

Comparative Analysis of Antimicrobial Activities of Essential Oils of Some Aromatic Plants against Some Pathogenic Bacteria and Fungi

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DOI: <https://doi.org/10.70382/hujhwsr.v7i3.012>

Key words:

Essential oils,
Aromatic plants,
Antimicrobial activities,
Salmonella typhi,
Cymbopogon citritus.

Abstract

Essential oils are one of the important natural products derived from aromatic plants for their various biological activities. However, the antimicrobial activities vary in potency and usage. Comparative antimicrobial activities of essential oils of lemon grass (*Cymbopogon citritus*), Orange (*Citrus sinensis*) peel Black plum (*Vitex doniana*), Ginger (*Zingiber officinale*) and Blue gum tree (*E. globulus*) against four bacterial species (*Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) and three fungal species (*Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*). The essential oils were extracted by steam

distillation and hydrodistillation. Antibacterial and antifungal activities were determined by paper disc diffusion technique and micro-dilution. The results showed all the essential oils exhibited strong antibacterial and antifungal activities against the test organisms with zones of inhibition ranging from 8.12mm to 26.00 mm. In comparison the inhibitory activities of the oils showed Ginger with 26.00mm against *Aspergillus niger*, while *E. globulus* has 16.50mm. *Vitex doniana* and *Z. officinale* had the lowest value of 10.60mm and 8.12mm against *Salmonella tyhi*. The minimum inhibitory concentration (MIC) indicated values ranging from; 0.12mg/ml to 0.82mg/ml. While minimum bactericidal (MBC) and minimum fungicidal concentration (MFC) had values from 0.20mg/ml to 0.110mg/ml. The result demonstrated that the five essential oils have high potential antimicrobial activities that can be developed into potent drugs. Further studies are needed to isolate the bioactive constituents and determine their mechanisms of action.

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 officinale* (Ginger) *Citrus sinensis* (Orange) among others (Kowalayzyk *et al.*, 2023)

Aromatic plants and extracts 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 this oil is used as sedative, 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.

Eucalyptus globulus is a medicinal plant, which leaves and bark are used for treatment of some disease. The chemical composition of *E. globulus* essential oil are α -pinene, 1,3- pinene, limonene, 1,8-cineole, α -terpinene, camphor and α -phellandrene (Negash *et al.*, 2006). According to Bachir and Benali, (2012) *E. globulus* essential oil showed significant antimicrobial activity against *E. coli* and *S. aureus*.

Vitex Doniana, The genus *Vitex* is important in traditional medicine and has been utilized for many years as an integral part of herbal medicines. Different phytochemical studies conducted on *Vitex* species revealed the presence of chemical compounds known to be pharmacologically active, such as diterpenoids, sesquiterpenoids, triterpenoids, lignans, iridoids, sterols and flavonoids (Mustafa, *et al.*, 2020).

Zingiber officinale is a rhizome popularly used as a spice. Several studies have shown that it has both antifungal and antibacterial activities against a number of pathogens. Essential oil extracted from this rhizome showed substantial antimicrobial activities. (Goa *et al.*, 2020).

In Nigeria, aromatic plants are 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, however, reports on comparative analysis are scanty.

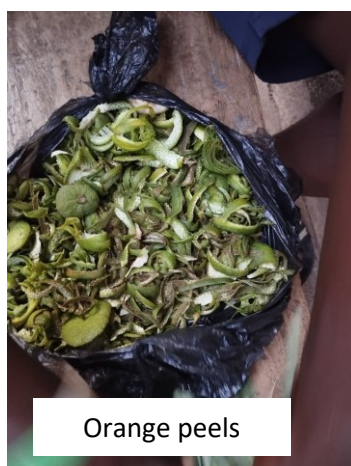
This study aimed at comparing antimicrobial activities of lemon grass, orange peels, Ginger, *E. globulus*, and *Vitex doniana* oils against *Salmonella*

typhi and *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Aspergillus flavus*, and *Aspergillus niger*.

Materials and Methods

Plant Materials

Fresh Ginger rhizomes were purchased from Lafia Modern market. Orange peels were obtained from orange vendors in Ombi I, Lafia, Nasarawa State. While fresh Lemon grass, *E. globulus*, and *V. doniana* were obtained from the garden in YMCA Headquarters, Lafia. All the plant materials were washed and rinsed with distilled water to remove debris. Each plant material was cut into pieces separately before extraction.



