Nd | Nd |

The different superscripts within the same row are different statistically at a 5% significance level. nd: Not detected.

Table 3: Antioxidant activity of *O. mollis* extracts.

| Antioxidant activity | EtOAc | MeOH | Water | Trolox | EDTA |
|---------------------|-------|------|-------|--------|------|
| Phosphomolybdenum   | 3.31 ± 0.03<sup>c</sup> | 2.01 ± 0.22<sup>b</sup> | 3.28 ± 0.14<sup>c</sup> | 1.05 ± 0.02<sup>a</sup> | — |
| DPPH scavenging     | 95.73 ± 0.55<sup>c</sup> | 3.33 ± 0.07<sup>b</sup> | 5.78 ± 0.45<sup>b</sup> | 0.29 ± 0.01<sup>a</sup> | — |
| ABTS scavenging     | > 20.00<sup>c</sup> | 2.30 ± 0.06<sup>b</sup> | 2.75 ± 0.28<sup>b</sup> | 0.26 ± 0.03<sup>a</sup> | — |
| CUPRAC reducing     | 3.96 ± 0.08<sup>d</sup> | 1.48 ± 0.01<sup>b</sup> | 2.30 ± 0.14<sup>c</sup> | 0.24 ± 0.01<sup>a</sup> | — |
| FRAP reducing       | 2.61 ± 0.10<sup>b</sup> | 0.79 ± 0.01<sup>b</sup> | 1.44 ± 0.06<sup>b</sup> | 0.10 ± 0.01<sup>a</sup> | — |
| Ferrous ion chelating | 2.58 ± 0.02<sup>c</sup> | 2.40 ± 0.18<sup>b</sup> | 1.01 ± 0.02<sup>b</sup> | 0.06 ± 0.004<sup>a</sup> | — |

TEs: troloxequivalent. EDTAEs: ethylenediaminetetraacetic acid (disodium salt) equivalent. The different superscripts within the same row are different statistically at a 5% significance level.
Table 4: Enzyme inhibition activity of O. mollis extracts.

| Enzyme inhibitory activity | EtOAc | MeOH | Water | Galantamine | Kojic acid | Acarbose |
|-----------------------------|-------|------|-------|-------------|------------|----------|
| AChE inhibition (IC<sub>50</sub> mg/mL) (mg GALAEs/g extracts) | 1.11 ± 0.03<sup>a</sup> | 1.41 ± 0.04<sup>b</sup> | 11.54 ± 1.08<sup>b</sup> | 0.004 ± 0.0003<sup>a</sup> | — | — |
| 2.75 ± 0.06<sup>b</sup> | 2.16 ± 0.06<sup>b</sup> | 0.26 ± 0.03<sup>c</sup> | — | — | — | — |
| BChE inhibition (IC<sub>50</sub> mg/mL) (mg GALAEs/g extracts) | 1.49 ± 0.01<sup>a</sup> | 6.72 ± 0.26<sup>b</sup> | >20.00<sup>c</sup> | 0.006 ± 0.0001<sup>a</sup> | — | — |
| 3.75 ± 0.04<sup>b</sup> | 0.83 ± 0.03<sup>b</sup> | 0.26 ± 0.01<sup>c</sup> | — | — | — | — |
| Tyrosinase inhibition (IC<sub>50</sub> mg/mL) (mg KAES/g extracts) | 3.66 ± 0.01<sup>a</sup> | 2.94 ± 0.19<sup>b</sup> | 4.54 ± 0.34<sup>c</sup> | — | 0.31 ± 0.01<sup>a</sup> | — |
| 73.34 ± 0.21<sup>b</sup> | 91.61 ± 5.98<sup>b</sup> | 59.26 ± 4.42<sup>b</sup> | — | — | — | — |
| α-Amylase inhibition (IC<sub>50</sub> mg/mL) (mgACEs/g extracts) | 2.63 ± 0.01<sup>b</sup> | 2.08 ± 0.04<sup>b</sup> | 16.88 ± 0.30<sup>c</sup> | — | — | 1.06 ± 0.03<sup>b</sup> |
| 399.44 ± 2.17<sup>b</sup> | 504.24 ± 9.45<sup>b</sup> | 59.80 ± 11.2<sup>c</sup> | — | — | — | — |
| α-Glucosidase inhibition (IC<sub>50</sub> mg/mL) (mgACEs/g extracts) | 1.07 ± 0.01<sup>a</sup> | 1.55 ± 0.02<sup>b</sup> | 4.12 ± 0.07<sup>c</sup> | — | — | 1.65 ± 0.07<sup>b</sup> |
| 1630.61 ± 10.78<sup>a</sup> | 1123.62 ± 15.90<sup>b</sup> | 423.06 ± 7.22<sup>c</sup> | — | — | — | — |

