Callistemon Citrinus (Curtis) Skeels as A Source of Antitubercular Compounds

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Research Article

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Abstract

Tuberculosis (TB) is still a principal cause of death in the world. There is considerable emphasis for search of novel drugs against TB due to increased Mycobacterium tuberculosis drug resistance and co infection with Human Immunodeficiency virus (HIV) that are challenging to treat. Callistemon citrinus (Curtis) skeels is one of the medicinal plants that are used in treatment of tuberculosis by local communities in Uganda. Though crude extracts from the plant have shown antimycobacterial activity in-vitro, the specific phytochemicals responsible for this activity are not known. The study aimed at isolating and characterizing compounds from C. citrinus and testing the compounds on different strains of M. tuberculosis.

Methods: Chromatographic and spectroscopic methods were used for isolation and characterization of pure compounds, while Microplate Alamar Blue Assay (MABA) was used to determine its efficacy on sensitive and resistant strains of M. tuberculosis.

Results: Betulinic acid, ursolic acid and epicatechin were isolated from C. citrinus. Epicatechin had minimum inhibitory concentration values of 3.4 µg/ml, 5.9 µg/ml and 3.9 µg/ml on the pan sensitive, rifampicin resistant and isoniazid resistant strains respectively. Betulinic acid and ursolic acid had MIC values of 29.3 µg/ml and 30.1 µg/ml respectively on the pan sensitive strain.

Conclusion: Our results substantiate the use of C. citrinus in traditional medicine for treatment of tuberculosis and this activity is related to presence of ursolic acid, betulinic acid and epicatechin.

Background

Tuberculosis (TB) is a highly virulent respiratory airborne disease caused by *Mycobacterium tuberculosis* [1].In the last decade, tuberculosis hasclaimed approximately twenty million people in the world and it is still the leading cause of death to date among infectious diseases[2].The high mortality of tuberculosis is aggravated by *M. tuberculosis*' capacity to genetically mutate thus becoming resistant to several formerly effective antibiotics. Tuberculosis that is resistant to Isoniazid and rifampicin, also known as Multi drug resistant TB (MDR TB) is characterized by mutations in the rpoB gene and katGor themabA-inhApromoter regions[3]while extensively drug resistant TB (XDR TB) that is resilient to both first and second line drugs[4]is in addition to the above mentioned mutations dominated with rrs and eis mutations[5].Treatment of *M. tuberculosis*drug resistant strains is problematic as it involves use of several toxicsecond line drugs for a stretched period of time resulting in cumulative adverse effects and non-adherence[6]. There is need for development of new therapies as one of the strategies toeliminate TB by the year 2050 in order to achieve the WHO sustainable development goals. New drugs such as bendaquiline and delamanid have been introduced in TB treatment and they have gone a long way in reducing MDR and XDRTB treatment duration[7]. However, there are fears of drug resistance to bedaquiline and delamanid. Studies have shown thatresistance to BDQ is acquired through a number of spontaneous chromosomal mutationsincluding; a BDQ target known as atpE, the MmpS5–MmpL5 efflux

pump (Rv0678 and Rv0677c), pepQ which encodes an Xaa-Pro aminopeptidase and mutations in the intergenic region between Rv0678 (efflux pump MmpL5) and Rv0677c (efflux pump MmpS5) that result in suppression of ATP synthase enzyme inactivity[8,9].There is thus a need for a continual investigation of leads that are active on resistant *M. tuberculosis*.

Plants still serve as a rich source of many novel biologically active compoundsyet very few have been thoroughly investigated. *Callistemon citrinus*(Curtis) Skeels,commonly known as crimson's bottle brush,is a plant species belonging tofamily Myrtaceae[10]. It is native to Australia however it is now spread all over the world. In addition to being used as an ornamental tree in African countries[11], *C. citrinus* is being used locally in management of cough and other cough related illnesses[12].InUganda, the plant is used locally in the treatment of tuberculosis[13, 14]. The efficacy of *C. citrinus*crude extracts on different strains of M. tuberculosis has been validated [15] however the phytochemicals responsible for this activity have not been investigated.In this study, we report isolation of phytochemicals from *C. citrinus* and investigation of their activity on different strains of *M. tuberculosis*.

