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PHYLLANTHUS AMARUS EXTRACT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND ITS CYTOTOXIC EFFECT ON HEP-2 CELL LINE

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ABSTRACT

Nanotechnology because of its wide applicability emerges out as an important technology of mankind. Metal nanoparticles have long been synthesized by wet chemical techniques which may be toxic. The recent technology of using plant extract is a green synthesis, and a single step environmentally friendly process for production of nanoparticles. The biomolecules present in the aqueous extract of the whole plant *Phyllanthus amarus* (Euphorbiaceae) is responsible for the reduction of silver nitrate to silver nanoparticles and also act as capping agent. Silver nitrate (10-3 M) was used with aqueous extract to produce silver nanoparticles. It has very good activity than its macro counterparts and reported to contain antibacterial effect. The surface Plasmon resonance (SPR) caused color change measured using UV-Vis spectrophotometer. The size of the silver nanoparticles were found to be spherical in range of 40 - 100 nm found from Scanning Electron Microscope. Fourier Transform Infrared spectroscopy (FTIR) was carried out to determine the presence of biomolecules in them. Its cytotoxic effect been studied on HEp-2 (Larynx) cancerous cell line and normal VERO cell line , MTT assay was done to test its optimal concentration and efficacy which gives valuable information for the use of silver nanoparticles for future cancer therapy.

Keywords: Nanotechnology, Silver Nanoparticles, Green Synthesis, Phyllanthus amarus, HEp-2 Cell Line

11 INTRODUCTION

Nanotechnology stands as an emerging interdisciplinary field which has gained substantial notice because researchers use it for applications across physics, chemistry, materials science, electrical engineering and nowadays it shows increasing importance in life sciences especially biomedical and biotechnological areas. New treatment methods for drugs and diagnostics alongside antimicrobial agents and cancer treatments can be developed using nanoscale material alteration (1-100 nm) [1]. The main benefit of nanotechnology stems from its ability to modify materials within the nanoscale (1-100 nm) range which grants them special physiological features along with electrical features and biological properties unlike bulk counterparts [2]. Effects of nanotechnology on biomedical sciences include enhanced reactivity along with greater surface-to-volume ratio and stronger mechanical properties and distinctive optical features [3].

Elaborate research about Silver nanoparticles (AgNPs) is underway because they show strong action against multiple bacteria and fungal pathogens and cancer cells. Numerous research studies examine AgNPs because these nanomaterials disrupt cell membranes thus causing threshold stress and blocking cellular signaling pathways to efficiently fight multidrug-resistant pathogens [4,5]. Silver nanoparticles show great potential in treating cancer since studies show they specifically destroy cancer cells without harming normal cells [6]. The production methods for AgNPs require physical and chemical techniques which use considerable amounts of energy alongside hazardous reducing agents that create safety issues for both the environment and biological compatibility [7].

Plant-mediated green synthesis of silver nanoparticles represents an environmentally sustainable and friendly method to overcome these production challenges. Plant-based bioactive compounds function as reducing stabilizing agents in this synthesis method so scientists do not need to use harmful chemicals nor expensive inputs of energy [8]. Medicinal plant-derived synthesis of nanoparticles benefits from secondary metabolites including flavonoids and terpenoids and polyphenols and alkaloids because these compounds assist in nanoparticle formation and improve their biological activity [9,10].

Multiple researchers have examined various medicinal plants regarding their ability to generate silver nanoparticles. Scientists have used Piper longum [11]. leaves as a source material to successfully create AgNPs that displayed strong cytotoxic action against HEp-2 (laryngeal cancer) cells. Cancer cell killing was proven to be an exceptional feature of AgNPs produced from Piper nigrum [12].leaf extract while demonstrating the therapeutic potential of plant-developed nanoparticles. Various plants like Azadirachta indica, additionally to Ocimum sanctum and Phyllanthus niruri along

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with Withania somnifera have successfully produced eco-friendly AgNPs with observable antimicrobial and anticancer effects [13].

Cancer remains globally significant as a health concern because medical experts need advanced therapeutic options which perform more effectively while generating minimum side effects. The medical world has explored silver nanoparticles as potential cancer treatment agents because they can induce apoptosis alongside cell growth suppression and reactive oxygen species (ROS) production in tumor cells [6,4]. Laboratory experiments demonstrate that green-synthesized AgNPs possess better biocompatibility with selective toxicity which confirms their potential as suitable agents to target cancer [10].

This investigation studies the AgNP synthesis through Phyllanthus amarus plants which possess strong anticancer and antibacterial and liver-protective effects [14]. The manufacturing process yielded nanoparticles that received HEp-2 (laryngeal cancer) cell line cytotoxicity measurements together with minimal toxic effects on normal VERO cell lines. This research examined the synthetized nanomaterials through a comparative method to determine their specific toxicity against target cells. Through the expected mechanism the nanoparticles would selectively target cancer cells for destruction yet remain non-harmful against healthy cells.

The method demonstrates high cost-effectiveness and preserves nanoparticle stability thus making it an excellent option for biomedical applications including cancer medicine. This research investigation extends the existing scientific knowledge about green nanotechnology along with its applications for performing safe and effective cancer treatments.

2. METHODOLOGY

2.1 Collection of plant material :

Researchers obtained the plant sample *Phyllanthus amarus* from the Arakkonam district farming areas before cleansing it with tap water. The Botanist at Central Research Institute of Siddha in Chennai identified the gathered material before storing its specimen in a herbarium. Multiple water washing cleansed the plant materials completely. The extract producers utilized the entire plant material.

2.2 .Procedure :

The plant pieces underwent chopping before weighing 25 g of plant matter into which 100 ml of distilled water was added. Next the plant material received a measurement. The mixture received heat at 90°c while boiling for twenty minutes. The mixture rested in room temperature until it reached a cool state. Whatman no: 40 filter paper was used to filter the solution three times. A single bottle received the filtrate which was collected during the procedure. Forthcoming utilization will require keeping the extract in refrigerator.

