

RESEARCH ARTICLE

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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING HIBISCUS SABDARIFFA FLOWER EXTRACT AND THEIR SYNERGISTIC ANTIMICROBIAL, ANTIOXIDANT, AND CYTOTOXIC ACTIVITIES

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Abstract

Multidrug-resistance in clinically relevant pathogens is a global problem, leading to renewed efforts for identifying and developing alternative therapeutic modalities that are not limited by the constraints associated with traditional antibiotics. Aims: To green fabricate silver nanoparticles (AgNPs) with Hibiscus sabdariffa leaf extract as a natural reducing and stabilizing agent. The nanoparticles synthesized were characterized using ultraviolet–visible spectroscopy, transmission electron microscopy, Fourier-transform infrared spectroscopy and X-ray diffraction, confirming formation of the particles with mostly spherical morphology and 45 nm mean particle size. In the microbiolog ical results, zones of inhibition against Escherichia coli and Staphyloco ccus aureus were shown for the extracts of 23 mm and 28 mm respectively, which were higher than those produced by the plant extract alone. Antioxidant analysis indicated high dose-dependent radical-scavenging activity of AgNPs(91% inhibition of 2,2-diphenyl-1-picrylhydrazyl at 150 µg/mL) compared with crude extract (75%). Cytotoxicity tests using the 3 (4,5 dimethylthiazol 2 yl) 2,5 diphenyltetr azolium bromide assay revealed a concentration-dependent response with half-maximal inhibitory concentration (IC_{50}) of 75 µg/mL (AgNPs), and 45 µg/mL for conjugated AgNPs; while the plant extract resulted in less toxicity (IC_{50} =180 µg/mL). In summary, the results reveal that H. sabdariffa mediated AgNPs exhibit effective antimicrobia l and anticancer properties. Furthermore, the study highlights green nanoparticle synthesis as a friendly-ecological, with sustainability alternative to traditional nanoparticle synthesis methods.

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Introduction:-

The remarkable physicochemical properties of nanoparticles (NPs), like a large surface area-to-sizeproportion, adjustable reactivity, and improvedimpactonecosystems, have enabled nanotechnology to play a pivotal role in today's biomedical science. Given these features, NPs can be successfully employed in drug delivery, antimicrobial treatment, diagnosis, biosensing, and tissue engineering.[1–3] Of the different metallic NPs, silver nanoparticles (AgNPs) have been the primary focus of interest owing to their strong antibacterial, antioxidant, antiviral, and

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anticancer activities. AgNPs act via membrane destruction, disruption of intracellular metabolic pathways, and oxidatation stress by producing reactive oxygen species (ROS),[4–6] representing a promising treatment for multidrug-resistant pathogens as well. Greenmanufacture of NPs has gotten significant interest because of their environmental friendliness compared with other chemical and physical methods. The fabrication of plant-based NPs offers several advantages over chemical and physical synthesis routes[7–9] predominantly because plant extracts are rich in biomolecules liketerpenoids, flavonoids, phenolics, proteins, and organic acids, which act as both stabilizing and reducing factors, thereby giving the synthesis of NPs without the use of toxic materials.[10] Such biosynthesized NPsexhibit better biocompatibility and improved biological activity.Hibiscus sabdariffa (Roselle) has emerged as an excellent botanical source for NP biosynthesis because of its rich phytochemical profile, including anthocyanins, flavonoids, polyphenols, and organic acids. These compounds demonstrate potent antibacterial, antioxidant, and anti-inflammatory activities, effectively facilitating NP reduction and stabilization.[10–12] Recent studies have confirmed the successful synthesis of AgNPs and zinc oxide NPs (ZnO-NPs) using H. sabdariffa extracts, reporting improved stability, reduced aggregation, and enhanced antimicrobial and antioxidant activities.[13–15]

Bioactive substances in H. sabdariffa act synergistically with metallic NPs, augmenting their biological activity in multiple ways, including enhanced ROS induction, membrane permeation, biofilm synthesis suppression, and quorum sensing disruption at the overall antimicrobial level.[16–18]H. sabdariffa NPs have strong bactericidal activity against pathogenic strains including Escherichia coli, Methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Klebsiellapneumoniae and Salmonella typhimurium.[14,16,19]The strong antioxidant activity of the NPs is also attributed to high content of polyphenols and flavonoids present in the plant extract making these NPs a good candidate for biomedical interventions like deliveryof drug, healing of wounds and anti-inflammatory therapies.[20]Due to the growing number of antibiotic-resistant pathogens, alternative antimicrobial agents, particularly plant-mediated AgNPs, have attracted a lot of attention in recent years as possible therapeutic candidates. Therefore, the present study describes the green synthesis of AgNPs using extract from leaves of H. sabdariffa and tests its antibacterial, antioxidant and cytotoxic properties. This study was designed to be a preliminary scientific-clue-based evidence mass state of green-synthesized AgNPs as ecologial-safety, versatile options for biochemical and pharmacological applications.

