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INVESTIGATING ANTIMICROBIAL EFFECTS OF DIFFERENT VARIETY OF FRESH AND DRIED BANANA PEEL EXTRACTS

V. Kavitha*[1] and Dr. G. Manonmani[2]

[1]Assistant Professor (SG), Dept of Costume Design & Fashion, Dr.N.G.P Arts and Science College.
[2]Assistant Professor, Dept of Home Science, Mother Teresa Women University.

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***Corresponding Author**
V. Kavitha
Assistant Professor (SG), Dept of Costume Design & Fashion, Dr.N.G.P Arts and Science College.

BACKGROUND

Banana is one of the valuable sources for maintaining human health. The use of fresh banana peel extractsfor antimicrobial properties can be of great significance in therapeutic treatments. Objective: This study aimed to evaluatethe antibacterial activity of both fresh and dried various banana peel extracts. Materials and Methods: Alcoholic extract of banana peel extracts was subjected to antibacterial efficacy against Gram-positive and Gram-negative bacteria by the well agar diffusion method. Results: The alcoholic extract of fresh and dried banana peel showed a various inhibitory effect against various microbial isolates. Highest inhibitory effects were observed on Nendran and Poovan against Staphylococcus aureus (13.55 ± 0.04), Bacillus subtilis (13.26 ± 0.02), and Pseudomonas aeruginosa (14.5 ± 0.00). Conclusion: Alcoholic peel extracts of fresh and dried banana could be considered as a good antibacterial agent against both Gram-positive and Gram-negative bacteria.

KEYWORDS: Alcoholic banana peel extract, Banana, Gram-positive and Gram-negative bacteria, Well agar diffusion.

INTRODUCTION

Nowadays screening of alternate effective and safe medicine from potential medicinal plants is led by the increasing antibiotic-resistant microbial infectious agent. The phytometabolites have substantial potential to *impede* bacteria, fungi, and virus. Various parts of the Plant such as bulb, gel, leaves, roots, barks, peels etc. were used for the extraction of phytometabolites (**Kinghorn***et al*., 2010). The present practice of medicine today has remodel a lot from its methods in medieval times. However, in India, we still use traditional methods for treatment of several diseases since Vedic period (**Surathu** and Kurumathur, 2011).

Banana one of the tropical fruit belonging to *Musaceae* family is grown in many regions of all over the world (**Shadma***et al.*, 2014). All parts of the banana plant such as flower, pulp, stem, and leaves have a medicinal application (**Imam** and Akter, 2011). The flowers of the banana is used to cure bronchitis and dysentery and ulcers; cooked flowers are utilised in the treatment of diabetics; the astringent plant sap are for hysteria, epilepsy, leprosy, fevers, hemorrhages, acute dysentery and diarrhea, and then it is applied to hemorrhoids, insect and other stings and bites; young leaves are kept on the poultices on burns and other skin diseases; the astringent ashes of the unripe peel are given in dysentery and diarrhea, and used for treating malignant ulcers (Girish and Satish, 2008); the roots are used to control digestive disorders, dysentery, and other ailments; banana seed mucilage is given in cases of diarrhea in India (**Bhat***etal*., 2010).

The previous studies have shown waste material of Banana peel has medicinal properties (**Shadma***et al.*, 2014, Imam and Akter, 2011). The bio diversified compounds such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids are present in banana peel which hasa pharmacological effect, especially as an antioxidant, antidiabetic, anti-inflammatory, and antibiotic (**Chabuck***et al.*, 2013). Phytocompounds extracted from various parts of the banana plant in which exhibited significant inhibitory effect towards the foodborne pathogens, hence banana plant should be considered to be a potential natural source of antimicrobial as well as antioxidant agent (**Fagbemi***et al.*, 2009). Therefore, the current study was focus to access the antimicrobial activity of fresh and dried Banana peel extracts against clinical pathogens as a comparative study.

MATERIAL AND METHODS

Selection of procurement of bacterial culture

The bacterial culture *S.aureus, Bacillus subtilis, P.aeruginosa*, and *E.coli* were obtained from Microbiological laboratory of Kovai Medical Centre and Hospital (KMCH), Coimbatore and the antibacterial assay was carried out in the Department of Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore.

