ABSTRACT

The hexane and ethylacetate fractions of Phyllanthus muellerianus demonstrated sensitivities against Mycobacterium tuberculosis strains H37Rv and local isolates. The metabolic profiling of the hexane fraction of the leaves of the plant using GC-MS analysis showed the presence of three fatty acids methyl esters (hexadecanoic acid methyl ester (8.25%), cis-13-octadecenoic acid methyl ester (4.48%), octadecanoic acid methyl ester (3.02%)), four fatty acid ethyl esters (Hexadecanoic acid ethyl ester (8.17%), linoleic acid ethyl ester (0.63%), ethyl oleate (12.94%), octadecanoic acid ethyl ester (6.11%)), a propyl ester (n-propyl-11-Octadecenoate (5.74%)), two butyl esters (Hexadecanoic acid butyl ester (3.28%), Octadecanoic acid butyl ester (1.49%)), a monoterpen (Bicyclo[3.1.1]heptanes-2,6,9-trimethyl (9.21%)) , an acyclic diterpene alcohol (Phytol (16.18%)), an unsaturated cyclic alcohol (3,6,6-Trimethylcyclohex-2-enol (1.30%)) and a triterpene polyunsaturated hydrocarbon (squalene (4.19%)). Phytol, with the highest percentage, and squalene had been reported in literature to show antimycobacterial activity. Some of the constituents are also known to possess antioxidant and antimicrobial properties which could have contribute to the therapeutic effect of the plant.

KEYWORDS: Mycobacterium tuberculosis, Phyllanthus muellerianus, antimycobacteria.

