Anticancer potential of musa × paradisiaca as cervical carcinoma and malignant melanoma

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Abstract

This study was performed to assess the anticancer potential of *Musa × pardisiaca* L. It has several traditional benefits due to the presence of various bioactive compounds. Ethyl acetate extract of leaves was prepared and characterized by Gas Chromatography-Mass spectrometry (GC-MS). Compounds (1-40) were isolated from leaves, out of which two major compounds i.e. eugenol and phytol are potentially responsible for anticancer activity and oxidative stress. *In vitro* cytotoxicity of extract showed significant inhibition of cells in cervical carcinoma and malignant melanoma when treated by MTT assay against Hela and A375 cell lines. Ethyl acetate extract of *musa × paradisiaca* L. shown anticervical carcinoma and anti-malignant melanoma activity in our study.

Introduction

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 [1]. In India, it is second most common cancer in females after breast cancer, with around a lakh new cases identified annually [2]. The highest incidence was recorded in Papumpare, Aizawl, Mizoram, accounting for a total of 27% of cancer cases amongst female in India [3, 4]. It accounted for 0.3% of all cancer patients and 27% of all female cancer patients seen [4]. Infection with human papillomavirus (HPV) is associated with more than 90% cases of cervix cancer [5]. Surgery, radiotherapy or chemotherapy, alone or in combination [6], can be used as therapeutic modalities for carcinoma cervix [7]. Malignant melanoma is the most dangerous form of skin cancer [8], being fifth most common tumor type among men and sixth in women in the United States [9]. 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 [10]. Ultraviolet (UV) radiation and number of moles more than 100 is the greater risk for melanoma [11].

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 [12]. 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 [13]. Approximately 60% of anticancer compounds and 75% drugs for infectious disease come from natural products or their derivatives [14]. One of them is Musa × paradisiaca L. commonly known as Plantain belonging to family musaceae (banana family) [15]. Native to the Mediterranean region. 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 [16]. due to the presence of anti-cancer activity Musa × paradisiaca L can be prove effective against cervical carcinoma and malignant melanoma [17].

Experimental

Material 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 Feb, 2018. The leaves were authenticated by Dr. Sunita Garg, Emeritus Scientist, National Institute of Science Communication and Information Resources (CSIR-NISCAIR) Delhi.

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 extraction [18]. 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 GC 7890 (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.

Evaluation of antioxidant activity by DPPH radical scavenging assay

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 [19]. In DPPH assay 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 [20]. 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

For cytotoxicity studies, 32 mg/ml stocks were prepared using methanol and serially three-fold dilutions were prepared from 320 µg/ml to 10 µg/ml using 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 Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 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 37^oC, in 5% CO_2 incubator Or with 5% CO_2 atmosphere.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 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. 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% CO_2 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^o C in 5% CO_2 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% (IC_{50}) value is generated from the dose-response curves for each cell line [21–23].

% Inhibition = 100 – (OD of sample/OD of Control) x 100.

Statistical analysis

IC_{50} Values for cytotoxicity tests were derived from a nonlinear regression analysis (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

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 (% area 10.96), phytol (% area 5.43), dibutyl phthalate (% area 5.08), palmitic acid (% area 3.80), phytane (% area 2.68), tetradocosone (% area 2.0), tetradecane (% area 1.72), docosane (% area 1.71), hexadeccanoic acid (% area 1.58) and caryophyllene (% area 1.40). Other compounds identified were acetyl eugenol, neophytadine, docodecane and heptacosano. The chemical structure and GCMS graph of Eugenol and Phytol are shown in Figs. 1 and 2, respectively.

Table 1
Detailed some specific biomedical applications of various natural gums

Peak	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	$C_{14}H_{28}O$	200	15.732	0.15
8	Tetradecane	$C_{14}H_{30}O$	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	$C_{12}H_{12}O_3$	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

Peak	Name of Compounds	Molecular formula	Molecular weight	Retention Time	Area%
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	$C16H_{32}O_2$	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

Evaluation of carcinoma cervix and malignant melanoma activity of extract by MTT assay

Musa × Paradisiaca L. leaves ethyl extract was tested for the evaluation of status of carcinoma cervix and malignant melanoma by MTT assay against Hela and A375 cell lines belonging to different tissue origin. The extract was tested at six different concentrations in triplet form i.e. 10, 20, 40, 80, 160, 320 µg/ml. The inhibition of Hela and A375 cell line (Figs. 4 and 6) was calculated as % inhibition and

graphical representation of result was done by plotting concentration of extract on X axis and % of inhibition on Y axis for the Dose-Response Curve of the assay in Fig. 3 and Fig. 5, respectively. IC_{50} Value was calculated from the dose curves. The MTT assay clearly indicates that the inhibition activity of extract was concentration dependent and the increase in the concentration of extract shows more efficiency in inhibition. Maximum inhibition of cell growth was found at 320 µg/ml. That was 54.35 and 55.97 respectively for Hela and A375 cell line. Therefore 320 µg/ml concentration of extract was used for further studies and it was found that IC_{50} value was 249.1 and 224.4, respectively.

