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Phytochemical Study of Achillea setacea Waldst. et Kit. with Anticholinesterase, Antidiabetic, Anti-Urease, and Anti-Tyrosinase Activities

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Abstract

This study focuses on the phytochemical composition and different biological activities of *A setacea* Waldst. et Kit, a wild species indigenous to the Ortau Mountain in the Karaganda region of the Republic of Kazakhstan. Utilizing the ultrasound-assisted extraction method, we evaluated the extraction efficiency through phytochemical analysis. Phenolic compounds in the extract were identified and quantified using a high-performance liquid chromatograph coupled with a diode array detector (HPLC-DAD). The major phenolic compounds identified in the methanol extracts of *Ach. setacea* were chlorogenic acids, coumarins, ellagic acids and apigenin in flowers (17.48, 13.84, 7.87 and 55.47 µg/g, respectively), aerial parts (73.41, 18.20 and 10.25, 3.76 µg/g, respectively) and roots (14.79, 9.95 and 4.02 µg/g, respectively). In comparison with the underground portion of the plant extracts, flowers and aerial parts exhibited higher total phenolic and flavonoid contents. For instance, chlorogenic acid, coumarin, myricetin, quercetin, apigenin, and ellagic acid show higher amounts in the flower and the plant's aerial part than the underground part. Furthermore, we found that hexane-based root extracts demonstrated superior cholinesterase and diabetic inhibitory activities compared to methanol extracts. On the contrary, methanol extracts exhibited higher uretic and tyrosinase inhibitory activities compared to those obtained from hexane extract.

Keywords: Phenolic and flavonoid contents; *Achillea setacea*; Anticholinesterase; Antidiabetic; Anti-tyrosinase activity.
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1. Introduction

Achillea setacea Waldst. et Kit. (*A. setacea*) is a herbaceous perennial plant that belongs to the genus, *Achillea*, and possesses numerous health benefits for mankind. It is one amongst 100 species within the genus found worldwide, predominantly recognized for its medicinal properties.

Typically, this plant thrives in rare forests and mountainous geographical regions. Extracts of *A. setacea* are known to contain flavonoids, phenolic acids, alkaloids, tannins, glycosides, vitamins, and essential oils.[1] The chemical composition of the plant parts often varies depending on the plant's geographical location and the time of harvest. In Kazakhstan, there are more than 10 species of the genus Achillea (Asteraceae), with *A. setacea* as one of the most common species. This wild plant is predominantly found in the mountains of Ortau located in the central region of the Republic of Kazakhstan, as well as in the steppes of Asia, North America, Australia, New Zealand, Western Europe, and as well as the southern part and Northwestern European region.[2] *A. setacea* has been studied for its natural products, which have shown potential in discovering new bioactive chemicals, including sesquiterpene γ-lactones. However, some bioactive secondary metabolites of *A. setacea* have not been extensively explored. The main reason why this plant species was chosen as the prime focus of our research. Recent studies have investigated the therapeutic benefits of *A. setacea* due to its bioactivities, including antimicrobial,[3] antioxidant,[4] and anti-inflammatory properties as observed in ethanolic extracts.[5] The lipophilic extract of *A. setacea* has shown

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hemostatic activity in cases of toxic animal hepatitis.[6] Previous studies conducted by several researchers found significant compounds in the essential oil extracts of *A. setacea* whereby major components such as borneol, eucalyptol, camphor,[7] and bisabolol oxide[8] were obtained. This plant was selected as the focus of our research due to its therapeutic and pharmacological benefits as compared with other agro-based waste biomass. These include antimicrobial[9] and antioxidant properties.[10] The pharmaceutical industry has been increasingly exploring herbal products and their natural bioactive compounds in recent years.[11] Natural herbal remedies offer a safe and effective alternative to pharmaceutical drugs, particularly in the treatment of diabetes mellitus, which increases the risk of retinopathy, neuropathy, and cardiovascular diseases, especially in type 2 diabetes.[12] Breakthroughs in research have highlighted the importance of these bioactive chemicals in the development of innovative medicinal approaches. The antidiabetic potential of certain plant-derived chemicals, for example, is highlighted in a study published in Chemistry & Biodiversity.[13] The study emphasizes modifying glucose metabolism and enhances insulin sensitivity.[13] The anti-inflammatory and antioxidant qualities of natural substances have also been studied by another research group where these qualities are critical in controlling inflammation and oxidative stress linked to diabetes and other chronic illnesses.[13] The use of natural chemicals in functional foods intended to improve health and prevent disease is covered in research published in Food Research International.[14,15] Indigenous knowledge has documented the therapeutic use of this plant in Alzheimer's disease, a progressive cognitive disorder. Acetylcholinesterase inhibitors are approved drugs for the treatment of mild to moderate Alzheimer's disease.[16] Urease plays a critical role in Helicobacter pylori-associated diseases, such as peptic ulcers and, in some cases, cancer, by promoting bacterial colonization during infection. Inhibition of urease offers a novel strategy to reduce bacterial colonization in the early stages of infection, particularly as antibiotic resistance diminishes its efficacy. Screening compounds and plant extracts for inhibitory effects on these enzymes is crucial for discovering potential treatments for these diseases.[17] Another intriguing topic related to skin disorder is the accumulation of melanin in the dermis, which can lead to skin pigmentation or melanogenesis.[18] Melanin, a dark pigment found in hair and skin, is produced in the dermis through melanogenesis.

