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Research Article

Phytochemical constituents from corn silk and antimicrobial activity of the isolates

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Abstract: Corn silk (*Stigma maydis*) is one of the traditional medicines for treating many microbial infections. However, there is little literature on the bioactive compounds responsible for these activities. This study was designed to investigate the phytochemical constituents present in corn silk and to screen the isolated compounds for antimicrobial activity. The pulverized plant sample of 1.14 kg was extracted with 3.6 L of methanol by cold maceration for 3 days. The extract was screened for phytochemicals, followed by isolation of constituent phytochemicals, characterization, and identification of isolated compounds. The isolates were screened for antibacterial and antifungal activities. The phytochemical screening revealed the presence of alkaloids, flavonoids, coumarins, tannins, reducing sugars, saponins, terpenoids, sterols, and cardiac glycosides. Further phytochemical investigation of the chloroformic subfraction of the methanolic extract of the silk led to the isolation of behenic acid and stigmasterol after running column chromatography as well as other chromatographic methods. The identity of the isolated compounds was established based on extensive spectroscopic analyses of their IR, 1D, 2D NMR data and comparing the data to the reported literature. Stigmasterol was active against *Staphylococcus aureus* and the fungal strain *Candida albicans* at 25 µg/mL while the mean minimum inhibitory concentration of behenic acid against *Staphylococcus aureus was* 100 µg/mL, *Tinea corporis* and *Klebsiella pneumoniae* were susceptible at 25 µg/mL. The study showed that plant secondary metabolites might be responsible for the reported biological activities of corn silk. It is the first report of behenic acid isolated from corn silk.

1. INTRODUCTION

In Ghana, maize (*Zea mays* L.,) is a dominant cereal in terms of cultivation and use. It is cultivated in all the agro-ecological zones in Ghana (Ragasa *et al*., 2014). The maize plant belongs to the family Poaceae. It contains husk, corn, and silk. Corn silk (*Stigma maydis*) is the shiny, soft thread-like, weak fibers about 10-20 cm long, which grow as part of the ears of corn and are enclosed in the husk. It is dark brown when dry (Fazilatun *et al*., 2012). Corn silk is the

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stigma of the plant, a waste from corn production, and it is very abundant (Maksimovic *et al*., 2004).

Medicinally, corn silk has been documented to possess the following activities: antioxidant (Eman, 2011; Ebrahimzadeh *et al*., 2008; Liu *et al*., 2011), antiprostatitis (Buhner, 2007), anti-diabetic (Guo *et al*. 2009), antiplasmodial (Tordzagla *et al*., 2022), antitumor (Habtemariam, 1998) and antiproliferative activities (Abirami *et al*., 2021). Moreover, corn silk has been reported for treating infection and cystitis (Steenkamp, 2003). In addition, it has been reported as a drug for treating kidney stones and other renal illnesses (Ribeiro *et al*., 1988; Tahraoui *et al*., 2007). Furthermore, hepatitis, tumor, hyperglycemia, and hypertension are treated traditionally using dry corn silk in China and some African countries (Li *et al*., 1995; Lukitaningtyas *et al*., 2020; Ma and Gao, 1998). It also possesses diuretic activity and immune-enhancing effects (Tang & Ding, 1995). Clinically, it is very potent against cystitis, prostatitis, urethritis, nephritis, and urinary infections (Yesilada *et al*., 1995; Grases *et al*., 1993). The medicinal properties of plants reside in the natural bioactive constituents in the plants. Natural bioactive constituents (phytochemicals) found in plants help to sustain plant life by acting as defense systems against diseases (Krishnaiah *et al*., 2007). These phytochemicals are namely primary and secondary phyto-constituents. Primary phyto-constituents are amino acids, proteins, sugars, and chlorophyll while examples of secondary ones are flavonoids, alkaloids, and terpenoids (Krishnaiah *et al*., 2007). Pharmacologically, flavonoids have antioxidant properties (Hollman, 2001) and a wide spectrum of antimicrobial activities, (Hanasaki *et al*., 1994). Isolated alkaloid compounds have *in vitro* and *in vivo* antiplasmodial activities (Nogueira & Lopes, 2011) while terpenoids are used to treat *Candida albicans* infection (Zore *et al*., 2011).

The silk of *Zea mays* was chosen for this study because it has been used extensively in traditional medicine and it is readily available. However, there is scanty literature on the bioactive compounds responsible for their reported medicinal activities, hence, the need for a phytochemical investigation of corn silk, also to screen the isolated compounds for antimicrobial activity.

2. MATERIAL and METHODS

2.1. Instruments/Equipment

The following equipment was employed; Rotavapor (Buchi, R-210) with V-710V(vacuum pump); water bath (Buchi, B-491); melting point apparatus (R000105350) from Stuart, UK/; balance Kern Sohn GmbH capacity =1000 g, D-72336 (Balingen, Germany). Jeol ECA, 500 MHz FT NMR spectrometer(NM/103508-10), Japan incorporating a NM50TH5T/FG2 probe; PerkinElmer ATR-FTIR spectrometer(A/0626/15) Waltham, USA; gravity column L 25 mm x 51 cm, Sigma-Aldrich, Merck Germany; pre-coated TLC plate (Macherey-Nagel, Germany)

2.2. Chemicals

The chemicals used included the following: methanol (BDH Chemical, UK), ethanol (Fischer Scientific, UK), chloroform (BDH Chemicals, UK), ethyl acetate (BDH Chemicals, UK), acetic acid (Needham Market Suffolk, UK), petroleum ether (VWR Chemicals, U.S.A.), hexane (BDH Chemicals, UK) and silica gel 70:230 mesh size (Merck, Germany).

2.3. Collection and Identification of Plant Material

Stigmas of *Zea mays* (corn silk) were collected at Ejisu in the Ashanti Region of Ghana in August 2016 then identified and authenticated by Mr. Clifford Osafo Asare, the horticulturist with the Department of Herbal Medicine, KNUST and a specimen was placed in the herbarium of the Department with a voucher number KNUST/HM/2016/001.

2.4. Extraction of Plant Sample

The dry corn silk was powdered by an electronic grinding mill and was extracted using methanol (CH_3OH). A 1.14 kg of the milled sample was cold macerated with 3.6 L of CH_3OH

for 3 days. After 72 hours, a whatman filter paper was used to separate the residue from the filtrate. The filtrate was concentrated to dryness using rotavapor and water bath at 40 ^{o}C.

Next, 10 g of the dry extract was first dissolved in 300 mL of distilled water, then poured into a separatory funnel and partitioned successively with 300 mL x 3 volumes of chloroform and ethyl acetate, respectively. The two organic fractions were also concentrated to dryness using the rotavapor. A freeze dryer was, however, used to dry the aqueous fraction. The concentrated chloroform fraction was used for the isolation of phytochemical constituents. Before the fractionation, phytochemical screening was done on the methanolic extract.

2.5. Phytochemical Screening

The methanolic extract was screened for possible phytochemicals by employing standard methods (Sofowora, 1993; Evans, 2002; Harborne, 1998).

2.5.1. *Test for flavonoids*

A preliminary test was done using a strip of filter paper. The paper was dipped in the liquid extract, subsequently dried, and exposed to a 2 M ammonia solution. A deep yellow color was formed, and the color disappeared after the filter paper was exposed to fumes of concentrated HCl, showing the presence of flavonoids.

For the confirmatory test, 2 mL of ethanol was used to dissolve about 0.4 g of the extract and 5 drops of concentrated HCl and magnesium turnings were added. A pink color developed, which confirmed the presence of flavonoids (Evans, 2002).

2.5.2. *Test for alkaloids*

The extract was treated with ammoniacal alcohol (ammonia: 95 % ethanol in the ratio of 1: 9 respectively) and filtered. The filtrate was evaporated, and 1% sulphuric acid was added to the residue to convert alkaloids to soluble salt forms. After filtration, the resulting solution was made alkaline with dilute ammonia partitioned in a separating funnel with chloroform, and then shaken for a few minutes. The chloroform layer was separated, and the filtrate evaporated, followed by the addition of 1% H_2SO_4 to the respective residue. Dragendroff's and Mayer's tests were carried out on the acidified residue (Evans, 2002).

2.5.2.1. Dragendroff's test. A solution of potassium bismuth iodide was added to the filtrate. The formation of red precipitate indicated the presence of alkaloids (Evans, 2002).

