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STUDIES ON THE ANTIMICROBIAL EFFECT OF SYZYGIUM AROMATICUM AND *LAVANDULA ANGUSTIFOLIA* ON SOME MICROBES

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ABSTRACT

Present investigation was undertaken to evaluate antimicrobial activity of *Syzygium aromaticum* (Clove) and *Lavandula angustifolia* (Lavender) and to determine its inhibition effect by different exposure times against some microbes. The volatile oil of Clove and Lavender was assessed for antimicrobial activity using the micro atmosphere method. An assay was performed to determine the exposure times of essential oil for the inhibition of microbes. The oil was tested against five microbe's namely- methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococc*i (VRE), *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Candida albicans*. The oil was bacteriocidal against all organisms except for VRE. There was considerable variability in size of zone of inhibition depending on amount of oil used and a statistical significant difference in antimicrobial activity was observed. *L.angustifolia* gave a total reduction in growth of microbes. *C. albicans* was also observed to be susceptible to *L. angustifolia* after three hours of exposure. Neither of the essential oils used were observed to be best against all microbes. Essential oils have the potential to be used as antimicrobial agents both for medical and for more commercial applications. Thus Clove and Lavender oil could serve as good antimicrobial agents.

KEYWORDS: Antimicrobial, Clove oil, Lavender oil, microbes.

INTRODUCTION

Essential oils have been traditionally used for treatment of infections and diseases all over the world for centuries.[1] Today the use of essential oils is a growing market and there are a considerable range of applications. The oils are used for example, in the food and beverages industry and as fragrances in perfumes and cosmetics, but the oils also covered a broad spectrum of biological activity which has lead to an increased interest among researchers. They can be synthesized in several plant organs such as buds, flowers, leaves, stem, branches, seeds, berries, roots, wood or bark, being stored in secretary cells, cavities, channels, epidermal cells or trichomes.[2] In recent years there has been extensive research to explore and determine the antimicrobial activity of essential oils. All oils tested to date have displayed some antimicrobial activity and some have been shown to be more effective than others. Essential oils are employed in aromatherapy and for the treatment of several diseases including cardiovascular disease, diabetes, alzheimer's and cancer.[3] Thymol, carvacrol, linalool and eugenol are main constituents of some plant essential oils that have been shown to have a wide spectrum of activity against microbes.[4,5] Members of this class are known to be either bactericidal or bacteriostatic, depending upon the concentration used. In the last decade there has also been an increased interest in essential oils and their antimicrobial activity due to the spread of antibiotic resistance. Essential oils have several biological properties such as larvicidal action,[6] antioxidant,[7] analgesic and anti-inflammatory,[8] Fungicide,[9] and antitumor activity. [10] Essential oils have tremendous business potential on the global market owing to their unique flavour and fragrance properties and also biological activities.[11,12]

Ever since the discovery of penicillin by Alexander Fleming in 1929 many new classes of antibiotics have become available for treatment of bacterial infections, but due to excessive and often unnecessary use of antibiotics in humans and animals, bacterial resistance has now been reported against every currently available antibiotic.[13] Microorganisms can be useful to combat various infectious diseases.[14] Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and resistant strains of *Pseudomonas* are examples of multi resistant bacteria that are becoming an alarming problem within the healthcare system. MRSA is probably the most common antibiotic resistant bacterium found in hospitals throughout the world and it naturally colonises skin and infects wounds. Today the prevalence of MRSA is between 25-50 percent in parts of the world, including the USA, Australia, South America and central parts of Europe.

Even in Scandinavian countries, where MRSA rates have been low, the frequency is beginning to rise.[15] VRE has also spread throughout the world since it was first discovered and isolated in the late 80's and can now be found in every continent. *Enterococci* can cause bacteraemia, wound infection and urinary tract infection, but serious infections of VRE usually occurs in patients with significantly compromised host defences.[16] *Candida* and *Pseudomonas* are other opportunistic pathogens that usually lead to serious infections in immunocompromised individuals. Therapies for *Candida* have been difficult because of the limited number of antifungal agents, and for *Pseudomonas* even drug-susceptible strains have considerable defences against antibiotics.[17,18]

The factors responsible for the spread of resistant bacteria do not differ so much compared to the ordinary strains of bacteria and they are most frequently seen in hospitals. The most common route of spread is through indirect transmission from the healthcare staff to their patients. Staff may carry the resistant bacteria on their hands or clothing and even equipment in the hospital can become contaminated and a source of infection.[19] New therapies are therefore necessary and of great value.