Melanin is essential for protecting the skin from UV radiation, preventing skin damage, and reducing the risk of skin cancer.[18] Researchers are also exploring the discovery, extraction, biosynthesis, and characterization of tyrosinase inhibitors from plants and medicinal herbs for skin whitening.[19] The composition of phenolic compounds and the potential bioactivity of *A. setacea* remains largely unexplored, with a dearth of accurate data on its anticholinesterase and anti-diabetic properties. Our study aimed to investigate the phenolic compound profiles and biological activities in hexane and methanol extracts derived from various parts of *A. setacea*, including flowers, aerial parts, and underground components. This study represents the first comprehensive investigation into the phenolic compound profiles and various bioactive properties, including α-glucosidase and α-amylase inhibitory activities, anticholinesterase effects, anti-uretic potential, and anti-tyrosinase activity of hexane and methanol extracts of *A. setacea*. Our findings provide a detailed assessment of their pharmacological relevance, particularly in terms of anticholinesterase, anti-diabetic, anti-uretic, and anti-tyrosinase activities.

2. Materials and methods

2.1 Plant material

The wild plants were sampled in June 2021, near the village of Ortau in the Karaganda region of the Republic of Kazakhstan. The plant was identified by Alibekov D.T., an expert in Ecology and currently a senior researcher at the 'Laboratory of Flora and Plant Resources' of the Astana Botanical Garden in Kazakhstan's capital. The sampled plant materials were air-dried at room temperature for a period of three weeks. Reference sample with registered № MUA 1012 was kept in the laboratory of the Department of Chemistry, Herbarium, Faculty of Science, Mugla Sitki Kocman University, Mugla, Turkey.

2.2 Chemicals

The following chemicals were used as received without any further purification: ethanol (C_2H_6O), hydrochloric acid (HCl), methanol (CH_3OH), hexane, sodium chloride (NaCl), 3,4-dihydroxy-D-phenylalanine (L-DOPA), fungal tyrosinase, Lugol's solution, horse serum butyrylcholinesterase (BChE), kojic acid, 4-N-nitrophenyl-α-D-glucopyranoside (PNPG), electricus electrophorus acetylcholinesterase (AChE), jack bean urease, α-glucosidase from Saccharomyces cerevisiae, galantamine, acarbose, porcine pancreas alpha-amylase. These chemicals were purchased from E. Merck (Darmstadt, Germany) and Sigma-Aldrich Chemical Co. (Steinheim, Germany).

2.3 Extraction

Different parts (flowers, aerial parts and roots) of *A. setacea*, dried and grounded in the shade, were weighed as 50 grams

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each and extracted separately with n-hexane and methanol solvents in an ultrasonic bath at 30 °C (30 min × 3 times), respectively. A rotary evaporator (Heidolph Instrument R215, Switzerland) was used to remove the solvents (hexane and methanol) from the extracts obtained. The hexane and methanol extracts were kept under refrigeration (+4 °C) until they were analyzed.

2.4 SEM Analysis

The surface morphologies of micro samples of *A. setacea* from the flowers and seeds were investigated by infrared scanning electron microscopy (SEM) [Zeiss Crossbeam 540, Germany] at 5-20 kV. The image was taken at different magnifications to better understand the surface morphology. For the SEM studies, samples of *A. setacea*. were taken from dried plants and transferred to a metal stub mounted on carbon using double-sided cello tape. A thin layer of gold was deposited on the surface using a Quorum Q 150T ES module automated sputter coater (10.0 nm).

2.5 HPLC-DAD phenolic contents of *A. setacea* extracts

A Shimadzu 20 AT series high-performance liquid chromatograph with a diode array detector (HPLC-DAD) (Shimadzu Cooperation, Kyoto, Japan) was employed to analyze the phenolic compounds. The phenolic content of extracts was determined as micrograms of pyrocatechol equivalents (PEs).[20,21] The extracts of the species were dissolved in water: methanol (80:20) solution and filtered through a 0.20 µm disposable LC filter disk. Separation was performed using an Intersil ODS-3 reverse phase C18 column. A sample with injection volume of 20 µL and a solvent flow rate of 1.0 mL/min was utilized. The mobile phase consisted of 0.5% acetic acid in water (A) and methanol (B). The elution gradient ranged from 0-10% B (0-0.01 min); 10-20% B (0.01-5 min); 20-30% B (5-15 min); 30-50% B (15-25 min); 50-65% B (25-30 min); 65-75% B (30-40 min); 75-90% B (40-50 min) 90-10% B (50-55 min). Detection was carried out by photodiode array detection (PDA) at a wavelength of 280 nm. A total of 26 phenolic and organic acid compounds were used and characterized using their specific retention times and UV data of the standards.[22] Quantitative analysis was performed using a calibration curve plotted with known concentrations of different standards (ranging from 0.0 to 1.0 ppm). To confirm the effectiveness of the chosen method, the analysis was repeated in triplicate and the results were expressed as mg/g of extract's dry weight.

