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ORIGINAL ARTICLE

In vitro Cytotoxic Activity using Fruits of *Annona muricata* and Aerial Parts of *Euphorbia tirucalli* against Lung and Oral Cancer Cell Lines

Swapna B, M. Raghavendra Rao[1], Satvik Kotha, Sagar. S[2], Ramachandra Siddamsetty Setty

Department of Pharmacology, Government College of Pharmacy, [1]Central Council for Research in Yoga and Naturopathy, Ministry of Ayush, HCG, [2]Department of Cell Biology, Skanda Life sciences, Bengaluru, Karnataka, India

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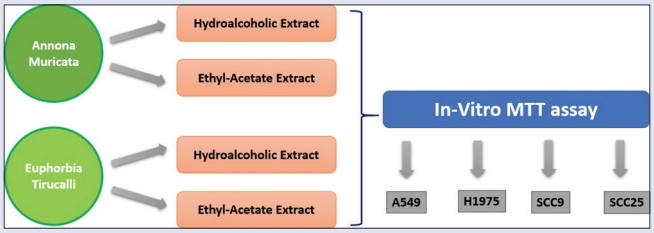
ABSTRACT

Aim: Cancer is one of the life-threatening diseases which cause severe pathological conditions, leading to mortality. The exhaustive data on the vital role of medicinal plants in combating cancer and related diseases are available since antediluvian times. **Materials and Methods:** In this present study, cell viability, morphological changes, and IC_{50} were evaluated by *in vitro* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertazoliumbromide) assay. Hydro-alcoholic and ethyl acetate extracts of fruits *Annona muricata* (AM) and aerial parts *Euphorbia tirucalli* (ET) against lung (A549, H1975) and oral (SCC9, SCC25) were used to investigate the potential antitumor activity. **Results:** Ethyl-acetate extract of AM and ET showed the highest IC_{50} value, 89.48 µg/mL and 119.2 µg/mL against lung cancer cell line A-549. Among four study extracts, the IC_{50} value of hydroalcoholic extract of AM showed 184.3 µg/mL against H1975 cell line. The hydroalcoholic extract of AM and ethyl acetate extract of EA showed the IC_{50} value of 149.7 µg/mL and 156.2 µg/mL against SCC9 cell line. Hydroalcoholic and ethylacetate extract of ET showed the highest cytotoxic potency against SCC25 139.2 µg/mL and 205.6 µg/mL compared to AM. Further, all the study extracts exhibited decrease in % cell viability in the dose-dependent manner. **Conclusion:** This comprehensive data obtained from the *in vitro* study indicate that the extracts that showed toxicity toward cancer cell lines can be considered potential chemotherapeutic agents. The data suggest to further confirm the anticancer potential by conducting *in vitro* and *in vivo* studies, so that the study plants may be adopted in the treatment of lung and oral cancer.

Key words: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertazoliumbromide) assay, *Annona muricata, Euphorbia tirucalli*, lung cancer cell lines, oral cancer cell lines

SUMMARY

- Hydroalcoholic and ethyl acetate extracts of fruits of *Annona muricata* and aerial parts of *Euphorbia tirucalli* exhibit cytotoxicity against lung and oral cancer cell lines. Based on IC_{50} and morphological changes observed from the result data, the study revealed that both extracts might have a role in cancer prevention. Further, studies can be carried out to explore the phytoconstituents responsible for cytotoxicity and its mechanism of action.



Abbreviations used: AM: *Annona muricata;* ET: *Euphorbia tirucalli;* HA: Hydroalchoholic; EA: Ethylacetate; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertazoliumbromide))µg/mL: microgram per milliliter; µM: micromolar; mM: millimolar; µl: microliter; DMEM: Gibco Dulbecco's modified eagle medium; RPMI: Roswell Park Memorial Institute medium; EDTA: Ethylenediamine tetraacetic acid; FBS: Fetal Bovine serum; DMSO: Dimethylsulfoxide; OD: Optical density; IC_{50}: Inhibitory concentration; FAK: Focal adhesion kinase.

Correspondence:

Dr. Ramachandra Siddamsetty Setty,
Department of Pharmacology, Government College of Pharmacy, Bengaluru, Karnataka, India.
E-mail: rssiddamsetty@rediffmail.com
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INTRODUCTION

Recent advancements and other life sciences have a significant impact on developing new approaches toward the prevention and therapy of cancer. Despite these advancements, there is a shortage of effective and safe drugs. There are reports that plants-containing various categories of phytoconstituents from all the geographical regions help alleviate cancer.[1] Plants from any parts of the world are considered to be potential sources for screening anti-cancer agents.[1] The strategy toward preventing cancer is targeting apoptosis/programmed cell death to maintain normal tissue homeostasis.[2] The lack of proper medicine for controlling the growth of cancer cells has accelerated long-standing interest in identifying the natural products for retarding progression and the resorption of tumors. The phytochemical-constituents present in natural products such as flavonoids, phenols, acetogenins, alkaloids, glycosides, triterpenoids, and esters are reported to possess anti-cancer properties.[3] In addition, a good number of molecules from herbs such as taxol, vincristine, vinblastine, paclitaxel, etoposide, podophyllotoxin, and vinca alkaloids have been adopted in treating cancer.[3] The researchers in our laboratory conducted a field survey and found two plants, *Annona muricata* (AM) and *Euphorbia tirucalli* (ET), claimed to possess anti-cancer activity.[4] Upon the literature survey, it is observed

