

A Study of Antibacterial Effect of Some Selected Essential Oils and Medicinal Herbs Against Acne Causing Bacteria

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ABSTRACT: Acne by definition is multifactorial chronic inflammatory disease of pilosebaceous units. *Propionibacterium acne* and *Staphylococcus epidermidis* are considered as the major skin bacteria that cause the formation of acne. Acne is one of the commonest skin disorders mainly affecting adolescents, (though it may present at any age) and though there are wide arena of anti acne agents in use, dermatologists are still struggling since years to treat it successfully. The aim of the present study was to investigate and evaluate anti acne efficacy of various essential oils and herbal extracts which possess antibacterial activity against the above bacteria. In the present study, 3 essential oils and two extracts were chosen and their Zone of inhibition, Activity index, Percent Inhibition was measured and compared against a known synthetic standard. Amongst the selected active materials, Cinnamon oil showed highest activity while Rosemary oil showed least.

Keywords—Acne, Antibacterial activity, Essential oil, Herbal extracts, *Propionibacterium acne*, *Staphylococcus epidermis*.

I. INTRODUCTION

Acne vulgaris is one of the commonest skin disorders, for which dermatologists are still struggling since years to treat successfully. It mainly affects adolescents, though it may present at any age.^[1] It is almost a universal disease occurring in all races and affecting 95 % of 16 year old boys and 83% of 16 year old girls to some degree. The incidence of severity of acne, peak at 40% in 14-17 year old girls and 35% in boys aged 16-19 year.^[2]

Acne by definition is multifactorial chronic inflammatory disease of pilosebaceous units^[3]. It affects the skin of the face, neck and upper trunk. These particular sebaceous follicles have capacious follicular channels and voluminous, multiacinar sebaceous glands. Acne develops when these specialized follicles undergo pathologic alteration that results in the formation of non inflammatory lesions (comedons) and inflammatory lesions (papules, pustules and nodules).^[4] *Staphylococcus epidermidis* (*S. epidermidis*) and *Propionibacterium acne* (*P. acne*) are considered as the major skin bacteria that cause the formation of acne. *P. acne*, a gram-positive anaerobic pathogen, plays an important role in the pathogenesis of acne.

It is implicated in the development of inflammatory acne by its capability to activate complements and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils^[5] *S. epidermidis*, an aerobic organism, is usually involved in superficial infections within the sebaceous unit.^[6] Modern acne therapy has been designed to interrupt the pathogenic pathway at one or more points. Topical or Systemic therapy is available for the treatment of acne, which includes comedolytic agents and antibiotics and various anti-inflammatory drugs and Systemic therapy includes antibiotics, zinc and hormones. The excessive use of antibiotics for long periods has lead to increased resistance in acne causing bacteria i.e. *P.acne* and *S. epidermidis* against a number of antibiotics used to treat acne.^[7]

To overcome the problem of antibiotic resistance, essential oils and medicinal plant extracts have been extensively studied as an alternative. Herbs are safe, efficacious and multifunctional. The ingredients in topical acne treatments, particularly herbs and naturally derived compounds have received considerable interest as they have fewer adverse effects than synthetic agents.^[8]

In the present study, 3 essential oils (Cinnamon Oil, Rosemary Essential Oil and Tea Tree oil) and 2 herbal extracts (Neem Extract and Daru Haldi extract) which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against *P. acne* and *S. epidermidis*. A

comparative study was also done for each material against a known synthetic anti-acne agent as Standard i.e Clindamycin.

1.1 Objectives:

1. To evaluate the in- vitro antibacterial activity of some selected essential oils and herbal extracts against *P. acne* and *S. epidermidis*.
2. To compare the antibacterial activity between each active material against a known synthetic anti-acne agent as Standard (Clindamycin).

II. MATERIALS AND METHODS

2.1 Selection of actives:

1) Cinnamon Oil:



Fig.1 Cinnamon Bark (*Cinnamomum zeylanicum*)^[9]

Cinnamon consists of the dried inner bark of the shoots of coppiced trees of *Cinnamomum zeylanicum* Nees (Syn. *Cinnamomum verum*) belonging to family Lauraceae.^[10]

It is native to tropical areas, including Sri Lanka, Southeastern India, South America, and the Caribbean. Cinnamon has been used as both a spice and a medicine since roughly 2000 B.C., and plays an important role in traditional Chinese medicine, where it is known as *gui zhi*.^[11]

Parts Used: Bark, Leaves.

