RESEARCH ARTICLE

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Phytochemical Screening and biological potentials of Musa × paradisiaca L. leaves extract as anticancer and antioxidant agents

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Abstract

Background Many of these plants, have therapeutic effects and can be extracted and used in preparation of drugs, used directly or in combination with other plant extracts for medication which is a common practice in developing counties. Unfortunately, many of those who utilize these plants therapeutically do not have adequate knowledge or training in the safe use of the products. For these reasons, natural plant products need to be standardized and preliminary studies done to evaluate possible risks such as undesirable side effects, overdose and toxicity.

Results Ethyl acetate extract of Musa × paradisiaca L shown anticervical carcinoma and anti-malignant melanoma activity in our study. Antioxidant activity demonstrated, that Musa × Paradisiaca L. leaves ethyl extract exhibited % inhibition at absorbance 517 nm with IC50 values = 3.70 to 45.50 at different concentration and compared with ascorbic acid as standard drug.

Conclusions The present study indicates the anticancer and antioxidant activity on the basis of biological and phytochemical screening of Musa × paradisiaca L leaves extract. Ethyl acetate extract of leaves was evaluated for its anticancer activity. In vitro anticancer activity of extract were estimated by measuring significant inhibition of HeLa and A375 cell lines by MTT assay. The MTT assay clearly indicates that the inhibition or inhibitory activity of the extract was concentration dependent. Maximum inhibition of cell growth was found at the concentration of 320 µg/ml which was 54.35 and 55.97, respectively for HeLa and A375 cell lines. Therefore, 320 µg/ml concentration of extract was used to study the IC50 value that was calculated as 249.1 and 224.4, respectively. Antioxidant activity demonstrated that, plant extract exhibited percentage inhibition with IC50 values = 3.70 to 45.50 at different concentration and compared with ascorbic acid as standard drug.

Key words Musa × paradisiaca L plant, phytochemical screening, anticancer activity, antioxidant activity, MTT assay

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Introduction

Many medicinal plants, have therapeutic effects and can be extracted and used in preparation of drugs, used directly or in combination with other plant extracts for medication which is a common practice in developing counties. Unfortunately, many of those who utilize these plants therapeutically do not have adequate knowledge or training in the safe use of the products. For these reasons, natural plant products need to be standardized and preliminary studies done to evaluate possible risks such as undesirable side effects, overdose and toxicity [1]. The use of traditional medicines by traditional healers has played an important role in the health care of millions of people [2].

Many commercially sold medicinal plants might contain chemical substances with potential mutagenic and/or carcinogenic properties as well as with antitumor properties, and the active extracts detected by screening methods should be subjected to accurate bioassays to determine their specific pharmacological activity [3]. Cancer had always been the most serious disease in humans around the world due to its high morbidity and mortality. Cancer is among the leading causes of death worldwide. In 2018, there were 18.1 million new cases and 9.5 million cancer-related deaths worldwide. By 2040, the number of new cancer cases per year is expected to rise to 29.5 million and the number of cancer-related deaths to 16.4 million. Cervical cancer is the third most common cancer of women after breast cancer and colorectal cancer [4]. Carcinoma cervix is a type of cancer that occurs in the cells of the cervix, the lower part of the uterus that connects it to the vagina. The incidence and mortality rate due to this cancer have declined in the developed countries over past few decades but still remains to be the most common gynecological cancer and fourth most common malignancy in women across developing countries. Death rates for female breast and cervical cancers, however, were considerably higher in transitioning versus transitioned countries (15.0 vs 12.8 per 100,000 and 12.4 vs 5.2 per 100,000, respectively) [5]. In India, it is second most common cancer in females after breast cancer, with around a lakh new cases identified annually [6]. The highest incidence was recorded in Papumpare, Aizawl, Mizoram, accounting for a total of 27% of cancer cases amongst female in India [7, 8]. It accounted for 0.3% of all cancer patients and 27% of all female cancer patients seen [8]. Infection with human papillomavirus (HPV) is associated with more than 90% cases of cervix cancer [9]. Surgery, radiotherapy or chemotherapy, alone or in combination [10], can be used as therapeutic modalities for carcinoma cervix [11]. Malignant melanoma is the most dangerous form of skin cancer [12], being fifth most common tumor type among men and sixth in women in the United States [13]. In addition, malignant melanoma is 1.4 times more common among men than in women. The incidence of both non-melanoma and melanoma skin cancers have been increasing over the past decades and are reported maximum in Australia, New Zealand, Switzerland, Denmark and Norway [14]. Ultraviolet (UV) radiation and number of moles more than 100 is the greater risk for melanoma [15].

