

Original Research Article

SENSITIVITY OF *PROPIONIBACTERIUM ACNES* TOWARDS COMMERCIAL ANTI-ACNE FORMULATIONS

Comment [PL1]: The usage of English is not really acceptable in this manuscript's current state, grammatical errors are found throughout this manuscript, and these errors can sometimes cause misunderstanding. I have corrected some but not all of the English in the manuscript. The authors should check again on the use of English in the manuscript.

Abstract

Propionibacterium acnes is a gram-positive anaerobe found prevalently in the sebum-rich follicles of the skin. They produce several pro-inflammatory substances that can trigger an immune response in the host by an influx of inflammatory leukocytes into the follicles, causing inflammatory lesions that leave behind scars. Repeated isolation of *Propionibacterium acnes* may reduce efficacy among the resistant types which clearly explains the importance of Acne lesions. The Counter acne therapies are often the first choice of treatment due to the convenience of cost and time ~~thanover~~ clinical appointments. However, not all of the commercially available anti-acne formulations are supported by clinical studies. The present study was conducted to test the efficacy of selected commercial anti-acne gel formulations. Microscopic observation and biochemical tests were performed and confirmation results were obtained. A sensitivity test was performed on all the isolates of *Propionibacterium acnes* by well diffusion technique, in which the selected over-~~the~~-counter anti-acne gel formulations failed to produce any inhibition zone.

Formatted: Font: Italic

Comment [PL2]: Please change into: "over-the-counter acne products"

Comment [PL3]: Confirmation results of what? Please rephrase the sentence.

Formatted: Font: Italic

Keywords: *Acne Vulgaris, Propionibacterium, facial acne lesions, Sensitivity test*

Introduction

Acne vulgaris is a common skin disorder of the pilosebaceous unit, with the severity

ranging from mild to chronic. The condition can most commonly be seen in 80% of adolescents and young adults (Khorvashet *al.*, 2012). Human skin ~~in is~~ one of the biggest organs present across the body, which consists of various tiny microorganisms ~~that are associated with skin, includes including~~ *Malassezia*, *Propionibacterium*, *Corynebacterium*, *Streptococcus*, and *Staphylococcus*. ~~Out of which~~ The most common human skin microbiota is a gram-positive anaerobic bacteria called *Propionibacterium acnes* (*P. acnes*) ~~a common human skin microbiota and also which controls colonizes~~ the pilosebaceous. The uniqueness of this propioni-bacteria is that they are capable in of maintaining skin through environmental niches which occupied by various pathogenic microbes. They produce bacteriocins, ~~short short~~-chain fatty acids, thiopeptides, and a few other molecules ~~which have a capability that are capable~~ of inhibiting ~~various other~~ organisms (Pochiet *al.*, 1988). *P. acnes* ~~P. aenes~~ and *P. granulosum* are commonly found in sebaceous gland-rich areas of skin while *P. acnes* can also ~~see be seen~~ in other parts of the body such as the gastrointestinal system, prostate, and ~~also found on the surface of~~ surface. The propioni-bacteria provide a support and maintain the microbial balance in the skin but they ~~are not so beneficial which~~ may also cause diseases ~~with under~~ improper ~~set of~~ conditions. The ~~disorder disease~~ in some cases may leaves permanent scars on the skin diminishing which causes negative effects on psychological and social well-being ~~leads to negative effects~~ in young adolescents such as discomfort, emotional stress (Fabbrocini *et al.*, 2010) anxiety, and embarrassment (Purvis *et al.*, 2006). In acne-prone skin, hyper-proliferation of the keratinocytes occurs and the abnormally desquamated corneocytes accumulate in the sebaceous follicle along with other lipids and debris, which blocks the follicle, and hence a non-inflammatory micro papule is formed. The pathogenesis of acne is multifactorial and the four main pathological factors involved ~~are increased which~~ includes sebum production, epidermal hyper-proliferation, irregular follicular desquamation, and bacterial proliferation and

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Comment [PL4]: This sentence is not understandable in English. Please rephrase it.

