

# Essential oils showing *in vitro* anti MRSA and synergistic activity with penicillin group of antibiotics

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**Abstract:** This study was planned in order to investigate effective essential oils to inhibit *in-vitro* growth of Methicillin resistant *Staphylococcus aureus* (MRSA). In this study using disc diffusion method anti MRSA activity of ten diverse essential oils extracted from traditional plants namely *Thymus vulgaris* L, *Mentha pulegium*, *Ocimum sanctum*, *Mentha piperita*, *Cymbopogon citratus*, *Rosmarinus officinalis* L., *Cortex cinnamom*, *Citrus nobilis x Citrus deliciosa*, *Origanum vulgare* and *Mentha sp.* was examined. All the essential oils inhibited growth of *S. aureus* to different extent, by exhibiting moderate to elevated zones of inhibitions. Essential oils of cinnamon (*Cortex cinnamomi*) and thyme (*Thymus vulgaris* L) were observed to be the most powerful against MRSA strains used in this study. At lowest concentration of 25µl/ml essential oils comprehensible zone of inhibition was found 9±0.085mm and 8±0.051mm respectively, and at elevated concentrations there was a total decline in growth of MRSA and a very clear zone of inhibition was observed. A synergistic effect of essential oils in amalgamation with amoxicillin a Penicillin group of antibiotic was also examined. Interestingly a strong synergism was observed with oregano (*Origanum vulgare*) and pennyroyal mint (*Mentha pulegium*) essential oils, which were not so effective alone driven out to be important synergistic candidate. Our results demonstrated that essential oils of cinnamon and thyme can be used as potential antimicrobial agent against the Methicillin-resistant *Staphylococcus aureus* infections and Amoxicillin antibacterial activity can be enhanced using active constituents present in oregano and pennyroyal mint essential oils.

**Keywords:** Methicillin-resistant, *Staphylococcus aureus* (MRSA), essential oils.

## INTRODUCTION

*S. aureus* belonging to the *Staphylococcus* genus is one of the superbug organism becoming troublesome bacterial strain of contemporary invasive medicine. Since 1960 methicillin-resistant *Staphylococcus aureus*, is considered to be a key pathogens responsible for many infections associated to health care that are usually difficult to treat (Bigos, 2008). Bacteria have developed resistance to antibiotics. Methicillin-resistant *S. aureus* strain is one such example that exist persistently within the hospital environment due to its simultaneous resistance to several groups of traditional antibiotics including beta-lactam antibiotics, aminoglycosides, lincosamides, tetracyclines, quinolones and rifampin. Microbial resistance to conventional antibiotics has led to find new sources as antimicrobial agents. In the last few decades there has been an exponential growth in the field of herbal medicines due to their natural origin and lesser side effects (Brahmachari, 2001). Plants have provided a good source of anti infective agents; tannins, terpenoids, alkaloids, flavonoids emetine, quinine and berberine remain highly effective tools in the fight against microbial infections (Marjorie, 1999). One area of interest in

offering antimicrobial drugs is the use of essential oils as some oils have been used for curing purposes in the past (Carson *et al.*, 1995). Essential oils have demonstrated their abilities of possessing antibacterial, antifungal, antiviral insecticidal and antioxidant and anticancer properties (Edris, 2007).

Active constituents found in essential oils, influence various biochemical processes in the pathogenic bacterial strains, exhibiting interactive cumulative antibacterial effects (Harris, 2003). Plants active constituents are considered a treasure as safe alternative source of antimicrobials (Sharif and Banik, 2006 and Doughari *et al.*, 2007). Many essential oils are already reported as having antimicrobial properties such as oregano, basil, peppermint and rosemary. But the data regarding antibacterial activities of essential oils of different medicinal plants against resistant strains of bacteria *i.e.*, Methicillin-resistant *Staphylococcus aureus* (MRSA) is very scanty in Pakistan. The present study has been conducted to screen essential oils of different medicinal plants for their antimicrobial potential alone and in synergy with amoxicillin antibiotic a penicillin class of antibiotics. Information obtained could be exploited for further medicinal uses.

