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Research Article

Antimicrobial activity of different fractions of roots of *Cassia fistula* Linn against various microbial strains

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Abstract

In the present investigation, the different fraction of roots of *Cassia fistula* Linn. was evaluated for antimicrobial activity against P. vesicularis, Streptococcus faecalis, Aeromonas hydrophilia, Salmonella typhae, Staphylococcus cohni, Serratia ficaria and E.coli at concentration of 12.5 mg/ml, 25 mg/ml, 37.5 mg/ml and 50 mg/ml. . The results indicate the different fraction of roots of *Cassia fistula* Linn. might be exploited as natural drug for the treatment of several infectious diseases caused by these organisms as it posses the antimicrobial action.

Key-Words: Antimicrobial activity, Streptococcus faecalist, E.coli, *Cassia fistula* Linn. etc.

Introduction

Today, infectious diseases are the second major cause of death worldwide and third leading cause of death in economically advanced countries. The ability of bacteria to deceive any kind of conventional therapy has become apparent and pathogens resistant to one or more antibiotics are emerging and spreading worldwide.

Resistant pathogens lead to higher expenditure on treatments due to extended stay in hospitals and expensive medicines. There is an urgent need for a sustainable supply of new, potential and safer antibacterial drugs having no cross-resistance to currently used antibiotics. Nature so far has been proved the treasure of potential remedies for diseases. It is more relevant for treatment of infectious diseases. Most of the antibacterials in use today could be discovered on the basis of information obtained from the study of natural products from microbes, marines and plants. Medicinal chemists do systematic studies and provide the tools for optimization of natural products to obtain drug molecules with improved pharmacokinetic, physicochemical and toxicological properties. Advance studies have been conducted on many classes in order to identify the drug target and mechanism of action. (Singh GS et al., 2011)

The emergence of resistance to antimicrobial agents continues to evolve substantially, influencing the evaluation and treatment of infections in nosocomial and health care– associated settings and in the community. (Luke FC et. al., 2011)

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Cassia fistula Linn. (Caesalpiniaceae) is commonly known as golden shower, Amaltas, Bahava, Amaha. The plant is 6-9 m. in height. trunk straight; bark smooth and pale grey when young, rough and dark brown when old; branches spreading, slender. Leaves 23-40 cm. long; main rhachis pubescent; stipules minute, linear-oblong, obtuse, pubescent. Leaflets 4-8 pairs, ovate or ovate.oblong, acute, 5.12.5 by 3.8-9.5 cm., bright green and glabrous above, paler and silvery-pubescent beneath when young, Pods 30-60 cm. long, 2.2.5 cm. diem., pendulous, cylindric, nearly straight, smooth, shining, brown-black, not torulose, indehisecnt, with numerous (40.100) horizontal seeds immersed in a dark coloured sweetish pulp, and completely separated by transverse dissepiments. Seeds broadly ovate, 8 mm. long, slightly less in breadth, and 5 rim. thick. The plant is found in all over India. It is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. It is an ornamental tree with beautiful bunches of yellow flowers (Kirtikar KR et al., 1999)

The root is useful in skin diseases, leprosy, tuberculous glands, syphilis; cures burning sensatfon. The root is generally given as a tonic and febrifuge. It has been found to act as a strong purgative. The plant is used by natives in Rhodesia as a remedy for malaria, blackwater fever, blood-poisoning, anthrax, and dysenteries. Every part of the plant is prescribed in combination with other drugs for the treatment of snake-bite and scorpion-sting (Wealth of India, 2007).

The roots are used in chest pain, joint pain, migraine and blood dysentery. The alcoholic fraction of the root lowered the blood sugar level up to 30 % after 2 hrs when tested on fasted albino rats. The roots contain 7-methylphyscion, betulinic acid, and β-sitosterol.

