Indian J. Applied & Pure Bio. Vol. 41(3), 1830-1837 (2026). **ISSN: 0970-2091**

Phytochemical Analysis and *In vitro* Antioxidant Activity of the Chloroform and aqueous Extract of *Sonchus arvensis* L.

[1]Syeda Ayesha Siddiqua, [1]T. D Karthik, [1]S Ravi kumar, [2]K Raagavalli and *[1]V. Krishna

[1]Department of Biotechnology, Kuvempu University, Jnana Sahydri, Shankaraghatta - 577451 (India)
[2]Department of Biotechnology, Sahyadri Science College, Shivamogga - 577203 (India)
*Corresponding author krishnabiotech2003@gmail.com.

Abstract

Sonchus arvensis L. is a medicinal plant widely recognized for its therapeutic properties owing to its diverse phytochemical constituents. The present study investigated the phytochemical composition and *in vitro* antioxidant potential of the chloroform (SCE) and aqueous (SAE) extracts of *S. arvensis*. Qualitative phytochemical screening revealed the presence of flavonoids, phenolics and terpenoids in both extracts, whereas tannins and cardiac glycosides were detected only in the chloroform extract. Quantitative analysis demonstrated that the chloroform extract contained higher total flavonoid (515 µg/g) and total phenolic (63.51 µg/g) contents than the aqueous extract (408.74 and 45.03 µg/g, respectively). Antioxidant activity was evaluated using DPPH, ABTS, hydroxyl radical scavenging, metal chelating, reducing power and phosphor molybdenum total antioxidant capacity assays. Both extracts exhibited concentration-dependent antioxidant activity; however, the chloroform extract showed superior antioxidant potential, as evidenced by lower IC_{50} values in the DPPH (327.36 µg/mL), ABTS (294.89 µg/mL), hydroxyl radical scavenging (360.87 µg/mL), and metal chelating (287.04 µg/mL) assays, together with higher total antioxidant capacity (600.66 mg AAE/g) and reducing power (653.8 mg QE/g). The enhanced antioxidant activity of the chloroform extract is likely associated with its higher phenolic and flavonoid contents. These findings suggest that *S. arvensis* is a promising natural source of antioxidant phytochemicals and supports its potential application in the development of plant-based therapeutic agents against oxidative stress-related disorders.

Key words : *Sonchus arvensis* L., phytochemicals, antioxidant activity, phenolic compounds, chloroform.

Medicinal plants have long served as valuable sources of therapeutic agents for the prevention and treatment of various diseases worldwide. Traditional medicine, particularly herbal remedies, continues to play a significant role in meeting the primary healthcare needs of populations in developing countries. The medicinal properties of these plants are attributed to the presence of biologically active phytochemicals that exert specific physiological and pharmacological effects on the human body[5]. Herbal medicines are increasingly preferred worldwide due to their affordability, biocompatibility, perceived safety and accessibility.[11]

Phytochemicals are broadly classified into primary and secondary metabolites. Primary metabolites, including carbohydrates, proteins, and chlorophyll, are essential for normal plant growth and development. In contrast, secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, and phenolic compounds are responsible for the diverse biological activities exhibited by medicinal plants. These compounds have attracted considerable attention because of their antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and anticancer properties[7].

Among the various extraction solvents used for phytochemical investigations, chloroform and aqueous were widely employed for isolating moderately non-polar and lipophilic phyto-constituents. Chloroform extracts are known to contain several pharmacologically important compounds, including alkaloids, flavonoids, terpenoids, steroids, and certain phenolic constituents. Furthermore, aqueous extraction is considered a simple, safe, environmentally friendly, and cost-effective method that closely resembles the traditional preparation of herbal medicines, making it highly relevant for evaluating the therapeutic potential of medicinal plants. These bioactive molecules have been reported to possess significant antioxidant, antimicrobial, anti-inflammatory, and anticancer activities, making chloroform extraction an important step in natural product research and drug discovery[3].

