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Evaluation of antioxidant and antinociceptive activities of leaves of *Cassia alata* l.

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Abstract

The ethanolic extract of leaves of *Cassia alata* L. (Family- Fabaceae)) was evaluated for antioxidant and antinociceptive activity. In the qualitative study, by spraying 0.02% DPPH solution of ethanol on TLC plate which was developed in medium polar, polar and nonpolar medium, the extract showed yellow spot that indicates the presence of anti-oxidant components in the leaves of *Cassia alata* L. In the quantitative study, by DPPH scavenging activity test, IC_{50} value of leaves of *Cassia alata* L. was found to be 69.18µg/ml and IC_{50} value of standard was 12.02µg/ml and their comparison [% inhibition vs. log conc.] showed that % inhibition value of leaves of *Cassia alata* L. is comparable to standard. The antinociceptive activity was investigated on animal model like Swiss albino mice. Ethanolic extracts of leaves of *Cassia alata* L. at the oral dose of 250 mg/kg($p < 0.02$) and 500 mg/kg($p < 0.01$) exhibited significant inhibition of writhing reflex (57.69% and 63.46% respectively) while for the standard drug diclofenac inhibition of writhing reflex was found to be 86.54%($P<0.001$) at a dose of 25 mg/kg body weight.

KEY WORDS: CASSIA ALATA L., ANTIOXIDANT ACTIVITY, ANTINOCICEPTIVE ACTIVITY, ACETIC ACID, DICLOFENAC SODIUM

Introduction

Cassia alata L. is an important medicinal tree as well as ornamental flowering plants in the subfamily Caesalpinioideae. *Cassia alata* L. is native to Mexico, and can be found in diverse habitats. In the tropics it grows up to an altitude of 1,200 meters. It is an invasive species in Austronesia. In Sri Lanka this is use an ingredient of Sinhala traditional medicine.

The shrub stands 3–4 m tall, with leaves 50–80 cm long. The inflorescence looks like a yellow candle. The fruit shaped like a straight pod is up to 25 cm long. Its seed are distributed by water or animals. The leaves close in the dark.

Cassia alata L.is often called the Ringworm Bush because of its very effective fungicidal properties, for treating ringworm and other fungal infections of the skin. The leaves are ground in a mortar to obtain a kind of "green cotton wool". This is mixed with the same amount of vegetable oil then rubbed on the affected area 2-3 times a day. A fresh preparation is made every day[1-2]. Its active ingredients include the yellow chrysophanic acid. Its laxative effect, due to its anthraquinone content, is also well proven.

Materials and Methods

Plant Material

The leaves of *Cassia alata* L. were collected from the Bayarbhanga , Batiaghata , Khulna. The time of collection was June, 2010 at the daytime. The fresh leaves were collected from the healthy host plants. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession number-35555). The collected plants were separated from undesirable materials or plants or plant parts and these were dried by shade drying for fifteen-twenty days to ensure the active constituents free from decomposition also to avoid any photochemical degradation.

The leaves were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

The leaves were extracted by cold extraction method. 200 gm grinded leaves powder was soaked in 500 ml of 98% of ethanol in a glass container for fourteen days accompanying regular shaking and stirring. The extract was separated from the plant debris by filtration by a piece of clean, white cotton material & it was done two times.

The filtrate (ethanol extract) obtained was taken into rotary evaporator to evaporate ethanol. Then this filtrate was taken into beaker, the opening of beaker was wrapped by a sheet of aluminum foil to which perforation was done for evaporation of the rest of the ethanol & was kept in dry & cool place for several days & at last evaporation was done under table fan until dried. It rendered concentrate of deep ash type. The concentrate was designated as crude extract of ethanol.

Animals

Young Swiss-albino mice of either sex, average weight 20-30 gm were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B). The mice were kept separately in plastic cages having dimension of (28 x 22 x 13) cm. Soft wood shavings were placed in the cages for housing of the mice.

