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CHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITIES OF THE VOLATILE OILS OF *CRINUM ZEYLANICUM* L AND *CRINUM ORNATUM* (AIT) BURY BULBS

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ABSTRACT

Crinum zeylanicum (*C. zeylanicum*) and *Crinum ornatum* (*C. ornatum*) have long history of used as food and medicine. This study aimed to determine the chemical constituents and examine the antimicrobial effects of the volatile oils of *C. zeylanicum* and *C. ornatum*. The volatile oils of the fresh bulbs of the plants were obtained by hydrodistillation method and their chemical constituents were analyzed by means of gas chromatography coupled to mass spectrometry (GC-MS). The inhibitory effects of these oils were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Candida albicans* and *Mycobacterium bovis* BCG using micro dilution broth method. From the GCMS results, twenty seven compounds were identified for volatile oil of *C.zeylanicum* characterized by high amount of monoterpenes (37.59%) and non-terpenes (25.11%). The main components were (R)-(+)-citronellic acid (33.06%), citronellol (4.53%), 9-octadecenamide (3.16%) and heptadecanal (2.92%). Eleven components were identified from *C. ornatum* with their main constituents being non-terpenes (81.68%) and sesquiterpenes (1.62%). The main components identified were hexatriacontane (39.21%), tetracosane (17.49%), decadienal (6.29%) and decane (4.36%). The two essential oils showed significant antimicrobial effect against the test microorganisms, with minimum inhibitory concentration ranging from 100 to 25µg/ml. The result showed that essential oils of *C. zeylanicum* and *C. ornatum* comprised higher proportions of non-terpenoid and monoterpene compounds with good antimicrobial effects.

KEYWORDS: *Crinum zeylanicum*, *Crinum ornatum*, volatile oil, citronellic acid, hexatriacontane.

INTRODUCTION

Medicinal plants are bioresources harnessed by humans to combat diseases and maintain healthy life. Plants remain the basis for development of modern drugs for the preservation of health.[1] Essential oils could be extracted from leaves, stems, flowers, roots, herbs, brushes, and trees through distillation. They have been used for medicinal and healing purposes for many years all over the world. Interest in essential oils has increased in recent decades with the popularity of aromatherapy, which claims that essential oils and other aromatic compounds have beneficial effects. They are widely used in perfumes, cosmetics, soaps, cleaning products and other products and also for flavoring of foods and drinks.[2]

The name *Crinum* originates from the Greek word '*krinos'* meaning trailing hair or Comet-tail.[3] This genus is related to a group of mostly southern African endemic genera, constituting the tribe Amaryllideae.[4] Amaryllidaceae is widely distributed containing about 90 genera and 1310 species. *Crinum* species have a considerable medicinal reputation as potent folkloric remedies. Their use extended from ancient times to nowadays especially in Africa, tropical Asia and South America.[5] Botanically, *Crinum* species are perennial herbaceous plants with giant bulbs larger than most other species of Amaryllidaceae. They can grow from 1-5 feet in height depending on the species and produce a neck or a pseudo stem made up of the sheathing old leaves. Flowers usually appear in May, June or August.[6] Crinums are large, showy plants with umbels of lily-like flowers. Worldwide, Crinums have a great economic

value as ornamentals due to their showy flowers. They are found in tropical and subtropical regions throughout the world, where for centuries, they have been used traditionally to cure ailments and diseases. Sometimes different species are prescribed for the same medicinal purpose. This would suggest that they contain some common ingredients. Phytochemical analysis yielded a vast array of compounds, including more than 150 different alkaloids. Huge amount of Crinums are traded for traditional medicines. Crinums attract considerable attention due to their various medicinal properties such as antitumor, immunostimulating, analgesic, antiviral, antimalarial, antibacterial and antifungal activities.[7] Furthermore, Crinum species have been used traditionally to cure ailments and diseases throughout the world and some of the most noted effects are analgesic, anticholinergic, antitumour and antiviral.[5]

Recently, galanthamine isolated from *Crinum* species has been registered as an acetylcholinesterase inhibitor, which is an important approach in treating Alzheimer's disease. Amaryllidaceae alkaloids are highly toxic, the use of *Crinum* species is not recommended for the novice, as a large dose could be fatal.[8] Since the 1950s, Crinums have been subjected to extensive chemical, cytological and pharmacological investigations due to their rich pharmacologically active principles.

