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DEVELOPMENT AND EVALUATION OF PHYTOSOMES OF *Artemisia vulgaris* L. FOR ANTICANCER ACTIVITY

SUNDARAGANAPATHY.R[*1], GAYATHRI.K[1], KUMUDHA.D[1], THABSEERA.N[1], UVAIS.T[1], SUREKA.M[1], SURYA PRAKASH.R[1], VELLAISAMY.R[1], VYSHNA.V[1] AND S MOHAN[2]

[1]Faculty of pharmacy, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu

[2]Karpagam College of Pharmacy, Coimbatore, Tamil Nadu

***Corresponding Author: E Mail: kumudhachem@gmail.com; Mobile: 9842713372**

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ABSTRACT

Cancer is a major public health issue associated with significant death and disability. Breast cancer is second and very common type of cancer in women worldwide. *Artemisia vulgaris* L. (Mugwort), family Asteraceae, has been used to treat various diseases. Since ancient times the Indians were used *Artemisia vulgaris* L. to treat imbalances that women may suffer such as Breast cancer, Premenstrual syndrome, Dysmenorrheal, Menopause and anti-fertility. The term "Phyto" means plant while "Some" means cell like. Phytosomes are little cell-like structure. Phytosomes are recently introduced herbal formulation which contains phytoconstituents that are better absorbed and as a result produce better bioavailability and pharmacological actions. Phytosomes are novel patented technology to develop and incorporate the standardized plant extract into phospholipids to produce lipid compatible molecular complexes. The Phytosomes are developed from the ethanolic extract of *Artemisia vulgaris* L. by anti-solvent precipitation method, characterized and evaluated for anticancer activity.

Keywords: *Artemisia vulgaris* L., Anticancer, Phytosomes, Breast Cancer

INTRODUCTION

Artemisia vulgaris L., (Asteraceace) is one of the several species of the genus Artemisia, also known as wormwood. Artemisia is a class of fragrant annual herb distributed widely in Asia, Europe, North America **[1]**. The plant is traditionally used to treat a wide range of conditions such as gastrointestinal disorders, headaches, nose bleeds, muscle spasms, epilepsy, circulatory problems, menopausal and menstrual complaints, fever, rheumatism, asthma, gout, infertility, bacterial infections, inflammation, malaria, warm infestation, psychoneurosis, depression, choleretic, hepatosis. Recently the use of essentials oils of Artemisia as medicinal agents because of anticancer potential. Artemisia species are responsible for their antiproliferative effects on cancer cells **[2, 3]**. Most of the bioactive constituents of herbal drugs are water soluble molecules. However, water soluble phytoconstituents like many flavonoids, tannins etc. are poorly absorbed either due to their larger or due to their poor miscibility with oils and other lipids. Severely limiting their ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine. Water-soluble phytoconstituent molecules (mainly polyphenoles) can be converted into lipid-compatible molecular complexes which are called Phytosomes **[4]**. Phytosomes are the novel formulation technology which helps to overcome these problems. In humans and other higher animals the phospholipids are also employed as natural digestive aids and act as carriers for both fat miscible and water miscible nutrients which are easily absorbed orally. Phytosome has been an emerging trend in delivery of herbal drug and nutraceuticals **[5]**. Phytosomes are more bioavailable as compared to simple herbal extracts owing to their enhanced capacity to cross the lipid rich biomembranes and finally reaches the blood. Phospholipids are complex molecules that are used in all known life forms to make cell membranes. The Chemical analysis indicates that in phytosome is usually a flavonoid molecule linked with at least one phosphatidylcholine (PC) molecule. A bond is formed between these two molecules, creating a hybrid molecule. This highly lipid-miscible hybrid bond is better suited to merge into the lipid phase of the enterocytes outer cell membrane **[4]**. Breast cancer is the most common female cancer in the world. Breast cancer incidence is rising all over the world at different rates. Breast cancer is ranked 2nd worldwide. It is estimated that globally 1.67 million new cancer cases diagnosed in

2012 by WHO and less than 1% of breast cancer develop in men **[6-8]**.

MATERIALS AND METHODS

Plant Collection and Authentication:

The aerial parts of the *Artemisia vulgaris* L. were collected from Thanjavur, Orathanadu, and Thirumangalakkottai east. The plant sample were identified and authenticated by Botanical Survey of India, Southern regional centre, Coimbatore.

