Antimicrobial Activity of Eucalyptus globulus and Prunus dulcis Oils Against Selected Pathogenic Strains

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Abstract: The antibacterial activity of essential oils of Eucalyptus globulus and Prunus dulcis (sweet almond) against quality control strains of Salmonella enterica, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis and Enterococcus faecum were assayed in this study. Paper disc diffusion method was employed in applying the oil on the various cultures. After incubation for 24hrs at 37ºC, the zones of inhibition at 100% concentration of Eucalyptus oil against the quality control strains were (8, 10, 10, 10, 8,10mm), 75% (8,8,8,8,8,mm) 50%(8,8,8,8,8,mm) and 25% (8,8,8,8,8,8mm) respectively. Antibiogram result showed that bacteria isolates were sensitive to CN and resistance to AMP except for E. faecalis which was sensitive to Amp. According to CLSI (2014), the strains were resistant to the brand of Eucalyptus oil used in this study. The quality control strains were also resistant to Sweet almond oil, at 100% concentration, the oil recorded zones of inhibition of (8,8,8,8,8,12, 8,10mm), 75% (8,8,8,8,8,8mm), 50%(8,8,8,8,8,6mm),25%(6,6,6,6,6,6) respectively. The low antibacterial activity of Eucalyptus oil in this study is in variance with numerous reports by researchers that the oil was an active antibacterial agent against broad-spectrum bacteria strains. This may possibly be due to the extraction process of Eucalyptus oil by the manufacturer, the type of strains used in this study, or low concentration of the major bioactive component that confers the oil with its antibacterial activity (i.e. 1, 8-cineole). While sweet almond oil is yet to be reported to have any antibacterial activity, the result of this study is in harmony with numerous reports by researchers. More researches on Eucalyptus globulus should be encouraged and the extraction of its bioactive compounds and its used in controlling pathogenic organisms.

Keyword: Antimicrobial, pathogenic, essential oil, Eucalyptus, Sweet almond, Inhibition Bioactive compounds.
1. INTRODUCTION

Medicinal plants have been used as a source of remedies for various kinds of diseases since ancient times. The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in treatment of various diseases. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including viral infections.

The use of essential oils as functional ingredient in, foods, drinks, cosmetics, and toiletries is gaining momentum, both for the growing consumers’ interest in the ingredients coming from natural sources, and also because of the increasing concern with harmful synthetic additives (Sacchetti et al. 2005). Due to their bioactive components, essential oils are indeed promising in view of their use as effective antibacterial, antifungal, and antioxidant agents. With the growing interest in essential oils in both food and pharmaceutical industries, systematic examination of the plant extracts has become increasingly important (Baratta et al. 1998). Examples of these essential oils include peppermint oil, thyme oil, clove oil, olive oil, almond oil, eucalyptus oil amongst others.

_Eucalyptus_ oil is obtained from different _Eucalyptus_ genus. The genus comprise of over 500 species of aromatic trees and shrubs. Of all eucalyptus, _Eucalyptus globulus_ labill., _myrtacea_ (Tasmanian Blue Gum) is the specie widely introduced and has been established especially throughout the Mediterranean region. Leaves of some eucalyptus species are steam distilled to extract eucalyptus oil to treat influenza, chest problems, cough and skin rashes while their vapor is inhaled to fight inflammation (Musyimi and Ogur, 2008). “Leaf extract of Eucalyptus” has been approved as a natural food additive and it is included among the antioxidants in the “List of Existing Food Additives in Japan” (Tyagi and Malik 2011). The leaf, bark extracts of Eucalyptus have shown various bio potent activity such as antioxidants, antibacterial, antifungal, or anti-hyperglycaemic (Takahashi et al., 2004) Oil of this plant is beneficial for curing lung infections and fungal infections (Adeniyi et al., 2006), large number of antibiotics have been produced, but it has been clinically threatened by exposure and emergence of various multidrug resistant pathogens (Bandow et al., 2003). World Health Organization (WHO) reported that medicinal plants would be the ideal source to obtain diverse drugs (Santos et al., 1995). _Eucalyptus_ oil is also very important in combating some bacteria species.

The almond is the most important nut in the world in terms of commercial production. Taxonomically, the almond tree, _Prunus dulcis_ is where sweet almond oil is extracted. It has been reported that almond oil has the ability to relieve inflammation, heal ulcers, alleviate cramps; it also has anti-viral, anti-bacterial and anti-fungal agents. Sweet almond oil can be applied on the skin and taken as a food supplement.

2. AIM AND OBJECTIVES

This work focuses on the systematic analysis of the antibacterial properties of eucalyptus and sweet almond oils on six (6) different bacteria strains.