GALAEs: galantamine equivalent. KAEs: Kojic acid equivalent. ACEs: Acarbose equivalent. The different superscripts within the same row are different statistically at a 5% significance level.

The EtOAc extract showed higher activity than others in all the tested assays except for the ferrous ion chelating activity test. The activity data exhibited by the MeOH extract in phosphomolybdenum, DPPH and ABTS radical scavenging, CUPRAC, and FRAP tests were 2.01, 3.33, 2.30, 1.48, and 0.79 mg/ml, respectively. The water extract exhibited higher ferrous ion chelating activity test activity than other extracts (1.01 mg/ml). It was followed by MeOH and EtOAc extracts, respectively (2.40 and 2.58 mg/ml, respectively). None of the extracts exhibited as high antioxidant activity as troloxy and EDTA used as positive control agents.

3.3. Enzyme Inhibitory Activity Potential of the Extracts. Table 4 shows the AChE, BChE, tyrosinase, α-amylase, and α-glucosidase inhibitory activity data. The results were given as IC<sub>50</sub> and the equivalent of galantamine, kojic acid, and acarbose. None of the extracts exhibited as high antioxidant activity as the positive control agents.

The EtOAc extract exhibited 1.11 and 1.49 mg/ml activity in the AChE and BChE inhibitory activity tests, respectively. It was followed by the MeOH extract (1.41 and 6.72 mg/ml, respectively). The water extract exhibited relatively weak inhibitory activity (11.54 and >20.00 mg/ml, respectively). In the case of tyrosinase inhibitory assay, the MeOH extract showed the highest activity (2.94 mg/ml). It was followed by EtOAc and water extracts, respectively (3.66 and 4.54 mg/ml, respectively). In the α-amylase inhibitory activity test, the MeOH extract showed the highest activity (2.08 mg/ml), while the α-glucosidase inhibitory activity test resulted in the superiority of the EtOAc extract (1.07 mg/ml). The water extract showed weaker inhibition in both activity tests than the others (16.88 and 4.12 mg/ml, respectively).

3.4. Correlation Coefficients between the Parameters. The correlation between total phenolic/flavonoid data and activity tests is given in Table 5. The same table also presents the correlation coefficients between biological activity parameters and phytochemicals found in high amounts in the extracts.

The correlation coefficients between the total phenolics/flavonoids of the extracts and the results obtained from the phosphomolybdenum, DPPH radical scavenging, CUPRAC, and FRAP assays were over 0.9. According to the data in the table, there was also a high correlation between the antioxidant activities of the extracts with apigenin 7-glucoside, luteolin 7-glucoside, rosmarinic acid, vanillic acid, pinocresinol, and apigenin. However, apart from the correlation between apigenin and tyrosinase inhibitory activity (0.924), no significant correlation was found between the phytochemicals in question and enzyme inhibitory activity.

4. Discussion

According to the literature research, it was determined that the chemical composition of O. mollis was investigated previously by both spectrophotometric and chromatographic methods [43]. The amounts of phenolic and flavonoid compounds reported in this report were considerably lower than those obtained from the current study. In addition, all of the phytochemicals found in high amounts in the present study were higher than those in the report of Tili et al. [43]. Although the same plant species were analyzed in both studies, it was seen that the plant species analyzed in these two studies were collected from different altitudes. This situation is thought to cause a difference in climatic conditions and, therefore, in chemical composition.

