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Green synthesis and sunscreen formulation of silver nanoparticles of corilagin and evaluation of antimicrobial and antioxidant activity

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Abstract
Corilagin, as a gallotannin, is a major active component of many ethnopharmacological plants such as *Phyllanthus Niruri* L., *P. emblica* L. and *P. urinaria* L., *etc*. from divi-divi (*Caesalpinia coriaria).* Corilagin was found to exert a variety of pharmacological effects, including anti-tumor, anti-microorganism, antioxidant, hepatoprotective, anti-inflammatory, neuroprotective and cardiovascular protective activities and has been found to be beneficial in managing type II diabetes.
Nanoparticles are particles of matter which particles size is 1-100 nm in diameter sometimes its large up to 500 nm. Nano particles are not simple molecules itself and therefore composed of three layers i.e. (a) The surface layer, which may be functionalized with a variety of small molecules, metal ions, surfactants and polymers.
(b) The shell layer, which is chemically different material from the core in all aspects, and (c) The core, which is essentially the central portion of the NP and usually refers the NP itself.
Creams are the topical preparations which can be applied on the skin. Creams are defined as "viscous liquid or semi-solid emulsions of either the oil-in-water or water- in-oil type" dosage forms which consistency varies by oil and water. Creams are used for cosmetic purposes such as cleansing, beautifying, improving appearances, protective or for therapeutic function. These topical formulations are used for the localized effect for the delivery of the drug into the underlying layer of the skin or the mucous membrane.
The study showed that formulation F2 was found to be more stable with high SPF value, proving a better sunscreen cream.

Keywords: Corilagin, extraction, Silver nanoparticles, sunscreen, antimicrobial, antioxidant

1. Introduction
Sunscreen are essential part of our daily skin care routine. UV radiation is essential to human health such that it helps in the intestinal absorption of calcium, phosphorous and for the production of vitamin D3. On the other hand, these radiations also harm our health by directly interacting with DNA, RNA proteins, lipids and thereby causing potential carcinogenic effects. The most efficient way to protect skin from harmful UV radiation is the topical application of any active molecule which has UV absorbing or reflecting properties. This is why the sunscreen has gained importance in the current scenario. We use silver nanoparticles of corilagin to synergistic effect and increase the antimicrobial as well as antioxidant properties of corilagin [1].
Silver nanoparticles are small particles of silver that are synthesized using chemical or green route methods. They have unique physicochemical properties and are cost- effective. Silver nanoparticles are known for their antimicrobial and antiviral activities, making them useful in various applications such as wound healing, protective surface coatings, textiles, and biomedical applications. Recently, biologically-mediated synthesis of nanoparticles have been shown to be simple, cost effective, dependable, and environmentally friendly approaches and much attention has been given to the high yield production of AgNPs of defined size using various biological systems including bacteria, fungi, plant extracts, and small biomolecules like vitamins and amino acids as an alternative method to chemical methods not only for AgNPs, but also for the synthesis of several other nanoparticles, such as gold and graphene. Several studies reported the synthesis of AgNPs using green, cost effective, and biocompatible methods without the use of toxic chemicals in biological methods. Anti- microbial activity

which inhibits the growth of or kills the micro-organisms, including bacteria, viruses, fungi etc. corilagin has been found to be inhibit the growth of bacteria. Antioxidants refers to a substance or molecule that inhibits the oxidation of other molecules, thereby preventing cell damage and promoting overall health. It linked to various health benefits including reducing inflammation, preventing chronic diseases, improving cognitive function and enhancing skin health [2].

2. Materials and Methods

2.1 Extraction of Plant Extract [3]

Fig 1: Soxhlet apparatus

Materials: *phyllanthus emblica* (amla) powder, methanol.
Method: *Phyllanthus emblica* fruits are typically dried, grund into powder and then extracted using a methanol as solvent. Extraction is done using Soxhlet apparatus. For the extraction we use Dry powder of Phyllanthus emblica and methanol as solvent. And arrangements made in Soxhlet apparatuses. And kept for 3 hours at 60o.collect the extract. The extract is filtered to remove solid particles, and may be centrifuged to further clarify the solution. The extract is filtered to remove solid particles, and may be centrifuged to further clarify the solution. The solvent is evaporated to concentrate the extract, often using a rotary evaporator. And prepared extract is used for further studies.

