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(RESEARCH ARTICLE)

Isolation and characterization of chemical constituents, cytotoxicity, antibacterial and antioxidant activity of the isolates, crude extract from *Hornstedtia scyphifera var* leaf

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Abstract

Hornstedtia scyphifera var belongs to ginger family, they are most of time used as spices for cooking, preservatives in food industry and colouring agents. It provides health-promoting effects to cutile certain diseases and ailment such as stomach-ache, Catarrh, asthma, diarrhoea, cold and cough, digestive disorder and rheumatism. In this study, we isolated phytochemicals and investigated the potentials of the leaf crude extract of hexane, dichloromethane, chloroform, ethyl acetate and methanol, cytotoxicity, antioxidant and antibacterial activity. The experiment was performed and two phytochemicals (quercetin and Dodecanoic acid) were isolated and characterised. Significant result was obtained from the isolated compounds and solvent crude extract with hexane and methanol have higher cytotoxicity of 35.462 and 34.059, chloroform and methanol with higher antioxidant of 34.46±0.32 and 35.33±0.210. There was an observed significant activity at 500 µg/mL in all the extracts against all the selected pathogen with zone of growth inhibition ranging from 07.70 ± 27.30 mm to 27.30 ± 0.10 mm in all the solvent extract. The result indicated that with the isolation of quercetin and dodecanoic acid from *Hornstedtia scyphifera var* Leaf extract is a potential plant medicine which can be harnessed as an agent for antioxidant and potential antibacterial. The compound was isolated for the first time from *Hornstedtia scyphifera var.*

Keywords: Isolation; Characterization; Constituents; Cytotoxicity; Antibacterial; Antioxidant; *Hornstedtia scyphifera*; GCMS; NMR; FTIR

1. Introduction

Numerous efforts by researchers have been directed towards the provision of empirical proof to back the use of tropical plants in traditional medicinal practice. Focus on medicinal plant research has increased worldwide and evidence abounds in the immense potentials of medicinal plants used in various traditional systems (Dahiru *et al*., 2010).

Various medicinal plants have been studied using different scientific approaches and results from these studies have revealed the potentials of medicinal plants in pharmacology (Fatahi *et al.,* 2003). These medicinal plants such as *Hornstedtia scyphifera var* are of great importance to the health of the individuals and communities to larger extend, and nutritional benefits are derived from these plants since they are commonly used as vegetables.

Hornstedtia scyphifera var belongs to ginger family, they are most of time used as spices for cooking, preservatives in food industry and colouring agents. However, this species *Hornstedtia scyphifera var* can provide health-promoting

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effects to cutile certain diseases and ailment such as stomach-ache, Catarrh, asthma, diarrhoea, cold and cough, digestive disorder and rheumatism (Jani *et al*., 2016).

There are more than ten species, including *H. scyphifera, H. ophiuschus* and *H. phaeochoana* found in Sarawak Malaysia (Hashim *et al*., 2014). The leaf of *Hornstedtia scyphifera var* leaf was consumed by the Javanese for flavouring, as well as to protect crops from insects by burning them. They are used as an external application to get relief from fever (Holtum, 1950).

The aim of this work is to isolate the phytochemicals and evaluate the potential of the leaves crude extract of its cytotoxicity, antimicrobial and antioxidant.

To the best of our knowledge, there has not been report on any isolated pure compound and bioactivities of this specie *Hornstedtia scyphifera var*. Here, we are reporting the isolated compound, cytotoxicity, antimicrobial and antioxidant properties of the leaves extracts of Malaysian *Hornstedtia scyphifera var.*

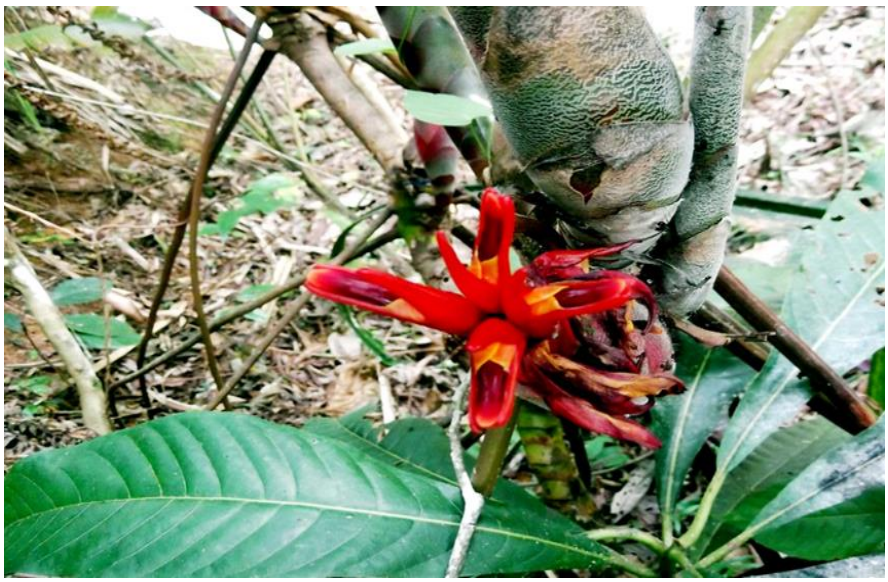


Figure 1 *Hornstedtia scyphifera var* showing the leaf, flower and stem-bark

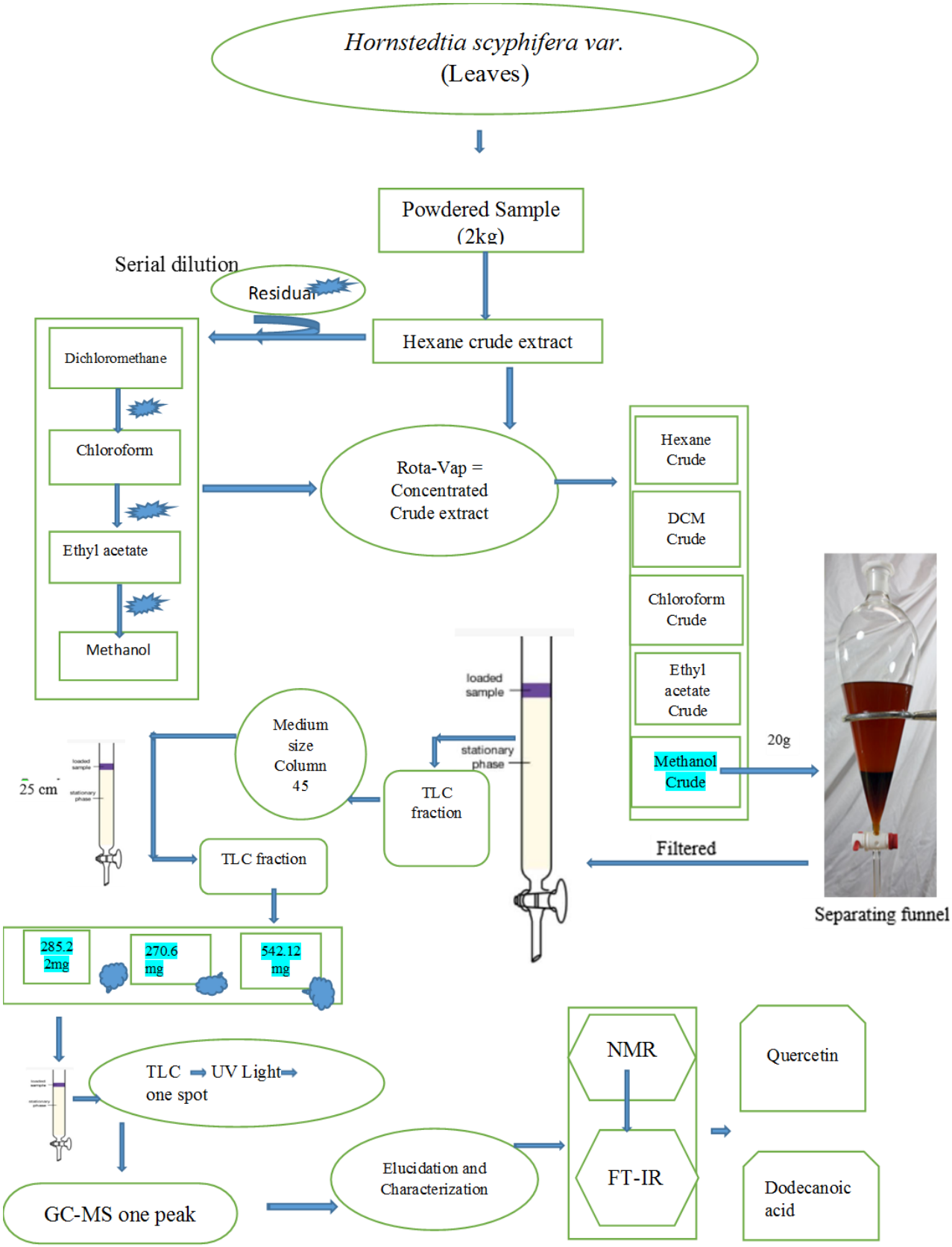


Figure 2 Flow chart for Isolation of pure Compounds

2. Material and methods

2.1. Plant Material

2.1.1. Sample Collection

The plant material was collected in Singh, Sarawak, Malaysia They were authenticated by a Botanist in the Universiti Malaysia Sarawak. The identified and certified plant materials were given a voucher number as IJU/2016/15010238 (*Hornstedtia scyphifera var.)* and The fresh leaves from the plant was carefully plucked and washed under running tap water. It was then air dried to be free of water and then spread in the laboratory and allow to dry at room temperature until they were fully dried.

2.1.2. Sample Preparation

Dried plant materials (leaves) were ground into fine powder form using laboratory pestle and mortar and electric grinder. The finely ground powdered samples (mesh 30) were packed into clean, dry sample containers and were labelled appropriately and kept for further use. Extraction was carried out by the conventional solvent extraction method described by Fasihuddin *et al.* (2010) and Isaac *et al*, (2018). This was achieved by soaking the ground plant material in solvents in the order of increasing polarity as shown in Figure 2. A total of 2 kg of the dried and ground powdered sample was extracted using cold soaking method with hexane. The sample were soaked in the hexane with the ratio of 1:3 (sample: hexane) in a 5 liters Erlenmeyer flasks at room temperature for 7 days. The resulting hexane solution was then filtered using Whatman filter paper No 4 and the residue was then re-extracted with fresh hexane for another 72 hrs and filtered. Both extracts were combined and evaporated to dryness with a rotary evaporator (Heidolph Laborota 4000 efficient) under reduced pressure below 50 ^{o}C to obtain the hexane crude extract. The residue was re-extracted using similar procedure with dichloromethane, chloroform, followed by ethyl acetate and methanol to obtain respective crude extracts. The dry weight and percentage yield of each crude extract were determined (simple percentage).

2.2. Isolation and Identification of Secondary Metabolites

2.2.1. Isolation and Purification

Column Chromatography

The basic principle of column chromatography is to separate a mixture of metabolites based on their molecular weight and polarity. A glass column of size 40/34 (large) was used for chromatography, and the sorbent used was silica gel 60 (Merck 70-230 Mesh ASTM 0.063 0.200 mm). Silica gel slurry was prepared by dissolving silica gel (150 g) with suitable solvent, usually hexane. The column was prepared by pouring a slurry mixture of silica gel and solvent, into a glass column and allow it to settle down (Firdous *et al.*, 2013). The packed column was left overnight before 4-10 g of sample was introduced onto the top of the packed column via wet-packing method or dry-packing method. The column was eluted with suitable solvent systems with increasing polarity (Fasihuddin *et al.*, 2010). The column's valve was then opened and about 10-30 mL fraction of the solvent coming out from the column was collected in test tubes (Patra *et al.*, 2012). The procedure was repeated by using different solvent systems, based on increasing polarity (Table 1). Samples from the column fractions were examined by using TLC plates in few suitable solvent systems to obtain the retention factor (R_f) of any components that appeared as spots. Fractions with similar R_f values were combined (Patra *et al.*, 2012). Fractions which contain more than one component were further isolated and purified by using smaller glass column of sizes 24/29 (medium) or 14/23 (small) with suitable solvent systems.

Fraction with single component (one spot) that appeared in TLC plate was treated as possible pure secondary metabolite. The combined fractions which contained the same single component was then allowed to air-dried or evaporated to dryness to obtain a pure secondary metabolite.

Table 1 Organic solvent systems used as eluting solvents for column chromatography

S/n	Solvent	Volume to volume ratio (v/v)	Volume (mL)
1	Hexane	1	200
2	Hexane: DCM	19:1	200
3	Hexane: DCM	15:1	200
4	Hexane: DCM	9:1	200
5	Hexane: DCM	5:1	200
6	Hexane: DCM	1:1	200
7	DCM	1	200
8	DCM: $CHCl_3$	19:1	200
9	DCM: $CHCl_3$	15:1	200
10	DCM: $CHCl_3$	9:1	200
11	DCM: $CHCl_3$	5:1	200
12	DCM: $CHCl_3$	1:1	200
13	$CHCl_3$	1	200
14	$CHCl_3$: EA	19:1	200
15	$CHCl_3$: EA	15:1	200
16	$CHCl_3$: EA	9:1	200
17	$CHCl_3$: EA	5:1	200
17	$CHCl_3$: EA	1:1	200
18	EA	1	200
20	EA: MeOH	19:1	200
21	EA: MeOH	15:1	200
22	EA: MeOH	9:1	200
23	EA: MeOH	5:1	200
24	EA: MeOH	1:1	200
25	MeOH	1	200

DCM: dichloromethane, $CHCl_3$: chloroform, EA: ethyl acetate, MeOH: methanol.

