

## A quest of Anti-acne Potential of Herbal Medicines for extermination of MDR *Staphylococcus aureus*

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**ABSTRACT :** Acne is a common disorder of pilosebaceous follicle. In the present study certain herbal drugs were chosen based on their antibacterial activity against MDR *Staphylococcus aureus*. Agar-well diffusion method with 0.5ml of extract was used. Neem extract, Tea tree oil, Lemon juice and Rose water (1:1), Orange Pulp extract, Orange peel extract, Aloe vera gel and Beet root extract showed anti *Staphylococcal* activity whereas there was no anti *Staphylococcal* activity observed for Chandan hot extract, Multani clay hot extract, Papaya extract, Saffron extract, Turmeric cold extract and Carrot extract. From the research conducted it was observed that some easily available plants and their parts have potential anti *Staphylococcal* activity for the treatment of acne. Study will help the pharmaceutical industry to formulate drug to tackle acne caused by MDR *Staphylococcus aureus*.

**KEY WORDS:** Acne, MDR Resistant *Staphylococcus aureus*, Herbal Medicine

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### I. INTRODUCTION

Acne, from the Greek word “Akme” means peak or apex, is genetic or acquired affections of the pilosebaceous units. The correct name for acne is *Acne vulgaris*. The 70%-80% of patients affected by this are aged 11-25 years old. *Acne vulgaris* is characterized by the formation of inflammatory and non-inflammatory lesions of the hair follicles and/or sebaceous glands commonly referred to as the pilosebaceous unit. A slight degree of acne is typical at puberty, but a serious case can cause unsightly appearance and leaves scarring in many cases even after treatment. Non-inflammatory lesions may be categorized as open comedons (blackheads) and closed comedons (white heads). Inflammatory lesions manifest themselves as papules, pustules, cysts and nodules (Daud et al., 2013; Sawarkar et al., 2010). *Staphylococcus aureus* is a ubiquitous organism that is found in both hospital and community settings. While *S aureus* colonizes the skin, it can also be responsible for localized cutaneous infections and life-threatening systemic infections. At one time, it was sensitive to many antibiotics and antimicrobial agents. However, because of its ability to adapt to these therapies and become resistant, clinical scenarios now exist in which few therapeutic options remain to treat this organism. Therefore, methicillin-resistant *S aureus* (MRSA) has become commonplace (Chambers and Deleo, 2009; Deleo et al., 2010).

Colonization with *S aureus* in general is relatively widespread, with approximately 40% to 50% of the population at large colonized, but, fortunately, in the community, MRSA organisms colonize fewer than 5% of the population. Patients with acne are generally young and healthy and are often exposed to antibiotics for extended periods because long-term antibiotic therapy, oral (e.g., tetracycline, erythromycin, trimethoprim-sulfamethoxazole) and/or topical (e.g., clindamycin or erythromycin), is a standard of care in the treatment of acne (Leyden, 1997, 2001; Margolis, 2006). Modern acne therapy has been designed to interrupt the pathogenic pathway at one or more points. Topical or systemic therapy is available for the treatment of acne. Topical therapy includes comedolytic agents and antibiotics and various anti-inflammatory drugs. Systemic therapy includes antibiotics, zinc and hormones (Kumar, 2004). With the excessive use of antibiotics for long periods has led to the increased resistance in acne causing bacteria i.e. *Staphylococcus aureus*, against a number of antibiotics used to treat acne. A long term therapy is required for the treatment of acne so there are more chances of occurrence of adverse effects due to this medication. Some of the reported adverse effects reported are lupus erythematosus, serum sickness like reaction, autoimmune hepatitis, pigmentation of skin, alveolar bone pneumonitis (Chaudhary, 2010). There is an increased interest among patients seeking alternative treatment for such conditions. World Health Organization (WHO) noted that majority of world's population depends on traditional medicine of primary healthcare.

Herbal therapy for skin disorders has been used for thousands of years. Even our biologically close relatives, the great apes, use herbal self-medication (Huffman 2001). Specific herbs and their uses developed regionally, based on locally available plants and through trade in ethnobotanical remedies. Systems of herbal use developed regionally in Europe, the Middle East (Ghazanfar 1994), Africa, India (Behl and Srivastava 2002), China, Japan, Australia, and the Americas. In India, records of Ayurvedic medicine date back to about 3000 BC. The system of Ayurvedic medicine combines physiological and holistic principles. It is based on the concept that the human body consists of five energy elements that also make up the universe: (1) earth, (2) water, (3) fire, (4) air, and (5) space. The interactions of these five elements give rise to the three *doshas* (forces), seven *dhatu*s (tissues), and three *malas* (waste products). All diseases are attributed to an imbalance among the three *doshas* (Bedi and Shenefelt 2002). Diagnosis is made by an elaborate system of examining the physical findings, pulse, and urine, as well as by an eightfold detailed examination to evaluate both the physical and mental aspects of the condition. The treatment is then tailored to suit an individual based on the findings (Routh and Bhowmik 1999). Present study is done to study the effect of some Indian herbal medicines on acne causing bacteria *S. aureus*.

