ANTIMICROBIAL ACTIVITY OF *CASSIA ROXBURGHII* SEEDS *IN VITRO*

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ABSTRACT

Antibacterial and antifungal activity of various extracts of *Cassia roxburghii* seeds was performed against six Gram positive, seven Gram negative bacteria and one fungus. By using Disc diffusion method, the zones of inhibitions of the extracts were determined. The seed extracts were found to be effective against various Gram positive, Gram negative bacteria and a fungi. The aqueous extract of *Cassia roxburghii* produced the antibacterial activity against 10 of 14 tested organisms with the zone of inhibition ranging from 8 to 13 mm, which was nearly 50% of standard antibiotic activity. MIC was determined by Broth dilution method for aqueous and alcoholic extracts, were found to be more active against *Bacillus cereus, Bacillus subtilis, Proteus vulgaris, Klebsiella aurogenes, Vibrio cholerae, Pseudomonas azotogensis and Candida albicans*..

Keywords: Seed, methanolic extract, disc diffusion method, MIC.

1. INTRODUCTION

Finding healing power in plants is an ancient idea. Due to problems like adverse effects, limited life span and misuse of traditional antibiotics efforts are currently underway to look for products of natural origin. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. Different classes of natural products, possessing potent physiological and pharmacological activities have been isolated from *Cassia* species, and they include anthracene derivatives, flavonoids and polysaccharides. Some of these compounds have been shown to possess considerable antimicrobial activity (Ayo and Amupitan, 2004; Abo et al., 1999; Dalziel, 1948). *Cassia* species are well known in folk medicine for their laxative and purgative uses. They are also widely used for treating skin diseases such as ringworm, scabies, eczema and wounds (Eluojoba et al., 1999; Benjamin, 1980; Irvine, 1961). The leaves and pods are normally used but we have selected its seed for screening its antimicrobial activity.

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2. MATERIALS AND METHODS

Plant Material

The seeds of *Cassia roxburghii* were collected from Thanjavur district, Tamilnadu, India and it was then authenticated by Dr. P. Jayaraman, comparing with the herbarium voucher specimen deposited at Plant Anatomy research Center (PARC), Chennai, bearing the number PARC/ 203/04. The seeds were washed with water and air dried under shade, powdered mechanically and stored in airtight containers. 100 g of fresh seeds were extracted successively (exhaustive) with various solvents in the increasing order of polarity viz., petroleum ether (60-80^{o}C), benzene, chloroform, acetone and methanol. Similarly aqueous extract was prepared by boiling the seed powder with distilled water. The extracts were concentrated by evaporation.

Antimicrobial Studies

Micro-organisms used

Microorganism used in this study were procured from American type culture collection (ATCC) and National collection of industrial microorganism (NCIM, Pune).The culture used were *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 9341), *Bacillus subtilis* (ATCC 6633), *Staphylococcus albus* (NCIM 2178), *Bacillus lentus* (NCIM 2466), *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 10536), *Proteus vulgaris* (NCIM 2027), *Klebsiella aerogenes* (NCIM 2239), *Vibrio cholera* (ATCC 11558), *Salmonella paratyphii* (ATCC 12176),

Pseudomonas azotogensis (NCIM 2075), *Pseudomonas aeruginosa* (NCIM 2945), and *Candida albicans* (NCIM 3466)(Table No:3).

Determination of antimicrobial activity

The disc diffusion method (Bauer *et al.,* 1966; Parekh and Chanda, 2006) was used to determine the antimicrobial activities with various extract of *Cassia roxburghii* seeds. Muller Hinton agar medium was prepared, sterilized and used as the growth medium for bacterial culture. 20ml of the sterilized medium was poured into each sterilized petri dish, covered and allowed to solidify. The plates were then seeded with the test microorganism (bacterial culture) by using sterile cotton swabs. For fungal culture Sabouraud Dextrose Agar (SDA) medium was prepared and transferred into sterile Petri plates and solidified. The medium plates were then swabbed with fungal culture. The sterilized paper discs were soaked in the prepared saturated solutions of extracts with different solvents and were dried at 50°C. The dried discs were then placed on both medium plates (Muller Hinton and Sabouraud agar) seeded with test microorganisms. The plates were then incubated at 37ÚC for bacteria and at room temperature for fungus. The zone of inhibition was measured after 24hrs for bacteria and 48hrs for fungus. In the present study ofloxacin 5µg/disc and clotrimazole of 25µg/disc were used as standard antibacterial and antifungal drug respectively.

