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Phytochemical Profiling and Identification of Bioactive Phyto-Compounds Present In the Rhizome of *Crinum Jagus*

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Abstract

Phytochemical screening of plant extracts is a promising approach that therapeutically examined bioactive compounds in various plant species. The present study was carried out to screen and identify the bioactive phytochemicals present in the rhizome of *Crinum jagus*. Phytochemical screening revealed the presence of alkaloids, steroids, tannins, flavonoids, saponins, terpenoids and glycosides. Gas chromatography mass spectrometry (GC-MS) results present a diverse array of compounds. Each compound was identified by its molecular formula, molecular weight, retention time, and peak area percentage, a total of ten major compounds: Mesitylene, Naphthalene, 1-methyl, Dibutyl phthalate, Linoleic acid ethylester, Bis(2-ethylhexyl)phthalate, 4-(4-Methoxyphenyl)-1-butanol, 1,3-Benzenedicarboxylic acid, 2-(4-Methoxyphenyl) ethanol, Squalene and Cyclononasiloxane octadecamethyl and ten most bioactive compounds: Alpha. -Terpineol, Piracetam, Dichloroxylenol, 2,4-Di-tert-butylphenol, 1,1'-Biphenyl, 3,3',4,4'-tetramethyl, Cholest-5-ene, 3-methoxy-, (3.beta.), Bis(2-ethylhexyl) phthalate, Stigmasterol, Gamma. -Sitosterol and Ergosta-4, 22-dien-3-one were identified from n-hexane and ethyl acetate fractions respectively. Phytochemical screening revealed the presence of phytochemicals that have demonstrated some biological activities. These compounds are recognized for their diverse biological activities, including antimicrobial and anti-inflammatory properties. Hence, their consistent detection across different solvents suggests that *C. jagus* could be a valuable source for pharmacological research due to its diverse phytochemical composition.

Keywords: *Crinum Jagus*, Bioactive, GC-MS, Phytochemical and Diverse

Introduction

Phytochemical screening of plant extracts is a promising approach that therapeutically examined bioactive compounds in various plant species [1]. Phytochemicals such as Alkaloids, phenols, steroids, tannins, flavonoids, saponins, coumarins, terpenoids and glycosides are phytochemicals that are found in plants including *Crinum jagus* species [2]. Alkaloids are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom. They also include some related compounds with neutral and even weakly acidic properties [3]. They are also poisonous active plant-derived chemical, examples; Ephedrine (A), Nicotine (B), Morphine (C), Quinine (D) and atropine (E) [4]. Tannins are bitter, astringent plant polyphenolic biomolecules that either bind to and precipitate proteins or shrink them. They are termed tannins as their uses as oak and other bark in tannin animal hides into leather, examples; ellagic acid (F), gallic acid (G) and Catechin (H) [5-7]. Flavonoids are flavone structural derivatives that are phenolic and water soluble and contain

conjugated aromatic systems [8]. They are usually coupled to sugar(s) as glycosides, examples; quercetin (I), apigenin (J) and kaempferol (K) [9]. Saponins are soap-like surface active agents that can be identified by their ability to generate foaming and haemolyse blood cells [9]. They are also referred to as triterpene glycosides with bitter taste usually toxic plant-derived organic chemicals that have a foamy quality when agitated in water [10]. They are widely distributed but found particularly in soapwort, a flowering plant, the soapbark tree and soybeans examples; betulinic acid (L), oleanolic acid (M), and lupane (N). Steroids are a man-made version of chemicals, known as hormones that are made naturally in the human body [11]. They are designed to act like these hormones to reduce inflammation [12]. Plant steroids that may or may not act as weak hormones in the body are known as Phyto steroids [13]. They have a similar fundamental ring structure to animal steroids, but chemical groups connected to the primary ring in different positions make them different, examples; testosterone (O), cortisol (P) and cholesterol (Q) [14]. Terpenoids otherwise known as isoprenoids, are a large and diverse class of naturally occurring compounds derived from five carbon isoprene units [15]. Terpenoids are essential for plant growth, development and contributed to the flavour, scents and colour of the plant's leaves, flowers and fruits, examples; carvacrol (R), menthol (S), linalool (T) and thymol (U). Cardiac glycosides (also known as cardenoloids) are medicines for treating heart failure and certain irregular heartbeats [16]. They are one of several classes of drug used to treat the heart and related conditions [17]. They also occur as a complex mixture in the same plant, examples; coumarin (V) and anthraquinone (W) [18].

