Biolife 2(3):747-752
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

# ANTIMICROBIAL ACTIVITY OF *CASSIA TORA* LINN. (LEAF) AGAINST SOME HUMAN PATHOGENIC MICROBES

**D. Sripriya**

*Department of Biotechnology, Dravidian University, Kuppam, A.P., India.*

E-mail: sripriyashok13@gmail.com

## ABSTRACT

Now days the pathogens microorganisms are very resisted by inorganic compounds. Different organic and aqueous extracts of leaves of *Cassia tora* L. (caesalpiniaceae) were screened for their antimicrobial activity against three human pathogenic bacteria and two fungal strains by disk diffusion method. The pattern of inhibition varied with the solvent used for extraction and the microorganisms tested. Among these extracts, methanol and aqueous extracts showed significant antimicrobial activity against most of the tested microbes. The most susceptible microorganism was *P. aeruginosa* (19 mm zone of inhibition in aqueous extract) followed by *Candida albicans* (7mm zone of inhibition in methanol extract). Preliminary photochemical analysis of different extracts revealed the presence of anthraquinones, carbohydrates, glycosides, steroids, flavonoids, saponins and alkaloids were absent in all the tested extracts.

**Key Words:** *Cassia tora* L. Antimicrobial activity, Human pathogens.

.

## INTRODUCTION

The tremendous progress in human medicines, infectectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance. During the last two well as the appearance of undesirable side effects of certain antibiotics has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected on their ethno-medicinal uses.

*Cassia tora* L. called as Cakramardah or Prapunnatah in Sanskrit, in Telugu Tantenu or Tagirisa and Trade name is Foetid Cassia. *Cassia tora* L. is belongs to family Leguminaceae and sub-family Caesalpiniaceae. It is an ayurvedic pant with huge medicinal importance. Leaves of *C.tora* plant have ethno medicinal importance like paste of leaves is external applied in healing wounds, itching, ring worms, skin diseases, leprosy, bronchitis and fevers and also it is very good laxative. Previous pharmacological investigations showed that *C.tora* leaf extracts have antibacterial, antifungal, antiplasmodial activity.

Moreover studies on this plant showed that the nature and amount of the plytochemical varies according to the season and geographical locations. In India, habit of this plant varies

greatly. It is found as parennual plant in South India (Andhra Pradesh, Tamil Nadu). But as a annual plant in North India. The previous results encouraged us to deepen the studies on antimicrobial properties of the leaves of *C.tora* L. by evaluating the inhibition zone by using agar disk diffusion method. In the present study on attempt was made to investigate the antimicrobial activity and preliminary plytochemical from the leaves of *C.tora* collected from Dravidian University Herbal Garden, Kuppam, Andhra Pradesh, India.

## MATERIALS AND METHODS

**Plant material:**
Leaves of *C.tora* were collected from the Dravidian University, Herbal Garden, Kuppam, Andhra Pradesh, India on December 2012. The plant specimen was botanically indentified and authenticated by comparing the herbarium specimen SVUBOT612 available in the Department of Botany, Tirupati, Andhra Pradesh, India.

**Preparation of the Extracts:**
The leaves were cleaned with deionized water, over dried at $50^0$c for 48 hour and powdered in a grinder. The plant material (250gms) was sequentially extracted with different solvents (Water, Methanol, Petroleum Ether, Benzene and Chloroform) (2500ml) according to their increasing polarity by using soxhlet apparatus for 24 hours at a temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatman No: 1filter paper and then concentrated under vacuum at $40^0$c by using a rotary evaporator. The extractive value of the extracts (percentage yield, water-soluble extractive and alcohol soluble extractive) was calculated. The residual extracts were stored in refrigerator at $4^0$c in small and sterile plastic bottles.

**Organoleptic Properties Determination:**
Organoleptic properties (colour, texture and odour) of the plant extract were determined in respective solvents in wet as well as dry conditions.

**Preparation of test samples:**
Test samples of the plant extract were prepared in DMSO (Dimethyl Sulfoxide) (400mg/ml).

**Tasted Microorganisms:**
Antimicrobial activity of leaves extract was investigated against three registered bacterial isolates and two fungal strains, which were obtained from the Microbial Type Culture Collection (MTCC) from Institute of Microbial Technology, Chandigarh. These included one gram-positive bacterium that is *Staphylococcus aureus* (MTCC3160), two gram-negative bacteria *Pseudomonas aeruginosa* (MTCC424) and *E.coli* (MTCC40) and two fungi *Aspergillus fumigates* (MTCC343) and *Candida albicans* (MTCC183). The tested microorganisms were cultured on Nutrient Agar (HiMedia, Mumbai) for bacteria at $35\pm2^0$c for 24 hours and Potato Dextrose Agar (PDA) (HiMedia, Mumbai) media for fungus at $28\pm2^0$c for 72 hours. The reference strains of bacteria and fungi were maintained on Nutrient agar (HiMedia, Mumbai) and on PDA (HiMedia, Mumbai) slants respectively. The cultures were sub-cultured regularly (every 30 days) and stored at $4^0$c as well as $-40^o$c by preparing suspensions in 10% glycerol.