2.2 Photochemical Screening of Amla Extract [4]

It can be performed for establishing profile of ethanol and aqueous extract for its chemical composition. The following tests were performed on extract to detect various phytoconstituents present in it.

1. Detection of alkaloids
Hager's test
1 ml of Hager's reagent with 1 ml of extract, yellow colour appeared.

2. Mayer's test
1 ml of Mayer's reagent with 1 ml of extract gives cream colour ppt.

3. Test for saponin
Foam test: 1 ml of extract with water forms foam.

4. Test for glycosides
Fehling's test
Few drops of Fehling's solution with 1 ml of extract appearance of yellowish ppt.

4. Test for flavonoids
Alkaline test
Few drops of NaOH solution with 1 ml of extract appearance of dark brown colour.

5. Detection of tannin
Ferric chloride solution test
To 1ml of the extract, ferric chloride solution was added. Appearance of green colour indicates the presence of tannins.

2.3 Green Synthesis of Corilagin [5]

Fig 2: Silver nanoparticles of corilagin

- **Materials:** Silver nitrate AgNO3, *Phyllanthus emblica* extract, distilled water, Glass beakers.
- **Method:** Synthesis of AgNPs by green synthesis process.

Preparation pf silver nitrate solution
1 mM silver nitrate solution in double distilled water was the source of silver.

Combine the silver nitration solution with herbal extract
Phyllanthus emblica fruit extract (0.5% (w/v)) was mixed drop by drop in aqueous
0.001 M AgNO3 solution and kept in boiling tube at around 25oC temperature till 12 h aging. Then the solution of a mixture of silver nitrate turns brown with increasing time interval. Synthesized silver nanoparticles were separated by centrifugation technique at 5000 rpm for 20 minutes. For the further settlement of particles, the supernatant material was transferred to a beaker and frequent centrifugation process was carried out to clean AgNPs. The obtained nanoparticle pellet was dry in an oven and stored for further study.
We prepared different preparation of silver nanoparticles with suitable concentration that we can incorporate in our formulation. The prepared concentration of silver nanoparticles is 0.1mg/mL, 0.2mg/mL, 0.5mg /mL. these different concentrations mixed according to get feasible product.

2.4 Formulation of Corilagin Sunscreen [7]

Sr no	Ingredients	F1	F2	F3
1	Corilagin	0.1 gm	0.2 gm	0.5 gm
2	Stearic acid	2.128 gm	2.128 gm	2.128 gm
3	Cetyl alcohol	0.87 gm	0.87 gm	0.87 gm
4	Methyl paraben	0.002 gm	0.002 gm	0.002 gm
5	Olive oil	3 ml	3 ml	3 ml
6	Starch	0.37 gm	0.37 gm	0.37 gm
7	Glycerine	1 ml	1 ml	1 ml
8	Sodium lauryl sulphate	1 ml	1 ml	1 ml
9	Rose oil	0.5 ml	0.5 ml	0.5 ml
10	Zinc oxide	0.5 gm	0.5 gm	0.5 gm
11	Distilled water	Q.s	Q.s	Q.s
	Toal quantity	10 gm	10 gm	10 gm

Method

Preparation of oil phase

The emulsifier (stearic acid) and other oil-soluble components (Cetyl alcohol and olive oil) were dissolved in the oil phase on a hot water bath.

Preparation of water phase

Preservatives and other water-soluble components (methylparaben, glycerol, SLS, starch, triethanolamine, extract) were dissolved in the aqueous phase and mixed and a sufficient quantity of water was added to the mixture on a hot water bath. Then both the phases were mixed, and continuous stirring was done till a homogenous product was obtained. At last UV filters like zinc oxide, Titanium Dioxide etc. is added. Creams were filled in a separate glass container and used for further evaluation.

Fig 3: Sunscreen formulation

2.5 Evaluation OF Sunscreen Formulation [8]

Physical parameters

- **Colour:** The colour of formulation was checked manually and observed.
- **Odour:** The Smell of Formulation was checked by applying preparation on hand and feel the fragrance

Determination of pH: The pH of sunscreens was determined using a pH paper. pH was measured after 1 gm of the formulation was dissolved in 100 ml of newly prepared distilled water for 2 hours. The purpose of this study was to guarantee that the pH of the produced herbal sunscreens is similar to the pH of the skin after 24 hours of use. The results were triple-checked, and S.D. was recorded.

