Variation in pre-extraction processes influences the differences in chemical constituent, quantity and biochemical activities of volatile oils from *Crinum jagus* (Th.) D.: Gas Chromatography-Mass Spectrometry analysis

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**ABSTRACT**

A comparative study performed on essential oil composition, cytotoxic and antioxidant activities of fresh and dried samples involving leaf and bulb of *Crinum jagus* (Th.) D. were reported. Qualitative and quantitative variations in the composition of oils were investigated using GC and GC-MS. The oil extracted from the leaf of *C. jagus* was characterized by high proportions of beta-ocimene (10.0–13.8%), followed by hexadecane (2.6–11.1%), tetramethylpentadecane (9.3–10.4%) and phytol (7.0–9.0%). Bulb oil is rich in 14-methylpentadecanoic acid methyl ester (20.6–22.5%), tetrahydroactone (7.9–10.0%) and 9,12-octadecadienoic acid (14.0–14.2%). Dried leaves and dried bulbs exhibited the highest cytotoxic activity (IC50 of 0.002 and 0.003 µg/mL, respectively) followed by fresh leaves 0.033 µg/mL and fresh bulb 0.55 µg/mL. The essential oils at 1.0 mg/mL displayed significant antioxidant activities. The level of antioxidant capacity varied according to samples. The chemical constituents and quantities significantly varied based on sample pre-extraction processing, thus affecting the biochemical activities.

1. Introduction

Volatile or essential oils from aromatic and medicinal plants via hydrodistillation have been known to possess biological activity, notably anticancer, antiviral, carminative, spasmyloytic, antimicrobial, antioxidant and hepatoprotective properties [1–3]. Owing to their aforementioned bioactive efficacy, they find extensive use in food and pharmaceutical industry.

The genus *Crinum* (Amaryllidaceae) contains about 160 species, mainly distributed in the tropical and subtropical regions of Africa, Asia, America and Australia. Their various species are acknowledged worldwide due to their high economical and medicinal values. Some are cultivated as ornamentals and for medical purposes [4]. The plants from this genus are reputable for their pharmacological properties, such as antitumor, antimicrobial, immunostimulating and analgesic, among others. *Crinum jagus* (Thompson) Dandy is a perennial bulb with tulip-like white flowers, which bloom during dry season on top of leafless stalks growing up to 1 m or more in height at maturity [5]. It is locally known as ‘bush onion’ and widely used among the traditional practitioners in Africa for treatment of diabetes, obesity, diarrhea, wound, memory loss, skin sore, asthma and snake bite. Previous phytochemical investigations on its extracts lead to the isolation of crinamine, lycorine, pseudolycorine, hamayne and tetrahydro-1, 4-oxazine. Others are calcium tetrata, 6-hydroxycrinamine and calcium oxalate [6]. The ethanol extract of *C. jagus* was shown to have antidiabetic, antioxidant and antimicrobial effects [6]. Despite the great potential of *Crinum* species as a valuable source of bioactive compounds, no scientific data regarding the chemical composition and biological potential of essential oil from *C. jagus* has been reported. Essential oil from the aromatic plant has been studied by many researchers using various drying methods, in order to increase the yield by reducing the moisture content [7–11]. However, far too little attention has been paid to the effect of the process on the constituent and bioactivity. In view of this, the present study focused on unearthing the chemical composition, antioxidant and cytotoxic potential of essential oil from *C. jagus* for the first time, which is found in abundance in the Southwest of Nigeria. Also, the effect of air-drying on the samples prior to hydrodistillation on their chemical composition and biological activity was reported.

2. Materials and methods

2.1. Plant material

Fresh leaves and bulbs of *C. Jagus* were collected in November 2013, at Ife road in Ibadan North Local Government Area of Oyo State, Nigeria. The identity
of specimens was confirmed at the Department of Plant Biology, University of Ilorin, Nigeria. A voucher specimen (number UIH 1022) was deposited in the herbarium. The leaves and bulbs were air-dried without sunshine until the weight was stable and then kept for subsequent use. Fresh samples were also kept for analysis.

