

## ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF *BLUMEA ERIANTHA DC DC* AGAINST SKIN PATHOGENS

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### ABSTRACT

In the current research work we have evaluated the antibacterial potential of essential oil of *Blumea eriantha DC*. The antimicrobial activity is studied against skin pathogens, mainly acne causing bacteria. The essential oil was extracted by hydrodistillation using Clevenger type apparatus. The oil showed significant antimicrobial activity against *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus pyogenes*. The antimicrobial activity was assessed by means of broth microdilution method and time kill analysis. The determined values of minimum inhibitory concentration and time kill analysis studies of the essential oil indicate that *Streptococcus pyogenes* is the most sensitive bacterium. The oil also showed significant activity against *Propionibacterium acnes* and *Staphylococcus aureus*. The obtained data highlight the potential of *Blumea eriantha DC* essential oil as a natural antibiotic in the treatment of skin infections.

**Keywords:** *Blumea eriantha DC*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pyogenes*.

### INTRODUCTION

Acne vulgaris is the most common disorder of human skin. It affects individuals of all races, covers 85% of teenagers, 42.5% of men and 50.9% of women between the age group of 20-30 years<sup>1,2</sup>. Acne has many different symptoms including seborrhea, comedones, pilosebaceous inflammation, inflammatory lesions and presence of bacteria *Propionibacterium acnes* (*P. acnes*), *Staphylococcus epidermidis* (*S. epidermidis*) in the follicular canal<sup>3</sup>. Inflammatory lesions of acne are of the greatest concern to patients as they may lead to acne scarring. *P. acnes* play a critical role in the development of inflammatory acne when it overgrows and colonizes the pilosebaceous unit<sup>4</sup>. This organism has been implicated over other cutaneous microflora i.e. *S. epidermidis*, *Staphylococcus aureus* (*S. aureus*) in contributing inflammatory acne due to its unusual ability to stimulate reticuloendothelial system<sup>5</sup>. *P. acnes* acts as immunostimulator, releases chemoactive factors that attract the immune system cells such as neutrophils, monocytes, and lymphocytes<sup>6</sup>. *P. acnes* stimulate the production of pro-inflammatory cytokines such as interleukins IL-1 $\beta$ , IL-8, IL-12, and tumor necrosis factor- $\alpha$ <sup>6,7</sup>. The major classes of therapeutic agents for treating acne vulgaris are topical and systemic retinoids, antimicrobial agents and systemic hormonal drugs<sup>8</sup>. Bacterial resistance to antibiotics is an ongoing problem in the treatment of such bacterial infections due to the indiscriminate use of commercial antimicrobial drugs<sup>9</sup>. Therefore new antibiotics that are fast and active in vivo with lesser side effects are essential.

The universal role of plants in the treatment of diseases is established by their extensive usage in all important systems of medicine such as Ayurveda and Siddha<sup>10</sup>. Medicinal plants are rich sources of secondary metabolites<sup>11</sup> such as essential oils, which are potential sources of useful drugs. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane disturbing the structures leading to death of the bacterial cells<sup>12</sup>. Being natural in origin they have minimum side effects compared to pharmaceutical drugs and hence may prove to be effective natural antibiotic agents.

*Blumea eriantha DC* (*B. eriantha*) is an aromatic, 1m tall, annual, erect herb, found abundantly along road sides and degraded forest lands. It is commonly known as 'Nimurdi' in Marathi and 'Kukronda' in Hindi. It is distributed in Bihar, Karnataka, Madhya Pradesh, Maharashtra, Orissa and Uttar Pradesh<sup>13</sup>. The juice of the herb is carminative. A warm infusion of the leaves is given as a sudorific while cold infusion is considered as diuretic and emmenagogue. The oil of the plant possesses significant antibacterial, antifungal and

insecticidal activities<sup>14</sup>. In the present study, the essential oil of *B. eriantha* was examined for antimicrobial property against acne inducing bacteria *P. acnes*, *S. epidermidis*, *S. aureus*. Along with that we have also studied its antimicrobial activity against *Streptococcus pyogenes* (*S. pyogenes*) which causes skin infections like impetigo, erysipelas, and cellulitis<sup>15</sup>.

### MATERIALS AND METHODS

#### Plant Material

The entire upper portion including aerial part, stem and leaves of *B. eriantha* were collected from Seawoods, Navi Mumbai, Maharashtra, India between the months of December 2010 to March 2011. The authentication of plant was carried out at Agharkar Research Institute, Pune, Maharashtra, India and the voucher specimen was deposited with the institute.

