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Full Length Article

Chemical Composition of *Gossypium herbaceum* Linn and its Antioxidant ,Antibacterial, Cytotoxic and Antimalarial Activities

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ABSTRACT

Background: *Gossypium herbaceum* (*G. herbaceum*), a plant commonly found in the wild in Nigeria, is said to possess some therapeutic activities. However, there is a dearth of information on its chemical constituents. Also, there is a need to investigate its therapeutic activities.

Objective: To investigate the qualitative and quantitative phytochemical components of the leaf extracts of *G. herbaceum*, as well as its antioxidant, antimalarial and cytotoxic activity.

Methods: Gas chromatography-mass spectrometry (GC-MS) analysis was used to determine the components of ethanol and hexane extracts of *G. herbaceum* leaf while 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide assays were used to determine the antioxidant potential. Malaria parasites viability was examined using parasite lactate dehydrogenase (pLDH) technique and HeLa cell was used for the cytotoxicity evaluation.

Results: Bioactive compounds identified in ethanol and hexane extracts of *G. herbaceum* were 37 and 30 kinds, respectively, with major components as linoleic acid (36.10% and 33.82%), vitamin E (7.15% and 5.98%) and caryophyllene (4.21% and 5.08%). It also has tannins, saponins, alkaloids, flavonoids steroids, phenols and terpenoids. In antibacterial test, significant inhibitory potentials against multidrug-resistant bacteria strains were present. *In vitro* antioxidant potentials (IC_{50}) of ethanol and hexane extracts were 3.33 and 4.12 μg/mL for DPPH assays, and 3.87 and 5.00 μg/mL for nitric oxide assays, respectively. Antiplasmodial activities (IC_{50}) were 9.99 and 9.76 μg/mL for ethanol and hexane extracts, respectively.

Conclusion: *G. herbaceum* leaf may provide novel plant-derived therapeutic agents, effective in treating infectious diseases arising from multiple drug-resistant bacteria and a target in the management of oxidative stress.

1. Introduction

The use of medicinal plants as a means of healing may be credited not only to sociocultural factors, but also to the ineffectiveness of some of the existing drugs. Lack of effective therapy in addition to antibiotic resistance has led to a search for novel plant-derived therapeutic agents (Berrino et al., 2009). Literature reveals that plants have antioxidant potentials as a result of the phenolic compounds they contain (Forni et al., 2017). These phenolic phytochemicals play a significant role in human with a broad spectrum of therapeutic activities such as antimicrobial, antimalarial, anti-allergic, antiviral, anti-cancer and cardiovascular potentials (Ksouri et al., 2007). Furthermore, these phenolic components thwart oxidative alteration through free radicals, oxygen scavenging, or disintegrating peroxides via the mechanism of their antioxidant actions (Nijveldt et al., 2001).

Gossypium herbaceum (*G. herbaceum*) is commonly referred to as Levant cotton. The plant is found in the semi-arid regions of sub-Saharan Africa. It grows in the wild in Nigeria, particularly in the southern

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and north central area of the country. It was originally cultivated in Southern Africa and initially domesticated in Arabia from where the plant spread to the western part of Africa and eastern part of India (Al-Snafi, 2018). *G. herbaceum* belongs to the family Malvaceae and is grouped under the division of Magnoliophyta. The species is one of the ancient groups of cultivated plants usually employed in the manufacturing of textile raw material for about 7000 years (Cope, 2018). Therapeutically, diuretic activity, antibacterial, anti-ulcer, antioxidant potencies, wound healing effect, anti-epileptic, anti-diabetic, anti-helminthic, and anti-urolithiatic activities have been ascribed to *G. herbaceum* in the literature (Narasimha et al., 2008; Sumalatha and Sreedevi, 2012; Niharika et al., 2018). However, there is paucity of information regarding the chemical constituents, antimalarial activity, and cytotoxic effect of other polar and non-polar solvent extracts of *G. herbaceum* leaf. Consequently, the present study examines the qualitative and quantitative phytochemical composition of both ethanol and hexane extracts of *G. herbaceum* leaf obtained from North Central Nigeria, as well as its antioxidant, antibacterial, cytotoxic and antimalarial activities.

2. Materials and Methods

2.1. Plant material

Matured leaves of *G. herbaceum* were obtained from their natural habitat in Egume area of Kogi State, Nigeria. Plant materials were identified and authenticated by Mr Ayegba Sule, a taxonomist in the Department of Plant Science and Biotechnology, Kogi State University, Anyigba, Nigeria. Voucher's specimen (Larayetan GH) was deposited at the University herbarium for record purposes.

2.2. Chemicals and reagents

All chemicals and reagents used for this study were purchased from Sigma Aldrich except for diethyl ether purchased from Merck, South Africa and dimethyl sulfoxide (DMSO) from Fluka Chemicals (Buchs, Switzerland).

2.3. Preparation of ethanol extract of G. herbaceum leaf

The powdered *G. herbaceum* dried leaves (200 g) was soaked in 700 mL of ethanol and the resultant mixture agitated on an orbital shaker (Model 420 Series, Thermo Fisher Scientific) at 200 rpm for 48 h. The mixture was filtered with Whatman No. 1 (320 mm, 4 μm) filtered paper, and concentrated at low pressure through a rotary vacuum evaporator (bath at 35°C). The concentrate, about 186.8 mg, was well-kept in a vial, labeled correctly, and stored in the refrigerator at 4°C until needed for analysis.

2.4. Preparation of hexane extract of G. herbaceum leaf

The powdered *G. herbaceum* dried leaves (200 g) was soaked in 700 mL of hexane for 48 h. The same procedure as described in Section 2.2 above was followed to get the hexane extract, 201.3 mg, which was also preserved in a vial and kept in the refrigerator until further analysis.

2.5. Gas chromatography/mass spectrometry analysis

An Agilent gas chromatograph (GC) (6890N) attached with a 5973 mass selective detector (MSD) and HP-5MS column (30 m × 250 μm × 0.25 μm) were employed for compound identifications and separations. The carrier gas used was Helium at 1 mL/min flow rate whereas the average velocity and nominal initial pressure were programed at 26 cm/sec and 13 psi correspondingly. The stationary phase for the HP-5MS column used was (5%-phenyl)-methylpolysiloxane. The ion source and quadruple temperatures were 230°C and 150°C respectively while the acquisition scan mass ranged from 50 to 500 amu. Starting temperature was set at 70°C (2 min hold) and conditioned at 30°C/min to 300°C, thus giving a total runtime of 49.67 min. The ethanol and hexane crude extracts (1 μL) were injected in a splitless mode at 250°C with a 50 mL/min purge flow (Adeyemi et al., 2021).

