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Biosynthesis of silver nanoparticles using *Andrographis Serpyllifolia (Rottler Ex Vahl) wight*. Leaf extracts there *In-Vitro* biological properties

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Abstract
facile green synthesis of silver nanoparticles biosynthesis is growing among varies field such as physicochemical and biological properties made a curiosity in the field of Nano biotechnology combined with green chemistry, has great potential for the development of novel and necessary products and they have a wide range of application. In pharmaceutical science, silver nanoparticles have a significant role. The plant extracts lead a significant role in biological activities such as antibacterial, antioxidant and anticancer activity the leaf extract of *A. serphyllifolia* was used to synthesis silver nanoparticles as a greener approach.

Keywords: antibacterial activity, antioxidant activity and anticancer activity

Introduction

Nanotechnology is the integration of science and technology involving the fabricationor synthesis, designand analys isofmaterial satthe nanometer scale the control of material sat the Nano meter scale allow susto adjust the properties of material for more specific applications. (Liddle J.A., Gallatin G.M., 2016) [1] Noble metal nanoparticles display size- dependent chemical and physical properties, including mechanical and biological characteristics, catalytic activity, thermal and electrical conductivity and remarkable optical properties. (HenzieJ. *et al*., 2006) [2]. These interesting properties have been considered for widespread applications in catalysis, electronics, optics, and environment and bio medicine. Metal nanostructures with highly controlled size, shape and optical properties are in use for biotechnological applications especially for diagnosis and biological imaging Nano biotechnology dealing with metal nanoparticles has Due to its cutting edge design and wide variety of applications in almost every area of science and technology, including biomedical sciences, it has attracted growing interest. (S.M, *et al*, 2013) [3]. The fields in which nano technology find sexten sive applications is Nano medicine, anemerging new field which is an out come off usi on ofnano technology and medicine (El-Sayed, *et al*. 2005) [4]. Medicine is no more physician job exclusively; the materials and devices designed at the level of Nano scale are for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and also in the overall preservation and improvement of health Synthesis of Ag NPs employing either microorganisms or plant extracts has emerged as an alternative approach (Duran, N., *et al*., 2007) [5]. These biosynthetic methods have a numbers of benefits they are simple, cost-effective, give high yields, and are environmentally friendly (S. Pal, *et al*., 2007) [6]. Silver nanoparticles can be dissolved in a liquid environment that prevents their agglomeration of entrapped in a matrix that utilizes special drug carrier systems (e.g. The drugis dissolved, entrapped, en capsulated or attached to a nano particles matrix). (Zhang Y. *et al*. 2013) [7]. These particles represent aninte resting candidate for research as micro bicides due to their effectiveness in small doses, minimal toxicity and side effects (Lara, *et al*. 2010) [8]. Cancer is a defect in the mechanism so fregulating of normal growth, proliferation and cell death. Cancer cells lose their control over the cell cycle and continue to proliferate in a continuous way, regard less of cellular messages and growth factors. Green chemistry is thus needed, including a smooth, non-toxic and environmentally-friendly technique for synthesizing nanoparticles. (Khatami, M. etal. 2017) [9]. A feasible, eco-friendly solution has been proposed in biological Nano-material production processes with the aid of microorganisms as a potential mediator. (C.Vetrivel.*et al*., 2019) [11]. However, widespread application of the Nano materials has also increased toxicity issues, raising concerns to human health and environment (J.P. *et al*. 2011) [21]. Biologically synthesized nanoparticles with antimicrobial, antioxidant, and anticancer properties are possible through the collaboration of different natural science sectors. The see nanotechnologies may provide novel resources. Forth evaluation and development of newer, safer, and effective drug formulations (Dipankar C, Murugan S., 2012) [22].

The naturally synthesized enzyme, protein, flavonoid, and antioxidant compounds from microorganisms and plant extract she lp in reducing and stabilizing the synthesized nanoparticles (Krishna raj *et al*., 2014, Sriram *et al*., 2012) [23, 24]. Some research studies have been reported to use silver nanoparticles as anti-cancer agents. The role of silver nanoparticles as an anti-cancer agent could open a new doors in the field of pharmaceutical as well as medical sciences. (Shaw key AM.*et al*., 2013) [25]. Green synthesis is defined as the use of environmentally compatible materials such as bacteria, fungi and plants in the synthesis of nanoparticles (Patra J K and Baek K H., 2014) [26]. Plant extracts contain number of secondary metabolite which plays a critical role during the nanoparticles synthesis by acting as reducing or capping agents. (Prasad R., 2014) [27].

