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FORMULATION AND CHARACTERIZATION OF ANTI-BACTERIAL HERBAL SILVER NANOPARTICLE

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ABSTRACT

Plant-based of nanoparticles has generated worldwide interest because of cost-effectiveness, eco-friendly nature and plethora of applications. In the present research work carried out antimicrobial potential of herbal silver nanoparticles of ethanolic extract of *Verbascum Thapsus* leaves has been investigated. Agar well diffusion method was used for determining antimicrobial activity of *Verbascum Thapsus* extracts. Phytochemical analysis of ethanolic extract of *Verbascum Thapsus* revealed the presence of tannins, saponins, steroids, alkaloids, flavonoids, and glycosides. Herbal silver nanoparticles synthesized using *Verbascum Thapsus* ethanolic extract, characterized by UV–Visible spectroscopy. Evaluation of the antimicrobial potential of herbal Silver Nanoparticle recorded the highest inhibitory activity against Staphylococcus Epidermidis. The results obtained indicated that the different crude extracts of *Verbascum Thapsus* plant as well as herbal Silver nanoparticle have a strong and effective antimicrobial potential that provide source for the development of new drug molecules of herbal origin which may be used for the welfare of humanity.

KEYWORD: *Verbascum Thapsus*, Silver nanoparticles, Antimicrobial.

INTRODUCTION

Silver nanoparticles are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. Silver nanoparticles have attracted increasing attention for the wide range of application in biomedicine. Tey are used as antimicrobial agents in wound dressings, as topical creams to prevent wound infection and as anticancer agents. Nano sized metallic particles are unique and can considerably change

physical, chemical and biological properties due to their surface to volume ratio, therefore these nanoparticles have been exploited for various purposes. Green synthesized nanoparticles show high yield, solubility and high stability. Among several synthetic methods for AgNPs biological methods seems to be simple, rapid, non-toxic, dependable and green approaches than can produce well-defned size and morphology under optimized conditions for traditional research.

Silver nanoparticles (AgNPs) are a class of materials with sizes in the range 1–100 nm. The interest in the study of AgNPs with respect to their various different behaviors has recently increased because of their unique and attractive physical, chemical, and biological properties.

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties. Due to their peculiar properties, they have been used for several applications, including as antibacterial agents, in industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopedics, drug delivery, as anticancer agents, and have ultimately enhanced the tumor-killing effects of anticancer drugs. Recently, AgNPs have been frequently used in many textiles, keyboards, wound dressings, and biomedical devices. Nanosized metallic particles are unique and can considerably change physical, chemical, and biological properties due to their surface-to-volume ratio; therefore, these nanoparticles have been exploited for various purposes. In order to fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Generally, conventional physical and chemical methods seem to be very expensive and hazardous. Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability. Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research. In the end, a green chemistry approach for the synthesis of AgNPs shows much promise.

The biological activity of AgNPs depends on factors including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration, and dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type, and the type of reducing agents used for the synthesis of AgNPs are a crucial

factor for the determination of cytotoxicity. The physicochemical properties of nanoparticles enhance the bioavailability of therapeutic agents after both systemic and local administration and other hand it can affect cellular uptake, biological distribution, penetration into biological barriers, and resultant therapeutic effects. Therefore, the development of AgNPs with controlled structures that are uniform in size, morphology, and functionality are essential for various biomedical applications.

2. METHODOLOGY

2.1 Collection of plant material

Leavess of *Verbascum thapsus*were collected from local area of Bhopal in the month of September, 2021.

2.2 Extraction by maceration process

Dried powdered leavess of *Verbascum Thapsus* has been extracted with Ethanol using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

2.3 Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

2.4 Phytochemical Screening

Leavess of *Verbascum Thapsus* extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of alkaloids, glycosides, phenols, flavonoids, Terpenoids, Saponins, Steroids.

2.5 Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method.

