Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

ANTIMICROBIAL ACTIVITY OF THE EXTRACTS OF *CASSIA FISTULA* AND *MILLETIA PINNATA* AGAINST AMPICILLIN RESISTANT STRAINS OF CLINICAL ORIGIN

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ABSTRACT

The antimicrobial activity of the extracts of leaves and bark of *Cassia fistula* and *Milletia pinnata* was evaluated against *Aspergillus niger, Candida albicans, E coli, Staphylococcus aureus,* and *Lacto bacillus.* The extracts showing good antimicrobial activity were further screened against Ampicillin resistant strains of *Escherichia coli,* and *Staphylococcus aureus.* The methanolic bark extract of *Cassia fistula* showed maximum activity against *E coli* and *S aureus* with zones of inhibition of 16 mm and 14 mm respectively. Also the methanolic leaf extract of *Milletia pinnata* showed good activity against *E* coli and *S aureus* with zones of inhibition of 13mm and 7mm respectively. This study concludes that these plants can be formulated into topical disinfectant formulations and used to control resistant microbes causing nosocomial and community acquired infections.

KEYWORDS

Herbal extracts; Antimicrobial agent; Ampicillin resistant strains.

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INTRODUCTION

Infections are increasing alarmingly and have emerged as a critical issue in hospital care outcome. Opportunistic microorganisms primarily cause nosocomial infections; and multidrug-resistant pathogens that are commonly involved in nosocomial infections are difficult to treat. Multidrug resistant infectious diseases of bacterial and fungal origin are leading killers and account for approximately 25% of global deaths[1]. The alarming rate at which the human pathogens like *Staphylococcus aureus* (Methicillin &

Multiantibiotic resistant), *Escherichia coli*, *Pesudomonas aeruginosa*, *Candida albicans* and *Cryptococcus neoformans* are evolving themselves as multidrug resistant "Superbugs" towards the newly generated classes of antibiotics, demands for exploration of new chemical sources from biodiversity and develop therapeutic regimes to combat infectious superbugs[2].

The hands of health care workers are the primary mode of transmission of these multidrug-resistant pathogens and infections to patients. Skin being the most exposed part of our body requires protection from skin pathogens. Hence it brings up the use of antiseptics for hand wash purposes[3]. Many of chemical antiseptics available in the market are alcohol based sanitizers. These formulations including soaps and solutions reduce health care associated transmission of contagious diseases but they have some short comings or adverse effects, their frequent use can lead to skin irritation and also resistance among pathogens[3]. Plants are rich in a wide variety of secondary metabolites, such as phenolic compounds, tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial properties. Hence there is an upsurge of developing herbal disinfectants and evaluate its efficacy[4]. The chemical analysis of different parts of *Cassia fistula* has been reported. It was found to contain Flavonoids, phenolic compounds, and proanthocyanidins[5]. Literature survey of this plant shows that it is reported to posses good antimicrobial properties[6, 7]. The chemical composition of *Milletia pinnata* was studied. It was reported to contain saponins, tannins, carbohydrates, alkaloids, sterols and flavonoidal glycosides[8]. The pharmacological properties of *Milletia pinnata* reported in literature also suggest it has good antimicrobial properties[9]. However there are no reports of their antimicrobial activities against resistant strains of microorganisms. The aim of the present study was to investigate the antimicrobial activity of the extracts of *Cassia fistula* and *Milletia pinnata* against the common organisms which cause nasocomial infections. The extracts showing good antimicrobial properties were further screened against Ampicillin resistant strains. If these extracts show good antimicrobial activities they can be further formulated into various formulations such as disinfectants, handwashes, etc and further evaluated.

MATERIALS AND METHODS

Plant material

The plants *Cassia fistula* and *Milletia pinnata* were collected from Mysore district, the specimen were authenticated at RRL, Bangalore. The leaves and bark were dried in hot air oven at 35^{o}C for three days, powdered to a mesh size of # 40 and stored in air tight containers.

Bacterial strains

*Aspergillus niger, Candida albicans, E coli, Staphylococcus aureus, Lacto bacillus,*were collected as clinical isolates from Department of Microbiology, BM Hospital, Mysore.

Preparation of plant extracts

150g of air dried powdered material of all the samples were extracted with 300ml of Petroleum ether, Chloroform, Ethyl acetate, Methanol, and 40% methanol, separately by refluxation for 8 hours. The obtained extracts were filtered and stored in air tight amber coloured glass containers in a cool place. Extracts were concentrated using vacuum evaporator and percentages of extractive values were calculated in terms of air-dried weight of plant material.

