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Isolation, Characterization and Antimicrobial Activities of Leaf and Stem extracts of *Guiera Seneglensis*

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Abstract
Guiera senegalensis is a medicinal plant used in Northern Nigeria for the treatment of malaria, acute gastroenteritis, leprosy and dysentery. The powdered leaves and stem was soaked in methanol for 72 hours with constant stirring. This was fractionated using modified kupchan method to obtain various fractions. Preliminary phytochemical screening of the fractions was carried out. The antimicrobial activity of the n-hexane, ethyl acetate and methanol fractions were investigated in vitro at various concentrations using agar well diffusion method. Preliminary phytochemical result for leaves and stem revealed the presence of tannins, terpenoids, flavonoids, alkaloids, anthraquinones, cardiac glycosides and phlobatannins. The antimicrobial activity of the fractions against ten pathogenic organisms revealed moderate to good inhibition for Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae and Candidas albicans. The minimum inhibitory concentration of fractions was determined using broth dilution method. The lower MIC value (12.5 mg/mL) was exhibited by the aqueous methanol and ethyl acetate fractions. With the help of HNMR, HQSC and CNMR which led to the isolation and characterisation of a proposed compound named β-sitosterol.

Keywords: antibacterial activity, antifungal activity, fractions, *Guiera senegalensis,* phytochemicals

INTRODUCTION
Interest in plants is on the increase due to their pharmaceutical, nutritional and cosmetic applications. They represent the source of active ingredients known long time ago by their traditional use for medical purposes (Marie *et al.,* 2017). Plants can be considered as an origin of natural ingredients useful in medicine and other purposes. Plants are rich in active compounds (secondary metabolites) such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are present in their organs such as leaves, flowers, bark, seeds, fruits, and root (Marie *et al.,*2017). Extraction processes of these metabolites are related to the difference in solubility of the compounds present in a mixture

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of solvent. The beneficial actions of these phytoconstituents typically come from the synergic role of these secondary products (Tonthubthimthong *et al*., 2001). Natural products are products from plants, microbes and animals. Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa *et al.,* 2006), The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan *et al*.,2006). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men have turned to ethnopharmacognosy (Sombié *et al.,* 2011) They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activities such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity have been reported from many plants extracts (Karuo *et al.,* 2005: Sasidharan *e*t *al.,* 2011).

Guiera senegalensis belong to the family of Combrataceae is one of the most important West African medicinal plants, often used to treat a variety of microbial infections. The most frequently used part of the plant is the leaf; due to their antimicrobial activities (Tijani *et al.* 2011). *Guiera senegalensis* is called Sabara or Barbata in Hausa language, in Yoruba is known as Olofun. It is a shrub and can grow to a height of 3 to 5 m depending on the area of habitat (Adama *et al.,* 2016). The plant is commonly distributed in the savanna region West and Central Africa, example include- Nigeria, Gambia, Mali, Niger, Senegal. The Hausa people in Nigeria use the plant for the treatment of fever (Onwulin *et al.,*2009). The leaves and roots have been reported to be effective against malaria parasites (Etkins,1988). The leaf infusion had clinically demonstrated to be effective in the treatment of acute gastroenteritis and dysentery. The leaf extract has anti-inflammatory, analgesic and antiemetic properties (Etkins *et al.*, 1982). The leafy decoction of *Guiera senegalensis* is used to relieve aches and pains and to treat fever antimalaria, (Somboro *et al*., 2011; Osibembe *et al.,*2018). The aqueous and methanol soluble extracts of the leaves were found to exhibit the growth *of Staphylococcus aureus, Bacillus subtilus Escherichia coli and Candida albicans* which are the most causative agents in wound and skin diseases, and gastrointestinal disorders (Sokomba *et al.,* 1983). Traditionally, the roots concoction is used to treat diarrhea, dysentery and microbial infection (Alshafei *et al*., 2016). *Guiera senegalensis* has reputation in prevention of leprosy, particularly when given to the new born child of leper (Narayana *et al.,*2011). A simple decoction of the leaves of *Guiera senegalensis* could also be used to treat the opportunistic diseases of AIDS patients (Abubakar *et al.,* 2013). The decoction or powder is mixed with fresh milk and given to pediatrics for treatment of diarrhea and skin rashes, where the residues can be applied external to the affected parts. However, the leaves extracts were found to exhibit broad activity against the clinical isolates of *Escherichia coli, Staphylococcus aureus and Klebsiella* species (Abubakar *et al.,* 2013). The leaves are also used as a poultice on tumors and against Guinea worm (Sule *et a*l.,2002.: Abubakar *et al.,* 2013). Other research work done showed the presence of alkaloids (Hyoscyamin I and solanine II, tannins, terpenoids menthol, coumarins, saponins, flavonoids (quercetin, III) cardiotonics and cyanogenic heterosides which were assayed in various organs of the plant leaves, stem bark, fruits and roots (Somboro *et al.,* 2011). Phytochemical components of *Guiera senegalensis* leaf extract based on crude, aqueous and ethanol extraction methods showed that tannin, flavonoid, saponins, steroid, triterpenes and glycoside were present while alkaloid was absent in all extraction methods (Ogbeba *et al.,* 2017; Ankit *et al.*,2012; Sule and Mohammed, 2009). Many research works have been carried out with the aim of evaluating antimicrobial and phytochemical constituents of medicinal plants as possible alternatives to antibiotics and other chemotherapeutic agents but resistance still

persists. The aim of this work was to evaluate the medicinal efficacy of the leaves and stem of *Guiera senegalensis* in order to isolate the possible bioactive compound(s).

MATERIALS AND METHODS

Sample Collection and Identification

Guiera seneglensis leaves and stems were collected in May, 2024 from Kiri town, Taura Local Government Area of Jigawa State. It was identified by a taxonomist in the Biological Science Department, Federal University Dutse, Jigawa State and the Voucher specimen number is FUD/BOT/005 was deposited in the herbarium. The leaves and stem were washed with water to remove dust and dirt. The residual water was evaporated at room temperature and they were allowed to dry under shade for one week with regular spreading and turning in order to prevent the growth of moulds. The dried samples was pulverized into fine powder using wooden mortar and pestle. The powdered sample was weighed using analytical balance and kept at room temperature until further use.

Extraction of Plant Material

Five hundred (500g) of the powder sample were weighed and transferred into a container. 500mL of Methanol (95%) was added to the powdered sample and was allowed to soak for 72 hours at room temperature with occasional shaking and stirring. The extract was then filtered using a clean fine cloth. The filtrate was evaporated using rotary evaporator (Gee and Bauder, 2018) and the weight of the crude extract was recorded.

Fractionation

Fractionation of crude methanol extract was done using modified 'Kupchan method of solvent-solvent partitioning as described by (Qureshi *et al.*, 2019). The extract (150 g) of the leaves and stem was redissolved with water. These were fractionated with the aid of separating funnel using solvents of increasing polarity of n-hexane, Ethyl acetate, butanol and aqueous methanol, All the three fractions were evaporated by the help of a rotary evaporator at reduced pressure The remaining aqueous fraction of methanol was then evaporated to yield residue. The aqueous methanol, n-hexane, ethyl acetate and butanol obtained were used for the phytochemical analyses and antimicrobial activities, respectively.

