

Total phenolic, flavonoid, protein contents and biological activities of wild mustard

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ORIGINAL RESEARCH PAPER

Received: April 9, 2023 • Accepted: May 25, 2023

Published online: July 24, 2023

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ABSTRACT

The nutritional and therapeutic benefits of plants are outstanding. Wild mustard (*Sinapis arvensis* L.) collected from different regions was analysed for its phenolic, flavonoid, protein, and biological activity levels. In this case, the plant ethanol extract was obtained using a soxhlet apparatus. Rel assay kits and the DPPH test were used to assess the plant's antioxidant activity. The agar dilution test was used to determine antimicrobial efficacy. A549 lung cancer cells were used in an MTT assay to measure antiproliferative activity. The Folin–Ciocalteu reagent was used to quantify the total phenolic content of the sample. The amount of flavonoids was determined using an aluminium chloride test. The amount of protein was calculated using the AOAC's standard technique. Based on the research conducted, it was shown that the maximum total antioxidant status (TAS) value for the ethanol extract of wild mustard from various places was $5.232 \pm 0.047 \text{ mmol L}^{-1}$. At a concentration of 2 mg mL^{-1} , DPPH activity was measured to be 82.06 ± 1.01 . The maximum levels of total phenolic, flavonoid, and protein were $80.57 \pm 2.19 \text{ mg g}^{-1}$, $154.07 \pm 2.79 \text{ mg g}^{-1}$, and $7.75 \pm 0.24\%$, respectively. Doses of $25\text{--}100 \text{ }\mu\text{g mL}^{-1}$ of plant extracts were effective against fungal strains, whereas doses of $50\text{--}200 \text{ }\mu\text{g mL}^{-1}$ were beneficial against bacterial strains. The plant extracts were shown to have potent antiproliferative properties.

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It was found that wild mustard's phenolic, flavonoid, protein, and biological activity levels varied according to the location from which it was gathered. It was also concluded that wild mustard had significant biological activity.

KEYWORDS

charlock mustard, field mustard, wild mustard, charlock, traditional medicine

1. INTRODUCTION

There are several biological processes that rely on plant components. Because of its many useful qualities, it has been put to use by humans for a very long time. Particularly trendy today are plants with medicinal uses. Antioxidant, anticancer, antimicrobial, antiaging, antiinflammatory, antiproliferative, hepatoprotective, and DNA-protective properties have all been demonstrated in plants (Selamoglu et al., 2017). The importance of identifying plant biological activity in this setting cannot be overstated. *Sinapis arvensis* L. was employed as a source material for this investigation.

S. arvensis, a member of the Brassicaceae family that may be found all over the world, prefers sunny, nitrified, and degraded open places. Mustard charlock is also known as wild mustard, field mustard, and charlock. The plant has significant monetary value. *S. arvensis* can grow to be between 20 and 80 cm long. The fruit is a tiki siliqua, the seeds are round, and the flowers are a sunny yellow. Young *S. arvensis* leaves are tasty. Typically, it is boiled before being ingested. The ground seeds can be used in place of mustard (Singh et al., 2022). The purpose of this research was to analyse the antioxidant (total antioxidant, total oxidant, DPPH), antimicrobial, and antiproliferative properties of the *S. arvensis* plant's aerial sections.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

Antalya, Hatay, Gaziantep, and Mersin provinces all contributed samples of their wild mustard plants (Türkiye). The plant samples were dried in a cool, well-ventilated location with indirect sunlight. Thirty grams of plant material was measured and extracted with 250 mL of ethanol at 50 °C for approximately 6 h. Heidolph Laborota 4000 Rotary Evaporator was used to remove the solvents from the final extracts.

2.2. Antioxidant tests

The DPPH test and Rel Assay kits were used to measure the antioxidant capacities of the plant samples. Total antioxidant status (TAS) was measured using the TAS kit, while total oxidant status (TOS) was measured with the TOS kit. The manufacturer's instructions for the kit were adhered to. Calibrators included Trolox (TAS) and hydrogen peroxide (TOS). By dividing the total oxidant stress (TOS) by the total antioxidant status (TAS), the oxidative stress index (OSI) was obtained (Sevindik, 2019).



