



## FORMULATION AND EVALUATION OF SILVER NANOPARTICLES OF HYDROALCOHOLIC EXTRACT OF *CUSCUTA REFLEXA*

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### Abstract

This study aims at formulation and evaluation of silver nanoparticles of hydroalcoholic extract of *Cuscuta reflexa*. The collected plant material was processed to get extract. Further the qualitative and quantitative estimation of the same was performed. Subsequently the AgNP was synthesized using plant extract and incorporated into gel and evaluated for various parameters. Results showed that the phytochemical analysis of *Cuscuta reflexa* extracts reveals the presence of phytoconstituents such as alkaloids phenol, proteins, carbohydrates, saponins, diterpenes and tannins, which are responsible for biological properties. The highest percentage yield and entrapment efficiency was observed to be  $76.65 \pm 0.14$  and  $0.769 \pm 0.009$  respectively which was associated with F3 formulation. The Average particle size and zeta potential of F3 formulation was found to be 220.5 nm and - 38.5 mV respectively. Further the formulated gel containing AgNP was evaluated for various parameters. The physical formulation of gel GF1, GF2, GF3 were brown in color, smooth in texture, good in washability, extrudability and Homogeneity. The viscosity of GF1, GF2, and GF3 were estimated  $3252 \pm 15$ ,  $3152 \pm 20$  &  $3065 \pm 24$  cp. Also, the highest flavonoid content was noted in GF2 which is  $0.728 \pm 0.032$  mg/100mg. From the results of In vitro drug release study, it was seen that in case of GF1 about 99.82 drug is released in 4 hours while in GF2 and GF3 about 98.87 and 89.65% drug is released in 4 hrs. The release kinetics regression values of formulation GF2 suggested that for zero order and first order the  $R^2$  value was estimated to be 0.955 and 0.827 respectively. The Antifungal activity was tested against *Candida albicans* using agar well diffusion method. The highest inhibition zone of the extract at 100 mg/ml concentration for *Candida albicans* was measured to be  $13 \pm 0.47$ mm, while that for silver nanoparticles gel (GF2) zone of inhibition was measured as  $17 \pm 0.75$ mm at the same concentration. From result of antimicrobial activity it can be interpreted that synergistic effect if Silver Nanoparticles Gel (GF2) was more efficient than *Cuscuta reflexa* extrac alone. The studies show that *cuscuta reflexa* extracts is a potent source of secondary metabolites. The use of the plant in the management of diseases is justified.

**Keywords:** *Cuscuta reflexa*, Phytochemiocals, Silver nanoparticle Gel, Antifungal, *Candida albicans*, AgNP

### Introduction

Ag is preferred as nanoparticle for the reason that it has antibacterial property and nontoxic to human beings. Various methods are used for preparation of silver nanoparticles like physical, chemical and biological. Demand of silver nanoparticle is increasing rapidly in many of the streams like in medical, pharmaceutical companies, healthcare, food, consumer, cosmetics etc. It has been used for its several applications like antibacterial properties, household, medical devices, and food industry, wound dressing, in diagnostic, orthopaedics and an



anticancer agent (Warthan *et al.*, 2010). Normally, physical and chemical methods are found to be costly and dangerous. But the nanoparticles that are prepared from biological method they show high yield, high solubility as well as high stability. Out of all three methods biological method is found to be simple, environmental, commercial and single step method and doesn't need elevated temperature, pressure, force and deadly chemicals. Different materials like leaf extract, bark, root, stem, leaf, fungi etc are used for the synthesis of nanoparticles (Ge *et al.*, 2014).

Medicinal plants have been using from ancient times, and their efficacy has been increasing day by day in the current world. Naturally, acquired compounds are considered as environmentally friendly and also more effective than the synthetic drug. These medicinal Plants represent a foundation for many pharmaceutical compounds since it is comprised of many secondary metabolites which have been used in the treatment of many human diseases. The phytochemical constituents present in plant materials serve as both reducing and capping agent in silver nanoparticle synthesis (Akhila *et al.*, 2012). The goal of the research was to prepare silver nanoparticles by using plant extract then formulate and evaluate various polymers with varying concentrations for the preparation of a safe and effective containing silver nanoparticles and to evaluate the in vitro evaluation and the antibacterial/antifungal activity for prepared formulations.

