http://doc.rero.ch

Published in "Natural Product Research 33(5): 642–650, 2019"
which should be cited to refer to this work.

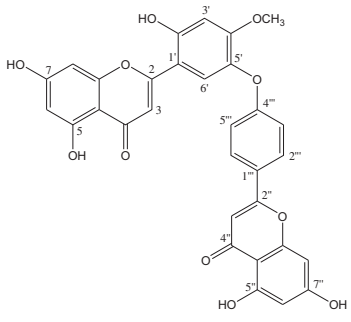
# Secondary metabolites from *Triclisia gilletii* (De Wild) Staner (Menispermaceae) with antimycobacterial activity against *Mycobacterium tuberculosis*

Eric Robert Tiam[a], Dominique Serge Ngono Bikobo[a,e], Auguste Abouem A Zintchem[a,b], Norbert Mbabi Nyemeck II[a,c], Esther Del Florence Moni Ndedi[d], Patrick Hervé Betote Diboué[d], Maximilienne Ascension Nyegue[d], Alex de Théodore Atchadé[a], Dieudonné Emmanuel Pegnyemb[a], Christian G. Bochet[e] and Ulrich Koert[c]

[a]Faculty of Science, Department of Organic Chemistry, University of Yaoundé I, Yaoundé, Cameroon; [b]Department of Chemistry, Higher Training College, University of Yaoundé I, Yaoundé, Cameroon; [c]Faculty of Chemistry, Philipps-Universität Marburg, Marburg, Germany; [d]Faculty of Science, Department of Microbiology, University of Yaoundé I, Yaoundé, Cameroon; [e]Department Chemie, Universität Fribourg, Fribourg, Switzerland

**ABSTRACT**

Triclisinone (**2**), a new ochnaflavone derivative, was isolated from the aerial parts of *Triclisia gilletii*, along with known drypemolundein B (**1**) and eight other known compounds. The chemical shifts of drypemolundein B (**1**) have been partially revised based on reinterpretation of NMR spectroscopic data. The eight other secondary metabolites are composed of: (+)-nonacosan-10-ol (**3**); stigmasterol (**4**), 3-*O*-β-D-glucopyranosylsitosterol (**5**), 3-*O*-β-D-glucopyranosylstigmasterol (**6**); oleanic acid (**7**); myricetin (**8**), quercetin (**9**) and 3-methoxyquercetin (**10**). Their structures were elucidated using IR, MS, NMR 1D and 2D, $^{1}H$ and $^{13}C$ and comparison with literature data. Furthermore, compounds **1**, **2**, **5, 6, 8, 9** and the crude extract were tested against *Mycobacterium tuberculosis*. Compounds **1, 2, 8** and **9** displayed moderate to very good activity against resistant strain (codified AC 45) of *M. tuberculosis* with minimum inhibitory concentrations MICs ranging from 3.90 to 62.5 µg/mL.




Triclisinone (2)

**KEYWORDS**

*Triclisia gelletii*; Menispermaceae; ochnaflavone derivative; NMR chemical shift analysis; antimycobacterial activity

**CONTACT** Dominique Serge Ngono Bikobo ngono_serge@yahoo.fr, sngono@uy1.uninet.cm

Supplemental data for this article

http://doc.rero.ch

## 1. Introduction

Despite the progress in tuberculosis (TB) diagnosis, treatment and prevention efforts, the epidemic remains one of the top 10 causes of the death worldwide, killing about 1.4 million people each year. In 2014, there were an estimated 10.4 million cases of tuberculosis with over 400 000 deaths resulting from TB disease among people living with HIV. The proportion of TB cases living with HIV was highest in the Africa Region where about 81% of people had been notified among men, women and children (WHO 2016). In 2015, there were an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100 000 people with rifampicin-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment. The aspiring objective of halving TB prevalence rates by 2015 as compared with the reference line of 1990 could not be completed in Africa (WHO 2014). Because of this increasing drug resistance incidence, there is an urgent need for new and effective anti-TB agents as an alternative to conventional drugs currently in use (Bergmann and Woods 1998). In this context, the search for new natural products from Cameroonian medicinal plants could provide new leads to antimycobacterial drugs.

