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Research Article

## In Silico Analysis of *Laccifer Lacca* as a Potential Therapeutic Agent for Cervical, Breast and Lung Cancers

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### Abstract

*Laccifer lacca* has generally been used as pigmenting, coloring agent and dying in chemical industry. Although, it has wide range of industrial applications, but inappropriately, due to lesser availability of data, it has been ignored. Keeping in mind, the wide application of *Laccifer lacca*, we tried to report the *in-silico* anti-cancer effects. The experimental techniques used to determine the structure was X-RAY diffraction. The reported resolution of this entry is 2.80 Å. Percentile scores (ranging between 0-100) for global authentication metrics of the record. In silico have a good pool to explore various parameters in molecular docking. We have performed in silico analysis of the active components of *Laccifer lacca* against the cervical, breast and lung cancer proteins and also found that lac extract enhances the production of anti-inflammatory markers and the increase is significant when compared to the standard vinblastine. It has been demonstrated by Lala and colleagues that a short lived molecule nitric oxide can result in the progression of human tumours. Therefore, the prominent antioxidant activity of phytochemical that can act as inhibitors of nitric oxide production can act as anticancer therapeutics. Both methanolic and aqueous extract shows significant anticancer effect on the hela, MCF-7 & A549 cells suggesting them as potential anticancer therapeutics for future.

**Keywords:** *Laccifer lacca*, In-vitro & In-silico analysis, Carcinogenesis, Anti-inflammatory, Molecular Docking.

## INTRODUCTION

Lac resin is the major component in lac. The qualitative and quantitative analysis of the lac resin carried out in the past 200 years showed that lac resin is a mixture of polyester, which consists of polyhydric fatty acid and sesquiterpenoid acid. Its average molecular weight is about 1,000 and each molecule contains four or five hydroxyls containing one free carboxyl, one aldehyde group, two or three ester radicals, and one unsaturated bond.<sup>1</sup> Lac resin is an acidic resin with a quite complicated chemical composition. Various acids can be obtained through basic hydrolysis of lac resin. Three chain aliphatic acids (aleuritic acid, kerrolic acid, and butolic acid) and eight cyclical terpenic acids (shellolic acid, jalaric acid, epishellolic acid, laksholic acid, epilaksholic acid, laccishellolic acid, epilaccishellolic acid and laccijalaric acid) had been separated.<sup>2,3</sup> Lac is a multipurpose resin which has been applied in industries of military, electric appliance, printing ink, leather, plastic, metallurgy, machinery, woodworking, food, and medicine, etc. However, its shortcomings, such as brittleness, low softening point and bad water resistance, etc, limited its application. In order to expand its application field, various formulations were developed, some additives were employed in the modification of lac properties, and many lac products with special functions were produced. Luk was historically employed as an anti-hyperlipidemic and anti-obesity agent. A hyperlipidemia clinical trial in luk was performed twice a day for three months in a dosage of three grams.<sup>4,5,6</sup> Lac is an

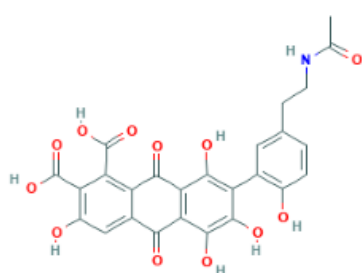
important drug of unani medicine and it has following medicinal properties like haemostatic, siccative, liver tonic, contraceptive, anti-inflammatory, anti-bilious, stomachic, aphrodisiac, detergent, deobstruent, anti-obesity, expectorant, kidney tonic, emmenagogues. And it is indicated to be used for following ailments, obesity, hyperlipidemia, renal, hepatic and spleen disorders, jaundice, ascitis, back ache, premature ejaculation, leprosy, cough, hemiplegia, asthma haemoptysis, epilepsy, chicken pox, ulcerations, palpitation etc.<sup>7</sup> Laccaic acid, commonly known as lac dye, is the soluble part of stick lac from insect origin *Coccus laccae* (*Laccifer lacca* Kerr). The use of lac dye in the dyeing of silk and leather seems to have been known to the Chinese some 4000 years ago. In the north and the northeast of Thailand, lac dye is widely used as a natural red dyestuff for cotton and silk dyeing. Moreover, lac dye has also been reported as a natural food coloring. Laccaic acids are the combination of anthraquinone derivatives present in lac dye. The wax portion has long chain esters, alcohols and hydrocarbons. There are many reports on the antioxidant activity of the anthraquinone derivatives.<sup>8,9</sup> Carminic acid is one of red dye which has the main constituent of an anthraquinone derivative same as the laccaic acid and it has been reported that carminic acid is as powerful antioxidant as ascorbic acid and even stronger than Trolox which indicates high antioxidant property.<sup>11</sup> Recently lac dye has proven its biological activity as it has a similar structure with the anti-cancer drug Adriamycin (ADR) as both are anthraquinone

