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GREEN SYNTHESIS OF SILVER NANOPARTICLE, FT-IR ANALYSIS AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF POLYHERBAL FORMULATION FROM CYMBOPOGAN CITRATUS,CITRUS AURANTIUM AND CURCUMA LONGA

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ABSTRACT

Green synthesis is an essential process for synthesizing nanoparticles through medicinal plant sources. For the synthesis of silver nanoparticles (AgNPs), an economically affordable and "green" approach has been concluded, which has been studied using UV-Visible spectroscopy. The aim of the present study was to evaluate the biosynthesis of silver nanoparticle (AgNPs) using polyherbal formulation from the ethanolic extract of three different plant parts of Cymbopogon citratus, Citrus aurantium and Curcuma longa . Characterization of AgNPs was performed using UV–visible spectroscopy and FT-IR analysis. Silver nanoparticles were synthesized and the presence of AgNPs was confirmed by UV-Visible spectrophotometer. Synthesis of silver nanoparticles was confirmed by visual observation in which the colourless solution gets changed to a brown-colored solution. Further characterization was done by UV-visible spectroscopy and FT-IR. The UV-Visible Spectroscopic results showed the maximum absorption peak at range of 449 nm. FT-IR analysis revealed that the presence of specific function group such as Aliphatic primary amine, Isothiocyanate and Alkene found in the AgNPs.of the ethanolic extract of poly herbal formulation. . The anti-bacterial activity of AgNPs.in the ethanolic extract of poly herbal formulation also evaluated against specific bacterial strains such as Ecoli and Staphylococcus aureus. Green synthesized silver nanoparticles have strong antioxidant and antibacterial activity due to the presence of active principles on the surface of silver nanoparticles. The antimicrobial assay showed that better antimicrobial activity found in poly herbal extract against two bacterial strains.

KEYWORDS

Polyherbal formulation -Ointment, Green synthesis, AgNPs. UV-Visible Spectroscopy, FT-IR, Anti-microbial assay, Well diffusion, Ecoli and Staphylococcus aureus

INTRODUCTION:

Nanotechnology is an emerging field as one of the large and thirst areas of research, offering specific features and extensive applications in various areas such as Food ,Agriculture, and Biomedicine.by *Erci, F. et al* [1]. The essential nanoparticles such as gold ,silver, platinum, copper, zinc, titanium, and magnesium, have gained appreciable attention for medical applications due to the presence of bioactive compounds in multifunctional aspects especially in medical field by Behboodi, S et al [2]. The most specialized nanoparticles such as silver and gold are used for various biomedical applications and in the emerging interdisciplinary nanobiotechnology by S Iravani[3].Metal nanoparticles are vital role for various applications in pharmacy and medicine. Green synthesis is an essential approach that has more advantages over the way, Nanoparticles are synthesized in physical and chemical ways . by S Qayyum *et al*[4] and M Oves *et al*[5] It acts as an important antimicrobial agents by showing antibacterial, antifungal, antiviral and anti-inflammatory activities . Silver nano particles are used as nano-carriers for antibiotics that help to enhance the antibiotic activity against resistant pathogens by NY Yount and MR Yeaman[6].

Metal ion such as silver has long been recognized as having inhibitory effect on pathogen in clinical and industrial field by Morones JR,. *et al*[7] and Lok C, *et al*[8]. The role of medicinal plants for synthesis of nanoparticles is very cheap and environment friendly, and a unique step method for biosynthesis process by Huang J *et al*[9]. Plant-mediated nanoparticles synthesis is focused as it is low cost, eco-friendly and safe for human medicinal use by Kumar.V et al.,[10].

Chemical reduction process which is the most generally used method for the synthesis of nanoparticles uses an organic solvent, like ethylene glycol by Wiley et al[11]. , and reducing agents, like hydrazine by Guzmán et al.[12] , sodium borohydride by Song et al[13]. , trisodium citrate Rashid et al. , and ascorbate by Qin et al[14].

In the present study, polyherbal extract was utilized for the reduction of silver nitrate to silver nanoparticles, and characterization of the synthesized nanoparticles was carried out by UV-visible spectroscopy, and Fourier transform infrared spectroscopy (FT-IR) Antimicrobial activity of the synthesized nanoparticles was detected against specific microorganism such as *Ecoli* and *Staphylococcus aureus* by well diffusion method.

MATERIALS AND METHODS :

Collection and Preparation of Sample :

The collection of sample such as Orange peel and turmeric were collected from the local market and the lemon grass was collected from ayurvedic botanical garden, Coimbatore Ayurvedic college ,Sulur, Coimbatore.

