Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Investigation of antimicrobial and antioxidant properties of extracts of the stem bark of *Drypetes floribunda* (Euphorbiaceae)

Alex Boateng[1], Joseph K. Adu[1*], Michael K. Baah[2]

Abstract

Background: The use of the stem bark of *Drypetes floribunda* in the treatment of microbial infections is common amongst the people of Kwahu Asakraka in the Eastern Region of Ghana. However not much studies have been done to establish the scientific basis of this and to isolate the bioactive principles from the stem bark. *Objectives:* To assess the antimicrobial and antioxidant properties of the stem bark and to isolate some bioactive principles and investigate their antimicrobial activity.
Methods: An antioxidant assay was conducted on the crude flavonoid extract and crude phenolic acid extract using ascorbic acid as control. A crude methanolic extract of the stem bark was obtained, its total phenolic content was determined and was further subjected to column chromatography. The fractions obtained were structurally elucidated using the various spectroscopic techniques and the High Throughput Spot Culture Growth Inhibition antimicrobial bioassay was conducted on it using six microbes (*Pseudomonas aeroginosa, Salmonella typhi, Escherichia coli*, *Streptococcus pyogenes, Staphylococcus aureus*, and *Candida albicans*).
Results: The crude flavonoid extract was found to have a higher antioxidant activity with an IC_{50} of 27.7843 µg/mL compared to the control (51.3925 µg/mL) and the crude phenolic acid extract (283.8573 µg/mL). The total phenolic content (TPC) of the crude methanolic extract varied from 34.56 ± 1.5997 mg/g to 68.79 ± 0.9568 mg/g. The crude with an MIC range of 125-250 µg/mL and the fractions (F_{10} and F_{14}) with MIC ranges of 125-500 µg/mL and 31.25-500 µg/mL respectively were found to be active against the microbes except for *Pseudomonas aeroginosa*, *Streptococcus pyogenes*.
Conclusions: The stem bark of *Drypetes floribunda* possesses antioxidant and antimicrobial activity.

Keywords: antioxidant; antimicrobial; cholestanamide; phenolic; phytosterols; steranes.

*Correspondence: Phone: (+233) (0)508265823; Email: jkadu2000@yahoo.com, jadu.pharm@knust.edu.gh; (Dr Joseph K. Adu)
Author's ORCID: 0000-0002-1544-5112 (Joseph K. Adu); 0000-0002-8739-6452 (Alex Boateng)

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana; [2]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

Citation on this article: Boateng A, Adu JK and Baah MK. Investigation of antimicrobial and antioxidant properties of extracts of the stem bark of Drypetes floribunda (Euphorbiaceae).Investigational Medicinal Chemistry and Pharmacology (2019) 2(2):32; Doi: https://dx.doi.org/10.31183/imcp.2019.00032.

Background

Over the years, efforts have been made to unravel several potential lead compounds with therapeutic activity to combat the menace posed by the rapid rise in disease conditions. Plants have been a rich source of most of these potential lead compounds especially those of antioxidant and antimicrobial activity. It is a scientific belief that about two thirds of the world's plant species have medicinal importance [1]. There are various phytochemical principles or secondary metabolites in traditional medicinal plants that do have some antimicrobial potential and thus prove useful for drug discovery [2, 3]. In the light of the increasing prevalence of antibiotic resistant bacteria as result of the overdependence and extensive use of conventional antibiotics in the eradication of infections and in instigating healing of wounds, interest in research into extracts obtained from traditional medicinal plants as potential sources of novel antimicrobial agents has been elevated [4, 5].
Myriad of plants belonging to this genus (Drypetes) are known to possess potent antimicrobial and antioxidant activity [6].

The plant *Drypetes floribunda* is a tree that can grow up to a height of 10 m and is commonly found in closed forest and in the Savanna in West African countries such as Ghana, Senegal, Ivory Coast and Nigeria. In Ghana, it is commonly grown in the secondary forest of Kwahu Asakraka. It is used in hut construction and as yam poles by the *Vhe* ethnicity of Ghana. The wood has a sweetish taste and commonly used as a chewing stick hence the Twi name, bedibesa meaning 'if you eat, it will soon finish'. It is characterized by the outgrowth of flowers from the stem.

Flavonoids are one of the prominent group of phytochemicals widely responsible for antioxidant potential of most plants. These phytochemicals are often utilized in plants to produce pigments which play a major role in flavoring and coloring the plants such as yellow, orange and other pigments. In addition, flavonoids have shown in some studies with the potential to exhibit some important anti-inflammatory, antimicrobial, anti-allergic and anti-cancer activities. They are also found to be powerful anti-oxidants and researchers are looking into their ability to prevent cancer and cardiovascular diseases [7].
Phytosterols are important group of phytochemicals or secondary metabolites found in plants, microorganisms such as algae and fungi and in animals (most notably cholesterol). However, rather different range of sterols are found in plants, algae and fungi (mainly ergosterol) compared to those found in animals. They constitute a group of triterpenes having a tetracyclic cyclopenta [a]phenanthrene structure and a side chain at carbon 17 [8].

Sterols are known to possess some antimicrobial properties and are wide-spectrum antibacterial agents. Thus, they have been shown in some studies to be able to inhibit the growth of some gram positive (example *Staphylococcus aureus*) and gram-negative organisms (example *Salmonella typhi*) and thus shown promise in the future to be further exploited in discovering new antibiotics [9].
Steranes (cyclopentanoperhydrophenanthrenes) can be defined as a group of 4-cyclic compounds derived from steroids or sterols via diagenetic and catagenetic degradation and saturation. Steranes have an androskeleton with a side chain at carbon-17. The framework of steranes constitute the core of sterols and some examples include cholestane and diasterane. Thus, they are sometimes employed as biomarkers in testing for the presence of eukaryotic cells [10].

One of the fractions, F_{10} (obtained from a solvent system of chloroform and ethyl acetate in a ratio of 90:10 during isolation on the crude methanolic extract) is an amide derivative of steranes with the derivatization occurring at C-25. It also has an additional Hydroxyl group at C-22. According to the nomenclature of steranes and derivatives, its name is 4, 4, 14-trimethyl-22-hydroxyl-24-norcholestanamide.
There has been a significantly disturbing rise in the evolution and constant wide spread of drug-resistant strains of pathogenic microbes. The WHO described antimicrobial resistance as the top (leading) magnificent menace in infection and pathogenic diseases today, posing a threat to both the developed and developing countries alike. However, enough attention and focus in terms of medical research have not been channeled to rapidly avert this menace [11, 12]. The 21st century scientific challenge is posed by the onset of extensively or extremely drug resistant (XDR) strains of *M. tuberculosis* as well as antimicrobial resistance among some fungal microbes (such as *Cryptococcus*, certain species of *Candida, Aspergillus, Trichophyton*) as well as the following bacteria namely: *E. faecium, S. aureus, K. pneumoniae, A. baumanii, P. aeroginosa and Enterobacter species* (ESKAPE).

Techniques that can help come out with new and relatively potent antibiotics and in the detection of antimicrobial resistance are as a result being very relevant and prominent in the health sector. The High Throughput Spot Culture Growth Inhibition (HT-SPOTi) antimicrobial bioassay was the antimicrobial bioassay model employed in this project. It is a rapid drug susceptibility test (DST) used to assess the resistance profiles of antibiotics and also to help in the discovery of novel compounds for anti-infective drug discovery. It is an antimicrobial bioassay that operates on the fundamental principle of the growth of an organism on agar medium containing different concentrations of drugs or inhibitors. Thus, in this assay, the MIC is the least concentration of the drug on the 96-well plate where there is no growth of the organism being exploited. This makes the assay the most recommended in assessing the antimicrobial potential of novel compounds as well as the antibiotic resistance profile of an organism or pathogen [13].

