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Research article

HRMS-based profiling of metabolites, metal ions content and *in-vitro* cholinesterase inhibitory activities of *Sonchus wightianus* DC plant parts ☆

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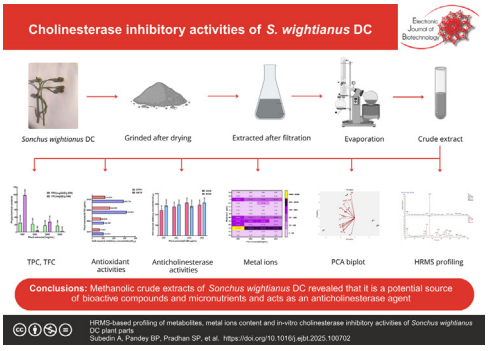
GRAPHICAL ABSTRACT

HRMS-based profiling of metabolites, metal ions content and *in-vitro* cholinesterase inhibitory activities of *Sonchus wightianus* DC plant parts



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ABSTRACT

Background: *Sonchus wightianus* DC is native to South Asia and has traditionally been known for its wide range of applications for the treatment of several human ailments. However, its application for the treatment of neurodegenerative diseases like Alzheimer's disease (AD) has not been studied yet. In this present study, comprehensive metabolite profiling of plant parts and *in-vitro* cholinesterase inhibitory potential was examined to see the efficacy of plant extract against AD.
Results: The potent antioxidant activity was demonstrated by the flower extract in both DPPH and ABTS assays, with IC_{50} values of 104.06 ± 2.05 μg/mL and 67.69 ± 1.58 μg/mL, respectively. The crude methanol extract of the leaf displayed the highest butyrylcholinesterase (BChE) inhibition potential with IC_{50} values of 281.09 ± 14.64 μg/mL. In contrast, the flower extract exhibited the strongest acetylcholinesterase (AChE) inhibition with IC_{50} values of 247.51 ± 11.15 μg/mL. Furthermore, the evaluated plant parts were a rich source of essential macro and micronutrients. Principal component analysis revealed the major

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contribution of total phenolic content (TPC), and total flavonoid content (TFC) in the plant extracts, which might be the prime reason for strong antioxidant and cholinesterase inhibition. Further, the HRMS profiling analysis revealed the presence of Linoleic acid, gingerol, kaempferol, genistein, daidzein, chlorogenic acid, fisetin and 12-oxo-phytodienoic acid.

Conclusions: The findings of this study suggest that *Sonchus wightianus* DC is a promising source of bioactive compounds and essential micronutrients, with notable potential as an anticholinesterase agent.

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1. Introduction

Medicinal plants and herbs have long been acknowledged for their potential health benefits in traditional medicine and remain a key source of novel therapeutic agents for the treatment of several diseases [1]. In many developing nations, plant-based medicines are essential to primary healthcare, often serving as the only accessible or affordable treatment option for significant portions of the population [2]. The majority of medicinal plant species are rich sources of secondary metabolites and have proven to be therapeutic agents for the treatment of various human ailments [3].

Alzheimer's disease (AD) is a neurological disorder characterized by a gradual loss of memory and cognitive function [4]. It has been estimated that over 55 million people worldwide suffer from dementia, with AD accounting for 60–70% of cases [5], and the number is projected to increase in the coming years. In Nepal, the number of people living with AD has increased significantly among the elderly population over the past few years [6]. Although several therapeutic agents are in use to treat AD, no complete cure has been achieved to date.

Among the known hypotheses, the cholinergic hypothesis accounts for the gradual loss of neurotransmitter molecules acetylcholine (AChI), which is essential for memory and learning. In most AD patients, the amount of AChI is significantly less; hence, enhancing the pool of AChI would help AD patients improve their memory. The AChI acts as a substrate for the enzyme cholinesterase; hence, the use of cholinesterase inhibitors is the alternative strategy to enhance the pool of AChI. Cholinesterase inhibitor drug molecules work for AD patients by blocking acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) to improve cholinergic signaling in the brain [7]. Natural products, including those from medicinal plants, have been demonstrated as potential cholinesterase inhibitors, offering an alternative to synthetic medications [8]. Of the known molecules galantamine, derived from the genus *Galanthus* spp. functions as an acetylcholinesterase inhibitor with further implications in AD treatment [9].

The genus *Sonchus*, under the Asteraceae family, is well-regarded in traditional medicine for its anti-inflammatory, diuretic, and wound-healing benefits [10]. Within this genus, *Sonchus*, which is native to South Asia, has gained scientific attraction for its therapeutic potential based on its traditional practices for the treatment of different diseases [11]. However, its biochemical characterization for the efficacy of neurogenerative diseases was not well-reported. Despite the immense potential of the *Sonchus* species, very little scientific evidence is available in the literature about its chemical composition, metal ions, and biological activities. In addition to that, the plant itself is a source of secondary metabolites, and its study is extremely essential for further drug development [12].

