

Anti-inflammatory activity of nutmeg oleoresin mediated silver nanoparticles- An *In-vitro* study

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ABSTRACT

Introduction and Aim: The most emerging area of research in nanotechnology deals with the synthesis of nanoparticles which are of great importance due to its use in various biological fields. *Myristica fragrans* is commonly known as “nutmeg”. It is popular as a spice and also possesses various therapeutic properties. It has a characteristic pleasant fragrance and a slightly warm taste. It has various therapeutic uses and is widely used. The aim is to assess the anti-inflammatory activity of nutmeg oleoresin mediated silver nanoparticles.

Materials and Methods: The nutmeg oleoresin mediated silver nanoparticles were synthesized and was confirmed by UV Vis spectroscopy. The anti-inflammatory property of the prepared nutmeg oleoresin mediated silver nanoparticles was assessed using albumin denaturation inhibitory assay technique.

Results: The nutmeg oleoresin mediated silver nanoparticles showed good anti-inflammatory activity with increasing concentration of the nanoparticles.

Conclusion: Although a variety of steroidal and non-steroidal anti-inflammatory drugs have been developed, researchers are focusing on natural substances to develop new anti-inflammatory agents. Nutmeg mediated silver nanoparticles showed a good range of inhibition and can be used against inflammation. The myristicin present in the nutmeg maybe responsible for its anti-inflammatory action. Increased albumin denaturation is reported in conditions like in rheumatoid arthritis, diabetes and cancer. Hence this may pay way to manage such conditions.

Keywords: *Myristica fragrans*; anti-inflammatory; nanoparticles; oleoresin; albumin denaturation assay technique.

INTRODUCTION

The most emerging area of research in nanotechnology deals with the synthesis of nanoparticles which are of great importance due to its use in various biological fields. The synthesis of nanoparticles is based on the chemical composition, mono-dispersity and dimensions (1, 2). Till date, metallic nanoparticles are mostly prepared from noble metals like gold, silver and platinum. The nanoparticles are used in the field of catalysis,

optoelectronics, diagnostic biological problem and these uncovered many significant findings.(1-4) Among the noble metals silver is the metal of choice in the field of biological system, living organism and medicine (5-7). The formation of nanoparticles include sol process, micelles, sol-gel process, chemical precipitation, hydrothermal method pyrolysis chemical vapor deposition, bio-based protocols etc (8). The bio-based protocols are currently under exploitation as it is cost-effective eco-friendly.

Myristica fragrans is commonly known as “nutmeg”. It is popular as a spice and also possesses various therapeutic properties. It has a characteristic pleasant fragrance and a slightly warm taste. It has various therapeutic uses and is widely used worldwide (9, 10). It is rich source of vitamin A, C and E, electrolytes (sodium and potassium) and minerals (manganese, zinc, copper, iron, calcium and phosphorus). It is used in flavoring many kinds of baked food, confections, puddings, meat, sausages and beverages (11).

M. fragrans has been used as a folklore medicine to treat diarrhea, mouth-sores and insomnia. The essential oil of nutmeg is used externally for sprains, rheumatism and paralysis, which possesses its analgesic and anti-inflammatory properties (12). Compounds isolated from the seeds of this plant have been reported to possess strong platelet anti-aggregatory activity (13,14). The myristicin found in *M. fragrans* has cytotoxic and apoptotic effects in neuroblastoma SK-N-SH cells with an accumulation of cytochrome and activation of Caspase 3 in the cytosol, which shows its anti-carcinogenic properties (15). In the present study, the anti-inflammatory activity of nutmeg oleoresin mediated silver nanoparticles was assessed using albumin denaturation inhibition assay.

MATERIALS AND METHODS

The nutmeg oleoresin was gifted by Synthite Industries Ltd, Kerala.

Synthesis of Nutmeg oleoresin mediated silver nanoparticles

90 mL of 1 millimolar of silver nitrate in water was mixed with 10 mL of the nutmeg oleoresin. The solution was kept in orbital stirrer for nanoparticles synthesis. The nanoparticles were centrifuged using lark refrigerated centrifuge at 8000 rpm for 10 minutes and the pellet is collected and was washed twice with distilled water. The final purified pellet was collected, dried at 60°C and stored in air tight Eppendorf tube (Fig 1, 2).

Inhibition of albumin denaturation assay

BSA (bovine serum albumin) was used as a reagent for the assay. Bovine serum albumin (BSA) makes up approximately 60% of all proteins in animal serum. It is commonly used in cell culture, particularly when protein supplementation is necessary, and the other components of serum are unwanted. BSA undergoes denaturation upon heating and starts expressing antigens associated with type III hypersensitive reaction which are related to diseases such as rheumatoid arthritis, glomerulonephritis, serum sickness and systemic lupus erythematosus (16). 2 ml of 1% bovine albumin fraction was mixed with 400 ml of plant crude extract indifferent concentrations (20-100 µg/ml) and the pH of reaction mixture was adjusted to 6.8 using HCL. The reaction mixture was incubated at room temperature for 20 minutes and then heated at 55°C for 20 min in a water bath. The mixture was cooled to room temperature and the absorbance value was recorded at 660 nm. An equal amount of plant extract was replaced with DMSO for control. Diclofenac sodium in different concentrations was used as a standard. The experiment was performed in triplicate (fig. 3).

% inhibition was calculated using the following formulae

$$\% \text{ inhibition} = \frac{\text{control O.D} - \text{sample O.D}}{\text{Control O.D}} \times 100$$

RESULTS AND DISCUSSION

The synthesis of nanoparticles is in the lime light in the modern nanotechnology. Amidst the drugs available outside, researchers are now focusing on drugs which can be obtained from natural products and hence, these natural products are under experimental process for the synthesis of nanoparticles (7).

