Development and Evaluation of GEL Incorporated with Synthesized Silver Nanoparticle from Aquilaria Malaccensis for the Treatment of Acne Vulgaris

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ABSTRACT

Acne vulgaris is one among the foremost prevalent skin diseases which affect almost 80% of adolescents within the world during their lifetime. Antibiotic resistance will Develop when we take the antibiotic during repeated treatment. Ancient time onwardsplants are utilised as medicine. Treatment of acne has been considered as a serious research area in pharmaceutical and private cosmetic care industries. The aim of this work was to guage the phytochemical composition of Aquilaria malaccensis , green synthesis of silver nanoparticle and to develop herbal topical gel formulation to treat acne. Aquilaria malaccensis is chosen supported its antibacterial activity. Phytochemical analysis revealed phytoconstituents like alkaloids, flavonoids, tannins and saponins are present within the extract. Silver nanoparticle was synthesized using 1 mM aqueous nitrate from the extracts of Aquilaria malaccensis and formation of silver nanoparticle was confirmed by UV spectroscopy and Functional groups are identified by FTIR analysis. Synthesized silver nanoparticles was incorporated into gel base and evaluated for its physical properties like pH, viscosity, spreadability and antibacterial activity against Propionibacterium acne, Staphylococcus aureus and Escherichia coli.The preparedformulation of this study showing no lumps, had uniform color dispersion and were free from any fibre and particle. it found that the formulation is easy to wash, better spreadability, pH was found to be 6.72 and 6.80 almost like pH of the skin. The developed formulation showed good antibacterial activity against Propionibacterium acne, Staphylococcus aureus and Escherichia coli. Synthesized silver nanoparticle of Aquilaria malaccensis showed higher activity than extract. Hence, silver nanoparticle of Aquilaria malaccensis in aqueous gel-base are often used as an appropriate formulation for treatment of acne.

Keywords: Acne vulgaris,Silver nanoparticle, antibacterial study.

INTRODUCTION:

Acne vulgaris is one among the foremost prevalent skin diseases which affect the young adults within the age bracket between 11 and 30 years. Continuous therapeutic application of antibiotic can develop an ineffectiveness of traditional antibiotic leading to resistance consequently resulting in incompetent effect against the actual disease. Thanks to the event of antibiotic opposition and therefore the availability of the massive number of medicinal plants, made the scientist to focus the scientific exploration to develop and identify an novelnatural antimicrobial drugs for the treatment. Antimicrobial nanoparticle in topical formulation is taken into account effective for treating acne. Among the varied metallic nanoparticles, silver has been considered as best one against

bacteria and virus1-3. The plant Aquilaria malaccensis possesses several phytoconstituents and having potential antibacterial activity against human pathogens. Taking into consideration of the value and straightforward availability of this medicinal plant, our present study was designed to prepareplant extract mediated nanoparticle followed by formulation and evaluation of topical gel and revealing its antibacterial activity.

MATERIALS AND METHODS:

Plant Materials:

The leaves of plant of *Aquilaria malaccensis* were collected from Tirunelveli district, Tamilnadu. It was identified and authenticated by V. Chelladurai, Research officer – Botany, (Retired) Central council for research in Ayurveda & Siddha. The healthy leaves were shade dried and powdered using electric blender to get a coarse powder.

Collection, authentication and preparation of extract; *The plant was collected from the surrounding areas of Bangalore and authenticated.Authenticated plant material was powdered and extracted with ethanol by hot continuous extraction followed by rotary evaporation.*

Phytochemical Analysis:

Ethanol extract was analyzed for its phytoconstituents such as saponins, anthraquinone glycosides, phyto steroids, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids.

Synthesis of Nanoparticle:

Silver Nanoparticle:

5 ml of leaf extract was mixed with 95 ml of 1 mM aqueous silver nitrate solution which is maintained at room temperature for 24 h in the dark. Silver nanoparticles was formed by reduction of pure silver ions and it will be monitored by measuring absorption of the reaction medium in the wavelength range of 300-700 nm using UV spectrophotometry. Then, the synthesized AgNPs were centrifuged at 10000 rpm for 15 min. The supernatant was allowed to settle the particles which is filtered,dried, purified and characterized the AgNPs.

