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Antioxidant Activity of *Lawsonia alba* Mediated Silver Nano-Particles

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Abstract

Introduction: *Lawsonia Alba* is commonly known as Henna and abundantly available in tropical and subtropical areas. *Lawsonia Alba* is cultivated for the medicinal and cosmetic value of its leaves. The shrub's stem bark, roots, flowers, and seeds have also been used in traditional medicine. Silver nanoparticles (AgNPs) have a wide range of medicinal and diagnostic uses. Silver is one of the most common metal nanoparticles, owing to its antimicrobial and pharmaceutical properties. It is in the medical industry as topical ointments to prevent infection against burn and open wounds. Antioxidants are compounds that may shield the cells from free radicals, which may cause heart disease, cancer, and other illnesses.

Aim: In the present study, we aim to evaluate the antioxidants activity of *Lawsonia Alba* mediated silver nanoparticles.

Materials and methods: The research involved preparing *Lawsonia Alba* extract and 20 milli molar silver. Both were mixed and stirred using a magnetic stirrer for the synthesis of nanoparticles. These were tested for antioxidant activity by DPPH assay.

Results: The present study shows that the antioxidant activity of *Lawsonia Alba* mediated silver nanoparticles was seen to be increased as the concentration increased in a dose-dependent manner, hence it can act as a good antioxidant.

Conclusion: *Lawsonia Alba* mediated silver nanoparticles were proved to possess a strong level of antioxidant activity. The results of the study reinforce the opinion that medicinal plants are promising sources of potent antioxidants that may be useful for therapy.

Keywords

Antioxidant activity • *Lawsonia alba* • Silver nano particle • DPPH assay • Innovative technique

Introduction

Nanotechnology is one of the foremost active research areas within modern material science. Nanotechnology is one of the fastest developing sciences over the past few years [1]. This is an emerging field of modern research dealing with the synthesis and designing of particle structures ranging from approximately 1-100 nm [2]. Nanotechnology being a nascent innovation has a lot of potential for medical and dental applications. It continues to influence several new developments and future advancements of orthodontics and dentistry [3]. Nanoparticles are known to decrease toxicity, increase bioactivity and also improve cell targeting. Nanoparticles of metal and metal oxides such as silver, zinc oxide, zirconium oxide, copper oxide, gold, selenium, hydroxyapatite, titanium oxide, and copper sulfide, have been used in many medicinal applications, in particular for cancer detection, screening purposes, drug delivery systems, antisense and gene therapy applications, and tissue engineering [4]. Silver nanoparticles (AgNPs) have a wide range of medicinal and diagnostic uses. Silver is one of the most common metal nanoparticles, owing to its antimicrobial and pharmaceutical properties [5–7]. New and innovative strategies are of potential interest for the synthesis of silver nanoparticles (AgNPs), which are used in a huge range of consumer products [8]. The utilization of nanoparticles as a medicine in the treatment of diabetes using the herbal mediated cerium oxide nanoparticles ($HMCeO_2$ NPs), herbal mediated silver nanoparticles (HMAg NPs) and Lawsonia inermis extract have been attempted [9]. *Lawsonia alba* is cultivated for the medicinal and cosmetic value of its leaves. The shrub's stem bark, roots, flowers, and seeds have also been used in traditional medicine [10]. Henna is the common name for this herb, which is widely distributed in tropical and subtropical regions. This plant is a worldwide known cosmetic agent used to stain hair, skin, and nails [11]. It is a small tree or a multi-branched shrub belonging to the family Lythraceae. It is typically 2-6 m in height [12]. Since antiquity, the *Lawsonia alba* plant's leaf paste has been used for dying blood, skin, and nails. Besides cosmaceutical usages, the plant also harbors a well-documented folklore history for treating convulsion, jaundice, and malignant ulcers [13]. *Lawsonia alba* was evaluated to have antimicrobial, antioxidant, anti-complementary activity, anti-sticking activity, and catalytic properties [14]. Studies on this important medicinal plant have resulted in the isolation and structure elucidation of more than 80 compounds up to 2011 and evaluation of biological activities of some of them. Lawsonia inermis (Lythraceae) commonly known as Henna is a well-known plant used in Indian medicine [15]. Traditional Indian medicine has used different parts of this herb. The plant has a wide range of phytochemicals [16]. Antioxidants are compounds that may shield the cells from free radicals, which may cause heart disease, cancer, and other illnesses [17,18]. When your body breaks down food or when you're exposed to cigarette smoke or radiation, free radicals are created [19]. Our team has extensive knowledge and research experience that has translate into high quality publications [20-39]. The research is novel and hence not much reference material available. Very few studies have been done on the use of *Lawsonia alba* as a source of silver nanoparticles.

