

Mouthwash Preparation Using Stevia and Neem Based Silver Nanoparticles and Its Cytotoxic Activity

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ABSTRACT

Introduction: Azadirachta indica (Neem) may be a rapid-growing plant even in severe drought which shed most of its leaves. The branches are wide and spreading. In this present investigation, we have prepared mouthwash using Stevia and Neem-based silver nanoparticles.

Materials And Methods: Azadirachta indica and Stevia rebaudiana plant powder form was taken. 1g of Neem and Stevia extract powder mixed with 100ml of distilled water and then it was boiled, cooled and filtered with filtered paper and allowed to settle and the extract mixture was found to be 50ml and filtered.

Results And Discussion : The plant had exhibited a potent cytotoxic effect at higher concentrations. Extracellular synthesis of silver nanoparticles using Neem and Stevia was primarily identified by visual identification; the appearance of silver and whitish colour in reaction mixture indicates nanoparticles.

Conclusion : The present study revealed that the silver nanoparticles can be synthesised in a simple eco-friendly method using Neem and Stevia .

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INTRODUCTION

Azadirachta Indica (Neem) may be a rapid-growing plant even in severe drought which shed most of its leaves. The branches are wide and spreading. The opposite, pinnate leaves square measure long, medium, and therefore the color is from dark inexperienced and it's about 3-8 centimeters (1.2-3.1 in) long. The petioles are short. The white and fragrant flowers square measure organized in more-or-less drooping axillary panicles (1). The plant Stevia rebaudiana has been used for quite 1500 years by the Guaraní peoples of South America, WHO referred to it as ka'a he'ê ("sweet herb") (2). These leaves are used historically for many years in each Brazil and Paraguay to sweeten native teas and medicines, and as a "sweet treat". The genus was named for the plant by Spanish biologist and Dr. Petrus Jacobus Stevus. In 1899, Swiss biologist Moisés Santiago Bertoni, while conducting analysis in jap Paraguay, 1st delineated the plant and therefore the sweet style thoroughly. Only restricted analysis was conducted on the subject till, in 1931, 2 French chemists isolated the glycosides that offer stevia its sweet style.

KEYWORDS:

Azadirachta indica,
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Cytotoxicity,
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Stevia is a herb from South America. They serve as sweeteners in foods. The leaf extract contains flavonoids, alkaloids, lipids, amino acids, etc. It is antihypertensive, Compounds of Stevia are pH and heat stable. It is a non-fermentable sweetener with fewer calories. It has antihyperglycemic, anti-diabetic, antihypertensive activities.

During recent years the development and use of silver nanoparticles (Ag-NPs) were rapidly increased due to their unusual optical, chemical, photoelectrochemical, catalytic, antibacterial, and biological labeling properties (3). Ag-NPs have attracted intensive research interest due to their advantageous and applications not only in biomedical, drug delivery, food industries, agriculture, textile industries, and water treatment as an antimicrobial and antifungal agent but also of their applications in catalysis and surface-enhanced Raman scattering. Plant extracts in the green synthesis process have received increasing attention for the development of metal nanoparticles (4). Many studies have also focused on the investigations of the reduction mechanism using plants; the biosynthesis of metal nanoparticles has also been achieved using enzymes, proteins, and amino acids.

In addition, the other major reasons for the development of biosynthesis of nanoparticles are the need for the environmentally benign process (5-7). There is a large body of references on the preparation of metal nanoparticles using some chemicals such as hydrazine, sodium borohydride, and dimethylformamide that have been considered to have some environmental and biological risk. Silver nanoparticles are being used in numerous technologies and incorporated into a wide array of consumer products that take advantage of the cytotoxicity properties. Ag-NPs are used in biosensors and numerous assays, where the Ag-NP materials can be used as biological tags for quantitative detection. Ag-NPs are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics for their antibacterial properties. Ag-NPs are used in conductive inks and integrated into composites. Various methods are administered for the synthesis of nanoparticles which include chemical, physical, and UV methods (8). However, the major drawback in their synthesis is the production of hazardous side products when chemical methods are administered (9). Hence, plants are considered as a better platform for the synthesis of nanoparticles since they produce eco-friendly by-products.

Indian trees and herbs, especially neem and Aloe vera, have been used from the prehistoric time because of its curative properties and it is an ingredient of various medicines available in the market (10). Moreover, silver nanoparticles (Ag-NPs) are proved to act as an efficient catalyst. In certain studies, it has been proved that lower nanoparticle concentration when used along with conventional therapeutic materials will give the best results Moreover, they have found that Ag-NPs are known

to be bactericidal as well as cytotoxic to mammalian cells (3). The nanoparticles may penetrate deep inside the cell wall, disrupting the phosphorus and sulfur compounds such as the DNA and other metabolic proteins present in the cell, also while attaching the cell wall, the nanoparticles will disrupt the cellular permeability as well as the cellular respiration. A nanoparticle or ultrafine particle is sometimes outlined as a particle of matter that's between one and one hundred nanometres (nm) in diameter (11).

