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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 with MIC (minimal inhibition concentration) of 15.6 mg/mL.

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

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. acne* 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

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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 would 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. ance,* 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 *P.ance* 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 scaring 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,

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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. aurous,* 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 creaming) was selected for in vitro testing.

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Table I Ingredients of the cream base

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

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 (MTCC737), 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).

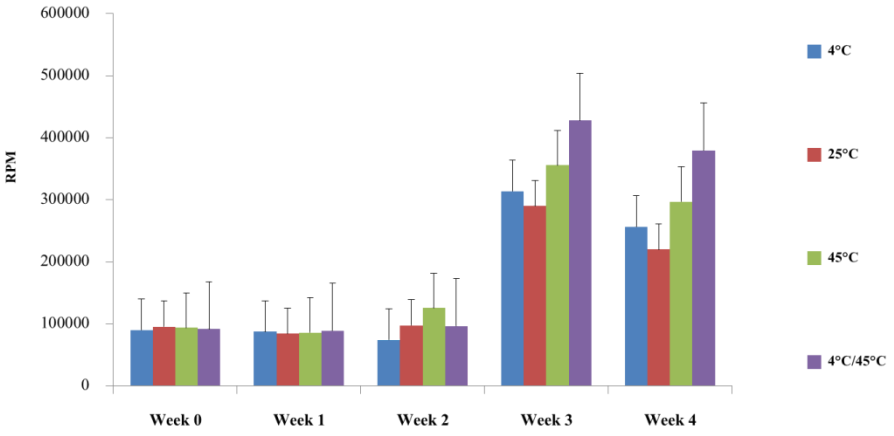

Figure 1. Viscosity of formulation in different temperatures during 4 weeks.

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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).

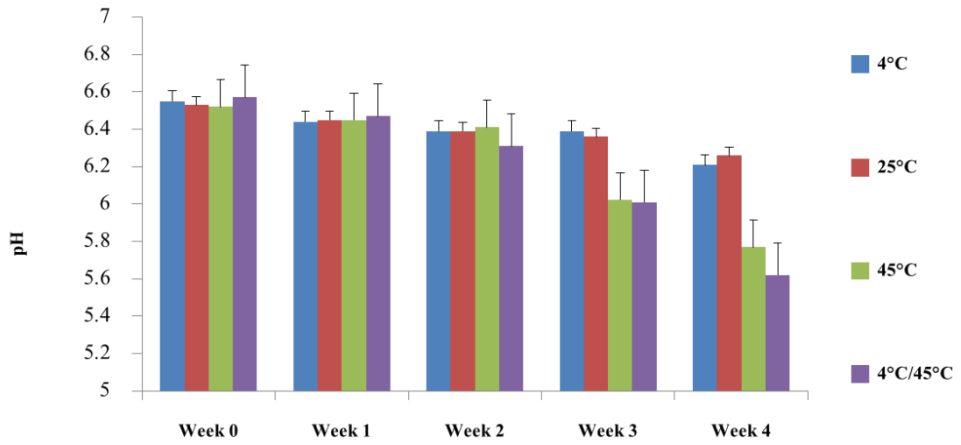

Figure 2. Stability test of pH in different temperatures from the first week to the fourth week

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).

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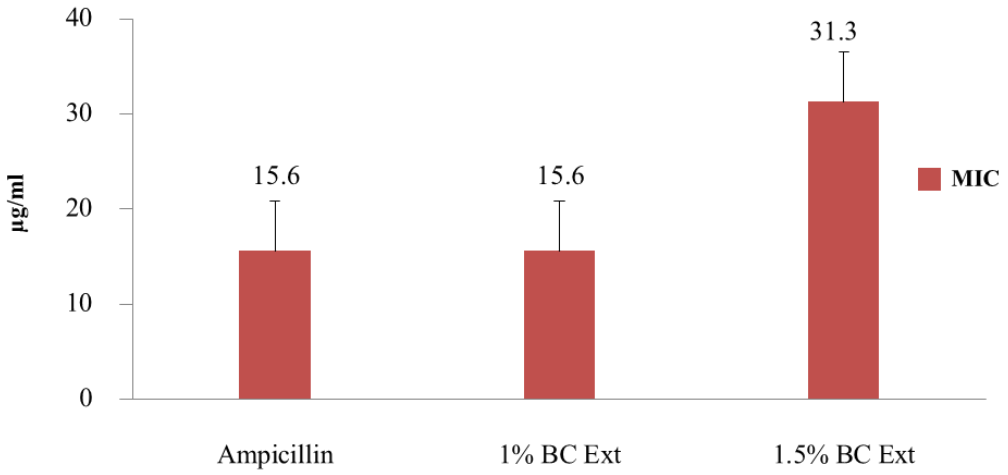