2.5.2.2. Mayer's test. The filtrate was treated with a solution of potassium mercuric iodide. The presence of alkaloids was confirmed by the appearance of a cream precipitate (Evans, 2002).

2.5.3. *Test for saponins*

About 2 mg of the extract was dissolved in 2 mL of distilled water and the mixture was shaken. Formation of a foam column not less than 1cm in height that persisted for at least 15 min, confirmed the presence of saponins (Evans, 2002).

2.5.4. *Tannins*

About 5 drops of iron (III) chloride ($FeCl_3$) solution were added to 2 mL of the extract. The appearance of blue-black color indicated the presence of tannins (Evans, 2002).

2.5.5. *Test for reducing sugars*

The methanolic extract was hydrolyzed with dilute HCl and then treated with 20 % NaOH. Finally heated with Fehling's solutions A and B. The appearance of brick-red precipitate confirmed the presence of reducing sugars (Sofowora, 1993; Evans, 2002).

2.5.6. *Test for coumarins*

The extract was first treated with chloroform and filtered. The filtrate was evaporated to dryness and hot distilled water was used to dissolve the residue and allowed to cool. The resulting solution was treated with 0.8 mL of 10 % ammonia and observed under UV light at 365 nm. The formation of a deep blue-green fluorescence under UV light indicated the presence of coumarins (Sofowora, 1993; Evans, 2002).

2.5.7. *Test for triterpenes/ terpenoids (Salkowski's test)*

A sample was extracted with chloroform and filtered. About 5 drops of concentrated H_2SO_4 were added to the filtrate and mixed thoroughly after shaking for a few minutes and allowed to stand. A brownish-red coloration showed the presence of triterpenes or terpenoids (Sofowora, 1993; Evans, 2002).

2.5.8. *Test for sterols (Libermann Burchard's test)*

Chloroform was added to a sample and filtered. The filtrate was then treated with about six drops of $(CH_3CO)_2O$ and boiled, then allowed to cool. A few drops of concentrated H_2SO_4 were then added and reacted to form a bluish-green coloration at the interface, which confirmed the formation of a steroidal ring (Evans, 2002; Harborne, 1998).

2.5.9. *Cyanogenetic glycosides*

About 0.2 g of the extract was placed in a dry test tube and a strip of sodium picrate paper was suspended using a cork in the neck of the test tube. The test tube and its contents were heated in a water bath. The color of the test paper changed to brownish red to indicate the presence of cyanogenetic glycosides because of the release of hydrocyanic acid (Sofowora, 1993; Evans, 2002).

2.5.10. *Cardiac glycosides*

A sample of the extract was treated with 70 % alcohol and filtered. About 10 mL of alcoholic filtrate was added to 2 mL of anhydrous CH_3COOH with 5 drops of iron (III) chloride solution. Concentrated H_2SO_4 was gently added by pouring it down the sides of the test tube using a dropping pipette. A reddish-brown coloration at the interface because of the presence of aglycone was observed. Hence, the presence of cardiac glycoside was confirmed (Evans, 2002).

2.6. Isolation of Compounds from Chloroform Fraction of the Methanol Extract of Corn Silk

The dry chloroform fraction of mass 10.0 g was dissolved in about 30 mL of chloroform with a small amount of the silica gel gradually added and stirred continuously to dryness. The homogeneous mixture was loaded onto the packed column ensuring uniform packing by gently tapping the side of the column. To prevent the mobile phase from disturbing the surface during solvent addition, a wad of cotton was placed on top of the packed sample in the column. The gradient elution was employed in developing the column using petroleum ether, ethyl acetate, and methanol. Elution was first started with 100 % petroleum ether. This was followed by petroleum ether/ethyl acetate, 100 % ethyl acetate, and ethyl acetate/ methanol. Various sub-fractions were collected and sub-fractions with similar TLC profiles were bulked and concentrated. A total of four distinct sub-fractions (C1-C4) were obtained.

Fraction C2 was eluted with petroleum ether /ethyl acetate (95:5) and precipitated as white-to-cream crystals after decanting. The solid obtained was thoroughly washed with 100 % petroleum ether to give colorless crystals labeled as compound **1** (38 mg).

Fraction C3 was obtained by eluting the column with petroleum ether /ethyl acetate solvent system (85:15) and precipitated as a colorless powdered solid after decanting off the green mother liquor. The colorless powder was thoroughly washed with pure petroleum ether, followed by 100 % methanol to obtain compound **2** (87 mg).

2.7. Identification and Structural Elucidation of the Isolates

The purity and identity of the isolates were confirmed using melting point determination, qualitative test, infrared spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectroscopy.

2.8. Melting Point Determination

Melting point data were obtained using a melting point apparatus with a capillary tube. The melting point range of the isolated compound was read as degrees centigrade (^{0}C). The reading was done thrice.

2.9. Chemical Tests

Investigations such as tests for alcohol and steroid were carried out on compound **2**.

2.9.1. *Test for alcohol*

About 2 g of $(NH_4)_2Ce(NO_3)_6$ was dissolved in a boiling tube containing 5 mL of 2 M nitric acid and then heated gently. A small quantity, about 5 mg of isolated compound **2**, was then dissolved in 1 mL of 1,4-dioxane. The resulting solution was added to 1 mL of $(NH_4)_2Ce(NO_3)_6$ and swirled. A yellow to red color developed indicating the presence of an alcohol functional group (Harborne, 1998).

2.9.2. *Test for steroid (Salkowski reaction)*

A little quantity of about 7 mg of the isolated compound **2** was dissolved in $CHCl_3$ and five drops of concentrated tetraoxosulphate (Vl) acid were added to the resulting solution and a reddish color was formed in the upper $CHCl_3$ layer (Evans, 2002).

2.9.3. *Liebermann-Burchard reaction*

A small quantity of about 7 mg of compound **2** was dissolved in 5 mL of $CHCl_3$ and about five drops of concentrated H_2SO_4 were added to it. Also, about three drops of $(CH_3CO)_2O$ were added. The steroidal ring formation was confirmed as a result of the development of a bluish-green coloration at the interface (Evans, 2002).

2.10. Infrared Spectroscopy

A Perkin-Elmer Fourier Transform Infrared (FTIR) Spectrophotometer (Waltham, USA/A/0626/15) which utilizes attenuated total reflectance with internal calibration was used in the infrared spectrophotometric analysis. About 2 mg of each isolate was put on top of the diamond crystal on the attenuated total reflectance plate and the probe was used to compress it. The infrared (IR) was measured over the wavelength range of 4000 to 400 cm^{-1}. It facilitated the identification of the functional groups in the isolated compounds.

2.11. Nuclear Magnetic Resonance (NMR) Spectroscopy

About 10 mg of each isolate was used in the NMR analysis for 1D and 2D spectra. The samples were dissolved in deuterated chloroform ($CDCl_3$) in a sample vial. The sample was vortexed and filtered into the NMR spectrophotometer tube and ran. Tetramethylsilane (TMS) was used as the internal standard. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively.

2.12. Test Organisms for Antibacterial and Antifungal Assays

The organisms for the assays were obtained from the Department of Biological Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. They included Gram-negative, Gram-positive, and fungal strains.

2.13. Inoculum Preparation of Test Microorganisms

The test bacteria and fungi strains were streaked on 20 mL sterile nutrient agar and Sabouraud dextrose agar plates, respectively. They were incubated at a temperature of 37 ^{o}C and their colonies were fished and suspended in sterile water of 10 mL in test tubes. The turbidity was

compared to 1.0 × 1 10^8 cells/mL, which is the same as 0.5 McFarland standard, and read with the eye.

2.14. Minimum Inhibitory Concentration (MIC) Determination for the Isolates

Minimum inhibitory concentrations of the test compounds were determined by the microdilution method using the method described by Andrews, (2001). Different concentrations of the test solutions of the compounds were prepared ranging from 12.5 to 400 µg/mL. The double strength-nutrient broth of 125 µL was used to fill 96-well (micro-titre plates). A microbial inoculum size of 25 µL was standardized with 0.5 McFarland turbidity solution added to each well. The antimicrobial activity of the standard drugs, as well as the test compounds, was determined against test organisms after incubating at 37 °C. After incubation for 24 h, the minimum inhibitory concentration was determined. The lowest concentration that inhibited the growth of microbes was observed after the addition of 20 µL of 1.25 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to the medium and then incubating for 30 min, at 37 °C (Andrews, 2001). Ciprofloxacin and clotrimazole were used as the standard or reference drugs for bacteria and fungi, respectively and concentrations ranging from 0.625 to 20.0 µg/mL were used. The results were validated by performing all the tests in triplicates.