*Cuscuta reflexa* Roxb. (Cuscutaceae a division of Convolvulaceae) is an extensive climber parasite. It occurs throughout the plains of India. It is more often called dodder in English. Traditional healers called in Hindi Akash bel in Tamil Akashavalli. Other names include hell weed, devil's gut, and beggar weed, strangle tare, scald weed, dodder of thyme, greater dodder, and lesser dodder. In Chinese, Cuscuta seeds are called tu si zi. It has no chlorophyll and cannot make its own food by photosynthesis. Some research studies say that the plant has very low levels of chlorophyll and can slightly photosynthesis. But other species of Cuscuta are entirely dependent on the host plants for nutrition. The stem is thread like filaments it is begin to grow and attach themselves to nearby host plants. The nature plants lives its entire life without attachment to the ground. It has long history of ethnomedicinal use. Cuscuta is a genus of about 100 – 170 species (Vijikumar *et al.*, 2011; Patel *et al.*, 2012).

Rural people of India used juice of *C. reflexa* for the treatment of jaundice, its warm paste is used to treat rheumatism and paste of whole plant is used for the treatment of headache (Siwakoti *et al.*, 1996). *C. reflexa* is used in the treatment of urination disorders, muscle pain and cough and also used as blood purifier. Seeds of *Cuscuta reflexa* have carminative and anthelmintic properties and used to treat bilious disorder (Khan *et al.*, 2010).



Considering above mentioned facts this study aims at formulation and evaluation of silver nanoparticles of hydroalcoholic extract of *Cuscuta reflexa*.

### Collection of plant material

Aerial parts of *Cuscuta reflexa* was collected from local area of Bhopal in the month of January, 2023.

### Extraction by maceration process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs (Jones and Kinghorn, 2005). 42 gm of dried powdered Aerial parts of *Cuscuta reflexa* has been extracted with hydroalcoholic solvent (ethanol : water; 80:20 v/v) using maceration process for 24 hrs, filtered and dried using vacuum evaporator at 40°C.

### Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Savithramma *et al.*, 2011).

### Biosynthesis of Silver nanoparticles

The biosynthesis of silver nanoparticles (AgNPs) using *Cuscuta reflexa* (CR) as a biological agent is a green and eco-friendly method of synthesizing nanoparticles. The process involves the reduction of silver ions to silver atoms in the presence of an active biological agent such as CR extract. The CR extract contains a variety of secondary metabolites, including amino acids, proteins, carbohydrates, and phenolics, which act as reducing and capping agents for the nanoparticles. The reduction of silver ions to silver atoms is triggered by the presence of the CR extract and its active agents. The silver atoms then begin to aggregate and form nanoparticles (Yilmaz *et al.*, 2011).

### Procedure

AgNO<sub>3</sub> powder was dissolved in distilled water to prepare 10 mM AgNO<sub>3</sub> stock solution from which a series of 1 mM, 2 mM and 3 Mm AgNO<sub>3</sub> solutions were prepared. The AgNO<sub>3</sub> solutions were mixed with the extract of aerial parts of *Cuscuta reflexa* 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 (Poulose *et al.*, 2014).

**Table 1: Different formulation of Silver nanoparticles**



Formulation Code	Extract (mg)	AgNO <sub>3</sub> (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. The % yield was estimated by dividing actual weight of product by total weight of dry sample multiplied by 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 (Patel *et al.*, 2019).

#### 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 IS/cm.



### Formulation development of gel

Measured amounts of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of aerial parts of *Cuscuta reflexa* were dissolved in about 100 ml of water in a beaker and stirred at high speed using mechanical stirrer (or sonicator) (Prabu *et al.*, 2017). 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
Silver nanoparticles of <i>Cuscuta reflexa</i>	500	500	500
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 silver nanoparticles 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

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.

A special apparatus was designed to study the formulations' spreadability. Spreadability is expressed in terms of the time taken by two slides in seconds to slip off the surface, put between them, under the application of a certain load. The less time required for two slides to separate, the greater the spreadability.



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### Method:

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 (Jain *et al.*, 2009).

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.

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

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.

### 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.

### 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<sub>3</sub> 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 (Singh *et al.*, 2023).