Test Organisms

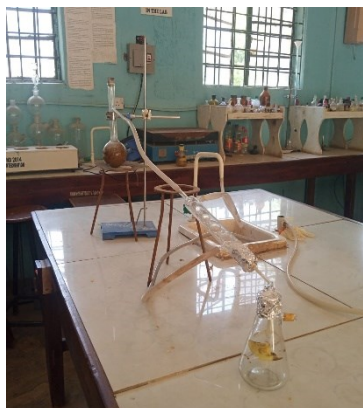
The test organisms *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Aspergillus flavus* and *Aspergillus niger* were clinical isolates obtained from the Federal University of Lafia Teaching Hospital, Lafia. The seven isolates were resuscitated and stock culture using Nutrient Agar after which they were incubated at 37°C for subsequent use.

Extraction of Essential Oils

Extraction of essential oil from Lemon grass, Ginger and Orange peels were done by steam distillation. While *E. globulus* and *V. doniana* were by hydrodistillation. A total of 6.5kg, of each of the plant material was used for the extraction. 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 oils were extracted from Ginger rhizome and Orange peels by the process of steam distillation using the cleverger apparatus. 500g of each of these plant materials were placed in a round bottom flask and filled with water to about three quarter full separately 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.

Extraction Apparatus



Extracted oils



Determination of Essential Oil Yield (%)

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

$$\text{"Percentage yield"} = \frac{\text{Volume of essential oil obtained}}{\text{Weight of the plant material}}$$

Determination of gravity

The density of oil obtained was determined using the equation.

$$\text{Density} = \frac{\text{Mass of oil sample}}{\text{Vol. of oil the beaker}}$$

Determination of Specific density

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 extracted from the five plant materials.

For the antibacterial and antifungal assay, the clinical isolates; *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *klebsiella pneumonia*, *Aspergillus flavus* 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°C and were diluted to 1×10^7 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 discs 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°C for 24 hrs for bacteria and 48hrs for fungi. Control discs were impregnated with streptomycin for bacteria and flucanazole for fungi to serve as positive control. Discs 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 discs 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 oil was diluted into various concentrations viz: 0.10mg/ml, 0.20mg/ml, 0.30mg/ml, 0.40mg/ml, 0.50mg/ml, 0.60mg/ml, 0.70mg/ml, 0.80mg/ml, 0.90mg/ml and 1.00mg/ml in sterile nutrient broth in test –tubes. Using a standard wire loop, a loopful of the bacteria or fungi culture was inoculated into test-tubes containing the various concentration of essential oil in nutrient broth. The test tubes were incubated at 37°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 essential oils concentration higher than MIC. These were smeared

on separate plates and incubated at 37°C for 24 hrs and 36 hrs. MBC and MFC were recorded as the lowest concentration of essential oil which gave no growth of bacteria or fungi.

Results and Discussion

The invitro antimicrobial activities of essential oils extracted from Lemon grass, Eucalyptus, Orange peel, Ginger and black plum were evaluated against four bacterial species (*Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*) and three fungi (*Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*).

Table 1 showed the yield in percentage for each of the 5 essential oils extracted. While Table 2, showed some physical properties of the oils. These include; Density, Specific density and Appearance.

Table 3, presents results of the mean values in Diameter of inhibition of the five essential oils extracted against the test organisms. All the five essential oil exhibited some levels of antibacterial and antifungal activity against the test organisms, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. The diameter of inhibition ranged from 12.50mm and 32.00mm. The zone or diameter of inhibition is interpreted as the antimicrobial activity of the essential oils. According to Handyayani *et al.*, (2019), antimicrobial strength is classified into three viz: Strong activity if it produces inhibition diameter (power) of more than 8mm, moderate activity if it produces 7-8mm zone of inhibition, and weak if it has inhibition power diameter less than 7mm

As shown in Table 3, of all the EOs evaluated, lemon grass and *Eucalyptus globulus* oils showed the highest antibacterial activities of 22.30mm and 25.00mm against *Staphylococcus aureus* respectively. Ginger oil has activity of 20.50mm against *Klebsiella pneumonia*. Lemon grass oil has activity of 22.30 against *Staphylococcus aureus*. Naick *et al.*, (2024) reported, EO of

Eucalyptus with inhibition diameter of 27.00mm and 24.00mm against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* respectively.

