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RESEARCH ARTICLE

EFFECT OF *CURCUMA LONGA* LOADED LAURIC ACID LIPID VEHICLE AGAINST *PROPIONIBACTERIUM ACNES* AND ITS CYTOTOXIC EFFECTS ON PBMC AND HACAT

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ABSTRACT

Acne vulgaris - a skin disease of the face and chest causing pimples, papules and scarring in adolescent children; has been found to be concomitant with the commensal skin bacteria Propionibacterium acnes**.** The commonly used drugs for the treatment of acne have many detrimental side-effects. For centuries *Curcuma longa* and lauric acid have been used in herbal medicines for various ailments including acne. The current study aimed to deliver *Curcuma longa* through the skin by a lipid vehicle lauric acid to inhibit acne more effectively than *Curcuma longa* or lauric acid being used as a single drug. *Curcuma longa* extract was prepared from the dried rhizomes and lauric acid was separated from coconut oil. The lipid vehicle was prepared by *Curcuma longa*, lauric acid and isopropanol. The Organoleptic properties of the lipid vehicle were studied. The antimicrobial properties of *Curcuma longa* loaded lauric-acid vehicle, along with the individual extracts of *Curcuma longa* and lauric acid were studied against *Propionibacterium acnes* by microdilution broth method. Cell viability assay on Peripheral Blood Mononuclear Cells by Dye Exclusion Method and the Cytotoxicity on HaCaT cell line by MTT assay were studied on the *Curcuma longa* loaded lauric-acid vehicle, as well as with the individual extracts of *Curcuma longa* and lauric acid.It was found in the study that the combination of *Curcuma longa* and lauric acid in a lipid form proved to be more beneficial than the individual extracts.

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INTRODUCTION

Acne vulgaris is a human skin disease predominantly found on the sebaceous follicles of the face and back. It begins in pre-pubertal children (teenagers). The commensal skin bacterium *Propionibacterium acnes* is considered as a cause for acne. *Propionibacterium* are Gram positive, anaerobic, non-spore forming slow-growing bacteria which make it ideal to grow and infect the sebaceous follicles. Acne is one of the most common skin diseases treated by Dermatologists world-wide. The affected patients who are mostly teens are self-conscious and hence are embarrassed, depressed, or withdrawn by the stigmatization associated to their acne infected skin (Shweta and Swarnlata, 2011). Acne is currently treated by doctors with variousmedicines like Benzoyl peroxide, Salicylic acid, Topical/oral antibiotics, Topical retinoid creams, Azelaic acid, Isotretinoin, and Low-dose birth control pills. Each medicine has a side effect like stinging, redness, irritation, peeling of the skin, dryness and sun sensitivity. Hormonal treatments for acne can cause irregular periods, headaches, blood clots, high blood pressure, and fatigue. Isotretinoin (a teratogenic drug) can cause serious birth defects. The spread of multiple drug resistant bacteria and the unfavorable side effects leads onto the growing need for new antimicrobial agents for an alternative safe treatment (Sanjay, 2011). The use of herbal preparations for the treatment of acne are more advantageous than the regularly used drugs. Some herbal extracts, oils or formulations have been used for decades for the treatment of acne. They are effective on many of Gram-positive and Gram-negative Bacteria and don't have any adverse effects (Patel *et al.*, 2015). One such is *Curcuma longa* (Turmeric). It has been used for centuries for its medicinal properties especially in Ayurvedic medicine. Turmeric's primary biologically active component is curcumin. Curcumin has compelling antioxidant, wound healing, and anti-inflammatory properties. Turmeric has been found innocuous when ingested or when used topically in medicinal preparations (Nita Chainani, 2003). Another important product of herbal importance is Coconut oil (*Cocos nucifera*). It possesses antimicrobial, antiviral and antiprotozoal properties, mainly because of its composition of fatty acids. It comprises 50% of Lauric acid and the remaining of capric acid, caproic acid, caprylic acid etc. Coconut oil has been found to be nature's largest source of lauric acid second to only breast milk. Lauric acid is well-known for its

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antimicrobial properties, it's the predecessor to monolaurin, a very powerful antimicrobial agent that can fight many viruses and bacterial infections. Hence the antimicrobial properties of lauric acid can be used to efficiently and harmlessly treat acne. Studies have shown that lauric acid can be used as an antibiotic treatment course against *Propionibacterium* bacteria (Shweta and Swarnlata, 2011). Although topical treatment is the most primary and promising method for treating skin disorders, the topical / oral treatment using herbal drugs curcumin and lauric acid as a combination for inhibiting *P. acnes* needs further research. In a study *In-vitro* and *in-vivo* experiments demonstrated that lipophilic drugs incorporated in micro emulsions were more efficient in delivery to the skin (Chi-Hsien and Hsin-Ying, 2013). Hence in this study, *Curcuma longa* was incorporated into lipid vehicle lauric acid in order to enhance delivery and inhibit the growth of *P. acnes* more efficiently. Having known the beneficial effects of *Curcuma longa* and lauric acid, this study was planned to study its effects in inhibiting *Propionibacterium acnes.*

MATERIALS AND METHODS

Preparation of *Curcuma longa* extract

Curcuma longa (turmeric) was procured from fields in Calicut, Kerala. The rhizomes were removed from the stalks and washed with running tap water and shade dried. Care was taken to avoid fungal contamination while drying. The dried rhizomes of *Curcuma longa* (500gms) were ground to a fine powder and kept in a sterile air tight container (Plate 1).

Plate 1. Dried *Curcuma longa* Rhizomes

Extraction of lauric Acid from coconut oil

For the extraction of lauric acid, organic virgin coconut oil was procured from Calicut, Kerala (Plate 2). Virgin organic coconut oil was preferred over refined coconut oil as the refinement process involves heating which leads to loss of a substantial amount of lauric acid. The Virgin organic coconut oil was kept in the refrigerator at 25^{o}c to separate the lauric acid. Coconut oil freezes at 25.1^{o}c whilst lauric acid freezes at only 44^{o}c, hence the liquid part was taken as this contained the most lauric acid.