C. zeylanicum (L.) is a medicinal plant used in western Nigeria for management of skin problems, injuries, convulsion and refractory ulcer.[9,10] The Hausas of northern Nigeria call it "Albassar Kwaadii" (Frog's Onion), while the Yorubas of the southern part of Nigeria call it "Isumeri". It is used in folk medicine for various ailments in different ethnic settings. In Sierra Leone, the leaf infusion is used for young children suffering from general debility and rickets.[11] *C. zeylanicum* possesses central nervous system depressant activity.[12] The ointment (1%, 5% and 10%) made from bulb methanol extract of *C. zeylanicum* was reported to have wound healing properties comparable to Gentamycin (1%) ointment. Phytochemical analysis of the methanol extract showed the presence of some secondary plant metabolites and volatile oils.[13] The efficacy of medicinal plants that are used for wound-healing purposes may be due to their direct action on the wound repair processes, anti-inflammatory and antimicrobial effects or a combination of these effects.[14] Previous studies carried out on wound-healing effects of medicinal plants have shown that these preparations were effective in wound care, facilitated rapid wound-healing with minimal pain, discomfort and scarring to the patient.[13] The aqueous and ethanolic extracts of *C. zeylanicum* were tested against intermediate hosts of schistosomiasis and fascioliasis. Both extracts showed remarkable mortality rates against *Biomphalaria pfeifferii* and *Lymnaea natalensis*.[5] Complex alkaloids in *C. zeylanicum* plant organs were recently revealed to include crinine, lycorine, 11-O-acetoxyambelline, ambelline, 6-hydroxybuphanidrine and 6-ethoxybuphanidrine. Crinine, 6-hydroxybuphanidrine and 6-ethoxybuphanidrine showed antiproliferative effects against human tumor cell lines, crinine being the most active.[15]

C. ornatum is known as Toad's Onion or "*Albasar kwaadi"* in Hausa language of Nigeria.[16] *C. ornatum* (Amaryllidaceae) is a bulbous plant with fleshy, wide-spreading, rich green and glaucous leaves, reaching 75 cm long by 6 cm wide. It grows well in damp site and is distinctly ornamental. The plant attracts considerable attention due to various medicinal properties as anti-tumor, immuno-stimulating, analgesic, anti-viral, anti-bacterial and anti-fungal.[17] Recently, phytoconstituents such as alkaloids, tannins, saponins, flavonoids, glycosides, cardiac glycosides, saponin glycosides, volatile oils and steroids were found to be present in the aqueous extract of *C. ornatum* bulbs. Similarly, the proximate composition was also reported together with the lethal dose (LD_{50}) of *C. ornatum* bulb being higher than 3000mg/kg for the test animals.[18] Two Amaryllidaceae alkaloids, haemanthamine and haymane were isolated from the bulb of *C. ornatum*[19] in addition to lycorine, crinamine, ornamine, ornazamine and ornazidine which were previously reported.[20] Lycorine and haemanthamine obtained from the crude extract of *C. ornatum* exhibited dose-dependent anticonvulsant effects using electrical stimulation test in rats. The ethanolic extract of *C. ornatum* bulbs as well as some isolated alkaloids such as lycorine, haemanthamine and crinamine showed significant DPPH scavenging effects.[19] Light yellow essential oil was isolated from the air-dried *C. ornatum* bulb comprising of 18 identified compounds representing 97.71% of the total essential oil. The major constituents identified were 14-Methylpentanedecanoic acid methylester (20.89%), n-hexanedecanoic acid (13.06%), 9, 12-Octadecadienoic acid (13.06%), tetratetracontane (10.45%), methyl benzene (5.49%), cis-decahydronaphthalene (5.49%), ethyl ester (5.22%) and 2, 6, 10, 15-Tetramethylheptanedecane (3.14%). *C. zeylanicum* and *C. ornatum* are the commonly found Crinum species in Nigeria.