Plant extract stock solutions with concentrations of 0.25, 0.5, 1, and 2 mg mL⁻¹ were prepared for the DPPH assay. DMSO was used to get the desired concentrations. After adding 50 µL of the stock solution to 160 µL of 0.039% DPPH, the mixture was incubated at room temperature for 30 min. After that, the absorbance at 517 nm was measured. All plant preparations were tested many times. The standard antioxidant used was ascorbic acid (Shimada et al., 1992). Finally, the percentage of DPPH free radical scavenging was calculated using the formula: % inhibition = [(Abs control–Abs sample)/Abs control] × 100.

2.3. Antimicrobial activity test

The agar dilution technique was used to determine the antimicrobial activity of the plant ethanol extract. The MICs of each extract were determined by testing them against reference strains of bacteria and fungi.

Bacteria - Gram positives: *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, Gram negatives: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 1960; and fungi - *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135, *Candida glabrata* ATCC 90030 were used in the study.

Mueller Hinton Broth medium was used to pre-cultivate bacterial strains, whereas RPMI 1640 Broth medium was used for pre-cultivating fungal strains. All extracts were diluted with distilled water to final concentrations in the range of 8.0–12.5 µg mL⁻¹ before being used in the experiments. Colony formation, expansion, and the lack of a colony were measured as examples of inhibition (Sevindik, 2019).

2.4. Total phenolic, flavonoid, and protein tests

A stock solution of 1 mg mL⁻¹ was obtained from the plant extract using distilled water. 250 µL of the stock solution was mixed with 1 mL of Folin–Ciocalteu reagent (1:9, v/v). After the addition of 0.75 mL of 1% Na₂CO₃, this solution was then incubated for 2 h at room temperature. The incubation period was followed by a measurement at 760 nm. Based on the gallic acid standard solution's calibration curve, the total phenolic content was calculated in mg g⁻¹ (Uysal et al., 2017).

Aluminum chloride analysis was used to calculate the plant's total flavonoid content (Chang et al., 2002). The mixture contained 0.1 mL 10% Al(NO₃)₃, 0.1 mL 1 M NH₄CH₃COO, 4.3 mL methanol, 0.5 mL quercetin, and 0.5 mL plant extract. After that, it spent 40 min in an incubator. The absorbance measurement at 415 nm was taken as the last step. Flavonoids were reported as mg g⁻¹.

Association of Official Analytical Chemists (AOAC Method No. 920.87, conversion factor 6.25) guidelines were used to calculate total protein content (AOAC, 1995). After adding the catalyst and 12 mL of sulfuric acid to the Kjeldahl flask, 5 g of the plant sample was added, and the heating was left on until the contents had cleared. After the combustion process was complete, 75 mL of distilled water was added to the apparatus. 50 mL of 4% boric acid solution was drawn into the distillate container at the other end of the system to retain the ammonia separated from the ammonium sulphate in the heated content by steam distillation. The ammonia volume was between 75 and 100 mL. Distillation was halted once around 150–200 mL of distillate had accumulated in the distillate container, and its contents were titrated with 0.1 N HCl solution.



2.5. Antiproliferative activity test

The MTT Cell Viability Assay was used to assess the antiproliferative efficacy of plant ethanol extracts against the A549 lung cancer cell line. Plant extract solutions were prepared at concentrations of 25, 50, 100, and 200 $\mu\text{g mL}^{-1}$. The cells were split using 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) once they reached 70–80% confluence, and then plated. After incubating for 24 h, the prepared solutions were added to the mixture. After that, it spent 24 h in an incubator. A 1 mg mL^{-1} MTT solution was added to the growth medium, and the mixture was incubated at 37 °C until a purple precipitate appeared in the supernatants. Dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) was added to MTT in order to facilitate its dissolution. Finally, an Epoch spectrophotometer (BioTek Instruments, Winooska, VT) was used to read the plates at 570 nm (Bal et al., 2017).

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity

Metabolic processes in living organisms produce free radicals particularly damaging to cells. Antioxidants mitigate or prevent the damage caused by free radicals. Oxidative stress arises when the body’s natural antioxidant defences are unable to prevent it. Oxidative stress has been linked to the development of several devastating illnesses in humans, including cancer, cardiovascular disease, dementia, and Parkinson’s disease. Oxidative stress can be mitigated with the use of supplementary antioxidants (Bal et al., 2019). In this study, the antioxidant potential of wild mustard plant collected from different regions was determined. The outcomes are displayed in Tables 1 and 2.

