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Research Article

PHYSICOCHEMICAL ASSESSMENT OF AgNPs INVIGORATED ANTIDERMATOPHYTIC CREAM FROM LEAVES OF *CRESCENTIA CUJETE L.* - A NANOPHARMACEUTICAL PLOY

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ABSTRACT

To combat pathogens, a wide variety of antibiotics has been developed and sold commercially through time. The proliferation of pathogenic microorganisms poses a threat to public health. However, a more serious problem arises when these pathogens develop resistance against commercial antibiotics. The research on screening and development of drugs for their activity is therefore, an unending process and there is hope of finding out anti-dermatophytic drugs from indigenous plants. In this study, the leaf extract of *Crescentia cujete L.* was employed as a green reducing agent to synthesise highly-stable antidermatophytic cream using invigorated *C. cujete* silver nanoparticles. Incorporation of nano-sized particles in cosmetic formulations can improve the stability of active constituents like vitamins, unsaturated fatty acids and antioxidants, thereby enhancing their therapeutic value. Specifically, metallic nanoparticles have attracted attention due to their application in the medical field as well as cosmetics, food, paint, and textile industry. The assessment involves physicochemical attributes that include grittiness, pH, rheological property, emulsion and stability tests.

Keywords: Antidermatophytic cream, AgNPs, *Crescentia cujete L.*, Physicochemical assays.

1. INTRODUCTION

Plants have been used in traditional medicine for several thousand years. Medicinal plants as a group comprise approximately 8000 species and account for about 50% of all the higher flowering plant species in India. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In a large number of countries, human population depends on medicinal plants for treating various illnesses as well as a source for livelihood. Cutaneous disorders are one of the major concerns in pharmaceutical and cosmetic sectors. The global market involving technologies for the treatment of skin diseases reached $17.1 billion in 2015, and is expected to reach $20.4 billion by 2020 [1]. Among the major pharmaceutical companies, innovative therapies for certain skin diseases have renewed interest in the market. Novel dermatological and cosmetic formulations are employed with antibacterial and antifungal potentials to combat various skin conditions and infections [2].

These formulations comprise of fragrance, oils and fats that can undergo auto-oxidation and chemical degradation when exposed to air, thereby becoming odourless. Addition of antioxidants can preserve them, increase their shelf life and also protect the skin from cellular damages.

Nanotechnology is a burgeoning field that has greater significance to revolutionize cosmetics and pharmaceuticals. Colloidal drug carriers like nanoparticles, have tremendous scope in cosmetic and dermatological sectors due to their nanometric size and potential applications [3, 4]. Modern cosmetic-oriented products consist of nano-sized particles, which are envisaged to replace conventional preservatives used in cosmetics [5]. They offer better skin penetration and UV protection, increased color and finish quality, long-lasting effects [6], and also eradicate or control the activity of various microorganisms [7]. Present study aims to evaluate physicochemical properties of formulated antidermatophytic cream using AgNPs obtained using leaf extracts of *Crescentia cujete* L.

2. MATERIAL AND METHODS

2.1. Preparation of Antidermatophytic cream

2.1.1. Preparation of leaf extracts

Fresh leaves of *Crescentia cujete L.* were washed with deionized water followed by distilled water to ward off any grime material. These were then subjected to dry in shaded condition for around 7 days and then pulverised to fine powder utilizing a clean mechanized kitchen grinder with stainless steel cutting edges. This powder was stored in airtight bottles and was utilized for preparing the extracts. The extract was made by adding 1 g of leaf powder to 50 ml of double distilled water and left overnight in refrigerator. The crude extract was filtered and pure extract was utilized for formation of nanoparticles.

2.1.2. Synthesis of AgNPs using fresh leaf extract of Crescentia cujete L.

To 30 ml of 1mM $AgNO_3$, 10 ml of leaf extract was added and at interval of every 20 minutes colorimetric readings were taken at 410nm to find the presence of nanoparticles. The colour change was an indication of formation of nano particles. These started forming within 20 minutes and continued up to 4-6 hours after that these particles were stable for about one month [8].

