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Bioanalytical, antibacterial and bioautographic assessment of spathe of Musa Sps.

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Abstract: ***Musa sps. are*** **perennial , widely distributed and well used plant varieties in Central Asia. Plant parts have various applications as religious, culinary preparations using stem, flower, fruits, and considered to be of medicinal and nutritional value. Research has been done on the plant parts - floral viz. spathe, flowers, stem, leaves. Bract (Spathe) has an important role in pollination and protection of floral parts. Earlier studies were carried out to assess the qualitative and quantitative composition of phytochemical constituents, antimicrobial and antioxidant properties. Tannins, phenols and alkaloids, were identified as some of the phytochemical compounds. The current study is aimed at analysing the antibiotic activity of the bract extract using the standard antimicrobial agar well method as well as bioautographic technique. The efficiency of the bract antimicrobial activity was checked with laboratory cultures as well as *Canine*fur microbes.**

Keywords: ***Musa sps*****, floral parts (bract), antimicrobial, bioautography, *Canine* microbes, and antioxidant.**

1. INTRODUCTION

Banana is one of the most familiar tropical fruits. *Musa* sps. belongs to family *Musa*ceae also known as Banana, a tropical fruit grown across continents in 122 countries and India being the largest producer. A largest herbaceous flowering plant and every part of the plant except the Rhizome is consumed as food, commercially important with considerable medicinal and nutritional properties. Banana in ripe or raw used as food and nutrition from ancient timesThe flowers are used in bronchitis and dysentery and on ulcers; cooked flowers are given to diabetics; the astringent plant sap used in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites; 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).Wide research was carried out on different banana species and different parts of the plant body especially fruits, leaves for their phytochemical composition, nutrient value and antioxidant activities.(Karuppiah, and Muhammed ,2013; Suraj ,2015; Uzama and John ,2015; Thomas ,2008; Pingyi et.al., 2005; Michele and Schmidt ,2015; Oliveira,2016 ; Okareh et.al.,2015 and Ehiowemwenguan, 2014; Pereira,2015).

The inflorescence is a complex structure with flowers that will develop into fruits which are protected by thick and colourful bract. It is supported by the aerial true stem, known as floral stem. The aerial true stem is produced by the terminal growing point on the rhizome. It grows the pseudostem and emerges at the top of the plant soon after the last cigar leaf. The female (pistillate) flowers appear first and the ovary develops into a seedless fruit by parthenocarpy (without being pollinated). As the female flowers develop into fruit, the distal portion of the inflorescence elongates and produces clusters of *male (staminate) flowers*, each subtended by a *bract.*

The medicinal parts used are fruits mainly as wellas peels, leaves and the juice. The root is antihelminthic and for reducing branchocele. The fruit has been used as part of anti-ulcer diet in combination with pineapple,blueberries, cloves, ginger and cinnamon. Antifungal and antibiotic principles are found in the peel and pulpof fully ripe banana. Although the antimicrobial activity of some medicinal plants is documented,their antimicrobial activitiesvary widely, depending on the type of spice or herb, test medium andmicro-organism.

Extensive research has been carried out on antimicrobial, antioxidant and medicinal properties of different varieties of *Musa* Sps. Banana fruits have several advantages due to the medicinal value such as Anti-diarrhoeal; anti-ulcerative; Hypoglycemic andhyperglycaemic controls, hypo-cholesterolaemic, antihypertensive, antioxidant, diuretic, antimalarial activity and effect on muscle (Akter,2011) were well studied (Sampath Kumar et.al.,2012). The aqueous extract of banana fruit peels puree has also reported to have bacteriostatic activity against *Bacillus cereus, B. coagulans, B. stearothermophilus, Clostridium sporogenes*. (Akter, 2011)

The current study is aimed at investigating antimicrobial activity of bract extract by using Bioautography assay and traditional agar well methodagainst the standard test micro-organism and microbes of animal origin. In future it can be further analysed and tested for the development of ecofriendly phytochemical based drugs.