derivatives. Antioxidant activity was investigated by DPPH assay, reducing power assay and the thiocyanate assay methods for laccaic acids and its aluminum lake.<sup>10</sup> Laccaic acids are also having a potent anti-cancer activity where it inhibits direct DNA competitive inhibitor of DNA methyltransferase I. Moreover, in order to evaluate its biological

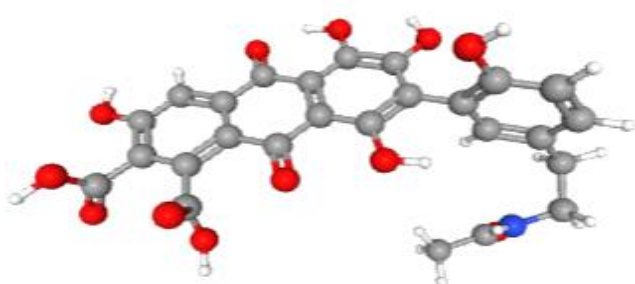
activity in broader spectrum, we have undertaken other cancer cell lines such as hela, MCF-7 & A549. In this paper, we report for the therapeutic potential of laccaic acid on three different cancer cell lines along with the isolation and characterization of the same with X-ray diffraction.



Figure 1: Laksha- *Laccifer Lacca*



2D structure of Laccaic acid



3D structure of Laccaic acid

Figure 2: 2D, 3D structure of Laccaic acid

## MATERIALS AND METHODS

**Chemical and reagents** Analytical grade reagents and chemicals were used for the study. Methanol, 0.22 $\mu$ M syringe filter, Mayer's reagent, Dragondroff's Reagent, Hager's Reagent, Ferric Chloride, Lead Acetate Solution, Trypsin, NaCl, KCl, Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O and KH<sub>2</sub>HPO<sub>4</sub>, 10% fetal bovine serum.

**Preparation of Methanolic extract (ME):** Firstly, the *Laccifer lacca* was grinded to a coarse powder and kept in thimble chamber of soxhlet apparatus. After that, it was extracted in soxhlet extractor with Methanol solvent. Extraction solvent was heated on a heating mantle for about 6-8 hour at 50°C. After cooling, the solvent was recovered by

distillation method and the remaining liquid extract was cooled. Then, the filtrate was dried on water bath and stored for further use.

**Preparation of Aqueous extract (AE):** 250 gm of dried resins were taken and crushed in an iron mortar to obtain a coarse powder. The obtained coarse powder is then soaked in 2 litre of purified water for 24 hours. After 24 hours, the whole mass was transferred to the distillation plant. The mixture was then heated at 100 degrees celsius for 6 hours. After 6 hours, 1 litres of AE Was obtained. Then it was cooled and stored in a tightly sealed container to protect it from light and moisture. The prepared extracts will be filtered through 0.22  $\mu$ M syringe filter (Millex) and stored at 4°C.