Materials And Methods

Plant collection,extraction and fractionation

Leaves of *C. citrinus*(Curtis) Skeels(Mwambala butonya in the local luganda language) were collected from Luwero District Makulubita sub county. It is located 48km from Kampala City Centre Uganda at latitude 32.7431° N and altitude 0.72269 ° E. The district is dominated by savanna and tropical grasslands receiving an average of 1,300mmof rainfall and mean annual temperature of between 15 ° C and 17.5 ° C. These conditions are favorable for *C. Citrinus* survival.

Permission to collect the plant material was obtained through ethical approval from the Uganda national council of Science and Technology under reference number HS 1288. Additionally, to comply with the institutional, n**ational, and international guidelines and legislation for conservation of plant species, small amounts of leaves were harvested (detached) from several mature healthy plants to ensure sustainability.**A voucher specimen was prepared identification and achieved under reference number BL014 in theMakerere Univeristy Department of Botany herbarium.

Air dried powdered leavesof*C. citrinus*(2.5kg) were extracted with 1:1 dichloromethane and methanol (10L) for a period of 48hours with occasional shaking. After filtration, the extract was concentrated (219g) in vacuum. It was thenpartitioned between water and*n*-hexane and then ethyl acetate solvents. Theethyl acetate fraction (20.34g)was subjected to silica gel open column chromatography using a gradient system of n-hexane-EtOAc andEtOAc-MeOH solvent systems. Fractions of 250 mlwere collected.Fractions CCC 3, Was fractionated as an oil and it was analysed using GC MS. Repetitive column chromatography was done on fractions CCC 10, CCC 13 and CCC 18 to obtain the other pure compounds

General experimental procedures

Preparative and analyticalTLC were performed respectively using silica gel 60 $PF_{254+366}$ andsilica gel60-F_{254}(Merck) precoated aluminum sheets (Merck) and the spots were visualized by heating after spraying with vanillin.

The volatile fractions were analysed using Agilent gas chromatography equipment (7890A) equipped with a quadruple Agilent 5975C inert XL mass selective detector and fitted with a HP-5 MS column (30 m x 0.25 mm, 0.25 µm)with helium as the carrier gas. The GC oven temperature was initially set at 100°C for 4 min, raised to 235°C at a rate of 7ºC min^{-1}, then to 300°C at 12 °C min^{-1} and maintained at that temperature for 10 min. This gave a run time of 38.702 min. The temperature of the injector was 290 °C and column head pressure was 72.553 kPa. Samples (1 µL) were injected in splitless mode. The mass spectrometer was operated in electron-impact (EI) mode and a full-scan mode was selected for identification purposes.

NMR spectra (1D and 2D)were recorded on Bruker DMX Avance 300 MHZinstrument equipped with an auto-tune probe and using the automation mode aided by internal standards,deuterated chloroform and methanol as solvents.

Mycobacterial testing

Susceptibility testing of extracts was determined using the microplatealamar blue assay(MABA)[16]. Three preserved strains of *M.tuberculosis*were used; a rifampicin resistant strain (TMC 331/ATCC35838), an isoniazid resistant strain (TMC 301/ ATCC 35820) and a fully susceptible pan sensitive strain (H37Rv) as a positive control.*M. tuberculosis* was revived on Middle brook 7H10 agar (Becton Dickinson Company), which was prepared according to manufacturer's instructions. Cells were scraped from freshly growing colonies (two weeks old) and introduced into 7H9 broth (10ml) (Difco, Detroit, Mich) supplemented with 0.2% (v/v) glycerol and 10% (v/v) OADC. The inoculum was incubated at 37°C for 24 hours. McFarland standard 0.5 was used and it was prepared using a nephlometer.