2.2.2. Thin Layer Chromatography (TLC)

The eluents collected from column chromatography, were subjected to thin layer chromatography (TLC) analysis. TLC was carried out using the method described by Isaac *et al*. (2019). A glass capillary tube was used to apply samples on the TLC plates (size 6.6 x 20 cm, 5 x 20 cm) repeatedly with a spot of about 0.3 mm in diameter. The TLC plate was then placed in a rectangular glass developing chamber with its lower marked edge (1 cm from the base) dipped into a developing solvent below the mark where the samples were spotted. The plates were allowed to develop to the level of upper mark (4 cm from the base) and then removed and dried. The TLC plates were then viewed directly for colored compounds, it was also viewed under UV box for UV fluorescent compound and stained with vanillin for compound that are neither visible nor UV fluorescence. Fractions containing similar characteristics were combined and dried.

2.3. Chemical Structure Elucidation

2.3.1. Gas Chromatography – Mass spectrometry (GC-MS)

Gas chromatography (GC) analysis of fractions that were obtained from TLC as single spot was performed using a Shimadzu GC-Mass Spectrometry model QP2010 plus, equipped with a BPX-5 column (5% phenyl polysylphenlenesiloxane) of 30 m in length, film thickness of 0.25 µm and internal diameter of 0.25 mm. The operating method was based on the method described by Umaru *et al*. (2019). Ionization energy of 70 eV was used in the electron ionization energy system of the GC-MS for detection and carrier gas, helium (99.999%) at a constant flow rate of 1 mL per min was used. Exactly 1 µL of purified sample was injected into the GC-MS using a syringe and sample was analysed using split mode with ration of 25:1. Injection temperature was set at 260 ^{o}C and the oven temperature was programmed from 60 ^{o}C with an increase of 10 ^{o}C per min, isothermal for 5 min, to 280 ^{o}C, ending with 10 min isothermal at 280 ^{o}C at 70 eV. Mass spectra was taken at a scan interval of 0.5 sec and fragments from 45 to 450 Da. By matching its average peak area to the total areas, the relative percentage quantity of each component was acquired. Compound identification was obtained by matching the retention times of the compounds and the mass spectral obtained from the library data of the corresponding compound.

2.3.2. Fourier Transform Infra-Red Spectrometry (FT-IR)

Fourier Transform Infra-Red (FT-IR) was performed using FTIR spectroscopy (Thermos Scientific, Nicolet iS10 SMART iTR) to detect the chemical bonds (functional groups) of the compounds. The operating system was based on the method described by Umaru *et al*., (2019). The liquid samples were introduced into the machine and scan range was set from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. Characteristic of the chemical bonds was read by spectrum produced through transmittance of wavelength of light. The chemical bond in a molecule were detected by interpreting the infra-red transmittance spectrum and the functional groups of the compounds were identified based on the Table of Characteristic IR absorptions published in Organic Chemistry (Janice, 2008).

2.3.3. Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectrometry was performed using JEOL JNM-ECA 500 Spectrometer. The operating system was based on the method described by Efdi *et al.* (2010). Sample was dissolved in 0.8 mL chloroform D1 ($CDCl_3$) or Acetone D6 and placed into NMR tube to a sample depth of 4 cm and the ^{1}H (500 MHz) and ^{13}C (125 MHz) spectra were measured. Chemical shifts were reported as δ units (ppm) with tetramethysilane (TMS) as internal standard and coupling constants (*J*) in Hz. Integration of the ^{1}H-NMR and ^{13}C-NMR data was performed by using DELTA version 5.0.4 software by JEOL. The identification of each ^{1}H-NMR and ^{13}C-NMR detected was based on the Table of Characteristic NMR absorptions published in Organic Chemistry (Janice, 2008) and with the guide of the possible proposed structure given by NIST library.

2.4. Melting Point

The melting point of the compounds isolated was determined using a melting point apparatus (Stuat model SMP3). Small amount of the sample was put into a small capillary tube and was inserted into the machine melting point heating bath. The heating process was monitored and the temperature at which the sample begins to melt and completely melted was recorded.

2.5. Antimicrobial Activity

2.5.1. Microorganisms

Bacterial strains *Escherichia coli* (*E. coli*), *Klebsielia pneumonia* (*K. pneumonia*), *Salmonella typhii* (*S. typhii*) and *Staphylococcus aureus* (*S. aureus*) were selected for the study. The bacterial strains were obtained from the Microbiology Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, and were used for the antibacterial activities. The stock cultures were incubated at 37 ^{o}C for 24 hrs on nutrient agar (Microcare Laboratory, Surat, India), and was stored at 4 ^{o}C. Plates containing Mueller-Hinton Agar (MHA) were used to grow the bacterial strains at 37 ^{o}C. The stock cultures were then kept at 4 ^{o}C until use.

2.5.2. Antibacterial assay

Antibacterial activity of leaf *Hornstedtia scyphifera var* was determined against four pathogenic bacterial strains *E. coli*, *K. pneumonia*, *S. typhii* and *S. aureus* using disk diffusion method as reported by various authors (Boyan *et al*., 2005; Prashanth *et al.,* 2006). The extract was dissolved using dimethyl sulfoxide (DMSO) and sterilized by filtration and stored at 4 ^{o}C until use. Standard antibiotics (tetracycline) was used for comparison of the zone of inhibition of the pure

strains of the bacteria. The extracts were then screened for their antibacterial activity against the bacterial strains. Set of five dilutions for antibacterial activity (25, 50, 100, 250, 500 µg/mL) of the leaf extracts of *Hornstedtia scyphifera var.* and standard drug (tetracycline) was prepared in distilled water. Sterile plates containing Mueller-Hinton agar were seeded with indicator bacterial strains and control experiment using tetracycline as standard drug were kept for 3 hrs at 37 ^{o}C. They were then incubated for 18 to 24 hrs at 37 ^{o}C, and the zones of growth inhibition around the disks were measured in mm. The antibacterial activity of the test organisms on the plant extracts were determined by measuring the diameter of the inhibitory zones on the surface of the agar around the disk, and the values <9 mm were considered as not active against the microorganism for antibacterial activity (Prashanth *et al.,* 2006). The experiment was carried out in triplicate and the mean values of the diameter of zones of inhibition was calculated using statistical software SPSS 22.

2.5.3. Brine shrimp (Artemia salina) Lethality Test

The LC_{50} of the plant extracts was determined using brine shrimp lethality test. The test was conducted using larvae of *Artemia salina* based on method developed by McLaughlin *et al.* (1991). One spatula full of brine eggs was placed into a 250 mL beaker containing 150 mL of sea water placed under light environment. A source of O_2 supply was connected to the beaker using water pump at reduced pressure and allowed for 72 hrs to hatch. The brine shrimp (nauplii) were then used for the test. Exactly 4 mg of each extract was dissolved in 200 µL of DMSO (RCI Labscan limited) and a lower series of chosen concentration was prepared by serial dilution with DMSO. The assay system was prepared with 5 mL of filtered seawater containing chosen concentration of extract and 1% yeast extract (for feeding) in a pre-marked 6-well microplate and 10 brine shrimps were carefully taken with micropipette and introduced into each microplate. This was done in triplicates making a total of 30 brine shrimps per concentration. Filtered seawater was added to DMSO and 10 brine shrimps in triplicates and this was used as the control groups. If the brine shrimp in these microplates shows a rapid mortality rate, then the test is considered invalid as the nauplii might have died due to some reasons other than the cytotoxicity of the extracts. The setup was allowed to remain for 24 hrs under constant illumination of fluorescent and number of survived nauplii were counted with a hand lens. Based on the data obtained, the average death of the brine shrimp at different concentrations of the extract and the LC_{50} of the extract was calculated using probit regression by statistical software SPSS 22 and the result was expressed as Mean + SD at the 95% level of confidence ($p < 0.05$).

2.6. DPPH (2,2-diphenyl-1-picryl-hydrazyl) Free Radical Scavenging Assay (Antioxidant)

The free radical scavenging assay of compound 2,2-diphenyl-1-pycryl-hydrazyl (DPPH) was used to evaluate the antioxidant properties of the crude extract. The measurement was based on the method described by Wang *et al*. (2008). The sample was prepared by diluting 6 mg of crude extract into 6 mL of methanol, producing a concentration of 1000 µg/mL. The stock solution was sonicated to ensure the homogeneity of the sample. Three other concentrations were prepared at 10, 50 and 100 µg/mL, diluted from the 1000 µg/mL stock solution. Sample of 5000 µg/mL was prepared separately by diluting 25 mg of crude extract into 5 mL of methanol.

Approximately 3 mL of 0.1 mM solution of 2,2-diphenyl-1-pycrylhydrazyl (DPPH) in methanol was each added into five series of prepared concentrations (10, 50, 100, 1000 and 5000 µg/mL) of sample solutions (1 mL). Analysis was done in triplicate. The solution was mixed vigorously and left to stand at room temperature for 30 minutes in the dark after which its absorbance was measured spectrophotometrically at 517 nm using Jasco ultra violet spectrophotometer model V-630. Methanol was used as blank (only methanol) and negative control (1 mL methanol mixed with 3 mL DPPH), while ascorbic acid (vitamin C) as the standard. The concentration of the sample required to inhibit 50% of the DPPH free radical was calculated as IC_{50} and the value was determined using Log dose inhibition curve which performed by using PRISM version 3.02 software, based on the calculated values of the DPPH scavenging activity (%) of the sample (Tailor & Goyal, 2014).

2.7. Physical Properties and Spectrometry Information of Pure Compounds

Quercetin (**1**) is a light yellow solid when dried with a melting point at 322-324 ^{o}C with molecular formula $C_{15}H_{10}O_{7}$.: IR Vmax cm^{-1}: 3347 cm^{-1}, 2973 cm^{-1}, 2880 cm^{-1}, 1655.81 cm^{-1}, 1453.73 cm^{-1}, 1328.68 cm^{-1} 1082.11 cm^{-1} 879.60 cm^{-1}, 646.30 cm^{-1}: MS *m/z* (% rel. int): 45 (8), 55 (9), 75 (100), 89 (5), 101 (2), 113 (3), 129 (2), 141 (1), 155 (1), 181 (1), 195 (1), 213 (1), 227 (1), 239 (1), 252 (1), 267 (1), 285 (1), 302 (30), 328 (1), 347 (1), 362 (1), 388 (1), 407 (1), 428 (1), 445 (1), 468 (1), 482 (1), 500 (1), 514 (1), 545 (1). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.94 (1H, s), 6.19 (1H, s), 6.12 (1H, d, =6.15), 0.70 (1H, s), 6.13 (1H, d, J=6.12), 6.91 (1H, s), 3.43 (1H, s), 6.71 (1H, t, J= 6.71), 6.93 (1H, t, J= 6.92): ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 146.35 (C-1), 137.09 (C-2), 175.24 (C-4), 104.28 (C-6), 159.95 (C-7), 90.62 (C-9), 164.13 (C-10), 94.42 (C-12), 157.59 (C-13), 121.81 (C-15), 115.98 (C-16), 145.29 (C-17), 148.35 (C-19), 116.29 (C-21), 121.63 (C-22).

Dedocanoic acid (**2**) is a light yellow solid when dried with a melting point at 44.6 ^{o}C with molecular formula $C_{12}H_{24}O_{2}$.: IR Vmax cm^{-1}: 3341.62 cm^{-1}, 2974.22 cm^{-1}, 1653.34 cm^{-1}, 1384.78 cm^{-1} 1086.68 cm^{-1} 1044.08 cm^{-1}, 878.61 cm^{-1}: MS *m/z* (% rel. int): 45 (19), 55 (70), 60 (99), 78 (100), 87 (51), 110 (15), 136 (22), 143 (42), 157 (34), 171 (54), 185 (30), 200 (15), 229 (10), 264 (18), 280 (8), 305 (6), 320 (6), 348 (4), 369 (3), 383 (5), 402 (5), 414 (5), 429 (5), 446 (6), 461 2), 474 (2), 498 (2), 510 (3), 529 (1). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 2.28 (2H, m), 1.63 (2H, m), 1.33 (2H, m), 1.32 (2H, m), 1.31 (2H, m), 1.31 (2H, m), 1.31 (2H, m), 1.31 (2H, m), 1.30 (2H, m), 1.37 (2H, m), 0.99 (3H, t, J=56), 9.47 (1H, s). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 177.13 (C-1), 34.35 (C-2), 24.81 (C-3), 28.93, (C-4), 28.96 (C-5), 28.96 (C-6), 28.96 (C-7), 28.96 (C-8), 29.06 (C-9), 31.65 (C-10), 22.94 (C-11), 14.02 (C-12).

3. Results and discussion

3.1. Purification and Structural Elucidation of Secondary Metabolites

3.1.1. Purification of Compounds 1, and 2 from methanol Hornstedtia scyphifera var Leaf extract

Compounds 1 and 2 were isolated from methanol crude extract of *Hornstedtia scyphifera var Leaf extract.* About 20 g of the crude extract was loaded using dry pack method into a column packed with silica gel in hexane (100%). The crude extract was then eluted from the column with solvent system in the sequence as indicated in Table 1.