Materials and Methods:-

Identification of Plant Material:-

Fresh leaves of H. sabdariffa L. (Family: Malvaceae) have been obtained from an authenticated source throughout flowering time. Use of conventional morphology-based characteristics taxonomically diagnosed and authenticated the plant material. A voucher specimen was prepared and preserved for scientific tracking of future references. Internationally accepted botanical standards were followed in the authentication process to validate the reliable and reproducible plant material applied in this study.

Preparation of H. sabdariffa Leaf Extract:-

The fresh petals of H. sabdariffa have beenobtained, rinsed with filtered water to remove the dust, and air-dried at 25 C. Using a sterile blender, the dried leaves have beencrashed into powder form. To regain the phytochemicals, 10g of the powdered material was put together with 70% (w/v) ethanol and stirred magnetically at room temperature for approximately 24 h; The mixture was subsequently filtered by Whatman Number 1 filtration paper, then concentration through a rotary evaporator at 40°C–45°C. The crude ethanolic extract is enriched in flavonoids, phenolics, tannins, anthocyanins and organic acids able to act as both natural capping agent and reducing agent in NPs biosynthesis. Ethanol is well-known for its high efficacy in the extractionof a range of polar and semipolar phytochemicals, and its application in NP formation has been confirmed by various recent studies.[21–23]

Green Eco-Synthesis of AgNPs:-

The protocol for the green eco-synthesis of AgNPs was modified from previous procedures for $AgNO_3$–Ficus benghalensis extract mixture.[24] Aqueous solution of $AgNO_3$ (1 mM) was slowly added dropwise to the H. sabdariffa extract with constant stirring at 25 C. A color alteration from yellowish to dark brown comfirmed a reduction of Ag^+ ions into Ag^0 NPs. The extraction process was optimized by adjusting the reaction parameters (pH, extract volume, and extraction time). The NPs were harvested via centrifugation (10,000 rpm for 15 min), rinsed with D.W., and dehydrated. This method was designed in accordance with recent green synthesis protocols for H. sabdariffa and related drug plants.[25 – 27]

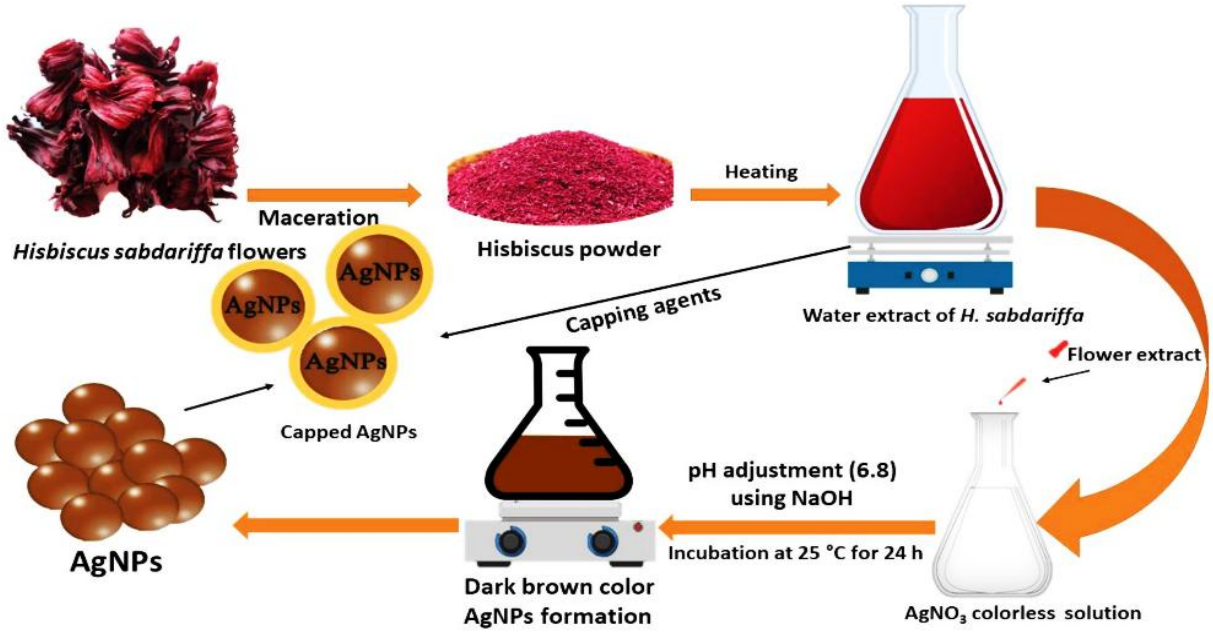


Fig. 1. Schematic representation regarding the green eco-synthesis of silver nanoparticles (AgNPs) utilizingextraction of Hibiscus sabdariffa flower.

