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An *in-vitro* studies on the primary phytochemical estimation and antibacterial potentiality of the peel extract of *Musa acuminata* against *Pseudomonas aeruginosa*

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Abstract
By this research work, we know the phytochemical analysis and antibacterial activity of the peel extract of *Musa acuminata*. For this studies, ethanol, methanol and aqueous solvent of extraction are used. This work was done by using ager well diffusion method and we know the antibacterial activity of the banana peel extract. Four concentrations are used for this study, these are 12.5 mg/ml, 25mg/ml, 50mg/ml, and 100mg/ml. Escherichia *coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterititis* of these test microorganisms are used in this study and by which we know the antibacterial activity of the peel extract of *Musa acuminata.* To study the phytochemical analysis of the peel extract of *Musa acuminata*, flavonoids, terpenoids, quinines and alkaloids are present in ethanol, methanol and aqueous extracts. So, phytochemical screenings are revealed. From the result, we know that banana peels shows the antibacterial activity against this test microorganisms. Banana peels extract play a great importance role in public health. The yellow banana peel shows a good antibacterial activity against Gram (+) and Gram (-) and replace the synthetic medicine in this diseases caused by this bacteria. From the result, we can see that *Pseudomonas aeruginosa* shows the maximum zone of inhibition (31.7±2.0) in the highest concentration of 100 mg/ml concentration. *Staphylococcus aureus* shows the minimum zone of inhibition (25.0±2.0) in 100 mg/ml concentration of ethanol extract. In lowest concentration (12.5 mg /ml), *Bacillus subtilis* shows the maximum zone of inhibition (19.0±2.0) and *Staphylococcus aureus* shows the minimum zone of inhibition (15.5+-2.0) in case of ethanol extract.

Keywords: phytochemicals, antibacterial, *Musa acuminata*, banana peel

Introduction
Musa acuminate (banana) is a most important food crop in our country. It is developed in various countries and grown in 122 countries. It is available in our country. Banana peel extract contain in many types of vitamin *viz* -vitamin B6, vitamin C, vitamin E and malic acid. Mainly peel is the waste part of various fruit. But the banana peel have some antibacterial activity. In case of commercial application, peels might be due to their unknown benefit. The potential application of banana peel depends on it's chemical composition. Fatty acids are present in the banana peel extract and it responsible for the antibacterial activity of the *Musaacuminata.* For this study, *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteritidis* of this bacterial culture are used and to know the antibacterial activity. Methanol, ethanol and aqueous solvents are used.100 mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentration are done in aqueous, ethanol and methanol extract and shows the zone of inhibition in each extracts.

Fig 1: *Musa acuminate plant*

Material and method

Collection of plant material

We *are* selected the fruit *Musa acuminata* for this study to know the antibacterial activity and phytochemical analysis. The fruit *Musa acuminata* was collected from our garden beside our home at Kotalpara village. This specimen was identified in our Microbiology Department, Champadanga, Hooghly. After identified this fruit, peels are collected from the banana fruit.

Fig 2: Musa acuminata peels extract

Preparation of the peel extract

At first, the collecting peels are washed in well with the hot water and cold water. Then it is dried in air. The dried peels are grinded in the blender machine. The mixture is collected in a closed jar for long storage.

Preparation of aqueous extract

5g of peels powder is added in the 25 ml of distilled water and made aqueous solution. This solution is made in a conical flask. The conical flask is bounded with tissue paper and rubber band. Then, some pore are done in the surface of the tissue paper which that air are passes. The solutions are kept in room temperature at 37^0C for 24 hours.

Preparation of ethanol extract

5g of peels powder is added in 25 ml of ethanol and made ethanol extract. This extract is made in a conical flask. The conical flask is bound with tissue paper and rubber band. Some pores are done for air passes. Then it is kept in 37^0C room temperature for 24 hours.

Preparation of methanol extract

5g of peel powder is added in the 25 ml of methanol in a conical flask. The conical flask is covered with the tissue paper and rubber band. On their surfaces some pore are done for air passing. Then this flask is kept in room temperature at 37^0C for 24 hours.

Collection of test microorganisms

The test microorganisms are *Bacillus subtitles (MTCC-441), Staphylococcus aureus* (MTCC-737), *Klebsiella pneumonia* (MTCC-432), *Pseudomonas aeruginosa* (MTCC-424), *Escherichia coli* (MTCC-443), *Salmonella enterititis* (MTCC -98) are collected. Those bacteria are grows on Muller Hinton ager and nutrients agar.

