International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:107-109
UGC Approved Journal No 48951

Original Article | Open Access

Inhibition of STX Virulence Factor Biosynthesis in *Staphylococcus aureus* by Thyme EO.

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Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Thool Vaishali U and Khobragade Kavita D (2018) Inhibition of STX Virulence Factor Biosynthesis in *Staphylococcus aureus* by Thyme EO., *Int. J. of Life Sciences*, Special Issue, A12: 107-109

ABSTRACT

Staphylococcus aureus is a nuisance pathogen and STX is the central eponymous feature of *Staphylococcus aureus*. To counteract with this global problem a more natural approach is required as antibiotic resistance is developing with the passage of time. The aim of this study was to study the antibacterial effect of Thyme EOs (EO) against STX producing *Staphylococcus aureus* isolates from various clinical samples. Thyme EO was found to be effective against all the S.aureus isolates (n=25). Antistaphylococcal activity of thyme oil showed the maximum inhibition zone of 22 mm. More such EOs should be explored for counteracting the increase in growing antibiotic resistance by STX producing staphylococcus isolates.

Key words: Antistaphylococcal activity, Thyme oil, *S. aureus*.

INTRODUCTION

Some strains of *S. aureus* are competent of producing a virulence factor STX (STX) - a carotenoid pigment which gives the pathogen a characteristic golden color. STX are antioxidants in nature and the biosynthetic pathways leading to its synthesis have been known. The carotenoid pigment has the ability to douse the singlet oxygen and helps the microbe to survive killing with reactive singlet oxygen used by the host immune system (*Krinsky,* 1993) the golden pigment is responsible for neutrophil killing and promotes virulence through its antioxidant activity. The antioxidant action of STX helps the microbe evade death by reactive oxygen species produced by the host immune system. (*Clauditz et al.,* 2006). Many researchers are working on drugs to inhibit the bacterium's production of the STX and these drugs may also weaken the activity of STX and renew its susceptibility to antibiotics (*Liu et al., 2005).* Also, a drug developed in the context of cholesterol-lowering therapy has shown to block *S. aureus* pigmentation and disease progression in a mouse infection model. (*Liu et al., 2008). In this context EOs can be explored in different ways as Methicillin Resistant Staphylococcus aureus and Vancomysin Resistant Staphylococcus*

aureus are emerging in India (Thool et al., 2012). EOs are composed of volatile aromatic compounds with strong odor and are produced by plants as secondary metabolites (Bakkali *et al*., 2008). The use of EOs in medicines, perfumes, cosmetics and food preservatives is known from long. Different classes of compounds present in EOs include 1- Terpenes which include: monoterpene and sesquiterpenes, 2-Oxygenated compounds including phenols, alcohols, aldehydes and ketones,esters. Thymus species have been shown to have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities. *Thymus vulgaris* L. (thyme), locally known"zaatar" or "zaitra', a member of the family Lamiaceae, is widely used in Morocco folk medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties. The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants all over the world. *Thymus* species are commonly used as herbal tea, flavoring agents and as medicinal plant. Hence this study was carried out to assess the thyme oil for arresting the STX producing *S. aureus*.

Materials and Methods

Antimicrobial agents and chemicals: The following commercially available compounds were purchased from the indicated manufacturers: Mueller–Hinton agar (MHA) and Mueller–Hinton broth (MHB) from Hi Media Laboratories, Mumbai, India. All standard chemicals were of analytical grade. For thyme oil, the compound (LR grade) was purchased from Burgyon Urbidges and Co., India.

Isolation of STX producing *S. aureus*: Clinical samples (pus, urine, catheters) were collected from various pathological laboratories of Gadchiroli district and inoculated into Tryptose soya broth. On inoculating the broth on Baird Parker Agar (BPA) and Mannitol Salt Agar (MSA) medium *S. aureus* were isolated and identified using morphological and cultural characteristics. Pigment production was studied by visual inspection of colonies grown on Nutrient Agar after 48 hrs of incubation.

Agar diffusion susceptibility testing : Thyme EO was assessed against all *S.aureus* strains (n=25). Antimicrobial susceptibility testing was performed on Mueller Hinton Agar (MHA) plates by the Kirby Bauer disc diffusion method and according to Clinical and Laboratory Standards Institute (CLSI) guidelines. (Bauer *et al.,* 1966; CLSI. 2006).

RESULT AND DISCUSSION

Consequently, the yellow pigment plays the key to the ability of *S. aureus* to survive immune system attacks. It may act as an antioxidant which prevents CCl_4 induced toxicity in liver, kidney and testis in mice (Kurjogi *et al.,* 2010). Production of pigment is influenced by the *rsbUVWsigB* system (Morikawa *et al.,* 2001).

Table 1 Antistaphylococcal activity of Thyme EOs against STX virulent factor producing *S.aureus*

Sr. No.	Sample No.	Zone of inhibition in mm diameter
1	O1	15mm
2	O2	**16mm**
3	O3	08mm
4	O4	10mm
5	O5	12mm
6	O6	08mm
7	O7	10mm
8	O8	13mm
9	O9	12mm
10	W1	12mm
11	W2	15mm
12	W3	13mm
13	W4	12mm
14	W5	14mm
15	C1	09mm
16	C2	**20mm**
17	C3	15mm
18	C4	09mm
19	C5	**16mm**
20	Y1	06mm
21	Y2	07mm
22	Y3	10mm
23	Y4	12mm
24	Y5	10mm
25	Y6	09mm

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

Katzif *et al.,* 2005 reported that pigment production is regulated by *CspA* through a *SigB* dependant mechanism. Lan *et al.,* 2010 indicates an intimate link between purine biosynthesis and oxidative phosphorylation for *in vivo* survival and pathogenesis of *S. aureus* and targeting purine biosynthesis is a promising strategy to develop anti *S. aureus* therapies. In this present study on Nutrient Agar pigment

production was seen after 48 hrs and 09 (36 %) orange colonies, 05 (20%) each of white and cream colonies and 06 (24%) Yellow colonies were noted. In the present study orange and cream pigment producing *S. aureus* isolates were more sensitive to *Thymus vulgaris* EO than other isolates. Zarringhalam *et al.,* 2013 indicated that Thyme EO can play a significant role in inhibition of *Escherichia coli O157: H7* and *Staphylococcus aureus.* Semeniuc *et al.,* 2017 stated that thyme EO exhibited strong antibacterial activity against *E.coli*, moderate against *S. typhimurium* and *B.*cereus, and mild inhibitory effects against *P. aeruginosa* and *S. aureus,* Combinations of lovage/thyme and basil/thyme EOs displayed antagonistic effects against all bacteria, parsley/thyme EOs against *B. cereus*, *S. aureus*, *P. aeruginosa*, and *E. coli*.

CONCLUSION

From this study it is concluded that Thyme oil was effective against STX pigment producing organism. In future drugs like thyme EO in combination with other drugs designed may be used to block the production of the STX and further may increase oxidant sensitivity and decrease whole-blood survival. This study offers a novel therapeutic approach in the treatment of complicated *S. aureus* infections.

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