Nanoparticle square measure is sometimes distinguished from microparticles. The properties of nanoparticles typically disagree markedly from those of larger particles of identical substance (12). Therefore, the properties of that surface layer might dominate over those of the majority material (13). This impact is especially studied for nanoparticles distributed in an exceedingly medium of various compositions since the interactions between two materials at their interface additionally becomes vital. The liquid neem tree extract is employed for the synthesis of varied nanoparticles like gold, silver, zinc oxide etc (14). Terpenoids and flavanones square measure the necessary phytochemicals in neem tree which might play a significant role in stabilising the nanoparticle and additionally act because of the capping and reductant (12,15). Our team has extensive knowledge and research experience that has translate into high quality publications (16-25). In this present investigation we have prepared mouthwash using Stevia and Neem based silver nanoparticles.

MATERIALS AND METHODS

Preparation of plant extract

Azadirachta indica and Stevia rebaudiana plant powder form was taken. 2.5g of Neem and Stevia extract powder mixed with 100ml of distilled water and then it was boiled, cooled and filtered with filtered paper and allowed to settle and the extract mixture was found to be 50ml and filtered (figure 1).

Synthesis of silver nanoparticles

50ml of plant extract 1mm of AgNO₃ is added and mixed with silver nanoparticles and then it is kept in a laboratory shaker for a few hours. For every 2 hours, the color change was observed. This reading of the synthesis of the nanoparticles was done using a UV spectrometer (5times the reading was taken and then colour change was observed and readings were recorded. The synthesis nanoparticles were then centrifuged Azadirachta Indica and Stevia rebaudiana mediated silver nanoparticles with proper synthesis were taken for studying the activity of mouthwash.

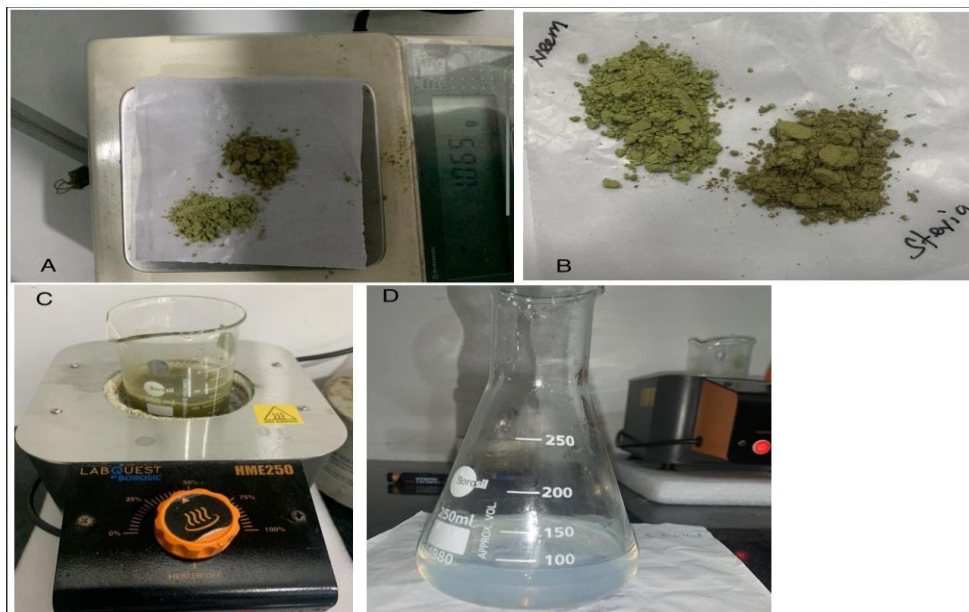


Figure 1: Image A representing 2.5gm of neem and Stevia was measured under the weighing machine. Image B representing 2.5gm of Neem was measured under the weighing machine. Image C representing the Neem and Stevia aqueous formulation and boiling aqueous extract of Neem and Stevia boiled for 10-20 minutes at 60 degrees Celsius. Image D representing the mixture of silver nanoparticles with an aqueous solution of neem and stevia

Preparation of mouthwash

In this study mouthwash was obtained from the given extract *Azadirachta indica* and *Stevia rebaudiana* and the mouthwash prepared from the nanoparticles .

**Brine Shrimp Lethality Assay
Saltwater preparation**

2g of iodine-free salt was weighed and dissolved in 200ml of distilled water.

6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well

(20µL,40 µL,60 µL.80 µL,100 µL). Validation of the procedure was done by nano research experts. Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for number of live nauplii present and calculated by using following formula,

number of dead nauplii/number of dead nauplii+number of live nauplii×100. Correlation analysis was done to analyze the cytotoxic activity by SPSS.

