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A Green Approach to Phytomediated Silver Based Nanoparticles Using Rhizome Extract of Acorus Calamus (Linn) And Their Antibacterial Activity

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KEYWORDS

Acorus caalmus(linn); antibacterial activity; photocatalytic degradation; silver nanoparticles

ABSTRACT:

Introduction: the synthesis of silver nanoparticles (AgNPs) has garnered considerable attention due to their diverse applications in medicine, catalysis, and electronics. However, the conventional methods for AgNP synthesis often involve the use of hazardous chemicals and energy-intensive processes, raising environmental concerns. In response to this, there is a growing interest in developing sustainable and green approaches for nanoparticle synthesis, utilizing plant extracts as reducing and stabilizing agents.Acorus calamus (Linn), commonly known as sweet flag, has been recognized for its pharmacological properties and bioactive compounds. The rhizome extract of Acorus calamus, rich in phytochemicals, presents an intriguing opportunity for the green synthesis of silver nanoparticles. This study aims to explore a novel eco-friendly approach to phytomediated synthesis, utilizing the reducing potential of Acorus calamus (Linn) rhizome extract for the fabrication of silver nanoparticles.

Objectives: objective of this research is to investigate the potential of Acorus calamus as a green and sustainable source for the synthesis of silver nanoparticles. Additionally, the study will delve into the antibacterial activity of these nanoparticles, assessing their efficacy against various pathogenic microorganisms. The eco-friendly synthesis and antibacterial properties of Acorus calamus-mediated silver nanoparticles hold promise for the development of environmentally benign and effective antibacterial agents, contributing to the advancement of green nanotechnology and sustainable materials.

Methods: Acorus calamus (Linn) rhizomes were harvested, cleaned, dried, and ground into a fine powder. Phytochemicals were extracted using an eco-friendly solvent, and the resulting extract was utilized for the green synthesis of silver nanoparticles. The reduction of silver ions was achieved by gradually adding the rhizome extract to a silver nitrate solution, with the reaction monitored until nanoparticle stabilization. Characterization using UV-Vis spectroscopy, FTIR, XRD, and TEM confirmed the successful synthesis, revealing the nanoparticles' size, morphology, and crystalline nature. The antibacterial activity of these green-synthesized silver nanoparticles was evaluated against pathogenic strains (e.g., Escherichia coli, Staphylococcus aureus) using standard assays, including agar well diffusion and microdilution methods. Statistical analysis was performed to ascertain the significance of the antibacterial results, highlighting the potential of Acorus calamus-mediated silver nanoparticles as effective and environmentally friendly antibacterial agents, thus contributing to the advancement of green nanotechnology and sustainable materials.

Results:The synthesized silver nanoparticles exhibited notable antibacterial activity against pathogenic strains, including Escherichia coli and Staphylococcus aureus. Agar well diffusion assays revealed distinct zones of inhibition, indicative of the nanoparticles' efficacy in restraining bacterial growth. Microdilution assays further supported these findings, with minimal inhibitory concentrations (MIC) demonstrating the potency of Acorus calamus-mediated silver nanoparticles against the tested bacterial strains.

Conclusions: The rapid formation and nucleation of AgNPs were evident, indicated by a color shift from light yellow to brownish yellow. This observed color change is attributed to the surface plasmon vibration effect and the conversion of Ag+ ions to Ag[o] in aqueous samples, providing explanation for the presence of the brown color. The findings underscore the biogenic synthesis process but also the multifaceted properties of AgNPs synthesized using Acorus calamus rhizome extract,

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1. Introduction

Lately nanotechnology has amplified substantial commendation in science and technology in variant ways [1-2]. The nanoparticles have unique possession that are broadly arrayed for solicitations in cosmetics eco-friendly remediation and biomedical expedients as well [3]. Nano particles found to have auspicious and distinctive properties like biological chemical and physical properties as well [4-5]. Nanoparticles assets and utility are reliant on shape and size. Subsequently to achieve a superior photochemical catalytic and an antibacterial the nanoparticles enhanced to resist shape and size explicitly[6-7]. Criterion size and shape can be which can be approach by applying variant stabilizers and reducing mediators Nevertheless the utilization of chemicals carriages were environment perilous and clusive therefore green blend are financial savvy and provides solitary approach [7-8].

