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Silver nanoparticles Synthesized using Asafoetida resin, Characterization of their broad Spectrum and Larvicidal Activity

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ABSTRACT

Plant secondary metabolites that aid in green synthesis were used to synthesize Ag-nanoparticles, and it is anticipated that such biological processes would have benefits such as being environmentally friendly and gaining popularity. *Ferula asafoetida* resin aquous extract was initially qualified and quantified by NMR and GC-Mass, Consequently its ability for silver nanoparticle (AgNPs) biosynthesis was studied. Accordingly, The synthesized asafoetida resin (As-AgNPs) were characterized,the absorption peak in UV-visible spectroscopy was at 460 nm. In SEM image, Ag NPs were spherical, rod and cuboid in shape with average size of 39 nm. FTIR spectrum of the Ag NPs bands at 2958,2924,2858,1463,1379 and 723 cm−1 silver ions were incorporated with asafoetida resin, as indicated by the presence of hydroxyl and carboxyl groups. Comparison of the FTIR spectrum of the Ag NPs with the essential oil revealed the presence of compounds responsible for reducing and capping the silver ions. XRD pattern AgNPs showed four large peaks at shows five diffraction peaks at 2θ = 38.90° (111) , 44.26° (200), 64.43° (220) and 78.38° (311) suggested the presence of crystal structure. *Ferula asafoetida* resin can be successfully used for biosynthesis of Ag NPs. When compared to the intended mosquito larval species, Asfoetida Ag NPs were less harmful to the non-target organisms studied. As a result, Asfoetida Ag NPs may be used to control mosquito vectors in the region.

Keywords
Ferula asafoetida resin, Sesquiterpenes, AgNPs, Energy Dispersive X-ray X-ray Diffraction and *Ae. aegypti*

Introduction

The use of nanomaterials is vast to enhance human health and the environment. The first recorded use of nanomaterials for human health was in the Indian method of Ayurveda medicine more than five thousand years ago, where nanoscience research was pioneered by modern science, and progress in this area has been growing rapidly all over the world [1]. Nanoparticles are particulate matter, composed of new nanometer sized materials including the creation of nanoparticles [2]. Nanoparticles are considered quickly evolving technologies with enormous potential for developing new materials with special properties and for manufacturing innovative and enhanced devices for various uses [3]. Natural bioactive compounds have the ability to convert metal ions into metal nanoparticles plant compounds to generate a biological synthesis of metallic nanoparticles have many benefits, such as readily accessible, safe to treat, and the availability of a wide variety of active plant biomolecules [4].

Biological species of plant extracts may be used as an alternative route to physical and chemical methods for the synthesis of nanoparticles at low cost in an environmentally safe manner [5]. Recent years have seen a rise in the ethnopharmacological significance of Ferula is mainly due to production of oleo resin resin, obtained from roots of *Ferula asafoetida*. This oleo resin resin is also recognized as Asafoetida, spice and a folk phytomedicine for centuries [6]. It is the dry latex which is extruded from a perennial herb rhizome growing 1 to 1.5 m tall, *Ferula asafoetida*. The genus *Ferula* (Apiaceae family) consists of more than170 species. *Ferula asafoetida* is a native plant, original to the Mediterranean region to Central Asia. In India it is grown in north east Punjab and Himalaya. In many South Indian households, Asafoetida resin is sulfurous odor and a bitter taste, traditionally used for digestive purposes and is a kitchen consumable.The resin like

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resin comes from the stem and roots removed from the dried sap and are used as a spice. When raw, the resin is grayish white but it dries to a dark amber colour. It gives foods a nice taste and is a strong antioxidant, with an anti inflammatory, antispasmodic, expectorant and stimulant [7]. Secondary metabolites produced by *Ferula asafoetida* include coumarins, alkaloids, glucosides and sulfur compounds [8].

