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Synthesis of Agnps by Plant Extracts and Detection of the Biological Application

Khalida F.AL-Azawi[1], Azhar M. Haleem[2], Nehia N. Hussein[3]
[1]Applied Chemistry Division, Department of Applied Sciences,[3] Biotechnology Division, Department of Biotechnology- College of science,[2]Environmental Research Center/ University of Technology- Baghdad- Iraq

100122@uotechnology.edu.iq

Abstract Silver nanoparticles (AgNPs) synthesized by alcoholic extracts to *Tribulus terrestris* and *Quercus infectoria* plants by hot plat stirrer at 30 minutes. AgNPs were identified by UV-visible, X-Ray, FTIR, and SEM. The effects of plant extracts, AgNPs, and mixture of both of them were investigated on chromosomes of the peripheral blood lymphocytes by evaluating Blastogenic Index (BI), Mitotic Index (MI), and Total Chromosomal Aberrations (TCAs).
Keyword: Green synthesis, Medicinal plants, AgNPs, chromosomal aberrations, cytogenetic.

1. Introduction

Tribulus terrestris is a medicinal plant employed for healing many illnesses like hypertension [1]. It belongs to the (Zygophyllaceae) family, it is characterized by being an annual plant seen in several moderate and tropical regions around the world [2]. *T. terrestris* is additionally known as Puncture Vine, it includes steroidal saponins, and act like a normal testosterone organizer. *T. terrestris* enhances testosterone activity by enhancing Luteinizing Hormone. There is much assurance that *T. terrestris* is valuable as a sexual improvement plant [3]. Locally *T. terrestris* is used in traditional medicine as astringent, analgesic, aphrodisiac, antihypertensive, stomachic, diuretic, lithotriptic, and anti-inflammatory agent of urinary tract infections. [4, 5]. *Q. infectoria* generally is known as gall oak. It is a small tree growing to 4 to 6 feet tall, crooked, by smooth and bright leaves, scaly, and downy (Figure1) [6]. Phytochemical analyses of *Q. infectoria* gall extracts showed the residence of tannins, phenolic, alkaloids, flavonoids, and saponins, these are active reducing factors due to their rich -OH-groups that improve their antioxidant and antibacterial action [7]. The creation of nanoparticles has increased excessive significance in the previous years due to their single properties and applications [8]. Nowadays, the creation of nanoparticles is created by living organisms like bacteria, fungi, yeast, algae, or by plant extracts [9-12]. This study amid to biosynthesis of silver nanoparticles AgNPs by *T. terrestris* and *Q.infectoria* galls alcoholic plant extracts and studding the toxicity effects by investigation of chromosomal aberration.

Figure 1. Plants appearance of (A) *T. terrestris* and (B) *Q. infectoria*

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2. Materials and methods

2.1. Preparation of plant extracts

Arial Plant parts were bought from the local market in Baghdad/Iraq in May 2020. Dried plants were extracted by the soxhlet with 70% ethanol. The extracts were fully removed by a rotary evaporator to obtain a semi-solid mass and then transferred to an oven to produce the crude extract. Portions were stored at 40 C until used [13].

2.2. Synthesis of AgNPs:

Ten milliliters of pre-prepared plant extracts have been added to 90 mL of 1 mM of AgNO3 at room temperature, then the mixture was stirring magnetically at 1000 rpm for 30 minutes [14].

2.3. Characterization of AgNPs:

2.4. The photoactivity of prepared AgNPs was detected using UV-Vis spectrophotometer (PG-T80+ UV/Vis spectrophotometer, England) from 350-700 nm wavelength range, while the crystallite space size was evaluated by XRD crests, Possibly free from irregular shapes, using the Scherrer equation[15].

