Sahel Journal of Life Sciences FUDMA (SAJOLS)
December 2023 Vol. 1(1): 169-188
ISSN: 3027-0456 (Print)
ISSN: xxxx-xxxx (Online)
DOI: *https://doi.org/10.33003/sajols-2023-0101-019*

Research Article

Isolation and Structural Elucidation of Antimicrobial Molecules from *Crinum ornatum* (Aiton) Rhizome of Dutsin-Ma

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Received: 3rd December, 2023 **Accepted**: 13th December, 2023 **Published**: 31st December, 2023

ABSTRACT

Crinum ornatum (*C. ornatum*) is a well-known traditional herb that belongs to the plant family Amaryllidaceae. It is used traditionally to treat various diseases including wounds, sores, vomiting, ear-aches, urinary tract infection, coughs and cold, renal and hepatic conditions, sexually transmitted diseases and backaches. The rhizomes of *Crinum ornatum* were collected, identified, air-dried and pulverized and subjected to cold extraction (maceration) using hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) respectively. Antimicrobial activity of the extracts was determined using standard antimicrobial tests with *Staphylococcus aureus, Salmonella typhi, Baccilus subtilis* and *Pseudomonas aeruginosa* as test microorganisms. The most active extract was thereafter subjected to Gas chromatography Mass Spectrometry (GCMS) analysis, the Structural elucidation of the isolated molecule was performed using advanced spectroscopic techniques, including nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), Results of the antimicrobial activity test of the extracts showed appreciable antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* (with MIC value ranging from 200 mg/ml to 25 mg/ml). The results demonstrated significant inhibitory effects, highlighting the potential of the compound as an antimicrobial agent. GCMS analysis identified 45 compounds in the most active (DCM) extract. Out of these, eight (8) compounds were most abundant (revealed >5% peak areas on GC chromatogram). These are: n-hexanedecanoic acid**,** hexadecanoic acid ethyl ester**,** 9,12-octadecadienoic acid (Z,Z),oleic acid**,** linoleic acid ethyl ester, ethyl oleate, squalene and tetrapentacontane. The antimicrobial activity of the isolated compound was assessed against a panel of pathogenic microorganisms, Antibacterial activity exhibited by the plant could be ascribed to the presence of the major phytocompounds in the plant and this provides scientific proof of some of ethnomedicinal uses of *Crinum ornatum* rhizome.

Keywords: GCMS; FTIR; NMR; Phytochemical; Antimicrobial; Dutsinma; *Crinum ornatum*

Citation: Riji, A. B., Rimi, A. D. and Sabo, A. S. (2023). Isolation and Structural Elucidation of Antimicrobial Molecules from *Crinum ornatum* (Aiton) Rhizome of Dutsin-Ma. *Sahel Journal of Life Sciences FUDMA*, 1(1): 169-188. DOI: *https://doi.org/10.33003/sajols-2023-0101-019*

INTRODUCTION

According to studies, humans have been using natural products like plants, animals, microbes, and marine organisms to eradicate and treat various diseases since. It has been estimated that humans have been using plants as remedies for at least 60,000 years. In some countries, such as South Africa and Ghana and many more. When early humans were searching for food, they occasionally ate poisonous plants, which could cause vomiting, diarrhea, comas, or other toxic reactions in the body system, and in some cases, death. As a result, early humans had the capacity to develop knowledge and skills about suitable materials and natural treatments (Gao *et al.,* 2007). The World Health Organization (WHO) estimated that due to poverty and lack of access to modern medication, 65 to 80 percent of the world's population lives in under developed nations and relies primarily on plants for

treatment (Awoyemi *et al.,* 2012; Kalayu *et al.,* 2013).

According to Jagtap (2014), the family Amaryllidaceae contains only one pantropical genus, *Crinum,* which has 111 taxa, 107 species, one subspecies, and three variations (Lekhak *et al.,* 2015). Bulbs of *Crinum ornatum* can grow up to 10 cm in diameter, frequently with a long neck, and spread vegetatively to create clusters. Bracts are persistent and have a greenish tinted red color. The flower has three to six petals and is dark greenish red in the bud (Yakandawala & Samarakoon, 2006). The plant commonly known as Toad's Onion in (English name), Albasar kwadi in Hausa, and Isumeri in Yaruba.

Crinum species have a high reputation as therapeutic plants in ethnopharmacology. They have been used for centuries and are still widely used today, especially in South America, tropical Asia, and Africa. (Refaat *et al.,* 2013).The bulb of several *Crinum* species is interesting since it is used to cure illnesses like urinary tract infections, coughs and colds, renal and hepatic ailments, ulcers, sexually transmitted diseases, backaches, as well as boost lactation in both people and animals, all over the world. It also has analgesic, immune-stimulating, antimalarial, antiviral, and antibacterial properties (Refaat *et al.,* 2012). In addition the *Crinum ornatum* bulbs are traditionally used in different countries to treat fever and respiratory illnesses such as coughs and colds. Cultural and spiritual uses, in southern African cultures. However, it is considered a sacred plant and is used in various cultural and spiritual ceremonies. For example, in the Zulu culture, the plant is used to communicate with ancestors and to protect against evil spirits (Cunningham, 2011).The bulb is said by herbalists to increase breastfeeding and reduce breast inflammation as well as other breast related illnesses when used in very modest doses. It is well known that the plants in this genus have analgesic, antimicrobial, immunostimulating, and anticancer effects (Ogunkunle & Olopade 2011).

MATERIALS AND METHODS

Materials and Chemicals

All chemicals used in this investigation were of analytical grade and were obtained from Sigma Chemical Co., St Louis, USA. Standard were obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK.

Plant collection and Preparation

The bulbs of *Crinum Ornatum* were collected at Dutsin-Ma Local Government Area, Katsina state, Nigeria. The bulbs was air-dried and ground into fine powder using mortar and pestle in the laboratory.

The fresh samples were air-dried. The dried plant material (bulb) were grounded into fine powder using laboratory pestle and mortar and electric grinder and packed into a clean sample container and labelled and kept for further use. Extractions was carried out through conventional method as described by Fasihuddin *et al.* (2010). This was achieved by soaking the powdered samples in solvent in the order of increasing polarity. A total of 1000g of the powdered sample was extracted using cold soaking method. This was achieved by soaking the powdered sample material in non-polar (n-hexane), medium polar (Dichloromethane and ethyl acetate) and polar (methanol) solvents in the order of increasing polarity. The dried bulbs of *Crinum Ornatum* was extracted. The sample was soaked in n- hexane in Bama bottles at room temperature for 72 hours. The resulting hexane solution was then filtered using filter paper and the residue was re-extracted with fresh hexane for another 72 hours and filtered. The extract was combined and concentrated using the rotary evaporator (model Heidolph Laborota 4000 efficient) under reduced pressure to obtain hexane crude extract. The residues were then re-extracted using similar/ procedure with dichloromethane, ethyl acetate, and methanol to obtain dichloromethane, ethyl acetate, and methanol crude extracts, respectively. At the end of the extraction process the dry weight and yield of each crude extracts were determined. However, dichloromethane (DCM) extract was used for the study.

Preliminary Phytochemical Screening

A few milligrams of the four different dried extracts were obtained from n-hexane Dichloromethane, ethyl acetate and methanol was first dissolved and the various solutions obtained were all subjected to phytochemical screening employing the standard screening test (Trease & Evan, 1996).

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of the extracts of the plants material. Immediate development of a red color indicates the presence of flavonoids

Test for Tannins

To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish-black precipitate indicates the presence of tannins (Trease &Evan, 1996).

Test for Alkaloids

Few drops of mayer's reagent were added to 1 mL of extract .A yellowish or white precipitate was formed, indicate the present of alkaloids (Trease & Evan, 1996).

Test for Terpenoids

To 5 ml of the extract add 2 ml of chloroform and 3 ml of H_2SO_4, conc., formation of a reddish brown ring confirms the presence of terpenoids (Trease & Evan, 1996).

Test for Carbohydrate

Few drops of molish reagent were added to 2 ml of extract later drops of concentrated H_2SO_4 were added along the walls of the test tube. At junction of two liquids, a violet colour ring appeared, indicating that carbohydrate were present

Test for Anthraquinones

A few ml of H_2SO_4 conc was added to 5 ml of extract, followed by 1 ml of diluted ammonia. The existence of anthraquinones is confirmed by the appearance of rose pink.