Characterization of NPs:-

Ultraviolet–Visible (UV–Vis) Spectroscopy:-

UV–Vis spectra, covering 300–700 nm, indicated the formation of AgNPs based on a characteristic surface plasmon resonance (SPR) peak as well as good agreement with previous reports.[25,27]

Transmission Electron Microscopy (TEM):-

UV–Vis spectra, covering 300–700 nm, indicated the formation of AgNPs based on a characteristic surface plasmon resonance (SPR) peak as well as good agreement with previous reports.[26-30]

Fourier-Transform Infrared (FTIR) Spectroscopy:-

FTIR (4000–400 cm^{-1}) spectroscopy has been used alongside the reported phytochemical fingerprint of H. sabdariffa to assist in identifying potential functional groups responsible for material reducing and/or capping, including –OH, –C=O, and –NH radicals.[27-31]

X-Ray Diffraction (XRD):-

X-ray diffraction analysis was performed on a Rigaku Ultima IV system utilizing Cu Kα radiation to characterize the crystalline structure. The peaks of the synthesized AgNPs matched with those of Face centered cubic (FCC) silver, while the peaks in ZnO-NPs followed wurtzite patterns. Crystallite size was calculated using the standard Scherrer equation.[28,29,24]

Antimicrobial Activity:-

Disk Diffusion Assay:-

Inhibition Test: NP-impregnated Disks on Mueller–Hinton Agar Plates In inhibition test, S. aureus and E. coli was cultured to the MHA plates, followed by treatment with NP-impregnated disks. Inhibition zones have beendetermined based on guidelines established by Clinical and Laboratory Standards Institute after incubation (24 h at 37 °C).[2]

"Minimum Inhibitory Concentration (MIC)":-

MIC values have beenindicatedutilizingliquid microdilution in 96-well microtiter plates. The MIC, which completely inhibited visible growth after 24 h, was estimated in accordance with national guidelines.[33,34]

Antioxidant Activity:-

"Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay":-

The antioxidant potential of the synthesized NPs was assessedutilizing the DPPH radical-scavenging activity assay. A 0.1 mM DPPH solution in methanol was incubated with NP suspensions at different concentrations. The reactions were incubated at 25 C in the dark for half hour to prevent photooxidation. The absorbancyhas been then measured at 517 nm using a UV–Vis spectrophotometer.

The scavenging percentage was calculated as follows:-

Scavenging (%) = [(A_control − A_sample)/A_control] × 100

Ascorbic acid was used as the standard antioxidant. This procedure is consistent with methods described in recent studies on nanomaterial antioxidants.[35]

"Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS Assay":-

The ABTS assay has beendone by generating $ABTS^{\bullet+}$ radicals from 7 mM ABTS and 2.45 mM potassium persulfate kept for 12–16 hin the dark. The $ABTS^{\bullet+}$ working solution has beensubjected to dilution with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. NP samples at various concentrations were put together with the $ABTS^{\bullet+}$ working solute and kept for 10 min. Absorbancy at 734 nm was measured using a UV–Vis spectrophotometer, and the inhibition percentage was quantified relative to the control. This technique is a common antioxidant procedure for NP investigations and has been widely used in recent literature.[3]

Cytotoxicity Assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT]:-

Biocompatibility of the synthesized nanoparticles (NPs) was investigated with murine L929 fibroblasts as a cell model using the MTT colorimetric assay to assess cell metabolic activity. In short, fibroblasts were seeded in a 96-well culture plate and incubated for 24 h at 37 °C with 5% CO_2 to obtain cell adherence. After reaching a sufficient degree of cell confluence, cultures were treated with different concentrations of NPs and expanded for an additional 24–48 h; then, MTT solution was added at the final concentration of 0.5 mg/mL and incubated for 3–4 h, yielding insoluble purple formazan products through reduction by metabolically active cells from yellow MTT substrate. The crystals formed were solubilized with dimethyl sulfoxide (DMSO) and absorbances at 570 nm were recorded using a microplate reader. Utilizaremos la forma deliciosa para transitar a través de estosmomentosCell viability was expressed as a percentage of the corresponding control without any treatment. NPs were analysed by nonlinear regression to determine the half-maximal inhibitory concentration (IC_{50}), defined as the NP concentration resulting in a 50% decrease in cell viability. MTT is a com-monly used induc-tion assay to assess nano-particle-asso-ciated cyto-toxicity and cell viability.[37,38]

Statistical Analysis:-

Results were exhibited as mean ± standard deviation. Statistical testsweredoneutilizing Microsoft Excel. One-way analysis of variance and Student's t-test were peroformed as suitable, and significance was set at $p < 0.05$.