The genus *Triclisia* comprises 20 species troughout the world with 12 in tropical Africa Region (Troupin et al. 1956). Plants belonging to that genus possess potential therapeutic values which have been used since very ancient times to cure various ailments and infectious diseases (Bouquet 1969; Disengomoka et al. 1983). *Triclisia gilletii* (Menispermaceae) is a robust creeper of up to 10 cm diameter, of the lowland dense rain-forest occurring from Liberia to Angola (Troupin et al. 1951, 1962). In some areas, the plant is used in the treatment of several ailments such as: malaria, venereal diseases, epileptic attacks, edema, anemia, diarrhea, stomach problems, leprosy, mental health problems, dysentery, respiratory diseases and convulsive coughing (Bouquet 1969; Kokwaro 1993; Burkill 1997; Neuwinger 1998; Mesia et al. 2008). According to the literature data, *Triclisia* species contains mainly bisbenzylisoquinoline (BBIQ) alkaloids (Schiff 1983; Murebwayire et al. 2006, 2009; Uche et al. 2017), morphinan alkaloids (Spiff et al. 1981) and other amide alkaloids (Murebwayire et al. 2006; Samita et al. 2017). Previous reports from *T. gilletii* revealed the presence of BBIQ (Tackie et al. 1973; Dwuma-Badu et al. 1975; Owusu et al. 1981). We herein report the isolation and elucidation of constituents present in the needles of *T. gilletii*. *In vitro* inhibitory activity of methanol extract and some isolated compounds was as well examined against a clinical isolate strain of *Mycobacterium tuberculosis* resistant to isoniazid and codified AC 45. Moreover, a few natural products like amide alkaloids, bisbenzylisoquinolines, pyrrolodiquinolines and vermelhotin were previously found to exhibit potent antimycobacterial activity against multidrug-resistant isolates of *M. tuberculosis* (Ganihigama et al. 2015; Sureram et al. 2012).

## 2. Results and discussion

The structural identifications of the compounds **3**, **4**, **5**, **6**, **7**, **8**, **9** and **10** were based on comparison of their physical and spectral data with authentic samples or those already reported (Figure 1). Their assignments were allowed as: (+)-nonacosan-10-ol (**3**) (Aarnoud et al. 2007), stigmasterol (**4**) (Budavari 1996), 3-O-*β*-D-glucopyranosylsitosterol (**5**) (Rubinstein et al. 1976), 3-O-*β*-D-glucopyranosylstigmasterol (**6**) (Ngono et al. 2014), oleanic acid (**7**)

