



## **In Vitro Anti-diabetic Activity of Clove and Lemongrass Extract-Mediated Strontium Nanoparticles**

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### **Abstract**

**Introduction:** Diabetes mellitus is a progressive metabolic disorder marked by chronic hyperglycemia and impaired carbohydrate metabolism. The inhibition of carbohydrate-hydrolyzing enzymes, especially  $\alpha$ -amylase, is a widely used strategy for reducing postprandial blood glucose. Medicinal plants such as clove (*Syzygium aromaticum*) and lemongrass (*Cymbopogon citratus*) contain bioactive compounds with antioxidant and antidiabetic potential. In recent years, green synthesis of nanoparticles has emerged as a promising approach to enhance the biological activity of plant extracts.

**Aim:** To evaluate the in vitro antidiabetic activity of clove and lemongrass extract-mediated strontium nanoparticles and compare their inhibitory potential with the herbal formulation and standard control.



**Materials and methods:** Clove and lemongrass extracts were prepared using aqueous extraction and used for the green synthesis of strontium nanoparticles. The antidiabetic activity of the herbal extract and synthesized nanoparticles was assessed by  $\alpha$ -amylase inhibition assay at different concentrations (10, 20, 30, 40, and 50  $\mu$ L). The percentage inhibition was determined spectrophotometrically and compared with the standard.

**Results:** Both the herbal extract and the nanoparticle formulation showed concentration-dependent  $\alpha$ -amylase inhibition. The clove and lemongrass herbal extract demonstrated inhibition ranging from approximately 47% to 86%, while the nanoparticle formulation showed inhibition from approximately 42% to 82%. The standard showed slightly higher inhibition across all concentrations.

**Conclusion:** Clove and lemongrass extract-mediated strontium nanoparticles exhibited significant in vitro antidiabetic activity. The findings suggest that this formulation may be a promising natural candidate for managing postprandial hyperglycemia, although further in vivo and mechanistic studies are required.

**Keywords:** Clove, lemongrass, strontium nanoparticles, green synthesis, antidiabetic activity,  $\alpha$ -amylase inhibition.

## **INTRODUCTION:**

Diabetes mellitus is one of the most common metabolic disorders and remains a major global health concern. It is characterized by chronic elevation of blood glucose levels resulting from defects in insulin secretion, insulin action, or both. Persistent hyperglycemia can lead to a wide range of complications, including cardiovascular disease, nephropathy, neuropathy, retinopathy, and impaired wound healing(1). Because of its chronic nature and increasing prevalence, diabetes places a substantial burden on patients, families, and healthcare systems.

One of the most effective therapeutic strategies for controlling postprandial hyperglycemia is the inhibition of digestive enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase. These enzymes catalyze the breakdown of complex carbohydrates into glucose(1,2). By suppressing their activity, carbohydrate digestion and absorption are slowed, leading to a reduction in the rapid rise of blood glucose after meals. Although synthetic enzyme inhibitors are available, long-term use may be associated with adverse effects, poor compliance, and limited therapeutic tolerance. This has encouraged the search for safer, natural alternatives(3).

Medicinal plants have long been used in traditional medicine for the management of diabetes. Clove and lemongrass are among the most widely studied medicinal plants because of their diverse pharmacological properties. Clove contains eugenol, flavonoids, tannins, and phenolic compounds that contribute to its antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities(4). Lemongrass contains citral, flavonoids, polyphenols, and other active constituents that have shown glucose-lowering and antioxidant effects. Together, these plants may provide synergistic therapeutic benefits(5).



Nanotechnology has introduced a new dimension in drug delivery and biomedical applications. Green synthesis of nanoparticles using plant extracts is especially attractive because it is eco-friendly, cost-effective, simple, and avoids toxic chemical reducing agents(6). Plant-derived nanoparticles often display improved surface reactivity, bioavailability, and biological efficacy. Strontium nanoparticles, in particular, have gained interest because of their biomedical relevance and potential pharmacological activity(7).

