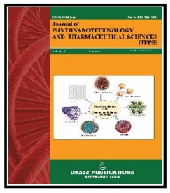
Journal of Phytonanotechnology and Pharmaceutical Sciences 3(2): 36-41, 2023

Online ISSN:2583-0376

http://jpps.ukaazpublications.com

DOI: http://dx.doi.org/10.54085/jpps.2023.3.2.6

Journal of Phytonanotechnology and Pharmaceutical Sciences



Review Article : Open Access

Phytochemical analysis of acetone extract of *Cassia siamea* L.

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

Department of Chemistry, Chaudhary Charan Singh Haryana Agricultural University, Hisar, 125004, Haryana, India

Article Info

Article history

Received 1 May 2023

Revised 17 June 2023

Accepted 18 June 2023

Published Online 30 June 2023

Keywords

Phytochemicals

Flavonoids

Phenolics

Antioxidants

Reducing sugar

Abstract

Phytochemicals are substances found in plants that are essential to their development and growth. Flavonoids, isoflavones, and other polyphenols are dietary phytochemicals that may have health advantages. Due to their high concentration of hydroxyl groups, polyphenolic compounds, the biggest family of phytochemicals, function as potent antioxidants. Kassod, also known as *Cassia siamea* L., is a plant with amazing medicinal and healing capabilities since it is a rich source of phytochemicals.

The goal of the current study was to assess the main phytochemical components of the acetone extract of dried *C. siamea* leaves, bark, and flower. Total sugars were determined using the Dubois method, reducing sugar by the Nelson method, total phenolic content was determined using the Folin-Ciocalteu method and flavonoids were determined by the $AlCl_3$ colorimetric test in acetone extract of leaves, bark, and flower.

A variety of phytochemicals are examined in acetone extract of leaves, bark, and flower, including total phenolic content (43.98 ± 0.80, 12.99 ± 1.02 and 56.35 ± 0.83 mg GAE/g), total flavonoids (12.84 ± 0.84, 4.38 ± 2.07 and 18.06 ± 0.72 mg CE/g), total sugars (27.65 ± 0.83, 7.87 ± 1.34 and 42.35 ± 0.79 mg/g), reducing sugars (9.95 ± 1.72, 4.09 ± 0.92 and 26.45 ± 2.03 mg/g), and non-reducing sugars (17.70 ± 0.60, 3.78 ± 0.45 and 15.90 ± 1.01 mg/g). These results confirm that *C. siamea* is a rich source of minerals and phytochemicals with pharmacological and health effects.

1. Introduction

The goal of phytochemical research is to discover new applications for using plants to produce pharmaceuticals. Phytonutrients have positive health effects (Moond *et al*., 2023). These have antibacterial, anticancer, anti-inflammatory, antihypertensive, and antidiabetic properties. People have employed various plant products as remedies since the beginning of human civilization (Moond *et al*., 2023). The medicinal value of plants is due to the phytochemical components that they produce, which have unique physiological activities. Primary constituents, which include sugars, amino acids, chlorophyll, and protein, *etc*., and secondary constituents, which include terpenoids, alkaloids, phenolic, flavonoids, saponins, essential oils, and tannin compounds, *etc*., are the two main categories of phytochemicals, which are chemical compounds derived from plants and these are non-essential nutrients (Murlidhar and Goswami, 2012; Moond *et al*., 2023).The main factor responsible for the antioxidant effects of phenolics and flavonoids is their redox properties, which are essential for scavenging and neutralizing free radicals (Aggarwal *et al*., 2022; Moond *et al*., 2023; Devi *et al*., 2020, 2023).

Cassia siamea L. (syn. *Senna siamea* L.) is an angiosperm. Southeast Asia, specifically India, Burma, Sri Lanka, Thailand, and Malaysia, is the native home of this tree. The Fabaceae family includes *C. siamea*. It has been used medically to treat rhinitis, liver problems, urticaria, and appetite loss brought on by digestive problems. Additionally, purgative and laxative properties are claimed to exist in it. The leaves are used to treat constipation, diabetes, and hypertension; the stem bark is used to treat skin disorders and hemorrhoids; and the root is used as an antipyretic in situations of fever (Parveen *et al*., 2010). The leaves are cleaned and then boiled in India. After filtering the decoction, honey is added. To combat anaemia and fever, this remedy should be taken in a quarter glass (150 ml) three times a day (Sati *et al*., 2010). The presence of numerous useful biochemicals, including p-cresol, 1,2-benzene dicarboxylic acid, butyl octyl ester, and heptadecanenitrile as significant constituents, led researchers to believe that *C. siamea* seed is a suitable feedstock for the creation of biofuels.

