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Research Article

INHIBITORY EFFECTS OF PLANTS EXTRACTS ON PROSTATE CANCER

Deepa Dahar and Aruna Rai*

Department of Botany, Smt. C. H. M. College (Affiliated to University of Mumbai), Ulhasnagar 421003, Thane, Maharashtra, India

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ABSTRACT

Cancer is a broad term. It describes the disease that results when cellular changes cause the uncontrolled growth and division of cells. Cancer is one of leading cause of death worldwide and sustained focus on development of novel anticancer agents from medicinal plants. Traditional medicinal systems are based on plants as a source of medicine for the treatment of life threatening diseases and disorders. Plants from Simaroubaceae family have been the subject of many studies regarding its chemical constitution and numerous compounds have been isolated for the treatment of many diseases. Among these are the quassinoids, alkaloids, triterpenes, steroids, coumarin, anthraquinones, flavonoids and other metabolites. Quassinoids can be considered a taxonomic marker of the Simaroubaceae family. Methanolic extracts of leaf, stem and stem bark of *Ailanthus excelsa,* Quassia amara and Simarouba glauca from Simaroubaceae family were used for anticancer potential using sulforhodamine B (SRB) cytotoxicity assay against human prostate cancer cell line PC-3. Adriamycin was used as a standard to compare the results. The median growth inhibition (GI50) concentration for methanolic extracts of Ailanthus stem and bark, Quassia stem and bark and Simaroubaleaf, stem and bark was <10 µg/ mlagainst Prostate cancer cell line, which indicates potential anticancer activity. It could be concluded that in vitro anticancer activity could be attributed to the presence of anticancerous phytochemicals like Quassinoid.

INTRODUCTION

Plants are very useful source of various bioactive compounds which have direct or indirect use in the treatment of various human ailments. From the time immemorial, human civilizations have been exploring and using various plants and plant products to cure the deadly diseases.

Among all diseases, cancer remains one of the leading causes of morbidity and mortality globally. Amongst the non-communicable diseases, cancer is the second leading cause of death, after cardiovascular disease. Cancer is responsible for one in eight deaths worldwide more than AIDS, tuberculosis and malaria together [1].

Cancer has been a constant battle globally with a lot of development in cures and preventive therapies. Current treatments include chemotherapy, radiotherapy and chemically derived drugs. Treatment such as chemotherapy can put patients under a lot of strain and further damage their health. Therefore, there is a focus on using alternative treatments and therapies against cancer. For many years herbal medicines have been used and are still used in developing countries as the primary source of medical treatment, plants have been used in medicine for their natural antiseptic properties. Thus research has developed into investigating the potential properties and use of terrestrial plant extracts for the preparation of potential nanomaterial based drugs for diseases including cancer.

However, it is difficult to engineer a chemically derived drug which is non-toxic to normal cells and is specific to cytotoxicity of cancer cells [2]. Therefore, development and research into naturally derived compounds to be used for anticancer treatment is becoming high in demand with a focus on those derived from plant species and their natural products.
Prostate cancer is the second most common cause of cancer deaths in men in most developed countries, and the incidence has increased significantly over recent years. Age is the most important risk factor. Prostate cancer is rare under the age of 40, and its incidence increases exponentially with age [3]. Present paper includes the study of plants from Simaroubaceae family which have been tested for their anticancerous activity on Human Prostate cancer cell line PC-3.

Corresponding author:* **Aruna Rai
Department of Botany, Smt. C. H. M. College (Affiliated to University of Mumbai), Ulhasnagar 421003, Thane, Maharashtra, India

The Sulforhodamine B (SRB) assay has been used since its development in 1990 [4]. to inexpensively conduct various screening assays to investigate cytotoxicity incell based studies [5]. 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. Given the treatments that are currently being used to fight prostate cancer and associated complications, drug resistance especially in metastatic prostate cancers, ever increasing costs of common treatments and increasing incidence of prostate cancer in both developing and developed countries, it is necessary to discover newer therapeutic approaches with higher efficacy to reduce the incidence and mortality of prostate cancer. In this regard, it is necessary to find cytotoxic plants against various cancers, especially prostate cancer, which, despite lower side effects, can replace chemotherapy and difficult treatments and also be used for treatment resistant cases [6].

