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Biological investigation and chemical constituents of *Croton nigritanus* scott elliot

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Abstract
Croton nigritanus is a member of the Euphorbiaceae family, which has about 1,300 species worldwide and is found in tropical climates. Many of these species have a long history of use as medicines in South America, Asia, and Africa. They have been used to cure a variety of illnesses, including malaria, ulcers, diabetes, constipation, and cancer. In this study, it was determined what new molecular structures were present in the compounds taken from *Croton nigritanus* leaves from Kaduna, Nigeria. The procedure involves liquid-liquid extraction using *n*-hexane and dichloromethane as solvents through the partition method, followed by solid-liquid extraction utilizing the maceration method with dichloromethane as the solvent. Thin-layer chromatography and gravity column chromatography (GCC) were used for separation and purification. FTIR and 1HNMR spectroscopy were used to identify the isolated chemical. The present compound was effectively isolated and examined in the investigation. Furthermore, *Croton nigritanus* crude extract shown action against both Yeast and *A. niger*, indicating its potential for treating a variety of fungal illnesses that impact living things.

Keywords: *Croton nigritanus* Scott Elliot, TLC, chemical constituents

Introduction
The plant *Croton nigritanus* is well recognized for its colorful leaves. It is one of six species of evergreen broadleaf perennials, shrubs, and small trees that make up the Euphorbiaceae family. According to research by [3], the specie is widespread in tropical areas of Asia, Africa, and the western Pacific region. Compact shrubs like *Croton nigritanus* are frequently used in tropical landscaping. This plant develops as a bushy shrub with numerous branches in its natural habitat, growing to a height of at least 10 feet. The produced versions, nevertheless, that are offered for sale as houseplants are often smaller than their wild counterparts. *Croton nigritanus* has big, leathery leaves that range in size from 2 to 12 inches. They come in a variety of shapes, from linear to round in some cases. *Croton nigritanus* foliage have a variety of hues, including green, white, pink, orange, red, yellow, or purple patterns. As the leaves mature, these hues may shift. These patterns can show up as blotches in various areas of the leaf or along the main veins. Additionally, some types have sprouts that are completely different from the parent plant in appearance. When *Croton nigritanus*' stems are cut, it releases a milky sap that, like that of many other Euphorbiaceae plants, can irritate sensitive people's skin. As stated by [3], it seldom ever flowers when grown indoors.

Antifungal Activity.
Croton nigritanus has big, leathery leaves that range in size from 2 to 12 inches. They come in a variety of shapes, from linear to round in some cases. *Croton nigritanus* foliage have a variety of hues, including green, white, pink, orange, red, yellow, or purple patterns. As the leaves mature, these hues may shift. These patterns can show up as blotches in various areas of the leaf or along the main veins. Additionally, some types have sprouts that are completely different from the parent plant in appearance. When *Croton nigritanus*' stems are cut, it releases a milky sap that, like that of many other Euphorbiaceae plants, can irritate sensitive people's skin. As stated by [3], it seldom ever flowers when grown indoors.

Isolation of Compounds from Croton Plant
According to [5] the following composites crotepoxide, lupeol and betulin, cis- clerodane, crotomacrine, 3β- Acetoxy tetraxer-14-en-28-oic acid, trachylina-19-oic acid, trachylina-18-oic acid are among the insulated composites from colorful corridor of *Croton macrostachyus*. (6) insulated new composites 5,14- dihydroxyrotundone- 9-(2)- methybut- (2)- enoate)

and (-) 5,14- dihydroxyrotundane-9-benzoate from *Croton hirtus* using spectroscopic ways. Phytochemical webbing of an ethanol - water excerpt from the dinghy of Croton guatemalensis led to the insulation and identification of eight composites including five entclerodane diterpenoids (junceic acid (1), 6 (s)-acetoxy (16), 14- trien-20-oic acid(crotoguatenoic acid A (2), 6 (s)- hydroxyoxy (16), 14-trien-20-oic acid(crotoguatenoic acid B (3), formosin F (4), bartsiifolic acid (5)), and three flavonoids(rutin (6), epicatechin (7), and quercetin (8)) [7].

