Antimicrobial activity of plant-based Essential Oils and Synergistic effect against selected Human Pathogenic Bacteria

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ABSTRACT

The occurrence of multidrug resistant by pathogens is a universal issue for providing tolerable treatment for various infectious diseases. The predictable anti-microbial proxies are relatively active against plentiful strains, still need of more drugs against multidrug resistant pathogens. Herbal drugs have potential against antimicrobial activity from ancient days and their treatment of pathogenic diseases is increasing for developing plant based natural products. Essential oils have combat against antibiotic resistant bacteria. Thus our study was focused on plant based essential oil against human pathogenic bacteria. The necessary oil extracted from *Curcuma longa* and *C. martini* revealed protuberant anti-microbial accomplishments against *B. subtilis*, *Escherichia coli*, *P. aeruginosa* and *S. aureus*. Low concentration of *C. longa* oil at 10 μl inhibited the growth of all strains *Bacillus subtilis* followed by *E. coli* and *S. aureus*. Maximum bustle was noted in *P. aerogenosa* at 40 μl in both *C. longa* and *C. martini* oils. Human pathogenic strain treated with *C. martini* oil showed maximum inhibition in *P. aerogenosa* followed by *B. subtilis*, *E. coli* and *S. aureus*. Synergistic activity of oils against the strains showed maximum inhibition at 40 μl in *P. aerogenosa*. 

Therapeutic florae and its foodstuffs are used as traditional medication for eras. As there are many synthetic medicines for diseases, still eighty percentage of the populace in the world depends on ethnomedicine for their therapeutic determinations (Palant and Steenkamp, 2008). More than nine thousand innate plants have been acknowledged and chronicled for their therapeutic possessions.

INTRODUCTION

Disease caused by infections are increasing day by day in recent years. It is addressed as a second foremost cause of death in the world population (Luqman et al., 2005). Resistance developed by many pathogens is the main reason for inactive antibiotics (Mothana and Lindequist, 2005). This resistance can be caused by many reasons such as unselective use of extensive field antibiotics, intravenous catheters, organ replacement, etc. (Selvamohan et al., 2012). About 2.22 million hospitalized and one lakhs six thousand patients expired in a solitary year because of adverse drug reactions (Joshi et al., 2011). Due to multidrug resistance and synthetic microbial drugs there is an urgent need to combat the issue by a potential antimicrobial substances from natural origin (Wigmore et al., 2016).

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Vital oils from therapeutic and aromatic plants are more aromatic in nature because of the availability of secondary metabolites such as aldehydes, terpenes, alcohols, ethers, esters, phenolic and ketones (Swamy and Sinniah, 2016). They remain employed for aromatherapy and many ailments such as cardiovascular disease, alzheimer, diabetes, malignancy etc, (Ali et al., 2015). Studies against pediatric patients also reveals no side effects (Man et al., 2019). It has been studied by some researcher regarding impact of essential oil against microbial strains (Safi and Al-Mariri, 2014).

*Curcuma longa* Linn is an herbaceous plant of Zingiberaceae family extensively used as Indian traditional remedy for, rheumatism, wound healing, indigestion and several eye ailments (Aggarwal et al., 2007). In India, China and South East Asia it is utilized as a food preservative and coloring agent in various aspects. It also used wide for its biological activities such as anti-inflammatory (Aggarwal and Harikumar, 2009), anti-bacterial activities (Naz et al., 2010).

*Cymbopogon martini* Roxb. commonly known as palmarosa, which shows more beneficial activity against central nervous system pathologies such as neuralgia, epileptic and anorexia (Buch et al., 2012). It has a potential as antimicrobial, antigenotoxic and antioxidant agent (Sinha et al., 2011; Thaker et al., 2009). Crucial oil from this herbal plants are produced by countries like India, Brazil and Madagascar for different purposes. The aim was to inspect the anti-microbial potential of *C. longa* and *C. martini* oils against human pathogenic bacterial strains and their potential on synergistic activity.