The Simaroubaceae family includes 32 genera and more than 170 species of trees and shrubs of pantropical distribution [7]. It is characterised by its content of bitter substances, mostly responsible for its pharmaceutical properties [8]. 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 [9]. Quassinoids can be considered a taxonomic marker of the Simaroubaceae family since it is the most abundant group of natural substances and their synthesis is almost exclusive. According to Polonsky [10] 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 excels 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 [11]. 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 [12].

Quassia amara or 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 is one of the bitterest substances found in nature and it has been used in management of type 2 diabetes [13]. Plant is shown to have activity against malaria. An HPLC method was employed for purification of the biologically active quassinoids; quassin and neo-quassin [14].

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 [15]. Root and leaves are used in diarrhoea, dysentery, intestinal worms and malaria [16]. Joshi and Joshi [17] speculated that the chemicals present in leaf, fruit, pulp and seed of *S. glauca* are known topossesthe medicinal properties such as analgesic, antimicrobial, antiviral, astringent, stomachic, vermifuge.

MATERIALS AND METHODS

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

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 prostate cancer cell line PC-3.

***Evaluation of Anticancer Activity Method*:** *In vitro* SRB assay for anti-cancer activity evaluation of methanolic 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 micro titer plates were incubated at 37°C, 5% CO_2, 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.

Table 1 Percentage growth of PC-3 cell line against various extract of *A. excelsa* and *Q. amara.*

2.00	Human Prostate Cancer Cell Line PC-3															
	% 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	1.2	-3.3	-4.4	2.7	-22.9	-17.8	-9.8	-11.6	-12.1	-12.4	-9.9	-6.8	-11.3	-11.2	-8.0	-5.2
AL	100.0	143.1	93.4	88.8	83.5	98.6	104.8	70.6	96.3	121.1	96.5	76.7	93.2	121.0	98.2	78.7
AS	-15.7	-12.2	-7.1	3.8	-30.1	-23.2	-25.3	-13.2	-30.1	-16.1	-19.0	-8.0	-25.3	-17.2	-17.1	-5.8
QB	39.1	32.2	5.9	-1.7	20.9	15.4	9.4	-10.9	9.6	4.3	-2.2	0.1	23.2	17.3	4.4	-4.2
QL	88.5	74.6	40.7	11.5	86.0	78.1	42.6	0.4	77.9	67.9	36.7	9.2	84.1	73.5	40.0	7.0
QS	41.4	8.8	29.9	31.3	24.1	14.9	19.6	22.6	18.6	6.8	20.2	32.8	28.0	10.2	23.3	28.9

Table 2 Percentage growth of PC-3 cell line against various extract of *S. glauca* and ADR

2.00	Human Prostate Cancer Cell Line PC-3															
	% 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	12.7	9.3	13.0	4.1	-14.2	-12.6	-15.9	-19.7	-3.6	3.8	-5.9	1.4	-1.7	0.2	-2.9	-4.7
SL	-4.9	-3.0	-4.8	-2.4	-11.3	-16.5	-11.5	-6.6	-19.2	-14.6	-9.7	-13.3	-11.8	-11.4	-8.7	-7.4
SS	61.9	28.3	5.0	0.0	50.4	26.7	-4.1	-3.2	46.6	37.7	2.4	-6.7	53.0	30.9	1.1	-3.3
ADR	-41.1	-61.2	-61.2	-49.2	-48.5	-52.6	-64.9	-54.1	-45.5	-51.2	-59.8	-47.3	-45.0	-55.0	-62.0	-50.2

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 (Skehan *et al*.,1990) (Vichai and Kirtikara, 2006).

RESULTS AND DISCUSSION

Anticancer efficacy of methanolic extract of leaf, stem and stem bark of *A. excelsa*, *Q. amara* and *S. glauca* were screened using Human prostate cancer cell line (PC-3) 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.