Characterisation of Nanoparticle:

The formation of silver using plant extract is monitored by various analytical techniques like UV–Visible Spectroscopy UV–Vis,Fourier-Transform Infrared Spectroscopy FT-IR.

Preparation of Topical Formulation:

Formulation of Topical gel was carried out by cold mechanical method using of carbopol-934. Carbopol 934 (polymer) 2gm was weighed separately and sprinkled slowly on surface of purified water. with vigorous stirring, distilled water was added and left overnight for dissolving the polymer. To the polymer solution, drug silver nanoparticles were added to the gel with continuous stirring. glycerol was added in required quantity and using magnetic stirrer it was mixed well. After proper dispersion, Sodium hydroxidewas added to adjust the gel neutral pH 7. With Distilled water, theformulation was made up to 100 g. The composition used in this study is tabulated in (Table 2).

Physicochemical Evaluation of Formulations:

Physical evaluation: Physical parameters such as color, appearance and consistency was Checked visually.
pH: Aqueous solution (1%) of the formulation was measured by using a calibrated digital pHmeter at constant temperature.
Viscosity: Brookfield Viscometer with spindle #C 50-1 is used to measure the viscosity of the formulated topical gel at a speed of 50 rpm in room temperature. The results were done in triplicate.
Spreadability: standard dimension (length of 6.0 cm) Glass slides areused where on the one side, the Topical gel formulation was placed sandwiched with the help of another slide. Excess gel on the outer surface of the glass slidesis removed by wiping. Slides are fixed in a stand that only upper slide to slip off freely without any disturbance by force of weight (20 g) tied to it. Time taken for the movement of upper slide to the distance of 6.0 cm will be measured. Measurement of spreadability will be done in triplicate and calculated by using the following formula:
Spreadability = (Weight×Length)/Time
Where, S=Spreadability

m=Weight of upper slide (20 g)
l=Length of the glass (6.0 cm)
t=Time taken in seconds

Preparation of inoculum:
For evaluation of antibacterial activity, 24 h fresh culture of bacteria such as *Escherichia coli (E.coli), Staphylococcus aureus* (*S. aureus*) and *Propionibacterium acnes (P.acnes).*is suspended in sterile water to obtain a uniform suspension of microorganism.

Determination of zone of inhibition:
agar well diffusion method was used to check the Antibacterial activity. In this method a previously liquefied medium will be inoculated with 0.1 mL Bacterial suspension having a uniform turbidity at temperature of 40°C. 20 mL of culture medium was poured into a sterile petri dish having an internal diameter of 8.5 cm. Care to be taken to produce a uniform thicknessof the medium in different plates. Wells aremade aseptically with cork borer having 6 mmdiameter after complete solidification of liquefiedinoculated medium. In each of these plate extract, silver and topical gel formulationwas placed carefully. Plates were kept for pre diffusion for 30 min at room temperature; thenthe plates were incubated at 37°C for 24 h and the zones of inhibition were measured.

RESULTS AND DISCUSSION:

Preparation of Extract:
The leaves of *Aquilaria malaccensis* were washed in water to remove the dust and foreign material from the surface then air dried under shade at room temperature. The air-dried plant material was coarse powdered and extracted by various solvents like Pet.ether, Chloroform,ethanol by hot continuous extraction and water by maceration followed by rotary evaporation. The various above extracts are subjected to preliminary phytochemical screening.

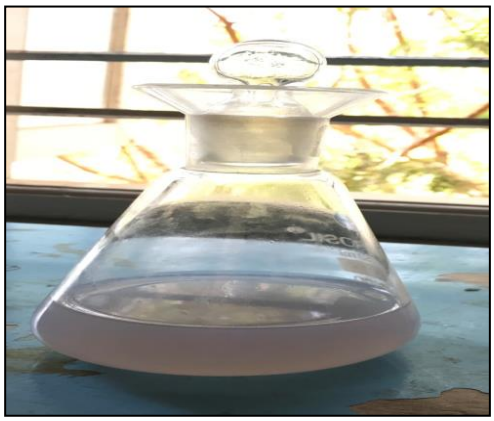
Phytochemical Analysis:
Plant extract are analysed for its phytoconstituents such as saponins, anthraquinone glycosides, phyto steroids, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids. (Table 1)

