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Aquatic fern (*Marsilea* sp.) assisted synthesis of Silver and Gold nanoparticles and evaluation of their anticancer properties

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Abstract: **This study aimed to evaluate the anticancer activity of bioinspired silver nanoparticles (Ag-NPs) and gold nanoparticles (Au-NPs) againstMCF-7 cells. Both Ag-NPs and Au-NPs were biologically synthesised using pteridophytes. In this work, synthesis of nanoparticles, characterisation and potential anticancer activity has been reported. *Marsilea* belongs to pteridophyte division can reduce HAuCl4 solution to Au-NPs and AgNO3to Ag-NPs due to the presence of secondary biological metabolites. Phytochemical examination reveals the presence of tannins, phenols, coumarins, flavonoids, anthro-quinones, terpenoids, saponins. These phytochemicals are responsible for the transformation and act as capping, reducing and stabilising agents. Formation of-NPs and Au-NPs were ascertained from UV-vis spectrometry, FTIR and XRD. The UV-visible study revealed the surface plasmon resonance. FTIR spectra confirmed the participation of functional groups. XRD reveals the peak positions and broadening, which indicates the size of particles. In both case sharped sharp peak was observed at 38° among 2θ values, the size distributions of Ag-NPs and Au-NPs were calculated by using Scherrer's formula. The cytotoxic potential of both SNPs was assayed against MCF-7 cells (breast cancer cell line) and HEK293 cell (human embryonic kidney cell line) by MTT assay. Results suggested that the NPs from *Marsilea* inhibits explicitly the viability of cancerous cell only and are non-cytotoxic to HEK293 cells in the tested concentration range. Based on these descriptions we can speculate that synthesised NPs may be used as promising anticancer agents, as the inhibits the viability of human breast cancer (MCF-7) cells and not detrimental to standard cell line.**

Keywords: **Silver Nanoparticles, Gold Nanoparticles, Aquatic pteridophytes, *Marsilea* sp., MCF-7 cells.**

I. INTRODUCTION

With an exponential increase in the advancement of the humanity, we have developed a bustling, and that has culminated in different diseases. Cancer has emerged as a life-threatening and lethiferous disease causing substantial morbidity and mortality and is perceived to be a significant health problem worldwide [1]. The current line of treatment is cumbersome, capital-intensive and full of side effects causing peril to the patient and the family alike. The emerging field of Nanoscience and technology holds prodigal promises in bio-medical sciences and bestows a ray of hope towards the treatment of cancer in future. Several noble metal nanoparticles such as silver, gold, platinum and copper were widely synthesised. These NPs have been shown to be effective against a broad range of human cancers cell lines such as Hep2 cell line [2,3], HT-29 cell lines [4], Vero cell line [5] and breast cancer line MCF-7 [6]. Nanoparticles with the size range between 1 to 1000 nm are mainly discovered for the diagnosis and treatment of human cancers which directed to the new discipline of nano-oncology [7].

Green Nanoscience and technology have opened a myriad of opportunities as it is eco-friendly, economically amenable and less cumbersome. In the past, biological systems like microbes (bacteria, fungi and actinomycetes), yeast, algae, gymnosperms and higher plants have been taken in to use for synthesising a vast plethora of nanoparticles showing biological/biomedical activities. The biological congeners being taken in to use for fabricating different nanomaterials are

Microorganisms (bacteria [8,9,10] fungi [11], actinomycetes [12], yeast [13], algae [14] and plants (bryophytes, pteridophytes [15], angiosperm [16], gymnosperm [17]. One added advantage of employing a plant congener having reported medicinal property for synthesising nanoparticles is that the left-over or un-utilised extract during the procedure of synthesis helps to encapsulate the burgeoned nanoparticles in one hand and functionalizes the same for much better efficacy. Amenability of scaling up is further an added advantage.

Marsilea sp. belongs to class Pteridophyta of the plant kingdom is described as 'swastika' being used as a divine drug in the traditional Indian system of medicine 'the Ayurveda'. In that system of medicine, this plant has been used for the treatment of various diseases like skin diseases and infections. The abundant presence of phytochemicals (Phenolic & Saponin) and nitrate reductase was reported in *Marsilea* [18,19] and these phytochemicals play an essential role in the bio-reduction of nanoparticles.

In the past, many efforts have been taken to utilise the widely distributed and highly promising treasure of pteridophytes for synthesising nanoparticles [20,21,22]. Here, in this new effort, we have synthesised silver and gold nanoparticles and have tried to assay their activity against MCF-7 cell line under in vitro condition.

II. MATERIAL AND METHOD

A. Preparation of the Plant Extract: -

Fresh fronds of *Marsilea* sp. were washed thoroughly with running tap water followed by50% Et-OH to remove the dust particles and dry it. 5 gm of fronds were grounded into a paste. This paste boiled with a mixture of 50 mL ethanol and 50 mL of distilled water and was placed on the boiling steam bath at 80 ºC for 15 to 20 min until the colour of the solvent changed. The extract was passed through a muslin cloth and filtered through Whatman Filter Paper No. 1 and used for the synthesis of nanoparticles.

