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Full Length Research Article

# Antiaging Cream Preparation and Evaluation of *Alpinia galangal* and *Morshella esculenta*

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## Abstract

The objective of the present investigation was to formulate and evaluate antiaging cream using *Alpinia galangal* rhizome and *Morshella esculenta* mycelia. Methanolic extraction of plants was carried out using maceration. Cream was formulated using dried methanolic extracts, borax, beeswax and mineral oil. Moreover, the cream was evaluated for physical parameters, pH, irritancy, washability, viscosity, thermal stability, diffusion of active ingredients on nutrient agar medium and microbial contamination on Potato Dextrose Agar (PDA) and Luria-Bertani (LB) agar medium. Microscopic analysis of cream was performed. The formulated cream (pH 6.43) exhibited yellow color, smelt odourless, was smooth and thick in texture, non-irritant on the skin and easily washable. Sudden changes in viscosity of the cream were not observed. Furthermore, results from thermal stability studies showed stability of cream up to 60 $^{0}$C**.** Besides, microscopic examination of the cream revealed no evidence of visual disturbances. Moreover, the cream was non susceptible to microbial growth. Finally, diffusion studies revealed no diffusibility of cream base with agar medium. In conclusion, cream prepared from *A. galangal* rhizome and *M. esculenta mycelia* might prove as an antiaging preparation. Our findings provoke strong requirement of future studies on these two plants and *in vitro* evaluation of antiaging formulation.

***Key words*****:** *Alpinia galangal, Morshella esculenta, A*ntiaging, Cream, Stability

Creams are described as semisolid emulsions which are water in oil (w/o) type or oil in water (o/w) type intended for external application [1]. It is applied on superficial part of the skin where it resides for longer durations at the site of application. The predominant function of a cream is to act as a skin barrier against different environmental conditions and provides soothing and various therapeutic effects to the skin. *Alpinia galangal* Wild. (Fam: Zingiberaceae) is an aromatic perennial rhizomatous herb used since Ayurveda in the name of sugandhamula, rasna, kulanjan and greater galangal, the genus is the most marked and ubiquitous in Zingiberaceae family that provides many useful products for food, condiments, dyes, perfume and aesthetics [2-4]. It is extensively found in Western ghats, Malabar coasts and Gujrat in India and produced by other Asian countries such as Thailand, China and Malaysia [4]. The rhizome of *Alpinia galangal* has therapeutic value and widely used as an antioxidant, antiaging, gastroprotective, antifungal, antihelminthic, antidiuretic, antidiabetic, antidementia, aphrodisiac, antimicrobial and antitumor [5-9].

*Morshella esculenta* (Pezizales, Ascomycota) is one of the costliest and predominantly found edible morel species in North-West Himalayas mainly, Kashmir and are renowned for their gastronomic quality due to their aroma, extraordinary flavour and meaty texture, indigenous and nutritional health benefits as functional food due to their anti-oxidative, anti-inflammatory, immunostimulatory properties, and high levels of protein, fibre and minerals. Morels are garnered in the wild majorly in India, China, Turkey, USA and Mexico where they grow abundantly [10-11]. Prasad *et al.* reported that *Alpinia galangal* and *Morshella esculenta* in *S. cerevisiae* showed antiaging aging properties attributed to the presence of principal antioxidants such as vanillic acid, rutin and caffeic acid. Furthermore, it was reported that *Alpinia galangal* and *M. esculenta* extracts were nontoxic and conferred viability to *S. cerevisiae* cells [12].

The present study was undertaken to develop and evaluate an antiaging polyherbal cream formulation using *Alpinia galangal* and *Morshella esculenta* methanolic extracts. The ingredient borax was employed in our formulation because it generates a stable product as it chemically reacts with free fatty acids in bee's wax. Another chief ingredient in our formulation was mineral oil, which assisted in inhibition of rancidity and ameliorated the stability of final product. Moreover, our study is the first study to report an antiaging cream formulation using *Alpinia galangal* and *Morshella esculenta* extracts synergistically.

## MATERIALS AND METHODS

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Materials used in this study comprised of methanolic extracts of *A. galangal* and *M. esculenta* as well as cream ingredients such as boric acid, beeswax and mineral oil.

*Collection of plant material*

*Alpinia galangal* rhizome was collected from Karnataka (India) and *M. esculenta* mycelia was collected from Uttarakhand (India).

*Extraction*

Samples were thoroughly cleansed with distilled water and air dried to remove moisture and grounded into coarse powder. For methanolic extraction, 10 g powder of all four plant samples were mixed with 40 ml solvent to attain a minimum concentration of 250 mg/ml followed by maceration with occasional shaking for 5 days at room temperature with 100% methanol. The extracts were filtered and evaporated to obtain the crude extracts. All the extracts were preserved at 4 °C for further [13].

