Formulation and Stability Determination of Anti-Acne Cream Containing Black Cumin Seed Oil and Kaolin Clay

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Abstract

Acne is the most common skin problem that could occur to any individual. *Nigella sativa* seed oil and kaolin as natural antimicrobial agents have been utilized in anti-acne cream formulated in this study. This study aimed at the development of anti-acne cream with antimicrobial properties using a crude extract of black cumin (*Nigella sativa* L.). Anti-acne creams had been formulated from cream-based agents with various percentages of crude black cumin seed extract and 1.0% (w/w) of mineral clay (Kaolin). Physical properties and stability of anti-acne cream at various storage conditions, including incubation at and freeze-thaw at 4, 40, and 45 °C for 28 days. The results showed that developed anti-acne cream containing a crude black cumin seed extract in 0.1 and 1.5% (w/w) had good physical stability. Therefore, the suitable formulation was then tested for anti-Propionibacterium acnes (*P. acnes*) susceptibility by the broth dilution method. From these results, it was found that 1.0% (w/w) of crude black cumin seed extract had the ability to inhibit *P. acnes* from MIC (minimal inhibition concentration) of 15.6 mg/mL.

Keywords: *Nigella sativa*, Kaolin clay, Acne, Propionibacterium acnes

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INTRODUCTION

Acne is the most common skin problem caused by chronic inflammation of a sebaceous follicle. It is usually observed in 80% of young adults and adolescents. The symptom may vary from mild to severe conditions. It can appear on the face, scalp, neck, chest, back, upper arms, and shoulders, where the sebaceous glands are widely present. This is one of the skin diseases that may affect not only the appearance but also result in discomfort and emotional stress. Many findings revealed the association of microbial infection with this disease. The common microbes found in pustular contents of acne are *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, and *Candida Albicans* (Achermann et al., 2014). *P. acnes* were found to be the main organism involved in acne development. It triggers the production of various inflammatory mediators and turns sebaceous triglycerides into fatty acids, which result in neutrophil recruitment. The removal and reduction of *P. acnes* is the main strategy to overcome this skin condition (Varma et al., 2014). The general treatments recommended for acne vulgaris are topical application and oral medication. The topical cream containing antimicrobial agents has been applied. Both natural and synthetic sources of antimicrobial agents have been added to cosmetic
formulations to inhibit the growth of bacteria as well as the inflammation caused by them. However, the microbes become resistant to the existing antibiotics most of the time (Nawarathne et al., 2019). Thus, the utilization of natural extracts as antimicrobial agents is one of the major concerns. In addition, the demands for a cosmetic product containing natural products are rapidly growing. Natural clay is of natural product which has been applied since prehistory for wound healing, reducing skin irritation. It is reported to use for skin inflammation as well as preservation of Egyptian mummies (Robertson et al., 1982). *Nigella sativa* seed oil is also reported as a potential antimicrobial agent against a variety of pathogens (Ali et al., 2001; Najah et al., 2012). The presence of Thymoquinone and other phytochemical compounds in its oil is also attributed to its antioxidant and anti-inflammatory properties, which are found to be very beneficial when applied to the skin. The *Nigella sativa* seed oil will not only be capable of inhibiting the growth of *P. acnes*, but it will also provide a gentle skin with the inflammation from acne scar is recovered. Therefore, kaolin clay and black cumin seed oil are incorporated in a cream formulation. The physical properties and stability of anti-acne cream at various storage conditions are performed. The antimicrobial property of anti-acne cream against *Pance* is evaluated.

LITERATURE REVIEW

*Acne vulgaris*

*Acne vulgaris* is one of the skin conditions that occur when chronic inflammation of the sebaceous follicle takes place. The formation of comedones, papules, pustules, inflamed nodules, superficial pus, field cysts, and deep scarring is a common characteristic of this skin condition. These skin conditions can be occurred due to many factors, including the release of inflammatory mediators into the skin, comedones development, the alteration of sebum production, as well as follicular colonization of *P. acnes* (Achermann et al., 2014). *P. acnes* is a gram-positive, anaerobic, and lipophilic bacterium known as a commensal bacterium colonize and inhabitant of human skin with *Staphylococcus, Corynebacterium, Streptococcus, and Pseudomonas spp.* It sometimes becomes an opportunistic pathogen when the anaerobic and lipid-rich condition within the pilosebaceous unit is attractive for its growth, especially when hair follicles are blocked by some dead skin cells, oil, and other bacteria. *P. acnes* may activate TLR-2 on macrophages which result in the production of IL-12 and IL-8 and recruitment of neutrophil. *P. acnes* are reported to produce biofilm formation, which triggers the chronic skin condition of acne vulgaris (Achermann et al., 2014).

