An Evaluation of the Chemical Compositions and Antifungal Activity of *Ocimum gratissimum* (Nchuanwu) leaves against some Plant Pathogens

Abstract

Aim: This work was carried out to determine the chemical compositions of *Ocimum gratissimum* leaves using GC-MS and its antifungal potential against some plant pathogenic fungi.
Study Design: The study was designed to determine its chemical compositions by GC-MS and to test the inhibitory ability of the plant extract on plant pathogens.
Place and Duration of Study: Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri and Department of Plant Science and Biotechnology, Imo State University,Owerri, Nigeria, between February toJuly2017.
Methodology: The leaf ethanol extract of *Ocimum gratissimum* was evaluated using GC-MS to determine the chemical compositions of the plant. The identification of compounds was done by comparing spectrum of the unknown component with the spectrum of the known components stored in the NIST library. The essential oil of the plant was used to analyze the antifungal potential of the plant. This was done against some plant pathogenic fungi using disc diffusion method and MIC using broth micro dilution method. **Results:** The GC-MS analysis revealed eight compounds with n- Hexadecanoic acid constituting the bulk of the oil (37.21 %), followed by Oleic acid (25.38 %) and Octadecanoic acid (16.19 %). Other compounds present in the plant are Glycyl alcohol (2.47 %), Methyl alpha –D- Glucopyranoside (8.33 %), Tetradecanoic acid (5.77 %), Palmitic amide (2.72 %) and d-Glucose, 2,3- diethyl-4,5-dithioacetyl (1.93 %). *Ocimum gratissimum* exhibited different degrees of antifungal activity against the mycelial growth of *Aspergillus niger, Botryodiploidia theobromae, Rhizopus stolonifer, Penicillium expansum* and *Colletotrichum spp* and *Fusarium oxysporium*. The maximum percentage degree inhibition of *Ocimum gratissimum* oil was observed on *A.niger* at different concentrations while the least inhibition was observed in *Colletotrichum spp* at different concentrations.

Analysis of some of the compounds found in *Ocimum gratissimum* such as Methyl alpha.-d-glucopyranoside, Oleic acid etc, reveals the rich pharmacological potential of this medicinal plant and the inhibitory potential of the plant against fungi justify the use of *Ocimums gratissimum* as a medicine traditionally.

Key words: *Ocimum gratissimum*, pharmacological activities, fungal growth

Introduction

Nigeria is blessed with several medicinal plants and *Ocimum gratissimum* plant is one of the medicinal plants used widely in herbal medicine and as spice in many delicacies. *Ocimum gratissimum* also called nchuanwu or scent leaves hails from Africa and is found throughout Hawaii and other tropical regions, it has many health benefits. It belongs to *Lamiaceae* family

and is widely known as clove basil or African basil, this plant is used by herbalists to treat a variety of diseases, from bacterial infections and diabetes to pain and liver damage [1]. *Ocimum gratissimum* is a herb used in making anti-bacterial medicines. It is a home grown plant and is also commercially cultivated .

The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhoea, headache, ophthalmic, skin diseases, pneumonia, cough fever and conjunctivitis [2]., *O. gratissimum* has been reported to be active against several species of bacteria and fungi [3].

Several studies have confirmed the efficacy of essential oil from *Ocimum gratissimum* in treating various diseases. This is largely credited to the plant's high concentrations of a phenylpropene compound called eugenol. Eugenol, an isolate from *O. gratissimum* has been reported to possess insecticidal properties, nematicidal and antihelminthic properties [4]

The antibacterial qualities of *Ocimum gratissimum* are perhaps the most studied and verified. Several studies have been performed that lend credence to herbalist use of this plant for treating diarrhea and other gastrointestinal infections. It was found that the leaf extract provided relief from diarrhea in lab rats and guinea pigs. It was found that the essential oil relaxed the small intestine in lab rats, furthering claims that the plant is beneficial in relieving gastrointestinal ailments.

