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ARTICLE

Antitubercular activities, antioxidant properties and GCMS fingerprinting of *Acacia hebecladoides*, *Acacia albida* and *Gmelina arborea*.

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Abstract

Background: It is a practice to use traditional medicine for curing various illnesses in West Africa. *Acacia hebecladoides*, *Acacia albida* and *Gmelina arborea* were selected based on their traditional belief for treating various ailments such as: hallucinations, inflammation and tuberculosis. The study sought to validate the antitubercular activity, antioxidant properties and phytochemical components of extracts and fractions of the leaves of selected plants.
Method: The antitubercular activities of the plants were evaluated against Mycobacterium tuberculosis (MTB), multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) and non-mycobacterium tuberculosis (NTM) using the Lowenstein Jensen (LJ) proportion method. Gas chromatography mass spectrometer (GCMS) assay and the antioxidant assays (1,1- diphenyl -2-picryhydrazyl (DPPH) radical assay, total phenolic content (TPC), β-carotene bleaching activity and nitric oxide scavenging activity) were all carried out.
Results: All three plant extracts used in the study inhibited *Mycobacterium tuberculosis* with *G. arborea* showing the lowest MIC value of 31.25μg/ml. Also, *G. arborea* inhibited MDR-TB with an MIC value of 125μg/ml. The most active plant, *G. arborea* was fractionated into four partitions, three (aqueous, n-hexane and ethyl acetate) fractions showed inhibitory activity against MTB with an MIC value of 62.5μg/ml. Also n-hexane and chloroform fractions of *G. arborea* showed inhibitory activities against MDR-TB with the lowest MIC value of 31.25μg/ml. The nitric oxide activity of the plant fractions showed that ethyl acetate fraction of *G. arborea* had high nitric oxide antioxidant activity. The ethanolic extract *A. hebecladoides* and *G. arborea*, while fractions of *G. arborea* (aqueous, ethyl acetate and chloroform) showed DPPH scavenging activity. The ethanolic extract *A. hebecladoides* and *A. albida*, while aqueous fraction of *G. arborea* had high total phenolic content. The ethanolic extract *G. arborea* and its fractions (aqueous and n hexane) showed β-carotene bleaching inhibition. Twelve compounds were found in the n-hexane fraction of *G. arborea* as 2,3-dihydro-3,5,-6-methy 4H-Pyran-4-one and 5-hydroxymethylfurfural were abundant with 35.81% and 19.03% respectively, followed by 3,5-dihydroxy-2-methy-4H-Pyran-4-one (9.82%) and n-Hexadecanoic acid (8.45%).
Conclusions: The study carried out showed that ethanolic extract and fractions of *G. arborea* possess inhibitory activities against MTB and MDR-TB. Also ethyl acetate and aqueous fractions of *G. arborea* showed high antioxidant activities.

Keywords: Tuberculosis, Lowenstein Jensen, Multidrug resistant, *Acacia hebecladoides*, *Acacia albida* , *Gmelina arborea*.

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1. Introduction

The bacterium *Mycobacterium tuberculosis* (MTB) is the cause of tuberculosis (TB) and has been a threat to public health for a number of years (Riccardo et al., 2020). In accordance with recent Global Tuberculosis Report (2020), about 10 million people developed TB and 1.4 million died in 2019, with the peak rates of death and infections mainly in low income and under developed countries (WHO, 2020). TB is a disease of poverty, vulnerability, marginalization, stigma and also classified as an hindrance for the stability of the general public health and economy of these countries, as it depletes financial resources that should've been invested in the economy (Reid et al., 2019).

Tuberculosis rates has been on the increase with a large gap of about 2.9 million people that were diagnosed newly with the 10 million people reported to have developed TB in 2019. This gap cut across underreporting of diagnosed TB patients and under diagnosis. Above half of this global gap was accounted for amongst the five countries: Philippines (7%), Pakistan (8%), Indonesia (10%), Nigeria (11%) and India (17%). (WHO, 2020).

Tuberculosis is a disease that can be prevented and cured when all measures are put in place. Due to the rise in resistance to the anti-TB drugs, there is an urgent need for the formulation of new TB drugs that entails shorter duration with little or no toxicity (Oladosu et al., 2013). The current front line TB therapy were developed over 50 years ago, and this has driven pharmaceutical companies into researching for new therapies and methods for antitubercular diagnostics and treatments. Commercial viability can be considered to aid accessibility and inclusion of the antitubercular treatment (Riccardo et al., 2020).

There is a surging threat to public health by drug-resistant TB. In 2019, globally, a record of 61% of patients with TB were rifampicin resistant, apart from the records of 2017 and 2012 (51% and 7% respectively). About 59% and 81% were tested for new and previously tested TB patients respectively. Multidrug-resistance TB or rifampicin resistance were detected and notified in 206,030 patients in 2019, which indicated a 10% increase from 186,883 that was notified in 2018 and 156,205 in 2018 (WHO, 2020).

Traditionally used plants as well as medicinal plants which contain biologically active agents are relevant and can have the potential to provide pharmacological activities. New mechanism of action of the chemical compounds present in these plants may possess inhibitory potential against the multi-drug resistant organisms (Pereira et al., 2005).

Acacia albida most times called *Faidherbia albida* belong to the family; Mimosaceae and is widely used in Africa. The plant is widely distributed from sub-Saharan Africa to Egypt, across northern Nigeria to Senegal and in East Africa to the Tranvaal (Eggeling et al., 1952). Commonly known as winter thorn or apple-ring acacia and called by the northern Nigerian (Hausa) as "Gawol". An infusion of the plant bark is used to treat fever, cough and in child birth (Dalziel, 1973). Also, a segment of the northern Nigeria (Fulani) uses a portion of the plant for the treatment of chest pains (Jackson, 1973). The presence of monosaccharides, cardiac glycosides and terpenes were attributed to the medicinal properties of the methanolic and aqueous extracts of *Faidherbia albida* (Abeer et al., 2016).