*Nigella sativa*

*Nigella sativa* Linn. is one of the aromatic plants belonging to the Ranunculaceae family. The plant can grow from 20 -25 cm tall. The color of flowers varies from white to pale blue with 5-10 petals. The bearing seeds are black with 2-3 mm long (Sudhir et al., 2016). Numerous phytochemical compounds were reported in many studies. The phytochemicals found in *Nigella sativa* are Thymoquinone, p-cymene, longifolene carvacrol, limonene, alpha-pinene,
thymol, 4-terpineol, t-anethole benzene, alpha-thujene, alpha-hederin, nigellone, nigellimine, nigericin. These bioactive compounds claimed to display the properties of anticancer, antidiabetic, antimicrobial, anti-inflammatory, antioxidant and wound healing activities (Gharby et al., 2015; Harzallah et al., 2011; Kooti et al., 2016; Sudhir et al., 2016). The antimicrobial properties of black cumin have been well addressed in many studies. In vitro antimicrobial studies indicated the efficacy of Thymoquinone and Nigella sativa seed oil against various types of bacteria, which caused an oral infection known as dental caries (Harzallah et al., 2011). A clinical study by Rafati et al. (2014) revealed that the Nigella sativa seed extract is effective against S. aureus, which can be comparable to the standard drug, mupirocin, when treated to neonates that encounter staphylococcal skin infections (Rafati et al., 2014). Many studies also reported that Nigella sativa seed was found to be more effective to gram-positive bacteria than gram-negative bacteria (Ali et al., 2001; Najah et al., 2012). Interestingly, the topical gel formulation containing Nigella sativa seed extract indicated a good antimicrobial property against acne-causing bacterial species (Nawarathne et al., 2019). The antimicrobial property of Nigella sativa seed is attributed to the Thymoquinone present in the Nigella sativa seed.

**RESEARCH METHODOLOGY**

**Plant material**
Black cumin seeds were collected from the local market of a local supplier (Bangkok, Thailand).

**Extraction of black cumin seed oil**
The blended of powdered black cumin seed 25 gram with ethanol 250 mL, at 4 °C for overnight. The obtained mixtures were then filtered, and the solvent was evaporated under a vacuum. The obtained oil and extract were refrigerated (4 °C) until further analysis. The oil yield can be calculated by using the following formula.

\[
\text{% yield of Black cumin seed oil} = \frac{\text{Weight after evaporation}}{\text{Weight before evaporation}} \times 100
\]

**Formulation of anti-acne cream using black cumin seed oil**
Acne cream formulation was two formulations of O/W cream with different compositions that were prepared with non-comedogenic ingredients (Table 1). Black cumin seed oil extracts were incorporated in the cream base in variable quantities of 0.1-10% (i.e. 0.1, 0.2, and 0.5 %). The formula with the most suitable rheological and physical properties (color, odor, and creasing) was selected for in vitro testing.
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Table I Ingredients of the cream base

