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RESEARCH ARTICLE
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EVALUATION OF THE CYTOTOXIC AND ANTI-CANCER ACTIVITIES OF *POLYSCIAS FRUTICOSA* (L) HARMS ROOT PHYTOSOME ON NEUROBLASTOMA CELL LINE

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Abstract
The present work focuses on the cytotoxic and anti-cancer studies of *Polyscias fruticosa* phytosome (PfrP) prepared from its root n-butanol 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. Pfr phytosome (40mcg/ml) showed effective cytotoxic action on the *Allium cepa* root tip cells demonstrated by the presence of cell division abnormalities as compared with standard reference Adriamycin (20mcg/ml). The anticancer activity is screened on neuro blastoma cell lines followed by MTT assay. The anticancer activity studies indicate that the Pfr phytosome at 125 mcg concentration (50.37% cytotoxicity) showed comparable activity with the reference standard etoposide at 50 mcg concentration (62.74 % cytotoxicity).

Keywords: PfrP (*P.fruticosa* root phytosome), Cytotoxic activity, Anti-cancer studies, neuro blastoma cell lines, MTT assay

INTRODUCTION

Phytosomes are advanced lipid-based delivery system that have a liposomes-related structure and can be used for the set up or entrapment of different categories of active phytoconstituents to enhance their absorption when administrated and also they act as lipid-based nanocarriers, in the enhancement of pharmacokinetic and pharmacodynamic properties of herbal-originated active compounds[7,8,10]. The advent of phytosome nanotechnology has a potential impact in the field of drug delivery and could transform the current state of administration of bioactive phytochemicals.

Phytosomes are usually prepared by mixing the active phytoconstituents with phospholipids, such as PC (phosphatidylcholine), PS (phosphatidylserine), and PE (phosphatidylethanolamine), in definite stoichiometric ratios under specific conditions. Solvent evaporation is a traditional and frequently used method for preparing phospholipid complexes. Shan and colleagues[14] reported the solvent evaporation method to prepare oleanolic acid-phospholipid complexes.
Phytosomes or Herbosomes are advanced form of botanicals and phyto-constituents 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, Anti-solvent precipitation process, Rotary evaporation process etc[7,8,9,10,11,12].

Several phytopharmacological studies on Polyscias root saponin extracts indicate that, it has got effective adaptogenic, free radical scavenging, anti-diabetic, immunostimulant, and cytotoxic activities[1,2,3,4,5,6]. In the present study, *P. fruticosa* roots saponin was extracted and prepared its phytosome by solvent evaporation technique. The phytosome was evaluated for particle size determination, zeta potential and SEM values. The prepared phytosome was screened for its cytotoxic activity on onion root tip cells and anti-cancer activity in neuroblastoma cancer cell lines.

2. EXPERIMENTAL

2.1. Collection and authentication of P. fruticosa roots

The roots of *P. fruticosa* were collected from Coimbatore, and authenticated at the Botanical survey of India (BSI/SRC/5/23/2023//Tech/974) and voucher specimens were deposited in the Herbarium of the Pharmacognosy Laboratory, PPG College of Pharmacy, Saravanampatti. (Herbarium accession number PPG/57/2023)

2.2. Preparation of the Plant saponin extract[16,17]

500g of the roots 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 26.5%.

2.3. Chemical Tests for Triterpenoids saponins[19]

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 [16,17]

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 500 mg /ml concentration of the saponin extract showed maximum hemolysis.