In this context, the present study was designed to evaluate the in vitro antidiabetic activity of clove and lemongrass extract-mediated strontium nanoparticles using an  $\alpha$ -amylase inhibitory assay.

## **MATERIALS AND METHOD:**

### **Study design**

The study employed a laboratory-based in vitro experimental design involving the preparation of aqueous extract from clove and lemon grass using a standardized protocol. Strontium nanoparticles were synthesized via a green synthesis method facilitated by the clove and lemon grass extract. The strontium nanoparticles were evaluated for their in vitro enzyme inhibition potential against diabetic enzymes. The inhibitory activity against  $\alpha$ -amylase enzymes was assessed using 3,5-dinitrosalicylic acid. Acarbose was used as the standard.

### **Preparation of clove and lemon grass extract**

The process involved taking fresh lemongrass leaves and dried clove buds, cleaning and drying it in the shade, and then grinding it into a coarse powder. A portion of this coarse powder, weighing 1.025 g, was measured and dissolved in 100 ml of distilled water, which was then heated to 90°C and allowed to boil for 15 minutes. Later, it was filtered through Whatman No. 1 filter paper.

### **Preparation of strontium nanoparticles**

Strontium nitrate (1.0 g) was measured and mixed with 50 ml of distilled water in a conical flask. Subsequently, 50 ml of boiled pomegranate peel extract was added to the strontium nitrate mixture using a magnetic stirrer. This mixture was then placed in an orbital shaker for a duration of 24 hours. After the incubation period, the contents were transferred into a 15 ml falcon tube and centrifuged at 8000 rotations per minute (rpm) for 15 minutes. Following centrifugation, the resulting pellets were extracted from the centrifuge tube and subjected to heating at 100°C in a hot air oven for a full day. The resulting dried clove and lemon grass-SrNPs were then examined for their potential against inhibition of carbohydrate hydrolyzing enzymes within in vitro conditions.

### **$\alpha$ -Amylase inhibition assay**



The inhibition of  $\alpha$ -amylase was carried out by the method described by Malik and Singh. Briefly, the reaction was initiated by the addition of 490, 470, and 450  $\mu$ L buffer to different volumes (10, 30, and 50 ML) of 30mg/mL of clove and lemon grass extract, synthesized strontium nanoparticles, stored (at room temperature, at 37°C and 4°C), and calcined (300°C, 500°C and 700°C) strontium nanoparticles samples, respectively, to make the total volume of 500  $\mu$ L reaction solution.

In the next step, 500  $\mu$ L  $\alpha$ -amylase was added, followed by the addition of 1,000  $\mu$ L of starch to the reaction vessels. Then, the reaction vessels were incubated in a water bath for 5 min at 100°C. Next to this step, 500  $\mu$ L of NaOH is added. The reaction was completed by the addition of 500  $\mu$ L of DNS, and then, the reaction vessels were again incubated for 5 min by putting them in a beaker with hot water. The color change from yellow to orange indicated  $\alpha$ -amylase inhibition activity.

For the preparation of blank, 30  $\mu$ L of clove and lemon grass extract was added into 1,500  $\mu$ L buffer, and all the steps were conducted in the same sequence as mentioned earlier except the addition of amylase and starch. The tubes were left to cool, and the solution. In the next step, 500  $\mu$ L  $\alpha$ -amylase was added, followed by the addition of 1,000  $\mu$ L of starch to the reaction vessels. Then, the reaction vessels were incubated in a water bath for 5 min at 100°C. Next to this step, 500  $\mu$ L of NaOH is added. The reaction was completed by the addition of 500  $\mu$ L of DNS, and then, the reaction vessels were again incubated for 5 min by putting them in a beaker with hot water.

The color change from yellow to orange indicated  $\alpha$ -amylase inhibition activity. For the preparation of blank, 30  $\mu$ L of clove and lemongrass extract was added into 1,500  $\mu$ L buffer, and all the steps were conducted in the same sequence as mentioned earlier except the addition of amylase and starch. The tubes were left to cool, and the absorbance was measured at 540 nm. The percentage inhibition of  $\alpha$ -amylase was calculated as  $((A_s - A_t)/A_s) \times 100$ , where  $A_s$  was the absorbance of the standard and  $A_t$  was the absorbance of the test samples.

