Section A-Research paper
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FORMULATION & EVALUATION OF ANTIFUNGAL TOPICAL GEL OF C.TORA SEED AND CAMELLIA SINENSIS EXTRACT

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ABSTRACT

To keep pharmacological or various effects of treatment to skin's surface, skin drug association is portrayed as usage of a response piece plan to not everlastingly fanned out to treat cutaneous issues or cutaneous indication of a general issue. Different pharmacological part structures, for instance, semisolids, liquid way of thinking, essential strong locales for showers, gels, creams, and fixes, are used in skin drug progress techniques. A relationship of cross-related polymers called gel makes when gotten down liquid. Coordinated effort between solid state polymer and liquid part has a significant impact on its characteristics. Gels don't have a flow that is constant.

One of the most frequent issues with dermatological illnesses is fungal infection of the skin. The most effective option for treating cutaneous infections is topical medication.

Therefore, we will employ Camellia sinensis and tora seed herbal plant extracts in this study. Numerous researches demonstrate the effective antifungal properties of camellia sinensis and tora seeds. As a result, some recent developments in formulation have been researched for the delivery of antifungal medications via the target region of the skin.

Key word: Topical Gel, Fungal infection ***C.tora, Camellia sinensis***

1 INTRODUCTION

The most common routes of drug delivery are the oral and Parenteral routes with the majority of small molecule drugs conventionally delivered orally [1,2]. The oral route has the advantage of pre-determined doses, portability and patient self-administration. For these reasons, the oral route remains the most convenient means of delivering medications [3,4]. However, most therapeutic peptides or proteins are not delivered by the oral route, due to rapid degradation in the stomach and size-limited transport across the epithelium [5]. The primary mode of

administering macromolecules is therefore via injection [5,6] which is not without limitations, such as the invasive nature of injections eliciting pain and lower acceptance/compliance by patients, in addition to the requirement for administration by a trained administrator [7]. Rationally, the conventional routes of medication delivery have many inherent limitations which could potentially be overcome by advanced drug delivery.

1.2 Herbal molecule for antifungal therapy

Antifungal medicines derived from natural plant extracts and oils may be a viable solution to this problem. Antifungal properties of many plants, including Vangueria infausta, Bucida buceras, Olinia ventosa, Breonadia salicina, Harpephyllum caffrum, and Xylotheca kraussiana, have been studied [8]. Cinnamon, peppermint, anise, citronella, pepper, clove, and camphor EOs, on the other hand, have been used in the creation of antimycotic medications because of their potent antifungal properties [9]. However, potential therapeutic effects are hampered by the poor solubility, stability, and bioavailability of these important chemicals as well as the gastric degradation that occurs in the gastrointestinal tract. To get over herbal extracts' drawbacks, new drug delivery systems have been developed as adaptable assemblies. Additionally, the resistance mechanisms could be avoided by encapsulating antimicrobial bioactive inside nanoparticles [10].

2 Methodologies

2.1 Collection and Authentication of the Plant

C.tora **Seeds** were collected from local market in Meerut and *Camellia sinensis* leaves were collected from CCS University Meerut. It was identified as ***C.tora*** seeds *and Camellia sinensis* and a specimen was authenticated by Botanical department of Chaudhary Charan Singh University Meerut.

2.2 Extraction of *C.tora* Seeds

The finely grounded seed of the shrub was subjected to extrication in Soxhlet apparatus to methanol by hot continuous percolation method. The collected extract was concentrated on rotary evaporator and was kept in vacuum dryer until used. Fifty gram of the desiccated extricate was diffused in 500 ml methanol to get final concentration of 10 mg/ml. Methanol extractive value was also calculated. The plant extract was also subjected to UV Spectrophotometric analysis to obtain absorption maxima. Qualitative analysis was done to find out the presence of glycoside, alkaloids, tannins, saponin and steroids.

2.3 Extraction of *Camellia sinensis*

A total of 20 g of the ground tea sample was extracted in a 1,000-mL glass beaker with 200 mL n-hexane in a supersonic water bath at 40°C for 30 min. The residues were separated from the solution by filtration on a filter paper (No. 102 filter paper, Xinhua Paper Mill) and then the residues were re-extracted two times as before. The filtrates were combined and evaporated at 40°C using a rotatory vacuum evaporator. to a final volume of

about 40 mL. The concentrated filtrate was transferred into a 50-mL volumetric flask and diluted to 50 mL using n-hexane.

2.4 Evaluation of the *Extract*

2.4.1 Characteristics of extracts

The ethanolic extracts of the ***C.tora* Seeds and *Camellia sinensis*** were evaluated for its physical state, color, odor, and taste.

