Evaluating the Anti-Lung Cancer activity of Berberis vulgaris in several in-vitro methods

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Research Article

Keywords: Anti-mitotic, BSL, Lethality, MTT, NCI-H460cell line, NSCLC, LCC etc

Posted Date: April 9th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6343316/v1

Additional Declarations: No competing interests reported.

Abstract

Background: Lung cancer, characterized by uncontrolled cell growth in lung tissue, poses a challenge to global health for its aggressive nature and poor prognosis. Despite advancements in medical research LC remains a leading cause of cancer-related deaths, highlighting the urgent need for innovative therapeutic strategies. This study investigates the effect of ***BV*** on the NSCLC, specifically LLC.

Methods: This study investigated the anti-cancer properties of ***BV*** using in -vitro models- Anti-mitotic assay, Brine Shrimp Lethality Assay and MTT Assay. In anti-mitotic assay the inhibition of mitosis cell division along with the root no & root length were observed in *Allium cepa*. The BSL model was performed to observe the lethality of BSL .The MTT Assay further confirmed the cytotoxic effects on NCI-H460cell lines, showing a dose-dependent reduction in cell viability. **Results:** In the Anti-mitotic assay, the same root no & length in the treatment groups with proper inhibition of mitosis cell division, In the BRS model 100 % lethality rate in test drug groups, in the MTT assay decreased rate of cell viability in the group of Test drug were observed .

Conclusion: The study demonstrates that ***BV*** exhibits its potential & promising anti-cancer properties by showing positive results in all models.

1. Introduction

Lung cancer is a leading cause of cancer-related deaths worldwide, characterized by the uncontrolled growth of abnormal cells in the lungs. It is broadly classified into two main types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) more than 80% lung cancer cases are non small cell lung cancer [1], with NSCLC being more prevalent. Smoking is the primary risk factor, but other contributors include exposure to second hand smoke, air pollution, occupational hazards, and genetic predisposition.

The disease often progresses silently, with symptoms like persistent cough, chest pain, and shortness of breath appearing in advanced stages. Early detection through screening and imaging is crucial for effective treatment, which may include surgery, chemotherapy, radiation, or targeted therapies.

Cancer arises from abnormal cell growth, affecting almost any part of the body. It is caused by genetic changes that result in uncontrolled cell division and tumor formation. Tumors can be benign (non-cancerous) or malignant (cancerous), with malignant cells having the potential to invade nearby tissues or spread throughout the body. Globally, cancer is a major health concern and a leading cause of mortality, with lung cancer being particularly prominent.[2]

A malignant tumor originating in the lungs, often caused by genetic damage to respiratory cells. Smoking and exposure to toxic substances are primary risk factors. Lung cancer is the second most common cancer worldwide, with a high mortality rate. Non-small cell lung cancer (NSCLC) constitutes the majority of cases, with late-stage diagnoses posing challenges for treatment. The plant Berberis vulgaris contains berberine the active herbal constituent which can prevent lung cancer. The root bark of

this plant contains highest amount of Berberine & this plant is the source of berberine, which contains highest amount of berberine, among all natural sources. Berberine can improve the tumor microenvironment of lung cancer & specially the angiogenesis condition of lung cancer.[3] The tumor microenvironment is the ecosystem that surrounds a tumor inside the body. It includes body's auto-defense cells, the fluid of outside of cell membrane,, blood receptacles, and other compartments, like fibroblasts.ME includes these for example as fibroblasts, auto-defense components, the fluid of outside of cell membrane, signalling proteins, advancing components, and internal secretions surrounding tumor compartments nourished by a capillary bed. [4].This plant has Angiogenesis, Cell cycle arrest, anti Metastsis. Autophasy, It has active anti-invasive property against cancerous cell ,it has also Anti-viral, Anti-oxidant, Anti-inflammatory,Cytotoxic activities, which is not only helpful to treat the cancer, but also for others related complications of Cancer.[5]Various factors are related with the tumor area similar area, among these our target was angiogenesis, This article determines, the anti-angiogenic property os *Berberis vulgaris*. This plant contain highest amount of Berberine, so it's possible to yield, more than required amount of Berberine from this plant. [6].This plant has Angiogenesis, Cell cycle arrest, anti Metastsis. Autophasy, It has active anti-invasive property against cancerous cell ,it has also Anti-viral, Anti-oxidant, Anti-inflammatory,Cytotoxic activities, which is not only helpful to treat the cancer, but also for others related complications of LC. [7, 8]As primary objectives, for in-vitro, the Anti-mitotic assay with *Allium cepa*, Brine shrimp lethality test, MTT Assay for Cell viability test the anti-lung cancer activity of *Berberis vulgaris* observed, with effective cancer inhibiting properties.