Antifungal activities of the EOs against fungal species *C.albicans*, *Aspergillus flavus* and *Aspergillus niger* are strong. The diameter of inhibition recorded for Ginger oil against the fungi ranged from 15.60mm to 26.00mm *Vitex doniana* showed antifungal activity of 18.00mm, 23.20mm and 21.00mm against, *Candida albicans*, *A. flavus*, *A. niger* respectively. While Lemon grass oil has 20.60mm, 19.80mm, and 17.50mm, Orange peel oil showed values of 16.30mm, 19.30mm, 18.30mm against *C. albicans*, *A. flavus* and *A. niger* respectively. These values are similar to those reported by Campano *et al.*, (2022).

Antimicrobial activity of botanicals is defined as good activity (MIC<100micrograms/ml), moderate activity (MIC100-500micrograms/ml), weak activity (MIC500-1000microgram/ml) and inactive >1000microgram/ml. (Naik *et al.*, 2024). In this study the MIC values in (mg/ml) for Ginger are; 0.12, 0.45, 0.82, 0.40, 0.32, 0.36, 0.42 against *S. aureus*, *S. typhi*, *K. pneumonia*, *P.aeruginosa*, *C. albicans* *A. flavus* and *A. niger* respectively. MIC values recorded for *V. doniana* showed 0.15, 0.06, 0.95, 0.45, 0.65, 0.68, and 0.50, while *E.globulus* showed MIC of 0.29, 0.30, 0.17, 0.22, 0.38, 0.35 and 0.26 against *S.aureus*, *S. typhi*, *K. pneumonia*, *P.aeruginosa* and *C. albicans*, *A. flavus* and *A. niger* respectively. The MIC observed for Orange peel oil against *S.aureus* 0.20, *S.typhi* 0.26, *K. pneumonia* 0.30, *P. aeruginosa* 0.45, *C. albicans* 0.18, *A. flavus* 0.40 and *A. niger* 0.60. For Lemon grass, MIC recorded are 0.26, 0.25, 0.35, 0.40, 0.23, 0.45 and 0.50 against *S.aureus*, *S. typhi*, *K. pneumonia*, *P. aeruginosa*, *C. albicans*, *A. flavus* and *A. niger*.

The MBC and MFC of the essential oils varied. Ginger showed the lowest MBC of 0.20mg against *S. aureus* while the Orange peels oil showed 0.25mg against *S. aureus*. Lemon grass 0.30mg, *E. globulus* 0.40mg, and *V. doniana* 0.35mg. MBC values against *S. typhi* indicated Ginger 0.50mg, *V. doniana* 0.70mg, *E. globulus* 0.35mg, Orange peels 0.45 and Lemon grass 0.30mg. MBC

values against *K. pneumonia* 0.90mg, 1.10mg, 0.35mg, 0.40mg and 0.40mg for Ginger, *V. doniana*, *E. globulus*, Orange peels and Lemon grass respectively. MBC values observed for essential oils against *P. aeruginosa* are Ginger 0.45mg, *V. doniana* 0.50mg, *E. globulus* 0.35mg, Orange peels 0.50mg and Lemon grass 0.45mg.

MFC for the different oils against the fungi pathogens tested exhibited the following values; *C. albicans*, *A. flavus*, *A. niger* has 0.40mg, 0.45mg and 0.48mg respectively for Ginger oil. While, *V. doniana* showed 0.78mg, 0.80mg and 0.80mg. MFC for *E. globulus* oil are 0.60mg, 0.40mg and 0.45mg. Orange peels oil showed MFC of 0.22mg, 0.45mg, and 0.65mg. While Lemon grass, gave values of 0.35mg, 0.50mg and 0.60mg.

A number of studies reported essential oils having significant antimicrobial activities with some demonstrating strong activity, moderate activity and weak activity, while few exhibit inactivity (Chouhan *et al.*, 2017). In this study, Ginger, Lemon grass, and Orange peels showed strong antibacterial activities of 0.12mg/ml, 0.20mg/ml and 0.26mg/ml against *S. aureus*, while *E. globulus* showed strong antibacterial activity of 0.17mg/ml against *K. pneumonia* and 0.22mg/ml against *P. aeruginosa*. Chouhan *et al.*, (2017), reported antimicrobial activity of *E. globulus* against *P. aeruginosa*, and *S. aureus* with MIC values of 3-4mg/ml which demonstrate that values obtained in this study are similar and showed that, the bacteria were sensitive to the essential oil. Ginger essential oil has been shown to be active against *S. aureus*, *K. pneumonia*, *P. aeruginosa*, *Listeria spp.*, *E. coli* and *Salmonella spp.* (Teles *et al.*, 2019).

