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Formulation and Evaluation of Peel-off face mask gel containing Bombax ceiba Silver Nanoparticles

Abstract-

Nanotechnology is arising as a field of modern science which finds its application in various fields such as medical, health care, food, industrial purposes, etc. due to their manufacturing of materials ranging in nanometres. The *B. ceiba* is a miraculous plant for the skin troubles and therefore it is selected for preparation of its nanoparticles. In topical formulations, antimicrobial nanoparticles are found effective for treating acne vulgaris and peel off formulations are recently trending in cosmetics**.** In the present study, biosynthesis of silver nanoparticles was achieved by reduction of silver nitrate using *Bombax ceiba* acetone extract**.** These biosynthesized silver nanoparticles were characterized by using UV- Vis spectroscopy, SEM and particle size analysis. Antioxidant and anti-microbial activity of nanoparticles and acetone extract was evaluated. The synthesized nanoparticles were then incorporated in the formulation and evaluated for its various physical parameters.

Keywords – Silver nanoparticles, Peel off mask, *Bombax ceiba*, Antioxidants, Green synthesis

I. Introduction-

Acne vulgaris is a common human skin disease, found in about 80% of young adults and adolescents. Along with pimples and scarring, patients also suffer from anxiety and embarrassment and can affect their physiological well-being (Dorland,2000). Various factors causes acne include genetics, youth, stress, comedogenic medications and pore clogging cosmetics. In the treatment of acne, the main goal is to treat existing lesions and prevent permanent scarring. There are variety of topical, systemic, and physical treatment available for treatment of acne. However, these methods can have their own side effects therefore, medicinal plants have been commonly used in patients since they have long history and shows low side effects (Fox L, et al., 2016). *Bombax ceiba* Linn. syn: *Salmalia malabarica* , also known as red cotton tree or shalmali belongs to Bombacaceae distributed in all the hotter parts of India up to 1500 meter or more. *B. ceiba*, is rich in many phytoconstituents such as steroids, tannins, triterpenoids, flavonoids, anthocyanins, lactone, xanthone, napthoquinones mangiferin, sterol and sesquiterpenoids (Kamble M, et al. 2017). Almost all parts of the tree are used for different medicinal properties such as gums for Diarrhoea, dysentery, roots are considered astringent, tonic, diuretic and aphrodisiac. Externally it is applied for rheumatic swelling, inflammations and eruptios. Flowers and fruits are also used in snake bite (Rastogi R.P. and Mahrotra B.M, 2001, Chaterjee A. 2003, Nadkarni A.K., Nadkarni K.R 1976, Chopra R.N, Nayar S.I. 1956, Kirtikar K.R. and Basu B.D, 2005, It has been studied that *Bombax ceiba* shows very good antioxidant and anti-acne activity (Patankar, 2005 , Gurunani , 2018). The thorns extract has already been a part of polyherbal anti-acne formulation which is clinically effective and safe for the treatment of acne vulgaris (Meena V, Chaudhary AK, 2017).

In topical formulations, antimicrobial nanoparticles are found effective for treating acne vulgaris. Various metals can be used for the preparation of nanoparticles out of which silver nanoparticles has gained more importance due to its effectivity against bacteria and viruses (Budiman A, 2017). Peel-off face mask gel is one of the popular forms of topical formulation which is applied on the face and is peeled off after few minutes of its application. The face mask has many advantages like giving moisturizing effects, cleanness, removing dead cells, relaxing the face muscles, skin toning etc. It is also used for the skin problems such as acne, wrinkles, ageing and to minimize pores (Prabhu L, 2017).

With this consideration, the present study has been aimed to achieve following objectives,

1. To develop a cost-efficient alternative to conventional methods by developing completely biogenic method of synthesizing silver nanoparticles of *B. ceiba*
2. To synthesize silver nanoparticle by using *B. ceiba* thorns extract and evaluating its physico-chemical properties.
3. To evaluate *B. ceiba* silver nanoparticle for antioxidant and anti-bacterial activity.
4. To formulate and evaluate topical peel-off face mask gel containing *B. ceiba* silver nanoparticles.