**Cell culture:** Cells were grown and maintained with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution in the Modified Eagle Medium (DMEM) of Dulbecco, and held in a humidified CO<sub>2</sub> incubator at 37 °C. When plates reached a 90% confluence with a 1:3 ratio, cells were subcultivated by scraping.

**Plating of cells in 96-well plate:** For plating, cells were taken out from CO<sub>2</sub> incubator in to laminar flow hood and treated with 2ml of IX PBS. Then 1ml of trypsin was added and cells were put in CO<sub>2</sub> incubator for 5 min. the solution was transferred in falcon tube and 1 ml of DMEM media was applied to it. Centrifugation of the tubes for 5 minutes at 1000 RPM. Supernatant was carefully discarded and 1 ml of media pellet dissolved. Approximately 5000 cells per well were poured in 96-well plate and left in CO<sub>2</sub> incubator for growth.

**MTT assay for cytotoxicity:** Cells were plated in a 96 well plate for the MTT assay at 5x10<sup>3</sup>cells/well. The MTT test was carried out for cell viability after 24 hour Extract treatment on the cells [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide]. The medias for each well had MTT (0.5 mg/mL) added and a 3-hour incubation was made for each plate. After 3 hours of removal, 100  $\mu$ l Dimethylsulfoxide (DMSO) were applied to each well to overcome violet formazan crystals produced by MTT

mitochondrial reduction of dehydrogenase. An ELISA plate reader read the absorption at 540 nm (ECIL).

**Passaging cell line:** To passage cells, T-flask containing confluent cell line was taken out from CO<sub>2</sub> incubator in to laminar air flow hood. After observing the cell growth in inverted microscope, supernatant was discarded and washing was provided with 2ml of 10% PBS. Supernatant was discarded using a pipette. Cells were treated with 1 ml of trypsin and incubated in CO<sub>2</sub> incubator for 5 min. Cells were transferred into new falcon tube and added with 1ml of DMEM media into it. Cells have been centrifuged for 5 min in 1000 RPM and supernatant is discarded, the pellet was dissolved properly into 1ml of DMEM media 2 new T-flask were taken into both flasks and both the flasks were put carefully in CO<sub>2</sub> incubator.<sup>30</sup>

## RESULTS

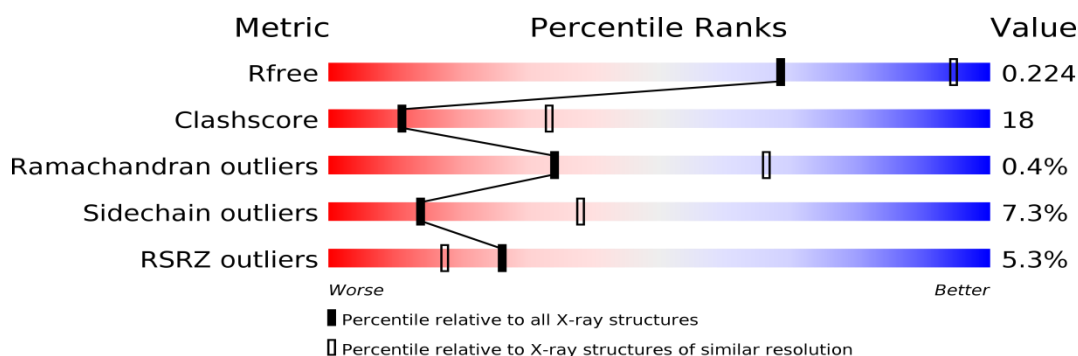
### Apoptosis measurement

Remove the cells from the cultivation plate and suspend in PBS or serum-free medium for the attached cells. Centrifugal

pellet cells at 600xg for five to six minutes. Remove the supernatant cells and replace the PBS cells. Make cell counts if needed. Remove the supernatant and dismantle it, pellet it again as before. Cell lysis, adding a sufficient volume of Lysis Buffer chilled e.g. 50µl, lysis of 1-5x 10<sup>6</sup> cells each. Vortex gently to suspend cells. Move into each well the buffer of 50µl 1x CasPASET<sup>TM</sup> Assay and 1X CasPASE<sup>TM</sup> Lysis Buffer . Into the suitable well, add 5µl cell lysate as shown in the table. Add 5µl ρNA Solution Substrate. Mix well content and read at zero time point (t = 0).5 Mix well content. . Incorporate the plate to 20-37 degrees C at 6. Measure the reaction at 405 nm each 30-60 minutes by reading the absorbance.

