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Research Article

Some biological activities of *Thalictrum minus* (Ranunculaceae)

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Abstract: Plants are vital natural resources that are used in a wide range of applications. Throughout history, these tools have proven to be valuable assets for individuals. We conducted a study to assess the biological activity of the aerial portions of *Thalictrum minus* L. In this particular situation, the plant's ethanol and methanol extracts were obtained using Soxhlet equipment. The Rel Assay kits were used to measure the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI). The antiproliferative effectiveness against the A549 lung cancer cell line was assessed using the MTT test. The anticholinesterase activity was assessed by measuring the activities of acetylcholinesterase and butyrylcholinesterase. The plant's ethanol and methanol extracts were analyzed, and their TAS values were determined to be 4.371±0.083 and 4.027±0.081, respectively. The TOS values were determined to be 11.816±0.121 and 13.580±0.176, respectively, whereas the OSI values were determined to be 0.271±0.007 and 0.337±0.009. The antiproliferative activity of the methanol extract of the plant was found to be greater than that of the ethanol extract. The ethanol extract had an anti-AChE value of 58.90±1.41, while the methanol extract had an anti-AChE value of 65.11±1.01. Similarly, the ethanol extract had an anti-BChE value of 72.25±0.79, while the methanol extract had an anti-BChE value of 85.79±0.68. Consequently, it was established that the plant has antioxidant, anticancer, and anticholinesterase properties.

1. INTRODUCTION

Throughout human history, individuals have utilized a variety of natural substances for various objectives. Individuals have employed natural substances for many functions, including generating warmth, constructing dwellings, procuring sustenance, manufacturing tools, and combating illnesses (Eraslan *et al.*, 2021; Mohammed *et al.*, 2023). Plants hold a significant

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position among natural commodities. Plants play a crucial role in the human diet because of their nutritional and aromatic characteristics (El-Chaghaby *et al.*, 2024). Plants are utilized by individuals not only for their nutritional attributes but also for their role in illness prevention and treatment. Several studies have shown that plants have many biological activities, such as antioxidant, antiproliferative, antiaging, anti-inflammatory, antimicrobial, anticancer, antitumor, DNA protective, and hepatoprotective properties (Selvi *et al.*, 2022; Aladı *et al.*, 2023; Kalkan *et al.*, 2023; El-Chaghaby *et al.*, 2024; Özcandır *et al.*, 2024; Seğmenoğlu and Sevindik, 2024; Yagi *et al.*, 2024; Zengin *et al.*, 2024). We conducted a study to assess the biological activities of *Thalictrum minus* L.

T. minus (Ranunculaceae) is commonly referred to as lesser meadow-rue. This plant species is widely distributed in various locations of the world and is known for its cosmopolitan nature. It thrives in several habitats including, gravel areas, coastal rocks, calcareous meadows, and rocky valleys. The plant reaches a maximum height of 30 cm, with upright stems and 3-4 sets of triple, hairy, and highly split leaves measuring 1 cm each. It is commonly employed for the management of diarrhea, elevated body temperature, and headaches (Popović *et al.*, 1992; Mushtaq *et al.*, 2016; Singh *et al.*, 2023).

2. MATERIAL and METHODS

We collected specimens from Kahramanmaraş, a city in Türkiye. The plant's aerial components were utilized for the process of extraction. The identification of the plant was made using Flora of Turkey and the East Aegean Islands, Volume 1 (Davis, 1965). The samples were desiccated in a controlled laboratory setting, shielded from direct sunlight. Weighed 30 grams of dry samples. Subsequently, it was extracted using 250 mL of ethanol in a Soxhlet apparatus, keeping at a temperature of 50 °C for a duration of 6 hours. The aforementioned procedure was replicated for the methanol extract. The solvents from the extracted substances were removed by evaporating them using a Buchi R100 Rotary Evaporator at a temperature of 40 °C. The crude extracts were refrigerated at +4 °C until the experiment was conducted.