II. Material and Methods

II.1. Collection and extraction of Thorns extract-

The thorns of *Bombax ceiba* were collected from wildly grown mature trees along the road sides of Nagpur region of Maharashtra state and authenticated in Department of Botany, R. T. M. Nagpur University, Nagpur (Voucher no. 10269). The dried thorns were coarsely powdered and then extracted by successive Soxhlet extraction method with increasing order of polarity of solvents such as petroleum ether, acetone and hydro-alcoholic solvent. All the extracts were concentrated and dried at room temperature.

II.2. Phytochemical analysis-

A plant may contain various compounds such as alkaloids, glycoside, volatile oils, tannins and flavonoids, etc which are termed as secondary metabolites and are responsible for therapeutic effects. Hence, all the extracts of *B. ceiba* thorns were subjected to phytochemical screening for identification of different constituents and plant metabolites present in the plant extract (Rangari VD, 2002).

II.3. Quantitative estimations:

The quantitative estimation is carried out for the determination of number of secondary metabolites present in the sample. The various estimations such as total flavonoid content by aluminium chloride and 2,4- dinitrophenyl hydrazine colorimetric method (Khadbadi SS, 2011), total phenolic contents by Folin- Ciocalteau method, estimation of total carbohydrate content (Sadashivam S,1996) was performed on extracts.

II.4. Preparation of Silver Nanoparticles-

The (SNAE) silver nanoparticles from acetone extract of *B. ceiba* thorns were prepared by different methods such as keeping overnight, mechanical stirring, shaking method (Chand K, 2019, Moodley JS,2018, Shriram T, 2014, Gomathi M, 2017). The preparation of nanoparticles with electromagnetic stirring method was selected for further studies. For the preparation of silver nanoparticles, acetone extract was added dropwise to 1mM aqueous solution of silver nitrate with constant stirring at 80 °C and the fabrication of SNAE is ascertained by the colour change of the solution from yellowish to dark brown colour. The SNAE was dried to powder using vacuum dry evaporator and is preserved until its use (Asadi S, 2018).

II. 5. Evaluation of Nanoparticles

II.5.1. UV Visible spectroscopy-

The silver nitrate solution and reduction of pure silver ions was monitored by UV-Vis spectrophotometer (Shimadzu R UV-1800, Kyoto double beam model) in 200-800 nm wavelength range. The samples were prepared in small aliquots with distilled water and spectra was observed (Behravan M, 2019).

II.5.2. SEM Analysis-

The surface morphology of the nanoparticles was characterized using a Scanning Electron Microscopy (SEM), Carl Zeiss, Supra 5- Germany (Telrandhe R, 2017).

II.5.3. Particle size analysis-

The particle size analysis of the silver nanoparticles was done by the Malvern. The dispersion medium used was water.

II.5.4. Antioxidant studies –

The antioxidant activity of the nanoparticles was carried out by DPPH scavenging activity method and was compared to pure extracts. The free radical scavenging activity of the extract was evaluated based on the ability to scavenge the synthetic DPPH (1,1-diphenyl- 2- picryl- hydrazyl). DPPH solution, standard ascorbic acid solution and stock solution of extract were prepared. 2ml of methanol solution of plant extract at different concentration was taken in a test tube and to it 3 ml of methanol solution of DPPH was added, incubated at room temperature for 30 min. in a dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against blank. The % inhibitory concentration was calculated and was plotted against concentration and from the graph IC_{50} was calculated (Ravichandran V, 2019).

