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RESEARCH ARTICLE

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SCREENING OF THE ANTI-PROLIFERATIVE ACTIVITY OF *Polyscias fruticosa* (L) HARMS LEAF AgNPs ON LUNG CANCER CELL LINE

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Abstract

The present work focuses on the acute toxicity and anti-proliferative studies of *Polyscias fruticosa* silver nanoparticles (Pfl AgNP) prepared from its leaf saponin extract (Pfls). The prepared nanoparticles are evaluated by particle size measurement, zeta potential, UV analysis 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, included in the same family and the therapeutic efficacy is well explored.

The results indicated that the prepared silver nanoparticles laid in the nanoparticle range (100-200nm) and exhibited characteristic zeta potential values, and SEM data. The Pfl AgNP showed UV λ_{max} at 420 nm. The LC_{50} was found to be 641mcg/ml & more than 1000mcg /ml for Pfl AgNP and Pfls respectively in *Brine shrimp assay* method. The anticancer activity is screened on lung cancer cell lines (A549) followed by MTT assay. The anticancer activity studies indicate that the Pfl AgNP at 125 mcg concentration (54.25% cytotoxicity) with reference to the standard etoposide at 50 mcg concentration (65.34 % cytotoxicity).

Keywords: Pfls, Pfl AgNP, Brine shrimp assay, A549 Lung cancer cell lines, MTT assay

INTRODUCTION

Plant secondary metabolites such as polysaccharides, proteins, polyphenols, flavonoids, terpenoids, tannins, alkaloids, ketones, aldehydes and amines which can act as reducing, stabilizing, and capping agents in the conversion of metal ions to metal nanoparticles with defined bio activities[1].

The synthesis of AgNPs using the triterpenoid saponins present in the leaf of *P. fruticosa*[2,3] by the green chemistry approach have several advantages. These procedures are economic, efficient, ecofriendly and cost-effective, results in less waste and safer products. The potentially active phytoconstituents involved in the plant mediated synthesis of nanoparticles are biocompatible for a wide range of biomedical applications[16,17].

Several phytopharmacological studies on *Polyscias* leaf saponin extracts indicate that, it has got effective adaptogenic, free radical scavenging, anti-diabetic, immunostimulant, and cytotoxic activities[6,7,8,9,10,11]. In the present study, *P. fruticosa* leaf saponin[4,5] is extracted and prepared its silver nanoparticles by green synthesis[12]. The prepared Pfl AgNPs are screened for its anti-cancer activity on human lung cancer cell lines (A549) by the MTT assay at different concentrations. The acute toxicity study is carried out using brine shrimp assay method.

Also, the study focuses on the particle size measurements and zeta potential of the nanoparticles by the use of particle size analyser, wavelength maximum (λ_{max}) values by UV spectrophotometer and the shape and morphological studies by Scanning Electron Microscope for the biosynthesised silver nanoparticles by *P. fruticosa* leaf saponins.

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. (Herbarium number PPG 58/2023)

2.2. Preparation of the Plant leaf saponin extract[4,5]

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 (Pfls). The percentage yield obtained was 16.5%.

2.3. Chemical Tests for Triterpenoids saponins[18,19]

2.3.1. Salkowski Test: A small quantity of the *P. fruticosa* leaf 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 [4,5]

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 leaf 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.

2.4.4. Preparation of Silver Nitrate Solution:

1mM AgNO3 solution was prepared by weighing 0.1699 g of AgNO3 and dissolve with distilled water and make up the solution up to 1000ml. After the completion of the preparation the formed nano particles are separated by using centrifuge and dried in an oven at low temperature.

2.5. Synthesis of Silver Nano Particles[12,13,14,15]

100 ml of leaf saponin extract (Pfls) is mixed with 900 ml of prepared 1mM AgNO3 solution in a 1000 ml glass flask at room temperature by using a magnetic stirrer. The solution was observed for colour changes. The prepared particles are designated as Pfl AgNP.

2.5.1. Characterisation of the prepared silver nanoparticles

In order to characterize the formation of silver nanoparticles UV spectroscopic method was used to observe the characteristic λmax values, 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. Fig.1, Fig.2, Fig.3, Fig.4, Fig.5

Colour transformation to dark brown was observed after three hours after mixing the plant extract and 1mM AgNO3 solution**.** This colour change is caused by the reduction of silver ions to Ag NPs and the occurrence of vibrations on the plasma surface. For AgNPs, the maximum absorbance was found to be at 420 nm after analysing the samples via the UV-Vis device.

