Annals of Phytomedicine 13(1): 1205-1213, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.1.130

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Estimation of phytochemicals and antioxidant capacity of leaves of *Cassia siamea* L.

Kamaljeet Saini, Sushila Singh◆, Monika Moond, Jyoti Rani, Anuradha Beniwal, Simran Kakkar, Yogita and Rajni Kant Sharma

Department of Chemistry, CCS Haryana Agricultural University, Hisar-125004, Haryana, India

Article Info

Article history
Received 6 January 2024
Revised 23 February 2024
Accepted 24 February 2024
Published Online 30 June 2024

Keywords
Flavonoids
Phenolics
Phosphomolybdenum
Phytochemicals
Tannins

Abstract

Cassia siamea L. (syn. *Senna siamea* L.) is a medium sized angiosperm. It is classified in the Fabaceae family. Medical professionals have used it to treat urticaria, liver issues, rhinitis, and appetite loss brought on by digestive issues. Furthermore, it is reported to have purgative and laxative properties. The current study's goal is to investigate proximate composition, phytochemicals and antioxidant activity of leaves of *C. siamea*. Proximate composition was estimated as w/w. Phytochemicals were analysed from methanol, aqueous and ethyl acetate extracts of leaves. Various phytochemicals such as reducing sugar, total sugar, non-reducing sugar, total phenolics and total flavonoids were present in significant amounts. Total antioxidant activity evaluated by phomolybdenum assay and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity.

1. Introduction

Finding new applications for the manufacture of pharmaceuticals from plants, is the goal of phytochemical research. A key source of discovery for chemicals that could be effective in the fight against infectious illness is the screening of plants using procedures that have been proven to work (Moond *et al*., 2023). Finding various extracts from traditional medicinal plants as possible sources of novel antibacterial and antioxidant compounds, has drawn increasing attention over the past twenty years (Devi *et al*., 2023; Moond *et al*., 2022; Dalal *et al*., 2022). The unregulated application of commercial antimicrobial drugs and chemicals for managing infectious diseases has recently resulted in the emergence of diverse forms of drug and chemical confrontation among both plant and human disease causing organisms. As a result, researchers have been working to create new, broad-spectrum antibiotics that can cure bacterial, parasitic, and fungal diseases (Goel *et al*., 2022; Moond *et al*., 2023). All around the world, medicinal plants have a long history of being used to prevent or treat disease. The healing advantages of plants reside in their bioactive phytochemicals, like phenolic, flavonoids compounds, anthracene, tannin derivatives, and essential oils, which elicit distinct physiological responses on people (Devi *et al*., 2023; Aggarwal *et al*., 2022).

Oxidative stress significantly contributes to the development of chronic and degenerative diseases such autoimmune disorders, cancer, rheumatoid arthritis, cataracts, ageing, neurological and cardiovascular problems. It has been demonstrated that a number of naturally occurring substances derived from medicinal plants contain antioxidant and/or radical-scavenging characteristics that shield the body from chronic illnesses (Moond *et al*., 2022; Poonam *et al*., 2023; Moond *et al*., 2023).

Cassia siamea L. is a medium sized angiosperm. This tree is native of India, Sri-Lanka, Burma, Malaysia and Thailand. *C. siamea* is classified in the Fabaceae family. It is 10-12 meters high as shown in Figure 1. The bole is tiny, and the crown first seems spherical and compact before becoming irregular. The complex, alternating leaves are 15-30 cm long, have 6-14 leaflets, and each one is finished with a tiny bristle (Kamagate *et al*., 2014). Medical professionals have used it to treat urticaria, liver issues, rhinitis, and appetite loss brought on by digestive issues. Additionally, purgative and laxative effects are said to be present. The stem bark is used to treat skin conditions and hemorrhoids, the roots are used as an antipyretic in cases of fever, and the leaves are used to treat constipation, diabetes, and hypertension.

Figure 1: *C. siamea* plant.

Corresponding author: Dr. Sushila Singh
Assistant Professor, Department of Chemistry, CCS Haryana Agricultural University, Hisar-125004, Haryana, India
E-mail: singhsushila999@gmail.com
Tel.: +91-8199939339

The objective of this study was to analyse the phytochemical constituents present in dried leaves of *C. siamea* through extractions using methanol, water, and ethyl acetate. The investigation revealed that the leaves were a noteworthy reservoir of total flavonoids, total phenolics, reducing sugars, total sugars, and non-reducing sugars.

2. Materials and Methods

2.1 Collection of plant material

At the CCS Haryana Agricultural University in Hisar, Haryana, leaves of the *C. siamea* plant were collected. The collected sample was verified by Dr. Anita, Asstt. Scientist, Department of Botany and Plant Physiology, CCS HAU, by using online platform (Tropicos & IPNI). Plant parts were cleaned, and then dried at room temperature in the shade.

2.2 Chemicals

High purity chemicals that were readily available were employed in various experimental processes. All of the chemicals and standards were bought from different places, including Sigma Aldrich, SISCO Research Laboratories, and Himedia Laboratories Private Limited in Mumbai.