inflammation (Dessiniotiet *al.*, 2010; Roselin *et al.*, 2010). The microflora present in a normal sebaceous follicle is qualitatively similar to that found in papules which includes three coexisting groups of bacteria namely coagulase-negative staphylococci, anaerobic diphtheroids, and lipophilic yeasts. The main goal of acne treatment is to control existing acne lesions, permanent scarring, limit the duration of the disorder, and minimize morbidity. A combination treatment that targets more than one of the mechanisms of acne pathogenesis is often successful. Few studies suggest the non-antibiotic agents are used for the treatment of mild to moderate acne which can be used as monotherapy or in combination with antibiotics to enhance the efficacy of treatment and also reduce the development of antibiotic resistance in *P. acnes*. Combined agents are found to be more effective, due to the synergistic effect; these combinations show antibacterial resistance in *P. acnes* and are much more effective in combination when they are used individually. Combination exerts bactericidal effects which are capable ~~in~~ of decreasing ~~in~~ *P. acnes* counts (Alexeyev *et al.*, 2012). Prolonged use ~~age~~ of antibiotics, especially by topical application, ~~in particular~~ results in the development of *P. acnes* resistant strains (Achermann *et al.*, 2014; Ramasamy *et al.*, 2019). Among various antibiotics over the counter (OTC) anti-acne formulations consist of antibiotics and non-antibiotics either as monotherapy or most often in combination, designed to target at least one of the pathogenic pathways that are reported to be involved in the development of acne. Similarly in the present study, we tried to ~~check~~ examine the efficacy of Commercial Anti-Acne formulations against *propionibacterium acnes*.

Comment [PL5]: This sentence needs a reference.

Comment [PL6]: This sentence is also not understandable in English. Please rephrase it.

Materials and Methods

Isolation of *P. acnes* aerobically

~~The~~ *P. acnes* was isolated from acne lesions. Three samples were randomly collected from ~~a~~ volunteers between the age group of 18 - 21 years olds. The samples were collected using a sterile Himedia swabs and were stored in a brain heart infusion broth

Comment [PL7]: Was these "healthy volunteers" or "patients"?

(BHI) and Nutrient broth (NB). 1 cm² area from the facial skin from three volunteers was smeared with sterile swabs and were stored in a test tube containing 10 ml of nutrient broth (NB) and was incubated ~~from~~ for 4 days in Anaero Gas Pak. The incubated samples were later streaked on nutrient agar and incubated at 37°C for 4 days. The obtained colony morphology was observed and stained using gram staining. (Hug *et al.*, 1999).

Staining and bacterial observation

Gram stain was performed as described previously with slight modifications. ~~From the collected samples, a~~ loop full of the samples was smeared on clean glass slides, air-dried, and heat-fixed. Crystal violet was added ~~on~~ to the samples and incubated for 5 min at room temperature. After incubation, the glass slides were gently rinsed under tap water in order to remove excess of crystal violet. ~~Further~~ Gram iodine ~~were was~~ then added and kept ~~it~~ for 2 min ~~and washed before washing~~ with tap water. The grams decolorizer was added in order to remove excess of crystal violet stain for about 30s and quickly rinsed under tap water. ~~The~~ A drop of Safranin stain was added and kept ~~it~~ for a 1 minute ~~and~~, followed by dehydration using 70% ethanol and coverslip were placed. (Bisenet *al.*, 2014; Abiola *et al.*, 2016).

Isolation and purification of *P.acnes* colony

In order to isolate the *P.acnes* bacteria from a cluster of bacteria's, 1 ml of culture nutrient broth ~~were was~~ spread on nutrient agar plates. The collected samples were serially diluted, 1 ml of 5-fold serial diluted samples were spread on nutrient agar plates. The culture plates were incubated at 37°C whereas mFC agar were incubated at 45°C for about 24 hours. The obtained colonies were further counted, characterized, and recorded. ~~Colonies were purified by twice subculturing using the streaking plate method.~~ The obtained colonies were further purified by repeated subculturing using the streak plate technique. The cultures were subjected to gram staining and were identified as ~~gram~~ gram-positive *P.acnes*. Further, the isolated bacteria were subjected to

Comment [PL8]: Did you use two different agar plates? Please identify "nutrient agar plates", "culture plates", and "mFC agar". Are these term all suggested the same agar?

biochemical identification tests.

Biochemical characterization of *P.acnes*

Catalase test

A loop of the colony was smeared on a clean glass slide and a few drops of 3% hydrogen peroxide were added. The production of air bubbles indicates the ~~present~~ presence of catalase and no air bubble indicates the absence of catalase. (Bisenet *et al.*, 2014).

Indole test

Indole test is used to determine the presence of *P.acnes*. The test organism was ~~cultures~~ cultured on Tryptone broth media ~~cultured in a~~ bijou bottle and incubated at 37°C for four days. To the media 0.5 ml Kovac's reagent ~~were was~~ added and gently shaken ~~and~~ until the obtained colored ring was observed (Abiola *et al.*, 2016).