However, to our knowledge, no comparative phytochemical investigations on *C. zeylanicum* and *C. ornatum* fresh bulb essential oils have been undertaken. The present study aims to evaluate the essential oil constituents and antimicrobial activities of *C. zeylanicum* and *C. ornatum* fresh bulb.

MATERIALS AND METHODS

Plant Materials

The plants, *C. zeylanicum* L. and *C. ornatum* (Ait) Bury, were collected in October 2017 from Chaza, Suleja, Nigeria. Identification and authentication of the plant material were done by a taxonomist at the Herbarium of the National Institute of Pharmaceutical Research and Development, Abuja, Nigeria.

Essential oil isolation

The fresh bulbs (500 g) were chopped into small pieces and hydrodistilled using a Clavenger-type apparatus. The extraction was carried out for 4 h. The colourless oil obtained was dried over anhydrous sodium sulphate. The oil was filtered through 0.22 microns filter and kept at 4^{o}C in sealed vials in dark until analysis.[21]

Gas Chromatography-Mass Spectral analysis

The oils were analysed by GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and Shimadzu GCMSsolution data system. The GC column was HP-5MS fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, length 30 m, internal diameter 0.25 mm and film thickness 0.25 µm. The carrier gas was helium with flow rate of 1.61 ml/min. The program used for GC oven temperature was isothermal at 60 ^{o}C, followed by 60-180 ^{o}C at a rate of 10 ^{o}C/min, then held at 180 ^{o}C for 2 minutes; followed by 180-280 ^{o}C at a rate of 15 ^{o}C/min, then again held at 280 ^{o}C for 4 minutes. The injection port temperature was 250 ^{o}C. The ionization of sample components was performed in the E.I. mode (70eV). Injector temperature was 250 ^{o}C while detector temperature was 280 ^{o}C. Helium was used as carrier gas at a flow rate of 1.61 ml/min. 1.0 µl of diluted sample (1/100 in hexane, v/v) was injected using autosampler and in the split mode. Split ratio was 10:90.[22]

Identification of the compounds

The individual constituents of the volatile oil were identified by referring to compounds known in literature [23] and also by comparing their mass spectra with known compounds in National Institute of Standards and Technology mass spectral library (NIST 11), and Flavour and Fragrance Natural and Synthetic Compounds mass spectral library database. Quantitatively, the area percentages of each component were reported as raw percentages based on the total ion current without standardization. Results are shown in Table 1.

Microbial strains

The following microorganisms were used in the evaluation of the antibacterial activity of the essential oil: Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923); Gram-negativebacteria, *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC13883), *Escherichia coli* (ATCC 10798); fungi *Candida albicans* (ATCC No. 2876) and *Mycobacterium bovis* BCG (ATCC 35737).

Antimicrobial activity

The minimum inhibitory concentration (MIC) values of the essential oils were determined by microdilution broth method in 96-well microplates.[24] The oil sample was dissolved in dimethyl sulfoxide (DMSO) followed by addition of sterile Mueller-Hinton nutrient broth for bacteria and Sabouraud-Dextrose nutrient broth for fungi, to achieve concentration of 200 µg/ml. The final DMSO concentration was 20% (v/v) and this solution was used as negative control. The inoculum was adjusted for each organism to yield a cell concentration of 2×10^7 colony forming units (cfu) per ml. Ciprofloxacin (Fidson, Nigeria) was used as positive control for bacteria and Fluconazole (Pfizer, UK) was used as the standard drug for fungi at stock concentration of 50 µg/ml. Controls of sterility for the Mueller-Hinton nutrient broth, control culture (inoculum), Ciprofloxacin, Fluconazole, essential oil and DMSO were performed. The microwell plates were closed and incubated aerobically at 37°C for 24 h.

