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Effect of different face washes on the bacterial pathogens of skin

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Abstract

Pathogens like Propionibacterium acnes, *Staphylococcus aureus*, *E. coli*, and *Candida* species are kept at bay by the skin's natural flora. There are face washes and cleansers on the market now that eliminate specific skin germs and keep healthy skin infection-free. Each volunteer's face was used to take a sample, the sample was taken by gently scraping every surface of the participant's face, including the popped-up Acne, using a sterile swab dipped in normal saline. The gathered samples were cultured on Nutrient Agar, and the organisms that had developed there were recognised by Gram staining and the appropriate biochemical identification and placedin different levels. The organism was then put to the test against several isolated organisms using a sensitivity test (Kirby-bauer Method) using diluted versions of 5 different face washes. *Staphylococcus aureus*, *Micrococcus* spp., *Bacillus* spp., *E. coli*, and *Klebsiella* spp., were among the predominant strains among the 40 different strains that were recovered from the 40 different volunteers .The antimicrobial effects of five commercially available face washes Vicco, Cetaphil, Himalayas, Neem and Tulsi and Ponds were tested on the isolated organisms at dilutions of 1:10. When compared to other face washes, HIMALAYA has the strongest antibacterial activity and the second- highest maximum zone of inhibition against the following microorganisms: *Staphylococcus aureus*, Micrococci spp., and *Klebsiella* spp., According to the experiment, face washes PONDS and CETAPHIL have the least antibacterial activity, no zone of inhibition, and are resistant to all five detected organisms.

Keywords: Face washes; Acne; Kirby-bauer Method; Zone of inhibition; Resistance; Grams staining; Biochemical reaction; Micrococci; *Klebsiella*

1. Introduction

The body's largest organ, the skin, acts as the first line of defense against pathogens that can cause aging and other ailments, such as bacteria, fungi, dust, UV radiation, and other pathogens. According to [1], excessive reactive oxygen species generation contributes to erythema, changes in skin elasticity and structure, trans epidermal water loss, and skin cancers in addition to the harmful effects of UV radiation on skin. A balanced nutritious diet rich in antioxidant flavonoids is the best line of defense against the harmful effects of free radicals as according to [2]. Acne vulgaris is a common skin disorder. Since acne is not an infectious disease, factors such as controlling sebum production and taking antibiotics are usually what cause it [3]. In addition to some of the most common strains identified by the microbium studies, we also included several additional pathogens that is formed and involved in skin infections such as *streptococcus pyogenes, bacilli (Bacillus subtilis, B. cereus, Bacillus megaterium) Enterococci (E. fecium, E. fecalis),Micrococci* (*M. luteus ,M. kocuria rosea)* and *acinetobacter john sonei* [4][5]. According to [6], the skin rash brought on by candidiasis causes pimples, ulcers, dry areas, burning, and itching.

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Flavonoids are used in cosmetic formulation for both therapeutic and cosmetic reasons[7]. According to [8], plants contain more than a thousand compounds with polyphenol or flavonoid structures that are generally engaged in defense against UV radiation, UV-induced inflammation, oxidative stress, DNA damage, and the risk of skin cancer or pathogen *aggression. These* polyphenolics/flavanoids have been shown to preserve blood vessels by improving skin microcirculation [9]. Melanin synthesis is inhibited [10], the release of the inflammatory mediator histamine [11], erythema is reduced, and platelet aggregation is inhibited [12], all of which have a beautifying effect on the face.For systemic treatment, which is normally reserved for treating more severe acne, oral tetracyclines, oxytetracyclines, doxycyclines, minocyclines, or lymecyclines are widely used; trimetroprim and beta lactams are discouraged due to rising resistance [13]. A variety of plant parts, including leaves, stems, roots, bark, and fruits, are used to make herbal skin care products [14][15], others *Melaleuca alterbifolia* tea tree oil extract in gel form has reportedly been shown to lower skin care [16][17].