Table 1: Preliminary phytochemical screening of dried powdered material and extracts of the leaves of Aquilaria malaccensis

S.No	Chemical Constituents	Powder	PE	$CHCl_3$	Ethanol	Water
1.	Carbohydrates	+	-	-	+	+
2.	Alkaloids	+	_	+	+	+
3.	Steroids	+	+	+	-	-
4.	Glycosides	+	-	-	+	+
5.	Saponins	+	+	-	+	+
6.	Flavanoids	+	-	+	+	+
7.	Tannins	+	-	+	+	+
8.	Phenolic Compounds	+	_	+	+	+
9.	Proteins	+	-	+	+	+
10.	Amino acids	+	-	+	+	+
11.	Gums & Mucilage	-	-	-	-	-
12.	Terpenoids	+	+	+	+	+

Synthesis of Nanoparticle:

Synthesis of Silver Nanoparticle:
In the single step green synthesis, 5 ml of Ethanolic leaf extract was added to 95 ml of 1 mM aqueous silver nitrate solution and kept in the dark place at room temperature for 24 h. A change in the solution color from pale yellow to dark brown was observed which indicates the reduction of silver ions and formation of silver nanoparticle.[3] Formation of silver nanoparticle is shown in (Figure 1).



1mM aqueous silver nitrate solution

Silver Nanoparticle

Figure 1: Formation of Silver nanoparticle

Characterisation of Nanoparticle:

UV-Visible:

The UV absorption spectrum of silver nanoparticles has shown a peak specific in the range between 400 and 450 nm. The UV spectrum of silver nanoparticle is shown in (Figure 2).

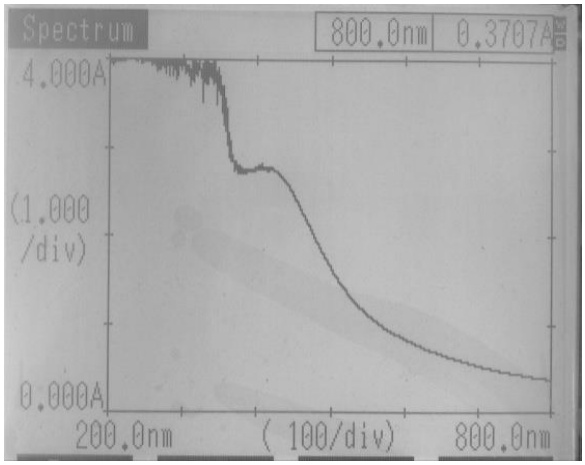


Figure 3: UV-Vis spectra for AgNPs

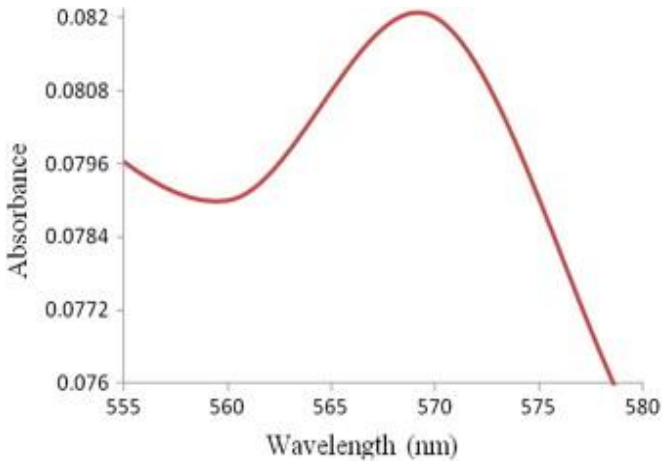


Figure 4: UV-Vis spectra for CuNPs

FTIR:

The representative ATR FTIR spectra of the ethanolic leaf extract of *Aquilaria malaccensis* and the stabilized silver nanoparticles and copper nanoparticles were shown in Figure 4, and 5. It can be seen that, in contrast to the ethanolic extract of *Aquilaria malaccensis* , the stabilized silver nanoparticles show significant changes in their respective vibrational spectra. The ethanolic leaf extract of *Aquilaria malaccensis* showed intense peaks at 3270.30 cm^{-1} and 1633.01 cm^{-1}(Figure 4). In stabilized silver nanoparticles the strong bands were observed at 3308.81 cm-1, 1637.29 cm^{-1}, 1437.62 cm^{-1}, 1312.11 cm^{-1}, 1140.97 cm^{-1}, 986.94 cm^{-1}, 901.37 cm^{-1} (Figure 5).broad and blend peaks were observed In the Plant extract, but after encapsulation of nanoparticles the peak was narrow and sharper. The absorption peak at 3270.30 cm^{-1} observed in control extract,is due to OH stretching vibration, 1633.01 cm^{-1} is due to C=O stretching, which indicates that the control extract may have the phenolic substances. These structural changes indicated that the reduction and stabilization of silver nanoparticles proceed via the coordination between the phenolic substances of the plant extracts with the silver ions. The FTIR studies have confirmed the fact that the Phenolic group has the stronger ability to bind metal indicating that the phenolic constituents could possibly form a layer covering the metal nanoparticles (i.e., capping of Silvernanoparticles) to prevent agglomeration and thereby stabilize the medium.

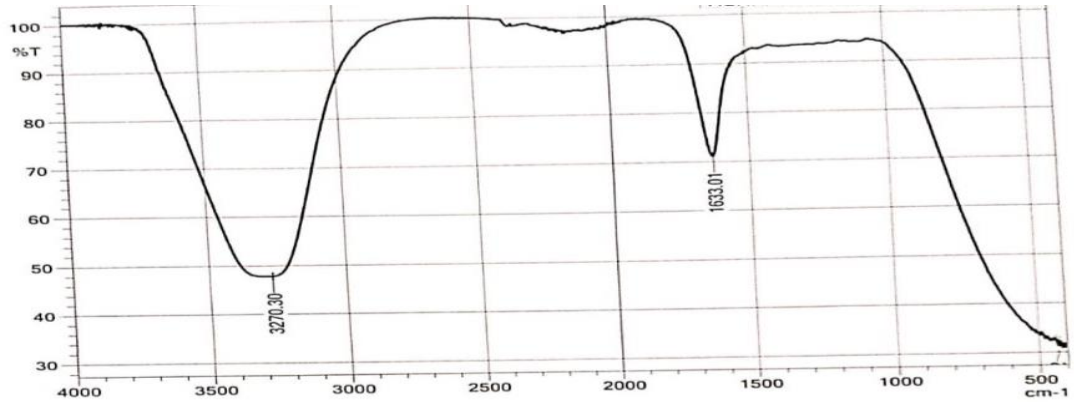


Figure 4: FTIR Spectra of ethanolic leaf extract of *Aquilaria malaccensis*

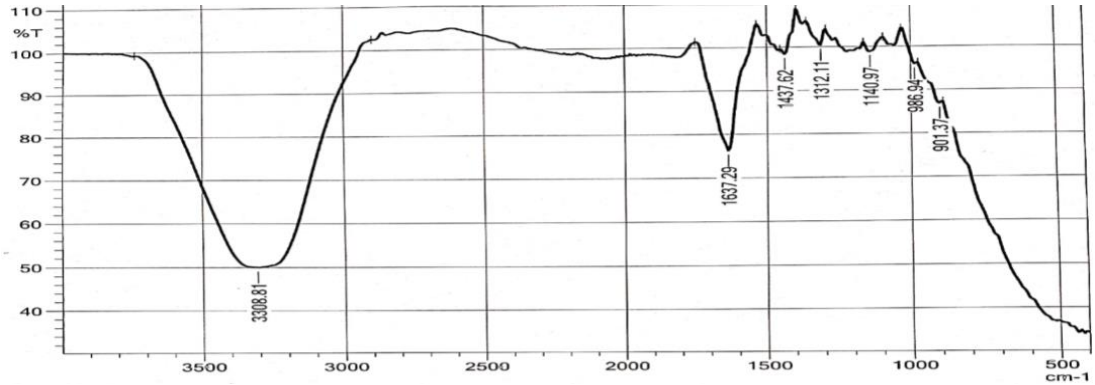


Figure 5: FTIR spectra of synthesized AgNPs synthesized

Preparation of Topical Formulation:

Formulation of Topical gel was carried out by cold mechanical method using of carbopol-934. Carbopol 934 (polymer) 2gm was weighed separately and sprinkled slowly on surface of purified water. with vigorous stirring, distilled water was added and left overnight for dissolving the polymer. To the polymer solution, drug silver nanoparticles were added to the gel with continuous stirring. glycerol was added in required quantity and using magnetic stirrer it was mixed well. After proper dispersion, Sodium hydroxide was added to adjust the gel neutral pH 7. With Distilled water, the formulation was made up to 100 g. The composition used in this study is tabulated in (Table 2).