2.3 Proximate composition

The proximate composition of *C. siamea* leaves (moisture, ash, crude fiber, crude fat, crude protein, and total carbohydrates) was evaluated in triplicate in accordance with the Association of Official Analytical Chemists (AOAC). The ash and moisture content were determined using the AOAC (1995) prescribed procedure. The estimation of crude fibre was carried out employing the Maynard method (1970). To determine the nitrogen content, the Micro-Kjeldahl method (AOAC, 1990) was employed. In order to calculate the crude protein concentration, the nitrogen percentage was multiplied by a factor of 6.25. Total carbohydrates content was calculated by difference as follows:

Total carbohydrates content (%) = [100 – (Moisture (%) + Ash (%) + Crude Fat (%) + Crude fiber (%) + Crude Protein (%))]

2.4 Mineral analysis

The mineral content was determined utilizing the approach outlined by Jackson (1973) and Ruig (1986). Using an atomic absorption spectrometer, the Fe, Zn, Mn, and Cu minerals in plant samples that had undergone acid digestion were measured. In accordance with the principles of atomic absorption spectrometry, the atoms of the metallic elements (Fe, Zn, Mn, and Cu) undergo excitation and energy absorption upon exposure to particular wavelength radiation. For each metallic element, a specific bulb is employed. The quantity of an element atoms present in a given volume is directly proportional to its radiation absorption. Atoms are able to absorb radiation regardless of temperature or wavelength.

2.5 Chemical analysis

2.5.1 Tannins

The tannin concentration was estimated using the Vanillin-HCl method of Burns (1971) as the catechin equivalent. The Vanillin-HCl method developed by Burns was used to calculate the tannin concentration, which was expressed as catechin equivalent (1971). In this procedure, a 25 ml test tube containing 200 mg of powdered *C. siamea* leaves was filled with 10 ml of methanol. The tubes were kept at temperatures between 25°C and 32°C overnight after being pith-corked closed and stirred occasionally. Subsequently, a 10 min centrifugation at 3000 rpm was carried out. The vanillin-HCl reagent was then added to a test tube along with 1ml of the clear supernatant solution in a ratio of 5:1 ml. The test tube solution was then allowed to incubate at 27°C to 30°C for 25 min. A UV-Vis double beam spectrophotometer (Model UV 1900, Shimadzu) was used to detect the resulting brownish-red colour absorbance at 525 nm, and this measurement was contrasted to one made with methanol in the blank. To determine the tannin amount in terms of mg catechin equivalent per gram (mg CE/g), a standard catechin curve was constructed, plotting concentrations ranging from 10 to 100 g/ml in methanol against the absorbance at 525 nm.

2.5.2 Alkaloids

The Harborne (1973) method was applied to determine the alkaloid content. Into a 250 ml beaker, 2.5 g of powdered *C. siamea* leaves samples were introduced, followed by the addition of 100 ml of 10% acetic acid in ethanol for each ml. After that, the mixture was covered and left to sit for 4 h. After this time, the mixture was filtered, and the resulting extract was then heated in a water bath to concentrate it into vials where it was then reduced to a quarter of its initial volume. The extract was gradually treated with concentrated ammonium hydroxide until precipitation was finished. Precipitate formed once the entire solution was given time to settle. After being cleaned with dilute ammonium hydroxide, this precipitate was then filtered. After that, the alkaloid that was left was dried and weighed.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100$$

2.6 Preparation of different solvent extracts of *C. siamea* leaves for phytochemical analysis

10 g of powdered *C. siamea* leaves samples were gathered and inserted into a Whatman No. 1 filter paper thimble. These prepared samples were then positioned within a standard Soxhlet apparatus, accompanied by a 500 ml round bottom flask. Around 300 ml of solvents (distilled water, methanol, and ethyl acetate) were introduced into the flask, reaching up to a level of 1.5 siphons. Depending on the specific solvent, extraction was performed under boiling temperatures. The resulting solvent vapours ascended through the column, condensed within the condenser, and subsequently flowed into the chamber containing the thimbles that held the *C. siamea* leaves samples. After the chamber has been entirely filled with the solvent containing the dispersed phytochemicals, the siphon mechanism is activated. This extract was extracted and sucked into the flask with a circular bottom. With the use of a siphon mechanism, the process continued for 5-6 h in the case of methanol and ethyl acetate as the solvent after seven to eight cycles were finished. In case of water the process continued for 7-8 h.

2.7 Determination of total sugars

Adapting Dubois *et al.* (1956) modified approach, the total sugars were measured. A phenol solution of 2.0 ml was combined with 1 ml of each extract. The reaction mixture was then added 5.0 ml of concentrated H_2SO_4 after which it was allowed to cool for 30 min. Using a UV-Vis double beam spectrophotometer, the absorbance of the reaction mixture at 490 nm was measured, and this measurement

was compared to a blank produced using the same technique but substituting the extract with the proper solvent.

2.8 Determination of reducing sugars

The Nelson method, as modified by Somogyi (1952), was used to calculate the amount of reducing sugars (Nelson, 1944). One milliliter of distilled water was added to a test tube containing 1ml of each extract made from *C. siamea* leaves. Alkaline copper reagent was added after complete mixing, and the resulting liquid was then heated in a water bath for 20 to 25 min. It took some time for the combination to cool to room temperature. The arsenomolybdate reagent was then added in a volume of 1 ml. Distilled water was used to further dilute the solution until it had a final volume of 10 ml. A UV-Vis double beam spectrophotometer was used to measure the reaction mixture's absorbance at 520 nm in comparison to a blank generated using the same procedure.

2.9 Determination of non-reducing sugars

The difference between the concentration of total sugars and that of reducing sugars was used to compute the non-reducing sugars.

Non-reducing sugars = Total sugars 6 Reducing sugars (Basra *et al*., 2005)

2.10 Determination of total phenolic content

The Folin-Ciocalteu method was used to determine the total phenolic content (Singleton and Rossi, 1965), with gallic acid acting as the standard reference. 1.0 ml of the extract, 1.0 ml of the 1 mol/L Folin-Ciocalteu reagent, and 2.0 ml of the 20%, w/v, Na_2CO_3 solution were mixed in a test tube to begin the procedure. After vigorous mixing, the volume was reduced with water to 10.0 ml. The reaction mixture was centrifuged for 10 min at a speed of 6000 rpm after an 8 min incubation time. The absorbance of the resultant supernatant at 730 nm was measured using a UV-Vis spectrophotometer. The results were then contrasted with a blank created using a similar process in which the extract was swapped out for the proper solvent. Using a regression equation created from the gallic acid standard curve, the total phenolic content was calculated as mg gallic acid equivalents per gram (mg GAE/g).

