PHYTOCHEMICAL EVALUATION, LIQUID CHROMATOGRAPHY-MASS SPECTROSCOPY AND ANTI DIABETIC, ANTI BACTERIAL ACTIVITY OF *MUSA X PARADISIACA* FLOWER EXTRACT

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ABSTRACT

Musa x *paradisiaca* flower extract are traditionally used to treat diabetes and its complications (inflammation and formation of reactive oxygen species) around the world. The present study investigates the antidiabetic and anti microbial acitivity. Liquid chromatography equipped with mass spectrometry (LC-MS) is deployed for the identification of various compounds in various crude extracts. On activity level, its ethyl acteate extract showed the highest inhibition towards α-amylase and α-glucosidase with an IC 50 of 655 µg/mL and 639 µg/mL respectively, inspected through the substrate-based method. LC-MS/MS result revealed the presence of 8 compounds in ethyl acteate extract belonging to flavonoid and phenolic categories. Identification of these compunds from *Musa* x *paradisiaca* and its biological implication also support the community-based usage of this plant and its medicinal value. The results of the antimicrobial activity by Disc diffusion method of flower of *Musa*x *paradisiacal* powder ethyl acetate and chloroform extract. The ethyl acetate extract was inhibited the growth of selected Gram positive bacterial strain *Bacillus subtilis* 13 mm and comparing standard Ampicilin 14 mm. Gram negative bacterial strain *Escherichia coli* in the range of 14 mm and comparing standard Gentamicin 15 mm. The chloroform extract was inhibited the growth of selected Gram positive bacterial strain *Bacillus subtilis* 12 mm and comparing standard Ampicilin 14 mm. Gram negative bacterial strain *Escherichia coli* in the range of 11 mm and comparing standard Gentamicin 15 mm. The flower extract of *Musa* x *paradisiacal* powder ethyl acetate extract in all gram positive and gram negative microorganism showed maximum good inhibition. So it is proved that the ethyl acetate extract of *Musa* x *paradisiacal* has good anti bacterial activity. This modest work indicates that the

Musa x *paradisiaca* flower extract have an important antidiabetic and microbial effect that can be well established treatment of diabetes and its complications.

Keywords:
Musa x *paradisiaca* , antidiabetic , LCMS, anti microbial activity.

INTRODUCTION

Diabetes mellitus (DM) is a leading cause of hyperglyce-mia, and carbohydrate-hydrolase inhibitors, such as inhibitors for α-amylase and α-glucosidase, offer an effective strategy to lower the level of postprandial hyperglycemia via control of dietary carbohydrates and glycogen breakdown[1] . Moreover, microbial infections have undermined the existing antibiotic-based treatment era and raised the mortal-ity rate in patients with higher medical expenses and extended hospital stays[2] . Around the world, about28,000 plant taxa have been known for their medicinal values and about 3000 plant species possess ethno pharmacological uses for the management of DM and others [3].

Plant products have played the important role in the development of new therapeutics with milder adverse side effects than commercial drugs[4]. Consequently, it is important to recognize and measure all the secondary metabolites to ensure the biological research reliability and reproducibility over the pharmacological benefits and/or hazards. Currently, liquid chromatography with high resolution mass spectrometry (LC-MS) has emerged as a leading tool for detecting and identifying pharmacologically compounds[5]. Nuclear magnetic resonance spectroscopy and mass spectrometry followed by chemometric tools are the most used analytical methods of annotation [6].Additionally, LC-MS is useful for exact mass measurement as well as for molecular formula generation of any unknown molecules, parent ions, and fragment ions in the plant extracts [7,8]. This work's main focus is highlight and reveal the hidden health benefit of the *Musa* x *paradisiaca* flower part usually been discarded by determining it active molecules (via LC-MS/MS) and their antidiabetic, and antimicrobial proprieties.

MATERIALS AND METHODS

COLLECTION OF MATERIALS

The flower of *Musa* x *paradisiaca* is obtained from local area Madurai, Tamilnadu. Sample was picked and dried at room temperature.

PROCESSING OF EXTRACT PREPARATION

200g of *Musa* x *paradisiaca* flower was ground using grinder to make fine powder after drying and sieved. Powdered sample was extracted in 500 ml chloroform and ethyl acetate for 72 hours at room temperature under cold maceration method. The beaker is covered with aluminium foil. Extraction was continued for 7 days. After 7 days the aluminium foil was removed and the extract was filtered with Whatman No.1filter paper and the ethyl acetate and chloroform filtrate was concentrated using hot plate to get a Brownish yellow and brownish green liquid extract.

The percentage yield of extracts and the colour of the extract was noted in Table 1.