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## MATERIALS AND METHODS

### *Plant material*

Aerial parts of all the tested plants including leaves and inflorescence were harvested from Herb Clonal Repository at Plant Genetic Resources Institute, National Agriculture Research Centre (NARC), Islamabad Pakistan. The dried Cinnamon was purchased from market.

### *Essential oil extraction*

300gm of dried and ground plant material was subjected to hydrodistillation for 2h using a modified Cleverger type apparatus according to the method outlined by the European Pharmacopoeia (Anonymous 1997).

### *Bacterial strains and antibiotic susceptibility test*

Fifteen different samples of *Staphylococcus aureus* were obtained from Clinical Microbiology Laboratory of Pakistan Institute of Medical Sciences (PIMS), These samples were from various clinical specimens. The disk diffusion method was used to investigate the specimens using nine different antibiotics according to (Finegold and Baran, 1996). The antibiotic discs from (Oxoid) Azithromycin (AZM, 15µg), penicillin (P, 10 units), Cefoxitin (FOX, 30µg) Sulphamethoxazole-Trimethoprim (SXT, 25µg), Vancomycin (VA, 30µg), Moxifloxacin (MXF, 5µg), Gentamycin (CN, 10µg), Chloramphenicol (C, 30µg), and Teicoplanin (TEC, 30µg) were used. The zones of inhibition in mm were interpreted as sensitive, moderate sensitive and resistant using the interpretation chart supplied by antibiotic discs manufacturers (Oxoid).

### *MIC determination of essential oils*

The Minimum Inhibitory Concentration of essential oils were conventionally determined in triplicate by macrodilution broth assay (Stalons and Thornsberry, 1975).

### *Screening of antibacterial activity*

Antibacterial activity of essential oils on bacterial growth was assessed in presence of different concentrations of essential oils using the agar diffusion technique (Rota *et al.*, 2004) against selected strain of MRSA. The volume of 0.1ml (approximately  $10^9$  cell/ml) of the tested microorganism grown in nutrient broth was spread onto the entire surface of petri plate containing nutrient agar. The sterile paper discs (Whatman No. 1) with 10 µl of essential oil from each concentration was applied onto the solidified nutrient agar plate inoculated with bacterial suspension. Amoxicillin was used as a positive control and DMSO was used as negative control. Inoculated plates were incubated at 37°C for 24 hr the zone of inhibition (mm) was measured. Sensitivity of each essential oil was classified by the diameter of zone as per the procedure of Ponce *et al* (2003) and Moreira *et al* (2007).

### *Synergistic effect of essential oils in combination with Amoxicillin*

Antibacterial activity of the antibiotic alone and its combinations with all essential oils were performed in presence of different increasing concentrations of essential oils using the agar diffusion technique. The concentrations of each oil and antibiotic were prepared as follows 1:1 v/v.

## RESULTS

Different antibiotics were tested against selected strain of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical samples of pus. The resistance profiling of the strain against various antibiotics as stated in table 1 clearly indicates the trends observed; Vancomycin formed a zone of 17mm and was recorded to be effective in inhibiting the strain. Other antibiotics which successfully inhibited the strain included Chloramphenicol, Teicoplanin and their zones of inhibition recorded were mostly between 17mm to 20mm. On the other hand, antibiotics which strain illustrated resistance towards included Sulphamethoxazole-trimethoprim, Gentamicin, Penicillin, Cefoxitin, Azithromycin and Moxifloxacin. The zones of inhibition measured for these were between 12mm to 16mm with Azithromycin, Cefoxitin and Penicillin forming no zones of inhibition. Antibacterial activity pattern of the essential oils against MRSA showed in fig. 1. The Minimum Inhibitory Concentration (MIC) could be defined as the concentration of essential oil at which half of the microbial population is inhibited. Lowest MIC was recorded for thyme essential oil as half of the MRSA population was inhibited at a concentration 8µl/ml. MIC for citrus peel, lemongrass and cinnamon essential oil was found to be 10µl/ml whereas, for rosemary and Holy basil MIC value was recorded to be 15µl/ml. For oregano essential oil, MIC was found to be between 20µl/ml to 25µl/ml. Mint asavi and pennyroyal mint MIC value was 25µl/ml.

