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Anti-bacterial activity and GC-MS analysis of ripened and un ripened Banana (*Musa x paradisiaca L*) cv. Bontha fruit pulp Extracts.

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ABSTRACT

Anti bacterial activity of ripened and un ripened ethanolic and hexane fruit pulp extracts of *Musa x paradisiaca L* cultivar bontha against pathogenic bacteria by Kirby-Bauer method as zone of inhibition and minimal inhibitory concentrations (MIC)were investigated. Further Gas chromatography- Mass spectrometry (GC-MS) was used for phytochemical analysis of unripe bontha ethanolic pulp extract .Both ethanolic and hexane pulp extracts of ripened and un ripened bontha showed anti bacterial activity against all tested organisms. Ethanolic pulp extracts of ripe and un ripe bontha showed more anti bacterial activity compared to ripe and un ripe bontha hexane pulp extracts. GC-MS analysis of un ripe bontha ethanolic pulp extract showed the presence of several bioactive compounds like aldehydes, ketones, alkaloids, furans and sugars that may be responsible for medicinal actions.

Keywords : Bontha banana *Musa x paradisiaca L* Anti bacterial activity GC-MS analysis Ripe and un ripe Food medicines

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INTRODUCTION

Wide spread use of antibiotics throughout world resulted in development of resistance by microbes .Further various side effects associated with the use of these drugs led to search for new antimicrobials from plant sources which are relatively safe with least side effects and cheaper[1]. Fruits are major part of food in developing countries and widely used in traditional medicinal systems to treat various diseases [2,3]. Several *in vitro* and clinical studies showed fruits are effective in controlling diabetes, cancer ,hypertension etc. [4-6]. Banana (*Musa x paradisiaca* L) cultivars are grown and banana fruits are consumed in many ways throughout the world .In traditional systems of medicine various parts of banana fruit are used in treatment of diseases like depression, ulcers, constipation etc.. Further *in vitro* studies of banana showed anti bacterial, anti viral and anti fungal activities [7-9]. Various phytochemicals present in plants are responsible for their anti bacterial activities [10]. Development of resistance by microbes to these phytochemicals may not be easier unlike existing medicines [11].

Different parts of different cultivars of banana have been subjected to GC-MS analysis for macromolecular composition by various workers. Elizabeth Adejoke Osibote *et al* carried out a structural characterization of aqueous and solvent extracts from *Musa paradisiaca* on a combined gas chromatograph-mass spectrometer [12]. Fatty acid compositions of the fixed oils obtained from the dried and fresh fruit peels of *Musa sapientum* var. Cavendishii (Musaceae) were examined by capillary GC-MS by Orhan İlkay *et al* [13]. The aril extracts of three Thai banana varieties, namely “Kluai Khai”(KK), “Kluai Namwa”(KN) and “Kluai Hom”(KH) were analysed by gas chromatography and mass spectrometry (GC-MS) by Rungnapa Meechaona *et al* [14]. Therefore as part of our search for new anti microbials from edible fruits which can serve as food medicines we investigated ripened and un ripened banana (*Musa x paradisiaca* L) cv. bontha fruit pulp extracts for anti microbial activity and bioactive phytochemicals by GC-MS method..

MATERIALS AND METHODS

Collection of ripe and unripe bontha bananas

A bunch (or more specifically, a hand) of freshly harvested, fully mature unripe bananas of bontha were obtained from the surrounding fields of Vaddeswaram, Guntur and local market, respectively. Few of the unripe bananas obtained were assayed. The remaining were left to ripen. When they were suitably ripe, they were assayed.

Preparation of banana cv. Bontha pulp extracts

Unripened and ripened pulp extracts of *Musa x paradisiaca* L. cv. Bontha prepared using polar and non-polar solvents ethanol and hexane. The extracts were obtained using a mortar and pestle, filtered, concentrated to dryness under vacuum and then collected. The left over powder was considered 100%. Different concentrations of the extracts such as 100, 250, 500, 750 and 1000 µg/ml were prepared by re dissolving the extract powder in the same solvent and tested.

