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# Phytochemical investigation of *Croton macrostachyus* crude extracts and evaluation of their antibacterial activity

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## Abstract

*Croton macrostachyus* is one of the most common traditional medicinal plants used in Ethiopia. The parts of this plant such as bark, twigs, fruits, leaves, roots, and seeds showed varieties of medicinal properties and cure various human and animal diseases and ailments. This paper reports the phytochemical constituents of the extracts from *Croton macrostachyus* plant parts grown in horo guduru wollega zone of Ethiopia and their antibacterial activities against different bacteria as well as isolation of a compound from the plant extract. Antibacterial activities of ethanol extracts of leaf, root, seed, and stem bark of *Croton macrostachyus* were screened against *Salmonella typhimurium*, *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* using disc diffusion method on Mueller – Hinton Agar (MHA). All the extracts showed inhibitory activity against *Salmonella typhimurium*, *Listeria monocytogenes,* and *Escherichia coli*. However no activity was recorded against *Staphylococcus aureus.* A compound, labeled as 1-18D, was isolated from the stem bark, and its structure was elucidated by $^{1}$H NMR, $^{13}$C NMR, FT-IR and compared with previously reported literature. The isolated compound showed antibacterial activity against *Salmonella typhimurium* and *Escherichia coli*, but no inhibitory activity against *Listeria monocytogenes* and S*taphylococcus aureus*. The finding of this study indicates that the ethanol extracts and the isolated compound is a promising antibacterial agent against several human pathogens.

**Keywords**: *Croton macrostachyus*, antibacterial activity, stem bark, root, seed.

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## Introduction

Though the use of plants in the treatment of ailments is common since ancient times currently an explosion of interest in the use of plants for their medicinal and therapeutic value on the treatment and prevention of various diseases is increasing in developing and developed countries due to several reasons (Ríos and Recio, 2005). For instance a study shows that more than 80% of the people in Africa mainly rely on plant-based treatments to complement lack of

trained health professionals and access to modern medication (Dubale *et al*., 2015). The phytochemicals isolated from medicinal plants have been considered useful alternatives to synthetic drugs. In addition to this, plants are regarded as a great laboratory of organic synthesis, as a result of millions of years of adaptation, providing an invaluable source of new molecules for researching anti-microbial activities (Gurib, 2006; Harvey, 2007; Rehecho *et al*., 2011). Before the advent of modern health sciences, traditional medicine is an ancient medical practice present within the communities. These ancient medical practices are the main basic information for the development of modern health sciences. Traditional medicine refers to any ancient and culturally based health care practice differing from scientific medicine, and is largely transmitted orally by communities of different cultures. The information about the traditional medicine comes from indigenous theories, beliefs and experience that are conserved down from generation to generations (Pagadala *et al*., 2015; Fokunang *et al*., 2011; Pan *et al*., 2014) and such information is basic for drug discovery (Megersa *et al*., 2013).

In some countries, the use of medicinal plants is often associated with witchcraft and superstition, because people do not have the scientific insight to explain and predict the curative action of plants. Especially majority of the population that lives in the rural and the poor people in urban areas rely mainly on traditional medicines to meet their primary health care needs, regardless of side effects of traditional medicine due to overdosing. It is noted that herbal medicines often have their own side effects and sometimes negatively interact with other herbs and medications (Megersa *et al*., 2013), therefore, supporting the traditional knowledge with scientific studies has paramount importance to minimize such unwanted effects and interactions.

Now a days, the studies on herbal medicines that appear under different names such as plant medicines, phytomedicines, natural products and under pharmacognosy usually referring to products processed from living organisms (plants, animals, insects, microorganisms and marine organisms). Findings from ethnobotanical and ethnomedicinal studies have shown correlation between medicinal use and laboratory results. Natural sources are usually the starting points for most pharmacological agents (Liu, 2011).

Ethiopia has a long history of traditional medicine and has developed ways to combat disease through it. Like other developing countries in Ethiopia medicinal plants have been used as

remedies for many years and still represent the main therapeutic tool in the traditional medicines. *Croton macrostachyus* belongs to the family Euphorbiaceae and genus Croton, which is one of the most commonly and widely used traditional medicinal plant (Alfred, 2017). Plants from Euphorbiaceae family are well known in different parts of the world as toxic and/or medicinal. A number of biologically active compounds (terpenes, flavonoids and alkaloids) have been isolated from it (Kapingu *et al*., 2000). Reports have also indicated that the parts of *Croton macrostachyus* have pharmacological and antimicrobial activities (Mahesh and Satish, 2008; Alfred, 2017).

