Comparative Study of Synergistic Antibacterial Potential of Clove and Eucalyptus Essential Oils

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Abstract

Wound infections are a significant public health concern, and the emergence of antibiotic-resistant bacteria has necessitated the search for alternative antimicrobial agents. This study evaluated the antimicrobial activity of cloves and *Eucalyptus*essential oil against the gram positive and gram-negative bacteria,including *Bacillus subtilis (ATCC-25920), Bacillus pumilus(MTC-160), Klebsiella pneumonia(NCTC-418) and Escherichia coli (ATCC25923).*The results showed that cloves and eucaltptus essential oil exhibited significant antimicrobial activity against all the tested bacteria along with antibiotics, with minimum inhibitory concentrations (nhibit B. pumilis growth and Amoxiciline +clove oils 50%) showed the highest inhibition against E. Coli. The study suggests that cloves and eucalyptus essential oil may be a useful natural remedy for the prevention and treatment of wound infections. Further studies are needed to evaluate the safety and efficacy of cloves essential oil for wound care

Key words: cloves essential oil, Eucalyptus oil, antimicrobial activity, antibiotics,

I. INTRODUCTION

Cloves (Syzygium aromaticum), the dried flower buds of the clove tree, have been usedfor centuries in traditional medicine for their antimicrobial, anti-inflammatory, andanalgesic properties [1].The increasing resistance of microorganisms to standard chemicals and drugs is a critical and obvious global trouble that has caused studies into the identification of recent biocides with wide activity. Plants and their derivatives, such as essential oils, are often used in folk medicine.Natural antibacterial derived from plant and bacterial metabolites as food additives are currently being promoted to replace synthetic antibacterialagents [2,3,4]The largest antibacterial components contained in *Syzygium aromaticum* (clove) and Eucalyptus essential oils are eugenol in clove and Cineole in eucalyptus, which have had antibacterial activity against foodborne pathogens [5,6,7]. In nature, essential oils play a vital role in the safety of plants. Essential oils contain an extensive variety of secondary metabolites that are able to inhibit or slow the growth of bacteria, yeasts and molds. Essential oils and their components have activity against a variety of targets, particularly the membrane and cytoplasm, and in some cases, they absolutely alternate the morphology of the cells [1]

Essential oils are also known as volatile oils, ethereal oils, or simply as the oil of the plant from which they were extracted, such as the oil of plant from which they were extracted, such as oil of clove. Essential oils are complex combinations of plant components that show higher activities than their isolated components; their final activities are due to the combine effects of several minor components. Thus, Essential oils contain multifunctional components that exert their activities through different mechanisms. Essential oils and their components may have new applications against diverse diseases of various origins (cancer, fungal, bacterial or viruses), because some of these complex diseases require more than one components and multifunctional therapies. The combination of essential oils with antibiotics therapeutic approach may lead to new ways to treat infectious diseases.

All parts of aromatic plants may contain essential oils as follows:

1. Flowers, including clove flower bud,
2. Leaves, including eucalyptus, mint, thyme, bay leaf, Savory, sage, pine needles, and roots.

The synergistic effect of components of essential oils is a promising field that could result in the optimisation of a given bioactivity. This phenomenon has been observed in activities like antimicrobials, antioxidants, analgesics and semi chemicals [8]The composition of every essential oil depends not only on the family but also on the part of the plants from which it is extracted, [9] Terpenoid and phenylpropanoid derivatives are the main components found in essential oils. Essential oil components belong mainly to the vast majority of the terpene family. Many thousands of compounds belonging to the family of terpenes have so far been identified in essential oils such as functionalized derivatives of alcohols (geraniol, α-bisabolol), ketones (menthone, p-vetivone) of aldehydes (citronellal, sinensal), esters (γ-tepinyl acetate, cedryl acetate), and phenols [10]. Essential oils have an excessive variability in their composition, each in qualitative and quantitative terms. This variability depends upon various factors which are grouped into two categories:

1. Intrinsic factors including plant and interaction with the environment (soil type, climate, etc) and the maturity of the plant at its harvest time,
2. Extrinsic factors including the extraction method and the environment.

The factors that determine essential oil yield and composition are numerous These parameters include the seasonal variations, plant organ and degree of maturity of the plant, geographic origin and genetics shown in table 1.