MRSA showed different range of susceptibility to each essential oil when tested in direct contact using disc diffusion technique. The zone of inhibitions sizes varied depending upon the oils and the concentration used. Analysis of variance showed highly significant differences among tested essential oils and their different concentrations for antimicrobial activity against MRSA strain as shown in figs. 2a and 2b.

The preeminent effectiveness was displayed by cinnamon at all tested concentrations *i.e.*, 25 µl/ml, 50 µl/ml, 75 µl/ml, 100 µl/ml and 125 µl/ml ( $9 \pm 0.085$ ,  $10 \pm 0.010$ ,  $16 \pm 0.0371$ ,  $18 \pm 0.034$ ,  $19 \pm 0.032$ , respectively) as shown in table 2. Thyme strongly inhibited the strain at 100µl/ml and 125µl/ml concentration and produced zones of inhibition measuring  $16 \pm 0.619$  and  $18 \pm 0.034$ mm,

respectively. Oregano and citrus peel also showed moderate efficacy. Results showed that mint asavi, rosemary, lemongrass and Holy basil were less effective against MRSA strain. The least effective oils included peppermint and pennyroyal mint which had the smallest mean values for the zones of inhibition even at 125µl/ml i.e., 10±0.015 and 10±0.011mm, respectively (table 2)

The study also focused upon analysing the effects resulting from applying synergy of different essential oils and antibiotic Amoxicillin. Each of the ten essential oils under report were used in synergy with Amoxicillin at a ratio of 1:1 v/v. Analysis of variance showed highly significant differences for essential oils and different concentrations when used in synergy with antibiotic for antibacterial activity. The mean values for synergistic antibacterial activity of selected essential oils in combination with amoxicillin in 1:1 (v/v) Oregano and amoxicillin at equal ratios of variable concentrations ranging between 25µl/ml to 125µl/ml showed the maximum size of zones (17±0.011 upto 30±0.160). *M. pulegium* which was not very active alone showed excellent results in synergy. Cinnamon and thyme were also highly effective in giving combinatory effects. Mint

asavi has also been found synergistic enhancer as it moderately inhibited MRSA. Citrus peel was the least effective in synergy, even at highest concentration zone of inhibition of 14±0.010mm diameter was observed.

## DISCUSSION

The study revealed that three of the ten essential oils demonstrated strong effectiveness against MRSA. Out of three, cinnamon essential oil exhibited highest activity at all the tested concentrations. At lowest concentration i.e., 25µl/ml, a clear zone of inhibition was obtained of 9±0.085mm diameter, while at higher concentrations, MRSA growth reduction was observed, with zone of inhibition of 19±0.032mm at 125 µl/ml. These findings are consistent with the results of Bowels *et al.* (1995) who reported that cinnamon oil inhibited the growth of *Staphylococcus aureus*, Matan (2006) who observed that cinnamon and clove oils had strong and reliable inhibitory effects against various pathogens. Hoque *et al.* (2007) also reported that cinnamon essential oil at 5% concentration showed highest antibacterial activity against *Staphylococcus aureus*. The thyme oil was also highly effective, and antibacterial activity of oregano and