2.6. Constituents' detection/identification

The crude ethanol and hexane extracts components of *G. herbaceum* leaf were detected from the GC-MS chromatogram. Analysis of the components of the extracts was carried out by comparison of their retention times (RT) with homologous series of n-alkanes in the NIST library 2014. Identity of the mass fragmentation patterns and calculated retention times of each compound was checked and compared with those available in the databases.

2.7. Qualitative screening ethanol and hexane extracts of G. herbeceum leaf

Bioactive tests for screening and detection of secondary metabolites in the ethanol and hexane extracts of *G. herbeceum* leaf were carried out using the technique of Yadav et al. (2014) with slight modification. Qualitative detection of the different secondary metabolite was carried out using Mayer's and Wagner's reagents (alkaloids), foam test (saponins), Salkowski and Liebermann Burchard's tests (steroids and terpenoids), ferric chloride (phenols and tannins) and lead acetate (flavonoids) tests.

2.8. Quantitative analysis of ethanol and hexane extracts of G. herbeceum leaf

2.8.1. Determination of total phenolic content

Total phenolic content was analysed via a spectrophotometric method (Kim et al., 2003). 1 mL of each filtrate was thoroughly mixed with 1 mL of Folin-Ciocalteu reagent followed by the addition of 10 mL of distilled water. The mixture was allowed to stand for 7 min before adding 10 mL of 7% Na_2CO_3 solution. The resulting solution was vortexed for about 30 sec and kept in a dark cupboard for colour improvement. Absorbance was read at 750 nm. The analysis was carried out in triplicate and results expressed in milligram per Gallic acid equivalent (GAE/100 g) using a standard calibration curve with application of a linear equation expressed as $y = 0.009x + 0.012$ ($R^2 = 0.999$), where x is the concentration and y is the gallic acid equivalent.

2.8.2. Determination of overall flavonoid content

The procedure of Ordonez et al. (2006) was used to quantify the overall flavonoid content of both ethanol and hexane extracts of *G. herbaceum* leaf. In each case, for 1 mL of either extract, 2 mL of distilled water was added separately. 0.4 mL of 5% aluminium chloride, 3 mL of sodium hydroxide and 0.5 mL of 10% sodium nitrate were mixed and vortexed thoroughly for about 40 sec, the mixtures were kept in a dark place at room temperature for an incubation period of 50 min. Absorbance was measured separately at 420 nm. The overall flavonoid contents of the two extracts were quantified as mg RE/100 g of rutin using the linear equation given as $y = 0.023x + 0.022$ ($R^2 = 0.982$), where x is defined as concentration dependant and y as the rutin equivalent scale.

2.8.3. Determination of total tannin content

Overall tannin content was evaluated according to the method described by Van Buren and Robinson (1969) with minor modifications using tannic acid as standard. 20 mg of the respective extracts were reconstituted in 30 mL of methanol. An aliquot (1 mL) each of the ensuing methanol extract was mixed with 10 mL of distilled water in separate test-tubes. The test-tube containing the mixtures were further treated with 2.5 mL (10-fold dilution equivalent) of 0.1 M $FeCl_3$ enmeshed in

0.1 M hydrochloric acid solution and potassium ferrocyanide solution (0.008 M). The mixtures above were vortexed for about 1 min and allowed to stay for 5 min before taking the reading of the absorbance at 605 nm alongside the blank solution. An evaluation of this calibration was based on a 7-point standard graduation curve of tannic acid (20, 40, 60, 80, 100, 140, and 200 mg/L) in deionized water. Overall tannin content was expressed as tannic acid equivalents (TAEs).

2.8.4. Determination of saponin content

The method of Obadoni and Ochuko (2002) was employed to determine overall saponin content of the ethanol and hexane extracts of *G. herbaceum*. 0.7 g of each plant extract was mixed with 100 mL of 80% aqueous ethanol. The ensuing mixtures were heated separately with uninterrupted stirring for 5 h. The new solutions obtained above were filtered and the residue were re-extracted with 100 mL of 80% aqueous ethanol. The resulting solutions were reduced using a rotavap (Buchi Model R153 Rotavapor) to a volume of 40 mL. The solutions were afterward defatted with diethyl ether (80 mL) each in a separating funnel thereby giving two distinct layers of aqueous and diethyl ether. Recovery of the aqueous layer from diethyl ether layer was done by gently pouring off the diethyl ether. 48 mL of n-butanol and 8 mL of 5% aqueous NaCl was added for additional purification, followed by evaporation to dryness of the ensuing solutions in a conical flask. The content of the saponins was calculated as a percentage.

2.8.5. Determination of steroid content

The method of Evans (2009) with slight modifications was used to evaluate the steroid content of the extracts in the present study. Stock solution was acquired by dissolving 1.25 g each of the two plant extracts (ethanol and hexane) in 25 mL of distilled water and dimethyl sulfoxide (DMSO) respectively in a 50 mL beaker, and agitated on an orbital shaker for 2 h. An aliquot of 3 mL of the solution obtained was washed with 4 mL of 0.1 M sodium hydroxide solution (pH=9). The solution was afterward mixed with 4 mL of chloroform and 2 mL of cold acetic anhydride, with a careful addition of three drops of concentrated H_2SO_4. The absorbance of the sample as against blank was obtained spectrophotometrically at 420 nm.

2.9. In vitro *antioxidant assay*

2.9.1. DPPH assay

Radical scavenging and antioxidant activity of ethanol and hexane extract of *G. herbaceum* were assessed against free radical DPPH. Six different concentrations (25–800 μg/mL) of both extracts and synthetic antioxidant vitamin C and E were incubated with DMSO solution of DPPH for about 30 min at room temperature in the dark. The solutions were mixed scrupulously with a vortex machine and the absorbance of each sample taken at 517 nm. Ethanol and hexane extract capabilities to scavenge DPPH free radicals in the solution was calculated using the equation below

$$\% \text{ Inhibition} = [(A_{control} - A_{vo}) / A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of DPPH+DMSO; A_{vo} is the absorbance of DPPH + ethanol/hexane extracts or the synthetic antioxidant agent. Dose-response curve was plotted and IC_{50} value of the synthetic antioxidant, ethanol and hexane extract was calculated (Larayetan et al., 2019).