### Overall quality at a glance

The following experimental techniques were used to determine the structure: X-RAY diffraction. The reported resolution of this entry is 2.80 Å. Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



**Figure 3:** Percentile ranks at difference metric versus values

### Cell counting using haemocytometer

During culturing with T-25, we have suspended 1 ml of trypsinized cells in 2 ml of full media (60-90% confluence). A 1.5 ml microfuge tube has been transmitted 200µl of cell suspension. 300µl PBS cells and 500µl Trypan Blue solution 0.4 percent (dilution factor 5) in the microfuge tube have been treated. The solution was thoroughly mixed and given 15 minutes to stand. A cover slip has been applied to a haemocytometer chamber with a small amount of trypanoblu cell solution. This was done by touching carefully the edge of the pipette tip on the cover and allowing a capillary action to fill the chamber. Cells were counted on a 1mm centre square and four edge squares

(non-viable cells stain blue as viable cells remain opaque). We had to maintain a separate number of viable and unsustainable cells (if more than 25 percent of cells are non-viable, the culture is being maintained on appropriate amount of media). Each square of haemocytometer represents a total volume of 0.1 mm<sup>3</sup> or 10<sup>-4</sup> cm<sup>3</sup>. Since 1 cm<sup>3</sup> is equal to 1ml, subsequent cell concentration per ml was calculated by using (Peter D Pioli, 2019):

Cells/ml= the average count per square x dilution factor x 10<sup>4</sup> (count to 10 square).

Total cell number = cells/ml x original volume of fluid from which sample was removed.

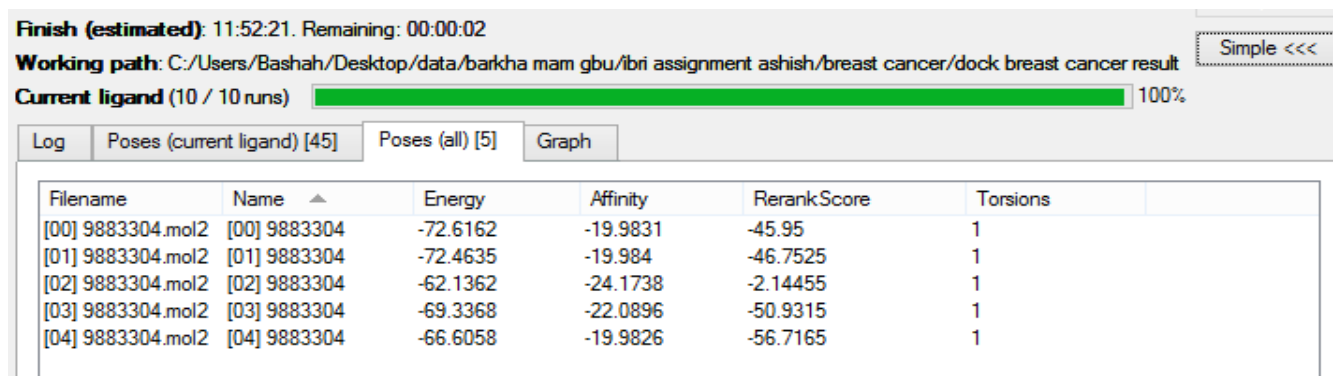
**Table 1:** Docking result of breast cancer

File Name	Energy	Affinity	Re rank Score	Torsions
9883304.mol2	-72.6162	-19.9831	-45.95	1
9883304.mol2	-72.4635	-19.984	-46.7525	1
9883304.mol2	-62.1362	-24.1738	-2.14455	1
9883304.mol2	-69.3368	-22.0896	-50.9315	1
9883304.mol2	-66.6058	-19.9826	-56.7165	1

