ejpmr, 2019,6(10), 640-649

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

METABOLIC PROFILING OF ANTIMYCOBACTERIAL HEXANE FRACTION OF THE LEAVES OF *PHYLLANTHUS MUELLERIANUS* KUNTZE (EXELL.) USING GC-MS ANALYSIS

Morufu Adisa Ademoye*[1], Labunmi Lajide[2], Bodunde Joseph Owolabi[3], Catherine Chizoba Onubogu[4]

[1,2,3]Chemistry Department, School of Sciences, Federal University of Technology, Akure. Nigeria.
[4]Centre for Tuberculosis Research, Nigerian Institute of Medical Research, Yaba. Nigeria.

***Corresponding Author: Dr. Morufu Adisa Ademoye**

Chemistry Department, School of Sciences, Federal University of Technology, Akure. Nigeria.

Article Received on 20/08/2019 **Article Revised on 10/09/2019** **Article Accepted on 30/09/2019**

ABSTRACT

The hexane and ethylacetate fractions of *Phyllanthus muellerianus* demonstrated sensitivity against *Mycobacterium tuberculosis* strains $H_{37}Rv$ 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 monoterpene (Bicyclo[3.1.1]heptanes-2,6,-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, gonorhoea, 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-o-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 antidiabetic 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-ethyloctyl) phthalate, bis (2-ethylicosyl) 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 microbiostatic 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 haemopoeitic ability in man ((Burkill, 1994). 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 falçiparum* (Ndjonka *et al*., 2010). Five compounds, bis(2-ethyloctyl)phthalate, bis(2-ethylicosyl)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-DFruf(1 2)]α-D-Fruf-(1 4)β-D-Glcp-(2 2)β-D-Fruf, was also isolated from the root bark of *P. muellerianus* (Ayegba *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 anti-nociceptive 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 $H_{37}R_V$ labeled PT_{12} and the local isolates labeled PT_{10} 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 tuberculosis 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, PT_{12} and the local strain, PT_{10}. 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^{-5}mg/ml and 10^{-3}mg/ml were prepared for inoculation. 0.1 ml of the 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 daychosen 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 0.320mm, column thickness 0.25µm. The initial temperature was 50°C, held for 2 minutes, flow rate 10°/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 $H_{37}Rv$ labeled PT_{12} and the local isolates from active TB patients labeled PT_{10}. 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	PT_{12}	PT_{10}	PT_{12}	PT_{10}	PT_{12}	PT_{10}	PT_{12}	PT_{10}	PT_{12}	PT_{10}
Hexane fraction	S	S	S	S	R	R	R	R	R	R
Ethylacetate fraction	S	S	S	S	S	S	S	S	R	R

Mtb: *Mycobacterium tuberculosis*; PT_{12}: $H_{37}Rv$ (Standard strain); PT_{10}: 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]-, Phenethylamine 2-methoxy-alpha-methyl-4,5-(methylenedioxy), 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 antiacme (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).