Phytochemistry of Croton Species

Croton oligandrus Pierre ex Hutch., *Croton megalocarpus* Hutch., Croton menyharthii Pax, Croton rivularis Mull. Arg., and *Croton megalobotrys* Mull. Arg. are five African Croton species. The chemical makeup of these species was elucidated by [8]. Ent-isopimara-7(8), 15-dien-19-yl octadecanoate, ent-isopimara-7(8), 15-dien-7-one, and ent-isopimara-7(8), 15-dien-16, 19-diol were isolated from the fruits of *C. oligandrum*, and ent-19-hydroxyisopimara-8(9), 15-dien-7-one was isolated from the species' leaves [8]. states that several spectroscopic methods were used to determine the compounds' structures.

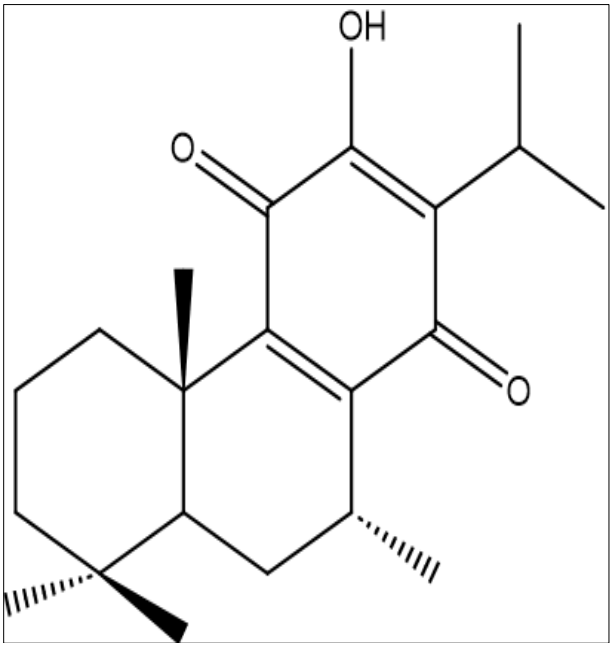

Fig 1: *Dehydroroyleanone*

The ent-isopimarane, ent-abietane, and ent-clerodane diterpenoids were confirmed to have the following absolute configurations: Epoxy-ent-clerodadiene,3,4:15,16-diepoxy-13 (16), 14-entclerodadien-17, 12 Solide, 3, 4:15, 16-diepoxy-8-hydroxy-ent-cleroda13(16),14-die Epoxychiromodine, -7,13-ent-abietadien-2-one, crotonolide E, furocrotinsulolide A, 3, 4: 15, 16-diepoxy-13 (16), 14-ent-clerodadiene, and crotohalimaneic acid [8, 9] found that in addition to other biologically active chemicals, *C. megalocarpus* also contains antiviral compounds. Eudesmane-type sesquiterpenes from *C. megalocarpus*, specifically 5-Hydroxy-8-methoxy eudesm-7 (11)-en-12, 8-olide (1), 5, 8-Dihydroxy eudesm-7 (11)-en-12, 8-olide, and 5-Hydroxy-8H-eudesm-7 (11)-en-12, 8-olide. Members of this plant family, according to [9], can create the ingredients for brand-new antivirals that can cure HIV. Alkaloids, diterpenoids, essential oils, flavonoids, furanoditerpenoids, and triterpenoids are some of the phytochemicals extracted from *Croton* species.