Figure 3. Comparison of different percentages of Black cumin Extract with Ampicillin (positive control) versus Minimal inhibition concentration of *P.acnes*

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

Abbasi, M. A., Kausar, A., Aziz ur, R., Saleem, H., Jahangir, S. M., Siddiqui, S. Z., & Ahmad, V. U. (2010). Preparation of new formulations of anti-acne creams and their efficacy. *african journal of pharmacy and pharmacology, 4*(6), 298-303.

Chaieb, K., Kouidhi, B., Jrah, H., Mahdouani, K., & Bakhrouf, A. (2011). Antibacterial activity of Thymoquinone, an active principle of Nigella sativa and its potency to prevent bacterial biofilm formation. *BMC Complementary and Alternative Medicine, 11*(1), 29.

Eady, E. A., & Ingham, E. (1994). Propionibacterium acnes - friend or foe? *Reviews in Medical Microbiology, 5*(3), 163-173.

Holland, K. T., Ingham, E., & Cunliffe, W. J. (1981). A review, the microbiology of acne. *J Appl Bacteriol, 51*(2), 195-215.

ISSN 2828-1500 (Online) | 2828-1985(Print)

McGinley, K. J., Webster, G. F., Ruggieri, M. R., & Leyden, J. J. (1980). Regional variations in density of cutaneous propionibacteria: correlation of Propionibacterium acnes populations with sebaceous secretion. *J Clin Microbiol, 12*(5), 672-675.

Misar, K. S., Kulkarni, S. B., & Gurnule, W. B. (2020). Formulation and evaluation of anti-acne cream by using Clove oil. *Materials Today: Proceedings, 29*, 1251-1258.

Robertson, A., Dodgson, J., Gormley, I. P., & Collings, P. (1982). An investigation of the adsorption of oxides of nitrogen on respirable mineral dusts and the effects on their cytotoxicity. *Ann Occup Hyg, 26*(1-4), 607-624.

Achermann Y, Goldstein EJ, Coenye T, Shirtliff ME, Shirtliffa ME: Propionibacterium acnes: From commensal to opportunistic biofilm-associated implant pathogen. *Clin Microbiol Rev* 2014;27:419-440.

Ali NA, Julich WD, Kusnick C and Lindequist U, Screening of Yemeni medicinal plants for antibacterial
and cytotoxic activities, J Ethnopharmacol, 2001, 74(2), 173-179.

Gharby, S., Harhar, H., Guillaume, D., Roudani, A., Boulbaroud, S., Ibrahimi, M., Charrouf, Z., 2015. Chemical investigation of Nigella sativa L. seed oil produced in Morocco. Journal of the Saudi Society of Agricultural Sciences 14(2): 172-177

Harzallah, H.J., Kouidhi, B., Flamini, G., Bakhrouf, A.,Mahjoub, T. 2011. Chemical composition, antimicrobial potential against cariogenic bacteria and cytotoxic activity of Tunisian Nigella sativa essential oil and Thymoquinone. Food Chemistry 129:1469-1474

Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N. Asadi-Samani, M., Ashtary-Larky, D. 2016. Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa) Chinese Journal of Natural Medicines 14(10): 0732-0745

Najah A. Mohammed, Effect of Nigella sativa L. extracts against Streptococcusmutans and Streptococcus mitis in Vitro, J Bagh College Dentistry Vol. 24(3), 2012 Effect of Nigella sativa Basic Sciences 154 -157.

Nakatsuji T, Kao MC, Fang JY, et al. Antimicrobial property of lauric acid against Propionibacterium acnes: its therapeutic potential for inflammatory acne vulgaris. J Invest Dermatol 2009;129:2480-8

Nawarathne, N. W., Wijesekera, K., Wijayaratne, W. M. D. G. B., Napagoda, M. 2019. Development of Novel Topical Cosmeceutical Formulations from *Nigella sativa* L. with Antimicrobial Activity against Acne-Causing Microorganisms", *The Scientific World Journal*, vol. 2019

Rafati S, Niakan M, Naseri M. Anti-microbial effect of Nigella sativa seed extract against staphylococcal skin Infection. Med J Islam Repub Iran 2014 (8 June). Vol. 28:42

ISSN 2828-1500 (Online) | 2828-1985(Print)