Fig-1
Colour transformation of the 1mM AgNO3 solution with the addition of *P. fruticosa* leaf saponin extract

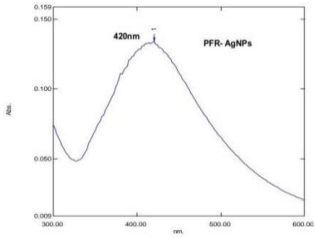


Fig-2
UV λ_{max} value of Pfl AgNP (420µm)

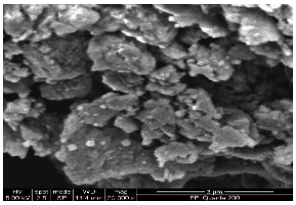


Fig-3.SEM of Pfl AgNP

Fig4: Pfl AgNP particle size data

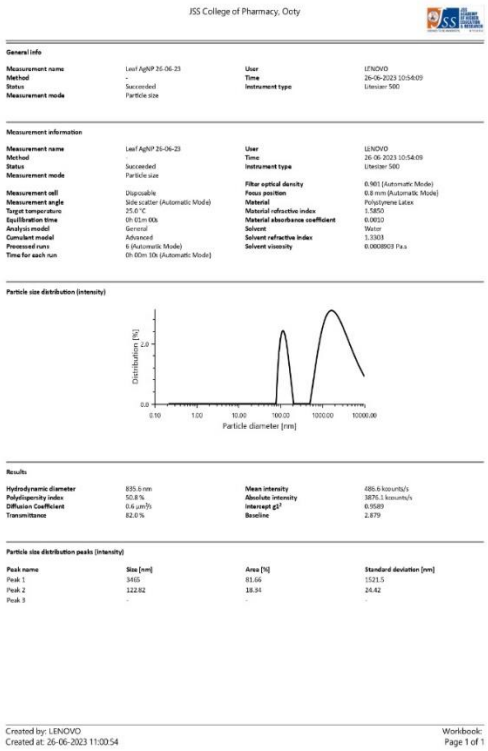
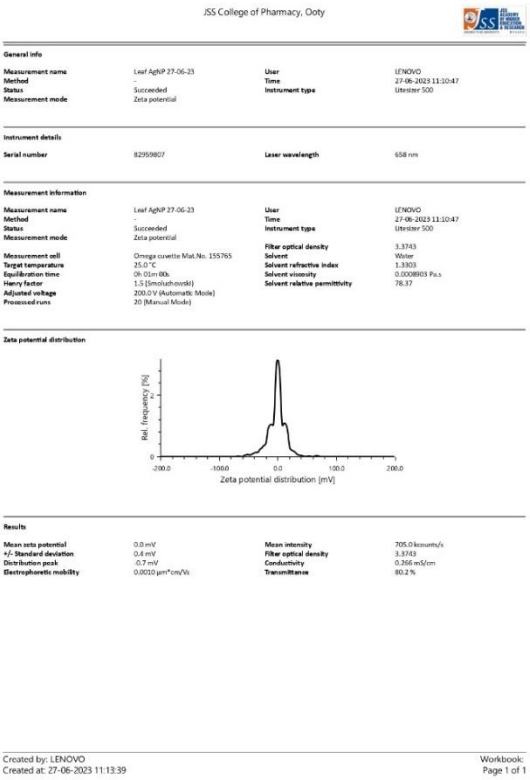


Fig 5: Pfl AgNP Zeta potential



2.6. ACUTE TOXICITY STUDIES

Brine Shrimp Assay Method [20,26]

Brine shrimp assay method was followed to find out the LC_{50} of the leaf saponin extract (Pfls) and PflAg NP (*P. fruticosa* leaf silver nanoparticles) respectively. 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[27]. The results are tabulated in Table.1, Fig.6

Table.1. Acute toxicity studies (Percentage deaths of *Brine shrimps* at 24 h)

Plant extracts	10 µg/ml	100 µg/ml	200 µg/ml	500 µg/ml	1000 µg/ml	LC_{50} µg/ml
Pfl AgNP	0	12	18	27	58	641
Pfls	2	5	20	18	34	>1000

Pfl AgNP: *P. fruticosa* leaf silver nanoparticles, Pfls: *P. fruticosa* leaf saponins