Surgery is the definitive treatment depending on number of factors, including the type of cancer, size of the lesion, anatomic location, available resources and patient preferences for early-stage melanoma, keeping or leaving medical management generally reserved as adjuvant treatment for advanced melanoma [16]. Moreover, nowadays doctors are exploring plant extracts to treat cancer, hypothesizing their traditional use because they can be used even in palliative care as well as to reduce the side effects associated with cancer treatment. The National Cancer Institute (NCI) has collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity. Over 3000 species of plants with potential antitumor activity have been reported [17]. Approximately 60% of anticancer compounds and 75% drugs for infectious disease come from natural products or their derivatives [18].

Antioxidants are the agents that neutralize free radicals, which scavenge reactive oxygen species may be high potent value in preventing the onset and propagation of oxidative diseases like neurovascular, autoimmune and cardiovascular diseases [19]. One of them is Musa × paradisiaca L. (**Fig. 1**) commonly known as Plantain belonging to family musaceae (banana family) [20]. It possesses several medicinal properties which include antilithiatic, antibacterial, antidiabetic, antiulcer, anti-diarrheal, hypocholesterolaemic, hepatoprotective, anti-snake venom, wound healing, hair growth promoting, anti-fungal and anti-menorrhagia activity [21].

Banana flower is a very good natural source for the development of an anticancer lead molecule with least side effects. Flower extract was checked for cytotoxicity on normal human peripheral lymphocytes. The anti-cancer activity of banana flower extract has evaluated on the cervical cancer cell line (HeLa) and displayed the highest cytotoxic activity [22]. Banana leaves acetone extract also presented vigorous activity against breast and liver hepatocellular tumor cell lines [23].

Based on aforementioned facts, we hereby report to evaluate the phytochemical screening, anticancer and antioxidant activities of Musa ×paradisiaca L plant leaves extract.

Materials and methods

Selection, collection and authentication of plant

Leaves of plant Musa × paradisiaca L. were selected for the present study and were collected from Herbal Garden of Maharshi Dayanand University, Rohtak, Haryana, India in the month of February, 2018. The leaves were authenticated by Dr. Sunita Garg, Emeritus Scientist, National Institute of Science Communication and Information Resources (CSIR-NISCAIR) Delhi (Ref no.-NISCAIR/RHMD/Consult/2018/3144-93).

Figure 1. Leaves of Musa × paradisiaca L. plant.

Table 1. Identified bioactive compounds of plant extract.

Sr No.	Name of Compounds	Molecular formula	Molecular weight	Retention Time	Area%
1	2-Ethylhexanol	$C_8H_{18}O$	130	7.992	0.20
2	Triethyl phosphate	$C_6H_{15}O_4P$	182	10.094	0.19
3	Octyl acetate	$C_{10}H_{20}O_2$	172	10.671	0.10
4	Docodecane	$C_{12}H_{26}$	170	11.858	0.26
5	Eugenol	$C_{10}H_{12}O_2$	164	15.059	10.96
6	Copaen	$C_{15}H_{24}$	204	15.514	0.09
7	Tridecanol	$C_{13}H_{28}O$	200	15.732	0.15
8	Tetradecane	$C_{14}H_{30}$	198	15.886	1.72
9	Caryophyllene	$C_{15}H_{24}$	204	16.372	1.40
10	Alpha caryophyllene	$C_{15}H_{24}$	204	17.032	0.20
11	Acetyl eugenol	$C_{12}H_{14}O_3$	164	17.979	1.93
12	β-Cadinene	$C_{15}H_{24}$	204	18.118	0.18
13	Octadecane	$C_{18}H_{38}$	254	18.937	0.26
14	Acetic acid, chloro- hexadecyl ester	$C_{18}H_{35}ClO_2$	318	19.303	0.59
15	Henicosane	$C_{21}H_{44}$	296	21.857	0.21
16	Hexadecyl iodide	$C_{16}H_{33}I$	352	22.176	0.43
17	1-Nonadecene	$C_{19}H_{38}$	266	22.515	0.32
18	Phytane	$C_{20}H_{42}$	282	22.623	2.68
19	Cyclohexane, 1-butylnonyl	$C_{18}H_{36}$	252	22.786	0.30
20	Neophytadiene	$C_{20}H_{38}$	278	23.166	1.32
21	Perhydrofarnesyl acetone	$C_{18}H_{36}O$	268	23.242	0.30
22	Phytol, acetate	$C_{22}H_{42}O_2$	338	23.521	0.39
23	Tetracontane	$C_{40}H_{82}$	562	24.709	0.33
24	Octacosane	$C_{28}H_{58}$	394	24.810	0.20
25	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	24.892	5.08
26	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	25.033	1.58
27	Tetracosane, 1-iodo-	$C_{24}H_{49}I$	464	25.110	0.41
28	Heptacosanol	$C_{27}H_{56}O$	396	25.418	0.33
29	Tetracosane	$C_{24}H_{50}$	338	25.509	2.00
30	Undecane, 4-cyclohexyl	$C_{17}H_{34}$	238	25.731	0.22
31	Heptacosano	$C_{27}H_{56}O$	296	26.709	0.84
32	Tetracontane	$C_{40}H_{82}$	562	26.855	0.59
33	Phytol	$C_{20}H_{40}O$	296	27.019	5.43
34	Pentatriacontane	$C_{35}H_{72}$	492	27.345	0.32
35	Phytol, acetate	$C_{22}H_{42}O_2$	338	27.491	0.55
36	3-Methyloctadecane	$C_{19}H_{40}$	268	27.786	0.34
37	Palmitic acid, butyl ester	$C_{20}H_{40}O_2$	312	27.953	3.80
38	Docosane	$C_{22}H_{46}$	310	28.147	1.71
39	Tetratriacontylheptafluorobutyrate	$C_{38}H_{69}F_7O$	690	28.237	0.31
40	Phytylpalmitate	$C_{36}H_{70}O_2$	-	28.296	0.30

