Role Of In- Situ Rutin Flavonoid Consisted Myristica Fragrans Silver Nano Particles (MF-Ag-Nps) Against Escherichia Coli and Bacillus Subtilis

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Abstract:
Most microbiological growth inhibitors are toxic and chemical solvents are harmful for human health. Copper, silver, and gold metallic nanoparticles have several uses in biotechnology and medicinal research. MF-Ag-NPs(silver nanoparticles)are potent anti-inflammatory, anti-proliferative, and anti-microbial agents. The purpose of this study was to biogenically generate silver nanoparticles by extracting rutin from nutmeg, a seed from the Myristica fragrans plant. In order to create Myristica fragrans MF-Ag-NPs, silver nitrate was dissolved in the extract. The transformation of the colour from dark brown to pale brown confirmed the conversion of Ag+ to Ag°. FTIR, EDX, and SEM were used to describe them. Images from scanning electron microscopy demonstrate the presence of silver nanoparticles between 20 and 300 nm in size. The presence of oxygen and silver, as well as the metal's oxidation state, were confirmed by EDAX analysis. The nanoparticles' distinctive functional groups were discovered using FTIR spectroscopy. The antibacterial activity of Myristica fragrans seed extract was assessed. The extract from the seeds of Myrsitica fragrans exhibited the strongest antibacterial properties. These findings imply that antibacterial MF-Ag-NPs phyto-formulated with nutmeg extracts might be used to treat microbial infections in the future.

Keywords: *Myristica fragrans, Rutin, Silver nanoparticles, Scanning Electron Microscopy*

1.Introduction:

Research and development on nanotechnology, as well as its uses in agriculture and medical, is a current and ongoing activity. Several other biological fields have successfully investigated this technology. The secret to nanoparticles' success is their small size, which makes it easy for them to penetrate matter and get to the desired cell. The number of bacterial strains that are resistant to various antibiotics has dramatically increased during the past few years, both locally and globally. In order to stop their growth and proliferation, the medical and pharmaceutical sectors have been forced to create alternative, innovative medications [1]. To battle tenacious and persistent fungal plant infections, the food and crop production sectors urgently require an alternative control technique to fungicides. Numerous resistant fungal strains have emerged as a result of the uncontrolled use of synthetic insecticides and fungicides. The agri-food sector is therefore investing heavily in research into alternative biologicals, such as greenly produced nanomaterials. The predicted food supply and agricultural output for the expanding global population have always been impeded by significant losses in food commodities caused by plant pathogenic fungus. Scientists are investigating and researching healthier alternatives that inhibit the growth of phytopathogens, enhancing agricultural yields and food production in response to the growing demand for a secure food supply [2,3]. Additionally, the food's nutritional value and quality must be maintained [3].

Innovative nanotechnology uses numerous metals, including zinc oxide, copper oxide, gold, and silver, to produce nanoparticles [4]. The use of biologically produced nanoparticles (NPs) has significantly increased recently compared to nanoparticles produced using more traditional techniques, such as chemical and physical approaches. In comparison to other approaches, the biological method of synthesis is more expedient, less expensive, and less harmful [5]. The biological components used in this process include fungi [6], algae [7], bacteria [8], plants [9], and biological compounds [10]. The green synthesis of NPs, which makes use of plant materials and their extracts, is one of them. Because it uses a variety of plant parts, including roots [11], stems [12], leaves [13], flowers [14], fruits [15], and seeds [16], for the fabrication while employing various metals and their oxides, green synthesis of NPs is sometimes referred to as phyto-fabrication. Plant resources are extremely suitable and dependable for the bio-fabrication of metallic NPs, according to prior studies on the green synthesis of NPs [17]. When compared to the standard antifungal fluconazole, MF-Ag-NPs made from

