ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Xylorious Biogenic Synthesis of Silver Nanoparticle and Their Cytotoxicity Effects against HT-29 Cell Line.

Varsha B, Arvindganth R, and Kathiravan G*.

Department of Biotechnology, Vels University, Pallavaram, Chennai - 600 117, Tamil Nadu, India.

ABSTRACT

Xylorious fungus isolated from curry leaves were used further biosynthesis of silver nanoparticles. The characterizations of nanoparticles like UV, IR SEM analysis were performed to study the structural morphology of the biosynthesized silver nanoparticles. The electron microscopy study revealed the formation of spherical nano-sized silver particles with different size. Thus, the results of the present study indicate that biologically synthesized silver and nanoparticle might be used to treat colon cancer; however, it necessitates clinical studies to ascertain their potential as anticancer agents.
Keywords: xylorious, silver nanoparticle, secondary metabolites and cytotoxicity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Endophytes have been found virtually in every plant studied, where they colonize the internal tissues of their host plant and can form a range of different relationships including symbiotic, mutualistic, commensalistic and trophobiotic.[1][2]. Most endophytes appear to originate from the rhizosphere or phyllosphere; however, some may be transmitted through the seed. Endophytes can promote plant growth and yield and also can act as biocontrol agents.[3] Endophytes can also be beneficial to their host by producing a range of bioactive compound that could be harnessed for potential use in medicine, agriculture or industry.[4] Endophytes from medicinal plants are a potential source of a diverse array of bioactive metabolites which can be used for the development of some potent drugs. [5]. Many authors have isolated endophytic microbes from various medicinal plants with antioxidant, antibacterial, antimicrobial. Further many more examples in which endophytes producing various secondary metabolites such as taxol, Asperagenase, Campothecin, as anticancer compounds and artimisinin as antimalarial etc.[6] Fungi that do not sporulate in culture do produce spores in nature, and can produce allergens, irritants and can cause hypersensitivity pneumonitis, dermatitis and systemic infection in immune compromised patients. A case of *aspergilloses* caused by a non-sporulating (in culture) strain of *Aspergillus fumigatus* was reported in a pregnant woman [7] and a case of an invasive infection with non-sporulating *Chrysosporium* species was reported in a patient who was treated with chemotherapy for relapsed acute lymphoblastic leukemia [8] Secondary metabolites may also affect the behavior of natural enemies of herbivorous species in a multi-trophic defense/predation association. For instance, terpenoid production attracts natural enemies of herbivores to damaged plants. These enemies can reduce numbers of invertebrate herbivores substantially and may not be attracted in the absence of endophytic symbionts. Multi-trophic interactions can have cascading consequences for the entire plant community, with the potential to vary widely depending on the combination of fungal species infecting a given plant and the abiotic conditions. [9] Taxol is an important anticancer drug used widely in the clinical field. Some endophytic fungi were isolated from selected medicinal plants and screened for the production of taxol. [10][11]. The effect of cytotoxicity of fungal taxol isolated from fungal endophytes was investigated by apoptosis method. The cell filtrate of endophytic fungi was determined by thin layer chromatography.[12] The fungal taxol isolated from the organic extract of six fungal cultures, had strong cytotoxic activity towards BT 220, H116, Int 407, HL 251 and HLK 210 human cancer cells in vitro.[13]. *Seimatoantlerium nepalense*, an endophytic taxol producing coelomycete from Himalayan yew (*Taxus wallichiana*). Mycotaxon.

MATERIALS AND METHODS

ISOLATION OF FUNGI FROM CURRY LEAVES

The infected leaves were collected and the infected areas are cut into pieces. It was surface sterilized by using mercuric chloride, 70% ethanol, and double distilled water. Infected leaf pieces were placed on the potato dextrose agar. Then it was kept for incubation at room temperature, for around 4 to 5 days. And subculture was done to isolate the required species.