Qualitative Phytochemical Analysis

The phytochemical constituents of *Guiera senegalensis* leaves and stem fractions were screened qualitatively using methods described.

Test for Flavonoids

Ferric chloride Test: About 5 mL of distilled water was added to the extract and boiled on water bath for about 2 mins and then filtered. 2mL of the filtrate was obtained in a test tube and few drops of 10% alcoholic ferric chloride solution were added. The formation of dark green colouration confirm presence of phenolic group (Trease and Evans, 2002: Saeed *et al.*, (2012).

Lead acetate Test: A small quantity of the extract was dissolved in water and filtered. Few drops of 10% lead acetate was added to 5 mL of the filtrate. A buff coloured, precipitate indicates the presence of flavonoids (Trease and Evans,2002).

Sodium hydroxide Test: about 2 mL of the filtered extract was dissolved in 10% NaOH to give a yellow-coloured solution. A change in color from yellow to colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids (Trease and Evans, 2002).

Test for Reducing Sugar
Fehling's Test: small amount of the powdered leaves was soaked in distilled water and allowed to extract for some time. The mixture was then filtered and the filtrate was heated with 5mL of equal volume of Fehling's solution A and B, for few minutes (5 mins). Formation of reddish precipitate of cuprous oxide indicates the presence of free reducing sugar (Trease and Evans, 2002).

Test for Cardiac Glycosides
5 mL of the extract was dissolved in pyridine and a few drops of sodium nitroprusside together with a few drops of 20% sodium hydroxide solution were added. The formation of deep red colour which fades to brownish yellow indicates the presence of cardenolides (Sofowora, 1982).

Test for Saponins
Frothing Test: The extract (0.5 g) was dissolved in 5 mL of distilled water and the shaken vigorously for 2 minutes in a test tube was shaken with distilled water in a test tube, frothing which persist on warming indicates the presence of saponins (Sofowora, 1982).

Test for Tannins
Ferric chloride test: To 0.5 g of the plant extract, 10 mL of distilled water was added and stirred. This was filtered and a 5 ml of 5% ferric chloride was added. A deep green coloration showed the presence of tannins. A second portion of the filterate was also added to iodine solution, a faint blue coloration formed confirming the presence of tannins (Trease and Evans, 2002).

Test for Alkaloids
Dragendorff's Test: small amount of the extract was stirred with 5mL of 1% aqueous hydrochloric acid on water bath and filtered. To the small portion of filtrate, a few drops Dragendorff's reagent was added, presence of an orange red precipitate indicates the presence of alkaloid (Trease and Evans, 2002: Sadiq *et al.*,2016).

Mayer's Test: Small amount of the extract was stirred with 5mL of 1% aqueous hydrochloric acid and filtered. To the small portion of filtrate, a few drops of Mayer's reagent was added. The formation of creamy precipitate indicates the presence alkaloid (Trease and Evans, 2002: Sadiq *et al.*,2016).

Test for Terpenoids
Salkowski Test: 2 mL of chloroform was added to 0.5 g of plant extract, 1 mL of acetic anhydride and 2 drops of concentrated H_2SO_4 was added. A peak colour which changes to bluish green on standing, which indicates the presence of terpenes (Trease and Evans, 2002).

Test for Steroids
Fractions of *Guiera senegalensis* leaves and stem bark (0.30 g) was reconstituted in 20 mL of ethanol, the mixture was extracted for 2 hours. 5 mL of the extract was added to 2 mL acetic anhydride followed with 2 mL of concentrated H_2SO_4. A violet to blue or green colour change in sample(s) indicates the presence of steroids.

Test for Phlobatannins
Fractions of *Guiera* senegalensis leaves and stem bark was treated with 3-4 drops of ferric chloride solution. Formation of bluish-black colour indicated the presence of phenols.

Test for Anthraquinone

Fractions of *Guiera* senegalensis leaves and stem bark (200 mg) was boiled with 6 mL of 1% HCl and filtered. The filtrate was shaken with 5 mL of benzene, filtered, and 2 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, violet, or red colour in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones.

Isolation of Compounds

Gravity Column Chromatography

Silica gel (200 g) was wet packed in a glass column (30 x35 cm) using n-hexane. Then ethyl fraction (3.0 g) was mixed with silica gel (60 – 230 mesh) then loaded onto column. The column was further packed with n-hexane and allowed to stabilize for four hours. The elution began with n-hexane 100 % and ethyl acetate was added gradiently from 0 to 100. Several fractions (10 cm^3 each) were collected and combined based on the TLC profile (n-hexane/ ethyl acetate in 1:3). Similar fractions (50 mL) were pooled together and concentrated in vacuo to give two major fraction, coded as compound 1and 2 having similar TLC each (10mg). Further purification of the compounds was carried out on micro column to afford the isolated compound coded DSLH1,2 (12 mg).

General and Spectroscopic Analysis

The isolated compound was subjected to Nuclear Magnetic Resonance (NMR) spectroscopic.

Nuclear Magnetic Resonance

The isolated compound (10 mg) was dissolved in 0.5 mL of deuterated chloroform and then subjected to 1D and 2D NMR analyses. All NMR spectra were obtained using pulsed Fourier transform methods on an Avance III HD Bruker 16.4 Tesla700MHz spectrometer (Bio Spin GmbH). Deuterium lock signals were obtained by using deuterated solvent ($CDCl_3$) (Cambridge Isotope Laboratories, Inc). Measurements were made at ambient probe temperature 296K in 5mm spinning tubes (Wilmad-541-PP-7). The chemical shift (δ) was reported in parts per million, (ppm) and the coupling constants (J values) are reported and measured relative to 85% tetramethyl silane (TMS) external standard. ^1H NMR at 700.13MHz spectra were obtained using a8.00us pulse, ^{13}C NMR at 176.07 MHz spectra were obtained using a 12.00s pulse. The NMR experiment was performed in Spectrophometric facility at H.E.J, Institute of Chemistry, International Centre for Chemical and Biological Sciences, University of Karachi, Pakistan.

Antimicrobial Analysis

The clinical bacterial isolates and fungal were screen, the bacterial include *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella tphi, Klebisiella pneumonia* while fungal isolate include *Candidas albicans, Candidas tropicalis, Aspergillus niger* and *Aspergillus flavus* were obtained from Department of Microbiology, General Hospital, Dutse, Jigawa State.

Preparation of Stock solution

A stock concentration of 200 mg/mL of each extract was prepared by dissolving 4m g of the extract in 2 mL of dimethyl sulfoxide (DMSO). Subsequently, 100, 50, 25 and 12.5 mg/mL concentrations were prepared from the stock using 2-fold serial dilution. The standard control (ciprofloxacin and fluconazole) for bacteria and fungi were also prepared by dissolving 0.5ml of each drug in 0.5ml distilled water (Garba *et al.,* 2019).

Standardization of Inocula

The Inocula were standardized by dissolving 1-2colonies of the test bacteria using a sterile wire from an overnight culture plate and dissolved in 10 ml of normal saline solutions contained in test tubes. The tubes were vortex mixed for homogeneity and compared with 0.5 McFarland standards (approximate cell count density: $1.5x10^8$) (Garba *et al.,* 2019).