2. MATERIALS AND METHODS

Sample collection:

Inflorescence of *Musa* sps collected from the local markets were considered for study. Bracts were separated from the florescence ad cut into small pieces with a scalpel, air dried for 3 days. It was then grounded into fine powder by grinding it into a grinder, packed in a sterile airtight bottle to avoid any effect of humidity and was stored at room temperature. Leaves of another medicinal plant *Schinus molle* commonly known as pink pepper were collected from local farm were treated similarly and extracts were considered for comparative study. The plant samples were identified by Dr.Bindu Gopala Krishnan of Botany department, Mithibai College.

Sample extraction:

70% (v/v) ethanol and distilled water were used for extraction of the samples. Approximately 1 gm of sample (powder) was mixed well to obtain the extracts in their respective solvents and kept for overnight in air tight chambers. Each extract was decanted, passed through muslin cloth, filtered through Whatman No. 1 filter paper and then lyophilized. The stock solutions were stored in sterile capped test tube and kept at 4 - 8°C

Proximal analysis: Aquatic extract of the bract were qualitatively and quantitatively assessed for carbohydrates and proteins using standard methods along with fibre content (Ranganna,1995)

Phytochemical analysis: The extracts were preliminary screened to identify phytochemicals such as alkaloids, carbohydrate, resin, flavanoids, terpenoids and steroids based on standard methods (Harborne, 1973) Qualitative tests for phytochemicals included alkaloids by Mayer's test and Wagner's tests; tannins-Ferric chloride test,Bromine water test and Lead sub-acetate test; saponins -Frothing test; flavonoids-Sodium hydroxide test and sterols by

Salwoski's test were performed (Jayaprada et.al.,2014 and Vanimakhal and Balasubramanian, 2016).

Quantitative Test for Phytochemicals:

Estimation of Alkaloids: Alkaloid determination by using Harborne (1973) method. 2.5 gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and its covered and allowed to stand for 4 h. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added by drop wise to the extract until the precipitate was collected and washed with dilute$NH_4OH$and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of total phenols: The total phenolic content of the extract was determined by the Folin-Ciocalteu method. Briefly, 200 micro litre of crude extract (1 mg/1 ml) were made up to 3 ml with distilled water, mixed thoroughly with 0.5 ml of Folin-Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for further 60 min in the dark, and absorbance was measured at 650 nm. Gallic acid was used as standard.

Estimation of Tannins: Tannins content was estimated by standard method as described byEspinosa and Santacruz, (2017). The methanolic extract (1 ml) was mixed with Folin-ciocalteau 1.25 mL of Folin-Ciocalteu reagent and 2.5 mL of sodium carbonate solution (5%). The resulting mixture was diluted to 25 mL with distilled water and the absorbance was measured after 30 minutes at 760 nm . Tannic acid was used as standard.

The sample used in above tests was weighed using Contech balance with a sensitivity of 0.1 gram.All the colorimetric analysis was performed using Equip-Tronics equipment model no.EQ650A.Each sample was analysedcolorimetrically in triplicates and the average was used for comparative purposes.

Anti Microbial analyses:

The antimicrobial activitity of the Musa bract extracts were analysed using methanol extracts using standard antimicrobial assay (agar cup method) and Chloroform and methanol extracts(2:1) for bioautography analyses. 1 gm of powdered sample of spathe as well as leaves of pepper mint (*Schinus molle*)was added to 10ml of solvent system and kept overnight. The supernatant was collected and used for antibiotic assays and analyses. The antimicrobial assays were carried out using laboratory microbial cultures, *Canine* microbiome cultures and isolated variants of canine fur microbes. The efficacy was compared with standard antibiotics used for humans, and medicinal plant i.e. pink pepper.

Bacterial species such as *Staphylococcus aureus*, *Bacillus subtilis* (gram-positive bacteria) and *Klebsiella pneumoniae* (gram-negative) opportunistic human pathogen were considered from the laboratory bacteria collection to study the antibacterial activity efficacy of Musa sps. Bract extracts extracted in different methanolic solvent concentrations.