Preparation of *Curcuma longa* loaded lauric-acid vehicle (Lipid Vehicle)

The lauric acid extract was mixed with isopropanol in the ratio of 1:2 of its weight, to enhance its water-solubility. The Lipid vehicle was made using the extracted lauric acid, isopropanol, and a surfactant (Tween 80). It was prepared by adding the ingredients drop-wise with gentle magnetic stirring for 10 min to form a single-phase and transparent lipid system. *Curcuma longa* powder extract (4 g/L) was then added to the prepared lipid vehicle and mixed well. The Lipid vehicle was filter sterilized and stored in the fridge in eppendorfs for further testing (Plate 3).

Plate 2. Virgin Coconut oil used for lauric acid extraction

Plate 3. Filter sterilized lipid vehicle

Organoleptic properties of lipid vehicle

Physical evaluation: Changes in organoleptic properties of the *Curcuma longa* loaded lauric-acid vehiclewere studied by visual inspection for color of extract, liquefaction and phase separation. These were evaluated over a period of 2 months at

specific time intervals. Physical tests including pH and centrifugation test were carried out on the lipid vehicle over a period of 2 months at specific time intervals. The pH of lipid vehicle was determined by a pH meter. Centrifugation was performed by using a centrifuge. The centrifuge was operated at 5000 RPM for 10mins, after which the contents were observed for phase separation.

Stability studies: Stability studies were conducted on the lipid vehicle over a period of 2 months at four different conditions: At 4°c in a refrigerator, At 25°c in an incubator, At 40°c in an incubator and At 40°c with relative humidity maintained at 75% in an incubator.

Revival of *Propionibacterium acnes* MTCC Strain

Propionibacterium acnes (MTCC # 1951) strain was obtained from Microbial Type Culture Collection and Gene Bank, CSIR – Institute of Microbial Technology, Chandigarh, India. It was revived from the lyophilized ampoules (Plate 4). The ampoule was opened aseptically and 0.5ml of Brain Heart Infusion (BHI) broth (HIMEDIA) with 1% glucose was added to the ampoule and carefully pipetted up and down for proper dissolving. A few drops of the suspension were streaked onto Blood agar plates. The plates were incubated under anaerobic conditions in an anaerobic jar along with BBL Gas Pak systems (HIMEDIA) for 48 hours (Plate 5).

Plate 4. *Propionibacterium acnes* (MTCC 1951)

Plate 5. *P.acnes*streaked onto Blood Agar plates and kept in anaerobic jar with gas pak

Antimicrobial activity on *P.acnes*

The *Curcuma longa* loaded lauric-acid vehicle, along with the extracts of *Curcuma longa* and lauric acid were tested against *Propionibacterium acnes (P. acnes)* by the microdilution broth method (Tsai *et al*., 2008). To evaluate their antimicrobial effects, *P. acnes* was incubated in Brain Heart Infusion (BHI) broth with 1% glucose for 72hrs under anaerobic conditions. $1x10^6$ CFU per ml of the incubated *P. acnes* cells were dissolved in BHI and used for the assay.

Table 1. Plate map for Determination of antimicrobial activity against *P.acnes*

	1	2	3	4	5	6	7	8	9	10	11	12	
A	200µl BHI broth	200µl C/L		20µl C/L			40µl C/L			80µl C/L			A
B	200µl BHI broth	200µl C/L		160µl C/L									B
C													C
D	200µl Solvent	200µl Curcuma		20µl C			40µl C			80µl C			D
E	200µl Solvent	200µl Curcuma		160µl C									E
F													F
G	200µl BCS	200µl Lauric acid		20µl L			40µl L			80µl L			G
H	200µl BCS	200µl Lauric acid		160µl L									H
	1	2	3	4	5	6	7	8	9	10	11	12	

*BHI broth = Brain Heart infusion broth, C/L = Curcumin loaded Lauric-acid vehicle, C = *Curcuma longa,* L = Lauric Acid, Solvent = Isopropanol, BCS = Bacterial cell suspension (Propionibacteria acnes)

In a sterile 96-well microtiter plate different concentrations (20, 40, 80 and 160 µg/ml) of the extracts were added to wells containing 20 µl of the bacterial suspension in broth. Controls of pure solvent (Isopropanol), Brain Heart Infusion broth, the extracts -*Curcuma long* loaded lauric-acid vehicles, *Curcuma longa* and lauric acid and the bacterial cell suspension *P. acnes* were also added to wells on the plate. BHI was used as the blank control. Triplicate samples for each test concentration were used (Table 1 and Plate 6). The plate was incubated at 37 °C under an anaerobic condition for 72 hours, microbial growth was evaluated by measuring absorbance at 600 nm using a micro plate reader.

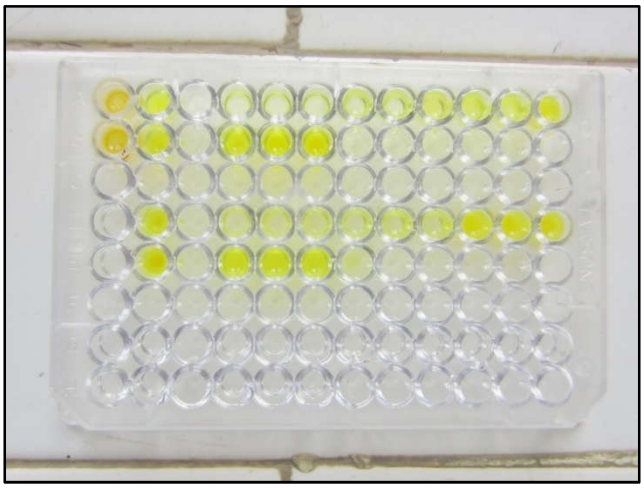

Plate 6. Determination of antimicrobial activity against *P.acnes* (Plate map)

Cell viability assay on Peripheral Blood Mononuclear Cells by Dye Exclusion Method