Table 2 Fractions collected from methanol crude leaf extract of *Hornstedtia scyphifera*

Code of Fraction	Weight of Fraction (mg)	Colour of Fraction
HSMtol 1	8.16	Colourless
HSMtol 2	10.39	Colourless
HSMtol 3	18.5	Light yellow
HSMtol 4	79.5	Light yellow
HSMtol 5	105.2	Brown
HSMtol 6	225.8	Brown
HSMtol 7	**180.6**	**Dark brown**
HSMtol 8	333.3	Dark brown
HSMtol 9	**220.9**	**Dark brown**
HSMtol 10	290.7	Dark brown
HSMtol 11	530.0	Dark brown
HSMtol 12	230.8	Dark brown

3.1.2. Purification and Structural Elucidation of Compound 1

Purification

Compound 1 was isolated from combined fraction HSMtol 9 of 220.9 mg dark brown. TLC analysis of the combined fraction HSMetol 9 was carried out in different solvent system. It was observed under UV light and recorded as shown in Table 3.

Table 3 R_f values of combined fraction HSMtol 9 in different solvent system under UV light

Solvent system (v/v)	Number of spots	Rf values
Hexane: DCM (7:3)	2	0.32
		0.11
Hexane: CHCl3 (8:2)	2	0.46
		0.40
Hexane: EA (4:1)	2	0.68
		0.28

Light colored spots seen under UV light with the same R_f values collected from fractions HSMtol 9-1 to HSMtol 9-15 were targeted and were combined, and it was labelled as HSMtol 9- A. Separation using small column of combined fractions HSMtol 9-A was performed and combined fraction HSMtol 9-A1 was obtained, it was then separated using small column and combined fraction HSMtol 9-A2 was obtained. Preparative TLC analysis of the combined faction HSMtol 9-A2 was performed in the solvent systems hexane: chloroform (8:2) which gave a good separation from the other spots present. The targeted spot was labelled as HSMtol 9-A3. TLC analysis of HSMtol 9-A3 was performed in different solvent systems and the result obtained under UV light and vanillin staining showed 2 spots as shown in Table 4.

Table 4 R_f values of combined fraction HSMtol 9-A3 in different solvent system under UV light

Solvent system (v/v)	Number of spots	Rf values
Hexane: CHCl3 (8:2)	2	0.56
		0.44
Hexane: EA (9:1)	2	0.60
		0.21

HSMtol 9-A3 was further purified in a smaller column using the solvent system hexane: chloroform (8:2) which gives a better separation as examined on the TLC profile. Fractions with similar R_f values of the targeted spots were combined and labelled as HSMtol 9-A4. TLC analysis of HSMtol 9-A4 was performed in different solvent systems and the spot obtained with its R_f value was recorded as shown in Table 5.

Table 5 R_f values of combined fraction HSMtol 9-A4 in different solvent system under UV light

Solvent system (v/v)	Number of spots	R_f values
Hexane: chloroform (8:2)	1	0.48

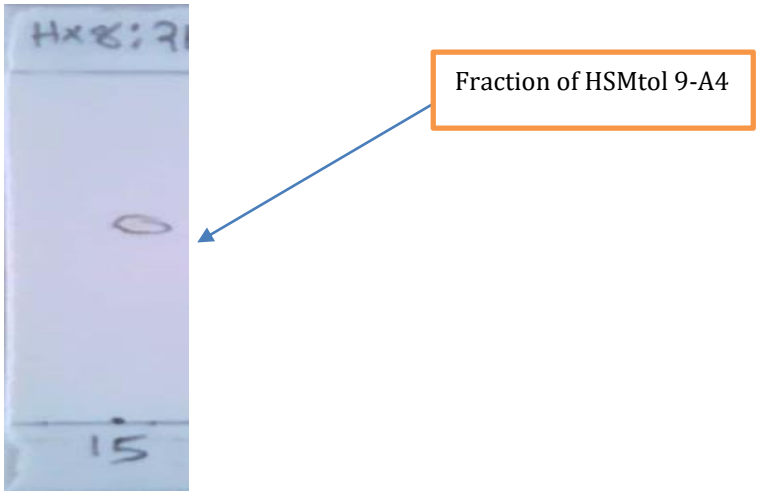


Figure 3 Shows the TLC profile for the combined fraction HSMtol 9-A4 in hexane: ethyl acetate (8:2) as a single spot which suggest that it is a pure compound.

GC analysis of the fraction of HSMtol 9-A4 was then carried out, and the result from the gas chromatogram (Figure 4) showed a single peak at a retention time of 24.48 min. This confirmed that HSMtol 9-A4 is a pure compound and it was renamed as Compound 1.

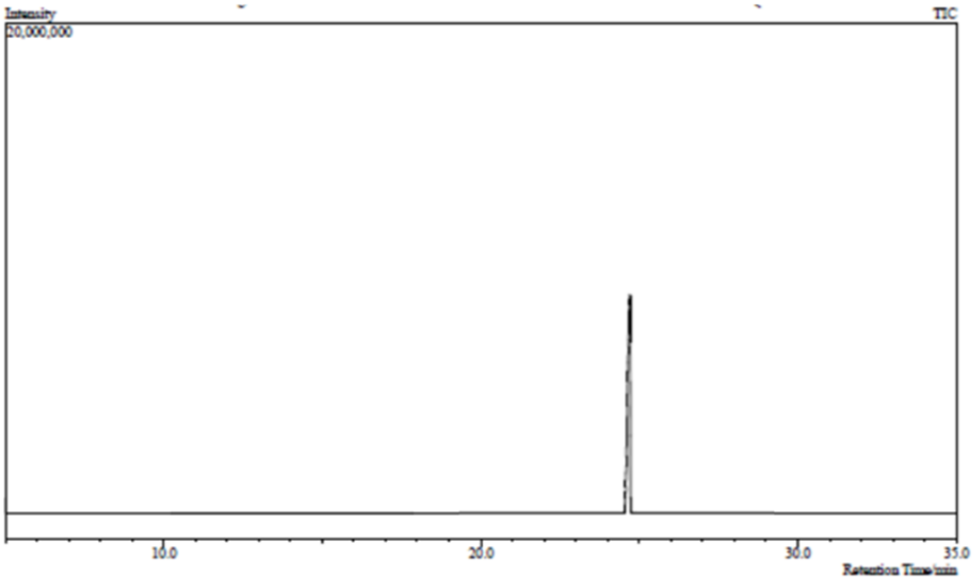


Figure 4 Gas chromatogram of Compound 1.

3.1.3. Structural Elucidation

Compound 1 was isolated from the methanol leaf crude extract of *Hornstedtia scyphifera,* with its physical appearance as a yellow crystal and a melting point at 322-324 ^{o}C with molecular formula $C_{15}H_{10}O_7$. The mass spectrum of Compound 1 in Figure 5 shows a similarity index of 98.9% with the mass spectrum of the suggested structure of Compound 1 by the NIST library in Figure 6. On the mass spectrum of Compound 1 one of its molecular ion peak was observed at *m/z* 302 which was found to correspond to the molecular ion peak and molecular ion weight of the suggested structure of Compound 1 by the NIST library which has a chemical formula of $C_{15}H_{10}O_7$. Figure 4 also shows base peak for Compound 1 at *m/z* 75 which was observed in the mass spectrum of the suggested structure for Compound 1.

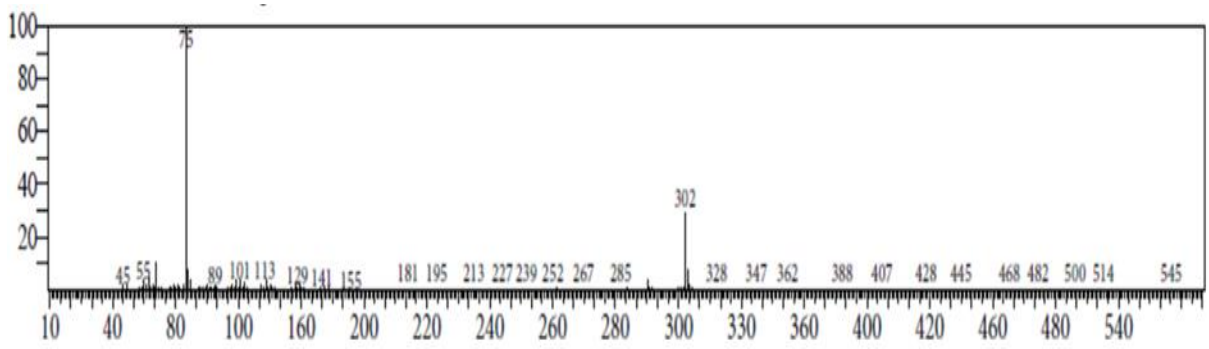


Figure 5 Mass spectrum of Compound 1.

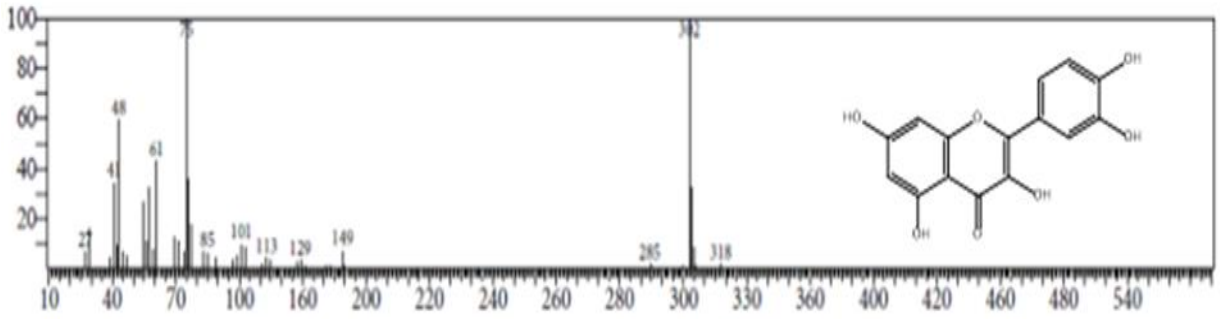


Figure 6 Mass spectrum of suggested structure of Compound 1 by NIST Library.

Chemical structure of Compound 1 consists of absorption bands of C-H which was observed at 2973 cm^{-1} and 2880 cm^{-1} in the IR spectrum (Figure 7) and indicated the presence of methyl alkyl carbon in the chemical structure. A signal indicating the presence of double bond was observed at 1655 cm^{-1} which also matched the double bonds of C=C in the ring, and another at 1453 cm^{-1} which may represent the C=C bond in the asymmetry ring in the suggested structure of quercetin (**1**). A signal was observed at 1082 cm^{-1} which was seen to represent the C-O bond stretching and single bond of C-C stretching at 879 cm^{-1} were observed in the IR spectrum of Compound 1 (Figure 7). IR spectrum of Compound 1 also showed similarity to IR of the same proposed compound reported by Duc *et al*. (2018).

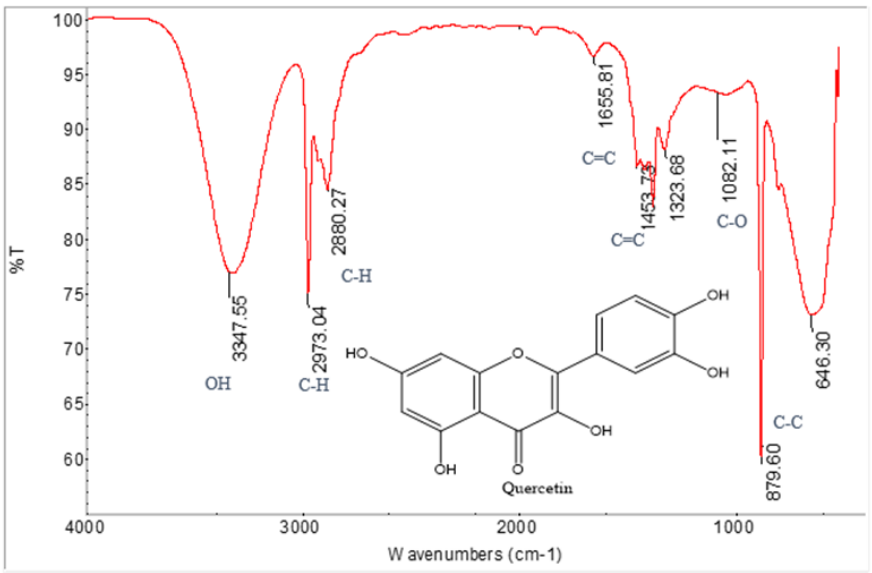


Figure 7 IR Spectrum of Compound 1

NMR analysis was further performed for the elucidation of the chemical structure of Compound 1, and the results are shown in Figure 8 (^{1}H-NMR) and Figure 9 (^{13}C-NMR). Based on the table of ^{1}H-NMR characteristics absorption and ^{1}H-NMR peaks splitting pattern as reported in Organic Chemistry by Janice (2008), the proton signals were all integrated and were assigned to every proton NMR of Compound 1 as the proposed chemical structure.

The result showed that ^{1}H-NMR spectrum of Compound 1 exhibited 9 proton resonates. The ^{1}H-NMR spectrum showed aromatic proton signals at δ H 6.71 (1H, d, J=6.71), 6.91 (1H, d, J=6.92), 6.93 (1H, d, J=6.92), two meta-coupled aromatic proton signals at δ H 6.12 (1H, d, J=6.15), 6.13 (1H, d, J=6.12,) and were assigned to H-21, H-16, H-22, H-9, and H-12, respectively.

Figure 9 shows the result of the ^{13}C-NMR spectrum of Compound 1. From the result every carbon NMR signal that was observed was assigned to the proposed chemical structure of Compound 1 which is based on the table of ^{13}C-NMR characteristics absorption reported in Organic Chemistry by Janice (2008).