2.1.3. Cream formulation using AgNPs

Base cream contains water and oil phases. The compositions and amounts of the formulation ingredients are shown in Table 1.

Table 1: The composition of antidermatophytic cream (%w/w)

Compound		Amount (%w/w)
Oil phase	Stearic acid	10.0
	Glycerine monosterate	5.0
	Cetyl alcohol	5.0
	Liquid paraffin	5.0
	Bees wax	5.0
	Spermaceti	5.0
Water phase	Glycerin	5.0
	Methyl paraben	2.0
	Propyl paraben	2.0
	Water	45
	Rose oil	QS
	Crescentia cujete L. AgNPs	10

* *QS= quantity sufficient*

In order to prepare the cream, different amount of ingredients were incorporated together, and then the required amount of the AgNPs was added. Ten (10g) sample of the cream was placed in a centrifuge tube (1cm diameter) and centrifuged at 2000rpm for 5, 15, 30 and 60mins. Then the phase separation and solid sedimentation of the samples were inspected.

2.2. Evaluation of the cream

The cream was evaluated for the following physical parameters to study formulation properties. The formulation properties of the cream were studied by visual appearance and characteristics.

2.2.1. Presence of foreign particles/grittiness

A small amount of cream is taken and spread on a glass slide free from greaseand observed against diffused light to check for presence of foreign particles and also feel of application was checked physically.

2.2.2. pH of the cream

The pH of formulation is determined by using digital pH meter. A suspension of each portion in 1% potassium nitrate solution was prepared and its pH was determined. A magnetic stirrer was used to produce homogeneity. The pH was determined at the interval of 48 h, one week, one month and three months after preparation. The measurement of pH of each formulation was done in triplicate and average values were calculated.

2.2.3. Rheological property -Viscosity (cps)

Using a Brookfield viscometer (model DV-I with No. 6 spindle) the rheologic behavior of the portion were studied. Sample was placed in a container and spindle velocity was raised gradually to maximum extent. Then the viscosity was determined at 0.3, 0.6, 3, 6 and 60 rpm. Student t-test (Microsoft excel software) was performed to compare test results with the control. $P<0.05$ was assumed as significant difference.

The rheological property was determined to know the flow behaviour of formulation. The rheological behaviour of the formulation was studied by taking 100 g of the cream in the beaker. The rate of shear was increased gradually from minimum to maximum and corresponding dial reading was noted; then, the rate of shear was decreased gradually to the lowest value and the dial reading was recorded. The graph was plotted between shear rate and viscosity and also viscosity as a function of time to determine type of flow.

2.3. Determination of type of emulsion

2.3.1. Dilution test

In this test, the emulsion is diluted either with oil or water. If the emulsion is o/w type and it is diluted with

water, it will remain stable as water is the dispersion medium but if it is diluted with oil, the emulsion will break as oil and water which are not miscible witheach other (w/o). Oil in water emulsion can easily be diluted with an aqueous solvent, whereas water in oil emulsion can be diluted with an oily liquid [9].

2.3.2. Dye solubility test

In this test, an emulsion is mixed with a water soluble dye amaranth and observed under the microscope. If the continuous phase appears red, it means that the emulsion is o/w type as the water is in the external phase and the dye will dissolve in it to give colour. If the scattered globules appear red and continuous phase colourless, then it is w/o type. Similarly, if an oil soluble dye Scarlet red C or Sudan III is added to an emulsion and the continuous phase appears red, then it is w/o emulsion [9].

2.4. Stability studies

2.4.1. Globule size

One mL of cream was diluted to 10 mL glycerine. A few drops of this were transferred onto a glass slide and was focused in a microscope. By using eyepiece micrometer, the diameters of 200 particles were determined randomly.

2.4.2. Phase separation

The formulated cream was kept intact in a closed container at 25-50°C not exposed to light. Phase separation was observed carefully every 24 hours for 30 days. Any change in phase separation was checked.

