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Study of Antioxidant and Antimicrobial Properties, Phytochemical screening and analysis of Sap Extracted from Banana (Musa acuminata) pseudostem

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ABSTRACT
Banana is the common name for herbaceous plants of the genus *Musa*. Bananas come in a variety of sizes and colors when ripe, including yellow, purple, and red. The banana plant is the largest herbaceous flowering plant. Plants are normally tall and fairly sturdy and are often mistaken for trees, but their main or upright stem is actually a pseudo stem that grows 6 to 7.6 meters (20 to 24.9 ft) tall, growing from a corm [3]. Each pseudo stem can produce a single bunch of bananas. After fruiting, the pseudo stem dies, but offshoots may develop from the base of the plant. The Banana pseudostem sap has some special properties relating to various phenomena such as browning of fruits after harvesting, permanent staining of cloth and fibers, antioxidant, antimicrobial, and antihemorrhagic properties. All the aqueous, methanolic and ethanolic extracts of pseudostem have been found to contain good amount of antioxidants along with different phytochemical compounds like carbohydrate, protein and phenolic compounds. The phytochemical screening and analysis of pseudostem sap indicated the presence of these carbohydrate, protein and phenolic compounds. The antimicrobial studies with different fungal and bacterial strains indicated the antimicrobial properties for the sap as well.

Keywords – Banana pseudostem sap**,** Antioxidant properties, Antimicrobial properties, Phytochemical screening, *Musa acuminata*

INTRODUCTION
Ayurveda stresses the use of plant based products like medicine. Herbs and human health can never be separated. The vegetables and the fruits are the herbs essential for good health because of the presence of certain essential enzymes, amino acids, alkaloids, vitamins etc. Medicinal plant spices and herbal remedies are known to Ayurveda in India since long time. The value of medicinal plants, herbs and spices as herbal remedies is being lost due to lack of awareness and deforestation [10]. As a result's several valuable healthful herbs are getting rare and precious information is lost. Several medicinal plants play very important role in as a key ingredient of many treatment procedure. Plants have been valuable source of natural products for various human beneficial products and maintaining human health, especially in the last decades, with more intensive studies for natural therapies. The use of plant extracts and phytochemical, both with known antimicrobial and antioxidant properties are often of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [7]. Many plants have been used because

of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [6].

Antimicrobials and antioxidant properties of plants origin have enormous therapeutic potential. They are effective in the treatment of the infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic compounds. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plants. In plants, such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, resins, fatty acids, and gums are mostly secondary metabolites [1] which are capable of producing definite physiological action on the body. Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever, bronchitis, etc. [11].

Banana is the common name for herbaceous plant of the genus *Musa*. Binomial nomenclature of banana plant is *Musa acuminata*. Bananas come in a variety of sizes and colours when ripe, including yellow, purple, and red. They are native to tropical Southeast Africa and are likely to have been first domesticated in Paupa New Guinea. Today, they are cultivated throughout the tropics and are full-grown in a minimum of 107 countries, primarily for their fruit and to a lesser extent to form banana wine, fiber, and as decorative plant.

The banana plant is the largest herbaceous flowering plant. The plants are normally tall and fairly sturdy and are often mistaken for trees; however their main or upright stem is really a pseudostem that grows 6 to 7.6 meters tall. Every pseudostem will turn out one bunch of bananas. After fruiting, the pseudostem dies, however offshoots could develop from the bottom of the plant. The banana pseudostem may be entirely green, green with maroon splotches or green on the top side and red purple below. It is used mainly to obtain a fiber employed in manufacturing paper. Banana fiber is extracted from dried petioles. The sap extracted from Banana pseudostem has some special properties relating to various phenomena such as browning of fruits after harvesting, permanent staining of cloth and fibers [8].

Here, in this study we have found significant antioxidant property, and a little antimicrobial activity of the sap extracted from the banana pseudostem. However, no antifungal activity has been reported but it was effective against some bacterial species. We have also reported some essential phytochemical in it as well.

