Res. Jr. of Agril. Sci. **11**(5): 1121-1127, Sept-Oct 2020

ISSN: 0976-1675

www.rjas.org

Research Journal of Agricultural Sciences

© Centre for Advanced Research in Agricultural Sciences

Research Paper

DI: 6200-0106-216

Phytochemical Analysis and Antimicrobial Activity of *Musa acuminata* in Methanol Extract

R Kowsalya*[1] and V Swetha[2]

[1]Department of Biochemistry,
D. G. Government Arts College for Women, Mayiladuthurai - 609 001, Tamil Nadu, India
[2]Department of Biochemistry, Government Arts College for Women, Krishnagiri, Tamil Nadu, India

Received: 01 June 2020; Revised accepted: 16 July 2020

A B S T R A C T

Banana is used widely because of its nutritional values. In the past, some studies show yellow banana peel (*Musa acuminata*) parts, and their fruits can be used to treat human diseases. Banana is a part of banana peel that also has antibacterial activity and antifungal activity against microorganisms but has not been studied extensively. Since, there are studies that relate the phytochemical analysis of qualitative, quantitative analysis, and microbial activity of antibacterial, antifungal activity of yellow banana peel against periodontal pathogens. Hence, this study aims to determine the phytochemical analysis and antimicrobial activity in methanol extract of yellow banana peel (*Musa acuminata*). The phytochemical result showed methanol to be a better solvent for the extraction of the bioactive agents in banana peels which include: alkaloids, flavonoids, saponins, tannins carbohydrate are (+) reaction but phenol and protein are (-) reaction. Antimicrobial activity both are activated against the gram-positive, gram-negative bacterial but maximum activity of antifungal.

Key words: *Musa acuminate,* Antimicrobial activity, Yellow banana peel, Methanol extract

All parts of the banana plant have medicinal applications (Amit and Shailandra 2006), the flowers in bronchitis and dysentery and on ulcers; cooked flowers are given to diabetics, fevers, hemorrhages, acute dysentery, and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites; young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and the leaves are taken in dysentery and diarrhea and used for treating malignant ulcers (Girish and Satish 2008); the roots are administered in digestive disorders, dysentery, and other ailments; banana seed mucilage is given in cases of diarrhea in India (Bhat *et al*. 2010).

Yellow banana peels (*Musa acuminata)* are not poisonous. They're edible and packed with nutrients. It contains high amounts of vitamin B_6 and B_{12}, as well as magnesium and potassium. There were analyses for minerals, nutritional and anti-nutritional contents. The mineral content indicates the concentrations (mg/g) of potassium, calcium, sodium, iron, manganese, bromine, rubidium, strontium, zirconium, and niobium. Banana peel is a waste product of banana (Shadma *et al*. 2014) and studies have to be yellow banana peel (*Musa acuminata)* also has medicinal properties (Imam *et al*. 2011). A bioactive compound such as flavonoids, tannins, phlorotannins, alkaloids, glycosides, and terpenoids are present in the banana peel. This bioactive compound is reported to exert a pharmacological effect, especially as an antioxidant, antidiabetic, anti-inflammatory, and antibiotic (Chabuck *et al*. 2013).

It has been observed that the antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannins, saponins, alkaloids, steroids, flavonoids, and carbohydrates (Singh and Bhat 2003). Gram-negative bacteria play a central role in the development of periodontal diseases, among them *Staphylococcus aureus* and *E. coli*) are major bioactive periodontal pathogens. (Bassam *et al*. 2006). Various

