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FORMULATION AND EVALUATION OF ANTIBACTERIAL CREAM CONTAINING NIGELLA SATIVA FOR TOPICAL USE

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ABSTRACT

The antimicrobial property of black seed was used in this formulation, the present work designed to form a topical herbal cream with safety and less side effects. This formulation contains black seed oil which contains active chemical components like thymoquinone, alkaloids, flavonoids, saponins, glycosides. Thymoquinone is the main chemical which shows the antimicrobial activity. The formulation contains Carbopol, steric acid, cetyl alcohol as an emulsifying and thickening agent. The formulation was studied for various evaluation parameters like pH, viscosity, spreadability, type of emulsion, particle size, drug content, antimicrobial test. By studying all the parameters from all the formulation, the batch no F4 and F5 formulation shows good result.

KEYWORDS: *black seed oil Carbopol, steric acid, cetyl alcohol etc*

INTRODUCTION

Cream it is a semisolid preparation intended for topical application. There are mainly two types of cream oil in water (O/W) and water in oil (W/O) [(1)]. Since the ancient era humans are using herbal plant as medicine to cure the diseases [(2)]. The cream containing various herbal plants as herbal oil, extract for the treatment of various disease and diseased state. Herbal medicinal plants are naturally containing many bioactive compounds which form backbone of traditional medicines. Herbs show many activities like antibacterial, antifungal, antiallergic, etc [(3)]. Antibacterial activity of cream is the ability of drug to kill or inhibit growth of bacterial cells [(4)]. *Nigella sativa* (Black seed) belongs to family Ranunculaceae, it is an annual herb with many pharmacological properties. NS contains many active chemical components such as Thymoquinone, alkaloids (Nigellicine, Nigellidine), flavonoids, proteins, saponins, flavonoids, proteins, fatty acids which shows good results on patients with different diseases. Thymoquinone is the most common and abundant constituent of black seed oil, which shows antioxidant, anti-inflammatory, antimicrobial, anticoagulant activity. Hence the black seed oil containing herbal cream is formulated and evaluated [(5,6).]

MATERIAL AND METHOD

MATERIALS

Black seed oil, glyceryl monostearate, cetyl alcohol, steric acid, propylene glycol, honey, tween 80, glycerine, HPMC, Carbopol, methyl paraben, propyl paraben, triethanolamine, water.

METHOD

The cream was prepared by mixing two phases oil and water phase. Make oil phase mixture add cetyl alcohol, glyceryl monostearate, steric acid and melt at 60°c on water bath add black seed oil into that oil phase (phase 1). Take required quantity of water add water soluble ingredients into that mix it heats it up to 60°c on water bath add weighed amount of Carbopol in water phase with constant stirring (phase 2). Add phase 1 (oil phase) in phase 2 (water phase) with continuous stirring followed by addition of perfume and triethanolamine to adjust pH until a smooth cream is formed and evaluated [(6).]

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FORMULATION TABLE

Sr. no.	Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
1.	Black seed oil	1	2	3	4	5
2.	Cetyl alcohol	4	4	4	4	4
3.	Steric acid	4	4	4	4	4
4.	Glyceryl monostearate	2	3	4	5	6
5.	Honey	3	3	3	3	3
6.	Tween 80	2	2	2	2	2
7.	Propylene glycol	0.5	1	1.5	2	2.5
8.	Glycerine	2.5	2.5	2.5	2.5	2.5
9.	Carbopol 940	0.4	0.4	0.4	0.4	0.4
10.	HPMC	0.6	0.6	0.6	0.6	0.6
11.	Triethanolamine	q. s	q.s	q. s	q. s	q. s
12.	Methyl paraben	0.3	0.3	0.3	0.3	0.3
13.	Propyl paraben	0.3	0.3	0.3	0.3	0.3
14.	Distilled water	q. s	q. s	q. s	q. s	q. s
15.	Flavour (rose oil)	q. s	q. s	q. s	q. s	q. s

Table No.1 Formulation Table Antimicrobial cream

EVALUATION OF CREAM

PHYSIOCHEMICAL PARAMETERS

The appearance, colour, odour, homogeneity, texture, grittiness of cream was evaluated manually [7].

pH

pH was determined by using digital pH meter. The range for topical preparation it is between 6.0-7.5. A total 10% of cream solution was prepared in distilled water and immersed in pH meter. The electrode was previously washed with distilled water and calibrated [7].