2.9.2. Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activities of ethanol and hexane extracts of *G. herbaceum* was determined based on the method of Rai et al. (2006). A separate mixture of 0.5 mL of various concentrations of *G. herbaceum* extracts and 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline were obtained and incubated in the dark at ambient temperature for 3 h. The reference control (synthetic antioxidant vitamins C and E) was used to determine NO scavenging activities in relation to the scavenging ability of *G. herbaceum* extracts in aqueous solution. At the expiration of the incubation period, a separate mixture of 1 mL of sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) and 0.5 mL of the reaction mixture was obtained. After 5 min of incubation, 1 mL of 0.1% naphthyl ethylene diamine dihydrochloride was added, mixed, and incubated for 30 min at 25°C. A separate absorbance of the chromophore obtained was read at 540 nm. The scavenging activities of the extracts brought about by nitric oxide were also measured using the Trolox standard curve. Results were expressed as mole Trolox equivalent (TE) antioxidant capacity per 100 g sample. In each case, all determinations were performed in triplicates.

2.9.3. Phosphomolybdate assay (total antioxidant capacity)

The overall antioxidant capacity of *G. herbaceum* extracts was analysed by phosphomolybdate method of Umamaheswari and Chatterjee (2008), using ascorbic acid as a reference standard. The procedure includes the addition of 0.1 mL of each sample extract, separately to 1 mL of the solution comprising 28 mM sodium phosphate, 0.6 M tetraoxosulphate (VI) acid, and 4 mM ammonium molybdate. The various tubes enclosing the mixtures were shielded with an aluminium foil and incubated in a water bath at 90°C for 85 min. Afterward, the test tubes were brought out from the water bath at the expiration of the incubation time and allowed to cool to room temperature. Absorbance of the mixture was then taken at 765 nm against the blank, using ascorbic acid as the standard.

2.10. In vitro *assessment of antiplasmodial activity*

Antimalarial potencies of the two extracts of *G. herbaceum* (ethanol and hexane) were examined by evaluating malaria parasite viabilities through parasite lactate dehydrogenase (pLDH) technique reported by Makler et al. (1993). A stock solution of 20 mg/mL of each extract was prepared in DMSO. An aliquot of 50 μg/mL each from the stock solutions previously prepared was added to the parasite cultures in 96-well plates. The resulting mixtures were incubated at 37°C in a CO_2 incubator for two days. After the expiration of the incubation period, 25 μL of each culture was removed from each of the wells and added to about 125 μL of a mixture of nitrotetrazolium blue chloride (NBT)/phenazine ethosulphate (PES) and Malstat solutions in new 96-well plates. Purple colour indicates the presence of pLDH. Absorbance at 620 nm of the mixtures in the new 96-well plates was taken to obtain the quantity of pLDH in each well plate. Dose-response assays were employed to determine IC_{50} of the extract that brought about a reduction in the parasite viabilities to less than 20%. Chloroquine (20 μM) was used as positive control (Rotimi et al., 2019).

A dose-response assay was carried out to determine the IC_{50} concentration of the extracts (concentration of the compound needed to kill 50% of the parasites in a culture). In principle, an extract with an $IC_{50} < 1$ μM can be considered a promising anti-malarial, while an $IC_{50} < 0.1$ μM is considered active. A standard drug like chloroquine or artemisinin with IC_{50} values of approx. 0.02 μM is used for comparison. Biologically active extracts with an IC_{50} values ≤ 20 μg/mL are promising, whereas those with IC_{50} values < 1 μg/mL are considered active. At any rate, percentage viability was required against Log (extract concentration). IC_{50} (50% inhibitory concentration) was determined from the ensuing dose-response curve by non-linear regression using Prism 5 for Windows, version 5.02 (Graph Pad Software, Inc) programme. IC_{50} of values of chloroquine were in the range 0.00001–100 μM. Samples were tested in a concentration range of 250 to 0.11 μg/ mL (3-fold dilutions) for antiplasmodial and from 125 to 0.057156 μg/mL (also in a 3-fold dilution series) for cytotoxic assays (Keusch et al., 1972).

2.11. Cytotoxicity assay with HeLa cell line

Cytotoxicity is mainly regarded as the potential of a compound or extract to bring about cell death. However, a promising antimalarial compound or extract is assumed non-toxic to the host cells. HeLa cell line were used to determine the cytotoxicity of the various extracts using the method of Keusch et al. (1972). Each extracts stock solution (20 mg/mL) was prepared in DMSO and afterward diluted with culture medium prepared from Dulbecco's Modified Eagle's Medium (DMEM) with 5 mM L-glutamine (Lonza). The medium was supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin/fungizone, PSF) to 50 μg/mL. The mixtures were incubated in duplicates along with 1×10^4 HeLa cells per well, dispensed in 96-well plates for 48 h at 37°C in a 5% CO_2 incubator (Fisher Scientific model 116885H). A standard indicator being a toxicology reagent known as resazurin-based (Sigma Aldrich) was applied (20 μL/well) with further incubation for about 3 h. Cells that remained active after subjection to the drug were resolved by mixing the resazurin-based reagent with the mixtures of extracts and HeLa cells. Living cells capable of changing resazurin into resorufin were quantified by a molecular device Spectramax M3 multi-mode microplate plate reader (MS-SC USA). The % cell viability was determined from the resorufin fluorescence in compound treated well, compared to untreated control (Larayetan et al., 2019).

2.12. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of ethanol and hexane extracts of *G. herbaceum* was assessed using micro-dilution technique by Collin et al. (1995). Briefly, 750, 800, 850, 900, and 950 μL of Mueller Hilton Broth (MHB) was dispensed into Eppendorf tubes and the stock solutions of both ethanol and hexane extracts of *G. herbaceum* (20 mg/mL) each were made using DMSO. A small portion of 250, 200, 150, 100, and 50 µL of the two extracts were poured into each Eppendorf tube prepared above to make a final volume to 1000 µL individually. Afterward, 25 μL from the inoculums' suspension of each bacteria strain (0.5 McFarland, ~1×10^8 cfu/mL) was added and vortexed to allow thorough mixing of both the extract and the broth. DMSO and ciprofloxacin were used as negative and positive control, respectively. The solution was incubated at 37°C for 24 h and was later carried out in duplicate. Minimum inhibitory concentration (MIC) of both extracts is described as the least concentration that shows no obvious growth when compared with control containing only MHB, whereas the minimum bactericidal concentration (MBC) was examined through pour plate of the entire tube content having no visible growth in the MIC method above. These were poured into another fresh Mueller-Hinton agar plates and the culture was thereafter incubated for 24 h at 37°C. The lowest concentration of both extracts that did not display any colony growth on the peripheral of the solid medium after an incubation period of 24 h was considered as the MBC.