MATERIALS AND METHOD

Source of Microorganisms

The test bacterial cultures used in this study were *Bacillus cereus, Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Staphylococcus citreus, Streptococcus feacalis, Klebsiella spp., Proteus mirabilis, Salmonella typhi and Pseudomonas aeruginosa.* The fungal cultures used were *Candida albicans, Candida parapsilosis, Cryptococcus sp., Aspergillus oryzae, Aspergillus flavus, Aspergillus niger, Trichophyton mentagrophytes, Penicillium sp., Trichoderma sp.,* and *Trichophyton rubrum.* These bacterial and fungal cultures were obtained from the Department of Microbiology, Genohelix Biolabs Bangalore India. The bacterial cultures were maintained on nutrient agar and MacConkey's agar slants and the fungal cultures on Potato Dextrose agar slants, respectively, at 4ºC throughout the study.

Source of chemicals and media

All the chemicals used were obtained from S.D. Fine Chem., Nice chemicals Pvt. Ltd., Karla, India. All the culture media used were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

Source of plant material

The plant material – pseudostem of Banana (*Musa acuminata*) used in the study was purchased from the local market in Bangalore city (Karnataka, India).

Preparation of Pseudostem extract

Thoroughly washed and chopped pseudostem was used to make four different kinds of extracts, which are crude aqueous extract, Crude 50% Methanol extract, Crude 90% Methanol extract and Crude 90% Ethanol extract. In each extract 300 gm of freshly chopped pseudostem was blended with 300 ml of respective medium in an electrical mixer. The mixture was filtered with mesh cloth; filtrate was collected and then centrifuged at 5000 rpm for 30

min at 4ºC. The supernatant was collected in sterile vial and used for further assays.

Total Antioxidant assay have been assessed with the phosphomolybdenum reduction assay technique according to Prieto *et al*. [9]. For qualitative phytochemical screening, standard Mayer's test, Wagner's test, Herger's test, Dragendroff test were done to determine the presence of Alkaloids. Similarly, standard Molish's test, Fehling's test, Barfoed test and Benedict's test, were carried out for Carbohydrate screening. For the determination of Saponin, standard Foam test was carried out. For the screening of Proteins, Millon's test, Biuret test, and Ninhydrin test were done. Standard Liebermann – Burchard's test was done for the screening of Phytosterols. To determine the presence of Phenolic compound in the sample, standard Ferric chloride test, Gelatine test, Lead acetate test, Alkaline reagent test, Magnesium and Hcl test were performed. The standard test for the screening of Gum and Mucilage was also done.

Quantitative estimation of the compounds found in the samples were also done by different methods. Determination of total Carbohydrate was estimated by standard Anthrone Method, Lowry's Method was used for the estimation of protein and Folin-Ciocalteau reagent based test was used for the estimation of Phenolic compounds.

The antimicrobial activities of aqueous extracts, methanolic extract and ethanolic extracts of Banana pseudostem were tested against various human pathogens (Bacterial and fungal) using the agar well diffusion as discussed by Fagbemi *et al.*,[2] and disc diffusion method as discussed by Mokbel and Hashinaga [5].

RESULTS AND DISCUSSION

Total antioxidant assay

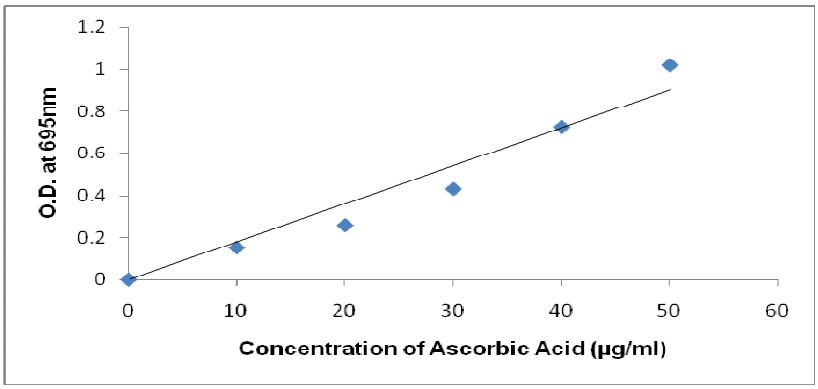
The total antioxidant capacity analyzed by spectrophotometric method revealed high amounts of antioxidants in all the extracts. The detailed results are presented in Table 1.

