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ANTIOXIDANT POTENTIAL AND ANTITUMOUR ACTIVITIES OF *MUSA ACUMINTA* ON BONE CANCER CELL LINE

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ABSTRACT

Banana belongs to *Musaceae* family and is believed to have originated in Indo-Malaysian region. Banana is rich in carbohydrate, fat, protein, vitamins, minerals, water; not only a part of human diet but it is also used as a feed for domestic animals. Banana is considered as one of the most important commercial crops in the world. The present study has focused on the *in vitro* antimicrobial, antioxidant and anticancer effect of crude. *Musa* pulp extract on bacteria (*S.aureus, P.aeruginosa, K.pneumoniae and E.coli*), antioxidant (DPPH assay and total antioxidant capacity) and anticancer activity against MG63 cell line. Five *Musa* varieties collected from Kerala, South India were used in this analysis. The fruit of the varieties were extracted with various solvents. *Musa accuminata* Colla (AA) (Kadali: S4) ethanol extract showed high antimicrobial and antioxidant activity. Bioactive compounds like flavonoids, tannins and polyphenolic compounds were present in the pulp extract. This sample was used in anticancer examination in the MG63 Cell line. *Musa* fruit extracts expressed a high level of cytotoxic effect on MG 63 bone tumor cell lines with a high scope of usage as an edible drug in cancer treatment.

KEYWORDS

MG-63, DPPH assay, Total antioxidant capacity, Bioactive compounds and Polyphenolic compounds.

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INTRODUCTON

Banana belongs to Musaceae family and the edible fruit is usually seedless, is believed to have originated in Indo-Malaysian region. It is the national fruit of Central African Republic. Banana is known to be the fourth largest fruit in the world[1]. It is also grown as ornamental or for fiber. Banana is rich in carbohydrate, fat, protein, vitamins, minerals, water. It is not only a part of human diet but it is also used as a feed for domestic animals too[2] which can also grow in a wide variety of soil at

any particular climatic condition. Babatunde[3] states that all the parts are used to feed livestock.

Musa is distributed among the regions in the north from Nepal, southern China and in the south of Southern islands of Indonesia and New Guinea, Western limits in India etc[4,5]. Banana is considered as one of the most important commercial crops in the world. The taxonomy of the approximately 50 species within the genus Musa remains poorly resolved, not least because of the widespread vegetative reproduction and natural occurrence of many hybrids.

Banana has been commonly used as traditional medicine for various intestine related disorders. Musa species have shown antioxidant, antibacterial and anticancer properties since they contain secondary metabolites like phenols, alkaloids and terpenoids[6,7]. Cancer is one of the major diseases and their death rate is high in the world. The abnormal cells can disturb normal cells and affect the entire system. Free radicals are naturally produced in our body due to environmental stress and other factors which play a major role in elevating diseases like cancers, Parkinson's diseases and Alzheimer's[8]. Banana antioxidants can reduce plasma oxidative stress as it contains dopamine, ascorbic acid and other antioxidant compounds[7].

Secondary metabolites are bioactive compounds which have applications in wound healing, antidiabetic, antimicrobial, antioxidant and anticancer activities. Musa varieties are one of the plant varieties in which the entire plant system is rich in phenolics and flavonoids[9]. The banana leaves have the ability to inhibit polymerization of human chromosomes and inhibit cancer cell growth[10]. Musa fruit have antimicrobial traits because of the presence of secondary metabolites. These natural products help to maintain a healthy and disease free system[11,10] has proved that *Musa* peel has antibacterial activity against gram positive bacteria. The fruit and vegetable have high potent antimicrobial, antioxidant and anticancer reactions. The fruits show higher antimicrobial and antioxidant activity than peel[12].

Recent studies have focused on the natural antioxidant compound isolation and characterization because these compounds have potentiality against disease resistance. Polyphenols present in Musa fruits are a potential source of antioxidant and anticancer activity. This study has been done to isolate bioactive compounds from *Musa* fruit using solvents and analyze their antioxidant and anticancer activities in MG63 cell lines.

MATERIAL AND METHODS

Five different banana fruits were collected from Attapady, Palakkad District, Kerala, India. The peel was removed from fruit, the pulp was collected, shade dried at room temperature for 40 days, powdered and stored at 4°C until further use. The samples were labelled as follows: S1: Musa acuminate Colla (AAA), S2: Musa X paradisiaca L. (AAB) S3: Musa X paradisiaca L. (AB), S4: Musa accuminata Colla (AA) and S5: Musa Pisanglilin (AA).