2.6 Anticholinesterase activity determination

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibition activity were analyzed spectrophotometrically using the Ellman method as described in the compound tests.[23] The reference compound used was galantamine.

2.7 Antidiabetic activity determination

Antidiabetic activity study was conducted to monitor the in vitro α-glucosidase and α-amylase inhibition assay efficiently. A starch iodine method with modifications was evaluated aiming at α-amylase inhibitory activity following the research conducted.[24,25] Porcine pancreas α-amylase and phosphate buffer (20 mM pH = 6.9 prepared with 6 mM NaCl) were used. 50 µL α-amylase and 25 µL sample solutions were mixed in a 96-well microplate and preincubated at 37 °C for 10 minutes. Then 50 µL starch solution (0.05%) was added and incubated for 10 minutes at 37 °C. HCl (0.1 M, 25 µL) and Lugol (100 µL) solutions were added, and absorbance was recorded at 565 nm.

The α -α-glucosidase inhibition activity was determined by the method of but it was further modified by Deveci *et al.* (2020).[26,27] In a 96-well plate, 50 mL of α-glucosidase enzyme obtained from Saccharomyces cerevisiae was used by adding 0.1 units/mL in phosphate buffer (0.01 M) at a pH of 6.0. To the above mixture, 25 mL of PNPG in phosphate buffer (0.01 M) at a pH of 6.9 was added. In addition, 50 mL of phosphate buffer (0.01 M) at pH 6.9 and 10 mL of the extract solution was prepared. The mixture underwent incubation for 20 minutes at 37 °C. In both analyses, acarbose was used as the main standard compound.

2.8 Anti-urease activity determination

To evaluate the inhibitory activity of the urease enzyme, the indophenol method was employed to measure the release of ammonia.[28] A 100 mM sodium phosphate buffer at pH 8.2 was used to prepare the urease solution. After adding *A. setacea*, 10 µL of each extract, 25 µL urease enzyme, and 50 µL of a 100 mM urea solution were combined and incubated at 30 °C for 15 minutes. Following incubation, 45 µL of 1% (w/v) phenol reagent and 70 µL of 0.005% (w/v) alkali reagent were added to each well. After an additional 50 minutes of incubation, the absorbance was measured at 630 nm using a microplate reader. Thiourea served as the reference compound, expressing the results as percentage inhibition at 100 µg/mL and as the 50% inhibition concentration (IC_{50}).

2.9 Anti-tyrosinase activity determination

To measure the inhibitory activity of the tyrosinase enzyme, the spectrophotometric method described by Masuda *et al.* (2005), with L-DOPA as the reaction substrate, was

employed.[29] In a typical measurement analysis, a mixture of 100 mM sodium phosphate buffer at pH 6.8 in 150 µL, 10 µL of extract, and 20 µL of tyrosinase enzyme solution in buffer at pH 6.8 was incubated at 37 °C for 10 minutes. After incubation, 20 µL of L-DOPA was added to the mixture. Absorbance was measured at a visible wavelength of 475 nm in a 96-well microplate. Kojic acid was used as the reference compound. Results were expressed as percentage inhibition (%) at 100 µg/mL and as 50% inhibitory concentration (IC_{50}).

2.10 IC_{50} and A 0.50 values

The results are given as 50% inhibition concentration (IC_{50}) in AChE, BChE, urease, tyrosinase, α-amylase, and α-glucosidase enzyme inhibition assays.

2.11 Statistical analysis

All data were analyzed in triplicate to confirm the effectiveness of the chosen method. Data were expressed as means ± standard deviation of three samples. Statistical analysis was performed with MINITAB 16. Differences were tested for significance by using the ANOVA (analysis of variance) procedure with a significance level of $p < 0.05$.

