EVALUATION OF SOME SELECTED OILS FOR THEIR ANTIOXIDANT ACTIVITY AND ANTI-MICROBIAL POTENTIAL AGAINST ACNE CAUSING BACTERIUM *PROPIONIBACTERIUM ACNE*

[1]Shalini, B.U., [2]Sushmitha, R.
[1]Associate Professor,
[1]Department of Botany,
Mount Carmel College (Autonomous), Bengaluru, Karnataka, India. Pin - 560 052.

Abstract: Acne is a skin disease which causes painful social and psychological effects on sufferers. It is a problem for many adults as well as for many teenagers. The primary factors involved in the formation of acne lesions are increased sebum production, sloughing of keratinocytes, bacterial growth and inflammation. *Propionibacterium acnes* (*P. acnes*), an anaerobic pathogen, plays an important role in the pathogenesis of acne. The excessive use of antibiotics for long periods leads to increased resistance in acne causing bacteria. To overcome the problem of antibiotic resistance, essentials oils and medicinal plant extracts have been extensively studied as an alternative. In the present study, 13 common oils were investigated for their anti-acne potential against *Propionibacterium acnes* and antioxidant activity. Antibacterial activity was investigated using well diffusion method. Among the oils investigated for antibacterial activity, Lemongrass oil showed the maximum zone of inhibition followed by Lemon oil. The least antibacterial activity was exhibited by Calamus oil. The free radical scavenging activity was estimated by 1, 1-Diphenyl-2-picryl-hydraxyl (DPPH) assay. Lemongrass oil showed the highest antioxidant activity and least was shown by Jojoba oil. There was a good correlation between the oils showing antioxidant activity and anti-acne activity. The oils which exhibited highest inhibitory effect against *Propionibacterium acnes* and anti-oxidant activity in the present study can be incorporated into the cosmetic formulation and can be used to treat acne.

***IndexTerms* – antioxidant, antiacne, *Propionibacterium*, oil, acne**

I. INTRODUCTION

The skin is the heaviest and largest organ of the body, with a total area of about 2 square metres and weighing between 3.5 and 10 kilos (7.5 and 22 pounds). The skin protects us from microbes and the elements, helps regulate body temperature and permits the sensations of touch, heat and cold. Skin is the also the most vulnerable part of the body. Day to day exposure to sun, dust leads to number of problems such as pimples, acne, sun burn marks and pigmentation (Aparijita *et al.*, 2014).

Acne vulgaris is a most common skin disorder or dermatologic diseases of pilo-sebaceous units in the world affecting almost everybody during the life (Scheinfeld, 2007). It affects over 80% of teenagers; persists beyond the age of 25 years in 3% of men and 12% of women. Acne is a polymorphic disorder that occurs on the face (99%), back (60%) and chest (15%) (Kapoor and Saraf, 2011). These are characterized by comedones, papules, pustules, cysts, nodules and often scars (Lalla *et al*., 2011). Several factors may induce acne production or increase its severity. Some of these factors include genetics, the male sex, youth, stress and smoking as well as comedogenic medications such as androgens, halogens, corticosteroids and pore clogging cosmetics. Past research suggests that genetic impudence combined with comedogenic hormones (especially androgens) produce abnormal volumes of sebum which contribute to acne lesions (Krautheim and Gollnick, 2001). It is also caused by hyperkeratosis, which retains keratin and sebum. The main microorganisms involved are *Propionibacterium acnes*, *Staphylococcus epidermis* and *Staphylococcus aureus.* The four main pathological factors involved in the development of acne are the increased sebum production, irregular follicular desquamation, *Propionibacterium acnes* proliferation and inflammation of area (Krautheim and Gollnick, 2001). Post acne scars are observed due to abnormal wood healing after damage that occurs in sebaceous follicle during acne inflammation (Holland and Jerency, 2005).

Propionibacterium acnes are relatively slow growing aero-tolerant anaerobic gram positive bacteria. This bacterium primarily lives on the fatty acid present in the sebaceous glands or sebum secreted by follicles. *P. acnes* play an important role in the pathogenesis of acne inflammation by producing polymorphonuclear leukocyte and monocyte or macrophage to produce pro-inflammatory mediators (Vowels *et al.*, 1995). On the contrary, *Staphylococcus epidermidis*, an aerobic organism, usually involves in superficial infections within the sebaceous unit (Burkhart *et al.*, 1999).The main components of pilosebaceous unit on the skin are the keratinocytes and sebocytes which can be activated by *P. acnes* leading to the production of pro-inflammatory products. These include lipases, proteases, hyaluronidases and chemotactic factors (Heymann, 2006). Among these products, lipase is an important factor in causing inflammation. They act by metabolising the sebaceous triglycerides into fatty acids, which chemotactically attracts neutrophils. As a consequence, neutrophils which are attracted to the acne lesion constantly release inflammatory mediators such as reactive oxygen species (Leyden, 1997). Although reactive oxygen species perform a useful function in the skin barrier against acne microbes (Boh, 1996), excess formation affects skin condition by activating neutrophil infiltration. The reactive oxygen species include singlet oxygen, superoxide radical anion, hydrogen peroxide, hydroxyl radical, lipid peroxide and nitric oxide. They play a critical role in irritation and disruption of the integrity of the follicular epithelium leading to the development of inflammatory acne as well as tissue injury (Jame *et al*., 1999). Therefore, the compounds for targeting *Acne vulgaris* should be able to inhibit *P. acnes*, reduce pro-inflammatory lipids in serum as well as reduce post acne scar formation (Nord and Oprica, 2006).