The reaction mixtures demonstrated a gradual increase in the color development from pale yellow to dark brown in color and exhibited a strong absorbance at 430 nm in UV visual analysis. This indicated the formation of nanoparticles. The reduction in the silver ions

present in aqueous solution of silver complex in the nutmeg extract demonstrated the change in color was due to the formation of silver nanoparticles. When the anti-inflammatory property of the nanoparticles was assessed, it was found that the percentage of inhibition kept increasing with the increase in concentration of the nutmeg silver nanoparticles. This proves the effect of nutmeg against inflammation.

The anti-inflammatory property of nutmeg was checked using the HRBC membrane stabilizing method. Nutmeg seed extract was reported to

have anti-inflammatory property. The anti-inflammatory activity of *M. fragrans* (Nutmeg) was carried out using HRBC membrane stabilizing method (17). Plants such as *B. racemosa* have also shown to have anti-inflammatory property by inhibiting albumin denaturation assay (18). Increased albumin denaturation is reported in conditions like in rheumatoid arthritis, diabetes and cancer (19). Similar studies are reported the anti-inflammatory activity of plant extract mediated silver nanoparticles (20).



Fig. 1: Visual observation

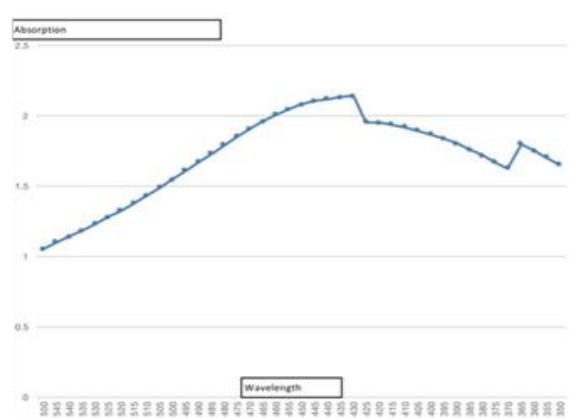


Fig. 2: UV Spectroscopy

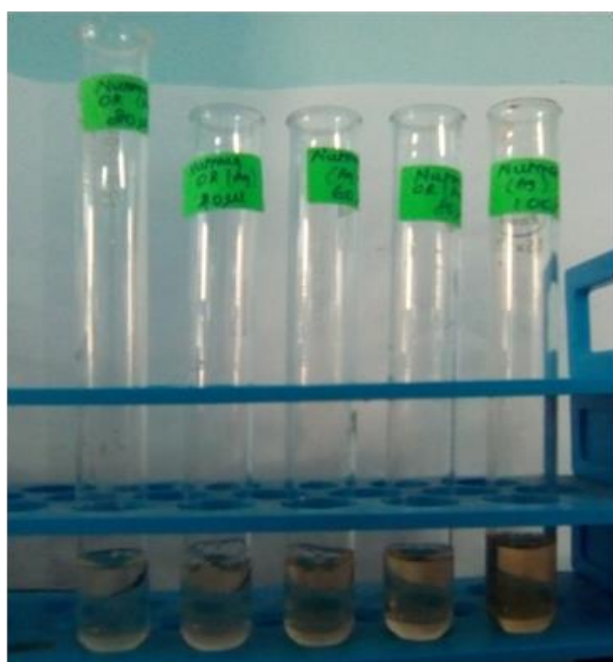
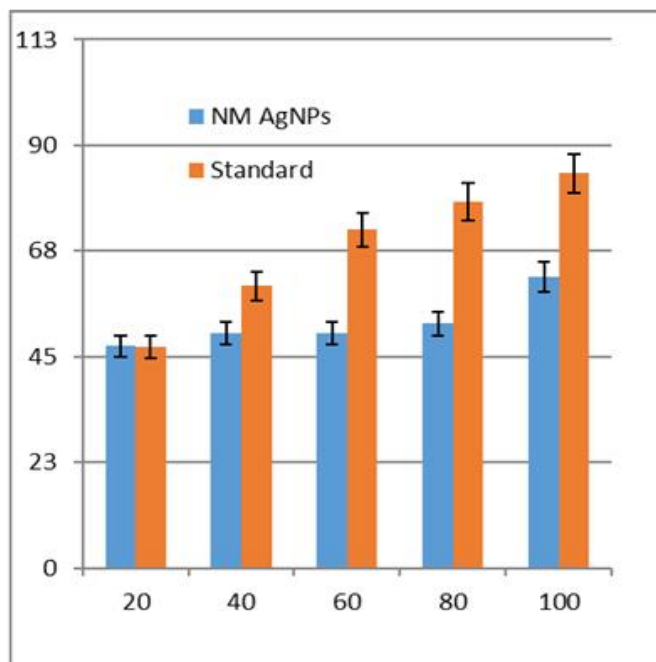


Fig.3: Antiinflammatory Activity



CONCLUSION

Although a variety of steroidal and non-steroidal anti-inflammatory drugs have been developed, researchers are focusing on natural substances to develop new anti-inflammatory agents. Nutmeg oleoresin mediated silver nanoparticles showed a good range of inhibition and can be used against inflammation. The oil present in nutmeg, myristicin is responsible for its anti-inflammatory action. Hence, this nutmeg oleoresin mediated silver nanoparticles may be used for conditions such as rheumatoid arthritis, cancer and diabetes.

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