Micro organisms

Escherichia coli (NCIM 2931), *Pseudomonas aeruginosa* (NCIM 5029), *Bacillus cereus* (NCIM 2106) and *Micrococcus flavus* (NCIM 2376) were obtained from National Chemical Laboratory, Pune, India.

Anti bacterial activity

Antibacterial activity was studied by Bauer AW *et al*. (Diffusion method) method [15-18].The minimum inhibitory concentration(MIC) was determined by Vander-Berghe Da and Vlietinck (Agar Dilution) method[19-21]. Triplicates of the assay were run for standardizing the result. Simultaneously the activity of standard antibiotic, Chloramphenicol (30µg/ml) and solvents (served as controls) was also tested against the microorganisms under study in similar conditions.

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Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Phytochemical analysis of the ethanol extract of unripe bontha was carried out by Gas Chromatography-Mass Spectrometry (GC-MS) unit of Agilent Technologies 6890, mass detector model 5973 run in spilt mode was used for assay with MS source at 230^{0}C, MS Quadrupole at 150^{0}C and Helium as carrier gas at 80 bar pressure.

Identification of components

The results of the GC-MS analysis were interpreted using the database of National Institute Standard and Technology (NIST, USA) having more than 62,000 patterns. The mass spectrum of each of the unknown compound was compared with the spectrum of the known compounds stored in the NIST library. The name, molecular weight, structure, nature of compound and activity of the compounds were determined.

RESULTS

Anti microbial activity as zone of inhibition of Musa x paradisiaca L. cv. Bontha

The anti microbial activity as zone of inhibition acquired by testing the solvent extracts – ethanol and hexane, of ripened and unripened pulp of *Musa x paradisiaca* L, cv bontha, at given concentrations against the test organisms are shown in Fig.1 and 2 respectively

Fig.1 shows the antimicrobial activity exhibited by ethanolic extract of ripened pulp of Bontha cultivar. Very good zones of inhibition against all the test organisms were obtained by the ethanolic extracts. The inhibitory activity was enhanced along with the increase of concentration. Zones of inhibition were observed at 100µg/ml against *B.cereus, M.flavus* and *P.aeruginosa*. Activity was observed on *E.coli* at 250µg/ml. Antimicrobial activity of ethanolic unripened pulp extracts of *Musa x paradisiaca,* cultivar Bontha can be also seen in Fig.1. Activity was seen at 250µg/ml against *M.flavus* and *P.aeruginosa*, at 500µg/ml against *E.coli* and at 1000µg/ml against all test organisms including *B.cereus*. The activity observed at 1000µg/ml against *E.coli* was almost equal to that of the control antibiotic and that on *P.aeruginosa* more than the standard