Gmelina arborea, a fast growing timber-yielding tree belonging to the family *Lamiaceae*, is one of the plants mentioned in ancient scriptures of Ayurveda (ancient Hindu system of healing) being used as folk remedies for jaundice, headache, hallucinations, fever and regulating physiologic functions (Chothani et al., 2014). The root, leaf, wood, fruits and tree bark are used in treatment of various ailments in folk medicine (Kulkarni et al., 2013).

The leaf and stem bark extracts has been reported to possess bioactive compounds (alkaloids, saponins, phenolics and tannins) which has showed antimicrobial activities against gram negative and gram positive microorganisms, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella dysenteriae*, *Salmonella typhia* and *Proteus mirabilis* (Idu et al., 2015).

Acacia hebeciadoides was implicated during a random respondent survey carried out in Kaduna South Local Government Area on locally used herbs in the treatment of tuberculosis (Faleyimu et al., 2009). The current study seeks to explore the efficacy of selected medicinal plants, in relation to their antioxidant and antitubercular potentials. Furthermore, the chemical analysis was substantiated by GCMS analysis.

2. Material and Methods

2.1 Plant material

Acacia hebecladoides, *Acacia albida* and *Gmelina arborea* were collected and authenticated at the Department of Botany, Ahmadu Bello University (ABU) Zaria, Nigeria with voucher numbers 02712, 02612 and 02512 and specimens were deposited in the Herbarium section of the Department of Botany, Ahmadu Bello University, Nigeria.

2.2. Crude extract preparation

The selected plants (leaves) were dried at room temperature and pulverized to powder. The grinded plants were soaked in ethanol with interval shaking for 48 hours at room temperature. Then the mixture was filtered to get the filtrate using a muslin cloth and a Whatman No.1 filter paper. A water bath set at 75° C was used to concentrate the filtrate to slurry.

2.3. Plant fraction preparation

Dried plant leaves material was sequentially extracted (Hostettmann, 1991) with n-hexane, n-butanol, chloroform, ethyl acetate and distilled water using the Soxhlet apparatus. Respective solvent (250 mL) was continuously refluxed with 50g of plant material for a period of 24-48 hours for proper extraction of the phytoconstituents. The fractions were then concentrated using a water bath at 70°C (for n-hexane, chloroform and ethyl acetate) while n-butanol and aqueous fractions were concentrated at 100°C and stored at room temperature.

2.4. Antioxidant assays

The antioxidant assays carried out on the plant fractions include: 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, nitric oxide scavenging activity, β-carotene bleaching assay and total phenolic content.

2.4.1. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH scavenging activity of the extracts was analyzed using the method of Xu and Chang (2007) with slight modifications. Briefly, a 50μl ethanolic solution of plant extracts and fractions (5mg/ml) was added to 180μl of 0.2mM DPPH solution (7.8mg, in 100ml 95% ethanol). 5mg/ml of ascorbic acid was prepared for use as the positive control while 50μl of ethanol was used as the negative control. The solution was mixed vigorously for 1 min and incubated at room temperature in the dark for 60 min.; Absorbance was taken at 492 nm. A 96 well plate was used to carry out the reaction, while the absorbance was read by a microplate reader. The assay was done in triplicate, while the absorbance was calculated as the change in absorbance and the scavenging activity percentage was calculated as follows: Scavenging activity (%) = [1- (Abs sample / Abs control)] × 100.

2.4.2. Nitric oxide (NO) scavenging activity

Nitric oxide activity was evaluated by Griess IlIosvoy reaction using the method of Garrat (1964) with modifications. For the experiment, 160μl of sodium nitroprusside (10mM), phosphate buffer saline (40μl) and 40μl of plant extract/standard were mixed in 96 micro titer well plates and incubated at room temperature for 120 min. Plant extract and ascorbic acid were dissolved in 95% ethanol at 5mg/ml concentration. The control was prepared using the same reaction mixture and equivalent amount of ethanol but without the extract. After incubating the mixture, 40μl of the reaction mixture was mixed with 160μl of Griess reagent [1% sulfanilamide, 2% phosphoric acid (H3PO4) and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride] and absorbance was read immediately at 492nm and change in absorbance was used. There is a formation of chromophore during diazotization of the nitrite with sulfanilamide, followed by coupling with Naphthylethylenediamine dihydrochloride. The standard antioxidant (ascorbic acid) and plants extracts/fractions inhibition of nitrite formation were calculated in relation to the control. Scavenging effect (%) = [(Abs control - Abs sample)/ Abs control] × 100.

2.4.3. β-carotene bleaching assay

The bleaching capacity of β-carotene was evaluated with the method of Nsimba et al. (2008) with modifications. To 1ml of chloroform, 2mg of b-carotene was added in a tube containing 0.2 ml of tween 40 and 0.02 ml of linoleic acid. Using a vacuum at 70°C for 20 min on a water bath and mixed with distilled water (50 ml), the chloroform was removed. 40 μl of standard and sample extracts were added to a 96 well plate. 200 μl of β-carotene emulsion was added to each of the samples, standard and control wells and was measured immediately at 450 nm. The plate was incubated in the dark for 60 min at 45°C and read at 450 nm and change in absorbance was used. Results were indicated as percentage of antioxidant activity measured on the basis of bleaching inhibition of β-carotene and calculated with the formula where AS is the absorbance of the sample and AC is the absorbance of the control: % AS = [1 - (AS (0) – AS (60)) / (AC (0) – AC (60))] x 100.

