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COMPARATIVE ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS OF CYMBOPOGON CITRATUS (LEMON GRASS) AND CITRUS SINENSIS (SWEET ORANGE) PEEL AGAINST SOME PATHOGENS

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Abstract

Essential oils are one of the important natural products derived from plant for their various biological activities, however, the antimicrobial activities vary in potency and usage. Comparative antimicrobial activities of essential oils of lemon grass (Cymbopogon citrtus, and Orange (Citrus sinensis) peel against three bacterial species (Salmonella typhy, Pseudomonas aeruginesa, and Staphylococcus aureus) and two fungal species (Candida albicans, and Aspergillus niger). The essential oils were extracted by steam distillation. Antibacterial and antifungal activities were determined by paper disc diffusion technique and micro-dilution. The results showed both essential oils of lemon grass and orange peels exhibited

Keywords;
Essential oils, *Cymbopogon citratus, Citrus sinensis, Antimicrobial activities, Salmonella typhi*

strong antibacterial and antifungal activities against the test organisms with zones of inhibition ranging from 15.00mm to 28.00 mm. Comparison of the inhibitory activities of the two oils showed orange had lower effect of 15.60mm as against lemon grass 19.00mm at concentration of 20µ/ml against salmonella typhi. Inhibitory activities was 28.30mm highest on Staphylococcus aureus for orange peel and 28.00mm for lemon grass oil. The minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) and minimum fungicidal concentration (MFC) indicated values20µl/ml and 10µ/ml against S.aureus respectively. The result demonstrated that both essential oils of lemon grass and orange have high potential antimicrobial activities that can be developed into potent drugs.

INTRODUCTION

Plants in nature have been the source of medical agents for thousands of years and since the beginning of man (Arshao *et al*., 2024). Among the array of medicinal products from plants, are essential oils, which are applied for treatment of various ailments including respiratory tract infections, inflammation, arthritis, rheumatism and abdominal gastrointestinal disorders. Essential oils have a plethora of medicinal values (Gao *et al*., 2020)

Essential oils (EOs) are complex mixture of natural volatile hydrophobic compounds obtained from Aromatic plants parts like flower buds, seed leaves, twigs, bark, fruits and roots, EOs are also referred to as ethereal oils. The primary functions of essential oils in plants are protection of the plants, provide scent, and flavor play an important communication role, attracting pollinators and repelling pests (Grazyna and Zofia, 2024)

Essential oils are known for their antiseptic bactericidal, fungicidal antiviral and other medicinal properties (Kowalayzyk *et al*., 2023) for centuries, EOs have been applied for food preservation, and attention is given to Eos currently in response to the growing interest to combat antibiotic resistance

and minimize adverse side effects of antibiotics. Some aromatic plants commonly used include, *Vitex doniana* (black plun), *Cymbopogon citratus* (lemon grass), *Eucalyptus globulus* (pole wire or blue gum tree) *Zingiber offinale* (Ginger) *Citrus sinensis* (orange) among others (Kowalayzyk *et al*., 2023)

In Nigeria, ginger, lemon grass and oranges are among the aromatic plants commonly used for treatment of some common disease including respiratory tract infections, typhoid fever, dysentery and urethral tract infections. Though several studies have been conducted on antimicrobial activities, the comparative analysis of the composition and antimicrobial properties are scanty.

Aromatic plants and extract prepared from them are known for their antiseptic, bactericidal, fungicidal antiviral and medical properties (Swamy *et al*., 2016),

Aromatic compounds derivatives of phenylpropane, occur as terpenes. According to Chanthaphore *et al*., (2008) essential oils are mixture of over a hundred compounds that can be approximated into three fractions; terpenes hydrocarbons, oxygenated compounds and non-volatile compounds. The terpene fraction can constitute from 50 to more than 95% of the oil.

(*Cymbopogon citratus* (OC) stap f, commonly known as lemon grass, is a perennial grass, with long leaves and is one of the main medicinal aromatic plants cultivated in Nigeria. Lemon grass is cultivated mainly for its essential oil in both tropical and subtropical regions of Asia, South America and Africa (Gao, *et al*., 2020). Lemon grass essential oil is used as antiseptic, anti-helminthic and to treat backache, sprain, and haemoptysis. Infusion of oil leaves are used in as sedation, antimicrobial and anti-inflammatory agents. The E.O is inhaled to relief symptoms of common cold. In some countries the E.O is used in treatment of diabetes (Mohammed *et al*., 2014).

Sweet Orange (*Citrus sinensis*) belongs to the family Rutaceae. The fruits are sweet rich in Vitamin C and mineral, and folic acid. Oranges are common fruit

in Nigeria. The orange peels are rich source of essential oil. However, the peels are discarded as waste in Nigeria, causing environmental pollution. With the high demand of essential oils, orange peels can serve as raw materials for production of essential oils.

