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Microbiological Property Evaluation of Natural Essential Oils Used in Green Cosmetic Industry

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Abstract

This study was designed to investigate antimicrobial properties of natural essential oils including; citronella, cinnamon, palmarosa, niaouli, clove, lavender, bergamot, lemon, grapefruit, petitgrain, peppermint, tea tree and eucalyptus from different oil sources. Datas taken from the disk diffusion assay indicate that cinnamon, niaouli, lavender, petitgrain and the tea tree oils have the most intense antibacterial effects on related microorganisms at the concentration %100 (direct usage). Endpoints confirm results reported in the past studies including the MIC assays on the related natural oils. Overall, natural oils have been used safely for many decades on the cosmetic formulations and this experiment emphasizes one more time the importance of the natural oils usage in industry.

Key Words: Antimicrobial effect, essential oil, raw material, disk diffusion, green cosmetic

1. Introduction

Since ancient times, essential oils are recognized for their medical value and effective chemical properties in cosmetic industry products. Essential oils have been used as perfumes, flavors for foods and beverages, or to body and mind. As the industry goes greener, today there is a great number of natural essential oils which mentioned about their properties and effective usage in green cosmetic formulations (Hammer and Carson, 1999; Wei and Shibamoto., 2010).

Essential oils are also have long been known to have antimicrobial properties (Boyle, 1955) and these properties have been reviewed in the past studies (Nychas, 1995) as having strong antimicrobial effects (Shelef, 1984). Wilkins and Board also reported that more than 1,340 plants are known to be potential sources of antimicrobial compounds. Furthermore, antimicrobial activity of plant extracts is frequently due to the essential oil fraction, or to sulfur-containing compounds in the aqueous phase. These compounds are also responsible for the characteristic aroma and flavor of the spices. (Gould, 1996). The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997).

Microbiological Disk Diffusion test is one of the microbial safety analyzes which has to be employed and give results about how natural essential oils effect the microorganisms. Microbiological analys of the natural raw materials used in green

cosmetics must be done in the quality control process and these tests should be done carefully (Santos and Corrêa, 2015).

Here we report the further investigation of the antimicrobial activity of tea tree oil against three clinically significant micro-organisms. The purpose of this study is to provide endpoints of the data on the antibacterial activity test of most common antibacterial oils including; citronella, cinnamon, palmarosa, niaouli, clove, lavender, bergamot, lemon, grapefruit, petitgrain, peppermint, tea tree, eucalyptus. This paper will focus chiefly on the antibacterial effect of natural essential oils on pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia Coli, Enterococcus faecalis, Bacillus subtilis, Salmonella typhimurium, Staphylococcus epidermidis and Enterococcus hirae.*

2. Materials and Methods

2.1. Essential Oils

Eugenia Caryophllus (Clove), *Lavandula angustifolia* (Lavender*),Cinnamomum verum* (Cinnamon), *Citrus aurantium* (Petitgrain), *Maleleuca viridiflora* (Niouli), *Mentha Piperita* (Peppermint) *Cymbopogon Nardus* (Citronella), *Cymbopogon martinii* (Palmarosa), *Citrus Aurantium SSP Bergamia* (Bergamot), *Citrus limonum* (Lemon*), Citrus paradisi* (Grapefruit) and *Eucalyptus globulus* (Eucalyptus) natural essential oils are kindly gifted from Art de Huile/Arın Derin Ltd. Co and *Melaleuca alternifolia* (Çay ağacı) is gifted from Arerko Co Ltd. Essential oils were tested at concentration at 100% to determine related effects.

2.2. Disk Diffusion Test

By the early 1950s, most clinical microbiology laboratories had adopted the disk diffusion method for determining susceptibility of bacteria to antimicrobials. Disk diffusion method is based on the principle that sample-impregnated disk, placed on agar previously inoculated with the test microbium. In this assay test microbiums are; *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia Coli, Enterococcus faecalis, Bacillus subtilis, Salmonella typhimurium, Staphylococcus epidermidis and Enterococcus hirae* respectively*.* Micro-organisms were obtained from the culture collections of the SFA R&D Ltd. Co*.* From a pure bacterial culture, colonies are taken with a wire loop and transfered to 5 ml of PBS, arranged to 0.5 MacFarland. Test suspension is inoculated to agar and leaved for 10 minutes to dry. After the penetration of inoculum disks are employed with the test product and placed on agar.