Preparation of Samples:

The samples were collected and dried, crushed and milled using laboratory blender in to fine powder for further analysis. Dried sample were added conical flask to 100 ml ethanol was added for maceration for 3 days and then mixture was filtered using filter paper .the filtrate was collected in china dish and kept in water bath until extract will make fine powder and stored in desiccator.

Polyherbal formulation Preparation – Ointment:

Paraffin wax was melted in china dish and wool fat. Cetosteryl alcohol and white soft paraffin was added to the mixture thoroughly. the ingredients are heated in boiling water bath at 50c until it get melted and fused together. The mixture was cooled and stirred well until a semi solid mass is formed and fused together. It was cooled and stirred well until semi solid mass is formed. then added the extract of the sample. Store in a well-closed container and keep in cool and dry place.

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extract were prepared by using simple maceration process to obtain a good yield of extract and were was no any harm to the chemical constituents and their activity. The levigation method was used to prepare ointment so that uniform mixing of the

herbal extract with the ointment base was occurred which was stable during the storage.

Formulated Ointment

Synthesis Of Silver Nano Particles Using Polyherbal Formulation:

Synthesis of silver nanoparticles using polyherbal extract 1 mM silver nitrate solution in double distilled water was the source of silver. Silver nitrate and poly herbal extract were mixed together in a ratio of 1:8. The reactive mixture was heated below the boiling point and continuously stirred at 900 rpm using magnetic stirrer. The mixture was converted into reddish brown in colour within 1 hour. The whole reaction was carried out in the dark. The obtained suspension of silver nano grandis was centrifuged at 20,000 rpm for 40 min. The pellet containing silver nanoparticles was washed 4-5 times with deionized water to remove silver ions and polyherbal extract residue. The precipitated nanoparticles were lyophilized and it was stored in a cool, dry, and dark place and their characterization process was carried out by UV-visible spectroscopy, and Fourier transform infrared spectroscopy (FT-IR)

Uv–Visible Spectroscopy Analysis:

Uv–visible spectroscopy study was used to evaluate the silver nanoparticle formation by the reduction of silver ions through the exposure of poly herbal plant extracts .Cittrarasu et al,[15] indicated the surface plasmon resonance sharp peak at 400–500 nm wavelength, which corresponds to the silver nanoparticles production. It was absorbed radiation at 445 nm wavelength due to the transition of electrons . Silver nanoparticle synthesis from polyherbal extracts and observed the absorbance at 445 nm using UV-spectrophotometer . In a study, maximum absorbance was reported at 445 nm in the NPs synthesis using poly herbal extract of different plant parts of *Cymbopogon citratus, Citrus aurantium* and *Curcuma longa*\

Ft- Ir Analysis:

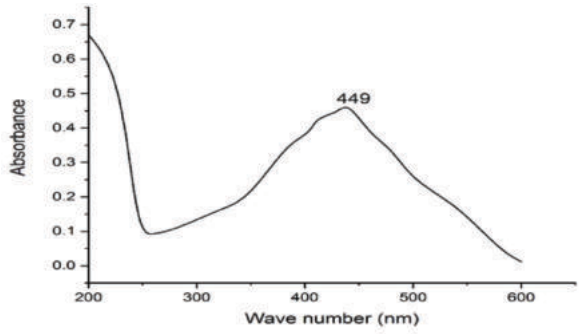
The functional groups capping in AgNPs were identified by using FTIR analysis. After centrifuging the colloidal solution for 15 minutes at 9000 rpm, the pellet was washed thrice with Milli Q water. Before FTIR analysis, the resultant suspension was fully dried in a freeze drier. The materials were dried before being combined with KBr powder and pelletized for FTIR; with wavelengths ranging from 4000 cm1 to 400 cm1 acquired using Perkin Elmer

The biomolecules connected to the silver and surface atoms of the capping agents of the nanoparticles have been mostly evaluated by using FTIR spectroscopy. It can give a good transmission and accuracy whether compounds are actively linked to the synthesis of nanoparticles. The functional groups play a vital role in capping as documented in the recent studies Niraimathi , K., et al [16] and. Prakash, P., et al[17]. The occurrence of peaks shows that the secondary metabolites of plants such as flavonoids, terpenoids, phenols, glycosides, and tannin role groups such as aldehydes, ketones, carboxylic acid were coated with the Nps.

