Developing an Effective Formulation for an Acne Treatment Cream with Ocimum Basilicum using Invasome

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Abstract/Summary

Cosmeceuticals formulations of Ocimum Basilicum are designed in an effective base for topical acne-causing inflammation medication using advanced drug delivery system. Acne vulgaris is a common disease found not only among the youth but also among adults. The present study suggests the use of Ocimum Basilicum as a cream for acne treatment. The anti-acne cream is formulated from herbal crude extracts for the antimicrobial and antioxidant activities of the formulation to prevent inflammation which causes acniform eruption. The study proposes that, along with the formulation of micro-emulsion, the use of invasome will be an effective advanced drug delivery system which can promise a great efficacy and stability performance, while simultaneously ensuring the anti-acne effect by Ocimum Basilicum.
Introduction

Cosmeceuticals refer to a combination of cosmetic products and pharmaceuticals for skin diseases (Jackson-Cannady, 2012). Development of natural products as cosmeceuticals leads to the treatment of diverse conditions as well as improvement of the skin appearances. Natural products are essential sources for biologically active drugs and for the modern phytocosmetics (Saleh, Dosari, Malik, Alsheikh, & Abdel-kader, 2009). Since synthetic chemical items can possess potential toxicity, researchers have been paying attention to natural products (Gupta & Sharma, 2006). The medicinal value these plants provide is based on bioactive phytochemical constituents, which generate definite physiological actions in the human body. Natural products from plants are often multifunctional, including photoprotection, moisturiser, and antiaging. Above all, herbal extracts are added to the cosmetic preparations predominantly for their antioxidant properties (Huang, Huang, Tsai, & Chang, 2004). Skin exposure to sunlight and other atmospheric conditions leads to reactive oxygen species (ROS), which can react with proteins, fatty acids, and DNA, resulting in an impairment of antioxidant system and oxidative damage (Chanchal & Swarnlata, 2008). This can cause an impairment of regulation pathways of skin and proceed photo-ageing and skin cancer development (Chanchal & Swarnlata, 2008). One of the consequences of oxidative stress is acne vulgaris, which can be treated with antioxidants according to emerging research implications (Arican, Kurutas, & Sasmaz, 2005). Acne is the 8th most common disease worldwide, having affected 633 million people globally (Aslam, Fleischer, & Feldman, 2015).

*Ocimum basilicum* L., also known as sweet basil, is a potential cosmeceutical product by functioning as an antioxidant and antimicrobial agent. Basil also has a good reputation for healing ringworms (Cosmetiqo, 2017). Patients with leukoderma or patches of white or light-coloured skin, as well as erythema activities, will also be able to find some relief thanks to *Ocimum basilicum* (Cosmetiqo, 2017). It can be used for skin care in various forms, broadly ranging from a cleanser to a moisturising cream.

Even though plant-derived extracts and compounds are regarded as a safer and sometimes more effective option due to enhancement in the immune system and detoxification than synthetic cosmeceuticals, there are some limitations such as efficacy. In order to overcome the limitation, this systematic review presents a formulation of a cream to prevent acne using sweet basil extract using a novel delivery system, invasome, for the first time. Invasomes are novel vesicles incorporating terpenes with enhanced penetration compared to the conventional liposomes. This paper contributes to the pharmaceutical field by developing a more effective formulation which can overcome the limitations that natural products may show through the implement of a novel delivery system, invasome.

Phytochemical Composition & Active Principals

There are varieties of *O. basilicum*. The phytochemical screening of *Ocimum Basilicum* has generally shown the presence of mucilage, gums, glycoside, proteins, tannins, amino acids, phenolic compound, triterpenoids steroids, flavones, flavonoids, sterols, and saponins in general (Bihari, Manaswini, Prabhat, & Kumar, 2011). Another study also showed that aqueous and ethanolic extracts of
*O. basilicum* augments the O6-methylguanine-DNA methyl transferase and glutathione S-transferase-P1 to a small extent, which are significant for antioxidant effects in human cells (Niture, Rao, & Srivenugopal, 2006). Each section of the plant plays significant roles, even the basil seeds, which contain various components important for acne treatment as shown in Table 1.

