https://dx.doi.org/10.4314/ijs.v26i3.2

GC-MS CHARACTERIZATION AND BIOACTIVITY STUDIES OF AERIAL PART OF *Hilleria latifolia* (LAM) EXTRACT AND FRACTIONS: ANTIOXIDANT AND ANTIBACTERIAL POTENTIALS

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(Received: 19th August, 2024; Accepted: 29th September, 2024)

ABSTRACT

This study sets out on identifying active compounds within *Hilleria latifolia* (Lam) extracts and fractions, with a specific emphasis on their potential antibacterial and antioxidant activities. GC-MS analysis was employed to characterize the chemical constituents in the n-hexane (*n*-Hex) and dichloromethane (DCM) fractions. The *n*-Hex and DCM fractions of the aerial part of *Hilleria latifolia* (Lam) revealed the presence of 45 compounds (27 in *n*-Hex and 18 in DCM), many of which are known for their antioxidant, anticancer, anti-inflammatory, and antibacterial properties. Antioxidant activity was evaluated through iron-reducing power (FRAP), nitric oxide (NO) inhibition, metal chelating ability (MC), total antioxidant capacity (TAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The EtOAc fraction showed the best activities in DPPH (IC_{50} 0.70±0.026 mg/mL), FRAP (478.503±8.607 mg AAE/g), MCA (0.199±0.085 mg/mL) and TAC (227.37±60.949 mg AAE/g). The sensitivity testing of the crude methanolic extract and fractions were carried out using the agar-well diffusion method against three gram-positive (*Bacillus stearothermophillus, Staphylococcus aureus, and Micrococcus luteus*) and five gram-negative (*Klebsiella pneumonia*e, *Pseudomonas aeruginosa, Escherichia coli*, *Proteus vulgaris, and Serratia marcescens*) bacterial strains. The crude methanol extract and aqueous fraction did not show activity towards all the bacterial strains. However, the *n*-Hex fraction exhibited moderate antibacterial activity particularly against *K. pneumoniae* (12 mm), *M. luteus* (9 mm), *P. aeruginosa* (12 mm), *B. stearothermophillus* (10 mm), and *S. aureus* (12 mm), the DCM fraction also showed effective antibacterial activity against *K. pneumoniae* (16 mm), *M. luteus* (12 mm), *P. vulgaris* (11 mm), *B. stearothermophillus* (12 mm), *E. coli* (12 mm) and *S. aureus* (11 mm). The ethyl acetate (EtOAc) fractions have shown considerable antibacterial activity against *K. pneumoniae* (12 mm), *M. luteus* (12 mm), *P. vulgaris* (11 mm), *B. stearothermophillus* (12 mm) and *S. aureus* (8 mm). The present findings demonstrate the antioxidant and antibacterial properties of *Hilleria latofolia* fractions, underscoring the need for further research to confirm their medicinal potential and explore its pharmaceutical applications.

Keywords: GC-MS, Antioxidant Activity, Antibacterial Activity, *H. latifolia*.

INTRODUCTION

Across the ages, people have turned to plants possessing medicinal properties as a valuable resource for treating a wide array of ailments. Among these botanical treasures is *Hilleria latifolia*, a member of the Phytolaccaceae genus, known by various vernacular names, including "Forest spinach" or "Pepper herb" (Otoo, 2014). In local communities, it goes by names such as "Aka ato" in Igbo and "ogo" in Yoruba, emphasizing its regional significance (Woode and Abotsi, 2012). This flowering plant is indigenous to tropical Africa and can be found from Guinea to Ethiopia, extending southward to South Africa, Angola, and Mozambique, with additional occurrences in Madagascar and Sri Lanka (Schmelzer, 2007). Within Nigeria, *Hilleria latifolia* thrives primarily in Ondo and Oyo States.

Hilleria latifolia has gained recognition for its diverse medicinal applications. In Cote d'Ivoire, its leaves are used to create a decoction for addressing food poisoning, while mashed leaves are applied locally to alleviate joint, muscle, and tendon pain (Odugbemi, 2008; Schmelzer, 2007). Moreover, it is customary to prepare an oral tea from the entire plant of *H. latifolia* to treat urethral discharges. In traditional Nigerian medicine, the leaves are simmered in broth to combat gonorrhoea. In Congo, a blend of leaves and stem sap from the *Costus afer* plant is employed for treating gynaecological conditions that require purging (Schmelzer, 2007). Asthma and bloody coughs find relief through the use of orange juice and flower petal pastes, respectively (Schmelzer, 2007).

In Ghana, steam baths infused with a decoction of *Hilleria latifolia* leaves and twigs are administered to

address jaundice and guinea worm infestations (Iwu, 1993). Additionally, the plant, whether in leaf form or as a whole, plays a role in treating breast cancer, often in conjunction with *Momordica charantia* (Curcubitaceae) (Abbiw, 1990; Odugbemi, 2008). Across East Africa, people employ decoctions of the entire plant for bathing, providing relief for various skin conditions. Furthermore, the plant's leaves, when transformed into lotion or used in their natural state, prove effective in soothing a variety of skin issues (Odugbemi, 2008; Schmelzer, 2007).

Despite the rich tapestry of folklore surrounding *Hilleria latifolia*'s therapeutic potential, there remains a paucity of information regarding chemical composition of the plant. As a result, this research endeavours to unravel the constituents of the *n*-hexane and dichloromethane fractions through GC-MS analysis.

MATERIALS AND METHODS

Solvent Used

Methanol, ethyl acetate, *n*–hexane, acetone and distilled water. All solvents used were analar grade from vendors that were still distilled to ensure purity.

Collection and Preparation of Plant Materials

Plant Collection, Authentication and Preparation

The aerial parts of *Hilleria latifolia* were collected in August 2018 in Akomu forest reserve in Ondo State. It was authenticated by Mr. Ademoriyo of the Herbarium section of the Department of Botany, Obafemi Awolowo University, Ile-Ife with voucher specimen (IFE 17980). The plant was air-dried for about two weeks and powdered using a pulverizing machine.

Extraction of the Plant Material

The powdered aerial parts of the plant (1 kg) were extracted in 8 L of 90% methanol at room temperature for 48 h and filtered with cotton fitted into a stainless-steel funnel. The filtrates were concentrated to dryness on rotatory evaporator (D-91126 Schwabach, Heldolph Instruments, Germany) at 40 ^{0}C. The crude extract obtained (101.38 g, 10.14 %) was stored in the refrigerator until required.

Solvent Partitioning of the Crude Extract

The crude extract (101.38 g) from the aerial parts of the plant was homogenized with 300 mL distilled water and partitioned in a 5 L separating funnel in turn with n – hexane *n*-hexane (3 × 800 mL), dichloromethane (3 × 800 mL), ethyl acetate (3 × 800 mL). The organic solvent layer of each solvent partitioning was evaporated to dryness on rotatory evaporator at 40 ^{0}C. This yielded four solvent fractions namely, the *n*–hexane fraction (24.53 g), dichloromethane fraction (2.41 g), ethyl acetate fraction (2.36 g) and aqueous fraction (48.68 g).