Water is now a common solvent in organic synthesis because it is readily available non-corrosive, non toxic, non flammable, inexpensive, and environmentally safe. In addition, water reactions have a peculiar reactivity and selectivity [9]. Breslow's work in the 1980s rediscovered the use of water as a green solvent in organic chemistry, highlighting how hydrophobic effects could dramatically increase the rate of certain organic reactions. In this impact, hydrophobic molecules in aqueous solution are accompanied by a cage of water molecules [10]. The minimization of the surface area of interaction between the hydrophobic and hydrophilic domains favors the condensation of individual molecules to form local domains composed of several hydrophobes surrounded by water cages. As a response, the hydrophobic surface area of the reactants should be related to the rate of a reaction accelerated by the hydrophobic effect. As an effect, the research community is paying close attention to the synthesis of chemical compounds useful reactions in water [11-15].

One of the most important fields of phytochemical science is the study of bioactive compounds in Asafoetida. The research entails isolating sulfur elements, separating them into individual molecules, and characterizing them. Asafetida resin is a complicated combination of sulphur compounds [16]. Since broad libraries of mass spectra are available for identification of components from many foreign organizations, gas chromatography coupled with mass spectrometer is the most widely used technique for quantitative measurement and qualitative identification of individual components from resin [17].

It has been a focus of research for more than a decade, 21st century that modern science initiated nanoscience research and development rapidly. Because of the presence of numerous bioactive molecules as reducing and capping agents without inducing toxicity, green engineering approach for nanoparticles synthesis has attracted a lot at present [18]. Thus, AgNPs' green engineering continues to be a useful technology for generating controlled nanoparticles form and scale using biological sources such as plants and microorganisms [19]. Developing an easy and eco-friendly process for the synthesis of nanomaterials would, however, help to foster greater interest in the application of metallic nanoparticles [20]. Silver ions and silver-based compounds are toxic to micro-organisms, making silver an ideal alternative for several positions in the medical industry [21]. To manufacture silver nanoparticles without the use of harmful chemicals, a fast green biological approach provides tremendous potential for the synthesis of nanoparticulated bioactive molecules [22].

Climate change, population increase, fragmentation, habitat invasion, and insecticide resistance have all played a role in the rise, reemergence, and spread of many vector-borne diseases in recent decades. Those spread by the mosquito Aedes aegypti (Diptera: Culicidae), which include dengue fever, yellow fever, chikungunya, and Zika, are among the most serious public health problems in a large area of our world, affecting hundreds of millions of people each year. *Ferula asafoetida* folkloric use and herbal validity, this study is set out to explore the develop a rapid

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method for extraction and characterization of bioactive molecules from aqueous extracts of *Ferula asafoetida* resin and evaluate the potential of Asafoetida as a larvicide against Ae. aegypti

Materials and Methods

Aqueous extract of essential oil from *Ferula asafoetida* resin

Asafoetida, raw chunk resin is obtained from the plant Ferula asafoetida resin. Hundred grams of raw chunk resin was purchased from a Soma Luna LLC supermarket in the Solsberry, IN 47459 of USA. They were washed thoroughly with tap and distilled water to remove unwanted fine particles and allowed to shade dried. The raw chunk resin was ground into a fine powder using mixer blender. Aqueous extracts were prepared by 5g of the fine powder was dissolved in 200 mL of Milli Q water, mixture was soxlet extraction for 4 hrs before being decanted, and was cooled then filtered through Whatman No.1 filter paper. The filtrate was designated as the aqueous extract of asafoetida and was used for used for the charcaterization of bioactive compounds.

Preliminary Phytocompounds analysis

Using normal phytochemical techniques, preliminary phytochemical analysis was conducted to assess various phytoconstituents present in freshly prepared resin extracts of Ferula asafoetida , such as phenol, tannins, flavonoids, triterpenoids, saponins, steroids, cardio glycosides, resin and alkaloids [23].

