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Full Length Research Paper

Extraction of Essential oil from Eucalyptus Leaves as Antibacterial Application on Cotton Woven Fabric

[1]Seid Yimer, [2]Manoharan and *[1]Omprakash Sahu

[1]Department of Chemical Engineering, KIOT Wollo University, Kombolcha (SW), Ethiopia
[2]Department of Textile Engineering, KIOT Wollo University, Kombolcha (SW), Ethiopia

*Correspondence author Email: ops0121@gmail.com

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Many aromatic and medicinal plants are available in Ethiopia from which the essential oil can be extracted and used for medicinal purpose. In this research project Eucalyptus leaves are extracted for essential oil by steam distillation process using soxhlet apparatus. The extracted oil has antimicrobial, antibacterial and anti fungal properties. The extracted oil is applied on cotton fabrics by pad-dry-cure method using padding mangle and value adding to the ordinary cotton fabric as Medical fabric. Extracted oil from these leaves shows 81.1% with acetic acid and 79.9% with citric acid resistance to antibacterial growth. The market value and demand of such medical fabrics are high.

Keywords: Bacterial**;** Composition; Essential oil; Growth rate; Microbes

INTRODUCTION

In Ethiopia large numbers of aromatic and medicated plants are available in most of the region. In which Eucalyptus plant is most common among them. The essential oil extract from the Eucalyptus leaves which contain compounds with an extremely broad range of biochemical effects as well as odor, flavor and functional properties. Antimicrobial, analgesic and anti-inflammatory properties of E. citriodora, E. globulus and E. teretcorni have been reported from different parts of the world (Ramezani et al., 2002; Silva et al., 2003). The leaves of E. citriodora contain about 1.36% essential oil that is predominately citronellal (57%) followed by citronellol (15.89%), citronellyl acetate (15.33%) and other compounds (Chalchat et al., 1997; Tian et al., 2005). This essential oil showed a wide spectrum of antimicrobial (Dellacassa et al., 1989; Hmamouchi et al., 1990; Hajji et al., 1993; Changriha et al., 1998), antifungal (Ramsewak et al., 2003; Ramezani et al., 2006), anticandidal (Dutta et al., 2007), antibacterial (Low et al., 1974; Cimanga et al., 2002), expectorant and cough stimulant activity (Oyedeji et al., 1999). Due to its disinfectant action, the essential oil is used externally, applied to cuts and skin infections but it has deleterious effect on the body in high doses (Whitman et al., 1994; Tibballs et al., 1995). Beside antimicrobial activity, the essential oil and its constituents have also been used for their herbicidal (Batish et al., 2006; Setia et al., 2007), insecticidal (Rudin, 2005; Park and Shin 2005), antihelmintic (Benne et al., 1996), anti-tumour (Takasaki et al., 1995) and anti-leech (Kirton, 2005) properties, as well as in integrated disease management against phytopathogenic fungi (Ramezani et al., 2006), nonspecific skin infections (Agarwal, 1997) and mastitis in animals (Pavneesh, 1996; Joshi et al., 1996). The Eucalyptus leaves have available in light green color on tall straight tree the picture is shown in Figure1.

Every year, 1.7 million HAIs cause almost 100,000 deaths in the United States (Pollack, 2010). Those that don't end in death can cause life-threatening illnesses. HAIs are generally caused by transmission of microbes between surfaces, patients and employees. Patients that are immune-suppressed are among the most susceptible to HAIs. In fact, any patient admitted into a healthcare facility for a medical transplant, disease, or critical trauma is at risk (Neely, 2000). One of the most common of these infections is Staphylococcus aureus, also known as "Staph." S. aureus is a facultative anaerobic gram positive coccus (spherical) bacterium, which indicates its spherical shape and lack of an outer membrane. Staph colonizes in both aerobic and anaerobic conditions and is a normal flora in about 30% of industrialized countrie's populations (Page, 2007). Many strains of Staph have the ability to develop a resistance to antibiotics. For example, methicillin resistant or multi-resistant Staphylococcus aureus (MRSA) was found in 50 % of Staph infection cases in

Figure 1. Eucalyptus Leaves (E. citriodora)

United States ICUs in 2007 (Rosenthal, 2009). Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterococcus have also developed antibiotic resistant strains that are difficult to control in clinical environments (Rosenthal, 2009). Clostridium difficle has become one of the leading causes of infection due to its spore forming bacterial cell wall. The dormant endospores of C. diff. allow it to undergo harsh conditions from antibiotics and antimicrobial surfaces (Pant, 2011). One of the reasons that these infections might find their way to immune deficient patients is contamination of the surrounding environment. For example, patients are in constant contact with bed linen during their hospital stay. The transfer of infectious disease through linen, although not highly recognized, is very common in clinical settings. Under certain temperatures and humidity, textiles such as bed linen are a perfect place for microbes to grow. (Gouveia, 2010).

