Anti-acne activity of *Casuarina equisetifolia* bark extract: A randomized clinical trial
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**Abstract**

The objective of this study was to evaluate the clinical efficacy of *Casuarina equisetifolia* bark extract (5% cream) in comparison with benzoyl peroxide as the standard drug for acne vulgaris. After exclusion, fifty patients were included in the study (n=50) with age group distribution between 17-34 years of age. Patients were distributed into Group 1 (5% cream) and Group 2 (benzoyl peroxide), and were advised to apply the herbal cream twice a day topically. The clinical efficacy of the herbal cream and severity of acne vulgaris was assessed by Cook’s acne grading scale. Results revealed that there was no significant difference found in both test and standard control groups. Further, remarkable improvement was found in acne grading in the test group.

**Introduction**

Acne vulgaris is a common chronic disease of skin with multi factorial etiology (Lalla et al., 2001). The onset of acne is earlier in females than males, but it is found that severe forms of acne are frequent in males (Adityan and Thappa, 2009). Predominantly, acne appears on the face (99%), back (60%) and chest (15%) (Burns et al., 2004; Hunter et al., 2003). *Propionibacterium acne*, an anerobic bacterium, found within the pilosebaceous follicle, is the most common pathogen being responsible for inflammatory acne. Whereas, superficial infections of the sebaceous units are mostly caused by *Staphylococcus epidermidis* (Burkhart et al., 1999; Kaur et al., 2010). Acne is managed with both topical and systemic drugs (Magin et al., 2006; Thiboutot et al., 1999). Benzoyl peroxide is commonly used as the first-line drug for mild and moderate acne, but it causes redness, itching and irritation in applied areas. The use of antibiotics to treat acne has increased antibiotic resistance (Eady et al., 1994). In such conditions, to overcome the ongoing problem of bacterial resistance, medicinal plants can be used as an alternative option (Chomnawang et al., 2005). Due to enormous beneficial capabilities, herbal therapies can effectively be used as modern alternatives in the management of acne (Shweta and Swarnalatha, 2011).

*Casuarina equisetifolia* L. family Casuarinaceae is an ornamental plant grown in Pakistan, India, tropical Africa and Sri Lanka. Due to the presence of amino acids, taraxerol, lupenone, lupeol, alicyclic acid, gallic acid and sitosterol, this plant is reported as antibacterial, antifungal, anti-inflammatory, anticancerous, antioxidant and analgesic (Vijayalakshmi et al., 2014). A polyherbal gel containing *C. equisetifolia* has shown anti-acne activity in rats (Thube and Patil, 2013). The present study is designed to assess the clinical efficacy and cutaneous...
tolerance of methanolic bark extract of *C. equisetifolia* (5% cream) in the management of acne vulgaris.

**Materials and Methods**

*The collection and authentication of plant material*

The bark of *C. equisetifolia* was collected from various regions of Karachi in 2011. The sample was authenticated by Prof. Dr. Ghazala H. Rizwani, Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. Voucher specimen (No 0091) was deposited in the Herbarium of Department of Pharmacognosy Herbal Museum, Faculty of Pharmacy, University of Karachi, Pakistan, for future reference.

*Extract preparation*

The plant material (2 kg) was cleaned, air dried under shade, coarsely grounded and extracted with 90% methanol for 15 days at room temperature with continuous shaking. After 15 days, the extract was filtered with Whatman No 1 filter paper and made solvent free using a rotary evaporator (Buchi, Switzerland) under reduced pressure at 40ºC. The dry extract (183 g) was obtained after complete evaporation of methanol (Ali et al., 2013).

*Formulation of 5% cream*

Contents constituting oil phase (cetostearyl alcohol 10 g, cetyl alcohol 3 g and cetomacrogol 2 g) were taken in a 100 mL beaker and were subjected to heating by using a magnetic stirrer with hot plate (Model # MM 31, Singapore) until melted at 60ºC (Table I). In another beaker, water phase contents were added (methyl paraben 0.25 g, propyl paraben 0.15 g and EDTA 0.1 g) and allowed to melt at the same temperature of 60ºC. When both oil and aqua phases achieved same molten conditions at 60ºC, then water phase was added into the oil phase with continuous stirring till temperature dropped and fine and homogenous cream base was obtained.

