

MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF CASSIA TORA L. AND CAPSICUM ANNUUM L. IN CHOSEN PATHOGENIC ORGANISMS

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ABSTRACT: Microbiologists have great interest to screening the medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The present study clearly indicate that the test plants (*Cassia tora* L. and *Capsicum annuum* L.) has high level of antimicrobial activities and control the bacterial population in an said area. Among the different extracts, ethanol extract showed more antibacterial activity. *Cassia tora* L. and *Capsicum annuum* L. showed maximum antibacterial activity, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) against two tested bacteria *Pseudomonas aeruginosa* and *Enterobacter* sp. than the other plant. Two bacteria were more susceptible to ethanol extract than the other organic extracts. Therefore, it may be recommended for the preparation of plant based drugs against human bacterial pathogens. *Cassia tora* L. has the anti-inflammatory, antihepatotoxic, antinociceptive, hypolipidemic and hypotensive properties. *Capsicum* and capsaicin both significantly prevent the increase of liver cholesterol levels. In future these plants can be subjected to isolation of the major constituent's of antimicrobials, antidiabetic, anticancer and to further pharmacological evaluation.

Keywords: *Cassia tora* L. and *Capsicum annuum* L. *Pseudomonas aeruginosa* and *Enterobacter* sp. anti microbial activity, MIC and MBC.

INTRODUCTION:

Cassia tora L. is an annual foetid herb with a height of 30 to 90 cm. In India, it is mainly found in the states of Uttar Pradesh and Madhya Pradesh. It has pinnate leaves, which are about 10 cm long. Each leaf has three pairs of leaflets that are opposite, ovate, oblong and oblique at the base. The yellow colour flowers are bearded in the axel of the leaves. The flowers comprises of five petals, each about half inch in diameter. *Capsicum annuum* L. is bell, sweet, or chilli pepper with cultivated varieties including bell, sweet, chilli, and paprika peppers. It is a perennial herbaceous plant originated in Central and South America and the Caribbean and was domesticated over 5,000 years ago. Peppers from *Capsicum annuum* have been developed into numerous varieties that are now cultivated around the world for sweet and hot varieties of green, yellow and red bell peppers. For a long period of time, plants have been a valuable source of natural products for maintaining human health especially, for the studies of natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. The use of antimicrobials of plant origin is gaining popularity among users, as more and more continue to derive the benefits of using these plants against a number of infectious diseases. The popularity of using plants for therapeutic purposes has been intensified especially at the onset where traditional health care using traditional medicine is being promoted (World Health Organization, 2010).

The search for antimicrobials of plant origin has been mainly stimulated by the fact that some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum. Now-a-days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Lakshmi Naidu *et al.*, 2006). Resistance in bacteria is most prevalent like methicillin resistant *Staphylococcus aureus* (MRSA) has become a huge problem worldwide to treat nosocomial infections since 1990s (Lee *et al.*, 2007).

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from 248 developed countries use traditional medicine, which has compounds derived from medicinal plants (Nascimento *et al.*, 2000). The value of medicinal plants is used in ayurvedic, allopathic and the homeopathic treatment. According to the World Health Organization, more than 80% of the world population still rely on herbal medicines as their primary source of health care. Medicinal Plants are being used by traditional healers either singly or in combination in the treatment of different types of diseases.

Antimicrobial activity of various extracts of *Cassia tora* and *Capsicum annuum* against human bacterial pathogens were reported by several workers (Sharma *et al.*, 2006; Saravanan *et al.*, 2008; Manjula *et al.*, 2009; Prasad *et al.*, 2009; Khan *et al.*, 2010 and Saurabh *et al.*, 2011). In the present investigation the two Indian plants *Cassia tora* L. and *Capsicum annuum* L. extracted with low polar to high polar organic solvents and against human bacterial pathogens.

MATERIALS AND METHODS:

Source of collection of plant material

Cassia tora L. and *Capsicum annuum* L. was chosen as a test plants. From this plants samples were isolated for screening the antibacterial activity. Small healthy leaves and stalks were collected during early morning period from the fully grown plants at Thiruthangal, Virudhunagar District, and Tamil Nadu.

