PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *MUSA ACUMINATA* EXTRACTS ON CERTAIN BACTERIAL PATHOGENS

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Abstract: Banana belongs to the family *Musaceae*, is one of the most popularly consumed fruits in the world. The present study was performed to evaluate the phytochemical potentials and antimicrobial activities of bract and pseudostem of *Musa acuminata* (Nenthran) against bacterial pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Solvents such as acetone, chloroform, ethanol and water were used for extraction of bract of inflorescence whereas fresh juice extract of pseudostem were used for antibacterial assay by well diffusion and disc diffusion methods. Significant activity was shown by all the extracts against the tested pathogens, whereas the phytochemical analysis of chloroform extracts of bract revealed the presence of saponins, tannins, coumarins, cycloglycosides, terpenoids, etc. This study demonstrated the effectiveness of *Musa acuminata* bract and pseudostem extracts against pathogenic bacteria with a conclusion that they can be used as a source of new antibacterial agents.

Index Terms: ***Musa acuminata*, Nenthran, bract, inflorescence, bacterial infections, antimicrobial activity, phytochemical screening.**

1. INTRODUCTION:

Nowadays, infectious diseases remain the major cause of mortality, accounting approximately one-half of all the deaths in tropical countries (Avery, 2006 and Tekwu *et al*., 2012). Prevalence of infectious diseases caused by bacteria is a major problem of global concern (Hamer *et al*., 2010 and Khan *et al*., 2013). In recent years, the emergence of multidrug resistant pathogenic bacteria has been drastically reported all over the world (Robin *et al*., 1998). This has been necessitated a quest for new antibacterial agents from other sources, and thereby, turning the attention of researchers on herbal medicines that leads to the development of better drugs against microbial infections (Bandow *et al.*, 2003). India is the largest producer of medicinal plants and has a rich heritage of knowledge on herbal products derived from these plants and their application to treat various ailments (Modak *et al.*, 2007 and Patra *et al.*, 2010). Many reports are available on the antimicrobial activity of plant extracts on human pathogenic bacteria (Babu *et al*., 2007). Plants produce certain chemicals known as phytochemicals which have protective or disease preventive properties (Kumar *et al*., 2009). Recent researches demonstrate that many phytochemicals can protect humans from diseases (Mamta *et al*., 2013).

Banana plant is the largest herbaceous flowering plant. Banana belongs to the family *Musaceae*. Its binomial name is *Musa acuminata* (Onyema *et al.*, 2016). Botanical classification of banana or "Nenthrapazham" is as follows:

Kingdom: *Plantae*
Division: *Magnoliophyta*
Class: *Liliopsida*
Order: *Zingiberales*
Family: *Musaceae*
Genus: *Musa*
Species: *acuminata*.

Musa acuminata grows to a height of six meters, but are perennial herbaceous plants with hard fibrous pseudostem composed of overlapping leaf bases. Leaves are large, arranged spirally around the stem*. Inflorescence emerges from the top of the pseudostem, large, horizontal or deflexed. Bracts of the inflorescence are red to dark purple, with both male and female flowers. Fruits are green but become yellow when fully ripe*. Banana plant is not only renowned for its nutritive values, but is also known for its medicinal uses. Apart from the banana fruit, all other parts of the banana tree have medicinal properties beneficial to mankind (Marikkar *et al.*, 2016). Banana is rich in potassium, calcium, iron and vitamins A, B, C and D. The roots, peel ashes, leaves and seed mucilage also serve medicinal purposes in some regions and cultures. The extracts of core of the stem are found to be useful in dissolving the stones in the kidney and urinary bladder and reducing the weight. To flush the urinary blocks, the inflorescence mixed with coconut oil and spices is used. Banana flower is traditionally used to treat many illnesses such as heart pain, diarrhea, asthma and stomach cramps (Sumathy *et al.*, 2011). Banana flowers are used as vegetables in several

cuisines, while bracts are used as food for cattles. Banana flowers are considered to be a good source of vitamin A and C, and are used in treatment of bronchitis, dysentery, constipation, ulcers and good for diabetics. It is traditionally believed to be beneficial as a lactating agent and helps to relieve painful menstruation. Traditionally leaves of banana plant are used to serve food and for wrapping food. The banana pseudostem can be utilized for its fiber. The pulp of the plant could be used to manufacture ropes, papers, place mats and other goods, but the pseudostem is often chopped and left in fields for its organic matter content (Sheng *et al*., 2010 and Sampath *et al*., 2012).

This research work was carried out to investigate the phytochemical potentials and antimicrobial activities of bract of inflorescence and pseudostem of *Musa acuminata*.

