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Evaluating the Anticancer Properties of *Musa acuminata colla* Ethanolic Extract through *In vitro* Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study examined the possible anticancer properties of a colla pulp extract derived from *Musa acuminata*. It employed three different cancer cell lines, MDA-MB-231 (breast cancer), HCT-116 (colon cancer), and MG-63 (osteosarcoma). We evaluated the antiproliferative properties using the MTT test and identified the bioactive components through GC-MS analysis. The GC-MS analysis showed that the ethanol extract contained several compounds that could be useful in the fight against cancer. These compounds included palmitic acid, linoleic acid, 5-hydroxymethylfurfural, and 5-Methyl-2-ethylamino-2-thiazoline. The extract showed remarkable cytotoxicity against the cancer cell lines, with IC_{50} values of 250 µg/ml, 175 µg/ml, and 320 µg/ml for MDA-MB-231, HCT-116, and

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MG-63 cell lines, respectively. Finally, it was determined that banana pulp contains a significant number of phenolic compounds, provitamin A, and antioxidants. The present study suggests that bananas could have promising uses in the field of cancer research, treatment, and prevention.

Keywords: Breast cancer; colon cancer; osteosarcoma; anticancer; antiproliferation; bioactive compound; breast cancer.

1. INTRODUCTION

Cancers are a significant contributor to human mortality. There have been approximately 14 million new cases of cancer reported worldwide, resulting in 8.2 million cancer-related deaths. The most frequently diagnosed types of cancer included lung, prostate, colorectal, stomach, liver, and breast cancer for both men and women. The number of cancer-related deaths is projected to increase to over 14 million in 2015 to 30 [1].

According to Comsa et al. (2015), breast cancer is a significant form of cancer affecting women globally, making up approximately 23% of all cancer cases. Breast cancer is a condition characterized by the uncontrolled growth of cells in breast tissue. The aesthetic appearance of the breast may be affected by surgical treatments for breast cancer, while chemotherapy can lead to significant side effects [2]. Colorectal cancer (CRC) is a major health issue. It is the third most common cancer in men and the second most common cancer in women around the world. 2030 it is expected to be 60% common [3]. Environmental factors, especially changes in lifestyle and food, are said to increase the risk of CRC [4].

Osteosarcoma is a disease that can be life-threatening and is commonly found in adolescents. Osteosarcoma is a frequently occurring malignant bone tumor characterized by immature bone and osteoid tissue formation. It primarily affects the long bones of the arms and legs. There is a higher incidence in males compared to females, and it can occur with or without underlying pathology. Swelling, joint dysfunction, and local pain are frequently observed in cases of it [5]. There have been notable advancements in treatment options and survivability because of extensive research and trials. The field of cancer diagnosis and treatment offers a range of therapeutic approaches, as highlighted by various studies [6,7,8].

There are several common approaches to treat this condition, including chemotherapy, radiotherapy, immunotherapy, enzyme therapy, antibiotics, and surgery. Nevertheless, the agents commonly used are cytotoxic and can cause varying degrees of nausea, vomiting, and other complications, which can sometimes be more severe than the disease itself. The importance in finding new therapeutic options for cancer treatment cannot be overstated. It is crucial to enhance effectiveness and specificity, overcome resistance, and adopt a personalized approach for every patient [9]. Therefore, extensive research is being conducted to address various types of cancers using phytochemicals, with the goal of reducing side effects and improving patient compliance [10]. Current studies on using fruit-derived phytochemicals to combat cancer are showing great promise in targeting cancer cells. The phenolic compounds discussed in the research are reported to have minimal side effects, can be easily sourced from plants, and are economically viable [11].