Canine microbiome:

St.test tubes with cotton swabs dipped insaline solution were used to collect the microbial colonies sample on the dog's skin .Samples were collected from fur of healthy dogs- stray as well as domesticated dogs. The skin sites included the head, neck, back and the paws of the dog. Each swab applicator was rubbed on the skin 3-4 times, while rotating each swab by one quarter for every 5-6 strokes. The swabs were stored in the same properly labelled tube and refrigerated at 4°C until further analysis.

Isolation and characterization

These swab samples were spread on 1% nutrient agar plate and incubated for 37^{O}C for 24 hours and growth of different colonies were observed. Different types of colonies were observed and their macroscopic characteristics were studied which included size, shape, surface, colour, elevation, margin and optical characteristics. Gram nature of the colonies was carried out to check whether they were gram positive or gram negative. 5 Gram negative colonies (Variants – A to E) were chosen along with the raw mixed bacterial cultures of domesticated and stray dog fur for antimicrobial sensitivity testing.

Preparation of bacterial suspension

The Gram negative colonies from the canine microbes as well as the unidentified bacterial culture suspension were further subcultured on sterile nutrient agar slants. Similarly*Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae*. *S. aureus and B. subtilis*are gram-positive bacteria and *K. pneumoniae* is gram-negative,opportunistic human pathogen were considered from the laboratory bacteria collection

Saline suspension of each sample (Gram negative/ positive colony/ the unidentified culture mixture) of bacterial suspension was prepared using sterile saline with a turbidity of 0.5 as per McFarland solutions.

Preparation of antibiotic solution:

Antibiotic stock solutions were prepared by using commercially obtained standard antibiotic solution for two different antibiotics (Gentamicin, Streptomycin). Known weight of antibiotic powder was diluted in sterile distilled water to obtain the stock solution of 0.05 g/10 ml.

Antimicrobial activityassessment:

1. Agar well diffusion method: Determination of the antimicrobial activity was performed using the agar well diffusion technique (Perez et.al.,1990). The *Canine*microbesisolated from the fur were considered to be tested against the standard antibiotics, *Musa* extract and the medicinal plant pink pepper extract. The microbes were inoculated in sterile nutrient

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agar and incubated for 24h at 37^{o}C.similarly a loop of inoculum was transferred into 5ml of nutrient broth, incubated for 2h at 37 ^{o}C. Well diffusion assay was carried out to check the antimicrobial activity of banana bract extract against *Canine*microbe strains on Muller Hinton agar plates. A sterile swab dipped in the canine microbe cultures was used to spread the coloniesonto the Muller Hinton agar plates to form a bacterial lawn. The inoculum was adjusted to standard McFarland 0.5 turbidity. Tenmillimeter diameter hollow sterile cork borer (flame sterilized) was used to prepare the wells on each plate. Gentamycin, streptomycin, pink pepper leaf extract, banana bract extract, were added into the respective wells on each plate. The plates were then kept for pre-diffusion at 4°C for 15-20 minute followed by overnight incubation at an incubation temperature of 37°C. The size of inhibition zone was measured by holding the measuring device.The zone of inhibition was measured in millimetres and the results were interpreted as Resistant, Intermediate or Sensitive for each antibiotic by comparing with the standard ranges listed on the Kirby-Bauer chart.(MM Suleimana, 2010)

2. Bioautography

The efficacy of plant phytochemicals against microbes was assessed using bioautography technique. The plant sample, aqueous extract and the choloform-methanol extract were loaded onto TLC plates in a narrow band and eluted using the mobile solvent system (petroleum ether and methanol). TLC of plant extracts were developed and plate was dipped into a *Canine* microbial suspension..An inoculum of absorbance of 0.5 at 560 nm was suggested. For bioautography the plate was incubated at 37^{0}C for 24 hours and visualized using tetrazolium salts treatment at 37^{0}C for 3-4 hours. Clear white zones against a purple background on the TLC plate indicate antimicrobial activity of the sample (Dewanjeea,2015)

