Synthesis of Silver Nanoparticles: Characterisation and Antimicrobial Activity of Synergistic Herbal Formulation Derived from *Cassia fistula* and *Manilkara zapota* Plants.

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Abstract: *The synergism of plants in herbal medicine has been more effective than the individual ones. Synthesis of silver nanoparticles was taken from the synergistic methanol extract of Cassia fistula flowers and leaves and Manilkara zapota leaves. This extract of plants was assessed for its major phytoconstituents and antimicrobial activity. Here in this investigation methanol concentrate of flowers and leaves of Cassia fistula and leaves of Manilkara zapota was used to synthesise silver nanoparticles. The silver nanoparticles were then characterised by the UV - visible spectrophotometer, FourierTrans form Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM). In the UV- visible spectroscopy, maximum absorption was obtained at 425 nm. In SEM analysis, the dimensions of the particle were irregular and ranged from 100nm - 150nm. In FTIR, different stretch of bonds was shown at 3332.16,2114.20,1635.70 and 637.27. The antibacterial assay of plant silver nanoparticles in case of Staphylococcus aureus, showed its maximum zone of inhibition at 17mm. Thus,the easily available ecofriendly plants and the synergistic consequence of the plants with the advanced nanoparticle technique will show revolution in health care and to cure various health issues .*

Keywords: Synergism, Nanoparticles, Characterisation, Synthesis, Antimicrobial activity.

Introduction

Plants and its health attributes are pondered as the most effective conventional system of medicine with many curing and healing properties. Various plantextracts and their phytoconstituents are a promising source for healing agents due to the existence of variety of active components, easy to access and low in side effects. The method of synergism, where the products with combined extracts of plants are considered more effective rather thanindividual ones. The activephytoconstituents of individual plants was spotted but aregenerally available insmall quantities, which is insufficient to produce the desiredtherapeutic action for curing wounds. In synergism, the co-mixture of plants results in efficiency of the medication, its cost-effective nature and reduced therapy time for treating the wounds [1]. The recent studies of synergism in plants are also used in TM prescriptions that is traditional medicine prescription where a single herb or combinations of herbs are used for the effective cure of a single target or multiple targets. This is called as network pharmacology. This will be a great help to the modern medicine [2]. Nano-biotechnology has raised as an important empiric of nanotechnology. The important aspect in the field of nanotechnology is the construction of a more consistent process for the fusion of nanomaterials more than a range of size (with good mono dispersity) and chemical composition [3]. In past, massive involvement and research efforts was led towards the evaluation and revaluation of metallic nanoparticles derived from new metals, such assilver and gold. Silver nanoparticles acts as the best antimicrobial agents. With this property silver nanoparticles were used as antibacterial vehicle in several healthcare, food and other industries [4]. Nanotechnology is the emerging trend in biotechnology. The plan of the investigation was to find outnanoparticle, which is efficient, cost effective, eco-friendly product for the researchers.Currently, the silver nanoparticles weresynthesised by the solvent methanol of the *Cassia fistula* flowers and leaves and leaves of *Manilkara zapota* and the characterisation studies were done. Further the silver nanoparticles underwent an antibacterial study against the desired organism. This nanoparticles with the effectiveness of the synergistic extract are combined with the silver nanoparticles for the future wound dressing. Das encapsulates the acute toxicological aspects of the *Euphorbiathymifolia*coupled with silver nanoparticles. His findings facilitated the evolution of safe and efficient Ag-ET nanoconjugate as an effective therapy against glucose mediated health disorders[5]. The present work concentrates on the combination of the plant silver nanoparticles with their extract, their characterisation, the antimicrobial test and animal dermal test of the prepared samples to ensure the safe and effective combination as wound dressing.

Materials and methods

Plant material:

Fresh leaves of *Manilkara zapota* and leaves and flowers of *Cassia fistula* were collected from Aranvayulkuppam, Thiruvallur, Tamil Nadu. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles.

Extraction method:

The dried powder of leaves and flowers of the plants was separately extracted successive by percolation method, using solvent methanol. The extracts were concentrated and freed of solvent under reduced pressure, using rotary evaporator. The weighed crude extract was stored in the refrigerator in air tight bottles.