Table 1

Essential oils	Major component	Standardpercentage of component
Eucalyptus oil	1,8-cineole (Eucalyptol)	78%
	Linalyl acetate	4.6% - 47%
	Linalool	28% - 37%
Clove oil	Beta – caryophyllene	9.77%
	Acetate eugenyle	80.26%
Cinnamon oil	E – cinnamaldehyde	0.33%
	Z – cinnamaldehyde	0.43%
	Trans-2-methoxycinnamaldehyde	87.32%
	Acetate cinnamyle	2.34%

The knowledge of composition of essential oils and their therapeutic properties have contributed to the improvement of their cultivation and markets. They are about 3,000 essential oils, out of which about 300 are commercially important and are traded in the world market [11]Essential oils are common natural products that can be used for various medical applications shown in figure 1,and in combination with the emergence of antimicrobial resistance, essential oils have been studied as potential antimicrobial agents.

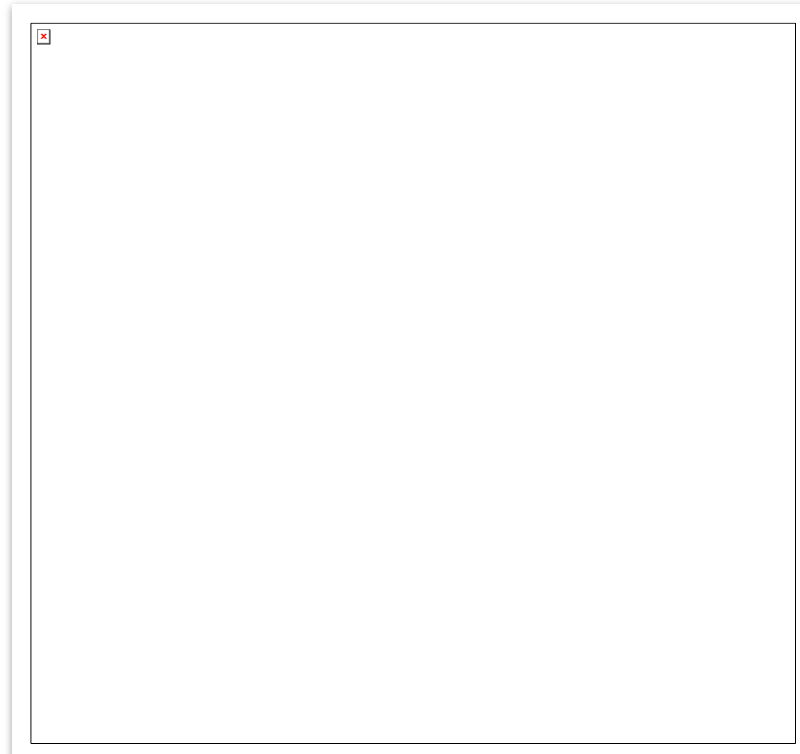

Figure1. Properties of Essential oils and their uses.

A synergistic effect is observed when the combination of substances is greater than the sum of the individual effects. Some studies have shown that the use of the whole essential oil provides an effect which is greater than that of the major components used together. This suggests that minor components are essential for activity and may have a synergistic effect.

Eucalyptus oil

Also known as *Eucalyptus tereticornis*, a genus of the plant family Myrtaceae native to Australia and cultivated worldwide. The leaves o selected Eucalyptus species are steam distilled to extract the eucalyptus oil. Eucalyptus oil has a history wide application, as a pharmaceutical, antiseptic, repellent, flavouring fragrance and industrial uses.

Composition – The main component is 1,8-cineole followed by cryptone, alpha-pinene, p-cymene, alpha-terpineol, trans-pinocarveol, cuminal, globulol, limonene, aromadendrene, spathulenol and terpinene-4-ol.