Antibiotics are most commonly used for the treatment of acne. The treatment of acne is directed towards correcting the altered pattern of follicular keratization, decreased sebaceous gland activity, decreasing the formation of causative bacteria *P. acnes* and finally leads to anti-inflammatory effect (Kumar *et al.*, 2005). Antibiotics such as macrolides (erythromycin, clindamycin, azithromycin and roxithromycin), fluoroquinolones (levofloxacin), tetracyclines (doxycycline, minocycline and lymecycline) and co-trimoxazole are used (Katsambas and

Papakonstantinou, 2004). Tetracyclines are very popular because they are effective and inexpensive. Doxycycline and minocycline are preferred because they cause less gastrointestinal irritation, and they are more lipids soluble, penetrating the pilo-sebaceous follicle more efficiently. The tetracycline family exhibits both anti-inflammatory as well as antibacterial properties. Additionally, less resistance in P. acnes have been reported with the tetracyclines than the macrolides (Webster, 2008). Erythromycin and clindamycin have little anti-inflammatory activity and mainly work by reducing the levels of *P. acnes*. Gram-negative folliculitis can occur as a complication of any long term topical or oral antibacterial treatment resulting in sudden onset of multiple pustules frequently localized around the peri-oral and peri-nasal areas. The minimum duration of treatment for acne either by using topical or oral anti acne agents is two–three months (Bowe and Logan, 2010). This long term expensive treatment increases the instances of alarming adverse effects. The increasing instances of bacterial resistances further developed the surge to adopt an alternative treatment. Among the alternative treatments, natural therapy is the most acceptable. The natural therapy lacking adverse effects is highly desirable with respect to its conceivable safety and rare *P. acne* resistance (Krautheim and Gollnick, 2001).

Natural treatments for *Acne vulgaris* have much to offer besides many mainstream dermatology treatment options available. All these prevailing treatments carry risks, and none is completely satisfactory. Moreover, the possibility of this topical approach may serve patients significantly by natural therapies despite continued resistance from mainstream dermatology (Yarnell, 2006). Many medicinal plants from ancient have been used as they have potential healing properties in Indian traditional medicines like Ayurveda, Unani and Siddha medicines. The therapeutic efficacies of many indigenous plants for various diseases have been described by the traditional herbal medicine practitioners. Therefore, natural alternatives are gaining greater research support and these naturally derived compounds, particularly those from herbs and their derivatives have been a good prospect for future development of anti acne products (Mallik *et al*., 2012).

During recent years, plant essential oils have come more into the focus of phytomedicine (Buckle, 1999). Their widespread use has raised the interest of scientists in basic research of essential oils. Especially, the anti-microbial and anti-oxidant activities of essential oils have been investigated (Dukic *et al*., 2009). Essential oils are complex natural mixtures which are used as flavouring agents in perfume, food and beverage. Additionally, they have been traditionally used as natural preservatives in medicine, food and cosmetics. Several reports have indicated antibacterial activities of essential oils (Burt, 2004).

Antioxidants are those substances which possess free radical chain reaction breaking properties. There has been an upsurge of interest in the therapeutic potential medicinal plants and their derivatives as antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad, 2006). Among the numerous naturally occurring antioxidants, ascorbic acid, carotenoids and phenolic compounds are more effective (Duh, 1999). They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions (Sudarajan *et al*., 2006).

The oils claimed for acne control should possess antibacterial, anti-inflammatory and anti-oxidant activities. Possessing antioxidant activity may be useful for relieving hypertrophic scar and keloid formation by suppressing the collagen synthesis of the fibroblasts from hypertrophic scars but not suppressing those of healthy normal tissues (Taniguchi *et al*., 1995). The present study aims to evaluate the *in-vitro* antibacterial activity of 13 selected oils against *Propionibacterium acnes*, to compare the antibacterial activity between each material against a known synthetic anti-acne agent as Standard (Clindamycin), evaluation of the anti-oxidant activity and comparison of the antioxidant and anti-bacterial activity of oils with high values.

II. MATERIALS AND METHODS

Material: Samples of pure oils for the present study were purchased from a Supplier Health and Glow, Bengaluru and also at Aroma World, Bengaluru and were used in the anti-acne and anti-oxidant studies. The list of oils is given in the Table 01.

