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Research Article

EFFICACY OF RIPENED AND UNRIPENED FRUIT EXTRACTS OF *MUSA* X *PARADISIACA* L. (BONTHA CULTIVAR) AGAINST HUMAN PATHOGENS

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ABSTRACT

Musa x paradiasaca L. var. *Bontha*, the plantain banana, one of the locally cultivated and the leading culinary banana in India[1] has been shown traditionally to have certain pharmaceutical uses but, its beneficial effects have never been attributed to the presence of antibiotic substances. The antibacterial activities of solvent extracts (aqueous, methanolic and ethanolic) of Musa spp. were evaluated by Kirby-Bauer method against bacteria viz, *Micrococcus flavus*, *Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli.* Phyto-chemical analysis revealed the presence of Phytochemicals that maybe responsible for the activities displayed by the extracts. This signifies the potential of banana plant as a source of therapeutic agents and may provide focus in the ongoing search for antimicrobial agents. The present investigation was undertaken to study antibacterial activity of aqueous, methanolic and ethanolic extracts of pulp of *Musa x paradisiaca* L. var *Bontha.* Phyto-analysis of extracts revealed the presence of chemicals such as alkaloids, flavonoids, glycosides, saponins, steroids, tannins and xanthoproteins.

Keywords: *Musa x paradisiaca* L. var *Bontha*, Antibacterial, Phytochemical, Gram-positive, Gram-negative, Chloramphenicol.

INTRODUCTION

The healing powers of plants have been known to man for generations. A discussion of human life on this planet would not be complete without a look at the role of plants[2]. Earlier used antimicrobials were derived from higher plants. But, discovery and subsequent extraction of effective antimicrobial compounds from other cheaper sources resulted in a shift of antimicrobial research from plants to the laboratories and then, synthetic compounds. Recently, though, interest in native plants' research has increased dramatically for a wide-range of reasons including an inability of many rural people and some governments to afford pharmaceutical care, revitalization of indigenous knowledge and "traditional" health systems, a greater appreciation for local and indigenous knowledge, international concerns for the conservation of biodiversity, and income-generating potential[3].

Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to these types of drugs. Plant drugs are frequently considered to be less toxic and free from side effects than the synthetic ones[4]. In the traditional medicinal systems of India, all the parts of *Musa* spp. (family Musaceae) are used for the treatment of various diseases[5]. Extensive investigations have proved the anti-ulcerogenic, ulcer healing activities[6] and wound healing activity[7] of plantain banana. But, its beneficial effects have never been attributed to the presence of antibiotic substances. Based on the above information, an attempt was made to study the antimicrobial spectrum of one of the extensively cultivated and consumed local cultivars of *Musa x paradisiaca* (Bontha) under *in vitro* conditions, in its ripe and unripe forms.

MATERIALS AND METHODS

Plant: *Musa* cultivar – Bontha

Medicinal uses of banana (Musa spp. in general)[5]: Flowers' extracts used to treat bronchitis, dysentery and on ulcers, Cooked flowers syrup used against Diabetes, Astringent plant sap used as a medication to cure hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and on hemorrhoids, insect and other stings and bites; whereas young leaves used as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and leaves works against dysentery, diarrhea and malignant ulcers; Roots are an age-old application in digestive disorders, dysentery and other ailments and Seed mucilage cures ophthalmic cataracts and diarrhea.

Previously isolated constituents from *Musa* spp. in general (Table 1)

Table 1: Major chemical constituents in edible portion of *Musa x paradisiaca* L.

Compound	Fruit
Alkaloids	Salsolinol
Terpenoids	Cycloeucalenol, Cycloeucalenone
Sterol	Cycloartenol,Obtusifoliol, Sitoindoside, Palmitate, Beta-Sitosterol, Campesterol, Isofucisterol, Stigmasterol
Flavonoids	Kaempferol, Quercetin, Rutin
Trace elements	Cadmium, Chromium, Cobalt, Copper, Iron, Manganese, Molybdenum, Nickel, Phosphorus, Rubidium, Selenium, Zinc

Alkaloids, Terpenoids, Sterol, Flavonoids and Trace elements[8].