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetyl Alcohol</td>
<td>0.1-5 %</td>
</tr>
<tr>
<td>Stearic acid (Enenorst)</td>
<td>0.1-5 %</td>
</tr>
<tr>
<td>PEG-150 Distearate</td>
<td>1-10 %</td>
</tr>
<tr>
<td>TSF 405</td>
<td>1-10 %</td>
</tr>
<tr>
<td>SF 1540</td>
<td>1-10 %</td>
</tr>
<tr>
<td>Finsolve TN</td>
<td>1-10 %</td>
</tr>
<tr>
<td>Glycerin (Colagate)</td>
<td>1-10 %</td>
</tr>
<tr>
<td>1,3Butylene Glycol</td>
<td>1-10 %</td>
</tr>
<tr>
<td>EDTA-Base</td>
<td>0.05-1 %</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>0.1-10 %</td>
</tr>
<tr>
<td>Kaolin</td>
<td>0.1-10 %</td>
</tr>
<tr>
<td>Bentonite</td>
<td>0.1-10 %</td>
</tr>
<tr>
<td>HydroxyethylAcrylate/SodiumAcryloyldimethyl Taurate Copolymer and Isohexadecane and Polysorbate 60</td>
<td>0.1-10 %</td>
</tr>
<tr>
<td>Allantoin</td>
<td>0.1-10 %</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>0.01-5 %</td>
</tr>
<tr>
<td>Aloe Vera Liquid</td>
<td>0.1-10 %</td>
</tr>
<tr>
<td>Black Cumin Extract</td>
<td>0.1-10 %</td>
</tr>
<tr>
<td>Phenoxyethanol and Methyl Paraben and Ethyl Paraben and Propyl Paraben and Butyl Paraben and Isobutyl Paraben</td>
<td>0.01-5 %</td>
</tr>
<tr>
<td>DI Water</td>
<td>to 100%</td>
</tr>
</tbody>
</table>

All the ingredients of phases A, B, C, D, and E were taken in separate beakers. Phase A and B were kept in a water bath till the temperature reached 75-80 °C, phase C was heated at 60 °C. Part A was mixed with a homogenizer at 5000-6000 rpm, then add Part B, Part C, respectively. After the temperature down to 45 °C, part D, E was added and mixed until all solution was homogeneous.
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Then the cream was allowed to cool down to room temperature and transferred to a suitable container.

Physical stability testing
The stability studies were carried out in all formulations at different temperature conditions (room temperature, heating-cooling cycle, and Cooling cycle) for 28 days. All the evaluation parameters, i.e., pH, viscosity, and phase separation studied at different time intervals of 4 weeks.

In vitro test of antimicrobial activity
Antibacterial/antifungal susceptibility was determined using the agar-well diffusing method according to the methodology established by the Faculty of Pharmacy, Mahidol University, Thailand. In this method, the activity of a compound is indicated by a clear zone around the 'cup,' and a hole is cut in the agar and filled with preparation under test. The zones of inhibition were measured. This method has the advantage of being reproducible and accurate comparisons between compounds can be made, and hence this method is selected for the present study. Anti-acne creams were evaluated for their antimicrobial activity against pure cultures of Pseudomonas aeruginosa (MTCC 1688), Staphylococcus aureus (MTCC 737), Candida albicans (MTCC 227), and Propionibacterium acnes (MTCC 1951).

FINDING AND DISCUSSION
The stability studies were characterized in terms of viscosity and pH in different temperature conditions (room temperature, heating-cooling cycle, and cooling cycle) for 28 days. As shown in Fig. 1, the results showed that the viscosity stability of the formulation containing 1% black cumin extract was increased after the third week in all temperature conditions. In addition, the higher temperature resulted in higher viscosities. This may be caused by water molecules being repelled when high temperature, allowing intermolecular interaction of ingredient and polymeric bases in formulation (Abbasi et al., 2010; Sharif et al., 2017).

![Figure 1. Viscosity of formulation in different temperatures during 4 weeks.](image-url)
Physical stability test results showed the level of acidity of the obtained formulation was stable at room temperatures and low temperatures during storage for 4 weeks, as shown in Fig. 2. However, the high temperature might induce the oxidation of active ingredients, leading to a decreased level of pH (Misar et al., 2020).

**Figure 2.** Stability test of pH in different temperatures from the first week to the fourth week

The results showed that 1% of Black cumin seed extract has minimal inhibition concentration of *P.acnes* at 15.6 µg/ml similarly as Ampicillin (positive control), while 1.5% of Black cumin seed extract has minimal inhibition concentration at 31.3 µg/ml, as shown in Figure 3(McGinley et al., 1980).
Figure 3. Comparison of different percentages of Black cumin Extract with Ampicillin (positive control) versus Minimal inhibition concentration of *P. acnes*

CONCLUSION AND FURTHER RESEARCH

From the experiments, 1% of the Black cumin extract formula has suitable physical stability; pH, viscosity, color, odor, emulsion dispersion instability compared with another formula (data not shown). This formula showed great minimal inhibition concentration similarity to Ampicillin as the positive control. The increasing amount of crude black cumin seed extract to 1.5 % (w/w) caused oily feeling and color instability which were clearly observed by naked eyes.

REFERENCES