Nitrate test

Nitrate broth is prepared and inverted Durham's tube is added into the medium without any appearance of air bubbles, and then a loop of the colony was inoculated into the medium and incubated at 37°C for four days. To the culture tube ~~add~~ 2 to 3 drops of nitrite reagent A and B ~~were added~~ and the reaction culture was observed (Moss *et al.*, 1967).

Sugar fermentation test

~~Purple~~ Two purple base broth was prepared ~~with an and added to two test tubes with~~ an inverted Durham's tube ~~added~~ without the appearance of air bubbles, one of the tubes is marked as control. A loop of the colony was inoculated into the medium and incubated at 37°C for about 24 hours and ~~the obtained a~~ yellow color ~~conforms confirms~~ the positive results of sugar fermentation test (Moss *et al.*, 1967).

Hemolytic test

To 1.25 ml of 5% defibrinated sheep blood was added and mixed, the prepared medium was poured into a Petri plate and allowed to solidify, after which the culture was

Comment [PL9]: This sentence is not understandable. What is added? And added to what?

inoculated on the medium by spread plate technique and kept for incubation at 37°C for four days (Moss *et al.*, 1967; Bakht *et al.*, 2011)

Gelatin hydrolysis

From the culture test bacterial plates, a loop of colony was stabbed into the gelatin media using a streaked as a single line and incubated at 37°C for 24 hours. To the plate an iodine solution ~~were~~was added to check the starch utilization (Moss *et al.*, 1967).

Methyl red test

The test organisms were culture ~~of in~~in MR broth and incubate at 37°C for about 48 hours. After incubation 1 ml of broth was transferredred into two test tubes, where one of the tubes is used as control. To these tubes 2 to 3 drops of methyl red ~~was~~were added, the formation of red color indicates the presence of positive methyl red test whereas yellow color indicates the negative results of methyl red test. (Abiola *et al.*, 2016)

Antimicrobial activity by well diffusion method

50 µl of bacterial samples were pipetted onto two solidified brain heart infusion agar plates and spread evenly on the surface using a glass rod until completely absorbed by the media. The two agar plates were then labeled as plate 1 and 2, each of which was divided and marked as four quadrants namely A, B, positive control (PC), and anti-acne gel (CI). Four wells were then made in the four quadrants of each plate using a cork borer (Valgaset *et al.*, 2007; Magaldiet *et al.*, 2004; Bakht *et al.*, 2011). Ampicillin was used as the positive control and therefore 200 µl of Ampicillin was pipetted into the (PC) labeled well of the two plates. 200 µl of ~~Distilled~~distilled water used for the preparation of stock solution was ~~poured~~added into well (A) as a negative control in both ~~the~~ plates. 200 µl of anti-acne gels from both the stock solutions C1 and C2 was added to well (CI) and well (B) of plate 1, and stock solutions B1 and B2 were pipetted into the wells marked as (CI) and B of plate 2 in the respective order. The plates were then ~~kept~~kept ~~for incubation~~ed at 37°C for 24 hours (Bakht *et al.*, 2011; Holder *et al.*, 1994).

Results and Discussion

Comment [PL10]: What anti-acne gels were used in this study? This should be stated in the Material and Methods section.

Comment [PL11]: What is stock solution C1 and C2? Do they mean different anti-acne gels? Please explain it clearly.

Comment [PL12]: Again, what is stock solution B1 and B2?

Isolation and culture of *P. acnes*

Propionibacterium acnes was collected from a surface swab of facial acne skin lesions and suspended in a nutrient broth; post aerobic incubation growth was seen by the appearance of biofilms; ~~and also the~~ turbidity was also found at the bottom of the tube which ~~conforms~~ confirms the presence of *P. acnes*.