2.4.3. In-vitro occlusivity test

Beaker of diameter 3.2 cm and height 4.6 cm was used. The test was performed by placing 10 g of distilled water in beaker and closing the open end with Whatman filter paper No. 1 on the upper surface of which 200 mg of the sample was evenly distributed. The beaker was then placed at prescribed conditions 37±2°C/607±5% RH for 48 h. The samples and a negative control where the filter paper was kept uncovered, were studied for the *in-vitro* occlusivity to determine the water flux. The whole set was performed in a desiccator provided with $Na_2Cr_20_2II_20$ [10, 11]. The occlusion factor F was calculated as

$$F = \frac{A - B}{A} X\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (Eq.\ 1.)$$

Where,

A= Water flux through uncovered filter (% water loss)

B= Water flux through filter when covered by test preparation (% water loss)

2.4.4. Tube Extrudability [13, 15, 16]

It is usual empirical test to measure the force required to extrude the material from a tube. The formulations were filled in standard caped collapsible tube and sealed. The tube was weighed and recorded. The tube was placed between two glass slides and was clamped. A 500 gm weight was placed over the glass slide and cap was opened. The amount of cream extruded were collected and weighed. The percent of cream extruded was calculated and grades were allotted (++++ Excellent, +++ Good, ++ Fair, + Poor).

2.4.5. Spreadability

Spreadability denotes the extent of area to which the formulation readily spreads on application to skin. The bioavailability efficiency of a formulation also depends on its spreading value. The spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load.

Lesser the time taken for separation of the two slides, better is the spreadability. Two glass slides of standard dimensions were taken. For this purpose, cream was applied in between two glass slides and they were pressed together to obtain a film of uniform thickness by placing 1000 gm weight for 5 minutes. Thereafter a weight (10 gm) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves over the lower plate to cover a distance of 10cm is noted. The spreadability (g.cm/sec) can be calculated according to Ashwini*et. al.* 2014 [12-16]. Spredability of the formulation may be determined by the following equation (Eq. 2.)

$$S = \frac{M \times L}{T} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (Eq.\ 2.)$$

Where,

M = Weight tied to upper slide

L = Length of glass slide

T = Time taken to separate the slides

2.4.6. Thermal cycle test

The portion were stored at 5°C for 48 h and then at 25°C for 48 hour. The procedure was repeated 6 times and then their stability and appearance were evaluated.

2.4.7. Thermal change test

Three portions, each of 20 g, of cream formulation were stored at 4-6°C, 25°C and 45-50°C respectively.

Their stability and appearance were evaluated after 24 hours, one month and three months respectively.

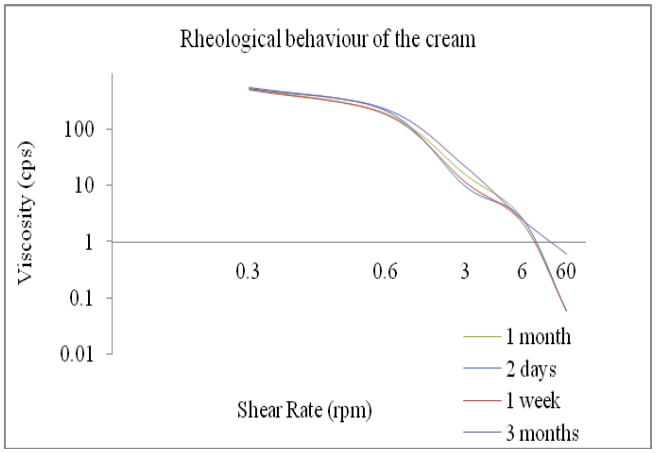
2.4.8. Freezing and thawing

Known amount of cream formulation (20 g) was stored periodically at 45-50°C and 4°C for 48 hours. The procedure was repeated six times and then the samples were checked regarding their appearance and stability. Sample was subjected through a series of extreme, rapid temperature changes that may encounter during normal shipping and handling processes.