2.5. Preparation of Phytosomes[10,11,12,13]

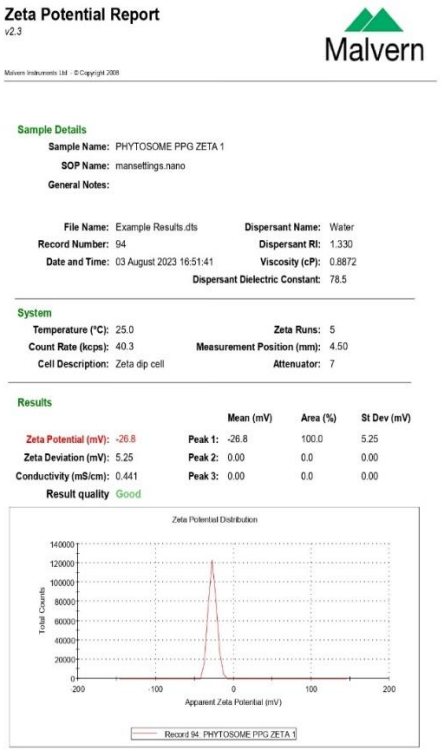
The Phytosome was prepared by solvent evaporation method by mixing 25 ml of root extract and added 50 g of soy lecithin at the ratio 1:2, in a 100 ml round bottom flask and refluxed with 20 ml of acetone (as aprotic solvent) at 50 -60 ^{o} C temperature for 2 hours The mixture was concentrated to 5-10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

2.5.1. Characterisation of the prepared Phytosome

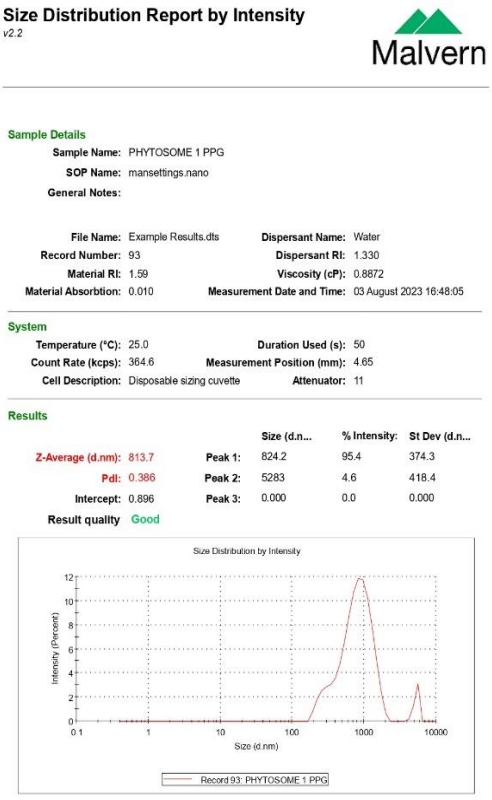
In order to characterize the formation of phytosome, Scanning Electron Microscopy (SEM) was used for determining the shape and morphological structure, and the particle size analyser for the determination of particle size distribution and zeta potential. Fig.1, Fig.2.

Fig 1: Pfr Phytosome particle size data and zeta potential

Pfr Phytosome particle size data



Pfr Phytosome Zeta potential



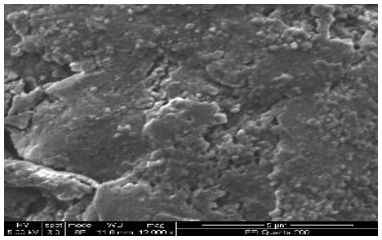


Fig 2: SEM of Pfr Phytosome

2.6. *Cytotoxic activity studies: Allium test*[20,21]

The cytotoxic effects of PfrP were observed on growing root tip cells of *Allium cepa*. Commercially available fresh onion bulbs were dried in sun shine and the scales were removed and the bulbs were allowed to sprout in wet land. When the root tips were about 1.0 to 1.5cm long they were placed separately for 24 hours in two different concentrations (20 & 40 mcg/ml) of the PfrP.
After treatment the root tips were washed thoroughly under tap water, harvested and fixed in acetic acid alcohol (1:3), acetocarmine squashes were prepared and observations were noted. For each test concentrations five slides were made and observed under Leitz orthopan microscope. Root tips kept in distilled water were used as the control.
Abnormalities like tropokinesis, stickiness, clumping of chromosomes and pycnotic nuclei were studied in the treated groups as compared to the standard (Adriamycin). The results are tabulated in Table 1. Fig.2, and Fig.3.

