Chemical Composition and Antifungal Activity of *Ocimum gratissimum* (Nchuanwu) leaves Against some Plant Pathogens

Abstract

Aim: This work was carried out to determine the chemical composition of *Ocimum gratissimum* ~~leaves~~ leaf ethanolic extract 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:** *Ocimum gratissimum* ~~leaves~~ leaf etholoic extract ~~collected from Owerri Municipal council~~ was evaluated ~~for chemical composition~~ using GC-MS . The chemicals from *Ocimum gratissimum* leaves harvested from Owerri…… were extracted with ethanol and subjected to GC/MS analysis. 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~~.~~ and its antifungal potential 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 %). *O.* ~~–~~*cimum 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. This study justifies the use of *O. gratissimum* as a medicine traditionally.

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

Introduction

Nigeria is known for its rich traditional value and leaves of *Ocimum gratissimum*~~, is~~are one of the medicinal plants used widely in herbal medicine and as spice in many delicacies. *Ocimum gratissimum*, which hails from Africa and grows throughout Hawaii and other tropical regions, has reputed health benefits. It is widely known as clove basil or African basil, this plant is used by herbalists to treat a variety of ~~maladies~~diseases, from bacterial infections and diabetes to pain and liver damage. *Ocimum gratissimum* is an herb used in making anti-bacterial medicines. It

belongs to *Lamiaceae* family. It is a home grown plant and is also commercially cultivated. The plant bears essential oil in its leaves and stems which is extracted to make several medicinal substitutes. The herbs possess qualities greatly beneficial for the health. The plant extracts can be used in relaxing intestinal muscles. The herbaceous plant has anti-nociceptive effects. It is effective in reducing blood glucose. It can reduce diabetes. It is helpful in preventing convulsions and seizures.

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 (Onajobi, 1986)., *Ocimum gratissimum* has been reported to be active against several species of bacteria and fungi (Nakaruma *et al.*, 1999).

Several studies have confirmed the efficacy of *Ocimum gratissimum* in treating various conditions after it is condensed into an essential oil. This is largely credited to the plant's high concentrations of a phenylpropene compound called eugenol. 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 (author if any). 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(author).

Studies suggest that *Ocimum gratissimum* effectively combats several types of invasive bacteria. These range from *Shigella* and *Salmonella* to *E*scherichia and *Proteus strains(author)*. The oils of the plant also were effective in fighting strains of *E. coli*, dysentery and typhoid(author). The oil is aromatic, yet deadly, it is used as mosquito repellant. Phytocemical screening of this plant has revealed the presence of many active ingredients, such as flavonoids, triterpenes, alkaloids, citral, saponins, eugenol, linaolol, methyl cinnamate, camphor, and thymol (authors). Eugenol, an isolate from *O gratissimum* has been observed to possess antihelminthic, nematicidal, and insecticidal properties (authors). The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity (Lamiaceae- Silva *et al*,(2010); Nweze E.I. and Eze E.E. (2009). A polyherbal preparation of a water extract obtained from the leaves of *Ocimum*

gratissimum showed analgesic activity (Oboh *et al*, 2009). The essential oil has potential for use as a food preservative, and is toxic to *Leishmania* (Nguefack *et al*, 2009). Extracts of the leaves are documented to possess antidiabetic properties (Aguiyi *et al*, 2000) anti-hyperlipidemic effect and ~~recently~~, it was shown to improve heamatological variables in experimental diabetes mellitus via its well reported antioxidant property (Egesie *et al*, 2006).

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 taxonomically identified and authenticated by Prof F.N Mbagwu (plant taxonomist)(The certification number from the National Herbarium of the State concerned), 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; ~~,~~ 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 chromatograph~~y~~ 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.

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 (Uchegbu *et al*, 2014).

Comment [AH1]: How do you get oil with ethanol extraction?

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 (Murray *et al*, 1995): the essential 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 procedure of (Uchegbu *et al*, 2017). The fungi isolates were obtained from dried and sterilized 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.

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

Determination of minimum inhibitory concentration (MIC)

This is described as the lowest concentration of the oil that reduced the growth of fungus and was done by broth dilution technique by following the procedure of (Gulluce *et al*; 2004).

The essential oil was added acetone to make 1000µg/ml. serial dilution was made to obtain concentrations of 125ng/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 contain PDA medium that were separately added 0.3g/ml mancozeb each were inoculated with different fungal spores 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	100	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
B. theobromae	40 + 0.40	60 + 0.31	75 + 0.31	100 + 0.07	100 + 0.01	41.20
R.stolonifer	37 + 0.71	54 + 0.4	68 + 0.05	100 + 0.01	95 + 0.21	55
Penicillium expansum	38 + 1.01	50 + 0.02	60 + 0.11	98 + 0.41	100(+0.31)	37
Colletotrichum spp.	23 + 0.01	37(+0.51)	44(+0.41)	70(+0.21)	100 + 0.04	70

Comment [AH2]: 100µg or 1000µg?