Isoniazid (95.5% purity) was prepared in sterile water while theextracts, pure compounds and rifampicin (98.0% purity) were prepared in DMSO.The final concentrations tested for the drugs and isolated compounds ranged between 0.063-32.5 µg/m.Sterile distilled water (200µl) was added to all outer perimeter wells of 96-well plates to minimize evaporation. The remaining wells received 100µl of 7H9 broth. One hundred microliters of drug solutions were added to the wells in rows B to G in column 2 and two fold serial dilutions were made through column 10. The wells in 11 were drug free and these acted as negative control wells. The wells were inoculated with 100µl of *M. tuberculosis*bringing the final volume to 200 µl per well. The plates were incubated at 37°C for 24 hours. The tests were prepared in triplicate for each of the strains used.Thirty micro liters of a freshly prepared alamar blue (Accumed International, Westlake, Ohio) reagent was added to well B11 and incubated at 37°C for 24 hours. If it turned color, then the dye was added to the remaining wells. The minimum inhibitory concentration values (MIC) was defined as the lowest drug concentration values which prevented a color change from blue to pink.

Results And Discussion

Antimycobacterial activity of fractions

Nineteen fractions, from the ethyl acetate extract were collected and tested on *M. tuberculosis*. Sixteen fractions showed activity on both the pan sensitive strain and isoniazid resistant strains (Table 1). Fraction 18 was the most active on the pansensitive strain and the Isoniazid resistant strain with minimum inhibitory concentration values of 0.01mg/ml and 0.09mg/mlrespectively.Fraction 10 had MIC valueof 0.07mg/ml, 0.09mg/ml and 0.09 on H37Rv,TMC 301 and TMC331 respectively and was the most active on the rifampicin resistant strain.Only four of the fractions (3,10,13 and 18) had activity on the rifampicin resistant strainwith MIC values of 0.45, 0.09, 0.07 and 0.07mg/ml respectively.

Table1: Minimum inhibitory concentration values of fractions from *C. citrinus* against pan sensitive, isoniazid resistant and rifampicin resistant M. tuberculosis

Minimum inhibitory concentration values (mg/ml)			
Fraction	H37Rv	TMC 301	TMC331
1	>50	>50	na
2	0.12	1.17	>50
3	0.49	0.59	0.45
4	0.29	0.59	>50
5	0.78	0.09	>50
6	1.25	0.09	>50
7	0.15	0.24	>50
8	0.24	0.24	>50
9	>50	>50	>50
10	0.07	0.09	0.09
11	0.09	0.15	>50
12	0.78	1.18	>50
13	0.19	3.13	0.07
14	0.09	0.14	>50
15	0.24	0.09	>50
16	0.19	0.09	>50
17	>50	>50	>50
18	0.01	0.09	0.07
19	1.56	2.34	na

H37rv = pan sensitive strain, TMC 301= Isoniazid resistant strain, TMC 331= rifampicin resistant stain, >50 were considered in active.

Gas chromatography Mass Spectroscopy determination

From the oily fraction three, seven compounds were identified using GC-MS analysis (Table 2).Dodecanoic acid 1, 2, 3 propanenetriyl ester, hexadecane and octadecane were the most abundant with relative abundance of 38.2%, 18.5% and 16.1% respectively. Phthalic acid (1.8%), pentadecane (1.8%), aspidinol (9.1%)and tetradecane (14.5%)were also detected. The oily fraction mainly comprised of carboxylic acids and hydrocarbons.

