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Polyacanthoside A, a new oleanane-type triterpenoid saponin with cytotoxic effects from the leaves of *Acacia polyacantha* (Fabaceae)

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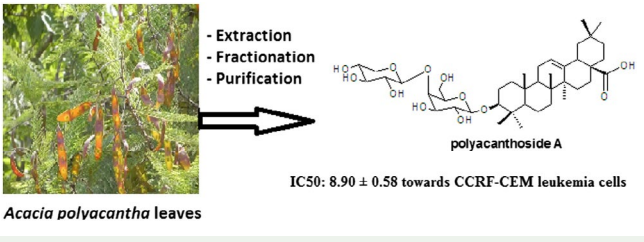
ABSTRACT

The chemical investigation of the leaves and stem bark of *Acacia polyacantha* (Fabaceae) led to the isolation of a new oleanane-type triterpenoid saponin named polyacanthoside A **1** together with fifteen known compounds. Their structures were established from spectral data, mainly HRESIMS, 1D NMR and 2D NMR and by comparison with literature data. The cytotoxicity of compound **1** and the analogues **8** as well as doxorubicin was determined in a panel of 9 cancer cell lines including sensitive and drug resistant phenotypes. Unlike the analogue **8,** compound **1** as well as doxorubicin displayed cytotoxic effects in all the 9 tested cancer cell lines with IC_{50} values ranged from 8.90 µM (towards CCRF-CEM leukemia cells) to 35.21 µM (towards HepG2 hepatocarcinoma cells) for compound **1** and from 0.02 µM (towards CCRF-CEM leukemia cells) to 66.83 µM (against CEM/ADR5000 leukemia cells) for doxorubicin.



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1. Introduction

Acacia species are used in folk medicine by rural population as a remedy for tuberculosis, leprosy, small pox, dysentery, cough, ophthalmic, toothache, skin ulcers, cancer, as astringents, antispasmodics, and aphrodisiac (Khidir 2009). *Acacia polyacantha* is one of the plants used by farmers to treat livestock diseases, such as Salmonellosis and gastrointestinal diseases in general (Koudoro et al. 2015**)**. The genus *Acacia* is known to be a rich source of triterpenoidal saponins (Youngwan et al. 2002; Jayatilake et al. 2003; Tchoukoua et al. 2017, 2018). Plants of this genus displayed a wide range of biological activities such as antibacterial, cytototoxic or enzyme inhibitory effects (Jæger et al. 2018). As part of our search of anti-cancer secondary metabolites from the Fabaceae family (Fotso et al. 2017) we have carried out the chemical investigation of the leaves and stem bark of *Acacia polyacantha*. The present work describes the isolation of a new oleane-type triterpenoid saponin from leaves of that plant together with the evaluation of the cytotoxicity of the new compound in a panel of 9 cancer cell lines including sensitive and drug resistant phenotypes.

2. Results and discussion

The intensive purification of the leaves extract of *A. polyacantha* resulted on the isolation of eight compounds among which a new oleanane-type triterpenoid saponin, named polyacanthoside A or 3-*O*-[*β*-D-xylopyranosyl-(1→4)-*β*-D-galactopyranosyl]-oleanolic acid (**1**) and seven known compounds including: oleanolic acid (**2**) (Kuete et al. 2007)**,** stigmasterol (**3)** (Chaturvedula and Prakash 2012)**,** stigmasterol-3-*O*-*β*-glucopyranosyl (**4)** (Freire et al. 2005)**,** epicatechin (**5)** (Adnyana et al. 2000)**,** quercetin-3-*O*-glucoside (**6)** (Xu et al. 2012)**,** 3-*O*-methyl-D-Chiro-inositol (**7)** (Sharma et al. 2016) and 3-*O*-[*β*-D- galactopyranosyl-(1→4)-*β*-D-galactopyranosyl]-oleanolic acid (**8**) (Chaturvedula et al. 2003). The same protocol on the stem bark of *A. polyacantha* afforded nine compounds identified as epicatechin (**5)** (Adnyana et al. 2000)**,** lupeol (**9)** (Fotso et al. 2014), *β*-sitosterol (**10)** (Chaturvedula and Prakash 2012)**,** betulin (**11)** (Fotso et al. 2014), betulinic acid (**12)** (Kuete et al. 2007), apigenin (**13)** (Fajriah and Megawati 2016), methylgallate (**14) (**Choi et al. 2009), 2,3-dihydroxypropyltetracosanoate (**15)** (Lee et al. 2000) and 2,3-dihydroxypropyl-24- hydroxytetracosanoate (**16)** (Lee et al. 2000). (Figure S1, Supplementary data).

Compound **1** was obtained as a beige crystal, mp: 216–217 °C, soluble in methanol. It showed positive Molish and Liebermann–Burchard reactions demonstrating its saponin nature (Zheng et al. 2010**)**. Its molecular formula $C_{41}H_{66}O_{12}$ was deduced from its HRESIMS in positive-ion mode which exhibited a pseudo-molecular ion peak $[M + Na]^+$ at m/z 773.4395 (calcd 773,4452 for $C_{41}H_{66}O_{12}Na^+$) (Figure S1, Supplementary data). Its IR spectrum (Figure S2, Supplementary data) displayed characteristic bands of bands for hydroxyl, carboxylic acid and alkene respectively at 3358, 1688 and 1637 cm^{-1} respectively. Based on the reported method (Tchoukoua et al. 2017) compound **1** was hydrolyzed to give an oleanolic acid identified by co-TLC with an authentic sample and the monosaccharides (D-galactose and D-xylose. This was in accordance with the molecular formula of **1**, $C_{41}H_{66}O_{12}$ corresponding to a triterpene (C-30) linked to a hexose (C-6) and a pentose (C-5). The ^1H and ^{13}C NMR data of **1** (Table S1, Supplementary data) coupled with its HSQC and DEPT spectra (Figures S2–S6, Supplementary data) displayed characteristic signals for oleanane triterpenoid (Krief et al. 2005) with seven angular methyl groups as singlets at δ_H/δ_C 0.81/16.3,