Phytochemical Screening

The crude extract of the plant was subjected to phytochemical screening using the method described by Ajayi *et al.* (2021).

Determination of Tannins

Two drops of 5% $FeCl_3$ were added to 1 mL of 0.625 mg/mL solution of the crude extract. A filthy, green precipitate indicated the presence of tannin.

Determination of Glycosides

Crude extract solution (1.0 mL) was pipette and 10 mL of 50% (v/v) H_2SO_4 was added. The mixture was heated for 5 minutes followed by the addition of 5 mL each of Fehling's solution A and B and then boiled. A brick red precipitate indicated the presence of glycoside.

Determination of Resins

A 2.5 mL of 0.5 M $CuSO_4$ solution was added to 2.5 mL solution of the crude extract. The mixture was vigorously shaken and allowed to settle. The appearance of green colour suggested the presence of resin.

Determination of Saponins (Frothing Test)

A 2 mL crude extract (0.625 mg/mL) was shaken in a test tube for two minutes. Frothing indicated the presence of saponins. The presence of saponins was suggested by the observed frothing behaviour.

Determination of Phlobatannins

A mixture of 5 mL of the crude extract solution (0.625 mg/mL) and 5 mL of distilled water is boiled with 2 mL of 1% HCl for two minutes. The appearance of a green colour suggested the presence of phlobatannins.

Determination of Flavonoids

The mixture is heated in a water bath and allowed to cool. Distinct layers were formed after cooling and a red coloured layer suggested the presence of flavonoids.

Determination of Steroids (Salkowski Test)

Concentrated H_2SO_4 (2 mL) was carefully added to 2 mL of 0.625 mg/mL solution of the extract. The formation of red precipitate suggested the presence of steroids.

Determination of Phenols

Equal volume of 20.0 mM $FeCl_3$ and 0.625 mg/mL solution of the crude extract were mixed together. A deep bluish green solution confirmed the presence of phenols.

Determination of Alkaloids

Concentrated H_2SO_4 (1 mL) was added to 3 mL solution of the crude extract. The mixture was then treated with Wagner reagent. A reddish-brown precipitate indicated the presence of alkaloids.

Determination of Carbohydrate (Fehling's test)

A 5 mL mixture of Fehling's A and B (1:1) in a test tube A 2 mL solution of the crude extract was added 5 mL mixture of Fehling's A and B (1:1) in a test tube. The resulting mixture was boiled for two minutes. A brick red precipitate suggested the presence of carbohydrate.

Determination of Terpenoid

A mixture of 2 mL $CHCl_3$ and 3 mL H_2SO_4 was added to 0.2 g sample of the crude extract. A reddish-brown interface developed suggested the presence of terpenoid.

Antioxidant Activities of the Plant Extract and Fractions

The following antioxidant parameters were determined on the crude extract and the four solvent fractions:

Determination of Total Phenol Content (TPC)

The method described by Singleton and Rossi, 1965 as modified by Gulcin *et al.* (2004) using the Folin-Ciocalteu's phenol reagent (as an oxidizing reagent) was used for the determination of TPC.

The standard solutions of varying concentrations were prepared by pipetting 0.2, 0.4, 0.6, 0.8, 1.0 mL from a 1 mg/mL stock solution of gallic acid solution in triplicate into clean test tubes and each test tube was made up to 1.0 mL with distilled water. A known amount (0.5g) of each fraction and crude extract was dissolved with 5.0 mL of 60% (v/v) aqueous methanol, filtered with Whatman filter paper and then made up to mark in a 25 mL standard flask with 60% (v/v) methanol. One mL of each of the reconstituted solutions of the fractions and crude extract was pipetted into clean dry test tubes. Thereafter, 1.5 mL of Folin-Ciocalteu's reagent (10 times diluted) was added to each test tube of the standard, fractions and the crude extracts. The mixture was incubated for 5 minutes at room temperature followed by the addition of 1.5 mL of 10% (w/v) $NaHCO_3$ solution to give a total reaction volume of 4.0 mL. The reaction mixtures were further incubated for 90 minutes and the absorbance was read at 725 nm using the UV spectrophotometer. The standard curve was obtained by plotting absorbance against concentration. The total phenol concentration of the fractions and the crude extracts were interpolated from the standard curve and expressed as milligram gallic acid equivalent per gram of extract (mg GAE/g).

Determination of Total Flavonoid Content (TFC)

Standard quercetin with varying concentration 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL was used as standard in comparison to the crude extract and fractions. This was carried out based on the aluminium chloride colorimetric assay method described by Miliauskas *et al.* (2004).

A 0.4 mL of distilled water was added to 0.1 mL of standard, crude extract, fractions. This was followed by 0.1 mL of 5% sodium nitrite. After 5 minutes, 0.1 mL of 10 % aluminum chloride and

0.2 mL of 5% sodium hydroxide was added and the volume was made up to 2.5 mL with distilled water. The absorbance at 510 nm was measured against the blank. The total flavonoid content of the plant crude extract and fractions, expressed as mg quercetin equivalents per gram of the plant extract is calculated according to equation below:

$$T = c \times \frac{V}{w}$$

Where:
T = Total content of flavonoid compound as quercetin equivalent
c = concentration of quercetin established from the standard curve
v = volume of extract (mL)
w = weight of the crude methanolic extract/fractions obtained.

DPPH Free Radical Scavenging Activity

The radical scavenging ability of the crude extract and fractions were determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) as described by Brand-Williams *et al.* (1995). The reaction of DPPH with an antioxidant compound which can donate hydrogen radical, leads to its reduction. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm.
A 1 mL of 0.3 mM DPPH in methanol was added to 1 mL of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) of crude extract, fractions or standard (vitamin C) in a test. The mixture was mixed and incubated in the dark for 30 mins after which the absorbance was read at 517 nm against a DPPH control containing only 1 mL methanol in place of the extract.

The percent of inhibition was calculated using Equation (3.2) below:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the crude extracts/fractions.

The sample concentration that corresponds to 50% inhibition (IC_{50}) was interpolated from the graph obtained by plotting the percentage inhibition (crude extract/fractions and standard) against their corresponding concentrations

Inhibition of Nitric Oxide (NO) Radical

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which was measured by Griess reaction.
The reaction mixture, containing 0.1 mL of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/mL) of the crude extract, fraction and 0.9 mL of sodium nitroprusside (2.5 mM) in phosphate buffer saline was incubated under illumination for 150 minutes. After incubation, 0.5 mL of 1% sulphanilamide in 5% phosphoric acid was added and incubated in the dark for 10 min., followed by addition of 0.5 mL 0.1% NED (N-1-napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was measured at 546 nm (Marcocci *et al.,* 1994). The percent of inhibition was calculated with Equation (3.3) below:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where
$A_{control}$ is the absorbance of the control reaction
A_{sample} is the absorbance of the crude extracts/fractions.

