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RESEARCH ARTICLE

ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *OCIMUM SUAVE* WILD [FAM. LABAIATAE] USING HYDRO – DISTILLATION AND SOLVENT EXTRATION METHODS

Izuagbe Gilbert. Osigbemhe* and Osaro Iyekowa

Department of Chemistry, Faculty of Physical Science, University of Benin, P.M.B 1154, Benin City, Nigeria
****Corresponding Author Email: gilbert.osigbemhe@uniben.edu***

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ABSTRACT

Ocimum suave aerial parts were acquired in Nigeria's Nassarawa State. Following that, the essential oil was extracted utilizing solvent extraction and hydro distillation techniques. The volatile constituents were identified by gas chromatography using the direct injection method. Staphylococcus aureus, Bacillus subtilis, streptococcus pneumoniae, pseudomonas aeruginosa, and E. coli were used to test certain volatile constituents for minimum bactericidal concentration (MBC) and minimum inhibition concentration (MIC). In Ocimum suave, forty-three compounds of terpenoids were identified with geranial (34.44, 36.16%), neral (32.67, 26.14%), β-caryophyllene (8.77, 10.15%), Linalool (6.54, and. 05%), allo-ocimene (4.23, 2.92%), Linalyl acetate (1.90, 4.85%), nerol (1.53, 2.06), geraniol (1.24, 1.35%) and β-bisabolene (1.17, 1.79%) respectively as the major constituents for hydro distillation and solvent extraction methods. The results of the extraction generally showed that hydro-distillation is better extraction method, and is also cheaper economically. The volatile constituents showed more some measure of activity against pathogens used.

KEYWORDS

Hydro-distillation, extraction, terpene, terpenoid, antimicrobial activity

1. INTRODUCTION

The use of plant derivative for bioactivity such as antiseptics, repellence, toxicity, antifeedant, preservative, Inhibition against pathogens etc. Was common in the tropics and other parts of world before the advent of synthetic chemicals. Additionally, attempts to describe these characteristics in a lab setting date back to the early 1990s (Martindale, 1910, Hoffman and Evans, 1911). Numerous plant species are being thoroughly studied and tested for their pharmacological qualities. The most significant suggested areas of essential oil use are in urology, dermatology, sleep and nervous disorders, laxatives, erosive gastritis, cardiac and vascular systems, immune modulating drugs, cold and cough, and more. The oils are put to the test in behavioral pharmacological trials, and their effects are tracked for any indications of toxicity as well as for use under a variety of settings. These ailments include tumor growth, stomach ulcer development, and disorders linked to stress. In addition, their effects on blood circulation, neurological system development, liver, protein, lipid, carbohydrate, and cholesterol metabolism, as well as any effects on the immune system and adrenal gland function, are tracked. The volatile oil from "Houttuynia cordate" was investigated for its antiviral properties against influenza, Hv-1, and the herpes simplex virus (Harrigan et al., 1994). It was proposed that the oil's antiviral properties might result from its interference with the virus envelope. Essential oil from various plants of the genus "Heracleum" had encouraging efficacy against influenza virus in another experiment (Dorman and Deans, 2000). There have also been reports of anticancer activity investigations (Shasany et al., 2000; Golob and Webley, 1980). The authors evaluated several cancer cell lines using cellular polypeptide assays and enzymes. It is possible to think about Ocimum suave volatile oil, Cyminum, Papever sammiferum, and Nigella salica as anti-carcinogenic agents. A thorough investigation of the antioxidant properties of plant essential oils was conducted in order to examine their potential to protect animal tissue's highly unsaturated lipids (Ayedoun et al., 1996). In aging hepatoprotective mammals, the oil has demonstrated its positive effects on PUFAs, namely the long chain C_{20} to C_{200} acids (DHA) levels in the retinas of aged rodents.