B. Synthesis of Nanoparticles

Freshly prepared extract solution was treated with an aqueous solution of HAuCl4 in 1:1 proportion. The mixture was allowed to react and warmed again in the steam bath for 20 min at 60 ºC, with vigorous shaking. Synthesis of Au-NPs was checked visually by determining the colour change from yellowish green to reddish colour. A similar procedure was followed for synthesis of Ag-NPs, indicated by a coffee brown colour formation. Confirmation was done from a characteristic peak obtained in the absorbance range of 500 – 600 nm for Au-NPs and 350 – 450 nm for Ag-NPs.

C. Nanoparticles Characterization

The formation of Au-NPs was confirmed by using a UV-Visible spectrophotometer (Lambda 950, Perkin Elmer). Approx.4mL of the diluted sample was placed in a quartz cuvette and inserted in AUV-Visible spectrophotometer in the wavelength range of 250–700nm to obtain the UV-Visible spectra of the sample. X-ray diffraction (XRD) analysis were done using Bruker AXS instrument (model Advance D8), with CuKα radiation. XRD is used for the characterisation of the crystal structure of the nanoparticles and phase identification [23]. The role of functional groups was analysed using a Fourier transform infrared (FTIR) spectrophotometer (Model Spectrum RX1, PerkinElmer Inc.) at a range of 4,000 to 500 cm−1. The surface morphology of the prepared nanoparticles was investigated by scanning electron microscopy (Carl Zeiss Microscopy). A thin layer of the samples was placed on a carbon tape, then coated with Platinum to fix on the stud for a detailed examination of Nanoparticles morphology.

D. Cell culture and cytotoxicity studies

To determine the cytotoxicity of NPs, cell viability study was done using MTT assay. In which, Human embryonic kidney (HEK293) and human breast cancer (MCF-7) cell lines were procured from National Centre for Cell Sciences, Pune, India. Dulbecco's modified Eagle's media (DMEM), antibiotic cocktail, TrypLE specific cell detachment enzyme and fetal bovine serum (FBS) were obtained from Gibco-life technologies, Thermo Fisher Scientific (USA). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) were purchased from Sigma (St. Louis, MO).

HEK293 and MCF-7 cells were maintained and cultured in DMEM media enriched with 10% heat-inactivated FBS and 1% penicillin, streptomycin solution in a humidified incubator (5% CO2) at 37°C. Cultures were regularly maintained and sub cultured not more than 30 passages.

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In order to evaluate the cytotoxicity of synthesised compounds a typical MTT assay was used as described earlier [24, 25]. Briefly, MCF-7 and HEK293 cells were seeded in a 96-well plate (9000-10000 cells/well) and allowed to grow overnight. On a subsequent day, cells were incubated with increasing concentrations (0–100 µg/ml) of synthesized nanoparticles (NP) in a final volume of 200 µl for 48 h at 37º C. After 48 h, the mixture of culture medium and NP were removed, cells were washed twice with phosphate buffer saline (pH 7.4) solution and 20 µl of MTT solution (from 5mg/ml stock solution in PBS) were added to each well. Additionally, 100 µl of DMEM was added to each well and plates were incubated for 4-5 h at 37ºC. Following the incubation, the supernatant was removed, and the purple coloured crystals of formazan (produced from MTT) were dissolved by adding 150 µl DMSO. The absorbance was measured at 570 nm on a multiple ELSIA reader (Bio-Rad). The percentage of cell viability was calculated and plotted as a function of concentration.

III. RESULT

A. Biosynthesis of Ag-NPs and Au-NPs

Plant extract has been used for a long time for different phyto pharmaceutics studies. We selected pteridophyta; *Marsilea* in this study. The Ag-NPs and Au-NPs were synthesised using leaves extract of *Marsilea* (pteridophyte). Change in colour of plant extract from greenish to red confirmed the ability of the plant extract to reduce HAuCl4 solution to gold nanoparticles. Au-NPs characterised spectrophotometrically using UV visible electromagnetic radiation. The extract fabricated gold particles were shown absorption spectra at 535 nm, which is the characteristics of surface plasmon absorption specific for gold nanoparticles. On the other hand, the natural coffee brown colour was formed on addition of the ofAgNO3 solution to the plant extract, indicating synthesis of Ag-NPs, which was confirmed by an absorption peak at 402 nm (Figure 1). These results indicate the successful synthesis of nanoparticles.

