

*Full Length Research Paper*

# **Efficacy of *Synadenium glaucescens*, *Symphytum officinale*, *Moringa oleifera*, and *Commiphora swynnertonii* extracts against tomato quality loss microorganisms**

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For centuries, plants extracts have served medicinal values against microbes including bacteria and fungi. This study investigated the antibacterial and antifungal efficacy of extracts from leaves, stem barks, and roots of *Synadenium glaucescens*, *Moringa oleifera*, *Symphytum officinale*, and *Commiphora swynnertonii* resin, against microbial contamination in the tomato value chain. Plant samples were sourced from Morogoro and Manyara regions, while the respective extracts were obtained through sequential maceration and extraction using ethyl acetate and methanol. Antimicrobial assays were achieved by agar well diffusion and minimum inhibitory concentrations (MIC) against *Citrobacter freundii*, *Klebsiella pneumoniae*, *Aspergillus niger*, and *Fusarium equiseti*. Among extracts, *S. glaucescens* stem bark showed inhibition activity (zones of inhibition 4.33-5.33 mm) against *K. pneumoniae* and *C. freundii*, respectively. The resin from *C. swynnertonii* demonstrated antifungal efficacy against all tested fungi, with MIC of 2.5-5 mg/ml phytochemical analysis led to the identification of heptadecanoic acid from *S. glaucescens*. Fractions and compounds in combination with crude extracts showed synergistic effects against fungi and bacteria. It is suggested that *S. glaucescens* stem bark extract has the potential for use as a bio-pesticide against spoilage and toxigenic microorganisms and may extend shelf life and enhance safety of tomato fruits. Further research is needed to confirm effectiveness and safety of the compound and extracts in real-world applications.

**Key words:** *Solanum lycopersicum*, crude extracts, fractions, heptadecanoic acid, antimicrobial activity.

## **INTRODUCTION**

Tomato (*Solanum lycopersicum*) is one of the most consumed fruit vegetables throughout the world (Hannah et al., 2023). It is a horticultural crop with great nutritional,

medicinal and economic importance, consumed either fresh or processed in different food products (juice, soup, paste, puree, ketchup, sauce, and salsa (Beckles, 2012).

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Unfortunately, the crops is highly perishable and with limited shelf life at ambient conditions (Haile and Safawo, 2018) particularly under tropical conditions (Arah et al., 2016). Spoilage, softening and ripening are critical quality deterioration problems of tomato fruits during storage and distribution (Ndirangu et al., 2017; Khalid et al., 2024).

Quality loss in tomatoes after harvest is mainly caused by environmental and pathogenic stresses (Lai et al., 2011). Microbial contamination of both fungi and bacteria is reported to be the main cause of tomato quality loss (Abdullahi et al., 2022). The occurrence of spoilage micro-organisms in fruits and vegetables is partly attributed to contamination during harvesting and successive handling processes. Produce surface contamination can be minimized through sanitization of fruits and vegetable surfaces to reduce latent infections (Caroline et al., 2018). Food grade sanitizing agents such as Sodium hypochlorite or potassium permanganate (KMnO<sub>4</sub>) have been used for the purpose against decay microbes including fungi and bacteria before packaging of fruits and vegetables (Caroline et al., 2018).

Chlorinated water at a concentration of 200 ppm has been considered efficient, cost-effective, and simple to use (Nielsen et al., 2022) and the most used method for fruits and vegetables sanitation (Hour et al., 2015; Arah et al., 2016). Despite the usefulness of chlorinated water for the purpose, ingestion of excessive levels of sodium hypochlorite can damage the respiratory system due to its bleach fumes (Peck et al., 2011). Additionally, cleaning solutions containing sodium hypochlorite (NaOCl) may react with organic matters during cleaning process to produce halogenated organic compounds, which are bad for aquatic life and serve as persistent environmental contaminants when adsorbed on activated carbon (Affrald, 2022). In addition to these challenges, the compound is not easily accessible to majority of the farmers and marketers in developing world. This call for the need to find a locally available alternative to replace the synthetic disinfectants with natural, effective, and nontoxic compounds (Oliveira et al., 2013; Macwan et al., 2016).

*Synadenium glaucescens* (mvunjakongwa), *Commiphora swynnertonii* (Oltemwai/ndemwai), *Moringa oleifera* (Mlonge), and *Symphytum officinale* (Comfrey) are among the medicinal plants used in Tanzanians to control microorganisms including fungi and bacteria (Mkangara et al., 2014; Al\_husnan and Alkahtani, 2016; Salehi et al., 2019). These plants contain secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, anthraquinones, steroids, and essential oils (Kandar, 2021; Mikail et al., 2022). Such compounds are reported to have activity against viruses, fungi and bacteria (Mabiki et al., 2013). Bacteria and fungi are known to be the major cause of fruits and vegetables decays and causing toxigenic effects to humans (Kereth et al., 2013; Machado-Moreira et al., 2019). Decay pathogens are reported to cause postharvest loss of 63%

(Kereth et al., 2013). Whereas the toxigenic microbes such as *Staphylococcus aureus*, *Salmonella* species, *Escherichia coli*, has been reported to cause diarrhea and typhoid fever if consumed (Machado-Moreira et al., 2019). Unfortunately, there is limited evidence on the effectiveness of the named plant extracts against the decay and toxigenic microbes found on tomato fruits. Therefore, the objective of this study was to determine the effectiveness of extracts obtained from leaves, stem barks, and roots of *S. glaucescens*, *M. oleifera*, roots, leaves and roots from *S. officinale*, and resin from *C. swynnertonii* against the decay and toxigenic microorganisms associated with quality loss of tomato fruits.

## MATERIALS AND METHODS

### Study area

The study was conducted in the chemistry and microbiology laboratories at Sokoine University of Agriculture.

### Collection of plant samples and preparation

The leaves of *S. glaucescens* were readily available and collected from Kola in the Morogoro municipal (-6.814435, 37.692935). The root and stem bark samples of mature *S. glaucescens* trees could only be accessed from the Gairo district (-6.134781°S, 36.989225°E) Morogoro region during that sampling period. *C. swynnertonii* resin was collected from the Mimerani- Simanjoro District in Manyara region (-3.574422, 36.999678). Roots and leaves of *S. officinale* were collected from Mgeta kibaoni (-7.079302, 37.581673) in the Morogoro region. The leaves, roots, and stem barks of *M. oleifera* were collected from Tungi (-6.779169, 37.693849) in Morogoro municipal. Plant samples, collected in November 2023, were identified by a botanist using morphological characteristics and other relevant identification tools.

Plant parts were washed with clean water and peeled to separate the bark and wood. Plant materials were dried at room temperature (25-30°C) under a shade at the old chemistry laboratory Mazimbu Compus Sokoine University of Agriculture in Morogoro Tanzania. Dry samples were grounded separately using a lab mill machine (Christy Hunt Engineering Ltd, England) and sieved to obtain approximately 2 mm particle size. Figure 1 shows the map of Tanzania, developed with QGIS 3.34.1, illustrating sampling areas in the Morogoro and Arusha regions.

### Reagents

In this study, ethyl acetate, petroleum ether, dichloromethane, and methanol (both analytical grade) were the solvents used for extraction, isolation, and dissolving samples. These chemicals were approved laboratory equipment suppliers for the Sokoine University of Agriculture. The positive control used in the study was sodium hypochlorite, prepared from a 3.5% v/v sodium hypochlorite solution.

### Extraction of plant

A sequential cold maceration (25-30°C) of the powdered stem bark samples (1000 g) was carried out three consecutive times using ethyl acetate and then methanol, according to John et al. (2018).