2. Materials & Methodology

2.1 Plant Collection and Identification

Berberis vulgaris root bark was purchased from the e-commerce site Yucca Enterprise and It was authenticated by the Faculty of Science, RK University.

2.2 Preparation of Methanolic extract of *Berberis vulgaris* by Soxhlation Process:

The root powder of BV used to make the extraction by soxhlation process, after soxhlation To make a semi-solid form of extraction solution the water bath was used. The extract solution which obtained from evaporator, placed on water bath at 40° c-50° c temp. After 2-2.5hrs the semi-solid form of dark Yellow-Green color extract achieved[9, 10], we also used ultra sonication process,After ultrasonication, filter the extract to removed solid particles, Concentrate the filtrate using Water bath. [11]

2.3 Preliminary Phytochemical Screening

The crude extract was analysed for detecting phytoconstituents- including Berberine, Phenol, Flavonoids, Sesquiterpene, Triterpenoids, using the standard method as referenced in sources by performing confirmatory tests. .[12–16]

2.4. UV spectrometric analysis:

Extract of Berberis vulgaris weighed & dissolved in water ,.Than observed the absorbance of 100µg/ml,200µg/ml,300µg/ml,400µg/ml,500µg/ml extract solution at the specific wavelength.[17–19]

2.5 HPLC Analysis:

Took Root Powdered extract of **Berberis vulgaris** dissolved in methanol ,Set the Baseline and set the ratio of Mobile Phase Water: Methanol: Acetonitrile (60:30:38, v/v/v),run the HPLC, and Took the result .[20–21]

2.6. IN-VITRO TEST:

2.6.1 Anti-Mitotic Test Allium cepa :

Onion put in water for 72hrs for developing uniform roots. The bulb which developed roots were selected for further studies. The root of onion placed on beaker filled with standard (MTX), test drug & control groups, with MTX – 0.1mg/ml & the test drug was 1 mg/ml,3mg/ml & 5mg/ml & water respectively for 24 hrs in room temperature (fig-1). After 24 hrs the root numbers & length were measured(fig-2). Then took 3cm of newly grown roots in watch glass for every group, added some 2–3 ml 1NHCL. Gently warm the glass not boil or can also keep in incubator for 20 mins at 60°c temp. After 15–20 mins, washed the root tips for 2times with water. Added Acetocarmine 1ml & put the root tips in this for 15–20 mins. Took the roots on the slides respectively for all groups & added 0.2 ml acetocarmine on it. Finally observed under Phase contrast microscope. The mitotic index was determined by using this equation, mitotic index = No of dividing cell /Total no of cells *100 [22]

2.6.3. Brine shrimp Lethality Test:

3GM OF BRS(Brine Shrimp) eggs & soaking the eggs in 150ml artificial sea water (20gm salt/l water. Lightening them with 50 w lamp & aerating them for 48hrs(fig-5-a,b)). Took 50 mg of the extract in 5ml DMSO-containing water,1000µl,800µL,500µl,250µl,100µl,50µl,25µl, 10µl from stock solution were inserted into vials. 5ml sea water added to each vial & used vortex to homogenize this.150 BRS Larvae added to each vial + 50µl yeast suspension as food. All the vials were kept under light for 72 hrs(fig-5-c). Then observed the vial at 6h,12h,24h,36h,48h,60h, & 72hrs. In each interval the dead larvae were counted under Photo contrast microscope. % Of Mortality = DEAD NAUPILLI/ Total NAUPILLI * 100, result determined according to this Eq. [23, 24]

2.6.4. Cell cultivation & cell seeding for MTT ASSAY [57]:

Cell Line Procurement

The NCI-H460 Cell line purchased from NCC, Pune(fig-10), India and cultured with standard procedure.