$D= 0.94 \lambda / \beta \cos \theta$…… (1)ط

2.5. Chromosomal Assay:

Chromosomal studies were carried out according to [16] by taking five millimeters of venous blood from healthy persons, unexposed to slightly direct contamination, nonsmokers, unexposed to any chemical or physical treatment with age from (25-35) years old. Chromosomal assay were carried out by mixing 0.5mL of peripheral blood to 4.5mL of complete tissue culture (RPMI-1640) provided with 10% of fetal calf serum, and 10 µg/mL of phytohemagglutinin (PHA) with different concentrations of synthesized AgNPs (0.0, 50, 100, 150, 200 and 250) µg/mL, in addition to one concentration of mitomycin MMC (0.65) µg/mL as a positive control. after 70 hours of incubation in the carbon dioxide incubator at 37º C, we added 10µg/mL of Colchicine solution to the test tubes and reincubated at 37º C for an extra two hours, then all tubes were centrifuged at 3000g for 10 minutes, the precipitated cells were mixed with 5 mL of hypotonic solution (KCl) and returned to the incubator for 45 min at 37º C. after that we got rid of the excess solution by centrifugation at 3000g and re-suspended the precipitated cells with an adequate amount of cool fixative solution (3:1 volume to volume of absolute methanol and glacial acetic acid), the final solution was added gradually with continuous mixing, After repeating this process several times, we obtained a transparent white precipitate of lymphocytes, a volume of this cellular deposit was dropped onto clean, precooled glass slides.. Blastogenic Index (BI) was calculated from 1000 transformed cells, and Mitotic Index also calculated as shown in the following equation

MI= Mitotic cells/ 1000 cells ×100 %.....(2)

While the total chromosomal aberration calculated in 25 mitotic cells.

2.6. Statistical analysis

Statistical Package for Social Science (SPSS) software version 16.0 was used to analyze the data, by analysis of variance ANOVA (I) and calculating least significant differences (LSD) at $p \leq 0.05$.A subsectionSome text.

3. Result and discussion

3.1. Biosynthesis of AgNPsCharacterization:

*T. terrestrisPure suspensions*was yellowish in color after expansion of AgNO3 and heated for 30 min, Alteration to deep greenish color was observed for the indication to formation of AgNPs

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[17]. Likewise in the case of the synthesis of AgNPs for the extract of alcoholic *Q. infectoria*, they were through the color change of the solution from a light yellow to a dark gray color and optimal synthesis conditions were at the acidic function pH 4.3 within 20 minutes, this result agreed with the findings of previous studies [18], some studies have indicated that an acidic neutral function is the best in the synthesis process (Figure 2) [19]. FTIR Spectral scanning of samples in Figure (3) was conducted within the range of 400-4000 cm^{-1}, and the results were compared with one of the studies [20].

The results showed that the alcoholic extract of the electrode plant has the presence of bundles at the range (3363.86 cm^{-1}), which refer to OH bars, as the presence of bundles at the range (2943.37 cm -1) indicates the presence of the C-H and the beams indicate at (1897.95 cm -1) on the presence of the double bond C = O, while the beams at (1622.19 cm-1) refer to double pinch C = N, while the beams at (1500.67 cm-1) refer to the presence of the C = C bond compared with the nanoparticles created by the*T. terrestris* plant extract. The phases CH disappeared and the phases O-Ag appeared at the bundle (688.61 cm -1), while all the bonds in the crude alcohol extract were found O-H, C = O, C = C and C = N at the range (3370.20-1820.86-1776.50-1591.33 cm -1) respectively. The spectral absorption results of the alcoholic extract of *Q. infectoria* showed the presence of bundles at the range (3730.45 cm-1), which indicates the presence of OH bars and the presence of the beams at the range (3203.87 cm -1) refer to the C-H aorta while the beams at the range (2360.95 cm- 1)refer to the SH bond, the beams at the range (1780.38 cm-1) indicate the presence of the double bond C = O, while the beams at (1666.55 cm-1) indicate the presence of the double bond C = N, and the beams at (1587.47 cm -1) on the presence of C = C, compared to the nanoparticles created by *Q. infectoria* extract, S-H disappeared and O-Ag appeared at the beam (50.736 cm-1), and all the elements present in the raw alcoholic extract O-H, CH, C = O, and C = N and C = C at the range (3600.41-3201.24-1770.32- 1622.49-1590.08 cm -1), respectively. When applying the Scherer equation In the XRD analysis in figure (4) the average size of the nanoparticles created by the *T. terrestris* plant extract was equal to 35.0 nanometers at the angle 2θ in degrees (29.75, 28.29, 27.31) fig. (4-31) and this is greater than the size reached [21], as it was found that the average size of the silver nanoparticles was 24.6 nm, and the average size of nanoparticles created by *Q. infectoria* extract was 12.6 nm at angle 2θ in degrees (26.30, 43.84, 65.67) figure (4-33) and this volume was less than it reached [18].Figure (5) shows theSEM analysis of the phenotypic properties for the nanoparticles created by alcoholic plant extracts are spherical and their average size is within the range (33-84) nm.

