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Der Pharmacia Lettre, 2016, 8 (1):331-337
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Antibacterial activity and GC-MS analysis of ripened and unripened banana (*Musa x paradiscia L*) cv. chakkarakeli fruit pulp extracts

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ABSTRACT

Anti bacterial activity and minimal inhibitory concentrations (MIC)of ripened and un ripened aqueous, ethanolic, methanolic and hexane fruit pulp extracts of Musa x paradisiaca L cultivar chakkarakeli against pathogenic bacteria Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus and Micrococcus flavus were investigated. Different ripened and un ripened chakkarakeli banana extracts showed broad inhibitory activities towards pathogenic micro-organisms. Ethanolic extracts showed good activity compared to other extracts with respect to similar bacteria. GC-MS analysis of ripened methanolic pulp extract showed the presence of several bioactive compounds like aldehydes, ketones, alcohols, furans , sulphur compounds , pyrimidines, nitrosamines, esters, eugenol and sugars that may be responsible for medicinal actions of bananas.

Key words: Anti bacterial activity, *Musa x paradisiaca L*, Chakkarakeli, GC-MS analysis, Ripened and un ripened banana, Pulp extracts

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INTRODUCTION

Treatment of diseases with locally available plant derived substances was part of cultures of various populations spread across globe [1]. But with introduction of fast acting modern medicines this traditional medical practice slowed down for some time due to lack of scientific basis. However due to limitations associated with the use of these drugs like emergence of resistance to most of the antibiotics, undesirable side effects , long incubation time , high cost of production search for new drugs from plant sources increased recently [2,3] . Non edible plants are extensively investigated for medicinal activities where as similar studies on edible plants are few [4]. Fruits are part of our food and contain many phytochemicals apart from known nutrients [5]. Further chemical constituents in ripe and unripe states in fruits are different. Banana (*Musa x paradiscia L*) is major fruit crop grown and consumed worldwide. Many cultivars of banana exist and several therapeutic values for these cultivars are well documented in indigenous systems of medicine [6]. Anti microbial activity in tropical fruits like guava, apple and in some banana cultivars was reported [7-9]. The phytochemicals of these fruits that occurs in ripe and unripe conditions contributes to their anti bacterial and other medicinal activities. Chakkarakeli a delicious banana cultivar grown widely in coastal areas of india is not studied for antimicrobial activity. Phytochemical compositon of *Musa x paradiscia* L extracts and three Thai banana extracts were characterized by using Gas chromatography- Mass spectrometry (GC-MS) analysis [10,11] . Hence the present investigation was undertaken to assess antibacterial activity of ripened and un ripened banana (*Musa x paradiscia L*) cv. chakkarakeli fruit pulp extracts and to identify phytomolecules by GC-MS analysis.

MATERIALS AND METHODS

Collection of ripened and un ripened chakkarakeli bananas
Freshly harvested, fully mature ripened and un ripened bananas of chakkarakeli were obtained from local market.

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.

Preparation of ripened and un ripened banana cv. Chakkarakeli pulp extracts
Ripened and un ripened pulp extracts of *Musa x paradisiaca* L. cv. Chakkarakeli were prepared using polar and non-polar solvents distilled water, ethanol, methanol 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.

Antimicrobial activity of ripened and un ripened chakkarakeli fruit pulp extracts
Antibacterial activity of pulp extracts was determined by Diffusion method [12-14]. Agar medium was prepared and poured into a conical flask and sterilized by autoclaving at 121^0C and 15 lb pressure for 20mins. The medium was then poured into Petri plates (10cm diameter) and let to stand for around 20 min for solidification. Whatmann No.1 Filter paper discs of 6mm diameter were made. The discs were taken and impregnated with different concentrations of pulp extracts. They were then dried and placed over agar plates (equidistance from each other and the circumference of the plate) with the test organisms and incubated at desired conditions of 37^0C in an incubator. Clear zones of growth inhibition around the discs stand for antimicrobial nature. Triplicates of the extracts were run for standardizing the result. The resulting Zone of Inhibition was measured in mm using a scale. The zones of growth inhibitions around the discs were measured and the area of inhibition was calculated. Simultaneously the activity of standard antibiotic, Chloramphenicol was also tested against the microorganisms under study in similar conditions, so as to compare the degree of inhibition exhibited by the pulp extracts of local cultivars. Discs fed with corresponding solvents served as controls. The Zone of Inhibition is the mean of the values obtained.

