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Synergistic Effect of Essential Oils of *Cymbopogon citratus* (Lemon Grass) and *Zingiber officinale* (Ginger) on *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*

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Abstract

Essential oils obtained from Lemon grass (*Cymbopogon citratus)* and Ginger (*Zingiber officinale)* are used in combined form as herbal for various diseases. Synergistic antibacterial activity of essential oils of *Cymbopogon citratus* (Lemon grass) and *Zingiber officinale* (Ginger) on *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* was determined. The study aimed to determine the synergistic antibacterial effect of combination of lemon grass and ginger essential oils by using different ratios. The essentials oils were obtained by steam distillation from each of the plants. The essential oils of each as well as their combinations were tested against the three bacteria by employing agar well diffusion techniques. The results showed that each oil separately exhibited antibacterial activity ranging from zone of inhibition of 8.35mm to 23.90mm with varying concentrations of the oils. Lemon grass oil showed higher antibacterial activity of 23.9mm against *Staph aureus* but only 9.00mm against *Salmonella typhi*. The combined synergistic effect achieved on Staph *aureus* was 45mm, using ratio 1:1 and *Salmonella typhi* 35.00mm. The MIC and MBC for *staph aureus* were 20% and *Escherichia coli* with 12% MIC and MBC respectively. While *Salmonella typhi* were 10% and 8% respectively of MIC and MBC. The combination of essential oils of lemon grass and ginger exhibited good synergistic potential which can be utilized in formulation of drugs and preservatives.

Keywords: Synergistic Effect, Antibacterial, Lemon Grass, Ginger, Essential Oil.

Introduction

Plant in nature has been a source of medicinal agents for thousands of years and since the beginning of man. In Nigeria, almost all plants are medicinal and the application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession (Arshao *et al.*, 2014).

Medicinal plants are found to be useful as pharmaceutical, nutriceutical, cosmetics and food supplements. Plants derived products have been used for medicinal purposes for centuries. In traditional Indian medicine *Zingiber officinale* (Ginger) *Allium sativum* (Garlic) and many others herbs have been used as medicine. With an increase in the antibiotic-resistant strains of microorganism, traditional plants are being investigated for their antibacterial and medical values (Chouchan *et al.*, 2017).

The medicinal properties of plants have received a great interest because of their low toxicity, pharmaceutical activities and economic viability (Elshafie *et al.*, 2015). Great consideration has been given to the effective use of EOs in clinical procedures (Elshafie and Camele, 2017).

Plant derived drugs remain an important resource in developing countries, to combat serious diseases including infectious disease. Approximately 65-80% of the world's population still relies on traditional medicine for the treatment of common illness. Essential oils have been shown to have a plethora of medicinal values (Gao *et al.,* 2020). Among compounds of natural origin, biological activities have been shown by essential oils from aromatic and medicinal plants and have received particular attention because of their medical scavenging properties (Chouhan *et al.,* 2017).

Presently in the developing countries, synthetic drugs are not only expensive and inadequate for treatment of disease but are also often with adulteration and side effects (Mustafa *et al.,* 2021). The search for reliable alternative medicines is inevitable. Aromatic plants are main providers of essential oils (EOs) and are employed in herbal therapies.

Essential oils are a complex mixture of natural volatile and aromatic compounds obtained from plants like flower birds, seeds leaves, twigs, bark, herb, wood, fruits and roots (Encyclopedia of Microbiology, 2018) and also known as ethereal oils, defined as the oil obtained by steam distillation of plants.

According to Mustafa *et al.,* (2020), essential oils are composed of liophilic and highly volatile secondary metabolites principally mono – and sesquiterpenes but other compounds such as allyl and iso allyl phenols may also be present.

Statement of the Problem

EOs have been used not only in monotherapy but in combination for many years. The interaction between EO compounds can produce four notable effects namely indifferent additive, antagonistic, or synergistic effects (Bassole and Julian, 2012). Generally, the antagonistic is attributed to the interaction between non-oxygenated and oxygenated monoterpene hydrocarbons. The additive and synergisms effects are associated with phenolic and alcoholic compounds (Bassole and Julian, 2012).