**Table 1 A summary of the components and roles of basil seeds**

<table>
<thead>
<tr>
<th>Components of Basil seeds</th>
<th>Roles in acne treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenolic falvonoids, mainly vicenin and orientin</td>
<td>Scavenge free radicals and prevent water loss, inhibiting acne eruption (Sabale, Kunjawani, &amp; Sabale, Formulation and in vitro evaluation of the topical antiageing preparation of the fruit of <em>Benincasa hispida</em>, 2011). These compounds showed anti-oxidant protection against radiation-induced lipid peroxidation in mouse liver (Peirce, 1999).</td>
</tr>
<tr>
<td>lutein, carotene, Vitamin A, Vitamin K, and zeaxanthin</td>
<td>They also have a protective role against oxygen-derived free radicals and ROS (Peirce, 1999).</td>
</tr>
<tr>
<td>minerals such as copper, manganese, potassium, magnesium, calcium, folates, and vitamins C</td>
<td>Potassium, as a vital component of cell and body fluids, can regulate blood pressures and heart rates (Hobbs, 1998). Manganese functions as a co-factor for superoxide dismutase, the antioxidant enzyme (Singh, 2018). Vitamin C, along with its active form L-ascorbic acid, presents very significant antioxidant ingredients of various topical formulations, as they can reduce ROS, which is vital for the acniform eruption improvement (Chiu &amp; Kimball, 2003).</td>
</tr>
<tr>
<td>citronellol, limonene, eugenol, linalool, citral and terpineol</td>
<td>Providing anti-inflammatory and antibacterial benefits (Singh, 2018).</td>
</tr>
</tbody>
</table>

The leaves which are distilled with water yield approximately 1.56% of yellow/green oil. The green leaves consist of high concentrations of oils, minerals and vitamins (Khare, 2007). At the doses of 200 and 400mg/kg body weight of hydroalcoholic extract of the fresh basil leaves is very efficient in increasing the antioxidant enzyme reactions through notably elevating the superoxide dismutase, hepatic glutathione reductase, and catalase activities (Rasul & Akhtar, 2011). A significant suppression of lactate dehydrogenase and lipid peroxidation formations is also correlated with the induction in antioxidant level (Dasgupta, Rao, & Yadava, 2004). Moreover, the essential oil contains more than 200 chemical components – Table 2 shows the main compositions (Kumar R., Shukla, Qidwai, Pandey, & Dikshit, Pharmacological studies of Ocimum Basilicum L. , 2017).

**Table 2 A table to show the main essential oil components of *O. basilicum* and there are many more, although not shown in table (Zamfirache, et al., 2011).**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Approximate portion (%)</th>
<th>Composition</th>
<th>Approximate portion (%)</th>
<th>Composition</th>
<th>Approximate portion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eugenol</td>
<td>19.22</td>
<td>germacrene D</td>
<td>8.55</td>
<td>α-bergamotene</td>
<td>3.96</td>
</tr>
<tr>
<td>tau-cadinol</td>
<td>15.13</td>
<td>δ-gurjunene</td>
<td>5.49</td>
<td>β-elemene</td>
<td>2.68</td>
</tr>
<tr>
<td>linalool</td>
<td>12.63</td>
<td>δ-cadinene</td>
<td>5.04</td>
<td>elixen</td>
<td>2.59</td>
</tr>
<tr>
<td>α-guaiene</td>
<td>2.33</td>
<td>bornil acetate</td>
<td>1.97</td>
<td>eucalyptol</td>
<td>1.79</td>
</tr>
<tr>
<td>cubenol</td>
<td>1.78</td>
<td>α-carophylene</td>
<td>1.67</td>
<td>tau muralol</td>
<td>0.96</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>0.95</td>
<td>β-cadinene</td>
<td>0.80</td>
<td>metil eugenol</td>
<td>0.76</td>
</tr>
<tr>
<td>epibiciclosesquiphelandrene</td>
<td>0.76</td>
<td>camphor</td>
<td>0.70</td>
<td>β-carophylene</td>
<td>0.61</td>
</tr>
<tr>
<td>β-farnesene</td>
<td>0.58</td>
<td>α-bisabolol</td>
<td>0.35</td>
<td>α-copaene</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Pharmacology & Cosmeceutical Use

Phytochemical compounds from *O. basilicum* are used for medicine with extensive ethnomedicinal applications (Kumar R., et al., 2016). Since the prehistoric time, it was applied for the treatment of digestive and nervous disorders, for antipyretic, anthelmintic, stomachic cardioprotective, nausea, paralysis, and cure for blood diseases (Bunrathep, Palanuvej, & Ruangrungsi, 2007). The essential oil of *O. basilicum* has also been reported to be antiviral, larvicidal, antimicrobial, antinociceptive (Shafique, et al., 2011) as proved by tests against bacterial strains such as *E. coli*, *S. aurens*, and *aeruginosa* (Kashyap, Ranjeet, Vikrant, & Vipin, 2011).