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that AM is being used as an anti-cancer agent by native practitioners of several ethnic groups in Peru, the USA, and other countries.[4] Further, the native practitioners in India are also using this plants for treating breast cancer. Fruits, leaves, seeds, and roots of the AM possess more than 100 anti-cancerous acetogenins and essential are muricin J, K, L, annonacin A, annopentacin A, B, C annonaine,[4] etc., The leaves of the same plant have been shown to possess anti-cancer properties against breast, lung, and pancreatic cancer cell lines.[4] Although fruits of this plant contain the same range of phytoconstituents, reports on the anti-cancer activity of fruits are significantly less; however, it has been reported a promising result on prostate cancer cell lines.[4]Ethanolic and water leaves extracts of AM reported to possess antioxidant properties and anticancer activity against *Ehrlich ascites* carcinoma. Apart from the anticancer activities, the methanolic and aqueous leaf extracts of the plant showed antibacterial activity,[5] anti-listerial activity using monocytogenes MTCC 657 model,[6] and antifungal activity against *Candida glabrata.*[7]

In addition, muricin A, B, C, D, E, F, and G a phytoconstituents from this plant have been patented.[8] Ethyl acetate extracts of AM leaves were studied on HT-29 and HCT-116 colon cancer cells. These findings demonstrated that the leaf extract has noticeable pro-apoptotic potential.[9] Further the same extract was studied against lung cancer A549 cells and concluded with evidence that extract inhibited the proliferation of A549 cells.[10]

Similarly, ET is claimed to possess anti-cancer activity by native practitioners. Latex of this plant exhibited anti-cancer activity against human breast cancer cell line.[11] Flower extract of *Euphorbia milii* (Other species) possesses chemopreventive and antioxidant activity against human breast cancer and colon cancer in mice.[12] Methanol and aqueous extracts of aerial parts of ET were screened for photochemical, antioxidant and anti-cancer activities and reported that extracts inhibited the growth toward MiaPaCa-2 pancreatic cancer cell lines.[13] Cytotoxic effects of high dilution (HD) latex of ET were investigated on melanocytes and human breast cancer cells (MCF7 cell line) and concluded with evidence that HDs interfered in the metabolism of cell lines.[14] Stems of ET were screened for *in vitro* anticancer activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertazoliumbromide) (MTT) assay, Real-time cell growth assay, Annexin V/PI apoptosis assay, cell cycle analysis by flow cytometry, caspase 3/7 activity, real-time PCR, and western blot. The results suggested that extracts induced cell cycle arrest.[15] The literature survey indicated that *Et al.* so includes the same range of phytoconstituents as Euphorbia Milli, i.e., terpenes, alcohol, eufol, sterols, alfaeuforbol, taraxasterol, *E. tirucalli*, tigliane, and inganen.[12] Further report also indicated that ET and *Euphorbia antiqorrum* exhibited antioxidant and hepatoprotective activity.[16,17] Keeping the available literature in view and as a preliminary step in this direction, the present study was carried out to assess the anti-cancer property of ethyl acetate and hydroalcoholic extracts of these study plants against lung (A-549, H1975) and oral (SCC9, SCC25) cancer cell lines. The ethyl acetate solvents extract polar and nonpolar constituents. The hydroalcoholic solvent extracts all the polar secondary metabolites. These two solvents are used for the extraction to ascertain the anticancer activity to a particular type of phytoconstituents.

The study's outcome may help to throw light on the usefulness of abundantly grown plants available at a hand stretch (AM and ET) in treating life-threatening diseases, cancer.

MATERIALS AND METHODS

Chemicals required: Gibco Dulbecco's modified eagle medium (DMEM), Roswell Park Memorial Institute medium (RPMI), Trypsin Ethylenediaminetetraacetic acid 0.05% (EDTA), Fetal Bovine Serum (FBS), and Penstrep was procured Gibco. MTT reagent and Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich. Potassium chloride, Disodium phosphate, and Monopotassium phosphate were purchased from Fisher scientific. Sodium chloride from SDFCL.[13,18-21]

Materials required: T25 flask was obtained from Falcon. Sterile 96 well and Filtration unit plate from Thermo Scientific. 200 and 1000 µl tips were obtained from Genaxy. Microcentrifuge tubes from × pet and Serological pipettes was procured from Eppendorf.

Preparation of test solutions

Standard vinblastine serial dilutions 100 µM to 3.125 µM were prepared from 10 mM stock using DMEM plain media for treatment. 50 mg/ml stocks test samples were taken to prepare 500 µg/ml to 15.625 µg/ml using DMEM primary media for treatment.

Cell lines and culture medium

The lung (A-549, H1975) and oral (SCC-9, SCC-25) cancer cell lines were obtained from American Type Culture Collection. C. Stock cells were cultured in DMEM/RPMI supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent.

Cell dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS) was used to dissociate the cells. The viability of the cells was checked, centrifuged, and 50,000 cells/well were seeded in a 96 wellplate incubated for 24 h at 37°C, 5% CO_2 incubator.

Procedure

Media-containing 10% FBS was used to trypsinze the monolayer of cell culture, and cell count was adjusted to 5 × 105 cells/ml. To each 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 discarded. washed once with medium, and 100 µl of different test drug concentrations were added on partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24 h in a 5% CO_2 atmosphere. After incubation, the test solutions were discarded, and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37°C in a 5% CO_2 atmosphere. The supernatant was removed and 100 µl of 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. The concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values was generated from the dose-response curves for each cell line.[17,22-25]

% Inhibition = ([OD of Control – OD of sample]/OD of Control) × 100.

IC_{50} values for cytotoxicity tests were derived from 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

The % viability and IC_{50} value of hydroalcoholic (AMHA, ETHA) and ethyl acetate study (AMEA, ETEA) extracts were studied using MTT assay against lung cancer (A549, H1975) and oral cancer cell lines (SCC9 and SCC25).