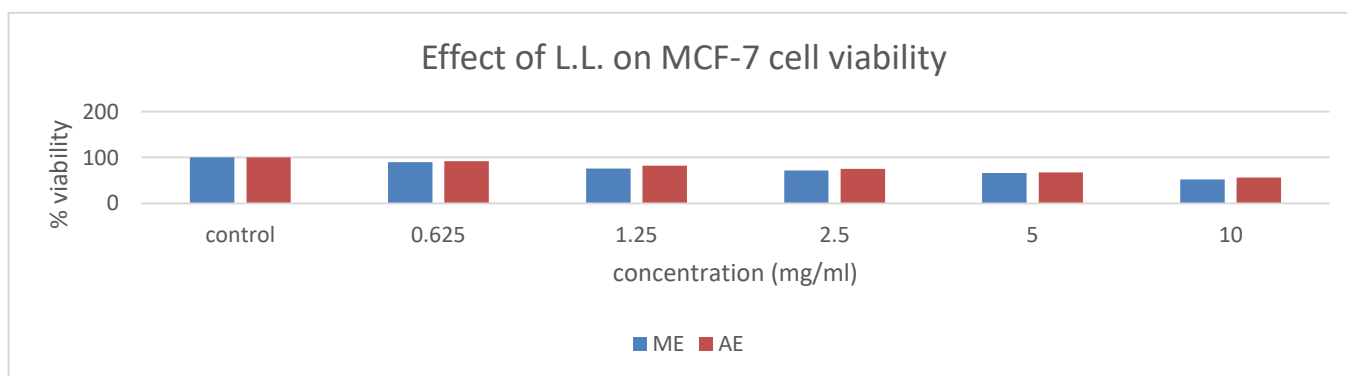


**Table 2:** Experimental Data Snapshot

Method:	X-RAY DIFFRACTION
Resolution:	3.21 Å
R-Value Free:	0.307
R-Value Work:	0.257
R-Value Observed:	0.260

**Figure 4:** Experimental data Snapshot of breast cancer

We found that Cell viability after treatment with the methanolic and aqueous extract of *Laccifer lacca* in the effect of L.L on MCF cell viability the methanolic and aqueous extract are same.

**Figure 5:** Cell viability after treatment with the methanolic and aqueous extract of *Laccifer lacca*. It is expressed in percentage in the ratio with the control cells.**Table 3:** Docking result of cervical cancer

File Name	Energy	Affinity	Re rank Score	Torsions	RMSD
9883304.mol2	-89.626	-20.6024	-73.5286	1	36.404
9883304.mol2	-90.3559	-19.7298	-80.6672	1	35.7628
9883304.mol2	-86.3342	-20.8158	-70.6973	1	35.8864
9883304.mol2	-86.6803	-20.5648	-58.105	1	35.8337
9883304.mol2	-85.4526	-20.6884	-75.9282	1	36.2304

**Table 4:** Experimental Data Snapshot

Method:	X-RAY DIFFRACTION
Resolution:	2.80 Å
R-Value Free:	0.294
R-Value Work:	0.243
R-Value Observed:	0.245

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Working path: C:/Users/Bashah/Desktop/data/barkha mam gbu/ibri assignment ashish/cervical cancer/dock result