2.1. Total Antioxidant and Oxidant Tests

The extracts' total antioxidant levels were quantified using the TAS kit. The TOS kit was used to measure the overall amounts of oxidants. Trolox served as a calibrator in the TAS test. The values were denoted in terms of molar concentration (mmol/L). Hydrogen peroxide served as a calibrator in the TOS test. The values were denoted in micromoles per liter (μmol/L). The kit manufacturer's methodology (Erel, 2004; Erel, 2005) guided the conduct of the tests. The OSI value was determined by comparing the unit of the TOS value with the unit of the TAS value, scaling the TOS value to match the TAS value, and then calculating the percentage (Sevindik, 2019).

2.2. Anticholinesterase Activity Tests

The anticholinesterase activity of the extracts was assessed using the Ellman method, as described by Ellman *et al.* in 1961. The extracts were evaluated for their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) within the context of anticholinesterase activity. Galantamine served as the study's benchmark. The extracts were diluted to generate stock solutions with concentrations ranging from 200 to 3.125 μg/mL. Next, we introduced 130 microliters of a 0.1 molar phosphate buffer with a pH of 8, 10 microliters of a stock solution, and 20 microliters of either AChE or BChE enzyme solution into the microplate. The sample then underwent a 10-minute incubation period at a temperature of 25 °C. Next, 20 μL of DTNB (5.5''-dithiobis-(2-nitrobenzoic acid)) solution and 20 μL of either acetylcholine iodide or butyrylcholine iodide substrate were added. The measurement was then taken at a wavelength of 412 nm. The extracts were analyzed in three repetitions. The IC50 values, representing the percentage inhibition of the samples, were given in units of micrograms per milliliter (μg/mL).

2.3. Antiproliferative Activity Test

The antiproliferative properties of plant extracts were assessed against the A549 lung cancer cell line using the MTT test. Stock solutions were generated by diluting the extracts to concentrations of 25, 50, 100, and 200 µg/mL. Subsequently, the cells reached a state of confluence, with 70-80% coverage. Subsequently, a 3.0 mL solution of Trypsin-EDTA (Sigma-Aldrich, MO, USA) was employed for the procedure of separation. Subsequently, the specimens were placed on a plate and subjected to incubation for a duration of 24 hours. Subsequently, stock solutions were introduced. Subsequently, the specimens were subjected to a 24-hour incubation period. Next, the liquid portion of the samples was mixed with the culture medium and substituted with a solution containing 1 mg/mL MTT. Subsequently, the samples were subjected to incubation at a temperature of 37 °C until the formation of a purple precipitate occurred. At this point, dimethyl sulfoxide (DMSO) from Sigma-Aldrich, MO, USA was introduced to the MTT solution. Ultimately, the plates were analyzed at a wavelength of 570 nm using an Epoch spectrophotometer (BioTek Instruments, Winooska, VT) (Bal *et al.*, 2017).

3. RESULTS and DISCUSSION

3.1. Antioxidant and Oxidant Status

Free radicals are reactive molecules formed during normal metabolic processes. Although low concentrations of these substances are not toxic, their amounts can lead to significant cellular damage as they increase (Krupodorova and Sevindik, 2020). The antioxidant defense system operates to mitigate the impact of free radicals. Occasionally, the antioxidant defense system may be inadequate in its ability to control the activity of free radicals (Bal *et al.*, 2019; Akkaya *et al.*, 2024). In this scenario, oxidative stress is present. Oxidative stress can lead to the development of various major diseases in humans, including diabetes, multiple sclerosis, Alzheimer's, Parkinson's, cardiological disorders, and cancer. Supplemental antioxidants can mitigate the impact of oxidative stress (Sevindik *et al.*, 2017; Korkmaz *et al.*, 2018; Baba *et al.*, 2020; Sudirman *et al.*, 2024). Plants possess significant natural antioxidant properties, making them highly valuable. In our work, we assessed the antioxidant capacity of *T. minus*. The Table 1 displays the TAS, TOS, and OSI values.

