Asian Journal of Green Chemistry 7 (2023) 258-268

SPC

Asian Journal of Green Chemistry

AJGC Asian Journal of Green Chemistry

Original Research Article

View Article Online | View Journal

Study of Anti-Thrombocyte Activity of *Cassia fistula* Seeds Extract and It's Total Phenolic and Flavonoid Content, *In Vitro* Antioxidant and Anti-Inflammatory Activities

Fazle Rabbi Shakil Ahmed[a], Mst. Jesmin Sultana[b,*], Afroza Sultana[a], Md. Ferdous Alom[a]

[a]*Department of Pharmacy, Khwaja Yunus Ali University, Enayetpur, Chouhali, Sirajganj-6751, Bangladesh*

[b]*Department of Materials Science and Engineering, University of Rajshahi, Rajshahi-6205, Bangladesh*

ARTICLE INFORMATION

Submitted: 20 May 2023
Revised: 22 June 2023
Accepted: 24 June 2023
Available online: 8 July 2023

Manuscript ID: AJGC-2305-1393
Checked for Plagiarism: Yes
Language Editor:
Dr. Fatimah Ramezani
Editor who approved publication:
Dr. Zeinab Arzehgar

DOI: 10.22034/ajgc.2023.398285.1393

KEYWORDS

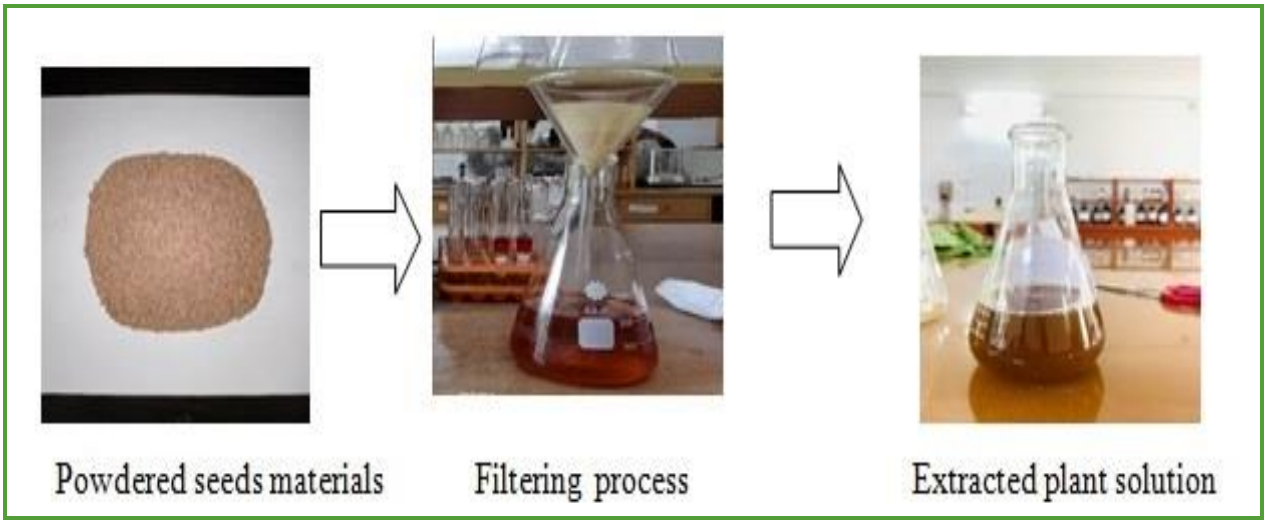
Cassia fistula
Phytochemicals
Anti-thrombocyte
Antioxidant
Anti-inflammatory

ABSTRACT

The purpose of this study was to measure the total phenol and flavonoid content and assess the antioxidant and anti-thrombocyte activity in ethanol extracts of *Cassia fistula* seeds. Aluminum chloride was used to calculate the amount of flavonoids, and Folin-Ciocalteu reagent was used to calculate the total amount of phenolic compounds using a spectrophotometric method. The *in vitro* antioxidant activity of the analyzed extracts was evaluated utilizing the DPPH approach. The total phenolics and flavonoid contents in the seeds extract *Cassia fistula* 246 ± 0.08 mg GAE/g dw and 118 ± 0.001 mg QE/g dw, respectively. The obtained results concluded that it may be considered a good amount of phenolic and flavonoid compounds. The *in vitro* anti-inflammatory properties showed the highest percentage inhibition of protein denaturation was 54% for 45 µg/ml and 70% for the reference drug diclofenac sodium at a similar dose. The minimum inhibition of hemolysis 58% was observed at 50 µg/mL of *Cassia fistula* seeds extract and 81% for the same dose of standard aspirin. The results presented that the ethanol seeds extract of *Cassia fistula* has potential anti-thrombocyte activity. IC_{50} values were used to express the antioxidant activity of the investigated extracts. The IC_{50} values was found to be 8.90 µg/ml for ethanol seeds extract which is comparable to that of ascorbic acid (IC_{50} = 6.73 µg/ml) a well-known standard antioxidant. Among these results, the lower the IC_{50} showed the higher the free radical scavenging activity. Among these findings, the stronger the free radical scavenging activity was seen the lower the IC_{50} indicated. Based on all of the findings, we concluded that the anti-thrombocyte antioxidant and anti-inflammatory activities may be caused by phytochemicals identified in *C. fistula* extract.