TABLE 1: Percentage yield and the colour of the extract of flower of *Musa x paradisiaca* powder

S.NO	EXTRACT	PERCENTAGE YIELD	COLOUR
1	Chloroform extract	15.5%	Brownish green
2	Ethyl acetate extract	20.3%	Brownish yellow

LIQUID CHROMATOGRAPHY–MASS SPECTROSCOPY[9]

Sample preparation was done, by adding 1 gm of sample with 10 ml of solvent (absolute methanol). Sample mixture was then incubated on a rocker shaker for 24 hours. Extract was then filtered through Whatman filter paper, and the extract was completely dried in oven at 40°C. Extract was then collected in micro centrifuge tube and stored for further ruse at 4°C.After sample preparation, instrument setup was done according to the manufacturer's instructions. Column installation for phytochemical identification was done which was C18, followed by mobile phase preparation which was solvent phase (A) – acetonitrile and solvent phase (B) – HCOONH4 (ammonium formate) buffer, and at last system equilibration was done. LC-MS parameters was then optimised such as – wavelength (280 nm), flow rate (0.2 ml/minute), stop time (40 minutes), injection volume (2 microliter), column temperature (35°C). After testing and calibration, sample was injected using auto sampler in LC-MS system. Spectra was recorded in negative and positive ionization mode between m/z 150 and 2000. Peak detection, integration, and quantification using appropriate algorithms was done and analytes along with their concentration was identified based on their retention time, mass-to charge ration and fragmentation patterns.

ANTIDIABETICACTIVITY

α-Amylase inhibition assay[10,11]

Different concentration of sample extract was added with 100 µl of 0.02 M sodium phosphate buffer (pH 6.9) and 100 µl of α-amylase solution (4.5 Units/ml/min) and pre incubated at 25°C for 10 min (Worthington, 1993). Then, 100 µl of 1% starch solution was added and incubated at 25°C for 30 min and the reaction was stopped by the addition of 1.0 ml of dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was diluted (10- fold) with distilled water and the absorbance was measured at 540 nm. The readings were related with the control, which contains buffer instead of extract and the percentage of α- amylase enzyme inhibition was calculated.

% Inhibition=[(Absorbance Control –Absorbance Sample)/ Absorbance Control] x 100

α-Glucosidase inhibition assay

Different concentration of the sample extract was taken with 100 µl of 0.1 M phosphate buffer (pH 6.9) and 100 µl of α-glucosidase solution (1 Unit/ml/min) and pre incubated at 25°C for 5 min (Worthington, 1993). Then, 100 µl of *p*-nitro phenyl-α-D- glucopyranoside (5 mM) was added and incubated at 25°C for 10 min. After the incubation period ,the absorbance readings were recorded at 405 nm and allegorized to a control that had 100 µl of buffer in place of the sample. The results were calculated and expressed on percentage basis.

% Inhibition=[(Absorbance Control –Absorbance Sample)/ Absorbance Control] x 100

ANTI BACTERIAL ACTIVITY[12-16]

The antimicrobial activity of the flower of chloroform and ethyl acetate extract of *Musa x paradisiacal* against *the microbial pathogens* was determined by Kirby and Bauer disc diffusion method. Mueller- Hinton agar plates inoculated with 1 ml of bacterial and fungal suspension of 1×10^6CFU/ml according to 0.5 Mc Farland standards was used for the antibacterial assay. Sterile filter paper discs of 6 mm dia were impregnated with 50 µl of crude extracts of lichen of varying concentrations such as 1 mg/ mL, 2 mg/mL and 5 mg/ml and after complete evaporation were placed on the surface of the inoculated agar plates. These plates were incubated at 37°c for 48 h. At the end of the incubation period, the antimicrobial activities were evaluated by measuring the zone of inhibition. In this assay, negative control was the respective pure solvent and the positive control was for *Bacilus subtilis* (Ampicilin), *Escherichia coli* (Gentamicin). All the tests were performed intriplicates.

RESULTS AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL INVESTIGATION

The obtained results showed the presence of tannins, saponins, flavonoids, alkaloids, glycosides, reducing sugars, polyphenols, HCN and terpenes in the flower of chloroform and ethyl acetate extract of *Musa* x *paradisiacal* (Table 2)

Table 2: Phytochemical analysis of flower of *Musa* x *paradisiacal* powder

S.NO	PHYTO CONSTIUTENTS	CHLOROFORM EXTRACT	ETHYL ACTEATE EXTRACT
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Triterpenoids	+	+
6	Proteins and aminoacids	-	-
7	Phenolic compounds	+	+
8	Flavanoids	+	+
9	Fixedoilandfats	-	-
10	Glycosides	+	+
11	Triterpenes	+	+
12	Phytosterols	+	+

LIQUID CHROMATOGRAPHY–MASS SPECTROSCOPY

The exact and observed mass of the compounds with high peaks are given in Table 3. The highest peak in positive mode was identified as 6-(Gamma, Gamma- Di methyl allyl amino) Purine Riboside at retention time 14.15. Some other significant positive ion molecules present in BPE were 1-Isothiocyanato-8-(Methyl sulfinyl)-Octane at retention time 1.27 and Cytidine-5'-Diphosphate Sodium Salt Hydrate at retention time

22.89. Similarly, in negative mode, the highest peak at retention time 24.74 was identified to be 2'-Deoxyinosine. Other compounds which gave high peaks were identified as Scopoletin at retention time 1.10, Quercetin-3-O-Alpha-L-Rhamnopyranosyl (1-2)-Beta-D- Glucopyranoside-7-O-Alpha-L-Rhamnopyranoside at 15.31, L-Saccharopine at 19.42 and Acacetin at retention time 30.93.