Test for Saponins

With a few ml of distilled water, 0.5 mg of extract was quickly shaken. For saponins, the production of foaming is a favourable sign.

Test for Steroids

The presence of steroids is shown by the emergence of red color and yellowish green fluorescence after mixing 2 ml of extract with 2 ml of chloroform and 2 ml of concentrated H_2SO_4, the appearance of red color and yellowish green fluorescence confirms the presence of steroids

Thin Layer Chromatography (TLC) for Bioautography

The two extracts were subjected to thin layer chromatography (TLC) using several solvent-system (Hexane per ethyl acetate (4:1), Hexane per ethyl acetate (5:3) and Hexane per ethyl acetate (3:2) to obtain the best solvent system that would give good separation for the compounds suitable for bioautography. The extracts were then spotted on TLC plates and allowed to dry. After drying, the plates were developed in an air-tight chromatographic tank using the perceived solvent system. The developed chromatograms were air dried and visualized; under normal day light using ultra violet light (254 nm & 366 nm).

Direct TLC Bioautography

For the bioautography study, the developed TLC plate containing crude extract spots with good separation was sprayed with fungal or bacterial suspension. The salmonella typhi bacteria were chosen for the study. The bioautogram was then incubated at 25 ℃ for 48 h under humid conditions for visualization of microbial growth, tetrazolium salts were used. These salts were converted by the dehydrogenases of living microorganisms to intensely colored formazen. These salts are sprayed onto the bioautogram and are reincubated at 25 ℃ for 24 h (Silva, *et al.,* 2005). A clear white zone against a purple background on the TLC plate indicated an antimicrobial activity of the sample. Afterwards, the bioautogram was re-view with ultraviolet light (254 nm & 366 nm).

Antibacterial Activity of the Extract (Methods)

Preparation of Inocula

The bacterial strains of *Bacillus* spp., *Pseudomonads* spp., *S. aureus* and *Salmonella* spp. from the culture plates were standardized by matching turbidity of culture to 0.5 McFarland standards which would then be diluted in fresh broth (peptone water) and incubated at 37^{o}C for 24 hours to achieve final inoculums (Ezouberi *et al.,* 2005)

Serial Dilution.

One (0.5 g) of the extract were measured using balance and dispensed into the clean and sterile test tube containing 2 ml of distilled water to obtained a concentration of 250 mg/ml, followed by transferring into another containing 1 ml to obtained 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml, and stored for further analysis.

Discs Preparation.

Filter paper was punched using puncher and obtained an approximately 6mm diameter, autoclaved at 1210c for 15 minutes and dispensed into each concentrations and allowed to absorb for 1 hour.

Sensitivity Test Using Disc Diffusion

Thirty nine grams of (MHA) were prepared and 0.2 ml of each bacteria was inoculated on to the solidified Mueller Hinton agar. The dish was left on bench set (Priya & Deepak, 2007). Discs containing different concentrations of 250 mg/ml, 125 mg/ml,62.5 mg/ml and 31.25 mg/ml were seeded and Ciprofloxacin would be set as control. The plate were then incubated at 37^{o}C for 24 hours and diameter zone of inhibition was measured (Priya & Deepak 2007).

Determination of Minimum Inhibitory and Minimum Bactericidal Concentration

Dilution tubes methods were used in varying concentration of the liquid medium and the extract in test tubes at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml, 0.1 ml of the standardized bacterial inoculum in the same tubes. The tubes were then incubated aerobically incubated at 37^{o}C for 24 hours, positive control were equal set up. The tubes with the least growth showed MIC (Fritsche *et al.,* 2006). The MIC were then sub- cultured into nutrient agar plates that contain no antibiotic, the lowest concentration of the chemotherapeutic agent that resulted in no growth of the subculture was noted. This refers as MBC of the chemotherapeutic agents (Ellof, 1998).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC–MS analysis of bioactive compounds from the DCM extracts of *Crinum ornatum* was carried out using Model 7890A (Agilent Technologies) interfaced with a mass selector detector model 5975°C. The electron ionization was kept at 70eV with anion source temperature of 250°C, using Helium as the carrier gas and HP-5MS (30 mm × 0.25 mm × 0.320 µm) as the stationary phase. The oven temperature was kept at 80°C held for 4 minutes and ramped to 270°C at the rate of 3.5°C/minutes holding for 6 minutes. 1 mg each of the extracts was dissolved in 1ml of acetonitrile. The mixture was vortexed and sieved through 0.4 millipore filter into a 5ml rotavapour flask and dried using rotavapour. 700 µl dichloromethane was added and transferred into screw cap tubes. 1 µl each of the prepared extracts was injected into the column at 300°C. The split mode was employed with a split ratio of 50:1. Relative quantity of the chemical compounds present in each of the extracts of *Crinum ornatum* was expressed in percentage based on the peak area produced in the chromatogram.

Fourier-Transform Infrared Spectroscopy (FTIR) Method

Fourier-Transform Infrared Spectroscopy is a technique used to identify and quantify materials based on their absorption of infrared light. The stages involves;

Sample Preparation: The sample under investigation is usually prepared in a form that allows for easy interaction with the infrared (IR) radiation. This could involve making a thin film, mixing the sample with a suitable matrix, or other methods depending on the nature of the sample.

Instrumentation: FTIR spectrometers use an interferometer to collect the entire infrared spectrum simultaneously. A source emits infrared radiation, which passes through the sample, and the resulting spectrum is obtained.

Data Collection: The FTIR instrument collects data across a range of infrared wavelengths. The resulting spectrum is a plot of the intensity of the transmitted or absorbed light as a function of wavelength.

Data Analysis: Interpretation of the obtained spectrum involves identifying characteristic peaks and patterns associated with functional groups present in the sample. Various software tools are available for spectral analysis.

Quantitative Analysis: FTIR can be used for quantitative analysis by correlating the intensity of specific peaks with the concentration of particular components in the sample.

RESULTS AND DISCUSSIONS

Extraction result revealed that methanolic extract has the highest yield, followed by the hexane extract, the ethyl acetate extract, and the DCM extract, which has the lowest mass as shown. These findings suggest that the amount of polar molecules (such as saponins and flavonoids, glycosides) removed from the plant's bulb may have been the highest, subsequently then the phytochemicals extracted from n-hexane solvent which recognize as non-polar (steroid, fatty acid, and triterpenes) and also DCM and ethyl acetate were used to extract molecules of intermediate polarity, such as anthraquinone, tannins, and phenolic acids.

Thus, phytocompounds such as alkaloids, flavonoids, saponins, glycosides, possessing polar groups moiety, These polar groups, if present, have been reported to have anti-inflammatory, antioxidant,

antimicrobial, anticancer and cardioprotective activity (Yang *et al.,* 2018).While for non-polar phycompounds such as steroids, and terpenes

Also these non-polar phytochemicals, if present, have been reported to have anti-inflammatory, antimicrobial, and anticancer properties (Calder, 2017, Chen *et al.,* 2018; Wang *et al.,* 2020 and Mao *et al.,* 2019).

Extracts were prepared based on their polarity nature which is Hexane, dichloromethane, ethyl acetate and methanol respectively. Phytochemical screening of the crude extracts of *Crinum ornatum* shown in (table 1), phytochemicals were tested in all extracts like flavonoids, saponin, alkaloid, anthraquinone, carbohydrate and tannin were absent while steroid, terpeniod were present in only n-hexane extract this happens because n-hexane solvent is non-polar it can only extract non-polar compounds and subsequently in dichloromethane extract it was examine that phytochemicals such as flavonoids, saponin, stereiod, alkaloid, anthraquinone, terpenoids tannins, were detected and carbohydarate were absent for ethyl acetate extracts saponin, stereiod, alkaloid, anthraquinone, terpenoids, tannins and carbohydarate were detected but flavonoids, tannins were absent while in methanolic extract phytocompounds like tannin, flavonoids, alkaloid, anthraquinone, saponin and carbohydarate were all present this arise due to high polarity of solvent and terpenoids, anthraquinones are completely absent in methanol extracts.