#### Extraction of essential oil

The fresh plant material including aerial part, stem and leaves of *B. eriantha* were chopped into small pieces. 750g of fresh plant material was subjected to hydrodistillation using Clevenger type apparatus of capacity 5 Liters. To this 3 liters of water was added. The mixture was heated on heating mantle at 85°C. The distillation was continued for three hours. The essential oil obtained was dried over anhydrous sodium sulphate and stored at 4°C in sealed vials until analysis.

#### Microorganisms used and their growth conditions

The essential oil was tested against four microorganisms. Reference strains were:

1. *Propionibacterium acnes*, MTCC No: 1951, Collection Acc. No. 1951, isolated from facial acne.
2. *Streptococcus pyogenes*, MTCC No 1925, Collection Acc. No. 1925 Isolated from Puerperal fever.
3. *Staphylococcus epidermidis*, MTCC No 435, Collection Acc. No.435 isolated from Skin lesion.

These type strains were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

4. *Staphylococcus aureus* ATCC 6538P was obtained from the Microbiology Department, G. N. Khalsa College, Mumbai, India.

*S. epidermidis* and *S. aureus* 6538P were maintained on nutrient agar while *P. acnes* and *S. pyogenes* were maintained on Brain heart Infusion agar supplemented with 5% blood.

*S. epidermidis*, *S. aureus* and *S. pyogenes* cultures were incubated at 37°C for 24 hours whereas *P. acnes* cultures were incubated at 37°C for 48 hours under anaerobic conditions.

#### Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

A broth microdilution method was used to determine the MIC and MBC of essential oil of *B. eriantha*. To overcome the insolubility of essential oil, the oil was initially emulsified in 0.85% NaCl saline containing 0.5% Tween -80<sup>16</sup>. Further, a serial two fold dilutions of emulsified oil were prepared over the range of 25%- 0.05% in sterile glass dilution tubes. Overnight broth cultures of each test organism were prepared in respective broth medium. The volume of each concentration of essential oil in sterile glass dilution tube was 250µl. To each of the tube 25 µl of 0.1 OD (at 540nm) adjusted culture was added. In case of *P. acnes* the preliminary culture density experiments indicated that the number of bacteria at 0.1 OD was high (confirmed by preliminary viable count experiments) hence the OD was adjusted to 0.05 (at 540nm). The ratio of volume of culture to broth medium was kept 1:10. The final concentration of bacterial cells in each dilution tube was approximately 10<sup>5</sup>- 10<sup>6</sup> CFU/ml and was confirmed by performing viable counts on respective agar media plates for each organism. Positive and negative growth controls were included in each test. The tubes were then incubated at respective growth conditions for each test microorganism for 24 hours. Turbidity due to *B. eriantha* essential oil makes the visual inspection difficult to determine the MIC. To determine the MIC and MBC, broth from each dilution tube was appropriately diluted and was spread on respective agar media plates of each test microorganism to determine the number of surviving organisms. The lowest concentration that maintained or reduced inoculum viability was the minimum inhibitory concentration whereas the minimum bactericidal concentration was the concentration where less than 0.1% of the initial inoculum survived<sup>17</sup>. All the MIC and

MBC determinations were carried out three times on three different days.

#### Time Kill Analysis

The time kill analysis was performed by measuring the numbers of CFU/ml of surviving bacteria over 2 hours for each test organism at MBC, ½ MBC and MIC concentrations of essential oil. The time kill analysis was performed using method described by Carson *et al*<sup>18</sup> with slight modifications. The stock concentration of essential oil was prepared in 0.85% NaCl saline containing 0.5% Tween -80 to overcome the insolubility of the essential oil. The MBC, ½ MBC and MIC concentrations (5ml each) were prepared in respective broth medium from the stock concentration.