Sample preparation (Synergism)

Leaves from the *Manilkara zapota* and leaves, flowers from *Cassia fistula* plants were taken in equal proportions (i.e) 1:1:1 were taken for the next level of study.

Synthesis of silver nanoparticles [6]

The plant silver nanoparticle was prepared by mixing aqueous solution of silver nitrate (1mM) and fresh methanol extract of the plants in the ratio of 1:9. The solution was incubated in dark at room temperature for 24 hrs. After 24 hrs, the colorless silver nitrate solution turned pale yellow to reddish brown. The synergistic plant mediatedAgNPs was centrifuged for 20 mins at 5000 rpm. The supernatant was discarded and the pellets were air dried.

Characterisation of nanoparticles

The depletion of silver metal ions to silver nanoparticle was analyzed by using UV-visible Spectrophotometer. The UV–vis analysis was performed by sampling the aqueous component at different time intervals and the absorption maxima was scanned over the300–800 nm wavelength range on a Perkin Elmer Lambda 25 spectrometer. The morphology and the size of the nanoparticles were characterised by SEM (Philips model CM 200). Magnifications were viewed in the range of 10000X to 30000X. The FTIR measurements (Perkin- Elmer) were carried out to identify the potential possible biomolecules of the herbal extract responsible for the conversion of the silver ions to silver nanoparticles.

Screening of antibacterial activity [7]

Nutrient agar was prepared and poured in the sterile petri dishes and allowed to solidify. 24 hours bacterial culture of *Staphylococcus aureus (MTCC 96)*was swabbed on it. The test sample with different concentrations were placed over the agar plate using sterile forceps. Tetracycline (25µL) was used as the control. The plates were then incubated at 37ºC for 24hours. After incubation the inhibition diameter was measured and units are mentioned in mm.

Results and discussion

Plant extracts contain polysaccharides and phytocomponents. The speed of the reaction reducing Ag^+ ions in the plant extract and the change in colour intensity are the main objective for the synthesis of silver nanoparticles [8]. In Fig 1, Distinct change in the colour of the sample with standard concentration of AgNO3 was observed but colour of the controls remained un-changed. The colour of sample turned light brown, which became deeper after 24hrs. Prior work of other authors has also reported similar colour change due to silver nanoparticle formation. The result of the synthesis of silver nanoparticles with Manilkara *zapota seed* extract, clearly specify the development of the silver nanoparticles [9]. Mohanta in the year 2016, found the methodology of the silver nanoparticle synthesis where the Cassia *fistula leaf* extract was used.He explained that *Cassia fistula* leaf extract with the silver nanoparticles can be used as the translational medicine [10].

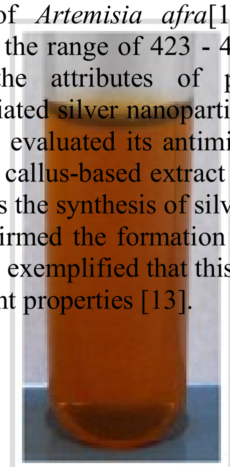


Figure 1: Synthesis of PlantSilver Nanoparticles. Colour Change in the tube after 24 hrs of the Reaction Time.

With the help of UV-Vis spectroscopy, we examined the absorption peak of the nanoparticle. Here the maximum absorption peak was noted as 425 nm (Refer Fig 2). The development of silver nanoparticle from the silver nitrate is observed by the colour change. Elias E. Elemike et al, tried the transfiguration of silver nanoparticle with the help of aqueous leaf extract of *Artemisia afra*[11]. Here the synthesised silver nanoparticles showed the SPR in the range of 423 - 438 nm. This result was compared with our work to confirm the attributes of phytochemical as the reducing agents.KeroJamal tried plant mediated silver nanoparticles with *Allophyllus serratus and* callus-based leaf extract and also evaluated its antimicrobial properties. The spectra of silver nanoparticles of leaves and callus-based extract was in the range of 440 - 445 nm [12]. Venugopal 2016, also reports the synthesis of silver nanoparticles from leaf extracts of *Commiphoracaudata.* He confirmed the formation of the silver nanoparticles by the UV spectroscopy method. He also exemplified that this type of silver nanoparticle works, best in antibacterial and antioxidant properties [13].