In most healthcare facilities, linens, scrubs and gowns are rented out by an industrial launderer. During the transportation of linen from the industrial laundry plant, it may be handled by up to eight different people before it is placed on the bed of a patient (according to healthcare personnel at Sierra Vista Regional Medical Center in San Luis Obispo, CA). Environmental personnel handling the linens may not be wearing protective gloves, allowing possible transfer of pathogens from handler to patient. In order to strive for more sanitary conditions, antimicrobial coatings and treatments of textiles can potentially be used to actively fight the growth of microorganisms after they've been washed. By eliminating pathogens in a patient's direct environment, antimicrobial linen can potentially reduce the risk of infectious disease transfer in the clinical environment.

Research and development are ongoing process in the functional finishes on the textile substrates**.** The production rate of fabric exceeds, but the market potential has limited scope which can be enhanced by value added finishing to textiles like antimicrobial, medicinal, herbal and fragrance finish, which will increase more value in the current scenario of market. Textile clothing and value added products increases gradually to cater the need of the customer to meet out the world trade market. The number Eucalyptus plant in Ethiopia is high especially in Wollo region due which an attempt has been made to use dry Eucalyptus leaves for antibiotic on fabric. The main aim of work is to extract the oil from leaves and applies on cotton fabrics as antibiotic material. The bacterial growths on fabrics after no of washing are also discussed.

MATERIAL AND METHODS

Material

The Eucalyptus leaves are arranged from university campus. The leaves are dried for 7days or the leaves falls down automatically are collected. It was crush manually with wooden arrangement and make in powder form, which shown in Figure 2.

Method

Extraction of oil was carried out by soxhlet apparatus and it's working on steam distillation process. The powdered leaves of 50 gm were added on 400 ml. of water, the working temperature was maintained at 100ºC

Figure 2. Steps for making the powder from leaves

Figure 3. Experimental setup

and distillated for 1 hour. Once the distillation started the sample start boiling within 5 min and vapour are formed. The vapour is cooled down with the help of condensed. The condensed material was collected on the other side of setup. The collected material is a mixture of oil and water. After the water was separated by the rotary evaporator, the Eucalyptus oil was purified, which used as antibiotic material in cotton fabric. The experimental arrangement is shown in Figure 3.

Analytical

Quality analysis of extracted oil

The major test was to know the percentage of 1, 8-cineole. According to British pharmacopeia standard if the1, 8-cineole content is greater than 70% the oil have not side effect on the skin. The essential oils were analyzed by GC/MS where the composition of the ten commonly found compounds in the oils were as follows by similar study in Tigray region on the same species: 1,8-cineole (66.28 - 75.36%), *cis*-ocimen (15.92 - 21.33%), α-terpineol acetate (2.70 - 3.39%), α-terpineol (1.51 - 2.26%), aromadendrene (0.69 - 2.85%), globulol (0.82 - 1.43%), β-pinen (0.96 - 1.24%), β-myrcene (0.66 - 1.00%), 4-terpineol (0.46 - 0.52%) and camphene (0.16 - 0.27%) as the main leaf oil components. The oils could be used for medicinal purpose if 1,8-cineole content is greater than 70% otherwise the oil needs purification and enrichment so as to make its 1,8-cineole content greater than 70%.

Table 1. Fabric details

S.No	Character	Parameters
1	Fabric	Full bleached fabric (from KTSC)
2	Oil application percentage	5%
3	Oil content	10ml
4	Acetic acid(cross linking/mordant)	100ml
5	Citric acid (cross linking/mordant)	100ml
6	pH	5
7	Liquor ratio	1:20
8	Wet pick up	85%
9	Application method	PAD –BATCH -- DRY – CURE method
10	Drying temperature/time	80^0 C for 5 minutes
11	Curing temperature/time	150^0 C for 3 minutes