A cell viability assay was performed on human peripheral blood mononuclear cells (PBMC) along with the *Curcuma longa* loaded lauric-acid vehicle and the extracts of *Curcuma longa* and lauric acidby using Tryphan blue dye exclusion method (DEM) to selectively stain dead cells blue. Human PBMC were isolated based on a density gradient centrifugation technique described by Boyum in 1968. 10 ml of venous blood was drawn from a donar and was de-fibrinated in a sterile 100 ml conical flask with stone beads by gently swirling the flask for 10 minutes. Blood was diluted with equal volume of RPMI 1640 media and carefully layered over histopaque in the ratio

of 1:2 (1 parthistopaque to 2 parts diluted blood). After centrifugation at 400 rpm for 20 minutes, the mononuclear cell layer seen at the interface was carefully transferred to a tube containing 5 ml RPMI 1640 medium. The cells were thoroughly mixed with the medium and washed by centrifugation at 500 rpm for 10 minutes. Washing was repeated twice. The final suspension was made into 5 ml RPMI 1640 medium with 10% Fetal Calf Serum (FCS). One drop of cell suspension (approximately 20 µl) and one drop of 0.4% tryphan blue solution (Sigma chemicals, U.S.A) were mixed, placed in a haemocytometer. Live and dead cells were counted under phase contrast objective. The cell concentration was adjusted to the desired number of viable mononuclear cells/ml of RPMI 1640 medium. Care was taken to obtain a cell suspension with 95% to 98% mononuclear cells with less than 5% to 7% contamination of erythrocytes, granulocytes, platelets and dead cells. The viable cell count was calculated by the formula

$$\text{Viable cell count} = \frac{\text{No. of cells counted}}{\text{Area counted} \times \text{Depth factor} \times \text{Dilution factor}} \times 10^3$$

The cell viability assay was performed on the isolated human PBMC along with various concentrations (20 µg, 40 µg 80 µg and 160 µg) of the *Curcuma longa* loaded lauric-acid vehicle, the extracts of *Curcuma longa* and lauric acid. The assay was performed in a 96 well tissue culture plate (Greiner, U.S.A), using various negative controls like plain media, complete media, vehicle, cell and extract. Positive controls like a known immunomodulator-PHA and a known cytotoxic compound-LPS were also maintained. After the addition of cell, media and extracts, the cultures were incubated in an incubator (TC2323, Shel lab, U.S.A) with 95% air, 5% CO_2 and humidified atmosphere at 37˚C.After the incubation period a suitable volume of a cell suspension (20-200µl) were taken from each extract in an appropriate tube and an equal volume of 0.4% Tryphan blue was added and gently mixed. It was allowed to stand for 5 minutes at room temperature. Live cells or tissues with intact cell membranes are not coloured and hence Tryphan blue is not absorbed but is absorbed in the membrane of a dead cell. Hence dead cells are shown as a blue under a microscope. 10 µl of stained cells were placed in a haemocytometer and the number of viable (unstained) and dead (stained) cells were counted. The average number of unstained cells in each quadrant were calculated and multiplied by 2×10^4 to find cells/ml. The percentage of viable cells is the number of viable cells divided by the number of dead and viable cells.

Cytotoxicity on HaCaT cell line by MTT assay

The cytotoxic effect of the *Curcuma longa* loaded lauric-acid vehicle, along with the extracts of *Curcuma longa* and lauric acidwere also studied on HaCaT (Human Keratinocytes) cell line by 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT) assay. The ability of the cells to survive a toxic insult was the basis of this Cytotoxicity assay. The assay was based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme Succinatedehydrogenase.

HaCaT cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM-HG supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and the assay was carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). 3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), DMEM-HG and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India. 10mg each of the lipid vehicle and the extracts were dissolved seperately in distilled DMSO and the volume was made with DMEM-HG supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration for each extract. Two fold serial dilutions were prepared. Monolayer HaCaT cells were trypsinized and cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM-HG containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed with medium once, 100 µl of different test concentrations of test drugs were added to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere and microscopic examination was carried out and observations were noted after every 24-hour interval. After 72 hours, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hours at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates and gently shaken to solubilize the formed formazan. Absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values was generated from the dose-response curves for each cell line.

RESULTS

Organoleptic properties

During the 2-month study period, no change in the color of lipid vehicle was seen, their pH was within the range 4 to 6. The refrigerator stored lipid vehicle became solid, whilst the other lipid vehicles stored at 25^{o}c, 40^{o}c and 40^{o}c+ relative humidity at 75% all were found with a slight phase separation which mixed evenly on shaking. Since no color change was observed, pH remained within the normal physiological range of 4-6 and liquefaction and phase separation was negligible during the stability studiesthe formulation can be considered stable (Table 2).

MTCC Strain revival

After 72 hours of anaerobic incubation, *Propionibacterium acnes (P. acnes)* growth was seen. The colonies appeared convex, semi-opaque, and glistening on blood agar plates.

Table 2. Organoleptic properties

Day	Color	pH	4°c	25°c	40°c	40°c + 75% relative humidity
1	Bright yellow	6	Solid	Liquid	Liquid	Liquid
5	Bright yellow	6	Solid	Liquid	Liquid	Liquid
10	Bright yellow	6	Solid	Liquid	Liquid	Liquid
15	Bright yellow	5.9	Solid	Liquid	Liquid	Liquid
20	Bright yellow	5.9	Solid	Liquid	Liquid	Liquid
25	Bright yellow	5.8	Solid	Liquid	Liquid	Liquid
30	Bright yellow	5.8	Solid	Liquid	Liquid	Liquid
35	Bright yellow	5.8	Solid	Liquid	Liquid	Liquid
40	Bright yellow	5.7	Solid	Liquid	Liquid	Liquid
45	Bright yellow	5.7	Solid	Liquid	Liquid	Liquid
50	Bright yellow	5.7	Solid	Liquid	Liquid	Liquid
55	Bright yellow	5.7	Solid	Liquid	Liquid	Liquid
60	Bright yellow	5.6	Solid	Liquid	Liquid	Liquid

Antimicrobial activity of lipid vehicle on *P. acnes*

Antimicrobial activities of the *Curcuma longa* loaded lauric-acid vehicle, along with the extracts of *Curcuma longa* and lauric acid were investigated to evaluate their inhibition effects by microdilution method. Various concentrations of extracts were incubated with *P. acnes* in Brain Heart Infusion broth for 24 hours to determine their optical density. *Curcuma long* loaded lauric-acid vehicles results showed that the concentrations 40µg/ml, 80µg/mL and 160 µg/mL inhibited more than 50% of *P. acnes*. *Curcuma long* showed that the concentrations 80µg/ml and 160 µg/ml inhibited more than 50% of *P. acnes*. Where as in lauric acid, 160 µg/ml concentration inhibited more than 50% of *P. acnes* (Fig 1). Results indicate that *Curcuma long* loaded lauric acid vehicles inhibit *P. acnes* more efficiently than *Curcuma longa*or lauric acid.

