World Journal of Agricultural Sciences 16 (4): 227-232, 2020
ISSN 1817-3047

DOI: 10.5829/idosi.wjas.2020.227.232

# Antimicrobial Activity of Leaf and Bark Extracts of *Cassia fistula*

[1]*S. Prabagar,* [2]*J. Nanthakumar,* [3]*S. Thuraisingam and* [4]*J. Prabagar*

[1]Industrial Technology Institute, Colombo 7, Sri Lanka
[2]Department of Botany, University of Jaffna, Sri Lanka
[3]Post Graduate Institute of Agriculture, University of Peradeniya, Sri Lanka
[4]Department of Chemistry, University of Jaffna, Sri Lanka

**Abstract:** *Cassia fistula* L is a medium sized tree and its parts are used in folk medicines for bacterial and fungal diseases. Aqueous extraction of plant materials such as leaf and bark were investigated for anti-microbial property without changing concentration. Results of this study revealed that all extracts had good inhibitory activity against gram positive and gram negative bacteria. The clear zone for *Staphylococcus aureus* and *E. coli* were found with the diameter of 2.5 cm and 2.9 cm respectively, though the inhibitory diameter of *Staphylococcus aureus* was smaller than the streptomycin control. Antifungal activity of bark and leaf extract of *C. fistula* against *Aspergillus* showed the highest inhibitory growth (64.3%). The findings exhibit that leaf and bark extracts have broad spectrum activity and there is a possibility in treatment of infectious diseases.

**Key words:** *Cassia fistula* · Leaf and Bark Extracts · Antimicrobial Activity

## INTRODUCTION

*Cassia fistula* L. is locally known as Konraiin tamil and Ehelain sinhala in Sri Lanka and golden shower [1] in India. The plant is native to India, Amazon and Sri Lanka where it is popularly planted as an ornamental and medicinal tree [2, 3]. It is shedding its leaves annually and 10m tall, often the plant is used as firewood. The heavy, strong and durable wood is fit for cabinetwork, posts, wheels, etc. The bark has been employed in tanning, often in combination with Avaram (*Cassia auriculata*). Since ancient times this medicinal plant is used in Ayurvedic medicine to cure disease caused by microbes. In modern medicine also these anti-microbial plant substances are isolated, purified and used as drugs. The drug is obtained from the pulp around the seed of Cassia fistula.

The leaves are used to treat skin diseases According to Hartwell the plants are used in traditional medicines for tumours of the abdomen, liver, stomach, throat and cancer. *C. fistula* has been described to be useful against skin diseases, tumours, hematemesis, diabetes, cough and retained excretions [1, 4, 5].

Fig. 1: *Cassia fistula* L. (a) leaves, (b) bark and (c) tree

**Corresponding Author:** S. Prabagar, Industrial Technology Institute, Colombo 7, Sri Lanka.

Previous investigation revealed that *C. fistula* has pronounced antibacterial activity against different varieties of bacteria. *C. fistula* was active against *Aspergillus niger* [6]. Perumalsamy *et al*. [7] have also made an effort to evaluate the antibacterial activity of *C. fistula* which showed significant antibacterial activity against *Escherichia coli, Klebsilla aerogenes, Proteus vulgaris* and *Pseudomonas aerogenes.* The aim of this investigation is to evaluate the aqueous extracts of *C. fistula* leaves and barks exhibit anti-microbial properties against several microbes.

## MATERIALS AND METHODS

**Sample Collection and Extract Preparation:** Leaf and bark of *C. fistula* was collected from the premises of University of Jaffna, Sri Lanka. For aqueous extract preparation, 2.5g of plant material was weighed and washed well in tap water. Then they were sterilized by giving a quick dip in alcohol and washed with sterilized water again. The weighed plant material was crushed with 10mL of sterile water and it was filtrated using Whatman'sfilter paper No.1. The filtrate was collected in sterile beaker.

