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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLE OF HYDROALCOHOLIC EXTRACT OF *CUSCUTA REFLEXA*

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ABSTRACT

The prevalence of infectious diseases has increased as a result of overuse and abuse of medications. AgNPs, along with other nanomaterials, have been studied in the defined post-antibiotic era to search for new agents that can help combat pathogenic microorganisms without promoting the appearance of new resistances. Additionally, green synthesis of AgNPs is preferred to offer a cost-effective, environmentally responsible, and cleaner synthesis method. Thus, this study deals with Green synthesis and characterization of silver nanoparticle of hydroalcoholic extract of *Cuscuta reflexa*. The AgNP combined with *Cuscuta reflexa* extract was synthesized & evaluated as per standard methods. Results showed that the entrapment efficiency for F3 Nanoparticle formulation was found to be 0.725±0.015. The Avg. particle size & Zeta potential of F3 was found to be 220.5 & - 38.5 mV respectively. The physical characteristics of F3 formulation was also touching idealistic parameters. From the three prepared formulations of gel the spreadibility & Viscosity for F2 was found to be 9.15±0.35 & 3025±8 respectively. Flavonoid content for F2 was found to be highest with value 0.728±0.032 mg/100mg while the pH of gel was noticed as 6.72±0.15. Results of in vitro drug release study also showed good results. The zero order & first order regression values of formulation F2 was found to be 0.953 & 0.914. The zone of inhibition at 100mg/ml of extract was found to be 12±0.47 while for same concentration the zone of inhibition for silver nanoparticle was found to be 15±0.75. This indicates that the cumulative antimicrobial effect of extract & AgNP is more appreciable.

Keywords: *Cuscuta reflexa*, AgNP, Antimicrobial, Green synthesis, *C. albicans*.

INTRODUCTION

Globally, the prevalence of infectious diseases has increased as a result of overuse and abuse of medications, which has led to microorganisms classified as multidrug resistant pathogens developing antibiotic resistance. These multi-drug resistant microorganisms cause severe health problems as well as financial harm. While being transported, food may become contaminated by pathogens that are multi-drug resistant. To combat these multidrug resistant food-borne infections, novel and natural antibacterial medicines are therefore necessary[1,2].

Since antibiotics are no longer effective in combating bacterial strains, it has become necessary to develop new bactericidal materials. Manufacturing these agents is crucial for solving these issues. Although antitoxin is smaller than any other nanoparticle, many antimicrobial agents have been developed with the aid of nanotechnology. The majority of the particles fall within the 5 to 10 nm size range, although some also fall within the 1 to 10 nm range. Nanotechnology makes things smaller and more compact, which increases the surface to volume ratio. However, the majority of the traditional techniques for making AgNPs call for numerous chemicals, which are not only expensive but may also leave behind hazardous residue. Therefore, a green synthesis of AgNPs is preferred to offer a cost-effective, environmentally responsible, and cleaner synthesis method [3,4].

Cuscuta reflexa is a parasitic plant which belongs to family Convolvulaceae. It is frequently referred to as the dodder plant. Throughout the ages, all societies have recognised and gradually shared information about potentially useful plants. Others are suggested by conventional healers, while some are used for self-medication. The use of plants as medicine includes both the direct administration of their leaves, seeds, bark, roots, and stems as well as the use of extracts and decoctions made from various plant components. Numerous Cuscuta species are common constituents of numerous folk medicinal systems because they are abundant sources of various phytochemicals. In conventional medicine, cuscuta species are used as purgatives, diaphoretics, anthelmintics, diuretics, tonics, and treatments for itching and bilious illnesses [5-6].

In the current study, silver ions from the silver nitrate were reduced using an aerial component extract from *Cuscuta reflexa* to create silver nanoparticles (AgNPs). The synthesis, optimisation, characterization, and assessment of these silver nanoparticles' antibacterial activity against *C. albicans* were the main goals of the current work.

MATERIALS & METHODS

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Aerial parts of *Cuscuta reflexa* were collected from local area of Bhopal in the month of September, 2022. Drying of fresh plant parts was carried out in sun but under the shade. Dried aerial parts of *Cuscuta reflexa* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Defatting of plant material

Aerial parts of *Cuscuta reflexa* were shade dried at room temperature. 75.8 gram dried powdered plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered aerial parts of *Cuscuta reflexa* has been extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC[7,8].

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. The $AgNO_3$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 mLin 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[9].

Table 1: Different formulation of Silver nanoparticles

Formulation Code	Extract (mg)	AgNO3 (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.

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[10].

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 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). 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[11].