The IC_{50} value of AMHA and ETHA extract was found to be 223.5 µg/mL and 232.6 µg/mL, correspondingly AMEA and ETEA were 89.48 µg/mL and 119.2 µg/mL against A549 lung cancer cell line [Table 1]. AMHA against H1975 was found to be 184.3 µg/mL, and ETHA extract was not

calculated due to less % inhibition, whereas AMEA exhibited 465 µg/mL and ETEA extract was 372.5 µg/mL [Table 2].

AMHA and ETHA extract against SCC9 was determined and was found to be 149.7 µg/mL and 455.8 µg/mL. AMEA and ETEA extracts were found to be 272.6 µg/mL and 156.2 µg/ml.

In continuation, the assay was performed against SCC25 cell line, AMHA, ETHA, AMEA, and ETEA extract showed 413.3 µg/mL,139.2 µg/mL,289.8 µg/mL, and 205.6 µg/ml, respectively. The values are tabulated in Tables 3 and 4.

Vinblastine drug was used as a standard against A549, H1975, SCC9, and SCC25 cell lines, and IC_{50} was found to be 17.6, 25.46, 23.45, and 21.8 µM correspondingly [Tables 1-4].

The study samples against lung (A549, H1975) and oral cancer cell lines (SCC9, SCC25) showed a significant reduction in the number of living cells in a dose-dependent manner [Tables 1-4]. These morphological changes against cell lines of lowest (15.625 µg/mL) and highest (500 µg/mL) concentrations of the extracts are depicted in Figures 3-6.

All the study extracts at high doses showed the decreased cell viability with slight variations in the morphology against lung and oral cancer cell lines as follows:

A549 lung cancer cell line

AMEA extract at high-dose showed complete cell membrane damage, cell shrinkage, and formation of apoptotic bodies. AMHA high dose showed clear cytoplasm, but cell synapses and focal adhesion kinase were lost. The cells were intact of both the extracts (AMEA, AMHA) at the low dose found clear margins among monolayers with bright cytoplasm. The standard and extracts graph showing % inhibition Vs concentration of MTT assay depicted in Figures 1 and 2.

ETEA and ETHA at high dose cell synapses and Focal adhesion kinase (FAK) were lost. Few cells were found apoptotic [Figure 3]; the remaining showed normal morphology with clear margins at a low dose.

H1975 lung cancer cell line

AMEA and AMHA extract at the high dose formed cell debris and blebs on the cell's surface; FAK and cell synapses were lost. Cells were intact, clear, and with bright cytoplasm at a low dose

ETEA extract at a high dose exhibited complete cell membrane damage with overall cell shrinkage; whereas; the ETHA section did not reduce the cell viability retained normal morphology at the low and high doses.

SCC9 and SCC25 oral cancer cell lines:

All the study extracts (AMHA, AMEA, ETHA, and ETEA) at higher doses appear to progress early apoptotic condition with cell debris and blebs on the cell's surface. Low dose did not show a reduction in the cell viability.

Vinblastine was used as standard and compared with all study extracts.

DISCUSSION

The earlier reports indicate the presence of muricin J, K, L, annonacin A, annopentacin A, B, C, and annonaine present in AM[4] and terpenes alcohol, eufol, sterols, alfaeuforbol, taraxasterol, *E. tirucalli*, tigliane, and inganen presentin ET.[12] Further, aqueous and ethyl acetate extracts of both the plants (AM, ET) were reported to possess antioxidant

Table 1: IC_{50} value of hydroalcoholic and ethyl acetate extract of *Annona muricata* and *Euphorbia tirucalli* against lung cancer cell line (A549)

Sample	Concentration (µM)	Mean inhibition±SEM	Percentage viability	IC_{50} (µM)
Control	0	0±0	100	
Vinblastine	3.125	6.35±0.7	93.64	17.6
	6.25	15.94±0.8	84.06	
	12.5	28.76±0.4	71.23	
	25	54.88±0.18	45.12	
	50	63.76±2.6	36.23	
	100	76.84±0.23	23.16	
Sample	**Concentration (µg/ml)**	**Mean inhibition±SEM**	**Percentage viability**	**Concentration (µg/ml)**
A. muricata-HA	15.625	13.65±0.33	86.35	223.5
	31.25	21.91±0.23	78.08	
	62.5	32.03±0.19	67.96	
	125	40.6±0.06	59.39	
	250	54.3±0.72	45.70	
	500	71.76±0.29	28.23	
A. muricata -EA	15.625	15.15±0.69	84.85	89.48
	31.25	24.03±0.11	75.96	
	62.5	35.66±0.05	64.34	
	125	56.34±0.21	43.66	
	250	68.27±0.22	31.73	
	500	77.15±0.21	22.85	
E. tirucalli-HA	15.625	11.88±0.53	88.12	232.6
	31.25	23.82±0.39	76.18	
	62.5	29.34±0.16	70.66	
	125	37.51±0.18	62.48	
	250	52.22±0.39	47.77	
	500	67.56±0.24	32.43	
E. tirucalli-EA	15.625	12.32±0.14	87.67	119.2
	31.25	24.83±0.39	75.17	
	62.5	30.09±0.41	69.91	
	125	43.13±0.51	56.86	
	250	57.13±0.1	42.86	
	500	66.19±0.44	33.81	

A. muricata: *Annona muricata*, *E. Tirucalli*: *Euphorbia tirucalli*, HA Hydroalcoholic, EA: Ethyl acetate, SEM: Standard error of the mean

Table 2: IC_{50} value of hydroalcoholic and ethyl acetate extract of *Annona muricata* and *Euphorbia tirucalli* against lung cancer cell line (H1975)