Studies have shown that essential oil obtained from the leaf of *Ocimum gratissimum* has shown marked antibacterial activity [5]

These range from *Shigella* and *Salmonella* to *Escherichia* and *Proteus strains.* The oil is aromatic, yet deadly, it is used as mosquito repellant [6]; [7]. A polyherbal preparation of a water extract obtained from the leaves of *Ocimum gratissimum* showed analgesic activity [8]. Extracts of the leaves are documented to possess antidiabetic properties [9], anti-hyperlipidemic effect and recently, it was shown to improve heamatological variables in experimental diabetes mellitus and it has antioxidant property [10].

In spite of the rich pharmacological potential of *Ocimum gratissimum*, so far the chemical constituents of the plant have not been fully documented, hence this study.

Materials and methods

Sample Collection / Preparation of Plants material. Fresh leaves of *Ocimum gratissimum* were collected from farm in Owerri Municipal council. The plant was identified and authenticated by Prof F.N Mbagwu, Department of Plant science and biotechnology, Imo State University, Owerri, Nigeria. The leaves were washed, allowed to drain, then pounded with mortar and pestle. The pounded leaves were soaked in ethanol for 48 hours and concentrated, 1ml of the extract was subjected to GC/MS analysis.

Experimental Procedure of Gas Chromatography – Mass Spectrometry (GC-MS).

The GC analysis were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV- 101) coated with polymethyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80- 200^{o}C held at 80^{o}Cfor 1 minute, rate 5^{o}C/min and at 200^{o}C for 20 min. FID temperature 300^{o}C, injection temperature of 250^{o}C and carrier gas nitrogen at a flow of 1ml /min, split ratio 1:75. GC- MS analysis was conducted using GCMS- QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 230^{o}C and carrier gas pressure of 100 Kpa. The column length was 30m with a diameter of 0.25mm and the flow rate of 50ml/min. the elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all analytical grade and were procured from MERCK, GERMANY [11].

Component Identification: Oil components were identified by matching the peaks with Computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks with those from literature [11].

Experimental Procedure of Antifungal Activity

Isolation of Essential oils. Fresh leaves of *Ocimum gratissimum* were subjected to hydro distillation using clevenger's apparatus for 8 hours. The distillate was extracted using diethyl ether and dried over anhydrous sodium sulphate. Antifungal activity of the essential oil was

performed using disc diffusion method as described by [12] the oil was added acetone and serial dilution was made to obtain a concentrations 1000, 750, 500, 250µg/ml. respectively.

Isolation and Culturing of the Pathogenic Fungi.

Following the procedures of [13], the fungi isolates were obtained from dried and sterized rotted yam discs (2x2mm) and cultured on potato dextrose agar (PDA) and incubated at 30^{o}C for 5days. About 3mm of each fungal culture were placed on the centre of sterilized Petri dish containing PDA. Then 10ml of each concentration of *Ocimum gratissimum* oil was placed inside each sterile paper disc (6mm diameter) and then placed on the PDA containing the fungi culture. Synthetic antifungal chemical, mancozeb acted as control. All the Petri dishes in 3 replications were incubated at 30^{o}C for 5days and monitor for growth inhibition.

$$\text{Percentage inhibition} = 100 \times \left[\frac{(1-\text{radial growth of treatment (mm)}}{\text{Radial growth of control (mm)}}\right.$$

Determination of minimum inhibitory concentration (MIC)

This is described as the lowest concentration of the oil that reduced the growth of fungus. It was done by broth dilution technique by following the procedure of [14].

The essential oil was added acetone to make 1000µg/ml. Serial dilution was made to obtain concentrations of 125µg/ml, 250µg/ml, 500µg/ml, 750µg/ml, 1000µg/ml. Then 1ml of the essential oil and 10µl spore suspension (80 spores /ml) of each fungus was inoculated in the test tubes in potato dextrose broth medium and incubated for 5days at 30^{o}C. The control tubes contained PDA medium that were separately added 0.3g/ml mancozeb. Each was inoculated with different fungal spore suspensions (80 spores/ml).