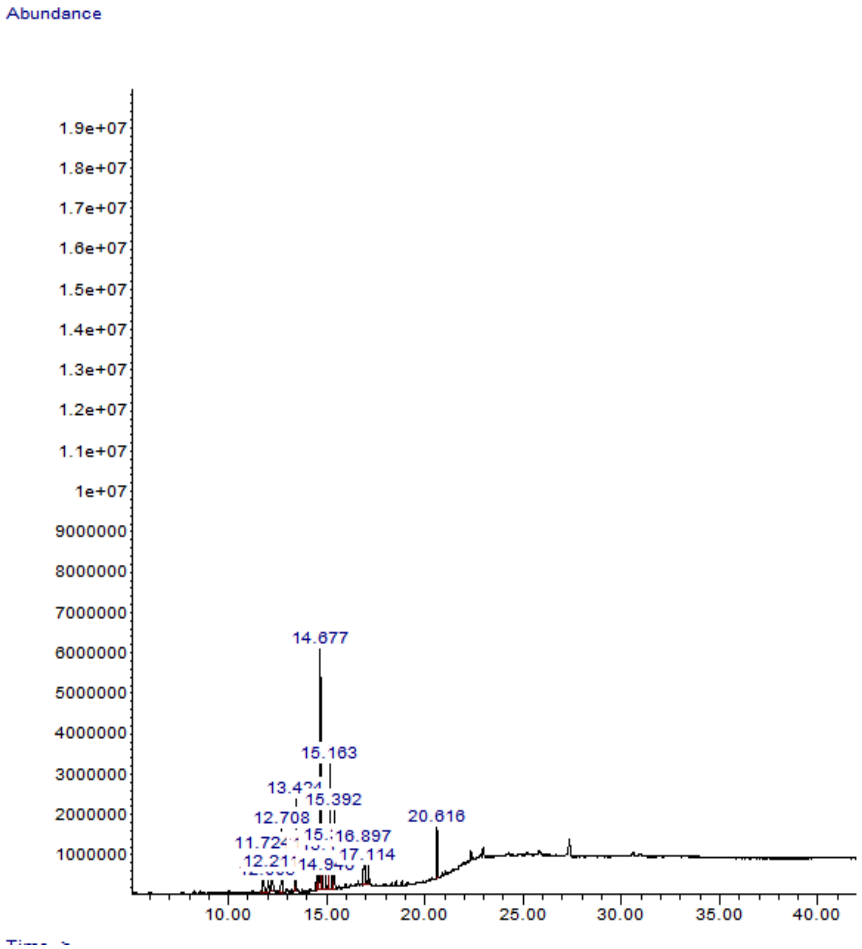
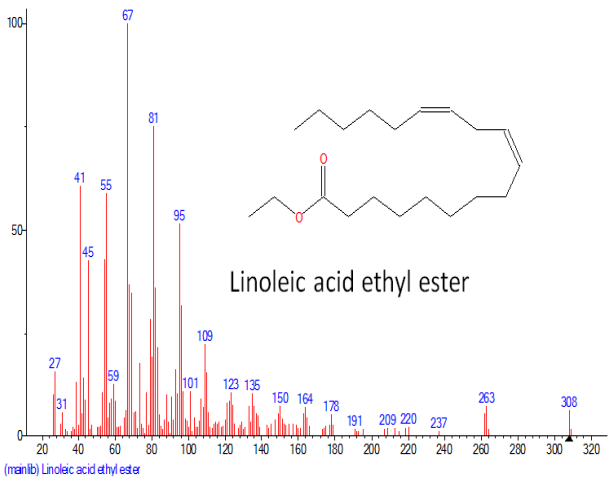


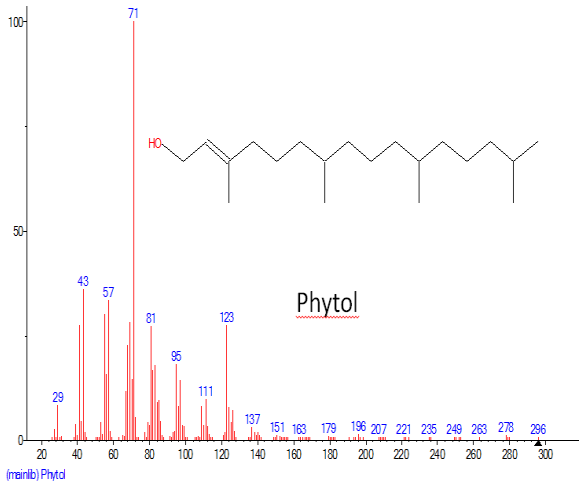
Figure 1: Chromatogram for Hexane fraction from *Phyllanthus muellerianus.*

Table 2: Compounds Obtained from GC-MS Analysis of Hexane Fraction From *Phyllanthus Muellerianus*.

S/N	Compound	RT	% abundance
1.	Bicyclo[3.1.1]heptanes,2,6,6-trimethyl	11.724, 12.005	9.21
2.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	12.211	3.02
3.	Hexadecanoic acid , methyl ester	12.708	8.25
4.	Hexadecanoic acid ethyl ester	13.424	8.17
5.	cis-13-Octadecenoic acid methyl ester	14.511	4.48
6.	Phytol	14.677	16.18
7.	Octadecanoic acid methyl ester	14.757	3.02
8.	3,6,6-Trimethyl-cyclohex-2-enol	14.946	1.30
9.	Linoleic acid ethyl ester	15.100	0.63
10.	Ethyl oleate	15.163	12.94
11.	Hexadecanoic acid butyl ester	15.318	3.28
12.	Octadecanoic acid, ethyl ester	15.392	6.11
13.	n-Propyl 11-Octadecenoate	16.897	5.74
14.	Octadecanoic acid butyl ester	17.114	1.49
15.	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl (all E)-	20.616	4.19

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 (Szczepiorkowski *et al*., 1994). However, ethyl oleate is cancer preventive, hypocholesterolemic, 5-alpha reductase inhibitor, antiandrogenic, insectifuge, anti-inflammatory, anemiagenic, 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).