Minimum Inhibitory Concentration (MIC) of pulp extracts
The minimum inhibitory concentration (MIC) of ripened and un ripened chakkarakeli fruit pulp extracts was determined by Agar Dilution method [15-17] . The different solvent extracts were tested for minimum inhibitory activity at different concentrations such as 100µg, 250µg, 500µg, 750µg and 1000µg. The Petri dishes were marked accordingly. One sterile nutrient agar plate without extract but with equal volume of the solvent served as the control plate. These plates were refrigerated for uniform diffusion of the extract throughout the media. The plates were dried at 37°C by keeping them in the incubator. One loopful (diameter-3mm) of an overnight grown nutrient broth culture of each test organism was placed in Petri dish. The inoculated plate was incubated at 37ºC for 24 hours and the MIC value obtained. MIC was determined from growth of organisms at each concentration. The experiment was repeated in triplicate and average values were recorded.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis
Phytochemical analysis of the methanol extract of ripe chakkarakeli pulp extract 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 230^0C, MS Quadrupole at 150^0C 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

The anti microbial activity of aqueous extracts of ripened and un ripened pulp of banana (*Musa x paradiscia L*) cv. chakkarakeli at different concentrations against the test organisms as zone of inhibition is shown in Fig.1. *B. cereus* and *M. flavus* were found to be susceptible to the ripened aqueous pulp extract at 750µg/ml concentration and *E. coli* at 1000µg/ml. *P. aeruginosa* showed no susceptibility to the extract at any concentration. Un ripened Chakkarakeli cultivar's aqueous pulp extract antimicrobial activity on the test organisms *E. coli* and *M. flavus* was observed at 1000µg/ml concentration and on *B. cereus* at 750µg/ml. No activity was observed on *P. aeruginosa* even at 1000µg/ml **.**

Figure.1. Antimicrobial spectrum of Ripened and Unripened aqueous pulp extracts of *Musa x paradisiaca*, cultivar Chakkarakeli

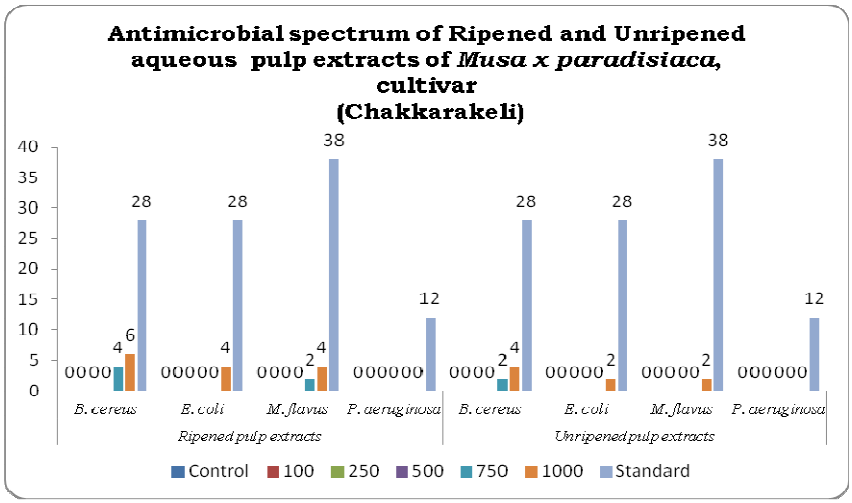
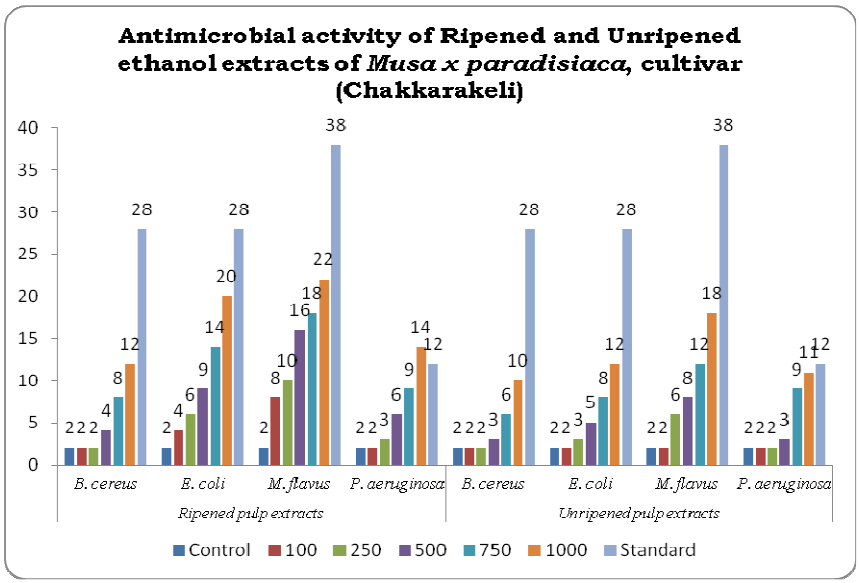


