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Antimicrobial activity of different solvent extracts of the leaves and stem of *Mappia foetida*

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ABSTRACT

Successive petroleum ether, chloroform and methanol extracts of *Mappia foetida* leaves and stem were tested (*in vitro*) for their antimicrobial activity. The stem extracts are more active and the chloroform extracts show a higher degree of inhibition.

KEYWORDS

Mappia foetida;
Leaves and stem;
Antibacterial activity;
Antifungal activity.

INTRODUCTION

Over the years the emergence of numerous resistant strains of pathogenic bacteria and fungi to a range of formerly efficient antibiotics constitutes a serious threat to public health. Traditionally the natural products have been utilized for the control of various diseases, because they are a pivotal source of many active compounds that show multiple therapeutic effects, in addition to constituting models for the synthesis of a large number of pharmaceuticals. Nevertheless, the use of extracts from various parts of the plant materials as a traditional remedy for microbial infections dates back to the ancient times. One such important tree is the *Mappia foetida* which belongs to the Icacinaceae family located in Ootacamund region of The Niligiri Hills[1] of TamilNadu in South India. The tree was identified by the local Badagar and the leaves and stem were collected during September 2012.

The aqueous extracts of *Mappia foetida* have a Folk-loric reputation for the treatment of a variety of ailments[2] among the Toda tribes of The Nilgiris District. Recently many researchers have reported the biological screening of the species for its promising anticancer activity[3-8]. The previously isolated compounds are Sitosterol, lupeol, alkaloids[9-20]. Due to the lack of the species and also the adequate scientific research and documentation, the medicinal properties of *Mappia foetida* still remain mostly in dark. The aim of the present study is to investigate the antibacetirial and antifungal activities of the stem and leaves of *Mappia foetida* against different resistance pathogenic microorganisms. Also, the antibacterial and antifungal activities of certain antibiotics commonly used in the treatment of infections caused by these resistance pathogenic antimicrobes were compared.

EXPERIMENTAL

Mappia foetida samples

Mappia foetida Miers.(=*Nothapodytes foetida* Blume.) Icacinaceae leaves and stem were collected from The Niligiri Hills[1] of South India during Sep. 2012

and identified by the curator of the Botanical Gardens. A voucher specimen is available in the Herbarium at the Botanical Survey of India, Coimbatore (No. 37005).

Preparation of samples

The air dried coarse powder of stem and leaves were successively extracted with petroleum ether, chloroform and methanol using the Soxhlet apparatus. The Extracts so collected were distilled off on a water bath at atmospheric pressure and the last traces of solvent were removed in vacuo.

Test organisms

All the extracts were screened for their antibacterial activities against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa (ATCC 9027) and Bacillus subtilis* (ATCC 6633) by using the Disc Diffusion Method. The extracts were also screened for their *in vitro* antifungal activities against *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), *Aspergillus fumigates, Fusarium oxysporum, Aspergillus flavus,* and *Alternaria macrospora.* Carbendazim and Ciprofloaxcin are used as standards.

Determination of Antibacterial Activity

Petroleum ether (5: 5.7 %), chlorofom (25.5:15 %) and methanol (38.5:20 %) obtained by soxhlet extraction from the stem:leaves respectively. Phytochemical screening showed the presence of steroids in pet. ether, alkaloids and flavanoids in chloroform and phenolics and alkaloids in methanol. All the extracts were screened for their antibacterial activities against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa (ATCC 9027) and Bacillus subtilis* (ATCC 6633) by using the Disc Diffusion Method.[21-23]. Bacteria were cultured in nutrient agar medium and used as inoculum for study. Bacterial cells were swabbed on to nutrient agar medium [prepared from NaCl (5.0 g), Peptone (5.0 g), beef extract powder (3.0 g), yeast extract powder (3.0 g), Agar (20.0 g) in 100 mL distilled water, pH =(7.5 ± 0.2)] in petri plates. The compounds to be tested were dissolved in dimethyl sulfoxide to a final concentration of 0.125 %, 0.25 % and 0.5% and soaked in filter paper discs of 5 mm diameter and 1 mm thickness. These discs were placed on the already seeded plates and incubated at 35 ± 2 ^{o} C for 24 h. The diameter (mm) of the inhibition zone around each disc was measured after 24 h. Streptomycin was used as standard for comparison.

Antifungal activity

The extracts were also screened for their *in vitro* antifungal activities against *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404), *Aspergillus fumigates, Fusarium oxysporum, Aspergillus flavus,* and *Alternaria macrospora.* The fungi were cultured in Czapek-Dox medium[24] and used as inoculums for study by using the Disc Diffusion method.[21-23] The inhibitory activities were compared with the commercial fungicide Carbendazim tested under similar conditions.