Figure 1: *C. siamea* Plant.

The current study's objectives were to determine the phytochemical components of an acetone extract made from dried *C. siamea* leaves,

Corresponding author: Dr. Sushila Singh

Assistant Professor, Department of Chemistry, Chaudhary Charan Singh Haryana Agricultural University, Hisar, 125004, Haryana, India

E-mail: singhsushila999@gmail.com

Tel.: +91-8199939339

bark, and flowers. This study found that leaves were a significant source of total phenolics, total flavonoids, total sugars, non-reducing sugars, and reducing sugars, as compared to bark and flower.

2. Materials and Methods

2.1 Collection of plant material

C. siamea plant components, including leaves, bark, and flowers, were gathered from the CCS Haryana Agricultural University botanical garden in Hisar, Haryana. Plant materials were cleaned before being air-dried in the shade.

2.2 Preparation of acetone extract of *C. siamea* leaves, bark, and flower for phytochemical analysis

10 g of powder from the leaves, bark, and flowers of *C. siamea* were collected in a thimble made of Whatman No. 1 filter paper. A 500 ml flask with a round bottom served as the main component of the typical Borosil Soxhlet apparatus in which this thimble was placed. Up to 1.5 siphons of acetone were added in a total volume of roughly 250 ml. As a result, acetone was used as the solvent in a Soxhlet device to percolate the powdered leaf sample. This acetone extract was used to measure the total phenolic content, total sugars, total flavonoids, non-reducing sugars, and reducing sugars capacity.

2.3 Quantitative analysis of phytochemicals determination of total phenolic content

Gallic acid was used as the reference standard to determine the total phenolic content using the Folin-Ciocalteu method (Singleton and Rossi, 1965). 2.0 ml of Na_2CO_3 (20%, w/v), 1.0 ml of 1 mol/l Folin-Ciocalteu reagent, and 1.0 ml of acetone extract were put in a test tube. Water was added after combining to make the final volume 10.0 ml. The reaction mixture was incubated for 8 min and then centrifuged for 10 min at 6000 rpm. The absorbance of the supernatant solution was measured at 730 nm using a UV-Vis spectrophotometer, and the results were compared to a blank made using the same method but with the right solvent in place of the extract. The total phenolic content of the acetone extract was determined using a regression equation created from the gallic acid standard curve, and the result was expressed as mg GAE/g.

2.4 Determination of total flavonoids

Catechin was used as a reference to calculate the total amount of flavonoids using an $AlCl_3$ colorimetric test (Marinova *et al*., 2005). A test tube containing 4.0 ml of distilled water was filled with 1.0 ml of acetone extract and 0.3 ml of 5% $NaNO_2$. Five minutes later, 0.3 ml of 10% $AlCl_3$ and 2.0 ml of 1M NaOH were added. Next, dilutions with distilled water up to a volume of 10.0 ml were added. A UV-Vis double beam spectrophotometer was used to measure the mixture's absorbance at 510 nm in comparison to a blank that was made using the same procedures but with acetone in place of the extract. The total amount of flavonoids in the extract was determined using a regression equation created from the catechin standard curve, and the result was expressed as mg CE/g.

2.5 Determination of total sugars

Total sugars were determined using a modified version of Dubois's technique (Dubois *et al*., 1956). In a test tube, the leaves, bark, and flowers of *C. siamea* were extracted in acetone with 1 ml and 2.0 ml of phenol solution was then added to it. 5.0 ml of concentrated H_2SO_4 was then added to the reaction mixture right away. The reaction mixture's absorbance at 490 nm was then compared to a blank that was made using the same procedure but with acetone in place of the extract using a UV-Vis double-beam spectrophotometer after the solution had been allowed to cool for 30 min. The total amount of sugars in the extract was determined using the regression equation created from the D-glucose standard curve, and the results were expressed as mg/g.

