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FORMULATION AND EVALUATION OF SILVER NANOPARTICLE OF *SCAPHIUM AFFINE* FOR ANTI MICROBIAL EFFECT

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ABSTRACT

This study focuses on the formulation and evaluation of a novel silver nanoparticle gel incorporating the hydroalcoholic extract derived from *Scaphium affine* fruits. The aim is to harness the antimicrobial potential of *Scaphium affine*, a plant renowned for its medicinal properties. The silver nanoparticle gel was meticulously formulated using hydroalcoholic extraction and nanoprecipitation techniques, and its physicochemical characteristics were comprehensively assessed. The antimicrobial efficacy was evaluated against a spectrum of pathogenic microorganisms, elucidating the potential of the developed gel as an antimicrobial agent. The results indicate successful synthesis of silver nanoparticles within the gel matrix, characterized by advanced analytical techniques. Furthermore, the gel demonstrated significant antimicrobial activity, showcasing its potential application in the development of pharmaceutical and cosmetic formulations with enhanced therapeutic benefits. This research contributes to the growing body of knowledge on plant-based antimicrobial agents and opens avenues for the utilization of *Scaphium affine* in the field of nanomedicine. The silver nanoparticle gel emerges as a promising candidate for further studies and potential applications in the realm of infectious disease management and skincare formulations.

Keywords: Silver nanoparticle gel, skincare formulations, *Scaphium affine,* hydroalcoholic extract, antimicrobial potential

INTRODUCTION

The earliest known form of life on Earth is microbes. They are commonly referred to as microorganisms because they are minuscule living entities. The only way to observe microbes is under a microscope. Fossils of microbes go back over 3.5 billion years. Garbage wouldn't break down and there wouldn't be as much oxygen available if microbial growth didn't occur. It's common to refer to microbes as "microscopic organisms." These organisms can be found practically anywhere on Earth, including in soil, rock, water, air, and even in plants, animals, and human tissue (Sarbeen & Gheena,2016).

Less than 1% of bacteria, for example, are dangerous microorganisms that can infect humans and cause illness. Infectious diseases like the measles and flu are caused by microbes. The infectious disease is caused by several bacteria colonizing the body. Numerous identical illness states might have a variety of etiological factors; for example, pneumonia can be brought on by viruses, bacteria, protozoa, or even fungi. Additionally, there is compelling evidence that bacteria may have a role in a number of

chronic non-infectious disorders, including coronary heart disease and certain types of cancer. Different kinds of microorganisms produce different diseases. Pathogens are microbes that cause disease (Sethi and Murphy, 2001; Hay and Morris-Jones; 2016).

The ability of bacteria to resist medications has led to a significant problem in clinical practice: microbial resistance to the existing antibiotics. This poses a hazard to public health. Because of spontaneous mutations and gene recombination, for example, the resistance profile of the microorganisms linked to the illness is closely correlated with their genetic profile. Furthermore, the overuse of antibiotics is a factor in the rise in this profile (Tacconelli *et al*., 2018).

With its use in science and technology to create novel materials at the nanoscale, nanotechnology is emerging as a quickly expanding area. Researchers are currently interested in metallic nanoparticles due to their large surface area to volume ratio and good antibacterial properties. This is because microbial resistance to metal ions, antibiotics, and the emergence of resistant strains is growing (Feynman, 1959).

The antimicrobial activity of silver nanoparticles is applied to various cell compartments and occurs through a variety of mechanisms, including physical interaction with the cell membrane, disruption of the lipid bilayer and/or release of cationic ions, and the formation of reactive oxygen species (ROS), which results in bactericidal action based on oxidative stress (Choi, *et al*., 2008; Lara, *et al*., 2010; Kim *et al*., 2007). Utilizing plants for nanoparticle synthesis can be advantageous over other biological entities since it can circumvent the laborious process of using microorganisms and maintaining their culture, which can cause the bacteria to lose their ability for nanoparticle synthesis. Therefore, in this sense, using plant extract for synthesis could have a huge impact in the upcoming decades (Jadoun, *et al*., 2020).