**Anti-Bacterial Activity:** The preliminary screening of antibacterial activity was done using well in agar method. *Bacillus sp, Staphyllococcus aureus, Klebsiella, E. coli* and *Pseudomonas* bacteria were selected for this study. These bacteria were streaked on pure nutrient agar plates separately and stored in refrigerator at 10°C with labelling. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121°C for 15min). Then the petridishes were used for the experiment. Peptone broth and agar standard solution were prepared and 100ppm streptomycin standard solution was used as positive control. The inoculum was spread in nutrient agar plates with bacterial strain and incubated at 37°C for 24 hours. Wells were prepared with UV sterile and wet heat sterile extracts, streptomycin solution and sterile water in agar plates for each bacterial speciesseparately. The diameter of the clear inhibitory zone around the well was measured.

**Anti-Fungal Activity:** *Candida sp, Aspergillus sp, Pencillium, Rhizopus* fungus were selected for this study. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121°C for 15min). The sterilized extracts and potato dextrose agar (PDA) media were mixed well and poured in petri dishes. They were incubated at room temperature for 2 days. After it had grown enough, disc with the diameter of 7mm were cut using the sterile cork borer. The disc of each fungus was placed on the middle of the plate, which contain herbal product with PDA by using sterile loop. Control plates were also maintained without plant extract. These plates were incubated at room temperature for 2 days. Then the diameter of the clear inhibitory zone around the well was measured.

## RESULTS AND DISCUSSION

**Anti-Bacterial Activity:** The extract of *Cassia fistula* found to be effective against the pathogenic bacteria. The diameter of the inhibition zone is shown in Table 1, has exposed the power against pathogenic bacteria.

RanjithVimalraj [8] stated that the inhibition zones for *Staphylococcus aureus* to alcoholic and aqueous extracts were found in the range of 0.7-1.2 cm and 0.7-1.16 cm. Some antimicrobial studies showed that methanolic bark extract with 0.5mg/mL showed maximum inhibition against starins *E. coli* (1.6cm) and *S. aureus* (1.4cm) [9, 10]. The clear zone for *Staphylococcus aureus* and *E. coli* were found with the diameter of 2.5cm and 2.9cm respectively, though the inhibitory diameter of *Staphylococcus aureus* was smaller than the streptomycin control. *C. fistula* inhibited the growth of *E. coli* and produced a clear zone with a large diameter. These results suggest that *C. fistula* can act as more resistant to the pathogenic bacteria of *S. aureus* and *E. coli* compared to other bacteria. The inhibition against the *Pseudomonas* was very low. It failed to show any inhibitory action against *Klebsiella*.

In present study, antibacterial activity on the concentration of all plant product extracts were maintained as 0.25g/mL of the four plates tested, *Cassia* has a wide range of anti-bacterial activity in these concentration. Bark extract of the plant showed higher activity thanleaf extract. However, there was no difference found in the antibacterial activities between the wet heat and UV sterilization.

**Anti-fungal Activity:** Many reports have revealed that some of the *Cassia* species contain antimicrobial substances and the results showed that the extracts were very effective to microbial activity of *Streptococcus pyogenes, Staphylococcus aureus, E. coli, Pencillium aeruginosa*, *Candida albicans Asperigilus niger* and *Aspergilus clavatus* [11-19].

Table 1: The diameter of the zone obtained from samples plant material under UV sterilization

| | Diameter of inhibitory zone (cm) | | |
|---|---|---|---|
| Bacteria | Leaf extract | Bark extract | Streptomycin |
| *E. coli* | 1.9 | 2.975 | 1.75 |
| *Bacillus sp 1* | 2.15 | 2.2 | 2.6 |
| *Bacillus sp 2* | 1.5 | 2.5 | 1.5 |
| *Pseudomonas* | Reduced growth | 1.75 | 1.9 |
| *Staphylococcus aureus* | 1.775 | 2.5 | 2.75 |
| *Klebsiella* | 0 | 0 | 1.875 |

Table 2: The diameter of the zone obtained from samples plant material under wet heat sterilization

| | Diameter of inhibitory zone (cm) | | |
|---|---|---|---|
| Bacteria | Leaf extract | Bark extract | Streptomycin |
| *E. coli* | 1.95 | 2.9 | 1.7 |
| *Bacillus sp 1* | 2.15 | 2.1 | 2.6 |
| *Bacillus sp 2* | 1.65 | 2.5 | 1.5 |
| *Pseudomanas* | Reduced growth | 1.75 | 1.95 |
| *Staphylococcus aureus* | 2.55 | 2.5 | 2.65 |
| *Klebsiella* | 0 | Reduced growth | 1.875 |