2.4.4. Total phenolic content

The Folin Ciocalteu's reagent (Ferreira et al. (2007) was used to evaluate the total amount of phenols in the plant extract/ fractions. The crude extract and fractions were prepared by dissolving 5 mg of the extracts in 1 ml of 95% ethanol to yield a concentration of 5mg/ml. Exactly 25μl of the extracts (5mg/ml) was combined with 100 μl of the Folin Ciocalteu reagent (serially diluted x10 in distilled water) in a 96-well microplate. The mixture was vigorously mixed and incubated at room temperature in the dark for 3 min. Then 100μl of sodium carbonate (20% w/v) was added, and gently mixed. After 60 min, a microplate reader was used to check the absorbance of the solution at 630nm. Changes in absorbance values were used.

2.5 Bacterial culture

The *Mycobacterium tuberculosis* (MTB) strains used were harvested from Zankli TB reference laboratory, Bingham University, Nasarawa state. The strains were from cultured active TB patient's samples who were drug sensitive and resistant to the first line anti TB drugs (Rifampicin, Ethambutol, Isoniazid and Pyrazinamide). These include: MTB sensitive strain (OSR 15), MTB resistant strain (745 & 141) and non- MTB strain (167).

2.5.1. Preparation of Lowenstein Jensen (LJ) media

Glass wares were autoclaved, raw eggs were soaked in mild detergent for 15 min and washed gently and further soaked in methylated spirit for 15 min. Eggs were blended and 18.6g of LJ powder was weighed in a conical flask, 8ml of glycerol was added and dimethyl sulfoxide (DMSO) was used to make up to 300ml. Exactly 500ml of the blended egg was added and mixed thoroughly. 5ml of prepared media was dispensed in a well labeled sample bottles and insipated at 85°C for 45min with loose cover. Allow cooling and proceed to quality checks of the media. Media prepared were preserved in the incubator till when they were used for inoculation as described by Canetti et al. (1969).

2.5.2. Preparation of drug containing LJ media

The control drugs used for this study were all first line and included isoniazid (INH), Rifampicin (RIF) and Ethambutol (EMB). The extracts and first line drugs were inserted separately into the LJ media and insipated. The insertion of the extracts, control drugs into the LJ media and inoculation of M. tuberculosis (reference strains) as well as the determination of the antitubercular activities were performed replicating standard protocols (Hans et al., 1998).

To prepare drug working concentrations for the first line control drugs of (INH, RIF and EMB), 0.1ml, 10ml and 1ml from stock solutions respectively were dissolved in 10ml of sterile distilled water (SDW) except for RIF which was dissolved in DMSO. Then 0.1ml, 0.2ml and 0.1ml using sterile tips were dispensed into a 50ml prepared plain LJ media to give a working concentration of 0.2μg/ml, 40μg/ml and 2.0μg/ml respectively for INH, RIF and EMB.

The plant extracts and fractions were prepared in varying concentrations of 31.25, 62.5, 125, 250, 500, 1000 and 5000μg/ml. Exactly 0.2ml of each prepared concentration was added aseptically to 50ml each of sterile LJ medium and shaken gently to mix thoroughly. Then 7ml of the medium was dispensed into 170mm sterile universal containers and then insipated to coagulate at 85oC for 45mins.

2.5.3. Inoculation

To the prepared LJ media, 0.1ml of bacterial suspension were used to inoculate. For growth controls labeled C1, C2 and C3 were inoculated with dilution (10-2, 10-4 and 10-6) respectively. Drugs containing media and plant extracts and fractions were inoculated bacterial dilution of 10-2, just as growth control C1. The media slopes were aligned in a slant angle from horizontal and placed in an incubator at 370C to aid inoculum cover the surface of the media. The slopes were in the incubator with loosely closed cap for 24hrs to evaporate the inoculums and then the caps were firmly closed and kept in the incubator at 370C for 6 weeks and the bacteria colony forming unit (cfu),% inhibition and MIC (Minimum Inhibitory Concentration) was enumerated.

2.5.4. Antitubercular susceptibility testing using LJ medium (Proportion method)

Normally mycobacteria grow slowly on LJ media. In this method of susceptibility testing, we monitor growth of Mycobacteria strains on drug-containing LJ slants and compare it with growth on drug free slants. The Critical Proportion for Resistance is 1%. The growth on the 10-4 control slant is 1% of the growth on the 10-2 control slant. The resistant strains were observed when the growth of a certain proportion of the inoculums (critical proportion) occurs on the culture media with a defined concentration as described by Canetti et al. (1969).

2.6. Gas chromatography mass spectrometry (GCMS) analysis of n-hexane fraction of A. digitata

The n-hexane fraction of *A. digitata* was selected for GCMS analysis as it showed the lowest MIC value. An Agilent USA hypherated to a mass spectrophotometer (5975C) with triple axis detector equipped with an auto injector (10μl syringe) was used. The carrier gas used was helium gas. The chromatographic partitioning was carried out on a capillary column with the specification: Thickness; 250μm, internal diameter; 0.2μm, Length; 30m, with phenyl methyl silox treatment. Other conditions include: Pressure; 16.2 psia, out time, 1.8mm, interface temperature; 300oC, ion source temperature, 250oC, 1 μl injector in split mode with split ratio 1:50 with injection temperature

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at 300°C the column temperature commenced at 35oC for 5mins and was raised to 150oC at the rate of 4oC/min, the temperature was further raised at the rate of 20oC/min and held for 5mins. At the end, the total time for elusion was 47.5 minutes. The mass spectra obtained were controlled by the software Ms Solution with the standard mass spectra from NIST library (NIST11).

2.7. Statistical analysis:
The One-way analysis of variance (ANOVA) was used in analyzing the data generated,
Also the significance of difference (SD) between the means were further analyzed by Dunnett's multiple-range test ($P < 0.05$) using Graph-pad prism 8.0 software. The values were expressed as means of triplicate determinations ± standard deviation.