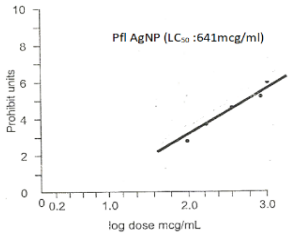


Fig-6. Dose- Response curve for Pfl AgNP

2.7. ANTI-PROLIFERATIVE ACTIVITY STUDIES [21,22,23,24,25]

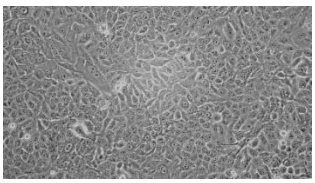
The level of cytotoxicity of the prepared Pfl AgNP to the lung cancer cells (A549) was evaluated by the MTT assay. The assay evaluated the cell ability to convert NADPH-dependent mitochondrial oxidoreductase enzymes to reduce the yellow-coloured tetrazolium dye MTT to its insoluble purple color formazan. The 2-(4, 4-dimethyl-2-thiazoyl)-2, 5-diphenyl-2, 4-tetrazolium salt (MTT) is converted into its formazan by viable metabolically active cells. The formazan formed is then solubilized with suitable solvent and the cell viability is measured in a microtitre plate reader. The absorbance was read at 650nm in a microtitre plate reader (Bio RAD, U.S.A.). The percentage viability of the cells in the treatment groups were calculated, compared to the control, using the formula. The results are tabulated in Table.2, Fig.7.

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

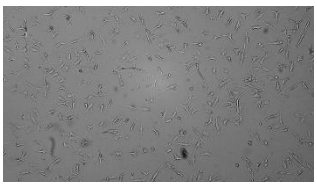
Table -2: Anti-cancer activity studies of Pfl AgNP on lung cancer cell line

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	65.34**
Pfl AgNP	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	54.25*
Pfls	100 µg	0.412	0.488	0.445	0.448	75.25	24.75
	125 µg	0.452	0.435	0.427	0.438	73.37	26.63

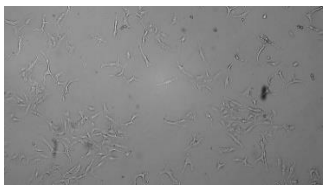
Pfl AgNP: *P. fruticosa* leaf silver nanoparticles, *Student t test:* ** → p value < 0.001, * → p value < 0.01
Pfls: *P. fruticosa* leaf saponins



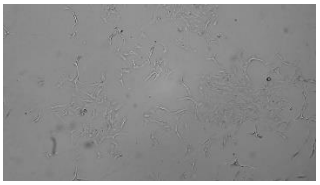
A549 Lung cancer cells (normal control)



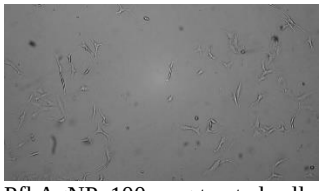
Pfl AgNP 25 mcg treated cells



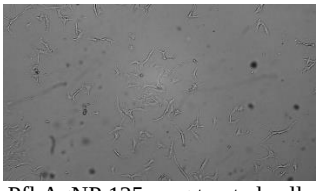
Pfl AgNP 50 mcg treated cells



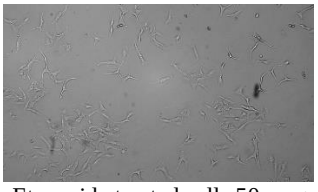
Pfl AgNP 75 mcg treated cells



Pfl AgNP 100 mcg treated cells



Pfl AgNP 125 mcg treated cells



Etoposide treated cells 50 mcg

Fig.7 Lung cancer cell lines treated with different concentrations of Pfl AgNP and standard drug Etoposide
Pfl AgNP: *Polyscias fruticosa leaf* silver nanoparticles

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 silver nanoparticles. The yield of the leaf n-butanol extract was found to be 16.5%. Pfl AgNP showed maximum absorbance at 420 nm in UV spectrophotometric analysis. The results for the particle size evaluation indicated that Pfl AgNP laid in the nanoparticle range (100-200nm) and exhibited characteristic zeta potential (-26.8 mV). The SEM data exhibited characteristic shape (oval or spherical) for Pfl AgNP.

The acute toxicity study data indicated that Pfl AgNP has an LC_{50} of 641mcg/ml in *Brine shrimp* assay method in comparison with the leaf saponin extract.

The anticancer activity studies indicated that the Pfl AgNP at 125 mcg concentration showed 54.25% cytotoxicity compared to the reference standard drug etoposide (50 mcg concentration, 65.34 % 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 AgNPs type of novel drug delivery systems and thereby useful in the chemotherapy of cancer along with conventional medicines.

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