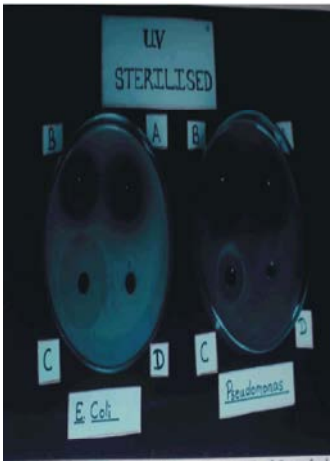


Fig. 2: Effect of UV sterilized extract of leaf (A) and bark (B) of *Cassia fistula* on growth of *E. coli, Pseudomonas*, (C; Streptomycin, D; sterile water)

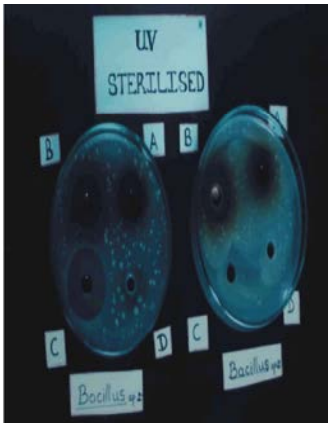


Fig. 3: Effect of UV sterilized extract of leaf (A) and bark (B) of *Cassia fistula* on growth of *Bacillus sp 1, Bacillus sp* 2, (C; Streptomycin, D; sterile water)

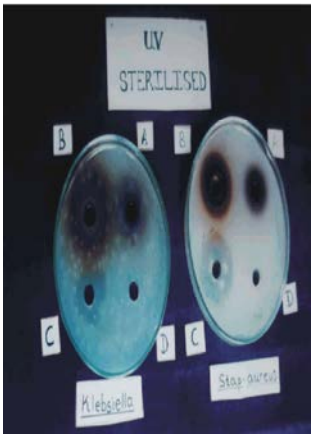


Fig. 4: Effect of UV sterilized extract of leaf (A) and bark (B) of *Cassia fistula* on growth of *Klebsiella, S. aureus*, (C; Streptomycin, D; sterile water)

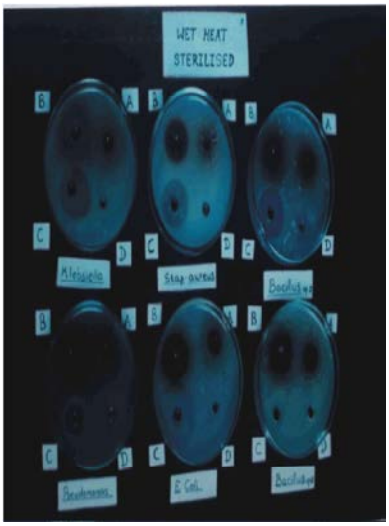


Fig. 5: Effect of wet heat sterilized extract of leaf (A) and bark (B) of Cassia fistula on growth of *Klebsiella, S. aureus, Bacillus sp1, Pseudomonas, E.coli, Bacillus sp 2*(C; Streptomycin, D; sterile water)

*Cassia fistula* has wide range of inhibitory effects on the tested fungi. The leaf extract of *C. fistula* showed lowest inhibition (10%) against yeast while stem bark showed 64% of inhibition. The leaf and bark extract exhibited 22% of inhibition in the growth of *Rhizopus*. Antifungal activity of bark and leaf extract of *C. fistula* against *Aspergillus* showed the highest inhibitory growth (64.3%). *Pencillium* also showed considerable effects as 26.3% by leaves and 36.8% by stem bark.

It has higher anti-microbial activities due to the plant contains phytochemicals such as of saponins, tannins, alkolids, flavonoids, steroids, glycosides, anthraquinones, coumarin, gum, mucilage [20, 21] that can be applied in folk medicine.

