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Research Article

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Formulation of Antifungal Herbal Shampoo Incorporating Silver Nanoparticles Synthesized from Biowaste

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ABSTRACT

Introduction: Nanoparticles are being used commercially to enhance the effects on the products for better results. Silver nanoparticles are gaining popularity due to their wide range of applications considering their antimicrobial and antifungal properties. This research work uses a quick and ecological method for formation of green synthesized silver nanoparticles and its application as an anti-dandruff shampoo.

Methods: Synthesis of silver nanoparticles from *Citrus limon* and *Allium cepa* aqueous peel extracts of 5mM and 10mM concentration. Characterization of the nanoparticles was done by FTIR (Fourier transform infrared spectroscopy), SEM (Scanning electron microscopy) and UV-spectroscopy. The formulation of herbal shampoo was carried out. The physical evaluation and antifungal properties (disc diffusion method) of the formulation were determined.

Results: UV-spectroscopy showed peaks at a range of 350-450 nm for both nanoparticles after 24hrs and 1 week. SEM analysis showed particle morphology of spherical shape in a range of 30-100nm for both Lemon and Onion nanoparticles. FTIR identified functional groups present in nanoparticles which showed presence of O–H, C=C and C–Br for *Citrus limon* nanoparticles and H-O-H bending in *Allium cepa* nanoparticles along with OH, C-Br and C-O groups. These nanoparticles were used to formulate an anti-fungal herbal shampoo using *Meethi, Amla, Reetha and Shikakai* extracts and anti-fungal activities were studied against the fungi *Candida balantits* and *Candida albicans*. The nanoparticles and nanoparticles incorporated shampoo formulation exhibited significant zone of inhibition stating its effectiveness against dandruff causing fungi.

Conclusion: The current study included the formulation of an anti-dandruff shampoo with reduced usage of silver nitrate, thereby less quantity of nanoparticles, hence decreasing the risk of toxicity.

Key Words: *Citrus limon, Allium cepa*, Anti-fungal, Silver nanoparticles, Herbal shampoo, Anti-dandruff.

INTRODUCTION

Scalp infections are mostly caused by fungus and lead to hair loss, rash and itchy scalp. Dandruff is a condition that leads to dry and flaky scalp, experienced by most people in their lifetime. It is mostly caused by Fungi like *Malassezia, Candida, and Tinea*. Nowadays a lot of shampoos and agents are developed as a treatment for dandruff, which includes Ketoconazole, coal tar, climbazole, etc. [1] Although, the resistance to these drugs is a concerning issue along with the chemical bases used which can be damaging to the scalp. This raises a need for the use of natural products embedded with equally efficient anti-dandruff mechanisms. Nanoparticles are made from metals or alloys and are sized about 5-100nm. Their properties are different as they have a large surface area to volume ratio as compared to other atoms and molecules of bulky nature or non-nanoparticles. Due to this, the optical, chemical, and physical property is enhanced which enables them to produce a quantum effect by limiting their electron movement. [2] Electrochemical processes, decomposition, and chemical and physical methods are all used for creating metallic nanoparticles. However, the chemicals utilized in such procedures are highly toxic, limiting their use in many places due to potential health dangers. Consequently, there is an increasing need to develop a safe and environmentally acceptable way of creating metallic nanoparticles. Currently, green synthesized nanoparticles are gaining a lot more attention due to their ability to reduce toxicity, and being environmentally and user-friendly. The synthesis of

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nanoparticles using leaves, flowers, fruit peels, and stems of plants involves the interconnection of Plant Biotechnology and Nanotechnology. Nanoparticles are formed because of metal ion reduction by plant extracts, which contain metabolites like terpenoids, alkaloids, flavonoids, and tannins.[3] Usage of capping agents in synthesis of nanoparticles precisely controls their development, aggregation, and physiochemical properties. Bio-capping nanoparticles with aqueous plant extracts are an effective approach to achieve controlled nanoparticle generation with minimal toxicity since they contain natural stabilizing agents as biomolecules. This research aims to develop a product or technology that is herbal and focuses on minimizing the wastage of beneficial plant material. Fruit peel extracts of C*itrus limon* and *Allium cepa* are used, which have an excellent anti-microbial properties and are rich in phytochemicals such as flavonoids, terpenoids, polyphenols, and tannins. Using Nanotechnology interlinked with natural ingredients, the formulation of an antifungal shampoo is studied to suppress organisms causing dandruff. Since both lemon and onion peel extracts are excellent antifungal agents, using them in nanoparticle formulation is proven to enhance their activity overall.