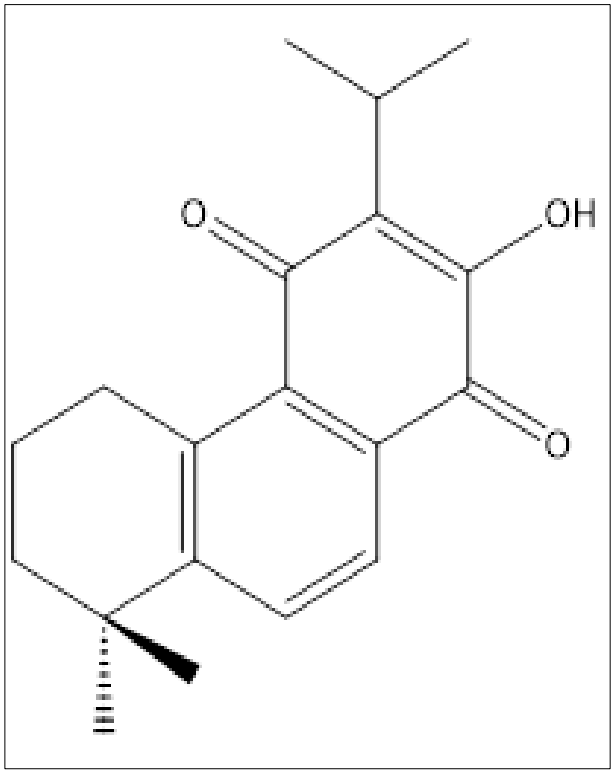

Fig 2: Structure of Salvialerione a diterpenoid

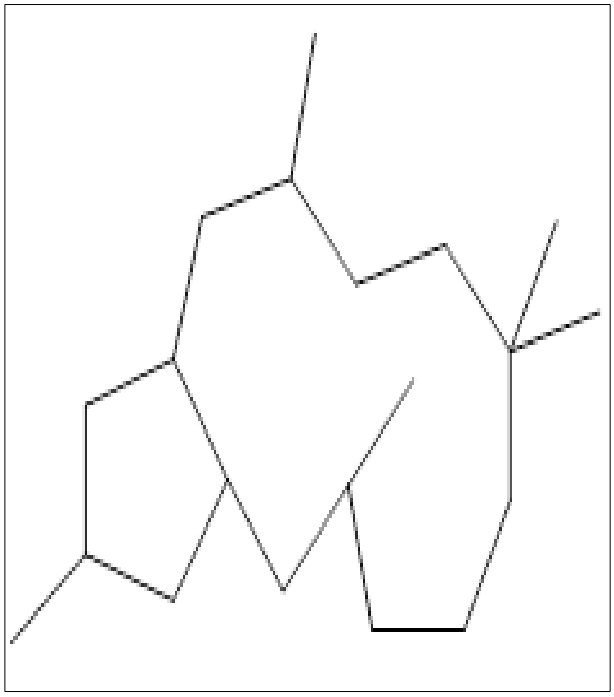

Fig 3: Jatrophane

Early pharmacological research indicates that isolated chemicals and extracts from Croton species have cytotoxic and antibacterial effects [10].

Along with other plant parts, the bark of *Croton macrostachyus* is employed as a treatment for a number of diseases in traditional Ethiopian medicine. On crude extracts from different plant sections, preliminary qualitative phytochemical testing revealed the presence of phenolic compounds, tannins, terpenoids, alkaloids, saponins, free anthraquinones, phytosterols, and polyphenols [5]. The leaf extracts of *C. gratissimus* in acetone and ethanol demonstrated that it can be used as a substitute source of therapeutic ingredients, according to [11].

