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Original Research Article

**Topical herbal formulation for the treatment of *Acne vulgaris***Pragathesh Singh K<sup>\*1</sup>, Latha D<sup>1</sup>, Monisha M<sup>1</sup>, Esther Monisha M<sup>1</sup>, Jayasree A S<sup>1</sup>, Muthulaxmi V<sup>1</sup>,  
Vijayalakshmi S<sup>2</sup> and Radhika Jevanand<sup>2</sup><sup>1</sup>School of Biological Sciences, CMS College of Science and Commerce, Coimbatore-049, Tamil Nadu, India<sup>2</sup>Department of Microbiology, D.G Vaishnav College, Arumbakkam, Chennai, India

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Coimbatore, Tamil Nadu- 049 India**\*Article History:****Received:** 15/06/2017**Revised:** 27/07/2017**Accepted:** 27/07/2017**DOI:** <https://doi.org/10.7439/ijbr.v8i7.4289>**Abstract****Background:** *Acne vulgaris* is the most common bacteria causing chronic inflammatory skin disorder of pilosebaceous unit that affects areas containing the largest oil glands, including the face involving abnormalities in sebum production. *Acne vulgaris* is characterized by both inflammatory (papules, pustules, and nodules) and non-inflammatory (comedones, open and closed) lesions. Acne is a universal disease occurring in all races and affecting 95% of boys and 83% of girls.**Methodology:** *Propioni bacterium acnes* and *Staphylococcus aureus* are common pus-forming microbes responsible for the development of various forms of *Acne vulgaris* and the same organisms were isolated from the human volunteer. This research mainly focuses on the treatment of acne using various herbs and making a poly herbal formulation. The comparative analysis of both solvent and aqueous extraction was done to reveal the fact that the selected ingredient can be used as a poly herbal face care powder for cosmetic purpose.**Result:** The aqueous and methanolic extraction shows better antibacterial activity against the isolated organisms. Thus in the present work, herbs having good properties for the face care powder formulation have been identified.**Conclusion:** Natural herbal extracts in combination can be effectively utilized for the treatment of Acne. Use of such products is far better than the use of synthetic ointments for the control of Acne.**Keywords:** *Staphylococcus aureus*, aqueous and methanolic extraction, anti-bacterial activity.**1. Introduction**

*Acne* begins just before puberty when the sebaceous glands enlarge and reaches a maximum prevalence in early adolescence when sebum output reach its peak.[1] An increase in circulating androgens at the onset of puberty stimulates the production of sebum into the pilosebaceous unit.[2] These events combine to create an environment within the pilosebaceous unit that is favorable for the colonization of the bacteria, *Propioni bacterium acnes*.[3] Sebum plays a central role in the pathogenesis of acne.[4,5]

From the period of ancient times, herbal therapies are used for the treatment of acne. This includes various herbal extracts, oil, herbal powder and their ayurvedic formulation.[6,7] There is a variety of medication for acne vulgaris including topical agents, oral antibiotics, oral

retinoid and oral hormonal therapies.[8] The use of natural remedies, particularly herbal medicine, dates back thousands of years.[9] Over the last decade, in view of increasing resistance to existing anti-microbial agents, side effects and sometimes high cost of treatment, interest in medicinal herbs has been progressively increased.[10]

The introduction of novel herbal formulations for the treatment of acne may produce many advantages over previously used therapies.[11] These herbal drugs are effective against a variety of Gram-positive and Gram-negative Bacteria.[12] The herbal extracts have negligible adverse effects compared with modern medicine for the treatment of acne. The effectiveness of the herbal agents in acne treatment is not only based on antimicrobial activity but also on the antioxidant and anti-inflammatory properties

by which neutrophil migration is inhibited.[13] Thus, natural substances, which are obtained from the medicinal plant, having antibacterial and anti-inflammatory activity, are commonly employed for the treatment of acne.[14] In the treatment of acne herbal extracts, herbal powder or oil may be used as monotherapy or in combination therapy.[15] The advantage of herbal cosmetics is their non-toxic nature, reduce the allergic reactions and time-tested the usefulness of many ingredients. The aim of this study was to evaluate the antibacterial activity of poly herbal extract against the isolated bacteria from pimple samples. The qualitative analysis of the antibacterial activity of both solvent and aqueous extract was determined.

## 2. Materials and Methods

### 2.1. Sample collection

Samples were collected from human face pimples by breaking open Acne pores on the face of patients. Swab sticks were used in collecting samples. The Samples were obtained by collecting Pus from the Pimples. The swabs were streaked on Mannitol salt agar plates. This process was repeated for all the samples. The streaked plates were incubated at 37°C for growth.

### 2.2. Subculture

The colonies from the plates were observed for the morphological difference. Each colony that differs morphologically from another was picked with a sterilized wire loop and inoculated in a freshly prepared nutrient agar slant bottles and incubated for 24 hours at 37°C in order to obtain pure colonies of the isolates.