Antalya’s sample of wild mustard had the highest TAS (5.232 ± 0.047), Gaziantep’s sample had the highest TOS (21.390 ± 0.184), and Gaziantep’s sample had the highest OSI

Table 1. TAS, TOS, and OSI values of wild mustard

Province	TAS (mmol L^{-1})	TOS ($\mu\text{mol L}^{-1}$)	OSI ($\text{TOS}/(\text{TAS} \times 10)$)
Antalya	5.232 ± 0.047	7.564 ± 0.153	0.146 ± 0.003
Hatay	4.780 ± 0.122	12.797 ± 0.135	0.268 ± 0.008
Gaziantep	4.411 ± 0.054	21.390 ± 0.184	0.485 ± 0.009
Mersin	4.567 ± 0.038	10.294 ± 0.125	0.233 ± 0.006

TAS: total antioxidant status; TOS: total oxidant status; OSI: oxidative stress index

Table 2. DPPH scavenging activity of wild mustard

Province	0.25	0.5	1	2 mg mL^{-1}
Antalya	29.80 ± 1.02	45.83 ± 1.29	76.14 ± 1.83	82.06 ± 1.01
Hatay	24.91 ± 0.86	43.65 ± 1.25	74.78 ± 1.32	80.35 ± 1.65
Gaziantep	21.60 ± 1.68	40.37 ± 0.62	70.51 ± 0.57	79.96 ± 0.55
Mersin	20.34 ± 0.59	39.74 ± 0.55	67.94 ± 0.37	78.88 ± 0.34
Ascorbic acid	82.18 ± 1.27	87.17 ± 0.83	94.93 ± 0.70	97.47 ± 0.45



(0.485 ± 0.009). There is no data in the literature regarding TAS, TOS, or OSI values of *S. arvensis*. TAS, TOS, and OSI values for various plant species have been reported; some examples include *Mentha longifolia* subsp. *longifolia* (TAS: 3.628, TOS: 4.046, OSI: 0.112), *Alcea kurdica* (TAS: 3.298, TOS: 8.312, OSI: 0.252), *Rumex scutatus* (TAS: 8.656, TOS: 4.951, OSI: 0.057), *Helianthemum salicifolium* (TAS: 9.490, TOS: 14.839, OSI: 0.157) and *Ferulago platycarpa* (TAS: 5.688, TOS: 15.552, OSI: 0.273) (Sevindik et al., 2017; Mohammed et al., 2020, 2021, 2022; Unal et al., 2022). All our samples of wild mustard had higher TAS than *M. longifolia* subsp. *longifolia* and *A. kurdica*, and lower TAS than *R. scutatus*, *H. salicifolium*, and *F. platycarpa*, as compared to this other research. The TAS value is representative of all components in a natural product that have antioxidant properties (Korkmaz et al., 2018). The wild mustard we tested seemed to have antioxidant properties. It has been hypothesised that this plant may serve as a useful antioxidant in this situation. The total oxidant potential (TOS) is a measure of all oxidising chemicals present in a product of nature (Korkmaz et al., 2018). The OSI level is calculated by dividing the sum of the TOS values by the sum of the TAS values. As the OSI value increases, it is recommended to limit the consumption of a natural product (Korkmaz et al., 2018). All wild mustard samples we tested showed better TOS and OSI values compared to *M. longifolia* subsp. *longifolia* and *R. scutatus*. In addition, it was found that the TOS and OSI values of certain samples of wild mustard from the province of Antalya were lower than those of *A. kurdica*, while the values of other provinces samples were higher. Wild mustard samples collected in Gaziantep had higher TOS values than those of *F. platycarpa* and *H. salicifolium*. Also, as compared to *F. platycarpa* and *H. salicifolium*, the TOS value of samples obtained in other places was lower. It was determined that the OSI value of wild mustard samples collected from Hatay, Gaziantep, and Mersin were higher than of *H. salicifolium*. In addition, it was determined that the OSI values of wild mustard collected from Gaziantep were higher than of *F. platycarpa*. In this context, we found that all wild mustard samples we tested showed promising anti-oxidant properties.