2.3.Synthesis of nanoparticles :

A mixture of 50ml 1mM (10-3 M) silver nitrate solution combined with 50ml plant extract solution. The solution was placed under microwave heat and dark and sunlit conditions before exposure. After the mixture receives exposure time it needs 10-minute centrifugation at 10000 rpm. Hot air oven conditions were used to obtain the pellet. Supernatant was stored in refrigerator. The analysis used the obtained pellet.

2.4. Microwave exposure :

Placing an equal mixture of plant extract and silver nitrate solution under microwave heating reached 90°c. The developer checked the surface plasmon resonance color formation through microwaves therefore recording the time needed for color development during 30sec intervals. In the closed space of the near infra red spectrum only the intended reaction occurs without interference from external sources.

2.5 Incubation in dark :

The mixture received dark room incubation at room temperature during twenty-four hours. The solution was checked at different time points for the development of dark brownish color.

2.6 . Sunlight exposure :

A glass beaker received the mixture with 33°c temperature reading obtained from Chennai Metrological Centre. The mixture underwent constant stirring for homogenization during the time it spent under direct sunlight exposure. The experiment proceeded until the reaction mixture reached dark brownish color.

2.7. UV-vis spectra analysis :

The detection of pure Ag+ ions reduction occurred through UV–vis analysis of overnight-incubated reaction medium after diluting an aliquot of the solution with distilled water. The appearance of color transformations in silver nanoparticles (AgNPs) solution became visible through distilled water observation. The substance exhibited a yellowish-brown color during particle synthesis. The production quantity of AgNP was measured using Systemics UV

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double-beam spectrophotometer (model 2201) at 1 nm resolution throughout 200 to 600 nm wavelength range with 10-mm-optical-path-length quartz cuvettes.

2.8. FTIR (Fourier-transform IR):

The presence of silver nanoparticles was confirmed through analysis done by Nicolet Impact 400 FTIR spectroscopy on the acquired samples.

2.9. SEM Analysis of Silver Nanoparticles :

JEOL 6380A (Tokyo, Japan) served as the scanning electron microscope to obtain pictures of the formulated AgNPs. **2.10. Cytotoxicity and anticancer activity :**

2.10.1. MTT assay :

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) product was acquired from Invitrogen based in USA. The remaining collection of fine chemicals originated from Sigma, Aldrich.

2.10.2. Cell culture :

VENO cells that NCCS (National Centre for Cell Science from Pune) distributed grew in Rose-well Park Memorial Institute RPMI medium with 10% fetal bovine serum together with penicillin/streptomycin (250 U/mL) and gentamycin (100 μ g/mL) and amphotericin B (1 mg/mL) from Sigma Chemicals based in MO USA. The conditioned incubation required all cell cultures to operate at 37°C under controlled conditions of 5% CO2 with saturated humidity. Cells achieved complete confluency within a period of 24 h before researchers used them.

2.10.3. Cell growth inhibition studies by MTT assay :

The MTT reduction assay as described by Mosmann (1983) served to measure cell viability. The experiment began by seeding HT29 cells at 5×103 cells/well density in 200 µL RPMI 10% FBS solution that made up the culture media in 96-well plates. The researchers transferred the culture supernatant before adding Yenamarugu zinc nanoparticles solutions (3.906–500 µg/mL concentration) for a 48-hour incubation period as a result. The treated cells received MTT solution (10 µL, 5 mg/mL) at 37°C before receiving DMSO at room temperature. The spectrophotometer analyzed plate readings through the wavelength of 595 nm using its scanning multi-well setup.

Cell viability (%) = Mean OD / Control OD $\times 100$

3. RESULTS AND DISCUSSION

3.1 Synthesis of silver nanoparticles :

The synthesis of silver nanoparticles uses plant extract as an elemental raw material. The nanoparticles formed through observations of color changes from light green to dark brownish hues. The observed color change correlates with the surface Plasmon vibrations of previous works in this method.

Table 1. Time taken for appearance of dark brownish color

Exposure Conditions <i>P.amarus</i> mediated AgNPSYield /100mlMicrowave70 seconds210mgDarkIncubated - 24 hrs150mgSunlight8 minutes20 mgMicrowave radiation causes the	<i>P.amarus</i> mediated AgNPSYield /100mlMicrowave70 seconds210mgDarkIncubated - 24 hrs150mgSunlight8 minutes20 mgMicrowave radiation causes the color modification while silver	Yield /100mlMicrowave70 seconds210mgDarkIncubated - 24 hrs150mgSunlight8 minutes20 mgMicrowave radiation causes the color modification while silver
nitrate reduction occurs under microwave-supported reactions where plant extracts serve to assist in this process. The microwave method produced 210mg of yield as the maximal output after operating for 70sec in <i>P.amarus</i> experiments (Table.1). The dark incubation resulted in observable yield after 24 hours of incubation yet the yield level under sunlight remained superior.	microwave-supported reactions where plant extracts serve to assist in this process. The microwave method produced 210mg of yield as the maximal output after operating for 70sec in <i>P.amarus</i> experiments (Table.1). The dark incubation resulted in observable yield after 24 hours of incubation yet the yield level under sunlight remained superior.	microwave-supported reactions where plant extracts serve to assist in this process. The microwave method produced 210mg of yield as the maximal output after operating for 70sec in <i>P.amarus</i> experiments (Table.1). The dark incubation resulted in observable yield after 24 hours of incubation yet the yield level under sunlight remained superior.
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3.2 UV-Vis spectroscopy analysis

UV-Vis spectroscopy served to verify the formation of silver nanoparticles. The λ max value appeared at 400 nm after scanning the samples between 200 -800 nm. The characteristic feature of silver determined that maximum absorbance occurred at 400nm and thus provided evidence for silver nanoparticles. The exposure conditions using microwave generated the highest absorbance during analysis.



Figure 1: UV-Vis spectra P.amarus extract mediated AgNPs

3.3 Scanning Electron Microscopy Analysis (SEM)

High Resolution Field Emission Electron Microscope analysis provided the information regarding nanoparticle dimensions and shapes. The analysis of solutions that were subjected to microwave exposure under dark conditions resulted in the determination of nanoparticle size and shape. UV-Vis absorption spectra analysis showed its connection to these results. SEM analysis yielded the calculations for size measurements. P.amarus mediated AgNPs exposed to microwave radiation yielded spherical nanoparticles between 35 - 70 nm according to Henglein A (1993) [15], measurements with mean diameter at 43 nm (Fig 2). The spherical nanoparticles (Fig 3) obtained in the dark environment displayed diameters ranging from 35 - 140 nm with a mean value of 75 nm.



