Chemistry International 10(2) (2024) 59-68

International Scientific Organization
http://iscientific.org/
Chemistry International
www.bosaljournals.com/chemint/

Physicochemical profiling of *crinum zeylanicum* oil: Antifungal activity of its bulb extracts in n-hexane, ethanol and water

Janet Moses Daben[1*], Dayil Albert Dashak[2] and Amina Mika Lohdip[2]

[1]Department of Science Laboratory Technology, Faculty of Natural Sciences, University of Jos, PMB 2084, Jos, Nigeria
[2]Department of Chemistry, Faculty of Natural Sciences, University of Jos, PMB 2084, Jos, Nigeria
*Corresponding author's E. mail: janetdaben1@gmail.com

ARTICLE INFO

Article type:
Research article
Article history:
Received November 2023
Accepted March 2024
April 2024 Issue
Keywords:
Aspergillus spcies
Ethno-medicine
Soap content
Solvent extracts
Susceptibility
Microorganisms

ABSTRACT

This study was performed to determine the quality and medicinal values of the oil and the anti-fungal activity of the extracts to buttress traditional medicinal practices. Soxhlet extraction of *n*-hexane, ethanol and water extracts were performed and antifungal activity was evaluated against *Yeast, Aspergillus niger, Aspergillus fumigates and Aspergillus flavus*. The oil percentage (3.71±1.03%), moisture content (57.65±1.16%), and refractive index (1.4667±0.06). Saponification value (205.62±0.58 mg/KOH/g), peroxide value (4.59±1.12 meq/kg), iodine value (129.74±0.39 mg/g), free fatty acid (5.93±1.02%), acid value (11.80±1.72 mg/KOH/kg), relative density at 25^{0}C (0.86kg/m^3), viscosity (0.94±0.93) was recorded, while soap content was not detected. The antifungal susceptible test of the water extract showed a marked inhibitory effect on all the test organisms especially on *A. niger* more than the control at 300 mg/mL, ($p < 0.0001$); having the least inhibitory activity on *yeast*. N-hexane and ethanol extracts have a significant effect on *A. niger* and the least on *A. fumigatus*. The Minimum inhibitory concentration (MIC) revealed n-hexane extract at 75 mg/mL having a potent effect on *Yeast, A. flavus, A. fumigates* and for water and ethanol extracts varied at 37.5 mg/mL to 150 mg/mL on *A. flavus* respectively. The minimum fungicidal concentration (MFC) of water extract remarkably showed resistance on *A. flavus* and the least action on *yeast* ranging from 7.5 to 150 (mg/mL). This study indicated that the oil has low susceptibility and potent anti-fungal activity suitable for health benefits.

Capsule Summary: The study revealed that the oil exhibits favorable chemical properties suitable for pharmaceutical exploration, while the bulb extracts demonstrate promising potential for antifungal activity.

Cite This Article As: J. M. Daben, D. A. Dashak and A. M. Lohdip. Physicochemical profiling of *crinum zeylanicum* oil: Antifungal activity of its bulb extracts in n-hexane, ethanol and water. Chemistry International 10(2) (2024) 59-68.
https://doi.org/10.5281/zenodo.11036094

INTRODUCTION

Crinum zeylanicum (L.) is a species of flowering plant in the family Amaryllidaceae (Yakandawala and Samarakoon, 2006). It is commonly called milk, wine or Ceylon swampy lily and beautiful crinum in English (Hutchinson, 1964; Burkill, 1985; Yakandawala and Samarakoon, 2006). This plant is known to be bulbous and can be grown from dormant bulbs gathered during the dry season in a warm

temperate region (Yakandawala and Samarakoon, 2006). In Plateau State, the Doemak people called it *Dau,* Mushere (*Leh'zipir*) (Daben and Dashak, 2016), Berom (*Juéébót)*, Gumai (*Ndan-murenang)* and other parts of Nigeria in Hausa (*gadali- Albassar kwadi*), Yoruba (*Isumeri*) (Burkill, 1985). Also, commonly found in Senegal to west Cameroun and throughout tropical Africa (Dalziel, 1956; Burkill, 1985) and the sub-tropical part of the world (Yakandawala and Samarakoon, 2006). The oil may not have been specifically extracted or isolated for any use but the whole bulb has been medicinally important. The plant parts have shown unique properties and have a long history of usage in traditional folklore medicine in Nigeria. Among the uses are; as surfactants in fractured bone setting and healing (Daben and Dashak. 2016); wound healing (Burkill, 1985; Tijani et al., 2012), convulsion, refractive ulcer (Inger and Rolf, 1982), ricket remedy in children (Agrahari et al., 2010), anti-inflammatory (Mukherjee, 2000), anti-proliferative against humane tumor cell (Strahil et al., 2011), remedy for ear ache and malaria (Tsuda et al., 1984; Strahil et al., 2011).

Few research or there could be nonelaborate physicochemical properties and anti-fungal activity on *C. zeylanicum* bulb owing to the high cost of chemotherapeutic drugs, there is a need for such scientific research for naturally occurring chemical components for health benefit. The physical and chemical constituents of oil as considered in this work can be used to investigate the quantitative and qualitative composition of oil. The terms lipid, fat and oil are used most often interchangeably but oil refers to those that are liquid at room temperature, while fat and oil belong to the wider group of naturally occurring substances called lipids (Aremu et al., 2015). Their flavor in foods is a desirable component and also serves as a source of oleo chemicals (Morrison et al., 1995). Vegetable oils as sources of protein, lipids and fatty acids help in the repair of worn-out tissues, and cell formation as useful energy potential (Aremu et al., 2015). Several researches have been reported on oil like the study on quality characteristics of *Luffa aegyptiaca* seed oil (Abayeh et al., 2013); Comparative analysis of physicochemical properties of extracted and collected palm oil and tallow (Abdulkadir and Jimoh, 2013); Chemical and physicochemical properties of moringa flours and oil (Abiodun et al., 2012); Effect of drying temperatures on physicochemical properties and oil yield of African star apple (Ajala and Adeleke, 2014); Physicochemical studies on oils from five selected Nigerian plant seeds (Akubugwo and Ugbogu, 2007) and many others. These findings and several others obtained from such research may lead to the validation of traditionally used medicinal plants which enable full usage of their properties.