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The synthesis of metal nanoparticles from herbal sources is also tedious and time-consuming. Previous literature majorly focused on Lawsonia inermis mediated silver nanoparticles and their antimicrobial activity, the present study aims to evaluate the antioxidant activity of *Lawsonia alba* mediated silver nanoparticles [40].

Materials and Methods

Extract preparation

1 gm of *Lawsonia Alba* was added in 100 ml of distilled water and boiled for 10-15 minutes at 70 degrees celsius. After boiling, the plant extract was filtered by Whatman No 1 filter paper. 90 ml of 1 millimolar silver nitrate is prepared in 250 ml of a conical flask; 40 ml of filtered plant extract was mixed to it and kept in a magnetic stirrer for nanoparticle synthesis (Figure 1). The synthesized nanoparticle was preliminarily analyzed using UV visible spectroscopy. Before the final step, the nanoparticle solution was centrifuged at 8000 rpm to prepare nanoparticle pellet powder; it was dried in a hot air oven at 80 degrees Celsius. The dried powder was sent for characterization. Finally, the leftover solution was taken to calculate antioxidant activity.

Antioxidant activity

DPPH method: A DPPH assay was used to test the antioxidant activity of biogenic synthesized silver nanoparticles. Diverse concentrations (2-10 µg/ml) of *Lawsonia alba* extract mediated silver nanoparticle were mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes (Figure 2). Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. Vitamin C was employed as control. The percentage of inhibition was determined from the following equation.

%inhibition=(Absorbance of control-Absorbance of test sample × 100)/ Absorbance of control

Figure 1. Preparation of *Lawsonia alba* mediated silver nanoparticle extract.

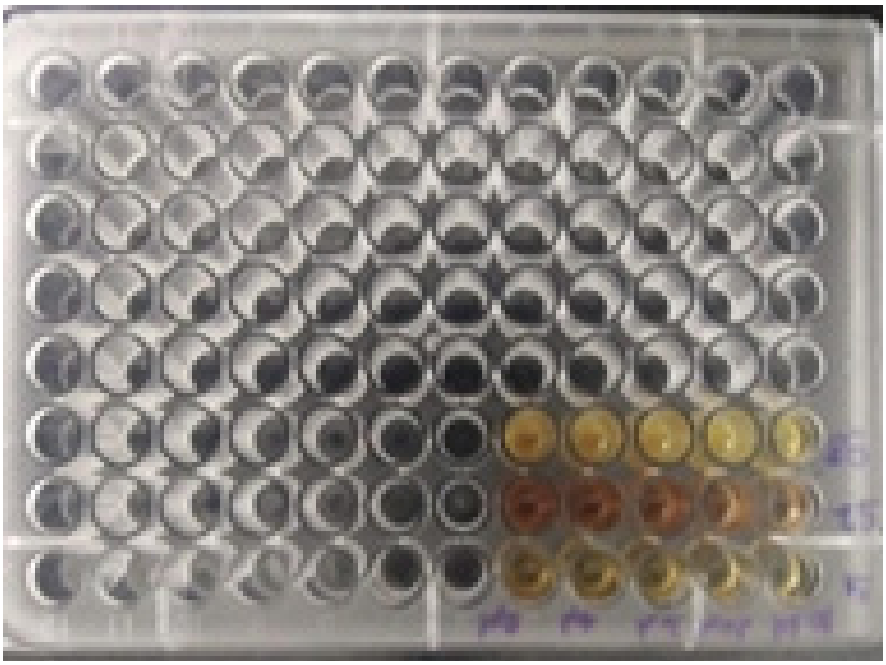
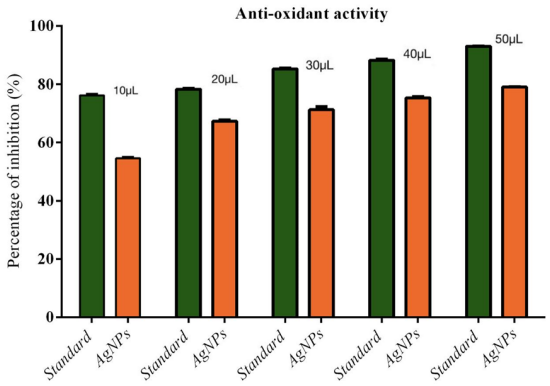


Figure 2. DPPH assay comparison ELISA plate wells with different concentrations of *Lawsonia alba* mediated silver nanoparticle for evaluation of its antioxidant activity.

