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Antibacterial Property of *Cayratia trifolia* L. as an Alternative Treatment for Boils

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Abstract

This study investigated the antibacterial property of the *Cayratia trifolia* L. alcoholic leaves extract (CALE) to boils caused primarily by *Staphylococcus aureus*. Microbiological tests like culture, Gram's stain and coagulase test were employed to isolate and identify the bacteria. CALE at concentrations of 25%, 50%, 75% and 100% were utilized to determine whether they could produce significant inhibition of the growth of the boil-causing bacteria. Results of the susceptibility test showed that the CALE concentrations exhibited antibacterial property with 25%, 50%, 75% and 100% recorded a mean zone of inhibition of 18.33 mm, 20.67 mm, 23.67 mm and 25.67 mm, respectively against a control drug. In conclusion, 75% and 100% CALE treatments possess a comparable antibacterial property as an alternative remedy for boils.

Introduction

Boils are painful, pus-filled bumps that form under the skin when bacteria infect and inflame one or more of the hair follicles [1]. Most boils are caused by *Staphylococcus aureus*, a type of bacteria commonly found on the skin and inside the nose. Boils sometimes develop at sites where the skin has been broken by a small injury or an insect bite, which gives the bacteria easy entry [2]. Drugs of plants origin are gaining popularity and investigating for the various diseases including boils. The objective of the present study was to evaluate the effectiveness of leaves extract in treating boils. *Cayratia trifolia (C. trifolia)* Linn. is commonly known as Fox grape in English. It is native to India, Asia and Australia. Flowers are small greenish white and brown in color [3]. Whole plant of *C. trifolia* has been reported to contain yellow waxy oil, steroids/terpenoids, flavonoids and tannins upon preliminary phytochemical screening. Leaves contain stilbenes (piceid, reveratrol, viniferin, ampelopsin). Stem, leaves, roots are reported to possess hydrocyanic acid and delphinidin. Several flavonoids such as cyanidins are reported in the leaves. This plant also contains kaempferol, myricetin, quercetin, triterpenes and epifriedelanol. Crude extract of this plant was tested in preliminary biological screening for their antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus* and *P. oxalium*. Precleaned extract was also investigated for their ability to inhibit protein kinase and tyrosine-specific protein kinase of epidermal growth factor [4].

Materials and Methods

1. Harvesting the Plant Material

Matured leaves of *Cayratia trifolia Linn.* were harvested from the herbal garden of the University. Then, the leaves were washed with running water prior to air-drying them under the shade for three (3) days. The air-dried leaves were oven-dried at 60^{o}C prior to powdering utilizing an electric grinder.

2. Extraction Method

Dried leaves of *Cayratia trifolia Linn.* were used for extraction. Extraction was made by shaking the plant material with ethanol (at a ratio of 1:10, w/v) for 3 days. The extract was filtered through three (3) layers of cheesecloth to remove plant debris and then through Whatman no. 1 filter paper. CALE concentrations of 25%, 50%, 75%, and 100% were prepared, using the following formula:

$$\% \text{ (v/v)} = \frac{\text{Volume of solute in ml}}{\text{Total volume of solution in ml}} \times 100$$

To prepare 25 percent crude alcoholic leaf extract of *Cayratia trifolia Linn.,* 0.25 ml of the plant extract was transferred into a beaker and distilled water was added up to 1-ml calibration mark. On the other hand, 0.50 ml and 0.75 ml of the plant extract were used to prepare the 50 percent and 75 percent concentrations, respectively. Distilled water was added up to the 1 ml calibration mark. The 100 percent concentration is the pure plant extract.

3. Microbiological Tests

Collection of discharge from a person with boils. Specimen collection for culture and sensitivity testing was carried out under standard precautionary techniques in order to avoid contamination of the specimen. Gloves, sterile specimen containers, careful preparation of culture site, and length of time between collection and actual laboratory preparation or testing could impact the results of culture growth. In addition, each organism's growth requirements, such as oxygen, moisture, temperature and nutrients, were considered. The researchers used cotton swab to collect a discharge from a person with boils that contain the bacteria *Staphylococcus aureus.*

Culture. Culture is defined as a laboratory test by which samples from body specimens are cultivated in a special growth medium in order to isolate the microorganisms that may be present. Culture is a highly effective laboratory method for identifying the microorganisms that cause the infectious disease and for obtaining definitive diagnosis. The researchers used Blood Agar Plate (BAP) for culturing the boil-causing bacteria.

Isolating bacterial colonies. The researcher inoculated the collected specimen using cotton swab in Fluid Thioglycollate (FT) to allow the initial growth of suspected bacteria, *Staphylococcus aureus*. Bacterial growth was indicated by turbidity. Using a sterile inoculating loop, a loopful of the bacteria from FT was transferred and inoculated onto a blood agar then incubated for 18-24 hours at 35°C. Presence of bacteria was observed after incubation. Bacterial growth was indicated by colonies.

Gram's stain. The Gram stain was employed to identify the bacteria [5]. *Staphylococcus aureus* is Gram-positive.

Coagulase test. To test for coagulase, a loopful of rehydrated coagulase plasma was placed on a clean slide. A loopful of water was added and a heavy suspension of the bacteria to be tested was prepared. Clumping of the bacterial cells was observed (clumping = coagulase - positive; no clumping = coagulase - negative) [6].

Susceptibility test. Selected colony from the blood agar culture was inoculated into Trypticase Soy Broth (TSB) to obtain a uniform suspension. The uniform suspension was prepared by emulsifying a loopful of bacterial colony into five (5) ml TSB [7]. Using Mueller Hinton Agar (MHA), the susceptibility test was performed using the agar diffusion method wherein 5 ml of bacterial suspension

(comparable to the 0.5 MacFarland standard) is added to melted Mueller Hinton agar and poured over solidified agar in a petri plate.

Forceps was sterilized by dipping in alcohol and burning off the alcohol which was then used to pick up disks impregnated with the control drug, 5 ug *oxacillin* and the paper disks dipped into the different CALE concentrations. These were placed on the surface of the agar. The disks were gently tapped to ensure better contact with the agar. There were three disks dipped into each of the treatment. One disk was assigned to each petri plate. Therefore, 3 petri plates each treatment were utilized. Three petri plates were also used for the control drug.

The following steps were then followed: incubated and inverted at 35^0C for 18-24 hours; measured the zones of inhibition in millimeters, using a ruler on the underside of the plate; recorded the zone size; computed the average zone of inhibition for the control drug and the experimental treatments [19]. The wider the zone of inhibition, the greater is the bactericidal activity of the CALE concentrations. The zones of inhibition of the six (6) disks dipped in the various concentrations of crude alcoholic leaf extracts were read and compared with the control drug.

4. Statistical Treatment

The researcher utilized the one-way ANOVA at .01 level of significance and the Tukey HSD test to determine the differences of the different CALE treatments.

Results and Discussion

The susceptibility test results of the 25%, 50%, 75%, 100% CALE and the control drug yielded a mean zone of inhibition of 18.33 mm, 20.67 mm, 23.67 mm, 25.67 mm, and 25.33 mm, respectively. In reference to the 75% and 100% CALE, there was no statistically significant difference (P.<.01) between the treatments and the control drug. This implies that these 2 treatments were comparable with the control drug in terms of antibacterial property.

Conclusion

The *Cayratia trifolia* L. leaves extract (75% and 100% treatments) exhibited antibacterial property against the boil-causing bacteria, *Staphylococcus aureus*.

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