Acne is the main disease impacting children. Active breakouts of acne are more irritating and irritable since the skin is being protected from acne and lessened its effects as well as other potential infections. The wounds they leave behind are also disturbing. A common benefit of facial cleansing is the removal of dirt, oil, and other unwanted debris. Throughout the day the skin on your face is continually covered with bacteria, pollutants, viruses, dirt, and old (dead) skin cells. Daily facial washing removes these impurities to give the skin a fresh look. The five commercially available, branded face cleansers utilized in this study were used to either remove or minimize the impact of germs on the skin. The organisms are separated from the face, and following gram staining and morphological comparisons, the separated organisms are identified using biochemical processes. To determine which facewash is most efficient against each specific organism, this study compares the antibacterial activity of various face washes against isolated germs.

2. Material and methods

The study was completed in the Microbiology Laboratory of the MMM College of Health Sciences in Mogappair, Chennai, between 1st Januaryand 31st December, 2022.

2.1. Isolation of microorganisms from face

Isolation of microorganisms from the face was accomplished using sterile swab sticks that had been dipped in sterile saline. These samples were then inoculated onto nutrient agar and incubated at 37 degrees Celsius for 24 hours. Gram staining was performed as a preliminary step before the most powerful isolates were chosen and inoculated into peptone water.

2.2. Methodology

Study media used

For this investigation, Muller Hinton Agar (MHA) and Nutrient Agar (NA) were both used.

Aim and Objectives

The study's objectives are to describe and isolate common components of the facial microflora and to assess the antimicrobial potency of various face wash brands available on the market.

- Isolation of microorganisms from the face is one of our objectives.
- Biochemical approaches for isolating isolates.
- Testing for antibiotic sensitivity in isolates.
- Assessing the effectiveness of certain commercially available face cleansers as antimicrobials against isolates.

2.3. Isolation of Microorganisms

- Face samples were collected using sterile swab sticks dampened with sterile saline and then inoculated into nutritional agar and incubated at 37˚ Cfor 24 hours.
- Gram staining was performed as a first step before the most potent isolates were chosen and inoculated into peptone water.

2.4. Assessment of Isolated Organisms

The pathogenic organisms were isolated and identified using a variety of biochemical assays, including the Indole, Methyl Red, Voges-Proskauer, Urease, Catalase, Citrate Utilisation, Oxidase, and Hanging Drop Motility Testing tests.

2.5. Grams Staining

Using the gram staining method, the distinct pathogenic bacterial colonies were distinguished microscopically as gram positive and gram negative.

2.6. Antimicrobial Activity of Facewashes

Face washes from several brands, including Himalayas, Vicco, Neem tulsi, Cetaphil, and Ponds, were bought from the local market and utilised to compare the antimicrobial activities in accordance with the accepted practise.

The test MHA plates were prepared, and 0.1 ml of a chosen bacterial culture was evenly distributed on the MHA plates to prepare grass culture. The single dilution was prepared by dissolving 0.1 ml of face wash in 10 ml of sterile distilled water.

Following solidification, wells were created, and each well contained 20 to 30 micro liter of diluted face washes. The wells were then incubated at 37 ˚C for 24 hours.

By measuring the diameter in millimetres of the zone to which the face washes restrict the growth, the zone of inhibition was identified.

3. Results

- Isolation and identification of microorganisms from the face.
- 40 volunteers are grouped into 4 categories as follows:
  - Category 1: normal unwashed students face at college premises.
  - Category 2: People exposed to heavy traffic.
  - Category 3:Heavy pimpled face
  - Category 4: Freshly washed face.

They were cultured on to nutrient agar, after overnight incubation, the colony characters were noted by gram staining.

Table 1 Colony Characteristics Of Isolated Organisms (Fig 1)

Sl. No	Isolates	On Nutrient Agar	Gram Staining
1.	1.	Circular, powdery colonies, slightly golden yellow	Gram positive cocci
2.	2.	Orange, slightly mucoid round colonies.	Gram positive cocci
3.	3.	Mucoid, Raised colonies	Gram negative bacilli
4.	4.	Mucoid, flat, opaque colonies.	Gram negative bacilli.
5.	5.	Circular raised powdery colonies.	Gram positive bacilli.