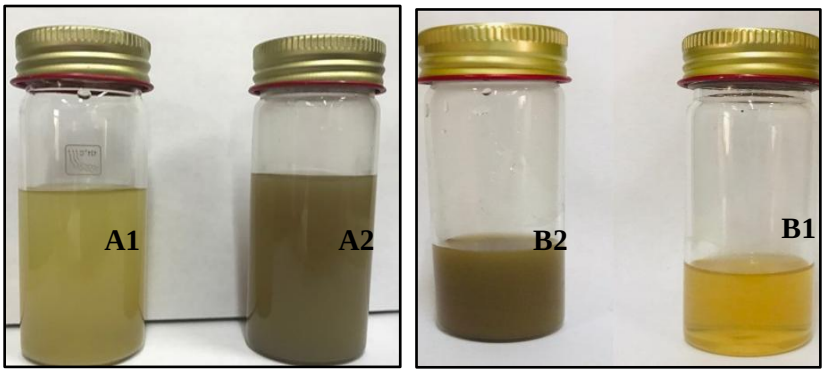

Figure 2: (A1)refer to *T. terrestris* plant extract (A2) refer to biosynthesis of AgNPs by *T. terrestris*, (B1) refer to *Q. infectoria* plant extract, and (B2) refer to biosynthesis AgNPs by *Q. infectoria*

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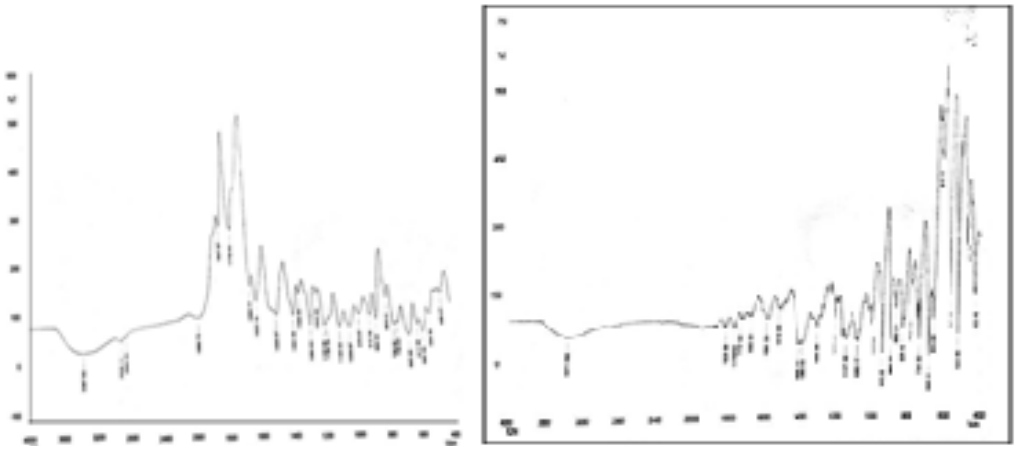
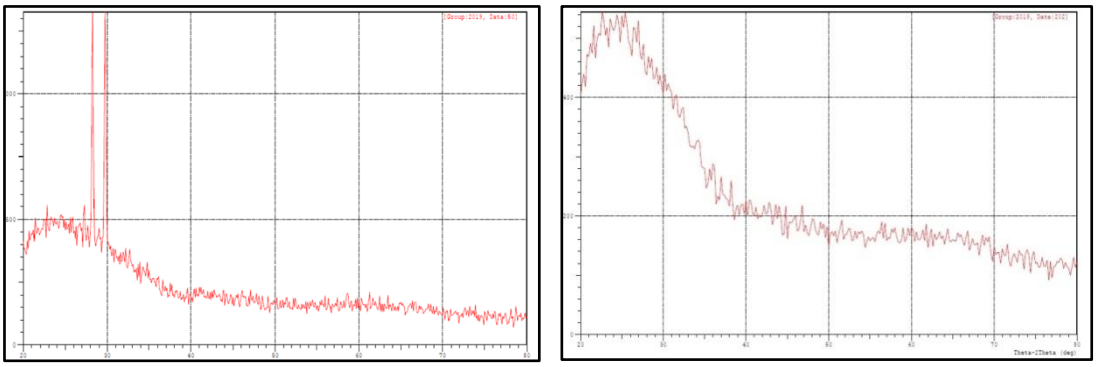
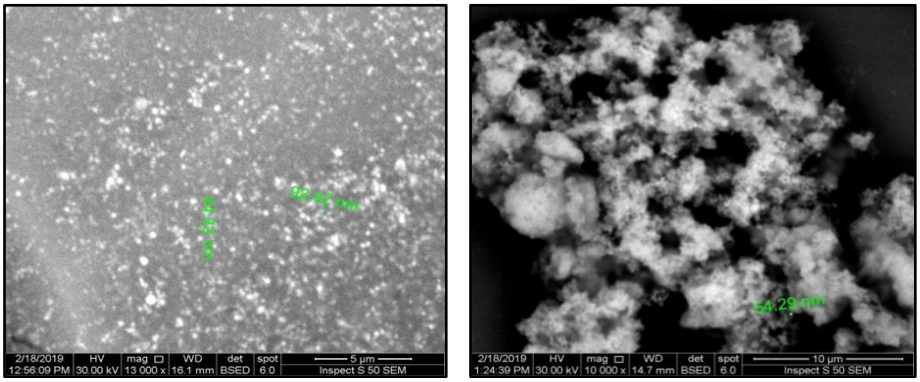