Seeding of Lung cancer cell (NCI-H-460) cells

Blended cells (70–80%) in Corning carafes were withdrawn employing a trypsin arrangement. The cells were re-suspended in RPMI − 1640 medium and numbered employing a hemacytometer. Cell densities

were balanced to 1x105 cells/ml. Suspension of cells (1000 ¼) was seeded into a pre-labelled Hi Media Tissue Culture Plate (12 wells of 24 wells).Three set for copy for each concentration of compound utilized were made.The plates were brooded at 37Â°C and 5% CO2 for 24 hours [23, 24]

2.6.5MTT ASSAY process :

After seeding the culture plates were put in incubator for 24 hrs.The cells were uncovered with RPMI-640 development medium containing test .Brooded for advance 24 hours at 37°C and 5% CO2.BV was tried in ten diverse concentrations for following 24 hours.100 µl of TT arrangement (5 mg/ml last concentration) was included to the wells and incubated for 1 hour at 37°C and 5% CO2.The mediums evacuated and two washes of saline water were utilized to evacuate over bundance color from well plate. At that point included of lysing buffer (200 µl) into each well. At that point the colour was measured at wavelength 562nm (ELISA peruser Lilac.)(fig-8).Concentration and length of the test details were chosen based on the preparatory metabolic push lists observed by MTT measure. .Cell Viability = No of Viable cells/Total no of cells * 100 [25, 26].

3. RESULT & DISCUSSION

3.1: Result of Anti-mitotic test:

Pre & Post root length & Root no observation of Allium cepa are mentioned in table-1, A& B respectively

3.2. Result of Brine Shrimp Lethality Test:

BSL Test result in no at different intervals, is mentioned in table-2

BSL test result in Percentage at different intervals,is mentioned in table-3

3.3 Result of MTT Assay:

Optical Density of different groups , Cell viability % of different groups are mentioned in table-4 a & b respectively

4. Discussion

4.1 IN-VITRO TEST :

4.1.1.Anti-mitotic Assay:

We performed this test with Allium cepa. At first, we observed the root no. & length with our test drug & standard drug groups. In all groups except the control, we observed the same root number & length(table-1 (graph-1). So in this test, our test drug showed a positive result, even in the low doses.

Then our next test was the Anti-mitotic test. Here we observed the mitosis cell division, then we also calculated the mitotic index for all groups. The mitotic index is defined as the percentage of cells in a population that are undergoing mitosis, which is the process of cell division.

So, which group showed us the lowest mitotic index, we can determine that, this group has highest anti-cancer activity. In control group we observed all phases of mitotic cell division(fig-3). The mitotic index of the standard drug was 28%(graph-2), here we observed multiple nuclei also within one cell wall(fig-4-c), it happens sometimes & in this type of study, this view is common because of incomplete cytokinesis. For incomplete cytokinesis, the cells fail to divide properly & sometimes the daughter nuclei can remain with a single cell, if cell division is disrupted, before the formation of the cell wall.

T1 didn't show any value because we couldn't observe any nuclei inside the cell(fig-4-a). The cause behind this might be that we observed the root when it was in the prophase phase. During the interphase, the nuclear envelope starts to disintegrate, and the nucleus becomes invisible, and we detected the microscopic view where we observed cells without cell nuclei.

T2- 49.25%(fig-4-b), T3- 42.15% (fig-4-c)

So, we observed a standard result from our test group, even in the lower dose .

4.1.2. Brine Shrimp Lethality test :

We observed the immortality in this test, at 6hr, 12hr,36hr, 48hr, 60hr, 72hr in different groups (fig-6,7). It was really a unique process to observe the lethality of Brine shrimp because we observed the lethality rate up to 72 hrs.

At 6hrs, the lethality rate was observed in, (table- 2,3)

Standard group- 60% & in our test drug group with the highest dose (1000µg) 56.66%.

