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GREEN SYNTHESIS, CHARACTERIZATION OF SILVER NANOPARTICLE AND ITS EVALUATION OF ANTI-OXIDANT, ANTI-INFLAMMATORY AND ANTI-MICROBIAL ACTIVITY OF POLYHERBAL GEL FROM AQUEOUS EXTRACT OF DIFFERENT PARTS OF *CENTELLA ASIATICA, CURCUMA LONGA, PIPER NIGRUM* AND *CITRUS SINENSIS*

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ABSTRACT

Green synthesis is an important 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 aqueous extract of four different plant parts from *Centella asiatica*, *Piper nigrum*, *Curcuma longa* and *Citrus sinensis*. Characterization of AgNPs was performed using UV–visible spectroscopy and FT-IR analysis. From this experiment that conducted on the polyherbal gel that made from the leaves of *Centella asiatica*, the seed of *Piper nigrum*, the rhizome part of *Curcuma longa* and the peel of *Citrus sinensis* shows a greater pharmacological effect in various aspects. The aqueous extract of each of the plants has the maximum phytochemicals when compared to other solvents such as in ethanol and methanol. The silver nanoparticles were incorporated to

the polyherbal gel and its confirmation was done through UV-VIS and FTIR analysis. The UV-VIS analysis shows a maximum absorbance at 429nm which confirms the synthesis of silver nanoparticles in the gel. The SOD activity in antioxidant assay gives GAE =127.524µg/ml, AAE=312.96µg/ml for the FRAP activity and 17.20% scavenging activity in DPPH assay. through all of these assays it can be confirmed that it has a good antioxidant activity. The anti-inflammatory activity was calculated through the albumin denaturation method and the result obtained was 40.90% which was excellent anti-inflammatory activity. The anti-microbial activity was confirmed through the well-diffusion method which shows it has higher anti-bacterial activity than the disc ampicillin. The disc has a zone of inhibition of 19mm while it as 32 for the polyherbal gel. in the three different strains *E. coli, pseudomonas aeruginosa* and *staphylococcus aureus* the result was same so that it can be confirmed that it has effect against both the gram negative as well as the gram-positive bacteria. The anti-fungal test was done on Aspergillus flavus the result obtained was 12mm zone of inhibition and the control fluconazole had 22mm zone of inhibition. The silver nanoparticle incorporated polyherbal gel had a significant anti-oxidant, anti-inflammatory as well as the anti-microbial activity.

KEYWORDS: Silver Nano Gel, Quercetin, Gallic acid, UV-VISIBLE Spectroscopy, FT-IR Antioxidant, DPPH, SOD, FRAP, Anti Inflammatory – Albumin Denaturation, Antimicrobial Assay- Well diffusion method.

INTRODUCTION

Polyherbal formulations represent a holistic approach in traditional medicine, leveraging the synergistic effects of multiple medicinal plants to enhance therapeutic efficacy. This method combines various bioactive compounds from different sources, potentially improving the overall health benefits compared to single-plant extracts. Each plant in a polyherbal formulation contributes its unique set of phytochemicals, which can act together to provide enhanced medicinal properties, including antimicrobial, anti-inflammatory, and antioxidant effects. This synergy not only maximizes the therapeutic potential of the individual plants but also minimizes the risks of side effects associated with high doses of single herbs.[1]

The medicinal herbs contain a plenty of bioactive compounds in it, which in turn shows a great activity when they are combined. For these combined polyherbal formulation has increasing demand in the market in recent times. Polyherbal formulations leverage synergistic effects, broader therapeutic activity, and improved bioavailability, allowing for reduced

dosages and side effects while being culturally relevant and customizable for individual needs. Their holistic approach not only aligns with traditional practices but also presents opportunities for innovation and cost-effective solutions in modern medicine.[2] One of the important advantages of using poly herbs over the single herbs are their synergically activity and minimum side effects when they are combined.[3] Silver nanoparticles are valued for their antimicrobial properties, making them effective in various applications such as medical devices, wound dressings, and water purification. Additionally, their unique optical and electrical characteristics make them promising in fields like electronics and drug delivery, paving the way for advancements in nanotechnology. So in this present study, green synthesis of silver nanoparticles is aimed to enhance the antioxidant and anti-microbial activity of the aqueous extract of the polyherbal formulation Mechanism using the environmentally friendly, affordable and less time-consuming technique.[4]