Table 1

List of oils used in anti-acne assay against *Propionibacterium acnes* and anti-oxidant assay

Sl. No.	Oil name	Botanical name	Family	Part used	Uses
01.	Calamus oil	*Acorus calamus* L.	Acoraceae	Rhizomes	It is mild tonic, counters hallucinations, rejuvenator for brain, aromatic, stimulant, expectorant, aphrodisiac and carminative.
02.	Coconut oil	*Cocos nucifera* L.	Arecaceae	Kernel of fruit	Helps in weight loss, reduces seizures, improves blood cholesterol level, hair toner and acts as skin moisturiser.
03.	Frankinscence oil	*Boswellia serrata* Roxb. ex Colebr.	Burseraceae	Gum	It soothes and calms the mind, relives pain in rheumatism and muscular aches, balances skin. It is also antiseptic, digestive and astringent.
04.	Gingergrass oil	*Cymbopogon martini* (Roxb.) J. F. Watson	Poaceae	Grass	It is essential component of aromatherapy. Soothes the mind, reduces stress and anxiety. Used in perfume making
05.	Geranium oil	*Pelargonium graveolens* L'Hér.	Geraniaceae	Leaves and flowers	It is mentally uplifting, refreshing, balances female hormones, complexion of skin, cures cold. flu, neuralgia, bruises and burns.
06.	Grapeseed oil	*Vitis vinifera* L.	Vitaceae	Seeds	It is used in controlling moisture of skin; used in aromatherapy, helps growth of hairs and strengthens it.
07.	Jojoba oil	*Simmondsia chinensis* (Link) C.K. Schneid.	Simmondsiaceae	Seeds	It controls oily skin and moistens, has anti-inflammatory and anti-bacterial properties. It controls hair fall and improve scalp health.
08.	Lemon oil	*Cirus limon* (L.) Burm. f.	Rutaceae	Peel of the fruit	It is good for oily skin and lightens skin pigment. It is antiseptic, astringent and antibacterial. Helps in bronchial problems, cold, fever, nervous tension and counteracts acidity.
09.	Lemongrass oil	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Grass	It has antiseptic, antibacterial and refreshing properties. Improves skin elasticity, stretch marks and scars. Also

					helps in curing throat and mild respiratory problems. Relieves shock, trauma, depression and stress.
10.	Olive oil	*Olea europea* L.	Oleaceae	Fruits	It acts as cleanser, moisturizer and has antibacterial property. It reduces Type 2 diabetes and prevents strokes, fights osteoporosis and skin cancer.
11.	Orange oil	*Citrus sinensis* (L.) Osbeck	Rutaceae	Peel of fruits	It has antibacterial, antispasmodic, anti-inflammatory properties. It is good diuretic and balances water retention and obesity, reduces tension and stress.
12.	Turmeric oil	*Curcuma longa* L.	Zingiberaceae	Rhizome	It cures pimples, improves skin complexion, shields cancer, lowers cholesterol, prevents strokes and improves digestion. It has anti-inflammatory, antibacterial, antioxidant and antiseptic properties.
13.	Ylang ylang oil	*Canaga odorata* (Lam.) Hook. f. & Thomson	Annonaceae	Flowers	It has antidepressant, antiseborrhoeic and antiseptic properties. Soothes the skin, helps in hair growth and lowers blood pressure and reduces cardiac problems.

Anti-acne studies: Acne is caused by two types of bacteria namely, *Propionibacterium acnes* and *Staphylococcus epidermis*. *Propionibacterium acnes* play a vital role in pathogenesis of acne inflammation. In the present study, a few medicinal plants oils were screened to check their antibacterial activity against *Propionibacterium acnes.* The pure culture of *Propionibacterium acnes* (Microbial Type Culture Collection, MTCC 1951) was obtained from IMTECH (Institute of Microbial Technology) Chandigarh, India. Brain Heart Infusion (BHI) Broth was procured form Hi-Media was used for inoculums preparation. Brain Heart Infusion (BHI) agar was used for maintenance and plating.

Culture method: *Propionioabacterium acne* is an anaerobe bacterium and requires anaerobic conditions for growth. An air tight glass jar with Gas pack was used to provide the anaerobic condition. Gas pack is a sachet which contains mixture of salts in powder form like cobalt chloride, citric acid, sodium bicarbonate and sodium borohydride. It also contains methylene blue in tablet form which is a chemical indicator that is colourless in absence of oxygen and oxidizes to blue colour in the presence of atmospheric oxygen.

Inoculum preparation: Brain Heart Infusion (BHI) broth was used to prepare the inoculum. Two to three loop full of *Propionibacterium acnes* were aseptically transferred into test tubes containing six mL using sterile broth. The tubes were incubated at 37˚C for 72 hours. After incubation the turbidity was adjusted to 0.5 McFarland Standard. Sterile broths were used in case the dilution of the inoculums density was needed to match the standard.

McFarland Standard: To standardize the density of the inoculums, 0.5 McFarland standard was prepared as described in National Committee for Chemical Laboratory Standards (NCCLS 1997). One percent V/V solution of Sulphuric acid was prepared by dissolving 1mL of concentrated Sulphuric acid in 99mL of water and mixed well. Solution of 1.175%W/V of Barium Chloride was prepared by dissolving 2.35g of dehydrated Barium Chloride in 200mL of distilled water. For the preparation of turbidity standard 0.5mL of the Barium Chloride solution was added to 99.5mL of 1% Sulphuric acid solution and mixed well. A small volume of the above turbid solution id transferred into a screw capped tube that is similar to the one used for preparing control inoculums and this is stored in dark at room temperature. With the help of spectrophotometer the turbidity of 0.5 McFarland standards was alternatively measured at 625nm.