At 12 hrs, Stnd- 66.66%, Test drug (1000 µg) – 80%

At 24hrs, Stnd- 73.33%, Test drug (1000 µg)-100%, here our drug showed, the better result than the standard drug.

At 36 hrs – the Stnd- 80%, the Test drug (1000µg)- 100%, after 36 hrs also the standard drug lethality rate was not equal to our test drug

At 48 hrs- the Stnd- 100%,the test drug (800 µg) -100%, the Test drug (1000 µg) 100%,

here 100% lethality observed in the standard drug group & in the test group, the 100% lethality was observed not only in 1000 µg test drug group, also in the group with 800 µg .

At 60 hrs, in the standard group – 100% & in test drug with 500 µg, 800 µg, 1000 µg showed 100% lethality rate.

At 72 hrs we observed the 100% lethality in all groups, except control, & test group with 10 µg & 25 µg groups.

So, according this result, our drug activity was remarkable & the 100% lethality also observed with our test drug which was earlier than the standard drug.

4.1.3.MTT Assay:

In MTT Assay,

OD (Optical Density)

OD measures the amount of formazan produced by metabolically active cells. Cells that are more metabolically active will reduce more MTT to formazan, resulting in a higher OD.

So, when the OD value decreases, that time we can determine the anti-cancer activity of the drug. For the standard drug, it has observed 0.09% & for our drug maximum dose (300µg) it showed 0.17% (graph-3 a), which is in the standard range of the lowest OD value(table-4,A).

Cell Viability test :

Cell viability in an MTT assay determines the metabolic activity of cells. Viable cells with active metabolism will reduce the MTT reagent to a colored formazan product.

So, anti-cancer property of the rug shows low cell viability. In the standard group, the cell viability was observed at 11.25% & with our drug's highest dose we observed 21.25% (graph-3, b) (table-4,b). According to this standard result, we can claim that our drug has exact anti-cancer activity.

5. Conclusion

A comprehensive analysis yielded noteworthy data concerning the phytochemical composition and anti-cancer potential of *Berberis vulgaris* extracts. We extracted significant quantities of bioactive chemicals using the Soxhlet and Ultrasonication extraction methods, with the latter generating a higher yield of 9% w/w. Phytochemical screening revealed the existence of anti-cancer chemicals such as phenols, sesquiterpenes, triterpenoids, flavonoids, berberine, and alkaloids. The extracts included significant levels of Berberine, as evidenced by HPLC analysis and UV spectroscopy. The anti-mitotic test utilizing *Allium cepa* indicated a significant reduction in the mitotic index, indicating effective anti-cancer activity. Furthermore, the brine shrimp mortality test revealed that the test medication was more lethal than the standard drug, particularly at higher doses. The indications of lower optical density and cell viability levels from the MTT assay served to confirm the anti-cancer action. All in all, our study showed that extracts from *Berberis vulgaris*, particularly those obtained via ultrasonication, are potential candidates for the development of anti-cancer drugs.

Abbreviations

LC- Lung Cancer

LLC-Large Cell Carcinoma

BSL-Brine Shrimp Lethality

RT PCR- Real-Time Polymerase Chain Reaction

DNA-Deoxyribose Nucleic Acid

MTX-Methotrexate

MTT- 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

OD-Optical density

UV spectroscopy- Ultraviolet Spectroscopy

HPLC- High-performance liquid chromatography

BV- *Berberis vulgaris*

AC-*Allium cepa*

Declarations

Acknowledgment

Not applicable

Author Contribution

All authors have studied the final manuscript for communication. The authors were responsible for study conceptualization complete literature search, protocol development, data gathering, statistical analysis, and development of the manuscript. The authors have read and approved the final version of the manuscript.

Funding

We would like to express our gratitude to the Student Startup Innovation Policy(SSIP) for funding and supporting our research work.

Availability of materials and data

The data used and analysed during the present study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate. Not applicable.

Competing interests

The authors have no competing interest

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Tables

Tables 1 to 4 are available in the Supplementary Files section

Graphs

Graphs 1-3 are available in the Supplementary Files section.

