Research Article

Development and evaluation of topical formulations of Ashwagandha for antibacterial and antifungal studies

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Abstract

Background: Ashwagandha (*Withania somnifera*) is widely used in Ayurvedic medicine, the roots of the plants are also known for its antimicrobial activity apart from adaptogenic, anti-inflammatory and stress relieving properties. It contains steroidal compounds known as withanolides including withaferin A and withanolide D. These have been reported to be responsible for most of the biological activity. **Objective:** The main objective of the present study was to design, develop and evaluate topical creams and gels of ashwagandha**. Materials and methods:** In the present investigation, various creams and gels containing ashwagandha root extract were developed. The creams were prepared by fusion method and gels were prepared by dispersion method. The formulations were evaluated for various parameters. Two cream formulations and one gel formulation were selected based on the viscosity, homogeneity, spreadability & pH. Antibacterial and antifungal studies were performed for the best selected formulations of creams & gels using agar well diffusion technique. **Results and conclusion:** All the selected formulations showed comparable zone of inhibition(zoi) with the standard. But it was observed that gel formulation showed greater zone of inhibition when compared to the cream formulations. This proved that the selected gel formulation G3 has antimicrobial property.

Keywords: Ashwagandha, *Withania somnifera*, antimicrobial, agar well diffusion, withanolides, antibacterial, antifungal

Introduction

The use of herbal medicines has gained popularity throughout the world in recent times. Often seen as natural alternatives with fewer side-effects than synthetic medicines, these medicinal plants and their phytochemical constituents are thought to have potential applications in the management of a wide range of health conditions (Saloni Khogta et al., 2020). *Withania somnifera*, known commonly as ashwagandha, Indian ginseng, poison gooseberry, or winter cherry, is an annual evergreen shrub in the Solanaceae or nightshade family that grows in India, the Middle East and parts of Africa. It is a well-known medicinal plant in Ayurvedic medicine. The principle active compounds include several withanolide-type compounds. Due to the nontoxic and high medicinal value of *W. somnifera*, it is widely used as a home remedy for numerous diseases. The roots of the plants are also known for its antimicrobial activity apart from adaptogenic, anti-inflammatory and stress relieving properties.

Topical skin infections commonly occur and often present therapeutic challenges to practitioners, despite the numerous existing antimicrobial agents available today. The necessity for developing new antimicrobial means has increased due to growing concerns regarding multidrug resistant bacterial, viral and fungal strains (Chen et al., 2016). Hence, in this present investigation, we proposed to formulate and evaluate topical anti-infective formulations using ashwagandha root extract.

Materials and methods

Materials

Ashwagandha root extract was a kind gift sample from Botanical Health Care Pvt. Ltd, Hyderabad, India. Stearic acid, lanolin, bees wax, liquid paraffin, glycerin, propylene glycol, isopropyl myristate, borax, methyl paraben, propyl paraben, Carbopol 934, HPMC K 100 M and triethanolamine were obtained from S.D fine chemicals, India. Glyceryl monostearate was purchased from a local bakery.

Formulation of creams

Six cream formulations (C1-C6) were prepared by fusion method with varying concentrations of stearic acid and bees

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Table 1. Composition of topical cream formulations of ashwagandha root extract

Ingredients	C1	C2	C3	C4	C5	C6
Ashwagandha root extract (gm)	1.5	1.5	1.5	1.5	1.5	1.5
Stearic acid(gm)	0.5	0.5	1.0	1.0	2.0	2.0
Bees wax(gm)	1.5	2.5	1.5	2.5	1.5	2.5
Liquid paraffin(gm)	4.0	4.0	4.0	4.0	4.0	4.0
Lanoline (gm)	0.5	0.5	0.5	0.5	0.5	0.5
GMS (gm)	1.5	1.5	1.5	1.5	1.5	1.5
Glycerin(ml)	2.0	2.0	2.0	2.0	2.0	2.0
Propylene glycol(ml)	2.0	2.0	2.0	2.0	2.0	2.0
Isopropyl myristate(ml)	1.0	1.0	1.0	1.0	1.0	1.0
Borax (gm)	0.1	0.1	0.1	0.1	0.1	0.1
Methyl paraben(gm)	0.015	0.015	0.015	0.015	0.015	0.015
Propyl paraben(gm)	0.03	0.03	0.03	0.03	0.03	0.03
Water to make(ml)	50	50	50	50	50	50
Perfume	q. s	q. s	q. s	q. s	q. s	q. s

wax and keeping the concentrations of drug and other excipients constant.

