Analysis of Oil Fraction from *Crinum Jagus* Bulb and Its Antibacterial Activity

Dupe Lyda Abiona, Oluwayemi Olanike Esther Onawumi and Sunday Olusegun Oladoye

***Abstract*—** Crinum jagus belongs to the *Amaryllidaceae* family. It is widely used in Africa as antidiabetic, antiobesity and antidiarrhoeal remedies. This study carried out gas chromatographic-mass spectrophotometric(gc-ms) analysis of oily ethylacetate fraction obtained from fractionating aqueous extract of *Crinum jagus* bulb using Agilent GC 7890A coupled with Agilent MSD 5975. The mass spectra of the compounds found were matched with the NIST, 2008 library. Agar diffusion method was used to evaluate the antibacterial activity of the oil in mm of inhibition zone against *Enterococcus faecium*, the most susceptible to the activity of the oil(6 -13mm), *Enterococcus spp*. was a bit sensitive to the oil(2- 6mm), while *Bacillus spp.*, *Staphylococcus aureus* and *Enterococcus fallacies* were resistant to the activity of the oil. The gas chromatographic-mass spectrophotometric analysis revealed predominantly the presence of n-hexadecanoic acid(24%) and 9,12-octadecadienoic acid (Z,Z)-(22.93%) in the oil. Others were butyl 9,12-octadecadienoate(6.12%), tetracosane(11.68%), nanocosane(4.05%), stigmasterol (5.12%) and γ-sitosterol(1.47%) amongst others.

***Keywords*—** Antibacterial, Crinum jagus bulb, gas chromatographic-mass spectrophotometric analysis, oil fraction

I. Introduction

Crinum jagus belongs to the *Amaryllidaceae* family. It is a widely distributed plant in the tropics and sub tropics. It is a tender perennial bulb that is native to tropical Africa with tulip-like white flowers, which bloom in clusters in dry season atop leafless stalks. It has strap-shaped green leaves and can grow up to 1m tall [22]. It is commonly called'ogede odo' in the southern western part of Nigeria. The common name asthma cough plant given to this plant is derived from its local use to relief asthma and related cough in some part of Nigeria [22]. It is widely used by traditional medicine practitioners in the western region of Cameroon as antidiabetic, antiobesity and antidiarrhoeal remedies [6]. It is used against mental trouble and snake bite [21]. It is employed in traditional medicine either singly or in combination with other plants in treating all forms of convulsive state [16], memory loss and other mental symptoms associated with ageing in southern Nigeria [24].

Alkaloidal extracts of the bulb was reported to show inhibition of acetylcholinesterase, an activity that was exploited therapeutically to raise the depressed levels of acetylcholine in brain associated with Alzheimer's disease [24], [15], [9], [23] . *Crinum jagus* bulb extract was reported to possess a significantly high antioxidant activity, an effect that was said to be more pronounced when compared with vitamin C at increased concentrations of 50-400 µg/ml [19]. Reference [3] research findings showed that the crude aqueous, ethanolic extracts and separated fractions of *Crinum jagus* bulb demonstrated significant anti-venom activity against rats injected with *Echis ocellatus* venom. Reference [14], reported that *C. jagus* ethanol/water extract was effective in decreasing glycemia, ameliorated the peripheral insulin sensitivity in induced diabetic rats and was not toxic, which they claimed justifies its use in the treatment of diabetes. The study carried out by[19], showed that methanolic *Crinum jagus* methanol bulb extract exhibited antioxidant and antimicrobial effects against some common wound contaminating microorganism both *in vitro* and *in vivo* in a wound healing model. Reference [18], showed that the acetone extract of crinum jagus bulbs may possess prophylactic and suppressive antiplasmodial activities which they said were dose-dependent. Reference [4] investigated the antimicrobial properties of the crude methanolic extract and chromatographic fractions from the bulb of *Crinum jagus* against clinical and laboratory isolates of bacteria and fungi using both agar well diffusion and agar dilution methods. The authors reported that the crude plant extract and its fractions demonstrated broad spectrum activity against all the bacteria and fungi isolates tested. Comparative assessment of the anticonvulsant activity of methanol extracts of *Crinum jagus* and *Solanum indicum* using mice and electroconvulsive shock equipment was carried out by [28]. The authors reported that all medicaments were protective against electrically induced convulsion, and that *C. jagus* showed protection after administration of 64.50 mg/kg body weight while *S. indicum* showed protection at 112.50 mg/kg body weight, showing that the bulb extract of *C. jagus* possess greater anticonvulsant effect than the fruit extract of *S. indicum.*

There is paucity of information about this bulb's oil fraction chemical components and bioactivity. Thus, this study carried out gas chromatographic-mass spectrophotometric (gc-ms) analysis of the oil fraction obtained from fractionating crude aqueous extract of *Crinum jagus* bulb, to identify the chemical compound present in the oil, and tested for its antibacterial activity.