extracts of thyme leaves and ginger rhizomes showed strong antifungal efficacy against Candida albicans [18]. Artocarpus heterophyllus Lam [19], Cuscuta japonica [20], Illicium verum [21], Trigonella foenum-graecum L [22], and Emblica officinalis [23] seed and fruit extracts were used to create silver and gold nanoparticles. In a prior study, MF-Ag-NPs created from aqueous Cuscuta japonica seed extracts shown strong antibacterial activity. Several bioactive chemicals discovered in the FTIR investigations of both extracts and MF-Ag-NPs of C. japonica were blamed for the potent bacterial suppression. The bioactive chemicals, including esters, alcohols, phenols, and carboxylic acids, that were discovered in the FTIR analyses of both the extracts and MF-Ag-NPs of C. japonica were responsible for the potent bacterial suppression. Similar to this, tiny MF-Ag-NPs (3–25 nm) with extremely potent antibacterial action against both gram-positive and gram-negative bacteria, such as Staphylococcus aureus, Bacillus subtilis, and Escherichia coli, were made using the seed powder extracts of Artocarpus heterophyllus Lam. As a result, phyto-fabrication is thought to be a great and straightforward method for creating NPs because it has paved the way for safer, more environmentally friendly, and more sustainable nanoproducts [24]. In addition, green synthesis is a widely sought-after method in biological synthesis since it doesn't require the use of growth culture media or particular environmental conditions to promote and sustain the growth of biological organisms. Additionally, plants themselves are a cheap and plentiful source of the raw materials used in green synthesis. Their comparatively quicker synthesis produces clean, safe, and eco-friendly nanoparticles with improved performance. The pharmaceutical business relies heavily on plants as vast repositories of unique chemical compounds to supply a variety of medications that are utilised as therapeutic agents for various diseases. Plant extracts include phytocompounds such alkaloids, flavonoids, terpenes, polyphenols, carbonyl compounds, and various proteins that function as capping, reducing, and stabilising agents during the formation of NPs [25].

Silver is the metal that is used the most frequently overall, particularly for making NPs. MF-Ag-NPs are already well-established and widely used in a variety of industries, including nanomedicine [26], drug delivery [27], anti-microbial goods [28], nanofertilizers [29], nanopesticides [30], nanomedicine [31], nanomedicine, and other biological applications. MF-Ag-NPs have gotten a great deal of approbation as an antibacterial, antifungal agent, and disinfectant notwithstanding a few findings that highlight their negative toxicity effects [32].

Tropical scented evergreen plant Myristica fragrans is a member of the Myristicaceae family. This plant's arils are referred to as mace, while the seed is usually known as nutmeg. The arils surround the seed in the form of a network. In many different cuisines, both the seed and the arils are frequently used as spices [33]. Nutmeg is frequently prescribed to treat peptic ulcers, flatulence, stomach discomfort, gastrointestinal problems, and anxiety in Unani, Ayurvedic, and Chinese traditional medicine [34,35]. Bactericidal activity of aqueous nutmeg extracts has only seldom been reported. Therefore, the objective of the current work was to investigate the antibacterial activity of silver nanoparticles synthesized using green synthesis from M. fragrans seed.

2.MATERIALS AND METHODS:

2.1. Plant material collection

Myristica fragrans (Nutmeg) dried ripe seeds were obtained from a regional place.After removing the dirt and any foreign elements, the area was cleaned with double distilled water.To obtain the active components in a constant size, the dried nutmeg seed was mashed with a mortar and pestle.

2.2. Chemicals

Silver nitrate, Sodium carbonate, Folin-Ciocalteu reagent, Methanol, Distilled water.

2.3. Nutmeg seed aqueous extract preparation:

8 grams dried nutmeg seed powder was added to 100 ml water, and the solution containing extract was agitated for 1 hour at 95°C. The solution was then allowed to cool before being filtered using Whatmann filter paper with a pore size of 25 m and centrifuged for 15 minutes at 8000 rpm. The supernatant produced after centrifugation was utilised as seed extract throughout.