Material and Methods

Plant Collection and Extract Preparation

The plants A. serpylli folia was collected from the local forest of Salem district, Tamil Nadu, India during the month of October 2016. The plant specimen was identified as A. serpyllifolia (Vahl) Wight Acanthaceae (Authentication No: BSI/SRC/5/23/2017/TECH/1672).

Systematic of Classification

Class: Dicotyledanae
Subclass: Gamopetalae
Order: Lamiales
Family: Acanthaceae
Genus: *Andrographis*
Species: *serphyllifolia* (Rottler ex Vahl) Wight.

Fig 1

Plant description

Habitat: peninsula (endemic)

Prostrate, trailing herb, branches, rising from a woody, stock; branch lets densely hairy leaves rotund orbicular, sub reniform, 2-3x 2-2.5 cm villous when young, glabrous at maturity except at margins, base obtuse or subcordate, margin entire, apex obtuse 0-1 mm, flowers axillary, solitary or few flowered, 2-3mm in fruit, bracts orbicular, 3x lip 6 mm, minutely bifid, lower lip purple, 7mm stamens 2;6mm, anthes unequal, bearded, capsule 1.5 cm, elliptic–oboviod, glabrous, with short, blunt acuminate. Occasional in hilly dry deciduous forests up to 600m altitude in middle and southern, eastern ghats fi: June –sept, Jan- mar, fr: Plant leaves were washed with running tap water and then rinsed thoroughly with de-ionized sterile water. In 200 ml deionizer water, 20 g of leaves were boiled, then filtered and centrifuged for 15 min at 4000 rpm. Then the extract was used for AgNPs synthesis.

Synthesis of AgNPs

A10mM of silver nitrate solution was prepared and store thin an amber bottle. A5ml of leaf extract was taken and was added to 50ml of silver nitrate solution drop wise with constant stirring at room temperature for 24 h and the colour change was observed. The colour change of the solution was checked periodically and then the conical flask was incubated at room temperature for 48h. The colour change of the solution from yellow to dark brown indicated the synthesized AgNPs. After synthesis, the solution was filtered through filter paper to remove the debris and then lyophilized to harvest then nanoparticles. (R. Madhan kumar. *et al*., 2019) [20].

Antibacterial activity

The synthesized AgNPs were tested for anti-microbial activity using the disc diffusion method following standards and guidelines from the Clinical Laboratory Standards Institute the antibacterial activity was done against the clinical pathogens *viz*, gram-positive bacteria and gram-negative bacteria by well diffusion method. Muller Hinton agar was prepared and autoclaved at 120_Cfor20 min and cooledto45_C. It was then seeded with microbial suspension using sterile swab. The wells were poured with different concentrations of AgNPs (25, 50, 75 lg/ml) along with the positive control ampicillin and were incubated at 37_C for 24 h. After incubation, the zone of inhibition was measured to determine the antibacterial potential of the AgNPs. (Azócar, M.I., *et al*., 2014) [15].

Antioxidant activity

DPPH ASSAY organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl. It is a crystalline powder of dark colour consisting of stable free-radical scavenging action.

HYDROXYL ASSAY the neutral form of the hydroxide ion (OH^-) is the hydroxyl radical, OH. Hydroxyl radicals are extremely reactive and often short-lived (easily becoming hydroxyl groups); they are, however, an important part of radical chemistry. The reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate was produced by the ABTS radical scavenging assay $ABTS^{\bullet+}$ cation radical (1:1),To reach an absorbance of 0.700 at 734 nm, the ABTS + solution was then mixed with methanol. Under aerobic conditions, NO interacts with oxygen to create stable products for nitric oxide scavenging operation. The amounts that can be measured using the reagent.

Anticancer activity

Cell treatment procedure

To make single cell suspensions, the monolayer cells were isolated with attempted sin-ethylene demine tetra acetic acid (EDTA) and viable cells were counted using a hemo cytometer and diluted with a medium containing 5 percent FBS to give a final density of 1x105 cells/ml. At the plating density of 10,000 cells/well, one hundred micro liters per well of cell suspension were seeded onto 96-well plates and incubated to enable cell attachment at 370C, 5 percent CO2, 95 percent air and 100 percent relative humidity. After 24 h, cells were treated with serial test sample concentrations. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots containing 100 µl of these numerous sample dilutions have been applied to the sufficient wells already containing 100 µl of the medium, resulting in the final sample concentrations required. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium without samples was used as a monitor and triplicate for all concentrations were retained.