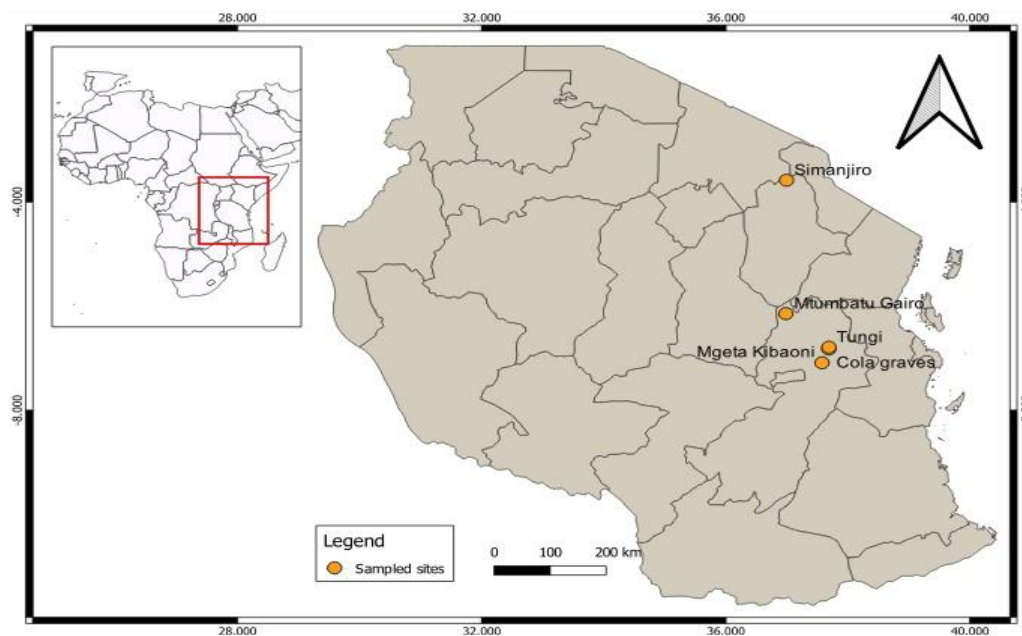


**Figure 1.** Map of Tanzania, developed with QGIS 3.34.1, illustrating sampling areas in the Morogoro and Arusha regions.

Extract filtration was performed using Whatman no1 filter paper (Maidstone-Kent, UK) every 72 h. The filtrates were concentrated using a rotary evaporator (Buchi R205 Labortetechnik, Flawil, Switzerland) with a water bath maintained at  $<60^{\circ}\text{C}$ . The dried extracts were stored in a refrigerator at  $4^{\circ}\text{C}$  until further use. The extraction yields for each plant sample were determined using a specified formula.

$$\% \text{Yield} = \frac{\text{Weight of extracts}}{\text{Weight of Sample}} \times 100 \quad (1)$$

where, %Yield is the yield of extracts obtained per weight of the sample used, weight of extracts is the weight of the dried crude extracts obtained, and weight of sample is the initial weight of the sample was measured.

### Isolation and purification of bioactive compounds

The isolation of pure compounds was achieved by column chromatography using solvent gradient elution. Sample profiling was done, ranging from 100% PE and DCM to 10% MeOH/DCM for the best column elution. A dry packing of sample slurry (40 g of EtOAc fraction) was prepared with 100% petroleum ether (PE). Thin Layer Chromatography (TLC) was used to monitor the solvent systems, characteristic fractions and purity of compounds. A single spot under both UV lamp (254/ 365 nm) and chemical treatment was considered a criterion for a pure compound isolated, the column solvent systems varied from 100% PE, DCM/PE (25, 40, 70 and 100% DCM). Then, the polarity of the solvent system was gradually increased to 5% MeOH in DCM, yielding 20 fractions (Fr. 63-83), and finally increased to 10% of MeOH in DCM, affording 22 fractions (Fr. 84-106).

On TLC analysis, the fractions were combined based on the retardation factor, that is, Fr. 1, Fr.2, Fr.3-4, Fr.5-6, Fr.7-8, Fr.9-11, Fr.16-19, Fr.20-22, Fr.23-26, Fr.27-29, Fr.30-34, Fr.35, Fr.36-38, Fr.39, Fr.40-44, Fr.45-51b, Fr.52, Fr.53-55, Fr.56-57, Fr.58-65,

Fr.66-69, Fr.70-77, Fr.78-95, and Fr.96-106. Upon settling at room temperature, fraction combinations Fr.1 and Fr.2 formed precipitates. The precipitates were filtered by washing with 100% MeOH and dried to obtain fraction combinations 1, and 2, respectively. Fr. 4,5-6,7-8,9-11,16-19,20-22, and the remains of 23 to 26 were mixed to make a total of 457 mg and subjected to a second column chromatography step. Dry packing 20% DCM/PE.

On TLC analysis fractions were combined based on the retardation factor Fr.2-3, Fr.4-8, Fr.9-14 Fr.15-21, Fr.22-30, Fr.31-32, Fr.33-42 upon settling at room temperature, fraction combinations were observed to form precipitates in the third (Fr.15-21, fourth Fr.31-32, and fifth Fr.43-51). The precipitates were filtered by washing with 100% MeOH and dried to obtain pure compounds 3, and fractions 4 and 5.

The purified compounds were analysed using a Bruker Avance 500 MHz Nuclear Magnetic Resonance (NMR) spectrometer in which the samples were dissolved in deuterated chloroform ( $\text{CDCl}_3$ ). The 1D ( $^1\text{H}$ ,  $^{13}\text{C}$ ) and 2D (HSQC and COSY) NMR spectra were recorded and compared with literature data to deduce the chemical structures of the isolated compounds (Supplementary Materials).

### Test bacterial and fungi species

*Citrobacter freundii*, *Klebsiella pneumoniae*, *Fusarium equiseti* and *Aspergillus niger*, which were surface microbial contaminants isolated using selective and differential media and confirmed by molecular characterization from tomato fruits were used in microbiology laboratory at Sokoine University of Agriculture. These species were selected because they are the illness-causing and decay-causing microorganisms in tomato fruits (Liu et al., 2016; Elijah et al., 2023).

### Preparation of crude extracts solutions

A stock concentration of  $20 \text{ mg mL}^{-1}$  crude extract from leaves, stem barks, and roots of *S. glaucescens*, *S. officinale*, *M. oleifera*,

and the resin of *C. swynnertonii* was prepared. This stock solution was used for all plant samples in agar well diffusion to determine the effectiveness of each extract in inhibiting the growth of bacteria and fungi. The Minimum Inhibitory Concentration (MIC) of each extract was determined from the stock concentration.

### Zones of inhibition determination by agar well diffusion method

Inhibition zones of tested plant samples against bacteria and fungi were determined according to Mirtaghi et al. (2016) with modifications. In brief, the bacterial and fungal suspension equivalent to 0.5 MacFarland standard turbidity ( $1.5 \times 10^6$  CFU mL<sup>-1</sup>) was prepared. A 20 mm plate containing 20 mL of Mueller Hinton Agar (MHA) was inoculated with the bacterial species, while a 20 mm plate containing 20 mL (PDA) was inoculated with fungi. Plates inoculated with the test organism had 6-mm wells cut into the surface of the agar using a cork borer dipped in alcohol and flamed. The wells were filled with 100  $\mu$ L of extract solutions. Additionally, 100  $\mu$ L of sodium hypochlorite was used as a positive control.

All bacterial plates were incubated (37°C) overnight, while the fungal plates were incubated at room temperature (25 to 30°C) for three to five days. After incubation, the diameters of any clear zones around the antimicrobial-containing wells were measured using a ruler. Each extract was tested in triplicate.

### MIC by broth dilution method

Assessment of antimicrobial effects was achieved by MIC values in a two-fold microdilution method according to Ochollah et al. (2022) and Msengwa et al. (2023), with modifications of the herb. In brief, sterile, 96-well polystyrene microtiter plates were first preloaded with 50  $\mu$ L of (MHA) for bacterial species and (PDA) for fungal species in each well followed by the addition of 50  $\mu$ L of extract solutions into the first well of each row to make a total volume of 100  $\mu$ L.

