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Research Article

ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *MUSA PARADISIACA* CV. PUTTABALE AND *MUSA ACUMINATE* CV. GRAND NAINE

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ABSTRACT

Banana is one of the most popular fruits distributed all over the world. Traditional, the fruit, Stem juice, flowers of banana plants were used for treating diarrhoea (unripe), dysentery, menorrhagia, diabetes, antilithic, antiulcerogenic, hypoglycemic, hypolipidemic , antioxidant actions and inflammation, pain & snakebite. In order to justify the ethnomedicinal claims. The corm ethanol extracts of *Musa acuminate* cv. Grand naine and *Musa paradisiaca* cv. Puttabale were screened for potential antibacterial activity using agar well diffusion method against 8 clinical strains. Extracts of *M. paradisiaca* showed a significant level of bacterial inhibition against *Proteus vulgaris* (19.78±0.40), *Pseudomonas aeruginosa* (19.67±0.41) and *Staphylococcus aureus* (17.44±0.50), moderate activity against *Salmonella typhi* (16.89±0.48), *Salmonella paratyphi* (16.67±0.37), *Klebsiella pneumoniae* (15.56±0.50), *Bacillus subtilis* (15.11±0.42) and very less against *Escherichia coli* (09.11±0.39). The extract of *M. acuminate* showed a significant level of bacterial inhibition against *Proteus vulgaris* (18.89±0.42), *P. aeruginosa* (13.44±0.47), *Staphylococcus aureus* (15.56±0.38), moderate activity against *Salmonella typhi* (15.67±0.44), *Salmonella paratyphi* (20.44±0.56), *Klebsiella pneumoniae* (14.33±0.33), *Bacillus subtilis* (14.00±0.41) and the very less against *Escherichia coli* (12.44±0.53).

Keywords: Antibacterial activity, Corm ethanol extracts, *Musa acuminate* cv. Grand naine and *Musa paradisiaca* cv. Puttabale.

INTRODUCTION

Medicinal plants have been used from centuries to treat infectious diseases as an alternative form of health care. In recent years there has been intensifying attention in the detection of new antimicrobial compounds; due to alarming raise in the rate of diseases with multi-drug resistant microorganisms (Bassam et al. 2006; Bhavani and Ballow, 2000) and due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control some bacterial diseases (Cowan, 1999). This shows the way of researchers to investigate the antimicrobial activity of the medicinal plants. Plants produce highly bioactive molecules that allow them to interact with other organisms in their environment.

Banana is one of the most popular fruits distributed all over the world. The production of this fruit in India is very high. FAO estimates that banana production was approximately 23,204,800 tons in the year 2008. Traditionally, the fruit, Stem juice, flowers of banana plants were used for treating diarrhoea (unripe), dysentery, menorrhagia, diabetes (Ghani, 2003; Yusuf et al., 2009), antilithic (Prasad, 1993, Khare, 2007), antiulcerogenic (Goel et al. 1986; Mukhopadhyaya et al. 1987; Lewis, 1999), hypoglycemic (Gomathy et al., 1990), hypolipidemic, antioxidant actions (Krishnan, 2005) and inflammation, pain & snakebite (Coe and Anderson, 1999).

Pharmacological investigations revealed that banana fruits, Stem juice, flowers are screened for antidiarrhoeal activity (Rabbani et al., 1999, 2001), antiulcerative activity (Pannangpetch et al. 2001; Goel and Sairam, 2002; Jain et al. 2007), antimicrobial activity (Richter and Vore, 1989; Ahmad and Beg, 2001; Mokbel and Hashinaga, 2005; Alisi et al., 2008; Fagbemi et al., 2009; Mumtaz Jahan, 2010), Hypoglycemic activity (Ojewole and Adewunmi, 2003; Mallick et al., 2006; Mallick et al., 2007; Singh et al., 2007); Hypocholesterolaemic activity (Vijayakumar et al.,2009), antioxidant activity (Yin et al., 2008), Diuretic activity (Jain et al., 2007), Wound healing activity (Agarwal et al. 2009), Anti-allergic activity (Tewtrakul et al., 2008), Antimalarial activity (Kaou et al., 2008), Anti-snake venom activity (Borges et al. 2005). Literature reviews indicated that banana fruits and flowers contain antibacterial principles and no reports available for antibacterial activities from corm of banana plants.

