ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *MUSA ACUMINATA* FLOWER EXTRACT

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Abstract

From recent years' fruits and vegetables started achieving high attention in research field due to their therapeutic value and also can act as an alternative for exiting antimicrobial agents. Due to safe and ecofriendly nature, bio-based agents can be used as antimicrobial agents with great significance. Theobjective of current research work was assessing the presence of antibiotic, antioxidant activity and also determines the phytochemicals that are present in *Musa acuminata* flower. On the basis of changes of colour, precipitation or turbidity of active compounds in the extract we can confirmed the presence of phytochemicals. From the Phytochemicals studies we can found many compounds liketannin, flavonoid,alkaloids, saponin, glycoside, terpenoid and steroid. The extract of*Musa acuminata* flower shown high antimicrobial activity and it confirmed by tested against the microorganisms with restricted a range of area (10mm to 20mm). Antioxidant activity of the banana extract also evaluated using DPPH free radical scavenging activity.

Keywords: Antibacterial; Antioxidant; Banana flower; *Musa acuminate*; Plant extraction;phytochemical screening

Introduction

Now a day's outbreak and spreading of disease become common and also the number of overcoming patients become less. The main reason for the disease outbreak was microorganism which becomes resistant to currently available antimicrobial agents. An alternative for this situation is introduction of a new antimicrobial agent which can replace all the side effects of existing one and also eco-friendly nature. This leads to development of antimicrobial agent using natural compounds from plant kingdom. These agents can inhibit the growth of microorganism and also safe in nature for mankind. From ancient times, different types of plants, vegetables, fruits and species has been used to treat many disorders due to their medicinal value. One of the main reason for choosing plant compounds as antibacterial agent is due to their easy availability (Sumathy*et al*.,2011).

Banana is alarge flowering plant in a genus *Musa* and also it has several medicinal values, its scientific name was *Musa sapientum*. It helps to neutralize the presence of acidity in gastric juice which helps to decrease gastric issues and intestinal disease (Kumar *et al*.,2012). In both medical and ornamental aspects, all part of banana plant was useful to human being. Banana contains lot of nutrient and vitamins which in strengthening the body. Normally banana parts has been consumed by mankind which helps to treat pain, asthma, arthritis, anemia and diabetes. Especially, flower helps to treat diarrhea, wounds, stomach cramp and control bleeding during menses time in women (Shruthi 2019;Kashyap*et al*.,2018). The flower of banana contains different useful compounds for our body like fibers, magnesium, iron copper, antioxidants and flavonoids. One of the major use of these flowers are it has been used as a bio-fertilizer and it is a very good organic material with high nutritional values (Mohapatra*et al*., 2010). Banana is considered as one of the prominent source for potassium which helps in proper working of muscles and also reduce the risk of stroke and also antioxidants such as Gallocatechin and dopamine. It also can be used as excellent diet in order to reduce weight. From recent years most of the research workers has been concentrate onhigh antioxidant and antibacterial property having natural compounds by their isolation and characterization. On the basis of research, banana has lots of biological and pharmaceutical uses such as antibacterial, anticancer, antihypertensive and anti-diabeticreactions because of the presence ofphytochemicals in the plant such as bioflavonoids, organic alkaloids, terpenoids and tannins (Singh *et al*.,2016; Vu *et al*.,2018).

Generally, plant act as an antimicrobial agent which was safe to mankind also environment. Mostly this plant can't allow the growth of any bacteria (both gram positive and negative) and also inhibit the growth of fungi and viruses. The main objective of our work includes the antimicrobial and antioxidant study of banana flower extract.

Materials and methods

Sample Collection

Musa Sapientum flower is easily available one and it purchased from local market. The flower was separated by remove the outer sap and an oven was used for drying(50°C for 48 hours) purpose of collected flowers. A grinding machine was used to make the dry sample into fine powder, and kept it in an air tight containerfor future use.

Extraction procedures

Ethyl alcohol (200ml), and Butanol (200ml) were used with the powders of dried flowers (100g) for extraction, the sample soaked in liquid (solvent) and kept in a sterile conical flask for softening then the macerated sample rotated with constant stirring overnight (Mumtaz*et al*.,2010). Sterile muslin cloth was used for the separation of extract and follow the filtration through sterile filter paper. DMSO was dissolved with the filtrates were dried. Sample was further stored at 4ºC for future use.

Screening of phytochemicals compounds

Phytochemical screening of flower extract was done by referred the paperof Ugochukwu*et al*., 2013.

Screening of alkaloids (Wagner's reagent method)

The extract of sample mixed well with 2-4 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and a reddish brown precipitate was formed to show positive result.

