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Antibacterial effect of plant extracts against clinical isolates of acne inducing bacteria

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Abstract:

Acne is one of the most common skin diseases, affecting more than half the individuals of a population. *Propionibacterium acnes* is a gram-positive human skin commensal that prefers anaerobic growth conditions and is involved in the pathogenesis of acne. *S. epidermidis* is a most frequently isolated species from human epithelia. It colonizes the axillae, head, and sebaceous areas such as the facial skin. The incidence of isolates among 100 patients screened in Allahabad (Prayagraj) region, where 65% of patients found to be positive for bacterial infection, with an incidence of 36% for *Propionibacterium acnes* and 30% for *Staphylococcus epidermidis*, which were identified respectively on the basis of cultural, morphological and biochemical characteristics. A number of demographic factors have been found responsible for the prevalence of acne, such as high humidity level, skin texture and high stress levels, hormonal changes. The age group of 15-25 years and gender, particularly females were found to be more susceptible to acne as compared to males. Furthermore the antibiotic susceptibility pattern test showed that the isolates; i.e. *S epidermidis* and *P acnes* were found to be multi drug resistant. These findings demonstrated that the high incidence of multidrug-resistant *P. acnes* and *S. epidermidis* species constitute on important potential threat to the human health. Control measures need to be taken to avoid contacting acne infection and prevent it from becoming extensively drug-resistant. Therefore plant extracts were tested against both the isolates, to observe the antimicrobial activity. The result showed that *Punica granuatum* had the maximum antimicrobial activity against both the isolates. Thus, the present study concludes that plant extracts containing bioactive compounds have antimicrobial properties which can be used in the treatment of acne.

Keywords:

Acne vulgaris, *Propionibacterium acnes, Staphylococcus epidermidis,* Multi drug resistant, Plant extracts

1. Introduction:

Acne vulgaris, a chronic inflammatory multi factorial, pleomorphic skin disease of the pilosebaceous follicles that has affected more than 85% of adolescents and young adults and is characterized by, non-inflamed (open and close comedones) and inflamed (macules, papules, pustules and nodules) lesions. It is the most common skin disorder of pilosebaceous unit that affects the areas containing the large oil glands, including the face, back and trunk (layden *et al.,* 1997). It is generally characterized by formation of seborrhoea, comedone, inflammatory lesions.

This disorder is generally caused by stress, hereditary factors, hormones, drugs. The action of sebum synthesized and secreted by androgen sensitive sebaceous glands, causes a increase in hormones called androgen in both girl and boy during puberty which is also responsible for this disorder. (Dorland *et. al.,* 2000).

The four major factors involved in the pathogenesis of Acne vulgaris are, increased sebum production, hypercornification of the pilosebaceous duct, abnormality of the microbial flora (especially colonization of the duct with *propionibacterium acnes*) and the production of inflammation. Although acne is not infectious, the three major organisms that have been isolated from the pilosebaceous ducts of acne patients including *staphylococcus epidermidis, malassezia furfur* and *propionibacterium acnes*.

Propionibacterium are usually nonpathogenic, spore forming, gram positive, anaerobic, pleomorphic rod whose end product of fermentation includes propionic acid. *Propionibacterium* resembles *Corynebactrium* in morphology and arrangement. *P. acnes* is considered an opportunistic pathogen, causing a range of infectious as well as being associated with a number of inflammatory conditions. (Chen and Yu., 1996)

It is implicated in the development of inflammatory acne by its capability to activate compliments and by its ability to metabolize sebaceous triglycerides into fatty acids, which chemo tactically attracts neutrophills. (Burkhart *et al.,* 1999)

S. epidermidis strains are natural colonizers of healthy human skin can easily grow on medical devices because of having strong attachment ability; they can easily be transmitted to medical device and colonize on the surface if it during an implantation process (Kaplan *et. al.,* 2004).

Antibiotics are a fundamental component for the treatment of acne owing to the role that P. *acnes* plays in its pathogenesis. However antibiotic resistance is a global issue with increasing prevalence over time. The antibiotics most frequently used for acne are topical erythromycin,

clindamycin, and oral tetracycline, which are bacteriostatic (inhibiting bacterial growth) rather than bactericidal (kill bacteria) (Brandon *et al.,* 2017)

Medicinal plants have a long history of use (Bahmani *et al.,* 2014) Nature has been a source of medicinal agents (Punjabi *et. al*., 2014). Plants and plant derivatives have been an integral part of healthcare system since very long and have been reported to possess very low side effects. Other than common cold and other such infectious diseases, they have proved to be useful in the prevention and treatment of a wide variety of diseases (Nasri *et. al,* 2015). Medicinal plants also possess the capacity to diminish drug induced adverse effects and even heavy metal and other toxicities, such as the protective effect of artichokes (*Cynarascolymus*) leaf extracts against lead toxicities in rats.

Novel 'bioactive' ingredients are being derived from the sea, earth and plant kingdom. Popular ingredients include Chinese herbs, vitamins, minerals, antioxidants, enzymes, hormones and a multitude of 'naturals'. The use of plants is as old as mankind and in the coming years, the market will see many new products containing natural oils and herbs. Plants have been once the main source and foundation of all cosmetics, before methods were discovered of synthesizing substances with similar properties.

Acne vulgaris drugs mostly possess adverse effects but medicinal plants might be considered as reliable sources for the development of new drugs. Herbal medicines are gaining increased popularity due to their advantages, such as better patient tolerance, long history of use, fewer side effects and being relatively less expensive (Rafieian., 2013) furthermore, they have provided good evidence for the treatment of a wide variety to cure diseases. These plants are used alone or in combination with synthetic drugs to treat diseases. More importantly, other than consumptions as preventive or treatment remedy, they might be accompanied with synthetic drugs to reduce their side effects. With no exception botanical drugs are also used accompanied by other methods or alone to treat Acne vulgaris. Many medicinal plants with anti-inflammation and anti-bacterial activities are used in different ways in the treatment of acne and other infective diseases.

Matricaria recutita, Calendula officinalis, Fragaria ananassa, Psidium guajava, Punica granatum, *Curcuma longa, Aloe vera* and *Triticuma estivumare* commonly used for acne treatment (Kraft *et. al.,* 2007). Creams or aqueous infusions made from these plants including astringents and composites such as tannins are used topically on skin after cleansing or a steam bath. *Hamamelis virginiana* has tannins and extraction of epidermidis is commonly used to treat acne because it is very safe for topical prescription.

To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatment for diseases. In the parent study some medicinal plants which have been traditionally used as antimicrobial and anti-inflammatory agents were examined for antimicrobial activities against microorganisms frequently in acne inflammation of *P. acnes* and *S. epidermidis*.

Therefore, in view of the above, the present study was carried out with the following objectives:

- To identify the *Propionibacterium* and *Staphylococcus* species selectively isolated from clinical samples.
- To assess the various demographic factors associated with prevalence of acne
- To evaluate antimicrobial susceptibility pattern of the isolates
- To evaluate the antimicrobial activity of selected plant extracts against the bacterial pathogens

2. Materials and methods:

2.1. Sample collection:

A total of 100 acne samples were collected aseptically from infected patients from local Hospital or Clinic of Allahabad city (Swaroop Rani Nehru Hospital, Bajaj Skin Clinic, Skin Care Center (Dr. Devendra Prasad), Skin Care Clinic (Dr. Sushil Kumar). Acne samples were collected in Thioglycollate broth (Appendix 1.1) media with the help of sterile cotton swabs and were transported to the laboratory. All the samples were stored at 4°C and processed within 4 hours.