MATERIALS AND METHODS

Plant material and extraction

The corm of *M. paradisiaca* cv. Puttabale and *M. acuminate* cv. Grand naine were collected from the villages near by Kuvempu University, Shimoga district, Karnataka, India. The corms were cleaned with deionized water and were shade dried, grounded porously by using mechanical blender and passes through 40-mesh sieve. About 1 kg of powder material was dipped in cold 95 % ethanol and incubated on rotary shaker at 80-120 rpm for 15 days at room temperature. The extracts were filtered (Whatman No.1 filter paper) and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) and then the extract was kept on water bath to obtain crude extract and subjected to phytochemical screening.

Bacterial strains and culture media

Various cultures of human pathogenic gram positive bacteria namely, *Bacillus subtilis*-NCIM-2063, *Staphylococcus aureus*-NCIM-2079, *Pseudomonas aeruginosa*-NCIM-2036 and gram negative bacteria namely, *Escherichia coli*-NCIM-2065, *Proteus vulgaris*-NCIM-2027, *Salmonell typhi*-NCIM- 2501, *Klebsiella pneumoniae*-NCIM-2957 and *Salmonella paratyphi*-MTCC-735 were obtained from National Chemical Laboratory, Pune and Microbial type culture collection and gene bank, Chandigarh, India and were used for screening of antibacterial activity of corm extracts of *M. paradisiaca* cv. Puttabale and *M. acuminate* cv. Grand naine. The microorganisms were repeatedly subcultured on sterile nutrient agar media in order to obtain pure isolates. A loop full test organism was inoculated on nutrient broth and incubated for 24 h at 37 ± 1°C and maintained in sterile condition.

Agar well diffusion assay

Antibacterial activity of leaf extract was screened against eight different bacterial strains by agar diffusion method. The culture plates were prepared with sterile nutrient agar media. 100 µl of bacterial culture (10^5cells ml) was inoculated on to the culture plate using sterile L-shaped glass rod to get uniform distribution of bacteria. Wells were created using a stainless steel sterilized cork borer (6.0 mm) under aseptic conditions. 50µl of the plant extract at different concentrations (100, 80, 60, 40, 20, 10 mg/ml) were aseptically loaded into wells. For comparative evaluation, Ciprofloxacin (BioChemika, ≥98.0% (HPLC) (Fluka)) was used as a positive reference standard and sterile distilled water as negative control. Then, the cultured plates were incubated for 24 h at 37°C. After incubation, inhibition of the bacterial growth was measured in mm. The result was statistical analyzed using one-way ANOVA and all the values are expressed as Statistical analysis mean ± S.E. (n = 9). Values of $P < 0.05$ were considered as significant.

Minimal inhibitory concentration determination

Minimal inhibitory concentration (MIC) values were determined by broth dilution method. Serial dilutions (final volume of 1 ml) of corm extracts of M. paradisiaca cv. Puttabale and M. acuminate cv. Grand naine (5.0 to 0.5 mg/ml) were performed with 0.9% saline. Following this, 9 ml of nutrient broth was added. Broths were inoculated with 100 µl of each bacterial suspension (5×10^4CFU) and incubated for 24 h at 37^0C. Ciprofloxacin was used as a positive control and 0.9% saline as negative control. After 24 h, bacterial growth was assayed by measuring absorbance at 625 nm. MIC was defined as the lowest concentration in mg of corm extracts of *M. paradisiaca* cv. Puttabale and *M. acuminate* cv. Grand naine to restrict the growth to ‹0.05 absorbance at 625 nm.

