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A Phytochemical Screening and *In-vitro* Antioxidant & Anti-inflammatory Potential Evaluations of Methanolic Extract of *Cassia siamea* (Lam.).

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Abstract

The core aim of this research study is to evaluate the antioxidant and anti-inflammatory properties of traditional Bangladeshi medicinal extracts and to examine these activities in relation to their antioxidant content. *Cassia siamea* (Lam.) (Family Fabaceae) is a medicinalplant of Bangladesh and Indian sub continent, which iswidely used as folk medicine for the treatment of many diseases.The aim in the present study was to screen the phytochemical profile and pharmacological activities of methanolic extract of *Cassia siamea* (Lam.) leaves. Because each part of *Cassia siamea* (Lam.) has different constituents, the pharmacological effects of the plant vary according to the part of the plant evaluated. To investigate pharmacological activities DPPH scavenging assay and HRBC membrane stabilization methods were done for antioxidant and anti-inflammatory potential respectively. The phytochemical analysis of methanolic extract ofplant leaves showed that they contained significant presence of flavonoids, phenols, saponins, terpenoids & triterpenes. Alkaloids, glycosides & tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content.The pharmacological studies revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation. The IC_{50} values by DPPH scavenging assay observed for standard & leaves were 99.65µg/ml & 491.15µg/ml respectively. There is also moderate anti-inflammatory activity. The IC_{50} values for anti-inflammatory acticity by standard & leaves were 24.68 µg/ml & 841.04 µg/ml respectively.

Keywords: *Cassia siamea* (Lam.); Phenols; Tannin Content; Antioxidant; Anti-Inflammatory; IC_{50} Values.

Introduction

Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants [1]. Medicines derived from plants are widely famous due to their safety, easy availability and low cost [2]. Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours and fragrances, and medicines [3]. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BC [4]. Among the substances that were used are oils of *Cedrus* species (cedar) and *Cupressuss empervirens* (cypress), *Glycyrrhizaglabra* (licorice), *Commiphoraspecies* (myrrh) and *Papaver somniferum* (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. In ancient Egypt, bishop's weed (*Ammimajus*) was reported to be used to treat vitiligo, a skin condition characterized by a loss of pigmentation [5-6]. More recently, a drug (_-methoxypsoralen) has been produced from this plant to treat psoriasis and other skin disorders, as well as T-cell lymphoma [6]. Alternative medicine – or fringe medicine – includes practices claimed to have the healing effects of medicine but which are disproven, unproven, impossible to prove, or are excessively harmful in relation to their effect; and where the scientific consensus is that the therapy does not, or cannot, work because the known laws of nature are violated by its basic claims; or where it is considered so much worse than conventional treatment that it would be unethical to offer as treatment [7]. Alternative therapies or diagnoses are not part of medicine or science-based healthcare systems [8]. Alternative medicine consists of a wide variety of practices, products, and therapies – ranging from those that are biologically plausible but not well tested, to those with known harmful and toxic ef-

fects. Contrary to popular belief, significant expense is paid to test alternative medicine, including over $2.5 billion spent by the United States government [9]. *Cassia siamea* (Lam.) is a very widespread medicinal and food plant cultivated in Southeast Asia and sub-Saharan Africa. Many traditional claims are reported as medical treatments on various diseases like constipation, malaria and associated diseases such as fevers and jaundice (9). Aerial parts of *C. siamea* are useful in ringworm and related skin diseases because of presence of anthraquinone derivatives. Ethno-botanical surveys also suggest antinociceptive and antiviral activities of aerial parts of *C. siamea* antioxidant and antihypertensive activity. A laxative activity, sedative activity and anti-inflammatory of stem bark extract of *C. siamea* were also reported (10). Such activities can be due to the presence of barakol, which has anxiolytic and CNS inhibitory effects.

Materials and Methods

Plant Material

Leaves, bark, seeds and flowers of C. siamea were collected from the University campus of University OF Chittagong, Bangladesh.

Total Phenolic Content (TPC) Determination

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu's reagent is used in this ionized phenolic solution, the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu's reagent is yellow and after the oxidation process the solution becomes blue. The intensity of the color change is measured in a spectrophotometer at 760 nm. The absorbance value will reflect the total phenolic content of the compound. [11].

Method of sample preparation

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, following the method described with slight modifications [12]. The test sample (0.2 mL) was mixed with 0.6mL of water and 0.2mL of Folin-Ciocalteu's phenol reagent (1 : 1). After5min, 1mL of saturated sodium carbonate solution (8%w/v in water) was added to the mixture and the volume was made up to 3mL with distilled water. The reaction was kept in the dark for 30min and after centrifuging the absorbance of blue color from different samples was measured at 760 nm. All determinations were carried out in triplicate.