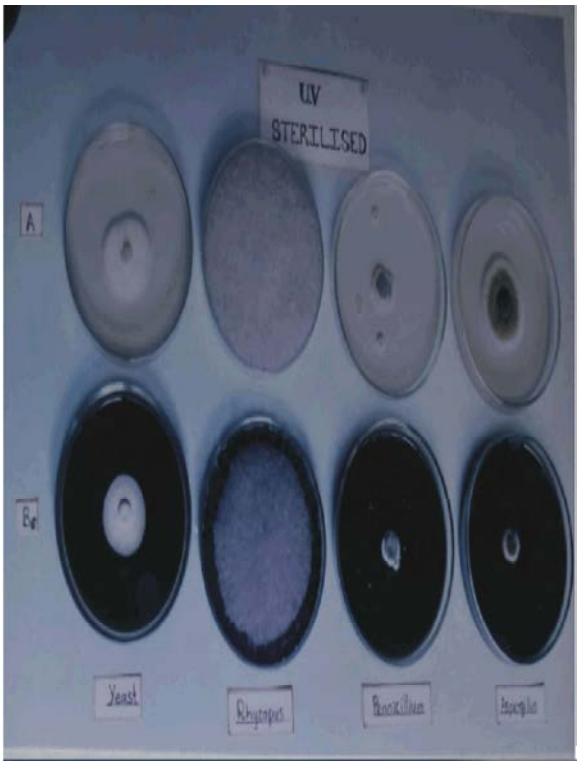


Fig. 6: Effect of UV sterilized leaf extract of *Cassia fistula* on mycelial growth of yeast, *Rhizopus, Pencillium* and *Aspergillus* (A; control, D; extract added)

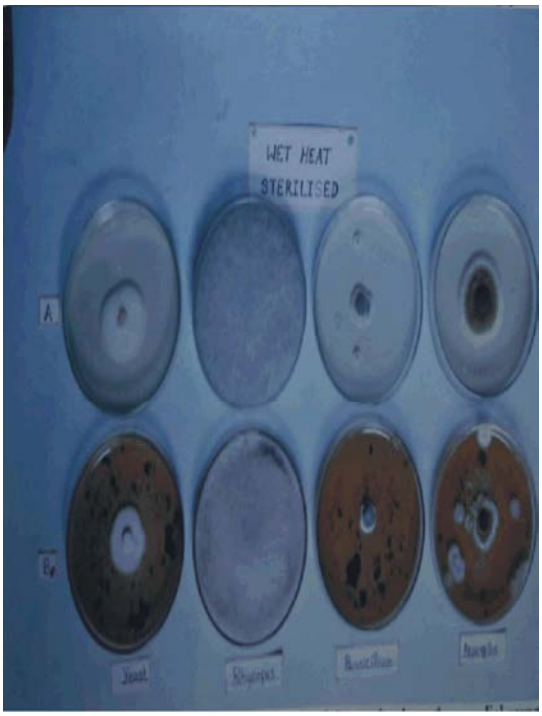


Fig. 8: Effect of wet heat sterilized leaf extract of *Cassia fistula* on mycelial growth of yeast, *Rhizopus, Pencillium* and *Aspergillus* (A; control, D; extract added)

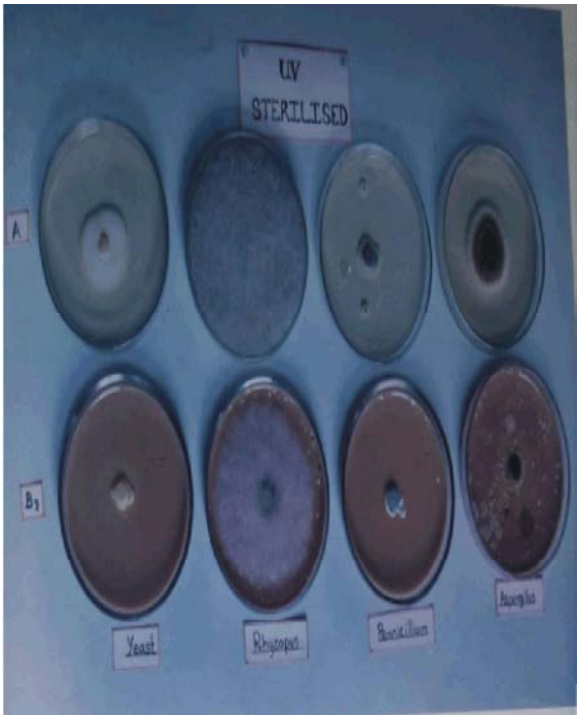


Fig. 7: Effect of UV sterilized bark extract of *Cassia fistula* on mycelial growth of yeast, *Rhizopus, Pencillium* and *Aspergillus* (A; control, D; extract added)