MTT assay

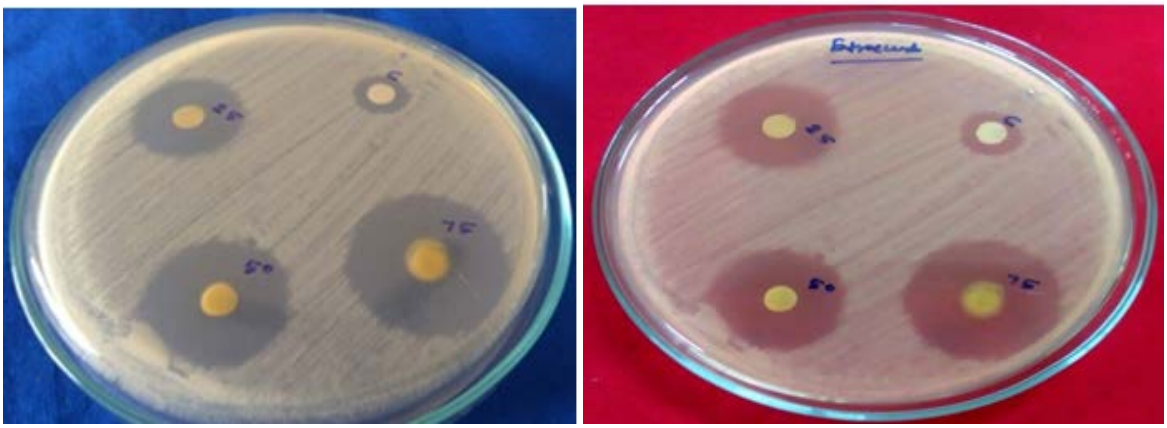
3-[4, 5-dimethylthiazol-2-yl] 2, 5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. The amount of

formazan produced is, thus, directly proportional to the number of cells that are viable. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

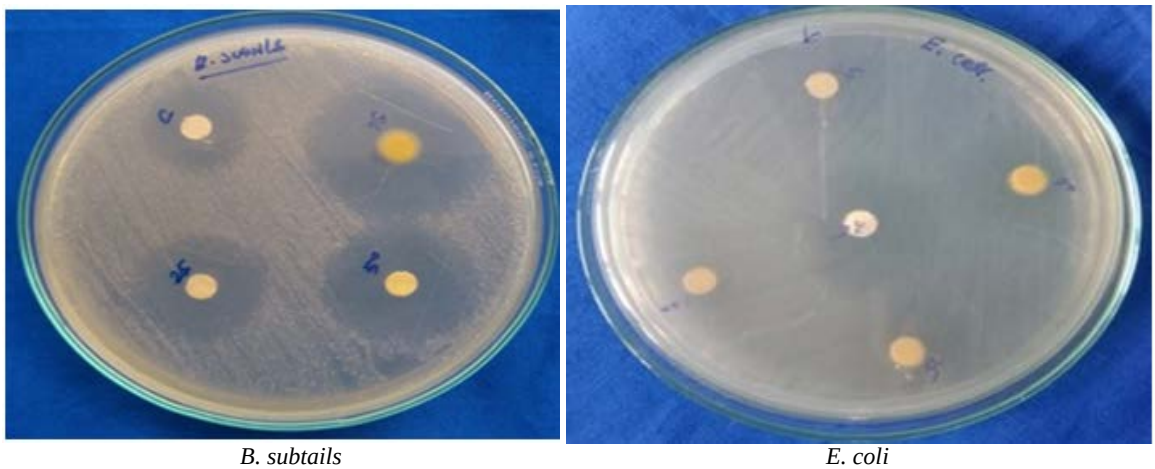
Result

Antibacterial activity

The antimicrobial properties of silver against a broad spectrum of microorganisms have been well known for decades. The Nano silver-based biomaterials and devices have found widespread.



