

SCREENING OF ANTIBACTERIAL ACTIVITY OF CRUDE LEAF EXTRACTS OF CASSIA TORA ON UTI PATHOGENS

PINKI RAJ SAHU AND M. P. SINHA*

Department of Biotechnology, Marwari College Ranchi – 834 001
University department of Zoology, Ranchi University Ranchi – 834 008
e-mail: m_psinha@yahoo.com

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*Corresponding author

ABSTRACT

Antibacterial effect of crude methanolic and aqueous extracts of leaves of *Cassia tora* against pathogenic bacteria *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumoniae* isolated from patients of U.T.I, were investigated using agar well diffusion method. Among the various concentration tested (ranging from 0.0625 to 6.0 mg/mL), 1.0-2.0 mg/mL of methanolic extract was found to be the minimum inhibitory concentration (MIC) for almost all the test organisms while aqueous extract showed MIC at > 6.0 mg/mL. *P. aeruginosa* and *K. pneumoniae* were resistant to aqueous extract. Methanolic extract was more effective over aqueous extract producing larger zone of inhibition ranging between 12-24mm. The traditional claims of leaves of *C. tora* as an antibacterial ability have been confirmed as the extracts displayed activity against the pathogens associated with UTI. This study indicates that the leaves of *C. tora* can be used as a source for new broad spectrum oral drug.

INTRODUCTION

Urinary Tract infections (UTI's) are the most common form of bacterial infections affecting people throughout their life span (Barnett *et al.*, 1997; Foxman, 2002). Leading etiological agent of UTI's include –*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus aureus* (Svanbory and Godaly, 1997). The incidence of UTI is greater in women as compared to men, which may be either due to anatomical predisposition or urothelial mucosal adherence to mucopolysachharide lining or other host factors (Schaeffer *et al.*, 2002). The incidence of acute uncomplicated UTI is estimated to exceed 0.5 episodes per annum among women between 18-30 years (Hooton *et al.*, 1996). The increasing drug resistance among these bacteria has made the therapy of UTI difficult and has led to a greater use of expensive broad- spectrum drugs. This resistance problem needs a re-new effort, resulting in searching effective antibacterial agents against pathogenic micro-organism resistant to current antibiotics (Soulsby, 2005). For centuries plants have been used throughout the world as drugs and remedies for various diseases (UNESCO report, 1988-1997). The herbal drugs serve as prototype to develop more effective and less toxic medicines. According to World Health Organization (WHO report, 2000), medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated for better understanding of their properties, safety and efficacy (Nascimento *et al.*, 2000). Many of the developing countries practice traditional medicine as its main source of healthcare, which is usually of plant

origin (Ahmad *et al.*, 2008; 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 (Pattnaik and Sharma, 2004; Qadrie *et al.*, 2009) 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 (Cowan, 1999; Alviano and Alviano, 2009). The review of literature revealed that considerable contributions have been made on medicinal plants by many workers (Dadsena *et al.*, 2013; Dandapat *et al.*, 2013; Kullu *et al.*, 2013; Kumar *et al.*, 2013; Kumar *et al.*, 2013a; Mahato *et al.*, 2013; Tabassum *et al.*, 2013; Toppo *et al.*, 2013). In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as very importance source for discovery of new agents for treating various ailments related to bacterial infections.

Cassia tora L, family *Leguminosae/ Caesa Ipinioidae* a herb being used in India as folk remedy in the form of decoctions and infusions to treat bacterial infections and also claimed to be an effective against variety of skin conditions like psoriasis, acne, wounds and urinary tract infection .

The present investigation has been carried out to study the unexplored area of this herb towards their antibacterial activity with respect to their traditional use in urinary tract infection.

Table 1: Zone of inhibition in mm of test organism for methanolic and aqueous extract in agar well diffusion Method

| Extract concentration (mg/mL) | Zone of inhibition | | | | | | | | | | | | | | | |
|-------------------------------|--------------------|----|-----|----|---------------------|----|-----|---|-----------------|----|-----|---|----------------------|----|-----|---|
| | <i>E.coli</i> | | | | <i>P.aeruginosa</i> | | | | <i>S.aureus</i> | | | | <i>K. pneumoniae</i> | | | |
| | Meth | | Aqu | | Meth | | Aqu | | Meth | | Aqu | | Meth | | Aqu | |
| | C | M | C | A | C | M | C | A | C | M | C | A | C | M | C | A |
| 1.00 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.00 | 0 | 18 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3.00 | 0 | 22 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 8 | 0 | 0 |
| 4.00 | 0 | 24 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 10 | 0 | 0 |
| 5.00 | 0 | 24 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 12 | 0 | 0 |
| 6.00 | 0 | 24 | 0 | 6 | 0 | 24 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 16 | 0 | 0 |
| > 6.00 | 0 | 24 | 0 | 12 | 0 | 24 | 0 | 5 | 0 | 24 | 0 | 8 | 0 | 20 | 0 | 5 |
| 30ug/mL chloramphenicol | 21 | | 0 | | | | 23 | | | | 0 | | | | | |