Figure 2 :SEM image of AgNPs synthesized by P.amarus extract - microwave exposure



Figure 3: SEM image of AgNPs synthesized by P.amarus extract – dark incubation

3.4 Fourier Transform Infrared Spectroscopy analysis (FTIR)

FTIR analysis provides excellent biomolecule detection capabilities because its results can reference standard IR fingerprint references. The stretching vibrations of O-H between 3100 - 3400 cm-1 in the broad spectrum stem from the hydroxyl group of phenol, alcohols (Fig 12). The intense band at 1640 cm-1 due to C=C stretching of aromatic ring plays a primary role for metal ion reduction in terpenoids (Fig 4 and 5). The presence of C=O ketones found in flavonoids could also be the reason behind these findings. Ultraviolet and visible absorbance spectra of silver nitrate solutions confirmed the presence of NO2 stretching quintessences. The C-N stretching vibration of aliphatic amides generates the bands between 1020 - 1220 cm-1. AgNPs surface interactions lead to many medium to weak bands

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between 1200-1300 cm-1 because COOH carboxylate group binds to the surface of the nanoparticles. The OH bending vibration from alcohol or phenol functional groups or from COOH provided both C-O stretching and OH bending signals. Stable silver nanoparticles depend on the protein molecule function as reducing agent and ligand that transforms silver nitrate into AgNPs. A significant proportion of reducing agents normally exists within plant extracts.



Figure 5: FTIR spectra of P.amarus mediated AgNPs (microwave exposed)

3.5 Cytotoxic assay (MTT)

A cytotoxicity study of plant extract nanoparticles focused on their effect against HEp-2 cell line and VERO cell line using MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) [16]. The cell lines received drugs of different concentrations during 37°c incubation which lasted 24 hrs in 5% CO2 conditions. The research evaluated drug toxicity using both HEp-2 Larynx cancerous cell line for LD 50 determination and VERO cell line for testing normal cell compatibility.

 Table 2. Invitro cytotoxicity effect of P.amarus mediated AgNPs (microwave exposed) and silver nitrate on HEp-2 cancerous cell line

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viability%SN				210.3533.334		
				9.2931251:20.		
				230.3936.505		
				4.92462.51:40		
				.250.4546.036		
				3.38531.251:8		
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Table 3: Invitro cytotoxicity effect of P.amarus mediatedAgNPs (microwave exposed) and silver nitrate on VERO

cell line

S.no	Concentration	Dilutions	Absorbance at 595		PM	SN
	(µg/ml)DilutionsAbsorb		nmPM cell		cell	cell
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		:10.250.27	.2738.4636	8.4636.493		
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2250	2501:10.250.2738.4636.4	1:10.250.2	0.250.2738	0.2738.463	38.4636.493125	36.4931251:20.3
	931251:20.340.3352.304	738.4636.	.4636.4931	6.4931251:		40.3352.3044.59
	4.59462.51:40.470.4572.	4931251:2	251:20.340	20.340.335		
	3060.81531.251:80.490.5		.3352.3044	2.3044.594		
	075.3867.57615.6251:16		.59462.51:			
			40.470.457			
			2.3060.815			
3125	1251:20.340.3352.3044.5	1:20.340.3	0.340.3352	0.3352.304	52.3044.59462.5	44.59462.51:40.
	9462.51:40.470.4572.306	352.3044.	.3044.5946	4.59462.51		470.4572.3060.8
	0.81531.251:80.490.5075	59462.51:	2.51:40.47	:40.470.45		1531.251:80.490
	.3867.57615.6251:160.52	40.470.45				.5075.3867.5761
						5.6251:160.520.
						528070.2777.81
						251:320.550.568
						4.6175.6883.906
462.	62.51:40.470.4572.3060.	1:40.470.4	0.470.4572	0.4572.306	72.3060.81531.2	60.81531.251:80
51:4	81531.251:80.490.5075.3	572.3060.	.3060.8153	0.81531.25	51:80.490.5075.	.490.5075.3867.
	867.57615.6251:160.520.	81531.251	1.251:80.4		3867.57615.625	57615.6251:160.
	528070.2777.81251:320.	:80.490.50	90.5075.38			520.528070.277

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	550.5684.6175.6883.9061					
	:640.590.6090.7681.089C					
	ell control-					
	0.650.74100100Testing					
	of cytotoxic effects for					
	silver nanoparticles					
1	produced from <i>P amarus</i>					
	extract used the MTT-					
	assay method on HEp-2					
	cell lines (Fig.6) A					
	substantial number of					
	cells died during					
	<i>P</i> amarus treatment when					
	exposed to 500 µg/ml					
	solution which resulted in					
	a 70% mortality rate (Fig					
	6). The lethal dose for					
	50% cells reached 62.5					
	ug/ml. Test results					
i	indicated that silver					
1	nitrate (AgNO3) brought					
	about 65% cell mortality					
,	when used at 500 µg/ml					
	concentration (Fig 7)					
,	while reaching its LD 50					
,	value at 250 µg/ml					
	concentration. The					
	P.amarus AgNPs treated					
	VERO cells exhibited					
1	maximum cell mortalities					
1	reaching 68% at 500					
	µg/ml (Fig.8) and reached					
1	the LD 50 of 48% at 125					
	µg/ml. The experimental					
	condition of 500µg/ml					
:	silver nitrate produced					
:	83% cell death while the					
]	LD 50 occurred at					
	62.5μg/ml. The					
1	gliomacells displayed this					
1	killing effect because of					
]	ROS generation within					
1	their structure. The three					
]	ROS radicals including					
:	superoxide radical and					
1	hydrogen peroxide and					
1	hydroxyl radical function					
1	to create cellular damage					
1	that ends in cell death					
	[17]. The killing effect of					
:	silver nanoparticles					
	against HEp-2 cells					
:	supports their potential					
1	use as cancer treatment					

