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EVALUATION OF IN VITRO ANTI CANCER ACTIVITY OF PLANTS FROM SIMAROUBACEAE FAMILY AGAINST HUMAN ORAL CANCER CELL LINE SCC-40

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ABSTRACT

Cancer is one of the most deadly type of disease, encompasses a broad group of syndrome involving unregulated cell population expansion and sustained focus on development of novel anticancer agents from medicinal plants. A number of plant derived compounds play important roles in treatment of cancer. India has the highest number of cases of oral cancer in the world and this is increasing. Because the aetiology of oral cancer is predominantly tobacco related, the immense public health challenge can be meliorated through habit intervention. Plants from simaroubaceae family has been the subject of many studies regarding its chemical constitution and numerous compounds have been isolated for the treatment of many diseases. Quassinoids can be considered a taxonomic markers of the simaroubaceae family. The leaf, stem and stem bark extracts of *Ailanthus excelsa*, *Quassia amara* and *Simarouba glauca* were used for anticancer potential using sulforhodamine B (SRB) cytotoxicity assay against human oral cancer cell line (SCC-40). Adriamycin was used as the standard to compare the results. The median growth inhibition (GI 50) concentration for extracts of *Ailanthus* stem and Bark, *Simarouba* leaf and Bark was <10µg/ml against human oral cancer cell line SCC-40 which indicates potential anticancer activity. It could be concluded that *in vitro* anti-cancer activity could be attributed to the presence of anti-cancerous phytochemicals like Quassinoids.

KEY WORDS: Anticancerous activity, Medicinal plants, Simaroubaceae, oral cancer.

INTRODUCTION

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use. The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations (WHO 1998).[1] Medicinal plants have proved their sole role in coping with a number of deadly diseases including cancer and the diseases associated with viral onslaught viz. Hepatitis, AIDS etc.[2]

Plants also have a long history of use in the treatment of cancer, more than 3000 plant species that have reportedly been used in the treatment of cancer.[3] Plants have played an important role as a source of effective anticancer agent, and it is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources including plants, marine organisms and micro-organisms.[4]

Cancer is one of the major heterogeneous diseases with high morbidity and mortality. Despite extensive research and considerable efforts for developing targeted therapies, it is still an alarming condition with a poor prognosis and high mortality. Studies have demonstrated that cancer cells are highly adapted to elevated levels of reactive oxygen species (ROS) by activating antioxidant pathways.[5] Thus targeting the ROS signalling pathways and redox mechanisms involved in cancer development are new potential strategies to prevent cancer.

The Indian subcontinent, especially India itself because of its large population, has long been regarded as the global epicentre of oral cancer.[6] About 90-95% of all new cases of oral malignancy in most populations, including that in India, are squamous cell carcinomas (SCC) arising from lining mucosa.[7] A number of plant derived compounds play important roles in treatment of cancer, some of the most promising drugs such as taxol, camptothecin, combrestatin, epipodophyllotoxin and the vinca alkaloids being derived from plant sources.[8]

Thus, the research of pharmacologically/ biologically active agents obtained by screening natural sources such as plant extracts had led to the detection of many pharmaceutically valuable drugs that play a key role in the treatment of human diseases. Present paper includes the study of plants from Simaroubaceae family which have been tested for their anti-cancerous activity on Human oral cancer cell line (SCC-40).

The Sulforhodamine B (SRB) assay has been used since its development in 1990.[9] to inexpensively conduct various screening assays to investigate cytotoxicity in cell based studies.[10] This method relies on the property of SRB, which binds stoichiometrically to proteins under mild acidic condition; thus, the amount of bound dye can be used as a proxy for cell mass, which can then be extrapolated to measure cell proliferation.