Our research showed that the wild mustard ethanol extract has increasing DPPH scavenging activity with increasing concentration. At the maximum dose of 2 mg mL^{-1} , we found that the standard (ascorbic acid) we utilised inhibited activity by 97.47 ± 0.45 percent. The wild mustard sample from Antalya province had the highest result of 82.06 ± 1.01 at a concentration of 2 mg mL^{-1} out of all samples tested. The ability of all plant samples to scavenge DPPH was found to be less than that of ascorbic acid. The DPPH value was found to be 78.25% in research using Iranian samples of *S. arvensis* (Tabaraki and Ghadiri, 2013). Several different parts of *S. arvensis* from Turkey were employed in yet another investigation. The DPPH value was found to be between 1.88 and $15.56 \mu\text{mol TE g}^{-1}$ (Basyigit et al., 2020). Our research found that the DPPH scavenging activity of wild mustard was higher than that seen in these other investigations. Therefore, it has been shown that wild mustard gathered in various locations has the ability to naturally catch free radicals.

3.2. Antimicrobial activity

The incidence of illnesses caused by microbes has risen in recent years. Unintentional antibiotic usage and the subsequent rise of drug-resistant bacteria are largely to blame. New antimicrobial medicines are needed in this setting. Natural antimicrobials have been studied as an alternative to synthetic ones because of concerns about their potential negative effects. In this work,



we looked at how well an ethanol extract of wild mustard from various places performed against test bacteria and fungi. Table 3 displays the acquired findings.

Essential oil from *S. arvensis* has been reported to be efficacious at doses between 25 and 250 µg mL⁻¹ against *Bacillus subtilis*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, and *P. aeruginosa* (Rad et al., 2013). Antimicrobial activity of *S. arvensis* against *S. aureus*, *E. coli*, *P. aeruginosa*, *Proteus vulgaris*, and *K. pneumoniae* was observed in a separate investigation at doses between 18 and 26 mg mL⁻¹ (Ali-Shtayeh et al., 2013). Our research showed that 50–100 µg mL⁻¹ of wild mustard extract from the Antalya and Hatay districts was efficient against bacterial strains, whereas 25–50 µg mL⁻¹ was beneficial against fungi. In addition, the concentrations of 50–200 µg mL⁻¹ against bacterial strains and 25–100 µg mL⁻¹ against fungi were observed in samples from Gaziantep and Mersin. At 50 µg mL⁻¹, all samples were most effective against *E. faecalis* and *A. baumannii*, while at 25 µg mL⁻¹, they were most effective against *C. glabrata*. In addition, the bactericidal concentrations of all plant materials ranged from 50 to 200 µg mL⁻¹. The concentration range in which it was effective against fungi was 25–100 µg mL⁻¹. The anti-fungal activity of plant extracts was often greater. Based on our findings, we conclude that wild mustard has antibacterial activity against the bacterial and fungal strains employed in this investigation.

3.3. Total phenolic, flavonoid, and protein contents

Secondary metabolites, such as phenolics and flavonoids, are produced by plants. Rather from being valued for their nutritional value, these chemicals are very significant from a medicinal standpoint. They're a good source of protein, too. Protoplasm in plant is rich in proteins. Their cellular roles are distinct from one another. In Table 4, we compare the total phenolic, flavonoid, and protein contents of wild mustard samples from various areas.

Table 3. Antimicrobial activity of wild mustard

Province	<i>S. aureus</i>	<i>S. aureus</i> MRSA	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. baumannii</i>	<i>C. glabrata</i>	<i>C. albicans</i>	<i>C. krusei</i>
Antalya	100	100	50	100	100	50	25	25	50
Hatay	100	100	50	100	100	50	25	50	50
Gaziantep	100	200	50	200	100	50	25	50	100
Mersin	200	200	50	100	100	50	25	25	100

Note: 25, 50, 100, 200 µg mL⁻¹ represents the lowest concentration that inhibits the growth of microorganisms.

