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Comparative study of antioxidant properties of aqueous leaves extract and biogenic silver nanoparticles using Hibiscus species & Trachyspermum species.

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Abstract
Objective: *To compare the antioxidant properties of aqueous leaf extracts and biogenic silver nanoparticles (AgNPs) synthesized from Hibiscus rosa sinensis and Trachyspermum ammi using green synthesis techniques.*
Method: *Aqueous leaf extracts were prepared using standard extraction methods, while AgNPs were synthesized by mixing leaf extracts with silver nitrate. Antioxidant activity was evaluated through the DPPH radical scavenging assay. Quantitative phytochemical analysis measured total flavonoid content (TFC) and total phenolic content (TPC).*
Findings: *The aqueous extracts exhibited superior antioxidant activity compared to their biogenic AgNP counterparts. The TFC and TPC were significantly higher in T. ammi extracts (31.12 ± 0.22 mg QE/g; 96.23 ± 0.31 mg GAE/g) than in H. rosa sinensis extracts (20.50 ± 0.15 mg QE/g; 62.42 ± 0.28 mg GAE/g). The DPPH assay showed dose-dependent antioxidant activity, with plant extracts outperforming AgNPs. The superior activity of plant extracts was attributed to their complex bioactive compounds acting synergistically. Conversely, AgNPs exhibited limited antioxidant potential, constrained by synthesis variability and stability issues.*
Novelty: *This study underscores the potential of traditional plant extracts as reliable and potent antioxidants compared to biogenic AgNPs. It also highlights the relevance of green synthesis and the comparative benefits of phytochemicals over nanoparticles in therapeutic applications.*
Keywords: *Antioxidant, plant extracts, biogenic silver nanoparticles, Hibiscus, Trachyspermum, green synthesis.*

INTRODUCTION

Antioxidants prevent oxidation, a process that generates free radicals and damages cells, contributing to chronic diseases like cancer, cardiovascular, and neurological disorders. Aqueous leaf extracts, rich in bioactive compounds like flavonoids, phenolics, and tannins, effectively neutralize free radicals, reducing oxidative stress [(1)]. They are safer, biocompatible, and less likely to cause side effects compared to synthetic antioxidants. Additionally, their extraction is economical, eco-friendly, and aligned with green chemistry principles. These extracts also possess anti-inflammatory, antibacterial, and anticancer properties, making them valuable for treating and preventing various illnesses.

Antioxidants protect cells by scavenging free radicals and promoting overall health. Plant extracts, rich in phytochemicals like tannins, phenolic acids, and flavonoids, are biocompatible, low in toxicity, and highly effective due to their synergistic bioactive compounds. Plants like *Hibiscus* and *Trachyspermum* species, abundant in antioxidants and secondary metabolites, are ideal for natural health products and play vital roles in defense, signaling, and environmental interaction [(2)]. Biogenic silver nanoparticles (AgNPs) synthesized using plant extracts have gained attention for their eco-friendly production via green synthesis. The nanoparticles exhibit enhanced antioxidant properties compared to plant extracts due to their increased surface area, altered physicochemical properties, and improved bioavailability, enabling more efficient free radical scavenging [(3)]. Nanoparticles, with

their high surface area-to-volume ratio, provide more interaction sites for free radical scavenging, enhancing antioxidant efficiency. Their ability to penetrate biological membranes ensures better cellular uptake and distribution of bioactive compounds, leading to more effective free radical neutralization. Nanoformulations enable controlled, sustained antioxidant release, prolonging their effect and reducing dosing frequency. They also enhance the synergistic action of phytochemicals, amplifying antioxidant activity, with promising applications in pharmacology, cosmetics, and medicine [4].

Despite the growing interest in biogenic silver nanoparticles (AgNPs) due to their enhanced stability, bioavailability, and targeted therapeutic potential, limited research has compared their antioxidant efficacy with traditional plant extracts. Additionally, there is a need to explore sustainable and eco-friendly approaches, consistent with green chemistry principles, to harness the antioxidant properties of natural resources effectively. A comprehensive evaluation of the antioxidant capabilities of biogenic AgNPs versus aqueous plant extracts remains unexplored, particularly for *Hibiscus* and *Trachyspermum* species. The primary objective of this study is to compare the antioxidant properties of aqueous leaf extracts and biogenic silver nanoparticles synthesized from *Hibiscus rosa sinensis* and *Trachyspermum ammi*. This research aims to evaluate their effectiveness and potential for developing sustainable antioxidant therapeutics.