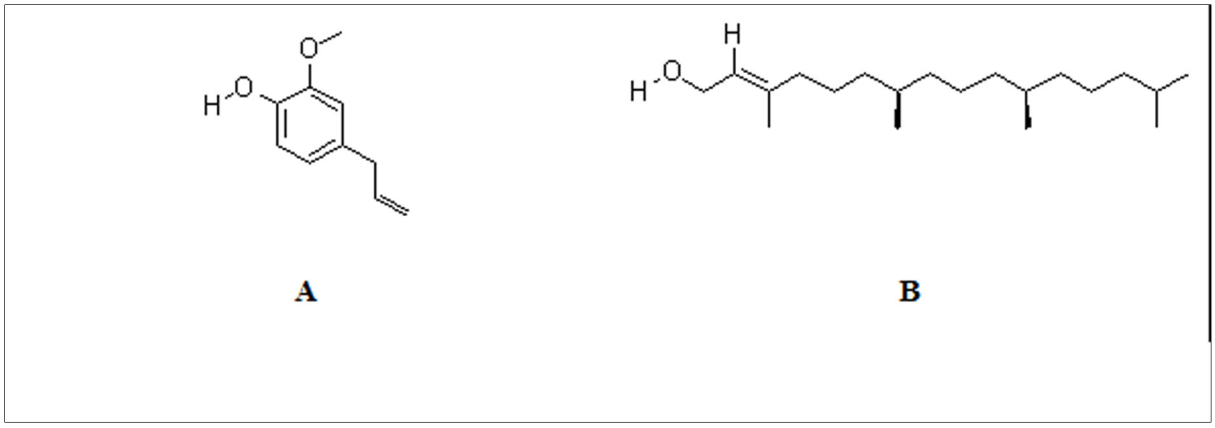

Figure 2. Structure of Eugenol (A) and Phytol (B).

Drying and powdering of plant material

The fresh leaves of Musa × paradisiaca L. were selected and washed under running and chopped into fine pieces and air dried in shade for 15 days. The dried leaves were crushed into coarse powder.

Preparation of extract

The coarse powder was extracted using Soxhlet apparatus [24]. The powder of dried leaves was defatted with petroleum ether for 6 h. The treated powder was further processed with ethyl acetate for 18 h and then it was concentrated by evaporating the solvent using water bath maintained at 60-80°C.

Qualitative analysis of extract by Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS Analysis of extract was carried out on a GC7890 (Agilent) comprising automatic liquid sampler and gas chromatograph interfaced to mass spectrophotometer (GC-MS) at Jawaharlal Nehru University, New Delhi, using Helium as a carrier and the injector temperature 270 °C. The oven temperature was programmed from 60 °C held for 15 min to 280 °C at 15° C/min.