Hence the present study was carried out to investigate the antimicrobial activity of commercial essential oils: Clove and Lavender and to determine their inhibition effect by different exposure times. The essential oils were tested against five different microorganisms: MRSA, VRE, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Candida albicans*.

MATERIALS AND METHODS

Essential oils used

The essential oils used in this study were: *Syzygium aromaticum* (Clove) and *Lavandula angustifolia* (Lavender).

Clove essential oil

Clove essential oil is extracted from *Eugenia caryophyllata* also known as *Syzygium aromaticum* of the Myrtaceae family. Although clove oil is a very potent that should be used with great care in aromatherapy. Clove oil has a warm, strong, spicy smell and the oil is colourless to pale yellow with a medium to watery viscosity. A native of Indonesia and Malacca Islands, it is an evergreen tree that grows to about 10 meters tall and has bright green leaves and nail-shaped rose-peach flower buds which turn, upon drying a deep red brown. These are beaten from the tree and dried. It was often used by the Greeks, Roman, and the Chinese to ease toothache and as a breath sweetener, especially when taking to the emperor. Clove oil can extract from the leaves, stem and buds. The main chemical components of clove oil are eugenol, eugenol acetate, isoeugenol and caryophyllene. The oil is a very potent and should be used with care. It may cause irritation to the skin of some individuals and can easily irritate the mucus membranes. It should be avoided during pregnancy. The therapeutic properties of clove oil are analgesic, antiseptic, antispasmodic, anti-neuralgic, carminative, anti-infectious, disinfectant, insecticide and stomachic. Clove oil can be used for acne, bruises, burns and cuts, pain killer and keeping infection at bay. It is also used for mouth sore, tooth ache, rheumatism, and arthritis. It is effective against digestive system, diarrhoea, parasites, as well as bad breath. Clove oil is valuable for relieving respiratory problems like bronchitis, asthma, and tuberculosis. It is also of use for skin problems, especially for skin sores and leg ulcers and as an insect repellent.

Lavender essential oil

Lavender oil extracted from *Lavandula angustifolia* of Lamiaceae (Labiatae) family. It has a light fresh aroma, is clear in colour and watery in viscosity. It is calming and relaxing oil, which combats stress and crisis. In addition to its antimicrobial property, it is traditionally believed to have sedative, anti-depressive and anti-inflammatory. It is excellent for asthma and migraines. It has a healing effect on skin. It was a favourite for strewing on the floor since it released an aroma when walked upon and it is often used these days in toilet water as an insecticide or in sachets to be placed between linen. It is also used to clean wounds and treat burns. The therapeutic properties of lavender oil are antiseptic, analgesic, anticonvulsant, anti-rheumatic, antispasmodic, anti-inflammatory, antiviral, bactericidal, carminative, deodorant, diuretic, hypotensive, nervine, sedative, and vulnerary. Lavender oil has a soothing and calming effect on the nerves, relieving tension, depression, panic, hysteria, and nervous exhaustion in general and is effective for headaches, migraines and insomnia. It is also very beneficial for problems such as bronchitis, asthma, colds, laryngitis, throat infections and whooping cough. It relieves pain when used for rheumatism, arthritis, lumbago, and muscular aches and pains especially those associated with sport. It is useful for all types of skin problems such as abscesses, acne, oily skin, boils, burns, sunburn, wounds, eczema, dermatitis, psoriasis, lice and insect bites. In vapour therapy, lavender oil can be useful for allergies, anorexia, dizziness, sleeplessness, hay fever, headaches, trauma, anxiety, fear, nightmares, irritability, nervous tension and as an insect repellent. It can be used as massage oil or diluted in the bath for abdominal pains, anorexia, bowel disorders, fatigue, insomnia, moodiness, and hysteria.

Experimental method

The method used in the present study for the estimation of essential oil activity is a well-tried method.[19] The essential oils were assayed against following microorganisms: MRSA, VRE, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Candida albicans*. MRSA, VRE and *Streptococcus pyogenes* were clinical strains obtained from Govt. Hospital. The bacteria were grown in nutrient broth and Mueller-Hinton agar. Sabouraud dextrose agar was used as

growing medium for *C. Albicans*. First a primary screening was performed to ensure that there were no differences in the bacterial growth between agar plates made out of glass and plastic due to the vapour from the essential oils. Positive controls were also used to ensure that MRSA and VRE were resistant against methicillin and vancomycin respectively. The methods and volumes used for these assays were the same as described below except for the antibiotic susceptibility test, where the activity was determined using the disc diffusion assay. Broth cultures of MRSA and VRE were spread on to different agar plates and antibiotic discs were then placed on to the surface of the agar. All media and discs-antibiotic as well as blank discs were purchased from Oxoid, UK. Commercial essential oils purchased from the manufacturer were used for the experiments.