SPREADABILITY

The spreadability of cream was determined by parallel plate method. In this method 2 glass slides were selected. About 1 gm of cream sample weighed and placed on one slide the other glass slides was placed on top of the cream. 1 gm of weight was placed on the slide so that cream was spread to form thin layer wait for some min. Weight was removed and the spread diameter was measured and note the time required to separate the glass slide. The spreadability was calculated by following formula

$$S = M \times L/T$$

Where, S is denoted by spreadability, M is denoted by mass tighed to upper slide in gm, L is length in cm, T is time required to separate the glass slides [8].

VISCOSITY

Viscosity was determined using Brookfield viscometer using spindle no. 63. The speed was kept 100 RPM. The formulated cream was directly immersed into the cream and viscosity was measured and values was recorded in centipoise [8]

TYPE OF EMULSION TEST

The type of emulsion was determined by dilution test and dye test. In a dye test a cream was mixed in water soluble dye (amaranth) and observed under microscope. If continuous phase was appearing red was considered as emulsion is o/w type and if the continuous phase was colourless was considered as emulsion were considered as w/o type. In the dilution test the cream was done to find out the oil in water emulsion. Take small amount of cream dissolve in small amount of water if the cream was completely dissolved in water then the emulsion is o/w type and if the cream is not dissolved in water the emulsion is w/o type (6).

PARTICLE SIZE DETERMINATION

The size of oil globule was determined using microscope by using Saglosoft image analyser virsion-2.

WASH ABILITY OF CREAM

A portion of cream was applied over the skin of the hand and allowed to flow under the force of flowing tap water for 10 min. The time when the cream completely removed was noted [1].

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DRUG CONTENT

The dose of drug was well below the saturation point. The amount of drug release after incorporation should be checked. Hence, the drug content was calculated by UV Visible spectrophotometer. 1gm of gel was dissolved in 10ml of ethanol with constant stirring. The solution was filtered through Whatman filter paper. Further dilutions are made to get required concentration and the absorbance solution was measured at 273nm by using systronics double bead spectrophotometer 2202 against black reagent ethanol [(9).]

The drug content was calculated using formula:

Drug content= Analysed content/ Theoretical content × 100

ANTIMICROBIAL TEST

Escherichia coli used for determination of the antimicrobial activity of various cream formulations. Standard well method was used for this study. The nutrient agar used as to make the inoculation plates. The sterilized nutrient agar solution was prepared and poured into previously sterilized petri plates upto 5mm thickness and plates were allowed to solidify for 5min. On each plate the E. coli culture was spread with a sterilized loop. The agar plates are inoculated with standard inoculum of the test microorganism. The cream sample dissolved into the dimethyl sulfoxide and a test solution is prepared. Then the filter paper disc containing the test component at a desired concentration are placed on agar surface and Petri dish kept for incubation at 37°C for 24hrs to allow the microorganism to grow. At the end of the incubation after 24hrs the zone of inhibition measured with the help of scale in cm [(10)].

RESULT AND DISCUSSION

PHYSICOCHEMICAL PROPERTIES

All the formulations were found to be white, homogenous, non-greasy, non-gritty.

Parameters	F1	F2	F3	F4	F5
Homogeneity	Yes	Yes	Yes	Yes	Yes
Grittiness	No	No	No	No	No
Grassiness	No	No	No	No	No
Consistency	Good	Good	Very good	Very good	Very good

Table no. 2 Physicochemical properties of F1 to F5

Fig no. 1 Physicochemical properties of cream

pH

The pH of cream was between range of 6.5-7. within the skin pH limit which indicates that cream is stable and safe for the skin. The pH of individual cream given in table no. 3

Formulation	pH
F1	6.5
F2	6.7
F3	6.5
F4	7
F5	6.8

Table no. 3 pH of F1 to F5

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SPREADABILITY

Spreadability parameter used to check how the cream spread on skin after application. The spreadability of all batches was between range of 60 to 100 gm.cm/sec. Batch which has good spreadability having a good solubility and efficacy. The spreadability of all batches given in table no. 4. The batch no F4 and F5 shows good spreadibility.