2.6 Total Phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

2.7 Biosynthesis of Herbal Silver Nanoparticle

$AgNO_3$ 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.[59] The AgNO3 solutions were mixed with the aqueous extract of leavess of *Verbascum Thapsus* 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.

2.7.1 Optimization of formulation of Herbal Silver Nanoparticles.

Table 2.1: Different formulation of Herbal Silver Nanoparticles.

Formulation Code	Extract (mg)	AgNO3 (mM)	Ratio
F1	500	1	1:1
F2	500	2	1:1
F3	500	3	1:1
F4	500	1	1:2
F5	500	2	1:2
F6	500	3	1:2

2.8 Characterization of Herbal Silver Nanoparticle

2.8.1 Microscopic observation of prepared Herbal Silver nanoparticles

An optical microscope with a camera attachment was used to observe the shape of the prepared silver nanoparticle formulation.

2.8.2 Percentage Yield

The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

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

2.8.3 Entrapment efficiency

Entrapment efficiency was determined by dialysis method. Herbal Silver nanoparticle entrapped extract were isolated from the free drug using dialysis method.

2.8.4 Surface charge and vesicle size

The particle size and size distribution and surface charge were obtained by Dynamic Light Scattering method. Zeta potential measurement of the Herbal Silver Nanoparticlewas based on the zeta potential that was estimated according to Helmholtz–Smoluchowsky from electrophoretic mobility.

2.9 Formulation development of gel

Measured amounts of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of leavess of *Verbascum Thapsus* were dissolved in about 100 ml of water in a beaker and stirred at high speed using mechanical stirrer. Then Carbopol 934 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 2.2: Formulation of gel.

Ingredients (mg)	F1	F 2	F3
Verbascum Thapsus extract	500	500	500
Carbopol 934	500	1000	2000
Glycerin	10	10	10
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

2.10 Evaluation of gel

A. Appearance and consistency

The physical appearance was visually checked for the texture of gel formulations and observations reported in table 3.7.

B. 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 3.7.

C. 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.

D. Determination of Spreadability

Principle

For gels an significant requirement is that it must have strong spreadability. Spreadability is a concept defined to denote the degree to which the gel applies readily to the skin upon application. A formulations medicinal potency also depends on its spread-value.

Spreadability was calculated by following formulation.

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

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.

E. Determination of pH

Digital pH meter had calculated the pH of the anti-acne gels. Measurements of pH were repeated twice for each formulation.

F. 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 %. The mixture was vortexed for 15s and allowed for the color production to stand at 40°C for 30 min, using a spectrophotometer the absorbance was measured at 420 nm.

G. 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.

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

In vitro diffusion experiments were performed using Franz diffusion cell for all formulations.

I. Antimicrobial activity of silver Nanoparticle gel

The well diffusion method was used to determine the antimicrobial activity of the Herbal silver Nanoparticle gel. There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram 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.

3. RESULTS AND DISCUSSION

3.1 Result of Percentage Yield

Table 3.1: % Yield of leaves of *Verbascum Thapsus.*

S. No.	Solvents	% Yield
1.	Ethanol	7.91

3.2 Phytochemical screening of extract

Small portion of the dried extracts was subjected to the phytochemical tests using standard methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract was suitably resuspended into the distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed in the table 3.2.

Table 3.2: Phytochemical screening of leaves extract of *Verbascum Thapsus.*

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

3.3 Results of estimation of total flavonoids and phenol content

Table 3.3: Estimation of total flavonoids and phenol content of leaves extract of *Verbascum Thapsus.*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic Extract	0.735	0.583

3.4 Characterization of Optimized formulation of silver nanoparticles

3.4.1 Percentage Yield

Practical yield of the prepared silver nanoparticles was in the range of 60.15±0.78 to 68.53±0.82.

Table 3.4: Determination of % yield of prepared formulations.