Methods

Plant material

Dried stem bark of *Drypetes floribunda* collected from the secondary forest of Kwahu Asakraka, Kwahu in the Eastern Region and authenticated by Mr. Asare of Herbal Medicine Department, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), KNUST, Kumasi and assigned a voucher number KNUST/HM1/2018/SB008.

Drug Samples
The profile of drug samples used are as stated in Table 1.

Equipment, Chemicals and Reagents

The equipment used in the research include Analytical balance (Kern, Germany), Melting point apparatus (Stusrt, UK), Water bath (Halo-Hollen, Denmark), Jenway UV Spectrophotometer 7315 (Jenway, UK), Bruker Biospin NMR Spctrophotometer F/NMR/A175 (Billerica, USA), Perkin Elmer IR Spectrophotometer A/0626/15 (Waltham, USA) and Multi pipette for HT-SPOTi Assay 492583F (ErgoOne, USA)

The reagents used in the project include analytical grades of Ethyl acetate, petroleum ether, methanol, chloroform (Fisher Scientific), Concentrated HCl (Fisher Scientific), NaOH pellets (Fisher Scientific) Dimethyl sulfoxide (Amresco LLC, USA) and Diethyl ether (Surechem Products Ltd, UK).

Microorganisms

The microbes used in this work were clinical strains obtained from the Microbiology section of the Department of Pharmaceutics, FPPS, KNUST, Kumasi. They are as stated in Table 2.

Extraction of the crude methanolic extract from the sample

2.5 kg of the dried powdered stem bark of the plant was extracted by means of cold maceration using 96% (v/v) methanol. The dried extract of 50 g representing 2.0% (w/w) was obtained after the extraction procedure. 7.5 g of the extract was taken through a phytochemical screening and an isolation process was conducted on 40 g of the extract using column chromatography. A total phenolic content determination was then conducted on it using the Folin Ciocateau method.

Extraction of the crude flavonoid extract

Crude flavonoid extract (an extract that contains flavonoids predominantly) was also prepared. The purpose of this was to investigate whether the flavonoids found in the stem bark of the plant contribute major to the overall antioxidant activity of the plant and to verify its antioxidant activity relative to that of phenolic acids which are the other group of antioxidant principles found in plants and the control which was ascorbic acid. Extraction was done using 62.5% (v/v) methanol on 50 g of the powdered sample. The extract was then acidified with 6 M HCl under 90 ºC for 2 hours to obtain flavonoid aglycones. A phytochemical screening to test for the presence of flavonoids was done on the extract. The IC_{50} was determined using the DPPH in-vitro antioxidant assay method and compared to that of the phenolic acid extract and the control (ascorbic acid) used.

Extraction of crude phenolic acid extract

Crude extract containing phenolic acids predominantly was also prepared for the same purpose as mentioned above in the extraction of the crude flavonoid extract. The phenolic acids come in three forms (free, esterified and glycosylated). They were all obtained separately. 80 % (v/v) methanol was used to extract the phenolic compounds from 50 g of the powdered sample at 80 ºC for 15 minutes and the organic solvent was made to vaporize thereby concentrating the extract. In extracting the free phenolic acids, water was used to prepare a suspension of the extract, and a HCl solution (6 M) was then utilized to control the pH of the solution to a pH of 2. The free phenolic acids were then extracted using diethyl ether. 20.00 mL of a solution of NaOH (2 M) was used in the neutralization and dissolution of the residue of the suspended extract for 4 hours. The extract was acidified again to pH of 2 after the alkaline hydrolysis. A Separating funnel was then employed in the isolation of the esterified phenolic acids derivatized by mixing with the diethyl ether. In releasing the phenolic acids from the glycosylated forms, 15 mL of 6 M HCl was added to the remaining aqueous fraction and the mixture kept in 100 ºC for 1 hour. Diethyl ether was then finally used to isolate the released phenolic acids [14, 15]. A phytochemical screening to test for the presence of phenols and absence of flavonoids was done.

Antioxidant capacity determination [Using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Model]

The evaluation of the antioxidant capacity on the phenolic acid extract, the flavonoid extract and using ascorbic acid was conducted as follows:
Concentrations (25-500 µg/mL) of the extracts (crude flavonoid and crude phenolic acid extracts) were prepared. Standard solutions of ascorbic acid were also prepared (12.5-200 µg/mL). To 1 mL of the extract, 3 mL of a 0.002% w/v DPPH solution was added. The set-up was allowed to stand for 30 minutes in a cool dark place. The absorbance of the resulting solution was measured at 517 nm and a dose-response curve was subsequently obtained and used to determine the IC_{50}. Procedure was repeated for the control as well.

Determination of the total phenolic content (TPC) of the crude methanolic extract of Drypetes floribunda

The TPC of the crude extract was determined as follows:
Concentrations of the extract (0.25 to 0.0125%w/v) were prepared. To 0.1mL of the extract in the test tube, 0.5 mL of Folin ciocalteu was added and after 15 minutes, 2.5 mL of 2% $NaHCO_3$ was also added. Set-up was placed in a cool dark place for about 15 minutes and the absorbance measured at 760 nm. Procedure was repeated twice for all the concentrations.
A Standard gallic acid curve was obtained using gallic acid solutions of the appropriate concentrations. The curve was then employed in estimating the TPC of the crude extract.

Isolation of bioactive principles from the crude methanolic extract

The column was packed with the silica using the wet packing technique after a cotton wool had been placed at the bottom of the column prior to the packing. A slurry of the crude methanolic extract was prepared by mixing the extract with silica and some amounts of methanol. The slurry was then poured into the column on top of the silica and cotton wool placed on it to reduce the impact that could have distorted the sample in the column on pouring the solvents into the column. The elution process was done starting from the non-polar solvents to the polar solvents as shown in Table 3. The fractions eluted were collected in glass bottles designated as F_N where N is the number of the successive fraction being collected into the bottle (say the 10^{th} fraction during the isolation procedure was collected in the bottle labelled F_{10}). The appropriate data was recorded. Thin Layer Chromatographic Techniques (TLCs) were conducted on the promising fractions (those with crystals and amorphous solids). The fractions were washed with ethylacetate, recrystallized with ethanol and the TLCs conducted on them in a repeated fashion till the results of the TLCs on such fractions gave just single spots for each of them when observed under the UV fluorescent lamp (both short and long wave). The fraction F_5 was obtained from a solvent system of 60% petroleum ether and 40% chloroform. The fraction F_{10} was obtained from the solvent system of 90% chloroform and 10% ethylacetate. The fraction F_{14} was obtained from a solvent system of 40% chloroform and 60% ethylacetate. The fraction F_{22} was obtained from a solvent system of 10% chloroform and 90% ethylacetate. The retardation factors for the TLC results of the various fractions were recorded. The fractions were then kept in the appropriate labelled vials for further structural elucidation techniques to be conducted on them. An antimicrobial bioassay was then conducted on the fractions F_{10} and F_{14} only as they were sufficient compared to the fractions which were in very limiting

amounts (less than 2 mg). The antimicrobial bioassay was also carried out on the crude methanolic extract.

Identification and structural elucidation

Thin layer chromatography

Thin-layer chromatography was conducted using plates precoated with silica gel 60 GF254. A ratio of 5:3:2 of chloroform, ethylacetate and methanol respectively was used to develop the TLC profile of the F_{10} fraction and for the F_{14} fraction, the same solvent system but in ratio of 5:4:2 respectively. The plates were dried for complete evaporation of solvents and detected under the UV lamp using the long wave and short wave. Spotting was done in triplicates and the R_f was determined and recorded appropriately as the mean and standard deviation.