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edite	or@ijprems.com	Vol. 05, 1	ssue 04, Apri	1 2025, pp : 4	-05-431	7.001
531. 251: 80.4 90.5 075. 3867 .576	medicines. Cancer treatment effectiveness can emerge from using lower concentration AgNPs since these nanoparticles possess harmful effects towards regular VERO cells. Studies should continue to clarify how you can use these nanoparticles in cancer treatment for humans and the biological factors leading to their effects. 31.251:80.490.5075.3867 .57615.6251:160.520.528 070.2777.81251:320.550. 5684.6175.6883.9061:64	1:80.490.5 075.3867. 57615.625	0.490.5075 .3867.5761 5.6251:160 .520.52807 0.2777.812 51:320.550 .5684.6175 .6883.9061 :640.590.6 090.7681.0 89Cell control- 0.650.7410 0100Testin g of cytotoxic effects for silver nanoparticl es produced from <i>P.amarus</i> extract used the MTT-assay method on HEp-2 cell lines (Fig.6). A substantial number of cells died during <i>P.amarus</i> treatment when exposed to 500 ug/ml	0.5075.386 7.57615.62 51:160.520 .528070.27	75.3867.57615.6 251:160.520.528 070.2777.81251: 320.550.5684.61	67.57615.6251:1 60.520.528070.2 777.81251:320.5 50.5684.6175.68
			500 μg/ml			



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	solution		
	which		
	resulted in		
	a 70%		
	mortality		
	rate (Fig 6).		
	The lethal		
	dose for		
	50% cells		
	reached		
	62.5 µg/ml		
	Test results		
	indicated		
	that silver		
	nitroto		
	$(\Lambda \alpha NO2)$		
	(AgNOS)		
	orougnt		
	about 65%		
	cell		
	mortality		
	when used		
	at 500		
	µg/ml		
	concentrati		
	on (Fig 7)		
	while		
	reaching its		
	LD 50		
	value at		
	250 µg/ml		
	concentrati		
	on. The		
	P.amarus		
	AgNPs		
	treated		
	VERO		
	cells		
	exhibited		
	maximum		
	cell		
	mortalities		
	reaching		
	68% at 500		
	µg/ml		
	(Fig.8) and		
	reached the		
	LD 50 of		
	48% at 125		
	µg/ml. The		
	experiment		
	al		
	condition		
	of		
	500µg/ml		



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	silver	
	nitrate	
	produced	
	83% cell	
	death while	
	the LD 50	
	occurred at	
	62.5µg/ml.	
	The	
	gliomacells	
	displayed	
	this killing	
	effect	
	because of	
	ROS	
	generation	
	within their	
	structure.	
	The three	
	ROS	
	radicals	
	including	
	superoxide	
	radical and	
	hydrogen	
	peroxide	
	radical	
	function to	
	cellular	
	damage	
	that ends in	
	cell death	
	[17]. The	
	killing	
	effect of	
	silver	
	nanoparticl	
	es against	
	HEp-2	
	cells	
	supports	
	their	
	potential	
	use as	
	cancer	
	treatment	
	medicines.	
	Cancer	
	treatment	
	effectivene	
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www.ijprems.com INTERNATIONAL JOURNAL OF PROGRESSIVE AND SCIENCE (IJPREMS) (Int Peer Reviewed Journal)					e-ISSN : 2583-1062 Impact Factor :	
edite	editor@ijprems.com Vol. 05, Issue 04, April 2025, pp : 405-431				05-431	7.001
615	15 6251:160 520 528070	1:160.520	emerge from using lower concentrati on AgNPs since these nanoparticl es possess harmful effects towards regular VERO cells. Studies should continue to clarify how you can use these nanoparticl es in cancer treatment for humans and the biological factors leading to their effects.	0.528070.2	8070 2777 8125	70 2777 81251-3
615. 6251 :160. 520. 5280	15.6251:160.520.528070. 2777.81251:320.550.568 4.6175.6883.9061:640.59	1:160.520. 528070.27	0.520.5280	0.528070.2 777.81251: 320.550.56	8070.2777.8125	70.2777.81251:3 20.550.5684.617 5.6883.9061:640 .590.6090.7681. 089Cell control- 0.650.74100100
77.8 1251 :320. 550. 5684 .617 5.68	7.81251:320.550.5684.61	1:320.550. 5684.6175 .6883.906	0.550.5684 .6175.6883 .9061:640. 590.6090.7 681.089Cel 1 control- 0.650.7410 0100Testin g of cytotoxic effects for silver nanoparticl es produced from <i>P.amarus</i> extract	0.5684.617 5.6883.906	84.6175.6883.90 61:640.590.6090 .7681.089Cell control- 0.650.74100100	75.6883.9061:64

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	used the	
	MTT-assav	
	method on	
	HEp-2 cell	
	lines	
	(Fig.6). A	
	substantial	
	number of	
	cells died	
	during	
	P.amarus	
	treatment	
	when	
	exposed to	
	500 µg/ml	
	solution	
	which	
	resulted in	
	a 70%	
	mortality	
	rate (Fig 6).	
	The lethal	
	dose for	
	50% cells	
	reached	
	62.5 μg/ml.	
	Test results	
	indicated	
	that silver	
	nitrate	
	(AgNO3)	
	brought	
	about 65%	
	cell mortality	
	when used	
	at 500	
	μg/m concentrati	
	on (Fig. 7)	
	while	
	reaching its	
	LD 50	
	value at	
	250 µg/ml	
	concentrati	
	on. The	
	P.amarus	
	AgNPs	
	treated	
	VERO	
	cells	
	exhibited	
	maximum	



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	cell	
	mortalities	
	reaching	
	68% at 500	
	ug/ml	
	(Fig.8) and	
	reached the	
	LD 50 of	
	48% at 125	
	μg/ml. The	
	experiment	
	al	
	condition	
	of	
	500µg/ml	
	silver	
	nitrate	
	produced	
	83% cell	
	death while	
	the LD 50	
	occurred at	
	62.5µg/ml.	
	The	
	gliomacells	
	displayed	
	this killing	
	effect	
	because of	
	ROS	
	generation	
	within their	
	structure.	
	The three	
	ROS	
	radicals	
	superoxide	
	hydrogen	
	nerovide	
	and	
	hydroxyl	
	radical	
	function to	
	create	
	cellular	
	damage	
	that ends in	
	cell death	
	[17]. The	
	killing	
	effect of	
	silver	