All the five essential oils demonstrated some level of antifungal activity with the value of 26.00mm Ginger oil against *A. niger* and 14.50mm for *E. globulus* against *A. flavus*. According Teles *et al.*, (2019), a study with essential oils against six fungi revealed that, Ginger exhibited potent antifungal activity.

Table 1: Percentage yield of essentials extracted from

Sample Material	Plant Mass of Plant Sample Used in (kg)	Essential oil obtained (ml)	Percentage (%) yield
Ginger	605	9.75	1.5
<i>Vitex doniana</i>	6.3	4.23	0.65
Lemon grass	6.5	4.6	0.71
Orange peel	6.5	5.0	0.77
<i>Eucalyptus globalus</i>	6.5	5.91	0.96

Table 2: Some physical properties of essential oils extracted

Essential oil	Density (kg/ml)	Specific Density	Odour	Appearance
Ginger	0.88	0.86	Fresh spicy sweet	Greenish-yellow
<i>Vitex doniana</i>	0.76	0.74	Sweet fruity odour	Light-yellow
Lemon grass	0.68	0.62	Sweet lemon scented	Bright-yellow
Orange peel	0.85	0.83	Tangy citrus scented	Amber-yellow
<i>Eucalyptus</i>	0.96	0.91	Minty scented	Colourless

Table 3: Mean values of zones of inhibition of essential oils in mm inhibition zones in mm.

Essential Oil	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>	<i>Klebsiella Pneumonia</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>
Ginger (offinale)	18.40	8.12	20.50	12.50	15.60	20.60	26.00
<i>Vitex doniana</i>	12.00	10.60	13.20	14.60	18.60	23.20	21.00
<i>Eucalyptus globulus</i>	25.10	20.20	18.30	18.60	15.00	14.50	16.60
Orange Peel	21.80	19.00	16.00	21.20	16.30	19.30	18.00
Lemon Grass	22.30	22.20	17.40	18.70	17.50	21.60	19.80
Ciprofloxacin	29.00	24.30	21.20	22.10	MD	ND	ND
Fluconazole	ND	ND	ND	ND	28.70	27.20	25.00

ND – Not determined.

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of Essential Oils in mg/ml

Essential Oil		<i>Staphylococcus Aureus</i>	<i>Salmonella typhi</i>	<i>Klebsiella Pneumonia</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>
Ginger	MIC	0.12	0.45	0.82	0.40	0.32	0.36	0.42
	MB	0.20	0.50	0.90	0.45	-	-	-
	C	-	-	-	-	0.40	0.45	0.48
	MF							
	C							
Vitex doniana	MIC	0.15	0.60	0.95	0.45	0.65	0.68	0.50
	MB	0.35	0.70	1.10	0.50	-	-	-
	C	-	-	-	-	0.78	0.80	0.80
	MF							
	C							
E. globulus	MIC	0.29	0.31	0.17	0.22	0.38	0.35	0.26
	MB	0.40	0.35	0.35	0.35	-	-	-
	C	-	-	-	-	0.60	0.40	0.45
	MF							
	C							
Orange Peel	MIC	0.20	0.26	0.30	0.45	0.18	0.40	0.60
	MB	0.25	0.45	0.40	0.50	-	-	-
	C	-	-	-	-	0.22	0.45	0.65
	MF							
	C							
Lemon Grass	MIC	0.26	0.25	0.35	0.40	0.23	0.45	0.50
	MB	0.30	0.30	0.40	0.45	-	-	-
	C	-	-	-	-	0.35	0.50	0.60
	MF							
	C							

Conclusion

The results showed the essential oils possess high inhibitory effects on the seven microorganisms. In comparison Ginger, *V. doniana*, Lemon grass oils showed higher antifungal activity. *E. globulus* and Orange peels showed moderate antibacterial activity. The positive effects exhibited on both gram positive and gram negative bacteria as well as fungi, is an indication that these essential oils are 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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