EXTRACTION OF SECONDARY METABOLITE

The fungal mate of Xylorious from curry leaves was removed. Then the equal volume of solvent Dichloromethane were added to the Secondary metabolite, it was mixed well and was separates using a separating funnel. The distillation process step is taken place. After this step we get partially purified samples of xylorious form of fungal extract. Then it was evaporated using rotary evaporator at 30°C. [14]

PREPARATION OF SILVER NANOPARTICLES

3.5 mM of $AgNO_3$ was prepared by dissolved 0.6 g in 100ml deionized water and was kept in magnetic stirrer under UV for overnight. This was kept in dark condition.

UV-VISIBLE ABSORPTION SPECTRA

The fraction was dissolved in chloroform and absorption spectrum was recorded between 200-400 nm using Perkin Elmer spectrophotometer. The λ max was determined.

IR-spectra

The functional groups present in CPT were determined by IR- spectra. The spectra were measured using Perkin Elmer PF2 800 in KBr.

Cell line and culture

Colon cancer- HT-29 cell lines was obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 50 μg/ml CO_2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyldiphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO)were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

In vitro assay for Cytotoxicity activity (MTT assay).

The Cytotoxicity of samples on HT-29 was determinedby the MTT assay [15]. Cells (1×10^5/well) wereplated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester,NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence ofvarious concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After4h incubation, 0.04M HCl/ Isopropanol was added. Viable cells weredetermined by the absorbance at 570nm .Measurements were performed and theconcentration required for a 50% inhibition of viability(IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer usingwells without sample containing cells as blanks. The effect of the amples on the proliferation of HT-29 was expressed as the % cell viability, using the following
FORMULA

% cell viability = A570 of treated cells / A570 of control cells.

RESULT AND DISCUSSION

ISOLATION OF FUNGI

Xylorious fungi isolated from curry leaves the mass cultured was maintain into M1D medium.

SECONDARY METABOLITE

The fungal mate of Xylorious was removed from curry leaves. Then the equal volume of solvent Dichloromethane were added to the Secondary metabolite, it was mixed well and separated by using a separating funnel. After the partially purified samples was evaporated by using rotary evaporator at 30̊ C.

UV SPECTROSCOPY ANALYSIS

The λmax with 270nm was observed in without $AgNO_3$. The Indicates the existence of an UV active chromophore, in the sample with $AgNO_3$ also UV spectra gives λmax value from 425 nm.

IR – SPECTRA ANALYSIS

The functional groups present in CPT were determined by IR- spectra. The spectra were measured using Perkin Elmer PF2 800 in KBr.

Both the control and treated have characteristic peaks to conform the existences of OH group CH_3 / CH_2 group as well as aromatic group.

The peak in the range 3402 refers to OH / NH group.

The peak in the range 2954 / 2922 / 2851 refers to the existence of SP^3 hybridized C – H stretching the peak at 1741 / 1742 refers to existence of carbonyl group.

The peak in the range 1659, 1463, refers to existence of aromatic group .
The peak at the 2174 the control shifted to 2336 in treated refers to the change W.R to C.N system.
All other peaks in the finger print region to characteristic peaks of C-H, O-H, N-H, system.