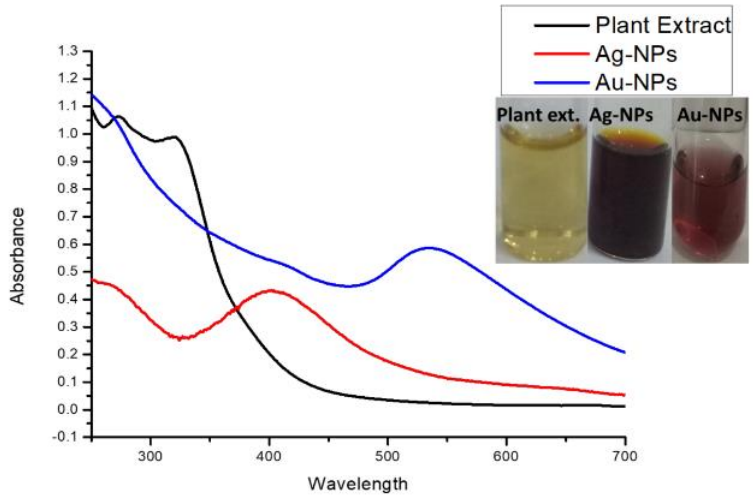

Fig 1: UV-visible spectra of Ag-NPs and Au-NPs synthesized using *Marsilea* leaf extract. Abbreviation: Ag-NPs-Silver nanoparticles, Au-NPs- gold Nanoparticles

Phytochemical examination reveals the presence of tannins, phenols, coumarins, flavonoids, anthro-quinones, terpenoids, saponins etc [26]. These phytochemicals are responsible for making the extract oxidant highly and thus might play a role in bio-reduction of gold and silver nanoparticles.

B. Physiochemical studies Ag-NPs and Au-NPs

XRD (X-ray diffraction): To further validate the synthesised nanoparticles performed by XRD. The size of NPs calculated by using Scherrer's formula; D =0.94 λ/β1/2 cos θ, where D represents the average crystalline domain size perpendicular to the reflecting planes, λ the X-ray wavelength, the full width of intensity at half maximum (FWHM), and θ represents the diffraction angle. The obtained XRD data was checked with the standard data for the respective samples COD No- 1509827 for Ag-NPs and COD No- 9012953 for Au-NPs.

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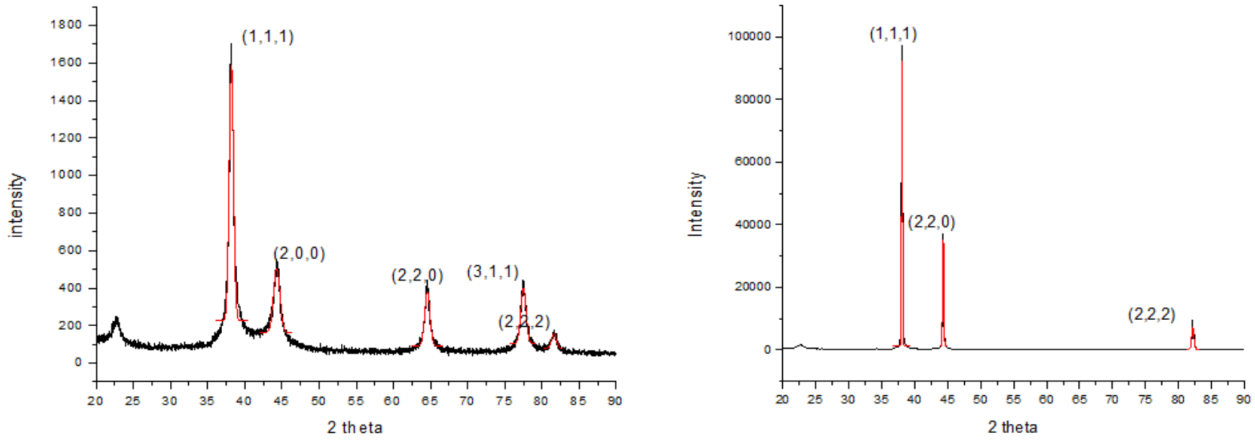

(a) **(b)**

Fig 2: X-ray diffraction pattern of silver and gold nanoparticles synthesized using *Marsilea* leaves extract. (a) Silver nanoparticles. (b) Gold nanoparticles.

Both Ag-NPs and Au-NPs showed cubic structure. In the case of Au-NPs, the lattice planes (111), (200), and (222), respectively. The intense peak obtained at 38° among 2θ values is the characteristics of Ag-NPs with lattice planes (111), (200), (220), (311) and (222) (Figures2(a) and 2(b). The average crystallite size for both the particles was calculated using Scherrer's formula ~28nm for Au-NPs and ~12nm for Ag-NPs (Table 1(a) and 1(b)). These results conform to the formation of colloidal silver, and gold nanoparticle successfully fabricated with extracts of *Marsilea*.