Alkaloids were also tested positive in the methanol extract (Table 1). Alkaloids have been reported to have wide range of pharmacological activities. Some alkaloids have antimalarial properties (e.g. quinine), anti-asthma activities (e.g.ephedrine), anticancer properties (e.g homoharringtonine), according to Kittakoop, 2014.Others are cholinomimetic (e.g. galantamine), according to Russo *et al.,* 2013.They also possess vasodilatory properties (e.g. vincamine), antiarrhythmic activities (e.g. quinidine), analgesic properties (e.g. morphine) as was reported by Raymond *et al.,* (2010). Antibacterial alkaloids (e.g.chelerythrine) were also reported by Cushnie and Lamb (2005). For this research eight phytocompounds were tested (tannins, flavonoids,saponins,steroids,alkaloids,terpenoids,anthraquinones and carbohydrate) using four different organic solvents (n-hexane, ethyl acetate, dichloromethane and methanol) .The presence of tannins, flavonoids,saponins,steroids,alkaloids,terpenoids,anthraquinones and carbohydrate from the rhizome of the plant could be the linked to the ethnomedicinal uses of the rhizome of *Crinum ornatum* in traditional medicine practices. This is because these secondary metabolites have been known scientifically to act as antioxidants, anti-inflammatory, anticancer and antimicrobial, anti-malarial agents (Yang *et al.,* 2018; Wang *et al.,* 2014 and Mao *et al.,* 2019). Therefore, the presence of these phytochemicals in the plants studied could be a scientific evidence for the traditional and biological uses of the plants.

The results of the antimicrobial studies of crude extracts against *Staphylococcus aureus, Salmonella spp, Bacillus spp., Pseudomonas spp* was discovered. This indicates that the plant could be utilized to treat ailments caused by the bacterium. Antibacterial activity of n-hexane, dcm, ethyl acetate, and methanolic extracts of *Crinum ornatum* against *Staphylococcus aureus, Salmonella spp, Bacillus spp.,Pseudomonas spp* was demonstrated, in this investigation, serial dilution was conducted 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, also it were discovered that increasing the concentration of the extracts increased the antibacterial activity of the extracts, as dcm extract exhibits more promising outcomes than the other extracts among all microorganisms except *Bacillus spp*, this could be because the solvent employed to extract the various elements had antibacterial properties, implying that dcm was the most effective solvent for extracting antibacterial chemicals from the chosen plant (*Crinum ornatum*). Against the bacteria examined, however, the methanol extract had little antibacterial action. The findings support Banso *et al.,* (1999).Findings that larger concentrations of antimicrobial material result in improved growth inhibition. The fact that the results of this study showed that extracts of *Crinum ornatum* have antibacterial properties justifies the plant's traditional use as a medicinal plant for skin infections, which means that the plant has activity against *Staphylococcus aureus,* more over it indication that the plant could be used in treating wound, ear infection, burn infection due to considering of bacillus spp. activity from the plant, further more in line with activity in urinary tract infection, anti-cancer these arise due to diagonistic activity of *Pseudomonas spp* from the plant

All the MICs, against the bacterial tested, for the crude extracts were 200mg/ml, 100 mg/ml, 50

mg/ml, and 25 mg/ml. at 25mg/ml and 50 mg/ml no activity throughout the extracts used except for n-hexane and ethylacetate at 50mg/ml but as the concentration increase it also increase the activity of the extract against the organisms. for ethyl acetate the extract revealed out activity at 100mg/ml to *Staphylococcus aureus*only while *Salmonella spp, Bacillus spp.,Pseudomonas spp*. Indicate no action throughout, also at the same crude extract 200mg/ml (MIC) has positive outcome against all the organisms (*Salmonella spp, Bacillus spp.,Pseudomonas spp, and Staphylococcus aureus).* However in dichloromethane (DCM) crude extract at 200 mg/ml and 100 mg/ml, the extract has action against all the organisms used except *Salmonella spp. And Bacillus spp* at 100 mg/ml which shows negative result. Nevertheless in n-hexane the crude extract indicate good activity at 200 mg/ml, 100 mg/ml, and 50 mg/ml with *salmonell spp.* Only, unlike *Bacillus spp., Pseudomonas spp, and Staphylococcus aureus* which recognize positive action of the extract at 200 mg/ml and 100 mg/ml (MIC) beside *Pseudomonas spp and Staphylococcus aureus* which has no effect at 100 mg/ml. even though, for methanolic crude extract, the extract has positive outcome at 200 mg/ml and 100 mg/ml in all the organisms except for *Staphylococcus aureus*, *Salmonella spp. And Bacillus spp* which has no activity at 100 mg/ml. Also for the MBC activity of the extract against the used organism increase with increase in concentration of the extract 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml throughout the system. It can also be observed from the result that the most active extracts are the moderate polar ones (either the ethyl acetate or dichloromethane), as shown above. This is not surprising because these extracts contain molecules with heteroatoms (like oxygen) that could confer pharmacological activity on them.

The bioautography experiments conducted in this study revealed that the tested sample had antibacterial activity against *salmonella typhi*. The presence of active compounds was observed as clear zones of inhibition on the TLC plates indicating that the sample contains compounds that are capable of inhibiting bacterial growth. The size of the inhibition zones varied depending on the concentration of the sample applied to the TLC plate, with higher concentrations resulting in larger zones. Findings from this study suggest that the antibacterial activity of the samples is concentration-dependent and that increasing the sample concentration could enhance its bioactivity. The studies were evaluated using, ethyl acetate and methanol extracts (Figure 1a & b). Ethyl acetate extract showed broad-spectrum antibacterial activity with clear zones observed on the TLC plate (Figure 1a). The size of the clear zones suggested that the extract has a higher concentration of active compounds. The methanol extract showed the broadest antibacterial activity with the largest clear zones observed on the TLC plate. The results suggested that the methanol extract contains the highest concentration of active compounds with the broadest range of antibacterial properties. However, the bioautography results imply that the methanol extract is the most promising extract for further investigation due to its broad-spectrum antimicrobial activity.

In the present study, four different crude extracts were obtained from the rhizome of *Crinum ornatum* through selective sequential extraction with different solvents, namely; n- hexanes, dichloromethane ethyl acetate and methanol. GC-MS analysis of dichloromethane crude extract were co*n*ducted (table 4.) revealed the present of fourty five (45) compounds, 2-Propenoic acid, 3-phenyl-, 1,7,7-trimet (0.08 %) has least Relative Area and 9,12-Octadecadienoic acid (Z,Z) (18.89 %) has most high Relative Area . In addition, out of 45 chemical compounds identified only eight (8) compounds are more bioactive compounds with high pharmacological activities. The eight (8) bioactive compounds are n-hexanedecanoic acid**,** hexadecanoic acid ethyl ester**,** 9,12-octadecadienoic acid (Z,Z),oleic acid**,** linoleic acid ethyl ester, ethyl oleate, squalene and tetrapentacontane.

However, major bioactive compounds such as Hexadecanoic acid has been used in skincare products due to its ability to improve skin hydration and elasticity. It has also been found to reduce inflammation in the skin (Yoo *et al.,* 2016), has a potential of biocontrol agent for plant diseases. Subsequently It has been found to inhibit the growth of several plant pathogens and may be a safer alternative to traditional chemical pesticides (D'Amico *et al.,* 2019),

9,12-Octadecadienoic acid (Z,Z) contain antimicrobial agent potential for topical applications, such as in wound healing and acne treatment. It has been found to inhibit the growth of bacteria and reduce inflammation (Johnson *et al.,* 2018) also 9,12-octadecadienoic acid (Z,Z) has been shown to have antibacterial activity against a wide range of gram-positive and gram-negative bacteria, including Staphylococcus aureus, Escherichia coli, and

Pseudomonas aeruginosa. It disrupts bacterial cell membranes and inhibits bacterial growth (Riaz *et al.,* 2019).Oleic acid has moisturizing properties and may help to improve skin hydration and reduce the appearance of fine lines and wrinkles (Fluhr, 2017). Linoleic acid ethyl ester has been investigated as a potential antimicrobial agent such as in wound healing and acne treatment. It has been found to inhibit the growth of bacteria and reduce inflammation (Li *et al.,* 2021). In additions Linoleic acid ethyl ester has been shown to have antibacterial activity against several gram-positive and gram-negative bacteria, including Staphylococcus aureus and Escherichia coli. It disrupts bacterial cell membranes and inhibits bacterial growth (Xu *et al.,* 2016).