2.6 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 one milliliter of an acetone extract of *C. siamea* leaves, bark, and flowers. The alkaline copper reagent was added, the mixture was thoroughly blended, and it was then cooked in a hot water bath for 20 to 25 min. After reaching room temperature, being thoroughly mixed, and being diluted with distilled water to a volume of 10.0 ml, the arsenomoly-bdate reagent was added to the boiling tubes. Using a UV-Vis double beam spectrophotometer, the absorbance at 520 nm of the reaction mixture was assessed in comparison to a blank that had been made in the same way but contained 1.0 ml of distilled water in place of extract. Using the D-glucose standard curve, the amount of reducing sugars in the acetone extract was calculated and the result was expressed as mg/g.

2.7 Determination of nonreducing sugars

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

Nonreducing sugars = Total sugars × Reducing sugars

3. Results

3.1 Quantitative analysis of phytochemicals

Quantitative analysis of the leaf, bark, and flower extract of *C. siamea* was performed to determine the amount of various phytochemicals, such as total phenolic content, total flavonoids, total sugars, reducing sugars, and non-reducing sugars. The results are shown in Table 1.

Table 1: Phytochemicals in acetone extract of the *C. siamea* leaves, bark, and flowers

S. No.	Phytochemicals	Leaves	Bark	Flowers
1.	Total phenolic content (mg GAE/g)	43.9 ± 0.80	12.99 ± 1.02	56.35 ± 0.83
2.	Total flavonoid (mgCE/g)	12.84 ± 0.84	4.38 ± 2.07	18.06 ± 0.72
3.	Total sugar (mg/g)	27.65 ± 0.83	7.87 ± 1.34	42.35 ± 0.79
4.	Reducing sugar (mg/g)	9.95 ± 1.72	4.09 ± 0.92	26.45 ± 2.03
5.	Non reducing sugar (mg/g)	17.70 ± 0.60	3.78 ± 0.45	15.90 ± 1.01

3.2 Total phenolic content

Using a standard curve with gallic acid as the reference, the total phenolic content of the leaves, bark, and flowers of *C. siamea* was determined.

The absorbance and gallic acid concentration were shown to be linearly related by the regression equation. Regression analysis was used to determine the total phenolic content of the acetone extract of leaves, bark, and flowers (y = 0.012 75x ×× 0.02521, R^2 = 0.9905) and it was determined to be 43.98 ± 0.80, 12.99 ± 1.02and 56.35 ± 0.83 mg GAE/g.

3.3 Total flavonoids

Using a standard curve with catechin as the reference compound, the total amount of flavonoids present in the acetone extract of *C. siamea* leaves, bark and flowers was determined (Figure 3).

The regression analysis revealed a linear relationship between catechin concentration and absorbance. Regression analysis was performed using the equation (y = 0.00106x + 0.00843, R^2 = 0.9902), 12.84 ± 0.84, 4.38 ± 2.07, and 18.06 ± 0.72 mg CE/g of total flavonoids were discovered in the acetone extract of the leaves, bark, and flower.

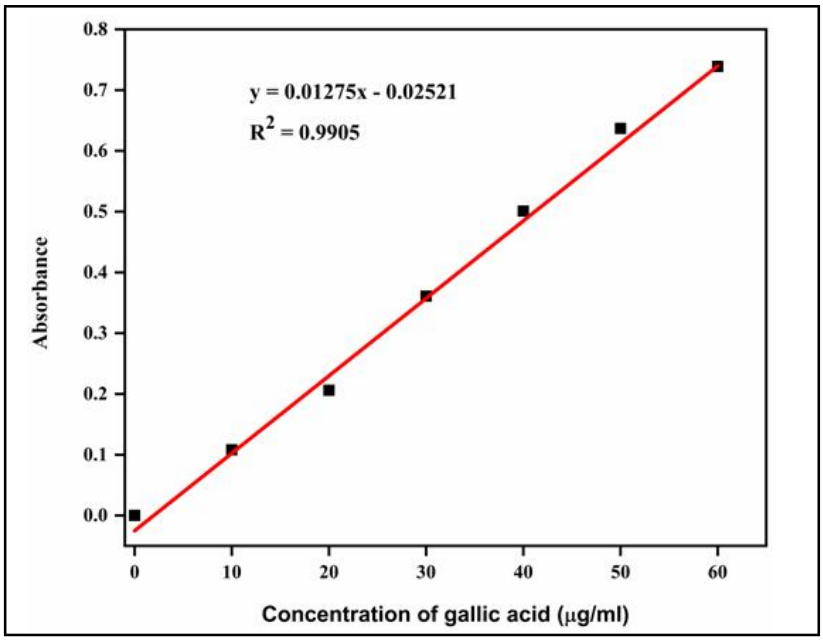


Figure 2: Standard curve of total phenols using gallic acid as a standard.

