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# STUDIES ON THE ACUTE TOXICITY AND ANTI-CANCER ACTIVITIES OF POLYSCIAS FRUTICOSA (L) HARMS LEAF PHYTOSOME ON LUNG CANCER CELL LINE

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## ABSTRACT

The present work focuses on the acute toxicity and anti-cancer studies of *Polyscias fruticosa* phytosome (PflP) prepared from its leaf aqueous extracts. The prepared phytosome is evaluated by particle size measurements, zeta potential and SEM studies. *P. fruticosa* growing in India included in the same family of ginseng (Araliaceae), contain large amounts of triterpenoid saponins in their leaves and roots. The Araliaceae family members are known for various therapeutic properties like adaptogenic, immunostimulant, antioxidant activities etc, as evidenced from the bioactivities of ginseng, a famous member of this family. The results indicated that the prepared phytosome laid in the nanoparticle range (50-200nm) and exhibited characteristic zeta potential, and SEM values. The $LC_{50}$ was found to be more than 1000 mcg/ml for Pfl phytosome in Brine shrimp assay method. The anticancer activity is screened on lung cancer cell lines followed by MTT assay. The anticancer activity studies indicate that the Pfl phytosome at 125 mcg concentration (51.45% cytotoxicity) showed comparable activity with the reference standard etoposide at 50 mcg concentration (67.32 % cytotoxicity).

**KEYWORDS:** *Polyscias fruticosa*, PflP (Pfl Phytosome), Brine shrimp assay, Lung cancer cell lines, MTT assay.

## INTRODUCTION

Phytosomes or herbosomes are advanced lipid-based drug delivery systems can be used for the entrapment of different categories of active compounds to improve absorption when administrated and also in the enhancement of pharmacokinetic and pharmacodynamic properties of active phytoconstituents.[1,2,4] The advent of phytosome technology has a potential impact in the field of drug delivery inorder to transform the current state of administration of bioactive phytochemicals.

Phytosomes are usually prepared by mixing the active phytoconstituents with phospholipids, in definite stoichiometric ratios under specific conditions. Solvent evaporation is a traditional and frequently used method for preparing phospholipid complexes. Shan and colleagues,[3] reported the solvent evaporation method to prepare oleanolic acid-phospholipid complexes.

Phytosomes or Herbosomes are advanced form of botanicals and phyto-constituent that are better absorbed both orally, and transdermally, when entrapped with phosphatidyl choline.

The phytosome technology forms a link between the traditional delivery system of phytoconstituents and novel drug delivery systems.

Normally phytosomes are prepared by various methods like Solvent evaporation method, mechanical dispersion method, sating out technique, lyophilisation method, Anti-solvent precipitation process, Rotary evaporation process etc.[5,6,7,8,9]

In the present study, *P. fruticosa* leaf saponin was extracted and prepared its phytosome by solvent evaporation technique. The phytosome was evaluated for particle size determination, zeta potential, and SEM values. Acute toxicity study was performed for the prepared leaf phytosome by brine shrimp assay method and anti-cancer activity in lung cancer cell lines.

Several phytopharmacological studies on Polyscias root saponin extracts indicate that, it has got effective adaptogenic, free radical scavenging, anti-diabetic, immunostimulant, and cytotoxic activities.[16,17,18,19,20,21]

## 2. EXPERIMENTAL

### *2.1. Collection and authentication of P. fruticosa leaves*

The leaves of *P. fruticosa* were collected from Coimbatore, and authenticated at the Botanical survey of India (BSI/SRC/5/23/2023//Tech/975) and voucher specimens were deposited in the Herbarium of the Pharmacognosy Laboratory, PPG College of Pharmacy, Saravanampatti. (Medicinal Garden accession number PPG 58/2023)

### *2.2. Preparation of the Plant leaf saponin extract*[10]

500g of the leaves of *P. fruticosa* were collected, washed free of extraneous impurities, coarsed and extracted with methanol and concentrated to dryness. The residue obtained was suspended in water and washed with diethyl ether to remove lipid impurities and then extracted with n-butanol. The vacuum dried n-butanol extract was subjected to various chemical tests to confirm the presence of saponins. The percentage yield obtained was 18.5%.