3. Results and discussion

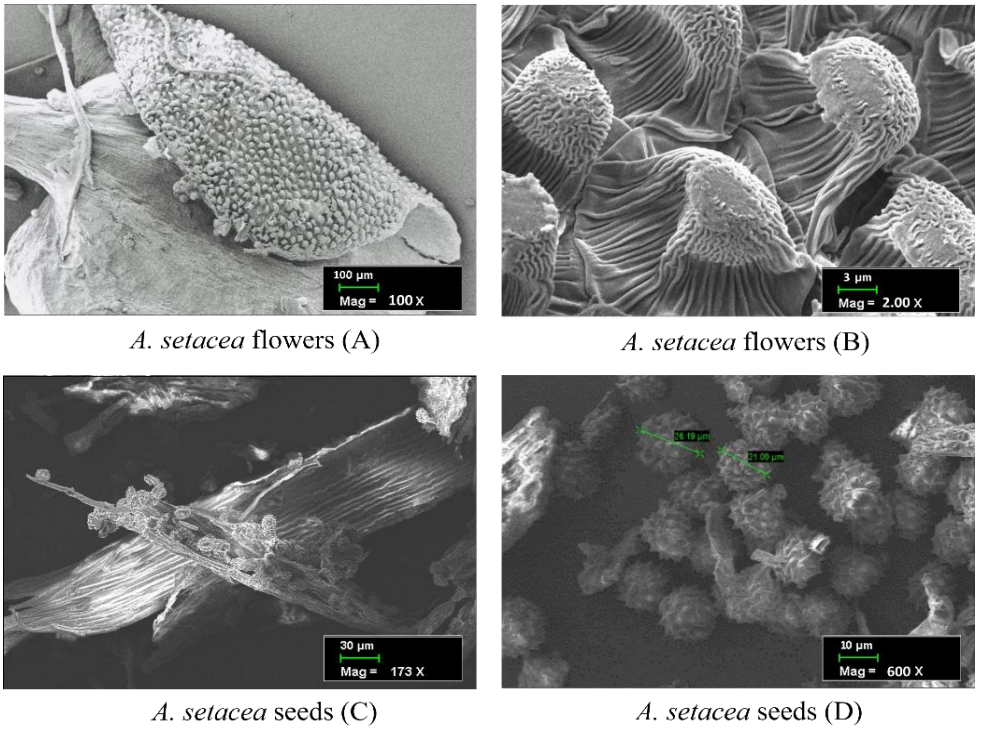
3.1 SEM and analysis

The surface structures of plants and their evolution integrate the chemistry and architecture of the surface with its functions and point to possible applications of biomimetics. The surface morphology of the different parts of the plant was studied using a SEM, and the results from Fig. 1 showed differences between the morphological parts of the flower and seeds of *A. setacea*. The flower results in Figs. 1(A&B) showed spheroidal and isopolar monads with subtriangular embay with different magnifications.

Different magnifications of morphologies obtained for seeds are reported in Figs. 1(C&D). The grain medium-size ranged between 21.00 and 26.19 µm in diameter, as shown in Fig. 1(D). The spines are conical and sharp. The ends of the spines are sharp.

3.2 Total Phenolic and Flavonoid Contents

Investigating the influence of modern extraction methods on phenolic compounds from different parts of *A. setacea* (flowers, aerial parts, and underground parts) is one of the important aspects of our research. High total phenolic content in plant extracts is valuable for functional foods and dietary supplements. Phenolic compounds are natural secondary metabolites with diverse chemical structures. These compounds have various biological and pharmacological properties that may protect against chronic diseases. The UE utilizes useful technologies for extracting phenolic compounds from plants. Extraction efficiency is determined by the concentration of phenolic compounds. The use of high-performance liquid chromatography with diode array detector (HPLC-DAD) analysis revealed the presence of phenolic compounds. Table 1 shows a total of 14 phenolic compounds



A. setacea flowers (A) *A. setacea* flowers (B)

A. setacea seeds (C) *A. setacea* seeds (D)

Fig. 1 SEM morphology images of flowers (A, B) and seeds (C, D) of *A. setacea* plant.