The specific objectives includes;

- To test for the purity of the various essential oil used in this work.
- To test for the susceptibility of the pathogenic strains against various antibiotics.

3. PHARMACOLOGICAL ACTIVITY

_Eucalyptus_ (Myrtaceae) is one of the world’s most important and most widely planted genera. In Australia, this genus is the second largest genus, after Acacia, and contains about 750 species. UAs an expectorant for symptomatic treatment of mild inflammation of the respiratory tract and bronchitis. Also for symptomatic treatment of asthma, fever and inflammation of the throat described in pharmacopoeias and in traditional systems of medicine. Treatment of cystitis, diabetes, gastritis, kidney disease (unspecified), laryngitis, leukorrhoea, malaria, pimples, ringworm, wounds, ulcers of the skin, urethritis and vaginitis uses described in folk medicine, but not supported by experimental or clinical data. Myrtaceous plants are known to be rich source of biologically active terpenoids and polyphenols, including flavonoids, phloroglucinol derivatives, and tannins. Previous phytochemical studies on the _Eucalyptus globulus Labill._ one new phloroglucinol derivative named eucalyptone G, together with nine known compounds. The antibacterial activity of the new compound has been studied. Eucalyptone G was found to be active against the Gram-positive _Bacillus subtilis_ and _Staphylococcus aureus_ and
caused an inhibition zone of 16 mm diameter after 24h of incubation at 37°C. Also, highly active against Gram-negative E. coli with an inhibition zone of 19 mm diameter (Gamal and Mohamed, 2007).

**Antihelmintic activity:** The present investigation concludes that E. globulus oil has an anthelmintic potential due to the presence of borneol, linalool, cineol, geranyl acetate, anethol, saffrol as phytocconstituents. Essential oil from E. globulus contains 1,8-cineole as the major component and is used in the treatment of pulmonary infections and also exhibits antibacterial activity (Taur et al., 2010).

**Wound healing activity:** Intra-dermal administration of the essential oils from the leaves of Eucalyptus hybrid and seeds of Seseli indicum increased cutaneous capillary permeability when tested in Evan’s blue treated rabbits. This effect may be beneficial in their probable wound healing activity (Sakar, 1994).

**Antibacterial and antifungal activity:** A 50% Ethanol extract of Eucalyptus globulus leaves have antibacterial activity against oral pathogenic microorganisms with MIC values ranging from 0.20 micrograms/mL to 6.25 micrograms/mL (Nagpal et al., 2010). A 50% EtOH-soluble material was extracted from the dried leaves of E. globules shows appreciable antibacterial activity against S. mutans Ingbrit and P. gingivalis ATCC 33277 (causes dental caries and periodontal disorders) with MICs values 12.5 and 6.25 μg/mL. Dried residue of methanolic extract of Eucalyptus globulus leaves showed antimicrobial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans with minimum inhibitory concentration of 5.0, 10.0, 10.0, 1.25 mg/ml respectively. Phloroglucinol- sesquiterpene coupled compounds, macrocarpals H, I, and J showed potent antibacterial activity and inhibitory effect of glucosyltransferase (Osawa et al., 1997). Freshly prepared camphor oil from Eucalyptus globulus with or without glycerol dilutions gave complete cure of human facial demodicidosis with concentrations of 100%, 75% and 50% 40. Eucalyptus globulus leaf extracts and oil showed antifungal property as they progressively inhibited the growth of Malassezia furfur on Sabouraud’s destrose agar medium (Vijayakumar et al., 2006).

**Antidiabetic (antihyperglycemic):** Eucalyptus globulus is used as a traditional treatment for diabetes. Incorporation of Eucalyptus globulus in the diet (62.5 g/kg) and drinking water (2.5 g/L) reduced the hyperglycemia and associated weight loss of streptozotocintreated mice. An aqueous extract of Eucalyptus globulus (AEE) (0.5 g/L) enhanced 2-deoxy-glucose transport by 50%, glucose oxidation by 60% and incorporation of glucose into glycogen by 90% in abdominal muscle of mice. In acute, 20 min incubation, 0.25-0.5 g AEE/L evoked a stepwise 70-160% enhancement of insulin secretion from the clonal pancreatic beta-cell line (BRIN-BD11). These data indicate that Eucalyptus globulus represents an effective antihyperglycemic dietary adjunct for the treatment of diabetes and a potential source for discovery of new orally active agent(s) for future therapy (Gray and Flatt, 1998).