MATERIAL AND METHODS

A) Collection of plant material

Lemon (C*itrus limon*) and Onion (*Allium cepa)*, obtained from local market and their peels were removed. Lemon peels were washed away of the dust particles. It was grinded into powdered form after exposure to sunlight for two days. Onion peels were sliced into tiny pieces and exposed to sunlight for 2 days without washing. Both the peels were stored in a moisture-free container at room temperature.

B) Preparation of extracts

10g of powdered lemon peels in 100ml of distilled water was heated for 10 mins. 4g of dried onion peels were added in 100ml distilled water and heated using a magnetic stirrer at 70°C for 1hr. After the mixture cooled down it was first filtered through muslin cloth to remove the solid particles and then through Whatman filter paper no.1 in order to produce a clear yellow aqueous extract. Once prepared the extract was kept at 4°C until usage.[4]

C) Green silver nanoparticles synthesis

0.16g and 0.085g silver nitrate powder was dissolved in 100 ml distilled water separately to form 10mM and 5mM silver nitrate solution respectively. 1.5ml of prepared extracts was added to 20ml Silver Nitrate ($AgNO_3$) solution of each concentration. For 15 mins, the bottles were placed directly in the sun. The synthesis of silver nanoparticles was verified by a colour shift in the colloidal suspension from colourless to reddish brown or golden brown. Onion Nanoparticles and Lemon nanoparticles were stored in Kesell bottles and kept for 24hrs in dark at room temperature.[5]

D) Formation of powdered nanoparticles

After incubation, lemon nanoparticles were centrifuged at 8000rpm for 15mins at 4°C and Onion nanoparticles at 8000rpm for 30mins at 4°C to obtain a pellet. The supernatant was discarded and 5ml distilled water was added and it was centrifuged again for 15 mins. Washings were given in between. Completely dry out the excess water, the pellet was placed in a crucible and heated to 100°C. Powdered nanoparticles were scraped off the crucible and stored in a sterile vial at room temperature.

E) Characterization of nanoparticles

UV-spectroscopy was done at a wavelength of 300nm to 800nm at varying time interims of 24hrs and 1 week of nanoparticles formation to monitor the reduction of Ag+ ions due to the formation of AgNPs. JSM-7600F machine model was used for SEM analysis to measure the size and shape of the AgNPs. With an accelerated voltage of 0.1 to 30 kV, the nanoparticles diameter was measured. On a staining mat, a drop of thin dispersion was applied and the carbon-coated grid was inserted into the drop with the coated side facing up. The extra solution was eliminated using filter paper and allowed to dry prior to measurements. FTIR was recorded at 400-4000 cm^{-1} to identify the quaternary ammonium compounds that were the capping agents in the prepared silver nanoparticles. FTIR was done using 3000 Hyperion microscopes with Vertex 80 FTIR system and the sample was used in powdered form.

F) Shampoo formulation

Herbal extracts were prepared using dried *Meethi, Retha, Shikakai and Amla* as per quantity. They were boiled in 200ml distilled water for 30-45mins (**Table 1**). The mixture was cooled down and filtered using a muslin cloth to obtain extracts. 1ml nanoparticles solution was added to 30ml herbal extracts each to provide anti-fungal properties. 1% Guar gum solution was added to maintain consistency and provide viscosity. [6]

Table 1: Composition of silver nanoparticles loaded herbal shampoo

Ingredients	Lemon nanoparticles shampoo	Onion nanoparticles shampoo
Meethi	2g	2g
Retha	2g	2g
Shikakai	1g	1g
Amla	1g	1g
Nanoparticles	1ml	1ml
1% Guar gum	2ml	2ml

G) Evaluation of shampoo

i) Physical appearance: The formulated shampoo was tested for their colour, odour and transparency.