In this study, metabolite profiling, metal ions, antioxidant and anti-cholinesterase properties of the flower, leaf, root, and stem of *Sonchus wightianus* DC were investigated to examine its applications for the treatment of AD.

2. Experimental setup

2.1. Chemicals used

Acetylcholinesterase (AChE) (CAS 9000-81-1), Butyrylcholinesterase (BChE) (CAS 9001-08-5), Acetylcholine iodide (AChI), Butyrylcholine iodide (BChI), 2,2-Diphenyl1-picrylhydrazyl (DPPH), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were acquired from Sigma-Aldrich (USA). Likewise, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO), nitric acid (HNO_3), perchloric acid ($HClO_4$), sodium bicarbonate (Na_2CO3), and sulfuric acid (H_2SO_4) were purchased from Fisher Scientific (India). All other chemicals were of analytical grade.

2.2. Collection of plant and extraction

Samples were gathered and collected from Nepal's Gulmi District (83°19′45.6″ E 28°05′44.9″ N) and labeled based on their parts as follows: SWF for flower, SWR for root, SWL for leaf, and SWS for stem. The collected plant parts were identified by Dr. Chitra Bahadur Baniya, Central Department of Botany, Tribhuvan University. The air-dried samples were smushed into powder and dissolved in absolute methanol (100%). In brief, 100 mL of methanol was used to macerate 10 g of powder samples, which were then shaken at 160 rpm for a whole night at room temperature. The entire mixture was filtered the next day. The crude samples were then evaporated using Rota Vapor and a Vacuum Centrifuge Condenser. After evaporation, the dried samples were stored at 4°C.

2.3. Determination of total phenolics and flavonoids

The total phenolic content (TPC) of various plant extracts was assessed using the Folin-Ciocalteu UV–VIS assay [13]. Each plant extract (10 μL, 1 mg/mL) was combined with 50 μL of 10% FC reagent in a 96-well plate and incubated in the dark for 5–6 min at room temperature. Afterward, 40 μL of 7% Na_2CO_3 was added, and the mixture was incubated in the dark for 1 h. Absorbance was measured at 765 nm using a UV–VIS spectrophotometer (Spectrostar Nano Mars, Germany), with gallic acid (4–10 μg/mL) as the standard. The total phenolic content was expressed as mg of gallic acid equivalent per g of dry weight (DW). Furthermore, total flavonoid content (TFC) was evaluated using the aluminum chloride ($AlCl_3$) colorimetric test, reported as mg of Quercetin equivalents per g of DW [14]. Briefly, plant extracts (20 μL) were diluted with 40 μL of distilled water, and then, 10 μL of 5% sodium nitrate ($NaNO_2$) and 10 μL of 10% $AlCl_3$ were added simultaneously. After 6 min, 20 μL of 1 M NaOH was introduced to reach a final vol-

ume of 200 µL with distilled water. Absorbance was measured at 510 nm using a 96-well plate after vigorous mixing.

2.4. Antioxidant assay

The *in vitro* 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay were used for the evaluation of antioxidant activities, following the standard protocol [15,16]. In brief, 136 µL of 100 µM DPPH and ABTS solution were mixed with different concentrations of plant extracts (10–160 µg/mL). After thorough shaking, the absorbance was measured at 517 nm following 30 min of dark incubation for measuring DPPH scavenging and at 734 nm after 10 min incubation for ABTS scavenging potency of the plant extract. The IC_{50} values were measured through extrapolation using curve fitting based on the dose–response data within the specified range.

The percentage of DPPH and ABTS scavenging was determined by using [Equation 1]:

$$\%\,\text{Radical Scavenging} = \frac{(Absorbance\ of\ Control - Absorbance\ of\ Test)}{Absorbance\ of\ Control} \times 100\% \quad (1)$$

2.5. Anti-cholinesterase assay

The assay was conducted using enzyme-substrate kinetics with Ellman's reagent [17], a colorimetric compound that quantifies thiol groups in a sample, also known as 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). DTNB is primarily utilized for pre-column derivatization of thiols through a thiol-exchange reaction, producing one equivalent of 5-thio-2-nitrobenzoic acid (TNB) and forming a thiol-TNB adduct. Samples (25–500 µg/mL) were combined with 0.05 U/mL AChE or 0.5 U/mL BChE to initiate the reaction, which was incubated for 15 min at 25°C. Following this, 0.5 mM DTNB and either 1 mM acetylthiocholine iodide or 1.5 mM butyrylcholine iodide were added. Two hundred microliters of sodium phosphate buffer (pH 8.0) was included to maintain a constant reaction volume. Absorbance was measured at 412 nm using a 96-well plate reader, with galantamine as the positive control and 1% DMSO as the negative control. Using [Equation 2], the percentage of inhibition of AChE and BChE was determined.