Sample	Concentration (µM)	Mean inhibition±SEM	Percentage viability	IC_{50} (µM)
Control	0	0±0	100	25.46
Vinblastine	3.125	19.27±0.34	80.72	
	6.25	28.72±0.38	71.27	
	12.5	40.42±0.4	59.58	
	25	53.81±0.33	46.19	
	50	66.56±0.36	33.43	
	100	81.23±0.27	18.77	
Sample	**Concentration (µg/ml)**	**Mean inhibition±SEM**	**Percentage viability**	**Concentration (µg/ml)**
A. muricata-HA	15.625	8.25±0.56	91.74	184.3
	31.25	18.47±0.2	81.53	
	62.5	29.99±0.31	70.00	
	125	38.17±0.24	61.82	
	250	56.43±0.35	43.56	
	500	72.03±0.41	27.97	
A. muricata-EA	15.625	4.4±0.45	95.60	465
	31.25	8.64±0.28	91.36	
	62.5	14.49±0.26	85.51	
	125	23.3±0.28	7670	
	250	35.04±0.28	64.96	
	500	52.96±0.24	47.03	
E. tirucalli-HA	15.625	3.98±0.29	96.02	IC_{50} was not calculated due to lesser percentage inhibition
	31.25	7.2±0.45	92.80	
	62.5	15.17±0.93	84.83	
	125	20.97±0.39	79.03	
	250	32.2±0.11	67.80	
	500	40.96±2.3	59.03	
E. tirucalli-EA	15.625	6.82±0.29	93.18	372.5
	31.25	11.86±0.34	88.14	
	62.5	19.45±0.29	80.55	
	125	32.24±0.28	67.75	
	250	47.41±0.35	52.58	
	500	68.51±0.57	31.48	

A. muricata: *Annona muricata*, *E. Tirucalli: Euphorbia tirucalli*, HA Hydroalcoholic, EA: Ethyl acetate, SEM: Standard error of the mean

Table 3: IC_{50} value of hydroalcoholic and ethyl acetate extract of *Annona muricata* and *Euphorbia tirucalli* against an oral cancer cell line (SCC9)

Sample	Concentration (µM)	Mean inhibition±SEM	Percentage viability	IC_{50} (µM)
Control	0	0±0	100	23.45
Vinblastine	3.125	10.57±0.56	89.43	
	6.25	23.26±0.05	76.73	
	12.5	37.66±0.25	62.33	
	25	50.71±0.56	49.29	
	50	63.36±0.22	36.64	
	100	82.49±0.23	17.51	
Sample	**Concentration (µg/ml)**	**Mean inhibition±SEM**	**Percentage viability**	**Concentration (µg/ml)**
A. muricata-HA	15.625	9.4±0.42	90.60	149.7
	31.25	14.57±0.5	85.42	
	62.5	28.21±0.52	71.79	
	125	39.19±0.48	60.80	
	250	53.69±0.4	46.31	
	500	67.1±0.21	32.90	
A. muricata-EA	15.625	6.66±0.07	93.34	272.6
	31.25	11.43±0.08	88.57	
	62.5	19.39±0.28	80.60	
	125	28.26±0.14	71.74	
	250	44.23±0.6	55.76	
	500	58.05±0.2	41.94	
E. tirucalli-HA	15.625	4.63±0.07	95.36	455.8
	31.25	10.08±0.84	89.92	
	62.5	15.84±0.06	84.16	
	125	23.08±0.46	76.92	
	250	34.69±0.57	65.30	
	500	51.93±0.37	48.07	

Contd...

Table 3: Contd...

Sample	Concentration (µg/ml)	Mean inhibition±SEM	Percentage viability	Concentration (µg/ml)
E. tirucalli-EA	15.625	8.9±0.55	91.09	156.2
	31.25	16.29±0.07	83.71	
	62.5	20.58±3.78	79.41	
	125	48.47±0.14	51.53	
	250	60.53±0.23	39.47	
	500	74.61±0.32	25.38	

A. muricata: Annona muricata, E. Tirucalli: Euphorbia tirucalli, HA Hydroalcoholic, EA: Ethyl acetate, SEM: Standard error of the mean

Table 4: IC_{50} value of hydroalcoholic and ethyl acetate extract of *Annona muricata* and *Euphorbia tirucalli* against an oral cancer cell line (SCC25)

Sample	Concentration (µM)	Mean inhibition±SEM	Percentage viability	IC_{50} (µM)
Control	0	0±0	100	
Vinblastine	3.125	13.12±0.47	86.88	21.89
	6.25	26.2±0.26	73.79	
	12.5	41.24±0.38	58.76	
	25	52.85±0.55	47.14	
	50	70.19±0.46	29.81	
	100	85.14±0.4	14.86	
Sample	**Concentration (µg/ml)**	**Mean inhibition±SEM**	**Percentage viability**	**Concentration (µg/ml)**
A. muricata-HA	15.625	5.05±0.71	94.95	413.3
	31.25	9.22±0.66	90.78	
	62.5	14.89±0.56	85.10	
	125	26.65±0.45	73.35	
	250	35.11±0.52	64.88	
	500	54.63±0.27	45.37	
A. muricata-EA	15.625	9.08±0.66	90.91	289.8
	31.25	13.03±0.29	86.96	
	62.5	24.43±0.67	75.57	
	125	31.88±0.69	68.11	
	250	45.58±0.27	54.41	
	500	63.23±0.48	36.77	
E. tirucalli-HA	15.625	5.27±0.56	94.72	139.2
	31.25	16.67±0.15	83.33	
	62.5	21.24±0.1	78.76	
	125	38.44±0.18	61.55	
	250	55.52±0.3	44.48	
	500	64.25±0.4	35.74	
E. tirucalli-EA	15.625	8.46±0.47	91.53	205.6
	31.25	12.1±0.5	87.90	
	62.5	25.31±0.73	74.68	
	125	41.77±0.39	58.23	
	250	59.11±0.23	40.89	
	500	77.2±0.17	22.79	

A. muricata: Annona muricata, E. Tirucalli: Euphorbia tirucalli, HA Hydroalcoholic, EA: Ethyl acetate, SEM: Standard error of the mean