Extraction of Plant Material:

The Plant material was dried under shade, powdered and then macerated in ethanol 70% (v/v) for 48 hours and then extracted by a percolator. The extracted solution were concentrated at 50̊ C under reduced pressure to dryness **[9]**.

Preparation of Phytosomes:

The specific amount of leaf extract and soya lecithin were taken into a 100ml round bottom flask and refluxed with 20ml of dichloromethane at a temperature not exceeding 60℃ for 2 hours. The mixture was concentrated to 5-10ml. Hexane (20ml) was added carefully with continuous stirring to get the precipitate which was filtered and collected and stored in vacuum desiccators overnight. The dried precipitate was crushed in mortar and sieved through #100 meshes. Powdered complex was placed in amber colored glass bottle and stored at room temperature by Anti-Solvent Precipitation Method **[7, 10]**.

Characterization of Phytosomes:

UV– Visible Spectrometry:

UV-Visible Spectroscopic studies were carried out on a Shimadzu UV-Visible double beam spectrophotometer over a wavelength range of 500-600nm.

Scanning Electron Microscopy:

Surface morphology was determined by the method SEM. In this, the formulation was analyzed by using SEM. These studies were mainly done to study the hollow nature of nanoparticles **[7]**.

Zeta Potential:

Zetapotential or ions on the slipping plane of vesicles is measured based on laser doppler electrophoresis technique using Malvernzeta-sizer nanoseries 30 (Nano-S90) instrument. Zeta potential is the electrical potential in the interfacial double layer at the location of the slipping plane versus appoint in the bulk fluid away from the interface. A value of 25m V (positive or negative) can be taken as the arbitrary value that separates low-charged surface for the highly charged surfaces. The zeta potential for the formulation was found out **[8]**.

Zeta Sizer:

Vesicle size determination of *Artemisia vulgaris L.,* phytosome were carried out using Malvern zeta-sizer nano series (Nano-S90) instrument. A quantity of 2ml prepared Extract were taken into the

cuvette and exposed to laser light diffraction at an angle of 90°. The average sizes of the formulations were also calculated **[8]**.

Anticancer Activity:

Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours. Add appropriate concentrations of the test agent. Incubate the plate for 24hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, take out the plates from incubator and remove spent media and add MTT reagent to a final concentration of 0.5mg/ml of total volume. Wrap the plate with aluminium foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. Then remove the MTT reagent and then add 100 µl of solubilization solution (DMSO). Gentle stirring in a gyratory shaker will enhanced is solution. Occasionally, pipetting up and down may be required to completely dissolve the MTT form a zan crystals especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength. The IC50 value was determined by using linear regression equation i.e. y= mx+c. Here, y=50, M and C values were derived from the viability graph **[10-13]**.

RESULT AND DISCUSSION

UV- Visible Spectroscopy:

The preliminary characterization of *Artemisia vulgaris* L. phytosome was monitored by UV–Visible Absorbance Spectra Analysis. The maximum absorbance study peak appears from the range at 500-600nm. This range indicates that the formed phytosomes have smaller particle size within the absorbance intensity **(Figure 1)**.

Scanning Electron Microscope Analysis:

Surface morphology of formulated phytosome was determined by SEM analysis which showed a small spherical shaped, discrete particle without aggregation and smooth texture in surface morphology **(Figure 2)**.

Zeta Sizer:

The prepared phytosome was subjected to particle size analysis using Zeta Sizer (nano ZS90, Malvern, UK). The formulations were sufficiently diluted with double distilled water prior to the measurement. The results showed that the particle size of prepared formulations were in the range at 400-800 nm with good PDI **(Figure 3)**.

From this result, the average size of the phytosome formulation of *Artemisia vulgaris* L., is around 732.4nm. The morphology and particle size were determined.

Zeta Potential:

Zeta Potential was determined using Malvern zeta-sizer nano series (Nano-S90) instruments. The instrument gives the measurement of ions on the slipping plane of the vesicles **(Figure 4)**.

The average negative potential value is -10.3mV which indicates that the phytosomal formulation of *Artemisia vulgaris* L. have better stability.

