Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(12):830-833

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Anti-tubercular activity of some bioactive compounds from *Laggera pterodonta* (Asteraceae) (DC.) Sch. BIP.

H. O. Egharevba[1*], P. Oladosu[2], K. S. Izebe[2], S. K. Okwute[3] and J. I. Okogun[4]

[1]*Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria*
[2]*Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria*
[3]*Department of Chemistry, University of Abuja, Gwagwalada, Abuja, FCT, Nigeria*
[4]*Pax Herbal Nigeria Limited, Ewu, Edo State, Nigeria*

ABSRACT

The plant Laggera pterodonta is a well-known tropical plant used in ethnomedicine for malaria fever and cough related ailments. Previous report on preliminary investigation had shown that the crude extract had anti-tubercular activity. This study investigated the anti-tubercular activity of some compounds isolated and characterized from the plant. Two of the compounds, taraxasteryl acetate and ethane-1,2-dieicosanoate exhibited significant activity against locally isolated strains of M. tuberculosis with MICs of 691.48 and 269.23 μM, respectively. These compounds may be responsible for the observed activity of the crude plant extract, suggesting that the plant could be used to develop an anti-tuberculosis herbal medicine.

Keywords: *Laggera pterodonta*, taraxasteryl acetate, ethane-1,2-dieicosanoate, anti-tubercular activity

---

INTRODUCTION

The plant, *Laggera pterodonta* (DC.) Sch. Bip. (Asteraceae/Compositae), is a commonly growing shrub in most parts of the tropics, including the wet land of Nigeria. The plant had been reported to be used in folklore medicine in many parts of the world especially in Asia and Africa [1, 2]. Broad spectrum antimicrobial activity and significant anti-tubercular activity of the crude extracts of the plant had earlier been reported [1, 2].

Previous phytochemical screening of the plant extracts revealed the presence of terpenoids, sterols and glycosides. Several compounds, mostly terpenoids had been isolated from the plant by some Asian researchers [3]. Egharevba *et al*. also reported some known compounds from extracts of the plant including taraxasteryl acetate (0.16%), pterodondiol (0.55%), ethane-1,2-dieicosanoate (0.17%) and eicosanoic acid (0.13%) [4-6]. Taraxasteryl acetate was reported for the first time in the plant by Egharevba *et al*. This study aimed at establishing the anti-tubercular activity of the isolated bioactive compounds of the aerial part of the plant which may have been responsible for the observed activity of the crude extract.

EXPERIMENTAL SECTION

Materials

Reagents used were of analytical grade and as specified by manufacturers. The compounds taraxasteryl acetate, di-eicosanyl glycol or ethane-1,2-dieicosanoate, eicosanoic acid and pterodondiol were isolated and characterized as reported by Egharevba *et al*. [4-6]. Fisher's 0.22 µm membrane filters (cat: 09-720-004, Lot: R4NA18053 expiring in 2017), and isolate cultures of *Mycobacterium tuberculosis* from Tuberculosis Research Unit of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria were used as filters and test organisms, respectively.

Anti-TB screening:

Tetrazolium microplate assay (TEMA)

Clinical *M. tuberculosis* collected from Tuberculosis Research Unit of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria was confirmed by Ziehl-Neelsen stain, grown on niacin media and arylsulphatase test which was positive after 14 days. The characterized plant's compounds (0.2 g) were each separately dissolved in 1 mL of dimethylsuphoxide (DMSO), further diluted in 4 mL of sterile distilled water and filter-sterilized with 0.22 µm membrane filter to give concentration of 40 mg/mL stock solutions. Varied concentrations were prepared from the stock solution in double strength Middlebrook 7H9 broth to give known concentrations. 50 µL of 7H9 Middlebrook broth was transferred into well 2-12 of the microplate. 100 µL of extract solution was dispensed each into the first column of the 96 micro-well plate in triplicate from where 50 µL was transferred into well 2 mixed thoroughly and subsequently continued to well 11 from where 50 µL was discarded. This was followed by addition of 50 µL prepared test organisms using suspensions of *M. tuberculosis* prepared by emulsifying growth from slants with 100 µL of Tween 80 into 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). The turbidity was adjusted to McFarland standard no. 1 (approximately 3×10^7 CFU/mL) by adding Tween 80 and bovine serum albumin. For Mycobacterium, after 5 days incubation at 37°C, 50 µL of the Tetrazolium-Tween 80 solution was dispensed into few wells for colour change to indicate growth and no colour change if there was no growth of the *Mycobacterium*. The least concentration at which there was no growth of *Mycobacterium* was taken as the minimum inhibitory concentration (MIC). Rifampicin was used as the standard antibiotic. Organism viability, extract, media and solvent sterility control were also set up and carried out.