Chemical and physical tests

Some basic chemical and physical tests were conducted on the fractions F_{10} and F_{14} to verify for the presence of some functional groups which might be present on the isolates and to know the solvents they are soluble in. The Ferric chloride test, litmus paper test and some solubility tests were thus conducted. Melting point determination was also conducted on the fractions.

Ultraviolent-visible analysis

A given solution of the fractions (F_5 and F_{10}) of concentration 0.015% w/v was prepared. A 0.0005%w/v solution of the fraction F_{14} was also prepared. 4 mL of this solution was transferred into a 1cm cuvette and the UV absorbance was measured using a Jenway 7315 spectrophotometer. Chloroform was run as the baseline for F_5 and F_{10} and methanol for F_{14}. The UV absorption spectrum was obtained for each fraction. And the wavelength of maximum absorption (λ_{max}) for each fraction was recorded.

High performance liquid chromatography

An apt chromatographic method was developed for the purpose of attaining a good chromatogram to be used in assessing or ascertaining the purity of the fraction F_{14}.
Column: ODS Supelco C-18 (10 x 4.6mm) 5 µm
Mobile phase: Acetate buffer: Methanol (pH 4.0) (70:30 v/v)
Wavelength of detection: 284 nm
Injection volume: 100 µL
Flow rate: 0.8 mL/min respectively
Column Temperature: 30 degrees Celsius

Infra-red (IR) analysis

2 mg of the fractions were used in the preparation of each respective disc which was placed in a PerkinElmer Spectrum Version 10.03.09 infra-red spectrophotometer and the infra-red spectrum was obtained.

Nuclear Magnetic Resonance (NMR)

10 mg of the sample was dissolved in a given volume of deuterated chloroform to produce a concentration of 10mg/mL in a vial. This was subsequently transferred into NMR tubes for 1H and C-13 analysis using the Bruker Biospin NMR.

Antimicrobial bioassay using the High Through-Put Spot Culture Growth Inhibition (HT-SPOTi)

All the materials and equipment used aside the samples, drugs and microbes were autoclaved at 115 ºC for 30 minutes. The organisms were sub-cultured twice at 24 hours each and used in preparing the appropriate solutions of organisms using normal saline as the diluent. After sub-culturing, about 0.10 mL of the organism were inoculated into 10.00 mL of the normal saline in a labelled falcon tube and the mixture well mixed using the vortex stirrer (mixer). 1.00 mL of the resulting solutions were then pipetted into another set of falcon tubes with 9.00 mL of normal saline and the procedure repeated to the last set of falcon tubes filled with 19.00 mL. A 50 mg/mL stock solutions of the standard drugs and samples were prepared in Eppendorf tube using DMSO (for the standard drugs, crude and fraction F_{14}) and chloroform (for the fraction F_{10}). The 10.00 mL pipette was used in pipetting 1.00 mL of the solvents used in preparing the solutions of the standard drugs and the samples, one to each Eppendorf tube. 100.00 µL of each solution were pipetted using the 200.00 µL pipette into the designated wells in the plate to be used as the library of preparations. 50.00 µL of the respective solvents were pipetted into the 10 wells of the remaining eleven for each drug/sample lane to be used in the dilution of the drugs and the samples. The multiple 200.00 µL pipette was then used to pipette 50.00 µL each of the various solutions from the wells in the lane 1 to the wells in the lane 2 for each horizontal drug lane, mixed with the solvent in the well of lane 2 and again 50.00 µL of the resulting solution was pipetted into the third well and the procedure was repeated in that fashion to the last lane of wells for each drug/sample. The agar was kept in the water bath and temperature maintained at 60 ºc to prevent the agar from solidifying at a lower temperature. A 2.00 µL pipette was used to pipette 2.00 µL of each solution from the plate into the correspondent well in another 96-well plate. The 198 µL of the agar were then added to the solutions in each well and 2.00 µL of the final solution of a particular organism were pipetted into the agar in the wells. The plate was then covered and incubated at 37 ºc for 24 hours in the incubator. The procedure was repeated for all six organisms each to one 96-well plate. The results were then checked after 24 hours and recorded appropriately. The above procedure is as illustrated in Figure 1.

Results

Antioxidant capacity evaluation

On conducting the antioxidant capacity testing on the extracts (crude phenolic acid and crude flavonoid extract) and the control (ascorbic acid) using the DPPH antioxidant capacity evaluation model, the crude flavonoid extract was found to have a higher antioxidant activity with an IC_{50} of 27.78 µg/mL compared to that of the control (51.39 µg/mL) and the crude phenolic acid extract (283.86 µg/mL) as shown in Figure 2a.

Total phenolic content determination (TPC)

In determination of the TPC of the crude methanolic extract, a calibration curve of gallic acid (the standard) was obtained and the TPC determined as a variation from 34.56 ± 2.32 mg/g to 68.79 ± 0.17 mg/g as illustrated in Figure 2b.

Isolation and TLCs

Isolation

The compounds were isolated as demonstrated in Figure 3. Compounds F_{57} and F_{58} were later found to be sugars thus proved irrelevant for further investigations. F_{22} was only sufficient for Infrared analysis to be conducted on and thus no further investigations could be conducted on it. F_{14} and F_{10} were the only fractions that were taken through the various spectroscopic analysis in an attempt to elucidate their unknown structures. An antimicrobial bioassay was further conducted on them.

TLCs

The following TLC results were obtained on trying several permutations of a solvent system of chloroform, ethyl acetate and methanol. A ratio of 5:3:2 of chloroform, ethyl acetate and methanol was used to develop the TLC profile of the F_{10} fraction and that employed for the F_{14} fraction was 5:4:2 of the solvents respectively. Samples were spotted alongside the crude on the TLC plate with Compound F_{10} and F_{14} having R_f of 0.58±0.11 and 0.85±0.01 respectively as shown in Figures 4 and 5.

Chemical and physical tests

The results of the physical and chemical tests conducted on the fractions F_{10} and F_{14} are as shown in Tables 5 and 6 respectively. The results of the melting point determination of fractions F_{10} and F_{14} are also as shown in Table 7.

Spectroscopy

Ultraviolet spectroscopy

The UV spectra were obtained for compounds F_5, F_{10} and F_{14} which showed peak of maximum absorption at 288 nm, 212 nm and 284 nm respectively.

HPLC

The HPLC chromatogram of the blank and the sample (fraction F_{14}) is as shown in Figures 6 (blank) and 7 (sample).

IR Spectroscopy

The Infrared spectra of the various fractions (F_5, F_{10}, F_{14} and F_{22}) obtained are as indicated in Figures 8, 9, 10 and 11.

NMR.

The 1H NMR and C-13 NMR results of the fraction F_{10} obtained are as shown in Figures 12, 13, 14, 15, 16, 17 and 18. An elaborate discussion and interpretation have been given in the final chapter.

HT-SPOTi antimicrobial bioassay

The results of the HT-SPOTi antimicrobial bioassay conducted on the crude and the fractions F_{10} and F_{14} is as illustrated in Table 9.

Discussion

Antioxidant bioassay and total phenolic content determination

There are several antioxidant bioassay models that can be resorted to in evaluating the antioxidant capacity of plant extracts. Some of which are in-vivo and others, in-vitro.