Corresponding authors, email: jssumi8@gmail.com, jesmin_mse@ru.ac.bd (M.J. Sultana).
Tel.: 088-01776800915.

Graphical Abstract



Introduction

As sources of remedies, medicinal plants are frequently employed as complementary therapeutic tools for the prevention or treatment of several diseases. Very common plant *Cassia fistula* L., noted for its therapeutic effects. *Cassia fistula* Linn, a member of the Caesalpiniaceae family and popularly named the "golden shower tree" (Bengali: Bandor lathi), is well-known for its therapeutic benefits. It is found in a number of areas, including Brazil, West Indies, China, South Africa, and Asia [1].

Cassia fistula is one of the herbs that are most frequently used in Unani and Ayurvedic medicine. It has been advised to utilize *Cassia fistula* to treat diabetes, leucoderm, pruritus, and haematemesis. *Cassia fistula* has also been found to be helpful for treating skin conditions, liver issues, and tuberculous glands [2].

Extracts relieve constipation, piles, and detoxifier [3].

Numerous biologically significant substances were isolated and identified from various plant parts [4, 5].

The observations were made utilizing various solvent extracts and plant components, and the plant extracts were found to have strong antibacterial, antifungal, anti-inflammatory, and antioxidant effects [6].

It has been reported on the chemical analysis of various *C. fistula* components. It was discovered to include proanthocyanidins, phenolic compounds, and flavonoids [7].

Several pharmacological activities of *C. fistula* extracts have been reported, including anti-inflammatory [8], wound healing properties [1] antioxidant [9], antibacterial [10], and anticancer activity [11].

However, there hasn't been any information published about a thorough pharmacological analysis of *Cassia fistula* seed extracts. Based on the literature review, the current study used seeds extract that had been subjected to phytochemical analysis. The same extracts were used for their anti-thrombocytic activity, anti-inflammatory effects, and antioxidant characteristics (which had not been explored in earlier investigations).

Furthermore, the total content of phenolic and flavonoid of *C. fistula* seeds extract. The seeds of *C. fistula* will gain valuable qualities as a result of these findings.

Experimental

Materials and Methods

Plant samples (seeds) collection and preparation for extract

Cassia fistula seeds were procured from Kamarkhand, Sirajganj on 1st June 2022. The plant was identified and verified by Professor Dr. AHM Mahbubur Rahman (Taxonomist), Department of Botany, University of Rajshahi, Bangladesh. The voucher number of the plant *Cassia fistula* is FK 239.

The collected seeds were cleaned, shade dried for two weeks, and ground by a mechanical grinder (Figure 1).

Figure 1. Processing of *Cassia fistula* seeds powder

Dried ground seeds were soaked in ethanol to extract the phytochemicals Whatman No. 1 filter paper and a Buckner funnel were used to filter the extracts. Using a rotary evaporator and lowered pressure at 40 °C, the filtrate was concentrated to dryness to determine the crude extract yield.

Phytochemical analysis

Phytochemical analysis was carried out utilizing accepted techniques from the literature [12, 13].

Using the test of different chemical groups found in the extract of *P. minima,* phytochemical screening was done [14].

Our study is now focused on the chemical groups that can be found in the seeds extract of *Cassia fistula,* specifically steroids, tannins, alkaloids, quinine, flavonoids content, saponins, terpenoids, glycosides, anthraquinone, proteins, phenols, carbohydrates, and gum are all present.

Determination of the total content of the phenolic compound

The total content of the phenolic compound of the tested seed extracts was identified spectrophotometrically with Folin-Ciocalteu reagent according to the aforementioned procedure [15] with slight modifications. Folin Ciocalteu reagent (5 mL) and 4 mL 7.5% Na_2CO_3 solution were mixed with dilute *Cassia fistula* seeds extract (1 mL) or Gallic acid (a common phenolic component).