Formulation	% Yield
FS1	64.71±0.39
FS2	63.86±0.38
FS3	68.53±0.82
FS4	62.69±0.67
FS5	60.15±0.78
FS6	65.91±0.67

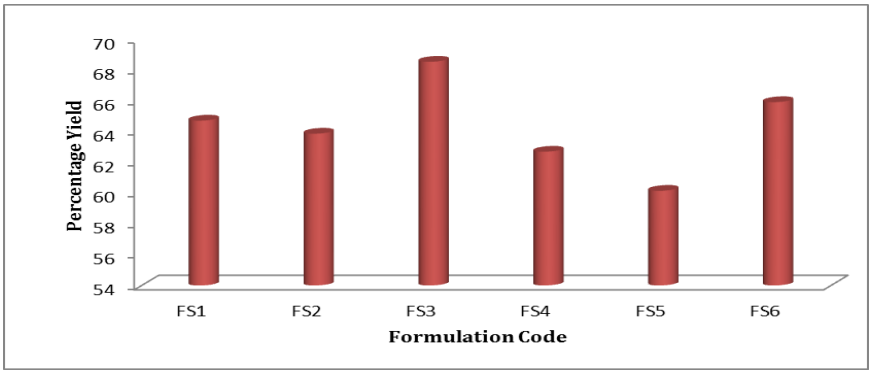

Figure 3.1: Graph of Determination of % yield of prepared formulation.

3.4.2 Results of % Entrapment efficiency

The EE was found to be in the range from 0.562±0.014 to 0.812±0.037%. It was observed that the encapsulation efficiency depends on the concentration of extract and silver nitrate ratio. On the basis of high yield, and encapsulation efficiency batch FS3 was observed as optimized batch for the preparation of silver nanoparticles.

Table 3.5: Determination of entrapment efficiency of prepared formulations.

Formulation	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
FS1	0.677±0.045
FS2	0.782±0.025
FS3	0.812±0.037
FS4	0.749±0.028
FS5	0.562±0.014
FS6	0.592±0.041

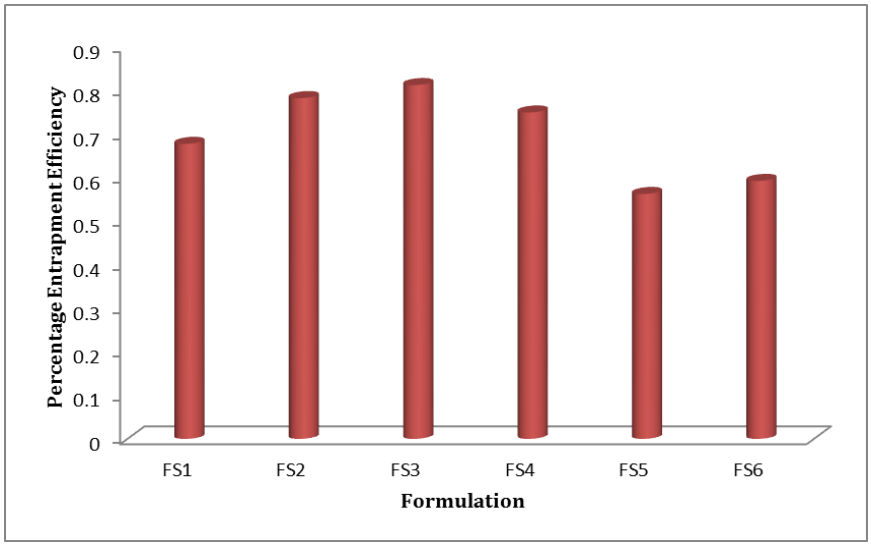

Figure 3.2: Graph of % Entrapment efficiency.