Antioxidant activity

The banana bract antioxidant activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. It is simple, rapid, reproducible, widely used and inexpensive methods to evaluate the antioxidant activity of fruits and vegetables. The plant extract was prepared using 1g of bract sample powder and added to the 10 ml of 50% methanol and kept overnight. The supernatant was used to assess the antioxidanat activity**.**

The antioxidant activity of the extract was determined using a free radical method to evaluate antioxidant activity based on the scavenging capacity of the DPPH free radicals.The absorbance of DPPH diluted in methanol was considered as control. The decrease in absorbance was measured at 517 nm using a spectrophotometer. The degree of stable DPPH decolourization to DPPHH (reduced form of DPPH) which is yellow indicated the scavenging efficiency of the extract. DPPH free radical scavenging assay was performed to determine the antioxidant activity of plant bract extract. DPPH (0.002%) was used as free radical. Equal volume of extracts and DPPH were mixed and the tubes were incubated at room temperature in dark for 30 minutesThe antioxidant capacity to scavenge the DPPH radical was calculated by the following equation:

Scavenging activity (%) = (A – B) / A x 100 Where, A is absorbance of DPPH and B is absorbance of DPPH and extract combination

3. RESULTS

The proximate analysis of spathe extract indidcated the presence of carbohydrates, proteins and fibre. Different tests for carbohydrates confirmed their presence. The reducing sugars concentrations was found to be 3.3mg/1gm. Proteins were assessed qualitatively and quantitatively by standard techniques such as Biuret method and Bradford method. The concentration of the proteins found to be 60 µg/mL (Biuret test) and 9 mg/mL (Bradford's test). Fiber content of the bract was found to be 3.7% per gm.

Table-1: Qualitative analysis of Phytochemicals

Phytochemical component	Aqueous extract	Methanolic extract
1. Alkaloids		
a. Mayer's test	Negative	Positive
b. Wagner's test	Negative	Positive
2. Tannins		
a. $FeCl_3$ test	Negative	Negative
b. Bromine water	Positive	Positive

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c. Lead sub-acetate	Positive	Positive
3. Saponins (Froth test)	Negative	Negative
4. Flavanoids (NaOH test)	Negative	Negative
5. Sterols		
Salwoski's test	Negative	Positive

The phytochemical analysis of bract was found to vary with the extraction solvent that has been considered. Qualitative tests were carried out to detect the presence of Phytochemicals viz. Alkaloids, Tannins, Saponins, Flavonoids and Sterols. The results indicated the presence of Alkaloids, Tannins and Sterols (Table-1).