**MATERIALS AND METHODS**

**Extraction of oil**

The healthy fresh leaves of *Curcuma longa* and *Cymbopogon martini* were collected during early hours from the field near Southern Western Ghats nearby Thiruvanaalai (krishnankovil), Virudhunager District, TN, India. The collected leaves of *C. longa* and *C. martini* were chopped finely and subjected to hydrodistillation for 4 hours in Clevenger kit. The CI-EO and Cm-EO separated were placid in a hygienic glass container. Water precipitations were detached using anhydrous Na₂SO₄ and the CI-EO, Cm-EO were kept at 4°C for the auxiliary studies.

**Microorganisms**

Bacterial strains *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus* acquired from “Microbial Type Culture Collection and Gene Bank” (MTCC), Chandigarh, India. The cultures were preserved by sub-culturing periodically in nutrient agar slants and preserved at 4°C.

**Screening of anti-bacterial activity**

Anti-bacterial activity was confirmed by well diffusion method (Mukherjee et al., 1995). The bacterial inoculum was prepared on tryptone soya agar for overnight. The nutrient media on sterile petriplates were kept for solidification. About 6mm diameter four wells were done by sterile micropipettes. The test organisms were swabbed into sterile nutrient medium petriplates. Different concentrations such as 10, 20, 30 and 40 ppm of *C. longa* and *C. martini* were used for the assay and the inoculated plates were gestated at 37°C for 24 hours. The diameter of the inhibition zone around each well was measured for anti-bacterial activity. Each investigation was completed in triplicate. The mean diameter of the inhibition zone was documented.

**Statistical Investigation**

Information from tests were exposed to investigation of and information were communicated as a mean of five recreates. Critical contrasts between treatment bunches were broke down by Tukey’s various range test (noteworthiness at P < 0.05) utilizing Minitab®17 programme.

**RESULTS**

Antimicrobial activity of *C. longa* and *C. martini* oils against human pathogenic bacteria including two gram positive (*S. aureus, B. subtilis*) and two gram negative strains (*E. coli, P. aeruginosa*) using well diffusion method exposed upright results. The result reveals that both the oils has its potential to effectively suppress the microbial growth of human pathogenic bacteria with variable potency. The activity of oils against pathogenic bacteria showed concentration dependent manner. The oil of *C. longa* was the virulent oil to retard microbial growth of all tested pathogenic bacteria at higher concentration. Retardation of microbial growth was maximum at 40 μl in *P. aerogenesa* followed by *E. coli, B. subtilis* and *B. subtilis*. Minimal activity was noted in *P. aerogenesa* at 10 μl followed by *S. aureus, B. subtilis* and *E. coli*. Results obtained was shown in Table 1 and Figure 1.

The activity of *C. martini* against human pathogenic bacteria reveals the *P. aerogenesa* was most susceptible than other strains. Most efficient activity of 42 mm was observed at 40 μl in *P. aerogenesa* followed by *B. subtilis, E. coli and S. aureus*. Lower inhibitory activity was observed at lowest concentration of 10 μl in *B. subtilis* followed by *S. aureus, E. coli and P. aerogenesa*. Results obtained was shown in Figure 2.
Figure 1: Antimicrobial activity of *Curcuma longa*

Figure 2: Antimicrobial activity of *Cymbopogon martinii*
Comparing the results obtained from Cl-EO, Cm-EO oils, the most notable activity was observed in C. longa treated bacterial strains. It showed maximum activity at concentration of 40 µl followed by 30 µl, 20 µl and 10 µl. Synergistic activity potential of Cl-EO, Cm-EO oils showed increased antimicrobial activity with lower concentrations. Results obtained was shown in Table 2 and Figure 3.

The usage of essential oils have been raised day by day and have wide range of claims in diverse areas such as beautifying, medication, therapeutic, agricultural and nutrition industries (Bakkali et al., 2008). Investigation of its biological activities has been one of the attractive research area in which the present researchers are affiliated.