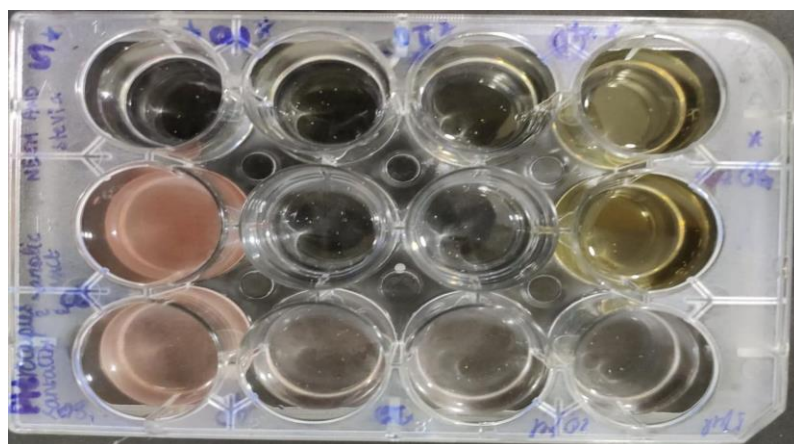


Figure 2: Cytotoxicity effect analyzed by counting the number of live nauplii in the microplate after 48 hours of incubation time. On day 2, at 5 µL and 10µL ten numbers of the nauplii were alive, at 20 µL and 40 µL nine nauplii were alive and at 80 µL six nauplii were alive.

RESULTS AND DISCUSSION

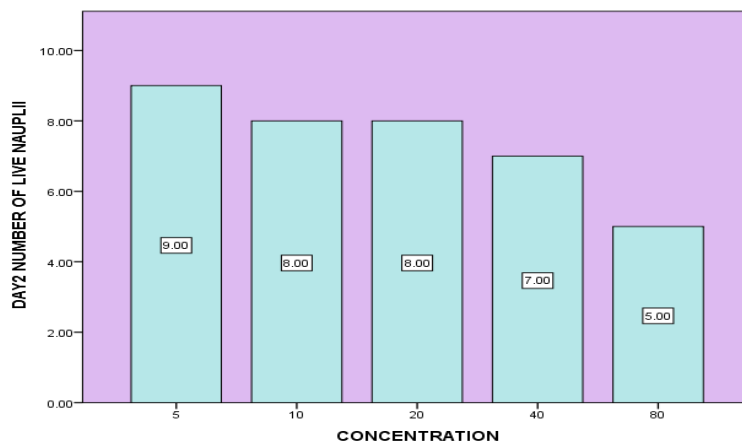


Figure 3: Cytotoxic activity of *Azadirachta indica* and *Stevia rebaudiana* showed a decrease in the number of live nauplii count with an increase in concentration

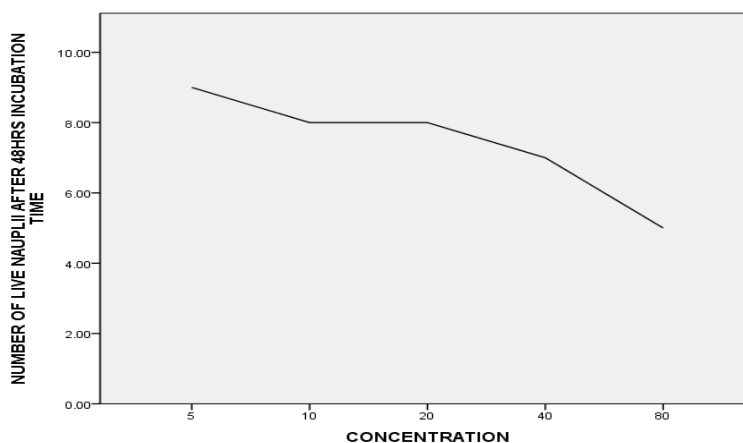


Figure 4: On day 2 there was a decreased number of live nauplii with increase in concentration.

