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# Antibacterial Activity of n-Hexane Extract of *Ocimum gratissimum* leaves

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***Abstract*:** *The antibacterial activity of n-hexane extract of Ocimum gratissimum leaves was investigated against gram negative bacteria ( Salmonella typhi) and gram positive bacteria (Staphylococcus aureus ) . Agar well diffusion and broth dilution methods were employed. The results revealed that both first and second n-hexane extracts (4days and 8 days n-hexane extract respectively) inhibited the microorganisms with the second being more active as it inhibited at 30mg/ml, 40mg/ml, 50mg/ml and 100mg/ml with zone of inhibition ranging from 8.5mm to 15.5mm in diameter. The first extract showed no inhibition at 30mg/ml and 40mg/ml respectively but showed inhibition at 50mg/ml and 100mg/ml with zone of inhibition ranging from 9mm to 12mm in diameter. The Minimum Inhibitory Concentration (MIC) of the second n-hexane extract ranged from 25mg/ml to 100mg/ml whereas, that for the first was at 100mg/ml. The result was compared to conventional drug (Gentamicin 20ug/ml) with zone of inhibition at 20mm in diameter. The Gas Chromatography Mass Spectrometry (GC/MS) identified antibacterial compounds which include Hexadecanoicacid, Phytol, Ricinoleicacid, Dehydrodihydrodiisoeugenol and 9-Octadecenamide. Based on the results it can be concluded that Ocimum gratissimum leaves extract possess antibacterial activity and can be applied in development of potent antibacterial drugs.*

***Keywords:*** *Ocimum gratissimum, n-hexane extract, antibacterial activity, Gas Chromatography Mass Spectrometry (GC/MS).*

## I. Introduction

Diseases are growing more resistant to synthetic drugs leading to researchers finding better alternatives from plant resulting to the comeback of 'green medicine'. *Salmonella typhi* a gram negative, rod shaped motile and anaerobic bacteria from genus Salmonella and family Enterobacteriaceae [1], the causal organism of typhoid fever. There has been resistance of this disease to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and streptomycin [2].*Staphylococcus aureus* gram positive cocci and anaerobic bacteria, causal organism of skin infections, pneumonia and food poisoning.

*Ocimum gratissimum* from the family Lamiacea and genus Ocimum is a perennial herb found in tropical and temperate region. It is the species commonly found in Nigeria. It is well known for its nutritional and medical properties. It has long been used in folkloric medicine to treat fever, skin infections and respiratory tract infections [3]. It is antifungal [4], [5], antibacterial [6], [7], antiviral [8], antileshmanial [9], antitrypanosomal [10], antidiabetic [11], anticonvulsant [12], antioxidant [13]. Phytochemical analysis [14] confirmed the presence of alkaloids, tannins, phenolics, saponins, glycoside, and resins. Steroids and terpenoids were also confirmed to be present in the plant [15]. GC/MS of its essential oil (ocimum oil) [16] showed eugenol to be major component, thymol, germacrene, bisabolene and other volatiles as minor component. This present study is aimed at determining the antibacterial activity of *Ocimum gratissimum* leaves.

## II. Materials And Method

### 2.1 COLLECTION AND EXTRACTION OF PLANT

*Ocimum gratissimum* leaves were collected from a vegetable garden along Zaria road in Jos North area of Plateau State in April 2013. It was identified by Mr Agyeno of Botany Department University of Jos, Plateau state Nigeria. The leaves were rinsed with water and shade dried at room temperature then pulverized. 50g of the powder was extracted for 4 days with 250ml research grade n-hexane with occasional stirring (twice a day). The marc was separated from the solvent by decantation and labeled first n-hexane extract of *Ocimum gratissimum* leaves ($OGH_1E$). Another 250ml of n-hexane was added to the marc and left for another 4 days with occasional stirring then decanted and labeled second n-hexane extract of *Ocimum gratissimum* leaves ($OGH_2E$). Both extracts (first and second n-hexane) were evaporated to constant weight using a steam bath.

### 2.2 ANTIBACTERIAL ASSAY

The microorganisms which were *Salmonella typhi* and *Staphylococcus aureus* were sourced from the Department of Pharmaceutical Science and Technology University of Jos. They were sub cultured in 5ml sterile nutrient broth then incubated at $37^0$C for 24hrs. 0.1ml of the 24hr broth culture was aseptically introduced into a

sterile nutrient agar, 20ml each was then transferred aseptically into petri dishes .Using a 3mm cork borer, holes were bored in each agar plate .The wells were filled with 30mg/ml, 40mg/ml, 50mg/ml and 100mg/ml of the extract dissolved in n-hexane. The plates were then incubated at $37^0$C for 24 hrs. The zone of inhibition of each well was obtained in millimeters. Gentamicin served as the positive control while n-hexane was used as the negative control.