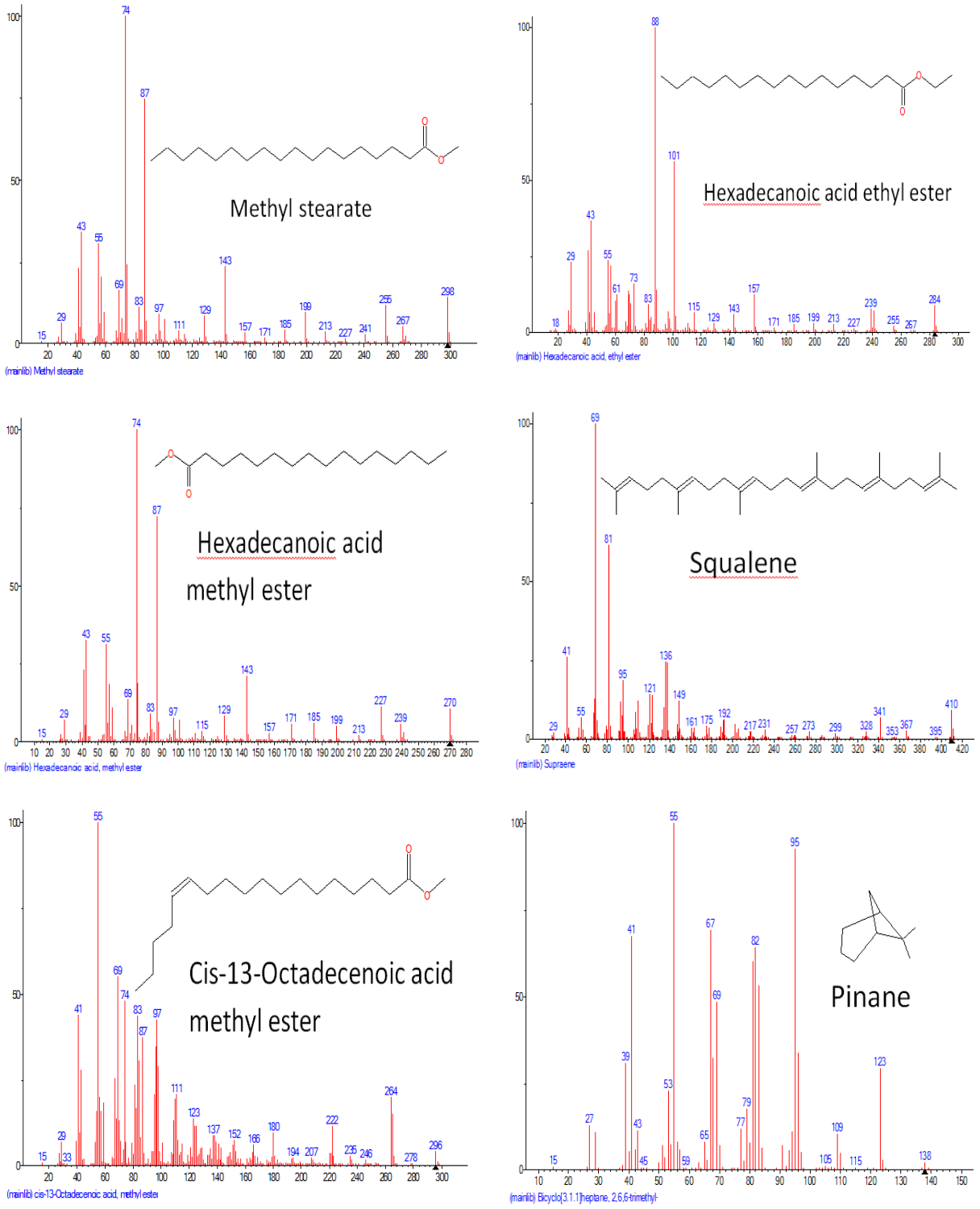


Figure 2: Some of the compounds from hexane fraction of *P. muellerianus.*

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-propyl11-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

antimycobacerium 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 phytol with the highest percentage, squalene and some other compounds discussed above. Phytol 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 phytol 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

