

**FORMULATION, DEVELOPMENT AND EVALUATION OF SILVER NANOPARTICLES OF
BLEPHARIS EDULIS FOR THE TREATMENT OF CANDIDIASIS****Pragya Pandey*, Dr. Shailendra Bindaiya, Dr. Shailendra Lariya****Radharaman College of Pharmacy, Bhopal***Corresponding Author's E mail: pragya8318@gmail.com

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<https://dx.doi.org/10.38164/AJPER/11.1.2022.99-108>**ABSTRACT**

Nanoparticles are gaining interest in biomedical applications due to its importance such as anti-bacterial, anti-fungal and anti-cancer agents. A conventional method for the synthesis of metal nanoparticles involves toxic reagents which produce harmful by-products and are hazardous to the environment. To overcome these limitations, green synthesis of nanoparticles was established. Eco-friendly methods using plant extracts are gaining popularity due to the abundance of raw materials and the production of non-toxic by-products threatening to the environment. Moreover, the nanoparticles synthesized from the plant extract are cost-effective. In addition, nanoparticles produced by green synthesis methods produce synergetic effect where both the nanoparticles as well as the natural bioactive constituents of the plant influence the biocidal properties. *Blepharis edulis* (*B. edulis*) is traditionally used as an antiseptic, purgative, aphrodisiac and anti-inflammatory agent. The present investigation evaluates phytochemical screening and green synthesis, characterization of silver nanoparticles. Characterization of silver nanoparticles performed by surface charge, vesicle size, entrapment efficiency and microscopic observation. The optimized formulation of silver nanoparticles was incorporated into a carbapol 940 gel base which was evaluated for drug content, pH, spreadability, washability, extrudability, viscosity and in vitro permeation. The phytochemical screening results unveiled the bearing of different phytochemicals viz., phenolics, proteins and amino acids, carbohydrates, saponins and diterpenes. The total phenolics content of seeds of aqueous extract was (1.125mg/gm). The characterization results of the prepared AgNPs displayed that the silver nanoparticles are formed and stabilized by plant phyto-constituents. Green synthesis process is a pivotal area in nanotechnology and usage of natural resources is the best choice for the making of NPs as a sustainable, eco-friendly, inexpensive and free of chemical contaminant method. These AgNPs have several potential biological and medical applications.

Keywords: *Blepharis edulis*, Phytochemical screening, Silver nanoparticles, Characterization, Carbapol 940.

INTRODUCTION

Fungal diseases contribute significantly to increased morbidity and mortality rates in immunosuppressed patients ¹, especially cases of HIV, cancer, transplanted organs, and individuals undergoing long-term

treatment with wide spectrum antibiotics². Likewise, implementation of medical devices such as peripheral and central vein catheters used for treatment of patients under chemotherapy, hemodialysis and parenteral nutrition, contribute significantly to colonization and fungal invasion by *Candida* spp³. *Candida* is yeast, whose species are commensals in the human body. Present in mucosa they are usually isolated from not only the oral cavity, but also the gastrointestinal and urogenital tracts⁴. However, *candida* species are opportunistic pathogens that can invade and cause diseases at mucocutaneous and systemic levels in case of immune system deficiency⁵. Currently, *candida* is considered by the National Nosocomial Infections Surveillance System (NNISS, USA) as the fourth blood pathogen at nosocomial level, with estimated mortality rate of up to 45 %⁶. The synthesis of nanomaterials is currently one of the most active areas in nanoscience. Special attention has been dedicated to nanomaterials that help improve the human quality life. A remarkable example is the silver nanoparticles (AgNPs) which are known by their inhibitory and bactericidal effects. AgNPs can be produced with various sizes and shapes depending on the fabrication method which can be physical, chemical, biological and hybrid. The chemical methods use toxic chemicals, which are not friendly to environment making them unsuitable for biomedical applications. Specifically, the widely used chemical reduction methods⁷ usually employ toxic and perilous chemicals that are responsible for various biological risks. On the other hand, physical methods are expensive and incompatible with sizeable production of nanoparticles. Therefore, to avoid toxic and hazardous chemicals, the green synthesis methods have been developed, attracting significant interest because they are environment friendly, rapid, facile and energy-efficient⁸. Green synthesis using huge biological molecules derived from plant extracts⁹ could facilitate size and morphology control of metal nanoparticles due to the presence of an innumerable quantity of biomolecules possessing bioreduction and biostabilization ability¹⁰. Specifically, many plants have been used for silver nanoparticles synthesis¹¹, such as stem bark of *Callicarpa maingayi*, *Terminalia cuneata*, *Illicium verum* (star anise) and pod extract of *Acacia nilotica*¹². *B. edulis* (family Acanthaceae) is a small gray pubescent or nearly glabrate perennial herb, found in Punjab, Western Rajasthan and malwa region of MP. In local Hindi language the plant is known as Utangan or Chaupatia where as in Ayurvedic system called as Shikhi. Stem is short and approximately 30cm length. Nature of stem is rigid and leaves appear in-group of fours at the nodes. Shape of leaf is upper pair 5cm x 1 cm and lower pair smaller with oblong or narrow elliptic. The flower appears in blue colour in storability inflorescence. The fruit capsules are 5cm long and two seeded¹³. The leaves and seeds are reported to be eaten. The herb forms a good fodder for sheep and camels. It has been identified as Uchchata- aphrodisiac drug in Ayurvedic. The leaves commonly sold in Indian market, are reported to be useful in wounds, ulcers, nasal hemorrhages, asthma, throat inflammation, purgative, disorders of liver and spleen. The root is considered diuretic and beneficial in