ii) Determination of pH: pH was determined using litmus paper by dipping the pH paper in each shampoo formulation. [7]

iii) Foaming Test: The foaming ability was detected using cylinder shake method. 100ml distilled water were mixed in 1ml of shampoo. 50ml of the diluted solution was shaken ten times. After 1min the foam content was measured. [8]

iv) Determination of percentage of solid contents: A clean and dry evaporating dish was weighed, and 4g of formulated shampoo was taken in order to estimate of the percentage of solid components. The liquid portion of the shampoo was evaporated by heating the evaporating dish. The weight and percentage of the solid contents of shampoo left after complete drying was taken to calculate the percentage of solid content.[8]

v) Surface tension measurement: A stalagmometer was used to test the surface tension using the drop count method after diluting 10ml of shampoo in 100ml of distilled water.[9]

vi) Dirt dispersion test: 10 ml of distilled water was put in a test tube with two drops of shampoo. It was shaken in the test tube ten times while adding 1 drop of India ink. The colour of the foam was measured as none, light, moderate and heavy.[10]

vii) Wetting Time test: for the disc to completely sink into the shampoo solution was measured using a stopwatch and recorded as the wetting time.[11]

viii) Anti-Fungal test: Sterile Plates 1% shampoo solution was formed. A canvas paper was used to create discs with a 1-inch diameter and an average weight of 0.44 g. The disc was positioned on the top of the shampoo and the time required were prepared using Sabouraud Dextrose Agar (SDA). The SDA agar was swabbed with the saline suspension of cultured fungus using a sterile cotton swab. Well diffusion method was used and 6mm wells were bored using a sterile cork borer. Saline suspension and 2% v/v ketoconazole served as positive and negative control. 20µl sample of silver nanoparticles and shampoo containing silver nanoparticles was added in different wells and the anti-fungal activity was checked against *C.albicans and Candida balantis* at 37 degree Celsius after 24hrs and 72hrs respectively. The inhibition zone was measured for the negative control, nanoparticles and nanoparticles incorporated shampoo.

RESULTS

Change of colour from light yellow to brown for lemon nanoparticles and light red to brown (**Fig 1**) for onion nanoparticles was observed due to reduction of Ag+ ions that indicated formation of silver nanoparticles.

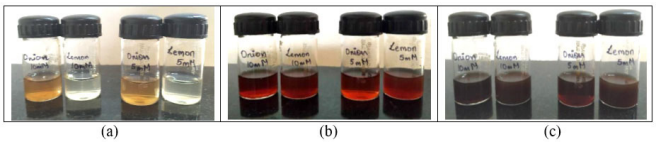


Figure 1: Lemon and onion extracts added in silver nitrate solution (a) before exposing to sunlight (b) After 15mins of exposure to sunlight, change of colour is observed due to reduction of Ag+ ions (c) After 24hrs in dark, Colour becomes darker due to bioreduction and saturation as the nanoparticles aggregate.

UV-Visible spectroscopy analysis done after 24hrs and 1 week showed peaks **(Table 2)**. Even after a week, the AgNPs remained stable, with very small changes in peak and symmetry. This is due to the phytochemicals stabilising and capping qualities found in the aqueous extract of red onion and lemon peels, which prevent the particles from deforming and decomposing while the AgNP is being produced. [12,13] Similar findings were reported by Rao et.al. 2014.[14] Depending on the size, shape, degree of aggregation, and surrounding dielectric medium of the particles, the spectrum may show a shift in wavelength as the time interval increases.[15]

Table 2: Peaks of UV-Vis spectroscopy of different concentrations of nanoparticles

Nanoparticles	Peak after 24hrs	Peak after 1 week
Lemon (10mM)	430nm	370nm
Lemon (5mM)	415nm	365nm
Onion (10mM)	465nm	450nm
Onion (5mM)	450nm	415nm

SEM image of *Citrus limon* nanoparticles show that the synthesized nanoparticles are spherical (**Fig 2**). Some are irregular but they are evenly distributed. The particle size ranges from 33nm-73.553nm which is in comparison with the previous work of Rao et.al., 2014 and Kumar et.al., 2015. [14,16] *Allium Cepa* silver nanoparticles showed some agglomerated spherical nanoparticles. The size ranges from 47.518nm-99.247nm, like in the studies carried out by other researchers. [17, 18]