MATERIALS AND METHODS

Preparation of Aqueous Extracts

The leaves were collected and thoroughly washed with distilled water. Then they were shade dried and grind into a fine powder (Figure 1). This fine powder was then soaked in distilled water (1:10 w/v) and heated at 60°C for 2 hours. The prepared leaf extract was then filtered using Whatman No. 1 filter paper. Further the aqueous leaf extract was stored at 4°C until further use.

Synthesis of Silver Nanoparticles (AgNPs)

The prepared aqueous leaf extract was mixed with a 1 mM solution of silver nitrate ($AgNO_3$) in a 1:1 ratio. The mixture was then incubated in dark conditions at room temperature for 24 hours. After monitoring the color change from yellow to brown, it was confirmed that the mixture solution is indicating the formation of AgNPs (Figure 2). For confirmation of its characteristics, the AgNPs were characterized using UV- Vis spectroscopy, X-ray Diffraction and Transmission Electron Microscopy (TEM).

Characterization of AgNPs

In order to display the full biological reduction of $AgNO^3$ to Ag-NPs following techniques were carried out for examining biogenically synthesised silver nanoparticles by Ahmad [5].

Microscopic Techniques (Scanning Electron Microscopy & Transmission Electron Microscopy). The size and shape of silver nanoparticles (AgNPs) are often determined through SEM & TEM.

Spectroscopic Techniques (UV-vis Spectroscopy, X-Ray Diffraction Technique)

- **UV-vis Spectroscopy** is the technique that offers information about the band gap energy and surface plasmon resonance of nanoparticles as well as information on their electrical structure and optical characteristics.
- **X-ray Diffraction (XRD)** is the method that identifies the crystal structure of materials, including nanomaterials. It includes exposing of the sample to X- rays and analyzing the diffraction pattern that results, which reveals the atomic configuration of the substance. The crystal phase, crystal size, lattice parameters, and crystallinity of nanomaterials can all be determined using XRD.

Figure 1 Preparation of plant extract from collected leaves

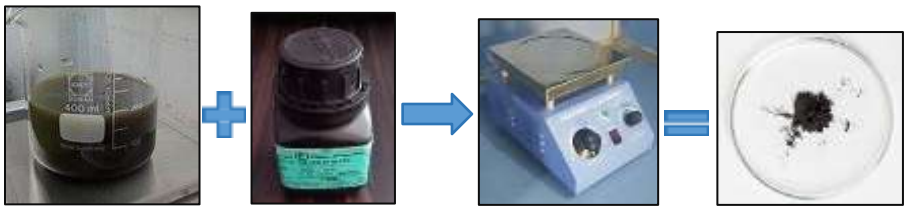


Figure 2 Synthesis of silver nanoparticles

Quantitative Phytochemical Analysis of the Plant Extracts
Total Flavonoid Content: Aluminium Chloride Colorimetric Assay Total Phenolic Content: Folin-Ciocalteu Spectroscopic Assay
Total flavonoid content was measured by the **aluminium chloride colorimetric assay**. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract [6-7].
Total phenolic content in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na2CO3) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract [8-9].
Antioxidant Activity Assays
Method: DPPH Radical Scavenging Assay
The aqueous extracts and formed silver nanoparticles were evaluated for their antioxidant activity by DPPH free radical scavenging activity assay. For determination of DPPH radical scavenging potential of the extracted samples 1,1-diphenyl 2-picryl-hydrazil (DPPH) method proposed by Alothman [10] was applied. The mixing of 100 µl. aliquot from compounds in different

concentrations (20-100 µl/L) was done in 3.9 ml. taken from 0.1 mM DPPH solution. Then blend was subjected to vortex and left for incubation in the dark for 30 min. OD was calculated at 517 nm while DMSO was used as blank.

The % radical scavenging activity was determined by the ratio = ($Ab_{control}$ – Absample / Abcontrol) × 100

Where $Ab_{control}$ is presenting the absorbance of the DPPH solution and absorbance of the DPPH solution with sample is denoted by Ab_{sample}.

Linear plot of concentration versus % inhibition was plotted and by this IC_{50} values were determined. The antioxidant potential of each extract was showed in form of IC_{50} (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of inhibition curve.

Statistical analysis

IC50 values were calculated by regression analysis using IBM SPSS 26.0 statistical software. Evaluation of significance was performed by one-way analysis of variance (one-way ANOVA). All statistical analyses were performed with Excel and IBM SPSS 17.0 statistical software.\

RESULTS

Evaluation of Antioxidant Activity

The research study was conducted with the objective of systematically determining the antioxidant activity of biogenic AgNPs and aqueous extracts of leaf *Hibiscus rosa sinensis and Trachyspermum ammi.* The crude aqueous extracts manifested significantly greater antioxidant potential than their respective biogenic silver nanoparticles for various assay parameters.