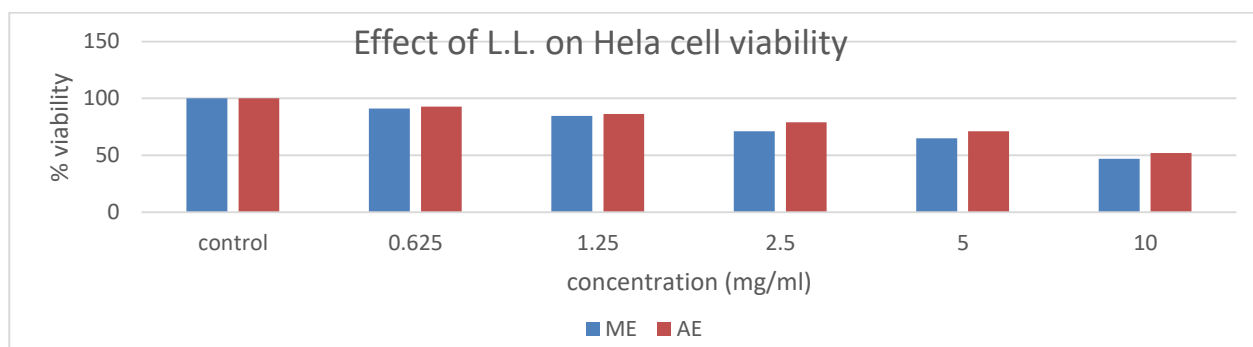
Current ligand (10 / 10 runs) 100%

Log Poses (current ligand) [36] Poses (all) [5] Graph

Filename	Name	Energy	Affinity	RerankScore	Torsions	RMSD
[00] 9883304.mol2	[00] 9883304	-89.626	-20.6024	-73.5286	1	36.404
[01] 9883304.mol2	[01] 9883304	-90.3559	-19.7298	-80.6672	1	35.7628
[02] 9883304.mol2	[02] 9883304	-86.3342	-20.8158	-70.6973	1	35.8864
[03] 9883304.mol2	[03] 9883304	-86.6803	-20.5648	-58.105	1	35.8337
[04] 9883304.mol2	[04] 9883304	-85.4526	-20.6884	-75.9282	1	36.2304

**Figure 6:** Experimental data Snapshot of cervical cancer

We found that Cell viability after treatment with the methanolic and aqueous extract of *Laccifer lacca* in the effect of L.L on Hela cell viability the aqueous extract has more effect with comparison in methanolic extract.



**Figure 7:** Cell viability after treatment with the methanolic and aqueous extract of *Laccifer lacca*. It is expressed in percentage in the ratio with the control cells.

**Table 5:** Docking result of lung cancer

File Name	Energy	Affinity	Re rank Score	Torsions	RMSD
9883304.mol2	-101.568	-23.0736	-6.8071	1	131.028
9883304.mol2	-87.9906	-22.3072	-63.0445	1	130.432
9883304.mol2	-88.9778	-22.2999	-66.1489	1	134.962
9883304.mol2	-84.9977	-23.1765	-60.3121	1	135.093
9883304.mol2	-82.0807	-20.5831	-71.7691	1	130.134

**Table 6:** Experimental Data Snapshot

Method:	X-RAY DIFFRACTION
Resolution:	2.80 Å
R-Value Free:	0.230
R-Value Work:	0.213
R-Value Observed:	0.213

Finish (estimated): 14:25:38. Remaining: 00:00:02

Working path: C:/Users/Bashah/Desktop/data/barkha mam gbu/ibri assignment ashish/lung cancer/dock result

