The Acne-treatment Potential of *Cinnamomum Camphora* Chvar. *Borneol* Essential Oil *in Vitro* and *in Vivo*

Shanshan Xiao  
Jiangnan University

Hang Yu  
Jiangnan University

Yunfei Xie  
Jiangnan University

Yahui Guo  
Jiangnan University

Jiajia Fan  
Chunjingziran Biotechnology Co.Ltd

Weirong Yao (✉ [yaoweirongcn@jiangnan.edu.cn](mailto:yaoweirongcn@jiangnan.edu.cn))  
Jiangnan University  https://orcid.org/0000-0003-4730-3976

Research

**Keywords:** Cinnamomum camphora chvar. Borneol essential oil (BEO), Acne, human keratinocytes (HaCaT) proliferation, network pharmacology

**Posted Date:** August 17th, 2021

**DOI:** [https://doi.org/10.21203/rs.3.rs-783519/v1](https://doi.org/10.21203/rs.3.rs-783519/v1)

**License:** [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/). Read Full License
Abstract

Background: Acne is one of the most common chronic inflammatory skin diseases, abnormal proliferation of keratinocytes can block the hair follicle sebaceous glands result in the formation of acne. Most drugs to treat acne can cause a variety of side effects, therefore, it is important to seek natural and safe complementary and alternative therapies.

Methods: The inhibitory effects of BEO were determined on the proliferation of human keratinocyte (HaCaT) cells induced by heat-inactivated Staphylococcus epidermidis and release of the inflammatory mediators. Further, a component-target-signal pathway for BEO’s effects on acne was constructed through network pharmacology and the mechanism of BEO action was studied in vivo through the rabbit ear acne model.

Results: BEO inhibited both cell proliferation, induced by heat-inactivated Staphylococcus epidermidis ($p < 0.0001$), and release of the inflammatory mediators TNF-α ($p < 0.0001$) and IL-1β ($p < 0.05$) in a dose-dependent manner ($r = -0.9952, -0.9492$), in a HaCaT cell-model of acne. A network pharmacology analysis of the chemical components of BEO characterized these effects as multi-component, multi-target and multi-pathway. All targets were mainly associated with metabolic pathways, the toll-like receptor signaling pathway and the NF-κB signaling pathway. BEO also reduced the severity of acne lesions, induced by intracutaneous injection of S. epidermidis in a rabbit ear acne model. The expression of inflammatory mediators and key signaling pathway components, including TLR2, AKT, P13K, NF-κB, TNF-α, IL-1β in rabbit ear, and TNF-α and IL-1 β in serum, were down-regulated ($p < 0.05$), indicating that BEO acts by inhibiting the pro-inflammatory TLR2/PI3K-AKT/NF-κB signaling pathway.

Conclusion: The current results showed that BEO has clear potential for development into a natural and safe anti-inflammatory skin preparation, which is an effective alternative to conventional treatments containing antibiotics and synthetic anti-inflammatory agents.

1. Background

Acne is one of the most common chronic inflammatory skin diseases [1], which is caused by an androgen-induced increase in sebum secretion, abnormal proliferation of keratinocytes and bacterial colonization of hair follicles [2]. Globally, more than 85% of young people are affected by acne [1]. Studies have shown that Staphylococcus epidermidis dysbiosis can cause infection of hair follicle sebaceous glands, which can lead to acne [3]. In addition, abnormal proliferation of keratinocytes can block the hair follicle sebaceous glands and result in the formation of acne [4]. Therefore, most drugs to treat acne (salicylic acid, antibiotics, and anti-inflammatory drugs) are targeted to the above two important factors, S. epidermidis infection and abnormal proliferation of keratinocytes [3]; however, these drugs can cause a variety of side effects and long-term use of antibiotics may result in antibiotic resistance in acne-causing bacteria [5]. Therefore, patients are gradually turning their attention to natural and safe complementary and alternative therapies, especially herbal medicines [6].
Essential oils are a natural, safe, effective and multifunctional alternative therapy [3]. Studies have shown that *Salvia eremophila* essential oil containing borneol, α-pinene, bornyl acetate, camphene, β-caryophyllene and and limonene [7] and *Tanacetum argyrophyllum* var. *argyrophyllum* essential oil containing borneol, camphor and camphene [8] showed antibacterial activity against *S. epidermidis* that causes acne. *Cinnamomum camphora* chvar. *Borneol* essential oil (BEO) is obtained by steam distillation of *Cinnamomum camphora* chvar. *Borneol* leaves. Its most abundant component is borneol (16.4%), followed by β-caryophyllene (10.7%), camphor (10.6%), α-pinene (7.45%), limonene (8.2%), camphene (4.4%) and bornyl acetate (0.3%) (Table S1) [9]. BEO has various biological activities, including significant anti-inflammatory effects [9], which has similar components with *Salvia eremophila* essential oil and *Tanacetum argyrophyllum* var. *argyrophyllum* essential oil, so its acne-treatment potential is worthwhile to investigate.