Each of the test sample materials was tested in duplicate. In the first well, the samples were mixed and 50  $\mu$ L was drawn from each well and transferred to the subsequent well until the last well. Then 50  $\mu$ L of the mixture from the last well was discarded. Thereafter, 50  $\mu$ L of the bacterial and fungi suspension equivalent to 0.5 MacFarland standard turbidity ( $1.5 \times 10^6$  CFU mL<sup>-1</sup>) was added to each well. An additional row containing 0.35 mg mL<sup>-1</sup> of sodium hypochlorite (NaOCl) (50  $\mu$ L) was used as a positive control. Wells containing (50  $\mu$ L) broth and micro-organisms were used as negative controls. One row on each plate contains only the broth was left as sterility control. Another row on each plate containing the broth, microorganisms, and NaOCl was left as the positive control. The plates were incubated at 37°C overnight for bacteria and at room temperature (25 to 30°C) for three to five days for fungi. MIC was determined visually, whereby the lowest concentration without growth of bacteria and fungi was considered as the MIC.

### Fractional inhibitory concentration (FIC)

The summation of fractional inhibitory concentration ( $\Sigma$ FIC) was calculated for each tested sample independently using, the following algebraic formula as specified by Kudumela et al. (2018), with slight modifications.

$$\text{FIC fractions} = \frac{\text{SSBE MIC Value in combination with fraction}}{\text{Fraction MIC value}} \quad (2)$$

and

$$\text{FIC compounds} = \frac{\text{SSBE MIC Value in combination with compound}}{\text{compound MIC value}} \quad (3)$$

where the combined effect was interpreted as synergistic if the FIC index  $\leq 0.5$ , additive if  $0.5 > \text{FIC index} < 4$ , or antagonistic if the FIC index  $\geq 4$ . This interpretation follows the conventional model suggested by Odds (2003) and Kassim et al. (2016).

### Data analysis

Data collected from laboratory investigations was recorded, screened, and coded using the microsoft excel 2021 program and analysed using R online software and GenStat 15 version software. Analysis of variance (ANOVA) was used to investigate the significant differences in inhibition zones of the plant crude extracts against the isolated microbes from the tomato fruits, and a p-value less than 0.05 was taken as statistically significant. Spectra data were processed in MestReNova version 14.2.0-26256.

## RESULTS

### Extraction yields of crude extracts

Extracted yields of crude extracts from the plant samples revealed that there is a variation in extraction yields depending on the solvents used. *Moringa* leaves had higher extraction yields in Ethyl acetate (9.99%) and lowest in methanol (MeOH) (3.93%). *Synadenium* stem bark had higher extraction yields in methanol 11.75% and lowest in Ethyl acetate 5.94%. The other plant sample and plant parts also demonstrated the variation in extraction yields when extracted sequentially using the two different solvent systems, ethyl acetate and methanol respectively as shown in Figure 2.

### Fractions and isolated compounds

The isolation of pure compounds was conducted in stages through the collection of fractions. Four fractions were obtained, with one pure compound, known as heptadecanoic acid, being successfully isolated. All fractions and compounds were derived from 100% ethyl acetate crude extracts, which indicated higher activity compared to others in a parallel study. Fraction 1 yielded 59 mg of white crystals that were UV negative, soluble in less polar and medium polar solvents, and insoluble in highly polar solvents. The average melting point of this fraction was 67.6°C. Fraction 2 contained 31 mg of white crystals that were also UV negative, soluble in less polar and medium polar solvents, and insoluble in highly polar solvents, with an average melting point of 69.7°C. Fraction 3 produced 15 mg of white crystals with similar properties; they were soluble in medium polar solvents and insoluble in highly polar solvents, and also UV negative, with an average melting point of 66.05°C.

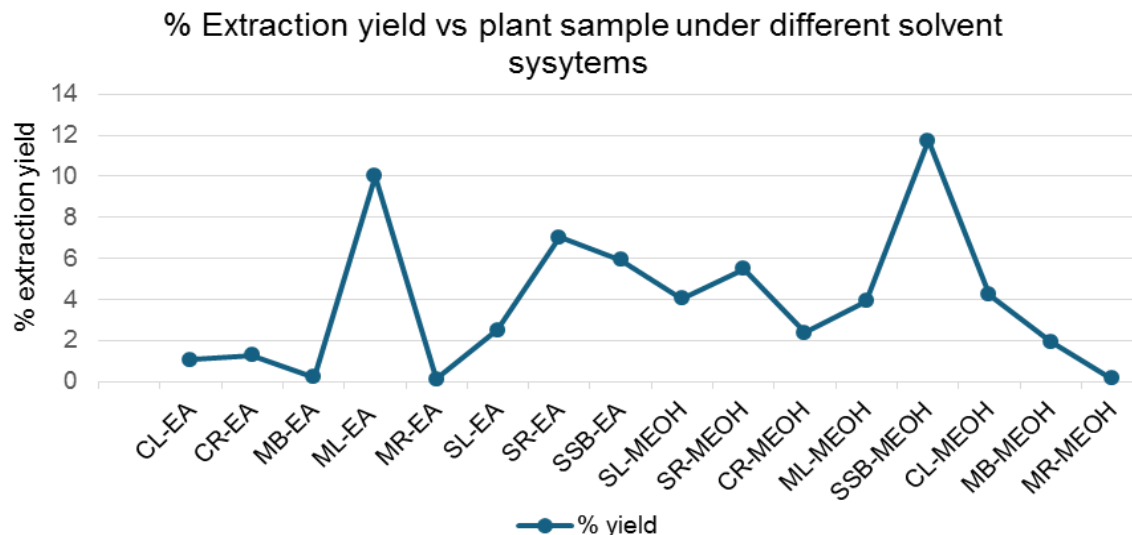


**Figure 2.** Extraction yield vs plant sample under different solvent systems.

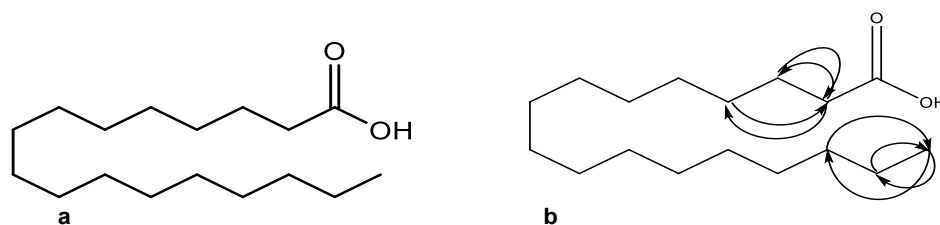


**Figure 3.** Structure of Heptadecanoic acid (a) and H-H correlation (HMBC) (b).

Fraction 4 produced 6 mg of a white powder that is UV negative, appears purple in a spraying medium and is insoluble in a highly polar solvent. The average melting point of this fraction was 75.65°C. Pure compound (heptadecanoic acid) produced 44 mg of a white substance that is soluble in a medium polar solvent and insoluble in a highly polar solvent. It is also UV-negative, with a melting point of 69.25°C.

#### Structure determination of heptadecanoic acid

The data from correlated Spectroscopy (COSY) revealed correlations among several protons: H-2 to H-3, H-3 to a long chain of CH<sub>2</sub>, and H-17 to H-16. Additionally, C-H correlations observed in the Heteronuclear Single Quantum Coherence (HSQC) data included H-17, H-16, H-2, H-3, and H-4 to H-15, corresponding to the long chain of CH<sub>2</sub>.

Together with the Heteronuclear Multiple Bond Correlation (HMBC) data, these findings confirmed that the pure compound is heptadecanoic acid. The structure of the compound is illustrated in Figure 3, and the spectral data are found in Table 1.

#### Inhibition zones of crude extracts against bacteria

The analysis of variance (ANOVA) indicated that the effectiveness of plant crude extracts in inhibiting the growth of *C. freundii* and *K. pneumoniae* varied significantly ( $P < 0.0001$ ). Further details regarding the efficacy of each plant crude extract on the bacteria are presented in Tables 2 and 3.