Gram positive and gram-negative bacteria are both rendered inactive by silver nanoparticles (AgNPs). Those certain AgNPs have indeed been integrated as better overall antibacterial properties in wastewater remediation by the adequate treatment [2,9-11]. Nanoparticles biosynthesized from Momordia charantia rhizome extract Adhatoda asica extract showed persuasive biocides against Pseudomonas aeruginosa [(5,8,12-14]. Chlorella vulgaris found to exhibit an antibacterial property] proficiently. Ample hypothesis was put forth for antibacterial activity nevertheless it is stagnant and contested. AgNPs spectacle catalytic assets for detoxification dye and its exclusion as well. Dyes detoxification can be experienced by variant methods of carbon adsorption UV light degradation redox treatments and flocculation as well (Dyes are broken down through photocatalysis) [15-18]. Biochemically produced nanoparticles are painstaking to overcome these ineffectual approaches of photocatalytic degradation with outstanding biocompatibility. Catalytic degradation of Rhodamine B revealed photocatalytic degradation due Solidago altissima by transforming silver to silver nanoparticles[19-22].

Biosynthesis of silver nanoparticles of the Acorus calamus rhizome aqueous extract is a current theme of research paper. Therapeutic assets of plant Acorus calamus are recognizable. Rhizomes of the plant Acorus calamus have been expansively spectulate for usage of variant diseases. The Acorus calamus rhizomes found to be treated against helminthic diseases [23-26]. Biosynthesis of extract of Acorus calamus AgNPs are inspected for detoxification of photocatalytic dyes and anti-bacterial activity as well.

2. Objectives

The objective of this study is to employ a sustainable and environmentally friendly approach for the synthesis of silver nanoparticles using the rhizome extract of Acorus calamus (Linn), with a focus on assessing their antibacterial activity. Through a green phytomediated synthesis, the aim is to explore the potential of Acorus calamus as a reducing and stabilizing agent for silver nanoparticles, ensuring a eco-friendly and cost-effective synthesis method. Furthermore, the study aims to investigate the antibacterial properties of the synthesized nanoparticles against pathogenic microorganisms, contributing valuable insights for the development of novel and sustainable antibacterial agents. To systematically evaluate and characterize the antibacterial activity of the synthesized silver nanoparticles, with a specific emphasis on their efficacy against a spectrum of pathogenic microorganisms, aiming to establish their potential as eco-friendly and efficient antibacterial agents for various applications

.Methods

The Acorus calamus rhizome was harvested in the Jalandhar Punjab region of India. For complete moisture removal Acorus calamus rhizomes were air dried for a week at room temperature in the shade. The bark was then divided into small pieces grounded into a fine powder and sieved. After being dissolved in 10g of distilled water the Acorus calamus powder was then boiled for 15 minutes before being cooled. Then filtered by using wattman filter paper. Filtrate obtained by the filtered solution was stored in a refrigerator[27-29].

Green synthesis of silver nanoparticles

1mM AgNO3 solution was made by dissolving 0.02 g of AgNO3 in 250 ml of distilled water.The Rhizome extract and silver nitrate were combined with a magnetic stirrer. The reaction mixture was continuously stirred at 800 rpm while being heated below the boiling point to 30 minutes The mixture's colour changed to a

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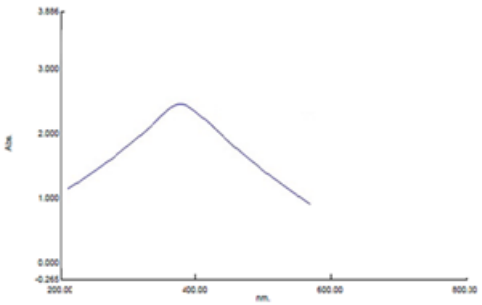
reddish brown. The entire reaction took place in the dark to avoid the oxidation . [30]Centrifuging was done on the Ag/Acorus calamus extract suspension for 50 minutes at 10000 rpm. To get rid of the silver ions and rhizome extract residue the silver nanoparticlecontaining pellet was washed three to four times with deionized water. The nanoparticles that had precipitated were lyophilized. After being lyophilized nanoparticles were kept in a cool dry and dark location while further characterization was done on them.[30-31].