2.13. Statistical analysis

Data was analyzed as mean ± standard deviation of triplicate determinations using Microsoft Excel. Furthermore, non-linear regression via Prism 5 for Windows, Version 5.02 (Graph Pad Software, Inc) program was employed to determine IC_{50} from the dose-response curve.

3. Results and discussion

3.1. Phytochemical components of G. herbaceum

Qualitative phytochemical test was carried out to ascertain the class of bioactive compounds present in both extracts of *G. herbaceum* by employing the procedure of Yadav et al. (2014). Secondary metabolites usually found in plants were seen in both ethanol and hexane extracts of *G. herbaceum* leaf as presented in Table 1. Tannins, saponins, alkaloids, flavonoids steroids and phenols are phyto-constituents identified in both extracts. Terpenoids, which have been earlier referred to as isoprenoids belonging to a huge and diverse group of naturally occurring bioactive organic chemicals, are also identified in the ethanol and hexane extracts of *G. herbaceum* leaf. Previous work on the phytochemical constituents of *G. herbaceum* revealed the presence of tannins, saponins, alkaloids, flavonoids steroids, phenols, and terpenoids (Shetti, 2010; Khaleequr et al., 2012), which corroborates the present findings. Secondary metabolites deposited in plants have been shown to elicit biological effects related to antimicrobial activities which can be employed to complement the therapeutic management of bacterial infections in humans (Doughari et al., 2009). It has also been shown that these secondary metabolites exhibit resistance to most microbial pathogens and thus may be responsible for the effective antibacterial activities recorded by both extracts of *G. herbaceum* leaf (Anibijuwon and Udeze, 2009). Some of the bioactive compounds detected in the plant extracts, such as alkaloids, have been documented to possess a wide range of pharmaceutical potentials such as antiasthma, antimalarial, and anticancer properties. Terpenoids were also reported for their antiviral, antimalarial, anti-inflammatory and antimicrobial potencies (Mahato and Sen, 1997; Odebiyi and Sofowora, 1978).

Table 1
Qualitative phytochemical analysis of ethanol and hexane extracts of *G. herbaceum* leaf.

Phytochemical components	Ethanol extract	Hexane extract
Tannin (Braymer's Test)	+	+
Saponins (Froth's test)	+	+
Alkaloid (Wagner's Test)	+	+
Flavonoids (Lead acetate Test)	+	+
Steroids (Salkowaski's Test)	+	+
Phenols (Ferric chloride)	+	+
Terpenoids (Salkowaski's test)	+	+

+: present.

The quantitative assessment of the bioactive metabolites in the ethanol and hexane extracts of *G. herbaceum* leaf is presented in Table 2. It is probable that extraction solvents like ethyl acetate, ethanol, methanol, water, and n-hexane have varying affinities for different secondary metabolites (Mahato and Sen, 1997; Odebiyi and Sofowora, 1978). There were high phenolic and flavonoids contents in *G. herbaceum* crude extracts relative to other secondary metabolites identified in the present investigation. Flavonoid content in both ethanol and hexane extracts ranked the second-highest as seen in Table 2 with values of 1013.3 ± 0.28 mg RE/100 g and 700.55 ± 0.77 mg RE/100 g, respectively. Literature has shown that flavonoids are a significant class of bioactive compounds with enormous antioxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic, and hepatoprotective potentials (Kumar and Pandey, 2013; Panche et al., 2016). This plant is remarkably known to be potent in the management of cancer,

Table 2
Quantitative phytochemical composition and antioxidant capacity of ethanol and hexane extracts of *G. herbeceum* leaf.

Extracts	Ethanol Extract	Hexane Extract
Total Phenolic Content (mg GAE/100 g)	3486.71 ± 0.71	1309.2 ± 0.14
Total Flavonoid Content (mg RE/100 g)	1013.3 ± 0.28	700.55 ± 0.77
Total Terpenoids (mg/100g)	502.15 ± 0.02	699.35 ± 0.03
Total Tannin Content (mg TAE/100 g)	498.59 ± 0.59	187.01 ± 0.04
Total Saponins Content (mg/100g)	175.96 ± 0.05	157.8 ± 0.26
Total Steroids Content (mg/100g)	157.50 ± 0.70	112.62 ± 0.07
Total Antioxidant Content (mg AAEQV/100g)	279.82 ± 0.26	163.08 ± 0.01
DPPH IC_{50} (mg/mL)	3.33	4.12
Nitric oxide IC_{50} (mg/mL)	3.87	5.00

DPPH, 2,2-diphenyl-1-picrylhydrazyl. Values are mean ± SD, $n = 3$

epilepsy, depression, radical scavenging, diabetes, bacterial infection, and malaria (Al-Snafi, 2018). It is probable that high phenolic (3486.71 ± 0.71 mg GAE/100 g and 1309.2 ± 0.14 mg GAE/100 g), flavonoid (1013.3 ± 0.28 mg RE/100 g and 700.55 ± 0.77 mg RE/100 g) and terpenoids (502.15 ± 0.02 mg/100g and 699.35 ± 0.03 mg/100g) contents in *G. herbaceum* leaf was responsible for its therapeutic ability. The estimated values of tannin in both ethanol and hexane extracts of the plant under study was 498.59 ± 0.59 mg TAE/100 g and 187.01 ± 0.04 mg TAE/100 g respectively, depicted in Table 2. Tannins are polyphenolic known to exhibit anti-inflammatory properties, antimicrobial and anti-inflammatory potentials (Okwu and Okwu, 2004). Hence, the total antioxidant capacities in both extracts were quantitatively estimated as 279.82 ± 0.26 mg and 163.08 ± 0.01 mg (Table 2).

3.2. Bioactive constituents of extracts

Hydro-distillation analyses carried out on both ethanol and hexane extracts of *G. herbaceum* leaf produced viscous dark brownish and brownish extracts with 37 and 30 bioactive compounds having percentage yields of 9.56% and 5.26% respectively. It is clear from the GC-MS results that the characterized compounds in both extracts are quantitatively different. The identified bioactive compounds, retention times and peak area (%), molecular weight (MW), molecular formula (MF), the nature of the chemical components and the total ion chromatograms of both extracts are given in Table 3 and Fig. 1A and B, respectively.