urinary discharges and dysmenorrhoea¹⁴. The seeds are considered to be diuretic, aphrodisiac, expectorant, deobstruent and useful in strangury and conjunctivitis. They yield bitter glycoside blepharin¹⁵ and other research workers found dl alantoin, saponin and tannin catechol in samples from Allahabad variety¹⁶. It also showed presence of phytosterol in seeds¹⁷. In general, the entire plant had utility as animal fodder due to increase in milk production in cattle and as effective treatment in earache¹⁸, contraceptive properties¹⁹. Its constituents include benoxazolone and blepharin²⁰, presence of galactose and fructose²¹, and novel 4'-O-diglycoside of decarboxyrosmarinic acid²². The present investigation evaluates phytochemical screening and green synthesis, characterization of silver nanoparticles.

MATERIALS AND METHODS

Materials:

Plant material

The seeds of *B. edulis* were collected from M. F. P. Park, Barkheda Pathani, Dist. Bhopal (M.P.).

Chemical reagents

Silver nitrate (AgNO_3) is purchased from Sigma-Aldrich Chemicals for this study. Dimethyl sulphoxide (DMSO) was purchased from Merck, India. The pH buffer tablets were purchased from Himedia. The aqueous solutions were prepared with triple distilled water. All the chemicals used in this study were of analytical grade.

Extraction of plant material

Powdered plant material (seeds) of *B. edulis* was shade dried at room temperature. The shade dried seeds was coarsely powdered and subjected to extraction with aqueous solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Qualitative phytochemical analysis of plant extract

The *B. edulis* seeds extract obtained were subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate^{23, 24}. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso *et al*²⁵. A volume of 2 ml of extracts or standard was mixed with 1 ml of Folin Ciocalteau 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 15min at 40°C for colour development. The absorbance was measured at 765 nm using

UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Green synthesis of silver nanoparticles

In the single step green synthesis, 5 ml of seeds of *B. edulis* extract was added to 95 ml of 1- 3mM aqueous silver nitrate solution and kept in the dark place at room temperature for 24 h ²⁶. Silver nanoparticles are formed by reduction of pure silver ions and it was monitored by measuring absorption of the reaction medium in the wavelength range of 300-700nm using UV spectrophotometry. The synthesized silver nanoparticle (AgNP) was purified by centrifugation at 10000rpm for 15min. The supernatant was transferred to a clean dry beaker for further settlement of particles and repeated centrifugation was carried using cooling microfuge to get dried, purified and characterized the AgNPs Table 1.

Table 1: Different formulation of silver nanoparticles

Formulation Code	Extract (mg)	AgNO ₃ (mM)	Ratio
F1	500	1	1:1
F2	500	2	1:1
F3	500	3	1:1

Evaluation of Silver nanoparticles

Microscopic observation of prepared silver nanoparticles

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared silver nanoparticle formulation.

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 silver nanoparticles.

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

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 Niosomes 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.

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 phenols could be obtained from the absorbance difference based on standard curve ²⁷.

Formulation development of silver nanoparticles gel

Measured quantity of methyl paraben, glycerin, polyethylene glycol and extract of *B. edulis* was dissolved in about 100ml of water in beaker and were stirred at high speed using mechanical stirrer (or sonicator). Then carbopol 940 (1, 1.5 and 2%) was added slowly to the beaker containing above liquid while stirring. Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed Table 2.

Table 2 Formulation of silver nanoparticles gel

Ingredients (%)	FG1	FG1	FG1
<i>B. edulis</i> extract	1gm	1gm	1gm
Carbopol 940	1 gm	1.5 gm	2 gm
Polyethylene Glycol	0.2ml	0.2ml	0.2ml
Methyl Paraben	0.08mg	0.08mg	0.08mg
Triethanolamine	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100ml	100ml	100ml

Evaluation of silver nanoparticles gel

Appearance and consistency:

The physical appearance was visually checked for the texture of gel formulations.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

Extrudability

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

Determination of spreadability

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. An apparatus in which a slide fixed on wooden block and upper slide has

movable and one end of movable slide tied with weight pan. To determine spreadability, 2-5 gm of gel placed between two slides and gradually weight was increased by adding it on the weight pan and time required with the top plate to face the distance of 10 cm on adding 80 g of weight was noted. Good spreadability shows lesser time to spread. It is determine by formula given below ²⁸.