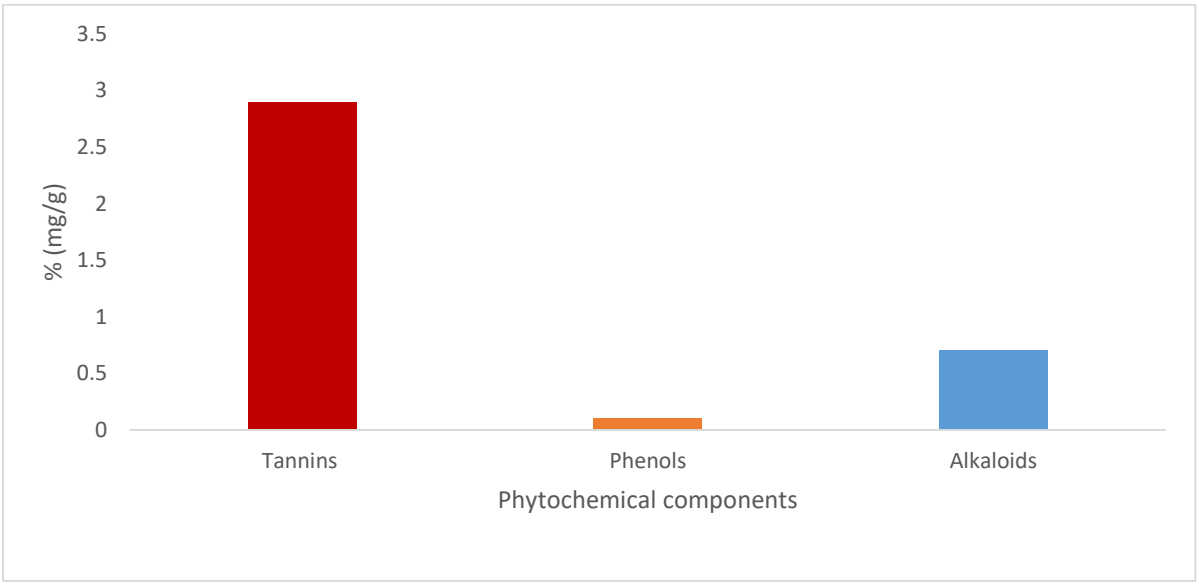

Fig.1 Phytochemical composition of *Musa* bracts

Quantitative tests for Alkaloids, Tannins and total Phenols was carried out to assess the concentration of phytochemicals. The results indicated a high concentration of Tannins (2.9 mg/ gm) followed by Alkaloids (0.71 mg/gm) and total phenols (0.1 mg / gm). (Fig.1)

Antimicrobial activity

The antibacterial activity of various extract of bract of *Musa* showed varying magnitudes of inhibition patterns with different bacterial species such as laboratory microbial populations and canine microbiomes as well as medicinal plant extract andstandard drugs Streptomycin and Gentacin a well-known broad-spectrum antibacterial agent. *Musa*species extracts (methanolic) were effective against both gram- positive and gram- negative test organisms.

Bacterial species such as *Staphylococcus aureus*, *Bacillus subtilis* (gram-positive bacteria) and *Klebsiella pneumoniae* (gram-negative) showed remarkable zones against the bract methanol extracts. Significant zones were observed against 80% methanol extracts and the sensistivity reduced with increasing concentration of the methanol concentration (Table-2).

Table 2: Inhibition of bacterial growth by methanolic extracts of *Musa* sps.

	Bract extract in differenct conc. Of methanol		
Bacterial species	20%	40%	80%
Staphylococcus aureus	12mm	22mm	22mm
Bacillus subtilis	22mm	20mm	24mm
Klebsiella pneumoniae	12mm	20mm	26mm

The stray *Canine* microbiome culture showed 11 mm and 12 mm inhibition zones against an alcoholic extract of banana bract and medicinal plant (pink pepper) extracts respectively; 25mm and 17mm against the standard antibiotics viz.,

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Gentacin and streptomycin respectively (Table-3). However, the domestic *Canine* microbe culture showed 10 mm and 16mm zone of inhibition the banana bract extract and pink pepper leaves extract. And 24mm and 21mm inhibition zone against the standard antibiotic respectively.

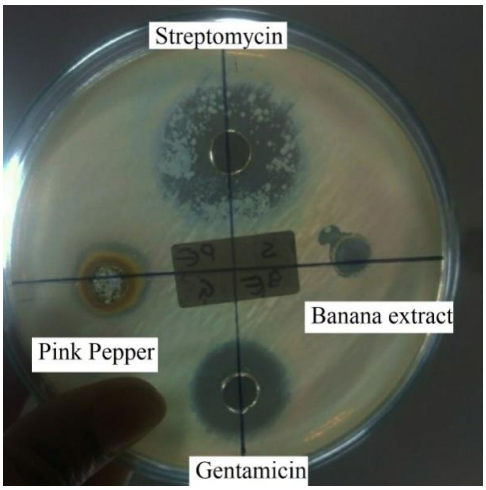

Fig.2 Antimicrobial activity of the Banana extracts against domesticated *Canine*microbe