3. RESULTS and DISCUSSION

3.1 Phytochemical Screening

The summary of the results from phytochemical screening of methanolic extract from corn silk is presented in Table 1.

Table 1. Phytochemical constituents of corn silk.

Test	Methanolic extract
Flavonoids	+
Alkaloids	
a. Dragendroff's test	+
b. Mayer's test	+
Tannins/phenolic compounds	+
Reducing sugar	+
Coumarins	+
Triterpenes/terpenoids	+
Sterols	+
Cyanogenetic glycosides	-
Cardiac glycosides	+

Key: (+) present and (-) absent

The phytochemical screening of the methanolic extract from corn silk showed the presence of flavonoids, alkaloids, coumarins, tannins, and triterpenes in the extract. Other phytochemicals detected were reducing sugars, sterols and cardiac glycosides. Many studies have established that thousands of isolated secondary plant metabolites possess excellent physiological properties, hence, they have been used as medicines (Bibiso & Anza, 2022; Iwu, 2014; Newman, 2008; Oluduro, 2012). The presence of these phytochemical constituents in corn silk may be responsible for the acclaimed traditional medicinal uses of this part of the plant.

3.2 Phytochemical Investigations of the Isolated Compounds

3.2.1 *Characterization of isolated compound 1*

Compound **1** was isolated as a colorless crystal with a melting point of 78-80 °C and R_f value of 0.32 (Pet. Ether/EtOAc: 9:1). The FTIR spectrum of the isolate showed a very strong peak at 1731 cm^{-1} indicating the presence of C=O (Figure 1) and quite intense bands at 1122 cm^{-1}

and 647 cm^{-1}, were attributed to hydroxyl group (OH), even though the normal broad band around 3200 to 3600 cm^{-1} was missing and this could mean that the (OH) was not free. The very strong peak at 2922 cm^{-1} was attributed to the stretching and bending vibrations of methyl groups. Bands at 2853 cm^{-1} and 1463 cm^{-1} were the vibrations of methylene groups. Also, a peak at 1217 cm^{-1} showed the presence of C-O functional group. In addition, a band at 1072 cm^{-1} represented C-C vibration. The assignments were in exact agreement with those values reported in the literature (Cruz-Castaneda *et al*., 2018).

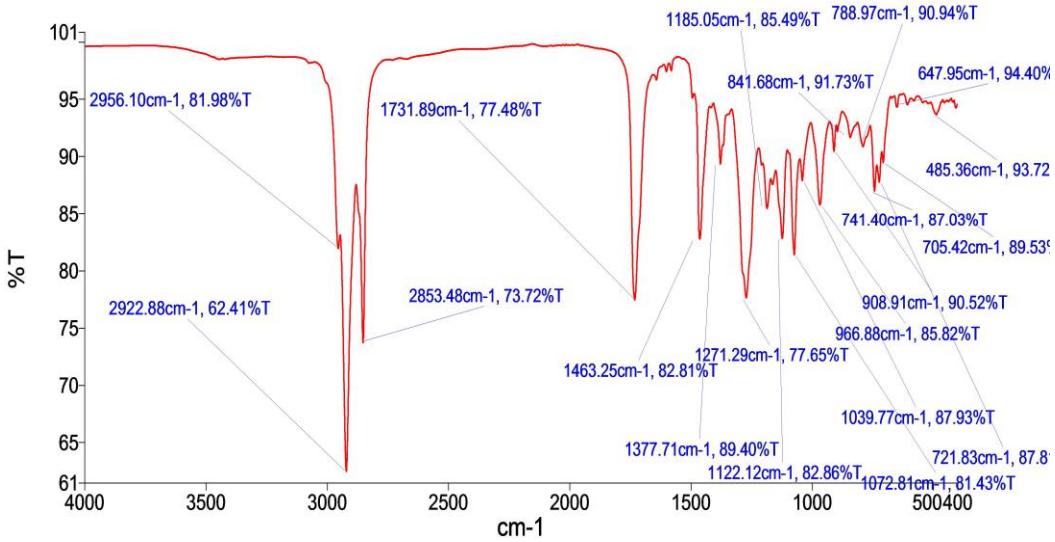

Figure 1. IR spectrum of compound **1** (behenic acid).

The proton NMR spectrum of saturated fatty acids has four distinct signals corresponding to protons on α-CH_2, β-CH_2, ω-CH_3 and the rest are the overlapping methylene (-CH_2) groups (Pietro *et al*., 2020). Signals at δ_H 2.26, 1.57, 0.84 and 1.21 are indicative of protons on α-CH_2, β-CH_2, ω-CH_3 and the rest of the methylene -CH_2 groups, respectively and these chemical shift values were compared with reported literature values for saturated fatty acid (Magritek, 2018). For ^{13}C NMR spectrum, the prominent signals at δ_C 176.7, 34.0 and 24.8 could be assigned to C-1, C-2 and C-3, respectively. Moreover, δc values at 31.8, 22.6 and 14.0 indicated the presence of C-20, C-21 and C-22, respectively. The carbonyl carbon atoms of the free fatty acids have distinct chemical shifts of 175.0-185.0 ppm. The rest of the carbon atoms which are sixteen methylene CH_2 groups appear in similar chemical environments with their chemical shift values close to one another and could be designated to the signals between δ_C 28 and 30 (Pietro *et al*., 2020). Based on detailed analysis of the 1D and 2D NMR spectra in Figures 2, 3, 4, 5, 6, and 7. These spectra were compared to reported data (Pietro *et al*., 2020; Magritek, 2018) and the structure of compound **1** was elucidated as behenic acid (Figure 8).

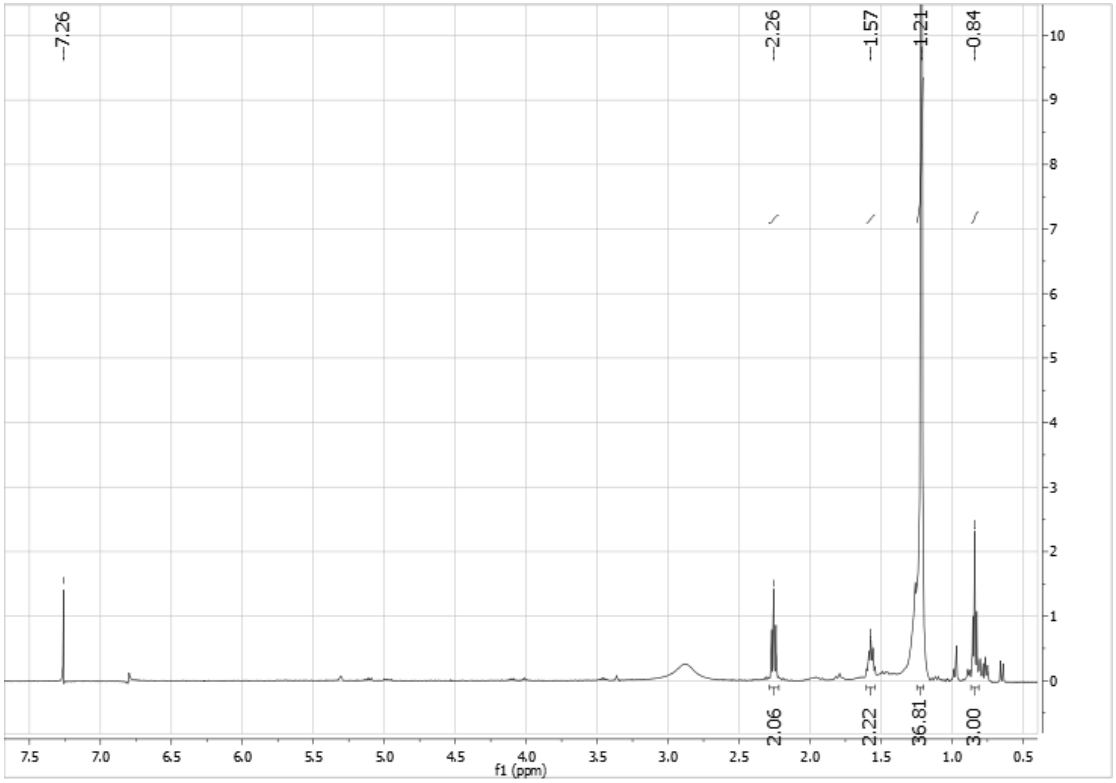

Figure 2. ^{1}H NMR spectrum of compound **1** (behenic acid) in $CDCI_3$ at 500 MHz.