The antimicrobial activity was determined using the micro atmosphere method. Agar plates were made at least one day before use and broth culture of bacteria was prepared fresh for each assay. The concentration of the broth culture was measured using a spectrophotometer and a standard plate count of viable microorganism was performed to ensure that a sufficient cell concentration was used. The plates were dried before 250µL of broth culture was spread on to the surface. Cultures were vortexed for 15 s before being pipetted on to the agar. A cover slip was fixed with a droplet of water on to each lid of the agar plates and blank discs (sets of 2, 4 and 6) were then transferred on to the cover slips. The cover slips were placed in the centre of the lids and discs were placed as close together as possible. 10 µL of essential oils were then pipetted on to each disc giving a volume range of 20, 40 and 60 µL between the agar plates. For control plates 10µL sterile water was pipette on to the discs. The agar plates were then sealed with parafilm and separated into plastic bags with only one kind of essential oil in each bag. After 18 hrs of incubation at 37^0 C, the results were recorded by measuring the clear inhibition zone with a slide calliper. For oils that had a good antimicrobial effect, an additional assay was performed to determine how the inhibition was effected by different exposure times to the essential oils. The microorganisms were exposed for 1 h, 3 h and 6 h respectively to 60µL of essential oil. The lids were changed after the exposure and then the plates were re-incubated overnight. The assays were completed in duplicate and repeated independently three times.

An assay was also performed to determine whether the antimicrobial effects were bacteriostatic or bacteriocidal. After incubation, a sterile loop was used to transfer bacteria from the agar plates exposed to 60 µL of essential oils on to fresh plates. If a total reduction in growth density was seen, colonies were taken randomly and spread on to the new plates. Otherwise the bacteria were taken from the inhibition zone. The fresh agar plates as well as the old were then incubated (37^0 C, 18 hrs). On the backside of the old plates a circle was drawn before incubation, marking the initial inhibition zone.

RESULTS

There were no differences in bacterial growth between the use of plastic and glass petridishes and the antibiotic susceptibility test confirmed that VRE and MRSA were resistant against vancomycin and methicillin respectively. The results of the micro atmosphere assay of two essential oils against five microorganisms are summarised in Table 1. *Pseudomonas aeruginosa* was the only bacterium not susceptible to either of the oils and was therefore excluded from further assays. The antimicrobial effects of the essential oils were bacteriocidal against all organisms except for VRE.

There was considerable variability in size of zone of inhibition depending on type of oil used and statistically significant differences in antimicrobial activity was observed by the use of different volumes. Greater zones of inhibition were observed when 60µL of essential oil was used rather than 40µL. No essential oils were observed to be the best against all organisms, with *Candida albicans* having constantly larger zones than the other microbes. Statistically significant differences in antibacterial activity were observed between MRSA and *Streptococcus pyogenes* against *Lavandula angustifolia*. *L. angustifolia* had the best effect against *Candida albicans* with a total reduction in growth. *Syzygium aromaticum* produced larger zones of inhibition than *Lavandula angustifolia* against MRSA ($p<0.001$). No inhibition was observed for MRSA after exposure the bacteria to *Syzygium aromaticum.*

Where inhibition zones although not clear was noticed after 6 hrs exposures, clear inhibition zones were observed for *Candida albicans* against *Syzygium aromaticum*. *Lavandula angustifolia* gave a total reduction in growth, but the reduction was not as great as after 18 hrs of exposure. *Candida albicans* was also observed to be susceptible to *Lavandula angustifolia* after 3 hrs exposures. No results were obtained for *Streptococcus pyogenes* because of growth problems following subculture. The most likely explanation is that the bacteria on the stock-plate were dead when they were transferred to the nutrient broth.

Table 1: Activity of essential oils against bacteria and fungus.

***Syzygium aromaticum* (Clove)**

MRSA	VRE	*C. albicans*	*Strep. pyogenes*
6x10 µl	20.1±1.9	30.9±1.6	q
4x10µl	13.25±1.5	27.6±1.9	q
2x10µl	q	19.6±1.9	q

***Lavandula angustifolia* (Lavender)**

MRSA	VRE	*C. albicans*	*Strep. pyogenes*
6x10µl	14.2±1.5	1+	22.6±4
4x10µl	q	1+/2+	q
2x10µl	4+	2+	q
Control (sterile water)	No inhibition was Recorded		

Results are presented as mean (±standard deviation) size of inhibition (in mm). Growth density, market cursive, is presented in a scale from 0 to 4+. With zero indicating no growth and 4+ indicating the growth of the control. q= Not a clear inhibition zone for one or several of the samples.