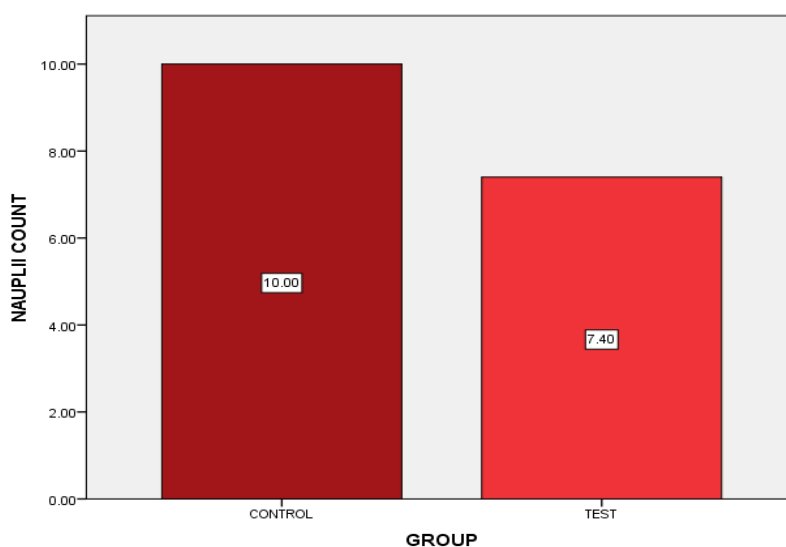


Figure 5: Test group showed a decrease in live nauplii count

Cytotoxicity Activity

The brine shrimp lethality assay is an important in the study of toxicity, that gives us information about cytotoxicity by a bioactive compound to cells. The viability of the nauplii was analyzed for different concentrations of nanoparticles that are synthesized from neem and stevia. After 24 hours, at 5 μ L, 10 μ L, 20 μ L, 40 μ L, and 80 μ L all nauplii were alive. After 48 hours, it was found that at a minimal concentration of 5 μ L and 10 μ L ten numbers of the nauplii were alive, at 20 μ L and 40 μ L nine nauplii were alive and at 80 μ L six nauplii were alive. Whereas the control showed 10 numbers of the nauplii to be alive.

Results are obtained from the graph where the cytotoxic activity is shown. In this present study it could be concluded that *Azadirachta Indica* and *Stevia rebaudiana* mediated silver nanoparticles has given a positive effect in the cytotoxic activity. The combination of nanoparticles with the plant is found to be effective and said to cause no side effects; being a medicinal plant it is reported as they can cure many types of the oral diseases. Plant mediated silver nanoparticles used as mouth wash reveal high positive activity when combined with this plant is said to exhibit better treatment and does not show any toxic effects in the body (26). The plant had exhibited a potent cytotoxic effect at higher concentrations. Extracellular synthesis of silver nanoparticles using neem and stevia was primarily identified by visual identification; the appearance of silver and whitish color in reaction mixture indicates nanoparticles. The present study indicated that the biosynthesis of nanoparticles can be made possible from Neem and Stevia and possess fewer cytotoxic effects. The high lethality of the brine shrimps caused by Stevia and Neem leaf is indicative of the presence of potent cytotoxic components. *Azadirachta* that is derived from the neem tree is a toxicant that affects brine shrimp lethality. In another study, the cytotoxic assessment was carried out using a brine shrimp lethality assay. The silver nanoparticles of the cytotoxicity to the brine shrimp did not show any effect. Nanoparticles were successfully synthesized using plant extract.

In a previous study, done by Nandita et al., it was observed that Stevia nanoparticles mediated mouthwash has given a positive result in cytotoxicity activities. There is research done in this plant but with different nanoparticles like selenium and copper etc. was used. This combination of nanoparticles with the plant is found to be effective and said to cause no side effects being a medicinal plant. It is reported to cure a number of diseases like mouth ulcer, mouth cancer, teeth cavities etc.. Plant mediated silver nanoparticles would give a supra additive effect when used. Phytochemical screening showed the presence of the carbohydrates, saponins, phytosterols and tannins in leaf is responsible for showing activities in this plant. It aids in fast healing and being a traditional medicine, it would definitely show its effect against any type of disease. Silver nanoparticles reveal high positive activity, when combined with this plant is said to exhibit better treatment and does not show any toxic effects in our body. A drug is said to be effective only when it produces a high effect in curing the disease without any damage to our normal system (27).

The plant has exhibited a potent cytotoxic effect at higher concentrations. Using probit analysis, the concentration of the lethal dosage was measured by 95% intervals. *Azadirachta indica* and *sativa*-mediated silver nanoparticles have shown an increased cytotoxicity rate at higher concentrations. There was a significant effect between the standard and plant extract mediated silver nanoparticles in the cytotoxicity effects. The limitation of the study is its anticancer property against premalignant lesions in the oral cavity has to be analyzed. We can further study using more volume of samples and this can be done as an *in vivo* study against oral ulcer, periodontitis, and oral cancer in future.

CONCLUSION

We can conclude that silver nanoparticles on Stevia and Neem extract have a considerable cytotoxicity effect. The present study revealed that the silver nanoparticles can be synthesized in a simple eco-friendly method using Neem and Stevia extract and showed up a successful outcome in assays therefore it can be used as a mouthwash to prevent ulcer, dental caries, plaque, and periodontal diseases

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CONFLICT OF INTEREST

The authors would like to declare no conflict of interest in the present study.

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