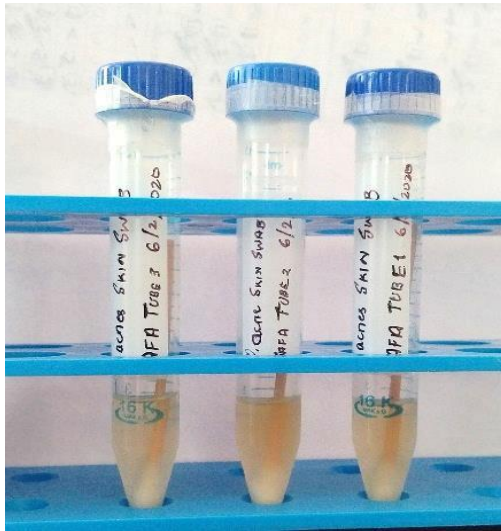


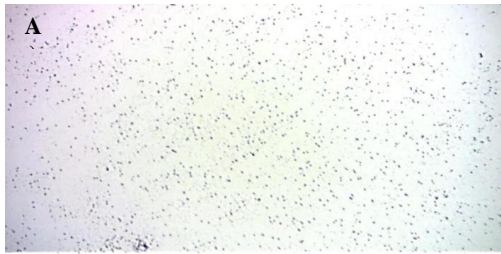
Figure 1: Formation of biofilms ~~conforms~~ confirms the presence of *P. acnes* after postincubation

Gram staining

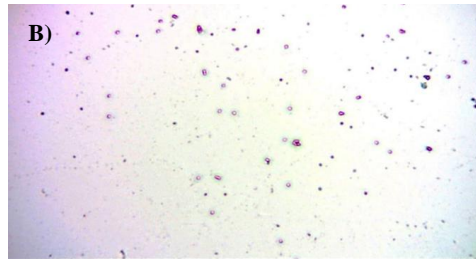
The obtained isolates were further examined using gram staining. Through this staining technique, it ~~conforms~~ confirms that the isolate consists of numerous gram-positive bacteria. The isolated Gram-positive bacteria were stained namely staphylococci, diplococci, tetrads, and streptococci were conformed under the magnification of 10x and 40x

Comment [PL13]: The definition of the word “confirm” and “conform” is different. Please use it carefully.

Comment [PL14]: This section is redundant to the study. Please remove this section, or put only the *P. acnes* results.



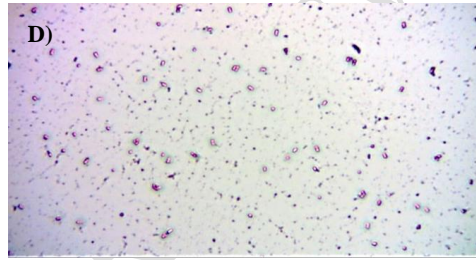
Staphylococci (10x)



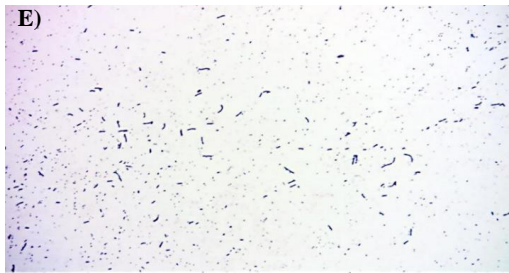
Staphylococci (40x)



Diplococci and tetrads (10x)



Diplococci and tetrads (40x)



Streptococci(10x)



Streptococci (40x)

Figure 2: Obtained gram-positive bacteria were stained namely *staphylococci*, *diplococci*, *tetrads*, and *streptococci*, where, figure A,C,E were observed under the magnification of 10x and figure B,D,F were observed under the magnification of 40x

Serial dilution

In order to obtain pure culture of *Propionibacterium acnes*, serial dilution was carried out using the spread plate method, and the obtained colonies were further

characterized and ~~conforms~~ confirms the presence of *P.acnes*. In the present study the obtained bacterial colonies were subcultured d and serially diluted in order to obtained a pure culture from the bulk samples. The samples were serially diluted ranging from 10⁻¹ to 10⁻⁵ and were ~~showed in~~ shown in table 1

Table 1: ~~Characteristics of the Sserially diluted bacterial colonies~~ Characteristics

Comment [PL15]: I don't think this table is needed to put in the manuscript. Please consider to remove this.

Dilution factor	Total No. of colonies	Colony forming unit (CFU)	Colony number	Form	Elevation	Margin	Color
10-1	638	638 x 10 ⁻¹	1	Irregular	Crateriform	Filamentous	Pale White
10-2	5	5 x 10 ⁻²	1	Circular	Convex	Entire	Yellow
10-3	27	27 x 10 ⁻³	1	Irregular	Convex	Filamentous	Pale White
10-4	0	0 x 10 ⁻⁴	-	-	-	-	-
10-5	3	3 x 10 ⁻⁵	1	Irregular	Crateriform	Filamentous	Pale White

Culture isolation and Purification of *P. acnes*

The serially diluted samples were further inoculated on nutrient agar plates using the streak plate method. The dilution was repeated for several times and the pure culture colony of *P. acnes* was further conformed using gram staining and biochemical characterization. The morphology, elevation, margin, and color conform the presence of *P. acnes* bacteria.