Post incubation, to indicate respiratory activity, to the plates were added 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT), incubated for another 2 h to indicate colour change in wells where there is no activity. MIC was defined as lowest concentration of essential oil at which the red formazan of MTT was not observed. All assays were carried out in triplicate.[22] Results are shown in Table 2.

Antimycobacterial Assay

Determination of antitubercular activity was carried out on *Mycobacterium bovis* BCG (ATCC 35737) using the broth dilution method previously described.[22] *Mycobacterium bovis* BCG cells were grown to an optical density of 0.2-0.3 at 650 nm in 7H9/ ADC/Tween consisting of Middlebrook 7H9 broth supplemented with 0.5% bovine serum albumin fraction V, 0.08% NaCl, 0.2% glucose, 0.2% glycerol and 0.05% Tween80. The essential oil sample was dissolved in dimethyl sulfoxide (DMSO), centrifuged for 20 minutes at 13,000 rpm, followed by addition of sterile 7H9/ADC/Tween to achieve concentration of 200 µg/ml solution. The final DMSO concentration was 4% (v/v) and this solution was used as a negative control. 50 µl of media was introduced into wells 2 to 12 of a 96-well micro-titre plate, while 100 µl of sample (200 µg/ml) was delivered into the appropriate first well of the 96-well plate. Two-fold dilutions were performed by sequential transfer of 50 µl of each sample from well 1 to 2; after thorough mixing, 50 µl was transferred from well 2 to 3. The process was repeated through to well 11 where 50 µl was discarded leaving column 12 for the negative control. 50 µl of inoculum prepared by diluting a 5-7 day old culture of *Mycobacterium bovis* BCG (OD 0.2-0.3) 1:1000 (by adding 50 µl of cell culture into 50 ml of 7H9/ADC medium) was added to all the wells and incubated for 14 days at 37°C, after which the growth or inhibition of growth was read by direct recording of visual growth. All of the minimum inhibitory concentration (MIC) determinations were done in duplicates. Post incubation was done to indicate respiratory activity. To the plates were added 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT), incubated for another 2 h to indicate colour change in wells where there is no activity. MICs were defined as lowest concentration of essential oil at which the red formazan of MTT was not observed. Isoniazid was used as positive control. Results are shown in Table 2.

Statistical analysis

All data were reported as mean of independent replicates. Data were analyzed by analysis of variance (ANOVA). $P<0.05$ was considered as significant level.

RESULTS

The volatile oil was extracted from the fresh bulbs of *C. zeylanicum* and *C. ornatum*. The gas chromatographs of these oils are shown in Figures 1 and 2. GC-MS profile of the two volatile oils from the fresh bulbs of *C. zeylanicum* and *C. ornatum* obtained by hydrodistillation resulted in the identification of 27 and 11 compounds respectively. The constituents are arranged in order of their retention indices from the HP-5MS column with their respective percentage compositions (Table 1).

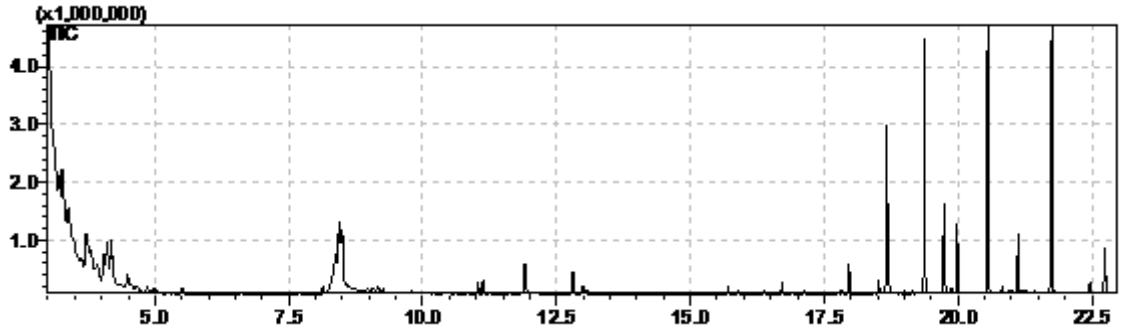

Figure 1: GC–MS chromatogram of *C. zeylanicum* analyzed on GC–MS (Shimadzu, Japan) using a capillary column (HP 5-MS) attached with mass detector. The chromatogram showed the presence of chemical components found in the *C. zeylanicum*.