#### Inhibition zones of crude extracts against Fungi

The analysis of variance (ANOVA) showed that the ability of various plant crude extracts to inhibit the growth of *F. equiseti* and *A. niger* significantly varied ( $P < 0.001$ ). Additional details regarding the effectiveness of each plant crude extract on the fungi are provided in Figures 4 and 5.

#### The MIC of crude extracts, fractions and compounds on *C. freundii*, *K. pneumoniae*, *A. niger* and *F. equiseti*

To determine the concentration of crude extracts

**Table 1.**  $^{13}\text{C}$  and  $^1\text{H}$  NMR Spectral Data of Compound C1. (Heptadecanoic acid).

| C    | C-13 (125 ppm)<br>Experimental | $^1\text{H}$ NMR (500 MHz) | References: Kamdem et al. (2022) |
|------|--------------------------------|----------------------------|----------------------------------|
| 1    | Not seen                       | -                          | $^1\text{H}$ NMR                 |
| 2    | 42.98                          | (2H) <i>t</i> , 2.31       | (2H), <i>t</i> , 2.22            |
| 3    | 32.06                          | (2H) <i>m</i> , 1.47       | -                                |
| 4    | 32.08                          | -                          | -                                |
| 5-14 | 29.86 (long)                   |                            |                                  |
|      | 29.83                          |                            |                                  |
|      | 29.81                          |                            |                                  |
|      | 29.77                          |                            |                                  |
|      | 29.64                          |                            |                                  |
|      | 29.58                          | (26H) <i>br, s</i> 1.18    | (26H) <i>m</i> , 1.28            |
|      | 29.52                          |                            |                                  |
|      | 29.49                          |                            |                                  |
|      | 29.43                          |                            |                                  |
| 15   | 24.05                          |                            |                                  |
| 16   | 22.85                          |                            |                                  |
| 17   | 14.29                          | (3H) <i>t</i> , 0.81       | (3H), 0.86 <i>t</i>              |

**Table 2.** Mean inhibition zones (mm) of plant crude extracts on *C. freundii*.

| Plant crude extract | <i>C. freundii</i> |
|---------------------|--------------------|
| SSB-EA              | 5.33 <sup>a</sup>  |
| C+                  | 5.00 <sup>a</sup>  |
| CR-EA               | 4.00 <sup>b</sup>  |
| ML-EA               | 4.00 <sup>b</sup>  |
| MB-MEOH             | 3.67 <sup>b</sup>  |
| SL-EA               | 3.67 <sup>b</sup>  |
| SR-EA               | 2.67 <sup>c</sup>  |
| SL-MEOH             | 2.33 <sup>c</sup>  |
| CR- MEOH            | 1.00 <sup>d</sup>  |
| MR-EA               | 1.00 <sup>d</sup>  |
| SSB-MEOH            | 1.00 <sup>d</sup>  |
| SR-MEOH             | 0.33 <sup>de</sup> |
| C-                  | 0.00 <sup>e</sup>  |
| CL- MEOH            | 0.00 <sup>e</sup>  |
| CL-EA               | 0.00 <sup>e</sup>  |
| MB-EA               | 0.00 <sup>e</sup>  |
| ML-MEOH             | 0.00 <sup>e</sup>  |
| MR-MEOH             | 0.00 <sup>e</sup>  |
| S1- MEOH            | 0.00 <sup>e</sup>  |
| e.s.e.              | 0.25               |

Means followed by the same letter in the same column are not significantly different at  $p \leq 0.05$ ). C+ Sodium hypochlorite, CR= comfrey root, ML= Moringa leaf, MR= Moringa root, SL= Synadenium leaf, SR=Synadenium roots, SSB= Synadenium stem bark, S1= resin from Commiphora tree, MB= Moringa stem bark, CL= comfrey leaf, MEOH= methanol, EA= ethyl acetate, C- saline water, e.s.e.= standard error of means

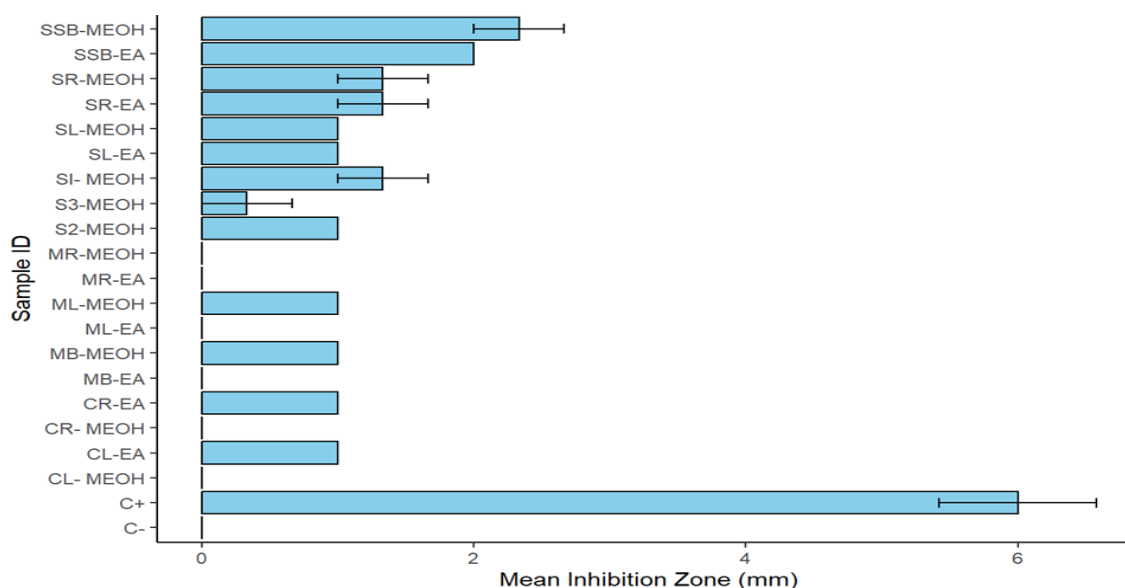
for antibacterial and antifungal activity, the MIC determination is used. The result of this study showed that *Synadenium* stems bark extract from ethyl acetate had a MIC value of 1.25 mg/ml for *C. freundii* and 2.5 mg/ml for *K. pneumoniae*. Also, the same extracts

demonstrated good MIC value in comparison with other extracts with a MIC value of 2.5 mg/ml for *A. niger* and 5 mg/ml for *F. equiseti*. The results in this study also indicated that despite crude extracts from *C. swynnertonii* resins having no effects on the bacteria, the extracts

**Table 3.** Mean inhibition zones (mm) of plant crude extracts on *K. pneumoniae*.

| Plant crude extract | <i>K. pneumoniae</i> |
|---------------------|----------------------|
| C+                  | 6.00 <sup>a</sup>    |
| SSB-EA              | 4.33 <sup>b</sup>    |
| SR-EA               | 3.67 <sup>bc</sup>   |
| MB-MEOH             | 3.33 <sup>c</sup>    |
| CR-EA               | 3.00 <sup>cd</sup>   |
| CR- MEOH            | 2.33 <sup>de</sup>   |
| SL-MEOH             | 2.00 <sup>ef</sup>   |
| CL- MEOH            | 1.33 <sup>fg</sup>   |
| MB-EA               | 1.33 <sup>fg</sup>   |
| ML-EA               | 1.33 <sup>fg</sup>   |
| SL-EA               | 1.33 <sup>fg</sup>   |
| SSB-MEOH            | 1.33 <sup>fg</sup>   |
| CL-EA               | 1.00 <sup>gh</sup>   |
| MR-EA               | 1.00 <sup>gh</sup>   |
| MR-MEOH             | 1.00 <sup>gh</sup>   |
| SR-MEOH             | 1.00 <sup>gh</sup>   |
| S1- MEOH            | 0.33 <sup>hi</sup>   |
| C-                  | 0.00 <sup>i</sup>    |
| ML-MEOH             | 0.00 <sup>i</sup>    |
| e.s.e.              | 0.28                 |

Means followed by the same letter in the same column are not significantly different at  $p \leq 0.05$ ). C+ Sodium hypochlorite, CR= comfrey root, ML= Moringa leaf, MR= Moringa root, SL= Synadenium leaf, SR=Synadenium roots, SSB= Synadenium stem bark, S1 = resin from Commiphora trees, MB=Moringa stem bark, CL= comfrey leaf, MEOH= methanol, EA= ethyl acetate, C- saline water, e.s.e.= standard error of means.