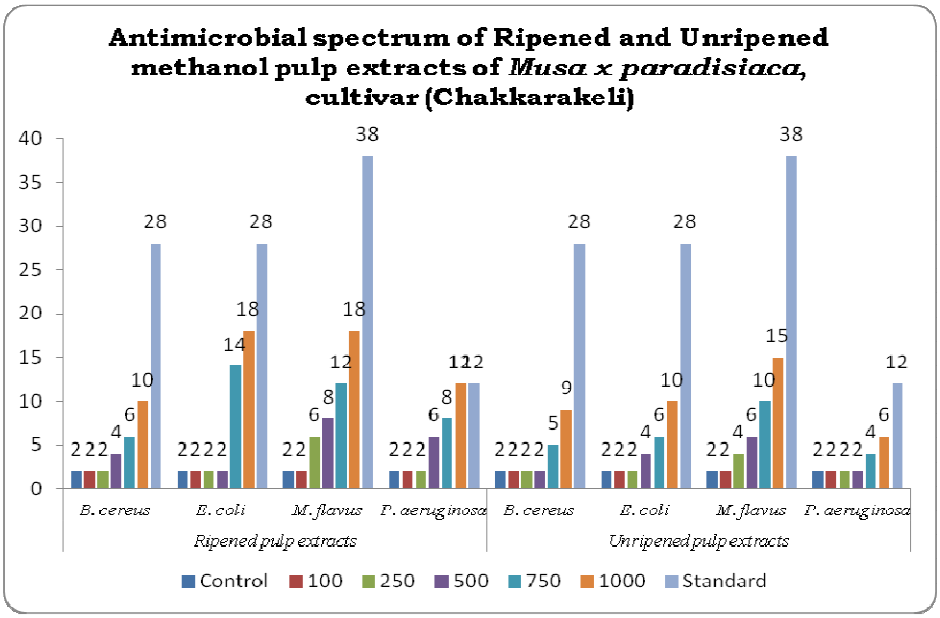
Fig.2.shows the activity exhibited by ripened ethanolic pulp extract of Chakkarakeli cultivar. Antimicrobial activity was seen at 100µg/ml against *E. coli* and *M. flavus*, at 250µg/ml on *P. aeruginosa* and on all the test organisms from 500µg/ml. The antimicrobial spectrum of ethanolic un ripened pulp extracts of *Musa x paradisiaca*, cultivar Chakkarakeli is shown in Fig.2. Very little zone of inhibition was seen at 250µg/ml on *E. coli* and *M. flavus* and at 500µg/ml on *B. cereus* and *P.aeruginosa*

Figure.2.Antimicrobial spectrum of Ripened and Unripened ethanol pulp extracts of *Musa x paradisiaca*, cultivar Chakkarakeli



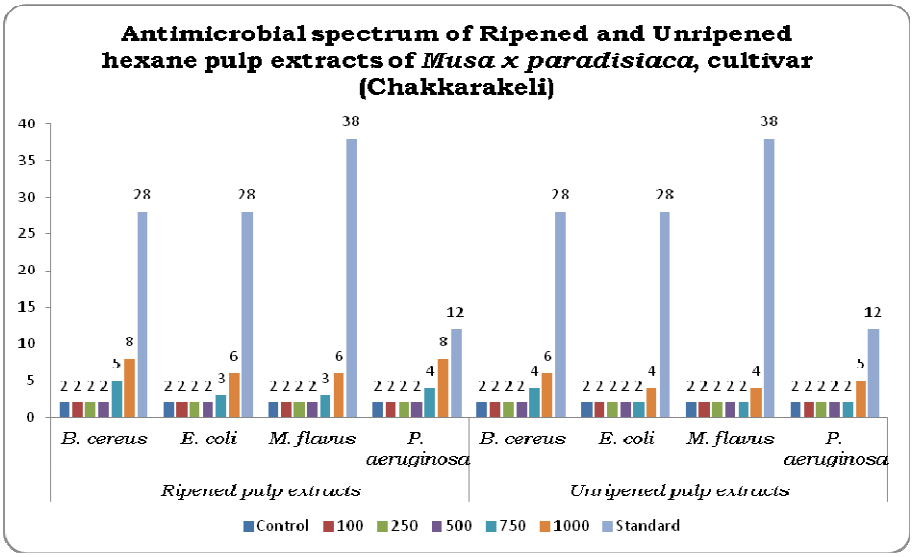
The antimicrobial spectrum of methanolic ripened pulp extracts of *Musa x paradisiaca*, cultivar Chakkarakeli is shown in Fig.3.. Zone of inhibition was seen at 250µg/ml on *M. flavus*, at 500µg/ml on *B. cereus* and *P. aeruginosa* and at 750µg/ml on *E. coli*. The antimicrobial spectrum of methanolic Unripened pulp extracts of same cultivar is shown in the above Fig. Zone of inhibition was seen at 250µg/ml on *M. flavus*, at 500µg/ml on *E. coli* and at 750µg/ml on *B. cereus* and *P. aeruginosa*

