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INVESTIGATIONS ON THE ANTI-NEOPLASTIC ABILITY OF *Polyscias fruticosa* (L) HARMS **ROOT CuNPs ON NEUROBLASTOMA CELL LINES**

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Abstract

The current study focuses on investigating the acute toxicity and anti-neoplastic properties of copper nanoparticles (Pfr CuNP) derived from the root saponin extract (Pfrs) of *Polyscias fruticosa*. Zeta potential, UV analysis, SEM examinations, and particle size measurement were used to assess the prepared copper nanoparticles. *P. fruticosa*, which grows in India is a member of the Araliaceae family like ginseng, has high concentration of triterpenoid saponins in its roots and leaves. The bioactivities of ginseng demonstrate many medicinal qualities of this family, which includes immunostimulant, antioxidant, and adaptogenic activities.

Based on the SEM data and the typical zeta potential values, the generated copper nanoparticles were found to lie within the nanoparticle range of 100-200 nm. UV λmax was observed at 570 nm for the prepared PfrCuNP. In the Brine Shrimp Assay Method, the LC50 values for PfrCuNP and Pfrs were analysed as 524 mcg/ml and more than 1000 mcg/ml, respectively. Neuroblastoma cell lines were used to screen anti-neoplastic activity, by the MTT assay method. The anticancer activity experiments indicated that PfrCuNP at 125 mcg/ml concentration showed 55.45% cytotoxicity while the reference standard etoposide showed 68.34% cytotoxicity at 50 mcg/ml concentration.

Keywords: Pfrs, PfrCuNP, Brine shrimp assay, neuroblastoma cell lines, MTT assay

INTRODUCTION

Plant secondary metabolites that can function as reducing, stabilizing, and capping agents in the transformation of metal ions into nanoparticles with specific bioactivities include polysaccharides, proteins, flavonoids, saponins, terpenoids, tannins, alkaloids, ketones, aldehydes, lignans and amines[1].

The green chemistry method of synthesizing CuNPs from the triterpenoid saponins found in *P. fruticosa* root solvent extract has a number of benefits. These processes provide economic, environmental friendly, cost-effective and safer products with less waste. For a variety of biomedical uses, the potentially active phytoconstituents used in plant-mediated nanoparticle production are biocompatible[16, 17].

Numerous investigations on the phytopharmacology of Polyscias root saponin extracts showed that the plant has potent cytotoxic, immunostimulant, anti-diabetic, adaptogenic, and free radical scavenging properties[6,7,8,9,10,11]. The current work aims to extract *P. fruticosa* root saponins[4,5] and employs green synthesis to create copper nanoparticles[12]. The brine shrimp assay method is used to conduct the acute toxicity investigation. The MTT assay is used to test the anti-neoplastic potential of the produced Pfr CuNPs at various doses on neuroblastoma cell lines. Additionally, the work focuses on the shape and morphological studies by Scanning Electron Microscopy, wavelength maximum (λmax) values by UV spectrophotometer, and particle size measurements and zeta potential of the nanoparticles by using a particle size analyser for prepared Pfr CuNPs.

2. EXPERIMENTAL

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

The Botanical Survey of India (BSI/SRC/5/23/2023//Tech/976) verified the authenticity of *P. fruticosa* leaves that were gathered from Coimbatore. Voucher specimens were subsequently placed in the Herbarium of the Pharmacognosy Laboratory at PPG College of Pharmacy, Saravanampatti. (Herbarium specimen number PPG 59/2023).

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

500g of *P. fruticosa* roots were collected, thoroughly cleaned to eliminate superfluous impurities, roughly crushed, dried and extracted with methanol. Diethyl ether was employed to remove fatty impurities, and the residue was then suspended in water and re-extracted using n-butanol. The vacuum-dried n-butanol extract was subjected to a number of chemical analyses in order to confirm the presence of saponins (Pfrs). The yield obtained was 21.5%.