Following a 15-minute standing period, the mixes were given a colorimetric analysis at 765 nm to measure their total phenolic content. Gallic acid solutions in methanol and water (50:50, v/v) at concentrations of 15.62, 31.25, 62.5, 125, 250, and 500 mg mL^{-1} were used to create the standard curve. On a spectrophotometer, in contrast to a blank (5 mL of Folin-Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3 were added in 1 mL of methanol); the absorbance was measured at 765 nm. The

total content of the phenolic compound of the samples was calculated using the calibration curve equation, and the findings were represented as mg of Gallic acid equivalents per g of dried weight extracts (mg GAE/g dw), which is a typical reference substance.

Determination of the total content of the flavonoid

The total content of the flavonoid compound of the extracts was determined using spectrophotometric analysis, and the process was based on the complexes that formed between the flavonoids and aluminum [16].

0.5 mL of a 1:10 mg mL^{-1} *Cassia fistula* seeds extract was combined with methanol (1.5 mL), 0.1 mL of 10% aluminum chloride, sodium acetate (0.1 mL of 1 M), and double distilled water (2.8 mL). It stayed at room temperature for thirty minutes, and a spectrophotometer was used to measure the reaction mixture's absorbance at 510 nm. Quercetin solutions in methanol were prepared at concentrations of 500, 250, 125, 62.5, 31.25, and 15.62 mg mL^{-1} to create the calibration curve.

In vitro anti-inflammatory effects

The protein denaturation approach was used to treat inflammation using the ethanoic seeds extract of *Cassia fistula* [17].

2.8 milliliters of phosphate-buffered saline (PBS), 0.2 milliliters of fresh hen egg albumin, and 2 milliliters of various test extract strengths with final concentrations of 15, 20, 25, 30, 35, 40, and 45 µg/mL were all included in the 5 mL reaction mixture. The same amount of double-distilled water was utilized as a control. The mixtures were then heated for five minutes at 70 °C after 15 minutes of incubation at 37 °C. After cooling, using a T60 visible spectrophotometer (PG Instruments Limited), their absorbance at 660 nm was assessed. To test the absorbance at a final concentration of (15, 20, 25, 30, 35, 40, and 45 µg/mL), diclofenac sodium was employed as a standard medication and treated uniformly [18].

By applying the formula below, the percentage (%) inhibition for protein denaturation was determined [19].

$$\text{Rotein denaturation inhibition percentage} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Determination of anti-thrombocyte activity

Five milliliters of blood from healthy human participants who had not taken any NSAIDs before the test was obtained for the experiment. Following that, it was combined with an equal volume of the anticoagulant ethylene-diamine-tetraacetic acid (EDTA), and centrifuged at 3000 rpm.

Isosaline was employed to clean the packed cells, and red blood cells were suspended at 10% v/v and used for observation. 0.5 mL of 10% HRBC, 1 mL of 0.15 M phosphate buffer (pH 7.4), 2 mL of hypotonic saline, and 0.5 mL of *Cassia fistula* or aspirin (for reference drug) at various concentrations (50, 100, 200, 400, and 800 µg/mL, respectively) made up the test solution. The test control solution consisted of 1 mL of phosphate buffer, 0.5 mL of 10% HRBC, and 2 mL of distilled water in isotonic saline.

Hemoglobin content was calculated spectrophotometrically at 560 nm after assay mixtures were centrifuged at 3000 rpm for 20 minutes and incubated at 37 °C for 30 min [20]. By assuming the control produced 100% of the hemolysis, the percentage of hemolysis was estimated. The formula below was used to project the hemolysis percentage:

$$\text{Percentage of hemolysis} = \frac{\text{Abs sample}}{\text{Abs control}} \times 100$$

According to the spectroscopic approach, the antioxidant effects of crude extracts and positive controls (ascorbic acid) on DPPH radicals were calculated [21].

An equal amount of the 60 mM DPPH solution and each aliquot of the crude extracts (1.5 mL each) were combined. All the combinations were vigorously mixed and allowed to stand for 20 minutes in the dark at room temperature.

The absorbance of the reaction solutions was determined spectrophotometrically at 517 nm. Ethanol was use as negative control. The percentage of DPPH decolorizations of the samples was calculated using the following equation:

$$\% \text{ Decolorization} = \frac{1 - (\text{ABS}_{\text{sample}})}{1 - (\text{ABS}_{\text{control}})} \times 100$$

The effective concentration at which 50% of the radicals in DPPH were scavenged was known as the IC_{50} value. This was determined by interpolating with a linear regression analysis. IC_{50} was determined from % inhibition vs. log conc. graph. A lower IC_{50} value denoted higher antioxidant activity.

