Symposium

In vitro anticancer potential of *Semecarpus anacardium* Linn

Abstract

Background: Keeping in view the toxicity of *Semecarpus anacardium* Linn. as reported in the traditional literature, the present study was carried out to evaluate the *in vitro* cytotoxic activity of ethanolic extract of *Semecarpus* on two different cell lines. **Materials and Methods:** The ethanolic extract of *Semecarpus* was prepared using cold extraction method. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay of ethanolic extract was carried out on HeLa and SiHa cell lines for determination of cytotoxicity. **Results:** The IC_{50} values of ethanolic extract of *S. anacardium* in HeLa and SiHa cell lines were 44.0 μg/ml and 57.0 μg/ml, respectively. The extract was standardized by thin-layer chromatography and Gas chromatography-mass spectrometry. **Conclusion:** The results showed good cytotoxic activity in the ethanolic extract of *S. anacardium* in both the cell lines may be due to the presence of toxic flavones.

Key words:
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, HeLa and SiHa, Semecarpus anacardium

Introduction

Cancer is a generic term for a large group of diseases that can affect any part of the body. Cancer arises from one single cell. The transformation from a normal cell into a tumor cell is a multistage process; cancer is an exception to the coordinated interaction among cell and organs. In general, the cells of a multicellular organism are programed for collaborations. Many diseases occur because the specialized cells fail to perform their assigned task. Cancer takes this malfunction one step further not only is there a failure of the cancer cell to maintain its specialized function but it also strikes out on its own; the cancer cell competes to survive using natural mutability. *Semecarpus anacardium* (family - *Anacardiaceae*) is a medium-sized tree found in moist deciduous forest in all over the country. It is commonly known as Bhilawa, Bladur in Unani system of medicine. It has been used in traditional system of medicine for different ailments and diseases. The fruits of the plant are used for their therapeutic beneficiary effect to cure the diseases. It is used as antiarthritis and cardiotonic agent. The biflavonoids, phenolics, bhilawanols, sterols, anacardic acid, and glycosides have been identified as constituents of *S. anacardium* nut extract. On the basis of chemical and spectral data, several biflavonoids, such as jeediflavanone, galluflavanone, nalluflavanone, semecarpetin, semecarpuflavanone, and anacardiflavanone, have been characterized.[1] The jeediflavanone also confer scavenging potential, semecarpuflavanone, semecarpetin, and

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galluflavanone have a 7-OH group that might contribute to the free radical-chelating activity of *S. anacardium*.[2] The thin-layer chromatography (TLC), high-performance liquid chromatography, and high-performance TLC analysis of the fruit extract confirmed the presence of the above compounds,[3-6] which show different pharmacological activity. Studies show that the drug has anti-inflammatory, hepatoprotective, antioxidant, antiarthritic,[7] anthelmintic,[8] and hypoglycemic activity[9] and it also act as a cardiotonic agent.[10] Therefore, in the present investigation, the nut milk extract has reported to have anticancer activity.[11] The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **(**MTT) assay of ethanolic extract was carried out on cervix cancer (HeLa and SiHa) cell lines for determination of the anticancer potential of *S. anacardium*.

Materials and Methods

Chemicals

Roswell Park Memorial Institute (RPMI-1640), fetal calf serum (FCS), and phosphate-buffered saline were procured from Gibco, USA. The trypsin-ethylenediaminetetraacetic acid, trypan blue, Penicillin-Streptomycin solution, Dimethyl sulfoxide (DMSO), and MTT were purchased from Sigma-Aldrich, USA. All other solvents and chemical used were of analytical grade and procured from Merck India Ltd.

Plant material

The drug samples were purchased from local market of Delhi, India, and the specimen (Ref. NISCAIR/RHMD/Consult/-2010-11/1563/161/27/10-10) authenticated by botanist Dr. H. B. Singh, Scientist F and Head Raw Material Herbarium and Museum, NISCAIR, New Delhi.

Preparation of hydroalcoholic extract of *Semecarpus anacardium*

The extraction was done by cold extraction method using percolator taking 500 g of powdered drug and extracting it using 70% alcohol as a solvent for 48 h. It was filled and evaporated to dryness under reduced pressure.

