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LIQUID CHROMATOGRAPHY MASS SPECTROMETRY PROFILING AND ANTIMICROBIAL ACTIVITIES OF THE METHANOLIC FRACTION OF *CRINUM JAGUS* RHIZOME FOUND IN DUTSIN-MA

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ABSTRACT

Rhizome of *Crinum jagus* was investigated to identify chemical substances of medicinal value to enable scientific validation of the plant in the treatment of pathogenic microorganisms' related diseases. The plant's rhizome, was subjected to cold extraction using methanol. The extract obtained was subjected to phytochemical screening, antimicrobial activities and liquid chromatography mass spectrometry analyses using standard procedures. The preliminary phytochemical screening of the crude extract revealed the presence of flavonoids, tannins, glycosides, steroids, triterpenoids, saponins and alkaloids. The Antimicrobial effects of the crude extract were tested against nine microorganisms namely: *Bacillus magisterium, Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans Trichophyton rubrum* and *Aspergillus* niger. The antimicrobial activities indicates that, the extract possess significant activity against *Staphylococcus aureus* (Zone of inhibition ranged from 09mm to 26mm at MIC ranges: 200 mg/ml). The result of the LCMS analysis of methanolic fraction revealed the presence of molecular fragmentation patterns at *m/z* 512.287. The findings of this research suggest the presence of a compound(s) with a parent molecular ion around 512, possibly indicating a molecular formula near $C_{30}H_{50}O$ or similar, characteristic of triterpenoids. The phytochemical constituent's and antimicrobial assay indicate that the plant could be used in the treatment of microorganisms related diseases.

Keywords: Antimicrobial, Chromatography, Fraction, Methanolic, Rhizome

INTRODUCTION

In traditional African medicine, various parts of the *Crinum jagus* plant have been used for medicinal purposes, including the roots, leaves, and flowers (Ogah *et al*., 2024). The plant is believed to have anti-inflammatory and analgesic properties, and is used to treat a range of ailments, including stomach disorders, fever, and skin infections (Ogbole *et al*., 2016). *Crinum jagus* is widely distributed in many parts of Africa, including West Africa, Central Africa, and East Africa. This species is found in a variety of habitats, including savannas, forests, and riverbanks (Oliveira *et al*., 2021). In West Africa, *Crinum jagus* is commonly found in Nigeria, Ghana, and Cameroon (Iwu *et al*., 2014). In Nigeria, it is found in many states including Katsina, Abia, Akwa Ibom, Cross River, Delta, Edo, Enugu, Imo, Ogun, Ondo, and Rivers (Adedapo *et al*., 2013). *Crinum jagus* rhizomes have been the subject of numerous studies aimed at identifying and characterizing the compounds responsible for their medicinal properties (Wang and Lee 2021). These compounds showed significant anti-inflammatory activity and may have potential as a natural anti-inflammatory agent. Another study investigated the antipyretic and anti-inflammatory effects of crude extracts of *Crinum jagus* rhizomes, the results showed that the extracts had significant antipyretic and anti-inflammatory effects in animal models, suggesting that they may be useful in the treatment of fever and inflammation (Ekor, 2014). *Crinum jagus*, a botanical specimen known by the vernacular name poison bulb, is recognized as a blossoming flora (Salawu *et al.*, 2020). *Crinum jagus*, a member of the Amaryllidaceae family, has a longstanding history in traditional medicine for addressing a range of ailments, notably cancer (Ogah *et al*., 2024). The bulbs of *Crinum jagus* (J. Thomps.) Dandy commonly known as St. Christopher lily or Harmattan lily and locally in Hausa "Farin Gadali" is used in northern part of Nigeria to treat ailments (Minkah and Danquah, 2021). *Crinum jagus* is a species of the genus Crinum, which is a member of the Amaryllidaceae family. The species is known for its medicinal properties and is used in traditional medicine for the treatment of various ailments (Odewale *et al*., 2016). The rhizomes of this plant have been widely used in traditional medicine to treat various ailments such as ulcer, typhoid, asthma, cough, fever, and pain (Alawode *et al.*, 2020). Rhizomes of *C. jagus* have been used in traditional medicine to treat various ailments, including malaria, snake bites, and abdominal pain. In recent years, there has been an increase in scientific interest in the therapeutic properties of this plant and its secondary metabolites (Wang *et al*., 2018). In recent years, there has been growing interest in the isolation and characterization of secondary metabolites from *Crinum jagus* rhizomes (Wang *et al*., 2018). These metabolites have been found to have various therapeutic properties, making this plant a potential source of natural drugs (Wang *et al*., 2018). Studies have shown that *C. jagus* rhizomes contain a wide range of secondary metabolites, including alkaloids, flavonoids, tannins, and phenolic compounds (Alawode *et al.*, 2020**)**. These compounds have been found to have various therapeutic properties, including antibacterial, antimalarial, antispasmodic, anti-inflammatory, and analgesic effects (Eze *et al*., 2014).The rhizome of this plant has been used for centuries and are still widely used today, especially inAfrica, for the treatment of various ailments such as ulcer, typhoid, asthma, cough and fever. However, further research is needed to fully identify and understand the potential medicinal applications of *Crinum jagus'* chemical constituents. Therefore, this research aimed to identify the chemical

