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Research Article

INVESTIGATION OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITIES OF FLOWERS AND FRUIT PEELS OF *MUSA SAPIENTUM*

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ABSTRACT

The methanol extract of flowers and fruit peels of *Musa sapientum* (family- Musaceae) has been investigated for the evaluation of phytochemical and antimicrobial activities. The phytochemical analysis of both extracts of *Musa sapientum* revealed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, coumarins, flavonoids, triterpenoids, tannins and phenolic compounds. The antimicrobial assays of *Musa sapientum* extracts were evaluated against number of different bacterial strains by the minimum inhibitory concentration and zone of inhibition by disc diffusion method. The methanol extract of flowers and fruit peels of *Musa sapientum* showed good antimicrobial activity as the minimum inhibitory concentration values better than control and zone of inhibition is almost same as the standard drug ciprofloxacin. The antimicrobial activities of methanol extracts may be due to the presence of phytoconstituents like flavonoids, tannins and phenolic compounds.

Keywords: *Musa sapientum*, antimicrobial, minimum inhibitory concentration, zone of inhibition, phytochemical.

INTRODUCTION

Musa sapientum also known as banana belonging to the Musacaeae family is a large, perennial, monocotyledonous herb 2–9 m in height that arises from large, subterranean rhizomes, from which the leaves emerge. The entire above-ground portion of the plant is not a true woody trunk, as in other trees, but a "false trunk" or "false stem" that consists of leaves and their fused petiole bases, referred to as a pseudo-stem. The pseudo-stem supports a canopy consisting of 6–20 (or more) leaves. In the center of the leaves, a growing point forms from the top of the rhizome, grows up and emerges as an overhanging inflorescence with a succession of reddish brown bracts. The bracts unfold from the base to the tip and fall off. Within the lower 1-12 bracts arise 14-18 female flowers in double rows which develop into parthenocarpic fruits. The next few bracts contains bisexual flowers that are rich in nectar but do not develop any further. In the upper bracts only male flowers are formed[1-4].

The banana originates in the Indomalayan area. By hybridization and domestication, about 300 varieties of banana have spread throughout the tropical and subtropical countries and are widely used for its nutritional values all over the world. The fruits as well as the other parts of the plant are used to treat different diseases in human in traditional medicine. The fruit of *Musa sapientum* is traditionally used in diarrhoea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), uremia, nephritis, gout, hypertension, cardiac disease. *Musa sapientum* is also used in the treatment of excess menstruation. Banana leaves (ashes) are used in eczema, as cool dressings for blister and burns. Flowers are used in dysentery and menorrhagia. Stem juice of fruited plant is used for treating diarrhoea, dysentery, cholera, otalgia, haemoptysis and flower is used in dysentery, diabetes and menorrhagia. The root is used as anthelmintic, blood disorders, venereal diseases. The plant is also used in inflammation, pain and snakebite[5-11].

All these above mentioned traditional uses indicate that there must be some antibacterial properties lying with the plant. In the present investigation both the methanol extracts of flowers and fruit peels of *Musa sapientum* were subjected for antibacterial activities.

MATERIALS AND METHODS

Plant material

The flowers and fruit peels of *Musa sapientum* were collected from local areas of Berhampur, Odisha, India, in the month of august 2017 and were identified by Dr. S. K. Dash, former professor, Department of Biosciences, College of Pharmaceutical Sciences, Mohuda, Dist- Ganjam, Odisha. The plant materials were cleaned with deionized water and were air-dried under shade, coarsely powdered, and kept in airtight container.

Preparation of extract

Methanol extract of flowers and fruit peels of *Musa sapientum* were prepared by soxhlet apparatus by successive extraction with petroleum ether (60–80∘C), chloroform, and methanol. Petroleum ether and chloroform were used in initial steps of extraction for defatting the plant materials. The methanol extracts were collected and dried using rotary vacuum evaporator followed by lyophilization and stored in desiccator until further use[12]. The type and extractive yield[13] of different extracts of *Musa sapientum* were observed and results of such observation are tabulated in table 1.

Phytochemical screening

Qualitative analysis of methanol extract of flowers and fruit peels of *Musa sapientum* were carried out based on standard protocols[14–17] and results of such observation are tabulated in table 2.