The room where the mice were housed was well ventilated for air and light. Husk and excreta were removed from the cages on every day. Fresh water and pellets of mice foods, prepared by ICDDR,B were given to the mice regularly. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol.

Drugs

Diclofenac sodium (Beximco Pharma Ltd, Bangladesh).

Pharmacological Studies

Antioxidant activity

Antioxidant is the type of molecule that neutralizes harmful compounds called free radicals that damage living cells, spoil food, and degrade materials such as rubber, gasoline, and lubricating oils. Antioxidants can take the form of enzymes in the body, vitamin supplements, or industrial additives. They are routinely added to metals, oils, foodstuffs, and other materials to prevent free radical damage. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods because of their potential in health promotion and disease prevention, and their high safety and consumer acceptability [3].

During investigation both qualitative and quantitative analysis was performed.

Qualitative assay

Test samples were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract of leaves of *Cassia alata* L.. The plates were dried at room temperature and were sprayed with 0.02% 1, 1-diphenyl-2-picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted[4]. DPPH forms deep pink color when it is dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants. Ascorbic acid was used as the positive control.

Quantitative assay

The method used was adopted with suitable modifications to our particular circumstance[5]. At first 9 test tubes were taken to make aliquots of 9 conc. (1.57, 3.13, 6.25, 12. 5, 25, 50, 100, 200 and 400) ìg/ml for plant extract and 9 test tubes were taken to make aliquots of 9 conc.(1.57, 3.13, 6.25,12.5, 25, 50,100,200,400) ìg/ml for ascorbic acid. Plant extract and ascorbic acid were weighed 3 times and dissolved in ethanol to make the required concentrations by dilution technique. Here ascorbic acid was taken as positive control. DPPH was weighed and dissolved in ethanol to make 0.004% (w/v) solution. To dissolve homogeneously magnetic stirrer was used. After making the desired concentrations, 6 ml of 0.004% DPPH solution was applied on each test tube by pipette and then 2 ml of different concentrations was mixed in each test tube. Test tubes were kept for 30 minutes in dark to complete the reactions. DPPH was also applied on the blank test tubes at the same time where only 2ml ethanol was taken as blank. After 30 minutes, absorbance of each test tube was determined by UV spectrophotometer at 517 nm. % of inhibition was calculated and IC_{50} was determined from % inhibition vs. log conc. graph.

The formula used for % inhibition ratio is:

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% inhibition=(Blank OD-Sample OD/Blank OD) X 100
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Antinociceptive activity

The antinociceptive activity of the crude extract was tested using the model of acetic acid induced writhing in mice[6-7]. Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group-IV consisting of 4 mice in each group. Each group received a particular treatment i.e. control, positive control and one dose of the extract. Group-I was served as the control and received only distilled water and tween-80. Group-II was received diclofenac sodium (25 mg/kg of body weight, IP), the standard drug for comparison of potencies. Group-III was administered orally with the crude extract suspensions at the doses of 250 mg/kg-body weight. The last group i.e. group-IV was administered orally at the dose 500 mg/kg-body weight Thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%, 10 ml/kg) was administered intraperitoneally

to each of the animals of a group[8]. After an interval of five minutes, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 15minutes.

Results

Antioxidant activity

In the TLC-based qualitative antioxidant assay using DPPH assay, leaves of *Cassia alata* L. showed the free radical scavenging properties indicated by the presence of strong yellow spot on a purple background on the TLC plate.

In DPPH based quantitative assay, Absorbance at 517 nm was determined by taking the crude extract of different concentration of leaves of *Cassia alata* L.

see Table 1.

see Table 2.

see Table 3.

see Figure 1.

see Figure 2.

Antinociceptive activity

The ethanolic extract of leaves *of Cassia alata* L. leaves exhibited significant antinociceptive effect in acetic acid induced writhing of white albino mice (Swiss-webstar strain). The extract produced 57.69% and 63.46% writhing inhibition at the doses of 250 and 500 mg/kg-body weight respectively.

see Table 4.

see Table 5.