### 2.3 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

This was done by the broth dilution method and turbidity of each tube was observed. The controls contained no microorganism only the extract .The lowest concentration showing no turbidity was the MIC.

### 2.4 DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The MBC was further carried out using the tubes that showed no turbidity and then smearing a loopful on a sterile nutrient agar plate, then incubating at $37^0$C for 24hrs and growth of microorganisms were observed.

### 2.5 CHEMICAL ANALYSIS OF EXTRACT

The n-hexane extract was chemically analyzed using a QP2010 PLUS SHIMADZU, JAPAN Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) instrument under the following conditions : AOC-20i auto injection , fused silica capillary column (RTX-5MS : 30m x 0.25mm I.D . d .f = 0.25µm)which was programmed as follows : flow rate of carrier gas(He) was 1ml/min , injection port temperature $200^0$C, initial temperature of column being $50^0$C increased and kept constant at $300^0$C for 9mins with heating rate of $8^0$C/min.The volume injected was 1µl with split mode. Identification of the extract constituents was made based on retention times and by comparison of mass spectra with the computer search using NIST05 library of mass spectral data.

## III. Result

The percentage yields of first and second n-hexane extract were 3% and 2% respectively. Antibacterial activity showed that n-hexane extract had zones of inhibition between 9 – 12 mm for the first extract (Table 1) and 8.5 – 15.5mm for the second extract (Table 4). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the first and second extracts are as shown in Tables 2, 3, 5 and 6. The chromatograms for $OGH_1E$ and $OGH_2E$ are shown in figures 1 and 2a respectively. The mass spectra of compounds responsible for antibacterial activity are presented in figures $2a_1$ to $2a_5$.

**Table 1: Antibacterial activity of first n-hexane extract of *Ocimum gratissimum* leaves ($OGH_1E$).**

| | Zones of inhibition of $OGH_1E$ (mm) / Concentration (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| Microorganisms | 30 | 40 | 50 | 100 | Gentamicin (20ug/ml) | n-Hexane |
| *Salmonella typhi* | - | - | 11 | 12 | 20 | - |
| *Staphylococcus aureus* | - | - | 9 | 12 | 20 | - |

- = No inhibition

**Table 2: Minimum Inhibitory Concentration (MIC) of the first n-hexane extract of *Ocimum gratissimum* leaves.**

| | MIC / Concentration (mg/ml) | | | | |
|---|---|---|---|---|---|
| Microorganisms | 6.125 | 12.5 | 25 | 50 | 100 |
| *Salmonella typhi* | + | + | + | + | - |
| *Staphylococcus aureus* | + | + | + | + | + |

\+ = Turbidity
\- = No turbidity

**Table 3: Minimum Bactericidal Concentration (MBC) of the first n-hexane extract of *Ocimum gratissimum* leaves.**

| | MBC / Concentration (mg/ml) | | | | |
|---|---|---|---|---|---|
| Microorganisms | 6.125 | 12.5 | 25 | 50 | 100 |
| *Salmonella typhi* | N.T | N.T | N.T | N.T | + |
| *Staphylococcus aureus* | N.T | + | + | + | + |

\+ = Growth
N.T = Not tested

**Table 4: Antibacterial activity of second n-hexane extract of *Ocimum gratissimum* leaves ($OGH_2E$).**

| | Zones of inhibition of $OGH_2E$ (mm) / Concentration (mg/ml) | | | | | |
|---|---|---|---|---|---|---|
| Microorganisms | 30 | 40 | 50 | 100 | Gentamicin (20ug/ml) | n-Hexane |
| *Salmonella typhi* | 9 | 10.5 | 12 | 15.5 | 20 | - |
| *Staphylococcus aureus* | 8.5 | 10 | 11 | 12 | 20 | - |

- = No inhibition

**Table 5: Minimum Inhibitory Concentration (MIC) of second n-hexane extract of *Ocimum gratissimum* leaves.**

| | MIC / Concentration (mg/ml) | | | | |
|---|---|---|---|---|---|
| Microorganisms | 6.125 | 12.5 | 25 | 50 | 100 |
| *Salmonella typhi* | + | + | + | - | - |
| *Staphylococcus aureus* | + | + | - | - | - |