A total of 15 carbon resonates was observed in the spectrum. The ^{13}C-NMR spectrum indicated that 5 has a flavonol skeleton with 15 carbons, including five aromatic CH; ten quaternary carbons (one carbonyl, five O bearing, and four aliphatic), suggesting that it is 3,5,7,3′,4′- pentahydroxyflavone, commonly known as quercetin. The down field region showed 13 carbon resonates at δ 146.35, δ 137.09, δ 175.24, δ 104.28, δ 159.95, δ 164.13, δ157.59, δ121.81, δ 115.98, δ145.29 δ 148.35, δ116.29 and δ121.63, respectively. They were assigned to C-1, C-2, C-4, C-6, C-7, C-10, C-13, C-15, C-16, C-17, C-19, C-21 and C-22. At up field two carbon signal was observed at δ 90.62 and δ 94.42, they were assigned to C-9 and C-12 respectively.

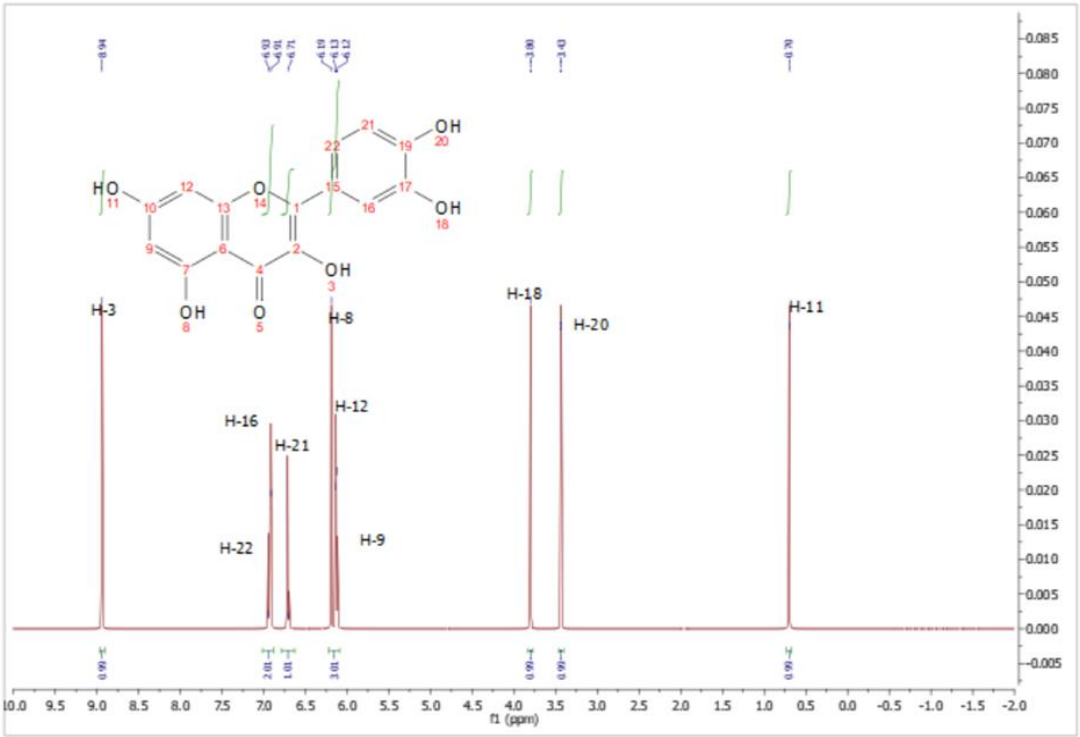


Figure 8 ^{1}H-NMR spectrum of Compound 1 (500 MHz, DMSO d6).

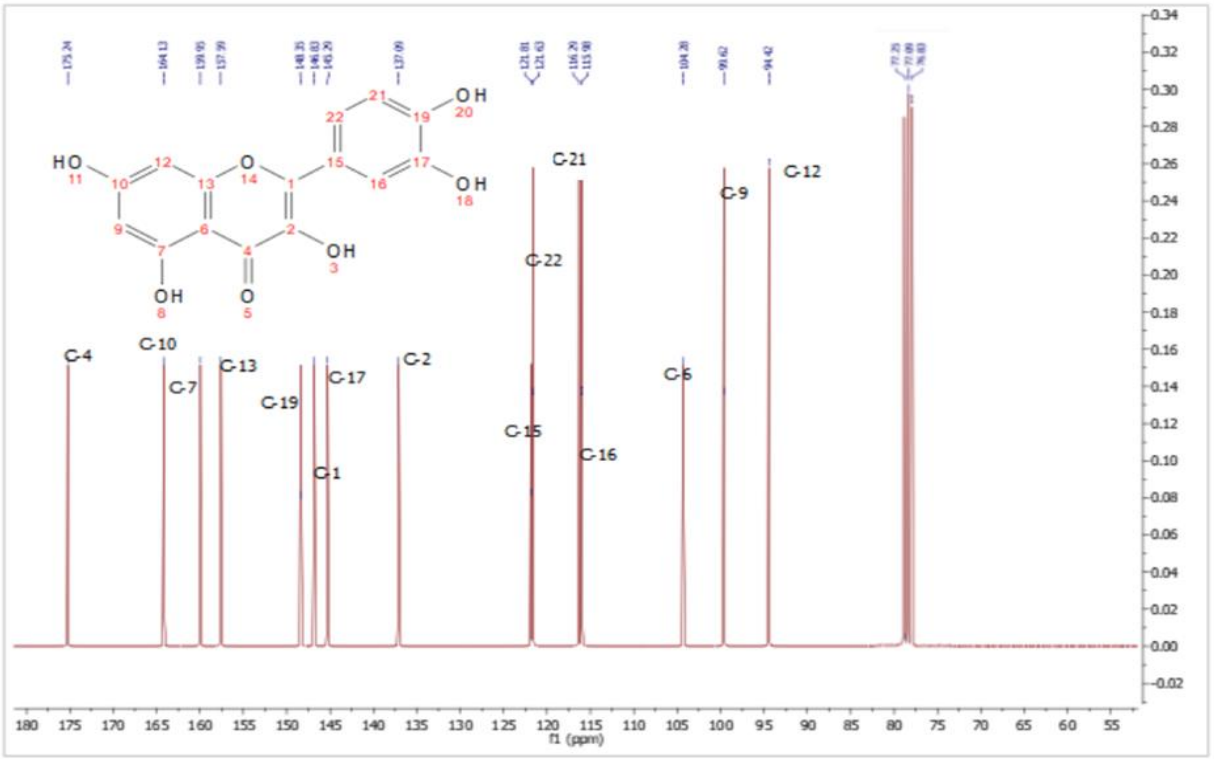


Figure 9 13C-NMR spectrum of Compound 1 (125 MHz, DMSO-d6)

Table 6 Proton NMR signal of Compound 1 and that reported by Duc *et al.* (2018).

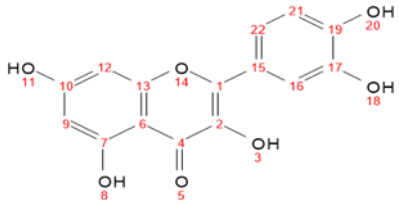
Proton assigned to Compound 1	Proton chemical shift (ppm) of Compound 1	Proton assigned to quercetin by Duc et al. (2018)	Proton chemical shift (ppm) of quercetin (Duc et al., 2018)
H-3	8.94 (1H, s)	H-3	OH
H-8	6.19 (1H, s)	H-8	OH
H-9	6.12 (1H, d, =6.15)	H-9	6.19 (1H, d, J=2.0)
H-11	0.70 (1H, s)	H-11	OH
H-12	6.13 (1H, d, J=6.12)	H-12	6.39 (1H, d, J=2.0)
H-16	6.91 (1H, s)	H-16	OH
H-20	3.43 (1H, s)	H-20	OH
H-21	6.71 (1H, t, J= 6.71)	H-21	7.74 (1H, d, J=2.0)
H-22	6.93 (1H, t, J= 6.92)	H-22	7.64 (1H, dd, J=2.0, 8.5)

Table 7 Carbon NMR signal of Compound 1 and that reported by Duc *et al.* (2018).

Carbon assigned to compound 1	Carbon chemical shift (ppm) of compound 1	Carbon assigned to quercetin (Duc *et al.*, 2018)	Carbon chemical shift (ppm) of quercetin (Duc *et al.*, 2018)
C-1	146.35	C-1	146.8
C-2	137.09	C-2	135.6
C-4	175.24	C-4	175.7
C-6	104.28	C-6	103.0
C-7	159.95	C-7	93.30
C-9	90.62	C-9	163.80
C-10	164.13	C-10	98.10
C-12	94.42	C-12	156.10
C-13	157.59	C-13	121.90
C-15	121.81	C-15	145.00
C-16	115.98	C-16	147.6
C-17	145.29	C-17	115.5
C-19	148.35	C-19	119.9
C-21	116.29	C-21	115.5
C-22	121.63	C-22	119.9

From the data obtained, the GCMS analysis of Compound 1 gave similarity index of 98.9% with the mass spectrum of the proposed compound by the NIST library which matched the characteristic of quercetin (**1)**, with chemical formula $C_{15}H_{10}O_7$. The melting point of Compound 1 is 322–324 ^oC Also, mass spectrum of Compound 1 is similar to the mass spectrum of the suggested structure by NIST library and is most probably identified quercetin (**1)**. IR data reported by Duc *et al.* (2018) could be seen to match the IR data of Compound 1 which was reported as quercetin (**1)**.

Based on mass spectrum, IR, ^1H-NMR and ^{13}C-NMR data and comparison with published literature (Duc *et al.,* 2018) Compound 1 was therefore identified as quercetin (**1).**



1

Quercetin (**1)** is one major active constituent of many herbal plants, such as *Kaempferia paviflora, Physalis peruviana linn, Piper caninum,* and *Leptospermum scoparium.* The compound demonstrated many beneficial pharmacological effects *in vitro*, including anti-inflammatory, antioxidant properties (Bei and An, 2016).

3.1.4. Purification and Structural Elucidation of Compound 2

Purification

Compound 2 was isolated from the combined fraction HSMtol 7 of 180.6 mg dark brown (Methanol crude leaf extract of *Hornstedtia scyphifera*) Table 2. TLC analysis of the combined fraction HSMtol7 was performed in different solvent systems and the result as observed under UV light was recorded as shown in Table 8.

Table 8 R_f values of combined fraction HSMtol 7 in different solvent system under UV light

Solvent system (v/v)	Number of spots	Rf values
Hexane: CHCl3 (3:2)	3	0.75
		0.61
		0.58
Hexane: EA (7:3)	3	0.62
		0.61
DCM: EA (7:3)	3	0.85
		0.81
		0.65

Fractions containing a light yellowish spot were targeted and combined, it was labelled as HSMtol7-A. Combined fraction HSMtol 7-A. was then further purified two successive times in a smaller column using the solvent system hexane: ethyl acetate (7:3), and each fraction collected (HSMtol 7-A1 and HSMtol 7-A2) were observed under UV light and those containing the light yellowish spot were combined and labelled as HSMtol 7-A3. The Combined fraction HSMtol 7-A3 was then tested using TLC and observed under UV light. The result is shown in Table 9.

Table 9 R_f values of combined fraction HSMtol7-A3 in different solvent system under UV light

Solvent system (v/v)	Number of spots	Rf values
Hexane: EA (7:3)	2	0.63
		0.60
DCM: EA (7:3)	2	0.85
		0.80

Combined fraction HSMtol7-A3 was further purified using small column and fractions containing the targeted spots from HSMtol 7-A3 were then combined and labelled as HSMtol7-A4. Combined fraction HSMtol 7-A4 was further purified using the solvent system hexane: ethyl acetate (7:3), which gives a better separation. TLC of the fractions collected was performed and examined under UV light. Fractions containing the target spots were combined and labelled as HSMtol7-A5. TLC of the combined fraction HSMtol7-A5 was performed in different solvent system and the result was again examined under UV light as well as vanillin staining. It showed a single sport as shown in Table 10.

Table 10 R_f values of combined fraction HSMtol 7-A5 in different solvent system under UV light.

Solvent system (v/v)	Number of spots	Rf values
Hexane: Ethyl acetate (7:3)	1	0.62

Figure 10 shows the TLC profile for the combined fraction HSMtol7-A5 in hexane: ethyl acetate (7:3) as a single spot which suggest that it is a pure compound

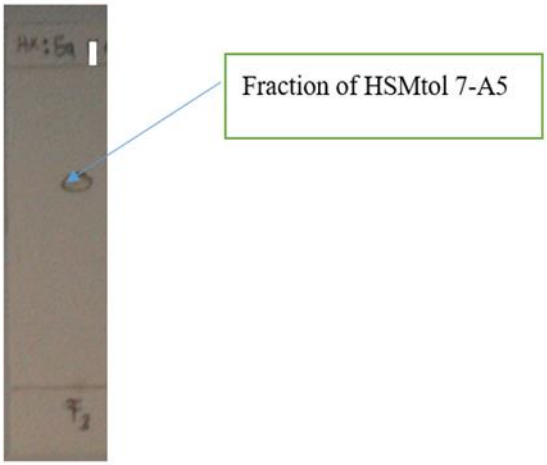


Figure 10 TLC plate showing the spot in combined fraction HSMtol 7-A5 in hexane: ethyl acetate (7:3)

Figure 10 show that combined fraction HSMtol7-A5 is a pure compound. GC analysis of the combined fraction HSMtol7-A5 was then carried out, and the result from the gas chromatogram (Figure 11) showed a single peak at a retention time of 19.020 min. This confirmed that HSMtol 7-A5 is a pure compound and it was renamed as Compound 2. It is a white compound and 15 mg was obtained.