K. pneumonia *Enterococcus*



B. subtails *E. coli*

Fig 2

Fig: 2 showed where, the $A_o$is absorbance of control reaction, A_1 is absorbance of test compound. Antimicrobial activity different solvent in highest inhibition zone for silver nitrate A. *serpyllifolia* extract the microorganisms for highest inhabitation zone (gram – positive) *Bacillus subtilis,* (9.66 ± 0.33) *Entrococus bacillus* (15.00 ±0.88) ((Gram-negative) *Klebsiella pnemonia,* (15.00 ± 0.57) *Escherichia coli,* (17.00 ± 2.30)

Antioxidant activity

Table 1

S. No	Antioxidant	Antioxidant Activity IC 50 Values µg/ml	
		Standard control L. Ascorbic acid	AgNPs of *Andrographis Serpyllifolia*
1	DPPH	183.97	208.56
2	Hydroxyl	226.34	244.52
3	ABTS	236.79	248.22
4	NitriOxide	225.87	248.72

Table 1: Showed DPPH ASSAY, organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free-radical scavenging activity for DPPHIC 50 values L. Ascorbicacids183.97 and AgNPs of *A. Serphyllifolia* 208.56. Hydroxylassay, The hydroxyl radical, OH, is the neutral form of the hydroxide ion (OH). Hydroxyl radicals are highly reactive (easily becoming hydroxyl groups) and consequently short-lived; however, they form an important part of radical chemistry Standard control L. Ascorbic acid 226.34 AgNPs *A. Serphyllifolia* 224.52 ABTS radical scaven gingassay ABTS•+ cationradical was Antioxidant Activity Standard control L. Ascorbic acid IC 50 Values µg/ml 236.79 AgNPs of *A. Serphyllifolia* 248.22.Nitric oxide scavenging activity under aerobic conditions, NO reacts with Oxygen to produce stable products (Nitrate and the quantities of which can be determined using reagent. Nitric oxide scavengers compete with oxygen, resulting in reduced nitrite ion production.

AgNPs in Cancer Control

As cancer therapeutics, AgNPs perform well because they can disrupt the mitochondrial respiratory chain, which induces reactive oxygen species (ROS) generation, and ATP

synthesis, which can induce DNA damage. (Swamy MK et. al2014) [18], (Nie Z, Liu KJ. *et al* 2007) [19].

Fig: Cytotoxicity Activity of Human Liver cell line using a *serphyllifolia* (Rottler ex Vahl) W. Leaf extract. In several literatures, cytotoxic effects of synthesized AgNPs against cancer cell lines were highlighted the anticancer efficacy of biosynthesized AgNPs against the MCF-7 cell line was studied in the current investigation and the percentage of cell viability of AgNPs was illustrated. The cytotoxicity effect was found to be increased with increase in concentration of AgNPs. The Ag-NPs Fig: 3 showed in habited the cell growth by 3.9, 11.8, 23.5, 56.9 and 72.8%atdosesof6, 12, 25, 50 and 100 g/ml, respectively a. *serphyllifolia*. Hence, the cyto- toxicity induced by biosynthesized AgNPs in the treated cells, correspondingly resulted in the inhibitory concentration (IC 50) value of 40g/ml after 24 h treatment. The morphological changes were examined under a phase contrast microscope in both untreated and treated MCF-7 cells. The untreated MCF-7 (control) cells were found to have high monolayer cell confluence and a smooth, flattened morphology with intact cell membrane. Whereas the cells treated with AgNPs exhibited retraction, rounding, and surface detachment and seemingly accumulated suspended cells.