Figure.3.Antimicrobial spectrum of Ripened and Unripened methanol pulp extracts of *Musa x paradisiaca*, cultivar Chakkarakeli



In Fig. 4, the zones of inhibition produced by hexane extract of ripened chakkarakeli are shown. The extract was potent to all test organisms at 750µg/ml. The antimicrobial activity of hexane extract of un ripened chakkarakeli extract is also shown in Fig.4 . The activity exhibited was very low was seen only on *B. cereus* at 750µg/ml. At the highest concentration of 1000µg/ml activity was seen on all test organisms. Even though the observed activity of ripened pulp extract was less, it was better than that of the un ripened extract .

Figure.4.Antimicrobial spectrum of Ripened and Unripened hexane pulp extracts of *Musa x paradisiaca*, cultivar Chakkarakeli



Minimum Inhibitory Concentration of chakkarakeli pulp extracts.
In Table.1.minimum inhibitory concentrations of ripened and un ripened chakkarakeli banana are presented.

GC-MS Analysis of ripe chakkarakeli methanol extract
Phytochemical composition of ripe chakkarakeli banana extract was analysed by GC-MS. The chromatogram obtained from this analysis is shown in Fig 5. The chromatogram has a total of twelve peaks at various retention times (RT) . A list of phytochemicals identified at the different retention times, their molecular mass, nature and activity are given in Table 2.

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

Cultivar	Extract		MIC value
	Ripe/ Unripe	Solvent	
Chakkarakeli	Ripe	Aqueous Ethanol Methanol Hexane	750 µg/ml (*B. cereus, M. flavus*),1000µg/ml(*E. coli*), No activity on *P. aeruginosa* 100 µg/ml (*E. coli and M. flavus)*, 250µg/ml, (*P. aeruginosa*), 500µg/ml(*B. cereus)* 250µg/ml (*M. flavus)* , 500µg/ml (*B. cereus and P. aeruginosa*),750 µg/ml (*E. coli*) 750µg/ml for all test organisms
	Un ripe	Aqueous Ethanol Methanol Hexane	750 µg/ml (*B. cereus , M. flavus*) , 1000µg/ml(*E. coli),* No activity on *P. aeruginosa* 250 µg/ml (*E. coli* and *M. flavus)*, 500µg/ml (*B. cereus, P. aeruginosa)* 250µg/ml (*M. flavus),* 500µg/ml (*E. coli*) , 750 µg/ml (*B. cereus* and *P. aeruginosa*). 750µg/ml for all test organisms