The leaves of *Centella asiatica*, the rhizome part of Curc*uma longa*, the seeds of *Piper nigrum* and the peel of *Citrus sinensis*. A polyherbal formulation combining *Centella Asiatica*, *Curcuma longa*, *Piper Nigrum*, and Orange Peel with silver nanoparticles offers potent wound-healing benefits. *Centella asiatica* stimulates collagen production, aiding in tissue repair and faster skin regeneration. Turmeric's curcumin and Piper nigrum's piperine provide powerful anti-inflammatory and antimicrobial effects, reducing infection and inflammation. Orange peel contributes with its rich antioxidant content, promoting skin rejuvenation. Silver nanoparticles enhance antimicrobial properties, preventing bacterial infections and accelerating the healing process, making this formulation highly effective for wound care.

MATERIALS AND METHODS

Collection of plant materials

The four different plant herbs and their parts such as the leaves part of *Centella asiatica*, the rhizome part of *Curcuma longa*, *the* seeds of *Piper nigrum* and the peel part of *Citrus sinensis* were collected from the local area of market surround Coimbatore, Tamil Nadu. The different part of the plants were prepared for different solvent extractions such as Aqueous, Ethanol, and Methanol for sample analysis.

Preparation of plant extract

25 gm of dried plant powder was dissolved in 250 ml of Aqueous, Ethanol, and Methanol for sample analysis. The plant extract was filtered through Whatmann No 1 filter paper and

residue was collected. The filter was concentrated using a rotator vacuum evaporator to get ethanol extract of the dried plant powder.

Soxhlet Extraction

Soxhlet extraction process is ultimately needed where the desired active compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.[5]

Phytochemical screening

Phytochemical screening were performed for observing the secondary metabolites present in the different extracts using procedures described by Trease and Evans.[6] The preliminary qualitative phytochemical studies were performed for testing the different active compounds present in different extracts.

Test for steroids

3ml of test solution and minimum quality of chloroform was added with 3 to 4 drops acetic anhydride and one drop of concentrated H_2SO_4. purple color formed which changes to green colour indicates steroids.

Test for lignin

Few drops 1% potassium permanganate and 3 drops of ammonium chloride was added to few ml of the test solution which was followed by 1 drop ammonium chloride. Brown precipitate indicates the presence of lignin.

Test for tannin

The extract is dissolved in 5 ml of distilled water after that neutral ferric chloride was added, the presence of dark green color indicates the presence of tannin.

Test for protein

To the solution add 2 drops 1% copper sulphate and equal volume of 40% sodium hydroxide. The appearance of violet color indicates the presence of protein.

Test for saponin

Take 1 ml of plant extract in to which 2 ml of distilled water was added and mix well using cyclo mixer. The presence of foam in it confirms the presence of saponin.

Test for phenols

Few ml test solution was taken. Add about 1 ml of water in to the extract. After add 3 drops of ferric chloride. Presence of Blue green or red colour presents.

Test for terpenoids

0.5 ml extract was mixed with 1 ml of chloroform. After that 1ml of sulphuric acid and 1ml of acetic acid was added. Formation of pink or violet colour is the presence of terpenoids.

Preparation of poly herbal formulations

Sample collection and plant extraction

Four different medicinal plants like turmeric, pepper, orange-peel, Centella asiatica were collected from Coimbatore Tamil Nadu India. The collected plant materials where dried under sunlight and was grinded into powder using Mortar and Pestle. 0.5 g of each powdered sample was weight and was dissolved in 10 ml of distilled water. The mixture was kept at shaking incubator for overnight for the release of bioactive constituents. After incubation the plant extract was filtered and was used for the poly herbal formulation.

Preparation of poly herbal gel

For the preparation of gel accurately weighed quantity of gelling agents such as carbapol 950 were dissolved in water, and then carboxymethyl cellulose was added drop by drop with constant stirring till pH was neutralized and gel was formed. Carboxymethyl cellulose was added slowly to the dispersion with continuous stirring until a stiff gel was formed. Then the measured quantity of aqueous extract of 4 different extract were added to gelling agent and mixed with continuous stirring for 30 mins. Polyethylene glycol was added as moistening agent, which was added in required quantities dissolved in water and added slowly with continuous stirring until a homogenous gel was formed, until a semisolid consistency was obtained. The consistency was checked every time to improve the viscosity of the preparation. Volume was made with water and stirred continuously till a uniform gel was obtained.[7]

Table 1: Composition of poly herbal gel.