Preparation of Plant Extract

Ten grams of dry fruit powder was dissolved in 100ml of solvent individually (ethanol, acetone and water) in a conical flask and kept for 24 hours in a magnetic rotatory shaker. The mixture was then filtered twice using Whatman No.1 filter paper and stored at 4°C for further analysis[13].

METHODOLOGY

Antibacterial Analysis

MHA (Muller Hinton Agar) plates were prepared and swabbed with individual test pathogens (*S.aureus, P.aeruginosa, K.pneumoniae and E.coli*). A well was cut in the middle of the plate and loaded with 10µl of fruit extract and dried. The plates were incubated at 37□ for 24 hrs. Zones of inhibition against test pathogens were observed after the incubation period[14].

Antioxidant assay

The antioxidant activity of extracts were analyzed using standard protocols.

Assay of DPPH radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging method is used as one of the parameters for antioxidant assay of the pulp extract[15]. 200µl plant extract of different strength (5, 25, 50, 100, 500µg/ml) was mixed with 2ml of 0.004% methanol

solution of DPPH. After 30 min, the absorbance was determined at 517nm using a UV spectrophotometer against a blank. Standard agent (Ascorbic acid) was used as control[16].

Determination of total antioxidant capacity

Total antioxidant capacity of the plant was analyzed by phosphomolybdenum method[6]. 0.3ml of extract was mixed with 3ml of reagents solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) and was incubated at 95°C for 90 min. The reduction of Mo (VI)-Mo (V) by the extracts followed by the formation of a green phosphate /Mo (V) complex at acid pH forms the basis of the method. After cooling, the reaction solution to room temperature, the absorbance was determined at 695nm using a UV spectrophotometer against a blank. Standard agent (Ascorbic acid) was used as control. The antioxidant activity of the extract was expressed as the number equivalent of ascorbic acid[8].

Cell line maintenance

Human MG63 cell lines were obtained from National Center for Cell Sciences (NCCS), Pune, India. Dulbecco's Modified Eagle Media (DMEM) was utilized for maintaining the cell line, which was enhanced with 10% Fetal Bovine Serum (FBS). Penicillin (100U/ml) and streptomycin (100µg/ml) were added to the medium to prevent bacterial contamination. The medium with cell lines was kept in a humidified environment with 5% CO_2 at 37°C.

Acridine orange and ethidium bromide staining

AO and EtBr staining with DNA allows visualization of the condensed chromatin of apoptotic cells. The control and extract-treated cells were seeded in a 6-well plate (3 × 104/well) and incubated in CO2 incubator for 48 h. The cells were fixed in methanol: glacial acetic acid (3:1) for 30 min at room temperature washed in PBS and stained with 1:1 ratio of AO/EtBr. Stained cells were immediately washed with PBS and viewed under a fluorescence microscope (Nikon, Eclipse TS100, Japan). The number of MG-63 cells expressing apoptotic features was counted and expressed as a fraction of the total number of cells present in the field 4.

RESULTS AND DISCUSSION

Antibacterial analysis

The ethanol extract of all the five Musa varieties exhibited maximum antimicrobial activity against all the test organisms which is given in Table No.1 and Figure No.1.

S1: *S.aureus, P. aeruginosa, K. pneumonia, E.coli*$^{q\text{-}t}$
S2: *S.aureus, P. aeruginosa, K. pneumonia, E.coli*$^{a\text{-}d}$
S3: *S.aureus, P. aeruginosa, K. pneumonia, E.coli*$^{e\text{-}h}$
S4: *S.aureus, P. aeruginosa, K. pneumonia, E.coli*$^{m\text{-}p}$

S4: *S.aureus, P. aeruginosa, K. pneumonia, E.coli*$^{i\text{-}l}$

Antioxidant Assay

The antioxidant activity of the fruit extract is given in Table No.2 and Table No.3 (Figure No.2 and Figure No.3).

The results showed that Musa accuminata Colla (AA) (S4) shows significant activities than that of other four varieties. The results are in accordance with the one reported by[17]. However, Pereira and Maraschin[23] reported that bioactive compounds like carotenoid, phenolic compounds and biogenic amines are present in banana pulp and peel which are the antioxidant potential compounds used for treatment of Parkinson's diseases. The antioxidant activity is found to be interconnected with the presence ofpolyphenolic compounds[19,20,21].