Preparation of Clindamycin: A stock solution of Clindamycin was prepared by dissolving 0.01mg of Clindamycin in 10 mL of Ethanol. With the help of stock solution, the first dilution was made by taking 0.1 mL of Clindamycin in 0.9 mL of Ethanol. From the first dilution the second dilution was prepared by taking 0.1 mL of Clindamycin from the first dilution and added to 0.9 mL of ethanol. Second dilution was used for further studies.

Antibacterial activity - Well diffusion method (modified Bauer *et al.*, 1966): *P. acne* was incubated in BHI broth for 72 hours under anaerobic conditions and adjusted to yield approximately 1.0×10^8 CFU/mL Brain Heart Infusion agar was used for plating. After solidification, 100µL of inoculums was spread or swabbed uniformly with the help of a sterile spreader and allowed to dry for five minutes. Fine wells were bored with the help of a sterile borer in the petriplates containing media. The oils were dissolved in 10% in aqueous dimethylsulfoxide (DMSO) with Tween 80 (0.5%) for easy diffusion. Under aseptic conditions 50µL of sample and standard Clindamycin was loaded into the well. All petriplates were sealed with parafilm and were left for 30 minutes at room temperature to allow easy diffusion of oils. The Petri plates with loaded wells were transferred into an air tight anaerobic jar containing a Gas pack with indicator tablet and kept in incubator maintained at 37˚C for 48 hours. At the end of incubation, the zone of inhibition, that appears as a clear zone around the loaded well was observed and measured in mm using HIMEDIA antibiotic zone scale. From this the activity index (A.I.) and Percent Inhibition (P.I.) were calculated for all oils and extracts using the following formula (Munazir *et al.*, 2012) (Plate 1)

$$\text{A.I} = \frac{\text{Mean zone of inhibition of each solvent extract}}{\text{Zone of inhibition obtained for standard}}$$

$$\text{P.I.} = \text{Activity index} \times 100$$

Plate 1

Antioxidant activity assay – DPPH radical scavenging assay (Mensor *et al.*, 2001)

The antioxidant activity was measured using the 2, 2-diphenyl-picryl-hydrazyl (DPPH) radical reduction assay. The assay was carried out by mixing 1.0 mL methanolic solution of each of the oils with 2.0mL of 0.02mM methanolic DPPH solution at two concentrations 50 µL/mL and 100 µL/mL of oils. The mixture was then incubated in the dark for 30 minutes at room temperature and absorbance at 517nm was recorded using spectrophotometer. The control was also carried out applying the same procedure to a solution without the test material and absorbance was recorded. The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ Inhibition} = 100 \times \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}}$$

III.RESULTS AND DISCUSSION

Down the ages, plant extracts and oils have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many diseases (Tepe *et al.*, 2004). World Health Organisation noted that majority of the world's population depends on traditional medicine for primary healthcare (Muttu *et al.*, 2006).

Anti-acne studies: In the present study, 13 oils were assayed for anti-acne activity against the bacterium *Propionibacterium acnes*. Of the 13 oils, 9 were essential oils namely Calamus oil, Frankinscence oil, Lemongrass oil, Lemon oil, Gingergrass oil, Geranium oil, Orange oil, Turmeric oil and Ylang Ylang oil; 3 were carrier oils namely Grapeseed oil, Jojoba oil and Olive oil; Coconut oil was a fixed oil. These oils were obtained from different plant parts like leaves, flowers, fruits, seeds and resins. The samples belong to 13 species of plants and 11 families (Table 1). Of all the oils studied, essential oils showed comparatively a good zone of inhibition against the *Propionibacterium acnes*. The zone of inhibition exhibited by standard (Clindamycin) was 11.5mm. Lemongrass oil showed the highest zone of inhibition (42.5mm) followed by Lemon oil (40mm) and Ginger grass oil (34mm) (Table 2, Figure 1, Plate 2). Similar studies were conducted by Luangnarumitchai *et al.*, (2007), Zu *et al.*, (2010) and Tsai *et al.*, (2011). The results revealed that lemongrass oil, lemon oil and ginger grass oil showed higher antibacterial activity against *P.acnes* than the previous study. The zone of inhibition exhibited by other oils were Orange oil (23mm), Geranium oil (22.5mm), Frankinscence oil (21.5mm), Grapeseed oil (19mm), Ylang Ylang oil (17.95mm), Coconut oil (13.95mm), Jojoba oil (13.30mm), Turmeric oil (13.25mm), Olive oil (13mm) and Calamus oil (11.6mm) (Table 2, Figure 1, Plate 2).

The activity index of the oils were as follows - Lemongrass oil 3.69, Lemon oil 3.47, Gingergrass oil 2.95, Orange oil 2.0, Geranium oil 1.95, Frankinscence oil 1.86, Grapeseed oil 1.65, Ylang Ylang oil 1.56, Coconut oil 1.21, Jojoba oil and Turmeric oil 1.15, Olive oil 1.13 and Calamus oil 0.99. The percentage inhibition of the oils were Lemongrass oil 369%, Lemon oil 347%, Ginger grass oil 295%, Orange oil 200%, Geranium oil 195%, Frankinscence oil 186%, Grape seed oil 165%, Ylang Ylang oil 156%, Coconut oil 121%, Jojoba oil and Turmeric oil 115%, Olive oil 113% and Calamus oil 99% (Table 3). Thus most of the oils used in the present study showed a comparatively higher percentage inhibition when compared with other studies.