2.4.2 Photochemical investigation of the extract [11]

Preliminary qualitative photochemical analysis was carried out to identify the active constituents present in the alcoholic extracts of ***C.tora* Seeds and *Camellia sinensis***. The following procedures were adopted to test for the presence of various constituents in the ***C.tora*** Seeds and *Camellia sinensis* extract.

1. Test for flavonoids

(a) Ferric chloride test

To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green color indicates presence of flavonoids.

(b) Lead acetate solution test

Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

2. Test for proteins

(a) Xanthoprotein test

Test solution treated with conc.nitric acid and on boiling gives yellow precipitate.

(b) Ninhydrine test

Test solution treated with Ninhydrine reagent gives blue color.

3. Test for amino acids

(a) Ninhydrine test

Heat 3ml extract with 3 drops of 5% Ninhydrine solution in boiling water bath for 10 minutes. Purple or bluish color appears.

(b) Test for Tyrosine

Heat 3ml extract with 3 drops of Million's reagent. Solution shows dark red colour.

4. Test for phenol

Ferric chloride test

To 2ml of extract add ferric chloride solution. Blue or red colour appears.

5. Test for organic acid

Adequate amount of sample is taken with 2M NaOH and add 5ml of silver nitrate solution. Light yellow precipitate indicates the presence of phosphoric acid.

2.5 Antifungal activity of the extract [12-14]

The following Standard cultures were used in the study

1. *Aspergillus*

2. *Fusarium*

3. *Candida albicans*

2.4.3 Preparation of bacterial suspension

To accomplish this serial dilution-agar plate technique was used. The method was performed by aseptically transferring 1 ml from the bacterial suspension tube to sterile water of known volume such that the culture has been diluted 10 times to 10-1 up to 10-10. Once diluted, the suspensions were poured with gentle rotation in to Agar media and incubated at 37^0C for 24 hrs and counted by using colony counter. Plates suitable for counting must contain no fewer than 30 or

more than 300 colonies. The total count of the suspension is obtained by multiplying the number of cells per plate by the dilution factor which is the reciprocal of the dilution.

2.6 Formulation and Optimization of Gelling Agent

Carbopol is a water-soluble polymer which acts as powerful, gelling thickener useful for making clear gels. In order to achieve desired gel consistency and spreadability, different concentrations of Carbopol 940 such 1%, 1.5% and 2% were tried and the concentration was optimized.

2.6.1 Formulation of Gel base:

Gelling agent was dispersed in sufficient quantity of water. Propylene glycol-400 which is used as humectant or plasticizer was added to the dispersion. Other excipients such as methylparaben and propyl paraben were added with continuous stirring. In Carbopol gels, pH of the vehicle was brought to neutral by using TEA (Triethanolamine).The final weight of the gel was adjusted to 60 gm with distilled water. Then the mixture was stirred by using using propeller for 2 hours at 500 rpm. After stirring, this homogenous gel appeared to be free of bubbles. It was kept at room temperature for 24 hours to check the consistency and stability of gel.

2.7 Formulation of antifungal gel containing C.tora seed and Camellia Sinensis Extract

Antifungal gel was prepared by incorporating ethanolic extract of C.tora seed and Camellia Sinensis liquid in to optimized**2.6 Formulation and Optimization of Gelling Agent**

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be homogenous and devoid of air bubbles. The prepared gel was kept at room temperature for 24 hours.

2.7 Evaluation of Antifungal Gel

The prepared polyherbal gel was subjected to physical characterization such as colour, appearance, pH, viscosity, spreadability. It was also evaluated for its stability property, antifungal activity and *in vivo* skin irritation study.

2.6.1 Physical appearance [15].

The formulated Polyherbal gel was inspected visually for their color, odor, homogeneity and consistency. All developed gels were tested for homogeneity by visual inspection after gels have been set in the container.

2.6.4 Spreadability [18,19]

Spreadability denotes the extent of area to which the gel readily spreads on application was placed over one of the slides. The other slides was placed on the top of the gel, such that the gel was sandwiched between the two upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide slip off freely by the force of weight tied to it. A 20gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times and the mean time taken for calculation.

Spreadability was calculated by using the following the formula:

They were tested for their appearance and presence of any aggregates.

2.6.2 Measurement of pH [16]

The pH of various formulations was determined by using digital pH meter. One gram of gel was dissolved in 100ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate.