This study aimed at comparing the chemical composition and antimicrobial activity of lemon grass and orange peels oils against *Salmonella typhi* and *Candida albicans, Pseudomonas aeruginosa, Staphylococcus aureus* and *Aspergilus niger*.

Materials and Methods

Plant Materials

The orange peels used were obtained from orange vendors in Ombi I, Lafia, Nasarawa State Nigeria. The orange peels were washed with distilled water to remove debris and then cut into pieces before the extraction commenced.

Lemon grass was obtained from the garden in YMCA Headquarters, Lafia. The fresh leaves were collected and washed after identification of species was done.

The lemon grass was cut into small pieces, before extraction.

Test Organisms

The test organisms *Salmonella typhi*, *Pseudomonas aeruginisa*, *Candida albicans*, *Staphylococcus aureus* and *Aspergillus niger* were clinical isolates obtained from the Federal University of Lafia Teaching Hospital, Lafia. The five isolates were resuscitated and stock culture using Nutrient Agar after which they were incubated at 37^{o}C for subsequent use.

Extraction of Essential Oils

Cut fresh leaves and rhizome of lemon grass were used for extraction by steam distillation. A total of 6.5kg, of fresh lemon grass was used. 200g pieces of lemon grass was loaded into the distillation flask, which is connected to a

round bottom flask containing water as described by Giwa *et al*, (2018). The flask was connected to a condensing unit with its tube. Heating mantle was used for heating the water in the round bottom flask. The essential oil was extracted as the steam passed through the lemon grass pieces in the distillation flask.

Similarly, essential oil was extracted from orange peels by the process of steam distillation using the clavenge apparatus. 500g of orange peels were placed in a round bottom flask and filled with water to about three quarter full as described by Obidi *et al*., (2013). The distillation apparatus was connected to the flask. A separating funnel was used to collect the essential oil as it floats on top of water. After distillation process, the product obtained was a mixture of oil and water. The separating funnel tap was carefully opened to separate water from the oil. The essential oil was dried over anhydrous sodium sulfate and stored in amber colored bottles.

Determination of Essential Oil Yield (%)

The essential oil yield was determined using formula as stated by Ashok *et al*., (2011) as follows.

$R = V/B \times 100\%$

Where R = essential oil yield (%)

V = Volume of essential oil obtained in (ml)

B = Weight of lemon grass, orange peels sample (grams)

Determination of gravity

To determine the gravity of the essential oil, an empty beaker was weighed and recorded. After which essential oil was poured into the beaker and weighed gain. The density of oil was obtained using the equation.

$$\text{Density} = \frac{\text{Volume of oil in the beaker}}{}$$

Determination of Specific density

This was done using a clear and dry gravity bottle. The specific gravity bottle was weighed and distilled water was poured into the bottle and weighed, similarly the same volume of essential oil was poured into the same bottle and weighed.

The specific gravity was obtained by the equation.

$$\text{Essential oil specific gravity} = \frac{\text{weight of volume of EO}}{\text{Weight of equal volume of water}}$$

Determination of Antimicrobial activities of essential oils of lemon grass and orange peel

For the antibacterial and antifungal assay, *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* were used. The antimicrobial activities of the essential oils were evaluated using paper disc diffusion technique (Al-Nabulsi *et al*., 2020). The test microorganisms were cultured in Agar Broth separately for 24 hr at 37^{o}C and were diluted to 1 x 10^{7} cfu/ml. The bacterial suspension was spread evenly on Muller-Hinton Agar, while fungal suspension was spread on potato dextrose Agar, and allowed to stand for 10 minutes. Absorbent Whatman No 1 sterile paper disc of diameter 5mm which was impregnated with the essential oil was then placed on the respective agar culture using sterile forceps and incubated at 37^{o}C for 24 hrs for bacteria and 48hrs for fungi. Control disc were impregnated with streptomycin for bacteria and flucanozole for fungi to serve as positive control. Disc without essential oil in them were used as negative control. The antimicrobial activities of the essential oils were assessed by comparing the zone of inhibition measured in mm. Clear zones of inhibition around the disc indicated the presence of antimicrobial activities.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal fungicidal concentration

MIC was determined using broth dilution method by Nagalakshmi *et al*., (2019) with slight modification. Each essential oils was diluted into various

concentrations viz: 10µg g/ml 20µg/ml, 40µg/ml, 60µg/ml 80µg/ml and 100µg/ml in sterile nutrient broth in test –tubes. Using a standard wire loop, a loopful of the bacterial or fungi culture was inoculated into test-tubes containing the various concentration of essential oil in nutrient broth. The test tube was incubated at 37^{o}C for 24 hour for bacteria and 48 hours for fungi. After which they were observed for turbidity or growth. The lowest concentration that inhibited growth was taken as the MIC

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by using culture medium from wells which had essentials oils concentration higher than MIC, were smeared on separate plates and incubated at 37^{o}C 24 hrs and 36 hrs. MBC and MFC was recorded as the lowest concentration of essential oil which lowest concentration of essential oil which gave no growth of bacteria or fungi. MBC was determined as the lowest concentration of essential oil which gave no growth of bacteria.