## II. MATERIAL AND METHODS

**Collection of Clinical Samples:** A total of 102 organisms were isolated from 63 skin swab samples from different patients showing evidence of acne on various parts of body such as face, neck, chest, and back and thigh region. These isolated bacterial cultures were transferred to microbiology laboratory for further processing. This was indeed collected from the patients not responding to routine antibiotic therapy of skin.

**Identification of Isolated Pathogens:** The isolated bacterial pathogens were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996) and the results were compared with Bergey's Manual of Determinative Bacteriology 9<sup>th</sup> edition. From 102 isolates, 38 were confirmed as *Staphylococcus aureus*.

**Preparation of Inoculum:** A loopful culture of each *Staphylococcus aureus* was inoculated in 5ml of sterile nutrient broth tube. The inoculated broth was incubated for 8 hours at 37<sup>o</sup>C and the turbidity was measured using 0.5 McFarland standards.

**Antibiotic Sensitivity Test:** All the *Staphylococcus aureus* were subsequently tested for antibiotic sensitivity patterns by disc diffusion method on Mueller Hinton Agar (Bauer et al., 1966). The five different antibiotic discs such as Teicoplanin (30 mcg), Vancomycin (30 mcg), Methicillin (5 mcg), Tobramycin (10mcg) and clindamycin (2mcg) were obtained from Hi-Media Laboratories Pvt. Ltd. Mumbai. The results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

**Selection of Antibiotic Resistant *Staphylococcus aureus*:** A total of 38 *Staphylococcus aureus* strains were tested against selected 5 different antibiotics. Out of this 16 isolates were found to be the multi-drug resistant *Staphylococcus aureus* which were then tested for the effect of herbal medicines on it.

### Preparation of herb extract:

**Aloevera extract, Beet root extract, Carrot extract, Neem extract and Papaya extract:** Aloevera leaves, Beet root, Carrot, Neem leaves and Papaya were firstly surface sterilized with 10 ppm of hypochloride solution, then peeled off and cut in small pieces. Juice was prepared by kitchen grinder and then filtered through Whatman filter paper no. 1 to get sterile extracts and it was then kept in sterile glass bottle.

**Chandan Powder extract and Multani clay hot extract:** A 1 gm each of Chandan powder and Multani clay was added in separate test tubes containing 10 ml sterile distilled water. Both the test tubes were boiled to reduce its volume upto 2.5 ml each respectively. Both the extracts were then filtered through Whatman filter paper no. 1 to get sterile extracts and it was then kept in sterile glass bottle.

**Lemon juice and Rose water (1:1):** A 2.5ml each Lemon juice and rose water was mixed with each other and filtered through Whatman filter paper no. 1 to get sterile extract and it was then kept in sterile glass bottle.

**Orange pulp extract:** Orange was firstly peeled off and then inner pulp was used for juice extraction by kitchen grinder and then filtered through Whatman filter paper no. 1 to get sterile extract and it was then kept in sterile glass bottle.

**Orange peel extract:** Orange was firstly peeled off and peel was then dried under shade till it completely dries. A 1 gm of dried orange peel was added in 10 ml sterile distilled water in a test tube and boiled to reduce its volume upto 2.5 ml. The extract was then filtered through Whatman filter paper no. 1 to get sterile extract and it was then kept in sterile glass bottle.

**Saffron extract and Turmeric extract:** The 250 mg each of saffron and turmeric powder was taken in 5ml of sterile distilled water in china dish and kept for 5hrs. The extracts were then filtered through Whatman filter paper no. 1 to get sterile extracts and it was then kept in sterile glass bottle.