2.6.3 **Determination of Viscosity [17]**

The measurement of viscosity of prepared gels was carried out with Brookfield viscometer (Brookfield viscometer) with spindle No.62.

to skin or the affected part. Two sets of glass slides of standard dimensions were taken. The gel formulation

slides in an area occupied by a distance of 6.0 cm along the slide.100gm weight was placed upon the

$$S = (\boldsymbol{M}\times\boldsymbol{L})/\boldsymbol{T}$$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length of the glass slide

T= Time (in sec) taken to separate the slides.

2.6.5 Extrudability [20-21]

The gels were incubated at room temperature for 2 h before measuring their extrudability using an HDP/FE forward extrusion cell of the TA-XT2 Texture Analyzer equipped with a 5 kg load cell. Prior to measurement, the gel was manually stirred and loaded (100 g) into the cell. The compression force was measured at the following conditions: pre-test speed 1 mm/s,

test speed 1 mm/s, trigger force 10 g, post-test speed 10 mm/s, compression distance 20 mm, and outlet diameter of extrusion cell 3 mm.

2.7 Stability Study [22,23]

Stability of the gel formulation was studied at different storage condition (80C and 400C) Samples were withdrawn at 7, 15 and 30 days and checked for their physical characteristics like appearance, homogeneity, pH, viscosity and spreadability.

2.8 Antimicrobial activity by cup plate method

The sterile Petri dishes were filled with Agar medium which was then inoculated with a apparatus was used. For making study, egg-membrane was fastened in between the donor and receiver compartment of the apparatus. The receptor compartment was maintained at a temperature of 37 ± 10 C and was filled with 10.0 ml of phosphate buffer ph 6.8. For testing, 0.1 g of gel formulation was placed over egg-membrane and solution of phosphate buffer ph 6.8 in the receptor compartment suitable dilution of a test organism (*Aspergillus Fusarium* and *Candida albicans*. Four cylinder or cups were made in the medium with the sterile borer in each plate. The formulated gel, standard disc and solvent control were prepared. A uniform amount of 0.2 ml solution was added to the cup and incubated at 37^{0}C for 24 hrs. The well diffusion test was performed in triplicates and antimicrobial activity was expressed as the mean of inhibition in diameter (mm).

2.9 In-vitro Drug Diffusion Study:

For deducing this parameter for all gel formulations, the Franz-Diffusion cell

with stirring at 50 rpm. Then the sample was withdrawn at regular time interval of 0, 1, 2, 3, 4, 5, 6 and upto 24 hrs. And diluted with 10.0 ml of blank solution and sink condition was maintained. Diffusion study of formulation was carried out in triplicate and average value ± standard deviation was calculated.

3. RESULT AND DISCUSSION

3.1 Evaluation of Extracts

3.1.1 Characteristics of Extracts

Table 1 Characteristics of the Extract

S.No	Characteristics	*C.tora*	*Camellia sinensis*
1	Physical State	Semi solid	Semi Solid
2	Colour	Green	Green
3	Odour	Characteristic	Characteristic
4	Taste	Characteristic	Characteristic

3.1.2 Photochemical Screening of Extract

Table 2 Photochemical Screening of C.tora*, and Camellia sinensis*

Constituents	Test	Observation
Flavonoids	Ferric chloride	++
	Lead acetate	++
Protein	Xanthoprotein	++
	Ninhydrin	++
Amino Acid	Ninhydrin	++
	Tyrosine	++
Phenol	Ferric chloride	++
Organic Acid	Phosphoric acid	++

++ Means Presence of Constituents

3.2 Evaluation of Antifungal Gel

Table 3 Preformulation evaluation of C. tora seed powder

S.No	Test	Result
1	Foreign matter	1.12±0.08
2	Moisture content	6.10± 0.21
3	Total Ash value	8.24±0.1
4	Water soluble ash value	2.85±0.12
5	Acid insoluble ash value	1.01±0.21

Table 4 formulated gel was checked visually for colour, appearance and homogeneity.

Parameter	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Physical Appearance	Sticky	Sticky	Sticky Gel	Sticky	Sticky	Sticky
Colour	Dark coffee	Dark coffee	Dark coffee	Dark coffee	Dark coffee	Dark coffee
Homogeneity	- Aggregates	- Aggregates	- Aggregates	- Aggregates	- Aggregates	- Aggregates

- **(Absence)**

Table 5 Evaluation of formulated gel

Parameter	**Formulation Code**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
pH	5.12±0.12	5.74±0.21	5.78±0.12	6.52±0.12	6.87±0.85	7.02±0.12
Viscosity(Poise)	298±25.12	378±14.12	485±28.14	587±39.01	602±17.01	612±31.14
Homogeneity	Fair	Fair	Fair	Good	Good	Very Good
Spredibility (gcm/sec)	35±0.19	35.45±0.14	36±0.14	36.7±0.22	37.85±0.24	38.98±0.3
% Drug Content	92.19 ± 0.12	92.76 ± 0.98	94.14 ± 0.78	95.71 ± 0.14	96.35 ± 0.14	97.12 ± 0.14

pH: The pH of all prepared formulation ranged from 5.12 -7.0 the pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin.