Determination of Viscosity: The Brookfield viscometer was used to test viscosity, with the proper number of spindles selected. A 50 ml beaker was used to hold 50 g of preparation until the spindle groove was dipped and the rpm was set. Sunscreen viscosity was measured at 5, 10, 20, 50, and 100 rpm. The viscosity was computed using the factor obtained from the reading.

Spreadability: The spreadability of sunscreens determined their therapeutic efficiency. The appropriate amount of sunscreen was applied between two slides, and under specified load directions, and the two sides took the time in seconds to slide off.

Spreadability was defined as the amount of time it took to separate two slides in less time. The formula for calculating it is

Spreadability

$$S = M \times L / t$$

Where, M = weight tied to the upper slide, L = length of glass slide, T = time taken to separate the slides

Washability: This test is carried out by simply washing applied sunscreen lotion with water.

Irritancy Test: Mark an area (one sq. cm) on the left hand dorsal surface. The lotion was applied to the specified area and time was noted. Irritancy, erythema, edema was checked if any for regular interval up to 24 hrs and reported.

Accelerated stability Testing: Stability testing of prepared formulation was conducted at room temp, studied for 20 days. And then the formulation was studied at45 ±1°C for 20 days. The formulation was kept both at room and elevated temperature and observed on 0th, 5th, 10th, 15th and 20th day for all the evaluation parameters.

2.6 Antimicrobial Activity [9]

Prepare nutrient agar plate and inoculated with the test organism with the depth of 4-5 mm and then allow it to solidify. Divide the NA plate into four equal portions. Then with the help of star borrower, make four cavities one in each portion. Then fill three cavities with the antibiotic solution and in one fill the standard solution. Slowly incubate the plates at 37 °C for 24 hours after incubation, measure the zone of inhibition.

2.7 Antioxidant Activity [10]

1. Stock solution of DPPH was prepared by dissolving 1.083 mg in 10 ml of methanol

2. Stock solution of formulation (100 ug/ml) was prepared by dissolving 1 ml of formulation in 10 ml of ethanol from this stock solution further dilution were prepared of concentration 10, 20, 30, 40, 50 ug/ml using ethanol
3. Similarly stock solution of standard ascorbic acid 1mg/ml was prepared by dissolving 10mg ascorbic acid in 10 ml ethanol. From this stock solution further dilution of concentration 1, 2, 3, 4, 5 ug/ml were prepared.
4. Absorbance of blank 5ml ethanol and 1ml DPPH as positive control was recorded using UV at 420nm.
5. Similarly, absorbance of formulation and comparative standard Eugenol were taken at 420 nm and recorded.
6. Since the colour of the formulation after adding DPPH was interfere with absorbance, absorbance of all concentration of formulation were taken before addition of DPPH and those subtracted from the final absorbance of all concentration to remove the error.

2.8 Protection Factor (Spf) Value [11]

The SPF value was measured using a UV spectrophotometer at a wavelength of 290-400 nm using a test solution concentration at 125 mg/L and ethanol as a blank. The absorption data were read at 5 nm intervals. The measurement was repeated three times. SPF values were counted using the following equation**.**

$$SPF = Cf \times \sum 400nm/\ 290\ nm\ EE\lambda\ I\lambda\ Abs\lambda$$

Where,
Cf: correction factor
I: intensity of the photon
EE: the Erythemogenic effect.
Abs: absorbance by the sample

Results and Discussion

Preliminary Phytochemical Screening of Amla Extract

Sr no	Phytochemical test	Test performed	Colour observed	Interference
1	Alkaloids	Mayer's test Hager's test	Cream colour ppt Yellow ppt	+
2	Glycosides	Fehling's test	Yellowish ppt	+
3	Flavonoids	Alkaline test	Dark brown colour	+
4	Tannins	Ferric chloride test	Green colour	+
5	Saponin	Foam test	Foam appeared	+

Evaluation Parameters of Sunscreen

Sr no	Parameters	F1	F2	F3
1	Appearance	Light yellow	White	Grey
2	Washability	Washable	Washable	Washable
3	Homogenicity	Homogenous	Homogenous	Homogenous
4	pH	6	7	8
5	Viscosity (cP)	194	192.4	183.2
6	Irritancy test	No irritancy	No irritancy	No irritancy
7	Spreadability	Good	Good	Good
8	Type of emulsion	o/w	o/w	o/w