Kaempferol and proanthocyanidin have been isolated from the acetone fraction of *C. fistula* flower. A bianthraquinone glycoside, fistulin together with kaempferol and rhein has been isolated from ethanol fractions of *C. fistula* flowers. Besides phenolics and their derivatives, a certain amount of alkaloids have also been reported in *C. fistula* flowers; traces of triterpenes have been observed in both flowers and fruits. A diterpene, 3B-hydroxy-17-norpimar-8(9)-en-15-one was isolated from the pods of *C. fistula*. (Duraipandiyan V et.al., 2011)

The bark and hardwood of *Cassia fistula* Linn. contain fistucacidin, an optically inactive leuco anthocyanidin. 3.4.7.8.4'~pentahydroxyflavan (I) along with barbaloin (II) and rhein (IJI). (Murn VK et.al., 1967)

Material and Methods

Procurement and authentication of plant

The root of the plant *Cassia fistula* Linn. was collected from fields of Walgaon Road, Amravati, Maharashtra and interiors of Bhopal, Madhya Pradesh respectively. The plant has been authenticated by Safia College of Science, Peer Gate, Bhopal, (Madhya Pradesh), and were given the voucher specimen number 160/Bot/Safia/2010. The authenticated roots were dried under shade and then coarsely powdered with the help of mechanical grinder. The powdered was passed through sieve no. 40 and stored in an airtight container for fractionation.

Preparation of flavonoids rich fraction from *Cassia fistula* Linn. roots

Fractionation of the powdered roots of the plant was carried out with the help of water by decoction method. Special care was taken while decoction process regarding temperature that it should not exceeding more than 40˚C ± 5˚C as it may cause the precipitation or crystallization of some phytoconstituents which will never solublized into any solvents in further process. Then this aqueous fraction was filtered and alcohol (Ethanol) was added slowly into this aqueous liquid fraction to precipitate out polysaccharides which are present into the roots of individual plant.

Then the solution was filtered and obtained filtrate was evaporated to 1/4th of the total volume. After evaporating 1/4th of the total volume of the solution, it was successively fractioned with equal amount of ethyl acetate with the help of separating funnel to get separate fraction of ethyl acetate fraction of the roots of the plant. Then the ethyl acetate fraction was acidified with 0.1 N HCL to increase the yield of fraction. Then this ethyl acetate fraction of root of the plant was evaporated to get precipitate which is then dissolved in methanol and evaporated slowly to get crystalline powder of flavonoids (Al – Meshal et al., 1985; Cibin et al., 2006). Finally obtained powder was further investigated for the presence of active phytoconstituents. During this investigation it was observed that it gave positive response to Shinoda test for the flavonoids.

Preparation of Saponins rich fraction from *Cassia fistula* Linn. roots

Same plant materials were used for getting saponins rich fraction. Pulverised plant materials was treated with ethanol: water (70:30) for maceration till seven days after defatting with petroleum ether (40 :60). Mixture was agitated at regular interval in this period. Obtained fraction after filtration with muslin cloth followed by filter paper was concentrated using rotary vacuum evaporator (40˚C), precaution was kept that fraction do not get powdered. Concentrated fraction was further treated with n-butanol to get n-butanol soluble fraction. n-butanol soluble fraction was further treated with chilled diethyl ether. After treating with chilled diethyl ether, precipitates were formed. This mixture with precipitate was kept at -20˚C for 24 hrs. Precipitates were further separated by centrifugation. These precipitate were further dissolved in methanol and methanol was evaporated slowly, to get crystalline powder.

Powder was further investigated for presence/absence of various phytoconstuents. It was observed hat powder gave positive results for froath test and hemolysis test, which ascertained presence of saponins in powder (Cabrini et al., 2008).

Antimicrobial Activity of different fractions by Well Diffusion Method

Antimicrobial activity of both the fraction from *Cassia fistula Linn* was carried out by the procedures given by (Indian Pharmacopoeia (2007), Bansal R et al., (2010), Jain NK (2005), with some modifications.

Preparation of sample stock solutions

Firstly the solubility of samples was tested in different solvent system. Those solvent systems were used in which the sample is completely soluble. A mother stock of 1mg/ml was prepared and then further dilutions of 50%, 37.5%, 25% and 12.5% were prepared.

Inoculation of Test Plates

Wells of 6mm were cut using a sterile cork borer. A sterile cotton swab is dipped into the 24 hrs growing bacterial culture. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surface of a nutrient agar plate is inoculated by spreading the swab over the entire sterile agar surface. This procedure is repeated by spreading two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed. The lid may be left open for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Application of Sample To Inoculated Agar Plates

80 µl of each dilution of each sample was poured in the respected well. The plates were then incubated at 37°C in an incubator for 16-18 hrs (for antifungal activity the plates were incubated for 22-24hrs).