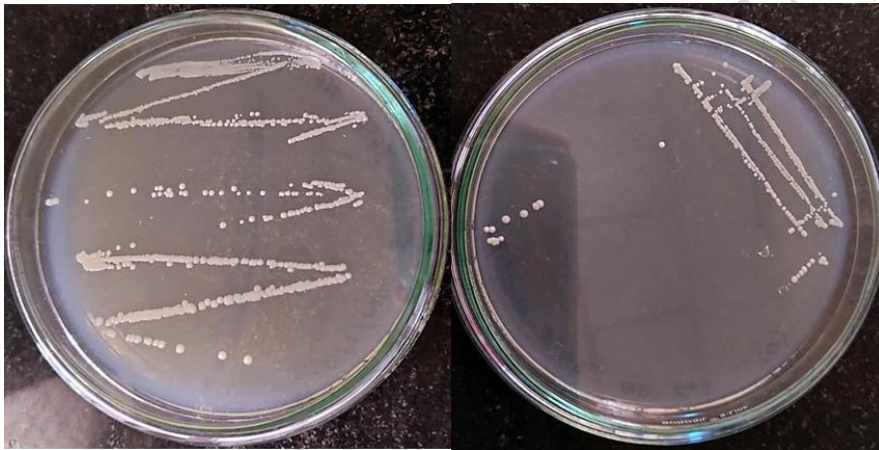


Figure 3: Sticking plate of *P. acnes* pure culture on brain heart infusion agar plate

Table2: Characteristics of the Isolated-purified culture of *P. acnes* colonies characters

Formatted: Font: Italic

Form	Elevation	Margin	Color
Circular	Convex	entire	white

Microscopic observation of *P.acnes* using Gram staining

The colonies stained by Gram staining were observed as Gram-positive bacilli.



Figure4: Gram-positive *P. acnes* were observed under the magnification of 40x

Biochemical characterization for *P.acnes*

The obtained bacterial colonies were further characterized and conforms the presence of *P.acnes* by biochemical analysis as described below.

Table 3: Biochemical characterization of *P. acnes*

No. of tests	Biochemical test	<i>P. acnes</i> result
1	Catalase test	+
2	Indole test	+
3	Nitrate reduction test	+

4	Sugar fermentation test	+
5	Hemolytic test	+
6	Gelatin hydrolysis test	+
7	Methyl red test	+

Antimicrobial activity by well diffusion method

The culture was further confirmed positive with biochemical tests characteristic of *Propionibacterium acnes*. The sensitivity of the isolated *Propionibacterium acnes* to commercial anti-acne gels was tested by a well diffusion method. The two selected anti-acne gels, namely 'Clindamycin phosphate Cleargel' and 'Biotique bio chlorophyll anti-acne gel' failed to produce any inhibition zones. The inhibitory zone of size 30mm (3cm) was seen only around well (PC) in both the plates and no inhibition zone was seen around well A, B, or C in either of the plates.

Formatted: Font: Italic

Comment [PL16]: 1. Was the experiment performed only once?

2. The authors collected 3 *P. acnes* samples from the volunteers. Was the experiment performed on all three *P. acnes* isolates?

Comment [PL17]: Please label the figure, which well is A, B, C1, and PC? Which is plate 1 and which is plate 2?