Antioxidant evaluation

The antioxidant activity of ethyl acetate extract of Musa × paradisiaca L. leaf and standard ascorbic acid was evaluated on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity by modified DPPH assay [25]. DPPH 0.1 mM in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of banana leaves extracts at different concentrations i.e. 5, 10, 20, and 40 µg/ml. Methanol was used as a blank control and allowed to stand at room temperature for 30 min. Ascorbic acid was used as a positive control and negative control contained the entire reaction reagent except for the extracts. In this test, DPPH act as free radical scavenger as it accepts hydrogen (H) from the scavenging molecule and changes color from purple to yellow. The absorbance of the sample was measured by UV spectroscopy at 517 nm [26]. The inhibition was calculated as % inhibition. The concentration of extract was taken on X-axis whereas percentage of inhibition was taken on Y-axis for plotting the graph. DPPH assay clearly indicate that extract was effective in scavenging of free radical with compare of ascorbic acid. The inhibition activity was concentration dependent and with the increase in the concentration of extract shows more efficiency in inhibition. The absorbance was noted and % inhibition was calculated as follows:

% Inhibition = [(Abs standard -Abs sample) / Abs standard x 100]

Absorbance (Abs)

Evaluation of extract by MTT assay

Preparation of test solutions

Preparation of stock solutions (32 mg/ml) of the test sample in methanol for anticancer activity and serially three-fold dilutions were prepared from 10, 20, 40, 80, 160, and 320 µg/ml using

Table 2. Antioxidant activity of ethyl acetate extract of Musa x paradisiaca L. leaves.

S. No.	Concentration (µg/ml)	Absorbance at 517 nm	% inhibition of extract	%inhibition of Ascorbic acid
1.	5	0.622	3.70 ± 0.75	6.70 ± 0.23
2.	10	0.527	16.94 ± 0.65	14.00 ± 0.76
3.	20	0.240	30.28 ± 1.23	40.50 ± 1.43
4.	40	0.057	45.50 ± 2.43	55.32 ± 2.64

Values are mean ± SD of 3 replicates.