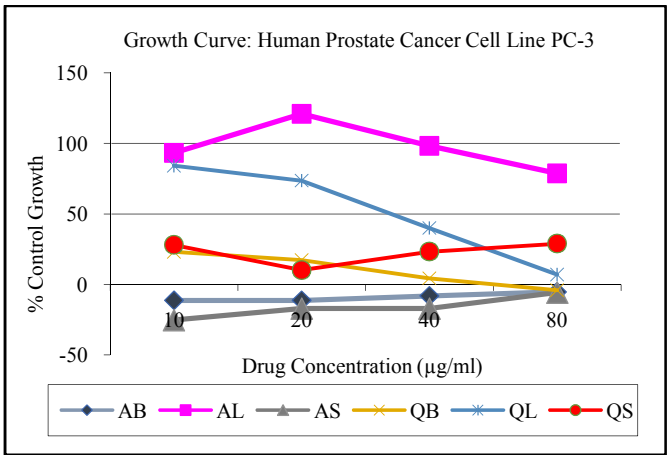
The lethal concentration value (LC50) of all extracts of plant samples are represented by NE (non-evaluable data) that means experiment needs to be repeated using different set of drugs and total growth inhibition (TGI) of SB was <10µg/ml and SS was 65.0µg/ml, whereas rest all samples data was non-evaluable. The median growth inhibition (GI50) concentration for extracts of AB (*Ailanthus* Bark), AS (*Ailanthus* Stem), QB (*Quassia* Bark), QS (*Quassia* Stem), SL (*Simarouba* Leaf), SB (*Simarouba* Bark) and SS (*Simarouba* Stem) was <10µg/ml against Human prostate cancer cell line PC-3 (Table 3), showing anticancer efficacy of methanolic extract.

However, median growth inhibition (GI50) concentration of AL (*Ailanthus* Leaf) data was non-evaluable and QL (*Quassia* Leaf) is 38.5µg/ml which indicated no anticancer effect of extract.

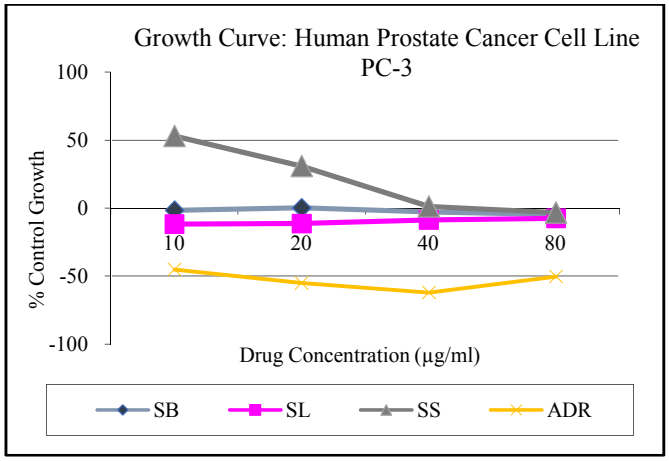
On the basis of above results it can be postulated that all the three plant possess anticancer activity against Human prostate cancer cell line PC-3. The results of SRB assay were compared with ADR (Adriamycin), a known drug available in the market as anti-cancer agent. Apparently, the promising active principle in *Ailanthus excelsa*, *Quassia amara* and *Simarouba glauca* inhibits prostate cancer 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.

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. Quassinoids are the main active group of chemicals in *Simarouba* which belongs to the triterpene chemical family (Iasmine *et al*.,2014). The presence of Quassinoid may be attributed to anticancer effect of methanolic extract of these plants.



Graph 1 Percentage growth curve of PC-3 cell line against plant extracts of *A. excelsa* and *Q. amara*.

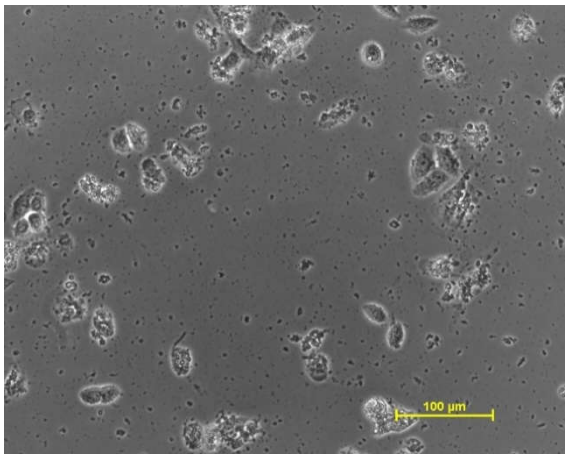


Graph 2 Percentage growth curve of PC-3 cell line against plant extracts of *S. glauca* and ADR.