Minimum Inhibitory Concentration: Broth Dilution Assay

MIC is the lowest concentration that inhibited the visible growth of the microorganisms and it is expressed in µg/ml. In this method serial dilutions of known concentration of aqueous and other extracts were made from the stock (250mg/ml) by using Muller Hinton broth and a control experiment was also set up for each organism for comparison. Strict aseptic condition was followed throughout the process. 100 µl of standardized inoculum was transferred into respective tubes. The inoculated tubes with bacterial strains were incubated at 37°C for 24hrs and for fungus inoculated tubes were kept at 25°c for 48hrs respectively. After incubation, the MIC was determined by comparing the turbidity produced in sample tubes with that of control.

3. RESULTS

Determination of antimicrobial activity by Disc diffusion method

The aqueous extract of *Cassia roxburghii* produced the antibacterial activity against 10 of 14 tested organisms with the zone of inhibition ranging from 8 to 13 mm, which was nearly 50% of standard antibiotic activity (Table 2). The petroleum ether extract was effective only against two cultures namely *Micrococcus luteus (*8mm) and *Pseudomonas azotogenesis* (13 mm). All the other cultures were resistant towards the extract. The extract with the solvent Benzene, which was used against the test culture showed similar result like aqueous extract. The maximum zone of inhibition was observed *Pseudomonas azotogenesis*, which was about 15mm in diameter, whereas the cultures of *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were resistant against the extract used. The chloroform extract was effective against nine cultures and the maximum zone of inhibition was noted in *Bacillus subtilis*, when compared to other cultures, which was measured about 11mm in diameter. The extract, which was obtained using acetone, against the 14 test organisms, ten strains were susceptible. Among which *Pseudomonas azotogensis* showed the maximal sensitivity with zone of inhibition of 13 mm. The zone of inhibition for the cultures of *Bacillus subtilis* and *Klebsiella aerogenes* were on par with each other, which were about 12mm respectively. With the alcohol extract almost all the cultures used were sensitive except five cultures and the maximum zone of inhibition was found to be in *Klebsiella aerogenes*, which was about 16mm. All the test cultures were susceptible for the standard used (ofloxacin) and the maximum zone of inhibition was observed with *Salmonella paratyphii* and diameter was about 23mm.

Minimum Inhibitory Concentration: Broth dilution assay

Based on the results of disc diffusion assay, MIC was determined for aqueous and alcoholic extracts against all Gram positive, Gram negative and one fungal standardized culture (Table 3). MIC was studied for the test organisms with aqueous and alcoholic extracts. The result showed the MIC with aqueous extract were effective against nine cultures namely *Bacillus cereus,*

Micrococcus luteus, Vibrio cholerae, Klebsiella aerogenes, *Pseudomonas azotogensis and Candida albicans,* which were all on par with each other measured about 6.25mg/ml, when compared to other culture which showed higher concentration. The effect of alcohol extract on the test organisms was found to be similar to that of aqueous extract. But *Bacillus cereus, Bacillus subtilis and Vibrio cholerae* showed the higher MIC in alcoholic extract, the concentration of which was ranged about 3.15mg/ml respectively.

Table 1 - List of microorganisms used for the microbial study

S. No	GRAM POSITIVE BACTERIA	GRAM NEGATIVE BACTERIA	FUNGI
1	*Bacillus cereus*(ATCC 11778)	*Escherichia coli*(ATCC 10536)	*Candida albicans* (NCIM 3466)
2	*Bacillus subtilis(ATCC 6633)*	*Proteus vulgaris* (NCIM 2027)	
3	*Bacillus lentus(NCIM 2466)*	*Klebsiella aerogenes* (NCIM 2239)	
4	*Micrococcus luteus*(ATCC 9341)	*Vibrio cholera*(ATCC 11558)	
5	*Staphylococcus albus*(NCIM 2178)	*Salmonella paratyphii*(ATCC 12176)	
6	*Staphylococcus aureus*(ATCC 29737)	*Pseudomonas azotogensis* (NCIM 2075)	
7		*Pseudomonas aeruginosa*(NCIM 2945)	

NCIM-National Collection of Industrial Microorganism, ATCC-American Type Culture Collection.