***Corresponding author:** Dr. R. Kowsalya, Assistant Professor, Department of Biochemistry, D. G. Government Arts College for Women, Mayiladuthurai - 609 001, Tamil Nadu

e-mail: kowsalyamouli@yahoo.com | **Contact:** +91- 9487586327

traditional plants and plant products have shown antimicrobial activity against periodontal pathogens (Palombo 2011). Antifungal and antibiotic principles are found in the banana peel of fully ripe bananas (Brooks 2008). The antibiotic acts against Mycobacteria (Omojasola and Jilani 2009). A fungicide in the peel is active against a fungus disease of tomato plants (Ponnuswamy *et al*. 2011). Norepinephrine, dopamine, and serotonin are also present in the ripe banana peel (Ratule *et al*. 2007). Therefore, the present study is to evaluate the presence of Phytochemical analysis to be a better solvent for the extraction of the bioactive agents in yellow banana peels (*Musa acuminata*) which include: Phytosterols, phenol glycosides, flavonoids alkaloids, tannins saponins, and Carbohydrate, but antimicrobial activity, the antibacterial activity of gram-positive, gram-negative bacterial activity and Anti-fungal activity minimum inhibitory concentration of methanol extract in antibacterial activity of banana peels (*Musa acuminata*) against some gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Sample collection and preparation

Yellow banana peels (*Musa acuminata)* were collected for this study. There are 1000 banana peels were collected in the local market of Seegalapalli, Krishnagiri (DT), Tamil Nadu, India. A separate sterile polythene bags from the area the cleaned, healthy Yellow banana peels materials are cut into small sections and dried under shade for three to four weeks. The dried material was grind into a fine powder then the entire mixture was homogenized in a blender. This homogenized mixture or slurry was left at room temperature for about 48 h.

Preparation of aqueous and ethanol extract

The 50g of powder was soaked in 250ml of methanol to prepare methanol extract respectively. After twenty-four hours, the solution was filtered and the clear filtrate was evaporated to dryness using a water bath at 400C. After completion of the reaction, the entire slurry was filtered to get banana peel extract (Edwards 1999). All extracts obtained were stored in a refrigerator until required for use. The Phytochemical Analysis extracts of *Musa acuminata* peels were qualitative analyzed for alkaloids, phenol, flavonoids, saponins, tannins, proteins, and carbohydrates. Quantitative analysis for alkaloids, flavonoids, phenolic, and tannins resins using standard procedures.

Phytochemical analysis: To identify the phytochemical in *Musa acuminata* peels extracts tests were carried out. The stock concentration of peel extracts 10 mg/ml was used.

Qualitative analysis of phytochemical components (Harborne 1998)

The chemical tests for screening and identification of bioactive chemical constituents in the methanol extract purified sample were carried out by a standard procedure.

Test for alkaloids: To the small quantity of the *Musa acuminata* peels extract was treated with few drops of dilute hydrochloric acid and filtered.

Mayer's test: The filtrate was treated with Mayer's reagent (potassium mercuric chloride) and observed a yellow colored precipitate.

Wager's test: The filtrate was treated with Wager's reagent (Iodine in Potassium iodide) and observed the brown, reddish precipitate.

Dragendroff's test: The filtrate was treated with Dragendroff's reagent (solution of potassium bismuth iodide) and was observed the red precipitate.

Hager's test: The extract was treated with Hager's reagent (saturated picric acid solution) presence of alkaloids confirmed by the formation of the yellow-colored precipitate.

Test for phenol

Ferric chloride test: To five mg of extract was taken and 0.5 ml of 5% ferric chloride was added and observed for dark bluish-black color.

Test for flavonoids

Alkaline reagents: The *Musa acuminata* peels extract was treated with few drops of sodium hydroxide solution and observed of intense yellow color.

Lead acetate: To *Musa acuminata* peels extracts were treated with few drops of lead acetate solution and observed a yellow color precipitate.

Test for saponins

Foam test: To the *Musa, acuminata* peels extract was 0.5g shaken with 2ml of water and observed for the produced persists for 10 minutes presences.

Test for tannins

Lead acetate test: To the *Musa, acuminata* peels extract 0.5ml of 1% lead acetate solution was added and the white precipitate was observed.

Test for proteins

To 2 ml of *Musa, acuminata* peels extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulfate were added; formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.