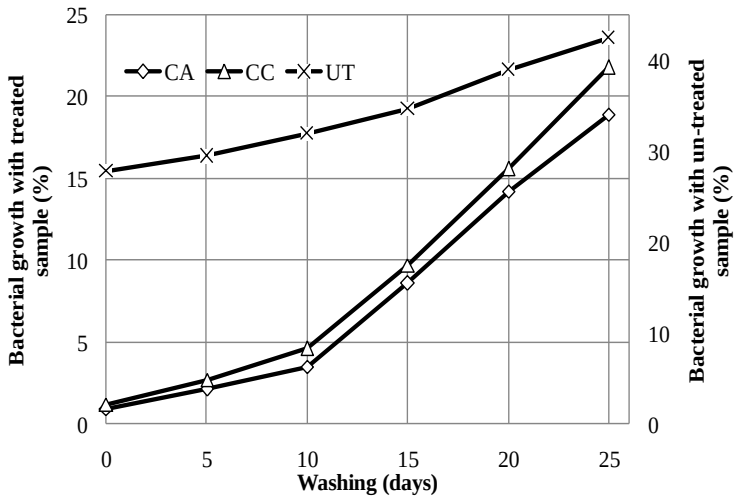

Figure 4. Effect of extracted oil on bacterial growth

Test Procedure

The test procedure was carried out by AATCC-147-1998 (USA) & AATCC-100-1998 (USA). The fabric characterization details are mention on the Table 1.
The procedures are following:

1. Cut 2x2 inches of untreated, treated fabric samples with acetic and citric fabric samples which have before wash and after 5, 10, 15, 20 and 25 washes.
2. Preparation of nutrient broth (water – 100ml, peptone- 0.2 gm, yeast- 0.2 gm, beef extract- 0.3 gm, NaCl- 0.5 gm).
3. The solution is sterilized for 15 minutes at 120 º C and it is allowed to cool. Then the fabric is immersed in broth solution for 24 hours and air dried.
4. Observation under microscope.

Bacterial growth

The bacterial growth are done by colony-forming unit (CFU), it's an estimate of viable bacteria or fungal numbers. They are multiply via binary fission under the controlled conditions and can rise to colony through multiphase. The purpose of plate counting is to estimate the number of cells present based on their ability to give rise to colonies under specific conditions of nutrient medium, temperature and time. The colony forming unit can be calculated by the equation (1)

$$\frac{CFU}{ml} = \frac{number\ of\ colonies * dilution\ factor}{volume\ of\ cutlture\ plate}$$

RESULT AND DISCUSSION

Effect of antibiotic material

The effect of antibiotic material was carried out with three sample i.e, Untreated fabrics (S1), Treated with acetic acid (S2), Treated citric acid (S3) for 25 washes. The bacterial growth was graphically represented in Fig.4. It was found that treated with acetic acid show maximum 81.1% resistance and only 18.9 bacterial growths after 25 washes. Fabrics which are treated with citric acid show 78.2% antibacterial resistance and 21.8% bacterial growth after 25 washes, with respect to untreated sample shows 57.6% antibacterial resistance and 42.5% bacterial growth respectively. The test results shows that the CFU value is less (96.4%) in fabric treated with acetic acid as mordant than treated with citric acid (95.7%). These clearly shows that the growth of bacteria inhibit in case of treated fabrics. Wash durability of treated fabrics are good, up to 20 washes (figure 4).

Table 2. Bacterial growth in laundry soap washing

S.No	Fabrics	After washing	Before washing
1	Average CFU of bra	412	46.67
2	Average CFU of towel	470	133.33
3	Average CFU of sock	545	59.33
4	Average CFU of panties	673.25	42.75