Determination of Antibacterial and antifungal Activity (Sensitivity Test)

Sterile swab sticks were immersed into the standardized Inocula contained in test tubes. Excess fluids were drained and swabbed onto prepared Mueller Hinton Agar (MHA)plates. Four wells of approximately 6 mm diameter were cut in each plate using a sterile cork borer. Aliquots of 100µl of various concentrations of the extracts were added into the wells using a micropipette. The plates were allowed to stand at room temperature for one hour for the extracts to diffuse into the agar and incubated for 24 hours at 37 ^oC. Antimicrobial activity was determined by measuring the diameter of inhibition zones in millimeter produced against the test bacterial and test fungal organisms. The experiments were performed in replicates and the mean values were calculated (Garba *et al.,* 2019).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined for the fractions using nutrient broth dilution method. Nutrient broth was prepared according to the manufacturer's instruction. McFarland turbidity scale was prepared to give turbid solution. Normal saline was prepared and dispensed into test tubes and the microorganisms were inoculated and incubated at 37^o C. Dilution of the test microorganisms in the normal saline was performed until the turbidity marched that of the Mac farlands scale by visual comparison. Two-fold serial dilutions of the fractions /extracts in the broth were performed to obtain the concentration for the fractions. 0 1 ml of the standard inoculum of the test microorganism in the normal saline was then inoculated into the different concentrations of the fractions. Incubation was made for 24 hours after which each broth was observed for turbidity. The lowest concentration of the fractions in the broth which showed no turbidity was recorded as the minimum inhibitory concentration (MIC) (Garba *et al.,* 2019).

Statistical Analysis The data obtained was statistically analyzed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the result was reported as mean± standard deviation.

RESULT AND DISCUSSION

The percentage yield of each Fractions is as follow; for n-hexane (8.0%), ethyl acetate (9.3%) and Methanol (9.67%) respectively. This implies that ethyl acetate and methanol fraction contain more constituents than n-hexane.

Phytochemical Screening

The result of the phytochemical screening of the n-hexane, ethyl acetate and methanol fractions of *Guiera senegalensis* for leaf and stem are presented in Table 1 and 2. The results showed the presence of some secondary metabolites.

Table 1: **Phytochemical's Screening of *Guiera senegalensis Leaf* extracts**

	Phytochemicals	Leaves			Stem		
		N-Hexane	Ethyl acetate	Methanol	N-Hexane	Ethyl acetate	Methanol
1.	Flavonoids	-	+	+	-	+	+
2.	Alkaloids	-	-	+	-	-	+
3.	Tannins	-	+	+	-	+	+
4.	Saponins	-	-	-	-	-	-
5.	Terpenoids	+	+	+	+	+	+
6.	Reducing Sugar	-	+	+	-	+	+
7.	Cardiac Glycoside	+	+	-	+	+	-
8.	Anthraquinones	-	+	+	-	+	+
9.	Phlobatannins	-	+	+	-	+	+

Key; - = absent **+ =** present

Antimicrobial Activity

The results of the antimicrobial activity of the fractions are presented in Figures 1,2,3 4, 5 and 6. the fractions inhibited the growth of the organisms at different concentrations.

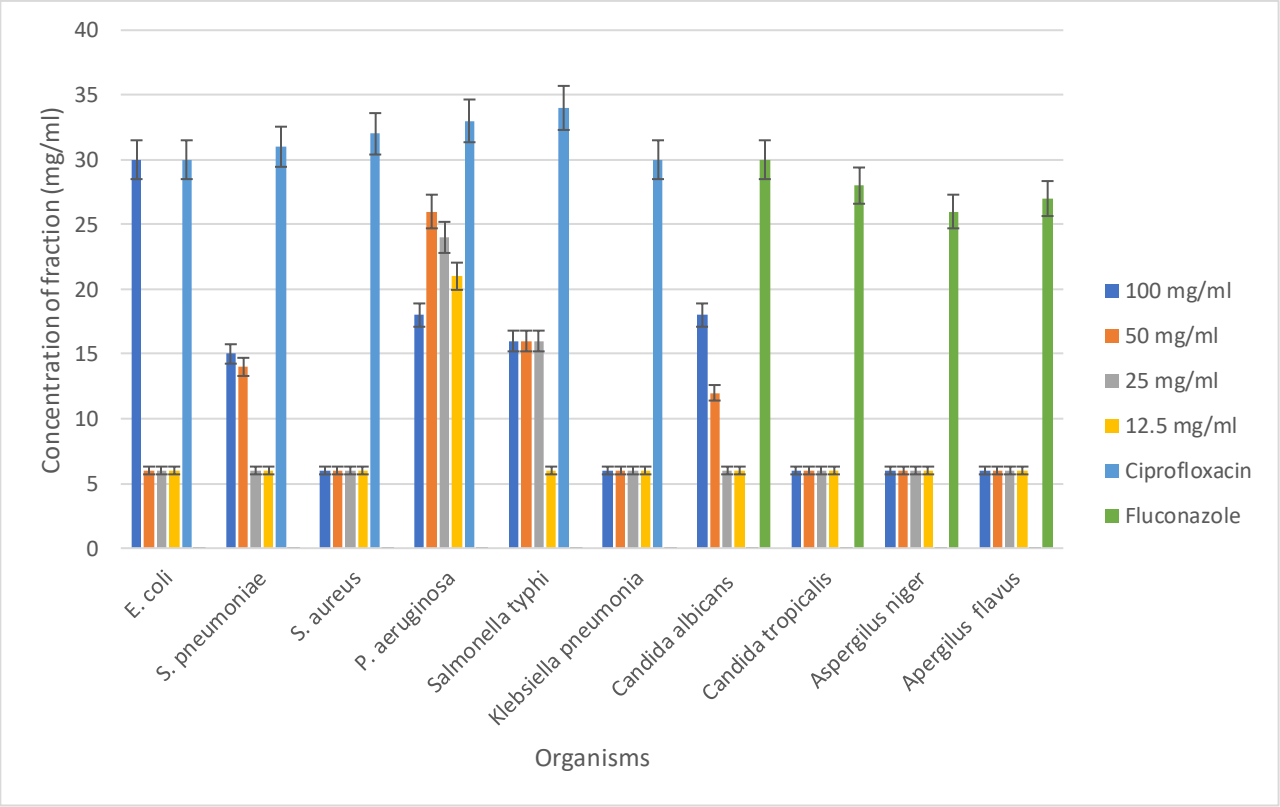


Fig. 1 : Antimicrobial activity of n-hexane fraction of *Guiera senegalensis leaf*