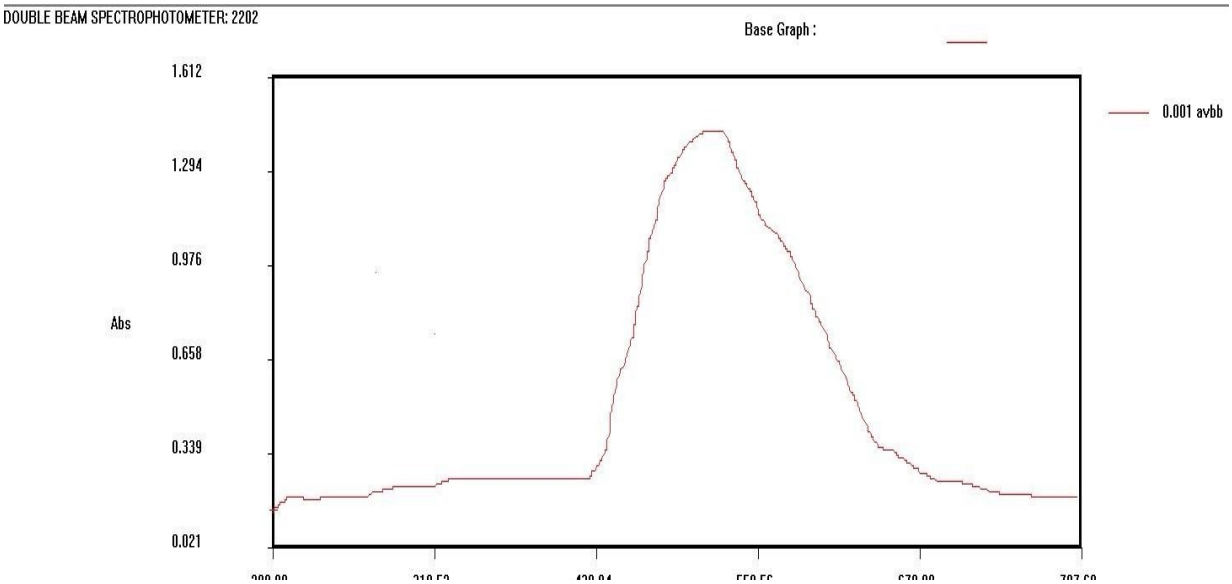


Figure 1: UV-Visible Graph of *Artemisia vulgaris* L . Phytosome

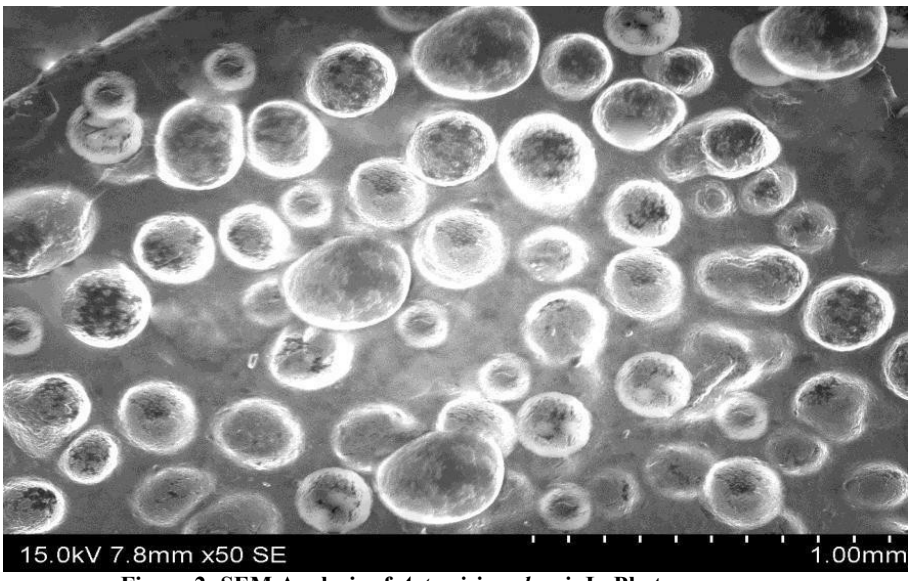


Figure 2: SEM Analysis of *Artemisia vulgaris* L. Phytosome

Results

		Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 732.4	Peak 1:	595.0	100.0	90.21
PdI: 1.000	Peak 2:	0.000	0.0	0.000
Intercept: 0.791	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

