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EVALUATING THE ANTIBACTERIAL EFFICACY OF TULSI, NEEM, AND ALOE VERA EXTRACTS AGAINST STAPHYLOCOCCUS AUREUS IN ACNE VULGARIS

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Abstract

Acne vulgaris, often involving Staphylococcus aureus and Propionibacterium acnes, presents treatment challenges, compounded by multi-drug resistant strains like Methicillin-Resistant Staphylococcus aureus (MRSA). This study investigates the antibacterial potential of Tulsi (Ocimum sanctum), Neem (Azadirachta indica), and Aloe Vera (Aloe barbadensis) extracts against Staphylococcus aureus. Through literature review, we explore their chemical composition and antimicrobial activities. Tulsi's eugenol and Neem's nimbin exhibit potent antibacterial effects, while Aloe Vera's aloin and emodin hinder biofilm formation. This research sheds light on herbal remedies as adjuncts for managing Staphylococcus aureus-related skin infections as a natural approach to combat drug-resistant strains.

Keywords: *Tulsi, Aloe vera, Neem, Staphylococcus Aureus, inhibition, chemical composition.*

INTRODUCTION:

Skin is the largest organ in the human body. Acne is a severe skin condition caused due to inflammation, clogged pores, production of excess sebum. Bacteria associated with Acne Vulgaris are – *Staphylococcus aureus* and *Propionibacterium acnes*. Both *Staphylococcus aureus* and *P. acnes* were identified as bacteria likely to cause *Acne vulgaris*[1]. Natural ingredients provide the skin with essential vitamins, minerals, and antioxidants. It heals breakouts and hyperpigmentation, combats UV rays, brightens up your skin, and hydrates and moisturises it. Skin and soft tissue infections (SSTIs) are the cause of considerable morbidity and cost to the community. Major causes of these infections include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*[2]. Keratin formation takes place due to the testosterone stimulation from the cells lining the follicular canal and due to sebaceous glands enlargement which leads to the production of more sebum. Pimples are produced and the canals are blocked due to overproduction of sebum. Blocked canals will lead to overgrowth of bacteria which is responsible for acne. These bacteria release the enzymes that cause breakage of the sebum which stimulates inflammation. The key factors in the formation of acne lesions are an increase in sebum production, sloughing of keratinocytes, bacterial growth, and inflammation[3]. For ages, herbs have been used in Ayurvedic treatments due to their medicinal properties.

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BACKGROUND INFORMATION:

Staphylococcus aureus - It is a gram-positive bacteria that causes a wide range of infections. Its treatment is challenging due emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant Staphylococcus aureus). These organisms can grow in up to 10% salts, and colonies are often golden or yellow. They can either grow aerobically or anaerobically. They are found in skin and mucous membranes[4].

Tulsi - Tulsi, scientifically known as, *Ocimum tenuiflorum* or *Ocimum sanctum*, is the holy basil from the family Lamiaceae and has been described as the "queen of plants" and the "mother medicine of nature" due to its medicinal properties. It has been traditionally used in India as a medicinal plant and every part of the plant has a therapeutic property. Tulsi's pharmacological effect is on the central nervous system, immune system, cardiovascular system, reproductive system, and gastric and urinary system.

Neem - Neem, aka, *Azadirachta indica* – tropical evergreen tree that is native to the Indian subcontinent. Throughout the years this tree has been used for various functions. It has played roles as pesticides, fertilisers, and antibiotics. Scientists are researching more about its properties in the fields of agriculture, food safety, dentistry, bacteriology, virology, and parasitology[5].

Aloe Vera - *Aloe barbadensis Mill*, is a short succulent herb resembling a cactus, with green dagger-shaped fleshy, spiny, and marginated leaves, filled with a clear viscous gel[6]. Aloe vera has been traditionally used to treat skin injuries (burns, cuts, insect bites, and eczemas) and digestive problems because of its anti-inflammatory, antimicrobial, and wound-healing properties[7]. Aloe juice is bacteriostatic against Staphylococcus aureus[8].

Ocimum sanctum

A LITERATURE REVIEW ON THE RATE OF INHIBITION OF *Staphylococcus aureus* BY THE EXTRACTS OF *Ocimum sanctum*:

Tulsi at concentrations 4.5 and 2.25% completely inhibited the growth of *S. aureus*[9].
The oil extracted from its leaves whenstudied against *S. aureus, E. coli* was seen to be equally effective against pathogenic gram-positive and gram-negative bacteria. Fresh leaves showed more antibacterial properties than dried leaves[10].