2.4.Silver nanoparticle biosynthesis:

Aqueous seed extract was employed to synthesise MF-Ag-NPs.In a 250 ml conical flask, 5 ml of aqueous seed extract was added to 95 ml of 1mm aqueous Silvernitrate solution and shaken for 24 hours at room temperature.The colour shift from pale brown to dark brown, as illustrated in Fig. 1, verified the bioreduction of silver nitrate to silver nanoparticles. After incubation, the MF-Ag-NPs were collected by centrifugation at 12000 rpm for 15 minutes. After being collected, the pellet was washed twice with double distilled water. The resulting silver nanoparticles were calcined at 250°C for 1 hour before being rinsed once with double distilled water to produce samples. [36,37]


A.**Without Silver nitrate** **B.With Silver nitrate**

Fig.1.Color change of Myristica fragrans seed extract from yellow to brown(A&B)

2.5.Characterization of MF-Ag-NPs:

SEM, elemental analysis, and Fourier transform infrared spectroscopy were used to characterise the silver nanoparticles that were manufactured. Scanning electron microscopy was used to analyse the surface shape and size of silver nanoparticles (SEM). SEM images of Silver nanoparticles generated by *Myristica fragrans* seed extract obtained by the bioreduction process are shown in Figures 2A&B. The Size of the silver nanoparticles were found in the range of 20µm and 300nm in size.

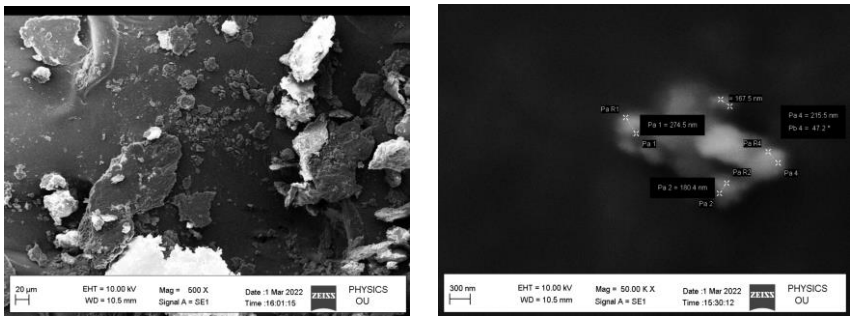


Fig.2 A&B- SEM micrograph of the Silver nanoparticles 20µm and 300nm

2.6.Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy:

Confirmation of silver nanoparticles through SEM-EDX data obtained shown below in Fig.3 Possibly omitted peak:2.170 ke V

Element	Weight(%)	Atomic(%)
C K	48.00	68.15
OK	26.02	27.74
Ag L	25.98	4.11
Total	100.00	

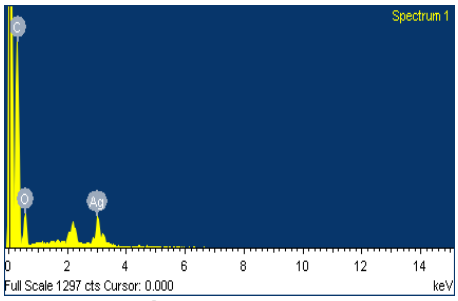


Fig.3 SEM-EDX

3.Results and Discussion:

The batch extraction process's physicochemical parameters were optimised.

3.1.Effect of varying solvent percentages on Rutin extraction:

When compared to monocomponent solvent systems, methanolic mixtures with varied quantities of water have demonstrated to be more successful in phenolic compound extraction.Small amounts of water added to an organic solvent generally results in a more polar media, which aids in the extraction of polyphenols.The dried *Myristica fragans*(nutmeg)seed powder was extracted with various percentages of the methanol(20%,40%,60%,80%,100%) were checked for the maximum extraction yield of Rutin and the results are shown in Fig 4.

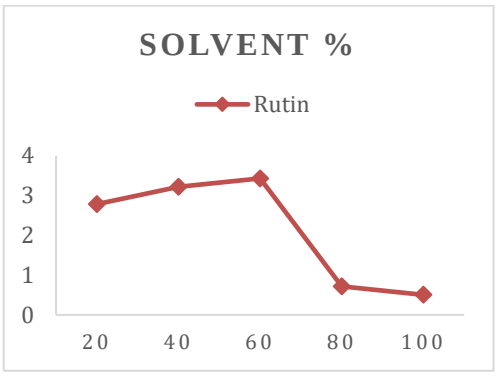


Fig .4 Concentration of Rutin vs Solvent percentage(%)

The results showed the maximum concentration of Rutin of 3.428 µg/ml was observed at 60% methanol.