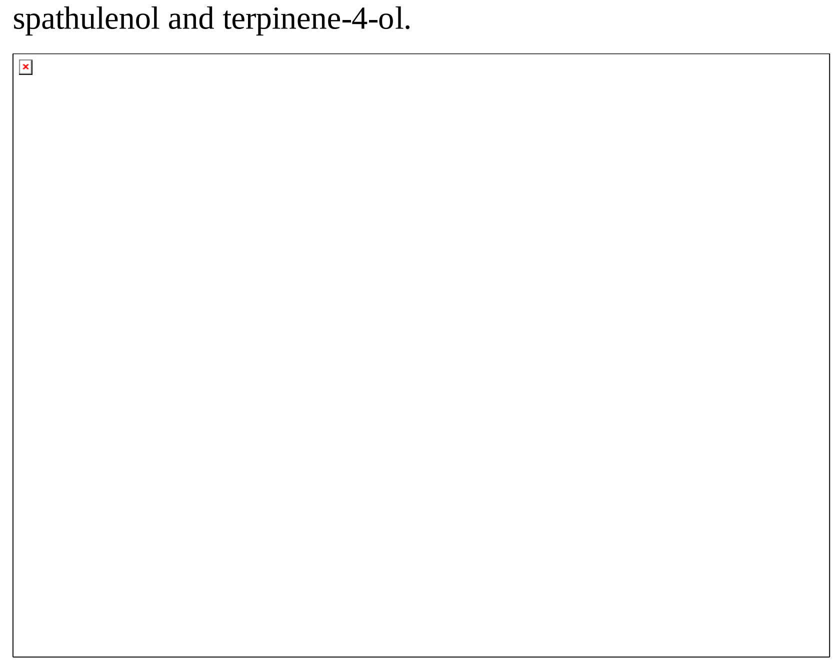

Figure 2. Eucalyptus oil and its main components

Clove oil

Syzygium aromaticum L. belong to the Myrtaceae family, Clove is an aromatic flower cultivated in Madagascar, Sri Lanka, Indonesia, and China. Clove essential oil contains a high amount of phenolic compounds with several biological activities, including antibacterial, antifungal, insecticidal, and antioxidant activities.

The FDA classifies clove essential oil as generally recognized as safe (GRAS); for this reason, it is used in perfumes, cosmetics, sanitary products, medicines, and foods [5].

Composition - Main constituents found in the clove bud oil are the phenypropanoids, eugenol, eugenyl acetate, carvacrol, thymol, beta-caryophyllene and 2-heptanone, when analyzed by gas chromatography. Less than 10% correspond to minor or trace components such as diethyl phthalate, caryophyllene oxide, cadinene, α-copaene, 4-(2-propenyl)-phenol, chavicol, and α-cubebene, among others [6].

Figure 3. Clove oil and its main components

II. MATERIAL AND METHODS

Essential Oils

1. Clove oil *(Syzygium aromaticum)*
2. Eucalyptus oil *(Eucalyptus tetricornis)*

Drugs

1. Amoxicillin (Himedia,India)
2. Nalidixic (Himedia,India)

DMSO (dimethyl sulfoxide), is used for negative control set-up.(From Merck,)

Bacterial strains

The bacterial strains were used were: *Bacillus subtilis (ATCC-25920), Bacillus pumilus(MTC-160), Klebsiella pneumonia(NCTC-418) and Escherichia coli (ATCC25923).*

The first two are gram-positive bacteria whilethe last two are gram- negative bacteria. These bacteria were revived in the Luria Bertini broth medium at 37°C for 16-24 hours.

Media

For preparation of Nutrient Media, Nutrient Broth and Agar, Type I were used. For preparation of nutrient broth media, 3.25 gm of broth was measured and added in 250 ml of distilled water. (pH – 7.5) and for Nutrient agar media, 7.5 gm of Agar and 6,5 gm of Nutrient broth was added in 500 ml of distilled water.

Methods

The method used in this study is **Agar Diffusion Method (ADM) and Minimum Inhibitory Concentration (MIC).** The agar diffusion method is the most widespread technique of antimicrobial activity assessment. The method is recognised as precise and reliable, even though it produces semi- quantitative results, and according to some authors, only qualitative and not always repeatable. However, it makes possible to estimate the degree of microorganism growth inhibition and their morphological changes in a simple way. According to this method, petri dishes of 5-12 cm diameter are filled with 10-20 ml of agar media and inoculated with microorganisms. There are two ways of essential oil incorporation, which are, on a paper disc or into the well (hole) made in the agar medium. In this study, essential oil incorporation is done through Wells. The most important parameters of this method are the diameter of Whattman's paper disc or the well, the amount of essential oil, as well the sort of dispersing solvent. Essential oil is rarely taken as pure liquid, usually its solutions are used. Series of petri plates with the same amount of essential oil solution at different concentrations are prepared. The plates are stored for some time to allow all the essential oil components to diffuse in the agar medium, then they are incubated.

