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Antishigellosis Activity of the Root Extracts of *Cassia tora* Linn

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Abstract: The root of *Cassia tora* exhibited substantial antishigellosis activity. The ethylacetate fraction of the crude extract showed maximum activity with the zone of inhibition ranging between 23-25 mm at the concentration of 200 μg disc^{-1}. The minimum inhibitory concentration (MIC) of ethylacetate, chloroform and ethanol extracts was found between 32-64 μg ml^{-1} whereas the methanol and petroleum fractions showed MIC values between 128-512 μg ml^{-1}. Thus the results suggest that the ethylacetate fraction may have some chemical constituents which could be useful as antishigellosis agents in modern clinical practice. Our effort is going on to isolate the potent antishigellosis constituents from the root extracts of *Cassia tora* with the aim of adding new therapeutic agents to fight against shigellosis problem in Bangladesh.

Key words: *Cassia tora*, antishigellosis activity

INTRODUCTION

Shigellosis, or bacillary dysentery, is an enteric infection caused by the bacterium *Shigella*. Shigellosis is endemic throughout the world, but more than 99% of the cases occur in the developing world, particularly in tropical zones[1]. About 10% of diarrheal episodes in children (less than 5 years of age) have visible blood in the stool. This dysentery accounts for about 15% of diarrhea-associated deaths in this age group world wide[2]. In developing countries, including Bangladesh, the problem is particularly acute. Among the children (1-4 years of age) in Bangladesh, shigella infection is most frequent and the fatality rate in this group is 3.5%[3]. Shigellosis shows a profound loss of blood from the ulcerated colon that continues even after the eradication of the pathogen with appropriate antibiotics[4].

In Bangladesh susceptibility to nalidixic acid was determined for isolates obtained after 1985. In 1983 13% of isolates were resistant to ampicillin, 23.5% to TMP-SMZ, and 0.8% to both drugs[5]. By 1990 51.2% of isolates obtained at the Diarrhea Treatment Centre were resistant to ampicillin, 47.7% to TMP-SMZ, and 40.5% to both drugs (for comparison with figures for 1983, P less than .001). Resistance to nalidixic acid increased from 0.8% in 1986 to 20.2% in 1990 (P less than .001). In 1990 71.5% of *Shigella dysenteriae* type 1 isolates were resistant to ampicillin, 68.5% to TMP-SMZ, 67.7% to both drugs, and 57.9% to nalidixic acid[5]. In Bangladesh ampicillin and TMP-SMZ are no longer useful for treatment of infection with any species of Shigella, and nalidixic acid is no longer useful for treatment of infections due to *S. dysenteriae* type 1[5]. Cefixime is ineffective in treating shigellosis in adults when used in the standard recommended dosage. Cephalexin and cephaloglycin, and the parenterally administered cephalosporin cefamandole are also ineffective in treating shigellosis[6-8].

Resistance to antibiotics appears to be increasing and the development of new drugs and preventive methods within economic reach of less developed countries are crucial for reduction of the disease and related deaths. In the continuation of this search for new antishigellosis components, we studied the root of *Cassia tora* which had been reported to use as tradition medicine in shigellosis. We found the antishigellosis activity of different organic extracts and further studies are continuing for exploring the single constituents from these organic extracts.

MATERIALS AND METHODS

Preparation of the extracts: The dry powder of the root of *Cassia tora* were extracted with hot ethanol in soxlet apparatus. The ethanol extract was then subjected to fractionation by petrolium ether followed by chloroform, ethylacetate and methanol.

Antishigella screening: *In vitro* Antbacterial screening is generally performed by disc diffusion method[9,10] for

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primary selection of the compounds as therapeutic agent. Disc diffusion method is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. Generally the more susceptible the organism the bigger is the zone of inhibition. The method is essentially a qualitative or semi quantitative test indicating sensitivity or resistance of microorganisms to the test materials as well as bacteriostatic or bactericidal activity of a compound[11]. The antishigellosis activity of the ethanol extract (Et. E), petroleum ether extract (PE), chloroform extract (CE), ethylacetate extract (EE) and methanol extract (ME) was determined at a concentration of 30 μg disc^{-1} and 200 μg disc^{-1} against five dysentery causing pathogens (*Shigella boydii*, *Shigella shiga*, *Shigella dysenteriae*, *Shigella flexneri* and *Shigella sonnie*). The diameters of zones of inhibition produced by the extracts were compared with the standard antibiotic, ciprofloxacin (CF) 30 μg disc^{-1}.