**Table 1:** Resistance profiling of MRSA strain

Antibiotic disks (OXOID)	Abbreviations	Zone of inhibition (mm)	Resistivity pattern
Gentamycin	CN (10 µg)	7	Resistant
Sulphamethoxazole-trimethoprim	SXT (25 µg)	16	Resistant
Moxifloxacin	MXF (5 µg)	12	Resistant
Penicillin	P(10 units)	No inhibition	Resistant
Teico planin	TEC (30 µg)	17	Sensitive
Vancomycin	VA (30 µg)	17	Sensitive
Chloramphenicol	C (30 µg)	20	Sensitive
Azithromycin	AZM (15 µg)	No zone	Resistant
Cefoxitin	FOX (30 µg)	No inhibition	Resistant

**Table 2:** Inhibition zone diameter (mm) of selected essential oils against MRSA

Essential oil	Concentration of E.O. in µl/ml				
	25	50	75	100	125
Citrus Peel	8±0.043	10±0.033	12±0.009	13±0.001	14±0.100
Thyme	8±0.051	10±0.075	14±0.086	16±0.619	18±0.034
Oregano	8±0.031	10±0.000	12±0.100	15±0.043	16±0.160
Holy Basil	8±0.054	9±0.071	10±0.000	11±0.083	12±0.031
Cinnamon	9±0.085	10±0.010	16±0.0371	18±0.034	19±0.032
Lemongrass	8±0.05	9±0.079	10±0.001	11±0.010	12±0.051
Rosemary	8±0.043	9±0.001	10±0.569	11±0.000	12±0.043
Pennyroyal	6±0.010	7±0.086	8±0.055	9±0.0173	10±0.011
Pepper Mint	6±0.173	7±0.011	8±0.000	9±0.085	10±0.015
Mint Asavi	8±0.055	9±0.084	10±0.075	11±0.035	12±0.000

Results are presented as mean (± standard deviation) size of inhibition (in mm)

citrus peel essential oil followed thyme oil. The zone of inhibition for thyme ranged from  $8 \pm 0.051$  mm to  $18 \pm 0.034$  mm. Present findings were supported by the results of Akgul and Kivanc 1988; Panizzi *et al.* 1993 and Nelson, 1997 who narrated strong antibacterial activities of thyme essential oil. It seems that the antimicrobial properties of thyme essential oils are mainly related to their high phenolic content,

In the current investigation, Rosemary, Mint Asavi, Lemongrass and Holy Basil oils have shown slightly less effectiveness than Cinnamon, Thyme and Oregano in inhibiting MRSA. But earlier studies reported broad spectrum antimicrobial properties of Lemongrass EO (Aiemsaard *et al.*, 2011). In the present investigation, among all tested plants Peppermint and Pennyroyal exhibited least inhibitory activity against MRSA. Sartoratto *et al.*, 2004 also reported antimicrobial activity of Peppermint particularly against drug resistant gram positive bacterial strains. The potential reason can be due to this fact the divergence in climate and growth conditions has undeviating effects on plants' active constituents (Ghasemi-Pirbalouti *et al.*, 2009) and thus the plants grown in diverse climate area should be screened for bioactive compounds (Motamedi *et al.*, 2009).

Occasionally, essential oils have been found to be synergistic enhancers in that though they may not produce any important inhibitory effects alone, but when they are used in mixture, the synergistic effect exceeds their individual presentation (Gibbons *et al.*, 2003). In our experiment, despite that some essential oils showed weak antimicrobial effects alone, the interactions between antibiotic and essential oil were merely additive against MRSA. Results indicate that Oregano and Pennyroyal exhibited strong synergistic activity. These results were in agreement with a previous report who mentioned a synergetic effect even these essential oils did not show any activity by themselves (Rodrigues *et al.*, 2009; Mahboubi *et al.*, 2010). There is proof in the literature that the essential oil of Oregano with dissimilar antibiotics such as fluoroquinolones, doxycycline, lincomycin and maquindox possessed modest to good synergistic effects against *E. coli* (Si *et al.*, 2008).