Preparation of different concentration

100 mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentration are used for this study.1g of peel mixture is measured and it added in 10 ml of DMSO and made 100 mg/ml concentration. This is called stock solution. For 50 mg /ml concentration,1 ml stock solution and 1 ml of DMSO are taken in a appendops tube.0.50 ml stock solution is added in 1.50 ml DMSO and prepare 25 mg/ml concentration. For 12.5 mg/ml concentration,0.25 ml stock is added with 1.75 ml DMSO. Each concentration are kept in a 2ml volume of appendops tube.6 nutrients ager plate are prepared and then it is inoculated with bacterial culture.3 plates are taken for 1 bacterial culture and another 3 plates are taken for another bacterial culture. Well are done in each ager plate with the borar.100 mg/ml and 12.5 mg/ml concentration are marked in two separate inoculated plate and given the concentration in the well from the particular tube. Another two inoculated plate are taken and then it is marked with 50 mg/ml and 25 mg/ml concentration and given concentration in the well from the particular tube. So, four plates are inoculated and given concentration. DMSO is given in another two plate.

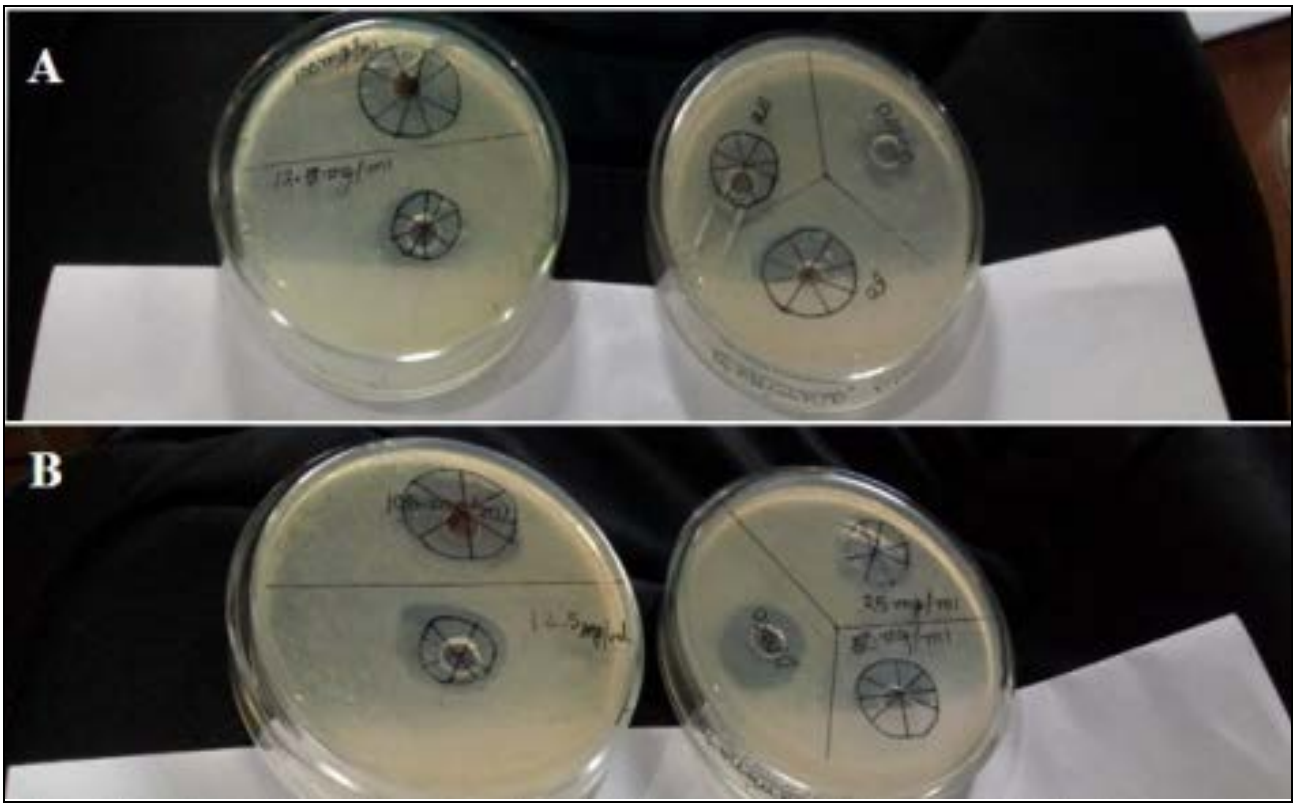

Fig 3: A. Antibacterial activity of agueous extract on *Klebiella pneumoniae* b. Antibacterial activity agueous extract on *Pseudomonas aeruginosa*

Result

Result obtained from this research work are summarized in the tables below.

