



Exploration of *Musa paradisiaca* L. Pseudostem Exudate and its antibacterial influence against the causative agents of urolithiatic Bacterial species

S. Sujatha² and Rajini¹

1-. Assistant Professor, Department of Biotechnology, Malankara Catholic College, Mariagiri-629153, Kanyakumari District, Tamilnadu, India.

2- Research Scholar, Department of Biotechnology, Interdisciplinary Research Unit, Malankara Catholic College, Mariagiri-629153, Kanyakumari District, Tamilnadu, India

Abstract

Musa paradisiaca Linn. is widely distributed throughout various tropical regions. Exudate (stem juice) of the pseudostem part of *M. paradisiaca* Linn. is highly nutritious and important naturally available medicinal valuable one for human. The present study was carried out to investigate qualitative and quantitative analysis of phytochemicals, nutritive and antibacterial analysis of *M. paradisiaca*. Qualitative phytochemical analysis was performed using standard protocols and three extracts were used for the study and the presence of alkaloids, saponins, cardiac glycosides, anthraquinones and flavanoids was indicated by the changes of colour, precipitation or turbidity. The determination of nutritional value in experimental exudate was investigated because they were used for multiple medicinal purposes particularly consequences of urolithiatic disease. Quantitative estimation of phytochemicals revealed that tannins are most abundant 28.04 ± 3.27 in raw *M. paradisiaca* exudates, 25.62 ± 2.16 in aqueous extract, 19.31 ± 2.92 in ethanol extract and 18.55 ± 1.26 in methanol extract. The mineral composition of *M. paradisiaca* exudate contained 18mg Iron, 71.9mEq/L Potassium, 32.5 mEq/L Sodium, 1.2mg Phosphorous and 9.6mg calcium in raw musa exudates. Antibacterial results revealed only a positive result in all the extract we have analysed. Among three solvent extract used for the study, Musa pseudostem raw exudate is significantly inhibited the microorganisms *E. aerogens*, *S. flexneri*, *Streptococcus pyogens*, *Klebsiella pneumoniae* at a concentration of

1000µg/ml. The present studies demonstrated that pseudostem extract of *musa paradisiaca* has high antibacterial activity. These results make it possible to infer further applications in the field of pharmacological area.

Key words: *Musa paradisiaca*, Pseudostem exudate, phytochemical analysis, urolithiatic agents

Introduction

Banana, a tropical fruit belonging to the Musaceae family, is cultivated in Saudi Arabia as well as in all world¹ and considered the second largest fruit product in the world (2) The parts of a banana plant including the pulp, leaves, and stem have a pharmaceutical value in several countries (3-4) Exudates are mixtures of organic molecules oozed by plants, predominating, but not always as a result of the injury. Exudates contain high carbon and hydrogen atoms and are also commonly known as sap although the word sap is utilized to characterize any liquid that exodus inside plants. In contrast, the exudate word refers to any such compound when it is oozed out of the plant (5) For the time being, known exudates are employed as constituents of medicines, cosmetics, perfumes, industrial, and food products, Previously, Imam and Akter³ reported significant antimicrobial activity of methanolic extract of fruit peels of *M. paradisiaca* against the pathogenic bacterial organisms such as *E.coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa* bacteria⁶. Several plants exude sticky ingredients as the result of a damage or injury, and this viscous ingredient often solidifies with time. Such ingredients have found religious, medicinal, and other practical and symbolic uses by humans⁷ Flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids as bioactive materials are found in the banana peel. These bioactive materials are reported to exert a pharmacological effect, particularly as an antioxidant, antidiabetic, anti-inflammatory, and antibiotic. The exudate of *Musa* sp. contains flavonoids and polyphenols with high concentration⁸ the antioxidant⁹ was detected. Efficiency of banana stem juice as a natural coagulant for treat spent coolant wastewater therapy stalk methanol and ethanol extract¹⁰. So far no other works have been done like the current research work, hence the present study was designed the following objectives assesses the phytochemicals along with its proximate compositions and its biological efficiency of Banana stem exudate juice.