Massive use of antibiotics has resulted in the emergence of resistance against them. *Salmonella typhi* species, coagulase negative *Staphylococcus*, *Escherichia coli* and *Pseudomonas aeroginosa* are amongst some of the main bacteria with multidrug resistance pathogens (Choucham *et al.,* 2017).

Cymbopogon citrates also known as lemon grass is an aromatic plant used commonly in Nigeria in combination with ginger for treatment of respiratory infection including common cold. Although several studies have confirmed the antimicrobial activities of lemon grass and ginger essential oil separately against species of microorganisms, but the synergistic effect of these two plants oils were not determined. In this study, the antimicrobial activities of Lemon grass and ginger essential oils against *Staphylococcus aureus*, *Salmomella typhi* and *Escherichia coli*, were investigated.

Aim

The study aimed at determining the in vitro synergistic effect of essential oils of widely used Lemon grass (*Cymbopogon citratus)* and Ginger (*Zingiber officinale*) on *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*.

Objectives

The objectives of the study are as follows:

i. To obtain essential oils from the plant materials through steam distillation.
ii. To test the antibacterial effect of the combined oils against *Salmonella typhi, Staphylococcus aureus*, and *Escherichia coli*
iii. To determine the minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC) of the oils against the test organisms.

Materials and Methods

The fresh lemon grass was obtained and identified by a Botanist from the Faculty of Agriculture Nasarawa State University Keffi (NSUK) Botanical Garden, while the Ginger was purchased from the Ombi II neighborhood market all in Lafia the Nasarawa State Capital.

Test Organisms

The test microorganisms used in this study (*Salmonella typhi*, *Staphylococus aureus* and *Escherichia coli*) were clinical isolates obtained from the Department of Microbiological Laboratory of the Dalhatu Araf Specialist Hospital, Lafia. The three bacterial isolates were stock using nutrient agar after confirmatory test and incubated at 37°c for subsequent use.

Preparation of the Essential Oils

The essential oils from plant samples were obtained using steam distillation process as described by Nyamath, and Karthikeyan, (2018). The Lemon grass leaves pieces (450g) were placed into the still chamber where steam was passed from the bottom. The essential oil from the plants sample was carried along with the steam from the top of the still and passed through the condenser and collected. Similarly, fresh ginger rhizome were cut into pieces and 450g used to obtain essential oil.

Each mixture was separated using separating funnel after it was allowed to stand for sufficient time to be clear as it remained on top of the water. The initial oil collected was then dried over anhydrous sodium sulphate and stored in an air tight container at temperature of 0°c until when required.

Antibacterial Activities

The antimicrobial activity of both the essential oil and the extract of Lemon grass (*Cymbopogo ncitratus)* and Ginger (*Zingiber Officinale*) was carried out using the punch hole diffusion technique described by Gao *et al*, (2020).

Each test organism broth was swabbed on the surface of prepared Muller Hinton agar plate separately and allowed to dry for about 5 munities. Holes (3mm) were punched in the center of the plate using micro-borers. Three drops of the essential oil were dropped into each of the hole at different concentrations, 10%, 20%, 30%, 40%, 50%. The oil was allowed to diffuse in the wells for one hour and plates were incubated at 37°C for 24-48hrs. The diameter of incubation was measured using a graduated scale in mm.

Determination of Synergistic Effect of the Essential Oils

The essential oils of lemon grass and ginger were combined in the ratio of 1:1, 1:2 and 2:1 respectively to determine synergistic effect as described by Mangalagirin *et al*., (2020). 1ml of active cell suspension of test organisms was spread on Muller Hinton agar uniformly and wells of 5mm diameter each were made. The oils were added into each well and incubated for 24-36hrs at 37°C, each zone of inhibition was recorded after incubation.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth dilution technique by CLSI (2018) serial dilution of the essential oil combined in different concentration was made (75%, 50%, 25%, 15%). These were tested against small inoculums of each test organisms. The lowest concentration of the essential to inhibit the growth of the pathogen was taken as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

Following the determination of MIC the MBC was obtained by using 0.5ml of oil from the test tubes in which there were no desirable growth. This was spread over the surfaces of agar plates and the lowest concentration of the oil that did not result in bacterial growth on the agar plate was recorded as the MBC as described by CLSI, (2021).