Table 1: Antibacterial activity of the *Musa acuminata*in different bacteria for aqueous extract in different concentration

Test microorganism	Concentration(mg/ml)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Escherichia Coli	27.25±2.0	22.25±2.0	18.25±2.0	17.25±2.0
Bacillus Subtilis	28.5±3.0	27.25±2.0	21.25±2.0	18.5±3.0
Staphylococcus aureus	36.1±3.0	27.4±3.0	23.7±2.0	20.0±2.0
Klebsiellapneumoniae	32.3±2.0	28.2±3.0	23.0±2.0	19.5±2.0
Pseudomonas aeruginosa	34.5±2.0	31.7±2.0	26.0±2.0	21.2±3.0
Salmonella enterotitis	29.0±2.0	26.2±3.0	23.1±2.0	19.4±3.0

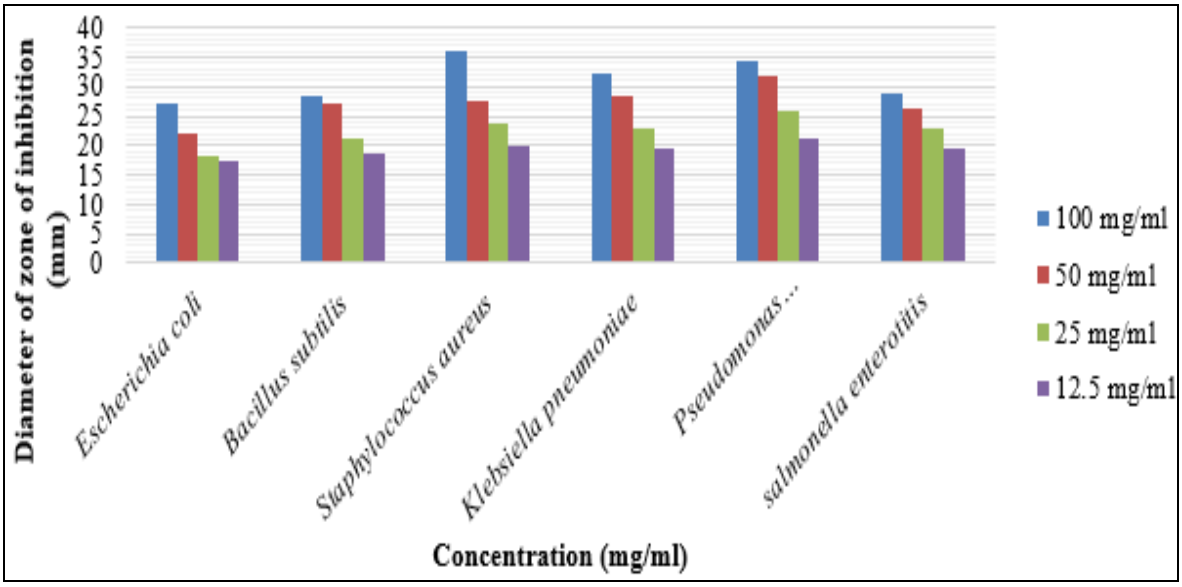

Fig 4

The antibacterial activity of *Musa acuminata* shows the highest zone of inhibition against Pseudomonas aeruginosa and the lowest zone of inhibition against Staphylococcus aureus.

Table 2: The antibacterial activity (inhibition zones in cm) of the peel extract of musa acuminate against different bactria for ethanolic extract in different concentration

Test microorganism	Concentration(mg/ml)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Escherichia Coli	31.6±3.0	27.5±2.0	23.0±2.0	18.2±2.0
Bacillus Subtilis	33.5±2.0	30.1±2.0	26.4±2.0	21.7±2.0
Staphylococcus aureus	29.4±2.0	25.7±2.0	21.3±2.0	18.0±2.0
Klebsiellapneumoniae	32.9±2.0	30.7±3.0	26.1±2.0	21.6±2.0
Pseudomonas aeruginosa	27.6±2.0	22.4±2.0	20.4±2.0	17.5±2.0
Salmonella enteritidis	30.0±2.0	27.4±3.0	24.5±2.0	18.7±3.0