Results and Discussion

Phytochemical analysis

An examination of the phytochemical composition of the seeds of *C. fistula* indicated the presence of nine significant phytoconstituents, including alkaloids, tannins, flavonoids, saponins, terpenoids, quinine, anthraquinone, phenols, carbohydrates, and proteins.

In addition, the absence of steroids, gum, and glycosides was noted in the outcome. This is depicted in Table 1.A similar result was also found for the genus of *Artemisia,* which indicated the presence of monoterpenes, sesquiterpene [22].

The total content of phenolic and flavonoids

By measuring the reducing capacity of the phenolic component, the total phenolics component concentration in the analyzed extracts was measured spectrophotometrically, and the results were derived using the equation for the standard curve: ($y = 0.0885x + 0.0049$, $R^2 = 0.9758$) obtained from the calibration curve (Figure 2) where, x represents the concentration of extract from *Cassia fistula* seeds and y represents absorbance at 750 nm.

The total phenolics content in the seeds extract of *Cassia fistula* is 246 ± 0.08 mg GAE/g dw (Table 2). It can be concluded from the data that there may be a sufficient amount of phenolic chemicals.

The production of chelates and the displacement of absorption bands as a result of a complex formed by flavonoids with metal ions, such as Al^{3+}, serve as the basis for estimating the total quantity of flavonoids present. The total amount of flavonoid was calculated with the crystalline aluminum chloride and crystalline sodium acetate reagent. Quercetin was used as standard. The total flavonoid content was determined as mg QE/g of dried plant material using the equation for the standard curve: $y = 0.0951x - 0.0091$, $R_2 = 0.9787$ where, Y represents the absorbance at 510 nm and x is the concentration of sample extract of *Cassia fistula* (Figure 3). The total flavonoid contents in the seeds extract of *Cassia fistula* 118 ± 0.001 mg QE/g dw (Table 2). It can be concluded from the data that there may be a sufficient amount of flavonoid compounds.

Table 1. Phytochemical analysis of *Cassia fistula* seeds extract

The phytochemicals' names	Ethanol extract	Name of the Phytochemicals	Ethanol extract
Glycosides	-	Gums	-
Flavonoids	+	Steroids	-
Saponin	+	Carbohydrates	+
Tannins	+	Quinone	+
Protiens	+	Alkaloids	+
Terpenoids	+	Anthraquinone	+

(+) = Presence, (−) = Absence

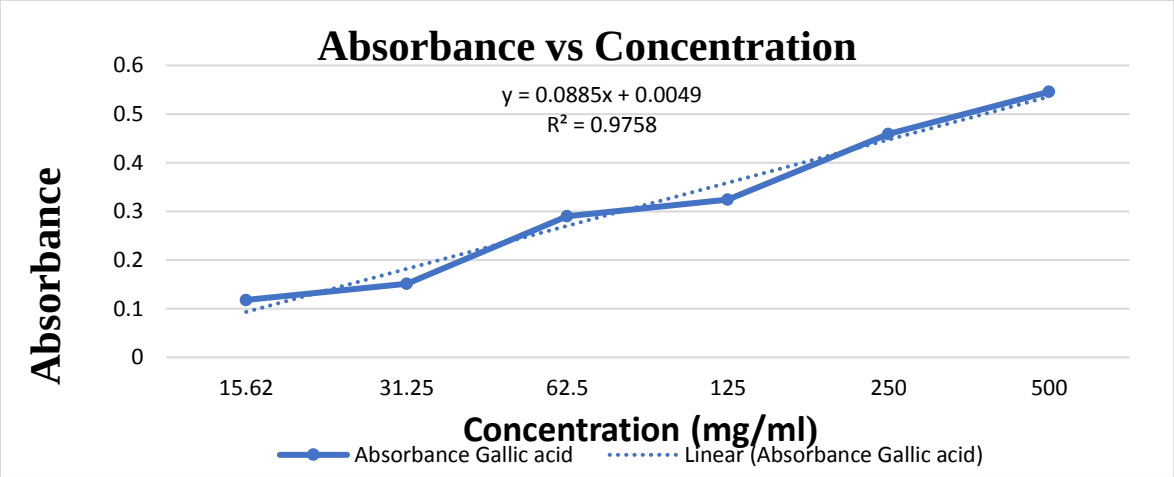


Figure 2. Graphical representation of absorbance of Gallic acid

Table 2. The total content of phenolic and flavonoids of the seed extract from *Cassia fistula*

Test Sample	Absorbance at 750 nm (Average ± SD)	Total phenolics content (mg GAE/g dw)	Absorbance at 510 nm (Average ± SD)	Total flavonoids content (mg QE/g dw)
Ethanol extract of *Cassia fistula* seed	0.223±0.08	246 ± 0.08	0.122±0.0011	118 ± 0.001

In vitro anti-inflammatory activity

Egg albumin was used to conduct *in vitro* anti-inflammatory activities. Using the seeds of *Cassia fistula*, the highest percentage of protein denaturation inhibition was observed as 54% for 45 µg/mL and 70% for the same dose of the standard medication diclofenac sodium. Figure 4 presents a summary of the outcomes.