2.11 Determination of total flavonoids

According to Marinova *et al*. (2005), the total flavonoid content was determined using the $AlCl_3$ colorimetric test, with catechin acting as the point of reference. 4.0 ml of distilled water, 1 ml of the extract, and 0.3 ml of 5% $NaNO_2$ were mixed together in a test tube. A 5 min break was followed by the addition of 2.0 ml of 1M NaOH and 0.3 ml of 10% $AlCl_3$. After then, water was used to bring the overall volume to 10.0 ml. After thoroughly blending the solution, the absorbance at 510 nm was measured against a blank using a UV-VIS double beam spectrophotometer (Model UV 1900, Shimadzu). The blank was created using the same methods as the regular catechin solution and includes the necessary solvent.

2.12 Antioxidant potential

2.12.1 Evaluation of DPPH free radical scavenging activity

The approach of Hatano *et al*. (1988) was modified for the measurement of DPPH free radical scavenging activity. Each extract was frozen to create a dry bulk that was then dissolved in the appropriate solvent to produce solutions with different concentrations. In a test tube containing 1 ml of each extract at the designated concentration, 2.0 ml of DPPH (0.1 mM in methanol) was then added. For 5 min, the liquid was forcefully mixed. The reaction mixture was then incubated at room temperature for 30 min in the dark. Using a UV-VIS double beam spectrophotometer (Model UV 1900, Shimadzu), the absorbance of the extract and the control were measured at 517 nm and compared to a blank containing the same sample. A graphical representation depicted the correlation between the extract concentration (g/ml) and the DPPH free radical scavenging activity (%). The equation $ax^2 + bx + c = 0$ was employed to determine the IC_{50} value, which was calculated by:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where,

$x = IC_{50}$ (µg/ml)

Calculation

The percentage of DPPH scavenged (% $DPPH^*_{SC}$) was calculated using:

$$\%\ DPPH^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where,

$A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.

2.12.2 Total antioxidant activity

According to Prieto *et al*. (1999), the phosphomolybdenum assay was used to measure the total antioxidant capacity, and the results were represented in milligrams (mg AAE/g) of ascorbic acid equivalents per gram. 0.3 ml of each extract solution and 3 ml of phosphomolyb denum reagent were put into glass vials, and the mixture was thoroughly blended before the vial lids were sealed. Following that, these vials underwent a 90 min incubation period at 95°C. The solution's absorbance was measured at 695 nm using a UV-VIS double beam spectrophotometer (Model UV 1900, Shimadzu) and a blank after the vial contents had cooled. Similar procedures were used to make a blank; however, this time the ascorbic acid solution was replaced with the suitable solvent.

2.13 Statistical analysis

For statistical analysis, the samples were collected in threes. The statistical software SPSS (Statistical Package for Social Sciences) version 23 was used to present the data regarding proximal composition and phytochemicals as mean standard error (SE). Using Origin 2018, the IC_{50} values for antioxidant activity were subjected to a regression analysis.

3. Results

3.1 Proximate composition

In proximate composition, the leaves of *C. siamea* consists of moisture content (7.51 ± 0.78), ash content (5.22 ± 0.63), crude fat (3.69 ± 0.38), crude fiber (12.67 ± 1.64), crude protein (20.72 ± 1.02) and total carbohydrate (51.19 ± 2.70).The data of proximate composition of leaves *C. siamea* is given in Figure 2.

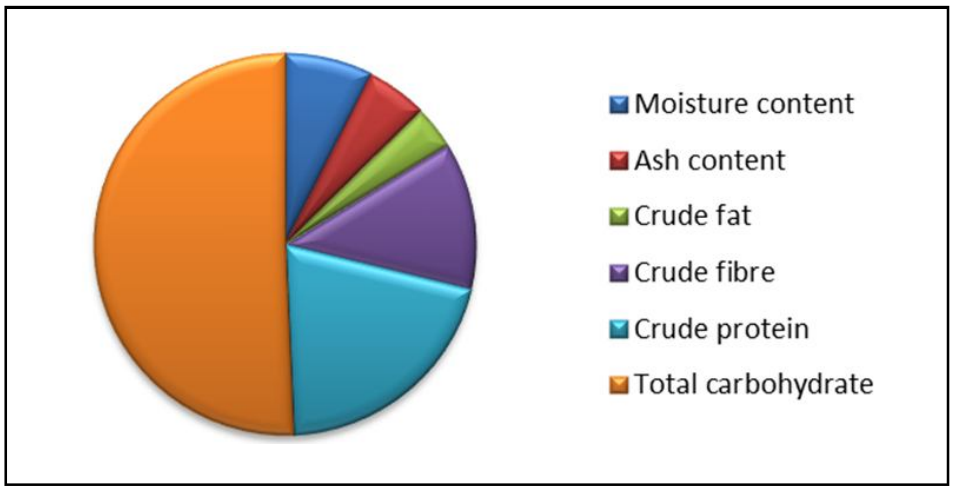

Figure 2: Proximate composition (%) of *C. siamea* leaves.

3.2 Mineral content

Mineral content of leaves was Cu (1.06 ± 0.09), Fe (119.32 ± 1.90), Mn (40.60 ± 1.75) and Zn (22.09 ± 0.53). The data of mineral analysis of leaves of *C. siamea* is presented in Table 1.