Based on the response of oil to the bacterium *Propionibacterium acnes*, oils were categorised mainly into Susceptible, Intermediate and Resistant effects (Daud *et al*., 2013). Among the oils studied, Lemon grass, Lemon, Ginger grass, Orange, Geranium, Frankinscence, Grape seed, Ylang Ylang oils showed Susceptible effect towards *P.acnes* whereas, Jojoba, Turmeric, Olive, Coconut and Calamus oils showed Intermediate effect towards *P.acnes*. The Standard Clindamycin showed Intermediate effect towards *P. acnes* (Table 4 and 5).

Generally, essential oils comprise of a large number of active components. The difference in antibacterial activity of the essential oils may be due to the difference in chemical compositions. The main components of essential oils exhibited high activity in this study were previously reported for their marked antibacterial activity against various types of bacteria. Citral, main component in lemongrass oil, showed activity against several microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (Hammer *et al*., 1998; Lertsatitthanakorn *et al.*, 2006 and Prabuseenivasan *et al*., 2006). Similarly in Lemon oil. Limonene, a main component showed antibacterial activity against microorganisms such as *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa and E.coli* (Prabuseenivasan *et al*., 2006). Therefore, antibacterial activity against *P. acnes* of these essential oils may be due to the presence of the key constituents. An increase in the concentration can cause an increase in the activity which can be assessed in future.

Antioxidant studies: The DPPH free radical scavenging activity was carried out for determining the antioxidant activity of oils. The antioxidant activity was estimated at two different concentrations namely at 50µL and 100µL. Among the oils studied, Lemongrass oil showed the highest DPPH scavenging potential at both the concentrations (80.99% at 50 µL and 85.87% at 100 µL). The antioxidant activity of the other oils at 50 µL concentration in the descending order is as follows; Geranium oil 81.05%, Lemon oil 78.85%, Frankinscence oil 79.65%, Gingergrass oil 78.53%, Calamus oil 76.45%, Grapeseed oil 76.38%, Turmeric oil 76.35%, Ylang Ylang oil 75.06%,Orange oil 67.54%, Coconut oil 58.29% , Olive oil 47.25% and Jojoba oil 41.54%. The antioxidant activity of the other oils at 100 µL concentrations in the descending order is as follows - Lemon oil 82.84%, Frankinscence oil 81.70%, Geranium oil 81.05%, Grape seed

oil 80.98%, Ginger grass oil 80.33%, Calamus oil 80.45%, Ylang Ylang oil 78.08%, Turmeric oil 77.64%, Orange oil 73.82%, Coconut oil 60.94%, Olive oil 64.94% and Jojoba oil 45.71%. (Table 6, Figure 2).

Amorati *et al.,* (2013) reviewed literature methods and assessed that many essential oils have antioxidant properties and use of these oils as natural antioxidants is growing interest as synthetic antioxidants are harmful to health. The present study also showed that the essential oils selected for the study showed good scavenging activity. Atolani *et al.,* (2012) studied the antioxidant activity of seed oils from edible fruits and results revealed that grapeseed oil, lemon oil and orange oil exhibited free radical scavenging activity similar to our present study. Tsai *et al.,* (2011) investigated the antimicrobial, antioxidant and anti-inflammatory activities of essentials and results revealed that *Cymbopogon martnii* (Gingergrass) showed good antioxidant activity similar to the present study.

Comparative study of anti-acne and anti-oxidant activity: There was a good correlation between the oils showing antioxidant activity and anti-acne activity namely Lemon grass oil, Lemon oil and Gingergrass oil (Figure 3). Those showing high antioxidant property showed higher inhibitory zone against *P. acnes*. Therefore it becomes necessary to use free radical scavengers for removal of ROS to reduce cell damage that occurs during acne inflammation. The antioxidant compounds have the ability to capture, deactivate and repair damage caused by free radicals. Thus oils having high antioxidant property can be used to treat acne thereby reduces free radicals released during acne formation.

Table 2
Laboratory evaluation of Anti-acne activity of selected oils against *Propionibacterium acnes*.

Sl. No	Samples	Zone of Inhibition in mm		
		Plate 1	Plate 2	Mean ± S.D
01.	Calamus	11	12.2	11.6±0.84
02.	Coconut	14.1	13.8	13.95±0.21
03.	Frankinscence	23	20	21.5±2.12
04.	Gingergrass	35	33	34±1.41
05.	Geranium	24	21	22.5±2.12
06.	Grapeseed	18.8	19.2	19±0.28
07.	Jojoba	12.7	13.9	13.3±0.84
08.	Lemon	41	39	40±1.41
09.	Lemongrass	42	43	42.5±0.70
10.	Olive	12.9	13.1	13±0.14
11.	Orange	21	25	23±2.82
12.	Turmeric	13	13.5	13.25±0.35
13.	Ylang Ylang	18.1	17.8	17.95±0.21
14.	Clindamycin	11.6	11.4	11.5±0.14

Table 3
Activity Index and Percentage Inhibition of selected oils against *Propionibacterium acnes.*

Sl.no	Samples	Activity Index	Percentage Inhibition
01.	Calamus	0.99	99
02.	Coconut	1.21	121
03.	Frankinscence	1.86	186
04.	Gingergrass	2.95	295
05.	Geranium	1.95	195
06.	Grapeseed	1.65	165
07.	Jojoba	1.15	115
08.	Lemon	3.47	347
09.	Lemongrass	3.69	369
10.	Olive	1.13	113
11.	Orange	2.0	200
12.	Turmeric	1.15	115
13.	Ylang Ylang	1.56	156

Table 4
Values to evaluate the bacterial response of each oil (Daud *et al*., 2013).