Determination of anti-thrombocyte activity

The RBCs hemolysis was significantly inhibited at all doses of *Cassia fistula* seeds extract (50, 100, 200, 400, and 800 µg/ mL) (Table 3). At different doses, the lowest inhibition of hemolysis 58% was observed 50 µg/mL of *Cassia fistula* seeds extract and 81% for the same dose of standard aspirin. The results presented that the ethanol seeds extract of *Cassia fistula* has potential Anti-thrombocyte activity.

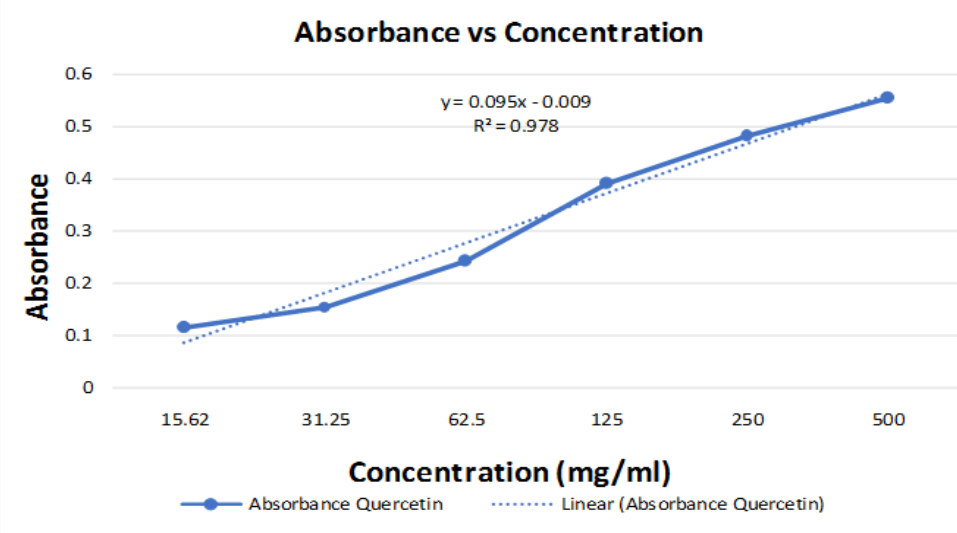


Figure 3. Graphical representation of absorbance of Quercetin

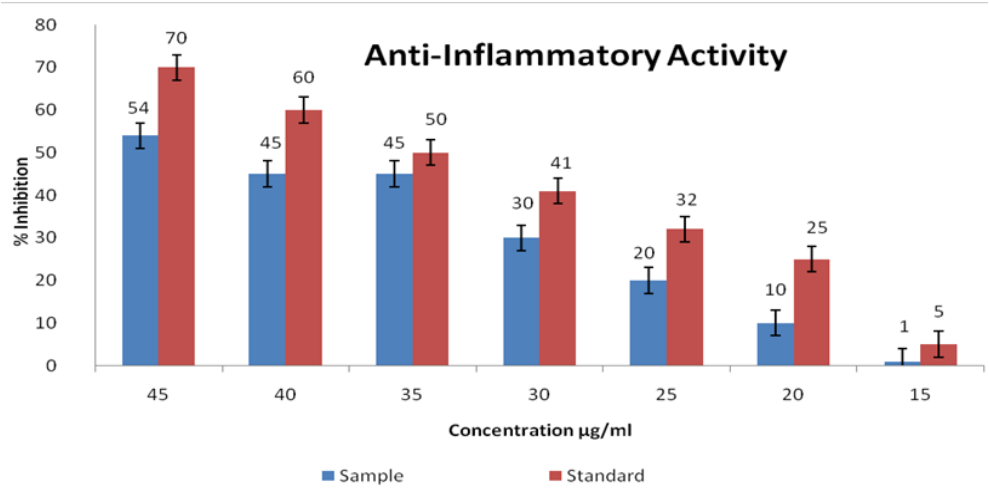


Figure 4. Inhibition of protein denaturation activity of *Cassia fistula* seeds extract. The data are shown as mean ± SEM ($p < 0.05$)

Table 3. Percentage of hemolysis inhibition at various concentrations

Percentage of hemolysis inhibition		
Concentration(µg/mL)	% Inhibition of *Cassia fistula* seeds extract	%Inhibition of Aspirin
50	58	81
100	68	87
200	77	90
400	84	94
800	88	96

The data are shown as the Mean ±SEM of three replicates (n=3). One-way ANOVA was used to evaluate the data. At P< 0.05, values were deemed significant.