DPPH Radical Scavenging Activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the free radical scavenging ability of the extracts. The principle of the method relies on the reduction of the DPPH molecule, which has an unpaired electron at the nitrogen atom. The free electrons and a hydrogen ion are donated to it by antioxidant substances, and the purple-colored DPPH radical is reduced to a yellow-colored hydrazine molecule. DPPH free radical scavenging activity was assessed at different concentrations (200, 400, 600, 800, and 1000 µg/mL) for both the plant species as depicted in Figures 3-6. It can be seen that a dose-dependent activity is well observed, where a higher concentration of samples showed an enhanced scavenging activity proportionally. This concentration-dependent activity is indicative of a systematic and predictable antioxidant activity.

Phytochemical Analysis

Total Flavonoid and Phenolic Content

The aqueous extracts of the plants have shown a significant scavenging activity, and this could be due to the presence of phenolic and flavonoid compounds in a considerably high amount, as seen in Figure 7. Detailed quantitative analysis of TFC and TPC was carried out for both the plant species involved, and these results are summarized in **Table 2.**

The Phytochemical analysis revealed:

1. ***Hibiscus rosa sinensis*** leaf extract:

- TFC: 20.50 ± 0.15 mg Quercetin equivalent/g
- TPC: 62.42 ± 0.28 mg Gallic Acid equivalent/g

2. ***Trachyspermum ammi*** leaf extract:

- TFC: 31.12 ± 0.22 mg Quercetin equivalent/g
- TPC: 96.23 ± 0.31 mg Gallic Acid equivalent/g

These results, graphically depicted in Figure 8, illustrate a major presence of flavonoids and phenolic compounds in the extracts. The higher values found in *T. ammi* may indicate a potentially greater antioxidant capacity than in *H. rosa sinensis.* **Mechanism of Action**

The flavonoids and significant levels of phenolic compounds in both plant extracts contribute to their antioxidant properties via multiple mechanisms:

1. Electron Donation: These compounds have a great electron-donating or hydrogen atom-donating capacity, thus neutralizing free radicals.
2. ROS Stabilization: They form less reactive complexes with ROS so that the damage incurred on the cell is not affected.
3. Metal Ion Chelation: Many flavonoids have been found to be useful as chelating agents; they deter metal ions from participating in free radical-generating reactions. **StatisticalAnalysis**

All experiments were performed in triplicate to ensure reliability and reproducibility. Values are presented as mean ± SD. The statistical significance was made via analysis of variance (ANOVA) with $P < 0.005$ and is considered to be statistically significant. The half-maximal inhibitory concentration (IC50) values were calculated for all test conditions for standardized antioxidant efficacy measurements.

Data Validation

We had low standard deviation values and coherent trends in multiple concentrations, which substantiate the validity of our results. The dose-response curves in Figures 3 **to 6** clearly showed a dependence on concentration linked with expected biochemical principles regarding antioxidant activity.

Comparative Analysis

From the comparison between the two plant species, TFC and TPC contents in *T. ammi* were significantly high than those in *H. rosa sinensis*. For the variations in antioxidant activity of the two plants, this difference in the composition of their phytochemicals might be attributed to them as indicated in Table 1. The higher phenolic and flavonoid contents present in *T. ammi* make it an excellent natural source of potent antioxidant activity.