Crude bark extract 5 g was firstly homogenized into fine particles in a mortar and pestle and then was soaked in 16 mL deionized water for 24 hours. After the specified time period 2 mL propylene glycol and tween 60 (0.5%) were added in the same mortar for proper trituration to yield smooth extract. Then this extract was firmly incorporated into the cream base with final concentration of 5% w/w (Allen, 2005).

*Skin irritation tests*

Two different types of skin irritation tests were conducted to ensure the safety profile of the product. Skin irritation tests were carried out by applying the cream on intact and broken skin of animals and humans.

*Patch test*

The cream was applied to animals (rats) and the human volunteers’ skin. The areas were marked and the results were checked after 48 hours for the presence or absence of edema and/or erythema (Tony et al., 2004).

*Prick test*

In prick test, the skin of sensitive individuals was prick-ed with a sterile needle and herbal cream was applied to it. The outcomes were noticed after 3-4 hours of skin examination (Tony et al., 2004).

*Anti-acne activity*

The clinical efficacy of herbal cream against acne vulgaris was assessed by Cook’s acne grading scale. Results revealed that there was no significant difference found in both test and standard control groups (Cook et al., 1979; Azad et al., 2012)

**Study locations**

To evaluate the efficacy of newly formulated cream from *C. equisetifolia* bark extract, clinical trials were conducted in the Outpatient Department of Institute of Skin Diseases, Sindh, Karachi and Barkat Clinic, Malir, Karachi, from 2013 to 2014.

**Study design**

This is a randomized and comparative clinical trial. The patients were randomly allocated into 5% cream of *C. equisetifolia* bark extract and benzoyl peroxide groups by using the lottery method.

**Study population**

Seventy patients were included in the study from both sexes, in the age group between 17 to 34 years. Out of

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Table I

| Ingredients            | Quantity per 100 g of cream |
|------------------------|----------------------------|
| Oil phase              |                            |
| Ceto stearyl alcohol   | 10 g                       |
| Cetyl alcohol          | 3 g                        |
| Cetomacrogol           | 2 g                        |
| Water phase            |                            |
| Methylparaben          | 0.25 g                     |
| Propelparaben          | 0.15 g                     |
| EDTA                   | 0.1 g                      |
| Water                  | QS (Quantity sufficient)    |
| *C. equisetifolia* crude bark extract | 5 g soaked in 16 mL of water |
| Propylene glycol       | 2 mL                       |
| Tween 60               | 0.5 mL                     |
these 20 patients were dropped out from study, finally 50 patients were randomly divided into two groups, 25 patients in each group. Group 1 received C. equisetifolia bark extract (5% cream) and Group 2 received benzoyl peroxide as the standard drug.

Inclusion criteria include a) males and non-pregnant or non-nursing females, b) patients of 17 years or older age, c) patients with acne vulgaris.

Exclusion criteria include a) patients with any mental and physical insufficiencies, genetic and endocrine disorders and other systemic illness, b) breast feeding mothers and pregnant women, c) acne patients except acne vulgaris (acne fulminans, acne rosaceous, acne necrotic), d) patients under 17 and above 34 years of age, e) patients who took any anti acne medication like isotretinoic acid, clindamycin gel or any oral antibiotic for acne treatment in last two months or any hormonal preparations, e.g. oral contraceptive pills.

**Study procedure**

At the start of the study, a detailed physical examination along with detailed history, including age, sex, socio-economical status, family history of acne, dietary pattern, previous illness, details of cosmetics or other skin products used as well as seasonal variation of acne was taken. Following satisfaction of entry criteria and screening procedures, baseline observations were noted on the proforma, especially designed for this study, which consisted of two parts, the 1st part was related to the personal information of the patients such as age, sex, occupation etc. while the 2nd part of the proforma was based on clinical history of the patients such as number of white heads, black heads, papules, pustules and nodules as well as duration of the treatment used.