http://doc.rero.ch

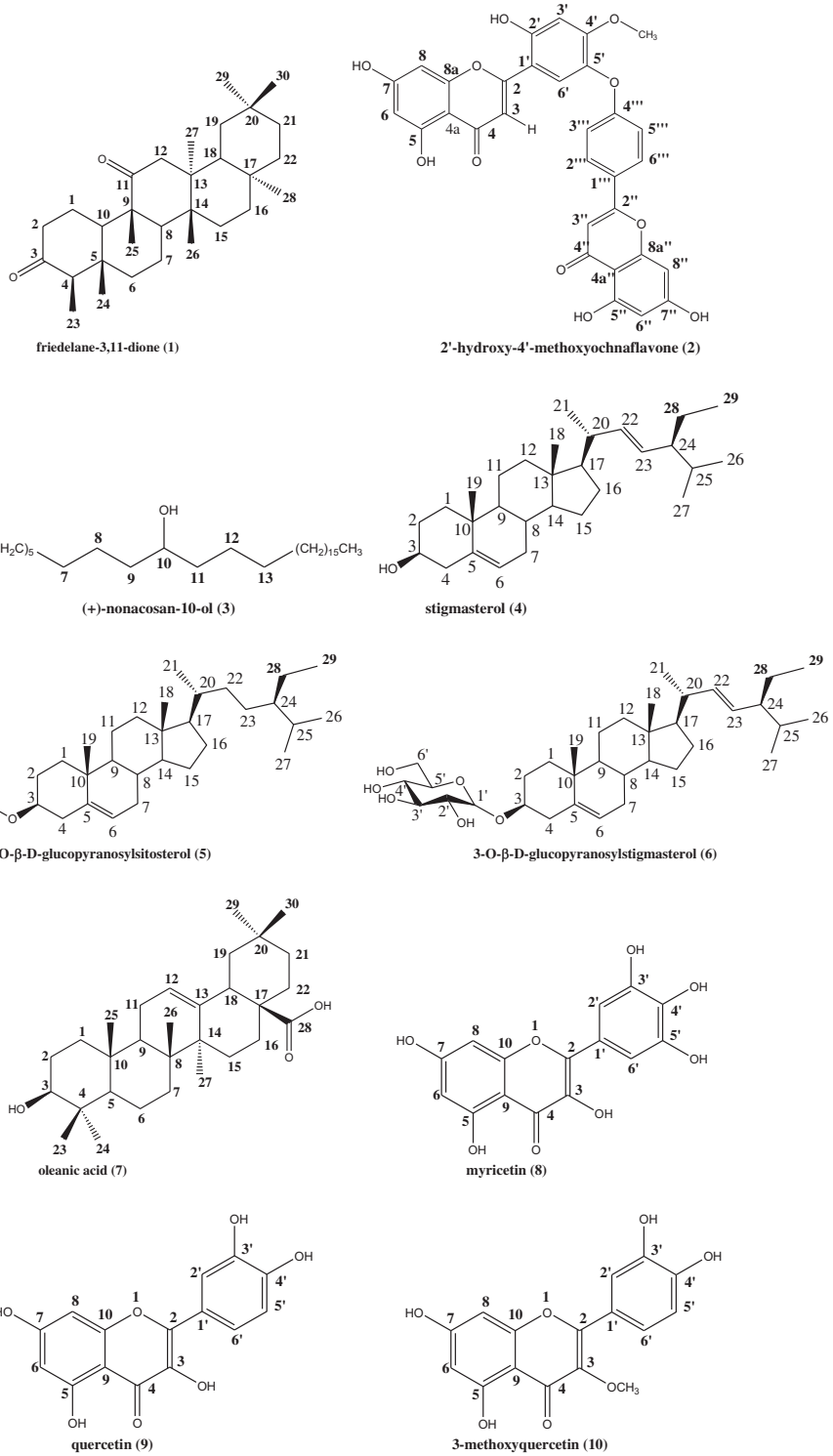



**Figure 1.** Compounds isolated from the leaves of *Triclisia gilletii.*

(Higuchi et al. 2008), myricetin (**8**) (Hinou et al. 1988), quercetin (**9**) (Güvenalp and Demirezer 2005) and 3-methoxyquercetin (**10**) (Peng et al. 2003).

### 2.1. Revision of compound 1

Compound **1** was isolated as a white solid from $CH_2Cl_2$/MeOH mixture, m.p. 281–283 °C. Its molecular formula was deduced to be $C_{30}H_{48}O_2$ (ESI-MS *m/z*: 439.4 $[M\text{-}H]^-$) which was confirmed by the LC-MS *m/z* 441 $[M + H]^+$. The IR spectrum showed absorption bands at $\upsilon_{max}$ 1718 and 1705 $cm^{-1}$, suggesting the presence and two carbonyl groups in **1**. This compound was previously isolated from the air-dried stems of *Drypetes molunduana*. The structure assignment appeared to be insufficiently detailed. This concern is highlighted by this study when the $^{13}$C-NMR data for the compound reported by Wandji et al. (2000) were compared to that reported for compound **1**.

As shown in Table S1, there was a significant mismatching of $^{13}$C-NMR shift values for drypemolundein B, with deviations of 4.1 ppm for the methine carbon (C-9) and 6.5 ppm for the nearest methine carbon (C-10). Consequently, the published values for drypemolundein B could not be accurate. Even though the 3 ppm gap between compounds analysed in the same solvent is considered acceptable (Garson et al. 2017), a re-evaluation of the $^{13}$C-NMR shift values suggested that the signals at 31.7 and 34.2 ppm, previously assigned to C-29 and C-30, respectively, were rather associated to C-30 and C-29 (Chiozem et al. 2009; Sun et al. 2009; Sousa et al. 2012 and from this study). Furthermore, NOESY correlations corroborated these revised chemical shift assignments in compound **1**, for example key NOESY correlations were observed between H-27 ($\delta_H$ 1.02) and methine at $\delta_H$ 1.41 and not 2.38 (H-8) as previously ascribed. However, no correlation was reportedly observed with H-24, H-25 and H-26 from H-28 and H-30. Therefore, it was speculated that D and E-rings of compound **1** adopted boat–boat conformation due to the lack of correlations between H-28 and H-30 (Figure S1), confirming the difference of stereochemistry of C-17 and other analogues (Chiozem et al. 2009; Sun et al. 2009; Sousa et al. 2012). These additional data have not been reported previously in the literature. We determined the structure of compound **1** to be friedelane-3,11-dione by comparing the NMR data to those from Wandji et al. (2000), so that the assignments of this compound were partially revised (Table S1).