2.2. Isolation:

All the samples was inoculated under aseptic conditions in selective medium *i.e.* Blood Agar (Appendix 1.3) and Mannitol Salt Agar (Appendix 1.4) to the isolation of *Propionibacterium acnes* and *Staphylococcus epidermidis*. At 37°C under aerobic conditions for isolation of *Staphylococci* and on blood agar at 37°C under anaerobic conditions in anaerobic jars for 3 to 7 days for isolation of *P. acnes.*

2.3. Identification:

The isolates were identified on the basis of cultural, morphological, and biochemical characteristics as per Bergey's Manual of Systemic Bacteriology (Holt *et al.,* 1984).

2.4. Cultural characteristics:

Plates were observed for the appearance and growth of the colonies on the medium surface on the basis of colony size, form, shape, elevation, opacity, type of margin *etc.*

2.5. Morphological characteristics:

Suspected colonies were picked from culture plates and smear was prepared on clean glass slide. Gram-staining was performed and observed under 100X objective for the identification and classification of Gram positive and Gram negative bacteria.

2.6. Biochemical characteristics:

Various biochemical tests were performed for the identification of *Propionibacterium acne and Staphylococcus epidermidis* as per the procedure given by Aneja, (2007).

2.7. Determination of antibiotic susceptibility pattern of the isolates:

Different antibiotics have different effects on different organisms. Some organisms may be completely resistant to a specific antibiotic while others are highly susceptible. The Kirby-Bauer or disk diffusion test was used to determine if an organism is susceptible or resistant to a selection of antimicrobial agents (Bauer *et al.*, 1966). This is a very useful procedure when trying to determine a therapeutic course against a particular infection. It can also be used to test the efficacy of a new antibiotic. Mueller Hinton agar (Appendix 1.7) plates were used for this test. Mueller-Hinton agar plates were poured to a depth of 4mm. After solidifying, the plates were swabbed/ inoculated for confluent growth. After the plates were inoculated, a variety of antibiotic discs was added. These discs would be infused with a specific amount of antimicrobial agent. The plates were then incubated for 18 – 20 h at 37°C. After incubation, there were "bacteria-free" circles of varying sizes around some of the discs. These are called zones of inhibition which indicated whether the organism was susceptible to the antibiotic or not. The larger the zone of inhibition surrounding an antimicrobial agent is, the more susceptible the organism is to the antibiotic. The Antibiotic susceptibility pattern of the isolates was performed accordingly and was interpreted with the CLSI (Wayne, 2003) standard and guidelines.

2.8. Antimicrobial activity of plant extract by well diffusion method:

Medicinal plants have a long history of use (Bahmani *et. al.*, 2014) and have been shown low side effects. Antimicrobial activity tests will be performed against medicinal plant extract (which is prepared already) by Well Diffusion method (Bauer and Kirby 1966). Mueller Hinton

agar plates were used for this test. Mueller-Hinton agar (Appendix 1.7) plates were poured to a depth of 4mm. After solidifying, the plates were swabbed/ inoculated for confluent growth. After the plates were inoculated, 4mm hole was cutted on agar plate by the help of borer and then 100 µl plant extracts was added on each hole. The plates were then incubated for 20 – 24 h at 37°C. After incubation, there were "bacteria-free" circles of varying sizes around the holes. These are called zones of inhibition which indicated whether the organism was susceptible to the extract or not. The larger the zone of inhibition surrounding an antimicrobial agent is, the more susceptible the organism is to the extract. The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993).

For conducting Well diffusion test, following plant extracts were used for *Propionibacterium acnes* and *Staphylococcus epidermidis:*

1. Fragaria vesca
2. Psidium guajava
3. Aloe vera
4. Punica granatum
5. Coleus forskohlii
6. Carica papaya
7. Curcuma longa

3. Results and discussion:

In the present study 100 patients were screened amongst which, 65% were found to be positive for bacterial infection with an incidence of 36% for *Propionibacterium acnes* and 30% for *Staphylococcus epidermidis*. The study showed a statistically non-significant differences when the data was analyzed in terms of incidences of different bacterial species ($P < 0.05$). Similarly, the data was analyzed in terms of incidences of *Propionibacterium acnes* and *Staphylococcus epidermidis* showed a statistically non-significant ($P < 0.05$) (Table. 4.1, Fig. 4.1)

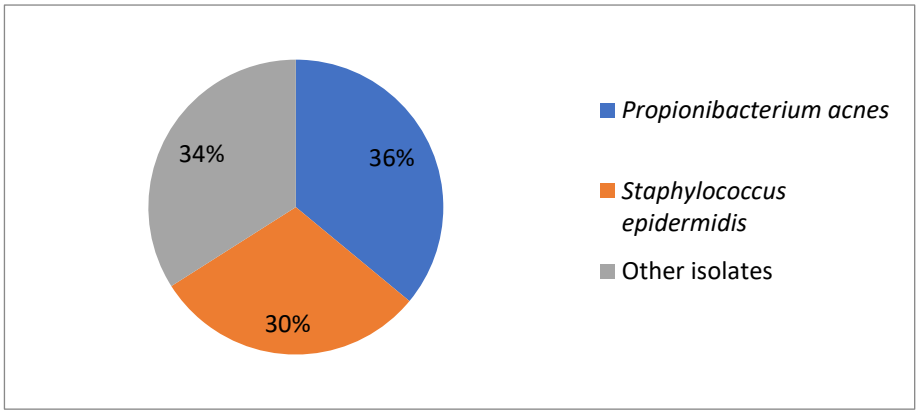

Figure. 4.1: Incidence of P. acnes and S. epidermidis in positive bacterial isolates

Table. 4.1: Distribution of bacterial pathogens in clinical samples positive for bacterial infection

No of patient positive for bacterial infection	Gram positive rods	Propionibacterium acnes
65	25 (38.46)	9 (36)
	Gram positive cocci	*Staphylococcus epidermidis*
	40 (61.53)	12 (30)

Figures in parenthesis indicates percentage

Z_{cal}=23.5, $Z_{cal}>Z_{tab}$ (5%) = 1.96

Z_{cal}=5.35, $Z_{cal}>Z_{tab}$ (5%) = 1.96

3.1. Identification of isolates:

The isolates were identified as *P acnes* and *S epidermidis* on the basis of characters given in Bergey's Manual of Systematic Bacteriology (Table. 4.2).