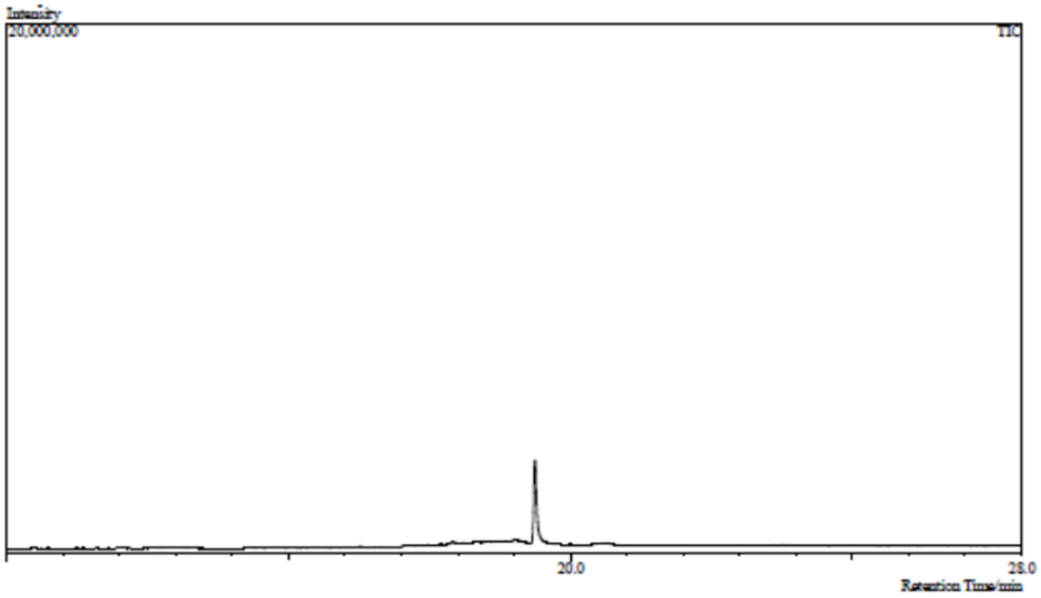


Figure 11 IR Spectrum of Compound 2

3.1.5. Structural Elucidation

Compound 2 was isolated from the methanol crude leaves extract of *Hornstedtia scyphifera,* its physical appearance as a light compound with a melting point of 44.6 ^{o}C. The mass spectrum of Compound 2 (Figure 12) show a similarity index of 96.5% with the mass spectrum of the compound suggested by the NIST library in Figure 13. The mass spectrum of Compound 2 showed an ion base peak which was observed at *m/z* 73 and a molecular ion peak of *m/z* 73 was also observed in the mass spectrum of the suggested structure of Compound 2. The mass spectrum of Compound 2 has one of its molecular ion peak observed at *m/z* 200, this corresponded to the same molecular ion peak and molecular ion weight of the suggested structure of Compound 2 by NIST library with a chemical formula of $C_{12}H_{24}O_{2.}$

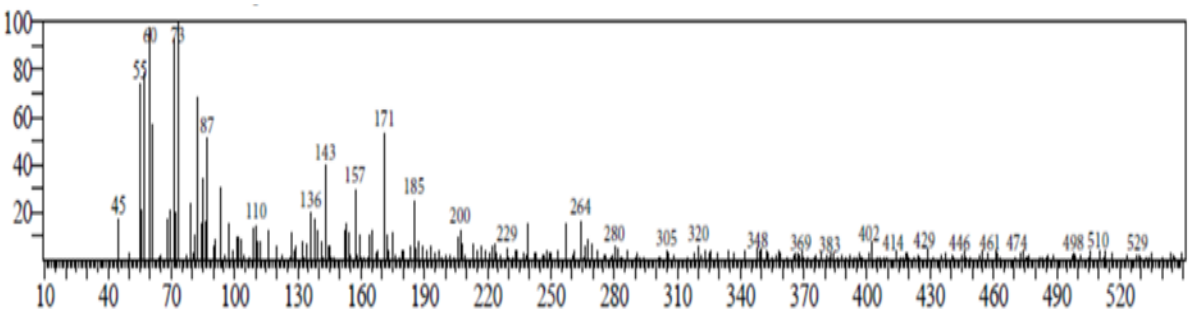


Figure 12 Mass spectrum of Compound 2

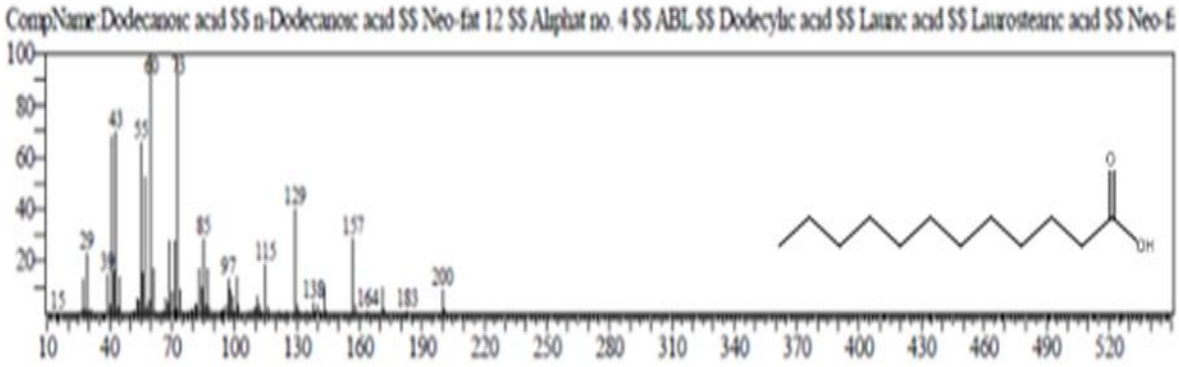


Figure 13 Mass spectrum of suggested structure of Compound 2 by NIST library.

The chemical structure of Compound 2 has functional group (O-H stretch) which appeared at 3341 cm^{-1} as illustrated in the IR spectrum (Figure 14). An absorption bands of C-H was observed at 2974 cm^{-1} which indicated the presence of methyl carbon in the chemical structure. A signal was observed at 1653 cm^{-1} which indicated the presence of C=O bond. At 1384 cm^{-1} a signal was observed which matched the double bonds of C=C in the suggested structure. Single bond C-C stretching was observed at 878 cm^{-1} in the IR spectrum of Compound 2.

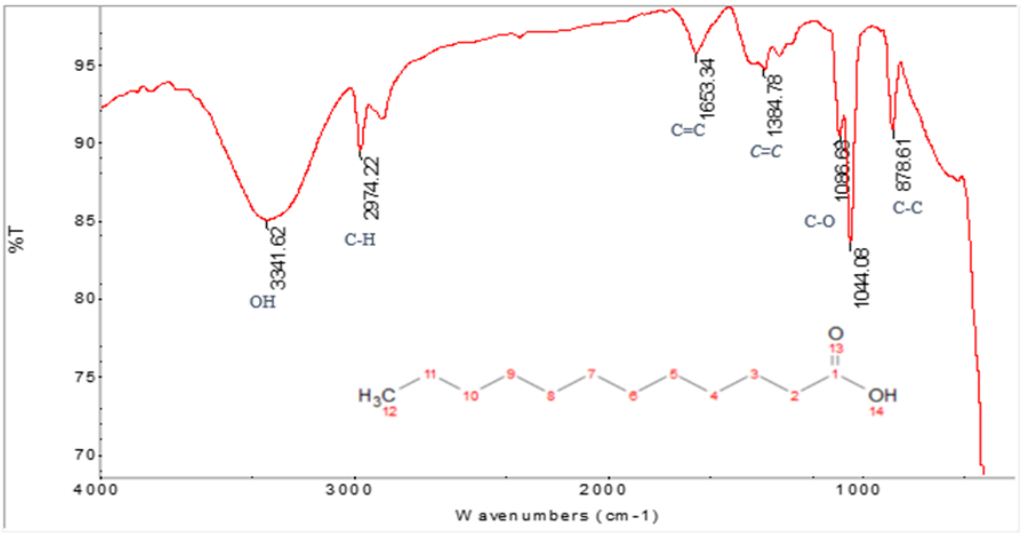


Figure 14 IR Spectrum of Compound 2

NMR analysis was further performed for the elucidation of the chemical structure of Compound 2. The results are shown in Figure 15 and Figure 16 (^{1}H-NMR), Figure 17 and Figure 18 (^{13}C-NMR). Based on the table of ^{1}H-NMR characteristics

absorption and ^{1}H-NMR peaks splitting pattern as reported in Organic Chemistry by Silverstein (2005), the proton signals were all integrated and were assigned to every proton NMR of Compound 2 as the proposed chemical structure.

The ^{1}H-NMR spectrum of Compound 2 exhibited 13 proton resonates. A singlet proton signal was observed at δ 9.47 (1H, s) indicating the presence of an OH group (hydroxyl) of the structure. A doublet proton signal was observed at δ 2.28, δ 1.63, δ 1.33, δ 1.32, δ 1.31, δ 1.31, δ 1.31, δ 1.31, δ 1.30 and δ 1.37, respectively. Indicating the presence of a methylene group of the long chain of Compound 2 and was assigned to H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10, and H-11. A multiplet proton signal was observed at δ 0.99 (3H, m) which correspond with a methyl group and was assigned to H-12.

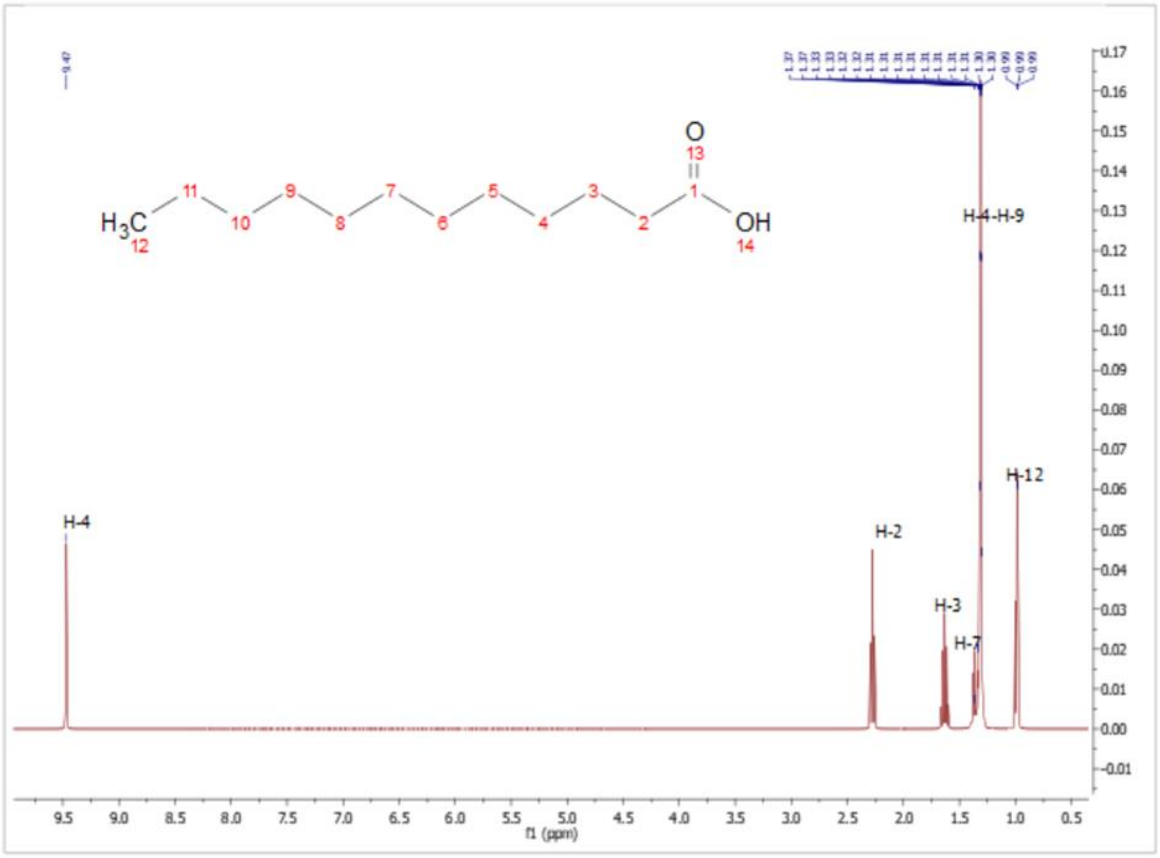


Figure 15 ^{1}H-NMR spectrum of Compound 2 from 0.5 to 9.5 (500 MHz, $CDCl_3$)