Aspergillus species are saprophytic and pore-forming molds infections found throughout most of the natural world (Stevens et al.,1999; Klich, 2007). Known to grow on plants, soil dead leaves, household dust, compost piles (Loudon et al., 1996; Marcelo et al., 2018) and many others common practices in developing countries. Though people breathe in *Aspergillus* spores without getting sick people with weakened immune systems or lung diseases are at high risk (Kimmerling et al., 1992; Natagawa et al., 1999; Bulpa et al., 2007). Other reported risks are patients with severe influenza virus infection, worsening asthma, subtle genetic immune defects, invasive and non-invasive aspergillosis and aspergilloma (Pasqualotto and Denning 2008; Janwal et al., 2018). Among the diseases caused by *Aspergillus* species none is as opportunistic as *A. fumigates and A. flavus* and no conclusive virulent factor have been identified (Kozakiewicz, 1995; Berkow et al., 2018). Therefore, this study was conducted to evaluate the physiochemical and anti-fungi activity to suit the purpose of pharmaceutical applications to improve the immune system of patients at risk.

MATERIAL AND METHODS

Sample collection and preparation

Crinum zeylanicum bulbs were obtained from an organic garden of a traditional health practitioner in Kopmabar village (Doemak District), Qua'an Pan Local Government Area (LGA) of Plateau State-Nigeria. The bulbs were conveyed to the laboratory in a sack, thereafter cleaned free of soil debris, chopped into smaller sizes and oven-dried at 60 °C. The dried sample was milled and stored in an airtight container for further analysis (Harborne, 1991).

Extraction and determination of oil content

The percentage oil content of the sample was determined according to Harborne, (1991). The sample (100 g) was weighed into a fat-free silk cloth and placed in a Soxhlet extractor of 500 mL capacity and 300 mL of n-hexane (68 ^{o}C boiling point) was poured into a 500 mL quick fit flask. The setup was heated at 65-68 ^{o}C steadily as the sample was continuously extracted for 3 hours. The mixture of the extraction containing the oil was heated in the water bath to evaporate the n-hexane from the oil. This was done in triplicate and the percentage of oil extracted was determined using equation 1.

$$\text{Oil yield } (\%) = \frac{W1 - W2}{Wt} \times 100 \quad (1)$$

Where $W1$ = Weight of sample before extraction, $W2$ = Weight of sample after extraction and Wt = Weight of sample

Determination of physicochemical parameters of the oil

The physical and chemical analysis content were moisture content, refractive index, Saponification value, peroxide value, iodine value, free fatty acid, acid value, relative density, viscosity and soap content. All procedures were done in triplicate for all the parameters, data was averaged and reported as mean± standard deviaition.

Moisture Content (MC)

Table 1: Physicochemical analysis of *C. zeylanicum* bulb oil

Parameters	Values
Oil yield	3.71±1.03%
Moisture content	57.65±1.16%
Refractive index	1.4667±0.06
Saponification value, mg/KOH/g	205.62±0.58
Peroxide value, meq/kg	4.59±1.12
Iodine value	129.74±0.39 mg/g
Free fatty acid	5.93±1.02%
Acid value, mg/KOH/kg	11.80±1.72
Relative density at 25 ^{0}C	0.86 kg/m^3
Viscosity	0.94 mPa^{-s}
Soap content	Nil

The moisture content was determined according to the procedures of Harborne, (1991): Briefly, 5 g of the fresh sample was oven dried at 105 °C for 24 hours with absence of moisture, transferred into a desiccator to cool after which it was weighed and the loss in weigh was expressed as percentage moisture content using equation 2.

$$\text{Moisture content } (\%) = \frac{W1 - W2}{Wt} \times 100 \quad (2)$$

Where, $W1$ = Weight of sample before drying, $W2$ = Weight of sample after drying and Wt = Weight of sample.

Refractive index (RI)

The refractive index was detected through a refractometer with two drops of the oil on the glass slide of the refractometer and water at 30 ^{o}C was circulated the glass slide for a uniform temperature. The dark portion view of the refractometer was adjusted in line with the intersection of the cross at no parallax error. The pointer on the scale was read and taken for the refractive index value (Muoka and Ibeh, 2018).

Saponification value (SV)

Two grams (2 g) of the oil was weighed into a 250 mL conical flask and 25 mL of 0.5 M ethanolic potassium hydroxide was added. The flask was corked and the mixture was refluxed to remove the fat for 30 mins and allowed to cool. Three (3) drops of phenolphthalein indicator were added to the mixture and titrated with 0.5M HCl until a pink endpoint was reached. A blank was determined with the same conditions. The saponification value was calculated using the formula in equation 3 as reported by Belay & Sisay, (2014); Tesfaye & Abebaw, (2016); Tsado, et al., (2018).

$$Saponification\ value\ (\%) = \frac{(B-S)\ x\ N\ x\ 56.10}{Wt} \quad (3)$$

Where B = Volume of HCl used to titrate the blank sample, S = Volume of HCl used to titrate the sample, N= Molarity of the acid, 56.1 = Equivalent weight of KOH and Wt = Weight of sample in grams.

Peroxide value (PV)

The oil (2 g) was weighted into a 250 mL conical flask which was dissolved in 30 mL of glacial acetic acid and chloroform in a mixture of 3:2 v/v ratios and 20 mL of 5% potassium iodide solution was added after cooling. Subsequently, 3 drops of starch indicator were added and slowly titrated to liberate I_2 in 0.02N $Na_2S_2O_3$, which gives the mixture a black coloration. Titration was also performed for blank. The peroxide value was expressed according to Equation 4 (Tesfaye & Abebaw, 2016; Tsado et al., 2018).

$$Peroxide\ value\ (\%) = \frac{S\ x\ N}{Wt} \times 100 \quad (4)$$

Where S = The volume of $Na_2S_2O_3$ used, N = The normality of $Na_2S_2O_3$ and Wt = Weight of oil sample.