Table 1: Total antioxidant capacity.
Standard stock concentration of ascorbic acid = 0.5 mg/ml

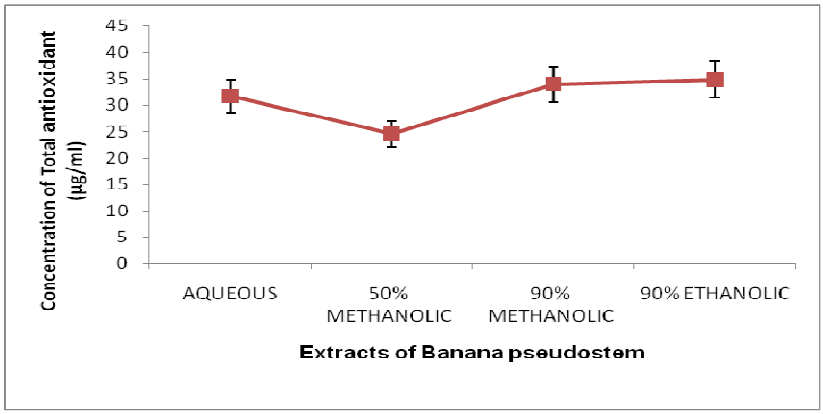
Sl. no.	Stock solution (ml)	Vol. of water (ml)	Reagent	Incubate at 95° C for 90 minutes	Cool to room temp.	OD at 695 nm	Conc. (µg/ml)
1	0.02	0.08	1 ml			0.152	10
2	0.04	0.06				0.258	20
3	0.06	0.04				0.431	30
4	0.08	0.02				0.724	40
5	0.10	0.00				1.017	50
S 1	0.01	-				0.576	31.68
S 2	0.01	-				0.446	24.53
S 3	0.01	-				0.618	33.99
S 4	0.01	-				0.633	34.815
BLANK	-	0.10				0.000	-

KEYS:-

S 1- Aqueous extract (pseudostem with water)
S 2- 50% Methanol extract
S 3- 90% Methanol extract
S 4- 90% Ethanol extract



Graph 1: Standard graph for Antioxidant Potential assay.



Graph 2: Total antioxidant concentration in Banana pseudostem extracts.

The 90% Ethanolic extract has shown maximum concentration of antioxidant (34.815 µg/ml) among all the pseudostem extracts.

Qualitative phytochemical screening

Table2: Phytochemical analysis of different extracts of Banana Pseudostem.

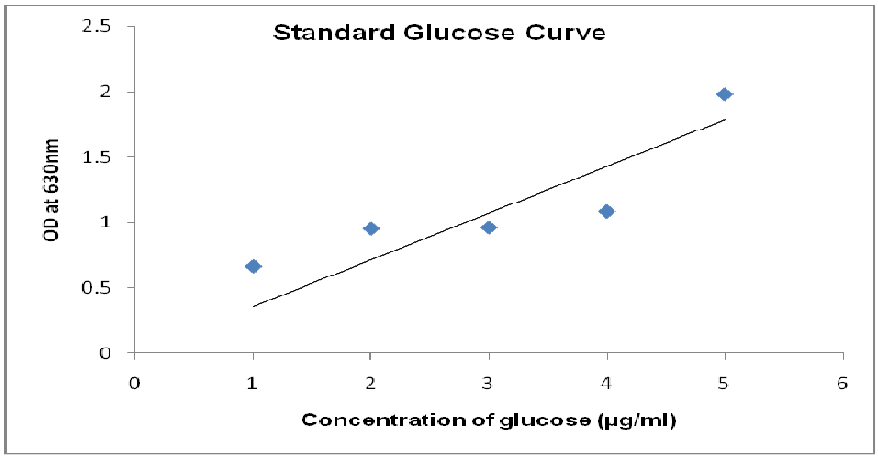
Sl. No.	Tests	Extracts		
		Aqueous	Ethanolic	Methanolic
1.	**Alkaloids**			
a.	Mayer Test	-	-	-
b.	Wagner Test	-	-	-
c.	Harger Test	-	-	-
d.	Dragendroff Test	-	-	-
2.	**Carbohydrates**			
a.	Molish Test	+	+	+
b.	Fehling Test	+	+	+
c.	Barfoed Test	+	+	+
d.	Benedict Test	+	+	+
3.	**Saponin**			
A	Foam Test	-	-	-
4.	**Protein and amino acid**			
a.	Million Test	+	+	+
b.	Biuret Test	+	+	+
c.	Ninhydrin Test	+	+	+