The tree species *Scaphium affine* belongs to the subfamily Sterculioideae of the family Malvaceae. It was formerly classified under the Sterculaceae, and synonyms for it include *Sterculia lychnophora* Hance. The Catalogue of Life recognizes no subspecies for this unique species to mainland Southeast Asia. Although S. affine has been shown to have a number of benefits, including anti-inflammatory, antioxidant, and ulcer-prevention properties, anticancer effects are still unproven. It has been demonstrated that β-sitosterol, a physiologically active component of numerous natural products, has anticancer properties. The seeds' decoctions are used to treat constipation, cough, menorrhagia, pharyngitis, laryngitis, and pain management. Both this species' and S. macropodum's seeds are used as a "coolant," for gastrointestinal issues, and to soothe sore throats in traditional Chinese and Indian medicine, Ayurveda. It has the ability to relieve lung heat, treat sore throats, neutralize toxins, and ease constipation (Phonsena and Wilkie, 2008; Oppong *et al*., 2018)

Considering the beneficial effect of silver nanoparticle and *Scaphium affine* this study deals with formulation and evaluation of silver nanoparticle of *Scaphium affine* for anti -microbial effect.

MATERIALS & METHODS

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Fruits of *Scaphium affine* were collected from Bhopal in the month of April, 2023. Drying of fresh plant parts was carried out in sun but under the shade. Dried fruits of *Scaphium affine* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

Defatting of plant material

Fruits of *Scaphium affine* were shade dried at room temperature. 40 gram dried 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 fruits of *Scaphium affine* has been extracted with hydroalcoholic solvent (ethanol: water: 75:25) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC (Mukherjee, 2007; Kokate, 1994).

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 fruits of *Scaphium affine* 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)	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

Characterization of synthesized silver nanoparticles formulations

Percentage yield

The silver nanoparticles, prepared with a size range of 200-300 nm, were gathered and quantified from various formulations. The calculated weight was then divided by the total quantity of all non-volatile components utilized in the microsphere preparation. (Vanaja *et al.,* 2013; Umashankari *et al.,* 2012).

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of the drug associated with the formulations to the total mass of the drug. The entrapment efficiency was assessed using the dialysis method, where the silver nanoparticle-entrapped extract was separated from the free drug. For this purpose, the aforementioned formulations were loaded into dialysis bags, and the free drug was dialyzed for 24 hours in 50 ml of buffer at pH 1.2.

The absorbance of the dialysate was measured against a blank buffer at pH 1.2, and the absorbance of the corresponding blank was measured under the same conditions. The concentration of free flavonoids was determined based on the absorbance difference using a standard curve (Banerjee *et al.,* 2014).

Surface charge and vesicle size

The particle size, size distribution, and surface charge were determined using the Dynamic Light Scattering method (DLS) with a Malvern Zetamaster, ZEM 5002 instrument from Malvern, UK, at SAIF RGPV Bhopal. Zeta potential measurements for the silver nanoparticles were conducted based on the Helmholtz–Smoluchowsky equation derived from electrophoretic mobility. For zeta potential measurement, a zetasizer was employed with a field strength of 20 V/cm in a large bore measurement cell. Samples were appropriately diluted with 0.9% NaCl and adjusted to a conductivity of 50 µS/cm.

Formulation development of silver nanoparticle gel

Precise quantities of methyl paraben, glycerin, polyethylene glycol, and hydroalcoholic extract of *Scaphium affine* fruits were dissolved in approximately 100 ml of water in a beaker. The mixture was vigorously stirred using a mechanical stirrer or sonicator, following the method described by Raut et al. in 2009. Subsequently, Carbopol 940 was gradually introduced into the beaker containing the aforementioned liquid while maintaining continuous stirring. The solution was neutralized by slowly adding a triethanolamine solution, stirring constantly, until the gel formation occurred.

Table 2: Formulation of gel

Ingredients (mg)	F1	F 2	F3
Scaphium affine extract	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 gel

Appearance and Consistency: The physical appearance and texture of gel formulations were visually inspected.

Washability: Formulations were applied to the skin and manually assessed for ease and degree of washing with water.

Extrudability Determination: Gel formulations were filled into aluminum collapsible tubes, sealed, and pressed to extrude the material. Extrudability of the formulation was noted.

Determination of Spreadability: Spreadability, a crucial factor for gel formulations, was evaluated using a specially designed apparatus. Two glass slides (6x2) were chosen, and the gel formulation to be tested was placed between them over a length of 6 cm. The time taken for the slides to separate under the application of a 20-gram load was recorded.

The experiment was repeated six times for each formulation, and the average was calculated.

Method: Two glass slides were selected, and the gel formulation was placed over one slide. The second slide was placed over the formulation, sandwiching it over a length of 6 cm. A 20-gram weight was applied, forming a thin layer. The time taken for the slides to separate under the weight was recorded.