### *2.3. Chemical Tests for Triterpenoids saponins*[11]

*2.3.1. Salkowski Test:* A small quantity of the *P. fruticosa* root extract in chloroform was treated with a few drops of conc. $H_2SO_4$; the solution turned yellow, then to red.

*2.3.2. Hirshorn Test*: A small quantity of the extract was heated with trichloroacetic acid, the solution turned to yellow color and finally changed to red.

*2.3.3. Lieberman Storch Morasky test*: 10-20mg of the saponin extracts were added to one drop of conc. $H_2SO_4$ on a slide. A characteristic sequence of color reactions beginning with yellow changing to red and finally to blue, green and violet were observed. This color reaction is characteristic for saponins in the extracts.

### *2.4. Quantitative Physical Analysis for Saponin Extract*[12,13]

*2.4.1. Fish Lethal Test*: Small fish were put into drug extracts. The presence of saponin in the extract was confirmed if 60% of fish were killed in the course of an hour.

*2.4.2. Foam Test*: 500mg/ml of the extract was shaken with water in a graduated cylinder for 15 seconds and allowed to stand for 15 minutes before the recording was made. A foam layer of 1.8cm (not less than 1 cm) was formed and persisted for 15 min.

*2.4.3. Hemolysis Test*: For this test three dilutions of the root saponin extract were added to 2.5% defibrinated blood in physiological salt solution. The hemolysis took place within 10 minutes and the blood suspension became transparent. The largest dilution of saponin causing total hemolysis is called hemolytic index. It was observed that 750 mg /ml concentration of the saponin extract showed maximum hemolysis.

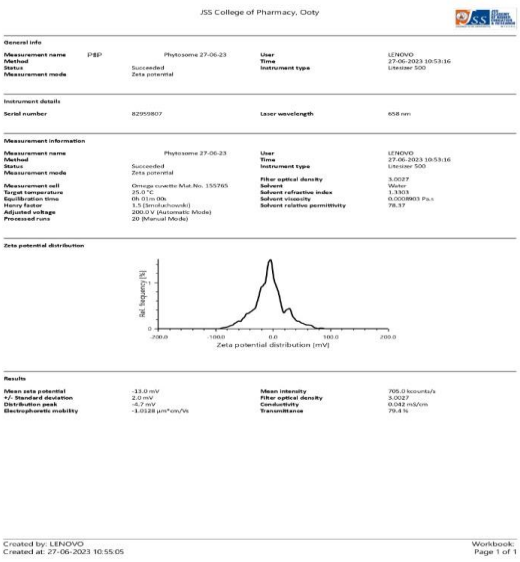
### *2.5. Preparation of Phytosomes*[4,6,7]

The Phytosome was prepared by solvent evaporation method by mixing 25 ml of leaf extract and added 50 g of soy lecithin at the ratio 1:2, in a 100 ml round bottom flask and refluxed with 20 ml of acetone (as aprotic solvent) at 50 -60 $^{o}$ C temperature for 2 hours The mixture was concentrated to 5-10 ml and the pprecipitate was collected and dried. The dried precipitate was stored in amber coloured bottle at room temperature and designated as PflP (*P. fruticosa leaf* Phytosome)

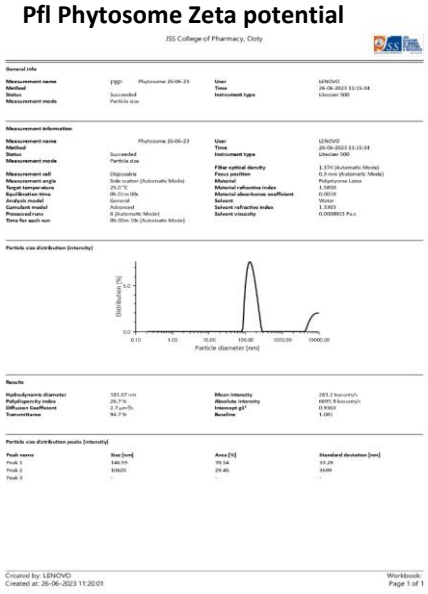
### *2.5.1. Characterisation of the prepared Phytosome*

In order to characterize the formation of phytosome, SEM device was used for determining the shape and morphological structure, and the particle size analyser for the determination of particle size distribution and zeta potential.