It showed good antibacterial properties against *Staphylococcus aureus*, *Bacillus pumilus*, and *Pseudimonas aeruginosa*, where S. aureus was the most sensitive organism. It was also examined whether the aqueous extract or the alcohol extract was more powerful. It was reported that the aqueous extract of *Ocimum sanctum* showed wide inhibition zones in *S. aureus* compared to the alcoholic extract[11].

CHEMICAL COMPOSITION OF Ocimum sanctum THAT MAKES IT AN ANTIBIOTIC:

The Tulsi leaves extract has some quantity of volatile oil which contains phytochemicals such as aldehyde, terpenes (sesquiterpenes, monoterpenes), and phenols and it also contains some quantity of saponins, tannins, glycosides, quinone, phlobatanin, Flavonoids (orientin & vicenin), steroids, coumarin and alkaloids[12]. The chemical constituent in tulsi is essential oil which has methyl chavicol, eugenol linalool, camphor, and methyl cinnamate. Besides oil, the plant also contains alkaloids, glycosides, saponins, and tannins[13].

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It was discovered that the leaves of *Ocimum sanctum* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol[14]. The high linoleic acid content in *Ocimum sanctum* fixed oil could contribute to its antibacterial activity[15].

Azadirachta indica:

A LITERATURE REVIEW ON THE RATE OF INHIBITION OF *Staphylococcus* aureus BY THE EXTRACTS OF *Azadirachta indica*:

When Azadirachta Indica's stem bark or leaves were experimented against *S.aureus*, the inhibition zone decreased as the dilution factor increased.

Ethanol extracts were the most effective. Neem extracts with low concentrations did not show the effect on *S. aureus* but by increasing the extract concentration, the effect was obtained as the dilution level decreased[16]. Several groups have tested the antibacterial activity of neem against *Staphylococcus aureus*. For example, Garg et al. (2015) demonstrated that methanol and chloroform neem extracts performed better than other plant extracts and better than several different antibiotics against both *S. aureus* and *P. aeruginosa*. a neem leaf ethanolic extract was found to inhibit *S. aureus* and methicillin-resistant S. aureus (MRSA) biofilm adherence at 62.5 and 125 µg/ml, respectively (Quelemes et al., 2015)[17].

The result of using different concentrations of ethanolic, methanolic, and aqueous extracts of neem leaf against the bacteria *Staphylococcus aureus* are as follows[18]:

Table 1: Inhibition zone Diameter of different concentrations of extracts in the presence of S.aureus

Inhibition zone diameter of respective extracts in the presence on S.aureus	Concentration of extract (mg/ml)							
	200	100	50	25	12.5	6.25	3.125	1.562
Ethanolic extract	22	19	17	14	14	11	11	0
Methanolic extract	20	17	16	13	12	11	8	0
Aqueous extract	18	16	15	13	12	11	11	0

Table 2: Minimum inhibitory concentration (mg/ml) of neem leaf extract

Minimum inhibitory concentration (mg/ml) of neem leaf extract			
Test organisnm	Water	Ethanol Extract	Methanol Extract
S. aureus	1.562	1.562	1.562

CHEMICAL COMPOSITION OF Azadirachta indica TO MAKE IT AN ANTIBIOTIC:

Leaves of Neem tree contain ingredients such as nimbin, nimbanene, nimbandiol, ascorbic acid, 6-desaacetylnimbinene, nimbolide, n-hexacosanol and amino acid, 7-desaacetyl-7- benzoylgedunin, 7-desaacetyl-7-benzoylazadiradione, 17-hydroxyazadiradione, and nimbiol . Quercetin and Beta-sitosterol are polyphenolic flavonoids that were purified from Neem fresh leaves and they are known to have antibacterial and antifungal properties. Seeds of the Neem tree hold valuable constituents including gedunin and azadirachtin. The active constituent of *Azadirachta indica* shows therapeutic implications in the modulation of cell signalling pathways involved in the management of cancer. Neem and its active ingredient play a role in the prevention and treatment of tumors due to its broader pharmacological activities[19].