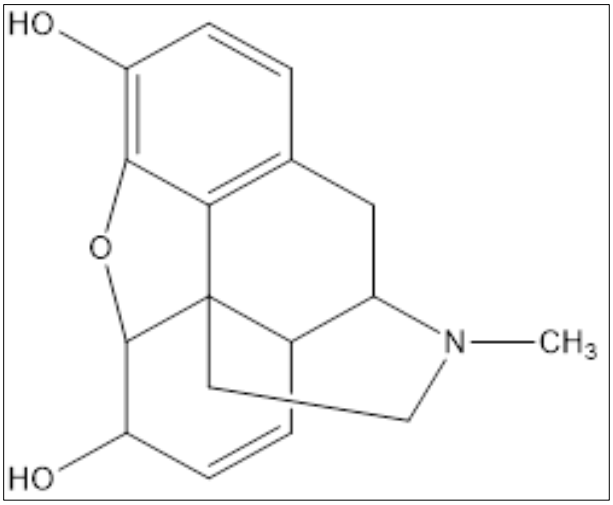

Fig 4: Morphine

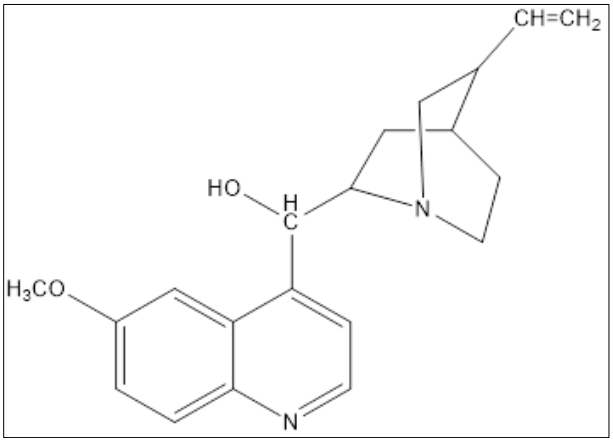

Fig 5: Quinine

Diterpenes, which include several types such clerodanes, cembranoids, halimanes, kauranes, labdanes, phorbol esters, trachylobanes, and sarcopetalanes, are often discovered chemicals in Croton. Due to the presence of volatile oils, several Croton species are fragrant [12].

Experimental

Materials and Method

Plant Materials: The plant component was procured in July 2023 from Savannah Woodland in Sakaru Village, Zaria, Kaduna State, at coordinates 11.0855 ºN and 7.7199 ºE. Dr. U.S. Gallah from the Department of Botany at Ahmadu Bello University in Zaria verified the sample's authenticity. *Croton nigritanus* leaves were packed in nylon bags with open mouths for aeration during transit to the Chemistry Research Laboratory of Pen Resource University Gombe, Nigeria. Voucher specimen ABU/H/13715 has been maintained at the herbarium of the University's Botany Department. The leaf fragments were air-dried for three weeks at room temperature before being crushed into tiny pieces in a Hammer Mill equipment. Until it was required for extraction, the resulting powdered plant material was kept in a tightly sealed polythene bag. Solvents of analytical quality were used for the extraction method.

Extraction of Plant Material

The maceration procedure was used to remove the dried and powdered plant material from the leaves (500 g) using 2.5 liters of CH_2Cl_2 [1]. Crude extracts were created by drying out the resultant extracts in a rotary evaporator at 50 degrees Celsius.

Phytochemical analysis

Dichloromethane extract from *C. nigritanus* leaves underwent a preliminary screening to look for common secondary metabolites. The techniques mentioned earlier in the analytical procedures were used for this screening [2].

Test for terpenes

A layer was created by combining 250 mg of ethanol extract with 2 ml of dichloromethane and 30 ml of concentrated H_2SO_4 [2]. A reddish-brown staining of the interface was noticed.

Test for flavonoids

A tiny amount of diluted NaOH was used to dissolve the dichloromethane extract (250 mg), and then 3 ml of strong HCl was added. The resulting yellow solution was seen, but on closer examination it became colorless [1].

Test for Tannins

The dichloromethane extract was diluted with water, then the mixture was heated on a water bath. A dark-green solution was seen when a few grams of solid $FeCl_3$ were added to the filtrate following filtration [3].

Test for alkaloids

2 cc of strong hydrochloric acid was added to 250 mg of the crude extract. Following [1] 's instructions, the liquid was filtered and then blended with a little amount of amyl alcohol at room temperature. The final combination was examined to determine whether the alcohol layer's coloring indicated the presence of alkaloids.

Test for saponins

30 minutes were spent shaking 250 mg of dichloromethane extract with 5 ml of distilled water. When the mixture reached boiling, it was looked for the presence of a creamy mixture with few bubbles [2].