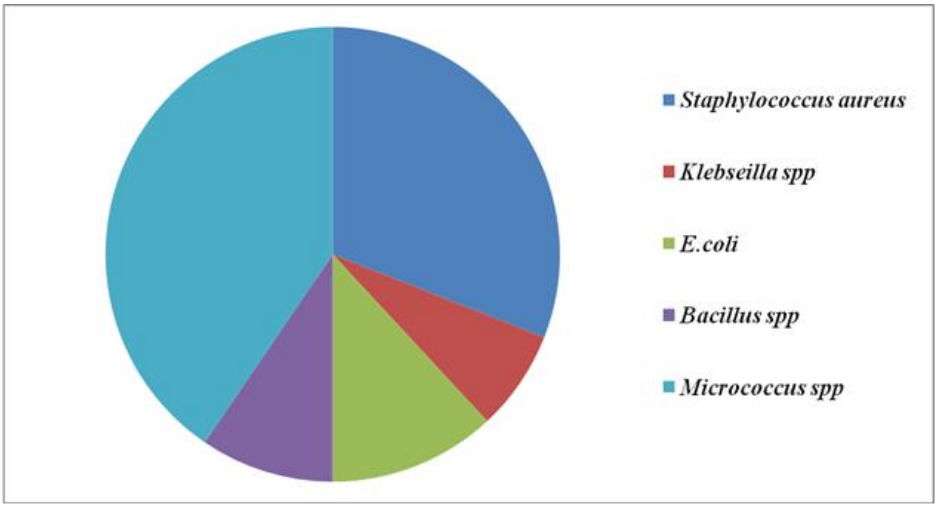

Figure 1 Number of samples containing the isolates

After overnight incubation five different colonies were observed. They are:

- Circular powdery colonies, slightly golden yellow, Gram positive cocci.
- Orange, slightly mucoid round colonies, gram positive cocci
- Mucoid, flat, opaque colonies, gram negative bacilli.
- Mucoid, raised colonies, gram negative bacilli.
- Circular raised powdery colonies, gram negative bacilli.

3.1. Biochemical reaction of the isolates

Different strains were further characterized by specific biochemical test were identified as shown in the table

Table 2 Biochemical reaction of the isolates

Isolates	Motility	BIOCHEMICAL TESTS							
		Indole	MR	VP	Citrate	Urease	Oxidase	Catalase	Organism identification
1.	Non-motile	-	-	+	+	+	-	+	*Klebsiella* spp.,
2.	Non-motile	-	+	-	-	+	-	+	*Staphylococcus aureus* (coagulase +ve)
3.	Non-motile	-	-	-	-	+	+	+	Micrococci spp.,
4.	Non-motile	-	-	+	+	-	-	+	*Bacillus* spp.,
5.	Motile	+	+	-	-	-	-	+	*E.coli*

+ --------Positive result; - ---------- Negative result

Different strains were further characterized by specific iochemical tests such as Indole, MR, VP, Citrate, Catalase, Coagulase, Oxidase, Urease and Motility . Based on these reactions the isolated organisms were identified as *klebsiella*, *Staphylococcus aureus*, *Micrococcus*, *E.coli* and *Bacillus*

- *Klebseilla* spp., is a non-motile gram negative *Bacillus*,it give positive results for VP, citrate, urease and catalase ,it gives negative results for indole, MR, oxidase tests.
- *Staphylococcus aureus* is a non-motile ,gram positive cocci arranged in grape like clusters,it shows positive for catalase, coagulase, urease and MR, it shows negative results for Indole, VP, citrate and oxidase

- *Micrococcus* spp., is a non-motile, gram positive cocci arranged in tetrads, it shows positive for urease, oxidase and catalase,it shows negative for citrate, VP, MR, Indole.
- *Bacillus* spp., is a non motile, gram positive bacilli,it gives positive results for catalase,citrate,and VP,it shows negative results for indole, MR, urease and oxidase.
- *E.coli* is a motile gram negative *Bacillus*,it shows positive for indole, MR, catalase and it shows negative for VP, Citrate, urease and oxidase