Iodine value (IV)

A known weight of 0.2 g of the oil was dissolved in 15 mL of CCl_4 (Tetrachloromethane) in a 100 mL glass stopper flask and 25 mL of Wijj's solution was added and shaken vigorously for a clear solution. The mixture was then allowed to stand for 30 minutes at room temperature in the dark when 20 mL of 10% aqueous KI solution was added to the mixture. The resultant solution was then titrated with an accurately standardized thiosulphate solution (0.1 M) in the presence of a starch indicator into a blue endpoint. A blank titration was also carried out with the same conditions (Muoka & Ibeh, 2018). The iodine value was calculated using the formula given in Equation 5.

$$\text{Iodine value } (\%) = \frac{(B-S)\ x\ N\ x\ 1.269}{Wt} \times 100 \quad (5)$$

Where B = Blank titer value, S = Sample titer value, N = Normality of $Na_2S_2O_3$, 1.269 = Equivalent weight of KOH and Wt = Weight of sample used.

Free fatty acid (FFA)

The sample 3 g was weighed and dissolved in 50 mL absolute ethanol, followed by heating to boiling point in a 250 mL conical flask. Then 3 drops of phenolphthalein indicator were added to the resultant solution and titrated against 0.1N of NaOH until the appearance of a permanent pink color endpoint was reached. The free fatty acid values was determined as stated in equation 6 (Abdulkadir & Jimoh, 2013).

$$\text{Free fatty acid } (\%) = \frac{T \times N \times 2.82}{Wt} \times 100 \quad (6)$$

Table 2: Susceptibility profile of fungi isolates to different concentrations of n-hexane extract

Concentration (mg/cm^3)	Mean ± SEM			
	Yeast	*A. Niger*	*A. Fumigatus*	*A. flavus*
300	22.00 ± 0.33^a	26.00 ± 0.58^a	20.00 ± 0.58^b	20.00 ± 0.58^a
150	16.00 ± 0.33^b	24.00 ± 0.58^b	17.00 ± 0.58^c	18.00 ± 0.58^b
75	13.00 ± 0.33^c	20.00 ± 0.58^c	15.00 ± 0.58^d	15.00 ± 0.58^c
37.5	11.30 ± 0.33^d	18.00 ± 0.58^d	13.00 ± 0.58^e	12.00 ± 1.58^d
18.75	9.00 ± 0.33^e	15.00 ± 0.58^e	10.00 ± 0.58^f	8.00 ± 0.58^d
Control	13.00 ± 0.33^c	27.00 ± 0.58^a	23.00 ± 0.00^a	21.00 ± 0.00^a
ANOVA	187. 200	68.000	63. 360	90.240
P-value	< 0.0001	< 0.0001	<0.0001	< 0.0001
	****	****	****	****

Values are means of triplicate reading. Means values were separated using Duncan's Multiple Range Test. Mean values with different superscripts in the same column are significantly different, **** = significant difference exists at p < 0.0001

Table 3: Susceptibility profile of fungi isolates to different concentrations of the aqueous extract

Concentration (mg/cm^3)	Mean ± SEM			
	Yeast	*A. Niger*	A. *Fumigatus*	*A. flavus*
300	17.33 ± 0.33^a	25.00 ± 0.58^a	20.00 ± 0.58^a	22.00 ± 0.00^a
150	15.67 ± 0.33^b	23.00 ± 0.58^b	17.00 ± 0.58^b	20.00 ± 0.58^b
75	12.67 ± 0.33^c	19.00 ± 0.58^{cd}	15.00 ± 0.58^c	16.67 ± 0.33^c
37.5	8.67 ± 0.33^d	18.00 ± 0.58^d	13.00 ± 0.58^d	16.67 ± 0.58^c
18.75	5.67 ± 0.33^e	10.00 ± 0.58^e	10.00 ± 0.58^e	16.00 ± 0.33^d
Control	13.33 ± 0.33^c	20.67 ± 0.33^{cd}	20.67 ± 0.33^a	20.33 ± 0.33^b
ANOVA	171.067	42.747	75.954	75.911
P-value	< 0.0001	< 0.0001	<0.0001	< 0.0001
	****	****	****	****

Explanation as given in Table 2

Where T= Titre value, N = The normality of NaOH, 2.82 = constant weight of oleic acid neutralized by 1 mg of NaOH) and Wt= Weight of oil used.

Acid value (AV)

In a 250 ml conical flask, 2 g of the oil was neutralized with a mixture of 25 mL of diethyl ether in alcohol 1:1 (v/v). The resultant solution was swirled then boiled for five minutes and allowed to cool before 3 drops of phenolphthalein indicator was added. This was titrated against 0.1M KOH until a pink-colored solution was obtained, indicating the endpoint alongside the blank. The number of milligrams of KOH needed to neutralize one gram of the oil was calculated as the acid value shown in equation 7 (SON, 2000).

$$\text{Acid value (\%)} = \frac{\text{T} \times \text{N} \times 56.1}{\text{Wt}} \times 100 \quad (7)$$

Where T = Volume of KOH used, N = The normality of standard KOH, 56.1 = molar mass of potassium hydroxide (KOH) and Wt = Weight of oil used.

Relative density (RD)

A cleaned dried pre-weighed relative density bottle of 10 mL capacity was filled with distilled water and maintained in a water bath until the temperature of the water reached 20 °C. The same bottle was emptied, dried and filled with samples to the same mark (10 mL) and the process was repeated as done for water. The RD of the sample was calculated using Equation 8 (Abdulkadir & Jimoh, 2013).

$$\text{Relative Density} = \frac{\text{M2}}{\text{M1}}[1 + \gamma(\text{T} - 20)\ ^\circ\text{C}] \quad (8)$$

Where RD = Relative density, $M1$= Mass of water, $M2$= Mass of oil, T= 20 °C and γ = Coefficient of cubical expansion for borosilicate glass (0.00001 k^{-1}).