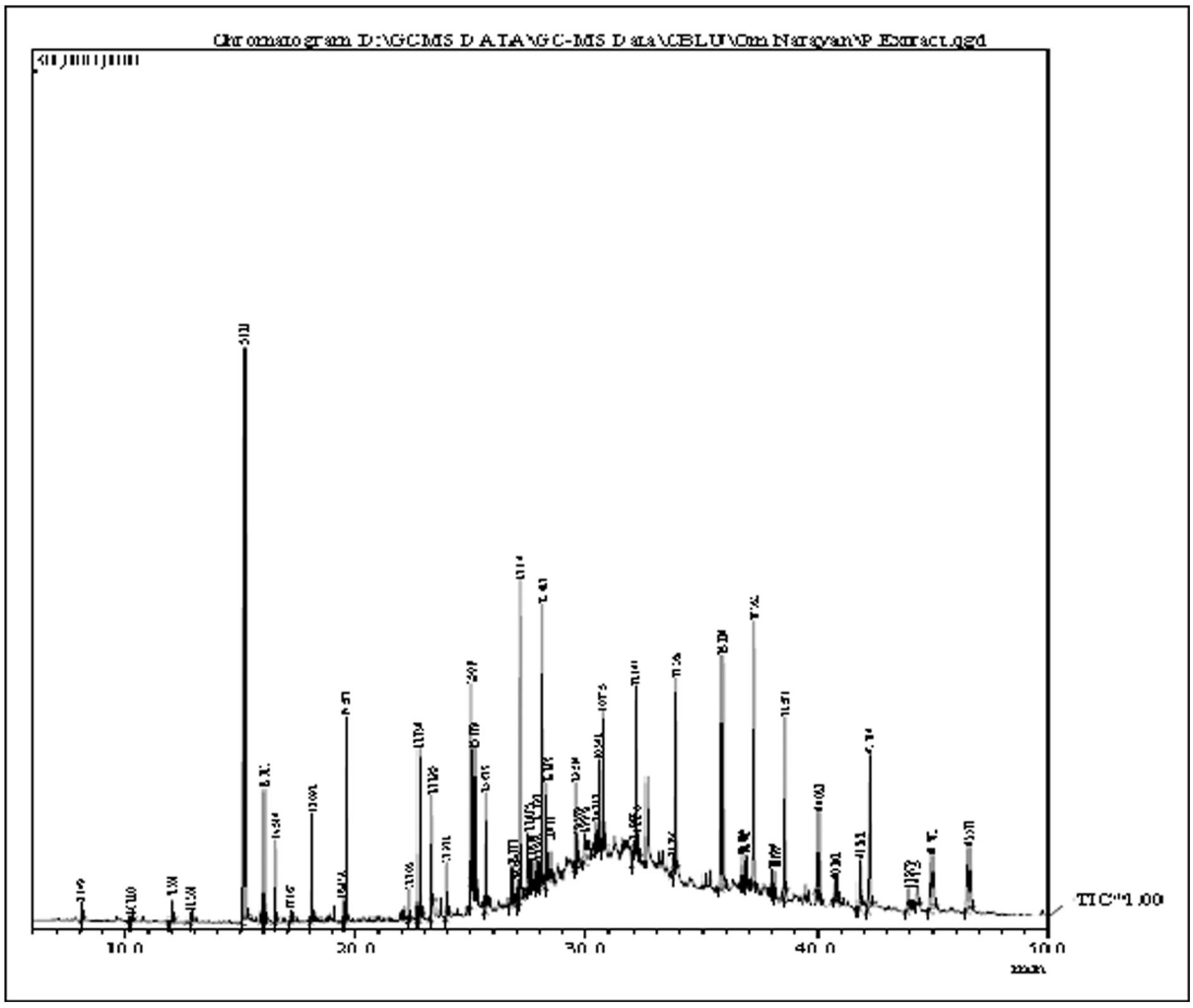

Figure 3. Chromatogram of GC-MS from ethyl acetate extract of Musa × paradisiaca L.

DMEM plain media for treatment.

Cell lines and culture medium

All the cell lines were procured from ATCC. Stock cells were cultured in DMEM, supplemented with 10 % inactivated Fetal

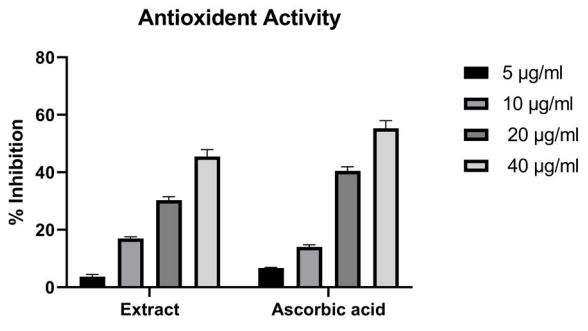

Figure 4. Graphical representation of antioxidant activity (% inhibition) (n±SD) n=3.

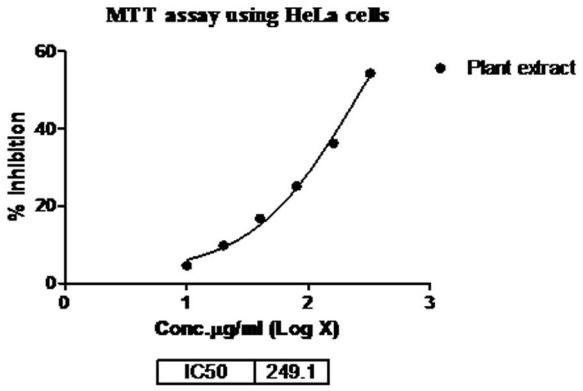

Figure 5. Dose response curve of extract against HeLa cancer cell lines(n±SD) n=3.

