ORIGINAL RESEARCH PAPER

Biochemistry

COMPARATIVE EVALUATION OF ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF THE GREEN SYNTHESIZED SILVER NANOPARTICLES AND HYDROETHANOLIC EXTRACT OF CINNAMOMUM VERUM: AN INVITRO STUDY

KEY WORDS: Cinnamomum verum, Hydroethanolic, Silver nanoparticle, Anti-oxidant, Anti-inflammatory.

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ABSTRACT

Cinnamomum verum (C.Verum) is known for its potent antioxidant and anti-inflammatory properties, attributed to its bioactive phytochemicals. **Aim:** This study compares the antioxidant and anti-inflammatory efficacy of hydroethanolic extracts and silver nanoparticle (AgNP) extracts of C.verum. **Materials & Methods:** Hydroethanolic extract of the powdered C.Verum was prepared by mixing 70 ml of distilled water with 30 ml of ethanol. 0.1ml of hydroethanolic extract of C.Verum was mixed with 50ml of 1mM aqueous silver nitrate solution and kept at room temperature for 8 hours to produce Ag nanoparticles. Antioxidant capacity was evaluated using DPPH assay, while anti-inflammatory effects were assessed through in-vitro protein denaturation inhibition. **Results:** Cinnamomum AgNPs exhibited superior antioxidant and anti-inflammatory activity compared to hydroethanolic extract, likely due to enhanced surface area, particle reactivity, and bioactive compound stability in the nanoparticle formulation. **Conclusion:** This study shows the potential of C.Verum based AgNPs as a novel therapeutic approach, offering enhanced efficacy over traditional extracts in managing oxidative stress and inflammation-related disorders.

INTRODUCTION

C.verum, commonly known as true cinnamon, is native to Sri Lanka and belongs to the Lauraceae family. Widely used as herbal medicine due to its wide range of various therapeutic effects (1). Rich in bioactive compounds such as cinnamaldehyde, cinnamic acid, and polyphenols, responsible for their anti-oxidant, anti-inflammatory, antidiabetic, antibacterial, and anticancer activities (2). Green synthesis of nanoparticles using plant extracts is a low-cost, environmentally friendly approach that produces stable nanoparticles (3), unlike chemical synthesis where particle stability decreases over time. Additionally, green synthesis of herbal drugs offers several advantages, including improved drug delivery, reduced toxicity, cost-effectiveness, and increased bioavailability (4).

MATERIALS AND METHODS

C. verum bark was procured from a certified seller from online store and authenticated by a botanist through macroscopic characterization.

Synthesis of crude extract and silver nanoparticle using C.Verum bark extract

For the preparation of C.verum extract, 2.5 g of powdered bark was mixed with 100 ml of distilled water and boiled for 5 minutes in a conical flask. The mixture was then cooled, filtered using Whatman filter paper, and stored at 4°C (5). To synthesize silver nanoparticles, 1 mL of C. verum extract was added to 50 mL of 1 mM aqueous silver nitrate ($AgNO_3$) solution and incubated at room temperature for 8 hours. Initially, the solution appeared yellowish, but upon reduction of Ag^+ ions to silver nanoparticles, it gradually changed to a darker color (6). The color change was monitored at hourly intervals to track the nanoparticle formation.

DPPH Assay for Anti-Oxidant activity

The antioxidant activity of extracts was measured by evaluating their ability to scavenge the stable radical DPPH (1,1-Diphenyl-2-Picrylhydrazyl). The DPPH, a purple free radical, is reduced to yellow 2,2-Diphenyl-1-Picrylhydrazine when it reacts with antioxidants. The antioxidant activity of C.Verum extract and silver nanoparticle extract was tested at concentrations of 50-500 µg/mL, while vitamin C at concentrations of 2,4,6,8 and10 µg/mL served as a standard. The scavenging activity was expressed as a percentage of free radical reduction, and absorbance was measured using spectrophotometry, with ascorbic acid as the positive control and solvents as the negative control. Mean values were taken from triplicate experiments. IC50 values of DPPH radical scavenging activity were determined by the following formula: Free Radical Scavenging % = [(A0- A1)/A0] x100. where, A0:-absorbance of control (negative control i.e. without extract) A1 :- absorbance of extract.

Anti-Inflammatory activity by Protein Denaturation assay

The anti-inflammatory activity of C. verum extract and its silver nanoparticle extract was evaluated using the albumin denaturation assay. Various concentrations of the extracts (100, 200, 300, 400, and 500 µg/mL) were added to a 1% aqueous solution of 0.45 mL of bovine serum albumin. The pH of the mixture was adjusted to 6.3 using 1N hydrochloric acid. Then, the samples were incubated at room temperature for 20 minutes, followed by heating in a water bath at 55°C for 30 minutes. After cooling, the absorbance was measured spectrophotometrically at 660 nm. Diclofenac sodium was used as the standard for this study. The percentage of protein denaturation was calculated using the following formula.

