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Research Article

ANTIMICROBIAL AND CYTOTOXIC PROPERTIES OF DIFFERENT EXTRACTS OF *MUSA SAPIENTUM* L. SUBSP. *SYLVESTRIS*

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ABSTRACT
The aim of this study was to evaluate the antimicrobial and cytotoxic activities of the different extracts of *Musa sapientum* L. subsp. *sylvestris* fruits. The methanolic extracts of *Musa sapientum* L. subsp. *sylvestris* peel (MSPE), pulp (MSPU) and seed (MSSE) were investigated for antimicrobial activity by disc diffusion method and for cytotoxic activity by Brine shrimp lethality bioassay. Good antimicrobial activity was shown by MSPU while moderate activity by MSPE against 5 gram-positive (*Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Staphylococcus aureus*) and 8 gram-negative (*Escherichia coli, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio mimicus, Vibrio parahemolyticus*) bacteria and 3 fungi (*Aspergillus niger, Candida albicans, Sacharomyces cerevaceae*). Antimicrobial activity of MSSE against the organisms was insignificant. The order of Brine Shrimp lethality was found as Vincristine sulphate > MSPU > MSSE > MSPE. These findings suggest the potentiality of finding novel compounds with antimicrobial property in the investigated fruit.
KEY WORDS: *Musa sapientum*, antimicrobial, cytotoxicity.

INTRODUCTION

Different species of *Musa* genus (Family: Musaceae), commonly known as Banana, are native to the Asian, Indo-Malaysian and Australian tropics and are now widely found throughout the tropical and subtropical areas. In Bangladesh, bananas grow almost everywhere in the country throughout the year especially in Rangamati, Barisal, Rangpur, Dinajpur, Noakhali, Faridpur and Khulna[1]. Traditionally the banana fruits are used in diarrhoea, dysentery, intestinal lesions in ulcerative colitis, diabetes, sprue, uremia, nephritis, gout, hypertension, cardiac disease[2,3]. Carbohydrates[4], norepinephrine, serotonin, dopamine[5], tryptophan, indole compounds [6], alkaloids, tannin, ascorbic acid, several flavonoids and related compounds (Leucocyanidin, quercetin and its 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) have been isolated from the pulp of plantain[7-9]. Sterols such as β-sitosterol, campesterol, stigmasterol were isolated from the fruit, peel and plant[10-12]. Sitoindoside-I, sitoindoside-II, sitoindoside-III and sitoindoside-IV possessing antiulcerogenic activity were isolated[10]. An antihypertensive principle, 7, 8-dihydroxy-3-methylisochroman-4-one, has been isolated from the fruit peel[13].

Musa sapientum L. subsp. *sylvestris*, commonly known as 'Bichi kola' or 'Aitta kola', is a treelike perennial herb that grows 5-9 m in height, with tuberous rhizome, hard, long pseudostem and big inflorescence with reddish brown bract. *Musa sapientum* L. subsp. *sylvestris* fruit is used (sometimes with seeds) in the treatment of diarhoea and dysentery, in excess menstruation[14]. The fruit of *Musa sapientum* have been found to possess significant antiulcerant[15], antibacterial[16], wound healing[17] and anti-allergic activity[18]. The flowers showed blood glucose and glycosylated haemoglobin reduction and hemoglobin increasing property[19]. The peel possesses diuretic property[20] and the juice of the inflorescence stalk possesses cholesterol and triglyceride lowering activity[21]. The plant also showed antihyperglycemic effect in hyperglycemic rabbit[22].

In the present study, methanolic extracts of *Musa sapientum* L. subsp. *sylvestris* fruit's peel (MSPE), pulp (MSPU) and seed (MSSE) were investigated for antimicrobial and cytotoxic activity in order to justify and support the folkloric use of the fruit in diarrhoea and dysentery.

MATERIALS AND METHODS

Plant material and extraction

Mature and unripe fruits of *Musa sapientum* L. subsp. *sylvestris* were collected from Roypur village in Jibannagar Upazila, Chuadanga, Bangladesh in January 2009. The botanical identification was done by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB: 33833). The separated peel, pulp and seeds were dried, powdered and then extracted by Soxhlet apparatus using methanol. The methanolic extract of the peel, pulp and seed were used for the experiments.