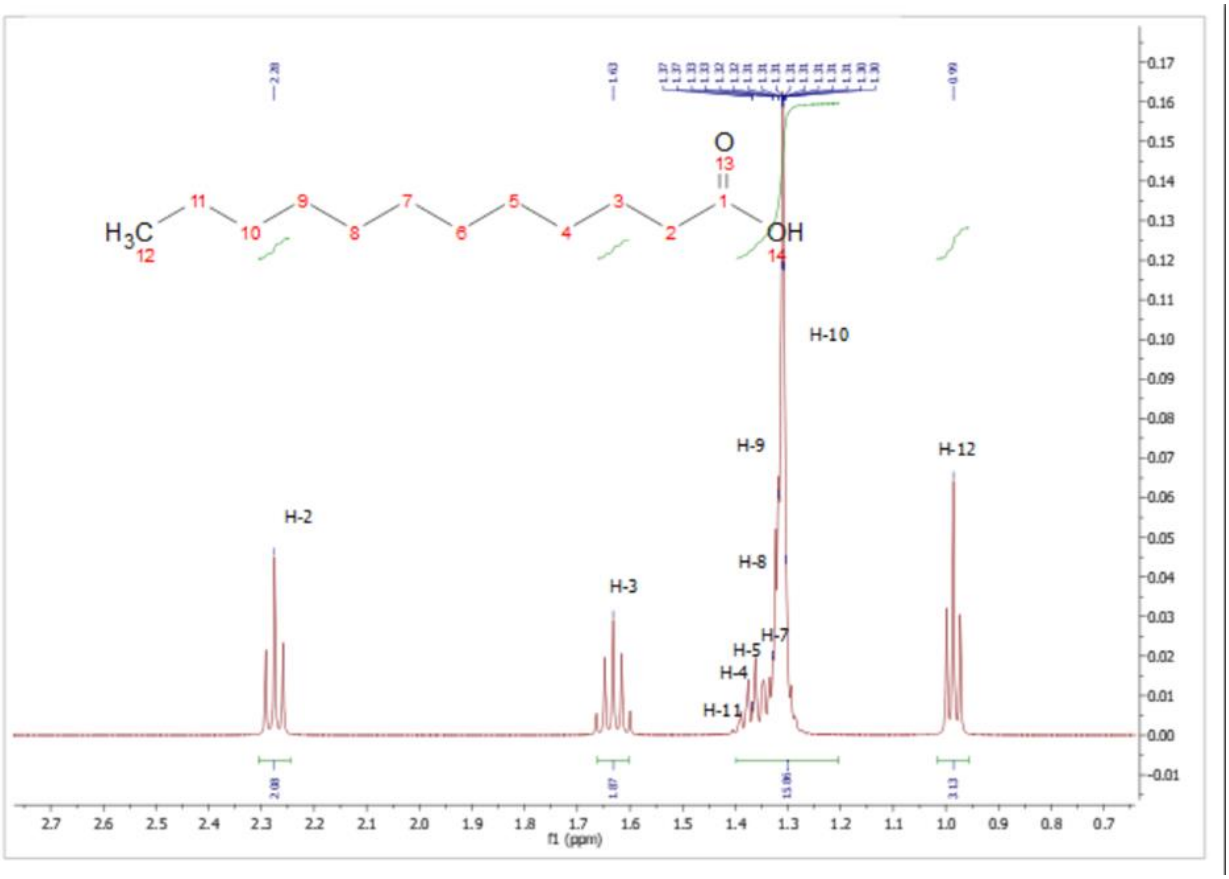


Figure 16 1H-NMR spectrum of Compound 2 from 0.7 to 2.7 (500 MHz, CDCl3)

From the result of the 13C-NMR spectrum of Compound 2 every carbon NMR signal that was observed was assign to the proposed chemical structure of Compound 2 which is based on the table of 13C-NMR characteristics absorption reported in Organic Chemistry Silverstein (2005).

A total of 12 carbon resonates were observed in the 13C-NMR spectrum of Compound 2. At the downfield region signals were observed at δ 177.13 and was assigned to C-1. A signal was observed at unfiled region at δ 34.35, δ 24.81, δ 28.93, δ 28.96, δ 28.96, δ 28.96, δ 28.96, δ 29.06, δ 31.65, δ 22.94 and δ 14.02 and was assigned to C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11and C-12, respectively.

Chemical shift of every proton and carbon NMR for Compound 2 is shown in Table 12 and Table 13 and comparison was made with NMR data of similar compound reported by Yamashita et al., (2015) and Zhu (2014).

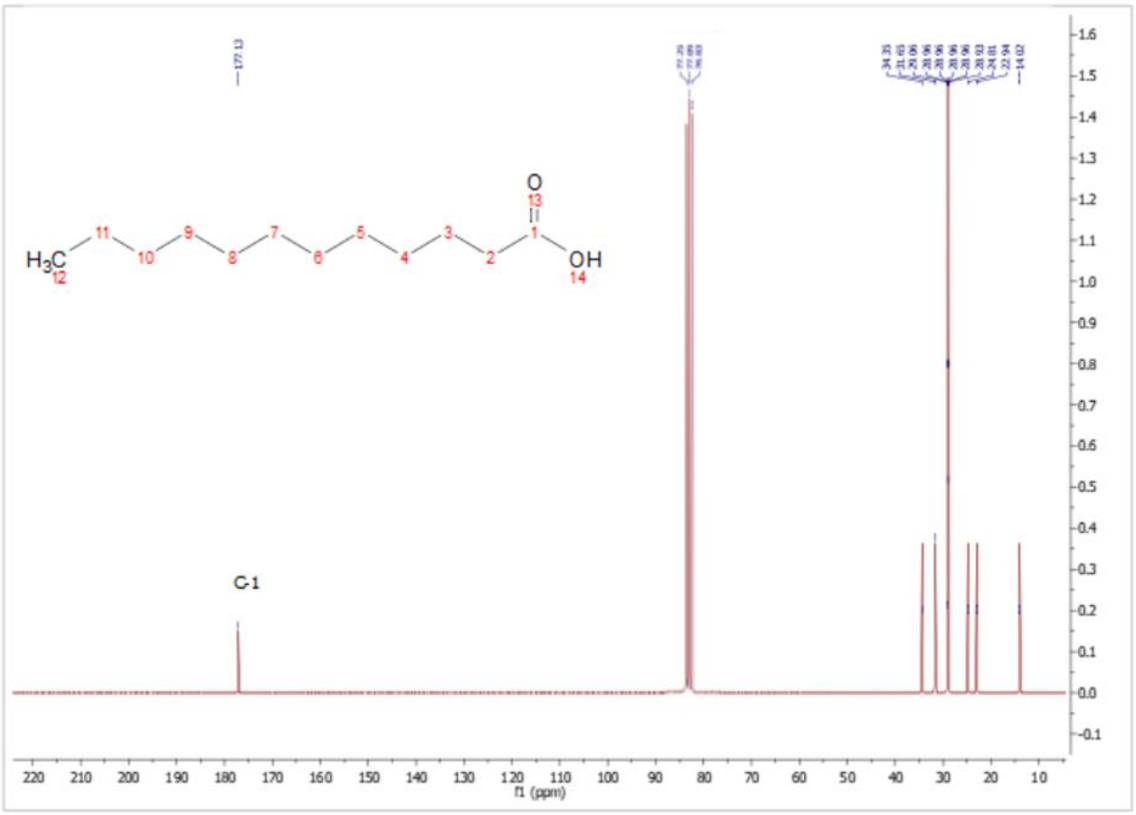


Figure 17 13C-NMR spectrum of Compound 2 from 10 to 220 (125 MHz, CDCl3)

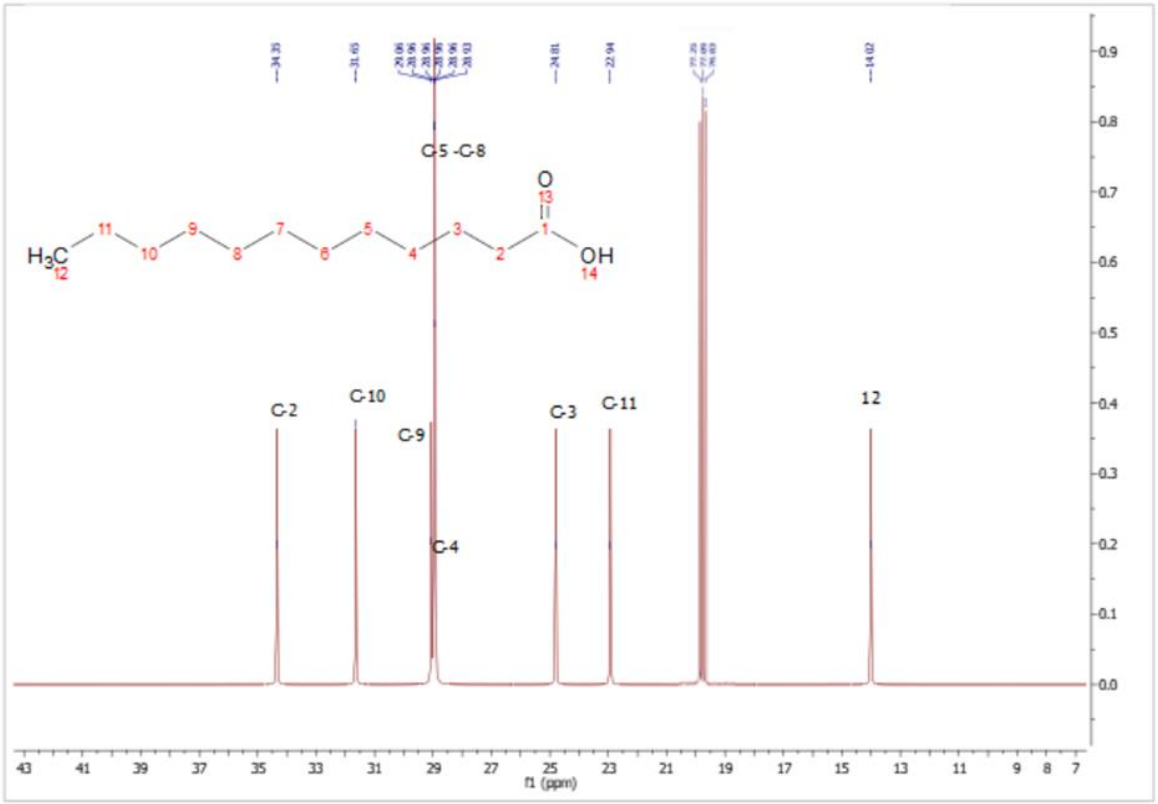


Figure 18 13C-NMR spectrum of Compound 2 from 13 to 35 (125 MHz, CDCl3)

Table 11 Proton NMR signal of Compound 2 and that reported by Yamashita *et al*., (2015).

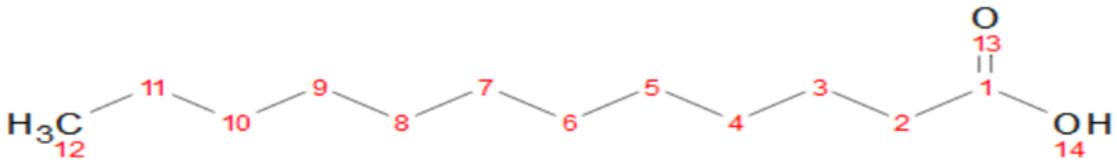
Proton assigned to Compound 2	Proton chemical shift (ppm) of Compound 2	Proton assigned to dodecanoic acid by Yamashita *et al*., (2015)	Proton chemical shift (ppm) of dodecanoic acid (Yamashita *et al*., 2015)
H-2	2.28 (2H, m)	H-2	2.30 (2H, m)
H-3	1.63 (2H, m)	H-3	1.52 (2H, m)
H-4	1.33 (2H, m)	H-4	1.29 (2H, m)
H-5	1.32 (2H, m)	H-5	1.26 (2H, m)
H-6	1.31 (2H, m)	H-6	1.26 (2H, m)
H-7	1.31 (2H, m)	H-7	1.26 (2H, m)
H-8	1.31 (2H, m)	H-8	1.26 (2H, m)
H-9	1.31 (2H, m)	H-9	1.29 (2H, m)
H-10	1.30 (2H, m)	H-10	1.29 (2H, m)
H-11	1.37 (2H, m)	H-11	1.31 2H, m)
H-12	0.99 (3H, t, *J*=56)	H-12	0.88 (3H, t, *J*=57)
H-14	9.47 (1H, s)	H-14	-

Table 12 Carbon NMR signal of Compound 2 and that reported by Yamashita et al. (2015).

Carbon assigned to Compound 2	Carbon chemical shift (ppm) of Compound 2	Carbon assigned to dodecanoic acid (Yamashita *et al*., 2015)	Carbon chemical shift (ppm) of dodecanoic acid (Yamashita *et al*., 2015
C-1	177.13	C-1	178.4
C-2	34.35	C-2	34.0
C-3	24.81	C-3	24.7
C-4	28.93	C-4	29.0
C-5	28.96	C-5	29.3
C-6	28.96	C-6	29.6
C-7	28.96	C-7	29.6
C-8	28.96	C-8	29.6
C-9	29.06	C-9	29.4
C-10	31.65	C-10	31.9
C-11	22.94	C-11	22.7
C-12	14.02	C-12	14.1

From the data obtained, the GC spectrum of Compound 2 gave a similarity index of 96.5% with the mass spectrum of the proposed structure by the NIST library, which matched the characteristic of dodecanoic acid (**2)** with chemical formula $C_{12}H_{24}O_2$. The melting point of Compound 2 is 44.6 ^{o}C. The proton and carbon NMR data of Compound 2 were mostly identical to match the NMR signal of dodecanoic acid (**2)** as reported by Zhu (2014).

Based on mass spectrum, IR, ^{1}H-NMR and ^{13}C-NMR data and comparison with published literature (Yamashita *et al*., 2015**;** Zhu, 2014) Compound 2 was identified as dodecanoic acid (**2).**



2

Dodecanoic acid (**1)** is a compound containing a carboxylic group attached to the terminal end of carbon one in the structure. The acid was report to have showed virucidal effects on enveloped RNA and DNA viruses. It was also observed to have inactivate bacteria, yeast, fungi, and enveloped viruses. It is also an active anti-microbial, that is alpha- and beta-MG. Also, it is mentioned that the anti-microbial effects of the dodecanoic acid are additive and total concentration is critical for inactivating viruses (Yamashita *et al*., 2015). Dodecanoic acid has greater anti-viral properties and many of the pathogenic organisms reported to be inactivated by these antimicrobial compound. It is known to be responsible for opportunistic infections in HIV-positive individuals. It can kill harmful pathogens like bacteria, viruses and fungi. This important compound can be beneficial to infants in reducing the cancer risk and future heart disease (Niknamian and Niknamian, 2015).