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			nanoparticl			
			es against			
			HEp-2			
			cells			
			supports			
			their			
			potential			
			use as			
			cancer			
			treatment			
			medicines.			
			Cancer			
			treatment			
			effectivene			
			ss can			
			emerge			
			from using			
			lower			
			concentrati			
			on AgNPs			
			since these			
			nanoparticl			
			es possess			
			harmful			
			effects			
			towards			
			regular			
			VERO			
			cells.			
			Studies			
			should			
			continue to			
			clarify how			
			you can use			
			these			
			nanoparticl			
			es in cancer			
			treatment			
			for humans			
			and the			
			biological			
			factors			
			leading to			
			their			
			effects.			
83.9	3.9061:640.590.6090.768	1:640.590.	0.590.6090	0.6090.768	90.7681.089Cell	81.089Cell
061:	1.089Cell control-	6090.7681	.7681.089C	1.089Cell	control-	control-
640.	0.650.74100100Testing	.089Cell	ell control-	control-	0.650.74100100	0.650.74100100
590.	of cytotoxic effects for	control-	0.650.7410	0.650.7410		
6090	silver nanoparticles	0.650.741	0100Testin	0100Testin		
.768	produced from P.amarus	00100Test	g of	g of		
1.08	extract used the MTT-	ing of	cytotoxic	cytotoxic		
	assay method on HEp-2	cytotoxic	effects for	effects for		
	cell lines (Fig.6). A	effects for	silver	silver		



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substantial number of	silver	nanoparticl	nanoparticl		
cells died during	nanopartic	es	es		
<i>P.amarus</i> treatment when	les	produced	produced		
exposed to 500 µg/ml	produced	from	from		
solution which resulted in	from	P.amarus	P.amarus		
a 70% mortality rate (Fig	P.amarus	extract	extract		
6). The lethal dose for	extract	used the	used the		
50% cells reached 62.5	used the	MTT-assay	MTT-assay		
µg/ml. Test results	MTT-	method on	method on		
indicated that silver	assay	HEp-2 cell	HEp-2 cell		
nitrate (AgNO3) brought	method on	lines	lines		
about 65% cell mortality	HEp-2	(Fig.6). A	(Fig.6). A		
when used at 500 µg/ml	cell lines	substantial	substantial		
concentration (Fig 7)	(Fig.6). A	number of	number of		
while reaching its LD 50	substantial	cells died	cells died		
value at 250 µg/ml	number of	during	during		
concentration. The	cells died	P.amarus	P.amarus		
P.amarus AgNPs treated	during	treatment	treatment		
VERO cells exhibited	P.amarus	when	when		
maximum cell mortalities	treatment	exposed to	exposed to		
reaching 68% at 500	when	500 ug/ml	500 µg/ml		
μ g/ml (Fig.8) and reached	exposed	solution	solution		
the LD 50 of 48% at 125	to 500	which	which		
ug/ml. The experimental	ug/ml	resulted in	resulted in		
condition of 500ug/ml	solution	a 70%	a 70%		
silver nitrate produced	which	mortality	mortality		
83% cell death while the	resulted in	rate (Fig 6).	rate (Fig 6).		
LD 50 occurred at	a 70%	The lethal	The lethal		
62.5µg/ml. The	mortality	dose for	dose for		
gliomacells displayed this	rate (Fig	50% cells	50% cells		
killing effect because of	6). The	reached	reached		
ROS generation within	lethal	62.5 µg/ml.	62.5 ug/ml.		
their structure. The three	dose for	Test results	Test results		
ROS radicals including	50% cells	indicated	indicated		
superoxide radical and	reached	that silver	that silver		
hydrogen peroxide and	62.5	nitrate	nitrate		
hydroxyl radical function	μg/ml.	(AgNO3)	(AgNO3)		
to create cellular damage	Test	brought	brought		
that ends in cell death	results	about 65%	about 65%		
[17]. The killing effect of	indicated	cell	cell		
silver nanoparticles	that silver	mortality	mortality		
against HEp-2 cells	nitrate	when used	when used		
supports their potential	(AgNO3)	at 500	at 500		
use as cancer treatment	brought	µg/ml	µg/ml		
medicines. Cancer	about	concentrati	concentrati		
treatment effectiveness	65% cell	on (Fig 7)	on (Fig 7)		
can emerge from using	mortality	while	while		
lower concentration	when used	reaching its	reaching its		
AgNPs since these	at 500	LD 50	LD 50		
nanoparticles possess	µg/ml	value at	value at		
harmful effects towards	concentrat	250 µg/ml	250 µg/ml		
regular VERO cells.	ion (Fig 7)	concentrati	concentrati		
Studies should continue	while	on. The	on. The		
to clarify how you can	reaching	P.amarus	P.amarus		



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use these nanoparticles in	its LD 50	AgNPs	AgNPs		
cancer treatment for	value at	treated	treated		
humans and the	250 ug/ml	VERO	VERO		
biological factors leading	concentrat	cells	cells		
to their effects.	ion. The	exhibited	exhibited		
	P.amarus	maximum	maximum		
	AgNPs	cell	cell		
	treated	mortalities	mortalities		
	VERO	reaching	reaching		
	cells	68% at 500	68% at 500		
	exhibited	µg∕ml	µg/ml		
	maximum	(Fig.8) and	(Fig.8) and		
	cell	reached the	reached the		
	mortalities	LD 50 of	LD 50 of		
	reaching	48% at 125	48% at 125		
	68% at	$\mu g/ml$. The	$\mu g/ml$. The		
	500 µg/ml	experiment	experiment		
	(Fig.8)	al	al		
	and	condition	condition		
	reached	of	of		
	the LD 50	500µg/ml	500µg/ml		
	of 48% at	silver	silver		
	125 µg/ml.	nitrate	nitrate		
	The	produced	produced		
	experimen	83% cell	83% cell		
	tal	death while	death while		
	condition	the LD 50	the LD 50		
	of	occurred at	occurred at		
	500µg/ml	62.5µg/ml.	62.5µg/ml.		
	silver	The	The		
	nitrate	gliomacells	gliomacells		
	produced	displayed	displayed		
	83% cell	this killing	this killing		
	death	effect	effect		
	while the	because of	because of		
	LD 30	nus	rus		
	at	within their	generation within their		
	62.5 u g/m	structure	structure		
	The	The three	The three		
	gliomacell	ROS	ROS		
	s	radicals	radicals		
	displayed	including	including		
	this	superoxide	superoxide		
	killing	radical and	radical and		
	effect	hydrogen	hydrogen		
	because of	peroxide	peroxide		
	ROS	and	and		
	generation	hydroxyl	hydroxyl		
	within	radical	radical		
	their	function to	function to		
	structure.	create	create		
	The three	cellular	cellular		
	ROS	damage	damage		