Conclusion

The present study concludes that the biological and phytochemical assessment of Musa × pardisiaca L extract, identifies it as an anticancer drug. Ethyl acetate extract of leaves was evaluated for its anticancer activity. In vitro cytotoxicity of extract showed significant inhibition of Hela and A375 cell lines in carcinoma cervix and malignant melanoma, 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 and 55.97 respectively for Hela and A375 cell lines. Therefore, 320 µg/ml concentration of extract was used for further studies and it was found that IC_{50} value was 249.1 and 224.4 respectively.

List Of Abbreviations

ATCC	**:**	**American type culture collection**
DMSO	:	Dimethyl sulphoxide
DPPH	:	1,1-diphenyl-2-picrylhydrazyl
FBS	:	Fetal bovine serum
GC-MS	:	Gas Chromatography-Mass spectrometry
MTT	:	3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide
NCI	:	National cancer institute
UV	:	Ultraviolet

Declarations

Availability of data and material - We have presented all our main data in the form of tables and figures.

Competing interests - The authors declare that they have no competing interests.

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Author's Contributions - ONU, RN, AD – designed and performed the experimental work, AKS - performed anticancer activity and SK, DW- designed the final manuscript. All authors read and approved the final version of the manuscript.

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Figures

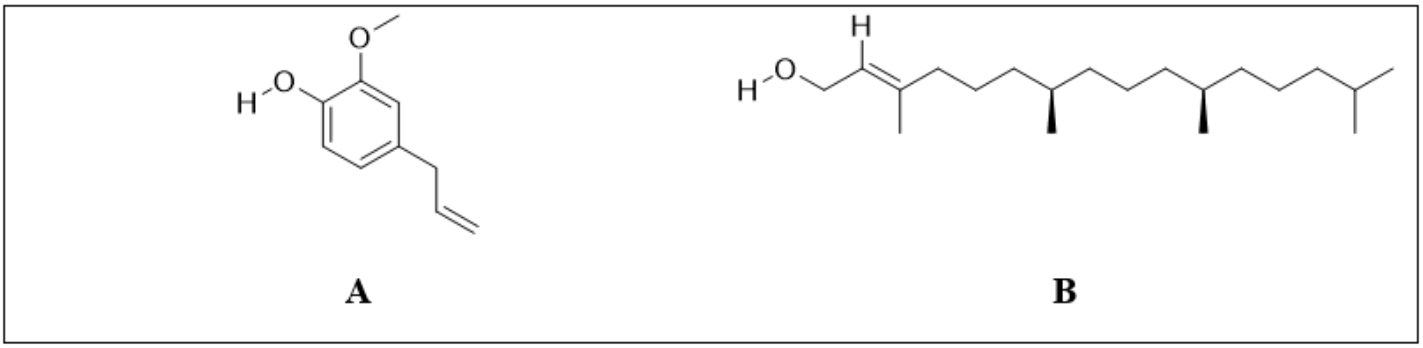

Figure 1

Structure of Eugenol (A) and Phytol (B)