Table 1. TAS, TOS and OSI values of *Thalictrum minus.*

Extract	TAS (mmol/L)	TOS (µmol/L)	OSI (TOS/(TASx10))
Ethanol	4.371±0.083	11.816±0.121	0.271±0.007
Methanol	4.027±0.081	13.580±0.176	0.337±0.009

Values are presented as mean ± SD

Previous studies have documented the antioxidant capacity of *T. minus* using various techniques (Karyagina et al., 2011; Malik et al., 2017; Mishra et al., 2021). In our study, the TAS, TOS, and OSI values of *T. minus* were determined for the first time. The literature contains recorded TAS, TOS, and OSI values for several plant species. The TAS values of *Ferulago platycarpa, Helianthemum salicifolium, Silybum marianum, Asparagus acutifolius, Galium aparine, Glycyrrhiza glabra*, and *Alcea kurdica* were reported as 5.688, 9.490, 5.767, 6.238, 5.147, 8.770, and 3.298 mmol/L, respectively, in these studies. Furthermore, these studies recorded the TOS values as 15.552, 14.839, 12.144, 13.892, 18.679, 14.590, and 8.312 mmol/L, respectively. The OSI values were recorded as 0.273, 0.157, 0.211, 0.221, 0.346, 0.167, and 0.252, respectively (Mohammed *et al.*, 2019; Mohammed *et al.*, 2020; Korkmaz *et al.*, 2021; Mohammed *et al.*, 2021a; Mohammed *et al.*, 2021b; Mohammed *et al.*, 2021c; Mohammed *et al.*, 2022). *T. minus* had lower TAS values in our study than *F. platycarpa, H. salicifolium, S. marianum, A. acutifolius, G. aparine*, and *G. glabra*. This was true for both ethanol and methanol extracts of the plant. However, the TAS values of *T. minus* extracts were higher than those of *A. kurdica*. The TAS value serves as a comprehensive measure of the collective antioxidant molecules generated in natural goods (Ahmad *et al.*, 2023). In our study, we observed that the ethanol extract of *T. minus* exhibited a greater TAS value. Furthermore, our study established the plant's antioxidant capabilities. The total oxidant status (TOS) is a quantitative measure of the collective amount of oxidant chemicals generated in natural products (Ahmad *et al.,* 2023). Our study assessed the total oxidant status (TOS) of the ethanol extract of *T. minus* and found it to be lower than that of *F. platycarpa, H. salicifolium, S. marianum, A. acutifolius, G. aparine*, and *G. glabra*. However, it was greater than *A. kurdica*'s TOS. The total oxidant status of the methanol extract was found to be lower than that of *F. platycarpa, H. salicifolium, A. acutifolius, G. aparine*, and *G. glabra*, while it was higher than that of *S. marianum* and *A. kurdica*. Upon analysis, it was shown that the OSI value of the ethanol extract of *T. minus* was lower than that of *F. platycarpa* and *G. aparine* but greater than that of *H. salicifolium, S. marianum, A. acutifolius, G. glabra*, and *A. kurdica*. Furthermore, the OSI value of the methanol extract was found to be greater than that of *F. platycarpa, H. salicifolium, S. marianum, A. acutifolius, G. glabra*, and *A. kurdica*, but lower than *G. aparine*. The OSI value quantifies the degree of reduction in oxidant chemicals present in natural goods due to the presence of endogenous antioxidant molecules (Ahmad *et al.,* 2023). The investigation revealed that *T. minus*, exhibited the capability to inhibit oxidant chemicals. Within this particular framework, it was determined that the plant possesses antioxidant capabilities.

3.2. Anticholinesterase Activity

Alzheimer's disease is a prevalent neurodegenerative illness in contemporary times. It is particularly prevalent among individuals aged 60 and above. Experts predict that the next few years will see the identification of over 80 million cases globally. One of the therapy techniques for this disease is the inhibition of cholinesterase enzymes (Sevindik *et al.,* 2024). In our investigation, we evaluated the anticholinesterase properties of *T. minus* ethanol and methanol extracts. Table 2 displays the results.