Oil in water emulsion cream was prepared by initially melting bees wax at 70-80°C and to the molten mass stearic acid, liquid paraffin, lanolin, isopropyl myristate, glyceryl monostearate and the root extract were added. Aqueous phase comprising of propylene glycol, borax, glycerin and water was heated to the same temperature as oil phase. Both the phases were mixed slowly with continuous stirring to form a homogenous dispersion. The preparation was cooled to 35°C and the perfume was added. Preservatives were added to the warm aqueous phase before mixing.

The composition of topical cream formulations of ashwagandha root extract is shown in table 1.

Formulation of gels

Four gel formulations (G1- G4) were prepared by dispersion method with varying concentrations of Carbopol 934 and HPMC K 100 M keeping the concentrations of drug and other excipients constant.

Required amount of gelling agent (Carbopol 934/HPMC K 100M) was weighed and dispersed in 50ml of water to form a gel. Drug and preservatives were added to propylene glycol at 50°C. The above solution was added to the gel with continuous stirring. Triethanolamine was added to adjust the pH of the formulation.

The composition of topical gel formulations of ashwagandha root extract is shown in table 2.

Evaluation of creams and gels

Physicochemical and organoleptic properties

The formulated creams and gels were inspected visually for their colour, homogeneity, consistency, texture and phase separation.

Determination of pH

One gram of each formulation was dispersed in 25 mL of deionized water, and the pH was determined using a pH meter (Systronics®. Ltd) at 25°C. Measurements were made in triplicate. The pH meter was calibrated with standard buffer solutions (pH 4 and 7) before each use.

Table 2. Composition of topical gel formulations of ashwagandha root extract

Ingredients	G1	G2	G3	G4
Ashwagandha root Extract(gm)	1.5	1.5	1.5	1.5
Carbopol 934(gm)	1.0	1.5	-	-
HPMC K 100 M(gm)	-	-	1.0	1.5
Propylene glycol(ml)	5.0	5.0	5.0	5.0
Methyl paraben(gm)	0.1	0.1	0.1	0.1
Propyl paraben(gm)	0.25	0.25	0.25	0.25
Purified water(ml)	50	50	50	50
Perfume(ml)	0.1	0.1	0.1	0.1
Triethanolamine(ml)	q. s	q. s	q. s	q. s

Spreadability

One gram of sample was placed within a circle of 1 cm diameter pre marked on glass plate over which a second glass plate was placed. A weight of 200g was allowed to rest on the upper glass plate for 5 minutes. The increase in the diameter due to spreading of test formulation was measured. Average of 3 readings was noted.

Viscosity

Viscosity was measured using Brookfield viscometer with spindle #64 at 25°C. The spindle was rotated at 10, 20,30,50, and 100 rpm values. At each speed, the corresponding dial reading was noted. All measurements were made in triplicate.

Anti-bacterial and anti-fungal studies

The anti-microbial studies were carried out on selected formulations. Anti-bacterial activity was studied using the bacterial strain of *Staphylococcus aureus* and antifungal activity was studied using fungal strain of *Candida albicans*. The anti-microbial studies were performed by agar well diffusion method.

Agar well diffusion method

Nutrient agar medium (20 ml) was prepared and allowed to solidify in the petri plate and seeded with the microorganism using pour plate method. Wells with a diameter of 6 to 8 mm were bored aseptically with a sterile cork borer, and an antimicrobial agent (20–100 µL) was introduced into the well. Then, agar plates were incubated at 37°C for 24 hours. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The diameter of zone of inhibition was measured.

Stability studies

The best selected formulations were subjected to accelerated stability studies at long term storage conditions

of 25°C ± 2°C/60% ± 5% RH for 3 months. The formulations were observed after each week for possible changes in colour, consistency and phase separation.

Results and discussion

In the experimental section, six cream formulations and four gel formulations of ashwagandha root extract were prepared and subjected to evaluation.

Physicochemical and organoleptic properties

Results showed that all the cream and gel formulations have a cosmetically agreeable appearance and smooth texture. They were all homogenous without any signs of phase separation.

Determination of pH

The values varied between pH 5.70±0.03 to pH 6.91±0.11 which are shown in the Table 3. The pH of the skin normally ranges from 4 to 6. The pH of all the creams and gels were found to be slightly acidic but non-irritant to the skin.

Spreadability

Spreadability is the ability of a cream to spread on the skin. It plays an important role in the administration of a standard dose of a medicated formulation to the skin and the efficacy of a topical therapy.