Soap content (SC)

In a mixture of 50 mL of 95% of acetone and 5 drops of bromophenol indicator, 30 g of the oil was added which gave a light-yellow color. The mixture was further shaken and allowed to stand, after which it was titrated against 0.01N HCl solution (A.O.A.C, 1990). The soap content was obtained using Equation 9.

$$\text{Soap content (\%)} \frac{\text{T} \times \text{N} \times 56.1}{\text{Wt}} \times 100 \quad (9)$$

Where T = Titre value, N = The normality of standard HCl, 30.44 = Soap constant and Wt = Weight of oil used.

Extraction of bulbs procedure

Three hundred and fifty grams (350 g) of the bulb sample was extracted using 750 mL of the solvent in a 1000 mL Soxhlet extractor with one gram (1 g) of anti-bumping granules and after 8 hours of continuous refluxing, the extracts were collected and then concentrated by the use of vacuum rotary evaporator at 50 °C (Harborne, 1991).

Antifungal activity

Test organisms and preparations

Clinical isolates of *Yeast, Aspergillus niger, Aspergillus fumigates, and Aspergillus flavus* having certified pure were obtained from the Department of Microbiology, University of Jos, Plateau State, Nigeria. The fungi were maintained on the slant potatoes dextrose agar (PDA, Difco- prepared according to manufacturer's instruction) and incubated at 25 °C for 48 hours.

Agar well diffusion method

The stock solution of the plant extracts was prepared by dissolving 3 g of the crude extract in 10 mL of distilled water to obtain 300 mg/mL as the highest stock solution. It was then serially diluted to obtain 150 mg/mL, 75 mg/mL, 37.50 mg/mL and 18.50 mg/mL (Atlas, 1995; Ochei and Kochatkar, 2007) while Nestatine at 20 mg/mL was included as positive control. Agar well diffusion method as described by (Sanchez et al., 2005) was used for the antifungal screening. From inoculation, the sterilized PDA at 25 °C with the organisms was set on the disinfected plates and equidistant wells were bored using a sterile cork borer of 4 mm diameter. 0.2 mL of prepared extracts of different concentrations as well as the standard drug was transferred into the made holes of the agar. The culture plates were allowed to stand for 30 mins for pre-diffusion and the fungi were incubated for 48 hours at 25 °C after which the zone of inhibition was examined.

Minimum inhibition concentration (MIC)

A serial double dilution technique was employed were solutions containing reconstituted extracts (300 mg/mL, 150 mg/mL, 75 mg/mL, 37.50 mg/mL and 18.50 mg/mL). These were incorporated in the potato dextrose broth with an accurate volume of 0.1 mL of the test fungi suspension prepared by McFarland's turbidity standard scale No. 7 of an overnight culture; and transferred to respective sets of the test tube except the control. After shaking to mix, the test tubes were incubated at 25 °C for 48 hours in an incubator. The test tubes were examined for the growth or death of organisms. The presence of the turbidity indicated growth in the test fungi. The highest concentration that inhibited the visible growth of the fungi was observed and recorded as the Minimum Inhibitory Concentration (MIC) of the extracts for that particular organism. The tube with the lowest concentration of the extract showing clear solution or death represents the Minimum Inhibitory Concentration. The test was conducted under aseptic conditions.

Minimum fungicidal concentration (MFC)

The Minimum Fungicidal Concentration (MFC) was determined by subculturing the inhibited discs of each fungal isolate on the PDA medium separately. Sub-culturing was done by streaking a loopful of the required MIC plates over the surface of the already set agar. This was incubated overnight at 25^0C for 48 hours. The MFC was recorded as the lowest concentration of the test extract without growth observed on the PDA plates more than 99.5% killing of the original inoculums.

Statistical Analysis

The results of the statistical analysis were produced in triplicate and the percentage, mean, and standard errors, were calculated by ibmSPSS version 23 and results were expressed as the % Mean ± SEM. Mean values were separated using Duncan Multiple Range Test and Comparison using Two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The results for the physical and chemical compositions of *C. zeylanicum* bulb oil showed that the bulbs have a high moisture content (57.65%). Considering the Refractive Index (RI) value (1.4667), Saponification Value (SV) (205.62 mg/KOH/g), Peroxide Value (PV) (4.59meq/kg) and Iodine Value (IV) (129.74gI_2/100g) showed values are within the range of acceptability (Table 5). The measured values of Free Fatty Acid (FFA) (5.93%), Acid Value (AV) (11.80mg/KOH/g) were higher while viscosity at room temperature (0.94mPa^{-s}), Relative Density (RD) (0.860kg/m^3) are lower than the recommended level and there was no trace of soap properties (Table 1).

The moisture content (57.65%) as compared to earlier reported (91.08%) of *C. zeylanicum* bulb (Dashak and Ano, 2007), showed that the lower moisture content in this study increases the resistible value or shelf life of the bulb. Further, this difference might be linked to the season of harvest; expectedly, higher during the rainy season than the dry season. To its merit, *C. zeylanicum* plant is propagated from dormant bulbs gathered during the dry season and such survival indicates that its moisture sap might be resistant to microbial development.

According to the CODEX standard for fats and oils from plant sources (1.4677-1.4707), the RI value (1.4667) obtained was within the range of acceptability limit for virgin, refined, and refined pomace oils (CODEX, 1999). However, the value was higher than many edible oils, such

as sunflower oil, palm oil, and garlic oil as reported by Rafe and Nadjafi (2014). Though the RI was lower than for moringa seed oil (Ogbungafor et al., 2011; Abiodun et al., 2012) and yellow pumpkin seed oil (Nwabanne, (2012), it has helped us to assume that this researched oil is less prone to rancidity; since rancidity is caused by oxidation of unsaturated fatty acids (Rafe & Nadjafi, 2014). Therefore, it can be distinguished for medicinal use.

Our finding for SV was within the range (195-205 mg/KOH/g) for palm oil as stipulated by NIS (1992); SON, (2000). It's worth noting that SV rises with volatility; as a result, a high SV indicates a high proportion of lower fatty acids (Pearson, 1976), whereas a low SV is likewise a high proportion of higher fatty acids, giving rise to non-edibility (Aremu et al., 2015).