II.5.5. Anti-microbial activity

The minimum inhibition concentration determines the lowest concentration of an antimicrobial agent that prevents the visible growth of the microorganisms. To determine the minimum inhibitory, silver nanoparticles of acetone extract of various concentration was taken and tested against strains of *E. coli* and *S. aureus (*Alyousef AA, 2019)

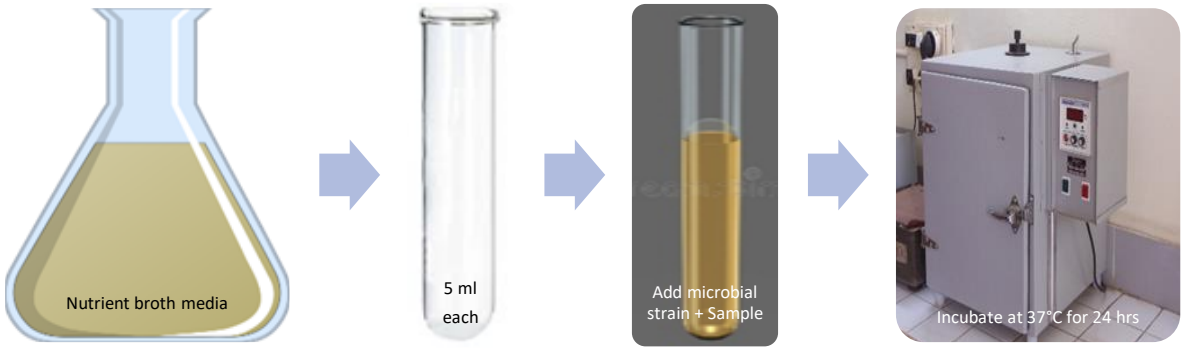


Fig 1 - Diagrammatic representation of Minimum Inhibitory Concentration

II.6. Preparation of Formulation-

The procedure of preparing the peel-off mask peel involves addition of six different phases. The composition of these phases is given in table no. 1. The phases are as follows;

Phase 1- This phase involves the addition of 14% of polyvinyl alcohol to distilled water (60%) in the beaker at 80°C temperature with a constant vigorous stirring. Further this mixture is allowed to cool down at 40° C

Phase 2- In this phase a mixture of Glycerine and PEG in the ratio of 3:1 is added to phase 1 at 40° C temperature and mix well.

Phase 3- Add (0.5%) polysorbate (tween 20). Add lemon oil and mix well.

Phase 4- Add methanol and add 0.5% distilled water with (0.1%) ascorbic acid and dissolve nanoparticles in 1% distilled water into the phase 3 mixture and mixed well.

Phase 5- Add activated charcoal and stirred well and cooled for few minutes (Kulkarni S, et al, 2018).

Table 1. Formulation Ingredient

Sr. No	Ingredients	Category	Formulation (mg/ml)
1	Polyvinyl Alcohol (PVA)	Film Former	14
2	Water	Base	50
3	Glycerine	Smoothing agent	3
4	Polyethylene glycol	Surfactant	1
5	Tween Twenty	Polymer	0.5
6	Methanol	Solvent	1
7	Activated Charcoal	Cleansing agent	1
8	Lemon Oil	Tightening agent	0.1
9	Nanoparticles	Active ingredient	10

II.7. Evaluation of Formulation

II.7.1. Physical Evaluation of Peel off mask gel - Physical parameters such as colour, odour and consistency were checked manually by applying it on the human skin.

II.7.2. Film weight- The weight of each prepared film was measured using Digital balance among the three film of every formulation and the average weight was calculated.

II.7.3. Thickness- The thickness of each film was measured using Micrometer Screw gauge at different points of film and average weight was calculated.

II.7.4. Homogeneity- After the gels have been set in container, all developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.

II.7.5. pH- The pH value of topical peel off gel was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurements of pH of the formulation were done in triplicate and average values calculated.

II.7.6. Drying time- The drying time test was performed by observing the time needed by the gel to dry, which starting from the gel application on the skin of the face until dry layer was created.

II.7.7. Erythema and Edema scoring method for skin reaction- The Erythema and skin irritation test were performed on healthy female volunteers.

