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DEVELOPMENT AND EVALUATION OF GEL INCORPORATED WITH SYNTHESIZED SILVER AND COPPER NANOPARTICLE FROM *ECLIPTA ALBA* 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 onwards plants 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 *Eclipta alba,* green synthesis of silver nanoparticle and to develop herbal topical gel formulation to treat acne. *Eclipta alba* is chosen supported its antibacterial activity. Phytochemical analysis revealed phytoconstituents like alkaloids, flavonoids, tannins and saponins are present within the extract. Silver nanoparticle and copper nanoparticle was synthesized using 1mM aqueous nitrate solution and 1mM aqueous copper sulfate solution from the extracts of *Eclipta alba* and formation of silver nanoparticle and copper nanoparticle was confirmed by UV spectroscopy and Functional groups are identified by FTIR analysis. Synthesized silver nanoparticles and copper nanoparticle 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 prepared formulation 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 *Eclipta alba* showed higher activity than copper nanoparticle and extract. Hence, silver nanoparticle and copper nanoparticle of *Eclipta alba* in aqueous gel-base are often used as an appropriate formulation for treatment of acne.

KEYWORDS

Acne vulgaris, Silver nanoparticle, Copper nanoparticle and Antibacterial study.

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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 novel natural antimicrobial drugs for the treatment. Antimicrobial nanoparticle in topical formulation is taken into account effective for treating acne. Among the varied metallic nanoparticles, silver and Copper nanoparticle has been considered as best one against bacteria and virus[1-3]. The plant *Eclipta alba* 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 prepare plant extract mediated nanoparticle followed by formulation and evaluation of topical gel and revealing its antibacterial activity.

MATERIAL AND METHODS

Plant Materials

The leaves of plant of *Eclipta alba* were collected from Tirunelveli district, Tamilnadu. It was identified and authenticated by V. Chelladurai, Research officer - Botany, (Retired) Central council for research in Ayurveda and 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[4,5]

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

5ml of leaf extract was mixed with 95ml of 1mM 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 300-700nm using UV spectrophotometry. Then, the synthesized AgNPs were centrifuged at 10000rpm for 15 min. The supernatant was allowed to settle the particles which is filtered, dried, purified and characterized the AgNPs[3].

Copper Nanoparticle

For the Cu nanoparticles synthesis, 1ml of *Eclipta alba* leaf extract was added to 100ml of 1mM aqueous $CuSO_4.5H_2O$ solution in a 250ml Erlenmeyer flask. Then it was maintained at room temperature overnight. The solution containing copper NPs was purified by repeated centrifugation at 12,000rpm for 15min followed by washed with water. Then the Cu nanoparticles were dried in oven at 80ºC[6].

Characterisation of Nanoparticle[3]

The formation of silver and Coppeer nanoparticles 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[7-9]

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 as well as copper 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 100g. The composition used in this study is tabulated in (Table No.2).

Physicochemical Evaluation of Formulations[7-9]

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 pH meter 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 50rpm in room temperature. The results were done in triplicate.

Spreadability

Standard dimension (length of 6.0cm) Glass slides are used 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 slides is 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 spread ability will be done in triplicate and calculated by using the following formula:

Spreadability = (Weight × Length)/Time

Where, S=Spreadability

m=Weight of upper slide (20g)

l=Length of the glass (6.0cm)

t=Time taken in seconds

Preparation of inoculums

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[10]

Agar well diffusion method was used to check the Antibacterial activity. In this method a previously liquefied medium will be inoculated with 0.1mL Bacterial suspension having a uniform turbidity at temperature of 40°C. 20mL of culture medium was poured into a sterile petridish having an internal diameter of 8.5cm. Care to be taken to produce a uniform thickness of the medium in different plates. Wells are made aseptically with cork borer having 6mmdiameter after complete solidification of liquefied inoculated medium. In each of these plate extract, silver and copper nanoparticle and topical gel formulation was placed carefully. Plates were kept for pre diffusion for 30 min at room temperature; then the 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 *Eclipta alba* 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[4,5]

Plant extract are analysed for its phytoconstituents such as saponins, anthraquinone glycosides, phyto steroids, tannins, flavonoids, carbohydrates, triterpenoids, polyphenol and alkaloids. (Table No.1).

Synthesis of Nanoparticle

Synthesis of Silver Nanoparticle

In the single step green synthesis, 5ml of Ethanolic leaf extract was added to 95ml of 1mM 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 No.1).