Table 3–Important Compounds identified by LC-MS in ethyl acetate extract of *Musa* x *paradisiaca*

Retention Time	CompoundName	Ion	Formula	Exact Mass	Observed Mass	Mass Diff
1.27	1-Isothiocyanato-8-(Methylsulfinyl)-Octane	Positive	C10H19NOS2	233.09	234.9277	1.8377
14.15	6-(Gamma,Gamma-Dimethylallylamino)Purine Riboside	Positive	C15H21N5O4	335.159	336.2207	1.0617
22.89	Cytidine-5'-Diphosphate SodiumSaltHydrate	[M+H]+	C9H15N3O11P2	403.18	403.4480	0.268
1.10	Scopoletin	Negative	C10H8O4	192.042	191.1840	0.858
15.31	Quercetin-3-O-Alpha-L-Rhamnopyranosyl(1-2)-Beta-D-Glucopyranoside-7-O-Alpha-L-Rhamnopyranoside	Negative	C33H40O20	756.211	755.5743	0.6367
19.42	L-Saccharopine	Negative	C11H20N2O6	276.132	277.3877	1.2557
24.74	2'-Deoxyinosine	Negative	C10H12N4O4	252.085	255.3946	3.3096
30.93	Acacetin	Negative	C16H12O5	284.068	283.5277	0.5403

ANTI DIABETIC ACTIVITY

α-amlase and α-glucosidase inhibition assay:

In the current study, flower of *Musa* x *paradisiacal* powder ethyl acetate and chloroform extracts were evaluated for their inhibitory effect on α-amylase and α glucosidase enzymes by the in-vitro method. Both extracts significantly demonstrated inhibition to α amylase and α-glucosidase enzymes in a dose-dependent manner. The ethyl acetate and chloroform extracts of *Musa* x *paradisiacal* (at a concentration g/ml) exhibited α amylase and α-glucosidase good inhibitory activity respectively. However, an ethyl aceate extract inhibited α amylase and α-glucosidase more than an chloroform extract. Moreover, the ethyl aceate extract of*Musa* x *paradisiacal* has shown higher enzyme inhibitory activity than the chloroform, with an IC50 value of 655 µL and 639 µL (α-amylase and α-glucosidase) (Table 4,5) .

Table 4. Anti diabetic activity of chloroform extract of *Musa* x *paradisiaca*

S.NO	NAMEOFTHE ASSAY	100 µ L	250 µL	500 µL	750 µL	1000 µL	IC50
1	α-Amylase inhibition	8.2%	17.6%	33.2%	48.1%	65.0%	768 µL
2	α-Glucosidase inhibition	8.4%	18.0%	35.1%	50.4%	66.9%	740 µL

Table 5. Anti diabetic activity of ethyl acetate extract of *Musa* x *paradisiaca*

S.NO	NAME OF THE ASSAY	100µL	250 µL	500 µL	750 µL	1000 µL	IC50
1	α-Amylase inhibition	9.0%	20.3%	38.6%	56.4%	75.8%	655 µL
2	α-Glucosidase inhibition	9.2%	21.0%	40.3%	58.1%	76.9%	639 µL

ANTI BACTERIAL ACTIVITY

The results of the antimicrobial activity by Disc diffusion method of flower of *Musa* x *paradisiacal* powder ethyl acetate and chloroform extract. The ethyl acetate extract was inhibited the growth of selected Gram positive bacterial strain *Bacillus subtilis* 13 mm and comparing standard Ampicilin 14 mm. Gram negative bacterial strain *Escherichia coli* in the range of 14 mm and comparing standard Gentamicin 15 mm. The chloroform extract was inhibited the growth of selected Gram positive bacterial strain *Bacillus subtilis* 12 mm and comparing standard Ampicilin 14 mm. Gram negative bacterial strain *Escherichia coli* in the range of 11 mm and comparing standard Gentamicin 15 mm (Table 6). The flower of *Musa* x *paradisiacal* powder ethyl acetate extract in all gram positive and gram negative microorganism showed maximum good inhibition. So it is proved that the ethyl acetate extract of *Musa* x *paradisiacal* has good anti bacterial activity.

Table 6: Anti microbial activity of flower of *Musa* x *paradisiacal* powder ethyl acetate and chloroform extract

S.NO	ORGANISMS	STANDARD	ETHYLACETATE EXTRACT	CHLOROFORM EXTRACT
1	**Anti bacterial Gram (+)** *Bacillus subtilis*	14	13	12
2	**Gram(-)** *Escherichia coli*	15	14	11

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