In many studies on the chemical composition of Onosma species, apigenin 7-glucoside [50–53], luteolin 7-glucoside [43, 50–53], rosmarinic acid [43, 50–55], vanillic acid [40, 50, 53], and pinocresinol [50–52] were found to be present in high amounts. Therefore, it was understood that the data obtained from the present study support the literature data.
|                      | Phosphomolybdenum | DPPH | ABTS | CUPRAC | FRAP | Ferrous ion chelating | AChE | BChE | Tyrosinase | α-amylase | α-glucosidase |
|----------------------|-------------------|------|------|--------|------|-----------------------|------|------|------------|------------|--------------|
| DPPH                 | 0.815             | —    | —    | —      | —    | —                     | —    | —    | —          | —          | —            |
| ABTS                 | 0.614             | 0.956| —    | —      | —    | —                     | —    | —    | —          | —          | —            |
| CUPRAC               | 0.883             | 0.988| 0.902| —      | —    | —                     | —    | —    | —          | —          | —            |
| FRAP                 | 0.911             | 0.967| 0.854| 0.991  | —    | —                     | —    | —    | —          | —          | —            |
| Ferrous ion chelating| -0.429            | 0.118| 0.398| -0.036 | -0.127| —                     | —    | —    | —          | —          | —            |
| AChE                 | 0.282             | -0.295| -0.557| -0.146 | -0.059| -0.979                | —    | —    | —          | —          | —            |
| BChE                 | -0.361            | -0.822| -0.949| -0.725 | -0.662| 0.786                 | —    | —    | —          | —          | —            |
| Tyrosinase           | 0.898             | 0.498| 0.229| 0.619  | 0.671| -0.767                | 0.667| 0.075| —          | —          | —            |
| α-Amylase            | 0.652             | 0.153| -0.138| 0.302  | 0.385| -0.963                | 0.897| 0.435| 0.905      | —          | —            |
| α-Glucosidase        | 0.079             | -0.483| -0.713| -0.343 | -0.260| -0.924                | 0.978| 0.895| 0.501      | 0.791      | —            |
| Total flavonoid      | 0.935             | 0.951| 0.820| 0.985  | 0.998| -0.189                | 0.006| -0.613| 0.720      | 0.444      | -0.197       |
| Total phenolic       | 0.931             | 0.842| 0.668| 0.915  | 0.933| -0.410                | 0.236| -0.402| 0.834      | 0.635      | 0.042        |
| Apigenin 7-glucoside | 0.973             | 0.810| 0.604| 0.889  | 0.927| -0.487                | 0.319| -0.335| 0.892      | 0.703      | 0.120        |
| Luteolin 7-glucoside | 0.972             | 0.826| 0.626| 0.901  | 0.937| -0.462                | 0.292| -0.361| 0.879      | 0.683      | 0.093        |
| Rosmarinic acid      | 0.973             | 0.818| 0.616| 0.895  | 0.932| -0.474                | 0.305| -0.348| 0.886      | 0.692      | 0.106        |
| Vanillic acid        | 0.809             | 0.994| 0.942| 0.987  | 0.976| 0.083                 | -0.270| -0.804| 0.499      | 0.182      | -0.457       |
| Pinoresinol          | 0.777             | 0.995| 0.965| 0.971  | 0.953| 0.178                 | -0.355| -0.856| 0.434      | 0.090      | -0.538       |
| Apigenin             | 0.954             | 0.736| 0.510| 0.831  | 0.877| -0.584                | 0.423| -0.225| 0.924      | 0.779      | 0.233        |
| Luteolin             | 0.678             | 0.973| 0.986| 0.931  | 0.896| 0.311                 | -0.482| -0.917| 0.305      | -0.049     | -0.647       |
The antioxidant activity of *O. mollis* was previously reported by Tili et al. [43]. As in the section above, where chemical composition data were discussed, the antioxidant activity data obtained from the present study were higher than the data in the literature. Tili et al. [43] reported the antioxidant activity of *O. mollis* in phosphomolybdenum, DPPH, and ABTS radical scavenging, CUPRAC and FRAP reducing activity, and ferrous ion chelating activity tests as 3.95, 14.22, 8.73, 4.38, 2.53, and 1.95 mg/ml, respectively. The main reason for the difference between the data obtained from the current study and the data published by Tili et al. [43] is that the polarity gradient of the extracts in the current study is wider. Unlike the previous study, EtOAc and water extracts were also isolated in the current study. While nonpolar phytochemicals are soluble in EtOAc extract, those with high polarity dissolve in water extract. Therefore, these phytochemicals exhibit different antioxidant activity than those with moderate polarity (MeOH extract).