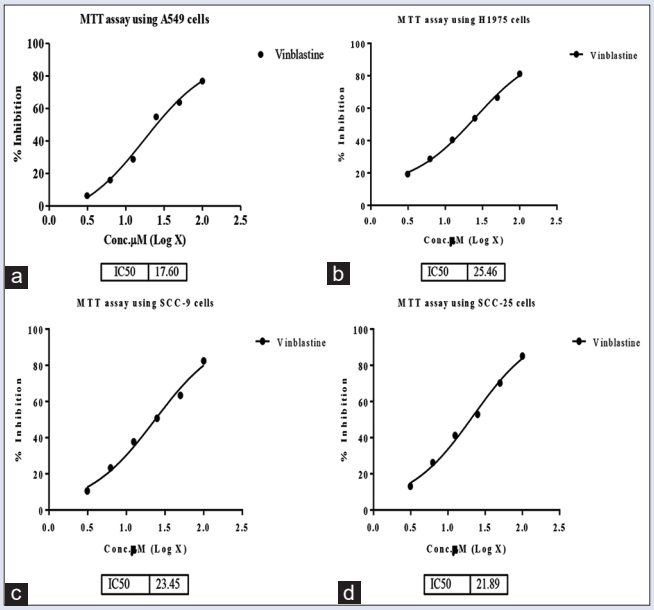

Figure 1: (a) Standard showing % inhibition of Vs concentration of MTT assay using A549 cell line. (b) Standard showing % inhibition of Vs concentration of MTT assay using H1957 cell line. (c) Standard showing % inhibition of Vs concentration of MTT assay using SCC-9 cell line. (d) Standard showing % inhibition of Vs concentration of MTT assay using SCC-25 cell line

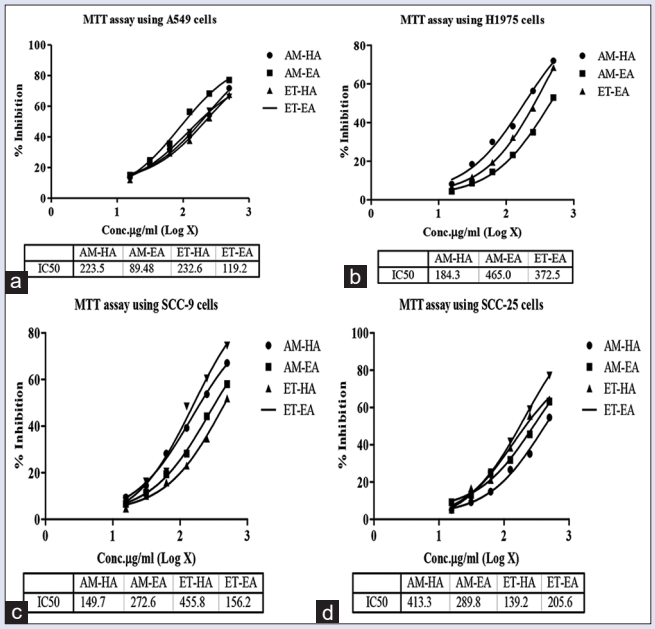

Figure 2: (a) Graph showing % inhibition vs. concentration of MTT assay using A549 cell line. (b) Graph showing % inhibition vs concentration of MTT assay using H1975 cell line. (c) Graph showing % inhibition vs concentration of MTT assay using SCC9 cell line. (d) Graph showing % inhibition vs concentration of MTT assay using SCC25 cell line

