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REGULAR ARTICLE

EVALUATION OF ANTIMICROBIAL POTENTIALITIES OF LEAVES EXTRACT OF THE PLANT *CASSIA TORA* LINN. (*LEGUMINOSAE/CAESALPINIOIDEAE*)

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SUMMARY

Cassia tora L. (Family *Leguminosae/Caesalpinioideae*), is a plant with enormous medicinal values. The chloroform, methanol and aqueous extract of leaves of *Cassia tora* L. showed antibacterial activity (0-5000 µg/ml) against 38, 58 and 29 bacterial strains respectively out of 120 various bacterial strains and also methanol extracts showed antifungal activity (0-64mg/ml) against 3 strains out of 4 strains. Five strains of *Shigella dysenteriae*, four strains of *Staphylococcus aureus*, and three strains of *Escherichia coli*, have shown sensitivity against in vitro treatment of the methanol extracts up to 2000 µg/ml concentration. The minimum inhibitory concentration (MIC) values ranges from 2–64 mg/ml for dermatophytes. Minimal Bactericidal Concentration (MBC) value lies in the range of 2000-2500 µg/ml against *Escherichia coli* ATCC25938 and *Shigella dysenteriae* 1. Phytochemical study indicates that the leaf extract contains flavonoids, saponins, resins, phytosterol, alkaloids and carbohydrates. The traditional claim of leaves of *C. tora* as an antimicrobial property have been confirmed as the extracts displayed activity against some bacteria and fungi which cause skin infection and gastro-intestinal disorder.

Key words: *Cassia tora* plant, Phytochemical study, Antimicrobial activity, MIC and MBC

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1. Introduction

Many of the developing countries practice traditional medicine as its main source of healthcare, which is usually of plant origin (Ahmad et al., 2008; Stephen Bent, 2008). Today, nearly 88% of the global populations switch to plant derived medicines as their first line of defense for maintaining health and combating diseases (Kintzios et al., 2006). Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases (Qadrie et al., 2009; Pattnaik and Sharma, 2004)., as a result the treatment failure and health care cost have raised day by day. This has encouraged the microbiologists all over the world to formulate new antimicrobial agents and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents (Alviano and Alviano, 2009; Cowan, 1999).

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season, climate and particular growth phase. Leaf is one of the highest accumulator plant parts of such compounds and people are generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Mukherjee and Wahile, 2006). They can inhibit the growth of microbes by binding their surface proteins, breaking the peptide bonds, acting as chelating agents, altering their biochemical systematic or by preventing utilization of available nutrients to the microorganisms.

Some compounds also cause lyses of microbial cells (Mukherjee and Wahile, 2006).

Cassia tora L, family *Leguminosae/ Caesalpinioideae* is known as Charota (Hindi), chakunda (Bengali), and in English it is called Foetid cassia (Oudhia, 2002). Traditionally reported that *Cassia tora* L. have medicinal properties, like laxative, antiperiodic, antihelmintic, ophthalmic, and effective for leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders, etc (Nadkarni, 1985; Chatterjee and Pakrashi, 1992)The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders (Oudhia, 2002). These herbs have been reported for their usefulness in the form of decoctions, infusions and tinctures in traditional system of medicines for treating skin diseases like psoriasis, leprosy etc. (Horvath, 1992; Zahra et al., 2000; Cordova et al., 2002; Harrison and Dorothy, 2003). *Cassia tora* L. have the hepatoprotective effects against carbon tetrachloride induced liver damage (Rajan et al., 2009). The root of *Cassia tora* L. exhibited substantial antishigellosis activity (Shahnaj et al., 2004). *Cassia tora* L. leaves have anthelmintic property for the presence of flavonoid (John et al., 2009). Aqueous extracts of seeds of *Cassia tora* L. exhibited better antibacterial activity (*Staphylococcus aureus* was more susceptible) as compared to petroleum ether, methanolic and ethanolic extracts (Roopashree et al. 2008).