constituents present in the rhizome of *Crinum jagus* and evaluate their antimicrobial activities.

MATERIALS AND METHODS

Collection and identification of plant material

Rhizomes of *Crinum jagus*, was collected and air dried in September 2024, from Dabawa of Dutsin-ma Katsina, Nigeria. The plant was authenticated by botanists in the Department of Biological Science, Faculty of life sciences, Federal University of Dutsin-ma, Nigeria.

Preparation, drying and pulverization of the plant material

The rhizome of the plant was sliced and air dried under shade at room temperature. The dried plant material was pulverized mechanically in to coarse powder using clean mortar and pestle. The homogenized plant material was then packed in polythene bag and stored for further use.

Extraction of plant material

The dried powder (200g) of the rhizome was macerated in methanol successively and exhaustively. The dried powdered plant material was first soaked in 800 ml of methanol for extraction with regular shaking at time interval and this continues for three (3) days then decanted. More methanol was added for continuous extraction until a colorless solvent was decanted. The crude extracts were concentrated individually using rotary evaporator (Chhabra *et al.*, 1980).

Materials and micro-organisms used in antimicrobial test

Petri-dishes (150 mm x 15 mm), cotton wool, Bunsen burner, parafilm, distilled water, incubator, microliter plates, test tubes, pipettes, autoclave, disinfectant, resazurin, sample bottle, dimethyl sulphoxide (DMSO) (10 %) in distilled water, Whatman filter paper, nutrient broth, dextrose agar and blood agar.

Gram-positive bacteria: *Bacillus cereus, Staphylococcus aureus, Streptococcus pneumonia* and *Staphylococcus saprophyticus.* Gram-negative bacteria: *Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas aeruginosa.* Fungi: *Candida albicans, Trichophyton redrum* and *Aspergillus niger*

Antimicrobial screening

The antimicrobial activity was determined against the following species of bacteria; four Gram-positive; *Bacillus cereus, Staphylococcus aureus, Streptococcus pneumonia* and *Staphylococcus saprophyticus*, four Gram-negative; *Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas aeruginosa* and three fungi species; *Candida albicans, Candida albicans, Trichophyton redrum* and *Aspergillus niger*. Clinical strains were obtained from the Department of Biological Sciences Ummaru Musa Yar'adua University Katsina. All the isolates were checked for purity and maintained in a slant of nutrient agar.

Culture media

The culture media used were Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB). All the media were prepared according to manufacturer's specifications.