Figures

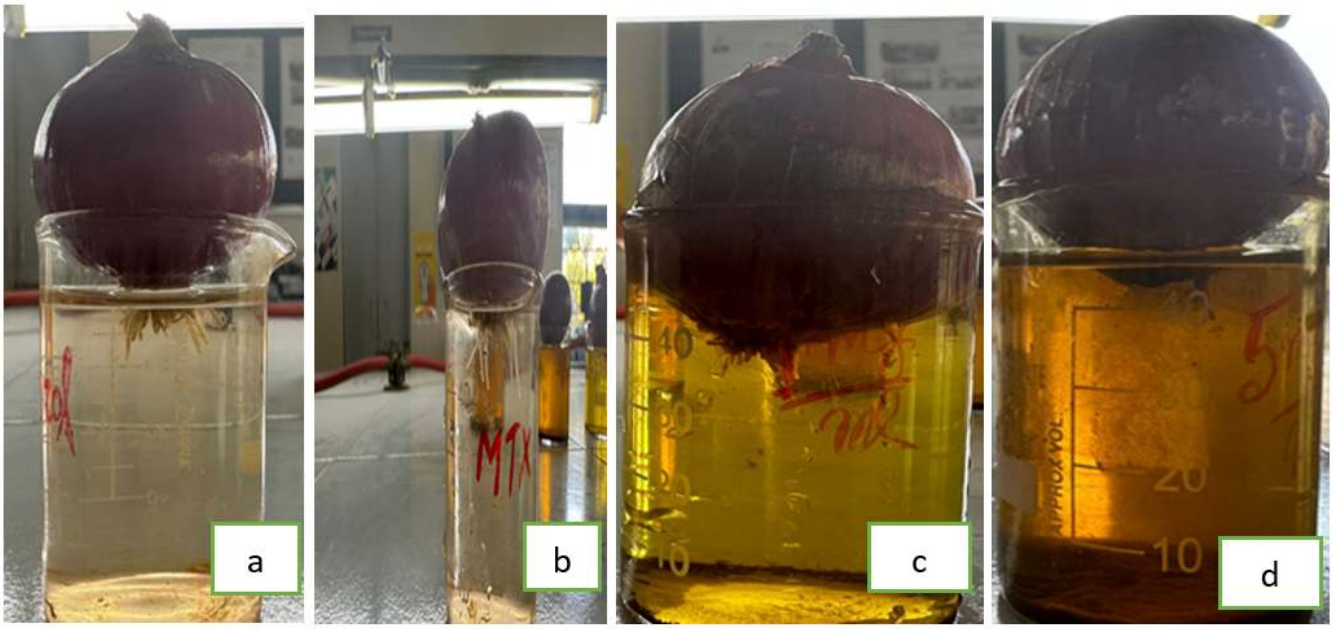

Figure 1

Growth of root in number & length in a) control, b) Stnd. drug, c)Test drug ,d)Test Drug