Reading Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers. This was held on the back of the inverted Petri plate.

Results and Discussion

Antimicrobial activity of both fractions i.e. flavonoid and saponins fraction of *Cassia fistula* Linn. was investigated against P. vesicularis, Streptococcus faecalis, Aeromonas hydrophilia, Salmonella typhae, Staphylococcus cohni, Serratia ficaria and E.coli at concentration of 12.5 mg/ml,

25 mg/ml, 37.5 mg/ml and 50 mg/ml. Antimicrobial effect was ascertained on the basis of zone of inhibition.

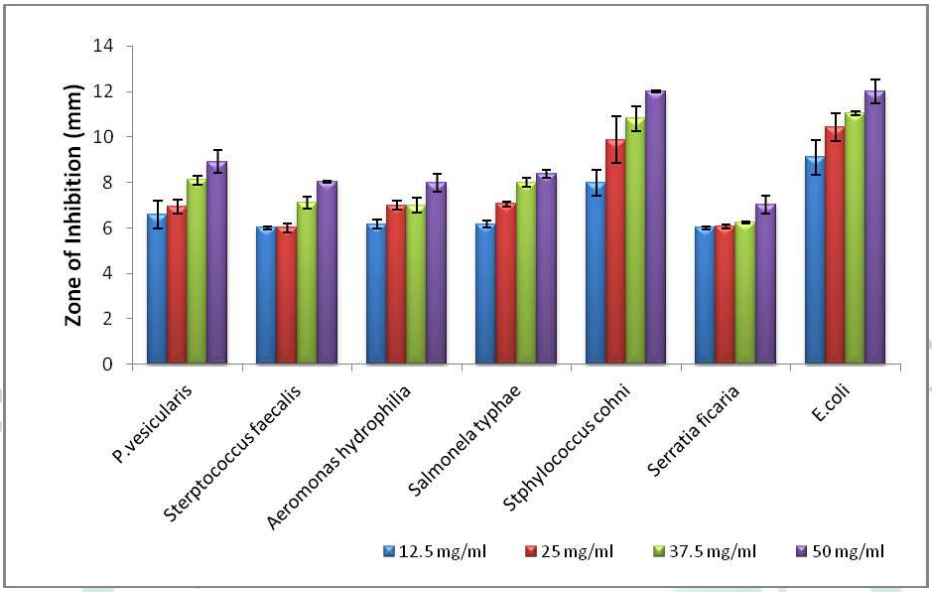

Fig 1: Antimicrobial activity of *Cassia fistula* Linn. Flavonoids rich fraction (CFF)

Microbial Strains	Fractions Concentration			
	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	6.60±0.62	6.95±0.30	8.10±0.20	8.92±0.51
Sterptococcus faecalis	6.00±0.05	6.00±0.21	7.12±0.25	8.02±0.05
Aeromonas hydrophilia	6.17±0.20	7.00±0.20	7.00±0.31	7.97±0.40
Salmonela typhae	6.17±0.17	7.05±0.10	8.00±0.20	8.37±0.18
Stphylococcus cohni	7.97±0.57	9.87±1.03	10.82±0.55	12.00±0.05
Serratia ficaria	6.00±0.07	6.07±0.09	6.25±0.05	7.02±0.38
E.coli	9.10±0.75	10.45±0.61	11.05±0.10	12.00±0.53

Table 1: Antimicrobial activity of *Cassia fistula* Linn. Flavonoids rich fraction (CFF)

