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Antimicrobial and antihyperglycemic activities of *Musa paradisiaca* flowers

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ABSTRACT

Objective: To screen the antimicrobial and antihyperglycemic activities of *Musa paradisiaca* (*M. paradisiaca*) flowers. **Methods:** The EtOH and EtOH: water (1:1) extracts of *M. paradisiaca* flowers were screened for antibacterial and antifungal activity against standard strains of *Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Staphylococcus aureus* (*S. aureus*), *Salmonella typhimurium* (*S. typhimurium*) and *Candida albicans* (*C. albicans*), *Cryptococcus albidus* (*C. albidus*) against amikacin and clotrimazole respectively. Both the extracts were also administered to normal and alloxan induced diabetic rats. The blood glucose levels were measured daily after oral administration of extracts at doses of 100, 250 and 500 mg/(kg·d). **Result:** The EtOH and EtOH: water (1:1) extracts exhibited antimicrobial activity with minimum inhibitory concentrations ranging from 5.62–25.81 and 7.60–31.50 μg/mL respectively. Both the extracts reversed the permanent hyperglycemia within a week in alloxan induced diabetic rats. The EtOH extract (250 mg/kg) was found to be 7.69% more potent hypoglycemic effect than standard oral hypoglycemic drug, glibenclamide 0.2 mg/kg b.w., respectively. **Conclusion:** The alcoholic extracts of *M. paradisiaca* flowers showed potent antihyperglycemic and moderate antimicrobial activities.

1. Introduction

Medicinal plants are used traditionally for the treatment of various ailments all over the world since the beginning of civilization[1]. Due to alarming incidence of antibiotic resistance in bacteria and fungi; there is requirement for new leads for bacterial and fungal infections[2–5]. Herbal drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects; hence explored for the discovery of potentially useful antimicrobial and antihyperglycemic leads[6–14]. The World Health Organization (WHO) has listed 21 000 medicinal plants; out of which 2 500 species belongs to India; hence known as botanical garden of the world[15].

Musa paradisiaca (*M. paradisiaca*) (family Musaceae) fruit commonly known as banana; used for its nutritional values all over the world. *M. paradisiaca* travelled from its native home Southwestern Pacific to India by about 600BC and latter spread all over the globe. Traditionally *M. paradisiaca* L. in India used for dressing of wounds and ulcers, eye diseases, anaemia, cachexia, haemorrhages, dysmenorrhoea, menorrhagia, inflammation and diabetes. It is locally known in Indian languages as kala, vana laxmi, kadali, rambha, kadalamu, valei, vala, bale hannu and in Eng. plantain or banana[16]. Fruits, leaves, peels, root, and stalks of *M. paradisiaca* L. have been used orally or topically as a medicine for treating diarrhea and dysentery, intestinal colitis[17], antilithic[18], inflammation, pain and snakebite[19–21], and protein metabolic disorders[22]. In addition it also posses uses as antimicrobial[23], antiulcerogenic[24], antihelmintic[25], hypoglycemic[26–28], antioxidant[29–30]. In the present study, *M. paradisiaca* flowers were studied for antimicrobial and antihyperglycemic activities.

2. Material and methods

2.1. Collection of flowers

Flowers of *M. paradisiaca* (Musaceae) were collected from Muradnagar, Ghaziabad, Uttar Pradesh, India in January,

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2010. The plant was identified by plant taxonomist Dr. A.K. Sharma, Department of botany, Multanimal Modi (P.G.) College, Modinagar, Ghaziabad (U.P.), India and voucher specimen (MMCM/02/021) was deposited for future reference.

2.2. Preparation and extraction of flowers

Fresh flowers were pulverized in electrical grinder. Powdered flowers (500 g) were heated with ethanol at 70 ℃ for 1 h to stop enzymatic reactions and kept for cold maceration for 7 d. The residue was further macerated with EtOH: water (1:1) for next 7 d. The weights of crude extracts were 40.9 g (=8.2%) & 51.7 g (=10.3%, w/w, yield) for EtOH and EtOH: water (1:1) extracts respectively.