Responses	Diameter of zone of inhibition(mm)
Resistant	10 or less
Intermediate	11-15
Susceptible	16 or more

Table 5
Bacterial response of each of the selected oils.

NAME OF THE OIL	RESPONSE OF THE OIL
Calamus	Intermediate
Coconut	Intermediate
Frankinscence	Susceptible
Gingergrass	Susceptible
Geranium	Susceptible
Grapeseed	Susceptible
Jojoba	Intermediate
Lemon	Susceptible
Lemongrass	Susceptible
Olive	Intermediate
Orange	Susceptible
Turmeric	Intermediate
Ylang Ylang	Susceptible
Clindamycin	Intermediate

Table 6
Laboratory evaluation of anti-oxidant activity of selected oils.

Sl. No.	Sample	Sample loaded (µL)	Antioxidant values in %		
			1	2	Mean ±S.D
01.	Calamus oil	50	77.5	75.40	76.45 ±1.4
		100	79.30	81.6	80.45 ±1.6
02.	Coconut oil	50	57.32	59.26	58.29±1.3
		100	61.63	60.25	60.94±0.97
03.	Frankinscence oil	50	79.20	80.10	79.65±0.63
		100	82.15	81.25	81.7±0.63
04.	Gingergrass oil	50	79.54	78.52	78.53±1.42
		100	80.42	80.25	80.33±0.12
05.	Geranium oil	50	81.2	80.9	81.05±0.21
		100	78.09	78.26	78.17±0.12
06.	Grapeseed oil	50	75.43	77.34	76.38±1.35
		100	80.53	81.43	80.98±0.63
07.	Jojoba oil	50	47.41	35.67	41.54±8.3
		100	41.17	50.25	45.71±6.42
08.	Lemon oil	50	77.85	79.86	78.85±1.42
		100	83.14	82.54	82.84±0.42
09.	Lemongrass oil	50	80.42	81.87	80.99±0.81
		100	82.2	89.55	85.87±5.19
10.	Olive oil	50	48.27	46.24	47.25±1.43
		100	64.65	65.24	64.94±0.41
11.	Orange oil	50	67.28	67.80	67.54±0.36
		100	71.4	76.25	73.82±3.42
12.	Turmeric oil	50	74.28	78.42	76.35±2.92
		100	75.46	79.82	77.64±3.08
13.	Ylang Ylang oil	50	75.84	74.28	75.06±1.10
		100	77.75	78.42	78.08±0.47