The experiment was conducted on the crude flavonoid extract and the crude phenolic acid extract alongside a standard drug (ascorbic acid). The IC_{50} of the two samples (extracts) were obtained and compared to that of the standard. A lower IC_{50} implies higher potency. This indicates that the flavonoids in the crude extract of the plant, *Drypetes floribunda* contributes more towards the overall antioxidant activity of the crude extract of the plant compared to the phenolic acids in the crude extract. This is because for similar concentrations of the crude flavonoid extract and crude phenolic acid extract, the crude flavonoid extract will give a higher antioxidant activity. This implies that for a given solution of the crude methanolic extract that contains both flavonoids and phenolic acids, its antioxidant activity will be mainly due to the presence of the flavonoids in the extracts. It can also be postulated that the crude extract is much potent in antioxidant activity compared to that of the ascorbic acid (standard) due to the presence of those flavonoids.

This has proven the scientific basis behind its use especially by the people of Kwahu Asakraka in the Eastern Region of Ghana in the treatment of some disease conditions and even infections mostly worsened by oxidative stress or overproduction of free radicals in the body. Some of these conditions include atherosclerosis, cancer, diabetes, rheumatoid arthritis, myocardial infarction to mention a few. This is because it has the propensity to mop out free radicals mostly produced during oxidative stress and also aids in the activation of specific enzymes to help clear out carcinogens which normally happens as part of the cascade events in the pathophysiology of such disease conditions especially cancer. Thus helping to alleviate some of the symptoms associated with such ailments as a result of oxidative stress and the carcinogens resulted from those diseases.

The TPC was done on the extract to determine the equivalent amount of total phenolic compounds present in the extract compared to that of gallic acid which was used in the determination. It is another way of evaluating the total antioxidant capacity of the extract. The TPC of the crude extract varies from 34.56 ± 1.60 mg/g to 68.79 ± 0.96 mg/g.

Isolation and Spectroscopy

TLC on the two sufficient fractions (F_{14} and F_{10}) were done in triplicates and the results (retardation factors) recorded as a mean and standard deviation as follows: 0.85± 0.01 (F_{14}) and 0.58 ± 0.011 (F_{10}). With reference to the solvent systems employed in the TLC as stated in the method and their R_f, it can be postulated the fraction F_{14} is relatively polar hence it was easily carried by the relatively polar solvent system to higher distance on the TLC plate (stationary phase) as it easily interacted with the solvent system or mobile phase employed. F_{10} can be said to be less polar as it was not effectively carried by the relatively polar solvent system to higher distance on the TLC plate (stationary phase).

The melting point range of the fractions F_{10} and F_{14} are 91-93 ºc and 207-209 ºc respectively. The melting point range for both isolates are sharp which shows that they are of good purity.

From the results of some basic chemical and physical tests conducted on the isolates, F_{10} is most likely to be a non-polar

basic compound as it was insoluble in methanol but soluble in chloroform, gave negative result for the ferric chloride test (non-phenolic) and turn red-litmus paper blue (basic). F_{14} on the other hand is most likely to be a polar phenolic compound as it was soluble in methanol but insoluble in chloroform, gave intense coloration for the ferric chloride test (phenolic) and turned blue litmus paper red which is characteristic feature of most phenolic compounds.

UV-VIS spectroscopy

UV spectrum of the fractions F_5, F_{10} and F_{14} were obtained. The λ_{max} of F_5 is 288 nm which indicates the possibility of a highly conjugated system present in the structure of the molecule. The λ_{max} of F_{10} is 212 nm which gives an indication that it could have a highly saturated system in the structure of its molecule. The λ_{max} of the prominent peak of F_{14} is 284 nm which also indicates the possibility of the presence of a highly conjugated system and unsaturation. Both fractions F_5 and F_{14} could have some degree of aromaticity in their respective structures. However same cannot be postulated about the possible structure of the fraction F_{10} as the λ_{max} falls almost outside the UV region and thus could be devoid of a conjugate system.

HPLC

This was done to have a fair idea of the level of purity or to know the number of components present in the sample of the fraction. Following the results of the TLC and the melting point (sharp range of 207-209 ºC) which all indicated that the fraction F_{14} could be a pure compound, the HPLC chromatogram of the fraction was obtained to verify and further substantiate the claim. This was conducted on the isolate F_{14} because it was the only isolate aside the isolate F_{10} left in sufficient amounts with which some could be used for the antimicrobial bioassay. This was also done on the isolate F_{10} however, there were no peaks as depicted from its chromatogram. This could have been because its wavelength of detection was closer to that of the solvent peak and hence its peak would be detected as part of the solvent front by the UV detector. The HPLC chromatogram was obtained to buttress the results of the melting point determination proving that the sample is of good purity.

This was done at various detection wavelengths in which there were no prominent peaks except at the detection wavelength of 284 nm, which gave a single prominent peak indicating the presence of the isolate at a retention time of 18.90 minutes. There were some very small peaks in the chromatogram, which were also present in the blank and thus indicating that there could be impurities from the solvent used in the preparation of the solution of the isolate for the analysis.

Infrared Spectroscopy

There are two regions in IR namely the finger print region and the functional group region. The finger print region is a distinctive feature or characteristic for every compound and it varies. Thus, they are mostly used in verifying the authenticity or originality of known compounds by comparing their finger print region to that from reported literature. The functional group region is the region normally resorted to in the structural elucidation of unknown compounds as it gives a fair idea the functional groups likely to be present in the compound. Thus, in the analysis of the IR results of the isolates, much attention was paid on the functional group region.

The IR spectrum of the fraction F_5 showed prominent peaks at 1632 cm^{-1}, 2850 cm^{-1}, 2918.66 cm^{-1} and 3485.73 cm^{-1}. The presence of a peak at 1632 cm^{-1} indicates the possibility of aromaticity or C=C bond as it falls within the range of 1500-1650. The presence of peaks at 2850 cm^{-1}, 2918.66 cm^{-1} depicts the possibility of aliphatic C-H stretching of C-C bonds as they fall within the range of 2850-3000 cm^{-1}. The broad band with a peak corresponding to 3485.73 cm^{-1} indicates the possibility of the presence of NH_2 or OH undergoing intermolecular Hydrogen bonding which is responsible for the broadening.

The IR spectrum of the fraction F_{10} revealed prominent peaks at 1741.58 cm^{-1}, 1703.24 cm^{-1}, 2851.3 cm^{-1}, 2920.75 cm^{-1} and 3473.57 cm^{-1}. The presence of peaks at 1703.24 cm^{-1} and 1741.58 cm^{-1} indicates the possibility of the presence of a C=O in the structure either in the form of an ester, amide or carboxylic acid. The peak at 2851.3 cm^{-1} and 2920 cm^{-1} shows the possibility of the presence of aliphatic and or cyclic C-H stretching of a C-C bond. The presence of a peak at 3473.57 cm^{-1} indicates the presence of either NH_2 or OH in the structure of the molecule.

For the IR spectrum of the fraction F_{14}, there were prominent peaks at 1518 cm^{-1} and 1647 cm^{-1} indicating the possibility of the presence of aromaticity or double bonds in the structure of the molecule. The peaks at 2849.78 cm^{-1} and 2917.89 cm^{-1} indicates the possibility of the presence of C-H stretching of C-C single bond. The presence of a broad peak at 3344.48 cm^{-1} shows that there could be NH_2 or OH groups undergoing a possible intermolecular hydrogen bonding resulting in the broadening of the peak.