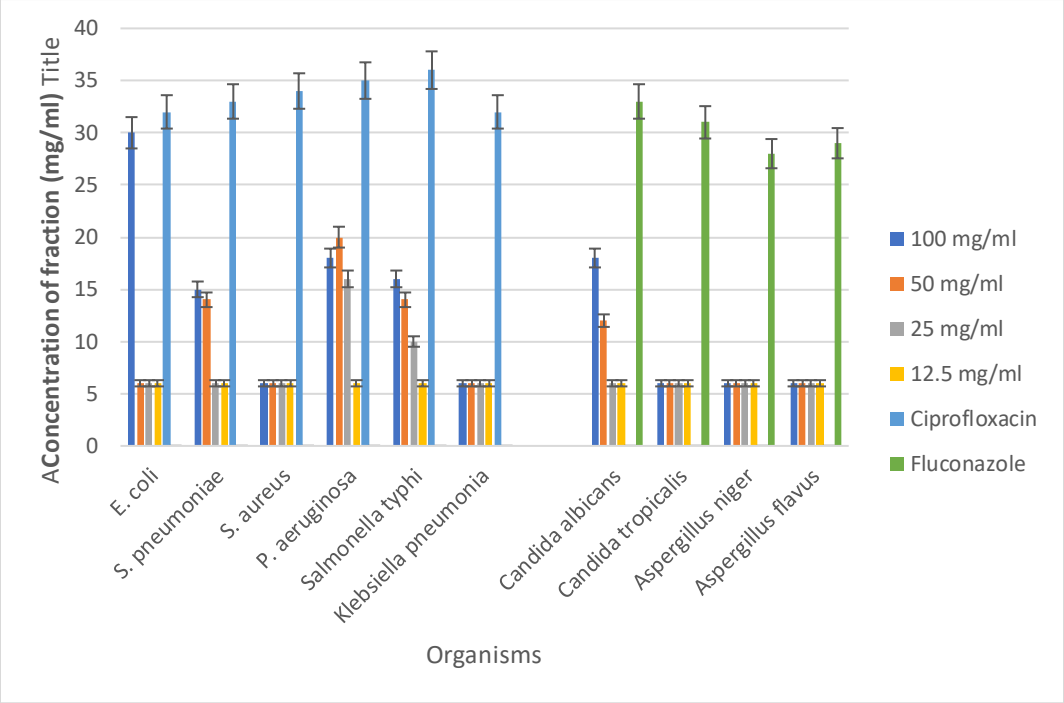
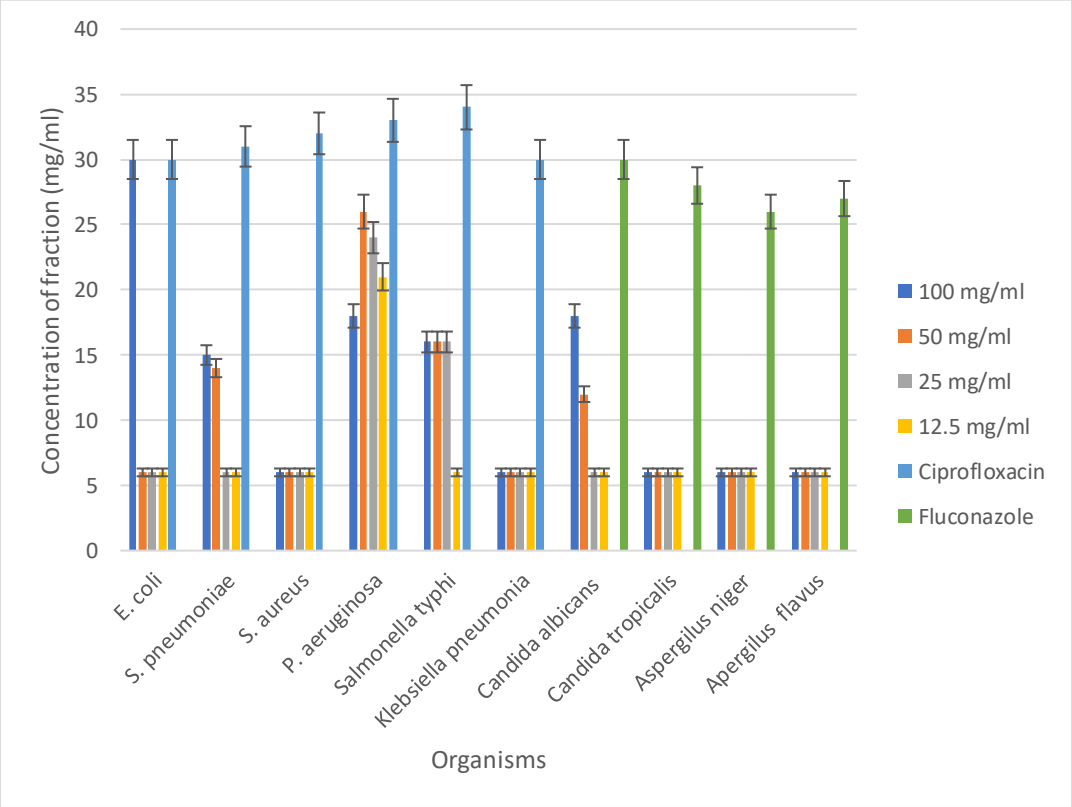


Figure. 2: Antimicrobial activity of n-hexane fraction of *Guiera senegalensis stem*



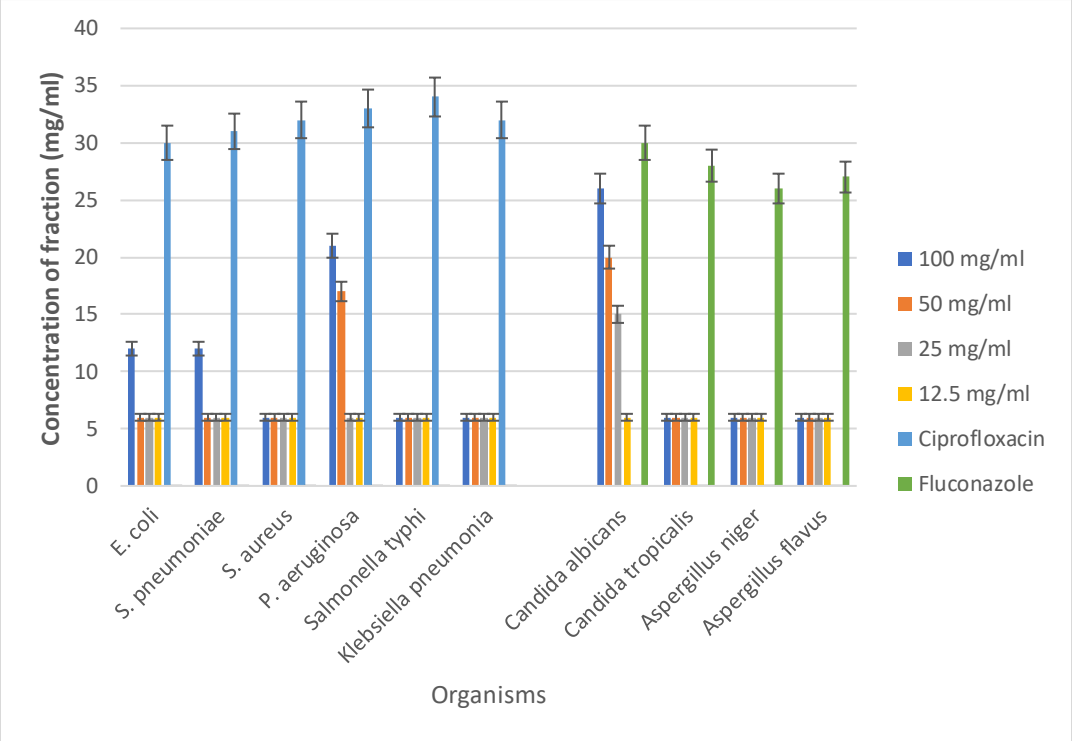


Figure 3: Antimicrobial activity of Ethyl acetate fraction of *Guiera senegalensis leaf*