The *Croton macrostachyus* has been widely used to treat many infectious diseases, especially in Horo Guduru Wollega Zone, Oromia Regional state, west Ethiopia *Croton macrostachyus* has many traditional applications in human beings and livestock. For instance, the mixture of fresh leaves of *Croton macrostachyus* and *Brucea antidyseanterica* in juice form are given to cattle for treatment of their stomach ache. The dried root in powdered form are also given to dogs for the treatment of rabies. Moreover the people around this area use fresh stem bark to reduce skin bleeding. It is also common to observe while people are using the juices or sap of the leaves and bark for treatment of tinea corporis which appeared on the face of human beings. Despite its numerous medicinal uses, and to the best of our knowledge, no studies has shown comparison of the antimicrobial activity of the different parts of the plant in the study area. Therefore, the purpose of this study is to compare the antimicrobial activities of *Croton macrostachyus* plant part extracts as well as to isolate the bioactive compound and compare its antimicrobial activity with crude extracts.

## Materials and Methods

### Collection and identification of the plant material

The leaf, stem bark, seed and roots of *Croton macrostachyus* (Figure 1) were collected from Horo district Oromia region, Ethiopia, which is located about 315 km away from the capital city, Addis Ababa. The fresh leaf, root, stem bark and seeds were transported to Debre Berhan University using plastic bag. Moreover the plant was identified by a botanist at the same university. Then fresh leaf, root, stem bark and seeds (about 1 Kg each) dried in an open air protected from direct exposure to sun light and ground using electric blender. The resulting powder was kept in polyethylene bag to avoid from certain environmental conditions (moisture, air and

other surrounding dusts) until used for further analysis.

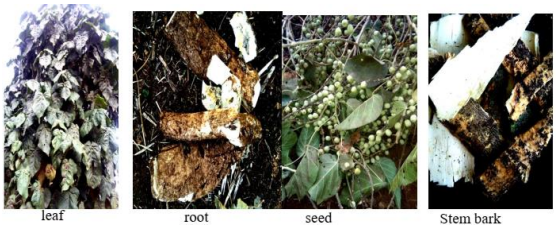



**Figure 1.** Parts of Croton macrostachyus (leaf, root, seed and bark)

**Chemicals, reagents and growth media**

The chemicals and reagents used in the study were: distilled water, organic solvents (ethanol, hexane, chloroform, methanol and ethyl acetate), appropriate media for bacteria (Muller Hinter Agar), silica gel, anhydrous sodium sulphate, sulfuric acid, vanillin reagent, gentamicin, streptomycin, concentrated and dilute hydrochloric acid, DMSO, 1% of aqueous Iron III chloride ($FeCl_3$), 10 % ferric chloride solution ($FeCl_3$), sodium chloride (NaCl, 2%) of methanoic potassium hydroxide, sodium hydroxide, concentrated sulfuric acid, Wagner's reagent (Iodine in Potassium Iodide), Barium chloride ($BaCl_2$).

**Instruments and apparatus**

Thin Layer chromatography was performed on pre-coated silica gel 60 F254 plates. Column chromatography packed with Silica gel 60-120 mm mesh size was used for isolation purpose. FT- IR spectra was recorded on FTIR, $^{1}H$ NMR and $^{13}C$ NMR spectra was recorded on Bruker (400MHz) in $CDCl_3$.

**Extraction of the plant materials**

The extraction of plant material was carried out following reported procedure (Alemu *et al*., 2017). The powdered 100 g of seed and stem bark of the plant was soaked with 600 mL of hexane for 72 h with occasional shaking, and then filtered. The air dried marc was kept for further soaking in ethyl acetate and ethanol respectively. Then powdered leaf, stem bark, seed and root samples of *Croton macrostachyus* (100 g each) were soaked with 600 mL ethyl acetate for 72 h with occasional shaking and filtered. The air dried marcs were further soaked in 600 mL of ethanol for 72 h at room temperature. All extracts were concentrated using rotary evaporator at 40 $^{0}C$, air dried and weighted, and kept for further work.

**Phytochemical Screening tests**

Detections of common secondary metabolites were performed on crude extracts of leaves, root, stem bark and seeds of C. *macrostachyus using* the preceding analytical procedures (Salem *et al*., 2013).

*Test for Terpenes (Salkowski test):* 2 mL of crude extract was mixed with 2 mL of chloroform and 3 mL of concentrated $H_2SO_4$ was added carefully to form a

layer. Reddish-brown coloration of the interface indicates the presence of terpenes.