Table 1: Mineral content and chemical analysis of *C. siamea leaves*

Mineral analysis	Cu (ppm)	1.06 ± 0.09
	Fe (ppm)	119.32 ± 1.90
	Mn (ppm)	40.60 ± 1.75
	Zn (ppm)	22.09 ± 0.53
Chemical analysis	Tannin content (mg CE/g)	0.67 ± 0.14
	Alkaloid content (%)	9.12 ± 0.70

3.3 Chemical analysis

The tannin and alkaloid content of leaves was 0.67 ± 0.14 (mg/g) and 9.12 ± 0.70%, respectively, as shown in Table 1.

3.4 Phytochemical analysis

3.4.1 Total sugars

Using a standard curve and D-glucose as the reference material, the total sugar content of the leaf extracts from the various solvents was quantified (Figure 3). Acidic conditions were applied to D-glucose to produce hydroxymethyl furfural, which then interacted with phenol to create a yellow-brown solution with a maximum absorbance (max) at 490 nm. The regression equation illustrated a linear correlation between the concentration of D-glucose and the measured absorbance. Employing this regression equation ($y = 0.0051x - 0.0215$, $R^2 = 0.9927$), the total sugar content within the methanol, aqueous, and ethyl acetate extracts of the leaves was computed to be 34.05 mg/g, 46.93 mg/g, and 6.99 mg/g, respectively, as shown in Table 2.

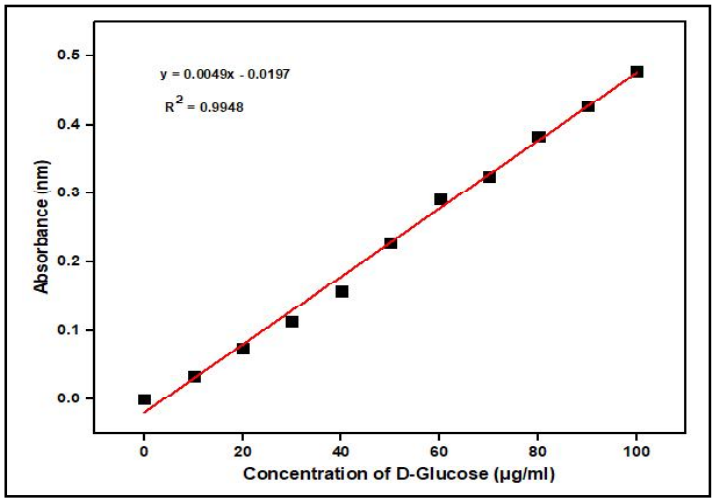

Figure 3: Standard curve of total sugar using glucose as standard.

3.4.2 Reducing sugars

The measurement of reducing sugars in the methanol, aqueous, and ethyl acetate extracts of the leaves was aided by using D-glucose as a reference standard to create a standard curve (Figure 4). Cuproic ions change into cuprous ions when heated in the presence of alkaline copper tartrate, which then results in the production of cuprous oxide. Molybdic acid undergoes reduction upon contact with cuprous oxide and arsenomolybdic acid, culminating in the creation of molybdenum blue. A UV-Vis spectrophotometer is then used to measure this transition at 520 nanometers. The results of regression analysis showed a linear relationship between the absorbance and the D-glucose concentration. The derived regression equation ($y = 0.0013x + 0.0029$, $R^2 = 0.9966$) illustrated this relationship. In the leaves, the content of reducing sugars (mg/g) within the methanol, aqueous, and ethyl acetate extracts were found to be 23.73, 33.29, and 4.59 mg/g, respectively (Table 2).

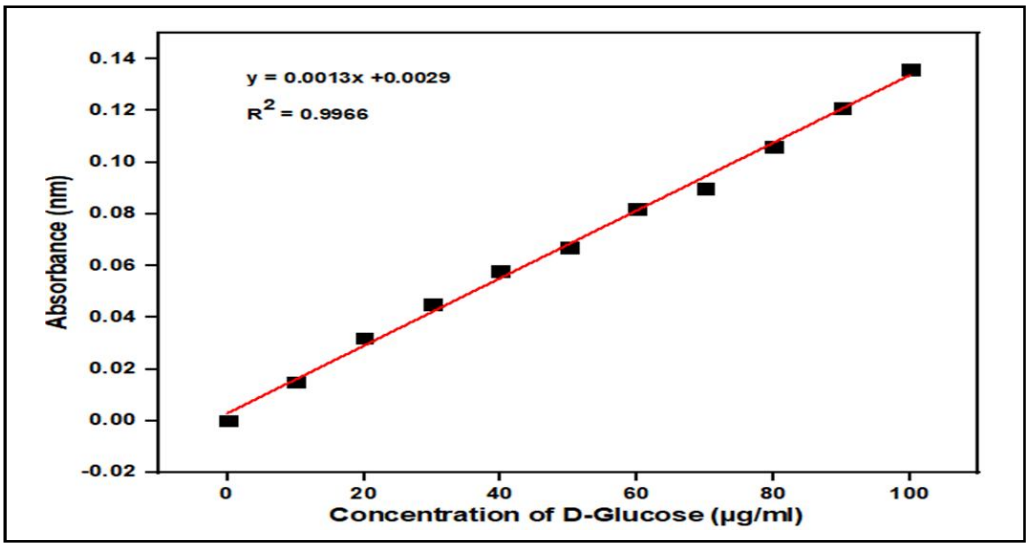

Figure 4: Standard curve of reducing sugars using glucose as standard.