2.3. Growth of Test Organisms

Cells used in all assays were twice passaged, in Triptic Soy Agar in case of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia Coli, Enterococcus faecalis, Bacillus subtilis, Salmonella typhimurium, Staphylococcus epidermidis and Enterococcus hirae* at 37°C for incubation.

3. Results

Observed results indicate that Tea Tree and Petitgrain natural essential oils have the most effective antibacterial property by the comparing endpoints. After these oils,

Cinnamon, Niouli, Lavender oils shows also strong antibacterial activity. Tea Tree oil has the most zone value as 30.6±1.52 mm on *Pseudomonas aeruginosa*, Petitgrain with 27.6 ±0.57 mm on *Staphylococcus epidermidis,* Cinnamon with 25.3±0.57 mm on *Enterococcus faecalis,* Niouli and Lavender with 35.0 ±0.57 mm, 30.0 ±1.00 mm *on Staphylococcus epidermidis,* respectively.

Eucalyptus oil showed the weakest effect on microorganismsm within the results such as 8.3±0.57 mm on *Enterococcus hirae* and 9.6±1.52 mm on *Pseudomonas aeruginosa.* Datas are showed in the Table 1 and given as mean ± standard deviation.

4. Discussion and Conclusion

Plant oils and extracts has been used for a wide variety of purposes for many thousands of years (Markham, 1999). The antimicrobial activity of natural essential oils and extracts has formed the basis of many applications, including raw and processed cosmetics preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997).

This study was designed with the Disk Diffusion Test method to examine the antimicrobial properties of the natural essential oils which has been using in related industries. The purpose of disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds. The pathogenic organism is grown on agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.

A wide variety of natural essential oils are known to have antimicrobial properties and in many cases this activity is due to the presence of active monoterpene constituents. Our endpoints show that Tea Tree oil has the most bacterial effect comparing to other natural essential oils. The essential oil of *Melaleuca alternifolia* (Tea Tree oil) has a long history of use as a topical antiseptic. In recent times Tea Tree oil has gained a reputation as a safe, natural and effective antiseptic. This has led to a resurgence in popularity and currently it is incorporated as the principal antimicrobial or as a natural preservative in many pharmaceutical and cosmetic products intended for external use. Examination of *Escherichia coli* cells using electron microscopy after exposure to tea tree oil revealed a loss of cellular electron-dense material and coagulation of cytoplasmic constituents, although it was apparent that these effects were secondary events that occurred after cell death. Tea tree oil also stimulates leakage of cellular potassium ions and inhibits respiration in *E. coli* cell suspensions, providing evidence of a lethal action related to cytoplasmic membrane damage (Ibukun and Toyin, 2006).

It is observed that Petitgrain oil has also strong antibacterial effect on microorganisms in our study. Datas are supported by the past studies. Previous results on Petitgrain has showed high antimicrobial activity with zone up to 20 mm diameters on *Pseudomonas aeruginosa* and showed *MIC* value as more than 2.0 on *Enterecoccus feacilis* bacteria (Hammer et al., 2007; Deans, 1987).