**Figure 4.** Mean inhibition zones of plant crude extracts on *Fusarium equiseti*.

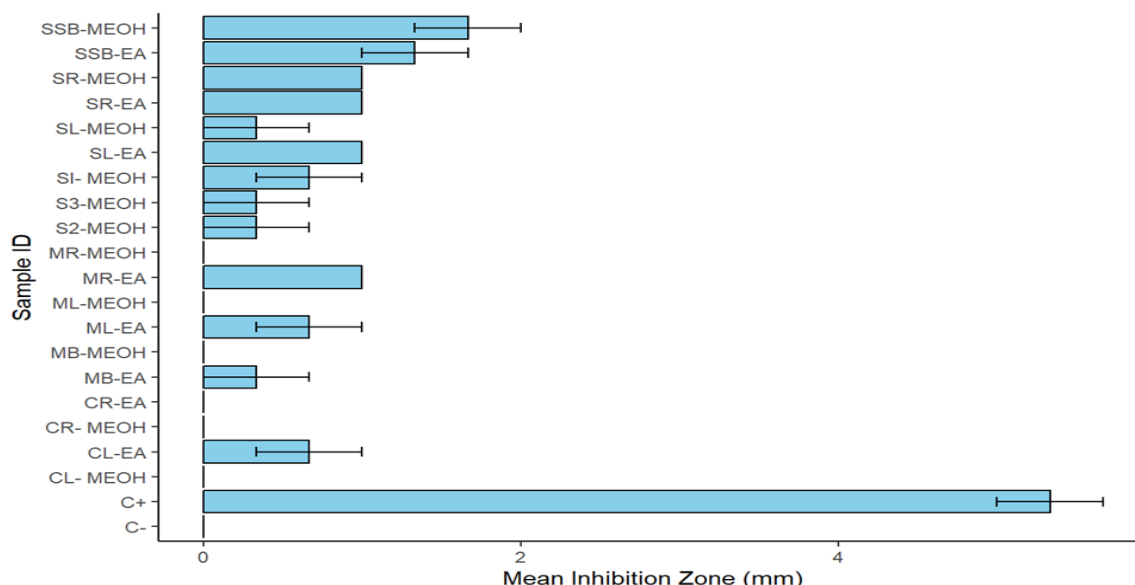


**Figure 5.** Mean inhibition zones of plant crude extracts on *Aspergillus niger*.

demonstrated good antifungal properties with an average MIC value of 2.5-5 mg/ml) for *Aspergillus* and *Fusarium* species, respectively. Sodium hypochlorite had a MIC value compared with plant crude extracts with a MIC value of 0.35 to 0.175 mg/ml for *F. equiseti*, *A. niger*, *K. pneumoniae*, and *C. freundii*. The MIC value for other plant species has been presented in Tables 4 and 5. The isolated compound and subfractions from Ethyl acetate fractions of *Synadenium* stem bark were also tested for antibacterial activity against *C. freundii* and *K. pneumoniae*, and antifungal activity against *A. niger* and *F. equiseti*. Compound and subfractions demonstrated low activity with MIC value range from (5 to 10 mg/ml) against the tested species compared to positive control (0.35 to 0.175 mg/ml) The MIC value for subfractions and compounds is presented in Table 6.

#### Fractions inhibition concentrations index (FIC) of combined fractions and compound

The FIC values were calculated, and the antibacterial and antifungal effects were summarized in Table 7. The results of this study indicated that all fractions, along with one pure compound (Heptadecanoic acid) in combination with ethyl acetate crude extracts of *S. glaucescens* stem bark, exhibited synergistic effects against *C. freundii*, *K. pneumoniae*, *A. niger*, and *F. equiseti*, with a cumulative (FIC) value of less than or equal to 0.5.

## DISCUSSION

The result of extraction yields of bioactive compounds

from this study reveals a variation in extraction yields among the solvents used in the extraction process. This variation in the extraction yields might be due to the difference in polarity among the solvents used. These results are consistent with Ng et al. (2020) who found that solvents with increasing polarity favour the extraction of phenolic antioxidants. The higher extraction yields of bioactive compounds observed in the *Moringa* leaf on ethyl acetate when used sequentially with methanol might be due to the higher solubility of bioactive compounds found on the *Moringa* leaf in ethyl acetate compared to methanol. These results align with Otero et al. (2018), who demonstrated that ethyl acetate is a better solvent for extracting fatty acids from freeze-dried *Galician Algae* compared to less polar (hexane) and more polar (ethanol and ethanol/water) solvents. Also, the results from this study demonstrate that despite the variation in the extraction yields of bioactive compounds between the ethyl acetate and methanol but seems like other bioactive compounds were soluble in both solvents. This correlates with the findings of Jaime et al. (2010), who studied the impact of ethanol and hexane solvents through the extraction yield of different pigments, such as chlorophylls and carotenoids from *Haematococcus pluvialis* microalgae by pressurized liquid extraction. Both pigment families were found in ethanol and hexane extracts. Zi et al. (2007) found the solubility of rutin, a type of flavonoid, was soluble in various solvents, including polar (methanol) and less polar solvents (ethyl acetate).

Shakeel et al. (2017) found a solubility of bioflavonoid apigenin under both less polar and polar solvents. This how that even with a high variation in extraction yields, some compounds are soluble in any solvent.

**Table 4.** Average MIC value of plant crude extracts on *C. freundii* and *K. pneumoniae*.

| Plant crude extract | <i>C. freundii</i> MIC value (mg/ml) | <i>K. pneumoniae</i> MIC value (mg/ml) |
|---------------------|--------------------------------------|----------------------------------------|
| CR-EA               | 5                                    | 5                                      |
| ML-EA               | 5                                    | 10                                     |
| MR-EA               | 5                                    | 5                                      |
| SL-EA               | 2.5                                  | 5                                      |
| SR-EA               | 2.5                                  | 5                                      |
| SSB-EA              | 1.25                                 | 2.5                                    |
| CR- MEOH            | 5                                    | 5                                      |
| MB-MEOH             | 2.5                                  | 10                                     |
| SL-MEOH             | 10                                   | 10                                     |
| SR-MEOH             | 1.25                                 | 5                                      |
| SSB-MEOH            | 5                                    | 5                                      |
| C+                  | 0.175                                | 0.35                                   |

C+ Sodium hypochlorite, Cr=comfrey root, Ml=Moringa leaf, MR=Moringa root, SL= Synadenium leaf, SR=Synadenium roots, SSB= Synadenium stem bark, EA= Ethyl acetate, MEOH methanol MIC= minimum inhibitory concentration.

**Table 5.** Average MIC value of plant crude extracts on *F. equiseti*, and *A. niger*.

| Plant crude extract | <i>A. niger</i> MIC value (mg/ml) | <i>F. equiseti</i> MIC value (mg/ml) |
|---------------------|-----------------------------------|--------------------------------------|
| SL-EA               | 5                                 | 7.5                                  |
| ML-EA               | 10                                | 10                                   |
| SSB-EA              | 2.5                               | 2.5                                  |
| MR-EA               | 5                                 | 7.5                                  |
| SR-EA               | 10                                | 5                                    |
| SSB- MEOH           | 10                                | 5                                    |
| MB-MEOH             | 5                                 | 5                                    |
| SR-MEOH             | 5                                 | 3.75                                 |
| S1-MEOH             | 2.5                               | 5                                    |
| ML-MEOH             | 10                                | 10                                   |
| C+                  | 0.35                              | 0.35                                 |

C+ Sodium hypochlorite, SL= Synadenium leaf, Ml=Moringa leaf, SSB= Synadenium stem bark, MR=Moringa root, SR=Synadenium roots MB=Moringa stem bark, S1=Resin extracted from Commiphora tree. EA= Ethyl acetate, MEOH= methanol, MIC= minimum inhibitory concentration.

