Development of an effective formulation for an acne treatment cream with *Ocimum basilicum* using invasomes

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Cosmeceutical formulations of *Ocimum basilicum* are designed in an effective base to medicate topical acne-causing inflammation using an advanced drug delivery system. Acne vulgaris is a common disease found not only in young people but also in adults. The present study suggests the use of *O. basilicum* as a cream for the treatment of acne. Anti-acne creams formulated from crude herbal extracts have long been used for their antimicrobial and antioxidant activities to prevent the inflammation that causes acneiform eruption. This study proposes that, along with the formulation of a micro-emulsion, the use of invasomes will offer an effective advanced drug delivery system that promises improved efficacy and stability in treating acne with *O. basilicum*.

Keywords: acne vulgaris; cosmeceuticals; facial cream; invasomes; micro-emulsion; *Ocimum basilicum*

Introduction

Cosmeceuticals refer to a combination of cosmetic products and pharmaceuticals for skin diseases [1]. Development of natural products as cosmeceuticals allows the treatment of diverse conditions as well as the improvement of skin appearance. Natural products are essential sources for biologically active drugs and modern phytocosmetics [2]. Since synthetic chemical products may possess potential toxicity, there is increased interest in natural products [3]. The medicinal value these plants provide is based on bioactive phytochemical constituents, which have specific physiological activities in the human body. Natural products from plants are often multifunctional, including photoprotective, moisturizing, and anti-aging effects. In particular, herbal extracts are added to cosmetic preparations for their antioxidant properties [4]. Skin exposure to sunlight

and other environmental conditions leads to the generation of reactive oxygen species, which can react with proteins, fatty acids, and DNA, resulting in an impairment of the antioxidant system and oxidative damage [5]. This can impair regulatory pathways in the skin and accelerate photo-ageing and the development of skin cancer [5]. According to emerging research, one of the consequences of oxidative stress is acne, which can be treated with antioxidants. Acne is the eighth most common disease worldwide, affecting 633 million people globally [6].

Ocimum basilicum L., also known as sweet basil, is a potential cosmeceutical product that functions as an antioxidant and antimicrobial agent. Basil also has a reputation for treating ringworm [7]. Patients with leukoderma, patches of white or light-colored skin, and erythema may also benefit from the application of *O. basilicum* [7]. It can be used for skin care in various forms, from cleansers to moisturizing creams.

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Although plant-derived extracts and compounds are regarded as a safer and sometimes more effective option, due to enhancing the immune system and detoxification, than synthetic cosmeceuticals, they still possess some limitations – namely, efficacy. In order to overcome the limitations, this systematic review suggests a possible formulation for a cream to prevent acne using sweet basil extract and a novel delivery system, invasomes, for the first time. Invasomes are novel vesicles incorporating terpenes with enhanced penetration compared to conventional liposomes. This paper will contribute to the pharmaceutical field by developing a more effective formulation to overcome the potential limitations of natural products through the implementation of a novel delivery system, invasomes.

Phytochemical composition and active principals

There are several varieties of *O. basilicum*. The phytochemical screening of *O. basilicum* has generally shown the presence of mucilage, gums, glycoside, proteins, tannins, amino acids, phenolic compound, triterpenoids, steroids, flavones, flavonoids, sterols, and saponins [8]. Another study also showed that aqueous and ethanolic extracts of *O. basilicum* augmented O6-methylguanine-DNA methyl transferase and glutathione S-transferase-P1 to a small extent, which are significant enzymes in the antioxidant pathways in human cells [9]. Each tissue of the plant plays significant roles, even the basil seeds, which contain various components important for acne treatment, as shown in Table 1 [10-14].

The leaves, when distilled with water, yield approximately 1.56% of yellow/green oil. The green leaves contain high concentrations of oils, vitamins, and minerals [15]. At the doses of 200 and 400 mg/kg body weight, hydroalcoholic extract of fresh

basil leaves has a strong antioxidative effect through notably increasing the activities of superoxide dismutase, hepatic glutathione reductase, and catalase [16]. A significant suppression of lactate dehydrogenase and lipid peroxidation formation is also correlated with the induction of an antioxidant effect [17]. Moreover, the essential oil contains more than 200 chemical components – Table 2 shows the major components [18], although there are many more which are not shown.