Characterization of synthesized silver Nano-particles

The UV spectra of Acorus calamus rhizome alcoholic

extract silver nanoparticle is given in Figure. The UV spectrum was not as significant as very dilute sample were done. The peak at 398 nm showed the formation of silver nanoparticles.[29,32].



Figur 1. The UV spectra of Acorus calamus rhizome alcoholic extract silver nanoparticle

Alcohols and phenols are depicted by the presence of the OH stretch in the FTIR band at 3433 cm1. The NH bend and CH bend are represented by the bands at 1638 cm1 and 1460 cm1 respectively. The spectra make it

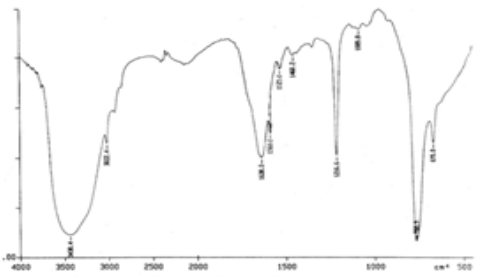


Figure2. The FTIR spectra of Acorus calamus rhizome alcoholic extract silver nanoparticle Fig 3 SEM image of the spherical AgNPs

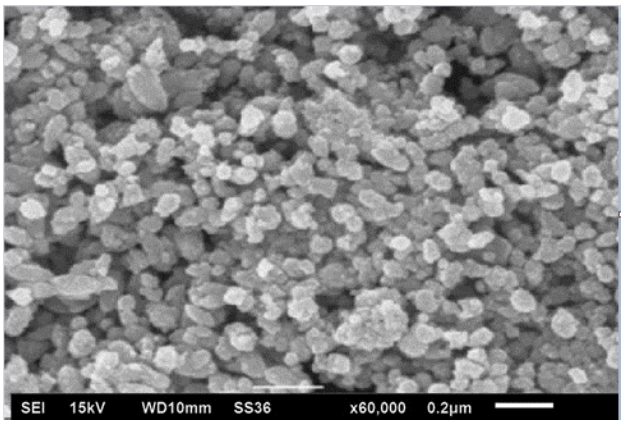


Figure 3. SEM image of the spherical AgNPs

Abundantly clear that the formation of AgNPs is caused by the presence of polyphenols specifically flavonoids phenols and tannins as well as a few compounds with amine groups.

SEM image of the spherical AgNPs produced through biological biosynthetic pathways using rhizome extract. In order to define the size of AgNPs alcoholic extract of rhizome of Acorus calamus the extract was undergo characterization of SEM. As a result the image of silver nanoparticles showed uniform distribution of silver nanoparticles all over the sample that is approx. 25 nm [16,33-34].

Optimization studies for silver nanoparticles of Acorus calamus

Ph – The pH values 4.0 5.0 6.0 7.0 and 8.0 were chosen for the optimization of Acorus calamus extract-AgNPs synthesis. As the pH increases UV–visible absorption spectra of Acorus calamus extract-AgNPs increased (Fig.1b) as also perceived from the color change of the reaction mixture from light brown to dark drown immediately after the addition of AgNO3. The spectrophotometric analysis revealed that the synthesis at pH 6.0 is proper as compared with pH .8 as at pH 8.0 agglomeration started after 20 min of exposure to sunlight. This suggested that the synthesis of Acorus calamus extract-AgNPs is supported by alkaline pH[16]. However, even after exposing the reaction mixture to pH 4.0 and 5.0 for 40 min, spectrophotometric analysis failed to detect a sharp absorption peak in the 400–500 nm range corresponding to the previously determined SPR of AgNPs. This indicated that AgNP generation is a pH-dependent

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process, and at acidic pH all functional groups carry a positive charge, which is important for NP biosynthesis. Therefore, Acorus calamus extract AgNPs, if formed, are not stable enough to avoid aggregation at acidic pH. In addition, functional groups become more bioavailable at alkaline pH, facilitating production. It shows a maximum absorption peak at 435 nm at pH 7.0 from the beginning of the reaction. [35-36].