2.2. Oil isolation

The oil samples codified as FL (fresh leaves), DL (dried leaves), FB (fresh bulbs) and DL (dried bulbs) were obtained by hydrodistillation using a distilled water on a Clevenger type apparatus for 3 h in accordance with the British Pharmacopeia specifications. About 100 g of each sample (fresh leaves, dried leaves, fresh bulbs and dried bulbs) was weighed prior to the hydrodistillation, with the fresh samples cut into smaller pieces (1–2 cm long) and the dried samples were pulverized. The essential oil was collected into well-capped glass vials and stored at 4°C until analysis.

2.3. Gas chromatography (GC) analysis

GC analysis of the oil was carried out using an HP 6890 Powered with HP ChemStation Rev. A09.01 [1206] software. Hydrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The GC oven temperature was kept at 50°C (Hold for 0 min), and programmed to reach 150°C at a rate of 5°C/min, then kept constant at 250°C for 2 min being the final hold time. The split ratio was adjusted to 20:1. The injector temperature was set to split injection with detector temperature 320°C. The hydrogen pressure is 22 psi while the compressed air is at 28 psi with FID detector and HP 5MS as the column type with dimensions 30 m × 0.25 mm × 0.25 µm.

2.4. Gas chromatography–mass spectrometry (GC–MS) and identification of constituent

The conditions for the GC were as described above. The detector interface temperature was 320°C, which is nearly equivalent to the actual temperature of the MS source. Mass spectra were acquired at 70 eV with mass range 50–300 m/z. The Data are reported as the mean value of two oil’s injections and analysed using HP ChemStation software.

Identification of constituents was done by comparing the retention times and the chromatography peaks with those of standards analysed under similar conditions. The peak assignment of the other volatile components was based on computer matching of the peaks obtained with the NIST08, Adams libraries, HP ChemStation and values from the literature.

2.5. Cytotoxicity assay

Brine shrimp lethality test (BST) protocol of McLaughlin and Rogers [12] was used for the evaluation of cytotoxicity effect. The brine shrimp (Artemia salina Leach) eggs were hatched in a small tank filled with sea water for 48 h at room temperature. Solution of the essential oil (FL, DL, FB, and DB) was made in dimethylsulfoxide (DMSO), at various concentrations (10 000, 1000, 100, 10, 1, 0.1, 0.01 µg/mL) and incubated in triplicates test tubes with the 10 brine shrimp larvae per test tube (30 shrimp per concentration). Control brine shrimp larvae were placed in a mixture of seawater and DMSO only. After 24 h, the average number of larvae that survived in each test tube was determined. The probit analysis to determine the LC50 values of 95% confidence intervals for statistically significant comparison of potencies were calculated using a Finney computer programme.

2.6. Free radical scavenger assays

The free radical scavenger activity was determined by hydroxyl radical (OH·−) scavenging and reducing power methods [13,14], each in triplicate and three independent experiments. In the hydroxyl radical (OH·−) scavenging assay, the stock solutions (2.0 mL) of the essential oils (FL, DL, FB, and DB) and positive control (Vit. C) were diluted to final concentrations of 1000, 800, 600, 400, and 200 µg/mL. Also, 0.6 mL of 8 mM ferrous sulfate, 0.5 mL of 20 mM H2O2 and 2 mL of 3 mM salicylic acid were mixed and incubated at 37°C for 30 min. Thereafter, 0.9 mL of distilled water was added to each vial. The resulting solution was centrifuged at 10,000 rpm for 10 min. After 10 min, the absorbance values were measured at 510 nm and converted into the percentage hydroxyl radical (OH·−).

In the reducing power method, 2.5 mL of 1% potassium hexacyanoferrate [K3Fe(CN)6], 2.5 µL of 0.2 M phosphate buffer (pH 6.6) and various concentrations (1000, 800, 600, 400, and 200 µg/mL) of essential oil extract (FL, DL, FB, and DB) suspended in 1 mL of distilled water and incubated at 50°C for 20 min. Thereafter, 2.5 µL of trichloroacetic acid was added to the mixture. This was centrifuged at 400 rpm for 10 min. after which 2.5 µL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% FeCl3.). The absorbance was immediately read at 700 nm.