5.	**Phytosterol**			
	Liberman –burchards method	-	-	-
6.	**Phenolic compounds**			
a.	Ferric chloride	+	+	+
b.	Gelatine	+	+	+
c.	Lead acetate	+	+	+
d.	Alkaline reagent	+	+	+
e.	Magnesium and HCl	+	+	+
7.	**Gum and mucilage**	+	+	+

The qualitative phytochemical estimation of different banana pseudostem extracts has shown the presence of carbohydrate, protein, phenol and gum and mucilage as shown in Table 2. Quantitative estimation for carbohydrate, protein and phenol were further carried out and corresponding results are shown below.

Table 3: Determination of total Carbohydrate by "Anthrone Method".
Table 3.1: Standard Glucose.

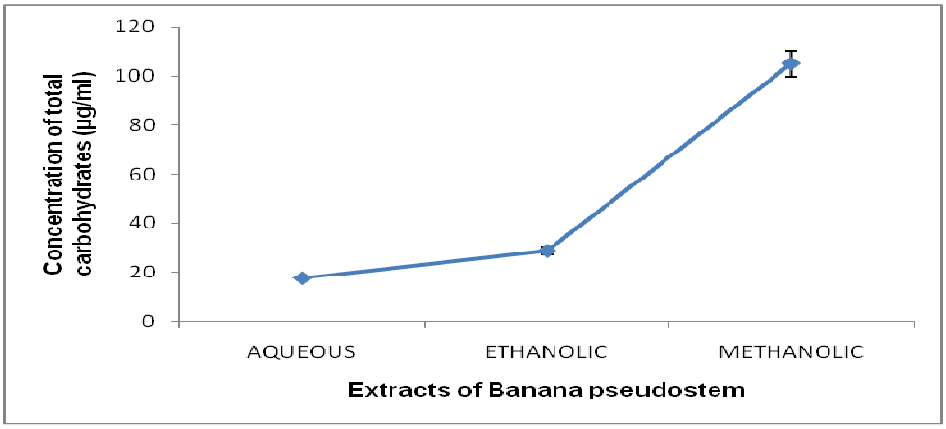
Sl. no.	Standard glucose(ml)	Distilled water(ml)	Conc. (µg/ml)	Anthrone reagent	Heat for 8 min. in boiling water bath.	OD at 630nm
1	0.0	1.0	0	4ml		0.000
2	0.2	0.8	20			0.664
3	0.4	0.6	40			0.952
4	0.6	0.4	60			0.960
5	0.8	0.2	80			1.083
6	1.00	0.0	100			1.980



Graph 3: Standard graph for estimation of Carbohydrate.

Table 3.2: Sample Extracts.

Sample Name	Sample (ml)	Anthrone reagent	Heat for 8 min. in boiling water bath	OD at 630nm	Conc. (µg/ml)
Aqueous	1.00	4ml		0.315	17.64
Ethanol	1.00			0.513	28.728
Methanol	1.00			1.874	104.944