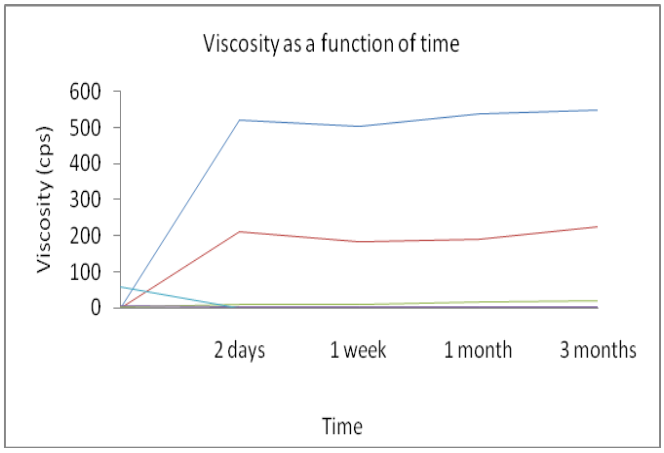
3. RESULT AND DISCUSSION

Cream is found to have a consistent colour intensity and odour which indicate the visual stability of cream ingredients. So it shows significant physical evaluation parameters. Formulations were evaluated micros-copically for the presence of any appreciable particulate matter which was seen under light microscope. Upon supplement of visual diffused light under microscopic observation, no particulate nature of cream was observed. Moreover there was smooth and consistent grittiness observed. Hence obviously the cream preparation fulfils the requirement of freedom from particulate matter and from grittiness as desired for any topical preparation. The pH of cream was determined to examine the possible side effects due to acidic or alkaline pH, which can leads to irritation of skin. Acidic or alkaline pH may cause irritation to the skin and influence the rate of hydration of polymer. There was no distinct change in pH of all the formulations and was found to be between 6.8±0.02 to 7.00±0.01 that is within the range.This also indicated that the selected ingredients of the formulation did not alter the pH of the formulation. Viscosity is the most important parameter in the evaluation of the cream. Viscosity governs the many properties of the product such as spread ability, pour ability of the product from the container etc. Viscosity was also noted and is found to be between 521.00±0.05 to 549±0.07; 211±0.01 to 226±0.05; 9.51±0.25 to 21.07±0.02; 2.54±0.04 to 2.43±0.01 and 0.06±0.01 to 0.6±0.2 when subjected to shear rate of 0.3, 0.6, 3.0, 6.0 and 60 rpm respectively for all time dependant observations. The results were statistically analysed with mean ± SD, n=3, $p < 0.05$.*

The tenuous structure of inorganic particles dispersed in water is disrupted by an applied shear stress. As shear stress is increased, more and more inter-particulate associations are broken, exhibited as a greater tendency to flow as viscosity decreases (Graph 1). Similarly for macromolecules dispersed in a solvent, the applied shear stress is tending to align the molecule in the direction of stress. The molecules straighten out, becoming less entangled as shear increases, lessening the resistance to flow.