Tested material

Aqueous, ethanolic and methanolic pulp extracts' residues of *Musa paradisiaca* L. var *Bontha*, when in un-ripened and ripened states. The extracts were dried in a flash evaporator for 30min and the left over powder was considered 100%. Different concentrations of extracts such as 100, 250, 500, 750 and 1000µg/ml [9] were prepared by redissolving the extract powder in the same solvent.

Studied activities

The minimum inhibitory concentration was determined by Vander-Berghe Da and Vlietinck (agar dilution) method [10, 11, 12, 13]. Antibacterial activity was studied by Bauer AW *et al* (Diffusion method) method[14, 15, 16, 17]. Preliminary Phytochemical analysis was carried out in all the evaporated solvent extracts by using standard color test procedure for detection of phyto-constituents[18, 19].

Used bacterial strains

Escherichia coli (NCIM 2931), *Pseudomonas aeruginosa* (NCIM 5029), *Bacillus cereus* (NCIM 2106) and *Micrococcus flavus* (NCIM 2376) obtained from National Chemical Laboratory, Pune, India. The characteristics of the test micro-organisms are given in (Table 2).

Table 2: Bacterial strains used in the present study

S. No	Microorganism	Characteristics	Diseases caused
1	*Escherichia coli* (NCIM 2931)	Gram negative rod-shaped bacterium, facultative anaerobe.	Urinary tract infections, gastroenteritis, neonatal meningitis, pneumonia
2	*Pseudomonas aeruginosa* (NCIM 5029)	Gram-negative, aerobic, rod-shaped bacterium with unipolar motility	Urinary tract infections, respiratory infections, dermatitis, soft tissue infections, opportunistic human pathogen.
3	*Micrococcus flavus* (NCIM 2376)	Gram - positive cocci, non - motile, aerobic	Urinary tract infections, respiratory infections
4	*Bacillus cereus* (NCIM 2106)	Gram-positive, rod-shaped, swarming motility, facultative anaerobe	Food-borne illnesses, nausea, vomiting and diarrhoea

RESULTS AND DISCUSSION

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. In the present study, aqueous, ethanolic and methanolic extracts of *Musa paradisiaca* var *Bontha*, were tested against selected Gram positive and Gram negative bacteria species.

Minimum Inhibitory Concentration

Table 3 shows the MIC values of the selected strains of bacteria against the aqueous and solvent extracts. At low concentrations (up to 750µg/ml), the aqueous extract was not inhibitory to the test organisms. Only at 1000µg/ml activity was observed. So, the MIC for aqueous ripened and un-ripened pulps' extracts of *Bontha* cultivar was determined to be 1000µg/ml. Ethanolic un-ripened 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.* Ripened ethanolic extracts were inhibitory to all the test organisms at 100µg/ml except *E. coli.* MIC of un-ripened methanolic extracts was found to be 100µg/ml for *M. flavus,* 250µg/ml for *E. coli,* 500µg/ml for *P. aeruginosa* and 1000µg/ml for *B. cereus.* For ripened extracts MIC values were found to be 100µg/ml for *M. flavus,* 250µg/ml for E. *coli* and *P. aeruginosa* and 500µg/ml for *B. cereus.*

Table 3: Evaluation of MIC of Unripened and ripened pulps of *Musa x paradisiaca* L. var *Bontha*.

Micro-organism	Type of extract		Concentration (µg/ml)				
			100	250	500	750	1000
B. cereus (NCIM 2106)	Unripe	aqueous	+	+	+	+	-
		ethanol	+	+	+	-	-
		methanol	+	+	+	+	-
	Ripe	aqueous	+	+	+	+	-
		ethanol	-	-	-	-	-
		methanol	+	+	-	-	-
E. coli (NCIM 2931)	Unripe	aqueous	+	+	+	+	-
		ethanol	+	+	-	-	-
		methanol	+	-	-	-	-
	Ripe	aqueous	+	+	+	+	-
		ethanol	+	-	-	-	-
		methanol	+	-	-	-	-
M. flavus (NCIM 2376)	Unripe	aqueous	+	+	+	+	-
		ethanol	+	-	-	-	-
		methanol	-	-	-	-	-
	Ripe	aqueous	+	+	+	+	-
		ethanol	-	-	-	-	-
		methanol	-	-	-	-	-
P. aeruginosa (NCIM 5029)	Unripe	aqueous	+	+	+	+	-
		ethanol	+	-	-	-	-
		methanol	+	+	-	-	-
	Ripe	aqueous	+	+	+	+	-
		ethanol	-	-	-	-	-
		methanol	-	-	-	-	-

** Values are mean of triplicates

'+' = Growth '-' = No growth

Determination of zone of inhibition of *Musa x paradisiaca* L. var *Bontha*

Ripened extracts

The Table 4 shows the antimicrobial activity exhibited by extracts 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.