Antimicrobial Evaluation

Sr no	Test culture	Standard	Zone of inhibition		
			Formulation		
		Ampicillin	F1	F2	F3
1	*E. coli*	10.0 mm	6.0 mm	8.0 mm	9.0 mm
2	*S.auereus*	8.0 mm	6.0 mm	7.0 mm	8.0 mm

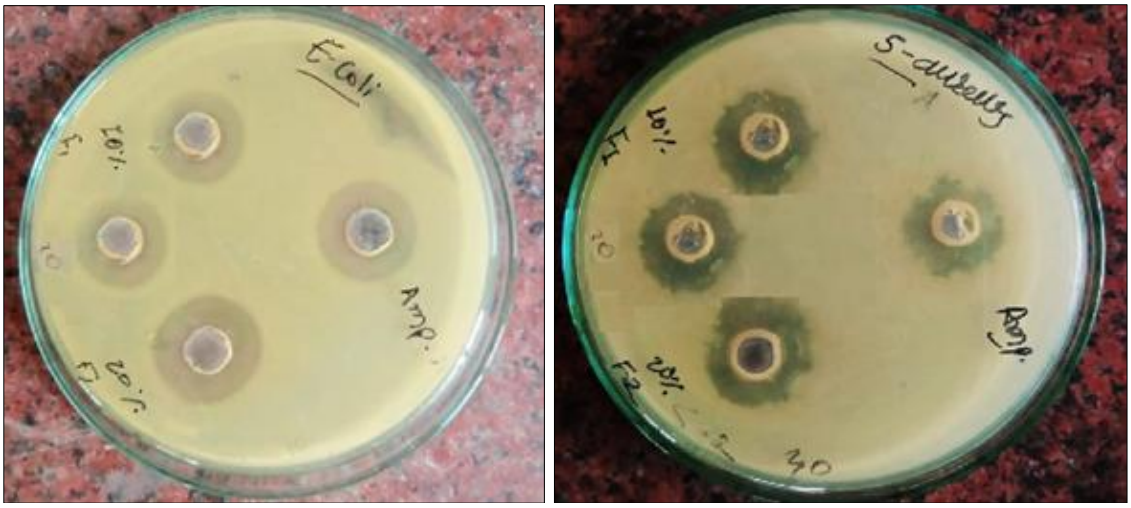
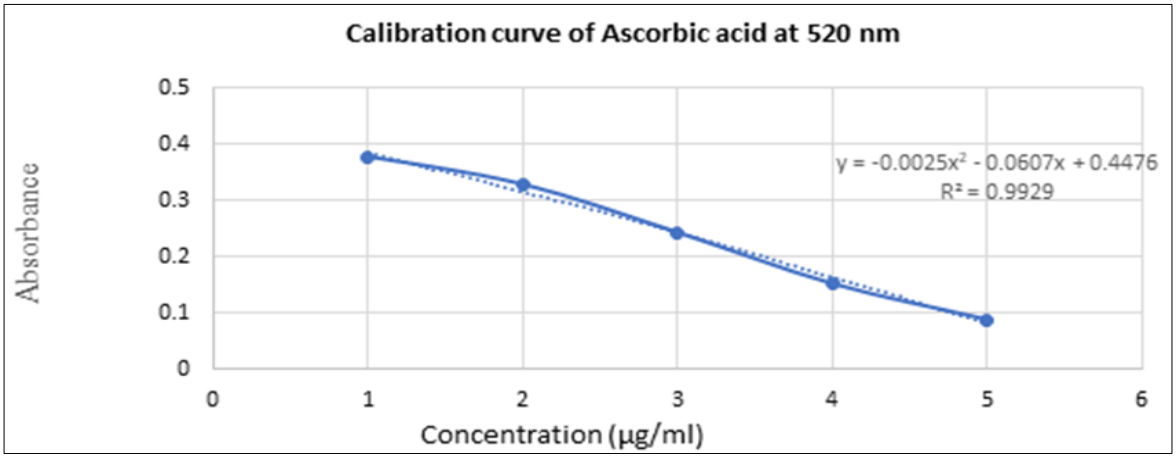


Fig 4: Antimicrobial evaluation

Evaluation of Antioxidant Activity

$$RSA\ \% = \frac{Acontrol - Asample}{Acontrol} \times 100$$

Antioxidant acitvity of cream fromulatipm by dpph
Antioxidant activity of ointment formulations by DPPH method (Control DPPH - 0.412…@520nm)
For standard_ Antioxidant activity of Ascorbic acid



