

# Silver Nanoparticles (Ag-Nps): Synthesis And Characterization

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## Abstract

Biosynthesis of nanoparticles is popular due to its efficiency, eco-friendliness, and low toxicity. This study examined the effect of aqueous Nutmeg seed extract on silver nitrate bioreduction to AgNPs. Silver nanoparticles (AgNPs) are effective because of their antimicrobial, anti-inflammatory, and anti-proliferative properties. The goal of this study was to produce silver nanoparticles conjugated with a flavonoid called Kaempferol using nutmeg, a seed from the plant, and to study their antifungal properties. Seed extract was used to dissolve AgNPs in order to create *Myristica fragrans* AgNPs and was described using FTIR, EDAX, and SEM analysis. Nanoparticles produced by this method contained distinct functional groups, as demonstrated by FTIR spectroscopy and showed protein molecules in the extract may act as a reducing and capping agent. The SEM image shows homogeneous particles. EDAX analysis confirmed the presence of Silver and oxygen, as well as the fact that silver had been oxidised. *Myristica fragrans* seed extract was tested for its antifungal properties using growth zone inhibition. The seed extract of *Myristica fragrans* has the best antifungal properties, according to the research. Antifungal AgNPs phyto-formulated with nutmeg extracts, according to these findings, could be used to treat fungal diseases

**Keywords:** *Myristica fragrans*; AgNP, SEM; FTIR; Antifungal activity; *Saccharomyces cerevisiae*.

## Introduction

Nanotechnology has branches in chemical, pharmaceutical, mechanical, and food processing industries [1,2]. Nanotechnology is used in computers, power production, optoelectronics, drug delivery, and environmental science. Traditional nanoparticle synthesis methods require long-term processing, high prices, time-consuming procedures, and in some cases, hazardous compounds. Due to these restrictions, most relevant research focuses on developing environmentally friendly and time-efficient nanomaterial synthesis processes. Material scientists have prioritised eco-friendly nanomaterial synthesis in recent years. Nanobiotechnology has applications in biotechnology, chemistry, medicine, and material science [3,4]. Green NP synthesis, using plant extracts, is a growing trend in green chemistry because it's simple, economical, and non-toxic. Green nanoparticles offer physical, chemical, and bioactivity advantages over standard approaches.

High density and surface area give metal oxide nanoparticles unique chemical and physical properties. Developing eco-friendly metal nanoparticle production technologies requires non-toxic chemicals [5]. The use of environmentally friendly materials for the synthesis of metallic nanoparticles, such as plant leaf extract, bacteria, fungi, and enzymes, has many advantages in terms of eco-friendliness and compatibility for pharmaceutical and biomedical applications. Researchers are interested in silver nanoparticles' electric [6], optical [7], catalytic [8], antioxidant [9], antimicrobial [10], antibiofilm [11], antifungal [12], anti-inflammatory [13], and anti-viral [14] properties, as well as their anti-angiogenesis [15], larvicidal effects [16], and anticancer activities [17]. *Jujuba seeds* [18], *Oryzae sativa*, *Helianthus annuus*, and *Zea mays* [19], *Jatropha curcas* [20], *Trianthema decandra roots* [21], *Ocimum sanctum stems and roots* [22], *Banana peel* [23], *Acacia leucophloea* [24], *Ficus sycomorus* [25], *Helianthus tuberosus* [26], and *Azardictha indica* [27]

In tropical climates, *Myristica fragrans* is grown as an evergreen shrub. Some call the seeds nutmeg and the arils mace. Nutmeg is a sweet and savoury spice. It's dried *Myristica fragrans* seed. It's antibacterial, antifungal, anti-inflammatory, antioxidant, anticarcinogenic, and anti-diarrheal. Camphene, elemicin, eugenol, isoelemicin, isoeugenol, methoxyeugenol, pinene, sabinene, safrol, myristic acid, and myristicin are chemicals in nutmeg extract that can act as reductants to form AgNPs [28]. This study investigated the bioactive potential of nutmeg components and tested biosynthesized AgNPs against *Saccharomyces cerevisiae*.