Screening of carbohydrate

2ml of samples was mixed with 2 or 3 drops of Molisch's reagent (alcoholic alpha napthol solution) and then few drops of concentrated H_2SO_4 were added along the wall of test tube. Formation of purple or violet colored ring indicate the presence of carbohydrate.

Screening of Terpenoids (Salkowki's test)

To form a mixture of 1ml chloroform and 2ml sample extractthen followed by adding 1 or 2 drops of concentratedH2SO4. Terpenoids in the extract gives a reddish brown precipitate.

Screening of Quinones

To form a mixture of extract and concentrated HCL produced a yellow precipitate, which indicates the presence of quinones.

Screening of sterols

The mixture of 1 ml extract, 2 or 3 drops of chloroform and concentrated H2SO4 showscolour change from bluish red to cherry red.

Screening of proteins (Millon's test)

Heating 2 drop of Millon's reagent to 2ml of sample gives a white precipitate further changed into red color and this is a symbolic representation of identification of protein presences.

Antioxidant assay

The in-vitro method (DPPH assay) was used topredict theanti-oxidant activity of banana flower.The modified procedure described by Ara and Nur, 2009 was referred for this. All the experiments were carried out in triplicate.

DPPH Radical Scavenging assay

By using DPPH for the identification of scavenging of DPPH capacity of the extracts. 0.004% DPPH solution was prepared in methanol (95%). The sample and methanol (95%) mix were taken as stock solution (10 mg/100mL). Newly prepared 0.004% DPPH solution was added to different concentrationof samples (0.5, 1,1.5, 2,2.5 mg) were prepared from the stock solution by serial dilution and kept for 10 minutes' interaction. Absorbance for test and standard (Ascorbic acid) solutions were determined against the blank (95% methanol) at 517nm with spectrometer (UV- visible). Control sample was free from extract. Following equation was used forDPPH free radical

% DPPH radical-scavenging = [(control absorbance - Test Sample absorbance) / (Control absorbance)] x 100

***In- vitro* screening**

Bacterial culture

The bacterial culture used for this studywere procured from Microtech, Microbiology Laboratory, Coimbatore.*E.coli, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus spp. Salmonella spp.*and *Enterococcus feacalis* are the used cultures. Collected isolates were inoculated onto Chromogenic agar media and colony morphology was observed after 24 hrs. Bergey's Manual of Systematic Bacteriology (Vol 2, Second Edition) was mainly used for the reference of bacterial culture procedure.

Well diffusion method

This test was completed on the basis of methods described in khan *et al*., 2011. The culture containing petri plate filled with 20 ml of sterilized MHA and allowed it to solidify. The swabbed plates were incubated with freshly prepared inoculums (100µL) using a sterile cotton swab, to confirm tested microbes were well spread on the plate surface. Sterile cork borer was used for making wells. Each well was filled with different concentration of extract and ampicillin (5µg/ml) was taken as control. Finally, antibacterial expression was found in the restricted zone of incubated plates(24 - 48hrs at 37°C).

Result and Discussion

The banana flower extraction prepared with various solvent such as ethyl alcohol, Butanol, Acetone and ethanol. From past few years the secondary metabolites present in the plants helps in increase in production of drug which cure various diseases. Main active constituents includesaponins, tannins, flavonoids,

alkaloids, terpenoids, and Phenols. Various method was used foe the isolation and identification of active ingredients from the crude extract such as Chromatography, especially High Performance Liquid Chromatography (HPLC). Phytochemical screening is considered as one of the easiest, cheapest and simplest method and it involves a qualitative study.

The phytochemical studies shown thatacetone crude extract of Musa acuminate flower contain more alkaloids, carbohydrates, flavonoids, saponins, phenols, terpenoids and proteins when compared to other three extractions (Table 1). All phytochemical compounds have its own biological activiesi.e Phenols exhibit some therapeutic effect against most of the diseases and considered as one of the active antimicrobial compounds. The banana leaf extractshave lots of pharmacological properties, the compound flavonoid in the extract is the main source these medicinal uses. It protects us from fungal infection, allergy, inflammatory diseases, diabetics,and cancer. Another compound saponins helps to cure epilepsy, excessive salivation, chlorosis and migraines (Ramu*et al*., 2014; Sadiq*et al*.,2015; Sadiq*et al*.,2017; Hoskin*et al*.,2008; Tan *et al*.,2015).

DPPH assay was heipful for evaluate the anti-oxidant activity of acetone extract which was shown in the Figure 1. The free radical present in the DPPH assay can decolorize the sample in the presence of antioxidants, is the basis of DPPH assay. The presence of purple colour was due to the presence of odd electron in DPPH radical. Antioxidant compounds donate electron which was accept by the DPPH then it get decolorized and quantified by measuring the change in absorbance (Ara and Nur, 2009; China *et al*.,2011). As the concentration of sample increases the scavenging activity (Table 2).