The sample concentration that corresponds to 50% inhibition (IC_{50}) was interpolated from the graph obtained by plotting the percentage inhibition (crude extract/fractions and standard) against their corresponding concentrations

Metal Chelating (MC) Activities (Ferrous Ion-chelating Ability Assay)

The ferrous ion-chelating (FIC) assay was carried out according to the method of Singh and Rajini (2004) with some modifications. The assay was carried out by preparing solutions of 2 mM $FeCl_2 \cdot 4H_2O$ and 5 mM ferrozine which were diluted 20 times. Briefly, an aliquot (1 mL) of different concentrations (10.0, 5.0, 2.5, 1.25, 0.625, 0.3125 mg/mL) of crude extract, and fractions were mixed with 1 mL $FeCl_2 \cdot 4H_2O$. After 5 min incubation, the reaction was initiated by the addition of ferrozine (1 mL). Each mixture was shaken vigorously and after a further 10 min incubation period, the absorbance of the solution

was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine – Fe^{2+} complex formation was calculated by using the formula given in equation 3.4 below:

$$\% \text{ Chelating effect} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ = absorbance of control sample (the control contains $FeCl_2$ and ferrozine, complex formation molecules) and A_{sample} = absorbance of a tested samples.

Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the crude extracts and fractions was carried out according to the method described by Prieto *et al.* (1999). The principle of this method is based on the reduction of Molybdenum (VI) to Molybdenum (V) by the crude extract/fraction and the subsequent formation of a green phosphate/molybdenum (V) complex in acidic medium.

Various concentrations of ascorbic acid standard were prepared (20, 40, 60, 80, 100 µg/mL) from a stock solution of 1 mg/mL. A known amount, 25 µL of 1 mg/mL solution of the crude extracts and fractions and the above standards were pipetted separately in triplicates into micro-plates and 250 µL of the reagent (prepared by dissolving 28 mM sodium phosphate and 4 mM ammonium molybdate with 0.6 M sulphuric acid and made up to mark in a 100 mL standard flask with the same acid) was added to each of the crude extracts, fractions and standards. The micro-plate was capped and incubated at 100 °C in an incubator (MK2000-2, Hangzhou Allsheng Instruments, China) for a period of 90 min. The mixture was then allowed to cool to room temperature before the absorbance was measured at 695 nm against a reagent blank. The reagent blank solution contained 250 µL of the reagent and 25 µL of absolute methanol in place of the test samples. The total antioxidant capacity was expressed as equivalence of ascorbic acid. Ascorbic acid equivalent (mg AAE/g fraction) of the test samples was calculated from the linear regression obtained from the plot of the mean absorbance of the standard against its concentration. The absorbance of the sample was interpolated from the standard curve and its concentration calculated.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method with absorbance measured with a spectrophotometer (Benzie and Strain, 1999). The principle of this method is based on the reduction of a colourless ferric-tripyridyltriazine complex to its blue ferrous coloured form owing to the action of electron donating in the presence of antioxidants.

A 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and 20 mmol/L $FeCl_3.6H_2O$ were mixed together in the ratio of 10:1:1 respectively, to give the working FRAP reagent. A 50 µL aliquot of the crude extract or fractions at 0.1 mg/mL and 50 µL of standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/mL) was added to 1 mL of FRAP reagent. Absorbance measurement was taken at 593 nm exactly 10 min. after mixing against reagent blank containing 50 µL of distilled water.

All measurements were taken at room temperature with samples protected from direct sunlight. The reducing power was expressed as equivalent concentration (EC) which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard.

Preliminary Screening for Antibacterial Activities of the Plant Extracts and Fractions

The sensitivity testing of the crude extract, fractions and isolated compounds was determined using agar-well diffusion method as described by Adegoke *et al.* (2010) with some modifications. The bacterial strains were first grown on nutrient agar for 18 h before use. The turbidity of the 18 h old culture was adjusted to 0.5 McFarland standards (10^6 cfu/mL) in sterile normal saline. The inoculum was then seeded onto sterilized Mueller-Hinton agar using sterile swab stick. Wells were then bored into the seeded plate using a sterile 6 mm cork borer. The wells were filled up with the prepared solution of the sample and care was taken to avoid spillage of the sample onto the surface of the medium. The plates were allowed to

stand on the work bench for 1 h to allow proper inflow of the solution into the medium before incubating in an incubator at 37 °C for 24 h. The plates were then observed for zones of inhibition. The effects of the sample on the bacterial strains were compared with standard antibiotic (streptomycin).

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening of the aerial parts showed that the phytochemicals in *H. latifolia*, constitutes tannins, resins, saponins, phlobatannins, flavonoids, phenols, alkaloids. This result agreed with the result obtained by Dapaah *et al.* (2016) and Imoh *et al.* (2017) but for the detection of cardiac glycoside in their result (Table 1). The ethnomedicinal uses of the plant may be associated with the presence of secondary metabolites such as alkaloids and flavonoids. Alkaloids have been known to exhibit antiplasmodial activity by blocking protein synthesis in *Plasmodium falciparum* (Muthaura *et al.,* 2007). Flavonoids have been reported to chelate the nuclei acid base pairing of the parasite (Muthaura *et al.,* 2007).

Table 1: Phytochemical Screening of the crude methanol extract of *H. latifolia.*

S/No	Phytochemicals	Observation
1.	Tannins	+++
2.	Glycosides	-
3.	Resins	++
4.	Saponins	++
5.	Phlobatannins	+
6.	flavanoids	++
7.	Sterols	-
8.	Phenols	++
9.	Carbohydrates	-
10.	Alkaloids	++
11.	Terpenoids	-

Key: +++ = very strong, ++ = strong, + = weak, - = undetected

Phenolic Constituents of the Crude Extract and Fractions of *Hilleria latifolia Lam.*

Plants phenolic compounds include phenolic acid, flavonoids etc. The presence and relative quantities of these phenolic compounds in the plant material were evaluated by the determination of the total phenolic content (TPC) and total flavonoid content (TFC) of the crude extract and the fractions. Although TFC and TPC, are not among antioxidant assays, but their determination provides information on the antioxidant potential as well as information on the levels of flavonoid and phenolic compounds present in plant extracts respectively (Olayiwola *et al.*, 2021). The result showed that the ethyl acetate has the highest total phenolic content of 775.172 ± 9.212 mg GAE/g while *n*–hexane has the least, 348.967 ± 9.212 mg GAE/g. The same trend is observed in the total flavonoid content, the highest total flavonoid content is found in the ethyl acetate (478.818 ± 18.409 mg QUE/g) while the least is found in *n*–hexane (203.577 ± 9.987 mg QUE/g). (Table 2).

Table 2: The Total Phenolic and Flavonoid Contents of the Crude Methanol Extract and Solvent Fractions of *H. latofolia.*

Samples	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QUE/g)
Crude Methanol	412.246 ± 0.000	298.706 ± 9.492
n-Hexane	348.967 ± 9.212	203.577 ± 9.987
DCM	633.724 ± 6.580	397.64 ± 12.556
EtOAc	775.172 ± 9.212	478.818 ± 18.409
Aqueous	382.468 ± 7.895	236.555 ± 58.986

Results are means of triplicate determination ± standard deviation

Antioxidant Activity of the Crude Extract and Fractions of *Hilleria latifolia*

The quantitative antioxidant potential of the crude extract and different solvent fractions of *Hilleria latifolia* as depicted (Table 3) were evaluated using different in vitro antioxidant complimentary assays. Four antioxidant assays were used for extract and the solvent fractions; DPPH Free Radical Scavenging, Nitric Oxide (NO) Inhibition, Metal Chelating Ability (MCA), Total Antioxidant Capacity (TAC) and Ferric Reducing Antioxidant Power (FRAP).