$$\%\,\text{Inhibition} = \frac{(Absorbance\ of\ Control - Absorbance\ of\ Test)}{Absorbance\ of\ Control} \times 100\% \quad (2)$$

2.6. Metal ion quantification

With a few modifications, the tri-acid mixture digestion method was used to quantify the major metal ions such as Ca, Fe, Mg, Zn, Cd, Na, Cr, Pb, Cu, Mn and K [18]. Briefly, 1 g of finely powdered and filtered materials was mixed with 25 mL of a 40:4:1 tri-acid solution (HNO_3, $HClO_4$, and H_2SO_4). At 80°C, the mixture was digested down until all of the vapors were expelled. Following cooling, the samples were diluted with 50 mL of deionized water and passed through the Whatman No. 42 filter paper. Using an Atomic Absorption Spectrometer (SavantAA, GBC, Australia) and the direct air-acetylene technique, the elemental composition of the samples was determined [19].

2.7. HRMS analysis

High-resolution mass spectrometry (HRMS) was used to analyze the secondary metabolites in the methanolic extract of plants, considering the results of antioxidant and enzyme inhibitory potential. A stock methanol sample solution containing 1 mg/mL was filtered using a 0.22-µm syringe filter and then diluted with methanol to 0.5–6 mg/mL. The LC-MS analysis was conducted using the HPLC-ESI-Q-TOF-MS system with a 5 µL injection volume, and spectra were recorded within a mass range of 70–1050 *m*/*z*. Mzmine software was used to perform the spectrometric analysis and to predict the chemical formula, including the accurate mass calculation [20]. The identified compounds were then drawn using ChemDraw Professional 16.0.

2.8. Statistical analysis

Experiments were conducted in triplicate, and results were reported as mean ± standard deviation. The inhibitory concentration at which absorbance is 50% (IC_{50}) values for antioxidant and enzyme inhibition activities were determined through linear regression analysis through GraphPad Prism 6.0. The Principal Component Analysis was performed in R Studio 2024.04.0 + 735.

3. Results and discussion

3.1. Total phenolic content (TPC) and total flavonoid content (TFC)

Our results revealed that methanol extracts from all parts examined showed noteworthy phenolic and flavonoid contents. The highest TPC was observed in the leaf extract (21.09 ± 1.6 mg GAE/g DW), followed by flower, stem, and root. Similarly, the flower extract revealed the highest flavonoid content (100.81 ± 2.1 mg QE/g DW), followed by root, leaf, and stem (Table 1). Flavonoids are generally the major constituents in the flower region of plants [21], which is also the case with our findings with *S. wightianus* DC. Phenolic and flavonoid molecules, which are widespread in many medicinal plants, are proven to have numerous biochemical implications, such as anti-inflammatory, antibacterial, antioxidant, and anticancer effects [22]. These phytonutrients play a crucial role in combating oxidative stress, diabetes, cardiovascular diseases, skin disorders, inflammation, cancer, neurological conditions, hypertension, and more [23–25].

3.2. Antioxidant activities

Antioxidant compounds from medicinal plants were known to help mitigate the risk of various diseases linked to reactive oxygen (ROS) and nitrogen species (RNS). Natural plant-based antioxidants, including flavonoids and phenolic compounds, offer a promising alternative to synthetic drugs [26]. The extract revealed the concentration-dependent inhibitory activities in DPPH and ABTS assays. (Fig. 1, Fig. 2). Our results revealed that methanol extracts of the flower showed strong antioxidant activities within both DPPH and ABTS assays with IC_{50} values of 104.06 ± 2.05 and 67.69 ± 1.58 µg/mL, respectively. The IC_{50} values of leaf, root, and stem samples for DPPH and ABTS were tabulated in Table 1.

3.3. Anticholinesterase activities

Finding the effective natural inhibitors of AChE and BChE is of growing scientific interest for AD management. Inhibiting cholinesterase is a common treatment approach for several conditions, including glaucoma, myasthenia gravis, and neurological dis-

Table 1
Total phenolic content, total flavonoid content, antioxidant activities, and enzyme inhibitory potential of the extract. Galantamine was used as a standard for AChE and BChE [48], and Gallic acid and Quercetin were used as a standard for DPPH and ABTS. Results were expressed as the mean ± standard deviation (Mean ± SD) of triplicate experiments.