Chemicals and animals

Dimethyl sulfoxide (DMSO) was purchased from Merck; vincristine sulphate was purchased from Sigma-Aldrich. The eggs of the brine shrimp (*Artemia salina* leach) were collected from a shop of University Market, Katabon, Dhaka, Bangladesh.

Phytochemical screening

Qualitative phytochemical tests were performed for the determination of presence of different class of constituents in the extract following standard procedures[2].

Test for antimicrobial activity

The antimicrobial activity test of the plant extracts were performed by the well accepted Bauer-Kirby method[23]. The activity of the extracts were tested against 5 gram-positive bacteria (*Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Staphylococcus aureus*) and 8 gram-negative bacteria (*Escherichia coli, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Pseudomonas aeruginosa, Vibrio mimicus, Vibrio parahemolyticus*) and 3 pathogenic fungi (*Aspergillus niger, Candida albicans, Sacharomyces cerevaceae*). The sterilized Potato Dextrose Agar (PDA) media was mixed with the suspension of bacteria and fungi and transferred to petridishes. The test disc containing the sample was prepared by incorporating 400 µg of extract in a sterilized 6 mm metrical filter paper disc (BBL, Cocksville, USA). Then the test disc, blank disc (negative control) and the Kanamycin standard disc (30 µg/disc, Oxoid Ltd.,UK) were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4^0C for about 24 h. Finally the plates were kept in an incubator at 37^0C for 24 h. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter.

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay was used for exploring the potential cytotoxic action of the extracts according to the methods described by McLaughlin (1982)[24]. Eggs of Brine Shrimp were hatched in a tank containing 3.8% NaCl solution. The plant extracts were dissolved in pure dimethyl sulfoxide (DMSO) and test solutions of different concentrations (1 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, 500 µg/ml) were prepared and were taken in vials containing 10 nauplii in 5 ml of simulated sea water. The toxic effect of the extract or reference drug on shrimp was observed after 24 h counting the surviving shrimps. Duplicate experiments were done for each concentration of extracts. The percent (%) mortality was calculated first and then LC_{50} values were calculated to determine the toxicity of the extracts. Vincristine sulphate was used as the reference cytotoxic drug[25].

Statistical analysis

LC_{50} of the cytotoxic activity test were calculated by regression analysis using SPSS 11.5.0 (Statistical Package for the Social Sciences) for windows (SPSS Inc., USA).

RESULTS

Phytochemical screening

Qualitative phytochemical analysis of the methanolic extract of *Musa sapientum* subsp. *sylvestris* fruit peel, pulp and seed revealed the presence of carbohydrates, alkaloids, steroids and glucosides in all extracts. Flavonoids were detected in pulp, saponin in peel and seed and tannin in peel and pulp extracts.

Antimicrobial activity

In the antimicrobial study it was observed that the methanolic extract of the *M. sapientum* subsp. *sylvestris* pulp showed good activity against all 13 gram-positive and gram-negative bacteria. The peel extract also showed significant activity against all organisms in the test. But the seed extract showed virtually no activity against any organism (Table 1). The standard drug, Kanamycin, showed 29-31 mm zone of inhibition against the gram-positive and gram-negative bacteria (Table 1). The pulp extract showed 19 mm zone of inhibition against *Shigella dysenteriae*, 18 mm zone of inhibition against *Bacillus subtilis* and *Salmonella paratyphi.* It showed 17 mm zone of inhibition against *Bacillus cereus, Bacillus megaterium, Escherichia coli, Shigella boydii, Vibrio mimicus* and *Vibrio parahemolyticus;* 16 mm against *Sarcina lutea, Pseudomonas aeruginosa, Salmonella typhi*; 15 mm against *Staphylococcus aureus*. The peel extract also showed activity against all the test organisms with a zone of inhibition of 7-9 mm. The pulp extract

was also found well effective in inhibiting the growth of the 3 pathogenic fungi (Table 2).