**Table 6.** Average MIC value in mg/ml of *S. glaucescens* Stem bark fractions and Heptadecanoic acid on *C. freundii*, *K. pneumoniae*, *F. equiseti*, and *A. niger*.

| EtOH stem bark fractions and compound | <i>C. freundii</i> | <i>K. pneumoniae</i> | <i>A. niger</i> | <i>F. equiseti</i> |
|---------------------------------------|--------------------|----------------------|-----------------|--------------------|
| Fraction 1                            | 10                 | >10                  | >10             | 10                 |
| Fraction 2                            | 5                  | 10                   | 10              | 5                  |
| Fraction 3                            | 10                 | 10                   | 10              | 10                 |
| Heptadecanoic acid                    | 5                  | 5                    | 10              | 10                 |
| Fraction 4                            | 5                  | 5                    | 10              | 10                 |
| NaOCl                                 | 0.175              | 0.35                 | 0.35            | 0.175              |

Under laboratory conditions, this study found that plant crude extracts from *S. glaucescens*, *M. oleifera*, *S.*

*officinale*, and resin from *C. swynnertonii* showed a significant variation in inhibiting the growth of

**Table 7.** Fractional inhibitory concentration Index (FIC Index) of combined fractions and compounds from ethyl acetate crude extracts of *S. glaucescens* stem bark tested against selected bacteria and fungi.

| Combination | <i>C. freundii</i> | <i>K. pneumoniae</i> | <i>F. equiseti</i> | <i>A. niger</i> |
|-------------|--------------------|----------------------|--------------------|-----------------|
| SSB EtOH+F1 | 0.125              | 0.125                | 0.25               | 0.125           |
| SSB EtOH+F2 | 0.25               | 0.25                 | 0.5                | 0.25            |
| SSB EtOH+F3 | 0.125              | 0.25                 | 0.25               | 0.25            |
| SSB EtOH+C1 | 0.25               | 0.5                  | 0.25               | 0.25            |
| SSB EtOH+F4 | 0.25               | 0.5                  | 0.25               | 0.25            |

SSB EtOH=ethyl acetate crude extracts of *S. glaucescens* stem bark, F1-F4= fractions, C1=Heptadecanoic acid.

microorganisms including *C. freundii* and *K. pneumoniae*. The antibacterial ability of these plant extracts was mainly because these plants contain secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, anthraquinones, steroids, and essential oils.

These compounds are reported to have antibacterial activity against viruses and bacteria (Mabiki et al., 2013). The antimicrobial activity of these compounds against fungi and bacteria occurs through several mechanisms, which include the following potential effects: promoting cell wall disruption and lysis, inducing the production of reactive oxygen species, inhibiting biofilm formation, obstructing cell wall construction, preventing microbial DNA replication, inhibiting energy synthesis, and preventing bacterial toxins from affecting the host (Mickymaray, 2019). *S. glaucescens* stem bark ethyl acetate fraction was effective against micro-organisms including *C. freundii* and *K. pneumoniae* isolated from the tomato fruits. Other studies have reported the medicinal value of *S. glaucescens* and *C. swynnertonii* against bacteria, fungi, and viruses (Credo et al., 2022; Ochollah et al., 2023). Also, Msengwa et al. (2023) found the effectiveness of *S. glaucescens* extracts from whole root and root bark against bacteria including *S. aureus*, *Streptococci* and *Enterococci* species, and moderate to weak activity against *E. coli*, and *Salmonella* spp. The efficiency of these extracts against the pathogenic bacteria species is critical because these bacterial species are responsible for human health risks (Khan et al., 2015).

Antifungal effect of crude extracts of *S. glaucescens*, *M. oleifera*, *S. officinale*, and resin from *C. swynnertonii* shows a significant variation in inhibiting the growth of *A. niger* and *F. equiseti* isolated from the surface of the tomato fruits. *S. glaucescens* stem bark and resin from *C. swynnertonii* shows a good performance in the inhibition of the growth of fungal colonies. This aligns with Madege et al. (2023) who reported the potential antifungal effects of *S. glaucescens* and *C. swynnertonii* in managing *F. oxysporum*. The variation in activity between the tested plant crude extracts might be due to the variation in the biochemical composition of the secondary metabolites which act against the fungal pathogen. *Synadenium* and

*Commiphora* species contain terpenoids, phenolics, flavonoids, saponins, diterpenes, and phorbol esters (Nyigo et al., 2016). These compounds interfere with the physiological activities of different pests, including fungi.

It was also established that the bioactivity of plant extracts against *C. freundii* and *K. pneumoniae*, *A. niger* and *F. equiseti* was highly dependent on the concentration of the extract. The lowest concentration observed on *S. glaucescens* stem bark was effective for both bacteria and fungi. This was in line with the other study which observed that extract of whole root, root bark, and leaves of *S. glaucescens* inhibited *S. aureus* at relatively low concentrations (Msengwa et al., 2023). While resin from *C. swynnertonii* had the lowest concentration against *Fusarium* species and *Aspergillus* species respectively. This was in line with a previous study which observed that resin from the *C. swynnertonii* was effective against *F. oxysporum* (Madege et al., 2023). The remaining extracts were effective at the higher concentrations ranging from 5-10 mg/ml in line with the previous study (Efenberger-Szmechtyk et al., 2021). The lowest activity of the other extracts against the pathogen isolated from the surface of tomato fruits might be due to active compounds in crude extracts being present in too low concentrations to show activity without enrichment (Altemimi et al., 2017), or antagonistic effect may hinder the activity, for example, by binding to the same receptors as the active compound (Silva et al., 2019).

The fractions and compounds that were isolated from *S. glaucescens* ethyl acetate fraction had low activity in inhibiting the growth of both fungi and bacteria isolated from tomato fruits in comparison to Sodium hypochlorite. The weak activity demonstrated by fraction and compound is related to previously reported findings on related compounds (Voukeng et al., 2017). These results showed that to potentiate the pure chemicals extracted from this plant, they require enrichment or structural changes.

The structure elucidation confirms that the pure compound isolated from the ethyl acetate crude extracts of *S. glaucescens* stem bark is heptadecanoic acid. This compound was previously isolated from the stem bark of

*Cola lateritia* (Kamdem et al., 2022). However, this is the first report of heptadecanoic acid in *S. glaucescens*. The antimicrobial properties of this compound were previously demonstrated by Kamdem et al. (2022).

The results from the fraction inhibition concentration studies indicate that both individual fractions and the pure compound heptadecanoic acid, isolated from ethyl acetate crude extracts of *S. glaucescens* stem bark, exhibited weak activity against the bacteria *C. freundii* and *K. pneumoniae*, as well as the fungi *A. niger* and *F. equiseti* when compared to its crude extracts. Previous studies have reported several synergistic effects from combinations of various plant herbs. For example, De Rapper et al. (2016) highlighted the synergy found in combinations of *Schkuhria pinnata* and *Commelina africana*, as well as *Dombeya rotundifolia* and *S. pinnata*, which demonstrated effectiveness against *P. aeruginosa* with fractional inhibitory concentration ( $\Sigma$ FIC) values of  $\leq 0.5$ . Additionally, Mbunde et al. (2019) showed a synergistic effect in the combination of *Bidens pilosa* and *Leonotis nepetifolia* extracts against *C. albicans*. These synergistic effects suggest that the antibacterial and antifungal activities of the crude extract are enhanced due to the cumulative effects of the individual fractions and pure compounds.