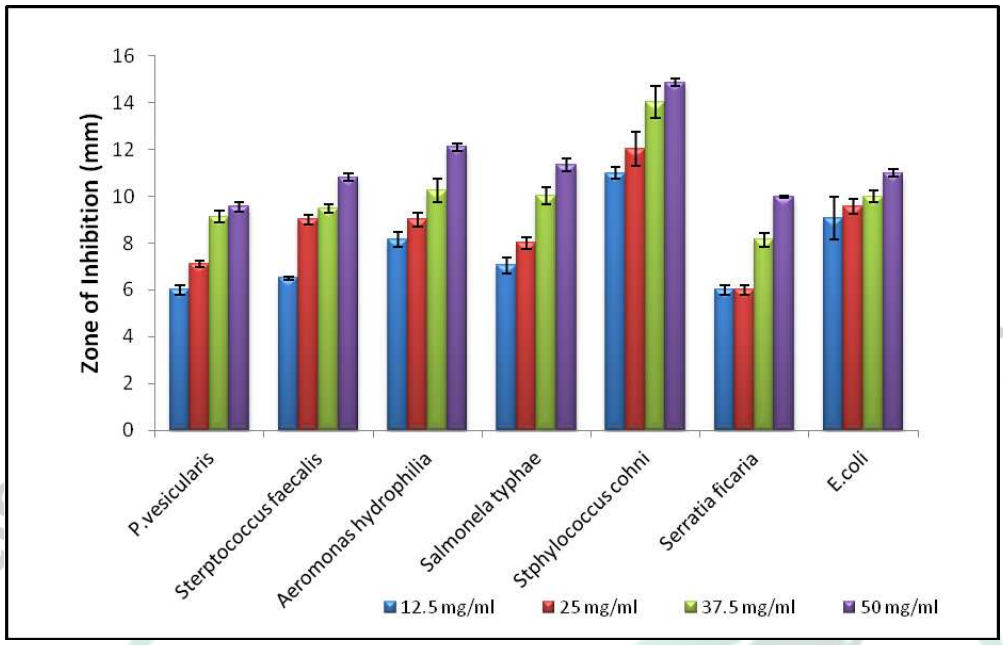

Fig 2: Antimicrobial activity of *Cassia fistula* Linn. Saponins rich fraction (CFS)

Microbial Strains	Fractions Concentration			
	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	6.00±0.20	7.10±0.14	9.12±0.25	9.55±0.19
Sterptococcus faecalis	6.50±0.08	9.00±0.21	9.47±0.17	10.82±0.14
Aeromonas hydrophilia	8.15±0.33	9.00±0.30	10.25±0.50	12.10±0.14
Salmonela typhae	7.05±0.33	8.00±0.24	10.02±0.36	11.35±0.26
Stphylococcus cohni	11.00±0.25	12.05±0.73	14.02±0.68	14.87±0.15
Serratia ficaria	6.00±0.20	6.00±0.21	8.15±0.30	9.97±0.05
E.coli	9.05±0.91	9.57±0.33	10.00±0.25	11.00±0.16

Table 2: Antimicrobial activity of *Cassia fistula* Linn. Saponins rich fraction (CFS)

In flavonoids rich fraction it was observed that maximum zone of inhibition was present against Staphylococcus cohni and E.coli (12.00±0.05 and 12.00±0.53 mm respectively) at 50 mg/ml and least effect against Serratia ficaria with zone of inhibition of 7.02±0.38 mm. Effect against Staphylococcus cohni and E.coli was near about same at 50 mg/ml but at 12.5 mg/ml effect against E.coli was significantly better ($P<0.05$) as compared to all microorganism. At 25 mg/ml, 37.5 mg/ml and 50 mg/ml no significant variation was observed in between zone of inhibition for Staphylococcus cohni and E.coli. Thus at 50 mg/ml antimicrobial effect of flavonoids rich fraction of *Cassia fistula* can be arranged in sequence of Staphylococcus cohni > E.coli > P. vesicularis> Salmonella typhae> Streptococcus faecalis> Aeromonas hydrophilia> Serratia ficaria. Saponin rich fraction *Cassia fistula* Linn. also showed significant effect as antimicrobial agent. Maximum effect was observed against Streptococcus cohni with zone of inhibition of 14.87±0.15 mm and least effect was observed against P.vesicularis with zone of inhibition of 9.55±0.19 mm at 50 mg/ml. At 12.5 mg/ml, 25 mg/ml, 37.5 mg/ml and 50 mg/ml effect against P.vesicularis was significantly better ($P<0.05$) as compared to effect on other test microorganism. According to results antimicrobial potential of saponin rich fraction of *Cassia fistula* Linn. can be sequenced in order - Streptococcus cohni> Aeromonas hydrophilia> Salmonella typhae> E.coli>, Serratia ficaria> P.vesicularis. Thus results demonstrated that flavonoid and saponin rich fractions of *Cassia fistula* Linn. possess significant antimicrobial potential against selected test microorganism. Effect of individual fraction on individual microorganism differed respectively as discussed above.