Preparation of the tested organisms

The lyophilized forms of different strains of microorganisms like *Bacillus licheniformis* (MTCC 429), *Escherichia coli* (MTCC 40), *Proteus vulgaris* (MTCC 426), P*seudomonas aeruginosa* (MTCC 424), *Shigella flexneri* (MTCC 1457), *Bacillus subtilis* (MTCC441), *Staphylococcus aureus* (MTCC 87), *Staphylococcus epidermidis* (MTCC 2639), *Shigella boydii*-8, *Salmonella typhimurium* NCTC-74, *Vibrio cholerae*-811 and *Klebsiella pneumoniae*-725 were collected from microbiology laboratory of Royal College of Pharmacy and Health Sciences, Berhampur, which were previously obtained from the Microbial Type Culture Collection and Gene bank (MTCC), Chandigarh, India and Division of microbiology, Jadavpur university, Kolkata. The bacterial cultures were maintained on Mueller-Hinton Agar (MHA) and were sub-cultured in the microbiology laboratory of the Royal college of Pharmacy and Health Sciences, Berhampur, Odisha, India. The average number of viable organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. About (108-109) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained. One loopful of an overnight grown nutrient broth culture of each test organism served as the inoculums for such antimicrobial activity determination. The average size of inoculums was about 106 cells contained in 3mm diameter of standard loop[18, 19].

Antimicrobial assay

Determination of the minimum inhibitory concentration (MIC)

Nutrient agar medium (250ml) was prepared and sterilized. Exactly 29 ml of media was dispersed in each of the 8 conical flasks, plugged with cotton and autoclaved. A Stock solution of methanol extract of flowers and fruit peels of *Musa sapientum* of 9 mg/ml was prepared. Measured quantities of the stock solution of extract were poured to the molten nutrient agar media to prepare concentration of 25, 50, 100, 200, 300 and 400µg/ml and then poured in Petri dishes. The Petri dishes were marked accordingly. One sterile nutrient agar plate without extract but with equal volume of the solvent served as the control plate. These plates were refrigerated overnight for uniform diffusion of the extract throughout the media. The plates were dried at 37°C by keeping them in the incubator. One loopful (diameter-3mm) of an overnight grown peptone water culture of each test organism was placed in petridish marked by the checker board technique. The spot inoculated plate was incubated at 37ºC for 24 hours and the MIC value obtained [20-22]. The experiment was repeated in triplicate and average values were disclosed in the Table no 3and 4.

Determination of zone of inhibition (ZOI)

For the determination of zone of inhibition, pure ciprofloxacin was taken as a standard antibiotic for comparison of the results. Two sets of three dilutions (100, 200 and 400 µg/ml) of methanol extract of flowers of *Musa sapientum* and methanol extract of fruit peels of *Musa sapientum* and ciprofloxacin (100 µg/ml) were prepared in double distilled water in Mc Cartney bottles. Sterile nutrient agar plates were prepared and incubated at 37ºC for 24hrs to check any sort of contamination. The sterile filter paper discs (Whatman no.1) of 6mm diameter were soaked in different dilutions of crude extract and placed in appropriate position on the surface of the flooded plate, marked as quadrants at the back of the Petri dishes. The Petri dishes were incubated at 37 ºC for 24 hrs and the diameter of the zone of inhibition were measured in mm. Similar procedure were adopted for the pure ciprofloxacin and the corresponding zone diameter were compared accordingly[20-22]. The experiment was repeated in triplicate and average values were written in the Table no 4.

RESULTS AND DISCUSSION

Phytochemical screening of the extracts

The percentage yield (w/w) of methanol extract of flowers of *Musa sapientum* was 17.08% and methanol extract of fruit peels of *Musa sapientum was* 15.64% (table 1). Preliminary phytochemical analysis of methanol extract of flowers and fruit peels of *Musa sapientum* revealed the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, coumarins, flavonoids, triterpenoids, tannins and phenolic compounds (table 2). Notably, both tannin and phenolics have been reported to possess antibacterial activity [23, 24].