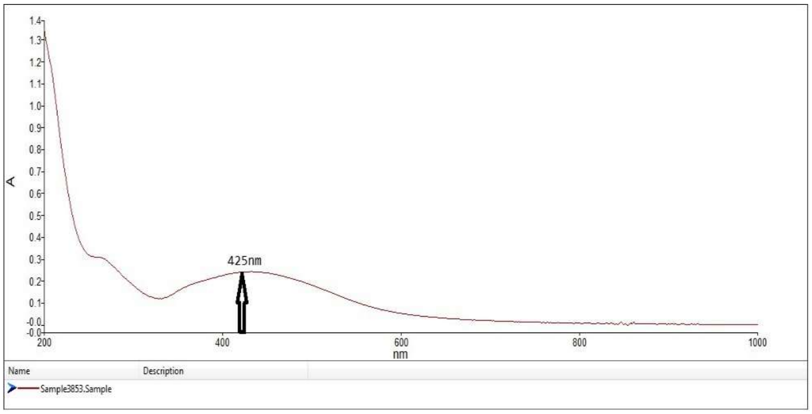
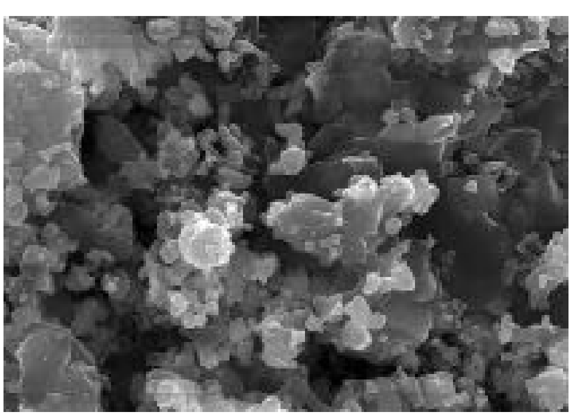


Figure2: UV-vis Spectroscopy of Silver Nanoparticles by Methanol Extract of *Cassia fistula* and *Manilkara zapota*. Absorption Peak was Noted to be at 425nm.

SEM is a quantitative analysis where the size of the particles can be conducted. It provides images of nanoparticles and their agglomerates in sufficient solution. SEM analysis shows high-density AgNPssynthesised by the synergistic herbal formulation. The figuredepicts the SEM characterisation of synergistic plant nanoparticles (*Cassia fistula flowers*and leaves:*Manilkara zapota leaves* in the ratio 1:1:1) in the magnification range of 30000x. Fig 3 Shows particles morphological structure, where the SNP are of irregular in size. They were equally dispersed in and around. The size of the nanoparticles ranged from 80nm - 150nm. It was shown that relatively irregular AgNPs were formed and the characterisation appears to have more pores. SEM image of silver nanoparticles described the surface topography and the composition of the sample. The biomolecules present in the nanoparticles confirm the presence of the plant's metabolites in the extract which is responsible for stabilizing the silver nanoparticle. Herbal plants like *Vasambu,Vilagam*silver nanoparticles SEM results showed the size which lies between 18.6 - 37.9 and 33 - 45.9 nm respectively. This confirms the SEM characterisation range of the plant silver nanoparticles [14]. In an inquiry on cytotoxic effect of bioactive AgNPssynthesised using *Cassia fistula* flower extract on breast cancer cell MCF-7, the FE-SEM outcomes was 50nm and the shape of the silver nanoparticles were spherical [15]. Our synergistic plant silver nanoparticles do fall in around this range which clearly states that *Cassia fistula* with the other plants can improve its efficiency in any translational medicines.