Sample collection

The eight variety of the banana peels namely Rasthali, Nendran, Kadali, Red banana, Karpooravalli, Robusta, Poovan and Pachainadan used for the study was acquired from the farmers in and around Coimbatore. Fifty percent of each peels were directly used for extraction and remaining fifty percent peel was air-dried and ground into powder with a mechanical blender. The powdered samples were stored in clean brown bottles at room temperature for further use.

Rasthali

It is a medium- sized and tall variety grown in Tamil Nadu, Andhra Pradesh, Kerala, Karnataka and Bihar. Its peculiar fruit quality has made Rasthali popular and a highly prized due to its demand. initially the fruit is in yellowish green throughout their development, then turn pale yellow to golden yellow after ripening. Fruit is delicious with a good smell. rasthali is expensive to grow because of long crop duration and requires a bunch cover to product fruits from sun cracking and formation of lumps in the fruit.

Nendran: this is very famous in kerala where it is used as a fruit and for processing. In recent times, nendran has been picked up in tamilnadu as well. Nendran is notable for its variety in plant height, pseudostem shading, presence or nonappearance of male hub, bundle size, and so on .Fruits have a distinct neck with thick green skin which turns yellow on ripening. Fruits remain as starchy even on ripening. Nendran is highly permits Banana Bract Mosaic Virus (BBMV), nematodes and borers.

Banana is an edible fruit, botanically a berry, produced by several kinds of large herbaceous, flowering plants in the genus Musa.In some countries, bananas used for cooking may be called plantains. The fruit is variable in size, color, and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind which may be green, yellow, red, purple, or brown when ripe.

Red Banana (AAA): Red banana is the most relished and costly variety of Kerala and Tamil Nadu, Karnataka, Andhra Pradesh and to some extent in Western and Central India. It has other names too,In Bihar and other regions, it is popular as LalVelchi while in Karnataka as Chandra Bale. The plant weighs upto 20-30 kg under good management practices. Fruits are delicious, orange yellow coloured and with a pleasant aroma.

Karpuravalli: It is the most popular variety of banana grown r in Central and Southern districts of Tamil Nadu and Kerala. In Bihar, cultivation is done in patches under the name 'Kanthali'. Karpuravalli is a tall, robust plant well suited to marginal soils, produced under low input conditions. Karpuravalli is occasionally seeded depending on the seasonal variability. It is ash coated golden in colour and sweet fruits have good keeping quality.

Robusta: It is a medium variety, grown mostly in Tamil Nadu Karnataka Andhra Pradesh and Maharashtra. It is a well developed fruit with high yield and has bunch in large size. Dull green natural products turn splendid yellow while maturing. Pack weighs around 25-30 kg requires propping. Organic producthave a snappy separate of mash in the wake of maturing consequently not appropriate for significant distance transportation.

Poovan: It is one of the commercial cultivar grown throughout the country specific ecotypes like palayankodan in Kerala, Poovan in Tamil Nadu, KarpuraChakkarakeli in Andhra Pradesh and Alpan in North Eastern Region. It is mostly cultivated as a perennial crop. Tamil Nadu is the leading producer of Poovan cultivar owing to its climatic and marginal soil condition. Poovan is also cultivated commercially for leaf industry throughout Tamil Nadu and in certain parts of Kerala. Fruit is moderately acidic, firm and has typical sour-sweet aroma. Fruits turn to attractive golden yellow on ripening. It is medium sized bunch which is closely packed in order to keep the good quality and resistant to fruit cracking is its plus points. But it is extremely susceptible to Banana Bract Mosaic Viral (BBMV) disease and Banana Streak Virus, (BSV), which cause considerable reduction in yield.

Pachanadan: It is a popular variety in Tamil Nadu grown especially for its cooling effects in hot tracts in summer. The variety can be cultivate well in marginal soils without any yield reduction. The bunch weight ranges from 12-15 kg (after 11-12 months.