RESULTS AND DISCUSSION

Disease causing microbes have always been considered a major cause for morbidity and mortality in humans. In recent years, the appearance of resistant microorganism paved the way to the occurance of infections that are only treated by a limited number of antimicrobaial agents[25]. The emergence of the resistance bacteria presents a major challenge for the antimicrobial therapy of infectious diseases and increases the incidence of the morbidity and mortality. Therefore there is much focus towards the microbial studies and that too from nature as mostly they do not have any side effects. The zone of inhibition formed due to various microbial pathogens (both bacterial and fungal) of the leaves and stem extracts of mappia foetida is tabulated in TABLEs 1 and 2. According to TABLE 1, though all the three leaf extracts, petroleum ether, chloroform and methanol showed moderate activity against all the tested pathogens, the chloroform extract showed some good zone of inhibition at all concentrations. However, the petroleum ether extracts showed smaller activity and sometimes no inhibition at all towards various fungicidal pathogens. Similar results were found in the case of stem extracts of different concentrations. From the results observed, as tabulated in TABLE 2 the compounds were found toxic to all the test bacteria and fungi at higher concentrations. Their activity decreases with dilution. On comparison the stem extracts showed some good zone of inhibition than the leaf extracts.

According to the observation, the toxicity increases with the increase in concentration of test solution containing the respective solvent extracts. Although all the extracts are active, except a few which is in lower concentration, they did not reach the effectiveness of the conventional bacterostatic streptomycin and fungicide Carbendazim.. The variation in effectiveness of different compounds against different organisms depend either on impermeability of cells of the microbes or diffusion in ribosomes of microbial cells.[26]

TABLE 1 : Antimicrobial Activities of Leaf Extract[a]

	Microorganism	Diameter of Zone of Inhibition in mm								
		Petroleum Ether			Chloroform			Methanol		
		0.5 %	1 %	2%	0.5 %	1 %	2 %	0.5 %	1 %	2 %
Antifungal Antibacterial data data	Staphylococcus aureus (ATCC 6538)	2	8	15	4	13	25	4	9	17
	Pseudomonas aeruginosa (ATCC 9027)	5	11	19	7	16	28	3	11	19
	Bacillus subtilis (ATCC 6633)	6	17	24	8	19	26	6	16	26
	Candida albicans (ATCC 10231)	3	10	21	6	18	29	4	14	25
	Aspergillus fumigatus	-	-	-	4	15	20	-	9	18
	Aspergillus niger (ATCC 16404)	-	-	-	7	15	19	4	14	22
	Aspergillus flavus	4	13	22	10	19	23	3	12	20
	Fusarium oxysporum	-	-	8	5	14	21	2	9	17
	Alternaria macrospora	-	5	13	6	13	20	4	10	18
	Carbendazim	6	14	21	9	16	21	7	13	18
	Ciprofloxacin	7	16	28	11	21	34	10	19	25

[a] Values of the mean of three replicates; - no inhibition

TABLE 2 : Antimicrobial Activities of Stem Extract[a]

	Microorganism	Diameter of Zone of Inhibition in mm								
		Petroleum Ether			Chloroform			Methanol		
		0.5 %	1 %	2%	0.5 %	1 %	2 %	0.5 %	1 %	2 %
Antifungal antibacterial data data	Staphylococcus aureus (ATCC 6538)	3	11	19	6	14	24	4	11	23
	Pseudomonas aeruginosa (ATCC 9027)	5	16	24	7	17	31	3	10	21
	Bacillus subtilis (ATCC 6633)	6	15	26	6	15	34	4	13	25
	Candida albicans (ATCC 10231)	3	8	12	5	13	26	5	14	28
	Aspergillus fumigatus	-	-	4	7	12	26	6	13	24
	Aspergillus niger (ATCC 16404)	-	-	7	17	26	34	8	14	19
	Aspergillus flavus	6	12	15	10	18	32	5	11	21
	Fusarium oxysporum	-	5	9	9	17	28	7	15	22
	Alternaria macrospora	4	9	14	6	15	24	3	10	17
	Carbendazim	6	14	21	9	16	21	7	13	18
	Ciprofloxacin	7	16	28	11	21	34	10	19	25

[a] Values of the mean of three replicates; - no inhibition

CONCLUSIONS

All the fractions of the *Mappia foetida* (stem and leaves) showed good activity against the tested organisms. The stem extracts are more active than the leaf extracts. An overall increase of inhibition was observed with increase in concentration. When compared to standard, the activity was less in petroleum ether and methanol extracts to that of chloroform extracts. Further screening and other potential biological studies are currently underway in our laboratory.

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