The Simaroubaceae family includes 32 genera and more than 170 species of trees and shrubs of pantropical distribution.[11] It is characterised by its content of bitter substances, mostly responsible for its pharmaceutical properties.[12] Since 1930, the Simaroubaceae family has been the subject of many studies regarding its chemical constitution and numerous compounds have been isolated and their structure has been elucidated; among these, quassinoids, alkaloids, triterpenes, steroids, coumarin, anthraquinones, flavonoids and other metabolites.[13] According to Polonsky,[14] the active component of plants in the Simaroubaceae family is a group of alkaloids known as quassinoids that gives out its distinct bitter taste.

Ailanthus excelsa Roxb. belonging to family Simaroubaceae is commonly known as Maharukha. It is large deciduous tree; bark slightly bitter and leaves pinnately compound. The traditional claims, phytochemical investigation, pharmacological evaluation and some ayurvedic formulations provide the backbone to make this tree, a plant of heaven.[15] Traditionally or in Indian system of medicine, *Ailanthus excelsa* Roxb. is used in treatment of asthma, cough, cancer, diabetes and also used as antispasmodic and bronchodilator.[16]

Quassia amara commonly known as bitter wood or Amargo is a small evergreen shrub growing only about 3 meter in height. It bears a small drupe, red flowers and compound, alternate leaves. *Quassia amara* Linn. being an ethnomedicinal plant proved to have anti-diabetic properties. Quassin constituents in it are one of the bitterest substances found in nature and it has been used in management of type 2 diabetes.[17]

Simarouba glauca, commonly known as 'Laxmitaru' or 'paradise tree'. The specific name *glauca* means covered with bloom which refers to the bluish green foliage. This is evergreen tree, grows to a height of 12-15 m with large circular crown. The bark and leaf extract of *Simarouba* is well known for its different types of pharmacological properties such as antihelmenthic, antiparasitic, antidysentric and anticancerous.[18] Joshi and Joshi.[19] speculated that the chemicals present in leaf, fruit, pulp and seed of *S. glauca* are known to posses the medicinal properties such as analgesic, antimicrobial, antiviral, astringent, stomachic and vermifuge.

MATERIALS AND METHODS

Collection of Plant Material: Fresh plant material of *Ailanthus excelsa*, *Quassia amara* and *Simarouba glauca* (leaf, stem and stem bark) were collected from different areas across Mumbai. Plant material was first washed and then sundried followed by oven drying at temperature 80^{o}C and then powdered using grinder.

Preparation of Extracts: Plant extracts were prepared by taking 80% of methanol as a solvent and kept in water bath for 2 hours at 60^{o}C. The extracts were filtered using filter paper. The filtered extracts were kept for complete evaporation of solvent in an oven at 40^{o}C and concentrated material was collected and weighed to obtain extracted values. The extracts obtained in concentrated form were used to test the anti-cancerous activity against human oral cancer cell line SCC-40.

Evaluation of Anticancer Activity Method: *In vitro* SRB assay for anti-cancer activity evaluation of plant extracts was done at Anti-cancer Drug screening facility (ACDSF) at ACTREC, Tata Memorial Centre, Navi Mumbai.

Experimental Procedure for SRB Assay

The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml with complete medium containing test article. Aliquots of 10µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90µl of medium, resulting in the required final drug concentrations i.e.10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the

plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10mM trizma base, and the absorbance was read on an plate reader at a wavelength of 540nm with 690nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells x 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: [Ti/C] x 100%

Percentage growth inhibition, total growth inhibition TGI) and LC50 was calculated. GI50 value of ≤10 µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤20 µg/ml is considered to demonstrate activity.