Total Tannin Content (TTC) Determination

Tannins are the complex organic, non nitrogenous derivatives of polyhydroxy benzoic acidswhich are widely distributed in the plant kingdom. They are present in aerial parts, e.g. leaves, bark, fruits and stem. They probably serve as a protective to the plant during growth and destroyed or deposited as end product of metabolism in some dead tissues of the mature plant. Tannins precipitate and combine with proteins. The protein-tannin complex is resistant to proteolytic enzymes. This property is known as astringent. During healing process of burns, the proteins of the exposed tissues are precipitated producing a mild antiseptic and protective layer under which the new tissues are regenerated. They are used as healing agents in inflammation, leucorrhoea, gonorrhea, burn, piles, and diarrhea and as antidote in the treatment of alkaloidal poisoning.[13]

Method of sample preparation

Fifty micro liters (µl) of tannins extract for each sample was taken in test tube and volume was made to 1.0 ml with distilled water. Then, 0.5 ml Folin Ciocalteu reagent was added and mixed properly. Then 2.5 ml 20 per cent sodium carbonate solution was added and mixed it and kept for 40 minutes at room temperature. Optical density was taken at 725 nm in spectrophotometer and concentration was estimated.[14]

Results and Discussion

Phytochemical screening

The following tests were done to find the presence of the active chemical constituents such as alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and triterpenes is shown in Table 3. Due to the different chemical compositions present in a Cassia siamea (Lam.). are obviously responsible for its different therapeutic and pharmacological activities. In this study,

Table 1. Total phenolic content (TPC) of *Cassia siamea* (Lam.).

Test sample	Absorbance	TPC (mg of GAE/g)	Average	TPC (mg of GAE/g) ± SEM
	0.302	**27.30**		
Leaves	**0.311**	**26.46**	**26.72**	**26.72 ± 0.37**
	0.309	**26.42**		

Total phenolic content (TPC) observed for leaves of Cassia siamea (Lam.). was 26.72 ± 0.37mg of GAE/g.

Table 2. Total tannin content (TTC).

Test sample	Absorbance	TTC (mg of TAE/g)	Average	TTC (mg of TAE/g) ± SEM
	0.298	1.769		
Leaves	0.296	1.801	1.785	1.785 ± 0.015
	0.295	1.785		

Total tannin content (TTC) observed for leaf of Cassia siamea (Lam.)was 1.785 ± 0.015mg of TAE/g.

Table 3. Different chemical compositions resent in plants.

Secondary metabolites	Name of the test	Results
Alkaloids	Wagner test	+++
Flavonoids	Specific test	++
Glycosides	General test	+++
Phenols	Litmus test	++
Saponins	Froth test	+++
Tannins	Ferric chloride test	+++
Terpenoids	General test	++
Triterpenes	Salkowski's test	++

Table 4. Average absorbance of control.

	Absorbance	Average
	0.365	
Control	0.359	0.362
	0.364	

Table 5. Spectroscopic Determination of Anti-inflammatory Activity of Leaves.

Concentration (µg/ml)	Absorbance	% Inhibition	Average	% Inhibition ± SEM	IC_{50} (µg/ml)
125	0.437	1.41	1.39	1.39 ± 0.6	
	0.435	0.79			
	0.433	1.98			
250	0.398	10.11	10.97	10.97 ± 0.2	841.04
	0.397	11.03			
	0.401	11.79			
500	0.350	47.03	47.45	47.45 ± 0.7	
	0.348	48.87			
	0.345	46.45			
1000	0.310	53.87	54.65	54.65 ± 0.5	
	0.316	54.67			
	0.313	55.43			

Table 6. Spectroscopic Determination of Anti-inflammatory Activity of Standard Compound (Diclofenac- Na).

Concentration (µg/ml)	Absorbance	% Inhibition	Average	% Inhibition ± SEM	IC50 (µg/ml)
125	0.342	46.99	47.36	81.65 ± 0.5	
	0.340	47.08			
	0.339	48.01			
250	0.238	65.65	66.43	87.54± 0.4	24.68
	0.239	66.34			
	0.236	67.32			
500	0.124	79.86	79.92	92.67 ± 0.5	
	0.119	80.23			
	0.120	79.08			
1000	0.071	89.76	90.27	96.49 ± 0.3	
	0.075	90.06			
	0.072	91.01			

the different constituents of the Cassia siamea (Lam.)which are found should have some relationship with domestic medicinal applications. It should be mentioned here that the presence of these kinds of chemical constituents, it is expected that the selective plant Cassia siamea (Lam.)should have Anti-inflammatory Activity and Anti oxidant activity.