## Conclusion

The stem bark extract of *S. glaucescens* is highly effective against the bacteria *C. freundii* and *K. pneumoniae*, as well as the fungi *A. niger* and *F. equiseti*. It exhibits the largest zones of inhibition and the lowest minimum inhibitory concentrations (MIC) for all tested microorganisms. In contrast, resin from *C. swynnertonii* shows no antibacterial activity against *C. freundii* and *K. pneumoniae*, but it does have some antifungal activity against *A. niger* and *F. equiseti*. Other resins demonstrate moderate antibacterial and antifungal activity against these same microorganisms. Structural analysis confirms that the pure compound isolated is heptadecanoic acid. The fractional inhibition concentration index (FIC) indicates a synergistic effect of the ethyl acetate crude extracts from *S. glaucescens* stem bark when compared to isolated fractions and pure compounds. In summary, the study indicated that *S. glaucescens* stem bark extract had potential compounds to serve as a bio-pesticide against spoilage and toxigenic microorganisms and might contribute to the extension of shelf life and safety of tomato fruits. This study is limited to in vitro; therefore, an in vivo study is required to validate the results. Further research is needed to confirm its efficacy and safety in real-world applications. These findings underscore the varying antimicrobial properties of different plant extracts against surface microorganisms isolated from tomato fruits, which could be of value for future research or practical applications.

## CONFLICT OF INTERESTS

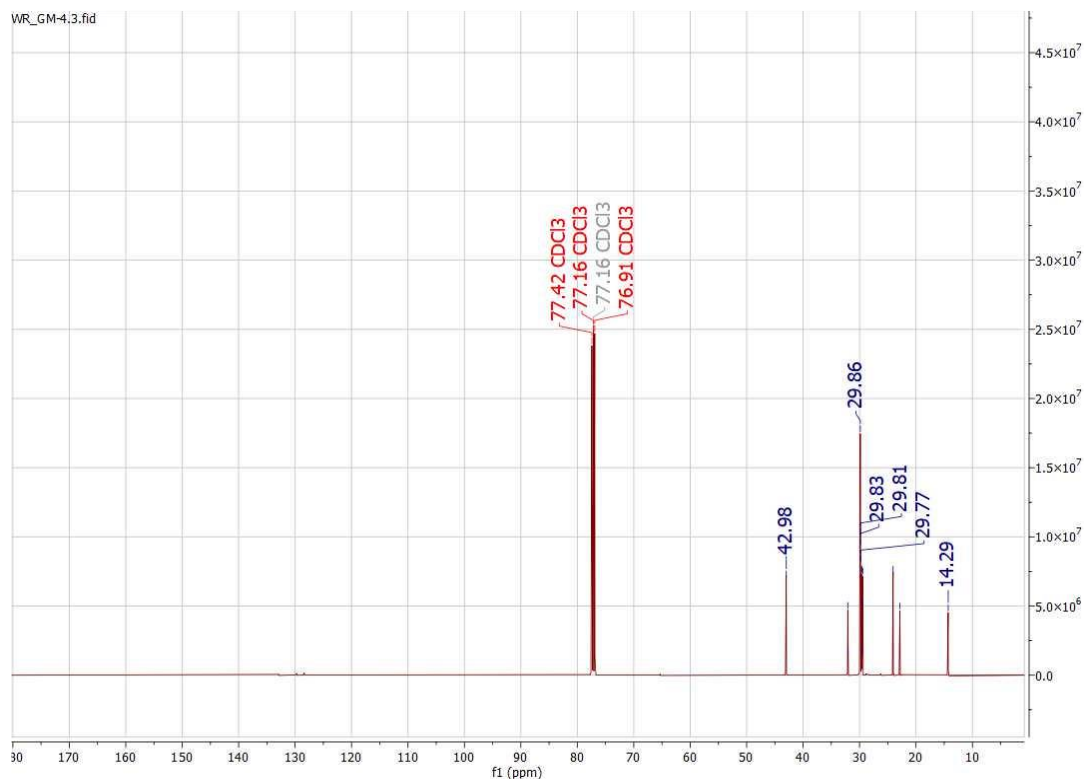
The authors have not declared any conflict of interests.

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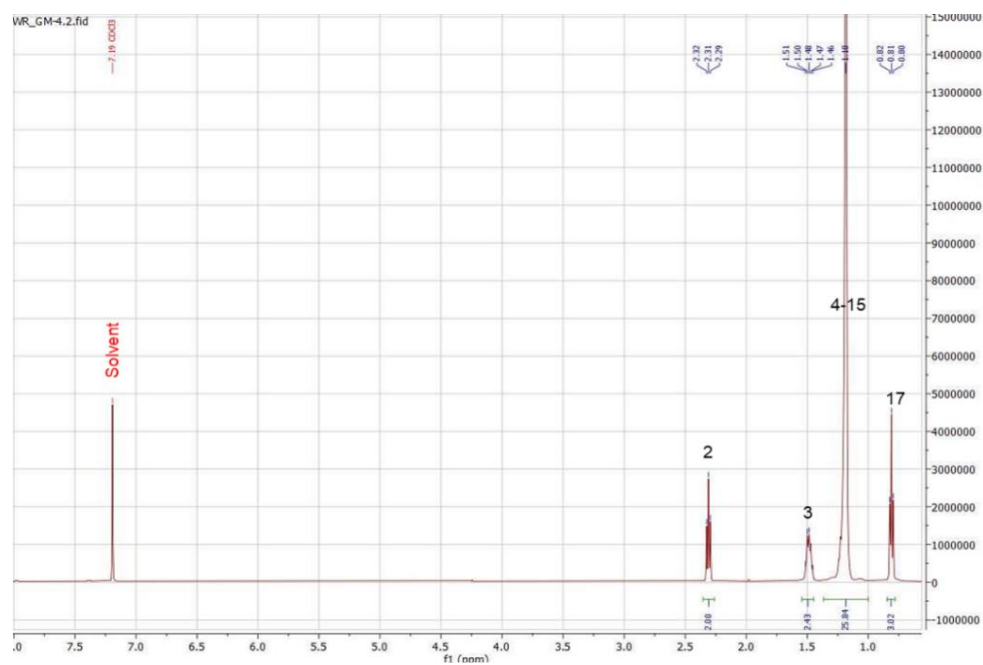
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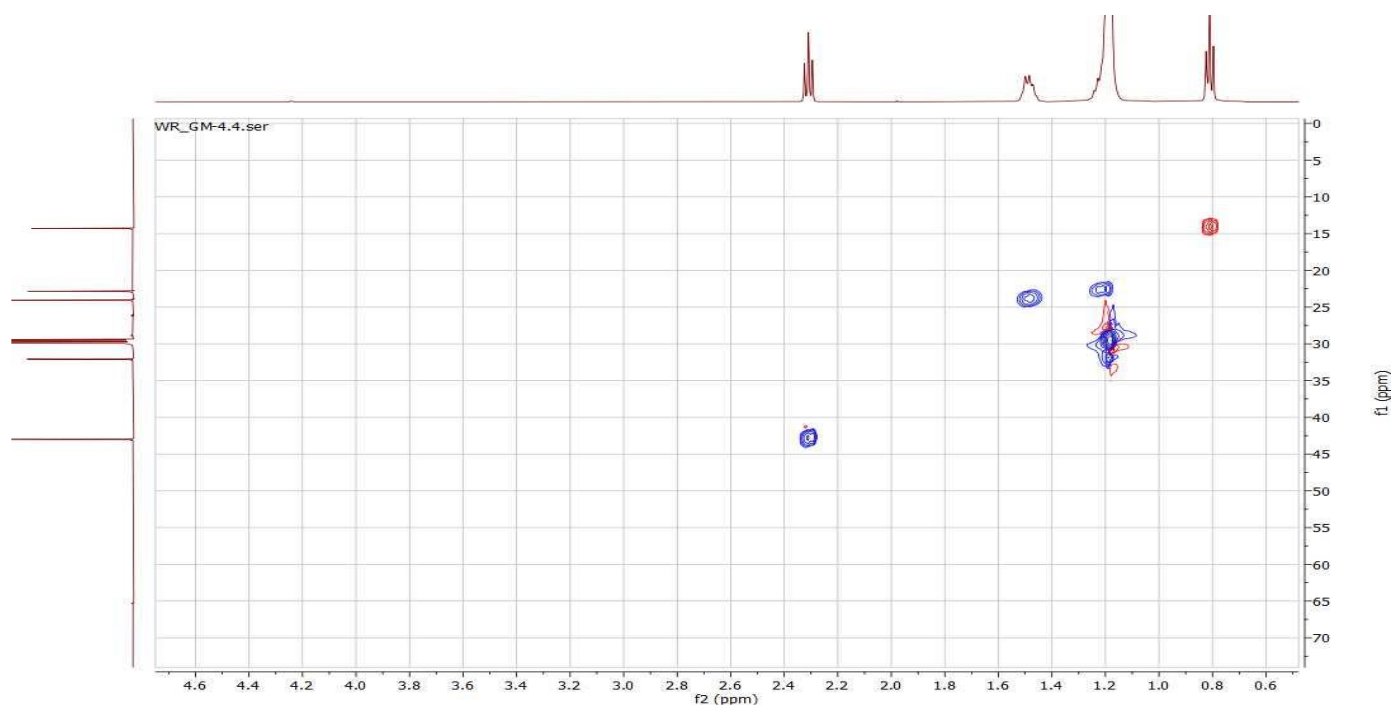
## LIST OF SUPPLEMENTARY MATERIALS