Bananas are an interesting plant which was extensively cultivated in tropical countries [11]. The banana (Musa spp.) plant produces elongated and edible fruit. There are two primary parthenocarpic species in banana, namely *M. accuminata Colla* and *M. balbisiana Colla*. The health benefits of *M. accuminata Colla* are numerous and can be attributed to its rich array of bioactive compounds. These compounds, such as phenolics, carotenoids, biogenic amines, phytosterols, and volatile oils, are found in various parts of the plant, including the stem, fruit, pseudostem, leaf, flower, sap, inner trunk, root, and inner core [12]. The phytochemical composition of banana fruit is highly diverse, encompassing polyphenols, fatty acids, phytosterols, flavonoids, carotenoids, steroids, and biogenic amines [13,14,15,16]. These compounds contribute to the nutritional value and health benefits of bananas. The research conducted by Mathew and Negi [17] revealed that bananas have a wide range of pharmacological activities. These include antioxidant, immunomodulatory, antimicrobial, anticancer, antiulcerogenic, hypolipidemic, hypoglycemic, leishmanicidal, and anthelmintic properties. The study conducted by Mondal et al.

[12] found that banana extracts have shown promising results in preventing and combating various types of cancers, including breast, cervical, esophageal, hepatic, oral, prostate, skin, and colorectal cancers. The study explored multiple mechanisms, such as cytotoxicity, cell cycle arrest, apoptosis of cancer cells, antioxidant, and anti-inflammatory effects. In addition, extensive research has been conducted on the impact of bananas and their phytoconstituents on colorectal cancer, both in laboratory settings and in living organisms [18,19,20]. The study conducted by Dahham et al. [21], demonstrated the effectiveness of banana peel extracts in inhibiting the growth of MCF-7 cells. The highest inhibition was observed in the 12.07% ethanol extract, highlighting its potential as an anticancer agent.

Our study focused on examining the *In vitro* cytotoxic and antiproliferative effects of the ethanolic extract of colla pulp on breast cancer (MDA-MB 231), colon cancer (HCT-116), and osteosarcoma (MG-63) cells.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The fruit of *M. acuminata colla* was collected from Kerala, India. The banana sample was thoroughly cleaned using water to remove foreign particles. A knife was used to slice the entire banana, which was then left to dry at room temperature under sunlight for several days. The dried samples were finely ground using a dry grinder and subsequently stored in an air-tight container in a freezer at -20°C until extraction. A 10% ethanol concentration was employed to extract the dried sample (10 g) over 36 hours, with gentle agitation at a temperature of 37°C. An extract solution was prepared by diluting the stock with sterile dimethyl sulfoxide, resulting in a concentration of 1 mg/mL [22].

Gas chromatography mass spectrometry (GC -MS) analysis: The Agilent 7890A (GC) and Agilent 5975C (inert) MSDs, both manufactured by Agilent Technologies and fitted with triple-axis detectors, were used by the researchers to conduct a GC-MS analysis of the extract. For the GC separation, a 30 m long, 0.25 mm thick J&W CP-Sil-8 GC column was used. The injection volume was 1 µl, and helium was injected at a rate of 1 ml/min using a carrier gas of methanol (5 mg/ml). When injecting the samples, they used a split-mode technique (1:20). The GC oven was made to heat up from 50 °C (held for 1 minute) to 120°C at 10°C/min, then to 280°C at 5°C/min (held for 2 min). The spectrum values and related bioactive components were interpreted, and the database of the obtained spectrum was maintained in the GC-MS-NIST (2008) library [23].

2.2 Cell Culture

The NCCS Cell Repository at the National Centre for Cell Sciences in Pune, India, included cell lines from many kinds of cancer. These cancers included breast (MDA-MB-231), colon (HCT-116), and sarcoma (MG-63) tumors. The selected cancer cells were maintained in Dulbecco's modified eagles medium (DMEM) supplemented with 2 mM L-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 gL^{-1} Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g L^{-1} glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to 1 ml. The cells were maintained at 37°C with 5% CO_2 in a humidified CO_2 atmosphere.