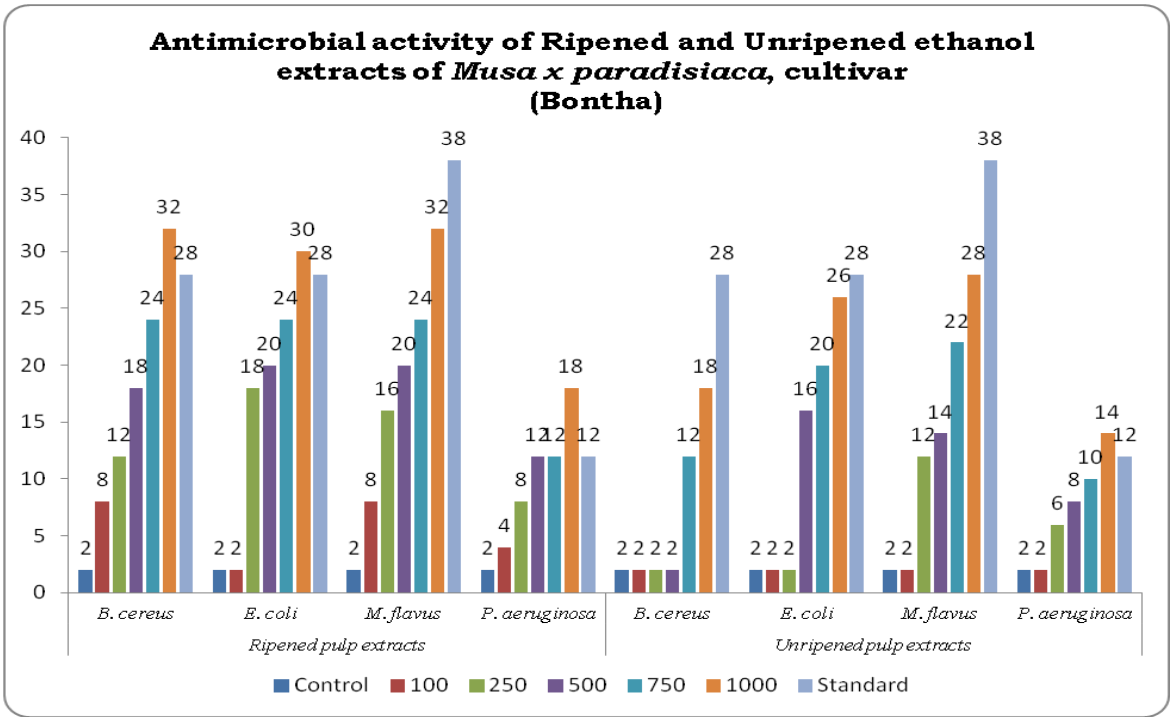

Figure 1: Antimicrobial spectrum of Ripened and Unripened ethanol pulp extracts of *Musa x paradisiaca*, cultivar Bontha

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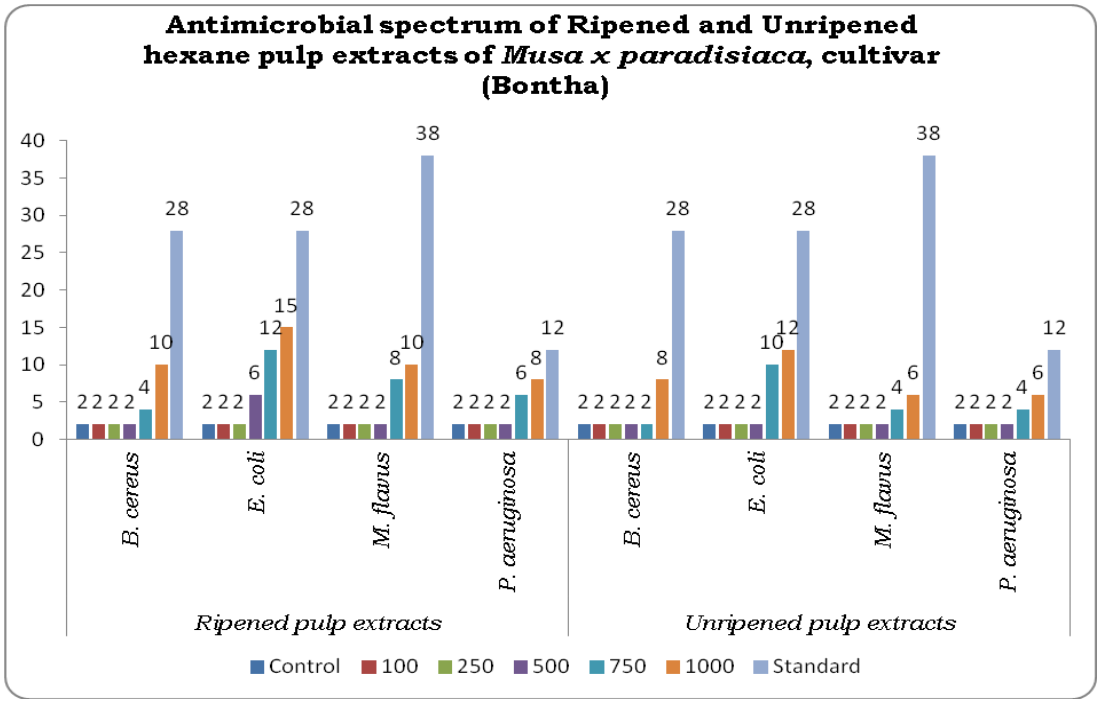