3.2.Effect of soaking time on Rutin extraction:

Soaking time is another important factor to consider when optimising Rutin extraction. The nutmeg seed powder was steeped in 60% methanol for 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 160 minutes, and the extraction result was observed as shown in Fig 5.

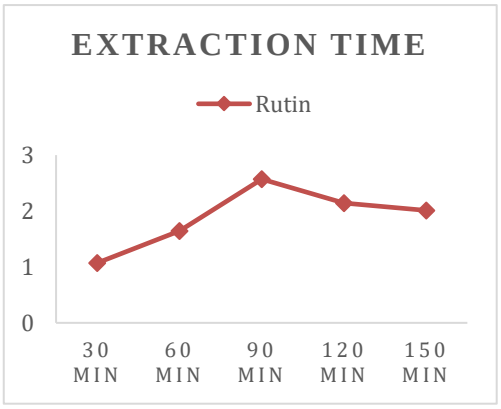


Fig 5.Concentration of Rutin vs Extraction Time(min)

Extraction times longer than 90 minutes resulted in rutin loss. It has been noticed that the extraction time is longer than the resulting polyphenol content. This might be due to the oxidation of phenolic chemicals, which then polymerize into insoluble molecules.

The results revealed that after 90 minutes of incubation of nutmeg seed powder in 60 percent methanol, a greater concentration of rutin of 2.57 g/ml was detected.

3.3.The Effect of pH on Rutin Extraction:

The extraction yield of components was detected at various pH values, including 5,6,7,8,9, and 10. Figure 6 depicts the results.

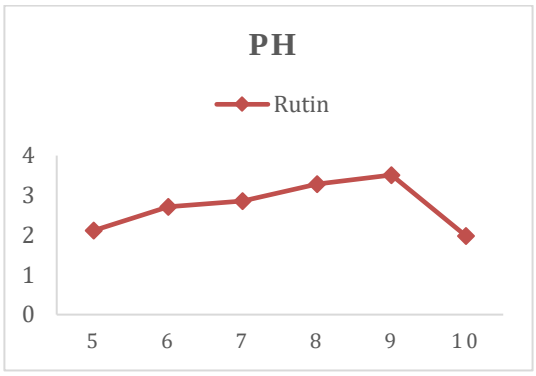


Fig 6. Concentration of Rutin vs pH

The greatest concentrations of Rutin, 3.285 g/ml, were achieved at pH-9 for 90 minutes using 60 percent methanol as a solvent.

3.4.The Effect of Temperature on Rutin Extraction:

The performance of the phenolic compound extraction process is strongly influenced by several parameters, the most important of which is the extraction temperature. The optimal yield of Rutin was extracted at multiple temperatures, including 32°C, 34°C, 36°C, and 38°C. Figure 7 depicts the outcomes.

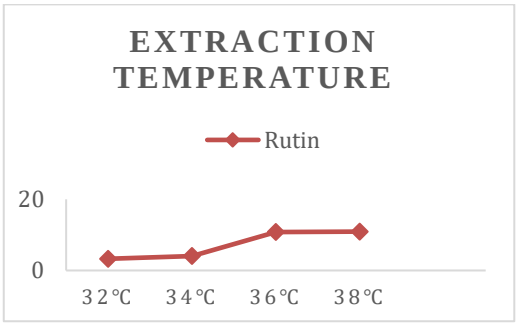
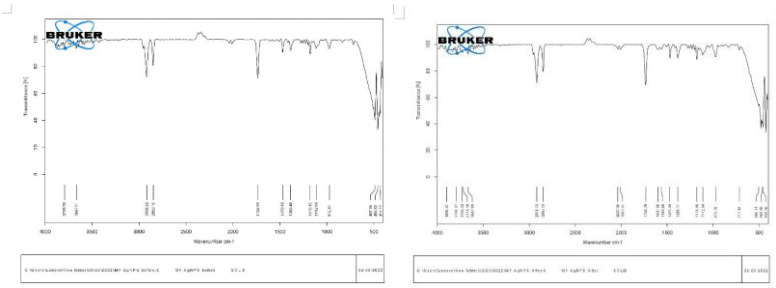