Table 2- Erythema and edema scoring method for skin reaction

Sr. No	Skin Reaction		Score
	A. Erythema and Eschar formation	B. Edema formation	
1	No erythema	No edema	0
2	Very slight erythema	Very slight edema	1
3	Well defined erythema	Slight edema	2
4	Moderate to severe erythema	Moderate edema	3
5	Severe erythema causing redness to eschar formation	Severe edema	4

II.7.8. Skin irritation Study- The formulated peel off should not produce any skin irritation or skin sensitization, after its application on the skin or else it will be unsuitable for application on the skin. Hence the peel off gel formulation was subjected to skin irritation study using Draize modified scoring technique.

Table 3 - Evaluation of Primary Skin Irritation Index (PII)

Sr. No	Evaluation	Score
1	Non irritant	0.0
2	Negligible irritant	0.1-0.4
3	Slight irritant	0.41-1.9
4	Moderate irritant	2.0- 4.9
5	Severe irritant	5.0- 8.0

III. Result and Discussion-

III.1. Phytochemical analysis-

Phytochemical screening of acetone extract and hydroalcoholic extract was performed and it revealed the presence of carbohydrates, flavonoids and tannins.

III.2.a. *Estimation of Total Flavonoid content*

The total flavonoid content in samples were determined by colorimetric method of aluminium chloride and 2,4 – dinitrophenyl hydrazine using Quercetin as an internal standard. The standard curve of Quercetin from 10-100 µg/ ml was prepared. The absorbance of all samples was measured at 415 nm using UV/VIS spectrophotometer. The results of total flavonoid content of *Bombax ceiba* thorns extract is given in the table 4.

III.2.b. Estimation of Total Phenolic content

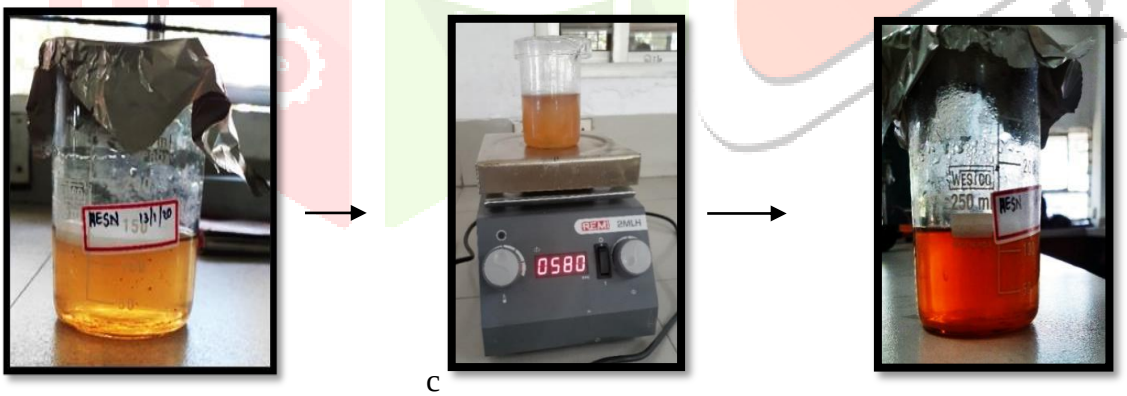
The total phenolic content in samples was determined by colorimetric method. By using Folin ciocalteau reagent and Gallic acid as an internal standard. The standard curve of Gallic acid from 10-100µg/ml was prepared. The absorbance of all samples was measured at 725 nm using UV/VIS spectrophotometer. The results of total phenolic content of *Bombax ceiba* thorns extract is given in the table 4.