Chemical analysis by Gas chromatography–mass spectrometry

Perkin Elmer instrument with a capillary column (HP-5MS, 30 m 9 0.32 mm (ID) 9 0.25 lm (FT)) GC analysis and Agilent Technologies (7890A-GC, 5975C-MS) mass system with capillary column ((ID) 9 0.25 lm film thickness, HP-5MS, 30 m 9 0.25 mm) were used for gas chromatography mass spectrometry (GC-MS) analysis. Initial temp 60°C for 2.80 min, ramp 10°C/min to 300°C, hold 6 min, InjAauto=260°C, Volume=0 µL, Split=10:1, Carrier Gas=He, Solvent Delay=2.20 min, Transfer Temp=230°C, Source Temp=230°C, Scan: 40 to 600Da, Column 30.0m x 250µm. Based on Wiley library data from a GC-MS system and a comparison of retention indices with previous reports, the components of asafoetida aqueous extracts were identified.

NMR Analysis

^{13}C Nuclear magnetic resonance spectrum of the essential compounds was recorded at 100.13 MHz on a Bruker AVANCE 400 Fourier Transform spectrometer operating, equipped with a 5 mm probe, in $CDCl_3$, with all shifts referred to internal tetramethylsilane. The following parameters were used to record ^{13}C-NMR spectra:4 s pulse width (flip angle 45°); 2.7 s acquisition time for 128 K data table with spectral width of 25 000 Hz (250 ppm.); CPD mode decoupling; digital resolution = 0.183 Hz/pt.The total number of scans for the sample under investigation was 50 (around 40 mg of the sample in 0.5 mL of CDCl3).In the experimental

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spectrum on chemical shifts of each carbon compared with the spectra of reference compounds compiled in a laboratory made ^{13}C NMR data library.

Asafoetida AgNPs by green synthesis

Silver nitrate ($AgNO_3$; Sigma-Aldrich, USA) was used as a source of reduction of Ag^{3+} ions into Ag^0 in experiment. Thus, prepare silver nitrate nanoparticles; 8.4 mg of silver nitrate (1 mmol/ml) was prepared freshly and dissolved in 50 mL of Milli Q water,to which 50 mL of asafoetida aquous extract was added and incubated in the dark for overnight at room temperature(to minimize the photo-activation of silver nitrate).The reaction mixture was monitored, reduction of Au^{3+} to Au^0 was confirmed by pale yellow reaction mixture became brown. Then residual mixture centrifuged at 16000 rpm for 15 min and then washed with distilled water consecutive three times and evaporated in an oven, sediment dried powder As-AgNPs and stored for further characterization.

Characterization of synthesized Asafoetida AgNPs
UV-Vis spectra of Asafoetida AgNPs

The maximal absorption of silver ions in the solution was scanned periodically by determining the absorption peak ranging from 400 to 1000 nm at regular time intervals by Double beam UV-visible spectrophotometer. It is noted that the surface Plasmon resonance peaks are about 400-450 nm strong. The absorption spectrum of spherical silver nanoparticles is stated to exhibit a range of 420 nm to 450 nm.

Fourier transform infrared (FTIR) spectra of Asafoetida AgNPs

In order to classify the potential biomolecules responsible for synthesising Ag NPs, the involvement of functional groups involved in the creation of silver nanoparticles and aqueous extracts was achieved. Asafoetida AgNPs aqueous extracts were properly mixed with potassium bromide (KBr) and made into a thin KBr disc under a pressure of 7845 KPa for 2 min and spectra were recorded by BRUKER FTIR spectrometer within a range of 500 to 4000 cm^{-1}.

Energy Dispersive X-ray spectrum of Asafoetida AgNPs

SEM (ICON - Quanta 200 Mark II SEM 30kV) had determined the surface morphology of the synthesised silver nanoparticles. In spot-profile mode, EDX spectra were obtained with the same instrument by concentrating the electron beam on a surface area filled with Asafoetida AgNPs and verifying the elementary composition of the samples with standard library.