Table 4. Total phenolic, flavonoid, and protein contents of wild mustard

Province	TPC (mg g ⁻¹)	TFC (mg g ⁻¹)	TPRO (%)
Antalya	80.57 ± 2.19	146.40 ± 3.55	7.75 ± 0.24
Hatay	73.05 ± 1.49	154.07 ± 2.79	6.68 ± 0.26
Gaziantep	72.17 ± 1.64	153.80 ± 0.90	7.00 ± 0.15
Mersin	70.34 ± 0.63	144.17 ± 1.24	6.91 ± 0.36

TPC: total phenolic content; TFC: total flavonoid content; TPRO: total protein content

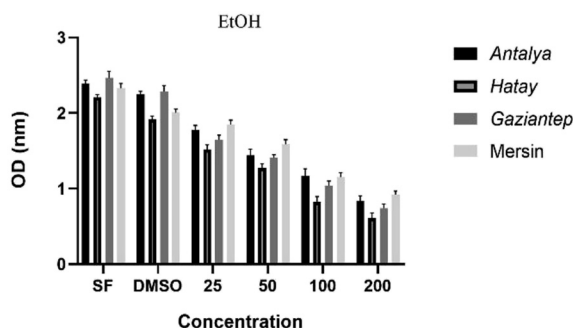


In this research, we analysed the total phenolic content of wild mustard samples from various locations and found that those from Antalya had the highest concentration (80.57 ± 2.19). The Mersin samples had the lowest total phenolic content (70.34 ± 0.63). Total flavonoid content ranged from 144.17 to 154.07 mg g^{-1} in the samples we analysed, with the highest concentration found in Hatay (154.07 ± 2.79) and the lowest in Mersin (144.17 ± 1.24). Antalya samples (7.75 ± 0.24) had the highest total protein level, whereas Hatay samples (6.68 ± 0.26) had the lowest. *S. arvensis* from Turkey has been found to have a total phenolic content of $6.42\text{--}22.25 \text{ mg g}^{-1}$ and a total flavonoid content of $0.76\text{--}7.39 \text{ mg g}^{-1}$ when analysing leaf, flower, root, fruit, and seed components (Basyigit et al., 2020). The total phenolic content of *S. arvensis* gathered in Iran was found to be between 1.62 and 51.86 mg g^{-1} in a separate research, which included data from the plant's leaves, roots, and flowers (Tabaraki and Ghadiri, 2013). Our analysis of wild mustard showed that its phenolic and flavonoid content was higher than that of the research cited above. The variations in climate between the locations where the plants are situated can be regarded as the primary cause.

3.4. Antiproliferative activity

From ancient times to the present, cancer has been a worldwide health concern. Herbal therapy has been suggested as an adjunct to conventional cancer treatment in a number of researches. Additionally, many cancer treatments' active components are extracted from plants and manufactured synthetically. Given this background, assessing the potential of herbal resources in the development of novel cancer therapies is crucial. Our research looked at the potential anti-cancer benefits of ethanol extracts of wild mustard from various geographic locations on the A549 lung cancer cell line. Figure 1 displays the research results.

In our investigation, it was established that the ethanol extract of wild mustard demonstrated substantial effects depending on the concentration rise in all samples. Samples taken in Hatay province showed the highest, while the samples taken in the province of Mersin showed the lowest levels of proliferation. Antiproliferative activity against the HCT116 colon cancer cell line has been observed for two *Sinapis* species (*Sinapis nigra* and *Sinapis alba*) in the literature (Boscaro et al., 2018). Our research is the first to examine the efficacy of *S. arvensis* against



SF: The group not treated with chemicals, only kept in the medium; DMSO: The group in which the medium and DMSO were applied; the group in which the extract was applied at 25, 50, 100 and 200 $\mu\text{g mL}^{-1}$ concentrations

Fig. 1. Antiproliferative activity of wild mustard



the A549 lung cancer cell line. Based on the results, it was concluded that this compound had potent antiproliferative properties.

4. CONCLUSIONS

In this study, the biological activities of wild mustard samples collected from different regions were determined. The levels of phenolics, flavonoids, and proteins were also calculated. The results show that wild mustard samples vary in their effects and composition depending on where they were collected. It also possesses powerful antioxidant and antiproliferative properties. The phenolic, flavonoid, and protein levels found in it indicate that those may be obtained from a natural source. Therefore, it has been shown that wild mustard, a plant that may be consumed, has significant biological activity and can be used as a resource.

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