2.3 MTT Assay (Cellular Viability)

The MTT experiment was conducted in accordance with the standard protocol followed by Harada et al. [24]. MG-63, HCT-116, and MDA-MB-231 are example of well-characterized and previously described cell lines. A control treatment or one containing 100 g/ml of MDA-MB-231, HCT-116, or MG-63 cells was applied to each well. Twenty-four hours were spent in an incubator at 37°C with MDA-MB-231, HCT-116, and MG-63 cells. Following incubation, the cells were exposed to various banana flesh extracts and CO_2 incubated for 48 h. The incubator's atmosphere consisted of 95% air and 5% CO_2. The drug-exposed cells were washed with fresh culture media, MTT (5 mg/ml in PBS) dye was added to each well, and the plates were incubated at 37°C for another four hours. After dissolving the purple precipitated formazan in 100 µl of concentrated DMSO, the absorbance at 540 nm was measured to determine cell viability. The numbers were reported as a proportion of unaltered cells against the control group. Over a period, the investigation of the optimal dose and half-maximal inhibitory concentrations (IC_{50}) was conducted.

$$\%\ \text{Inhibitory of cell proliferation} = \frac{Mean\ absorbance\ of\ the\ sample}{Mean\ absorbance\ of\ the\ control} \times 100$$

A sample of ethanolic extract of pulp and dose response curve was used to calculate the IC_{50} values, and cytotoxicity inhibition at 50% was calculated when compared with control cells. Each concentration was used as triplicate.

2.4 Acridine Orange/Ethidium Bromide (AO/EB) Staining Method

Three different cell lines, MDA-MB-231, HCT-116, and MG-63, were each seeded at a density of 5 x 10^4 in a 6-well plate and then incubated for 24 hours. After 24 hours of treatment with 100 to 500 µg/ml ethanol extract of colla pulp, cells were isolated, washed twice with cold PBS, and stained with a combination of AO (100 µg ml) and EB (100 µg ml) at a ratio of 1:1 for 5 minutes at room temperature. The plates were stained for five minutes with a mixture of acridine orange and ethidium bromide (1:1 ratio; 100 µg/ml) and then seen under a fluorescence microscope at 20x magnification. When counting the cells in the field, researchers took note of how many showed apoptotic characteristics as a percentage.

2.5 Statistical Analysis

Each experiment was repeated three times, and the results are shown as means and standard deviations. One-way ANOVA and significance testing were performed with the help of the 7.0 GraphPad Prism program

3. RESULTS AND DISCUSSION

3.1 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Fig. 1 indicates the results of the GC-MS Chromatogram, which detected a total of 21 compound peaks in the ethanolic extract of *M. acuminate colla* fruit. These peaks cover 100% of the area percentage and have varying retention times (Table 1). Several compound fragments were observed in various areas, including Glycerol. beta. -palmitate (27.02%), GUANOSINE (14.71%), 5-Methyl-2-ethylamino-2-thiazoline (10.49%), 3-Deoxy-d-mannoic lactone (9.23%), 5-Hydroxymethylfurfural (8.97%), 2-Ethylbutyric acid, eicosyl ester (4.79%), Di(1-methyl-1-silacyclobutyl) amine (3.55%). Similarly, Kumar et al. [25] reported the identification of 15 bioactive components utilizing the crude extract of *M. acuminate colla* fruit. In their study, Jordan et al. [26] identified a total of 43 volatile chemicals in the fruit of *M. acuminate colla*. The ethanol extract exhibited elevated levels of fatty acids, fatty acid esters, and various sterols, a few of which have been investigated for their potential role in cancer prevention.

Table 1. Bioactive compound in ethanol extract identified through by GC-MS

S. No	Name	R.Time	Area%
1	5-Methyl-2-ethylamino-2-thiazoline	7.995	10.49
2	ETHYL CAPRYLATE	8.269	3.08
3	N-(1,1-Dimethylpropyl)-2,2,3-trimethylaziridine-1-carboxamide	9.416	2.53
4	5-Hydroxymethylfurfural	9.841	8.97
5	ETHYL CAPRINATE	11.099	2.49
6	PENTADECANE	11.159	1.04
7	Di(1-methyl-1-silacyclobutyl)amine	11.318	3.55
8	GUANOSINE	13.010	14.71
9	TETRADECANE	13.233	1.88
10	HEXADECANE	13.680	0.86
11	3-Deoxy-d-mannoic lactone	15.578	9.23
12	HEXADECANE	16.050	0.98
13	ETHYL PALMITATE	19.431	1.69
14	ETHYL LINOLATE	23.327	0.59
15	ETHYL MARGARATE	24.067	0.55
16	Heptadecyl acetate	24.391	0.62
17	2-Ethylbutyric acid, eicosyl ester	29.967	4.79
18	Glycerol .beta.-palmitate	30.269	27.02
19	2-methyloctacosane	31.639	0.50
20	Glycerol .beta.-palmitate	33.693	2.40
21	.Beta.-Sitosterol	44.525	2.02