**Pfl Phytosome particle size data**




**Pfl Phytosome Zeta potential**




**Pfl Phytosome SEM analysis**

### 2.6. Acute Toxicity Studies

***Brine Shrimp Assay Method*[14]**

Brine shrimp assay method was followed to find out the LC50 of PflP (*P. fruticosa* leaf Phytosome). The brine shrimp eggs were hatched in a rectangular chamber and filled with artificial sea water and ten members each were transferred to vials using a 9-inch disposable pipette. The survival rate of the shrimps was observed after 24h for various concentrations of the saponin extracts. The $LC_{50}$ was found out from the dose – response graph.

**Percentage deaths at 24 h**

| Plant extracts | 10 µg/ml | 100 µg/ml | 200 µg/ml | 500 µg/ml | 1000 µg/ml | $LC_{50}$ µg/ml |
|---|---|---|---|---|---|---|
| PflP | 0 | 12 | 15 | 17 | 42 | >1000 |

**PflP: *P. fruticosa* leaf Phytosome**

### 2.7. Anti-Cancer Activity Studies[15]

MTT Assay: The extent of cytotoxicity of the synthesized sample to the cancer cells was determined by the MTT dye reduction assay as described by Igarashi and Miyazawa.[16]

***Principle***

The 2-(4, 4-dimethyl-2-thiazoyl)-2, 5-diphenyl-2, 4-tetrazolium salt (MTT) is converted into its formazan derivative by live cells and the amount of formazon formed is a measure of number of viable cells. The formazan formed is then solubilized with suitable solvent and the cell viability is measured in a microtitre plate reader.

***Reagents***

PBS (phosphate buffered saline), MTT - 3mg/ml in PBS, Isopropanol in 0.04N HCl

***Procedure***

100µl of treated cells were incubated with 50µl of MTT at 37ºC for 3 hours. After incubation, 200µl of PBS was added to all the samples and aspirated carefully to remove excess MTT. 200µl of acid-propanol was added and left overnight in the dark for solubilization.

The absorbance was read at 650nm in a microtitre plate reader (Bio RAD U.S.A.). The optical density of the control cells was fixed to be 100% viable and the percent viability of the cells in the other treatment groups were calculated using the formula. The results are tabulated in table.2

$$\text{Percent viability} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \text{ X } 100$$

**Table 2: Anti-cancer activity studies of Pfl phytosome lung cancer cell line.**

| Samples | Concentrations | OD values (triplicate)- 24hrs | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | Average | % of viability | % of cytotoxicity |
| Control cells (without treatment) | | 1.684 | 1.683 | 1.683 | 1.683 | 100% | No toxicity |
| Etoposide (standard drug) | 50 µg | 1.132 | 1.132 | 1.134 | 1.133 | 32.67 | 67.32 |
| Pfl Phytosome | 25 µg | 0.426 | 0.425 | 0.426 | 0.426 | 74.68 | 25.31 |
| | 50 µg | 0.533 | 0.532 | 0.537 | 0.534 | 68.27 | 31.72 |
| | 75 µg | 0.648 | 0.644 | 0.648 | 0.647 | 61.55 | 38.44 |
| | 100 µg | 0.756 | 0.755 | 0.755 | 0.755 | 55.13 | 44.86 |
| | 125 µg | 0.869 | 0.869 | 0.861 | 0.866 | 48.54 | 51.45 |