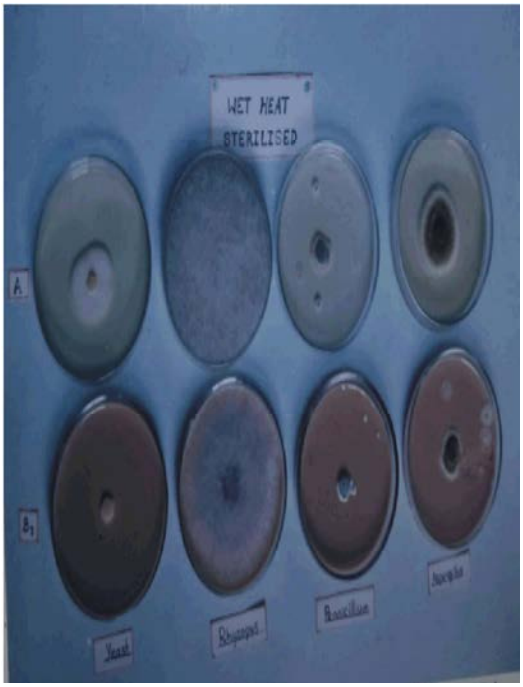


Fig. 9: Effect of wet heat sterilized bark extract of *Cassia fistula* on mycelial growth of yeast, *Rhizopus, Pencillium* and *Aspergillus* (A; control, D; extract added)

Table 3: The diameter of the Mycelial disc in cm and % of growth reduction of samples obtained from *C. fistula* under UV sterilization

| | Diameter of inhibitory zone (cm) | | | | |
|---|---|---|---|---|---|
| Fungus | Leaf extract | Bark extract | Control (without plant extract) | % of growth reduction in leaf extract | % of growth reduction in bark extract |
| *Yeast* | 3.0 | 1.2 | 3.35 | 10 | 64 |
| *Rhizopus* | 7.0 | 7.0 | 9.0 | 22 | 22 |
| *Aspergillus* | 1.5 | 1.5 | 4.2 | 64.28 | 64.2 |
| *Pencillium* | 1.4 | 1.2 | 1.9 | 26.3 | 36.8 |

Table 4: The diameter of the Mycelial disc in cm and % of growth reduction of samples obtained from *C. fistula* under wet heat sterilization

| | Diameter of inhibitory zone (cm) | | | | |
|---|---|---|---|---|---|
| Fungus | Leaf extract | Bark extract | Control (without plant extract) | % of growth reduction in leaf extract | % of growth reduction in bark extract |
| *Yeast* | 3.0 | 1.2 | 3.35 | 10 | 64.1 |
| *Rhizopus* | 7.0 | 7.0 | 9.0 | 22 | 22 |
| *Aspergillus* | 1.5 | 1.8 | 4.2 | 52.6 | 57.14 |
| *Pencillium* | 1.4 | 1.2 | 1.9 | 26.3 | 36.8 |

## CONCLUSION

The present study revealed that the extracts obtained from leaf and bark of *Cassia fistula* figure out strong activitiesagainst the gram negative and gram positive bacteria. And it has a wide range of anti-fungal activities. This study leads to the possibility for the treatment of infectious diseases, however further studies need to be conducted to find isolation and antimicrobial active constituents from the plant.