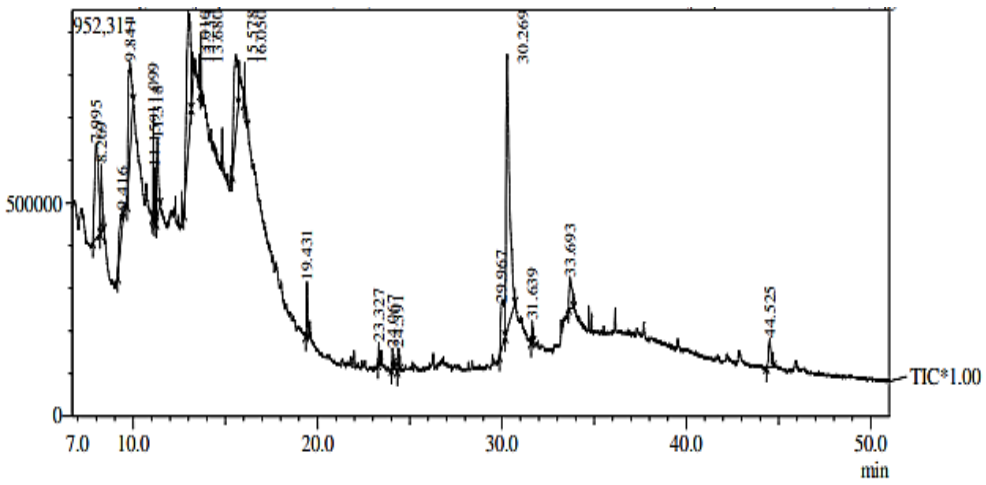

Fig. 1. GC-MS analysis of the ethanolic extract of *Musa acuminate colla*

3.2 MTT Assay

Cancer cells reveal unregulated cellular proliferation. Various herbal extracts have been demonstrated to impede the growth of cancer cells, as evidenced by multiple studies [27]. The cytotoxic effects of the ethanolic extract of *M. acuminate* colla were assessed on MDA-MB-231 (breast cancer cell), HCT-116 (colon cancer cell), and MG-63 (osteosarcoma cell) using the MTT bioassay for 24 hours (as shown in Figs. 2, 3, and 4). The ethanol extract had the highest level of antiproliferative activity across all cancer cell lines investigated. This finding demonstrates that the ethanolic extract of *M. acuminate colla* effectively suppressed the proliferation of MDA-MB-231, HCT-116, and MG-63 cancer cells in a manner that was dependent on the dosage. The IC_{50} values for the ethanolic extract of *M. acuminate colla* against MDA-MB-231 breast cancer cell lines were 250 µg/ml (Fig. 2), HCT-116 colon cancer cell lines were 175 µg/ml (Fig. 3), and MG-63 cancer cell lines were 320 µg/ml (Fig. 4). Following a 48-hour incubation period, banana extracts were analyzed using the MTT test to determine their ability to inhibit cell growth in MDA-MB-231 (breast), HCT-116 (colon), and MG-63 (osteosarcoma) cells (Fig. 5). The ethanol extract of *M. acuminate colla* showed inhibitory efficacy against three human lung cancer cell lines. The cytotoxicity seen in this study can be attributed to the phytocomponents present in the ethanolic extract of bananas.