Test for anthraquinones

500 mg of the dichloromethane extract were filtered after being briefly heated in strong hydrochloric acid in a water bath. After allowing the filtrate to cool, the same amount of $CHCl_3$ was added. The mixture received a few drops of ammonia before being cooked in a water bath. A rose-pink tint was seen to develop [2].

Table 1: Phytochemical screening test of Dichloromethane extract of *Croton nigritanus*

Plant constituent	Observation
Alkaloids	+
Flavonoids	+
Terpenes	+
Tannins	-
Saponins	+
Anthraquinones	+

(+) detected (-) not detected.

Column Chromatography Packing of Crude Extract

After being thoroughly cleaned, the glass column was firmly fastened upright to a retort stand. At the foot of the sparkling glass column, cotton wool was layered. To ensure adequate packing, the prepared slurry was gently poured into the

column while being lightly tapped. The crude extract was then applied to the column in the predetermined amount. Beginning with CH_2Cl_2, the elution proceeded with n-hexane (9:1), (8:2), (7:3), (6:4), and (1:1); ethyl acetate (6:4), (7:3), (8:2), and (9:1); and, finally, 100% ethyl acetate. 200 mL of several solvent systems with increasing polarities were used to wash the column. The final fractions were collected and allowed to dry in a chamber before being placed in 10 mL vials. The eluents were also subjected to thin-layer chromatography (TLC), with a 100% n-hexane solvent system. In this step, the purity of each eluent was evaluated, along with its component count and, when practical, compositional analysis.

Thin Layer Chromatography

Using silica gel plates as the stationary phase, thin-layer chromatography of the active polar CH_2Cl_2 fraction from the crude extract was carried out. These chromatoplates were placed in a developing chamber and allowed to proceed in a CH_2Cl_2: n-hexane (9:1, 8:2, 7:3, 6:4, 1:1) mobile phase system before being exposed to 100% CH_2Cl_2 and 100% n-hexane. The TLC plate was removed after it had been developed, dried by air, and examined under a UV lamp using spray reagent (10% sulphuric acid in water), which was then heated with a hairdryer.

Isolation and Purification

Eluents that had similar components, as confirmed by their Rf values obtained under experimental settings utilizing thin-layer chromatographic methods, underwent additional purification. Different column diameters (varying from 1 to 3 cm in diameter) packed with silica gel (Merck Art. 5554) in particular solvent systems were used for column chromatography purification. Two fractions with the identical Rf values were produced throughout the purification process and mixed, displaying a yellowish tint. A single spot for the fraction was also found using thin-layer chromatography. The isolate, known as CNDR-1, underwent various spectrum analyses.

Characterization Procedure

Spectroscopic examination was used to pinpoint the structures. The Royal Botanic Garden, Kew, a cooperating laboratory, received the separated chemicals for proton NMR investigation. A 400MHz Bruker AVANCE III NMR spectrometer was used for the NMR investigations. The central line at around 7.26 in the 1H NMR spectrum was sometimes used to reference CDCl3 when spectra were taken in that solvent. Software called Delta NMR was used to process the spectra. The isolated chemical needs additional investigation to determine its exact structure.

Test Microorganisms

The Department of Microbiology at Gombe State University in Gombe, Nigeria, acquired and authenticated clinically produced laboratory resistant strains of *Aspergillus Niger* and yeast.

Antifungal activity

In this experiment, an RPMI 1640 agar plate with three wells containing different antifungal drug concentrations- itraconazole at 4 g/ml, voriconazole at 2 g/ml, and posaconazole at 0.5 g/ml - and one well devoid of any medication - was used. After adding yeast and *Aspergillus Niger* to the plate, their growth was tracked for 48 hours. The isolates had established susceptibility [14].