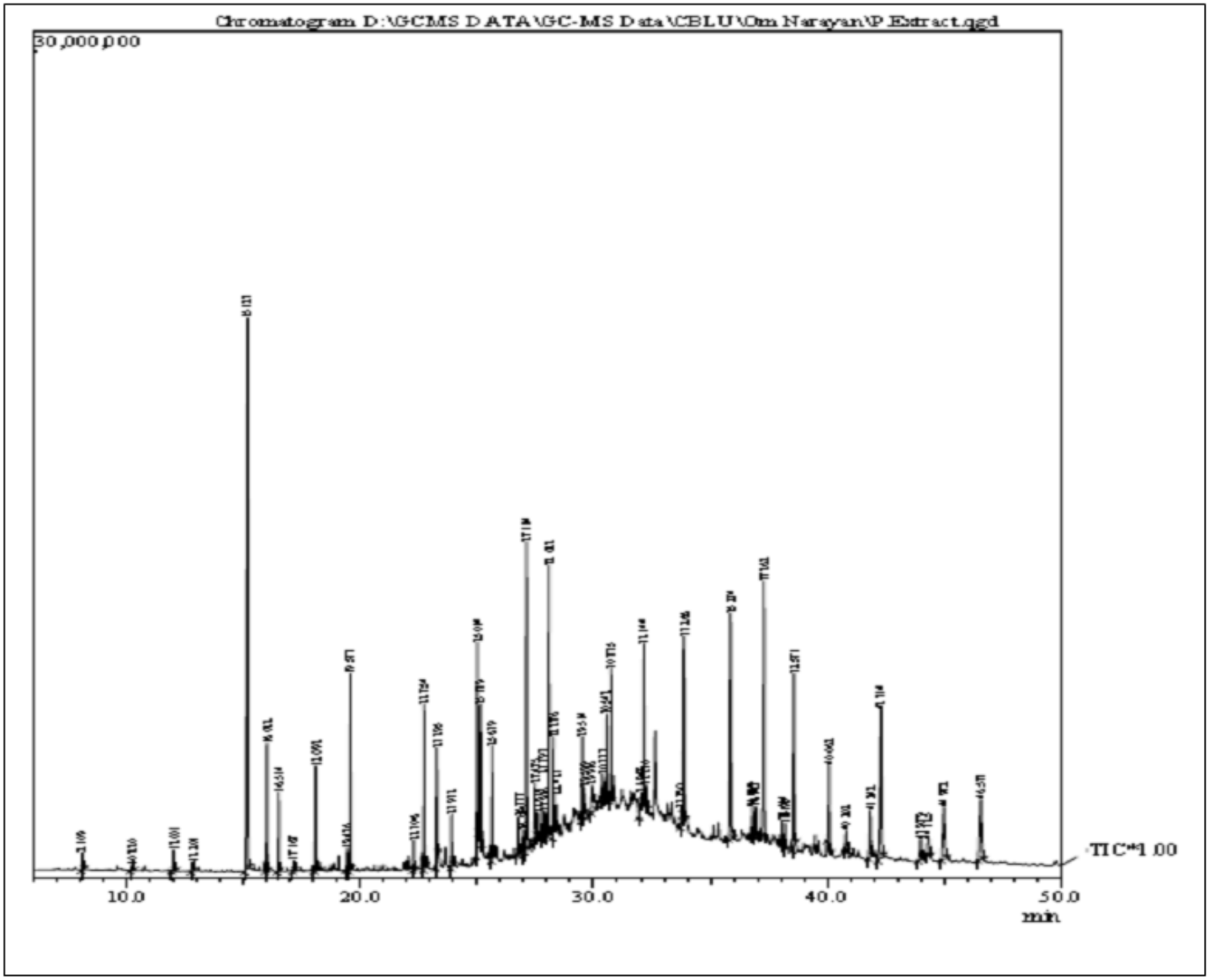

Figure 2

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

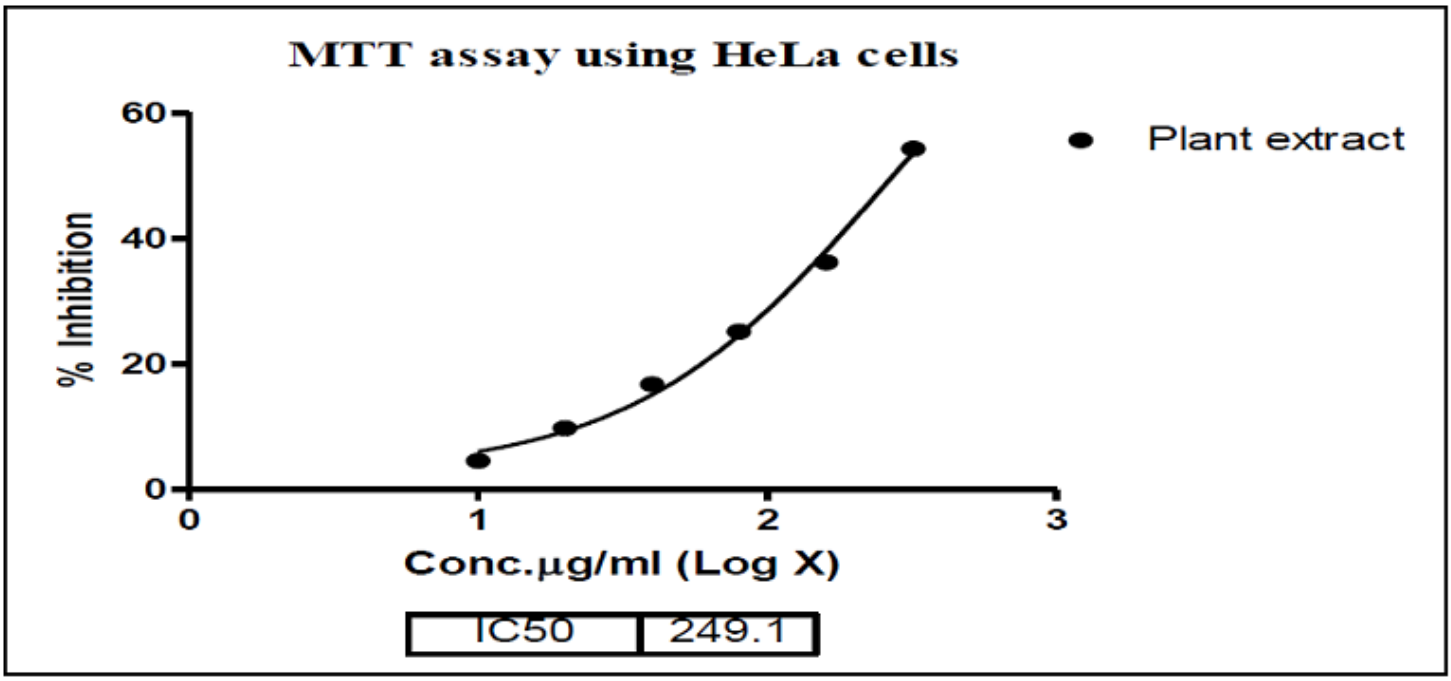