This study aimed to use the heat-inactivated *S. epidermidis*-induced HaCaT cell proliferation and inflammation model to study the acne-treatment effect of BEO in vitro, and construct a “component-target-signaling pathway” network of acne regulated by BEO, through a network pharmacology approach, to analyze the potential mechanism of BEO for acne treatment. An *in vivo* rabbit ear acne model was established to probe the relationship between the Toll-like receptor signaling pathway and the anti-inflammatory effect of BEO on acne in vivo.

### 2. Materials And Methods

#### 2.1 Materials and Reagents

BEO was provided by *Chunjingziran* Biotechnology (Shaoxing, Zhejiang Province, China), and was obtained by steam distillation of fresh branches and leaves of *Cinnamomum camphora* chvar. *Borneol*, a voucher specimen (768133), was deposited at South China Institute of Botany, Chinese Academy of Science (Guangzhou, Guangdong). The essential oil was dehydrated by adding anhydrous Na$_2$SO$_4$, and collected in a brown bottle, then stored at 4°C until future use. The BEO components were previously identified by our research group using gas chromatography mass spectrometry; BEO contains 43 components, the most abundant being borneol (16.4%) [9] (Table S1).

Reagents used were: Dulbecco's Modified Eagle's Medium (DMEM; GIBCO/Thermo Fisher Scientific, Waltham MA); fetal bovine serum (FBS; GIBCO); penicillin/streptomycin (GIBCO); dimethyl sulfoxide (DMSO; *Solarbio*, Beijing, China) and enzyme-linked immunosorbent assay (ELISA) kits (*SenBeiJia* Biotechnology, Nanjing, China), which were used to assay rabbit serum tumor necrosis factor (TNF-α) and interleukin (IL)-1β, rabbit tissue TNF-α, IL-1β, nuclear factor kappa B (NF-κB), phosphoinositide 3-kinase (PI3K), protein kinase B (AKT), Toll-like receptor (TLR) 2 and HaCaT cellular supernatant (TNF-α and IL-1β).

#### 2.2 Bacterial Strains and Culturing
Staphylococcus epidermidis (S. epidermidis) ATCC 12228 was cultured aerobically on nutrient broth (NB) and incubated at 37 °C for 24 h.

2.3 Anti-human keratinocytes (HaCaT) proliferation and inflammation in vitro

2.3.1 Cell culture

Human keratinocytes (HaCaT) were cultured in DMEM, supplemented with 10% FBS and penicillin/streptomycin at 37 °C in a humid 5% CO₂ atmosphere.

2.3.2 Cell Viability Assay of S. epidermidis-induced HaCaT cells

The viability of HaCaT cells was determined as described previously, with some modifications [10]. Cells were treated with heat-killed S. epidermidis (wet weight 200 µg/mL) and different concentrations of BEO (0, 0.08, 0.16, or 0.4 mg/mL), for a 24 h incubation, and were analyzed with a CCK-8 kit (Beyotime, Shanghai, China). HaCaT cells were seeded into a 96-well plate at 5000 cells/well. The absorbance of wells was measured using a microplate reader (Epoch 2, Bio Tek Instruments, USA) at 450 nm.

2.3.3 Effects of BEO on Cell inflammation of S. epidermidis-induced HaCaT cells

HaCaT cells were induced as described above (2.3.2), for a 24 h incubation. Cell-free supernatants were collected and the concentrations of TNF-α and IL-1β were analyzed with the respective ELISA kits. Non-induced cells were used as the controls.

2.4 Network pharmacology analysis

The chemical composition of BEO was determined previously by our research group [9] (Table S1). Simplified Molecular Input Line Entry System (SMILES) strings of the components were obtained by searching the Traditional Chinese Medicine Integrated Database (http://www.megabionet.org/tcmid/) and imported into the Swiss Target Prediction database (STP; http://www.swisstargetprediction.ch/) to identify potential targets of BEO components. The STP database can predict the targets of active molecules based on the chemical structure of the molecules, ligand similarity and by cross validation and arrangement analysis [11]. The predicted targets of all BEO components were obtained from limited search species in humans. Next, the DisGeNET database (http://www.disgenet.org/web/DisGeNET/menu/home) was used to screen potential targets for acne treatment. Then, the targets of BEO components and the targets of acne treatment were intersected to identify potential targets that could be used in the treatment of acne by BEO.