Figure 2

Root no & Length observation

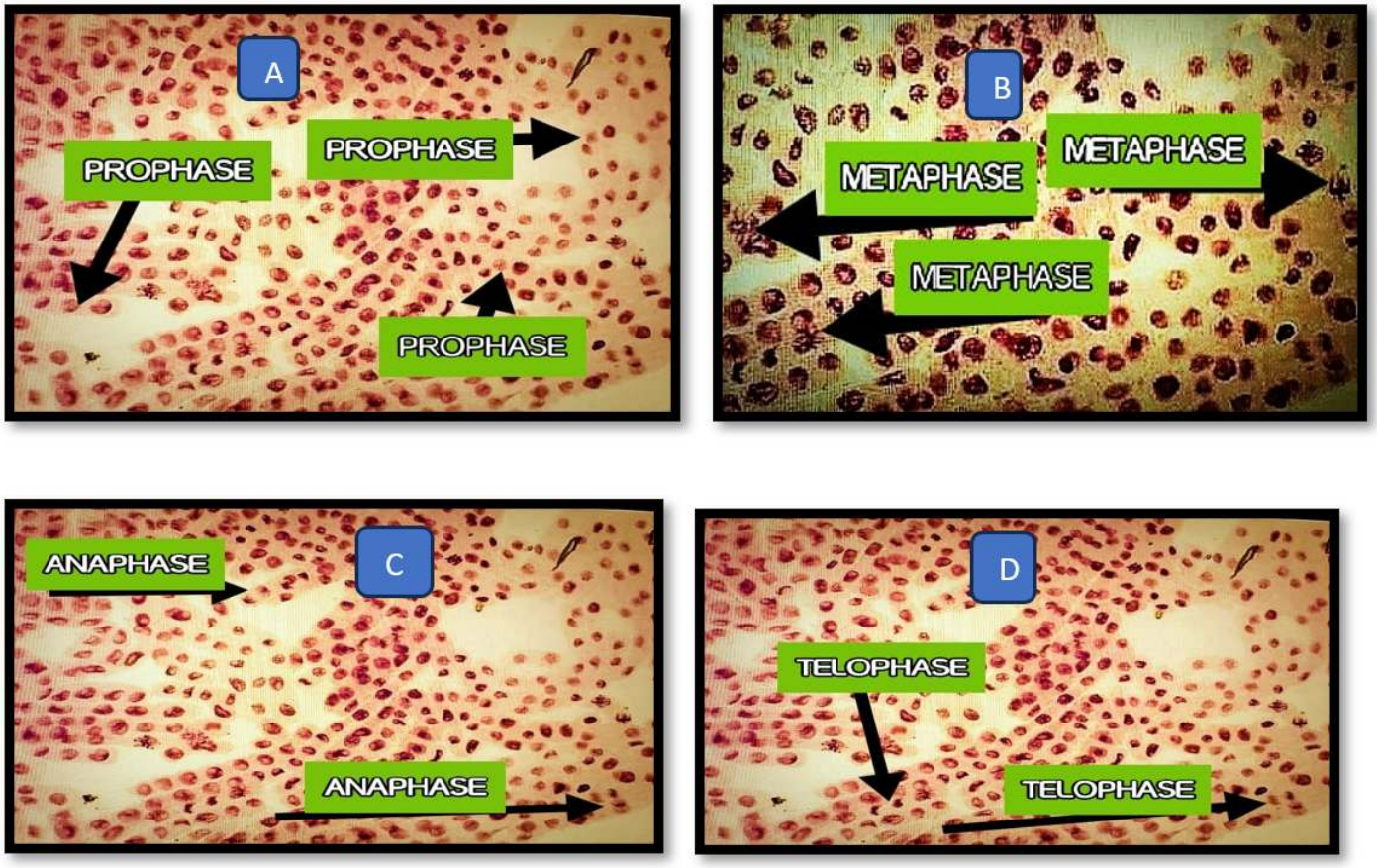

Figure 3

A- Prophase,B-Metaphase,C-Anaphase,D-Telophase cell Division in control group

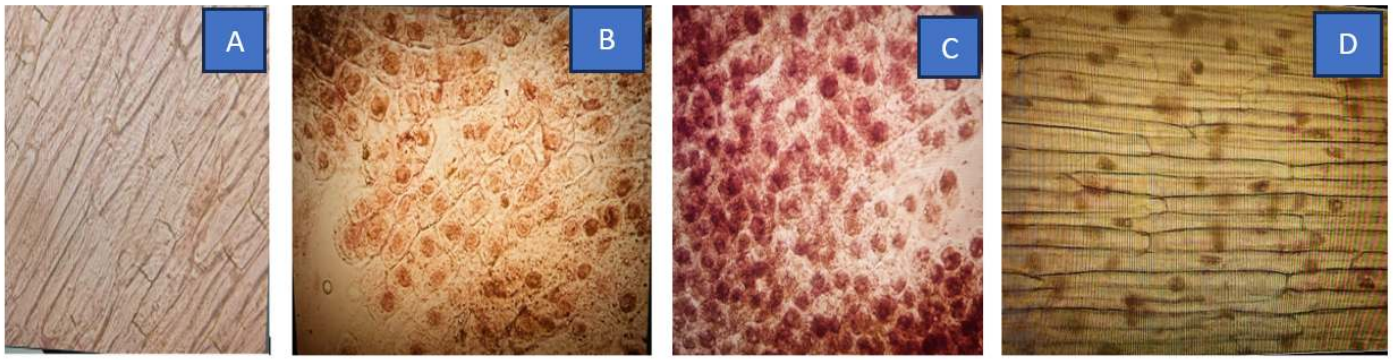

Figure 4

Phase contrast microscopic view in a-T1 group,B-T2 group,C-T3 group, D-stnd group