Table 1: a) Various sizes of Ag-NPs b) Various sizes of Au-NPs by *Marsilea* leaves extract using Scherrer's equation

Peak no.	Peak position	FWHM	Crystalline size (nm)
1	38°	0.69	12.66
2	44°	1.02	8.77
3	64°	0.82	11.93
4	77°	0.83	12.79
5	82°	0.78	14.00

(a)

Peak no.	Peak position	FWHM	Crystalline size (nm)
1	38°	0.19	15.82
2	44°	0.21	41.06
3	82°	0.40	27.05

(b)

FTIR: FTIR spectroscopy was used to identify the biological groups that bound on the gold and silver surface and involved in the fabrication of nanoparticles. Different peaks are shown different stretches of different bonds. The major peaks observed in FTIR of the sample were 3450, 2928, 1640, 1431, 1167, 1048, 882, 672 and 617 cm−1(figure 3). The peak at 3450 cm−1 is attributed to O-H and N-H stretch in phenols [27]. This peak indicates the presence of a phenolic compound [28]. Phytochemical evaluation of *Marsilea* also reports the presence of phenols [29]. The peak at 2928 cm−1 was due to C-H stretching vibration [30]. Peaks at 1640 cm−1 are credited to C=N and C=C, peaks at 1431, 1167 and 1048 cm−1 attributed to C-C, C-O, and C-N stretching vibrations [31,32,33]. The peak at 672 cm−1 can be credited CH=CH [34]. As seen in the case of FTIR for AuNPs, the peak at 1111 cm−1 had diminished entirely; this suggests that the extract might be bound to the AuNPs through or by replacing –COC-group. In FTIR of Ag-NPs presence of a peak at 1382 cm−1 correspond to amide II group.

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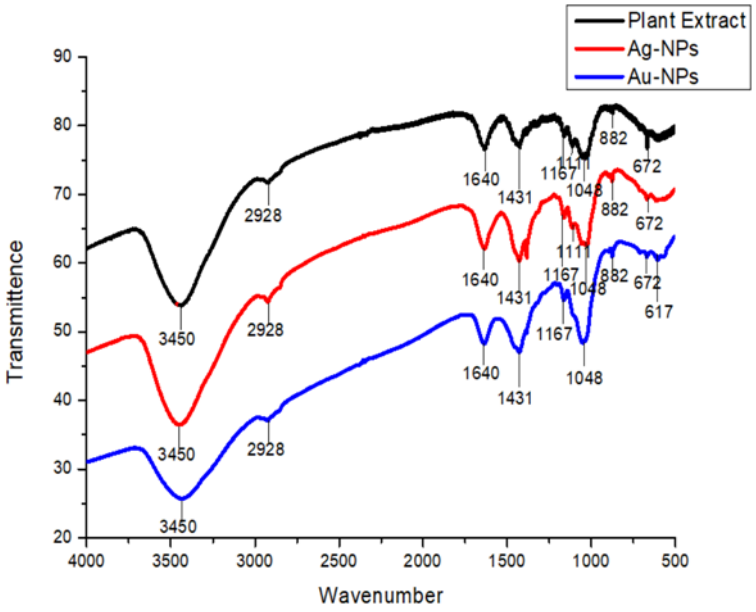

Fig 3: FTIR spectra for *marsilea* extract, the Ag-NPs and Au-NPs

These functional groups are water soluble compounds; such as flavonoids, terpenoids, and phenols are present in the *Marsilea* extract [19], seems to be responsible for the synthesis of nanoparticles and their stabilisation. Other scientists also reported that proteins or polysaccharides are present in the extract as biological material cap and stabilise nanoparticles [35]. These interactions may occur between free amino groups, a carboxylate group, or cysteine residues in the proteins [36].

SEM analysis of nanoparticles: The SEM analysis of nanoparticles was used to determine the surface morphology and shape of nanoparticles. In this study SEM images (Figure 4) has shown individual nanoparticles as well as many aggregates. The Fig. (a) revealed that the shape of Ag-NPs was found approximately spherical and Fig. 4(b) showed gold nanoparticles were predominantly cubical with uniform shape.