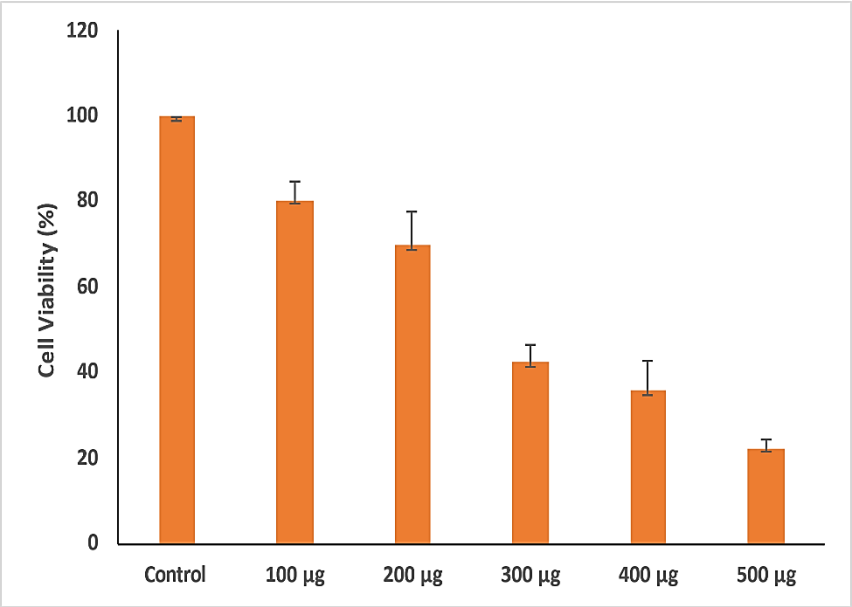

Fig. 2. The antiproliferative potential of banana flesh ethanol extract in MDA-MB 23

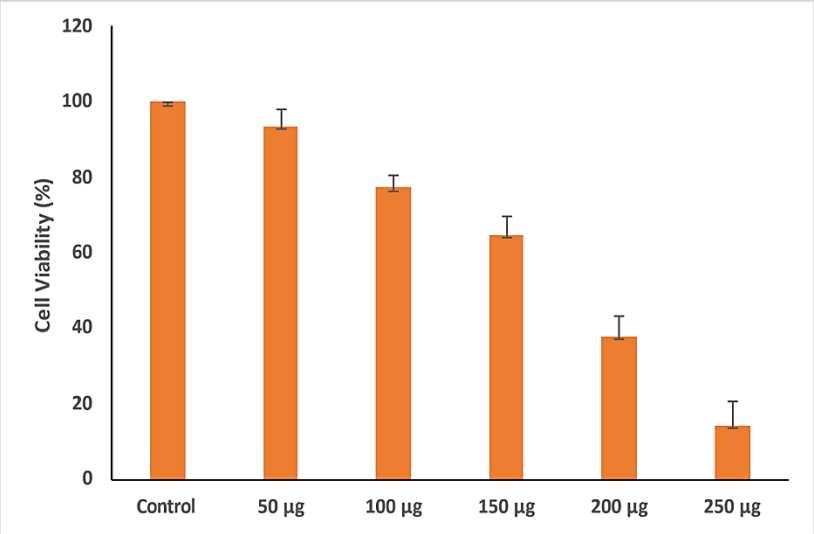

Fig. 3. The antiproliferative potential of banana flesh ethanol extract in HCT-116