## 2.1. Plant material Collection

The dried, ripe seeds of *Myristica fragrans* (nutmeg) were purchased from a local source. The material was twice rinsed with double distilled water after being cleared of the flith and other foreign debris. Following the removal of the dirt and

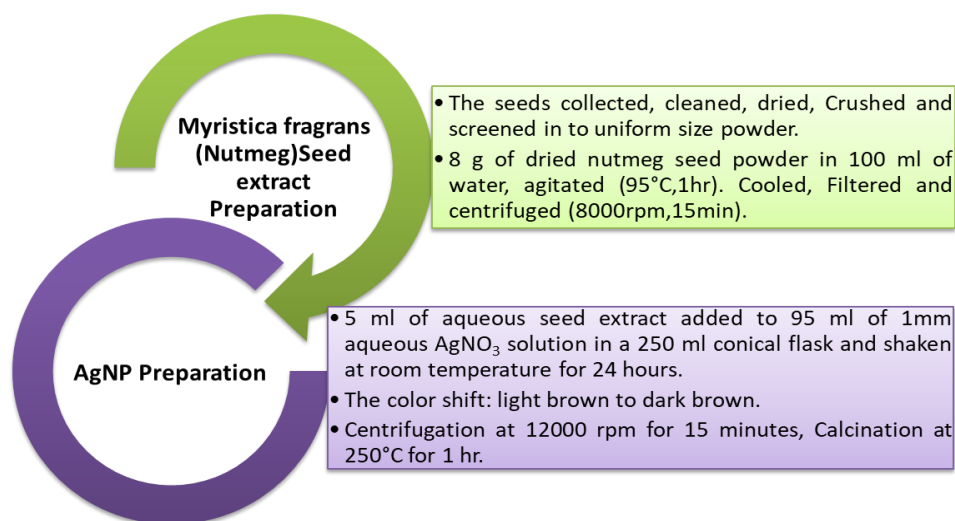
other foreign objects, double-distilled water was used to rinse the equipment. To obtain a uniform size for the extraction of the active plant components, the dried plant material was ground using a mortar and pestle.

**2.Chemicals**

The chemicals utilised were all of the analytical variety. From Sigma-Aldrich, silver nitrate (AgNO<sub>3</sub>) was bought. Throughout the tests, deionized water was used.

**2.3. Nutmeg seed extract preparation**

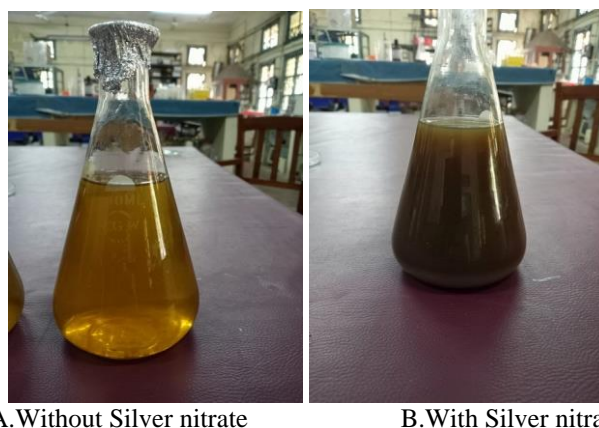
100 ml of water were used to dissolve 8 g of dried nutmeg seed powder. The aqueous solution was then stirred at 95°C for one hour. After cooling, the solution was filtered through Whatmann No. 1 filter paper with a particle size of 25 microns. It was then centrifuged at 8000 rpm for 15 minutes. The *Myristica fragrans* seed extract used for the production of silver nanoparticles was the supernatant solution created after centrifugation.



**Fig.1: Schematic description of the Green synthesis process**

**2.4. Green synthesis and Characterization of AgNPs**

For the biosynthesis of AgNPs, 95 ml of 1 mM aqueous AgNO<sub>3</sub> solution and 5 ml of aqueous seed extract were combined in a 250 ml conical flask and agitated for 24 hours at room temperature. Silver nitrate was bioreduced to silver nanoparticles as evidenced by the change in colour from light brown to dark brown(Fig.2A&B) Centrifugation at 12000 rpm for 15 minutes was used to collect the AgNPs. The pellet was collected and then given two washes in double-distilled water. The produced AgNPs underwent a 1-hour calcination process at 250 °C.