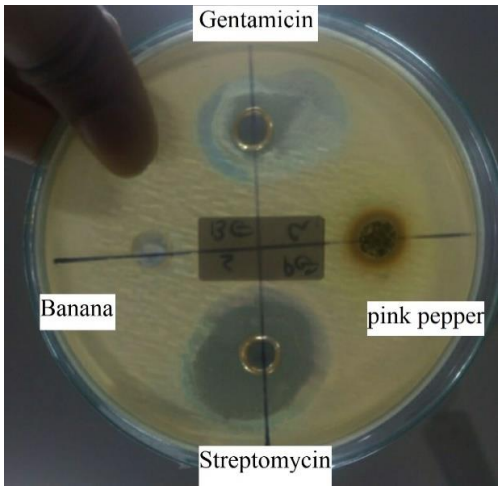

Fig.3 Antimicrobial activity of the Banana extract against stray *Canine*microbe

The *Musa* leaf extract prepared in ethanol shows antibacterial activity against the domesticated *Canine* microbe (Fig.2.) than the microbes of stray dogs (Fig.3.) and in comparison with the standard antibiotics or the medicinal plant such pink pepper leaf extract it is less effective.

The isolated colonies of canine microbes Isolate-A, Isolate-B, Isolate- C, Isolate- D and Isolate- E were sub-cultured and used for the antibiotic activity testing against the methanol: chloroform extracts of bracts of banana inflorescence and pink pepper showed remarkable results (Table-3).

Table 3: Antibacterial efficacy of the standard antibiotics in comparison with phytal extracts

Type of bacterial variant	Chloroform:Methanolic extract of *Musa* sps.	Chloroform :Methanolic extract of *schinus molle*	Streptomycin	Gentacin
Pet dog	---	16mm	24mm	21mm
Stray dog	---	12mm	25mm	17mm
Isolate A	---	20mm	35mm	34mm
Isolate B	26mm	28mm	32mm	34mm
Isolate C	12mm	16mm	30mm	22mm
Isolate D	11mm	22mm	31mm	22mm
Isolate E	12mm	19mm	30mm	24mm

Mixed cultures or the raw fur microbiome samples of both pet and stray dogs showed no response against musa bract extract. In contrast, more efficient results were noticed with phytal extract of pink pepper and the two standard antibiotics. Isolate B was found to be more sensitive against all the extracts as well as antibiotics. Streptomycin was more efficient against the canine fur microbiomes than the other phytal extracts or Gentacin (commercial antibiotic) However, the results of this study showed less antibacterial activity than earlier studies which can be attributed to the cultivar of banana and geographical distribution.

Bioautogram

In the direct bioautography assays, the antibacterial activity of the compounds separated on TLC was determined and the result was evident by the significant clear zone of inhibition on a purple background confirming the antimicrobial activity of the *Musa* bract extract. (Fig. 4)

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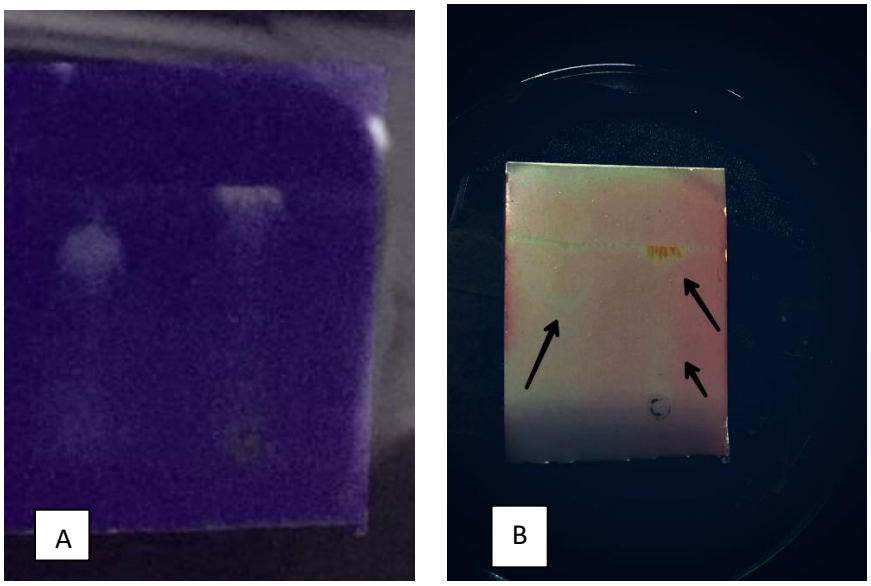

Fig.4 Bioautogram: A. Separation by TLC followed by incubation with microbe B. After the treatment with tetrazolium salts.