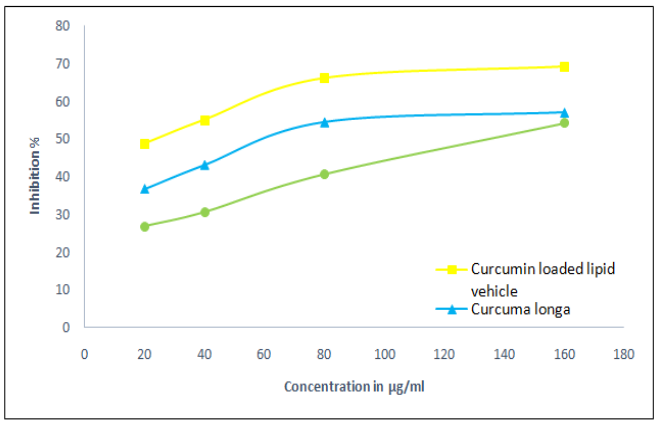

Fig. 1. Inhibition % due to antimicrobial activity

Cell viability assay on Peripheral Blood Mononuclear Cells (PBMC) by DEM

To assess the cell viability, the *Curcuma longa* loaded lauric-acid vehicle, along with the extracts of *Curcuma longa* and lauric acid were treated on Human Peripheral Blood Mononuclear cells (PBMC) by Dye Exclusion Method (DEM). When *Curcuma long* loaded lauric-acid vehicle was tested on PBMC by DEM, it was found to be non-toxic for the concentrations 20µg, 40µg, 80µg and 160µg even after 72-hours exposure (Table 3 and Fig 2).

Table 3. Effect of *curcuma longa* lauric-acid vehicle on PBMC by DEM

S.No	Concentration of the extract in µg	Time exposure 24 hours	48 hours	72 hours
1	20	Non toxic	Non toxic	Non toxic
2	40	Non toxic	Non toxic	Non toxic
3	80	Non toxic	Non toxic	Non toxic
4	160	Non toxic	Non toxic	Non toxic

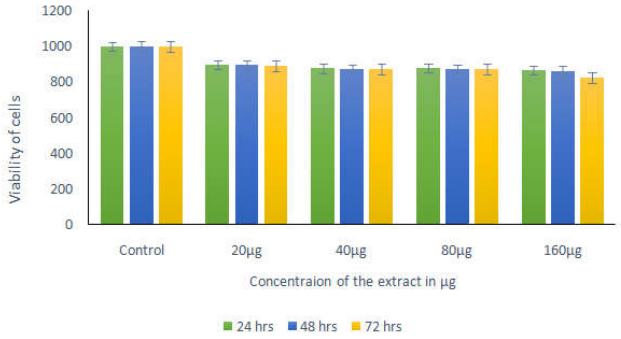

Fig. 2. Effect of *curcuma longa* lauric-acid vehicle on PBMC by DEM

When *Curcuma longa* was tested on PBMC by DEM, it was also found to be non-toxic for concentrations 20µg, 40µg, 80µg and 160µg even after 72-hours exposure (Table 4 &Fig 3).

Table 4. Effect of *curcuma longa* on PBMC by DEM

S.No	Concentration of the extract in µg	Time exposure 24 hours	48 hours	72 hours
1	20	Non toxic	Non toxic	Non toxic
2	40	Non toxic	Non toxic	Non toxic
3	80	Non toxic	Non toxic	Non toxic
4	160	Non toxic	Non toxic	Non toxic

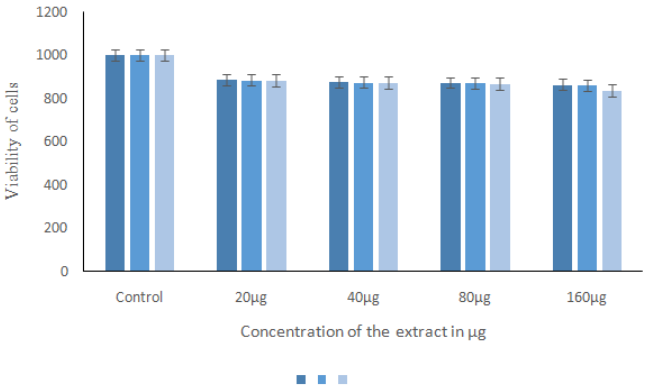

Fig 3:Effect of *curcuma longa* on PBMC by DEM

When lauric-acid was treated on PBMC and tested by DEM, it was found to be non-toxic for concentrations 20µg, 40µg and 80µg. At 160µg concentration it was found to be cytotoxic to PBMC when exposed for 72 hours (Table 5 &Fig 4).

Cytotoxicity on HaCaT cell line by MTT assay

HaCaT cell line on treatment with *Curcuma long* loaded lauric acid vehicle, percentage of inhibition was found to be almost similar from concentrations 250µg/ml to 1000µg/ml when compared to lower concentrations. Almost similar results were obtained for *Curcuma longh*and for lauric acid (Fig 5& Plate 7, 8, and 9).