The GC-MS components of both extracts revealed a multifaceted mixture of compounds that belong to numerous chemical classes like monoterpenes, sesquiterpenes, diterpenes, triterpenoids, fatty acids, steroids (sterols), esters, ethers, etc. The major components found in both the polar and non-polar crude extracts of *G. herbaceum* leaf in this study are linoleic acid (36.10% & 33.82%), palmitic acids (18.16% and 16.63%), vitamin E (7.15% and 5.98%), stearic acid (6.06% and 4.02%) caryophyllene (4.21% and 5.08%). The bioactive components appearing in considerable low quantities are phytol (2.28% and 4.26%), squalene (3.75% and 6.02%), γ-sitosterol (3.98% and 2.04%), and α-amyrin (2.12% and 1.06%). Six bioactive components were observed to have appeared in the ethanol extract of *G. herbaceum* leaf but were not detected in the hexane extract. These compounds are cadina-1(10),4-diene (1.13%), caryophyllene oxide (0.74%), humulene epoxide (0.17%), loliolide (0.17%), 2, 4, 4-trimethyl-2-but-2-enolide (0.27%), nerolidol (0.46%), and glycerol β-palmitate (1.28%).

The pharmacological activities of these bioactive components emanating from cotton species of *G. herbaceum* leaf have been reported with numerous health benefits to humans. Various classes of fatty acids are thought to elicit specific biological effects reported earlier by their epidemiological studies. Palmitic acids (C16:0) are in the group of saturated fatty acids which are partly harmful when consumed owing to their enhancement of low-density lipoprotein (LDL) cholesterol *in vivo*. LDL cholesterol are often specifically referred to as the 'bad' cholesterol due to the evidence of its accumulation in the arterial walls of the blood vessels thus increasing the chances of health-related problem such as heart failure, atherosclerosis or stroke (Okwu and Okwu, 2004). Dietary stearic acids (C18:0) are thought to function in favor of a healthy heart, as they are known to reduce LDL cholesterol moiety *in vivo* and also aid

Table 3
Bioactive profiles of ethanol and hexane extracts of *G. herbaceum* leaf.

Retention time (min)	Bioactive constituents	*G. herbaceum* % Composition			Class of Compounds	
		Ethanol extract	Hexane extract	MF	MW	
13.40	α-Copaene	0.99	1.26	$C_{15}H_{24}$	204	Sesquiterpenes
14.05	Caryophyllene	4.24	5.08	$C_{15}H_{24}$	204	Sesquiterpenes
14.52	Humulene	1.66	1.84	$C_{15}H_{24}$	204	Sesquiterpenes
15.26	Cadina-1(10),4-diene-	1.13	-	$C_{15}H_{24}$	204	Sesquiterpenes
15.69	Lauric acid	0.18	0.92	$C_{12}H_{24}O_2$	200	Fatty acid
16.15	Caryophyllene oxide	0.74	-	$C_{15}H_{24}O$	220	Oxygenated sesquiterpenoid
16.48	Humulene epoxide	0.17	-	$C_{15}H_{24}O$	220	Cyclic ethers
18.01	Myristic acid	0.37	0.22	$C_{14}H_{28}O_2$	228	Fatty acid
18.32	Loliolide	0.17	-	$C_{11}H_{16}O_3$	196	Monoterpenoids lactone
18.37	5-methyltridecane	0.29	0.55	$C_{14}H_{30}$	198	Hydrocarbon
18.69	2, 4, 4-trimethyl-2-but-2-enolide	0.27	-	$C_7H_{10}O_2$	126	Ester
18.82	Neophytadiene	1.10	1.05	$C_{20}H_{38}$	278	Diterpene
18.88	2-phytene, isomer 1	0.29	0.25	$C_{20}H_{38}$	280	Hydrocarbon
19.27	11-Hexadecen-1-ol, acetate, (Z)	0.24	0.15	$C_{18}H_{34}O_2$	282	Ester
19.37	n-Cetyl alcohol	0.23	0.25	$C_{16}H_{34}O$	242	Fatty alcohol
19.94	Palmitoleic acid	0.82	0.84	$C_{16}H_{30}O_2$	254	Fatty acid
20.29	Palmitic acid	18.96	16.33	$C_{16}H_{32}O_2$	256	Fatty acid
20.44	Ethyl palmitate	0.41	0.22	$C_{18}H_{36}O_2$	284	Esters
20.77	Nerolidol	0.46	-	$C_{15}H_{26}O$	222	Oxygenated sesquiterpenes
21.11	Margaric acid	0.30	0.21	$C_{17}H_{34}O_2$	270	Fatty acid
21.48	Methyl elaidate	0.21	0.17	$C_{19}H_{36}O_2$	296	Ester
21.57	Phytol	2.28	4.26	$C_{20}H_{40}$	296	Diterpenoid
22.04	Linoleic acid	36.10	33.82	$C_{18}H_{32}O_2$	280	Fatty acid
22.22	Stearic acid	6.06	4.02	$C_{18}H_{36}O_2$	284	Fatty acid
23.26	Tricosane	0.16	3.37	$C_{23}H_{48}$	324	Hydrocarbon
23.83	Arachidic acid	0.27	0.14	$C_{20}H_{40}O_2$	312	Fatty acid
24.92	Heneicosane	0.27	1.88	$C_{21}H_{44}$	296	Hydrocarbon
25.07	Glycerol β-palmitate	1.28	-	$C_{19}H_{38}O_4$	330	Fatty acid ester
26.45	Heptacosane, 1-chloro	0.68	0.11	$C_{27}H_{55}Cl$	414	Hydrocarbon Chloride
27.51	Squalene	3.75	6.02	$C_{30}H_{50}$	410	Triterpene
27.90	Nonacosane	0.58	2.46	$C_{28}H_{60}$	408	Hydrocarbon
29.22	γ-Tocopherol	0.49	0.12	$C_{28}H_{48}O_2$	416	Phenol lipids
29.67	Nonacosene	0.58	5.01	$C_{29}H_{58}$	406	Hydrocarbon
30.02	Vitamin E	7.15	5.98	$C_{29}H_{50}O_2$	430	Isoprenoid chromanols
31.50	Stigmasterol	0.72	0.22	$C_{29}H_{48}O$	412	Sterols
32.35	γ-Sitosterol	3.98	2.04	$C_{29}H_{50}O$	414	Sterols
33.70	α-Amyrin	2.12	1.06	$C_{30}H_{50}O$	426	Triterpenols
	Total	99.7%	99.85%			

MF, molecular formula; MW, molecular weight.