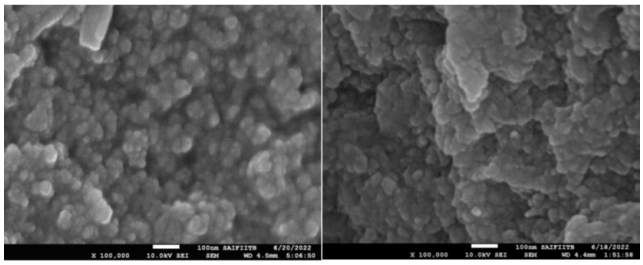
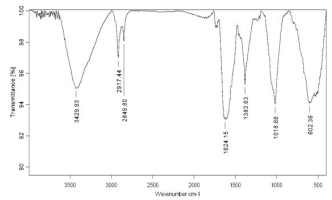


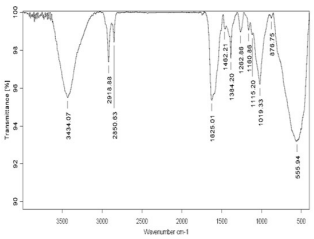
Figure 2: SEM image of *Citrus limon and Allium cepa* silver nanoparticles observed at 100nm.

FTIR analysis for lemon peel (**Fig 3**) and Onion peel (**Fig 4**) nanoparticles showed absorption peaks which indicated different functional groups.



Sr No	Wavenumber (nm)	Functional Group	Description
1.	1624.15	C=C	Medium Bond, Alkene, Aromatic Ring
2.	1383.63	O-H	Strong, bondd, Alcohol
3.	3429.93	O-H	Medium Bond, Alcohol
4.	2917.44	C-H	Medium Bond, Alkane
5.	2849.80	C-H	Medium Bond, Alkane
6.	602.36	C-BR	Strong Bond

Figure 3: FTIR analysis showing peaks of functional compounds and description of peaks present in *Citrus limon* nanoparticles.



Sr No	Wavenumber (nm)	Functional Group	Description
1.	1625.01	C=C	Strong, alkene
2.	3434.07	O-H	strong, broad, Alcohol
3.	2918.88	C-H	Medium Bond, Alkane
4.	2850.63	C-H	Medium Bond, Alkane
5.	1462.21	C-H	Medium Bond, Alkane
6.	1384.20	O-H	Medium, phenol
7.	1262.86	C-O	Strong, aromatic ester
8.	1160.86	C-O	Strong, tertiary alcohol
9.	1115.20	C-O	Strong, secondary alcohol
10.	555.94	C-I, C-Br	Strong Bond

Figure 4: FTIR analysis showing peaks of functional compounds and description of peaks present in *Allium cepa* nanoparticles.

The presence of strong and medium bonds of alcohols, phenols, alkanes, alkenes, and halogens were observed. When comparing the current research on lemon nanoparticles to earlier studies conducted by researchers on lemon peel extracts. [19,20] The shift in the position of bands is seen, confirming that phytochemicals (flavonoids, alkaloids, coumarins, and phenolics) may be involved in reducing the silver salt to Ag+ and that these substances act as the stabilising and capping agents while reduction of Ag+ ions. The presence of strong C-I, and C-Br bonds indicates stability in onion nanoparticles. Along with that Strong and medium alkenes, alcohol and aromatic esters were present.

Evaluation of the nanoparticles incorporated in shampoo

The results of the visual inspection indicated that all the shampoos had consistent colour, odour and characteristics. The foaming ability was fair due to the *Reetha* extract and the texture was smooth without any grainy particles. pH was found to be 6 for all the shampoos, hence it is able to minimize eye irritation and stabilize the scalp's ecological balance. It is also close to the skin pH to avoid harm. The percentage of solids is satisfactory since more solids cannot be easily washed and work in hair. If the percentage is below 10 then the shampoo is considered too watery. Surface tension measurement should be less than that of water (72.8 dynes/cm) as an ideal surfactant should be able to break the surface tension of water as a part of it is hydrophobic in nature and hence sticks to the oil and dirt and acts on it. The dirt dispersion test resulted in light colour foam which indicated that the dirt which is in the ink remains in the water and not in the foam; hence the shampoos have good cleansing action. The wetting time test required the canvas paper discs to sink for between 130 and 150 seconds, which is appropriate and proves the efficacy of surfactant as indicated in **Table 3**.[21]