Anti-Denaturation activity(%) = (Absorbance of control – Absorbance of sample /Absorbance of control) x 100

RESULTS:

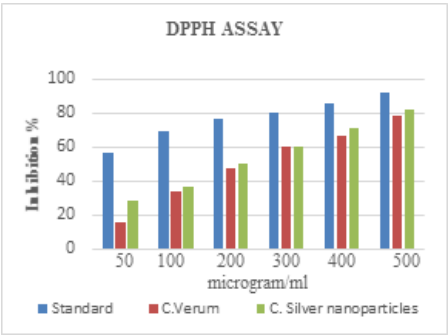
Anti- Oxidant activity

The in-vitro antioxidant potential of C. verum extract and cinnamomum silver nanoparticles is illustrated in Graph 1. Both the hydroethanolic extract and C. verum silver nanoparticles exhibited the highest radical scavenging activity at a concentration of 500 µg/mL.

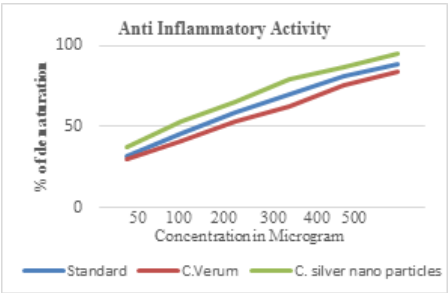
Anti-Inflammatory activity

The in-vitro anti-inflammatory activity was highest at 500 mg for both the hydroethanolic extract and the cinnamon silver nanoparticle extract. However, the cinnamon silver

nanoparticle extract demonstrated greater anti-inflammatory activity compared to the hydroethanolic extract.



Graph1: Illustrates anti-oxidant activity of C. verum and Cinnamomum silver nanoparticles compared with Standard



Graph 2: Illustrates anti-inflammatory activity of C.Verum and Cinnamomum Silver nanoparticles compared with Standard

DISCUSSION:

The antioxidant activity of C. verum is attributed to its ability to scavenge free radicals, reduce oxidative stress, and inhibit lipid peroxidation. The presence of polyphenols and flavonoids in C.Verum extract enables it to donate hydrogen atoms and electrons, thereby neutralizing reactive oxygen species (ROS) (7). Studies indicate that C.Verum extract exhibits strong DPPH assay results, demonstrating its potent antioxidant potential. On the other hand, silver nanoparticles synthesized using C. verum extract have shown enhanced antioxidant activity compared to the crude extract. This enhancement is likely due to the increased surface area and reactivity of nanoparticles, which allows better interaction with free radicals. The bioactive compounds from C.Verum involved in nanoparticle synthesis may also stabilize silver nanoparticles, synergistically improving their free radical scavenging ability (8). Several reports suggest that AgNPs exhibit superior antioxidant activity in comparison to C.Verum extract, possibly due to the controlled and prolonged release of active compounds from the nanoparticle matrix.

C.verum has been extensively studied for its anti-inflammatory properties, primarily mediated through the inhibition of pro-inflammatory cytokines, suppression of NF-KB signaling, and downregulation of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (9). The cinnamaldehyde and eugenol present in C.Verum play crucial roles in alleviating inflammation by reducing the production of nitric oxide (NO) and other inflammatory mediators (10). Silver nanoparticles synthesized using C.verum have demonstrated superior anti-inflammatory effects in comparison to crude extracts. The nanoparticles can modulate immune responses by reducing oxidative stress-induced inflammation, suppressing pro-inflammatory cytokine production, and inhibiting cellular inflammatory pathways. Additionally, AgNPs exhibit direct interactions with inflammatory cells, leading to reduced macrophage activation and cytokine secretion. The nano formulation enhances the bioavailability and targeted delivery of active compounds, thus amplifying their therapeutic efficacy. Moreover, in comparison to aqueous extract, hydroethanolic solvent appears to perform better in terms of total phytochemical constituents and antioxidant activity which could be attributed to the polarity of solvent used for extract preparation (11).

CONCLUSION

Both C.verum extract and its silver nanoparticles exhibit potent antioxidant and anti-inflammatory activities, but the latter shows a significant enhancement in efficacy. The nano formulation of silver with C.Verum derived phytochemicals results in a synergistic effect, improving the biological activities through increased surface area, controlled release, and enhanced interaction with biological molecules. This comparison underscores the potential of silver nanoparticles synthesized using C. verum as a novel therapeutic approach for managing oxidative stress and inflammatory disorders.

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