Preparation of inoculums of test organisms

McFarland turbidity standard scale 1 was used to standardize the organisms. The scale was prepared by adding 1 % sulphuric acid (9.9 mL) to 1 % barium chloride (0.1 mL). Suspension of the organisms were made in sterile distilled water and compared with the McFarland standard, until the opacity matched with the scale number 1, which corresponds to 1.5×10^6 CFU/mL

Sensitivity test of the crude extracts

The agar well diffusion method was used as reported by Nostro *et al.,* (2000). The antimicrobial activity of the n-hexane, ethyl acetate, dichloromethane and methanol extracts of the rhizome of *Crinum jagus* was determined using McFarland standard stock concentration (100 mg/mL). The standardized inoculate of the isolates were uniformly streaked unto freshly prepared Mueller Hinton agar plates with the aid of a sterile stick. Using a sterile corn borer (6 mm in diameter), five appropriately labelled wells were bored into each agar plate. Appropriate extract concentration (0.2 mL) was placed in each well and then allowed to diffuse into the agar. An extra plate was streaked with the inoculate isolate and ciprofloxacin standard (10 ug/disc) was placed on it. The plates were incubated at 37 ^{o}C for 24 hours, while for the fungi, Sabouraud Dextrose Broth was used and the incubation period was 48 hours at 25 ^{o}C. The antimicrobial activities were expressed as diameter zones of inhibition produced by the plant extracts.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the extracts was determined using the broth dilution method as reported by Awla *et al.,* (2017). Different concentrations of the extract that exhibited antimicrobial activity against the test organisms were in test tubes containing Mueller Hinton broth (MHB). The organisms were inoculated into each tube containing the diluted extracts. The tubes were incubated at 37 ^{o}C for 24 h for bacteria and 25 ^{o}C, 48 h for fungi. The lowest concentration in the series showing no visible growth of the test organisms was considered to be the minimum inhibitory concentration (MIC).

Minimum bactericidal/fungicidal concentration (MBC/MFC)

Minimum bactericidal/fungicidal concentrations were determined by assaying the content of the test tubes in the MIC determinations. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 ^{o}C for 24 h and 25 ^{o}C for 48 h, for bacteria and fungi respectively after which it was observed for microbial growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal/fungicidal concentration (Awla *et al.,* 2017).

RESULTS AND DISCUSSION

Table 1: Qualitative Phytochemical Screening of the Extract

Test compounds	Methanol Extract
Saponins	+
Alkaloids	+
Anthraquinones	-
Steroids	+
Terpenoids	+
Flavonoids	+

Table 2: Zone of inhibition of methanol extract of the rhizome of *Crinum jagus* against tested microorganisms in millimeter (mm)

Test pathogens	Methanol extract concentration in (mg/mL)				Control (ug/mL) Cipro. /Fluc.
	200	100	50	25	20
S. aureus	26	18	14	12	22
S. epidermidi	19	14	13	10	22
B. magisterium	16	15	13	11	21
E. coli	00	00	00	00	22
S. typhi	13	10	10	09	22
P. aeruginosa	15	14	13	11	22
C. albicans	18	14	13	10	22
T. redrum	14	12	12	12	0.0
A. niger	00	00	00	00	22

Key: ND = Not detected: *B. magisterium = Bacillus magisterium, S. aureus= Staphylococcus aureus, S. pneumonia= Streptococcus pneumonia, E. coli= Escherichia coli, S. typhi= Selmonella typhi, P. aeruginosa= Pseudomonas aeruginosa, C. albicans= Candida albicans T. rubrum= Trichophyton rubrum* and *A. niger = Aspergillus niger*

Table 3: Minimum inhibition concentration (MIC) of crude extracts against tested microorganisms in (mg/ml)

Test Pathogens	Methanol extract
S. aureus	100
S. epidermidi	200
B. magisterium	ND
E. coli	ND
S. typhi	200
P. aeruginosa	200
C. albicans	200
T. redrum	200
A. niger	200