The reason antioxidants are important for human physical well-being is because metabolic activity can change oxygen into more reactive forms like superoxide hydroxide peroxide, singlet oxygen, and hydroxyl radicals—collectively known as active oxygen. These chemicals are produced by various metabolic mechanisms in living cells, and the generation of free radicals is aided by certain chemicals, the contamination from burning fossil fuels and tobacco smoke, radiation, and pollutants like carbon dioxide. One enzyme, superoxide dismultase, can potentially pass through all cellular membranes and convert superoxide into H2O2.These compounds are thought to be responsible for a large amount of damage because they can change into highly reactive oxidants, such as the hydroxyl radical. It takes traces of catalytic metal ions, primarily iron and copper, to form OH from O_2. The ability of the copper ion/H_2O_2 system to cause damage to DNA and proteins is well known. Since the hydroxyl radical reacts with practically every molecule present in living cells, it is extremely reactive. Oxizable substrates in live cells include proteins, lipids, carbohydrates, and DNA. Apoptosis stimulation, lysosomal instability, and modifications to membrane structure, permeability, and fluidity are examples of secondary events. Superoxide and hydrogen peroxide can stimulate growth in a variety of malignant mammalian cell types, and they may play a significant role as extracellular messengers for cell growth and viability. Other occurrences of oxygen free radicals include lipid peroxidation and fluidity, lysosomal destabilization, and stimulation of apoptosis. Finally, lipid peroxidation results in loss of memory function and integrity, which leads to cell necrosis and death. Hydroxyl radicals can also react with bases in the DNA and cause mutations.

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Many compounds have been suggested to have antioxidant properties in vivo. These include vitamin E in flammatories, beta carotene, albumin, mono acid, oestrogen, polyamines, flavonoids, ascorbic acid, and plant phenolic. By reducing their cytostatic chemicals to tumor cell lines, they can stabilize the membranes and perhaps provide new antiproliferative agents.

Using a thiobarbituric acid (TBA) experiment, they examined *Pelargnium sp. Monarda citriodora var. citriodora, Myristica fragrans*, and *Origanum vilgare ssp hirtum* for their antioxidative properties (Dorman and Deans, 2002). The oils showed potent antioxidant properties even at very low dilution levels. Rosemary has long been known to include antioxidant molecules, which have been identified as rosmarinic acid, carnasol, sarsolic acid, carnasol, and rosmaridiphanol. These compounds are found in ethanol-soluble fractions. The volatile oil fraction also contains antioxidant qualities. It is crucial to remember, though, that in some situations, antioxidants can act as pro-oxidants and even trigger free radical reactions (Dorman and Deans, 2002). This is because of a variety of factors, including genetics, geography, crop and post-harvest processing, crop and post-harvest conditions, and the nutritional status and chemotypes of the plant, among others, that can affect the composition of the oil (Lawless, 1995). Due to their specificity and potential low toxicity to mammals, insect repellents and antifeedants provide hope for protecting stored grain from insect attack (Charles and Simon, 1990). Repellants and antecedents that are more efficient, long-lasting, and cost-effective than currently available synthetics are still needed to help protect stored goods from insect attack. Over 1400 of these chemicals have been identified chemically after being isolated from plants (Lawless, 1995). Some native plants in Pakistan have been used to protect food, wool, and insects from people (Olonisakin et al., 2005). As seen in plate 2.5, *Ocimum suave* wild is an upright, branching shrub that grows up to 1 mm in height. Guinea, West Cameroons, and other parts of Africa, East Africa, and Tropical Asia are typical places to find it. On open waste areas, it is common. In East Africa's upland woodland regions, it is widespread. It is used as traditional medicine to cure influenza, coughing, and stomachaches. Additionally, it is utilized as a grain protectant, perfume, and insect repellent—especially against mosquitoes. When tested in an olfactometer an hour after application, the oil demonstrated repellent qualities against S.zeamais. Mono- and sesquiterpenoids, as well as eugenol, are its main components (Poitou et al., 1996).

2. Materials and Methods

2.1 Sample collection and Identifications

Two plants materials used for this work were collectedfrom Keffi Market, Nassarawa State, Nigeria. The aerial partof Ocimum suave wild [Fam. Labaiatae] was purchased in Keffi market. Following that, botanists from the Faculty of Natural and Applied Sciences at Nassarawa State University in Keffi, Nigeria, and the Faculty of Life Sciences at the University of Benin in Benin City, Nigeria, identified the plant material.

2.2 Extraction of the essential oils

2.2.1 Hydrodistillation

Prior to extraction, To maximize the surface area, fresh aerial sections of the *Ocimum suave* plant were cut into small pieces after being cleaned of sand and other contaminants. Using all-glass Clevenger equipment, 200g of it was hydro-distilled for two hours. After being reduced into powder, the other two plants underwent a 4-hour hydrodistillation process. The resulting oils were placed in a sample bottle and refrigerated until they were ready for analysis after being dried with anhydrous sodium sulphate.

2.2.2 Solvent Extraction

17g of powdered samples of Ocimum suave were packed each into the Soxhlet extractor using petroleum ether as solvent and this was refluxed for about six hours in the Chemistry Department of Nassarawa State University, Keffi. Each of the essential oil obtained from this solvent extraction were steam dried to ensure that little solvent that may possibly be present is driven off through evaporation. The essential oil gotten from each was stored in a refrigerator until ready for use.