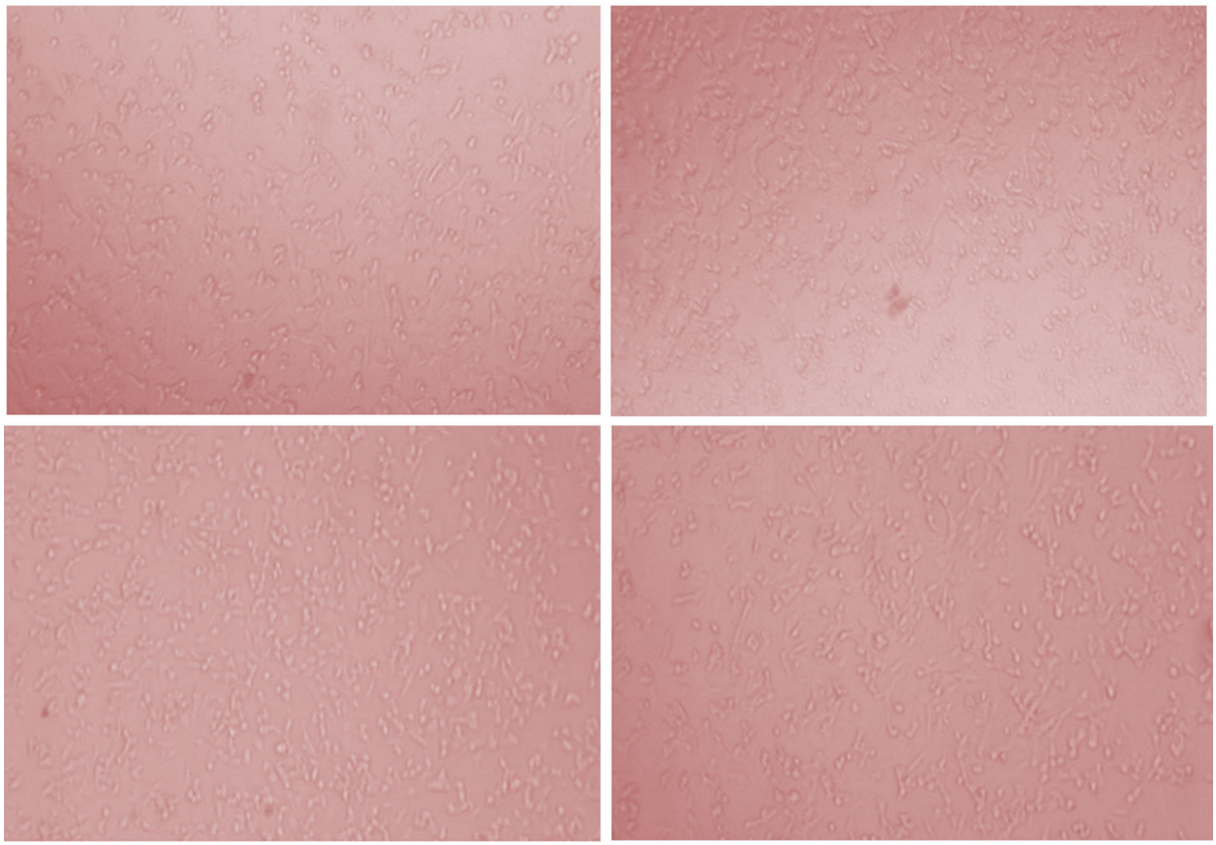

Figure 6. Growth inhibition against HeLa cancer cell line.

Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5 % CO2 at 37 oC until confluent. The cells were dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells was checked and centrifuged. Further, 50,000 cells per well were seeded in a 96 well plate and incubated for 24 h at 37oC, in 5 % CO2 incubator or with 5% CO2 atmosphere.

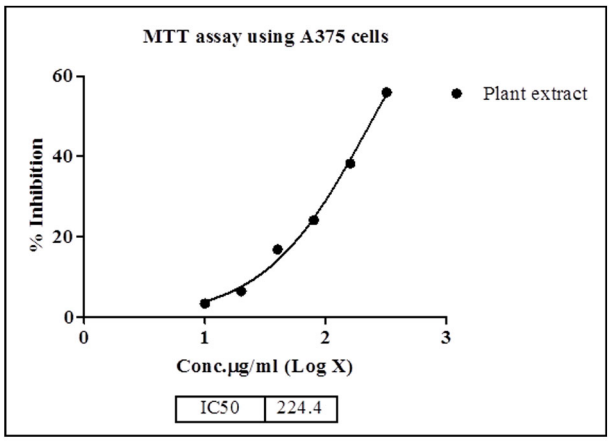

Figure 7. Dose response curve of extract against A375 cell lines (n±SD). n=3.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000 cells/well) was added. The extract was tested at six different concentrations in triplet form i.e. 10, 20, 40, 80, 160, and 320 µg/ml. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37 oC for 24 h in 5% CO2 atmosphere. After incubation, the test solutions in the wells were discarded and 100 µl of MTT (6 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37 oC in 5 % CO2 atmosphere. The supernatant was removed and 100 µl of dimethyl sulphoxide (DMSO) was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50 % (IC50) value is generated from the dose-response curves for each cell line [27-29].

% Inhibition = 100 – (OD of sample/OD of Control) x 100 where OD is the optical density.