Table 2: Phytoconstituents of *C. siamea* leaves

S.No.	Extracts	Phytochemicals				
		Total sugars (mg/g)	Reducing sugars (mg/g)	Non-reducing sugars (mg/g)	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)
1.	Methanol	34.05 ± 0.92	23.73 ± 1.01	10.32 ± 0.29	78.77 ± 2.24	25.19 ± 2.31
2.	Aqueous	46.93 ± 1.18	33.29 ± 1.29	13.64 ± 0.60	73.98 ± 0.80	22.84 ± 0.94
3.	Ethyl acetate	6.99 ± 0.46	4.59 ± 0.50	2.40 ± 0.10	15.28 ± 1.38	3.73 ± 0.22

3.4.3 Non-reducing sugars

The determination of non-reducing sugars in the diverse solvent extracts of the leaves was accomplished by calculating the disparity between the total sugar concentration and that of reducing sugars. In the leaves, the methanol, aqueous, and ethyl acetate extracts displayed non-reducing sugar contents of 10.32 mg/g, 13.64 mg/g, and 2.40 mg/g, respectively (Table 2).

3.4.4 Total phenolic content

The total phenolic content of the various leaf extracts was calculated using a standard curve with gallic acid as the standard (Figure 5). The oxidation of phenolic compounds was accomplished using the Folin-Ciocalteu reagent. Following the oxidation of phenols, phosphotungstic and phosphomolybdic acids underwent reduction to produce blue tungsten and molybdenum oxides. The amount of phenolic chemicals in the extract directly correlated with the colour intensity produced, with the blue colour's peak absorption occurring around 730 nm.

The regression equation showed a linear relationship between absorbance and gallic acid content. This regression equation ($y = 0.0126x - 0.0022$, $R^2 = 0.9971$) was used to calculate the total phenolic content of the leaves across different solvent extracts. Total phenolic content was found to be 78.77 mg GAE/g, 73.98 mg GAE/g, and 15.28 mg GAE/g in the methanol, aqueous, and ethyl acetate extracts of the leaves, respectively as shown in Table 2.

3.4.5 Total flavonoids

By using a standard curve with catechin as the standard, the amount of flavonoids present in the various solvent extracts of leaves was calculated (Figure 6). This methodology is based on the concept that a stable complex is formed between $AlCl_3$ and the hydroxyl groups located at positions C-3, C-5, and the keto group at position C-4 within flavones and flavonols. This complex formation allows for the determination of total flavonoids. Additionally, contact with $AlCl_3$ results in the formation of labile acid complexes because orthodihydroxyl groups on the A or B ring of flavonoids are involved.

Regression analysis revealed a linear relationship between the catechin concentration and absorbance. The flavonoid content in the leaf extracts of methanol, water, and ethyl acetate was shown in Table 2.

The aggregate flavonoid content in the leaf extracts of methanol, water, and ethyl acetate was determined to be 25.19 mg CE/g, 22.84 mg CE/g, and 3.73 mg CE/g, respectively, using the regression equation ($y = 0.0025x + 0.1304$, $R^2 = 0.9981$).

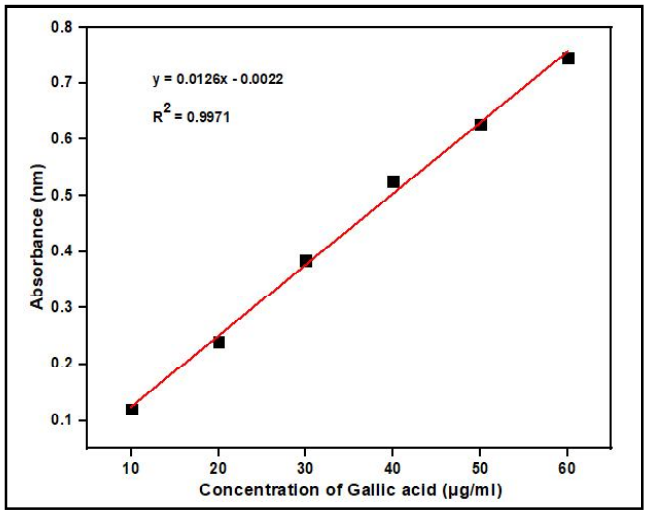

Figure 5: Standard curve of total phenols using gallic acid as a standard.

3.5 Antioxidant potential

DPPH free radical scavenging activity and total antioxidant activity were used to assess the antioxidant capacity of *C. siamea* leaf tissue.

3.5.1 Evaluation DPPH free radical scavenging activity

The assessment of the capacity of antioxidants to counteract free radicals is commonly conducted using the stable free radical termed DPPH. As the concentration of diverse solvent extracts derived from leaves was elevated, the percentage of DPPH free radical scavenging activity displayed a corresponding increase as shown in Table 3. The most substantial level of DPPH free radical scavenging activity within the methanol extract was observed at 350 µg/ml. Additionally, percentages of 57.73%, 51.96%, 43.37%, 34.31%, 25.43%, and 9.57% were documented at concentrations of 300, 250, 200, 150, 100, and 50 µg/ml, respectively. The greatest DPPH activity for the aqueous extract was determined to be 61.85% at 350 µg/ml, while the next highest values were 54.62%, 47.17%, 40.97%, 28.11%, 20.21%, and 7.32% at 300, 250, 200, 150, 100, and 50 µg/ml. The maximal DPPH activity for the ethyl acetate extract was 70.04% at 3500µg/ml, whereas the subsequent highest values were 65.98%, 62.19%, 53.03%, 43.69%, 31.18, and 12.91% at 3000, 2500, 2000, 1500, 1000, and 500 µg/ml, respectively.