*Preparation of polyherbal moisturizing cream*

Bees wax pellets were melted using water bath at 75 °C in a small borosilicate beaker. It was further heated in microwave for 50 s followed by continuous agitation using a magnetic stirrer along with continuous addition of mineral oil and boric acid for 10 s at 70 °C until a semisolid mass was formed. It was further cooled to bring down at room temperature. *A. galangal* and *M. esculenta* crude methanolic extracts (1 ml) were dried to evaporate the organic solvent and the dried extracts were added to the freshly prepared cream and mixed [14]. The formulation for the prototype moisturizing cream shown in (Table 1).

Table 1 Formulation of cream

| S. No | Ingredients | Quantity (g) |
|---|---|---|
| 1 | Boric acid | 0.08 |
| 2 | Bees wax | 1.5 |
| 3 | Mineral oil | 5 |

*Evaluation of moisturizing cream*

*Physical evaluation*

The prepared cream was evaluated for physical parameters such as color, odour, texture and state.

*Color*: The color of the cream was noted by visual examination.

*Odour*: The odour of cream was examined by sniffing.

*Consistency*: The formulation was evaluated by rubbing cream on hand manually.

*State*: The state of cream was determined visually [15].

*pH*

0.5 g cream was dispersed in 40 ml distilled water and then pH was measured by using digital pH meter, standardized with pH 4.0 and 7.0 standard buffers before usage and average of triplicates were noted [16].

*Irritancy*

The cream was applied on the left-hand dorsal surface area (1 $cm^2$) and the time was noted. Then it was examined for itching, spotting, edema and erythema if any for a time period of 2 h and recorded [15-16].

*Washability*

A small quantity of cream was applied on the hand and washed with warm water excluding usage of soap and ease of extend of washing was checked [15-16].

*Changes after two weeks*

The cream formulation was kept under observation, undisturbed for two weeks and was examined for any noticeable changes in the formulation.

*Viscosity*

Prepared cream was heated until melted and then the melted cream was transferred to a volumetric flask and was filled in a graduated cylinder marked up to 4 ml. It was allowed to stand at room temperature and cooled. A small piece of marble was carefully dropped on the cream into the flask and the time for the piece of marble to sink through the cream from the initial volume of 4 ml was noted [17].

*Thermal stability*

Investigational cream was melted and then transferred to a graduated cylinder marked up to 4 ml. A small piece of marble was carefully dropped on the cream into the cylinder. Subsequently, the cylinder was placed in a water bath wherein the cream was subjected to, at different temperatures initially varying from low to high temperatures (40°C - 70°C), at a definite time interval of 30 min for each temperature. The temperature at which the piece of marble started to move down through the cream from the initial volume of 4 ml was noted.

*Microscopic analysis*

Cream formulation of 0.05 ml was placed on glass slide and examined under different magnifications of compound microscope to check for cluster preparations or any visual disturbances.

*Microbial contamination test*

PDA (0.1 g potato extract, 1 g dextrose and 0.2 g agar in 25 ml of distilled water) and LB (0.25 g peptone, 0.125 g yeast extract, 0.25 g sodium chloride, 0.4 g bacteriological agar in 25 ml of distilled water) were prepared. Upon solidification of the media, a small amount of investigational cream was taken and spread on the solidified media followed by observation for 1 week at 37 °C in Bio-Oxygen Demand incubator.

*Diffusion of active ingredient*

Nutrient agar medium was prepared using yeast extract 0.125 g, peptone 0.25 g, sodium chloride 0.25 g, agar 0.4 g and distilled water 25 ml. Subsequently, medium was poured into a petri dish and two holes were made on the medium and the cream was then applied to one of the holes while the other hole contained mineral oil as standard compound, since, mineral oil is standard fat in which the cream base was prepared and was observed for 1 hour to check for diffusion [18].

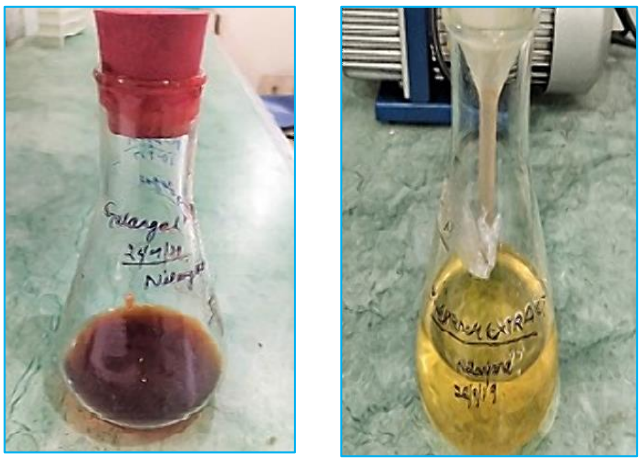

Fig 1 Methanolic extracts (A) *A. galangal* (B) *M. esculenta*

## RESULTS AND DISCUSSION

*Extraction of plants*

*A. galangal* and *M. esculenta* extracts were prepared in methanol using maceration method. The concentration of the methanolic extracts were found to be 250 mg/ml. The results are shown in (Fig 1).