Many researchers agree that there is a significant relationship between the chemical composition of plants and their antioxidant activities [56–58]. Some reports in the literature showed apigenin 7-glucoside, luteolin 7-glucoside, rosmarinic acid, vanillic acid, and pinoresinol, which were found in high concentrations in the current study, may contribute to antioxidant activity [50–53, 59]. These literature data support the data obtained from the current study.

Considering the data in Table 2, it is thought that apigenin 7-glucoside in EtOAc extract may contribute to cholinesterase inhibitory activity. There are no reports in the literature that this phytochemical has cholinesterase inhibitory activity. However, some reports indicate that some *Onosma* species containing high amounts of apigenin 7-glucoside exhibit significant cholinesterase inhibitory activity [50–53]. Therefore, it is considered that both the AChE and BChE inhibitory activities of the compound in question should be evaluated in future studies.

As can be seen from the section, where the chemical compositions of the extracts were discussed, the MeOH extract was found to be rich in apigenin 7-glucoside, luteolin 7-glucoside, rosmarinic acid, vanillic acid, and pinoresinol. Thus, these phytochemicals were thought to be possible compounds contributing to activity. To have more convincing information about the tyrosinase inhibitory activity potential of these compounds, it is helpful to look at some literature data. A study conducted by Aghraz et al. [60] reported that *Bubonic imbricatum* and *Cladanthus arabicus* exhibited significant tyrosinase inhibitory activity, and both aglycon and glycosidic forms of apigenin were among the main components. In another study conducted by Sarikurkcü et al. [50], the MeOH extract of *O. ambiguens* exhibited higher tyrosinase inhibitory activity than other extracts and was rich in apigenin 7-glucoside and luteolin 7-glucoside. There are also some reports in the literature that plant extracts rich in rosmarinic acid [61–63], vanillic acid [64, 65], and pinoresinol [66] show significant tyrosinase inhibitory activity.

It is thought that the superiority of the MeOH extract in the α-amylase inhibitor activity test may be due to the compounds indicated in Table 1 and also expressed in the sections above. There are some reports in the literature that these compounds themselves or extracts rich in these compounds have high α-amylase inhibitor activities. In a study conducted by Witkowska-Banaszczak et al. [67], it was reported that *Succisa pratensis* rich in luteolin and apigenin in glycosidic forms exhibited significant α-amylase inhibitory activity. In another study carried out by Uysal et al. [61], it was reported that the α-amylase inhibitor activity of *Salvia ceratophylla* might be due to luteolin and rosmarinic acid. In a study in which the α-amylase inhibitory activities of different *Brassica oleracea* subspecies collected from north-central Italy were analyzed, it was reported that vanillic acid could be one of the compounds contributing to the activity [68]. However, in a study conducted by Mwakalukwa et al. [69], it was reported that the α-amylase and α-glucosidase inhibitory activity of pinoresinol was weak. There is also evidence in the literature that vanillic acid exhibits more than 40% inhibitory activity on glucosidase [70]. This finding is consistent with the data obtained from the current study.

To the best of our knowledge, AChE, BChE, and α-glucosidase inhibitory activity of *O. mollis* have not previously been reported elsewhere. Therefore, data presented on these parameters could be assumed as the first report for the literature.