Table. 4.2: Identification of P acnes on the basis of Cultural, Morphological and Biochemical characteristics

Organism	***Propionibacterium acnes***
Cultural characteristics	
Colony color on nutrient agar	Cream
Colony color on Blood agar	White to Cream
Surface	Smooth
Elevation	Convex
Margin	Entire

Morphological characterisitics	
Gram's reaction	Positive
Shape	Rods
Size	0.5-0.8µm
Biochemical characteristics	
Catalase activity	Positive
Indole hydrolysis test	Positive / Negative
Methyl red test	Negative
Voges-proskauer test	Negative
Citrate utilization test	Negative
Urease test	Negative
Gelatin liquefaction test	Positive
TSI	Negative
Nitrate reduction test	Positive / Negative
Coagulase test	Negative
Carbohydrate fermentation	
Glucose	Positive
Mannose	Positive
Lactose	Negative
Xylose	Negative
Inositol	Negative
Arabinose	Negative
Sucrose	Negative
Fructose	Positive
Raffinose	Negative
Galactose	Positive

A^+= Acid production; A^-= no acid production; G^+= gas production; G^-= no gas production

Table. 4.3: Identification of S. epidermidis on the basis of Cultural, Morphological and Biochemical characteristics

Organism	***Staphylococcus epidermidis***
Cultural characteristics	
Colony color on nutrient agar	White
Colony color on Mannitol salt agar	White
Surface	Smooth
Elevation	Convex
Margin	Entire
Morphological characterisitics	
Gram's reaction	Positive
Shape	Cocci
Size	0.5-1.5µm
Biochemical characteristics	
Catalase activity	Positive
Oxidase activity	Negative
Methyl red test	Negative
Voges- proskauer test	Positive
Citrate utilization test	Negative
Urease test	Positive
Gelatin liquefaction test	Negative
Nitrate reduction test	Positive
TSI	Positive
Coagulase test	Negative
Carbohydrate fermentation	
Glucose	Positive
Maltose	Positive
Lactose	Positive
Mannitol	Negative
Fructose	Positive
Sucrose	Positive

Mannose	Positive
Xylose	Negative
Arabinose	Negative
Raffinose	Negative

A^+= Acid production; A^-= no acid production; G^+= gas production; G^-= no gas production

3.2. Demographic factors associated with prevalence of acne:

On the basis of age group, the study population was divided into two group's *viz.* 15-25 years and 25-35 years. The number of patients positive for bacterial isolates was high in the age group of 15-25 years as compared to the age group comprising patients of 25-35 years. The data was statistically analyzed and found to be non- significant (P >0.05). Similarly, In case of *P. acnes* and *S. epidermidis*, the incidence was high in the age group of 15-25 years, compared to other group. (Table. 4.4, Fig. 4.2)

Table. 4.4: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to age

Age group (in years)	***Total number of patients***	***Number of patients positive for bacterial isolates***	***Number of patients positive for P. acnes***	***Number of patients positive for S. epidermidis***
15-25	49	37(75.51)	6(16.21)	7(18.91)
26-35	51	28(54.90)	3(10.71)	5(17.85)

Figures in parenthesis indicates percentage

Z_{cal}= 2.77, $Z_{cal}>Z_{tab}$ (5%) = 1.96

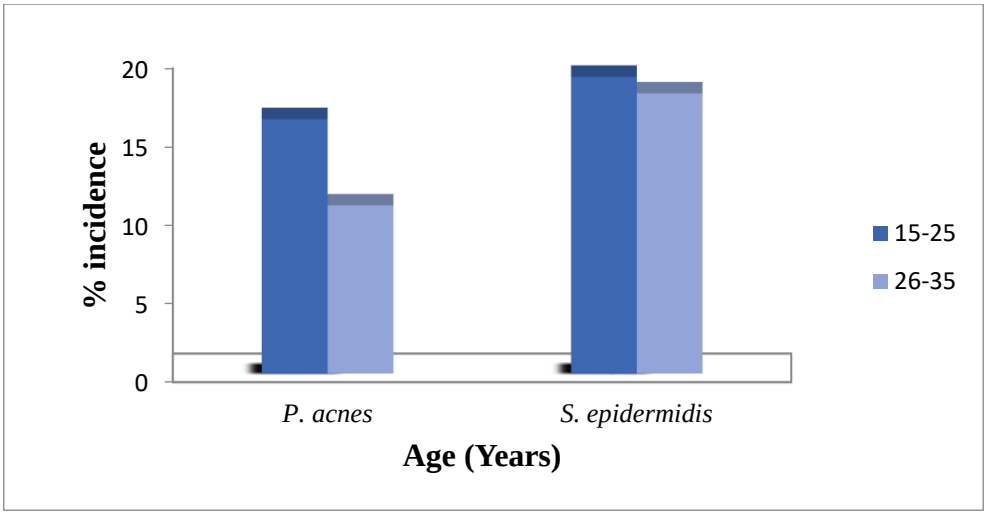

Figure. 4.2: Distribution of `P. acnes and S. epidermidis with respect to age

On the basis of gender, the study population was grouped into two categories *viz.* male and female. The number of patients positive for bacterial isolates was slightly higher in male as compared to females. The data was statistically analyzed and found to be non- significant (P >0.05).In case of *P. acnes* and *S. epidermidis*, the incidence was high in females as compared to males. (Table. 4.5, Fig. 4.3)

Table. 4.5: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to gender

Gender	***Total number of patients***	***Number of patient positive for bacterial isolates***	***Number of patient for P. acnes***	***Number of patient for S. epidermidis***
Male	37	25(67.56)	2(8)	4(16)
Female	63	40(63.49)	7(17.5)	8(20)

Figures in parenthesis indicates percentage

Z_{cal}= 23.27, $Z_{cal}>Z_{tab}$ (5%)= 1.96

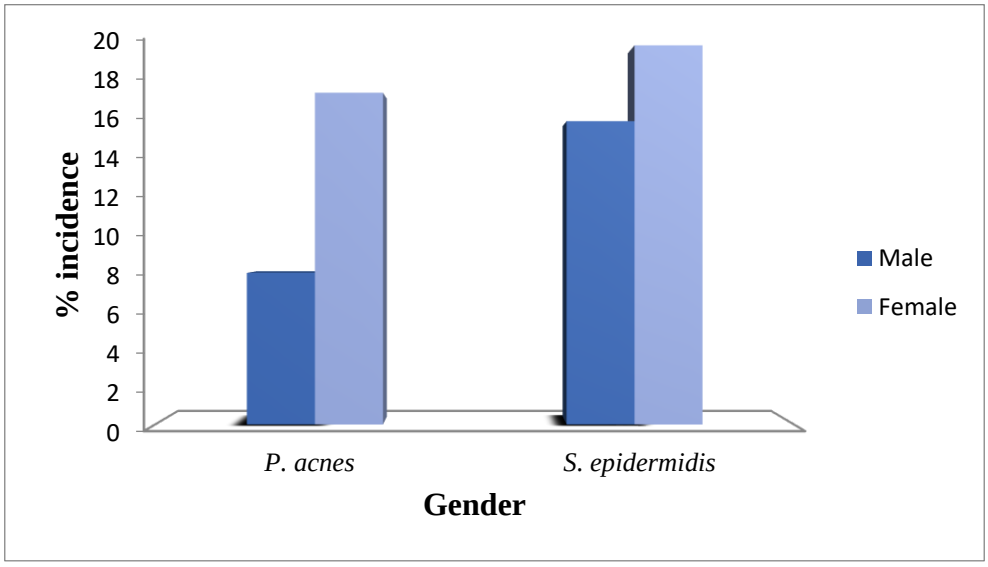

Figure. 4.3: Distribution of `P. acnes and S. epidermidis with respect to gender

On the basis of seasonal variation, the study population was divided into three categories *viz*. monsoon, summer and winter. The number of patients positive for bacterial isolates was high in monsoon season as compared to other seasons. The data was statistically analyzed and found to be non- significant (P >0.05). Similarly, In case of *P. acnes*, the patients had a higher level of incidence during the monsoon followed by summer, whereas winter showed the least but in case of *S. epidermidis*, the incidences during summer and monsoon was almost same and least during winter. (Table. 4.6, Fig. 4.4)

Table. 4.6: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect toseasonal variation

Seasonal variation	*Total number of patients*	*Number of patient positive for bacterial isolates*	*Number of patient positive for P. acnes*	*Number of patient positive for S. epidermidis*
Monsoon	72	52(72.22)	8(15.4)	10(19.23)
Summer	20	10(50)	1(10)	2(20)
Winter	8	3(37.5)	0(0)	0(0)