1. Ada FB, Ayotunde EO, Sunday KJ. Haemo-Immune System Remediation Potential of Phyllanthus muellerianus Root Extracts in Clarias gariepinus exposed to 2,4-Dimethyl amine. *International Journal of Innovative Environmental Studies Research*, 2018; 6(2): 1-16.
2. Adeleye AI, Onubogu CC, Ayolabi CI, Isawumi AO, Nshiogu ME. Screening of twelve medicinal plants and 'wonder-cure' concoction used in Nigeria unorthodox medicine for activity against Mycobacterium tuberculosis patients sputum. *African Journal of Biotechnology*, 2008; 7(18): 3182-3187. DOI. 10.4314/ajb.v.7i18.59256.
3. Adesida GA, Girgis P, Taylor DAH. Friedelin derivatives from *Phyllanthus muellerianus*. *Phytochemistry*, 1972; 11: 851–852. 10.1016/0031-9422(72)80070-0
4. Agyare C, Lechtenberg M, Asase A, Niehues M. An ethnopharmacological survey and *In vitro* confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *Journal of Ethnopharmacology*, 2009; 125(3): 393-403. DOI: 10.1016/j.jep.2009.07.024
5. Agyare C, Lechtenberg M, Deters A, Petereit F, Hensel A. Ellagitannin from Phyllanthus muellerianus (Kuntze) Exell.: Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. *International Journal of Phytotherapy and Phytopharmacology*, 2010; 18(7): 617-624.
6. Agyare C, Lechtenberg M, Deters A, Petreit F, Hensel A. Ellagitannins from Phyllanthus muellerianus (Kuntze) Exell.: Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. *Phytomedicine*, 2011; 18: 617-624
7. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment; *Chemical Biology & Drug Design*, 2012; 80(3): 434-439. doi: 10.1111/j.1747-0285.2012.01418.x
8. Arora S, Kumar G, Meena S. Screening and evaluation of bioactive components of Cenchrus ciliaris L by GC-MS analysis. *International Research Journal of Pharmacy*, 2017; 8(6): 69-76.
9. Arun T, Senthilkumar B, Purushothaman K, Aarthy A. GC-MS Determination of Bioactive Components of Phyllanthus amarus (L) and its Antibacterial Activity. *Journal of Pharmacy Research*, 2012; 5(9): 4767-4771
10. Ayegba OE, Usman PU, Ibrahim SM, Jephthah OO. UV, IR and NMR Characterization of a novel pentasaccaride isolated from the root bark of Phyllanthus muellerianus (Kuntze) Excell (EUPHORBIACEAE). *Journal of Natural Product and Plant Resources*, 2015; 5(1): 11-25.
11. Azevedo LG, Muccillo-Baisch AL, Filgueira D, Boyle RT. Comparative cytotoxic and anti-tuberculosis activity of Aplysina caissra marine sponge crude extracts. Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology, 2008; 147(1): 36-42.
12. Balamurugan A, Michael Evanjaline R, Parthipan B, Mohan VR. GC-MS Analysis of Bioactive Compounds from the Ethanol Extract of Leaves of *Neibuhria apetala* Dunn. *International Research Journal of Pharmacy*, 2017; 8(12): 72-78.
13. Belakhdar G, Benjouad A, Abdennebi EH. Determination of some bioactive chemical constituents from *Thesium humile* Vahl. *Journal of Materials and Environmental Science*, 2015; 6(10): 2778-2783.
14. Boakye-Gyasi E, Kasenga EA, Biney RP, Mensah KB, Agyare C, Woode E. Anti-Nociceptive Effects of Geraniin and an Aqueous Extract of the Aerial Parts of Phyllanthus muellerianus (Kuntze) Exell. in