X-ray Diffraction (XRD) analysis of Asafoetida AgNPs

The XRD patterns of bioreduced metallic silver nanoparticles observed using an X-ray diffractometer.The synthesized Asafoetida AgNPs were spreaded onto glass substrate and subjected to diffraction at an operating voltage of 30 kV, a current of 30 mA and scan rate of 10°/min with Cu-Kα radiation in a 2θ configuration from 20-80°.Crystal size and structure can be

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further confirmed by observing the sharp Bragg's reflection in the XRD patterns. The mean crystallite size of the Asafoetida AgNPs was calculated by Debye-Scherrer's formula for the reflection peak according to D is the crystallite domain size perpendicular to the reflecting planes, K is the Scherrer constant (0.89), λ is the X-ray wavelength of silver (1.5406Å), β is the full width at half maximum of the peak in radians and θ is the Bragg's diffraction angle. To validate the crystalline structure of the AgNPs, the findings were compared with the standard JCPDS library.

Larvicidal Assays against *Aedes aegypti*

We used the WHO-recommended technique to test the larvicidal efficacy of the aqueous extract and synthesized AgNPs of Asafoetida. Every bioassay had two experimental groups (one for each concentration tested) and a control group. The specific study unit (i.e. technical replicate) for each of these groups was a plastic well containing 25 third instar larvae (Institute of Vector Control and Zoonoses, Hosur,Tamilnadu) in either 50 mL of the test solution at the desired concentration (for experimental groups) or distilled water (for control groups).During bioassays, larvae were held at normal insectary temperatures (28°C, 80% relative humidity, and a 12 hour light/12 hour darkness photoperiod). During this time, no food was given. The death rate was measured 24 hours after the start of each bioassay. Specimens were rendered dead if they did not move at all or moved slowly after being stimulated by contact and were unable to rise to the surface of the rearing medium.

Results and Discussion

This analysis looks into the biosynthesis of *Ferula asafoetida* silver nanoparticles (Asafoetida-AgNPs) and used spectrum analysis to characterize the ability of nanoparticles made from the aqueous resin extract of *Ferula asafoetida*.

Phytochemical Analysis

The phytochemical constituents responsible for the reduction and capping of silver nanoparticles in the aqueous resin extract of *Ferula asafoetida* were qualitatively examined. As seen in Table 1, the phytochemical screening of the *Ferula asafoetida* aqueous resin extract revealed that this was a strong source of secondary metabolites (Figure.1).

Table 1. Preliminary Phytochemical compounds of Aqueous resin extracts of *Ferula asafoetida*

Phytoconstituents	**Reaction**	**Observation**
Phenol	Blue/black colour	Presence
Tannins	Brownish green colour	Presence
Flavonoids	Pink tomato red colour	Presence
Triterpenoids	Reddish brown ring colour	Presence
Saponins	Foaming appearance	Presence
Steroids	Green colour	Presence
Cardio glycosides	Brown ring colour	Presence
Resin	Light blue colour	Presence

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Alkaloids	Orange red colour	Presence

These phytochemicals may be responsible for silver reduction as well as serving as a capping agent to avoid nanoparticle accumulation and provide stabilization. The bulk of phytochemicals derived in polar solvents are polar, and they play an important role in nanoparticle synthesis, reported similar results [24].

Figure 1. Phytoconstituents Reactions

GC chromatogram

Figure 2 shows the chromatogram of the *Ferula asafoetida* aqueous resin and the constituents are Selinene (36.09%) was the most abundant compound among 48 constituents that were identified followed by Oxirane, 4-(1,1-dimethylethyl)ph enoxy]methyl (26.73 %), Silane, trichlorooctadecyl (15.73 %), Azulene, 1,2,3,3a,4,5,6,7-octahydr o-1,4-dimethyl-7-(1-methylethenyl) (10.23%), Undecanoic acid (5.77%) and Tetradecadiene (5.43%), reported similar results [25].