3.4.3 Microscopic observation of prepared silver nanoparticles optimized formulation FS3

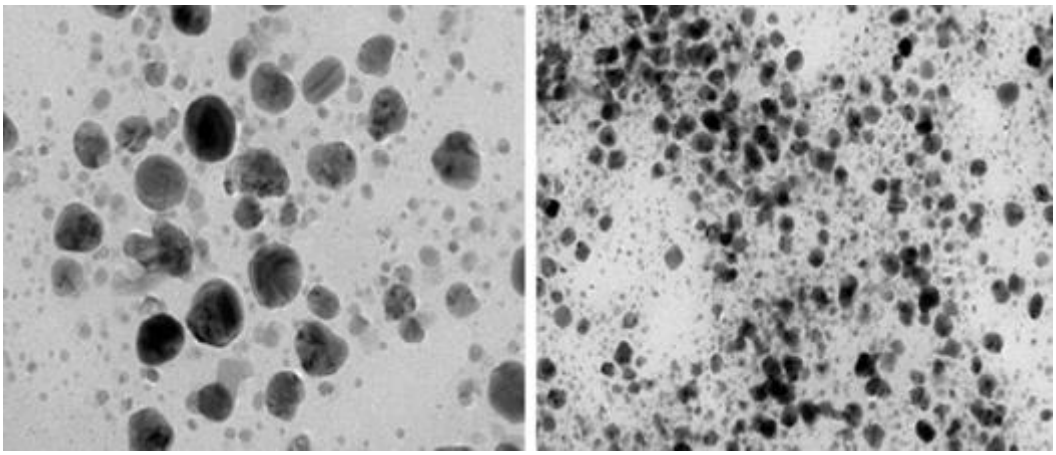

Figure 3.3: Microscopic observation of prepared silver nanoparticles optimized formulation FS3.

3.4.4 Result of Average particle size and zeta potential

Table 3.6: Characterization of Optimized formulation of silver nanoparticles for average particle size and zeta potential.

Formulation	Average Particle size (nm)	Zeta Potential (mV)
FS3	225.1 nm	-39.7 mV

3.5.1 Evaluation of gel Formulation

Table 3.7: Results of Physical Characteristics.

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

3.5.2 Results of Spreadability

Table 3.8: Results of spreadability of gel.

Formulation	Spreadability* (gcm/sec)
F1	10.25±0.11
F2	8.92±0.26
F3	7.63±0.45

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

3.5.3 Results of Viscosity

Table 3.9: Results of Viscosity of gel.

Formulation	Viscosity* (cp)
F1	3379
F2	3127
F3	3018

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

3.5.4 Results of flavonoid Content

Table 3.10: Results of flavonoid content in gel using $AlCl_3$ method

Formulation	Flavonoid Content (mg/100mg)
F1	0.621±0.064
F2	0.917±0.072
F3	0.849±0.055

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

3.5.5 Results of pH

Table 3.11: Results of pH of gel

Formulation	pH
F1	6.97±0.02
F2	7.00±0.01
F3	6.95±0.02

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

3.5.6 Results of *In Vitro* Drug Release Study

3.5.6.1 *In vitro* drug release study of prepared gel formulation

Table 3.12: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release		
		F1	F2	F3
1	0.25	23.59	21.14	14.63
2	0.5	37.72	36.56	25.79
3	1	48.25	47.98	33.36
4	1.5	62.58	57.74	46.62
5	2	82.82	69.95	55.27
6	2.5	92.28	80.98	64.48
7	3	96.65	91.23	76.72
8	4	97.90	99.82	88.73