Hence, the present investigation was carried out to evaluate the antibacterial activity of *Cassia tora*, leaves extract using different solvents and their aqueous extracts against several bacteria that can cause skin diseases and gastro-intestinal disorders in man.

2. Materials and Method

Extraction

The leaves of *Cassia tora* L. was collected from the Jalpaiguri district, West Bengal, India during the month of May and June, 2009. Extraction was carried out at room temperature under normal conditions. The leaves were shade dried for seven days and powdered and weighed 100 gm. Dried powdered leaves of *Cassia tora* L. were macerated in a 500 ml conical flask at room temperature using pet-ether, chloroform, methanol, and water successively for 72 hrs each, which was the modified method of CSIR protocol(CSIR Protocol, 1997). Before extraction with the next solvent the powder was air dried to remove the adhering solvent. The extracts were filtered and the solvent was evaporated to dryness under reduced pressure in an Eyela Rotary Evaporator at 40–45°C. All the extracts were used for Phytochemical and antibacterial study.

Phytochemical evaluation

Phytochemical examinations were carried out for all the extracts as per the standard methods (in Table 1) (Ellen and Sydney, 1990; Brain and Turner, 1975; Evans, 1996).

Microorganisms

The total of 120 strains of bacteria out of which 4 Gram positive and 8 Gram negative genera and 4 types of fungal strains were used in this study. All these bacterial strains and fungal strains are preserved in the Division of Microbiology, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata-32, West Bengal, India. The bacterial strains were grown in blood agar or MacConkey agar plates at 37 °C and maintained on nutrient agar (Hi-media) slants while the fungi were grown at 30 °C and maintained in Saboraud glucose agar slants.

Preparation of inoculums: McFarland Nephelometer Standard (Roopashree et al., 2008) method was followed for the preparation of suspension of organisms. A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was controlled in such a way that each ml of suspension contained approximately 1.5×10^8 cells/ml. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05ml of 1.175% of barium chloride and 9.95 ml of 1% sulphuric acid.

Table -1. Different biochemical tests with respect to different Phytochemicals

Phytochemicals	Name of test performed	
Alkaloids	a)	Mayer's Test
	b)	Dragendorff's Test
	c)	Wagner's Test
	d)	Hager's test
Carbohydrates	a)	Molisch's Test
	b)	Benedict's Test
	c)	Fehling's Test
Glycosides	a)	Modified Borntrager's Test
	b)	Legal's Test
Saponins	a)	Froth Test
	b)	Foam Test
Phytosterols	a)	Salkowski's Test
	b)	Libermann Burchard's Test
Phenolic compounds	a)	Ferric Chloride Test
Tannins	a)	Gelatin Test
Flavonoids	a)	Alkaline Reagent Test
	b)	Lead acetate Test
	c)	Shinoda Test
	d)	Zinc hydrochloric acid reduction Test

Antimicrobial assay

Sensitivity tests were performed by disk diffusion method, as per (NCCLS, 1993) protocol. The Mueller Hinton agar (Hi-media) plates, containing an inoculum size of 10^6 cfu/ml of bacteria or 2×10^5 yeast cells or fungal spores on Saboraud glucose agar (Hi-media) plates, were used. Previously prepared extract impregnated discs at concentrations of 0–5000 µg/ml for bacteria and 0–64 mg/ml for yeasts and fungi were placed aseptically on sensitivity plates with appropriate controls (Chattopadhyay et al., 1998b). All the plates were then incubated at 37 °C overnight for bacteria yeasts and at 30 °C for 3 days in the case of fungi. The sensitivity was recorded by measuring the clear zone of growth inhibition on agar surface around the discs.