Thin-layer chromatography fingerprinting analysis

The hydroalcoholic extract (28 mg) of *S. anacardium* was dissolved in 1.0 mL of methanol and HPTLC was performed using toluene: Ethyl acetate: Formic acid (8:2:0.5 v/v/v) as solvent system for performing TLC fingerprinting.

Gas chromatography-mass spectrometry analysis

The dried hydroalcoholic extract (5 mg) was sonicated in 5.0 mL of hexane for 30 min at room temperature and filtered and then made up the final volume up to 10 mL for gas chromatography-mass spectrometry (GC-MS) analysis.

Cytotoxicity assay of *Semecarpus anacardium* extract

Sample preparation for in vitro activity

The *S. anacardium* extract (500 mg) was dissolved in DMSO and volume was made up to 10 mL in a volumetric flask. The solution was passed thorough 0.45 µ membrane filter and stored at 4°C until used. The previously prepared 50 µg/ml stock solution was diluted 50 times using RPMI-1640 media (1.0–50 mL) to get concentration of 1000 µg/ml of hydroalcoholic extract. It was passed through 0.22 µ membrane filter before using *in vitro* studies.

Cytotoxicity study

The cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL using medium containing 10% FCS. To each well of the 96 well microtiter plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells in 100 µL) was added. After 24 h, when the cell is adhered, media was discarded, and an equal volume of fresh media and drug (100 µL each) was added. The plates were incubated at 37°C in 5% CO_2 incubator for 24, 48, and 72 h. After 24 h, the drug solutions in the wells were discarded, and 25 µL of MTT was added to each well. The plates were gently shaken and again incubated for 3–4 h at 37°C in 5% CO_2 incubator. After 4 h, the supernatant was removed, and viable cell showed the formation of formazan crystal, which was formed by adding MTT. The formed formazan crystal was dissolved by adding 100 µL of DMSO in each well, followed by incubation at 37°C for 30 min. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The experiment was repeated after 48 and 72 h.

Results and Discussion

Thin-layer chromatography fingerprinting

The TLC fingerprinting of hydroalcoholic extract of *S. anacardium* was developed on silica gel. The extract showed a maximum number of UV active compounds and thus detected at 254 nm, with (11) number of spots present in them with their respective R_f values [Figure 1a].

Gas chromatography-mass spectrometry analysis

The recorded spectra were compared with MS library and or reference standard. By comparing with the NIST library, 11 compounds were detected and identified. GC-MS chromatogram of *S. anacardium* was shown in Figure 1b and Table 1.

Cytotoxicity assay

The cytotoxicity of hydroalcoholic extract of *S. anacardium* on cervix cancer cell lines was determined by MTT assay. The results of HeLa and SiHa cell lines cytotoxicity assay of extract are (IC_{50} ranged from 44 µg/ml and 57 µg/ml at 72 h) similar to results of 24 h and 48 h summarized in Table 2, show dose response curve in Figure 2 and microphotograph of HeLa cell and SiHa cells after treatment with hydroalcoholic extract of *S. anacardium* [Figure 3].

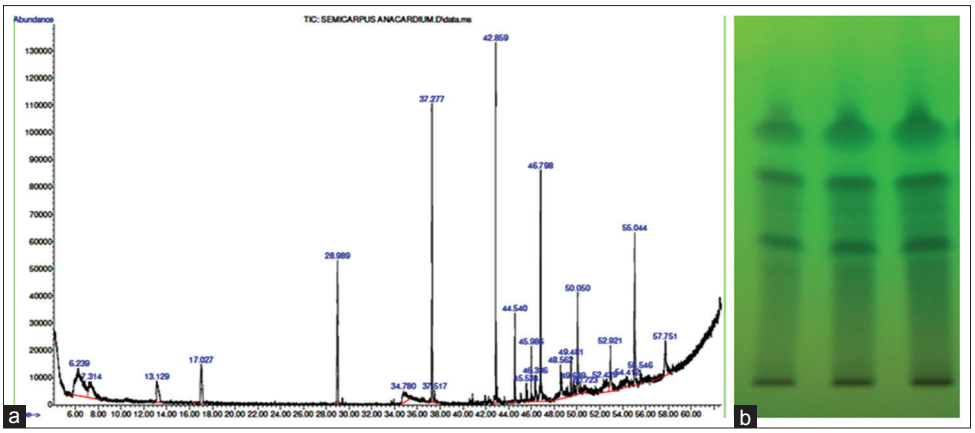

Figure 1: Figure showing gas chromatography-mass spectrometry peak analysis (a) and thin-layer chromatography chromatogram (b) of hydroalcoholic extract of *Semecarpus anacardium*