Table 3. pH, spreadabilty and viscosity values of all the cream and gel formulations

Formulation code	pH	Spreadability (cm)	Viscosity (cps)
C2	**6.67±0.04**	**5.0±0.01**	**3184±4.02**
C3	**6.91±0.11**	**4.8±0.05**	**1952±4.04**
C4	6.63±0.06	3.8±0.03	784±2.23
C5	6.68±0.03	3.0±0.01	1472±4.23
C6	6.71±0.07	3.0±0.06	1752±3.26
G1	5.70±0.03	4.7±0.02	1164±3.52
G2	5.78±0.04	4.5±0.02	3247±4.01
G3	**6.82±0.02**	**5.0±0.01**	**5303±3.22**
G4	6.23±0.09	3.7±0.01	5045±3.90

#All values are mean±SD of three determinations. SD: Standard deviation

Table 4. Zone of inhibition of the selected cream and gel formulations against

Microorganism	C2	Standard	C3	Standard	G3	Standard
Staphylococcus aureus	28mm	38mm	30mm	38mm	38mm	40mm
Candida albicans	25mm	30mm	25mm	30mm	38mm	40mm

Spreadabilty of all the formulations was determined and it was observed that the formulations can spread upto 5cm. Among all the cream formulations C2 and C3 showed greater spreadability and out of all the gel formulations G3 showed good spreadability. The spreadability values of all the formulations are shown in the table 3.

Viscosity measurement and Rheological behavior

Viscosity of all the formulations was determined using Brookfield viscometer (Brookfield, India). The spindle was rotated at 10, 20,30,50, and 100 rpm. At different rates of

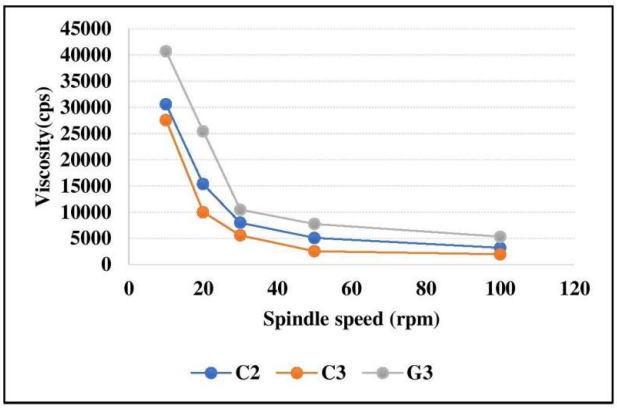

Figure 1. Graph indicating viscosity curves of the selected formulations

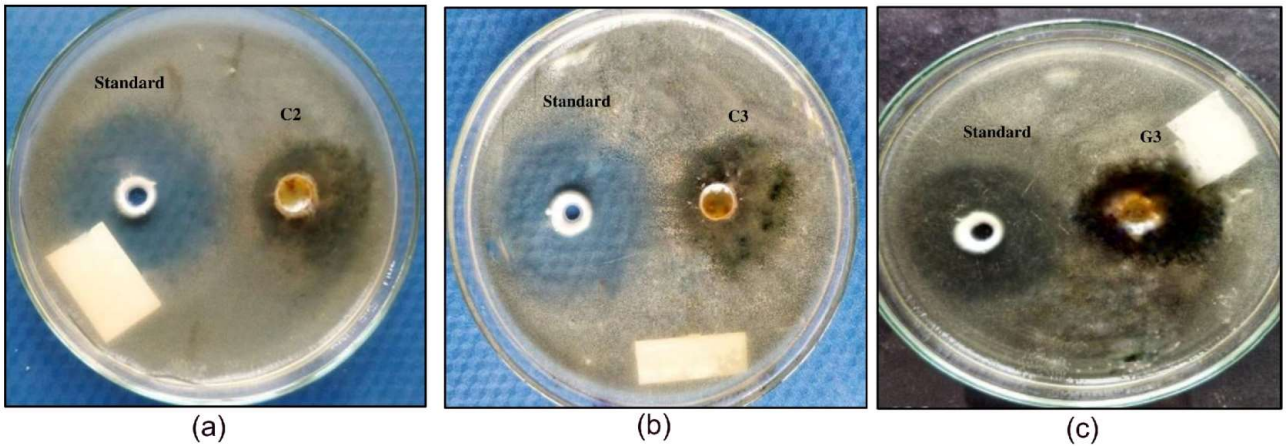

Figure 2. ZOI of formulations against *Staphylococcus aureus*: (a) C2 and standard (b) C3 and standard (c) G3 and standard