F.oxysporium	48 ± 0.01	56(±1.01)	60(±0.01)	100(±0.61)	100 ± 0.31	38

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

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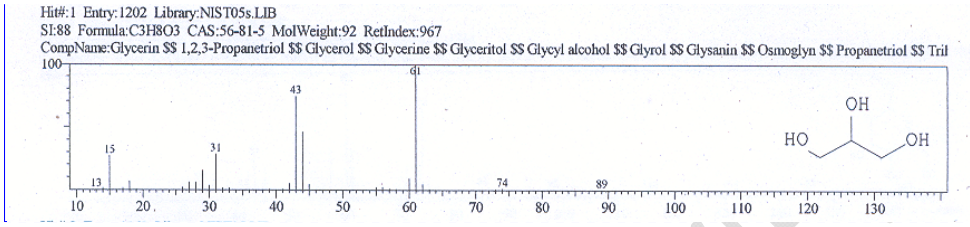

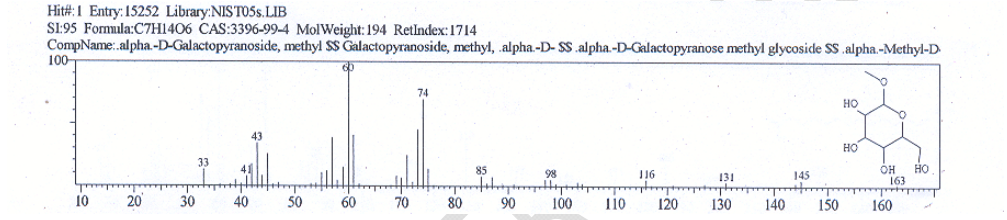

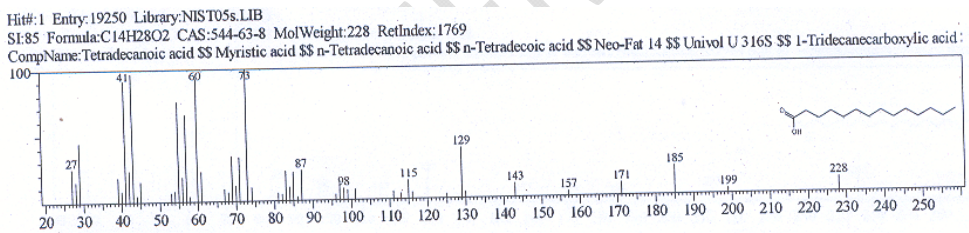

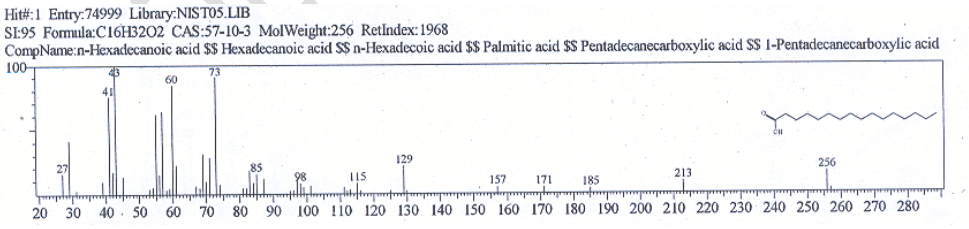

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Comment [AH4]: These curves could if necessary be added as annexes. Provide a table please.

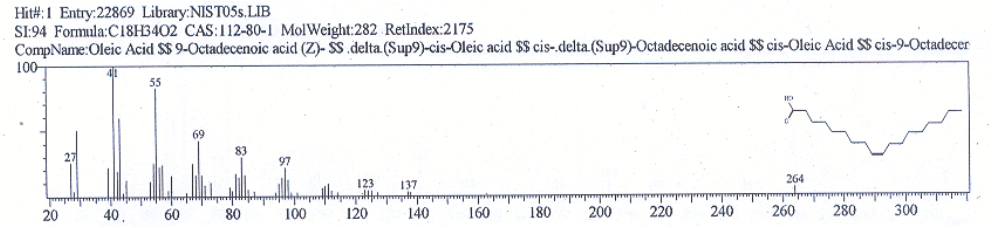

Fig 1: Structures of some of the compounds obtained from GC-MS Analysis of *Ocimum gratissimum*

The ethanol extracts of *Ocimum gratissimum* leaves contains rich phytochemical constituents which resulted in the identification of eight different compounds by GC/MS analysis. The chromatograms 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 (Teras *et al*, 2008 in Uchegbu *et al*, 2017).
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 (Aparna *et al* 2012). Ethyl .alpha.-d-glucopyranoside has antituberculous activity, antioxidant ,alpha amylase inhibitory activity, Hypolipemic activity, Anticonvulsant (Rane Zab *et al*, 2012).

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 is consistent with the report 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. Okoi and Afuo, 2009), *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*. (Adebolu and Salau, 2005).

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 (Okigbo and Emoghene, 2003). Also Okoi and Afuo, (2009) 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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