Table 2: MIC values of the crude leave extracts of *C. tora*

| S No | Organisms | Methanolic extract MIC(mg/mL) | Zone of inhibition (mm) | Aqueous extract MIC (mg/mL) | Zone of inhibition (mm) |
|------|-----------|-------------------------------|-------------------------|-----------------------------|-------------------------|
| 01 | E1-E2 | 10.75 | 12mm-16 mm | 65 | 6mm-6mm |
| 02 | P1-P2 | 22 | 12mm-16mm | 2420 | 5mm-6mm |
| 03 | S1-S2 | 21.5 | 8mm-12mm | 1412 | 8mm-12mm |
| 04 | K1-K2 | 32 | 8mm-12mm | 2016 | 5mm-7mm |

E1,P1,S1 and K1 are the pure stains while E2,P2,K2 and S2 are the isolated stains

bacteria. It is in contradiction to the data presented in this study. Our findings are similar to De and Ifeoma (2002) as these did not show any antibacterial activity with the aqueous leaf and bark extract of neem against test bacteria. The zones of inhibition recorded for the methanolic and acetone extracts by De and Ifeoma (2002) were also smaller in size than those obtained in the present study. Several factors are known to influence yield and biological activities of plant based products, including the age of the plant, time of harvest, drying and processing of the materials, methods of extraction and the solvents used (El-Mahmood *et al.*, 2010). This may be the reason of difference in results in the present study. Roopashree *et al.* (2008) reported that aqueous extract of *C.tora* inhibited *S. aureus*, *P. aeruginosa* and *E.coli* at the concentration of 100ug/mL, 200ug/mL and 250ug/mL respectively which also contradict our findings as among all the test organisms *P. aeruginosa* showed resistance to all of the tested concentration of aqueous extract and other pathogens *E. coli*, *S. aureus*, did not responded at this lower concentration but responded better in concentration >6.0 mg/mL. Similarly the low MIC value of methanolic extract of *C. tora* leaves found in the present study were different from the findings of Roopashree *et al.* (2008) with high MIC value for *S. aureus* and *E. coli* i.e. 64mg/mL. Our findings were also vary with the reports of Nadkarni *et al.* (1982), Brown and Dattner (1998), Grover and Yadav (2004) and Christopher *et al.* (2005), as they reported that aqueous extract exhibited high antibacterial activity than methanolic extracts, in term of zone of inhibition for the same test organism.

Amongst the test bacteria, *E.coli* (E1) was the most susceptible, closely followed by *P. aeruginosa* (P1) and *S. aureus* (S1) whereas *K. pneumoniae* (K1) was less susceptible. Several authors have reported that plant extracts are more effective against gram-positive than gram-negative bacteria and attributed this to the differences in their cell wall structures (Rabe and Van Staden, 1997, Parekh and Chanda, 2006) El-Mahmood *et al.* (2010), which contradict our finding as *E.coli*

a gram negative bacteria showed maximum susceptibility than *S.aureus* a gram positive bacteria. Our report is also not in agreement with those of Patel and Patel (1957), Awal *et al.* (2004) and Gaurang *et al.* (2010) who reported *S.aureus* susceptibility of higher than all the same test bacteria against the crude plant extract. Control bacteria were more susceptible to the toxic effects of the crude extracts than the test bacteria though the sensitivity also varied according to strains, (El-Mahmood *et al.* 2010).

The standard antibiotic chloramphenicol, (30ug/mL) demonstrated highest activity than the crude extracts for *E. coli* and *S. aureus* (Table 1). This is because the antibiotic is in pure state and has undergone some refining processes that have established it as standard antibiotic.

The present study stipulated that methanolic extract of *C.tora* leaves possess antibacterial activity against UTI pathogens, and supports the finding of Dewenjeet *et al.* (2007).

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