Purva Mimaansa | Refereed | Peer Reviewed
ISSN: 0976-0237 | Volume 13, Issue September 2022 | Impact Factor 5.004

IN-VITRO ANTI-CANCER EVALUATION OF SQUALENE SEPARATED FROM LANTANA CAMARA LEAF EXTRACT AGAINST A549 AND HELA CELL LINES

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ABSTRACT

Worldwide cancer is the leading cause of death. Over the years, the use of same type drugs for certain class of cancers develops resistance while some drugs start showing heavy side effects on the body. Therefore, finding novel anti-cancer drugs is theforemost area of interest in academia as well as in industry. *Lantana camara* is well known for its anticancer properties but very few studies have done on compounds separated from the extract of this plant. The present study was designed to carry out the anticancer activity of separated terpene-based Squalene from ethyl acetate extract of this plant.MTT bio-assay method was used for determining the anticancer activity using A549 (Human Lung Carcinoma) cell line and HeLa (Human Cervix Carcinoma) cellline. Squalene aliquots were taken at different concentrations (10µg, 20µg and 30µg) as test samples for this bioassay. Doxorubicin (30µg/ml), a well-known anti-cancer drug was used as a standard reference compound and blank as control. Absorbance and % cell lysis was calculated at the end of the experiment. Squalene showed promising anticancer activity as % Cell lysis evaluated was in the range almost nearer to the reference standard for both the cell lines. The IC_{50} value was found to be 30µg against A549 cell line and less than 10µg against HeLa cell line. These findings support the fact that *Lantana camarais a good source of* anti-cancer agents.

KEYWORDS: *Lantana camara*, MTT assay, A549 cell line, HeLa cell line, Squalene.

INTRODUCTION

Cancer is the leading cause of deaths worldwide and it ranks second in India causing about 0.3 million deaths per year. Cancer development is a dynamic, long term and multistage process which involves many complex factors through critical steps of initiation, promotion and progression, which lead to an uncontrolled growth of cancerous cells throughout the body[1, 2].

In such a multistage carcinogenesis, promotion stage is closely linked to oxidative and inflammatory tissue damage. A substance with pronounced antioxidant and anti-inflammatory effect is anticipated to act as an anti-cancer agent. Natural products have always been the source of many active ingredients in medicines[3, 4].

One of the areas where plants and their constituents have had a major impact on longevity and quality of life is in the chemoprevention of cancer. Natural products with diverse chemical structures still remain the most important source of discovery for new and potent drug molecules.There are many nutritive and non-nutritive plants and naturally inspired sources currently under investigation for their potential cancer chemopreventive effects. Market is full with many a new anti-cancerdrug but most of these chemopreventive drugs have limited efficacies. Chemotherapy suppresses the immune system so that conventional drugs related to plant and animal kingdom staging a comeback for the treatment of cancers[5].

Indian traditional and folklore from time immemorial depends upon plants for cure and care needs due to vast forest cover with enriched plant biodiversity. Plants and plant derived components have been used as folklore medicine[6].

A wide variety of terpenoids derived from different species of family *Verbenaceae* have been reported to have marked antioxidant and anti-inflammatory effects which contribute to their cancer chemopreventive potential. Most of the members of *Verbenaceae* are used in folklore medicine around the world. The present study was thus initiated with *Lantana camara* plant belonging to *Verbenaceae* which is traditionally used in medicinal practices even today. It was revealed from the study to the full extent that *Lantana camara* show immense potential in cancer treatment. It is suggested to have potential anticancer properties prominent in occurrence as the plant is rich in anticancer compounds such as sesquiterpenes, triterpenes etc[7, 8]

Terpenes, especially, triterpenes have basically been recognized as potent anticancer agents that selectively target diseased and unhealthy human cells, while sparingnormal and healthy cells[9]. From our previous column chromatographic experiment Squalene was separated and characterized from ethyl acetate extract of this plant and our objective of the present work is to evaluate the anti-cancer activity of this separated compound against A549 and HeLa cell lines.

MATERIALS AND METHOD

Lantana camara **(**Family: *Verbenaceae*) commonly known as Wild sage, is an evergreen aromatic shrub found in yellow-orange, pink-violet, yellow and white shades of flowers ornamentally planted at house gardens (Figure 1). It is a medium sized branched deciduous plant distributed throughout India and a native of tropical, sub-tropical America, wild or cultivated. It requires dry, hot and temperate climate to grow.Its leaves are bitterminty tasting, easy to digest, anti-phlogisitic, anti-dermatosesand Anti-pyretic[10]. Plant of *Lantana camara was* collected from outskirts of Gondia, Maharashtra, India. The collected plant was authenticated from Department of Environmental Science, Institute of Science, Nagpur by renowned taxonomist Dr. (Mrs.) Rani V. Choubey.