**Fig.2. Color change of Myristica fragrans seed extract from yellow to brown(A&B)**

**2.5 Characterization of AgNP:**

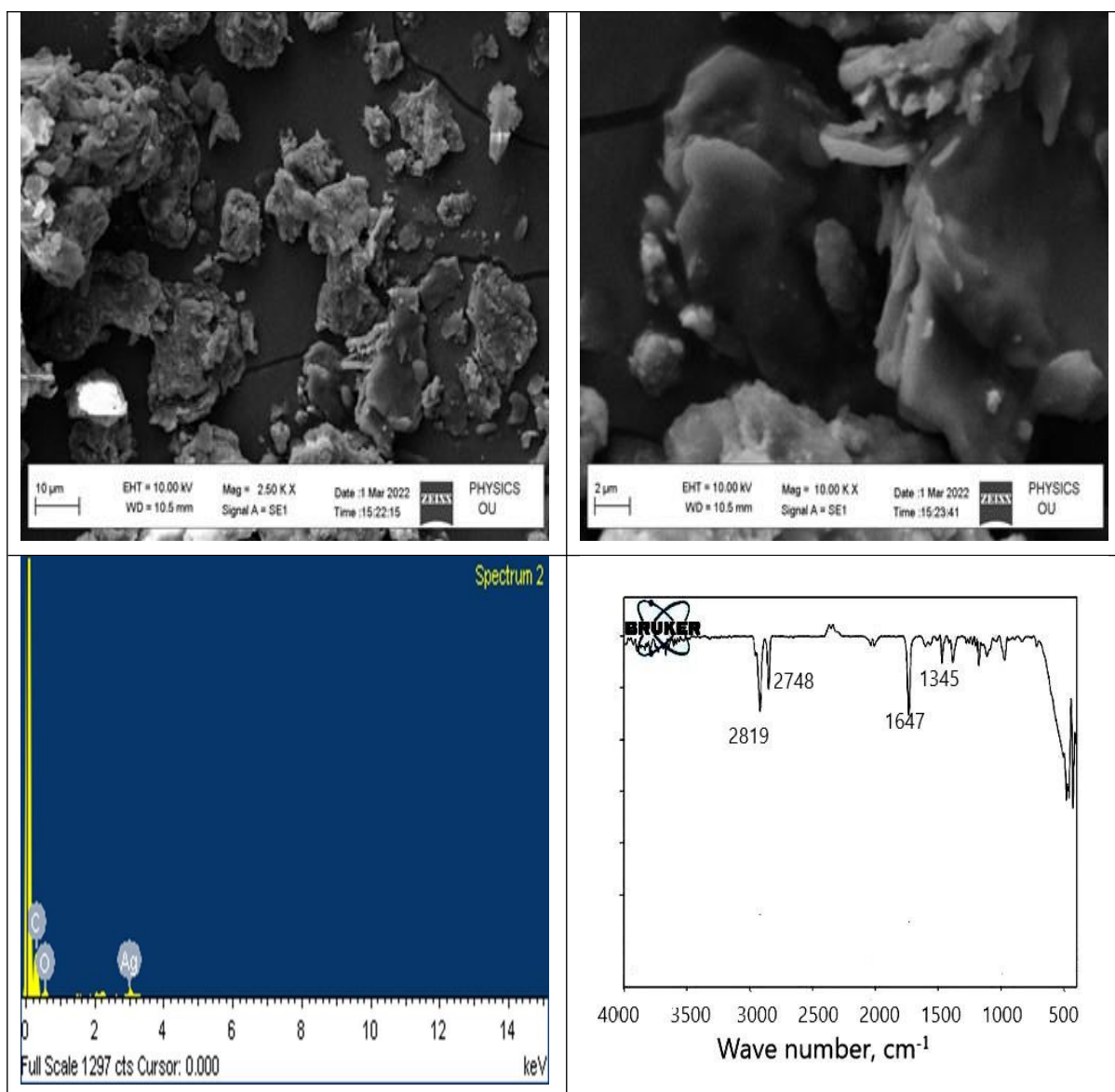
A physical alteration in hue will make the bioreduction of Ag<sup>+</sup> ions in solution visible. The functional groups that were present in the sample could be identified and recorded using a Bruker FT-IR spectrophotometer with a resolution of 2 cm<sup>-1</sup> extending from 4000 to 400 cm<sup>-1</sup>. Studies using scanning electron microscopy will show the size and shape of the

produced nanoparticles. The elemental makeup of the produced AgNPs is ascertained by an energy dispersive X-ray analysis, or EDAX. The investigation's purpose called for the use of a Carl Zeiss FE-SEM.