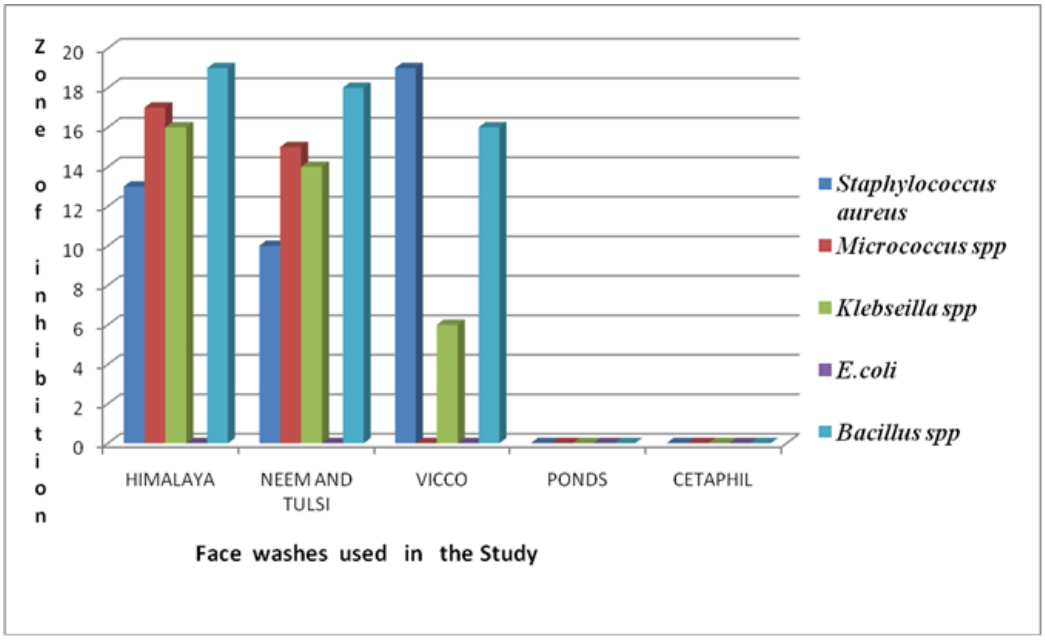

Figure 2 Antibiotic Susceptibility Test Of The Isolates

- *Staphylococcus aureus*was isolated from 13 samples.
  - It shows 13mm zone of inhibition to Himalaya face wash.
  - It shows 10mm zone of inhibition to Neem and Tulsi face wash.
  - It shows 19mm zone of inhibition to Vicco face wash.
  - It shows 0 mm zone of inhibition to ponds and cetaphil face wash.
- *Micrococcus* spp.,WAS ISOLATED FROM 17 SAMPLES
  - It shows 17mm zone of inhibition to Himalaya face wash
  - It shows 15mm zone of inhibition to Neem and tulsi face wash.
  - It shows 0mm zone of inhibition to vicco ,cetaphil and ponds face wash.
- *Klebsiella* spp., WAS ISOLATED FROM 3 SAMPLES
  - It shows 16mm zone of inhibition to Himalaya face wash.
  - It shows 14mm zone of inhibition to Neem and tulsi face wash
  - It shows 6mm zone of inhibition to Vicco face wash/
  - It shows omm zone of inhibition to Ponds and cetaphil.
- *E. coli* WAS ISOLATED FROM 5 SAMPLES
  - It shoes 0mm zone of inhibition to all face washes.
- *Bacillus* spp., WAS ISOLATED FROM 4 SAMPLES
  - It shows 19 mm zone of inhibition to Himalayas face wash.
  - It shows 18 mm zone of inhibition to Neem and Tulsi.
  - It shows 16mm zone of inhibition to Vicco face wash.
  - It shows 0mm zone of inhibition to ponds and cetaphil.

3.2. Biostatistical Inference

Table 3 Biostatistical Inference

Organisms	Himalaya	Neem And Tulsi	Vicco	Ponds	Cetaphil
Staphylococcus aureus	12±1.73	10.6±1.15	1.76±1.15	_	_
Micrococcus spp.	16.3±1.15	13.6±1.5	_	_	_
Klebsiella spp.	15.6±0.57	13.3±1.5	5.3±0.57	_	_
Bacillus spp.	18.3±1.15	16.6±1.15	15±1.73	_	_
E.coli	_	_	_	_	_