2.7. Data analysis and statistics

All experimental data were expressed as means ± SD (standard deviation) of four independent experiments except otherwise stated. Two-way Analysis of Variance was used and p < .05 was considered statistically significant. The antioxidant activity of OH·− scavenging assay was calculated by using the formula:

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\text{Percentage OH·−} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]
The absorbance of the mixture with oil and the absorbance of the oil alone.

3. Results

3.1. Chemical composition of essential oil

The yields of the essential oil obtained by hydrodistillation of the fresh leaves, dried leaves, fresh bulbs and dried bulbs of *C. jagus* were 0.38%, 0.21%, 0.26% and 0.40% (v/w), respectively (Table 1). The chemical compositions of the extracted oils were determined using Gas Chromatography coupled to Mass Spectrometry (GC-MS) which are summarized in Table 2. The oils obtained were colourless. In addition, oils from the leaves (FL and DL) gave a floral odour.

A total of 57 and 56 compounds representing 99.8% and 99.9% of the total oil profile were identified from the FL and DL, respectively. In both oils, the principal constituent was beta-ocimene (10.0–13.8%), followed by hexadecane (2.6–11.1%), tetramethylpentadecane (9.3–10.4%), phytol (7.0–9.0%), hexacosane (10.1–10.7%), nonacosane (9.7–10.3%), heptacosane (6.7–8.3%), hexahydrofarnesyl acetone (6.0–8.8%), 2,4-dimethylhexane (2.9–7.0%), and pentadecane (4.6–6.4%).

In the bulb, a total of 43 compounds were identified from both FB and DB representing 99.8% of the total oil. The essential oils are dominated by oxygenated monoterpene, but high content of aliphatic hydrocarbons, which drastically reduced after air drying. The oils were characterized by a low content of mono- and sesquiterpene and other aliphatic hydrocarbons.

A comparative analysis of volatile chemical profiles of the leaves and bulbs of *C. jagus* showed a significant difference in the constituents of the volatile oils (Table 2). The oils from leaves contained more variety of compounds than the bulb’s oil. Beta-ocimene was found to be most abundant in the oils from leaves while bulbs’ oils were characterized by a high level of 14-methylpentanedicanoic acid methyl ester.

In the leaf, FL and DL gave essential oils containing identical constituents with exception of methyl benzene in DL, but the quantitative composition differed significantly. Drastical reduction in the amounts of benzyl alcohol, car-2-ene, myrcene, cis-ocimene, D-limonene, allo ocime, alpha-pinene, alpha-thujene, gamma-terpine was observed on drying the leaves and similar trends were noticed for fenchone, neral, geranial, isoartemisia, 1,8-cineole, geraniol, linalool, α-terpinol, terpinen-4-ol and β-caryophyllene. Other constituents with significant reduction in quantity were thymyl methyl ether, linalyl acetate, ethyl cinnamate, γ-cadinene, β-elemene, β-pinene, β-cadinene, hexadecane, α-muurolene and trans-decahydroxynaphthalene. However, percentage composition of β-ocimene, 2,4-dimethylhexane and bisabolen increased (about 16%) drastically after air drying; similar increments (10%) were recorded for hexahydrofarnesyl acetone, tetracosane, heptacosane, nonacosane and phytol.

For the bulb, chemical profiles of FB and DB are the same, though quantitative differences can be seen for some individual compounds, such as tetratriacontane, trans-decahydroxynaphthalene and hexadecanoic acid, which drastically reduced after air drying. The oils were characterized by a low content of mono- and sesquiterpene, but high content of aliphatic hydrocarbons.

Generally, analysis of the four oils showed that they were predominantly oxygenated monoterpen, sesquiterpene and other aliphatic hydrocarbons. Although, the latter group of compounds was quantitatively the major constituents. There are 41 compounds which are common in both leaf and bulb oils, however,

