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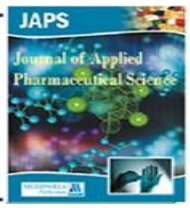

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Assessment of antibacterial, thrombolytic and cytotoxic potential of *Cassia alata* seed oil

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ABSTRACT

Cassia alata is a pan tropical shrub. It has been reported to have medicinal activities like laxative effect and active against ringworm, skin diseases and ulcer. The leaf extract of this plant has shown antibacterial activity. In this study, seed oil extracted from *Cassia alata* , was studied for Antibacterial, Thrombolytic and Cytotoxic activity by using *in vitro* techniques. Chloroform extract of the seed oil was tested for Antibacterial activity using disc diffusion method and that extract showed potent antibacterial activity against both Gram(+) and Gram(-) bacteria. DMSO extract of the seed oil was tested for Brine shrimp lethality bioassay using Brine shrimp nauplii. The LC_{50} of DMSO extract of *Cassia alata* seed oil was found to be 250µg/ml, indicates that the oil has moderate pharmacological action. The DMSO extract of the seed oil was also tested for *in vitro* thrombolytic activity. The extract showed reasonable thrombolytic activity against negative control (water).Further investigation on the plant is required to confirm their pharmacological activity and thereby utilizing them as useful medicinal plant.

Keywords: *Cassia alata,* Cytotoxicity, Oil, Thrombolytic, Streptokinase, Antibacterial.

INTRODUCTION

Medicinal plants play a dominant role in the treatment of varieties of human diseases from the twilight of the human civilization (Nostro et al., 2000). Obsession on modern medicinal system leads people to an alternative approach to improve and maintain good health is increased tremendously by using medicinal herb over last centuries. Many of the modern days important drugs and processed medicines are of plant origin (Thomas et al., 2008). Medicinal plants contain different therapeutic agents which may have thrombolytic activity, antimicrobial activity, cytotoxic effect etc. Working with different medicinal plants extract showed that they can lyses thrombus as streptokinase (Sweta et al., 2006). The plant sap can act against microorganisms by preventing the growth of microbial colony (Hammer et al., 1999). Some of the plant extract also increase lethality of the cell due to their known cytotoxic effect. Brine shrimp lethality bioassay is performed for evaluating the level of toxicity (Pintusorn et al., 2009). The plant is differently named in different countries .*Cassia alata* locally known as dadmardan in Bangladesh and India. Chinese: chi jia jue ming, Spanish: bajagua, mocoté. It is also known as Candelbra Bush, Empress Candle plant ,Ringworm tree or Candle tree in English. It is a pan tropical shrub, native to tropical Americas. It is widely distributed from tropical America to India and Bangladesh (Kirtikar *et al.*, 1975), Fiji (Smith *et al.*,1979), Indonesia and Malaysia (Corner, 1952). The sap or the extract of the plant has been reported to posses some medicinal value, for example, the leaves have been

reported to posses some medicinal value, for example, the leaves have been reported to have a laxative effect and are also used against ringworm, scabies, ulcers and other skin diseases (Seaforth, C.E.,1962).Used to treat ringworm, herpes, liver diseases and gastrointestinal problems as a laxative (Perry et al.,1980). Decoction of flowers , bark and wood are also reported to treat skin diseases such as pruritis, eczema, itching, bronchitis and asthma (Kirtikar, K.R. Basu, B.D.,1975). Leaf extracts has been reported to have antimicrobial activity (Ibrahim, D. Osman, H., 1995). There are reports on use in Leprosy (Bokemo *et al.,* 1984)**.** In subcontinent *Cassia alata* leaf extract was used to treat ring worm infection and in order to get a maximum result, a little amount of lime was sometimes mixed with the extract.