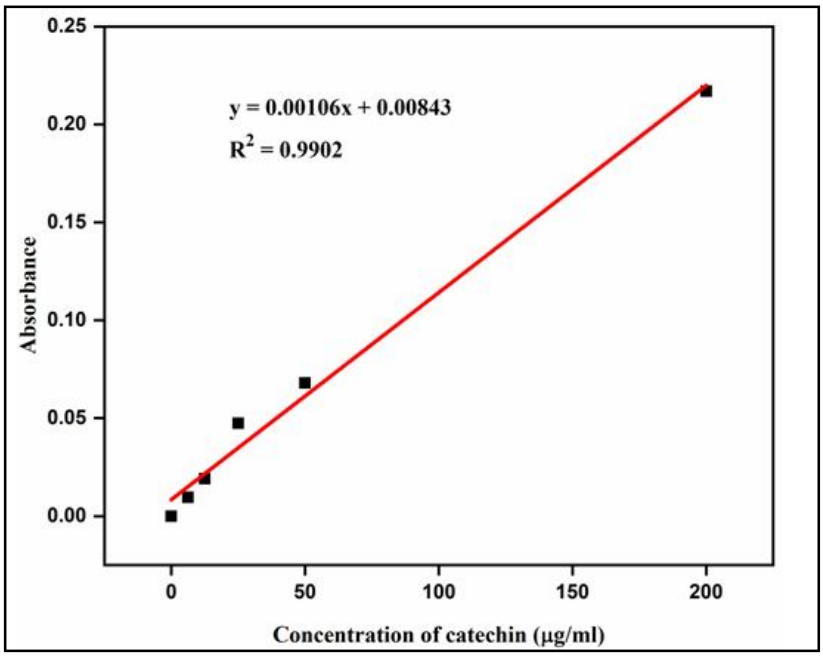


Figure 3: Standard curve of total flavonoids using catechin as a standard.

3.4 Total sugars

The total sugars in the acetone extract of the *C. siamea* leaf, bark, and flowers were calculated using a standard curve and D-glucose as the reference substance (Figure 4). D-glucose concentration and absorbance were shown to be linearly related by the regression equation. The total sugar content of the acetone extract of the leaves, bark, and flower was calculated using the regression equation ($y = 0.00543 \times 0.00759$, $R^2 = 0.9979$), and it was determined to be 27.65 ± 0.83, 7.87 ± 1.34 and 42.35 ± 0.79 mg/g.