Figure 2: Antimicrobial spectrum of Ripened and Unripened hexane pulp extracts of Musa x paradisiaca, cultivar Bontha

The range of anti microbial activity of ripened and unripened hexane extracts of Bontha can be seen from Fig 2.. The hexane extract of ripened Bontha cultivar inhibited the growth of all test organisms including *B.cereus* at 750µg/ml . The highest activity was on *E.coli* at 1000µg/ml. The antimicrobial activity of hexane pulp extract of unripe bontha can be also seen in Fig.2.Zone of inhibition was seen at 750µg/ml on *E. coli, M. flavus* and *P. aeruginosa* whereas on *B.cereus*, activity was seen only at highest concentration of 1000µg/ml.

Minimum inhibitory concentrations (MIC)

In Table.1 MIC of ripened and unripened pulp extracts of bontha are presented. Ripened ethanolic extracts were inhibitory to all the test organisms at 100µg/ml except *E. coli.*. Ethanolic unripened extracts were active against *M. flavus* and *P. aeruginosa* at 250µg/ml concentration, 500µg/ml for *E. coli,* where as 750µg/ml against *B. cereus.*. The ripened hexane extracts of Bontha showed a MIC of 500µg/ml for *E.coli* and 750µg/ml for the remaining test bacteria. For unripened hexane extracts, the MIC was fixed at 750µg/ml for *E. coli, M. flavus* and *P. aeruginosa*, whereas for *B.cereus* it was found to be 1000µg/ml .

Table 1: Minimum Inhibitory Concentration (MIC) values of unripened and ripened pulp extracts of *Musa x paradisiaca* L. cv Bontha

Cultivar	Extract		MIC value
	Ripe/ Unripe	Solvent	
Bontha	Ripe Unripe	Ethanol Ethanol	100µg/ml for all except E.*coli*, 250µg/ml for E.*coli*. 250µg/ml for*M. flavus* and *P. aeruginosa,* 500µg/ml for *E. coli,* 750µg/ml for *B. cereus.*
	Ripe Unripe	Hexane Hexane	500µg/ml for *E. coli* , 750µg/ml for others 750µg/ml for all except *B. cereus*, 1000µg/ml for *B. cereus*

GC-MS Analysis of Unripe bontha ethanol extract

*P*hytochemical composition of unripe bontha banana extract was analysed by GC-MS. The chromatogram obtained from this analysis is shown in Fig 3. The chromatogram has a total of ten peaks at

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various retention times. A list of phytochemicals identified at the different retention times, their molecular mass, nature and activity are given in Table 2. Several bioactive compounds like aldehydes, ketones, alkaloids, furans and sugars are identified in ethanolic extract of unripe bontha cultivar.

Table 2: GC-MS analysis of Unripe Ethanolic pulp extracts of Musa x paradisiaca L cultivar Bontha

S No	RT (mins)	Phyto-component	Mol formula	MW	Compound nature	Activity
1	4.590	2(5H)-Furanone, 5-ethyl	$C_6H_8O_2$	112.12	Furans	Flavor
2	4.991	2,5 Piperazinedione	$C_4H_6N_2O_2$	114.10	Ketone	Antitumor, Cyclic peptide
3	5.100	Benzeneacetaldehyde	C_8H_8O	120.14	Aldehyde	Aromatic compound, Antibiotic
4	5.386	Cyclohexanamine N-3-butenyl-N-methyl-	$C_{11}H_{21}N$	167.29	Alkaloid	--
5	5.563	Pyridine,2,3,4,5-tetrahydro-	C_5H_9N	83.13	Alkaloid	Flavor (pungent)
6	6.129	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	Ketone	Aroma compound
7	6.919	2-furancarboxaldehyde,5-(hydroxymethyl)-	$C_6H_6O_3$	126.11	aldehyde	Antimicrobial, preservative
8	10.684	Alpha-1-rhamnopyranose	$C_6H_{12}O_5$	164.15	Sugar	Nutrient
9	15.508	Methylenebis(2,4,6-triisopropylphenyl phosphine)	$C_{31}H_{50}P_2$	484.67	--	--
10	8.933	Sucrose	$C_{12}H_{22}O_{11}$	342.29	sugar	Nutrient