**Antiplaque:** Eucalyptus globulus may be useful in inhibiting dental plaque formation (Sato et al., 1998).

**Respiratory Diseases:** Eucalyptus globulus have been used in traditional medicine in the treatment of bronchitis, asthma and other respiratory diseases (Vigo et al., 2004). Cutaneous application of essential oils of Eucalyptus globulus to mice suppressed the cellular inflammation of skin. This suggests that essential oils using in aromatherapy massage may suppress the inflammatory symptoms related with neutrophil accumulation and edema (Maruyama et al., 2004).

**Antimalarial:** Intragastric administration of a hexane leaf extract to mice (100mg/kg body weight) did not inhibit the growth of Plasmodium berghei . Furthermore, administration of an aqueous (3.48 g/kg body weight) or chloroform (264mg/kg body weight) leaf extract to chickens by gastric lavage did not inhibit the growth of P. gallinaceum . An ethanol–water extract27of the leaves inhibited the growth in vitro of P. falciparum at a concentration of 75mg/ml (Njoroge and Bussman, 2006).

**Antioxidant:** The methanol extracts of Eucalyptus globulus showed efficiency in preventing the oxidation process. Hyperglycemia in diabetes has been associated with increased formation of reactive oxygen species (ROS) and oxidative damage to tissue compounds. The aim of this study was to evaluate the effects of eucalyptus in the diet (20 g/Kg) and drinking water (2.5 g/L) on lipid peroxidation, protein oxidation and antioxidant power in plasma and liver homogenate, as well as glycated-Hb (HbA1C) of blood in streptozotocin-induced diabetic rats for a period of 4 weeks. Diabetes induced in rats by a single intraperitoneal injection of streptozotocin (STZ, 65 mg/Kg). At the
end of the treatment period, the level of plasma glucose, plasma and liver malondialdehyde (MDA, the main product of lipid peroxidation), protein carbonyl (PC, one of the protein oxidation products) and HbA1C increased and ferric reducing antioxidant power (FRAP) decreased in diabetic rats compared to normal rats. Eucalyptus administration for 4 weeks caused a significant decrease in the plasma glucose levels, plasma and liver MDA, PC and HbA1C, also a concomitant increase in the levels of FRAP in diabetic treated rats (Sugimoto et al., 2006).

4. MATERIALS AND METHOD

MATERIALS: Materials and equipment used in this study were Bunsen burner, autoclave, antibiotics, susceptibility discs, pipette, test tubes, incubator universal container, sterile distilled water, test samples (almond and eucalyptus oil), cork borer, weighing balance, conical flask.

OIL SAMPLES: The essential oils used in this study are sweet almond oil (EL HAWAG product) and eucalyptus oil (Hemani product) used in this study were obtained from Jumia Nigeria.

MICROBIAL STRAINS AND CULTURE: The microbical strains used in this study were Salmonella enterica (ATCC 14028), Enterococcus faecalis (ATCC 19433), Enterococcus faecum (ATCC 19434), Eschericia coli (ATCC 29214), Staphylococcus aureus (ATCC 12598), and Pseudomonas aeruginosa (ATCC 29853). These strains were obtained from South Africa.

PREPARATION OF SAMPLES AND CULTURING: The media used for analysis were Tryptone soya broth and Mueller-Hinton agar (both obtained from Oxoid, United kingdom). These media were prepared according to manufacturer instruction.

TRYPTONE SOYA BROTH (TSB): Tryptone soya broth is a highly nutritious multipurpose medium that supports the growth of many fastidious organisms as a result of the inclusion of broth tryptone and soya peptone. It was prepared by adding 30g of commercially produced agar in 1 liter of distilled water and thus was properly dissolved to obtain a homogenous mixture. It was then sterilized by autoclaving at a temperature 121°C at 15psi for 15 mins and was then cooled at 45°C and aseptically dispensed into sterile petri dishes.

MUELLER HINTON AGAR: Mueller Hinton agar is a non-selective, non-differential medium that is used majorly for antimicrobial susceptibility testing. It is the standard medium for the Bauer Kirby method. It was prepared by suspending 38g of the medium in 1 liter of distilled water and was dissolved properly to get a homogenous mixture. It was then autoclaved at a temperature of 121°C at 15psi for 15 minutes and was cooled at 45°C. The molten agar was aseptically dispensed into sterile petri dishes.

PREPARATION OF OIL CONCENTRATION: The oils used in this study were dissolved in tween 80 to obtain varying concentrations (100%, 75%, 50% and 25%) of the oils were prepared in stoichiometric quantity using 10ml as the standard volume. A 100% was achieved by aseptically pipetting 10ml of each oil into a sterile universal bottle. 75% concentration was achieved by measuring 7.5ml of oil and dissolving with 2.5ml of Tween 80 (as solvent). 50% concentration of each oil was attained by dissolving 5ml of oil into 5ml of Tween 80. 25% was achieved by dissolving 2.5ml of oil into 7.5ml of Tween 80.