**Inoculums Preparation:**
A loop full of isolated colonies was inoculated into 4ml peptone water and incubated at $37^0$c for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately $1\text{-}2x10^8$ colony forming units per millilitre (cfu/ml). This 2hours grown suspension was used for further testing.

**Antimicrobial Inoculation:**
The antimicrobial activities of the extracts were determined by the Kirby-Bauer Agar Diffusion Method according to NCCLS standards. Muller Hinton Agar Media (MHAM) (HiMedia, Mumbai) was used for the antimicrobial activity test. Under aseptic conditions in the Bio safety Chamber, 15ml of MHA Media was dispensed

into pre-sterilized petridishes to yield a uniform depth of 4mm and inoculated by the bacterial and fungal culture, respectively. The sterile discs Diameter 6mm were impregnated with 8mg/ disc concentration of the extract and dried for 10-15 minutes. The dried discs were placed on MHA Media surface with flamed forceps and gently pressed down to ensure contact with the agar surface. Streptomycin for bacteria and Ketocanzole for fungus (10µg) were used as positive controls and DMSO was used as a negative control. The discs were spaced for enough to avoid reflections wave from the edges of the petridishes and overlapping rings of inhibition. Finally, the petridishes were incubated for 18 to 24 hours at $35\pm2^0$c for bacteria and $28\pm2^0$c for 48 to 72 hours for fungus. The diameter of zone of inhibition (mean of triplicates ±SD) as indicated by clear area which was devoid of growth of microbes was measured.

**Determination of activity index:**
The activity index of the cured plant extract was calculated as
Activity index (A.I)=Mean of zone of inhibition of the extract/Zone of inhibition obtained for standard antibiotic drug.

**Determination of proportion index:**
The proportion index was calculated as
Proportion Index (P.I)=Number of positive results obtained for individual extract/Total number of tests carried out each extract.

Preliminary analysis of alkaloids, saponins, carbohydrates, glycosides, amino acid, anthraquinones, tannins, phenolic compounds and flavonoids were carried out by using the method of Harbone (Rajeshwar and Lalita, 2013).

**Statistical evaluation:**
The antimicrobial activity was determined by the measuring the diameter of zone of inhibition that is the mean of triplicates ±SD of three replicates.

## RESULTS

**Physical Characterization of Herbal Extracts:**
Among all the plant extracts water extract was found to have maximum extractive yield followed by the methanol and petroleum ether extract (Table-1). Methanol and aqueous extracts were found to have maximum alcohol soluble and water soluble extractive values.

**Table-1: Extravtive value of plant in different solvents**

| Plant extracts | % yield of extracts | % alcohol soluble extracts | % water soluble extracts |
|---|---|---|---|
| Aqueous | 7.0% | 3.5% | 6.32% |
| Methanol | 6.2% | 4.13% | 5.22% |
| Petroleum ether | 5.5% | 2.0% | 1.75% |
| Benzene | 1.7% | 1.22% | 0.57% |
| Chloroform | 1.9% | 0.50% | 0.23% |

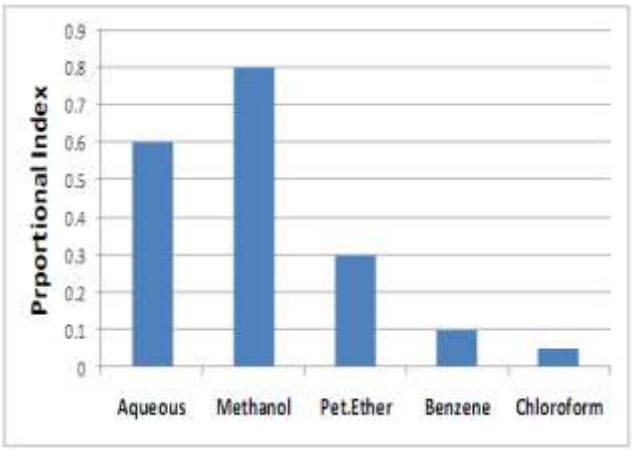
**Table-2: Organoleptic properties of leaf extracts of *Cassia tora* L**

| Plant extract | Wet extract | | | Dry extract | | |
|---|---|---|---|---|---|---|
| | Colour | Texture | Odor | Colour | Texture | Odor |
| Aqueous | Brown | Highly foamy when shaken | Smoke like | Dark brown | Powdery | Light sweet odor |
| Methanol | Brownish red | foamy when shaken | Alcohol | Reddish brown | Gummy | Leafy smell |
| Petroleum ether | Thick green | Water like | Pungent | Black | Gummy | Unpleasant odor |
| Benzene | Black | Not foamy | Sweet | Dark black | Resinous | Unpleasant odor |
| Chloroform | Blackish brown | Not foamy | Pungent | Black | Thick gummy | Bad odor |