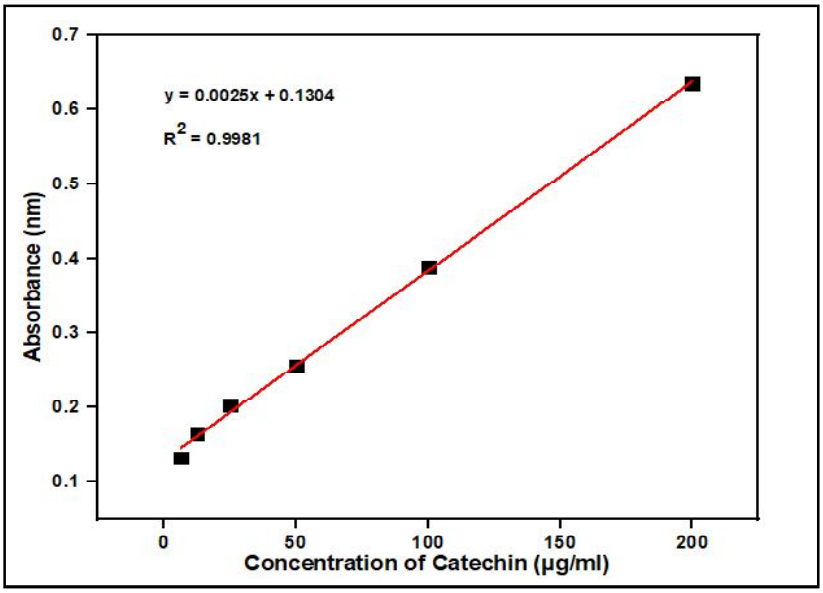

Figure 6: Standard curve of total flavonoids using catechin as a standard.

Table 3: DPPH free radical scavenging activity (%) among different solvents extracts of *C. siamea* leaves

S. No.	Conc. (µg/ml)	DPPH free radical scavenging activity (%)							IC_{50} (µg/ml)
		50	100	150	200	250	300	350	
1.	Methanol extract	9.57	25.43	34.31	43.37	51.96	57.73	66.74	243.59
2.	Aqueous extract	7.32	20.21	28.11	40.97	47.17	54.62	61.85	272.80
3.	Ethyl acetate extract	12.91	31.18	43.69	53.03	62.19	65.98	70.04	2086.35

3.5.2 Total antioxidant activity

Employing ascorbic acid as a benchmark, a standard curve was employed to gauge the comprehensive antioxidant capability of distinct solvent extracts derived from *C. siamea* leaves (Figure 7). The antioxidants present in the extracts induce the conversion of molybdenum (VI) to molybdenum (V).

The green molybdenum (V)-phosphate complex, also known as the phosphomolybdenum complex, is created when the molybdenum

(V) species combines with the phosphate group in sodium phosphate under acidic circumstances. This reaction exhibits a pronounced dependence on time, and the intensity of this complex is quantified utilizing a spectrophotometer at its specific wavelength of maximum absorption (λ_{max}).

The regression equation demonstrated a linear relationship between ascorbic acid concentration and absorbance($y = 0.0028x - 0.0281$, $R^2 = 0.9923$). The total antioxidant content of the leaves was 122.25, 109.41, and 23.08 mg AAE/g in methanol, aqueous, and ethyl acetate extracts, respectively (Figure 8).

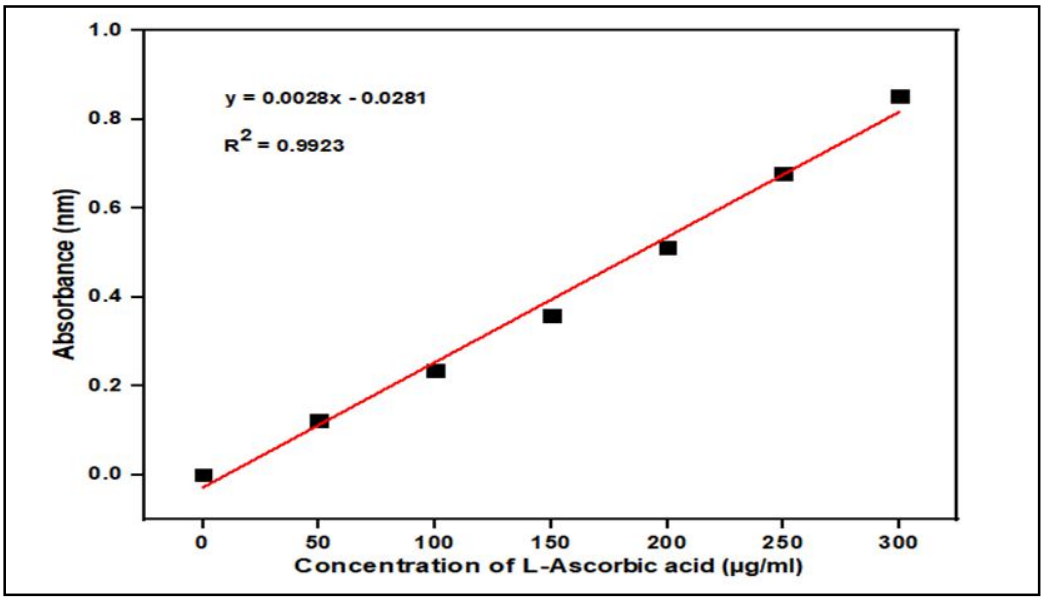

Figure 7: Standard curve of total antioxidant activity using ascorbic acid as a standard.