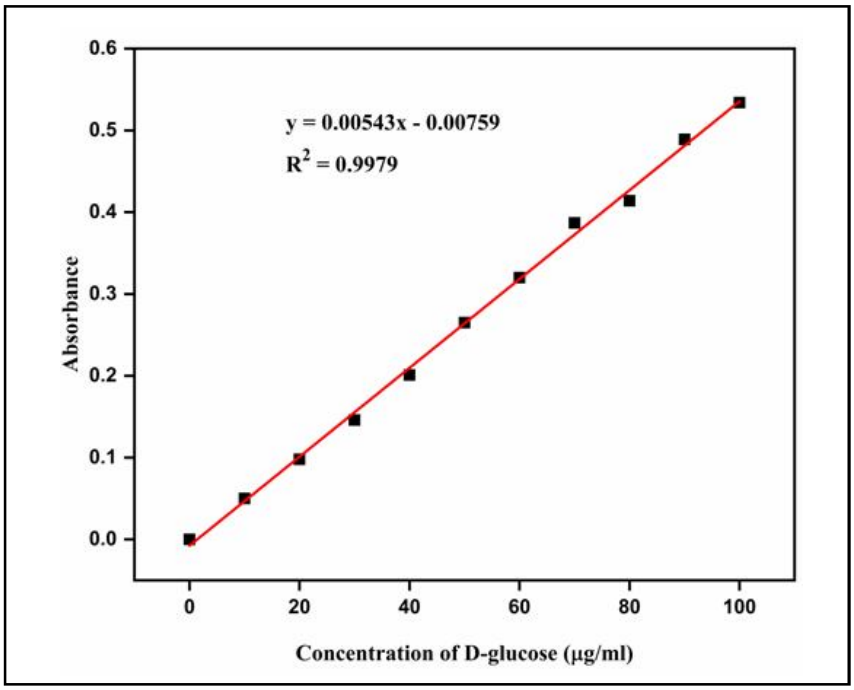


Figure 4: Standard curve of total sugar using glucose as standard.

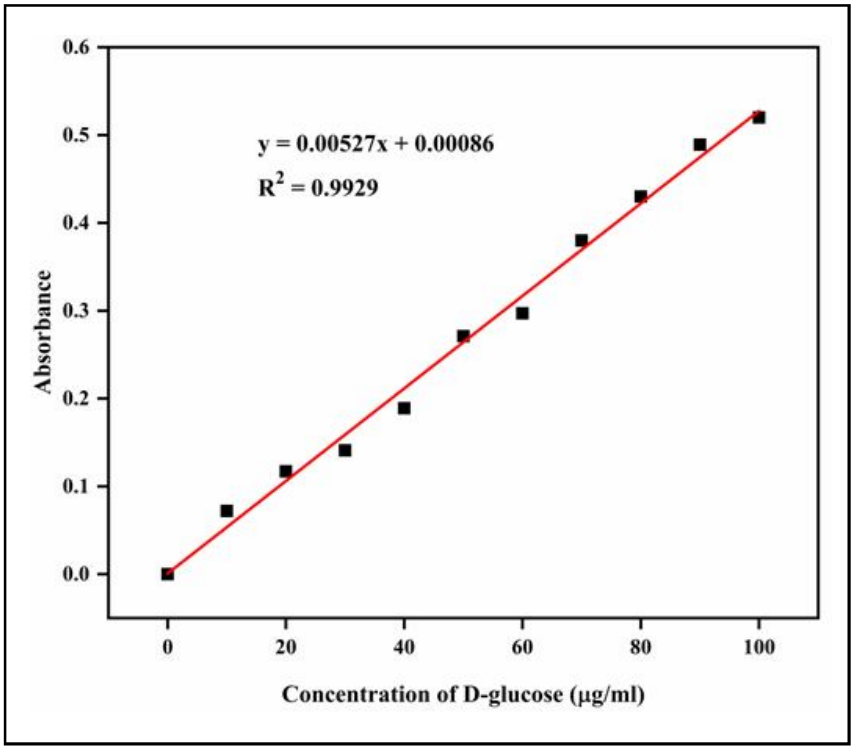


Figure 5: Standard curve of reducing sugars using glucose as standard.

3.5 Reducing sugars

D-glucose was used as a reference to create a standard curve to calculate the amount of reducing sugars in the acetone extract of *C. siamea* leaves, bark, and flowers (Figure 5).

The results of the regression analysis showed a linear relationship between absorbance and D-glucose concentration. Regression analysis was used to determine the total sugar content of an acetone extract of leaves, bark, and flowers ($y = 0.00527x + 0.00086$, $R^2 = 0.9929$). It was determined to be 9.95 ± 1.72, 4.09 ± 0.92, and 26.45 ± 2.03 mg/g.

3.6 Non-reducing sugars

The difference between the concentration of total sugars and that of reducing sugars was used to evaluate the non-reducing sugars in the acetone extract of the *C. siamea* leaves, bark, and flower. It was found to be 17.70 ± 0.60, 3.78 ± 0.45, and 15.90 ± 1.01 mg/g.

4. Discussion

Medicinal plants are widely acknowledged as a significant source of natural antioxidants. Plants can be used to make medicines due to their phytochemicals, which have specific physiological effects on humans.

Results of the present investigation were in close agreement with the estimation of Chanda *et al*. (2012) who reported 75.81, 53.81 and 72.12 mg GAE/g of total phenolic content was found in methanol, acetone and aqueous extract of leaves, respectively. Chanda *et al*. (2012) reported 16.57, 46.25 and 1.40 mg CE/g of total flavonoid content in methanol, acetone and aqueous extract of leaves, respectively.