Figures in parenthesis indicates percentage

X^2_{cal}=105.02, X2cal>X^2_{tab} (5%) = 5.991

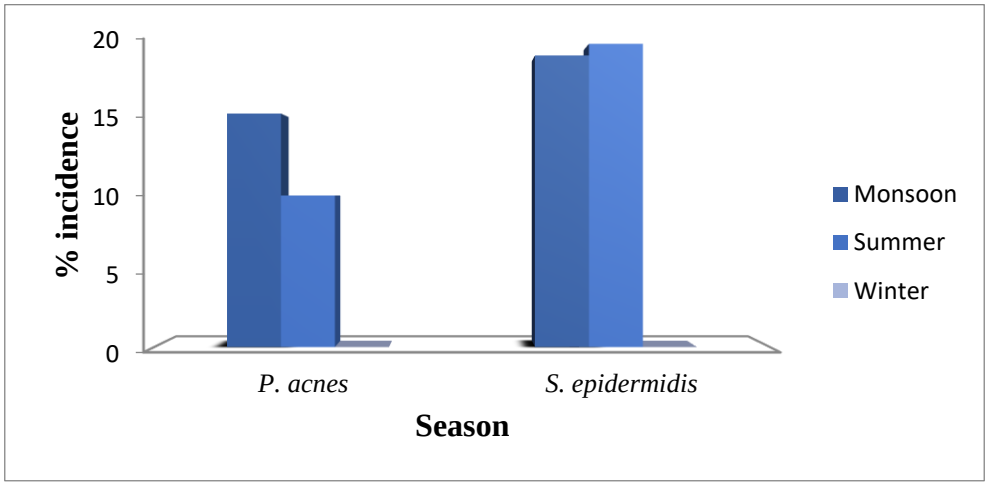

Figure. 4.4: Distribution of `P. acnes and S. epidermidis with respect to seasonal variation

On the basis of economic status governed by the annual income of the family, the study population was divided into three categories *viz.* upper class, middle class and lower class. The number of patients positive for bacterial isolates was higher in middle class patients as compared to other classes. The data was statistically analyzed and found to be non- significant (P >0.05). Similarly, In case of *P. acnes* and *S. epidermidis* was found high in middle class patients as compared to other classes. (Table. 4.7, Fig. 4.5)

Table. 4.7: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to economic status governed by the annual income of the family

Economic status	*Total number of patients*	*Number of patients positive for bacterial isolates*	*Number of patients positive for P. acnes*	*Number of patients positive for S. epidermidis*
Upper class	16	3(18.75)	0(00)	0(00)
Middle class	52	43(82.7)	7(16.3)	9(20.93)

Lower class	32	19(59.4)	2(10.5)	3(15.78)

Figures in parenthesis indicates percentage

X^2_{cal}= 14.65, $X^2_{cal} > X^2_{tab}$ (5%) = 5.991

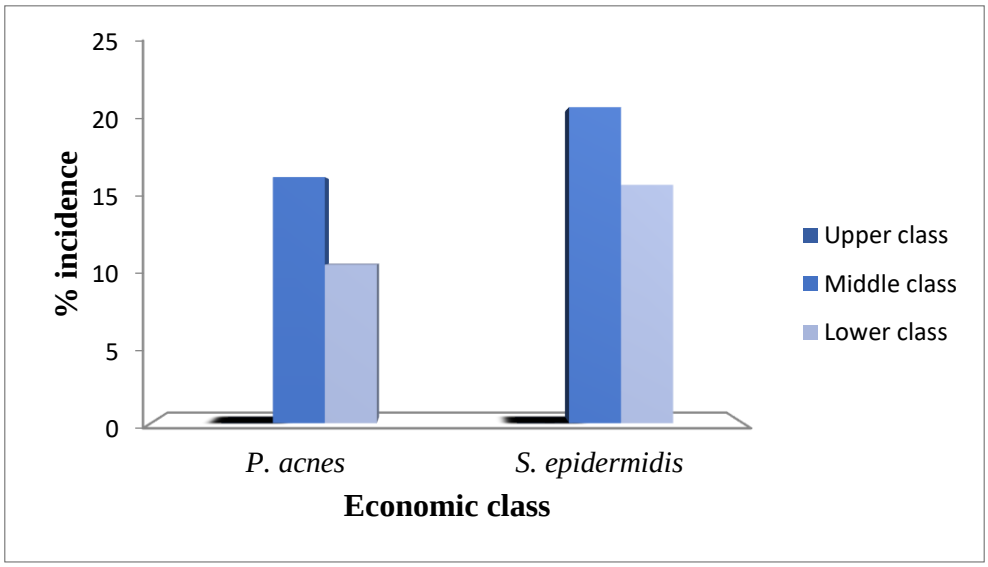

Figure. 4.5: Distribution of `P. acnes and S. epidermidis with respect to economic class

On the basis of skin texture, the study population was divided into three group's *viz.* oily skin, dry skin and normal skin. The number of patients positive for bacterial isolates was highest among the patients having oily skin compared to other group of patients. The data was statistically analyzed and found to be non- significant (P >0.05). Similarly, the incidence of *P. acnes* and *S. epidermidis* was slightly highest among the patients having oily skin, followed by normal skin and negligible in the case of patients having dry skin. (Table. 4.8, Fig. 4.6)

Table. 4.8: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to skin texture

Skin texture	***Total number of patients***	***Number of patients positive for bacterial isolates***	***Number of patients positive for P. acnes***	***Number of patients positive for S. epidermidis***
Oily	49	37(75.5)	7(18.9)	9(24.3)
Dry	21	13(61.9)	0(0)	0(00)
Normal	30	15(50)	2(13.3)	3(20)

Figures in parenthesis indicates percentage

X^2_{cal}= 38.28, $X^2_{cal} > X^2_{tab}$ (5%) = 5.991

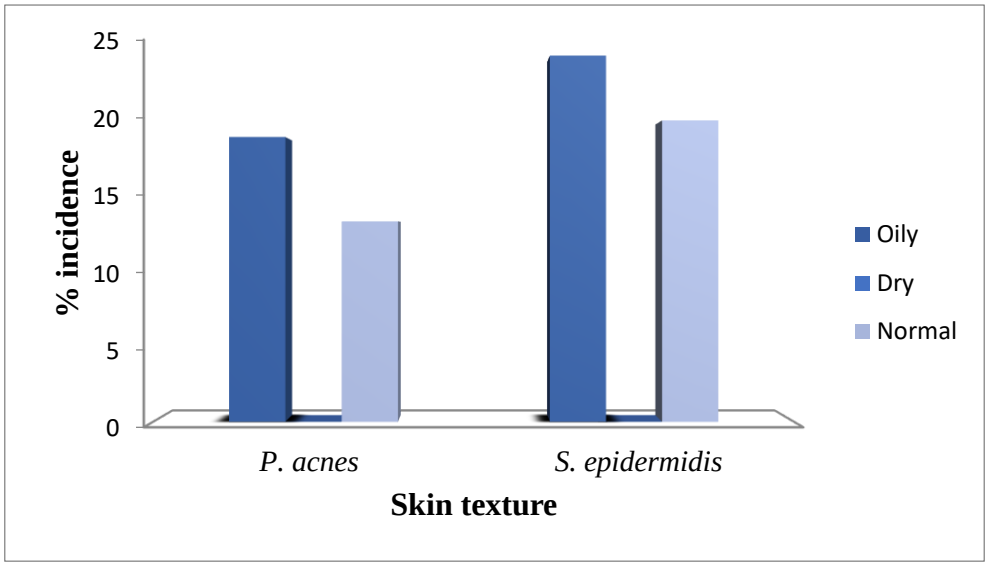

Figure. 4.6: Distribution of `P. acnes and S. epidermidis with respect to skin texture