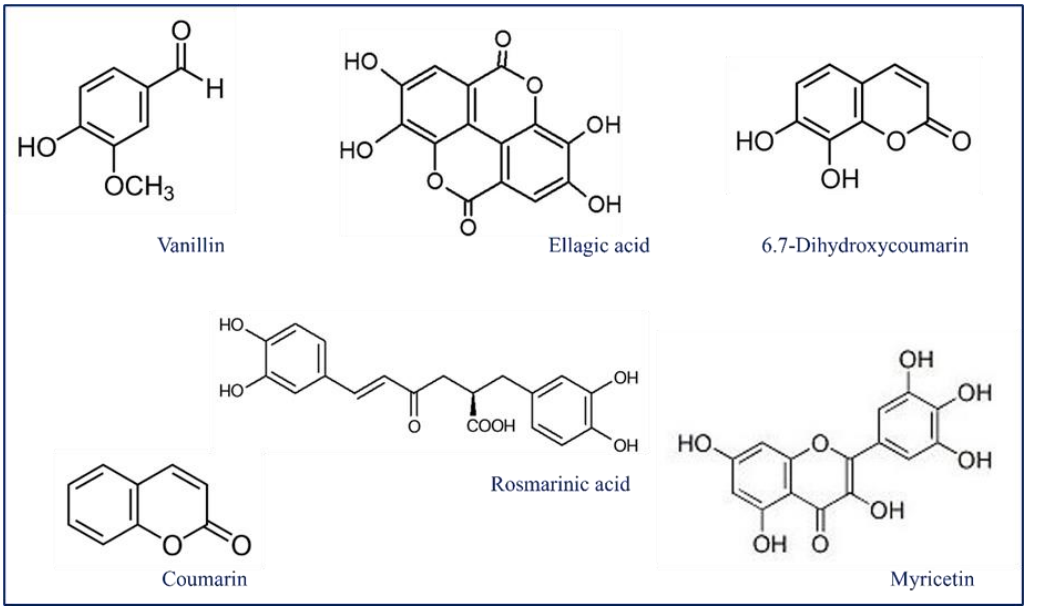


Fig. 2 The most important phenolic compounds identified in the extracts of *A. setacea* species.

Table 1. Phenolic composition of *A. setacea* (µg/g extract) by HPLC- DAD.

Phenolic compounds	RT (min)	*A. setacea*		
		(flower)	(aerial part)	(underground)
Protocatechuic acid	8.75	2.51±0.23	3.75±0.12	1.22±0.09
Catechin	10.68	nd	2.24±0.10	0.56±0.02
Chlorogenic acid	12.35	17.48±0.51	73.41±0.28	14.79±0.35
6.7-Dihydroxy coumarin	14.10	3.54±0.30	3.61±0.43	1.08±0.17
Vanillin	17.78	2.42±0.17	4.27±0.24	0.75±0.05
p-Coumaric acid	20.56	nd	2.88±0.34	3.56±0.20
Coumarin	24.49	13.84±0.66	18.20±0.26	9.95±0.41
Rutin	25.30	nd	3.14±0.10	nd
Ellagic acid	26.11	7.87±0.72	10.25±0.55	4.02±0.23
Rosmarinic acid	26.77	8.33±0.18	2.11±0.38	nd
Myricetin	27.35	8.64±0.27	6.48±0.27	nd
Quercetin	30.83	2.58±0.22	1.46±0.21	0.68±0.05
Luteolin	31.70	10.06±0.53	nd	nd
Apigenin	33.77	55.47±0.75	3.76±0.19	nd

[a] Values expressed are means ± S.E.M. of three parallel measurements ($p < 0.05$). nd: not detected.

identified in the flowers, aerial part and underground parts of *A. setacea* extracts.

These include protocatechuic acid, catechin, chlorogenic acid, 6.7-dihydroxy coumarin, vanillin, p-coumaric acid, coumarin, rutin, ellagic acid, rosmarinic acid, myricetin, quercetin, luteolin, apigenin. The structures of the most important compounds that were identified in the extracts of the *A. setacea* species are shown in Fig. 2. Phenolic compounds were detected in the flowers at retention times ranging from 8 to 34 minutes. Notably, apigenin, a yellowish crystalline core phenolic compound, exhibited the highest concentration at 55.47±0.75 µg/g. Apigenin is a widely studied compound known for its potential therapeutic effects in treating various disorders and pharmacological diseases, including anticancer, anti-inflammatory, anti-toxicity, and Alzheimer's disease. Humans commonly utilize it in the form of extracts for medicinal purposes.[30] A higher level of apigenin was detected in the flowers of the *A. setacea* plant, which underscores the potential therapeutic benefits of this plant in treating various chronic diseases and disorders. Conversely, only 3.76±0.19 µg/g of apigenin was detected in the aerial parts of the plant, and none was found in the underground parts. Chlorogenic acid emerged as the second most abundant compound detected in the flowers of *A. setacea*, with a concentration of 17.48±0.51 µg/g recorded at a retention time of 12.35 minutes. Interestingly, a higher concentration of 73.41±0.28 µg/g was