Chemical constituents:

Cinnamon bark contains 0.5 to 1.0% of volatile oil, 1.2% of tannins, mucilage, calcium oxalate, starch and the sweet substance known mannitol. The volatile oil is the active constituent of the drug. It is light yellow (when freshly distilled) in color and changes to red on storage. It contains 60-70% of cinnamaldehyde, 5-10 % eugenol, benzaldehyde, cuminaldehyde and other terpenes like phellandre, pinene, caryophyllene, etc.^[10].

Properties:

Cinnamon has anti-inflammatory and antibacterial properties helpful for treating acne.^[11] Cinnamon is known to possess anti fungal, anti microbial and clotting properties. It has been shown to suppress *E.coli*, *Staphylococcus* and *Candida albican*. Cinnamon can kill bacteria as well as fungi and viruses.^[12]

Procurement of sample: Pure cinnamon essential oil was purchased from the supplier Katyani exports, Mumbai.

2) Rosemary essential oil



Fig.2: Rosemary Plant (*Rosmarinus officinalis*)^[13]

It is the volatile oil obtained from fresh flowering tops of plant *Rosmarinus officinalis* Linn. From family Labiatae. Native to Mediterranean, main oil producing countries are France, Spain and Tunisia. ^[14]

Parts Used: Flowers and leaves

Chemical constituents: The flowers contain about 1.0% of volatile oil, resin, ursolic acid and bitter principle. While leaves, in addition to oil, also contain tannins. Volatile oil contains mainly 10-15% of borneol, 2.5 - 3% of bornyl acetate, camphor, eucalyptol, pinene, d-camphene, cineol and 45% of terpenes. ^[15]

Properties

Rosemary oil possesses analgesic, anti microbial, anti oxidant, antiseptic, fungicidal and astringent properties. It is useful for treating Acne, Dermatitis, Eczema, etc. ^[15]

Procurement of sample: Blossom Kocher's Aroma Rosemary essential oil was used for the study.

3) Tea tree oil:



Fig.3: Tea tree leaves (*Melaleuca alternifolia*) ^[16]

It is taken from the leaves of the Australian native plant *Melaleuca alternifolia* from family Myrtaceae, native to Australia. ^[17]

Parts Used: Leaves

Chemical Constituents

Among over 98 compounds contained in the oil, terpinen-4-ol is responsible for most of the antimicrobial activity. ^[17] Other compounds present are - Terpinene 10-28%, α terpinene 5-13%, 1 δ cineole, α terpinolene 1.5-5%, α -terpineol 1.5-8%, α -pinene 1-6%, p-cymene 0.5-8%. ^[18]

Properties

Tea Tree oil has anti microbial, anti inflammatory, anti infectious, anti septic, anti viral and bactericidal properties. Useful to treat Abscess, Acne, Blisters, Insect bites, Rashes, Wounds etc. ^[19]

The components of the oil penetrate deep into skin and support regeneration of skin damaged by skin diseases caused by germs, fungus and acne causing bacteria. ^[20]

Procurement of sample: Tea Tree essential oil used for study was purchased from the supplier Ghaziabad aromatics, Ghaziabad.

4) Neem



Fig.4: Neem leaves (*Azadirachta indica*)

It consists of all aerial parts of plant known as *Azadirachta indica*, family *Meliaceae*.^[21]
It is a native of India, Myanmar and Japan.

Parts Used: Leaves Bark.

Chemical constituents:

It contains diterpenes - stigmasterol, triterpenes, number of cyclic tri and tetrasulphides. Nimbidin Margolone, margolonone and isomargolonone are the main constituents of Neem leaves.^[22]

Properties: Neem leaves have known to possess diverse pharmacological properties like anti inflammatory, anti pyretic and anti microbial.^[23]

Preparation of extract: Hydro-alcoholic Extract was prepared in house by the following method- 10 gm of fresh Neem leaves (dried in oven) were taken and refluxed in round bottom flask using reflux condenser for 3 hrs using water and ethanol mixture as a solvent in 7:3 ratio. After cooling, the extract was filtered and used for further study.

5) Daru Haldi



Fig.5: Daru Haldi (*Berberis aristata*)

Berberis aristata DC, Family *Berberidaceae*. Commonly known as Daru Haldi, This shrub is found growing wild in the Sub-Himalayan tract. It also grows in the Nilgiris and in Ceylon^[24].

Parts Used: Bark, Fruit, Root, Stem and Wood.