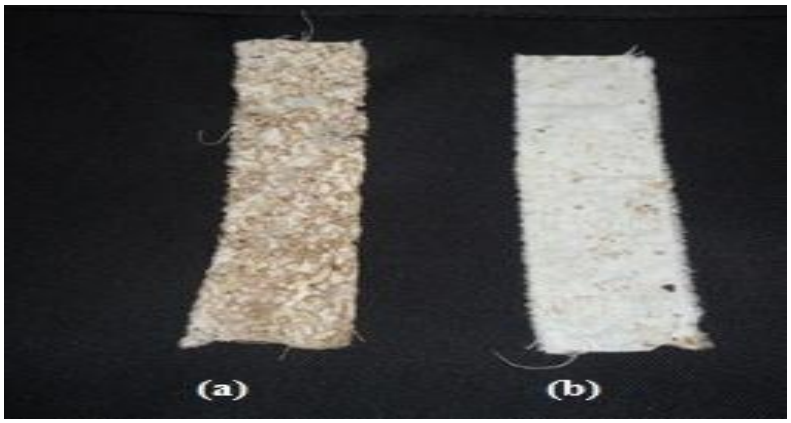

Figure 5. Bacterial growth (a) untreated (b) treated fabrics

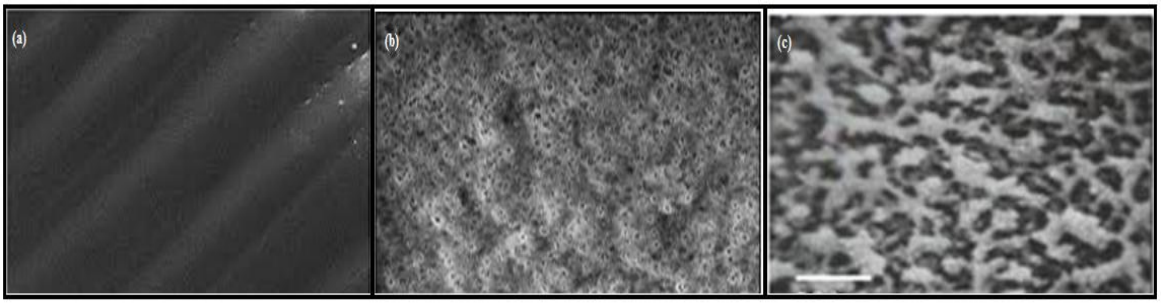

Figure 6. Scanning electron microscopic (a) treated (b) 10 wash (c) 20 wash

For discussion we took the comparison data from second hand clothes CFU study in Nairobi, Kenya before washing and after laundry soap washing, which are mention in Table 2.

Bacterial growth

Test sample size of 1.5" x 6" is prepared and both treated and untreated samples are subjected to soil burial test to analyze the antifungal activity. The test samples are buried in soil as per the procedure for 2 weeks. The temperature maintained is 28º C ± 2º C and moisture content at 25 ± 5 %. The incubation clearly shows that fungal activity is least in treated fabrics than untreated fabric. It was found that untreated sample surface have yellow color layer was occurred that indicated the presence of bacterial. For the second sample which was treated with acetic acid there was no layer was found, this indicated the effect of antibacterial activity on fabric material. The image of experiment is shown in Figure 5.

Scanning Electron micrograph

To study the bacterial growth of the fabric treated with acetic acid before wash, after tenth washes, and twenty-fifth washes was carried out, which has shown Figure 6(a)-(c). It was found that before washing there was no bacterial growth was observed. The fabric seems to very clean there is no dots are found. Fig (b) after tenth washes it was found that little bacterial growth was

occurred (3.5%), which can be seen like dotes foam. In the Figure (c) It was observed that bacterial growth was increase 18.9% after 25th washes. From this study it was proofed that Eucalyptus leaves are very much effective for antibiotic application.

CONCLUSION

This research work has given a new idea in finishing of cotton fabric with essential oil extracted from eucalyptus for antimicrobial activity. The washing fastness tests revealed that the treated fabrics with eucalyptus oil with acetic acid showed only 18.9% bacterial growths and 21.8% bacterial growth with citric acid after 25 washes as well holds good washing fastness more than 25 washes. As a result of this they are found to be very hygienic with less fungi, bacteria and microbes. Moreover, it can be value add as medical fabrics which gains high market potential.

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REFERENCE

Agarwal AK (1997). Therapeutic efficacy of an herbal gel for skin 33. affection in dogs. Indian Veterinary J. 74(5):417-19.

Batish DR, Singh HP, Setia N, Kaur S, Kohli RK (2006). Chemical 27. composition and phytotoxicity of volatile essential oil from intact and fallen leaves of Eucalyptus citriodora. Z Naturforsch. ;61(78):465-71.

Bennet-Jenkins E, Bryant C (1996).. Novel sources of anthelmintics. 30. Int J Parasitol. 26(8/9):937-47.

Chalchat JC, Muhayimana A, Habimana JB, Chabard JL (1997). 13. Aromatic plants of Rawanda II. Chemical composition of essential oils of ten Eucalyptus species growing Ruhande Arboretum, Butare Rwanda. J Essent Oil Res. 9(2):159-65.

Changriha N, Cherif YF, Baailouamer A, Meklati BY (1998). 17. Antimicrobial of Algerian cyprus and eucalyptus essential oils. Rivista Italiana EPPOS. 1998;25:11-16.

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totte J, Pieters L, Vlientinck AJ (2002).. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol. 79(2):213-20.

Dellacassa E, Menendez P, Moyna P (1989). Cerdeiras. Antimicrobial 16. activity of eucalyptus essential oils. Fitoterapia. 60(6):544-46.

Dutta BK, Karmakar S, Naglot A, Aich JC, Begam M (2007). 20. Anticandidial activity of some essential oils of a mega biodiversity hotspot in India. Mycoses. 50(2):121-24.

Gouveia IC (2010).. Nanobiotechnology: A new strategy to develop non-toxic antimicrobial textiles for healthcare applications. J. Biotechnol.. 150-S.