Above three parameters were calculated only when the level of activity was observed. The values were expressed as greater or less than maximum or minimum concentration tested when the effect was not reached or exceeded.[9,10]

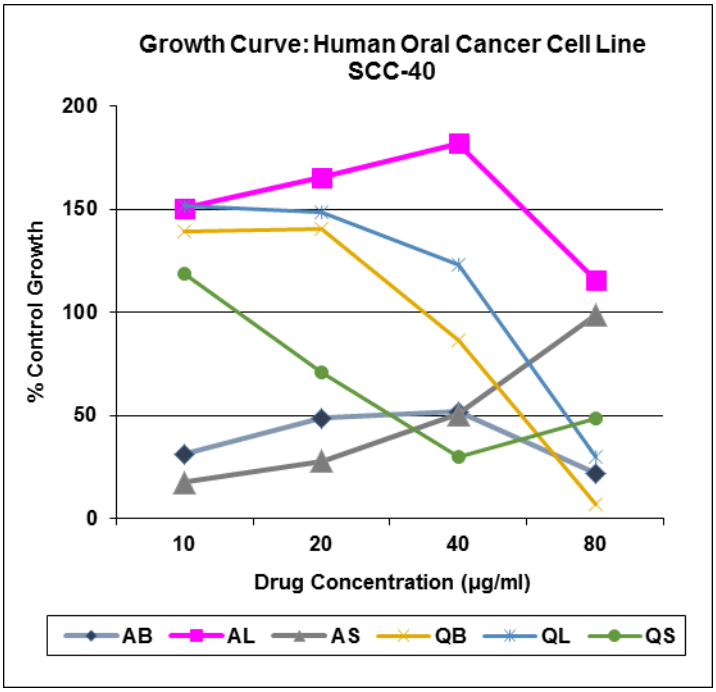
RESULTS AND DISCUSSION

The extract of leaf, stem and stem bark of *A. excelsa*, *Q. amara* and *S. glauca* were screened for anticancer activity against human oral cancer cell line (SCC-40) at 10, 20, 40 and 80 (µg/ml) concentrations (Table 1 & 2). SRB assay was used for evaluation and absorbance was read on a plate reader at a wavelength of 540nm with 690nm reference wavelength.

The lethal concentration value (LC50) of all extracts of plant samples are represented by NE (non-evaluable data) that means experiment needs to be repeated using different set of drugs and total growth inhibition (TGI) of QB (*Quassia* bark) and SS (*Simarouba* stem) was found to be >80µg/ml and SB (*Simarouba* bark) was 71.9 and SL (*Simarouba* leaf) was 6.7 whereas rest all samples data was non-evaluable. The median growth inhibition (GI50) concentration for extracts of AB (*Ailanthus* Bark), AS (*Ailanthus* Stem), SB (*Simarouba* Bark) and SL (*Simarouba* Leaf) was <10µg/ml against human oral cancer cell line SCC-40 (Table 3) showing anticancer efficacy of plant extracts. However median growth inhibition (GI50) of QL (*Quassia* leaf), QS (*Quassia* stem), QB (*Quassia* bark) and SS (*Simarouba* stem) indicated no anticancer effect of extracts against SCC-40 cell line. Whereas AL (*Ailanthus* leaf) represents non-evaluated data.

Table 1: Percentage growth of SCC-40 cell line against various extract of *A. excelsa* and *Q. amara.*