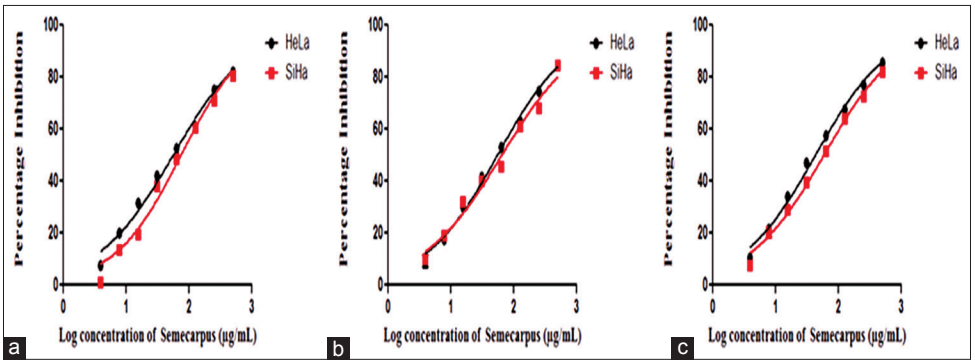

Figure 2: Dose response curve to determine the IC_{50} values of *Semecarpus anacardium* extract in HeLa and SiHa cells after 24 h (a), 48 h (b), 72 h (c)

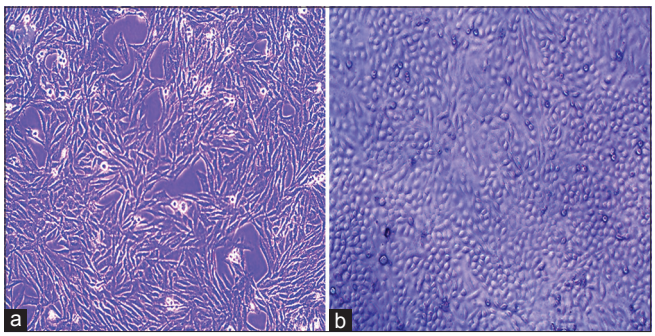

Figure 3: Microphotograph of HeLa cell (a) and SiHa cells (b) after treatment with hydroalcoholic extract of *Semecarpus anacardium*

Conclusion

The results showed good cytotoxic activity in the hydroalcoholic extract of *S. anacardium* in both the cell lines may be due to the presence of toxic flavones, biflavonoids, phenolics, bhilawanols, sterols, anacardic acid, and glycosides have been identified as constituents of *S. anacardium* nut extract.

Table 1: Results of gas chromatography-mass spectrometry analysis of hydroalcoholic extract of *Semecarpus anacardium*

Component name	RT	Peak area (%)
3,5,5-trimethyl-2-cyclohexenone	13.127	2.93
n-dodecanol	17.026	3.48
Cyclododecane	28.991	6.89
1-hexadecene	37.278	10.18
Octadecylene	42.858	9.91
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	44.539	2.75
3,4-dihydro-7,12-dihydroxy-7,12-di methylbenz[α]anthracene	45.984	1.69
1,2-benzenedicarboxylic acid, butyl decyl ester	46.337	1.68
Trifluoroacetoxy hexadecane	46.799	7.98
Diisooctyl maleate	49.483	1.58
1-docosene	50.053	4.10
1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester	55.044	5.81

RT – Retention time

Table 2: Results of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide of hydroalcoholic extract of *Semecarpus anacardium*

Cancer type	Cell line	IC_{50} (μg/mL)		
		24 h	48 h	72 h
Cervix	HeLa	58.0	52.0	44.0
	SiHa	75.0	64.0	57.0

Acknowledgment

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Nil.

Conflicts of interest

There are no conflicts of interest.

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