Extract preparation

Extraction, as the term used pharmaceutically, comprises the breaking up the active components of the banana peel from the inactive portions by using the ethanol in standard extraction procedure. The preparation of the plant extract was executed in three stage: drying, grinding and extraction. The collected fresh and mature banana peels was coarsely chopped, 50% of the peel used for extraction without drying and 50% was allowed to dry in the shade to avoid breakdown of important compounds. Dry grinding of the dry banana peel was done in grinder mixer. After that the dry powder was sieved to separate the dirt and lump particles.

Fresh banana peels and dried banana peels powder were kept in 70% ethyl alcohol for extraction. The extraction consists solvent penetration into the herbal cells, solubilisation of secondary particles and in the end, releasing the dissolved secondary particles in the solvent of extraction.

Then, the entire mixture was homogenized in blender and left at room temperature for about 48 h. As the

reaction continued, the yellow transparent liquid turned to amber and later to an opaque black liquid that served as the indicator for completion of the reaction. After reaction is complete, the entire slurry was filtered through Whatman filter to obtain banana peel extract (**Edwards**, 1999). The filtrate was exposed to revolving vacuum evaporator to get strong dissolvable free curd remove and put away for additional bioassay.

***In vitro* antibacterial assay**

A loop full of bacterial cultures were inoculated into nutrient broth incubated at 37 °C for 18 hours and checked the purity. The log phase bacterial suspensions were diluted with sterile nutrient broth to adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU / ml. The sterile cotton swab was dib into the standardized bacterial culture to make lawn culture on Mueller-Hinton agar surface of plates and the plates were left for 5-15 minutes at room temperature to dry. Sterile corkborer was used to cut well (6mm diameter) on lawn cultured plates. Solvent-free banana peel extracts were dissolved in Dimethyl sulfoxide (DMSO), from this 0.1ml was added to the well. DMSO and chloramphenicol were utilized as negative and positive control individually.The plates were incubated at 37 °C for 18-24h and the size of the zone of inhibition was measured. Each experiment was carried out in triplicate.

Determination of Minimum Inhibitory Concentrations (MICs)

The banana peel extracts were subjected into Determination of the minimum inhibitory concentration (MIC) using the tube-dilution technique (**Murray,** 2007). A two-fold serial dilution was made using Muller Hinton broth (MHB). The following concentrations were obtained: 1025mg/ml, 512.5mg/ml, 256mg/ml, 128mg/ml, 64mg/ml, 32mg/ml, 16mg/ml and 8mg/ml. Equal volume of extract and Muller Hinton broth (2ml) was dispensed into sterilized test tubes. A quantity (0.1ml) of standardized inoculum (1.5×10^8cfu/ml) was added to each of the test tubes which were incubated aerobically at 37^{o}C for each 24h. A tube containing broth and inoculum without extract similarly tube with broth and extract without inoculum served as organism control and extract control respectively. The lowest concentration of the extracts which inhibited microbial growth (no turbidity) was recorded as the minimum inhibitory concentration (MIC).

Statistical analysis

Each experiment was done in triplicate, and the data were expressed as mean± standard error of mean.

RESULTS AND DISCUSSION

Totally eight dried peel extract and eight fresh various variety of the banana peel extracts were used for the present study. The antibacterial efficacy of both fresh and dried banana peel extracts against clinical isolated were tested and the results were organised(Table 1 and 2). The fresh peel extract of Nendran showed great activity against *S.aureus*(13.55±0.04) and *P.aeruginosa* (14.5±0.00) and showed moderate activity against *B.subtilis* (12.5±0.04) and *E.coli* (10.51±0.02), whereas the dried peel extract of Nendran did not show any activity against *S.aureus* and showed moderate activity against other organisms. Similarly, fresh peel extract of poovan showed significant activity than chloramphenicol used as positive control. Whereas, dried poovan peel extract not showed acceptable activity against clinical pathogens.

Other new and dried banana (Pachainadan, Rasthali, Robusta, Kadali, Rasthali and karpooravalli) strip extricates indicated least viability against the microorganisms.

Table 1: Antibacterial activity of dried banana peel sample.