Chemical constituents:

The plant contains berberine, oxyberberine, berbamine, aromoline, karachine, palmatine, oxyacanthine and taxilamine. *Berberis aristata* contains protoberberine and bis isoquinoline type of alkaloid. Root of plant *Berberis aristata* contains alkaloid which are berbamine, Berberine, oxycanthine, epiberberine, palmatine, dehydrocaroline, jatrorrhizine and columbamine, 5, 6 karachine, 7 dihydrokarachine, taximaline, 8 oxyberberine, aromoline.^[24]

Properties

The major alkaloid berberine responsible for antimicrobial activity. It also acts as anti inflammatory, wound healing and anti oxidant.^[24, 25]

Preparation of extract

Hydro-alcoholic Extract was prepared in house by the following method- 20 gm of dried stems of Daru Haldi were taken, grinded coarsely and were refluxed in round bottom flask and reflux condenser for 3hrs using ethanol and water mixture as a solvent in 7:3 ratio. After cooling, the extract was filtered and was used for further study.

2. 2 Clindamycin

Clindamycin is in a class of medications called lincomycin antibiotics^[26].

Properties: Treatment of acne vulgaris with clindamycin inhibits the growth of *P. acnes*, normalizes desquamation, prevents follicular plugging, and may provide anti-inflammatory effects. It possesses broad range of anti-inflammatory properties.^[27]

It was taken as a standard for comparison with essential oils and herbal extracts.

2. 3 Microorganism and media:

- The test organism used in study was *P. acne* (MTCC No.1951) which was purchased from MTCC Chandigarh and *S. epidermidis* (ATCC No.12228) which was purchased from National Chemical Laboratory, Pune, India.

- All media used were purchased from Hi-media Laboratories.

2. 4 Antibacterial Assay

‘Well Diffusion Method’ was adopted for evaluation of antimicrobial activity:

- *P. acne* was incubated in brain heart infusion medium (BHI) with 1% glucose for 48 hrs under anaerobic conditions and adjusted to yield approximately 1.0×10^8 CFU/ml. Aliquots of molten BHI with glucose agar were used as the agar base.
- A prepared inoculum was added to the molten agar, mixed and poured over the surface of the agar base and left to solidify.
- *P. acne* inoculum was spread into a sterile petriplate with the help of sterile swab stick.
- A well approximately 9 mm in diameter was bored on the surface of agar medium using a sterile cork borer.
- Then the sample was introduced into the well. Clindamycin ($10 \mu\text{g/ml}$) was used as the standard.
- The plates were then incubated at 37°C for 48 hrs under anaerobic conditions in an anaerobic jar (Hi-Media) with gas pack and indicator tablet and the jar was kept in an incubator for 48 h at $35.5 \pm 1^\circ\text{C}$.
- Anaerobic gas pack - a disposable oxygen absorbing and carbon dioxide generating agent for use in anaerobic jar was used to maintain and check the anerobiosis. Anaerobic tablet having pink color was used, on introduction into the jar color remains pink indicating anaerobic conditions (original pink color if change to purplish-blue indicating aerobic condition because of the absorption of oxygen).^[28] (Refer Fig.6)



Fig. 6: Anaerobic Chamber

- *S. epidermidis* was incubated in soybean casein digest medium (TSB) for 24 hrs at 37°C and adjusted to yield approximately 1.0×10^8 CFU/ml. The procedure was the same as performed above, except the plates were incubated at 37°C for 24 hrs under aerobic conditions.
- All the Well Diffusion tests were performed in duplicate and antibacterial activity was expressed as the mean of inhibition diameters (mm). (Refer TABLE 1).

2. 5 Measurement of zone of inhibition

The zone of inhibition for each sample was observed, measured and expressed in mm. From this the activity index (A.I.) and Percent Inhibition (P.I.) were calculated for all oils and extracts using the following formula.^[29] (Refer TABLE 1).

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

P.I. = Activity index x 100

Table no 1: Zone of inhibition, Activity Index, Percent Inhibition Of essential oils against *P. acne* & *S. epidermidis* after 24hrs.

Sr . no .	Name of Sample	<i>Propionibacterium acne</i>					<i>Staphylococcus epidermidis</i>				
		Zone of inhibitions in mm		Mean (2)	Activity Index	% inhibition	Zone of inhibitions in mm		Mean (2)	Activity index	% inhibition
1	Cinnamon oil	32	33	32.5	1.75	175	31.5	35	33.2	1.68	168
2	Tea tree oil	18	17.5	17.75	0.95	95	19	18.5	18.75	0.94	94
3	Rosemary oil	12	13.5	12.75	0.68	68	14.75	15	13.5	0.68	68
4	<i>Berberis aristata</i>	18.5	19	18.75	1.01	101	19.5	20.2	19.85	1.00	100
5	Neem extract	15	15.2	15.1	0.81	81	16	16.5	16.25	0.82	82
6	Clindamycin	18	19	18.5	-		20	19.5	19.75	-	

Following table shows values to evaluate the bacterial response to each compound.