Antimicrobial assay

The results regarding the antibacterial activity of methanol extract of flowers and fruit peels of *Musa sapientum* are indicated in Table-3, 4 and 5. The MIC and ZOI were carried out by using twelve different bacterial strains of Gram +ve and Gram –ve microorganism. The MIC of test compound compared with control group and ZOI values with standard ciprofloxacin. From the results of MIC values it indicates that the methanol extract of flowers and fruit peels of *Musa sapientum* showed significant antibacterial properties against Gram +ve and Gram –ve bacteria by agar dilution technique.

The MIC results of methanol extract of flowers of *Musa sapientum* (Table-3) shows that *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Escherichia coli* were inhibited at the concentration of 25µg/ml and were highly sensitive to the extract. The strains *Bacillus Licheniformis*, *Shigella flexneri*, *Shigella boydii* and *Vibrio cholerae* were inhibited at the concentration of 50 µg/ml and were moderately sensitive. The remaining bacterial strains were found to be inhibited within the concentration range of 100-200µg/ml and were less sensitive to the extract. All the bacterial strains were inhibited within the concentration range of 25 µg/ml to 200 µg/ml.

The MIC results of methanol extract of fruit peels of *Musa sapientum* (Table-4) shows that *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* were inhibited at the concentration of 25µg/ml and were highly sensitive to the extract. The strains *Bacillus Licheniformis*, *Proteus vulgaris* and *Escherichia coli* were inhibited at the concentration of 50 µg/ml and were moderately sensitive. The remaining bacterial strains were found to be inhibited within the concentration range of 100-300µg/ml and were less sensitive to the extract. All the bacterial strains were inhibited within the concentration range of 25 µg/ml to 300 µg/ml.

Basing on MIC results the antibacterial activity studies further carried out by ZOI and the result was depicted in table no 5. The zones of inhibitions produced by the methanol extract of flowers of *Musa sapientum* were ranged from 7-13.5 mm at the concentration range of 100-400µg/disc. While the zones of inhibition produced by methanol extract of fruit peels of *Musa*

sapientum were ranged from 6.5-11.5 mm at the concentration range of 100-400µg/disc. The zones of inhibition produced by ciprofloxacin were 13.50-17 mm at the concentration of 100µg/disc. Both the extracts were showed significant antibacterial activity as that of standard drug ciprofloxacin. But the methanol extract of flowers of *Musa sapientum* showed more antibacterial activity than that of methanol extract of fruit peels of *Musa sapientum.*

Table 1: Types and percentage yield of different extracts of methanol extract of flowers and fruit peels of *Musa sapientum*

Sl. no.	Plant part	Solvent used for extraction	Colour of the extracts	Physical appearance of the extracts	%Yield (w/w)
1	Flowers of *Musa sapientum*	Petroleum ether	Reddish Brown	Sticky mass	7.52
		Chloroform	Reddish brown	Dried powder	9.01
		Methanol	Light brown	Dried powder	17.08
2	Fruit peels of *Musa sapientum*	Petroleum ether	Black	Sticky mass	6.85
		Chloroform	Blackish Brown	Dried powder	8.5
		Methanol	Brown	Dried powder	15.64

Table 2: Preliminary phytochemical investigation of different extracts of *Musa sapientum*

Group of phytoconstituents	Methanol extract of flowers of *Musa sapientum*	Methanol extract of fruit peels of *Musa sapientum*
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+
Cardiac glycosides	-	-
Saponin glycosides	+	+
Proteins and Amino acids	+	+
Tannins and phenolic compounds	+	+
Triterpenoids	+	+
Flavonoids	+	+
Coumarins	+	-
Steroids	-	-
Fats and oils	-	-

Table 3: Determination of the MIC of methanol extract of flowers of *Musa sapientum*

Sl. No.	Name of the Bacteria	Concentrations of methanol extract (µg/ml)						
		0	25	50	100	200	300	400
1	*Bacillus Licheniformis*	+	+	-	-	-	-	-
2	*Bacillus subtilis*	+	+	+	+	-	-	-
3	*Proteus vulgaris*	+	-	-	-	-	-	-
4	*Pseudomonas aeruginosa*	+	-	-	-	-	-	-
5	*Shigella flexneri*	+	+	-	-	-	-	-
6	*Shigella boydii*	+	+	-	-	-	-	-
7	*Escherichia coli*	+	-	-	-	-	-	-
8	*Staphylococcus aureus*	+	+	+	-	-	-	-
9	*Staphylococcus epidermidis*	+	-	-	-	-	-	-
10	*Salmonella typhimurium*	+	+	+	+	-	-	-
11	*Vibrio cholerae*	+	+	-	-	-	-	-
12	*Klebsiella pneumoniae*	+	+	+	-	-	-	-

'0 concentration' stands for plain nutrient agar without the drug serving as control '+' stands for growth and '-' stands for no growth.