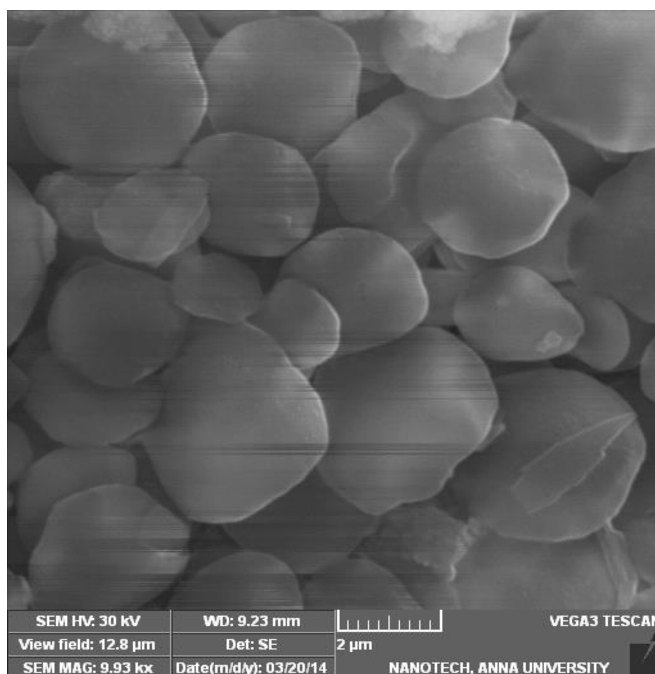


Plate - 1: SEM image of Silver nanoparticle synthesis from Xylorious fungi

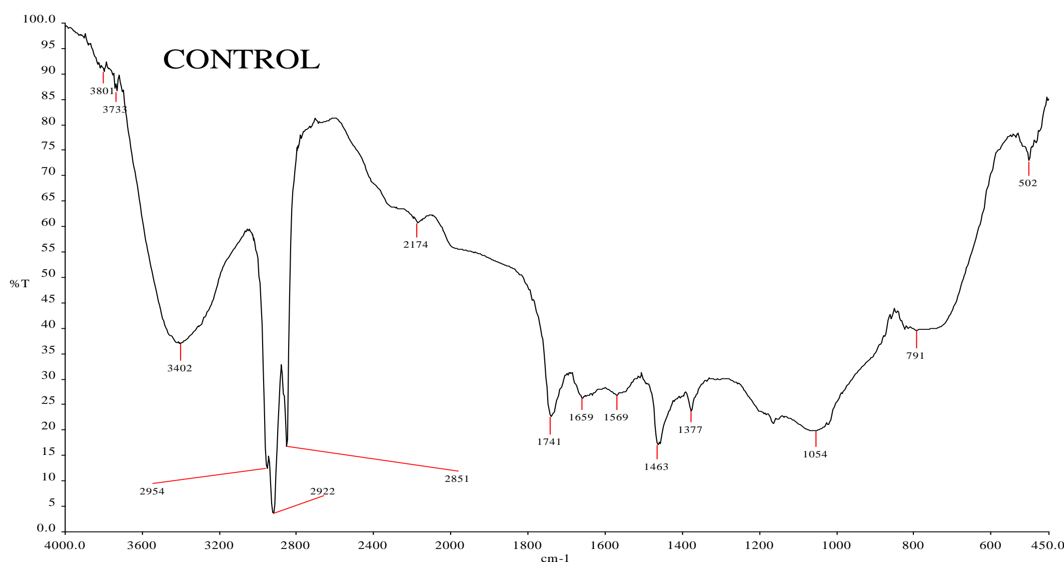


Fig.1: IR SPECTRUM SHOWING WITHOUT NANO PARTICLE TAXOL

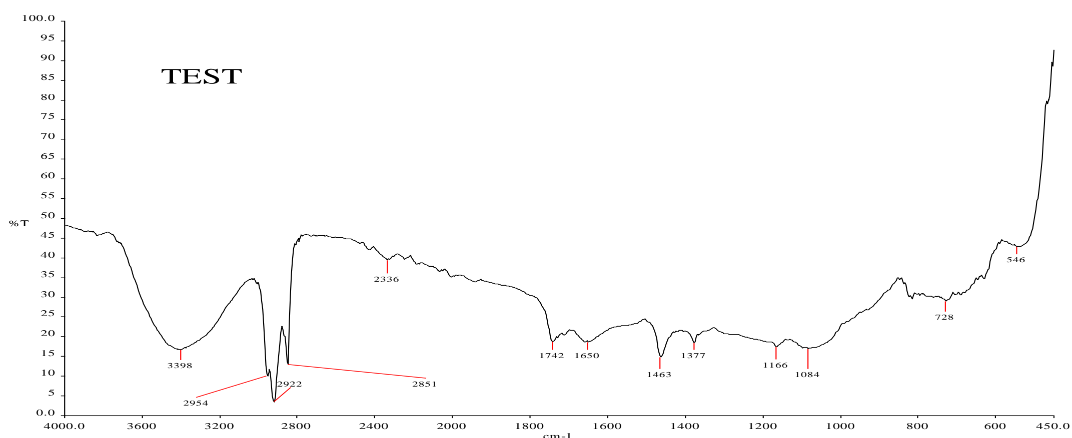


Fig. 2: TAXOL IR SPECTRUM SHOWING WITH NANO PARTICLE

Table. 1: Anticancer effect of control (without nano particle)- on HT-29 cell line(Dichloromethane Extract)