*Formulation of cream*

Moisturizing cream (6.58 g) was prepared using bees wax, mineral oil and boric acid as shown below in (Fig 2).

Fig 2 Moisturizing cream

Fig 3 Viscosity test of cream

*Physical evaluation of cream*

The formulated cream was evaluated for physical parameters such as color, odour, consistency and state as well as for other parameters such as pH, irritancy etc. The results are represented in (Table 2).

Table 2 Results of moisturizing cream

| S. No. | Parameters | Results |
|---|---|---|
| 1 | Color | Yellow |
| 2 | Odour | Odourless |
| 3 | Consistency | Smooth and thick |
| 4 | State | Semi solid |
| 5 | pH | 6.43 |
| 6 | Irritancy | Non irritant |
| 7 | Washability | Easily washable |
| 8 | Changes after two weeks | Unchanged |

*Viscosity*

The piece of marble remained at its initial position (4 ml) in the graduated cylinder and did not flow through the cream as shown below in (Fig 3). Therefore, the cream showed no sudden changes in viscosity at room temperature.

*Thermal stability*

At 40 °C and 50 °C, the piece of marble showed no movement through the cream. However, at 60 °C, the cream started to melt and the piece of marble moved down at the bottom of the cream in the graduated cylinder. Hence, the thermal stability of cream was found to be up to 60 °C.

*Microscopic analysis*

The cream showed small cluster formations at 40x. However, there were no signs of visual disturbances such as unclarity, blurriness etc. as given in (Fig 4).