Test for carbohydrate

Benedict's test: To 1ml of the test solution and equal volume of Benedict's reagent was added. Orange-red color precipitate indicates the presence of carbohydrates.

Fehling's test: 1ml of Fehling's A and B solutions were mixed with a small quantity of *Musa acuminata* peels extract and boiled for 1minute in boiling water bath for 5-10

minutes and tubes were observed for a yellow, then brick-red precipitate, indicated the presence of carbohydrate.

Quantitative phytochemical analysis

Determination of total alkaloid: The *Musa acuminata* peels extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2N HCl, and filtered. This solution was transferred to separating funnel, 5ml of bromocresol green solution, and 5ml of phosphate buffer were added. The mixture was shaken with 4ml chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470nm with a UV/Visible spectrophotometer. The total alkaloid content was expressed as mg /g of extract. By using a standard atropine calibration curve, measured the concentration of alkaloid content in atropine equivalents using unit's mg/g of Gallic acid (GAE) (Fazel *et al*. 2008).

Determination of total phenolic content: Total phenolics in extracts were determined by the Folin-Ciocalteu method (Ranalli et al., 2006). Briefly, 0.5 mL (0.1%, w/v) of each sample was mixed with 2.5 mL of a 10fold diluted Folin-Ciocalteu reagent followed by 2 mL of 7.5% sodium carbonate. The tubes were covered with parafilm (American National Can, Chicago) and allowed to stand for 30 min at room temperature before the absorbance was recorded at 760 nm (U-1800, Spectrophotometer, Hitachi, Japan). Different concentrations of gallic acid (0.1 to 0.60 mg/mL) were prepared in methanol for the preparation of the standard curve. All determinations were analyzed in triplicate and results expressed in mg gallic acid equivalents (GAE)/g dried extract.

Determination of total flavonoid content: The total flavonoid content of *Musa acuminata* peels extracts were estimated according to the method described by (Zhishen *et al*. (1999). 1.0 mL (0.1%, w/v) of sample was mixed with 4 mL of distilled water and subsequently with 0.3 mL of $NaNO_2$ solution (10%, w/v). After allowing the mixture to stand for 5 min, 0.3 mL AlCl3 solution (10%, w/v) was added followed~ 449 ~Journal of Pharmacognosy and Phytochemistry by 2.0 mL of (1%, w/v) NaOH solution. The mixture was thoroughly mixed immediately and absorbance was determined against blank at 510 nm. The standard curve of quercetin (Sigma Aldrich USA) was prepared in a concentration ranging from 0 to 12 mg/mL and the results were expressed as quercetin equivalents (mg quercetin equivalents/g dried extract).

Determination of total tannin content: The tannin content in the sample was determined using insoluble polyvinyl- polypirrolidone (PVPP) which binds tannins (Makkar *et al*. 1993). Briefly, 1 mL of extract (0.1%, w/v) dissolved in methanol in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, kept for 15 min at 4°C and then centrifuged for 10 min at 3000 rpm. In the clear supernatant, tannin phenolics were determined. Tannin content was calculated as a phenolic content read at 450nm.

Source of test microorganisms: The Pure cultures of pathogenic bacteria such as Staphylococcus aureus, *E. coli*, and *Aspergillus* were all culture was obtained from the Department of Microbiology, Muthayammal arts and Science College, Rasipuram, Namakkal, Tamil Nadu, India. They were gram stained and subjected to biochemical tests to confirm their identity (Cheesbrough 2000). The organisms were subcultured in nutrient agar plates and stored in nutrient agar slants at 4O0C until needed for use. Antibacterial Activity Assay The determination of antibacterial activity.

Preparation of inoculums: Bacterial inoculums were prepared by growing cells in Muller-Hinton broth (MHB) (HI media) for 24 hours at 37 c. The filamentous fungi were growth on sabouraud dextrose agar (SDA) slants at 28 c for 10 days and the spores were collected rings sterile doubled distilled water and homogenized. Yeast was growth on sabouraud dextrose broth (SDB) at 28 c for 48 c.