Processing of medicinal plants

The leaves and stalks were collected, washed with water to remove soil and dust particles. Then they were dried in thoroughly shaded place, and blended to form a fine powder and stored in airtight containers.

Human pathogenic bacteria species

The human pathogenic bacteria such as *Pseudomonas aeruginosa* and *Enterobacter sp.*, were obtained from Vivek Laboratory, Nagercoil, and Tamilnadu.

Preparation of plant extracts

The fresh leaves and stalks (10g) were taken and it is grind with 5% ethanol with the help of mortar and pestle. The extract was filtered with the help of muslin cloth.

Dry plant extract

The 10g of powder is taken and 25ml of ethanol is added and it is kept in a shaker for 2hrs. The extract was filtered with the help of muslin cloth.

Disc preparation

What man No-1 filter paper was used to prepare disc with the help of punching machine. The discs were sterilized in autoclave and loaded with suitable concentration of test extracts.

Disc diffusion method

Filter paper disc diffusion technique in agar was followed to determine antimicrobial activity by the procedure of Garg and Jain, 1998. The sterile nutrient agar plate was prepared and dried. The pathogenic cultures of gram negative bacteria (*Pseudomonas aeruginosa* and *Enterobacter sp.*) were selected for analysis. The bacterial strains sub cultured in sterile Nutrient broth periodically (maintain purity) and incubate at 37°C for 18-24 hours. Each young cultures lawn into dried sterile nutrient agar plates separately. Sterilized filter paper No.1 discs were loaded with the extracts of *Cassia tora* L. and *Capsicum annuum* L. using different solvents. The amount of extracts loaded in each disc was in the concentration viz., 25 µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml. Similarly discs were prepared for standard antibiotics penicillin (w/v) and cefotaxime (w/v) and were impregnated in the filter paper discs in different concentrations 25 µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml. The plates were incubated for 24 hrs at 37°C. After incubation period the diameter of inhibitory zones was formed. Similarly instead of plant extracts a standard antibiotic, penicillin and cefotaxime used to detect antibacterial activity and it was compared to plant extracts.

Result

Screening of anti bacterial activity

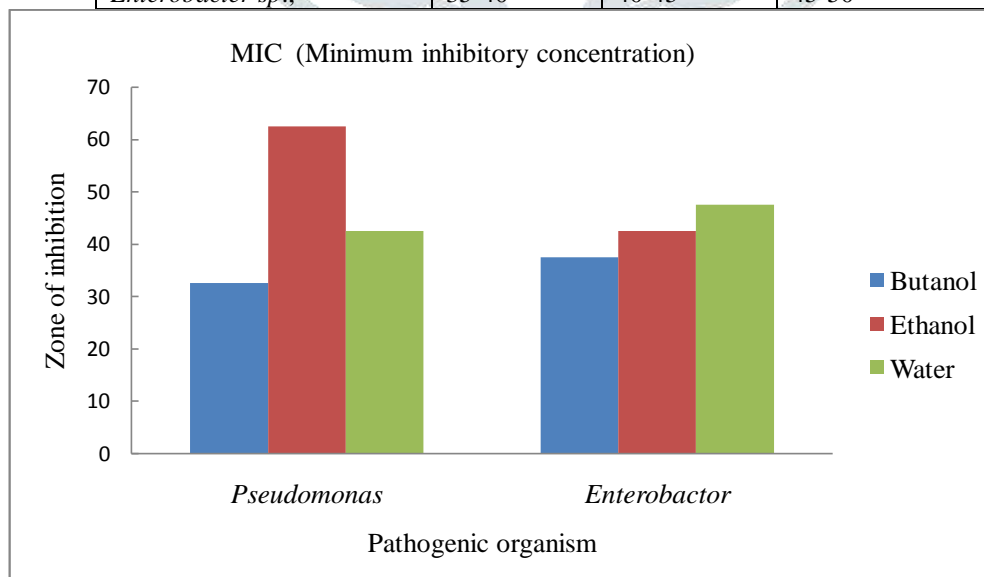
Antimicrobial screening of the leaf extracts were analysed in MIC (Minimum Inhibitory Concentration) and MBC (Minimum bactericidal concentration) methods.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of various extracts of *Cassia tora* L. and *Capsicum annuum* L. against bacterial pathogen was given in Table 1.