Acridine orange and ethidium bromide staining

The AO/EtBr assay used to detect apoptotic effects of bioactive compounds in Musa pulp crude extract. These staining methods are used to identify early, late apoptotic cells and morphological variations in the cell[22]. The ethanol crude pulp extract showed 26.53% to 90.23% inhibition of cell proliferation and also showed a high cytotoxic effect of pulp ethanol extract in the MG63 cell line (Figure No.4). In the previous studies[23,20], banana peel showed moderate cytotoxic effect in MCF-7 and HepG2 cell line. It is clear that the bioactive compounds in Musa prohibit cancer cell growth by antioxidation and inhibition of cell proliferation, inducing cell cycle arrest and induction of apoptosis[23-26,20]. The Musapulp extract exhibits cytotoxic and apoptosis effects in bone marrow cell line MG63. Several studies have reported the IC50 value in banana extract in different cancer cell lines[23,27,28,20] has

shown cytotoxic effects in breast cancer cell lines. The banana peel and pulp is a potent agent of antioxidant and anticancer and it is also included in dietary supplements against bone cancer.

Table No.1: Anti-microbial activity of *Musa* species

S.No	Pathogen	Positive control	Aqueous extract						Acetone extract					
		Chloramphenicol	Distiled water (-ve control)	*S1*	*S4*	*S5*	*S3*	*S2*	Acetone (-ve control)	*S1*	*S4*	*S5*	*S3*	*S2*
1	Escherichia *coli*	15	No zone	No zone	No zone	No zone	No zone	1	No zone	16	10	13	15	17
2	Pseudomonas *aeruginosa*	10	No zone	No zone	No zone	No zone	No zone	1	No zone	15	15	17	20	15
3	Staphylococcus *aureus*	10	No zone	No zone	No zone	No zone	No zone	1	No zone	15	12	15	13	17
4	Klebsiella *Pneumoniea*	20	No zone	No zone	No zone	No zone	No zone	No zone	No zone	13	16	12	12	14

Table No.2: DPPH Antioxidant activity of Musa species

S.No	Concentration of extract	Percentage of inhibition standard	Percentage of inhibition sample (Ethanol extract)				
			S1	S2	S3	S4	S5
1	0.2mg/ml	41.93%	20%	34%	28%	36%	26%
2	0.4mg/ml	51.61%	34%	46%	38%	42%	46%
3	0.6mg/ml	67.74%	42%	54%	46%	52%	56%
4	0.8mg/ml	70.96%	58%	66%	66%	68%	64%
5	1mg/ml	87.09%	70%	76%	72%	80%	76%

Table No.3: Phospho Molybdenum method analysis of fruit extracts

S.No	Concentration of extract	Absorbance of standard at 695nm	Absorbance of sample at 695nm (Ethanol extract)				
			S1	S2	S3	S4	S5
1	0.2mg/ml	0.44	0.32	0.39	0.38	0.42	0.36
2	0.4mg/ml	0.68	0.48	0.52	0.58	0.66	0.60
3	0.6mg/ml	0.88	0.62	0.68	0.70	0.78	0.72
4	0.8mg/ml	1.08	0.78	0.80	0.85	0.98	0.81
5	1mg/ml	1.28	0.88	0.94	0.92	1.1	1.01

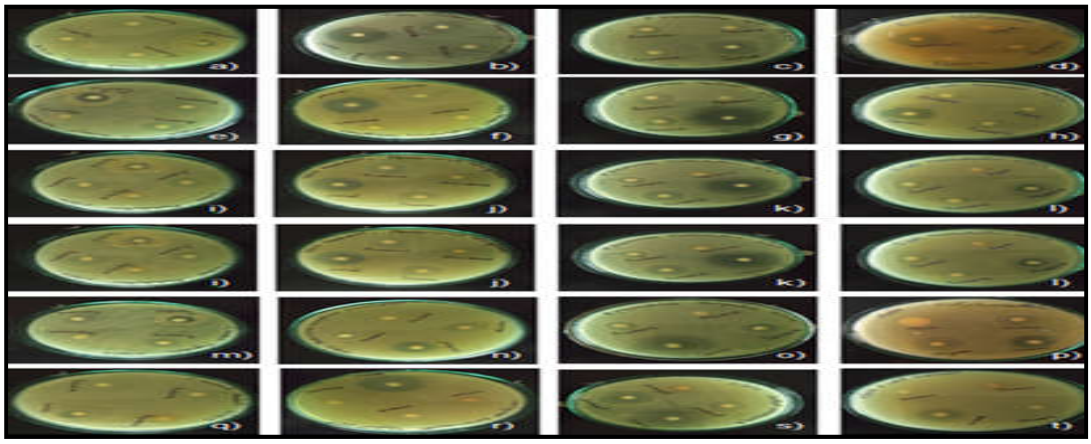

Figure No.1: Antibacterial activity of banana fruit extracts using disc diffusion method