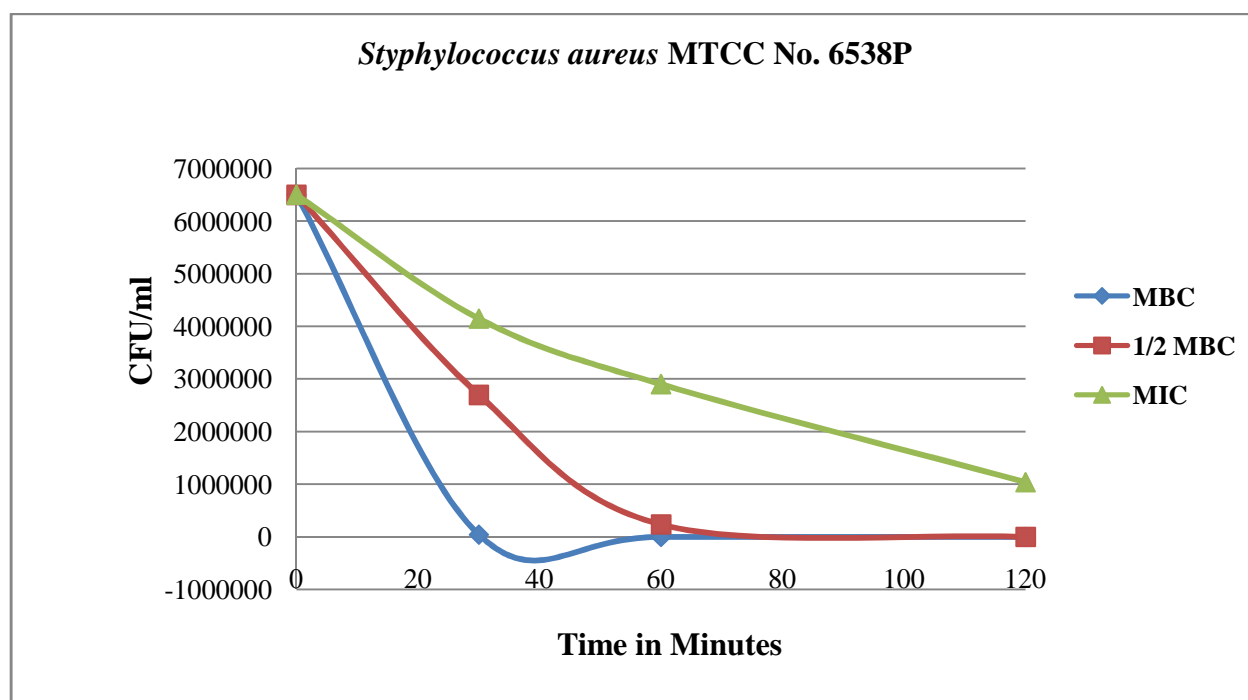
The culture conditions and volume of culture added were same as that of MIC procedure. The final concentration of test microorganism in each concentration tube was in the range of 10<sup>5</sup>- 10<sup>6</sup> CFU/ml, which was confirmed by viable count method. The solution without essential oil was used as a control. 0.5ml of samples were removed at 30, 60 and 120mins, serially diluted and plated on respective agar media plates and incubated at respective growth condition to determine the no. of surviving microorganisms. This test was repeated twice. Time-kill curves were constructed by plotting the average number of (CFU/ml) surviving bacteria against time (min). In case of *Streptococcus pyogenes* where rapid killing was observed the time intervals chosen were 5, 10, 20, 30, 60 and 120mins.

#### RESULT AND DISCUSSION

The hydrodistillation method was successfully utilized for extraction of essential oil of *B. eriantha*. With this method a separate upper layer of light green colored essential oil was obtained in the collection tube of Clevenger type apparatus. The oil turned light yellow in color on storage. The yield of essential oil ranged from 0.14% to 0.25% (v/w) on a fresh weight basis.

**Table 1: Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of *Blumea eriantha* DC essential oils against the test skin pathogens.**

	<i>Propionibacterium acnes</i>	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>
MIC (%v/v)	0.39%	1.56%	0.19%	0.09%
MBC (%v/v)	1.56%	6.25%	0.78%	0.39%