INTRODUCTION

Phyllanthus muellerianus (Kuntze) Exell, which belongs to the family Euphorbiaceae, is widely distributed in West Africa. P. muellerianus is used in folkloric medicines to treat infectious diseases (both bacterial and viral), inflammatory disorders and skin diseases (Mathias, 2018). The stem bark of the plant is used in Cameroon as a remedy for wound healing and tetanus. In Guinea, the leaves are boiled with palm fruits and administered to women undergoing labor. In Ghana, the root is cooked with maize meal and used for treating chronic dysentery, and in Togo, Côte d'Ivoire, and Zambia, the roots and leaves are boiled and administered to children for treatment of eruptive fever. It is also used to manage menstrual disturbances, pain, dysentery, gonorrhoea, and stomach sores. Freshly ground leaves are applied to boils, wounds, and also used for treatment of menstrual disorders, fevers, and skin eruptions in Sierra Leone, Ghana, Nigeria, and Cameroon (Agyare et al., 2009). The plant is used for treating constipation, bronchitis, and for relieving urethral discharges (Chopra et al., 1986; Sriram et al., 2004). P. muellerianus is used in Nigeria for treating jaundice, skin diseases, stomach problems, fever, cough, insomnia and dysentery (Odugbemi, 2008). The traditional use against wound infections and tetanus is validated by its activity against S. pyogenes and C. sporogenes (Ayegba et al., 2015). The extracts of P. muellerianus exhibits both immune-boosting and immunosuppressing actions at different doses and can be employed in both immunodeficiency and over reactive immune conditions at appropriate doses (Mathias, 2018). The antimicrobial property of the essential oil from the plant was reported by Brusotti et al., 2012. P. muellerianus was shown to exhibit stimulating activity on dermal fibroblasts and keratinocytes, leading to increased cell proliferation, barrier formation and formation of extracellular matrix proteins thereby justifying the traditional use of the plant for wound healing (Agyare et al., 2011). The root decoction of the plant is useful in alleviating anemia and the results lend credence to its use in traditional medicine in the management of anemia (Lwanga et al., 2017). Again, the anti-cancer properties of some phyllanthus species were evaluated (Tang et al., 2013). Nitidine, a benzophenantridine alkaloid, was isolated and characterized from the plant and was reported to be responsible for the antibacterial activity of the plant (Cesari et al., 2015). The bark of P. muellerianus has been shown to contain 22β-hydroxyfriedel-1-ene, 1β, 22β-hydroxyfriedel-1-ene (Adesida et al., 1972). In
addition, geraniin, furosin, corilagin, isoquercitrin, astragalin, rutin, phaselic acid, gallic acid, methylgallate, caffeic acid, chlorogenic acid, 3,5-dicaffeoylquinic acid have been isolated from the leaves and aerial parts of *P. muellerianus* (Agyare et al., 2010). The presence of corilagen and furosin explains the use of the plant in wound healing. Astragalin is a bioactive constituent of various traditional medicinal plants such as *Cuscuta chinensis*. This multifaceted compound is well known for its diversified pharmacological applications such as anti-inflammatory, antioxidant, neuroprotective, cardioprotective, antiobesity, antiosteoporotic, anticancer, antiulcer, and anti diabetic properties. It carries out the aforementioned activities by the regulation and modulation of various molecular targets (Riaz et al., 2018). Geraniin induces apoptotic cell death in human lung adenocarcinoma A549 cells *in-vitro* and *in-vivo* (Li et al., 2013). Saleem et al., 2009, also isolated bis (2-ethylloctyl) phthalate, bis (2-ethylcylosyl) phthalate, 3-friedelanone, methylgallate, β-sitosterol from the leaves of the plant. Agyare et al., 2011, reported that the aqueous leaf extract of *P. muellerianus* and its major isolate, geraniin, stimulate cellular activity, differentiation, and collagen synthesis of human skin keratinocytes and dermal fibroblasts. The time-kill kinetics study of extracts of *P. muellerianus* showed the extracts may act as microbistatic agents, anti-inflammatory, and antioxidant activities (Boakye et al., 2016). The antioxidant activity of *P. muellerianus* may enhance its wound-healing activity (Boakye et al., 2018). The methanol extract of the stem of *P. muellerianus* was found to be cytotoxic bioactive with a positive lethality (LD50 4.867 μg/ml) (Onocha and Alli, 2010). The plant extract had been observed to possess haemopoetic ability in man ((Burkill, 1997). It could be used to cure some blood disorders in fish. For efficient growth and metabolism especially during therapy, root extract of this plant could be used for recovery of damaged blood tissue in fish.(Ada et al., 2018). The methanol crude extract and the methanol fraction of the leaves of *P. muellerianus* have been shown to have promising antimicrobial activities against various isolates of *S. aureus*. The combined extract of methanol fraction with the standard drug, ciprofloxacin, produced synergistic effect in many of the combination ratios against the bacteria and this has a lot of therapeutic implications in the treatment of infections caused by *S. aureus* (Ofokansi, et al., 2012). The antimicrobial property of the essential oil from the plant had been reported by Brusotti et al., 2012. The defatted methanol extract, inactive against *S. aureus*, *E. coli*, *C. albicans*, exhibited a very interesting activity against *C. sporogenes* and *S. pyogenes* (MIC 100 μg/ml and 300 μg/ml respectively), which seems to validate the use of this plant in pygmies traditional medicine for the treatment of tetanus and wound infections. The activity found against Streptococcus mutans (300 μg/mL), aetiological agent of caries, may suggest a possible use of this plant as natural remedy to prevent dental diseases (Brusotti, et al., 2011). In Sierra Leone and southern Nigeria, the fresh juice of the plant is used to treat eye infections and skin diseases (Dalziel, 1937). A leaf infusion is used as an eye lotion and as a wash for fever, malaria, skin eruptions and wounds. Ethanol leaf extract has been found to be active against chloroquine-resistant Plasmodium falciparum (Ndjonka et al., 2010). Five compounds, bis(2-ethylloctyl)phthalate, bis(2-ethylcylosyl)phthalate, 3-Friedelanone, β-sitosterol, methyl gallate have been isolated and characterized from *P. muellerianus* (Saleem et al., 2009). 3-Friedelanone is a pentacyclic triterpenoids. It has been reported to show selective antibacterial activity (Kuete et al., 2011). A novel pentasaccharide, α-D-Glcp-(2 6)-[α-D-Fruf(1 2)]β-D-Glc-Fru-(1 4)β-D-Glcp-(2 2)β-D-Fruf, was also isolated from the root bark of *P. muellerianus* (Ayeogba et al., 2015). The polysaccharides extracted from medicinal plants had been shown to possess significant anticancer, antioxidant and immunomodulatory activities (Zhang et al., 2018). The traditional usage of the plant in management of pain had been examined. Aqueous extract and geraniin obtained from the aerial parts of *P. muellerianus* possess both peripheral and central antinociceptive effects in murine models of chemical nociception (Boakye-Gyasi et al., 2016). The methanolic leaf extract of *P. muellerianus* inhibited castor oil-induced diarrhoea, magnesium sulphate-induced diarrhoea, and also inhibited small intestinal propulsion and distal colonic propulsion (John-Africa, 2009).