Ingredients	Gram/ ml
Turmeric extract	2
Centella asiatica extract	2
Orange extract	2
Pepper extract	2

PEG	3
Carboxymethyl cellulose	0.5
Carbopol 950	3

Green synthesis of silver nanoparticle in herbal formulation

1mM of silver nitrate was added to the 4 different medicinal plants extract mixture. Then the mixture was incubated at dark room condition for overnight. As a result, a brown-yellow solution was formed, indicating the formation of silver nanoparticles. The synthesized silver nanoparticles were incorporated into the prepared herbal formulation and further characterization was carried out.[8]

UV visible study

Principle

UV-Vis spectrophotometry operates on the principle that molecules absorb ultraviolet and visible light at specific wavelengths, causing electronic transitions. The intensity of light absorbed by the sample is measured and is proportional to the concentration of the absorbing molecules, following the Beer-Lambert Law. By analyzing the absorbance spectrum, the spectrophotometer helps identify compounds and determine their concentrations, with the characteristic absorption peaks providing valuable information about the molecular structure.[9]

Procedure

The bio-reduction of the Ag+ ions in solutions was monitored by sampling of aliquots (1 mL) of the aqueous component and measuring the UV-Vis spectra of the solution and characteristics surface resonance peaks were noted. UV-VIS spectra of these aliquots were monitored on a Labtronics LT 291 microprocessor UV spectrophotometer in 400–600 nm range operated at a resolution of 10 nm.

FT-IR Analysis

Principle

FT-IR (Fourier transform infrared) is based on the interaction between infrared radiation and matter. When a molecule absorbs infrared light, its bonds vibrate at characteristic frequencies. These vibrations depend on the nature of the bonds (single, double, triple) and the types of atoms involved. By measuring how much light is absorbed at different frequencies, FTIR can generate a unique spectrum that acts as a molecular "fingerprint" for a compound.

Procedure

Fourier transform infrared (FTIR) spectra of the synthesized silver nanoparticles were recorded in the range 4000–450 cm−1 using a shidamzu FTIR spectrometer to identify the functional groups involved in the stabilization of the AgNPs.[10] **Table 8**. zone inhibition of different bacteria.

Antioxidant activity

DPPH Assay

Principle: The DPPH assay is based on the principle of measuring the antioxidant activity of a substance by its ability to scavenge the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). DPPH has a deep violet color in its radical form, which absorbs light at a specific wavelength (usually around 517 nm). When an antioxidant donates an electron or hydrogen atom to DPPH, it neutralizes the radical, causing the solution to change color from violet to yellow or colorless. The decrease in absorbance at 517 nm is measured spectrophotometrically, and the antioxidant activity is expressed as a percentage of scavenging activity. The percentage is calculated using the formula Scavenging Activity (%) = (Control absorbance – Sample absorbance)\Control absorbance* 100

A higher percentage of scavenging activity indicates a stronger antioxidant potential of the tested sample.

Procedure

1 ml of plant herbal formulation was taken in a test tube. To each tube, 0.2mL of 0.1mM DPPH is added and mixed. Then incubated for 5 min. After the incubation, 0.4mL of 50mM Tris HCl was added and following a period of around 30 minin a dark environment, the absorbance was measured at 517nm. The percentage of inhibition was determined using the formula typically employed for assessing anti-inflammatory properties. Ascorbic acid served as positive control. Combination of ethanol and DPPH as the negative control.[11]

Percentage of scavenging activity= Ac- As/ Ac× 100

Superoxide Dismutase (SOD) Assay

Principle

The SOD (Superoxide Dismutase) assay evaluates antioxidant activity based on the enzyme's ability to catalyze the dismutation of the superoxide radical (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2). In the assay, a superoxide-generating system (such as xanthine

and xanthine oxidase) produces superoxide radicals, which react with a detection reagent like nitroblue tetrazolium (NBT), forming a blue-colored compound. The presence of antioxidants (including SOD or other antioxidant compounds) inhibits this reaction by scavenging the superoxide radicals, reducing the blue color intensity.