Many research groups have gone one step further and either isolated and identified the structure of flavonoids that possess antibacterial activity, or quantified the activity of commercially available flavonoids. Examples of such flavonoids are apigenin, galangin, pinocembrin, ponciretin, genkwanin, sophoraflavanone G and its derivatives, naringin and naringenin, epigallocatechin gallate and its derivatives, luteolin and luteolin 7-glucoside, quercetin, 3-O-methylquercetin and various quercetin glycosides and kaempferol and its derivatives. Other flavones, flavone glycosides, isoflavones, flavanones, isoflavanones, isoflavans, flavonols, flavonol glycosides and chalcones with antibacterial activity have also been identified. (Tim Cushnie TP et. al., 2005) Saponins are glycosides of triterpenes, steroids or steroidal alkaloids; they are regarded as natural antimicrobial compounds with very diverse biological activities whose roles in food, animal feed stuffs and pharmaceutical applications have been useful to man. Saponins have been implicated as bioactive antibacterial agents of plants containing them. Another study indicates the antibacterial sensitivity of saponins more on gram negative than gram positive. Although variations in the chemical nature of the saponins from different plants reported in the literature, may perhaps explain the variation in activity against various pathogens. Saponin compounds in plant are believed to naturally protect it against attack from pathogens, which would account for their antimicrobial activity. The mode of action of antibacterial effects of saponins may involve membranolytic properties, rather than simply altering the surface tension of the extracellular medium (Aliyu AB et.al., 2011).

Conclusion

The present study exhibited the antibacterial effect of various extracts of *Cassia fistula* L. The inhibitory effect of the extract justified the medicinal use of *Cassia fistula* L. and further study is required to find out the active component of medicinal value.

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References

1) Aliyu AB, Musa AM, Abdullahi MS, Ibrahim MA, Tijjani MB, Aliyu MS and Oyewale AO, 2011. Activity of saponin fraction of Anisopus mannii against some pathogenic microorganisms, Journal of Medicinal Plants Research 5(31), 6709-6713.

2) Al – Meshal, IA, Tariq M, Parmar NS, Ageel A.M, 1985. Anti - inflammatory activity of the flavonoids fraction of khat (Catha edulis Forsk). Agents and Action 17, 3 – 4.

3) Bansal R, Bansal M, 2010. Pharmaceutical microbiology 1, 199-216.

4) Cabrini DA, Castardo JC, Prudente AS, Ferreira J, Guimaraes CL, Monache FD, Filho VC, Otukia MF, 2008. Anti-inflammatory effects of hydroalcoholic extract and two biflavonoids from Garcinia gardneriana leaves in mouse paw oedema. Journal of Ethnopharmacology 118, 405–411.

5) Cibin, TR, Srinivas G, Gayathri Devi, G, Srinivas, P, Lija Y, Abraham A, 2006. Antioxidant and antioproliferative effect of flavonoids from Emilia

sonchifolia Linn. On human cancer cells. International journal of pharmacology 2, 520 – 524.

6) Duraipandiyan V, Ignacimuthu S, 2007. Antibacterial and antifungal activity of Cassia fistula L. An ethnomedicinal plant. Journal of Ethnopharmacology 112, 590–594.
7) Indian Pharmacopoeia, 2007. Govt of India Ministry of Health & Family Welfare.1: 45-46.
8) Jain NK, 2005. Pharmaceutical microbiology 2, 294-305.
9) Kirtikar KR, Basu, BD, 1999. Indian medicinal plant, International book seller and publisher, India, 856-860, 867- 868.
10) Luke FC, Chopra T, Kaye KS, 2011. Pathogens Resistant to Antibacterial Agents, Med Clin N Am 95, 647–676.
11) Murn VK, Rao TVP and Venkateswarlu V, 1967. Chemical examination of Cassia fistula. Tetrahedron 23, 515 – 518.
12) Singh GS, Pandeya SN, 2011. Natural products in discovery of potential and safer antibacterial agents. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry, 63-101.
13) Tim Cushnie TP, Lamb AJ, 2005. Antimicrobial activity of flavonoids International. Journal of Antimicrobial Agents 26, 343–356.
14) Wealth of India, 2007, NISCAIR, CSIR, India, 1: [A-Ci], 222 – 224.