Sample	TPC (mgGAE/g DW)	TFC (mgQE/g DW)	IC_{50}(µg/mL)			
			DPPH	ABTS	AChE	BChE
SWF	20.81 ± 0.73	100.81 ± 2.1	104.06 ± 2.05	67.69 ± 1.58	247.51 ± 11.15	283.99 ± 17.005
SWL	21.09 ± 1.6	3.69 ± 0.6	105.79 ± 3.34	80.92 ± 3.5	275.34 ± 2.17	281.09 ± 14.64
SWR	16.3 ± 3.12	26.01 ± 3.1	316.38 ± 2.67	165.18 ± 3.43	316.61 ± 6.68	294.13 ± 3.02
SWS	16.92 ± 1.75	2.45 ± 0.7	289.77 ± 4.13	118.85 ± 1.63	293 ± 4.19	309.77 ± 11.65
Gallic acid	–	–	11.5 ± 2.4	33.61 ± 2.45	–	–
Quercetin	–	–	104.06 ± 2.05	67.69 ± 1.58	–	–
Galantamine	–	–	–	–	1.09 ± 0.02	26.27 ± 1.41

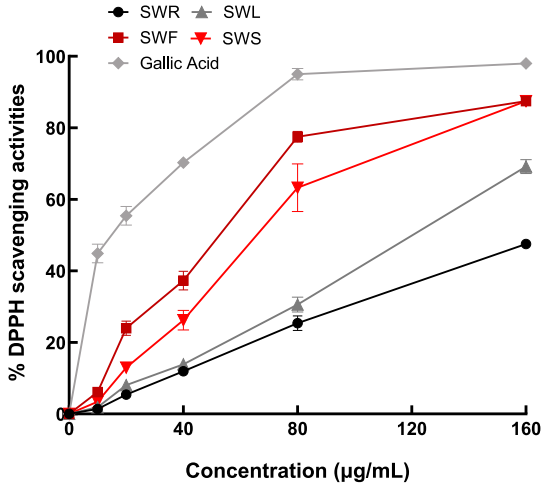

Fig. 1. Percentage scavenging activity of extracts (DPPH).

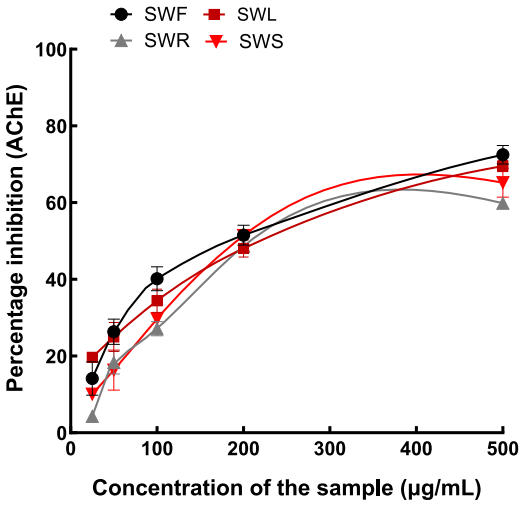

Fig. 3. Percentage of AChE inhibition by extracts.

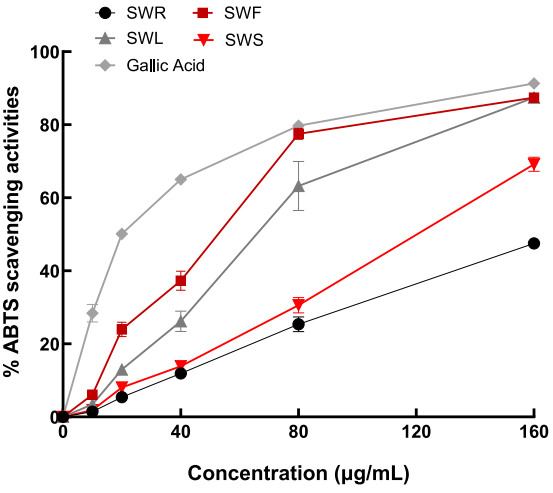

Fig. 2. Percentage scavenging activity of extracts (ABTS).

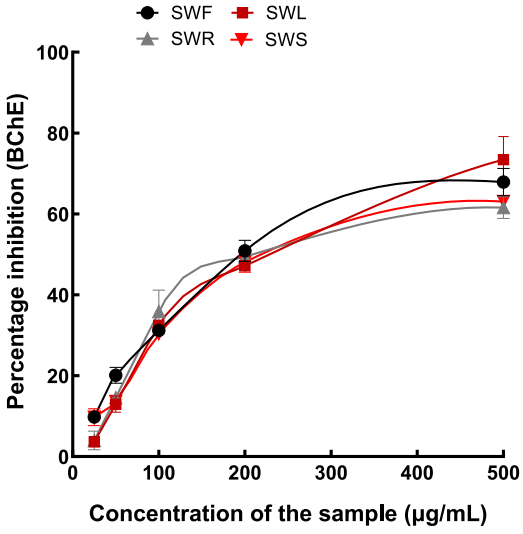

Fig. 4. Percentage of BChE inhibition by extracts.