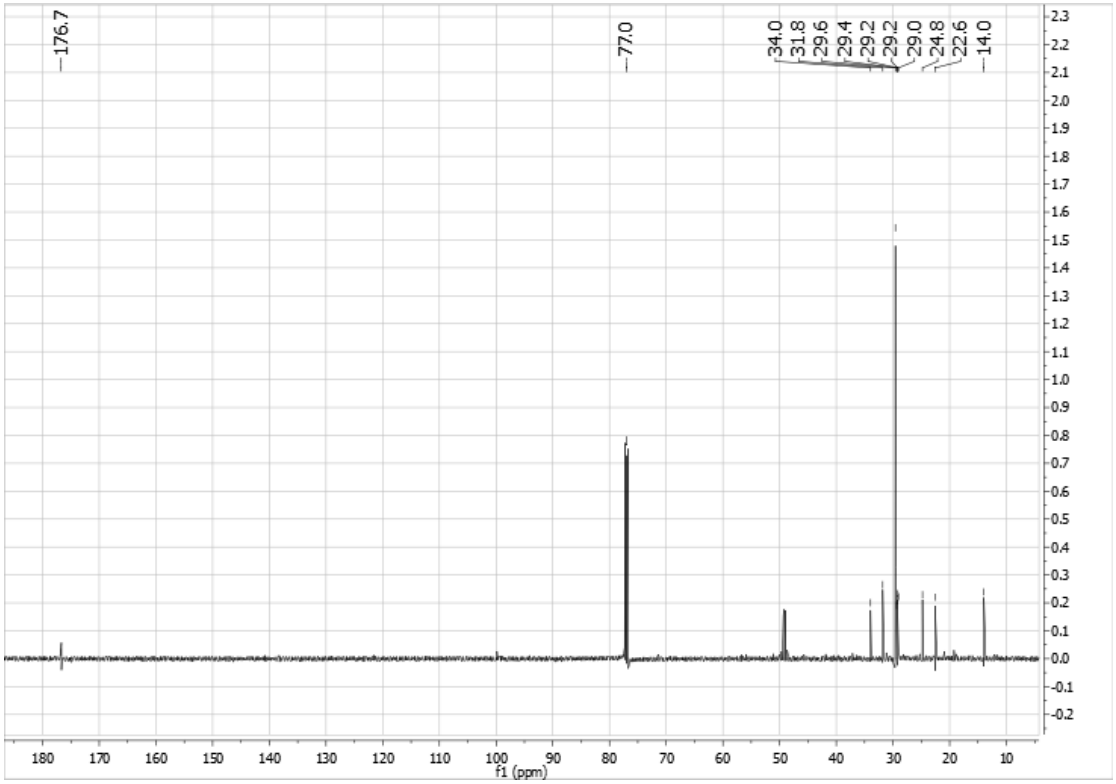

Figure 3. ^{13}C NMR spectrum of compound**1** (behenic acid) in $CDCI_3$ at 125 MHz.

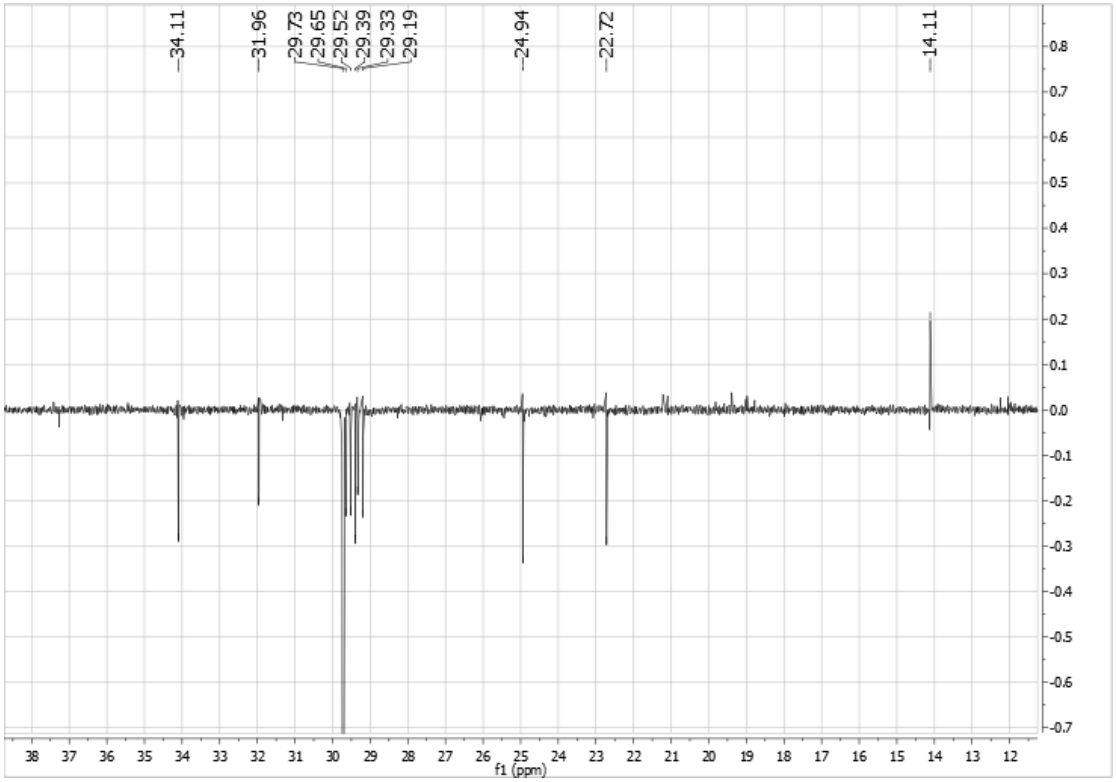

Figure 4. DEPT-135 spectrum of compound**1** (behenic acid) in $CDCI_3$ at 500 MHz.

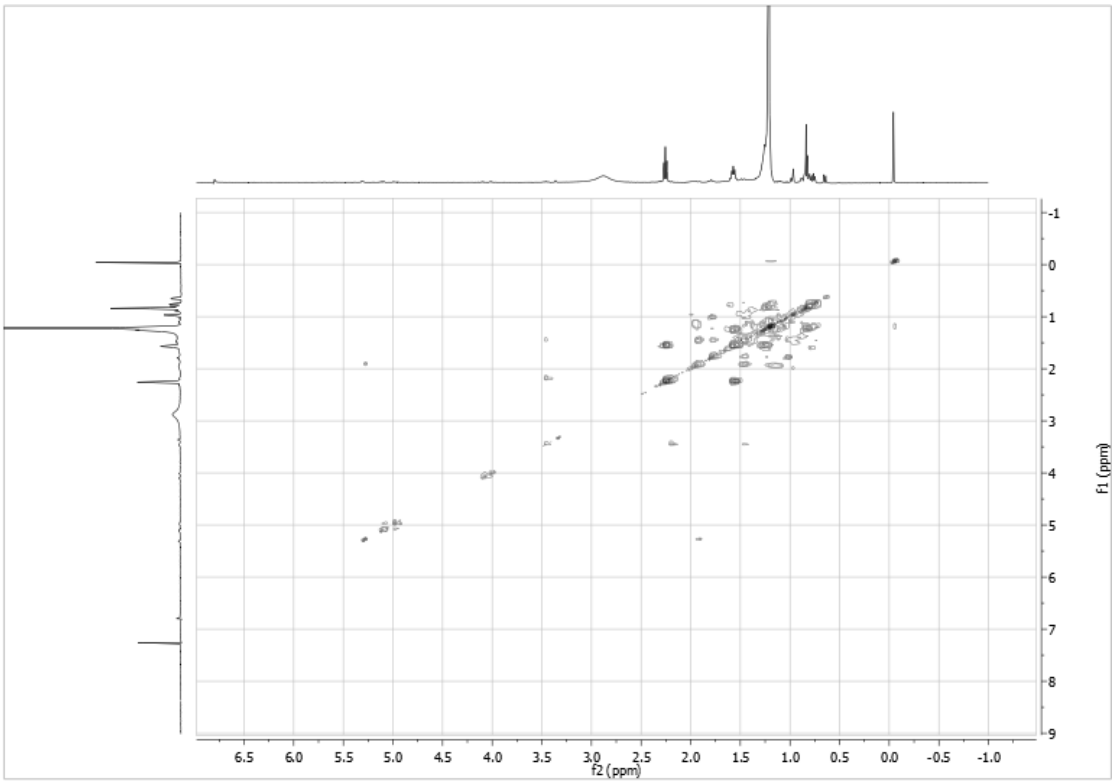

Figure 5. COSY spectrum of compound**1** (behenic acid) in $CDCI_3$ at 500 MHz.