On the basis of occupational stress level, the study group was divided into two categories *viz.* severe and mild. The number of patients positive for bacterial isolates was higher in severe stress condition as compared to other group. The data was statistically analyzed and found to be non- significant (P >0.05). The patients under severe stress condition showed higher level of incidence of *P. acne* as well *S. epidermidis* as compared to patients with mild stress level. (Table. 4.9, Fig. 4.7)

Table. 4.9: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to occupational stress level

Stress level	***Total number of patients observed***	***Number of patients positive for bacterial isolates***	***Number of patients positive for P. acnes***	***Number of patients positive for S. epidermidis***
Severe	42	33(78.57)	5(15.15)	7(21.21)
Mild	58	32(55.17)	4(12.5)	5(15.62)

Figures in parenthesis indicates percentage

Z_{cal}= 7.39, $Z_{cal} > Z_{tab}$ (5%) = 1.96

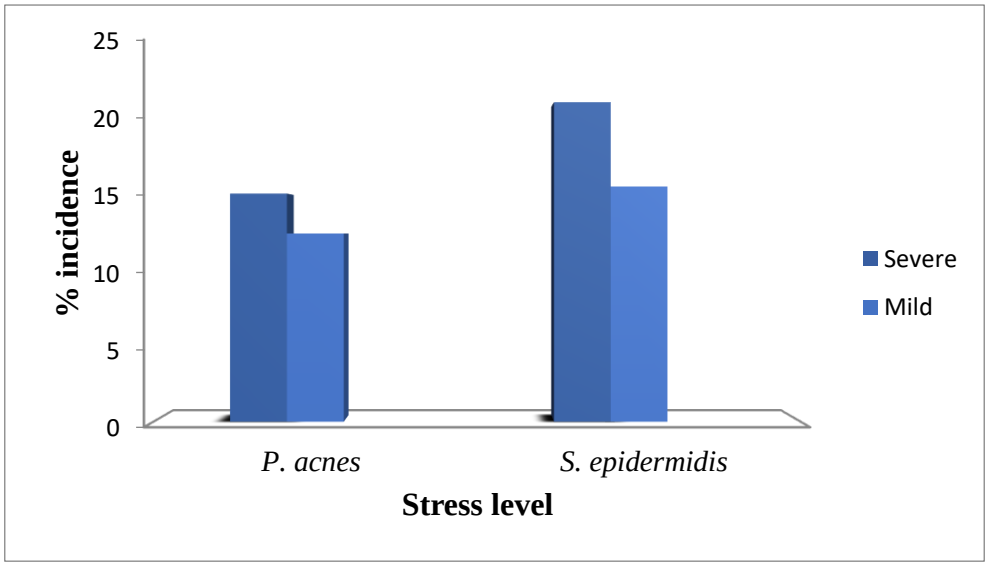

Figure. 4.7: Distribution of `P. acnes and S. epidermidis with respect to occupational stress level

On the basis of hygiene condition, the study population was divided into two categories *viz*. hygienic and non-hygienic. The number of patients positive for bacterial isolates was slightly higher on those patients who maintained good hygiene condition as compared to the patients who maintained lower or non-hygienic condition. The data was statistically analyzed and found to be non- significant (P >0.05). In the case of *P acnes* and *S. epidermidis*, the patients who maintained lower or non-hygienic condition had a higher incidence as compared to the patients who maintained good hygiene. (Table. 4.10, Fig. 4.8)

Table. 4.10: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to hygiene condition

Hygiene condition	***Total number of patients***	***Number of patients positive for bacterial isolates***	***Number of patients positive for P. acnes***	***Number of patients positive for S. epidermidis***
Hygienic	76	50(65.8)	3(06)	4(08)
Non- hygienic	24	15(62.5)	6(40)	8(53.3)

Figures in parenthesis indicates percentage

Z_{cal}= 3.06, Z_{cal}>Z_{tab} (5%)= 1.96

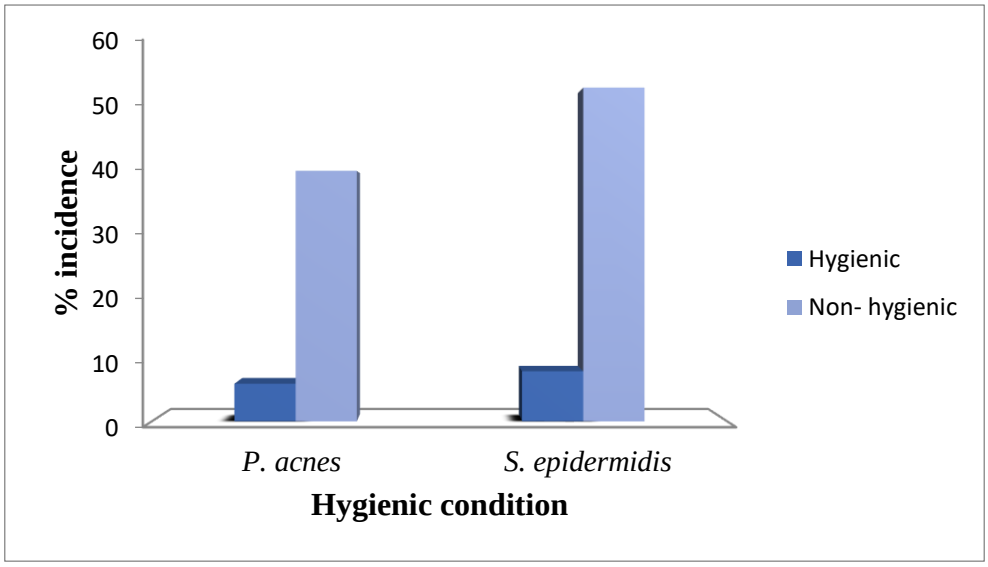

Figure. 4.8: Distribution of `P. acnes and S. epidermidis with respect to hygiene condition

On the basis of use of cosmetic products, the study population was divided into two categories *viz*. herbal products and chemical based products. The number of patients positive for bacterial isolates was higher among the individuals who had been using chemical products compared to individuals using herbal products. The data was statistically analyzed and found to be non-significant (P >0.05). A similar trend was observed for *P. acnes* and *S. epidermidis* (Table. 4.11, Fig. 4.9)

Table. 4.11: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to use of cosmetic products

Cosmetic products	***Total number of patients***	***Number of patients positive for bacterial isolates***	***Number of patients positive for P. acnes***	***Number of patients positive for S. epidermidis***
Herbal products	42	23(54.76)	2(8.69)	2(8.69)
Chemical based products	58	42(72.41)	7(16.66)	10(23.80)