Copper Nanoparticle

For the Cu nanoparticles synthesis, 5ml of 5ml of Ethanolic leaf extract was added to 95ml of 1mM aqueous $CuSO_4.5H_2O$ solution in a 250ml Erlenmeyer flask. The flask then kept overnight at

room temperature. On mixing plant extract with the $CuSO_4.5H_2O$ solution, a change in the solution color from blue to light brownish was observed which indicates the reduction of copper ions and formation of copper nanoparticle[6]. Formation of copper nanoparticle is shown in (Figure No.2).

Characterisation of Nanoparticle[3]

UV-Visible

The UV absorption spectrum of silver nanoparticles has shown a peak specific in the range between 400 and 450nm. The UV spectrum of silver nanoparticle is shown in (Figure No.3). The UV absorption spectrum of copper nanoparticles has shown a peak specific in the range between 572-582nm. Peak specific in this region might be due to Mie scattering effect24. The UV spectrum of Copper nanoparticle is shown in (Figure No.4).

FTIR

The representative ATR FTIR spectra of the ethanolic leaf extract of *Eclipta alba* and the stabilized silver nanoparticles and copper nanoparticles were shown in Figure No.5, Figure No.6 and Figure No.7. It can be seen that, in contrast to the ethanolic extract of *Eclipta alba*, the stabilized silver nanoparticles show significant changes in their respective vibrational spectra. The ethanolic leaf extract of *Eclipta alba* showed intense peaks at 3270.30cm^{-1} and 1633.01cm^{-1} (Figure No.5). In stabilized silver nanoparticles the strong bands were observed at 3308.81cm-1, 1637.29cm^{-1}, 1437.62cm^{-1}, 1312.11cm^{-1}, 1140.97cm^{-1}, 986.94cm^{-1}, 901.37cm^{-1} (Figure No.6). In stabilized Copper nanoparticles the strong bands were observed at 3308.81cm^{-1}, 1637.29cm^{-1}, 1437.62cm^{-1}, 1312.11cm^{-1}, 1140.97cm^{-1}, 986.94cm^{-1} (Figure No.7). 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 3270.30cm^{-1} observed in control extract, is due to OH stretching vibration, 1633.01cm^{-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 Silver and copper nanoparticles) to prevent agglomeration and thereby stabilize the medium.

Preparation of Topical Formulation[7-9]

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 as well as copper 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 100g. The composition used in this study is tabulated in (Table No.2).

Physicochemical Evaluation of Formulations[7-9]

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 and Copper 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 6640cps 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 the Copper Nanoparticle and *Eclipta alba* Plant extract against all the pathogens. The antibacterial activity of the study results is shown in (Figures No.8a-8c).

Table No.1: Preliminary phytochemical screening of dried powdered material and extracts of the leaves of *Eclipta alba*

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 and Mucilage	-	-	-	-	-
12	Terpenoids	+	+	+	+	+