Result of NO, MCA, DPPH, FRAP and TAC Activities of *Hilleria latifolia*

In the DPPH free radical scavenging potential of the ethyl acetate shows the best activity with the IC_{50} of 0.70 ± 0.026 mg/mL followed by dichloromethane fraction with IC_{50} of 0.927 ± 0.032 mg/mL. This same trend was also observed in the in the MCA, TAC and FRAP assays (Table 3). The NO inhibitory activity show that the crude extract has the highest activity (IC_{50} of 0.551 ± 0.035 mg/mL) followed by ethyl acetate (IC_{50} of 0.591± 0.047 mg/mL).

Table 3: Antioxidant Activities of Crude Methanol Extract and Fractions.

Samples	DPPH (IC_{50}) (µg/mL)	MCA (IC_{50}) (µg/mL)	NO (IC_{50}) (µg AAE/g)	FRAP (mg AAE/g)	TAC (mg AAE/g)
Crude	1.052±0.058	2.147±0.883	0.551±0.035	233.040±9.5	29.794±4.32
n-Hexane	1.252±0.055	0.934±0.052	0.931±0.001	69.648±5.03	26.319±4.75
DCM	0.927±0.032	0.703±0.060	0.743±0.007	342.218±2.8	153.404±9.42
EtOAc	0.70±0.026	0.199±0.085	0.591±0.047	478.503±8.6	227.370±11.8
Aqueous	0.946±0.037	0.609±0.166	0.963±0.108	80.190±6.87	15.022±3.21
Standard	0.008±0.54	$0.124\pm3x10^{-3}$	0.0634 ± 10^{-3}	135.8 ± 8.9^{bc}	341.2 ± 16.4^{a}

Results are means of triplicate determination ± standard deviation

GC-MS of Bioactive *n*–Hexane and DCM Fractions of *H. latifolia*

GC-MS analysis of the chemical constituents of *Hilleria latifolia* from the *n*-hexane and dichloromethane fractions were fatty acid esters, glycosides, ketone, saturated and unsaturated fatty acids, alcohols, and sterols (Tables 5 and 6). These constituents were presented according to the fractions from which they were identified. Figure 1 and 2 showed the GC-MS chromatogram showing the retention time of the identified compounds and their relative abundance in both the *n*-Hex and DCM fractions.

The *n*-hexane and dichloromethane fractions obtained from the partitioning of crude methanol extracts of *Hilleria latifolia* exhibited significant antibacterial and antioxidant activities. These

fractions were subjected to GC-MS analyses to identify their constituents. The *n*-hexane fraction shows a total of 27 compounds out of which 9 were major compounds; *n*–hexadecanoic acid (palmitic acid) (15.13%), oleic acid (13.81%), phytol (10.04%), stigmasta-3,5-diene (9.42%), 9-octadecenoic, methyl ester (8.16%), 9-octadecenoic, ethyl ester (5.64%), pentacosane (5.06%), hexadecanoic acid, methyl ester (3.45%) and octadecane (3.14%) (Table 4). The other compounds have concentrations <3%. The dichloromethane fraction shows a total of 18 constituents out of which 10 compounds were major; *n*-hexadecanoic acid (20.75%), 9,12-octadecadienoic acid, (17.66%), and (+)–spathulenol (11.75%), 9-octadecenoic, methyl ester (7.90%), phytol (5.23%), estra-1,3,5(10)-trien-17β-ol (4.83%), 9-octadecenoic, ethyl ester (4.20%), stigmasts-3,5-diene (4.18%), γ-sitosterol (4.09%) and oleic acid (3.99%) (Table 5). Other compounds have concentrations < 3%. All the identified constituents have been reported to possess various biological activities such as antioxidant, antimicrobial, anticancer, anti-inflammatory and antibacterial activities (Kumar *et al.,* 2010, Aparna *et al.,* 2012, Sivakumar *et al.*, 2011, Chandrasekaran *et al.,* 2011). Table 6 gives other reported biological activities of some of the compounds.

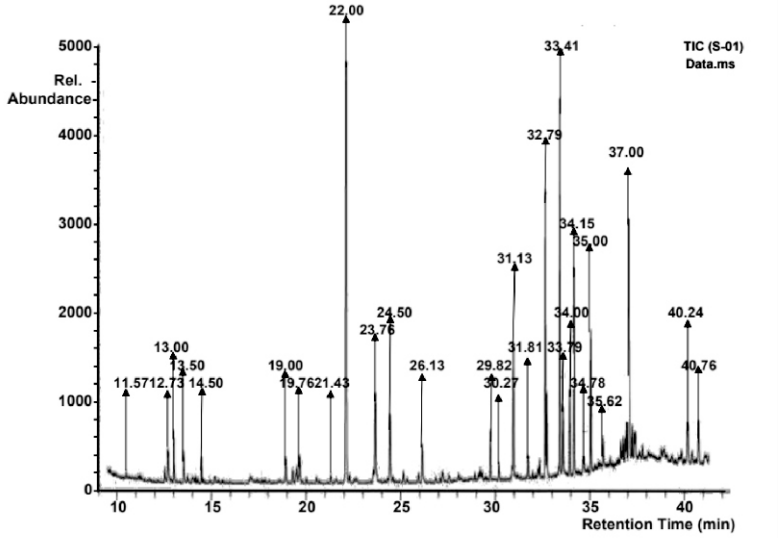

Figure 1: GC-MS Chromatogram of *n*-Hexane Fraction of *H. latifolia.*

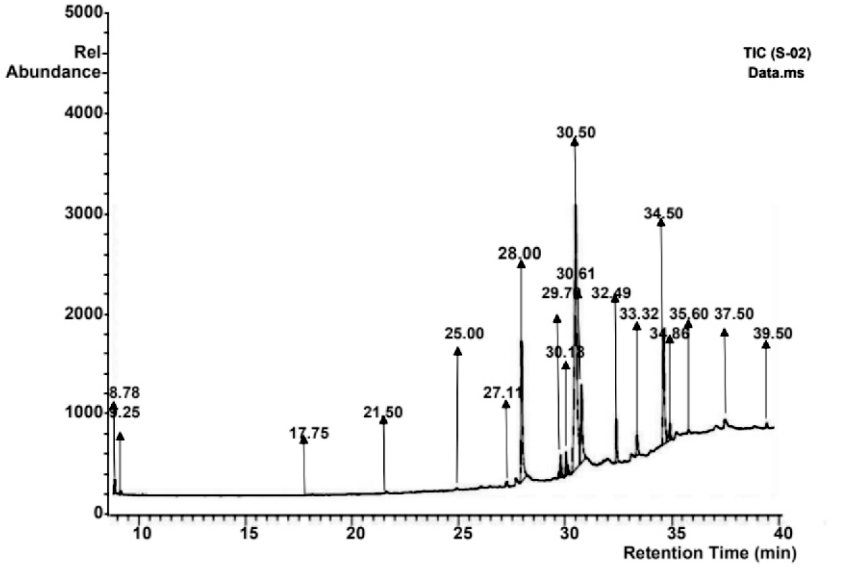

Figure 2: GC-MS Chromatogram of Dichloromethane (DCM) Fraction of *H. latifolia.*

Table 4: Phytocompounds Identified by GC-MS Analysis of the *n*-Hexane Fraction of *H. latifolia*.