Antimicrobial Assay:

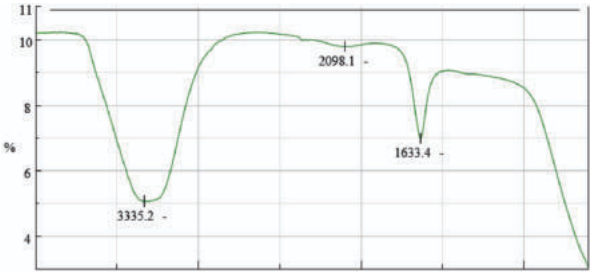
The antibacterial activity of poly herbal extracts was determined by well diffusion method .MHA plates were prepared by pouring 25ml of agar medium into sterile petri plates. After solidification of media, 20-30 suspension of bacterial inoculums was swabbed uniformly. Sterile paper discs were dipped into required solvents then placed in agar plates. Then different concentration (40,60,80 and 100 µg) of plant extract was poured into the wells. After that, the plates were incubated at 37°C for24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. Well were put into the agar medium using sterile forceps. plant extract were poured on to wells. Then plates were incubated at 37° for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm. Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against Gram positive and Gram negative bacteria. Polyherbal extraction contains AgNPs showed excellent antibacterial activity due to their stability and size of the particle by Singh, M., et al[18].

RESULTS AND DISCUSSION:



Graph -1- Characterization of Silver Nanoparticles by UV-Visible Spectroscopy:

The UV-visible spectrophotometric analysis study revealed that the presence of silver nanoparticles. found in the range at 449 nm. Graph - 1 showed a UV-Vis spectra of AgNP's by the peak representation at 400 - 450 nm.



Graph -2- Identification of functional groups by FT-IR Analysis

Table -1: Identification of active functional group by FT-IR Analysis.

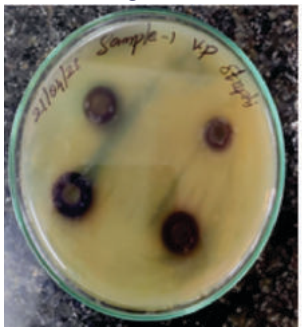
S.NO	Charecteristic Absorbption	Intensity	Functional group
1	3335.2-	Stretching vibration, Medium	Aliphatic primary amine
2	2098.1-	Stretching vibration, Strong	Isothiocyanate
3	1633.4-	Stretching vibration, Medium	Alkene

The FT -IR assay studies reveal that the 3 peak value in different wavelength that indicates different functional groups such as aliphatic primary amine, isothiocyanate, alkene it showed the stretching vibration in strong and medium intensity. The ethanolic extract of polyherbal formulation possess the essential organic functional group for analysis .

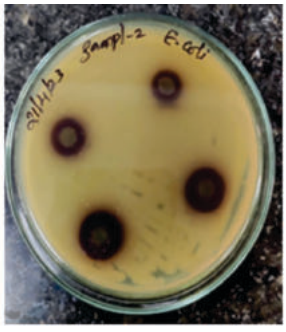
Antibacterial Activity:

s. no	Microorganism	Zone of inhibition(mm)			
		Concentration (ug/ml)			
		40	60	80	100
1	Staphylococcus aureus	3mm	5mm	8mm	10mm
2	Ecoli	4mm	7mm	10mm	12mm

Table 2 : Antibacterial activity of ethanolic extracts of poly herbal formulation against bacteria



Staphylococuss



Ecoli

CONCLUSION :

Synthesis of silvernano particles found in the poly herbal extract of different plant parts of *Cymbopogon citratus, Citrus aurantium* and *Curcuma longa* by UV-Visible spectroscopy at the range between 400 to 500 nm and also evaluated that the identification of specific active functional group found as different characteristic peak values by FT-IR analysis, The antimicrobial assay studies showed that polyherbal formulation has potential antibacterial activity against Ecoli and Staphylococcus aureus The maximum diameter of the inhibition zone was observed in higher concentration of polyherbal formulation based on the dose dependent manner The diameter of the inhibition zone level is 12mm E.coli and 10mm in Staphylococcus aureus by well diffusion method in a maximum concentration in 100 µg .

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The ethanolic extract of polyherbal formulation of ointment has exhibited better antibacterial activity in different bacterial strains due to the presence of secondary metabolites such as flavonoids, alkaloid, tannin, phenol, carbohydrate. The poly herbal extract has possess the silvernano particle and effective active functional group compounds have been evaluated by different techniques. The result revealed that the poly herbal formulation of the plant drug as ointment might be better activity for for anti-inflammatory and wound healing studies for future research in animal model.

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Conflict Of Interest:

None declared.

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