The results of statistical analysis are as follow:

see Figure 3.

see Figure 4.

Discussion

Plants are employed as important source of medication in many traditional medications[9-11]. Since *Cassia alata* L. is a medicinal plant, widely grown in domestic and public gardens and has a beautiful inflorescence in yellow, red and orange, part of the plant constituents may be polar in nature. Ethanol was used which has a wide range of solubility in both polar and nonpolar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness[12].

Phytochemical screening showed the presence of flavonoid, tanin, alkaloid in the plant extract during investigation. Multiple biological effects, including antioxidant activity commonly found in plants containing polyphenolic compounds, like flavonoids, tannins and phenolic acids [13]. Tannic acid present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action. It was shown that the percentage (%) scavenging of DPPH radical was increased significantly with increasing dose, $P< 0.001$. IC50 value of the extract was found to be very fairly significant 69.18µg/ml) when compared to the IC50 value of the reference compounds ascorbic acid 12.02µg/ml respectively.

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function[14]. Antinociceptive activity of the ethanolic extract of leaves of *Cassia alata* L. was tested by acetic acid induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [15]. Increased levels of PGE2 and PGF2á in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid[16]. The extract of *Cassia alata* L. produced significant writhing inhibition comparable to the standard drug diclofenac sodium . The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result, it can

be concluded that the ethanolic extract of leaves of *Cassia alata* L. might possess antinociceptive activity.

Conclusion

Finally, it could be suggested that the ethanolic extract of *Cassia alata* L. of leaves possesses antioxidant and antinociceptive activities. These facts indicate the scientific basis of *Cassia alata* L. being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

Acknowledgments

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Blank Solution	1st reading	2nd reading	Average
	0.945	0.944	0.945

Table 1: UV reading for blank solution

Conc. (extract) (µg / ml)	Absorbance 1	Absorbance 2	Average	% inhibition	log Conc.
1.57	0.798	0.786	0.792	16.19	0.195
3.13	0.798	0.786	0.790	16.40	0.495
6.25	0.767	0.770	0.769	18.62	0.795
12.5	0.703	0.705	0.704	25.50	1.096
25	0.615	0.685	0.650	31.22	1.397
50	0.600	0.595	0.598	36.72	1.698
100	0.346	0.344	0.345	63.49	2.00
200	0.171	0.172	0.172	81.80	2.301
400	0.105	0.105	0.105	88.88	2.602

Table 2: DPPH Scavenging Assay of leaves *of* leaves *Cassia alata* L.

Conc. (ascorbic acid) µg / ml	Absorbance 1	Absorbance 2	Average	% inhibition	log conc.
1.57	0.740	0.745	0.743	21.38	0.195
3.13	0.741	0.743	0.742	21.48	0.495
6.25	0.585	0.588	0.587	37.88	0.795
12.5	0.453	0.455	0.454	51.69	1.096
25	0.038	0.040	0.039	95.87	1.397
50	0.030	0.032	0.031	96.72	1.698
100	0.028	0.028	0.028	97.03	2.00
200	0.029	0.027	0.028	97.03	2.301
400	0.027	0.028	0.028	97.03	2.602