2. MATERIALS AND METHODS:

2.1. Collection and preparation of plant material:

Fresh, healthy inflorescence and pseudostem of *Musa acuminata* were collected from Parassala, Trivandrum district, South Kerala. The bracts from the inflorescence of *Musa acuminata* were separated and washed in water to make them free from dust, dried at 50°C for 48 hours in hot air oven and were coarsely powdered using an electric blender, sieved and stored in air tight polythene bags at 4°C for further analysis.

The pseudostem of *M. acuminata* was washed in water, then chopped into pieces, grinded and the juice extract obtained was filtered through Whatmann No.1 filter paper. The filtrate obtained was collected and used for further studies.

2.2. Solvent extraction:

10 gm of the powdered bract were taken separately and extracted using 50 ml of distilled water, ethanol, chloroform and acetone. After two days of storage at room temperature, the contents were stirred well and filtered using Whatmann no: 1 filter paper. The filtrates obtained were evaporated using water bath, labelled and stored in refrigerator at 4°C for further studies.

2.3. Collection of test organisms:

Pure cultures of pathogenic bacteria were obtained from the stock culture of our microbiology laboratory. The bacterial isolates include *Staphylococcus aureus, Streptococcus pyogenes*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The test organisms were streaked on selective media to confirm their identity. The pathogens were sub cultured onto nutrient agar slants and then stored at 4°C until when required for use.

2.4. Antimicrobial activity assay:

2.4.1. Well diffusion method:

The antibacterial activity of bract of inflorescence and pseudostem of *Musa acuminata* was evaluated by agar well diffusion method. 24 hours grown bacterial cultures were swabbed on the Mueller Hinton agar plates using sterilized cotton swabs. 6mm diameter wells were bored over the agar plates and 50µl of each extract of *M. acuminata* were loaded into the wells. The plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for zones of growth inhibition and the results were recorded (Umamaheswari *et al*., 2017).

2.4.2. Disc diffusion method:

The antibacterial activity of bract of inflorescence and pseudostem of *Musa acuminata* was evaluated by disc diffusion method 24 hours grown bacterial cultures were swabbed on the Mueller Hinton agar plates using sterilized cotton swabs. 50 µl of extract loaded filter paper discs were placed aseptically on the agar plates and the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for zones of growth inhibition around the discs and the results were recorded (Senthil Kumar and Parameshwari, 2017).

2.5. Phytochemical Analysis:

The extracts of *M. acuminata* were analyzed for the presence of phytochemicals using standard procedures (Gunavathy *et al*., 2014).

2.5.1. Detection of carbohydrates:

To a small quantity of the extract, 2 ml of Molish's reagent and 2 ml of concentrated sulphuric acid was added. Formation of a reddish ring indicated the presence of carbohydrates.

2.5.2. Detection of alkaloids:

A little of the extract was stirred with Mayer's reagent. Formation of cream colored precipitate indicated the presence of alkaloids.

2.5.3. Detection of saponins:

About 2 ml of the extract was diluted with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. The presence of saponins was indicated by the formation of layer of foam.

2.5.4. Detection of tannins:

To 2 ml of the extract, few drops of 1% lead acetate were added and the formation of yellowish precipitate indicated the presence of tannins.

2.5.5. Detection of flavonoids:

A small quantity of the extract was treated with dilute sulphuric acid. The appearance of orange color indicated the presence of flavonoids.

2.5.6. Detection of terpenoids:

To 2 ml of extract, 2 ml of acetic acid and sulphuric acid were added. Formation of bluish green ring indicated the presence of terpenoids.

2.5.7. Detection of phlobotannins:

2 ml of the extract was boiled with 1% hydrochloric acid. Deposition of red precipitate indicated the presence of phlobotannins.

2.5.8. Detection of coumarins:

3 ml of 10% sodium hydroxide was added to 2 ml of extract. Formation of yellow color indicated the presence of coumarins.

2.5.9. Detection of cycloglycosides:

To 5 ml of extract, 2 ml of acetic acid, 1 drop of 1% ferric chloride and 1 ml of sulphuric acid was added. Formation of greenish ring indicated the presence of cycloglycosides.

2.5.10. Detection of phenol:

To a small quantity of the extract, 3-4 drops of ferric chloride solution was added. Formation of deep blue color indicated the presence of phenol.

2.5.11. Detection of quinones:

The extract with 5 ml of hydrochloric acid resulted in yellow precipitate, indicating the presence of quinones.

2.5.12. Detection of anthraquinones:

2 ml of the extract was treated with 2 ml of 10% ammonium hydroxide solution. Formation of pink color indicated the presence of anthraquinones.

2.5.13. Detection of steroids:

2 ml of the extract was dissolved in 2 ml of chloroform. To this equal volume of acetic acid and concentrated sulphuric acid was added by the sides of test tube the formation of bluish green indicated the presence of steroids.

