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STUDY OF ESSENTIAL OILS FOR THEIR ANTIMICROBIAL PROPERTIES AGAINST MICROORGANISM

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Abstract – Essential oils are highly trending due to their wide scope of properties. Essential oils of cinnamon, clove, palmarosa and tea tree, obtained from a local licensed distiller, were tested against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative bacteria (*Enterobacter sp.* and *Escherichia coli*). The essential oils were diluted to four different concentrations each (100%, 75%, 50% and 25%) and tested. Cefotaxime (50mcg, 30mcg, and 10mcg) and 0.5% Tween20 (v/v) were chosen as positive and negative control respectively. It was observed that out of all the four oils, cinnamon essential oil showed the highest antimicrobial activity, followed by that of clove. Cinnamon and clove showed antimicrobial activity in all the concentrations. *Enterobacter sp.* was observed to have the highest susceptibility towards cinnamon essential oil. Palmarosa oil showed the lowest antimicrobial activity and was not ith effective at lower concentrations. Cinnamon has potential to be used in therapeutics to overcome antibiotic drug resistance and as a natural alternative to synthetic therapeutics, preservative.

INTRODUCTION

Ayurveda and Unani are well-known traditional practice of medicine. Ethnomedicine reflects the practice of medicine with the help of ethnic knowledge. It is a term synonymous to traditional medicine. The world has started shifting its focus onto products that are more "natural" in nature. This concept has gained demand in sectors such as cosmetics and skin-care, food preservation, pest control and medicine. Every now and then a new natural-based product is coming up as an alternative to the synthetic products. Essential oils have been popularised with the concept of aromatherapy and these products are major constituent of in cosmetic and body-care products. These are mixtures of various highly complex chemical compounds, characterised for being volatile, aromatic and biologically active. The biologically active compounds collectively making up the essential oils can be categorised into phenolics, phenolic acid and quinones, tannins, glycosides, alkaloids, terpenoids and terpenes (Voon *et al*., 2012), and are generally termed as secondary metabolites. They have very good properties of antioxidation, antiinflammation and spasmodic action (Swamy *et al*., 2016; Singh and Maurya, 2005). They have potential apoptosis activity against tumour cells and are oestrogen stimulant (Ali *et al*., 2015). Apart from the above, they are popularly used in aromatherapy, linked with cosmetic, medical and psycho-therapy (Ali *et al*., 2015). Essential oils usually do not show genotoxicity (Bakkali *et al*., 2008) but their major mode of action involves destabilisation of cellular architecture, disruption of cell membrane integrity, giving rise to increased permeability (Swamy *et al*., 2016; Tongnuanchan and Benjakul, 2014). The essential oils of cinnamon (*Cinnamomum verum*), clove (*Syzygium aromaticum)*, tea tree (*Melaleuca alternifolia* var *Cheel*) and palmarosa (*Cymbopogon martini)* have been studied for their antimicrobial activity, for a very long time. These products are well-known and have been used by major ethnic groups around the world. They are chief ingredients to many home-remedies. Apart from being home-remedies, their properties are utilised in fields of cosmetology, dentistry, and have the potential to replace conventional therapeutics, preservatives and pesticides. Their antimicrobial action was studied against gram-positive bacteria (*Bacilus subtilis*, *Staphylococcus aureus*), and gram-negative bacteria (*Escherichia coli*, *Enterobacter sp.*) here.

MATERIALS AND METHODS

Microorganisms

Cultures of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Enterobacter sp.* were used from their respective preserved cultures at ASPEE Shalkilam Biotechnology Institute, Navsari Agricultural University, Surat, Gujarat. The purified cultures were transferred to pre-sterilised nutrient broth with the help of metallic wire-loop under aseptic conditions, the cultures were incubated at 37 degree Celsius, 120 rpm for 24 hrs.

Essential Oils

The essential oils of cinnamon (*Cinnamomum verum*), eucalyptus (*Eucalyptus globulus*), tea tree (*Melaleuca alternifolia* Cheel) and palmarosa (*Cymbopogon martini*) collected from local distillers specialized in preparing Unani Medicine (Hakim's Chi Chi and Hakim's Natural, Surat, Gujarat). The oils were claimed to be extracted using steam distillation. Diluted emulsion of each essential oil was prepared.

