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FORMULATION AND CHARACTERIZATION OF AGNPs OF *OPERCULINA TURPETHUM* FOR ANTI- MICROBIAL ACTIVITY

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ABSTRACT

Operculina turpethum, a medicinal plant with a rich history in traditional medicine, has gained attention for its potential antimicrobial properties. In this study, we aimed to harness the antimicrobial attributes of *Operculina turpethum* by formulating and characterizing silver nanoparticles (AGNPs) using an eco-friendly approach. The synthesized AGNPs were thoroughly characterized to understand their physicochemical properties, and their antimicrobial activity was evaluated against selected microbial strains. The synthesis of AGNPs was achieved through a green and sustainable method, ensuring minimal environmental impact. Antimicrobial assays against a panel of selected microbes revealed the potency of AGNPs of *Operculina turpethum*. The AGNPs exhibited significant inhibition zones, indicating their effectiveness against the tested microbial strains. This antimicrobial activity holds potential for diverse applications in pharmaceuticals, cosmetics, and medical devices, where the need for safe and effective antimicrobial agents is paramount. This study not only highlights the successful formulation and characterization of AGNPs from *Operculina turpethum* but also underscores their role as promising antimicrobial agents. The eco-friendly synthesis and potent antimicrobial properties of these AGNPs open new avenues for their utilization in various products and healthcare solutions.

Key words: *Operculina turpethum,* silver nanoparticles, Formulation, Characterization

INTRODUCTION

In the field of nanotechnology, the synthesis and characterization of nanoparticles have emerged as a compelling area of research with far-reaching applications. Among these, silver nanoparticles (AGNPs) have garnered significant attention for their antimicrobial properties, making them promising candidates for a wide range of biomedical and industrial applications. As the world faces escalating challenges in managing microbial infections and developing safe antimicrobial agents, the role of AGNPs derived from natural sources becomes especially pertinent.

Operculina turpethum, commonly known as "Indian Jalap" or "Turpeth Root," is a medicinal plant deeply rooted in traditional Ayurvedic medicine (Singh *et al.,* 2017). It has long been recognized for its therapeutic potential, including its purgative and anti-inflammatory properties (Khosla *et al.,* 2015). Moreover, *Operculina turpethum* possesses compounds with antimicrobial attributes, making it a valuable source for the synthesis of AGNPs with inherent antibacterial and antifungal activities (Vanaja *et al.,* 2012).

The synthesis of AGNPs from natural sources is gaining prominence due to its eco-friendly nature and sustainable practices (Raveendran *et al.,* 2003). The synthesis methods involve the reduction of silver ions using biologically active compounds present in plant extracts. This eco-friendly approach not only reduces the environmental footprint but also promotes the utilization of indigenous flora with medicinal significance.

Silver nanoparticles are known for their unique physicochemical properties, such as size-dependent optical and catalytic attributes, which contribute to their antimicrobial efficacy (Rai *et al.,* 2009). The application of AGNPs in healthcare settings includes wound dressings, drug delivery, and medical devices, where their ability to combat microbial infections without the adverse effects associated with conventional antibiotics is of paramount importance (Durán *et al.,* 2005).

This study endeavors to harness the antimicrobial potential of *Operculina turpethum* by synthesizing AGNPs using an environmentally friendly approach. The synthesized AGNPs will be comprehensively characterized to understand their size, morphology, and stability. Furthermore, their antimicrobial activity will be assessed against selected microbial strains.

MATERIALS & METHODS

Extraction by maceration process

Defatted dried powdered leaves of *Operculina turpethum* has been extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC (Mukherjee, 2007; Kokate, 1994).

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Audu *et al.,* 2007).

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method (Gaur Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Gaur Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Biosynthesis of Silver nanoparticles

$AgNO_3$ powder was dissolved in distilled water to prepare 10 mM $AgNO_3$ stock solution from which a series of 1 mM, 2 mM

and 3 Mm $AgNO_3$ solutions were prepared (Ponarulselvam *et al.,* 2012). The $AgNO_3$ solutions were mixed with the extract of leaves of *Operculina turpethum* at a ratio of 1:1, and 1:2 (v/v) to a volume of 50 mL in a flask. The flask was wrapped with an aluminum foil and was then heated in a water bath at 60°C for 5 hours. Furthermore, the mixture was stored in the refrigerator for the further use.

Table 1: Different formulation of Silver nanoparticles

Formulation Code	Extract (mg)	$AgNO_3$ (mM)	Ratio
F1	250	1	1:1
F2	250	2	1:1
F3	250	3	1:1
F4	250	1	1:2
F5	250	2	1:2
F6	250	3	1:2

Characterization of synthesized silver nanoparticles formulations

Percentage yield

The prepared silver nanoparticle with a size range of 200-300nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres (Vanaja *et al.,* 2013; Umashankari *et al.,* 2012).