measured in the aerial parts of the plant, while 14.79±0.35 µg/g was found in the underground parts. Chlorogenic acids are naturally occurring phenolic compounds known for their biological properties and can be found in various plant species.[31] They also aid in modifying glucose-6-phosphatase in glucose metabolism and contribute to fighting obesity. Based on the results, a higher amount detected in the aerial part of the plant demonstrates the medicinal advantages of this aspect of the plant over the flowers and the underground parts. Another significant phenolic compound found in the plant at a retention time of 24.49 minutes is coumarin, a vital compound used in medicine and pharmacy. The highest amount, 18.20±0.26 µg/g, was measured in the aerial part of the plant, while 13.84±0.66 µg/g and 9.95±0.41 µg/g were respectively measured from the flowers and the underground part of the plant. This phenolic compound also serves as a precursor in synthesizing many coumarin derivatives possessing pharmacological activities.[32] Luteolin, an important flavonoid, was obtained from the flowers at a retention time of 31.70 minutes, with a concentration of 10.06±0.53 µg/g, while no amount was detected in the aerial and underground parts. Luteolin is known to inhibit inflammatory pathways in endothelial tissues, as reported by previous studies.[33] An antiproliferation and antioxidant phenolic compound, ellagic acid.[34] has been detected in the extract at a retention time of 26.11 min with a concentration (7.87, 10.25 and 4.02 µg/g) respectively, for flowers, aerial part, and underground part of the studied plant. The highest amount was obtained in the aerial part, followed by the flowers and the underground part of the plant. This acid is also present in raspberries, grapes, strawberries, pomegranates, *etc*.[35] Other important phenolic compounds were detected in trace amounts and are reported in Table 1, with some phenolic acids or flavonoids falling below the detection limit in certain parts of the plant. In general, phenolic acids and flavonoids were predominantly found in the plant's aerial parts (leaves and stems), while in the underground parts, phenolic acids, coumarin, and flavonoids were detected. The prominent compounds observed include chlorogenic acid (73.41 µg/g), apigenin (55.47 µg/g), coumarin (18.20 µg/g), ellagic acid (10.25 µg/g), and luteolin (10.06 µg/g). However, chlorogenic acid was found in higher concentrations in the methanolic extract of the aerial parts compared to the flowers and roots, whereas the apigenin content was higher in the flowers, as depicted in Figs. 3A to 3C. Major peaks obtained from HPLC-DAD results were in accordance with results presented in Table 1 and as well compared with the 26 standard phenolic compounds18 which include protocatechuic acid, catechin, 6.7-dihydroxycoumarin, vanillin, p-coumaric acid, rutin, rosemarinic acid, and various flavonoids such as myricetin, quercetin, and syringetin.

3.3 Anticholinesterase activity

Alzheimer's disease is the leading cause of dementia worldwide. It is a neurological disorder characterized by the

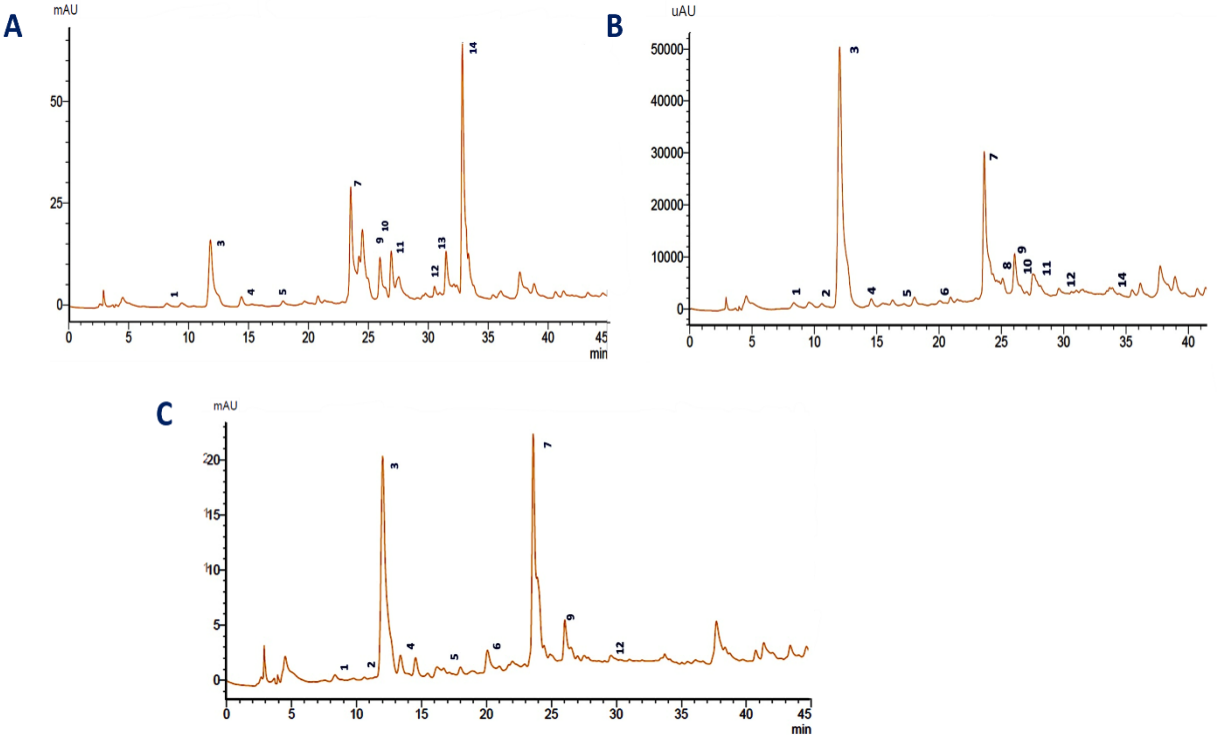


Fig. 3 HPLC-DAD Chromatogram of phenolic compounds *A. setacea* (flowers (A), roots (B), and underground (C)).