In a study reported by Rayan and McDonnell (2014), that dietary supplementation of dodecanoic acid in maternal mice enhances resistance to giardia duodenalis infection in suckling Neonatal pups.

Yamashita reported the identification of self-growth-inhibiting compounds of dodecanoic acid from *Helicobacter pylori* (Yamashita *et al*., 2015).

3.2. Cytotoxicity, Antioxidant and Biological activity of Hornstedtia scyphifera var leaf solvent Crude extract

3.2.1. Cytotoxicity of Hornstedtia scyphifera var leaf extract using Brine Shrimp (Artemia salina)

Result in Table 13 shows the leaf crude extract of hexane, dichloromethane. Chloroform, ethyl acetate and methanol fraction. Highest brine shrimp lethality was observed in methanol crude and hexane crude extract with LC_{50} value of 34.059 µg/mL, 35.462, respectively. Ethyl acetate crude extract exhibited the lowest activity with LC_{50} value 62.220 µg/mL when compared to the test control thymol of 1.16 µg/mL. There was an observed concentration dependent increment in mortality rate of the brine shrimp. The isolated compound as shown in table 14, dodecanoic acid exhibited higher toxicity of LC_{50} of 46.23 µg/mL and Quercetin of LC_{50} 56.66 µg/mL.

In toxicity evolution of plant extracts by brine shrimp lethality bioassay, LC_{50} values lower than 1000 µg/mL are considered active (Umaru *et al*., 2018). This has been well utilized for screening and fractionation of physiologically active plant extracts and has also been demonstrated to correlates reasonably well with cytotoxic and other biological properties (Isaac *et al*., 2018). Brine shrimp bioassay has been established as safe, practical and economical method for determination of bioactivities of synthetic compounds (Almeida *et al*., 2002). It has established a significant correlation with *in-vitro* growth inhibition of human solid tumor cell where it shows the value of this bioassay as a pre-screening tool for antitumor drug research (Anderson *et al*., 1991). Which support the results obtained from crude and isolated compound of the leaf extract of *Hornstedtia scyphifera var* as an agent to be considered.

Table 13 Average death of brine shrimp (*Artemia salina*) at different concentrations of the *Hornstedtia scyphifera var* leaf extract

Plant part	**Solvent system**	**LC_{50} (µg/mL)**				
		5	**10**	**50**	**100**	
Leaf	-ve Control	0	0	0	0	0
	+ve Thymol	5 ± 0.55	7 ± 0.55	10 ± 0.55	10 ± 0.55	1.16
	Hexane	3 ± 0.57	4 ± 0.55	5 ± 0.55	7 ± 0.55	35.462
	Dichloromethane	3 ± 0.57	3 ± 0.57	4 ± 0.57	5 ± 0.00	47.722
	Chloroform	3 ± 0.56	3 ± 0.56	4 ± 0.56	5 ± 0.10	46.924
	Ethyl acetate	2 ± 0.55	3 ± 0.55	4 ± 0.55	4 ± 0.00	62.220
	Methanol	3 ± 0.54	4 ± 0.54	4 ± 0.54	7 ± 0.54	34.059

The result is Mean ± SD. N = 30

There was an observed concentration dependent increment in mortality rate of the brine shrimp.

Table 14 Average death of brine shrimp (*Artemia salina*) at different concentration of isolated compounds

Solvent system	**Average death of *Artemia salina* Concentration (µg/mL)**						**LC_{50} (µg/mL)**
	1	10	25	50	100	500	
-ve Control (DMSO)	0	0	0	0	0	0	-
+ve Thymol	5.00±0.57	7.00±0.58	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	1.16
Quercetin	1.00±0.00	3.57±0.58	3.10±0.00	3.31±0.58	4.23±0.58	4.57±0.58	56.66
Dodecanoic acid	2.00±0.13	3.00±0.12	3.33±0.55	4.18±0.57	4.54±1.15	5.13±0.58	46.23

The result is Mean±SD. N = 30

There was an observed concentration dependant increment in mortality rate of the brine shrimp

3.2.2. Antioxidant Activity of Hornstedtia scyphifera var leaf crude extract

Many medicinal plants as well as the pure bioactive isolates have demonstrated tremendous beneficial therapeutic potentials, and many herbs were reported to contain antioxidant properties, and most of these activities are largely attributed to the phytochemicals (Aqil *et al*., 2006). Antioxidants are substances that possess free radical chain reaction breaking properties (Isaac *et al*., 2018), antioxidant activity is a fundamental important property for life (Velioglu *et al.,* 1998) and has been shown to reduce oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006).

Free radical scavenging activities of leaf *Hornstedtia scyphifera* from different solvent systems of varying polarity as while as the isolated compounds were evaluated using DPPH a stable free radical method that is sensitive in determining the antioxidant activity of plant extracts and vegetables. It has been reported that natural antioxidant presents in plants scavenge harmful free radicals from human body and exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzyme or by chelating trace elements (Asok Kumar *et al*., 2009).

The antioxidant effect of *Hornstedtia scyphifera* is shown in Table 15 and Figure 19 for crude extract and Table 16 and Figure 20 for isolated compounds. The results of the study showed that methanol and chloroform fractions of the leaf extract exhibit strong antioxidant activity with IC_{50} values of 34.43 $\pm$ 0.110 µg/mL and 34.46±0.32 µg/mL, respectively. The strong antioxidant activity exhibited by the methanol extract in this study is congruent to the work of Mariya and Reena (2007) in which they reported that methanolic extract was found to be most effective antioxidant property. It was observed also that hexane and dichloromethane fractions exhibited weak antioxidant activity with IC_{50} values 63.4±0.11 and 51.91±0.921 when compared to the standard ascorbic acid of 17.27±10.16µg/mL.

Table 16 and Figure 19 shows the dodecanoic acid to have higher antioxidant potential of 30.63 and lower was observed with dodecanoic acid of 39.47 when compared to the control ascorbic acid of Figure 20.

Table 15 IC_{50} values of leaves crude extract of *Hornstedtia scyphifera*

	Crude extract	**IC_{50} (µg/mL)**	**R2**
Leaves	Control	17.27.4±0.16	0.9937
	Hexane	63.4±0.11	0.9824
	Dichloromethane	51.91±0.921	0.9362
	Chloroform	34.46±0.32	0.9886
	Ethyl acetate	44.26±0.26	0.9949
	Methanol	35.33±0.210	0.9838

Table 16 DPPH IC50 (µg/mL) Value of Isolated Compound

Standard and compound	**R2 and**	**IC50 (µg/mL)**
	R2	IC50 (µg/mL)
Standard Vitamin C	0.9657	17.27
Quercetin	0.9070	30.63
Dodecanoic acid	0.9703	39.47

IC_{50} = Half maximal inhibitory concentration

3.2.3. Antibacterial Activity of Leaves extract of Hornstedtia scyphifera

Over the years, there has been a tremendous increase on the report of antimicrobial properties of medicinal plants by researchers worldwide and this has contributed enormously to the understanding and discovery of natural agents that could be effective in combating microorganism in human health delivery. Table 17 and Table 18 show the mean values of the zone of growth inhibition of leaf extract and isolated compound of *Hornstedtia scyphifera* against bacterial strains in mm compared to tetracycline.

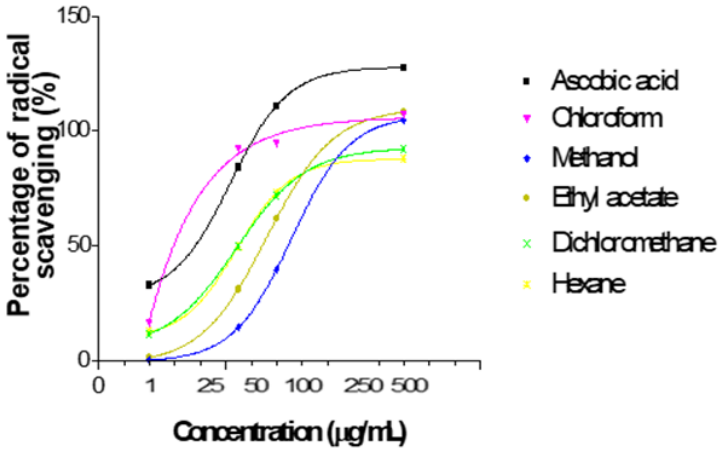


Figure 19 Radical scavenging activities of leaves extract in different solvents at absorbance of 517 nm.

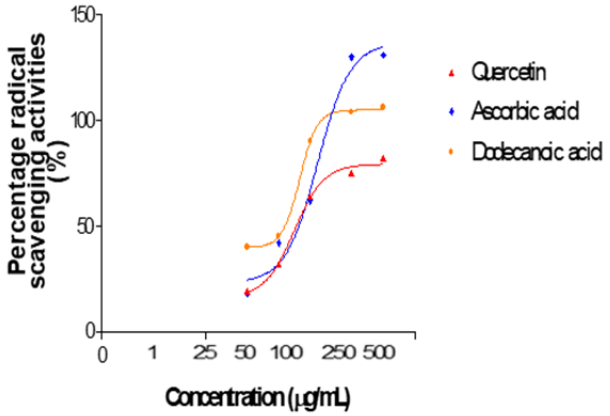


Figure 18 Radical scavenging activities of Isolated pure compounds at absorbance of 517 nm**.**

In this study, the leaf extracts and the isolated compounds quercetin and dodecanoic acid of *Hornstedtia scyphifera* showed significant bactericidal effects against the test bacterial strains; *Escherichia coli (E. coli)*, *Salmonella typhi (S. typhi), Klebsiella pneumonia (K. pneumonia)* and *Staphylococcus aureus (S. aureus)*, as shown in Table 17 and Table 18.

There was an observed significant activity of the crude extract at 500 µg/mL in all the extracts against the selected pathogen with zone of growth inhibition ranging from 07.70 ± 27.30 mm to 19.30 ± 0.10 mm. The activity was observed in *Salmonella typhi* ranged from 7.87 ± 0.06 mm in ethyl acetate extract to 17.97 ± 0.06 mm in methanol extract. The activity against *E. coli* was observed in all the extract which ranged from 7.47 ± 0.15 mm in chloroform extract to 19.73 ± 0.06 mm in hexane extract, as compared to standard drug tetracycline with zone of growth inhibition of 19.77 ± 0.38 mm.

The activity against *S. aureus* was observed in all the extract range from 9.67 ± 0.06 mm methanol to 18.06 ± 0.06 mm methanol at 500 µg/mL. The activity of leaf extract of *Hornstedtia scyphifera* on this pathogen *K. pneumonia* range from 10.0 ± 0.00 mm ethyl acetate to 16.10 ± 0.10 mm ethyl acetate. However higher inhibition was observed with ethyl acetate and methanol crude on Escherichia coli with inhibition value of 20.80 ± 0.10 mm and 20.80 ± 0.10 mm, respectively. Lower inhibition was generally observed with hexane crude extract as shown in the Table 17.

Table 17 Mean values of the zone of growth inhibition of extract of Leaves extract of Hornstedtia scyphifera of different concentration.

		Crude extract				
		Diameter of the zone of inhibition in mm				
Concn (µg/mL)	**Control and Organism**	**Hexane**	**Dichloromethane**	**Ethyl acetate**	**Chloroform**	**Methanol**
	Tetracycline (control)	19.77 ±0.38	19.77 ± 0.38	19.77 ± 0.38	19.77 ± 0.38	19.77 ± 0.38
25	*Salmonella typhi*	8.63 ± 0.06	8.55 ± 0.07	7.87 ± 0.06	8.67 ± 0.15	7.87 ± 0.12b
	Escherichia coli	8.43 ± 0.06	8.47 ± 0.06	7.57 ± 0.06b	7.47 ± 0.15	8.57 ± 0.06b
	Staphylococcus aureus	11.73 ±0.06	11.60 ± 0.00a	11.67 ± 0.06	9.67 ± 0.06a	9.73 ± 0.23
	Klebsiella Pneumonia	11.60 ±0.00	12.57 ± 0.06	10.0 ± 0.00b	8.63 ± 0.06	10.63 ± 0.12
50	*Salmonella typhi*	9.70 ± 0.00	10.63 ± 0.15	10.87 ± 0.06a	8.67 ± 0.06	11.00 ± 0.20
	Escherichia coli	18.60 ± 0.00	17.50 ± 0.00	17.70 ± 0.10	12.73 ± 0.06b	13.70 ± 0.00
	Staphylococcus aureus	13.93 ± 0.15	14.90 ± 0.10	13.77 ± 0.06	12.80 ± 0.10	13.60 ± 0.00
	Klebsiella Pneumonia	11.73 ± 0.06	11.70 ± 0.00	12.83 ± 0.06	10.70 ± 0.17	14.80 ± 0.10
100	*Salmonella typhi*	11.73 ± 0.06	10.67 ± 0.15	11.83 ± 0.12a	10.73 ± 0.21	14.93 ± 0.06
	Escherichia coli	19.73 ± 0.06	19.70 ± 0.10	20.80 ± 0.10	11.83 ± 0.06b	20.80 ± 0.10
	Staphylococcus aureus	16.97 ± 0.06	15.00 ± 0.10	14.87 ± 0.06	13.90 ± 0.20	15.83 ± 0.06
	Klebsiella Pneumonia	12.80 ± 0.10a	12.77 ± 0.06	13.90 ± 0.00	10.83 ± 0.06a	15.77 ± 0.21b
250	*Salmonella typhi*	14.83 ± 0.12	15.93 ± 0.15b	15.80 ± 0.17b	12.77 ± 0.66	16.70 ± 0.20
	Escherichia coli	16.73 ± 0.06*	17.93 ± 0.06*	18.83 ± 0.12*	14.00 ± 0.10	23.03 ± 0.06b
	Staphylococcus aureus	11.03 ± 0.06	16.10 ± 0.10	15.97 ± 0.06	15.03 ± 0.12a	17.03 ± 0.06
	Klebsiella Pneumonia	12.87 ± 0.06	11.97 ± 0.06	14.00 ± 0.00	11.03 ± 0.12	15.97 ± 0.06b
500	*Salmonella typhi*	12.87 ± 0.23	17.77 ± 0.12a	17.90 ±0.10	13.87 ± 0.15	17.97 ± 0.06b
	Escherichia coli	14.83 ± 0.06	16.03 ± 0.06	16.20 ± 0.10	15.07 ± 0.06*	27.30 ± 0.10b*
	Staphylococcus aureus	18.10 ± 0.10a	18.23 ± 0.06*	18.03 ± 0.06	15.03 ± 0.06	18.06 ± 0.06
	Klebsiella Pneumonia	13.97 ± 0.06	14.00 ± 0.10	16.10 ± 0.10	14.13±0.06ab	14.07 ± 0.06

Values are Mean ± SD for three determinations

*Significantly ($p < 0.05$) higher compared to different plant part at the same concentration in each column

Concentration of standard is 30 µg/mL of tetracycline

The finding is congruent to studies reported on the effects of solvent medicinal plants extract on *S. typhi, E. coli, S. aureus and K. pneumonia* by some authors (Daljit *et al.,* 1999; Prashanth *et al.,* 2006; Apu *et al.*, 2010; Nayan *et al.,* 2011).