Determination of Minimum Inhibitory Concentrations (MICs)

The procedure described by [14] was used to determine the Minimum Inhibitory Concentrations (MICs) of fractions generated from endophytic fungal extracts. The same microorganisms that showed activity during the initial antimicrobial screening were used to evaluate these MICs. Following the 2018 CLSI recommendations, the MICs of the fungal extracts were calculated using the agar dilution method. For each sample, a stock solution of 5 mg/ml was first made. These solutions were then serially diluted twice to produce 400, 300, 200, and 100 g/mL concentrations. Then, sterile Petri plates holding 1 mL of the various sample dilutions were filled with 4 mL of molten double-strength MHA and SDA (for bacterial and fungus isolates, respectively). After being grown in broth for an overnight period, the test isolates were adjusted to the McFarland 0.5 standard and streaked onto the surface of agar plates containing various extract dilutions. MHA plates were incubated for 24 hours at 37 °C and SDA plates for 3 days at 27 °C. All plates were checked for growth after incubation. The Minimum Inhibition Concentration (MIC) was defined as the lowest dilution (or concentration) of the samples that entirely prevented each organism's development.

Result and Discussion

Table 2: Result of FTIR spectrum of *Croton nigritanus* isolate

S/N	Peak	Functional group
1	3541.53cm^{-1} Broad	-OH group present
2	2853.25 cm^{-1}	Sp^3 -CH
3	2955.59 cm^{-1}	Sp^2 -CH
4	1738.78 cm^{-1} medium	-C=C present
5	1634.82 cm^{-1} Strong and sharp	-C=O stretch
6	Weak medium peak at 1658.32 cm^{-1}	Aromatic overtone
7	Medium sharp peaks at 1640.07 cm^{-1} and 1620.63 cm^{-1}	

Table 3: Spectral data of Compound 1, 1H NMR (400 MHz; CDCl3).

Position	1H NMR (δ ppm)
1.	-
2.	-
3.	-

4.	-
5.	-
6.	-
7.	-CH_2(C)
8.	-CH_2(CH)
9.	-CH(OH)
10.	-CH(O)CH_3
11.	-CH(Ar)
12.	-CH_2(C=O(CH_3)
13.	-CH_3(CH)
14.	-
15.	-CH_3(C=O)
16.	-
17.	-OH
18.	-
19.	-CH_3(O)
20.	-CH_3(Ar)
21.	-H(Ar)
22.	-H(Ar)

The values can be interchangeable within the identical compound. (CDCl3, 400 MHz).

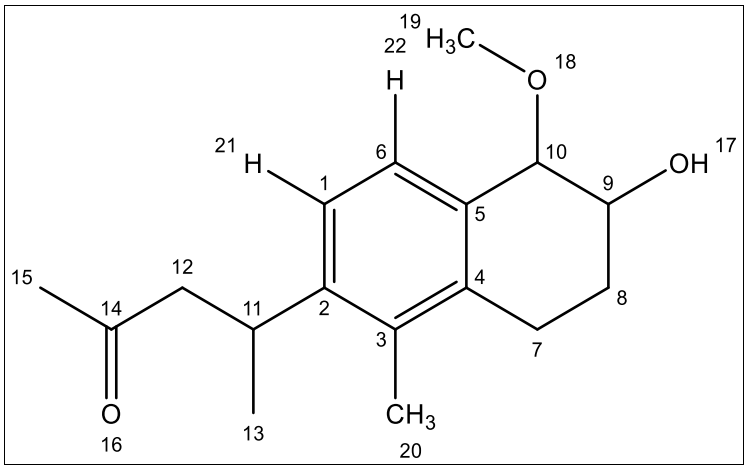

Fig 6: The suggested configuration of the compound.

Table 4: Antifungal Sensitivity test result of crude extract of *Croton nigritanus*

S/N	Organism	Concentrations				Control
		400 µg	**300 µg**	**200 µg**	**100 µg**	**Aug 30 µg/Keto 100 µg/ml**
1.	Yeast Sp	15 mm	11 mm	8 mm	7 mm	Keto 100 µg/ml 31 mm
2.	*A. niger*	16 mm	8 mm	7 mm	6 mm	Keto 100 µg/ml 22 mm

Keyword: mm = millimeter, Keto = Ketoconazole 100 µg/ml, Aug = Augmenting, 30 µg/ml = microgram (µg).