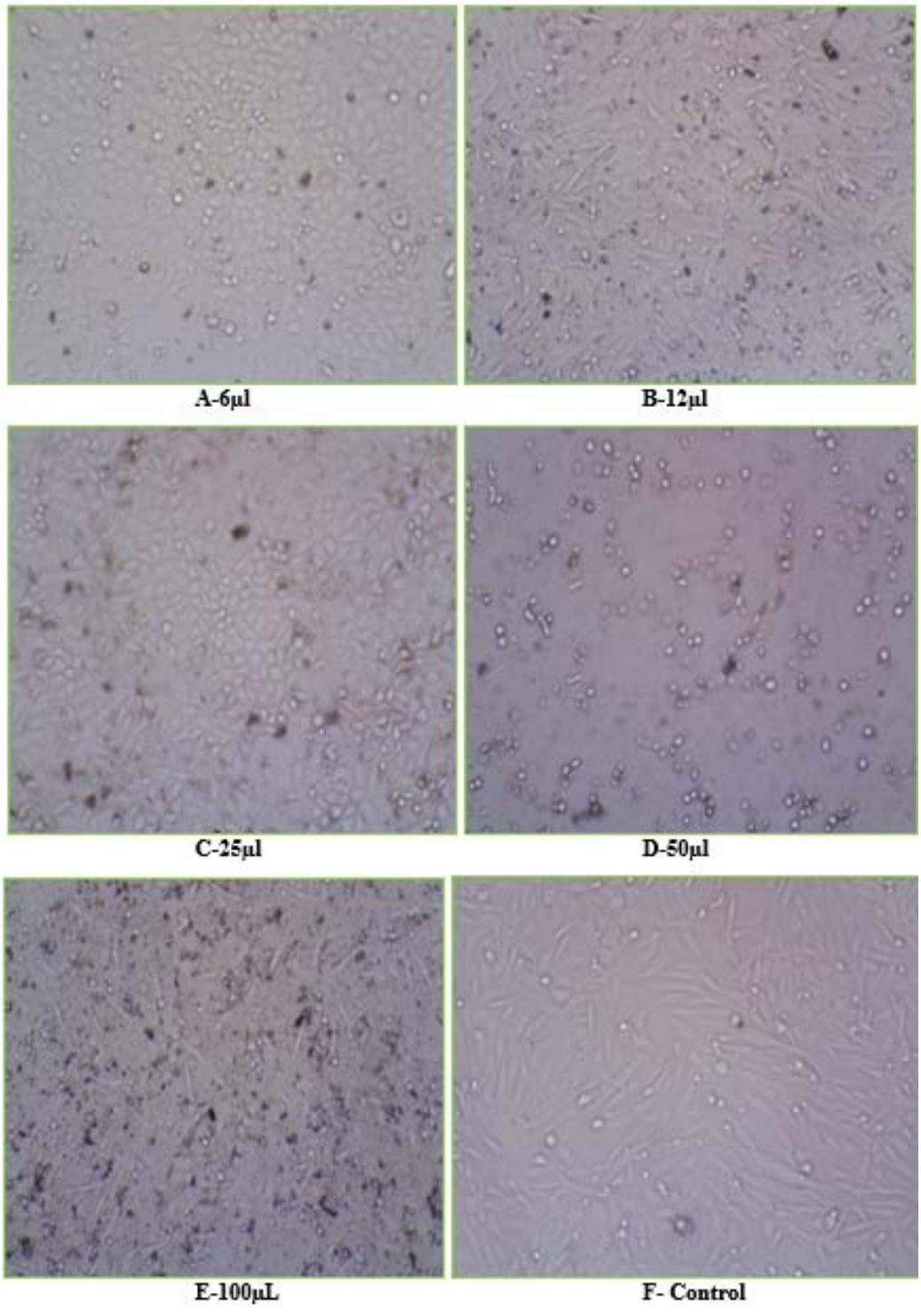


Fig 3

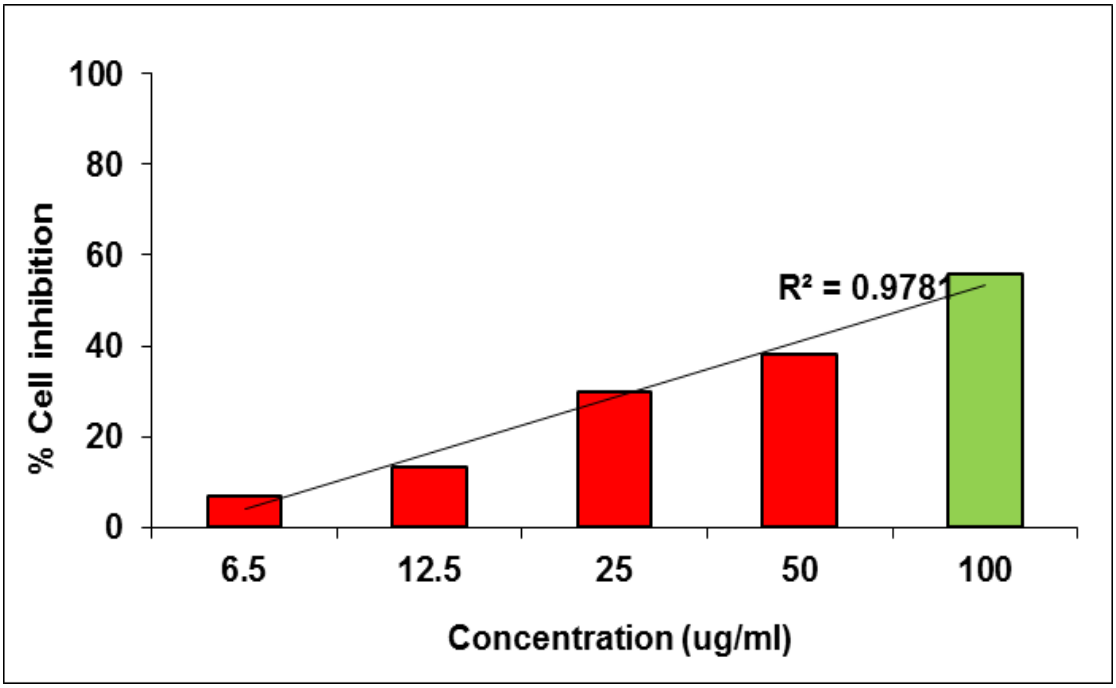


Fig 4: Cytotoxicity Activity of human Liver cell line *A. Serphyllifolia* (Rottler ex Vahl) W.

Table 2

Conc. (µg/ml)	% Cell inhibition		
6.5	6.74		
12.5	13.25	IC 50	87.93 µg/ml
25	29.66	R^2	0.978
50	37.97		
100	55.95		

Table 3

Conc	6.5 µg	12.5 µg	25 µg	50 µg	100 µg	Cont.
ABS	0.415	0.385	0.311	0.275	0.195	0.445
	0.415	0.387	0.314	0.277	0.196	0.447
	0.417	0.386	0.315	0.276	0.197	0.445
Avg	0.415667	0.386	0.313333	0.276	0.196	0.445667

Discussion

Where, the Ao is absorbance of contro lreaction, A1 is absorbance of test compound. Antimicrobial activity different solvent in highest inhibition zone for silver lived; however, they for man important part of radical chemistry Standard control L. *Nitrate A. serphyllifolia* extract the microorganism for highest inhabitation zone (Gram positive) Bacillus sub tilis, (9.66±0.33) Entrococus bacillus (15.00 ±0.88) ((Gram-negative) Klebsiella pneumonia, (15.00 ± 0.57) Escherichia coli, (17.00 ± 2.30) The organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl. It is a dark- colored crystalline powder composed of stable free-radical scavenging activity for dpphic 50valuesL. Ascorbic acids 183.97 and AgNPs of *A. Serphy llifolia* 208.56. H The hydroxyl radical, OH, is then eutral form of thehy droxidei on (OH). Hydroxyl radicals are highly consequently short Ascorbic acid 226.34 AgNPs *A. Serphyllifolia* 224.52 ABTS radical scaveng in gassay ABTS•+cation radical was Antioxidant Activity Standard control L. Ascorbic acid IC50Valuesµg/ml236.79AgNPsof*A.serphyllifolia*248.22The cytotoxiceffects of synthesized AgNPs against cancer celllines were high lighte dinseveralliteratures. In