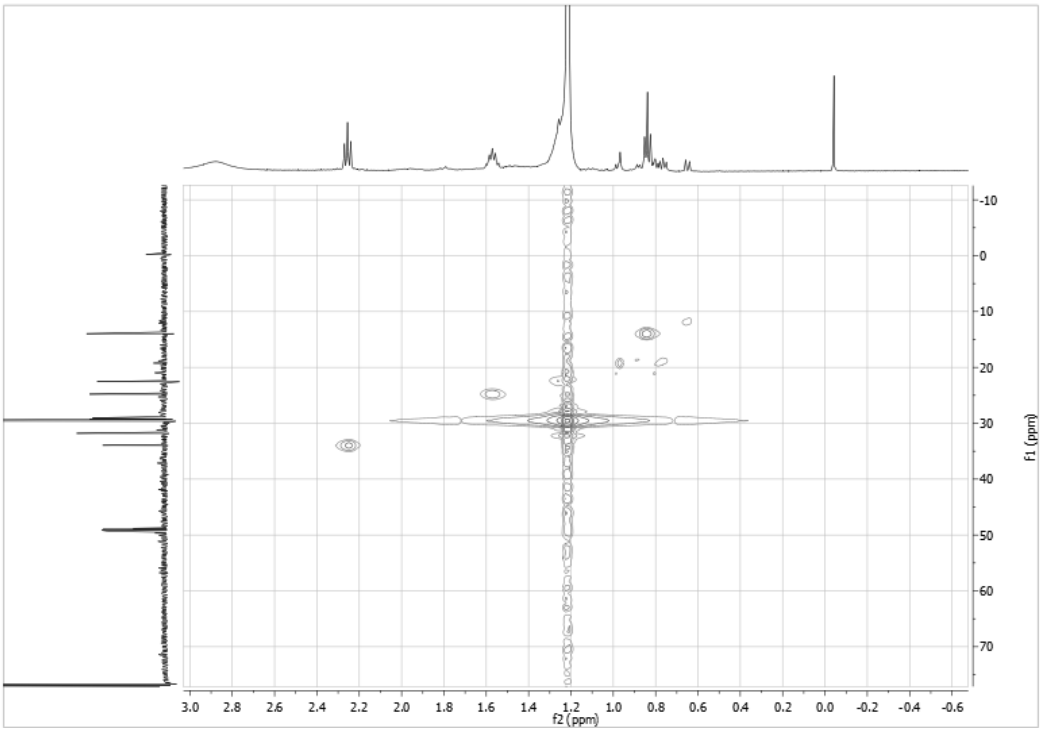

Figure 6. HSQC spectrum of compound**1** (behenic acid) in $CDCI_3$ at 500 MHz.

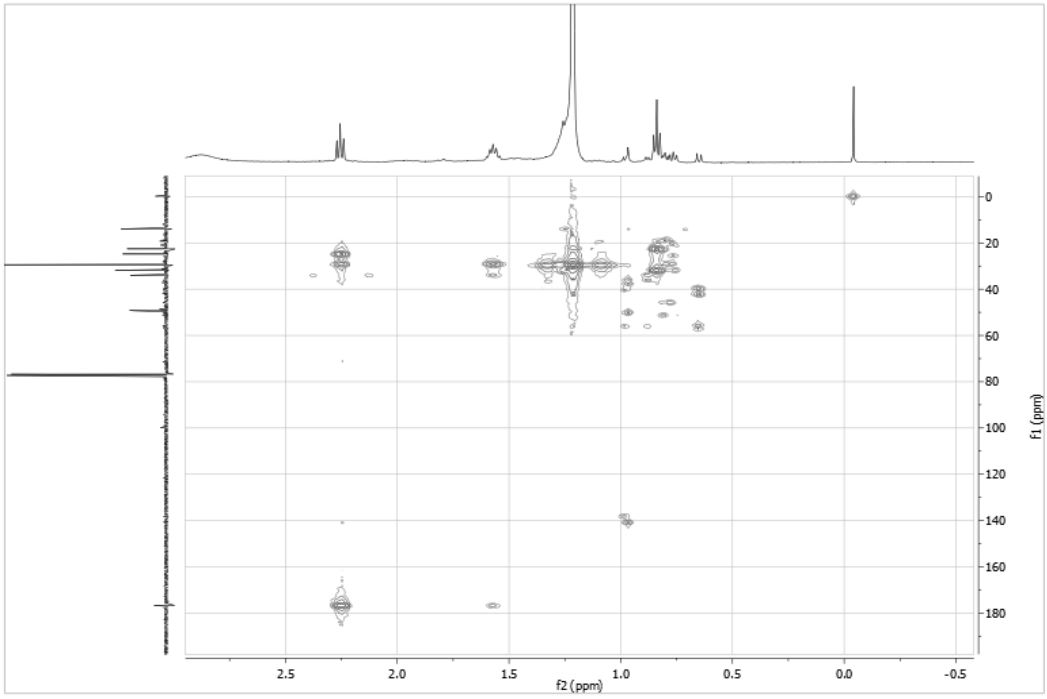

Figure 7. HMBC spectrum of compound**1** (behenic acid) in $CDCI_3$ at 500 MHz.

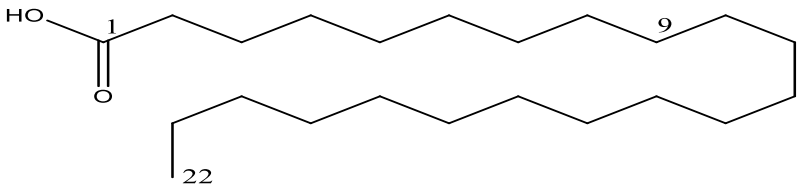

Figure 8. Chemical structure of behenic acid.

3.2.2. *Characterization of compound 2*

Compound **2** was isolated as a colorless powder, and it gave positive tests for steroids and alcohol. The melting point was determined to be 169-171 ^{0}C and the R_f value of 0.52 (Pet. Ether/EtOAc: 6:2). The FTIR spectrum of the compound depicted a broad band at 3361 cm^{-1} and quite intense bands at 1192 and 626 cm^{-1} were ascribed to hydroxyl functional group. A weak band at 1702 cm^{-1} was attributed to C=C stretch of alkenes. This particular band is different from C=O band, in that the carbonyl band has a very intense peak. The out-of-plane C-H vibration of the unsaturated portion was shown at 838 cm^{-1}. The strong band at 2933 cm^{-1} was ascribed to stretching and bending vibrations of the methyl groups. Bands at 2849 cm^{-1} and 1463 were the vibrations of the methylene groups. Also, the vibrational carbon-to-carbon sigma bond was recorded at 1042 cm^{-1}.