RESULTS AND DISCUSSION

The extraction of 1000 g of corm powder in cold ethanol yielded 28 g of *M. paradisiaca* cv. Puttabale and 32 g of *M. acuminate* cv. Grand naine respectively. The preliminary phytochemical screening of the corm extracts gave positive tests for the presence of flavonoids, glycosides, terpenoids and tannins (Table. 1). The antibacterial activity of ethanol extract of corm of *M.paradisiaca* and *M. acuminate* showed varying magnitudes of inhibition patterns with standard drug Ciprofloxacin a well-known broad-spectrum antibacterial agent. The mean inhibitory zone of ethanol extracts and the standard drug Ciprofloxacin against eight bacterial species is summarized in Table 2. In the agar diffusion method, corm extract of *M. paradisiaca* showed a significant level of bacterial inhibition against *P. vulgaris* (19.78±0.40), *P. aeruginosa* (19.67±0.41) and *S. aureus* (17.44±0.50). It showed moderate activity against *S. typhi* (16.89±0.48), *S. paratyphi* (16.67±0.37), *K. pneumoniae* 15.56±0.50, *B. subtilis* (15.11±0.42), and very less against *E.coli* (09.11±0.39). The extract of *M. acuminate* showed a significant level of bacterial inhibition against *P. vulgaris* (18.89±0.42), *P. aeruginosa* (13.44±0.47) and *S. aureus* (15.56±0.38). It showed moderate activity against *S. typhi* (15.67±0.44), *S. paratyphi* (20.44±0.56), *K. pneumoniae* (14.33±0.33), *B. subtilis* (14.00±0.41) and the very less against *E. coli* (12.44±0.53).

The lowest concentration of the corm extracts, requisite for hampering the growth was considered as the MIC of the extracts against bacterial strains. The MIC value determined by broth dilution method indicated that significant antibacterial activity of corm ethanol extracts at 0.5 to 5 mg/ml against the eight tested bacterial strains was presented in Table 3. It was found that the data obtained from the extracts of *M. paradisiaca* cv. Puttabale and *M. acuminate* cv. Grand naine required relatively lesser quantity for inhibiting the growth of tested microorganisms. Ahmad and Beg, 2001 investigated the alcoholic extracts of *M. paradisiaca* banana fruit peel showed better activity against the Staphylococcus (Gram-positive) and Pseudomonas species (Gram-negative) than banana leaf extract. However, the alcoholic stem extract showed no activity against *Staphylococcus aureus, Salmonella paratyphi, Shigella dysenteriae, Escherichia coli, Bacillus subtilis, Candida albicans*. This is may be presence of phytoconstituents at very lower concentration than banana fruit peel and leaf extracts. Similar report by Fagbemi et al., 2009 with ethanolic and aqueous extract of unripe *M. sapientum* fruit showed good activity against *S. aureus, S. aureus, Salmonella paratyphi, Shigella flexnerii, E. coli, Klebsiella pneumoniae, B. subtilis* and *Pseudomonas aeruginosa* with minimum inhibitory concentration (MIC) ranged at 2-512 mg/ml and 32-512 mg/ml respectively. The result indicated that ethanolic extracts showed significant antibacterial activity than water extracts. But ethyl acetate extract of *M. sapientum* seeded banana peel and pulp exhibited significant higher antibacterial activity than the ethanolic extract (Preeti Jain, et al., 2011). This signifies that ethyl acetate can dissolve the more active phytochemicals than ethanol. In the present study, extraction of phytochemicals from cold ethanolic of both M. paradisiaca cv. Puttabale corm showed higher activity against P. vulgaris-NCIM-2027 (19.78±0.40), *P. aeruginosa* -NCIM-2027 (19.67±0.41) and lowest activity against *E. coli* -NCIM-2065 (09.11±0.39) and *M. acuminate* cv. Grand naine corm showed good antibacterial activity against *S. paratyphi*-MTCC-735 (20.44±0.56) and lowest against *E. coli* -NCIM-2065 (12.44±0.53). This is due to presence of alkaloids, tannin, steroid and flavonoids may be collectively or individually responsible for the observed antimicrobial activities. Subrata Kumar Biswas, et al., 2011 reported that ethanol extract of the root of Musa Paradisiaca Lam. showed moderate in-vitro antibacterial activity against both gram positive (*B. Megaterium, B. Subtilis, S. aureus*) and gram negative (*E. coli, P. aeruginosa, S. dysenteriae, S. typhi, Vibrio cholerae and S. flexneri*) bacteria with the zones of inhibition ranging from 10.53 ± 0.37 to 12.42 ± 0.85 mm at concentration of 500 µg/disc. But in this study corms extraction showed better antibacterial activity than root extract and may be it depends on the cultivar of banana and geographical distribution.