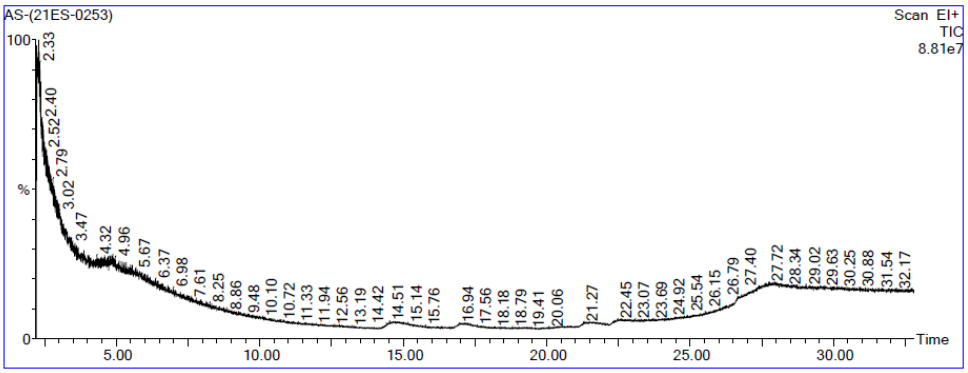

Figure 2. GC chromatogram showing phytoconstituents of Aqueous resin extracts of *Ferula asafoetida*

NMR spectrum

Ferula asafoetida aqueous resin compound Fnarthexol was also determined to be a sesquiterpene by the presence of diagnostic peaks in the 13C- NMR spectra. The 13C- NMR spectra of compounds displayed signals due to six aromatic methines at δH/ δC [{6.21 (d, J = 9.6 Hz)/58.87, CH-3}, {6.80 (br. d, J = 8.4 Hz)/40.62, CH-6}, {6.78 (br. s)/39.35, CH-8}, {7.33 (br d, J = 8.4 Hz)/18.64, CH-5} and {7.61 (d, J = 9.3 Hz)/18.20, CH-4}] (Figure 3) , reported similar results [26].

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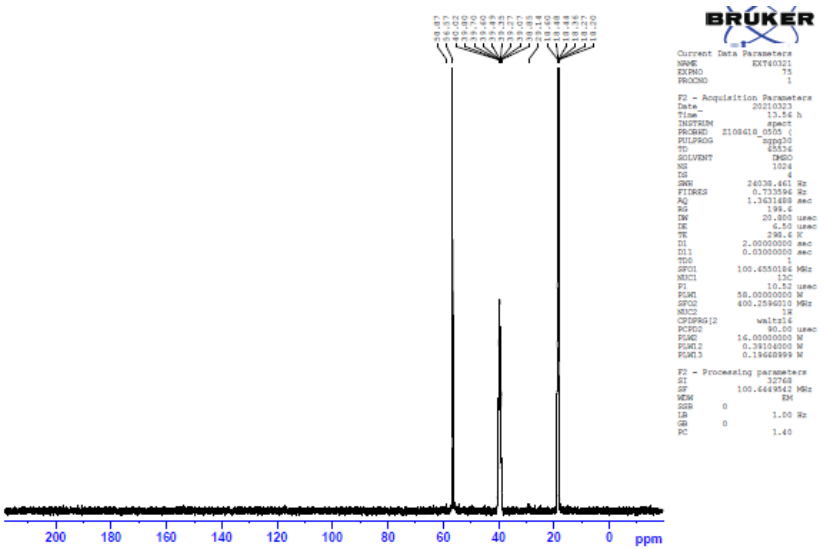

Figure 3. NMR chromatogram showing phytoconstituents of Aqueous resin extracts of *Ferula asafoetida*

Asafoetida AgNPs UV-Vis spectrum

Bioreduction of silver ions into silver metal was visually confirmed by a change in colour from white to reddish-brown for silver nanoparticles during the exposure time in asafoetida resin aqueous extract, although no change of colour was observed as control of silver nitrate solution without asafoetida resin aqueous extract.The asafoetida resin aqueous extract was light yellow in colour and the colour of the mixture turned dark brown due to surface Plasmon Resonance (SPR) within 24 h, suggesting the development of silver nanoparticles, after applying colourless AgNO3 solution. At 460 nm, a high absorption peak was observed at various time intervals 24 h in the UV-Vis absorption range (Figure.4).