Retention Time (min)	Compound Detected	Molecular Formula	Molecular Weight	Comp % Wt.	% Match
11.57	3-Hexen-1-ol	$C_6H_{12}O$	100	1.64	95.0
12.73	p-Xylene	C_8H_{10}	106	2.38	87.5
13.000	Nonane	C_9H_{20}	128	2.41	90.0
13.50	Thymol	$C_{10}H_{14}O$	150	1.49	90.0
14.50	N,N Dimethyltryptamine	$C_{12}H_{16}N_2$	188	1.08	83.6
19.00	Eugenol	$C_8H_8O_3$	164	1.01	96.0
19.76	3-Octadecene, (E)-	$C_{18}H_{36}$	252	1.48	96.0
21.43	m-phenethyl-Benzonitrile,	$C_{15}H_{13}N$	207	1.35	83.1
22.00	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.33	97.0
23.76	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	1.84	93.5
24.50	Neophytadiene	$C_{20}H_{38}$	278	1.16	77.0
26.13	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.19	94.2
29.82	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.16	90.0
30.27	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252	1.93	90.6
31.13	Pentacosane	$C_{25}H_{52}$	352	4.49	93.8
31.81	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	256	1.85	82.0
32.79	Phytol	$C_{20}H_{40}O$	296	7.79	98.0
33.41	Oleic acid	$C_{18}H_{34}O_2$	282	10.32	98.4
33.79	Octadecane	$C_{18}H_{38}$	254	2.73	95.9
34.00	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.95	98.6
34.15	9-Octadecenoic aci d (Z) -, methyl ester	$C_{19}H_{36}O_2$	296	5.40	97.5
34.78	Octadecanal	$C_{18}H_{36}O$	268	2.28	96.4
35.00	9-Octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310	2.92	98.0
35.62	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	2.84	98.3
37.00	Stigmasta-3,5-diene	$C_{29}H_{48}$	396	10.94	91.7
40.24	Squalene	$C_{30}H_{50}$	410	3.83	90.4
40.76	γ-Sitosterol	$C_{29}H_{50}O$	414	3.07	89.5

Table 5: Phytocompounds Identified by GC-MS Analysis of the Dichloromethane Fraction of *H. latifolia*.

Retention Time (min)	Compound Detected	Molecular Formula	Molecular Weight	Comp % Wt.	% Match
8.78	3-Hexen-1-ol	$C_6H_{12}O$	100	3.08	96.5
9.25	p-Xylene	C_8H_{10}	106	1.42	97.5
17.75	Nonane	C_9H_{20}	128	2.35	92.90
21.50	Thymol	$C_{10}H_{14}O$	150	2.07	88.0
25.50	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.81	93.5
27.11	3-Octadecene, (E)-	$C_{18}H_{36}$	252	1.48	96.0
28.00	(+)-Spathulenol	$C_{15}H_{24}O$	220	12.41	70.6
29.70	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252	2.28	82.0
30.10	Neophytadiene	$C_{20}H_{38}$	278	0.94	71.8
30.50	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.66	97.0
30.61	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	7.60	94.0
32.49	Phytol	$C_{20}H_{40}O$	296	5.31	98.7
33.32	Oleic acid	$C_{18}H_{34}O_2$	282	4.16	98.5
34.50	9,12-Octadecadienoic acid, (Z, Z)	$C_{18}H_{32}O_2$	280	8.26	84.0
34.86	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	256	2.71	86.4
35.60	γ-Sitosterol	$C_{29}H_{50}O$	414	3.31	80.0
37.50	9-Octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310	2.37	97.5
39.50	Stigmasta-3,5-diene	$C_{29}H_{48}$	396	4.18	89.5

Table 6: The Reported Activities of Some of the GC-MS Identified Compounds

Compounds	**Reported Activities**
Thymol	Antibacterial (Didri *et al.,* 1994), antifungal, anti-inflammatory (Aeschbach *et al.*, 1994), antioxidant (Yanishlieva *et al.*, 1999), anti-mutagenic (Zahin *et al.*, 2010), larvicidal (Pavela, 2009), analgesic (Ozen *et al.*, 2011), anti-microbial (Karpanen *et al.,* 2008), acaricidal (Araujo *et al.,* 2015), anticonvulsant, antiepileptogenic (Sancheti *et al.,* 2014), wound healing (Riella *et al.,* 2012), anti-hemolytic (Alinezhad *et al.,* 2013), antiphlogistic (Anamura, 1989), antileishmanial (Robledo *et al.,* 2005) and radioprotective (Archana *et al.,* 2011) properties.
Eugenol	Antimicrobial, antioxidant and anti-inflammatory activities (Anna *et al.,* 2017)
3-Octadecene	Antimicrobial and antioxidant (Ameera *et al.,* 2015)
n-hexadecanoic acid	Anti-inflammatory (Aparna *et al.,* 2012), Antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor (Awa *et al.,* 2012), potent mosquito larvicide (Rahuman *et al.,* 2000)
Neophytadiene	Antimicrobial, treatment of headache, rheumatism and some skin disease (Suresh *et al* ., 2010)
Tetradecanoic acid	Larvicidal and repellent activity antiepileptogenic (Sivakumar *et al.,* 2011)
3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	anticancer, anti-inflammatory, antimicrobial and antioxidant activities (Rijstenbil *et al.,* 1998). Sedative and anxiolytic effects (Neepal *et al.,* 2019) antioxidant effects (Santos *et al.,* 2013), Antimicrobial activity (Ghaneian *et al.,* 2015), component of chlorophyll and vitamin E and K.
Pentacosane	Antioxidant and antimicrobial activity (Mihailovi *et al.*, 2011)
13-Heptadecyn-1-ol	Antimicrobial antiinflammatory, antifungal (Al-Rubaye *et al.,* 2017) Anticancer, Antioxidant and Cytotoxic Activities (El Mokni *et al.,* 2016)
Estra-1,3,5(10)-trien-17β-ol	Anti-arryhthmic activities (Neepal *et al.,* 2019)
Oleic acid	Antibacterial (Awa *et al.*, 2012)
Hexadecanoic acid methyl ester	Antibacterial and antifungal (Chandrasekaran *et al.,* 2009)
9-Octadecenoic acid (Z)-, methyl ester	Antioxidant, antimicrobial, cancer enzyme inhibitors in pharmaceutical, cosmetics, and food industries (Albergoni *et al.*, 1980, Sayik *et al.,* 2017)
9-Octadecenoic acid, ethyl ester	Perfumery activity (Harborne and Baxter, 1983; Ross, 2003).
Squalene	Skin emollient, antioxidant, and antitumor activities (Zih-Rou Huang *et al.,* 2009)
γ-Sitosterol	Antimicrobial, anticancer anti-inflammatory anti-asthma, and diuretic antiarthritic (Jegajeevanram *et al.,* 2014
Spathulenol	Antifungal and antibacterial activities (Kamilla *et al.,* 2017)