The KEGG Mapper tool of the KEGG database (http://www.kegg.jp/) was used to find enriched pathways for the targets, and Cytoscape 3.2.1 was then used to construct an “active components-targets-signal
pathways” network, in which nodes representing BEO active components, potential targets and associated signal pathways were connected by lines [12]. The size of the nodes represents the degree of influence of components, or the degree of effect on the targets and pathways. The larger the node, the greater the degree. The thickness of the connecting grey lines represents the combined score; the thicker the line, the greater the combined score.

2.5 In vivo studies

2.5.1 Experimental animals

All experimental animal procedures were approved by the Ethics Committee of the Experimental Animal Center of Jiangnan University (Wuxi, Jiangsu Province, JN. No. 20190430R0420715[98]). Care and use of laboratory animals were conducted in accordance with national and international guidelines (Directive 2010/63/EU). Adult New Zealand rabbits (1.5–2.5 kg) were bred in an environment kept at 22 ± 2°C, with 55% ± 15% relative humidity and 12-h light/dark cycles. They had free access to water and food. The experiments were performed after the animals had adapted to the experimental environment for at least 5 d.

2.5.2 Establishment of the rabbit ear acne model

The rabbit ear acne model was established as described previously [13] with slight modifications. New Zealand rabbits (42 total; seven per group) were randomly divided into control, model control, negative control (GTCC, 200 mg/kg), positive control (Clindamycin hydrochloride gel, 200 mg/kg, calculated for the daily dose of a patient) and BEO treatment groups. Except for the control group, the inner ear-tube opening of each rabbit’s right ear was evenly coated with 0.5 mL of oleic acid, covering an area of 2 cm × 2 cm, once a day for 14 consecutive days. On days 7–12, the right ear was injected intracutaneously with *S. epidermidis* (30 µL,10^8 CFU/mL) every day and the left ear was left untreated as a control. After successful establishment of the model, BEO (25, 50, 100 mg/kg, diluted with GTCC) and clindamycin hydrochloride gel (200 mg/kg) were administered once a day for 14 consecutive d. Twenty minutes after the last administration, the rabbits were anesthetized and blood was taken, which was centrifuged at 3,000 × g for 10 min, and the serum was isolated.

2.5.3 Hematoxylin and eosin (HE) staining

The rabbits were euthanized after anesthesia, and freshly excised rabbit auricle tissues were fixed with 4% paraformaldehyde for 24 h, then dehydrated, embedded in paraffin wax, sectioned, and stained with HE. The tissue slides were observed with an inverted fluorescence microscope.

2.5.4 Western blotting

Rabbit auricle tissues were lysed in RIPA Lysis Buffer (Beyotime Biotechnology, Shanghai, China) containing protease/phosphatase inhibitors. A total of 60 µg protein was loaded in each lane. Primary antibodies, including Armenian hamster anti-TNF-α (1:1,000; SC-12744; Santa Cruz Biotechnology, Dallas, TX, USA) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:1,000; ab125247; Abcam,
Horseradish-peroxidase-conjugated secondary antibodies were also used. The protein bands were visualized using a Chemiluminescence Kit (Bio-Rad, Hercules, CA, USA). Protein bands were quantified by densitometry (Image Lab; Bio-Rad).

## 2.6 Data analysis

Prism 6 software (GraphPad, San Diego, CA) and OriginLab-9.0s (Origin Lab, Northampton, MA) were used for data analysis and plotting. The results are expressed as the mean ± standard deviation. The data were analyzed using one-way analysis of variance with Dunnett’s multiple comparisons test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 were considered as statistically significant.