Materials and Methods

Reagents and Solvents

HCL, H₂SO₄, FeCl₃, NaOH, ethyl acetate, n-hexane, dichloromethane and methanol of analytical grade were used.

Plant Collection and Identification

Rhizomes of *Crinum jagus*, were collected and air dried in June 2023, from Dabawa of Dutsin-maKatsina, Nigeria at latitude of 12°27'18"N 7°29'29"E. The plant was authenticated by botanists at Department of biological science, Federal University of Dutsin-Ma Katsina State Nigeria.

Extraction of Plant Material

The dried powder (500 g) of the rhizome was macerated in n-hexane, dichloromethane, ethyl acetate and methanol successively and exhaustively. The dried powdered plant material was first macerated in 1200 ml of n-hexane for extraction with regular shaking at time interval and this continues for three (3) days then decanted. More n-hexane was added for continuous extraction until a colorless solvent was decanted. Same procedure was employed for dichloromethane, ethyl acetate and methanol. The crude extracts were concentrated using rotary evaporator and dried in a vacuum desiccator [19].

Preliminary Phytochemical Screening:

The crude extracts of n-hexane, DCM, ethyl acetate and methanol were subjected to preliminary qualitative screening of secondary metabolites using standard methods as described by [20].

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) assessment was executed utilizing a unified 7890A gas chromatographic system (Kaduna NAFDAC) in conjunction with a mass spectrophotometer, which was equipped with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with a 5675C Inert MSD featuring a Triple-Axis detector. Helium was employed as the carrier gas, which was calibrated to a column velocity flow rate of 1.0 ml/min. Additional GC-MS parameters included ion-source temperature maintained at 250 °C; interface temperature set at 300 °C; pressure regulated to 16.2 psi; out time fixed at 1.8 mm; and a 1 µl injector operating in split mode with a split ratio of 1:50 and an injection temperature of 300 °C. The column temperature commenced at 36 °C for duration of 5 minutes and subsequently escalated to 150 °C at a rate of 4 °C/min. The temperature was further elevated to 250 °C at an increased rate of 20 °C/min and sustained for an additional 5 minutes. The overall elution duration was recorded at 47.5 minutes. The relative percentage of each constituent was determined by juxtaposing its average peak area against the cumulative areas [1].

Results

Preliminary Phytochemicals Screening of the Extracts

Qualitative phytochemical screening of the rhizome of *Crinum jagus* was conducted using four organic solvents (n-hexane, dichloromethane (DCM), ethyl acetate, and methanol) presented below.

Test Compounds	n-Hexane Extract	DCM Extract	Ethyl acetate Extract	Methanol Extract
Saponins	+	+	+	+
Cardiac glycosides	-	-	+	
Alkaloids	-	+	+	+
Anthraquinones	-	-	-	-

Steroids	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	-	+	-	-
Tannins	-	-	+	+
Key: (+) = Present and (-) = Absent				