Also Ethyl oleate has been shown to have anti-inflammatory effects on the skin and may be useful in treating various skin disorders. It revealed that ethyl oleate reduced inflammation and improved skin barrier function in a mouse model of atopic dermatitis (Kim *et al.,* 2016). It found that topical application of ethyl oleate reduced acne lesions and improved skin appearance in human volunteers (Chiu, 2012). Squalene has been used in cosmetic formulations as a moisturizer and skin conditioning agent due to its ability to penetrate the skin and enhance its natural barrier function. It discover as a potential In treatment for skin disorders such as acne, atopic dermatitis, and skin aging. (Lin *et al.,* 2018) in additions Squalene has been shown to have immune-boosting effects and may help improve the body's response to infections. Squalene has been shown to inhibit the proliferation of various cancer cell lines, including prostate, breast, lung, and colon cancer. It works by inducing apoptosis, inhibiting cell cycle progression, and reducing the expression of oncogenes (Chen et al,. 2021).While for the Tetrapentacontane exhibits antioxidant activity, which helps to reduce oxidative stress in the body. It scavenges free radicals and protects cells from damage caused by oxidative stress, making it a potential candidate for the treatment of diseases associated with oxidative stress such as cancer, diabetes, and cardiovascular disease (Alam*,* 2015).

From FTIR study the Strong peaks in the region of 2800-3000 cm suggest the presence of aliphatic C-H stretching vibrations, indicating the extraction of hydrocarbons. Lack of peaks in the carbonyl (C=O) and hydroxyl (O-H) stretching regions indicates the absence of polar compounds. Hexane is generally effective at extracting non-polar substances like fats, oils, and some pigments. A sharp peak around 1735 cm^{-1} indicates C=O stretching vibrations, suggesting the presence of esters or other carbonyl-containing compounds. the appearance of carbonyl-related peaks distinguishes ethyl acetate from hexane, suggesting a different set of extracted compounds with more polarity. Peaks in the 700-800 cm^{-1} range indicate C-Cl stretching vibrations, suggesting the presence of chlorinated compounds. Similar to hexane and ethyl acetate, peaks in the 1375-1475 cm-^{1} and 1725 cm^{-1} ranges indicate C-H bending and carbonyl stretching, respectively. A broad peak in the region of 3200-3600 cm^{-1} indicates O-H stretching vibrations, suggesting the presence of alcohol or phenolic groups. Methanol itself contains an O-H group, contributing to the peak.The integration values in the 1H NMR spectrum provide information about the relative number of protons contributing to each signal. The integration values (the area under each peak) in the 1H NMR spectrum provide information about the relative number of protons contributing to each signal. The distance between split peaks (coupling constants) provides information about the coupling between neighboring protons and can help determine the connectivity of the atom.

The presence of these bioactive compounds with antimicrobial properties suggests the potential of *Crinum Ornatum* as a sources many pharmacological activities. Base on medicinal uses of bioactive compounds identified from GC-MS of dichloromethane extract from *Crinum ornatum Rhizome* with best peak, it revealed out that the plant has antimicrobial activity which is the main target of the research.

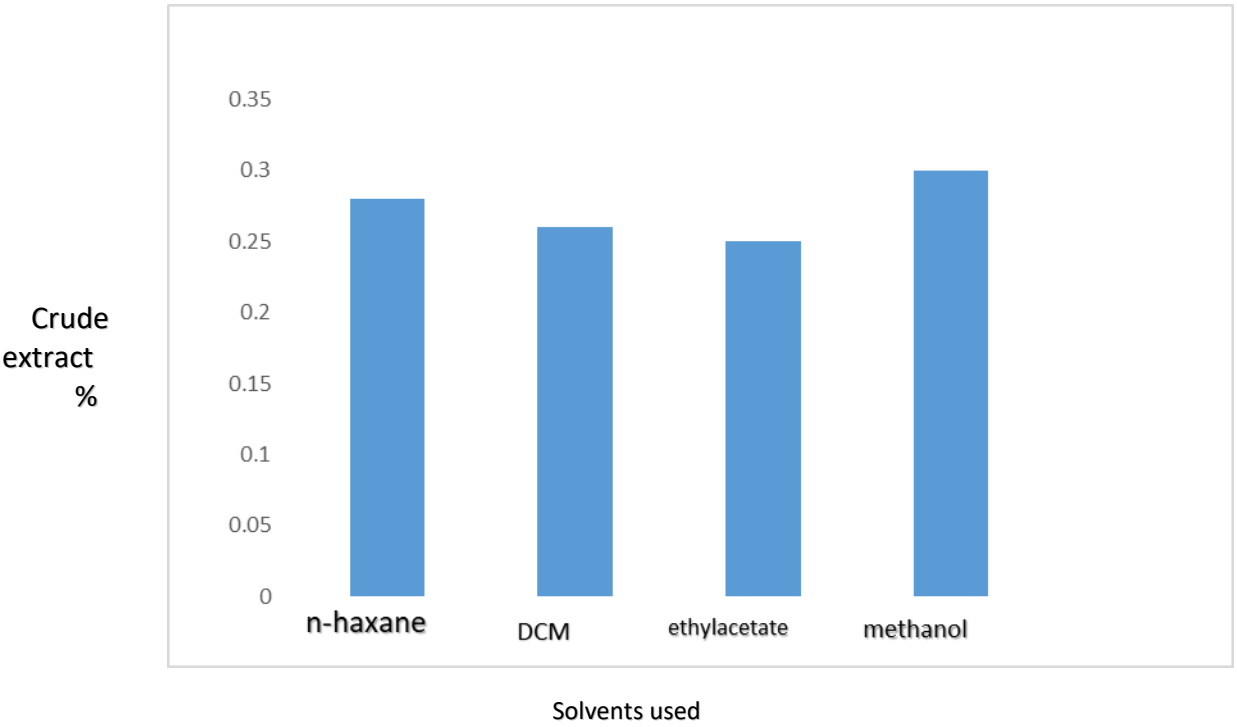


Figure 1. Percentage Yield (%) of Crude Extract result

Table 1. *Crinum ornatum* Phytochemical Screening of the Crude Extracts

Phytochemicals	n-hexane	CH_2Cl_2	EtOAc	MeOH
Tannins	-	+	-	+
Flavonoids	-	+	+	+
Saponins	-	+	+	+
Steroids	+	+	+	-
Alkaloids	-	+	+	+
Terpenoids	+	+	+	-
Anthraquinones	-	+	+	+
Carbohydrate	-	-	+	+

Key: += present, **-** =absent MeOH= Methanol extract, CH_2Cl_2 = Dichloromethane extract, n-hex= Hexane extract, EtOAc= ethyl acetate extract