Table 4 – Quantitative estimation of extracts of *Bombax ceiba*

Sr. No	Phytochemicals	Acetone extract	Hydroalcoholic extract
1	Total Flavonoids content	4.61	3.34
2	Total Phenolic content	57.98	32.83

III.3. UV- Visible spectroscopy-

An interesting property in metal nanoparticles is its optical property that changes proportional to the shape and size of nanoparticles. In Fig 2, the colour change from yellow to dark brown was observed which is primary indication of formation of silver nanoparticles. The UV- Visible spectroscopy of nanoparticles prepared from acetone extract of thorns of *Bombax ceiba* was performed. The nanoparticles sample displayed the absorbance peak of about 422 nm as shown in Spectrum 1. The peak arises due to the absorption phenomenon of metallic silver nanoparticles as a result of the Surface Plasma Resonance (SPR).



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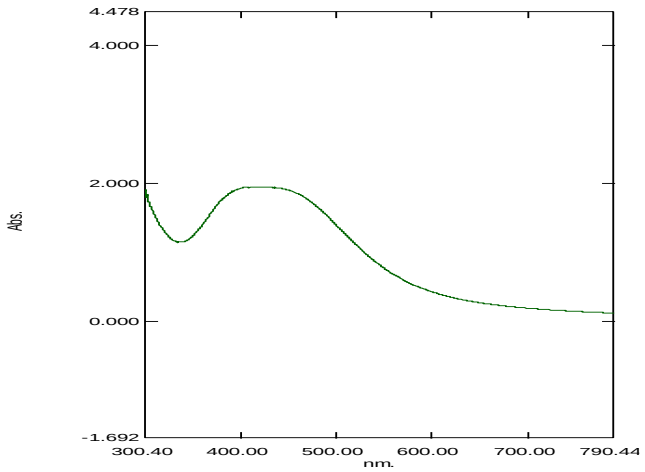
a) Sample before heating **b) During Heating** **c) Sample after heating**

Figure 2 - Preaparation of silver nanoparticles



Spectrum 1 - UV Visible spectra of nanoparticles of hydroalcoholic extract

III.4. SEM Analysis-

The scanning electron microscopy (SEM) was performed for studying the morphology of the nanoparticles. It was observed that nanoparticles were in aggregated form which made it difficult to study its exact shape. From figure 3, it was observed that the nanoparticles were roughly spherical in shape.