Sonchus arvensis L., commonly known as perennial sow thistle, belongs to the family Asteraceae. Although it is often regarded as an invasive agricultural weed, the species has been widely utilized in traditional medicine because of its diverse therapeutic properties. The plant is characterized by an extensive creeping root system, enabling it to thrive under a wide range of environmental conditions. Several studies have demonstrated that S. arvensis is a rich source of biologically active phytochemicals, including phenolic acids, flavonoids, sesquiterpene lactones, terpenoids, and other secondary metabolites that contribute to its medicinal value[9,14].

Previous pharmacological investigations have reported that *S. arvensis* possesses remarkable antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, antidiabetic, and anticancer activities.[14] These biological effects are primarily associated with its abundant phenolic and flavonoid constituents, which effectively scavenge reactive oxygen species and protect cells against oxidative damage. Despite these promising findings, comprehensive phytochemical characterization of the chloroform extract remains limited.

Therefore, the present study was

undertaken to qualitatively and quantitatively evaluate the phytochemical constituents of the chloroform extract and aqueous extracts of *Sonchus arvensis* L. Furthermore, its antioxidant potential was assessed using different in vitro antioxidant assays, including DPPH, ABTS, hydroxyl radical scavenging, metal chelating, reducing power, and total antioxidant capacity assays. The findings of this study will contribute to understanding the phytochemical composition and antioxidant potential of the chloroform and aqueous extracts and may support its future pharmaceutical and therapeutic applications.

Plant Material Collection and Preparation of Extracts :

Whole plants of *Sonchus arvensis* L. were collected from kuvempu university, thoroughly washed with tap water to remove adhering soil and debris, and shade-dried at room temperature until a constant weight was obtained. The dried plant material was finely powdered using a mechanical grinder and stored in airtight, moisture-free containers until further use.

The powdered plant material was sequentially extracted using solvents of increasing polarity in a Soxhlet extraction apparatus. The obtained extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland) according to the method described by Alara *et al*.[1] The plant extract was further fractionated by silica gel column chromatography (60–100 and 100–200 mesh) using appropriate solvent systems to obtain enriched phytochemical fractions.

Phytochemical Screening :

Qualitative Phytochemical Analysis :

Qualitative phytochemical screening of the extracts of *Sonchus arvensis* L. was carried out to detect the presence of major classes of secondary metabolites using standard phytochemical procedures described by Yadav *et al*.[16]. The extract was screened for alkaloids, flavonoids, tannins, saponins, phenolic compounds, cardiac glycosides, and terpenoids.

Quantitative Analysis of Phytochemicals:

Determination of Total Flavonoid Content (TFC):

The total flavonoid content (TFC) of the extract was determined using the modified aluminum chloride colorimetric method described by Ojha *et al*.[8]. Briefly, different concentrations of the extract were mixed with 6 µL of 5% sodium nitrite solution and incubated for 5 min. Subsequently, 6 µL of 10% aluminum chloride solution was added, followed by incubation for another 5 min. Thereafter, 40 µL of 1 M sodium hydroxide and 48 µL of distilled water were added to the reaction mixture. The absorbance was measured at 510 nm using a UV–Visible spectrophotometer against an appropriate blank. The total flavonoid content was calculated from a quercetin standard calibration curve and expressed as quercetin equivalents (QE, mg/g dry mass).

Determination of Total Phenolic Content (TPC):

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method

described by Waterhouse *et al*.[8]. Briefly, 50 µg of each extract (1 mg/mL) was mixed with 50 µL of diluted Folin–Ciocalteu reagent (1:10 dilution) and incubated for 5 min. Subsequently, 40 µL of 7.5% sodium carbonate solution was added, and the reaction mixture was incubated at 50°C for 10 min. The absorbance was measured at 750 nm using a double-beam UV–Visible spectrophotometer. The total phenolic content was calculated using a gallic acid standard curve and expressed as gallic acid equivalents (GAE, mg/g dry mass).

In vitro Antioxidant Activity :

DPPH Radical Scavenging Assay :

The free radical scavenging activity of the plant extracts were evaluated using the modified DPPH method described by Floegel *et al*.,[6]. Different concentrations of the extract were mixed with 100 µL of 0.004% DPPH solution prepared in ethanol and incubated in the dark at room temperature for 30 min. The absorbance was recorded at 517 nm using a UV–Visible spectrophotometer, with butylated hydroxytoluene (BHT) serving as the reference standard.