3. RESULT AND DISCUSSION:

The antimicrobial activity of bract of inflorescence of *M. acuminata* against four bacterial pathogens was evaluated by agar well diffusion and disc diffusion methods and the results were recorded on table 1 and 2.

a) **Agar well diffusion method**: Ethanolic extract of bract showed higher activity against test organisms such as *Staphylococcus aureus* and *Streptococcus pyogenes* with an inhibition zone of 22 mm and 17 mm. Acetone extract showed significant activity against *Staphylococcus aureus* and *Streptococcus pyogenes* with an inhibition zone of 14 mm and 12 mm, respectively. Chloroform extract showed significant activity against *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with an inhibition zone of 11 mm, 12 mm and 10 mm, respectively.

b) **Disc diffusion method**: Ethanolic extract of bract showed significant activity against *Staphylococcus aureus* with an inhibition zone of 12 mm. Acetone extract showed significant activity against *Staphylococcus aureus* and *Streptococcus pyogenes* with an inhibition zone of 12 mm and 11 mm, respectively. Chloroform extract showed significant activity against three test pathogens such as *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with an inhibition zone of 10 mm, 11 mm and 9 mm, respectively.

Distilled water extracts showed no activity against any of the test pathogens in both agar well diffusion and disc diffusion methods. The antimicrobial activity of pseudostem juice extract of *M. acuminata* against four bacterial pathogens was evaluated by agar well diffusion method and disc diffusion methods, and the results were recorded on table 3 and table 4. Significant activity was showed by pseudostem juice extracts in well diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* with an inhibition zone of 14 mm, 11 mm and 10mm, respectively whereas no activity was shown against *Klebsiella pneumoniae*. The pseudostem juice extracts in disc diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* exhibited an inhibition zone of 10 mm, 9 mm and 8 mm, respectively whereas no activity was shown against *Klebsiella pneumoniae*.

The phytochemical analysis of chloroform extracts of bract of *M. acuminata* by adopting various chemical tests revealed the presence of phytochemicals such as saponins, tannins, terpenoids, coumarins, cycloglycosides, quinones and steroids. The results of this study showed the bract and pseudostem of *M. acuminata* has a broad spectrum of activity against tested pathogens. This may be due to the presence of phytochemicals present in them.

Table 1: Antimicrobial activity of *Musa acuminata* bract by well diffusion method

Sl.no:	Extract	Zone of Inhibition formed against test organism in diameter			
		***Streptococcus* sp.**	***Staphylococcus* sp.**	***Klebsiella* sp.**	***Pseudomonas* sp.**
01.	Acetone	12 mm	14 mm	-	-
02.	Chloroform	-	11 mm	12 mm	9 mm
03.	Ethanol	17 mm	22 mm	-	-
04.	Dis. water	-	-	-	-

Table 2: Antimicrobial activity of *Musa acuminata* bract by disc diffusion method

Sl.no:	Extract	Zone of Inhibition formed against test organism in diameter			
		***Streptococcus* sp.**	***Staphylococcus* sp.**	***Klebsiella* sp.**	***Pseudomonas* sp.**
1.	Acetone	11 mm	12 mm	-	-
2.	Chloroform	-	10 mm	11 mm	9 mm
3.	Ethanol	-	12 mm	-	-
4.	Dis. water	-	-	-	-

Table 3: Antimicrobial activity of pseudostem of *Musa acuminata* by well diffusion method

Sl.no:	Extract	Zone of Inhibition formed against test organism in diameter			
		***Streptococcus* sp.**	***Staphylococcus* sp.**	***Klebsiella* sp.**	***Pseudomonas* sp.**
1.	Juice extract	10 mm	14 mm	-	11 mm

Table 4: Antimicrobial activity of pseudostem of *Musa acuminata* by disc diffusion method

Sl.no:	Extract	Zone of Inhibition formed against test organism in diameter			
		***Streptococcus* sp.**	***Staphylococcus* sp.**	***Klebsiella* sp.**	***Pseudomonas* sp.**
1.	Juice extract	8 mm	10 mm	-	9 mm

4. CONCLUSION:

Banana is popularly called as “Kalpataru” because of the multiple uses of all parts of it. It is used as an alternative medicine in Ayurveda, and other traditional medicines throughout the world. This study showed the efficient activity of bract and pseudostem extracts of *Musa acuminata* against many pathogens. Our study also reveals the presence of many phytochemicals in *Musa acuminata* bract extracts, which may contribute its therapeutic potentials. Thus the study supported the traditional use of *Musa acuminata* against various ailments. More studies should be performed to characterize the phytochemicals present in them which will provide an alternative to modern medicines in future.

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