Graph 1: Rheological behaviour of Cream



Graph 2: Formulation viscosity parameter as a function of time

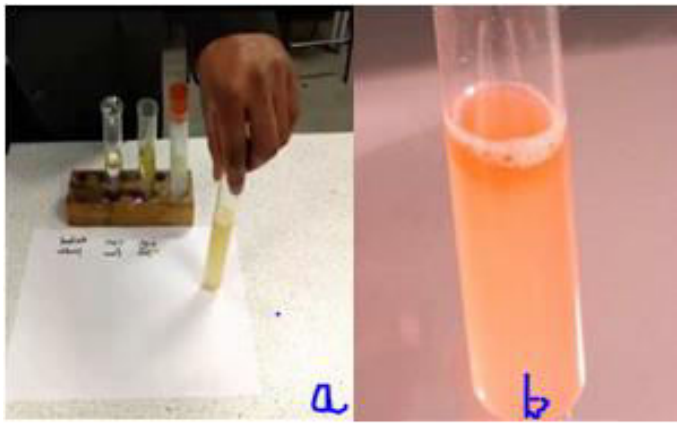


Fig. 1: Cream emulsion type: a. Solubility test b. Sudan III - w/o type

Stability studies are essential to ensure that the product is stable throughout its designated shelf life. Tube extrudability test is the measure of the force required to extrude the material from a collapsible tube when the certain amount of force has been applied on it in the form of weight. In the present study the quantity in percentage of cream extruded from the tube on application of certain load shows that the formulation has good extrude ability. The good extrude ability and Spread ability is required for the therapeutic efficiency of the formulation. The therapeutic potency of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from cream which is placed in between the slides under the direction of certain load. The spread ability of formulated cream was determined which indicates good spreading of cream when applied to the skin. In case of thermal test, the cream formulation is found stable and consistent though out the time dependent observation results. Formulation had increasing viscosity value after storage in temperature dependent condition. All samples were water-in-oil creams; hence, their water content might lose at fluctuated temperatures. Therefore, the suggested storage condition for these products should be at constant temperature. The formulations with suitable viscosity could provide more adhesiveness and spreading efficacy. No phase separation and changing in colour as well as odour were observed in all samples after stability test; however, they seemed to be more viscous. From the results it is observed that the given formulations are relatively stable at accelerated temperature and humidity.

Table 2: Physicochemical evaluation of cream

Properties	Conditions		Time dependent Observations			
			After 2 days	After 1 week	After 1 month	After 3 months
Colour	Visual		Light yellow	Light yellow	Light yellow	Light yellow
Odour	---		Pleasant odour	Pleasant odour	Pleasant odour	Pleasant odour
Foreign Particles	Diffused light		No particulate observed	No particulate observed	No particulate observed	No particulate observed
Grittiness	---		Smooth	Consistent	Consistent	Consistent
pH	1% KNO_3, Homogenous		7.0 ± 0.01	7.0 ± 0.22	6.9 ± 0.51	6.8 ± 0.02
Viscosity* (cps)	Shear rate(rpm)	0.3	521.00 ± 0.05	503 ± 0.24	537 ± 0.13	549 ± 0.07
		0.6	211 ± 0.01	184 ± 1.19	192 ± 0.58	226 ± 0.05
		3	9.51 ± 0.25	11.32 ± 0.01	15.65 ± 1.09	21.07 ± 0.02
		6	2.54 ± 0.04	2.19 ± 0.5	2.66 ± 0.02	2.43 ± 0.01
		60	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.001	0.6 ± 0.2

mean ± SD, n=3, p< 0.05

Table 3: Stability of cream formulation

Evaluation parameter	Condition	Observation
Globule size	Microscopic observation	Homogenous mixture
Phase separation	25 – 50°C for 24 hours	No phase separation (w/o)
*In-vitro*occlusivity test (occlusion factor -F)	37±2°C/607±5% RH for 48 h	32.49 ± 0.81
Extrudability	500 gm weight (fixed mass)	+++ (good)
Spreadability (gm.cm/sec)	---	14.37 ± 0.76
Thermal cycle test	---	Stable
Thermal change test	---	Stable
Freezing and thawing	---	Stable

4. CONCLUSION

Personal care industry is currently more concentrated on herbal based formulation compounds as now a day it is a fast growing segment with a vast scope of manifold expansion in coming years. The use of bioactive ingredients in pharmaceutical cream formulation influence biological functions of skin and provide nutrients necessary for the healthy skin with its

influence of antiseptic, antibacterial or anti dermatophytic activities. There is tremendous scope to launch numerous herbal formulations for treatment of skin diseases using appropriate bioactive ingredients with suitable phytoactive constituents and additives. It is mandatory that adequate safety testing should be conducted according to existing rules and well documented along with the ingredients composition.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be constructed as a potential conflict of interest.

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