$$A. S = \frac{m \cdot l}{t}$$

B. Where, S=Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams),

C. l= length of glass slide (6cms), t = time taken is seconds.

Determination of pH

The pH of the gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Drug content

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 2 ml of stock solution 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 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

RESULTS AND DISCUSSIONS

The crude extracts so obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the seeds of the plants using water as solvents was found to be 5.173% w/w. Phytochemical analysis of aqueous extracts of seeds sample of *B. edulis* showed the presence of phenolics, proteins and amino acids, carbohydrates, saponins and diterpines Table 3. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of aqueous extract of *B. edulis* seeds showed the content values of 11.57 Table 4. The % yield, average vesicle size (nm), % entrapment efficiency and zeta potential (mV) of prepared formulation was given in Table 5. Average vesicle size, % entrapment efficiency and zeta potential of optimized formulation of silver nanoparticle (F2) was found to be 185.65±2.32, 72.25±1.25and -35.45±0.1respectively. Prepared gel of silver nanoparticles loaded with aqueous extract was prepared and evaluated for viscosity, pH, % drug content, extrudability, spreadability and drug release study. From the psychorheological characteristics studies of formulation showed that all of them have clear colour, No clogging, good homogeneity and smooth texture Table 6. The results of washability, extrudability, spreadability, pH, viscosity was given in Table 7. Extrudability study was performed by gel formulations were filled into aluminium collapsible tubes,

the formulation have average extrudability. The skin irritation test performed showed no signs of sensitivity, erythema and edema. So the prepared formulations were considered to be non-irritant. In the all formulation of different gels the percentage of phenol content was found maximum in FG2 Table 8.

Table 3 Phytochemical screening of *B. edulis* Seeds extract

S. No.	Constituents	Aqueous extract
1.	Alkaloids	
	Dragendroff's test	-ve
	Hager's test	-ve
2.	Glycosides	
	Legal's test	-ve
3.	Flavonoids	
	Lead acetate	-ve
	Alkaline test	-ve
4.	Phenolics	
	FeCl ₃	+ve
5.	Proteins	
	And Amino acids	
	Xanthoproteic test	+ve
6.	Carbohydrates	
	Fehling's test	+ve
7.	Saponins	
	Foam test	+ve
8.	Diterpenes	
	Copper acetate test	+ve
9.	Tannins	
	Gelatin Test	-ve

Table 4 Total Phenolic content of *B. edulis*

S. No.	Extract	Total Phenol (GAE)
1.	Seeds	1.125 mg/100mg

Table 5 Determination of % yield, average vesicle size (nm), % entrapment efficiency and zeta potential (mV) of prepared formulation

Characterization	% Yield	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F1	69.45±0.45	220.3±3.45	68.45±3.74	- 20.32±0.2
F2	73.32±0.25	185.65±2.32	72.25±1.25	-35.45±0.1
F3	67.78±0.36	236.65±1.14	65.98± 1.35	-28.85±0.2

Table 6 Results of Psycho Rheological Characteristics

Formulation	Colour	Clogging	Homogeneity	Texture
FG1	Yellowish brown	Absent	Good	Smooth
FG2	Yellowish brown	Absent	Good	Smooth
FG3	Yellowish brown	Absent	Good	Smooth

Table 7 Results of washability, extrudability, spreadability, pH, viscosity

Formulation	Washability	Extrudability* (g)	Spreadability (gcm/sec)	pH	Viscosity (cps)
FG1	Good	152±4	13.25±0.45	6.95±0.05	2985±15
FG2	Good	165±3	12.56±0.35	7.01±0.02	2565±10
FG3	Average	162±5	11.12±0.26	6.58±0.01	2345±12

*Average of three determinations (n=3)

Table 8 Results of phenolic content using FCR method

Formulation	Phenol Content (mg/100mg)
FG1	0.985±0.002
FG2	1.015±0.012
FG3	0.875±0.014

*Average of three determinations (n=3)

CONCLUSION

The present investigation involved the screening of *B. edulis* seeds crude extracts for the phytochemicals. Green synthesis of AgNPs using aqueous seeds extract and characterization of synthesized nanoparticles were also carried out in the study. The optimized formulation of silver nanoparticles was incorporated into a carbapol 940 gel base which was evaluated for drug content, pH, spreadability, washability, extrudability, viscosity and *in vitro* permeation. Outcome of all the experiments carried out suggests the existence of most of the phytochemicals in the seeds and are having some important biological activities. Further work is needed to isolate, purify and identify the exact active principle which is the cause for the biological activities. The Green synthesis is a simple, low cost and ecofriendly approach without any huge inputs in terms of energy. This is the first report of green synthesis of silver nanoparticles for this plant. Being exhibiting greater antimicrobial activity, phytochemical based nanoparticles may stand as a potential remedy in developing drugs against antibiotic resistant bacteria.

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