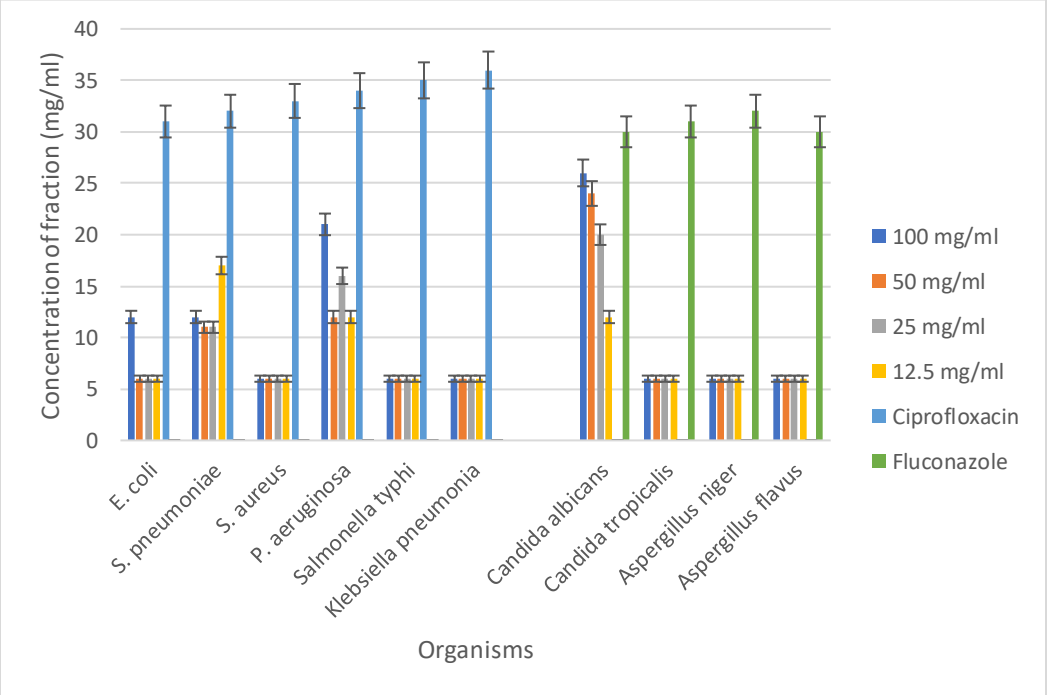


Figure. 4: Antimicrobial activity of Ethyl acetate leaf fraction of *Guiera senegalensis*

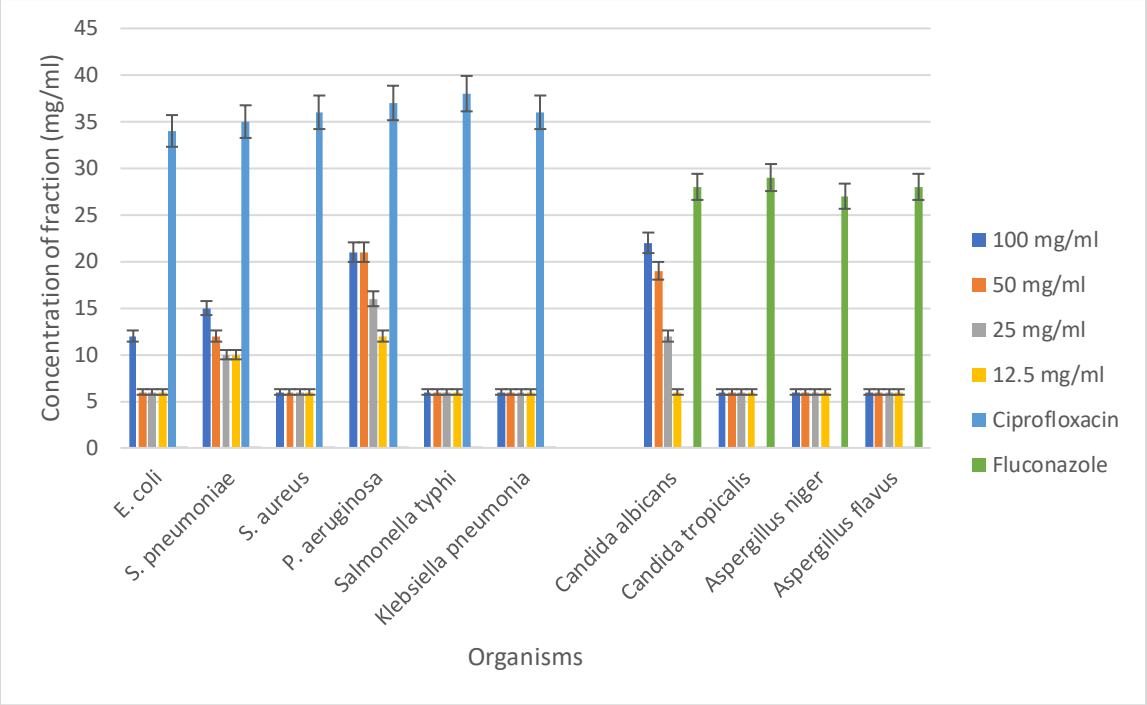


Figure. 5: Antimicrobial activity of methanol fraction of *Guiera senegalensis stem*

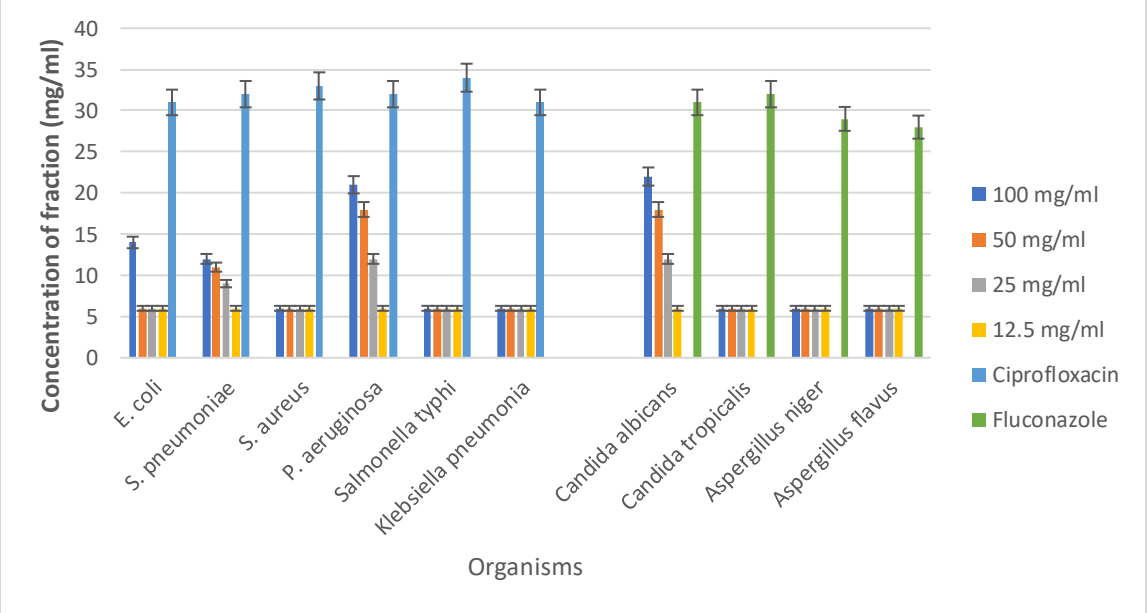


Figure. 6: Antimicrobial activity of methanol fraction of *Guiera senegalensis stem*