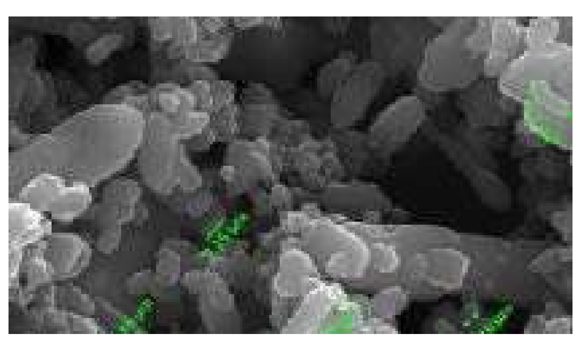


Figure 3: Scanning Electron micrograph of Silver Nanoparticles by Methanol Extract of *Cassia fistula* and *Manilkara zapota*. Good Dispersion of the Nanoparticle in the Solution and Size Ranges from 80nm - 100nm

FTIR spectroscopy was useful in probing the chemical composition of the surface of the silver nanoparticles and the local molecular environment of the capping agents on the nanoparticles. Results of FTIR analysis of this study showed differentstretch of bonds shown at different peaks; 3332.16 which showed the alcohol, aliphatic primary amine and secondary amine compound group frequencies of. “polymeric” O-H stretch indicating the presence of hydroxyl groups in the reducing agent [13]. 2114.20 showed frequencies of C triple bond C indicating the presence of alkyne group. 1635.70 alkene group frequencies of alkyl C=C stretch [14]. 637.27 halo compound frequencies of C-Br stretch and C-Cl stretch. This result explains the ability of the biomolecules coating the nanoparticles, which prevents from agglomeration and stabilizes the nanoparticle. The FTIR spectroscopic study certifies that the synergistic extract has the potential to perform dual functions of reduction and stabilisation of silver nanoparticles. Fig 4, shows the spectrum of the silver nanoparticles synthesised by the synergistic herbal formulation of *Cassia fistula* and *Manilkara zapota.*

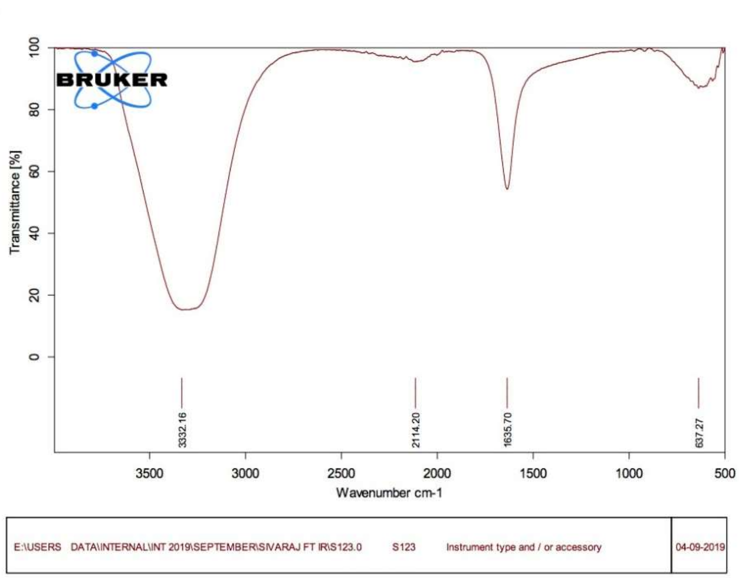


Figure 4: FTIR Spectrum of Silver Nanoparticles Synthesised by Methanol Extract of *Cassia fistula* and *Manilkara zapota*

The antibacterial screening of the silver nanoparticles in opposition to *Staphylococcus aureus* (*MTCC 96*) was performed by Disc diffusion method. Table 1, shows the maximum zone of retardation at 17 mm for *Staphylococcus aureus.* Fig 5 shows the zone of inhibition of the extract. The synthesis of silver nanoparticles by *Cassia fistula* silver nanoparticles showed the maximum zone of retardation against *Staphylococcus aureus* comparatively to the other microorganisms.AgNPs were efficient against Staphylococcus *aureus alike* theprevious work[15]. Thus, the synergistic plantAgNPs provides the best bactericidal effect and provides less wound healing time than the other drugs [16]. These studies were in accordance with the former studies that examined the similar concept [17].