### 5. Conclusion

In this study, the *in vitro* antioxidant and enzyme inhibitory activities of the extracts obtained from the aerial parts of *O. mollis* were analyzed. It was concluded that the extracts have antioxidant activity comparable to positive control agents, and therefore, they could be assumed as one of the sources rich in antioxidants. In addition, it was concluded that MeOH and EtOAc extracts exhibited α-amylase and α-glucosidase inhibitory activity comparable to acarbose and, therefore, could be the source of new and alternative compounds in the treatment of diabetes. However, it is thought that more detailed analysis should be carried out to determine the phytochemicals responsible for the activity in these extracts.

### Data Availability

The data used to support the findings of this study are included within the article.

### Conflicts of Interest

The author declares that there are no conflicts of interest.

### Supplementary Materials

The supplementary file contains details of the chromatographic, antioxidant, enzyme inhibitory activity, and statistical analysis performed in the current study. (Supplementary Materials)

### References

[1] L. Baselga-Escudero, V. Souza-Mello, A. Pascual-Serrano et al., “Beneficial effects of the Mediterranean spices and...
aromas on non-alcoholic fatty liver disease,” *Trends in Food Science & Technology*, vol. 61, pp. 141–159, 2017.

[2] K. Patra, S. Jana, D. P. Mandal, and S. Bhattacharjee, “Evaluation of the antioxidant activity of extracts and active principles of commonly consumed Indian spices,” *Journal of Environmental Pathology, Toxicology and Oncology*, vol. 35, no. 4, pp. 299–315, 2016.

[3] H. Ene-Obong, N. Onuoha, L. Aburime, and O. Mbah, “Chemical composition and antioxidant activities of some indigenous spices consumed in Nigeria,” *Food Chemistry*, vol. 238, pp. 58–64, 2018.

[4] Y. Yesiloglu, H. Aydin, and I. Klic, “In vitro antioxidant activity of various extracts of ginger (*Zingiber officinale* L.) seed,” *Asian Journal of Chemistry*, vol. 25, no. 7, pp. 3573–3578, 2013.

[5] I. Choi, H. Cha, and Y. Lee, “Physicochemical and antioxidant properties of black garlic,” *Molecules*, vol. 19, no. 10, pp. 16811–16823, 2014.

[6] K. Srinivasan, “Antioxidant potential of spices and their active constituents,” *Critical Reviews in Food Science and Nutrition*, vol. 54, no. 3, pp. 352–372, 2014.

[7] X. Bi, J. Lim, and C. J. Henry, “Spices in the management of diabetes mellitus,” *Food Chemistry*, vol. 217, pp. 281–293, 2017.

[8] M. Serafini and I. Peluso, “Functional foods for health: the interrelated antioxidant and anti-inflammatory role of fruits, vegetables, herbs, spices and cocoa in humans,” *Current Pharmaceutical Design*, vol. 22, no. 44, pp. 6701–6715, 2016.

[9] N. A. Samah, M. R. Mahmood, and S. Muhamad, “The role of nanotechnology application in antioxidant from herbs and spices for improving health and nutrition: a review,” *Selangor Science & Technology Review (SeSTeR)*, vol. 1, no. 1, pp. 13–17, 2017.

[10] A. Yashin, Y. Yashin, X. Xia, and B. Nemzer, “Antioxidant activity of spices and their impact on human health: a review,” *Antioxidants*, vol. 6, no. 3, p. 70, 2017.

[11] V. V. Kandale, S. N. Mujawar, P. J. Welasy et al., “Development of integrated database of neurodegenerative diseases (IDND),” *Review of Research*, vol. 2, no. 9, pp. 1–5, 2013.

[12] L. S. Schneider, F. Mangialasche, N. Andreassen et al., “Clinical trials and late-stage drug development for Alzheimer’s disease: an appraisal from 1984 to 2014,” *Journal of Internal Medicine*, vol. 275, no. 3, pp. 251–283, 2014.

[13] L. E. Hebert, P. A. Scherr, L. A. Beckett et al., “Age-specific incidence of Alzheimer’s disease in a community population,” *The Journal of the American Medical Association*, vol. 273, no. 17, pp. 1354–1359, 1995.