Table 3: DPPH Scavenging Assay of Ascorbic acid

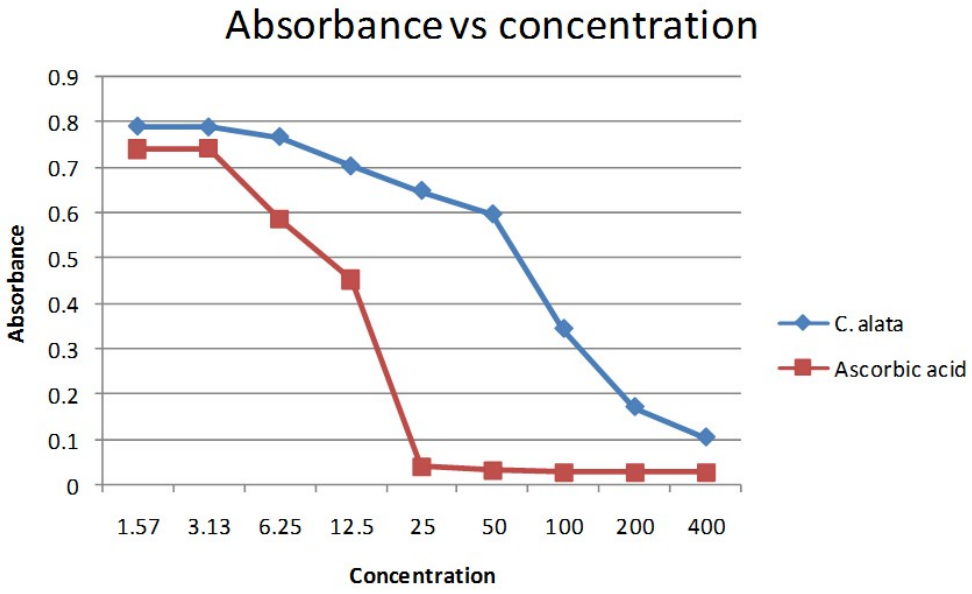

Figure 1: DPPH Scavenging Assay of *Cassia alata* L. leaves(Absorbance vs Conc.)

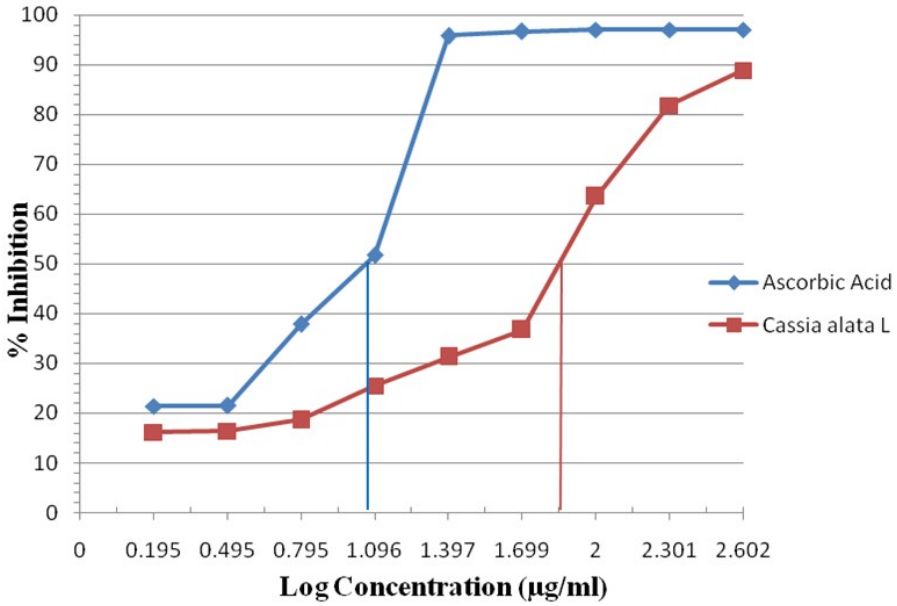

Figure 2: DPPH Scavenging Assay of *Cassia alata* L. leaves (% inhibition vs log Conc.)

Administered (Dose)	Group	Numbering of mice	Weight(gm)	Dose (ml)	Total writhing	Average Writhing
Control	I	1	26	.26	17	13
		2	32	.32	10	
		3	27	.27	11	
		4	30	.30	14	
Diclofenac (25 mg/kg)	II	1	40	.40	0	1.75
		2	28	.28	5	
		3	34	.34	1	
		4	35	.35	1	
Extract (250 mg/kg)	III	1	31	.31	2	5.5
		2	31	.31	9	
		3	27	.27	7	
		4	30	.30	4	
Extract (500 mg/kg)	IV	1	35	.35	2	4.75
		2	31	.31	8	
		3	28	.28	6	
		4	32	.26	3	

Table 4: Effects of the crude extract of *Cassia alata* L. at the doses of 250 and 500 mg/kg-body weight on acetic acid induced writhing of mice.