Table 1: Phytochemical screening of ethanol extract of M. paradisiaca cv. Puttabale and M. acuminate cv. Grand naine.

Sl.No.	Test	Procedure	Observation	*M. paradisiaca* cv. Puttabale	*M. acuminate* cv.Grandnaine
1	Alkaloids	Extract + Dragondroffs reagent	No orange ppt.	–	–
		Extract + Mayer's reagent	No white ppt.	–	–
		Extract + Hager's reagent	No yellow ppt.	–	–
2	Sterols	Extract + Liebermann test	change in color	+	+
3	Flavonoids	Shinodaw's test	Red color	+	+
		Zn-HCl acid reduction test	Magneta color	+	+
4	Glycosides	Extract + Anthrone + H_2SO_4 +Heat	Purple color	+	+
5	Terpenoids	Extract + chloroform + con. H_2SO_4	Lower layer turns yellow	+	+
6	Tannins	Extract + lead acetate + water	White ppt.	+	+
7	Quinones	Extract + conc. H_2SO_4	No red color	+	+
8	Saponins	Extract + water + Shake well	Formation of stable froth	–	–

+ = Present, – =Absent

Table 2: Antibacterial activity of ethanol extract of *M. paradisiaca* cv. Puttabale and *M. acuminate* cv. Grand naine against various bacterial strains by agar diffusion method.

Test bacterial strains	Gram stain	Ciprofloxacin	Musa paradisiaca cv. Puttabale (mg/ml)	Musa acuminate cv. Grand naine (mg/ml)
B.subtilis- NCIM-2063	+	23.17±0.31	15.11±0.42	14.00±0.41
S.aureus-NCIM-2079	+	23.67±0.33	17.44±0.50	15.56±0.38
E.coli -NCIM-2065	–	25.00±0.26	09.11±0.39	12.44±0.53
S.typhi-NCIM- 2501	–	23.00±0.26	16.89±0.48	15.67±0.44
S.paratyphi-MTCC-735	–	21.67±0.42	16.67±0.37	20.44±0.56
P.vulgaris-NCIM-2027	–	23.17±0.17	19.78±0.40	18.89±0.42
K.pneumoniae-NCIM- 2957	–	22.50±0.62	15.56±0.50	14.33±0.33
P.aeruginosa -NCIM-2027	+	23.50±0.50	19.67±0.41	13.44±0.47

Concentration of extract - 40 mg/disk

Note: Zone of inhibition in mean± SE mm (n=9)
Values * p < 0.05 are considered significant compared to standard.

Table 3: MIC values of bacterial strains

Sl. No.	Test bacterial strains	Musa paradisiaca cv. Puttabale (mg/ml)	Musa acuminate cv. Grand naine (mg/ml)
1	*P.vulgaris*-NCIM-2027	0.5	0.5
2	*P.aeruginosa* -NCIM-2027	0.5	3.0
3	*S.aureus*-NCIM-2079	1.5	2.0
4	*S.typhi*-NCIM- 2501	1.0	1.5
5	*S.paratyphi*-MTCC-735	1.5	0.5
6	*K.pneumoniae*-NCIM- 2957	1.5	2.0
7	*B.subtilis*- NCIM-2063	1.5	2.0
8	*E.coli* -NCIM-2065	4.0	3.0

CONCLUSION

The ethanol extracts of *M. paradisiaca* cv. Puttabale and *M. acuminate* cv. Grand naine extracts showed the broad spectrum of antibacterial activity on the tested microorganisms with high inhibitory potency against *P. vulgaris* and *S. paratyphi*. The phytochemical analysis showed the presence of effective biological compounds like glycosides, flavonoids, Terpenoids, tannins. These derivatives could be potential control of clinical pathogenic bacteria. Fractionation and characterization of these active compounds will be the future work to investigate.

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