3. Results

3.1 Anti-tubercular activity
3.1.1. Plant extracts anti-tubercular activity: The antitubercular activities of the selected plant extracts and the reference drugs presented as MIC are shown on Table 1.1. Plant ethanol extracts inhibited MTB growth, but none of the plant's extracts inhibited the NTM (M. fortuitum) growth. The ethanolic extracts of *G. arborea* showed activity against M. tuberculosis with a lower MIC value of 31.25μg/ml compared to A. albida (62.5 μg/ml) and *A. hebecladodies* that showed no activity. However, the MIC values of all the plant extracts against *M. tuberculosis* were lower than that of the reference MTB drug, Rifampicin (40 μg/ml).

Gmelina arborea showed good antitubercular activity against multi-drug resistant isolates (MDR-TB) with an MIC value of 125μg/ml, while *A. albida* showed activity with an MIC value of 250 μg/ml and A. hebecladodies had an MIC value of 1000μg/ml. In comparing extracts tested against MDR-TB with the reference to first line drugs (INH and RIF) the extracts showed very good activity as the reference drugs were unable to resist the bacterial growth (Table 1.1).

3.1.2. Plant fractions anti-tubercular activity: The antitubercular activities of the selected plant fractions and the reference drugs presented as MIC are shown on Table 1.2. Aqueous, n-hexane and ethyl acetate fractions of *G. arborea* inhibited growth of MTB with an MIC value of 62.5μg/ml while chloroform fraction inhibited MTB growth with MIC value of 250μg/ml. The n-hexane and chloroform fractions of *G. arborea* showed activity with an MIC of 31.25μg/ml against MDR-TB while ethyl acetate and aqueous fractions had MIC values of 62.5μg/ml and 125μg/ml respectively.

3.2 Antioxidant activity

3.2.1. Nitric oxide scavenging activity (%) of plant extracts and fractions
The result for nitric oxide scavenging activity (%) of the ethanolic extracts was shown in Fig 1.1. The positive (standard) control used was ascorbic acid which showed antioxidant activity of 73%, while the extracts showed activity at A. albida (38.8%), A. hebeciadoides (21.9%) and *G. arborea* (5.0%). The nitric oxide scavenging activity in the *G. arborea* fractions produced better result than the extracts as shown in Fig 1.1. Ethyl acetate fraction of *G. arborea* was the highest with 80.9%, followed by aqueous fraction (79.6%), while n-Hexane and chloroform did not show nitric oxide scavenging activity.

3.2.2. 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant capacity of extracts and G. arborea fractions
The DPPH antioxidant capacity expressed in percentage of the plant (ethanolic) extracts was shown in Fig 1.2 . The value of the standard control (ascorbic acid) used for the assay was 93.3%, *A. hebecladodies* and *G. arborea* extracts had the highest value of 69.1 and 64.2% respectively, followed by A. albida (25.8%). Among the four fractions of *G. arborea* tested, the aqueous fraction showed the highest value of 95.8% for DPPH antioxidant activity.

3.2.3. Total phenolic content (TPC) of ethanol extracts and G. arborea fractions
The values of TPC were defined as mg of gallic acid per gram of dry weight (mg GAE/g) and shown in Fig 1.3. Standard control used for the assay is gallic acid and the value obtained was 62.5 mg GAE/g. Thus the values obtained for the extracts were as follows; *A. albida* (86.1 mg GAE/g), *A. hebecladodies* (66 mg GAE/g) and *G. arborea* (6 mg GAE/g). The TPC values for the fractions of *G. arborea* revealed that aqueous fraction had the highest value of 46 mg GAE/g followed by ethyl acetate fraction with 28.5 mg GAE/g.

3.2.4. β-carotene bleaching inhibition of ethanol extracts and G. arborea fractions
The β-carotene bleaching inhibition of plant extracts expressed as percent inhibition is shown in Fig 1.4. Ascorbic acid was the standard control and had 65.43% inhibition. *G. arborea* had the highest value of 95% and the only plant that showed activity above 50%. The result for the β-carotene bleaching inhibition of plant fractions are shown in Fig 1.4. The n-hexane and aqueous fractions of *G. arborea* (77.3% and 55.5% respectively) had better result as both showed inhibition above 50%.

3.3. GCMS analysis of n-hexane fraction of G. arborea
The n-hexane fraction of *G. arborea* showed that twelve (12) volatile compounds (Table 2.1) were present in the plant. The compounds in abundance include: 2,3-dihydro-3,5,-6-methy 4H-pyran-4-one (35.81%), 5-hydroxymethylfurfural (19.03%), 3,5-dihyroxy-2-methy-4H-pyran-4-one (9.82%) and n-hexadecanoic acid (8.45%). Although seven (7) minor compounds were present, hexadecenoic acid (1.75%) is the least amongst the others in the n-hexane fraction of *G. arborea.*

4. Discussion

The ethanol extract of the 3 plants shows antitubercular activity in various degrees when compared to the standard drug used (Rifampicin). Based on their MIC values the most active ethanol extract against the multi-drug resistance TB was *G. arborea* (125μg/ml) followed by A. albida (250 μg/ml) and then *A. hebecladodes* (1000μg/ml) as shown in Table 1.1. Gmelina arborea was further separated into fractions based on scarce reports on its anti-tubercular activities. Also, the plant fractions showed activity with the n-hexane and chloroform fractions of *G. arborea* possessing the lowest MIC values of 31.25μg/ml, followed by ethyl acetate (62.5μg/ml) and aqueous fractions of *G. arborea* with an MIC value of 125μg/ml (Table 1.2).

Tuberculosis is involved in inflammatory processes in which nitric oxide is one of the free radicals produced. In relating with reactive oxygen species (ROS), nitric oxide (NO) is known as a contributor to anti-TB properties. NO synthase in macrophages, synthesizes NO and NO synthase deficient mice have been shown to exhibit susceptibility to the bacterium M. tuberculosis (Idh et al., 2017). As stated by Idh (2017) active pulmonary TB patients have been found to have high levels of NO in their lungs. The compound sodium nitroprusside will react with oxygen to produce nitrite (NO) at physiological pH in an aqueous solution. Ethyl acetate and aqueous fractions of *G. arborea* displayed NO scavenging activities by inhibiting the nitrite formation.