Determination of minimum inhibitory concentration (MIC)

MIC was determined both by agar and broth dilution methods (Chattopadhyay et al., 1998a). A 2-fold serial dilutions (0–5000 µg/ml for bacteria and 0–64 mg/ml for fungi) of the extracts, with appropriate antibiotic control were prepared in Mueller Hinton broth for bacteria (Chattopadhyay et al., 1998b) and Saboraud glucose broth for fungi (Ibrahim and Osman, 1995). For agar dilution assay previously prepared sensitivity plates, using serial 2-fold dilutions of the extracts and control antibiotics as above, were spot inoculated (2×10^6 cfu per spot for bacteria and 2×10^5 spores per spot for fungi). The inoculated plates were then incubated at 37 °C for 24 hrs (bacteria) and 30 °C for 96 hrs (fungi). For broth dilution tests, 0.1 ml of standardized suspension of bacteria (10^6 cfu/ml) or fungal spores (5×10^5 spores/ml) were added to each tube (containing crude extracts at a final concentration of 0–5000 µg/ml for bacteria and 0–64 mg/ml for fungi) and incubated at 37 °C for bacteria and at 30 °C for fungi for 24 hrs or 96 hrs respectively. The lowest concentration of the tube or plate which did not show any visible growth after macroscopic evaluation was considered as the MIC.

Determination of minimal bactericidal concentration (MBC)

MBC was determined by broth dilution method. Previously prepared drug dilutions (0–3000 µg/ml) of the crude drug (methanol extract) were added in Mueller Hinton broth. The mixtures were then incubated at 37 °C for 18 hrs with shaking on a platform shaker at 200 rpm. The concentrations were added to the mid-logarithmic phase of growth of

the organism and aliquots of 1.0 ml were withdrawn at 2 hrs intervals for the determination of OD_{540} and colony count (Chattopadhyay et al., 1998a). The lowest concentration of crude drug(methanol extract) of the tube which did not show any visible growth after colony count was considered as the MBC (Chattopadhyay and Maiti, 2001).

Statistical analysis

The results for each assay were expressed as the mean ± standard deviation of three individual experiments. Differences in the mean values amongst the treatment groups were analyzed by analysis of variance (ANOVA). Statistical analyses were performed using the software INSTATE. $P \leq 0.05$ was considered to be statistically significant.

3. Results

Phytochemical investigation of pet ether, chloroform, methanol and aqueous extracts of the plant *Cassia tora* revealed differences in their phytoconstituents. Extracts of the same drug obtained using different solvents also exhibited differences in their constituents. Phytochemical compounds such as alkaloids, carbohydrates, flavonoids, phytosterol and saponins were detected in *Cassia tora* L (Table 2). Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts obtained using same herb with different solvents will be different (Kirtikar and Basu, 1999).

Table 2. Results of different phytochemical tests

Extracts	Alkaloid	Carbohydrate	Glycoside	Saponin	Phytosterol	Resin	Tannin	Flavonoid
1) Pet. ether extracts	–	–	–	–	+	–	–	–
2)chloroform extracts	+	–	–	–	+	–	–	–
3)Methanol extracts	+	+	–	–	–	–	–	+
4)Aqueous Extracts	–	+	–	+	–	–	–	+