Morphological characterization was done for all the clinical isolates and it was given in the Table 3. By using well-diffusion method the antibacterial activity of banana flower could be confirmed. For bacterial growth identificationMuller-Hinton agar was used as medium and it confirmed by the presence ofinhibited zone. Most of the anti-microbial activity against all the isolates showed byacetone extract of banana flower. In the case of bacterial culture, *S.aureus, E.coli, K.pneumoniae,* and*P.mirabilis*showed high zone of inhibition in seven isolates that indicates the antibacterial efficacy of banana flower extract. The anti-microbial compound present in the cell membrane leads to the change in the structure and function of cell, loss of cytoplasmic content and finally cell lysis happens (Chabuck*et al*.,2013; Sitthiya*et al*.,2018).

Conclusion

Among different solvents used for the extraction of banana flower, acetone extracts were considered as a good antibacterial and antioxidant agent. Acetone extract inhibit the growth of all the clinical isolates. The phytochemical screening of active compounds helps to development of new drugs with safe nature and more efficiency. Plant is considered as store house of natural antioxidant and it can help in balancing the free radicals and antioxidants ratio in our body and prevent tissue damage. Banana flower extract used for the treatment of disease such as cancer, ulcers, diarrhea and Alzheimer's, etc. This work suggests that banana flower extract can consider as a huge source of bioactive materials with extreme anti-bacterial and anti-oxidant activity.

S. No	Phytochemicals test	Acetone Extract	ethanol extract	Butanol Extract	Ethyl acetate
1	Alkaloids	+	+	-	-
2	Carbohydrate	+	-	+	+
3	Flavoniods	+	+	-	-
4	Phenols	+	-	-	-
5	Saponins	+	-	-	-
6	Tannin	-	-	-	-
7	Terpenoids	+	+	-	+
8	Quinon	-	-	-	-
9	Sterols	-	-	-	-
10	Proteins	+	-	-	-

Table 1: Phytochemicals analysis of Banana flower

S.No	Solvents	Con. of sample and antioxidant activity in %				
		0.5mg	1mg	1.5mg	2mg	2.5mg
1.	Acetone	12.0	33.6	44.5	59.7	77.1
2.	Ascorbic acid	79.8	82.4	84.2	87.7	90.3

Table 2: Antioxidant activity of Banana flower extract

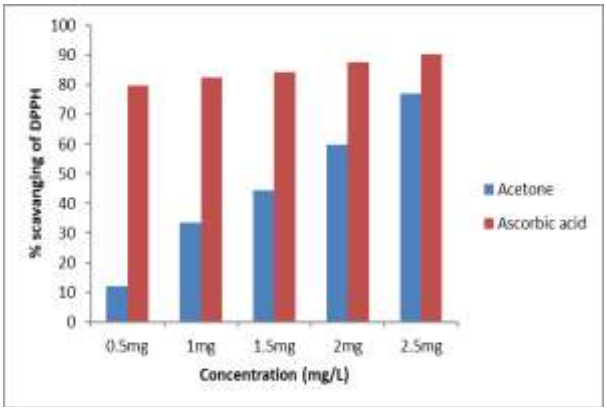

Figure 1: Antioxidant activity of Banana flower extract

S.No	Bacterial isolates	Morphological character
1.	*E.coli*	**Chromogenic media** : Pink colour and small colony
2.	*P.aeruginosa*	**Chromogenic media**: white colonies with mucoid
3.	*K.pneumoniae*	**Chromogenic media**: Blue colour and mucoid colony
4.	*S.aureus*	**Chromogenic media**: Dry White colour colony
5.	*E.faecalis*	**Chromogenic media**: Blue colour, small colony
6.	*P.mirabilis*	**Chromogenic media**: Light brown colony
7.	*Salmonella spp*	**SS agar media:** Black colonies

Table 3: Morphological characterization of clinical isolates

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S. no	Isolates	Zone of inhibition in mm					
		Conc. of extract					
		1	2	3	4	Acetone	Amp
1.	*E.coli*	-	12	15	18	-	-
2.	*K.pneumoniae*	-	12	15	18	-	-
3.	*P.aeruginosa*	10	13	15	17	-	-
4.	*S.aureus*	-	13	17	20	-	-
5.	*E.faecalis*	-	-	13	17	-	-
6.	*Salmonella pp*	-	-	11	13	-	-
7.	*P.mirabilis*	-	13	15	18	-	-

Table 4: Antimicrobial activity of acetone extract of Banana flower extract

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