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ESSENTIAL OILS A PROMISING THERAPY FOR STAPHYLOCOCCAL INFECTIONS

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ABSTRACT

In the milieu of managing staphylococcal infections, health departments are facing a slow down to curb the antibiotic menace. This is in concordance with the scenario that in the last decade Vancomycin Intermediate Staphylococcus aureus (VISA) are emerging around the globe and is necessitating the need to find alternative treatments. In the present study effect of various essential oils was studied against S.aureus isolates from various clinical samples. Comparative activities of different essential oils was recorded. Thyme essential oil was found to be effective against most of the S.aureus isolates. All the S.aureus isolates (n=25) exhibit sensitivity to all the essential oils, however clove oil and thyme oil showed excellent antibacterial activity. Essential oils offer a novel prophylactic approach for controlling Staphylococcal infections. The use of essential oils may prevent the development of infections and will minimize antibiotic use, prevent development of resistance as well as promote healing.

KEY WORDS

Antibiotic resistance, Essential oils, S.aureus

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a ubiquitous bacterium that can cause minor skin and soft-tissue infections. They are also associated with diseases that have high morbidity and mortality such as toxic shock syndrome (TSS) and necrotizing pneumonia. *S. aureus* also produce a large number of virulence factors that contribute to colonization and lesion progression. These secreted virulence factors are primarily expressed during post-exponential and stationary phase and include a large group of exoenzymes such as glycerol ester hydrolase (lipase) and proteases. A large group of exotoxins are also secreted by *S. aureus*, including highly inflammatory cytolysins (mainly α, β, γ, and δ toxins) and super antigens (SAgs) such as enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1).

Staphylococcus aureus cause a wide spectrum of clinically significant Hospital and Community acquired infections in human, including skin and soft-tissue infections and life threatening systemic infection (Daum, 2008; Pacheco et al., 2011; Karamatsu et al., 2012). The frequency of methicillin resistant S. aureus (MRSA) infections has now been declared a public-health imperative (Okesola, 2011; Dhand & Sakoulas, 2012; Stefani *et al*., 2012). Of additional concern is the fact that these organisms are resistant to most available antibiotics, and therapeutic options for treatment and control of MRSA infections are very limited (Nuno et al., 2012).

An alternative strategy that is now gaining interest Is to inhibit the synthesis of bacterial virulence factor that are essential for bacterial growth and/or survival in infected host (Wang et al., 2007; Escaich, 2008; Oh et al., 2010; Artini et al., 2011; Mitchell et al., 2012). Staphyloxanthin, a yellowish-orange carotenoid pigment, is one of the important virulence factors of *S.*

aureus (Liu & Nizet, 2009). Lennette et al., 1985, reported that 90 % of *S.aureus* isolates from human infections are pigmented. The carotenoid Pigment provides integrity to its cell membrane (Mishra *et al*., 2011). Staphyloxanthin has been associated with enhancement of bacterial survival in harsh environments and during infections (Giachino et al., 2001; Kahlon et al., 2010). The membrane pigment promotes resistance to reactive oxygen species (ROS) such as O_2, H_2O_2 and HOCl generated by host neutrophils (Langet *et al*., 2000; Clauditz *et al*., 2006; Song *et al*., 2009b).

Numerous studies have discovered promising novel antimicrobial candidates from plant-derived essential oils (EOs). EOs are particularly interesting as some oils have been used by native groups for curative purposes in the past (Saravolatz *et al*. 1982; Burt 2004). Also, research data indicate that many EOs have antimicrobial activity. In previous studies, the antimicrobial activities of other EOs have also been investigated, and their actions against various pathogens, including clinical MRSA isolates, have been demonstrated (Cox et al., 1998; Elsom and Hide 1999; Hammer *et al*. 1999; May *et al*. 2000; Takarada *et al*. 2002; Edwards-Jones *et al*. 2004; Brady et al. 2006; Prabuseenivasan et al. 2006; Chao et al. 2008; Doran et al. 2009; Tohidpour et al. 2010). There are also several clinical studies and case reports noting the successful use of EOs in treating MRSA nasal carriage and wound infections (Caelli et al. 2000; Sherry et al. 2001; Dryden et al. 2004; Sherry and Warnke 2004).

MATERIAL AND METHOD

The EOs like Thyme oil, Clove oil, Lemon oil, Nilgiri oil and Sesame oil were used as antibacterial component against *S. aureus*.

Microorganism and Media

The microorganism employed were *S.aureus* isolates (n=25) of clinical origin (pus and urine). *S.aureus* isolates were stored in glycerol solution at -20^{0}c. *S.aureus* were screened on Mannitol salt Agar, Baired Parker Agar and by Coagulase test. These isolates were also studied for staphyloxanthin pigment production. Antibiotic susceptibility test was done for each isolate by Kirby Bauer disc diffusion Method using EOs like Thyme oil, Clove oil, Lemon oil, Nilgiri oil and Sesame oil (CLSI 9th ed, 2006).