Cytotoxic activity

The peel, pulp and seed extracts were found lethal to brine shrimp with a LC_{50} value of 304.4 µg/ml, 112.4 µg/ml and 212.02 µg/ml respectively, while the standard Vincristine sulphate showed a LC_{50} value of 0.66 µg/ml. The pulp showed better lethality to the shrimps than the peel and seed extract (Table 3). The order of potential lethality of the extracts is: Vincristine sulphate> Pulp> Seed> Peel.

DISCUSSION

The results suggest the wide range of antibacterial activity by the fruit pulp extract against a number of pathogenic bacteria, especially those cause diarrhoea and dysentery. The alkaloids present in the extracts may contribute to the antibacterial activity[26] due to the inhibition of topoisomerase to intercalate DNA and to inhibit DNA synthesis[27], cell lysis and morphological changes[28]. The tannin content of the pulp may also contribute to the antimicrobial activity due to their astringent property that can induce complexation with microbial enzymes or substrates, iron deprivation, hydrogen bonding or nonspecific interaction with microbial enzymes, toxic action on microbial membranes, complexation of metal ions[29]. The antimicrobial activity may also be due to the presence of flavonoids[30], saponins[31] and steroids[32].

The brine shrimp lethality bioassay can detect a broad spectrum of bioactive principles in crude samples for the front-line screening. This method is very useful and is been used in predicting cytotoxic, antitumor and pesticidal activity of extracts[33]. The phytochemicals present in the extracts such as alkaloids, steroids may be responsible for the cytotoxic activity of extracts[34]. The cytotoxic activity of the pulp and peel extracts further supports the antibacterial activity of the extracts.

CONCLUSION

The findings of the present study demonstrate that the methanolic extracts of *Musa sapientum* L. subsp. *sylvestris* fruit possess good antimicrobial activity against a number of gram-positive and gram-negative bacteria as well as against pathogenic fungi and affirm the traditional use of the fruit in the treatment of dysentery and diarrhoea. However, further studies are required to study the bioactive compounds and elucidate the mechanisms of pharmacological activities of the plant.

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Table 1: Antibacterial activity of methanolic extracts of *Musa sapientum* subsp. *sylvestris*.

Bacteria	Diameter of zone of inhibition (mm)			
	MSPE (400 µg/disc)	MSPU (400 µg/disc)	MSSE (400 µg/disc)	Kanamycin (30 µg/disc)
Gram-positive bacteria				
Bacillus cereus	9	17	-	30
Bacillus megaterium	8	17	-	30
Bacillus subtilis	9	18	-	31
Staphylococcus aureus	8	15	-	30
Sarcina lutea	7	16	-	31
Gram-negative bacteria				
Escherichia coli	8	17	4	30
Pseudomonas aeruginosa	8	16	-	29
Salmonella paratyphi	9	18	-	30
Salmonella typhi	7	16	-	29
Shigella boydii	8	17	4	30
Shigella dysenteriae	8	19	5	30
Vibrio mimicus	8	17	4	30
Vibrio parahemolyticus	8	17	4	31

MSPE = *Musa sapientum* subsp. *sylvestris* peel extract, MSPU = *Musa sapientum* subsp. *sylvestris* pulp extract, MSSE = *Musa sapientum* subsp. *sylvestris* seed extract.

Table 2: Antifungal activity of methanolic extracts *Musa sapientum* L. subsp. *sylvestris*.

Fungi	Diameter of zone of inhibition (mm)			
	MSPE	MSPU	MSSE	Kanamycin
Aspergillus niger	8	18	-	30
Candida albicans	8	19	-	30
Sacharomyces cerevaceae	7	16	-	28

MSPE = *Musa sapientum* subsp. *sylvestris* peel extract, MSPU = *Musa sapientum* subsp. *sylvestris* pulp extract, MSSE = *Musa sapientum* subsp. *sylvestris* seed extract.

Table 3: Cytotoxic activity of methanolic extracts of *Musa sapientum* subsp. *sylvestris.*

Extract	LC_{50} (µg/ml)
MSPE	304.4
MSPU	112.4
MSSE	212.02
Vincristine sulphate	0.66

MSPE = *Musa sapientum* subsp. *sylvestris* peel extract, MSPU = *Musa sapientum* subsp. *sylvestris* pulp extract, MSSE = *Musa sapientum* subsp. *sylvestris* seed extract.

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