Pharmacology and cosmeceutical use

Phytochemical compounds derived from *O. basilicum* are used in medicine with extensive ethnomedicinal applications [18]. Since prehistoric times, it has been used in the treatment of digestive and nervous disorders, nausea, and paralysis, and as an antipyretic, an anthelmintic, a stomachic, a cardioprotective, and a cure for blood diseases. The essential oil of *O. basilicum* has also been reported to have antiviral, larvicidal, antimicrobial, and antinociceptive activities [19] as shown by its efficacy against bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [20].

There are currently several cosmeceutical topical preparations of *O. basilicum*, which are mainly used for anti-aging and whitening, as insecticides, and for treating erythema, acne, and fungal skin infections.

There are various factors contributing to acne. However, the two main root-causes, oxidative stress and microbial activity, can be resolved by the use of *O. basilicum*. Oxidative stress, which may also be caused by microbial activity, activates various transcription factors, e.g. NF- κ B, AP-1, and p53, causing the expression of over 500 different genes, including inflammatory cytokines, chemokines, cell cycle regulatory molecules, and anti-inflammatory molecules [21]. It has been reported that

Table 1. Summary of the components of basil seeds and their roles in acne treatment

Component of basil seeds	Role in acne treatment	Reference
Polyphenolic flavonoids, mainly vicenin and orientin	Scavenge free radicals and prevent water loss, inhibiting acne eruption These compounds showed anti-oxidant protection against radiation-induced lipid peroxidation in mouse liver	[10,11]
Lutein, carotene, vitamin A, vitamin K, and zeaxanthin	These also have protective roles against oxygen-derived free radicals and ROS	[11]
Minerals such as copper, manganese, potassium, magnesium, calcium, folates, and vitamin C	Potassium, as a vital component of cell and body fluids, can regulate blood pressure and heart rate Manganese functions as a co-factor for superoxide dismutase, the major antioxidant enzyme Vitamin C, along with its active form L-ascorbic acid, is a significant antioxidant ingredient of various topical formulations, as it can reduce ROS, which is vital for the improvement of acneiform eruption	[12-14]
Citronellol, limonene, eugenol, linalool, citral, and terpineol	Anti-inflammatory and antibacterial benefits	[13]

ROS, reactive oxygen species.

Table 2. Major components of the essential oil of *Ocimum basilicum*

Component	Approximate proportion (%)
Eugenol	19.22
Tau-cadinol	15.13
Linalool	12.63
α -guaiene	2.33
Cubenol	1.78
α -terpineol	0.95
Epibiclosiqui phelandrene	0.76
β -farnesene	0.58
Germacrene D	8.55
δ -gurjunene	5.49
δ -cadinene	5.04
Bornil acetate	1.97
α -cariophyllene	1.67
β -cadinene	0.8
Camphor	0.7
α -bisabolol	0.35
α -bergamotene	3.96
β -elemene	2.68
elixer	2.59
Eucalyptol	1.79
τ -muralol	0.96
Metil eugenol	0.76
β -cariophyllene	0.61
α -copaene	0.33

the release of inflammatory factors occurs as the first event in the formation of acne [22]. One study revealed that production of hydrogen peroxide (an inflammatory molecule) was 43% higher in acne patients compared to that in healthy controls [23]. Although the exact mechanism by which oxidative stress influences 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 method

Current topical preparations include creams, lotions, foam-cleansers, and wash gels. Emulsions (Fig. 1), creams, and lotions are the most prevalent types of topical formulation based on *O. basilicum* that are currently available. This lesion is considered for the selection of the formulation type.

An emulsion refers to a mixture of two immiscible liquids, generally water and an organic oil, in which one liquid (the continuous phase) holds the other (the dispersed phase), which is

dispersed in the form of microscopic droplets [24]. Emulsions also consist of emulsifiers, which lower the energy necessary to break the dispersed phase into droplets and prevent it from assembling with a repulsive force or a physical barrier between droplets by concentrating at the phase interface to lower the interfacial tension [24]. 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 [25]. Emulsions formed by dispersing aqueous droplets into a continuous oil phase are termed water-in-oil which have low hydrophilic-lipophilic balance (HLB) values ranging from 4 to 6, while the converse are termed oil-in-water (o/w) with HLB values between 8 and 18 [26]. More complex “triple” emulsions also exist. 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. Other ingredients are often added, including active compounds or extracts, coloring agents, fragrances, and preservatives.