RESULT AND DISCUSSION

The continued emergence of multiple Drug resistant *S.aureus* originating from community and nosocomial sources necessitates the development of new and improved antimicrobial agents for the prevention and treatment of these Life threatening infections (Hall et al., 2003). Rosenbach isolated the pigment producing pathogen in pure culture and proposed the name *S.aureus* (aureum-golden, in Latin) and stated that it is one of the Virulence factor responsible for producing pathogenesis (Rosenbach, 1884). The golden pigment is responsible for Neutrophil killing and promotes virulence through its antioxidant activity (Liu et al., 2005). Pigment production is also associated with harsh environmental conditions and increased staphylococcal pathogenicity and impairing neutrophil killing (Liu et al., 2005)

Table No.1: Antistaphylococcal activity of various EOs against *S.aureus*

Sr. no.	Sample No.	Clinical Sample	Clove Oil	Thyme Oil	Lemon Oil	Nilgiri Oil	Sesame oil
1	O1	Pus	10mm	15mm	9mm	10mm	4mm
2	O2	Pus	12mm	16mm	8mm	9mm	3mm
3	O3	Pus	9mm	8mm	7mm	7mm	4mm
4	O4	Pus	13mm	10mm	5mm	6mm	4mm
5	O5	Pus	11mm	12mm	6mm	9mm	5mm
6	O6	Urine	6mm	8mm	4mm	5mm	2mm
7	O7	Urine	8mm	10mm	5mm	8mm	3mm
8	O8	Urine	10mm	13mm	7mm	10mm	5mm
9	O9	Urine	7mm	12mm	5mm	7mm	3mm
10	W1	Pus	10mm	12mm	8mm	9mm	4mm
11	W2	Pus	9mm	15mm	7mm	10mm	3mm
12	W3	Pus	10mm	13mm	6mm	11mm	5mm
13	W4	Pus	13mm	12mm	10mm	12mm	6mm
14	W5	Pus	11mm	14mm	7mm	10mm	3mm
15	C1	Urine	2mm	9mm	6mm	8mm	2mm
16	C2	Pus	10mm	20mm	6mm	12mm	3mm
17	C3	Pus	16mm	15mm	5mm	13mm	8mm
18	C4	Urine	15mm	9mm	8mm	11mm	3mm
19	C5	Urine	13mm	16mm	8mm	13mm	5mm
20	Y1	Urine	9mm	6mm	4mm	6mm	4mm
21	Y2	Urine	10mm	7mm	3mm	5mm	3mm
22	Y3	Urine	7mm	10mm	7mm	5mm	2mm
23	Y4	Urine	8mm	12mm	6mm	10mm	3mm
24	Y5	Urine	9mm	10mm	4mm	7mm	4mm
25	Y6	Pus	10mm	9mm	6mm	6mm	3mm

Note: O – Orange color pigment, W- White color Pigment, C- Cream color Pigment, Y- Yellow Color Pigment

Although there are many investigations on the *E. coli* (ATCC25923) susceptibility to EOs, study relating to EOs extracted from dried leaves of parsley, lovage, basil, and thyme. In previous studies the sensitivity of *E. coli* (ATCC 25923) was tested with EOs from fruits of lovage (Mirjalili MH et al., 2010) from aerial parts of basil (Hussain AI, Anwar F et al., 2008) and commercially available EOs of basil and thyme (Stefan M, Zamfirache MM, Padurariu C et al., 2013). Against B. cereus (ATCC 11778), commercially available EOs of parsley, basil, and thyme (Dobre AA, Gagiu V, Petru N et al., 2011) and the EO from aerial parts of basil (da Silveira SM, Cunha Junior A, Scheuermann GN et al., 2012) were tested. The sensitivity of *S. aureus* (ATCC 6538P) was tested with commercially available EOs of basil and thyme (Bors, MD, Tofana M, Suharoschi R, et al., 2016). Against *P. aeruginosa* (ATCC 27853), EOs from aerial parts of basil and thyme (Beatovi_c D, Krsti_ et al., 2015) as well as commercially available EOs of parsley, basil, and thyme (Gutierrez J, Rodriguez G, Barry-Ryan C, et al., 2013) were tested. The sensitivity of *S. typhimurium* (ATCC 14028) was tested with EOs from aerial parts of basil (da Silveira SM, Cunha Ju´ nior A, Scheuermann GN et al., 2012) leaves of basil (Eriotou E, Anastasiadou K, Nikolopoulos D, Koulougliotis D et al., 2015) flowers and leaves of thyme (Lu F, Ding YC, Ye XQ, Ding YT et al., 2011) and commercially available EOs of parsley, basil, and thyme (Gutierrez J, Rodriguez G, Barry-Ryan C, Bourke P et al., 2008).

From above result it is concluded that EOs (Thyme oil, Clove oil, Lemon oil (LR grade) Purchased from Burgoyn Urbidges and Co, India and Nilgiri oil and Sesame oil purchased from medical shop at Gadchiroli, Maharashtra India and Sesame oil) are very effective against staphyloxantin pigment producing organism. From this study it is concluded that Thyme oil is highly effective against *S.aureus* which gives maximum inhibition zone of 20mm for C2 isolate. Among the 5 EOs which were used Thyme oil is highly effective than clove oil inhibits the virulent factor of *S.aureus* than nilgiri oil. Lemon oil and sesame oil is not showing any inhibitory effect against *S.aureus.*

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