Statistical analysis

IC50 Values were derived from a nonlinear regression analysis

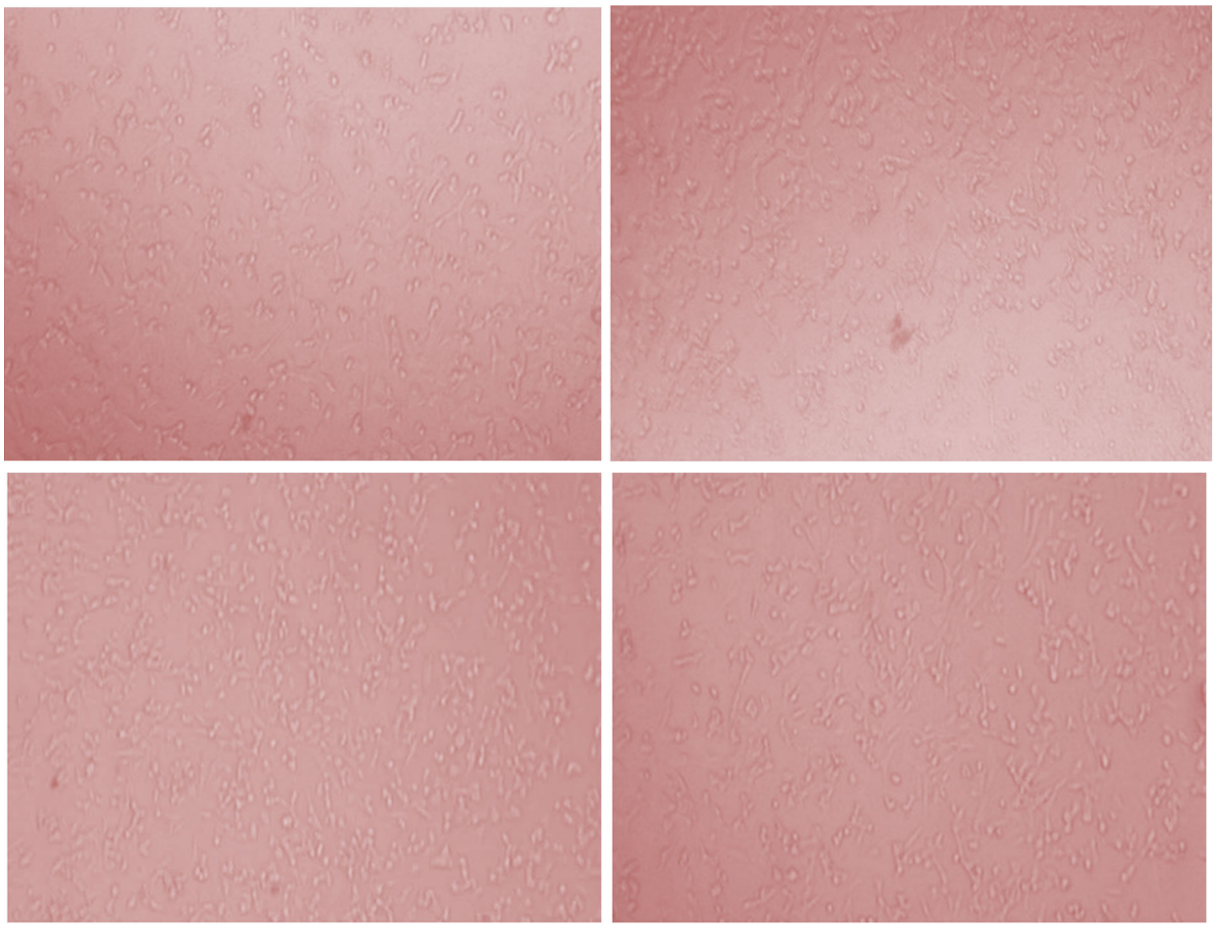

Figure 8. Growth inhibition against A375 cell line.

(curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, San Diego, CA, USA).

Results and discussion

Qualitative analysis of extract by GC-MS

Banana flower is a very good natural source for the development of an anticancer lead molecule with least side effects. Flower

Table 3. Anticancer screening results of plant leave extract on HeLa cell line.

Cancer cell (HeLa)	Anticancer screening results - MTT assay			
	Conc. (µg/ml)	OD at 590nm	% Inhibition	IC_{50} (µg/ml)
Control	0	0.582 ± 0.02	0.00	-
Leave extract	10	0.555 ± 0.01	4.56	249.1
	20	0.525 ± 0.01	9.77	
	40	0.484 ± 0.04	16.75	
	80	0.435 ± 0.02	25.19	
	160	0.371 ± 0.03	36.20	
	320	0.265 ± 0.01	54.35	

Experimental results are expressed as mean ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($P < 0.05$).

Table 4. Anticancer screening results of plant leave extract on A375 cell line.

Cancer cell (A375)	Anticancer screening results – MTT assay			
	Conc. (µg/ml)	OD at 590nm	% Inhibition	IC_{50} (µg/ml)
Control	0	0.681 ± 0.04	0.00	-
Leave extract	10	0.659 ± 0.02	3.32	224.4
	20	0.638 ± 0.006	6.43	
	40	0.567 ± 0.03	16.82	
	80	0.517 ± 0.02	24.11	
	160	0.421 ± 0.04	38.20	
	320	0.300 ± 0.01	55.97	

Experimental results are expressed as mean ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($P < 0.05$).