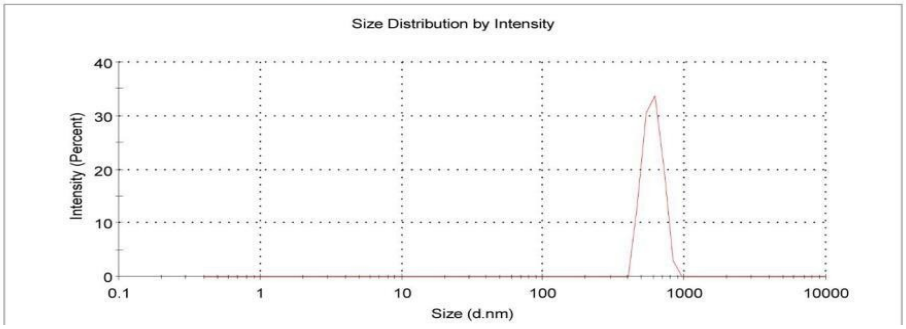


Figure 3: Zeta Sizer of Phytosome

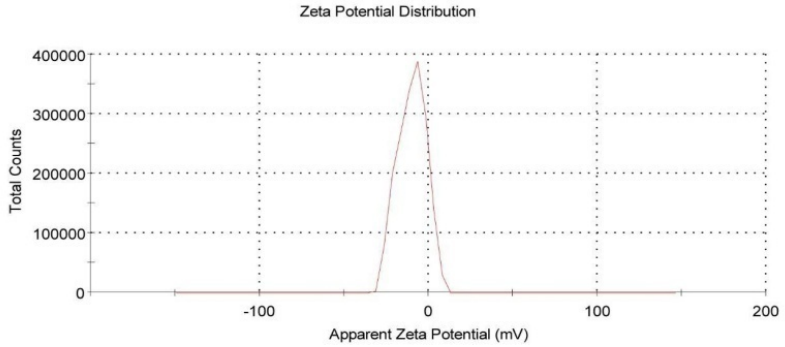
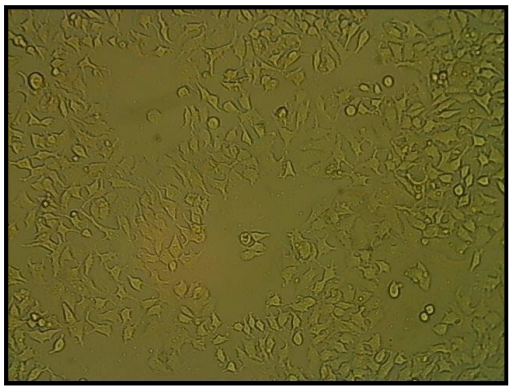
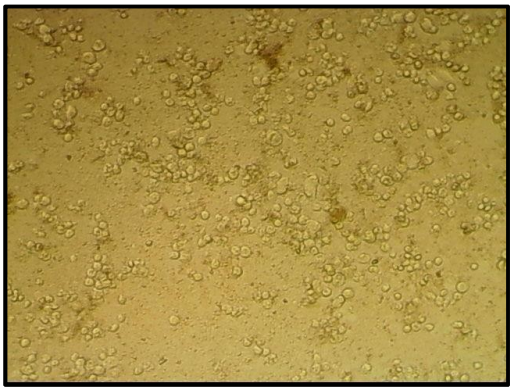


Figure4: Zeta Potential of Phytosome

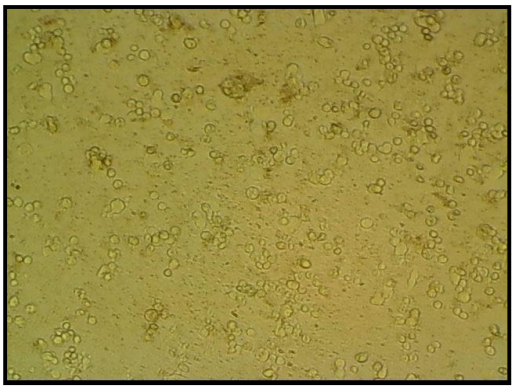
Cell line Studies:



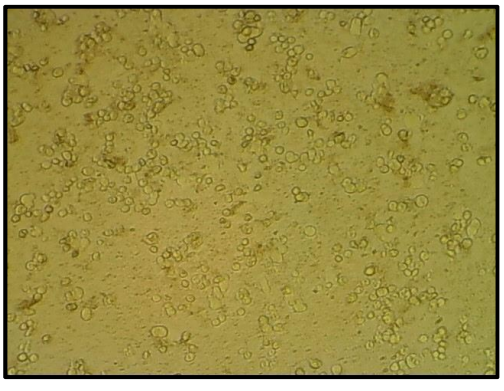
100µg/ml



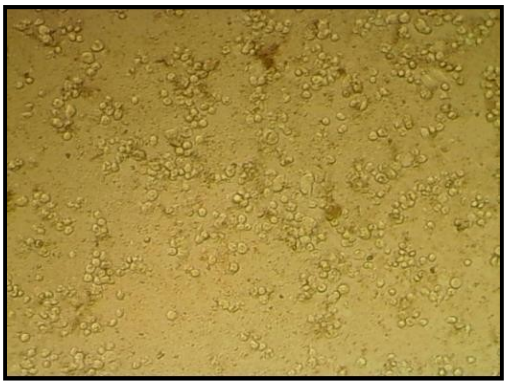
200µg/ml



300µg/ml



400µg/ml

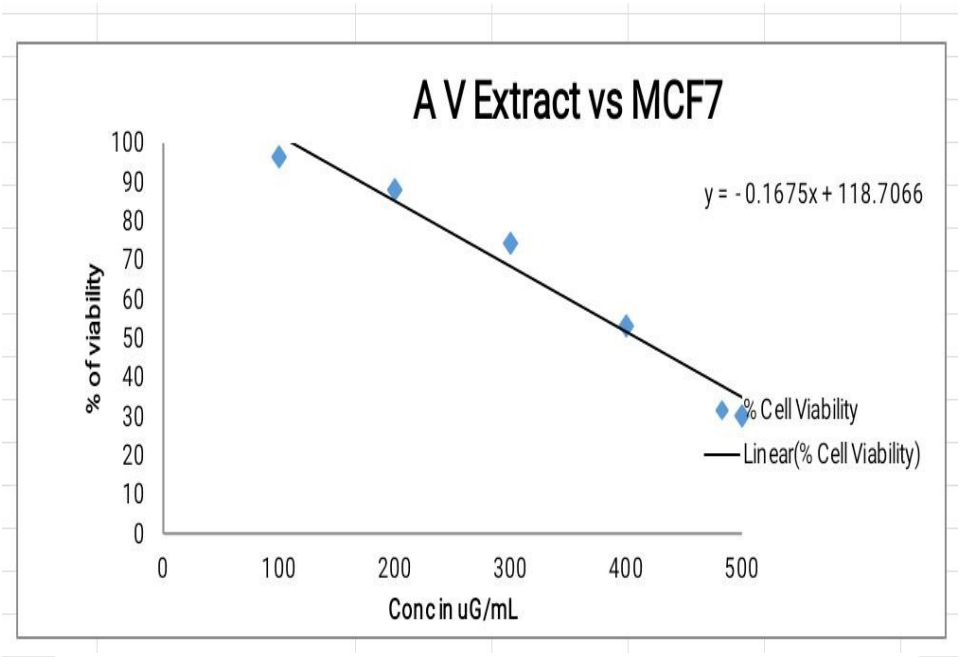


500µg/ml

Figure 5: *Artemisia vulgarisl.* Extract VS MCF 7

Table 1: *Artemisia vulgaris* L. Extract VS MCF7

Anticancer activity Incubation:24hrs								
Observation	**Blank**	**Untreated**	**STD (25µg/ml)**	**100 µg/ml**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
Reading 1	**0.04**	**0.841**	**0.428**	**0.8**	**0.74**	**0.606**	**0.452**	**0.278**
Reading 2	**0.05**	**0.846**	**0.425**	**0.8**	**0.74**	**0.607**	**0.454**	**0.281**
Reading 3	**0.05**	**0.641**	**0.315**	**0.61**	**0.58**	**0.554**	**0.398**	**0.241**
Mean	**0.05**	**0.776**	**0.389**	**0.75**	**0.68**	**0.589**	**0.434**	**0.266**
Mean OD- Mean B		**0.729**	**0.343**	**0.70**	**0.64**	**0.5423**	**0.388**	**0.22**
Standard Deviation		**0.117**	**0.064**	**0.11**	**0.09**	**0.0303**	**0.0317**	**0.02227**
Standard Error		**0.083**	**0.046**	**0.08**	**0.06**	**0.0214**	**0.0224**	**0.01575**
Viability%		**100**	**46.98**	**94.98**	**88.07**	**74.36**	**53.199**	**30.1645**