DPPH free radical scavenging capacities of the plant extracts and fractions are presented in Figure 1.2. DPPH is known to be a stable free radical. On binding with DPPH, antioxidants either transfer hydrogen atoms or electrons, thereby neutralizing the free radical present (Naik et al., 2003). Although, *G. arborea* and *A. hebecladodies* extracts displayed DPPH scavenging activity, only aqueous fraction of *G. arborea* had higher antioxidant activity as compared to ascorbic acid, and this could mean that the anti-tubercular activity of *G. arborea* is linked to its antioxidant properties.

Table 1.1: Percent inhibition and MIC determination of plant extracts against *M. tuberculosis* isolates

Extract	**Isolates**	**Mean Colony forming units (Cfu) on media**							**Percentage inhibition %**						**MIC**
Treatment Conc. (µg/ml)		**Control**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**	
A. hebecladoides	OSR-15	190	180	173	165	157	150	143	5	9	13	17	21	30	-
	461	180	175	166	161	120	95	87	3	8	11	33	47	52	1000
A. albida	OSR-15	195	110	67	44	39	26	22	44	66	77	80	87	89	62.5
	461	190	122	115	111	95	91	84	36	39	42	50	52	56	250
G. arborea	OSR-15	195	95	40	31	20	15	10	51	79	84	90	92	95	31.25
	461	185	120	125	85	35	25	20	35	32	54	81	86	89	125
Rifampicin	OSR-15	180	33	28	24	20	17	13	82	84	87	89	91	93	31.25
	461	180	120	117	113	110	108	100	33	35	37	39	40	44	-
Isonazid	OSR-15	185	27	24	20	16	12	10	85	87	89	91	94	95	31.25
	461	190	130	123	119	111	107	101	32	35	37	42	44	47	-

Note: (-) means % inhibition below 50, OSR-15 – *Mycobacteria tuberculosis*, 461 – Multi-drug resistant *mycobacteria tuberculosis*,

MTB =Mycobacterium tuberculosis, cfu = colony forming units,

% inhibition= $\frac{Cc - Ct}{Cc}$ X100, Cc = No of colony in the control medium, Ct = No of colony in test media

Table 1.2: Percent inhibition and MIC determination of *G. arborea* fractions against *M. tuberculosis* isolates

Extract / Standard Drugs	Isolates	Mean Colony forming units (Cfu) on media							Percentage inhibition %						MIC (µg/ml)
Treatment Conc. (µg/ml)		Control	31.25	62.5	125	250	500	1000	31.25	62.5	125	250	500	1000	
Aqueous fraction	OSR-15	195	167	37	33	25	16	10	14	81	83	87	92	95	62.5
	745	190	120	100	43	31	24	12	37	47	77	84	87	94	125
n-hexane fraction	OSR-15	195	171	73	64	61	54	48	12	63	67	69	72	75	62.5
	745	190	50	42	23	14	11	9	74	78	89	93	94	95	31.25
Chloroform fraction	OSR-15	195	156	133	112	50	31	25	20	32	43	74	84	87	250
	745	190	73	66	47	29	18	13	62	65	75	85	91	93	31.25
Ethyl acetate fraction	OSR-15	195	100	89	71	65	61	53	49	54	64	67	69	73	62.5
	745	190	150	100	58	41	26	15	25	50	69	78	86	92	62.5
Rifampicin	OSR-15	187	36	28	21	16	12	6	81	85	89	91	94	97	31.25
	745	180	139	131	124	117	113	97	23	27	31	35	37	46	-
Isonazid	OSR-15	190	29	24	17	13	10	8	85	87	91	93	95	96	31.25
	745	182	141	135	128	122	116	112	23	26	30	33	36	38	-

Note: (-) means % inhibition below 50, OSR-15 – *Mycobacteria tuberculosis*, 745 – Multi-drug resistant *mycobacteria tuberculosis*,
MTB = Mycobacterium tuberculosis, cfu = colony forming units,

$$\% \text{ inhibition} = \frac{Cc - Ct}{Cc} \times 100$$

Cc = No of colony in the control medium, Ct = No of colony in test medium

Table 2.1: GCMS analysis of hexane fraction (nHF) of *Gmelina arborea*

S/N	Retention time	Compounds in n-hexane fraction	Area (%)	Mass (g/mol)
1	5.187	2,3-dihydro-3,5,-6-methy 4H-Pyran-4-one	35.81	144.12
2	5.349	L-Leucine,	5.18	131.17
3	5.675	3,5-dihyroxy-2-methy-4H-Pyran-4-one	9.82	142.11
4	6.288	5-hydroxymethylfurfural	19.03	126.11
5	6.838	1,2-Benzenediol	3.71	110.10
6	7.501	Hydroquinone	3.10	110.11
7	19.718	Hexadecanoic acid, methyl ester	2.97	270.45
8	20.487	n-Hexadecanoic acid	8.45	256.43
9	23.421	Methyl stearate	4.97	298.50
10	23.659	9-Octadecenoic acid	3.29	282.47
11	24.100	Z-7-Tetradecenoic acid	1.93	226.35
12	29.676	Hexadecenoic acid	1.75	254.41

The activities of antioxidants could inhibit the bleaching process of b-carotene, which in turn reduces the chain reactions initiated by lipid peroxidation and changing the reactive species end product to become more stable (Nithiyanantham *et al*., 2013). The bleaching inhibition of *G. arborea* showed high antioxidant activity than the positive control which was ascorbic acid. Also, n-hexane and aqueous fractions of *G. arborea* had higher bleaching inhibition than ascorbic acid. The presence of carotenoid plays an important role in the decrease of the free radical concentration (Nithiyanantham *et al*., 2013).