Sample name	Zone of inhibition in mm			
	S.aureus	***B. subtilis***	***P.aeruginosa***	***E.coli***
Rashthali	9.53±0.47	9.5±0.04	12.51±0.02	9.51±0.2
Nendran	0	9.15±0.11	10.48±0.02	9.09±0.11
Kadali	0	13.5±0.04	12.26±0.17	9.09±0.12
Red banana	0	9.16±0.12	14.49±0.00	9.02±0.02
Karpooravalli	9.51±0.02	9.4±0.15	9.5±0.00	0
Robusta	9.52±0.02	10.53±0.2	10.53±0.04	9.51±0.01
Poovan	0	11.5±0.08	11.53±0.04	10.5±0.02
Pachainadan	9.15±0.12	10.5±0.08	0	11.49±0.04
Chloramphenicol	9.03±0.04	13.02±0.02	14.49±0.03	20.0±0.04

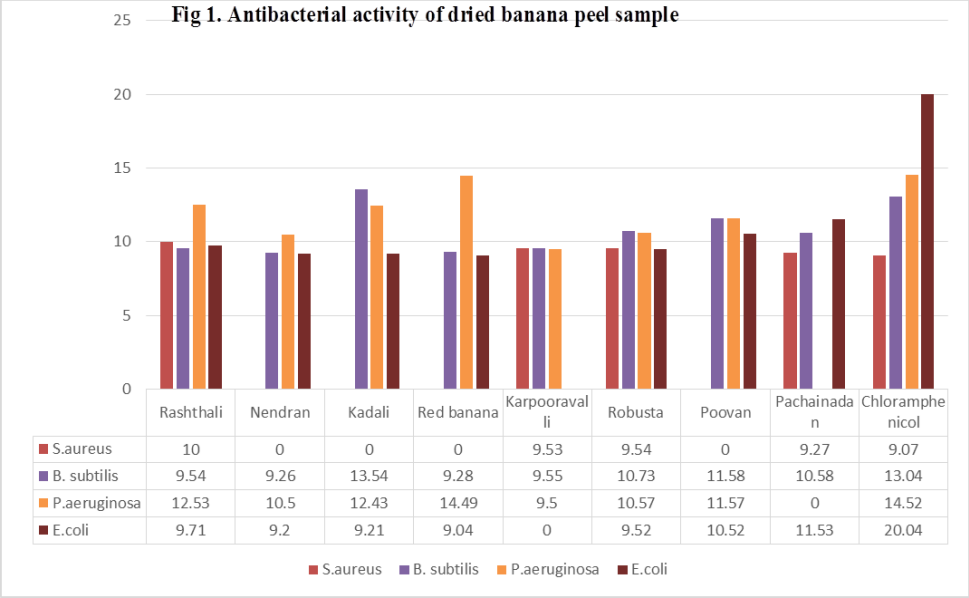
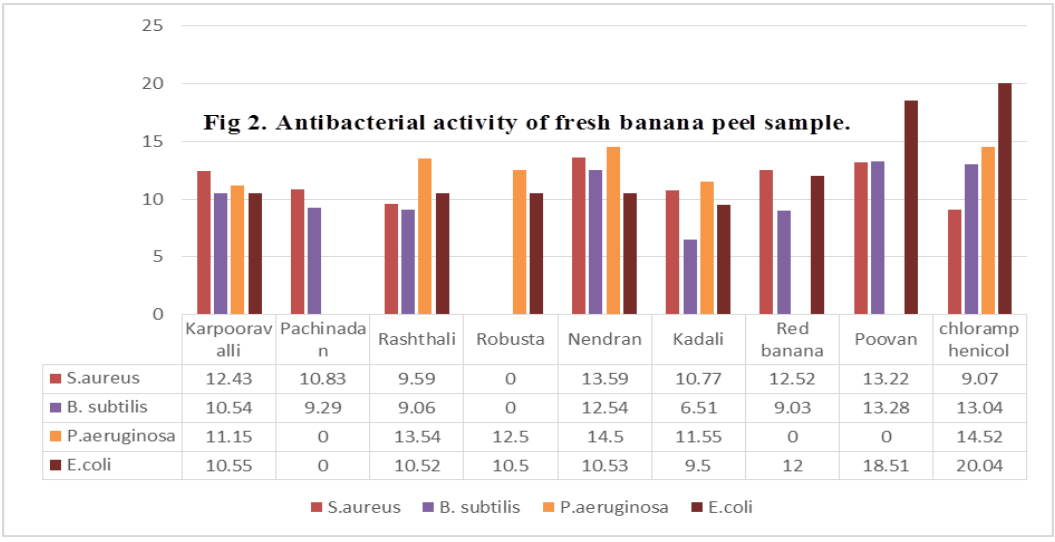