Table No.2: Composition of the formulation

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

Figure No.1: Formation of Silver nanoparticle

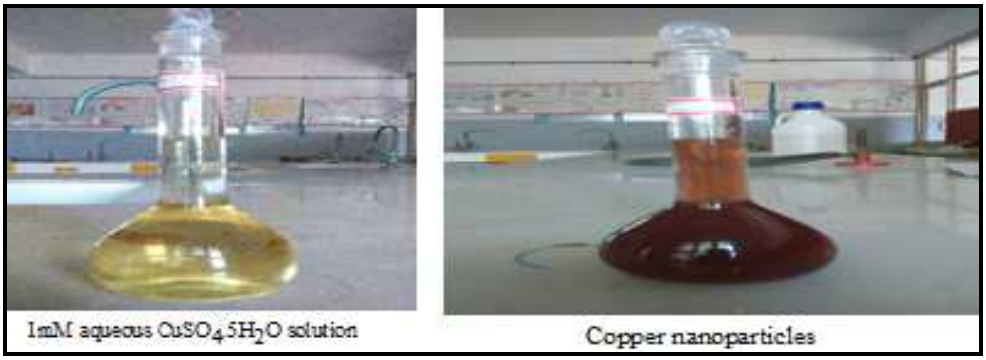

Figure No.2: Formation of copper nanoparticle

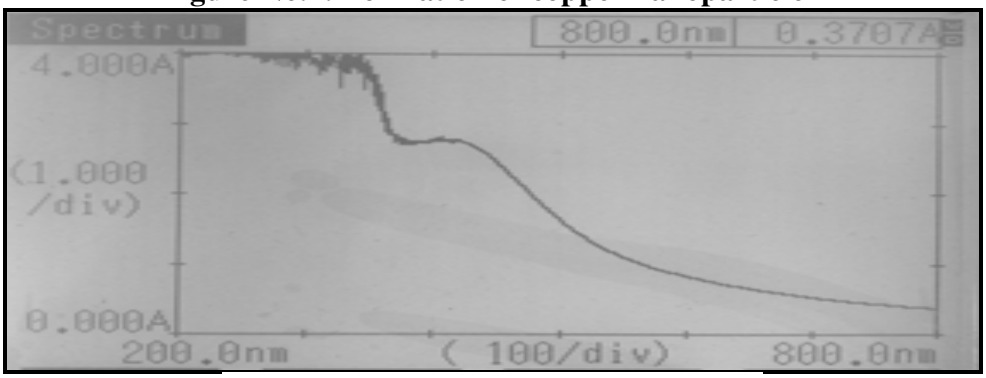

Figure No.3: UV-Vis spectra for AgNPs

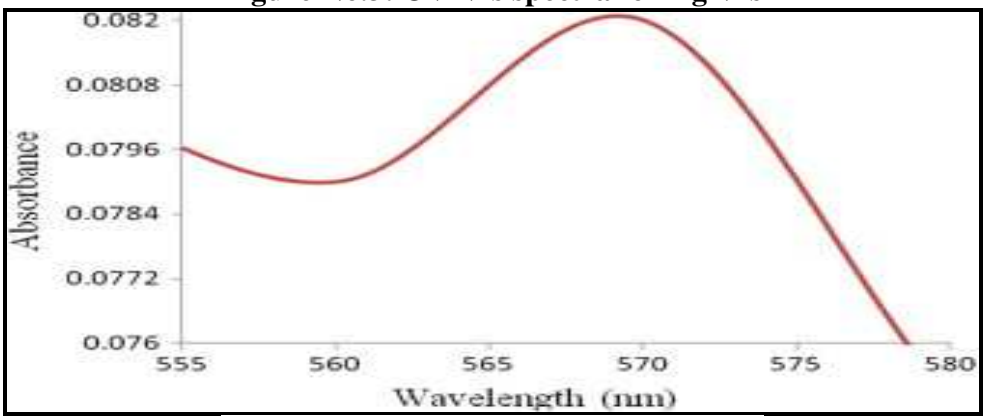

Figure No.4: UV-Vis spectra for CuNPs

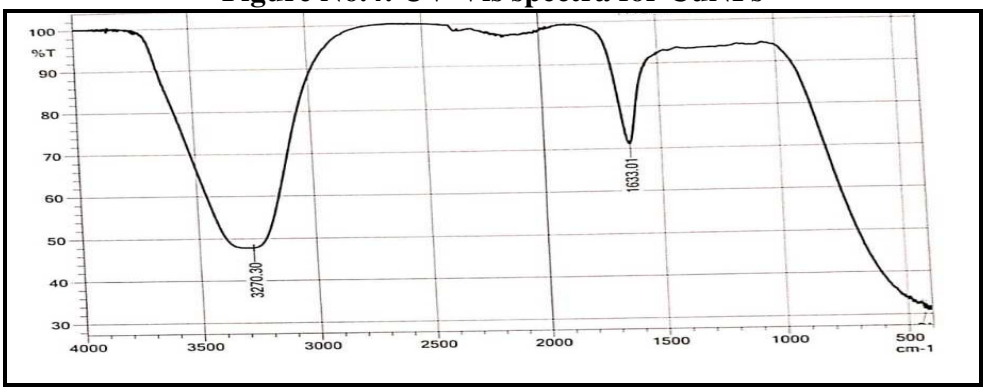

Figure No.5: FTIR Spectra of ethanolic leaf extract of *Eclipta alba*

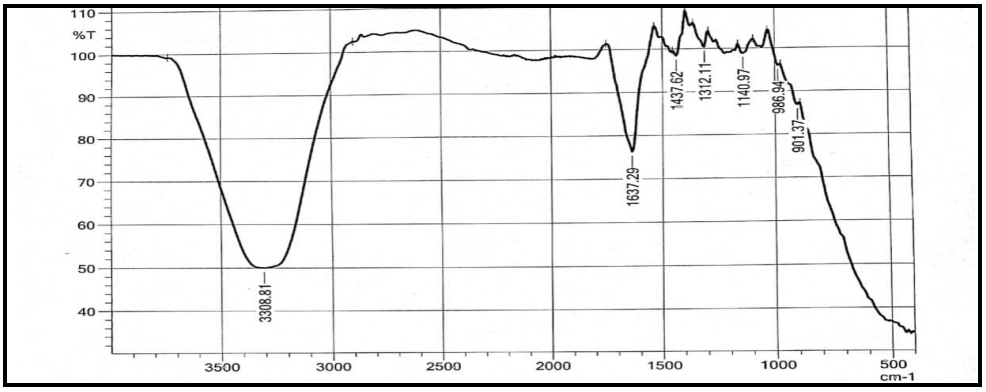

Figure No.6: FTIR spectra of synthesized AgNPs synthesized

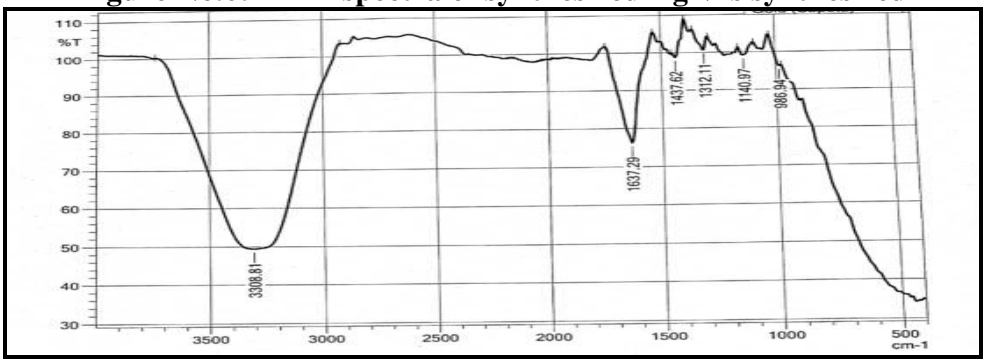

Figure No.7: FTIR spectra of synthesized CuNPs synthesized

Figure 8a: Antibacterial activity against *E. coli*

Figure No.8b: Antibacterial activity against *S. aureus*

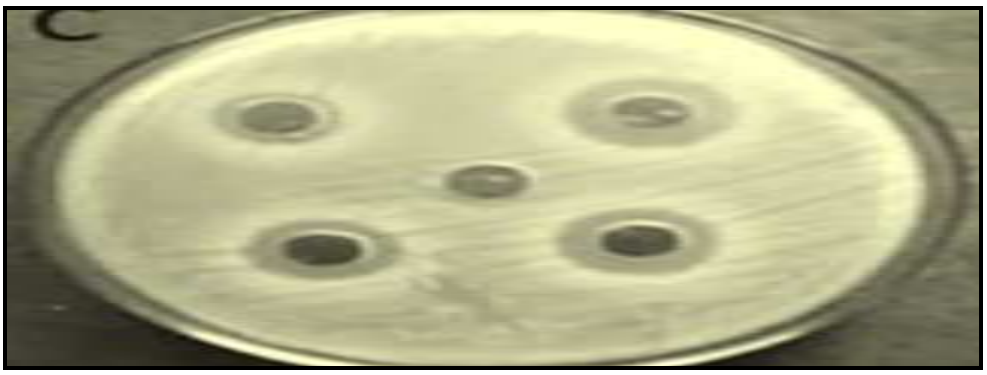

Figure No.8c: Antibacterial activity against *P. acne*

CONCLUSION

Concerning the environmental protection, green synthesis of nanoparticle has gained friendly and growing demand. Among the different metal nanoparticle, AgNPs and CuNPs 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 remedy because of different metabolites and its chemical constituents. These phytoconstituents and metabolites can reduce the silver ions and assist synthesize of AgNPs and CuNPs from plant extracts. The present study reveals a simple, rapid and economical method to synthesize AgNPs silver nanoparticle and CuNPs from *Eclipta alba*. From the results, it was found that the synthesized AgNP silver nanoparticle using *Eclipta alba* leaves extract showed higher activity than the extract. AgNP of *Eclipta alba* in a gel base can be used as an appropriate formulation for the treatment of acne vulgaris.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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