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. Silver nanoparticle entrapped extract were isolated from the free drug using dialysis method. The above said formulations were filled into dialysis bags and the free drug dialyzed for 24 hr. into 50 ml of buffer pH 1.2. The absorbance of the dialysate was measured against blank buffer pH 1.2 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free flavonoids could be obtained from the absorbance difference based on standard curve (Banerjee *et al.,* 2014).

Surface charge and vesicle size

The particle size and size distribution and surface charge were obtained by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the silver nanoparticles was based on the zeta potential that was estimated according to Helmholtz–Smoluchowsky from electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm.

Formulation development of silver nanoparticle gel

Measured amounts of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of leaves of *Operculina turpethum* were dissolved in about 100 ml of water in a beaker and stirred at high speed using mechanical stirrer (or sonicator) (Raut *et al.,* 2009). Then Carbopol 940 was slowly added to the beaker which contained above liquid while stirring. Neutralized the solution by adding a slow, constantly stirring triethanolamine solution until the gel formed.

Table 2: Formulation of gel

Ingredients (mg)	F1	F 2	F3
Operculina turpethum silver nanoparticles	250	250	250
Carbopol 940	250	500	750
Polyethylene Glycol 600	0.2	0.2	0.2
Methyl Paraben	0.08	0.08	0.08
Triethanolamine	1.0	1.0	1.0
Distilled Water	100 ml	100ml	100ml

Evaluation of gel

Appearance and consistency:

The physical appearance was visually checked for the texture of gel formulations and observations reported in table.

Washability

Prepared formulations were added to the skin and then manually tested for ease and degree of washing with water, and findings were recorded in table.

Extrudability determination of formulations

The gel formulations were filled into aluminium collapsible tubes and sealed. The tubes were pressed to extrude the material and the extrudability of the formulation was noted.

Determination of Spreadability

Two normal dimensional glass slides (6x2) were chosen. The gel formulation the spreadability of which had to be determined was placed over one of the diapers. The second slide was mounted over the slide in such a way as to sandwich the formulation over the slide over a length of 6 cm between them. The upper slide had 20 grams of weight, so that the gel formulation between the two was placed uniformly to form a thin layer (Ruiz-Baltazar *et al.,* 2017).

The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cm and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each formulation.

$$\boldsymbol{Spreadability} = \frac{m * l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 gram)

l= length of glass slide (6cm).

t = time taken is seconds.

Viscosity

The viscosity of the prepared gel was determined by a Brookfield digital viscometer. The viscosity was assessed using spindle no. 6 at 10 rpm at ambient room temperature of 25-30^{o}C. Reasonable large bottle for the mouth loaded the correct volume of gel. Usage of large mouth container to allow viscometer spindle within the jar. Viscosity value was noted down after stable of reading. Gel samples were allowed to settle more than 30minutes before the measurements at the constant room temperature.

Drug content

The composition of the medication was measured by taking 1gm of gel mixed with methanol in 10 ml volumetric flask. 3 ml of stock solution has been mixed with 1 ml $AlCl_3$ solution of 2 percent. The mixture was vortexed for 15s and allowed for the color

production to stand at 40°C for 30min, using a spectrophotometer the absorbance was measured at 420 nm (Ajitha *et al.,* 2015).

Determination of pH

Digital pH meter had calculated the pH of the gels. One gram of gel was dissolved in 25 ml of purified water and the electrode was then dipped into gel solution until steady reading was achieved. Measurements of pH were repeated twice for each formulation.

***In vitro* diffusion profile (*In vitro* permeation in rat skin)**

In vitro diffusion experiments were performed using franz diffusion cell for all formulations. Locally assembled as an open-ended cylindrical tube with an area of 3.7cm^2 and a height of 100 mm with a diffusion area of 3.8 cm^2. Phosphate buffer (pH 7.4) was used as substrate for receptors. Rat abdominal skin used as membrane for dialysis. The skin was tied to the diffusion cell (donor cell) such that the stratum corneum side of the skin was in intimate contact with the release surface of the formulation in the donor cell. Isotonic phosphate buffer solution, pH 7.4 (100 ml) was added to a donor compartment prior to be mounted on the diffusion cell. A weighed quantity of formulation equivalent to 1g of gel was taken on to the rat skin and was immersed slightly in 100 ml of receptor medium, which was continuously stirred. The whole network had been held at 37±1^{o}C. At different time intervals of up to 4 hours, an aliquot of 5 ml was extracted, and spectrophotometrically measured at 295 nm. The diffusion media was replaced with an equal volume of fresh diffusion medium after each withdrawal. For each time period the total percent release was measured.