Key: ND = Not detected: *B. magisterium = Bacillus magisterium, S. aureus= Staphylococcus aureus, S. pneumonia= Streptococcus pneumonia, E. coli= Escherichia coli, S. typhi= Selmonella typhi, P. aeruginosa= Pseudomonas aeruginosa, C. albicans= Candida albicans T. rubrum= Trichophyton rubrum* and *A. niger = Aspergillus niger*

Mass spectra of the liquid chromatography mass spectrometry (LC-MS) from methanol fraction

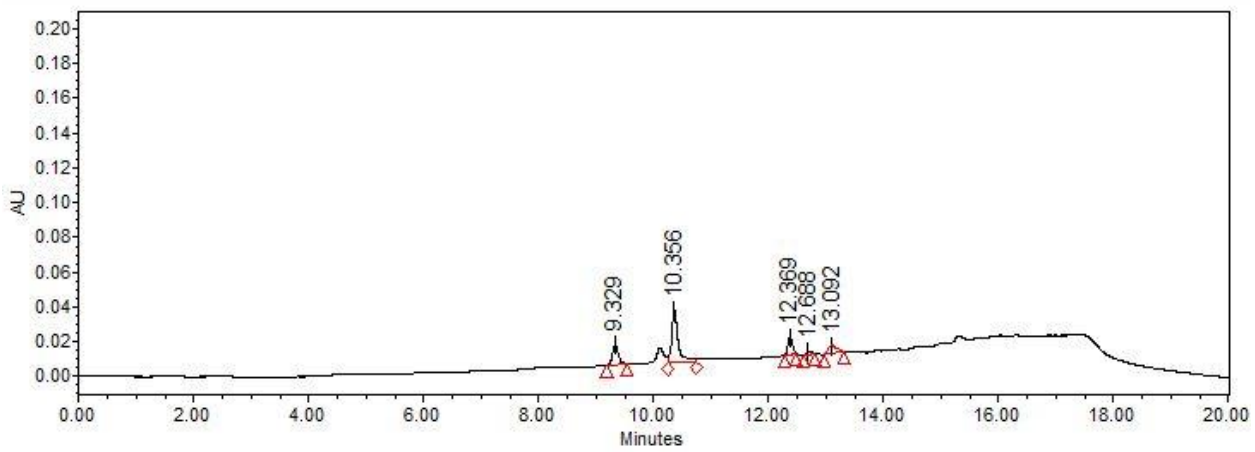

Figure 1: Total chromatogram of methanol fraction

Figure 2: Molecular fragmentations of methanol fraction

Discussion

The qualitative phytochemical analysis of the methanol extract revealed the presence of steroids, terpenes, saponins, flavonoids, and alkaloids while anthraquinones were absent (Table 1). Alkaloids were detected in the extract which are a significant group of phytochemicals in *Crinum jagus*. According to a study by Verma *et al*. (2021), alkaloids such as crinine and lycorine are present in the plant. Lycorine, in particular, has been studied for its potential in cancertherapy due to its ability to inhibit protein synthesis in cancer cells (Chen *et al*., 2020). Saponins were found in the extract. Saponins are another major group of phytochemicals identified in *Crinum jagus*. These compounds have been linked to various pharmacological activities, including antidiabetic and antioxidant effects (Ndiaye *et al*., 2022). Saponins' ability to modulate glucose absorption and enhance insulin sensitivity makes them relevant for diabetes management (Gao *et al*., 2023). Flavonoids were found to be present. Flavonoids are known for their antioxidant properties and have been identified in *Crinum jagus*. The antioxidantof flavonoids can help protect cells from oxidative stress and reduce the risk of chronic diseases (Kumar *et al*., 2023). In *Crinum jagus*, flavonoids such as quercetin and kaempferol have been reported, contributing to its overall antioxidant potential (Jiang *et al*., 2022). Tannins were found in the extract. Tannins are polyphenolic compounds with significant astringent and antimicrobial properties. In *Crinum jagus*, tannins contribute to the plant's ability to treat gastrointestinal issues and exhibit antimicrobial activity (Olaniyi *et al*., 2024). Their astringent properties help in the treatment of diarrhea and other digestive disorders. Terpenoids and steroids were present in the extract. Terpenoids and steroids in *Crinum jagus* have shown promise in various biological activities, including antimicrobial and anti-inflammatory effects. These compounds, such as those found in the essential oils of the plant, have been shown to exhibit activity against a range of pathogens (Singh *et al*., 2023). These phytochemicals found gave a preliminary idea about the relationship between the biological activity and phytochemicals present in the plant's extracts.