Table 1: Cytotoxic activity studies of Pfr Phytosome on *Allium cepa* root tip cell mitosis

Conc. of the phytosome mcg/ml	Todal number of dividing cells	Cell abnormalities %				Percentage of Mitotic Abnormality (%)
		Tropokinesis	Pycnotic Nucleus	Ribbon Shaped Nucleus	Clumping of Chromosomes	
PfrP 20	1328±8.2	2.58±0.952	16.2±1.45	19.35±0.05	6.45±0.87	58.04
PfrP 40	892±10.13	11.77±0.026*	22±2.6*	15±2.5	9±0.45	69.2*
Adriamycin 20	890±10.7	10.5±0.94	30.41±0.85	28.5±0.76	8.6±0.28	58.93
Adriamycin 40	1265±12.24	14.2±3.45*	35.08±4.76**	47.5±0.35**	11.6±1.65*	85.37**

PfrP: *P. fruticosa* root Phytosome, Student t test: ** → p value < 0.001, * → p value < 0.01

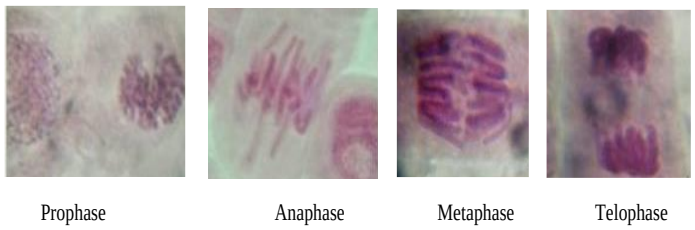


Fig.2. Stages of mitosis in *Allium cepa* root tip cells

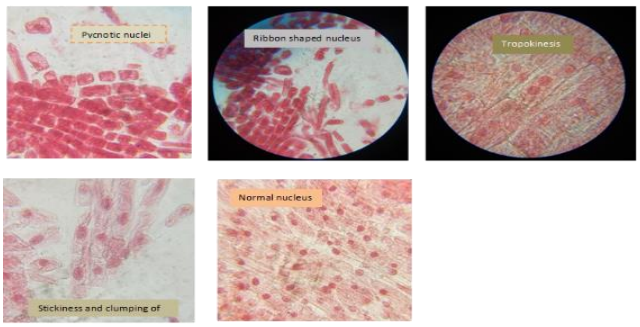


Fig.3. Observed cell abnormalities

Tropokinesis (two phases in one cell), *Pycnotic nucleus* (one cell contains three or more nucleus), *Ribbon shaped nucleus*, *Clumping of chromosomes* (chromosomes are in group formation)

2.7. *Anti-Cancer Activity Studies*[22]

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, Fig.4, and Fig.5.

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

Table 2. Anticancer activity studies using neuroblastoma cell lines

Samples	**Concentrations**	**OD values (triplicate)- 24hrs**					
		1	**2**	**3**	**Average**	**% of viability**	**% of cytotoxicity**
Control cells (without treatment)		1.345	1.342	1.341	1.342	100%	No toxicity
Etoposide (standard drug)	50 µg	0.843	0.843	0.841	0.842	37.25	62.74**
PfrP	25 µg	0.356	0.372	0.384	0.371	72.35	27.64
	50 µg	0.442	0.452	0.431	0.442	67.06	32.93
	75 µg	0.544	0.523	0.514	0.527	60.73	39.26
	100 µg	0.608	0.598	0.595	0.600	55.29	44.70
	125 µg	0.671	0.682	0.675	0.676	49.62	50.37*

PfrP: *P. fruticosa* root Phytosome, Student *t test:* ** → p value < 0.001, * → p value < 0.01