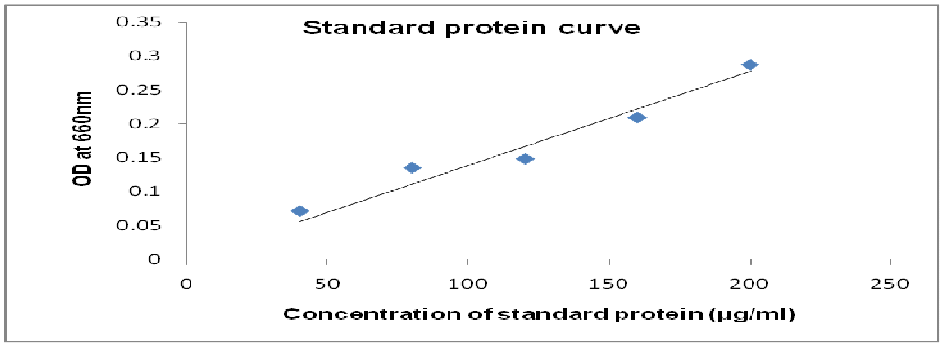
Graph 4: Total carbohydrate concentration in Banana pseudostem extracts.

90% methanolic extract has shown maximum concentration of total carbohydrates (104.994µg/ml) among all the pseudostem extracts. The detailed results have been presented in Table 3.2.

Table 4: Estimation of protein by "Lowry's Method".

Table 4.1: Standard Protein.

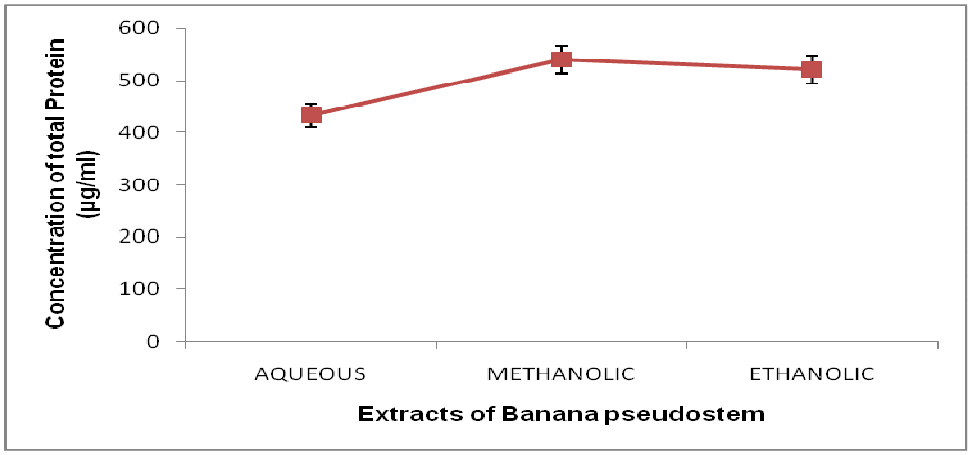
Sl. no.	Standard protein (ml)	Distilled water (ml)	Conc. (µg/ml)	Alkaline Cu solution	Incubate for 10 min at room temp.	FCR reagent	Incubate in dark for 30 min at room temp.	OD at 660nm
1	0.00	1.00	000	5ml		0.5ml		0.000
2	0.2	0.8	040					0.071
3	0.4	0.6	080					0.135
4	0.6	0.4	120					0.148
5	0.8	0.2	160					0.209
6	1.00	0.00	200					0.287



Graph 5: Standard graph for estimation of Protein.

Table 4.2: Sample extracts.

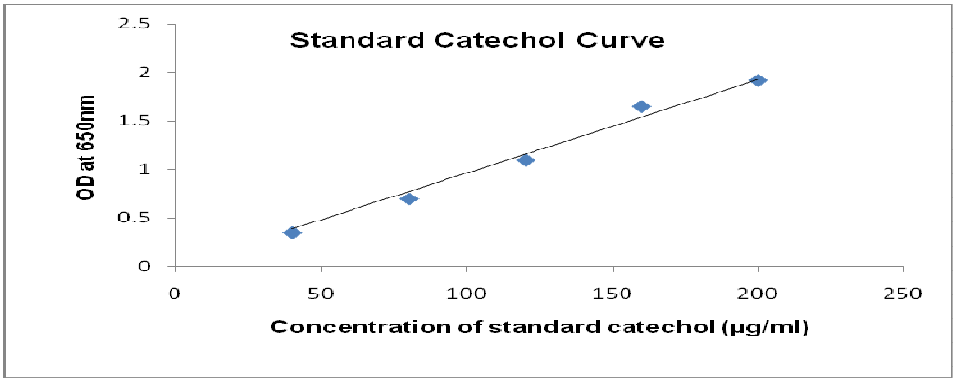
Sl. no.	Standard protein (ml)	Distilled water (ml)	Alkaline Cu solution	Incubate for 10 min at room temp.	FCR reagent	Incubate in dark for 30 min at room temp.	OD at 660nm	Conc. (µg/ml)
1	0.2 A	0.8	5ml		0.5ml		0.601	432.72
2	0.2 M	0.8					0.750	540.00
3	0.2 E	0.8					0.723	520.56