The percentage of radical scavenging activity was calculated using the following equation: % Inhibition = $[(A_{control} - A_{test}) / A_{control}] \times 100$. The IC_{50} value was calculated from the concentration-response curve.

ABTS Radical Scavenging Assay :

The ABTS radical scavenging activity was determined according to the method described by Re *et al*.[10]. The $ABTS^{\bullet+}$ radical cation was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate (1:1) and incubating the mixture in the dark at 27°C for 16 h. The resulting solution was diluted with ethanol until an absorbance of 0.700 ± 0.02 was obtained at 734 nm. Subsequently, 160 µL of the $ABTS^{\bullet+}$ solution was mixed with different concentrations of the extract, and the absorbance was measured at 734 nm after 30 min.

The percentage inhibition was calculated using the following equation: ABTS Radical Scavenging (%) = [(AB − AA) / AB] × 100

Hydroxyl Radical Scavenging Assay :

Hydroxyl radical scavenging activity was determined following the modified method of Chimi *et al*.[4]. The reaction mixture consisted of the plant extract, iron–EDTA solution, 15 µL of 0.018% EDTA, and 30 µL of 0.85% DMSO prepared in phosphate buffer (pH 7.4). The reaction was initiated by adding 15 µL of 0.22% ascorbic acid and incubated at 80–90°C for 15 min. The reaction was terminated by adding 30 µL of 17.5% trichloroacetic acid (TCA), followed by the addition of 100 µL of Nash reagent. After incubation for an additional 15 min, the absorbance was measured at 412 nm.

The hydroxyl radical scavenging activity was calculated as percentage inhibition, and the IC_{50} value was determined accordingly.

Metal Chelating Activity :

Metal chelating activity was evaluated according to the modified method described by Alavi *et al*.[2]. Different concentrations of the extract were mixed with 10 µL of 2 mM ferrous chloride solution followed by 40 µL of

5 mM ferrozine solution. The reaction mixture was incubated in the dark for 10 min, and the absorbance was measured at 562 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the reference standard. The percentage inhibition was calculated using the following equation: % Inhibition = $[(A_{control} - A_{test}) / A_{control}] \times 100$

Reducing Power Assay :

The reducing power of the plant extract was determined using the method described by Vijayalakshmi *et al*.,[13]. Different concentrations (10–50 µg/mL) of the extract were mixed with 50 µL of 20 mM phosphate buffer (pH 6.6) and 50 µL of 1% potassium ferrocyanide solution. The reaction mixture was incubated at 50°C for 30 min, followed by the addition of 50 µL of 10% trichloroacetic acid and 10 µL of 0.1% ferric chloride. After incubation for 10 min, the absorbance was recorded at 700 nm. Ascorbic acid and phosphate buffer served as the standard and blank, respectively.

Determination of Total Antioxidant Capacity:

Total antioxidant capacity was estimated by the phosphor molybdenum method described by Untea *et al*.,[12]. Briefly, 100 µL of the extract was mixed with 50 µL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixture was incubated at 95°C for 90 min and then cooled to room temperature. The absorbance was measured at 695 nm against a methanol blank. Ascorbic acid was used as the reference standard, and the results were expressed as milligrams of ascorbic acid equivalents (AAE) per gram of dry extract.

Table-1. Phytochemical Analysis

Tests	Qualitative analysis		Quantitative analysis	
Extracts	SCE	SAE	SAE	SCE
Alkaloids	-	-		
Flavonoids	+	+	408.74 µg/g	515 µg/g
Tannins	+	-		
Saponins	-	-		
Phenolics	+	+	45.03 µg/g	63.51 µg/g
Cardiac Glycosides	+	-		
Terpenoids	+	+		

SCE- Chloroform extract, SAE- Aqueous extract.

The qualitative and quantitative phytochemical analyses of the chloroform (SCE) and aqueous (SAE) extracts of *Sonchus arvensis* L. are presented in Table-1. Qualitative screening revealed the presence of flavonoids, phenolics, and terpenoids in both extracts. Tannins and cardiac glycosides were detected only in the chloroform extract, whereas alkaloids and saponins were absent in both extracts. Quantitative estimation showed that the chloroform extract contained higher levels of total flavonoids (515 µg/g) and total phenolics (63.51 µg/g) than the aqueous extract, which contained 408.74 µg/g flavonoids and 45.03 µg/g phenolics.