The *in vitro* antimicrobial activity of rhizome's extract of *Crinum jagus* was evaluated via agar well method against nine pathogenic strains of bacteria; *Bacillus magisterium*, *Staphylococcus aureus, Streptococcus epidermidi are* (Gram positive bacteria) and *Escherichia. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* (Gram negative bacteria) and three fungal isolates; *Candida albicans, Aspergillus niger* and *Trichophyton rubrum* and compared to ciprofloxacin and fluconazole as standard antibiotics and antifungal drugs respectively. Table 1 recorded the activity index of methanol crude extract. The result showed highest activity of the extract against gram positive bacterium (*S. aureus)* and also greater than the commercial drug, this is in agreement with the report of many researchers that, methanol extract of *Crinum* species is highly active against gram positive bacteria than gram negative bacteria (Cech *et al.,* 2012; Avinash *et al.,* 2015; Siregar *et al.,* 2019) and agrees with the ethnomedicinal importance of plant in the treatment of diarrhea and urinary tract infections (Dawurung *et al.,* 2019).

The minimum inhibitory concentration (MIC) results indicate the antimicrobial potency of crude extract against various test microorganisms. The methanol extract demonstrated inhibitory effects at 200 mg/ml for all tested microorganisms except *B. magisterium* and *E. coli*, which showed no inhibition (ND).

The methanol extract (2.0 g) was chromatographed on silica gel column eluting with hexane/ethyl acetate mixture (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90), as a solvent system to give 50 fractions. Twenty (20) fractions were pooled together based on similarity in their TLC profile to give 1 sub-fractions. Repeated chromatography of the sub-fractions 1 gave 6 sub-fractions. Sub-fractions 3-5 consisted

of just one spot were pooled together and subjected to liquid chromatography mass spectrometry (LCMS). The result of the LCMS analysis of methanolic fraction revealed the presence of molecular fragmentation pattern at *m/z* 512.287 (Figure 3) suggest the presence of a compound with a parent molecular ion around 512, possibly indicating a molecular formula near $C_{35}H_{48}O_2$ or similar, characteristic of triterpenoids or steroidal structures. The consistent base mass around 512 m/z supports the likelihood of a lupeol derivative, a known pentacyclic triterpenoid molecule. Given this, the compounds present may include structurally related triterpenoids, possibly with minor modifications such as hydroxylation or esterification. This metabolite identified from the methanolic fraction was reported in the existing literature to likely be responsible for antimicrobial activities against some microorganisms such *Staphylococcus aureus, Streptococcus epidermidi are* (Gram positive bacteria) and *Escherichia. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* (Ibrahim and Bashir 2022).

CONCLUSION

The present study shows potential of the methanolic extract of *Crinum jagus* based on the antimicrobial assay. LCMS analysis of methanolic fraction revealed the presence of molecular fragmentation patterns at *m/z* 512.287. The findings of this research suggest the presence of a compound(s) with a parent molecular ion around 512, possibly indicating a molecular formula near $C_{30}H_{50}O1$ or similar, characteristic of triterpenoids or steroidal structures. The phytochemical constituent's and antimicrobial assay indicate that the plant could be used in the treatment of microorganisms related diseases.

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