### 2.2. Characterisation of compound 2

Compound **2** was isolated as a yellow amorphous powder from $CH_2Cl_2$/MeOH mixture, m.p. 289–291 °C. Its molecular formula was deduced to be $C_{31}H_{20}O_{11}$ (ESI-MS *m/z*: 567.2 $[M\text{-}H]^-$). The IR spectrum showed absorption bands $\upsilon_{max}$ at 3100, 1653, 1601, 1164 and 1028 $cm^{-1}$, compatible with the presence of hydroxyl group, conjugated carbonyl, aromatic ring, C–*O*–C stretching vibration and C–*O*–H vibration, respectively. The $^1$H-NMR spectrum of **2** exhibited two aromatic proton signals displaying an AB system at $\delta_H$ 6.16 (d, *J* = 2.0 Hz, H-6) and 6.48 (d, *J* = 2.0 Hz, H-8), and also two singlet aromatic proton signals (typical pattern of a 1,2,4,5-tetrasubtituted benzene) at $\delta_H$ 6.82 (s, H-3′) and $\delta_H$ 7.79 (s, H-6′). A flavone-type proton was observed at $\delta_H$ 7.14 (s, H-3) for this sub-structure, suggesting the presence of an isoetin moiety in this compound. Meanwhile, an AA′BB′ system was noticeable based on signals at $\delta_H$ 7.96 (d, *J* = 8.6 Hz, H-2‴/6‴) and 6.90 (d, *J* = 8.6 Hz, H-3‴/5‴); another AB system was deduced from aromatic proton signals at $\delta_H$ 6.33 (d, *J* = 2.0 Hz, H-6″) and $\delta_H$ 6.53 (d, *J* = 2.0 Hz,

http://doc.rero.ch

H-8′′) when a supplementary flavone proton at $\delta_H$ 6.87 (s, H-3) was observed (Table S2). We noted also the presence of one methoxy signal at $\delta_H$ 3.78 (3H, s, $OCH_3$). The $^{13}$C-NMR and DEPT 135 spectra showed 29 signals, consisting of 1 methoxy at $\delta_C$ 56.6, 10 methines including two carbon signals at $\delta_C$ 103.3 and $\delta_C$ 108.0, 18 quaternary carbons including two carbonyl groups at $\delta_C$ 182.2 and 182.1. The HMBC spectrum exhibited correlations between proton H-3′ ($\delta_H$ 6.82) and carbon atoms C-1′ ($\delta_C$ 109.5), C-2′ ($\delta_C$ 155.0), C-4′ ($\delta_C$ 157.3) and C-5′ ($\delta_C$ 133.4); another remarkable correlation was observed between the methoxy group $\delta_H$ 3.78 (*s*, $OCH_{3-4}$′) and upfielded carbon at $\delta_H$ 157.3 (C-4′). Else, referring to the position of the methoxy group, the analysis of NOESY spectrum indicates correlation between that group and the proton at $\delta_H$ 6.82 (*s*, H-3′), emphasising a close relationship of this B-ring with the one of ochnaflavone (Okigawa et al. 1973); moreover, compound **2**′s B-ring bears a supplementary hydroxyl group which induced the upshield field of carbon signals at $\delta_C$ 102.1 (C-3′) and $\delta_C$ 109.5 (C-1′). In addition, HMBC correlations between H-3 ($\delta_H$ 7.14) and carbon atoms C-1′ ($\delta_C$ 109.5), C-2 ($\delta_C$ 182.2) and C-4a ($\delta_C$ 104.7) are in accordance with the presence of a substituted apigenin with benzenetriol ring, i.e. isoetin (Figure S**2**). The other partial unit of compound **2** was determined using HMBC spectrum; supplement analysis enables correlations between: H-2′′′ ($\delta_H$ 7.96) and C-1′′′ ($\delta_C$ 122.6), C-3′′′ ($\delta_C$ 116.2), C-4′′′ ($\delta_C$ 157.6) and C-2′′ ($\delta_C$ 164.4), then H-3′′ ($\delta_H$ 6.87) with C-1′′′ ($\delta_C$ 122.6), C-4′′ ($\delta_C$ 182.1) and C-4a′′ ($\delta_C$ 103.5) (Table S2), confirming its nature as an apigenin moiety. The two flavonoid units could therefore be connected after analysis of the NOESY spectrum (Figure S2). The cross-peaks observed between H-3′′′ ($\delta_H$ 6.90) and $OCH_3$ ($\delta_H$ 3.78) as already mentioned and H-6′ ($\delta_H$ 7.79) and H-5′′′ ($\delta_H$ 6.90) enabled to locate the $C_5$′–*O*–$C_4$′′′ bond between isoetin and apigenin units, respectively, Additional data from the IR spectrum at $\upsilon_{max}$ 1028 and 1010 $cm^{-1}$ also confirmed the presence of a C–*O*–C bond in this molecule**.** Consequently, the structure of **2** was concluded to be 2′-hydroxy-4′-methoxyochnaflavone named triclisinone.