Lotions, with a high water content and low viscosity, are mainly applied in daily facial skin care; however, they have a reduced moisturizing effect compared to creams because they evaporate rapidly [27]. Creams exhibit increased viscosity and can readily penetrate into the deeper layers of the skin, depending on their active ingredients [10]. To ensure the preparation of a consistent quality product, emulsion-based pharmaceutical formulations are analyzed by various techniques usually implemented for the characterization of colloids. The characteristics investigated 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 [24].

Various methods have been implemented for the dehydration of plant material and extraction of active ingredients from *O. basilicum* [28]. Extracting essential oils from *O. basilicum* usually involves one of three techniques: extraction in a microwave field, sonication, or maceration. The most effective extraction technique is maceration using ethanol with ethyl ether (1:1 v/v) as the extraction solvent system, which gives a continuous, high yield process, while minimizing the time and solvent required [29]. There are five typically used drying methods: sun, microwave, contact, shaded-open atmosphere, and oven drying. The drying performance (final moisture content and drying time), color analysis, drying kinetics, and essential oil analysis have been assayed for all drying methods [30]. An air temperature of up to 45°C to 55°C was found to be the most suitable for drying

Fig. 1. Various components of an emulsion in a drug product. API, active pharmaceutical ingredients.

basil [30].

The affinity to the base and the viscosity of the preparation influence the release of an active principle. When prepared in aqueous Methanol (MeOH), an remarkably increased antimicrobial activity of basil oil solutions can be observed, compared to that in liquid paraffin, showing the lipophilic affinity of the oil for liquid paraffin, which inhibits the release of its active elements into the more hydrophilic agar medium. Although aqueous MeOH solvent does not improve the antimicrobial activity, it enhances the diffusibility of the active components by acting less viscously than the pure oil. Basil essential oil in lipophilic semisolid bases, such as petrolatum, showed significantly reduced activities compared to an equivalent formulation in the more hydrophilic macrogol blend ointment base. Preparations of the oil in a sodium laurate monostearin cream base demonstrated significantly greater activity compared with that of the macrogol cream base, which has no observable activity at an oil concentration of 2% [31]. The intrinsic antimicrobial properties of sodium lauryl sulfate resulted in notable activity of the monostearin cream formulation [31].

The current prevalent delivery system of sweet basil oils for acne treatment uses a micro-emulsion, as this effective delivery vehicle can improve the efficacy of the *O. basilicum* oils for acne treatment [28]. Micro-emulsions are selected because of their enhancement of transdermal drug transport by altering the skin barrier function. They also exhibit a high physical stability, low preparation costs, and good production possibilities [28]. Micro-emulsions are thermodynamically stable and are spontaneously formed through mixing water and oil phases with a surfactant/co-surfactant mixture [28]. The low viscosity of micro-emulsions can be overcome using a thickening agent in the aqueous phase. Micro-emulsions are the most efficient way of treating acne as they increase skin attachment, and hence augment the accumulation of the antimicrobial agent at the target site [28]. In addition, stability can be improved through an increase in the essential oil concentration, as well as by increasing the phase inversion temperature for more stable systems, even at lower temperatures [32]. The stability of an emulsion is directly related to the viscosity, and its increase can give a more stable emulsion [33].

There has long been interest in developing the most suitable advanced delivery system for acne treatment formulation, to improve efficacy through enhancing the load capability for active pharmaceutical ingredients and avoid side effects. Liposomes have been one of the most widely applied nanocarriers for cosmeceuticals, since they are capable of incorporating *O. basilicum* compounds and deliver them into the deeper layers of the skin [34]. Liposomes can enhance drug deposition within the skin at the action site and reduce systemic absorption and side effects, hence offering a localized effect. They are regarded as safe and are composed of phospholipid bilayers, which are natural materials. Their capacity to carry both hydrophilic and hydrophobic compounds into the skin is their main advantage [35]. Liposomes offer improved absorption, stability, and penetration, together with the enhanced pharmacological activities of phytochemicals and reduced side effects. However, they have stability issues due to oxidation reactions. 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 insufficient research related to *O. basilicum* incorporated into advanced delivery systems. However, a recent study into invasomes has shown a novel type of liposomal vesicles consisting of small amounts of terpenes or terpene mixtures with ethanol, which are potent carriers with enhanced skin penetration. Invasomes demonstrate a greater skin penetration rate compared to liposomes and can provide benefits including enhanced drug efficacy and improved patient compliance and comfort. Invasomes are suspension-like semisolids that can be formulated into creams, which can be readily applied to skin for better efficacy. The mechanism of penetration through these vesicles is expected to be by disturbing lipid packaging of the stratum corneum and by disrupting bilayer stacking [36]. Given that this advanced delivery system provides better stability and can be administered as a semisolid form (gel or cream), the formulation of cream using *O. basilicum* is likely

to be suitable and even increase patient compliance.

