International Journal of Research in

Engineering and Bioscience

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# IN-VITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL INVESTIGATION ON LEAF EXTRACTS OF *CASSIA FISTULA*

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**Abstract**

Phytochemical constituents of the plants are effectively used to prepare wide range of ailments and antibiotics. In this present study methanol, chloroform and aqueous extracts of *Cassia fistula* leaf were subjected for antimicrobial activity by well diffusion method against six bacterial strains like *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis.* The methanol and chloroform extract exhibited strong inhibitory activity against all the tested organisms. The phytochemical screening of plant extracts showed the presence of carbohydrates, proteins, alkaloids, flavanoids, steroid, saponins and tannins.

**Keywords:** Medicinal plant, *Cassia fistula,* Antimicrobial activity, Phytochemical constituents

A R T I C L E I N F O : Received: 10.04.2013 Received in revised form: 24.04.2013
Accepted: 24.04.2013

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## Introduction

Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. Medicinal values of many plants still remain unexplored for its enumerable activity of compounds responsible for later. Pharmogonostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents (Anushia *et al*., 2009). Over 60% of the world human population, 80% in developing countries depends directly on plants for their medicinal purposes, since ancient times medicinal plants have been harvested from the wild (Dhillon *et al*., 2002).

Plant produces phytochemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently (Argal and Pathak, 2006). Many plant extracts have been shown to inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in defense reactions of plants, towards infections by pathogenic microorganisms (Fawcett and Spencer, 1976).

*Cassia fistula* L., (Fabaceae, Caesalpinioideae), a very common plant known for its medicinal properties is a semi-wild in nature. It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil (Prashanth Kumar *et al.,* 2006). 1. It is commonly known as Amultas and in English popularly called "Indian Laburnum" has been extensively used in Ayurvedic system of medicine for various ailments. It is a deciduous tree with greenish grey bark, compound leaves, leaf lets are each 5-12 cm long pairs, a semi-wild tree known for its beautiful bunches of yellow flowers and also used in traditional medicine for several indications (Gupta, 2010). In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and its use in the treatment of rheumatism,

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hematemesis, pruritus, leucoderma, and diabetes (Alam *et al.*, 1990).

The WHO (World Health Organization) considers phytotheraphy in its health programs suggested basic procedures for validation of drugs from plants origin in developing countries. Nowadays in medicinal plants are rarely used as antioxidants in traditional and modern medicines. In the present study, an attempt has been made to investigate the antibacterial activity of different solvent extracts of leaf of *C. fistula* obtained by sequential extraction method, against Gram-positive and -negative bacteria. In addition to this, presences of phytochemical constituents in the respective extracts were also carried out.

## Materials and Methods

### Plant sample

Leaves and flowers of *Cassia fistula* were collected from Marthandam, Kanyakumari District. It was rinsed severally with clean tap water to make it dust and debris free and subjected to drying in a dark place at room temperature for few days. The dried leaves were ground in electric chopper to get fine powder form for further use.

### Preparation of extracts

The powder was subjected to successive extraction with methanol, Chloroform and aqueous using soxhlet apparatus and cold extraction using the same in the shaking condition. After solvent evaporation, each of these extract was weighed and preserved in room temperature until further use.

### Antimicrobial activity assay

Four bacterial cultures (two Gram positive and two Gram negative) namely *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* were used in this investigation. The media used for antibacterial test were Nutrient Broth (NB) and Muller Hinton agar (MHA). The test bacterial strains were inoculated into NB medium and incubated at $37^0$C for 24hrs. After the incubation period, the culture tubes were compared with the turbidity standard.

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Antimicrobial activity was carried out by Agar well diffusion method. Fresh bacterial cultures of 0.1ml having 108 CFU were spread onto MHA plate using sterile cotton swab. The wells were punched off into MHA medium with sterile well puncture. Each well filled with 50µl of each plant extracts by using micro pipette in aseptic condition. The plates were then kept in a refrigerator to allow pre-diffusion of extract for 30 minutes and further incubated in an incubator at $37^0$C for 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition.

**Preliminary phytochemical screening**

All the plant extracts were subjected to systematic phytochemical screening for the presence of chemical constituents (Kokate, 1993; Hosamani *et al*., 2011).

**Tests for Carbohydrates**

***Benedict's test*** **-** In a boiling test tube, equal volume of Benedict's reagent and plant extracts was taken then heated in boiling water bath for 5 min. Solution may be appeared green, yellow or red depending on amount of reducing sugar present in test solution.

**Tests for Proteins**

***Biuret test*** **-** with 3 ml plant extracts, add few drops of 4% sodium hydroxide and 1% copper sulphate solution formation of violet color indicates the presence of proteins.