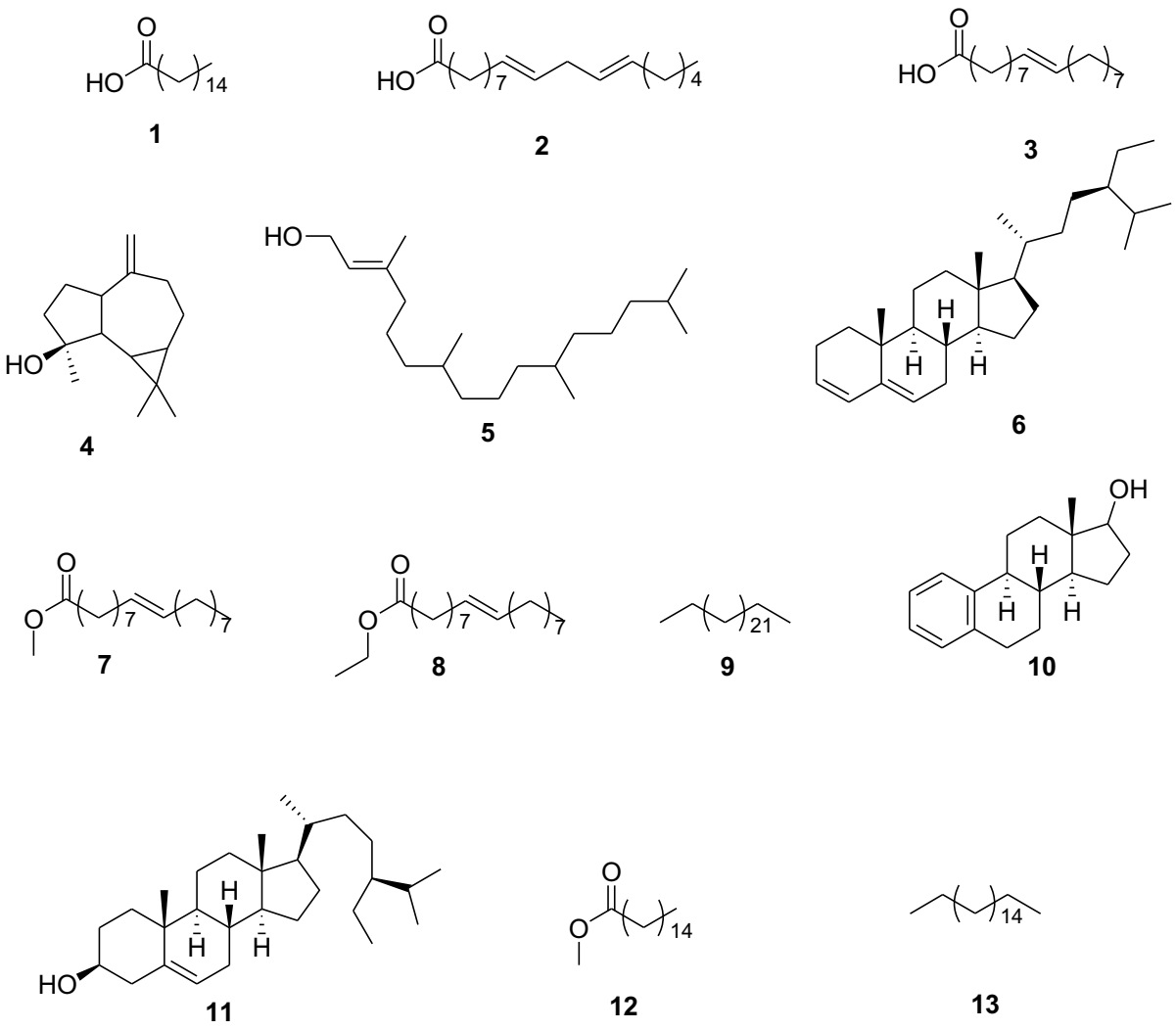

Figure 3: Chemical structures of compounds identified by GC-MS of *n*-hexane and dichloromethane fractions of the aerial part of *H. latifolia.*

Antibacterial Activity of the Crude Methanol Extract and Solvent Fractions

The crude methanol extract and solvent fractions from the plant were subjected to antibacterial sensitivity test against eight bacteria strains which are three gram +ve strains (*Bacillus stearothermophillus, Staphylococcus aureus, and Micrococcus luteus)* and five gram –ve strains (*Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli and Serratia marcescens*). The measured zone of inhibition was the diameter of the circle about the wall where there no bacterial growth. The crude methanol extract and aqueous fraction did not show activity towards all the bacteria strains. The ethyl acetate fraction shows activity against the three gram +ve strains at 10 mg/mL with inhibition zone of 12 mm, 8 mm and 12 mm for *B. stearothermophillus, S. aureus, and M. luteus* respectively and also against one gram –ve strain, *K. pneumonia* at a concentration of 10 mg/mL with inhibition zone of 12 mm. The *n*–hexane fraction showed activity against two gram +ve strain, *Micrococcus luteus* and *Bacillus stearothermophillus* at 15 mg/mL with inhibition zone of 9 mm and 10 mm respectively. It also showed activity against three gram–ve strain at the same concentration with the inhibition zone of 12 mm each for *K. pneumonia, P. aeruginosa,* and *E. coli.* The dichloromethane fraction showed activity against three gram+ve strains at 15 mg/mL with inhibition zone of 12 mm, 12 mm and 11 mm for *M. luteus, B. stearothermophillus* and *S. aureus* respectively. It is also active against three gram –ve strain at the same concentration with inhibition zone of 16, 11, and 12 mm for *K. pneumonia, P. vulgaris,* and *E. coli* respectively (Table 7).

Table 7: Zone of Inhibition in mm of the Crude Methanol Extract and Solvent Fractions from the Aerial parts of *H. latifolia*

Isolate	Crude 30 mg/mL	EtOAc 10 mg/mL	Hex 15 mg/mL	Aq. 15 mg/mL	DCM . 15 mg/mL	Strept 1 mg/mL	Microorganism
NCIB 418	-	12	12	-	16	24	*Klebsiella pneumoniae*
NCIB 1377	-	-	-	-	-	21	*Serratia marcescens*
NCIB 196	-	12	9	-	12	21	*Micrococus luteus*
NCIB 950	-	-	12	-	-	-ve	*Pseudomonas aeruginosa*
NCIB 67	-	11	-	-	11	22	*Proteus vulgaris*
NCIB 8222	-	12	10	-	12	20	*Bacillus stereothermophilus*
NCIB 86	-	-	12	-	12	19	*Escherichia coli*
ATCC 43300	-	8	-	-	11	18	*Staphylococ aureus*

Legend: National (NCIB)Ethyl acetate fraction (EtOAc); *n*-Hexane fraction (Hex); Dichloromethane fraction (DCM); Aqueous fraction (Aq.); Streptomycin (Strept.);

CONCLUSION

This study has established that the crude methanol extract and the fractions from *Hilleria latifolia* exhibit varying degrees of antibacterial and antioxidant activities and could be effective in the management of infectious and oxidative stress-related diseases. The results from the antioxidant assays obtained from this study showed that *H. latifolia* possessed antioxidant capacity. However, the ethyl acetate fraction possessed the highest antioxidant potential, which could be due to the polar nature of its constituents. The various compounds identified by the GC-MS in the *n*-hexane and dichloromethane fractions could play a significant role in all the activities observed and could be responsible for its significant activity especially against *K. pneumoniae*, *E. coli*, *Pseudomonas aeruginosa* and *S. aureus*. These observed activities could serve as the basis for the medicinal use of the plant in traditional medicine. Further study should be carried out with a view to isolate and characterize the active compounds responsible for the various activities.