Although a film hydration technique can be used for the preparation of invasomes, a mechanical dispersion technique is preferred due to its simplicity and superior efficiency. The drug and terpene are dissolved in an ethanoic phospholipid solution. The mixture is vortexed for 5 minutes before sonication for 5 minutes to produce a clear solution. In the solution, phosphate buffered 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 several times [37]. It is essential to elevate the pressure and reduce the temperature, or lower the pressure while increasing temperature for higher yields [29]. Alpha-tocopherol can be added to stabilize the invasome membranes, while a low concentration of parabens can be added as preservatives [38]. Producing the final product involves the invasome suspension mixed with light mineral oil, in similar method to that with liposomes containing essential oils, as invasomes are a type of liposome.

Formulation efficacy

In Viyoch et al.'s study [28], micro-emulsions were formulated with basil oils in concentrations according to the minimum inhibitory concentration (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 deionized water was combined with the oil phase (respective amounts are shown in Table 3). The emulsion was slightly heated to transparency. Hydroxyethylcellulose (HEC) 0.5% w/v was added to the premixed micro-emulsions to enhance viscosity [39]. The final systems were then mixed until homogeneous dispersions were achieved.

After application to the skin surface, although the water phase may evaporate, the favorable properties of the micro-emulsion will begin to cotransport the lipophilic components of the *O.*

Table 3. Formulation of the micro-emulsions containing *Ocimum basilicum* oil

Micro-emulsion	Purpose	Version 1	Version 2
Sweet basil oil (% v/v)	Acne Treatment	2.0	3.0
Isopropyl myristate (% v/v)	Polar emollient used in cosmetic and topical medicinal preparations where effective absorption into the skin is desired	8.0	7.0
Polysorbate 80 (% v/v)	Non-ionic surfactant and emulsifier	29.2	29.2
1,2-Propylene glycol (% v/v)	Safe additive	5.8	5.8
Deionized water (% v/v)	Maximizes product shelf-life and minimizes contaminants from water	55.0	55.0

Values are presented as number only.

basilicum oils into the deeper layers of the stratum corneum and the base of the infected sebaceous glands, making the formulation effective [28]. Moreover, the persistence of the antimicrobial efficacy of the formulation is good, since the zone of inhibition (mm) measured to test the antibacterial activity of *O. basilicum* against *Propionibacterium acnes* was 35.3 ± 1.5 , showing very high susceptibility, at a concentration of 2.0% v/v (MIC) [28]. Viyoch et al. [28] demonstrated that the antibacterial activity of this plant oil resulted from the high percentage of methyl chavicol.

In addition, Matiz et al. [40] found that *O. basilicum* was effective as an alternative to the previous antibiotic treatment to overcome antimicrobial resistance, which develops in bacterial strains involved in the pathogenesis of acne. The experimental study involved 28 volunteer patients in four groups with seven patients each. Treatments were administered daily for eight weeks and each group was assigned either antibacterial essential oils, essential oils mixed with acetic acid, keratolytic medication, or keratolytic medication with acetic acid. The results highlighted the improvement of acne in all groups, reporting a maximum of 75% clearance of lesions with a minimum of 43% [40]. This study described the physical and chemical stability of the essential oil formulations during application, as shown by gas chromatography where no change was evident in the composition profiles of either essential oils alone or in acetic acid [40]. The high satisfaction recorded by patients with symptom improvements of up to 75% was likely due to the joint keratolytic and antiseptic activities.

Conclusion

O. basilicum can be used to prepare a successful cream with effective antimicrobial and antioxidant activities to treat acne. Based on the above-described characteristics of *O. basilicum* in vitro, topical creams in the o/w form with invasomes can be prepared for acne treatment. The production of the finalized cream formulation involves three steps: firstly, maceration is recommended for the extraction of the active ingredients from *O. basilicum*, using ethanol with ethyl ether; secondly, *O. basilicum* is dried before formulation; lastly, an advanced delivery system with invasomes is used for the successful o/w cream to overcome the barrier properties of stratum corneum. Invasomes are a promising tool to deliver drugs through the skin with increased permeation. Hence, they can open up new opportunities and challenges for the development of novel improved therapies.

Conflicts of interest

The author has nothing to disclose.

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