Agar diffusion method

All the extracts were prepared in the concentration of 0.5mg/ ml in Dimethyl Sulfoxide (DMSO) and screened against selected microorganisms. Mueller-Hinton medium and Potato Dextrose agar medium was used for culture of bacteria and fungi respectively. The cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto the petri plates. By using a sterile borer the medium was bored and the prepared extracts were taken and 0.2 ml of each extract was added in bores. The plates were then incubated at 37°C and 25°C for bacteria and fungi respectively for 24h. All the results were compared with the standard antibiotic Ampicillin in the concentration of 40ug/ml for bacteria. Fluconazole in the concentration of 125 mg/ml was used as standard for fungi. The antimicrobial activity

was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in duplicate and the diameter of the inhibition zones was calculated and tabulated in Table No.1.

Determination of Minimum Inhibitory Concentration (MIC)

MIC determination of the extracts was carried out by serial dilution method. Media used was Muller-Hinton broth and Potato dextrose broth for bacteria and fungi respectively. Organisms used were *Candida albicans, E coli,* and *Staphylococcus aureus.* MC Farland constant selected was 0.5 which contains an approximate cell density of $1x10^8$cells/ml. The test tubes were serially diluted and different concentrations of extracts were added along with the inoculum. The tube with inoculum without extract served as positive control whereas the tube without inoculum served as negative control. The results are tabulated in Table No.2.

Phytochemical screening

The leaf and bark extracts of *Cassia fistula* and *Milletia pinnata* showing good activity were subjected to phytochemical screening tests to identify the presence of tannins, carbohydrates, sterols, saponins, alkaloids and aglycones. The results are tabulated in Table No.3.

Selection of resistant strains

Ampicillin resistant strains of *E coli* & *S aureus* were obtained as clinical isolates from patients at Microbiology department, BM Hospital, Mysore.

Anti microbial Screening against resistant strains

The active extracts showing good activity in the preliminary antimicrobial screening were further screened against ampicillin resistant strains and the results are tabulated in Table No.4.

RESULTS AND DISCUSSION

Agar diffusion method

The initial susceptibility testing of the leaves and stem bark extracts was done. The bark extracts of *Cassia fistula* and leaf extracts of *Milletia pinnata* showed considerable antimicrobial activity in terms of good zones of inhibition. The leaf extracts of *Cassia fistula* showed no inhibition zones whereas the methanolic and ethyl acetate leaf extracts of *Milletia pinnata* showed inhibition zones at 0.5 mg/ml concentration. The results are tabulated in Table No.1.

Determination of Minimum Inhibitory Concentration (MIC)

MIC determination of the extracts was carried out by serial dilution method. Results showed that the ethyl acetate and methanolic extracts of *Cassia fistula* showed MIC values in the range of 0.5mg/ml whereas the ethyl acetate and methanolic leaf extracts of *Milletia pinnata* showed MIC of 1mg/ml and 0.5 mg/ml respectively. The results are tabulated in Table No.2.

Phytochemical screening

Results showed that the leaf and bark extracts of *Cassia fistula* contain anthraquinones, saponins, carbohydrates, phytosterols and tannins. The leaf extracts of *Milletia pinnata* showed the presence of alkaloids, sterols and saponins. The results are tabulated in Table No.3.

Anti microbial Screening against resistant strains

The results of zones of inhibition of extracts against resistant strains were comparatively significant. The methanolic bark extract of *Cassia fistula* showed maximum zones of inhibition against resistant strains (E *coli*=16mm, *S aureus*=14mm). The methanolic leaf extract of *Milletia* pinnata showed maximum zone of inhibition against *E* coli. (13mm).The results are tabulated in Table No.4.

DISCUSSION

Cassia fistula and *Milletia pinnata* are known to contain flavonoids, phenolic compounds, proanthocyanidins[5] and saponins, tannins, alkaloids, sterols, flavonoid glycosides respectively[8]. Literature survey indicates that *Cassia fistula* exhibit various pharmacological properties such as antibacterial[6], antifungal[7], antioxidant[10], hepatoprotective[11], antitumor[12], antiinflammatory[13], hypocholestremic[14] and wound healing[15] activities. The pharmacological properties of *Milletia pinnata* reported in the literature include antimicrobial[9], antidiabetic[16], hypolipedemic[17], antiulcer[18], analgesic and anti-inflammatory activities[19].