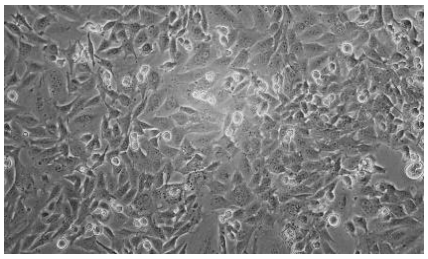
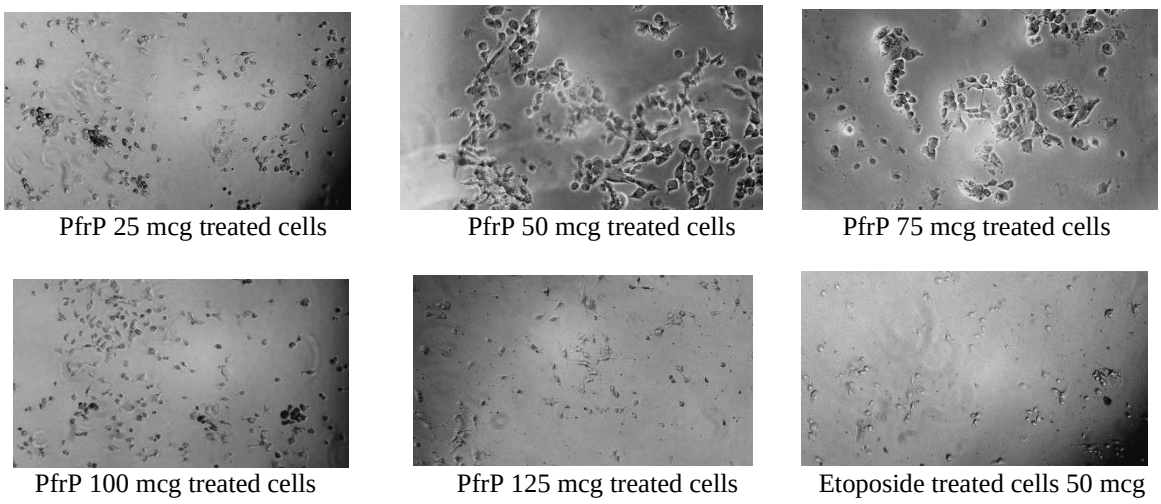


Fig.4. Neuroblastoma cells (Normal control)

Fig.5. Neuroblastoma cell lines treated with different concentrations of PfrP with standard drug Etoposide



PfrP 25 mcg treated cells | PfrP 50 mcg treated cells | PfrP 75 mcg treated cells

PfrP 100 mcg treated cells | PfrP 125 mcg treated cells | Etoposide treated cells 50 mcg

PfrP: *Polyscias fruticosa root Phytosome*, Standard drug: Etoposide
Normal control*:* Neuroblastoma cells

3. RESULTS AND DISCUSSION

The preliminary phytochemical analysis revealed the presence of triterpenoid saponins present in the root 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 root n-butanol extract was found to be 26.5%. The results for the particle size evaluation indicated that Phytosome (PfrP) laid in the nanoparticle range (50-200nm) and exhibited characteristic zeta potential (-26.8 mV). The SEM data exhibited characteristic shape and size for the phytosomes prepared from the root saponins of *P. fruticosa.*

The cytotoxic effects induced by the *P. fruticosa* root phytosome may be due to different means, ultimately disturbing nucleic acid metabolism, thereby inhibiting protein synthesis and finally resulted in range of abnormalities at the nuclear and chromosome levels of *Allium cepa* root tip meristem.

The data for the antimitotic activity in the root tip meristem of *Allium cepa* indicated that Pfr Phytosome at 40mcg/ml concentration produced significant level of cytotoxicity (69.2%) in Allium test as indicated by the number of cell division abnormalities like pycnotic nuclei, stickiness and clumping, tropokinesis, nuclear lesions etc. The standard drug Adriamycin at 40mcg/ml produced 85.37% mitotic abnormality.

The anticancer activity studies indicated that the Pfr phytosome at 125 mcg concentration showed 50.37% cytotoxicity compared to the reference standard drug etoposide (50 mcg concentration, 62.74 % cytotoxicity).These findings throw light towards the supportive potential of these saponin compounds present in *P. fruticosa* roots 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 conventional medicines.

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