REFERENCES

Abbiw, D. K. (1990). Useful Plants of Ghana West African Uses of Wild and Cultivated Plants. Intermediate Technology Publications Ltd., London, UK, 154-157.

Abotsi, W. K., Ainooson, G. K. and Woode, E. (2012). Anti-inflammatory and antioxidant effects of an ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). *African Journal of Traditional, Complementary and Alternative Medicines*, 9(1): 138-52. doi: 10.4314/ajtcam.v9i1.19

Abulude, F. O. (2007). Phytochemical screening and mineral contents of leaves of some Nigerian woody plants. *Research Journal of Photochemistry*, 1(1): 33-39.

Abulude, F. O., Akajagbor, C., and Dafiewhare, B. H. (2001). Distribution of trace minerals, phosphorus and phytate in some varieties of mushrooms found in Nigeria. *Advances in Food Sciences*, 23(3): 113-116

Adegoke, A. A., Iberi, P. A., Akinpelu, D. A. Aiyegoro, O. A. and Mboto, C. I. (2010). Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*, 3(3): 6-12.

Aeschbach, R., Loliger J., Scott, B. C., Murcia, A., Butler, J., Halliwell, M. (1994). Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chemical Toxicology*, 32: 31–36. doi: 10.1016/0278-6915(84)90033-4

Ajayi, O. S., Balogun, O. S., Olawuni, I. J., October, N., Adigun, R. and Akinlade, I. G. (2021). Alpha amylase inhibition and antioxidant activities of bicyclic diterpenoid lactones from Andrographis paniculata. *Tropical Journal of Natural Product Research*, 5(6): 1110-1117. doi: 10.26538/tjnpr/v5i6.22.

Alinezhad, H., Azimi, R., Zare, M., Ebrahimzadeh, M. A., Eslami, S. and Nabavi, S. F., (2013). Antioxidant and antihemolytic activities of ethanolic extract of flowers, leaves, and stems of *Hyssopus officinalis* L. Var. angustifolius. *International Journal of Food Properties*, 16: 1169–1178. doi: 10.1080/10942912.2011.578319

Al-Rubaye, A. F., Kadhim, M. J. and Hameed, I. H. (2017). Determination of Bioactive Chemical Composition of Methanolic Leaves Extract of *Sinapis arvensis* Using GC-MS Technique. *International Journal of Toxicology and Pharmacological research*, 9(2): 163-178. doi: 10.25258/ijtpr.vi9i02.9055

Anamura, S. I. (1989). Effects of phenolic dental medicaments on arachidonic acid metabolism and their antiinflammatory action. *Hiroshima Daig saku Shigaku Zasshi*, 21: 147–162.

Anna, M., Ramona, B., Erika, C., Ilkay, E., Maria, D., Seyed, F., Morteza, I., Mohammad, A., Seyed M. and Marjan, A. (2017). Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. *Critical review in microbiology*, 43(6): 668-689. doi: 10.1080/1040841X.2017.1295225

Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical Biology and Drug Design*, 80: 434–439. doi: 10.1111/j.1747-0285.2012.01418.x

Araujo, L. X., Novato, T. P., Zeringota, V., Matos, R. S., Senra, T. O. and Maturano, R., (2015). Acaricidal activity of thymol against larvae of *Rhipicephalus microplus* (Acari: Ixodidae) under semi-natural conditions. *Parasitology Research*, 114: 3271–3276. doi: 10.1007/s00436-015-4547-3

Archana, P. R., Nageshwar, R. B. and Satish, B. S. (2011). Modulation of gamma ray-induced genotoxic effect by thymol, a monoterpene phenol derivative of cymene. *Integrated Cancer Therapy*, 10: 374–383. doi: 10.1177/1534735410387421

Benzie, I. F. and Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 299: 15-27. doi: 10.1016/s0076-6879(99)99005-5

Brand-Williams, W., Cuvelier, M. E. and Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1): 25-30. doi: 10.1016/S0023-6438(95)80008-5

Chandrasekaran, M., Senthilkumar, A. and Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from leaves of *Sesuvium portulacastrum*. *European Review for Medical and Pharmcological Science*, 15: 775–780.

Dapaah, S. O., Agyare, C., Boakye, Y. D. and Appiah, T. (2016). Modulatory effects of *Hilleria latifolia* and *Laportea ovalifolia* on activity of selected antibiotics. *Journal of Medicinal Plants Research*. 10(25), 360-366. doi: 10.5897/JMPR2016.6122

Didri, N., Dubreuil, L. and Pinkas, M. (1994). Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharmaceutical Acta Helvetiae*, 69: 25–28. doi: 10.1016/0031-6865(94)90027-2

El Mokni R., Hammami S., Dall'Acqua S, Peron G., Faidi K., Braude J. P., Sebei H and El Aouni M. H. (2016). Chemical Composition, Antioxidant and Cytotoxic Activities of Essential Oil of the Inflorescence of *Anacamptis coriophora* subsp. Fragrans (Orchidaceae) from Tunisia. *Natural Product Communications*, 11(6): 857-860. doi: 10.1177/1934578X1601100640

Ghaneian, M. T., Ehrampoush, M.H., Jebali, A., Hekmatimoghaddam, S., and Mahmoudi, M. (2015). Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. *Environmental Health Engineering and Management Journal*, 2(1): 13–16.

Gulçin, Ý., Sat, I. G., Beydemir, S., and Kufrevioglu, O. I. (2004). Evaluation of the *in vitro* antioxidant properties of *Brassica oleracea* extracts. *Italian Journal of Food Science*, 16(1): 17-30.

Imoh, S. J., Ette O. E., and Jude E. O. (2017). In vivo antiplasmodial activities of ethanolic leaf extract and fractions of *Hilleria latifolia*. *Journal of Medicinal Plants Studies*, 5(4): 118-122.

Iwu, M. M. (1993). *Hilleria latifolia*, Handbook of African medicinal plants. CRC Press Taylor and Francis, 236.

Jegajeevanram, P., Alhaji N. M. and Kumarave, S. (2014). Identification of pesticide compounds of *Cynodon dactylon* by GC-MS analysis. *International Journal of Pharmacy and Biological Sciences*, 5: 604-608.