Aqueous extracts (Ripened) showed zone of inhibition at 1000µg/ml concentration on the test bacteria *B. cereus, E. coli, M. flavus* and *P. aeruginosa* at 8mm, 14mm, 8mm and 6mm respectively. Even then, the activity observed at 1000µg/ml was very less compared to other extracts. Up to 750µg/ml no zone of inhibition was exhibited on all test organisms by aqueous extract.

Ethanolic extracts (Ripened) exhibited antibacterial activity starting at 100 µg/ml on all the test organisms except *E. coli*. The activity of the tested concentration of extract was found to be ranging from 8mm – 32mm each for *B. cereus* and *M. flavus* and 4mm – 18mm for *P. aeruginosa.* The inhibition of growth of *B. cereus*, *E. coli* and *P. aeruginosa* at 1000µg/ml by ethanolic extract was greater than that of the standard antibiotic, Chloramphenicol at 30µg/ml concentration.

M. flavus was found to be most susceptible to Methanolic extract at 100µg/ml and a zone of inhibition of 10mm. *E.coli* and *P. aeruginosa* yielded to the methanolic extract at 250 µg/ml with inhibition zones of 10mm and 12mm respectively. Inhibition activity against *B.cereus* was observed at 500µg/ml with a susceptibility zone of 16mm. The methanol extract could act against the test organism *P. aeruginosa* on par with the control antibiotic, Chloramphenicol (30µg/ml) at as less a concentration as 250µg/ml.

Table 4: Determination of zone of inhibition of pulp extracts of Ripened *Musa x paradisiaca* L. var *Bontha.*

Type of extract	Micro-organism	Zone of Inhibition (mm)						
		Control	Concentration (µg/ml)					Std*
			100	250	500	750	1000	
Aqueous	*B.cereus*	-	-	-	-	-	8	28
	E.coli	-	-	-	-	-	14	28
	M.flavus	-	-	-	-	-	8	38
	P.aeruginosa	-	-	-	-	-	6	12
Ethanol	*B.cereus*	2	8	12	18	24	32	28
	E.coli	2	2	18	20	24	30	28
	M.flavus	2	8	16	20	24	32	38
	P.aeruginosa	2	4	8	12	12	18	12
Methanol	*B.cereus*	2	2	2	16	22	26	28
	E.coli	2	2	10	14	20	26	28
	M.flavus	2	10	16	18	28	32	38
	P.aeruginosa	2	2	12	16	18	24	12

*Standard: Chloramphenicol (30 µg/ml)

** Values are mean of triplicates

Un-Ripened extracts

The antimicrobial activity exhibited by un-ripened pulp extracts of *Bontha* cultivar can be seen in Table 5. Activity was observed only at 1000µg/ml concentration for aqueous extract, but, it was slightly less than the activity exhibited by the ripened aqueous extract. The growth inhibitory zones observed for aqueous extract were 6mm for *B. cereus*, 10mm for *E. coli*, 4mm for *M. flavus* and 4mm for *P. aeruginosa* as against the values of 8mm, 14mm, 8mm and 6mm against *B. cereus, E. coli, M. flavus* and *P. aeruginosa* respectively.

Antimicrobial activity of ethanolic extracts of unripe *Musa x paradisiaca* L. var *Bontha* was revealed to be starting at 250µg/ml against *M. flavus* and *P. aeruginosa* with zone of inhibition of 12mm and 6mm, respectively. Susceptibility to extract was seen at 500µg/ml by *E. coli* (16mm) and at 1000µg/ml against all test organisms including *B. cereus* (12mm). 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 reference standard, Chloramphenicol at 30µg/ml.