Figure 3

Dose response curve of extract against Hela cell lines

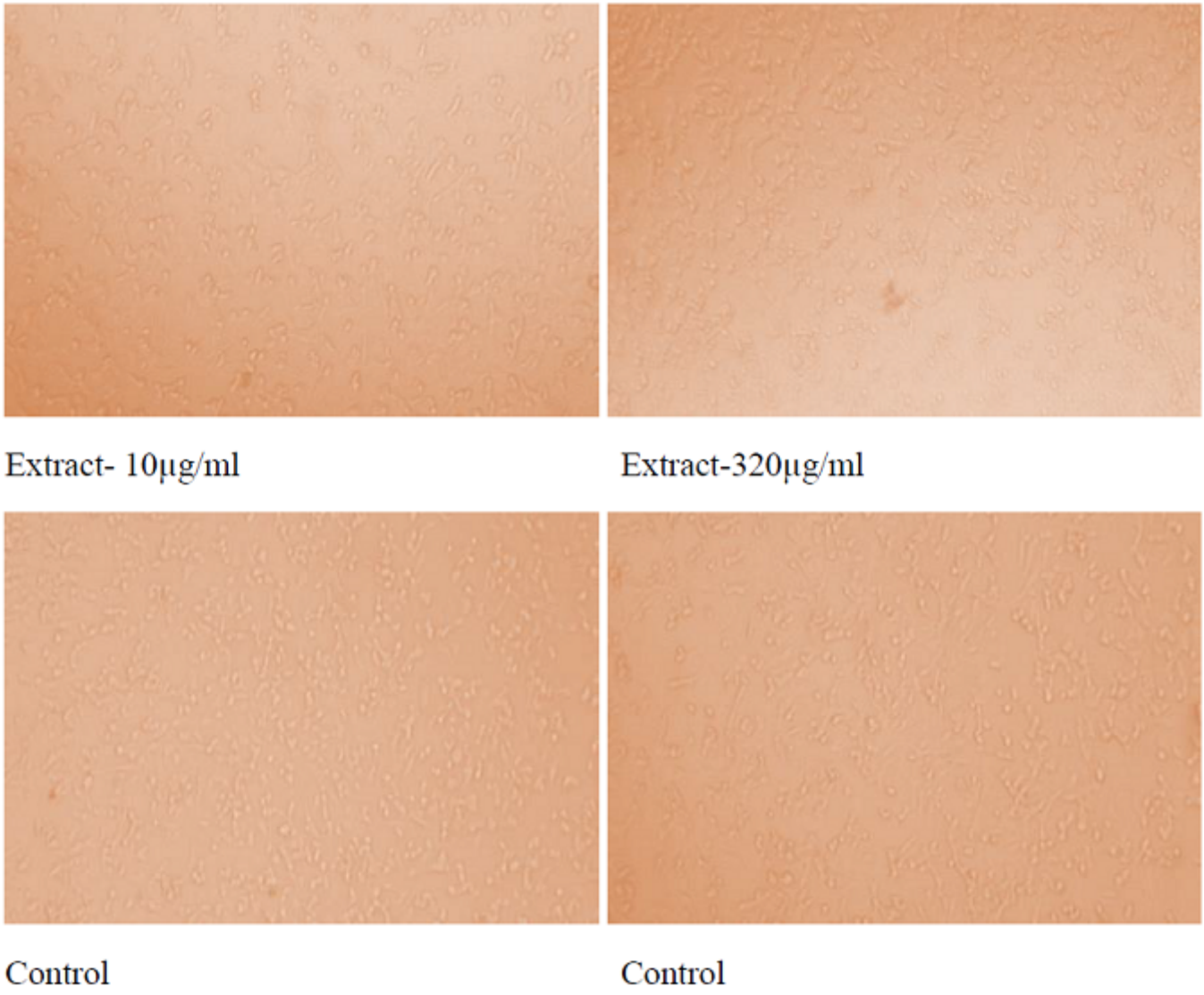