orders like dementia and AD [27]. Our results revealed that the stem, flower, root, and leaf of *S. wightianus* DC were potential sources of cholinesterase inhibitors to serve as a reference for future research in natural chemical compounds (Fig. 3, Fig. 4). The crude flower extract showed the inhibitory potential toward AChE with IC_{50} values of 247.51 ± 11.15 µg/mL whereas the leaf extract revealed good inhibitory potential toward enzyme BChE with IC_{50} values of 281.09 ± 14.64 µg/mL (Table 1). The lower the IC_{50} value, the more potent the substance is in displaying its inhibiting property toward the enzyme [28]. Further purification and fractional separation of the metabolites will significantly increase the cholinesterase inhibitory activities. We next performed the metal ion analysis as well as profiling of the major chemical constituents responsible for such biological activities.

3.4. Metal ions

Metal ions, including both macronutrients and micronutrients, play a crucial role in human health. Hence, proper quantification of major metal ions in the herbal extract is crucial for the management of AD as well [29]. Furthermore, heavy metal contamination in the herbal extract was reported to have adverse health complications. Hence, proper quantification of essential micro and macro nutrients, along with heavy metals, was equally important along with biological activities [30]. Quantification of major ions was carried out using AAS. Results revealed the highest abundance of Ca in all the plant parts evaluated. Flower showed the maximum concentration of essential micronutrients like Ca > K > Na > Mg > Zn > Fe > Mn > Pb > Ni > Cu > Co > Cr > Cd in the given order, whereas root revealed Ca > Fe > K > Mg > Na > Zn > Mn > Pb > Ni > Cu > Co > Cr > Cd in the given order. Furthermore, the leaf exhibited the highest levels of essential micronutrients in the following order: Ca > K > Fe > Na > Mg > Mn > Zn > Cu > Pb > Ni > Co > Cr > Cd. The results were summarized in Table 2. Fe chelation has demonstrated the potential to minimize oxidative damage and slow the progression of the disease [31]. Chelators targeting Cu can disrupt Aβ-Cu interactions, mitigating plaque formation and neurotoxicity [32]. Disruptions in Zn homeostasis can affect synaptic dysfunction and neurotoxicity, emphasizing the importance of its regulation through chelation [33].

Furthermore, Fe is also needed for numerous biological activities, such as gene regulation, electron transport in mitochondria, oxygen supply, and DNA synthesis [34]. One essential nutrient that aids in preserving proper intracellular fluid levels is potassium. In addition, magnesium is the second most common intracellular cation in the human body and is necessary for more than 300 enzymatic processes [35]. Trace amounts of chromium are necessary to promote healthier brain function, better metabolic processes in the human body, and control of blood sugar. Copper is a necessary metal that the human body needs for the proper functioning of the blood, bone, and many enzyme processes [36].

Lead is a widely recognized environmental pollutant, mainly originating from human activities, including industrial emissions, vehicle exhaust, and the application of lead-containing paints and pesticides [37]. Cadmium is another heavy metal of concern, frequently linked to agricultural activities like the use of phosphate fertilizers and industrial waste releases [38]. The elevated levels of Pb and Cd found in the sample suggest substantial absorption of these heavy metals from the environment, raising alarms about potential impacts on both ecological systems and human health. The European Pharmacopoeia guidelines for herbs consumed by humans set the permissible limit for Pb at 10.0 mg/kg, reflecting a moderate threshold for heavy metal safety. For Cd, a stricter limit of 0.3 mg/kg is established, emphasizing its higher toxicity and potential health risks even at low concentrations [39].

3.5. Principal components

Principal Component Analysis (PCA) was carried out to see the contribution of key variables of flowers, leaves, stems, and roots of *S. wightianus* DC based on the findings of TPC, TFC, DPPH radical scavenging, anti-cholinesterase assay, and metal ion quantification. Two among the three obtained principal components (PCs) with an eigenvalue greater than one were chosen based on the Kaiser-Criterion to account for the total contribution of all the results in the sample [40]. The amount of variance that each PC captures is indicated by its eigenvalue; a greater value depicts a greater amount of variance in the data. With an eigenvalue of 11.97, PC1 explains the largest variance in the data, accounting for 63% of the variance.

Variables such as TPC, Na, Mg, and Pb exhibit a strong positive contribution along PC1, indicating their significant role in differentiating the plant samples along this axis (Fig. 5). Conversely, variables like DPPH, ABTS, Cr, and AChE co-contribute to the negative side of PC1, suggesting an inverse relationship or trade-off between these two sets of variables. Along PC2, variables such as Mn, Cu, Zn, and BChE exert a strong influence, emphasizing their importance in distinguishing the samples along the vertical axis. SWL is heavily influenced by Mn, Cu, Zn, and BChE, aligning in the upper region of the plot. TPC correlates strongly with Na and Mg, indicating that sample SWF might possess higher phenolic content and mineral levels, which is also the case in our data findings. The clustering of antioxidant activities (DPPH and ABTS) suggests they share a common role in plant bioactivity, with a strong relationship to TPC.