Activity exhibited by the methanolic pulp extracts of *Bontha* against the test organisms was seen at the lowest concentration of 100µg/ml against *M. flavus* and an inhibition zone of 8mm, at 250µg/ml against *E. coli* with inhibition of 8mm, at 500µg/ml and a measured susceptibility of 14mm against *P. aeruginosa* and 1000µg/ml against all test organisms including *B. cereus* (zone of inhibition – 18mm). The activity of methanol extract against *P. aeruginosa* was found to be very much greater (almost two times) than the reference standard, Chloramphenicol (30µg/ml) at the highest extract concentration of 1000µg/ml.

Table 5: Determination of zone of inhibition of pulp extracts of Un-Ripened *Musa x paradisiaca* L. var *Bontha.*

Type of extract	Micro-organism	Zone of Inhibition (mm)						
		Control	Concentration (µg/ml)					Std*
			100	250	500	750	1000	
Aqueous	*B.cereus*	-	-	-	-	-	6	28
	E.coli	-	-	-	-	-	10	28
	M.flavus	-	-	-	-	-	4	38
	P.aeruginosa	-	-	-	-	-	4	12
Ethanol	*B.cereus*	2	2	2	2	12	18	28
	E.coli	2	2	2	16	20	26	28
	M.flavus	2	2	12	14	22	28	38
	P.aeruginosa	2	2	6	8	10	14	12
Methanol	*B.cereus*	2	2	2	2	2	18	28
	E.coli	2	2	8	12	16	22	28
	M.flavus	2	8	14	17	24	28	38
	P.aeruginosa	2	2	2	14	16	22	12

*Standard: Chloramphenicol (30 µg/ml)

** Values are mean of triplicates

High inhibitory activity by ethanolic extract was seen on the Gram negative test organism *E. coli* and the Gram-positive test organisms, when compared to the activity on *P. aeruginosa*.

Methanolic extract displayed more activity on *E. coli* and *M. flavus* than on B. *cereus* and *P. aeruginosa*. The aqueous extract inhibited very little growth of all the test-organisms.

Phytochemical Studies (Preliminary)

The preliminary phyto-chemical screening of ethanolic and methanolic extracts of the selected *Musa x paradisiaca* L. var *Bontha* indicated the presence of certain secondary metabolites as shown in the Table 6. Ethanolic extract was found to contain alkaloids, flavonoids, steroids, tannins, xanthoproteins and glycosides,

whereas the methanolic extract revealed the presence of alkaloids, saponins, xanthoproteins and glycosides.

The accountability of antimicrobial activity exhibited by the extracts can be attributed to these chemicals present in the respective extracts.

Table 6: Chemical constituents found in Ethanolic and Methanolic extracts of both Unripened and ripened fruit pulp of *Bontha*.

Phytochemical Constituents	Ethanolic Extract	Methanolic Extract
Alkaloids	Present	Present
Carboxylic acids	Absent	Absent
Coumarins	Absent	Absent
Fixed oils	Absent	Absent
Flavonoids	Present	Absent
Phenols	Absent	Absent
Quinones	Absent	Absent
Resins	Absent	Absent
Saponins	Absent	Present
Steroids	Present	Absent
Tannins	Present	Absent
Xanthoproteins	Present	Present
Glycosides	Present	Present

The results showed that for the extracts of both ripened and Unripened pulps, the Gram- negative bacteria *Escherichia coli* and Gram-positive bacteria *Micrococcus flavus* were more sensitive than the other bacteria, at all concentrations. Antibacterial activity of extract of different solvents varied related to the test organisms. The most active of the concentrations was 1000µg/ml concentration inhibiting completely the growth of all the Gram-negative and Gram-positive bacteria. While Cos *et al*., 2006[20] reported that Gram-negative bacteria are generally more resistant compared to the Gram-positive ones in the case of natural products.

The aqueous Un-ripened and ripened pulp extracts of *Musa x paradisiaca* L. var *Bontha* were found to be less active against all the test organisms. Ethanolic ripened and un-ripened extracts showed highest inhibitory activity to all the test organisms. Methanolic extracts of both ripened and un-ripened suppressed the growth of all the test organisms.

Scott *et al* [21] in a previous work, presented the antifungal activity exhibited by solvent extracts (aqueous, methanol and petroleum-ether) obtained from the pulp and skins of green, naturally ripened, and ethylene-ripened bananas. They also showed appreciable antibacterial activity in extracts from the pulp and skins of ripe bananas.