Antimicrobial activity of silver nanoparticle gel

The well diffusion method was used to determine the antimicrobial activity of silver nanoparticle gel prepared from the leaves of *Operculina turpethum* using standard procedure (Bauer *et al.,* 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

RESULTS AND DISCUSSION

The phytochemical study revealed the presence of flavonoid, phenol, protein, carbohydrate, saponin & diterpene. Total flavonoid & phenol content in hydroalcoholic extract of *Operculina turpethum* was found to be 0.826 and 0.474 mg/ 100 mg respectively. Further six different formulations of nanoparticle was created, among them the maximum % efficiency was observed in F3 formulation with entrapment efficiency of 0.712±0.013 while the % yield was found to be also maximum for F3 which is 76.65±0.32%. Further, the average particle size & zeta potential for F3 was estimated to be 220.5nm & - 38.5 mV respectively. Further the nanoparticle were incorporated into gel. About three formulations of gel were created. All was observed to be brown in color, with good washability & extrudibility. The texture of gel was smooth with absence of clogging and zero homogeneity.

The spreadibility for F1, F2 & F3 was found to be 10.23±0.15, 9.65±0.22 & 8.78±0.16 gcm/sec. The viscosity for F1, F2 & F3 was calculated to be 3365±15, 3215±10 & 3162±16 cp respectively. The flavonoid content was found to be 0.665, 0.712 & 0.698 in F1, F2 & F3 formulation respectively.

The pH of gel for F1 & F3 was observed to be 7.25±0.02 and for F2 was found to be 7.05±0.01. The % Cumulative drug Release was observed to be maximum for F1 which is 99.85 % while for F2 & F3 it was 98.85 % & 86.65% respectively.

The *in vitro* drug release data for gel F2 was then conducted as the maximum flavonoid content was associated with F2. Additionally the release kinetics for F2 formulation was then conducted. The regression value for zero order & first order reaction was found to be 0.968 and 0.863.

Finally the antimicrobial activity for Extract coated with AgNP & Gel incorporated with AgNP was performed against *Staphylococcus aureus*. The zone of inhibition for 25, 50 & 100 mg/ml of extract was observed to be 9±0.15, 10±0.20 & 11.3±0.17mm. While the zone of inhibition with Silver nanoparticles gel at same concentration was observed to be 11±0.12, 13±0.10, and 15.8±0.15mm.

Table 3: Phytochemical screening of extract of leaves of *Operculina turpethum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam Test	-ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	-ve

Table 4: Estimation of total flavonoids and phenol content of leaves of *Operculina turpethum*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.826	0.474

Table 5: Determination of % yield of prepared silver nanoparticles formulations

Formulation code	% Yield
F1	64.25±0.12
F2	68.850±0.25
F3	76.65±0.32
F4	70.12±0.18
F5	69.85±0.32
F6	68.78±0.11

Table 6: Determination of entrapment efficiency of prepared formulations

Formulation code	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	0.612±0.012
F2	0.658±0.015
F3	0.712±0.013
F4	0.652±0.012
F5	0.589±0.019
F6	0.557±0.013

Table 7: Results of physical characteristics

Formulation code	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
F1	Brown	Absent	Good	Smooth	Good	Good
F2	Brown	Absent	Good	Smooth	Good	Good
F3	Brown	Absent	Good	Smooth	Good	Good

Table 8: Results of spreadability of gel

Formulation code	Spreadability* (gcm/sec)	Viscosity* (cp)	Flavonoid Content (mg/100mg)	pH
F1	10.23±0.15	3365±15	0.665	7.25±0.02
F2	9.65±0.22	3215±10	0.712	7.05±0.01
F3	8.78±0.16	3162±16	0.698	7.25±0.02

*Average of three determinations (n=3 ±SD)

Table 9: *In-vitro* drug release data for gel F2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.25	0.5	-0.602	22.36	1.349	77.64	1.890
0.5	0.707	-0.301	33.36	1.523	66.64	1.824
1	1	0	45.58	1.659	54.42	1.736
1.5	1.225	0.176	59.98	1.778	40.02	1.602
2	1.414	0.301	67.74	1.831	32.26	1.509
2.5	1.581	0.398	79.98	1.903	20.02	1.301
3	1.732	0.477	86.65	1.938	13.35	1.125
4	2	0.602	98.78	1.995	1.22	0.086

Table 10: Release kinetics regression values of formulation F2

Formulation code	Zero order	First order
F2	0.968	0.863

Table 11: Antimicrobial activity against selected microbes

S. No.	Name of drug	Microbes	Zone of inhibition		
			25 mg/ml	50 mg/ml	100 mg/ml
1.	Extract	*Staphylococcus aureus*	9±0.15	10±0.20	11.3±0.17
2.	Silver nanoparticles gel		11±0.12	13±0.10	15.8±0.15

CONCLUSION

This research contributes to the development of potent, eco-friendly antimicrobial agents in the form of AGNPs derived from *Operculina turpethum*. The successful synthesis and characterization of these AGNPs open new avenues for their utilization in healthcare products, cosmetics, and other industries requiring effective and safe antimicrobial agents. As the demand for sustainable and efficient antimicrobial solutions continues to grow, the findings of this study hold significant promise. The results presented here are a testament to the potential of nature-inspired nanomaterials in addressing contemporary healthcare challenges, and they invite further research to explore the full range of applications for AGNPs in diverse sectors.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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