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	radicals	that ends in	that ends in		
	including	cell death	cell death		
	superoxid	[17]. The	[17]. The		
	e radical	killing	killing		
	and	effect of	effect of		
	hydrogen	silver	silver		
	peroxide	nanoparticl	nanoparticl		
	and	es against	es against		
	hydroxyl	HEp-2	HEp-2		
	radical	cells	cells		
	function	supports	supports		
	to create	their	their		
	cellular	potential	potential		
	damage	use as	use as		
	that ends	cancer	cancer		
	in cell	treatment	treatment		
	death [17].	medicines.	medicines.		
	The	Cancer	Cancer		
	killing	treatment	treatment		
	effect of	effectivene	effectivene		
	silver	ss can	ss can		
	nanopartic	emerge	emerge		
	les against	from using	from using		
	HEp-2	lower	lower		
	cells	concentrati	concentrati		
	supports	on AgNPs	on AgNPs		
	their	since these	since these		
	potential	nanoparticl	nanoparticl		
	use as	es possess	es possess		
	cancer	harmful	harmful		
	treatment	effects	effects		
	Geneer	towards	towards		
	tractment	VEDO	VERO		
	offootivon				
		Studios	Studios		
	emerge	should	should		
	from	continue to	continue to		
	lising	clarify how	clarify how		
	lower	VOIL Can lise	voll can lise		
	concentrat	these	these		
	ion	nanoparticl	nanoparticl		
	AgNPs	es in cancer	es in cancer		
	since	treatment	treatment		
	these	for humans	for humans		
	nanopartic	and the	and the		
	les	biological	biological		
	possess	factors	factors		
	harmful	leading to	leading to		
	effects	their	their		
	towards	effects.	effects.		
	regular				
	VERO				
	cells.				

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		Studies				
		should				
		continue				
		to clarify				
		how you				
		can use				
		these				
		nanopartic				
		les in				
		cancer				
		treatment				
		for				
		humans				
		and the				
		biological				
		factors				
		leading to				
		their				
		effects.				
9Cel	Cell control-		0.650.7410	0.7410010	100100Testing	100Testing of
1	0 650 74100100Testing	0 650 741	0100Testin	0.7410010 OTesting of	of cytotoxic	cytotoxic effects
cont	of cytotoxic effects for	0.050.741 00100Test	g of	cytotoxic	effects for silver	for silver
rol-	silver nanonarticles	ing of	cvtotoxic	effects for	nanoparticles	nanoparticles
0.65	produced from <i>P</i> amarus	cytotoxic	effects for	silver	produced from	produced from
0.05	extract used the MTT-	effects for	silver	nanonarticl	P amarus	P amarus
	assay method on HEn-?	silver	nanonarticl	es	extract used the	extract used the
	cell lines (Fig.6) Δ	nanopartic	es	produced	MTT-assay	MTT-assay
	substantial number of	les	produced	from	method on HEn-	method on HEn-
	cells died during	produced	from	P amarus	2 cell lines	2 cell lines
	<i>P amarus</i> treatment when	from	P amarus	extract	(Fig 6) A	(Fig 6) A
	exposed to 500 µg/ml	P amarus	extract	used the	substantial	substantial
	solution which resulted in	extract	used the	MTT-assay	number of cells	number of cells
	a 70% mortality rate (Fig	used the	MTT-assav	method on	died during	died during
	6). The lethal dose for	MTT-	method on	HEp-2 cell	P.amarus	P.amarus
	50% cells reached 62.5	assav	HEp-2 cell	lines	treatment when	treatment when
	ug/ml. Test results	method on	lines	(Fig.6). A	exposed to 500	exposed to 500
	indicated that silver	HEp-2	(Fig.6). A	substantial	ug/ml solution	ug/ml solution
	nitrate (AgNO3) brought	cell lines	substantial	number of	which resulted	which resulted
	about 65% cell mortality	(Fig.6). A	number of	cells died	in a 70%	in a 70%
	when used at 500 µg/ml	substantial	cells died	during	mortality rate	mortality rate
	concentration (Fig 7)	number of	during	P.amarus	(Fig 6). The	(Fig 6). The
	while reaching its LD 50	cells died	P.amarus	treatment	lethal dose for	lethal dose for
	value at 250 µg/ml	during	treatment	when	50% cells	50% cells
	concentration. The	P.amarus	when	exposed to	reached 62.5	reached 62.5
	P.amarus AgNPs treated	treatment	exposed to	$500 \mu g/ml$	µg/ml. Test	ug/ml. Test
	VERO cells exhibited	when	500 µg/ml	solution	results indicated	results indicated
	maximum cell mortalities	exposed	solution	which	that silver nitrate	that silver nitrate
	reaching 68% at 500	to 500	which	resulted in	(AgNO3)	(AgNO3)
	µg/ml (Fig.8) and reached	µg/ml	resulted in	a 70%	brought about	brought about
	the LD 50 of 48% at 125	solution	a 70%	mortality	65% cell	65% cell
	µg/ml. The experimental	which	mortality	rate (Fig 6).	mortality when	mortality when
	condition of 500µg/ml	resulted in	rate (Fig 6).	The lethal	used at 500	used at 500
	silver nitrate produced	a 70%	The lethal	dose for	µg/ml	µg/ml
	83% cell death while the	mortality	dose for	50% cells	concentration	concentration