Graph 6: Total Protein concentration in Banana pseudostem extracts.
90% methanolic extract has shown maximum concentration of protein (540µg/ml) among all the pseudostem extracts. The detailed information has been shown in Table 4.2.

Table 5: Estimation of Phenol.
Table 5.1: Standard Catechol.

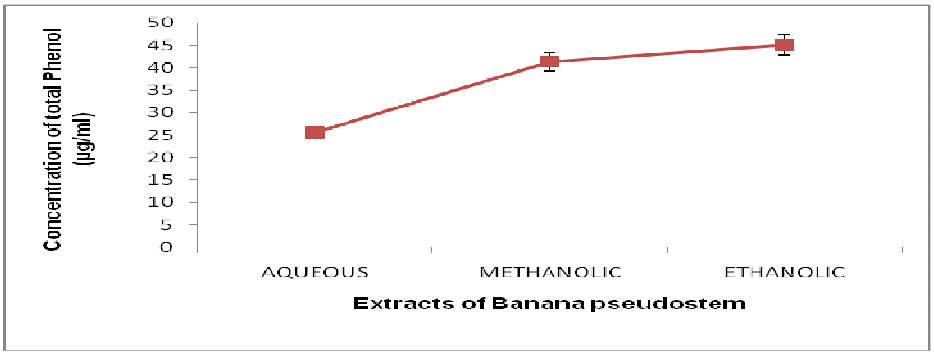
Sl. no.	Standard catechol (ml)	Distilled water (ml)	Conc. (µg/ml)	FCR reagent	Incubate for 3 min at room temp.	20%Na_2CO_3	Incubate in boiling water bath for 1 min.	OD at 650nm
1	0.0	5.0	000	0.5ml		2.0ml		0.000
2	0.4	4.6	40					0.345
3	0.8	4.2	80					0.693
4	1.2	3.8	120					1.092
5	1.6	3.4	160					1.644
6	2.0	3.0	200					1.912



Graph 7: Standard graph for estimation of Phenol.

Table 5.2: Sample extracts.

Sample	Distil water (ml)	FCR	Incubate for 3 min at room temp.	20% Na_2CO_3	Incubate in boiling water bath for 1 min.	OD at 650nm	Conc. (µg/ml)
Aqueous	5ml	0.5ml		2ml		0.244	25.62
Methanol						0.394	41.37
Ethanol						0.429	45.045



Graph 8: Total Phenol concentration in Banana pseudostem extracts.

90% ethanolic extract has shown maximum concentration of phenol (45.045µg/ml) among all the pseudostem extracts. The detailed information is presented in Table 5.2.

Antimicrobial assays

The antimicrobial activities of aqueous extracts, ethanolic extract and methanolic extracts of Banana pseudostem were tested against various human pathogens (Fungal and Bacterial) using the agar well diffusion and disc diffusion method.

Table 6: Zone of inhibition for extracts of Banana pseudostem against different fungal pathogens.

Sl. No.	Fungal strains used	Diameter of inhibition (in mm)				Antifungal Disc (Fluconazole)
		Aqueous	Methanol 50%	Methanol 90%	Ethanol 90%	
1.	*Aspergillus flavus*	-	-	-	-	8 ± 0
2.	*Aspergillus niger*	-	-	-	-	15 ± 0.5
3.	*Aspergillus oryzae*	-	-	-	-	10 ± 0
4.	*Candida albicans*	-	-	-	-	12 ± 0
5.	*Candida parapsilosis*	-	-	-	-	10.5 ± 0.5
6.	*Cryptococcus*	-	-	-	-	24 ± 1
7.	*Trichophyton mentagrophytes*	-	-	-	-	13 ± 0
8.	*Trichophyton rubrum*	-	-	-	-	14.5 ± 0.5
9.	*Trichoderma sp.*	-	-	-	-	11 ± 0.5
10.	*Penicillium sp.*	-	-	-	-	9 ± 0

In the present study none of the extracts were effective against the test fungal pathogens with both the disc diffusion and well diffusion methods.