Table 2: Antibacterial activity of fresh banana peel sample.

Sample name	Zone of inhibition in mm			
	S.aureus	***B. subtilis***	***P.aeruginosa***	***E.coli***
Karpooravalli	12.23±0.20	10.5±0.04	11.07±0.08	10.51±0.04
Pachinadan	10.53±0.3	9.17±0.12	0	0
Rashthali	9.55±0.04	9±0.06	13.5±0.04	10.5±0.02
Robusta	0	0	12.47±0.03	10.5±0.00
Nendran	13.55±0.04	12.5±0.04	14.5±0.00	10.51±0.02
Kadali	10.55±0.22	6.1±0.41	11.52±0.03	9.5±0.00
Red banana	12.51±0.01	9±0.03	0	12.0±00
Poovan	13.13±0.09	13.26±0.02	0	18.5±0.01
chloramphenicol	9.03±0.04	13.02±0.02	14.49±0.03	20.0±0.04



Effect of plant constituents can combat human and plant pathogenic bacteria, fungi and viruses without toxic side effects and environmental hazards (**Hsieh***et al.*, 2001). The consumption of banana is good because of its nutritional value. It is used in anemia, stroke [**Roy and Saraf**, 2006] depression, stress [**Girish** and Satish, 2008] heartburn, [**Mokbel** and Hashinaga, 2005] etc., Banana peel which is an outer shell of banana also have been studied for the treatment of mosquito bites, [**Odebiyi** and Sofowora, 1978] gastrointestinal disorders, [**Pannangpetch,** 2001], and nipple fissures caused by Staphylococcus aureus.

Previous study reported the antifungal and antimicrobial properties of yellow banana fruit peel and found that it is effective against different Gram positive and negative bacteria (**Sumathy**, 2011).

In our current study, we concentrated on various fresh and dried banana peel extract to screen the efficacy on clinical pathogens as a relative study. In this study Gram positive and Gram negative microorganisms were exposed to evaluate the impact of banana peel extracts against infectious agent. The previous studies used either dried or fresh peel extracts only but in our present study both extract were examined their efficacy. This study revealed fresh nendran banana peel extract significant activity than chloramphenicol which used as positive control.

The higher measure of more bioactive mixes were extricated with ethanol 70% because of its higher extremity than unadulterated ethanol. In the current investigation additionally 70% ethanol was utilized for the extraction of dynamic mixes from the banana strip, it could be the explanation behind the movement of the nendran strip removes which show the natural solvents like ethanol one of the separating dissolvable to extricate the phytocompounds.

The ethanolic fresh banana peel extracts was evaluated MIC ranging from 8µg/ml to 1025µg/ml (Fig.1). The least MIC 128 µg/ml of Nendran and Poovan against S.aureus and moderate MIC (256µg/ml) against B.subtilis and P.aeruginosa was observed. Other banana peel extracts showed highest MIC against the clinical pathogens.

Certain studies conclude that that banana peel extract not only inhibit the non-spore forming bacteria but also unidentified substance extracted from banana skin has been shown to inhibit spore formation of bacteria by using plate biological assay, the unknown substance demonstrate inhibitory effects at pH values as high as 7.5 (**Aldean**, 2010).

CONCLUSION

Alcoholic peel extracts of fresh and dried banana could be considered as a good antibacterial agent against both Gram positive and negative bacteria to replace the synthetic medicines in treatment of diseases caused by bacteria. In our present study the fresh banana peel extract showed optimum level of inhibition against some clinical pathogens.