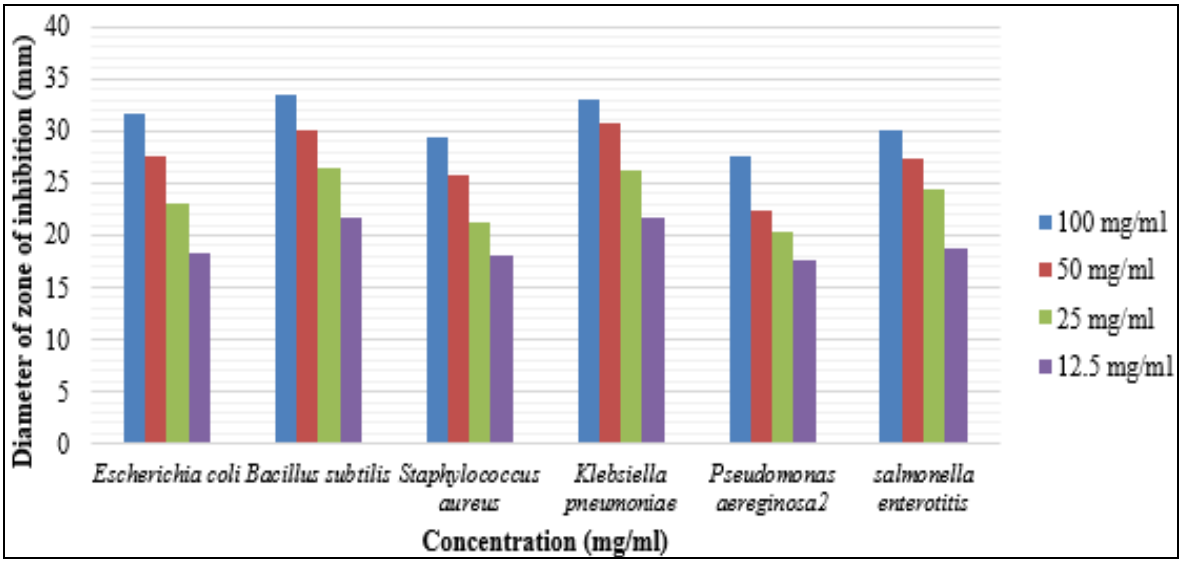

Fig 5

Pseudomonas aeruginosa shows the maximum zone of inhibition and *Escherichi coli* shows the minimum zone of inhibition.

Table 3: The antibacterial activity (inhibition zones in cm) of the peel extract of musa acuminate against different bactria for methanolic extract in different concentration

Test microorganism	Concentration(mg/ml)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Escherichia coli	26.1±2.0	22.3±2.0	20.4±2.0	16.6±2.0
Bacillus Subtilis	29.3±2.0	26.7±2.0	23.4±3.0	19.0±2.0
Staphylococcus aureus	25.0±2.0	21.2±2.0	18.7±2.0	15.5±2.0
Klebsiellapneumoniae	29.4±2.0	25.0±2.0	21.5±2.0	16.2±2.0
Pseudomonas aeruginosa	31.7±2.0	24.5±2.0	20.3±3.0	17.0±2.0
Salmonella enteritidis	28.5±2.0	25.9±3.0	21.4±2.0	18.7±3.0

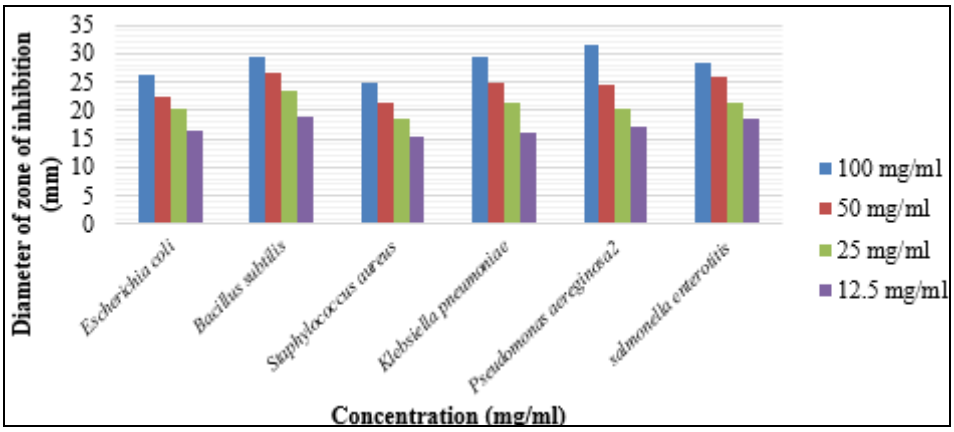

Fig 6

Pseudomonus aeruginosa shows the maximum zone of inhibition (31.7 ±2.0) and *Staphylococcus aureus* shows the minimum zone of inhibition (15.5±2.0).