The equation below was used to determine the different concentrations of the oil:

\[ \frac{x}{2} \times 100 = z \times x \]

\[ \text{X}= \text{volume of oil} \]
\[ \text{Y}= \text{volume of solvent (Tween 80)} \]

10ml was in this case regarded as the assumed final volume which is equal to x+y.

The required concentration which could be 100%, 75%, 50%, 25% is taken as A

\[ \frac{x}{2} \times 100 = A \]

ACTIVATION OF MICROBIAL STRAINS: Bacterial strains used in this experiment were obtained from micro biolgies. The lyophilized organisms were activated according to manufacturer’s specification. The unopened KWIK-STIK™ pouch was allowed to equilibrate at room temperature before the open at the notch to remove KWIK-STIK™ the pull-tab portion on the label was turned off and attached to the primary culture plate (care was taken not to disassemble the device during hydration). The ampoule at the top the KWIK-STIK™ was pinched once just below the fluid meniscus of the ampoule found in the cap to release the hydrating fluid. KWIK-STIK™ was held vertically and tapped on a hard surface to facilitate the flow of fluid through the shaft into the bottom unit containing the pellet. The pellet in the fluid was crushed during the pinching action at the bottom of KWIK-
STIK™ unit until the pellet suspension became homogenous swab was transferred to agar medium. The primary culture plate(s) inoculated by gently rolling of the swab over one-third of the plate, a sterile loop was used to streak to facilitate colony isolation after which it was incubated for 24hours at 37°C.

**STANDARDIZATION OF INNOCULUM:** The test organisms were sub cultured into fresh plates of Mueller Hinton medium and incubated for 24hours at 37°C. the agar plates were stored at 4ºC until required. Over night cultures from these plates were suspended in Mueller Hinton broth for antimicrobial assay. They were adjusted to a turbidity matching the 0.5 Mcfarland standards.

4. RESULTS

Antimicrobial activities of *Prunus dulcis* and *Eucalyptus globulus* essential oils was analyzed using disc diffusion method against pathogenic bacterial strains such as *Salmonella enterica* ATCC 14028, *Pseudomonas aeruginosa* ATCC 29853, *Escherichia coli* ATCC 29214, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 19433, *Enterococcus faecum* ATCC 19434. The essential oil of *Eucalyptus globulus* had the highest antimicrobial activity against all pathogenic strains as compared to that of *Prunus dulcis*.

4.1 Antibiotic sensitivity test on bacterial strains

The antibiotic sensitivity test using antibiotics viz: Ciprofloxacin, Ampicillin, Amoxicillin, Gentamicin, Erythromycin, Vancomycin, Ceftriazon, Cephalozin, Cefuroxine And Cefixine was tested against the pathogenic bacterial strains. The results of the antibiotics sensitivity test are presented in table (3) below.

**Data Analysis on APPENDIX (8)**

5. DISCUSSION

The antibacterial activity of Eucalyptus oil which has been reported by numerous researchers to have a broad-spectrum antimicrobial action, making it an attractive alternative to pharmaceuticals was evaluated also with sweet almond oil in this study. According to Betts (2000), the major bioactive components of Eucalyptus oil includes 1, 8-cineole, α-pirene, myrcene, α-Terpineol. Eucalyptus was not only reported by Betts (2000) to have an antibacterial activity, but also immuno-stimulatory, anti-inflammatory, anti-oxidant, analgesic, spasmylic effect. The major component of Eucalyptus oil that is responsible for its antibacterial action was reported by Damjanovic-Vrtnica, et al. (2011) to be 1, 8-cineole (85.8%).

The antibacterial activity of Eucalyptus oil which was reported by Damjanovic-Vrtnica et al. (2011) to be very high against bacteria strains like *Staphylococcus aureus* was evaluated in this study. However, the result of this study (figure 2.) was low compared to the report of Damjanovic-Vrtnica et al. (2011). Sadlon and Lamson (2010) also reported the high antibacterial activity of Eucalyptus oil. Nagpal et al. (2010) also reported that Eucalyptus oil extracted from dried leaves had high antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The low antibacterial activity of Eucalyptus oil in this study may possibly be due to the extraction method used by the manufacturer of the brand of Eucalyptus oil used in this study. It may also be due to the strain of bacteria used in this study. It may also be due to low concentration of the bioactive component of Eucalyptus oil (1, 8-cineole) that gives it its antibacterial property.