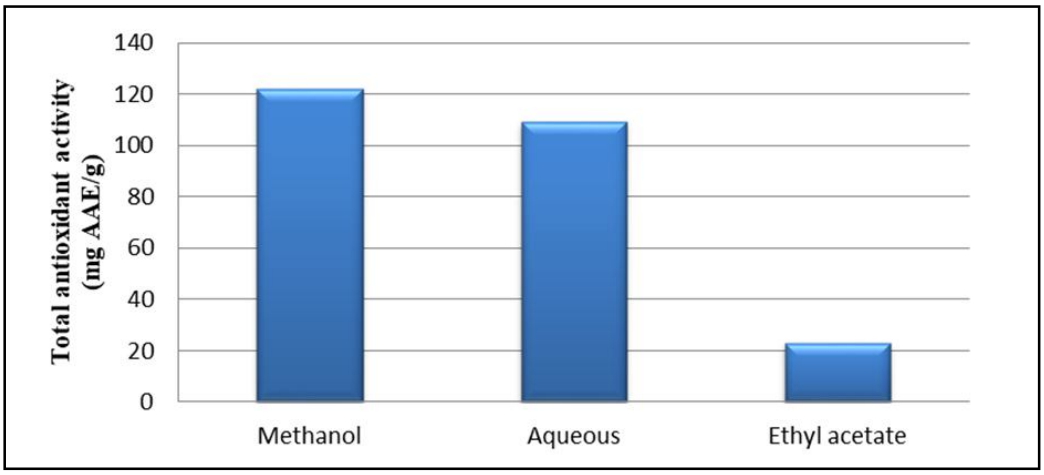

Figure 8: Total antioxidant capacity (mg AAE/g) among different solvent extracts of *C. siamea* leaves.

3. Discussion

Medicinal plants are widely acknowledged for their substantial contribution as valuable reservoirs of natural antioxidants. Due to their phytochemical constituents, which elicit well-defined physiological impacts on humans, plants hold notable medicinal importance. Within the realm of these bioactive botanical compounds, alkaloids, tannins, flavonoids, and phenolic compounds stand out as particularly pivotal. Because they have a high potential for antioxidant protection, phenolic compounds are crucial in scavenging free radicals. Flavanoids are plant byproducts that act as antioxidants and in cell signalling and they are regarded to have a good impact on health. Hence, the present investigation encompasses the determination of the proximate composition and mineral content, alongside the evaluation of phytochemical characteristics, within the methanol, aqueous, and ethyl acetate extracts of *C. siamea* leaves powder. Additionally, the study involves the assessment of antioxidant potential through the utilization of the DPPH scavenging assay and the phospho-molybdate assay.

In leaves of *C. siamea*, the moisture content (7.51 ± 0.78), ash content (5.22 ± 0.63), crude fat (3.69 ± 0.38), crude fiber (12.67 ± 1.64), crude protein (20.72 ± 1.02) and total carbohydrates (51.19 ± 2.70) were examined in present research. Esievo *et al.* (2016) reported 7.33% moisture content and 6.46% ash content in leaves. Christiana *et al.* (2018) reported 3.0% crude fat, 13.00% crude fiber and 21.88% crude protein. Findings of present work was in close agreement with other researchers. Total phenolic content in leaves was 78.77 mg

GAE/g, 73.98 mg GAE/g, and 15.28 mg GAE/g in the methanol, aqueous, and ethyl acetate extracts, respectively. Chanda *et al*. (2012) reported 75.81 and 72.12 mg GAE/g of total phenolic content was found in methanol and aqueous extract of leaves, respectively. The IC_{50} values of leaves in different solvent (methanol, aqueous and ethyl acetate) used are 243.59, 272.80 and 2086.35 µg/ml. Chanda *et al*. (2012) reported that methanol extract of leaves had IC_{50} value of 305 µg/ml. Findings of present work was in close agreement with other researchers. Some difference in data is due to change of location and other environmental factors.

4. Conclusion

The results of this study suggest that the phytochemical components found in the various solvent extracts of *C. siamea* leaves may be crucial in preventing the effects of oxidative stress-causing chemicals. Gaining insights into the pharmacological impacts of *C. siamea* leaves necessitates a quantitative exploration of its phytochemical makeup and its capacity for antioxidant activity. Subsequent investigations are imperative in order to explore the potential applications of *C. siamea* leaves within the realms of medical, pharmaceutical, and nutraceutical industries, given their abundant reserve of phytochemical compounds. Further, research is essential to precisely identify the constituents comprising the antioxidant system, enabling the development of applications for both the pharmaceutical and food sectors.

Ackowledgements

The Department of Chemistry at Chaudhary Charan Singh Haryana Agricultural University, Hisar, provided the necessary resources, for which the authors are grateful.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Aggarwal, P.; Singh, S.; Sangwan, S.; Moond, M. and Devi, P. (2022). Effect of extraction solvents on antioxidant potential of *Prosopis cineraria* (L.) leaves. Ann. Phytomed., **11**(1):426-431.

AOAC (1990). Official methods of analysis. Association of Official Analytical Chemists: Washington, DC. Chemistry, **146**:299-307.

AOAC (1995). Official Methods of Analysis. 16 Ed. A.O.A.C. Virginia,DC, U.S.A.

Basra, S. M. A.; Farooq, M.; Tabassam, R. and Ahmad, N. (2005). Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). Seed Science and Technology, **33**(3):623-628.

Burns, R. E. (1971). Method for estimation of tannin in grain sorghum. Agronomy Journal, **63**:511-512.

Chanda, S.; Kaneria, M. and Baravalia, Y. (2012). Antioxidant and antimicrobial properties of various polar solvent extracts of stem and leaves of four Cassia species. African Journal of Biotechnology, **11**(10):24-90.