There are cosmeceutical topical applications of *O. basilicum*, mainly used for anti-aging, insecticidal effects, erythema treatment, whitening, acne treatment, and healing fungal skin infections.

There are various factors contributing to acne; however, the two main root-causes can be solved by the use of *O. basilicum*, which are oxidative stress and microbial activities. Oxidative stress, also caused by microbial activities, activates various transcription factors, e.g. NF-κB, AP-1, and p53, causing the expression of over 500 different genes, including inflammation cytokines, chemokines, cell cycle regulatory molecules and anti-inflammatory molecules (Reuter, Gupta, Chaturvedi, & Aggarwal, 2010). It has been reported that the release of inflammatory factors happens as the first event in the acne process (Saint-Leger, Bague, Lefebvre, Cohen, & Chivot, 1986). There was one study, which revealed that hydrogen peroxide (inflammatory chemical) production was 43% higher in acne patients compared to healthy controls (Bunrathep, Palanuvej, & Ruangrungsi, 2007). Although exact mechanism of oxidative stress in the pathogenesis of acne remains elusive, many researchers believe that it plays a causative role and that clinical intervention with topical agents against oxidative stress are beneficial in acne treatment.
Formulation Approach

Current topical items are present in creams, lotions, foam-cleansers, wash gels, etc. Emulsions (Figure 1), creams and lotions, are the most prevalent topical formulation types developed based on *O. basilicum*, which are on the market. This lesion is considered for the selection of the formulation type.

![Figure 1 Different emulsion types in a drug product](image)

An emulsion refers to a mixture of two immiscible liquids – generally water and an organic oil - in which one liquid (continuous phase) holds the other (the dispersed phase) which is dispersed in the form of microscopic droplets (Particle Sciences, 2009). Emulsions also consist of emulsifiers, which lower the energy necessary to break the dispersed phase into droplets and prevent them from assembling with a repulsive force or a physical barrier between them by concentrating at the phase interface for lowering the interfacial tension (Particle Sciences, 2009). These compounds exhibit hydrophobic and hydrophilic properties in balance, and they are separated into non-ionic, cationic, and anionic emulsifiers, which are used in combination to produce more stable emulsions (Tadros, 2013). Emulsions formed by dispersing aqueous droplets into a continuous oil phase are called water-in-oil (w/o) which have low HLB (Hydrophilic – Lipophilic Balance) values ranging from 4 to 6, while the opposite is termed oil-in-water (o/w) with a HLB value ranging between 8 and 18 (Alexander & Johnson, 1949). There are also more complex “triple” emulsions. Thickening agents are often added to the system to increase the stability further, improving the viscosity and avoiding the
aggregation of the disperse phase droplets. There are other ingredients supplemented, including active compounds or extracts, colouring agents, fragrances and preservatives (Faculdade de Farmácia, 2018).

Lotions, with a high-water content and low viscosity, are mainly applied for a daily facial skin care; however, they have less moisturising effects than cream types as they evaporate rapidly (Bimakr, et al., 2016). Creams exhibit an increased viscosity, and they can readily penetrate into the deeper layers of the skin depending on their active ingredients (Sabale, Kunjawani, & Sabale, Formulation and in vitro evaluation of the topical antiaging preparation of the fruit of Benincasa hispida, 2011). For the preparation of a consistent quality product, emulsion-based pharmaceutical formulations are featured by various techniques usually implemented for the characterisation of colloids. The aspects include: particle size distribution, electrical charge of the droplets via zeta potential measurements, phase separation by eye, creaming or sedimentation rate via Turbiscan, and rheology (Particle Sciences, 2009).

In terms of the preparation of the active ingredients from O.basilicum, various methods have been implemented for the dehydration and extraction of the plant (Viyoch, et al., 2006). Extracting essential oils from O.basilicum usually involves three different techniques: extraction in microwave field, sonication, and maceration. The most effective extraction technique is maceration, with the best extraction solvent system ethanol with ethyl ether (1:1 v/v), which can contribute to a continuous, less time- and solvent-consuming process, as well as high yields (Sarker, Latif, & Grays, 2006). There are five typically used drying methods including sun, microwave, contact, shaded-open atmosphere and oven drying (Polatc & Tarhan, 2009). The drying performance (final moisture content and drying time), colour analysis, drying kinetics, and essential oil analysis are carried out for all drying methods (Bilal, et al., Phytochemical and Pharmacological studies on Ocimum Basilicum Linn-A review, 2012). Drying basil with air temperature of up to 45-55°C is suitable (Bilal, et al., Phytochemical and Pharmacological studies on Ocimum Basilicum Linn-A review, 2012).