Figure 4

Growth inhibition against Hela cell line

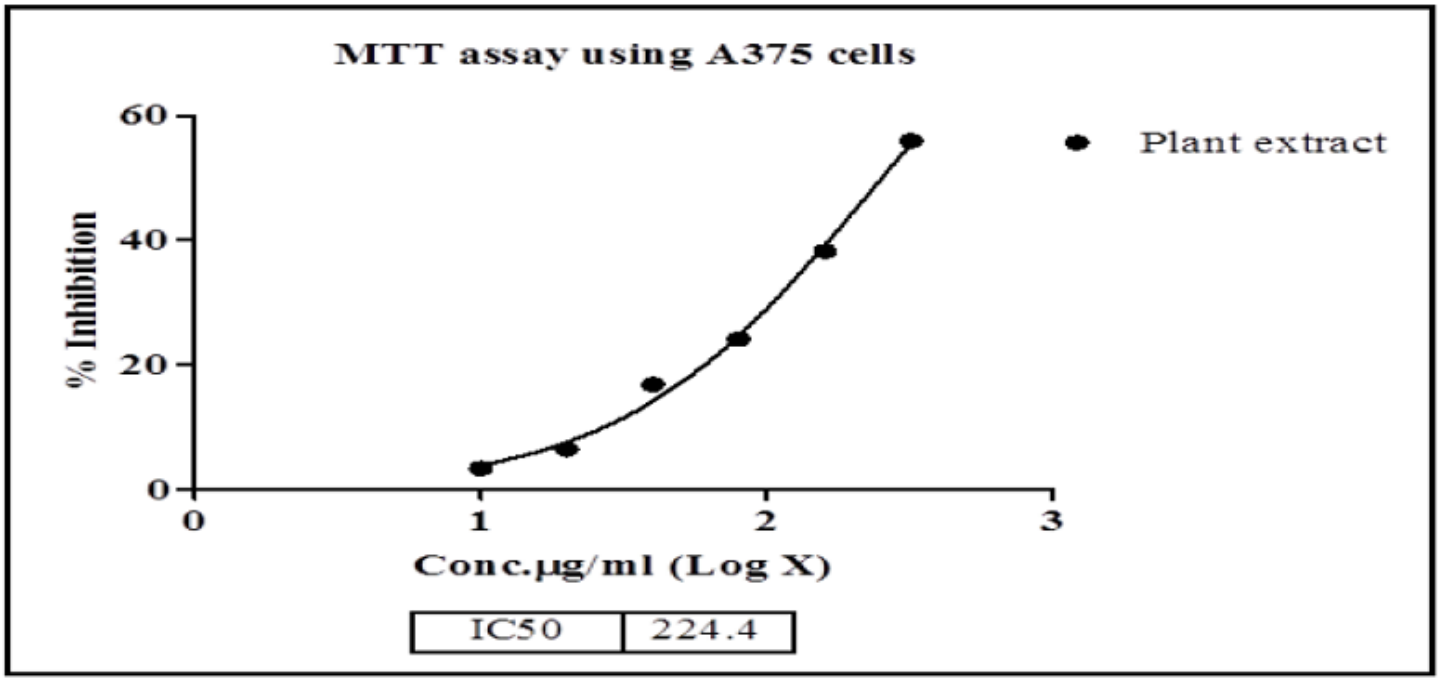

Figure 5

Dose response curve