**Fig. 1: Time kill curve for *Staphylococcus aureus* over 2 hours at MIC, ½ MBC and MBC.**

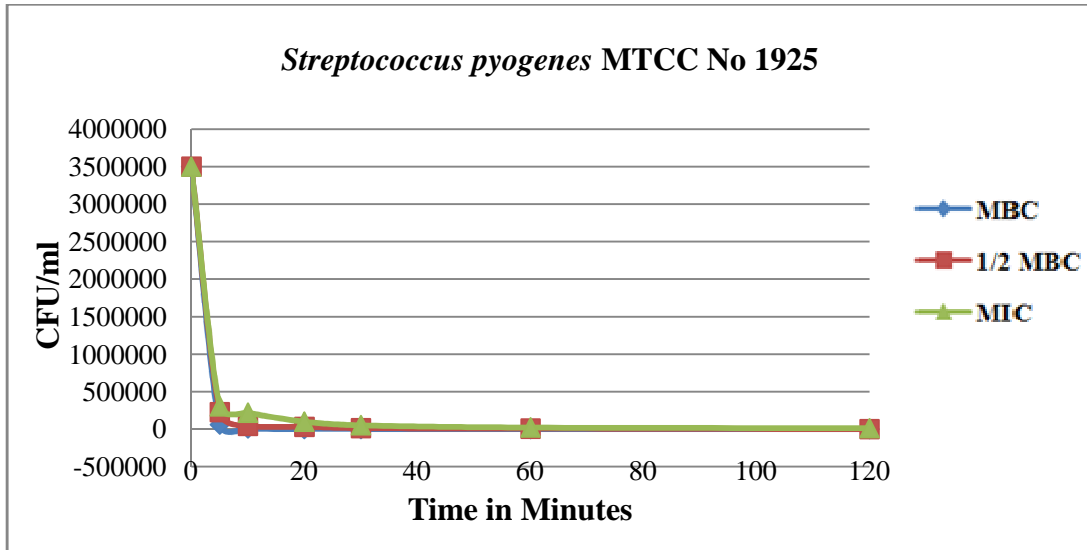


Fig. 2: Time kill curve for *Streptococcus pyogenes* over 2 hours at MIC, 1/2 MBC and MBC.

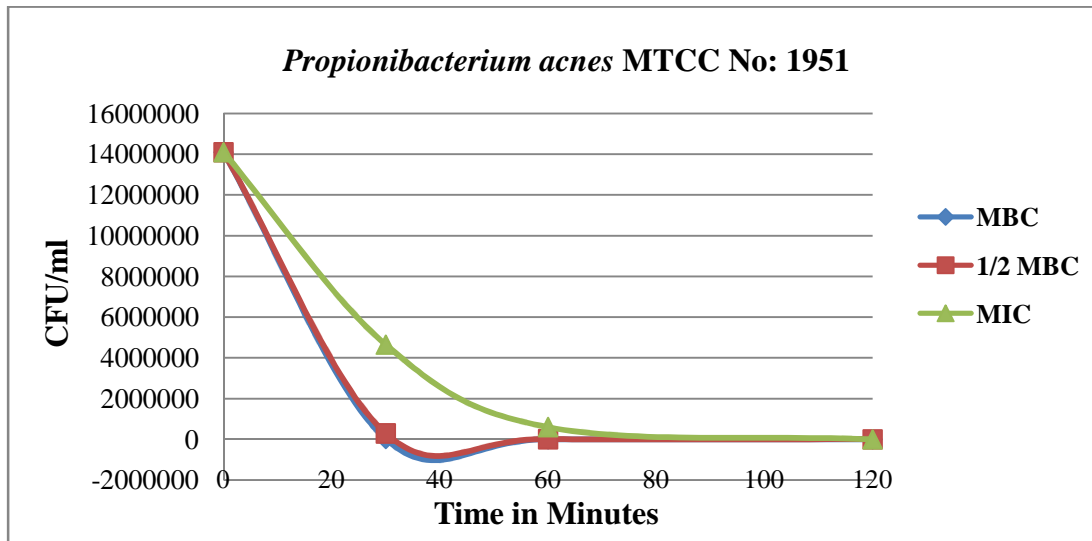


Fig. 3: Time kill curve for *Propionibacterium acnes* over 2 hours at MIC 1/2 MBC and MBC.