2.3 Identification of the Chemical component of the essential oils using Gas Chromatography

The chemical ingredient was identified using gas chromatography with direct injection in the split mode at Multi-Environment Management Consultancy Limited in Lagos, Nigeria, under the following circumstances: The HP Chemstation Rev A09.01(120B)-powered Hewlett Packard 6890 had a quartz capillary column and a flame ionization detector (FID); it measured 30 m x 0.25 mm x 0.25 µmm. The carrier gas was hydrogen, which flowed at a rate of 1.0 ml/min; the initial oven temperature was 40°C. To reach 200°C, ramp at 5°C/min. Do a 2-minute run at 200°C. 150°C for the injection detector and 300°C for the detector. One HP 5MS column type, split ratio, compressed air pressure of 28 psi, and hydrogen pressure of 22 psi. An region of the detector's signal was integrated using a digital integrator. Each peak ended with an automatic printout of the integrate area, retention time, and percentage composition. The retention time and mass spectra of each constituent were compared to the library's in order to qualitatively identify them.

2.4 Antimicrobial Test of the essential oils

The bacteria used in this investigation were generated at the University Benin Teaching Hospital (UBTH), located in Benin City. These included clinical isolates of *Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pneumonia, Escherichia coli, and Staphylococcus aureus.*

2.5 Media

This study made use of nutritional broth and nutrient agar, both of which are manufactured by Himedia Laboratories in Mumbai, India. The medium was made up of 3.0g of beef extracts, 5.0g of peptone, 8.0g of sodium dioxide, and 15g of agar.

2.6 Agar Well Diffusion Assay

The agar well diffusion technique was used to assess the extracts' antibacterial activity. The MBC for nutrients was noted. Each bacterial culture (10^6 CFU/ml) was seeded into 0.1 ml of agar plates overnight. Each bacterium's 24-hour broth culture was seeded on sterile molten agar at 45°C, allowed to set, and then placed in a well-made sterile standard cork borer (6.0 mm in diameter) with 200 ml (0.2 ml) of an extract solution containing 15 mg/ml. Following a 24-hour incubation period at 37°C, the diameter of the inhibitory zones on the plates was measured.

2.7 Determination of Minimum Inhibition Concentration (MIC)

Each extract's MIC values were calculated by preparing concentrations of 50, 25, 12.5, 6.3, 3.2, 1.6, 0.8, 0.4, and 0.2 mg/ml using a two-fold micro-dilution. A drop of bacterial suspension that had been previously diluted to around 10^6 (CFU/ml) was added to molten nutrient agar along with 1 ml of each extract and 10 ml of distilled H2O, and the mixture was let to set. For twenty-four hours, the plates were incubated at 37°C. The MIC for each test organism was determined by taking the lowest concentration that prevented observable growth. For every extract concentration, these tests were conducted in triplicate, with controls against the bacterial isolates. Distilled water served as the negative control, and amoxicillin, streptomycin, gentamycin, and ampicillin served as the positive controls.

2.8 Determination of Minimum Bactericidal Concentration (MBC)

On the base of the nutrient agar plates, each region was labeled with the corresponding concentration. The content of each MIC plated in the corresponding plate sections was plated out using these. Following a 24-hour incubation period at 37°C, the MBC was measured on the plates.

3. Result and Discussion

3.1 Chemical Composition of *Ocimum suave*

The essential oil analysis yielded both qualitative and semi-qualitative results. The constituents of *O. suave* identified by Gas Chromatography analysis are summarized in Table 1 and 2. For the chemical constituent of *Ocimum suave* in table 1 obtained by solvent extraction method, forty-three components were identified with; geranial (36.16%), neral (26.14%), β-caryophyllene (10.15%), linalool (7.05%), linalyl acetate (4.85%), allo ocimene (2.92%), nerol (2.06%), β-bisabolene (1.79%) and geraniol (1.35%). The chemical constituent of *Ocimum suave* in table 2 represent the ones obtained using the hydro-distillation method with geranial (37.4%), neral (32.67%), Linalool (6.54%), beta caryophyllene acetate (1.53%) and beta bisabolene (1.17%) as major terpenoids in the oil.