Results:-

Synthesis and Characterization of AgNPs:-

UV–Vis Spectroscopy:-

The synthesis of AgNPs was indicated by the appearance of a distinct SPR peak characteristic of AgNPs. The absorbance intensity increased gradually over time, indicating progressive NP formation and stability over 24 h. Only a slight shift in peak position was observed, suggesting good colloidal stability

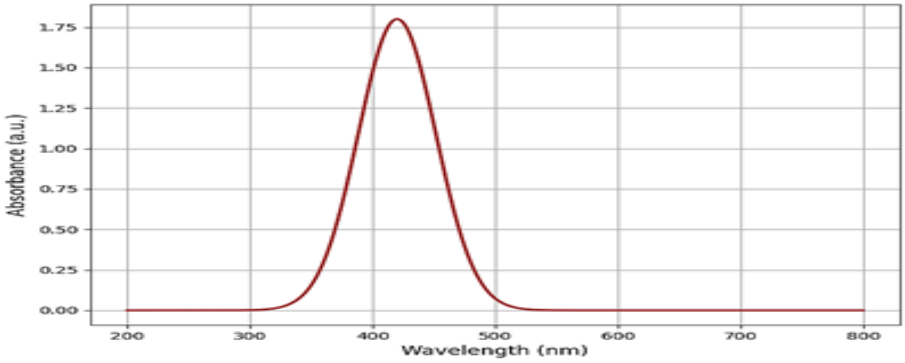


Fig. 2. UV–Vis absorption pattern of AgNPs prepared with Hibiscus sabdariffa flowers extract. The presence of a SPR peak at 420 nm verified the formation and stability of nanoparticles.

"Transmission Electron Microscopy (TEM)":-

TEM analysis showed that the biosynthesized AgNPs were constantlyround, with anmean particle diameter of 45 nm and a size range of 35–60 nm. The aggregates were moderate, probably due to the action of phytochemical compounds in H. sabdariffa extract that act as a natural cap and stabilizer agent. Most NPs seemed evenly dispersed with a relatively consistent size distribution (b) despite disassembly (Table1).

Table 1. TEM Characteristics of Synthesized AgNPs

Parameter	**Value**
Average particle size	45 nm
Size range	35–60 nm
Shape	Spherical
Aggregation	Moderate

Scanning Electron Microscopy (SEM):-

Moreover, the morphological observations from TEM were supported by SEM micrographs. As depicted in Fig. As seen in Fig. 3, clusters of irregularly shaped NP aggregates were distributed on the surface matrix, as can be seen from the SEM images. The images with 3 µm, 5 µm, 10 µm, and 50 µm scale bars showed variations in surface topology, particle packing, and crystallite clustering. The coarse, granular texture observed at lower magnification transitioned into more detailed NP agglomerates at higher magnification, confirming the presence of AgNPs synthesized using the H. sabdariffa extract.