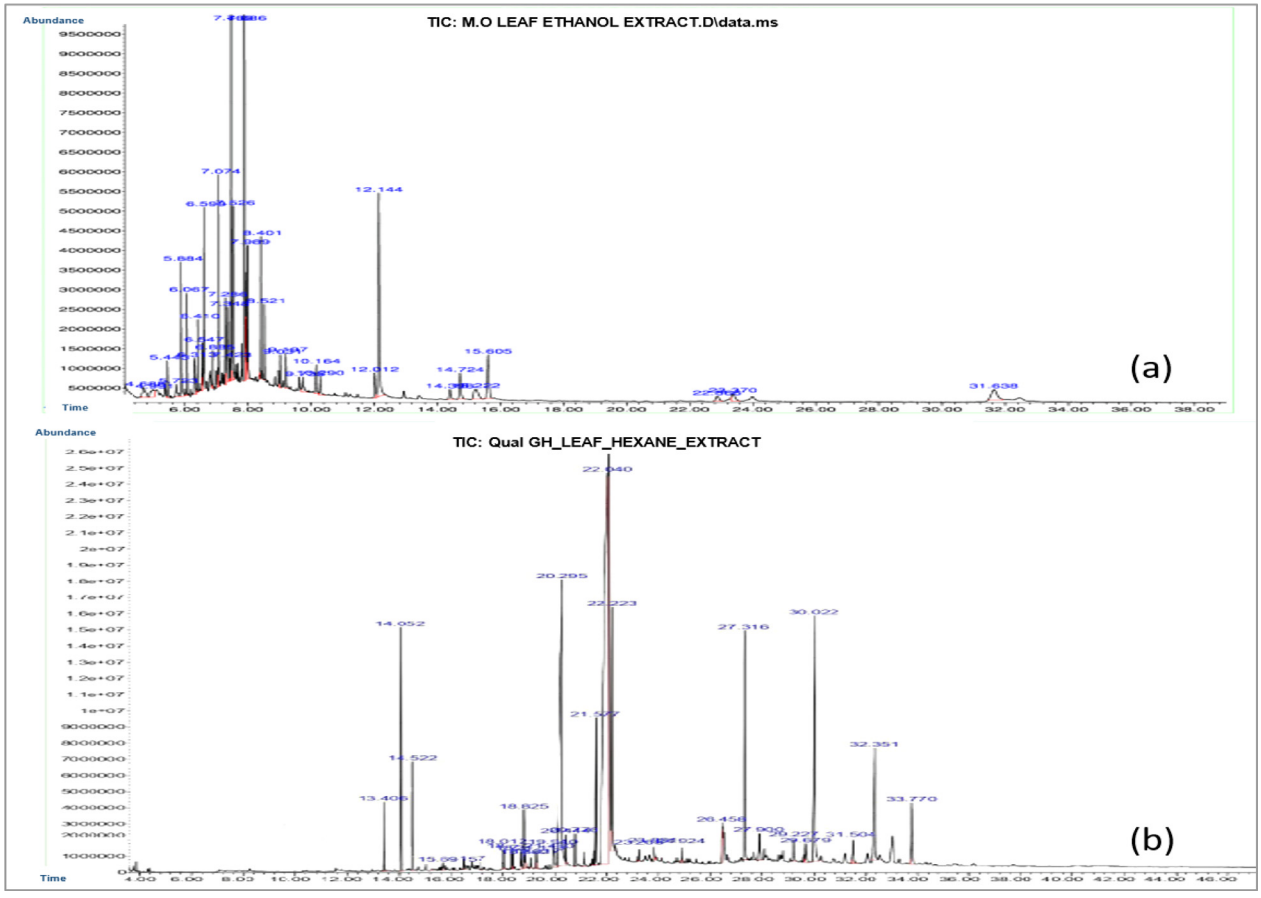

Fig. 1. GC/MS chromatogram of ethanol (a) and hexane (b) extract of *G. herbaceum*.

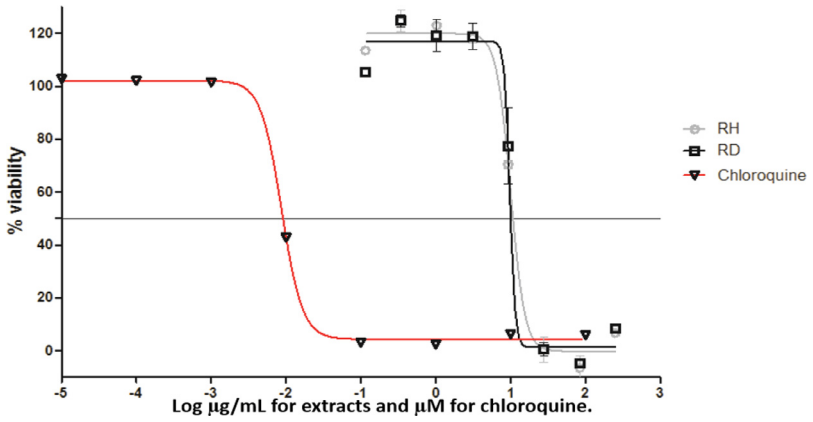

Fig. 2. Dose-response Curve of pLDH of Ethanol (RH) and Hexane extracts (RD). Values are mean ± SD, n =3. Time: 48 h. Concentration: 50 µg/mL for extracts and 20 µM for chloroquine. Method: parasite lactate dehydrogenase (pLDH) technique. Cell Model: HeLa cell line.

in facilitating the buffering ability of the blood. Thus, epidemiological evidence and inductive pharmacological activities *in vivo* are the major variance between (C16:0) and (C18:0) as documented in the literature (Senyilmaz-Tiebe et al., 2018). Caryophyllene on the other hand is a natural bicyclic sesquiterpenes found as one of the major bioactive components in both plant extracts of *G. herbaceum* leaf. Reports on this compound affirmed its anti-inflammatory, antibiotic, anticarcinogenic, antihyperglycemic, and antioxidant properties (Legault and Pichette, 2007). Phytol belongs to the class of bioactive components known as diterpenes, a constituent of chlorophyll, with several pharmacological potentials such as antihyperalgesic, anti-inflammatory, and anti-arthritic effects. Cytotoxic activity against certain cancer cell lines, like MV4-11 and HeLa, breast (MCF-7), prostate (PC-3) and lungs (A-549), have been reported (Pejin et al., 2014; Gliszczynska et al., 2017). Squalene, being a polyunsaturated hydrocarbon have been earlier shown to prevent oxidative damage to the striatum in a mouse experiment and reduce toxicity of 6-hydroxydopamine (Kabuto et al., 2013). Also, sterols, such as gamma sitosterol, compound in GC-MS bioactive profile of *G. herbaceum* leaf, have been shown to initiate de novo cholesterol synthesis in both liver and intestinal cell lines (Ho and Pal, 2005).