Figures in parenthesis indicates percentage

Z_{cal}= 28.63, Z_{cal}>Z_{tab} (5%)= 1.96

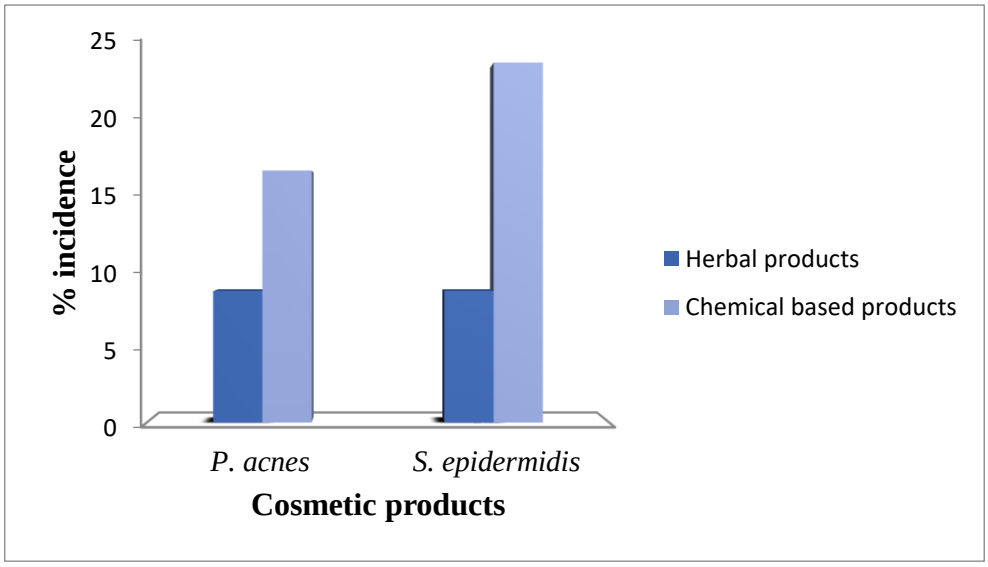

Figure. 4.9: Distribution of `P. acnes and S. epidermidis with respect to use cosmetic products

On the basis of family history of individual who had acne, the study population was divided into two categories *viz*. the ones who had family history of acne and other who did not. The number of patients positive for bacterial isolates was high in who had no reported family history of acne as compared to other group. The data was statistically analyzed and found to be non-significant (P >0.05). In case of P. acnes, the patients who had reported family history of acne had a higher incidence as compared to individuals having a no family history of acne. However, in the case of *S. epidermidis*, both the population had the similar incidence. (Table. 4.12, Fig. 4.10)

Table. 4.12: Distribution of P. acnes and S. epidermidis in patients positive for bacterial infection with respect to family history of acne

Family history of acne	***Total number of patients***	***Number of patients positive for bacterial isolates***	***Number of patients positive for P. acnes***	***Number of patients positive for S. epidermidis***
Yes	18	11(61.11)	3(27.27)	2(18.18)
No	82	54(65.85)	6(11.11)	10(18.51)

Figures in parenthesis indicates percentage

Z_{cal}= 72.38, $Z_{cal}>Z_{tab}(5\%)$= 1.96

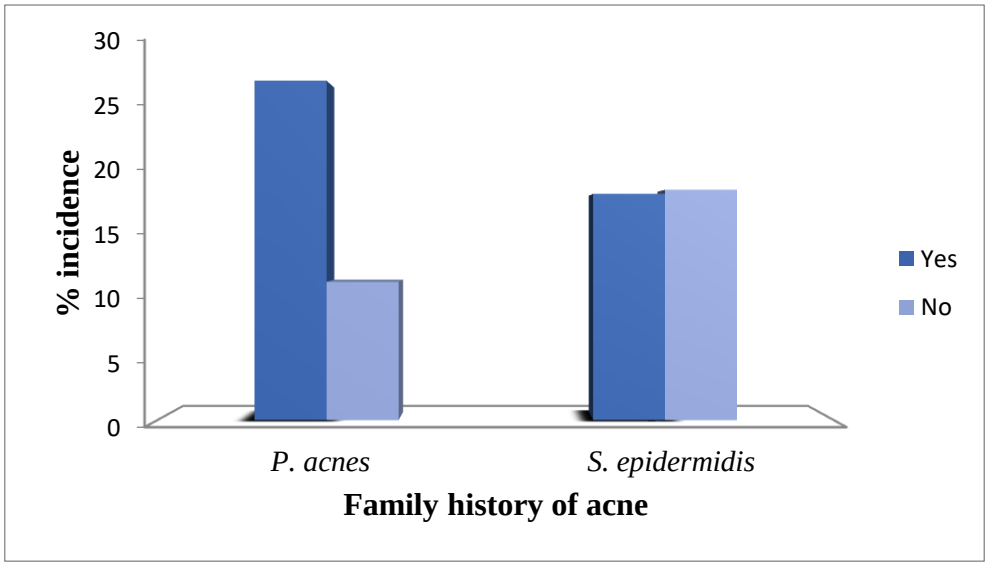

Figure. 4.10: Distribution of `P. acnes and S. epidermidis with respect to family history

3.3. Determination of antibiotic susceptibility pattern of P. acnes and S. epidermidis:

Antibiotic susceptibility of all the isolates was performed by the disc diffusion (modified-Kirby Bauer disc diffusion method) according to CLSIs guidelines. Results revealed an increasing trend towards development of antibiotic resistance. The results showed that all isolates of *P. acnes* were completely susceptible to Erythromycin, Gentamicin, Levofloxacin, Vancomycin. On the other hand, isolates showed resistance to antibiotics that included Rifampicin (22%), Cefoxitin (33%), Tobramycin (33%), Chloramphenicol (44%), Cephalexin (67%), Amikacin (22%), Tetracycline (22%) making them multiple drug resistance (MDR). Methicilin, Penicillin, Ampicillin were found to be 100% resistance towards all the tested isolates. (Table. 4.4, Fig. 4.2)

In case of *S. epidermidis*, results showed that all the isolates were completely susceptible to Clindamycin, Tobramycin. The isolates showed resistance to antibiotics that included Rifampicin (33%), Trimethoprim (75%), Nitrofurantoin (42%), Vancomycin (33%), Teicoplanin (42%), Tetracycline (33%) making them multiple drug resistance (MDR). Methicilin, Cephalothin and Oxacilin were found to be 100% resistance towards all the isolates of *S. epidermidis.* (Table. 4.13, Fig. 4.11)

Table. 4.13: Antibiotic susceptibility pattern of P. acnes isolates

S.No	***Antibiotic***	***Disc Concentration (µg)***	***Interpretation (Isolate No)***									***Percentage Resistance (%)***
			1	2	3	4	5	6	7	8	9	
1.	Clindamycin (cd)	20	S	S	S	S	S	IR	S	S	S	11
2.	Rifampicin (r)	05	IR	IR	S	S	S	S	S	S	S	22
3.	Erythromycin (e)	15	S	S	S	S	S	S	S	S	S	0
4.	Cefoxitin (cn)	30	1R	S	S	S	IR	IR	S	S	S	33
5.	Vancomycin (va)	30	S	S	S	S	S	S	S	S	S	0
6.	Methicilin (m)	05	R	R	R	R	R	R	R	R	R	100
7.	Tobramycin (tb)	10	S	S	S	R	S	R	R	S	S	33
8.	Levofloxacin (le)	05	S	S	S	S	S	S	S	S	S	0
9.	Chloramphenicol (cip)	30	S	IR	IR	IR	S	S	S	R	S	44
10.	Ampicillin (amp)	10	R	R	R	R	R	R	R	IR	R	100
11.	Cephalexin (ce)	10	IR	IR	IR	S	S	R	S	R	R	67
12.	Penicillin (p)	10	R	R	R	R	R	R	R	R	R	100
13.	Amikacin (ak)	30	S	S	S	S	S	S	R	R	S	22
14.	Gentamicin (gen)	10	S	S	S	S	S	S	S	S	S	0
15.	Tetracycline (te)	30	S	IR	IR	S	S	S	S	S	S	22