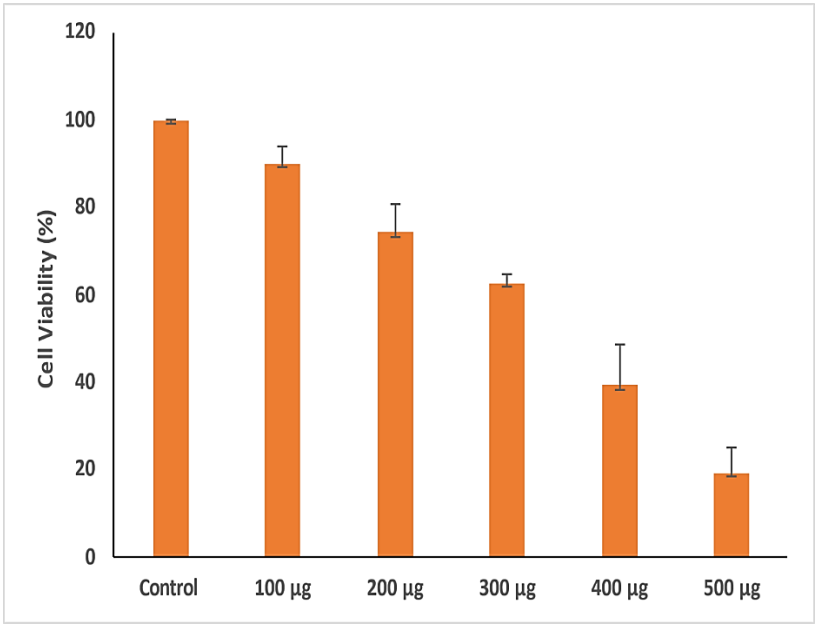

Fig. 4. The antiproliferative potential of banana flesh ethanol extract in MG-63

Several studies have documented the inhibitory effects of banana extracts on cell proliferation. Kim et al. [28] recently discovered that banana flesh extract demonstrated cytotoxic and apoptotic effects in PANC-1 human pancreatic cancer cells and MDA-MB-231 human triple-negative breast cancer cells. In a study conducted by Kamal et al. [29], it was discovered that extracts derived from banana peels exhibited *in vivo* anticancer and radioprotective characteristics. Consistent with our discovery, the ethanolic extract of banana (*M. paradisiaca*) showed anti-cancer properties against the HeLa cervical cancer cell line [30]. A study found that the hexane extracts of *M. sapientum* (Banana) fruit had the strongest inhibitory effects on the growth of HCT116 and MCF-7 cells, compared to ethanol and water extracts [21]. Additionally, it was reported that the extract of banana flour demonstrated anti-proliferative effects by inducing apoptosis in human colorectal cancer HCT116 and SW480 cells [31]. The research indicates that the phytochemical contents of banana extracts differ depending on the species, portions of the banana, and procedures used for extraction.

3.3 Acridine Orange/Ethidium Bromide (AO/EB) Staining

Colla pulp (*M. acuminate colla*) ethanolic extract prevents EB/AO staining in MDA-MB-231, HCT-116, and MG-63 cancer cell lines, respectively. The fluorescence microscopic analysis was carried out to the effect of apoptogenic activity of the Colla pulp) ethanol extract on cancer cells. Fig. 6 shows MG-63, HCT-116, and MDA-MB-231 cancer cells in the absence of Colla pulp extract (negative Control) and in the presence of ethanolic extract of colla pulp with inhibitory concentrations given in Figs. 6 (a, b & c). These results show that the untreated cancer cells (control) showed no significant variation. The treatment with colla pulp extract resulted in the transformation of green-colored cells to orange/red, indicating apoptosis and nuclear condensation. This effect is attributed to the action of colla pulp ethanolic extract at concentrations of 250 µg/ml for MDA-MB-231 breast cancer cell lines, 175 µg/ml for HCT-116 colon cancer cell lines, and 320 µg/ml for MG-63 cancer cell lines.

The results of our study align with prior research indicating that the ethanol extract of banana, along with its phenolic components, can trigger apoptotic cell death by activating caspases, cleaving PARP, and causing DNA fragmentation [21,32]. A recent study by Lavudi et al. [33] has demonstrated the strong anti-tumorigenic effects of Musa floral extracts on breast cancer cell lines MCF-7 and MDA-MB-231, as observed in *In vitro* experiments. A recent scientific investigation examined the effects of anthocyanin derived from *M. acuminata* on MCF-7 cell lines, which are associated with breast cancer. The study found that anthocyanin had potent anticancer properties [34,35,36]. Additional experimental findings, conducted on cervical carcinoma (HeLa), demonstrated an enhanced impact that varied in intensity based on the dosage when exposed to extracts derived from the rhizome of *M. acuminata* [37,38].

The bioactive components mentioned in the study by Salama et al. [39] have the potential to prevent cancer through many mechanisms, such as antioxidant activity, suppression of cell proliferation, activation of apoptosis, inhibition of cell invasion, and modulation of intracellular signalling networks [40-42]. Therefore, it can be concluded that *M. acuminata* colla possesses significant pharmacological capabilities, making it highly promising in the field of medicine.