### 3. Results

#### 3.1 The inhibitory effect of BEO on HaCaT cell proliferation and inflammation

##### 3.1.1 The effect of BEO on the proliferation of HaCaT cells induced by heat-inactivated *Staphylococcus epidermidis*

Three non-cytotoxic concentrations of BEO (0.08, 0.16, 0.4 mg/mL), determined previously by our research group [9], were selected to test its effect on the proliferation of HaCaT cells induced by heat-inactivated *S. epidermidis* and the cell viability was tested by CCK-8. Compared with the control cells, heat-inactivated *S. epidermidis* significantly increased the proliferation of HaCaT cells, to a survival rate of 155.5 ± 0.01% (Fig. 1A). The cell survival rates after BEO treatment at 0.08, 0.16, or 0.4 mg/mL were 150.7 ± 0.03%, 146.9 ± 0.05% and 97.6 ± 0.03%, respectively. Therefore 0.4 mg/mL BEO significantly inhibited (by 40.96 ± 1.77%) and essentially normalized the excessive proliferation of HaCaT cells (p < 0.01) (Fig. 1A), indicating that BEO has positive effects on excessive keratinocyte proliferation in acne.

##### 3.1.2 The effect of BEO on inflammatory mediator production by HaCaT cells induced by heat-inactivated *Staphylococcus epidermidis*

BEO was applied to *S. epidermidis*-treated human keratinocyte (HaCaT) cells in a cell-based acne model. The content of TNF-α and IL-1β in the cell supernatant was determined (Fig. 1B, C). Compared with control, heat-inactivated *S. epidermidis* significantly increased the release of the inflammatory mediators (p < 0.0001). BEO (0.08, 0.16, or 0.4 mg/mL) significantly inhibited the release of TNF-α and IL-1β caused by heat-inactivated *S. epidermidis* (p < 0.05), in a dose-dependent manner (r = -0.9952, r = -0.9492, respectively). These data indicate that BEO has an anti-inflammatory effect in the *in vitro* acne model.

### 3.2 Network pharmacology analysis
3.2.1 Components and targets

BEO components with potential activity against acne were screened from the database, and a BEO “active component-target-signaling pathway” network was constructed. (Fig. 2A). A total of 18 targets, corresponding to 35 BEO ingredients was obtained and of these, limonene (10), β-caryophyllene (10) and borneol (9) were associated with nine or more targets. Thus, these compounds can be considered as the main components of BEO that are active against acne. The target genes Cytochrome P450 (CYP)19A1, androgen receptors (AR), estrogen receptor (ESR)1 and CYP17A1 were associated with 35, 31, 28 and 23 BEO components, respectively (Table S2).

3.2.2 Targets and pathways

Analysis of the KEGG signaling pathways revealed that the main pathways associated with the 18 BEO targets identified by network pharmacology analysis (Fig. 2B) were: Pathways in cancer (involving seven targets); Metabolic pathways, Human cytomegalovirus infection pathway (five targets); IL-17 signaling pathway, Cytokine-cytokine receptor interaction and Toll-like receptor signaling pathway (three targets) and nine other pathways, indicating that the targets of BEO active components are widely distributed and each component interacts with multiple pathways (Table S2).

3.3 The effects of BEO in the rabbit ear acne model

3.3.1 Histopathology

Macroscopic observation of the treated rabbit ears (Fig. 3A) showed that the distribution of the hair follicle openings in the control group was fine and flat, whereas those in the model control and negative control groups were enlarged, the skin was rough and raised, and there were pimples and pustules. The positive control group and the BEO treatment group had markedly fewer pimples.

Histopathological observations (Fig. 3B) showed that there were no visible abnormalities in rabbit ears from the control group, i.e., no inflammatory cell infiltration and clear and complete boundaries between each layer of the epidermis. In the model control group, there was visible thickening of the spinous cell layer, large areas of tissue necrosis, a large number of cell necrotic fragments in the necrotic foci and inflammatory cell infiltration. All of these manifestations are similar to human acne and consistent with a previous report [14], indicating that the rabbit ear acne model is a valid representation of human acne. The BEO treatment and positive control groups significantly reduced the severity of the acne. The proliferation of the spinous cell layer and the degree of inflammatory infiltration were markedly improved, and the overall appearance was similar to that of the control group.

3.3.2 Inflammatory mediator production in the rabbit ear acne model

Based on network pharmacology analysis, the contents of TNF-α and IL-1β in rabbit auricle tissues and serum were further determined, which is closely related to the occurrence of acne. The results are shown
in Fig. 4. The contents of TNF-α and IL-1β in the serum and auricle tissues of the model control group were significantly up-regulated ($p<0.0001$), indicating that the model was working well (Fig. 4). Compared with the model group, the levels of TNF-α and IL-1β in auricle and serum of the positive control and BEO groups were significantly down-regulated ($p<0.01$) in a dose-dependent manner. The $r$ values of TNF-α and IL-1β were $-0.9689$ and $-0.998$ in auricle, respectively, and $-0.9998$ and $-0.9824$ in the serum, respectively, and their effects were comparable to that of the positive control. Western blotting was performed to assess the release of TNF-α in the control and treatment groups (Fig. 5A). The expression of TNF-α protein in the model control group was significantly up-regulated, which was in agreement with a previous report [15]. Compared with the model control group, the expression of TNF-α protein was significantly down-regulated after BEO treatment ($p<0.0001$, Fig. 5B), indicating that BEO modulated TNF-α expression in acne lesions and would contribute to mitigating the progression of acne lesions.