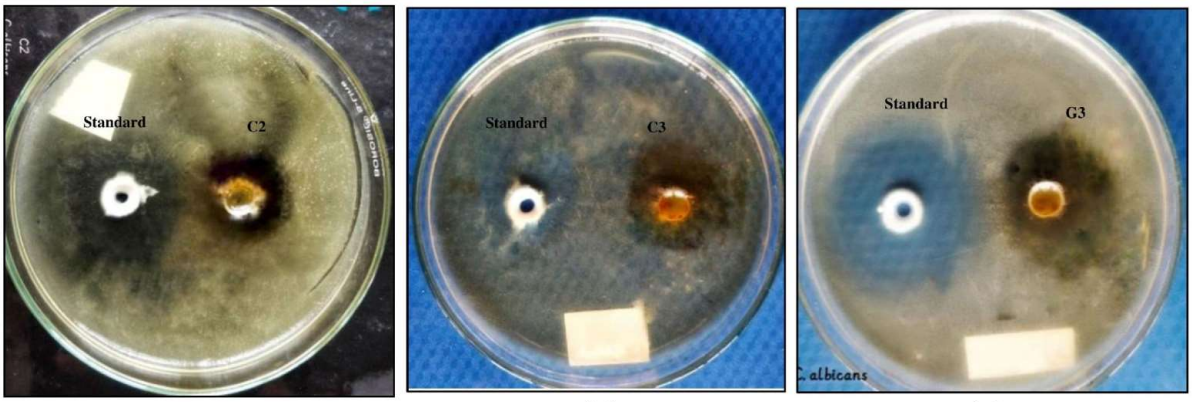

Figure 3. ZOI of formulation against *Candida albicans*, (a) C2 and standard (b) C3 and standard (c) G3 and standard

shear, the corresponding stress values were noted and the viscosity of all the formulations were found in the range of 767± 4.56 to 5303± 3.22 cps at 100 rpm. The viscosity values of all the formulations are shown in the table 3.

The selected formulations C2, C3 & G3 were checked for their rheological behavior and it was observed that the viscosity decreased with the increase in shear rate confirming the pseudoplastic behavior. The pseudoplastic behavior of the selected formulations is indicated in the graph figure 1.

Formulations **C2, C3** and **G3** showed better results in comparison to all the other formulations in terms of pH, viscosity and spreadability. Hence, two cream formulations (C2&C3) and one gel formulation (G3) were selected for further studies.

Antimicrobial studies

The in vitro antibacterial and antifungal study was performed by measuring and comparing the diameter of zones of inhibition (in mm) for the selected formulations. The zone of inhibition can be defined as the clear region around the well that contains an antimicrobial agent. It is known that the larger the zone of inhibition, the more potent the antimicrobial agent. The antibacterial and antifungal activity of the selected formulations was determined using agar well diffusion method. The antimicrobial activity of the formulations was compared to the marketed formulation (standard) against *Staphylococcus aureus* and *Candida albicans.* Clopinate GM® cream which has both antibacterial and antifungal properties was used as the standard. Clopinate GM® cream contains clobetasol and gentamicin as antibacterial and miconazole as antifungal.

The results indicated that all the selected formulations showed comparable ZOI with standard Clopinate GM®. But it was observed that the gel formulation G3 showed greater ZOI (i.e., 38mm) against both *Staphylococcus aureus* (Figure 2) and *Candida albicans* (Figure 3) when compared to the two cream formulations C2 & C3. The values of ZOI are shown in the table 4.

Stability studies

The optimized formulations were subjected to stability studies at room temperature 25°C ± 2°C for 3 months. The formulations were observed after each week for possible changes in colour, consistency, phase separation and pH. All the three selected formulations C2, C3 & G3 were found to be physically stable at room temperatures. The stability studies data is shown in table 5.

Table 5. Stability studies of the selected formulations

S. No	Parameters	Formulation code	Conditions (25°C±2°C)	
			0 Day	3 Months
1	Colour	C2	Light brown	Light brown
		C3	Light brown	Light brown
		G3	Dark brown	Dark brown
2	Texture	C2	Smooth	Smooth
		C3	Smooth	Smooth
		G3	Smooth	Smooth
3	Homogeinity	C2	Homogenous	Homogenous
		C3	Homogenous	Homogenous
		G3	Homogenous	Homogenous
4	pH	C2	6.67±0.04	6.77±0.04
		C3	6.91±0.11	6.83±0.11
		G3	6.82±0.02	6.90±0.02
5	Phase seperation	C2	No	No
		C3	No	No
		G3	No	No

Conclusion

The topical preparations of ashwagandha root extract were formulated and evaluated for its antimicrobial activity. All the selected formulations showed comparable zone of inhibition when compared to the standard Clopinate GM®. This proved that the selected formulations have potential antimicrobial property.

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