2.3. Chemical Tests for Triterpenoids saponins

The preliminary phytochemical analysis[18,19] of Pfrs by Salkowski Test and Lieberman Storch Morasky test showed positive response for the presence of triterpenoid saponins

2.4. Quantitative Physical Analysis for Saponin Extract [4,5]

The root extract (Pfrs) produces 1.8 cm persistent foam which complied with the accepted standards in foam test[4]. Hemolysis Test: Haemolytic index refers to the highest dilution of saponins that results in total haemolysis and maximum haemolysis was observed at a concentration of 750 mg/ml of the saponin extract Pfrs.

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

Preparation of Copper Nitrate Solution: Cu $(NO_3)_2$ (1 mM) was made by weighing 0.241 grams of copper nitrate, and dissolving it in 1000 millilitres of distilled water. 100 ml of root extract (Pfrs) is mixed with 900 ml of the prepared 1mM Cu $(NO_3)_2$ solution in a 1000 ml flask at room temperature and well mixed by using a magnetic stirrer. The solution was observed for colour changes. After the completion of the preparation the formed nano particles are separated by centrifugation and dried in an oven at low temperature and stored in a desicator. The prepared particles were designated as PfrCuNP.

2.5.1. Characterisation of the prepared copper nanoparticles

In order to characterize the formation of copper nanoparticles, UV spectroscopic method was used to observe the characteristic λmax values, SEM device 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 brownish yellow was observed after three hours of mixing the plant extract with 1mM Cu (NO3)2 solution**.** This colour change is caused by the reduction of copper ions to Cu NPs and the occurrence of vibrations on the plasma surface. For CuNPs, the maximum absorbance was found to be at 570 nm with the UV-Vis spectrophotometer.

Fig-1

Colour transformation of the 1mM Cu $(NO3)_2$ solution with the addition of *P. fruticosa* root saponin extract

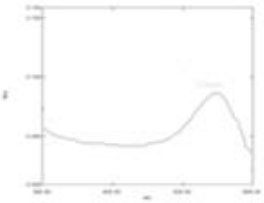

Fig-2

UV λ_{max} value of Pfr CuNP (570µm)

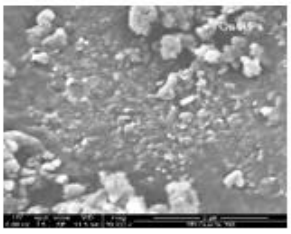

Fig-3

SEM of Pfr CuNP

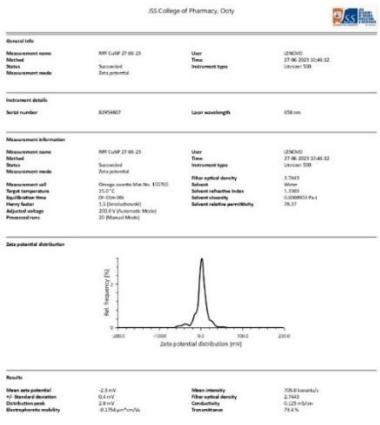

Fig4: Pfr CuNP particle size data

Fig 5: Pfr CuNP Zeta potential

2.6. ACUTE TOXICITY STUDIES

Brine Shrimp Assay Method [20]

The LC50 values of the Pfrs and Pfr CuNP were determined using the brine shrimp assay method. Ten brine shrimp eggs were transferred to vials using a disposable 9-inch pipette after the eggs were hatched in a rectangular container filled with artificial sea water. After 24 hours, the shrimps' survival rate for different saponin extract concentrations were noted. The LC50 was determined from the dose-response graph. The results are tabulated in Table 1 and 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
Pfr CuNP	0	12	18	27	58	524
Pfrs	2	5	20	18	34	>1000

Pfr CuNP: *P. fruticosa* leaf copper nanoparticles, Pfrs: *P. fruticosa* root saponins