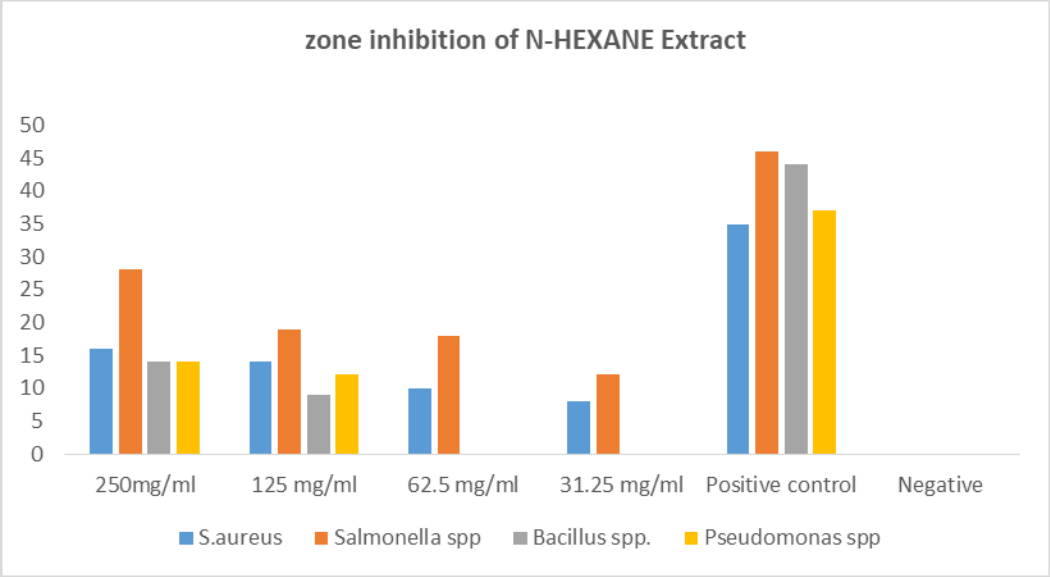


Figure 2: n-Hexane Crude Extract Antibacterial Test

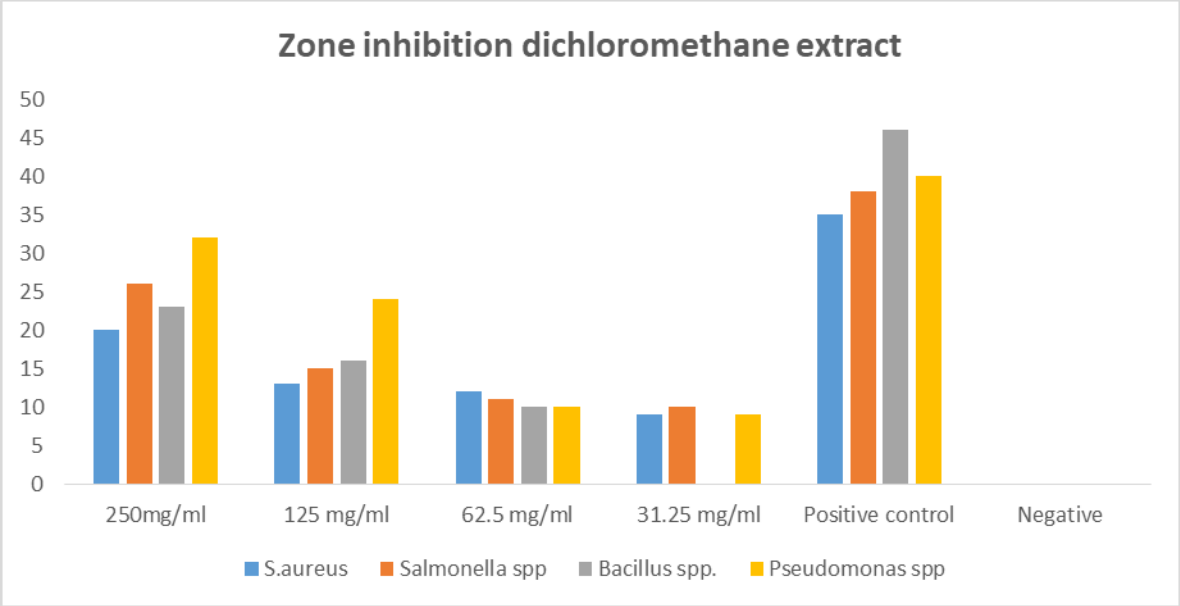


Figure 3. Dichloromethane Extract Antibacterial Test

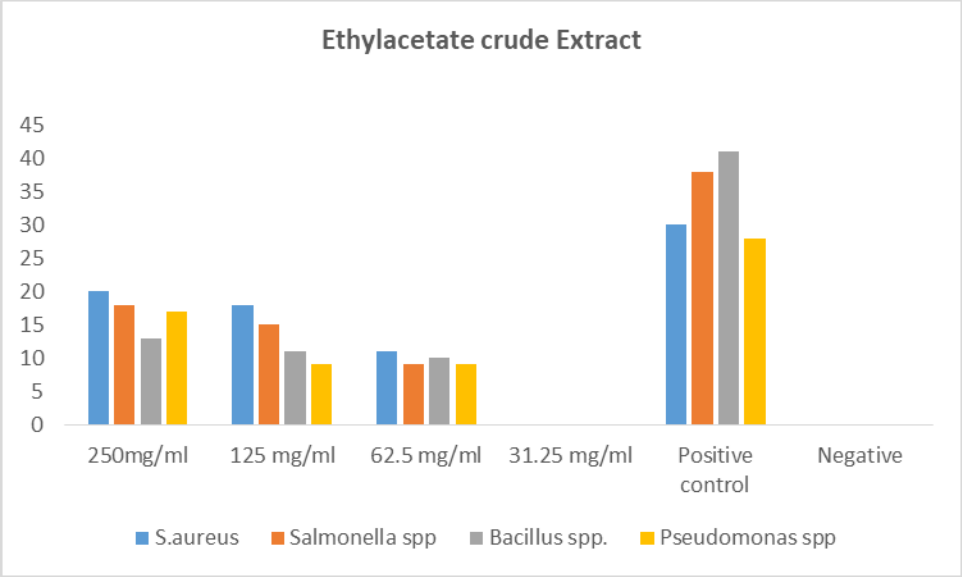


Figure 4. Ethyl Acetate Crude Extract Antibacterial Test

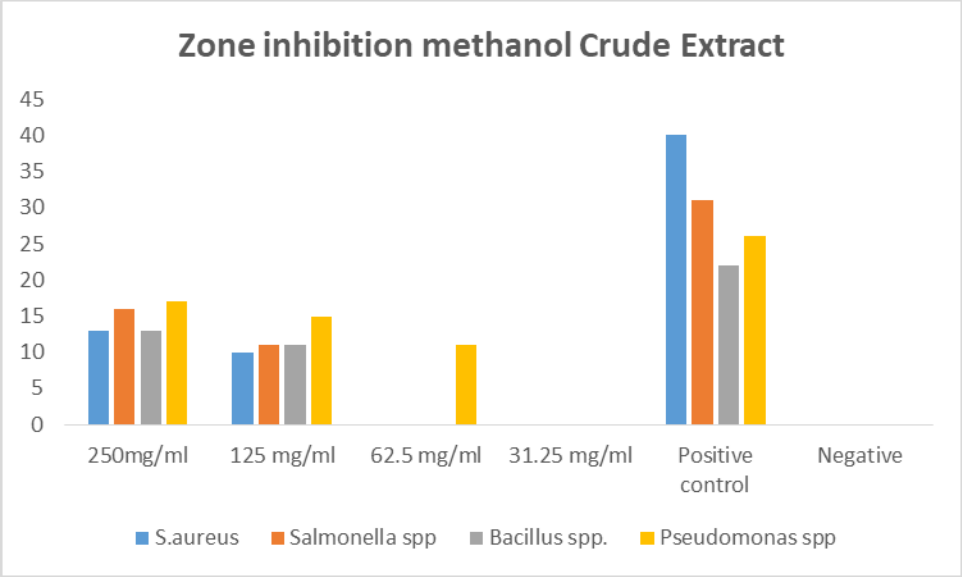


Figure 4. Ethyl Acetate Crude Extract Antibacterial Test

Table 2. Minimum Inhibitory Concentration (MIC) of *Crinum Ornatum*

Bacteria	200mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Positive control	Negative
Ethyl acetate						
Staphylococcus aureus	+	+	+	-	+	-
Salmonella spp	+	-	-	-	+	-
Bacillus spp.	+	-	-	-	+	-
Pseudomonas spp	+	-	-	-	+	-
Dichloromethane						
Staphylococcus aureus	+	+	-	-	+	-
Salmonella spp	+	-	-	-	+	-
Bacillus spp.	+	-	-	-	+	-
Pseudomonas spp	+	+	-	-	+	-
N-Hexane						
Staphylococcus aureus	+	-	-	-	+	-
Salmonella spp	+	+	+	-	+	-
Bacillus spp.	+	+	-	-	+	-
Pseudomonas spp	+	-	-	-	+	-
Methanol						
Staphylococcus aureus	+	-	-	-	+	-
Salmonella spp	+	-	-	-	+	-
Bacillus spp.	-	-	-	-	+	-
Pseudomonas spp	+	+	-	-	+	-

+ = absence of turbidity

- = Presence of turbidity.