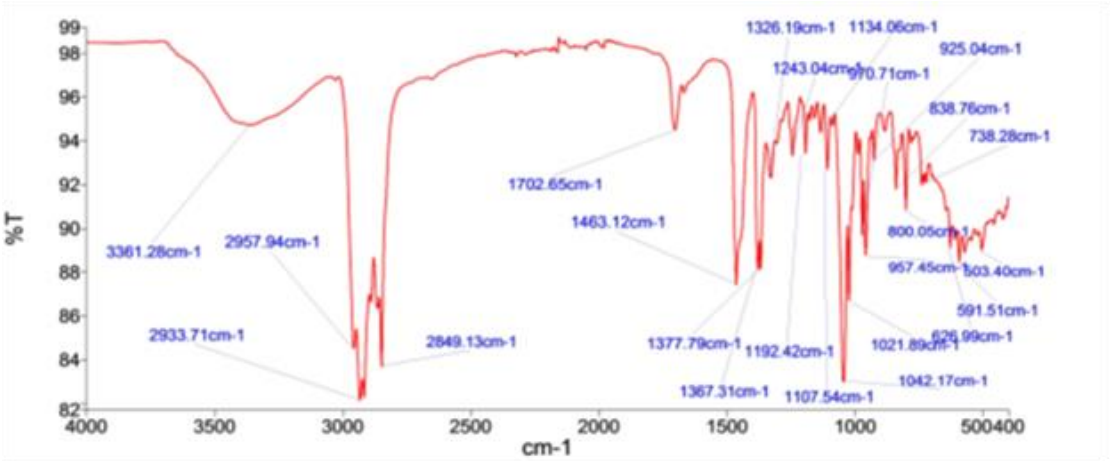

Figure 9. IR spectrum of compound **2** (stigmasterol).

The ^{1}H NMR spectrum ($CDCI_3$, 500 MHz) signals at δ_H 0.69, 0.72, 0.80, 0.85, 1.00, and 1.15 indicated the presence of protons of six -CH_3 groups. Also, the signal at δ_H 3.51 was indicative of a hydroxyl (-OH) group attached to carbon while chemical shift values at δ_H 5.04, 5.14, and 5.34 confirmed the presence of olefinic protons. The NMR spectrum for carbon-13 gave 29 signals including three quaternary carbons. Information from DEPT-135 was useful in distinguishing between carbon-13 signals. DEPT-135 gave twenty-six signals and could be assigned to eleven methine, nine methylene, and six methyl groups. Prominent among carbon-13 NMR signals included δc values at 140.8 and 121.6; 138.4 and 129.4 that could be designated to the double bonds of C-5 and C-6; C-22 and C-23 respectively. In addition, the melting point recorded for compound **2** was compared with the literature values of 169-171 ^{0}C (Woldeyes *et al*., 2012). The ^{1}H NMR and ^{13}C NMR values were designated as a result of HSQC, HMBC, and COSY spectra interpretations (Figures 10, 11, 12, 13, 14, and 15). Figure 16 shows COSY and HMBC correlations of stigmasterol. Compound **2** was identical to stigmasterol (Figure 17).

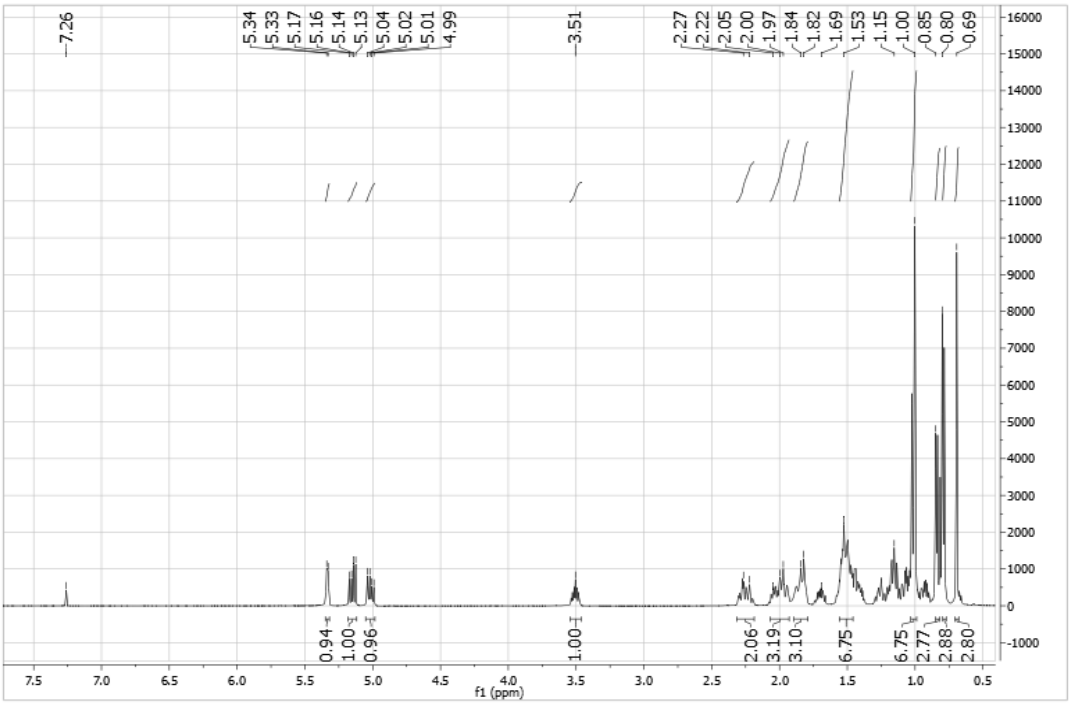

Figure 10. ^{1}H NMR spectrum of compound **2** (stigmasterol) in $CDCI_3$ at 500 MHz.

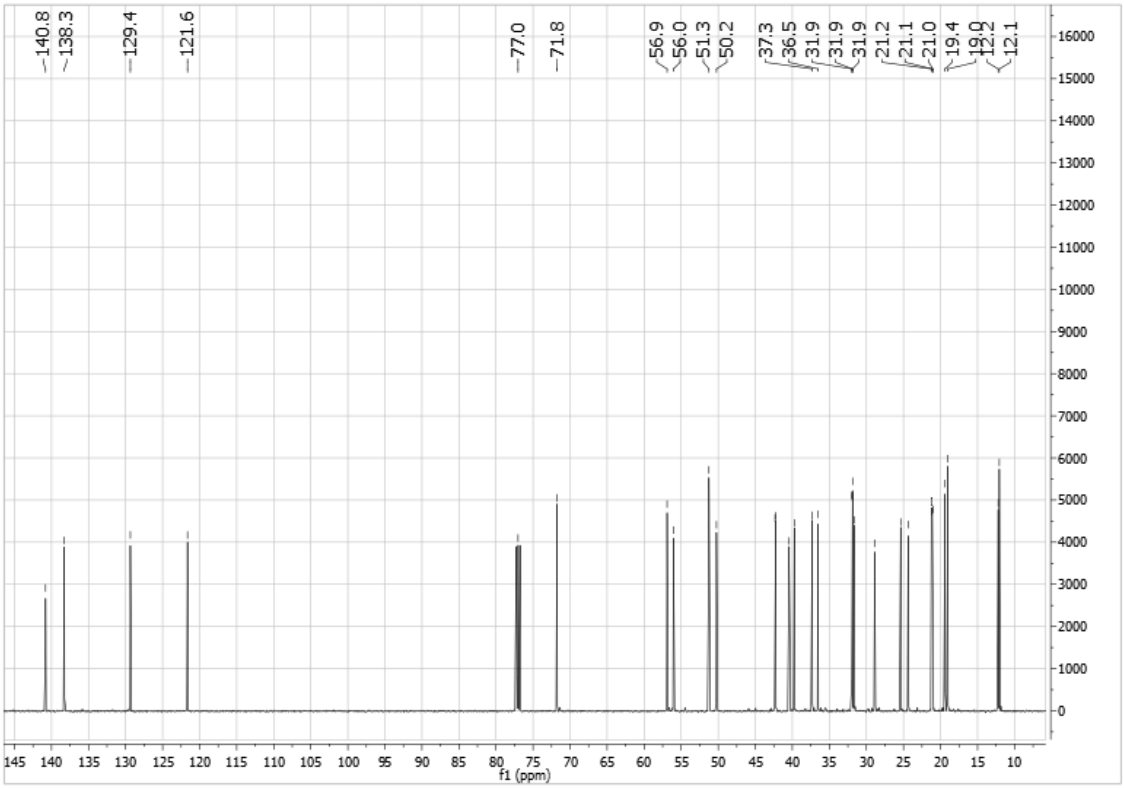

Figure 11. ^{13}C NMR spectrum of compound **2** (stigmasterol) in $CDCI_3$ at 125 MHz.