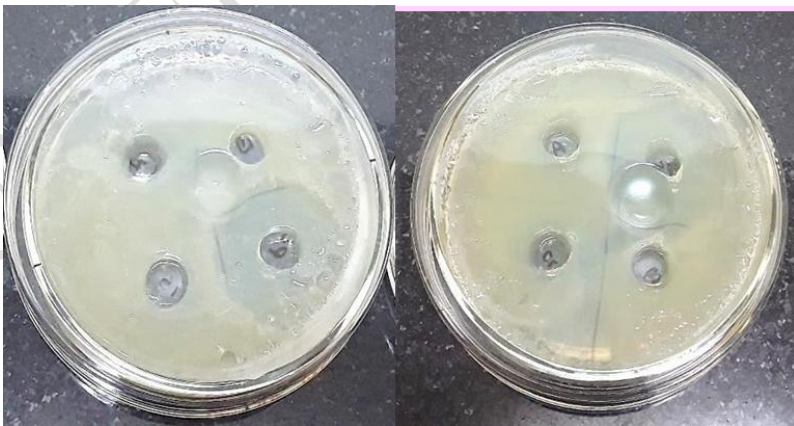


Figure 4: Antimicrobial activity of *P. acnes* against the selected drugs

Conclusion

The limited presence of clinically supported over-the-counter topical anti-acne treatments makes it difficult for the consumer to find an effective treatment from a wide range of products. These treatments are mainly designed to target the reduction in bacterial colonization of the skin to reduce inflammation induced by the organism. The most probable organism among the skin commensal that can proliferate in the anaerobic condition of the plugged follicle is *Propionibacterium acnes*, making it the most efficient target of topical anti-acne treatments. Antibiotics like macrolides, tetracyclines, and antimicrobial non-antibiotic agents like benzoyl peroxide and zinc that can inhibit *Propionibacterium acnes* are most commonly used.

The colonies conform the shape of cocci by staining and microscopy observation.

Propionibacterium acnes was then isolated anaerobically by taking a facial skin swab of acne lesion, in a brain heart infusion broth using an Anaerobic Gas Pak jar. After incubation for 4 days, a sample from the broth was stained and observed microscopically as Gram-positive bacilli. The culture was further confirmed positive with biochemical tests characteristic of *Propionibacterium acnes*. The sensitivity of the

isolated *Propionibacterium acnes* to commercial anti-acne gels was tested by a well

diffusion method. The two selected anti-acne gels, namely 'Clindamycin phosphate Cleargel' and 'Biotique bio chlorophyll anti-acne gel' failed to produce any inhibition

zones. It was concluded that Over the Counter 'Clindamycin phosphate Cleargel' and

'Biotique bio chlorophyll anti-acne gel' was unable to inhibit the growth of isolated *Propionibacterium acnes*. Further susceptibility tests of *Propionibacterium acnes* towards

other Over Counter anti-acne gels containing different macrolides and tetracyclines as monotherapy or in combination with non-antibiotic agents like benzoyl peroxide will be

Comment [PL18]: What bacteria are you referring to? *P. acnes* is rod-shaped.

Formatted: Font: Italic

Comment [PL19]: The authors should also discuss about the following:

1. Do these products contain antibiotics? If so, the authors should discuss why these products failed to inhibit the bacteria.

2. I am also wondering, was the *P. acnes* sample isolated from healthy donors or patients? This may affect the results as the isolates may have very different resistance profiles.

carried out in the future.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

- Abiola C, Oyetayo VO. Isolation and Biochemical Characterization of Microorganisms Associated with the Fermentation of Kersting's groundnut (*Macrotyloma geocarpum*). *Research Journal of Microbiology*. 2016;11(2):47-55.
- Achermann Y, Goldstein E, Coenye T, Shirtliff M. *Propionibacterium acnes*: from Commensal to Opportunistic Biofilm-Associated Implant Pathogen. *Clinical Microbiology Reviews*. 2014;27(3):419-440.
- Adityan B, Kumari R, Thappa DM. Scoring systems in acne vulgaris. *Indian J Dermatol Venereol Leprol* 2009;75:323-6.
- Ajayi AA, Oniha MI, Atolagbe OM, Onibokun EA. Studies on *Staphylococcus aureus* isolated from pimples. *Pakistan Journal of Biological Sciences*. 2017.
- Akaza N, Akamatsu H, Numata S, Yamada S, Yagami A, Nakata S, Matsunaga K. Microorganisms inhabiting follicular contents of facial acne are not only *Propionibacterium* but also *Malassezia* spp. *The Journal of dermatology*. 2016 Aug;43(8):906-11.
- Alexeyev O, Jahns A. Sampling and detection of skin *Propionibacterium acnes*: Current status. *Anaerobe*. 2012;18(5):479-483.

Ali MJ, Obaid RF. Antibacterial Activity for Acne Treatment through Medicinal Plants Extracts: Novel Alternative Therapies for Acne. *J Pure Appl Microbiol.* 2019;13(2):1245-50.

Bakht J, Islam A, Shafi M. Antimicrobial potential of *Eclipta alba* by well diffusion method. *Pak. J. Bot.* 2011 Dec 1;43:161-6.

Balouiri M, Sadiki M, Ibensouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis.* 2016 Apr 1;6(2):71-9.

Bisen PS. *Microbes in Practice.* IK International, New Delhi. 2014:139-155

Christensen GJ, Scholz CF, Enghild J, Rohde H, Kilian M, Thürmer A, Brzuszkiewicz E, Lomholt HB, Brüggemann H. Antagonism between *Staphylococcus epidermidis* and *Propionibacterium acnes* and its genomic basis. *BMC genomics.* 2016 Dec;17(1):152.