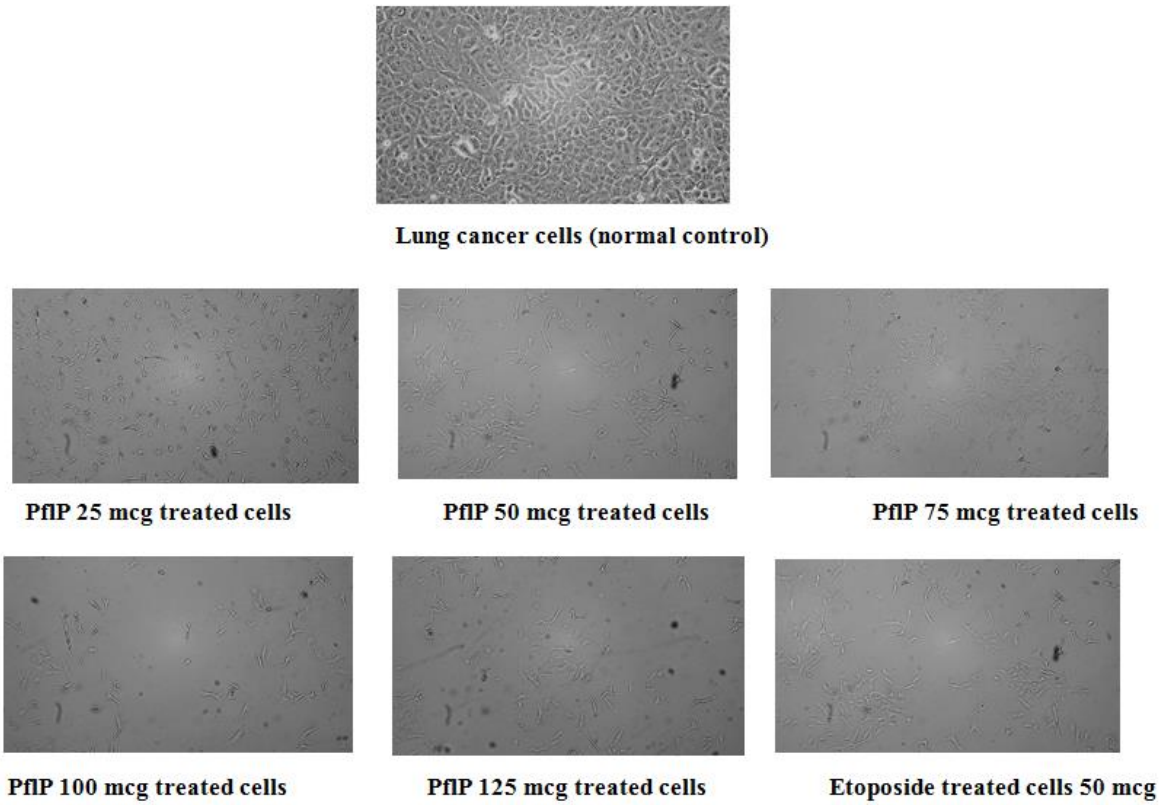



**Fig. 2: Lung cancer cell lines treated with different concentrations of PflP with standard drug Etoposide PflP: *Polyscias fruticosa leaf Phytosome*, Standard drug: *Etoposide*, Normal control: lung cancer cells**

## 3. RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed the presence of triterpenoid saponins present in the leaf extract of *P. fruticosa.* The oleanolic acid related triterpenoids are the therapeutically active compounds utilised in the work for making the plant phytosome. The yield of the leaf n-butanol extract was found to be 18.5%. The results for the particle size evaluation indicated that Phytosome (PflP) laid in the nanoparticle range (50-200nm) and exhibited characteristic zeta potential (-13. mV). The SEM data exhibited characteristic shape and size for the phytosomes prepared from the leaf saponins of *P. fruticosa.*

The acute toxicity study data indicated that *P. fruticosa* leaf phytosome (PflP) has an $LC_{50}$ more than 1000 mcg/ml in Brine shrimp assay method.

The anticancer activity studies indicated that the Pfl phytosome at 125 mcg concentration showed 51.45% cytotoxicity compared to the reference standard drug etoposide (50 mcg concentration, 67.32 % cytotoxicity) on lung cancer cell line studies.

These findings throw light towards the supportive potential of these saponin compounds present in *P. fruticosa* leaves for the preparation of phytosome type of novel drug delivery systems and thereby useful in the chemotherapy of cancer with reduced side effects along with modern medicines.

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