Micronutrients such as Mn, Cu, and Zn cluster positively along PC2, likely reflecting their connection to enzymatic or metabolic processes, such as those involving BChE. Antioxidants like DPPH and ABTS appear to function synergistically in managing oxidative stress, potentially balancing their interaction with phenolic content. Similarly, minerals like Mn, Cu, and Zn likely support enzymatic functions as cofactors, explaining their alignment with BChE. The relationship between TPC and minerals points to an interplay between antioxidant and phenolic pathways with nutrient profiles in these plants.

In brief, PC1 primarily distinguishes variables based on antioxidant activity and phenolic content, while PC2 highlights the contribution of enzymatic activity and micronutrients. The positioning of the plant samples reflects their distinct biochemical compositions and profiles.

Table 2
Results for the quantification of main elements. All studies were conducted in triplicate, and findings are provided as mean ± standard deviation (Mean ± SD).

Metals (ppm)	SWF	SWR	SWL	SWS
Co	11.8 ± 0.6	5.08 ± 0.46	9.26 ± 0.27	6.5 ± 2.2
Zn	59.4 ± 1.05	28.6 ± 2.5	68 ± 0.39	25.16 ± 2.65
K	2613.67 ± 49.96	781.1 ± 31.1	900.68 ± 42.6	904.36 ± 36.7
Mn	40.5 ± 0.80	37.2 ± 3.85	143.36 ± 21.66	22.35 ± 0.25
Cu	25.2 ± 2.07	27.38 ± 2.58	29.8 ± 5.7	20.91 ± 0.07
Cr	6.93 ± 0.46	4.55 ± 0.35	5.86 ± 0.20	3.68 ± 0.25
Ni	45.2 ± 0.98	22.53 ± 2.5	25.71 ± 6.4	15.58 ± 0.02
Pb	51.06 ± 1.006	15.66 ± 1.13	22.63 ± 3.17	13.51 ± 0.45
Na	1934 ± 56	717.217 ± 54.6	705.7 ± 67.20	688.05 ± 16.88
Fe	1209.53 ± 12.52	1288.67 ± 74.5	1253.83 ± 30.03	350.483 ± 2.61
Cd	1.26 ± 0.11	0.41 ± 0.02	0.56 ± 0.07	0.38 ± 0.02
Mg	1315.5 ± 29.04	339.4 ± 21.9	387.43 ± 20.7	368.25 ± 28.5
Ca	52116.2 ± 919.09	12703 ± 685.713	20403.2 ± 220.037	16717.1 ± 213.9

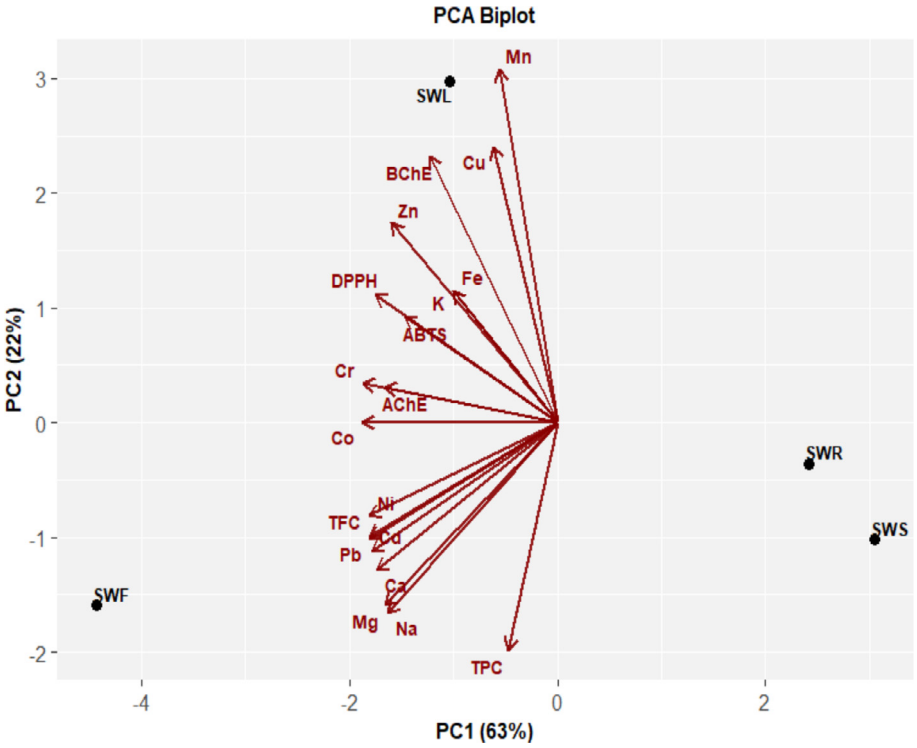

Fig. 5. Bi-plot of PCA analysis.