Phenolic compounds exhibit antioxidant activities and some plants with antioxidant properties have been shown to have antitubercular activities such as *Piper imperiale* (Diaz *et al*., 2012), *Leucas marrubioides* (Gowrish *et al*, 2016) and *Piper sarmentosum* (Hussain *et al*., 2009). *Acacia albida* and *A. hebecladodies* showed high phenolic content as well as anti-tubercular activity as shown in (Figure 1.3, Table 1.1), this might indicate the relationship between antioxidant properties and anti-tubercular activities.

Gas chromatography-mass spectrometry assay of the n-hexane fraction of the plant revealed a number of phytochemicals, of which most were semi volatile and volatile organic compounds. The compound 9-octadecenoic acid which is known as oleic acid was present in the n hexane
fraction of *G. arborea* and has the attributes of a lipophilic compound (Patel et al., 2013). As described by Mohamad (2018) that the hydrophobic outer membrane of M. tuberculosis could be penetrated by compounds with lipophilic nature to exert inhibitory effects.

The compound 2, 3-dihydro-3, 5-dihydroxy-6-methyl 4H-Pyran-4-one, has been classified as fragment of flavanoids which when synthesized by plants termed as phytoestrogens was present in n-hexane fraction of *G. arborea*. It has displayed numerous biological activities including radical scavenging (Takara et al., 2007). 2, 3-dihydro-3, 5-dihydroxy-6-methyl 4H-Pyran-4-one compounds are saponins with proven antioxidant and antitumor potentials (Cˇ echovská et al., 2011).

Unsaturated fatty acids inhibited growth of bacterial as reported by (Ohta et al., 1995). The anti-tuberculosis properties of the ink extract of cuttlefish and *Sepiella inermis* indicated that fatty acids played a pivotal role (Ravitchandirane et al., 2013). A consistent fatty acid found in n-hexane fraction of *G. arborea* was methyl esters of Hexadecanoic acid (Palmitic acid) (8.45%) and n-Hexadecanoic acids (2.97%) as shown in Table 2.1. Palmitic acid has been found to show antimicrobial, antitumor and antioxidant activities (Pinto et al., 2017). A report showed that the oil extract from Moringa oleifera seed showed antitubercular activity and this was linked to the presence of palmitic acid and oleic acid (Egharevba et al., 2015). In addition, it has been reported that unsaturated fatty acids inhibit the growth of mycobacterium in a short time (Kanetsuna, 1985). Hexadecenoic acid methyl ester has been reported to possess antibacterial properties (Wei et al., 2011). Based on these reports, there could be a synergistic link between the saturated fatty acid (hexadecanoic acid) and other unsaturated fatty acids of *G. arborea* to inhibit the growth of the *M. tuberculosis*.

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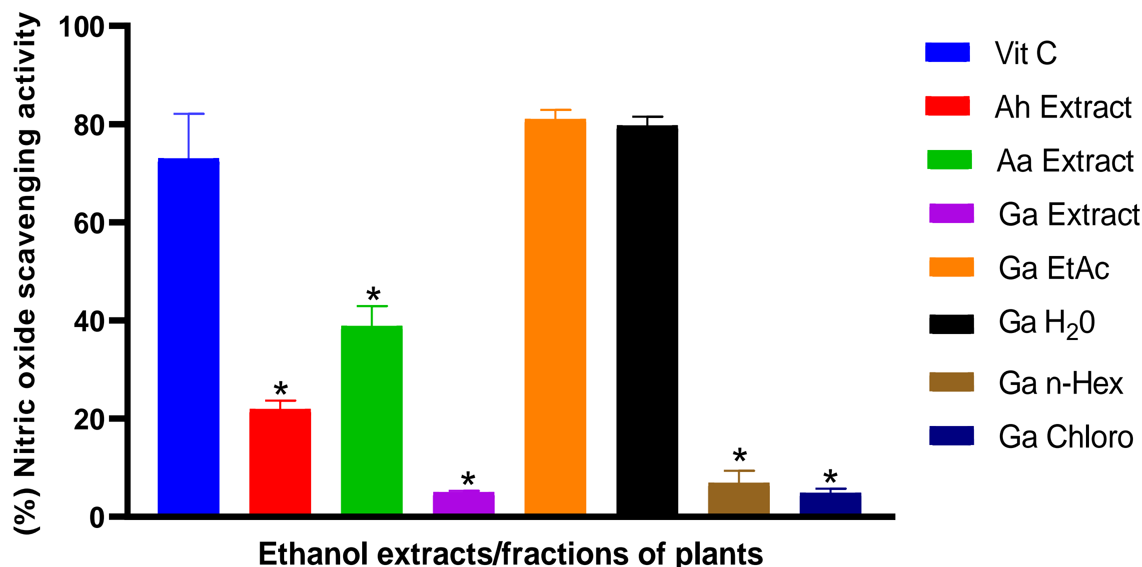

Figure 1.1: Nitric oxide scavenging activity (%) of plant extracts/fractions
Values are means of triplicate determinations ± standard deviation. Mean values followed by (*) superscript in the same column are significantly ($P < 0.05$) different for the standard reference (Vit C), Ascorbic acid. Ah, *A. hebeciadodies*; Aa, *A. albida*; Ga, *G. arborea*; EtAc, ethyl acetate; H_20, aqueous; n-Hex, n-hexane; Chloro, chloroform.