Table: 3. Minimum Bacterial Concentration (MBC) of *Crinum Ornatum* Crude Extract

Bacteria	200mgml	100 mg/ml	50 mg/ml	25 mg/ml	Positive control	Negative
Ethylacetate						
Staphylococcus aureus	+	+	-	-	+	-
Salmonella spp	+	-	-	-	+	-
Bacillus spp.	-	-	-	-	+	-
Pseudomonas spp	+	-	-	-	+	-
Dichloromethane						-
Staphylococcus aureus	+	-	-	-	+	-
Salmonella spp	+	-	-	-	+	-
Bacillus spp.	+	-	-	-	+	-
Pseudomonas spp	+	-	-	-	+	-
N-Hexane						-
Staphylococcus aureus	+	-	-	-	+	-
Salmonella spp	+	+	-	-	+	-
Bacillus spp.	+	+	-	-	+	-
Pseudomonas spp	+	-	-	-	+	-
Methanol						-
Staphylococcus aureus	+	-	-	-	+	-
Salmonella spp.	-	-	-	-	+	-
Bacillus spp.	-	-	-	-	+	-
Pseudomonas spp	+	-	-	-	+	-

Key: + = Absence of growth on the media, - = Presence of growth on the media

Plate 1: TLC showing plates a) before bioautography, and b) after bioautography respectively

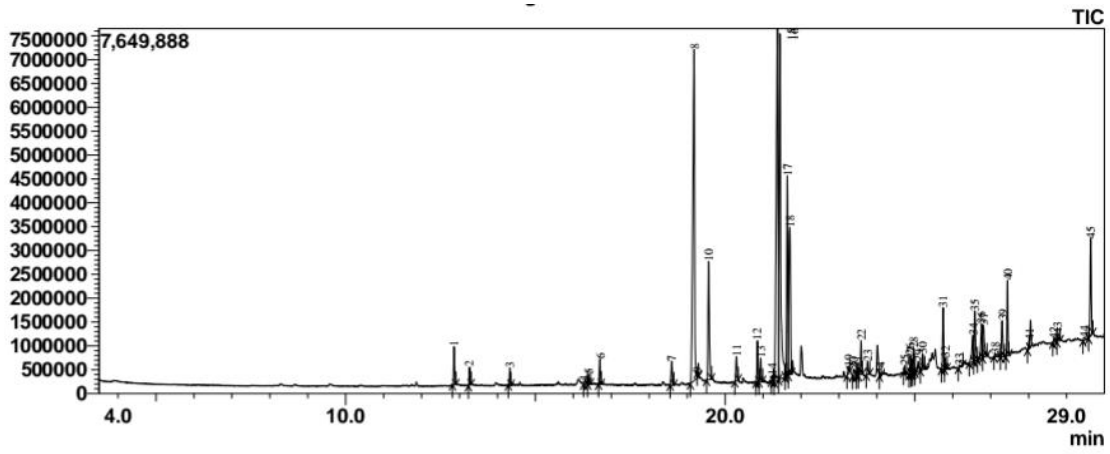
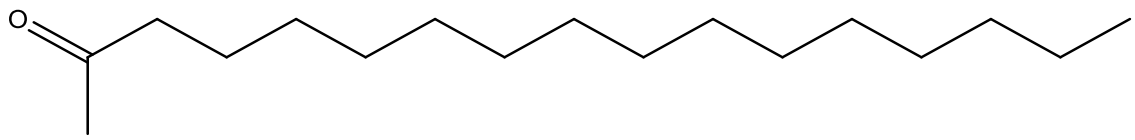


Figure 5. GC-MS chromatogram of dichloromethane extract of *Crinum Ornatum Rhizome*

Table 4. Bioactive Components Identified in Dichloromethane Extract of *Crinum Ornatum Rhizome* by GC-MS

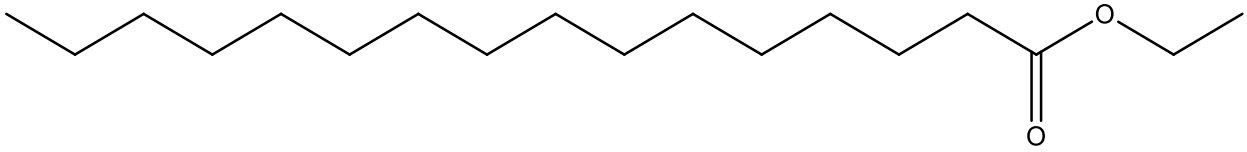
Peaks	Compounds	Mol. Formula	Mol. Weight	Retentio.Time (min)	Relative Area (%)
1	2-Cyclohexen-1-one, 2-hydroxy-3-methy	$C_{10}H_{16}O_2$	168	12.865	1.15
2	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	13.265	0.51
3	1-Heptadecene	$C_{17}H_{34}$	238	14.326	0.45
4	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	16.285	0.16
5	Spiro[4.5]decan-7-one, 1,8-dimethyl-4-(1	$C_{15}H_{26}O$	222	16.401	0.29
6	1-Nonadecene	$C_{19}H_{38}$	266	16.696	0.73
7	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	18.592	0.88
8	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.184	17.91
9	Hexadecane-1,2-diol	$C_{16}H_{34}O_2$	258	19.275	0.01
10	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	19.564	4.48
11	5-Hepten-3-one, 2-(5-ethenyltetrahydro-	$C_6H_8N_2O_2$	140	20.297	0.87
12	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	20.853	1.30
13	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	20.935	0.79
14	Methyl stearate	$C_{19}H_{38}O_2$	298	21.246	0.15
15	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	21.379	18.89
16	Oleic Acid	$C_{18}H_{34}O_2$	282	21.446	15.22
17	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	21.638	6.59
18	Ethyl Oleate	$C_{20}H_{38}O_2$	310	21.705	7.06
19	Octadecanamide, N-(2-hydroxyethyl)-	$C_{20}H_{41}NO_2$	327	23.254	0.24
20	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466	23.385	0.35
21	2,4,4-Trimethyl-3-(3-oxobutyl)cyclohex-2	$C_{13}H_{20}O_2$	208	23.489	0.25
22	2-(t-Butylphenyl)-5-(4-biphenylyl)-1,3,4-o	$C_{24}H_{22}N_2O$	354	23.583	1.18
23	Eicosanoic acid	$C_{20}H_{40}O_2$	312	23.751	0.46
24	Decane, 1-iodo-	$C_{10}H_{21}I$	268	24.095	0.10
25	1-Monopalmitin, 2TMS derivative	$C_{25}H_{54}O_4Si_2$	474	24.729	0.18
26	1H-Indene, 1-hexadecyl-2,3-dihydro-	$C_{25}H_{42}$	342	24.880	0.59
27	Octacosanol	$C_{28}H_{58}O$	410	24.915	0.43
28	Nonacosane	$C_{29}H_{60}$	408	24.966	0.71
29	Hexadecanoic acid, 2-hydroxy-1-(hydrox	$C_{19}H_{38}O_4$	330	25.086	0.42
30	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	25.207	0.49
31	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	25.746	1.99
32	Etracosane	$C_{24}H_{50}$	338	25.796	0.33
33	2-Propenoic acid, 3-phenyl-, 1,7,7-trimet	$C_{19}H_{24}O_2$	284	26.165	0.08
34	Benzenepropanol, 4-methoxy-	$C_{10}H_{14}O_2$	166	26.530	1.37

35	Pentatriacontane	$C_{35}H_{72}$	492	26.581	1.97
36	5,6-Dihydroergosterol	$C_{28}H_{46}O$	398	26.762	1.57
37	Stigmasterol	$C_{29}H_{48}O$	412	26.809	1.52
38	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	27.105	0.24
39	Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	426	27.301	1.43
40	Squalene	$C_{30}H_{50}$	410	27.442	2.13
41	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	28.005	0.31
42	Trenbolone Acetate	$C_{20}H_{24}O_3$	312	28.660	0.19
43	Octacosanol	$C_{28}H_{58}O$	410	28.768	0.39
44	3(2H)-Pyridazinone, 6-chloro-	$C_4H_3ClN_2O$	130	29.450	0.12
45	Tetrapentacontane	$C_{54}H_{110}$	758	29.635	3.55



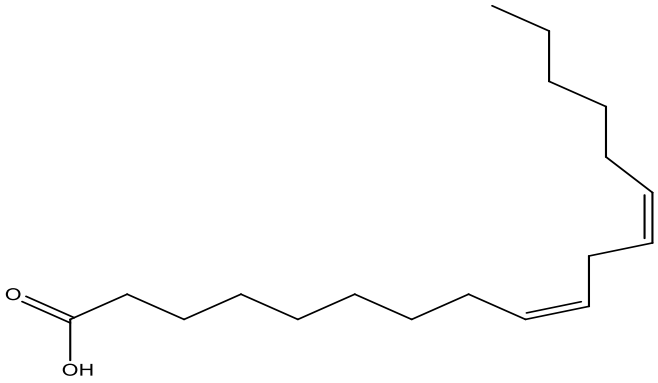
(A) n-hexanedecanoic acid

Hexadecanoic acid, also known as palmitic acid, is a saturated fatty acid that has various medical uses such as



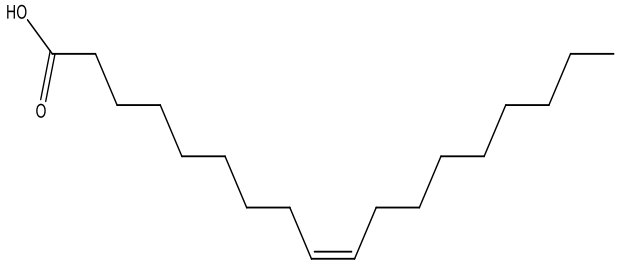
(B)- Hexadecanoic Acid Ethyl Ester

Hexadecanoic acid, ethyl ester, also known as ethyl palmitate, is a common fatty acid ester found in many natural sources such as vegetable oils, animal fats, and waxes.