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.06	13.3
	500	1:1	0.11	24.4
3	250	1:2	0.18	40.0
4	125	1:4	0.22	48.8
5	62.5	1:8	0.25	55.5
6	31.2	1:16	0.30	66.6
7	15.6	1:32	0.34	82.9
8	7.8	1:64	0.39	86.6
9	Cell control	-	0.45	100

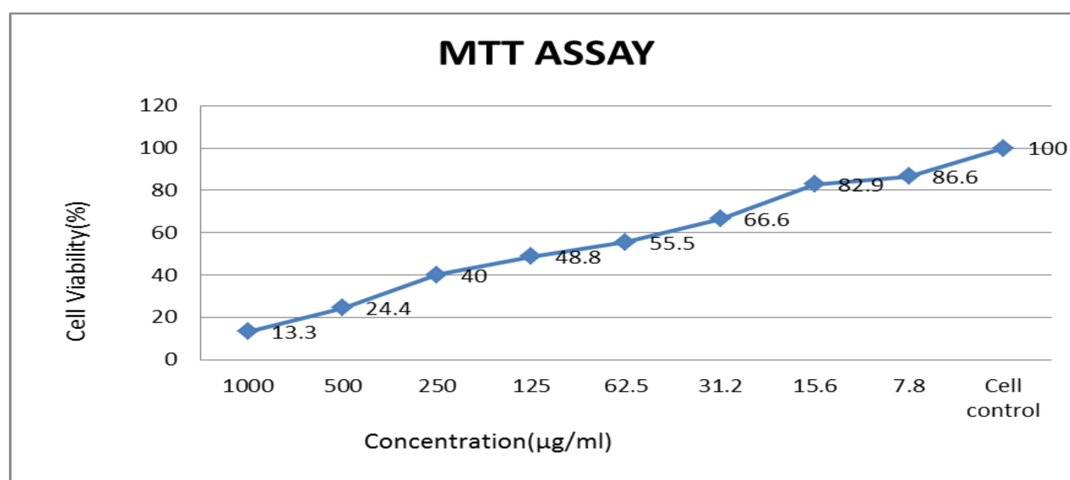
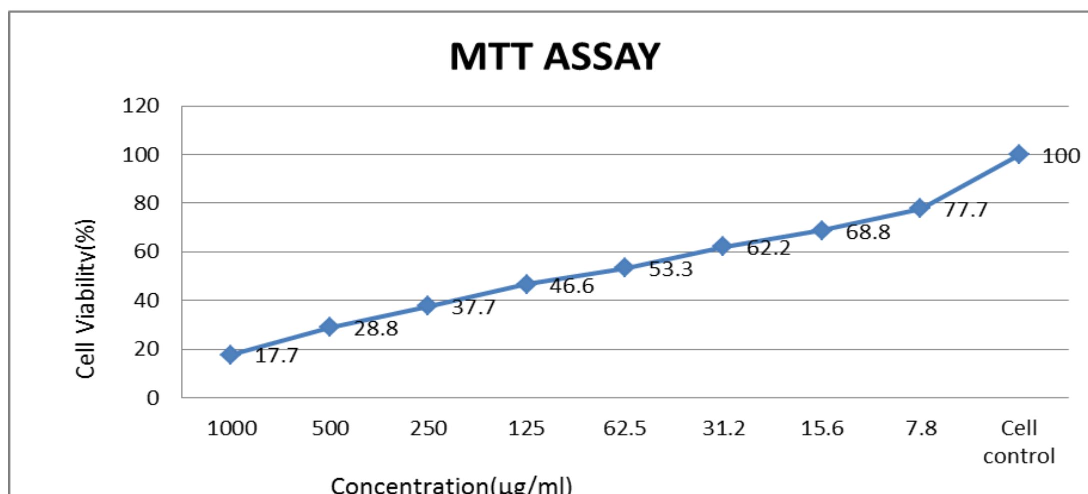