**Organoleptic Properties:**
The colour, texture and odor of the plant extracts in different solvents in both wet and dried conditions were characterized (Table-2). The methanolic extracts were better than corresponding aqueous and other organic extracts in retaining the natural fragrances of the plant. This may be due to the preservative ability of methanol (Reducing breakdown of organic compounds by microorganisms). Is enhanced extraction capability or a combination of both. Dried extracts obtained by Lypholization generally appeared darker and more turbid than the wet extracts.

**Antimicrobial activity:**
Among all tested extracts, methanol and water extracts were found to be most active than corresponding organic extracts (Table 3,4). Methanol extract was found to be active against three tested bacteria (*P. aeruginosa, S. aureus & E. coli*) on the other hand, the aqueous extract was effective against one out of three tested bacteria (*P. aeruginosa*) and fungus (*C. albicans*). Aqueous extract was found to have maximum zone of inhibition against *P. aeruginosa* (15mm) while the minimum zone of inhibition was against *E.coli.* (4mm).

**Figure-1: Proportion Index of Antimicrobial Activity of Leaves Extract in Different Solvents**




The petroleum either and Chloroform extracts of the leaves of *C.tora* were effective against *S. aureus* respectively while benzene extract was found to be very inactive against all tested aqueous (*P. aeruginosa)* to the minimum 0.15 for methanol (*E. coli)* extract. The proportion index for the antimicrobial activity among different extracts varied from '0' (benzene) to 0.85 (methanol). All active extracts were stable at $4^0$c in both DMSO and in dry state up to several months and did not show and reduction

**Table-3: Antibacterial activity of leaves extracts of *Cassia tora* L.**

| | Plant extracts (8mg / disc) | | | | | |
|---|---|---|---|---|---|---|
| Tested bacterial strains | Aqueous DIZ/AI | Methanol DIZ/AI | Petroleum either DIZ/AI | Benzene DIZ/AI | Chloroform DIZ/AI | Streptomycin 10µg DIZ |
| *P. aeruginosa* | 15±0.75/0.70 | 6±0.65/0.4 | -/0 | -/0 | -/0 | 23 |
| *S. aureus* | 2±0.1/0 | 10±0.5/0.45 | -/0 | -/0 | -/0 | 22 |
| *E. coli* | -/0 | 9±0.8/0.30 | 4±0.22/0.20 | -/0 | -/0 | 20 |

*DIZ = Diameter of zone of inhibition in mm (mean ±SD), AI=Activity Index*

**Table-4: Antifungal activity of leaves extracts of *Cassia tora* L.**

| | Plant extracts (8mg / disc) | | | | | |
|---|---|---|---|---|---|---|
| Tested bacterial strains | Aqueous DIZ/AI | Methanol DIZ/AI | Petroleum either DIZ/AI | Benzene DIZ/AI | Chloroform DIZ/AI | Streptomycin 10µg DIZ |
| *A. fumigatus* | -/0 | -/0 | -/0 | -/0 | -/0 | 10 |
| *C. albicans* | 4±0.45/0.4 | 7±0.56/0.6 | -/0 | -/0 | -/0 | 12 |

*DIZ = Diameter of zone of inhibition in mm (mean ±SD), AI=Activity Index*

**Table-5: Phytochemical analysis of *Cassia tora* L.**

| Phytochemicals tested | Test used | Leaves extracts | | | | |
|---|---|---|---|---|---|---|
| | | aqueous | methanol | petroleum either | benzene | chloroform |
| Alkaloids | 1. Mayer's test | - | - | - | - | - |
| | 2. Wagner's test | - | - | - | - | - |
| Anthraquinones (free, combined & carbohydrates) | 1. Molish's test | ++ | + | + | - | - |
| | 2. Fehling's test | +++ | + | + | - | - |
| | 3.Barfoed's test | ++ | + | + | - | - |
| | 4.Benedioct's test | + | + | + | - | - |
| Glycosides | Borntager's test | + | + | + | - | - |
| Proteins & Amino acids | 1.Biuret's test | ++ | ++ | + | + | + |
| | 2.Ninhydrin test | ++ | ++ | + | + | + |
| Saponins | Foam test | + | + | + | - | - |
| Phenolic compounds & Tannins | Ferric chloride test | + | + | - | - | - |
| Flavonoids | 1.Alkaline reagent test | + | + | - | - | - |
| | 2. Lead acetate test | + | ++ | + | - | - |

of activity against the sensitive bacteria as compared to the activities of the first day.