Table 7: Zone of inhibition for extracts of Banana pseudostem against different bacterial pathogens.

Table 7.1: Well diffusion.

Sl. No.	Baterial strains used	Diameter of inhibition (in mm)				Antibiotic disc
		Aqueous	Methanol 50%	Methanol 90%	Ethanol90 %	
1.	*Bacillus sp.*	-	-	-	-	8.5 ± 0.5*
2.	*Escherichia coli*	-	-	-	7.5 ± 0.5	25.5 ± 0.5
3.	*Klebsiella sp.*	6.5 ± 0.5	-	-	-	31.5 ± 0.5
4.	*Proteus mirabilis*	-	-	-	-	24.5 ± 0.5
5.	*Pseudomonas aeruginosa*	7.5 ± 0.5	-	-	-	32.5 ± 1.5
6.	*Salmonella typhi*	-	-	-	-	33.5 ± 0.5
7.	*S. aureus*	-	-	-	-	32.0 ± 1.0
8.	*S. citreus*	-	-	-	-	24.5 ± 0.5
9.	*Streptococcus faecalis*	6.5 ± 0.5	7.0 ± 1.0	5.5 ± 0.5	9.5 ± 0.5	36.5 ± 0.5
10.	*Serratia marcescens*	-	-	-	-	36.0 ± 3.0

Keys:-

- Antibiotic discs of Ampicillin and Ciprofloxacin were used as control for gram positive and Gram negative bacteria respectively.
- * Data given are mean ± standard deviation, n=2.

Table 7.2: Disc diffusion.

Sl. No.	Baterial strains used	Diameter of inhibition (in mm)				Antibiotic disc
		Aqueous	Methanol 50%	Methanol 90%	Ethanol 90%	
1.	*Bacillus sp.*	-	-	-	-	9.5 ± 0.5*
2.	*Escherichia coli*	-	-	-	-	24.5 ± 0.5
3.	*Klebsiella sp.*	-	-	-	-	31.5 ± 0.5
4.	*Proteus mirabilis*	-	-	-	-	24.5 ± 0.5
5.	*Pseudomonas aeruginosa*	-	-	-	-	31.5 ± 1.5
6.	*Salmonella typhi*	-	-	-	-	33.5 ± 0.5
7.	*S. aureus*	-	-	-	-	30.0 ± 1.0
8.	*S. citreus*	-	-	-	-	24.5 ± 0.5
9.	*Streptococcus faecalis*	-	-	-	-	34.5 ± 0.5
10.	*Serratia marcescens*	-	-	-	-	36.0 ± 3.0

Keys:-

- Antibacterial discs of Ampicillin and Ciprofloxacin were used as control for gram positive and Gram negative bacteria respectively.
- * Data given are mean ± standard deviation; n=2.