Resistance developed by many pathogens against antibiotics are now become a global threat. Multidrug resistant (MDR) developed by pathogens of many infectious diseases occurs due to repeated usage of most common drugs for their multiple role of action. About ninety to ninety five percentage of human pathogenic strain S. aureus are impervious to penicillin and seventy to eighty percentage against human pathogenic strain methicillin (Casal et al., 2005). In past, a successful antibiotics which are developed should have only the capability to control the infectious disease. But in present days number of factors to be achieved or overcome such as resistance of microbes, persistence level, mode of action, non-development of cross resistance, eco-friendly etc.

Resistance to antimicrobial drug therapy can be acquired by signal transmission within bacterial species (Blair et al., 2015). The resistance mechanism can be occur by transformation in the target

### Table 1: Antimicrobial screening test of oil extracts against human pathogenic bacterial strains

| Plant species         | Gram positive pathogenic bacteria |  |  |  |  |  |  |  |  |
|-----------------------|-----------------------------------|---|---|---|---|---|---|---|---|
|                       | B. subtilis                       | S. aureus                      |  |  |  |  |  |  |  |
|                        | Concentration                     | 10 µl | 20 µl | 30 µl | 40 µl | 10 µl | 20 µl | 30 µl | 40 µl |
| Curcuma longa          | 10 ± 15 ± 20 ± 24 ± 10 ± 14 ± 22 ± 26 ± 0.05 0.8 0.65 0.48 0.59 0.82 0.90 0.85 |
| Cymbopogon martini    | 1 ± 3 ± 9 ± 17 ± 6 ± 10 ± 14 ± 20 ± 0.11 0.8 0.55 0.39 0.65 0.75 0.86 0.86 |
| Plant species         | Gram negative pathogenic bacteria |  |  |  |  |  |  |  |  |
|                       | E. Coli                           |  |  |  |  |  |  |  |  |
| Curcuma longa          | 10 ± 14 ± 20 ± 26 ± 8 ± 18 ± 22 ± 0.52 0.66 0.75 0.66 0.68 0.92 0.88 0.58 |
| Cymbopogon martini    | 6 ± 10 ± 18 ± 26 ± 10 ± 16 ± 26 ± 0.48 0.62 0.68 0.59 0.87 0.87 0.69 0.62 |
| Plant species         | P. aerogenosa                     |  |  |  |  |  |  |  |  |
|                       |  |  |  |  |  |  |  |  |  |

### Table 2: Synergistic antimicrobial activity of oil extracts against human pathogenic bacterial strains

| Plant species         | Gram positive pathogenic bacteria |  |  |  |  |  |  |  |  |
|-----------------------|-----------------------------------|---|---|---|---|---|---|---|---|
|                       | B. subtilis                       | S. aureus                      |  |  |  |  |  |  |  |
|                        | Concentration                     | 10 µl | 20 µl | 30 µl | 40 µl | 10 µl | 20 µl | 30 µl | 40 µl |
| Curcuma longa          | 11 ± 16 ± 22 ± 26 ± 12 ± 15 ± 23 ± 28 ± 0.95 0.52 0.28 0.66 0.69 0.99 0.55 0.75 |
| Cymbopogon martini    | 2 ± 3 ± 11 ± 17 ± 7 ± 12 ± 16 ± 21 ± 0.55 0.38 0.88 0.74 0.65 0.97 0.55 0.77 |
| Plant species         | Gram negative pathogenic bacteria |  |  |  |  |  |  |  |  |
|                       | E. Coli                           |  |  |  |  |  |  |  |  |
| Curcuma longa          | 11 ± 15 ± 21 ± 28 ± 10 ± 19 ± 24 ± 61 ± 0.88 0.58 0.66 0.79 0.29 0.77 0.82 0.60 |
| Cymbopogon martini    | 7 ± 12 ± 20 ± 27 ± 12 ± 17 ± 27 ± 45 ± 0.29 0.86 0.77 0.63 0.38 0.29 0.83 0.99 |
Figure 3: Synergestic activity of *Curcuma longa* and *Cymbopogon martinii*

Microorganisms can create obstruction by means of extracellular medication efflux interceded by efflux siphons, target change and enzymatic corruption of medications (Ullah et al., 2013).