**Preliminary Plytochemical Analysis of the Extract:**

Preliminary phytochemical analysis of the plant extracts (aqueous, methanol, petroleum either, benzene, chloroform) showed the presence of anthraquinones, carbohydrates, glycosides, amino acids, saponins, phenolic compounds, tannins and flavonoids while alkaloids are absent in all of the tested extracts (Table-5).

**Discussion:**

Aqueous and methanolic extracts of the leaves of *Cassia tora* were most effective against the tested microorganisms. This is the first attempt to investigate the extract in different solvent on polarity basis. Organoleptic properties and detailed preliminary phytochemical study of the leaf extract of this plant. Antimicrobial activity of the extracts of *Cassia tora* was first time investigated against *P. aeruginosa, S. aureus, E. coli* and *C. Albicans*. However, our results showed remarkable variation in the effectiveness of the leaf extracts against *E. coli.*

The chemical constituents of plants vary depending on the species, variety and part of the plant, with conditions of growth (Environmental factors) and with the age of the plant. However, according our investigation, aqueous and methanolic extract were found to contain both flavonoids and steroids. However, alkaloids were absent in all tested extracts.

At the same time antimicrobial nature of *Cassia tora* L. was also observed the mechanism of action of the antimicrobial activity of the sub-family Casilpinianceae to which *Cassia* belongs may be explained in terms of their ability to induce of ions.

## CONCLUSION

Overall, the present study indicated the antimicrobial properties of leaves extract of *Cassia tora* L. and provides some idea about phytochmical evaluation on *Cassia tora* L. This

study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity.

## REFERENCES


1. Abo KA,Adeyemi AA, Jegede IA,1998. Evaluation of Cassia sieberiana, Cassia alata and Cassia occidentalis for anthraquinones content and antimicrobial activity. Proceedings of the 1st International Workshop on Herbal Medicinal Products,Ibadan,Nigeria,22-24.
2. Arora DS, Kaur GJ, 2007. Antibacterial activity of some Indian medicinal plants. Journal of Natural Medicine, 61: 313-317.
3. Bauer AW, Kirby WM, Sherris JC Turck M, 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 493-496.
4. Bouamama H, Noel T, Villard J, Benharref A, Jana M, 2006. Antimicrobial activities of the leaf extract of two Moroccan Cistus L species. Journal of Ethnopharmacology, 104: 104-107.
5. Chaudhury RR,1999. Herbal medicine for human health. World Health Organization,Geneva,CBS Publishers and Distributors Ltd, New Delhi,130.
6. Harbone JB, 1988. Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd edition, chapmen and Hall, London,1-138.
7. Kong WJ, Wang JB, Jin C, Zhao YL, Dai CM,Xiao XH, Li ZL,2009. Effect of emodin on Candida albicans growth investigated by microcalorimetry combined with chemometric analysis. Applied Microbiology and Biotechnology,83:1183-1190.
8. Mailard JY,2002. Bacterial target site for biocide action. Journal of Applied Microbiology,92:16-27.
9. NCCLS, 2000. Performance standards for antimicrobial disk susceptibility tests: Approval standard M2-A7 7th edition. Pennsylvania: Clinical and Laboratory Standards Institute.
10. Okemo PO, Bais HP, Vivanco JM, 2003. In vitro activities of Maesa lanceolata extracts against fungal plant pathogens. Fitoterapia, 74: 312-316.
11. Oladunmoyem MK, Akinyosoye FA, Adetuyi FC,2007. Comparative studies on the amount of protein, sodium and potassium ions released by methanolic extracts from six cassia species. Asian Journal of Cell Biology,2:29-33.
12. Rajeshwar Y and Lalitha R. 2013. Prelimiminary Phytochemical Screening And *In Vitro* Anthelmintic Effects Of *Acmella Paniculata* Plant Extracts. Biolife. 1(3), 106-112.
13. Samy RP,Lgnacimuthu S,2000. Antibacterial activity of some folklore medicinal plants used bybtribals in Western Ghats of India, journal of Ethnopharmacology,69:63-71.
14. Singh B, Sahu PM, Sharma MK, 2002. Anti-inflamatory and antimicrobial activities of triterpenoids from Strobilanthes callosus Nees. Phytomedicine, 9:355-359.
15. Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, Miert SV, pieters L, Totte J, Viletinck AJ, 2004. In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. Journal of Ethnopharmacology, 93: 27-32.
16. Zampini IC, Cuello S, Alberto Mr, Ordonez RM, Almeida RD, Solorzano E, Isla MI, 2009. Antimicrobial activity of selected plant species from the Argentine puna against sensitive and multiresistant bacteria. Journal of Ethnopharmacology, 124: 499-505.


*DOI: http://dx.doi.org/10.17812/blj2345*
*Received: 14 July 2014;*
*Accepted; 30 August 2014;*
*Available online : 17 September 2014*