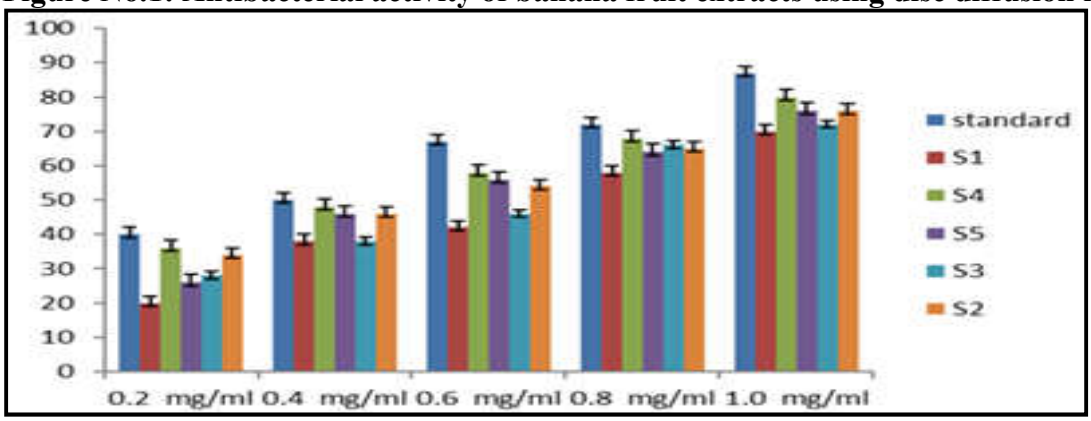

Figure No.2: DPPH Antioxidant activity of Musa species

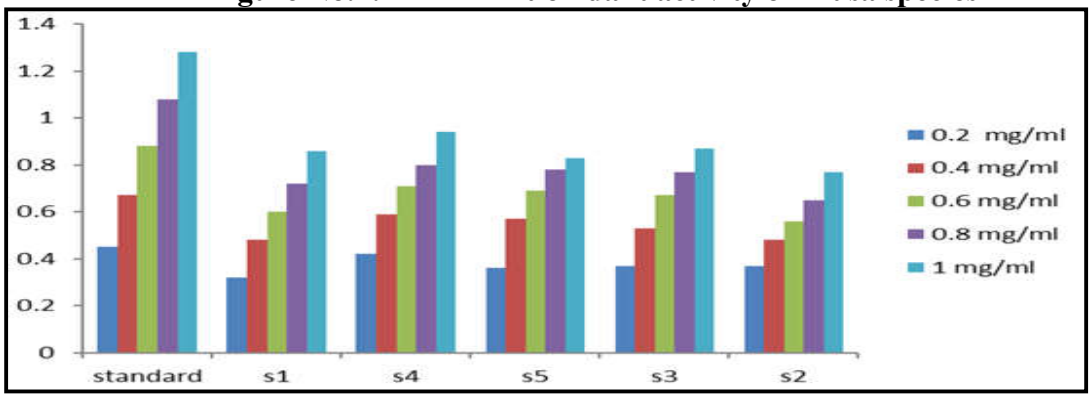

Figure No.3: Phosphomolybdenum Antioxidant activity of Musa species

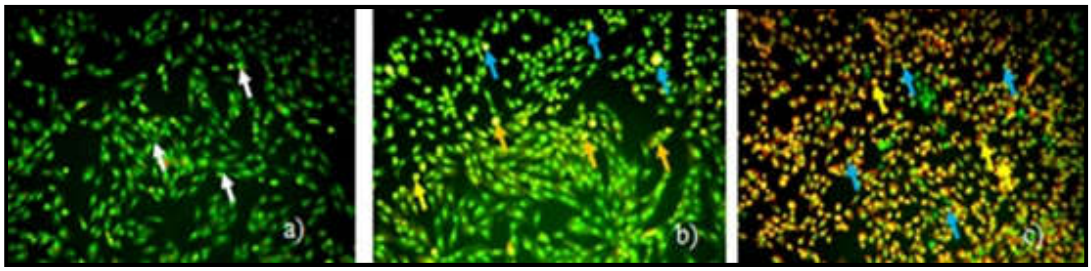

Figure No.4: Effect of extract induces apoptotic incidence. MG-63 cells treated within control and extract various concentrations at 24h, stained with dual dye EB/AO and then analyzed by fluroscence microscopy. a) Control, b) 100µg. White arrow indicates green florescence; orange arrow indicates apoptotic bodies; Blue arrow indicates apoptotic cells; Yellow arrow indicates necrotic cells

CONCLUSION

The present study has proved that the banana pulp is a natural antioxidant and anticancer agent because of the presence of provitamins, carotenoids and polyphenolic compounds. The banana is a richest source of bioactive compounds and micronutrients. These characters were used to produce drugs against different types of cancers. Detailed studies are needed for the identification of specific compounds, their mechanism and action in tumor suppression.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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