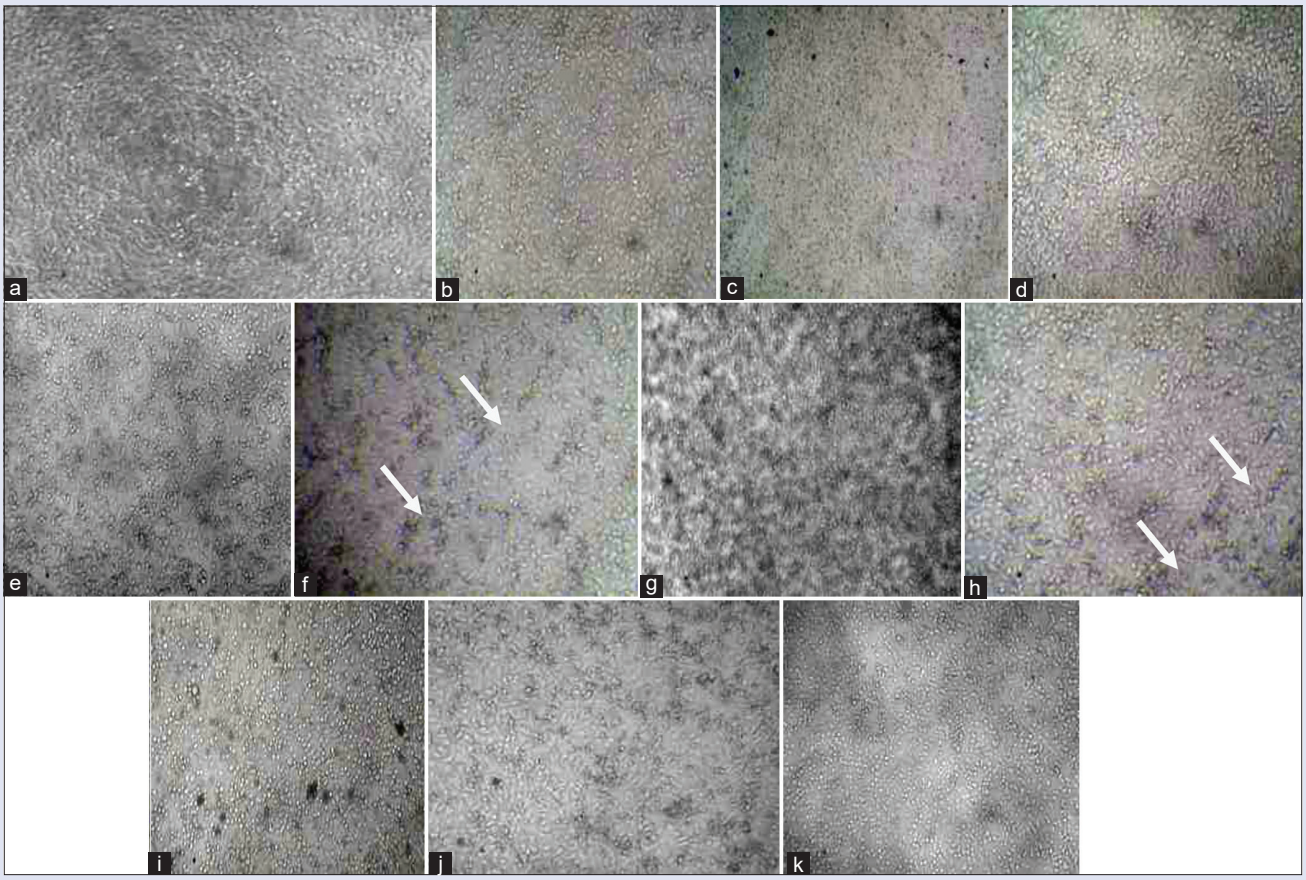

Figure 3: Comparative cytotoxicity effect of AM and ET extracts (hydroalcholic, ethylacetate) on cell viability of lung cancer cell lines (A549). (a) Control group A549 cell line. (b) AM ethylacetate extract 15.625 µg/mL. (c) AM ethylacetate extract 500 µg/mL. (d) AM hydroalcoholic extract 15.625 µg/mL. (e) AM hydroalcoholic extract 500µg/mL. (f) ET ethylaceate extract 15.625µg/mL. (g) ET ethylacetate extract 500µg/mL. (h) ET hydroalcoholic extract15.625 µg/mL. (i) ET hydroalcoholic 500 µg/mL. (j) Vinblastine 3.125µg/mL. (k) Vinblastine 100µg/mL

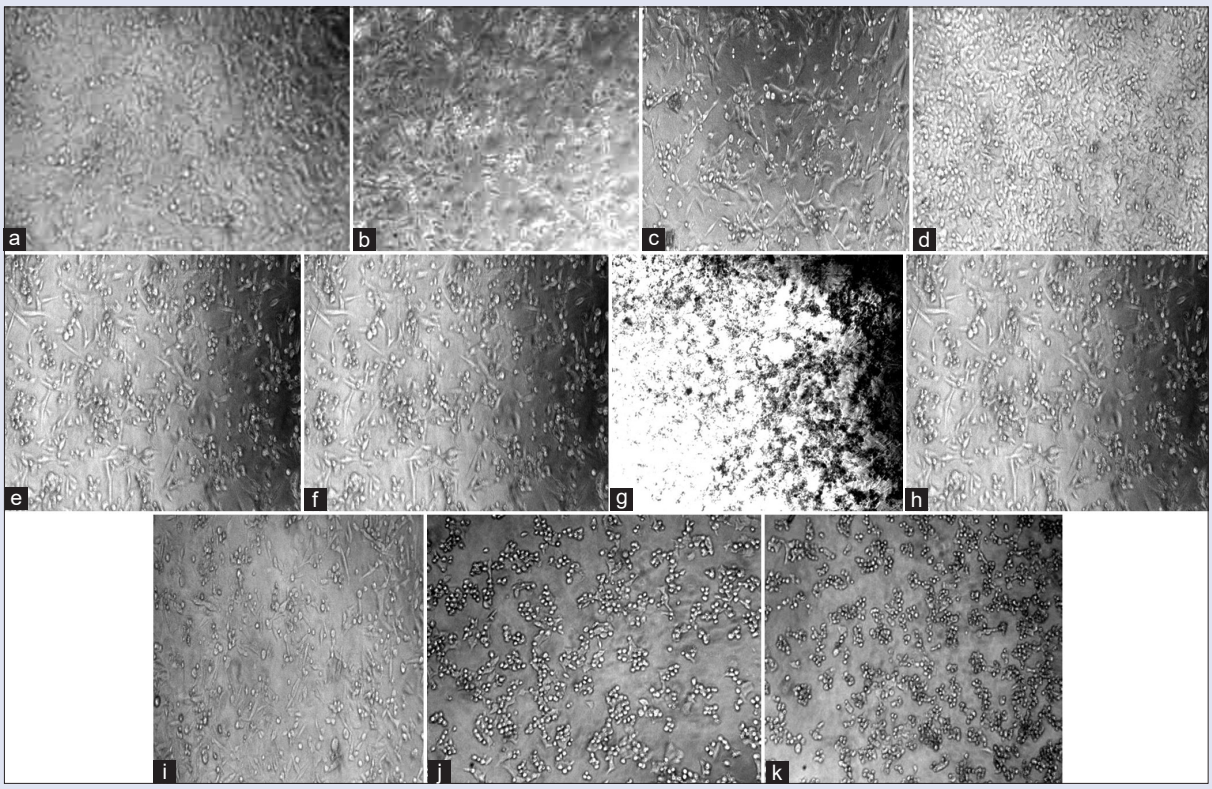

Figure 4: Comparative cytotoxicity effect of AM and ET extracts (hydroalcholic, ethylacetate) on cell viability of lung cancer cell lines (H1975). (a) control group (H195). (b) AM Ethylacetate extract 15.625µg/mL. (c) AM Ethylacetate extract 500µg/mL. (d) AM hydroalcoholic extract 15.625 µg/mL. (e) AM hydroalcoholic extract 500µg/mL. (f) ET ethylacetate extract 15.625µg/mL. (g) ET ethylacetate extract 500 µg/mL. (h) ET hydroalcoholic extract 15.625 µg/mL. (i) ET hydroalcoholic 500 µg/mL. (j) Vinblastine 3.125 µg/mL. (k) Vinblastine 100 µg/mL