Table 18 showed quercetin to have higher growth inhibition value of 20.7±0.04 mm on *Klebsiella pneumonia,* dodecanoic acid showed growth inhibition of 18.00±0.0 mm at the same concentration (100 µg/mL) on Salmonella typhi. Lower inhibition was observed at 25 µg/mL on *Salmonella typhi* of 9.20±0.02 mm and 9.00 ±0.00 mm, respectively when compared to the control tetracycline of 19.8 ±2.20 mm.

However, at lower concentrations of the crude extract, activity was observed but they are not significant as compared to the standard drug as while as to the standard inhibition where inhibition < 9 is considered inactive reported by Jan Hudzicki, (2009).

Table 18 Antibacterial activity of isolated pure compounds

Concentration (µg/mL)	Bacteria	Control Tetracycline (30µg)	Quercetin	Dodecanoic acid
	Escherichia coli	17.30±1.6	11.4±0.05	12.6 ±0.43
25µg/mL	*Klebsiella pneumonia, ST*	19.80 ±2.2	11.7 ±0.16	13.3±0.16
	Salmonella typhi	25.1 ±3.40	9.20±0.02	9.00 ±0.00
	Escherichia coli	17.3±0.30	15.7±0.04	12.7 ±0.16
50µg/mL	*Klebsiella pneumonia, ST*	19.8 ±2.20	16.2±0.02	17.3±0.16*
	Salmonella typhi	25.1 ±3.40	16.2±0.02	14.3±0.16
	Escherichia coli	17.32±1.6	16.9±0.05	12.9 ±0.28*
100µg/mL	*Klebsiella pneumonia, ST*	19.8 ±2.20	20.7±0.04*	15.5 ±0.14
	Salmonella typhi	25.1 ±3.40	15.7±0.04	18.00±0.0*

Higher inhibition =*,

4. Conclusion

In this study, we extracted, isolated and characterized two pure compound quercetin and dodecanoic acid from *Hornstedtia scyphifera* var leaf, a series of biological activity was experimented, demonstrating the necessity of the crude extract as an agent for proper function as an antioxidant and antibacterial potential. These results help to explain the high sequence of medicinal activity deposited and observed at this position in all the solvent extract and the isolated pure compounds. We subsequently found that the crude extract, because of the composition of the phytochemical deposit such as quercetin and dodecanoic acid, the plant extract and its isolate could be used as an agent in pharmaceutical industry. To the best of our knowledge this known compound was first isolated in this plant *Hornstedtia scyphifera* var leaf.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing of interest.

References

[1] Dahiru D, Mamman DN and Wakawa HY. (2010). *Ziziphus mauritiana* fruit extract inhibits carbon tetrachloride-induced hepatotoxicity in male rats. Pakistan Journal of Nutrition, 9(10), 990-993.

[2] Fatahi M, Rashidabady T and Fetahi-Hassanabad Z. (2003). Effects of *Crocus sativa* petal extract on rat blood pressure on response induced by electrical field stimulation in the rat isolated vas deferner and guinea pig's ileum. Journal of Ethno pharmacology, 84, 119-203.

[3] Jani NA, Ibrahim N, Hashim ST and Mohd H. (2016). Antimicrobial and Antioxidant activities of *Hornstedtia leonurus* Retz. Extracts. *Journal of Science and* Technology, 1-9.

[4] Hashim SE, Sirat HM and Yen KH. (2014). Chemical Compositions and Antimicrobial Activity of the Essential Oils of *Hornstedtia havilandii* (Zingiberaceae). Natural Product Communications, 9(1), 119-120.

[5] Holtum RE. (1950). The Zingiberaceae of the Malay Peninsula, Volume 13, Garden's Bulletin Singapore, Singapore.

[6] Fasihuddin BA, Sallehuddin NKNM and Assim Z. (2010). Chemical constituents and antiviral study of Goniothalamus velutinus. Malaysian Journal of Fundamental and Applied Sciences, 6(1), 73-76.

[7] Isaac JU, Fasihuddin, AB, Zaini, BA and Hauwa, AU. (2018). Antibacterial and cytotoxic actions of chloroform crude extract of *Leptadenia hastata* (pers) Decnee. Clin Med Biochemistry, 4, 1-4.

[8] Firdous AM, Fayaz AL and Mushtaq AS. (2013). Isolation of active components derived from rhizome of *Euphorbia wallichii Hook*. International Journal of Ayurvedic and Herbal Medicine, 3(3), 1173-1183.

[9] Patra JK, Gouda S, Sahoo SK and Thatoi HN. (2012). Chromatography separation, 1H NMR analysis and bioautography screening of methanol extract of *Excoecaria agallocha* L. from Bhitarkanika, Orissa, India. Asian Pacific Journal of Tropical Biomedicine, 2(1), S50-S56.

[10] Isaac JU, Fasihuddin BA and Hauwa AU. (2019). Extraction, Elucidation, Characterization and Evaluation of Antibacterial Activity of Four Pure Compound from Barringtonia racemosa Leaf Extract. World Journal of Pharmacy and Pharmaceutical Sciences, 8(8), 184-223.

[11] Umaru IJ, Badruddin BA and Umaru HA. (2019). Extraction, Isolation and Characterization of New Compound and Anti-Bacterial Potentials of the Chemical Constituents Compound from *Leptadenia Hastata* Leaf Extract. ChemRxiv, 1-42.

[12] Kalaiselvan A, Gokulakrishnan K and Anand T. (2012). Gas chromatography mass spectrum analysis of bioactive components of the ethanol extract of *Andrographis paniculata*. Journal of Pharmaceutical and Biomedical Science, 20(15), 1-3.

[13] Shalini S and Sampathkumar P. (2012). Phytochemical screening and antimicrobial activity of plant extracts. International Journal of Current Science, 20, 209-218.

[14] Janice GS. (2008). Key concepts - Mass spectrometry, infrared spectroscopy and nuclear magnetic resonance spectroscopy.Organic Chemistry 2nd Edition. New York, McGraw-Hill, 485-525.

[15] Efdi M, Fujita S, Inuzuka T and Koketsu M. (2010). Chemical studies on *Goniothalamus tapis Miq*. Natural Product Research, 24(7), 657-662.

[16] Boyan B, James H and Judicael P. (2005). Principles of accessing bacterial susceptibility to antibiotics using agar diffusion method, *Journal of Antimicrobial Chemotherapy*, 61, 1295-1301.

[17] Prashanth VK, Chauhan NS, Padh H and Rajani M. (2006). Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology, 107(2), 182-188.

[18] McLaughlin JL, Rogers LL and Anderson JE. (1991). The use of biological assays to evaluate botanicals. Drug Information Journal, 32(2), 513-524.

[19] Wang HX, Liu CM, Liu Q and Gao K. (2008). Three types of sesquiterpenes from rhizomes of *Atractylodes lancea*. *Phytochemistry*, 69(10), 2088-2094.

[20] Tailor CS and Goyal A. (2014). Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. leaves. American *Journal of Ethnomedicine*, 1(4), 244-249.

[21] Duc LV, Thanh TB and Hau TN. (2018). Flavonoids from *Dicliptera chinensis* (L) Nees Grown in Vietnam and their Anti-Inflammatory Activities. *Asian Journal of Biomedical and Pharmaceutical Sciences,* 8(64), 6-13.

[22] Bei D and An G. (2016). Pharmacokinetics and tissue distribution of 5, 7-dimethoxyflavone in mice following single dose oral administration. Journal of Pharmaceutical and Biomedical Analysis, 119, 65-70.

[23] Silverstein RM, Webster FX and Kiemle DJ. (2005). Spectrometric Identification of Organic Compound.ISBN 978-0-471-39362-7.

[24] Zhu CL. (2014). Chemical constituents of n-BuOH extract from *Phyllanthus matsumurae*. Zhong yao cai= Zhongyaocai. *Journal of Chinese Medicinal Materials*, 37(4), 608-610.

[25] Niknamian S and Niknamian S. (2016). Dodecanoic-Acid in Extra Virgin Coconut Oil, May Reduce the Incidence of Heart Disease and Cancer in Humans. *International Journal of Science and Research,* 5(1), 792-797.

[26] Rayan P1 and McDonnell PA. (2014). Dietary Supplementation of Dodecanoic Acid in Maternal Mice Enhances Resistance to *Giardia duodenalis* Infection in Suckling Neonatal Pups. *Pharmacognosy Communications*, 4(4), 83-89.

[27] Yamashita S, Igarashi M, Hayashi C, Shitara T, Nomoto A, Mizote T and Shibasaki M. (2015). Identification of self-growth-inhibiting compounds lauric acid and7-(Z)-tetradecenoic acid from *Helicobacter pylori*. Microbiology, 161, 1231-1239.

[28] Umaru IJ, Badruddin FA and Umaru HA. (2018).Cytotoxicity Brine Shrimp Activity of *Leptadenia hastata* (Per) Decne Leaves, Stem-bark and Roots Extract. *International Journal of Biochemistry and Physiology,* 3(2), 01-09.

[29] Isaac JU, Fasihuddin AB, Zaini BA and Hauwa AU. (2018). Cytotoxicity (Brine shrimp Lethality Bioassay) of *Barringtonia racemosa* Leaves, Stem-Bark and Root Extract. *Journal of Biotechnology and Bioengineering*, 2(2), 1-6.

[30] Anderson KM, Odell PM, Wilson PW and Kannel WB. (1991).Cardiovascular disease risk profiles. *American Heart Journal*, 121(1), 293-298.

[31] Aqil F Ahmad I and Mehmood Z. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish Journal of Biology*, *30*(3), 177-183.

[32] Isaac JU and Fasihuddin AB. (2018). Antioxidant and Biological Activities of *Leptadenia Hastata* ethyl acetate Roots Extracts, *Medicinal & Analytical Chemistry International Journal,* 2(1), 1-5.

[33] Velioglu YS, Mazza G, Gao L and Oomah BD. (1998).Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, *46*(10), 4113-4117.

[34] Pourmorad F, Hosseinimehr SJ and Shahabimajd N. (2006). Antioxidant activity phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*, *5*(11), 1142 – 1145.

[35] Asok Kumar K, Uma Maheswari M, Sivashanmugam AT, Subhadra Devi V, Subhashini N and Ravi TK. (2009). Free radical scavenging and antioxidant activities of *Glinus oppositifolius* (carpet weed) using different *in-vitro* assay systems. *Pharmaceutical Biology*, *47*(6), 474-482.

[36] Mariya S and Reena L. (2017). Evaluation of pigments as antioxidant and antibacterial agents from *Beta vulgaris* Linn. *International Journal of Current Pharmaceutical Research,* 9, 33-37.

[37] Daljit SA and Jasleen, K. (1999). Antimicrobial activities of spices. *International Journal of Antimicrobial Agents 12*, 257-262.

[38] Prakash V, Kishor MP and Meenakshi M. (2009). Screening of medicinal plant extracts for antioxidant activity. *Journal of Medicinal Plants Research*, *3*(8), 608-612.

[39] Apu AS, Muhit MA, Tareq SM, Pathan AH, Jamaluddin ATM and Ahmed M. (2010). Antimicrobial activity and brine shrimp lethality bioassay of the leaves extract of *Dillenia indica* Linn. *Journal of Young Pharmacists*, *2*(1), 50-53.

[40] Nayan BR, Nariya PB and Shukla VJ. (2011). Antibacterial and antifungal activity from flower extracts of *Cassia fistula* L.: An ethnomedicinal plant. *International Journal of PharmTech Research*, *3*(1), 160-168.

[41] Jan Hudzicki (2016). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *American Society of Microbiology,* 1-23.

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