Table-2. *In vitro* anti-oxidant activities of plant extracts

Activity	Conc	% of inhibition		IC_{50} values		
		SAE	SCE	SCE	SAE	Standard drug
DPPH	100	23.15	32.15	327.36	338.055	124.87
	200	36.91	44.69			
	300	48.87	48.25			
	400	55.62	54.98			
	500	59.48	68.06			
Metal Chelating	100	7.046	17.98	287.04	360.87	109.21
	200	22.35	37.54			
	300	32.07	44.22			
	400	50.19	55.28			
	500	60.96	65.60			
ABTSAssay	100	14.07	10.88	294.89	338.92	111.9
	200	24.87	24.23			
	300	38.89	40.20			
	400	54.71	67.34			
	500	61.28	77.64			
Hydroxyl radical scavenging assay	100	7.43	9.91	360.87	382.51	282.7
	200	19.00	21.48			
	300	38.01	42.97			
	400	49.56	52.06			
	500	60.33	65.28			

SCE- Chloroform extract, SAE- Aqueous extract

The *In vitro* antioxidant activities of the chloroform and aqueous extracts were evaluated using DPPH, ABTS, hydroxyl radical scavenging, metal chelating assays and the results are presented in Table-2. Both extracts exhibited concentration-dependent antioxidant activity in all assays. In the DPPH assay, the chloroform extract showed a lower IC_{50} value (327.36 µg/mL) than the aqueous extract (338.06 µg/mL), indicating slightly stronger radical scavenging activity. Similarly, the chloroform extract demonstrated better hydroxyl radical scavenging activity (IC_{50} = 360.87 µg/mL) than the aqueous extract (382.51 µg/mL). In contrast, the aqueous extract exhibited greater metal chelating activity (IC_{50} = 287.04 µg/mL) and ABTS radical scavenging activity (IC_{50} = 294.89 µg/mL) than the chloroform extract, which showed IC_{50} values of 360.87 µg/mL and 333.96 µg/mL, respectively. However, in all assays, the standard antioxidant exhibited significantly lower IC_{50} values in both extracts, indicating good antioxidant activity.

Table-3. Total Antioxidant Capacity and Total Reducing Power of plant extracts

Tests	SAE	SCE
Total Antioxidant	407.33 mg AAE/g	600.66 mg AAE/g
Total Reducing power	422.9 mg QE/g	653.8 mg QE/g

SCE- Chloroform extract, SAE- Aqueous extract

The total antioxidant capacity and total reducing power of the aqueous (SAE) and chloroform (SCE) extracts of *Sonchus arvensis* L. are presented in Table-3. The chloroform extract exhibited higher total antioxidant capacity (600.66 mg AAE/g) than the aqueous extract (407.33 mg AAE/g). Similarly, the total reducing power of the chloroform extract (653.8 mg QE/g) was markedly higher than that of the aqueous extract (422.9 mg QE/g), indicating a greater electron-donating ability and antioxidant potential of the chloroform extract.

The antioxidant activity exhibited by the chloroform and aqueous extracts of *Sonchus arvensis* L. can be attributed to the presence of bioactive phytochemicals, particularly phenolic compounds, flavonoids, tannins, and terpenoids, which are known to possess free radical scavenging properties. Both extracts demonstrated concentration-dependent antioxidant activity in all assays, indicating their ability to neutralize reactive oxygen species through different antioxidant mechanisms. Although the chloroform extract contained higher total phenolic and flavonoid contents, than the aqueous extract. the chloroform extract showed comparatively better antioxidant activities, suggesting that lipophilic antioxidant constituents extracted by chloroform also contribute significantly to the overall antioxidant potential. Similar findings have been reported in previous studies on *Sonchus arvensis* and other medicinal plants, where phenolics and flavonoid-rich extracts exhibited strong antioxidant activities due to their hydrogen-donating and metal-chelating abilities. These findings suggest that *S. arvensis* L. is a valuable source of natural antioxidants and support its potential application in the development of therapeutic agents for the management of oxidative stress-related disorders.