Table 2: Compounds found from Gc Ms analysis of *Callistemon citrinus* fraction three

Compound name	Retention time (min)	Relative composition %	Molecular Formula	Molecular Weight	Compound nature
Tetradecane	10	14.5	$C_{14}H_{30}$	196.39	Long chain alkane
Phthalic acid	23.0	1.8	$C_8H_6O_4$	166.13	Carboxylic acid
Hexadecane	12.9	18.2	$C_{16}H_{34}$	226.44	hydrocarbon
Aspidinol	17.3	9.1	$C_{12}H_{16}O_4$	224.25	Carboxylic ester
Pentadecane, 7 methyl	15.4	1.8	C_6H_{34}	226.44	Hydrocarbon
Dodecanoic acid 1,2,3 propanenetriyl ester	29.1	38.2	$C_{39}H_{74}O_6$	639.00	Carboxylic ester
Octadecane	15.5	16.4	$C_{18}H_{38}$	254.5	hydrocarbon

Isolated compounds

Spectral data

Two pentacyclic triterpenoids including ursolic acid (**1**) and betulinic acid (**2**) plus a flavonoid epicatechin (**3**)(Fig 1) were isolated from the leaves of *C. citrinus.*Their proton and Carbon 13 spectral data is shown below.

Compound **1**white powder^{1}H NMR (300MHz, MeOH) δH (m, J in Hertz)0.87 (t, J=15Hz), 0.95(d, J=18Hz),2.17(d, J=9Hz),3.3brd,5.5(t, J=2.55Hz)

NMR (300MHz, MeOH) δC 38.4(CH_2), 27.8(CH_2), 78.3(CH), 38.4(C), 55.3(CH), 18.1(CH_2), 32.9(CH_2), 39.3(C), 48.3(CH), 36.7(C),22.9(C), 125.4(CH_2), 138.2(CH_2), 41.8(CH), 27.8(C), 22.9(CH_2), 48.0(C), 52.9(CH), 39.0(CH), 38.4(C), 30.3(CH_2), 36.7(CH_2), 27.8(CH_3), 14.9(CH_3), 16.4(CH_3), 18.0(CH_3), 22.9(CH_3), 180.1(C), 18.0(CH_3), 27.3(CH_3)ESI-MS ($[M]^+$ at *m/z* 456.4) Calcd for$C_{30}H_{48}O$

Compound **2** white amorphous powder^{1}H NMR (300MHz, $CDCl_3$) δH(m, J in Hertz)4.428(d J=4.5Hz), 2.798 (d,J=3.6Hz),1.23(t, J=10.2), 1.05s, 1.05s, 0.94s, 1.05s, 1.23s, 0.94s, 1.0s.

NMR (300MHz, cdcl3) δC 38.2(CH_2), 27.2(CH_2), 82.4(CH), 38.22(C) 54.52(CH), 34.3(CH_2), 40.9(C) 50.9(CH), 22.1(CH), 24.7(CH_2), 38.2(CH_2), 42.4(CH), 30.6(C), 31.5(CH_2), 54.5(C), 46.2(CH), 50.9(CH),

150.4(C), 29.4(CH_2), 34.0(CH_2), 29.4(CH_3), 27.9(CH_3),15.3(CH_3), 19.5(CH_3) 19.5(CH_3), 182.8(C), 113.1(CH_3), 19.5(CH_3) ESI-MS ($[M]^+$ at *m/z* 438) Calcd for $C_{30}H_{50}O$

Compound **3** yellow solid ^1H NMR (300MHz, $CDCl_3$) δH(m, J in Hertz);4.7(br), 2.83(d, J=4.8Hz), 2.68(dd, 19.8Hz), 5.85(t, J=9Hz), 6.9(s), 6.72(dd, J=14.7)

NMR (300MHz, cdcl3) δC 80.2(CH), 67.62(CH), 29.38(CH), 158.1(C), 96.6(CH), 157.7(C), 96.61(CH), 157.4(C), 100.3(C), 132.4(CH), 115.5(CH), 145.9(CH), 145.8(CH), 115.5(CH),119.6(C)ESI-MS ($[M]^+$ at *m/z* 290.5) Calcd forC_{15} H_{14} O_6

Anti-mycobacterial activity of compounds

Isolated compounds (**1**-**3**) were tested on both pan sensitive and resistant strains of *M. tuberculosis*using MABA. Among these, epicatechin (**3**) was found to be active on all the strains of *M.tuberculosis.*It had minimum inhibitory concentration values of 3.4µg/ml, 5.9µg/ml and 3.9µg/ml on the pan sensitive, rifampicin resistant and isoniazid resistant strains respectively (Table 3).