presence of plaques and neurofibrillary tangles in specific brain regions.[36] Two main enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are involved in Alzheimer's disease. Table 2 shows the specific anticholinesterase activities of hexane and methanol extracts against AChE and BChE. The current commercial drugs for Alzheimer's disease (donepezil, rivastigmine, and galantamine) are AChE inhibitors. AChE rapidly breaks down acetylcholine in the nervous system, thereby stopping impulse transmission, [37] while BChE is classified as a depolarizing neuromuscular blocking agent, an enzyme that targets the metabolism of succinylcholine in patients. All patients deficient in BChE are prone to prolonged neurological disorders due to mutation in the gene.[38] As shown in Table 2, the hexane extract of *A. setacea* leaves and stems exhibited the highest cholinesterase inhibitory activity among the species studied for both AChE and BChE. The highest inhibition value, 44.87±0.17 µg/mL, was observed in the hexane extract of leaves and stems for BChE, while for the same enzyme, the methanol extract exhibited a value of 39.16±0.59 µg/mL. Comparatively, for the AChE enzyme, the highest inhibition values were 33.47±0.22 µg/mL and 32.13±0.45 µg/mL, respectively, for the hexane and methanol extracts of leaves and stems. In the flowers, inhibition values of 38.71±0.29 µg/mL and 33.03±0.14 µg/mL were obtained for the BChE enzyme with the hexane and methanol extracts, respectively. For the AChE enzyme, values of 30.13±0.62 µg/mL and 27.36±0.76 µg/mL were obtained with the hexane and methanol extracts, respectively. Surprisingly, the root extract showed a higher inhibition amount of 36.40±0.56 µg/mL in the methanol extract compared to 31.64±0.92 µg/mL detected in the hexane extract for the BChE enzyme. Meanwhile, for the AChE enzyme, values of 22.80±0.86 µg/mL and 17.67±0.25 µg/mL were respectively detected in the hexane and methanol extracts. Overall, hexane extract concentrations exhibited higher activity compared to methanol extracts. To date, there has been no investigation of the anticholinesterase activity of extracts from *A. setacea* species. Therefore, the inhibitory activities of extracts from different parts of *A. setacea* against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were investigated for the first time.

3.4 Antidiabetic activity

Diabetes is a condition characterized by high blood glucose levels due to impaired insulin secretion or dysfunction in biological processes. It affects a large number of people worldwide.[39] Herbal products that inhibit key enzymes in diabetes offer a cost-effective and efficient treatment approach. To achieve this, we need natural resources that are more effective, have fewer side effects, and contain more specific inhibitors than current drugs. The enzymes alpha-glycosidase and alpha-amylase break down carbohydrates into glucose. By inhibiting these enzymes, type 2 diabetes can be effectively treated by lowering blood glucose levels. Table 2 displays the α-amylase and α-glucosidase inhibitory activities of the extracts. The hexane extracts of *A. setacea* exhibited significant inhibitory activity against α-amylase compared to the methanol extracts. At a concentration of 200 µg/mL, the hexane root extracts showed the highest α-amylase inhibitory activity, with a result of 55.20±0.87 µg/mL, while for the methanol extract, 23.34±0.25 µg/mL was detected. In comparison, the α-glucosidase activity of the hexane root extract yielded a value of 54.66±0.28 µg/mL, while for the methanol extract, 18.48±0.21 µg/mL was measured. The extracts obtained from the leaves and stems exhibited inhibitory activity of 32.73±0.74 µg/mL and 29.35±0.95 µg/mL, respectively, for α-amylase in the hexane and methanol extracts. For α-glucosidase, values of 15.18±0.11 µg/mL and 11.97±0.69 µg/mL were respectively detected in the hexane and methanol extracts. Similarly, the hexane and methanol extracts obtained from the flowers showed inhibitory activity of 28.61±0.32 µg/mL and 25.23±0.35 µg/mL, respectively, for α-amylase. For α-glucosidase, values of 22.87±0.19 µg/mL and 13.27±0.43 µg/mL were respectively detected in the hexane and methanol extracts. Thus, the present study is the first to investigate the α-glucosidase and α-amylase inhibitory activities of extracts from different parts of *A. setacea.*

3.5 Anti-urease activity

Urease is an enzyme that hydrolyzes urea to ammonia and carbamate and then breaks down carbamate to release more ammonia and carbon dioxide. It is important in infections caused by helicobacter pylori in the gastric tract and proteus and related species in the urinary tract.[40] The importance of discovering new urease inhibitors is magnified, as they could serve as potential drugs to treat ulcers. The test samples demonstrated high activity in inhibiting urease, with the methanol extracts showing the highest percentage inhibition across all plant parts compared to the hexane extracts. An IC_{50} value of 73.45±0.55 µg/mL was obtained in the stems and leaves with the methanol extract, whereas in the hexane extract, a value of 24.87±0.92 µg/mL was detected. Table 2 shows the results. The second highest inhibition activity was observed in the flower extracts, with a percentage inhibition of 67.85±0.93 µg/mL in the methanol extract and 25.30±0.71µg/mL in the hexane extract. In the roots extract,

Table 2. Antidiabetic, cholinesterase, urease and tyrosinase inhibitory activities of the samples a.