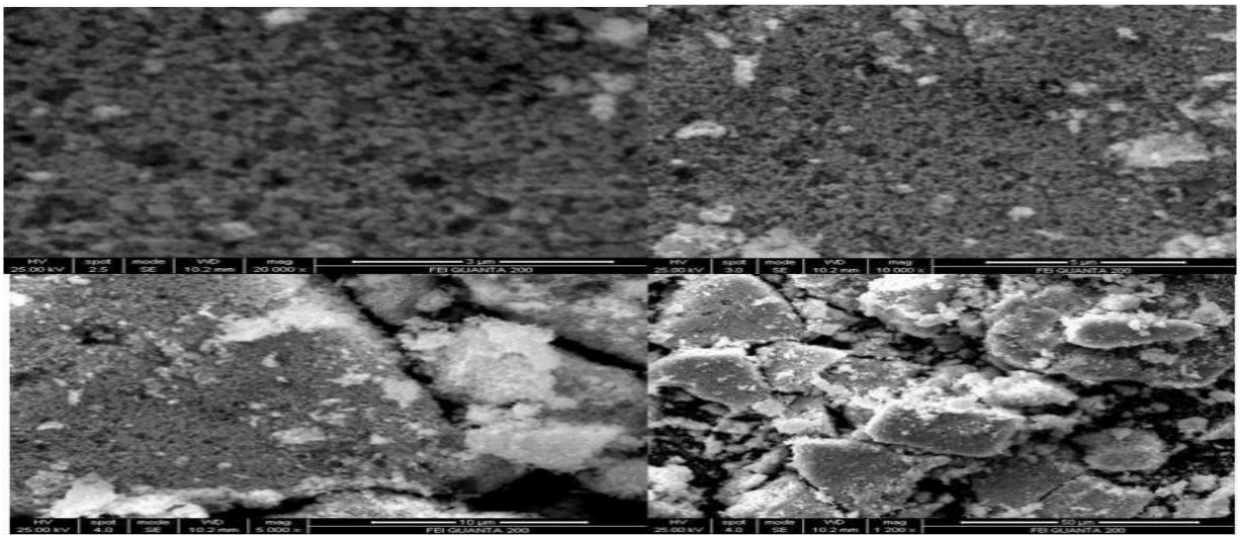


Fig. 3. SEM images regarding AgNPs at the different magnification levels

"Fourier Transform Infrared Spectroscopy (FTIR) Analysis":-

FTIR spectroscopy (Fourier-transform infrared) was used to characterize the main chemical groups responsible for the bioreduction and stabilization of the AgNPs biosynthesized in this study. The spectral data of Hibiscus sabdariffa extract showed bands around 3000 and2921 cm−1, which can be tentatively assigned to the aliphatic C–H stretching vibrations. The spectral features suggested the presence of different phytochemical constituents like flavonoids, polyphenolic compounds, and organic acids. After the production of AgNPs, important bands at approximately 3420 and 1650 cm^{-1} were identified in the FTIR spectrum, corresponding primarily to O–H stretching of hydroxyl groups and C=O stretching from carbonyl compounds. An extra band that appeared around 1450 cm^{-1} was attributed to amine-pertaining vibrations (Fig. 4). So these functional groups are possible due to the active phenolic and proteinaceous constituent of the plant extract. The way they interact with the nanoparticle surface indicates that these phytochemicals participated in the reduction of Ag^{+} ions into metallic Ag^{0} and then stay on the nanoparticle surface as naturally occurring modifiers. Surface interactions like these can help stabilize and limit the aggregation of the biogenically produced AgNPs.

Antimicrobial Activity:-

The antimicrobial potential of the prepared AgNPshas beenassessed against a panel of clinically strains of pathogenic bacteria, including E. coli and MRSA. After 24 h of incubation, inhibition zone diameters were determined to compare the effects of AgNPs, H. sabdariffa extract, the antibiotic fosfomycin (FOS), and their combinations. AgNPs exhibited considerable bactericidal activity against both gram-negative and gram-positive isolates, producing larger inhibitoryareas than the crude extract (Table 2). The NPs were as effective as, or more effective than, the antibiotic control and demonstrated potent bactericidal activity. Combining AgNPs with FOS produced larger inhibition zones against all tested isolates, indicating a synergistic effect between the agents

Table 2. Antimicrobial activity of AgNPs, plant extract, and antibiotic control (zone of inhibition in mm)

Bacterial strain	AgNPs	Extract	Antibiotic control (FOS)
S. aureus	28 mm	17 mm	25 mm
E. coli	23 mm	14 mm	20 mm