RESULTS AND DISCUSSION

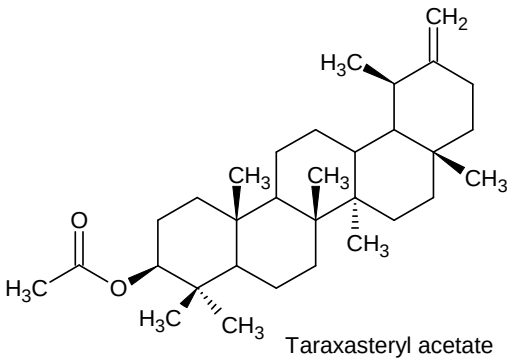
The result of anti-tubercular analysis is as shown in Table1. The result of screening of the four compounds showed activities for taraxasteryl acetate and di-eicosanyl glycol or ethane-1,2-dieicosanoate, while there was no activity for eicosanoic acid, pterodondiol (5βH,7βH,10α-epi-eudesmane-4α,11-diol or 5βH,7βH,10α-epi-cryptomeridiol) at the tested concentrations.

Table 1: Result of anti-microbial screening – Minimum inhibitory concentration (MIC)

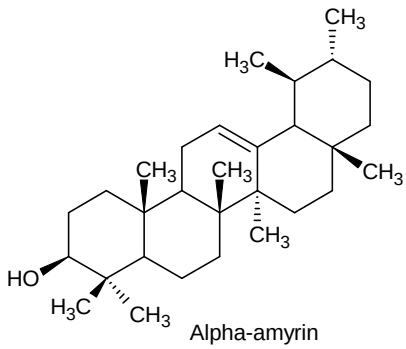
S/N	Tested Compounds	MIC (µM)
1	Taraxasteryl acetate	691.48±0.00
2	Di-eicosanyl glycol	269.23±0.00
3	Eicosanoic acid	NA
4	Pterodondiol	NA

MIC for Rifampicin was 109.36 µM; NA means no activity; MIC = mean ± SEM of triplicate study.

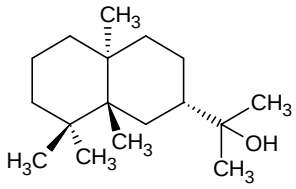
The MICs for taraxasteryl acetate and ethane-1,2-dieicosanoate activity were 691.48 µM and 269.23 µM, respectively. Rifampicin had an MIC of 109.36 µM, which is within the range (0.063 - 0.5 µg/mL i.e. 76.55 – 607.53 µM) reported by Ocheretina *et al.* for some strains of *M. tuberculosis* in Haiti [7]. Esters of taraxasterol and its analogues had been reported to exhibit several biological activities notable among which include anti-cancer, and antiviral activities [8-12.



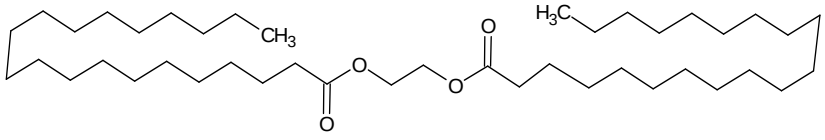
Taraxasteryl acetate



Alpha-amyrin



Pterodondiol



ethane-1,2-dieicosanoate

Taraxasterol is a pentacyclic triterpenoids of ursane family. The mode of antibacterial action has not been understood. However, according to Tenover, the modes of action of antibacterial may include interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, and inhibition of a metabolic pathway [13]. Inhibition of cell wall synthesis are mainly exhibited by the β-lactams, such as the penicillins, cephalosporins, carbapenems, monobactams and the glycopeptides, including vancomycin and teicoplanin [14, 15]. β-lactam agents act by interfering with the enzymes required for the synthesis of the peptidoglycan layer [15]. However, the glycopeptides interfere with cell wall synthesis by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis. The common antibacterial drug combination of trimethoprim, a folic acid analogue, plus sulfamethoxazole (a sulfonamide) inhibits two steps in the enzymatic pathway for bacterial folate synthesis. Disruption of bacterial membrane structure may be a fifth mechanism of action, although less well characterized. It is postulated that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents [16, 17].

Betulinic acid, a pentacylic triterpenoid and a potent antiphlogistic agent acting via inhibition of the enzymes in the arachidonic acid pathway had been well studied. Its anti-viral activity had been suspected to be through combination with the protein coat of the virus and thus hinder its binding to the cellular membrane of the host cell. The exact mode of action of betulinic acid and some other triterpenoid acids that inhibit HIV 1 replication is still unclear [18, 19]. However, it has been established that pentacyclic triterpenoids such as amyrins and ursolic acid and their glycosides, possess receptor binding activities and are able to bind with a number of specific binding sites. Stigmasterol glycoside, a steroidal pentacyclic tritepenoid had been demonstrated to bind to serotonin receptors [20]. Taraxasteryl derivatives may act in the same was as some of these pentacyclic triterpenoids. Its mode of action which may be through membrane permeability or receptor binding hindrance, DNA synthesis inhibition, etc., need to be properly investigated.