Antioxidant activity

Antioxidant Activity by DPPH Radical Scavenging Assay: Phenolic compounds are important fruit constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. The DPPH radical scavenging activity of Banana bract extracts was determined. The extract showed the scavenging ability of 11.1%.

4. CONCLUSION

Various studies were undertaken to assess the medicinal, molecular biology aspects, nutritional value, and phytochemical analysis of *Musa acuminate* or *M.paradisica.* The bracts of the plants may have potential bioactive compounds which can be exploited for the developments of new products due to their pharmacological properties which in turn may help in the development of new bio products (Gunavathy,2014). The phytochemicals play many important roles as control of bacteria and maintain the balance of the microbial composition in the gastric systems.

In the current study an attempt was made to identify the compositon of phytochemicals qualitatively and quantitatively of the bract of *Musa* sps. Variation in the composition of phytochemicals was observed with change of solvent system. The results indicated the presence of alkaloids, tannins, and sterols in the methanolic extracts. Only tannins were observed in the aquatic extracts of bracts.

Similar results were reported from the *Musa paradisica* and *M.acuminata* sps. Bracts of *M. paradisica* reported to have alkaloids, flavanols, lignin (Abbas et.al.,2015),and Anthocyanin (Swati et.al.,2011). However, in the case of *M.acuminata* , alkaloids and cycloglycoside (Petroleum Ether), no secondary metabolites in Chloroform extraction, Flavonols (Ethyl acetate); tannins, coumarins and total phenols (water) and Alkaloids, saponins, tannins, flavonols, terpenoids, Coumarins, cycloglycosides, total phenols and steroids (methanol) (Gunavathy et.al., 2014; Uma maheshwari et.al., 2017) were reported as phytochemical constituents in the earlier studies.

Quantitatively, the phytochemical compostion of methanol extract of bract comprised predominantly by tannins Alkaloids and total phenols were observed in the next order as chief phytochemical constituents of bracts. The TLC of bract extract showed 3 bands representing the phytochemical constituents of bract.

Musa sps (banana bract) although a very minimum but does exhibit antibacterial properties. The ethanol extracts of *Musa* extracts showed the broad spectrum of antibacterial activity on the laboratory as well as *Canine* microorganisms. However for the extraction of *Musa* sps chloroform is more suitable as the extract of *Musa* spp bract prepared in chloroform showed good antimicrobial activity as seen in bioautography.

Bioassay techniques such as bioautography is a simple and inexpensive tool for simultaneous chemico-biological screening of natural sources. The bioautography is a preferred tool in detecting the presence of antimicrobial compounds in extracts at the earliest stages of down streaming. Screening of antimicrobial compounds produced by banana bract extract was followed by their antimicrobial properties assessment indicated that the phytochemical constituents play an important role in maintaining the bract from microbial degradation and any types of diseases that attack them. Simultaneously they have good scavenging activity which found to be 11.1% ascorbic acid based on by DPPH method which was similar to that of the previous studies. (Baskar et.al., 2011) . Anti-oxidant, anti-microbial and toxicological properties of *Schinus molle* were well studied (Maria,2014). However, the comparative studies of anti microbial properties with that of *Musa* sps indicated that the pink pepper proved to be better in its activity.

The bracts of Musa sps. are of great value due to the anthocyanin component which help the plant parts as well as rich in phytochemical constituents which can be used as neutraceuticals. The future of medicine is largely depends on the nutraceuticals of plant sources. The difference in the efficacy of these plants products could be due to stage of collection of plant sample, method of extraction, geographical location, plant variety. Future studies are directed towards the development of purified bioactive compounds and quantitative determination of safe concentrations that can be used to improve existing drugs or to create new agents against *Canine* microbes.

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