Murine Models of Chemical Nociception. *Iranian Journal of Pharmaceutical Sciences*, 2016; 12(3): 17-30.
15. Boakye YD, Agyare C, Hensel A. Anti-infective Properties and Time-Kill Kinetics of Phyllanthus muellerianus and its Major Constituent, Geraniin. *Journal of Medicinal Chemistry*, 2016; 6: 95-104.
16. Boakye YD, Agyare C, Ayande GP, Titiloye N, Asiamah EA, Danquah KO. Assessment of Wound-Healing properties of medicinal plants: The case of *Phyllanthus muellerianus*. *Frontiers in Pharmacology*, 2018; 9: 945.
17. Brusotti G, Frassà G, Cesari I, Grisali P. Antimicrobial properties of stem bark extracts from Phyllanthus muellerianus (Kuntze) Excell. *Journal of Ethnopharmacology*, 2011; 135(3): 797-800 DOI: 10.1016/j.jep.2011.03.042
18. Brusotti G, Cesari I, Gilardoni G, Tosi S, Grisoli P, Picco AM, Caccialanza G. Chemical composition and antimicrobial activity of Phyllanthus muellerianus (Kuntze) Excel essential oil. *Journal of Ethnopharmacology*, 2012; 142(3): 657-662. doi: 10.1016/j.jep.2012.05.022
19. Burkill, H. M. The Useful Plants of West Tropical Africa. 2nd Edition vol.2, Royal Botanical Gardens, Kew, 1994; 1: 385- 387.
20. Cesari I, Grisoli P, Paolillo M, Milanese C, Massolini G, Brusotti G. Isolation and characterization of the alkaloid Nitidine responsible for the traditional use of *Phyllanthus muellerianus* (Kuntze) Exell stem bark against bacterial infections. *Journal of Pharmaceutical and Biomedical Analysis*, 2015; 105: 115-120. doi: 10.1016/j.jpba.2014.11.051
21. Chen J-J, Shieh P-C, Lin W-J, Sung P-J. A new long chain alkene and antitubercolosis constituents from the leaves of Pourthiaea lucida. *Chemistry & Biodiversity*, 2010; 7(3): 717-21. DOI: 10.1002/cbdv.200900198.
22. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian Medicinal Plants (Including Supplement).* New Delhi: *Publisher Council of Scientific and Industrial Research*, 1986; 235–286.
23. Dalziel JM. The Useful Plants of West Africa. Crown Oversea Agent for the Colonies, London, 1937; 158.
24. Ge F, Zeng F, Liu S, Guo N, Ye H, Song Y *et al*., In vitro synergistic activity between 8-methoxypsoralen and ethambutol, isoniazid, and rifampin when used in combination against *Mycobacterium tuberculosis*. *World Journal of Microbiology and Biotechnology*, 2010; 26(4): 623–8. doi: 10.1007/s11274-009-0214-0.
25. Gideon VA. GC-MS analysis of phytochemical components of *Pseudoglochidion anamalayanum* Gamble: An endangered medicinal tree. *Asian Journal of Plant Science and Research*, 2015; 5(12): 36-41.
26. Gold B, Pingle M, Brickner SJ, Shah N, Roberts J, Rundell M, Bracken WC, Warrier T, Somersen S, Venugopal A, Darby C, Jiang X, Warren JD, Fernandez J, Ouerfelli O *et al*., Non steroidal anti-inflammatory drug sensitizes *Mycobacterium tuberculosis* to endogenous and exogenous antimicrobials, *Proceedings of the National Academy of Sciences, USA*, 2012; 109(40): 16004-11. DOI: 10.1073/pnas 1214188109.
27. Gupta VK, Kumar MM, Bisht D, Kaushk A. Plants in our combating strategies against *Mycobacterium tuberculosis*: progress made and obstacles met. Pharmaceutical Biology, 2017; 55(1): 1536-1544 doi: 10.1080/13880209.2017.1309440
28. Hu C, Ma S. Recent development of lipoxygenase inhibitors as anti-inflammatory agents. *MedChemComm*, 2018; 9: 212-225. DOI:10.1039/C7MD00390K
29. Huang ZR, Lin YK, Fang JY. Biological and Pharmacological activities of squalene and related compounds: potential uses in cosmetics dermatology. *Molecules*, 2009; 14(1): 540-554 doi: 10.3390/molecules14010540
30. Jemimma HL, Arumugasamy K, Kumar NR, Kafoor AH. GC-MS Analysis of Root and Aerial Parts Ethanolic Extract of *Phyllanthus vasuki* parthipan Et Al., Sp. Nov (Phyllanthaceae). *International Journal of Ayurvedic and Herbal Medicine*, 2017; 7(4): 2672-2684. DOI: 10.18535/ijahm/v7i4.06
31. John-Africa L, Adzu B, Dzarma S, Gamaniel KS. Anti-diarrhoeal activity of the methanolic leaf extract of Phyllanthus muellerianus. *International Journal of Biological and Chemical Sciences*, 2009; 3(5): 1021-1029
32. Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP. Fatty Acids and Derivatives as Antimicrobial Agents. *Antimicrobial Agents and Chemotherapy*, 1972; 2(1): 23-28
33. Kanimozhi D, Ratha Bai V. Analysis of Bioactive Components of Ethanolic Extract of *Coriandrum Sativum* L. *International Journal of Research in Pharmacy and Science*, 2012; 2(3): 97-110.
34. Laposata M, Hasaba A, Best CA, Yoerger DM *et al*., Fatty acids ethyl esters: recent observations. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 2002; 67(2–3): 193–196. doi:10.1054/plef.2002.0418
35. Li J, Wang S, Yin J, Pan L. Geraniin induces apoptotic cell death in human lung adenocarcinoma A549 cells in vitro and in vivo. *Canadian Journal of Physiology and Pharmacology*, 2013; 91(12): 1016-24 doi: 10.1139/cjpp-2013-0140
36. Lopes MA, Ferracioli KRC, Siqueira VLD, de Lima Scodro RB, Cortez DAG, da Silva RZ, Cardoso RF (2014). In vitro interaction of eupomatenoid-5 from *Piper solmsianum* C. DC. var. *solmsianum* and anti-tuberculosis drugs. *International Journal of Tuberculosis and Lung Disease*, 18(12):1513–5. doi: 10.5588/ijtld.14.0229.
37. Lwanga GB, Sijumbila GM, Nyirenda J, Muzandu KM. Efficacy of the aqueous root extract of *phyllanthus muellerianus* in alleviating anemia in