Formulation	Spreadability (gm.cm/sec)
F1	60
F2	90
F3	63.3
F4	100
F5	95

Table no. 4 Spreadability of F1 to F5

VISCOSITY

The viscosity of formulation was determined by using Brookfield viscometer. Among all the batches the batch no. F5 shows higher viscosity. The viscosity of all batches is given in table no.5

Formulation	Viscosity (cps)
F1	2088
F2	2428
F3	2072
F4	2448
F5	2782

Table no. 5 Viscosity of F1 to F5

TYPE OF EMULSION

Dilution test: the dilution test was performed cream was taken and dissolved in distilled water-soluble cream was completely dissolved in water which confirmed that the cream was o/w type.

Dye test: the dilution test was performed cream was taken and dissolved in water soluble dye amaranth which is observed under a optical microscope which shows the continuous phase is red confirmed that the cream was o/w type

PARTICLE SIZE DETERMINATION

The average size of globule was between 2.446 µm to 4.439 µm. The oil globule size of all batches is given in table no. 6 and the globules observed under Saglosoft image analyser was given in Fig no. 2

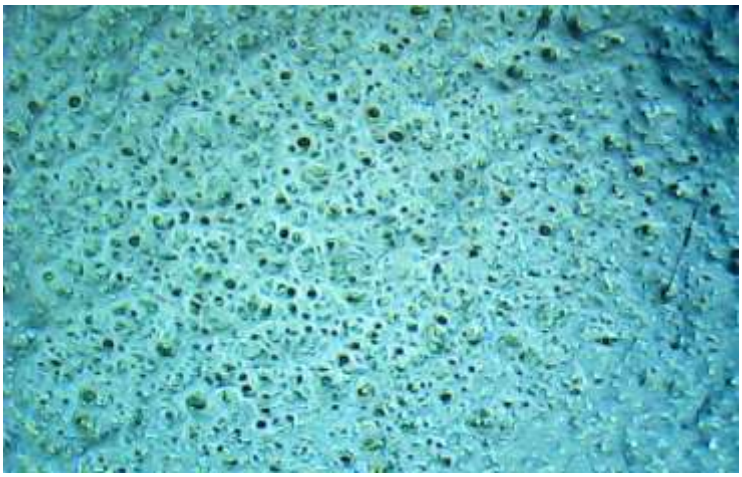

Fig no. 2 Particle size of cream

Formulation	Globule size (µm)
F1	3.389
F2	4.439
F3	3.193
F4	2.446
F5	2.553

Table no. 6 Particle size determination of F1 to F5

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CALIBRATION CURVE OF BSO OIL

The black seed oil show solubility in N hexane. The 1 gm of oil dissolved in 100 ml of n hexane to get concentration of 10 mg/ ml. from the above stock solution aliquots of 0.2, 0.4, 0.6, 0.8, 1 ml was removed and diluted up to 10ml with n hexane to get concentration of 2, 4, 6, 8, 10 µg/ml respectively. the solution was scanned under the UV range between 200-400 nm and wavelength was found to be 273nm by taking n hexane as blank. The absorbance of prepared dilutions was taken at 273nm using double beam systronics UV Visible spectrophotometer. The calibration curve of black seed oil given in fig no.3. It was found that the solution of black seed oil in N hexane show the linearity of 0.9923 in absorbance at concentration of 2- 10 µg/ml.

Sr no.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.11
3	4	0.202
4	6	0.269
5	8	0.352
6	10	0.484

Table no. 7 Calibration curve reading

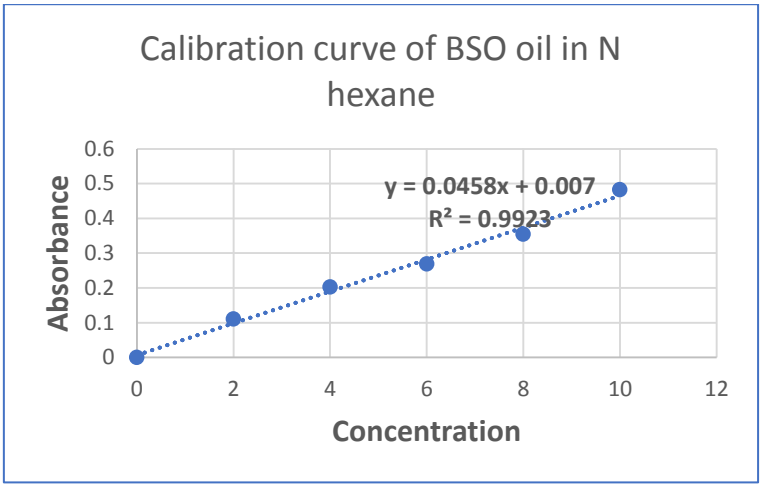

Fig no. 3 Calibration curve graph

DRUG CONTENT

The drug content of all batches was between range of 85% to 96 % given in table no. 8. The batch no. F3=95 and F5=96 show higher % of drug content.