### *2.3. Evaluation of antimycobacterial activity*

From the antimycobacterial test results (Table S3), it appears that all the tested compounds exhibited good antitubercular activity with a Minimum Inhibitory Concentration (MIC) ranging from 3.90 to 62.5 µg/mL (Table S3). According to Cantrell et al. (2001), isolated compounds that exhibit a MIC of 64 µg/mL or lower are considered promising. For crude extracts, the MIC should be equal to or lower than 125 µg/mL (Gu et al. 2004). Among the isolated metabolites, compounds **1** and **8** were the most potent antitubercular agents possessing the MIC values of 3.90 and 7.81 µg/mL, respectively, more than the standard drug, Rifampicin (MIC value of 0.976 µg/mL) (Table S3). The methanol extract of *T. gilletii* showed poor inhibitory activity against *M. tuberculosis,* exhibiting a MIC and MBC of 1250 and 5000 µg/mL, respectively (Table S3), suggesting that there are other components within the extract which might have antimycobacterial effect. In addition, mycobacteria have a lipid-rich hydrophobic cell wall and are often susceptible to less polar compounds (Pauli et al. 2005). Flavonoids, bisbenzylisoquinoline, alkaloids, steroids and triterpenoids showed promising activity or can act as template for new antitubercular natural products (Okunade et al. 2004; Copp and Norrie Pearce 2007; Sureram et al. 2012; Erickson et al. 2014). For triterpenoids, the presence of polar substituents showed a decreased inhibitory potency for antimycobacterial effect; this is exemplified in compound **1** (MIC = 3.90 µg/mL, the least polar isolated compound tested) corroborating on the one hand Pauli's assertion and on another hand previous report

http://doc.rero.ch

on antitubercular triterpenes (Jiménez-Alleranes et al. 2007; Bamuamba et al. 2008) and/or other non-polar compounds which demonstrated higher lipophilicity (Lirio et al. 2014). For significant antitubercular activity concerning the flavonoid classes, this can be explained by the presence of polyhydroxyl substitutions which might produce remarkable improvements in antitubercular activity (Myricetin, **8**) (MIC = 7.81 µg/mL), whereas the presence of methyl exemplified by 2′-hydroxy-4′-methoxyochnaflavone (**2**) (MIC = 62.5 µg/mL) or glycosidic residues in these substitution patterns may led to decreased activity against the test organism (compound **2** was 8 times less active than **8**). The antimycobacterial activity detected for **1, 5, 6, 8** and **9** is in agreement to that previously reported (Erickson et al. 2014; Ebeh Messanga et al. 2017). According to Peterson and Shanholtzer (1992), bacteriostatic activity has been defined as a ratio of MBC to MIC of > 4. Thus, all tested compounds exhibited bactericidal activity.

### 2.4. Chemotaxonomy

To our knowledge, compound **1** is reported for the first time from *Triclisia* genus and it suggests that the genus can be considered as a potential source of triterpenes. Nevertheless, some triterpenoids have already been isolated within the Menispermaceae family, especially from *Tinospora crispa* (Kongkathip et al. 2002). This latter species is also a provider of flavonoids (Kalsom and Noor 1995) as well as *Cissampelos capensis* (Menispermaceae) (Babajide et al. 2015) which contains an analogue of quercetin (**8**), found in the present study from the *Triclisia* genus for the first time. Moreover, the appearance of compound **3** in this study is closely related to the molecular data from previous study in *Stephania abyssinica* (Menispermaceae) (Gautam et al. 2005). Even though some biflavonoids have already been described from *Stephania tetrandra* (Menispermaceae) (Si et al. 2001), this survey displays the first occurrence of this class of compounds within the *Triclisia* genus.

From this study, it is evident that the chemistry of *Cissampelos* and *Stephania* is more similar to *Triclisia* genus regarding the structures of compounds.

## 3. Experimental section (also in supplemental data)

### 3.1. Plant material

The leaves of *T. gilletii* were collected at Eséka (Koumoul) near Yaoundé in the Centre Region of Cameroon in March 2014 and identified by the botanist Mr Victor Nana. A voucher specimen (N° 64296 HCN) was deposited at the National Herbarium in Yaoundé, Cameroon.

### 3.2. Friedelan-3, 11-dione (1) $C_{30}H_{48}O_2$

White amorphous powder; m.p 281–283°; TLC $R_f$: 0.85 ($CH_2Cl_2$/MeOH; 25/1); $[\alpha]_D^{20} = -8.018°$ ($c = 0.05$, $CDCl_3$); IR (KBr) $cm^{-1}$: 2954, 2860, 1718, 1705, 1460, 1068; $^1$H- and $^{13}$C-NMR spectral data (500, 125 MHz, $CDCl_3$), see Table S1; ESI-MS *m/z*: 439.4 $[M-H]^-$; LC-MS *m/z* 441 $[M + H]^+$ (calcd for $C_{30}H_{48}O_2$, 439.38; 441, respectively).

http://doc.rero.ch

### 3.3. 2′-hydroxy-4′-methoxyochnaflavone (2) $C_{31}H_{20}O_{11}$

Yellow amorphous powder; m.p 289–291°; TLC $R_f$: 0.38 ($CH_2Cl_2$/MeOH; 10/1); IR (KBr) $cm^{-1}$: 3100, 2890, 1653, 1601, 1164, 1028, 1010; $^1$H- and $^{13}$C-NMR spectral data (500, 125 MHz, DMSO-$d_6$), see Table S2; ESI-MS *m/z*: 567.2 $[M-H]^-$ (calcd for $C_{31}H_{20}O_{11}$, 567.18).