#### Statistical analysis

The data obtained for  $\alpha$ -amylase inhibition assays were analyzed by Student's t-test using SPSS software (IBM Corp., Armonk, NY). For statistical analysis, the triplicate values of each single concentration were taken into account and a comparison was done between nanoparticle and standard. The % inhibitory effect ( $n=3$ /concentration) was represented as mean  $\pm$  SEM for triplicates.  $P < 0.05$  is considered as significant.

**RESULTS:**

**FIGURE 1: Preparation steps involved in clove and lemongrass extract-mediated synthesis of strontium nanoparticles.**

1: boiling of clove and lemongrass extract; 2: Filtration of clove and lemongrass extract  
3: Strontium Nitrite 4: clove and lemon grass mediated strontium solution.

**Effect of Clove and lemongrass-Strontium NPs on  $\alpha$ -amylase activity:**



The nanoparticle formulation also showed a concentration-dependent increase in inhibition, from approximately 42% at 10  $\mu\text{L}$  to 82% at 50  $\mu\text{L}$ . The standard produced inhibition values ranging from 52% to 88%.

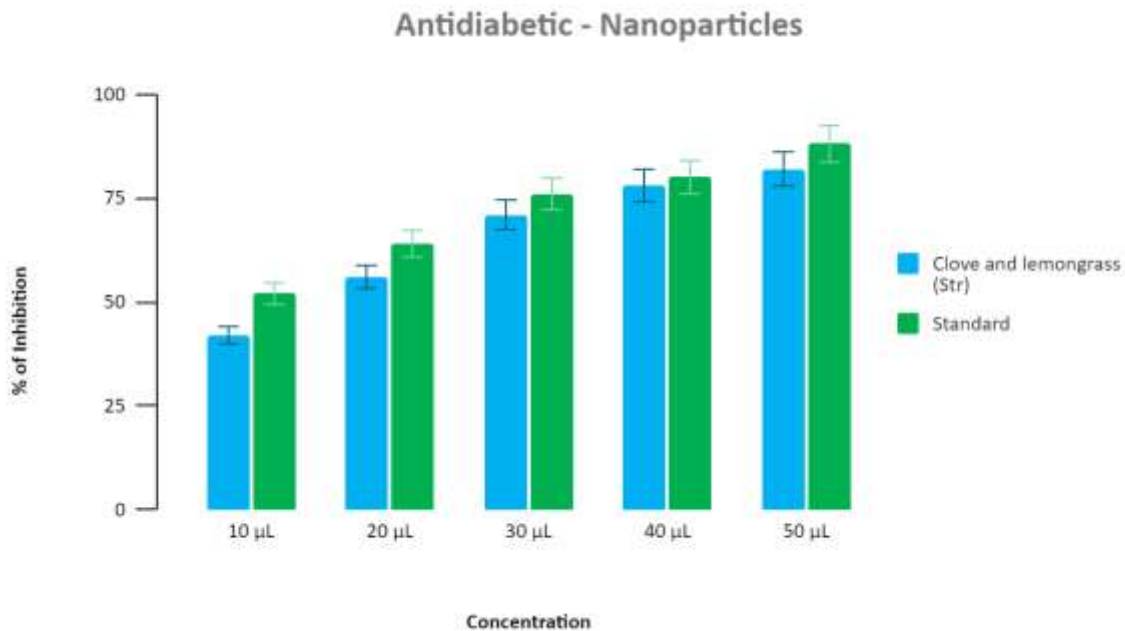


Table 1 represents The nanoparticle formulation demonstrated notable antidiabetic activity with increasing concentration. Although slightly lower than the standard, the formulation achieved substantial inhibition at higher concentrations, indicating effective enzyme inhibitory potential.



### Anti diabetic activity of herbal extract:

The herbal extract exhibited increasing inhibitory activity with increasing concentration, ranging from approximately **47% inhibition at 10  $\mu$ L to 86% inhibition at 50  $\mu$ L**. The standard showed slightly higher inhibition values (**52–88%**) across all concentrations. The herbal extract demonstrated activity comparable to the standard, particularly at higher concentrations.