Table 3: Minimum Inhibitory Concentration (MIC)

Fractions/organisms	**Concentration (mg/ml)**					
	100	80	60	40	20	10
E.coli						
n-hexane	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	+
Methanol	-	-	-	-	-	+
S.pneumoniae						
n-hexane	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	+	+
Methanol	-	-	-	-	+	+
P.aeruginosa						
n-hexane	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	+	-
Methanol	-	-	-	-	-	+

Compound I

White powder, IR (KBr) Vcm^{-1}): 3330 (OH):2954- 2916 (CH_2); 1684 (C=C): 1452(CH_3); Mp,135-140 ^{0}C; UV 275 nm, TLC: Rf 0.61 in n-hexane/ethyl acetate (8:2),^{1}HNMR:δH(ppm).5.50(m),5.30(m)3.50(m),2.24(m),2.14(m),1.97(m),1.82(m),1.64(m),1.67(m),1.56(m),and1.34(m).^{13}CNMR(175MHz,$CDCl_3$)δ,15.65(C1),15.70(C2),71(C3),40.61(C4).125(C5),140,87(C6),17.56(C7),18.37(C8),21.43(C9),23.28(C10),23.38(C11),26.62(C12),27.28(C13),28.11(C14),28.14(C15),28.77(C16),31.27(C17),32.94(C18),33.77(C19), 38.79(C20),19.3(C21), 32.62(C22), 26.6(C23), 41.54(C24), 29.17(C25), 21.0(C26), 21.6(C27), 23.0(C28),12.5(C29).

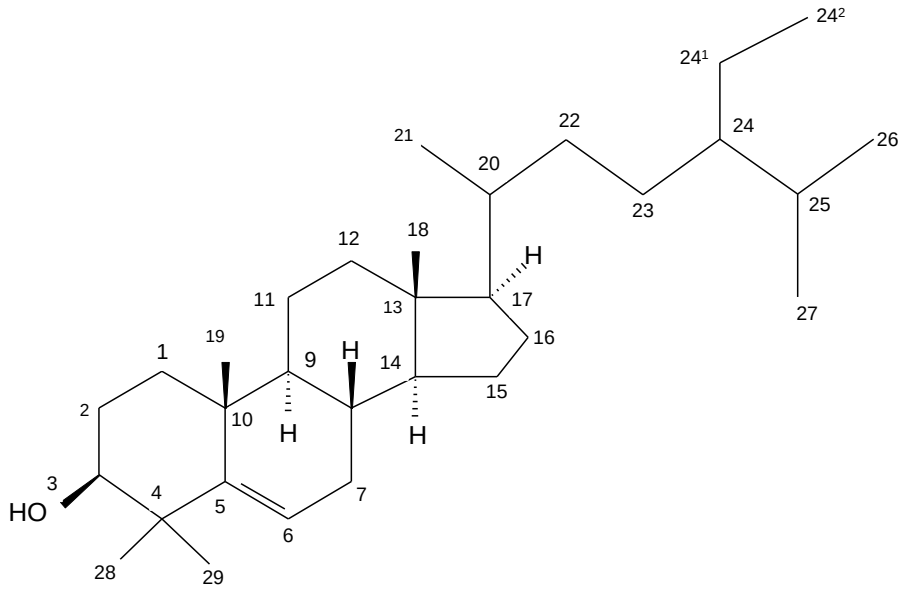


Figure 7: Propose Structure of compound I

DISCUSSION

The result of phytochemical analysis of the leaves and stem of *Guiera senegalensis* was presented in Table 1. The phytochemical screening showed that the leaves fraction contained alkaloid only in methanol fraction by Dragendorff's test and absence of saponins in all fractions. Tannins, flavonoid, terpenoids and reducing sugar were also found to be present. The results of this study was similar to the finding of Mamman *et al.* (2013) which showed that the leaves extract contained anthraquinones, tannins and phlobatannins, flavonoid while saponins were not found in the leaves extract. The result was compared to Onwulin *et al.*,

(2009) which shows that *Guiera senegalensis* contained anthraquinones (free anthraquinones and combined anthraquinones), carbohydrates, cardiac glycosides, flavonoids, saponins, terpenes, phenolic compounds and tannins. The presence of this phytochemicals is very important, because they play a vital role as antimicrobial, antidiarrhea and anti-helminthic agent. The presence of alkaloid has anti-diarrhoea effect; Terpenes, which is anti-malarial, antibacterial, antiviral as well as antifungal; Tannins is also known to be anti-tumour; Anthraquinones, which is anti-hemorrhagic; flavonoids, which inhibits *Vibrio cholerae, Strept. Mutans,* shigella and viruses (Trease and Evans, 1989).

The leaves and stem of *Guiera senegalensis* fractions ~~were~~ tested against some Gram positive and negative bacteria namely: *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *Streptococcus pneumonia, Pseudomonas aeruginosa, Salmonella typhi, klebsiella pneumonia,* while fungal species are *Candidas albicans, Candidas tropicalis, Aspergillus niger and Apersgillus flavus* respectively.

The chart in figures 1,2,3,4,5 and 6 explained the antimicrobial activity of three fractions of leaves and stem at different concentrations so as to established whether the stem can also inhibit the growth of Test-organisms and was also compared with standard antibiotics. In figure 1, n- hexane fraction, showed significant inhibitory effect against some of the microorganisms. While in this present research *Escherichia coli was* found to have the highest inhibition of growth at concentration of 100 mg/mL. For *Streptococcus pneumoniae* shows zones of 15 mm and 14 mm at 100, 50 mg/mL concentration. However, it was reported by Onwulin *et al*., (2009) that *Escherichia coli* was found to be sensitive to the extracts of all concentrations. While *pseudomonas aeruginosa* shows zone of inhibtion which are moderate implies that as the concentration increases the zone of inhibition increases. Also, *Candidas albicans* inhibited the growth of organisms at 18 and 12mm, the others test organism shows insignificant inhibitory growth and standard drug, C Ciprofloxacin inhibited all bacterial species except fungal species no growth at all and Considering the bar chart in figure 2, the chart shows that *Escherichia coli was* found to be have the highest inhibition of growth of 30 mm at concentration of 100 mg/mL. For *Streptococcus Pneumoniae* shows zones was moderate at 50 and 25 mg/mL concentration. While *Pseudomonas aeruginosa* shows zone of inhibtion implying that is significant at 100, 50 and 25 mg/mL respectively. Likewise, *Salmonella typhi* gave zone of inhibition that significant at concentration of 100, 50, 25 mg/mL. Also, *Candidas albicans* inhibited the growth of organisms at 18 and 12mm, the others test organism shows insignificant inhibitory growth and standard drug, Ciprofloxacin inhibited all bacterial species except fungal species no growth at all while fluconazole inhibited the growth of fungal organism at 27 mm.