Table 1:Antibacterial Activity ofPoly Herbal Silver Nanoparticles (1:1:1) against*Staphylococcus aureus*

Pathogen	Concentration / Zone of inhibition (mm)				
	250µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL	Standard 25 µL (Tetracycline)
Staphylococcus aureus	13	15	16	17	14

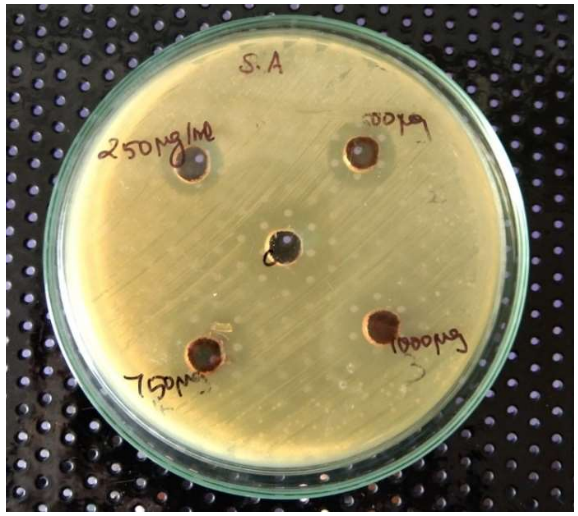


Figure 5: Zone of Inhibition of PlantSilver Nanoparticle against *Staphylococcus aureus.*

Previous studies of silver nanoparticles with *Cassia fistula* leaf extract were characterised by UV-visible, FT-IR, SEM, EDX and AFM. The antimicrobial activity was tested against six different human pathogens and it was found that AgNPs were more effective against gram positive bacteria. Due to its vital functions, the silver nanoparticles synthesised by the synergistic plant extract were used as bactericidal, anti-cancerous and anti-inflammatory in various medical applications. This led to vast scale synthesis of nano material which is used in wound healing and other biomedical applications [18]. It also confirms that antibacterial and antioxidant activities of extracts of C*assia* species and SNPs synthesised using their extracts were reported in the literatures[19]. Ruchi Mathur 2017 observed that natural antioxidants drug from herbal plants are safe without any side effects. These drugs are used for both internal and external medical applications. The author used the silver nanoparticles of the leaf and stem of the *Manilkara zapota*[20]. The author Rajakumar in his paper explained that the AgNPssynthesised by *Manilkara zapota* leaf extract showed acaricidal activity at LC50 values of 16.72 and 3.44 mg/L against *Rhipicephalus (Boophilus) microplus* [21].Hence with the above information the *Manilkara zapota* leaf was tested for the bactericidal activity and confirmed that the leaf plays a vital role in resisting the bacteria. Above information, confirms that the synergistic effect of the *Cassia fistula* leaves, flowers and *Manilkara zapota* leaves has the effect to work as a plant-based medicine for the wound healing studies. The silver nanoparticles along with the synergistic plant extract would have been working vitally as an extra boost to our concept. With these results, further research will be carried outusing this synergised plant-based silver nanoparticles in health care or translational medicines.

Conclusion

In this study, we successfully demonstrated the ability of *Cassia fistula flowers*, *Cassia fistula leaves* and Manilkara *zapota leaves* extract to synthesiseAgNPs. This synthesis of silver nanoparticles with the synergistic plants created an effective SNP for our future wound studies. Unlike pharmaceutical formulations, herbal medicines contain many phytoconstituients which causes null ill effects on its action. Thus, this paper informs that the ancient medicinal herbs were always better than the synthetic medicines. Synergistic herbal formulation doubles up the effect of what we were working on. The characterisation methods of nanoparticles like UV - Vis spectroscopy, SEM analysis and FT-IR confirmed that our synergistic silver nanoparticle was a stabilised nanoparticle which can be further used for variousapplications. The antibacterial activity of the synergistic silver nanoparticle by disc dif- fusion method justifies our work in resisting and killing the microbe which is responsible for wound and other related studies. Results conclude that the above synergised herbal based silver nanoparticle took a stand effectively against the microorganism like *Staphylococcus au- reus* and *Aspergillus niger*which are one of the causes for the skin diseases and allergies. In future, the formulation will be applied and concentrated for wound related studies.

Abbreviation

AgNPs- Silver Nanoparticles
U-Vis - Ultraviolet Visible Spectroscopy
SEM - Scanning Electron Microscope
FTIR - Fourier Transform Infrared Spectroscopy
SNP - Silver Nanoparticle

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