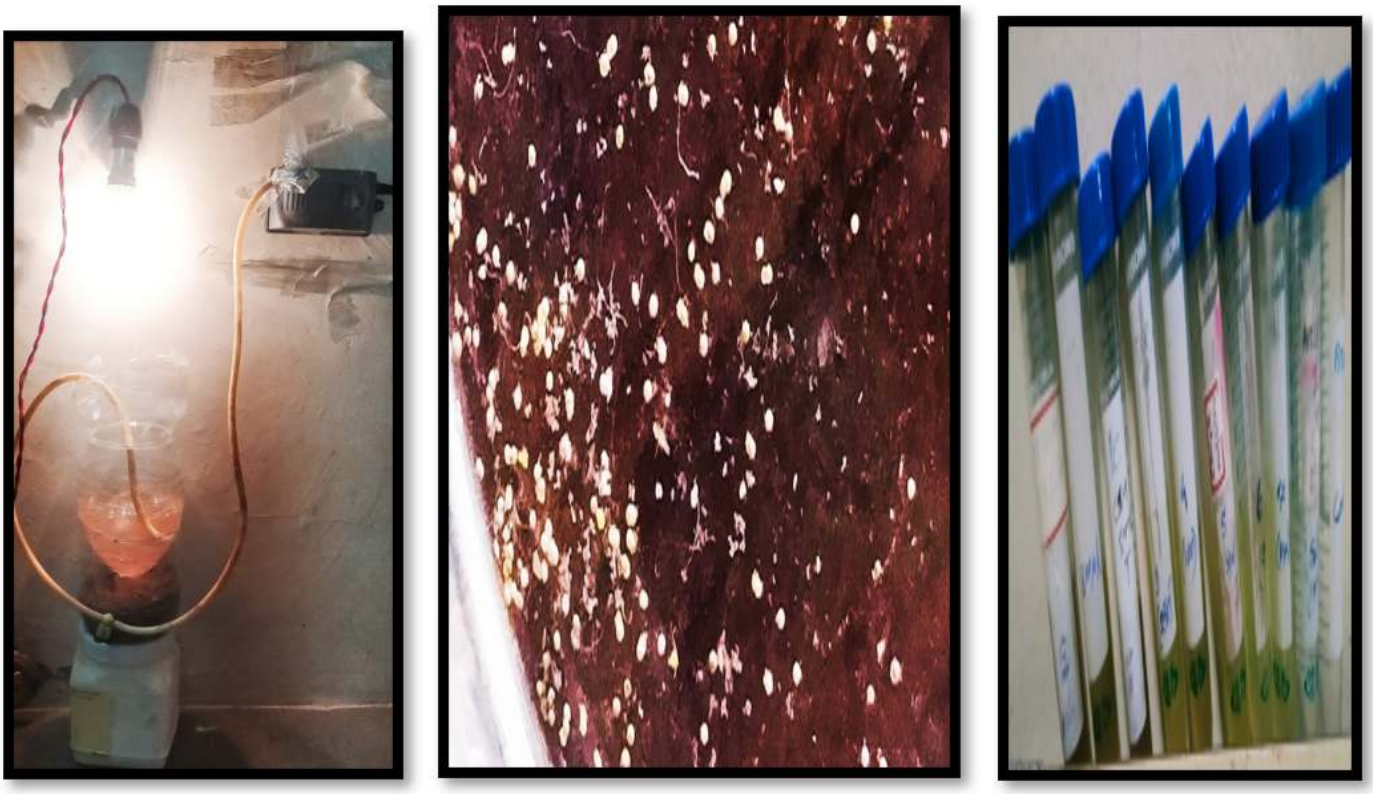

Figure 5

a)Larvae Production, b)Microscopic view of Larvae, c)Solution preparation with Larvae