Tuberculosis is an airborne infectious disease. The burden of drug-resistant TB in Nigeria is high (Onyedun et al., 2017). The severity of adverse effects experienced by TB patient forces the discontinuation of the antibiotic schedule. This in turn facilitates the emergence of drug resistant strains of MTB (Sarkar et al., 2016). The knowledge gained regarding the use of medicinal plants in TB and the promising results obtained from earlier studies warrant the use of medicinal plants as an immunomodulator or in using them as a supplement to currently used anti-TB drugs (Gupta et al., 2017). Researchers are interested in compounds that can display synergistic activity with efficacious anti-TB drugs in order to increase both therapeutic efficacy and reduce toxicity commonly observed by the drugs (Ge et al., 2010; Lopes et al., 2014).

This research was done with the intent to discover the effect of the plant fractions on the *Mycobacterium tuberculosis* and to identify compounds present in the active fractions.

**MATERIALS AND METHODS**

**Plant Collection**

The leaves of *P. muellerianus* were obtained from Mushin market in Lagos state. The plant was identified by Mr T. K. Odewo, formerly of the Forestry Research Institute of Nigeria, (FRIN), Ibadan.

**Plant Extraction**

300ml of 80% ethanol solution was added to 60 g of the dried powdered sample of the plant. The mixture was
kept at room temperature for 72 h with gentle and intermittent shaking and thereafter was filtered. The filtrate was dried at 42.5°C. Sequential extraction was carried out on the ethanol extract to obtain the hexane fraction.

**MYCOBACTERIUM TUBERCULOSIS SENSITIVITY TEST**

**The Test Organisms**
The reference *Mycobacterium tuberculosis* strain H37Rv labeled PT12 and the local isolates labeled PT10 were used. The local isolates were isolated from TB patients using standard methods (Salami and Oluboye, 2002). The organisms were sub-cultured in Middle Brook 7H9 broth supplemented with OADC at 37°C for 21-28 days and were confirmed acid fast gram positive bacillus using Ziehl Nelson stain

**Preparation of Plant Samples for Mycobacterium Sensitivity Test**
The antimycobacterial test was done using proportion method. Each 20ml of homogenized egg LJ medium of plant sample concentrations 2.5mg/ml, 1.25mg/ml, 0.75mg/ml, 0.5mg/ml and 0.25mg/ml was duplicated to serve both the standard Mtb strain, PT12 and the local strain, PT10. Standard drugs, isoniazid and rifampicin, at 0.2 µg/ml and 0.4µg/ml respectively, were added to LJ media accordingly. The media were slanted to form slopes. The LJ slopes without extracts and drugs were used as control. The slopes were insipissated at 85°C for 45 minutes, cooled and stored in a refrigerator at 4°C. Sterility and viability check were carried out before inoculation.

**Bacterial Innoculation and Reading of Results**
Bacterial dilutions 10^3 mg/ml and 10^3 mg/ml were prepared for inoculation. 0.1 ml of The universal containers were loosely closed with caps to allow evaporation and were incubated at 37°C. The specimens were checked on the 7th, 14th, and 21st days to ensure no contaminations. Readings were done on the 28th day chosen bacterial dilutions were inoculated into all the labeled LJ slopes (Adeleye et al., 2008).

**GC-MS Analysis**
Constituents in the hexane extracts and hexane fractions of the plant were elucidated using GC-MS performed on Agilent Technologies 7890 A GC coupled with Agilent Technologies 5975 C MS. Helium was used as carrier gas and sample was injected in split less mode at 70ev in a column HP 5 MS, length 30meters, internal diameters 0.32mm, column thickness 0.25µm. The initial temperature was 50°C, held for 2 minutes, flow rate 10³/min, final temperature 240°C, held for 6 minutes. The resulting GC-MS was analyzed using commercially available standards.