3.3. *Antiplasmodial action of* G. herbaceum *leaf*

Ethanol and hexane crude extracts of *G. herbaceum* leaf were evaluated for a dose-response test. The percentage viabilities against varying concentrations of the extracts at 250 to 0.11 µg/mL corresponding to a 3-fold dilutions, and the IC_{50} (50% inhibitory concentration) were obtained from the dose-response curve by non-linear regression in Fig. 2.

Chloroquine, an anti-malarial drug, was used as a comparative reference drug of choice (IC_{50} value of 3.19×10^{-3} µg/mL). The IC_{50} values of ethanol and hexane extracts were 9.99 and 9.76 µg/mL. By convention, antiplasmodial activities of any given crude extracts from plants is expected to exhibit an IC_{50} below 100 μg/mL (Cos et al., 2006). It has also been documented that most promising antimalarial extracts exhibit IC_{50} values under 10 µg/mL (Krettli, 2009). Furthermore, the antiplasmodial activity of *Gossypium arboreum* Linn from literature shows poor activity with an IC_{50} value of 197.9 µg/mL (Ajaiyeoba et al., 2005). In this present study, the two extracts examined exhibited IC_{50} that is less than 10 µg/mL indicating they might be good candidates for antiplasmodial drug.

3.4. Cell viability of HeLa cells

Cell viability, which represents the capability of cell existence, survival and development, was evaluated using Hela cells. The two extracts of *G. herbaceum* leaf were subjected to cytotoxicity assay using HeLa cells at a concentration of 50 µg/mL. The cytotoxic effect at 50 µg/mL shows non reduction in the viability of HeLa cells to below 50% as the percentage cell viability was experimentally greater than 70%.

3.5. Antimicrobial potentials of the extracts

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both extracts from *G. herbaceum* leaf are presented in Table 4. These bioactive compounds could be acting synergistically or singly with each other to exert their activities against the microorganisms employed in the study (Yusuf et al., 2018). It is interesting to note that both extracts (ethanol and the hexane) displayed noteworthy inhibitory potentials against the four multidrug-resistant reference strains, *Vibro alginolyticus* (DSM 2171), *Escherichia coli* (ATCC 35150), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853). The extracts equally demonstrated inhibitory activities against other variants of multidrug-resistant bacteria, which are *Salmonella typhi* (ACC), *Aeromonas hydrophila* (ACC), *Staphylococcal enteritis* (ACC) and *Listeria monocytogenes* (ACC). MIC values of the ethanol extract 9.00 ± 0.01 mg/mL, 9.00 ± 0.02 mg/mL, 4.50 ± 0.01 mg/mL and 4.50 ± 0.00 mg/mL exhibited effective antibacterial activities than the hexane extract comparatively. The MIC values recorded for hexane extract under the condition of our investigation were 18.00 ± 0.01, 36.00 ± 0.03, 36.00 ± 0.02, 9.00 ± 0.03 and 36.00 ± 0.02 (mg/mL) against *Aeromonas hydrophila* (ACC), *Salmonella typhi* (ACC), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and *Listeria monocytogenes* (ACC), respectively. The crude extracts of the plant under study were observed to have the same MIC values, of 9.00 ± 0.02 mg/mL and 18.00 ± 0.00 mg/mL, respectively, for three of the microorganisms employed, *Escherichia coli* (ATCC 35150), *Vibro alginolyticus* (DSM 2171), and *Staphylococcal enteritis* (ACC). Interestingly, the MBC of ethanol and hexane extracts of *G. herbaceum* leaf demonstrated good bactericidal potencies against *Staphylococcal enteritis* in comparison with ciprofloxacin with MBC value of 18.00 ± 0.00 mg/mL, Likewise, the ethanol extract of this plant showed high antibacterial activity against all the Gram negative bacteria used *Aeromonas hydrophila* (ACC), *Escherichia coli* (ATCC 35150), *Vibro alginolyticus* (DSM 2171), *Salmonella typhi* (ACC), *Pseudomonas aeruginosa* (ATCC 27853). The MBC values in their order of appearance were 18.00 ± 0.02, 4.50 ± 0.01, 18.00 ± 0.00, 18.00 ± 0.02 and 18.00 ± 0.02 mg/mL respectively. There was, however, a contrary antibactericidal activity shown by hexane extract with lower MBC values compared to ethanol extract as depicted in Table 3. This observation could be attributed to the hydrophilic lipopolysaccharide component of the external complex membrane of Gram negative bacteria which tends to produce a barricade towards hydrophobic chemicals, making gram-negative bacteria more tolerant toward hydrophobic antibacterial agents (Perussi, 2007; Iravani, 2011; Thatoi and Patra, 2011).

Table 4
Antibacterial activities of both Ethanol and Hexane extracts.