The PV (4.59 meq/kg) of the oil in this study compared to the standard range for seed and nut oil (≤ 10 meq/kg) by NIS (1992); SON, (2000), was higher than some medicinal and industrial oils reported by other authorities, such as Black turtle bean oil (3.2) (Audu et al., 2013); African star apple seed oil (1.80-1.98) (Ajala and Adeleke, 2014) and soya beans oil (3.5) (Akanni, et al., 2005). The researched oil on the other hand is lower than yellow calabash seed oil, sesame seed oil, and African pear oil as reported by previous researchers (Olaofe et al., 2012; Mohammed and Hamza, 2008; Ogbonna and Ukaan, 2013; Akubugwo and Ugbogu, 2007; Ajayi and Adesanwo, 2009), respectively.

Additionally, based on the IV obtained (129.74 gI_2/100g), the oil can be used in various ways as a semi-drying agent (< 130 gI_2/100g); drying agent (≥130 gI_2/100g) and it can be a used for alkyl resins in paints, varnish, and production of ink (Aremu et al., 2015; Tsado et al., 2015). However, the IV level was within the limit that might not cause vulnerability to oxidative rancidity; similar to that was measured by Abayeh et al., (2013) for luffa gourd at 130 gI_2/100g; Akanni et al., (2005) for African pear seed oil at 130 gI_2/100g; but less than 140 and 150 gI_2/100g previously reported for sunflower (Fakhri and Qadir, 2011) and white melon seed oil (Olaofe et al., 2012) respectively.

Nevertheless, the amount of FFA in this investigation was higher than the acceptable level. High-quality oils contain a minimal amount of FFA so the study oil needs to be refined for edibility. This is uneconomical in crude vegetable oil because it causes large losses of the neutral oil during refining, lowers the smoke point, causes popping during cooking, and shortens the oil's shelf-life (Aremu et al., 2015). Others attributed it to a low melting point and a low pH level (Kamalu & Ogbome, 2008; Muoka and Ibeh, 2018). However, there is a possibility for the oil to be commercialized according to specifications.

The AV in our investigation (11.80 mg/KOH/g) showed a higher value above the allowed edible limit (0.6 mg/KOH/g) (A.O.C.S. 1990), notwithstanding, it is comparable to 10.70 for cashew nut seed (Evbuowman et al., 2013) and 12.60 for pumpkin seed oil (Bwade et al., 2013). On the other hand, lower to the findings by Belewu et al., (2010); Abayeh et al., (2013) reported 18.76, and 20.62 for Curcas seed oil and Luffa gourd respectively. Even though the value is above the edible limit, it could be modified as a therapeutic oil for topical uses.

Viscosity (V) was determined by the chemical properties of the triglycerides (TGs) contained in the oil, such as chain length and saturation and/or unsaturation. The viscosity (0.94 mPa^{-s}) at room temperature is low comparable to the recommended American Standard for Testing of Material – ASTM (Knothe and Dunn, 2003). Since the viscosity of oil determines how well it may be used as a lubricant (Belewa et al., 2010); there might be a possibility to improvement for biodiesel or lubricant oil.

Again, the RD was slightly lower than the SON (2000) approved for the density standard range (0.898-0.907g/mL). This was closely related to Danguguwa, (1983); Vaugham, (1990); Kamalu and Ogbome (2008) who reported a range of 0.717-0.924, implying a high anti-oxidant level and strong lubricating characteristics (Nangbes et al., 2013).

The absence of soap characteristics in this study also revealed that the sample oil has high unsaponifiable matter that cannot be converted into soap which agrees with the low saponification, iodine, and high acid values suggesting that the oil may not be a good soap material.

Studying the fungi toxic effects of the plant extracts of *C. zeylanicum* bulb in Table 2-7, indicate significant differences in their inhibitory effects on the test organisms at various concentrations of the plant extracts. It goes to show therefore that experimentally; the extracts can be an important source of antifungal compounds that may provide a renewable source of antifungal drugs. This agrees with the conclusion of other researchers stating that plant sources have been an important natural active constituent (Fabricant and Farnsworth, 2001; Wuzella et al., 2011) of antimicrobial substances (Yinusa et al., 2007; Okon and Echeme, 2018), antifungal activities (Ali-Shtayeh and

Table 6: Minimum inhibitory concentration (MIC) of the extracts

Yeast	75.00±0.19	75.00±1.13	75.00±1.75
A.Niger	37.50±0.06	75.00±0.43	75.00±0.02
A.Fumigatus	75.00±0.24	75.00±2.01	75.00±1.84
A.Flavus	75.00±1.57	150.00±0.13	37.50±2.91

Table 7: Minimum fungicidal concentration (MFC) of the extracts

Yeast	150.00±1.12	150.00±0.32	150.00±0.13
A.Niger	75.00±0.75	150.00±0.17	75.00±0.41
A.Fumigatus	300.00±1.23	300.00±0.04	75.00±0.47
A.Flavus	300.00±0.18	150.00±0.07	37.50±0.05

Suheil, 1999; Ayanbimpe et al., 2005) including the antibacterial activities of the *C. zeylanicum* bulb (Daben et al., 2017).

In Table 2, *yeast* was more susceptible to n-hexane extract at ≥75mg/mL than the control and other organisms used. The n-hexane extract constituents do not vary significantly with that of the volatile oil as reported to have antibacterial effects (Okhale et al., 2018) making it a broad-spectrum antibiotic to yeast infections commonly candidiasis. This result may be attributed to differences in the nature and/ or concentration of chemical inhibitors in the n-hexane extract (Ali-Shtayeh and Suheil, 1999). This resistance effect of n-hexane extract is not a cause for concern since the oil anti-pathogen mechanism is through triglyceride components, a natural component that kills micro-organisms by rupturing the cell membrane (Ayimbimpe et al., 2005).

The ethanol extract in Table 3, showed that the inhibition of *yeast* reduced to ≥150 mg/mL and *A. flavus* at ≥300 mg/mL was included to have an inhibitory effect more than the control. It appears that ethanol extract is superior in inhibition than n-hexane extract (Table 5) in this case owing to the inhibition of *A. flavus* known to be the second leading cause of *Aspergillus* species infections (Pasqualotto and Denning, 2018). *A. flavus* which have been known to produced cyclopiazonic acid a major contributor to the degeneration and necrosis of various organs has low oral toxicity (Amaikes and Keller, 2011) and Aflatoxin a major problem in developing country, contaminant maize responsible for the death of hundred people in Kenya in recent years and dogs in the U.S. in 1998 and 2005-2006 (Pitt and Hocking, 2009).