*'–' shows absent of phytochemical
*'+' shows present of phytochemical

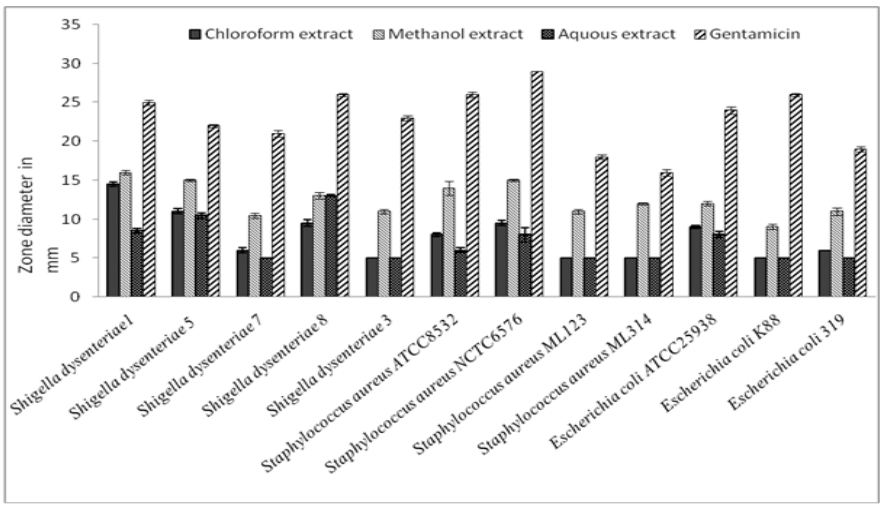
The antimicrobial activity tests of methanolic extracts, as shown in Table- 3, revealed that the growth of 30 strains of *Escherichia coli*, 2 strains of *K. pneumonia*, 5 strains of *S. typhi,* 8 strains of *Vibrio cholera,*6 strains of *Bacillus subtilis,* 4 strains of *Pseudomonas aerugenosa* , 2 strains of *Klebsiella* sp., 27 strains of *Shigella dysenteriae* and 30 strains of *Staphylococcus aureus*, as well as 3 species of dermatophytes were affected by the extract, as the MIC was <5000 µg/ml (for bacteria) and <64 mg/ml (for fungi). However, each strain of *Shigella dysenteriae* and *Escherichia coli* had the MIC of 500 and 750 µg/ml respectively against the methanol extract. In case of aqueous extract, as shown in Table-3, 15 strains of *Shigella dysenteriae,* 13 strains of *Escherichia coli,* 2 of *Vibrio cholerae,* 6 strains of *Bacillus subtilis* and 4 strains of *Staphylococcus aureus* show the MIC in <5000 µg/ml. On the other hand chloroform extract of leaves of *Cassia tora* L demonstrates 15 strains of *Shigella dysenteriae,* 4 strains of *Staphylococcus aureus,* 8 of *Vibrio cholerae,* 4 of *Pseudomonas aeruginosa,* 6 strains of *Bacillus subtilis* and 13 strains of *Escherichia coli* show the MIC in <5000 µg/ml (Table-3). Petroleum ether extract had no such antimicrobial activity. These results indicate that the different extracts of the herbs under study

exhibit antibacterial activity and among the various extracts, methanolic extracts have shown better activity as compared to other extracts with respect to similar bacteria. This indicates that methanolic extracts are better as compared to other extracts.

Table-3. Antimicrobial activities of different leaves extracts of *Cassia tora* L.

Name of bacteria	*No of strains tested*	Minimum inhibitory concentrations (µg/ml)				
		Chloroform extracts	Methanol extracts	Aqueous extracts	Amoxicillin	Gentamicin
Staphylococcus aureus	4	3000	1000	5000	0.5	1
Staphylococcus aureus	13	>5000	3000	>5000	0.5	2
Staphylococcus aureus	7	>5000	4000	>5000	1	16
Staphylococcus aureus	6	>5000	5000	>5000	4	1
Bacillus subtilis	6	4000	3000	4000	1	1
Pseudomonas aerugenosa	4	4000	4000	>5000	8	64
Escherichia coli	1	3000	750	4000	64	1
Escherichia coli	12	5000	2000	5000	128	2
Escherichia coli	17	>5000	4000	>5000	128	1
Vibrio cholerae	2	4000	3000	4000	256	1
Vibrio cholerae	6	5000	4000	>5000	>256	1
Salmonella typhi	5	>5000	5000	5000	>256	1
Klebsiella pneumoniae	2	>5000	5000	>5000	256	4
Klebsiella.pneumoniae	3	>5000	>5000	>5000	256	4
Proteus vulgaris	5	>5000	>5000	>5000	>256	64
Shigella dysenteriae	1	2000	500	2000	>256	1
Shigella dysenteriae	14	3000	2000	3000	>256	8
Shigella dysenteriae	12	>5000	4000	>5000	>256	16

Name of Fungi	*No of strains tested*	Minimum inhibitory concentrations (mg/ml)			
		Chloroform extracts	Methanol extracts	Aqueous extracts	Nystatin
Candida albicans	1	64	16	32	1
Candida lichinoformis	1	>64	32	>64	4
Cryptococcus neoformans	1	>64	32	64	0.5
Trichophyton mentagrophytes	1	>64	>64	>64	1



‘ I ‘ Values are in terms of Mean ± SEM of results done in triplicate.