Table 1: Phytochemical Constituents of Crinum Jagus Extracts

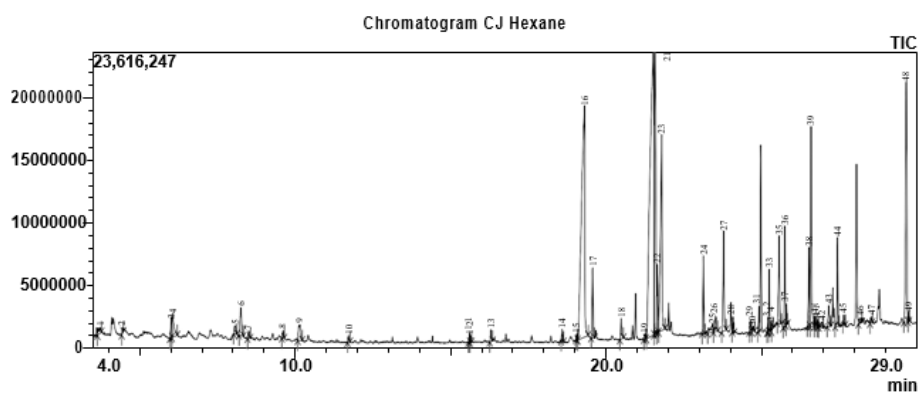


Figure 1: Gas Chromatography Mass Spectrometry (GC-MS) Of N-Hexane Fraction

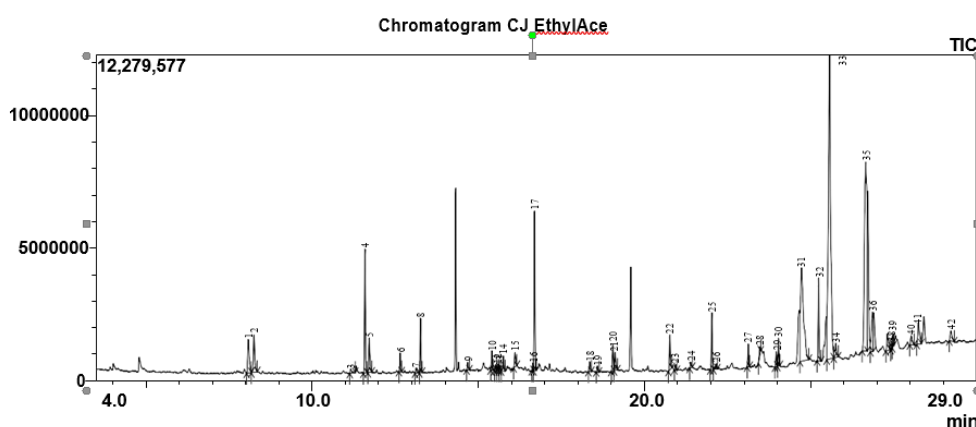


Figure 2: Gas Chromatography Mass Spectrometry (GC-MS) Of Ethyl Acetate Fraction

No.	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	R. Time (minutes)	Peak Area (%)	Structure of Compounds
1	Mesitylene	C ₆ H ₁₂	120.2	3.730	0.29	
2	Naphthalene,1-methyl	C ₁₁ H ₁₀	142.2	10.146	0.75	
3	Dibutyl phthalate	C ₁₆ H ₂₄ O ₄	278.3	19.054	0.11	
4	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308.5	21.651	1.66	

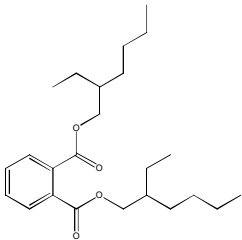
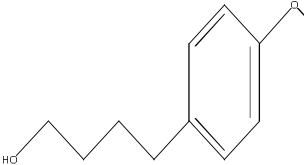
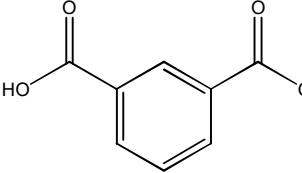
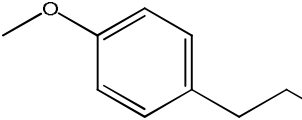