Meanwhile figure 3 and 4 (bar chart) revealed the growth of inhibtion, ethyl acetate of leave and stem- While *pseudomonas aeruginosa* shows zone of inhibtion which is good at 100 and 50 mg/mL respectively implies that as the concentration increases the growth also increase. Also *Escherichia coli was* found to be have zone of inhibtion of growth at high concentration 100 mg/mL. while, *Candidas albicans* inhibited the growth of organisms with 26 mm while others were negative.for the stem in figure 4, the result shows that *Candidas albicans* inhibited the growth of organisms at 26 and 20, and 12 mm respectively.

Considering the chart in figure 5 and 6 showing zone of inhibtion for methanol fraction both leaves and stem. In the case of figure 5, revealed the growth of inhibtion for leave: *Pseudomonas aeruginosa* shows zone of inhibtion that is excellent at all concentration. Also *Escherichia coli was* found to be have zone of inhibtion at 22 mm with concentration 100 mg/mL. while,

Candidas albicans inhibited the growth of organisms and others were insignificant. while *Streptococcus pneumonia* shows inhibition that was good all throughout. respectively.

In figure 6, revealed the growth of inhibtion methanol fraction for stem: P*seudomonas aeruginosa* shows zone of inhibtion which is excellent implies that methanol extract contains metabolites that are responsible for the above activity. Also *Escherichia coli was* found to have moderate zone of inhibtion at higher concentration. while, *Candidas albicans* inhibited the growth of organisms that was good. while *Streptococcus pneumonia* shows inhibition that was moderate at all concentration. While *Pseudomonas aeruginosa* shows zone of inhibtion that was excellent, implies that as concentration increases the inhibition increases likewise. All others test organisms shows no inhibition. Meanwhile the Ciprofloxacin was sensitive to the bacterial species while no inhibition to fungal species but the Fluconazole was sensitive to all the fungal species but also insensitive to bacterial organisms.

But in the present research there are bacterial and fungal that was not sensitive to all the fractions at varying concentration. The ethyl acetate and methanol fractions showed high efficacy on almost all the test organisms. The activity of the fractions was shown to increase with the increase in concentration. However higher concentration is required in order to produces significant effect of these leaf fraction on *Escherichia coli. Guiera senegalensis* possess considerable antimicrobial activity as evidence from their action on the different test organisms. It has been reported to cure skin diseases, leprosy, gonorrhea, stomach pains, asthma, diarrhoea and dysentery (Onwulin *et al.,* 2009). The table 5, 6, 7 and 8 revealed the minimum inhibitory concentration of the three different leaf extracts on the test organisms. The MIC of *Streptococcus pneumoniae* was 20 mg/ml for the ethyl acetate and methanol extracts. *Pseudomonas aeruginosa* were 20 mg /ml for methanol and ethyl acetate extracts while 40 mg/ml for ethyl acetate. While the *Escherichia coli* was 10 mg /ml for the three extracts. While *Staphylococcus aureus* was 80 mg/ml for the ethyl acetate and methanol extracts. These results implies that all the extracts could attained therapeutic concentration in living host.

White crystalline powder, Mp135-140 ^{0}C. The melting point of the compound is within the range of 135-140 ^{0}C. The IR spectrum suggested that it contained a hydroxyl group at (3330 cm^{-1}), a terminal double bond at (2954,2954,2916, 1682, and 1452 cm^{-1}).

^{1}HNMR: δH (ppm).5.50(m),5.30(m) 3.50(m), 2.24(m), 2.14(m),1.97(m), 1.82(m), 1.64(m), 1.67(m) 1.56(m) and1.34(m). The spectrum showed a cluster of signals between δH 0.8 to 1.2, which are characteristics of methyl hydrogen (CH_3). Also other δH were observed between 1.2 to 1.5 which are characteristic of methylene (CH_2). The 1HNMR showed six methyl (CH_3) signals at δH (ppm) 0.90 (3H), 0.91 (3H), 0.98 (3H), 0.99 (3H),1.04 (3H) and 1.05 (3H) with different multiplicities indicative of different electronic environments. This suggests that there are six methyl groups present in the compound. The presence of down field signals at δH(ppm) 5.33 (1H, br.) suggest the presence of olefinic proton.

^{13}CNMR Spectrum of Compound I

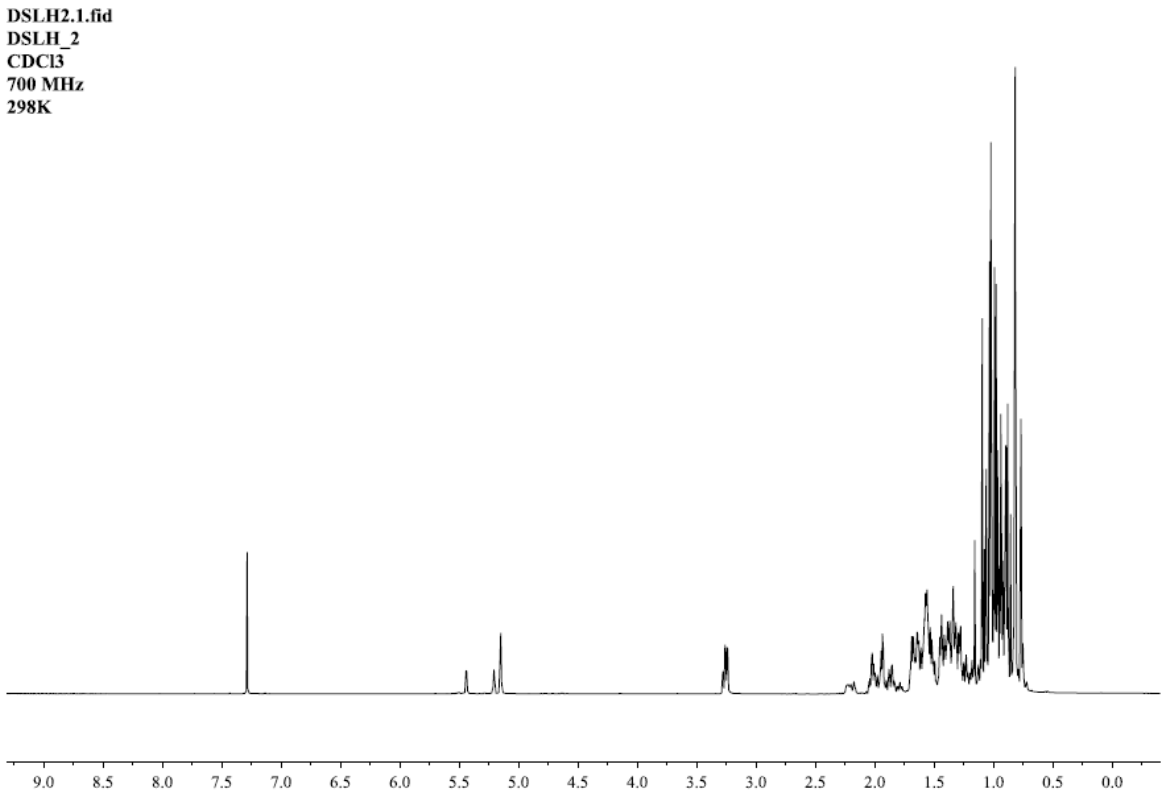
Several signals were observed in the spectrum. They include δc (ppm) 140.7, 125 and 76.7. Other signals were observed between the range of δc (ppm) 15 to 59 which are characteristic of methyl (CH_3), methylene (CH_2), and methine (CH) carbons.