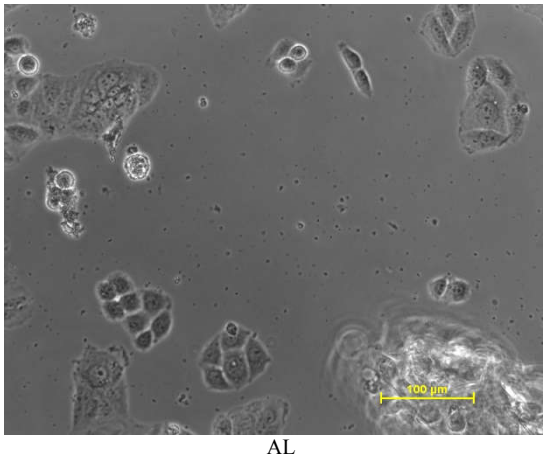
Table 3 Results of SRB assay of PC-3 cell line against various plant extracts

Drug concentrations (µg/ml) calculated from graph			
PC-3	**LC50**	**TGI**	**GI50***
AB	NE	NE	<10
AL	NE	NE	NE
AS	NE	NE	<10
QB	NE	NE	<10
QL	NE	NE	38.5
QS	NE	NE	<10

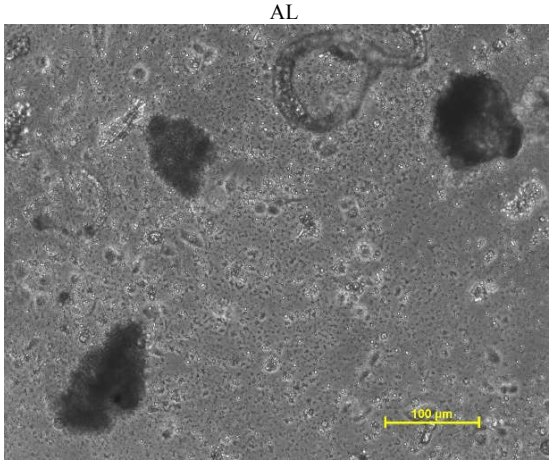
Drug concentrations (µg/ml) calculated from graph			
PC-3	**LC50**	**TGI**	**GI50***
SB	NE	<10	<10
SL	NE	NE	<10
SS	NE	65.0	<10
ADR	<10	<10	<10