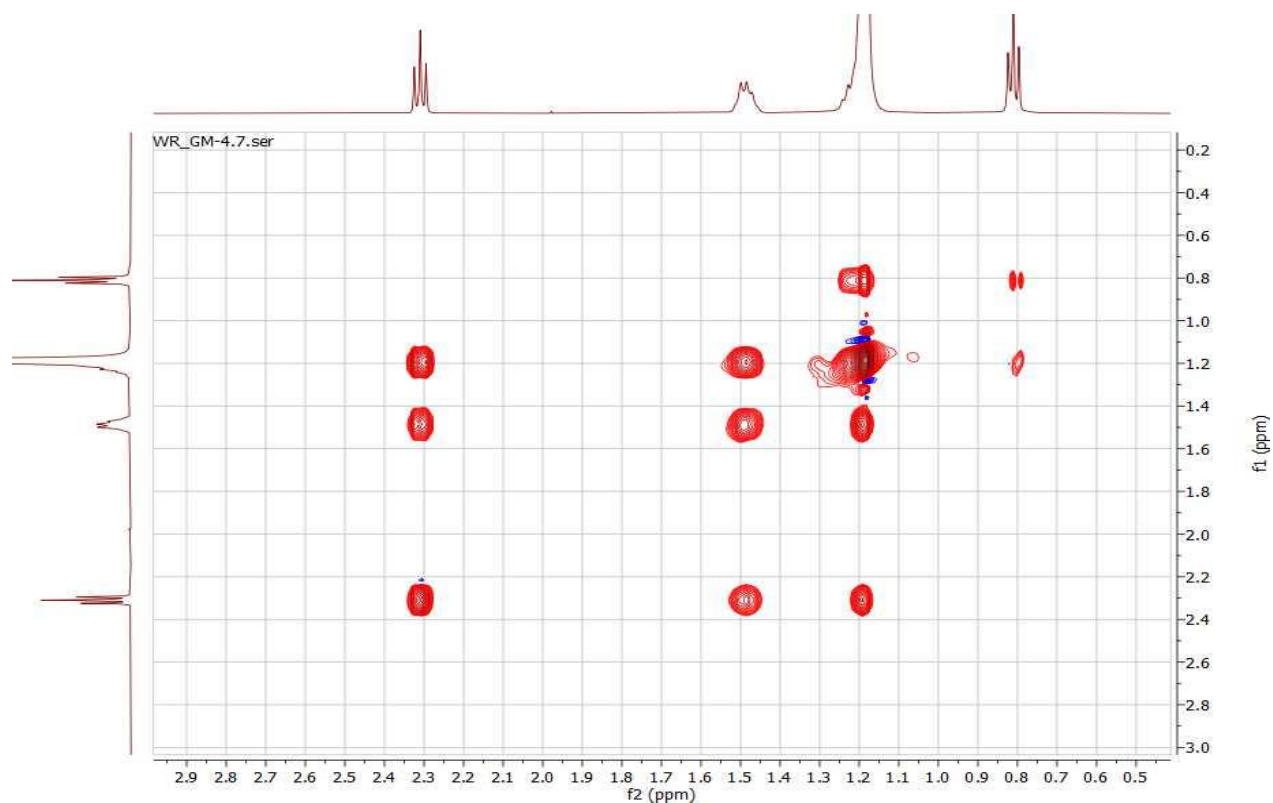
**Supplementary Figure 1.**  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ) spectrum of compound C1(Heptadecanoic acid).



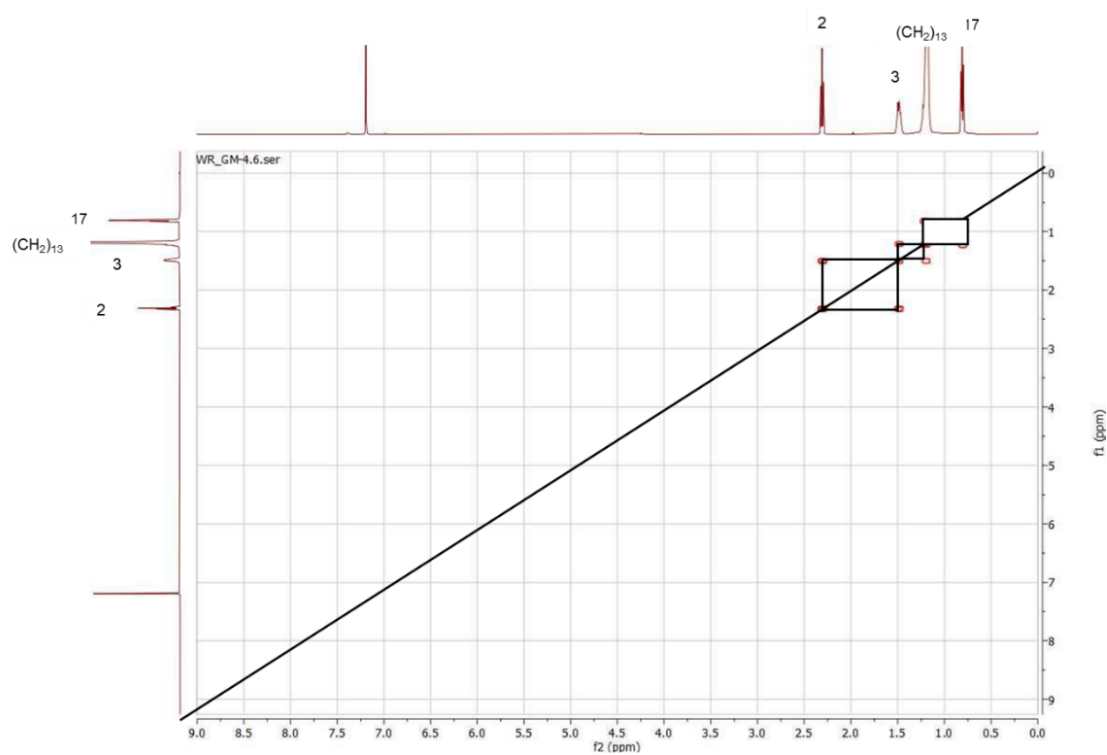
**Supplementary Figure 2.**  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ) spectrum of compound C1(Heptadecanoic acid)



**Supplementary Figure 3.** HSQC NMR spectrum for compound C1(Heptadecanoic acid).



**Supplementary Figure 4.** HMBC NMR spectrum of compound C1(Heptadecanoic acid).



**Supplementary Figure 5.** H: H Correlation (COSY spectrum of compound C1 (Heptadecanoic acid).