Table 3: Evaluation of silver nanoparticle shampoo formulation

Parameters	Lemon Nanoparticle shampoos	Onion Nanoparticles shampoos	Ideal Shampoo
Colour	Brown	Brown	Brown, white, Green, yellow.
Odour	Good	Good	Good
pH	6	6	5-7
Foam ability (ml)	87	88	80-115
Solid content %	15%	16.75%	10-30%
Surface tension	38.34dyn/cm	38.24dyn/cm	30-40dyn/cm
Wetting time test	136seconds	143seconds	>170seconds
Dirt dispersion	Light	Light	Light and moderate

The anti-fungal test showed promising results against *Candida albicans* and *Candid balantis* as significant zone of inhibition was formed around herbal Shampoo with nanoparticles and nanoparticles solution. Ketoconazole showed a maximum zone of inhibition of nearly 20-30mm as indicated in **Table 4**. It showed more effectiveness against *Candida balantis* than *Candida albicans*. Both the shampoos and nanoparticles of lemon (**Fig 5**) and onion (**Fig 6**) showed almost similar results to different concentrations. This is due to the strong antifungal properties of silver, lemon, onion, *meethi, reetha, amla and shikakai*. This also indicated that the use of 5mM silver nitrate is preferred over 10mM as it involves less usage of the chemical silver nitrate, providing effective results considering the ratio of silver nanoparticles to herbal shampoo in ml was 1:30.

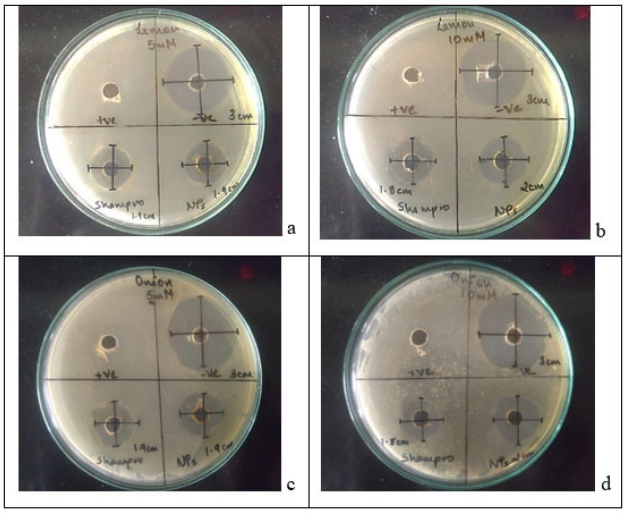


Figure 5: Activity on *Candida balantis:* (a)&(b)5mM & 10 mM shampoo containing *Citrus limon* nanoparticles (c) & (d) 5mM & 10mM *Citrus limon* nanoparticles

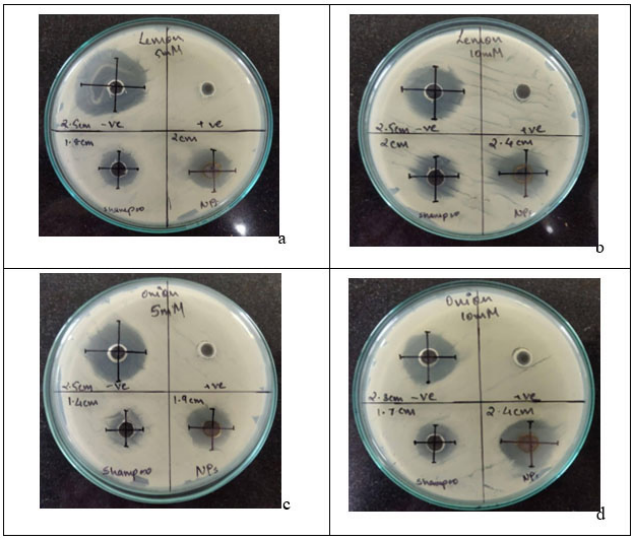


Figure 6: Activity on *Candida balantis*: (a) &(b) 5mM & 10 mM shampoo containing *Allium cepa* nanoparticles (c) & (d) 5mM & 10mM *Allium cepa* nanoparticles

Table 4: Zone of inhibition of Nanoparticles incorporated Shampoo and Nanoparticles against *Candida balantis* and *Candida albicans*.