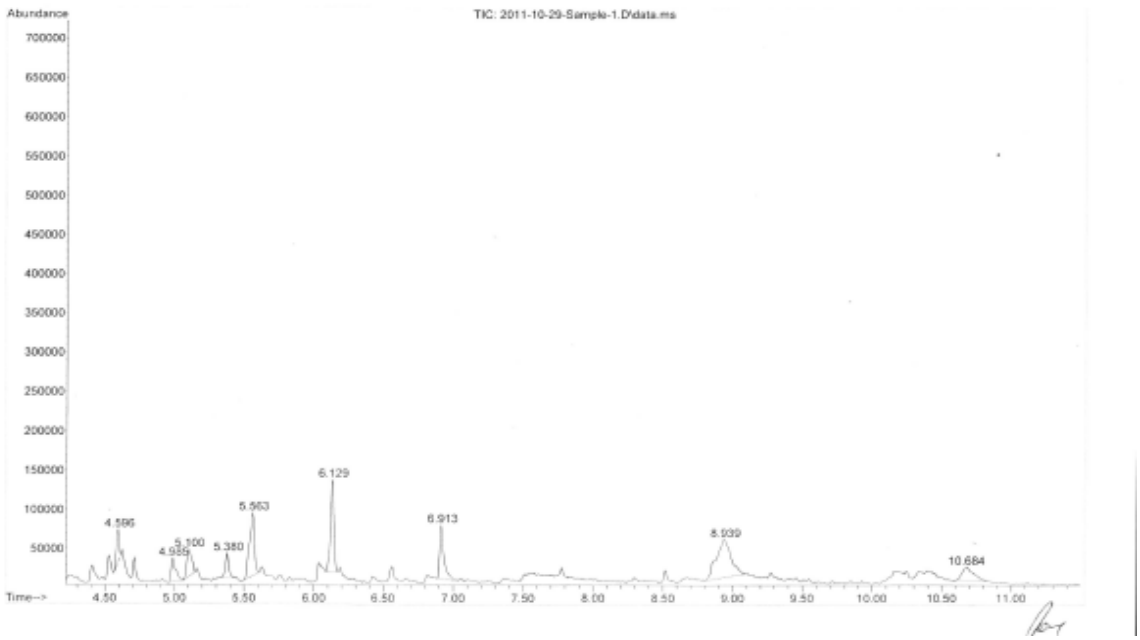

Figure 3: GC-MS chromatogram of ethanol extract of Unripe Bontha

DISCUSSION

The results of the present study suggest that the ethanolic and hexane extracts of the cultivar bontha posses antibacterial activity and among the extracts ripe ethanolic extract show better activity compared to other extracts against bacteria tested. Hence ripe and unripe bontha bananas can act as potential source of antimicrobial agents of food origin. Various medicinal activities of bananas may be due to the presence of

phytochemicals identified by GC-MS analysis. Aldehydes detected in ethanolic bontha fruit extracts may be responsible for the antimicrobial activity because aldehydes inhibit microbial growth by interacting with nucleic acids and proteins(22). Further studies are needed to characterize more potent phytomolecules which can act against many pathogenic bacteria.

CONCLUSION

This study indicates that bontha bananas of ripe and unripe can serve as food medicines due to their anti microbial action and as a source of new anti microbials from edible fruits.. Further studies involving isolation and characterization of phyto molecules with wide antimicrobial activity and other medicinal properties which may act as novel drugs are needed.

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