Table 2: Formulation of gel

Ingredients (mg)	F1	F 2	F3
Silver nanoparticles of *Cuscuta reflexa*	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

RESULTS & DISCUSSION

The phytochemical screening revealed the presence of flavonoid, phenol, protein, carbohydrate, saponin, diterpene, tannin. The total flavonoid & phenol content was found to be 0.732 mg/ 100 mg & 0.495 mg/ 100 mg. The highest percentage yield of78.85±0.15 was obtained with formulation F3. Also, the entrapment efficiency for F3 was found to be 0.725±0.015. The Avg. particle size & Zeta potential of

F3 was found to be 220.5&- 38.5 mV respectively. The physical characteristic of F3 formulation was also touching idealistic parameters. The spreadibility & Viscosity for F2 was found to be 9.15±0.35&3025±8 respectively. Flavonoid content for F2 was found to be highest with value 0.728±0.032mg/100mg while the pH of gel was noticed as 6.72±0.15. Results of in vitro drug release study also showed good results. The zero order & first order regression values of formulation F2 was found to be 0.953&0.914. The zone of inhibition at 100mg/ml of extract was found to be 12±0.47 while for same concentration the zone of inhibition for silver nanoparticle was found to be 15±0.75. This indicates that the cumulative antimicrobial effect of extract &AgNP is more useful.

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

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager'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
	Benedict'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 *Cuscuta reflexa*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.732	0.495

Results of optimized formulation of silver nanoparticles

Table 5: Determination of % yield of prepared formulations

Formulation code	% Yield
F1	65.58±0.25
F2	69.98±0.32
F3	78.85±0.15
F4	70.23±0.23
F5	68.87±0.18
F6	67.45±0.21

Table 6: Determination of entrapment efficiency of prepared formulations

Formulation code	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	0.705±0.015
F2	0.698±0.023
F3	0.725±0.015
F4	0.615±0.014
F5	0.632±0.013
F6	0.695±0.014

Table 7: 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

Table 8: 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 9: Results of spreadability of gel

Formulation code	Spreadability* (gcm/sec)
F1	11.23±0.25
F2	9.15±0.35
F3	8.79±0.21

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

Table 10: Results of Viscosity of gel

Formulation code	Viscosity* (cp)
F1	3145±10
F2	3025±8
F3	2898±5

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

Table 11: Results of flavonoid content in gel using $AlCl_3$ method

Formulation code	Flavonoid Content (mg/100mg)
F1	0.715±0.054
F2	0.728±0.032
F3	0.705±0.042

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

Table 12: Results of pH of gel

Formulation code	pH
F1	7.10±0.10
F2	6.72±0.15
F3	6.75±0.20

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

Table 13: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release		
		F1	F2	F3
1	0.25	30.25	26.65	23.65
2	0.5	36.65	32.25	29.98
3	1	52.23	48.85	46.65
4	1.5	69.98	63.32	62.23
5	2	80.23	71.12	69.98
6	2.5	89.98	82.23	76.65
7	3	98.12	92.65	82.23
8	4	99.45	98.78	86.65

Table 14: *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	0.500	-0.602	26.65	1.426	73.35	1.865
0.5	0.707	-0.301	32.25	1.509	67.75	1.831
1	1.000	0.000	48.85	1.689	51.15	1.709
1.5	1.225	0.176	63.32	1.802	36.68	1.564
2	1.414	0.301	71.12	1.852	28.88	1.461
2.5	1.581	0.398	82.23	1.915	17.77	1.250
3	1.732	0.477	92.65	1.967	7.35	0.866
4	2.000	0.602	98.78	1.995	1.22	0.086

Table 15: Release kinetics regression values of formulation F2

Formulation code	Zero order	First order
F2	0.953	0.914

Table 16: Antifungal activity against *Candida albicans*

S. No.	Name of drug	Microbes	Zone of inhibition		
			25 mg/ml	50mg/ml	100 mg/ml
1.	Extract	*Candida albicans*	8±0.50	11±0.74	12±0.47
2.	Silver nanoparticles gel		11±0.25	13±0.15	15±0.75

Conclusion

In this study, AgNPs were synthesized without the usage of any hazardous chemicals by using *Cuscuta reflexa* extract as a reducing and capping agent. This approach is also simple, quick, and environmentally friendly. Our findings further demonstrate that the synthetic AgNPs have good physical characteristics and beneficial biological outcomes. Additionally, AgNPs have antibacterial properties and can be used instead of antibiotics. The synthesized AgNPs had substantial biological effects that indicated that research be done to determine the medicinal value of AgNPs.

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