The absorbance of the reaction mixture is measured, and the inhibition of the color formation is proportional to the antioxidant capacity of the sample. When the antioxidant activity is evaluated in terms of gallic acid equivalence, the sample's scavenging ability is compared to that of gallic acid, a standard antioxidant. The results are expressed as the equivalent amount of gallic acid (GAE), calculated from a standard curve created using gallic acid at known concentrations.

The stronger the antioxidant activity, the greater the inhibition of the blue color, and the results are reported in terms of the equivalent concentration of gallic acid that would provide the same level of antioxidant protection.

- SOD reaction mixture 2.
- Test sample.

Procedure

1ml of SOD reaction mixture I was taken in test tubes Containing sample The reaction mixture was prepared by adding 1 ml of 50m phosphate buffer solution, 0.075 ml of EDTA, 20M L-Methionine and 0.04 ml of 10mM Hydroxyamide hydrochloride. Then the mixture was kept at pre-incubation at 37°C for 10 mins. 50µl of 50mM Riboflavin was later added. The tubes were mixed well and exposed for 5 mins under UV fluorescent light. After the exposure time, 1 ml of SOD reaction mixtures II was added and the absorbance of the colour formed was read at 543nm. SOD reaction mixtures 2 was freshly prepared by mixing 1% sulphanilamide in 5% phosphoric acid. Gallic acid was used as the standard. The SOD activity was expressed as µg/ml.[12]

FRAP Assay

Principle

The FRAP (Ferric Reducing Antioxidant Power) assay is based on the principle that antioxidants can reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) under acidic conditions. In the FRAP assay, the reduction of ferric-tripyridyltriazine (Fe^{3+} -TPTZ) complex to the

ferrous form (Fe^{2+} -TPTZ) results in the formation of an intense blue color, which absorbs light at 593 nm.

The antioxidant compounds in the sample donate electrons to reduce the ferric ions to ferrous ions, and the increase in absorbance is proportional to the antioxidant capacity of the sample.

When the result is expressed in terms of ascorbic acid equivalence, the antioxidant activity of the sample is compared to that of ascorbic acid (vitamin C), a standard antioxidant. The antioxidant power of the sample is quantified by creating a standard curve using known concentrations of ascorbic acid, and the results are expressed as the equivalent concentration of ascorbic acid that would provide the same reducing power as the sample.

The higher the FRAP value, the stronger the antioxidant activity.

Procedure

FRAP assay were carried out by the plant extracts in respective solvent were mixed with phosphate buffer (1 ml) and 1% ferric cyanide (1 ml). This mixture was kept at 50°C in water bath for 20 min. After cooling, 2.5 ml of 10% trichloro acetic acid was added and was mixed with distilled water (2ml) and a freshly prepared 0.1% ferric chloride solution (0.25ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. The reducing power was estimated by using ascorbic acid standard.[13]

Anti-inflammatory activity

Principle

The albumin denaturation method for analyzing anti-inflammatory activity is based on the principle that inflammatory agents cause the denaturation (unfolding) of proteins, like albumin, which can be prevented or reduced by anti-inflammatory substances. In this method, albumin is exposed to heat or chemicals that induce denaturation, and the test sample (suspected to have anti-inflammatory properties) is added. If the sample prevents or reduces the denaturation, it demonstrates anti-inflammatory activity. The extent of inhibition is measured spectrophotometrically, with a higher percentage of inhibition indicating stronger anti-inflammatory potential.

Procedure

The albumin denaturation method was performed to analyse the anti-inflammatory property of plant extracts. 1ml of herbal formulations extracts were taken in test tubes. To the tubes, 3

mL of PBS and 1mL of egg albumin are added and mixed and incubated at 37°Cfor 20min. Denaturation was induced by increasing the temperature to 90°C for 2-3 min. Absorbance was taken at 660nm after cooling down the mixture. As a positive control, diclofenac was utilized, while distilled water serves as the negative control.[14] The percentage of denaturation inhibition is calculated by.