**Tea tree oil:** Essential oil of tea tree was purchased from the market.

**Susceptibility Testing of Herbal Extracts:** Hi-sensitivity test broth was prepared and sterilized at 15lbs for 15 minutes and inoculated with the previously screened antibiotic resistant bacteria *S. aureus* aseptically. A 0.5 ml of 6-8 hours old test organism was inoculated on solidified sterile Hi-sensitivity test agar plates and spread with sterile spreader. Wells were cut in previously solidified sterile Hi-sensitivity test agar plates with the help of 6 mm borer. A 0.5ml of previously prepared plant extracts were added in the plates. A 0.1 ml of Gentamycin at a concentration of 300µg /ml was taken as standard reference. Plates were immediately kept at 4<sup>o</sup>C in refrigerator for 1hr diffusion. After diffusion the petridishes were incubated at 37<sup>o</sup>C for 24 hr and zone of inhibition was observed and measured (Chaudhary, 2010).

### III. RESULTS

Acne can have important negative psychosocial consequences for the affected individual, like diminished self-esteem, social withdrawal due to embarrassment and depression. A total of 102 organisms were isolated from 63 skin swab samples from different patients showing evidence of acne on various parts of body such as face, neck, chest, and back and thigh region. From 102 isolates, 38 were confirmed as *Staphylococcus aureus*. Further, antibiotic susceptibility test by the disc diffusion method of 38 *Staphylococcus aureus* was performed against as Teicoplanin (30 mcg), Vancomycin (30 mcg), Methicillin (5 mcg), Tobramycin (10mcg) and clindamycin (2mcg). Out of this 16 isolates were found to be the multi-drug resistant *Staphylococcus aureus* (MRSA) which were then tested for the effect of herbal medicines on it. Aloe vera gel, Beet root extract, Carrot extract, Chandan hot extract, Lemon juice and Rose water (1:1), Multani clay hot extract, Neem extract, Tea tree oil, Papaya extract, Orange peel extract, Orange Pulp extract, Saffron extract and Turmeric cold extract were tested for anti *Staphylococcal* activity. Zone of inhibition for herbal extracts was interpreted for resistant and sensitive bacteria with reference to protocol of Johnson et al., (1995); Daud et al., (2013) (Table 1). Beet root extract and Tea tree oil were found to be effective while Carrot extract, Chandan hot extract, Multani clay hot extract, Papaya extract, Saffron extract and Turmeric cold extract were non-effective against all (100%) isolates of MRSA. All MRSA were moderately sensitive to Alovera gel. On the other hand, 87.5% MRSA were sensitive to Neem extract, followed by 75% to Lemon juice and Rose water, 50% each to Orange Peel extract and Orange Pulp extract (Table 2) (Figure 1).

### IV. DISCUSSION

Herbal medication are considered safer than allopathic medicines as allopathic medicines are associated with side effects such as contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, etc. (Sawarkar et al., 2010). Tea Tree oil has anti microbial, anti inflammatory, anti infectious, anti septic, anti viral and bactericidal properties. Useful to treat Abscess, Acne, Blisters, Insect bites, Rashes, Wounds etc. (Lawless, 1995). Among over 98 compounds contained in the oil, terpinen-4-ol is responsible for most of the antimicrobial activity. Other compounds present are - Terpinene 10-28%,  $\alpha$  terpinene 5-13%, 1  $\beta$  cineole,  $\alpha$  terpinolene 1.5-5%,  $\alpha$ -terpineol 1.5-8%,  $\alpha$ -pinene 1-6%, and p-cymene 0.5-8%. (Daud et al., 2013). The components of the oil penetrate deep into skin and support regeneration of skin damaged by skin diseases caused by germs, fungus and acne causing bacteria (Junemann and Lutejoham, 1998). Shemesh and Mayo (1991); Caelli et al., (2000) also reported that Tea tree oil possess anti Staphylococcal activity. Neem leaves have known to possess diverse pharmacological properties like anti inflammatory, anti pyretic and anti microbial (Arora et al., 2008). It contains diterpenes - stigmaterol, triterpenes, number of cyclic tri and tetrasulphides. Nimbidin Margolone, margolonone and isomargolonone are the main constituents of Neem leaves (Biswas et al., 2002). Beetroot is rich in anti-oxidants. They help the skin from oxidation processes by free radicals. Beetroot is a natural detoxifier. It purifies the blood which makes the skin glow. Beetroot juice has been significantly beneficial against boils and pimples. Consumption of beetroot juice also reduces skin inflammation. It also works as an anti-aging substance. Beetroot is a source of lycopene which is a powerful anti-oxidant. Lycopene helps in maintaining skin elasticity and protects skin from harmful sun radiations. Beetroots have characteristic intense color which is associated with the presence of phenolic compounds and antimicrobial activity (Benavente-Garcia, 1997).