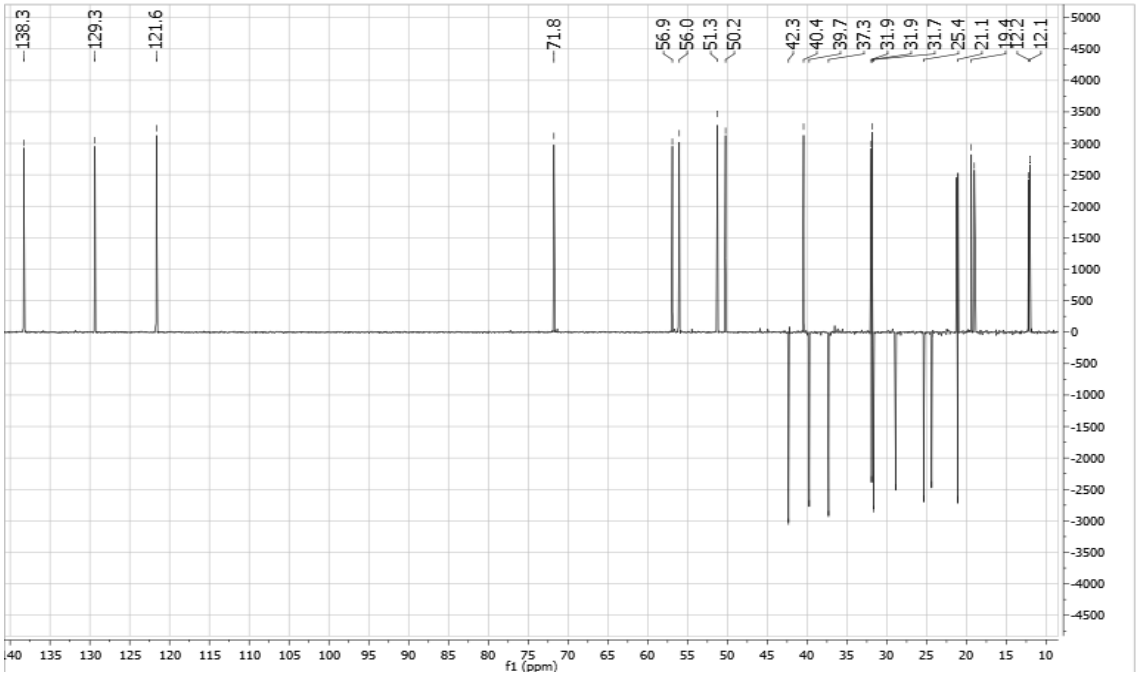

Figure 12. DEPT-135 spectrum of compound **2** (stigmasterol) in $CDCI_3$ at 500 MHz.

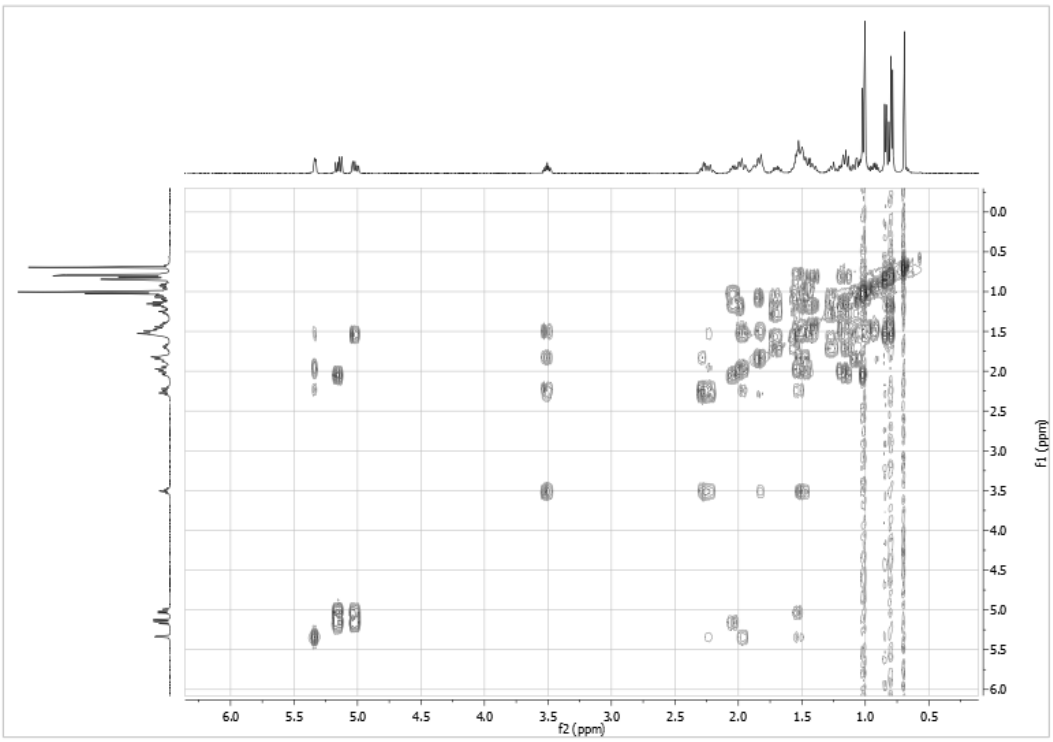

Figure 13. COSY spectrum of compound **2** (stigmasterol) in $CDCI_3$ at 500 MHz.

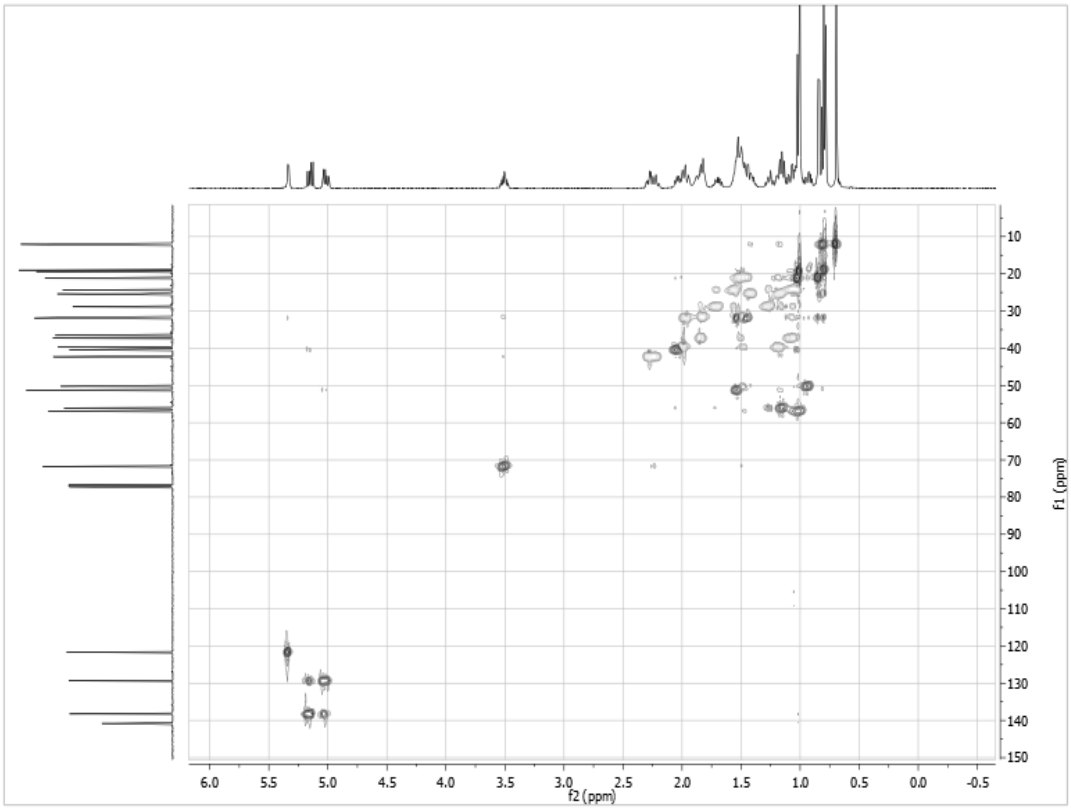

Figure 14. HSQC spectrum of compound **2** (stigmasterol) in $CDCI_3$ at 500 MHz.