### Table 1. Cytotoxic test using Brine shrimp lethality test (BST).

| Samples             | LC50 (µg/mL) | Yield (%) | Upper confidence limit | Lower confidence limit |
|---------------------|--------------|-----------|------------------------|------------------------|
| *C. jagus* fresh leaves | 0.033        | 0.38      | 1.1153                 | %1.701412E + 38         |
| *C. jagus* dried leaves | 0.002        | 0.21      | 0.9488                 | %133342820000.00        |
| *C. jagus* fresh bulbs | 0.550        | 0.26      | 1.1792                 | %10336600.0000          |
| *C. jagus* dried bulbs | 0.003        | 0.40      | 0.3544                 | %93739450.0000          |
β-caryophyllene (1.8–2.7%) and 2,4-dimethylhexane (3.9–7.0%) were only common constituents of the studied oils which occurred in appreciable quantity. The leaf oils were characterized by the abundance of β-ocimene, which is not detected in bulb oils. Methyl benzene, cis-decahydronaphthalene and other major compounds in the bulbs’ oils are detected in very low amount in the leaf oil. Notably the oils from the leaf lack eicosane, similarly, oils from bulb showed absence of car-2-ene, myrcene, cis-ocimene, D-limonene, allo ocimene, α-pinene, α-thujene, β-terpine, β-caryophyllene, fenchone, nerol, and geranial which were all monoterpenes.
Figure 1. Scavenging activity (hydroxyl radical) of C. jagus’s volatile oils.

Figure 2. Scavenging activity (reducing power) of C. jagus’s volatile oils.

hydrocarbons. Other noteworthy oxygenated monoterpenes compounds absent were geraniol, nerol and bornanol. The bulb oils contained more (majorly) sesquiterpenes hydrocarbons and other higher hydrocarbons than the leaf oils. C-20 hydrocarbon, eicosane, which is abundant in bulb oils is absent in leaf oils. The occurrence of β-ocimene in appreciable quantities (10–14%) in the leaves could be responsible for the floral odour.

The chemical constituents seen in the DB oil of this specimen was similar to that previously reported for DB oil from Crinum ornatum (Ait.) Bury, a different species [15]. Though only 18 compounds were identified in the species, representing 97.71%, however, the DB described here, differed with the presence of monoterpenes and sesquiterpenes hydrocarbons. These noted similarities are often ascribed to the existence of specific chemotypes which would be a research of interest for further study. The results obtained clearly indicated that air drying of the plant samples influence the quantitative composition of the oil. This variability in composition was also observed in our previous work involving Spondias mombin Linn [16], where an increment in the oxygenated monoterpenes contents and a decrease in the amount of sesquiterpenes hydrocarbon, was observed on air-drying the leaves. In addition, the oil obtained from fresh leaves was more bioactive than that obtained from dried leaves. Research such as that conducted by Asekun et al. [17] has shown that different drying methods before extraction has significant effect on the quality and quantity of the essential oils from Mentha longifolia L. A possible explanation for this might be that, some compounds which were dragged to the surface of the leave by the evaporating water got lost completely or partially, during the drying process [18].

There are well-documented cases showing a remarkable correlation between brine shrimp toxicity and 9KB (human epidermoid carcinoma of nasopharynx) cytotoxicity ($p = .036$ and kappa = 0.56) and this lead to the discovery of many new antitumor and pesticides from natural products by using this bioassay BST [12]. All the examined oils using BST were highly active, though oil obtained from DL and DB were the most potent. Recent evidence suggests that bioactivity of an essential oil may not be always attributed to their major constituent(s), there could be internal synergy among their constituents facilitating or enhancing the putatively toxic (active) principles [19].

The ability of the oils to scavenge different free radicals is noteworthy. This is an indication that the oils may be useful in preventing or minimizing oxidative damage effect of free radicals on cells in relation to certain diseases such as cancer, cardiovascular and neurodegenerative diseases. From the data in Figure 1, it is apparent that the DL and FB oils displayed better antioxidant capacity than vitamin C, in the hydroxyl radical (OH•) scavenging assay. This may not be unconnected to the presence of phytochemicals (such as β-ocimene and β-pinene) which are known to be good antioxidants.

Some differences in bioactivity of these oils may be expected. Several studies have documented monoterpenoids to exhibit a diverse ray of therapeutic properties, so infraspecific variation in monoterpenoids content and quantity could be expected to result in corresponding differences in their bioactivities or efficacies.