**Tests for Alkaloids**

A small portion of the extract is stirred with few drops of 1% Hydrochloric acid and filtered. The filtrate is treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

**Detection of Flavonoids**

***Ferric chloride test*** **-** The plant extracts were treated with few drops of 5% ferric chloride solution. The formation of blackish green confirmed the presence of flavonoids.

**Test for Saponins**

***Foam test*** **-**The extract was diluted with 20ml distilled water, the suspension was shaken well in graduvated cyclinder for

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15 minutes formation of foam indicates the presences of saponins.

**Tests for Steroids**

***Leibermann- Burchards test*** **-** The plant extract was treated with 50% sulphuric acid and a few drops of acetic anhydride are added. The development of reddish brown ring indicates the presence of steroids.

**Tests for Tannins**

A portion of plant extracts (2-3ml) add 5% ferric chloride solution, development of blue green color indicates the presence of tannins.

**Result and Discussion**

In this present study, the methanol extract of *Cassia fistula* leaves exhibit strong antimicrobial activity against all the tested organisms. *Bacillus cereus* (18mm), *Staphylococcus aureus* (14mm), *Escherichia coli* (16mm), *Klebsiella pneumoniae* (16mm), *Pseudomonas aeruginosa* (12mm) and *Proteus mirabilis* (16mm). The chloroform extract showed good activity against *Bacillus cereus* (18mm), *Staphylococcus aureus* (14mm), *Escherichia coli* (14mm), *Klebsiella pneumoniae* (14mm), *Pseudomonas aeruginosa* (10mm) and *Proteus mirabilis* (12mm). Finally the aqueous extracts showed moderate activity against *Bacillus cereus* (10mm), *Staphylococcus aureus* (10mm), *Klebsiella pneumoniae* (8mm) and *Proteus mirabilis* (8mm).

The results (Table 1) revealed that the Gram positive bacteria are strongly inhibited by all the extracts of *Cassia fistula* (L) than the Gram negative bacteria and it shows minimum activity against *Pseudomonas aeruginosa.* This study was supported by previous studies; Perumal Samy *et al*. (1998) reported moderate antibacterial activity of *C. fistula* against a wide spectrum of bacteria such as *E. coli, Bacillus mycoides, B. subtilis, Mycobacterium smegmatis, Klebsiella aerogenes, Pseudomonas aerogenes,* and *Proteus vulgaris*. Valsaraj *et al*. (1997) studied the antibacterial activity of *C. fistula* seeds by broth dilution method. Their observation at 12.5 mg/ml concentration, *E. coli* and *B. subtilis* were inhibited while *S.*

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*aureus* was inhibited at a concentration 6.25 mg/ml. Antibacterial activity of Fruit of *C. fistula* was observed by Kumar *et al*. (1966) using agar dilution streak method. Only *E. coli* was moderately inhibited, whereas no inhibition was found in case of *B. subtilis* and *S. epidermidis*. Antibacterial activity of aqueous and alcoholic extract of stem bark of *C. fistula* was done by Vimalraj *et al*. (2009). In their study, the alcoholic extracts recorded greater inhibition against *S. aureus* compared with aqueous extract.

**Table: 1 Zone of inhibitions of *Cassia fistula* leaves extracts**

| SL. No | Test organisms | Methanol | Chloroform | Aqueous |
|---|---|---|---|---|
| 1 | *Bacillus cereus* | 18 | 18 | 10 |
| 2 | *Staphylococcus aureus* | 14 | 14 | 10 |
| 3 | *Escherichia coli* | 16 | 14 | - |
| 4 | *Klebsiella pneumoniae* | 16 | 14 | 8 |
| 5 | *Pseudomonas aeruginosa* | 12 | 10 | - |
| 6 | *Proteus mirabilis* | 16 | 12 | 8 |

Zone of inhibition in 'mm'; '-'no activity

The Phytochemical analysis of *Cassia fistula* leaves showed the presence of carbohydrate, protein, alkaloids flavanoids, saponin and tannin (Table 2). Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores (Bonjar, 2004). Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection (Marjorie, 1999). Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes (Raquel, 2007). Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al*., 1996). Tannins bind to proline rich proteins and interfere with the

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protein synthesis (Shimada, 2006). Plants are the source of inspiration for novel drug compounds as plant-derived medicines have made significant contribution toward human health. Isolation of bioactive compounds from plant material largely depends on the type of solvent used in the extraction procedure (Sujogya *et al*., 2011).