Spreadability Formula: S=m×ltS=tm×l Where, SS = Spreadability (gcm/sec), mm = weight tied to the upper slide (20 grams), ll = length of the glass slide (6 cm), tt = time taken in seconds.

Viscosity: The viscosity of the gel was determined using a Brookfield digital viscometer with spindle no. 6 at 10 rpm and at a room temperature of 25-30°C. Measurements were taken after allowing the gel samples to settle for more than 30 minutes.

Drug Content: The drug content was measured by dissolving 1g of gel in methanol in a 10 ml volumetric flask. A mixture of 3 ml of stock solution and 1 ml AlCl3 solution (2%) was vortexed, and the color production was allowed to stand at 40°C for 30 minutes. Absorbance was measured at 420 nm using a spectrophotometer.

Determination of pH: The pH of the gels was measured using a digital pH meter. One gram of gel was dissolved in 25 ml of purified water, and the electrode was dipped into the gel solution until a steady reading was obtained. pH measurements were repeated twice for each formulation.

***In vitro* diffusion profile:** In vitro diffusion experiments were conducted using Franz diffusion cells. Rat abdominal skin was used as the membrane for dialysis, tied to the diffusion cell. Isotonic phosphate buffer solution (pH 7.4) served as the substrate for receptors. A weighed quantity of the formulation equivalent to 1g of gel was applied to the rat skin, and aliquots were withdrawn at different time intervals, measured at 295 nm. The total percent release was calculated for each time period, and the diffusion media were replaced with fresh medium after each withdrawal.

In vitro antimicrobial activity of silver nanoparticle gel

The antimicrobial activity was performed by well diffusion method. The antimicrobial activity of gel was tested against *Bacillus subtilis* and *Klebsiella pneumoniae.* The gel was made into concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml. In well diffusion method, first the nutrient agar media was prepared and autoclaved. After that the sterile media was poured in petri plate. Once it got solidified the inoculation of microbe was performed. Further four wells are created by using gel puncture of which three wells for three concentration and one as control. The concentrations and control is then added to respective wells. The plates are incubated at 37 degrees for 24 hrs. After that the antimicrobial activity was measured by estimating the zone of inhibition.

RESULTS AND DISCUSSION

The phytochemical tests indicated the presence of alkaloid, flavonoid, phenol, protein, carbohydrate, saponin. The quantitative estimation of total phenol and falavonoid content suggested that about 0.56 mg/100mg of flavonoid and 0.82mg/100mg of phenol is present in the sample. The entrapment efficiency of silver nanoparticle was observed to be 0.715±0.025 mg/100mg in case of F3 formulation. The % yield was seen to be again highest in F3 which is 78.85±0.15% and lowest in F1 which is 65.58±0.25%. The particle size and zeta potential for F3 was observed to be 220.50nm and - 38.5 mV respectively. Smaller particles have a higher surface-area-to-volume ratio than large particles, which makes them more rapidly oxidized. This is another method of releasing Ag ions from smaller particles more quickly than from large particles. Here, the rate at which particles release Ag ions—that is, the surface area to volume ratio—plays a significant role in both the cytotoxicity and antibacterial activity of AgNPs.

Further the evaluation of gel was carried out. The colour of all formulation was observed to be brown with good homogeneity, Extrudability and washability with no net clogging. The spreadibility for F1, F2 and F3 was observed to be 10.23±0.32, 9.85±0.25 and 6.95±0.15 respectively. The viscosity was seen to be 3355±10, 3265±15 and 3145±18 cp for F1, F2 and F3 respectively. The gel's viscosity rose as the carbopol concentration rose. The cross-linked polyacrylate polymer known as Carbopol® 934 polymer contains ionized carboxyl groups that undergo conformational changes in the polymer chain, causing the polymer matrix to swell and releasing the medication. It generates dense formulations for opaque gels and provides exceptional stability at high viscosities.

The highest flavonoid content was estimated in F2 formulation which is 0.752±0.018mg/100mg. The pH for F1, F2 and F3 formulation was noted as 6.85±0.02, 6.80±0.01 and 6.95±0.02. The % Cumulative Drug Release in 4 hours was estimated to be 99.65, 99.02 and 88.98 respectively. By considering all the parameters the F2 formulation was elected as optimized formulation.

The Release kinetics regression values of formulation F2 indicated that R^2 for zero order as 0.992and first order as 0.792.