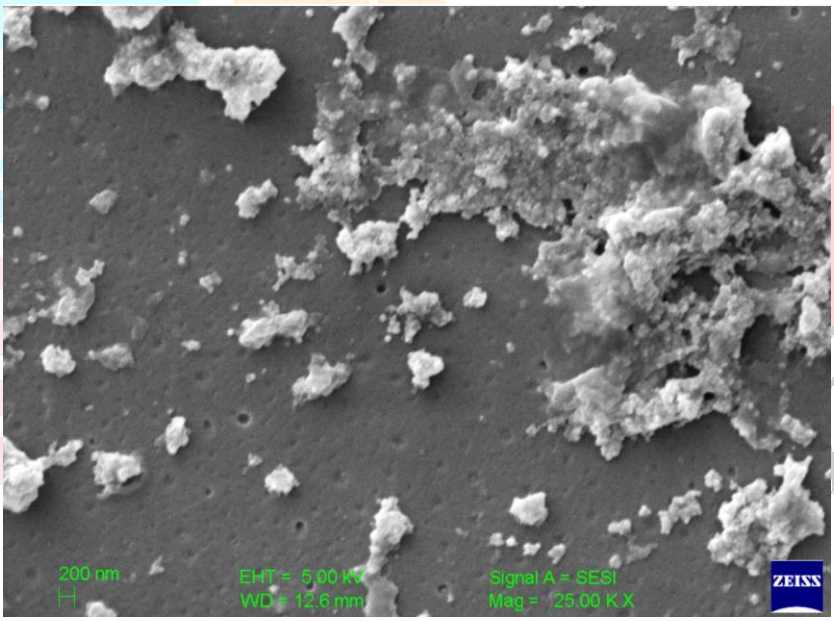


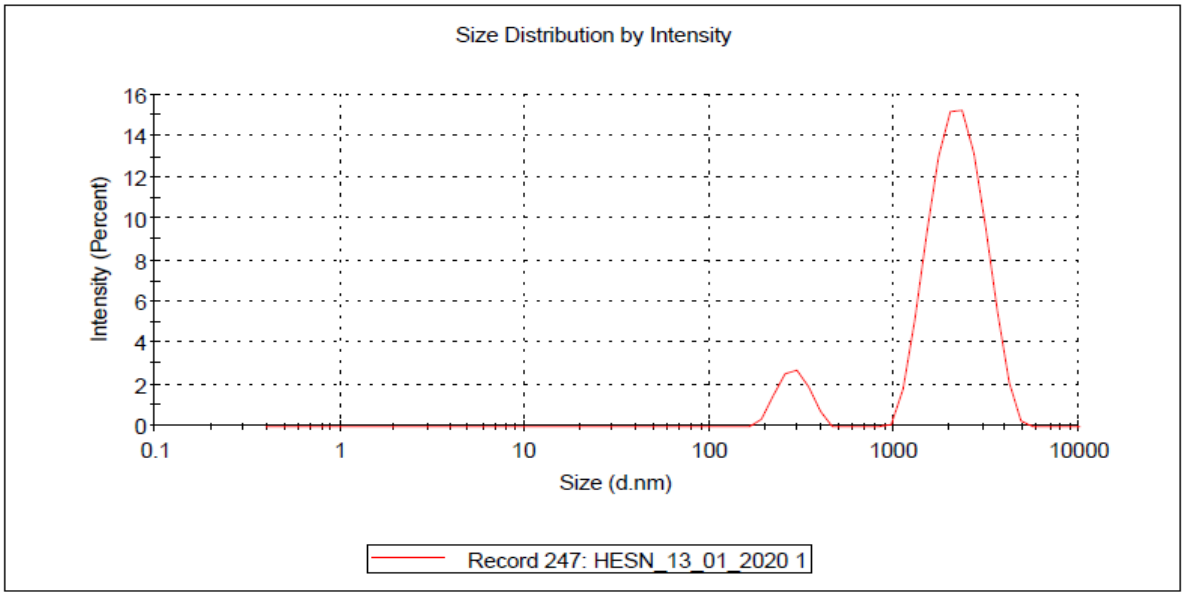
Figure 3 – SEM image of nanoparticles from Acetone extract of *Bombax ceiba*

III.5. Particle size analysis-

The particle size analysis of nanoparticles of *B. ceiba* acetone extract was performed. From spectrum 2, it was observed that two peaks were obtained, peak 1 is at 226.4 nm and peak 2 at 286.2 nm. The Z- average was found to be 1504 nm.