Out of fifty patients, twenty five patients were allocated to the herbal formulation (5% cream) and 25 patients to benzoyl peroxide. Each patient in Group 1 was provided with herbal formulation in a 30 g container. The patients were thoroughly explained about the method of application of herbal formulation. They were instructed to apply the cream twice daily on the acne affected areas and then wash with lukewarm water. Patients were advised not to take any other drugs. The duration of the treatment was 45 days. Patients on benzoyl peroxide (Group 2) were directed to apply the drug once daily at night. Each patient was advised to report the clinic every week. Visual observation was done to evaluate the efficacy of the drug, as the visual appearance of the skin considered as one of the basic parameters in comparing the efficacy of different treatments. Photographs of the patients were taken during every follow up and at the baseline level by using a digital camera in order to determine the reduction in the presence of black and/or white heads, inflamed papules and/or pustules, cysts and nodules (Spittle et al., 1980).

**Statistical analysis**

Statistical analysis was done by using SPSS program (Version 20) Man Whiteney U-test and Wilcoxon Syned rank test were used to calculate the significance and probability of baseline and six-week readings.

**Results**

The demographic observation in the study showed the highest rate of occurrence (62%) of acne in the age group of 17-22 years, whereas the lowest rate of occurrence (6%) was observed in the age group of 29-34 years. Thirty two (64%) patients were females and eighteen (36%) patients were males (Table II).

| Age distribution of patients and incidence of acne | 17-22 (years) | 23-28 (years) | 29-34 (years) |
|----------------------------------------------------|---------------|---------------|---------------|
| Male                                               | 12 (24%)      | 5 (10%)       | 1 (2%)        |
| Female                                             | 19 (38%)      | 11 (22%)      | 2 (4%)        |
| Total                                              | 31 (62%)      | 16 (32%)      | 3 (6%)        |

The results of the skin irritation tests did not show any kind of irritation like itching, erythema and burning sensation which shows that neither the plant extract nor the excipients used in the formulation of the cream produce any kind of allergic reactions.

In the test group, 9 (18%) patient exhibited grade 8, 11 (22%) patients exhibited grade 6, 16 (32%) patients exhibited grade 4, 14 (28%) patients exhibited grade 2, and no patient exhibited grade 0 at baseline grading. Whereas in the control group, 8 (16%) patients exhibited grade 8, 13 (26%) patients exhibited grade 6, 17 (34%) patients exhibited grade 4, 12 (24%) at baseline. Baseline grading of acne vulgaris of 5% cream and the benzoyl peroxide group was compared with Man Whitney test. There was no statistical significant difference between two groups. Remarkable response, in acne grading, in both test and control groups, was observed at the end of the treatment. Twenty five (50%) patients exhibited grade 0, 18 (36%) patients showed grade 2, 5 (10%) patients showed grade 4, only 2 (4%) patients showed grade 6, and no patients exhibited grade 8 after treatment in the test group (Table III). Similarly, in benzoyl peroxide group, 27 (54%) patients exhibited grade 0, 18 (36%) patients exhibited grade 2, 4 (8%) patients exhibited grade 4, 2 (4%) patient exhibited grade 6, and no patients exhibited grade 8 after the treatment (Table III).