Table 3: Minimum Inhibitory Concentration values ofthe ursolic acid, betulinic acid and epicatechin on pan

sensitive, rifampicin resistant and isoniazid resistant *M. tuberculosis*

Compound	Minimum inhibitory concentration values (µg/ml)		
	Pan sensitive strain ($H_{37}Rv$)	Rifampicin resistant strain (TMC 331)	Isoniazid resistant strain (TMC 301)
Ursolic acid (1)	30.1± 1.9	>32.5	>32.5
Betulinic acid (2)	29.3±2.0	>32.5	>32.5
Epicatechin (3)	3.4± 0.8	5.9±3.5	3.9±1.8
Isoniazid	2.0±0.0	4.0±2.5	>32.5
Rifampicin	4.0±0.0	-	2.0±0.0

Discussion

Tuberculosis treatment has become complex due to development of new resistant M. tuberculosis strains to cheap easily available and effective first line and second line anti TB drugs. Efforts are underway to have an arsenal of drugs to tackle the new strains and for the first time in about fifty years, new strides

have been made in anti TB drug development with the introduction of delamanid and bedaquinine novel agents in treatment of drug resistant strains of *M. tuberculosis.*Delamanid and bedaquininecontaining regimens have gone a long way to dramatically shorten the treatment duration of MDR and XDR TB from the original 24 months to between 9-12 months. However,there are increasing fears of drug resistance to these novel drugs as well [17] 26. Also, there is still need to reduce the treatment period further and minimize toxic effects.

In this study, we isolated and tested the antimycobacterial activity of fractions and compounds from *Callistemon citrinus*. We isolated three compounds from *C. citrinus* leaves namely; epicatechin, betulinic acid and ursolic acid. Betulinic acid and Ursolic acid have previously been isolated from a number of plant species including*Vitex negundo* Linn,Alnus incana, Curtisia *dentate*but typically from theBetulaceae family, particularly *Betula alba* [*18,19,20,21,22].*The NMR data (^{1}H and ^{13}C) reported by these previous studieswas compared to ours to confirm the compound structures reported here. Epicatechin has also been previously isolated from*Trichilia emetica* whole Seeds**,** *Boerhaavia erecta* L. leaves and*Pterocarpus marsupium* however it has not been previously reportedinC. citrinus[23,24,25].

Epicatechin was active on both susceptible and single drug resistant strains of *M. tuberculosis.M*ycobacterium *tuberculosis* is characterized by a unique highly lipophilic cell wall possessing mycolic acids that have up to C90 α alkyl and β hydroxyl long chain fatty acids that provide a highly impermeable barrier for most agents thus prolonging the treatment period [26]. The high lipophilicity of epicatechin could have provided it an advantage agnaist *M. tuberculosis* however more studies are needed to verify its mechanism of action. Epicatechin has also been reported to; enhance the antibacterial activity of B lactams on methicillin resistant *staphylococcus aureus* [27], have neuroprotective activity[28] and antihypertensive activity [29] however its antitubercular activity is reported here for the first time.

Ursolic acid (**1**) and betulinic acid (**2**) showed a low activity on the pan sensitive strain with minimum inhibitory concentration values of 29.3 µg/ml and 30.1 µg/ml respectively. This is in agreement with previous studies [30]. However, a study done byFadipe et al [21], reported that modification of ursolic acid and betulinic acid to3-O-acetyl-Ursolic acid and 3-O-acetyl- betulinic acid respectively, lowered the MIC values to 3.4 µg/ml and 19.8 µg/ml respectively, indicating that there is potential for further development of plant derived compounds into better leads that can be developed into new drugs.Earlier studies have also shown that *M. tuberculosis* is susceptible to several weak acids owing to their ability to disrupt membrane potential and function[31]. This could possibly further explain the anti TBactivity of betulinic acid, ursolic acidand fraction 3 agnaist *M. tuberculosis.*

Conclusions

In conclusion, bioassay guided isolation of *C. citrinus* yielded threebioactive compounds including; epicatechin, betulinic acid and ursolic acid. In spite of the fact that these compounds have been isolated earlier from other plants, their activity on resistant strains of *M. tuberculosis* had not been investigated.