### 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°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.

#### ***In vitro* diffusion profile (*In vitro* permeation study)**

*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<sup>2</sup> and a height of 100 mm with a diffusion area of 3.8 cm<sup>2</sup>. Phosphate buffer (pH 7.4) was used as substrate for receptors. Semipermeable membrane used as for dialysis. 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 Semipermeable membrane and was immersed slightly in 100 ml of receptor medium, which was continuously stirred. The whole network had been held at 37±1°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. The total percent release was measured.

#### **Antifungal activity of silver nanoparticle gel**

The well diffusion method was used to determine the antifungal activity of silver nanoparticle gel prepared from the aerial parts of *Cuscuta reflexa* using standard procedure. There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in studies. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 25°C for 48 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug (Kumar *et al.*, 2022).

#### **Results and Discussion**

AgNP synthesis has been the subject of increased research recently in an effort to develop a number of applications, including imaging, biosensing, catalysis, and antibacterial activity. An alternate technique called "green synthesis" was created to create metal nanoparticles with organic substances or parts of plants. These methods spare the environment from the toxicity of chemicals. NPs have been synthesized by algae, bacteria, fungus, and plants without the application of extra stabilizing and reducing agents. Functional substances found in plant extracts, such as sorbic acid, citric acid, euphol, cyclic peptides, polyhydroxy limonoids, ascorbic acid, retinoic acid, tannins, ellagic acid, and gallic acid, are thought to be essential for the bioreduction and stabilization of nanoparticles.

The phytochemical analysis of *Cuscuta reflexa* extracts reveals the presence of phytoconstituents such as alkaloids phenol, proteins, carbohydrates, saponins, diterpenes and tannins, which are responsible for biological properties.





The % yield ranged from  $63.32 \pm 0.25$  to  $76.65 \pm 0.14\%$  while the entrapment efficiency ranged from  $0.587 \pm 0.014$  to  $0.769 \pm 0.009$ . The highest percentage yield and entrapment efficiency was observed to be  $76.65 \pm 0.14$  and  $0.769 \pm 0.009$  respectively which was associated with F3 formulation. The Average particle size and zeta potential of F3 formulation was found to be 220.5 nm and - 38.5 mV respectively.

Further the formulated gel containing AgNP was evaluated for various parameters. The physical formulation of gel GF1, GF2, GF3 were brown in color, smooth in texture, good in washability, extrudability and Homogeneity. The viscosity of GF1, GF2, and GF3 were estimated  $3252 \pm 15$ ,  $3152 \pm 20$  &  $3065 \pm 24$  cp. The gel base created by Carbopol 940 has a pH of 6.72 and 6.80, respectively, which is close to skin pH and is hence compatible with skins. The formulation was found to be easily washable.

Also the Spreadability was found to be  $13.32 \pm 0.15$ ,  $11.14 \pm 0.25$  and  $10.52 \pm 0.21$  gcm/sec respectively. The pH of GF1, GF2, GF3 were detected as  $7.20 \pm 0.15$ ,  $6.85 \pm 0.22$  &  $6.71 \pm 0.23$ . Also, the highest flavonoid content was noted in GF2 which is  $0.728 \pm 0.032$  mg/100mg. From the results of In vitro drug release study, it was seen that in case of GF1 about 99.82 drug is released in 4 hours while in GF2 and GF3 about 98.87 and 89.65% drug is released in 4 hrs. The release kinetics regression values of formulation GF2 suggested that for zero order and first order the  $R^2$  value was estimated to be 0.955 and 0.827 respectively.

The Antifungal activity was tested against *Candida albicans* using agar well diffusion method. The highest inhibition zone of the extract at 100 mg/ml concentration for *Candida albicans* was measured to be  $13 \pm 0.47$ mm, while that for silver nanoparticles gel (GF2) zone of inhibition was measured as  $17 \pm 0.75$ mm at the same concentration. From result of antimicrobial activity it can be interpreted that synergistic effect if Silver Nanoparticles Gel (GF2) was more efficient than *Cuscuta reflexa* extract alone. AgNPs' effectiveness against the test organisms was average. The well diffusion procedures revealed that the AgNPs biosynthesised with *Cuscuta reflexa* had comparable activity on all of the examined fungi. Despite a large body of research examining AgNPs antibacterial properties, the exact mechanism of action remains unclear. According to several studies, their ability to enter and accumulate in the fungal cell wall is correlated with their antifungal properties. Furthermore, smaller AgNPs inhibited the fungal cells more because their bigger surfaces allowed them to engage with the cell wall.