Materials and methods

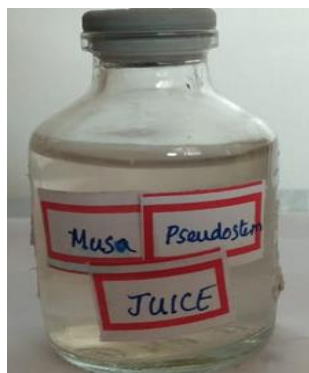


Fig:1- Experimental *M. paradisiacca* pseudostem Exudate

Qualitative Analysis of Phytochemicals in *M. paradisiacca* L. Pseudostem Exudate

Phytochemical screening

The experimental sample has been screened for the presence of alkaloids, saponins, tanins, carbohydrates, flavonoids, anthraquinones, cardiac glycosides, gum and mucilages steroids and terpenes using standard methods as described by Harborne (1998).(11)

Preparation of culture media

The culture media used namely nutrient agar and nutrient broth (Oxiod, England), were prepared according to the manufacturer's instruction, sterilized in an autoclave at 121°C for 20 min and allowed to cool to about 45°C.

Preparation of inoculum

Potent promising extract alone was subjected to this study following modified methods microdilution method (12)

Susceptibility testing

The agar well diffusion method was used to carry out this test. 0.1 ml of 1:1000 dilution of test organism for Gram-positive and 1:5000 dilution of test organism for Gram-negative were introduced into labelled sterile Petri dishes. Twenty millilitres of the cooled nutrient agar medium was aseptically poured into each Petri dish and gently swirled to mix. The plates were allowed to set and wells were created using sterile 4-mm cork borer. Different

concentrations (200–12.5 mg/ml) of the extract diluted with water was introduced into the different wells and labelled appropriately. The Petri dishes were allowed to stand for 20 min before incubation at 37°C for 24 h. The diameters of the zones of growth inhibition were measured in millimetres (mm).

Antibacterial screening of Pseudostem exudates from *M. paradisiaca*

Spread Plating

The number of bacteria in solution can be readily quantified by using the spread plate technique. In this technique, the sample is appropriately diluted and small aliquots transferred to an agar plate. The bacteria are then distributed evenly over the surface by a special streaking technique. After colonies are grown, they are counted and the number of bacteria in the original sample calculated.

Tested organism

In vitro antibacterial activities were examined from the experimental extracts against kidney stone causative bacterial pathogens (five) bacteria (Gram- positive and negative) were investigated by the agar gel puncture method . Pathogenic microorganism were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh . The bacterial strains were *Escheritia coli*, *Klebsiella*, *Bacillus* sp., *Enterobacter* sp.. The bacterial cultures were maintained in nutrient broth as turbid growth and kept in refrigerator at 4°C for further analysis.

Antibacterial Assay: Antibacterial activities of extracts were studied by the agar gel punching method .Lawnes of each organism were prepared on nutrient agar plates. 0.2 ml of fruit juice extract was added to each well. The plates were then incubated for 24 hours at 30°C. After incubation the degree of sensitivity was determined by measuring zone of inhibition of growth around the well.

Antibacterial activity of kidney stone causative bacterial pathogens were analyzed by the plating the diseased part extracts in agar plates and more dominant microbial colonies formed were inoculated in nutrient broth and used for further antibacterial analysis.

Statistical Analysis: Results of the present study data showed the means of four parallel measurements and were calculating by Microsoft Excel -2019. Analysis of Varince (ANOVA) was used to define differences in samples. The resulted data differences were finded as significant at $P < 0.05\%$.