**RESULTS AND DISCUSSION**
The hexane and ethylacetate fractions from ethanol extract of the leaves of *P. muellerianus* were screened for antimycobacterium test using H37Rv labeled PT12 and the local isolates from active TB patients labeled PT10. The two fractions showed antimycobacterium activity with the ethylacetate fraction demonstrating stronger activity with MIC of 0.5mg/ml and hexane fraction with MIC 1.25mg/ml as shown in Table 1. The GC-MS analysis of the antimycobacterium tuberculosis hexane fraction from the plant showed Phytol (16.18 %), Ethyl oleate (12.94 %), Hexadecanoic acid methyl ester (8.25 %), Hexadecanoic acid ethyl ester (8.17 %), cis-13-Octadecenoic acid methyl ester (4.48 %), Hexadecanoic acid butyl ester (3.28 %), Octadecanoic acid ethyl ester (6.11 %), n-propyl 11-Octadecenoate (5.74 %), Octadecanoic acid butyl ester (1.49 %), 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-(all E)-squalene (4.19 %).

| Table 1: Results of antimycobacterium tuberculosis tests of fractions from ethanol extract of *P. muellerianus.* |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Concentration    |                  |                  |                  |                  |                  |                  |
|                  | 2.5mg/ml         | 1.25mg/ml        | 0.75mg/ml        | 0.5mg/ml         | 0.25mg/ml        |                  |                  |
| Mtb strains      | PT12 PT10        | PT12 PT10        | PT12 PT10        | PT12 PT10        | PT12 PT10        | PT12 PT10        | PT12 PT10        |
| Hexane fraction  | S S S R R R R R  |                  |                  |                  |                  |                  |                  |
| Ethylacetate fraction | S S S S S S S S R |                  |                  |                  |                  |                  |                  |

Mtb: *Mycobacterium tuberculosis*; PT12: H37Rv (Standard strain); PT10: Local isolate of Mtb; S: Sensitive (Mtb growth inhibited); R: Resistance (Mtb growth is not inhibited).

Some of these compounds were reported by Jemimma et al., 2017 to be present in another Phyllanthus plant, *Phyllanthus vasukii*. The compounds are n-propyl 11-octadecenoate, ethyl oleate, octadecanoic acid ethyl ester, squalene, hexadecanoic acid methyl ester and hexadecanoic acid ethyl ester. In addition, *Phyllanthus vasukii* contained phytol acetate and 9-octadecenoic acid methyl ester as there are phytol and 13-octadecenoic acid methyl ester in *P. muellerianus*. In contrast Arun et al., 2012, reported the presence of benzene-1, 2 – dimethoxy– 4-[(4-methylphenyl) sulfonyl]-methyl]-1, Phenethylamine 2-methoxy-alpha-methyl-4,5-(methyleneoxy), phenanthylamine-2-methoxy, cyclopentane pentyl, 3-(3-(1-Axirdinyl) propoxy)-2,5-dimethylpyrazine, and 3-(Cyclopropylamino) propionitrile in ethanol extract of *Phyllanthus amarus*. These compounds are completely different from those identified in *P. muellerianus*. The Figure 1 showed the chromatogram for the active hexane fraction of *P. muellerianus* while the Table 2 showed the compounds identified in the hexane fraction of *P. muellerianus* using GC-MS. Figure 2 showed the spectra and structures of some of the compounds identified in the fraction. Phytol has the highest percentage abundance. Long-term intake of phytol had been reported to possess...
beneficial effects on insulin resistance, obesity, and diabetes via improvement of lipid metabolism. Phytol is anti-diabetes. It induces insulin secretion (Matsuda et al., 2018). *P. muellerianus* reduces blood glucose level and improves lipid profile (Ndeingang et al., 2019). E-phytol constituent from hexane fraction of *Morinda citrifolia* showed pronounced antitubercular activity (Saludes et al., 2002). Chen et al., 2010, also reported that E-phytol exhibited antitubercular activity against *Mycobacterium tuberculosis* H37Rv. The crude methanol extract of *Leucas volkensii* showed antimycobacterial activity against Mtb and the principal active component of the extract, E-phytol, showed MIC of 2µg/ml (Rajab et al., 1998). The anti-inflammatory effect of some of the saturated and unsaturated fatty acids could have synergistic effect with phytol to establish the discovered mycobacteriocidal effect. Phytol demonstrated a strong antioxidant effect *in vitro* in its capacity to remove hydroxyl radicals and nitric oxide as well as to prevent the formation of thiobarbituric acid reactive substances (TBARS) (Santos et al., 2013). The Thiobarbituric Acid Reactive Species (TBARS) were significantly increased in TB. The presence of oxidative stress was found to be profound in the TB (Rajopadhye et al., 2011). Oxidative stress conditions exist in the guinea pig model of tuberculosis similar to what is seen in humans (Palanisamy et al., 2011). Phytol attenuates the inflammatory response by inhibiting neutrophil migration that is partly caused by reduction in IL-1β and TNF-α levels and oxidative stress (Silva et al., 2014). Gold et al., 2012, reported that non-steroidal anti-inflammatory drug sensitizes *Mycobacterium tuberculosis* to endogenous and exogenous antimicrobials. Antioxidant and antimicrobial activities of phytol could have contributed to the demonstrated antimycobacterium effect of the fraction. Esters of long chain fatty acids had been reported to possess antimicrobial activities (Kabara et al., 1972). Linoleic acid ethyl ester is hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, 5-alpha reductase inhibitor, antihistaminic, anticoronary, insectifugal, antieczemic and antiacne (Sudha et al., 2013). Hexadecanoic acid methyl ester is a fatty acid ester. Its biological activity include antioxidant, antimicrobial, hypocholesterolemic, antiandrogenic, hemolytic and 5-alpha reductase inhibitor (Sujayil and Dhanaraj, 2016).