Table 4 varied significantly and observed to inhibit the growth of completely the test organisms at varying concentration than the control but at no significant difference than the control .This evident show that water extract may contain more than one bioactive component that was different from n-hexane and ethanol extracts; especially the inhibition of *A. fumigatus, A. flavus* and *A. niger* produce a variety of effects on humans ranging from illness to allergic reaction to mild pneumonia to overwhelming generalized infection (Pitt and Hocking, 2009 ;Amaikes and Keller, 2011).

The results of the MIC and MFC in Table 6 and 7 respectively concerted with the inhibitory and fungicidal activities of the plant extracts on the test organisms. Showing that the lowest concentration of the plant extracts capable of inhibiting the growth of the fungi was: *Yeast* and *A. fumigatus* (≥ 75mg/ml), *A. flavus* (37.5- 150 mg/ml) and *A. niger* (37.5mg/ml). The MFC also produced greater inhibitory action against *A. flavus* (37.5), *A. fumigatus* and *A. niger* (≥ 75mg/ml) and *Yeast* (≥ 75mg/ml) in the case of water extract but higher concentration was obtained for n-hexane and ethanol extracts. From history, traditional health practitioners prepare health extracts from water and it has been noted that 80% of modern medicine which were derived from traditional plants sources; had a traditional use identical or related to the current use of active elements of the plants (Fabricant and Farnsworth, 2011; Amekyeh et al., 2024; Kabiraj and Deshmukh, 2024; Portella et al., 2024; Purwono et al., 2023).

CONCLUSIONS

This research is embarked upon in view to add to existing literature to validate the potency most especially for bone fracture healing. The analysis of the oil was determined to evaluate the quality, stability and applicability in comparison to the specified standard for food and medicines. The results showed that the bulb could be valid for medicinal uses considering its values for refractive index, saponification, peroxide and iodine with no trace of soap properties. However, the anti-fungal activity is clear that the plant's bulb could be used as remedy for *Aspergillus* infectious diseases. This work can be analyzed further on the efficacy of the oil on bone fracture healing in a system.

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interest.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of the Office of the Research Development (ORD) University of Jos Plateau State, Nigeria and the professional personnel in Department of Microbiology of the same institution for the antifungal activity.

REFERENCES

Abayeh, O.M., Garba, I.H., Adamu, H.M., Abayeh, O.J., 2013. Quality characteristics of *Luffa aegyptiaca* seed oil. International Journal of Scientific and Engineering Research 4(4), 11-16.

Abdulkadir, A.G., Jimoh, W.L.O., 2013. Comparative analysis of physicochemical properties of extracted and collected palm oil and tallow. Chemical Search Journal 4(2), 44 – 54.

Abiodun, O.A., Adegbite, J.A., Omolola, A.O., 2012. Chemical and physicochemical properties of moringa flours and oil. Global Journal of Science Frontier Research Biological Sciences 12(5), 12-18.

Agrahari, A.K., Panda, S.K., Meher, A., Padhan, A.R., 2010. Screening of wound healing activity of *Curculigo orchioides* Gaertn root tubers methanolic extract. International Journal of Pharmaceutical & Applied Sciences 1(1), 1-5.

Ajala, A.S., Adeleke, S.A., 2014. Effect of drying temperatures on physicochemical properties and oil yield of African star apple (*Chrysophyllum alibidum*) seeds. Global Journal of Engineering, Design and Technology 3(3), 12-16.

Ajayi, I.A., Adesanwo, O., 2009. Comparative study of the mineral element and fatty acid composition of *Dacryodes*

edulis pulp and seed. World Journal of Agricultural Sciences 5(3), 279-283.

Akanni, M.S., Adekunle, A.S., Oluyemi, E.A., 2005. Physicochemical properties of some non-conventional oil seeds. Journal of Food Technology 3(2), 177-181.

Akubugwo, I.E., Ugbogu, A.E., 2007. Physicochemical studies on oils from five selected Nigerian plant seeds. Pakistan Journal of Nutrition 6(1), 75-78.

Amaike, S., Keller, N.P., 2011. *Aspergillus flavus.* Annual Review of Phytopathology 49, 107-133.

Amekyeh, H., Kumadoh, D., Adongo, D.W., Orman, E., Abubakar, S., Dwamena, A., Aggrey, M.O., 2024. Evaluation of packaging, labels, and some physicochemical properties of herbal antimalarial products on the Ghanaian market. Heliyon 10, e27032.

AOAC., 1990. Official Methods of Analysis, (15th edn.) Association of Official Analytical Chemists. Washington DC. USA.

AOCS., 1990. Official Methods and Recommended Practices of the American Oil Chemists Society, (4th edn.), American Oil Chemists' Society, Champaign, IL.

Aremu, M.O., Ibrahim, H., Bamidele, T.O., 2015. Physicochemical characteristic of the oils extracted from some Nigeria plant food. Chemical & Process Engineering Research 32, 36-52.

Audu, S.S., Aremu, M.O., Lajide, L., 2013. Effect of processing on physicochemical and antinutritional properties of black turtle beans (*Phaseolus vulgaris* L.) seeds flour. Oriental Journal of Chemistry 29(3), 979 – 989.

Ali-Shtayeh, M.S., Suheil, I.A., 1999. Antifungal activity of plant extracts against dermatophytes. Mycoses 42, 665-672.

Atlas, R.M., 1995. Microorganisms in our World (2nd edn). Mosby Publishers Inc. Baltimore, pp. 765.

Ayanbimpe, G.M., Afolabi, E.A., Kim, E.G., Ajiji, R.T., 2015. The anti-fungal activities of virjin coconut oil against some human pathogenic fungi. African Journal Natural Sciences 6, 7-10.