## REFERENCES


1. Bindhu R. Kamath and Sabeena Kizhedath, 2019. *In vitro* antibacterial activity of *Cassia fistula* Linn methanolic leaf extracts. International Journal of Basic & Clinical Pharmacology, 2: 270-274.
2. Amit Kumar Dutta and Preeti Madharia, 2012. *In- vitro* Evaluation of Antibacterial Activity of *Cassia fistula* Against Different Gram-Positive and Gram-Negative Bacteria, Biomedical & Pharmacology Journal, 5(1): 185-188.
3. Aarti V. Pawar, Sayali J. Patil, Suresh and G. Killedar, 2017. Uses of *Cassia fistula* Linn as a Medicinal Plant. International Journal of Advance Research and Development, 2(3): 85-91.
4. Asolkar, L.V., K.K. Kakkar and O.J. Chakre, 1992. Second supplement to glossary of Indian medicinal plant with active principles. New Delhi: Publication and Information Directorate, CSIR, pp: 414.
5. Kirtikar, K.R. and B.D. Basu, 2006. Indian Medicinal Plants, International Book Distributors, 2: 856-860.
6. Valsaraj, R., P. Pushpangadan, U.W. Smitt and A. Adsersen, 1997. Antimicrobial screening of selected medicinal plants from India. Journal of Ethnopharmacology, 58(2): 75-83.
7. Perumalsamy, R., S. Ignacimuthu and A. Sen, 1998. Screening of Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacology, 62(2): 173-181.
8. Ranjith Vimalraj, T., S. Saravanakumar, S. Vadivel, S. Ramesh and S. Thejomoorthy, 2009. Antibacterial effect of *Cassia fistula* extract on pathogenic bacteria of veterinary importance. Tamilnadu Journal of Veterinary & Animal Sciences, 5(3): 109-113.
9. Zeeshan Afsar and Salma Khanam, 2013. Antimicrobial activity of the extracts of *Cassia fistula* and *Milletia pinnata* against Ampicillin resistant strains of clinical origin. Asian Journal of Phytomedicine and Clinical Research, 1(4): 245-252.
10. Deepikapriyadharshini, S. and V. Sujatha, 2013. Determination of four *Cassia* species for their antioxidant and antimicrobial properties. Indian Journal of Applied Research, 3(3).
11. Agarkar, S.V. and D.R. Jadge, 1999. Phytochemical and pharmacological investigations of genus Cassia: a review. Asian Journal of Chemistry, 11: 295-299.
12. Caceres, A. B.R. Lopez, M.A. Giron and H. Logeman, 1991. Plants used in Guatemala for treatment of dermatophytic infections. Screening for antimycotic activity for 44 plant extracts. J. Ethnopharmacol., 31: 263-276.

13. Caceres, A., B.R. Lopez, X. Juarez, J. Del Aguila and S. Garcia, 1993. Plants used in Guatemala for treatment of dermatophytic infections. Evaluation of antifungal activity of seven American plants. J. Ethnopharmacol., 40: 207-213.
14. Crockett, C.O., F. Guede-Guina, D. Pugh, M. Vangah Manda, J. Robinson, J.O. Qlubadewo and R.F. Ochillo, 1992. *Cassia alata* and the preclinical search for therapeutic agents for the treatment of opportunistic infections in AIDS patients. Cell Mol. Biol., 35: 505-511.
15. Fuzellier, M.C., F. Mortier and P. Leetard, 1982. Antifungal activity of *Cassia alata* L. Ann. Pharm. Fr., 40: 357-363.
16. Jayalakshmi, T. and M.G. Amarnath Reddy, 2018. Antibiotic sensitivity test against medicinal plant. International Journal of Pure and Applied Mathematics, 119(12): 1319-1330.
17. Nafisa Hassan Ali, Shaheen Faizi and Shahana Urooj Kazmi, 2011. Antibacterial activity in spices and local medicinal plants against clinical isolates of Karachi, Pakistan. Pharmaceutical Biology, 49(8): 833-839.
18. Somchit, M.N., I. Reezal, I. Elysha Nur and A.R. Mutalib, 2003. *In vitro* antimicrobial activity of ethanol andwater extracts of *Cassia alata*. J. Ethnopharmacol., 84: 1-4.
19. Villasenor, I.M., A.P. Canlas, M.P.I. Pascua, M.N. Sabando and L.A.P. Soliven, 2002. Bioactivity studies on *Cassia alata* Linn. leaf extracts. Phytother. Res., 16(1): S93-S96.
20. Senthamil Pavai1, B. Thamarai Selvi and R. Anisha, 2019. Antimicrobial and antioxidant activity of the golden shower, *Cassia fistula*, Bioscience Biotechnology Research Communications, 12(2): 496-503.
21. Sharma, D.N. and Seema Rai, 2019. Antibacterial and antifungal activity of *Cassia fistula* and *Bauhinia variegata*. International Research Journal of Pharmacy, 10(5).