*Test for Flavonoids:* 2 mL of the extract was mixed with a few drops of dilute sodium hydroxide. An intense yellow color was inspected in the plant extract, which become colorless on addition of a few drops of dilute acid indicates the presence of flavonoids.

*Test for saponins* (Froth Test): To 2 mL of each extract, 5 mL of distilled water was added in a test tube. Then, the solution was shaken vigorously and observed for a stable persistent froth. Formation of froth indicates the presence of Saponins.

*Test for Tannins*: 5 mL of various crude extract was mixed in 10 mL distilled water and filtered. 1% aqueous Iron chloride ($FeCl_3$) solution was added to the filtrate. Dark-green solution indicates the presence of tannins.

*Test for alkaloids* (Wagner's Test): Extracts was dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

*Test for Phenols*: To 1 mL of various crude extracts of sample, 2 mL of distilled water followed by a few drops of 10 % aqueous ferric chloride solution were added. Formation of blue or green or blue black color indicated the presence of phenols.

**Antibacterial test of the extracts**

*Microorganisms and inoculum preparation*

The microorganisms used in this study were *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*. Inoculum of these bacteria were prepared in nutrient broth medium to obtain isolated colonies. After incubation at $35^0$C overnight, well-isolated colonies were selected with an inoculating needle or loop, and transferred to a tube of sterile saline and vortex thoroughly. The bacterial suspension was then compared to the 0.5 McFarland standards (prepared by adding 0.5 mL of 0.048 M $BaCl_2$ to 99.5 ml of 0.36N $H_2SO_4$). The turbidity standard was agitated on a vortex mixer immediately prior to use. When bacterial suspension does not appear to be the same density as the McFarland 0.5 standard, the turbidity were reduced by adding sterile saline or increased by adding more bacterial growth. Within 15 minutes after adjusting the turbidity of the inoculum suspension, by using sterile cotton swab the bacteria was dipped into standardized bacterial suspension. Pressing firmly against the inside wall of the tube just above the fluid level, the swab rotated to remove excess liquid. The swab was streaked over the entire surface of the agar medium four

times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums. Finally, swab all around the edge of the agar surface. The surface of the medium was allowed to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture. The antibacterial disks were applied to the plates after 10 minutes of inoculation (Belay *et al*., 2011; Fekam *et al*., 2003).

*Antibacterial activity test*

Antibacterial activity of hexane, ethyl acetate and ethanol extracts of stem bark and the ethanol extracts of root, leaf, seed extracts and the isolated pure compound of *Croton macrostachyus* were evaluated by using the paper disc diffusion method against two gram-positive bacteria (*Staphylococcus aureus* and *listeria monocytogenes*) and two gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). The paper disks which is prepared from the Whatmann filter paper number 4 (6mm in diameter) and sterilized in autoclave for 1 h were placed individually with sterile forceps, and then gently pressed down onto the agar. 20 µL of the concentrations (25, 50 and 100 mg/mL) of each samples were pipetted to the discs in three replications. Antibiotic discs containing Gentamycin and Streptomycin were used as positive controls. Then the plate was inverted and incubated at 35°C for 24 h. After incubation, the diameter of the zones of complete inhibition (including the diameter of the disk) was measured and recorded in millimeters. The measurement was made with a ruler on the undersurface of the plate without opening the lid (Belay *et al*., 2011).

**Column fractionation, compound isolation and elucidation**

Separation and purification of the various constituents of the crude extract were mainly done by column chromatography and recrystallization processes. Powdered sample (5 g) was applied to a column packed with silica gel using n-hexane (100 %) as solvent. The different solvent system (formed by mixing organic solvents) was used as eluent. A total of 23 fractions were collected eluting with the following solvent systems: hexane-chloroform (90:10), hexane-chloroform (70:30), hexane-chloroform (50:50): fraction 1-6, hexane-chloroform(30:70), hexane-chloroform(10:90), hexane-chloroform (0:100): fraction 6-10, chloroform-ethyl acetate (80:20), chloroform-ethyl acetate (60:40): fraction 10-18, chloroform-ethyl acetate (40:60), chloroform-ethyl acetate (20:80): fraction 18-23. Totally 23 fractions were collected and fraction 1-5 are 20 mL each, fraction 6-12 are 15 mL each and

fraction 13-23 each are 18 mL each . All the fractions exhibited varieties of spots on TLC except fraction 4. The fraction with pure spot was concentrated by rotary evaporator and white powder 140 mg was obtained, labeled and subjected to spectral analysis as 1-18D.