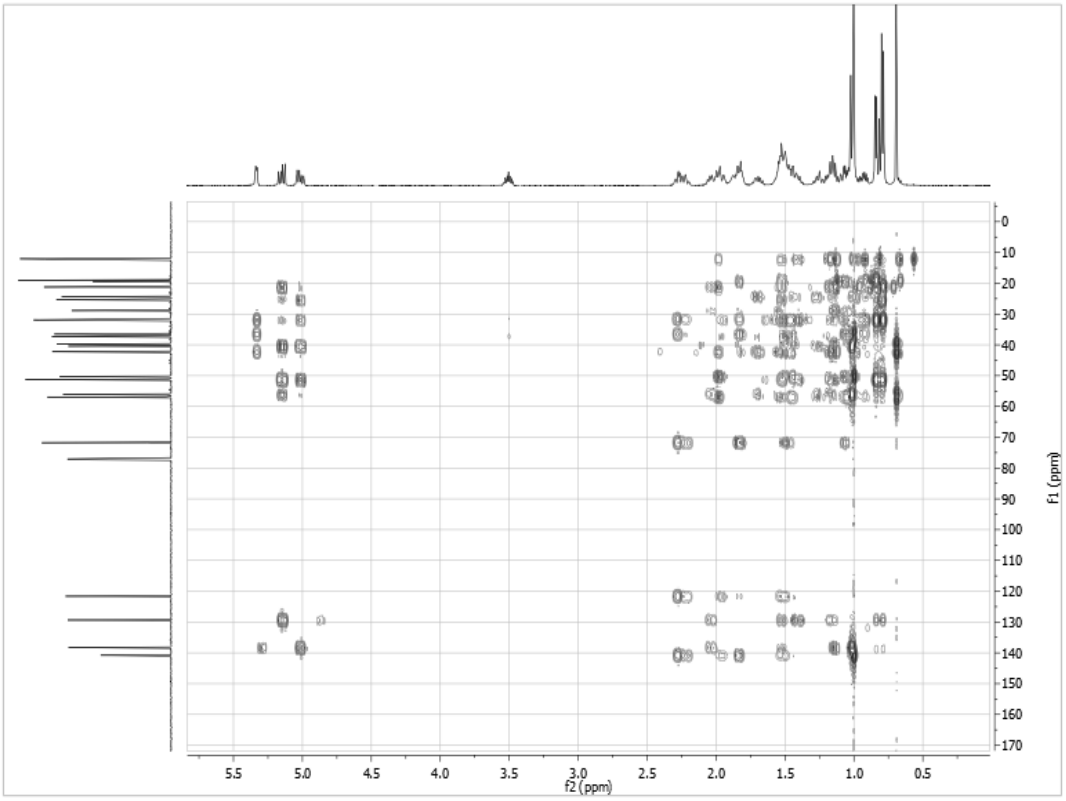

Figure 15. HMBC spectrum of compound **2** (stigmasterol) in $CDCI_3$ at 500 MHz.

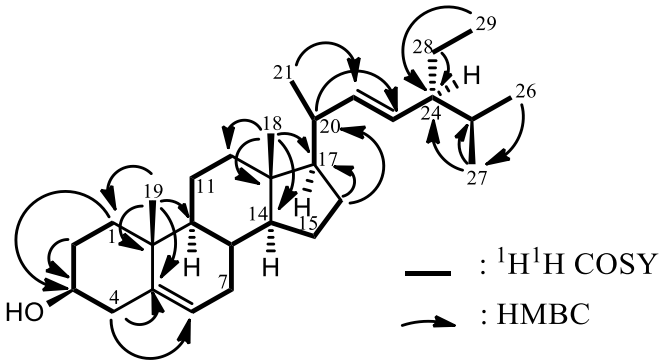

Figure 16. COSY and HMBC correlations of stigmasterol

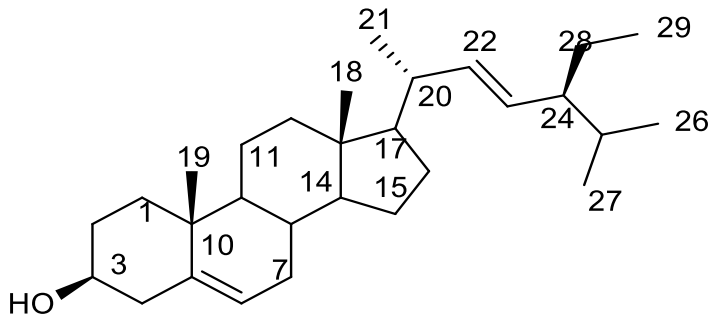

Figure 17. Chemical structure of stigmasterol.

Table 2. Minimum inhibitory concentrations of behenic acid, stigmasterol and standard drugs on the test microbes.

Compound/ Drug	Minimum inhibitory concentrations (MIC) values (µg/mL)					
	Sa	*Ec*	*Kp*	*Pa*	*Ca*	*Tc*
Behenic acid	100.00	>400.00	25.00	200.00	>400.00	25.00
Stigmasterol	25.00	200.00	25.00	200.00	25.00	25.00
Ciprofloxacin	1.25	1.25	1.25	2.50	NA	NA
Clotrimazole	NA	NA	NA	NA	1.25	1.25

The results are by means of triplicate experiments. Ec=*Escherichia coli* (ATCC 25922), Pa=*Pseudomonas aeruginosa* (ATCC 27853), Kp= *Klebsiella pneumonia* (700603), Sa= *Staphylococcus aureus* (ATCC 25923), Ca= *Candida albicans* (10031), Tc= *Tinea corporis.* NA= Not applicable.

Results of behenic acid revealed clear differences in susceptibility with respect to *Candida albicans and Staphylococcus aureus.* The mean minimum inhibitory concentration of behenic acid against *Staphylococcus aureus was* 100 µg/mL with no effect on *Candida albicans*. However, its fungus counterpart *Tinea corporis* was susceptible at 25 µg/mL, which was similar to s *Klebsiella pneumoniae* at the same concentration. However, the compound exhibited low activity against *Pseudomonas aeruginosa* at a relatively high concentration of 200 µg/mL. Also, there was no activity against Gram negative, *Escherichia coli* and a fungal strain *Candida albicans* even at a very high concentration of 400 µg/mL, hence the most resistant in this trend against behenic acid.

From Table 2, *Klebsiella pneumoniae, Staphylococcus aureus, Candida albicans,* and *Tinea corporis* were the most susceptible to stigmasterol with MIC of 25 µg/mL. *Pseudomonas aeruginosa and Escherichia coli* were only sensitive at very high concentrations with a minimum inhibitory concentration of 200 µg/mL, which is eight times, that of the sensitive

organism; revealing the inherent resistance of the Gram negatives to most antimicrobial agents. This scientific research has confirmed the antimicrobial activity of stigmasterol as reported by Gbedema, (2014) that a phytosteroid isolated and determined to be stigmasterol was inactive against *Pseudomonas aeruginosa* but demonstrated moderate antibacterial properties against *Staphylococcus aureus*. Moreover, isolated stigmasterol from *Sida rhombifolia* was active against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* (Woldeyes *et al*., 2012).

Overall, the most resistant organism across all isolates was *Escherichia coli* with MIC range of 200-400 µg/mL. The most susceptible organisms across the isolates were *Klebsiella pneumoneae* and *Tinea corporis* with MIC of 25 µg/mL. Stigmasterol was the active compound against the most resistant *Escherichia coli* with MIC of 200 µg/mL. Also, stigmasterol was very active against *Staphylococcus aureus* and *Candida albicans* at 25 µg/mL.

4. CONCLUSION

This study has shown that the methanolic extract of the silk of *Zea mays* (corn silk) possesses plant secondary metabolites such as alkaloids, flavonoids, coumarins, cardiac glycosides, tannins, saponins, reducing sugars, terpenoids, and sterols. Further phytochemical investigation of the chloroform fraction of the methanolic extract, led to the isolation of behenic acid and stigmasterol. In addition, it is the first report of behenic acid being isolated from corn silk.

Screening the two isolated compounds against the selected microbes, revealed that the most resistant microbe across all isolates was *Escherichia coli* and the most susceptible organisms across the isolates were *Klebsiella pneumoneae* and *Tinea corporis*. The two compounds demonstrated varying degrees of antimicrobial activity and therefore could be useful as leads for future structure optimization towards development for the treatment of some infectious diseases.

Data Availability

The raw data from the experimental study are available with the corresponding author and are available upon request. Previous reports that were used to support this research work have been cited correctly in the text.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Nestor Tordzagla and **Isaac Ayensu** participated in the experimental work, preparation of the manuscript, data analysis, and proofreading; **James Oppong-Kyekyeku** took part in the data analysis and proofreading.

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