Table No.1: Zones of inhibition (mm) of leaf and bark extracts of *C fistula* in the concentration of 0.5 mg/ml against different organisms

Extracts	*Aspergillus niger*	*Candida albicans*	*E coli*	*Staphylococcus aureus*	*Lacto bacillus*
Standard	16.0	18.0	18.0	12.0	16.0
CB-PET	14.0	__	8.0	6.0	16.0
CB-CHL	16.0	__	14.0	__	16.0
CB-ETH	8.0	__	10.0	10.0	8.0
CB-MOH	14.0	__	14.0	12.0	20.0
CB-40MOH	10.0	__	12.0	8.0	6.0
CL-PET	__	__	__	__	__
CL-CHL	__	__	__	__	__
CL-ETH	__	__	__	__	__
CL-MOH	__	__	__	__	__
CL-40MOH	__	__	__	__	__
MB-PET	__	12.0	6.0	10.0	12.0
MB-CHL	__	12.0	12.0	8.0	16.0
MB-ETH	__	8.0	16.0	12.0	18.0
MB-MOH	__	14.0	16.0	12.0	16.0
MB-40MOH	__	6.0	12.0	10.0	16.0
ML-PET	__	10.0	10.0	__	8.0
ML-CHL	__	7.0	4.0	__	6.0
ML-ETH	__	12.0	8.0	__	8.0
ML-MOH	__	12.0	6.0	__	10.0
ML-40MOH	__	10.0	6.0	__	12.0

Note: CB= *Cassia fistula* bark, CL= *Cassia fistula* leaf, MB= *Milletia pinnata* bark, ML= *Milletia pinnata* leaf. PET= Petroleum ether, CHL= Chloroform, ETH= Ethyl acetate, MOH= Methanol, 40MOH= 40% Methanol. __indicates no activity

Table No.2: Minimum Inhibitory Concentrations of extracts

S. No	Extract	MIC (mg/ml)		
		C albicans	***E coli***	***S aureus***
1	CB-PET	--	2.0	1.0
2	CB-CHL	2.0	1.0	1.0
3	CB-ETH	1.0	0.5	0.5
4	CB-MOH	1.0	0.5	0.5
5	CB-40MOH	1.0	1.0	1.0
6	CL-PET	--	1.0	1.0
7	CL-CHL	--	1.0	2.0
8	CL-ETH	1.0	2.0	0.5
9	CL-MOH	2.0	1.0	0.5
10	CL-40MOH	2.0	2.0	1.0
11	MB-PET	--	--	--
12	MB-CHL	--	1.0	1.0
13	MB-ETH	2.0	2.0	2.0
14	MB-MOH	1.0	1.0	1.0
15	MB-40MOH	2.0	2.0	2.0
16	ML-PET	2.0	1.0	2.0
17	ML-CHL	2.0	1.0	2.0
18	ML-ETH	1.0	1.0	0.5
19	ML-MOH	1.0	0.5	0.5
20	ML-40MOH	2.0	2.0	1.0

Table No.3: Phytochemical evaluation of the active extracts

S.No	Extract	Alkaloids	Carbohydrates	Anthraquinones	Saponins	Phytosterols	Tannins
1	CB-ETH	--	--	+	--	+	--
2	CB-MOH	--	+	+	+	--	+
3	CL-ETH	--	+	--	+	+	--
4	CL-MOH	--	+	+	+	--	--
5	ML-ETH	+	--	--	+	+	--
6	ML-MOH	+	+	--	+	--	+

Table No.4: Zones of inhibition (mm) of leaf and bark extracts of *C fistula* at concentration of 0.5 mg/ml against resistant strains

Extracts	Zones of inhibition (mm)	
	E coli	***Staphylococcus aureus***
CB-ETH	7.0	6.0
CB-MEOH	16.0	14.0
CL-ETH	6.0	6.0
CL-MEOH	11.0	12.0
ML-ETH	—	6.0
ML-MEOH	13.0	7.0

CONCLUSION

In our study the extracts of these plants were screened for antimicrobial activity in order to confirm the literature survey. Preliminary anti microbial screening by agar diffusion method was carried out for the different extracts of the leaf and bark. Among all the 20 extracts 06 extracts were found to possess good activity against the organisms *Candida albicans*, *E coli*, and *S aureus* at a concentration of 0.5mg/ml. Further MIC of all the extracts was determined using serial dilution method. The extracts were found to exhibit MIC in the range of 0.5 mg/ ml. These extracts were further screened on ampicillin resistant strains of *E coli*, and *S aureus*. The ethyl acetate and methanolic bark and leaf extracts of *Cassia fistula* and leaf extracts of *Milletia pinnata* exhibited antimicrobial activity against ampicillin resistant strains. Hence these drugs may find their way to treat bacterial and fungal resistant infections as well as formulated in various antimicrobial formulations such as antiseptics, disinfectants, hand washes, dentifrices and other health care products.

ACKNOWLEDGEMENT

I would also like to thank Farooqia College of Pharmacy, Mysore, India for provided the necessary facilities to carry out this work.

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