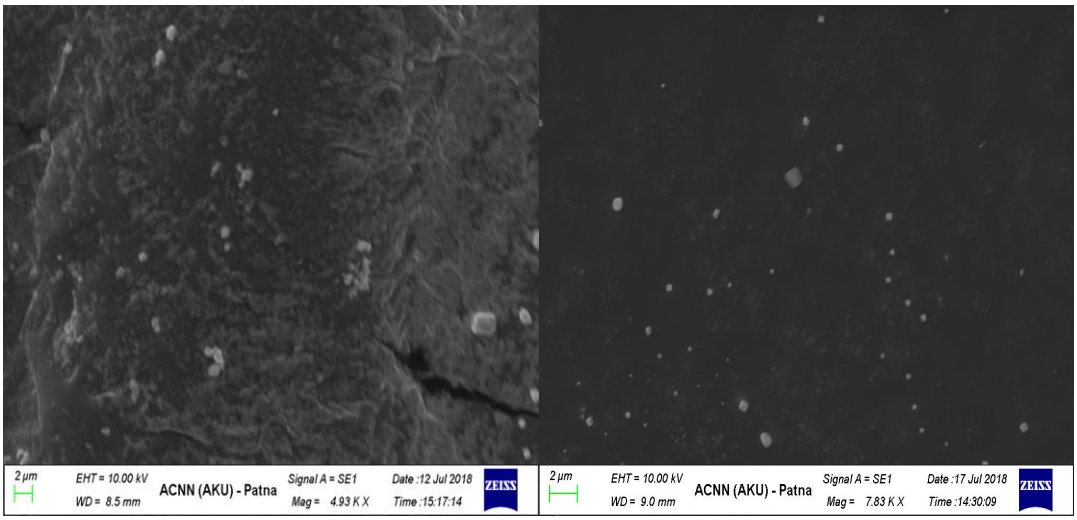

Fig 4: SEM images of Ag-NPs and Au-NPs synthesized using *marsilea* leaf extract. (a) Silver nanoparticles. (b) Gold nanoparticles. Abbreviation: SEM- Scanning electron microscope, Ag-NPs-Silver nanoparticles, Au-NPs- gold Nanoparticles

Cell viability studies: To evaluate the viabilities of cancer and normal cell lines in the presence of synthesised NPs, HEK293 and MCF-7 cells have been selected. For these cell viability studies, the synthesised NPs were evaluated on

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selected cell lines using MTT assay. MTT assay is a widely used method to estimate the number of viable cells in a particular format of treatment. It is based on the observation that viable cells are having active metabolism convert MTT into purple coloured crystals of formazan that absorb at 570 nm, on the other hand, dead cells lose the capability to convert MTT into formazan [37]. The NPs were screened in the concentration range of 0-100 µg/ml, and treatments were given for 48 h. Synthesized NPs, considerably inhibits the viability of MCF-7 cells (cancerous one) (Figure 5(a) and 5(b)) and on the other hand these NPs doesn't affect the viability of HEK293 cell (normal cells) even at 100 µM concentration (Figure 5 (c) and 5 (d)).