An imperative finding of this study was the fact that Oregano has very strapping combinatory effects against MRSA, this may be due to the presence high content of carvacrol in oregano. Another possible reason is the fact that synergistic effects of essential oils are more effective against gram-positive than gram-negative bacteria (Su *et al.*, 2012). Past studies carried out on the synergistic effect of essential oils against MRSA are restricted which makes it difficult to compare and confirm results. In the case of Peppermint, Cinnamon and Holy basil, good synergetic effect was noted with the zone diameter of 20 mm. Lemongrass and Mint Asavi showed

very less synergistic effect. As Mint Asavi has not been completely investigated, therefore, more studies need to be conducted to search for active compounds of this plant, so that it can be used in therapeutic treatment. Moreover, results of synergy are variable which shows the diversity research presents and scope of the essential oils.

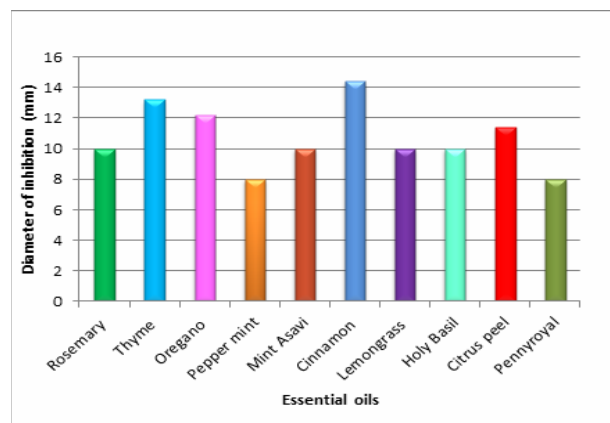


Fig. 1: Antibacterial activity of the essential oils against MRSA.

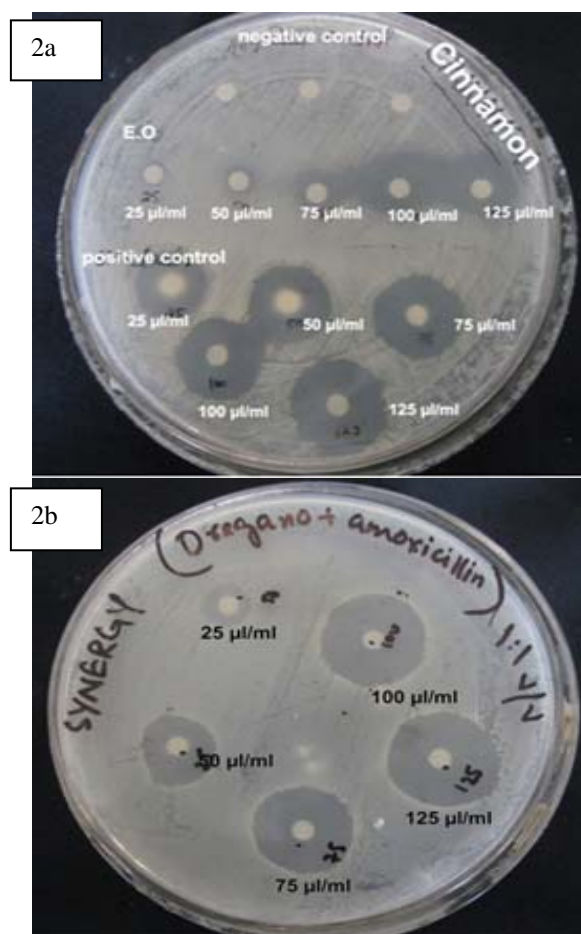


Fig. 2: (a) Screening of antibacterial activity of Cinnamon, (b) Synergistic activity of Cinnamon.

## CONCLUSION

The current study supports the notion that essential oils possess strong antimicrobial activity or potential. It has been examined that all the essential oils possess both bacteriostatic and bactericidal activity a lot higher than that of synthetic antibiotics when tested *in vitro*. It could be concluded that Cinnamon and Thyme could be the best antimicrobial agents to kill test pathogen. There are heaps of possibilities for the essential oils to be employed in combination with antibiotics as novel treatment modalities to the bacterial infections.

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