Table 4: Determination of the MIC of methanol extract of fruit peels of *Musa sapientum*

Sl. No.	Name of the Bacteria	Concentrations of methanol extract (µg/ml)						
		0	25	50	100	200	300	400
1	*Bacillus Licheniformis*	+	+	-	-	-	-	-
2	*Bacillus subtilis*	+	+	+	-	-	-	-
3	*Proteus vulgaris*	+	+	-	+	+	-	-
4	*Pseudomonas aeruginosa*	+	-	-	-	-	-	-
5	*Shigella flexneri*	+	+	+	-	-	-	-
6	*Shigella boydii*	+	+	+	-	-	-	-
7	*Escherichia coli*	+	+	-	-	-	-	-
8	*Staphylococcus aureus*	+	+	+	-	-	-	-
9	*Staphylococcus epidermidis*	+	-	-	-	-	-	-
10	*Salmonella typhimurium*	+	+	+	+	+	-	-
11	*Vibrio cholerae*	+	+	+	-	-	-	-
12	*Klebsiella pneumoniae*	+	+	+	+	-	-	-

'0' stands for plain nutrient agar without the drug serving as control '+' stands for growth and '-' stands for no growth.

Table 5: Determination of ZOI of methanol extract of fruit peels of *Musa sapientum*

Sl. No.	Name of the Bacteria	Ciprofloxacin µg/ml	methanol extract of flower of *Musa sapientum* µg/ml			methanol extract of fruit peels of *Musa sapientum* µg/ml		
		100	100	200	400	100	200	400
1	*Bacillus Licheniformis*	14.00	7.00	8.50	11.00	7.00	8.00	9.50
2	*Bacillus subtilis*	15.50	8.00	9.00	11.50	7.50	9.50	10.00
3	*Proteus vulgaris*	13.50	7.50	8.50	9.50	6.50	7.50	8.50
4	*Pseudomonas aeruginosa*	16.00	7.50	9.50	12.00	7.00	9.00	11.50
5	*Shigella flexneri*	14.50	8.50	10.00	13.00	8.00	9.50	11.00
6	*Shigella boydii*	15.00	8.00	9.50	12.50	7.50	9.00	11.00
7	*Escherichia coli*	14.00	8.50	9.50	11.00	7.00	8.00	9.00
8	*Staphylococcus aureus*	16.50	8.00	9.00	12.00	7.50	8.50	11.00
9	*Staphylococcus epidermidis*	14.50	8.00	9.00	11.50	7.00	8.50	10.00
10	*Salmonella typhimurium*	17.00	8.00	9.50	11.50	7.50	10.50	9.00
11	*Vibrio cholerae*	14.50	8.50	10.00	13.50	8.00	9.50	11.50
12	*Klebsiella pneumoniae*	16.00	8.00	9.00	11.50	7.50	8.50	10.50

Values are Zone of inhibition (mm); tests were done in triplicate.

CONCLUSION

The results of the present work provide evidence that methanol extract of flowers and fruit peels of *Musa sapientum* have antimicrobial activity. These extracts exhibited antibiotic potential on Gram +ve and Gram –ve microorganisms in a dose dependent manner. The phytochemical study showed that the extract contains phytochemicals like alkaloids, carbohydrates, glycosides, saponins, proteins, coumarins, flavonoids, triterpenoids, tannins and phenolic compounds. Presence of both tannin and phenolics may be responsible for potent antibacterial activity. On the basis of the outcomes of the present study, it is concluded that methanol extract of flowers and fruit peels of *Musa sapientum* has potential antimicrobial activities and the result scientifically justifies their use in the folklore remedies.

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