S= sensitive, R=resistant, IR= intermediate resistance

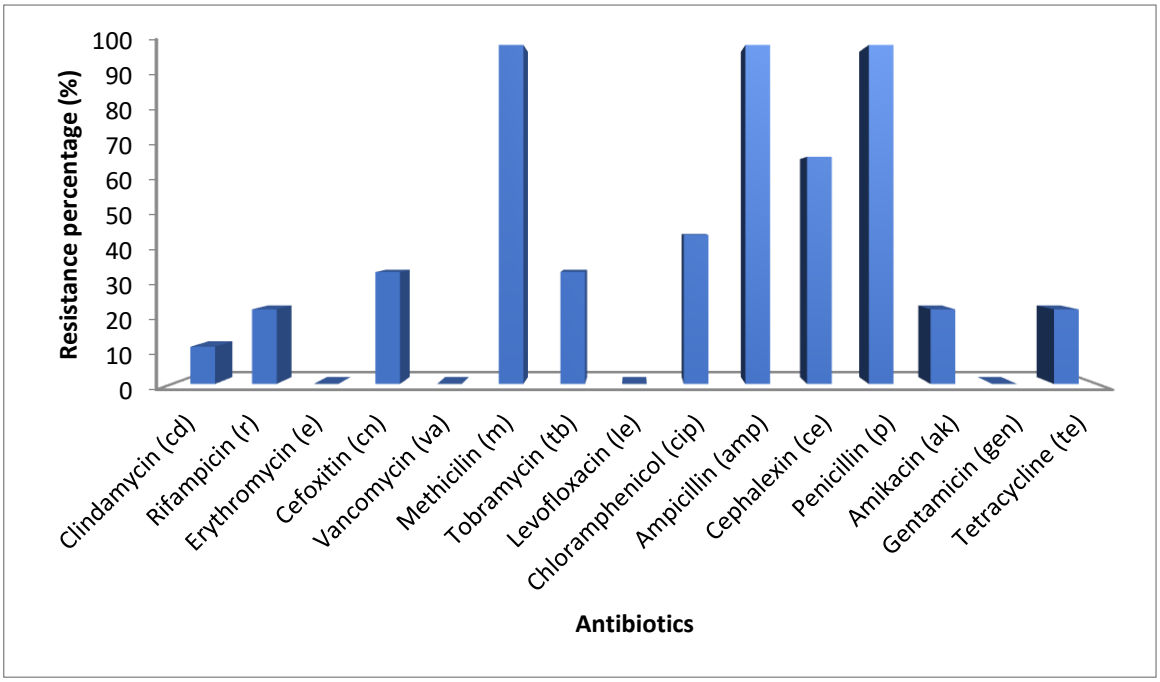

Figure. 4.11: Resistance of clinical isolate of P. acnes against antibiotics

Table. 4.14: Antibiotic susceptibility pattern of S. epidermidis isolates

S.No	*Antibiotic*	*Disc Concentration (µg)*	*Interpretation (Isolate No)*												*Percentage Resistance (%)*
			1	2	3	4	5	6	7	8	9	10	11	12	
1.	Clindamycin (cd)	20	S	S	S	S	S	S	S	S	S	S	S	S	0
2.	Rifampicin (r)	05	IR	IR	IR	S	S	S	S	S	S	S	S	IR	33
3.	Trimethoprim (tr)	12	R	R	R	S	S	IR	R	R	R	R	S	IR	75
4.	Nitrofurantoin (nf)	300	S	S	S	S	S	R	R	IR	S	R	R	S	42
5.	Vancomycin (va)	30	S	S	S	S	IR	IR	S	S	S	R	R	S	33
6.	Methicilin (m)	05	R	R	R	R	R	R	IR	IR	IR	S	R	R	100
7.	Tobramycin (tb)	10	S	S	S	S	S	S	S	S	S	S	S	S	0
8.	Cephalothin (cep)	30	R	R	R	R	R	R	R	R	R	R	R	R	100
9.	Oxacillin (ox)	1	R	R	R	R	R	IR	IR	R	R	R	R	R	100

10.	Teicoplanin (tei)	30	S	S	S	S	R	R	S	IR	S	IR	S	R	42
11.	Linezolid (lz)	30	S	S	S	S	S	IR	IR	S	S	S	S	S	17
12.	Gentamicin (gen)	10	IR	IR	S	S	S	S	S	S	S	S	S	S	17
13.	Tetracycline (te)	30	S	S	S	S	R	R	R	S	S	S	S	R	33

S= sensitive, R=resistant, IR=intermediate-resistance

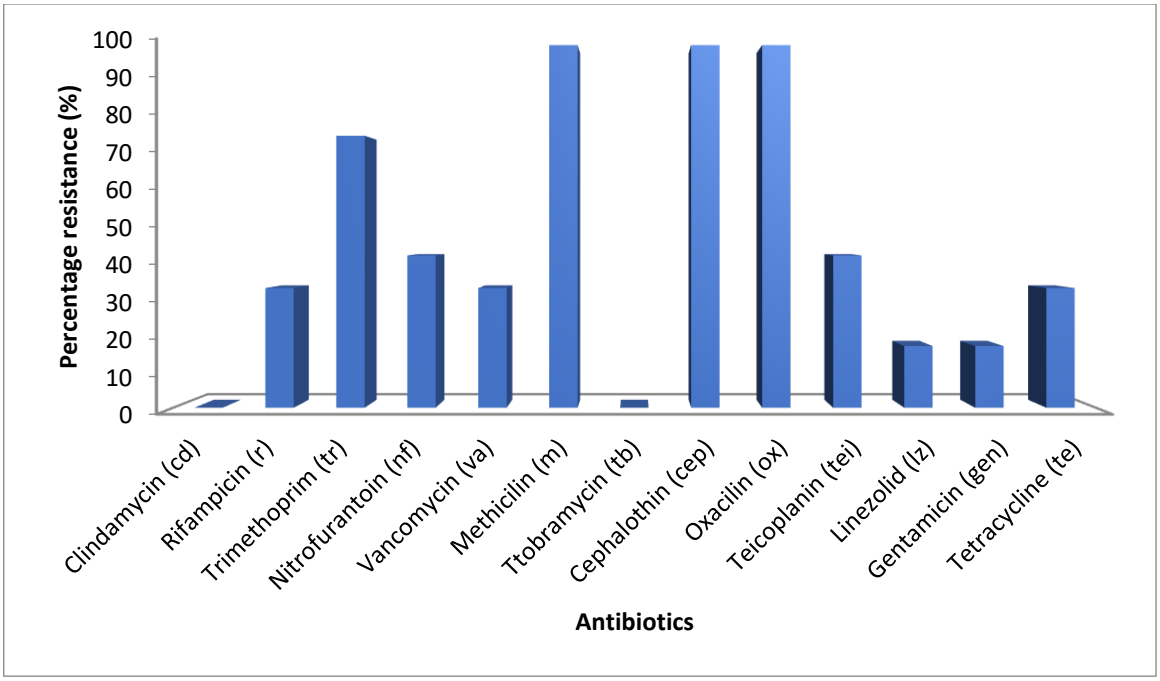

Figure. 4.12: Resistance of clinical isolate of S epidermidis against antibiotics