Minimum inhibitory concentration (MIC) determination: The minimum inhibitory concentration of the extracts (Et.E, PE, CE, EE and ME) was determined against the five pathogenic bacteria (*Shigella boydii*, *Shigella shiga*, *Shigella dysenteriae*, *Shigella flexneri* and *Shigella sonnie*) by serial dilution technique[11]. The results were compared with the standard antibiotic, ciprofloxacin.

RESULTS AND DISCUSSION

Antishigellosis activity: Shigellosis is a fatal disease in developing countries like Bangladesh and scientists are now engaged to explore proper treatment to cure this disease[12-14]. In the continuation of this strategy of new drug development in shigellosis, we studied the root of *Cassia tora* and found substantial activity against defferent *Shigellla* species. The ethylacetate (EE) and chloroform (CE) fraction of the crude extract of *Cassia tora* showed excellent activity against the tested *Shigellla* species at the concentration of 200 μg disc^{-1} (Table 1). At this concentration (200 μg disc^{-1}) the chloroform and ethylacetate extract gave the zone of inhibition between 19-25 mm. The petroleum ether fraction did not show any activity against the *Shigella* species on the otherwise methanol extract showed poor activity and ethanol extract showed moderate antishigellosis activity. These findings suggest that the maximum antishigellosis components present in the ethylacetate and chloroform fractions.

The MIC values of the extracts (Table 2) suggest that the chloroform (CE), ethylacetate (EE) and ethanol extracts (Et. E) of *Cassia tora* root having lower MIC values of 32-64 μg ml^{-1} are more active against the tested *Shigella* species than the petroleum (PE) and methanol (ME) extracts having higher MIC values of 128-512 μg ml^{-1}. The higher activity of the extracts (CE, EE and Et. E) may be due to glycosides present in these extracts but further studies are required to explore the highly active components present in these extracts.

Table 1: *In vitro* antishigellosis activity of the root extracts of *Cassia tora* and standard ciprofloxacin (CF)

	Diameter of zone of inhibition (in mm)										
	Et.E		PE		CE		EE		ME		CF
μg/disc	30	200	30	200	30	200	30	200	30	200	30
Shigella boydii	09	11	00	00	00	21	10	23	00	14	29
Shigella shiga	00	18	00	00	00	19	09	23	00	10	30
Shigella dysenteriae	00	20	00	00	09	22	10	24	00	00	31
Shigella flexneri	00	19	00	00	00	20	09	25	00	10	28
Shigella sonnie	09	19	00	00	00	21	00	23	00	00	30

Table 2: Minimum inhibitory concentration (MIC) values of the root extracts of *Cassia tora* and standard ciprofloxacin (CF)

	Minimum inhibitory concentration (μg ml^{-1})					
Test organisms	Et.E	PE	CE	EE	ME	CF
Shigella boydii	64	512	64	32	128	4
Shigella shiga	32	512	32	32	128	4
Shigella dysenteriae	32	256	64	32	128	2
Shigella flexneri	64	256	32	64	128	2
Shigella sonnie	64	512	32	64	256	4

This is interesting findings of antishigellosis activity for the organic extracts and it is indicative of the presence of active antishigellosis components in the root of *Cassia tora*. We did not find any report of antishigellosis components from the root of *Cassia tora* in the previous literature so; we were interested to investigate the potent antishigellosis components from the root of *Cassia tora*. Further studies are going on in our laboratory to establish the boiactive chemical constituents from this medicinally important root of *Cassia tora* which may lead to discover of new antishigellosis principles. The isolated antishigellosis components may be helpful for their clinical implications.

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