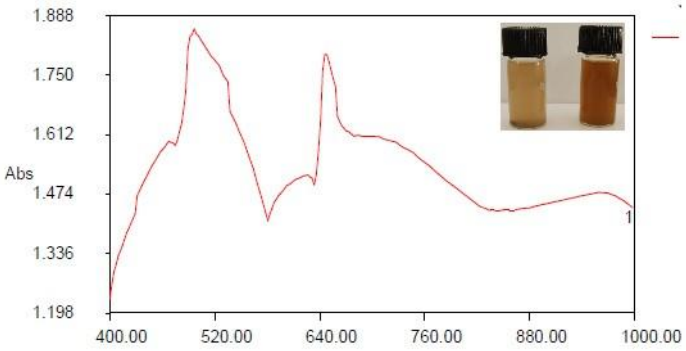

Figure 4. Spectrum of As-AgNPs at 460 nm with range 400-1000nm

The increase was due to the vast number of molecules that were uniformly dispersed in the solution of the mixture, supporting our findings previously stated. With the same experimental process replicated, the stability of the synthesised As-AgNPs was measured. The synthesis process was tracked over a period of time 24 h through visual inspection by a shift of colour and agglomeration. To test stabilisation, the synthesised As-AgNPs were characterised by UV-Vis spectroscopy and observed the same absorption peak at 460 nm.The observed result indicated that synthesised As-AgNPs are stable, reported similar results [27].

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Previously it was stated that the process of reducing silver ion (Ag+) to silver atom (Ag0) nanoparticles was probably due to NADPH-dependent dehydrogenases operation.The resin extracts mixed with an aqueous AgNO3 solution were documented to result in yellow to brown colour due to the reduction of silver ions in the reaction mixture, and confirmed silver nanoparticles development. The absorbance peaks can be used simultaneously to determine particle size and stability [28-30].

Asafoetida AgNPs FTIR spectrum

The FTIR spectral analysis was used to identify the presence of functional groups in both aqueous extract and synthesized silver nanoparticles. Figure 5a shows the The FTIR spectrum bands at 3340, 2976, 2899, 1647, 1384,1095,1045,879 and 642 cm−1 for the properties of the aqueous extract. The properties of the synthesized AgNPs were studied by using FTIR spectroscopy.The FTIR spectral peaks confirmed that the bands at 2958,2924,2858,1463,1379 and 723 cm−1 silver ions were incorporated with asafoetida resin, as indicated by the presence of hydroxyl and carboxyl groups (Figure 5b).

The strengths of this study include having data from a large previous studies peaks of synthesised silver nanoparticles for ApAgNPs were observed at 3446, 2914, 2851, 1633, 1265, 1255, 1090, 1030, 803 and 607 cm-1; for MoAgNPs 3449, 2924, 1631, 1385 and 1033 cm-1; for AgNPs 3424, 2925, 2360, 1628, 1383, 1271, 1051 and 670 cm-1. The peaks at 3446 cm-1 and 2360 cm-1 reflect the amine group N-H stretch, with 3449 cm-1 stretching phenol group. In the range of 3424 cm-1 is expressed the O – H stretching of the alcohol group which binds the silver ions with the molecules. The stretching of peaks at 2914 and 2925 cm-1 represents the alkanes group C – H bonds, while aldehydes represent 2851 cm-1. The C = C alkene stretch corresponds to the 1633 cm-1, reported similar results [31-32].