3.6. Secondary metabolites

Profiling of the major metabolites was carried out using high-resolution mass spectrometry (HRMS). HRMS chromatogram revealed the presence of diverse secondary metabolites. The analysis of the flower, leaf, stem and root of *S. wightianus* DC in both positive and negative ion modes revealed the presence of diverse secondary metabolites (Fig. S1–S9). Our results revealed the presence of different flavonoids and isoflavone molecules such as kaempferol (*m*/*z*: 286.0472), genistein (*m*/*z*: 270.0523), daidzein (*m*/*z*: 254.0567) and fisetin (*m*/*z*: 286.04695) in the examined plant parts. In addition to these flavonoids, other major metabolites including linoleic acid (*m*/*z*: 280.2393), chlorogenic acid (*m*/*z*: 354.09334), gingerol (*m*/*z*: 294.18221), and 12-oxo-phytodienoic acid (*m*/*z*: 292.20291) were also detected in the extract (Table 3, Fig. 6).

The flavanol fisetin has been consistently observed to reverse cognitive decline in AD in mouse models [41]. Several AD-related characteristics, such as Aβ plaque buildup, tau hyperphosphorylation, neuroinflammation, and oxidative stress, have been identified as key targets for drug development [42]. Kaempferol and its derivatives have been scientifically proven to counteract Aβ-induced damage, thereby alleviating symptoms of neurodegenerative diseases [43]. Genistein has been found to have neuroprotective effects and can cross the blood–brain barrier [44]. Daidzein is known to prevent the degradation of dopamine receptor-stimulating neurons in the brain, improving neurodegenerative disease symptoms in mice [45,46]. Gingerol is crucial in improving memory performance, promoting long-term hippocampal enhancement, and accelerating neural growth by upregulating nerve growth factor (NGF) expression [47]. Additionally, 12-oxo phytodienoic acid suppresses neuroinflammation by inhibiting p38 MAPK and Nf-κB signaling, which are the hallmarks of AD, in LPS-activated cells [48]. Linoleic acid prevents the neuroinflammation presented in both in vitro and in vivo models by delaying the neurodegeneration of dopaminergic neurons in mice [49]. Chlorogenic acid has demonstrated neuroprotective effects in rat brain homogenates by reducing the activity of acetylcholinesterase and butyrylcholinesterase [50]. A total of 32 phytochemicals, including chlorogenic acid, luteolin, and various flavonoid classes, were also identified previously, in the different tissues of *S. oleraceus* [51]. The phytochemicals in *Sonchus* could serve as a valuable

Table 3
Bioactive secondary metabolites detected in flower, leaf, stem and root extract of *S. wightianus* DC, where –ve is the negative electrospray ionization technique in HRMS.

Compound Detected	Molecular formula	Expected weight	Observed weight	Error (ppm)	RT (min)	Mode (ESI)	Ref
Linoleic acid	$C_{18}H_{32}O_2$	280.2402	280.2393	−3.32	22.428	−ve	[52]
Gingerol	$C_{17}H_{26}O_4$	294.1831	294.18221	−3.04	17.08	−ve	[53]
Kaempferol	$C_{15}H_{10}O_6$	286.0477	286.0472	−1.78	11.825	−ve	[54]
Chlorogenic acid	$C_{16}H_{18}O_9$	354.0950	354.0933	−4.93	8.395	−ve	[55]
Genistein	$C_{15}H_{10}O_5$	270.0528	270.0523	−1.74	12.737	−ve	[56]
Daidzein	$C_{15}H_{10}O_4$	254.0579	254.0567	−4.88	11.467	−ve	[56]
Fisetin	$C_{15}H_{10}O_6$	286.0477	286.0469	−2.74	11.832	−ve	[57]
12-Oxo phytodienoic acid	$C_{18}H_{28}O_3$	292.2038	292.2029	−3.18	18.1	−ve	[57]