In another previous study, Mokbel *et al* [22] showed that ethyl acetate extract of green banana peel recorded significant antimicrobial activities against *S. aureus, B. subtilis*, *B. cereus, S. enteritidis* and *E. coli*. Fagbemi *et al*'s [23] investigation on the potency of unripe banana (*Musa sapientum* L.), was carried out against pathogens like *E. coli*, *S. aureus*, *B. subtilis* and *S. paratyphi*. It was revealed that the minimum inhibitory concentration (MIC) of unripe banana ranged between 2 and 512 mg/ml depending on the isolate and extracting solvent.

Mohamed *et al*[24] investigated the antimicrobial activity of ripe and unripe banana (*Musa sapientum* var *Montel*) solvent extracts (Petroleum ether, chloroform, and ethanol) against Gram-positive bacteria, Gram-negative bacteria, yeast and fungi (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Lactobacillus bulgaricus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonelli typhi, Saccharomyces cerevisiae, Candida lypolytica, Rhizopus* spp., *Aspergillus niger* and *Chlamydomucor* spp) using both the filter paper disc diffusion and tube dilution assays. Unripe banana showed activity against all the bacteria except towards *P. vulgaris*.

According to a report submitted by CS Alisi *et al*[25], the aqueous extract from the unripe fruit peels and leaves of *Musa paradisiaca* var *sapientum* exhibited activity against pathogenic bacteria like *Staphylococcus* and *Pseudomonas* species.

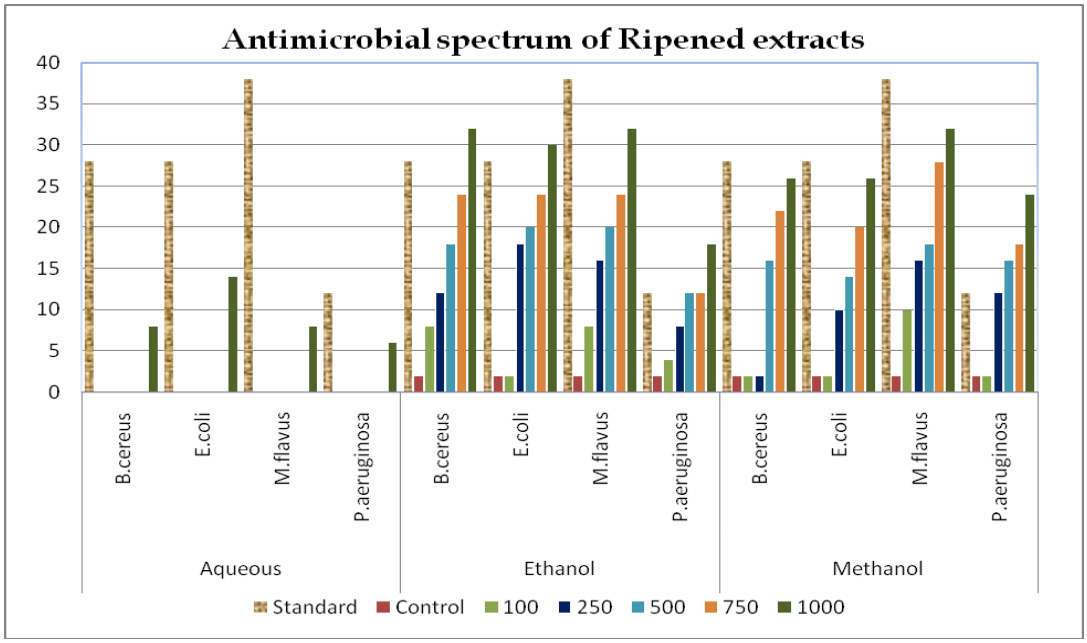

Fig. 1: A comparative graphical representation of different Ripened extracts of *Musa x paradisiaca* L. var *Bontha*