The IR spectrum of the fraction F_{22} gave peaks at the following wave numbers: 1642.2 cm^{-1}, 1740.48 cm^{-1}, 2852.03 cm^{-1}, 2921.14 cm^{-1} and 2954.28 cm^{-1}. The presence of a peak at 1642.2 depicts some aromaticity or unsaturation such as C=C bond in the structure of the molecule. The peak at 1740.48 cm^{-1} indicates the presence of C=O most likely to be an ester. The peaks at 2852.03 cm^{-1}, 2921.14 cm^{-1} and 2954.28 cm^{-1} depicts the possibility of an aliphatic C-H stretching of C-C bonds in the structure of the molecule.

These are the possible predictions of the various functional groups likely to be present in the isolates. For absolute certainty of the structure of the molecule and its configuration, further spectroscopic techniques such as the UV and NMR were conducted on the fractions.

NMR Spectroscopy

1H NMR and C-13 NMR spectroscopy were conducted on the fractions F_{10} and F_{14}. The fraction F_{10} has been fully elucidated from the results obtained. However, work is currently on going in the interpretation of the results of the fraction F_{14} and thus, it is yet to be elucidated.

The 1H NMR gives an indication of the presence of the various functional groups present in the molecule and also in some instances tells the saturation pattern of the compound as in the case of the 1H NMR of sterols, stanols, steranes and derivatives of cholestanoic acid in which the peaks from chemical shifts of δ_H 0.6-2.2 ppm shows a high degree of saturation and hence appear shady.

With reference to the fraction F_{10}, the region from around δ_H 0.6 ppm to 2.2 ppm showed a high degree of saturation as it appeared shady which is a characteristic feature of the 1H NMR of phytosterols, phytostanols and steranes in general referencing a classical example of β-sitosterol. The peaks at δ_H 3.15 ppm and 3.47 ppm indicate the presence of deshielded methylene groups

resonating further downfield of 1.3 as a result of the close proximity of their environment to that of the C=O. The one further downfield at δ_H 3.47 ppm is due to the protons from the methylene group closer to the C=O. Peaks at δ_H 4.1 ppm and 4.41 ppm are indicative of the protons from the OH group (occur between δ_H 0.5-5 ppm). The amide consists of a carbonyl and an amino group. The carbonyl has the likelihood to deshield the protons in the amino group causing it to resonate at a further downfield compared to that of the protons of the OH group. Thus, the amide peak occurs between δ_H 5-9 ppm and hence most likely to be the peak at δ_H 5.11 ppm.

COSY 2D 1H NMR was done to show the correlation, the presence of cross peaking of the protons on adjacent carbons in the various groups to help in predicting the most likely configuration of the various groups relative to each other. The main protons are located on the diagonal and cross peaking between groups can be predicted by placing a vertical line on a proton in a particular group in the diagonal and moving another horizontal line up the vertical line to meet another proton in the vertical lane of the proton in the diagonal. This indicates protons from the group in the diagonal or group of interest undergoes cross peaking with the protons in the other group in its vertical lane. Which implies that both groups might be in close proximity to each other. As illustrated in Figure 13 and in Figure 18 on the structure, the following correlations occurred amongst protons with the following resonance frequencies: δ_H 2.30 ppm and 1.6 ppm, δ_H 3.15 ppm and 3.47 ppm and δ_H 4.41 ppm and 1.6 ppm. This was used to estimate the configuration of the various groups of the molecule relative to each other in the molecule.

The C-13 NMR was also done on the isolates and the results are interpreted as follows:

Peaks from δ_C 10-35 ppm are indicative of the presence of methyl groups in the molecule. Those from δ_C 15-50 ppm indicate the presence of methylene (R-CH_2) groups within the molecule. Which implies that some peaks due to the CH_3 groups in the molecule and those due to the R-CH_2 groups in the cyclic framework structure and on the side chain at C-23 and C-24 overlap in their resonant frequencies and there are a lot of them in the molecule and hence accounting for the shady region from δ_C 15-45 ppm in the C-13 NMR. The peaks between δ_C 30-40 ppm could also indicate the presence of quarternary carbon at C-4, C-10, C-13 and C-20. Some peaks within the δ_C 20 to 60 ppm range could also indicate the presence of tertiary carbon at C-5, C-9, C-14 and C-17 in the structure of the molecule. The peak at 70 ppm could be due to the C-O at C-22 or deshielded methylene carbons at C-23 and C-24.

Peaks between δ_C 100 to 150 ppm are indicative of the presence of double bonds within the molecule. Thus, the peaks at δ_C 122.10 ppm and 144.21 ppm could be due to the double bond between C-20 and C-22. The presence of a peak between δ_C 165-175 ppm shows there could be an amide group within the molecule. Thus, the peak at δ_C 173.75 ppm indicates the most likely possibility of the amide group at the terminal end of the structure of the molecule of the fraction. The C-13 NMR showed 28 carbon atoms.

The HMBC shows the correlation between the proton NMR and the C-13 NMR. It gives the correlation between protons and carbons separated by two, three or four bonds and is useful in conjugate systems. As illustrated in the Figure 18, there is a correlation between δ_H 2.30 ppm (C-17 proton) and δ_C 25 ppm (C-21 Carbon) and 173.75 ppm (C=O) on the side chain. There are two more correlations with one between δ_H 1.60 ppm and δ_C 30 ppm and the other between δ_H 1.20 ppm (C-18 protons) and δ_C 144.21 ppm (C-20 carbon). The HSQC as illustrated in Figure 16 gives information on the proton carbon single bond correlation. The DEPT NMR as illustrated in Figure 17 helps to differentiate between the CH_3 group, the CH_2 and the CH group and even quarternary carbons. The DEPT NMR showed a total of 11 methylene groups as seen in the negative phase of the DEPT 135 NMR with the remainder on the positive phase denoting CH_3 and CH groups within the structure of the molecule apart from the quarternary carbons and the carbon accounting for the chemical shift at δ_C 173.75 ppm which did not show on the DEPT NMR as the carbon (C=O) accounting for that shift has no hydrogen and thus further buttress the possibility of it being a carbonyl aside the other proofs from the proton NMR. This helps to narrow down several permutations alongside with the COSY to facilitate in arriving at an absolute or closer to the absolute structure and configuration of the molecule.

Proposed structure of the fraction F_{10}

The proposed structure of the fraction F_{10} is as illustrated in Figure 19. The results of the various identification tests including chemical, physical tests and the spectroscopic analysis of the compound with UV, IR, 1H and C-13 NMR analysis were interpreted and put together to enable the structural elucidation of fraction F_{10}. The proposed structure of fraction F_{10} has been subjected to SciFinder CAS 2018 edition search and no matching has been found. The Log P value of the fraction was found to be 5.61 and it means the drug would be best administered sublingually to exert its pharmacological action fully. The Log P value (Partition coefficient) helps estimate the biopharmaceutical properties of the drug and helps in designing the appropriate dosage forms and means of administration of the drugs to improve upon its onset of action and to increase the duration of action.

Antimicrobial bioassay using the HT-SPOTi model

The crude methanolic extract of the stem bark of *Drypetes floribunda* and the fractions (F_{14} and F_{10}) obtained from it showed significant antimicrobial activity against some micro-organisms implicated in causing infections of common occurrence such as typhoid, Urinary Tract Infections (UTIs), boils and some fungal infections. The assay was conducted using four (4) standard drugs namely amoxicillin, ciprofloxacin, flucloxacillin and fluconazole for comparative analysis of the potency of the crude methanolic extract and the fractions with the standard drugs as well.

The crude extract showed activity against all the organisms with the exception of *P. aeroginosa* and *Streptococcus pyogenes*.

The fraction F_{10} showed activity against *S. typhi* (125 µg/mL), *E. coli* (250 µg/mL) and *C. albicans* (500 µg/mL).