The affinity towards the base and viscosity of the preparation are the factors which influence the release of an active principle (Florence and Attwood, 1990; oyedele et al., 2000). In aqueous MeOH, an impressively higher activity of basil oil solutions can be noticed compared to the liquid paraffin, showing the lipophilic affinity of the oil for liquid paraffin which weakens the release of its active elements into the more hydrophilic agar medium. Although aqueous MeOH solvent does not improve the antimicrobial activity, it enhances diffusibility of the active components by acting less viscously than the pure oil. Oil in lipophilic semisolid bases such as petrolatum showed activities at a much less extent relative to its formulation in the more hydrophilic macrogol blend ointment base. Preparing the oil in sodium laurate monostearin cream base demonstrated significantly greater activity compared with the macrogol cream base which indicated no activity at 2% oil concentration (Orafidiya, et al., 2002). Intrinsic antimicrobial property of sodium lauryl sulphate resulted in a notable activity of the monostearin cream formulation (Orafidiya, Oyedele, & Elujoba, 2002).

The currently prevalent delivery system of sweet basil oils for acne treatment is the design of micro-emulsions as this effective delivery vehicle can improve the efficacy of the O.basilicum oils for acne treatment (Viyoch, et al., Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion formulas against Propionibacterium acnes, 2006). Micro-emulsions are selected because of their enhancement in transdermal drug transport by changing the skin barrier function. They also exhibit a high physical stability, low costs of preparation and good production possibilities (Viyoch, et al., Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion
formulas against *Propionibacterium acnes*, 2006). Micro-emulsions are stable thermodynamically, which are spontaneously formed through mixing water and oil phases and a surfactant/co-surfactant mixture together (Viyoch, et al., Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion formulas against *Propionibacterium acnes*, 2006). The low viscosity of micro-emulsions can be overcome using a thickening agent to the aqueous phase. It is the most efficient way of treating acne as it increases the skin attachment, and hence augments the accumulation of antimicrobial agent to the target site (Viyoch, et al., Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion formulas against *Propionibacterium acnes*, 2006). Also, stability can be improved through an increase in the essential oil concentration as well, by a possible intensification of the phase inversion temperature for more stable systems even at lower temperatures (Engels, Forster, & Rybinski, 1995). The stability of an emulsion is directly related to the viscosity, and its increase can give a more stable emulsion (Garrett, 1965).

In Viyoch, et al. (2006)’s study, the micro-emulsions were formulated with the basil oils in concentrations according to the MIC values through mixing with isopropyl myristate (oil phase). The mixture of water phase containing polysorbate (non-ionic surfactant), 1,2-propylene glycol (co-solvent), and deionised water is combined with the oil phase (respecting amounts shown in Table 3). The emulsion is slightly heated for a transparency. Hydroxyethylcellulose (HEC) 0.5% w/v was added to the premixed micro-emulsions for the viscosity enhancement (Florence & Whitehill, 1982). The final systems were then mixed until homogeneous dispersions were achieved.

Although the formulation can lead to an evaporation of the water phase of the micro-emulsion, the favourable properties of the micro-emulsion will still start to cotransport the lipophilic components of the *O. basilicum* oils into the deeper layers of the stratum corneum, as well as to the base of the infected sebaceous glands, which makes the formulation effective (Viyoch, et al., Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion formulas against *Propionibacterium acnes*, 2006). Moreover, the persistence of the anti-microbial efficacy of the formulation is good, given that the zone of inhibition (mm) measured to test the antibacterial activity of *O. basilicum* against *Propionibacterium acnes* showed a very high susceptibility at a concentration of 2.0% v/v (MIC) (Viyoch, et al., Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion formulas against *Propionibacterium acnes*, 2006). The zone of inhibition was 35.3 ± 1.5** (Viyoch, et al., Evaluation of in vitro antimicrobial activity of Thai basil oils and their micro-emulsion formulas against *Propionibacterium acnes*, 2006). Experiments conducted by Viyoch, et al. (2006) revealed that the antibacterial activity of this plant oil resulted from the high percentage of methyl chavicol.