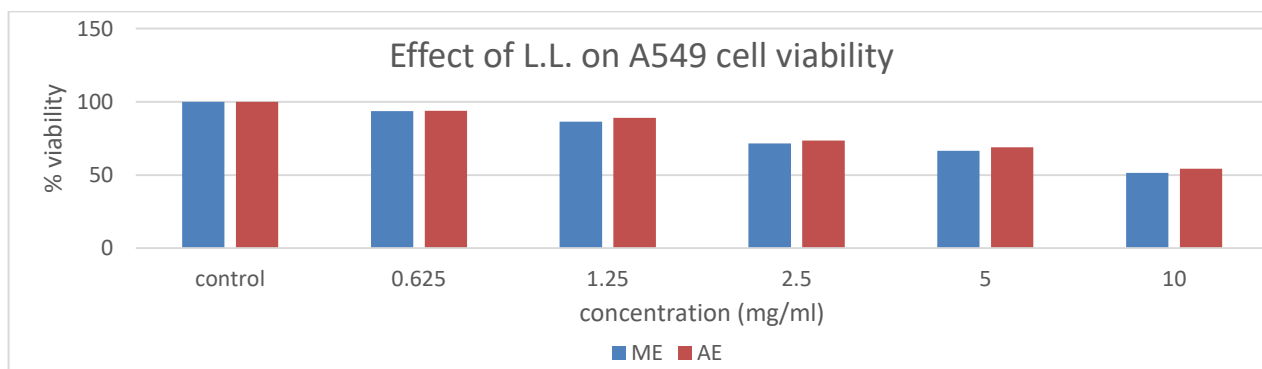
Current ligand (10 / 10 runs) 100%

Log Poses (current ligand) [47] Poses (all) [5] Graph

Filename	Name	Energy	Affinity	RerankScore	Torsions	RMSD
[00] 9883304.mol2	[00] 9883304	-101.568	-23.0736	-6.8071	1	131.028
[01] 9883304.mol2	[01] 9883304	-87.9906	-22.3072	-63.0445	1	130.432
[02] 9883304.mol2	[02] 9883304	-88.9778	-22.2999	-66.1489	1	134.962
[03] 9883304.mol2	[03] 9883304	-84.9977	-23.1765	-60.3121	1	135.093
[04] 9883304.mol2	[04] 9883304	-82.0807	-20.5831	-71.7691	1	130.134

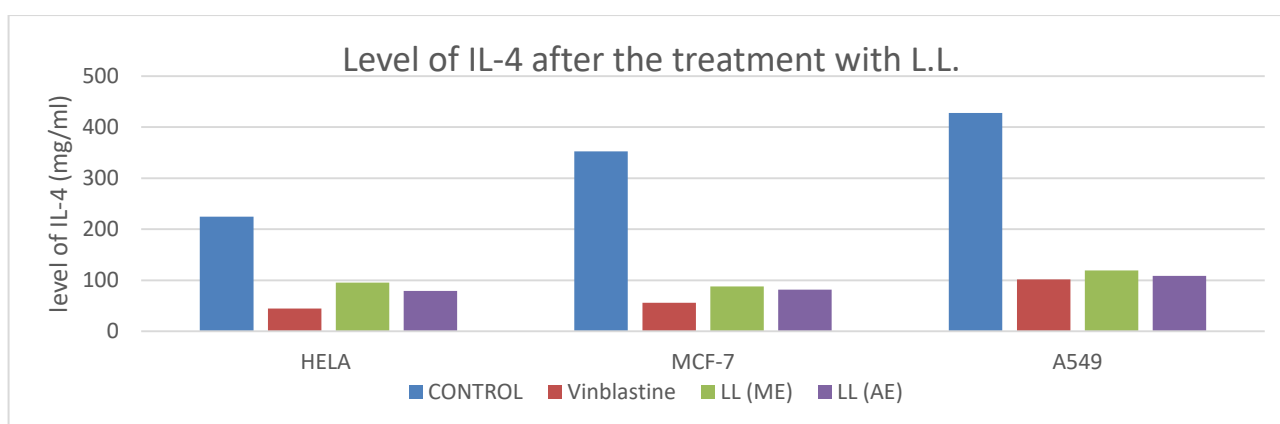
**Figure 8:** Experimental data Snapshot of lung cancer

We found that Cell viability after treatment with the methanolic and aqueous extract of *Laccifer lacca* in the effect of L.L on Hela cell viability the aqueous extract has more effect with comparison in methanolic extract.