Table 1 : Identified Chemical Constituents in the essential oil *Ocimum suave* using solvent extraction method

Compound	Retention Time (min)	Concent (%)
Isoamyl acetate	2.95	0.14
Tricyclene	4.13	0.14

Table 1 (cont) : Identified Chemical Constituents in the essential oil *Ocimum suave* using solvent extraction method		
Camphene	4.78	0.14
Sabinene	6.93	0.18
Limonene	7.79	0.33
Alpha Pinene	9.82	0.18
Beta Pinene	11.21	0.14
Benzyl Alcohol	11.44	0.34
Cis Ocimene	12.90	0.33
Myrcene	12.99	0.12
Allo Ocimene	13.19	2.92
Alpha Thujene	14.20	0.16
Gama Terpinene	14.92	0.37
Fenchone	15.11	0.33
Neral	15.29	26.14
Geranial	15.38	36.16
Isoartemisia	16.44	0.14
1,8-cineole	16.54	0.34
Geraniol	17.19	2.06
Nerol	17.44	1.35
Linalool	17.69	7.05
Borneol	17.83	0.29
Alpha Terpineol	18.67	0.15
Terpinen-4-ol	18.77	0.16
Thymyl methyl ether	19.72	0.19
Linalyl Acetate	20.79	0.26
Ethyl Cinnamate	21.39	0.34
Borneol Acetate	21.60	0.42
Linalyl Acetate	21.79	4.85
Beta Bisabolene	21.85	1.79
Beta Caryophyllene	22.42	10.15
Trans-Alpha-Bergamotene	22.89	0.29
Beta Elemene	23.35	0.08
Germacrene D	24.02	0.53
Bicyclogermacrene	24.66	0.27
Alpha Copane	24.78	0.14
Acetyleugenol	26.94	0.12
Elemicin	27.06	0.07
Benzyl benzoate	27.75	0.23
Viridiflorol	29.56	0.14
Torreyol	29.68	0.14
Tetra Decanoic Acid	29.99	0.28
Hexa Decanoic Acid	30.85	0.04

Table 2: Identified Chemical Constituents in the essential oil Ocimum suave using Hydro distillation extraction method		
Compound	**Retention Time (min)**	**Concent (%)**
Isoamyl Acetate	2.96	0.70
Tricyclene	4.14	0.07
Camphene	4.79	0.07
Sabinene	6.95	0.10
Limonene	7.80	0.55
Alpha Pinene	9.82	0.10
Beta Pinene	11.21	0.07
Benzyl Alcohol	11.45	0.18
Cis Ocimene	12.91	0.18
Myrcene	12.99	0.06

Table 2 (cont): Identified Chemical Constituents in the essential oil Ocimum suave using Hydro distillation extraction method		
Allo Ocimene	13.19	4.24
Alpha Thujene	14.21	0.08
Gama Terpinene	14.93	0.20
Fenchone	15.11	0.15
Neral	15.08	32.67
Geranial	15.29	37.44
Isoartemisia	15.43	0.18
1,8-cineole	16.45	0.18
Geraniol	16.54	1.54
Nerol	17.20	1.13
Linalool	17.38	6.54
Borneol	17.70	0.15
Alpha Terpineol	17.84	0.08
Terpinen-4-ol	18.66	0.08
Thymyl methyl ether	18.78	0.10
Linalyl Acetate	20.12	0.14
Ethyl Cinnamate	20.80	0.18
Borneol Acetate	21.40	0.23
Linalyl Acetate	21.61	1.90
Beta Caryophyllene	21.86	8.77
Trans-Alpha-Bergamotene	22.42	0.15
Beta Elemene	22.85	0.04
Germacrene D	23.34	0.18
Bicyclogermacrene	24.66	0.14
Alpha copane	24.66	0.14
Acetyleugenol	24.66	0.06
Elemicin	24.78	0.03
Benzyl Benzoate	26.96	0.12
Viridiflorol	28.77	0.06
Torreyol	26.69	0.07
Tetra Decanoic Acid	29.99	0.15
Hexa Decanoic Acid	30.44	0.02

Earlier research done on Ocimum suava in keffi, Nassarawa state shows sabinene bergamol (4.4%) and limonene (4.07%) as major constituents (Olonisakin et al., 2005). But the major constituents of this research are largely different except for caryophyllene and limonene that are similar in both researches. The observed variations could likely be caused by genetic factors, season, crop and post-harvest processing, various chemotypes, plant nutritional condition, and other variables that can affect the composition of the oil (Paakkon et al., 1990; Charles and Simon, 1990).