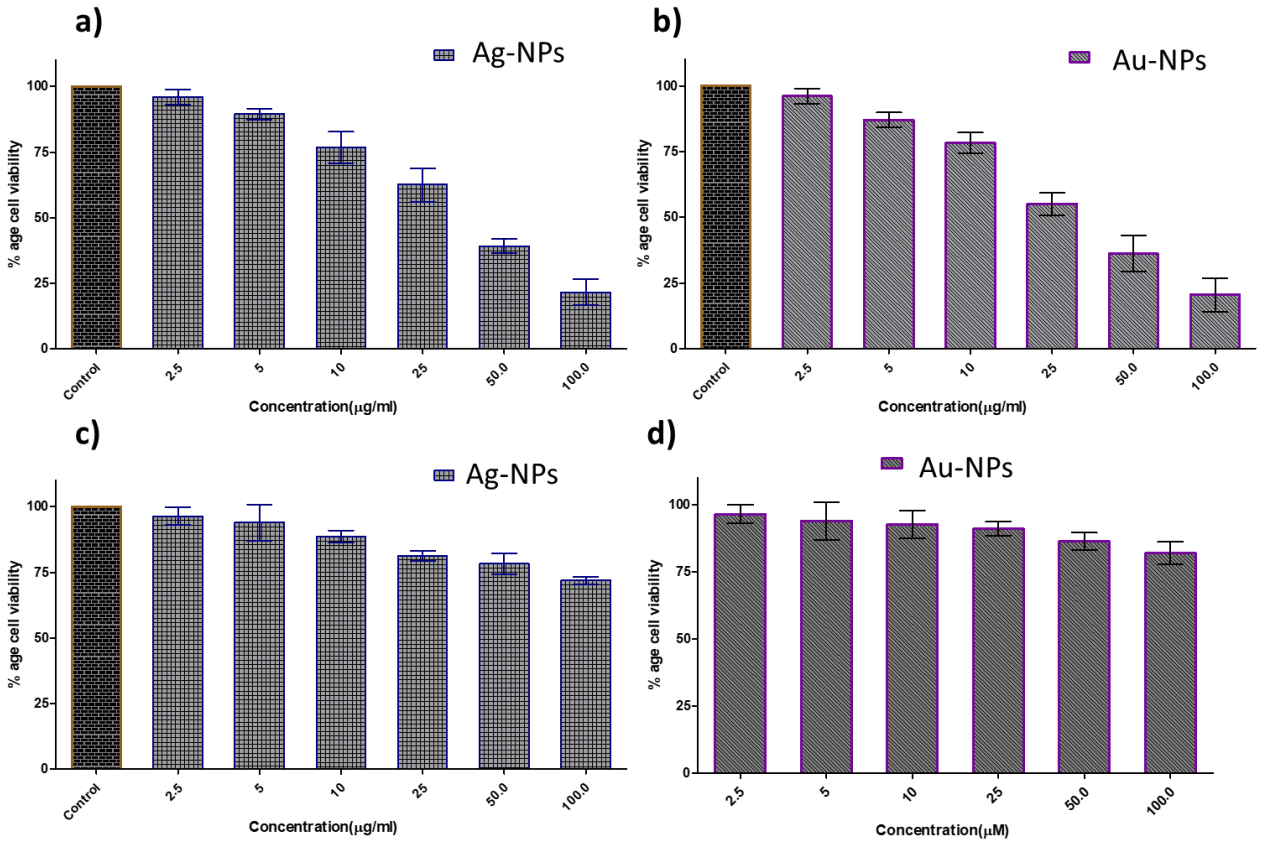

Fig 5: Cell cytotoxicity studies of *marsilea* mediated silver and gold NPs: Cell viability of (a) MCF-7 cells, (c) HEK-293 cells in presence of increasing concentrations of synthesized Ag-NPs. Cell viability of (b) MCF-7 cells, (d) HEK-293 cells in presence of increasing concentrations of synthesized Au-NPs studied with the help of MTT assay. Percentage cell viability was determined with respect to the control cells (cells treated with media only) and plotted as a function of concentration. Abbreviation: Ag-NPs-Silver nanoparticles, Au-NPs- gold Nanoparticles, MCF- Michigan cancer foundation (Breast cancer cell line), HEK-Human embryonic kidney, MTT-(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide).

These results suggested that the synthesised NPs inhibits explicitly the viability of cancerous cell only and are non-cytotoxic to HEK293 cells in the tested concentration range (Figure 5). Based on these descriptions we can speculate that synthesised NPs may be used as promising anticancer agents, as they inhibit the viability of human breast cancer (MCF-7) cells and bear no toxicity towards normal human cells.

IV. DISCUSSION

Plants can survive in stress conditions by developing different mechanisms, one of them is the antioxidant system. *Marsilea* also has an antioxidant activity which can play an essential role in chelating transitional metals [38]. This is due to the presence of secondary metabolites. It was hypothesised that these secondary metabolites like tannins, ascorbic acid, quercetin and kaempferol are responsible for the reduction of Ag & Au ions to Ag & Au nanoparticles and the proteins encapsulated these nanoparticles which is the reason for its stability (Figure 6).

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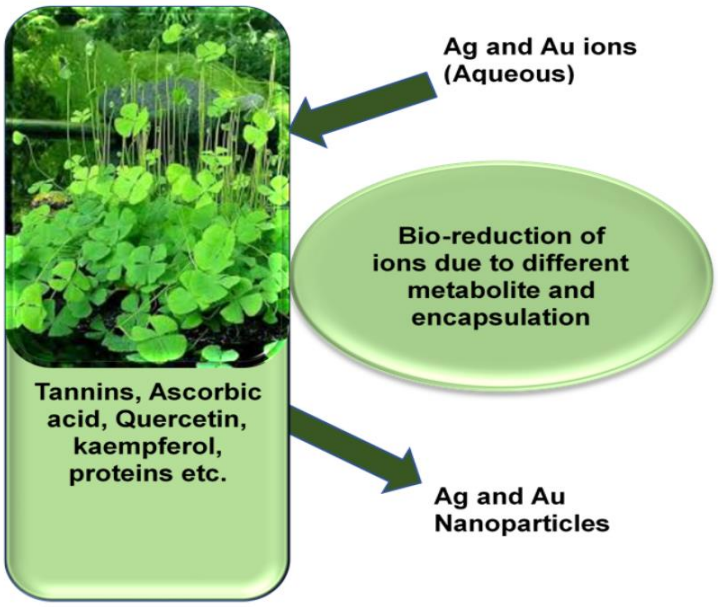

Fig 6: Role of metabolites in bio-reduction silver and gold nanoparticles.

The aquatic fern *Marsilea* with many bioactive compounds useful as reducing agent in the fabrication of Ag & Au nanoparticles. The nanoparticles of silver and gold showed remarkably significant activity against cancer cells in vitro because they get functionalized by the unused metabolites. However, an optimisation may be carried out in this regard, and our work in that direction is on.

V. CONCLUSION

In conclusion, both Ag-NPs and Au-NPs were successfully synthesised using *Marsilea* leaves extract. Ag-NPs were found to be spherical while Au-NPs exhibited cubic morphology. The anticancer activity of *Marsilea* extract synthesised Ag-NPs and Au-NPs was investigated for the first time against cancer cells. MTT assays have indicated the synthesised NPs inhibits explicitly the viability of cancerous cell only and are non-cytotoxic to normal cells. However, these nanomaterials are more biocompatible and are found to be the least toxic. The overall report emphasises cost effective, single step and eco-friendly synthesis of NPs. Hence, the green synthesised nano-biomaterials may have promising therapeutic potentials, and their application has been extended to cancer diagnosis and treatment shortly.