Results and Discussion

DPPH has been widely used as a stable free radical to test reducing substances as well as a useful reagent for investigating the component's free radical scavenging behavior. From the results obtained it has been inferred that there is a consistent increase in the antioxidants activity with *Lawsonia Alba* mediated silver nanoparticles. At 10 µl concentration 54.8% is observed by silver nanoparticle and 76.56% by standard, 67.8% antioxidant activity was observed by AgNPs and 78.52% by the standard at 20 µl concentration, at 30 µl concentration AgNPs showed 71.9% antioxidant property and 85.63% by standard, at 40 µl concentration 75.7% was observed by AgNPs and 88.68% by standard, at 50 µl concentration 79.2% and 93.15% is observed by AgNPs and standard respectively. Hence maximum antioxidant activity is observed at 50 µl concentration. The present study shows that the antioxidant activity of *Lawsonia alba* mediated silver nanoparticles was seen to be increased as the concentration increased in a dose-dependent manner, hence can act as good antioxidant control. The graph represents a comparison of antioxidant activity of *Lawsonia alba* mediated silver nanoparticles at different concentrations. From the above graph it has been observed that the antioxidant activity of *Lawsonia alba* mediated silver nanoparticles was seen to be increased as the concentration increased in a dose-dependent manner, hence can act as good antioxidant control (Graph 1).



Graph 1. Anti-oxidant activity.

DPPH radical scavenging assay was used to determine AgNPs' major antioxidant capacity. The BHT (butylated hydroxytoluene) has been used as the standard. Kharat (2016) analyzed the antioxidant behavior of the synthesized nanoparticles using the DPPH assay and detected the antioxidant ability of photosynthesized nanoparticles [41]. They suggested that photosynthesized NPs could be used as possible free radical scavengers. The color difference was caused by the reduction of Ag+ into silver nanoparticles when exposed to the *Lawsonia alba* extract. The extract was yellow when it was freshly prepared. The extract turned dark brown after being treated with $AgNO_3$ and incubated. The surface plasmon resonance effect results in color variations in aqueous solutions [42]. Plants including *Cymbopogon citratus*, *Garcinia mangostana* bark extract, *Mucuna pruriens* seed, *Kalanchoe pinnata* leaf, *Acorus calamus* root and *Chrysanthemum Indicum* have previously been reported to produce AgNPs [43–48]. The prior studies indicated that the antioxidant property may be due to the existence of flavonoids and tannins in leaves and phytochemicals such as phytol, sterols, and fatty acids [49]. Both the amounts of oxidants and antioxidants in tissues should be controlled. If ROS oxidants are present at high levels, oxidative stress is produced in the body by destroying DNA, carbohydrate, and protein repair, thereby disrupting the regular metabolism of the body [50]. Yet it also tends to stop aging. Antioxidants play an important role in the neutralization of free radical species generated as end products of natural biochemical processes in the body, thereby regulating the aging process and other degenerative diseases [51]. In a previous study done by Anand findings indicated that, relative to vitamin C, AgNPs have greater antioxidant activity [51]. In terms of DPPH radical scavenging, Patra in his study showed high antioxidant activity (IC50 385.87 g/mL). The findings suggest that AgNPs can be used as natural antioxidants to protect against various types of oxidative stress linked to degenerative diseases. In particular, AgNPs must be tested for antioxidants before being used *in vivo* models or human applications [52]. The Ag-NPs coating is used to secure the NPs by causing electrostatic and electrostatic repulsions between them. The coating also defends against the cytotoxicity of Ag-NPs, according to several studies. Nanoparticles may help with vascular changes, particularly endothelial dysfunction caused by oxidative stress [53]. This syndrome can cause a decrease in Nitric Oxide (NO) bioavailability, which can affect vascular tone control and endothelial dysfunction, the first stage of cardiovascular disease. Thus, the antioxidant nanoparticles produced in this study may be used to treat vascular disease caused by hypertension, diabetes, or atherosclerosis (Figures 3 and 4) [54].