The results show how responsive the tested organisms are to various antifungal agent doses. The antimicrobial disc's inhibition zone measures the efficiency of the tested concentration against the organism in millimeters (mm) units. Greater sensitivity or effectiveness of the antifungal drug is indicated by a broader inhibition zone. The control data offer a basis for comparison by illuminating the organisms' anticipated sensitivity to the used control agents.

Table 5: Minimum Inhibitory Concentration (MIC) of the test organisms

S/N	Organism	Concentrations			
		400 µg	**300 µg**	**200 µg**	**100 µg**
1	Yeast Sp.	-	-	-	+
2	*A. niger*	-	-	+	+

Keyword: (-) = Negative not detected, (+) = Positive Detected, S/N = Serial Number

A positive symbol (+) in the MIC values denotes a substance concentration that successfully inhibited the growth or activity of the tested organism, whereas a negative symbol (-) denotes a substance concentration that had no such inhibitory effect. The MIC stands for the lowest concentration of a drug required to effectively inhibit the development of a bacterium. In this case, concentrations of 100 g effectively prevented the growth of yeast and *A. niger*ia. Additionally, *A. niger*'s growth was successfully stopped by concentrations of 200 g and 100 g. However, 400 g and 300 g concentrations failed to stop the organisms. It is significant to highlight that without further information regarding the compounds under study, a thorough interpretation of the MIC results is difficult.

Table 6: Minimum Bacterial Concentration (MBC) of the test Organisms

S/N	Organism	Concentrations			
		400 µg	300 µg	200 µg	100 µg
1	Yeast Specie	-	-	+	+
2	*A. niger*	-	+	+	+

Keyword: (-) = Negative not detected, (+) = Positive Detected, S/N = Serial Number

At doses of 200 g and 100 g, growth inhibition in yeast and *A. niger* was evident. These findings imply that the tested substance's inhibitory effects on various organisms at various concentrations varied. According to [13] results, *A. niger* was particularly sensitive, showing growth reduction at the majority of the tested doses, underscoring *A. niger*'s elevated sensitivity among the tested fungi. However, only quantities of 200 g and 100 g of yeast showed inhibition. The results highlight how susceptible the yeast species is to antifungal medications. A significant inhibition zone was visible for the control drug ketoconazole (31 mm), demonstrating the strong sensitivity of yeast species to this antifungal medication. Higher concentrations resulted in smaller sensitivity and bigger inhibition zones. *A. niger*'s sensitivity to the tested antifungal drugs was also shown by the results. Ketoconazole (22mm), the control drug, displayed a comparatively broad inhibitory zone, demonstrating *A. niger*'s high sensitivity to this antifungal medication. Larger inhibition zones were produced as a result of the antifungal drugs' decreasing efficiency as their concentrations rose. These findings show that the examined organisms exhibit various levels of antifungal agent sensitivity. The control results serve as a baseline for comparison and show how sensitive the organisms should be to the applied control agents. These results can help medical professionals choose the best antifungal medications for infections brought on by these organisms while taking into account the antifungal medicines' potency at various concentrations. According to the research by [1], Bacillus subtilis, Staphylococcus aureus, and other bacteria were moderately to strongly inhibited by the DCM/Methanol fraction (95:5). MIC values for Candida albicans and Escherichia coli range from 0.03 to 1 mg/mL. However, it showed no inhibition against Salmonella typhi and *Aspergillus Niger*.

Conclusion

Croton nigritanus dichloromethane extract demonstrated the presence of alkaloids, flavonoids, terpenes, saponins, and anthraquinones in preliminary phytochemical study. The study confirmed its traditional therapeutic uses against fungal illnesses by revealing the presence of plant chemicals that prevent the growth of particular fungus, like Yeast and *A. niger*. Spectroscopic examination also reveals the proposed structure of the substance CNDR-1. Furthermore, it is essential to fully characterize the isolated molecule.

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Conflicts of Interest: The authors assert that they do not possess any conflicts of interest.

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