Compound 1-18D: white solid, 1H NMR (400MHz, CDCl3): 1.269 (2H, m, H-1),1.957 (2H, m, H-2), 3.188 (1H, dd,J1=4.8,J2=10.8, H-3),1.342 (1H, m, H-5),1.902 (2H, m, H-6), 1.692 (2H,m,H-7), 1.669 (1H,m, H-9), 1.512 (2H,m, H-11), 1.372 (2H,m, H-12), 1.512 (1H,m, H-13), 1.302 (2H,t, H-15), 1.932 (2H,t, H-16), 1.924 (1H,m, H-18), 2.371 (1H,m, H-19), 1.480 (2H,m, H-21),1.398 (2H, m, H-22), 0.978 (3H, s, H-23), 0.771 (3H, s, H-24), 0.839 (3H, s, H-25), 1.041 (3H,s, H-26), 0.956 (3H,s, H-27), 0.80 (3H,s, H-28), 1.693 (3H,s, H-30), 4.579 (1H,s, H-29a), 4.701 (1H,s, H-29b). 13C NMR (400 MHz, CDCl3), 38.751 (C-1), 27.439 (C-2), 78.98 (C-3), 38.884 (C-4), 55.33 (C-5), 18.358 (C-6), 34.32 (C-7), 40.855 (C-8), 50.464 (C-9), 37.191 (C-10), 20.966 (C-11), 25.167 (C-12), 38.081 (C-13), 42.852 (C-14), 27.481 (C-15), 35.617 (C-16), 43.023 (C-17), 48.327 (C-18), 48.004 (C-19), 150.909 (C-20), 29.88 (C-21), 40.034 (C-22), 28.033 (C-23), 15.422 (C-24), 16.158 (C-25), 16.015 (C-26), 14.588 (C-27), 18.044 (C-28), 109.376 (C-29), 19.358 (C-30).

IR νmax (KBr) cm-1:3310, 3070, 2956, 2868, 1632, 1481, 1367, 1039, 837, 522.

## Results and discussion

### Phytochemical screening test

The ethanol and ethyl acetate extracts of C*roton macrostachyus* were subjected to phytochemical screening test to analyze the presence of different chemical constituents in each part of *Croton macrostachyus* (leaf, bark, seed and root). The results of the phytochemical screening test are presented in Table 1. The screening test of the crude ethyl acetate and ethanol extract indicated the presence of alkaloids in each part of the plant (Stem bark, leaf, seed, and root). However, phenols and tannins are not observed in ethyl acetate extract of all parts of the plant as shown in Table 1. The screening test also exhibited the presence of terpenoids in the stem bark, seed, and root, but not in the leaf. According to the test, flavonoid was detected in ethyl acetate extract of the stem bark than that of the seed. Tannin is only present in crude ethanol extracts of the leaf of *Croton macrostachyus*. Report also showed the presence of alkaloids, phenolic compounds, tannins, terpenoids, saponins, and

flavonoids in hydroalcoholic crude extracts of the seed and root of *Croton macrostachyus* (Mekonnen, 2015).

**Isolation and characterization of compound 1-18D**

The ethyl acetate crude extract of stem bark of *croton macrostachyus* was subjected to chromatographic purification and recrystallization. This lead to the isolation of compound 1-18D (Figure 3). The structure of 1-18D was elucidated by FTIR (Figure 2), and Nuclear Magnetic Resonance (NMR, proton 1H and carbon 13C) (Table 2, Figure 3) and compared the data with literature.