	Candidabalantis		Candida albicans	
	Shampoo	Nanoparticles	Shampoo	Nanoparticles
Citrus limon (5mM)	19mm	19mm	18mm	20mm
Citrus limon(10mM)	18mm	20mm	20mm	24mm
Allium cepa (5mM)	19mm	19mm	14mm	19mm
Allium cepa (10mM)	18mm	20mm	17mm	24mm

DISCUSSION

The development of antifungal shampoo by using rapid and efficient biological methods could be a viable path to nanoparticles uses that could be ecologically friendly. Since they are smaller in size their ability to showcase smaller volume to larger surface area ratio and great dispersion force can be used in anti-fungal products.[21] Antifungal properties were imparted to the shampoo formulation due to the use of Herbal ingredients and silver nanoparticles. Plants were selected because of their use as traditional medicine for treating dandruff and other infections of the scalp caused due to fungi.[22] A peak was observed after UV-Vis spectroscopy at a range of 430nm-465nm for lemon and Onion nanoparticles after 24hrs of incubation and at 370nm-450nm after 1 week of incubation respectively. UV-Visible spectroscopy detected the formation of nanoparticles as a change in colour was observed with the intensities of brown hue increasing in direct proportion to the incubation period. The reduction of silver nitrate ($AgNO_3$) and excitation of the surface plasmon resonance (SPR) phenomenon leads to the colour change.[23] FTIR Spectroscopy revealed the functional groups due to O–H stretching, aldehydic CHO stretching, C–C and, C=C, C–O strcm^{-1}r *Citrus limon* nanoparticles. Wavenumber 3434.07 indicated H_2O $(cm^{-1}$I bending) in cm^{-1} *cepa* nanoparticles and 2918.88 and 1625.01 corresponds to C=C, C-H and C-O groups hence confirming the action of phytochemicals of the plant extracts as capping agents and SEM analysis showed the spherical shape of both the nanoparticles with size range of 33nm to 99nm. Size of the nanoparticles determines its interaction with other compounds. According to Aziz et.al., 2019, nanoparticles used in cosmetics should range from 10-200nm for uniform distribution on the skin, enhancement of active component delivery and skin feel.[24] Crude extracts were prepared, incorporated with silver nanoparticles, and tested against *Candida balantis* and *Candida albicans* by agar well diffusion method and the zones of inhibitions were measured. The finding of this research has showed extensive anti-fungal properties of silver nanoparticles prepared by *Citrus limon* and *Allium cepa* peels. The cause for this anti-fungal property is stated by many researchers as destruction of the membrane integrity but the exact mechanism is yet to be understood.[25] However, it is believed that the phytochemicals included in *Citrus limon* peel suppress the mycelial growth of fungus. The peels of *Citrus limon* are abundant in many nutrients and phytochemicals, such as flavanones (hesperidin, eriocitrin, narirutin, naringin, and didimin), which belong to the phenolic group.[26,27] Flavones contain C-glycosides that include diosmetin-7-rutinoside, 6,8-di-C-glucopyranosylapigenin, and 6,8-di--Cglucopyranosyl-luteolin.[28] Lemon peels include flavonols such as rutin, quercetin, myricetin, isocitrol, limocitrol, and limocitrin. These compounds might also be responsible for the antifungal activity of methanolic extract

of lemon peel. Along with that the shampoo formulation and evaluation was done which included less concentration of silver nitrate hence it can be considered less harmful for scalp while providing good results.

CONCLUSION

The study concludes that green synthesis of Silver nanoparticles using *Citrus limon* and *Allium cepa* peels provides good anti-fungal activity against *Candida balantis* and *Candida albicans* both as nanoparticles solution and as shampoo solution. The process of synthesis proves to be rapid, environment friendly, cheap and non-hazardous. Even the less concentrated nanoparticles exhibit findings those are nearly identical to those of the concentrated ones, demonstrating their effectiveness and recommending the usage of less concentrated nanoparticles to lessen skin toxicity. Extracts of *Meethi, Amla, Reetha, shikakai,* Lemon and Onion also contributed to strengthening the antifungal effect. The shampoo formulation uses a very less amount of silver nanoparticles solution which is in an aqueous medium, as compared to the herbal extracts and hence can be used on the scalp for the prevention of dandruff. Therefore, antifungal activity of lemon and onion silver nanoparticles containing shampoo formulation creates an opportunity for new range of cosmetics and pharmaceutical products.

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