Table 1. Comparative zone results of natural oils in disc diffusion test

	Name of Microorganisms							
Sample name	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Escherichia coli***	***Enterococcus faecalis***	***Bacillus subtilis***	***Salmonella typhimurium***	***Staphylococcus epidermidis***	***Enterococcus hirae***
Citronella oil	11.6±0.57 mm	11.6±0.57 mm	14.6 ±0.57 mm	14.6±0.57 mm	12.0 ±1.00 mm	10.0 ±1.00 mm	31.0±1.00 mm	15.6±0.57 mm
Eucalyptus oil	9.6±1.52 mm	12.0±1.00 mm	15.3±0.57 mm	11.6 ±0.57 mm	11.0±1.00 mm	14.6±0.57 mm	12.0 ±1.00 mm	8.3±0.57 mm
Cinnamon oil	17.6 ±0.57 mm	23.3 ±0.57 mm	15.0±1.00 mm	25.3±0.57 mm	21.3±0.57 mm	23.0±1.00 mm	17.0±1.00 mm	25.0±1.00 mm
Palmarosa oil	10.3 ±1.52 mm	11.0±1.00 mm	15.6±0.57 mm	16.6±0.57 mm	12.0 ±1.00 mm	11.0±1.00 mm	20.6 ±0.57 mm	15.3 ±0.57 mm
Lavender oil	15.6±1.52 mm	20.0±1.00 mm	17.6±0.5 mm	19.6±0.57 mm	16.0 ±1.00 mm	14.3±0.57 mm	30.0 ±1.00 mm	20.6±1.52 mm
Bergamot oil	13.6 ±0.57 mm	11.0±1.00 mm	14.6±0.57 mm	26.0 ±1.00 mm	19.6±1.52 mm	11.0±1.00 mm	16.0±1.00 mm	9.3±0.57 mm
Lemon oil	15.6±1.52 mm	15.6±1.52 mm	15.6±1.52 mm	15.6±1.52 mm	13.6 ±0.57 mm	8.3±0.57 mm	14.6±0.57 mm	10.3 ±1.52 mm
Grapefruit oil	12.0±1.00 mm	12.0±1.00 mm	11.0±1.00 mm	15.6±1.52 mm	12.0 ±1.00 mm	8.3±0.57 mm	13.6 ±0.57 mm	10.3 ±1.52 mm
Petitgrain oil	27.0±1.00 mm	26.3 ±0.57 mm	19.0 ±1.00 mm	22.0 ±1.00 mm	25.6 ±0.57 mm	8.0 ±1.00 mm	27.6 ±0.57 mm	19.6 ±0.57 mm
Peppermint oil	18.0±1.00 mm	12.0 ±1.00 mm	14.6±1.52 mm	17.0 ±1.00 mm	26.0 ±1.00 mm	10.0 ±1.00 mm	25.0 ±1.00 mm	14.3±0.57 mm
Tea tree oil	30.6±1.52 mm	26.3 ±0.57 mm	24.6±1.52 mm	23.6±0.57 mm	24.6±1.52 mm	24.6±1.52 mm	26.0 ±1.00 mm	13.6 ±0.57 mm
Niouli oil	13.0±1.00 mm	20.0±1.00 mm	21.0±1.00 mm	15.0±1.00 mm	17.0±1.00 mm	15.0±1.00 mm	35.0 ±0.57 mm	13.0±1.00 mm

In a study including Cinnomons antibacterial effect claims that, employed material indicates antibacterial effect against 25 genera of bacteria with strong effect. Cinnamon use in food products and cosmetics could be a good strategy to reduce or avoid bacterial degradation and thus to reduce the incidence of infection caused by cosmetics. Cinnamon oil is also not harmful when used in correct conditions. In addition, cinnamon could be used to treat infectious disease with the light of previous suppportive antimicrobial study results (Seyed et al., 2015).

After our observation we found that Niaouli oil also has strong antibacterial effect which is supported by the data as an effective immunostimulant essential oil that increases the antibody and white blood cell count making it ideal for boosting the immune system and as a recuperative oil. When used as an inhalation, niaouli essential oil can be used to treat respiratory infection of many types, and can be gargled at the onset of a cold or sore throat.

Lavender essential oil shows one of the most strong effect from our essential oils. In a past study, antimicrobial activity of Lavender was determined by establishing the minimum inhibitory concentration (MIC) using a series of microdilutions and claimed that in vitro study indicate a significant effect of Lavender essential oil on the inhibition of microbial growth. These results encourage further studies on a larger scale that will confirm antimicrobial efficiency and define the mechanisms of action of *Lavandula angustifolia* essential oil and its individual components (Koulivand et al., 2003).

After all these endpoints, we can summerize that essential oils are gaining popularity within the supported antimicrobial results. As personal care and naturopathic remedies continue to provide alternative solutions for people desiring to take charge of their own health care, it is important to have a firm understanding of essential oils.

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