![Figure 1](image_url): Chromatogram for Hexane fraction from *Phyllanthus muellerianus*. 
Table 2: Compounds Obtained from GC-MS Analysis of Hexane Fraction From *Phyllanthus Muellerianus*.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compound</th>
<th>RT</th>
<th>% abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bicyclo[3.1.1]heptanes,2,6,6-trimethyl</td>
<td>11.724</td>
<td>9.21</td>
</tr>
<tr>
<td>2.</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>12.211</td>
<td>3.02</td>
</tr>
<tr>
<td>3.</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>12.708</td>
<td>8.25</td>
</tr>
<tr>
<td>4.</td>
<td>Hexadecanoic acid ethyl ester</td>
<td>13.424</td>
<td>8.17</td>
</tr>
<tr>
<td>5.</td>
<td>cis-13-Octadecenoic acid methyl ester</td>
<td>14.511</td>
<td>4.48</td>
</tr>
<tr>
<td>6.</td>
<td>Phytol</td>
<td>14.677</td>
<td>16.18</td>
</tr>
<tr>
<td>7.</td>
<td>Octadecanoic acid methyl ester</td>
<td>14.757</td>
<td>3.02</td>
</tr>
<tr>
<td>8.</td>
<td>3,6,6-Trimethyl-cyclohex-2-enol</td>
<td>14.946</td>
<td>1.30</td>
</tr>
<tr>
<td>9.</td>
<td>Linoleic acid ethyl ester</td>
<td>15.100</td>
<td>0.63</td>
</tr>
<tr>
<td>10.</td>
<td>Ethyl oleate</td>
<td>15.163</td>
<td>12.94</td>
</tr>
<tr>
<td>11.</td>
<td>Hexadecanoic acid butyl ester</td>
<td>15.318</td>
<td>3.28</td>
</tr>
<tr>
<td>12.</td>
<td>Octadecanoic acid, ethyl ester</td>
<td>15.392</td>
<td>6.11</td>
</tr>
<tr>
<td>13.</td>
<td>n-Propyl 11-Octadecenoate</td>
<td>16.897</td>
<td>5.74</td>
</tr>
<tr>
<td>14.</td>
<td>Octadecanoic acid butyl ester</td>
<td>17.114</td>
<td>1.49</td>
</tr>
<tr>
<td>15.</td>
<td>2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl (all E)</td>
<td>20.616</td>
<td>4.19</td>
</tr>
</tbody>
</table>