Christiana, K. A.; Gidado, A. A.; Bertha, D. A.; Zakari, A. and Amina, A. (2018). Phytochemical screening, proximate and elemental analyses of *Senna siamea* (Lam.) leaves from Kashere metropolis of Gombe State, Nigeria. Inter. Res. J. Chem. Sci., **5**(1):82-85.

Dalal, R.; Singh, S.; Sangwan, S.; Moond, M. and Beniwal, R. (2022). Biochemical and molecular mechanism of plant-mediated synthesis of silver nanoparticles: A review, mini-reviews in Organic Chemistry, **19**(8):939-954.

Devi, D.; Singh, S.;Moond, M.; Matoria, P.; Kumari, S.; Saini, K. and Sharma, R.K. (2023). Phytochemical analysis and antioxidant potential of *Albizia lebbeck* (L.) seeds. Ann. Phytomed., **12**:502-507.

Devi, R.; Singh, S.; Moond, M.; Beniwal, R.; Kumar, S.; Kumari, S. and Sharma, R.K. (2023). Proximate composition, phenolic-flavonoid contentand antioxidant potential of *Syzygium cumini* (L.) Skeels seed powder. Ann. Phytomed., **12**(1):508-513.

Dubois, M.; Gilles, K.A.; Hamilton, J. K.;Rubers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugar and related substances. Analytical Chemistry, **28**(3):350-356.

Esievo, K. B.; Fatokun, O. T.; Ibrahim, J. A. and Kunle, O. F. (2016). Pharmacognostic studies of the leaf of *Senna siamea* (L.) Irwin & Barneby Family: Caesalpiniaceae Drugs, **21**:22.

Goel, N.; Kumari, S.; Singh, S.; Moond, M.; Panghal, M.; Rani, I.; Sangwan, V. and Bhardwaj, K.K. (2022). Mineral content, bioactive ingredient identification and antioxidant activity of *Argemone Mexicana* L.flowers extracts. Ann. Phytomed., **11**(2):478-483.

Harborne, J. B. (1973). Phytochemical methods. Chapman and Hall, London.

Hatano, T.; Kagawa, H.; Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root, their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin. **36**:2090-2097.

Jackson, M.L.C. (1973). Soil chemical analysis Prentice-Hall of India, Private Limited, New Delhi.

Kamagate, M.; Koffi, C.; Kouamé, N. M.; Akoubet, A.; Yao, N. A. R. and Die-Kakou, H. M. (2014). Ethnobotany, phytochemistry, pharmacology and toxicology profiles of *C. siamea* L. The Journal of Phytopharmacology, **3**(1):57-76.

Marinova, D.; Ribarova, F. and Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy, **40**:255-260.

Maynard, A.J. (1970). Methods in Food Analysis. Academic Press, NewYork, pp:176.

Moond, M.; Singh, S.; Devi, R.; Beniwal, R.; Matoria, P.; Kumari, S. and Sharma, R.K. (2023). Proximate and mineral analysis of *Trigonella foenum-graecum* (Fenugreek) seeds and leaves of variety HM444. Ann. Phytomed., **12**(1):546-552.

Moond, M., Singh, S., Sangwan, S. Devi, P., Beniwal, A., Rani, J., Kumari, A. and Rani, S. (2023). Biosynthesis of silver nanoparticles utilizing leaf extract of *Trigonella foenum-graecum* L. for catalytic dyes degradation and colorimetric sensing of Fe^{3+}/Hg^{2+}. Molecules, **28**: 951.

Moond, M.; Singh, S.; Sangwan, S.; Devi, R.; Rani, J.; Beniwal, A. and Beniwal, R. (2023). Synthesis, optimization and characterization of ailver nanoparticles using aqueous seed extract of *Trigonella foenum-graceum* L. for catalytic reduction of p-nitrophenol. Chemistry Select, **8**(29):2365-6549.

Moond, M.; Singh, S.; Sangwan, S.; Rani, J.; Beniwal, A.; Matoria, P.; Saini, K. and Sharma, R.K. (2023). Proximate composition, phytochemical analysis and antioxidant potency of *Trigonella foenum-graecum* L. seeds Ann. Phytomed., **12**(1):486-491.

Moond, M.; Singh, S.; Sangwan, S.; Rani, S.; Beniwal, A.; Rani, J.; Kumari, A.; Rani, I.; Devi, P (2023). Phytofabrication of silver nanoparticles using *Trigonella foenum-graceum* L. leaf and evaluation of its antimicrobial and antioxidant activities. Int. J. Mol. Sci., **24**:34-80.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for determination of glucose. Journal of Biological Chemistry, **153**: 375-380.

Poonam, Singh, S., Moond, M.. Sangwan, S. and Devi , P. (2023). Phytochemicals and antioxidant potential of *Andrographis paniculata* Nees. Ann. Phytomed., **12**(2):769-775.

Ruig, W.G.D. (1986). Atomic absorption spectrophotometric determination of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc in animal feeding stuffs, interlaboratory collaborative studies. Journal of Association of Official Analytical Chemists, **69**:1009.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, **16**(3):144-158.

Somogyi, M. (1952). Notes on sugar determination. Journal of Biological Chemistry, **195**:19-23.

Prieto, P.; Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry, **269**:337-341.

Citation **Kamaljeet Saini, Sushila Singh, Monika Moond, Jyoti Rani, Anuradha Beniwal, Simran Kakkar, Yogita and Rajni Kant Sharma (2024). Estimation of phytochemicals and antioxidant capacity of leaves of *Cassia siamea* L. Ann. Phytomed., 13(1):1205-1213. http://dx.doi.org/10.54085/ap.2024.13.1.130.**