The data collected were subjected to statistical analysis using analysis of variance (ANOVA) method according to Duncan multiple range test (DMRT) and treatment means were separated

using fishers least significant difference (LSD) at 5% level of propability, using statistical package for social science (SPSS) software, version 11.5, Chicago. IL. USA.

Results and Discussion

Table1; Percentage inhibitions of fungal pathogens, 5 days after inoculation with *Ocimum gratissimum* oil and Mancozeb, incubated at 30^0 C and their MIC values.

	Concs. of *Ocimium* oil (µg/ml**)**					
Fungal Pathogens	250	500	750	1000	Mancozeb 0.3g/100ml	MIC µg/ml
Aspergillus niger	60±2.01	84 ± 1.01	98 ± 0.01	100 ± 0.02	100 ± 0.23	34 ± 0.03
B. theobromae	40 ± 0.40	60 ± 0.31	75 ± 0.31	100 ± 0.07	100 ± 0.01	41.20± 0.01
R.stolonifer	37 ± 0.71	54 ± 0.4	68 ± 0.05	100 ± 0.01	95 ± 0.21	55 ± 0.25
Penicillium expansum	38 ± 1.01	50 ± 0.02	60 ± 0.11	98 ± 0.41	100 ± 0.31	37 ± 0.02
Colletotrichum spp.	23 ± 0.01	37±0.51	44 ±0.41	70 ± 0.21	100 ± 0.04	70 ± 0.01
F.oxysporium	48 ± 0.01	56 ±1.01	60 ± 0.01	100 ± 0.61	100 ± 0.31	38 ± 0.04

N.B: Values in brackets are the standard errors of treatments

The ethanol extracts of *Ocimum gratissimum* leaves contain rich phytochemical constituents which resulted in the identification of eight different compounds by GC/MS analysis. The chromatogram of the GC/MS analysis is given in figure 1. The individual names of compounds identified. Compounds revealed include n- Hexadecanoic acid constituting the bulk of the oil (37.21 %), followed by Oleic acid (25.38 %) and Octadecanoic acid (16.19 %). Other compounds present in the plant are Glycyl alcohol (2.47 %), Methyl alpha –D- Glucopyranoside (8.33 %), Tetradecanoic acid (5.77 %), Palmitic amide (2.72 %) and d-Glucose, 2,3- diethyl-4,5-dithioacetyl (1.93 %).

Oleic acid is used as emollients, small amount of oleic acid is used as an excipient in pharmacy, and consumption of oleate in olive oil has been associated with a decreased risk of breast cancer and reduction of blood pressure [15] in [13].

n-Hexadecanoic acid was also found to be present in *Ocimum gratissimum.* In India, medicated oils rich in n-Hexadecanoic acid are used in the treatment of rheumatism and inflammation [16]. Ethyl alpha.-d-glucopyranoside has antituberculous activity, antioxidant alpha amylase inhibitory activity, Hypolipemic activity, Anticonvulsant [17].

This result differs from the result of the analysis carried out by [1] and [18]. According to them, the Phytochemical screening of the aqueous extract of *Ocimum gratissimum* revealed the presence of many active ingredients, such as flavonoids, triterpenes, alkaloids, citral, saponins, eugenol, linaol, methyl cinnamate, camphor, and thymol.

The results of antifungal activity of *Ocimum gratissimum is shown in* Table 1. Different concentrations of the essential oil from *O.gratissimum* exhibited different degrees of antifungal activity against the mycelial growth of *Aspergillus niger, Botryodiploidia theobromae Rhizopus stolonifer, Fusarium oxysporium, Penicillium expansum* and *Colletotrichum spp.* The maximum percentage degree inhibition of *Ocimum gratissimum* oil was observed on *A.niger* at different concentrations while the least inhibition was observed in *Colletotrichum spp* at different concentrations. *A. niger* exhibited least MIC value (34 µg/ml), this is followed by *Fusarium oxysporium* (38 µg/ml) while the highest MIC value was seen in *Colletotrichum spp* (70 µg/ml).