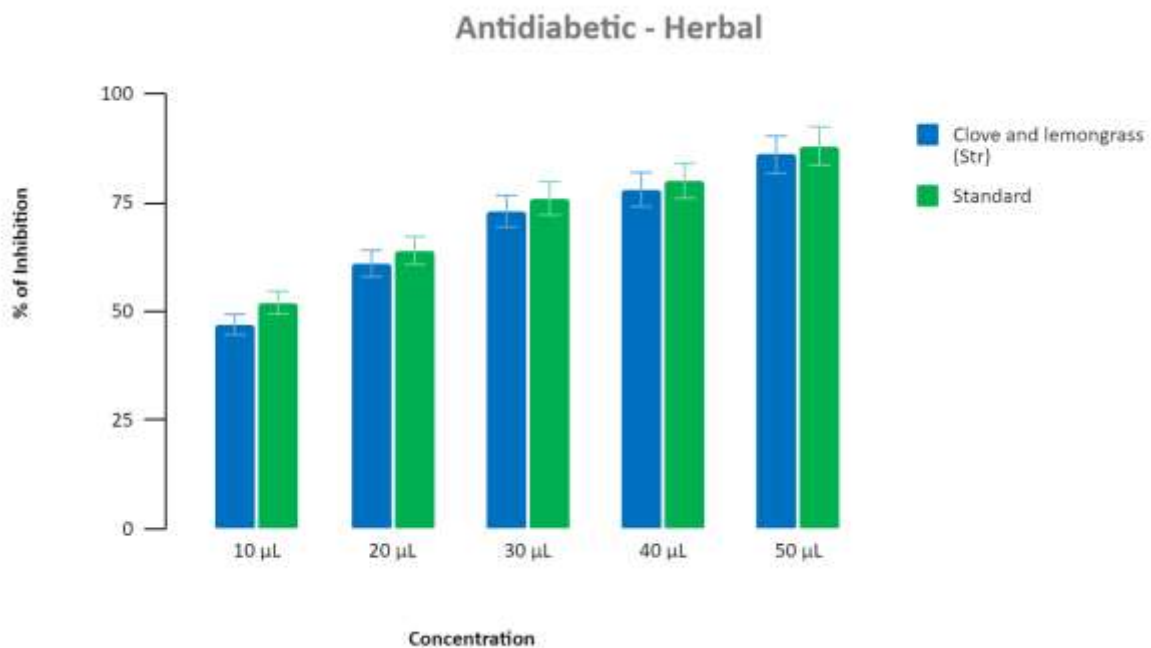


Table 2 represents The herbal extract exhibited significant antidiabetic activity, with inhibition increasing in a dose-dependent manner. At 50  $\mu$ L, the extract achieved approximately 97–98% of the activity of the standard, indicating strong inhibitory potential.



### **DISCUSSION:**

The present study evaluated the in vitro antidiabetic activity of clove and lemongrass-mediated strontium nanoparticles using the  $\alpha$ -amylase inhibitory assay. The results demonstrated a concentration-dependent increase in enzyme inhibition, with the synthesized nanoparticles exhibiting 42%, 56%, 71%, 78%, and 82% inhibition at concentrations of 10, 20, 30, 40, and 50  $\mu$ L, respectively. Although the inhibitory activity was slightly lower than that of the standard drug, the nanoparticles exhibited substantial antidiabetic potential, indicating their possible role in controlling postprandial hyperglycemia.(8)

The findings of the present study are consistent with those reported by Royapuram Parthasarathy et al., (9)who investigated the antidiabetic activity of pomegranate peel extract-mediated strontium nanoparticles. Their study demonstrated concentration-dependent inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes, with maximum inhibition values of 79.28% and 76.17%, respectively, at the highest tested concentration. The authors concluded that plant-mediated strontium nanoparticles could effectively suppress carbohydrate-hydrolyzing enzymes and may be useful in reducing postprandial blood glucose levels(10). Similar to their observations, the clove and lemongrass-mediated strontium nanoparticles in the present study exhibited marked inhibition of  $\alpha$ -amylase activity, supporting the therapeutic relevance of strontium nanoparticle formulations in diabetes management.