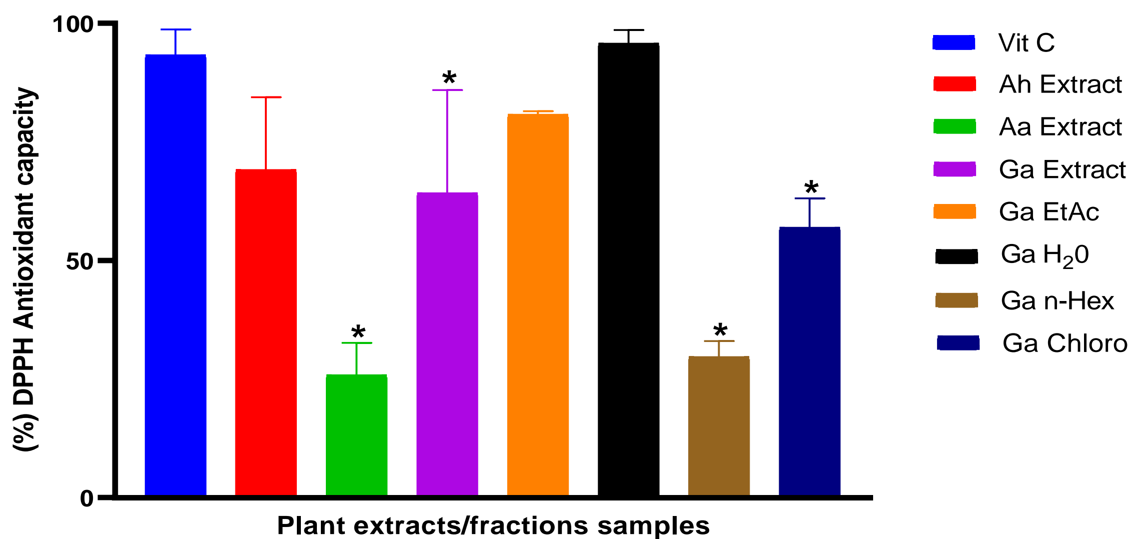

Figure 1.2: DPPH antioxidant capacity (%) of plant extracts/fractions
Values are means of triplicate determinations ± standard deviation. Mean values followed by (*) superscript in the same column are significantly ($P < 0.05$) different for the standard reference (Vit C), Ascorbic acid. Ah, *A. hebeciadodies*; Aa, *A. albida*; Ga, *G. arborea*; EtAc, ethyl acetate; H_20, aqueous; n-Hex, n-hexane; Chloro, chloroform.

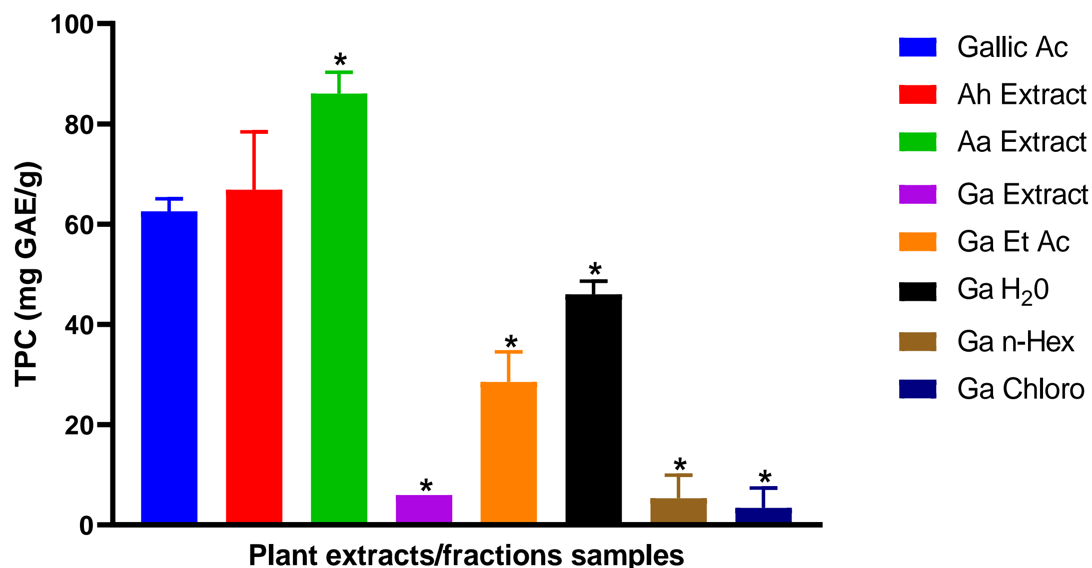

Fig 1.3: Total phenolic content (TPC) of plant extracts/fractions

Values are means of triplicate determinations ± standard deviation. Mean values followed by (*) superscript in the same column are significantly ($P < 0.05$) different for the standard reference (Gallic Ac), Gallic acid. Ah, *A. hebeciadodies*; Aa, *A. a*

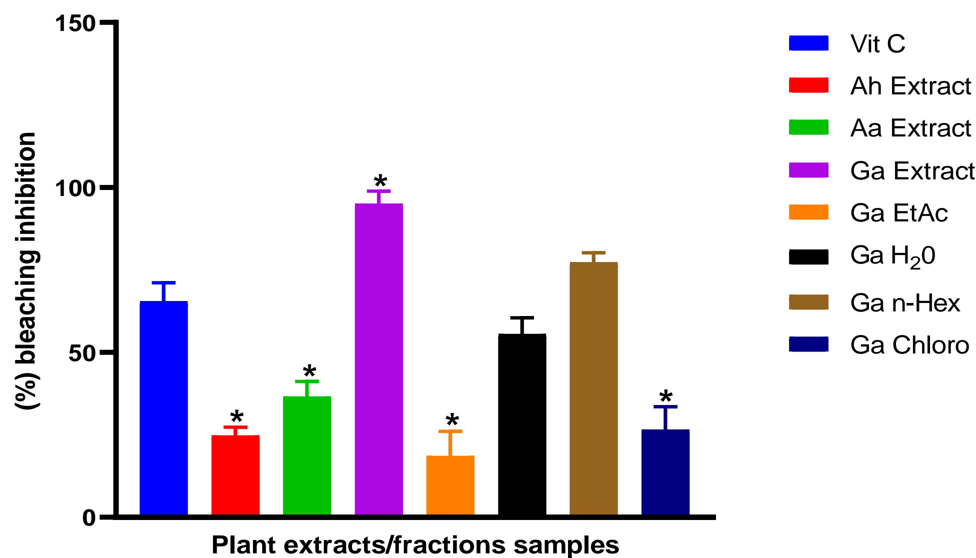

Fig 1.4: β-carotene bleaching inhibition of plant extracts/fractions.