Table 5. Effect of lauric acid on PBMC by DEM

S.No	Concentration of the extract in µg	Time exposure 24 hours	48 hours	72 hours
1	20	Non toxic	Non toxic	Non toxic
2	40	Non toxic	Non toxic	Non toxic
3	80	Non toxic	Non toxic	Non toxic
4	160	Non toxic	Non toxic	Cytotoxic

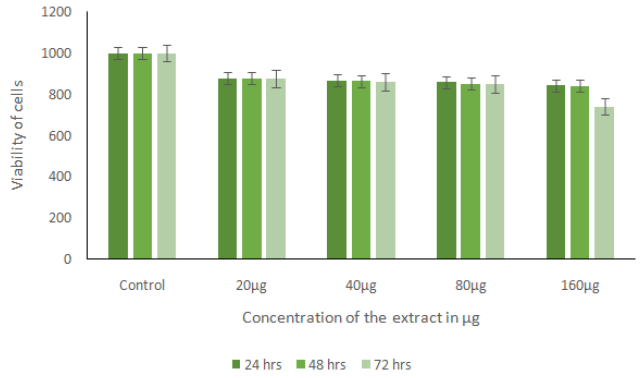

Fig. 4. Effect of lauric acid on PBMC by DEM

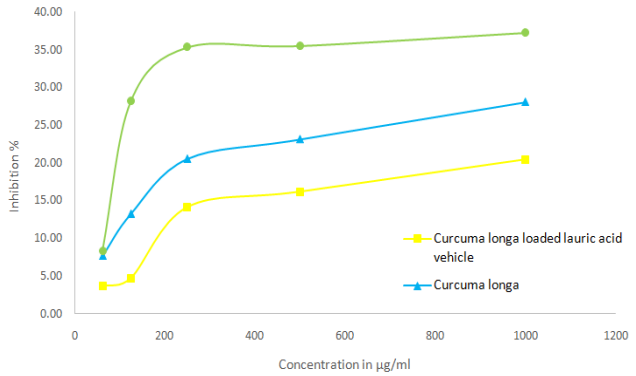

Fig. 5. Cytotoxicity on HaCaT

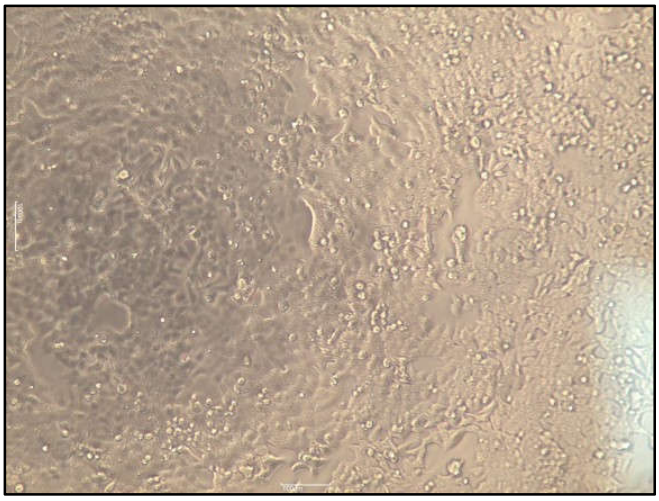

Plate 7. HaCaT cell line – control

DISCUSSION

Curcuma longa and lauric acid (extracted from virgin coconut oil) both famous for their antimicrobial activity for centuries, are regularly used as an antioxidant, antibacterial and anti-inflammatory agent.

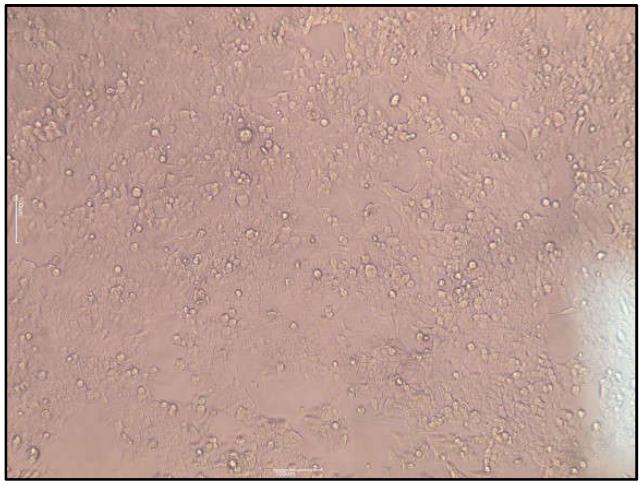

Plate 8. HaCaT cell line treated with 500µl/ml of extract

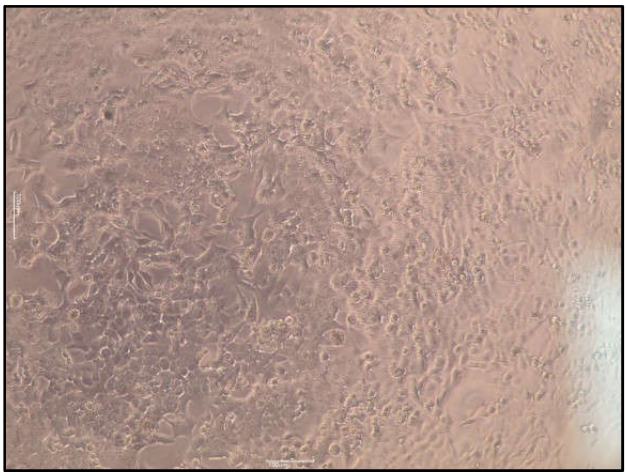

Plate 9. HaCaT cell line treated with 1000µl/ml of extract

Curcuma longa and lauric acid are both hydrophobic which limits their delivery and absorption. Lipid vehicles were found to enhance transcutaneous absorption of *Curcuma longa* and lauric acid. Lipid vehicles are composed of an aqueous phase, an oil phase, and surfactant or cosurfactant. Lauric acid is a saturated fatty acid which is not water soluble, isopropanol acts as the cosurfactant to increase the solubility of lauric acid and bring about the stability of the oil–water interface. Hence lauric acid was the oil phase in the system to accommodate *Curcuma long* along with Tween 80 as the surfactant and isopropanol as the cosurfactant (Chi-Liu *et al.*, 2013). The effects of *Curcuma long* loaded Lauric-acid vehicles, *Curcuma longa*, and lauric acid on in-vitro inhibition of *P. acnes* were evaluated. *Curcuma long* loaded lauric-acid vehicles strongly inhibited microbial growth compared to *Curcuma longa* and lauric acid used seperately.