Epicatechin demonstrated promising activity on all the tested strains; if further modified could be a lead compound in the development of novel drugs against tuberculosis.The results of this study further emphasize that bioassay guided isolation though cumbersome is still valuable in the process of drug development.

Declarations

*Ethical approval*and consent to participate

The research was approved by the Uganda National council of Science and Technology (HS 1288) and also by theMakerere University, College of Health sciences higher degrees committee. This study was carried out mainly as an experimental study that did not involve any human participants therefore there was no need for seeking consent.

Consent for publication

This was mainly an experimental study which did not need consent for publication.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no competing interests.

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Authors' contributions

BL collected the plants, was involved in the preparation of the extracts and bio-assay testing of crude extract, fractions and isolated compounds and NMR and MS analysis. GWF was involved in the isolation, NMR and MS analysis as well as the structural elucidation of the isolated compounds and writing of the manuscript. SOY and PW did the conception, sought for funding, supervised the work and corrected the final manuscript. All authors read the manuscript, contributed in correcting it, and approved its final version

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Abbreviations

TB: Tuberculosis, MDR: Multi drug resistance, XDR Extensive drug resistance, HIV: Human immune Virus, AIDS: Acquired immune deficiency syndrome, WHO: World Health Organisation, JCRC: Joint Clinical research centre, NMR: Nuclear Magnetic resonance, MABA Micro plate alamar blue assay MIC: Minimum inhibitory concentration, TLC: Thin layer chromatography.

References

1. Miggiano R, Rizzi M, Ferraris D M. Mycobacterium tuberculosis pathogenesis, infectionprevention and treatment pathogens. 2020; 9: 385;doi:10.3390/pathogens905038
2. World Health Organization.Global Tuberculosis Report (2020). World Health Organization: Geneva, Switzerland, 2020
3. Rosales-Klintz S, Jureen P, Zalutskayae A, Skrahina A, XuB, Hu Y, Pineda-Garcia L, Merza MA, Muntean I, Bwaga F, Joloba M, Hoffner SE, Drug resistance-related mutations in multidrug-resistant Mycobacterium tuberculosis isolates from diverse geographical regions, Int J Mycobacteriol.2012; 1; 124-130, https://doi.org/10.1016/j.ijmyco.2012.08.001
4. Bahizi, G., Majwala, R.K., Kisaka, S. Nyombi A, Musisi K, Kwesiga B, Bulage L, RiolexusA A, Turyahabwe S. Epidemiological profile of patients with rifampicin-resistant tuberculosis: an analysis of the Uganda National Tuberculosis Reference Laboratory Surveillance Data, 2014–2018. Antimicrob Resist Infect Control 2021;10: https://doi.org/10.1186/s13756-021-00947-2
5. Liu Q, Yang D, Qiu B, Martinez L, Ji Y, Song H, Wang J.Drug resistance gene mutations and treatment outcomes in MDR-TB: A prospective study in Eastern China. PLoS Negl Trop Dis 2021; 15(1)https://doi.org/10.1371/journal.pntd.000906
6. Zhang, MW., Zhou, L., Zhang, Y,Chen B, Peng Y, Wang F, Liu Z, Wang Xand Chen STreatment outcomes of patients with multidrug and extensively drug-resistant tuberculosis in Zhejiang, China. Eur J Med Res. 2021; 26:31. https://doi.org/10.1186/s40001-021-00502-0
7. Riccardi N, Del Puente F, Magnè F, Taramasso L, Di Biagio A. Bedaquiline: A New Hope for Shorter and Better Anti-Tuberculosis Regimens. Recent Pat Antiinfect Drug Discov. 2018;13(1):3-11. doi: 10.2174/1574891X12666170619101904. PMID: 28625141.