**Table 3: Phytochemical screening of aerial parts of *Cuscuta reflexa***

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

**Table 4: Determination of % yield and Percentage entrapment efficiency of prepared formulations**

Formulation code	% Yield	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	63.32±0.25	0.685±0.015
F2	68.85±0.32	0.712±0.012
F3	76.65±0.14	0.769±0.009



F4	71.12±0.16	0.587±0.014
F5	69.98±0.14	0.665±0.017
F6	70.12±0.18	0.678±0.013

**Table 5: Characterization of average particle size and zeta potential**

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F3	220.5	- 38.5 mV

### Results of gel Formulation

#### Evaluation of gel formulation of gel

**Table 6: Results of physical characteristics**

Formulation code	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
GF1	Brown	Absent	Good	Smooth	Good	Good
GF2	Brown	Absent	Good	Smooth	Good	Good
GF3	Brown	Absent	Good	Smooth	Good	Good

**Table 7: Results of Viscosity and spreadability of gel**

Formulation code	Viscosity* (cp)	Spreadability* (gcm/sec)
GF1	3252±15	13.32±0.15
GF2	3152±20	11.14±0.25
GF3	3065±24	10.52±0.21

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

**Table 8: Results of pH and flavonoid content in gel using AlCl<sub>3</sub> method**

Formulation code	pH	Flavonoid Content (mg/100mg)
GF1	7.20±0.15	0.715±0.054
GF2	6.85±0.22	0.728±0.032
GF3	6.71±0.23	0.705±0.042

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

**Table 9: *In vitro* drug release study of prepared gel formulation**



S. No.	Time (hr)	% Cumulative Drug Release		
		GF1	GF2	GF3
1	0.25	44.65	33.65	22.25
2	0.5	69.98	48.85	32.15
3	1	85.65	59.98	39.98
4	1.5	96.25	63.32	46.65
5	2	99.05	72.23	59.98
6	2.5	99.15	76.65	68.87
7	3	99.47	88.85	79.98
8	4	99.82	98.87	89.65

**Table 10: *In-vitro* drug release data for gel GF2**

Time (h)	Square Root of Time(h) <sup>1/2</sup>	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.25	0.5	-0.602	33.65	1.527	66.35	1.822
0.5	0.707	-0.301	48.85	1.689	51.15	1.709
1	1	0	59.98	1.778	40.02	1.602
1.5	1.225	0.176	63.32	1.802	36.68	1.564
2	1.414	0.301	72.23	1.859	27.77	1.444
2.5	1.581	0.398	76.65	1.885	23.35	1.368
3	1.732	0.477	88.85	1.949	11.15	1.047
4	2	0.602	98.87	1.995	1.13	0.053

**Table 11: Release kinetics regression values of formulation GF2**

Formulation code	Zero order	First order
GF2	0.955	0.827

**Table 12: Antifungal activity against *Candida albicans***

S.	Name of drug	Microbes	Zone of inhibition
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No.			25 mg/ml	50 mg/ml	100 mg/ml
1.	Extract	<i>Candida albicans</i>	9±0.50	12±0.74	13±0.47
2.	Silver nanoparticles gel (GF2)		12±0.25	15±0.15	17±0.75

## Conclusion

Environmental conservation has led to an increasing demand for environmentally acceptable nanoparticle production. AgNPs, one type of metal nanoparticle, have a non-toxic effect on human cells, which makes them a good antibacterial agent. Since ancient times, people have utilized medicinal plants as a natural cure because of their wide range of metabolites and phytoconstituents. These metabolites and phytoconstituents have the ability to lower silver ions and aid in the synthesis of AgNPs from plant extracts. Strong binding affinities between these AgNPs and several functional groups in the plant extracts are observed.

Silver nanoparticles are now extremely used as an anti-bacterial, apart from this AgNPs have their uses as anti-viral, anti-inflammatory, gene therapy, anti-fungal, in diagnostic and imaging purpose and many more. The rapid biological synthesis of silver nanoparticles using *Cuscuta reflexa* Hydroalcoholic extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The formulated AgNP gel have synergistic antifungal effect of *Cuscuta reflexa* extract and AgNP.

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