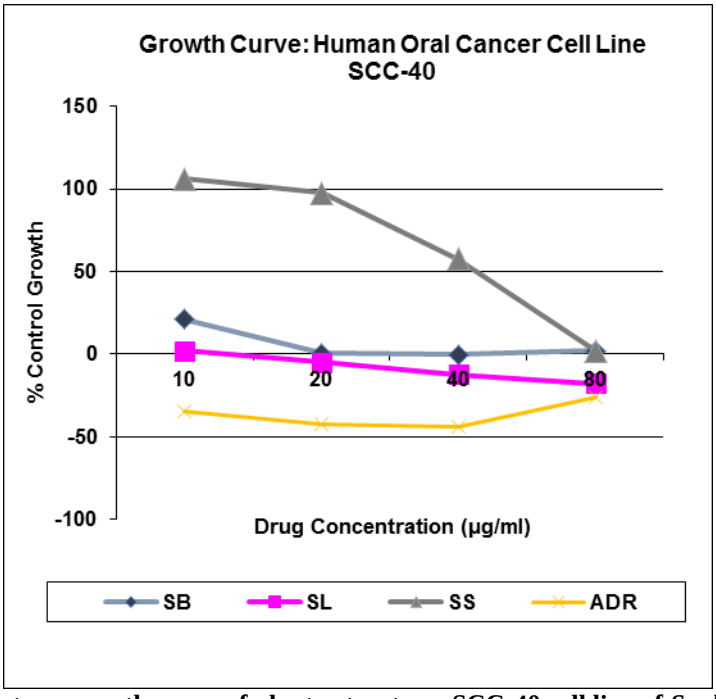
4.00	**Human Oral Cancer Cell Line SCC-40**															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				**Experiment 2**				**Experiment 3**				**Average Values**			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
AB	98.8	169.4	166.7	56.3	-3.0	-9.0	-8.2	-6.1	-2.1	-14.0	-2.6	15.5	31.2	48.8	51.9	21.9
AL	241.2	246.5	295.5	149.1	101.1	132.1	124.2	94.2	109.8	118.0	126.1	104.5	150.7	165.5	181.9	115.9
AS	107.8	135.2	144.2	135.5	-29.4	-24.0	6.7	87.3	-25.6	-28.0	1.2	73.3	17.6	27.8	50.7	98.7
QB	232.0	254.2	191.3	28.9	92.3	95.2	41.4	0.1	94.2	71.4	27.4	-8.8	139.5	140.3	86.7	6.7
QL	238.8	239.7	205.5	52.9	115.8	105.5	93.6	18.9	101.4	101.4	70.5	18.7	152.0	148.9	123.2	30.1
QS	176.2	157.7	97.9	78.5	91.4	42.3	5.7	40.4	88.0	13.0	-13.0	26.2	118.5	71.0	30.2	48.4



Graph 1: Percentage growth curve of plant extracts on SCC-40 cell line of *A. excelsa* and *Q. amara.*

Table 2: Percentage growth of SCC-40 cell line against various extract of *S. glauca* and ADR.

3.00	Human Oral Cancer Cell Line SCC-40															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
SB	34.1	10.7	-0.3	8.5	20.4	-2.0	15.1	5.7	8.7	-7.6	-15.3	-7.6	21.1	0.4	-0.2	2.2
SL	5.6	2.6	-5.5	-17.2	10.7	-1.2	-10.1	-21.0	-9.7	-16.3	-20.9	-15.6	2.2	-5.0	-12.2	-17.9
SS	110.2	105.1	68.4	5.9	114.5	101.1	61.6	1.3	94.2	86.8	42.8	-0.7	106.3	97.7	57.6	2.2
ADR	-35.6	-38.6	-37.1	-23.7	-24.5	-35.4	-39.5	-12.6	-44.6	-53.2	-54.4	-41.9	-34.9	-42.4	-43.7	-26.1

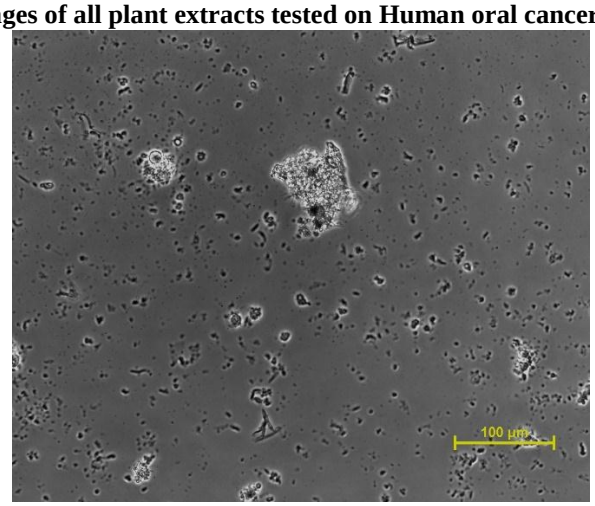


Graph 2: Percentage growth curve of plant extracts on SCC-40 cell line of *S. glauca* and ADR.