The antibacterial activity of *Curcuma longa* is due to inhibition of the cytokinetic Z-ring assembly. The Z-ring assembly plays a vital role in cell division. Lauric acid inhibits Gram-positive cocci by disrupting the cell membrane (Chi-Liu *et al.*, 2013). The combination of two agents having destructive properties may result in more inhibitory effects than a single agent. Our results indicate that the combination of lauric acid and *Curcuma longa* in the vehicle carrier might provide a new route for *P. acnes* therapy. A study by Darren *et al.*, (2009) evaluated the antimicrobial activity of various fatty acids and its liposomal derivatives against *P. acnes*. They studied the

antimicrobial effect of three free fatty acids (lauric acid, palmitic acid and oleic acid) and found lauric acid had the strongest bactericidal activity against *P. acnes*. The only setback waslauric acid had poor water solubility. Lauric acid was incorporated into a liposome formulation to enhance absorption and antimicrobial activity. Further studies found that the antimicrobial activity of lauric acid -loaded liposomes (LipoLA) could fuse with the membranes of *P. acnes* and the lauric acid directly affected the bacterial membranes hence killing the bacteria effectively. In the study to assess the anti-proliferative activity and immunomodulatory effect on Human Peripheral Blood Mononuclear cells (PBMC) by Dye Exclusion Method (DEM) it was found that *Curcuma longa* loaded lauric acid vehicle and *Curcuma longa* were non-toxic to PBMC for all concentrations for 72 hours. Whilst lauric acid showed immunomodulatory effect from 160µg after 72 hours. In a previous study by Grace *et al*., (2010), the immunomodulatory activities of the polar fractions of *Curcuma longa* by hot water extracts were investigated on human peripheral blood mononuclear cells (PBMC). Results showed that the high polarity fraction of the hot water extract showed stimulatory effects on PBMC proliferation. The study of cytokine productions (TGF-β, TNF-α, GM-CSF, IL-1α, IL-5, IL-6, IL-8, IL-10 and IL-13, etc.) were modulated by a polysaccharide-enriched fraction. The findings revealed the potential use of *Curcuma longa* crude extract as an ideal adjuvant supplement for cancer patients, whose immune activities were suppressed during chemotherapies.

Toxicity of *Curcuma longa* loaded lauric acid vehicle against HaCaT by MTT assay showed similar inhibition percentage from 250-1000µg/ml indicating the extracts had only a basic and minimal effect on the HaCaT keratinocytes. A study conducted by Teruaki *et al*., (2009) for the cytotoxicity of lauric acid on the human immortalized sebaceous gland cell line SZ95. In the study SZ95 sebocytes were incubated with lauric acid at various concentrations for 18 hours at 37^{o}c and cell viability was determined. It was found that lauric acid did not affect sebocyte viability at the concentrations used. Ayurveda which is almost 6000 years old, has abundant anti-inflammatory agents used for centuries to treat chronic diseases. More than 200 Ayurvedic plants have been identified that exhibit anti-inflammatory activities (Bharat *et al*., 2011). Amongst them *Curcuma longa* has a long history of use in Ayurvedic medicine in treatment for inflammatory conditions. Lauric acid from coconut oil also has a very important place for treating wounds, inflammation, skin diseases. Currently use of natural compounds, especially of plant origin; has come to the forefront as their efficacy has been well studied and are believed to be safe for humans. Many Indian herbs are being used for centuries in traditional practices to cure various human ailments. Furthermore, in future, the isolated principles from these extracts need to be evaluated in scientific manner using various innovative experimental models and clinical trials to understand its mechanism of action, in search of other active constituents, so that its other therapeutic uses can be widely explored.

Conclusion

Curcuma longa and lauric acid extracted from virgin coconut oil were studied for their antimicrobial potential on *Propionibacterium acnes*, that causes acne. The chosen extracts have been used for hundreds of years in Ayurveda for their diverse medicinal properties. The combination of *Curcuma longa* and lauric acid in a lipid form called *Curcuma longa* loaded lauric acid vehicle proved to be more beneficial than the individual extracts as was observed in most of the experiments performed in this study. *Curcuma longa* loaded lauric acid vehicle was found to strongly inhibited microbial growth of *P. acnes*. It was also found to be non-toxic to human PBMC and HaCa Thuman keratinocyte cells. Further studies have to be done to develop an injectable or ingestible drug to treat acne and help avoid the serious side effects of the current drug treatments that are being used.

REFERENCES

Adeline, F., Deyrieux and Van, G. 2007. Wilson. In vitro culture conditions to study keratinocyte differentiation using the HaCaT cell line. *Cytotechnology.,* 4: 77–83.

Adityan, B., Kumari, R., Thappa, D.M. 2009. Scoring systems in acne vulgaris. *Indian Journal of Dermatology*, *Venereology and Leprology*, 75: 323-326.

Andrew, P. Desbois, and Keelan, C. Lawlor. 2013. Antibacterial Activity of Long-Chain Polyunsaturated Fatty Acids against Propionibacterium acnes and Staphylococcus aureus. *Marine drugs*., 11: 4544-4557.

Bensky, D. and Gamble, A. 1986. Chinese Herbal Medicine Materia Medica, Seattle. *East land press*., 390-391.

Bharat, B. Aggarwal, Sahdeo Prasad, Simone Reuter, RamaswamyKannappan, Vivek R. Yadav, Byoungduck Park, Ji Hye Kim, Subash C. Gupta, KanokkarnPhromnoi, ChitraSundaram, Seema Prasad, Madan M. Chaturvedi and Bokyung Sung. Identification of Novel Anti-inflammatory Agents from Ayurvedic Medicine for Prevention of Chronic Diseases. *Current Drug Targets.* 2011; 12(11): 1595–1653.

Bruggemann, H., Henne, A., Hoster, F., Liesegang, H., Wiezer, A., Strittmatter, A., Hujer, S., Durre, P., and Gottschalk, G. 2004. The complete genome sequence of Propionibacterium acnes, a commensal of human skin. Science (New York). 305(5684): 671-673.

Carpo, B.G., Verallo-Rowell, V.M., and Kabara, J. 2007. Novel antibacterial activity of monolaurin compared with conventional antibiotics against organisms from skin infections: an in vitro study. *Journal of Drugs in Dermatology.* 2007; 6(10): 991-998.