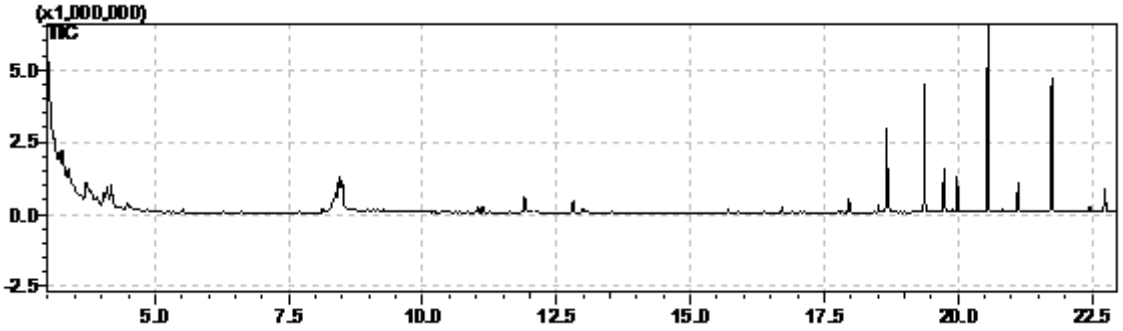

Figure 2: GC–MS chromatogram of *C. ornatum* analyzed on GC–MS (Shimadzu, Japan) using a capillary column (HP 5-MS) attached with mass detector. The chromatogram showed the presence of chemical components found in the *C. ornatum*.

Table 1: Percentage of compounds in the volatile oil of the fresh bulbs of *C. zeylanicum* and *C. ornatum*.

Compounds RI[a] LRI[b]			% Composition	
			C. zeylanicum	*C. ornatum*
Decane	1000	999	-	4.36
1,2,3-trimethyl benzene	1025	1018	-	4.14
3,5-dimethyl cyclohexanol	1030	1025	0.37	-
2,6,8-trimethyl decane	1110	1104	0.33	-
2-Furfuryl-5-methylfuran	1163	1160	1.27	-
Decanal	1201	1204	0.48	-
Citronellol	1232	1228	4.53	-
Undecan-2-one	1296	1291	1.55	-
Citronellic acid	1300	1303	33.06	-
2,4-E,E-decadienal	1320	1314	-	6.29
Ethyl decanoate	1390	1394	0.89	-
β-Caryophyllene	1412	1417	0.37	1.62
Tridecan-2-one	1500	1505	1.51	-
Hexadecane	1558	1600	0.55	-
Tridecan-1-ol	1579	1575	-	0.35

Heptadecanal	1685	1682	2.92	-
Heptadecane	1696	1700	0.45	-
4,7-dimethyl benzofuran	1737	1743	0.64	-
cis-9-Hexadecenal	1803	1800	0.82	-
Heptadecan-4-one	1902	1905	0.71	-
Heptadecan-3-one	1905	1900	0.49	-
Oxacycloheptadec-8-en-2-one	1922	1925	1.43	-
Methly palmitate	1932	1926	0.63	-
Eicosane	2000	2000	0.86	Tr
Octadecanal	2022	2021	0.78	-
Nonadecan-2-one	2108	2106	0.73	-
9-Octadecenamide	2393	2398	3.16	5.94
Tetracosane	2400	2400	-	17.49
2-Methyltetracosane	2465	2462	-	0.7
n-Docosanol	2505	2501	0.51	-
2-Methylhexacosane	2665	2662	1.36	-
Supraene	2829	2835	1.53	3.2
Nonacosane	2905	2900	1.14	-
Hexatriacontane	3596	3600	-	39.21
TOTAL			63.07%	83.3%

[a]Retention indices on HP-5MS column; [b]Literature retention indices;[21] Tr, trace amount < 0.1%; - not identified**.**

Antimicrobial efficacy

The volatile oils of *C. zeylanicum* and *C. ornatum* bulbs were analysed for their antimicrobial efficacy. The volatile oil considerably inhibited the growth of the tested organisms which were measurably compared with the positive control and were found to be relatively less active (Table 2).