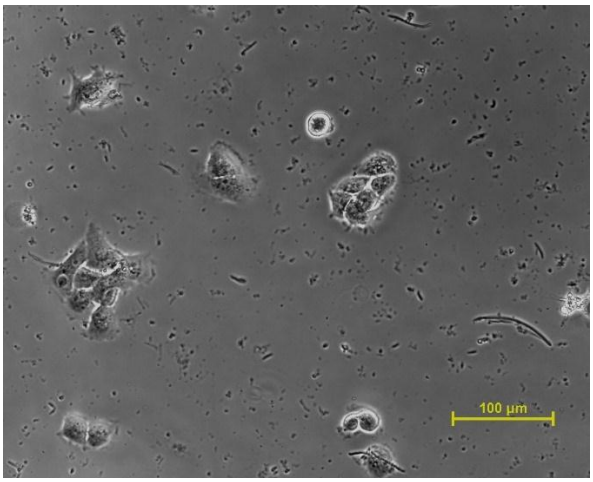
Table 3: Results of SRB assay against SCC-40 of various plant extracts.

	Drug concentrations (µg/ml) calculated from graph		
SCC-40	**LC50**	**TGI**	**GI50***
AB	NE	NE	<10
AL	NE	NE	NE
AS	NE	NE	<10
QB	NE	>80	59.0
QL	NE	NE	72.6
QS	NE	NE	58.1
	Drug concentrations (µg/ml) calculated from graph		
SCC-40	**LC50**	**TGI**	**GI50***
SB	NE	71.9	<10
SL	NE	6.7	<10
SS	NE	>80	47.9
ADR	<10	<10	<10

Images of all plant extracts tested on Human oral cancer cell line SCC-40



AB



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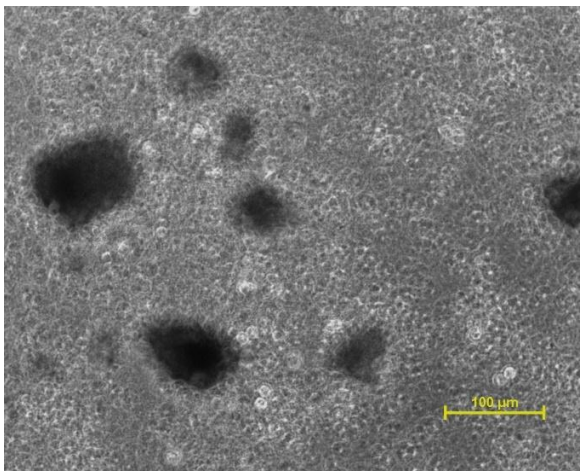