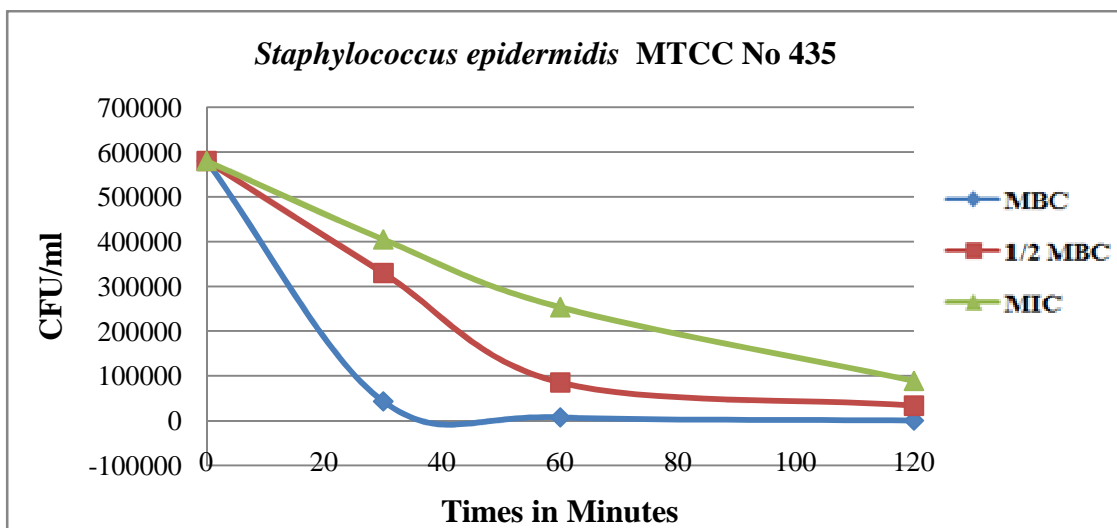


Fig. 4: Time kill curve for *Staphylococcus epidermidis* over 2 hours at MIC, 1/2 MBC and MBC.

The agar disc- or well diffusion assay is the easiest microbial susceptibility assay to perform and is frequently used in the determination of antimicrobial activity. However, this method is not ideal for water insoluble compounds such as essential oils. The oil components set partitioned themselves through the agar according to their affinity with water<sup>19</sup>. The hydrophobicity of the essential oil creates difficulty in obtaining a stable dispersion of the oil in aqueous media and also creates problem in diffusion of lipophilic component through agar. This leads to improper diffusion of oil through the agar media. Hence broth microdilution method was used as it provides more precise data on the antimicrobial properties by way of determinations of bacteriostatic and bactericidal concentrations. The minimum inhibitory concentrations and minimum bactericidal concentrations (in % v/v) of the essential oil of *B. eriantha* against the four test skin pathogens are shown in **Table 1**. The essential oil showed a variable degree of antimicrobial activity against the different skin pathogenic bacteria tested. *S. pyogenes* was the most sensitive microorganism with lowest MIC and MBC values 0.09% and 0.39% respectively. The MIC and MBC values for *S. epidermidis* were found to be highest (MIC 1.56% and MBC 6.25%) hence it is the least sensitive microorganism to the essential oil. *S. aureus* and *P. acnes* showed intermediate sensitivity to the essential oil. The sensitivity of the essential oil decreased in the order of *S. pyogenes* > *S. aureus* > *P. acnes* > *S. epidermidis*. Although the MICs and MBCs results varied between organisms tested, the MBC value is approximately four times the MIC value for each test pathogen.

The time-kill curves for all four skin pathogens are shown in **Figure 1, 2, 3, 4**. The essential oil showed strongest bactericidal activities against *S. pyogenes* at MBC concentration (0.4%) 98% of the bacteria were killed within 5 min and complete reduction occur within 20minutes. The **Figure 2** indicates that the essential oil showed similar time kill kinetics against *S. pyogenes* at MBC, ½ MBC and MIC concentrations. In case of *S. aureus* and *P. acnes* 99% of the bacteria get killed within 30minutes at MBC concentration i.e 0.8% and 1.56% respectively. The time kill curve for *S. aureus* [**Figure 1**], indicate marked differences in kill kinetics at MBC, 1/2MBC and MIC concentrations over two hours. *P. acnes* [**Figure 3**] showed similar kill kinetics at MBC and ½ MBC concentrations compared to kill kinetics at MIC concentration. The time kill curves for *S. epidermidis* [**Figure 4**] indicate lowest bactericidal activity oil. The oil takes 120 minutes for complete reduction of *S. epidermidis*, at MBC concentration (6.25%).

## CONCLUSION

In the present study we investigated the effectiveness of essential oil of *B. eriantha* "in vitro" on survival and growth of selected skin pathogens. The MIC and MBC values and time kill analysis data indicate that *S. pyogenes* is the most sensitive organism to *Blumea eriantha DC* essential oil among all the tested skin pathogens. Although the oil showed less effectiveness against *S. epidermidis*, it effectively reduces the growth of the other two acne inducing bacteria i.e. *S. aureus* and *P. acnes*. These results confirm the potential use of essential oil of *B. eriantha* as an effective natural antibiotic agent in the treatment of acne and other skin infections caused by tested skin pathogens. The oil can also be used in skin cosmetics as natural antibacterial agent.

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