Antioxidant activity of Cassia fistula seeds extract

The antioxidant activity of the seeds of *Cassia fistula* extracted in 95% ethanol was examined using the DPPH free radical scavenging test technique. The most mentioned method for evaluating the antioxidant activity of numerous plant-based medications is the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay.

This approach is based on the decrease of the colored free radical DPPH in ethanolic solution by various sample concentrations. IC_{50}, or 50% oxidative inhibitory concentration, was used to measure antioxidant activity. Ten different concentrations of extract (1, 1.95, 3.90, 7.81, 15.62, 31.25, 62.2, 125, 250, and 500 µg/mL) were prepared in ethanol solvent for this experiment. Ethanol without extract served as the control, and ascorbic acid served as the standard. Using a UV-Visible spectrophotometer, absorbance was determined at wavelength 517 nm. Each experiment was carried out three times. The percentage oxidative inhibition values of seed extract evaluated at various doses are shown in Figure 5 along with the results. IC_{50} was determined from % inhibition vs. log conc. Graph (Figure 6).

These results make it evident that the ethanol seed extract of *Cassia fistula* has an IC_{50} value of 8.90 µg/mL, which is equivalent to ascorbic acid's IC_{50} value of 6.73 µg/mL, as a well-known standard antioxidant. A similar result was also found for the methanolic extracts of *Ephedra sarcocarpa* which showed the high antioxidant activities and the IC50 value in the DPPH assay 4.6 mg/mL [23].

On the other hand, *Artemisia kulbadica* extracts was exhibited the moderate values DPPH radical scavenging activity IC50= (422.4 ± 2.4) µg/mL [24].

Among these results, thus the free radical scavenging activity was higher the lower the IC50 revealed. However, this was noticed that the extract has a lower antioxidant activity of *Cassia fistula* seeds extract than standard ascorbic acid.

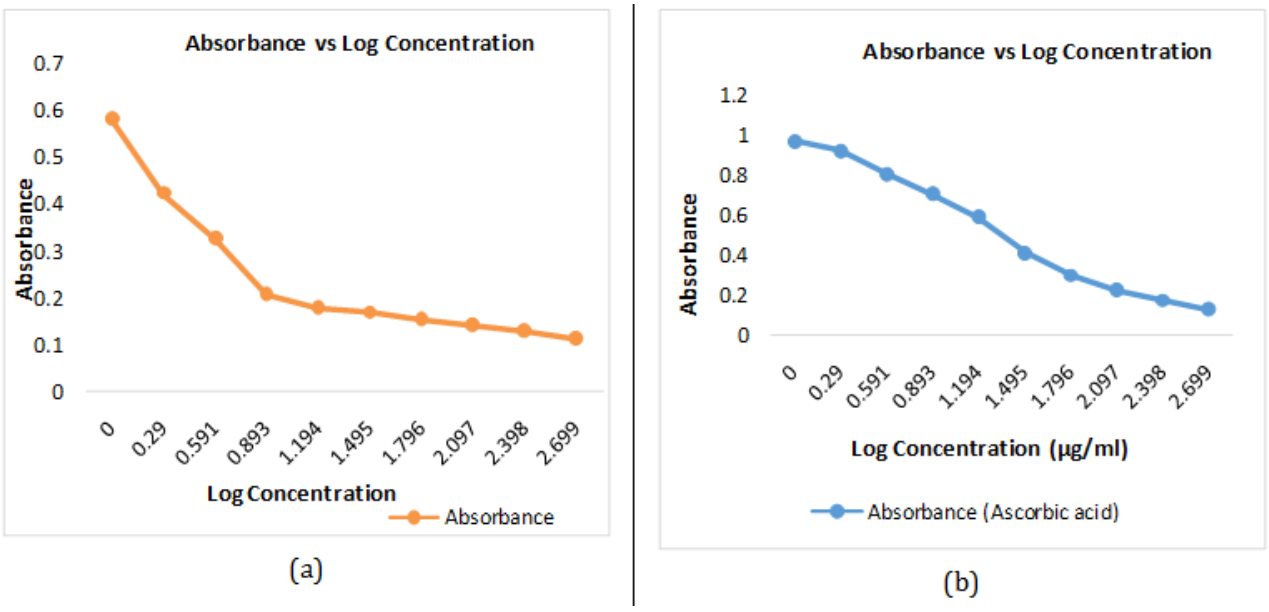


Figure 5. Absorbance vs. concentration graph for (a) ethanol extract of *Cassia fistula* and (b) standard (Ascorbic acid)