Claudel JP, Auffret N, Leccia MT, Poli F, Corvec S, Dréno B. *Staphylococcus epidermidis*: A Potential New Player in the Physiopathology of Acne?. *Dermatology.* 2019;235(4):287-94.

Decker A, Graber EM. Over-the-counter Acne Treatments: A Review. *The Journal of clinical and aesthetic dermatology.* 2012;5(5):32-40.

Dessinioti C, Katsambas AD. The role of *Propionibacterium acnes* in acne pathogenesis: facts and controversies. *Clinics in dermatology.* 2010 Jan 1;28(1):2-7.

EA, Segre JA. The skin microbiome. *Nature Reviews Microbiology.* 2011 Apr;9(4):244-53.

Fabbrocini G, Annunziata MC, D'arco V, De Vita V, Lodi G, Mauriello MC, Pastore F, Monfrecola G. Acne scars: pathogenesis, classification and treatment. *Dermatology research and practice.* 2010;2010.

Fabbrocini G, Annunziata MC, D'arco V, De Vita V, Lodi G, Mauriello MC, Pastore F, Monfrecola G. Acne scars: pathogenesis, classification and treatment. *Dermatology research and practice.* 2010;2010.

Fitz-Gibbon S, Tomida S, Chiu BH, Nguyen L, Du C, Liu M, Elashoff D, Erfe MC, Loncaric A, Kim J, Modlin RL. Propionibacterium acnes strain populations in the human skin microbiome associated with acne. *Journal of investigative dermatology*. 2013 Sep 1;133(9):2152-60.

Fox L, Csongradi C, Aucamp M, Du Plessis J, Gerber M. Treatment modalities for acne. *Molecules*. 2016 Aug;21(8):1063.

Grice EA. The intersection of microbiome and host at the skin interface: genomic-and metagenomic-based insights. *Genome research*. 2015 Oct 1;25(10):1514-20.

Hazarika N. Acne vulgaris: new evidence in pathogenesis and future modalities of treatment. *Journal of Dermatological Treatment*. 2019;;1-9.

Holder IA, Boyce ST. Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns*. 1994 Oct 1;20(5):426-9.

Holland KT, Cunliffe WJ, Eady EA. Intergeneric and intrageneric inhibition between strains of Propionibacterium acnes and micrococcaceae, particularly Staphylococcus epidermidis, isolated from normal skin and acne lesions. *Journal of medical microbiology*. 1979 Feb 1;12(1):71-82.

Hug DH, Dunkerson DD, Hunter JK. The degradation of l-histidine and trans and cis-urocanic acid by bacteria from skin and the role of bacterial cis-urocanic acid isomerase. *Journal of Photochemistry and Photobiology B: Biology*. 1999 May 1;50(1):66-73.

Jelić D, Antolović R. From erythromycin to azithromycin and new potential ribosome-binding antimicrobials. *Antibiotics*. 2016 Sep;5(3):29.

Khorvash F, Abdi F, Kashani HH, Naeini FF, Narimani T. Staphylococcus aureus in acne pathogenesis: a case-control study. *North American journal of medical sciences*. 2012 Nov;4(11):573.

Leyden JJ. Effect of topical benzoyl peroxide/clindamycin versus topical clindamycin

and vehicle in the reduction of *Propionibacterium acnes*. *Cutis*. 2002 Jun 1;69(6):475-80.

Magaldi S, Mata-Essayag S, De Capriles CH, Perez C, Colella MT, Olaizola C, Ontiveros Y. Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases*. 2004 Jan 1;8(1):39-45.

Moss CW, Dowell VR, Lewis VJ, Schekter MA. Cultural characteristics and fatty acid composition of *Corynebacterium acnes*. *Journal of bacteriology*. 1967 Nov 1;94(5):1300-5.

Muizzuddin N, Giacomoni P, Maes D. Acne – a multifaceted problem. *Drug Discovery Today: Disease Mechanisms*. 2008;5(2):e183-e188.

Nishijima S, Kurokawa I, Kawabata S. Sensitivity of *Propionibacterium acnes* isolated from acne patients: comparative study of antimicrobial agents. *Journal of international medical research*. 1996 Nov;24(6):473-7.