Figure 3 .FTIR spectrumof (A1) *T. terrestris*, (B1) *Q. infectoria* plant extract and (A2) AgNPs by *T. terrestris*,and (B2) AgNPs by *Q. infectoria*.



A **B**

Figure 4. XRD examples recorded from drop-covered films on glass substrate of AgNPs blended by treating (A) *T. terrestris* extract, and (B) *Q. infectoria* with AgNO3 aqueous solution.



A **B**

Figure 5. The spherical shape of nanoparticles by SEM (A) for *T. terrestris*, and (B) for *Q. infectoria*

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3.2. Chromosomal analyses:

Both Blastogenic Index (BI) and Mitotic Index (MI) are considerable bioindecator for examination, for many types of physical and chemical agents on living cells especially peripheral blood lymphocytes (PBL), since of their sensitive to such variables & the ability of measuring the impacts on their incidence and recurrence rate. From findings that were described in table (1), the Mitotic index (MI) and Blastogenic index (BI) reduced significantly in PBLs treated with different concentrations of AgNPs prepared by medicinal plants *T. terrestris*, and *Q. infectoria* with increasing the concentrations of AgNPs prepared in both plants However, this decrease remained less than the value of the decrease resulting from the treatment of PBLs with 0.65 µg/mL of mitomycin C (MMC) that known for its mutagenic impacts on many types of cells including PBLs, on the other hand Total Chromosomal Aberrations (TCAs) has been increased gradually with (AgNPs prepared in both *T. terrestris*, and *Q. infectoria*respectively. The genotoxic and DNA damage associated with AgNPs might be causes from its unique characteristics for mineral nanoparticles such as high surface area, small size, shape, coverings, and charge of particles surface. Nanoparticles react chemically with biomolecules and produce reactive oxidative Stress (ROS) such as hydroxyl radicals and hydrogen peroxide conclude superoxide radical (O^{2-}), induce an inequality in the macrobiomolecules causes irreparable and irreversible damage to important parts of the cell, such as the cell membrane, and the manufacture of proteins, enzymes, and the DNA molecule [22, 23].

Table 1. Chromosomal analyses of AgNPs prepared by *T. terrestris*, and *Q. infectoria* for peripheral blood lymphocytes (PBL).

Q. infectoria				*T. terrestris*			
Concentration µg/mL	BI	MI	TCA	Concentration µg/mL	BI	MI	TCA
0.0	33.12±0.55	0.66±0.02	0.18±0.01	0.0	33.12±0.55	0.66±0.02	0.18±0.01
50	32.8±0.61*	0.63±0.011*	0.18±0.08*	50	*31.22±2.1	0.62±0.13*	0.19±0.01*
100	32.77±0.22*	0.58±0.02*	0.21±0.05*	100	*31.15±1.1	0.59±0.06*	0.21±-.01*
150	31.15±0.31*	0.57±0.05*	0.25±0.06*	150	30.77±0.22*	0.55±0.22*	0.21±0.02*
200	30.67±0.44*	0.51±0.04*	0.28±0.22*	200	30.58±0.13*	0.51±0.11*	0.23±0.11*
250	30.12±0.28*	0.51±0.11*	0.32±0.12*	250	30.44±0.34*	0.47±0.113*	0.28±-.02*
0.65 MMC	22.15±0.02*	0.12±0.01*	0.55±0.18*				