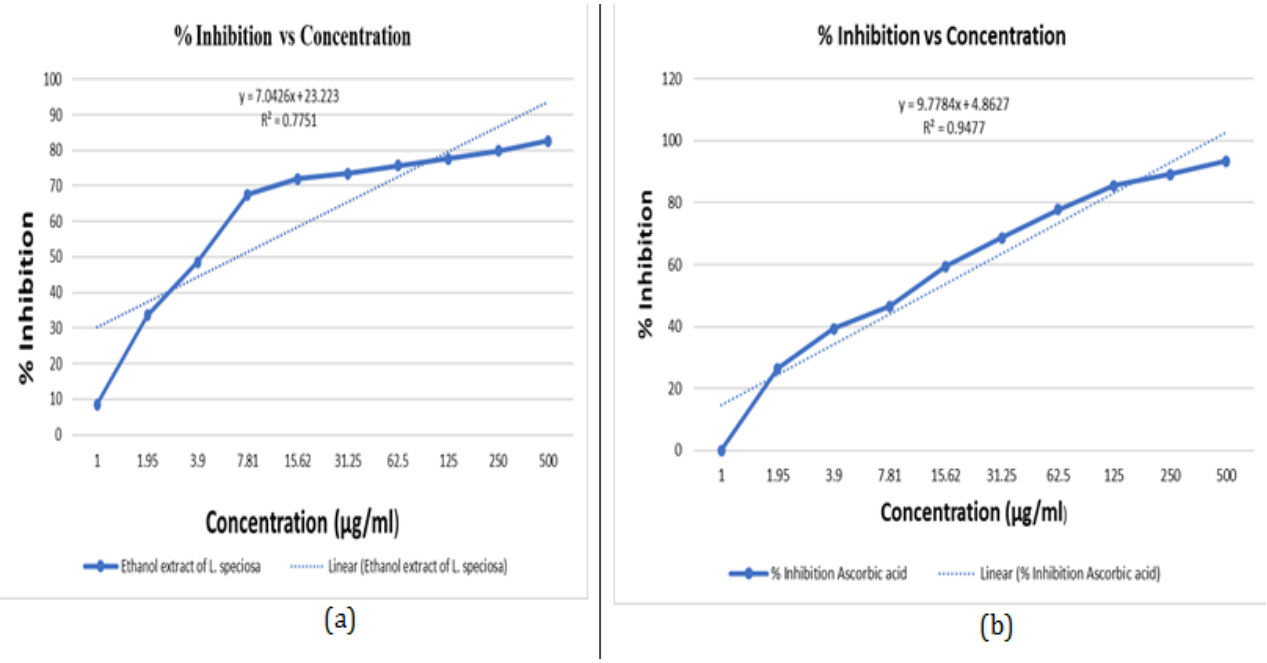


Figure 6. % inhibition vs. concentration graph for (a) ethanol extract of *Cassia fistula seed* and (b) standard (Ascorbic acid)

Conclusion

According to the research that was given, *C. fistula* seed extracts have a high concentration of phenolic and flavonoid components, which directly contributes to their significant antioxidant activity. These findings demonstrate the efficacy of traditional medicinal practices and point to the possibility for *C. fistula* seeds to be used as an antioxidant and anti-inflammatory agent. It is obvious that the seeds of C. *fistula* may contain some novel compounds that have the potential to be sources of novel anti-inflammatory, anti-thrombocyte, and antioxidant drugs based on the research into the effects of anti-inflammatory, anti-thrombocyte, and antioxidant behaviour of ethanol seed extract.

Acknowledgements

The authors would like to thank Khwaja Yunus Ali University, Sirajganj, Bangladesh. Also, they present their sincere gratitude to the Department of Materials Science and Engineering, University of Rajshahi, Rajshahi, Bangladesh. Furthermore, they would like to thank Md. Rubel Hossain (Department of Library Science, Khwaja Yunus Ali University, Sirajganj, Bangladesh) for checking similarity using their software.

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the article and agreed to be responsible for all the aspects of this work.