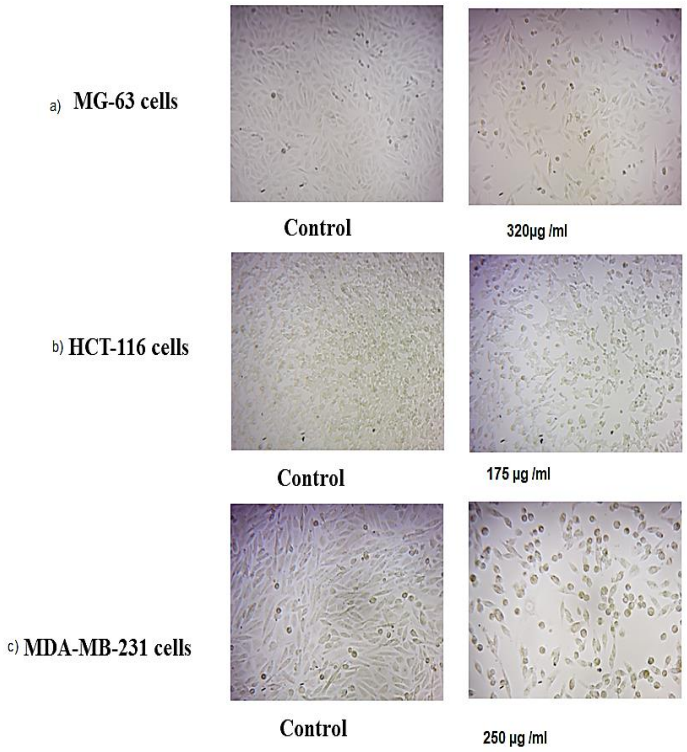

Fig. 5. Effects of ethanolic extract of banana flesh on the clonogenic ability of breast cancer (MDA-MB 231), Colon cancer (HCT-116), Osteosarcoma (MG-63) cell line

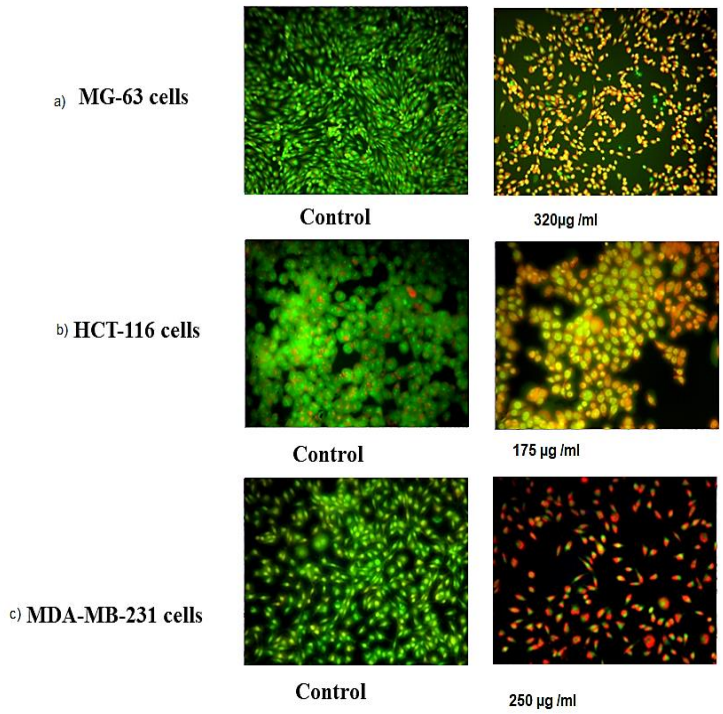

Fig. 6. Fluorescence microscopic analysis by ethidium bromide/avridine orange (EB/AO) staining of breast cancer (MDA-MB 231), Colon cancer (HCT-116), Osteosarcoma (MG-63) cell line

4. CONCLUSION

The ethanolic extract of banana (colla) pulp exhibits a more pronounced inhibitory effect on cancer cells growth. Additionally, the study found that the ethanol extract from bananas demonstrated an anti-proliferative effect by triggering apoptosis in human breast cancer (MDA-MB 231), colon cancer (HCT-116), and osteosarcoma (MG-63) cells. The study indicates that the ethanol extract of *M. acuminate* colla pulp shows promise as a potential source for the development of an anticancer agent.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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