Synthetic antifungal chemical (Mancozeb) compared favourably with *O.gratissimum* oil in inhibiting the mycelial growth of all the fungal plant pathogens.

This result agrees with the report of [18] who reported that *O. gratissimum* is among important plants whose extracts are capable of checking the spread of many fungal diseases of food crops such as *R. stolonifer, F. culmorum, S. Sclerotiarum* and *P. expanum* associated with the post harvest decay of carrots, in vitro., *Ocimum gratissimum* was reported to inhibit the growth of S*taphylococcus aureus, Escherichia coli, Salmonella typhi and Salmonella typhimurium,* pathogenic bacteria that cause diarrhea. and the minimum inhibitory concentration (MIC) ranged from 0.1% for *S. aureus* to 0.01% for *E. coli* and *S. typhimurium*, and 0.001% for *S. typhi*. [20].

O. gratissimum leaf extract effectively protected maize seeds from seed borne infection of *Fusarium moniliforme* and completely inhibited conidial germination of *Mycosphaerella fijiensis* that cause sigatoka disease of banana [21]. Also [19] reported that crude extracts of *O. gratissimum* effectively exhibited antifungal activity on *Cercospora arachidicola*, the causal organism of leaf spot disease of groundnut.

Conclusion

This study revealed that ethanol extract of *Ocimum gratissimum* contains compounds that can be used to treat different diseases. It exhibited different degrees of antifungal activity against some plant pathogenic fungi. Hence the oil might be used as natural antifungal agents replacing synthetic fungicides for the control of some fungal plant pathogens.

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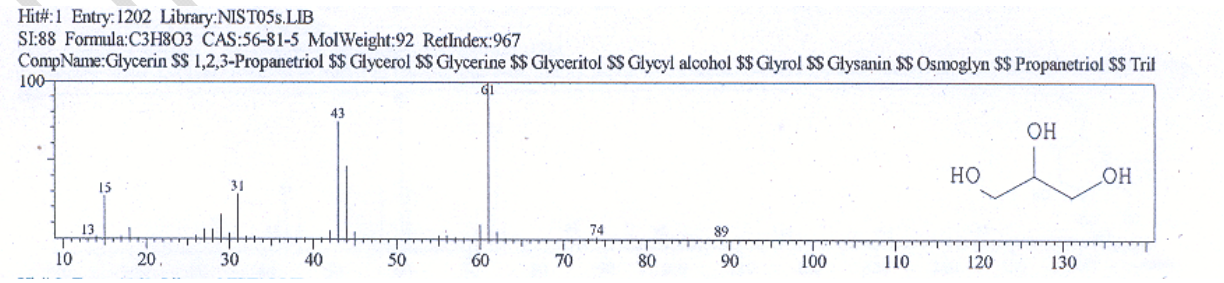