* Significant at p≤0.05, Data represented as M±SD

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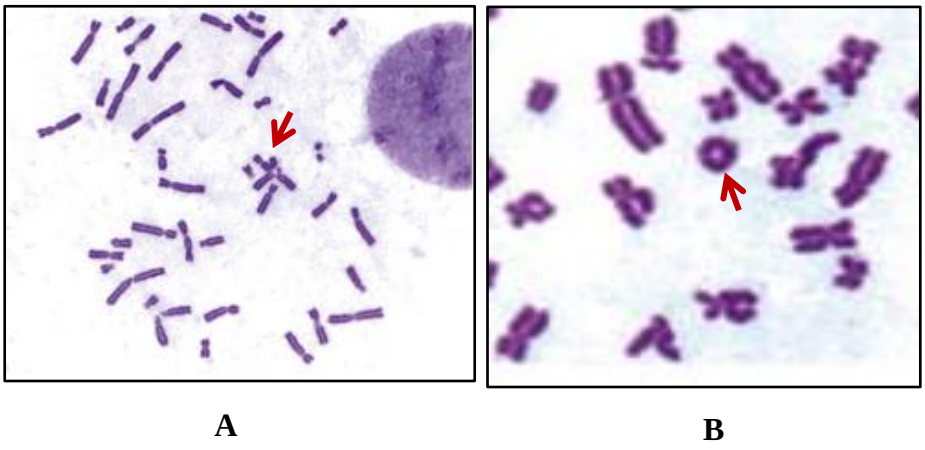

A B

Figure 6. Chromosomal aberrations (A) chromatid exchange induced by 100µg/mL of AgNPs (40X) (B) ring chromosome induced by 150µg/mL of AgNPs prepared by *T. terrestris* (100X)

Conclusion

From the results we can conclude that AgNPs can be prepared by biosynthesis methods by using plant materials, but these nanometals can be induced considerable DNA damage in the PBLs this may lead to mutagenic effects and causes irreversible destruction in the genome, Therefore, many studies must be conducted on different biological systems to determine the level of damage resulting from exposure to such nanoparticles.

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Conflict of interest statement

None.

References

[1] **Mothana** RA, Lindequist U, Antimicrobial activity of some medicinal plants of the island Soqotra. J. Ethnopharmacol, 2005, 96(1- 2):177–181.
[2] **Abeywickrama**K, Bean GA, Toxigenic *Aspergillus flavus* and aflatoxins in Sri Lankan medicinal plant material, Mycopa-thologia, 1991, 113: 187–190.
[3] **Gauthaman** K, Ganesan AP, Prasad RN, Sexual effects of puncturevine (*Tribulus terrestris*) extract (protodioscin): an evaluation using a rat model, Journal of Alternative and Complementary Medicine, 2003, 9 (2): 257–265.
[4] **Majeed** SH, Mahmood MJ, Herbs and Medicinal Plants in Iraq between Traditional Medicine and Scientific Research, 1st Ed. Baghdad: Dar Al-Thaowra for Publishing., 1988, p. 40. (in Arabic).
[5] **Aldein** S, Medicinal Herbs, 1st Ed. Baghdad: Dar Al-Shoun AlThaqafia Al-Aama for Publishing.1986, p. 70. (in Arabic).
[6] **Ullah Khan** , F. ., & Hussain , N. . (2020). NH Serological and Molecular Based Diagnosis of Toxoplasma gondii in Galliformes by using ToxPK1 gene. *Journal of Scientific Research in Medical and Biological Sciences*, *1*(2), 116-122. https://doi.org/10.47631/jsrmbs.v1i2.58
[7] **Dhiman** AK. Ayurvedic drug plants. Delhi, India: Daya Publishing House. 2006.
[8] **Zaki** S, ElKady M, Farag S, Abd-El-Haleem D, Determination of the Effective Origin Source for Nanosilver Particles Produced by Escherichia coli Strain S78 and Its Application as

Antimicrobial Agent, Materials Research Bulletin, 2012, 47, 4286-4290.

[9] **Garlapati** VK, Vundavilli RP, BanerjeeRE,valuation of lipase production by genetic algorithm and particle swarm optimization and their comparative study, Appl Biochem Biotechnol, 2010, 162(5): 1350-1361.