### 3.3.3 Key mediators of inflammation signaling pathways

Based on network pharmacology analysis, Toll-like receptor signaling pathway was further investigated in vivo. The results are shown in Fig. 4. Compared with the control group, the levels of NF-κB, PI3K, AKT and TLR2 in the auricle tissues of the model control group were significantly increased ($p<0.0001$). After BEO treatment, the levels of NF-κB, PI3K, AKT and TLR2 in rabbit auricle tissues were significantly down-regulated ($p<0.05$) in a dose-dependent manner ($r=-0.9975$, $r=-0.9007$, $r=-0.976$, $r=-0.9323$), compared with the model control group. This suggests that the acne amelioration effect of BEO may be mediated by regulation of the TLR2/PI3K-AKT/NF-κB signaling pathway (Figs. 4 and 6). Our findings indicated that amelioration of acne by BEO may be achieved by regulating the TLR2/PI3K-AKT/NF-κB signaling pathway.

### 4. Discussion

*Curcuma longa* L. essential oil (at 0.1%, 0.3%, or 1%) [16], had a similar composition to BEO, whereas BEO (at 0.01%, 0.02%, or 0.05%) had similar anti-inflammatory activities at lower concentrations.

Further network pharmacological analysis results showed that *CYP19A1* and *CYP17* were closely related to the occurrence of acne; a deficit in the aromatase enzyme encoded by the *CYP19A1* gene caused acne [17], and the existence of increases in the *CYP17* gene increased the risk of acne in humans [18]. ARs mediate hyperkeratosis of keratinocytes, and increase the inflammatory response of macrophages and neutrophils, resulting in the initiation and progression of acne [19]. There are polymorphisms in genes such as ESR1 and matrix metallopeptidase (MMP)1, which are suspected to be the cause of acne scars [20]. In addition, BEO components are related to the target genes, tumor necrosis factor (TNF), and prostaglandin-endoperoxide synthase (PTGS) 2 (Fig. 2A). BEO significantly reduces expression of TNF-α in serum and tissues with xylene-induced auricular inflammation in mice [9], and its main component, borneol, can inhibit TNF-α and PTGS2 [21] production in tissues of mice with acute inflammations. Limonene has been reported to have anti-inflammatory effects by reducing the content of serum TNF-α and down-regulating the expression of NF-κB in inflammatory rats, and has been used in the treatment of...
acne [22]. The β-caryophyllene significantly reduces the mRNA expression levels of ESR1 in the Alzheimer’s disease cell injury model [23], indicating that the above target genes and components are of great significance in BEO treatment of acne.

Acne is a metabolic disease of humans [24]. Combined with network pharmacology analysis, it was found that the pathogenesis of acne was related to human cytomegalovirus infection [25], the cytokine-cytokine receptor interaction pathway [26] and the NF-κB signaling pathway [27]. In addition, the Toll-like receptor signaling pathway is a potential therapeutic target for treatment acne [28], which is consistent with findings from human clinical trials [29].

In the present study, the contents of TLR2, PI3K, AKT, NF-κB, IL-1β and TNF-α were measured in the rabbit ear acne model. The results showed that BEO treatment of acne inhibited the activation of the TLR2/PI3K-AKT signaling pathway mediated by S. epidermidis, which is consistent with the results reported by previous studies showing that upregulation of the TLR2/PI3K-AKT signaling pathway is an important stage in the pathogenesis of acne [30]. In addition, when microorganisms are recognized by Toll-like receptors on the surface of skin cells, they up-regulate the expression of TLRs in the cells, which can also activate downstream signals, such as NF-κB and promote the expression of inflammatory mediators, which stimulates sebum production by hair follicles [31]. Human studies have shown that NF-κB and its downstream pro-inflammatory cytokines (TNF-α, IL-1β) are activated in inflammatory acne lesions [29]. It indicates that BEO acne-treatment may be achieved by regulating the TLR2/PI3K-Akt/NF-κB signaling pathway.