Table 1. The Minimum Inhibitory Concentration of various extracts of *Cassia tora* L. and *Capsicum annuum* L. (µg/ml) against human pathogenic bacterial organisms.

Pathogenic organisms	Butanol	Ethanol	Water
<i>Pseudomonas aeruginosa</i>	30-35	60-65	40-45
<i>Enterobacter sp.</i>	35-40	40-45	45-50



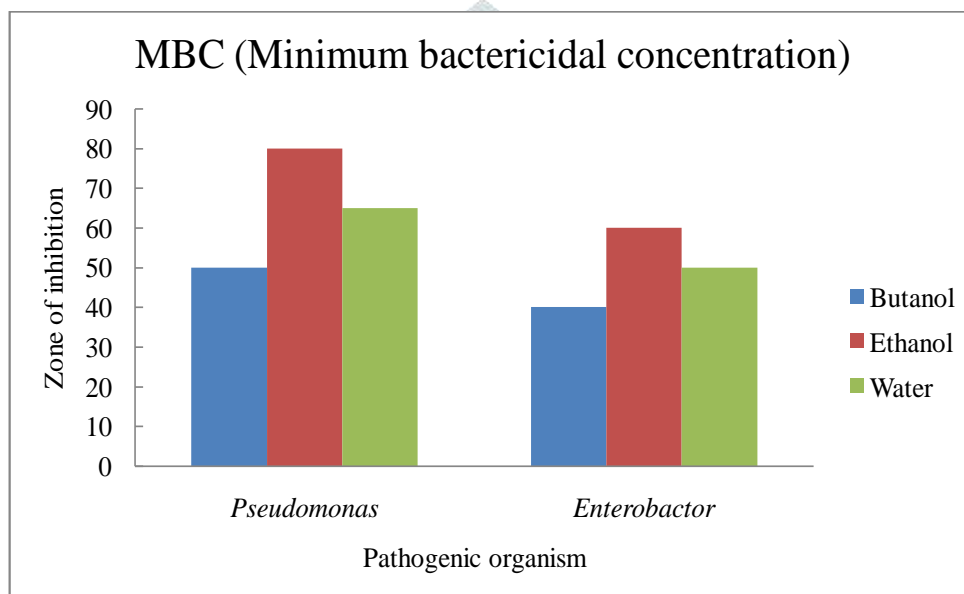
In the present study among the various extracts, the minimal inhibitory concentration of *Cassia tora* L. and *Capsicum annuum* L. against bacterial pathogens, the ethanol extract showed highest activity than all other solvent extracts. The MIC value was found to be in the ranges from 60-65 and 40-45 µg/ml against *Pseudomonas aeruginosa* and *Enterobacter sp.*, respectively. However, the MIC of butanol extracts showed the minimum effect in *Pseudomonas aeruginosa* and *Enterobacter sp.*, around 30-35 and 35-40 µg/ml. The MIC activity of water extract the *Cassia tora* L. and *Capsicum annuum* L. showed least effect in *Pseudomonas aeruginosa* (40-45 µg/ml) and highest effect in *Enterobacter sp.*, (45-50 µg/ml) were noticed. (Similar findings due to leaf extract of *Achyranthus aspera* were reported by Mohan *et al.*, 2008 and Alam *et al.*, 2009).

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the various extracts of *Cassia tora* L. and *Capsicum annuum* L. for the four bacterial pathogen was enumerated (Table 2).

Table 2. The minimum bactericidal concentration of various extracts of *Cassia tora* L. and *Capsicum annuum* L. (µg/ml) against human bacterial pathogens.

Pathogenic organisms	Butanol	Ethanol	Water
<i>Pseudomonas aeruginosa</i>	50	80	65
<i>Enterobacter Sp.</i>	40	60	50



The minimum bactericidal concentration of the various extracts of *Cassia tora* L. and *Capsicum annuum* L. the butanol extract showed highest activity against *Pseudomonas aeruginosa* (50 µg/ml) and the lowest value in *Enterobacter Sp.*, (40 µg/ml) (Table 2). In ethanol extract of *Cassia tora* L. and *Capsicum annuum* L. the MBC value was highest in *Pseudomonas aeruginosa* (80 µg/ml) and lowest in *Enterobacter Sp.*, (60 µg/ml). The water extract of *Cassia tora* L. and *Capsicum annuum* L. exhibited highest activity in *Pseudomonas aeruginosa* (65 µg/ml) and least activity in *Enterobacter Sp.*, (50 µg/ml).