Discussion

Banana is cheap fruit and readily available that is consumed by different people around the world. Because it have high nutritional properties. In recent time, it had been reported that this peels are not altogether useless as many of the bioactive plant components reside in them. Therefore, the project work was done to ascertain the antimicrobial efficacy of banana peels against clinical isolates. From the phytochemicals analysis of ethanol and aqueous extracts of banana peel, we know that flavonoids, terpenoids, quinines and alkaloids are present in both ethanol and aqueous solvents, and tannins, saponins are absent in both solvents. Flavonoids are known as effective antimicrobial substance against a wide array of microorganisms. It is synthesized by plant in response to microbial attack. Their activity is probably due to their ability to react with extracellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria. Tannins have various physiological effects like anti-irritant, antimicrobial and antiparasitic effects. Tannins containing plant are used for treat nonspecific diarrhoea, inflammation of mouth and throat and slightly injured skins. From this research work, we are found that which bacteria have a good antibacterial activity. We are followed that *Staphylococcus aureus* is a susceptible bacteria species and it's zones of inhibition is 36.1+-3 in 100 mg/ml for aqueous extract. So,Lt shows a good antibacterial activity in highest concentration for aqueous extract. And lowest concentration (12.5 mg/ml) the zone of inhibition is 20.0+-2. *Pseudomonas aeruginosa* and *Klebsiellapneumoniae* is very sensitive in aqueous extract. In case of ethanol extract, *Bacillus subtilis* show a good antibacterial activity in highest concentration. So, the range of zone inhibition is 33.5+-2.0 in 100 mg/ml concentration. *Klebsiellapneoniae* and *Escherichia coli* is very sensitive in ethanol extract and range of zone of inhibition are 32.9+-2.0 and 31.6+-3.0 in 100 concentration. In methanolic extract, *Pseudomonas aeruginosa* the range of zone inhibition is 31.7+-2.0 in 100 mg/ml. So, it shows a good antibacterial activity. *Bacillus subtilis a*nd *Klebsiellapneumoniae*is very sensitive in methanol extract. The zone of inhibition are 29.3+-2.0 and 29.4 +-2.0 in 100 mg/ml.

Phytochemicals estimation

The peel extract of *Musa acuminata* were analysed for alkanoids, tannins, glycosides, steroids, flavonoids, saponins, votatile oil and resins using standard procedures.

Test for Glycosides

To 1ml of extract was taken, then it is mixed with 2 ml of acetic acid and then cooled in an ice bath at 4℃.1ml of sulfuric acid is added in drop wise to this mixture. An oillayer formation is seen on top of the solution that is indicated the presence of glycosides.

Test for alkaloids

1ml of 1% HCL was added in 3ml of the extract. Then, the mixture is treated with few drop's of Meyer's reagent. If a creamy white precipitation is appeared so, we can identified the presence of alkaloids.

Test for saponins

5 drops of olive oil was mixed in 2 ml of plant extract and this mixture shaken vigorously. A stable emulsion is formed that indicates the presence of saponins.

Test for tannins

2 drops of 5% ferric chloride was added in 1 ml of the plant extract. A dirty -green precipitate is appeared which indicated the presence of tannins.

Test for flavonoids

To 1 ml of the extract was mixed in 3 drops of ammonia solution followed by 0.5 ml of concentrated HCL.A pale brown colour is formed in the mixture that indicated the presence of flavonoids.

Test for steroids

1ml of concentrated tetraoxo sulphate (vi) acid was added in 1ml of the plant extract. A red colouration is formed that confirmed the presence of steroids.

Test for Resins

To 5ml of the extract was added in 5 ml of copper acetate solution. The mixture was shaken vigorously and allowed to separate. A reddish-brown precipitation is made and it indicates the presence of resins.

Table 4: Phytochemical analysis of ethanol and aqueous extracts of *Musa acuminata* peel

Phytochemicals	Solvents		
	Ethanol	Aqueous	Methanol
Flavonoids	+	+	+
Tannins	-	-	-
Terpenoids	+	+	+
Saporins	-	-	+
Quinines	+	+	-
Alkaloids	+	+	+

Conclusion

In case of the ethanolic and aqueous extract of banana peels, the antibacterial properties have found to be considerably high in this research work. The test organism were highly resistant antibiotics and they were found to susceptible in the banana peels extract. A yellow banana peels have an good antibacterial activity against both gram (+) and gram (-) and it is also known as good antibacterial agent. We are concluded that banana peel have a good anti bacterial activity in public health Musa acuminata the wild species of banana is a plant of the tropical and subtropical regions. All parts of the plant including fruit, peels, leaves, roots are used in the treatment of many disease in traditional medicine. The pharma cologicable activities of musaacuminata include antioxidant, antidiabetic, anticancer and antimicrobial especially anti-HIV activity. From phytochemicals study, we can know the traditional use of different parts of musaacuminata in various diseases.

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