Additionally when the antimicrobial activity of extract only was performed the zone of inhibition in case of *Bacillus subtilis* and *Klebsiella pneumoniae* at 100 mg/ml was measured to be 11.5±0.47mm and 12.8±0.50mm respectively. Same extract when conjugated with AgNP and incorporated in gel which is our F2 formualtion found to have zone of inhibition of 13.1±0.57mm and 15.45±0.25mm at 100 mg/ml respectively. Thus, from results Silver nanoparticles gel (F2) is more effective against *Klebsiella pneumoniae* than *Bacillus subtilis* The propposed mechanism of antibacterial action of AgNP is taht AgNPs, tiny particles have the ability to enter cells and destroy the bacterial membrane. A cell's particles and ions can also attach to proteins and DNA, which can induce the generation of reactive oxygen species (ROS). These mechanisms even cause cell lysis by rupturing organelles and denatureing the cell membrane. Thus it can be interpreted that the plant extract

containing AgNP gel is more effective than extract alone. This can be due to he vast surface area of the AgNP gel which provides an active adsorbing hydrophilic surface for the microorganism in such a trap to contact AgNPs, enhancing the interaction between the microbes and AgNPs, which could account for the enhanced antibacterial activity of Plant extract-AgNPs gel material. These findings demonstrate the the Formualted AgNP could be employed in treating bacterial infections for longer protective effect.

Table 3: Determination of % yield and entrapment efficiency of prepared silver nanoparticles formulations

Formulation code	% Yield	Percentage entrapment efficiency (Flavonoid mg/100mg Quercetin equivalent)
F1	65.58±0.25	0.658±0.025
F2	69.98±0.32	0.687±0.032
F3	78.85±0.15	0.715±0.025
F4	70.23±0.23	0.652±0.032
F5	68.87±0.18	0.621±0.036
F6	67.45±0.21	0.687±0.025

Table 4: Characterization of average particle size and zeta potential of optimized formulation F3

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F3	220.50	- 38.5 mV

Table 5: 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 6: Results of viscosity and spreadability of gel

Formulation code	Viscosity* (cp)	Spreadability* (gcm/sec)
F1	3355±10	10.23±0.32
F2	3265±15	9.85±0.25
F3	3145±18	6.95±0.15

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

Table 7: Results of pH and flavonoid content in gel

Formulation code	pH	Flavonoid Content (mg/100mg)
F1	6.85±0.02	0.612±0.015
F2	6.80±0.01	0.752±0.018
F3	6.95±0.02	0.605±0.014

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

Table 8: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release		
		F1	F2	F3
1	0.25	32.25	30.25	22.32
2	0.5	45.65	38.85	31.15
3	1	58.98	46.65	40.32
4	1.5	65.85	57.75	49.95
5	2	81.12	63.32	56.65
6	2.5	96.65	76.65	69.98
7	3	99.05	84.45	73.32
8	4	99.65	99.02	88.98

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	30.25	1.481	69.75	1.84
0.5	0.707	-0.301	38.85	1.589	61.15	1.79
1	1	0	46.65	1.669	53.35	1.73
1.5	1.225	0.176	57.75	1.762	42.25	1.63
2	1.414	0.301	63.32	1.802	36.68	1.56
2.5	1.581	0.398	76.65	1.885	23.35	1.37
3	1.732	0.477	84.45	1.927	15.55	1.19
4	2	0.602	99.02	1.996	0.98	-0.01

Table 10: Release kinetics regression values of formulation F2

Formulation code	Zero order	First order
F2	0.992	0.792

Table 11: Antimicrobial activity against selected microbes

S. No.	Microbes	Zone of inhibition		
		25 mg/ml	**50 mg/ml**	**100 mg/ml**
		Extract		
1.	*Bacillus subtilis*	7.5±0.50	9.9±0.50	11.5±0.47
2.	*Klebsiella pneumoniae*	9.8±0.94	11.8±0.86	12.8±0.50
		Silver nanoparticles gel (F2)		
1.	*Bacillus subtilis*	9.8±0.15	11.6±0.74	13.1±0.57
2.	*Klebsiella pneumoniae*	10.5±0.5	12.3±0.86	15.45±0.25

CONCLUSION

The *Scaphium affine* extract conjugated with AgNP embedded into gel, and the gel's efficacy was evaluated both in vitro for the purpose of treating infections. Maintaining the particle size, the AgNPs conjugated with plant extract were evenly dispersed throughout the gel and gradually released at body temperature. Significant antibacterial qualities against *Bacillus subtilis* and *Klebsiella pneumoniae*, was demonstrated by the AgNP–plant extract embedded gel. Consequently, the F2 formualtion of gel may show promise for clinical use by effectively treating microbial infections.

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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