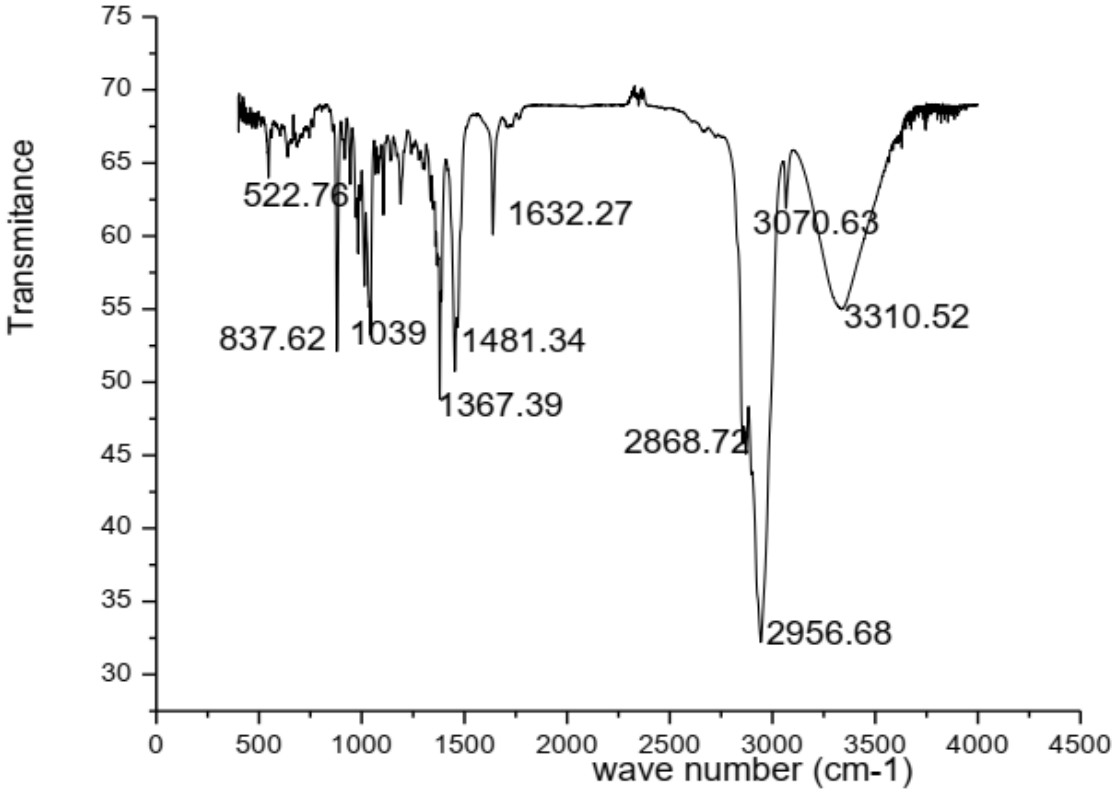
**Table 1**. Phytochemical constituents of ethanol and ethyl acetate extract of *Croton macrostachyus.*

| Secondary Metabolites | Crude extracts | Plant parts | | | |
|---|---|---|---|---|---|
| | | Stem bark | Seed | Leaf | Root |
| Terpenoids | Ethyl acetate extract | + | + | - | + |
| | Ethanol extract | + | + | + | + |
| Flavonoids | Ethyl acetate extract | + | + | - | - |
| | Ethanol extract | + | + | + | + |
| Saponins | Ethyl acetate extract | - | + | - | + |
| | Ethanol extract | - | - | + | + |
| Tannins | Ethyl acetate extract | - | - | - | - |
| | Ethanol extract | - | - | + | - |
| Alkaloids | Ethyl acetate extract | + | + | + | + |
| | Ethanol extract | + | + | + | + |
| Phenols | Ethyl acetate extract | - | - | - | - |
| | Ethanol extract | - | - | + | + |
| Sterols | Ethyl acetate extract | - | + | + | + |
| | Ethanol extract | + | + | - | + |

*+ = Present - = absent*

The $^{1}H$ NMR spectral data (Table 2) showed two broad singlet peaks at δ 4.579 and δ 4.702, each integrating for one proton, correspond to the methine protons of an olefinic methylene group. A doublet of doublet peak at δ 3.188 integrating for one proton corresponded to the methine proton which was assigned to C-3 carbon atom with $J_1$=4.8,$J_2$=10.8Hz. The different J values indicate neighboring protons found at the opposite side and the proton which is opposite side to proton at position 3 has more J value because the distance between them high. A multiplet peak at δ 2.399 integrating for one proton corresponded to

the methine proton which was attached to C-19 carbon atom. A singlet peak at δ 2.384 integrating for one proton corresponds to the –OH proton. The $^{1}$H NMR experiment also exhibited signals due to seven methyl protons (all singlet), with their peaks at δ 0.772, δ 0.801, δ 0.84, δ 0.957, δ 0.979, δ 1.042, and δ 1.693, each integrating for three protons, corresponded to the carbons (C-24,C-28,C-25,C-27,C-23,C-26,C-27) respectively. However, peaks did not appear at C-4, C-8, C-10, C-14, C-17 and C-20 since the carbons are quaternary i.e. they are not connected to hydrogens.