Kumar, P. P., Kumaravel, S. and Lalitha, C. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African Journal of Biochemical Research*, 4: 191–195.

Lipowska, J., Miks, C. D., Kwon, K., Shuvalova, L., Zheng, H., Lewiñski, K., Cooper, D. R., Shabalin, I. G. and Minor, W. (2019). Pyrimidine biosynthesis in pathogens–structures and analysis of dihydroorotases from Yersinia pestis and Vibrio cholerae. *International Journal of Biological Macromolecules*, 136, 1176–1187. doi: 10.1016/j.ijbiomac.2019.05.149

Marcocci, P. L., Sckaki A. and Albert, G.M., (1994). Antioxidant action of *Ginkgo biloba* extracts EGP761. *Methods Enzymol.* 234: 462–475. doi: 10.1016/0076-6879(94)34117-6

Mihailovi, V., Vukovi, N., Niforovi, N., Soluji, S., Mladenovi, M., Maškovi, P. (2011). Studies on the antimicrobial activity and chemical composition of the essential oils and alcoholic extracts of *Gentiana asclepiadea*. *Journal of Medicinal Plant Research*, 5(7): 1164-1174.

Miliauskas, G., Venskutonis, P. R., and Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, 85(2): 231-237. doi: 10.1016/j.foodchem.2003.05.007

Muthaura, C.N., Rwukunga, G.M., Chabbra, S.C, Omar, S.A., Guantai, A.N. and Gathirwa, W. (2007). Antimalarial activity of some plants traditionally used in Meru district of Kenya. *Phytotherapy Research,* 21: 860-867. doi: 10.1002/ptr.2170

Neepal, I. A., Nidaa, A. A., Khansaa, A. A. S. and Zina, K. A. B. S (2019). Analysis of bioactive phytochemical compound of (Cyperus alternifolius L.) By using gas chromatography – mass spectrometry. Institute of physics Conference Series: *Materials Science and Engineering*, 571: 1-20. doi: 10.1088/1757-899X/571/1/012047

Novakovic, D., Feligioni, M., Scaccianoce, S., Caruso, A., Piccinin, S. and Schepisi, C. (2013). Profile of gantenerumab and its potential in the treatment of Alzheimer's disease. 7:1359-1364. doi: 10.2147/DDDT.S53401

Odugbemi, T. (2008). Outline and pictures of medicinal plants from Nigeria. Vol. 10 University of Lagos Press, Lagos, Nigeria, 95.

Olayiwola, M. O., Ajayi, O. S., Balogun, O. S., Olawuni, I. J., Adeyemo, S. M. & October, N. (2021). Isolation, Identification and Biological Activities of the Constituents of Hibiscus rosa-sinensis Leaf Extract. *Ife Journal of Science and Technology*, 5 (1), 33-48.

Ozen, T., Demirtas, I. and Aksit, H. (2011). Determination of antioxidant activities of various extracts and essential oil compositions of Thymus praecox subsp. skorpilii var. skorpilii. *Food Chemistry*, (124): 58–64. doi: 10.1016/j.foodchem.2010.05.103

Pavela, R. (2009). Larvicidal property of essential oils against Culex. Industrial Crops Products, 30: 311–315. doi: 10.1016/j.indcrop.2009.06.005

Rahuman, A. A., Gopalakrishnan, G., Ghouse, B. S., Arumugam, S. and Himalayan, B. (2000). Effect of Feronia limonia on mosquito larvae. *Fitoterapia*, 71: 553–555. doi: 10.1016/S0367-326X(00)00164-7

Riella, K. R., Marinho, R. R., Santos, J. S., Pereira-Filho, R. N., Cardoso, J. C. and Albuquerque-Junior, R. L. (2012). Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from Lippia gracilis, in rodents. *Journal of Ethnopharmacology*, 143: 656–663. doi: 10.1016/j.jep.2012.07.028

Robledo, S., Osorio, E., Munoz, D., Jaramillo, L. M., Restrepo, A. and Arango, G., (2005). *In vitro* and *in vivo* cytotoxicities and antileishmanial activities of thymol and hemisynthetic derivatives. *Antimicrobial Agents Chemotherapy*, 49: 1652–1655. doi: 10.1128/aac.49.4.1652-1655.2005

Ross, I. A. (2003). Medicinal plants of the world. Vol.1. Chemical constituents, traditional and modern medicinal uses. 2 nd Edition (2003). Humana press Inc., Totowa, 455.

Rossi, G. C., Brown, G. P., Leventhal, L., Yang K., and Pasternal, G. W. (2005). Novel Receptor Mechanisms for Heroin and morphine-6-glucuronide analgesia. *Neuroscience Letters*, 216(1): 1-4. doi: 10.1016/0304-3940(96)12976-1

Sancheti, J., Shaikh M. F., Chaudhari, R., Somani, G., Patil, S. and Jain, P. (2014). Characterization of anticonvulsant and antiepileptogenic potential of thymol in various experimental models. *Naunyn-Schmiedebergs Archive Pharmacology*, 387: 59–66. doi: 10.1007/s00210-013-0917-5

Schmelzer, G. H. and Gurib-Fakim, A. (2007). Resources of Tropical Africa 11(1) – Medicinal plants 1. PROTA Foundation, Wageningen, Netherlands / Backhuys Publishers, Leiden, Netherlands / CTA, Wageningen, Netherlands. 791.

Singh N. and Rajini P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85(4): 611-616. doi: 10.1016/j.foodchem.2003.07.003

Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3): 144-158. doi: 10.5344/ajev.1965.16.3.144

Sivakumar, R., Jebanesan, A., Govindarajan, M. and Rajasekar, P. (2011). Larvicidal and repellent activity of tetradecanoic acid against Aedes aegypti (Linn.) and Culex quinquefasciatus (Say.). *Asian Pacific Journal of Tropical Medicine*, 4: 706–710. doi: 10.1016/S1995-7645(11)60178-8

Soetan, K. O., and Aiyelaagbe, O. O. (2009). The need for bioactivity-safety evaluation and conservation of medicinal plants – A review. *Journal of Medicinal Plants Research* 3(5): 324-328.

Steinman, V. M., Milliken, W. K., and Baracat, A. (2010). Interactive Key and Information Resources for Flowering Plants of the Neotropics. *Neotropikey*, 5: 145-149.

Suresh L., Veerabah, R. M. and Gnanasingh, S. R. (2010). GC-MS analysis of ethanolic extract of Zanthoxylum rhetsa spines. *Journal of Herbal Medical Toxicology*, 4:191-192.

Woode, E. and Abotsi, W. K. (2011). Antinociceptive effect of an ethanolic extract of the aerial parts of Hilleria latifolia (Lam.) H. (Phytolaccaceae). *Journal of Pharmacy and Bioallied Sciences*, 3(3): 384-396. doi: 10.4103/0975-7406.84445

Zih-Rou Huang, Yin-Ku Lin, and Jia-You Fang (2009). Biological and pharmacological activities of squalene and related compounds: Potential uses in cosmetic dermatology. *Molecules*, 14: 540-554. doi: 10.3390/molecules14010540