Preparation of active bacterial suspension: The nutrient broth was made up to 100ml (1.95g in 100ml of distilled water) and was transferred into different boiling tubes and they were kept for sterilization for comparison. Evaluation of the antimicrobial activity of selected *Musa acuminata* peels extracts 30 minutes in the autoclave. This broth was cooled. Active culture for experiments was prepared by transferring a loopful of stock cultures to 25 ml of nutrients broth and incubating aerobically at 37 cover night for bacterial proliferation.

Preparation of fungal cultures: Potato dextrose broth was prepared by dissolving potato dextrose agar (3.9g) in 300 ml of distilled water and was filtered and the filtrate was transferred into four different boiling tubes and they were sterilized in an autoclave at 121 c for 30 minutes. This broth was cooled. A loop full of fungal culture was transferred to the broth and incubating aerobically at 37 c for hours for fungal proliferation.

Preparation of medium: Potato dextrose agar (3.9g) was mixed with 300ml of distilled water along with agar type 1 (3.75g). It was then sterilized in an autoclave for 30 minutes and then allowed to cool but not solidify. Then they were transferred to twelve different sterile Petri dishes and the medium was left in the laminar airflow chamber for 30-60 min. After solidification 20ml of the fungal cultures were inoculated to the Petri plates and incubated at 30 c. Freshly prepared cultures were inoculated to the Petri plates and incubated at 30 c. Freshly prepared sterilized cotton swabs were used to swab the developed fungal cultures on to the solidified medium. Then the filter paper disks immersed in the extract which was serially diluted (sample 1g/100ml of solvent was prepared). Five test tubes with 0.5ml of fungal

ethanol were taken 0.5ml of the prepared extract was dissolved in the test tubes numbered a one and 0.5ml of *Musa acuminata* peels extract was taken and dissolved in test tube numbered five. Hence five different serial dilutions were used. Then they were kept on to the particulates and observed zone of inhibition for 24-28 hours. The antifungal was assayed by measuring the zone of inhibition for the respective plant extract potato dextrose agar plate without adding cultures that were used as control (Mase *et al*. 2008).

RESULTS AND DISCUSSION

Qualitative analysis

All the results of the phytochemical analysis are showed in (Fig 1). The results of the qualitative analysis of *Musa acuminata* peel extract. (Table 1) showed the preliminary phytochemical screening of methanol extract of *Musa acuminata* banana peel revealed the presence of alkaloids, flavonoids, saponins, tannins, and carbohydrates, are (+) but phenols and proteins are (-). The present study also carried out found to be rich in the solvents used methanol extract of *Musa acuminata* peel phytoconstituents are the key factors for the pharmacological activity of a medicinal plant.