2.3. Phytochemical analysis

Flowers extracts were subjected to preliminary quantitative tests for the presence of carbohydrate, protein, steroid, glycoside, saponin, alkaloid, tannin, phenolic compound and flavonoid according to standard quantitative and qualitative methods[31–32].

2.4. Microorganisms

Bacterial and fungal strains were obtained from I.T.S Paramedical College (Pharmacy) and I.T.S Center for Dental Studies and Research, Muradnagar, Ghaziabad, U.P., India. Mueller Hinton agar and Saboraud's dextrose agar (SDA) were procured from Himedia Laboratories (India).

2.5. Disc diffusion method

Antibacterial activity against standard strains of *Bacillus subtilis* (*B. subtilis*) (MTCC–121), *Bacillus cereus* (*B. cereus*) (MTCC–430), *Escherichia coli* (*E. coli*) (MTCC 443), *Klebsiella pneumoniae* (*K. pneumoniae*) (109), *Proteus mirabilis* (*P. mirabilis*) (MTCC–1429), *Staphylococcus aureus* (*S. aureus*) (ATCC 25923), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 9027), *Salmonella typhimurium* (*S. typhimurium*) (MTCC–98), *Streptococcus pneumoniae* (*S. pneumoniae*) (MTCC–2672) using amikacin as reference, and antifungal activity against standard strains of *Candida albicans* (*C. albicans*) (MTCC–183), *Cryptococcus albidus* (*C. albidus*) (MTCC–2661) using clotrimazole as reference were evaluated using disc diffusion method. Sterile filter paper discs (Whatman No. 1, diameter 5 mm) were loaded with 100 μL of flowers extracts (5 mg/disc), reference drugs; amikacin (10 μg/disc; for bacteria) and clotrimazole (20 μg/disc; for fungi). The discs were completely saturated with the extracts and dried. The bacteria and fungi were cultured in Muller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) respectively and incubated at 37 ℃ for 24 h. Then, the active cultures were inoculated into 10 mL of cultures (MHB/PDB) and incubated at 37 ℃ for 15 h. Microorganisms were diluted with MHB/PDB to obtain bacterial/ fungi count of 5–10×105 CFU/ mL. The loaded discs were placed on tryptone agar plates (for bacteria) and Saboraud's dextrose agar plates (for fungi) inoculated on the surface with each microorganism culture

(0.01 mL) and incubated at 37 ℃, for 24–48 h. The discs were tested in triplicate, including blank and zones of inhiition (Table 1) were measured[33–35].

2.6. Minimum inhibitory concentration

Minimum inhibitory concentrations (MIC's) of all the extracts were determined by micro dilution method[36]. It is carried out by the disc diffusion test of different concentration of the extracts. The minimum concentration of extracts that inhibits the growth of bacteria and fungi were noted as MIC values (Table 2).

2.7. Experimental rats

Male albino rats (Wistar strain) (150–250 g) of age 8–12 weeks old were procured from Institutional Animal House (Reg. No. 1044/c/07/CPCEA), I.T.S Paramedical (Pharmacy) College, Muradnagar, Ghaziabad, Uttar Prasesh, India. Animals were housed under standard conditions (25 ℃, 12 h light and 12 h dark cycle, 60% humidity, water ad libitum), and acclimatized to the laboratory conditions for 6 d.

2.8. Blood glucose level determination

Levels of glucose in blood (mg/100 mL) were measured with Accu–Check active (Roche Diagnostic GmbH, Germany), based on the glucose oxidase method. Blood samples were collected from tip of the tail at defined time intervals.

2.9. Acute toxicity studies

M. paradisiaca flowers extracts were tested for their acute and short– term toxicity in albino Wistar rats. For determining acute toxicity of a single oral administration of herbal drug, the OECD guidelines (OECD/OCDE 2001, 423, Annex 2c) were followed[37]. Stepwise doses of extracts from 300 mg/kg b.w. up to the dose 5 000 mg/kg b.w. were administered orally. Animals were kept under observation continuously for the initial 4 h and intermittently for next 6, 24, and 48 h following drug administration. Parameters like grooming, sedation, and hyperactivity, loss of righting reflex, respiratory rate and convulsion were observed. No considerable signs of toxicity were observed in tested animals. On the basis of above study, following doses 100, 250 and 500 mg/kg were selected for present aim.