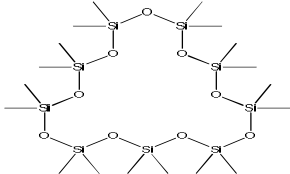
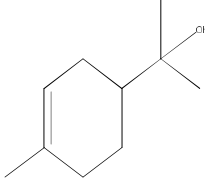
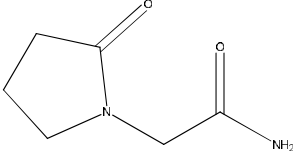
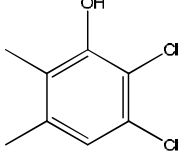
5	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	25.249	1.16	
6	4-(4-Methoxyphenyl)-1-butanol	$C_{11}H_{16}O_2$	180.2	26.527	1.7	
7	1,3-Benzenedicarboxylicacid	$C_8H_6O_4$	166.1	26.933	0.25	
8	2-(4-Methoxyphenyl) ethanol	$C_9H_{12}O_2$	152.2	27.167	0.58	
9	Squalene	$C_{30}H_{50}$	410.7	27.447	1.7	
10	Cyclononasiloxane,octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.4	27.650	0.17	

Table 2: Gas Chromatography Mass Spectrometry (Gc-Ms) Analysis of N-Hexane Fraction

No.	Name of Compound	Molecular Formula	Molecular Weight (g/mol)	R. Time (minutes)	Peak Area (%)	Structure of Compounds
1	Alpha. -Terpineol	$C_{10}H_{18}O$	154.2	8.256	2.13	
2	Piracetam	$C_6H_{10}N_2O_2$	142.2	11.160	0.24	
3	Dichloroxylenol	$C_8H_8Cl_2O$	156.6	11.725	1.53	

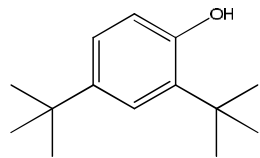
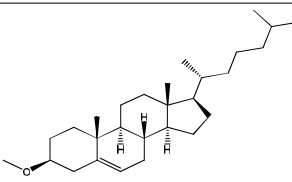
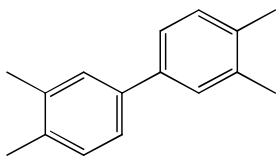
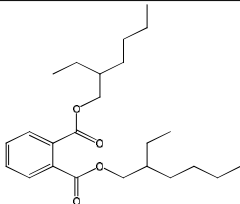
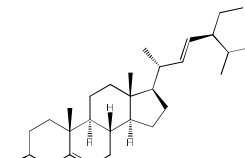
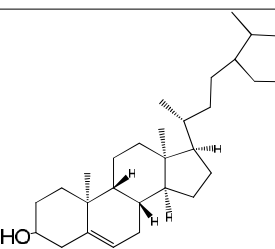
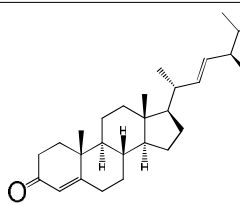
4	2,4-Di-tert-butyl-phenol	C ₁₄ H ₂₂ O	206.3	13.263	1.74	
5	1,1'-Bi-phenyl,3,3',4,4'-tetramethyl	C ₁₆ H ₁₈	210.3	15.410	0.60	
6	Cholest-5-ene,3-methoxy-, (3. beta.)	C ₂₈ H ₄₈ O	400.7	24.726	11.29	
7	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390.6	25.240	2.83	
8	Stigmasterol	C ₂₉ H ₄₈ O	412.7	25.569	28.00	
9	Gamma. -Sitosterol	C ₂₉ H ₅₀ O	414.7	26.861	2.49	
10	Ergosta-4,22-dien-3-one	C ₂₈ H ₄₄ O		28.026	0.60	

Table 3: Gas Chromatography Mass Spectrometry Gc-Ms) Analysis of Ethyl Acetate Fraction

Discussion

The Phytochemical Screening of Various Extracts

The phytochemical screening of various extracts (n-hexane, dichloromethane (DCM), ethyl acetate, and methanol) of *Crinum jagus*'s rhizome revealed a diverse profile of secondary metabolites, which are crucial for their therapeutic potential. Saponins were detected in all extracts (n-hexane, DCM, ethyl acetate, and methanol). These compounds are known for their surfactant properties and have been associated with various health benefits, including cholesterol-lowering effects and immune system enhancement [21]. Cardiac glycosides were found only in the ethyl acetate extract. These compounds are crucial in treating heart conditions due to their ability to improve cardiac contractility [22]. The selective presence of cardiac glycosides in this extract suggests a targeted therapeutic potential for cardiovascular diseases [23]. Alkaloids were present in both DCM and ethyl acetate extracts but absent in n-hexane and methanol extracts. Alkaloids are well-known for their pharmacological activities, including analgesic and antitumor effects [24].