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Table 3: Chemical composition of Neem

Sr. No	Source	Chemical Composition	Uses
1	Seed oil	Nimbidin	Anti-inflammatory, Anti-arthritics,Hypoglycemic, Antipyretic, Spermicidal, Antifungal, Antibacterial, Diuretic
2	Seed oil	Azadirachtin	Antimalarial
3	Seed oil	Nimbin	Spermicidal
4	Seed oil	Nimbolide	Antimalarial, Antibacterial
5	Seed oil	Gedunin	Antimalarial,Antifungal
6	Seed oil	Mahmoodin	Antibacterial
7	Bark	Gallic Acid and Catechin	Antibacterial
8	Bark	Margolone, Margolonone, and isomargolonone	Antibacterial
9	Leaf	Cyclic trisulphide and Cyclic tetrasulphide	Antifungal
10	Leaf	Polysaccharides	Anti-inflammatory
11	Bark	Polysaccharides G1A, G1B	Antitumor
12	Bark	Polysaccharides G2A	Anti-inflammatory
13	Bark	NB-2 Peptidoglucon	Immunomodulatory

Aloe barbadensis–

A LITERATURE REVIEW ON THE RATE OF INHIBITION OF *Staphylococcus aureus* BY THE EXTRACTS OF *Aloe barbadensis*:

The largest inhibition zone was recorded against *S. aureus* and confirmed by scanning electron microscope. The plant extract could also block the biofilm formation by most *S. aureus* strains. In conclusion, the current results may support the use of aloe vera extract as an antibacterial agent against methicillin-resistant *S. aureus* infections[20].

One of the compounds attributed to antibacterial activity against *Staphylococcus aureus* is aloe-emodin[21] which acts by inhibiting biofilm development and extracellular protein production[22].

Staphylococcus aureus had the quiet zone of inhibition diameter on both aloe leaf and root extract respectively at 100 µl, whereas the ethanol extract had a significant effect on the zone of inhibition of *S. aureus*. It was found that at 100 µl concentration of aloe vera leaf and root extract showed similar sensitivity as that of standard antibiotics used against all bacterial isolates Gram-negative as well as Gram-positive, respectively[23].

CHEMICAL COMPOSITION OF ALOE VERA TO MAKE IT AN ANTIBIOTIC:

It contains an enzyme called Bradykinase which helps in reducing excessive inflammation[24] when applied to the skin topically. Aloin and emodin act as antibacterials[25].

It provides 20 of the 22 human-required amino acids and 7 of the 8 essential amino acids. It also contains salicylic acid which possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances the penetrative effect of the other ingredients into the skin. Saponins which are soapy substances form about 3% of the gel and have cleansing and antiseptic properties.

Its anti-inflammation mechanism works as Aloe vera inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production from arachidonic acid. Recently, a novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts[26].

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Aloe vera contains antimicrobial properties, which can be valuable in controlling causative agents of mastitis such as *S. aureus*, *Streptococcus agalactiae*, and *E. coli*[27]. some reports have described that intramammary formulations[28] of Aloe vera and other plants show an anti-inflammatory effect.[29]

The Chromones present have anti-inflammatory properties. It contains salicylic acid that possesses anti-inflammatory and antibacterial properties. The hormones – auxins and gibberellins have anti-inflammatory action. It contains 6 antiseptic agents: Lupeol, Salicylic acid, Ureanitrogen, cinnamomic acid, phenols, and sulfur. They have an inhibitory action on bacteria[30].

The antibacterial properties of *Aloe-emodin* work by slowing the transfer of electrons in the mitochondrial respiratory system, where phenolics are compounds derived from phenol and can change the nature of bacterial proteins, by increasing the permeability of cells which causes cells to become blocked and damaged[31].

RESULTS:

Leaf extracts of both *A. indica* and *O. sanctum* have shown antimicrobial activity against *E. coli* and *S. aureus*. *A. indica* has higher antimicrobial activity against *S. aureus*. In contrast, the *O. sanctum* was more effective against *E. coli*. (indicated by the zone of inhibition)[32]. Eugenol (l-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum sanctum*, is responsible for its therapeutic properties. Ursolic and Carvacrol are responsible for the antimicrobial properties in the *Ocimum sanctum.* It has maximum anti-microbial activity at 4% concentration level[33].

Ethanol extract of neem leaves exhibits in vitro antibacterial activity against both staphylococcus aureus starting at 50% concentration and MRSA starting at 75% concentration[34]. Aloe vera's constituents: Lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols, and sulfur have inhibitory action towards staphylococcus aureus[35].

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