Spreadability: denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The spreading was expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides,

under certain load. Lesser the time taken for separation of the two slides, better the spreadability. Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides. Spreadability of different gel formulation were studied. The formulation F6 produced good spreadability than the other formulation.

The prepared gel formulation was subjected to the percentage of drug content study. The results pointed out that all formulation do not show marked variation in their drug content. The drug content found to be 92.19 ± 0.12 to 97.12 ± 0.14 respectively the formulation F6 fond to be give maximum drug content due to different concentration of extract.

Viscosity:

Viscosity is a crucial characteristic of fluids that characterises a liquid's resistance to flow and is associated with internal fluid friction. This rheological characteristic aids in determining consistency and the pace at which a medication diffuses from a gel. A Brookfield viscometer with spindle number 62 was used to test the viscosity of the produced gel.

The outcomes were displayed in Table No. 12. The benefits of more attractive aesthetic qualities and simplicity of correct application through better flow and pourability are realised by maintaining the viscosity below roughly 15,000 cps.

Stability Studies: On the basis of above study it is found that there is no major variation when we stored the formulation for long period of time at different different humidity and temperature as per provided by ICH. So we can say that the optimized formulation is safe and effective for long time.

Activities by cup plate method :

The sterile Petri dishes were filled with Agar medium which was then inoculated with a suitable dilution of a test organism (*Aspergillus Fusarium* and *Candida albicans*. Four cylinder or cups were made in the medium with the sterile borer in each plate.

The antifungal activity testing is performed by measuring and comparing the diameter of zones of inhibition (in mm). 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 antifungal agent.

The formulated gel (F6) is observed for its antifungal property towards the organism such as *Candida albicans, Aspergillus, Fusarium* and it is also compared with standard such as Fluconazole(25-mcg). From the result it was observed that it showed good zone of inhibition but lesser when compared to standard.

Table 6 In vitro Antifungal activity

Formulation	Drug Content	Zone of Inhibition	
		Fluconazole	**Antifungal Gel**
F1	92.19 ± 0.12	**15.21**± 0.21	**11.21**± 0.24
F2	92.76 ± 0.98		**12.02**± 0.14
F3	94.14 ± 0.78		**11.85**±0.24
F4	95.71 ± 0.14		12.24±0.25
F5	96.35 ± 0.14		**12.85**±0.65
F6	97.12 ± 0.14		**14.19**±0.85

Table 7 *In vitro* Dissolution study

F. Code Time(Hrs)	F1	F2	F3	F4	F5	
0.5	13.21±0.05	12.65±002	13.01±01	12±01	13.85±01	14.24±02
1	16.33±12	18.89±09	17±01	16.45±01	19.89±02	20.87±01
2	23.25±14	26.52±004	29.58±04	25.85±01	36.52±02	38.85±03
4	29.02±01	35.48±04	38.98±01	36.12±02	46.12±03	48.17±02
6	39.25±05	43.58±02	46.89±001	48.12±03	57.1±07	60.4±05
8	45.58±001	49.85±001	56.4±014	58.1±01	69.1±08	75.3±04
10	52.98±004	59.25±01	63.85±02	66.85±01	77.1±002	85.4±001
12	60.25±001	66.12±023	68.99±01	69.99±02	84.4±001	94.6±003
24	71.24±004	76.52±01	79.98±01	75.52±001	95.24±001	97.24±001

Conclusion:

As many traditional healers are using this c.tora seed and camellia sinensis extractfor treating number of fungal and bacterial infections, we made a formulation by using the c.tora seed and camellia sinensis extract extractions.No change was observed in its pH and other physical parameters and skin irritation studies were observed with all the five formulations. Along with the above the gel formulation is also have good antifungal activity. The antifungal activity of the .tora seed and camellia sinensis *herbal* gel formulations shows dose dependent zone of inhibitions in exponential manner i.e F5 formulations shows **14.19**±0.85cm zone of inhibition it is very much greater than the remaining four formulations and the standard . When compared with the standard drug our formulation gels are showing better antifungal activity. By this study results we are concluding that all these five formulations are best in their stability and antifungal activity so we can use this formulation for treating fungal infections.

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