The chemical constituent of *Ocimum suave* obtained by solvent extraction and hydro-distillation show different number of peaks. The major constituents were the same, but differ in the percentages distribution as shown in table 1 and 2. Apart for geranial, neral and allo ocimene that showed higher percentages with the hydro-distillation method, the other six major constituents were more in percentages when compared with the ones obtained by solvent extraction method. This might mean that more of geranial, neral and allo-ocimene can be extracted using hydro-distillation method and vice versa.

3.2 Antimicrobial activity of the essential oil

Table 3's results demonstrate the essential oils' ability to combat *Streptococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Bacillus aureus, Bacillus subtilis*, and *Staphylococcus aureus*. The oil's actions are dose-dependent. According to solvent extraction techniques, *Ocimum suave* exhibited activity ranging from 5.0 to 10.3 mm for the essential oil and from 5 to 6.5 mm for the essential oil by hydro-distillation. Ocimum suave essential oil extracted using a solvent exhibited more activity than Ocimum suave essential oil extracted through hydro-distillation.

Table 3: Antibacterial activity of the *Ocimum suave* essential oil

Bacterial Isolate	**A1 (zone of inhibition mm)**	**A2 (zone of inhibition mm)**
S. aureus	6.5	10.3
B. subtilis	—	5.0
S. pneumoniae	—	8.0
P. aeruginosa	5.0	8.0
E. coli	3.0	5.0

- A1 – hydro-distillated essential oil
- A2 – solvent extracted essential oil

The essential oil of *Ocimum suave* from both methods was not active against organisms used except *Ocimum suave* by solvent extraction that was active against *S. aureus*. These findings are consistent with the report by (Olonisakin et al., 2005). In the study, the essential oil from solvent extraction had higher activities than the essential oils from the hydro-distillation; this may be due to the active compounds present in solvent extraction method than the hydro-distillation method. According to the study, the activities of oil should be related to the plant volatile oils' respective composition, the structural arrangement of their constituent parts and their functional groups, and any potential synergistic interactions between them (Dorman and Deans, 2000).

Table 4: MIC values (in mg/ml) of each essential oil		
Bacteria IIsolates	**A1**	**A2**
S. aureus	25	12.5
B. subtilis	—	12.5
S. pneumoniae	—	12.5
P. aeruginosa	50.0	3.2
E. coli	25.0	3.2

- A1 – hydro-distillated essential oil
- A2 – solvent extracted essential oil

The results obtained from the Minimum Inhibition Concentration (MIC) shown in table 4, which means that this minimum amount in mg/ml will be required to make the microorganism inactive or inhibited. The MIC values for *Ocimum suave* by hydro-distillation ranges from 25 – 50.0mg/ml and 3.2 – 12.5mg/ml for *Ocimum suave* by solvent extraction. The *Ocimum suave* by hydro-distillation was not effective against *B. subtilis* and *S. pneumoniae*. The *Ocimum suave* by solvent extraction made *P. aeruginosa* and *E. coli* inactive at 3.2mg/ml.

The Minimum Bactericidal Concentration (MBC) shown in table 5, that is, at this concentration the essential oil will kill the organisms used. From table 5 *Ocimum suave* by solvent extraction and *Ocimum suave* by hydro-distillation were 6.3 – 50.0mg/ml and 0.0 – 50.0mg/ml respectively.

Table 5: MBC Values (mg/ml) of each essential oil		
Bacterial Isolates	**A1**	**A2**
S. aureus	50.0	25.0
B. subtilis	—	50.0
S. pneumoniae	—	50.0
P. aeruginosa	50.0	6.3
E. coli	25.0	25.0

- A1 – hydro-distillated essential oil
- A2 – solvent extracted essential oil

The result conforms to the trend observed in the MIC result for the various essential oils. The essential oils obtained by solvent extraction were generally more effective than the ones by hydro-distillation.

4. Conclusions

From the result of the chemical composition, it is possible that factors other than geographical, environmental, crop and post crop processing do affect the percentage yield, and the kind of chemical constituents present. This is because in comparing this research with previous one; done in the same Nassarawa State, Nigeria; differences were noticed in the percentage composition of the essentials oils present. This might be attributable to the season of the year the plants were harvested since the plants share other factors alike.

Also from the research work using the hydro-distillation and solvent extraction methods it showed that the method of extraction used do greatly affect both the yield and the percentage distribution of the chemical composition. The solvent extraction method had a higher yield and higher activity against the organisms used. However what can be deduced is that some compounds that are active against these organisms must have been more extracted using the solvent extraction method.

Recommendations

It was observed that hydro-distillation is also a better method of extraction and is also cheaper economically. It is therefore recommended that hydro-distillation should be used for extraction.

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