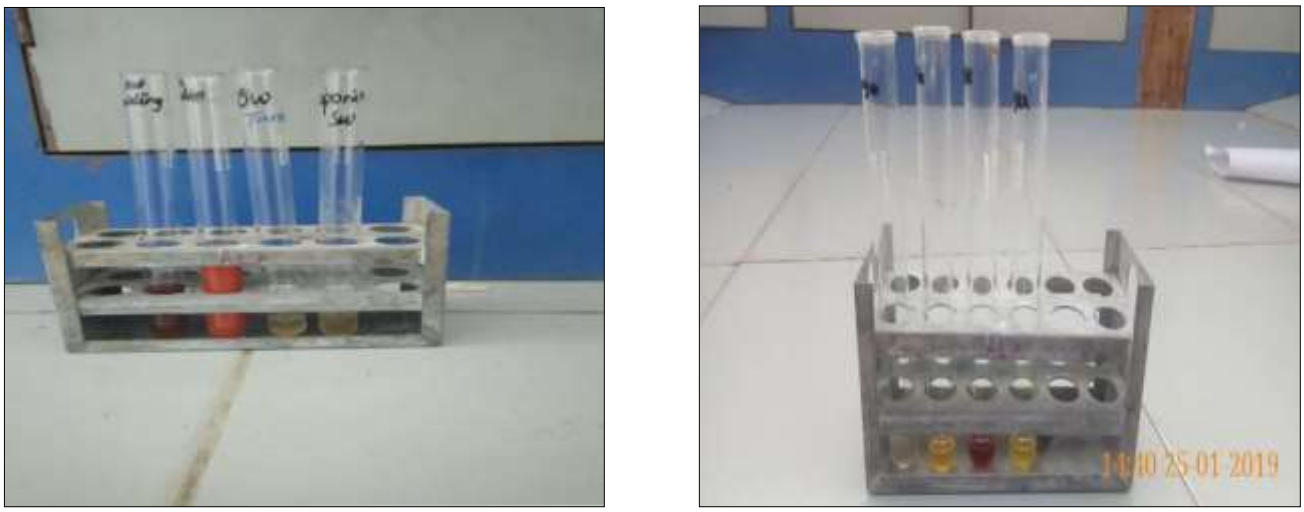

Fig 1 Represents the phytochemical analysis of banana peel in methanol extract of *Musa acuminata*

Table 1 Represent the phytochemical analysis of banana peel in methanol extract of *Musa acuminata*

Compounds	Result
Alkaloids	+
Phenols	-
Flavonoids	+
Saponins	+
Tannins	+
Proteins	-
Carbohydrates	+

Quantitative analysis of phytochemical constituents of Musa acuminata peel extract

The results of the quantitative screening of phytochemical presented in (Fig 2). Total phenol compounds are present in methanol extract of *Musa acuminata* samples values obtained for the concentration was found to be 100 to 500 µg/ml in the examined extract ranged from 0.05 ± 0.13 µg catechol/g. optical density at 650 nm.

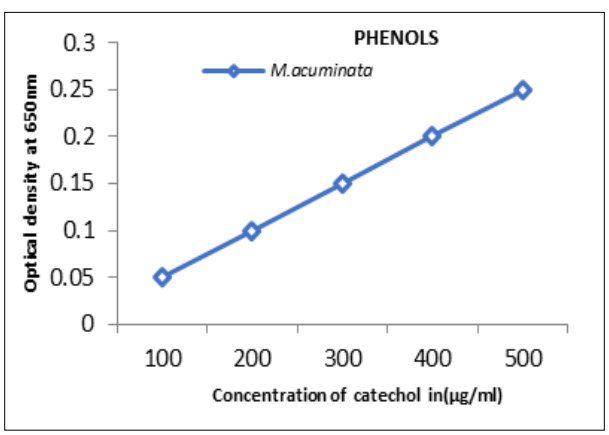

Fig 2 Total phenolic content in the banana peel in methanol extract of *Musa acuminate* expression

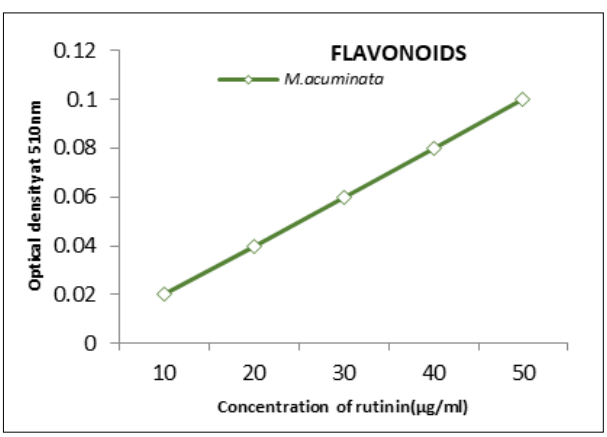

Fig 3 Total flavonoid content in the banana peel in methanolic extract of *Musa acuminata* expression

Determinations of total flavonoids in Musa acuminata peel extract

The results of the quantitative screening of phytochemical presented in the flavonoids in plant extract of *Musa acuminata* peel was determined by using concentration was10 to 50 µg/ml expressed in terms of equivalent as 0.02 ± 0.06mg rutin/g (Fig 3).