The present study demonstrated that both the chloroform and aqueous extracts of *Sonchus arvensis* L. possess valuable phytochemical constituents and significant *in vitro* antioxidant activity. Qualitative phytochemical screening confirmed the presence of flavonoids, phenolic compounds, and terpenoids in both extracts, while tannins and cardiac glycosides were detected only in the chloroform extract. Quantitative analysis revealed that the chloroform extract contained higher levels of total phenolics and flavonoids and exhibited superior performance in all antioxidant assays, including DPPH, ABTS, hydroxyl radical scavenging, metal chelating activity, total antioxidant capacity, and reducing power, compared with the aqueous extract. These findings indicate that the antioxidant potential of *S. arvensis* is also influenced by the chloroform-soluble phytochemicals and their ability to act through multiple antioxidant mechanisms. Overall, the results suggest that *S. arvensis* is a promising natural source of bioactive compounds with considerable antioxidant potential and supports

its traditional medicinal use. Further studies involving the isolation, characterization, and pharmacological evaluation of the active constituents are warranted to explore their therapeutic applications.

The authors extend their appreciation to the Chairman of the Department of Biotechnology for offering the necessary facilities to carry out the research.

Fundings :

The authors express their sincere gratitude to the Karnataka Science and Technology Promotion Society (KSTePS), Department of Science and Technology, Government of Karnataka, India, for providing financial support.

References :

1. Alara, O. R., N. H. Abdurahman, C. I. Ukaegbu, and N. A. Kabbashi, (2019). *Journal of taibah university for science, 13*(1): 414-422.
2. Alavi, M., and N. Karimi (2018). *Artificial cells, nanomedicine, and biotechnology, 46*(8): 2066-2081.
3. Al-Oqail, M. M., E. S. Al-Sheddi, M. A. Siddiqui, J. Musarrat, A. A. Al-Khedhairy, and N. N. Farshori, (2015). *Pharmacognosy Magazine, 11*(Suppl. 4), S598–S605.
4. Chimi, H., J. Cillard, P. Cillard, and M. Rahmani, (1991). *Journal of the American Oil Chemists' Society, 68*(5): 307-312.
5. Edeoga, H. O., D. E. Okwu, and B. O. Mbaebie, (2005). *African Journal of Biotechnology, 4*(7): 685–688.
6. Floegel, A., D. O. Kim, S. J. Chung, S. I. Koo, and O. K. Chun, (2011). *Journal of food composition and analysis, 24*(7): 1043-1048.
7. Krishnaiah, D., R. Sarbatly, and A. Bono, (2007). *Biotechnology and Molecular Biology Reviews, 1*(4): 97–104.
8. Ojha, S.B., S. Roy, S. Das, and G. Dhangadamajhi (2019). *Food and Nutrition Sciences, 10*(08): 900.
9. Patel, D., M. Maithani, P. Ghosal, M. Yadav, V. Gupta, and R. Sharma, (2025). *International Journal of Creative Research Thoughts, 13*(11):
10. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, (1999). Free radical biology and medicine, *26*(9-10): 1231-1237.
11. Samal, J. (2016). *Indian Journal of Health Sciences and Biomedical Research kleu, 9*(1): 14-19.
12. Untea, A., A. Lupu, M. Saracila, and T. Panaite, (2018). Comparison of ABTS, DPPH, phosphor molybdenum assays for estimating antioxidant activity and phenolic compounds in five different plant extracts.
13. Vijayalakshmi, M., and K. Ruckmani, (2016). *Journal of Pharmacology, 11*(3): 570-572.
14. Wahyuni, D. K., E. Silas, A. S. Pratama, R. K. Asyahidah, A. J. Syukriya, C. T. Rahmawati, and S. Subramaniam, (2026). *Journal of Pharmacy & Pharmacognosy Research, 14*(2): 2429.
15. Waterhouse, A. L. (2002). *Current protocols in food analytical chemistry, 6*(1): I1-1.
16. Yadav, R. N. S., and M. Agarwala, (2011). *Journal of phytology, 3*(12):