Tween20 was used to enhance solubility and stability of essential oils in emulsion suspension. Tween20 also enhances diffusion of essential Oils through the medium (Raeisi *et al*., 2015).

Disks of 6mm were punched out of whatman paper no. 5. The disks were dried and sterilised in autoclave under 121 psi for 15 mins. 20-25 disks were soaked in each of the essential oils for 1-2 minutes.

Control

0.5% v/v Tween20 was chosen to act as negative control for the experiment performed. 5% and 3% (w/v) cefotaxime was chosen as negative control.

The solutions were vortexed at 3000 rpm to make an evenly distributed emulsion. 10-15 sterilized whatman paper no. 5 disks (6mm) were soaked in each vortexed negative and positive control solutions.

Analysis of Antimicrobial Activity

Nutrient Agar was prepared and poured into clean test tubes. Filled test tubes were sterilised in autoclave under 121psi for 15 minutes. Test tubes were let to cool down for 5 minutes before inoculation of bacterial cultures. 1000 μL from broth cultures (incubated for 24 hours at 37 ^{o}C) of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Enterobacter sp.* were pipetted using a micropipette. Pipetted culture was inoculated into test tube containing molten cooled nutrient agar. Test tubes were gently rotated to evenly distribute the inoculated culture in the medium. The content of test tubes was transferred into sterilized petri plates. The medium was let to solidify before impregnation of essential oil disks.

Disk Diffusion Method

Test Plate: Solidified culture plate (consisting bacterial culture inoculated nutrient agar) was impregnated with essential oil disk immediately after soaking them in the emulsions. The disks were slightly tapped on sterilised bloating paper to remove excess fluid. Four disks of different concentrations (100%, 75%, 50% and 25%) of the same oil were impregnated in each plate.

Control Plate: Solidified culture plate (consisting bacterial culture inoculated nutrient agar) was impregnated with positive control (cefotaxime) and negative control (Tween20) immersed disk. The disks were slightly tapped on sterilised bloating paper to remove excess fluid. Each plate consisted of four disks, 3 positive control (50mg, 30mg and 10mg cefotaxime) and 1 negative control.

All the plates were incubated overnight at 37 ^{o}C.

RESULTS

It was observed that essential oil of cinnamon showed the highest level of overall antimicrobial activity. Zone of inhibition for 25% cinnamon essential oil was clear and visible for all the test organisms. The inhibition zone from cinnamon was also observed to be the largest amongst all the tested essential oils for virtually all the concentrations. essential oil of cinnamon was followed by that of clove. The clove essential oil also showed antimicrobial activity at 25% for all the test organisms.

Lowest antimicrobial activity was observed for palmarosa essential oil. 25% and 50% Palmarosa essential oil showed antimicrobial activity in cultures of *Bacillus subtilis* and *Escherichia coli* only. Cefotaxime positive control was highly active against all of the test microorganisms.

Table 1. represents the overall result obtained

from the experiment. It represents the zone of inhibition (in mm) observed for different concentration of the treatments used against the selected microorganisms.

DISCUSSION

In this study conducted, it is well observed that cinnamon essential oil has a very good and reliable effect on microorganisms. Here, cinnamon essential oil showed the highest antimicrobial activity in comparison to clove, palmarosa and tea tree essential oils. Saad Rashad El-Zemity and his team in 2018, studied antimicrobial activities of 13 essential oils extracted from plants well-known for their antimicrobial properties. Study was conducted against two major plant pathogen, *Agrobacterium tumefaciens* and *Erwina carotovora*. Out of the 13 essential oils, cinnamon essential oils showed the highest activity, followed by clove essential oil (El-Zemity *et al*., 2008). The bioactive compound cinnamldehyde is the major contributor towards the oils antimicrobial activity but the effect is higher when it works with its other counter-compunds present in the essential oil (Raeisi *et al*., 2015).

A study conducted on 21 essential oils extracted from different plant sources also found Cinnamon essential oil to be the most effective out of all the other oils. The same study also found Cinnamon oil to be more effective than streptomycin at certain concentrations (Prabhuseenivasan *et al*., 2006). The zone of inhibition obtained through this study seems to be less wide than theirs, which can be due to variation in dilution method, use of different nutrient media or due to the difference in quality of the essential oils.