5. Conclusions

The inhibitory effects of BEO were determined on the proliferation of HaCaT cells induced by heat-inactivated Staphylococcus epidermidis (inhibition by 41%) and release of the inflammatory mediators, TNF-α and IL-1β (inhibition by 44% and 10.5%, respectively), demonstrating BEO’s potential as an acne treatment. A component-target-signal pathway for BEO’s effects on acne was constructed through network pharmacology and the mechanism of BEO action was studied in vivo through the rabbit ear acne model. The main mechanism of BEO’s action against acne appears to be through regulation of the TLR2/PI3K-AKT/NFκB signaling pathway. BEO therefore, has clear potential for development into a natural and safe anti-inflammatory skin preparation, which is an effective alternative to conventional treatments containing antibiotics and synthetic anti-inflammatory agents.

Abbreviations

Cinnamomum camphora chvar. borneol essential oil (BEO); nature crystalline borneol (NCB); Staphylococcus epidermidis (S. epidermidis); lipopolysaccharide (LPS); human keratinocyte (HaCaT); dimethyl sulfoxide (DMSO); tumor necrosis factor (TNF)-α; interleukin (IL)-1β; mitogen activated protein kinase (MAPK); nuclear factor kappa B (NF-κB); Toll-like receptor (TLR); Phosphoinositide 3-kinase (PI3K); Protein kinase B (AKT); enzyme-linked immunosorbent assay (ELISA); nutrient broth (NB); fetal bovine
serum (FBS); triglyceride caprylate (GTCC); matrix metallopeptidase (MMP); half maximal inhibitory concentration (IC₅₀); Cytochrome P450 (CYP); Standard Error of Mean (SEM); Androgen receptors (AR); estrogen receptor (ESR); prostaglandin-endoperoxide synthase (PTGS); Peroxisome Proliferator-Activated Receptor-α (PPAR-α); cannabinoid receptor 2 (CNR2); transient receptor potential cation channel (TRPV1); component target weight (CTW); component pathway weight (CPW).

Declarations

Acknowledgements
We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

Authors’ contributions
Shanshan Xiao: conceptualization, methodology, software, investigation, data curation, and writing the original draft; Hang Yu: resources, writing the review and editing, and supervision; Yunfei Xie: validation, formal analysis, visualization, and supervision; Yahui Guo: resources and project administration; Jiajia Fan: resources and funding acquisition; Weirong Yao: conceptualization, validation, formal analysis, visualization, writing the review and editing, supervision, and data curation.

Funding
The work described in this article was supported by the National Key R&D Program of China (2018YFC1602300).

Availability of data and materials
The datasets used during the current study are available from the corresponding author upon reasonable request.

Ethical Approval and Consent to participate
All experimental animal procedures were approved by the Ethics Committee of the Experimental Animal Center of Jiangnan University (Wuxi, Jiangsu Province, JN. No. 20190430R0420715[98]).

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.
References

1. Sun P, Zhao L, Zhang N, Wang C, Wu W, Mehmood A, Zhang L, Ji B, Zhou F: Essential Oil and Juice from Bergamot and Sweet Orange Improve Acne Vulgaris Caused by Excessive Androgen Secretion. *Mediators Inflamm* 2020, **2020**:8868107.

2. Williams HC, Dellavalle RP, Garner S: *Acne vulgaris*. *Lancet* 2012, **379**(9813):361-372.

3. Taleb MH, Abdeltawab NF, Shamma RN, Abdelgayed SS, Mohamed SS, Farag MA, Ramadan MA: *Origanum vulgare L. Essential Oil as a Potential Anti-Acne Topical Nanoemulsion-In Vitro and In Vivo Study*. *Molecules* 2018, **23**(9).

4. Chen LW, Chung HL, Wang CC, Su JH, Chen YJ, Lee CJ: Anti-Acne Effects of Cembrene Diterpenoids from the Cultured Soft Coral *Sinularia exibilis*. *Mar Drugs* 2020, **18**(10).

5. Zaenglein AL, Pathy AL, Schlosser BJ, Alikhan A, Baldwin HE, Berson DS, Bowe WP, Graber EM, Harper JC, Kang S *et al.*: Guidelines of care for the management of acne vulgaris. *J Am Acad Dermatol* 2016, **74**(5):945-973 e933.

6. Azimi H, Fallah-Tafti M, Khakshur AA, Abdollahi M: A review of phytotherapy of acne vulgaris: perspective of new pharmacological treatments. *Fitoterapia* 2012, **83**(8):1306-1317.