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	LD 50 occurred at	rate (Fig	50% cells	reached	(Fig 7) while	(Fig 7) while
	62.5µg/ml. The	6). The	reached	62.5 µg/ml.	reaching its LD	reaching its LD
	gliomacells displayed this	lethal	62.5 µg/ml.	Test results	50 value at 250	50 value at 250
	killing effect because of	dose for	Test results	indicated	µg/ml	µg/ml
	ROS generation within	50% cells	indicated	that silver	concentration.	concentration.
	their structure. The three	reached	that silver	nitrate	The <i>P.amarus</i>	The <i>P.amarus</i>
	ROS radicals including	62.5	nitrate	(AgNO3)	AgNPs treated	AgNPs treated
	superoxide radical and	µg∕ml.	(AgNO3)	brought	VERO cells	VERO cells
	hydrogen peroxide and	Test	brought	about 65%	exhibited	exhibited
	hydroxyl radical function	results	about 65%	cell	maximum cell	maximum cell
	to create cellular damage	indicated	cell	mortality	mortalities	mortalities
	that ends in cell death	that silver	mortality	when used	reaching 68% at	reaching 68% at
	[17]. The killing effect of	nitrate	when used	at 500	500 µg/ml	500 µg/ml
	silver nanoparticles	(AgNO3)	at 500	µg/ml	(Fig.8) and	(Fig.8) and
	against HEp-2 cells	brought	µg/ml	concentrati	reached the LD	reached the LD
	supports their potential	about	concentrati	on (Fig 7)	50 of 48% at	50 of 48% at
	use as cancer treatment	65% cell	on (Fig 7)	while	125 μ g/ml. The	125 μ g/ml. The
	medicines. Cancer	mortality	while	reaching its	experimental	experimental
	treatment effectiveness	when used	reaching its	LD 50	condition of	condition of
	can emerge from using	at 500	LD 50	value at	500µg/ml silver	500µg/ml silver
	lower concentration	µg/ml	value at	250 µg/ml	nitrate produced	nitrate produced
	AgNPs since these	concentrat	250 μg/ml	concentrati	83% cell death	83% cell death
	nanoparticles possess	10n (Fig 7)	concentrati	on. The	while the LD 50	while the LD 50
	harmful effects towards	while	on. The	P.amarus	occurred at	occurred at
	regular VERO cells.	reaching	P.amarus	AgNPs	$62.5\mu g/ml$. The	62.5μ g/ml. The
	Studies should continue	its LD 50	AginPs	treated	gliomacells	gliomacells
	to clarify now you can	value at 250 waves			uispiayed this	uispiayed this
	use these hanoparticles in	250 µg/III		owhibitod	bacausa of POS	haceuse of POS
	humans and the	ion The	evhibited	maximum	generation	generation
	biological factors leading	P amarus	maximum	cell	within their	within their
	to their effects	A gNPs	cell	mortalities	structure The	structure The
		treated	mortalities	reaching	three ROS	three ROS
		VERO	reaching	68% at 500	radicals	radicals
		cells	68% at 500	ug/ml	including	including
		exhibited	µg/ml	(Fig.8) and	superoxide	superoxide
		maximum	(Fig.8) and	reached the	radical and	radical and
		cell	reached the	LD 50 of	hydrogen	hydrogen
		mortalities	LD 50 of	48% at 125	peroxide and	peroxide and
		reaching	48% at 125	µg/ml. The	hydroxyl radical	hydroxyl radical
		68% at	µg/ml. The	experiment	function to	function to
		$500 \ \mu g/ml$	experiment	al	create cellular	create cellular
		(Fig.8)	al	condition	damage that	damage that
		and	condition	of	ends in cell	ends in cell
		reached	of	$500 \mu g/ml$	death [17]. The	death [17]. The
		the LD 50	500µg/ml	silver	killing effect of	killing effect of
		of 48% at	silver	nitrate	silver	silver
		125 µg/ml.	nitrate	produced	nanoparticles	nanoparticles
		The	produced	83% cell	against HEp-2	against HEp-2
		experimen	83% cell	death while	cells supports	cells supports
		tal	death while	the LD 50	their potential	their potential
		condition	the LD 50	occurred at	use as cancer	use as cancer
		0İ 500(1	occurred at (2.5 m)	62.5μg/ml.	treatment	treatment
			0∠)U2/MI.	THE	medicines.	medicines.



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		silver	The	gliomacells	Cancer	Cancer
		nitrate	gliomacells	displayed	treatment	treatment
		produced	displayed	this killing	effectiveness	effectiveness
		83% cell	this killing	effect	can emerge from	can emerge from
		death	effect	because of	using lower	using lower
		while the	because of	ROS	concentration	concentration
		LD 50	ROS	generation	AgNPs since	AgNPs since
		occurred	generation	within their	these	these
		at	within their	structure	nanoparticles	nanonarticles
		62.5 u g/m	structure	The three	nanoparticles	nanoparticles
		$02.5\mu g/m$	The three		offooto towardo	offooto towarda
		aliomaaall		rodicala	require VERO	regular VERO
		gnomacen	KUS modicala	including	alla Studias	acilla Studioa
		S d:1	radicals		cens. Studies	cells. Studies
		displayed	including	superoxide	should continue	should continue
		unis 1-:11:	superoxide	radical and	to clarify how	to clarify how
		K1111ng	radical and	nyarogen	you can use	you can use
		effect	nydrogen	peroxide	tnese	tnese
		because of	peroxide	and	nanoparticles in	nanoparticles in
		ROS	and	nydroxyl	cancer treatment	cancer treatment
		generation	hydroxyl	radical	for humans and	for humans and
		within	radical	function to	the biological	the biological
		their	function to	create	factors leading	factors leading
		structure.	create	cellular	to their effects.	to their effects.
		The three	cellular	damage		
		ROS	damage	that ends in		
		radicals	that ends in	cell death		
		including	cell death	[17]. The		
		superoxid	[17]. The	killing		
		e radical	killing	effect of		
		and	effect of	silver		
		hydrogen	silver	nanoparticl		
		peroxide	nanoparticl	es against		
		and	es against	HEp-2		
		hydroxyl	HEp-2	cells		
		radical	cells	supports		
		function	supports	their		
		to create	their	potential		
		cellular	potential	use as		
		damage	use as	cancer		
		that ends	cancer	treatment		
		in cell	treatment	medicines.		
		death [17].	medicines.	Cancer		
		The	Cancer	treatment		
		killing	treatment	effectivene		
		effect of	effectivene	ss can		
		silver	ss can	emerge		
		nanopartic	emerge	from using		
		les against	from using	lower		
		HEp-2	lower	concentrati		
		cells	concentrati	on AgNPs		
		supports	on AgNPs	since these		
		their	since these	nanoparticl		
		potential	nanoparticl	es possess		
		use as	es possess	harmful		