Table 2: Anticancer effect of *Silver conjugate*- on HT-29 cell line(Dichloromethane Extract)

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.08	17.7
	500	1:1	0.13	28.8
3	250	1:2	0.17	37.7
4	125	1:4	0.21	46.6
5	62.5	1:8	0.24	53.3
6	31.2	1:16	0.28	62.2
7	15.6	1:32	0.31	68.8
8	7.8	1:64	0.35	77.7
9	Cell control	-	0.45	100



CONCULUSION

The present study showed xylorious secondary metabolites synthesis of AgNP with an average particle size in range. The IR and UV results showed that the bioreduction of silver occurred when the silver ions interacted with the functional groups of the mycelial surface of the test fungi. In cytotoxicity studies against HT-29 cell line also shows good result. Thus it indicated that the xylorious can be utilized for the production of AgNPs which have wide application in pharmaceutical and medical fields.

REFERENCES

[1] Furukawa H, Wu TS, Onta T, Kuoh, CS. Chem Pharm Bull 1985; 35: 4122- 4138.
[2] Gopalan C, Ramasastri BV, Balasubramanian SC. Nutritive value of Indian foods National Institute of nutrition Hyderabad. Indian council of medical research New Delhi; 1984: 66 - 117.
[3] The Wealth of India CSIR New Delhi; 1962: 448 – 452.
[4] Satyavathi GV, Raina MK, Sharma M. Indian Medicinal Plants New Delhi; 1983: 289 – 299.
[5] Chopra RN, Glossary of Indian Medicinal Plants Council of Scientific and Industrial Researc New Delhi; 1956.
[6] Itoigawa M, Kashiwada Y, Furukawa H, Bastow KF, Lee KH, J.Nat. Prod 2000; 63 (7): 893-897.
[7] Khan BA, Abraham A, Leelamma S. Indian J. Expt. Biol 1997; 35(2):148–150.
[8] Khanum F, Anilakumar KR, Sudarshana KKR, Viswanathan KR, Santhanam K. Plant Foods Hum Nutr 2000; 55: 347–355.
[9] Bary DA, Morphologie physiologie der Pilze Flechten, Myxomyceten. Hofmeister's handbook of physiological botony Leipzig, Germany 1866; 2.
[10] Stone JK, Bacon CW, White JF, Bacon CW, White JF, editors. Microbial edophytes. Marcel Dekker Inc., New York, USA 2000; 01: 03-29.
[11] Roa K, Kirishna MB, Srinivas N. Trop J Pharm Res 2004; 3(1): 305-59.
[12] Noor HA, Modh AS, Mawardi R, Atan MS, Kaida K, Umi KY. The Malaysian Journal of Analytical Sciences 2007; 11: 173-76.
[13] Zhang B, Salituro G, Szalkowski D, Li Z. Sci 1999; 284: 974-77.
[14] Strobel GA. 2003; Infect 5: 535-544.
[15] Masman T, Immunol J. Methods 1983; 16: 65(1-2) 55-63.