II. Materials And Methods

A. Sample Collection And Sample Preparation

Crinum jagus bulbs were purchased at herbal shops at Oje,

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one of the local markets in Ibadan North local government area, Ibadan, Oyo state, Nigeria, West Africa. The scaly leaves of the bulbs were removed and washed with clean water and then with distilled water. The adherent water on the bulbs was allowed to drain off completely.

B. Preparation of Crude Aqueous Extract

Five hundred (500g) of *Crinum jagus* bulb was weighed, cut into pieces and soaked in 1.5 liter of distilled water for 72 hours. The crude aqueous extract was filtered after 72 hours. The distilled water was removed using rotary evaporator at 50^{o}C.

C. Column Chromatography

The column chromatographic separation of the crude extract into its components was done using the wet method. A slurry of the stationary phase (silica gel) was prepared with the eluent, and then carefully poured into the column. Care was taken to avoid air bubbles. 10g of the extract was weighed and dissolved in ethylacetate to form a slurry. It was carefully introduced on top of the stationary phase in the column. This layer was topped with cotton wool to protect the shape of the organic layer from the velocity of newly added eluent. The mobile phase was slowly passed through the column in graded manner to advance the sample components. The eluents were collected in a series of fractions. The oil fraction was obtained from 100% ethylacetate solvent as mobile phase.

D. Gas Chromatographic-Mass Spectrophotometric Analysis

The GC-MS analysis was carried out using Agilent Technologies 7890 equipped with 5975 MSD with HP -5ms capillary column (30m x 250 µm x 0.25µm). Helium was used as carrier gas at constant flow rate of 0.5ml/min. Injector temperature was set at 250^{o}C. The injection volume was 1µl, and was done in splitless mode. The oven temperature was programmed from 110^{o}C for 4minutes, then at 20^{o}C/min to 240^{o}C for 4min, and then at 20^{o}C/min to 280^{o}C for 74min. Run time is 90.5min.

E. Identification of Components

The identification of compounds was done by comparing the spectra data of sample with reference spectra in spectral libraries of National Institute of Standard and Technology (NIST), 2008.

F. Antimicrobial Analysis

The antimicrobial activity of the oil was determined using agar diffusion method as described by reference [2],. The tested organisms were *Bacillus species*, *Staphylococus aureus*, *Enterococcus* species, *Enteroccocus faecalis*, *Entero ferecium*. Prepared sterile Mueller-Hinton agar was inoculated with standardized organisms of a day old culture. Four concentrations of 150mg/ml, 75mg/ml, 36.5mg/ml, and 18.75mg/ml of the oil were prepared. Sterile cork borer of 5mm was used to make four ditches on each plate. The ditches were then filled with 0.5ml of each of the oil concentrations, and labeled appropriately. The oil was allowed to diffuse into the medium and the plates were incubated at 37^{0}C for 24hrs. After incubation, the plates were observed for the zone of clearance around the ditches. The zones were measured in millimeter (mm). The actual zones of inhibition were calculated by subtracting the diameter of respective holes from the diameter of zones around the holes.

III. Results

The Chromatogram of the oil is presented in Fig.3. (a), and the mass spectra of the major components of the oil are presented in Fig. 3.(b) and Fig. 3.(c) as shown below:

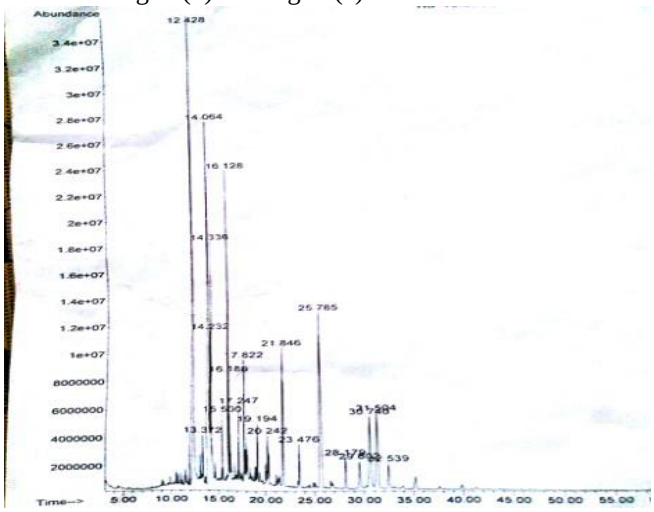

Fig. 3. (a). The Chromatogram of the Oil

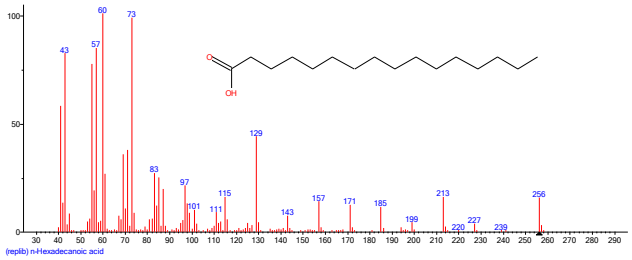

Fig. 3. (b). N-Hexadecanoic acid Mass Spectrum

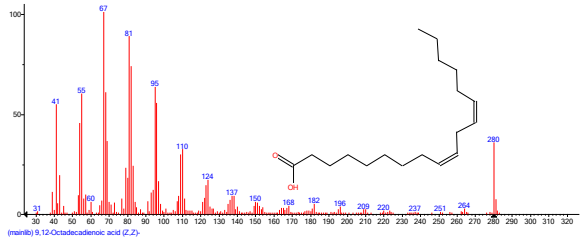

Fig . 3. (c). 9,12-Octadecadienoic acid (Z,Z)- Mass Spectrum

TABLE I: Major Compounds Identified in Oil Fraction

S/N	Compound	Molecular mass	Retention Time	Area%
1	N-Hexadecanoic acid	256	12.428	24.00
2	9,12-Octadecadienoic acid (Z,Z)-	280	14.062	22.93

TABLE II: ANTIMICROBIAL ANALYSIS RESULT EXPRESSED IN MILLIMETER (MM) OF ZONE OF INHIBITION

Oil Fraction Concentration (mg/ml)	*Bacillus Spp.*	*Enterococcus spp.*	*S. aureus*	*E. fallacies*	*E. faecium*
150	0	6	0	0	13
75	0	4	0	0	11
37.5	0	2	0	0	10
18.75	4	2	0	0	6

0 implies no zone of inhibition was observed

IV. DISCUSSION

The gc-ms analysis revealed predominantly the presence of n-hexadecanoic acid (24%) and 9,12-octadecadienoic acid (Z,Z)- (22.93%) in the oil as shown in Table I. The comparison of the mass spectral of the two compounds with NIST, 2008 spectral library gave 100% and 95% match respectively. N-hexadecanoic acid is a saturated fatty acid, and has been reported to have anti-inflammatory property [29],[8]. 9,12-octadecadienoic acid (Z,Z)- is a polyunsaturated omega-6 fatty acid, an essential fatty acid that must be consumed for proper health [13]. The comparison of the mass spectral of other compounds identified with NIST, 2008 spectral library was less than 50% match, though the molecular weights and the structures matched. Amongst these compounds are the esters of n-hexadecanoic acid and 9,12-octadecadienoic acid (Z,Z)-, which include, hexadecanoic acid, butyl ester, butyl 9,12-octadecadienoate and 9,12-Octadecadienoic acid (Z,Z)-, methyl ester. These have various bioactivities which includes: antifungal, antioxidant, hypocholesterolemic, nematicide haemolytic, 5-alpha reductase inhibitor, antimicrobial, hepatoprotective , and antieczemic were reported of these compounds[1], [17]. Also identified were stigmasterol(5.12%), γ-sitosterol(1.47%) and campesterol(1.41%).These are phytosterols generally known to possess cholesterol lowering properties which may reduce the risk of heart diseases [17], [10], [11], [12] and have been reported to possesses antioxidant, hypoglycemic, anti-cancer and anti-inflammatory properties [25], [26], [27]. Saturated alkanes such as tetracosane, nanocosane were also identified.

The antibacterial activity test as presented in Table II, revealed that *Enterococcus faecium* was the most susceptible to the effect of the oil, with the zone of inhibition range of 6-13mm. *Enterococcus spp*. was a bit sensitive to the oil, with the zone of inhibition range of 2-6 mm, while *Bacillus spp*., *Staphylococcus aureus* and *Enterococcus fallacies* were resistant to the activity of the oil. The antibacterial activity of the oil against *Enterococcus faecium and Enterococcus spp*. might be due to the predominant acidic components of the oil.

V. CONCLUSION

The oil of *Crinum jagus* bulb is a source of significant bioactive compounds which can be therapeutically employed, especially in the treatment of infections caused by *Enterococcus faecium, and also of Enterococcus spp*. probably at higher concentration.

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