The effectiveness of essential oil is demonstrated by the size of the zone of microorganism's growth inhibition around the disc or well, and it is usually expressed as the diameter (rarely as radius) of this zone (in mm), including or not the disc or the well diameter.

The agar diffusion method is considering as inappropriate for essential oils as there volatile components are likely to evaporate with the dispersion solvent during the incubation time while their poorly soluble components do not diffuse well in the agar broth. Still, it is the most common technique of the antibacterial and antifungal essential oil activity assessment because it is easy to perform and requires only small amounts of essential oils. It can be recommended as a pre- screening method for a large number of essential oils, so as the most active ones may be selected for further analysis with the help of more sophisticated methods

Theminimum inhibitory concentration is the lowest concentration of chemical usually a drug, which prevents visible in vitro growth of bacteria or fungi [7]. The MIC is determined by preparing a dilution series of the chemical, adding agar or broth, then inoculating with bacteria and incubating at a suitable temperature. MICs can be determined on plates of solid growth medium called agar or broth dilution methods after a pure culture is isolated.

The minimum inhibitory concentration of the antibiotic is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth.

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The value obtained is largely dependent on the susceptibility of the microorganism and the antimicrobial potency of the chemical, but other variables can affect results too. The MIC is often expressed in micrograms per millilitre or milligrams per litre (mg/L).

An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. The first step in drug discovery is often measurement of the MICs of biological extracts, isolated compounds etc against bacteria and fungi of interest. MIC values provide a quantitative measure of an extract or compounds antimicrobial potency. The lower the MIC, the more potent the antimicrobial. When *in vitro* toxicity data is available, MICs can also be used to calculate selectivity index values, a measure of off-target to target toxicity.

MIC scores are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Clinicians use MIC scores to choose which antibiotics to administer to patients with specific infections and to identify an effective dose of antibiotic. This is important because populations of bacteria exposed to an insufficient concentration of a particular drug or to a broad spectrim antibiotic can evolve resistance to these drugs. Therefore, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains.

III. RESULTS & DISCUSSION

In the present study antibacterial property of various oils and drugs administered alone and in combination with various drugs was confirmed by Agar Diffusion Method (ADM) and Minimal Inhibitory Concentration (MIC) and growth inhibition zones were measured. Results are presented in triplicate recordings in Tables below.

The clove essential oil showed better antibacterial activity compared to the Eucalyptus essential oil with MIC values ranging from 0.158 mg/ml to 0.640 mg/ml and 0.130 mg/ml to 0.656 mg/ml respectively.

With regard to the effectiveness of the natural compounds used in association with (EO-EO) or in combination (EO - Antibiotic), the most active synergies toward four bacterial strains were observed for Amoxicillin + Eucalyptus oil.

BacterialStrains& E.O	**Different concentrations of Antibiotics and their inhibitory activity (in mm)**			
Escherichiacoli	**1 mg**	**2 mg**	**3 mg**	**DMSO**
Nalidixic	-	-	15	-
Amoxicillin	18	18.5	19	-
Klebsiellapneumonia				
Nalidixic	-	17	18	-
Amoxicillin	16	16.5	24	-
Bacillus pumilus				
Nalidixic	27	29	29	-
Amoxicillin	20	20	21	-
Bacillus subtilis				
Nalidixic	34	36	35	-
Amoxicillin	-	-	-	-

Table2. Zone of inhibition in effect with antibiotics

Figure. Zone of inhibition in Effect with Antibiotics.

Bacterial Strains& Essential oils	**Zone of inhibition indifferent concentrationsof essential oils (in mm)**				
	25 %	**50 %**	**75 %**	**DMSO**	**Drug**
Escherichia coli					
Clove	22	19	18	-	14
Eucalyptus	16	-	-	-	-
Klebsilla pnuemoniae					
Clove	20	18	23	-	20
Eucalyptus	18	-	-	-	-
Bacilluspumilus					
Clove	27	24	20	-	17
Eucalyptus	15	15	-	17	20
Bacillus subtilis					
Clove	20	20	19	13	12
Eucalyptus	12	-	-	12	14

Table 3. Zone of inhibition in effect with essential oils

Figure 4. Zone of inhibition in effect with different Essential oils**.**

Bacterial Strains, E.O& Antibiotic	**Zone of inhibition by different concentrationsOf EssentialOils & Antibiotics (in mm)**