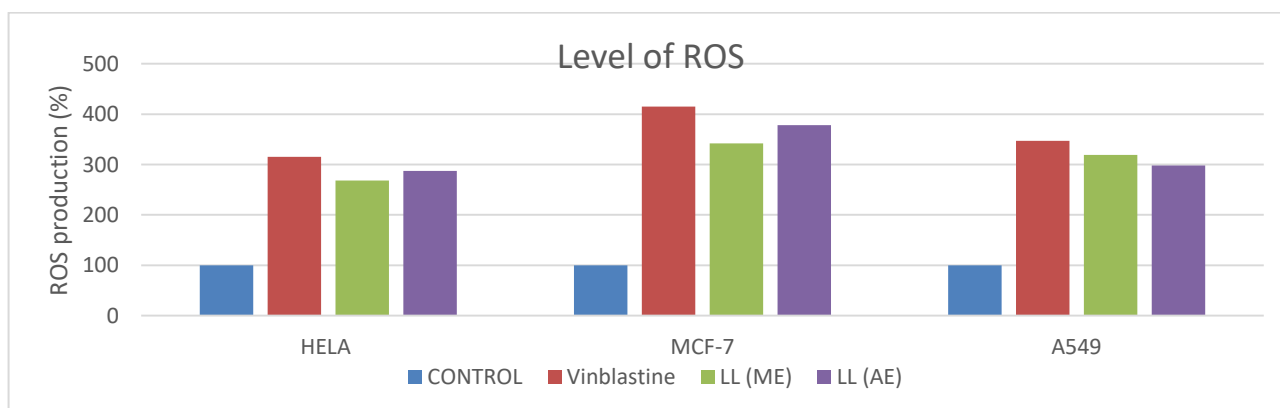
**Figure 9:** Cell viability after treatment with the methanolic and aqueous extract of *Laccifer lacca*. It is expressed in percentage in the ratio with the control cells.

We found that the cytokines levels were compared with the control and vinblastine that control group has more level of cytokines which is mg/ml.



**Figure 10:** Cytokine measurement by kit based ELISA. The levels of cytokines are measured in mg/ml. The cytokines levels were compared with the control and standard vinblastine.

We found that the level of ROS in vinblastine group is more than methanolic extract and aqueous extract group of *Laccifer lacca*.



**Figure 11:** Measurement of reactive oxygen species production in the cells after the treatment with the standard drug and test extracts for 24 hours. The production is expressed as %.

## DISCUSSION

We were determined to analyse the therapeutic potential of the lac as anticancer agent and for that we prepared two preparation, one being aqueous and other is methanol extract. The treatment of cancer cells with lac resulted in the inhibition of cell proliferation in MTT assay. Methanolic extract is more efficient in the inhibition of cell proliferation when compared to the aqueous extract. It has been stressed enough that balance between pro- and anti- inflammation is very crucial for the body's health as well to fight off risks like

cancer. The heightened pro-inflammatory milieu in our body may weaken the immune response and thus resulting in an increased risk of cancer. However, not all inflammation is bad for our body, inflammation is also important to stimulate the body's immune response and signal it to fight against the cancer cells. Therefore, now it has been considered that anti-inflammatory properties are an addition to the anti-cancer properties of the drug. Plants foods are known to have anti-inflammatory constituents as well as anti-oxidants. We analysed our drug lac for its anti-inflammatory properties against the cancer cell lines. We

measured the levels of anti-inflammatory markers IL-4 & IL-10 in the cell supernatant after treating them with extracts of lac and standard drug vinblastine.

## CONCLUSION

In conclusion, we found out that *laccifer lacca* extract enhances the production of anti-inflammatory markers and the increase is significant when compared to the standard vinblastine. It has been demonstrated by Lala and colleagues that a short lived molecule nitric oxide can result in the progression of human tumours. Therefore, the prominent antioxidant activity of phytochemical that can act as inhibitors of NO production can act as anticancer therapeutics. We measured the levels of nitric oxide (NO) in the treated cells and it was found that the levels of NO were decreased after treatment. Both methanolic and aqueous extract shows significant anti-cancer effect on the hela, MCF-7 & A549 cells suggesting them as potential anticancer therapeutics for future.

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## CONFLICT OF INTEREST:

Authors report no conflict of interest in publishing this research work.

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