Results and Discussion

Phytochemical Analysis: The phytochemical screening showed the presence of some secondary metabolites which includes alkaloids, saponins, tannins, cardiac glycosides, terpenes, deoxy sugar, flavonoids and carbohydrates. The result was presented in Table-1. Phytochemical analysis of *M. paradisiaca* pseudostem exudate was performed by using standard procedure. Fifteen phytochemicals were analysed, and the result showed that Alkaloids, tannin, flavonoids, phytosterols, protein, cardiac glycosides, steroids, quinones, coumarins, and anthraquinones were present in all the four extracts such as ethanol and methanol extract. Phlobatannin was absent in ethanol extract and present in all other extract. Saponin was present in raw and aqueous extract and absent in ethanol and methanol extract. Reducing sugars was present only in ethanol extract and terpenoids were absent in all the experimental extracts that was analysed result has been represented in Table-1

Quantitative analysis of Phytochemicals: The current results of quantitative estimation of phytochemical compositions from the three different extracts showed that statistically significant level of phyto-constitution, foremost maximum amount has been noticed the phytocompounds named as tannins (28.04 ± 3.27) followed by glycosides (20.14 ± 1.05) alkaloids (19.21 ± 0.32), saponins (16.90 ± 1.22). Moreover, least level of quantity also been observed in Anthraquinone (12.08 ± 0.31) and Phlobatannins (11.51 ± 0.11) respectively. Among all the seven phytocompounds only two compounds are present in optimum level. Apart from the research clearly showed insignificantly reduced when the experimental sample mixed with other water methanol and ethanol solvents. The overall results of the phytochemical compositions depicted from the three experimental exudate of *Musa* contained supreme quantity as following orders such as tannins > glycosides > saponins were abundance in only raw *Musa* pseudo stem exudate than other experimental samples of methanol and ethanol along it mixed with water too. Though, Anthraquinone, Phlobatannins are more or less similar amount has been noticed in the same sample. However, significantly quite minimum quantities of phytochemicals also observed other three experimental solvent exudate extract when compared with raw exudate sample

Table 1 Qualitative analysis of phytochemicals in *Musa paradisiaca* L. pseudostem exudate fluid mixed with water and methanolic extract

SL.NO	PHYTOCHEMICAL ANALYSIS	Exudate fluid from <i>M. paradisiaca</i>		
		E	M	Exudate (original Stem juice)
1.	Test for Alkaloids	+	+	++
2.	Test for Phlobatannins	-	+	+
3.	Test for saponiin	-	-	+
4.	Test for terpenoids	-	-	-
5.	Test for tannin	+	+	+
6.	Test for flavanoids	+	+	++
7.	Test for phytosterols	+	+	+
8.	Test for protein	+	+	+
9.	Test for saponin	-	-	+
10.	Test for Reducing sugars	++	-	-
11.	Test for Cardiac glycosides	+	+	++
12.	Test for steroids	+	+	+
13.	Test for quinones	+	+	+
14.	Test for coumarins	+	+	+
15.	Test for anthraquinones	+	+	++

Present in high concentration, -Absent, +-Present in low concentration, ++- Present in moderate concentration, +++

Table- 2. Quantitative estimation of phytochemicals present in exudate fluid mixed with water, ethanol and methanolic extract (g / 100 g)

Sl.NO	Name of the phytochemicals	Expmtal sample of Musa pseudo stem exudate (µg/ml)	Expmtal sample mixed with water (µg/ml)	Expmtal sample mixed with ethanol (µg/ml)	Expmtal sample mixed with methanol (µg/ml)
1	Alkaloids	19.21±0.32	14.65±1.32	10.13±2.58	11.22±3.21
2	Anthraquinone	12.08±0.31*	08.11±0.02 ^{is}	07.32±01.00	06.57±1.43
3	Flavonoid	18.01±1.31	13.25±2.85*	10.22±1.56*	08.35±1.44 ^{is}
4	glycosides	20.14±1.05**	15.82±3.14	11.25±2.33*	11.07±2.62
5	Phlobatannins	11.51±0.11	07.40±0.11 ^{is}	08.73±2.76	10.37±2.33
6.	Tannins	28.04±3.27**	25.62±2.16**	19.31±2.92	18.55±1.26*
7.	Saponin	16.90±1.22*	12.15±3.57	10.15±3.67	10.18±0.64

** - Denotes Statistically significant at 5% level of significance

* - Denotes

Table- 3:- Antibacterial activity of *M. paradisiaca* exudate fluid against Selected urolithiatic disease supportive clinical Pathogenic Organisms