**Table: 2 Phytochemicals of *Cassia fistula* leaves extract**

| SL. No. | Chemical constituents | Methanol | Chloroform | Aqueous |
|---|---|---|---|---|
| 1 | Carbohydrates | + | + | + |
| 2 | Protein | + | - | + |
| 3 | Alkaloids | + | + | - |
| 4 | Flavanoids | + | + | - |
| 5 | Steroids | - | + | - |
| 6 | Saponin | + | + | - |
| 7 | Tannin | + | + | + |

'+' presence of compounds; '-'absence of compounds

## Conclusion

The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Plant herbs were widely used in traditional medicine and have been reported to posses hepatoprotective, anti-inflammatory anti-microbial properties. Above this aspect, in the present study *Cassia fistula* leaf extracts was screened to detect the presence or absence of several bioactive compounds, which are reported to cure different diseases and ailments. It was evidence that the presence of antimicrobial substances in the medicinal plant *Cassia fistula* and further study is required to find out the active component of medicinal value.

## Acknowledgement

The authors thank the Noorul Islam Centre for Higher Education President Dr. A.P. Majeed Khan, Vice chancellor Dr. R. Perumal Samy and Advisor Dr. Siva Subramanian for the constant support and providing necessary facilities.

## References

Agarry O O., Olaleye M T and Bello-Michael C O., Comparative Antimicrobial Activities of *Aloe vera* gel and leaf. Afr. J. Biotechnol, 4(12): 1413-141 (2005).

International Journal of Research in

Engineering and Bioscience

IJREB

Journal home page: www.ijreb.org

Alam MM., Siddiqui MB., Hussian W., Treatment of diabetes through herbal drugs in rural India. Fitoterapia, 61:240–242 (1990).

Anushia C., Sampathkumar P and Ramkumar L., Antibacterial and antioxidant activities in *Cassia auriculata*. Global Journal of Pharmacology, 3 (3): 127-130 (2009).

Argal A and Pathak AK., CNS activity of *Calotropis gigantean* roots. J Ethnopharmacology, 106: 142-145 (2006).

Bonjar GHS., Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J Biol Sci 2004; 4: 405-412.

Dhillon SS., Svarstad H., Amundsen C., Bugge., Bioprospecting, effects on development and environment, AMBIO, 31: 491-493 (2002).

Fawcett C H and Spencer D M., Plant chemotherapy with natural products. Ann. Rev. Phytopathol, 8: 403- 418 (1976).

Gupta R.K., Medicinal and Aromatic plants, CBS publishers & distributors, 1st edition, 116-117 (2010).

Hosamani P A., Lakshman H C., Sandeepkumar K and Rashmi C Hosamani., Antimicrobial Activity of Leaf extract of *Andrographis paniculata* Wall Science Research Reporter, 1(2): 92 – 95 (2011).

Kokate C K., Practical Pharmacognosy (4th Ed.) Vallabh Prakashan 1993; pp 107-111, 178-181.

Kumar A., Pande CS., Kaul RK., Chemical examination of *Cassia fistula* flowers. Indian J Chem, 4:460 (1966).

Marjorie C., Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews, 12: 564-582 (1999).

Perumal Samy R., Ignacimuthu S., Sen A., Screening of 34 Indian medicinal plants for antibacterial properties, J Ethnopharmacol, 62:173–82 (1998).

Prashanth Kumar V., Chauhan N.S., Padh H. and Rajani M., Search for antibacterial antifungal agents from selected Indian medicinal plants, J. Ethnopharmacol, 107, 182-188 (2006).

Raquel F. Epand., Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. Biochimica et Biophysica Acta, 2500–2509 (2007).

International Journal of Research in

Engineering and Bioscience

IJREB

Journal home page: www.ijreb.org

Shimada T., Salivary proteins as a defense against dietary tannins. J. Chem. Ecol, 32 (6): 1149-1163 (2006).

Sujogya K. Panda, Padhi L P., and Mohanty G., Antibacterial activities and phytochemical analysis of *Cassia fistula* (Linn.) leaf. J Adv Pharm Technol Res, 2(1): 62–67 (2011).

Valsaraj R., Pushpangadan P., Smitt UW., Adsersen A., Nyman U., Antimicrobial screening of selected medicinal plants from India. J Ethnopharmacol, 58:75–83 (1997).

Vimalraj TR., Saravanakumar S., Vadivel S., Ramesh S., Thejomoorthy P., Antibcaterial effects of *Cassia fistula* extracts on pathogenic bacteria of veterinary importance. Tamilnadu J Veter Anim Sci, 5:109–1 (2009).

Zablotowicz RM., Hoagland RE., Wagner SC., Effect of saponins on the growth and activity of rhizosphere bacteria. Adv Exp Med Biol, 405:83-95 (1996).