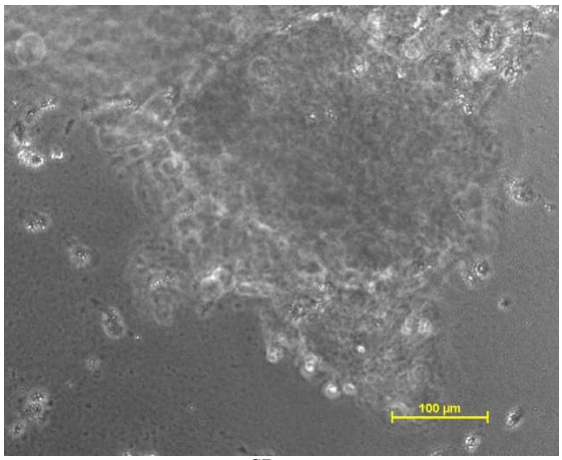
AB



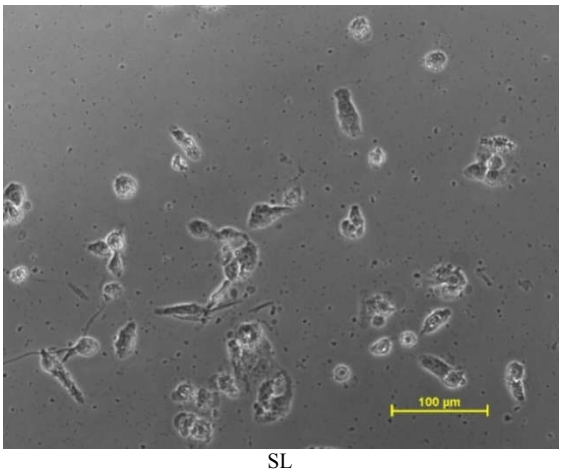
AL



AS



SB



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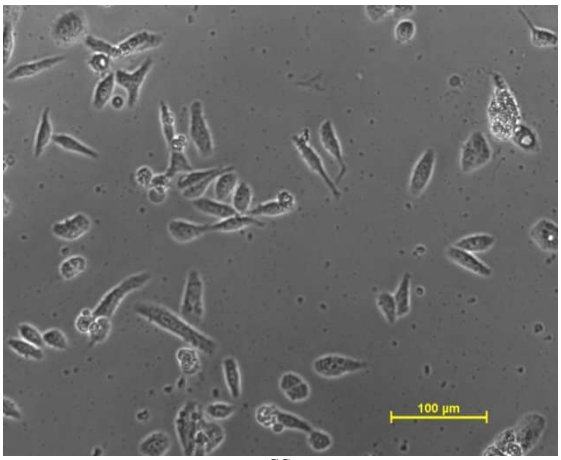
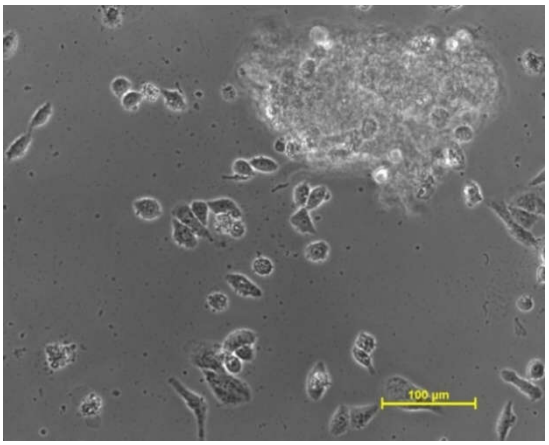
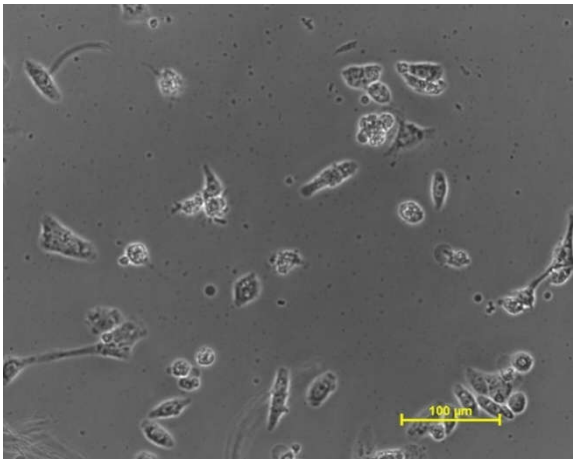


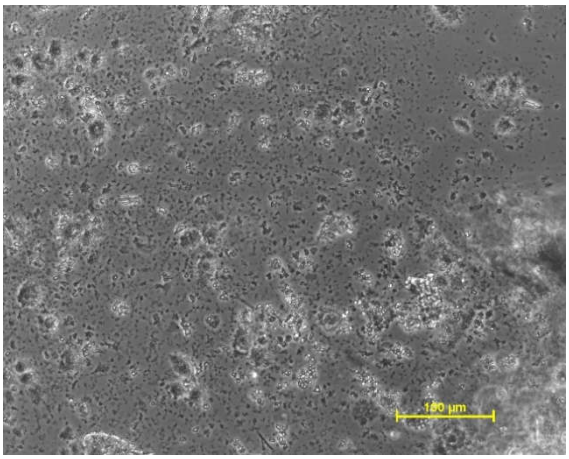
SS



QB



QL



QS

Photoplate 1 Images of all plant extracts tested on Human prostate cancer cell line PC-3.

CONCLUSION

The present study was carried out to explore anticancer potential of methanolic extract of plants from Simaroubaceae family. Selected plants *Ailanthus excelsa*, *Quassia amara* and *Simarouba glauca* showed promising anticancer activity against Human prostate cancer cell line PC-3 which might be correlated to its Quassinoid content and other anticancerous sphytochemicals. In future, work will be needed to isolate bioactive constituents of plants parts extracts to locate potential anticancer phytochemical.

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