Taxonomy of Plant	
Kingdom	Plantae
Order	Lamiales
Family	Verbenaceae
Genus	Lantana
Species	*camara*

Figure 1. General plant profile of *Lantana camara.*

Preparation of Extract and Column Chromatographic Component separation: Dried coarsely powdered leaves of *Lantana camara* 500 g were subjected to extraction using ethyl acetate as solvent. The weighed plant materials, fresh/dried, were put in a well-sealed brown glass bottles with 750 ml solvent and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter get dissolved. The extract thus obtained was decanted and filtered. The clear extract was subsequently concentrated using rotary vacuum evaporator. The crude residual mass of extract was concentrated, stored and preserved (2-8°C) [11].The preserved extract was further subjected to column chromatography for separation of components.

Cancer cell-lines:

A549 Lung carcinoma cell line and HeLa Cervix cell line for MTT bioassay were procured from the cell repository of the National Center for Cell Science (NCCS) at Pune.

Revival of frozen cells and maintenance of cell culture:

Ampules were removed with the help of a pair of forceps from the liquid nitrogen containers to immerse instantly in 37 °C water bath and shaken constantly for fast thawing. Then ampules were wiped with alcohol and opened by breaking the upper neck region. The contents were transferred to appropriately marked tubes. Cell lines were grown and maintained in Minimal Essential Medium (MEM) supplemented with 5.5 g/L Glucose, 2.5 ml L-Glutamine and 10% Fetal Bovine Serum (FBS) (growth medium) at 37 °C in 5% CO_2.

MTT ASSAY[12-18]

Requirements:

Growth medium with 10 % FCS, 5mg/ml MTT, 3-(4, 5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide solution sterilized by filtration dissolved in PBS, Dimethyl sulfoxide (DMSO), Dulbecco's modified eagle's medium (DMEM).

Experimental procedure:

Cells were seeded on to 96 well plates at a cell density of 1×10^5 mL^{-1} per well and allowed to grow in a CO_2 incubator for 24 h at 37 °C, 5% CO2. After 24 hours, the medium is

removed and replaced by fresh medium with Squalene of 10, 20 and 30 µg/ml dilutions made by dissolving in DMSO which is added to each well and incubated for 48 hours. The control groups received the same amount of DMSO. Doxorubicin was used as positive control. Cells were incubated for 24 h at 37 °C, 5 %CO2. Then, 100 µL MTT ([3- (4, 5-dimethylthiazol-yl)-2, 5-diphenyltetrazoliumbromide]) solution (0.5 mg/mL in Dulbecco's modified eagle's medium) was added toeach well and incubated for 4 hours.

The MTT assay converts yellow tetrazolium salt-MTT topurple-formazan crystals by metabolically active cells which quantitatively determines presence of viable cells. The formazan product of MTT reduction was dissolved in DMSO. The medium was removed and100 µL DMSO was added to each well to dissolve the MTT metabolic product. Then the plates were shaken at 150 rpm for 5 min and the optical density was measured at 540 nm.

Reading of results and calculations: Absorbance of wells containing cells and blanks was measured at 540 nm. Mean Value of absorbance of wells was calculated with all treatments after subtracting of blank absorbance. The results were normalized by considering control wells as 100%expressing the results as percentage of controls. The O.D. is directly proportional to cell lysis. An increase in O.D. signifies increased cell lysis. Due to increased cell lysis, O.D. increases as turbidity increases. Three concentrations viz., 10, 20 and 30 µg/ml of compound was used. IC_{50} value and % lysis cell at each concentration was calculated. Two cell lines were used for performing anticancer activity i.e. A549, HELA. The percentage cell lysis of each cancer cell line was calculated by using following equation:

% cell lysis= [100 - (absorbance of test wells/absorbance of control wells) 100]

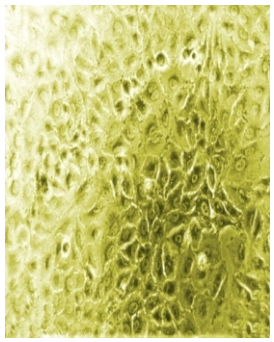
RESULT

The effect of Squalene separated from ethyl acetate extract of *Lantana camara* was studied by using MTT assay.

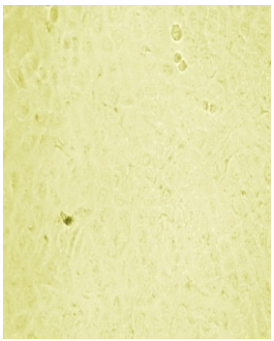
Against A549-Human lung carcinoma cell line: The IC_{50}value of compound against A549-Human lung carcinoma cell line was found to be 30 µg/ml and the results are mentioned in Table 1, Figure 2, Figure 3 and Figure 4.