2.10. Induction of diabetes

Diabetes was induced in rats by a single intraperitoneal injection of alloxan monohydrate (CDH, Bombay) in normal saline (120 mg/kg) after overnight fasting for 12 h. The fasting blood glucose level was measured after 48 h of injection. The rats with effective and permanent elevated blood glucose levels (⩾ 300 mg/100 mL) were selected.

2.11. Effect of flowers extracts on glucose–loaded normal rats

Oral glucose tolerance test was carried after overnight

fasting (16 h) of normal rats. Vehicle (distilled water), EtOH extracts of flowers of *M. paradisiaca* at three doses (100, 250 and 500 mg/kg) and standard oral hypoglycemic drug, glibenclamide (Daonil® Sanofi Aventis Pharma. Ltd. Mumbai, India) (0.2 mg/kg) were administered to six different groups of rats (*n*=6). Glucose (4 g/kg) was fed to rats; 60 min after treatment with flowers extracts. Blood samples were withdrawn from tip of the tail after 0, 30, 60, 90, 120, 240 and 360 min from normal control and experimental animals for measurement of blood glucose levels.

2.12. Measurement of blood glucose level in diabetic rats after 7 d treatment

Albino rats were divided randomly in four groups of 6 rats each. After overnight fasting; diabetic rats were treated orally with vehicle, EtOH extracts of *M. paradisiaca* flowers (100, 250 and 500 mg/kg) and glibenclamide (0.2 mg/kg) daily up to 7 d. Blood samples were collected from tip of the tail daily from control and experimental animals.

2.13. Statistical analysis of results

Results were analyzed on the GraphPad instat version 5 software using Student's *t* test for paired data and one way ANOVA using Dunnett's multiple comparison test. A difference in the mean values of $P<0.05$ were considered significant.

3. Result

Phytochemical screening of *M. paradisiaca* flowers revealed the presence of carbohydrates, steroids, glycosides, tannins, flavonoids in the ethanolic extract and carbohydrates, glycosides, tannins, flavonoids, saponins in the EtOH: Water (1:1) extract respectively. The antibacterial and antifungal properties of flowers extracts against medically important pathogens were measured by the presence or absence of zones of inhibition and the MIC values. The antibacterial activity of the EtOH and EtOH: water (1:1) extracts of the *M. paradisiaca* flowers were very effective against most of the bacteria tested and especially against Bacillus species

and *P. aeruginosa*. The EtOH extract was shown to have significant antifungal activity against *C. albicans, C. albidus.*

However petroleum ether extract did not inhibit the growth of any of the tested bacterial and fungal species. Since EtOH and EtOH: water (1:1) extracts were shown to be effective, hence MICs of these extracts were performed. The EtOH and EtOH: water (1:1) extracts showed MICs of 5.62–25.81 and 7.60–31.58 μg/mL respectively (Table 2). EtOH extract was highly effective against *P. aeruginosa, B. subtilis* and *B. cereus* with an MIC of 5.93, 6.82, 7.95 μg/mL. Both extracts showed effective inhibition towards *C. albicans* and *Cryptococcus albidus.* MIC studies further supported the data obtained by agar diffusion assay indicating zones of inhibition (Table 1). This study proves the traditional use of *M. paradisiaca* as antibacterials.