Results and Discussion

Screening of lemon grass and ginger essential oils for antibacterial activities against *Staphylococcus aureus*, *Salmonella typhi* and *E. coli*.

Both lemon grass and ginger EOs showed antibacterial activity separately as shown in table 1.

Table 1: Antibacterial activities of Lemon grass and Ginger essential oils

Test organism	Zone of Inhibition (mm)									
	10%		20%		30%		40%		50%	
	Lg	G	Lg	G	Lg	G	Lg	G	Lg	G
Staph.aureus	14.5	13.00	18.00	17.50	19.5	17.4	23.9	20.5	23.00	21.00
E.coli	12.5	11.67	15.30	15.00	20.6	18.00	20.5	19.0	21.0	19.5
Salm. typhi	9.00	8.35	10.5	11.5	13.6	12.5	18.0	17.5	19.5	18.5

Keys: Lg = Lemon grass oil, G = Ginger oil

Table 2 showed that the combined effect of ginger and lemon grass Eos, with values ranging from 26mm to 45mm zone of inhibition. The combination of the two oils in ratio of 1:1 showed a zone of inhibition of 45, 41, and 31mm against *Staph aureus*, *E. coli* and *Salmonella typhi* respectively.

Table 2: Shows the Antibacterial effect of the combined essential oils of Lemon grass and Ginger

Test organism	Zone of inhibition (mm)		
	1:1	1:2	2:1
Staph.aureus	45.00	32.00	29.10
E.coli	41.10	36.00	28.00
Salm. typhi	35.00	32.30	28.50

Table 3: shows the maximum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Test organism	MIC (%)	MBC (%)
Staphylococcus aureus	20	20
Escherichia coli	12	12
Salmonella typhi	10	8

Discussion

The Zone of inhibition representing the synergistic antimicrobial activity of the essential oils from Lemon grass (*Cymbopogon citratus*) and ginger (*Zingibe rofficinale*) on *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* after 24 hours of incubation are presented in Table 1-2.

The zones of inhibition were measured in millimeter (mm) and compared to the diameter of the plates used which was 90mm. From the results presented' it was observed that the effect of the essential oil of Lemon grass and Ginger combined shows highest antimicrobial activity against *Staphylococcusaureus* followed by *Escherichia coli* as seen in Table 1.

Also as seen in Table 3: When the two oils of Lemon grass and Ginger were used against the test organisms the synergistic effect was also seen prominently on *Staphylococcus aureus with the highest zone of* MIC & MBC, followed by *Escherichia coli.* The results are similar to those obtained by Gao *et al.*, (2020),

From all indications in the two tables, *Staphylococcus aureus* showed indications of high susceptibility to EOs oils of the two plants. It was also observed that the synergistic effect of the oils are highly inhibitory such that they inhibits growth absolutely of the microorganisms after 24 hours of incubation.

The synergistic antibacterial activity of essential oils of lemon grass and ginger combined is in agreement with other studies that revealed essential oil inhibit both gram positive and

gram negative bacteria; Mangalagiri *et al*., (2020) concluded that essential oils of lemon grass has appreciable activity against some gram positive and gram negative bacteria.

Conclusion

The oils of these plants are seen to possess amazing inhibitory effects on the three microorganisms. The combined Lemon grass and Ginger oils possess synergistic effect on all three test organisms, there was no antagonistic effects observed between the two plants EOs during the study.

Recommendations

Based on the findings in this study, it is recommended that;

i. The oils can be utilized in formulating drugs.
ii. Further studies be conducted on the appropriate ratios of combination of the oils for food preservation.

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