Belay, K., Sisay, M., 2014. Phytochemical constituents and physicochemical properties of medicinal plant (*Moringa oleifera*) around Bule Hora. Chemistry Material Research 67, 61-71.

Berkow, E.L., Nunnally, N.S., Bandea, A., Kuykendall, R., Beer, K., Lockart, S. R., 2018. Detection of $TR_{34/L98H}$ CYP51A mutation through passive surveillance of azole-resistance *A. Fumigatus* in United State from 2015-2017. Antimicrobial Agents chemotherapy 62, e02240-17.

Burkill, H.M., 1985. The useful plants of west tropical Africa. Families (A-D), (2nded.). Royal Botanical Gardens Kew, pp 62-67.

Bwade, K.E., Aliyu, B., Kwaji, A.M., 2013. Physicochemical properties of pumpkin seed oil relevant to bio-diesel production and other industrial application. International Journal of Engineering business and Enterprises Applications 4(1), 73-78.

Bulpa, P., Divem A., Sibille, Y., 2009. Invasion pulmonary *Aspergillosis* in patients with chronic obstructive pulmonary diseases. European Respiratory Journal 30, 782-800.

Codex Alimentarius, 1999. Codex standards for named vegetable oils. 8, pp. 12-22.

Daben, J.M., Dashak, D.A., 2016. Extraction, isolation and mass spectral analysis of *Crinum zeylanicum* (Beautiful crinum) bulbs. Eweman Journal of Herbal Chemistry and Pharmacological Research 3(1), 2-58.

Daben, J.M., Dashak, D.A., Daben, M.R., Jock, A.A., Onaji. J.O., 2017. An assessment of the proximate and antibacterial activity of *Crinum zeylanicum* bulb extracts used for the treatment of bone setting and healing. IOSR-Journal of Applied Chemistry 10 (6), 55-60.

Dalziel, J.M., 1956. Useful plants of West tropical Africa. Crown Agents for Overseas Government, London, pp 242-248.

Danguguwa, A.A., 1983. Cultivation of Tiger nut in Bauchi Samaru Agricultural Newsletter 5, 86-87.

Evbuowman, B.O., Lawson, J.N., Atuka, M.M., 2013. Some physicochemical properties of cashew nut (*Anacardium occidentale*) and palm kernel (*Elaeis guineensis*) oil using straight run gasoline. International Journal of Science & Engineering Investigations 2(20), 82 – 84.

Fabricant, D.S., Farnsworth N.R., 2001. The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives 109(1), 69–75.

Fakhri, N.A., Qadir, H.K., 2011. Studies on various physicochemical characteristics of some vegetable oils. Journal of Environment Science and Engineering 5, 844-849.

Harborne, J.B., 1991. Phytochemical methods. A guide to modern techniques of plant analysis. (2nd edn). Chapman and Hall, London, pp. 17-20.

Hutchinson, J., 1964. The genera of flowering plants. Dicotyledons. Vol. 1. The Claredon press: Oxford University Press, London pp. 516.

Inger, N., Rolf, W., 1982 Studies in the *Crinum zeylanium* complex in East Africa. Nordic Journal of Botany 2(5), 465-473.

Kamalu, C.I.O., Ogbome, P., 2008. Extraction and characterization of tiger nut oil. Journal of Chemical Society of Nigeria 33(1), 79-87.

Kimmerling, E.A., Fedrick, J.A., Tenholder, M.F., 1992. Invasion. *A. Niger* with fatal pulmonary oxalosis in chronic obstructive pulmonary diseases, Chest 101, 870-872.

Klich, M.A. 2007. *A. flavus*: The major producer of aflatoxin. Molecular Plants Pathogen 8, 713-722

Knothe, G., Dunn, R., 2003. Dependence of oil stability index of fatty compounds on their structure and concentration and presence of metals. Journal of the American Oil Chemists' Society 80, 1021-1026.

Kabiraj, A., Deshmukh, R., 2024. A review on chinese herbal medicine used as carminative. Pharmacological Research - Modern Chinese Medicine 11, 100409.

Loudon, K.W., Coke, A.P., Burnie, J.P., Shawa, A.J., Oppenheim, B. A., Morris, C. O., 1996. Kitchens as a source of *A. niger* infection. Journal of Hospital Infection 32,191-197.

Mercelo, V.G., Gilson, P., Camila, B.M., Bernardi, A.O., Copetti, M.V., 2018. Fungi in spices and mycotoxigenic potential of some *Aspergilli* isolates. Food Microbiology 73,93-9.

Mohammed, M.L., Hamza, Z.U., 2008. Physicochemical properties of oil extracts from *Sesamum indicum* L. seeds grown in Jigawa State-Nigeria, Journal of Applies Science Environmental Management 12(2), 99-101.

Morrison, W.H., Hamilton, R.J., Kalu, C., 1995. Sun flower seed oil, In: Hamilton, R. J(ed). Developments in oils and fats. Blackie Academic and Professional, Glasgow, pp.132-152.

Mukherjee, P.K., 2000. The evaluation of wound-healing potential of *Hypericum hookerianu* leaf and stem extracts. Journal of Alternative and Complimentary Medicine 6, 61-69.

Muoka, R.O., Ibeh, G.U., 2018. Extraction, characterization and utilization of oil from the seed of *Moringa oleifera* from Amichi in Nnewi, South LGA of Anambra State. Journal of Laboratory Science 5(1), 51-60.

Nangbes, J.G., Nvau, J.B., Buba, W.M., Zukdimma, A.N., 2013. Extraction and characterization of castor (*Ricinus Communis*) seed oil. The International Journal of Engineering and Science 2(9), 105–109.

Natagawa, T., Shimazu, K., Ebihara, M., Nakagawa, K., 1999. *A. niger* pneumonia with fatal pulmonary oxalosis. Journal of Infections and Chemotherapy 5, 97-100.

NIS., 1992. Standard for Edible Vegetable Oil pp. 5–12.

Nwabanne, J.T., 2012. Kinetics and thermodynamics study of oil extraction from fluted pumpkin seed. International Journal of Multidisciplinary Sciences and Engineering 3(6), 11–15.