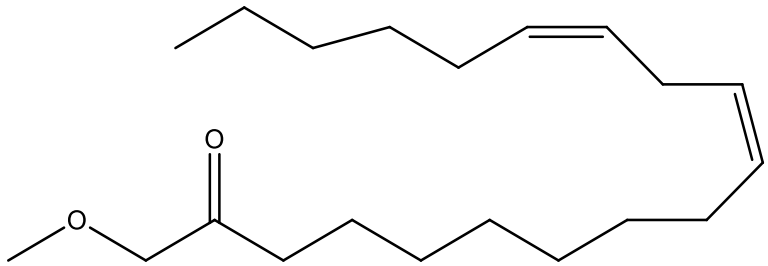
(C)- 9,12-octadecadienoic acid (Z,Z)

9,12-octadecadienoic acid (Z, Z), also known as linoleic acid, is an essential omega-6 fatty acid that cannot be synthesized by the human body and must be obtained through diet it contain biological uses such as;



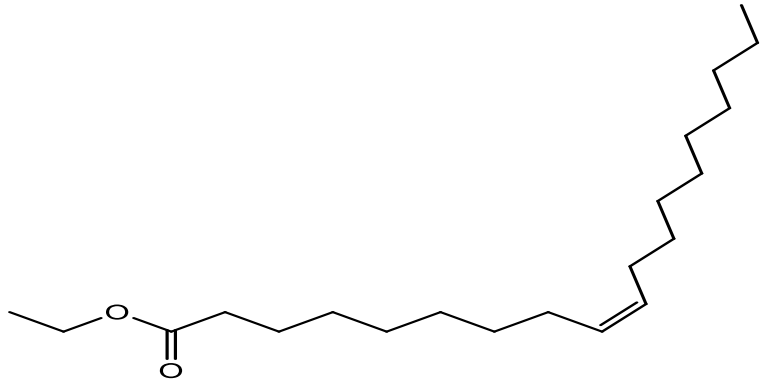
(D)- Oleic Acid

Oleic acid is a monounsaturated fatty acid that is commonly found in many vegetable oils, such as olive oil, and animal fats.



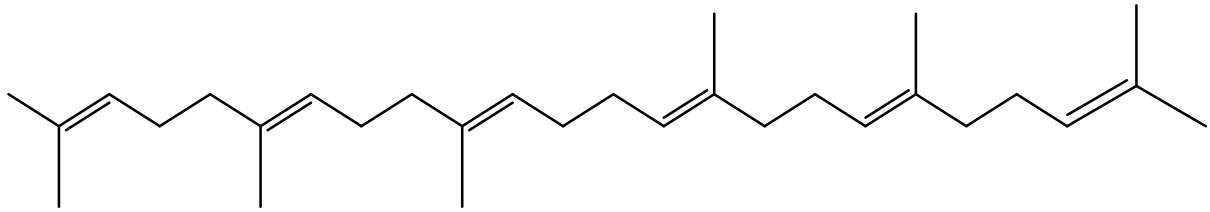
(E)Linoleic Acid Ethylester

Linoleic acid ethyl ester is an ethyl ester derivative of linoleic acid, which is a polyunsaturated omega-6 fatty acid



(F)-Ethyl Oleate

Ethyl Oleate is an ester of oleic acid, a monounsaturated omega-9 fatty acid. Here are some of the medicinal uses of ethyl oleate.

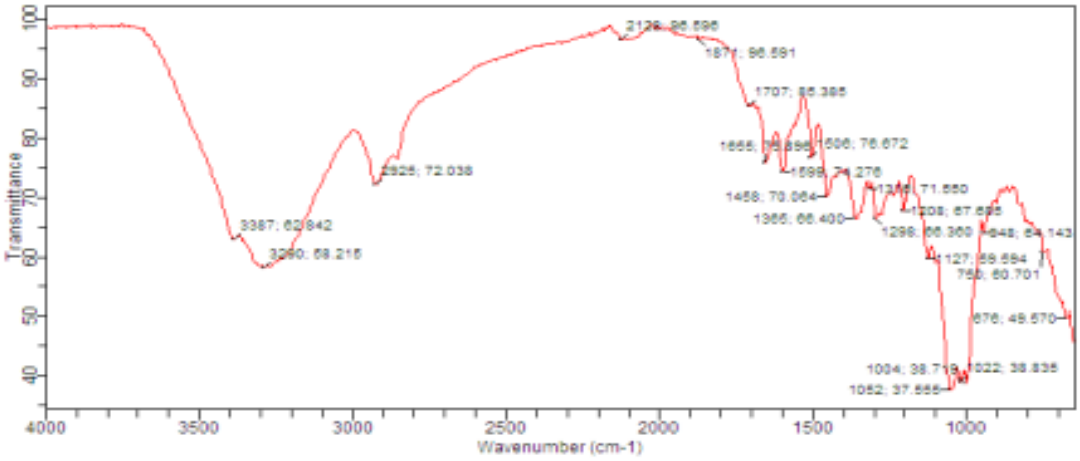


(G) SQUALENE

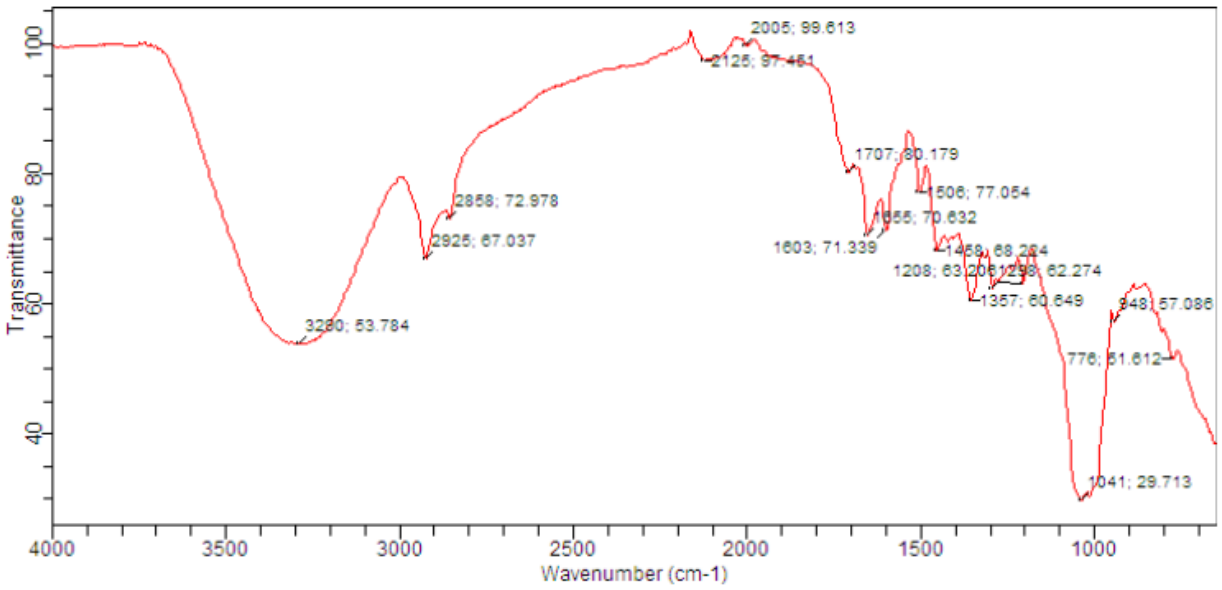
Squalene is a natural organic compound that is found in high concentrations in shark liver oil