of extract against A375 cell lines

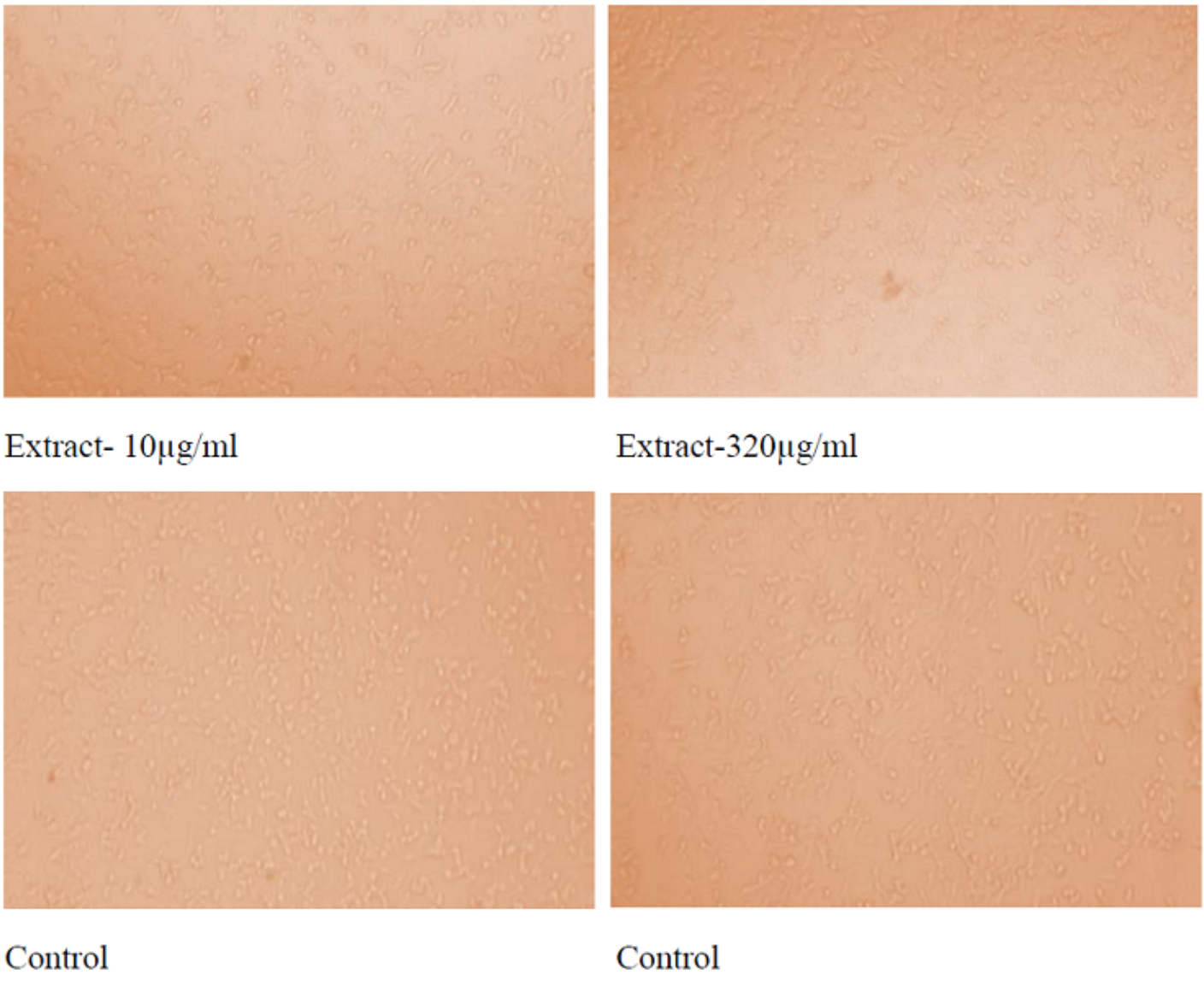

Figure 6

Growth inhibition against A375 cell lines