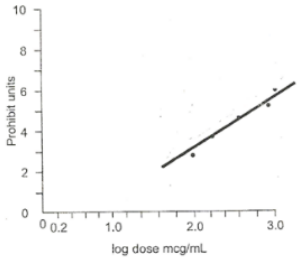

Fig.6: Pfr CuNP LC_{50}:524 mcg/ml

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

The level of cytotoxicity of the prepared Pfr CuNP to the neuroblastoma cells was evaluated by the MTT assay. The assay measured the cell's capacity to decrease the yellow-colored tetrazolium dye MTT to its insoluble purple colour formazan by means of NADPH-dependent mitochondrial oxidoreductase enzymes. Viable, metabolically active cells transform the 2-(4, 4-dimethyl-2-thiazoyl)-2, 5-diphenyl-2, 4-tetrazolium salt (MTT) into its formazan. After solubilizing the formazan with an appropriate solvent, the cell viability is assessed using a microtiter plate reader. The absorbance was measured using a Bio RAD microtitre plate reader (U.S.A.) at 650 nm. Using the formula, the percentage viability of the cells in the treatment groups was determined in relation to the control group. The results are tabulated in Table.2 and Fig.7.

$$\% \text{ Viability} = \frac{\text{Control OD} - \text{Sample OD} \times 100}{\text{Control OD}}$$

Table -2: Anti-cancer activity studies of Pfr CuNP on neuroblastoma 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	68.34**
Pfr CuNP	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	55.45*
Pfrs	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

Pfr CuNP: *P. fruticosa* root copper nanoparticles, Student t test: ** → p value < 0.001, * → p value < 0.01, Pfrs: *P. fruticosa* root saponins

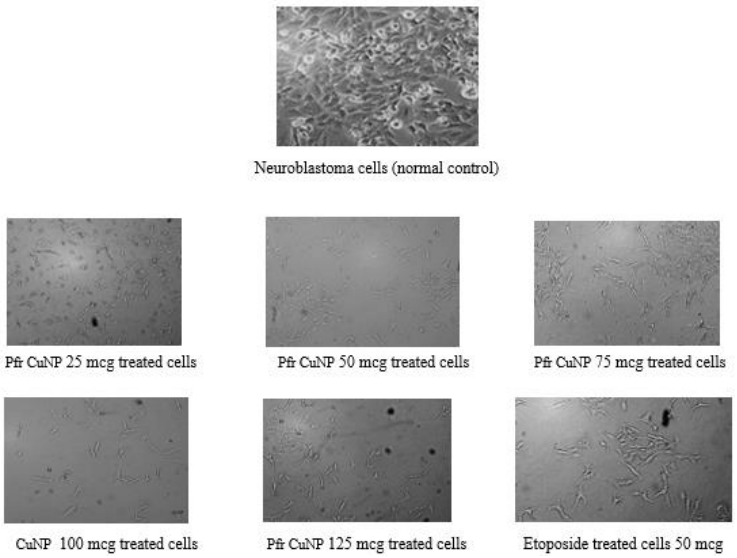

Fig.7. Neuroblastoma cell lines treated with different concentrations of Pfr CuNP with standard drug Etoposide
PfrCuNP: *Polyscias fruticosa* root copper nanoparticles, Standard drug: Etoposide, Normal control: neuroblastoma cells

3. RESULTS AND DISCUSSION

Preliminary phytochemical investigations proved the presence of triterpenoid saponins in *P. fruticosa* root extract. The medicinally active substances used in the process of creating plant copper nanoparticles are oleanolic acid derived triterpenoids. It was found that the leaf n-butanol extract produced an yield of 21.5 percentage. PfrCuNP demonstrated highest absorption in UV spectrophotometric measurement at 570 nm and showed a typical zeta potential (-26.8 mV). The prepared PfrCuNP was found to be in the nanoparticle range (100-200 nm) according to the results of the particle size evaluation.

The SEM findings showed that Pfr CuNP had an oval or spherical shape. The acute toxicity study data indicated that Pfr CuNP has an LC_{50} of 524 mcg/ml in Brine shrimp assay method in comparison with the root saponin extract Pfrs.

The reference standard drug etoposide at 50 mcg concentration, produced 68.34% cytotoxicity, while Pfr CuNP at 125 mcg concentration demonstrated 55.45% cytotoxicity, in the antineoplastic activity screening studies using neuroblastoma cell lines. These results throw light towards the potential of saponin compounds found in *P. fruticosa* roots, to design innovative drug delivery systems, such as CuNPs, which can be used in cancer chemotherapy with fewer adverse effects when combined with conventional medications.

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