[14] M. J. Knapp, D. S. Knopman, P. R. Solomon et al., “A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer’s disease,” *The Journal of the American Medical Association*, vol. 271, no. 13, pp. 985–991, 1994.

[15] J. E. Mintzer and P. Kershaw, “The efficacy of galantamine in the treatment of Alzheimer’s disease: comparison of patients previously treated with acetylcholinesterase inhibitors to patients with no prior exposure,” *International Journal of Geriiatric Psychiatry*, vol. 18, no. 4, pp. 292–297, 2003.

[16] M. Boada-Rovira, H. Brodaty, P. Cras et al., “Efficacy and safety of donepezil in patients with Alzheimer’s disease: results of a global, multinational, clinical experience study,” *Drugs & Aging*, vol. 21, no. 1, pp. 43–53, 2004.

[17] V. J. Hearing and M. Jiménez, “Mammalian tyrosinase—the critical regulatory control point in melanocyte pigmentation,” *International Journal of Biochemistry*, vol. 19, no. 12, pp. 1141–1147, 1987.

[18] M. Jiménez, K. Tsukamoto, and V. J. Hearing, “Tyrosinases from two different loci are expressed by normal and by transformed melanocytes,” *Journal of Biological Chemistry*, vol. 266, no. 2, pp. 1147–1156, 1991.

[19] K. Tsukamoto, I. J. Jackson, K. Urake, P. M. Montague, and V. J. Hearing, “A second tyrosinase-related protein, TRP-2, is a melanogenic enzyme termed DOPAchrome tautomerase,” *The EMBO Journal*, vol. 11, no. 2, pp. 519–526, 1992.

[20] C. Jiménez-Cervantes, F. Solano, T. Kobayashi et al., “A new enzymatic function in the melanogenic pathway. The 5,6-dihydroxyindole-2-carboxylic acid oxidase activity of tyrosinase-related protein-1 (TRP1),” *Journal of Biological Chemistry*, vol. 269, no. 27, pp. 17993–18000, 1994.

[21] A. Hashimoto, “The mechanism of depigmentation by hydroquinone; a study on suppression and recovery processes of tyrosinase activity in the pigment cells in vivo and in vitro,” *The Japanese Journal of Dermatology*, vol. 94, no. 7, pp. 794–804, 1984.

[22] S. Akiu, Y. Suzuki, T. Ashara, Y. Fujinuma, and M. Fukuda, “Inhibitory effect of arbutin on melanogenesis—biochemical study using cultured B16 melanoma cells,” *The Japanese Journal of Dermatology*, vol. 101, no. 6, pp. 609–613, 1991.

[23] T. Katagiri, “Inhibitory effect of esculin on melanogenesis,” *The Japanese Journal of Dermatology*, vol. 104, pp. 1367–1372, 1994.

[24] V. Kahn, “Effect of kojic acid on the oxidation of DL-DOPA, norepinephrine, and dopamine by mushroom tyrosinase,” *Pigment Cell Research*, vol. 8, no. 5, pp. 234–240, 1995.

[25] Y. Kumano, T. Sakamoto, M. Egawa, I. Iwai, M. Tanaka, and I. Yamamoto, “In vitro and in vivo prolonged biological activities of novel vitamin C derivative, 2-O-.ALPHA.-D-Glucopyranosyl-L-Ascorbic acid (AA-2G), in cosmetic fields,” *Journal of Nutritional Science and Vitaminology*, vol. 44, no. 3, pp. 345–359, 1998.

[26] K. Tasaoka, T. Kamei, S. Nakano, Y. Takeuchi, and M. Yamato, “Effects of certain resorcinol derivatives on the tyrosinase activity and the growth of melanoma cells,” *Methods and findings in experimental and clinical pharmacology*, vol. 20, no. 2, pp. 99–110, 1998.

[27] M. Ichihashi, Y. Funasaka, A. Ohashi et al., “The inhibitory effect of DL-α-tocopheryl ferulate in lecithin on melanogenesis,” *Anticancer research*, vol. 19, no. 5A, pp. 3769–3774, 1999.