Table 2: Minimum inhibitory concentration of the volatile oilsfrom the bulb of *C. zeylanicum* and *C. ornatum.*

S/No.	Microorganisms	Minimum inhibitory concentrations	
		Essential oil (µg/ml) Cz Co	Standard drug (µg/ml)
1*	*Pseudomonas aeruginosa* (ATCC 27853)	100 50	0.05
2*	*Klebsiella pneumonia* (ATCC 13883)	100 100	0.39
3*	*Escherichia coli* (ATCC 10798)	100 100	0.39
4*	*Staphylococcus aureus* (ATCC 25923)	25 100	0.09
5**	*Candida albicans* (ATCC 2876)	100 50	6.25
7***	*Mycobacterium bovis* BCG (ATCC 35737)	50 25	0.07

*Bacterial strain; **Fungal strain; ***Mycobacterium strain. Ciprofloxacin (standard drug for bacterial strains); Fluconazole (standard drug for fungi strain); Isoniazid (standard drug for Mycobacterium strain).*Crinum zeylanicum* (Cz) and *Crinum ornatum* (Co).

DISCUSSION

Recent phytochemical review confirmed the presence of essential oils in the bulb of *C. zeylanicum*[13] and *C. ornatum*.[18] The present study identified the various components present in the fresh bulb volatile oils. The essential oils obtained were colourless. The oils were further analysed by GC-MS which resulted in the identification of 27 compounds from *C. zeylanicum* and 11 from *C. ornatum* (Table 1) representing 63.07% and 83.3% of the constituents respectively. The percentage oil yield of *C. zeylanicum* was 0.15% w/w while that of *C. ornatum* was 0.12% w/w. Essential oil formation is dependent on climatic conditions, especially temperature, number of hours of sunshine and frequency and magnitude of precipitation; and in some cases by the time of harvesting.[25]

The *C. zeylanicum* volatile oil comprises of a large amount of citronellic acid (33.06%), an acyclic monoterpene acid, citronellol (4.53%) as acyclic monoterpene alcohol as well as 9-octadecenamide (3.16%) as an aliphatic amide. The non-terpene constituents amounted to 25.11%, monoterpenes 37.59% and cyclic sesquiterpenes 0.37%. The non-terpene constituents were aliphatic hydrocarbons (6.85%), aliphatic alcohol (0.88%), aliphatic ketone (4.99%), aliphatic aldehyde (5.0%), aliphatic ester (0.89%), aliphatic amide (3.16%) and heterocyclic compound (3.34%). Some monoterpenes such as β-citronellol have been reported to possess antimicrobial activity.[25]

The *C. ornatum* volatile oil was mainly composed of hexatriacontane (39.21%), tetracosane (17.49%), decadienal (6.29%) and 9-octadecenamide (5.94%). This

result contrasted with previous study where main components of the dried bulb were 14-methylpentanedecanoic acid methylester (20.89%), heneicosane (13.14%), n-hexanedecanoic acid (13.06%) and 9,12-octadecadienoic acid (13.06%), making up a total of 18 compounds[17] while the present study identified a total of 11 essential oil compounds. The percentage composition of caryophyllene and eicosane in the fresh bulb sample in the present study were about the same as that reported in the dried sample.[17] *C. ornatum* consisted mainly of non-terpenoid (81.68%) and sesquiterpenes (1.62%). The non-terpenoid was further grouped into aliphatic hydrocarbon (61.76%), aliphatic aldehyde (6.29%), aliphatic amide (5.94%), aromatic hydrocarbon (4.14%), cyclic aliphatic (3.2%) and aliphatic alcohol (0.35%). Furthermore, *C. ornatum* bulb volatile oil also demonstrated cytotoxicity against breast cancer cells *in vitro*.[25] *C. ornatum* is rich in phytochemicals including alkaloids, saponins and flavonoids.[26]