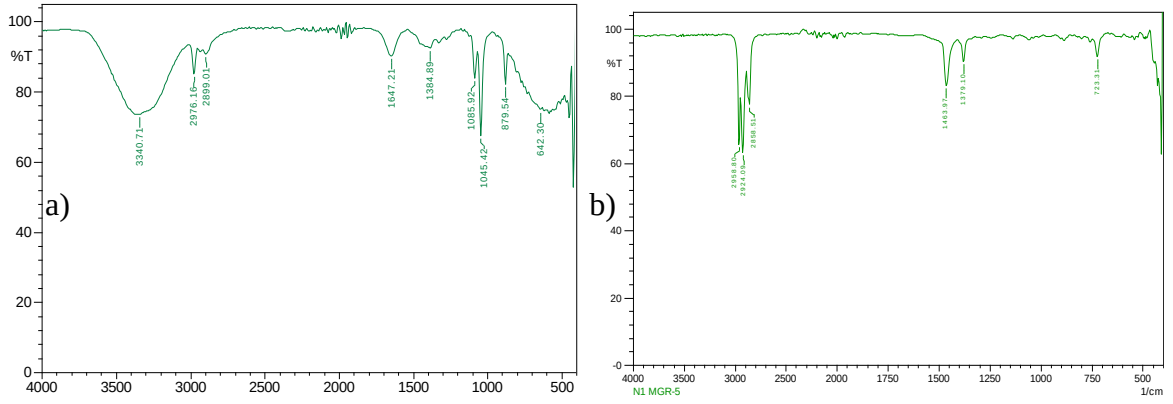

Figure 5. FT-IR spectral analysis: a) asafoetida resin aqueous extract b) synthesised asafoetida AgNPs

EDX image of asafoetida AgNPs

The shape of the asafoetida AgNPs fabricated by using aqueous extracts was found to be spherical, rod and cuboid topology documented under scanning electron microscopy.The average size of the synthesized asafoetida AgNPs was found between 36-65 nm (Figure 6a). The EDX

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image of asafoetida AgNPs showed the strong silver signal in the range of 2–4 keV and it showed weak peaks of C, K, Na, Mg, O. (Figure 6b) , reported similar results [33-34].

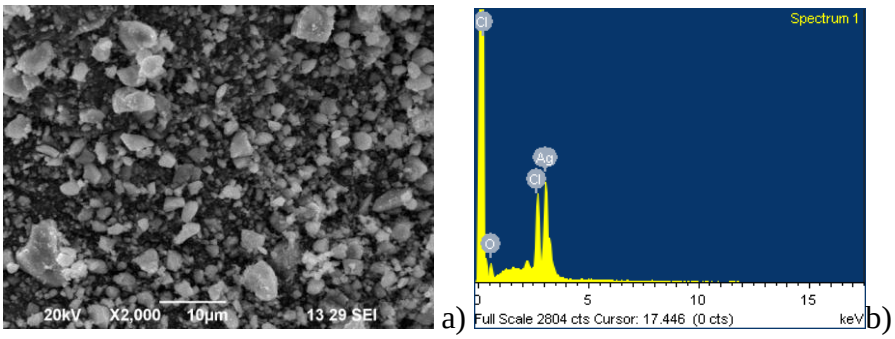

Figure 6. a) SEM images of synthesized asafoetida AgNPs b) Energy dispersive X-ray (EDX) spectrum of AgNPs exhibited the strong signal.

Asafoetida AgNPs XRD spectrum

The spectral results for asafoetida synthesised AgNPs showed six large peaks at 2 volume values of 40 and 48degree corresponding to planes (Figure 7) XRD patterns at 2theta with corresponding values were 38.90 (111) , 44.26 (200), 64.43 (220) and 78.38 (311) respectively, indicating the crystalline nature of the Asfoetida Ag NPs.The small difference in peak positions suggested the presence of strain in the crystal structure, which is a characteristic of nanocrystallites.