Sl. No.	Name of the microorganism	Zone of inhibition in average (mm in size)			
		Types of extract			Ciprofloxacin
		Negative control	Positive control (Exudate)	Ethanol mixed with Exudate Extract	
1.	<i>Bacillus subtilis</i> (MTCC 441)	-	0.9±0.00	1.04±0.05*	2.45
2.	<i>Klebsiella pneumoniae</i> (MTCC 4030)	-	0.4±0.00	1.5±0.1*	5.61
3.	<i>Vibrio cholerae</i> (MTCC 3906)	-	0.3±0.00	0.82±0.17*	4.13

4.	<i>Streptococcus pyogenes</i> (MTCC 442)	-	1.1±0.00	1.15±0.1*	3.47
5.	<i>Shigella flexneri</i> (MTCC 1457)	-	0.6±0.00	1.15±0.05*	3.25
6.	<i>Enterobacter aerogenes</i> (MTCC 2822)	-	0.5±0.00	1.31±0.1	4.42
7.	<i>Escherichia coli</i> (MTCC 443)	-	0.4±0.00	0.83±0.05 ^{ln}	3.24

** Ciprofloxacin disc (25 µg) as a positive reference standard; Values are mean inhibition zone(mm) ± S.D of three replicates, * denote as significant at 0.05% level of significance.

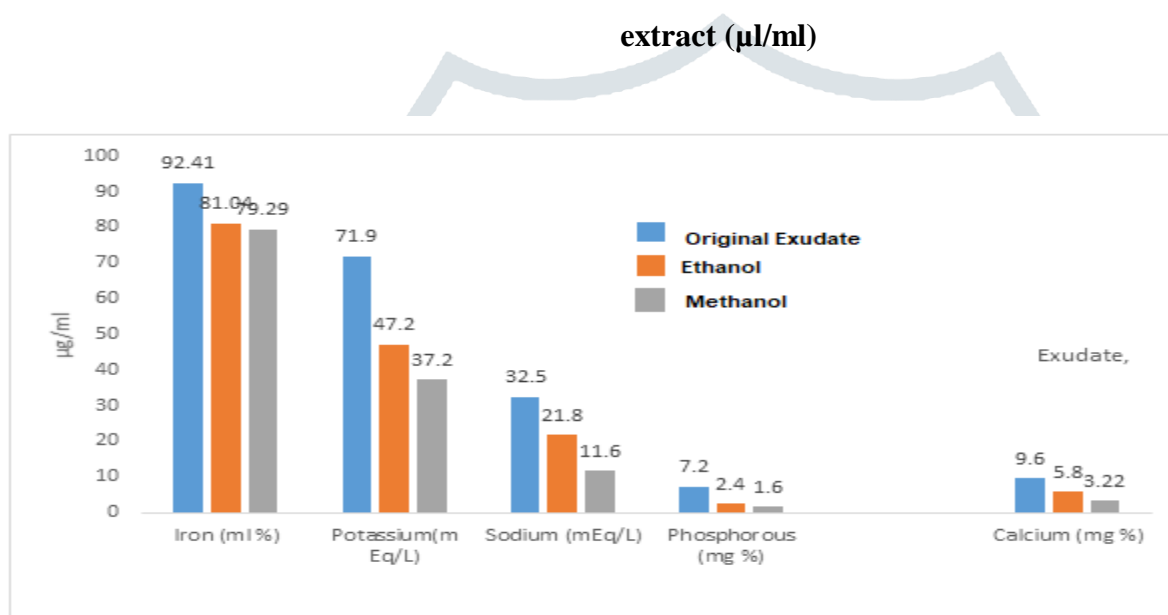
Antibacterial activity: Antibacterial activity of *Musa* exudate was analysed against urolithiatic disease supportive organisms. Seven organism were selected for the present study. From this study it was clearly identified that the exudate exhibited highest activity against *Enterobacter aerogenes* (MTCC 2822) (5.0±1.21; 4.5±0.00; 3.31±0.1) in original exudate ethanol extract and methanol extracts respectively. From the present result of antibacterial activity was clearly revealed that maximum zone of inhibition has been noticed against *S. pyogenes* followed by *B. subtilis* in original exudate. Though, minimum and optimum range of antibacterial response has been noticed in the same extract against *E. aerogenes*, *V. cholera* organisms. Moreover equal range of zone of inhibition also observed against *E. coli* and *K. pneumonia*. Similarly ethanol extract showed the maximum antibacterial activity was *K. pneumonia*