Bacteria Strains	*Gossypium herbaceum* extracts				Positive & Negative controls				DMSO
	Ethanol extract		Hexane extract				Ciprofloxacin		
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	0.4 mL
Aeromonas hydrophila ACC -	9.00 ± 0.01 Bactericidal NVG	18.00 ± 0.02 Bactericidal NVG	18.00 ± 0.01 Bactericidal NVG	9.00 ± 0.01 Bactericidal NVG	9.00 ± 0.01 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	VG
Escherichia coli ATCC 35150 -	9.00 ± 0.03 Bactericidal VG	4.50 ± 0.01 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	36.00 ± 0.01 Bacteriostatic VG	18.00 ± 0.02 Bactericidal NVG	18.00 ± 0.02 Bactericidal NVG	VG
Vibro alginolyticus DSM 2171 -	18.00 ± 0.00 Bactericidal NVG	18.00 ± 0.00 Bactericidal NVG	18.00 ± 0.00 Bactericidal NVG	36.00 ± 0.03 Bacteriostatic VG	4.50 ± 0.01 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	VG
Salmonella typhi ACC -	9.00 ± 0.01 Bactericidal NVG	18.00 ± 0.02 Bactericidal NVG	36.00 ± 0.03 Bacteriostatic VG	18.00 ± 0.02 Bactericidal NVG	18.00 ± 0.00 Bactericidal NVG	18.00 ± 0.02 Bactericidal NVG	VG
Pseudomonas aeruginosa ATCC 27853 -	9.00 ± 0.02 Bactericidal NVG	18.00 ± 0.02 Bactericidal NVG	36.00 ± 0.02 Bacteriostatic VG	36.00 ± 0.03 Bacteriostatic VG	9.00 ± 0.01 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	VG
Staphylococcal enteritis ACC +	18.00 ± 0.02 Bactericidal NVG	36.00 ± 0.01 Bacteriostatic VG	18.00 ± 0.02 Bactericidal NVG	18.00 ± 0.02 Bactericidal NVG	4.50 ± 0.01 Bactericidal NVG	4.50 ± 0.02 Bactericidal NVG	VG
Staphylococcus aureus ATCC 25923 +	4.50 ± 0.01 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	9.00 ± 0.03 Bactericidal NVG	18.00 ± 0.02 Bactericidal NVG	4.50 ± 0.02 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	VG
Listeria monocytogenes ACC +	4.50 ± 0.00 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	36.00 ± 0.02 Bacteriostatic VG	36.00 ± 0.04 Bacteriostatic VG	4.50 ± 0.00 Bactericidal NVG	9.00 ± 0.02 Bactericidal NVG	VG

NVG, no visible growth; VG, visible growth; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; ACC, (AEMREG) culture collection centre; ATCC (American type collection centre); Values are mean ± SD, n = 3.

Extract obtained from ethanol displayed higher antibacterial activities against the microorganisms employed in this study. This may be due to a higher secondary metabolites composition of this extract compared to hexane extract. This observation may be linked to the nature of the secondary metabolites whose potencies may be higher in the presence of more polar solvents since it had been documented that polar solvents have better and higher ability to pull out these secondary metabolites relative to non-polar solvents (Dhawan and Gupta, 2017).

3.6. *Antioxidant potentials of* G. herbaceum *leaf extracts*

Determination of the antioxidant potentials of ethanol and hexane extracts of *G. herbaceum* leaf was carried out *in vitro* via two radical models (DPPH and NO). Remarkable antioxidant capacities were documented for both plant extracts with inhibition display of IC_{50} values of 3.33 and 4.12 μg/mL for DPPH assay, and 3.87 and 5.00 μg/mL for the NO assay. The demonstrated antioxidant potentials might be ascribed to the presence of terpenoids, like caryophyllene, α-copaene, cadina-1(10), 4-diene-, caryophyllene oxide, in the extracts. Terpenoids are widely distributed in plants and have been shown to exert many therapeutic activities as well as free radical scavenging abilities, anti-inflammatory and anti-cancer activities (Han and Bakovic, 2015). DPPH solution is an established radical solution, known to interact with any substance that is capable of giving hydrogen atom or with another radical present in a radical solvent system. NO, on the other hand, is produced in biological tissues by nitric oxide synthase (NOS), in ideal physiological status in an organism in the process of metabolizing arginine to citrulline through a five-electron oxidative reaction sequence (Knowles et al., 1989). Overproduction of NO accounts for a compromised structural and functional behavior of several cellular components. Phytochemicals like terpenoids, phenols and flavonoids have been documented to be responsible for antioxidative activities in biological systems, scavenging singlet oxygen and free radicals in the system (Iqbal et al., 2015; Ajayi et al., 2017a, 2017b). Vitamin E is a fat-soluble compound with unique antioxidant activities. In addition to its antioxidant capacity, it acts in immune function and in the regulation of gene expression and other metabolic processes (Traber and Atkinson, 2007). It is found in various foods, nuts, seeds, and vegetable oils. Natural vitamin E occurs in eight chemical groups namely; alpha-, beta-, gamma-, and delta-tocotrienol and alpha-, beta-, gamma-, and delta-tocopherol, with different degrees of therapeutic actions (Traber and Atkinson, 2007). It is, however, worthwhile to note that out of these eight classes of vitamin E, only α-tocopherol is known to meet the requirement of human need. Vitamin E as an antioxidant compound is capable of inhibiting reactive oxygen species (ROS). It also shields the biological membrane moiety of the cells from oxidative destruction of reactive free radical molecules, having an unshared electron (Verhagen et al., 2006).

4. Conclusion

This study investigated the chemical constituents, antiplasmodial actions, antioxidant potencies and cytotoxicity effects of polar and non-polar solvents of the crude extract of *G. herbaceum* leaf. The result of GC-MS revealed 37 and 30 phytochemical compounds in ethanol and hexane extracts respectively with major component being linoleic acid. Results also revealed significant inhibitory potential against plasmodial and multidrug-resistant reference strains with no sign of cytotoxicity against HeLa cells. Notable antioxidant capacity was documented for both plant extracts. The GC-MS profiles and secondary metabolites screening for both extracts of this plant revealed significant presence of phytochemicals linked to its various medicinal properties judging from the pharmacological activities exhibited on different assay models. Hence, structural elucidation of the lead compound(s) responsible for the bioactivity of *G. herbaceum* and their toxicity profile is recommended for further studies.

Ethical approval

Not applicable.

Data availability

Nil.

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Declaration of Competing Interest

No conflicts of interest by any of the author as regard this research work.

CRediT authorship contribution statement

Rotimi Abisoye Larayetan: Conceptualization, Methodology, Data curation, Investigation, Writing – original draft. **Gideon Ayeni:** Investigation. **Abdulrazaq Yahaya:** Investigation, Methodology, Writing – original draft. **Abayomi Ajayi:** Investigation, Methodology, Writing – original draft. **Sunday Omale:** Investigation. **Umar Ishaq:** Investigation. **Dauda Joseph Abiodun:** Investigation, Formal analysis. **Chijioke Olisah:** Investigation, Writing – original draft. **Julius Aigbogun:** Formal analysis. **Swesme Enyioma-Alozie:** Investigation, Methodology, Writing – original draft.

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Supplementary Materials

Nil.

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