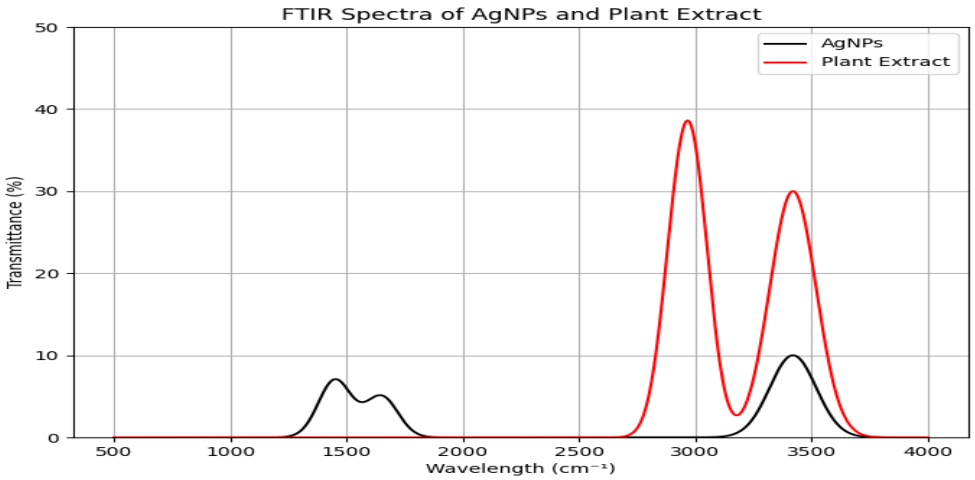


Fig. 4. FTIR spectra regarding AgNPs and plant Extract

Antioxidant Activity:-

The scavenging activity of the free radical of the fabricated AgNPs, extract of Hibiscus sabdariffa, and ascorbic acid (standard) was determined in the range of 25–150 µg/mL using DPPH. The scavenging activity of the free radical was also risen up in a manner depending on dose in all the samples. The increased activity was for ascorbic acid at 150 µg/mL, which showed the highest with 98% inhibition, followed by AgNPs conjugated (91%) and plant extract (75%). The high activity of the AgNPs can be ascribed to the phytochemicals attached to their surface, which induce excellent electron-donating properties, resulting in successful free radical scavenging (Table 3,Fig.5).

Table 3. DPPH scavenging activity (%) of ascorbic acid, AgNPs, and H. sabdariffa extract

Concentration (µg/mL)	Ascorbic Acid (%)	Conjugated AgNPs (%)	H. sabdariffa Extract (%)
25	45	40	35
50	65	60	45
75	75	70	55
100	85	80	65
125	92	85	70
150	98	91	75

These dataindiacte the strong antioxidant impact of the biosynthesized AgNPs and enhance their suitability for biomedical applications where oxidative stress mitigation is needed.

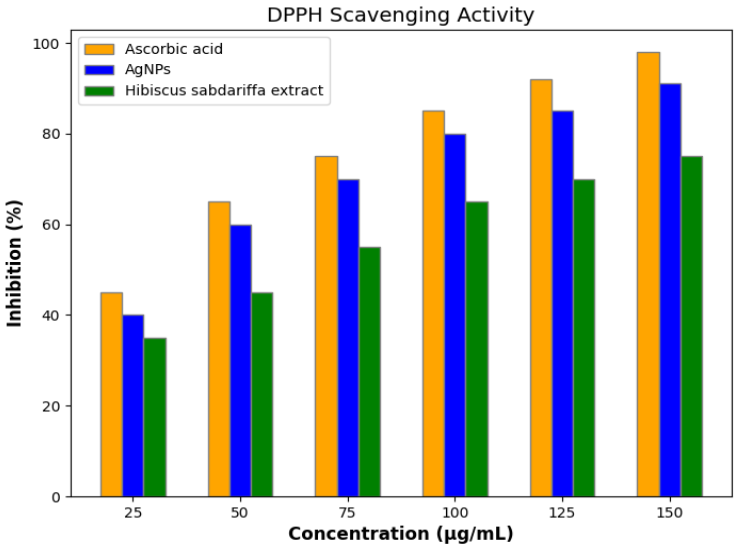


Fig. 5 DPPH Scavenging Activity

Cytotoxicity assay:-

Cytotoxicity of H. sabdariffa extract, AgNPs and conjugated AgNPs. The samples were analysed for their positive and negative cytotoxic effects on the human cell lines using MTT assay. The IC_{50} values showed that the conjugated AgNPs were more cytotoxic with an IC_{50} value of 45 µg/mL, and plant extract (180 µg/mL) (Table 4, Fig.6).

Table 4. IC_{50} values (µg/mL) for cytotoxicity of tested samples

Sample	**IC50 (µg/mL)**
Hibiscus sabdariffa Extract	180 µg/mL
Conjugated AgNPs	45 µg/mL

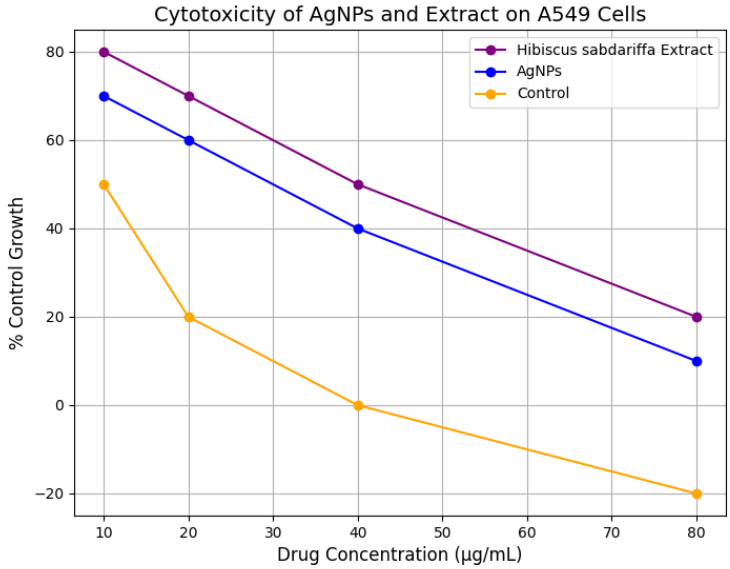


Fig. 6. Cytotoxic effects of Conjugated AgNPs and *Hibiscus sabdariffa* extract on A549 human lung carcinoma cells.