Sl. No.	Concentration of ascorbic acid µg/ml	Absorbance of blank	Absorbance of ascorbic acid	Percentage of free radical inhibition
1	1	0.411	0.378	8.03%
2	2		0.349	15.09%
3	3		0.243	40.88%
4	4		0.152	63.02%
5	5		0.088	78.59%

Antioxidant activity of formulation 1

Sl. No.	Concentration of Sample F1 µg/ml	Absorbance of blank	Absorbance of sample	Percentage of free radical inhibition
1	10	0.411	0.371	9.73%
2	20		0.321	21.90%
3	30		0.233	43.31%
4	40		0.144	64.96%
5	50		0.078	81.02%

Antioxidant activity of formulation 2

Sl. No.	Concentration of Sample F3 µg/ml	Absorbance of blank	Absorbance of sample	Percentage of free radical inhibition
1	10	0.411	0.321	21.90%
2	20		0.298	27.49%
3	30		0.226	45.01%
4	40		0.125	69.59%
5	50		0.046	88.81%

Antioxidant activity of formulation 3

Sl. No.	Concentration of Sample F2 µg/ml	Absorbance of blank	Absorbance of sample	Percentage of free radical inhibition
1	10	0.411	0.355	13.63%
2	20		0.312	24.09%
3	30		0.221	46.23%
4	40		0.131	68.13%
5	50		0.072	82.48%

SPF Determination

$$SPF = Cf \times \sum^{400nm/\ 290\ nm} EE_\lambda\ I_\lambda\ Abs_\lambda$$

Where,
Cf: correction factor
EEλ: Erythemogenic effect
Iλ: intensity of the photon
Absλ: absorbance by the sample

Calculation
Cf = 10
EE λ x I λ x Abs λ of individual formulation is calculated in the table. Then at last their summation is added to calculate the SPF of individual formulation

Wavelengt h	I λ	EE λ	Abs λ λ x Abs	EE λ x I λ	Abs λ x Abs λ	EE λ x I λ	Abs λ Abs λ	EE	λ x I λ x
290	0.041	0.015	1.843	0.02764	2.995.0.0449		3.2733	0.49099	
295	0.083	0.030	1.448.	0.1183	4.739	0.3872	3.4743.	0.28385	
300	0.153	0.050	0.837	0.2405	1.735	0.4997	0.9246	0.266099	
305	0.243	0.070	1.423	0.4665	2.964.	0.9716	1.0413	0.34133	
310	0.351	0.100	0.872.	0.1625	1.925.	0.3588	3.1486	0.58689	
315	0.473	0.120	1.205	0.1009	1.975	0.1653	2.8856.	0.023961	
320	0.610	0.150	1.294	0.0233	2.839.0.0511		3.0563.	.054946	
			Total: - 1.139		Total: - 2.4786		Total: -1.606		
			SPF: - 11.39		SPF: - 24.786		SPF: -16.06		

Discussion

The present study focused on the development of herbal sunscreen from the silver nanoparticles of Corilagin, a ellagotannin compound found in *Phyllanthus emblica* (Amla) known for its antimicrobial and antioxidant properties. For preparing the silver nanoparticles of corilagin we used the green synthesis method which is simple, cost effective and environmental friendly. To assess the sunscreen we evaluated the antimicrobial and antioxidant activity of the formulations.

Among the different formulations developed, F2 was found to be the most desirable characteristics for the sunscreen in terms of stability, texture and other properties. The successful development of this sunscreen formulation highlights its potential as an effective skin care product.

Conclusion

The study successfully demonstrated extraction of herbal extract (Amla) and green synthesis of silver nanoparticles (AgNPs) using corilagin. AgNPs enhanced antimicrobial and anti-oxidant properties of the formulation. Anti-oxidant activity is also significant, protecting skin from oxidative stress. The sunscreen cream is used to protect the skin from UV radiation of the sun which cause skin damage and tanning. Sunscreen formulation will show stability and consistency. Evaluation of antimicrobial and antioxidant activity. These Corilagin sunscreen might provide cost effective, truly broad-spectrum sunscreen product with skin protective effect.

The study aimed to formulate and develop herbal sunscreen (cream) using Corilagin. The formulation was prepared by varying the composition & evaluated for their physicochemical properties and SPF.

The Study showed that Formulation F2 was found to be more stable with high SPF value, proving a better sunscreen cream.

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