Essential oils of *C. longa* and *C. martini* against human pathogenic bacteria exhibited superior antimicrobial activity. As it is from plant based herbal oil it can be a safest tool for protection. A report from a review reveals herbal based extract exhibited higher antimicrobial activity when compared with synthetic antimicrobial agents (Ayaz et al., 2019).

As our oils showed better results, their action may be breakage of cell membrane, coagulation of organelles, binding and blocking of intake of nutrient sources. It is evident by the report that essential oil involves in commotion of the cell membrane and infuriating cell compound seepage, congealing of cell compounds and interfaces with ATPase membrane proteins, this leads to penetrable of molecules or ions, and inhibit synthesis of enzymes or the nutritional absorption, which cause bacterial death (Cristani et al., 2007).

Cl-EO and Cm-EO showed its potential to inhibit the growth of human pathogenic organism by showing highest activity in *P. aerogenosa*. Similarly, oils from *A. clavenae* (Skočibušič et al., 2004), *S. sclarea* (Cui et al., 2015), *Satureja cuneifolia* (Oke et al., 2009), *Salvia lavandulifolia* (Jirovetz et al., 2007), *Struchium sparganophora* (Kasim et al., 2014), *Syzygium cumini* (Mohamed et al., 2013) showed inhibitory effect against *P. aeruginosa*.

At 40 μl the essential oils against *E. coli* was markable in their inhibitory activity. Comparable with the results obtained from oils of *Warionia saharae* (Sellam et al., 2014), *Verbena officinalis* (Shan et al., 2011), *Thuja plicata*, *Thuja occidentalis* (Jirovetz et al., 2007), *Thymus kotschyanus* (Ahmadi et al., 2015), *Syzygium cumini* (Mohamed et al., 2013) also implies the similar results.

Cl-EO showed prospective activity against *B. subtilis* at higher concentration when compared with *P. aeruginosa*, *E. coli* and *S. aureus*. Result obtained from extracted oils of *Dracocephalum foetidum* (Saet et al., 2007), *Fortunella margarita* (Bozin et al., 2006), *Myrtus communis* (Ait-Ouazzou et al., 2012), *Ocimum kilimandscharicum* (Lawal et al., 2014), *Oreganum vulgare* (Amatiste et al., 2014; Bozin et al., 2006) showed prominent activity against *B. subtilis*.

Synergistic activity of oils showed good inhibitory level when compared with individual oils activity. Thus it has good inhibition ability to control human pathogenic bacteria in minimal level than individual oils. Correspondingly the synergistic effect of two or additional indispensable oils against humanoid pathogens deliberate by numerous scholars also proclaims the same results (Nazzaro et al., 2013).

Combined activity of oils showed effective antimicrobial activity at lower concentrations. This
demonstrates the beneficial synergistic potential of *C. longa* and *C. martini* oils. Similarly, antibiotics neomycin and amikacin reduction was observed when combined with *Lippia origanoides* against *Staphylococcus aureus* (Barreto *et al.*, 2014). In the same way the combination of *A. triphylla* and *L. alba* oils against *Aeromonas* spp reduced effective antimicrobial concentrations (de Souza *et al.*, 2017).

Even though many antibiotics had developed and are presently in markets, there are still requirements of new novel drugs to suppress the microbial pathogens because of drug resistance. The usage of synthetic drugs are limited because of its carcinogenic effects, acute toxicity (Swamy *et al.*, 2016). In this aspect the natural plant based oils can be used to control epidemic multi drug resilient pathogenic microorganisms (Mulyaningsih *et al.*, 2010).

**CONCLUSION**

The essential oils from *C. longa* and *C. martini* showed significant antimicrobial activity, but its inhibitory level increased with lower concentration through synergism. This reduced dosage can contribute decreased bacterial resistance. Thus efficacy of traditional antibiotics can be governed by our essential oils. Further work has to be focused on synergistic activity of essential oils with conventional antibiotics.

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**Conflict of Interest**

The authors declare that there is no conflict of interest for this study.

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