DISCUSSION

Earlier studies carried out on the inhibitory effect of essential oil vapours are limited which makes it difficult to compare and confirm results. Most research that has been performed is based on the agar diffusion method and hasn't considered the possible antimicrobial effect by the vapour from the essential oils.

The mechanism of action is still unclear but some studies suggest that compounds penetrate the cell, where they interfere with cellular metabolism. Other studies suggest that phenols such as carvacrol and eugenol disturb the cellular membrane and react with active sites of enzymes.[19] Antimicrobial effect usually refers to *in vitro* conditions.[20]

Essential oils mainly include two biosynthetic groups, all characterized by low molecular weight, including aromatic and aliphatic constituents and terpenes and terpenoids.[21] Although linalool presents important antioxidant and antimicrobial effects,[22] it must be noted that the antimicrobial effect of an essential oil depends on all of its chemical components. The antimicrobial activity of some essential oils could be explained by the significant amount of linalool, which is an oxygenated monoterpenoid.[23] Present study has shown that the vapours from *Lavandula angustifolia* and *Syzygium aromaticum* have an antimicrobial effect. The only organism that was not susceptible to any of the tested oils was *Pseudomonas aeruginosa*. Many research works confirm that *P. aeruginosa* is more resistant against essential oils because of the cell wall structure. Gram-negative bacteria have an outer lipopolysaccharide wall that can work as a barrier against toxic agents.[24] *S. aromaticum* consists of phenolic structures (eg. eugenol and carvacrol), which have been confirmed by many studies to have a good antimicrobial activity.[25] In previous studies, essential oils with predominant alcoholic compounds have been shown to be slightly less active than compounds containing phenolic structures.[26] However the correlation between the composition and activities of essential oils have not been brought to satisfying conclusion, yet the good antimicrobial effect of lavender oil against MRSA, VRE and *S. Pyogenes* have been confirmed by Moon et. al., 2006.[27] Some essential oils including the lavender oils have been demonstrated to have *in vitro* activity against VRE using disk diffusion assays.[28] It has been noted that the inhibitory effect of essential oil can differ between the volatiles and the direct contact with microorganism and the inhibitory effect of some essential oils on fungi have been reported to be greater when the volatile oils are used. *C. albicans* was susceptible to both the tested essential oils and was the only organism where good antimicrobial activity was observed after been exposure to essential oils for 6 hrs. The antifungal activity of the volatile phase of essential oils has been reviewed by Cavanagh, 2007[29] and confirms that many oils possess strong activity against a wide range of fungi. It is unclear exactly how the volatiles are inhibiting fungal growth and why some essential oils have better activity against fungi than against bacteria. The exact mechanism of action of essential oil on fungi is unclear but the majority of reports agree that oil volatiles result in morphological changes to the hyphae.

One explanation could be the fact that the antimicrobial activity of volatile compounds result from the combined effect of direct vapour absorption on microorganisms and indirect effect through the medium that absorbed the vapour.[20,30] Fungi grow mainly on the surface of the agar medium and might be more susceptible to direct vapour contact while the antimicrobial effect against bacteria might be more dependent on the vapour accumulation into the agar. This could explain why no clear zones of inhibition were observed after 6 hrs of incubation with essential oil against MRSA and VRE. Another explanation is that some fungi are more susceptible to essential oils than bacteria. In the present study a range of sterile discs were used to allow comparison of antimicrobial effects using different volumes of essential oils. It was proven to be difficult to place and keep the sterile discs close together, which could have increased the variance of the results. In further studies it may be better to use sterile discs of different sizes rather than placing several discs next to each other.

In conclusion the present study has demonstrated that the volatile from the tested essential oils have good antimicrobial effect and that a longer exposure time of 6 hrs is necessary to obtain good results against bacteria. The result suggests that essential oils have the potential to be used as antimicrobial agents both for medical and for more commercial applications. The good effect against MRSA for some of the oils should be further investigated. Essential oil volatiles have the advantage that they can treat large areas and do not require direct contacts with liquid oils which can make it suitable for use as disinfectant of rooms and as a cleaning products. The oils might also be used as inhalation therapy against bacterial respiratory tract pathogens as *S. plyogenes* which can cause pharyngitis. Before applying vapour therapy of essential oil in clinical practise further studies are required to determine the range of the possibilities, important factors such as the minimal exposure time for efficacy, applicability and the possibility of toxicity needs to be further evaluated.

Although promising results have been obtained, further investigation is required for identification, isolation, biological evaluation, and exact mechanism of action of

active component in the essential oil responsible for antimicrobial activity.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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