The EtOH and EtOH : water (1:1) extracts of *M. paradisiaca* flowers at the doses of 100, 250 and 500 mg/kg reduced the blood glucose level significantly after 30 min in glucose loaded rats; which was comparable to the glibenclamide (0.2 mg/kg) ($P<0.05$)(Table 3). Further, treatment of diabetic rats up to 7 d with flowers extracts at doses of 100, 250 and 500 mg/(kg.d), p.o. significantly reversed the permanent hyperglycemia induced by alloxan monohydrate. The highest anti–hyperglycemic effect was observed by the EtOH extract of flowers at 100 mg/kg. The EtOH extract (250 mg/kg) was found to be 7.69% more potent than standard oral hypoglycemic drug, glibenclamide 0.2 mg/kg b.w., respectively (Table 4). The LD_{50} for *M. paradisiaca* extracts were found to be >5 000 mg/kg (p.o.) in albino wister rats. Morbidity and sign of toxicity was not observed in any of the five normal rats tested with extracts.

The present antimicrobial and antihyperglycemic study is the first systematic positive report on the efficacy of EtOH and EtOH: water (1:1) extracts of *M. paradisiaca* flowers. The polar extracts might be effective due to the fact that the majority of the traditional medicines were prepared using water as the medium.

4. Discussion

The EtOH and EtOH: water (1:1) extracts showed antimicrobial activity towards both Gram–positive, Gram–

Table 1.
Antimicrobial activity of extracts of *M. paradisiaca* flowers by disc diffusion assay (*n*=3).

Microorganism	Zone of inhibition (mm)			
	A	B	Amik	Clotr
B. cereus (MTCC–430)	13.7±0.8	10.3±0.5	16.9±0.6	NC
B. subtilis (MTCC–121)	11.9±0.4	13.2±0.8	13.7±1.4	NC
E. coli (MTCC– 443)	3.6±0.5	6.7±1.3	2.7±0.8	NC
K. pneumonia (MTCC–109)	5.2±0.7	5.9±1.3	6.4±0.5	NC
P. mirabilis (MTCC–1429)	3.4±0.9	5.6±1.2	3.2±1.4	NC
P. aeruginosa (ATCC–9027)	11.1±0.9	9.4±1.7	12.8±1.1	NC
S. typhimurium (MTCC–98)	1.3±1.6	1.4±0.6	13.3±0.2	NC
S. aureus (ATCC–25923)	7.3±1.8	5.1±0.8	13.8±0.3	NC
S. pneumonia (MTCC–2672)	4.7±1.3	1.6±1.2	4.7±0.9	NC
C. albicans (MTCC–183)	8.3±0.7	1.5±0.8	NC	12.9±1.2
C. albidus (MTCC–2661)	5.5±1.2	3.8±2.6	NC	13.5±0.8

Each value represents mean ± SEM; A – EtOH extract; B – Ethanol: water (1:1) extract; Amik – Amikacin; Clotr – Clotrimazole; NC – Not Carried.

Table 2.
Minimum inhibitory concentrations of extracts of *M. paradisiaca* flowers (*n*=3).

Microorganism	MIC (μg/mL)			
	A	B	Amik	Clotr
S. aureus (ATCC−25923)	11.36	19.83	0.89	NC
E. coli (MTCC− 443)	20.37	31.58	3.77	NC
P. aeruginosa (ATCC−9027)	5.62	12.45	0.93	NC
B. subtilis (MTCC−121)	6.82	14.83	0.65	NC
B. cereus (MTCC−430)	7.95	19.57	3.12	NC
K. pneumonia (MTCC−109)	19.59	21.32	0.59	NC
P. mirabilis (MTCC−1429)	20.78	22.13	5.68	NC
S. typhimurium (MTCC−98)	25.81	15.27	0.94	NC
S. pneumonia (MTCC−2672)	21.89	24.86	0.58	NC
C. albicans (MTCC−183)	8.62	9.88	NC	0.53
C. albidus (MTCC−2661)	6.49	7.61	NC	0.59

Each value represents mean ± SEM; MIC−Minimum Inhibitory Concentration; A − EtOH extract; B − EtOH: Water (1:1) extract, NC Not carried; Amik − Amikacin; Clotr − Clotrimazole.

Table 3.
Antihyperglycemic effect of *M. paradisiaca* flowers extracts in glucose loaded normal hyperglycemic rats (*n*=6).