Ac- As/ Ac× 100

Antimicrobial activity by agar well diffusion method

Principle

The agar well diffusion method for analysing antimicrobial activity is based on the principle that when a substance with potential antimicrobial properties is introduced into a well cut in an agar plate inoculated with microorganisms, it diffuses radially through the agar. As the substance spreads, it inhibits microbial growth in the area around the well. The effectiveness of the antimicrobial agent is indicated by the zone of inhibition, a clear area around the well where bacterial growth has been prevented. The larger the zone, the more potent the antimicrobial activity of the tested substance.

Antibacterial activity

The antibacterial activity of the poly herbal formulation and biosynthesized -AgNPs was determined using the disc diffusion method. A bacterial inoculum suspension was spread uniformly on solidified Muller–Hinton Agar (MHA) using a sterile swab. The bacterial strains used in this study were Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginisa* and *E. coli*. A fixed volume of about 25 µLeach of the herbal formulation, silver nanoparticle incorporated herbal formulation was added into different wells made with cork borer and placed in Petri plates for incubation at 37 °C for 24 h. Antimicrobial activity of ethanolic extract of polyherbal formulation from *Cymbopogan citratus, Citrus aurantium* and *Curcuma longa* have possessed the antibacterial activity against gram positive and negative bacteria.[15]

Antifungal Activity

For the antifungal study malt extract agar was prepared (39gm in 1000ml of distilled water, followed by autoclaving) after the addition of antibiotic for the prevention of bacterial growth poured to sterilized petri plate. After solidification 80 µl of fungal culture of *A.flavus* was added and spread, wells were made using a sterile cork borer (6 mm in diameter) and the samples were added to the respective wells, then the plates were incubated 3-5 days at 300C.

5 µl of Fluconazole was used as a standard (10mg/ml), After incubation zone of inhibition was measured in mm.[16]

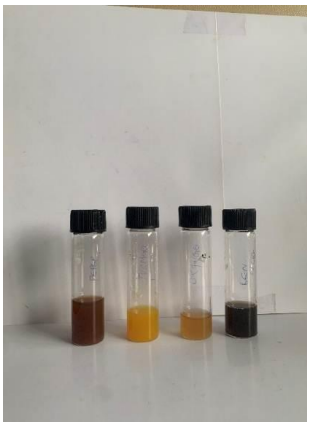
RESULTS AND DISCUSSION

Table 2: Phytochemical analysis.

SL. No		*Centella asiatica*			*Curcuma longa*		
	Phytocompounds	**Aqueous**	**Ethanol**	**Methanol**	**Aqueous**	**Ethanol**	**Methanol**
1	Alkaloids	++	++	++	++	++	-
2	Flavonoids	++	-	-	++	-	-
3	Terpenoids	++	++	-	++	-	-
4	Saponins	++	++	++	++	++	++
5	Tannins	-	-	++	-	-	-
6	Lignin	-	-	-	++	-	-
7	Steroids	-	++	-	-	-	-
8	Glycosides	++	-	-	++	-	-
9	Phenols	++	-	++	++	++	-
10	Proteins	-	-	-	-	++	-

Table 3: Phytochemical analysis.

		Piper nigrum			*Citrus sinensis*		
SL NO	**Phytocompounds**	**Aqueous**	**Ethanol**	**Methanol**	**Aqueous**	**Ethanol**	**Methanol**
1	Alkaloids	++	++	++	++	++	-
2	Flavonoids	++	-	-	++	++	-
3	Terpenoids	-	++	-	++	-	-
4	Saponins	++	++	++	++	++	++
5	Tannins	-	++	-	-	++	-
6	Lignin	++	-	-	-	-	-
7	Steroids	-	++	-	-	-	-
8	Glycosides	++	-	-	++	-	-
9	Phenols	++	++	++	++	++	++
10	Proteins	++	-	-	-	-	-