8. Ghajavand H, Kamakoli MK, Khanipour S, Dizaji SP, Masoumi M, Jamnani FR, et al. High prevalence of bedaquiline resistance in treatment-naïve tuberculosis patients and verapamil effectiveness. Antimicrob Agents Chemother 2019;63: e02530-18.
9. Nguyen TVA, Anthony RM, Bañuls AL, Nguyen TVA, Vu DH, Alffenaar JWC.Bedaquiline resistance: its emergence, mechanism, and prevention. ClinDis 2017;66:1625–30.
10. Goyal PK, Jain R, Jain S, Sharma A. A Review on biological and phytochemical investigation of plant genus Callistimon Asian Pac. J. Trop. 2012; S1906-S1909
11. Sutar N, Sutar R, Kumar M. *Callistemon citrinus* (bottle brush) an important medicinal plant: a review of its traditional uses, phytoconstituents and pharmacological properties. Indian Research Journal of Pharmacy and Science.2014; 1: 69-77
12. Tugume P, Kakudidi EK, Buyinza M, Namaalwa J, Kamatenesi M, Mucunguzi P, Kalema J. Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. Ethnobiol. Ethnomed.2016;12: 1-28
13. Bunalema L, Obakiro S, Waako p, Tabuti JRS. Knowledge on plants used locally for TB treatment in Uganda. J Ethnopharmacol. 2014; 151: 999-1004
14. Tabuti JRS, Kukunda CB, Waako P. Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda. J Ethnopharmacol 2010; 127: 130–136
15. Bunalema L, Tabuti JRS, Sekagya Y, Ogwang S, Waako P, Anti-tubercular activity of Callistemon citrinus and Piptadenistrum africanum on resistant strains of Mycobacterium tuberculosis using micro plate alamar blue assay. Spatula DD.2015; 5: 235-240
16. Franzblau SG. Witzig RS, McLaughlin JC. Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate alamar blue assay. J of Clin Microbiol, 1998; 36:362– 366
17. Polsfuss S, Hofmann-Thiel S, Merker M, Krieger D, Niemann S, Rüssmann H, Schönfeld N, Hoffmann H, Kranzer K. Emergence of low-level delamanid and bedaquiline resistance during extremely drug-resistant tuberculosis treatment. Clin Infect Dis. 2019; 69(7):1229-1231.
18. Dehelean CA, Şoica C, Ledeţi I, Aluaş M, Zupko I, Gǎluşcan A, Cinta-Pinzaru S, Munteanu M. Study of the betulin enriched birch bark extracts effects on human carcinoma cells and ear inflammation. Chem Cent J 2012; 6:137.
19. Ladda PL, Magdum CS. Antitubercular activity and isolation of chemical constituents from plant Vitex negundo Linn. Iran J Pharm Res. 2018;17(4):1353-1360.
20. Li H, Webster D, Johnson JA, Gray CA. Anti-mycobacterial triterpenes from the Canadian medicinal plant Alnus incana. J Ethnopharmacol. 2015; 165:148-51. doi: 10.1016/j.jep.2015.02.042
21. Fadipe VO, Mongalo NI, Opoku AR, Dikhoba PM and Makhafola TJ. Isolation of anti-mycobacterial compounds from Curtisia dentata (Burm.f.) C.A.Sm (Curtisiaceae). BMC Complement Altern Med. 2017; 17: 306. https://doi.org/10.1186/s12906-017-1818-9