Treatment	Mean blood glucose concentration ±SEM (mg/dL)						
	0 min	30 min	60 min#	90 min	120 min	240 min	360 min
Control	96.35±2.67	95.90±3.21	94.49±1.83	158.38±3.13	134.29±3.59	112.27±3.51	100.92±2.74
Glib (0.2 mg/kg)	95.63±2.54	81.23±3.39**	67.84±3.62**	92.47±2.23**	80.54±2.22**	65.65±3.62**	75.36±3.62**
A (100 mg/kg)	96.46±2.32	96.64±1.75	93.56±2.67	149.39±5.11*	137.93±3.87	103.66±3.64	98.93±2.75
A (250 mg/kg)	94.65±1.99	92.58±2.72	87.63±2.11*	129.83±4.57**	118.75±3.03**	98.39±2.86*	94.74±2.22
A (500 mg/kg)	98.57±1.93	96.59±1.55	89.88±2.64*	133.63±4.06*	121.46±2.99*	99.36±2.57*	96.52±2.69
B (100 mg/kg)	93.83±2.59	94.57±2.38	92.89±1.25	138.44±2.66*	130.56±3.53	104.89±2.84*	101.22±4.87
B (250 mg/kg)	96.37±1.47	94.49±2.76	93.38±1.59	137.39±2.83*	129.92±3.39	102.67±4.66*	99.09±2.39
B (500 mg/kg)	97.45±1.68	94.48±2.30	92.34±1.61	135.88±2.05*	125.56±3.09	100.36±4.57*	98.29±2.78

SEM − Standard error of the mean; #Glucose load (4 g/kg), *$P<0.01$, significantly different compared to control, **$P<0.001$, significantly different compared to control, Glib. − Glibenclamide, A − EtOH extract; B − EtOH: water (1:1) extract.

Table 4.
Antihyperglycemic effect of *M. paradisiaca* flowers in diabetic rats up to 7 d (*n*=6).

Treatment#	Mean blood glucose concentration ±SEM (mg/dL)				
	0 day	1st day	3rd day	5th day	7th day
Control	98.76±3.50	97.84±2.21	94.03±2.59	97.37±1.08	96.42±3.08
Diabetic control	311.36±3.86	315.90±2.16	319.05±2.11	315.86±6.57	313.69±3.61
Glib (0.2 mg/kg)	318.49±3.63	281.32±7.77*	237.28±5.52*	169.86±4.54**	92.76±3.61**
A (100 mg/kg)	314.26±4.29	278.38±3.86*	245.66±5.71**	161.85±4.35**	98.28±3.46**
A (250 mg/kg)	319.28±5.97	281.72±4.92**	239.47±5.29**	152.23±3.37*	85.62±6.75**
A (500 mg/kg)	310.57±3.78	284.68±4.63**	241.39±3.06**	157.96±4.43*	97.31±4.73**
B (100 mg/kg)	314.74±2.87	289.46±6.83*	241.36±5.32*	165.48±3.92*	105.32±3.33*
B (250 mg/kg)	319.52±3.28	287.56±7.24**	243.93±4.85**	163.54±6.68**	102.29±6.37**
B (500 mg/kg)	317.96±2.37	285.50±5.22**	240.46±4.07**	161.58±5.75**	101.22±3.30**

#mg/(kg.d) for 7 d; *$P<0.01$, significantly different compared to diabetic control, **$P<0.001$, significantly different compared to diabetic control.

negative organisms and the fungi which support the traditional use of this plant in the Ayurvedic treatise and the Charka Samhita (100 A.D.). The present study also suggests that the EtOH and EtOH: water (1:1) extracts of *M. paradisiaca* flowers have potent anti−hyperglycemic properties. Treatment up to week with these extracts reversed the permanent hyperglycemia induced by alloxan. Phytochemical screening showed the presence of glycosides, flavonoids, saponins and tannins in flowers extracts of *M. paradisiaca*. Many reports suggested the hypoglycemic properties of flavonoids[38−41]. This may be the reason for antidiabetic properties of *M. paradisiaca* flowers. Further biochemical, toxicological and pharmacological investigations are required to better characterize the active principle(s) responsible for antimicrobial and antidiabetic properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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