Results and Discussion

The essential oils of lemon grass and orange peels were evaluated after extraction. The two oils each exhibited characteristics as shown in table 2. Table 1 showed the percentage yield of essential oil of lemon grass 0.6% while orange peels gave yield of 0.77% from a total of 6.5kg of materials used for the extraction. The color of essential oil of lemon grass was a light golden yellow while orange was bright yellow. Orange and lemon grasses are aromatic plants that are commonly used in aromatherapy.

Antibacterial and antifungal activities of the two oils are shown on table 3. The agar paper disc diffusion technique is commonly and extensively used to evaluate the antimicrobial effects of natural substances such as essential oils. The antimicrobial effects of essential oils of lemon grass and orange peels were evaluated against *Salmonella typhi*, *Pseudomonas aeruginasu*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. The zones of

inhibition range for different concentrations of 40µl, 60µl, and 80µl as indicated in table 3.

It was observed that *Staphylococcus aureus* showed the highest susceptibility to orange peels and lemon grass oils of 28.30mm and 35.20mm respectively. *Salmonella typhi* and *Candida albicans* showed the least antimicrobial susceptibility of values 15.60 and 15.80 for volume 20µl. According to . Handyayni *et al*., (2019), antimicrobial strength is classified into 3, namely, strong activity if it produces inhibition diameter (power) of more than 8mm, moderate activity if it produces 7-8mm zone of inhibition diameter, and weak if it has inhibition power diameter less than 7mm. In this study, the essential oils of orange peels and lemon grass showed strong inhibition power diameters. Handyayni *et al*., (2019) reported that essential oil of orange peel at some altitudes showed strong antimicrobial activity of 18mm against *Staphylocuccus aureus* and 19mm against *Candida albicans*. This study shows antimicrobial activity of essential oils of orange peel on *Salmonella typhi,* 23.50mm *Staphylococcus aureus*, 28.30mm, *Pseudomonas aeruginasa,* 24.20mm, *Candida albicans*, 19.30mm and *Aspergillus niger* of 20.40mm. lemon grass oil showed antimicrobial values at concentration of 20µl, *Salmonella typhi*, 26.30mm *Pseudomonas aeruginasa*, 26.20 *Staphylolcoccus aureus*, 28.00mm *Candida albicans*, 23.5mm and *Aspergillus* antibacterial and antifungal activity.

Table 1: Yield of essential oil in percentage

	Mass of material	Essential oil obtained	Percentage (%)
Orange peels	6.5kg	5ml	0.77
Lemon grass	6.5kg	4.6ml	0.56

Table 2: Some physical properties

	Density	Specific density	Appearance

Orange peels	0.85	0.83	Bright yellow
Lemon grass	0.68	0.62	Golden yellow

Table 3: Susceptibility of Bacterial and Fungi to essential oils of lemon grass and Orange peels Zone of inhibition in mm

Essential Oil	*Salmonella typhi*	*Psuedomonas aeruginesa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
Orange peels					
20µl	15.60	17.80	20.40	15.80	16.80
40µl	18.70	21.20	21.80	16.30	18.00
60µl	21.50	22.50	24.60	18.40	19.60
80µl	23.50	24.20	28.30	19.30	20.40
Lemon grass					
20µl	19.00	18.70	21.40	17.5	19.80
40µl	22.30	22.30	23.60	19.6	20.60
60µl	24.70	24.50	27.00	21.4	22.50
80µl	26.30	26.20	28.00	23.5	24.60
Streptomycin	31.20	33.30	35.20	-	-
Flucanozole				26.50	27.80

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal and Fungicidal concentration (MBC/MFC) volumes for essential oils in µl MIC and MBC/MFC in µl/ml

Essential Oil	*Salmonella typhi*	*Psuedomonas aeruginesa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
Orange peels	MIC 16 µl	18 µl	20 µl	15 µl	18 µl
Lemon	MIC 20 µl	15 µl	10 µl	15 µl	18 µl
	MBC 20 µl	10 µl	10 µl	15 µl	18 µl

Conclusion

The results showed the two essential oils possess highly inhibitory effects on the five microorganisms. In comparison the two essential oils exhibited closely similar effects. The positive effect exhibited on both gram positive, and gram negative bacteria as well as fungi, is an indication that these potential therapeutic agents that can be developed into potent drugs. Further studies are needed to isolate the bioactive constituents and

determine the mechanisms of action. In addition, the toxicity, anti-inflammatory, antioxidant and anti-tumor properties of the different essential oils need to be investigated.

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