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REFERENCES

[1] S. Rajeshkumar, Anticancer activity of eco-friendly gold nanoparticles against lung and liver cancer cells, Journal of Genetic Engineering and Biotechnology, (2016), http://dx.doi.org/10.1016/j.jgeb.2016.05.007

[2] J.S. Devi and B.V. Bhimba, Anticancer Activity of Silver Nanoparticles Synthesized by the Seaweed Ulva lactuca Invitro, Sci. Rep. 1 (4) (2012) 242-244 http://dx.doi.org/10.4172/scientificreports.

[3] F.S. Rosarin, V. Arulmozhi, S. Nagarajan, S. Mirunalini, Antiproliferative effect of silver nanoparticles synthesised using amla on Hep2 cell line.Asian Pac J Trop Med. 6(1): (2013) 1-10. doi: 10.1016/S1995-7645(12)60193-X.

[4] J.S. Devi, B.V. Bhimba, K. Ratnam, Invitro anticancer activity of silver nanoparticles synthesised using the extract of Gelidiellasp. Int. J. Pharm. Pharm. Sci. 4 (4), (2012) 710–715.

[5] K. Renugadevi, D. Inbakandan, M. Bavanilatha, V. Poornima. Cissusquadrangularis assisted biosynthesis of silver nanoparticles with antimicrobial and anticancer potentials, Int. J. Pharm. Bio. Sci. 3 (3) (2012) 437–445.

[6] N. Thangaraju, R.P. Venkatalakshmi, A. Chinnasamy, K. Pandian, Synthesis of silver nanoparticles and the antibacterial and anticancer activities of the crude extract of Sargassum polycystum C, Nano Biomed. Eng. 4 (2) (2012) 89–94.

[7] M.V. Yezhelyev, X. Gao, Y. Xing, A. Al-Hajj, S. Nie, R.M. O'Regan, Lancet Oncol. 7 (2006) 657–667.

[8] K Prasad, A K Jha, AR Kulkarni, Lactobacillus assisted synthesis of titanium nanoparticles, Nanoscale Research Letters, Volume 2, Issue 5, (2007) Pages 248.

[9] A K Jha, K Prasad, AR Kulkarni, Synthesis of TiO2 nanoparticles using microorganisms, Colloids and Surfaces B: Biointerfaces, Volume 71, Issue 2, (2009) P- 226-229.

[10] R. Sanghi and P. Verma, Microbes as green and eco-friendly nanofactories. Green Chem Environ Sustainable;15: (2010) 315–39

[11] G.S. Dhillon, S. K. Brar, S. Kaur, M. Verma, Green approach for nanoparticle biosynthesis by fungi: current trends and applications. Crit Rev Biotechno; 32: (2012) 49–73.

[12] M. Sastry, A. Ahmad, M. I. Khan, R. Kumar, Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci;85: (2003)162–70.

[13] A K Jha and K Prasad, Yeast mediated synthesis of silver nanoparticles, International Journal of Nanoscience and Nanotechnology, V-4, Issue 1, (2008) P 17-22.

[14] A K Jha and K Prasad, Biosynthesis of gold nanoparticles using bael (Aegle marmelos) leaf: mythology meets technology, International Journal of Green Nanotechnology, V-3, Issue 2, (2011) P 92-97.

[15] A K Jha and K Prasad, Aquatic Fern (Azolla Sp.) Assisted Synthesis of Gold Nanoparticles, International Journal of Nanoscience. 15,(2016)1650008 https://doi.org/10.1142/S0219581X16500083.

[16] A K Jha and K Prasad, Green synthesis of silver nanoparticles using Cycas leaf, International Journal of Green Nanotechnology: Physics and Chemistry, V-1, Issue 2, (2010) P110-P117.

[17] Mugdha Rao, Babita Jha, Anal K Jha, Kamal Prasad, Fungal Nanotechnology: A Pandora to Agricultural Science and Engineering, Fungal Nanotechnology, (2017) P 1-33.

[18] M. J. Mithraja, J. M. Antonisamy, M. Mahesh, Z. M. Paul, and S. Jeeva, Inter-specific variation studies on the phytoconstituents of Christella and Adiantum using phytochemical methods, Asian Pacific Journal of Tropical Biomedicine, vol. 2, no. 1 (2012) pp. 40–45.

[19] Gopalakrishnan K, Udayakumar R. Phytochemical content of leaf and stem of Marsileaquadrifolia (L.). J Plant Sci Phytopathol. 1: (2017) 026-037. https://doi.org/10.29328/journal.jpsp.1001003.

[20] D.G. Sant, T.R. Gujarathi, S.R. Harne, S. Ghosh, R. Kitture, S. Kale, B.A. Chopade, and K.R. Pardesi, (2013) Adiantum phillipense L. Frond Assisted Rapid Green Synthesis of Gold and Silver Nanoparticles. Journal of Nanoparticles, pp. 1–9. doi: 10.1155/2013/182320.

[21] .A K Jha and K Prasad, Understanding mechanism of fungus mediated nanosynthesis: a molecular approach, Advances and Applications Through Fungal Nanobiotechnology, (2016) P 1-23.