The fraction F_{14} showed activity against *S. typhi* (250 µg/mL), *E. coli* (500 µg/mL), *S. aureus* (125 µg/mL) and *C. albicans* (31.25 µg/mL).

The standard drugs exhibited relatively higher activities against most of the organisms. However, with the exception of ciprofloxacin, none of them showed activity against *Salmonella typhi*.

Ciprofloxacin showed activity against *S. typhi* at an MIC of 250 µg/mL, which is relatively higher compared to that of F_{10} proving that F_{10} is more potent (lower MIC hence more potent) against *S. typhi* than the standard, ciprofloxacin, which is normally resorted to in the treatment of infections caused by *S. typhi*.

The fraction F_{10} showed higher activity against gram-negative organisms such as *E. coli* and *S. typhi* compared to the

fraction F_{14}. The fraction F_{14} showed higher activity against gram-positive organisms.

In the light of the menace posed by the rapidly increasing resistance of *Salmonella typhi* to fluoroquinolones, the extract from this plant serves as a good candidate to resort to in unearthing potential lead compounds such as the fraction F_{10} which can even be further modified and developed into more potent potential lead compounds to help get more drugs with higher significant activity against *S. typhi*. Moreover, this eventually can help curb the menace of *S. typhi* resistance to fluoroquinolones such as ciprofloxacin and levofloxacin.

The fraction F_{10} could also be used as a template to develop more potent potential lead compounds that can be used in the discovery of more anti-infective drugs against coliforms and other gram negative organisms as well as fungi such as those of the *Candida* species.

The fraction F_{14} also proven to be a promising candidate to be used as a template in the development of more potent antibiotics against gram-positive organisms.

Table 1. Profile of drug samples used

Drug	Batch number
Amoxicillin.$3H_2O$	170114141
Ciprofloxacin HCl	HB OON1704016
Flucloxacillin Na	P/FCX-0213/17
Fluconazole	3A18160301
Ascorbic acid	201701004

Table 2. Microorganisms and their culture type

Microorganism	Culture type
Pseudomonas aeroginosa	Clinical strain/ ATCC 4853
Salmonella typhi	Clinical strain
Escherichia coli	ATTC 25922
Streptococcus pyogenes	Clinical strain
Staphylococcus aureus	ATCC 25923
Candida albicans	Clinical strain

Table 3. Table of the column chromatography elution under gravity

Fraction (F)	Solvent system	Colour
1	Petroleum ether (100%)	Light green
2	Petroleum ether (100%)	Colourless
3	Petroleum ether + $CHCl_3$ (8:2)	Colourless
4	Petroleum ether + $CHCl_3$ (6:4)	Colourless
5	Petroleum ether + $CHCl_3$ (6:4)	Light yellow
6	Petroleum ether + $CHCl_3$ (6:4)	Colourless
7	Petroleum ether + $CHCl_3$ (4:6)	Colourless
8	Petroleum ether + $CHCl_3$ (2:8)	Colourless
9	Chloroform (100%)	Colourless
10	$CHCl_3$ + Ethylacetate (9:1)	Bright yellow
11	$CHCl_3$ + Ethylacetate (9:1)	Colourless
12	$CHCl_3$ + Ethylacetate (8:2)	Colourless
13	$CHCl_3$ + Ethylacetate (7:3)	Colourless
14	$CHCl_3$ + Ethylacetate (6:4)	Pale yellow
15	$CHCl_3$ + Ethylacetate (6:4)	Light yellow
16	$CHCl_3$ + Ethylacetate (6:4)	Colourless
17	$CHCl_3$ + Ethylacetate (5:5)	Colourless
18	$CHCl_3$ + Ethylacetate (4:6)	Pale yellow
19	$CHCl_3$ + Ethylacetate (4:6)	Colourless
20	$CHCl_3$ + Ethylacetate (3:7)	Colourless
21	$CHCl_3$ + Ethylacetate (2:8)	Colourless
22	$CHCl_3$ + Ethylacetate (1:9)	Pale Orange
23	$CHCl_3$ + Ethylacetate (1:9)	Pale Orange
24	$CHCl_3$ + Ethylacetate (1:9)	Pale Orange
25	$CHCl_3$ + Ethylacetate (1:9)	Colourless
26	Ethylacetate (100%)	Pale orange
27	Ethylacetate (100%)	Pale Orange

28	Ethylacetate (100%)	Pale Orange
29	Ethylacetate (100%)	Pale Orange
30	Ethylacetate (100%)	Colourless
31	Ethylacetate + CH_3OH (9:1)	Light orange
32	Ethylacetate + CH_3OH (9:1)	Light orange
33	Ethylacetate + CH_3OH (9:1)	Light orange
34	Ethylacetate + CH_3OH (9:1)	Light orange
35	Ethylacetate + CH_3OH (9:1)	Light orange
36	Ethylacetate + CH_3OH (9:1)	Colourless
37	Ethylacetate + CH_3OH (8:2)	Bright orange
38	Ethylacetate + CH_3OH (8:2)	Bright orange
39	Ethylacetate + CH_3OH (8:2)	Pale orange
40	Ethylacetate + CH_3OH (8:2)	Pale orange
41	Ethylacetate + CH_3OH (8:2)	Colourless
42	Ethylacetate + CH3OH (7:3)	Deep orange
43	Ethylacetate + CH3OH (7:3)	Orange
44	Ethylacetate + CH3OH (7:3)	Pale orange
45	Ethylacetate + CH3OH (7:3)	Pale orange
46	Ethylacetate + CH3OH (7:3)	Colourless
47	Ethylacetate + CH3OH (6:4)	Deep orange
48	Ethylacetate + CH3OH (6:4)	Deep orange
49	Ethylacetate + CH3OH (6:4)	Deep orange
50	Ethylacetate + CH3OH (6:4)	Deep orange
51	Ethylacetate + CH3OH (6:4)	Deep orange
52	Ethylacetate + CH3OH (6:4)	Deep orange
53	Ethylacetate + CH3OH (4:6)	Deep orange
54	Ethylacetate + CH3OH (4:6)	Deep orange
55	Ethylacetate + CH3OH (4:6)	Deep orange
56	Ethylacetate + CH3OH (4:6)	Deep orange
57	Ethylacetate + CH3OH (4:6)	Deep orange
58	Ethylacetate + CH3OH (4:6)	Deep orange
59	Ethylacetate + CH3OH (4:6)	Deep orange
60	Ethylacetate + CH3OH (3:7)	Deep orange
61	Ethylacetate + CH3OH (2:8)	Deep orange
62	Ethylacetate + CH3OH (1:9)	Deep orange
63	Methanol (100%)	Deep orange

Table 4. Results for the TPC determination

Crude methanolic extract concentration (%w/v)	**Gallic acid equivalent [X] (mg/g)**			**Mean± SD (mg/g)**
	X_1	**X_2**	**X_3**	
0.100	34.78	32.50	36.40	34.56±1.5997
0.250	69.81	67.51	69.05	68.79±0.9568

Table 5. Chemical and physical test results of fraction F_{10}

Test	**Observation**	**Inference**
Solubility test	Dissolved completely in chloroform but insoluble in methanol	Fraction may be non-polar
$FeCl_3$ test	No intense coloration was observed	Phenols may be absent
Litmus paper test	Red litmus paper changed to blue	Fraction may be basic
	Blue litmus paper remained blue	

Table 6. Chemical and physical test results of fraction F_{14}

Test	**Observation**	**Inference**
Solubility test	Dissolved completely in methanol but partially soluble in chloroform	Fraction may be polar
$FeCl_3$ test	Intense purple coloration was observed	Phenols may be present
Litmus paper test	Blue litmus paper changed to red	Fraction may be acidic
	Red litmus paper remained red	