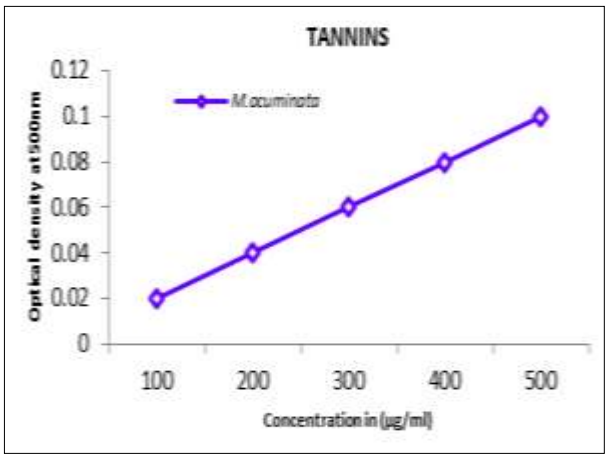

Fig 4 Total tannin content in the banana peel in methanolic extract of *Musa acuminate* expression

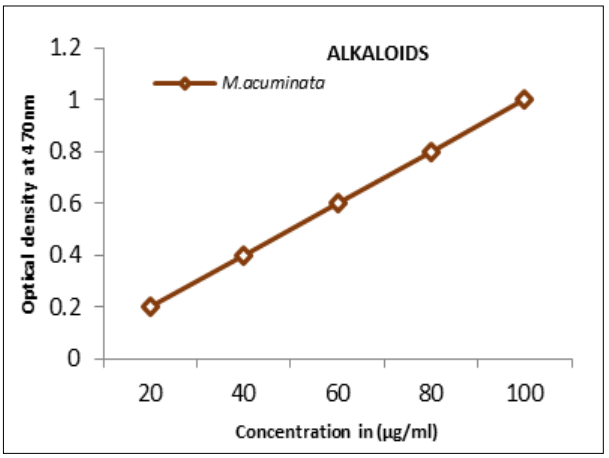

Fig 5 Total alkaloid content in the banana peel in methanol extract of *Musa acuminate* expression

Determinations of total tannins in Musa acuminata peel extract

The results of the quantitative screening of phytochemical presented in the tannins content were examined in *Musa acuminata* peel extract using the Folin-Ciocalteu's reagent is expressed in terms of rutin equivalent. The values obtained for the concentration of 100 to 500µg/ml tannin contents are expressed as 0.02±0.06 mg of rutin/g of methanol extract (Fig 4).

Determination of total alkaloids in Musa acuminata peel extract

The results of the quantitative screening of phytochemical presented in the alkaloid content were examined in *Musa acuminata* peel extract and expressed in terms of atropine equivalent as mg of AE/g of extract. The concentration of 20 to 100 µg/ml alkaloid was measured by 0.2 ± 0.6 equivalent/g in methanol extract (Fig 5).

Table 2 Antimicrobial activity of methanol extract of banana peel (*Musa acuminata*) against the human pathogen

Staphylococcus *aureus*	Zone of inhibition
10	11 mm
15	13 mm
20	14 mm
30	16 mm

Anti-bacterial gram-positive Staphylococcus aureus activity of Musa acuminata Banana peel extract

The results showed banana peel Methanol extract of *Musa acuminata* use of this peel in antimicrobial resistance. Antibacterial antibodies and the chemotherapeutic agents have to be of value in controlling many infections but they depend on judicious use to minimize the incidence of resistance. *Staphylococcus aureus* inhibition whereas the concentration of 10µl to 30µl in zone of inhibition 11mm to 16mm *Musa acuminata* Banana peel extract was increased in a concentration-dependent manner (Fig 6, Table 2).