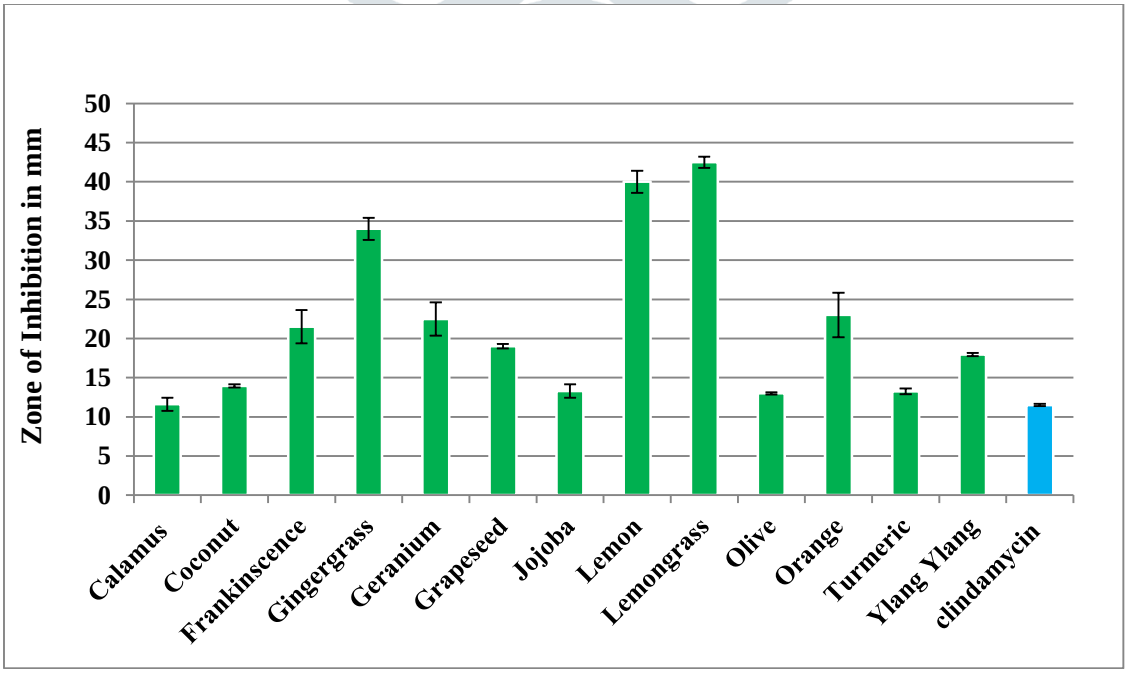


Figure 1

Anti-acne activity of selected oils against *Propionibacterium acnes.*

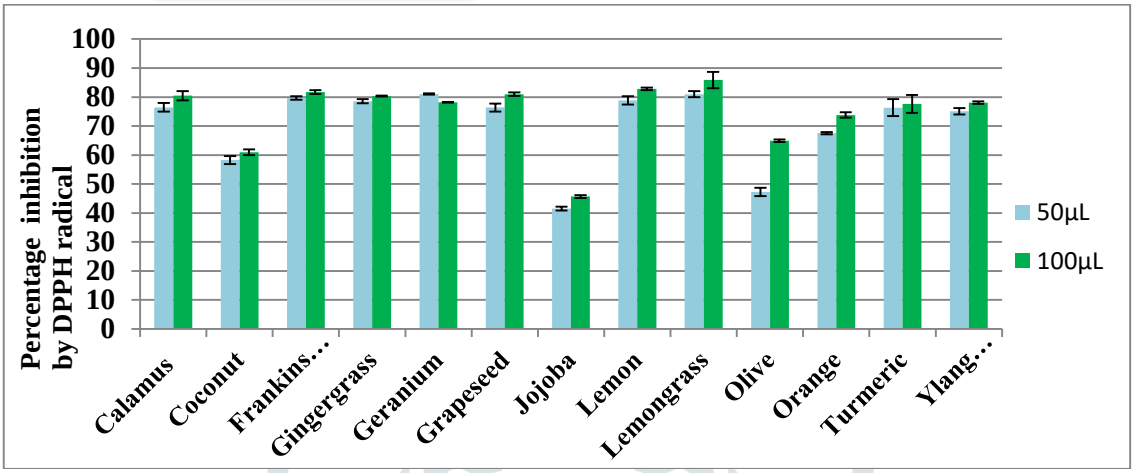


Figure 2
Anti-oxidant activity of selected oils used for anti-acne study.