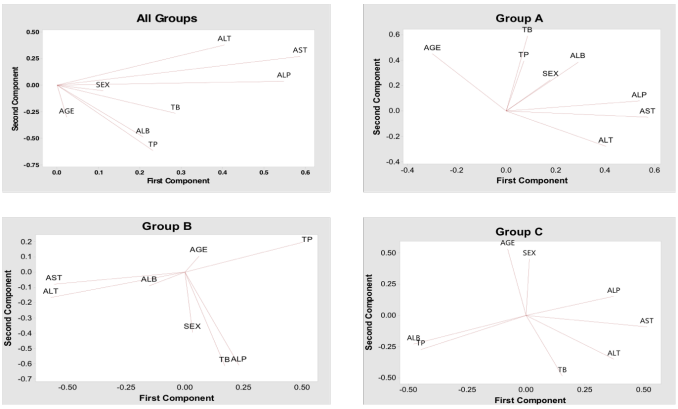


Figure 3. A loading plot showing the relationship among variables. Note: In the loading plot, the high positive correlation between two variables leads to two vectors that are very close to each other, forming a small angle. The non-correlation leads to two vectors out of phase by 90o, while the anti-correlation or negative correlation leads to two vectors that are out of phase by 180o. Seropositive HIV-1 subjects on ART (Group A); healthy population control group with no overt aetiology (group B) and seropositive HIV-1 subjects yet to commence ART (group C). Plots reveals only the first and second components. Abbreviations: ALT: Alanine Aminotransferase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; TP: Total protein; ALB: Albumin; TB: Total Bilirubin.

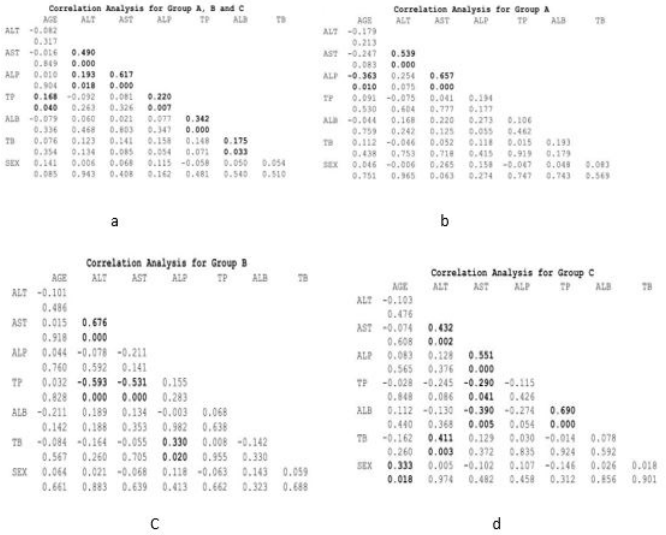


Figure 4. Correlation tables for liver function protein in the different study groups as shown above. Note: Group A=seropositive HIV-1 on antiretroviral drug. Group B=healthy control group. Group C=seropositive group not yet on anti-retroviral drug. In bold are significant correlations of interest. Each protein has 2 data set, top is the r-value and bottom are the p-value. Correlations in (4i) is combination of A, B and C (independent of study groups). Abbreviations: ALT: Alanine Aminotransferase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; TP: Total Protein; ALB: Albumin; TB: Total Bilirubin.

Conclusion

The current study was limited to the use of DPPH assay for evaluating antioxidant activity. Other conclusive methodologies like ABTS assay, FRAP assay, FOX assay and FTC assay need to be employed for further evaluation. Future research into silver nanoparticles examining their biological properties such as ant diabetic, anti-inflammatory, and antimicrobial activities both in vitro and *in vivo* will lead to the production of Nano-formulations as therapeutics in various diseases. *Lawsonia Alba* mediated silver nanoparticles were proved to possess a strong level of antioxidant activity. The results of the study reinforce the opinion that medicinal plants are promising sources of potent antioxidants that may be useful for therapy.

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