Values are means of triplicate determinations ± standard deviation. Mean values followed by (*) superscript in the same column are significantly ($P < 0.05$) different for the standard reference (Vit C), Ascorbic acid. Ah, *A. hebeciadodies*; Aa, *A. albida*; Ga, *G. arborea*; EtAc, ethyl acetate; H_20, aqueous; n-Hex, n-hexane; Chloro, chloroform.

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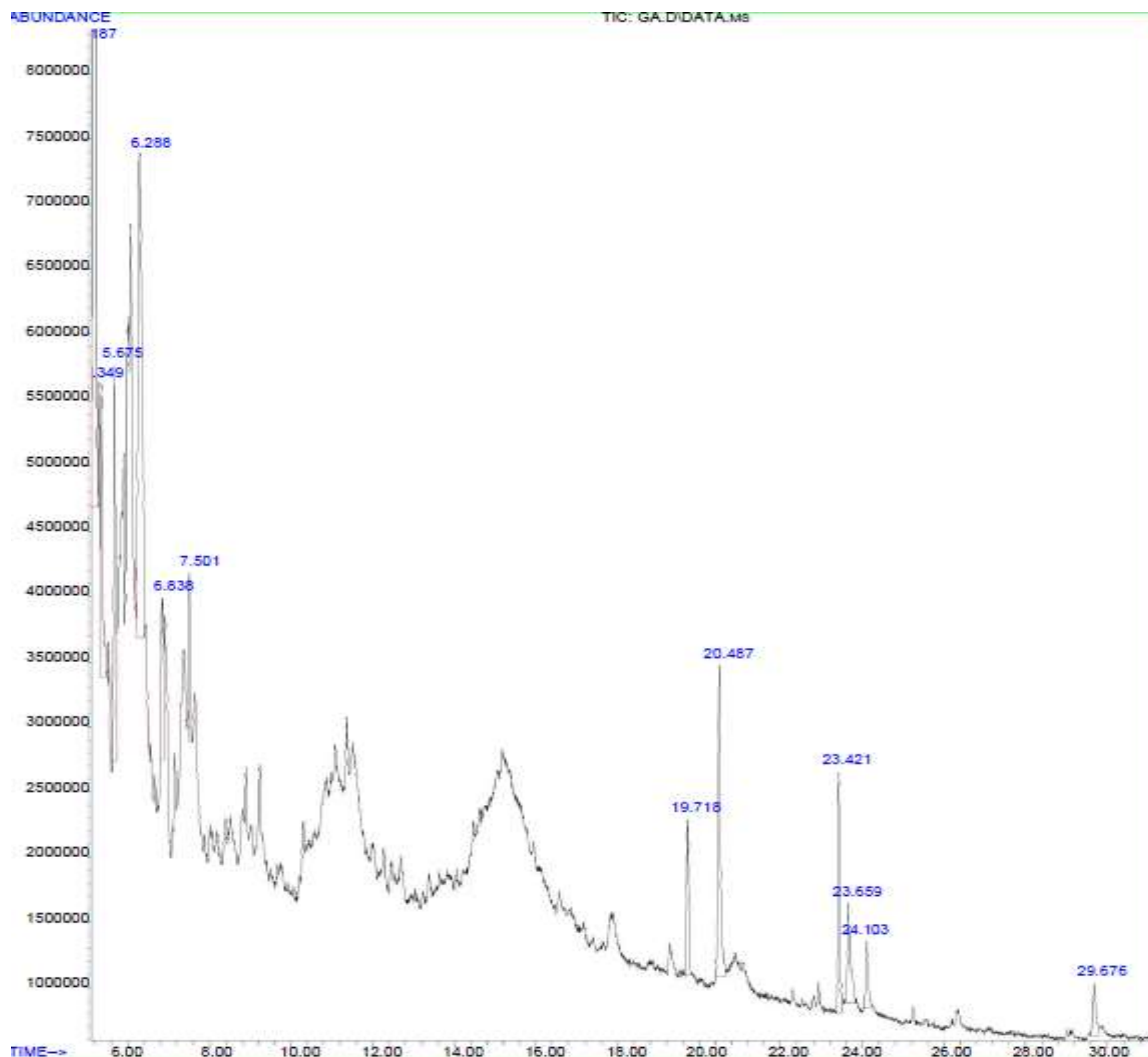

Figure 2.1: GCMS analysis of hexane fraction of *Gmelina arborea*

5. Conclusion

In summary, the study showed that ethanolic extract of *Gmelina arborea* showed good antitubercular activities against the *M. tuberculosis* and MDR M. tuberculosis. The most active fractions of *G. arborea* based on their M IC values were n- hexane and ethyl acetate fractions which inhibited the growth of *M. tuberculosis* and MDR M. tuberculosis. Also, *G. arborea* ethanol extract and fractions (aqueous and ethyl acetate) showed good antioxidant properties and might be good competitors of free radicals.

Author Contributions

Paul T. Olonishuwa: Conceptualization, Writing- Original draft preparation, Data curation, Formal analysis
Gabriel O. Anyanwu: Supervision, Methodology, Writing- Reviewing and Editing, Software
Uju D. Ejike: Supervision, Validation.

Conflict of Interest

Authors declare no conflict of interest.

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