7. Ebrahimabadi AH, Mazoochi A, Kashi FJ, Djafari-Bidgoli Z, Batooli H: Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss. from Iran. *Food Chem Toxicol* 2010, **48**(5):1371-1376.

8. Polatoglu K, Demirci F, Demirci B, Goren N, Can Baser KH: Antimicrobial activity and essential oil composition of a new *T. argyrophyllum* (C. Koch) Tvzel var. argyrophyllum chemotype. *J Oleo Sci* 2010, **59**(6):307-313.

9. Xiao S, Yu H, Xie Y, Guo Y, Fan J, Yao W: The anti-inflammatory potential of *Cinnamomum camphora* (L.) J.Presl essential oil in vitro and in vivo. *J Ethnopharmacol* 2021, **267**:113516.

10. Sasaki T, Kano R, Sato H, Nakamura Y, Watanabe S, Hasegawa A: Effects of staphylococci on cytokine production from human keratinocytes. *Br J Dermatol* 2003, **148**(1):46-50.

11. Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V: SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res* 2014, **42**(Web Server issue):W32-38.

12. Tan W, Li Y, Wang Y, Zhang Z, Wang T, Zhou Q, Wang X: Anti-coagulative and gastrointestinal motility regulative activities of *Fructus Aurantii Immaturus* and its effective fractions. *Biomed Pharmacother* 2017, **90**:244-252.

13. Miao MS, Li Y, Wang T, Wang T, Bai M, Miao JX, Gong B: Effects of motherwort alkaloids on rat ear acne. *Bangl J Pharmacol* 2016, **11**:S26-S30.

14. Wang Q, Jiang C, Liu W, Chen J, Lin X, Huang X, Duan X: A new optical intra-tissue fiber irradiation ALA-PDT in the treatment of acne vulgaris in rabbit model: improved safety and tolerability. *An Bras Dermatol* 2017, **92**(3):350-355.
15. Fang F, Xie Z, Quan J, Wei X, Wang L, Yang L: Baicalin suppresses Propionibacterium acnes-induced skin inflammation by downregulating the NF-kappaB/MAPK signaling pathway and inhibiting activation of NLRP3 inflammasome. *Braz J Med Biol Res* 2020, 53(12):e9949.

16. Kumar A, Agarwal K, Singh M, Saxena A, Yadav P, Maurya AK, Yadav A, Tandon S, Chanda D, Bawankule DU: Essential oil from waste leaves of Curcuma longa L. alleviates skin inflammation. *Inflammopharmacology* 2018, 26(5):1245-1255.

17. Unal E, Yildirim R, Tas FF, Demir V, Onay H, Haspolat YK: Aromatase Deficiency due to a Novel Mutation in CYP19A1 Gene. *J Clin Res Pediatr E* 2018, 10(4):377-381.

18. He L, Yang ZJ, Yu HW, Cheng BR, Tang WR, Dong YL, Xiao CJ: The relationship between CYP17-34T/C polymorphism and acne in Chinese subjects revealed by sequencing. *Dermatology* 2006, 212(4):338-342.

19. Bharti S, Vadlamudi HC: A strategic review on the involvement of receptors, transcription factors and hormones in acne pathogenesis. *J Recept Signal Transduct Res* 2021, 41(2):105-116.

20. Gold MH, Manturova NE, Kruglova LS, Ikonnikova EV: Treatment of Moderate to Severe Acne and Scars With a 650-Microsecond 1064-nm Laser and Isotretinoin. *J Drugs Dermatol* 2020, 19(6):646-651.

21. Wu HY, Tang Y, Gao LY, Sun WX, Hua Y, Yang SB, Zhang ZP, Liao GY, Zhou QG, Luo CX et al.: The synergetic effect of edaravone and borneol in the rat model of ischemic stroke. *Eur J Pharmacol* 2014, 740:522-531.

22. Angellotti G, Murgia D, Presentato A, D’Oca MC, Scarpaci AG, Alduina R, Raimondi MV, De Caro V: Antibacterial PEGylated Solid Lipid Microparticles for Cosmeceutical Purpose: Formulation, Characterization, and Efficacy Evaluation. *Materials* 2020, 13(9).

23. Zhang Y, Wu Y, Fu Y, Lin L, Lin Y, Zhang Y, Ji L, Li C: Anti-Alzheimer's Disease Molecular Mechanism of Acori Tatarinowii Rhizoma Based on Network Pharmacology. *Med Sci Monit Basic Res* 2020, 26:e924203.