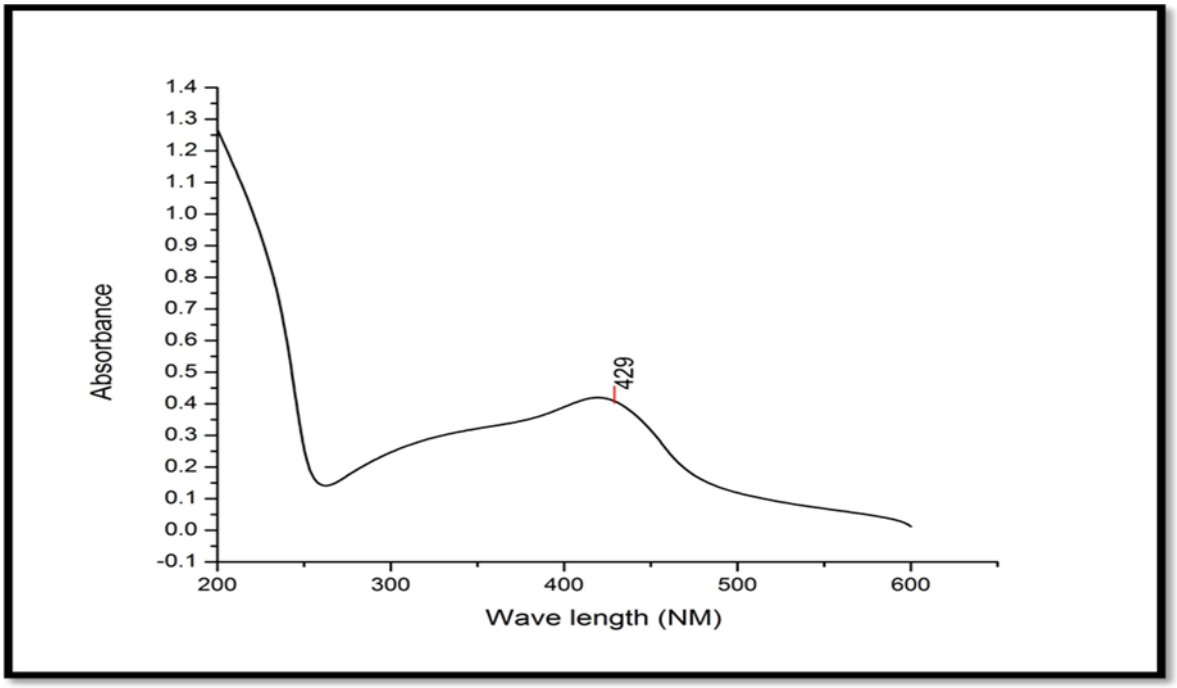


Plant extract

Poly Herbal Gel

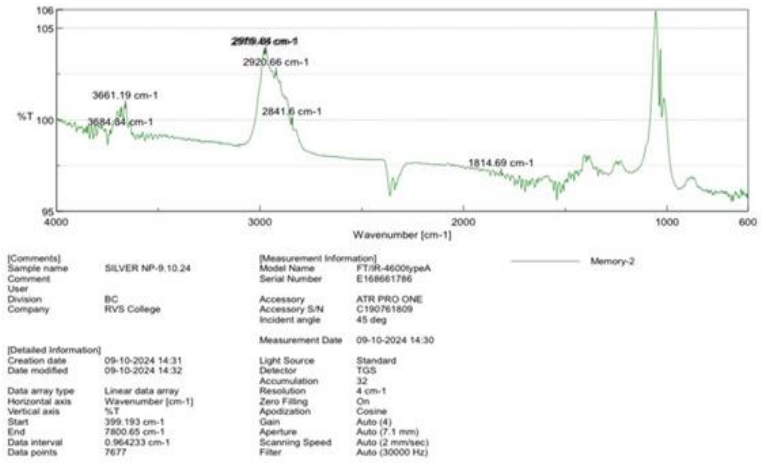
Green synthesis of silver nanoparticles

Characterization of silver nanoparticle



Graph -1: Uv-Visible Spectroscopy Study.

The formation of silver nanoparticles in the formulation is confirmed through the absorption at the UV-VISIBLE Spectroscopic study, they show a peak absorbance at a range from 400-450nm. Here in the study it was shown that the sample has peak absorbance at 429nm which confirms the formation of silver nanoparticles.