## 4. Conclusion

The results from this study suggest that the bioactive principles present in the extract belong to triterpen and flavonoid skeletons such as friedelane 3,11-dione (**1**) and myricetin (**8**) and appear to be useful templates for the development of new anti-tuberculosis drugs.


## Acknowledgements

We thank Mr V. Nana for the collection and identification of the plant material. We thank Mr Felix Fehr, Department of Chemistry, University of Fribourg, and Koert's team of Philipps-Universität Marburg for spectral analysis.


## Disclosure statement

No potential conflict of interest was reported by the authors.


## Funding

The authors gratefully acknowledge financial support from the Swiss National Science Foundation (SNSF) [grant number IZK0Z2-157272] for research fellowships in Switzerland to D. S. Ngono Bikobo.


## References


Aarnoud D, Jennifer W, Kerstin K, Wilhelm B, Thomas K. 2007. Synthesis of nonacosan-10-ol, the major component of tubular plant wax crystals. Eur J Org Chem. 21:3508–3511.

Babajide JO, Mabusela WT, Green IR. 2015. Some alkaloids and flavonoids from *Cissampelos capensis*. J Med Plants Res. 9:16–29.

Bamuamba K, Gammon DW, Meyers P, Dijoux-Franca MG, Scott G. 2008. Anti-mycobacterial activity of five plants species used as traditional medicine in the western Cape Province (South Africa). J Ethnopharmacol. 117(2):385–390.

Bergmann JS, Woods GL. 1998. *In vitro* activity of antimicrobial combinations against clinical isolates of susceptible and resistant *Mycobacterium tuberculosis*. Int J Tuber Lung Dis. 2(8):621–626.

Bouquet A. 1969. Fetish and traditional medicine of Congo (Brazzaville). O.R.S.T.O.M. Paris. 36:177–178.

Budavari S. 1996. The merk index: an encyclopedia of chemicals, drugs and biologicals, 12th ed. White House Station: Merck & Co. Inc.; p. 694.

Burkill HM. 1997. The useful plants of West Tropical Africa. 2nd ed. vol 4. Richmond: Families M-R. Royal Botanic Gardens; p. 969.

Cantrell CL, Franzblau SG, Fischer NH. 2001. Antimycobacterial plant terpenoids. Planta Med. 67(8):685–694.

Chiozem DD, Van-Dufat HT, Wansi JD, Mbazoa Djama C, Fannang VS, Seguin E, Tillequin F, Wandji J. 2009. New friedelane triterpenoids with antimicrobial activity from the stems of *Drypetes paxii*. Chem Pharm Bull. 57(10):1119–1122.

Copp BR, Norrie Pearce A. 2007. Natural product growth inhibitors of *Mycobacterium tuberculosis*. Nat Prod Rep. 24:278–297.