<table>
<thead>
<tr>
<th>Micro-emulsions</th>
<th>Purpose</th>
<th>Version1</th>
<th>Version2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sweet basil oil (% v/v)</strong></td>
<td>Acne Treatment</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Isopropyl myristate (% v/v)</strong></td>
<td>Polar emollient used in cosmetic and topical medicinal preparations in which good absorption into the skin is desired</td>
<td>8.0</td>
<td>7.0</td>
</tr>
<tr>
<td><strong>Polysorbate 80 (% v/v)</strong></td>
<td>Non-ionic surfactant and emulsifier</td>
<td>29.2</td>
<td>29.2</td>
</tr>
<tr>
<td><strong>1,2 – Propylene glycol (% v/v)</strong></td>
<td>Safe additive</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td><strong>Deionised water (% v/v)</strong></td>
<td>Maximises shelf-life of the product and minimises contaminants in the water</td>
<td>55.0</td>
<td>55.0</td>
</tr>
</tbody>
</table>

*Table 3 The formulations of the micro-emulsion containing O.basilicum oil*
There has been a long investigation among researchers to find the most suitable advanced delivery system to acne treatment formulations, to improve their efficacy through enhancing the load capability of APIs and avoid side effect. Liposomes have been one of the most widely applied nanocarriers for cosmeceuticals, since they have the capacity of incorporating *O. basilicum* compounds and deliver them into the deeper layers of the skin (Ghanshyam, Sahu, Sharma, & Jha, 2014). They can enhance drug deposition within the skin at the action site, lowers systemic absorption, and reduces the side effects hence offering localised effect. They are regarded to be safe, composed of phospholipid bilayers which are natural materials. Their ability of carrying both hydrophilic and hydrophobic compounds into the skin is their main advantage (Monteiro, Martins, Reis, & Neves, 2014). An improved absorption, stability and penetration, together with the enhanced pharmacological activities of phytocompounds and reduced side effects can be experienced. However, they possess stability issues due to their reaction towards oxidation. Antioxidant compounds and chelators are thus combined and storage conditions are critical to overcome this problem. In order to restrict any microbial growth, preservatives are also added, to the liposomes, which are water-based formulations.

Until now, there was not sufficient research indications related to *O. basilicum* incorporated with advanced delivery system. However, a recent study on invasomes has shown a novel type of liposomal vesicles consisting of small amounts of terpenes/terpene mixtures with ethanol which are potent carriers with enhanced skin penetration. Invasomes demonstrate a greater skin penetration rate compared to liposomes. Invasomes can provide benefits including enhanced drug efficacy, and improved patient compliance and comfort. Invasomes are suspension-like semisolids which can be formulated into creams which can be readily applied to skin for a better efficacy performance. Given that this advanced delivery system provides better stability and its ability to be administered as semisolid form (Gel or cream), the formulation of cream using *O. bacilium* will be suitable and will even increase patient compliance.

Although film hydration technique can be used for the preparation of invasome, mechanical dispersion technique is selected due to its simpler steps and better efficiency. Drug and terpene get dissolved in ethanoic phospholipid solution. The mixture is vortexed for 5 minutes before sonication for 5 minutes which can produce a clearer solution. In the solution, phosphate buffer saline (pH 7.4) is added under constant vortexing, which is continued for extra 5 minutes. Then the extrusion of multilamellar vesicles is performed through polycarbonate membranes of varying pore sizes. The invasome dispersions are extruded through each polycarbonate membrane for a few times (Badran, Kuntsche, & Fahr, 2009). It is essential to elevate the pressure and lower temperature, or lower the pressure while increasing temperature for higher yields (Sarker, Latif, & Grays, 2006). Alpha-tocopherol added can be used for stabilising the invasome membranes, while a low concentration of parabens can be added as preservatives (Quinn, 2012). Producing the final product involves in the invasome suspension mixed with light mineral oil, like liposomes containing essential oils, as invasomes are types of liposomes (Chatzinasiou, 2016).
Conclusion

O. basilicum can be potentially used to prepare a successful cream for effective antimicrobial and antioxidant effects to treat acne. Based on the above and the characteristics of topical creams from O. basilicum which can function as an antioxidant and antimicrobial agent in vitro, an o/w cream with invasomes can be prepared for acne treatment. The production of the finalized cream formulation involves three steps: firstly, maceration technique is selected for the extraction of the active ingredients from O. basilicum, using ethanol with ethyl ether; secondly, O. basilicum is dried before formulation; Lastly, the advanced delivery system, invasomes, is used for the successful o/w cream to overcome the barrier properties of SC. Invasome is a promising tool for delivering drugs through the skin with increased permeation. Hence, it can open up new opportunities and challenges for the development of novel improved therapies.

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Bibliography


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