IC_{50} Value=411.37µg/ml

Graph 1: *Artemisia vulgaris* L. VS MCF7

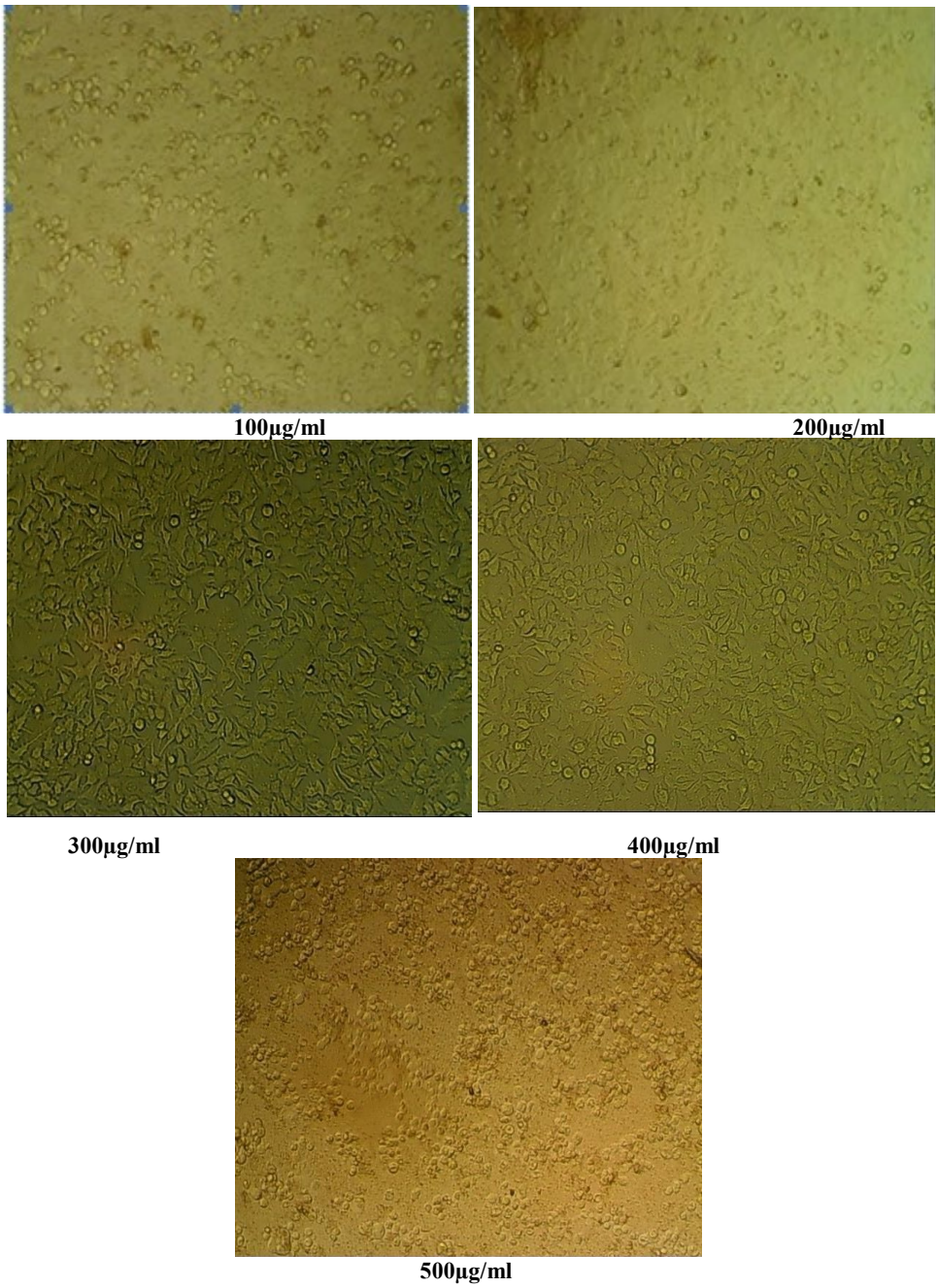
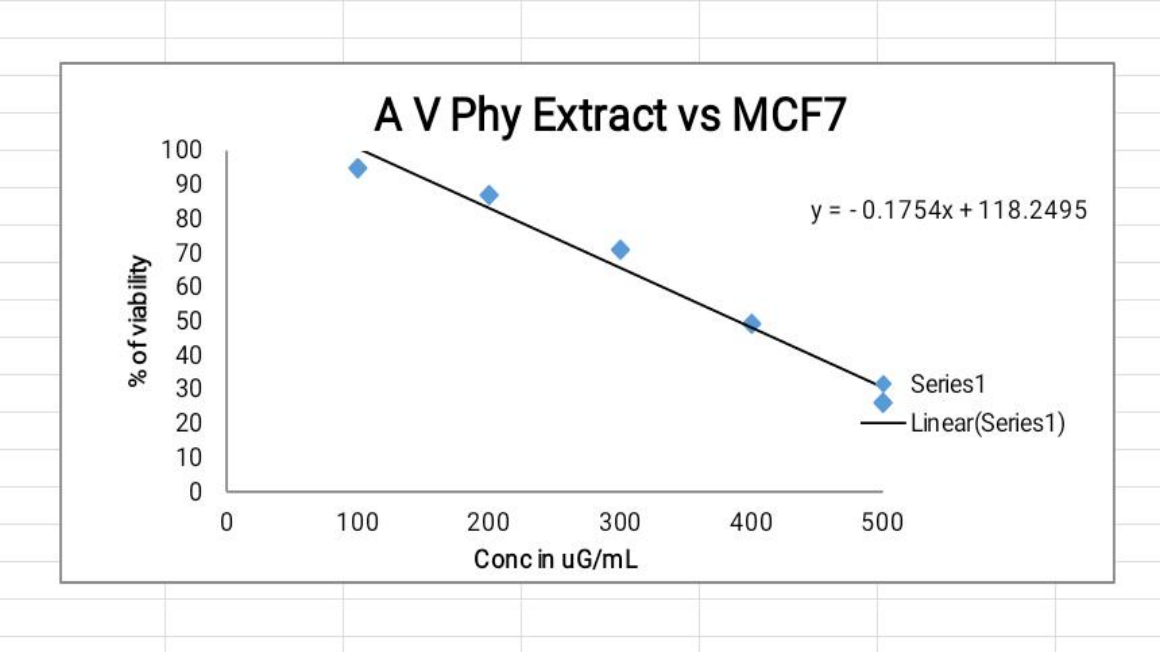