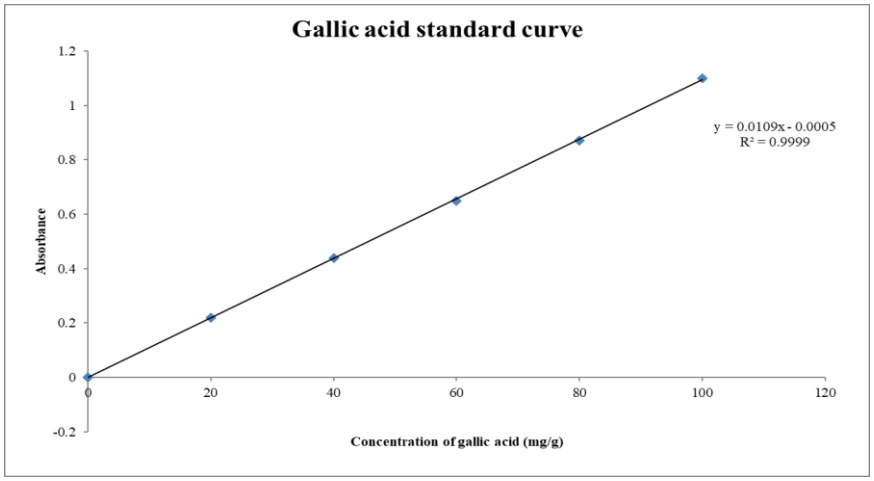
Graph- 2: FTIR analysis.

S NO	CHARACTERISTIC ABSORPTION	INTENSITY	ASSIGNMENT	FUNCTIONAL GROUPS
1.	3684.34 cm^{-1} 3661.19 cm^{-1}	Stretching vibration, strong	O-H stretching	alcohols or phenols
2.	2979.48 cm^{-1} 2969.84 cm^{-1}	Stretching vibration, strong	C-H stretching	alkanes, hydrocarbon chains
3.	2920.66 cm^{-1} 2841.6 cm^{-1}	Stretching vibration, strong	C-H stretching band	aliphatic compounds
4.	1814.69 cm^{-1}	Stretching vibration, strong	C=O stretching	esters, ketones, o carboxylic acids

The presence of these peaks suggests organic functional groups commonly used in the synthesis or stabilization of silver nanoparticles. The O-H and C-H stretches indicate the possible use of natural or polymeric stabilizers, while the C=O stretch might relate to carbonyl-containing compounds used in the process.

Anti-oxidant activity

SOD Activity



Graph -3: SOD Enzyme Activity.

gallic acid standard curve

The OD of test = 1.39

From the equation from the graph

The concentration of gallic acid in the sample =

Y= 1.39

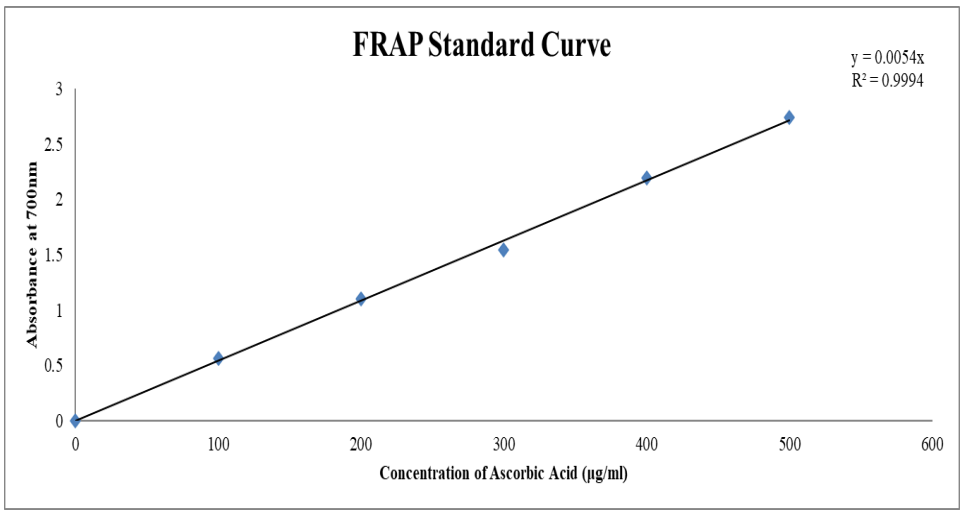
M=0. 0109

C=0.0005

X=127.524

GAE =127.524µg/ml

FRAP activity



Graph – 4: FRAP Assay.