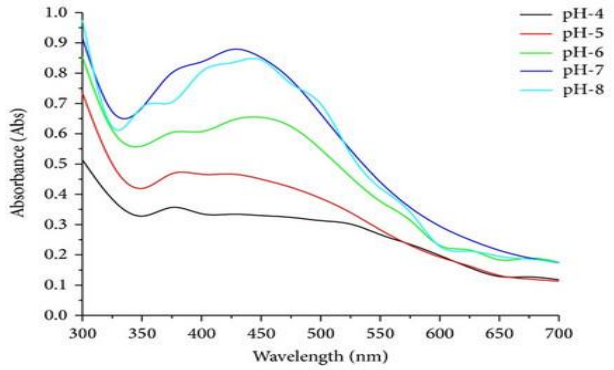

Figure4. UV-visible absorption spectra of Acorus calamus extract AgNPs at varied pH values.

Concentration – Optimization can be also regulated by variant concentration of AgNO3 for the further reaction. AgNPs suspension absorbance was attained.of extract of rhizome of Acorus calamus and silver nanoparticles. By therapeutic plant rhizome extracts with 1, 2, 3 and 4 mM AgNO3, the effect of varying silver nitrate concentrations was optimized for nanoparticle synthesis. All reactions were analyzed by UV-Vis spectroscopy. The suspension obtained by the reaction was further undergone investigation by UV spectroscopy [20]

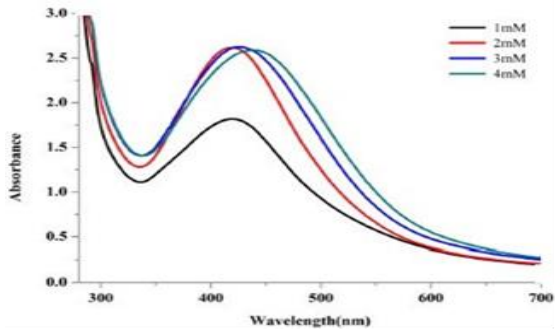

Figure5. UV-visible absorption spectra of AgNPs from Acorus calamus extract at distinct molar concentrations.

Temperature – Optimization can also be determined by variant temperature ranges by keeping the concentration of extract of Acorus calamus rhizome and AgNO3 solution. The suspension obtained was further investigated by UV spectroscopy[25].

Time

The reaction solution was monitored by periodically sampling the reaction mixture at regular time intervals using UV-Vis spectroscopy. The synthesized AgNps exhibited a maximum absorption peak at 435 nm (Figure 7). The AgNps formed were very stable up to 30 min after reaction [22].

The formation of monodispersed nanoparticles can be controlled by the right conditions for AgNO3 solution and Acorus calamus rhizome extract.[37-38]. The solution's colour changes from light yellow to dark brown when silver ions are reduced. The peak near 435 nm demonstrated the formation of AgNPs. It is vibrant from the graph that AgNPs yield increases with the concentration of the Acorus calamus rhizome extract.

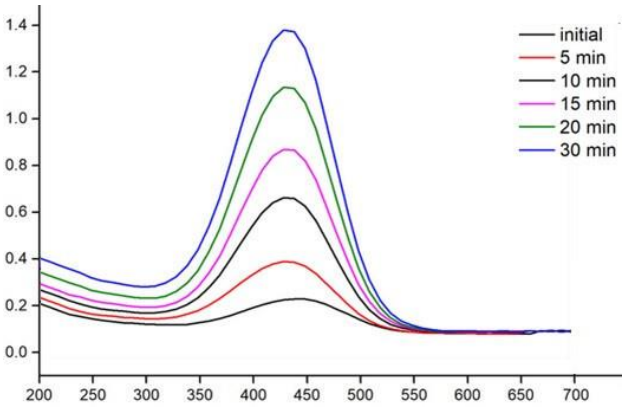

Figure6.UV–visible absorption spectra of Acorus calamus extract-AgNPs at different time

Antibacterial activity [26,39]

Salmonella typhimurium Escherichia coli Penicillium chrysogenum and Aspergillus niger were selected as the four general pathogenic microorganisms to test the anti-microbial effectiveness of synthesized silver nanoparticles. Following the recommended procedure in the literature the disc diffusion method was used to conduct an in vitro study of anti-bacterial activities against Gram-negative bacteria (Escherichia coli Salmonella typhimurium) [40]. After being autoclaved at 160°C for 1 hourWhattman no. 1 filter paper discs

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were destroyed. The dry discs were then deposited with test compounds in varying concentrations. The plates were incubated at 32°C for 24 hours with the stow disc placed on the medium at appropriately spaced intervals.