The present study was done to observe the antibacterial, cytotoxic and thrombolytic activity of *Cassia alata.*

MATERIALS AND METHODS

Cytotoxic Activity Test

Brine shrimp lethality bioassay was used for probable cytotoxic action (Meyer *et al.,* 1982) (Persoone, G., 1988). The eggs of Brine shrimp (Artemia salina Leach) were collected and hatched in a tank at a temperature around 37°C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solutions of the samples were prepared by dissolving required amount of extracts in specific volume of pure dimethyl sulfoxide (DMSO). Four ml of seawater was given to each of the vials. Then specific volumes of sample were transferred from the stock solution to the vials to get final sample concentrations of 1, 5, 10, 20, 50, 100, 200 and 500 µg/ml. In the control vials same volumes of DMSO(as in the sample vials) were taken. With the help of a Pasteur pipette 10 living nauplii were put to each of the vials. After 24h the vials were observed and the number of nauplii survived in each vial was counted. From this, the percentage of lethality of Brine Shrimp nauplii was calculated for each concentration of the extract.

Antibacterial assay

The antimicrobial assay was performed by using the disc diffusion method (Bauer et al., 1966*;* Barry et al., 1980). Eight pathogenic bacteria were used as test organisms for antibacterial activity of dried sample extracts. The tested bacterial strains were collected from Molecular Genetics Laboratory, Department of Genetic Engineering & Biotechnology, University of Chittagong.

Herbal preparation for thrombolytic activity

100 mg extract was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. 100 µl of this aqueous preparation of herbs was added to the alpine tube containing the clots to check thrombolytic activity (Sweta et al., 2006).

Clot lysis

Experiments for clot lysis were carried as reported earlier (Sweta et al., 2006). Venous blood was drawn from healthy volunteers (n = 10) and transferred in different pre-weighed sterile alpine tube (500 µl/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone). Each alpine tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. As a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The test was repeated ten times.

Statistical analysis

The significance of % clot lysis by herbal extracts by means of weight difference was tested by the paired t-test analysis. Data are expressed as mean □+standard deviation.

RESULT

Assay for antibacterial activity of *Cassia alata* seed oil extract

Antibacterial activity of *Cassia alata* seed oil extract was studied on three Gram positive and Five Gram negative bacteria by disc diffusion method. Antibacterial activity of *Cassia alata* seed oil extract was measured at 0.1 ml/disc concentration and found activity against Gram (+) bacteria and Gram (-) bacteria at 0.1 ml/disc. More specifically, *Cassia alata* seed oil extract showed-13mm, 9mm and 8mm zone of inhibition against three tested Gram (+) bacteria like *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus* respectively, and 12mm,10mm,9mm,11mm and 9mm zone of inhibition was observed against tested Gram (-) bacteria like *Vibrio cholerae, E. coli , Salmonella typhi, Pseudomonas aeroginosa, Shigella sonnei.*

Table 1: *In-vitro* antibacterial activity of *Cassia alata* seed oil extract.

Bacterial type	Test organism	Diameter of zone of inhibition (mm)	
		Cassia alata seed oil extract (0.1 ml/disc)	Kanamycin (30µg/disc)
Gram (+)	*Bacillus cereus*	9	28
	Bacillus subtilis	13	27
	Staphylococcus aureus	8	38
Gram (-)	*E. coli*	10	25
	Salmonella typhi	9	27
	Vibrio cholerae	12	22
	Pseudomonas aeroginosa	11	35
	Shigella sonnei	9	30

Assay for Cytotoxicity of *Cassia alata* seed oil Extract

The DMSO extracts of *Cassia alata* seed oil was tested for Brine shrimp lethality bioassay using brine shrimp nauplii(Mc laughin *et al.,*1988). The DMSO-oil extract show positive result on brine shrimp lethality bioassay. So they are pharmacologically

active. The LC50 value for the extract was obtained from the table 3.2.Control was used to see whether DMSO has any effect on brine shrimp lethality (Mc laughin *et al.*,1988). The control group of brine shrimp nauplii with and without DMSO exhibited no mortality. For the extract, the number of nauplii died and percent of mortality were counted. The result is shown in the following table 2.

Table 2: Result of Brine shrimp Lethality Bio-assay of *Cassia alata.*

Volume of sample (ml)	Concentration of The sample, C(µg/ml)	Log Concentration (log c)	% of mortality	LC50(µg/ml)
2.00	1000	3.00	100	
1.50	750	2.87	90	**250**
1.00	500	2.70	70	**(2.39)**
0.50	250	2.39	50	
0.20	100	2.00	30	
0.050	25	1.39	10	
0.025	12.5	1.09	10	
Control	0	0	0	

Table 3: Thrombolytic activity of different agent.