Table 1. In- vitro evaluation of IC_{50} against A549 cell line by MTT assay.

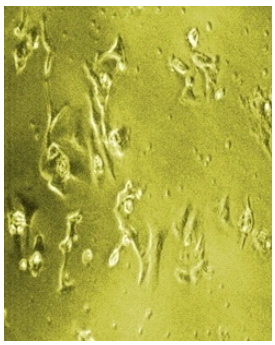
Sr. No.	Compound	Concentration (µg/ml)	O.D. at 540 nm	%of cell lysis	IC $_{50}$ value
1.	Squalene	10	0.469	25%	30 µg/ml
2.		20	0.545	35%	
3.		30	0.671	50%	
4.	Doxorubicin	30	0.990	65%	
5.	Control	-	0.351	No lysis	



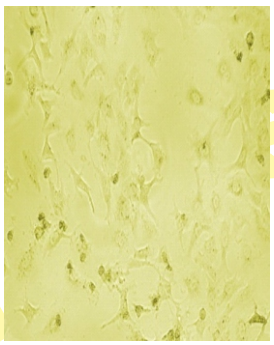
(a) Control



(b) DMSO



(c) Squalene



(d) Doxorubicin

Figure 2. Activity of Squalene against A549 cells.

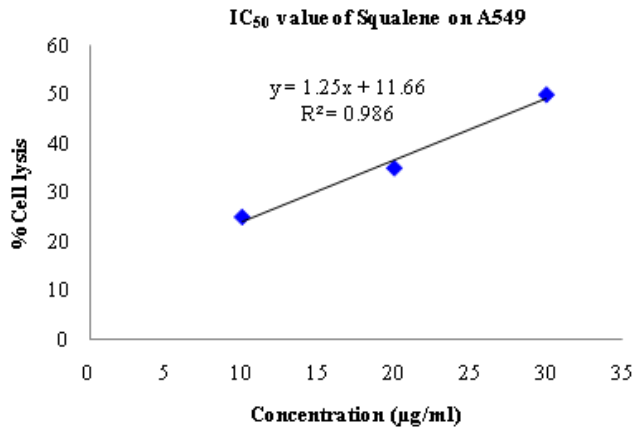


Figure 3. Effect of Squalene at various concentrations against A549 cell line.

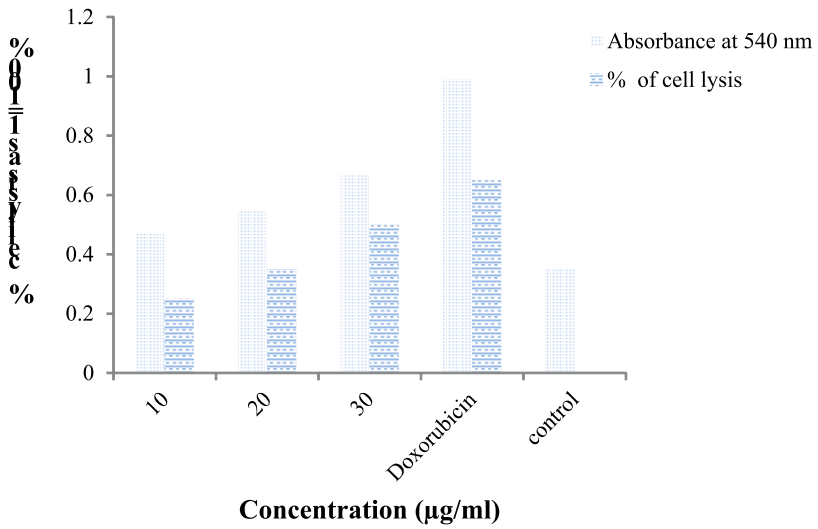


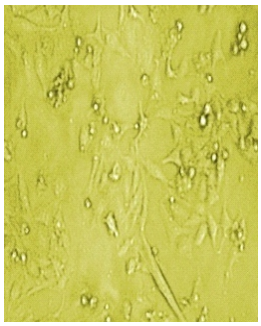
Figure 4. The comparison of test sample with standard against A549 cell line.

Against HeLa-Human cervix carcinoma cell line: The IC_{50} value of compound against HeLa-Human cervix carcinoma cell line was found to be less than 10 µg/ml and the results are mentioned in Table 2, Figure 5, Figure 6 and Figure 7.

Table 2. In- vitro evaluation of IC_{50} against HeLa cell line by MTT assay.