### 2.3. Gram staining:

A drop of distilled water was placed on a clean grease free glass slide and a colony in isolates was picked with a sterilized wire loop and emulsified. The glass slide was passed over the flame three times to heat fix. The smear was flooded with crystal violet for 60 seconds and rinsed with distilled water. Gram's iodine was added, then decolorized with ethanol and rinsed immediately with distilled water. The smear was counter stained with Safranin for 1-2 minutes and rinsed with distilled water. The smear was air dried and viewed under the compound microscope.

### 2.4. Biochemical tests

#### a. Coagulase test:

A drop of physiological saline was put on a clean glass slide, followed by making a smear of 24 hours old isolate of the test organism. Then a drop of human plasma was added into it to make a suspension. Clumping indicated that the test organism has the ability to produce coagulase.

#### b. Catalase test:

One drop of H<sub>2</sub>O<sub>2</sub> solution and a loopful of inoculum were placed on a clean glass slide; the presence of gas bubbles indicated a positive result.

#### c. Citrate test

This detects the ability of an organism to use citrate as the sole source of carbon. Simmon citrate agar was prepared and test organism was inoculated and incubated at 37°C for 24 hours.

#### d. Triple Sugar Iron agar (T.S.I Agar)

The medium contains three sugars: glucose, sucrose, and lactose. The homogenized agar was dispensed into test tubes. It was then allowed to solidify in slanting position. The organism was inoculated by streaking into medium and incubated at 37°C for 24 hours. Fermentation of any of the sugars is indicated by the change in colour from red to yellow and crack or raise in the medium indicates gas production is a positive result, while no change is a negative result.

#### e. Indole test:

The test organism was inoculated in peptone water and incubated at 37°C for 24 hours, 0.5ml Kovac's indole reagent was added and gently shaken. In a positive test, indole (present in the culture) dissolves in the reagent which then becomes pink or red and forms a layer at the surface of the medium.

#### f. Methyl red test (MR Test):

The test organism was inoculated into MR-VP broth and incubated at 37°C for 24 hours. Few drops of methyl red were added to the culture. MR positive test is indicated by red colour formation while no change is negative test.

#### g. Voges- Proskauer test (VP Test):

The test organism was inoculated in MR-VP broth and incubated at 37°C for 24 hours. After incubation Barrit's reagent A and B in the ratio 3:1 is added mixed well. The presence of red colour indicates a positive test for Voges-proskauer.

**Table 1: Herbal plants name & botanical name**

Plants name	Botanical name
Korai kizhangu	<i>Cyperus rotundus</i>
Kasturi manjal	<i>Curcuma aromatic</i>
Poola kizhangu	<i>Amorphophallus paeoniifolius</i>
Avaram poo	<i>Senna auriculata</i>
Thulasi	<i>Ocimum tenuiflorum</i>

### 2.5. Herbal extraction

The herbs were procured from the local market (Table 1). The collected herbs were dried, powdered and then passed through sieve No. 100, mixed geometrically.

The dried powder of combined form had passable flow property which is suitable for a face pack. The particle size of the powder was found to be 20 -25µm. The microscopical characters of dried powder of combined form were noted.

Fresh leaves of *Cyperus rotundus*, *Curcuma aromatic*, *Amorphophallus paeoniifolius*, *Senna auriculata*, *Ocimum tenuiflorum* was shadow dried at 37°C. Drying was done to reduce the moisture content of leaves to less than 20%. Dried leaves were grounded to make fine powder for the extraction of desired materials. The fine powdered material was extracted to obtain the active substances with the suitable solvent (methanol). 10 grams of powdered leaves were extracted in 100ml of 80% methanol for 18 hours under shaking condition.[13]

#### 2.6. Antibacterial activity:

The extract was poured on the Muller Hinton agar swabbed with the culture. After 24 hours incubation, the zone of inhibition was observed. It indicated that the herbal extraction has the ability to prevent the growth of organisms.

### 3. Results

From the research we have isolated the Gram-positive rods and cocci from the pimple samples of individuals using sterile swab sticks. The organisms include *Staphylococcus aureus*. It was found that *Propionibacterium acnes* have the highest percentage frequency of (43.8%) followed by *Staphylococcus aureus* (39.5%). The biochemical analysis conducted showed positive results for catalase, coagulase, Indole, TSI, Voges Proskauers test. In that, the result *Propionibacterium*, and *Staphylococcus aureus* were identified. The antibacterial activity of herbal extraction was measured. The results showed that the herbal extractions were effective but specifically the 80% concentration of aqueous herbal extract was good. The zone of inhibition and Minimum inhibitory concentration (MIC) was increased on *Propionibacterium acnes*.

### 4. Conclusion

In future, further optimization studies are required in this study to find the useful benefits of face care powder on human use as the cosmetic product. Also, further studies like organoleptic evaluation, physicochemical characterization, general powder, microscopical characters, chemical evaluation, etc., is to be done.

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