Fig 6 *Musa acuminata* banana peel extract in *Staphylococcus aureus*

Anti-bacterial gram-negative E. coli activity of Musa acuminata banana peel extract

The results showed banana peel Methanol extract of *Musa acuminata* use Among the good activity against *E. coli*. There are different 10µl to 30µl of peel extract. The zone of inhibition (20mm, 23mm, 25mm, 27mm). Whereas extract of *Musa acuminata* banana peel was more promising against *E. coli* when compared to 30µl plant extract it showed higher activity (Fig 7, Table 3).

Table 3 Antimicrobial activity of methanol extract of Banana peel *(Musa acuminata)* against the human pathogen

E. coli	Zone of inhibition
10	20 mm
15	23 mm
20	25 mm
30	27 mm

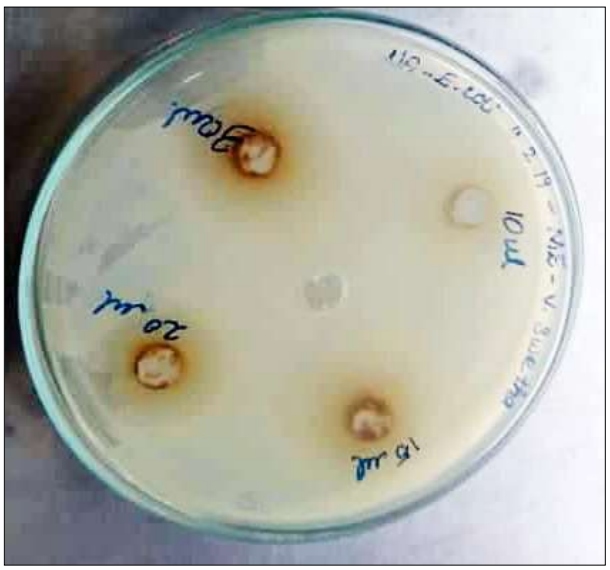

Fig 7 *Musa acuminata* banana peel extract in *E. coli*

Table 4 Antimicrobial activity of methanol extract of banana peel (*Musa acuminata*) against human pathogen

Compound *Aspergillus*	Zone of inhibition
10	12 mm
15	18 mm
20	23 mm
30	29 mm

Anti-fungal gram Aspergillus activity of Musa acuminata banana peel extract

The results showed banana peel methanol extract of *Musa acuminata* Antifungal activity was studied by poisoned food technique (Nene and Thapliyal 1993). The pure culture of three fungal pathogen were obtained from formed the standard plant extract solution (100%). All these plant extracts are known to retain their fungal toxicity after a thermal treatment at 70° C for 10 min (Kurucheve *et al*. 1997). Different fractions were taken in concentration range (10-30 µl). The Zone of inhibition of 12mm to 29mm Compound was as found to be more effective against aspergillus considered as bioactive (Fig 8, Table 4).

Fig 8 *Musa acuminata* banana peel extract in *Aspergillus*

Antimicrobial activity

In-vitro antibacterial and antifungal activity of *Musa acuminata* banana peel extract was investigated by the agar well diffusion method. The methanol peel extracts of the yellow banana peel were tested against two bacterial strains i.e. *Staphylococcus aureus* and *Escherichia coli* and two fungal strains i.e. *Aspergillus*. The results of antibacterial activity showed that methanol extract was an effective significant activity for bacteria. The methanol extract of *Musa acuminata* peel showed maximum antifungal activity. The results showed yellow banana peel Methanol extract of *Musa acuminata* use of this in treating microbial infection and new potent antimicrobial agents.

Both gram-positive, gram-negative bacterial and anti-fungal showed sensitivity against a methanol extract of yellow banana peel (*Musa acuminata*). Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug-resistant microorganisms pose an enormous threat to public health. The use of medicinal plants with antimicrobial activity need to be given more attention to arrest the situation. The phytochemical result showed methanol to be a better solvent for the extraction of the bioactive agents in banana peels which include: Phytosterols, phenol, glycosides, flavonoids, alkaloids, tannins saponins, and carbohydrate but antimicrobial activity both are activity against gram-positive, gram-negative bacterial but maximum activity of antifungal.