The $^{13}$C NMR spectral data of the isolated compound showed 30 signals, suggesting triterpenoidal nucleus as indicated in Table 2 and Figure 3. The peaks appeared at δ 150.90 and 109.37 are due to the olefinic carbons of the exocyclic double bond. Moreover the DEPT spectral data of the compound showed the presence of 7 methyl, 11 methylene, 6 methine and 6 quaternary carbons (Table 2). We also compared the $^{13}$C NMR spectral data of the isolated compound with reported NMR spectral data (Reynolds *et al*., 1986), and all the data of the isolated compound 1-18D are found to be consistent with the chemical shifts reported in literature suggesting that the isolated compound closely looks like lupeol. There is an exact match with $^{13}$C NMR

values of all 30 carbons chemical shifts as shown in the Table 3. The compound 1-18D and the lupeol have the same carbon types. They have 11 methylene groups, seven methyl groups, six quaternary carbons and six methine groups. Based on the information of the spectral data (NMR and FT-IR) and reported literature, the isolated compound coded 1-18D is proposed to be lupeol as indicated in Figure 3.

**Table 2.** Proton NMR, $^{13}C$ NMR and DEPT (400 MHz, $CDCl_3$) spectral data of the isolated compound 1-18D.

| Carbon | 1-18D | | | | Lit. (Reynolds et al.,1986), |
|---|---|---|---|---|---|
| | $\delta_H$ (multiplicity) | $\delta_C$ | DEPT | Carbon type | $\delta_C$ |
| C (1) | 1.269,2H, (m) | 38.751 | 38.748 | $CH_2$ | 38.67 |
| C (2) | 1.957,2H, (m) | 27.439 | 27.438 | $CH_2$ | 27.35 |
| C (3) | 3.188,1H, (dd) | 78.98 | 78.979 | CH | 78.94 |
| C (4) | - | 38.884 | - | C (Q) | 38.81 |
| C (5) | 1.342,1H(m) | 55.33 | 55.331 | CH | 55.25 |
| C (6) | 1.902,2H, (m) | 18.358 | 18.356 | $CH_2$ | 18.28 |
| C (7) | 1.692,2H, (m) | 34.32 | 34.317 | $CH_2$ | 34.23 |
| C (8) | - | 40.855 | - | C (Q) | 40.78 |
| C (9) | 1.669(m) | 50.464 | 50.462 | CH | 50.38 |
| C (10) | - | 37.191 | - | C (Q) | 37.11 |
| C (11) | 1.512(m) | 20.966 | 20.964 | $CH_2$ | 20.89 |
| C (12) | 1.372(m) | 25.167 | 25.165 | $CH_2$ | 25.08 |
| C (13) | 1.512(m) | 38.081 | 38.079 | CH | 38.00 |
| C (14) | - | 42.852 | - | C (Q) | 42.78 |
| C (15) | 1.302(m) | 27.481 | 27.481 | $CH_2$ | 27.41 |
| C (16) | 1.932(m) | 35.617 | 35.616 | $CH_2$ | 35.54 |
| C (17) | - | 43.023 | - | C (Q) | 42.59 |
| C (18) | 1.924(m) | 48.327 | 48.325 | CH | 48.24 |
| C (19) | 2.371(m) | 48.004 | 48.004 | CH | 47.94 |
| C (20) | - | 150.909 | - | C (Q) | 150.88 |
| C (21) | 1.480(m) | 29.88 | 29.878 | $CH_2$ | 29.8 |
| C (22) | 1.398(m) | 40.034 | 40.034 | $CH_2$ | 39.96 |
| C (23) | 0.978(s) | 28.033 | 28.032 | $CH_3$ | 27.95 |
| C (24) | 0.771(s) | 15.422 | 15.432 | $CH_3$ | 15.35 |
| C (25) | 0.839(s) | 16.158 | 16.156 | $CH_3$ | 16.09 |
| C (26) | 1.041(s) | 16.015 | 16.013 | $CH_3$ | 15.94 |
| C (27) | 0.956(s) | 14.588 | 14.586 | $CH_3$ | 14.51 |
| C (28) | 0.80(s) | 18.044 | 18.044 | $CH_3$ | 17.97 |
| C (29) | 4.579,4.701(2H, s) | 109.376 | 109.376 | $CH_2$ | 109.31 |
| C (30) | 1.693(s) | 19.358 | 19.352 | $CH_3$ | 19.28 |

*Lit: literature, 1-18D: isolated compound in this study*

FT-IR was used to further confirm the functional group present on the isolated compound as indicated in Figure 3. In the FT-IR spectrum of the compound 1-18D, the broad absorption band at 3310cm$^{-1}$ showed the O-H stretching indicating the presence of a hydroxyl group. The absorption band at 3070 cm$^{-1}$ showed the presence of the C-H stretching of the olefin. The strong absorption band at 2956 cm$^{-1}$ showed the presence of the C-H stretching for methyl groups. The absorption band at 2868cm$^{-1}$ showed the presence of C-H stretching of the methylene groups. The absorption band at 1632 cm$^{-1}$ showed the presence of the olefinic C=C stretching. The absorption band at 1039 cm$^{-1}$ showed the presence of the C-O bond stretching.