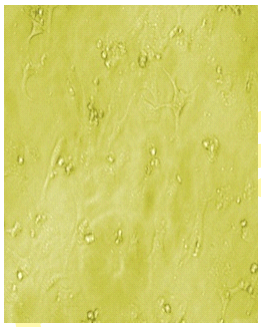
Sr. No.	Compound	Concentration (µg/ml)	O.D. at 540 nm	%of cell lysis	IC $_{50}$ value
1.	Squalene	10	0.811	70%	Less than 10 µg/ml
2.		20	0.821	75%	
3.		30	1.050	100%	
4.	Doxorubicin	30	0.850	80%	
5.	Control	-	0.155	No lysis	

(a) Control



(b) DMSO

(c) Squalene



(d) Doxorubicin

Figure 5. Activity of Squalene against HeLa cells.

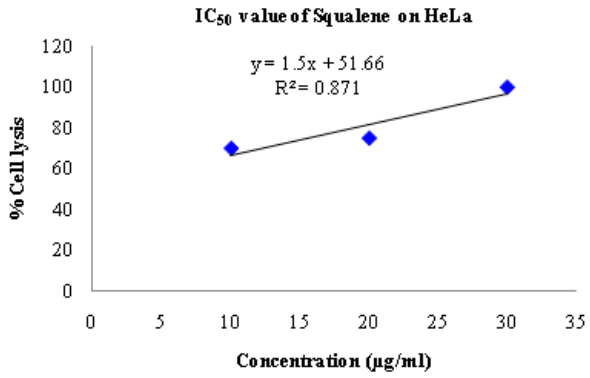


Figure 6. Effect of Squalene at various concentrations on HeLA cell line.

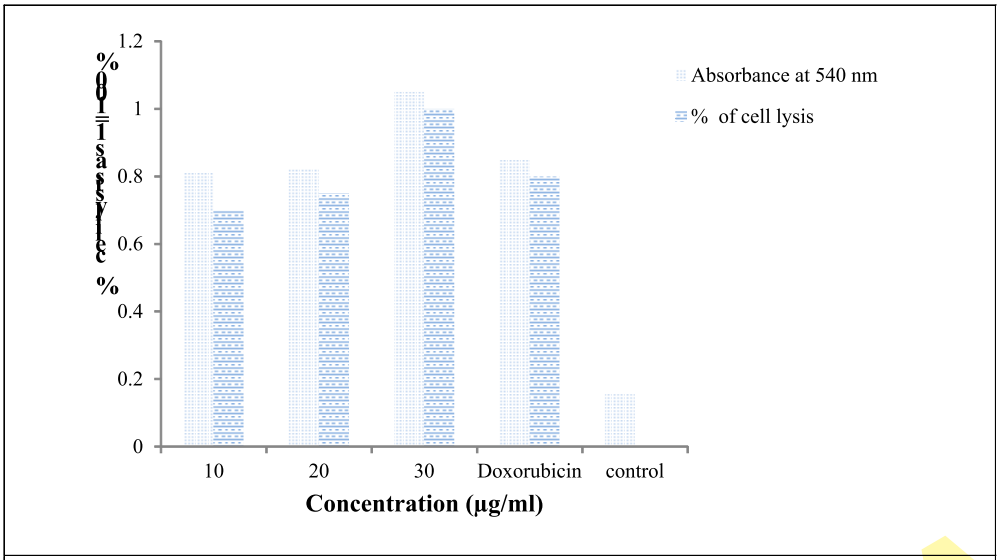


Figure 7. The comparison of test sample with standard against HeLa cell line.

DISCUSSION

Table 1and Table 2 depicts the anticancer activity of Squalene against A549 cell line and HeLa cell line using MTT assay as represented by optical density and % cell lysis. IC_{50} values give the clear idea about the potency of test sample as compared to standard drug.

The optical density for test sample at lowest concentration is more than control and at highest concentration is nearly equal to standard drug indicating that the compound possesses anticancer activity.

Graphical representation of the data is shown in Figure 3 & Figure 4 for A549 and Figure 6 & Figure 7 for HeLa cell line. The image of micro-plates represents the effect of test samples on the cells in comparison to control and the standard drug (Figure2 & Figure 5) for A549 and HeLa cell lines respectively.

CONCLUSION

The present study confirms the anticancer activity of separated triterpene, Squalene, from ethyl acetate extract of *Lantana camara* leaves against A549- human carcinoma cell line and HeLa- human cervix carcinoma cell line. It was further concluded that Squalene acts more effectively against HeLa cells as indicated by IC_{50} value of two cell lines. It also signifies the importance of terpenes, especially, triterpenes as excellent anticancer agents.

ACKNOWLEDGEMENT

The authors extend sincere thanks to Director, Institute of Science Nagpur for providing sophisticated botany and environmental science laboratory facilitates to complete the plant analysis.

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