		Cholinesterase inhibitory activity				Anti-diabetic activity							
		AChE		BChE		α-glucosidase		α-amylase		Urease inhibitory		Tyrosinase inhibitory	
Plants	Extracts/ Standards	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
A. setacea (flower)	Hexane	>200	30.13±0.62	>200	38.71±0.29	>200	22.87±0.19	>200	28.61±0.32	>200	25.30±0.71	>200	12.35±0.37
	Methanol	>200	27.36±0.76	>200	33.03±0.14	>200	13.27±0.43	>200	25.23±0.35	85.11±0.80	67.85±0.93	>200	28.11±0.19
A. setacea (aerial part)	Hexane	>200	33.47±0.22	>200	44.87±0.17	>200	15.18±0.11	>200	32.73±0.74	>200	24.87±0.92	>200	15.80±0.63
	Methanol	>200	32.13±0.45	>200	39.16±0.59	>200	11.97±0.69	>200	29.35±0.95	76.05±0.86	73.45±0.55	>200	31.52±0.24
A. setacea (underground)	Hexane	>200	22.80±0.86	>200	31.64±0.92	132.4±0.70	54.66±0.28	130.7±1.24	55.20±0.87	>200	14.52±0.21	>200	10.43±0.14
	Methanol	>200	17.67±0.25	>200	36.40±0.56	>200	18.48±0.21	>200	23.34±0.25	>200	32.48±0.86	>200	19.17±0.25
Standards	Galantamine	5.50±0.20	89.25±0.48	42.20±0.35	79.43±0.60	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*
	Acarbose	*NT*	*NT*	*NT*	*NT*	128.5±0.62	56.70±0.75	32.50±0.45	82.10±0.27	*NT*	*NT*	*NT*	*NT*
	Thiourea	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	8.20±0.36	87.37±0.52	*NT*	*NT*
	Kojic acid	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	23.50±0.44	83.54±0.56

[a] Values represent the means ± SEM of three parallel sample measurements (p < 0.05). NT: Not Tested

the urease inhibition activity was measured at 32.48±0.86 µg/mL and 14.52±0.21 µg/mL, respectively, for the methanol and hexane extracts. The urease inhibitory activity of the methanol extract of the stem and leaves was like that of the reference compound thiourea (87.37±0.52%). Thus, the urease inhibitory activities of methanol and hexane extracts from different parts of *A. setacea* were investigated for the first time in the present study.

3.6 Anti-tyrosinase activity

The type, distribution, and degree of melanin pigment in a person determine the color of their hair and skin. Tyrosinase is the key enzyme creating melanin.[41] Concentration-dependent inhibition of the tyrosinase activity was observed for all the extracts of *A. setacea* and is presented in Table 2. The methanol extract showed the most potent inhibitory activity with an IC_{50} of 31.52±0.24 µg/mL. The hexane extracts showed IC_{50} values of 15.80±0.63 µg/mL but lower activity than the reference kojic acid (83.54±0.56%). In the flowers of *A. setacea*, the percentage of tyrosinase inhibition detected for the methanol and hexane extracts were 28.11±0.19% and 12.35±0.0.37%. Similarly, the roots extract exhibited tyrosinase inhibitory activity of 19.17±0.25 µg/mL and 10.43±0.14 µg/mL, respectively, for the methanol and hexane extracts. Thus, the present study is the first to investigate the tyrosinase inhibitory activity of methanol and hexane extracts of different parts of *A. setacea*.

4. Conclusion

The phenolic compounds of *A. setacea* were extracted using an ultrasound-assisted extraction method and subsequently analyzed by SEM and HPLC-DAD. This analysis identified chlorogenic acid, coumarin, vanillin, ellagic acid, rutin, quercetin, myricetin, and other flavonoids as the main phenolic compounds. Among these compounds, chlorogenic acid, apigenin, and coumarin were found to be the most abundant in the studied plant. Historically, extracts from this flavonoid-rich plant have been used to treat inflammations and disorders in humans. In this study, we thoroughly investigated the phytochemical content and activities of extracts from the

flowers, leaves, stems, and roots of *A. setacea*. Specifically, this work targets their anti-cholinesterase, anti-diabetic, anti-urease, and anti-tyrosinase properties, which have been explored for the first time. The extracts demonstrated promising biological properties, suggesting potential pharmaceutical, cosmetics, and food applications. Thus, further research is needed to isolate and identify antidiabetic components from these species.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

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