[28] K. Shimizu, R. Kondo, and K. Sakai, “Inhibition of tyrosinase by flavonoids, stilbenes and related 4-substituted resorcinols: structure-activity investigations,” *Planta Medica*, vol. 66, no. 1, pp. 11–15, 2000.

[29] A. Mitraokou, D. Kelley, M. Mokan et al., “Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance,” *New England Journal of Medicine*, vol. 326, no. 1, pp. 22–29, 1992.

[30] D. Porter Jr, “Clinical importance of insulin secretion and its interaction with insulin resistance in the treatment of type 2 diabetes mellitus and its complications,” *Diabetes*, vol. 17, no. 3, pp. 181–188, 2001.

[31] A. D. Baron, “Postprandial hyperglycaemia and α-glucosidase inhibitors,” *Diabetes Research and Clinical Practice*, vol. 40, pp. S51–S55, 1998.

[32] R. B. Goldberg, D. Einhorn, C. P. Lucas et al., “A randomized placebo-controlled trial of repaglinide in the treatment of type 2 diabetes,” *Diabetes Care*, vol. 21, no. 11, pp. 1897–1903, 1998.

[33] K.-T. Kim, L.-E. Rioux, and S. L. Turgeon, “Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by fucoidan obtained from *Fucus vesiculosus* and *Ascophyllum nodosum*,” *Phytochemistry*, vol. 98, pp. 27–33, 2014.
[63] G. Zengin, R. Ceylan, K. I. Sinan et al., “Network analysis, chemical characterization, antioxidant and enzyme inhibitory effects of foxglove (Digitalis cariensis Boiss. ex Jaub. & Spach): a novel raw material for pharmaceutical applications,” Journal of Pharmaceutical and Biomedical Analysis, vol. 191, Article ID 113614, 2020.

[64] J. Z. Liu, X. H. Xu, R. Jiang et al., “Vanillic acid in Panax ginseng root extract inhibits melanogenesis in B16F10 cells via inhibition of the NO/PKG signaling pathway,” Bioscience Biotechnology and Biochemistry, vol. 83, no. 7, pp. 1205–1215, 2019.

[65] T. Rodboon, S. Okada, and P. Suwannalert, “Germinated riceberry rice enhanced protocatechuic acid and vanillic acid to suppress melanogenesis through cellular oxidant-related tyrosinase activity in B16 cells,” Antioxidants, vol. 9, no. 3, p. 247, 2020.

[66] H. Azhar ul, A. Malik, M. T. H. Khan et al., “Tyrosinase inhibitory lignans from the methanol extract of the roots of Vitex negundo Linn. and their structure-activity relationship,” Phytomedicine, vol. 13, no. 4, pp. 255–260, 2006.

[67] E. Witkowska-Banaszczak, V. Krajka-Kuzniak, and K. Papierska, “The effect of luteolin 7-glucoside, apigenin 7-glucoside and Succisa pratensis extracts on NF-kappa B activation and alpha-amylase activity in HepG2 cells,” Acta Biochimica Polonica, vol. 67, no. 1, pp. 41–47, 2020.

[68] A. Mollica, A. Stefanucci, G. Zengin et al., “Polyphenolic composition, enzyme inhibitory effects ex vivo and in vivo studies on two Brassicaceae of north-central Italy,” Biomedicine & Pharmacotherapy, vol. 107, pp. 129–138, 2018.

[69] R. Mwakalukwa, Y. Amen, M. Nagata et al., “Postprandial hyperglycemia lowering effect of the isolated compounds from olive mill wastes—an inhibitory activity and kinetics studies on alpha-glucosidase and alpha-amylase enzymes,” ACS Omega, vol. 5, no. 32, pp. 20070–20079, 2020.

[70] G. Y. Chen, H. Zhang, and F. Q. Yang, “A simple and portable method for beta-glucosidase activity assay and its inhibitor screening based on a personal glucose meter,” Analytica Chimica Acta, vol. 1142, pp. 19–27, 2021.