Noguera-Julian A, Monsonis M, Ludwig G, Moreno-Romo D, Gené-Giralt A. Osteoarticular infections: Blood as a determinant factor in the isolation of *Kingella kingae*. *Journal of microbiological methods*. 2019 Jun 1;161:8-11.

Perry A, Lambert P. *Propionibacterium acnes*. *Letters in Applied Microbiology*. 2006;42(3):185-188.

Pochi PE. Acne: Androgens and microbiology. *Drug Development Research*. 1988;13(2-3):157-68.

Polugari R, Marla SR, Shailaja D. Isolation and Molecular Characterization of acne causing *Propionibacterium acnes*. *International Journal of Scientific and Research Publications*. 2016 Jun 6(6): 2250-3153.

Puhvel S, Sakamoto M. The Chemoattractant Properties of Comedonal Components. *Journal of Investigative Dermatology*. 1978;71(5):324-329.

PUHVEL S. *Corynebacterium Acnes*. *Archives of Dermatology*. 1966;93(3):364.

Purvis D, Robinson E, Merry S, Watson P. Acne, anxiety, depression and suicide in

teenagers: A cross-sectional survey of New Zealand secondary school students. *Journal of paediatrics and child health*. 2006 Dec;42(12):793-6.

Ramasamy S, Barnard E, Dawson T, Li H. The role of the skin microbiota in acne pathophysiology. *British Journal of Dermatology*. 2019;181(4):691-699.

Roselin P, M.Shailaja R, Dasetty S. Isolation and molecular characterization of acne causing *Propionibacterium acnes*. 2016;6(6):809-814.

Shaheen B, Gonzalez M. A microbial aetiology of acne: what is the evidence?. *British Journal of Dermatology*. 2011 Sep;165(3):474-85.

Swinyer LJ, Baker MD, SWINYER TA, Mills Jr OH. A comparative study of benzoyl peroxide and clindamycin phosphate for treating acne vulgaris. *British*

Tan HH. Topical antibacterial treatments for acne vulgaris. *American journal of clinical dermatology*. 2004 Apr 1;5(2):79-84.

Thiboutot DM. The role of follicular hyperkeratinization in acne. *Journal of dermatological treatment*. 2000 Jan 1;11(2):5-8.

Toombs EL. Cosmetics in the treatment of acne vulgaris. *Dermatologic clinics*. 2005 Jul 1;23(3):575-81.

Tschen EH, Katz HI, Jones TM, Monroe EW, Kraus SJ, Connolly MA, Levy SF. A combination benzoyl peroxide and clindamycin topical gel compared with benzoyl peroxide, clindamycin phosphate, and vehicle in the treatment of acne vulgaris. *Cutis*. 2001 Feb 1;67(2):165-9.

Tucker SB, Tausend R, Cochran R, Flannigan SA. Comparison of topical clindamycin phosphate, benzoyl peroxide, and a combination of the two for the treatment of acne vulgaris. *British Journal of Dermatology*. 1984 Apr;110(4):487-92.

Valgas C, Souza SM, Smânia EF, Smânia Jr A. Screening methods to determine antibacterial activity of natural products. *Brazilian journal of microbiology*. 2007 Jun;38(2):369-80.

Wang Q, Cui S, Zhou L, He K, Song L, Liang H, He C. Effect of cosmetic chemical preservatives on resident flora isolated from healthy facial skin. *J Cosmet Dermatol*. 2019 Apr;18(2):652-658.

Weber N, Biehler K, Schwabe K, Haarhaus B, Quirin KW, Frank U, Schempp CM, Wölfle U. Hop extract acts as an antioxidant with antimicrobial effects against *Propionibacterium acnes* and *Staphylococcus aureus*. *Molecules*. 2019 Jan;24(2):223.

Winston M, Shalita A. Acne Vulgaris: Pathogenesis and Treatment. *Pediatric Clinics of North America*. 1991;38(4):889-903.

Witkowski JA, Parish LC. The assessment of acne: an evaluation of grading and lesion counting in the measurement of acne. *Clinics in dermatology*. 2004 Sep 1;22(5):394-7.

Wright TE, Boyle KK, Duquin TR, Crane JK. *Propionibacterium acnes* susceptibility and correlation with hemolytic phenotype. *Infectious Diseases: Research and Treatment*. 2016 Jan;9:IDRT-S40539.

Xu H, Li H. Acne, the skin microbiome, and antibiotic treatment. *American journal of clinical dermatology*. 2019 Jun 1;20(3):335-44.

Xu H, Li H. Acne, the skin microbiome, and antibiotic treatment. *American*