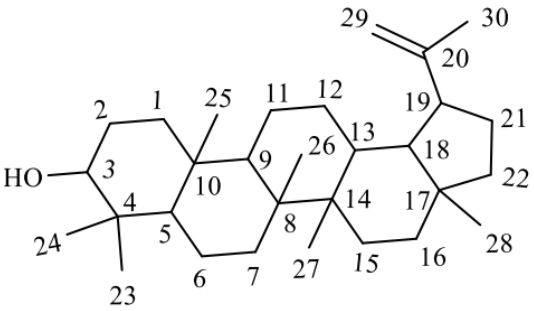



Figure 3. The proposed structure of compound 1-18D.

**Antibacterial activity test**

In this study, we also performed antibacterial test for the crude ethanol extracts (with concentration 25, 50, 100 mg/mL) from leaf, bark, seed and root of *Croton macrostachyus* against four bacteria (*Salmonella typhimurium, Listeria monocytegene, and Escherichia col*i) and then compared with antibacterial activity of isolated compound 1-18D. Gentamicin and Streptomycin were used as positive control. The antibacterial test for all crude extracts are tabulated in Table 3. Crude ethanol extract of *Croton macrostachyus* stem bark with concentration of 100 mg/mL showed a zone of inhibition of 11.5 $\pm$ 0.5 for *Salmonella typhimurium* gram-negative bacteria, however, gentamicin (positive control) showed 19 mm. the same extract demonstrated 8.5 $\pm$ 0.5 zone of inhibition against *Escherichia coli* and *Listeria monocytogenes* bacteria. In all cases the zone of inhibition is lower compared to positive controls, but still can be considered as a moderate activity. Besides, the extract showed no activity against *Staphylococcus aureus*, gram-positive bacteria, but the control drug, gentamicin, showed a zone of inhibition of 19 mm (Table 3). This indicates that *Croton macrostachyus* ethanol stem bark extract is not active against *Staphylococcus aureus*, gram-positive bacteria. The crude extract of *Croton macrostachyus* seed was subjected to antibacterial activity and the results were tabulated in Table 3. Gentamicin was used as a positive control. The crude extract showed antibacterial activity against *Salmonella typhimurium, Listeria*

*monocytogenes*, and *Escherichia coli* bacteria with a zone of inhibition ranging from 7-10 mm, but no zone of inhibition against *Staphylococcus aureus* bacteria.

**Table 3.** Antibacterial activity of ethanol crude extracts of the seed, root, leaf and steam bark of *Croton macrostachyus* against *Salmonella typhimurium (St), Listeria monocytogene (Lm), Staphylococcus aureus (Sa) and Escherichia coli (Ec).*

| BS | Conc. | Zone of inhibition (mm), *Croton macrostachyus* | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | (mg/mL) | Seed | Root | leaf | bark | 1-18D | Gm | Stm |
| *St* | 25 | 7.33±0.45 | 10±00 | 8±00 | 9.5±0.5 | 10±1.41 | | |
| | 50 | 9.33±0.45 | 10±0.81 | 8.5±1.22 | 10±1 | 12.5±0.5 | 19 | 23 |
| | 100 | 10.33±0.45 | 12±0.81 | 11.33±0.47 | 11.5±0.5 | 12.5±1.22 | | |
| *Lm* | 25 | 8±00 | 10.5±0.5 | 9±00 | 7±1 | - | | |
| | 50 | 8±00 | 10.5±0.5 | 9±1 | 7.5±0.5 | - | 18 | 23 |
| | 100 | 10±00 | 11±1 | 10.5±0.5 | 8.5±0.5 | - | | |
| *Ec* | 25 | 7.66±0.47 | 10.33±1.24 | 9±00 | 6.5±0.5 | 10±1 | | |
| | 50 | 8±00 | 11±0.81 | 10.5±0.5 | 7.5±1.5 | 11±00 | 18 | 24 |
| | 100 | 9±00 | 12±0.81 | 11±1 | 8.5±0.5 | 12.5±0.5 | | |
| *Sa* | 25 | - | - | - | - | - | | |
| | 50 | - | - | - | - | - | 19 | 22 |
| | 100 | - | - | - | - | - | | |