Their presence aligns with previous studies that highlighted the rich alkaloid content in *C. jagus*, particularly hippadine, which has shown cytotoxic activity against cancer cell lines [25]. No anthraquinones were detected in any

of the extracts. This absence is noteworthy as anthraquinones are often associated with laxative effects and potential anticancer properties [26]. Steroids were found across all extracts, indicating a broad spectrum of potential biological activities, including anti-inflammatory and immunomodulatory effects [27]. The presence of steroids can enhance the therapeutic profile of *C. jagus* as they are commonly used in various medical treatments. Terpenoids were also present in all extracts. These compounds are recognized for their diverse biological activities, including antimicrobial and anti-inflammatory properties [2]. Their consistent detection across different solvents suggests that *C. jagus* could be a valuable source of terpenoid compounds for pharmacological research. Flavonoids were detected only in the DCM extract. Known for their antioxidant properties, flavonoids contribute to the protective effects against oxidative stress-related diseases [24]. Tannins were present exclusively in the ethyl acetate extract. These polyphenolic compounds are recognized for their astringent properties and potential health benefits, including antimicrobial activity and cancer prevention [27].

Gas Chromatography Mass Spectrometry (Gc-Ms) Results

Gas chromatography mass spectrometry (GC-MS) results provided in (Table: 2) present a diverse array of compounds, a total of ten (10) major compounds were identified from the n-hexane fraction each characterized by its molecular formula, weight, retention time, and peak area percentage. The following bioactive compounds were present in the GC-MS analysis carried on n-hexane fraction of *Crinum jagus* rhizome are: Mesitylene, Naphthalene, 1-methyl, Dibutyl phthalate, Linoleic acid ethylester, Bis(2-ethylhexyl)phthalate, 4-(4-Methoxyphenyl)-1-butanol, 1,3-Benzenedicarboxylic acid, 2-(4-Methoxyphenyl) ethanol, Squalene and Cyclononasiloxane octadecamethyl. GCMS results of ethyl acetate fraction presented in (Table: 3) present a various number of compounds, ten (10) major bioactive compounds were identified from the ethyl acetate fraction each characterized by its molecular formula, weight, retention time, and peak area percentage. The following bioactive compounds were present in the GC-MS analysis carried on ethyl acetate of *Crinum jagus* rhizomes are: Alpha-Terpineol, Piracetam, Dichloroxyleneol, 2,4-Di-tert-butylphenol, 1,1'-Biphenyl, 3,3',4,4'-tetramethyl, Cholest-5-ene, 3-methoxy-, (3. beta.), Bis(2-ethylhexyl) phthalate, Stigmasterol, Gamma. -Sitosterol and Ergosta-4, 22-dien-3-one. The chromatogram of n-hexane and ethyl acetate were presented in figure 1 and 2 respectively.

Conclusion

Phytochemical screening revealed the presence of Alkaloids, steroids, tannins, flavonoids, saponins, terpenoids and glycosides. Gas chromatography mass spectrometry (GC-MS) results of the rhizome of *Crinum jagus* present a diverse array of compounds, a total of ten (20) major compounds were identified from the n-hexane and ethyl acetate fractions each characterized by its molecular formula, weight, retention time, and peak area percentage. Phytochemical screening revealed the presence of phyto-compounds that have demonstrated some biological activities. These compounds are recognized for their diverse biological activities, including antimicrobial and anti-inflammatory properties. Hence, their consistent detection across different solvents suggests that *C. jagus* could be a valuable source for pharmacological research due to its diverse phytochemical composition.

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Authors' Contributions

All the authors have been contributed in the design and preparing the manuscript.

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