	75%	50%	25%	DMSO
Escherichiacoli				
Amoxicillin + Clove	22	25	23	-
Amoxicillin + Eucalyptus	26.5	26	25	-
Nalidixic + Clove	26	25	31	-
Nalidixic + Eucalyptus	28	30	30	-
Klebsillapneumonia				
Amoxicillin + Clove	33	24	22	-
Amoxicillin + Eucalyptus	28.5	23	22	-
Nalidixic + Clove	37	25	28	-
Nalidixic + Eucalyptus	27	31	29	-
Bacilluspumilus				
Amoxicillin + Clove	31	24	19	-
Amoxicillin + Eucalyptus	23	23	22	-
Nalidixic + Clove	30	24	19	-
Nalidixic + Eucalyptus	22	26	26	-
Bacillussubtilis				
Amoxicillin + Clove	27	27	26	-
Amoxicillin + Eucalyptus	19	18	17	-
Nalidixic + Clove	30	30	17	-
Nalidixic + Eucalyptus	17	25	19	-

Table 4. Zone of inhibition in effect with combination of essential oils with antibiotics.

Figure5. Zone of inhibition in effect with combination of essential oil and antibiotics.

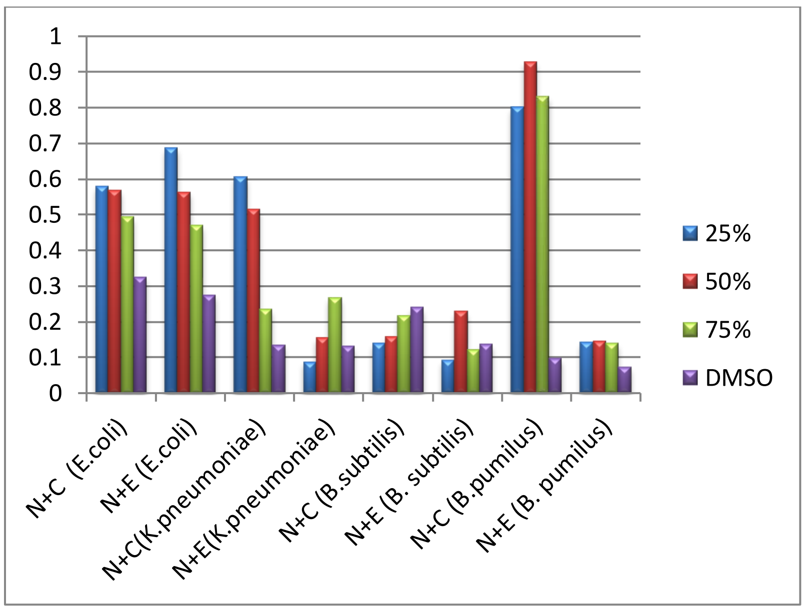

Figure6. Synergistic effect of combination of antibiotic and oil with different bacteria.

The results show that the antibacterial activity of Clove and Eucalyptus essential oils with Amoxicillin and Nalidixic antibiotic administered alone and in combination in different concentration resulted in different zone of inhibitions (ZOI).

The antibacterial activity when administered with different essential oil using Agar Diffusion Method shows that the maximum zone of inhibition was obtained in *Bacillus pumilus* (2.7 cm at 25%) with Clove essential oil.

When administered with essential oil then maximum zone of inhibition was obtained in *Bacillus subtilis* with eucalyptus oil (0.983 mg/L at 75%).

When administered with combination of essential oil and antibiotic then maximum zone of inhibition was obtained in Amoxicillin + Clove oil in *Bacillus pumilus* (0.931 mg/L AT 50%) by the MIC method.

From this study, we can see that all Essential oils showed antibacterial activity against different bacterial strains but at different concentrations. All the tested bacteria were more or less sensitive to the two essential oils.

There has been an increasing interest in the exploration of antibacterial plant products having mechanism of action different from those of the conventional chemical drugs. This aspect is effectively evaluated by the evaluation of zone of inhibition of different essential oils against different bacteria at different concentration and the combination of essential oils with antibiotics to show the synergistic effect with agar diffusion method and MIC method.

IV. CONCLUSION

From our study, we observed that *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis and Bacillus pumilus* has significant antibacterial activity against all the four bacteria when combined with different antibiotics and essential oils.

Hence based on the result we can conclude that essential oils or antibacterial plants and their products may serve as a valuable source for antibiotic production. Further studies are required to explore novel active compounds.

Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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