Table 7. Melting point of fraction F_{10} and fraction F_{14}

Compound	**Melting point (ºC)**		
Fraction F_{10}	91-93	91-92	91-93
Fraction F_{14}	206-208	207-209	207-209

Table 8. Peak table of the IR spectrum of F_{22}

X (cm^{-1})	Y (%T)
2954.28	82.45
2921.14	59.34
2852.03	70.85
1740.48	94.61
1642.2	95.94
1463.04	84.18
1377.15	90.67
1260.68	88.33
1185.39	92.07
1081.66	87.97
1020.83	88.21
968.18	92.38
800.65	85.17
720.48	89.51

Table 9. HT-SPOTi antimicrobial bioassay MIC results

Drug/Sample	Minimum Inhibitory Concentration [MIC] (µg/mL)					
	C. albicans	*E. coli*	*S. pyrogenes*	*P. aeroginosa*	*S. aureus*	*S. typhi*
Crude	250	250	-	-	250	125
F_{10}	500	250	-	-	-	125
F_{14}	31.25	500	-	-	125	250
Amoxicillin	3.91	15.63	250	31.25	31.25	-
Ciprofloxacin	1.95	7.81	3.91	1.95	1.95	250
Flucloxacillin	125	62.5	500	62.5	15.63	-
Fluconazole	250	500	-	250	125	-

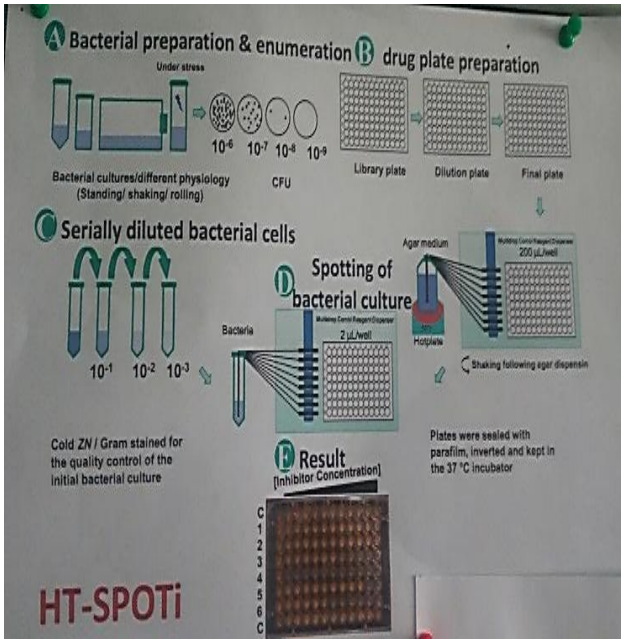
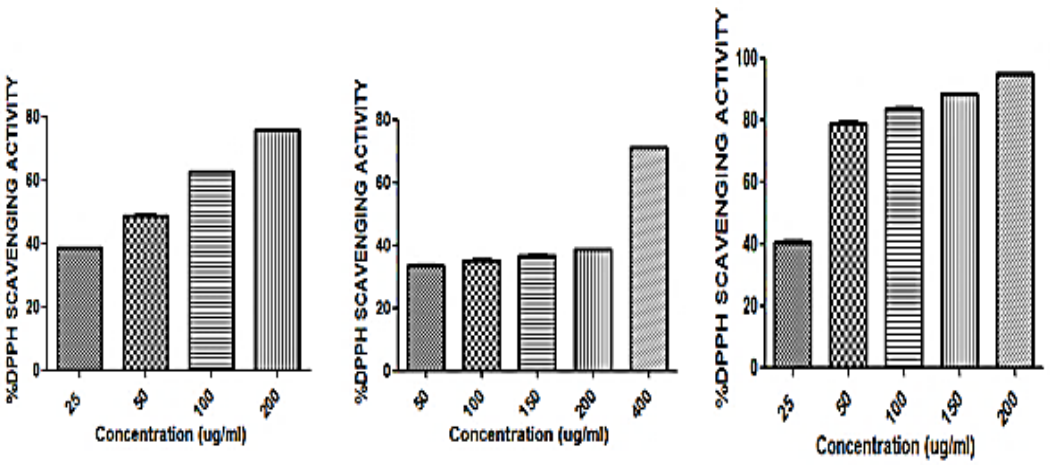


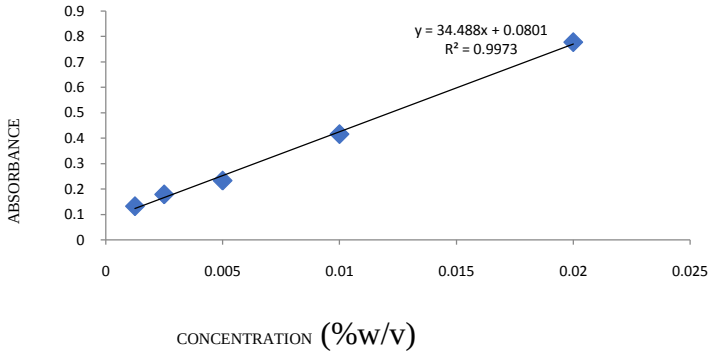
Figure 1. HT-SPOTi antimicrobial bioassay scheme [13]

CONTROL PHENOLIC ACID FLAVONOID



IC_{50} = 51.39 µg/mL **IC_{50} = 283.86 µg/mL** **IC_{50} = 27.78 µg/mL**

Figure 2a. Antioxidant capacity of crude extracts and control



The TPC varied from 34.56 ± 2.32 mg/g to 68.79 ± 0.17 mg/g

Figure 2b. Calibration curve for TPC of gallic acid (Standard)