Ochei, J., Kochatkar, A., 2007. *Medical Laboratory Science*, Theory and Practice (3rd edn.). Tata McGraw-Hill Ltd, pp. 795-817.

Ogbonna, P.E., Ukaan, S.I., 2013. Chemical composition and oil quality of seeds of sesame accessions grown in the Nsukka plains of south eastern Nigeria. African Journal of Agriculture Research 8(9), 797 – 803.

Ogbunugafor, H.A., Eneh, F.U., Ozumba, A.N., Igwo-Ezikpe, M. N., Okpuzor, J., Igwilo, I.O., Adenekan, S.O., Onyekwelu, O. A., 2011. Physico-chemical and antioxidant properties of *Moringa oleifera* seed oil. Pakistan Journal of Nutrition 10(5), 409 – 414.

Okhale, S.E., Buba, C.I., Oladosu, P., Okoro, I.J., Ugbabe, G.E., Ibrahim, J.A., Egharevba, H.O., Kunle, O.F., 2018. Chemical constituents and antibacterial activities of the volatile oils of *Crinum zeylanicum L* and *crinum ornatum* (Ait) bury bulbs. International Journal of Modern Pharmaceutical Research 2(2), 1-7.

Okon, M.O., Echeme, J.O., 2018. Preliminary phytochemical screening and in-vitro anti-microbial effects of methanol extract of the whole plant of *Ipomoea involucrate* (Morning glory). Journal of Laboratory Science 5(1), 46-50.

Olaofe, O., Ogungbenle, H.N., Akhadelor, B.E., Idris, A.O., Omojola, O.V., Omotehinse, O.T., Ogunbodede, O.A., 2012. Physicochemical and fatty acids composition of oils from some legume seeds. International Journal of Biology Pharmacy and Allied Sciences 1(3), 355-363.

Pasqualotto. A.C., Denning, D.W., 2008. An Aspergilloma caused by *A. flavus*. Medical Mycology 46(3), 275-278.

Pearson, D., 1976. The chemical analysis of foods, (7th Edn), Church Hill, Livingstone Longman Grp Ltd., London. pp.813.

Pitt, J.I., Hocking, A.D., 2009. Fungi and food spoilage, Springer, United States.

Portella, C.F.S., Silva, I.D.A., Simões, R.S., Ghelman, R., Baracat, E.C., Júnior, J.M.S., Sorpreso, I.C.E., 2024. An overview of systematic reviews of medicinal plants and herbal formulations for the treatment of climacteric symptoms. Journal of Herbal Medicine 45, 100863.

Purwono, S., Nisa, U., Astana, P.R.W., Wijayaningsih, R.A., Wicaksono, A.J., Wahyuningsih, M.S.H., Kertia, N., Mustofa, M., Wahyuono, S., Fakhrudin, N., 2023. Factors Affecting the Perception of Indonesian Medical Doctors on Herbal Medicine Prescription in Healthcare Facilities: Qualitative and Quantitative Studies. Journal of Herbal Medicine 42, 100747.

Rafe, A., Nadjafi, M.S., 2014. Physicochemical characteristics of garlic (*Allium sativum L*) oil: effect of extraction procedure. International Journal of Nutrition and Food Science 6(1), 1-5.

Sanchez, N.R., Garcia, D.A., Shiavini, M.S., Nakamura, C.V., Filho, B.P.D., 2005. An evaluation of antibacterial activities of Psidiumguajava. Brazilian Journal of Biotechnology 48, 429-436.

SON, 2000. Standard Organization of Nigeria: Standards for edible refined palm oil and its processed form. pp. 2–5.

Stevens, D.A., Virginia L.K. Marc, A.J., Vicki, A.M., Stephen, D., David, W.D., John E. B., Thomas, J.W., Thomas, F. P., George, A. P., 1999. Practice guidelines for diseases caused by *Aspergillus.* Clinical Infectious Diseases 30, 696–709.

Strahil, B., Stefani, R., Maria, H., Frances, V., Carles, C., Georgi, M., Iliana, I., James, B., 2011. Anti-proliferative alkaloids from Crinum zeylanicum. Phytother Research 25, 1686-1692.

Tesfaye, B., Abebaw, A., 2016. Physicochemical characteristics and level of some selected metal in edible oils. Advances in Chemistry 3, 1-8.

Tijani, A.Y., Salawu, O.A., Good-luck, J., Mbah, C.C., Madara, A. A., Ikwuka, A.C., Chindo, B.A., 2012. Wound healing activity of *Crinum zeylanicum* L. (*Amaryllidaceae*). Phytopharmacology 3(2), 319-325.

Tsado, D.B., Ndamitso, M.M., Ajai, A.I., 2018. Determination of Physicochemical Properties and Fatty Acid Profile of Oil Extract of *Blighia sapida* Fruit from Selected Areas in Niger State, Nigeria. Nigerian Journal of Chemical Research 23(1), 21-34.

Tsuda, Y., Kashiwaba, N., Kumar, V., 1984. The alkaloidal constituents of Goda Manel (*Crinum zeylanicum*). Chemical Pharmaceutical Bulletin 32, 3023-3027.

Vaugham, D.C., 1990. The Structure and Utilization of Oil Seeds. Chapman & Hall Ltd, London. pp.187.

Wuzella, G., Mahendran, A.R., Bätge, T., Jury, S., Kandelbauer, A., 2011 Novel, binder-free fibre reinforce, composites based on a renewable resource from the reed-like plant Typha. Industrial Crops and Products 33 (3), 683-689.

Yakandawala, D.M.D., Samarakoon, T.M., 2006. An empirical study on the taxonomy of *Crinum zeylanicum* L. and *Crinum latifolium* L., *Amaryllidaceae*, occurring in Sri Lanka. Ceylon Journal of Science Biology 35(1), 53-72.

Yinusa, I., Ndukwe, I.G., Amupitan, J.O., 2007. Phytochemical and antimicrobial screening of aerial parts of *Indigofera pulchra.* Chemclass Journal 5, 162-166.

Visit us at: http://bosaljournals.com/chemint
Submissions are accepted at: editorci@bosaljournals.com