**3. RESULTS AND DISCUSSION:**

**3.1 Characterization of AgNP:**

By examining the colour changes brought on by the reduction of silver ions into silver particles, the production of AgNPs is proven. After the seed extract was added to the AgNO<sub>3</sub> solution, a paler brown hue was noticed, and after 24 hours, the color's intensity increased to a darker brown. AgNPs are being formed when a dark brown colour appears in the reaction mixture; this is caused by the surface plasmon vibration being excited during the synthesis process. The silver nanoparticles' SEM picture was captured and is displayed in Fig. 3. (a,b). The SEM image demonstrates unequivocally that the particles are present in a homogenous state, and that the homogeneity of nanoparticles is crucial to the various functions they perform. EDAX analysis was used to examine the nanoparticles, and the results showed that elemental silver was present. The silver components are associated to the peaks around 3 keV. (Fig.3c). The outcomes agree with earlier research[28]. FTIR analyses of the created silver nanoparticles were conducted (Fig. 3(d)) to learn more about potential interactions between proteins and silver nanoparticles. The peaks at 2819cm<sup>-1</sup> are due to phenols' OH stretching vibrations. The peaks corresponding to wavenumbers 2748, 1647, and 1345cm<sup>-1</sup> show evidence of C-H stretching. The 1647 cm<sup>-1</sup> peak further supports the existence of aldehyde groups in the methanolic extract of Myristica fragrans, which are in charge of capping and stabilising the green-produced silver nanoparticles. Functional groups like alkene, carbonyl, and amine are confirmed by the spectrum[29,30].

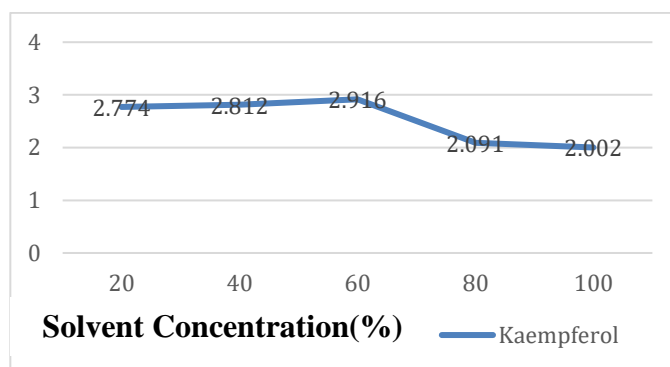


**Fig.3: a) b) SEM images c) EDAX analysis d) FTIR graph of AgNP**

**3.2. Optimization results of physicochemical parameters for the batch extraction process.**

**3.2.1. Effect of different solvent percentages on the extraction of Kaempferol:**

Mixtures of methanol with different proportions of water have shown to be more effective in extracting phenolic compounds compared to mono-component solvent systems. The addition of a small quantity of water to organic solvent usually leads to a more polar medium, which facilitates the polyphenols extraction. 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 Kaempferol and the results are shown in Fig 4.

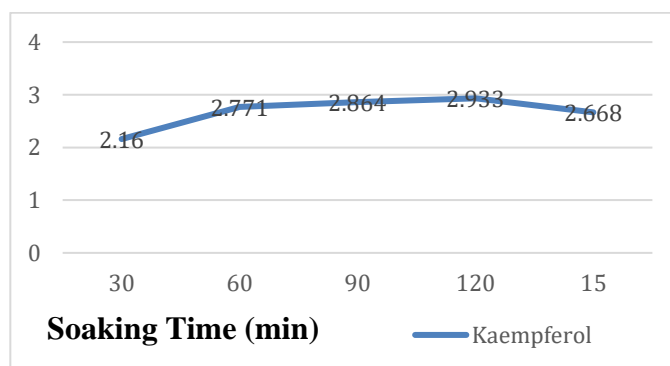


**Fig .4** Concentration of Kaempferol vs Solvent percentage(%)

The results showed the maximum concentration of Kaempferol of 2.916 µg/ml was observed at 60% methanol.

**3.2.2. Effect of soaking time on the extraction of Kaempferol:**

Soaking time represents another key parameter in optimizing Kaempferol extraction. The nutmeg seed powder was soaked in the 60% methanol for different periods like 30 min,60 min,90 min,120 min,160 min, and observed the extraction result as shown in Fig 5.



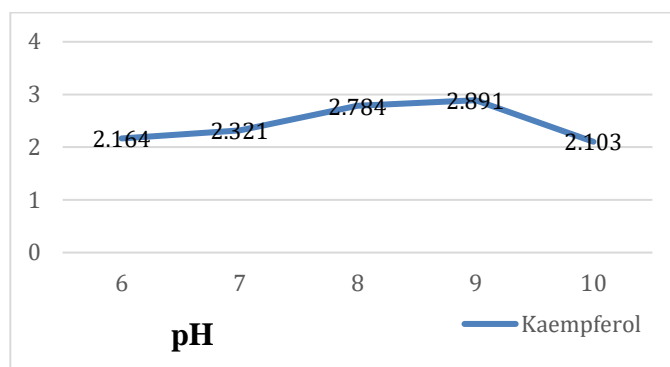
**Fig 5.** Concentration of Kaempferol vs Extraction Time(min)

Extraction time beyond 120 minutes induced loss of Kaempferol content. The more the extraction time is, the lesser the content of polyphenol obtained. This could be the result of loss of phenolic compounds, via oxidation, which might polymerize into insoluble compounds.