Chang, H.M. and But, P.P. 1987. Pharmacology and Applications of Chinese MateriaMedica. *World Scientific.,* 2: 936-939.

Chi-Hsien Liu, and Hsin-Ying Huang. 2013. In Vitro Anti-Propionibacterium Activity by Curcumin Containing Vesicle System. *Chemical and Pharmaceutical Bulletin.*, 61(4): 419–425.

Chinampudur, V. Chandrasekaran, Kannan Sundarajan, Jothie R Edwin, Giligar M Gururaja, Deepak Mundkinajeddu and Amit Agarwal. 2013. Immune-stimulatory and anti-inflammatory activities of Curcuma longa extract and its polysaccharide fraction. *Pharmacognosy Research.* 2013; 5(2): 71–79.

Cogen, A.L., Nizet, V., and Gallo, R.L. 2008. Skin microbiota: a source of disease or defence. *The British Journal of Dermatology.,* 158(3): 442–455.

Darren Yang, Dissaya Pornpattananangkul, Teruaki Nakatsuji, Michael Chan, Dennis Carson, Chun-Ming Huang and Liangfang Zhang. The Antimicrobial Activity of Liposomal Lauric Acids Against Propionibacterium acnes. Biomaterials. 2009; 30(30): 6035–6040.

DebMandal, M. and Mandal, S. 2011. Coconut (Cocos nucifera L: Arecaceae): in health promotion and disease prevention. *Asian Pacific Journal of Tropical Medicine*. 4(3): 241-247.

Ditte, M.S. Lundvig, Sebastiaan, W.C., Pennings, Katrien, M. Brouwer, Matilda Mtaya-Mlangwa, Emeria Mugonzibwa, Anne Marie Kuijpers-Jagtman, Frank ADTG Wagener, Johannes W Von den Hoff. 2015. Cytoprotective responses in HaCaT keratinocytes exposed to high doses of curcumin. *Experimental Cell Research*., 336: 298–307.

Downing, D.T., Stewart, M.E., Wertz, P.W., Colton, S.W., Abraham, W. and Strauss, J.S. 1987. Skin lipids: an update. *The Journal of Investigative Dermatology.*, 88: 2-6.

Duggi Shrishail, Handral Harish, Handral Ravichandra, G. Tulsianand and S D Shruthi. Turmeric: Nature's precious medicine. *Asian Journal of Pharmaceutical and clinical research*. 2013; 6(3): 10-16.

Elizabeth, A. Grice and Julia, A. 2011. Segre. The skin microbiome. *Nature Reviews Microbiology*. 9(4): 244–253.

Fitz-Gibbon, S., Shuta, T., Bor-Han, C., Nguyen, L., Du, C., Minghsun, L., Elashoff, D., Erfe, M.C., Loncaric, A., Kim, J., Modlin, R.L., Miller, J.F., Sodergren, E., Craft, N., Weinstock, G.M. and Li, H. 2013. Propionibacterium acnes Strain Populations in the Human Skin Microbiome Associated with Acne. *Journal of Investigative Dermatology*., 133(9): 2152–2160.

Francis, D. and Rita, L. Rapid. 1986. Colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *Journal of Immunological Methods*, 89: 271-277.

Grace, G.L. Yue, Ben C.L. Chan, Po-Ming Hon, Kwok-Pui Fung, Ping-Chung Leung, and Clara B.S. Laua. 2010. Immunostimulatory activities of polysaccharide extract isolated from Curcuma longa. *International Journal of Biological Macromolecules,* 47(3): 342–347.

Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J. and Young, A.C. 2009. NISC Comparative Sequencing Program, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML and Segre JA. Topographical and Temporal Diversity of the Human Skin Microbiome. Science (New York). 324(5931): 1190–1192.

Grice, E.A. 2014. The skin microbiome: potential for novel diagnostic and therapeutic approaches to cutaneous disease. *Seminars in Cutaneous Medicine and Surgery.,* 33(2): 98–103.

Hannigan, G.D. and Grice, E.A. 2013. Microbial ecology of the skin in the era of metagenomics and molecular microbiology. Cold Spring Harbor Perspectives in Medicine. 3(12).

Hina Shrestha, RajniBala, and Sandeep Arora. 2014. Lipid-Based Drug Delivery Systems. *Journal of Pharmaceutics*. 2014.

Huang, Y.C., Yang, C.H., Li, T.T., Zouboulis, C.C., Hsu, H.C. 2015. Cell free extracts of Propionibacterium acnes stimulate cytokine production through activation of p38 MAPK and Toll-like receptor in SZ95 sebocytles. *Life Science*. 139: 123-31.

Intahphuak, S., Khonsung, P. and Panthong, A. 2009. Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. *Pharmaceutical Biology*, 48(2): 151-157

Jon, J. Kabara, Dennis M Swieczkowski, Anthony J Conley, and Joseph P Truant. 1972. Fatty Acids and Derivatives as Antimicrobial Agents. *Antimicrobial Agents Chemotherapy*. 2(1); 23-28

Kirschbaum, J.O. and Kligman, A.M. 1963. The pathogenic role of Corynebacterium acnes in acne vulgaris. *Archives of Dermatology*. 1963; 88: 832–833.

Kojima, H., Yanai, T. and Toyota, A. 1998. Essential oil constituents from Japanese and Indian Curcuma aromatica rhizomes. *Planta Medica*. 64: 380-381.

Larsen, K., Lock, J.M., Maas, H., Maas, P.J.M. 1998. Zingiberaceae. The families and genera of vascular plants. *Springer*. 4: 474-495.

Leyden, J. 2003. A review of the use of combination therapies for the treatment of acne vulgaris. *Journal of the American Academy of Dermatology.,* 49(3): 200–210.

Luangnarumitchai, S., Lamlertthon, S., and Tiyaboonchai, W. 2007. Antimicrobial Activity of Essential Oils Against Five Strains of Propionibacterium acnes. Mahidol University *Journal of Pharmaceutical Sciences,* 34(1-4): 60-64.