Figure 6: ***Artemisia vulgaris*** **LINN, Phytosome Formulation VS MCF7**

Table 2: *Artemisia vulgaris* L. phytosome formulation VS MCF7

Anticancer activity Incubation:24hrs								
Observation	**Blank**	**Untreated**	**STD 25 µg/ml**	**100 µg/ml**	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
Reading 1	**0.04**	**0.841**	**0.428**	**0.8**	**0.7**	**0.568**	**0.412**	**0.236**
Reading 2	**0.05**	**0.846**	**0.425**	**0.8**	**0.7**	**0.571**	**0.407**	**0.234**
Reading 3	**0.05**	**0.641**	**0.315**	**0.62**	**0.6**	**0.554**	**0.398**	**0.241**
Mean	**0.05**	**0.776**	**0.389**	**0.74**	**0.7**	**0.564**	**0.406**	**0.237**
Mean OD-Mean B		**0.729**	**0.343**	**0.69**	**0.6**	**0.518**	**0.359**	**0.1903**
Standard Deviation		**0.117**	**0.064**	**0.1**	**0.1**	**0.009**	**0.007**	**0.0036**
Standard Error		**0.083**	**0.046**	**0.07**	**0.1**	**0.006**	**0.005**	**0.0025**
Viability%		**100**	**46.98**	**94.9**	**87**	**70.98**	**49.22**	**26.0968**



IC_{50}Value=389.71µg/mL
Graph 2: *Artemisia vulgaris* L. Phytosome Formulation VS MCF7

Based on the cell line study, *Artemisia vulgaris* Linn., phytosome has shown an enhanced cytotoxic activity with IC50 value of 389.71µG/mL that the herbal extract with IC_{50}value of 411.37µg/mL. The cell viability also decreased up to 26.09%.

CONCLUSION

The cell line study was carried out for both herbal extract and its phytosome formulation for the comparison of therapeutic activity. The result was remarkable with an enhanced Anticancer activity in case of phytosome formulation than herbal extract. The phytosome formulation of the *Artemisia vulgaris* Linn.,

showed a promising effect in the treatment of Cancer.

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