http://doc.rero.ch

Disengomoka I, Delaveau P, Sengele K. 1983. Medicinal plants used for child's respiratory diseases in Zaire. Part 2. J Ethnopharmacol. 8:265–277.
Dwuma-Badu D, Ayim JSK, Tackie AN, Knapp JE, Slatkin DJ, Schiff PL Jr. 1975. Additional alkaloids of *Triclisia patens* and *Triclisia subcordata*. Phytochemitry. 14:2524–2545.
Ebeh Messanga R, Ngono Bikobo DS, Abouem àZintchem A, Mbabi Nyemeck II N, Moni Ndedi EDF, Betote Diboué P, Nyegue MA, Atchadé AT, Pegnyemb DE, Bochet CG, Koert U. 2017. Rauvolfianine, a new antimycobacterial glyceroglycolipid and other constituents from *Rauvolfia caffra*. Sond (Apocynaceae). Nat Prod Res. doi:10.1080/14786419.2017.1356832.
Erickson MP, Dietmar G, Karsten K, Scott GF, Allan PGM. 2014. Anti-tubercular flavonol derivatives from *Uvaria rufa*. Res J Pharm Biol Chem Sci. 5(6):856–859.
Ganihigama DU, Sureram S, Sangher S, Hongmanee P, Mahidol T, Mahidol C, Ruchirawat S, Kittakoop P. 2015. Antimycobacterial activity of natural products and synthetic agents: Pyrrolodiquinolines and vermelhotin as anti-tubercular leads against clinical multidrug resistant isolates of *Mycobacterium tuberculosis*. Eur J Med Chem. 89:1–12.
Garson MJ, Hehre W, Pierens GK, Suciati. 2017. Revision of the Structure of Acremine P from a Marine-Derived Strain of *Acremonium persicinum*. Molecules. 22:521–525.
Gautam LN, Awale S, Kalauni SK, Shreshta K, Gewaji MB. 2005. Phytochemical and biological studies on *Podocarpus neriifolius D. Don*: A Himalayan conifer of Nepal. Sci World. 3:22–24.
Gu JQ, Wang Y, Franzblau SG, Montenegro G, Yang D, Timmermann BN. 2004. Antitubercular constituents of *Valeriana laxiflora*. Planta Med. 70:509–514.
Güvenalp Z, Demirezer O. 2005. Flavonol glycosides from *Asperula arvensis* L. Turk J Chem. 29:163–169.
Higuchi CT, Sannomiya M, Roberto S, Sacramento LVS, Sato DN. 2008. Triterpenes and anti-tubercular activity of *Byrsonima crassa*. Quim Nova. 31(7):1719–1721.
Hinou J, Lakkas N, Philianos S. 1988. Les constituants polyphénoliques de *Myrtus communis* L. Med Plant Phytother. 22:98–103.
Jiménez-Alleranes A, Meckes M, Torres J, Herrera-Luna J. 2007. Antimycobacterial triterpenoids from *Lantana hispida* (Verbenaceae). J Ethnopharmacol. 111:202–205.
Kalsom YU, Noor H. 1995. Flavone *O*-glycosides from *Tinospora crispa*. Fitoterapia. 66(3):280.
Kokwaro JO. 1993. Medicinal plants of East Africa. 2nd ed. Nairobi: Kenya Literature Bureau; p. 401.
Kongkathip N, Dhumma-upakorn PD, Kongkathip B, Chawananoraset K, Sangchomkaeo P, Hatthakitpanichakul S. 2002. Study on cardiac contractility of cycloeucalenol and cycloeucalenone isolated from *Tinospora crispa*. J Ethnopharmacol. 83:95–99.
Lirio SB, Macabeo AP, Paragas EM, Knorn M, Kohls P, Franzblau SG, Wang Y, Aguinaldo MA. 2014. Antitubercular constituents from *Premna odorata Blanco*. J Ethnopharmacol. 154(2):471–474.
Mesia GK, Tona GL, Nanga TH, Cimanga RK, Apers S, Cos P, Maes L, Pieters L, Vlietinck AJ. 2008. Antiprotozoal and cytotoxic screening of 45 plant extracts from Democratic Republic of Congo. J Ethnopharmacol. 115(3):409–415.
Murebwayire S, Diallo B, Luhmer M, Vanhaelen-Fastré R, Vanhaelen M, Duez P. 2006. Alkaloids and amides from *Triclisia sacleuxii*. Fitoterapia. 77:615–617.
Murebwayire S, Ingkaninan K, Changwijit K, Frédérich M, Duez P. 2009. *Triclisia sacleuxii* (Menispermaceae), a potential source of acetylcholinesterase inhibitors. J Pharm Pharmacol. 61:103–107.
Neuwinger HD. 1998. Afrikanische Arzneipflanzen und Jagdgifte Chemie, Pharmakologie, Toxikologie. 2nd Ed. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH; p. 960.
Ngono Bikobo DS, Mosset P, Abouem A, Zintchem A, Atchadé AT, Balemaken Missi M, Mbabi Nyemeck II N, Pegnyemb DE. 2014-15. Campylospermine, an *N*-hydroxy alkaloid from the leaves *of Campylospermum densiflorum* (Ochnaceae). Int J Pharm Phytochem Res. 6:719–728.
Okigawa M, Kawano N, Aqil M, Rahman W. 1973. The structure of ochnaflavone, a new type of biflavone and the synthesis of its pentamethyl ether. Tetrahedron Lett. 14(22):2003–2006.
Okunade A, Elvin-Lewis MP, Lewis WH. 2004. Natural antimycobacterial metabolites: current status. Phytochemistry. 65:1017–1032.
Owusu PD, Slatkin DJ, Knapp JE, Schiff PLJ. 1981. Constituents of West African medicinal plants. 28. Additional alkaloids of *Triclisia gilletti*. J Nat Prod. 44:61–66.
Pauli GF, Case RJ, Inui T, Wang Y, Cho S, Fischer NH, Franzblau SG. 2005. New perspectives on natural products in TB drug research. Life Sci. 78:485–494.