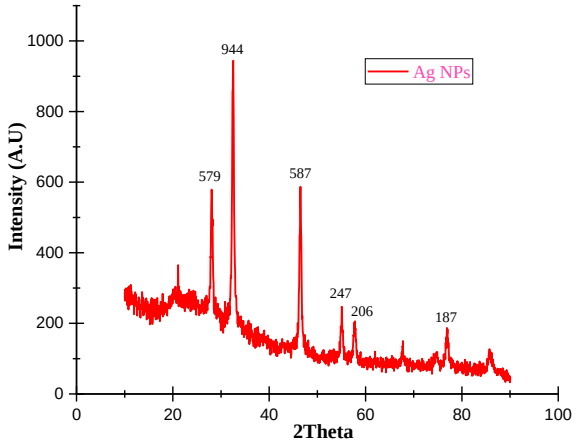

Figure 7. XRD pattern of biosynthesized asafoetida AgNPs exhibiting the facets of crystalline silver.

Thus the XRD pattern clearly suggested that nanocrystalline in nature was found to be the synthesised AgNPs by green process. By studying the strong reflection of the Bragg in the XRD spectra crystallite domain size perpendicular to the reflective planes, the crystal size and composition were further established, K being the Scherrer constant 0.89, reported similar results [35-37].

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Larvicidal Activity of Aqueous Extract and Synthesized AgNPs

AgNPs. Mortality values observed following all bioassays are shown in Table 1. Both the aqueous extract and the *Asafoetida* synthesized AgNPs showed a dose dependent toxic effect against *Ae. aegypti* larvae. No mortality was observed in the control groups. Several studies have reported on the larvicidal activity of *Asafoetida* of against *Ae. aegypti* (Figure 8).

Figure 8. larvicidal activity of *Asafoetida* of against *Ae. aegypti*

Larvicidal activity of aqueous extracts and silver nanoparticles synthesized using *Asafoetida* against *Aedes aegypti* third instar larvae [39-41]. The silver nanoparticles had the greatest larvicidal activity, according to the findings in Table 2.

Table 2. Larvicidal activity of aqueous extracts and silver nanoparticles synthesized using *Asafoetida* against *Aedes aegypti*

Mosquito species	Concentration (µg/ml)	Mortality (%)± SDa	LC50 (µg/ml) (LCL-UCL)	LC90 (µg/ml) (LCL-UCL)	Slope	Regression equation	$\chi 2$ (d.f.)
Ae. aegypti	50	22.8±0.8	153.23 (134.12-170.22)	303.12 (283-312.25)	2.76	y= 6.1+0.27x	3.084 (4) n.s.
	100	41.2±0.3					
	150	64.1±0.2					
	200	82.1±0.6					
	250	96.1±0.2					

a Values are mean ±SD of five replicates, No mortality was observed in the control, SD = standard deviation.
LC50= a lethal concentration that kills 50% of the species exposed.
LC90= a lethal concentration that kills 90% of the species exposed
UCL= The upper confidence limit is set at 95%.
LCL= 95% lower confidence limit.
$\chi 2$= chi square, *d.f.* = degrees of freedom, n.s. = not significant (α=0.05).

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Conclusion

Based on the above results, it can be concluded that *Ferula asafoetida* resin can be successfully used for green synthesis of asafoetida Ag NPs with proper characteristics. Its involvement in the synthesis of AgNPs led the silver nanoparticles to exhibit good characterization properties. This is the characterization report related to the asafoetida mediated green synthesis of silver nanoparticles and their characterization confirmed by UV-Visible spectrum and it revealed strong intense peaks at 460 nm and size of silver nanoparticles obtained were spherical ranging from 36-65 nm for asafoetida AgNPs. In conclusion, the synthesized AgNPs from *Ferula asafoetida* resin confirmed the formation of silver nanoparticles, it can also be suitably scaled up for large scale synthesis of nanoparticles. These findings suggest that Asafoetida aqueous extract and green-synthesised silver nanoparticles made from such extracts could be transformed into effective alternative tools for controlling Aedes aegypti populations.

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Conflicts of Interest

The authors declare no conflict of interest.

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