+ = Turbidity
- = No turbidity

**Table 6: Minimum Bactericidal Concentration (MBC) of second n-hexane extract of *Ocimum gratissimum* leaves.**

| | MBC / Concentration (mg/ml) | | | | |
|---|---|---|---|---|---|
| Microorganisms | 6.125 | 12.5 | 25 | 50 | 100 |
| *Salmonella typhi* | N.T | N.T | N.T | - | - |
| *Staphylococcus aureus* | N.T | N.T | + | - | - |

- = No growth
+ = Growth
N.T = Not tested

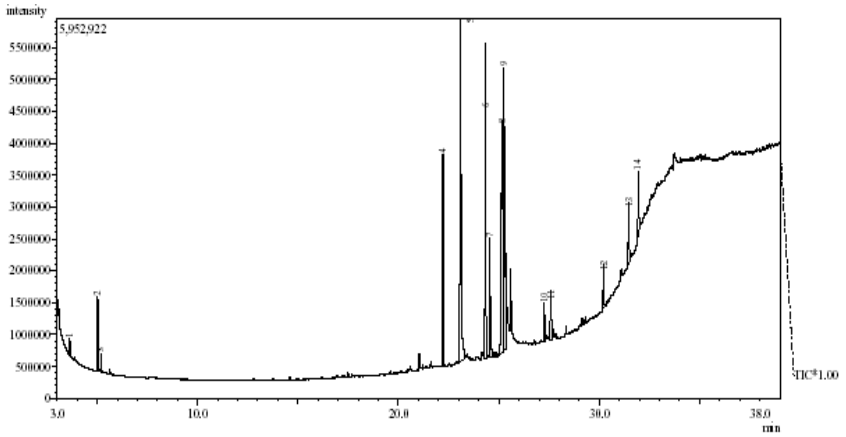



**Figure 1: Chromatogram of $OGH_1E$**

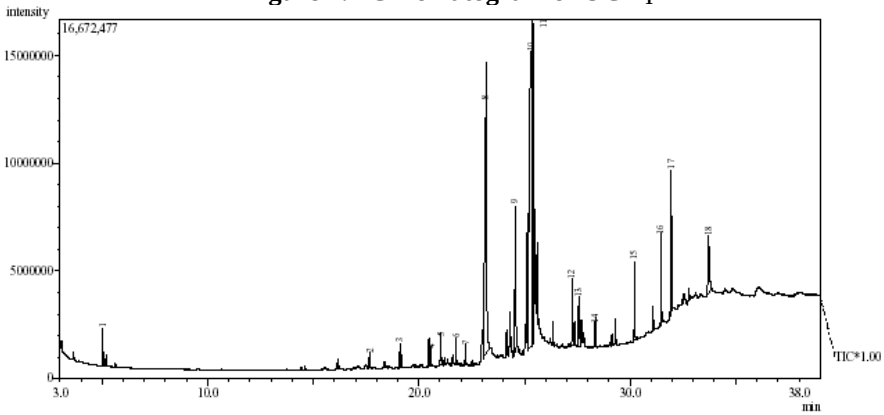
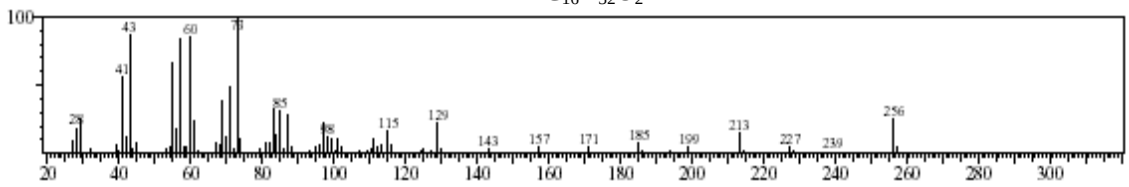