[22] X. Baskaran, A.V.G. Vigila, T. Parimelazhagan, Muralidhara-Rao, S. Zhang, Biosynthesis, characterisation, and evaluation of bioactivities of leaf extract-mediated biocompatible silver nanoparticles from an early tracheophyte, Pteris tripartita Sw. International Journal of Nanomedicine, 11, (2016) pp. 5789–5805. doi: 10.2147/IJN.S108208.

[23] S. Sun, C. Murray, D. Weller, L. Folks, A. Moser, Monodisperse FePt nanoparticles and ferromagnetic FePt nanocrystal superlattices. Science, (2000) 287:1989–92.

[24] P. Khan, S. Rahman, A. Queen, S. Manzoor, F. Naz, G.M. Hasan, S. Luqman, J. Kim, A. Islam, F. Ahmad, M.I. Hassan, Elucidation of Dietary Polyphenolics as Potential Inhibitor of Microtubule Affinity Regulating Kinase 4: In silico and In vitro Studies, Sci Rep 7(2017) 9470.

[25] Queen, P. Khan, D. Idrees, A. Azam, M.I. Hassan, Biological evaluation of p-toluene sulphonylhydrazone as carbonic anhydrase IX inhibitors: An approach to fight hypoxia-induced tumours, Int J Biol Macromol 106(2018) 840-850.

[26] Yuan Zhang, Hai-Yan Tian, Ya-Fang Tan, Yuk-Lau Wong, Hoi Yan Wu, Jun-Feng Jia, Guo-En Wang, Jing-Jing Gao, Yi-Fang Li, Hiroshi Kurihara, Pang-Chui Shaw & Ren-Wang Jiang: Isolation and identification of polyphenols from Marsileaquadrifolia with antioxidant properties in vitro and in vivo, Natural Product Research: Formerly Natural Product Letters, (2015) DOI: 10.1080/14786419.2015.1062377.

[27] P. Devaraj, P. Kumari, Chirom Aarti, and A. Renganathan, Synthesis and Characterization of Silver Nanoparticles Using Cannonball Leaves and Their Cytotoxic Activity against MCF-7 Cell Line," Journal of Nanotechnology, vol. 2013, Article ID 598328, 5 pages, (2013). https://doi.org/10.1155/2013/598328.

[28] M. J. Mithraja, J. M. Antonisamy, M. Mahesh, Z. M. Paul, and S. Jeeva, Phytochemical studies on Azolla pinnata R. Br., Marsilea minuta L. and Salvinia molesta Mitch, Asian Pacific Journal of Tropical Biomedicine, (2011) S26-S29, DOI: 10.1016/S2221-1691(11)60116-0.

[29] Santhoshkumar S and Nagarajan N: Biological Synthesis of Silver Nanoparticles of Adiantum Capillus-Veneris L. And Their Evaluation of Antibacterial Activity against Human Pathogenic Bacteria. Int J Pharm Sci Res; 5(12): (2014) 5511-18.doi: 10.13040/IJPSR.0975-8232.5 (12).5511-18.

[30] S.Marimuthu, A. A. Rahuman, G. Rajakumar et al., "Evaluation of green synthesized silver nanoparticles against parasites," *Parasitology Research*, vol. 108, (2011) no. 6, pp. 1541–1549.

[31] N. Johnston, S. Krimm, An infrared study of unordered poly-L-proline in CaCl2 solutions, Biopolymers 10 (1971) 2597–2605.

[32] S.Y. Venyaminov, N.N. Kalnin, Quantitative IR spectrophotometry of peptide compounds in water (H2O) solutions. I. Spectral parameters of amino acid residue absorption bands, Biopolymers 30 (1990) 1243–1257.

[33] M.S. Braiman, D.M. Briercheck, K.M. Kriger, Modeling vibrational spectra of amino acid side chains in proteins: effects of protonation state, counterion, and solvent on arginine C\N stretch frequencies, J. Phys. Chem., B 103 (1999) 4744–4750.

[34] A. M. Priya, R. K. Selvan, B. Senthilkumar, M. K. Satheeshkumar, and C. Sanjeeviraja, "Synthesis and characterization of CdWO4 nanocrystals," *Ceramics International*, vol. 37, no. 7, (2011) pp. 2485–2488.

[35] . T.C. Prathna, Lazar Mathew, N. Chandrasekaran, Ashok M. Raichur and Amitava Mukherjee, Biomimetic Synthesis of Nanoparticles: Science, Technology & Applicability, Biomimetics Learning from Nature, Amitava Mukherjee (Ed.), ISBN: (2010), 978-953-307-025-4.

[36] S.P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, M. Sastry, Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract, Biotechnol. Progr. 22 (2006) 577–583.

[37] T. Mosmann, a Rapid colourimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J Immunol Methods 65(1983) 55-63.

[38] W. Zheng and S.Y. Wang, Antioxidant activity and phenolic compounds in selected herbs. J Agric. Food Chem., 49: (2001) 5165-5170.