Qualitative evaluations showed significant presence of flavonoids, phenols, saponins, terpenoids, & triterpenes.Alkaloids, glycosides & tannins are also moderately present in the methanolic extract of leaves of Cassia siamea (Lam.)

Anti-inflammatory Activity

Percent inhibition of protein denaturation was calculated as follows[15]:

% inhibition = (Control - Sample)/(Control)) X 100

The method of HRBC membrane stabilization was chosen to evaluate anti-inflammatory effect. It is already proved that membrane stabilization of RBC is as effective as healing inflammation in provoking delayed hypersensitivity. It revealed that the plant extracts may have moderate anti-inflammatory effect which is probably mediated by HRBC membrane stabilization. The secondary metabolites such as phenolic compounds and tannins which were found in preliminary phytochemical screening might be responsible for such type of activity.

By analyzing the above data, it revealed that the plant extracts may have moderate anti-inflammatory effect which is probably mediated by HRBC membrane stabilization.

Antioxidant activity

The free radical-scavenging activity of extracts was evaluated with the DPPH assay based on the measurement of the reducing ability of antioxidants toward the DPPH radical[16].By analyzing the above data, it revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation.

By analyzing the above data, it revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation.

Table 7. Comparative study based on IC_{50}.

Test Sample	IC_{50}
Leaves	841.04
Standard	24.68

Table 8. Average absorbance of control.

	Absorbance	Average
	0.913	
Control	0.915	0.918
	0.927	

Table 9. Spectroscopic Determination of Antioxidant Activity of Leaves.

Concentration (µg/ml)	Absorbance	% SCV	Average	% SCV ± SEM	IC50 (µg/ml)
62.5	0.961	11.65	11.89	8.76 ± 0.6	
	0.954	11.98			
	0.950	12.05			
125	0.754	26.89	26.86	23.01 ± 0.3	
	0.749	26.67			
	0.751	27.03			
250	0.514	53.95	53.96	53.97 ± 0.6	491.15
	0.509	54.85			
	0.511	53.07			
500	0.312	69.59	69.05	69.05 ± 0.5	
	0.309	68.18			
	0.311	69.39			
1000	0.114	89.98	90.50	90.5 ± 05	
	0.098	90.87			
	0.099	90.65			
2000	0.087	98.56	98.57	98.57 ± 0.60	
	0.088	97.98			
	0.086	99.18			

Table 10. Spectroscopic Determination of Antioxidant Activity of Standard Compound (L- Ascorbic Acid).

Concentration (µg/ml)	Absorbance	% SCV	Average	% SCV ± SEM	IC50 (µg/ml)
62.5	0.349	61.79	61.92	61.92 ± 0.35	
	0.349	61.60			
	0.338	62.54			
125	0.260	71.35	70.78	70.78 ± 0.36	
	0.268	70.09			
	0.267	70.88			
250	0.196	78.25	78.87	78.87 ± 0.50	99.65
	0.188	79.85			
	0.195	78.50			
500	0.118	86.79	87.26	87.26 ± 0.30	
	0.119	87.36			
	0.119	87.68			
1000	0.047	94.79	94.52	94.52 ± 0.2	
	0.056	94.27			
	0.045	94.56			
2000	0.029	97.68	96.94	96.97 ± 0.6	
	0.030	97.27			
	0.033	95.88			

Table 11. Comparative study based on IC_{50}.

Test Sample	IC50
Leaves	491.15
Standard	99.65

Conclusion

From my this research work it was found that qualitative evaluations show significant presence of flavonoids , phenols , saponins , terpenoids& triterpenes. Alkaloids, glycosides & tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content.There is an excellent antioxidant activity in the methanolic extract. There is also moderate anti-inflammatory activity in the methanolic extract ofleaves. Each part of *Cassia siamea* (Lam.) has different constituents, the pharmacological effects of the plant vary according to the part of the plant evaluated. Alkaloids, glycosides and tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content. The IC_{50} values by DPPH scavenging assay observed for standard and leaves were 99.65 µg/ml and 491.15µg/ml respectively. So, there is an excellent antioxidant activity in the methanolic extract. There is also moderate anti-inflammatory activity in the methanolic extract of leaves. The IC_{50} values for anti-inflammatory activity by standard and plant leaves were 24.68 µg/ml and 841.04 µg/ml respectively.

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