Sweet almond oil, the other essential oil used in this study has been reported by Ahmad (2010) to contain high percentage of monosaturated oleic aid (omega-9) and linoleic acid (PUFA omega-6). Josse et al. (2007) also reported that sweet almond oil contains vitamin E, vitamin A, Zinc, and other minerals. Ahmad (2010) further reported that sweet almond oil helps against constipation, decreases irritable bowel syndrome symptoms and has anti-inflammatory properties.

Gupta et al. (2008) in their study on some herbal oils including sweet almond oil reported no antibacterial activity of the oil. This is in consonance with the result of this study on the antibacterial activity of sweet almond oil (figure 1.), as no antibacterial activity against the quality control strains
were recorded. This may be due to the absence of bioactive components that confer antibacterial property in other herbal oils in sweet almond oil.

6. CONCLUSION

Eucalyptus oil has antibacterial activity due to the presence of some bioactive components in the oil, although the susceptibility of the quality control strains (zones of inhibition) was low compared to previous studies done on this oil. Sweet almond oil does may not possess any antibacterial activity.

7. REFERENCES


8. APPENDIX

![Figure 1: Antibacterial activities of Prunus dulcis essential oil at different concentrations on various pathogenic bacteria.](image)

According to CLSI, (2014) ≥20= SENSITIVE, 15-19= INTERMEDIATE, ≤14= RESISTANCEs

Table 1: Resistance of bacterial pathogens to different concentration of Prunus dulcis (sweet almond) essential oil.

<table>
<thead>
<tr>
<th>Antimicrobial susceptibility Concentration (%)</th>
<th>100</th>
<th>75</th>
<th>50</th>
<th>25</th>
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<tbody>
<tr>
<td>Isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. enterica</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>P. aeruginosa</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>E. coli</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>S. aureus</td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>E. faecalis</td>
<td>R</td>
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<tr>
<td>E. faecium</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tbody>
</table>
**Figure 2:** Antibacterial activities of *Eucalyptus globules* essential oil at various concentrations.

**Legend:** According to CLSI, (2014) ≥20 = SENSITIVE, 15-19 = INTERMEDIATE, ≤14 = RESISTANCE

**Table 2:** shows the resistance of bacteria pathogens to different concentrations of Eucalyptus globules essential oil.

<table>
<thead>
<tr>
<th>Zones of inhibition (mm)</th>
<th>Concentration (%)</th>
<th>100</th>
<th>75</th>
<th>50</th>
<th>25</th>
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<tbody>
<tr>
<td><strong>Isolates</strong></td>
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<tr>
<td><em>S. enterica</em></td>
<td></td>
<td>R</td>
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<td>R</td>
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<tr>
<td><em>P. eruginosa</em></td>
<td></td>
<td>R</td>
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<tr>
<td><em>E. coli</em></td>
<td></td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td><em>E. faecalis</em></td>
<td></td>
<td>R</td>
<td>R</td>
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<tr>
<td><em>E. faecium</em></td>
<td></td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tbody>
</table>

Table 3: Antibiotic sensitivity test in bacterial strains.

<table>
<thead>
<tr>
<th>Zones of inhibition (mm)</th>
<th>Isolates</th>
<th>CIP</th>
<th>AMP</th>
<th>AML</th>
<th>CN</th>
<th>E</th>
<th>VA</th>
<th>CRO</th>
<th>KZ</th>
<th>CAM</th>
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<tbody>
<tr>
<td>S. enterica</td>
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<tr>
<td>P. aeruginosa</td>
<td>S R R S R R I R R R R</td>
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<tr>
<td>E. coli</td>
<td>S R R S R R I R S S R</td>
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<tr>
<td>S. aureuas</td>
<td>S S S S I S R S S R</td>
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<tr>
<td>E. faecalis</td>
<td>S R S S R R I R I S</td>
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<tr>
<td>E. faecum</td>
<td>R R R R R I R I I</td>
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</table>

KEY: R= RESISTANCE, I= INTERMEDIATE, S=SENSITIVE

Figure 3: Multiple Antibiotic Resistance index (MAR) of isolated pathogenic strains.

Table 4: Microbial purity of Eucalyptus and almond oils

<table>
<thead>
<tr>
<th>Samples</th>
<th>incubation time (days)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (%)</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Almond oil</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>100</td>
<td></td>
<td>+</td>
<td>+</td>
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<td>75</td>
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<td>25</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Eucalyptus oil</td>
<td>100</td>
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<td>-</td>
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<tr>
<td>75</td>
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<td>50</td>
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<td>25</td>
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</tbody>
</table>

KEY: += PRESENCE OF CONTAMINANTS
- = ABSENCE OF CONTAMINANTS

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