Figure 3 HRS AgNPs

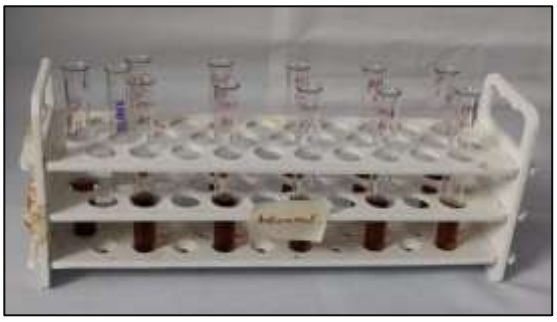


Figure 4 HRS Leaf Extract

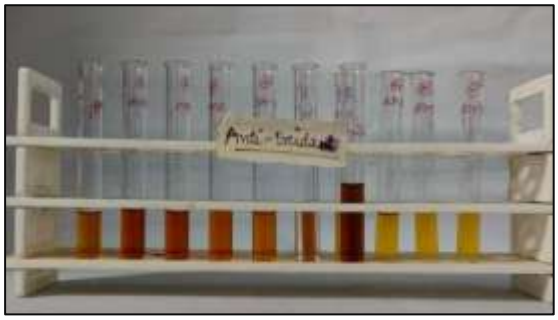


Figure 5 TA AgNPs

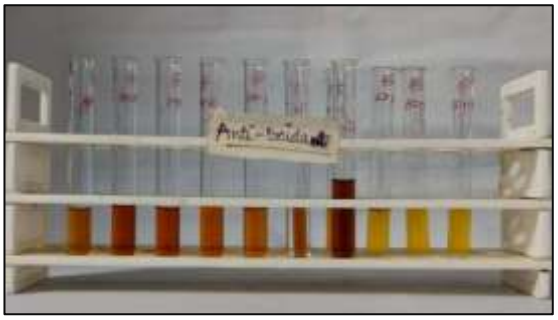


Figure 6 TA Leaf Extract

Name of samples	% Free radical scavenging activity at different concentrations (µg/ml)					
Sample name	200	400	600	800	1000	IC50 value (µg/ml)
HN	11.95±0.20	15.27±0.18	17.96±0.23	20.26±0.25	21.98±0.20	3193.99±27.74
AN	13.46±0.20	14.69±0.23	17.49±0.18	20.36±0.20	24.84±0.23	2841.94±27.72
AJP-1	43.61±0.20	44.98±0.25	46.65±0.13	49.26±0.24	49.60±0.10	990.27±18.70
HRP-2	38.57±0.09	39.92±0.25	42.00±0.24	44.30±0.33	47.89±1.65	1264.77±126.87

Table 1: % Free radical scavenging activity at different concentrations (µg/ml) by DPPH Assay

Sample	Total Phenolic Content (mg/g.)	Total Flavonoid Content (mg/g.)
AJP	96.23±7.86	31.12±1.87
HRP	62.42±6.54	20.50±2.25

Table 2: Quantitative phytochemical analysis of the plant extracts (TPC & TFC)

10	HN	AN	AJP-1	HRP-2
200	11.95	13.46	43.61	38.57
400	15.27	14.69	44.98	39.92
600	17.96	17.49	46.65	42
800	20.26	20.36	49.26	44.3
1000	21.98	24.84	49.6	47.89

Fig.7 Graphical representation of % Free radical scavenging activity at different concentrations (µg/ml) by DPPH Assay

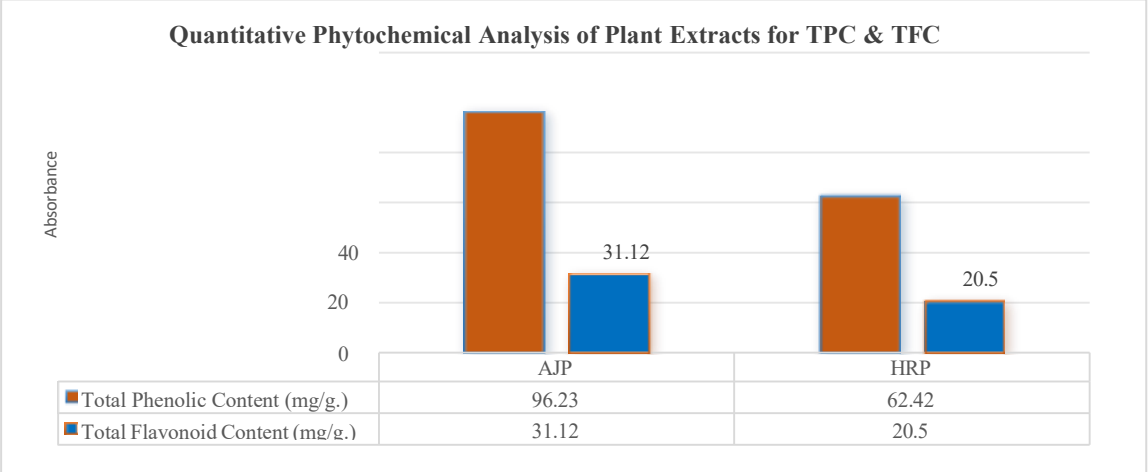
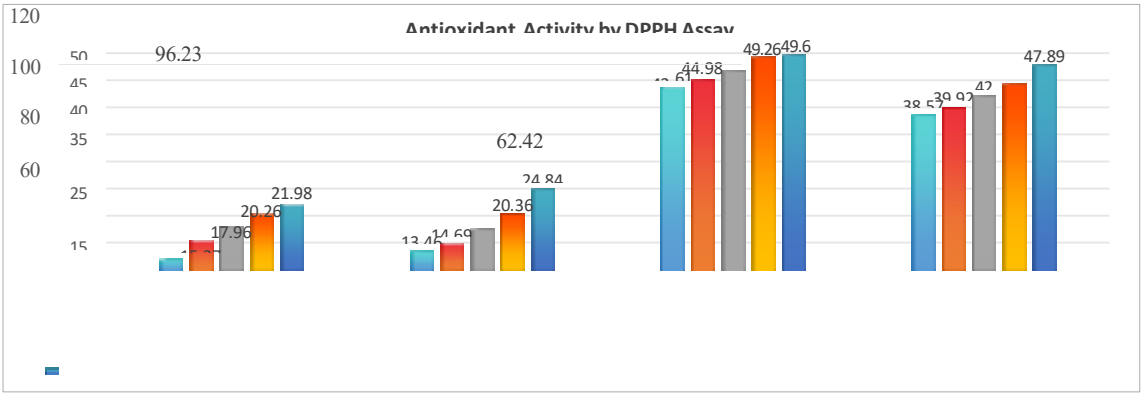