Animal group	Writhing count				Mean	% Writhing	SD	SE	% Protection	T-test (value of p)
Control	17	10	11	14	13	100	2.74	1.58	0	
Diclofenac (25mg/kg)	0	5	1	1	1.75	13.46	1.92	1.11	86.54	**5.83 (p<.001)**
Extract (250 mg/kg)	2	9	7	4	5.5	42.31	2.69	1.55	57.69	**3.39 (p<.02)**
Extract (500 mg/kg)	2	8	6	3	4.75	36.54	2.38	1.37	63.46	**3.95 (p<.01)**

Table 5: Statistical evaluation of the results shown in table.

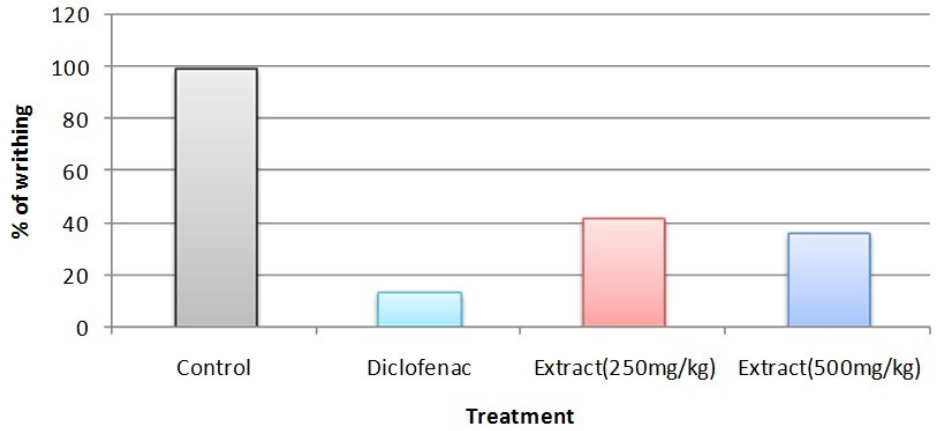

Figure 3: Effect of crude extract of *Cassia alata* L. on acetic acid induced writhing of mice.

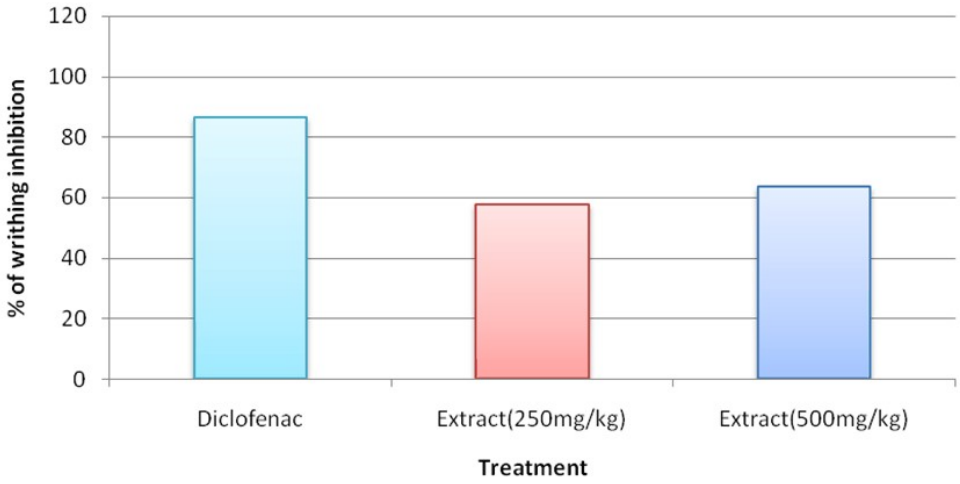

Figure 4: Percent writhing inhibition of crude extract of *Cassia alata* L. on acetic acid induced writhing of mice.