Table 2. Anti-AChE and anti-BChE values of *Thalictrum minus.*

Extract	AChE	BChE
Ethanol	58.90±1.41	72.25±0.79
Methanol	65.11±1.01	85.79±0.68
Galantamine	9.84±0.15	16.39±0.20

Values are presented as mean ± SD

The existing literature lacks any research on the acetylcholinesterase and butyrylcholinesterase activities of *T. minus*. We conducted this study for the first time. According to the literature, some plant species have anticholinesterase activity, as documented by Adewusi *et al.* (2010) and Mohammed *et al.* (2024). Our investigation found that the ethanol extract of *T. minus* exhibited higher levels of both anti-AChE and anti-BChE activities compared to the methanol extract. Furthermore, we used galantamine as a benchmark and found that the extracts exhibited reduced effectiveness. The existence of enzymes that contribute to the development of illnesses, as well as the inhibition of these enzymes, are critical for therapy approaches (Sevindik et al., 2024). Our investigation suggests that *T. minus* possesses both acetylcholinesterase and butyrylcholinesterase activity, making it a potential natural source in this setting.

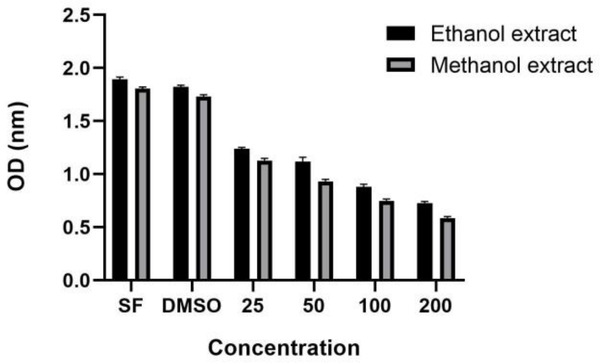
3.3. Antiproliferative Activity

Currently, a wide array of cancer kinds is observed. Novel therapeutic approaches have been devised to combat these specific forms of cancer. Currently, researchers have developed a multitude of therapy modalities to combat various forms of cancer. Furthermore, the utilization of supplements may play a crucial role in achieving victory in the battle against cancer (Karaltı et al., 2021; Esparza et al., 2024). Plants serve as valuable resources for other natural products.

We conducted a study to assess *T. minus*'s inhibitory effect on the growth of A549 lung cancer cells. Figure 1 displays the results.

The literature does not contain any reports on the antiproliferative activity of T. *minus*. According to a paper by Li *et al.* (2016), the species *T. foliolosum* has been found to have cytotoxic effects on various cancer cell lines, including MCF-7 (human breast cancer), PC-3 (human prostate cancer), HL-60 (human leukemia), and U937 (pro-monocytic model).

Figure 1. Antiproliferative activity of *Thalictrum minus*



We conducted a study to examine the impact of ethanol and methanol extracts from *T. minus* on A549 lung cancer cells. The study concluded that the methanol extract had a greater impact than the ethanol extract. Furthermore, it was established that the extracts displayed potent cytotoxic properties that were directly proportional to the concentration rise. Consequently, our investigation determined that *T. minus* may possess natural anticancer properties.

4. CONCLUSION

This study determined the antioxidant, anticholinesterase, and antiproliferative activities of ethanol and methanol extracts of *T. minus*. The obtained results indicated the plant's potential as a natural antioxidant agent. Additionally, the plant showed potential for use in pharmacological designs to combat Alzheimer's disease. The plant also demonstrated strong cytotoxic effects on A549 lung cancer cells. Consequently, the biological activities of *T. minus* demonstrated its effectiveness.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Mustafa Sevindik: Fundings, Conception, Materials, Data collection and processing, Analysis and Interpretation. **Oguzhan Koçer**: Fundings, Analysis and Interpretation, Supervision, and Writing. **Nuh Korkmaz**: Fundings, Design, Analysis and Interpretation and Literature review. **Mehmet Ali Yüzbaşıoğlu**: Statistics and Design. **Imran Uysal**: Fundings, Analysis and Literature review.

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