*- Indicates no inhibition zone; Gm: gentamycin; 1-18D: isolated compound; Stm: Streptomycin; BS: bacterial strain*

The crude extract of *Croton macrostachyus* root was subjected to antibacterial activity and the results are tabulated in Table 3. Gentamicin was used as a positive control. The crude extract showed antibacterial activity against *Salmonella typhimurium*, *Listeria monocytogene*, and *Escherichia coli* bacteria with a zone of inhibition ranging from 10-12 mm, whereas the extract showed no inhibitory activity in all concentrations against *Staphylococcus aureus* bacteria. The antibacterial activity of methanolic crude root extracts of *Croton macrostachyus* was reported by Ayele *et al.* (2022) which is consistent with our study.

Similarly the crude extract of leaf of *Croton macrostachyus* was subjected to antimicrobial activity using Gentamycin and Streptomycin as positive control. The crude extract showed antibacterial activity

against *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli* bacteria with zone of inhibition ranging from 8-11mm, but like other extracts no inhibition zone recorded against *Staphylococcus aureus* bacteria. We also carried out the antibacterial activity for the isolated compound 1-18D. Surprisingly, this compound was found to be active against gram-negative bacteria only (*Salmonella typhimurium* and *Escherichia coli*) with inhibition zones ranging from 10-12.5mm. However no activity was observed against gram-positive bacteria (*Listeria monocytogene* and *Staphylococcus aureus*) for this compound. Difference in activities were observed among antibacterial test of the extracts. These differences could be due to the variations in the chemical composition of the extracts. Previously the methanol leaf extracts of *Croton macrostachyus* induced growth inhibition against gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria. Report exhibited that the methanol extracts of this plant also showed highest inhibition zones compared to the positive control (tetracycline) against *S. aureus* and suggested that the ingredients of the plant extract are more potent than the antibiotics (Tala *et al*., 2013). In this study, we used the ethanol extracts of the different parts of *Croton macrostachyus* (stem bark, root, seed and leaf) and all extracts exhibited antibacterial activity against all bacteria tested (*Salmonella typhimurium*, *Listeria monocytogene)* except *Escherichia coli* at all concentrations (25, 50, 100mg/mL). Report showed the broad antimicrobial activity of Lupeol from C. macrostachyus against several important human pathogens *E. coli, S. typhi, K. pneumoniae, C. albicans E. aerogenes* and *L. monocytogenes* (Minyamer and Belay, 2018).]. However, in our findings the isolated compound which were suggested to be lupeol revealed inhibition against gram-negative bacteria but no inhibitory activity shown against *L. monocytogenes* and *Staphylococcus aureus* (gram-positive bacteria). In general, the results show that there is variation in susceptibility to extracts between the bacterial strains. Compared to the ethanol extracts of leaf, root, seed and stem bark of *Croton Macrostachyus*, the isolated compound showed better activity on gram-negative bacteria (*Salmonella typhimurium* and *Escherichia coli*) with zone of inhibitions 12.5+0.81 though no inhibitory activity was recorded against gram-positive bacteria (*Listeria monocytogene* and *Staphylococcus aureus*). Therefore the finding shows the all ethanol extracts are moderately effective against gram-negative and gram-positive bacteria suggesting their broad spectrum antibacterial activity. This

is might be due to the presence of phytochemicals terpenoids, flavonoids, saponins, alkaloids, phenols and sterols. However the isolated compound is effective against gram-negative bacteria only, indicating it has no broad spectrum activity compared to crude ethanol extracts (inhibiting both gram positive and gram negative bacteria).

## Conclusion

In this study, the phytochemical screening tests of each plant parts revealed the presence of different class of phytochemicals including terpenoids, flavonoids, saponins, tannins, alkaloids, phenols and sterols. The ethanol crude extracts and the isolated compound from the stem bark extracts of *Croton macrostachyus* exhibited antibacterial activity against *Salmonella typhimurium*, *Listeria monocytogene* and *Escherichia coli* but not against *Staphylococcus aureus.* No significant antibacterial susceptibility variation was observed among the crude extracts though the isolated compound, which is proposed tobe lupeol, showed a little better activity against gram-negative bacteria (*Salmonella typhimurium* (12.5±1.22mm) and *Escherichia coli* (12.5±0.5mm)) compared to crude extracts. Therefore the extracts of *Croton macrostachyus* parts and the isolated compound could be a potential candidate for antibacterial agent against several human pathogens.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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