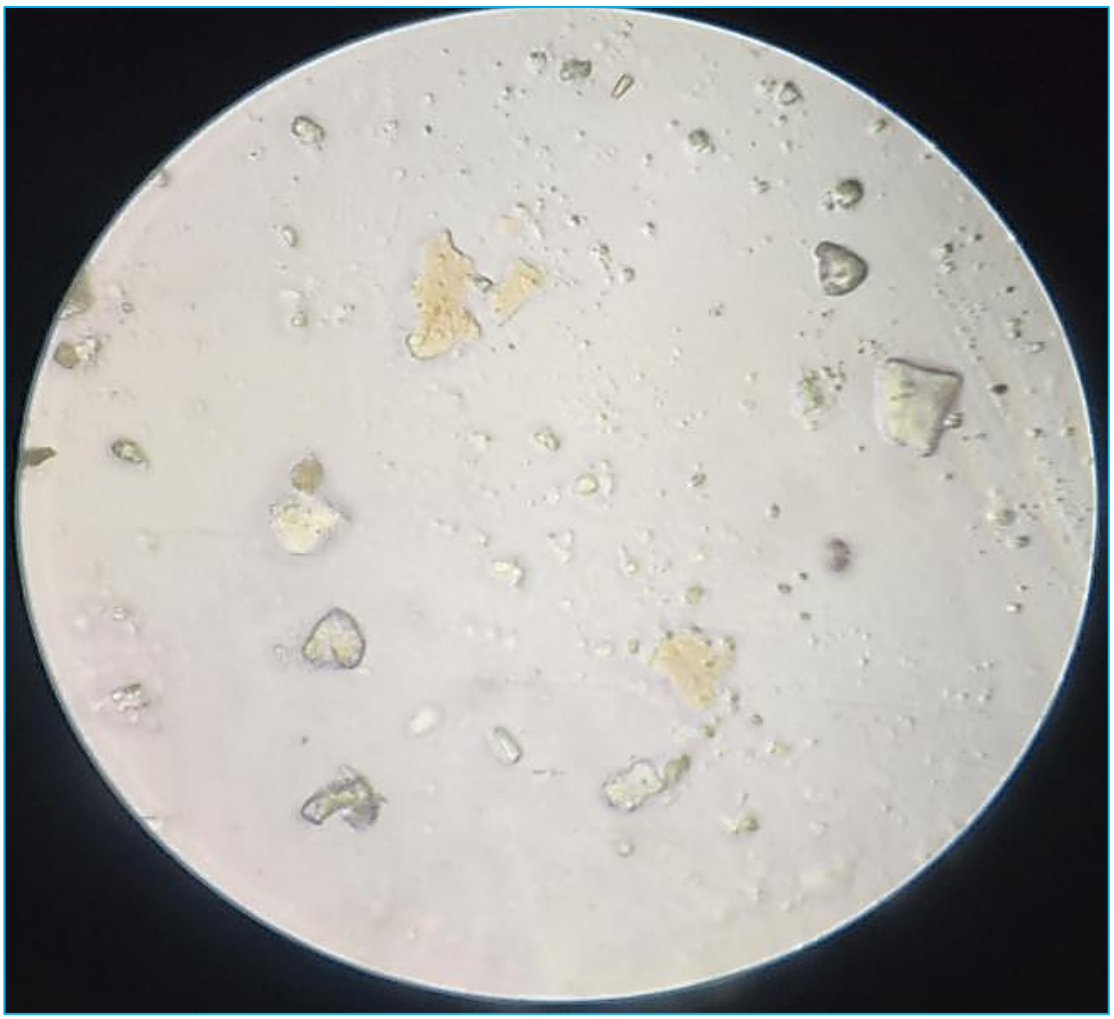

Fig 4 Microscopic image of cream at 40x

*Microbial contamination*

No microbial growth was observed on LB agar and PDA media. The results are shown below in (Fig 5).

Fig 5 No microbial growth on (A) LB agar medium (B) PDA medium. The cream sample was spread onto the LB agar and PDA and incubated for one week at 37 °C

*Agar diffusion test*

There was no amount of cream base which was diffused with the nutrient agar media observed for 1 hour as shown in (Fig 6).


Fig 6 Agar diffusion test showing no diffusion of cream with media

The present study focused on the formulation and evaluation of polyherbal antiaging cream using ingredients such as boric acid, bees wax and mineral oil with *Alpinia galangal* and *Morshella esculenta* crude methanolic dried extracts being the predominant sources of herbs for cream formulation. The formulated cream consisted of 37.99 mg/ml of plant extracts. Several studies reported usage of borax, beeswax and mineral oil for cream formulations which provided good stability to formulated cream [14-15]. Various antioxidants such as phenolic acids and flavonoids were present in our formulation such as caffeic acid, vanillic acid and rutin which contributed to antiaging property of the cream [12]. In addition, a study reported the presence of flavonoids in red and white galangal creams [19]. Our formulated cream displayed yellow color, smelt odourless, appeared smooth and thick in texture and showed non-irritancy on the skin. Furthermore, microscopic analysis of the cream at 40x showed no evidence of visual disturbances.

Microbial contamination studies revealed non susceptibility of the cream to microbial growth on LB agar and PDA media upon incubation at 37 °C for 1 week. Diffusion of antioxidants present in cream with agar medium was studied by using mineral oil as a positive control because mineral oil is standard fat. Previous studies reported the use of viscometers for determining viscosities of creams [20-22] whereas in our study viscosity of the formulated cream was examined by determining the time required for a piece of marble to sink to the bottom of graduated cylinder. Results from our study presented no sudden changes in viscosity of the cream which indicated stability of formulated cream at room temperature while results obtained from thermal stability studies indicated stability of cream up to 60 °C. This can be explained on the fact that viscosity relies on the shape, size of the particles in the liquid and attraction between them. Liquids which are less viscous flow quickly rather than liquids which are highly viscous which flow slowly.

A study reported formulation of antifungal creams from rhizome extract of *Alpinia galangal* using stearic acid, triethanolamine etc., with an acidic pH between 4.5 – 6.5 which is quite consistent with pH of our formulated cream which is 6.43 [23]. In addition, another study reported antifungal cream formulation from the same using bees wax and vegetable oils with no signs of irritancy on the skin which are quite congruous with our results [24]. Furthermore, results from diffusion studies revealed unfavourable results which showed no diffusibility of cream base with agar medium which strongly renders a promising scope of future studies on diffusion of the respective cream under study by alteration of experimental conditions such as reduced or increased amount of antioxidants, clinical trials etc. along with simultaneous studies on exogenous and endogenous effects on cells.

Future prospective includes *in vitro* evaluation of the topical antiaging formulation of the rhizome of *Alpinia galangal* and mycelia of *Morshella esculenta*. Thus, the antiaging cream prepared from *Alpinia galangal* rhizome extract and *Morshella esculenta* mycelia extract might prove to be a potent antiaging formulation and can be used for decelerating the symptoms of aging. The outcomes obtained in the present investigation are at most directional in view and supplementary investigation can be made on this basis to retrieve additional information about the plants and synergistic effects of various plants extracts can also be studied on skin aging.

## CONCLUSION

From the above study, it can be concluded that, ethnomedicinal plants *Alpinia galangal* and *Morshella esculenta* possessed antiaging potential which could be formulated into an antiaging cream formulation due to the presence of principal antioxidants, using the formula consisting of boric acid, bees wax and mineral oil. The formulation has been found to be safe to use on the skin, thus, might prove to be an effective antiaging formulation. We are blessed with plethora of magical natural ingredients from nature, it solely depends upon us to scientifically explore them for societal benefits.

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