LITERATURE CITED

Amit R and Shailandra S. 2006 Ethnomedicinal approach in biological and chemical investigation of phytochemicals as antimicrobials. *Indian Journal of Pharmaceutical Science* **41**: 1-13.

Bassam A S, Ghaleb A and Naser J. 2006 Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant Staphylococcus aureus. *Turkish Journal of Biology* **30**: 195-198.

Bhat M S, Prabhakar A, Rama K R R, Madhu G M and Rao G H. 2010. Statistical optimization and neural modeling of amylase production from banana peel using *Bacillus* subtilis MTCC 441. *International Journal of Food Engineering* **56**: 34-45.

Brooks A A. 2008. Ethanol production potential of local yeast strains isolated from ripe banana peels. *African Journal of Biotechnology* **7**: 3749-3752.

Chabuck Z A G, Al-Charrakh A H, Hindi N K K and Hindi S K K. 2013. Antimicrobial effect of aqueous banana peel extract, Iraq. *Research Gate Pharmacy Science* **1**: 73-75.

Cheesbrough M. 2000. *Microbiological Test: District Laboratory Practice in Tropical Countries*. *In*: (Eds) Cremer A. and Evan G. Cambridge University Press, UK. pp 1-226.

Fazel S, Monsef H, Ghamooshi R and Verdian-rizi M. 2008. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai Journal of Pharmaceutical Science* **32**: 17-20.

Girish H V and Satish S. 2008. Antibacterial activity of important medicinal plants on human pathogenic bacteria: A comparative analysis. *World Application of Science Journal* **5**(3): 267-271.

Harborne J B. 1998. *Phytochemical Methods a Guide to Modern Techniques of Plant Analysis*. *Springer Science and Business Media* **12**: 4-9.

Imam M Z and Akter S. 2011. *Musa paradisiaca* L. and *Musa sapientum* L. A phytochemical and pharmacological review. *Journal of Applied Pharmacy Science* **1**: 14-20.

Makkar H P S, Bluemmel M, Borowy N K and Becker K. 1993 Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of Science, Food and Agriculture* **61**: 161-165.

Mase K, Adjih C and Baccelli E. 2008. A common framework for auto configuration of adhoc networks draft-mase-autoconf-framework. MANET Autoconfiguration (AUTOCONF) *Internet-Draft Versions*,; 00 01 02 03 04.

Omojasola P F and Jilani O P. 2009. Cellulose production by Trichoderma longi, Aspergillus niger and saccharomyces cerevisae cultured on plantain peel. *Research Journal of Microbiology* **40**: 67-74.

Palombo E A. 2011.Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. *Evid Based Complement Alternat Medicine.* pp 680354.

Ponnuswamy V, Lekshmi V D and Prakash S G V. 2011. Bio-processing of banana peel for amylase production by Sp. Penicillium. *Asian Journal of Biological Science* **2**: 257-264.

Ranalli A, Contento S, Lucera L, Di Febo M, Marchegiani D and Di Fonzo V. 2006. Factors affecting the contents of iridoid oleuropein in olive leaves (*Olea europaea* L.). *Journal of Agriculture and Food Chemistry* **54**: 434-440.

Ratule M T, Osman A, Saari N and Ahmad S H. 2007. Microstructure of peel cell wall and selected physic-chemical characteristics of Berangan banana (Musacv. Berangan [AAA]) ripened at high temperature. *Asia Pacific Journal of Molecular Biology and Biotechnology* **15**: 8-13.

Shadma A, Sundaram S and Rai G K. 2014. Nutraceutical application and value addition of banana peel: A review. *International Journal of Pharmaceutical Science* **6**: 81-85.

Singh B and Bhat T K. 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. *Journal of Agriculture and Food Chemistry* **51**: 5579-5597.

Zhishen J, Mengcheng T and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* **64**: 555-559.