3.4. Antimicrobial activity of plant extract by well diffusion method:

In the case of *P. acnes*, extracts of *Punica granuatum*, *Aloevera and Carica papaya* shows maximum (12mm, 9mm, 10mm respectively) antimicrobial susceptibility pattern but extract of *Coleus forskohlii* and *Psidium guajava* shows minimum (5mm, 8mm respectively) antimicrobial susceptibility pattern and extract of *Curcuma longa* and *Fragaria vesca* does not shows antimicrobial susceptibility pattern. The data was found statistically non-significant. (Table. 4.15, Fig. 4.13)

In the case of *S. epidermidis*, extracts of *Carica papaya, Aloevera, Psidium guajava* and *Punica granuatum* shows maximum (13mm, 14mm, 12mm, 14mm) antimicrobial susceptibility pattern but extracts of *Coleus forskohlii, Curcuma longa and Fragaria vesca* does not show any antimicrobial susceptibility pattern. The data was found statistically non-significant. (Table. 4.16, Fig. 4.14)

Table. 4.15: Antimicrobial activity of P. acnes against different plant extracts

S. No.	***Plant Extracts***	***Average mean value (mm)***
1	*Curcuma longa*	00
2	*Carica papaya*	10
3	*Aloe vera*	9
4	*Punica granuatum*	12
5	*Fragaria vesca*	00
6	*Coleus forskohlii*	5
7	*Psidium guajava*	8

Figures in parenthesis indicates percentage

$F_{(cal)} = 65535$, $F_{(cal)} > F_{(cal)} = 8.9$

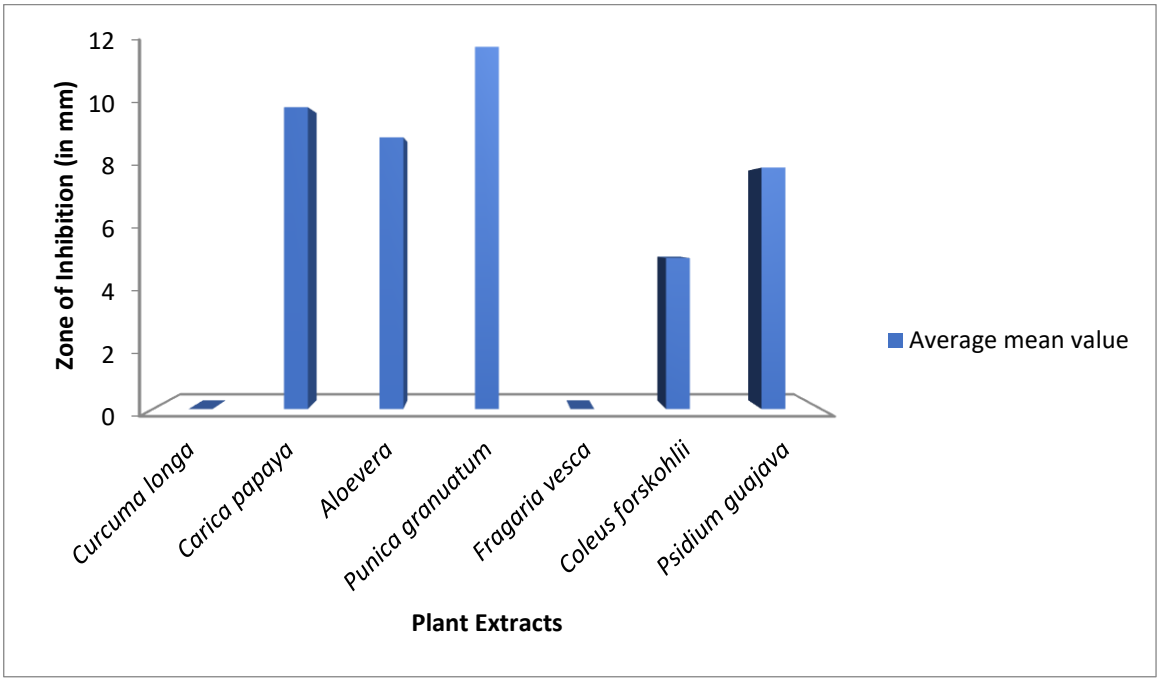

Figure. 4.13: Resistance of clinical isolate of P. acnes against different plant extracts

Table. 4.16: Antimicrobial activity of S epidermidis against different Plant extracts

S. No.	***Plant Extracts***	***Average mean value (mm)***
1	*Curcuma longa*	00
2	*Carica papaya*	13
3	*Aloe vera*	14

4	*Punica granuatum*	14
5	*Fragaria vesca*	00
6	*Coleus forskohlii*	00
7	*Psidium guajava*	12

Figures in parenthesis indicates percentage

$F_{(cal)}$ = 65535, $F_{(cal)} > F_{(tab)}$ =8.9

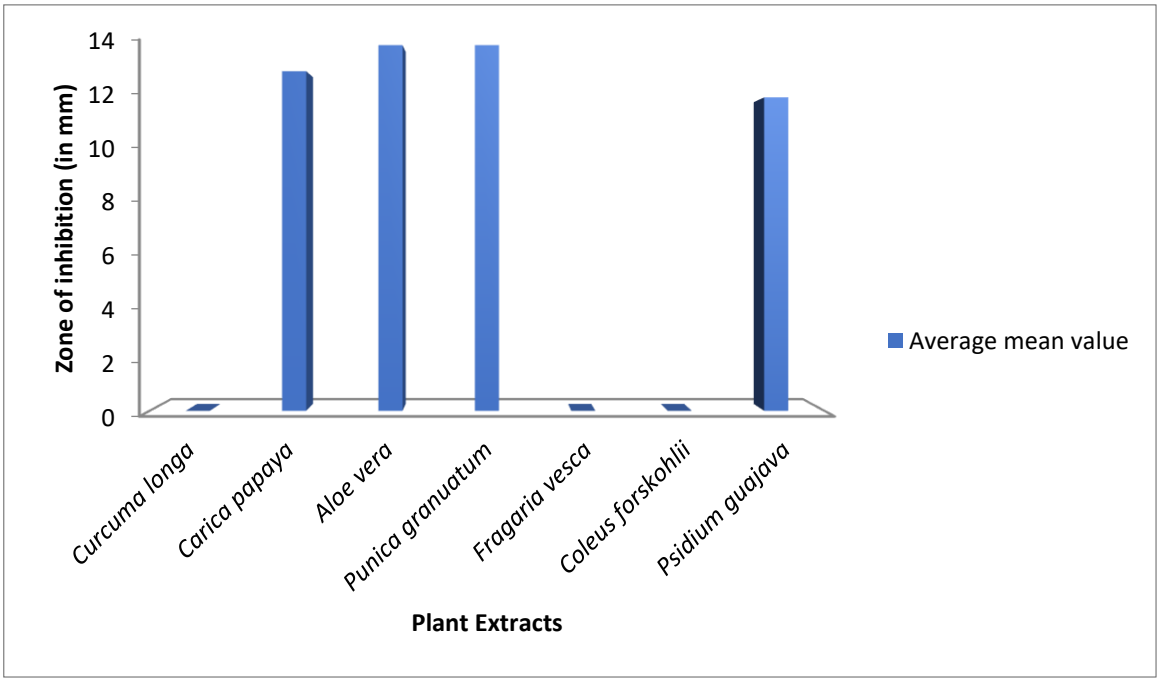

Figure. 4.14: Resistance of clinical isolate of S epidermidis against different plant extracts

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