Table 2: Composition of the formulation

S.No	Components Gel A (using Silver Nanoparticle)
1	Carbopol 2 g
2	Glycerin 2 g
3	Silver nanoparticle 0.02 g
4	Water upto 100 g

Physicochemical Evaluation of Formulations:

Physicochemical parameters such as homogeneity of color, presence of any foreign particle and fibers, washing ability, pH and viscosity are evaluated. prepared topical gel formulation has uniform color distribution and free from any lumps, fibres and foreign particles. Formulation was easily washable and the pH was found to be 6.49 and 6.51 for gel prepared by Silver Nanoparticle as gel base which is near to the pH of the skin and hence is found to be compatible with skin. Viscosity was found to be 6640 cps and 6842cps for gel prepared by silver and Copper Nanoparticle.

Antibacterial Activity of the Formulation:

The antibacterial activity study results of the formulated herbal gel showed antibacterial activity against acne causing bacteria such as *Escherichia coli (E.coli), Staphylococcus aureus (S. aureus) and Propionibacterium acnes (P. acnes)*. The antibacterial study reveals that the silver nanoparticle showed higher activity than *Aquilaria*

malaccensis Plant extract against all the pathogens. The antibacterial activity of the study results is shown in (Figures 8a-8c).

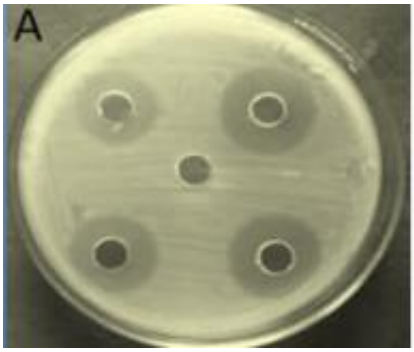


Figure 8a. Antibacterial activity against E. coli

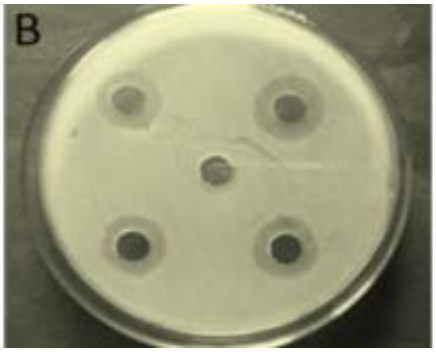


Figure 8b. Antibacterial activity against S. aureus

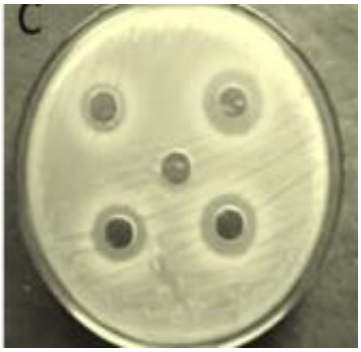


Figure 8c. Antibacterial activity against P. acne

CONCLUSION:

Concerning the environmental protection, green synthesis of nanoparticle has gained friendly and growing demand. Among the different metal nanoparticle, AgNPs has an excellent antibacterial agent due to its non-toxic effect on the human cells. from ancient time Medicinal plants are widely used as a home remedybecause ofdifferent metabolites and its chemicalconstituents. These phytoconstituents and metabolites can reduce the silver ions and assist synthesize of AgNPs from plant extracts. The present study reveals a simple, rapid and economical method to synthesize AgNPs silver nanoparticle from *Aquilaria malaccensis* . From the results, it was found that the synthesized AgNP silver nanoparticle using *Aquilaria malaccensis* leaves extract showed higher activity than the extract. AgNP of *Aquilaria malaccensis* in a gel base can be used as an appropriate formulation for the treatment of acne vulgaris.

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