22. Ayatollahi AM, Ghanadian M, Afsharypour S, Abdella OM, Mirzai M, Askari G. Pentacyclic triterpenes in *Euphorbia microsciadia* with their T-cell proliferation activity. Iranian Journal of Pharmaceutical Research. 2011;10 (2): 287-294
23. Petrus AJA, Hemalatha SS, Suguna G. Isolation and characterisation of the antioxidant phenolic metabolites of Boerhaavia erecta L. leaves. J Pharma Sci and Res. 2012; 4: 1856 – 1861
24. Usman A, Thoss V, Nur-e-Alam M. Isolation of (-) - Epicatechin from Trichilia emetica Whole Seeds. Org Chem. 2016; 6(3): 81-85
25. Chakravarthy BK, Gode KD. Isolation of (-)-Epicatechin from Pterocarpus marsupium and its Pharmacological Actions. Planta Med. 1985;51(1):56-9. doi: 10.1055/s-2007-969393.
26. Maitra A, Munshi T, Healy J, Martin LT, Vollmer W, Keep NH, Bhakta S. Cell wall peptidoglycan in Mycobacterium tuberculosis: An Achilles' heel for the TB-causing pathogen, FEMS. Microbiol Rev, 2019; 43:548–575,
27. Qin R, Xiao K, Li B, Jiang W, Peng W, Zheng J, Zhou H. The combination of catechin and epicatechin callate from Fructus Crataegi potentiates beta-lactam antibiotics against methicillin-resistant staphylococcus aureus (MRSA) in vitro and in vivo. Int J Mol Sci. 2013;16;14(1):1802-21. doi: 10.3390/ijms14011802.
28. Nichols M, Zhang J, Polster BM, Elustondo PA, Thirumaran A, Pavlov EV, Robertson GS. Synergistic neuroprotection by epicatechin and quercetin: Activation of convergent mitochondrial signaling pathways. Neuroscience. 2015; 12:75-94. doi: 10.1016/j.neuroscience.2015.09.012.
29. Jackson D, Connolly K, Batacan R, Ryan K, Vella R, Fenning A. (-)-Epicatechin reduces blood pressure and improves left ventricular function and compliance in deoxycorticosterone acetate-salt hypertensive rats. Mol. 2018; 23(7):1511. doi:10.3390/molecules 23071511.
30. Cantrell CL, Franzblau SG, Fischer NH. Antimycobacterial plant terpenoids. Planta Medica. 2001; 67: 685–69
31. Zhang Y, Zhang H, Sun Z.Susceptibility of Mycobacterium tuberculosis to weak acids J Antimicrob Chem. 2003; 52: 56-60.

Figures

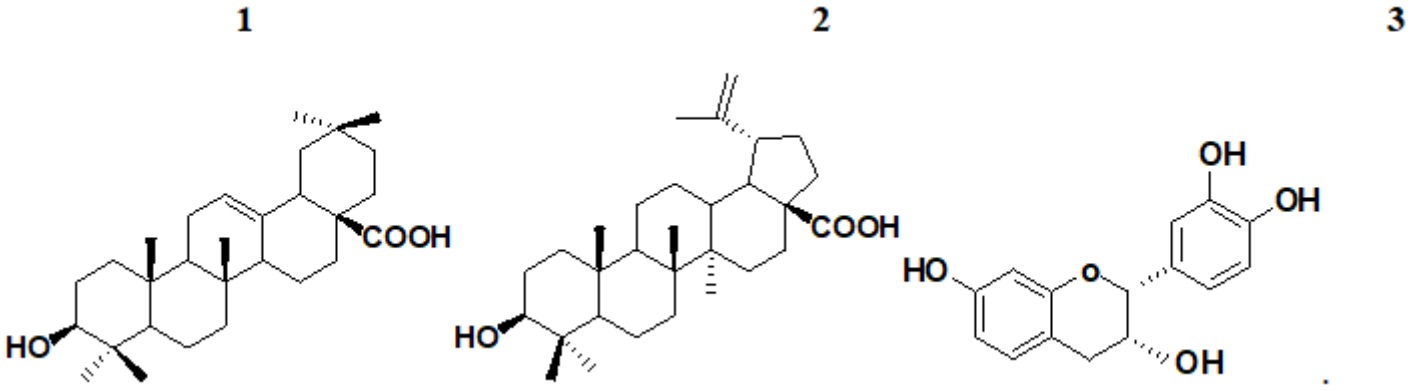

Figure 1

Chemical structures of compounds 1-3

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