Values are in Mean ± Standard deviation

- *Staphylococcus aureus* shown maximum mean value of (12), for Himalaya facewash, followed by neem and Tulsi facewash and a lowest mean value for vicco facewash (1.76).
- *Micrococcus* spp. shown maximum mean value of (16), for Himalaya facewash, followed by least mean value of (13.6) for Neem and Tulsi facewash.
- *Klebsiella* spp. Shown a maximum mean value of (15.6) for Himalaya facewash, followed by neem and Tulsi facewash, with least mean value of (5.3) for vicco facewash.
- *Bacillus* spp. Shown a maximum mean value of (18.3) for Himalaya facewash, followed by neem and Tulsi face wash with least mean mean value of (15) to vicco facewash.

4. Discussion

A wide range of microorganisms are associated with skin infections of every human beings most notoriously in acnes. These pathogenic organisms can be removed by using antibacterial facewashes, body washes, soaps etc. According to my study, face washing is effective method to tone down these skin infections. In my study 40 different strains were isolated from 40 different volunteers, among 40 strains the major strains were tentatively identified as *Staphylococcus aureus*, *Micrococcus* spp.,*Bacillus* spp., *E. coli*, *Klebsiella* spp .,The isolated organisms were subjected to antimicrobial activity of 5 commercially available facewashes viz, Vicco , Cetaphil, Himalayas, Neem and Tulsi, Ponds in 1:10 dilutions. Among the face washes used, HIMALAYA has the highest antibacterial activity with maximum zone of inhibition against Staphylococcus, Micrococci and *Klebsiella*, and NEEM TULSI FACEWASH has the second maximum zone of inhibition against the following organisms. Face washes PONDS and CETAPHIL has the least antibacterial activity with no zone of inhibition and being resistant to all the five isolated organisms, which is confirmed from the investigation.

The study conducted by Mundi KS et al., is about the combined antibacterial activity of face cleansing agents and Psidiumguajavaleaf extract on MRSA. The face cleansing agent did not have any activity against MRSA isolates, while two of the undiluted forms of the face cleansing had slight activity against sensitive non-MRSA laboratory strains. Furthermore, after incorporating the face cleansing agents with the Psidiumguajava leaf extract, it produced a synergistic antimicrobial activity against MRSA. This is in line with the report that the combination of the antimicrobials and plants produced synergistic antibacterial activity against resistant bacteria. Also the result of synergism of antimicrobials and Methanolic extract of Psidiumguajava leaf extract was reported and this indicated that these combinations can be employed to fight or reduce drug resistance for disease caused by MRSA.

Another study conducted by PG Kareuet al., showed that T.diversifolia soap exhibited the highest activity against *E. coli* . This was demonstrated by the increased inhibition of *E. coli* at all the T.diversifolia extract concentration in the herbal soap. The aloe and the neem soap had comparitive activity against *E. coli*. Inhibition of C.albicans by the T.diversifolia soap was ineffective below 9% concentration of thithonia extract in the soapsand had the least effect against the test fungus when compared to the other soaps. These results justified the traditional use of T.diversifolia in the treatment of skin infection. The A.secundiflora and neem extract were earlier reported to be used for the skin conditions. The reported antimicrobial properties of the neem and the A. Secundiflora plant extract were confirmed in this investigation. However, the activity of the herbal soaps on the test organisms were significantly different and dependent on the extract concentration.

5. Conclusion

From this study, *E.coli, Staphylococcus spp.,, Klebsiella spp.,, Micrococci spp.,, Bacillus spp.,* have been isolated with predominance of *Staphylococcus aureus and Micrococcus spp.* Among the face washes used, HIMALAYA has the highest antibacterial activity with maximum zone of inhibition against *Staphylococcus, Micrococci* and *Klebsiella* ,and NEEM and TULSI FACEWASH has the second maximum zone of inhibition followed by VICCO against the following organisms.Face washes PONDS and CETAPHIL has the least antibacterial activity with no zone of inhibition and being resistant to all the five isolated organisms, which is confirmed from the investigation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Hereby the authors stating that 'The present research work does not contain any work performed with the live samples from animals/humans subjects"

Statement of informed consent

Authors stating that "Informed consent was obtained from all the individual participants included in the study.

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