The predominant group of the volatile oils found in *C. zeylanicum* were the hydrocarbons with the highest component being citronellic acid (33.06%) and lowest being 2,6,8-trimethyldecane. The highest compound in *C. ornatum* bulb volatile oil was hexatriacotane (39.21%) and the lowest was β-caryophellene (1.62%). In a GC-MS evaluation of *C. ornatum* dried bulb volatile oil, the predominant phytochemical was hydrocarbons with 14-metylpentanedecanoic acid methylester (20.89%) as the most prominent compound.[17]

The most abundant constituent in *C. zeylanicum* bulb essential oil was citronellic acid, a well-known acyclic monoterpene carboxylic acid. Citronellic acid was reported from several essential oils such as the *Pelargonium* species, *Pelargonium graveolens*, *Chamaecyparis taiwanesis, Eucalyptus citriodora*.[22] It was reported to have antimicrobial activity against *Fones annosus* and *Mycobacterium tuberculosis*.[27]

The major volatile oil constituents of the two species were hydrocarbons. β-caryophyllene, 9-octadecenamide, suprene and eicosane were the compounds present in both *C. zeylanicum* and *C. ornatum*. *C. zeylanicum* showed percentage composition of (0.37%, 0.86%, 3.16%, and 1.53%) while *C. ornatum* showed (1.62%, trace, 5.94% and 3.2%) for β-caryophyllene, eicosane, 9-octadecanal and suprene respectively. The chemical variation observed in the two species may be due to intrinsic variation with the exception of extrinsic variation, because both the species were obtained from the same geographical location. Genetic background such as chemotypes and growth stage may be likely factors that could account for the chemical variation within the species.

The susceptibility of some microorganisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Mycobacterium bovis* BCG were tested against the volatile oils obtained from the fresh bulbs of *C. ornatum* and *C. zeylanicum* (Table 2). The MIC values were determined as the lowest concentration of essential oil capable of inhibiting the growth of these microorganisms. The organisms were susceptible to the volatile oils of both plants, making the oils broad spectrum antibiotics to gram positive, gram negative microbes and fungus (Table 2). *C. ornatum* volatile oil was more effective against *Pseudomonas aeroginosa* (MIC 50 µg/ml), *Candida albida* (MIC 50 µg/ml) and *mycobacterium bovis* (MIC 25 µg/ml) in comparison with *C. zeylanicum* (MIC 100 µg/ml). *C. zeylanicum* volatile oil had MIC of 25 µg/ml against *Staphylococcus aureus*, compared to100 µg/ml for *C. ornatum*. The two species showed the same activities against *Klebsiella pneumonia and E. coli* (MIC 100 µg/ml). The tested microorganisms are pathogens or opportunists for man, animal and plants, and they cause food spoilage, as well as contamination of water and air.

Methanol extract of *C. zeylanicum* bulb had been used as a wound healing ointment.[13] The extract contained balsam, sterols, terpenes, resins, carbohydrate, tannins, phlobatannins, saponins, flavonoids, alkaloids and volatile oils.[13] Phytoconstituents like tannins are known to for wound healing ability due to their astringent and antimicrobial property. The wound healing potency of *C. zeylanicum* is believed to be due to the phytoconstituents present in it, which may be acting separately or synergistically. The extract possibly fastened wound healing through contraction and epithelization process.[13] *C. zeylanicum* essential oil contained hexanal, (E, E)-2, 4-decadienal, nonanal and furfural, which had been reported to be bioactive against root knot nematode *Meloidogyne javanica*.[28]

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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