The mucilaginous jelly from the parenchyma cells of the peeled, spineless leaves of the plant is referred as Aloe vera gel. The gel is a watery-thin, viscous, colorless liquid that contains anthraquinone glycosides, glycoprotein, gamma-lanoline acid, prostaglandins and mucopolysaccharides that are mainly responsible for the antibacterial, antifungal as well as its antiviral activity (Shafi et al., 2000). Subramanian et al., (2006) also observed remarkable antibacterial activities with ethanolic extracts of Aloe vera gel even at low concentrations compared with the standard antibiotics and supported the view that Aloe vera is a potent antimicrobial agent compared with the conventional antibiotics. Mangena (1999); Agarry et al., (2005); Kaithwas et al., (2008) reported Aloe vera gel shows greatest inhibitory effect on the *Staphylococcus aureus*. Especially the agents which are involved to prevent or destroy the bacterial or fungal cells and help to attain the normal growth and functions of the body are cinnamonic acid, salicylic acid, traumatic acid, phenol, allantoin, campesterol, lectins and gibberellins that are found to have effective as antimicrobial properties .

Antibacterial activity of Citrus fruits such as Lemon and Orange has been reported in Ayurveda which showed good antibacterial activities against gram positive and gram negative microorganisms (Waidulla et al., 2010). Citrus is one of the most important commercial fruit crops grown in all continents of the world. Oranges and Lemon are an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts of Lemon against clinically significant bacterial strains has been reported (Kawaii, 2000). Orange peel is one of the important dietary sources of antioxidant phenolics (Jayaprakasha et al., 2008). The citrus peels are rich in nutrients and contain many phytochemicals, these can be efficiently used as drugs or as food supplements. The peel of Citrus fruits is a rich source of flavonoid glycosides, coumarins,  $\beta$  and  $\gamma$ - sitosterol, glycosides and volatile oils. Many polymethoxylated flavones have several important bioactivities, which are very rare in other plants (Ahmad et al., 2006; Shahnah et al., 2007). Many studies have reported antioxidant and antibacterial effect of juice and edible parts of lemon and oranges of different varieties. As far as the peel is concerned, extracts from this part of the fruit were found to have a good total radical anti-oxidative potential (Farak et al., 2009). Herbal plants and its parts are effective alternatives used to treat acne. Herbal medicines are recommended since these are powerful cleansers that could clear your body of unwanted harmful toxins and enrich the body with useful nutrient minerals (Pandey et al., 2010). Further research into the efficacy, safety, optimal uses, and standardization of herbal remedies is clearly needed. Many herbal therapies have been used for centuries, which show good anecdotal results. A few randomized, controlled trials have also demonstrated significant results in the use of herbal therapies for the treatment of dermatologic disorders. Some countries, such as Germany, now require standardization of herbal preparations and specific recommendations as to the use and efficacy of herbs in the treatment of disease. It is important to know what common herbal alternatives exist and which potential adverse effects or interactions can occur to permit more effective counseling of patients.

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Table 1: Interpretation Chart for Zone of Inhibition of Herbal Extract

Interpretation	Zone of Inhibition
Resistant (R)	10 mm or less
Intermediate (I)	11-15mm
Sensitive (S)	16mm or more
No Zone (NZ)	-

Table 2: Zone of inhibition of Herbal Extract against Methicillin Resistant *Staphylococcus aureus*

Herb Used	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12	S-13	S-14	S-15	S-16
Aloe vera gel	13	14	13	14	13	14	13	14	14	13	14	14	13	14	14	14
Beet root extract	23	23	20	19	18	23	20	23	24	20	19	18	23	20	23	24
Carrot extract	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chandan hot extract	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lemon juice and Rose water (1:1)	43	43	22	25	15	20	28	44	43	22	25	15	20	28	44	43
Multani clay hot extract	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neem extract	23	23	20	20	15	20	20	23	22	20	20	15	20	20	23	22
Orange peel extract	14	15	18	18	19	14	25	15	14	18	18	19	15	25	14	15
Orange Pulp extract	15	15	18	18	19	15	25	15	15	18	18	19	15	25	15	15
Papaya extract	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saffron extract	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tea tree oil	22	22	30	30	18	28	18	21	22	30	30	18	28	18	21	22
Turmeric cold extract	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gentamycin	27	26	27	27	24	27	23	23	26	27	27	24	27	23	24	26

Where, S-1 to S-16= *Staphylococcus aureus* isolates

Figure 1: Herbal Sensitivity Pattern against Methicillin Resistant *Staphylococcus aureus*