Pseudostem exudate has been providing a significant energy and nutritional requirements to human beings. The carbohydrates, proteins, and fats are the nutrients found in *Musa* plants, as well as minerals, play a vital role in creating a healthier organ control system in human beings. The analysis for five micro- and macro-elements in the plants indicated that these were present in an experimental samples which are responsible for curing different types of diseases.

Present result showed that the five different minerals were identified named as iron, potassium, sodium, phosphorous and calcium. Among the five minerals maximum (92.41) and minimum (9.60) percentage has been noticed in iron and calcium respectively. Moreover potassium and sodium were present as optimum and moderate

level. Though, phosphorous content was very low level observed in raw pseudostem exudate. Similarly other two solvent extracts also revealed that all the identified five minerals were significantly reduced when compare with the original without any mixture stem exudate. While compare between two solvent with exudate all the five minerals clearly showed that methanol solvent possessed two fold minimized the analysed minerals than the ethanol solvent. Apart from the present study significantly expressed the original pseudostem juice contains more accurate amount of minerals than when mixed with other solvents (Fig-2).

Fig-2: Quantitative Analysis of Minerals present in exudate fluid mixed with water, Ethanol and methanolic



M. paradisiaca Linn. Pseudostem exudate was highly nutritious mainly used for medicinal purpose in human society. Exudates are complex mixtures of organic compounds oozed by plants, often, but not always as a result of injury. These products are rich in carbon and hydrogen atoms and are also commonly called “sap” although the word “sap” is used to describe any fluid that travels inside plants. In contrast, the word “exudate” refers to any such material when it is oozed out of the plant. Medicinal plants have attracted considerable global interest in recent years, *M. paradisiaca* is a medicinal plant with diverse pharmacological activities. The main pharmacological activities of this plant are typically potential traditional medicine against the disease of antiurolithiatic or kidney stone diseases. The results show that the stem extrudes of *Musa paradisiaca* contains tannins and glycosides in abundance followed by alkaloids, saponins, flavonoids, polyphenols and reducing sugars were present in moderate quantities while reducing sugars and terpenoids were absent in original exudate of pseudostem of *M. paradisiaca* tree. Phytochemicals are known to occur in various parts of plants with diverse functions which include provision

of strength to plants, attraction of insects for pollination and feeding, defence against predators, provision of colour, while some are simply waste products (13) When ingested by animals these secondary metabolites exhibit varied biochemical and pharmacological actions depends with different phytochemicals and its nutritional and toxic effects including reduced feed intake, growth, feed efficiency and net metabolizable energy (14-15). The plants are characterized by the different characteristics. Quantitative estimation of the phytochemicals revealed that tannins are the most abundant in all the extract 28.04 ± 3.27 in musa pseudostem, 25.62 ± 2.16 in aqueous extract, 19.31 ± 2.92 in ethanol extract and 18.55 ± 1.26 in methanol extract.