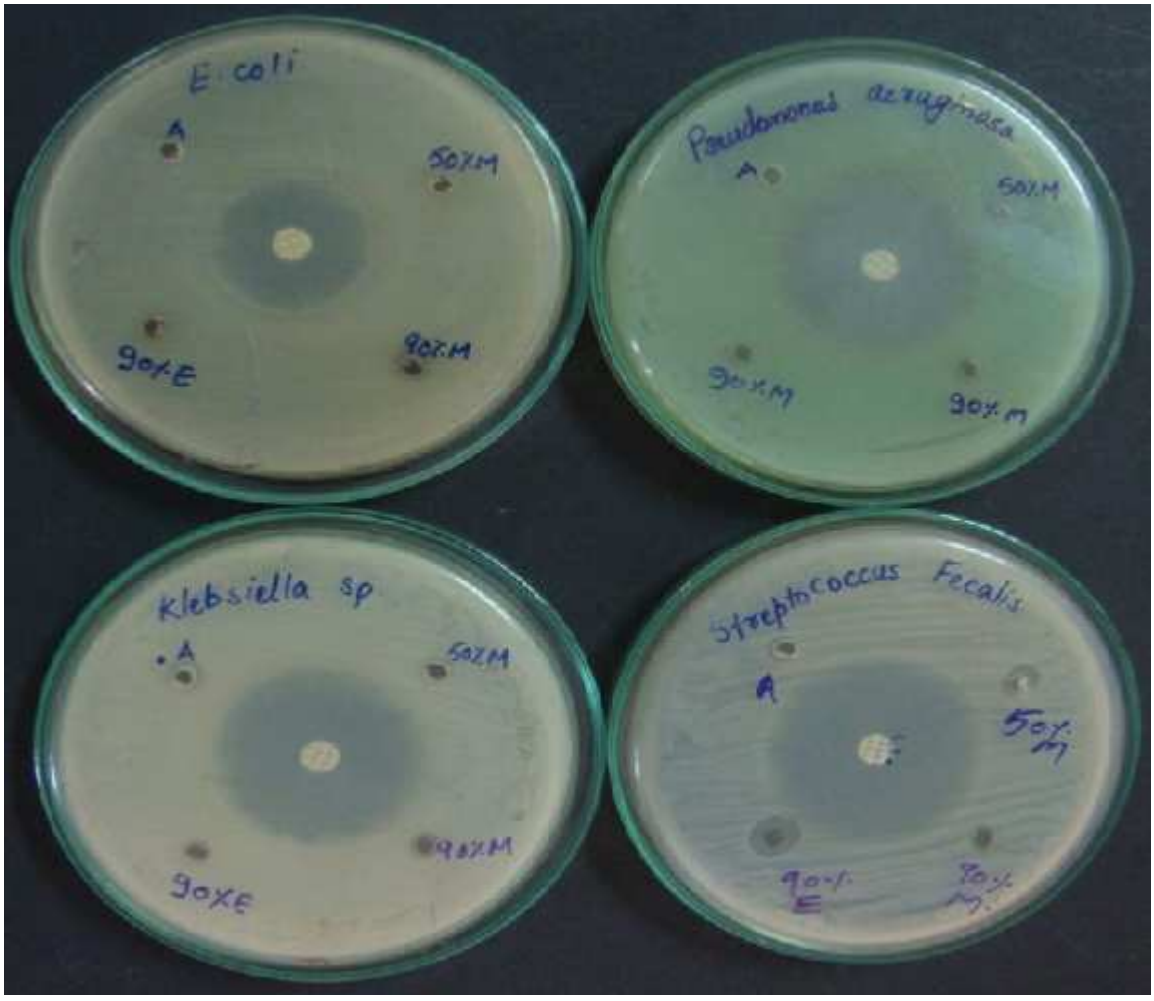


Figure 2: Zone of inhibition for *Escherichia coli, Klebsiella sp., Pseudomonas aeruginosa* and *Streptococcus faecalis.*

Well diffusion method shown zone of inhibition for *Escherichia coli, Klebsiella sp., Pseudomonas aeruginosa* and *Streptococcus faecalis* whereas no zone of inhibition has been found in Disc diffusion method.

CONCLUSION

In the present study, an attempt has been made to identify the antioxidant, phytochemical and antimicrobial properties of banana pseudostem sap. All the aqueous, methanolic and ethanolic extracts of pseudostem have been found to contain significant amount of antioxidants along with carbohydrate, protein and phenolic compounds. The study also indicated that none of the extracts were effective against fungal pathogens and hence no zone of inhibition was observed, whereas pseudostem extracts were effective against some of the bacterial strains namely *Escherichia coli*, *Klebsiella sp.*, *Pseudomonas aeruginosa* and *Streptococcus faecalis.* The results of the study suggest that the sap of banana pseudo stem may be used for the extraction and preparation of various antioxidant and antibacterial formulations in pharmaceutical industries.

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