Figure .5. GC-MS chromatogram of methanol extract of Ripe Chakkarakeli

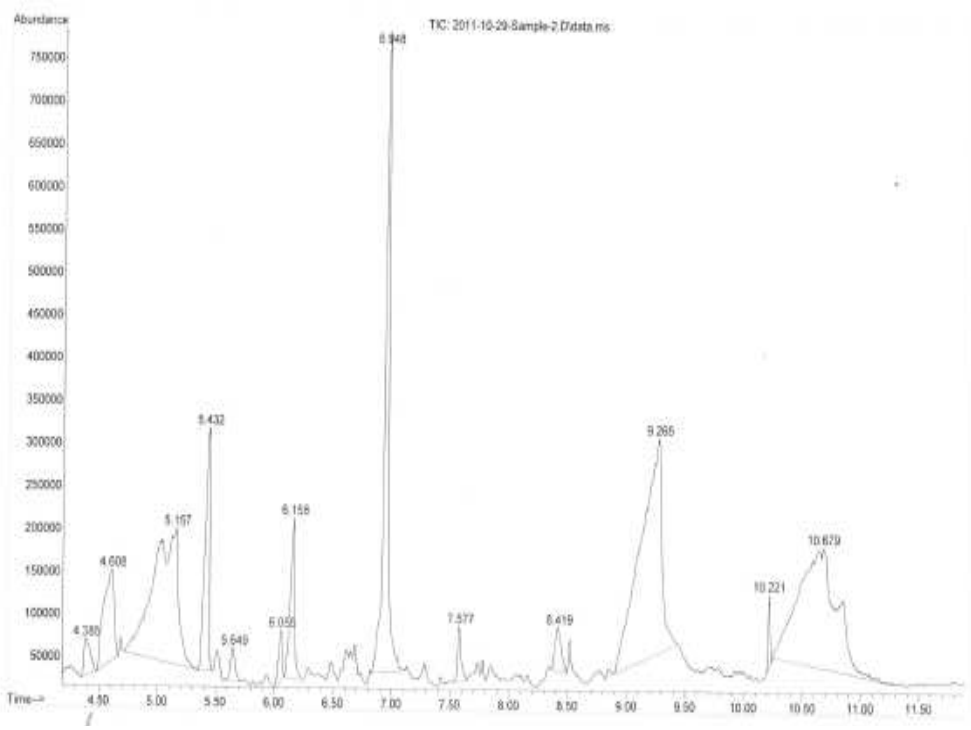


Table.2. GC-MS analysis of Ripe Methanol pulp extracts of *Musa x paradisiaca* L cultivar Chakkarakeli

S No	RT (mins)	Phyto-component	Mol formula	MW	Compound nature	Activity
1	4.396	Cis-3-methyl-2-n-propyl thiophane	$C_8H_{16}S$		Sulfur-containing	Odour
2	4.608	Butanedial	$C_4H_6O_2$	86.08	Aldehyde	Precursor for synthesis of atropine alkaloid
3	5.146	Glycerin	$C_3H_8O_3$	92.09	Alcohol	Lubricant, humectants
4	5.432	Thymine	$C_5H_6N_2O_2$	126.11	pyrimidine	--
5	5.649	Sec-butyl nitrite	$C_4H_9NO_2$	103.12	Nitrous acid ester	Intermediate for perfumes and anti-freeze preparations
6	6.055	Ethanamine, N-ethyl-N-nitroso	$C_4H_{10}N_2O$	102.135	Nitrosamines	gasoline & lubricant additive; antioxidant; stabilizer.
7	6.158	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.125	Ketone	Aroma compound
8	6.948	2-furancarboxaldehyde,5-(hydroxymethyl)-	$C_6H_6O_3$	126.11	aldehyde	Antimicrobial, preservative
9	8.419	Acetic acid, 2-propyltetrahydropyran-3-yl ester	$C_{10}H_{18}O_3$	186.24	Ester	--
10	9.260	Sucrose	$C_{12}H_{22}O_{11}$	342.29	Sugar	Nutrient
11	10.22	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_3$	194.22	Eugenol (Phenol)	Flavoring agent, Antioxidant
12	10.650	3-deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162.14	--	--

Several bioactive compounds like aldehydes, ketones, alcohols, furans , sulphur compounds , pyrimidines, nitrosamines, esters, eugenol and sugars are identified in methanolic extract of ripe chakkarakeli cultivar.

DISCUSSION

In this study four types of chakkarakeli banana pulp extracts were screened for anti bacterial action against four types of pathogenic bacteria. The results indicate that different ripe and un ripe chakkarakeli banana extracts exhibit broad inhibitory activities towards pathogenic micro-organisms. Further among extracts ethanolic extracts showed good activity compared to other extracts with respect to similar bacteria as seen from the values of zones of inhibition. Both ripened and un ripened *Musa x paradisiaca* L. cv chakkarakeli ethanol fractions were potent extracts. The medicinal values of bananas may be due to the phytochemicals present in the methanolic extract of ripe chakkarakeli as identified by GC-MS analysis. Among various phytochemicals aldehydes in methanolic chakkarakeli fruit extracts may be responsible for the antimicrobial activity because aldehydes inhibit microbial growth by interacting with nucleic acids and proteins [18]. This indicate that chakkarakeli bananas are promising potential antibacterial agents from natural plant sources [19]. Further studies are needed to characterize useful bioactive molecules which can serve as precursors for the generation of new antimicrobials as well as new drugs.

CONCLUSION

Ripened and un ripened banana (*Musa x paradisiaca L*) cv chakkarakeli fruit pulp aqueous , ehanol, methanol and hexane extracts showed potential anti bacterial activity against pathogenic bacteria**.** Ethanolic extracts of ripened and un ripened pulp were more potent against bacteria among all other extracts. Various phytochemicals identified in ripened methanolic pulp extract by GC-MS analysis may be responsible for medicinal properties of bananas including anti microbial activity. Further isolated phytochemicals may serve as lead molecules for development of new drugs.

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