Studies of Prabuseenivasan, 2006 and Saad Rashad El-Zemity, 2008 and their respective teams also found clove essential oil to be an effective antimicrobial following the effects of cinnamon. Eugenol is the major contributor of antimicrobial property of clove essential oil (Bhuiyan *et al*., 2010). Time-kill studies of *E. coli, P. aeruginosa* and *S. aureus* state that within 10 minutes, clove essential oil has the potential to exponentially decrease the percentage of microbial survivors, from 100% to 0.0001% (Nuñez *et al*., 2012). In comparison with sodium propionate (standard food preservative), clove essential oil gives much satisfactory results against many food borne microbes (Gupta *et al*., 2008). Failure of antimicrobial activity of clove essential oil against clinically isolated strains have been reported in a recent studies (Packyanathan and Prakasam, 2017). Also, clove essential oil imparts toxicity to human body if consumed abnormally

Table 1. Antimicrobial activity of Selected Essential Oils against Test Microorganisms by Disk-Diffusion method

Treatment and its concentration used		Zone of Inhibition of Growth Observed for Each Test Organism(mm)			
		Bacillus subtilis	*Enterobacter sp.*	*Escherichia coli*	*Staphylococcus aureus*
Cinnamon Essential Oil	25%	8	11	9	9
	50%	9	12.5	9.5	11.5
	75%	11.5	17.5	13.5	13
	100%	16.5	19	14.5	16
Clove Essential Oil	25%	8.5	7.5	8	7.5
	50%	9	7	7.5	8.5
	75%	11	11.5	10	9.5
	100%	13.5	13.5	11.5	14
Palmarosa Essential Oil	25%	7	0	8.5	0
	50%	7	0	9	0
	75%	8	7.5	9.5	7.5
	100%	9	7.5	10	8
Tea Tree Essential Oil	25%	0	7.5	6.5	7
	50%	7	7	7	8
	75%	7	8	7.5	8
	100%	9	9.5	15	10.5
Control	Blank	0	0	0	0
	10mcg	19	28	11	11.5
	30mcg	26	31	17	17.5
	50mcg	28	34	19	20.5

(Dibazar *et al*., 2014; Prashar *et al*., 2006).

On contratry, palmarosa and tea tree showed comparatively lower activities of antimicrobial action. The results of other comparative analysis also state that the two oils show a relatively smaller zones of inhibition when studied besides oils of cinnamon, clove, lime, thyme etc. In studies against plant bacterial wilt, palmarosa essential oil serves as a good soil treatment chemical effective against bacterial wilt (Pradhanang *et al*., 2003). As mentioned by M. H. Lodhia and his team in a study conducted in 2009, palmarosa was found to be more effective against *Escherichia coli* than *Staphylococcus aureus* (Lodhia *et al*., 2009). This information matches the result obtained in the current experiment. Yet, palmarosa oil was more effective in their study as the zone of inhibition in their results are wider and the oil showed good microbial activity in lower concentrations as well. In spite of significantly lower antimicrobial properties, palmarosa essential oil has been proven to be a very effective insect repellant, especially against mosquito when tested on human volunteers topically (Das *et al*., 2003; Maia *et al*., 2011).

Tea tree essential oil did show expected results in the experiment conducted according to the repost published by Prabuseenivasan, 2006. In a report, Tea Tree oil performed better than antibiotic cefapime when tested against *Bacillus subtilis, Streptococcus agalactiae, Aromonas hydrophilia* and *Pseudomonas aeruginosa* (Mumu, 2017). Tea tree Tree may have shown comparatively lower antimicrobial activity against bacteria, but it has been reported that the Oil has better anti-fungal properties (Agarwal *et al*., 2010; Ergin and Arikan, 2002; Mertas *et al*., 2015).

CONCLUSION

From the experiment and all the reading done regarding it, it can be well concluded that cinnamon essential oil has strikingly good antimicrobial properties. This is followed by clove. Tea tree and palmarosa have comparatively lower antimicrobial properties. The results of this experiment can be compared and are similar with various journal articles. It was well observed that both gram-npositive as well as Gram-negative bacteria, were susceptible to the essential oils. Under few treatments, gram-negative bacteria showed higher susceptibilty towards the essential oils than the gram-positive bacteria.

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