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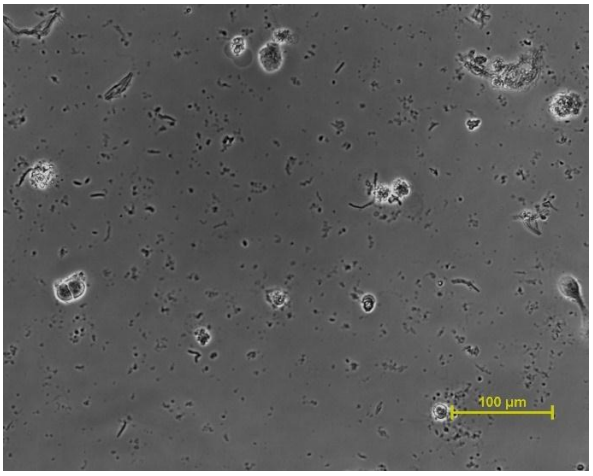
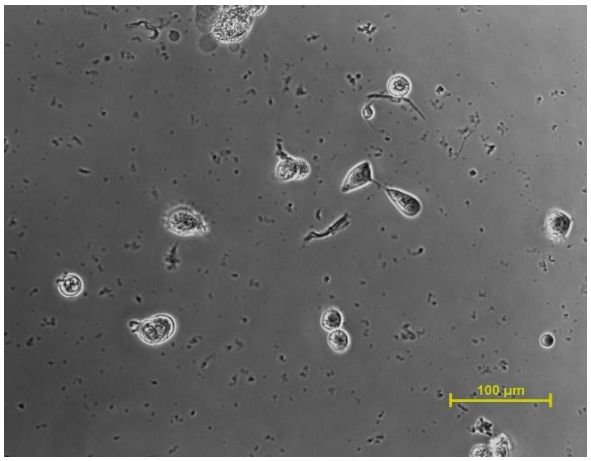
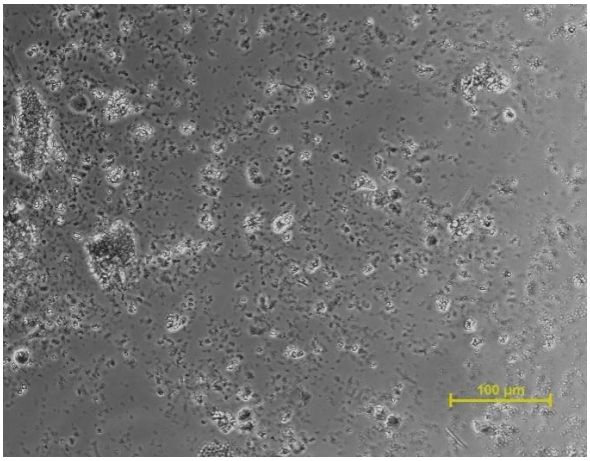