[10] **Philip** D, Unni C, Aromal S, Vidhu V, Murraya koenigii Leaf-Assisted Rapid Green Synthesis of Silver and Gold Nanoparticles, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2011, 78, 899-904.

[11] **Meah** RK, Jaaffar AI, Al-Azawi**,** preparation of juniperus extract and detection of its antimicrobial and antioxidant activity, Iraqi Journal of Agricultural Sciences, 2019, 50(4):1153-1161.

[12] **Al Majeed** RM, Hussein NN, AL-Azawi KF, Detection the antimicrobial activity of agnps synthesized by quercus infectoria plant, Plant Archives, 2020, 20 (1), 579-584.

[13] **Maeah RK**, Hasoon BA, Abd-Alwahab AI, AL-azawi KF, Hameedi WB, Biosynthesis of silver nanoparticles using Hibiscus sabdariffa and their biological application,2020, Eurasia J Biosci 14, 3377-3383 .

[14] **Nehia** NH, Raghad KM, Zahraa AS, Aasoon B, Sulaiman GM, Ayad A, Taha AA, Jwad KH, Cytotoxic, Antioxidant and antibacterial activities of crude extract of *Syzygium aromaticum* plant, Biochem.Cell.Arch. 2018, 18,2,1721-1726.

[15] **Suliman** GM, Mohammad AW, Abdul-wahed HE, Ismail MM, Biosynthesis ,antimicrobial and cytotoxic effects of silver nanoparticles using *Rosmarinus officinalis* extract. Digest Journal of Nanomaterials and Biostructures. 2013, 8, 1, 273 – 280.

[16] **Jeevan** P, Ramya K, Rena AE, Extracellular biosynthesis of silver nanoparticles by culture supernatant of *Pseudomonas aeruginosa*, Indian Journal of Biotechnology. 2012,11, 72-76.

[17] **Verma**, R, Babu A, Human chromosomes: Manual of Basic techniques. Pregramon press, **New** York, 1989.

[18] **Lu** F, Gao Y, Huang J, Sun D, Li Q, Roles of Biomolecules in the Biosynthesis of Silver Nanoparticles: Case of *Gardenia jasminoides* Extract. Chinese Journal of Chemical Engineering, 2014, 22, 706-712.

[19] **Heydari**R, Rashidipour M, Green Synthesis of Silver Nanoparticles Using Extract of Oak Fruit Hull (Jaft): Synthesis and In Vitro Cytotoxic Effect on MCF-7 Cells Hindawi Publishing Corporation International Journal of Breast Cancer, 2015, 846743, p6-8.

[20] **Cynthia**A, Vivekanandhan S, Misra M, Mohanty AK, Switch grass (*Panicum virgatum*) extract mediated green synthesis of silver nanoparticles, World Journal of Nano science and Engineering, 2012, 2, 47-52.

[21] **Pham** T, Sassaroli A, Blaney G, Fantini S, New near-infrared spectroscopy method for local measurements of cerebral blood flow. In European Conference on Biomedical Optics (2019, June). Optical Society of America.

[22] **Gopinatha** V, MubarakAlib D, Priyadarshinia S, Meera N, Priyadharsshinia NT, P. Velusamya P, Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: A novel biological approach. Colloids and Surfaces B: Biointerfaces, 2012, 69-74.

[23] **Haleem** AM, Abbas RH, Jawad, MA, Alberaqdar F, Cytotoxic Effects of Titanium Dioxide Nanaoparticles Synthesized by Laser Technique on Peripheral Blood Lymphocytes and Hep-2 Cell Line. Toxicol. Environ. Health Sci, 2019,11, 219–225.

[24] **Kumar** V., Sharma N., Maitra S. S. ," In vitro and in vivo toxicity assessment of nanoparticles" International Nano Letters. 2017, 7,243–256.

[25] **Ubaoji**, K., Nwosu, O., Agu, K., Nwozor, K., Ifedilichukwu, N., & Okaka, A. (2020). Gas Chromatographic Analysis of the Phyto-Constituents and the Assessment of the Anti-Microbial Properties of the Leave Extracts of Nigeria-Grown Gingko biloba. *Journal of Scientific Research in Medical and Biological Sciences*, *1*(2), 45-56. https://doi.org/10.47631/jsrmbs.v1i2.57