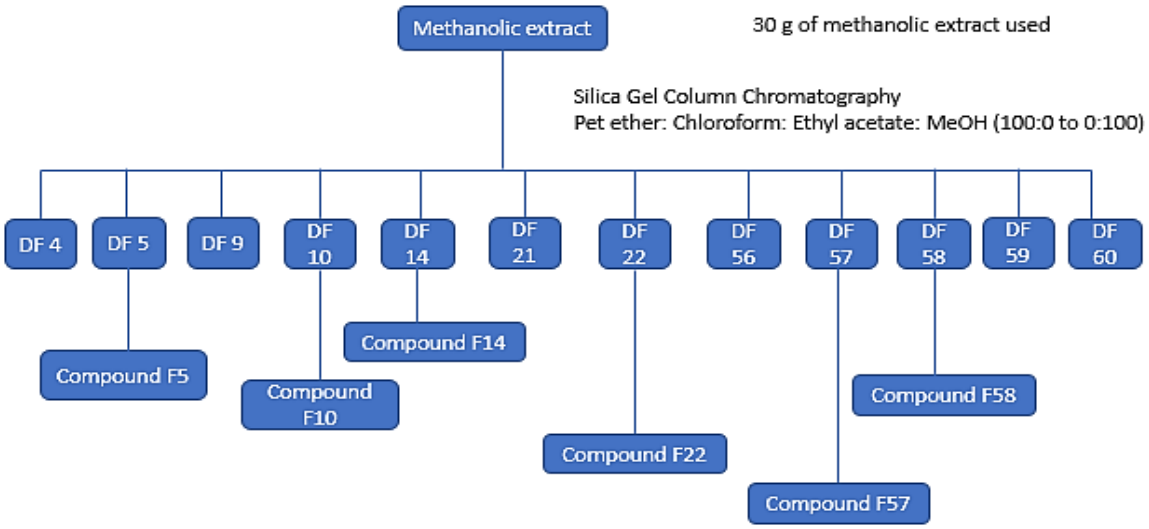


Figure 3. Isolation of compounds from the crude methanolic extract

Compound F_{10}
R_f = 0.85±0.01

Figure 4. TLC of F_{10}

Compound F_{14}
R_f = 0.58±0.11

Figure 5. TLC of F_{14}

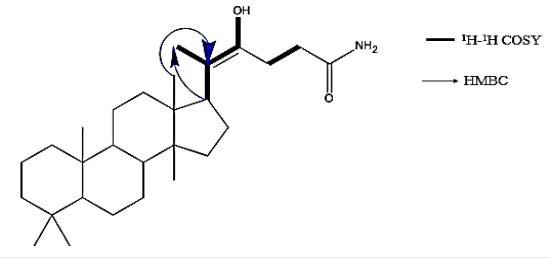
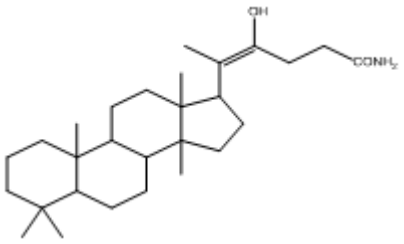


Figure 18. Selected ^{1}H-^{1}H COSY and HMBC 2D NMR correlations on the structure of fraction F_{10}



4, 4, 14-trimethyl-22-hydroxyl-24-norcholestanamide

Figure 19. Proposed structure of fraction F_{10}

Conclusions

The flavonoids in the plant contribute predominantly towards the overall antioxidant potential of the plant as the IC_{50} of the crude phenolic acid extract and the crude flavonoid extract obtained were 283.86 µg/mL and 27.78 µg/mL respectively. The total phenolic content of the crude methanolic extract of the stem bark varied from 34.56 ± 1.60 mg/g to 68.79 ± 0.96 mg/g. Concerning the proposed structure of the fraction F_{10} obtained from the crude methanolic extract, it is a cholestanamide as it has the cholestane framework with an amide at the terminal. The proposed structure has been proven to be novel as no match was found on running the structure through the SciFinder CAS 2018 Edition. The Log P value of the fraction F_{10} is 5.61 which means it is about 5.6 times more soluble in octanol (non-polar) than in water (polar). The

fraction F_{14} is yet to be fully characterized. The crude methanolic extract, the fractions F_{10} and F_{14} showed promising antimicrobial activity against *E. coli, C. albicans, S. aureus* and *S. typhi*. The fraction F_{10} showed higher significant activity as the crude methanolic extract against *S. typhi* at an MIC of 125 µg/mL compared to the standard drugs used of which with the exception of ciprofloxacin showed no activity against the *S. typhi*.

Additional file

Figures 6 to 17 are as illustrated in the supporting additional information file. Available at: https://www.investchempharma.com/imcp32-supporting-information/

Abbreviations

TLC: Thin layer chromatography; UV-Vis: Ultra-violet -Visible radiation spectrophotometry; IR: Infra-Red Spectrophotometry; HPLC: High Performance Liquid Chromatography; HT-SPOTi: High Throughput Spot Culture Growth Inhibition; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; COSY: Correlation Spectroscopy; DEPT: Distortionless Enhancement Polarization Transfer; HMBC: Heteronuclear Multiple Bond Correlation; HSQC: Heteronuclear Single Quantum Correlation.

Authors' Contribution

All authors contributed effectively to sourcing materials and equipment, standard operating procedures (SOPs) and performance of protocols as well as the collation and analysis of data and manuscript finalization. All authors read and approved the final manuscript.

Acknowledgments

The authors thank the technicians and staff of the Department of Pharmaceutical Chemistry for their immense contribution. We gratefully acknowledge the Department of Chemistry, KNUST for their support.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Article history:

Received: 5 August 2019
Received in revised form: 25 August 2019
Accepted: 25 August 2019
Available online: 26 August 2019

References

1. Krishnaiah, D., Rosalam, S., and Rajesh, N. 2011. A review of the antioxidant potential of medicinal plants species. Food Bio Production Process. 89: 217-233.
2. Savoia, D. (2012). Plant-derived antimicrobial compounds: Alternatives to antibiotics. Future Microbiol. 7: 979-990.
3. Amor, I.L.B., Boubaker, J., Ben Sgaier, M., Neffati, A., Kilani, S., Bouhlel, I. et al 2009. Phytochemistry and Biological activities of Phlomis species. Journal of Ethnopharmacology.125: 183-202.
4. Hossain, K., Hassan, M., Nazma, P., Siddiqul, I., Mahmudul, H., Ahsanul, H. 2012. Antimicrobial, cytotoxic and thrombolytic activity of *Cassia senna* leaves (Family: Fabaceae). J. Applied Pharm. Sci., 2: 186-190.
5. Riffel, A., Medina, V., Santos, R.C., Bizani, D. and Brandelli, A. 2002. In vitro antimicrobial activity of a new series of 1, 4-naphthoquinones. Brazilian Journal of Medical and Biology Research. 35: 811-818.
6. Daffodil, M., Packia, L., Tresina, E.D, and Mohan, P.S. 2014. Estimation of Total Phenolics and Flavonoids Content and In-Vitro Antioxidant Activity of *Drypetes sanitaria* stem. Ethnopharmacology Unit. U. D Chidambarum College. Tamil Nadu.
7. Cazarolli, L.H., Zanatta, L., Alberton, E.H., Figueiredo, M.S., Folador, P., Damazio, R.G. et al 2008. Flavonoids: Prospective Drug Candidates. Mini Review Med Chem. Department de Bioquimìca, Centro de Ciências Biològicas, Campus Universitário, UFSC, Bairro Trindade, Cx. Portal 5069. CEP: 88040-970, Florianòpolis, SC, Brazil. Nov; 8 (13): 1429-1440.
8. Ferrer, A., Altabella, T., Arró, M., and Boronat, A. 2017. Emerging roles for Conjugated Sterols in plants. Progress in Lipid Research. 67: 27-37.
9. Sharma, R.K. (1993). Phytosterols: Wide Spectrum Antibacterial Agents. Cited by 30.
10. www.eaps.miy.edu/geobiology/biomarkers/steroids.html. Retrieved on 12/08/2018 at 22:10 GMT.
11. axminarayan, R., Duse, A., Wattal, C., Zaidi, A.K., Wertheim, H.F., Sumpradit, N. et al 2013. Antibiotic resistance-the need for global solutions. Lancet Infectious Diseases. 13: 1057-1098.
12. Bragginton, E.C., and Piddock, L.J. 2014. UK and European Union Public and Charitable fund-raising from 2008 to 2013 for Bacteriology and antibiotic research in the UK: An observational study. Lancet Infectious Diseases. 14: 857-868.
13. Danquah, C.A., Maitra, A., Gibbons, S., Faull, J., and Bhakta, S. 2016. HT-SPOTi: A rapid drug susceptibility test (DST) to evaluate antibiotic resistance profiles and novel chemicals for anti-infective drug delivery. Curr. Protoc. Microbiol. 40: 17.8.1-17.8.12.
14. Weidner, S., Amarowicz, R., Karamać, M. and Dąbrowski, G. 1999. Phenolic acids in caryopses of two cultivars of wheat, rye and triticale that display different resistance to pre-harvest sprouting. European Food Research and Technology. 210: 109-113.
15. Stalikas, C.D. (2007). Review: Extraction, Separation and Detection methods for Phenolic acids and Flavonoids. Journal of Separation Science