Figure 6

Microscopic view of Lethality of Brine Shrimp In Control group

Figure 7

Microscopic view of Lethality in Test group

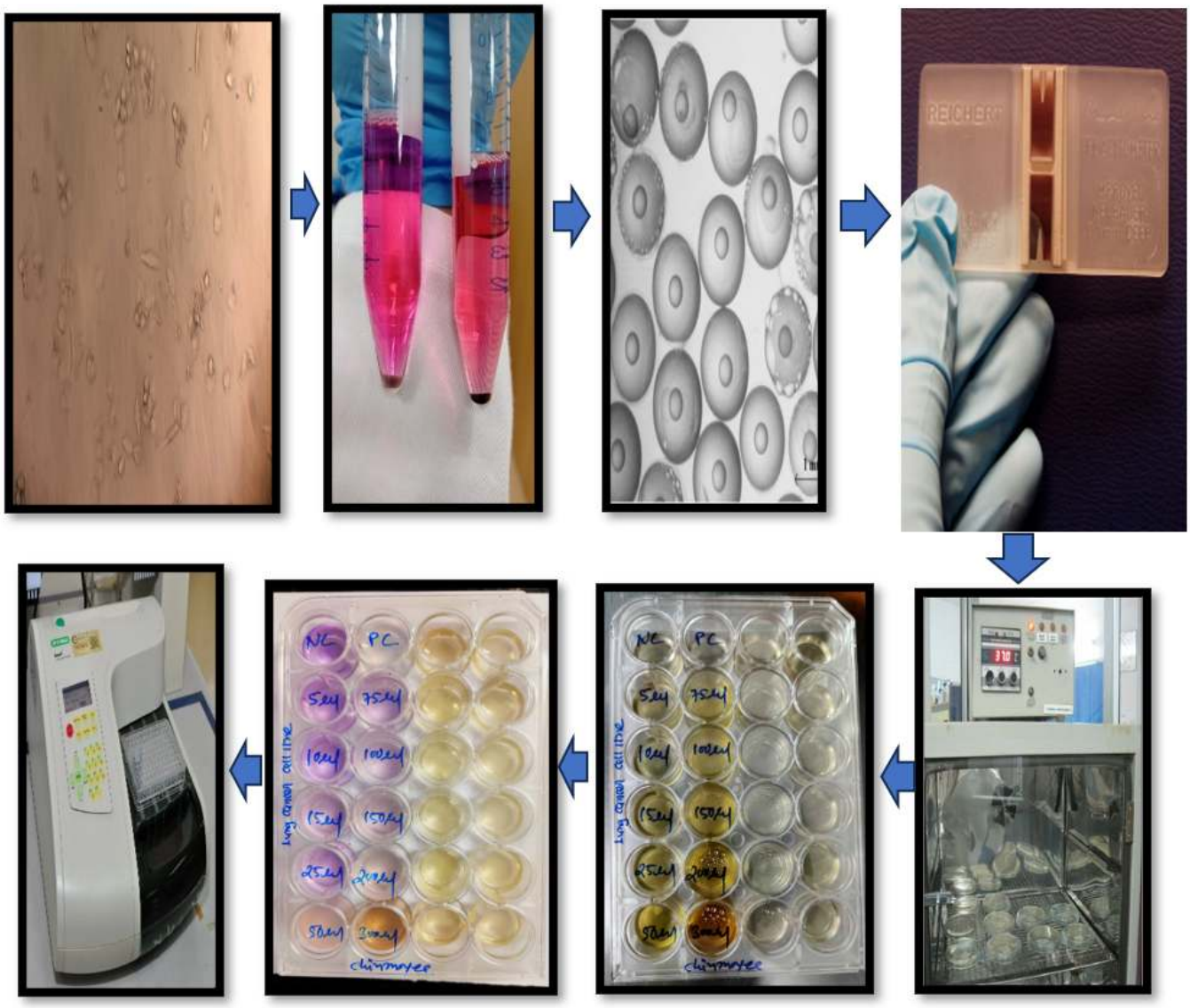

Figure 8

MTT Assay Process

Supplementary Files

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