Photocatalytic degradation [41-43]

Aqueous methylene blue solution was used to test the photocatalytic activity of an extract of silver nanoparticles from Acorus calamus rhizome in the presence of sunshine. In the dark, at room temperature, 100 mL of methylene blue stock solution (15 mg/l) were combined with 15 mg of AgNPs and adjusted to pH 9.0 [52]. After that, a 5 mL aliquot of the suspension was obtained and centrifuged to remove the suspended AgNPs at predetermined intervals (every 15 min). Using a UV-vis spectrophotometer set to 664 nm, the absorption spectra was measured to quantify the rate of dye degradation. On the basis of the formula, the effectiveness of photocatalytic degradation was evaluated.

$$\% \text{ Degradation efficiency} = \frac{C_0 - C}{C_0} \times 100$$

Where, C_0 is the initial concentration of Methylene blue, C is residual Methylene blue concentration after time t. [44-46]. Then, energy source-driven (Sunlight) processes followed the photocatalytic degradation activities. The second phase involved a six-hour suspension in the sun, which was seen as the methylene blue's peak intensity decreased as it degraded. [47-49]. The suspension mixture was measured after centrifugation at regular intervals to validate dye Photodetoxification at various times.Ag nanoparticle adsorption on the methylene blue solution started out gradually and grew over time[50–52]. Methylene blue's signature absorption peak was identified at 664 nm.

The lowering of the peak intensity at 664 nm) during the dye's degradation in the presence of Ag nanoparticles was evidence of this.

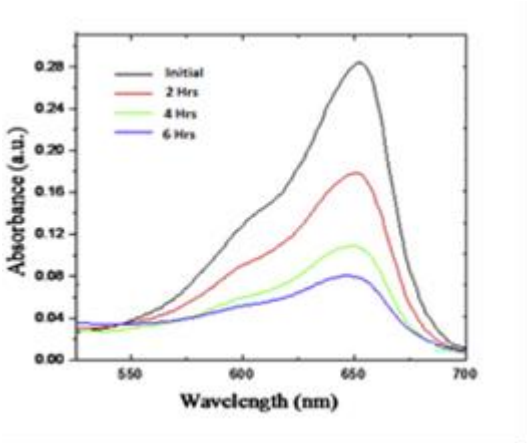

Figure7. UV spectra of Photocatalytic degradation of Methylene blue using silver nanoparticles synthesized from Acorus Calamus.

3. Results

It has been concluded the biogenic formation of AgNPs by using ethanolic extract of rhizome of Acorus calamus. It has been also depicted that phytochemicals contempt in the alcoholic extract aided an efficient and capping agents and reducing agent. Additionally the study designated the antibacterial efficacy of AgNPs that further support the solicitation as an effectual antimicrobial mediator. The silver nanoparticles can also aided the high photocatalytic of the green methodology in this paper that can enhance the eco-friendly environment.

Table 1. Summarization of the inhibition zone was measured. It is evidenced from the table that the nanoparticles synthesized are virtuous candidates for the drug as an anti-bacterial. The data represent the mean ± SE.

Strains in Bacteria	Inhibition Zone (mm)Second
Klebsiella neumonia	2400 ± 0.58
Bacillus subtilis	26.67 ± 0.87
Salmonella typhi	33.00 ± 1.30
Proteus vulgaris	22.67 ± 0.60

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4. Discussion

It has been concluded the biogenic formation of AgNPs by using ethanolic extract of rhizome of Acorus calamus. It has been also depicted that phytochemicals contempt in the alcoholic extract aided an efficient and capping agents and reducing agent. Additionally the study designated the antibacterial efficacy of AgNPs that further support the solicitation as an effectual antimicrobial mediator. The silver nanoparticles can also aided the high photocatalytic of the green methodology in this paper that can enhance the eco-friendly environment.

AgNPs quickly formed and nucleated as evidenced by the colour shift from light yellow to brownish yellow that was visible. The surface plasmon vibration effect and the conversion of Ag^{+} ions to $Ag^{[o]}$ by aqueous samples can both be used to explain why brown colour appears to be present.

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