extract was checked for cytotoxicity on normal human peripheral lymphocytes. The anti-cancer activity of banana flower extract has evaluated on the cervical cancer cell line (HeLa CCL-2) and displayed the highest cytotoxic activity [22]. Forty bioactive compounds were identified in qualitative analysis by Gas Chromatography-Mass spectrometry (GC-MS) from ethyl acetate extract of Musa × paradisiaca L. The name of compound, molecular formula, molecular weight, retention time and percentage of the identified component were ascertained (**Table 1**). The nine major compounds having highest peak in chromatogram was eugenol (10.96 % of area), phytol (5.43 % of area), dibutyl phthalate (5.08 % of area), palmitic acid (3.80 % of area), phytane (2.68 % of area), tetradocosone (2.0 % of area), tetradecane (1.72 % of area), docosane (1.71 % of area), hexadeccanoic acid (1.58 % of area) and caryophyllene (1.40 % of area). Other compounds identified were acetyl eugenol, neophytadine, docodecane and heptacosano. Phytol and Eugenol shows higher antioxidant and anticancer activity. The chemical structure and GCMS graph of Eugenol and Phytol are shown in **Fig. 2** and **3**, respectively. Although much remains to be learned in each of anticancer and antioxidant research area that no doubt will have an impact on the management of cancer, it is already possible to identify new directions in cancer and oxidant treatment. The approaches that are being pursued at present study are essentially for (a) the development of specific or highly selective anticancer agents (b) the leaves extract show the significant inhibition on cancer cell line (c) the plant extract also show the antioxidant results with % inhibition at absorbance 517 nm.

Antioxidant screening results

Antioxidant activity demonstrated, that Musa × Paradisiaca L. leaves ethyl extract exhibited % inhibition at absorbance 517 nm with IC50 values = 3.70 to 45.50 at different concentration and compared with ascorbic acid as standard drug. At the concentration of 10 µg/ml, extract showed the better activity as compared to the standard drug. The presented results are showing in **Fig. 4** and **Table 2**.

Anticancer screening results

Musa × Paradisiaca L. leaves ethyl extract was tested for the evaluation of status of anticancer screening by MTT assay against HeLa and A375 cancer cell lines. The extract was tested at six different concentrations in triplet form i.e. 10, 20, 40, 80, 160 and 320 µg/ml. The MTT assay indicates that the inhibition activity of the extract was dependents on the concentration of the plant extract because increases the concentration of extract shows the more efficiency in inhibition. Maximum inhibition of cell growth was found at concentration 320 µg/ml (54.35 % inhibition and 55.97% inhibition, respectively for HeLa and A375 cancer cell line). Therefore, 320 µg/ml concentration of extract was used to study the IC50 value that was calculated as 249.1 and 224.4, respectively. The presented results (IC50 value) are showing in **Table 3**, **Figs. 5, 6** (for HeLa cancer cell) and **Table 4, Figs. 7, 8** (for A375 cancer cell).

Out of various GC-MS constituents of Musa × paradisiaca L leaves extract, Phytol and Eugenol shows higher antioxidant and anticancer activity [30-31].

Conclusion

The biological and phytochemical screening of Musa × paradisiaca L leaves extract indicated its anticancer and antioxidant potential. Ethyl acetate extract of leaves was evaluated for its anticancer activity. In vitro anticancer activity of extract showed significant inhibition of HeLa and A375 cancer cell lines, when evaluated by MTT assay. The MTT assay clearly indicates that the inhibition or inhibitory activity of the extract was concentration dependent. Maximum inhibition of cell growth was found at the concentration of 320 µg/ml which was 54.35 % inhibition and 55.97 % inhibition for Hela and A375 cell lines and IC50 value was 249.1 and 224.4 respectively. Antioxidant activity demonstrated, plant extract exhibited percentage inhibition with IC50 values = 3.70 to 45.50 at different concentration and compared with ascorbic acid as standard drug. Various GC-MS constituets of Musa × paradisiaca L leaves extract shows great antioxidant and anticancer activity, these are Eugenol, tetradecane, caryophyllene, alpha caryophyllene, acetyl euggenol, octyl acetate, neophytadiene, phytol, palmitic acid, and phytyl palmitate. Out of these constituents Phytol and Eugenol shows higher antioxidant and anticancer activity.

Ethics approval and consent to participate

None.

Funding

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Author contributions

Aakash Deep, Om Narayan Upadhyay, Priya, Sanjiv Kumar– designed and performed the experimental work; Arun Kumar Sharma- performed anticancer activity; Nitin Bansal and Harish Kumar performed the characterization of data and Surender Kumar, Deepak Wadhwa- designed the final manuscript.

Competing interests

All authors disclose no competing interests.

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