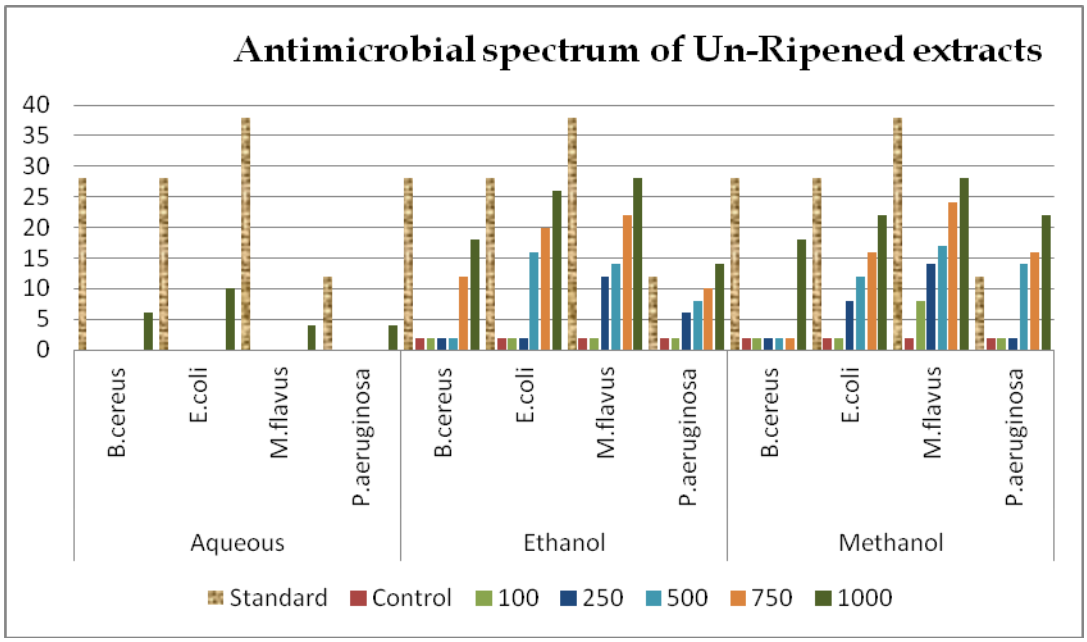

Fig. 2: A comparative graphical representation of different Un-Ripened extracts of *Musa x paradisiaca* L. var *Bontha*

Preliminary phyto-chemical screening of plant is very useful for determination of the active constituents in different solvents and their yields. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds[26]. Flavonoids and tannins present in the ethanol extract may be responsible for the antibacterial activity. Tannin is known to show the antibacterial activity by precipitation the microbial proteins. Flavonoids are produced by the plants for the defense against the infection. So, use of the crude ethanol extract of this plant as an agent to control microbial pathogens needs further extensive research for their better economic and therapeutic utilization. Further phyto-chemical studies are required to determine the purified fractions/bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antimicrobial agents[27].

In our study, a wide range of human pathogenic microorganisms were examined, including Gram-positive and Gram-negative bacteria, The results obtained indicate that the different extracts of the cultivar under study exhibit antibacterial activity and among the various extracts, ethanolic extracts have shown better activity as compared to other extracts with respect to similar bacteria. This may partly indicate that the pulp extracts of *Musa x paradisiaca* L. var *Bontha* have broad inhibitory activities to pathogenic micro-organisms and are promising to act as potential antibacterial agents from natural plant sources[27].

CONCLUSION

The ever-growing resistance of microbes to existing drugs and also the inefficiency of the drugs have given rise to an era of research wherein plants are the primary source of new and potent antimicrobial compounds [28]. *Musa x paradisiaca* L. var *Bontha*, the common pomological and commercially important plant of India, is a rich source of bioactive compounds with diverse chemical structure. As of now, little work has been done on the biological activity and plausible medicinal applications of the phytochemical compounds and hence extensive investigation is needed to exploit the bioactive principles of local varieties of *Musa x paradisiaca* L. var *Bontha* for therapeutic utility. In the present study antibacterial activity of *Musa x paradisiaca* L var *Bontha* pulp extract residues towards significant pathogenic microbes has been investigated. Further detailed investigations may lead to development of new antibiotics of high potency. The present study suggests that the ethanol extract of *Musa x paradisiaca* L. var *Bontha* plants is a potential source of natural antibacterial agents. After this screening experiment, further work should be performed to describe the antibacterial activities in more detail as *in vivo*. Also phyto-chemical studies will be necessary to isolate the active constituents and evaluate the antibacterial activities against a wide range of bacterial population[29].

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