Table 5 - Size and % Intensity of Nanoparticles

	Size (d. nm)	% Intensity	Std Dev
Peak 1	2264	90.3	711.7
Peak 2	286.2	9.7	5.93

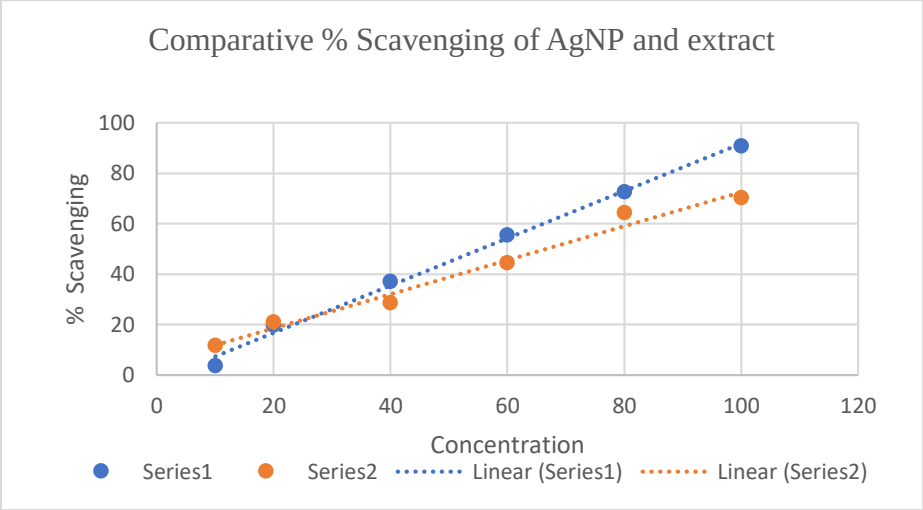


Spectrum 2 - Size distribution of Acetone extract AgNPs by Intensity

III.6. Antioxidant activity-

The demonstrated modified spectrophotometric method makes use of the 2,2-diphenyl-picryl hydrazyl (DPPH) radical and its specific absorbance properties. The absorbance decreases when the radical is reduced by antioxidants. In contrast to other investigations, the absorbance was measured at a wavelength of 517 nm. This wavelength enabled the measurements of the stable free DPPH radical. For estimating the antioxidative potential of chemical components different experimental approaches were used. Most of them require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results.

The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening of many samples for radical scavenging. By using ascorbic acid as an internal standard. And the IC50 is the concentration of an inhibitor where the response (or binding) is reduced by half. The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. The degree of discoloration of violet color of DPPH, as it gets reduced, indicated the radical scavenging potential of the antioxidant. Results of the DPPH scavenging activity of the studied samples, expressed as IC_{50} value that represent the concentration of the sample required to scavenge 50% of DPPH radical. The results of free radical scavenging activity DPPH of acetone extract and its nanoparticles were presented in table 6. The IC50 value of acetone extract was found to be 66.63 and IC50 value of nanoparticeswas found to be 51.18. It was observed that acetone extract nanoparticles shows better anti-oxidant activity than acetone extract.



Graph 1- Comparative % Scavenging of AgNPs and Acetone Extract

III.7. Anti-microbial activity

The Minimum inhibitory concentration of the extracts and their nanoparticles was performed against *E.coli* and *S. aureus* strains of bacteria..It was observed that acetone extract shows MIC at 110mg/ml whereas acetone extract nanoparticles shows MIC at 100mg/ml against *E.coli* and *S.aureus* bacteria .

Table 6 - MIC Concentration and Antioxidant activity of extracts and Nanoparticles

Samples	Minimum Inhibitory Concentration (mg/ml)		IC 50 Value
	E. coli	*S. aureus*	
Acetone Extract	110	110	66.63
Acetone extract nanoparticles	100	100	51.18

III.8. Peel off Formulation-

The peel off gel formulation was prepared with incorporation of acetone extract silver nanoparticles and various physical parameters were evaluated. The reults are depicted in table 7 and fig. 4 shows the peel-off mask obtained when applied on skin and allowed to dry. It was observed that the formulation was black in colour with semisolid consistency and no odour, it was homogenous in nature. Its pH was 7..5 with spreadibility of 17mm and 17.6 minutes of drying time. There was no sign of skin irritation or edema or erythema formation. The formulation was kept in air thight container for 3 months, it was stable and no physical changes were observed

Table 7 - Evaluation of formulation

Sr. No	Parameters	Observation
1	Colour	Black
2	Consistency	Semisolid
3	Odour	Odourless
4	Homogenenity	Homogenous
5	Spreadibility	17 mm
6	pH	7.5
7	Drying time	17.6 mins
8	Skin irritation study	0.0
9	Erythema and edema fomation	0

Fig 4– Peel- off mask preparation and application

IV. Conclusion-

Green synthesis of silver nanoparticles has gain importance because other conventional method of synthesis of nanoparticles are expensive and toxic. Silver nanoparticles has gained importance due its unique properties and enhanced antibacterial activity of the extract. The present study reveals that the nanoparticles from extracts of thorns of *Bombax ceiba* can be prepared by synthesis at higher temperature, which shows better anti-oxidant activity and anti-bacterial activity than the simple extracts. The silver nanoparticles prepared from *Bombax ceiba thorns* extract can be further used in various formulations for different pharmacological properties.

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