http://doc.rero.ch

Peng ZF, Strack D, Baumert A, Subramaniam R, Goh NK, Chia TF, Tan SN, Chia LS. 2003. Antioxidant flavonoids from leaves of *Polygonum hydropiper L*. Phytochemistry. 62:219–228.
Peterson LR, Shanholtzer CJ. 1992. Tests for bactericidal effects of antibacterial agents: technical performance and clinical relevance. Clin Microbiol Rev. 5:420–432.
Rubinstein I, Goad LJ, Clague ADH, Mulheirn LJ. 1976. The 220 MHz NMR spectra of phytosterols. Phytochemistry. 15:195–200.
Samita F, Ochieng CO, Owuor PO, Manguro LOA, Midiwo JO. 2017. Isolation of a new β-carboline alkaloid from aerial parts of *Triclisia sacleuxii* and its antibacterial and cytotoxicity effects. Nat Prod Res. 31:529–536.
Schiff PLJ. 1983. Bisbenzylisoquinoline alkaloids. J Nat Prod. 46:1–43.
Si D, Zhong D, Sha Y, Li W. 2001. Two biflavonoids from the aerial part of *Stephania tetrandra*. Phytochemistry. 58:563–566.
Sousa GF, Duarte LP, Alcântara AFC, Silva GDF, Vieira-Filho SA, Silva RR, Oliveira DM, Takahashi JA. 2012. New Triterpenes from *Maytenus robusta*: Structural Elucidation Based on NMR Experimental Data and Theoretical Calculations. Molecules. 17:13439–13456.
Spiff AI, Zabel V, Watson WH, Zemaitis MA, Ateya AM, Slatkin DJ, Knapp JE, Schiff PLJ. 1981. Constituents of West African medicinal plants. XXX. Tridictyophylline, A new morphinan alkaloid from *Triclisia dictyophylla*. J Nat Prod. 44(2):160–165.
Sun CR, Hu HJ, Xu RS, Yang JH, Wan TH. 2009. A new friedelane type triterpene from *Euonymus hederaceus*. Molecules. 14:2650–2655.
Sureram S, Senadeera SPD, Hongmanee P, Mahidol C, Ruchirawat S, Kittakoop P. 2012. Antimycobacterial activity of bisbenzylisoquinoline alkaloids from *Tiliacora triandra* against multidrug-resistant isolates of *Mycobacterium tuberculosis*. Bioorg Med Chem Lett. 22(8):2902–2905.
Tackie AN, Dwuma-Badu D, Okarter T, Knapp JE, Slatkin DJ, Schiff PL. 1973. Trigilletine and tricordatine: two new bisbenzylisoquinoline alkaloids from *Triclisa* species. Phytochemistry. 12:2509–2511.
Troupin G. 1962. Monographie des Menispermaceae africaines. Mémoires in-8. Académie Royale des Sciences d'Outre-Mer, Classe des Sciences Naturelles et Médicales, Nouvelle série. 8(2):313.
Troupin G, Robyns W, Staner P, Demaret F, Germain R, Gilbert G, Hauman L, Homès M, Jurion F, Lebrun J, et al. (Editors). 1951. Menispermaceae. In: *Flore du Congo belge et du Ruanda-Urundi Spermatophytes*. Volume 2. Brussels, Belgium: Institut National pour l'Étude Agronomique du Congo belge; pp. 202–255.
Troupin G, Turrill WB, Milne-Redhead E. (Editors). 1956. Menispermaceae. In: Flora of Tropical East Africa. London: Crown Agents for Oversea Governments and Administrations; p 32.
Uche FI, Abed MN, Abdullah MI, Drijfhout FP, McCullagh J, Claridge TWD, Richardson A, Li WW. 2017. Isochondodendrine and 2'-norcocsuline: additional alkaloids from *Triclisia subcordata* induce cytotoxicity and apoptosis in ovarian cancer cell lines. RSC Adv. 7:44154–44161.
Wandji J, Wansi JD, Fuendjiep V, Dagne E, Mulholland DA, Tillequin F, Fomum ZT, Sondengam BL, Nkeh BC, Njamen D. 2000. Sesquiterpene lactone and friedelane derivative from *Drypetes molunduana*. Phytochemistry. 54:811–815.
WHO, 2014. Global tuberculosis report. Geneva: World Health Organization. [Accessed on 2015 December 15]. http://www.who.int/tb/publications/global_report/gtbr14_main_text.pdf.
WHO, 2016. Global tuberculosis report. Geneva: World Health Organization. [Accessed on 2017 July 3]. http://www.who.int/tb/publications/global_report/gtbr2016_main_text.pdf.

http://doc.rero.ch