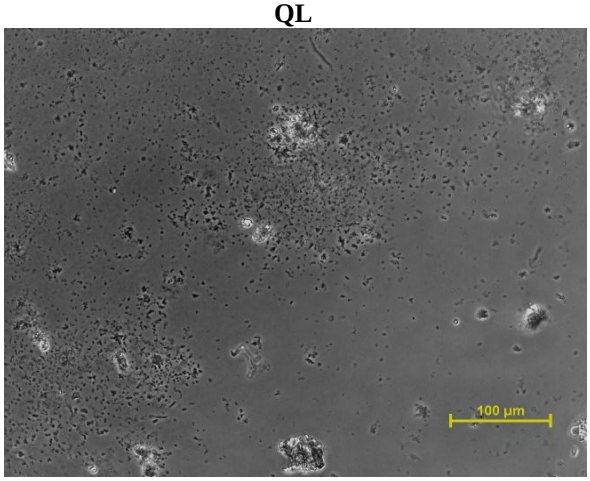
QB



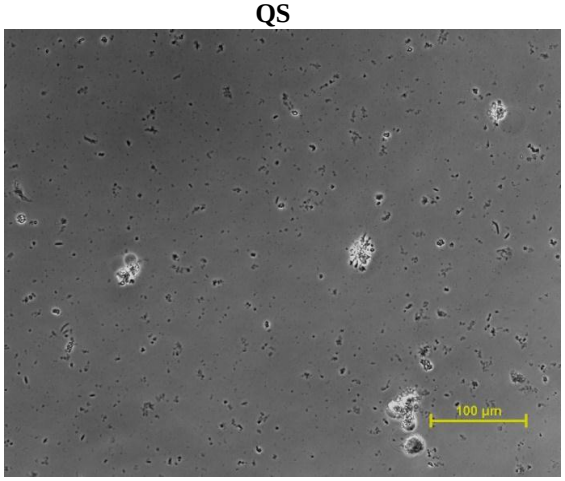
QL



QS



SB



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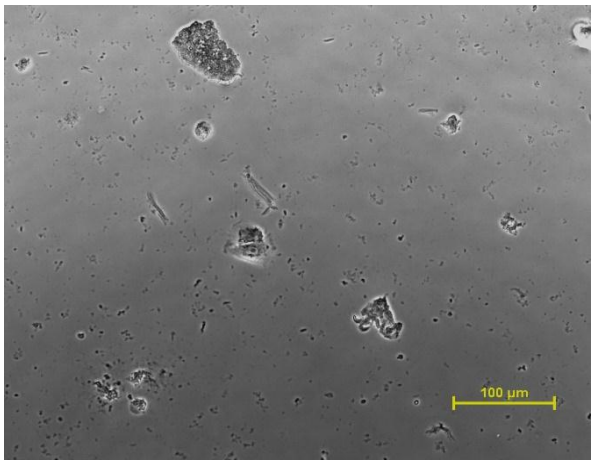

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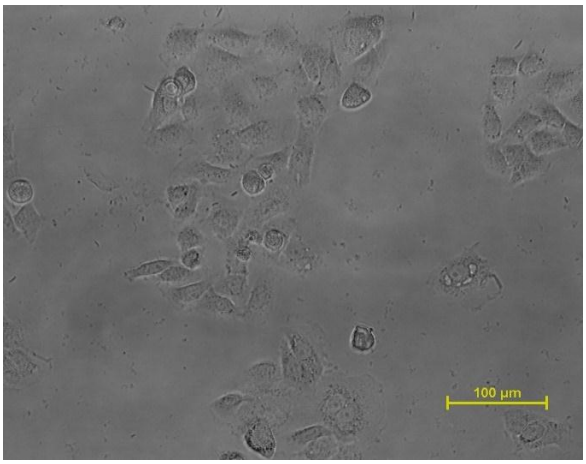
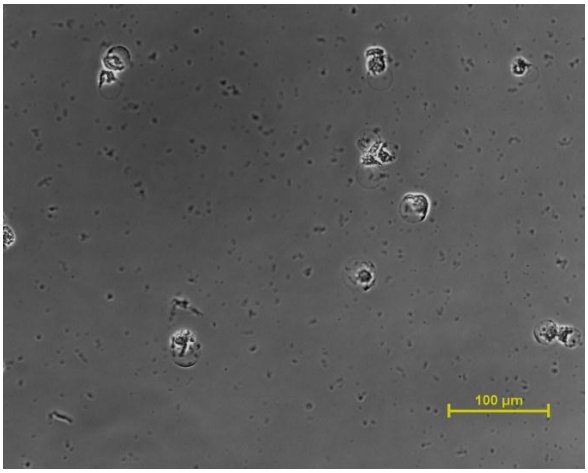


Control



Positive Control

On the basis of above results it can be postulated that *Ailanthus excelsa* and *Simarouba glauca* plant possess anticancer activity whereas *Quassia amara* was found to have no anticancer activity against human oral cancer cell line SCC-40. The results of SRB assay were compared with ADR (Adriamycin), a known drug available in the market as anti-cancer agent. Several previous studies showed that plant extract contains abundant number of phytochemicals which possess anticancer properties and might be responsible for anticancer effect of these plant extracts. Though the plants have anticancerous properties with other cancers but no reports available for oral cancer**.**

Apparently, the promising active principle in *Ailanthus excelsa* and *Simarouba glauca* inhibits oral cancer cell line (SCC-40), indicating need to investigate underlying mechanism by which this activity was exhibited. Further, all these plant extracts need to be screened against different cell lines apart from the selected cell line to confirm the activity.

CONCLUSION

The present study was carried out to explore anticancer potential of plants from Simaroubaceae family. Selected plants *Ailanthus excelsa* and *Simarouba glauca* showed promising anticancer activity whereas *Quassia amara* did not showed anticancer activity against human oral cancer cell line SCC-40. The anti-cancerous activity might be correlated to its Quassinoid content and other anti-cancerous phytochemicals. Furthermore, these data can also pave the way for future development of therapeutic opportunities against cancer.

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