Fig 7. The concentration of Rutin vs Extraction Temperature

The highest concentrations of Rutin 10.92 g/ml were produced using 60 percent methanol as a solvent for 90 minutes at 38°C temperature and pH-9.

3.5.FTIR (Fourier Transform Infrared Spectroscopy):

Nutmeg nanoparticle FT-IR spectra for MF-Ag-NPs shown in (Fig.8 A,B)revealed bands at 3338.20 cm corresponding to -OH stretching, proving the presence of carboxylic acid4 OH; 2956.25 cm corresponding to C-H stretching of alkane C-H; 1738.78 cm corresponding to C = O stretching of the ester group; 1478.64 cm' for Esters, phenolic compounds, and alcohols were found to be present in green-synthesised nutmeg nanoparticles, similar to findings from a previous study[36]. Myristica fragrans seals were found to contain carbonyl groups, alkane groups, and ether groups as functional groups of biological compounds such as alkaloids, steroids, tannins, flavonoids, phenolics, and glycosides, all of which are known to have antimicrobial properties[38]. The antimicrobial properties of these functional groups have been determined to be a result of this conclusion.



A.Before **B.After**

Fig.8 A&B FT-IR analysis: Green synthesized MF-Ag-NPs before &after extraction

3.6.Antibacterial activity analysis:

Using a well-diffusion process, we determined the antibacterial properties of synthesised silver nanoparticles against E.coli (Figure 9)and B. subtilis (Figure 10). MF-Ag-NPs synthesised with Myristica fragrans (nutmeg) had ZOIs of 1.08 ± 0.073 cm and 1.23± 0.039 cm against E.coli and Bacillus subtilis, respectively, at concentrations of 10 g/mL.One of the positive controls for the experiment was Gentamicin, A ZOI of 2.12±0.047 cm was found. According to these findings, the nutmeg seed extract used in the biosynthesis of the MF-Ag-NPs was more effective at inhibiting bacteria than the extract alone[26]

Fig.9.Zone of Inhibition of MF-Ag-NPs for gram negative bacteria *Escherichia coli*

Fig.10. Zone inhibition of MF-Ag-NPs for gram positive bacteria *Bacillus subtilis*

4.Conclusion:

Microbiological growth can be inhibited by the use of chemical solvents. On the other hand, chemical solvents pose a health hazard to users and have only weak ar bacterial properties. In biotechnology and biomedicine, nanoparticles made of metallic elements (such as coppe silver, and gold) have many applications. Silver nanoparticles (MF-Ag-NPs) are effective because of their antimicrobial, anti inflammatory, and anti-proliferative properties. The goal of this study was to produce silver nanoparticles using nutmeg, a seed from the Myristica fragrans plant and extracting the bioactive component called rutin and to study their antibacterial properties. Seed extract was used to dissolve Myristica fragrans MF-Ag-NPs in order to create Myristica fragrans MF-Ag-NPs and was described using FTIR. EDX, and SEMalysis. The diameters of these MF-Ag-NPs are between 20µm and 300 nm, according to SEM analysis. EDX analysis confirmed the presence of silver carbon and oxygen, as well as the fact that silver had been oxidised. Nanoparticles produced by this method contained distinct functional groups, as demonstrated by FTIR spectroscopy. Myristica fragrans seed extract was tested for its antibacterial properties using growth zone inhibition. The seed extract of Myrsitica fragrans has the best antibacterial properties, according to the research. Antibacterial MF-Ag-NPs phyto-formulated with nutmeg extracts, according to these findings could be used to treat bacterial diseases.

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DOI: https://doi.org/10.15379/ijmst.v10i4.3680