(H) –Tetrapentacontane

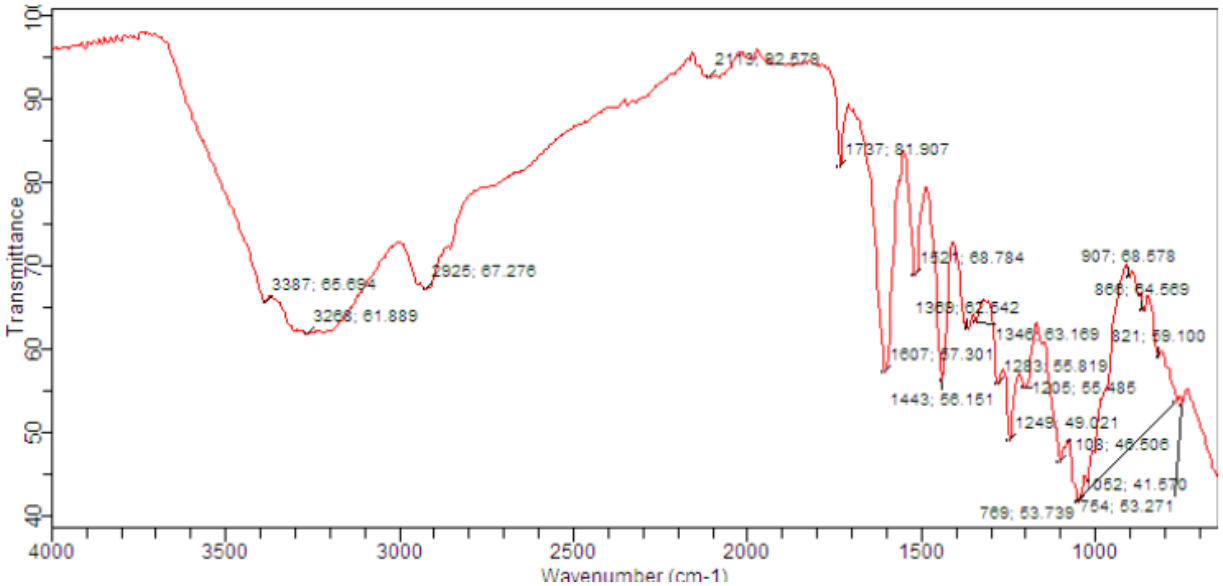
Base on medicinal uses of bioactive compounds identified from Gc-Ms of dichloromethane extract from *Crinum ornatum Rhizome* with best peak, it revealed out that the plant has antimicrobial activity which is the main target of the research.



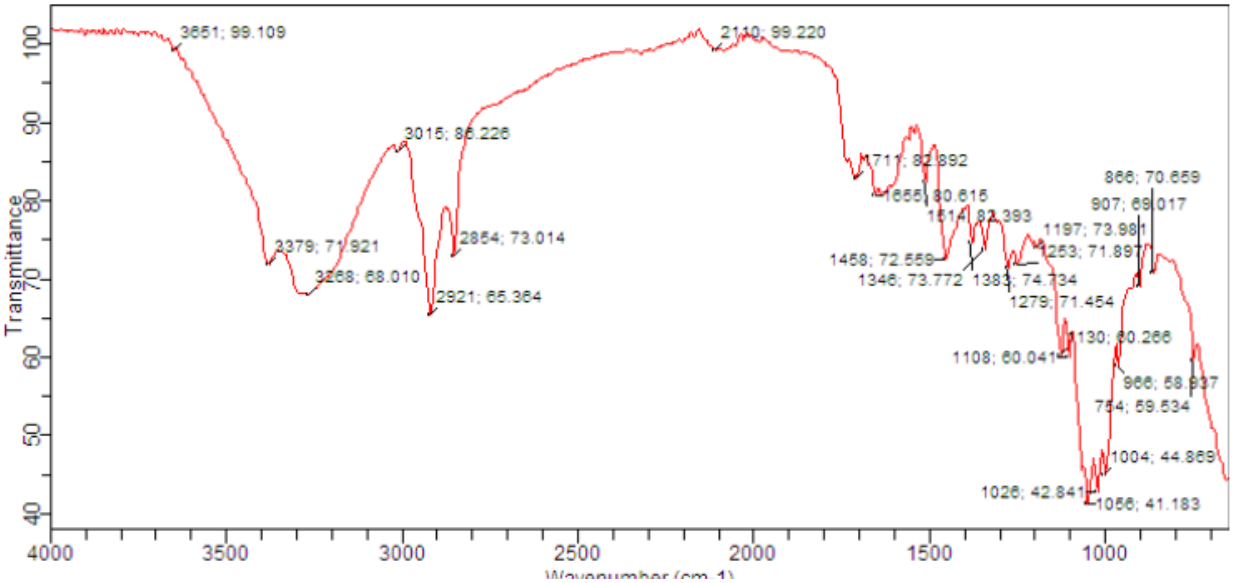
n-hexane crude extract



Dichloromethane crude extract

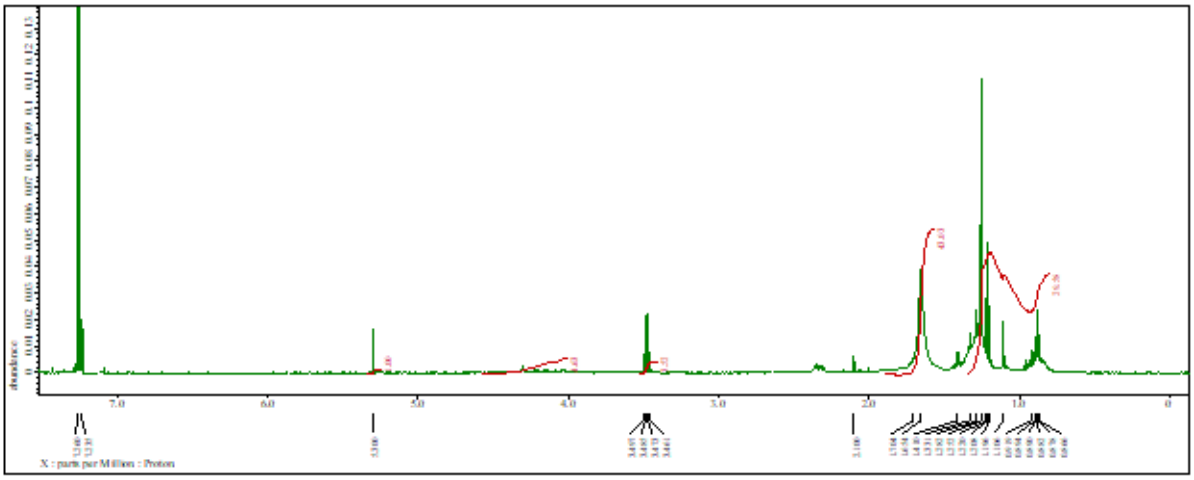


Ethyl acetate crude extract



Methanol crude extract

T.L.C Plate of the isolate



Nuclear Magnetic Resonance Proton NMR of the Isolate

CONCLUSION

The presence of these bioactive compounds with antimicrobial properties suggested the potential of *Crinum ornatum* as a sources many pharmacological activities. Base on medicinal uses of bioactive compounds identified from GC-MS of dichloromethane extract as shown. Subsequently from bioautography study revealed that methanol extract contains the highest concentration of the active compounds with the broadest range of antibacterial properties which imply that the methanol extract is the most promising extract with highest anti-typhoid activity using tested bacteria (*Salmonella typhi*), Therefore, the presence of phytochemicals in the plants studied could be a scientific evidence for the traditional and biological uses of the plants, the isolation process effectively extracted bioactive compounds from the plant, demonstrating the efficacy of the chosen extraction method. The obtained molecules exhibited notable antimicrobial activity, affirming the traditional medicinal uses of *Crinum ornatum* in folk medicine. Thin-Layer Chromatography and column chromatography served as crucial steps in the isolation process, NMR, played a pivotal role in elucidating the molecular structures of the isolated compounds. The identification of antimicrobial molecules from *Crinum ornatum* opens avenues for potential therapeutic applications, particularly in the field of infectious diseases and natural product chemistry. Future studies could focus on detailed bioactivity assays to assess the specific antimicrobial mechanisms and potential synergistic effects of the isolated compounds. Optimization of extraction techniques might enhance the yield of bioactive

compounds, providing a foundation for sustainable pharmaceutical applications. In vivo studies and toxicity assessments are essential to evaluate the safety and efficacy of the isolated molecules, paving the way for their eventual integration into pharmaceutical or therapeutic interventions.

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