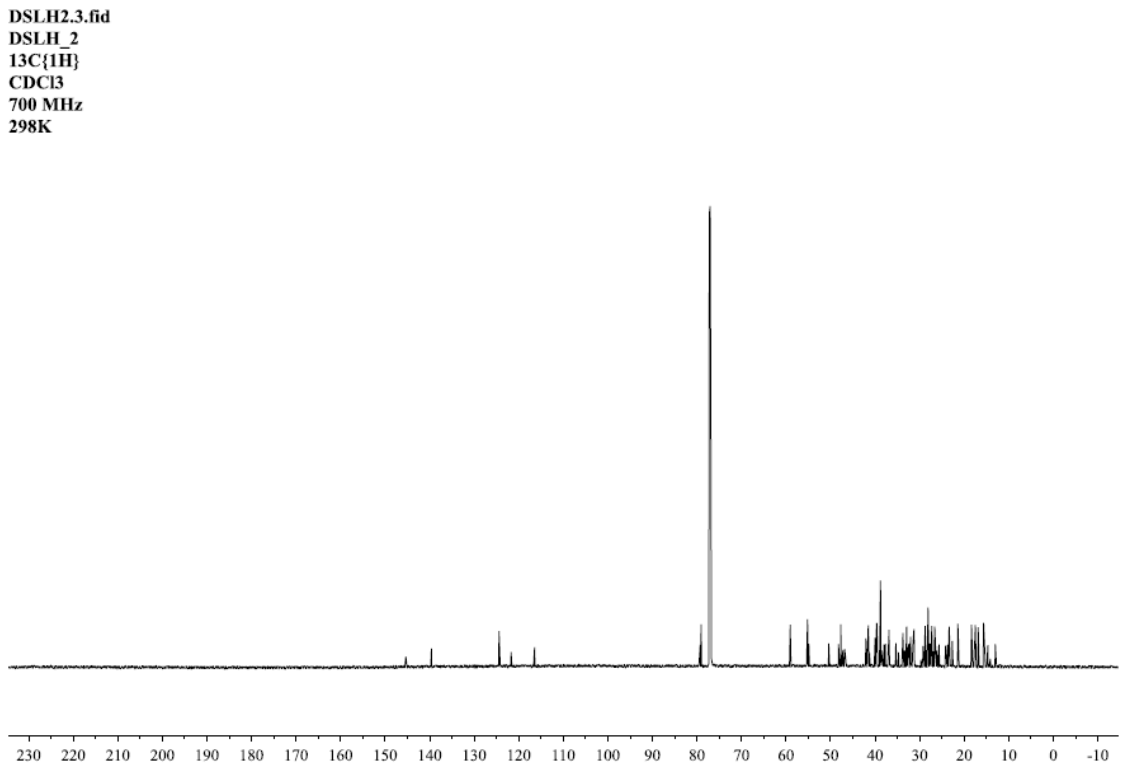
From the Nuclear Magnetic Resonance (NMR), Heteronuclear Single Quantum Correlation (HSQC) which compare well with available literature (Pateh *et al.,* 2009), Compound I was isolated as a white crystalline powder, highly soluble in chloroform; with a melting point range of 136 – 140 ^{o}C, the signal at δc (ppm) 125 confirms the presence of an olefinic carbon

(C-5). The Carbon NMR of compound I revealed 29 carbon signals. This suggests that the compound may consists of 29 carbon atoms. The signal at δc (ppm) 140 indicates the presence of a quarternary carbon (C-6) atom. The carbons at δc (ppm) 71 confirm the presence of carbon (C-3) bearing the hydroxyl group. This data is consistent with the earlier work published by Pateh *et al.,* (2009) and it is a characteristic of β-sitosterol Fig. 4. The structure of compound I is suggested to be β-sitosterol (22, 23-dihydrostigmasterol). This information is also consistent with work of Pateh *et al* (2009) and it is a characteristic of β-sitosterol.

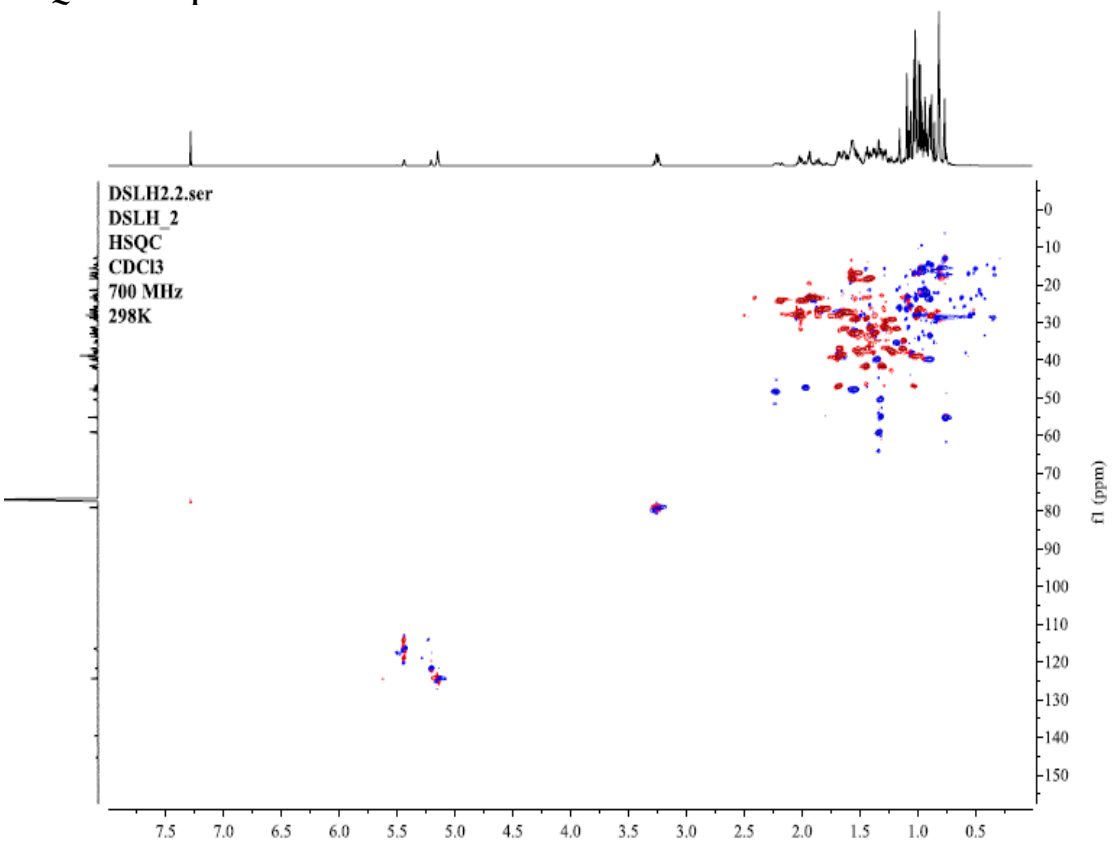
CONCLUSION

The phytochemical screening showed that the leaves fraction and stem contained alkaloid only in methanol fraction and absence of saponins in all fractions. Tannins, flavonoid, terpenoids and reducing sugar were also found to be present. The ethylacetate and methanol fractions inhibited the test organisms mostly at all concentration. The leaf fraction gave a possible compound known as β-sitosterol.





HSQC of comp I



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