The observed antidiabetic activity may be attributed to the phytochemical constituents present in clove and lemongrass. Clove is rich in eugenol, gallic acid, flavonoids, and tannins, which have been reported to possess antioxidant and antihyperglycemic properties(11). Eugenol has been shown to enhance glucose utilization, improve insulin sensitivity, and reduce oxidative stress associated with diabetes. Lemongrass contains citral, geraniol, flavonoids, and phenolic compounds that contribute to its antioxidant and enzyme inhibitory effects. These bioactive compounds may interfere with carbohydrate digestion by inhibiting  $\alpha$ -amylase activity, thereby delaying glucose absorption(12).

Several studies have reported similar findings with plant-mediated nanoparticles. Bagyalakshmi and Haritha demonstrated that silver nanoparticles synthesized using *Pterocarpus marsupium* exhibited significant  $\alpha$ -amylase inhibitory activity comparable to acarbose. Likewise, Thirumal and Sivakumar reported potent antidiabetic activity of silver nanoparticles synthesized from *Cassia auriculata* leaves. These studies support the concept that nanoparticle



synthesis enhances the biological efficacy of medicinal plants by improving surface reactivity and interaction with target enzymes.

Badmus et al., reported that silver nanoparticles synthesized using *Annona muricata* leaf extract exhibited remarkable inhibition of both  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes(13). Similarly, Prasad et al. (14)demonstrated highly selective  $\alpha$ -glucosidase inhibition by green-synthesized zinc oxide nanoparticles derived from *Citrus limon*. These findings further support the growing evidence that plant-mediated nanoparticles can serve as effective enzyme inhibitors and potential antidiabetic agents(15).

The antidiabetic activity observed in the present study may also be related to the unique physicochemical properties of strontium nanoparticles(16). Nanoparticles possess a high surface-area-to-volume ratio, which enhances their interaction with biological molecules. Previous investigations by Mukherjee et al. demonstrated that strontium-based nanomaterials improved glucose metabolism and insulin regulation in diabetic experimental models. The authors suggested that strontium nanoparticles may influence carbohydrate metabolism pathways and improve glucose homeostasis(17). The present findings support this hypothesis and suggest that strontium nanoparticles synthesized using clove and lemongrass extracts retain significant biological activity.

Overall, the findings of the present investigation are in agreement with previous studies demonstrating the antidiabetic potential of plant-derived nanoparticles(18). The concentration-dependent inhibition observed suggests effective suppression of  $\alpha$ -amylase activity and highlights the potential application of clove and lemongrass-mediated strontium nanoparticles as natural antidiabetic agents.

### **Limitations**

This study is limited to in vitro enzyme inhibition. The results provide preliminary evidence of antidiabetic potential, but they do not confirm in vivo efficacy, safety, bioavailability, or molecular mechanism. Further studies should evaluate the formulation using cell-based assays, animal models, and detailed characterization techniques such as UV-visible spectroscopy, FTIR, SEM, and XRD. Toxicity studies are also needed before any therapeutic application can be considered.



### **Conclusion**

Clove and lemongrass extract-mediated strontium nanoparticles exhibited significant in vitro antidiabetic activity through  $\alpha$ -amylase inhibition. Both the herbal extract and the nanoparticle formulation showed a concentration-dependent increase in inhibitory effect, with the herbal extract reaching about 86% inhibition and the nanoparticle formulation reaching about 82% inhibition at 50  $\mu$ L concentration. The standard showed slightly higher activity, but the test formulation demonstrated strong promise.

These findings suggest that clove and lemongrass-based strontium nanoparticles may serve as a potential natural strategy for diabetes management, particularly in controlling postprandial hyperglycemia. However, further in vivo, toxicological, and mechanistic investigations are necessary to confirm their therapeutic value.

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