24. Clatici VG, Voicu C, Voaides C, Roseanu A, Icriverzi M, Jurcoane S: Diseases of Civilization - Cancer, Diabetes, Obesity and Acne - the Implication of Milk, IGF-1 and mTORC1. *Maedica (Bucur)* 2018, 13(4):273-281.

25. Kawara S, Miyake M, Oiso N, Kawada A: Pityriasis rubra pilaris with preceding cytomegalovirus infection. *Dermatology* 2009, 219(4):350-352.

26. Chen B, Zheng Y, Liang Y: Analysis of Potential Genes and Pathways Involved in the Pathogenesis of Acne by Bioinformatics. *Biomed Res Int* 2019, 2019:3739086.

27. Grange PA, Raingeaud J, Calvez V, Dupin N: Nicotinamide inhibits Propionibacterium acnes-induced IL-8 production in keratinocytes through the NF-kB and MAPK pathways. *Journal of Dermatological Science* 2009, 56(2):106-112.

28. Cheon D, Kim J, Jeon D, Shin HC, Kim Y: Target Proteins of Phloretin for Its Anti-Inflammatory and Antibacterial Activities Against Propionibacterium acnes-Induced Skin Infection. *Molecules* 2019, 24(7).
29. Kang S, Cho S, Chung JH, Hammerberg C, Fisher GJ, Voorhees JJ: Inflammation and extracellular matrix degradation mediated by activated transcription factors nuclear factor-kappaB and activator protein-1 in inflammatory acne lesions in vivo. *Am J Pathol* 2005, 166(6):1691-1699.

30. Melnik BC: FoxO1 - the key for the pathogenesis and therapy of acne? *J Dtsch Dermatol Ges* 2010, 8(2):105-114.

31. Jin S, Lee MY: Kaempferia parviflora Extract as a Potential Anti-Acne Agent with Anti-Inflammatory, Sebostatic and Anti-Propionibacterium acnes Activity. *International Journal of Molecular Sciences* 2018, 19(11).

**Figures**

Fig. 1 Shanshan Xiao et al.

**Figure 1**
Antiproliferative effects of BEO on human keratinocyte cells (HaCaT) (A) and effects of BEO on the production of TNF-α (B) and IL-1β (C) in heat-killed Staphylococcus epidermidis-treated HaCaT cells. Data are expressed as the mean ± Standard Error of Mean (SEM). n = 6. *p < 0.05, **p < 0.001, ****p < 0.0001 compared with Heat-killed S. epidermidis alone.

Figure 2

Acne pharmacology network of the “compounds-targets-pathways” regulated by BEO. Yellow rhomboidal nodes represent compounds, orange round nodes represent targets, and pink hexagonal nodes represent pathways. 1. Cytokine-cytokine receptor interaction; 2. Toll-like receptor signaling pathway; 3. MAPK
signaling pathway; 4. Renin-angiotensin system; 5. Pathways in cancer; 6. Metabolic pathways; 7. Ovarian steroidogenesis; 8. Salmonella infection; 9. Human cytomegalovirus infection; 10. IL-17 signaling pathway; 11. NF-κB signaling pathway; 12. TNF signaling pathway; 13. Endocrine and other factor-regulated calcium reabsorption; 14. Neuroactive ligand-receptor interaction

Figure 3
Effects of BEO on histopathological changes in the rabbit ear acne model (hematoxylin and eosin staining, original magnification 40×; scale bar 500μm)

**Figure 4**

Effects of BEO on the production of TNF-α and IL-1β in serum and TNF-α, IL-1β, NF-κB, PI3K, AKT and TLR2 in auricle tissues in the rabbit auricle acne model Data are expressed as mean ± SEM (n = 6), *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, compared with the model control. “C”: control group; “M”: model control group; “N”: negative control group; “P”: positive control group.
Figure 5

Western blotting analysis on the effect of BEO on the expression of TNF-α in the rabbit ear acne model. The protein levels of TNF-α and GAPDH in the auricle tissues of the rabbits were assessed for each group. The levels of TNF-α were normalized to the levels of GAPDH proteins, respectively. GAPDH was used as the internal loading control for TNF-α. Data are expressed as mean ± SEM from at least three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, compared with the Model Control.
Figure 6

Possible mechanism of action of BEO treatment of rabbit ear acne – inhibition of the Toll-like receptor/NF-κB signalling pathway

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.docx