Table 2 - Anti-microbial Activity of *Cassia roxburghii* by Disc Diffusion Method

Name of the organisms	Zone of Inhibition in mm							
	Aqueous	Successive Extracts					STD (OFN)	STD (CLOZ)
		PER	BEN	CHL	ACE	MEOH		
Bacillus cereus	-	-	13	-	12	-	22	-
Micrococcus luteus	11	8	8	7	8	10	18	-
Bacillus substilis	12	-	-	11	-	12	20	-
Staphylococcus albus	13	-	8	8	9	12	21	-
Bacillus lentus	12	-	9	8	10	13	20	-
Staphylococcus aureus	-	-	-	-	-	-	22	-
Escherichia coli	11	-	-	-	-	13	19	-
Proteus vulgaris	8	-	8	7	9	12	20	-
Klebsiella aerogenes	13	-	10	10	12	16	19	-
Vibrio cholerae	12	-	7	8	9	14	21	-
Salmonella paratyphii	8	-	8	9	9	14	23	-
Pseudomonas azotogensis	-	13	15	-	13	-	20	-
Pseudomonas aeruginosa	-	-	-	-	-	-	20	-
Candida albicans	8	-	7	9	8	11	-	19

Table 3 - Minimum Inhibitory Concentration values for Aqueous and Alcoholic Extracts against various Microorganisms

S.No	Name of the organisms	MIC of Aqueous Extract(mg\ml)	MIC of Alcohol Extract(mg\ml)
1.	*Bacillus cereus*	6.25	3.15
2.	*Micrococcus luteus*	6.25	12.5
3.	*Bacillus substilis*	6.25	3.15
4.	*Staphylococcus albus*	12.5	6.25
5.	*Bacillus lentus*	12.5	12.5
6.	*Staphylococcus aureus*	12.5	12.5
7.	*Escherichia coli*	12.5	6.25
8.	*Proteus vulgaris*	6.25	6.25
9.	*Klebsiella aerogenes*	6.25	6.25
10.	*Vibrio cholerae*	6.25	3.15
11.	*Salmonella paratyphii*	12.5	12.5
12.	*Pseudomonas azotogensis*	6.25	6.25
13.	*Pseudomonas aeruginosa*	12.5	12.5
14.	*Candida albcans*	6.25	6.25

4. DISCUSSION

Since ancient times, plants have been a veritable source of drugs. However, man tends to ignore the importance of herbal medicine. Recently, much attention has been directed towards extracts and biologically active compounds isolated from popular species. Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. The use of medicinal plants play a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Munoz-Mingarro *et al.,* 2003). Some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent, but we found in this study that seed extracts prepared with methanol as solvent provided more consistent antimicrobial activity. This might be due to the solubility of the compounds in the methanol as also reported earlier (Allero and Afolayan, 2006; Parekh and Chanda, 2007 a). Multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs that are commonly used in the treatment of infectious diseases, making it a global growing problem. There is an urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants, which may be less toxic to humans and possibly with a novel mechanism of action. There are numerous examples of antimicrobials of plant origin that have an enormous therapeutic potential (Parekh and Chanda, 2007 b). Several phytoconstituents like flavanoids (Tsuchiya et al., 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya et al., 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes (Goren, 1996) etc., are effective antimicrobial substances against a wide range of microorganisms. *Cassia roxburghii* seed extracts were tried out for antimicrobial properties. The Anti microbial study of the seed was found to be effective against various gram positive, gram negative bacteria and a fungus. From their MIC values the aqueous and alcoholic extracts were found to be more active against *Bacillus cereus, Bacillus subtilis, Proteus vulgaris, Klebsiella aurogenes, Vibrio cholerae, Pseudomonas azotogensis and Candida albicans.*

5. CONCLUSION

The antimicrobial activity of *Cassia roxburghii* may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect to inhibition of microbes. The work carried was a basic approach to find out the antimicrobial activity in *Cassia roxburghii* seeds. Further works on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes.

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