the present investigation, the anticancer efficacy of biosynthesized AgNP swas studied liver cancer cellline and the percentage fcell viability of AgNP sareillustratedin. The cytotoxicity effect was found to be increased with increase in concentration of AgNPs. Atdoses of 6, 12, 25, 50 and 100? g/ml, respectively *A. serphyllifolia*. Hence, the cyto-toxicity induced by biosynthesized AgNPs in the treated cells, correspondingly resulted in the inhibitory concentration (IC50) value of 40g/ml after 24 h treatment. Morphological variations were studied under a phase contrast microscope in both untreated and treated MCF-7 cells. These results suggest that AgNP scan induce cell deathinMCF-7cellsand our finding is reliable with the earlier report.

Conclusion

In conclusion, the present study shows promising reducing property of *A. serpyllifolia* leaves bioreduction of AgNO3 (Ag+toAg0). An ecofriendly and fast facile synthesis of AgNPs by A. serpyllifolia leaf extract is established. Further surface modification is done to conjugate with standard drug resveratrol. AgNPs synthesized from leaf ethanol extracts of *A. serpyllifolia* showed can be successfully used for the synthesis of AgNPs that exhibit effective antioxidant, antibacterial as well as cytotoxic activity against a liver cancer cellline. The improved cytotoxic effect of A. serpyllifolia may be attributed to the presence of bioactive compounds and fast entry inside the cells. Future investigation need to focus on possible mechanisms underlying the cytotoxicity activity this study has given a perception for the novel drugmanipulative after directing tests on the *in vivo* models.

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