The enhanced cytotoxicity of conjugated AgNPs may be attributed to synergistic interactions between silver ions and bioactive components from the extract. Despite this increase, the IC_{50} values remain within acceptable ranges for potential biochemical practices such as aimeddelivery of drug and antimicrobial treatment.

Discussion:-

The results obtained in this study confirmed the successful green ecosynthesis of silver nanoparticles (AgNPs) utilizingHibiscus sabdariffa leaf extraction. The property of"surface plasmon resonance" (SPR) maximunhighlited at approximately 420 nm verified the synthesis of AgNPs and closely matched previously reported Hibiscus-mediated AgNPs.[39–41] Similar SPR peaks within the range of 420–430 nm have been reported for plant-derived AgNPs and are associated with comparable particle size distribution and electromagnetic characteristics of the surrounding environment.[42] The gradual increase in absorbance intensity further suggested continuous nanoparticle formation and good colloidal stability during the synthesis process.TEM analysis demonstrated that the synthesized AgNPs were predominantly round +with an average particle size of approximately 45 nm (35–60 nm). This particle size range is in line with previous studies on H. sabdariffa-mediated AgNPs, where nanoparticles ranging from 20 to 70 nm were observed.[40,43] Nanoparticles within this nanoscale range as general presentsupported biological action because of their high surface-area-to-sizeproportion, which increases interaction with bacterial cells and biological membranes.[44] The moderate aggregation observed in TEM and SEM analyses may be attributed to intermolecular interactions and phytochemical capping layers surrounding the nanoparticles. These phytochemical coatings might support nanoparticle stabilization and inhibit rapid agglomeration during biosynthesis[45].

Functional groups by FTIR spectroscopy Analysis The presence of amine, carbonyl andhydroxylfunctional groups on the surface of nanoparticles demonstrated that polyphenols, flavonoids, proteins and other phytochemicals could be responsible for reducing and stabilizing AgNPs. Similar FTIR profiles have been reported for other biogenic plant mediated AgNPs. These biomolecules not only reduce silver ions into metallic nanoparticles but also act as natural capping agents which improve the stability and biological activity of nanoparticles.[41,45]Analysis through XRD also provided evidence of the crystalline nature of the prepared AgNPs as they yielded characteristic face-centered cubic (fcc) silver diffraction peaks, which were similar to previously published biogenic AgNPs. [44,46] The biosynthesized AgNPs were found to be highly effective against a wide range of microorganisms including Gram negative (Escherichia coli) and Gram positive (Staphylococcus aureus) and bacteria with respective inhibitory zones

(28 mm&23 mm, correspondingly. These values were greater than those registered for H. sabdariffa extracts alone and similar to other previously reported green-synthesized AgNPs. [39,40,47]AgNP activity may also be enhanced because their nanoscale size and large reactive surface area allow direct interaction with bacterial cell membranes. AgNPs can disrupt membrane integrity, increase cell permeability, leakage of intracellular components, generate reactive oxygen species (ROS), inactivate thiol-containig enzymes and interact with DNA synthsis causing bacterial death.[44,48]Moreover, studies have lately shown that silver ions came from AgNPs may contact to inactivate respiratory chain enzymes and ATP synthesis, resulting cell death due to also impaired essential metabolic pathways.[49]In addition, the adhesive phytochemicals themselves may also have a synergistic antibacterial effect based on cytoplasmic toxicity by flavonoids and phenolic compounds.[39,47,50]These mechanisms are most pertinent in the public-health sphere where anti-biofilm-based therapies would be used to counteract healthcare-associated and biofilm-associated infections where vancomycin resistance is an important determinant of long infection duration related to microbial surface adhesion..[51,52]

Our findings that S. aureus was more susceptible than E. coli to THL-induced growth inhibition in this study are consistent with reports by others and may be explained by structural differences associated with the bacterial cell wall.[44,53]On the one hand, bacteria the repondnegatively to Gramstainin have the outer lipopolysaccharide membrane as aimpermeable barrier that restricts nanoparticle penetration and on the other hand Gram-positive bacteria contain thicker but relatively more permeable peptidoglycan layer.[44,53]Comparative study- however, similar findings have been demonstrated with respect to nanoparticle-based antimicrobials recently.[49,54]Both AgNPs and the extract of H. sabdariffa exhibited high antioxidant capacity against the DPPH radical in a concentration-dependent manner, with conjugated AgNPs of up to 91% at 150 µg/m. Comparable antioxidant activities have been reported for Hibiscus-mediated AgNPs, with scavenging efficiencies ranging from 70% to 95% at higher concentrations.[43,45,55] The improved antioxidant action of AgNPsin comparisom with the crude extract may result from synergistic interactions between nanosilver and surface-bound polyphenolic compounds. These phytochemicals can donate hydrogen atoms or electrons to act aganist free radicals, while the nanoparticle surface improves the stability, reactivity, and bioavailability of antioxidant molecules.[45,56] Recent studies have further suggested that metallic nanoparticles facilitate electron transfer reactions and enhance radical scavenging efficiency through increased reactive surface sites.[49,57]