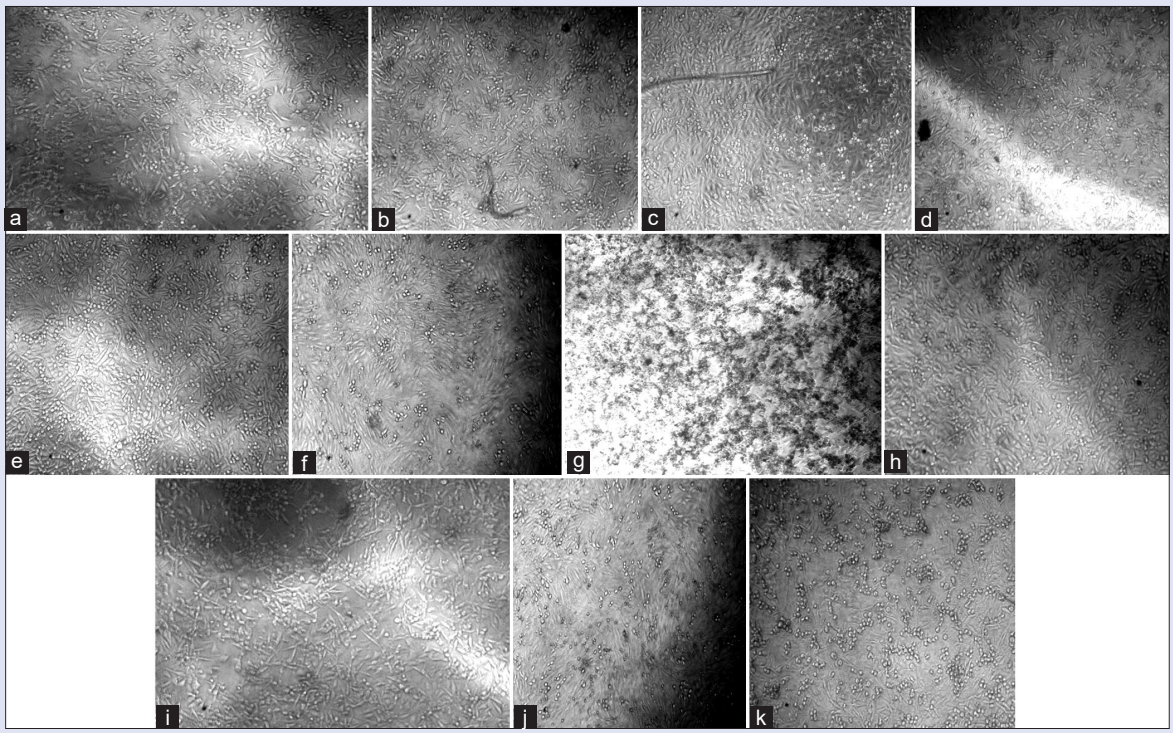

Figure 5: Comparative cytotoxicity effect of AM and ET extracts (hydroalcholic, ethylacetate) on cell viability of oral cancer cell line (SCC9). (a) Control group (SCC9). (b) AM Ethylacetate extract 15.625 µg/mL. (c) AM Ethylacetate extract 500 µg/mL. (d) AM hydroalcoholic extract 15.625 µg/mL. (e) AM hydroalcoholic extract 500 µg/mL. (f) ET ethylaceate extract 15.625 µg/mL. (g) ET ethylacetate extract 500 µg/mL. (h) ET hydroalcoholic extract15.625 µg/mL. (i) ET hydroalcoholic 500 µg/mL. (j) Vinblastine 3.125µg/mL. (k) Vinblastine 100µg/mL