Table no.2: Values to evaluate the bacterial response to each compound^[30]

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

3. FIGURES



Fig 7: Zone of inhibition of Cinnamon oil against *S. epidermidis*

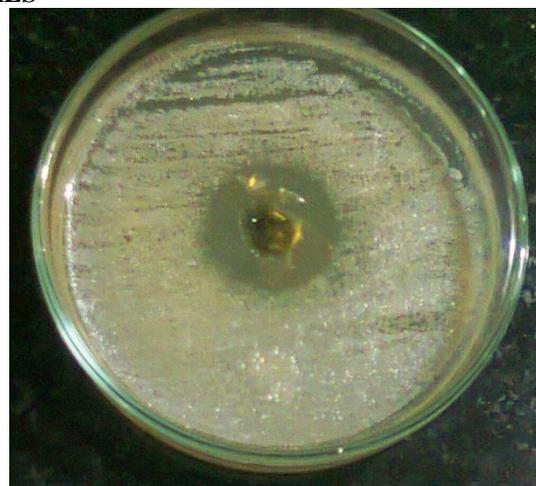


Fig 8: Zone of inhibition of Cinnamon oil against *P. acne*



Fig 9: Zone of inhibition of Daru Haldi extract against *S. epidermidis*



Fig 10: Zone of inhibition of Daru Haldi extract against *P. acne*

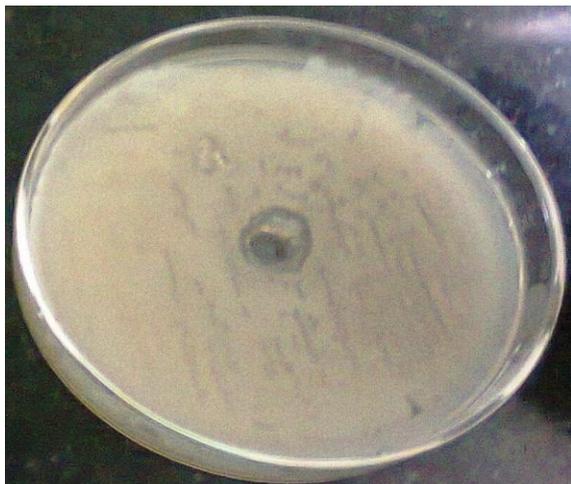


Fig 11: Zone of inhibition of Clindamycin against *S. epidermidis*



Fig 12: Zone of inhibition of Clindamycin against *P. acne*

IV. RESULT

- In the present study 3 essential oils and 2 extracts were examined for antimicrobial activity against *P. acne* and *S. epidermidis*.
- Amongst the 3 essential oils, results showed that Zone of Inhibition of Cinnamon oil was found to be highest against *S. epidermidis* and *P. acne* followed by Tea tree oil and Rosemary oil when compared with standard i.e. Clindamycin. This indicates that Rosemary oil shows least antibacterial activity.
- In case of extracts, Daru Haldi extract showed highest Zone of Inhibition as compared with Neem extract and Standard. (Refer Fig.7 to12)
- The Activity index and percent inhibition was highest in Cinnamon oil and lowest in Rosemary oil.

V. DISCUSSION

Acne can have important negative psychosocial consequences for the affected individual, like diminished self-esteem, social withdrawal due to embarrassment and depression. Herbal medication are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, etc. [31] The present research work deals with evaluation of antibacterial activity of 3 essential oils and 2 extracts against acne causing bacteria. Cinnamon bark oil, Tea tree oil, Rosemary oil and hydro alcoholic extract of Daru Haldi and Neem leaves were used. Zone of inhibition, Activity index and Percent inhibition was calculated and results were noted. It was found that Cinnamon oil showed maximum Antibacterial activity while Rosemary oil showed least. Similarly amongst the extracts Daru Haldi extract showed higher inhibition than Neem extract.

VI. CONCLUSION

The Cinnamon bark oil, Berberis aristata extract, Tea tree oil and Neem extract were found to be susceptible against the bacteria *P. acne* and *S. epidermidis* while Rosemary oil gave an intermediate effect against both the bacteria when compared with the values given in Table no. 2.

It was thus be concluded from above study that natural actives have a better if not comparable inhibitory action against acne causing bacteria when compared with known synthetic antibacterial agent. Amongst the actives selected, the order of activity was found as follows-

For Oils – Cinnamon oil > Tea tree oil> Rosemary oil

For Extracts - Daru Haldi extract > Neem extract

For All Actives– Cinnamon oil > Daru Haldi extract> Tea tree oil > Neem extract> Rosemary oil.

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