Formulation	Drug content (%)
F1	85
F2	87
F3	95
F4	89
F5	96

Table no. 8 Drug content of F1 to F5

ANTIMICROBIAL TEST

The in vitro antimicrobial study was performed by measuring and comparing the zone of inhibition. The zone of inhibition is defined as the clear region around the well that contains an antimicrobial agent. The larger the zone the antimicrobial agent is more potent. The zone of inhibition for different batches given in table no. 9. The batch no. F1 and F4 show the greater zone of inhibition.

Formulation	Zone of inhibition
F1	3 cm
F2	2.5 cm
F3	2.8 cm
F4	3cm

Table no. 9 Antimicrobial test of F1 to F4

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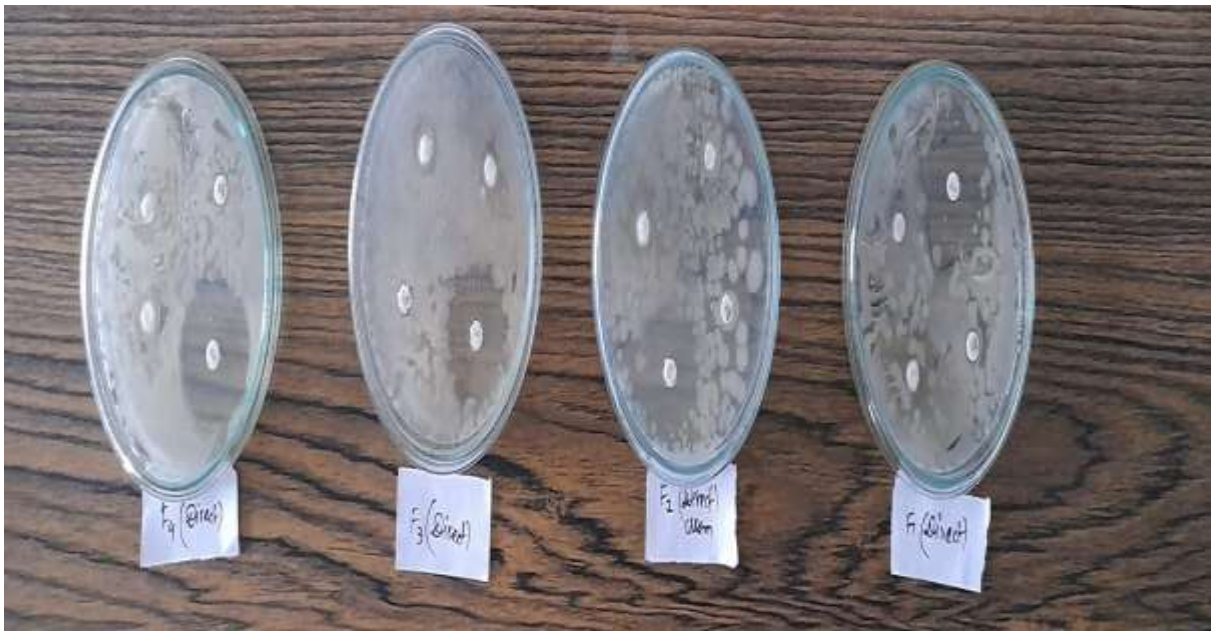

Fig no. 4 Antimicrobial test of F1 to F4

CONCLUSION

As herbal products are safe in use and has less side effects their demand in market is increasing. A topical herbal cream with antimicrobial activity was prepared in this formulation. The formulation was optimized and important parameters like pH, spreadability, viscosity, wash ability, antimicrobial test by well method, content uniformity shows good result within acceptable range and a good cream was formed. The F4 and F5 batch shows good results.

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