Fig. 1. Different bacterial strains exhibit different zone diameter (in mm) in their MIC value of different extracts of *C. tora* L. leaves with respect to gentamicin disk (values are mean ± SEM; $n = 4$)

Different bacterial stains exhibit different zone diameter (in mm) in their MIC value of chloroform extract, methanolic extract and aqueous extract of *C. tora* L. with respect to gentamicin disk in Fig. 1 chloroform, methanolic and aqueous extract exhibit highest zone of inhibition were 14.5 mm, 16 mm and 13 mm against the strain of *Shigella dysenteriae* 1, *Shigella dysenteriae* 1 and *Shigella dysenteriae* 8 respectively. In the other hand, methanolic extracts showed highest zone diameter (10.2mm) against *Candida albicans* (Fig.2).

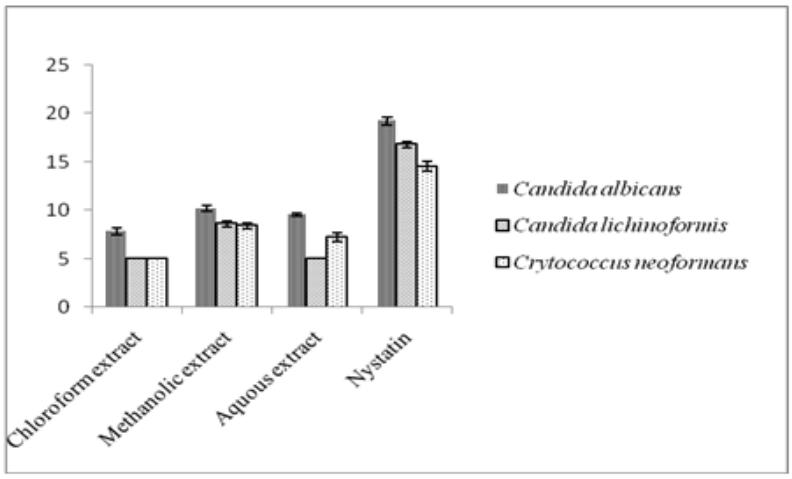


Fig. 2. Different extract of *C. tora* L. exhibit different zone of inhibition in different fungal strains with respect to Nystatin in MIC value (values are mean ± SEM; $n = 4$).

MBC was always found to be 3 to 4-fold higher than MIC values. The results also revealed that the extract exhibited bacteriostatic activity at lower concentrations but bactericidal at higher concentrations. The inhibitory effect of the extract of *C. tora* L. was on susceptible bacterial species (*Escherichia coli* ATCC 25938 and *Shigella dysenteriae* 1) at different concentrations showed that the growth of these organisms were decreased by increasing concentration of the extract and were inhibited at their MBC values which were 2500µg/ml and 2000µg/ml of *Escherichia coli* ATCC 25938 and *Shigella dysenteriae* 1 respectively. The inhibitory effect of the extract was also demonstrated by the time dependent *in vitro* growth (Fig. 3).