5. Conclusion

The volatile chemical composition of C. jagus is being reported for the first time. Up to now, research tended to focus on the oil yield rather on the effect of the process. The obtained data clearly revealed that variation in the pre-extraction process (i.e. drying process) influences the chemical constituent and quantity, which could be responsible for the disparity in their bioactivities. The oils are potent and displayed good antioxidant activity. This may be useful in the food and pharmaceutical industries in the treatment of oxidative related ailments. It would be interesting to assess the cytotoxic effects of the oils on various cancer cell lines. Overall, this study strengthens the idea that various pre-extraction methods should be employed prior to hydrodistillation, in order to get optimum conditions needed for the respective study.
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References

[1] Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytother Res. 2007;21(4):308–323.
[2] Mukazayire MJ, Tomangi JC, Stevigny C, et al. Essential oils of four Rwandese hepatoprotective herbs: Gas chromatography-mass spectrometry analysis and antioxidant activities. Food Chem. 2011;129(3):753–760.
[3] Russo A, Formisano C, Riganò D, et al. Chemical composition and anticancer activity of essential oils of Mediterranean sage (Salvia officinalis L.) grown in different environmental conditions. Food Chem Toxicol. 2013;55:42–47.
[4] Tram NT, Titorenkova TV, St. Bankova V, et al. Crinum L. (Amaryllidaceae). Fitoterapia. 2002;73:183–208.
[5] Ogunkunle AJ, Olopade OR. Studies on the asthma coughs plant Crinum jagus L. (Amaryllidaceae) in Nigeria. Afr J Plant Sci. 2011;5(2):108–114.
[6] Udegbunam SO, Okoli R, Kene C, et al. Evaluation of wound healing potential of methanolic Crinum jagus bulb extract. J Intericult Ethnopharmacol. 2015;4(3):194–201.
[7] Ante I, Aboaba S, Siddiqui H, et al. Essential oils of the leaf, stem-bark, and nut of Artocarpus Camansi: gas chromatography- mass spectrometry analysis and activities against multidrug-resistant bacteria Camansi: gas chromatography-mass spectrometry. J Herbs Spices Med Plants. 2016;22(3):203–210.
[8] Innocent E, Hassanali A. Constituents of essential oils from three plant species used in traditional medicine and insect control in Tanzania. J Herbs Spices Med Plants. 2015;21(3):219–229.
[9] Oladimeji AO, Aliyu MB, Ogundajo AL, et al. Identification and comparison of the volatile constituents of fresh and dried leaves of Spondias mombin found in North-central Nigeria: in vitro evaluation of their cytotoxic and antioxidant activities. Pharm Biol. 2016;54(11):2674–2678.
[10] Oladosu I, Oladimeji A. Volatile constituent of sea purse, dioecia reflexa root. Elixir Org Chem. 2012;45:7738–7740.
[11] Russo A, Cardile V, Graziano ACE, et al. Comparison of essential oil components and in vitro anticancer activity in wild and cultivated Salvia verbenaca. Nat Prod Res. 2015;29(17):1630–1640.
[12] McLaughlin JL, Rogers LL. The use of biological assays to evaluate botanicals. Drug Inf J. 1998;32:513–524.
[13] Oyaizu M. Studies on products of browning reaction: antioxidative activities of product of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;44(4):307–315.
[14] Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. 1989;28(4):1057–1060.
[15] Oloyede GK, Oladosu IA, Shodia AF. Chemical composition and cytotoxicity of the essential oils of Crinum ornatum (Ait.) Bury. Afr J Pure Appl Chem. 2010;4(3):35–37.
[16] Oladimeji AO, Babatunde O, Musa RT, et al. GC-MS analysis and cytotoxic activity of essential oils from the leaves of Abrus precatorius L. Gaertn. Asian Pac J Trop Dis. 2016;6(5):372–375.
[17] Asekun OT, Grierson DS, Afolayan AJ. Effects of drying methods on the quality and quantity of the essential oil of Mentha longifolia L. subsp. Capensis. Food Chem. 2007;101(3):995–998.
[18] Charalambous G. Spices, herbs and edible fungi. Amsterdam: Elsevier; 1994.
[19] Isman MB. Pesticides based on plant essential oils: phytochemical and practical considerations. Washington (DC): ACS; 2016.