rats. *The International Journal of Multi-Disciplinary Research*, CFP/182/2117, 2017; 1-10.
38. Matthias A. Immunomodulatory Effects of Phyllanthus muellerianus: A Mechanistic Approach. *Journal of Clinical & Cellular Immunology*, 2018; 9(5): 565.
39. Matsuda H, Suzuki D, Asakura M, Ooi S, Saitoh R *et al*., Effects of Dietary Phytol on Glucose Uptake and Insulin Secretion *In Vitro* and *In Vivo*. *Food and Nutrition-Current Research*, 2018; 1(1): 29-37.
40. Matthias A. Immunomodulatory Effects of Phyllanthus muellerianus: A Mechanistic Approach. *Journal of Clinical & Cellular Immunology*, 2018; 9(5): 565.
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.
42. Ndjonka D, Agyare C, Luersen K, Hensel A, Liebau E. In vitro Anti-leishmanial Activity of Traditional Medicinal Plants from Cameroon and Ghana. *International Journal of Pharmacology*, 2010; 6(6): 865-871
43. Odugbemi T. A Textbook of Medicinal Plants from Nigeria. *University of Lagos Press, Akoka, Yaba-Lagos, Nigeria*, 2008; 73.
44. Ofokansi KC, Attama AA, Uzor PF, Ovri MO Antibacterial Activities of the Combined Leaf Extract of *Phyllanthus muellerianus* and Ciprofloxacin against Urogenital Isolates of *Staphylococcus aureus*. *Clinic Pharmacology and Biopharmaceutics*, 2012; 1: 106. doi:10.4172/2167-065X.1000106
45. Onocha PA, Alli MS. Antileishmaniasis, phytotoxicity and cytotoxicity of Nigerian Euphorbiaceous Plants 2: *Phyllanthus amarus* and *Phyllanthus muellerianus* Extracts. *African Scientist*, 2010; 11(2): 79-83.
46. Onyedum CC, Alobu I, Ukwaja KN Prevalence of drug-resistant tuberculosis in Nigeria: A systematic review and meta-analysis. *PLoS ONE*, 2017; 12(7): e0180996. https://doi.org/10.1371/journal.pone.0180996
47. Palanisamy GS, Kirk NM, Ackart DF, Shanley CA, Orme IM, Basaraba RJ. Evidence for Oxidative Stress and Defective Antioxidant Response in Guinea Pigs with Tuberculosis. *PLoS ONE*, 2011; 6(10): e26254. https://doi.org/10.1371/journal.pone.0026254
48. Rajab MS, Cantrell CL, Franziblau SG, Fischer NH. Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study. *Planta Medica*, 1998; 64(1): 2-4.
49. Rajopadhye SH, Mukherjee SR, Chowdhary AS, Dandekar SP. Oxidative Stress Markers in Tuberculosis and HIV/TB Co-Infection. *Journal of Clinical and Diagnostic Research: JCDR*, 2017; *11*(8): BC24–BC28. doi:10.7860/JCDR/2017/28478.10473
50. Riaz A, Rasul A, Hussain G et al., Astragalin: A Bioactive Phytochemical with Potential Therapeutic Activities. *Advances in Pharmacological Sciences*, 2018, 9794625, 2018; 15.
51. Salami AK, Oluboye PO. Hospital prevalence of pulmonary tuberculosis and co-infection with Human-Immuno Deficiency virus in Ilorin: a review of nine years (1991-1999). *West African Journal of Medicine*, 2002; 4: 24-27