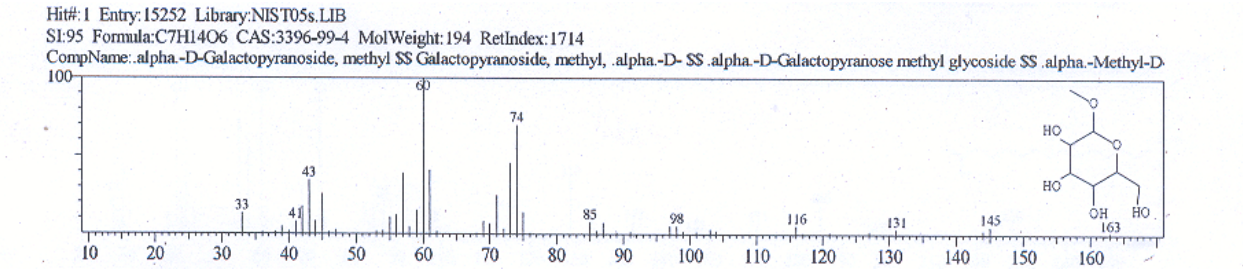

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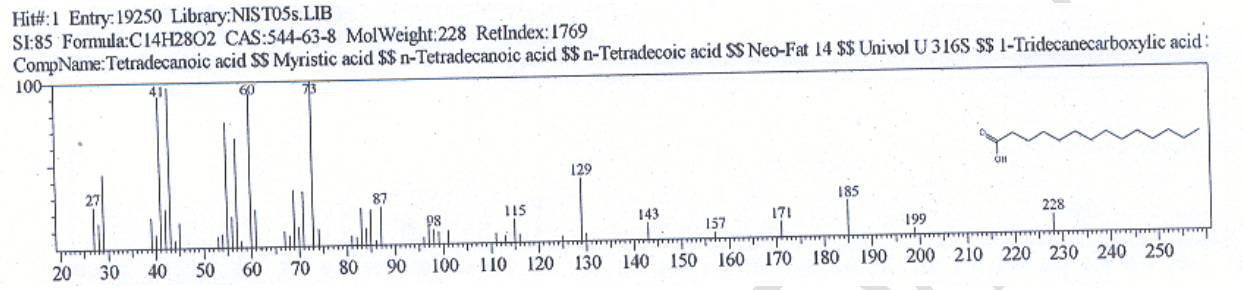

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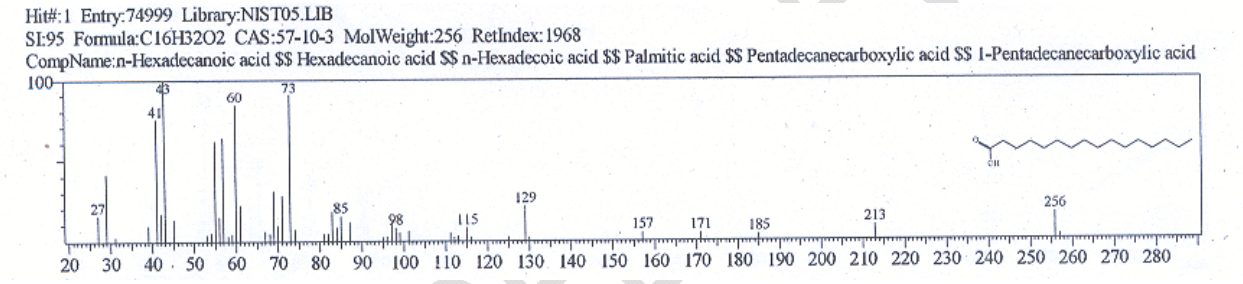

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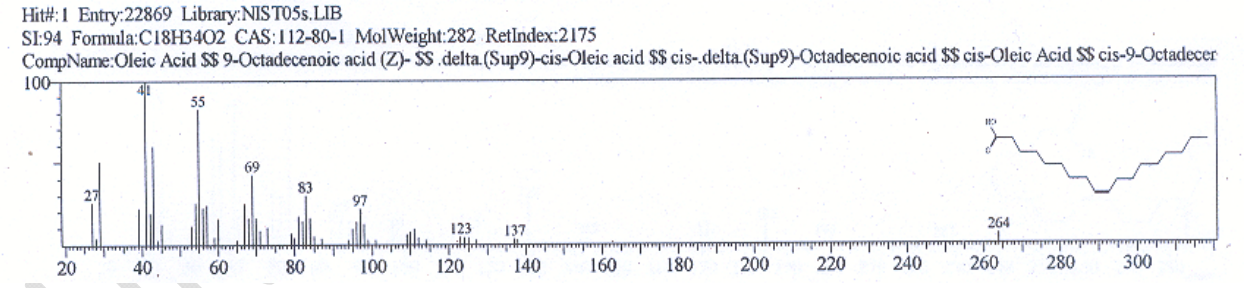

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184 Fig 1: Structures of some of the compounds obtained from GC-MS Analysis of *Ocimum*
185 *gratissimum*

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Authors' contributions

This work was carried out in collaboration between all authors.

Author RIU designed the study, performed the chemical composition, wrote the protocol, and wrote the first draft of the manuscript.

Author JNA determined the fungi associated with deterioration and wrote the second draft of the manuscript

Author CES did the analysis

The work is an Original Research Article