The results showed higher concentration of Kaempferol of 2.933 µg/ml was observed at 120 minutes of incubation of nutmeg seed powder in 60% methanol.

**3.2.3. Effect of pH on the extraction of Kaempferol:**

The extraction yield of components was observed at different pH values namely 5,6,7,8,9 and 10 were used. Results are shown in Fig 6.

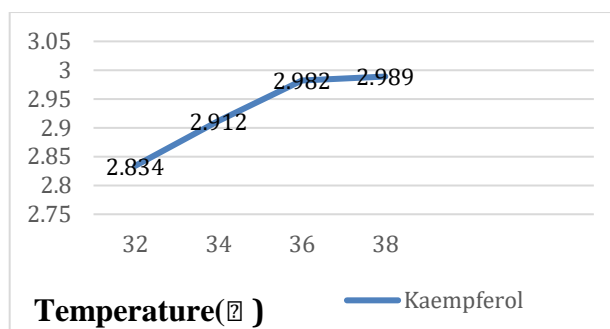


**Fig 6.** Concentration of Kaempferol vs pH

The results showed the maximum concentrations of Kaempferol 2.891 µg/ml, was obtained at pH-9 using 60% methanol as a solvent for 90 minutes.

**3.2.4.Effect of Temperature on the extraction of Kaempferol:**

The effectiveness of the extraction process of phenolic compounds are largely regulated by different parameters and mainly by the extraction temperature. Extraction at different temperatures such as 32°C,34°C,36°C,38°C were used to extract the optimum yield of Kaempferol. The results were shown in Fig 7.



**Fig 7.** Concentration of Kaempferol vs Extraction Temperature

The results showed the maximum concentrations of Kaempferol 2.989 µg/ml, was obtained at 38°C temperature and pH-9 using 60% methanol as a solvent for 90 minutes

**3.3 Antifungal activity Analysis:**

The antifungal effects of *Myristica fragrans* seed extract on a yeast strain called *Saccharomyces cerevisiae*. Using a well-difusion process, we determined the antimicrobial properties of synthesized Silver nanoparticles against *Saccharomyces cerevisiae* (Fig.8). One of the positive control for the experiment was Gentamicin. A ZOI of 2.12 0.047 cm was found. The plate was then kept at 30°C for 48 hours of incubation. AgNPs synthesized with *Myristica fragrans* (nutmeg) had Zone of Inhibition (ZOIs) of 1.06 0.072 cm against yeast strain. The Zone of Inhibition was determined as the lowest extract concentration that, when compared to control, resulted in an 80% reduction in discernible growth, as well as when the mic value was 100 g/ml or less. With an increase in seed extract concentration (mg/ml), the extracts' antifungal properties grew. The methanolic extract of *Myristica fragrans* was found to be ineffective against the test bacteria. The outcomes demonstrated that *Saccharomyces cerevisiae* is sensitive to these silver nanoparticles potent dose-dependent antifungal activity.



**Fig:8:** Zone of inhibition of *Myristica fragrans* AgNPs against *Saccharomyces cerevisiae*.

**4. Conclusion:**

Silver nanoparticles (AgNPs) are effective because of their antimicrobial, anti-inflammatory, and anti-proliferative properties. The goal of this study was to produce silver nanoparticles conjugated with a flavonoid called Kaempferol using nutmeg, a seed from the *Myristica fragrans* plant, and to study their antifungal properties. Seed extract was used to dissolve AgNPs in order to create *Myristica fragrans* AgNPs and was described using FTIR, EDAX, and SEM analysis. The diameters of these AgNPs are homogenous, according to SEM analysis. EDAX analysis confirmed the presence of Silver 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 antifungal properties using growth zone inhibition. The seed extract of *Myristica fragrans* has the best antifungal properties, according to the research. Antifungal AgNPs phyto-formulated with nutmeg extracts, according to these findings, could be used to treat fungal diseases. This method can therefore be used for the quick, affordable, and environmentally friendly green synthesis of silver nanoparticles for use in industry and medicine.

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