Fig.8 Graphical representation of TPC & TFC for given plant extracts



DISCUSSION:

This study's findings align with earlier investigations highlighting the antioxidant potential of both plant extracts and nanoparticles. Zarshenas et al, demonstrated the therapeutic versatility of *Trachyspermum ammi* [11]. emphasizing its rich phytochemical composition, including terpenoids, tannins, and glycosides, which contribute to its industrial and medicinal applications. Its traditional role in managing obesity and related disorders underscores the significance of its antioxidant and pharmacological properties.

Similarly, Buarki et al, reviewed the therapeutic potential of *Hibiscus rosa sinensis*, showcasing its antioxidant, antimicrobial, antidiabetic, and anti-inflammatory properties [12]. This study supports the utilization of *Hibiscus* in enhancing health and preventing disease through its bioactive compounds, which play a crucial role in reducing oxidative stress. Chahardoli et al, highlighted the utility of green synthesis in creating nanoparticles with bioactive compounds. Their findings that *Nigella arvensis* leaf extract-based gold nanoparticles displayed moderate antioxidant activity, though lower than crude extracts, parallel our observations regarding the limited yet promising capabilities of biogenic AgNPs[13]. Similarly, Pilaquinga et al, demonstrated a significant reduction in phenolic content during nanoparticle synthesis, [14]. affirming that while nanoparticles are useful, they often have lower

antioxidant potency compared to plant extracts.

Willian et al and Sivakumar et al, emphasized the advantages of nanoparticle synthesis using eco-friendly methods, showing enhanced antibacterial and antioxidant activity [15-17]. However, both studies noted variability in the efficacy of nanoparticles depending on synthesis methods, consistent with our results regarding the limitations of AgNP stability and bioavailability. Jabeen et al, explored nanosuspensions of *T. ammi*, demonstrating increased phenolic content and antioxidant activity in nanosuspensions compared to raw extracts[18]. This highlights the potential for advanced formulations to enhance therapeutic outcomes, although the study reaffirmed the efficacy of traditional plant extracts.

Finally, Wang et al, and Smith et al, discussed industrial applications of plant extracts and nanoparticles, from nutraceuticals to cosmetics, emphasizing their versatility[19-21]. This study reinforces the importance of combining traditional and modern approaches to maximize antioxidant benefits while adhering to sustainable practices.

Our findings support the superiority of aqueous plant extracts, particularly those from *T. ammi* and *H. rosa sinensis*, in delivering robust antioxidant activity due to their complex bioactive compounds. However, biogenic nanoparticles, while less potent, hold promise for future applications with further optimization in synthesis and stability. This dual approach bridges traditional remedies with modern nanotechnology, offering sustainable solutions to combat oxidative stress and related disorders.

CONCLUSION

Our study demonstrates that aqueous extracts of *Hibiscus rosa sinensis* and *Trachyspermum ammi* possess superior antioxidant capabilities compared to biogenic silver nanoparticles (AgNPs). The synergistic action of diverse phytochemicals in plant extracts, such as polyphenols and flavonoids, provides a robust antioxidant mechanism, surpassing the limited and variable efficacy of AgNPs. While green nanotechnology holds promise, plant extracts remain a more reliable, stable, and potent source of antioxidants for therapeutic applications. These findings highlight the enduring importance of traditional plant-based antioxidants in both modern medicine and future research.

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