Nakatsuji, T., Kao, M.C., Fang, J.Y., Zouboulis, C.C., Zhang, L., Gallo, R.L. and Huang, C.M. 2009. Antimicrobial Property of Lauric Acid Against Propionibacterium acnes: Its Therapeutic Potential for Inflammatory Acne Vulgaris. *The Journal of investigative dermatology.,* 129(10): 2480–2488.

Nikkari, T., Schreibman, P.H. and Ahrens, E.H. 1974. Jr. In vivo studies of sterol and squalene secretion by human skin. *Journal of Lipid Research,*15: 563-573.

Nita Chainani-Wu, 2003. Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa). *Journal of Alternative and Complementary Medicine*, 9(1): 161-168.

Parfene, G., Hornicar, V., Tyagi, A.K., Malik, A. and Bahrim, G. 2013. Production of medium chain saturated fatty acids with enhanced antimicrobial activity from crude coconut fat by solid state cultivation of Yarrowialipolytica. Food Chemistry. 2013; 136(3-4): 1345-1349.

Patel, S. D, Shah, S. and Shah, N. 2015. A Review on Herbal Drugs Acting Against Acne Vulgaris. *Journal of Pharmaceutical Science and Bioscientific Research,* 5(2): 165-171.

Preuss, H.G., Echard, B., Dadgar, A., Talpur, N., Manohar, V., Enig, M., Bagchi, D. and Ingram, C. 2005. Effects of essential oils and monolaurin on Staphylococcus aureus: in vitro and in vivo studies. *Toxicology Mechanisms and Methods*., 15: 279-285.

Ramasastry, P., Downing, D.T., Pochi, P.E. and Strauss, J.S. 1970. Chemical composition of human skin surface lipids from birth to puberty. *The Journal of Investigative Dermatology.,*54: 139-144.

Rambir Singh, Ramesh Chandra, Mridula Bose and Pratibha Mehta Luthra. Antibacterial activity of Curcuma longa rhizome extract on pathogenic bacteria. *Current Science.* 2002; 83(6): 737-740.

Roth, R.R. and James, W.D. 1988. Microbial ecology of the skin. *Annual Review of Microbiology.,* 42(1): 441–464.

Rui Zhao, Bei Yang, Linlin Wang, Peng Xue, Baocheng Deng, Guohua Zhang, Shukun Jiang, Miao Zhang, Min Liu, Jingbo Pi and Dawei Guan. Curcumin Protects Human Keratinocytes against Inorganic Arsenite-Induced Acute Cytotoxicity through an NRF2-Dependent Mechanism. *Oxidative Medicine and Cellular Longevity*. 2013.

Sanjay, K. 2011. Rathi. Acne Vulgaris treatment: The current scenario. *Indian Journal of Dermatology*, 56(1): 7–13.

Sasikumar, B. 2005. Genetic Resources of Curcuma: Diversity characterization and utilization. *Plant Genetic Research,* 3(2): 230-251.

Schoop, V.M., Mirancea, N. and Fusenig, N.E. 1999. Epidermal Organization and Differentiation of HaCaT Keratinocytes in Organotypic Coculture with Human Dermal Fibroblasts. *Journal of Investigative Dermatology,* 112(3): 343-353.

Shahiquz Zaman and Naveed Akhtar. 2013. Effect of Turmeric Extract Cream on Human Skin Sebum Secretion. *Tropical Journal of Pharmaceutical Research,* 12(5): 665-669.

Shweta Kapoor and Swarnlata Saraf. 2011. Topical Herbal Therapies an Alternative and Complementary Choice to Combat Acne. *Research Journal of Medicinal Plant,* 5(6): 650-669.

Singh, S. and Aggarwal, B.B. 1995. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane). *Journal of Biological Chemistry,* 270: 24995–5000.

Teruaki Nakatsuji, Mandy C Kao, Jia-You Fang, Christos C Zouboulis, Liangfang Zhang, Richard L Gallo and Chun-Ming Huang. 2009. Antimicrobial Property of Lauric Acid Against Propionibacteriumacnes: Its Therapeutic Potential for Inflammatory Acne Vulgaris. *Journal of Investigative Dermatology,* 129(10): 2480–2488.

Thody, A.J. and Shuster, S. 1989. Control and function of sebaceous glands. *Physiological Reviews,* 69: 1-4.

Todar, K. 2008. Normal Bacterial Flora of Humans Todar's Online Textbook of Bacteriology.

Tsai, T.H., Tsai, T. H., Chien, Y. C., Lee, C. W. and Tsai, P. J. 2008. In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. *Food Chemistry*., 110: 859–864.

Uta Jappe. 2003. Pathological Mechanisms of Acne with Special Emphasis on Propionibacterium acnes and Related Therapy. *ActaDermatoVenereologica*., 83: 241–248.

Wen-Cheng Huang, Tsung-Hsien Tsai, Lu-Te Chuang, You-Yi Li, Christos C. Zouboulis and Po-Jung Tsai. 2014. Anti-bacterial and anti-inflammatory properties of capric acid against Propionibacterium acnes: A comparative study with lauric acid. *Journal of Dermatological science*, 73(3): 232-240.

Whitney, A. Fisk, Hadar A. Lev-Tov and Raja K. Sivamani. 2014. Botanical and Phytochemical Therapy of Acne: A Systematic Review. *Phytotherapy Research*, 28(8): 1137-1157.

Wilson, V.G. 2013. Growth and Differentiation of HaCaT Keratinocytes. *Methods in Molecular Biology*, 1195: 33-41.

Yang, D., Pornpattananangkul, D., Nakatsuji, T., Chan, M., Carson, D., Huang, C.M. and Zhang, L. 2009. The Antimicrobial Activity of Liposomal Lauric Acids Against Propionibacterium acnes. Biomaterials, 30(30): 6035–6040.

Zhao, G.J., Lu, Z.Q., Tang, L.M., Wu, Z.S., Wang, D.W., Zheng, J.Y. and Qiu, Q.M. 2012. Curcumin inhibits suppressive capacity of naturally occurring CD4+CD25+ regulatory T cells in mice in vitro. *International Immunopharmacology*. 14(1): 99-106.

Zouboulis, C.C. 2004. Acne and sebaceous gland function. Clinics in Dermatology, 22: 360-366.

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