Orcid

Mst. Jesmin Sultana
https://orcid.org/0009-0009-9316-9609

References

[1]. Kumar V.P., Chauhan N.S., Padh H., Rajani M. *J. Ethnopharmacol*., 2006, **107**:182 [Crossref], [Google Scholar], [Publisher]
[2]. Dutta A., De B. *Indian. J. Pharmacol. Sci*., 1998, **60**:388 [Publisher]
[3]. Agarwal S.S., Paridhavi M. *Ahuja Publishing House*, 2005, 281 [Publisher]
[4]. Thirumal M., Surya S., Kishore G. *Crit. Rev. Pharmaceut. Sci*., 2012, **1**:43 [Publisher]
[5]. Sultana M.J., Ahmed F.R.S., Alam M.T. *Asian J. Green Chem*., 2019, **3**:518 [Crossref], [Google Scholar], [Publisher]
[6]. Gupta R.K. *CBS publishers and distributors, 1st edition*., 2010, 116
[7]. Luximon R.A., Bahroun T., Soobrattee M.A., Aruoma O.I. *J. Agric. Food. Chem*., 2002, **50**:5042 [Crossref], [Google Scholar], [Publisher]
[8]. Sharma D.K., Sharma S.K., Lonare M.K., Kaur Rajdeep, Dumka V.K. Pharmacological Evaluation of Antipyretic, Analgesic and Anti-inflammatory Activities of Ethanolic Extract of *Cassia fistula*. *Indian Journal of Animal Research*, 2022, **56**:483 [Crossref], [Google Scholar], [Publisher]
[9]. Irshad M., Singh M., Zafaryab M., Rizvi M.M.A. *Int. J. Med*., 2012 [Crossref], [Google Scholar], [Publisher]
[10]. Irshad M., Aijaz A., Zafaryab M., Ahmad F., Manzoor N., Singh M., Rizvi M.M.A. *Nat Prod Commun*., 2013, **8**:261 [Crossref], [Google Scholar], [Publisher]
[11]. Irshad M., Mehdi S.J., Al-Fatlawi A.A., Zafaryab M,. Ali A., Ahmad I., Singh M., Rizvi M.M.A. *J. Biol. Active. Prod. Nat*., 2014, **4**:158 [Crossref], [Google Scholar], [Publisher]
[12]. Trease G.E., Evans W.C. *13th edn. Bailliere Tindall, London*, 1989, 176 [Google Scholar], [Publisher]
[13]. Govind P., Madhuri S. *Indian. Drugs*., 2006, **43**:869 [Google Scholar], [Publisher]
[14]. Ahmed F.R.S., Sultana A., Sultana M.J. Saha A. *Bull. Natl. Res. Cent*., 2022, **46**:94 [Crossref], [Google Scholar], [Publisher]
[15]. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. *In Methods in Enzymology*, 1999, **299**:152 [Crossref], [Google Scholar], [Publisher]
[16]. Chang C., Yang M., Wen H., Chern J. *J. Food Drug Analaysis*, 2002, **10**:178 [Crossref], [Google Scholar], [Publisher]
[17]. Sahoo A.K., Dash U.C., Kanhar S., Mahapatra A.K. *Toxicol*, 2017, **4**:274 [Crossref], [Google Scholar], [Publisher]
[18]. Sultana M., Nibir A.I.S. Ahmed F.R.S. *Beni-Suef Univ. J. Basic. Appl. Sci*., 2023, **12**:26 [Crossref], [Google Scholar], [Publisher]
[19]. Dineshkumar G., Radha V., Sri Kumaran N. *Int. J. Green. Pharm*., 2018, **12**:S528 [Crossref], [Google Scholar], [Publisher]
[20]. Hossain R., Rahman M.A., Rafi M.K.J., Siddique T.A., Noman A.A., Makki A., Alelwani W., Hajjar D., Tangpong J. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 2020, **48**:1155 [Crossref], [Google Scholar], [Publisher]
[21]. Myint, Phyu P., May T.S., Htet H.H. *Journal of Pharmacognosy and Photochemistry,* 2017, **6**:528 [Crossref], [Publisher]
[22]. Rustaiyan A., Ezzatzadeh E. *Asian. J. Chem*., 2011, **23**:1774 [Google Scholar], [Publisher]
[23]. Rustaiyan A., Javidnia K., Farjam M.H., Aboee-Mehrizi F., Ezzatzadeh E. *Journal of Medicinal Plants Research*., 2011, **5**:4251 [Crossref], [Google Scholar], [Publisher]
[24]. Ezzatzade E., Farjam M.H., Rustaiyan A. *Asian. Pacific. J. Tropical. Disease*., 2012, **2**:S431 [Crossref], [Google Scholar], [Publisher]

How to cite this manuscript: Fazle Rabbi Shakil Ahmed, Mst. Jesmin Sultan, Afroza Sultana, Md. Ferdous Alom. Study of Anti-thrombocyte Activity of *Cassia fistula* Seeds Extract and It's Total Phenolic and Flavonoid Content, *In Vitro* Antioxidant and Anti-Inflammatory Activities. *Asian Journal of Green Chemistry*, 7(4) 2023, 258-268.
DOI: 10.22034/ajgc.2023.398285.1393