**Figure 2a: Chromatogram of $OGH_2E$**

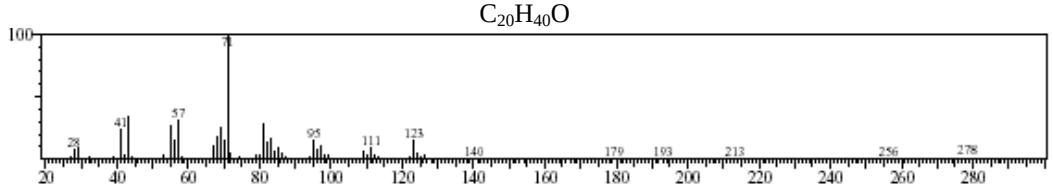
R.T 23.108min Hexadecanoic acid

$C_{16}H_{32}O_2$



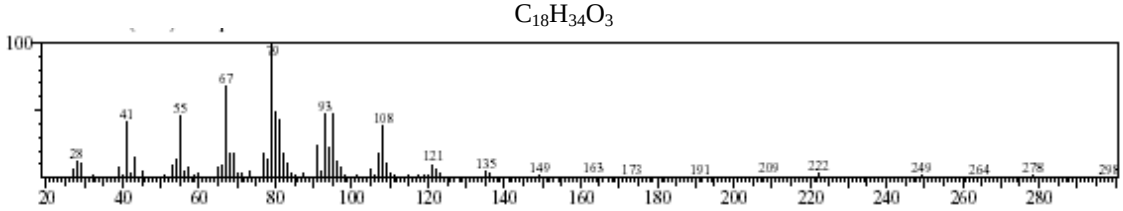
R.T 24.558min Phytol

$C_{20}H_{40}O$



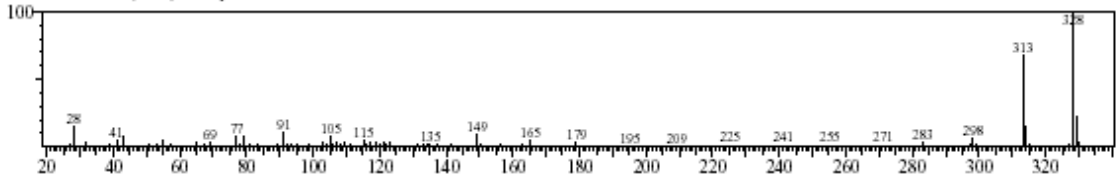
R.T 25.400min Ricinoleic acid

$C_{18}H_{34}O_3$



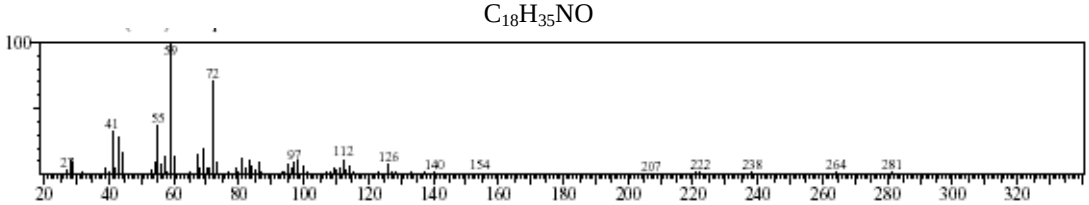
R.T 27.258min Dehydrodihydrodiisoeugenol

$C_{20}H_{24}O_4$



R.T 27.575min 9-Octadecenamide

$C_{18}H_{35}NO$



**Figures $2a_1$ to $2a_5$: Mass spectra of compounds responsible for antibacterial activity according to their retention times (R.T).**

## IV. Discussion

The present study showed that the n-hexane extract of *Ocimum gratissimum* leaves inhibited the growth of both microorganisms. It can also be seen that the extract was both bacterostatic and bactericidal.

From chromatogram of $OGH_1E$, Peak 5 which is the highest is hexadecanoic acid with R.T 23.108min.Peak 7 is phytol with R.T 24.550min. Dehydrodihydrodiisoeugenol is peak 10 with R.T 27.267min. 9-Octadecenamide is peak 11 with R.T 27.600min. From chromatogram of $OGH_2E$, peak 8 is hexadecanoic acid also in high proportion with R.T 23.175min. Peak 9 is phytol higher than that in $OGH_1E$ with R.T 24.558min. Peak 11 being the highest peak is ricinoleic acid with R.T 25.400min. Peak 12 is dehydrodihydrodiisoeugenol higher than that in $OGH_1E$ with R.T 27.258. 9-octadecenamide is peak 13 with R.T 27.575min.From the chromatograms it can be inferred that the retention times of the compounds are similar and increase in dehydrodihydrodiisoeugenol, phytol and especially the presence of ricinoleic acid in $OGH_2E$ lead to increase in antibacterial activity and inhibition of bacterial strains at low concentrations. Hexadecanoic acid has been shown to be a potent antibacterial compound [17], [18]. Phytol has been shown to possess activity against *Salmonella typhi* and resistant gonorrhea [19]. Eugenol and isoeugenol has also been observed to possess antibacterial activity [20], so dehydrodihydrodiisoeugenol being a phenolic compound could exhibit certain antibacterial activity. Ricinoleic acid being the major component of castor oil also possesses antibacterial and antifungal properties [21].

## V. Conclusion

The data analysis of this work has shown *Ocimum gratissimum* to be effective against gram positive and gram negative strains of bacteria making it a broad spectrum antibacterial drug and provides the possibility of treating various bacterial infections by applying it in production of pharmaceuticals.

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