From the overall observations of the present investigation, among the various extracts, the ethanol extract of *Cassia tora* L. and *Capsicum annuum* L. was found to have the highest activity (MIC and MBC) than all other extracts for the tested pathogens. It is interesting to note that regarding minimum inhibitory concentration value of the various extracts of *Cassia tora* and *Capsicum annuum* against tested pathogens, the ethanolic extract was found to inhibit good and that bacteria only at the concentration of 80, 60 µg/ml and thereby it wouldn't affect the beneficial micro flora of intestine. Therefore, it may be recommended for the preparation of plant based drugs against human bacterial pathogens.

5. DISCUSSION

In developing countries the peoples very often suffer from diabetes and the major cause of this disease is uncontrolled diet with the aim to wiping out the problem of diabetes, which leads an increasing stage in developing countries, the World health Organization (WHO, 1964) has constituted a diabetic disease control program which includes studies on traditional medicinal practices, together with evaluation of health education and prevention approaches (Anonymous, 1979 and Syder and Merson, 1982). In traditional system of medicine, plant materials are used as stomachic, demulcent, emetic and also used in diabetes, diarrhoea, gonorrhoea, eye troubles, liver and kidney complaints (Kiritkar and Basu, 1935 and Nadkarni and Nadkarni, 1976). On the basis of traditional use of the plants as a potent antibacterial and antidiabetic agent, the present study was carried out with ethanol extract of *Cassia tora* L. and *Capsicum annuum* L. plant materials to substantiate the folklore claim using different experimental models.

MIC (Minimum Inhibitory Concentration)

In the present study among the various extracts, the minimal inhibitory concentration of *Cassia tora* L. and *Capsicum annuum* L. against bacterial pathogens, the ethanol extract showed highest activity than all other solvent extracts. (Table 1).

Similar observations due to different plants were reported by various workers (Akunyili *et al.*, 1993 and Nascimento *et al.*, 2000). The highest MIC effect was seen in *Pseudomonas aeruginosa* and the least activity in *Staphylococcus aureus* due to ethanol and water extract of *Tamarindus indica* reported by Doughari, 2006 corroborated the result of the present study.

MBC (Minimum Bactericidal Concentration)

The minimum bactericidal concentration of the various extracts of *Cassia tora* L. and *Capsicum annuum* L. the ethanol extract showed highest activity against *Pseudomonas aeruginosa* (50 µg/ml) and the lowest value in *Enterobacter Sp.*, (40 µg/ml). The result of the present study coincided with the report of Sabahat Saeed and Parveen Tariq, 2005 and they reported the leaves of *M. piperata* exhibited highest antibacterial activity against *Pseudomonas aeruginosa*. In ethanol extract of *Cassia tora* L. and *Capsicum annuum* L. the MBC value was highest in *Pseudomonas aeruginosa* 80 µg/ml and lowest in *Enterobacter Sp.*, 60 µg/ml. The present investigation confirmed the previous works carried out by Ellof, 1998, Nascimento, 2000 and Doughari, 2006. (Table 2).

From the overall observations of the present investigation, among the various extracts, the ethanol extract of *Cassia tora* L. and *Capsicum annuum* L. was found to have the highest activity (MIC and MBC) than all other extracts for the tested pathogens. The highest MIC and MBC values of pseudomonas is an indication that either the plant extracts are less effective on some gram negative bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication of the efficiency of the plant extracts. It is interesting to note that regarding minimum inhibitory concentration value of the various extracts of *Cassia tora* L. and *Capsicum annuum* L. against tested pathogens, the ethanolic extract was found to inhibit good and that bacteria only at the concentration of 80 and 60 µg/ml and thereby it wouldn't affect the beneficial microflora of intestine. In preliminary toxicity study it is safe; however chronic toxicity evaluation will be required for human use. Therefore, it may be recommended for the preparation of plant based drugs against human bacterial pathogens.

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