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	cancer	harmful	effects		
	treatment	effects	towards		
	medicines.	towards	regular		
	Cancer	regular	VERO		
	treatment	VERO	cells.		
	effectiven	cells.	Studies		
	ess can	Studies	should		
	emerge	should	continue to		
	from	continue to	clarify how		
	using	clarity how	you can use		
	lower	you can use	tnese		
	concentrat	tnese	nanoparticl		
	10n	nanoparticl	es in cancer		
	AgNPs	es in cancer	freatment		
	since	for hymon	for numans		
	neneratio	for numans	hiele sizel		
	las	and the	factors		
	nes	factors	lading to		
	barmful	leading to	their		
	effects	their	effects		
	towards	effects	eneets.		
	regular	circets.			
	VERO				
	cells.				
	Studies				
	should				
	continue				
	to clarify				
	how you				
	can use				
	these				
	nanopartic				
	les in				
	cancer				
	treatment				
	for				
	humans				
	and the				
	biological				
	factors				
	leading to				
	their				
	effects.				

Testing of cytotoxic effects for silver nanoparticles produced from *P.amarus* extract used the MTT-assay method on HEp-2 cell lines (Fig.6). A substantial number of cells died during *P.amarus* treatment when exposed to 500  $\mu$ g/ml solution which resulted in a 70% mortality rate (Fig 6). The lethal dose for 50% cells reached 62.5  $\mu$ g/ml. Test results indicated that silver nitrate (AgNO3) brought about 65% cell mortality when used at 500  $\mu$ g/ml concentration (Fig 7) while reaching its LD 50 value at 250  $\mu$ g/ml concentration. The *P.amarus* AgNPs treated VERO cells exhibited maximum cell mortalities reaching 68% at 500  $\mu$ g/ml (Fig.8) and reached the LD 50 of 48% at 125  $\mu$ g/ml. The experimental condition of 500 $\mu$ g/ml silver nitrate produced 83% cell death while the LD 50 occurred at 62.5 $\mu$ g/ml. The gliomacells displayed this killing effect because of ROS generation within their structure. The three ROS radicals including superoxide radical and hydrogen peroxide and hydroxyl radical function to create cellular damage that ends in cell death [17]. The killing effect of silver nanoparticles against HEp-2 cells supports their potential use as cancer

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treatment medicines. Cancer treatment effectiveness can emerge from using lower concentration AgNPs since these nanoparticles possess harmful effects towards regular VERO cells. Studies should continue to clarify how you can use these nanoparticles in cancer treatment for humans and the biological factors leading to their effects.



(a)Normal HEp-2 cell line??????(b) High conc. 500µg/ml



(c) LD 50 (62.5 µg/ml)

Figure 6. Invitro cytotoxic effect of P.amarus mediated AgNPs (microwave exposed). On HEp-2 cell line



a) Normal HEp-2 cell line ??????b) High conc. 500µg/ml



c)LD 50 (250ug/ml)

Figure 7. Invitro cytotoxic effect of silver nitrate at various conc. on HEp-2 cell line





a) Normal VERO cell line

???? b) High conc. 500µg/ml



c)LD 50 (125 µg/ml)

Figure 8. Invitro cytotoxic effect of *P.amarus* mediated AgNPs (microwave exposed) at various conc. on VERO cell line.



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(a) Normal VERO cell line	b) High conc. 500µg/ml		



#### (c)LD 50 (62.5 µg/ml)





Figure 10: Invitro cytotoxicity effect of P.amarus, mediated AgNPs (microwave exposed) and silver nitrate on HEp-2 cell line (MTT assay)



Figure 11: Invitro anticancer effect of *P.amarus* –AgNPs (microwave exposed) -comparison between VERO and HEp-2 cell lines



Figure 12: Invitro anticancer effect of silver nitrate- comparison between VERO and HEp-2 cell lines

#### 4. CONCLUSION

The plant extract Phyllanthus amarus mediated synthesis of silver nanoparticles was found to be more effective and the plant itself has got the medicinal property so tapping these resources seems to be ecofriendly and easy to produce these nanoparticles. Bioreduction of Ag+ ions to silver nanoparticles by the plant extracts occur. The plant aqueous

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extract act as reducing and capping agent of silver nanoparticles. The SEM analysis and FTIR analysis gave details about the size and also the presence of biomolecules. Among several physical conditions microwave treatment gave maximum yield and the colour change occur within 70 seconds at faster rates. Already reports are available on the usage of Nanoparticles for antibacterial activity; hence in our present study we put an innovative step in using these AgNPs for invitro cytotoxic effect on HEp-2 cell line. Several compounds present in the plant extracts acts as a ligand in the reduction process, which would be involved in the stabilization of these AgNPs. P.amarus extract mediated AgNPs exposed to microwave was cytotoxic and LD50 was at 62.5 µg/ml on cancerous cell line and at the same concentration in normal VERO cell line 73% viable cells were present. The cytotoxic assay was also carried on normal VERO cell line and it was toxic at very high concentration but at low concentration it was nontoxic to normal cell line. This makes the use of these plant mediated AgNPs for cancerous treatment. Thus green synthesis seems to be a very effective, innovative step and the nanoparticles produced by this method can be used to treat cancer.

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