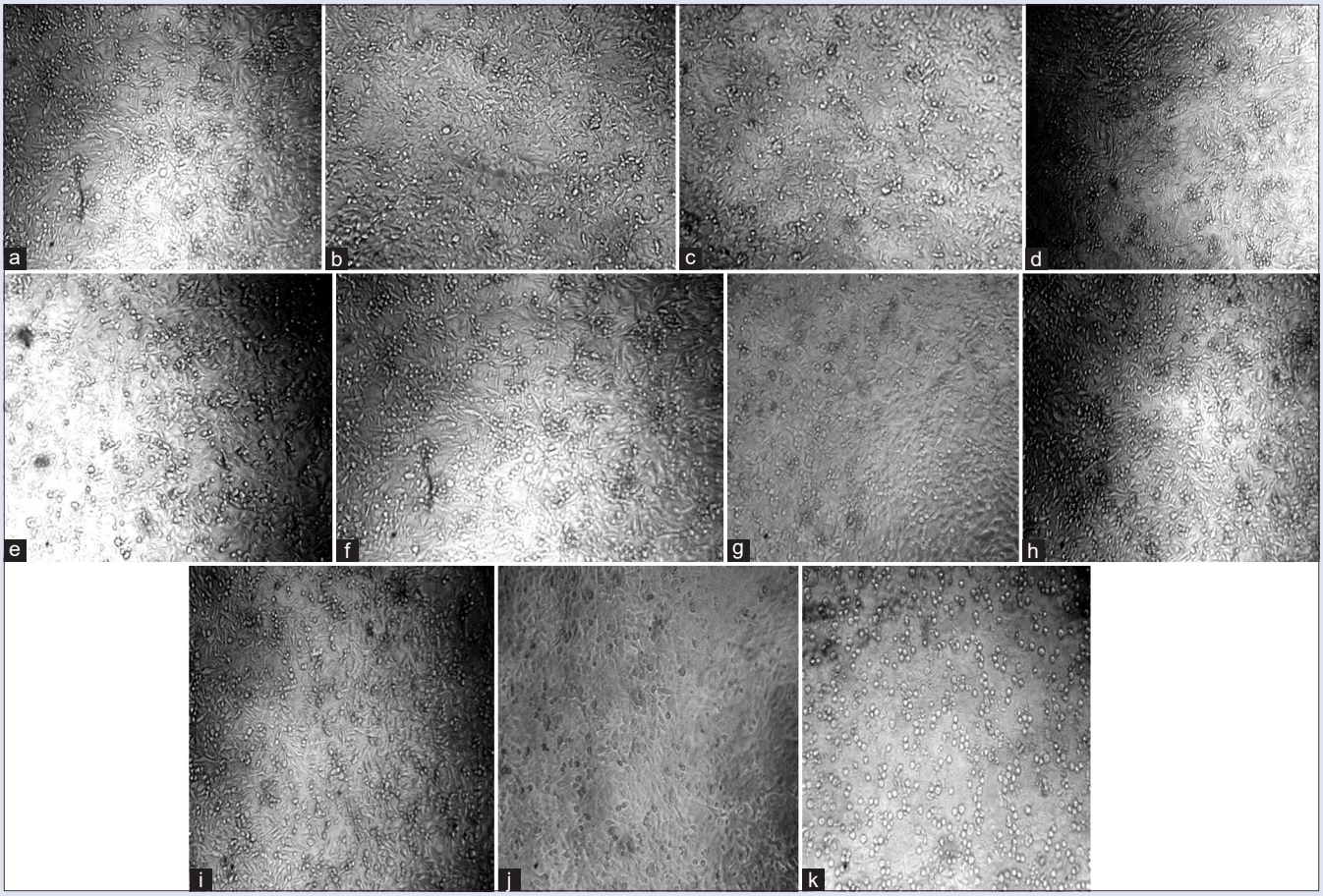

Figure 6: Comparative cytotoxicity effect of AM and ET extracts (hydroalcholic, ethylacetate) on cell viability of oral cancer cell line (SCC25). (a) Control group (SCC25). (b) AM Ethylacetate extract 15.625 µg/mL. (c) AM Ethylacetate extract 500 µg/mL. (d) AM hydroalcoholic extract 15.625 µg/mL. (e) AM hydroalcoholic extract 500 µg/mL. (f) ET ethylaceate extract 15.625 µg/mL. (g) ET ethylacetate extract 500 µg/mL. (h) ET hydroalcoholic extract 15.625 µg/mL. (i) ET hydroalcoholic 500 µg/mL. (j) Vinblastine 3.125 µg/mL. (k) Vinblastine 100 µg/mL

and anticancer activity aginst various cancer cell lines.[5,9,13,26] Hence, extracted the study plant materials with these solvents and were used in the *in vitro* MTT cytotoxic assay against lung cancer and oral cell lines to assess their cytotoxic potency. The cytotoxic potency measured as inhibitory concentration 50 (IC_{50}) and IC_{50} value is inversely proportional to cytotoxic potential. The lung (A-549, H1975), and oral cancer cell lines (SCC9, SCC25) have been considered a common form of cancer with few therapeutic options. Hydro-alcoholic and ethyl acetate extracts of both the herbs (AM, ET) tested against the above cell lines showed a different extent of cytotoxicity. Ethyl acetate extract of AM and ET showed a promising cytotoxic effect against the A549 cell line. AM hydroalcoholic extract showed better activity in H1975. Similarly, hydroalcoholic extract of AM was cytotoxic against the SCC9 oral cancer cell line, and ET hydroalcoholic extract was effective against SCC25. The data was evident by further analyzing pictures of cell viability. The study extracts of AM and ET at high doses (500 µg/mL) showed remarkable cell membrane damage with loss of cell synapses, destroying the cell communication.

FAK proteins are essential mediators of cell growth signaling, cell proliferation, cell survival, and cell migration.[27] (FAK) proteins were denatured in all the study extracts. The denaturation of FAK proteins indicates that the cell viability, destruction of cell membrane and inhibition of cell proliferation. The present study establishes the evidence for the hypothesis that inhibition of FAK proteins might serve as an excellent therapeutic target in treating cancer. The study extracts showed the total flavonoid, phenol content, and antioxidant activity (DPPH, nitric oxide scavenging activity) of AM and ET.[28,29] The Radical scavenging potential by phenols and flavonoids of the study extracts may also play a vital role in preventing and remission a tumor. The unpublished data from our laboratory indicating various secondary metabolites of study plants showed minimum glide score and protein-ligand binding stability with the multiple proteins telomerase, CDK2, Bcl-2, CYP3D, EGFR, VEGF, MMPK, P53, and NEK-2 reported to be involved in the causation of lung and oral cancer. Hence, the cytotoxicity associated with lung and oral cancer cell lines may be attributed to inhibition of carcinogenic potential by the secondary metabolites present in the study extracts. Further, based on the pictures of cancer cells, the reduced viability and destruction of FAK enzymes warrant the cytotoxic investigation.

In conclusion, the prepared extracts can be taken forward to understand the effect of the plant inhibiting/evokes multiple pathways involved in cancer genesis, growth, spread, and ascertain the contribution of each constituent in this regard.

CONCLUSION

Hydro alcoholic and ethyl acetate extracts of AM and ET found to possess anticancer potential against human lung and oral carcinoma.

Further studies like growth kinetics, apoptosis analysis, gene expression studies would confirm the potential activity of these plant samples.

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Conflicts of interest

There are no conflicts of interest.

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