52. Saleem M , Nazir M, Akhtar N, Onocha PA, Riaz N, Jabba A. New Phthalates from Phyllanthus muellerianus (Euphorbiaceae). *Journal of Asian Natural Products Research*, 2009; 11(11): 9747. doi: 10.1080/10286020903341388-.
53. Saludes JP, Franzblau SG, Garson MJ, Aguinaldo A. Antitubercular constituents from hexane fraction of *Morinda citrifolia* Linn (Rubiaceae). *Phytotherapy Research*, 2002; 16(7): 683-5. DOI: 10.1002/ptr.1003.
54. Santos CC, Salvadori MS, Mota VG *et al*., Antinociceptive and Antioxidant Activities of Phytol In Vivo and In Vitro Models. *Neuroscience Journal*, 2013; 2013: 949452.
55. Sarkar S, Ganguly A, Sunwoo HH Current Overview of Anti-Tuberculosis Drugs: Metabolism and Toxicities. *Mycobacterial Diseases*, 2016; 6: 209. doi:10.4172/2161-1068.1000209
56. Silva RO, Sousa FB, Damasceno SR, Carvalho NS *et al*., Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. *Fundamental & Clinical Pharmacology*, 2014; 28(4): 455-464. doi: 10.1111/fcp.12049
57. Sriram S, Patel MA, Patel KV, Punjani HN. *Compendium on Medicinal Plants.* Ahmedabad: GAU, 2004.
58. Sudha T, Chidambarampillai S, Mohan VR. GC-MS analysis of bioactive components of aerial parts of *Fluggea leucopyrus* Willd, (Euphorbiaceae). *Journal of Applied Pharmaceutical Science*, 2013; 3(5): 126-130. DOI: 10.7374/JAPS.2013.3524
59. Sujayil TK, Dhanaraj TS. Determination of bioactive compounds Evolvulus alsinoides leaf extract using GC-MS technique. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, 2016: 31-38.
60. Szczepiorkowski, Z. M., G. R. Dickersin, and M. Laposata. Incorporation of fatty acid ethyl esters into human hepatoblastoma cells results in decreased cell proliferation and protein synthesis. Gasteroenterology, 1994; 108: 512-522
61. Tan MA, Takayama H, Aimi N, Kutajima M, Franzblau SG, Nonato MG. Antitubercular triterpenes and phytosterols from *Pandanus tectorius* Soland. Var. laevis. *Journal of Natural Medicines*, 2008; 6(2): 232-235. doi: 10.1007/s11418-007-0218-8.

62. Tang Yin-Quan, Jaganath I, Manikam R, Sekaran SD. Phyllanthus Suppresses Prostate Cancer Cell, PC-3, Proliferation and Induces Apoptosis Through Multiple Signalling Pathways (MAPKs, P13K/AKE, NKκB and Hyposia. *Evidence-Based Complimentary and Alternative Medicine*, volume 2013, Article ID 609581, 2013; 13. http://dx.doi.org/10.1155/2013/609581
63. World Health Organization (WHO) (2008). Squalene-based adjuvants in vaccine. https://www.who.int/vaccine_safety/committee/topics/adjuvants/squalene/questions_and_answers/en/
64. Zhang L, Khoo CS, Koyyalamudi SR, de Pedro N, Reddy N. Immunostimulatory and Anticancer Activities of Polysaccharides Extracted from Traditional Anticancer Chinese Medicinal Herb. *Pharmacologia*, 2018; 9: 18-29. DOI: 10.3923/pharmacologia.2018.18.29