Taraxasteryl derivatives are structurally related to amyrin, an ursane analogue and may act the same way. This suggests that the compound could possess amyrin or ursane type activities. Eicosanoic acid derivative, ethane-1,2-dieicosanoate, which was 200% as active as taraxasteryl acetate, probably exert its effect through the glycol functional group which has been reported to possess antibacterial activity [21], and through membrane disruption by substituting cell membrane components leading to cellular leakage and death.

CONCLUSION

The study showed that the compounds taraxasteryl acetate and di-eicosanyl glycol or ethane-1,2-dieicosanoate may be partly responsible for the activity of crude extract against *Mycobacterium tuberculosis* which was earlier reported. These compounds may be acting independently or in synergy with other compounds to exert the observed biological effect. However, the mode of action of these compounds needs to be investigated and established in order to guide the drug discovery process. Since taraxasteryl derivatives are a structural analogue of amyrin, they may be involved in the biosynthetic pathways of other biologically active compounds such as avenacine, centellosides, glycyrrhizin or ginsenosides [22], and this may open up a new horizon of biosynthetic route and drug development.

REFERENCES

[1] HO Egharevba; MS Abdullahi; SK Okwute; JI Okogun, Bip. (Aerial Part). *Res.*, **2010,** 2(10), 35-40.
[2] HO Egharevba; O Peters; SE Okhale; I Iliya; OF Kunle; SE Okwute; JI Okogun, *J. Med. Plant Res.*, **2010**, 4(12), 1235-1237.
[3] XC Li; CH Huo; QW Shi; H Kiyota, *Chem. Biodivers.*, **2007**, 4, 105-111.
[4] HO Egharevba; SK Okwute; JI Okogun; J Igoli, *Nig. J. Chem. Res*., **2009**, 14, 8-16.
[5] HO Egharevba; SK Okwute; JI Okogun; J Igoli, *Int. J. Nat. Prod. Res.*, **2012**, 1(3), 45-53.
[6] HO Egharevba; SK Okwute, *Nat. Sci.*, **2014**, 12(1), 79-86.
[7] O Ocheretina; VE Escuyer VE; MM Mabou; G Royal-Mardi; S Collins; SC Vilbrun; JW Pape; DW Fitzgerald,. *PLoS ONE*, **2014** 9(3), e90569. doi:10.1371/journal.pone.0090569
[8] T Kuljanabhagavad; R Suttisri; T Pengsuparp; N Ruangrungsi, *J. Health Res*., **2009**, 23(4), 175-177.
[9] CY Ragasa; MJ Apuada; JA Rideout, *NRCP Res. J.*, **2009**, 10(1), 17-26.
[10] M Takasaki; T Konoshima; H Tokuda; K Masuda; Y Arai; K Shiojima; H Ageta, *Biol. Pharm. Bull*., **1999**, 22(6), 602-605.
[11] M Takasaki; T Konoshima; H Tokuda; K Masuda; Y Arai; K Shiojima; H Ageta, *Biol. Pharm. Bull*., **1999**, 22(6), 606-610.
[12] ML Villarreal; L Alvarez; D Alonso; V Navarro; P Garcia; G Delgado, *J. Ethnopharmacol*., **1994**, 42(1), 25-29.
[13] FC Tenover, *The Am J Med.*, **2006**, 119(6A), 3–10
[14] MC McManus, *Am. J. Health Syst. Pharm.* **1997**, 54, 1420-1433.
[15] HC Neu, *J. Sci.*, **1992**, 257, 1064-1073.
[16] DR Storm; KS Rosenthal; PE Swanson, *Annual Rev. Biochem.*, **1977**, 46, 723-763.
[17] J Patocka, *J. Appl. Biomed*., **2003**, 1, 7 – 12.
[18] MA Fernandez; B de las Heras; MD Garcia; MT Saenz; A Villar, *J. Pharm. Pharmacol.*, **2001**, 53, 1533–1539.
[19] C Ma; N Nakamura; H Miyashiro; M Hattori; K Shimotohno, *Chem. Pharm. Bull*. (Tokyo), **1999**, 47, 141–145.
[20] WB Mors; MC do Nascimento; BMR Pereira; NA Pereira, *Phytochem.*, **2000**, 55, 627-642.
[21] TM Nalawade; K Bhat; SH Sogi, *J. Int. Soc. Prevent. Communit. Dent.*, **2015**, 5, 114-119.
[22] L Hernandez-Vázquez; M Bonfil; E Moyano; RM Cusido; A Navarro-Ocaña; J Palazón, *Biotechnol. Lett.* **2010**, 32(2), 94-104.