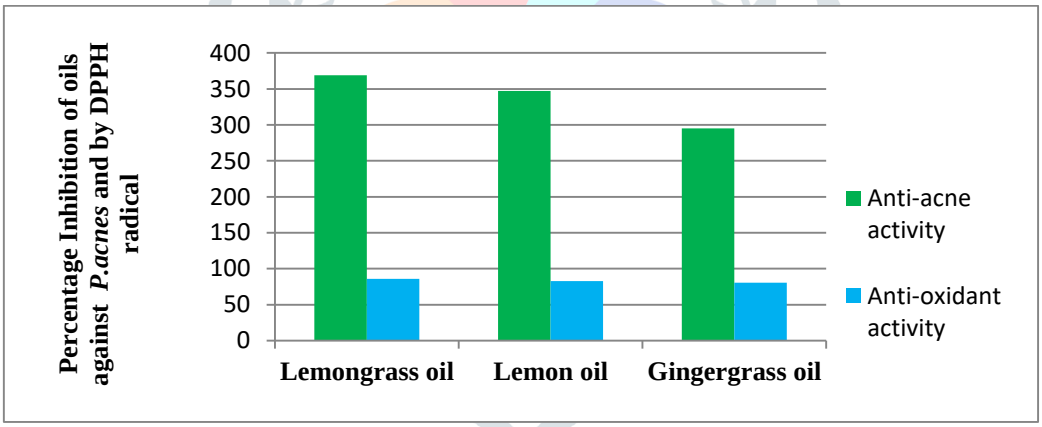
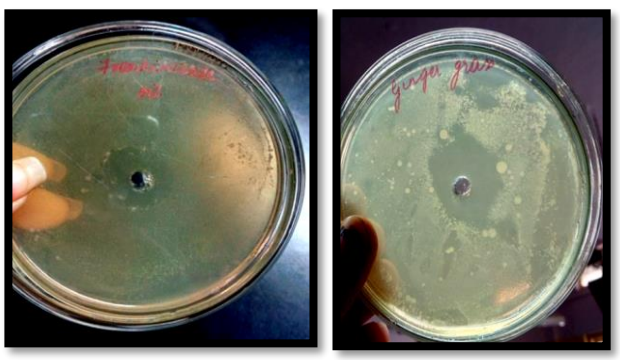
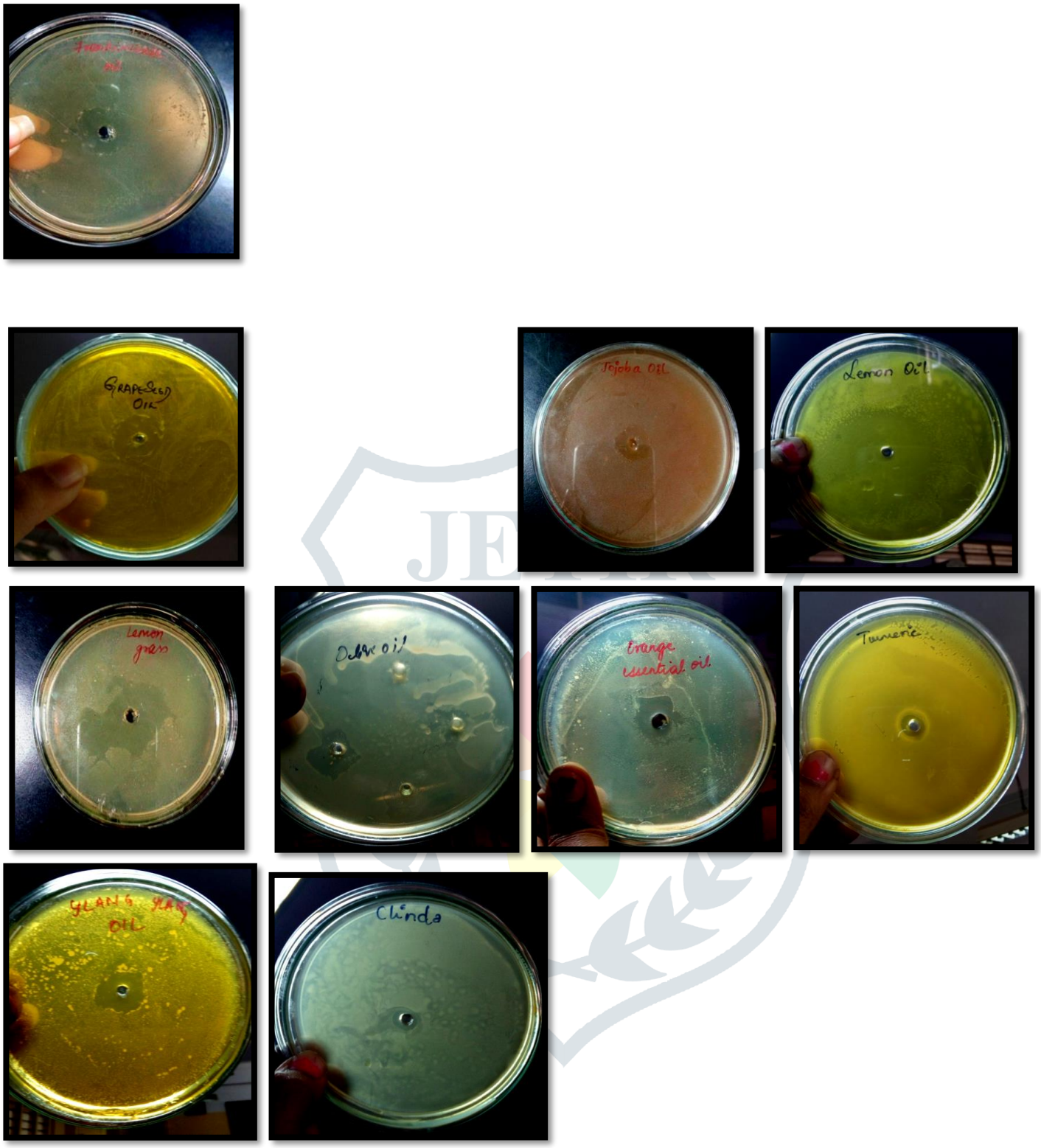


Figure 3
Correlation of Anti-acne and Antioxidant activity of oils which showed best results in the present study.

Plate 2 Antibacterial activity of Oils against *P.acnes*





Calamus oil, Coconut oil, Frankinscence oil, Gingergrass oil, Geranium oil, Grapeseed oil, Jojoba oil, Lemon oil, Lemon grass oil, Olive oil, Orange oil, Turmeric oil, Ylang Ylang oil and Clindamycin (Left to right)

IV. CONCLUSION

The targeting molecules should be able to inhibit *Propionibacterium acnes*, reduce pro-inflammatory lipids in sebum as well as reduce post acne scar formation. Among the oils investigated for antibacterial activity, Lemongrass oil showed the maximum zone of inhibition which was followed by Lemon oil. The study also revealed that Lemongrass, Lemon, Gingergrass, Orange, Geranium, Frankinscence, Grapeseed, Ylang Ylang oils showed susceptible effect towards *P.acnes* whereas, Jojoba, Turmeric, Olive, Coconut and Calamus oils showed intermediate effect towards *P.acnes*. The antioxidant activity of carrier oils and fixed oil ranged between 45%-65% whereas, essential oils exhibit a good free radical scavenging activity ranging from 70-85%. Lemongrass oil showed the highest antioxidant activity and least was shown by Jojoba oil. There was a good correlation between the oils showing antioxidant activity and anti-acne activity. Those showing high antioxidant property showed highest inhibitory zone against *P.acnes*.

The oils namely lemongrass, lemon oil and gingergrass oil which exhibited higher inhibitory effect against *Propionibacterium acnes* and anti-oxidant activity in the study can be useful inclusion into the cosmetic formulation or skin care products. It can thus be concluded from

present study that natural actives have a better if not comparable inhibitory action against acne causing bacteria when compared with known synthetic antibacterial agent. Skin irritation tests are necessary for their safety use and *in vivo* model study is necessary for their efficacy.

V. ACKNOWLEDGMENT

The authors would like to thanks the Management and Principal, Mount Carmel College for providing necessary facilities to conduct this research work.

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