Wilcoxon Signed rank test for paired data was used to analyse the improvement in acne grading in both groups. The base line grading and grading at the end of the study showed a significant difference with $p<0.001$. The results of the improvement in acne grading, obtained by using a five point scale, demonstrated that
in 5% cream (test group), excellent response was observed in 16 (32%) patients, good response in 31 (62%) patients, poor response in 2 (4%) patients and no response in 1 (2%) patient. Whereas in benzoyl peroxide (control group), excellent response was seen in 13 (26%) patients, good response in 26 (52%) patients, poor response in 7 (14%) patients and no response in 4 (8%) patient (Table IV). The safety and cutaneous tolerance of the cream and standard drug was assessed by erythema, burning, pruritus, dryness and scaling. In 5% cream group, no obnoxious adverse effects were seen during and after the study, and the cream was well tolerated by the patients. However, 17% patients of the benzoyl peroxide group complained of irritation and redness along with swelling of skin.

Discussion

*C. equisetifolia* bark extract (5% cream) was checked for *in vivo* anti-acne activity. Drug response and clinical analysis were assessed by using five-point grading scale and Cook’s acne grading scale respectively. Both types of drugs were found to be effective against acne vulgaris; however, remarkable improvement was found in the group 1 in acne grading at p<0.001. Decline in acne grading was observed in both groups, although 5% cream was well accepted by the patients because of significant results without any burning, itching and other side effects. Patients with non-inflammatory acne (black and white comedones) were cured in 2-3 weeks while participants with inflammatory acne (papules, pustules and nodulo-cystic acne) were cured in 4-6 weeks depending on the severity of acne flare.

As *C. equisetifolia* has shown to possess anti-inflammatory, anti-oxidant, anti-bacterial and anti-fungal potential. The synergism between these activities might have lead to decline in acne grading. However, the exact mechanism for inhibition of *propionibacterium acne* and *Staphylococcus epidermidis* is not known, but it may be due to the components of the plant which make complex with extracellular and soluble proteins and with bacterial cell wall. *C. equisetifolia* bark extract (5% cream) has shown statistically significant result and could be used as an alternative in dermatological settings in the management of acne vulgaris.

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Ethical Issue

The diagnosed patients of acne vulgaris were registered in the study after receiving their written informed consents. The patients who refused to fill in the consent forms were excluded from the study. This research was approved and registered by

### Table III

| Grade | Number of patients | Baseline | 1st Week | 2nd Week | 3rd Week | 4th Week | 5th Week | 6th Week |
|-------|--------------------|----------|----------|----------|----------|----------|----------|----------|
| Casuarina equisetifolia extract (5% cream) | | | | | | | | |
| 0 | | 3 | 5 | 14 | 19 | 25 (50%) | |
| 2 | 14 (28%) | 14 | 12 | 15 | 13 | 20 | 18 (36%) | |
| 4 | 16 (32%) | 16 | 15 | 12 | 9 | 5 | 5 (10%) | |
| 6 | 11 (22%) | 11 | 11 | 9 | 6 | 3 | 2 (4%) | |
| 8 | 9 (18%) | 9 | 9 | 9 | 8 | 3 | | |
| Benzoyl peroxide (Control) | | | | | | | | |
| 0 | | 5 | 5 | 14 | 21 | 27 (54%) | |
| 2 | 12 (24%) | 12 | 10 | 13 | 11 | 20 | 18 (36%) | |
| 4 | 17 (34%) | 14 | 17 | 14 | 9 | 7 | 4 (8%) | |
| 6 | 13 (26%) | 15 | 9 | 10 | 8 | 3 | 2 (4%) | |
| 8 | 8 (16%) | 8 | 8 | 7 | 6 | 3 | | |

### Table IV

| Number of patients | Response | Test | Control |
|--------------------|----------|------|---------|
| 0 | Excellent | 16 (32%) | 13 (26%) |
| 2 | Good | 31 (62%) | 26 (52%) |
| 4 | Poor | 2 (4%) | 7 (14%) |
| 6 | No response | 1 (2%) | 4 (8%) |
| 8 | Worse | 0 (0%) | 0 (0%) |
the ethical committee of the Board of Advanced Studies and Research University of Karachi Vide Resol No. 16. The specifications given in the declarations of Helsinki 1964 and 2013 were followed during the experimentation.

Conflict of Interest

We declare that there is no conflict of interests regarding the publication of this article.

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