***In-vitro* drug release study of F1, F2 and F3**

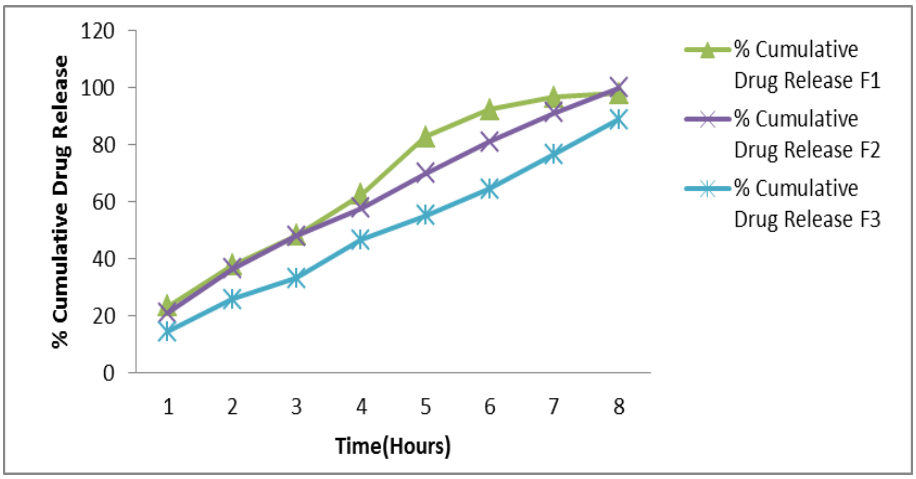

Figure 3.4: Graph of gel formulation F1, F2 and F3.

3.5.6.2 Release Kinetics of gel F2

Table 3.13: Release Kinetics Regression values of formulation F2.

Formulation code	Zero order	First order
F2	0.981	0.875

3.5.7 Antimicrobial activity of extract and prepared silver nanoparticles gel

Table 3.14: 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 Epidermidis	9±0.26	14±0.83	22±0.52
2.	Silver nanoparticles gel		9±0.94	15±0.71	24±0.86

SUMMARY AND CONCLUSION

AgNPs are also known to have unique properties in terms of toxicity, surface plasmon resonance, and electrical resistance. Based on these, intensive works have been conducted to investigate their properties and potential applications for several purposes such as antimicrobial agents in wound dressings, anticancer agents, electronic devices, and water treatment. Small portion of the dried extracts was subjected to the phytochemical tests using standard methods. Phytochemical screening reveals that presents of various phytoconstituents such as saponins, flavonoids, phenol, tannin, carbohydrates and separately for Ethanolic extract of *Verbascum Thapsus*. Total flavonoids and phenol (mg/100mg) was found in Ethanolic extract of *Verbascum Thapsus* 0.735 *and* 0.583 mg/100mg respectively. Practical yield of the prepared silver nanoparticles was in the range of 60.15±0.78 to 68.53±0.82. The yield of nanoparticles decreased with increasing the concentration of extract and silver nitrate, which might be due to generation of stickiness by extract. The EE was found to be in the range from 0.562±0.014 to 0.812±0.037%. It was observed that the encapsulation efficiency depends on the concentration of extract and silver nitrate ratio. On the basis of high yield, and encapsulation efficiency batch F3 was observed as optimized batch for the preparation of silver nanoparticles.

Average particle size of nanoparticles was found to be 225.1 nm. The zeta potential is defined as the electrical potential between the medium and the layer of the fluid attached to the dispersed particles. Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles and is one of the fundamental parameters known to affect stability.

The Optimized gel formulation F2 release approx 21.14 percent drug within 15 minutes and approx 99.82 percent of drug release in 4 hours. When the regression coefficient values were compared, it was observed that 'R^{2}' values of first order were maximum i.e. 0.875 hence indicating drug releases from formulation follow first order release kinetics. In low

concentrations, silver has been indicated as non-toxic material to humans, and it has been assessed as a promising material in pharmaceutical and biomedical fields. Although silver nanoparticles have been investigated for their superior physical, chemical, and biological properties, some issues related to synthesis methods, potential risks to health and the environment and scale-up production still require future works to promote a safer and more efficient use of the nanoparticles. Antibacterial study of the developed formulation showed higher inhibitory activity against *Propionibacterium acnes,* when compared to the extract. The results of our study concluded that silver nanoparticle of *Verbascum Thapsus* in aqueous gel base may be used for the treatment of acne vulgaris.

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