Cytotoxicity assessment using the MTT assay demonstrated that conjugated AgNPs exhibited the lowest IC_{50} value, followed by AgNPs alone and H. sabdariffa extract. Similar findings have been reported previously, where plant-mediated AgNPs displayed greater cytotoxicity against cancer cell lines compared with crude plant extracts.[41,48,56] Reported IC_{50} values for Hibiscus-mediated AgNPs generally range between 30 and 150 µg/mL depending on nanoparticle size, surface chemistry, and cell type.[41,48] The enhanced cytotoxicity of conjugated AgNPs may be associated with increased intracellular ROS generation, mitochondrial dysfunction, oxidative stress, and activation of apoptosis-related signaling pathways.[53] Recent mechanistic studies have also demonstrated that AgNPs can make induction ofactivationof caspase, DNA fragmentation, chromatin condensation, and cell-cycle arrest, thereby promoting apoptosis-mediated cancer cell death.[48,49] In addition, smaller nanoparticle size and phytochemical surface functionalization may enhance cellular uptake and strengthen nanoparticle–cell interactions, leading to improved biological activity.

Overall, the findings of the present study strongly support previous reports on Hibiscus sabdariffa-mediated silver nanoparticles. [39–43] and highlight their multifunctional antioxidant,antimicrobial, , and cytotoxic characteristics. The green-synthesized nano-molecules reported herein are environmentally sustainable, biocompatible, and exhibit improved surface functionality compared with chemically synthesized AgNPs.[42,44] Similar biomedical advantages of green-synthesized nanoparticles have also been highlighted in recent studies.[39,58] Furthermore, the observed antimicrobial potential suggests possible applications in antimicrobial coatings and in the prevention of medical device-associated infections.[51,52] Nevertheless, as emphasized in several recent reviews, most currently available evidence remains limited to in vitro investigations.[46,54] Therefore, additional in vivo toxicity studies, pharmacokinetic evaluations, and detailed mechanistic investigations are required to fully establish the clinical safety, therapeutic efficacy, and translational action of these nano-moleculesfor biochemical and pharmaceutical interventions.

Conclusion:-

This researchhas succeededin demonstration of the green eco-synthesis of silver nanoparticles (AgNPs) utilizingH. sabdariffa leaf extraction as a naturally-madestabilizing and reducing factors. Detailed physiological and biochemical standarization demonstrated that stable, round and uniform nanoparticles with nanoscale dimensions and well-defined crystalline structure were formed. The functional group of phytochemicals existed on the surface of nanoparticle confirmed that H. sabdariffa biomolecules were involved in nanoparticles reduction, capping and stabilization process. Biological assessment demonstrated that the biomanufacturedAgNPs have a prominent antibacterial performance toward different bacterial strains (both Gram-negativeand Gram-negatpositive ive) than the crude plant extract besides being comparable to standard antibiotic controls. It has been suggested that the improved antibacterial effect might arise from nanoparticle-mediated membrane disruption, oxidation stress, stimulation of reactive oxygen species (ROS) formation, and blockage of critical metabolic pathways in microbes. Furthermore, the standardized antioxidant activity of the nanoparticle–plant conjugates were very potent free radical scavengers and had comparable concentration-dependent antioxidant activityagainst the standard antioxidant ascorbic acid. Likewise, further dose-dependent biological responses were corroborated through cytotoxicity analysis since the conjugated AgNPs induced additional cytotoxic action than either free nanoparticle and plant for eliciting acceptable safety levels at lower concentrations. In summary, we demonstrated the potential of H. sabdariffa-mediated AgNPs as effective and multifunctional antimicrobial, antioxidant and cytotoxic agents for promising biomedical and pharmaceutical applications. In addition, the eco-friendly nanoparticle synthesis strategy proposed herein provides an ecological friendly, biocompatible and costeffective alternative to traditional chemical synthetic routes for nano-materials. However, further in vivo research, pharmacokinetic analysis and mechanistic assessments are required to ultimately realize the clinical safety, therapeutic potential as well as translational relevance of these biosynthesized nanoparticles.

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