Hajji F, Tetouani SF, Tantaui EA (1993). Antimicrobial activity of twenty-14. one eucalyptus essential oils. Fitoterapia. 64(1):71-77.

Hmamouchi M, Elarakas A, Eantoui A, Sati NE (1990). Agoumi 15. A. Elucidation of antibacterial and antifungal properties of essential oils of Eucalyptus. Plantes Med Phytother. 24(4):278-89.

Joshi HC, Kumar M, Saxena MJ, Chhabra MB (1996). Herbal gel 35. for the control of subclinical mastitis. Indian J Dairy Sci. 49(9):631-34.

Kirton LG (2005). Laboratory and field test of the effectiveness 32. of the lemon-eucalyptus extract, Citriodiol, as a repellent against land leeches of the genus Haemadipsidae. Ann Trop Med Parasitol. 99(7):695-714.

Low D, Rawal BD, Griffin WJ (1974). Antibacterial action of the 22. essential oils of some Australian Myrtaceae with special references to the activity of chromatographic Fractions of oil of Eucalyptus citriodora. Planta Med. 26(2):184–89.

Neely Alice N, Matthey P (2000). Maley. Survival of Enterococci and Staphylococci on Hospital Fabrics and Plastic. J. Clin. Microbiol. 724-726.

Oyedeji AO, Ekundayo O, Olawore ON, Adeniyi BA (1996). 23. Koenig WA. Antimicrobial activity of the essential oils of five Eucalyptus species growing in Nigeria. Fitoterapia. 70(5):526-28.

Page K, Robert GP, Ivan PP, Michael W, Shelley LPS, Alan V (2007). Chadwick. Titania and silver-titania composite films on glass: potent antimicrobial coatings. Journal of Materials Chemistry. 95-104.

Pant C, Thomas JS, Abhishek D, Anil M (2011). Clinical approach to sever Clostridium difficle infection: Update for the hospital practitioner. Eur. J. Internal Med...

Park IK, Shin SC(2005). . Fumigant activity of plant essential oils 29. and components from garlic (Allium sativum) and clove bud (Eugenia caryophyllata) oils against the Japanese termite (Reticulitemes speratus Kolbe). J Agric Food Chem. 153(11):4388-92.

Pavneesh M, Pandey SK, Chhabra MB, Saxena MJ (1996). Efficacy 34. of a tropical herbal gel for mastitis control. Int J Animal Sci. 1996;11(2):289-91.

Pollack A (2010).. Rising Threat fo Infections Unfazed by Antibiotics. *The New York Times*, February 27: B1.

Ramezani H, Singh HP, Batish DRO, Kohli RK (2002). Antifungal 9. activity of volatile oil of Eucalyptus citriodora. Fitoterapia. 2002;73:261-62.

Ramezani H (2006). Fungicidal activity of volatile oil from Eucalyptus 18. citriodora Hook against Alternaria triticana. Common Agric Appl Bio Sci. 71(3B):909-14.

Ramsewak RS, Nair MG, Stommel M, Selanders L (2003). In 19. vitro antagonistic activity of monoterpenes and their mixtures against toe nail fungus pathogens. Phytother Res.;17(4):376-79.

Rosenthal Victor D, Dennis G Maki, Silom J, Eduardo A (2009). Medeiros. International Nosocomial Infection Contro Consortium (INICC) report, data summary for 2003-2008, issued June 2009. Am.J. Infection Contr.. 95-104.

Rudin W (2005). Protection against insects. 28. Ther Umsch. 62(11):713-18.

Setia N, Batish DR, Singh HP, Kohli RK (2007). Phytotoxicity of 26. volatile oil from Eucalyptus citriodora against some weedy species. J Environ Biol. 28(1):63-66.

Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL (2003). Matos 11. FJA. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. J Ethnopharmacol. 89:277-83.

Takasaki M, Konoshima T, Kozuka M, Tokuda H (1995). Anti-tumor-31. promoting activities of euglobals from Eucalyptus plants. Biol Pharm Bull. 18(3):435-38.

Tian Y, Liu X, Zhou Y, Guo Z (2005). Extraction and determination 12. of volatile constituents in leaves of Eucalyptus citriodora. Chinese J Chromatography. 23(6):651-54.

Tibballs J (1995). Clinical effects and management of eucalyptus 24. oil. Ingestion in infants and young children. Med J Aust. 163(4):177-80.

Whitman BW, Ghazizadeh H (1994). Eucalyptus oil (from Eucalyptus 25. spp.including Eucalyptus globulus): Therapeutic and toxic aspects of pharmacology in human and animals. J Paediatr Child Health. 1994;30(2):190-91.