Previously, ¹⁶ reported that, saponins possess antimicrobial properties and could function as precursors of several steroidal substances with diverse physiological roles. The discovery of saponins in the peels extract of plantain also renders it ideal for utilization in the preparation of medicinal soap given that saponin could function as a forming agent. Refreshingly, plantain peels seem to be a promising source of nutrients for animal feeds production.¹⁷ Tannins in plants have been shown to confer antidiarrhoeic and anti-haemorrhagic properties on plants.¹⁸ This is consistent with the traditional use of the sap of *M. paradisiaca* for the treatment of diarrhoea, fresh wounds, cuts and insect bites. Saponins have been reported to have antifungal properties.¹⁹ as well as serve as an expectorant and emulsifying agent.²⁰ Moreover, alkaloids, flavonoids and tannins have been known to show medicinal activity as well as exhibiting physiological activity.²¹ Flavonoids are known to have antioxidant effects and have been shown to inhibit the initiation, promotion and progression of tumors.²² The presence of these phytochemicals in the sap of *M. paradisiaca* confers medicinal properties on the plant and this explains the use of this plant for treatment of different ailments. The findings of this study is consistent with reports of the presence of these phytochemicals in various parts of the *Musa paradisiaca* plant as documented.³ The result of phytochemical assay indicated that the exudate of *M. paradisiaca* L. pseudostem contained various types of phytochemical active compounds including alkaloids, flavanoids, anthraquinone, cardiac glycoside, and phlobatannins (Table-1) similar findings also been agreed by several authors.^{23,6,9} The obtained result of quantitative phytochemicals in raw original exudates possessed maximum quantity, the same observations also been agreed with other studies^{21,24,12}. Previously, reported that the exudate of a plantain (*Musa* spp) stem contained total phenol and tannin.²⁵

In this regard, *M. paradisiaca*, commonly known as plantain was studied to extract the active components of the stem exudate carry out phytochemical screening of the crude extract and identify the secondary metabolites present.²⁶ Bioactive compounds found to be present in this plant include alkaloids, saponins, tannins, cardiac glycosides, terpenes, deoxy sugar, flavonoids and carbohydrates as presented in Table-3. The current results were in agreement with the other findings of where pseudostem banana extract shows an antibacterial activity against *P. aeruginosa* and *S. aureus*.²⁶ Several studies reported that flavonoids are responsible for the antimicrobial activity. Additionally aqueous, ethanolic, and methanolic extracts of *M. paradisiaca* L pseudostems against some pathogenic fungi too portrayed.²⁸

The results of the antibacterial activity of *M. paradisiaca* L. pseudostem exudate against the test organisms particularly urolithiatic disease causative pathogens. The obtained present results also been agreed with other researchers (24,6)pharmacological activity against Gr^{+ve} in a dose-dependent manner such a measurable value in the form of zone of inhibition remained found to be minimum to maximum result was noticed against *S. aureus* and *E. coli* exudate respectively. *P. aeruginosa* and *L. acidophilus* were not inhibited with low concentrations. However, Ahmad and Beg reported that alcoholic extract of *M. paradisiaca* stem showed no activity against *S. aureus*, *S. paratyphi*, *S. dysenteriae*, *E. coli*, *B. subtilis*, and *C. albicans* ²⁹.

CONCLUSION

The current study was designed to evaluate the therapeutic benefits of *Musa paradisiaca* L. pseudostem exudate including investigate the phytochemical activity both qualitatively and quantitatively, Quantitative Analysis of Minerals present in exudate fluid, and antibacterial activities against the important clinical pathogens in vitro. Fifteen phytochemicals were analysed, and the result showed that Alkaloids, tannin, flavonoids, phytosterols, protein, cardiac glycosides, steroids, quinones, coumarins, and anthraquinones were present in all the four extracts such as ethanol and methanol extract. Apart from the present result, it was clearly showed the overall results depicted from the three experimental exudate of *Musa* contained supreme quantity of phyto-compounds possessed as the following orders such as tannins > glycosides > saponins were abundance in only raw *Musa* pseudo stem exudate than other experimental samples of methanol and ethanol along with mixed with water too. Moreover, Seven organism were selected for the present study. From this study it was clearly identified that the exudate

exhibited highest antibacterial activity against the *E. aerogens* (MTCC 2822) (5.0 ± 1.21 ; 4.5 ± 0.00 ; 3.31 ± 0.1) in original exudate ethanol extract and methanol extracts respectively. In the present research clearly indicated that raw freshly taken *M. paradisiacca* exudates are possessed medicinal property particularly urolithaiatic causative agents. Hence its remained a better natural and potential medicinal potential against kidney stone diseases

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