N-hexadecanoic acid is an inhibitor of phospholipase A(2) and hence it possesses anti-inflammatory property (Aparna et al., 2012). Hexadecanoic acid methyl ester is anti-oxidant (Balamurugan et al., 2017). Ethyl oleate is one of the fatty acid ethyl esters (FAEE) that is formed in the body after ingestion of ethanol. It is the mediator of ethanol-induced organ damage (Laposata et al., 2002) and its concentration in the body is used as an alcohol biomarker. Fatty acid ethyl ester, FAEE, reconstituted in low density lipoprotein (LDL) particles can be incorporated into HepG2 cells and subsequently decrease their rate of cell proliferation and protein synthesis (Szczerpiorkowski et al., 1994). However, ethyl oleate is cancer preventive, hypocholesterolemic, 5-alpha reductase inhibitor, antiandrogenic, insectifuge, anti-inflammatory, anemiogenic, dermatitigenic and choleretic (Jemimma et al., 2017). Hexadecanoic acid butyl ester is antimicrobial and antioxidant (Belakhdar et al., 2015). Octadecanoic acid ethyl ester is known as stearic acid ethyl ester (ethyl stearate). It is the neutral, more lipid soluble form of the free acid. Ethyl stearate perturbs the cell cycle and induces apoptosis in HepG2 cells and is a marker of excessive alcohol consumption that can be isolated from an individual's hair (Kanimozhi and Ratha Bai, 2012). Another compound of interest identified is 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl (all E). This is squalene. Squalene, the main component of skin surface polyunsaturated lipids, shows some advantages for the skin as an emollient and antioxidant, and for hydration and its antitumor activities. It is also used as a material in topically applied vehicles such as lipid emulsions and nanostructured lipid carriers (NLCs) (Huang et al., 2009). An adjuvant using squalene is Seqirus' proprietary MF59, which is added to influenza vaccines to help stimulate the human body's immune response through production of CD4 memory cells (WHO, 2008).
Squalene resulted in marked increases of cellular and non-specific immune functions and enhancement of host resistance to tumor challenge in dose-dependent manner. Squalene is a lipoxygenase inhibitor (Gideon, 2015). The inhibition of LOX can reduce leukotrienes LT, thereby producing an anti-inflammatory effect (Hu and Ma, 2018). Thus it could be possible that squalene contributes to the reported anti-inflammatory activity of *P. muellerianus*. Squalene inhibited the growth of Mycobacterium tuberculosis H37Rv with MICs 100µg/ml (Tan *et al.*, 2008). There is no reported activity for n-propy111-Octadecenoate. The acid of Cis-13-Octadecenoic acid methyl ester is used in surgery (Arora *et al.*, 2017). The GC-MS analysis of the hexane fraction of *P. muellerianus* revealed the presence of phytol and squalene which has been reported to have

Figure 2: Some of the compounds from hexane fraction of *P. muellerianus*. 
antimycobacterium capacity. Azevedo et al., 2008, reported that the removal of lipid fraction diminish antitubercular capacity of Aplysina caissara marine sponge crude extract. Thus, the other bioactive fatty acids esters in the fraction could be suggested to enhance the antimycobacterium activity of phytol and squalene in the hexane fraction of the P. muellerianus.

CONCLUSION
The hexane and ethylacetate fractions from the ethanol extract of the leaves of P. muellerianus inhibited the growth of M. tuberculosis. The GC-MS analysis of the active hexane fraction showed the presence of bioactive phyтол with the highest percentage, squalene and some other compounds discussed above. Phyтол and squalene had been reported in literature to demonstrate antimycobacterium activity. Some other compounds identified in the fraction like ethyl oleate, Hexadecanoic acid methyl ester, Hexadecanoic acid ethyl ester, Cis-13-Octadecenoic acid methyl ester and Octadecanoic acid ethyl ester possessed biological activities which could play supportive role to the antimycobacterium property of phyтол and squalene. Further research work is required to isolate, identify and characterize compounds present in the active ethylacetate fraction of the plant in order to possibly suggest the component(s) responsible for the antimycobacterium activity of the fraction.

Conflict of Interest
The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT
The authors sincerely appreciate the management of Nigerian Institute of Medical Research, NIMR, Yaba, for granting the permission to use their tuberculosis laboratory facilities. Our sincere gratitude goes to Mr Nshiogu Michael, a member of staff of NIMR, for his technical assistance.

REFERENCES


37. Lwanga GB, Sijumbila GM, Nyirenda J, Muzandu KM. Efficacy of the aqueous root extract of *phyllanthus muellerianus* in alleviating anemia in
41. Ndeingang EC, Deeh PBC, Watch P, Kamanyi A. Phyllanthus muellerianus (Euphorbiaceae) Restores Ovarian Functions in Letrozole-Induced Polycystic Ovarian Syndrome in Rats. Evidenced Based Complimentary and Alternative Medicine, 2019, Article ID 2965821, 2019; 16.
52. Saleem M., Nazir M. Akhtar, Onocha PA, Rajab MS, Jabba A. New Phthalates from Phyllanthus muellerianus (Euphorbiaceae). Journal of Asian Natural Products Research, 2009; 11(11): 9747. doi: 10.1080/10286020903341388-