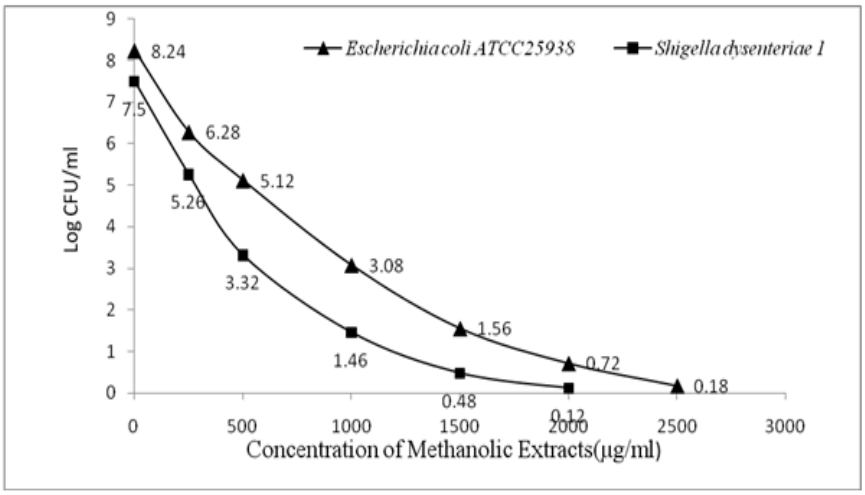


Fig. 3. Log CFU/ml against different concentration of methanolic extracts of *Cassia tora* L.

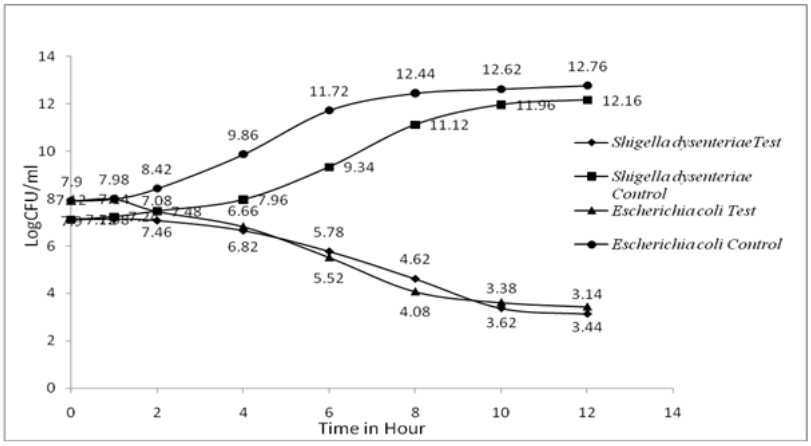


Fig. 4. Time dependent in vitro growth curve of *S. dysenteriae* 1 and *Escherichia coli ATCC25938* at their MIC values against methanolic extract of *C. tora* L. leaves

4. Discussion

Our preliminary investigation showed that all chloroform, methanol and aqueous extracts of ethno medicinal plant *Cassia tora* L. (leaves) were active against human pathogens (120 bacterial strains and 4 fungal strains) used in this study. Phytoconstituents present in plants namely flavonoids, alkaloids, tannins and triterpenoids are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms (Dewenjee et.al., 2007)s

Literature also reveals that, *S. aureus* and *S. dysenteriae* being the most susceptible organism, this drug could be more effective in infections related to *S. aureus* and *S. dysenteriae* rather than other bacterial infections (Patel and Patel, 1957; Awal et al., 2004).

The MIC value of the active plant extracts obtained in this study were lower than the MBC values suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration.

Traditionally *Cassia tora* L. is used externally to treat fungal infections in Malaysia, (Abdul Rahim 1996). The results from this current study showed that methanolic extract of *Cassia tora* L. had antifungal properties on *Candida albicans*, *Candida lichinoformis and Cryptococcus neoformans*. Graybill (1988) reported that many commercial antifungal drugs induce adverse drug reactions/toxicities that include liver, kidney and gastrointestinal toxicities. Increase of hepatic enzymes up to 7% in patients that received continuous antifungal drug therapy (Restrepo et al. 1986). Herbal remedies often do not induce adverse effects (Perry, 1980). Alternative medicine has become a popular remedy to various types of aliments.

All these findings indicate that *Cassia tora* L extracts possess antibacterial activity and they cause lysis and eradicate bacteria by degrading bacterial cell walls. By the findings and purification of the active agent that is present in the extract of *C. tora* L., it will be possible to discover new natural drugs serving as chemotherapeutic agents for treatment with nosocomial pathogens. Other different studies are required to understand the mechanism of this herbal extracts (which is cheaper, easily available and safe in comparison to other synthetic antimicrobial drugs) for the treatment of various infections, skin diseases and intestinal disorders.

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