The determination of phenolic compounds is required to oxidize Folin-Ciocalteu reagent. Phosphotungstic acid and phosphomolybdic acid are reduced to a mixture of blue tungsten and molybdenum oxides, which is the reagent, after oxidizing phenols. The amount of phenolic compound found in the extract is closely correlated to the intensity of the blue coloring that is created, which has a maximum absorption region of around 730 nm.

Total flavonoids are calculated based on the hypothesis that $AlCl_3$ and the C-3, C-5 hydroxyl group and C-4 keto group of flavones and flavonols form an acid stable complex. Orthodihydroxyl groups on the A or B rings of flavonoids can also form acid-labile complexes by interacting with $AlCl_3$.

The evaluation of total sugars is based on D-glucose was dehydrated in an acidic medium to yield hydroxymethyl furfural, which was then combined with phenol to create a yellow-brown solution with a maximum absorbance at 490 nm.

During determination of reducing sugar alkaline copper tartrate causes cupric ions to be converted into cuprous ions when reducing sugars are heated, which produces cuprous oxide. When cuprous oxide and arsenomolybdic acid are combined, molybdic acid is transformed into molybdenum blue, which is detectable with a UV-Vis spectrophotometer at 520 nm.

5. Conclusion

The current study found that the acetone extract of *C. siamea* leaves, bark, and flower included phytochemicals that may be essential in scavenging species that cause oxidative stress. Understanding the pharmacological effects of *C. siamea* leaves might benefit from the quantitative investigation of phytochemicals. Future studies into the uses of *C. siamea* flowers in the medical, pharmacological, and nutraceutical industries are required because they are a richer source of phytochemicals than the leaves and bark. More research is required to identify the precise components of the antioxidant system and develop applications for the pharmaceutical and food industries.

Ackowledgements

For providing the necessary resources, the authors are grateful to the Department of Chemistry at Chaudhary Charan Singh Haryana Agricultural University, Hisar.

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 the antioxidant potential of *Prosopis cineraria* (L.) leaves. Ann. Phytomed., **11**(1):426-431.

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**(1):1-6.

Devi, P.; Singh, S.; Sangwan, S.; Dalal, P. and Moond, M. (2020). Effect of pH on antioxidant and phytochemical activities of Mulhatti roots (*Glycyrrhiza glabra* L.). Journal of Agricultural Science and Technology A, **11**:276-282.

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.

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.

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* L. (Fenugreek) seeds and leaves of variety HM444. Ann. Phytomed., **12**(1):1-7.

Moond, M.; Singh, S.; Devi, R.; Pinki and Rajita. (2023). Estimation of phytochemicals and antioxidant potency of *Trigonella foenum-graecum* leaves (Variety HM444). Agricultural Science Digest. doi: 10.18805/ag.D-5677.

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 *Trigonellafoenum-graecum* L. for catalytic dyes degradation and colorimetric sensing of $Fe3^+$/$Hg2^+$. Molecules. **28**: 951.

Moond, M.; Singh, S.; Sangwan, S.; Rani, S.; Beniwal, A.; Rani, J.; Kumari, A.; Rani, I.; Devi, P.(2023). Phytofabrication of silver nanoparticles using *Trigonellafoenum-graceum* L. leaf and evaluation of its antimicrobial and antioxidant activities. Int. J. Mol. Sci., **24**:3480.

Murlidhar, M. and Goswami, T. K. (2012). A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. Journal of Food Processing and Technology, **3**(9):220.

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

Parveen, S.; Shahzad, A. And Saema, S. (2010). *In vitro* plant regeneration system for *C.siamea* L., a leguminous tree of economic importance. Agroforestry Systems, **80**:109-116.

Sati, S. C.; Nitin, S.; Rawat, U. and Sati, O. P. (2010). Medicinal plants as a source of antioxidants. Research Journal of Phytochemistry, **4**(4):213-224.

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.

Citation **Anuradha Beniwal, Sushila Singh, Kamaljeet Saini, Jyoti Rani, Simran Kakkar, Monika Moond and Yogita (2023). Phytochemical analysis of acetone extract of *Cassia siamea* L.. J. Phytonanotech. Pharmaceut. Sci., 3(2):36-41. http://dx.doi.org/10.54085/jpps.2023.3.2.6**