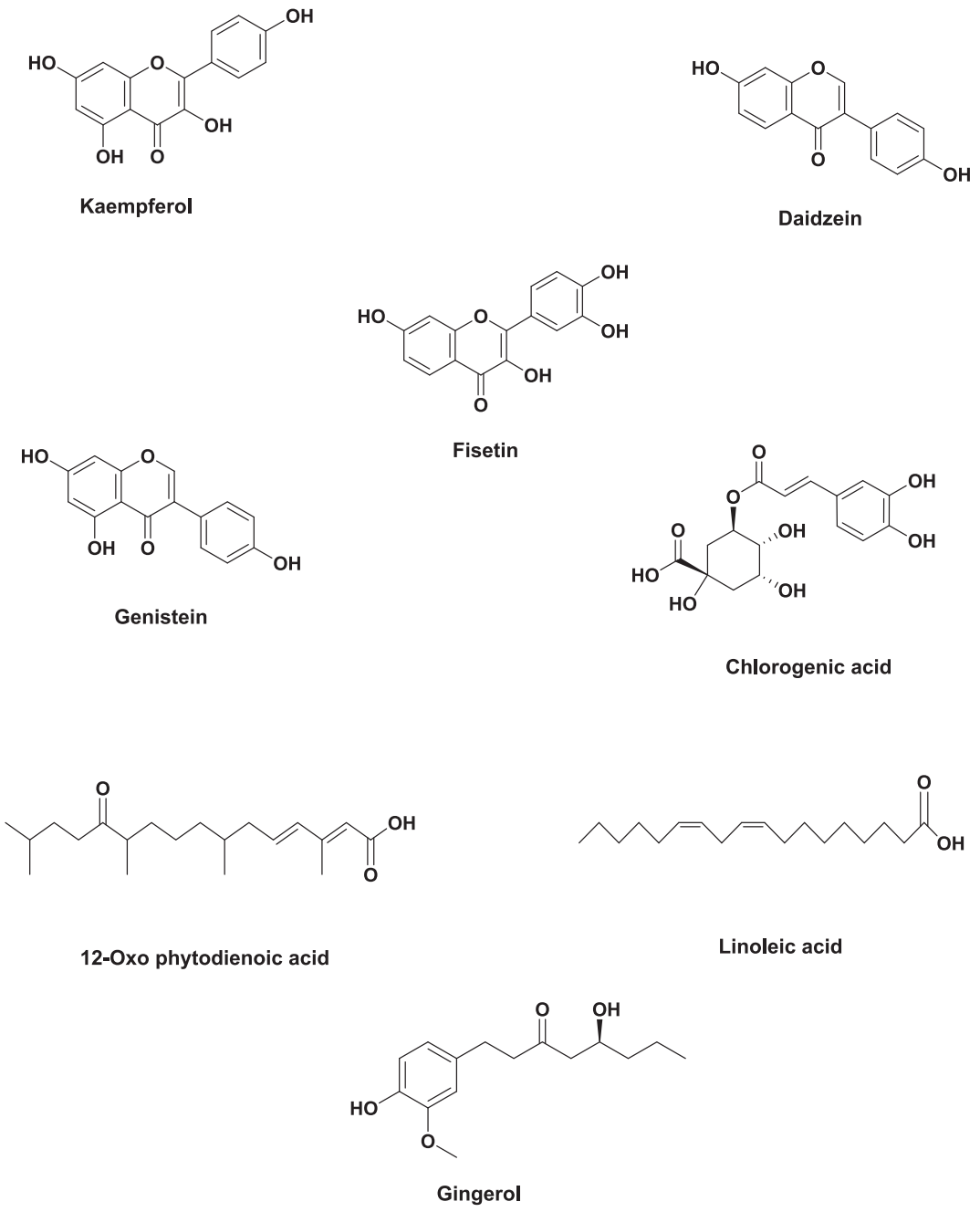

Fig. 6. Major metabolites found in the S. wightianus DC.

source for developing natural, multi-targeted therapeutics against neurodegenerative disorders, leveraging their diverse neuroprotective actions to counteract key pathological features of AD.

4. Conclusions

This study explored the biochemical properties and determined the elemental composition of different parts of *S. wightianus* DC. Among all parts, extracts from flowers and leaves exhibited good potential as antioxidants and cholinesterase enzyme inhibitors. The analyzed plant parts were a rich source of essential metal ions, highlighting their health benefits. Additionally, eight different secondary metabolites, mostly flavonoids, were identified, which were also reported to be effective against AChE and BChE; hence, further purification and isolation of pure compounds is equally important to identify the most effective metabolites for cholinesterase inhibitors. Our research opens up the possibilities in the future to further investigate *S. wightianus* DC against AD.

CRediT authorship contribution statement

Abhimat Subedi: Writing – original draft, Investigation, Formal analysis. **Bishnu Prasad Pandey:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Suman Prakash Pradhan:** Investigation, Conceptualization. **G.C. Ashok:** Investigation. **Sumit Bhattarai:** Investigation. **Ankita Dahal:** Investigation. **Era Tuladhar:** Supervision. **Anupama Chapagain:** Investigation. **Mukti Ram Aryal:** Project administration. **Gopal Prasad Ghimire:** Supervision.

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Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary material

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Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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