Agent	% of clot lysis
Dis.water	3.2%
Streptokinase	81.20%
C. alata seed oil extract	37.92%

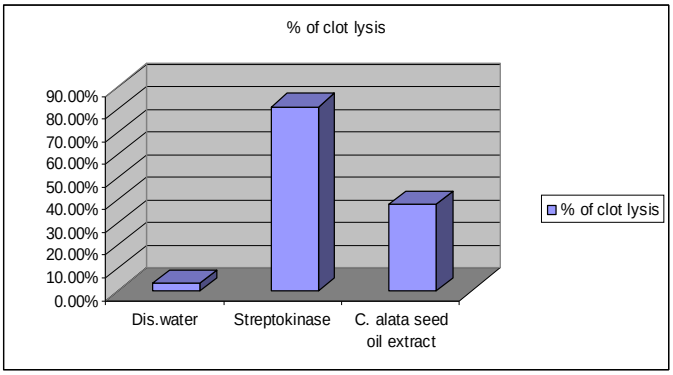

Fig.1: Clotlysis by Streptokinase, Distilled water and herbal preparation.

DISCUSSION

Antibacterial activity of C*assia alata* seed oil extract

Antibacterial activity of *Cassia alata* seed oil extract (0.1ml/disc) was studied on three Gram positive and five Gram negative bacteria by disc diffusion method and compared with the standard antibiotic disc kanamycin (30 µg/disc).

Antibacterial activity of *Cassia alata* seed oil extract was measured at 0.1ml/disc concentration and found activity against Gram (+) bacteria and Gram (-) bacteria at 0.1 ml/disc. More specifically, *Cassia alata* seed oil extract showed- 15mm, 9mm and 8mm zone of inhibition against three tested Gram (+) bacteria like *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus* respectively, and 17mm,10mm,9mm,11mm and 9mm zone of inhibition was observed against tested Gram (-) bacteria like *Vibrio cholerae, E. coli , Salmonella typhi, Pseudomonas aeroginosa, Shigella sonnei*. On the other hand, standard antibiotic kanamycin (30µg/disc) showed significant antibacterial activity against all tested Gram (+) and Gram (-) bacteria (Table 1). This results indicate that *Cassia alata* seed oil extract have potent antibacterial activity against both Gram(+) and Gram(-) bacteria.

Cytotoxic activity of *Cassia alata* seed oil extract:

Brine shrimp lethality bioassay was used to assess the cytotoxicity of *Cassia alata* seed oil extract. Brine shrimp lethality is a general bioassay, which is an indication of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions (MacLaughin et al., 1991).

In cytotoxicity, the LC50 value of the extract was found significant (250 µg/ml) which indicates that the DMSO extract of *Cassia alata* seed oil has high pharmacological actions (Gupta et al., 1996). It also indicates that the plant might have the potentiality to kill cancer cells (MacLaughin et al., 1991).

In vitro thrombolytic effect of *Cassia alata* seed oil extract:

Atherothrombotic diseases occur as serious impacts of the thrombus formed in blood vessels. Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels; but these drugs are not above limitations and can lead to serious and sometimes fatal consequences. The present study was carried out to investigate the thrombolytic activity of the *Cassia alata* seed oil extract. An *in vitro* thrombolytic method was used to investigate the thrombolytic activity of plant extracts in blood sample from healthy human volunteers, along with streptokinase as a positive control and water as a negative control. (Sweta et al. 2007). SK, a known thrombolytic drug (Tillet W.S., Garner R.L., 1933) is used as a positive control. The comparison of positive control with negative clearly demonstrated that clot dissolution does not occur when water was added to the clot. On the basis of the result obtained in this present study we can say that the *Cassia alata* seed oil extract have moderate thrombolytic activity compared to negative control (water).

CONCLUSION

In summary, pharmacological evaluation of *Cassia alata* seed oil extract shows interesting activities like antibacterial, cytotoxic and thrombolytic. However, further studies are necessary to elucidate the mechanism behind these effects. This report may serve as a footstep to use this plant as a new source of medication.

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