OD of test = 1.69

The reducing power was estimated using ascorbic acid standard

From equation of the graph

Y=1.69

M=0.0054

X=312.96

AAE=312.96µg/ml

DPPH Assay

% of scavenging activity/ anti-oxidant activity

(ac-at)/ac*100

absorbance of control (ac)-0.93

absorbance of test (at)- 0.77 % of scavenging activity = 17.20%

The anti-oxidant study of the polyherbal formulation indicates that the polyherbal formulation show a greater SOD and FRAP activity while in DPPH it is less when compared to others. over all the polyherbal formulation shows a high anti-oxidant activity , and this property is due to the presence of the bioactive components such as phenols and glycosides in it.

Anti-inflammatory activity

Table 4: OD of test and control.

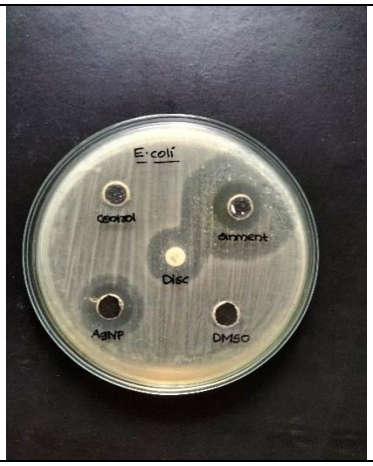
Components	OD @ 660nm
Blank	0.0
Control	0.33
Sample	0.195

The percentage of denaturation inhibition is calculated by:(absorbance of control-absorbance of test)/absorbance of control*100 From the equation given, the percentage of denaturation inhibition of the gel is 40.90%. The anti-inflammatory assay of the polyherbal formulation indicates that it has a strong anti-inflammatory property. This activity of the formulation is due to the presence of various bioactive components such as phenols, alkaloids, flavonoids etc.

Anti-microbial activity

Anti-bacterial activity

Activity against S.aureus



Activity against E.coli

Activity against P. Aeruginos

Zone of inhibition

Table 5: – Zone of Inhibition against different bacterial strains.

Bacteria	Gel	AgNP	Disc
Pseudomonas aeruginisa	32mm	16mm	19mm
Staphylococcus aureus	33mm	17mm	16mm
E. coli	31mm	19mm	17mm

The anti-microbial assay studies show that the polyherbal formulation has a potential anti-bacterial activity against *E. coli*, *pseudomonas aeruginosa* and *staphylococcus aureus*. The diameter of the zone of inhibition was 31mm, 32mm and 33mm respectively, while for the Disc it was 17, 19 and 16 mm.

The studies that conducted on the different strain indicates that it has anti-bacterial effect because of the presence of the bioactive components such as alkaloids, flavonoids, tannins, saponins etc.

Anti-fungal activity

Anti-fungal activity against *Aspargilus flavus*.

The positive control fluconazole shows zone of 22mm and the gel shows a 12mm which confirms that it has anti-fungal activity.

SUMMARY AND CONCLUSIONS

The green synthesis of silver nanoparticles (AgNPs) using a polyherbal formulation has demonstrated promising biological activities, particularly in anti-inflammatory, antioxidant, and antimicrobial assays. The anti-inflammatory activity of the formulation, as determined by the inhibition assay, showed a 40% inhibition, indicating a moderate potential for reducing inflammation. The antioxidant properties, evaluated using the Superoxide Dismutase (SOD) assay, revealed a significant activity, with 127 µg/mL in terms of Gallic Acid Equivalent (GAE), and the Ferric Reducing Antioxidant Power (FRAP) assay showed 312 µg/mL in terms of Ascorbic Acid Equivalent (AAE). These results highlight the formulation's robust ability to counteract oxidative stress. The DPPH scavenging activity further supported this with a 17.20% free radical inhibition, although it was comparatively lower than other assays. In anti-microbial assay the gel shows a 12mm zone inhibition in anti-fungal test, fluconazole was taken as the positive control where as it shows a 22mm zone inhibition. In anti-bacterial assay there shows the zone from 32mm, 33mm and 31mm *for pseudomonas aeruginosa*, *staphylococcus aureus* and *E. coli* respectively.

The green-synthesized silver nanoparticles in this polyherbal formulation exhibit moderate anti-inflammatory, strong antioxidant, and potential antimicrobial activities, making it a valuable candidate for further investigation in therapeutic applications. These findings emphasize the efficiency of green synthesis approaches in nanotechnology and their potential for developing eco-friendly biomedical formulations.

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CONFLICT OF INTEREST

None declared.

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