0.84/15.6, 0.90/32.2, 0.93/22.6, 0.94/14.5, 1.05/27.1 and 1.15/25.0; a double bond between C-12 and C-13 in the aglycone at δc 122.2 and 143.8 respectively; The HSQC spectrum of **1** also displayed the signals of two anomeric protons at δ 4.83 (d, J = 7.8 Hz, 2H) correlating with the anomeric carbon signals at δ 104.0 and 105.1. Based on the high coupling constants of the anomeric protons and the chemical shifts of the anomeric carbons, the anomeric configurations of the sugar moieties were determined as β for the two sugars. The high chemical shift of C-28 (δc 180.5) also indicated that **1** is a monodesmodic type triterpenoidal saponin with the two sugars units linked at C-3 (Debella et al. 2000). At this point, the structure of **1** was closely similar to the one of Polyfoliolide A or 3-O-β-D-galactopyranosyl-(1→4)-β-D-xylopyranosyloleanolic acid isolated from the infrutescences of *Polyscias amplifolia* (Chaturvedula et al. 2003). However, the HMBC spectrum of **1** (Figures S7c and S7d, Supplementary data) showed correlations between H-1′ of Gal (δ_H 4.34) and C-3 of the aglcone (δc 89.5) and between H-3 of the aglycone (δ_H 3.16) and C-1′ of Gal (δc 105.1). In addition, the two diastereotopic protons of the D-xylose at δ_H 3.26 and 3.90 were shown to correlate with the second anomeric carbon at δc 104.0 and C-4″ of Xyl at δc 69.5. This allowed us to suggest that **1** has a terminal β-xylopyranosyl and a 4-substituted β- galactopyranoxyl unit linked to the aglycone at C-3. This was in accordance with the high chemical shift of C-4 of Gal (δc 79.5) due to the effect of the *O*-glycosylation. The connectivity of the two sugar units was confirmed by the HMBC spectrum of **1** (Figures S7c, Supplementary data) in which a correlation was observed between H-4′ of Gal (δ_H 3.50) and C-1″ of Xyl (δc 104.0). This was also confirmed by the HRESIMS spectra of **1** (Figures S1, Supplementary data) in positive mode in which the ion fragment $[M- CO_2-$ xylose + $2H]^{++}$ resulting from the lost of the terminal xylose was observed at m/z 575. The ion fragment $[M - CO_2-$ xylose–galactose + $H]^+$ at m/z 409 coming from the lost of the second sugar unit was also observed. To further confirm the terminal xylopyranosyl group, the partial hydrolysis of **1**with oxalic acid was performed and afforded the known 3-*O*-β-D-galactopyranosyloleanolic acid (Elujoba et al. 1990) (Figures S8–9, Supplementary data). Compound **1** was then univocally characterized as 3-*O*-[β-D-xylopyranosyl-(1→4)-β-D-galactopyranosyl]-oleanolic acid. To the best of our knowledge **1** is a new triterpenoid saponin to which the trival name polyacanthoside A was given.

Compound **1** as well as doxorubicin displayed cytotoxic effects in all the nine tested cancer cell lines (Table S2, Supplementary data). The recorded IC_{50} values ranged from 8.90 ± 0.58 μM (towards CCRF-CEM leukemia cells) to 35.21 ± 1.51 μM (towards HepG2 hepatocarcinoma cells) for compound **1** and from 0.02 ± 0.00 μM (towards CCRF-CEM leukemia cells) to 66.83 ± 2.20 μM (against CEM/ADR5000 leukemia cells) for doxorubicin. Significant cytotoxic activity with IC_{50} values 10 μM (Kuete and Efferth 2015) was obtained with compound **1** towards CCRF-CEM cells. Hypersensitivity (degree of resistance or D.R. below 0.90) (Mbaveng et al. 2017) of MDA-MB231*/BCRP* breast adenocarcinoma cells compared to it sensitive parental cell line MDA-MB231 cells was noted with compounds **1** (Table S2, Supplementary data**)**; Besides, the D.R. of this compound towards the resistant cells HCT116*(p53$^{-/-}$)* cells*, U87MG.ΔEGFR* cells was also below 1. In addition, the D.R. of compound **1** in all tested resistant cancer cell lines was lower than that of doxorubicin. This is an indication that this compound could be helpful in the fight against multi-drug resistant (MDR) cancer cells. Though the selectivity index of this compound was below 1, it was still better than that of the reference drug, doxorubicin. The overall data highlight the possibility of using compound **1** to fight drug sensitive and resistant cancers. For a comparative study of

1 with compound **8**, a preliminary study was performed in the sensitive CCRF-CEM leukemia cells; however this compound rather showed low cytotoxic effects with a recorded IC_{50} values 35.16 µM; Consequently it was no further tested in other cancer cell lines; This clearly indicates that the presence of galactopyranosyl sugar unit (Compound **8**) instead of xylopyranosyl (Compound **1**) significantly reduce the cytotoxicity towards CCRF-CEM cells (Table S2, Supplementary data).

3. Experimental (Supplementary data)

3.1. 3-O-[β-D-xylopyranosyl-(1→4)-β-D-galactopyranosyl]-oleanolic acid

Beige crystals; mp: 216–217 °C; $[\alpha]_D^{25}$: +23.2° (c 1.25, MeOH); IR (KBr, v, cm^{-1}): 3358, 2940, 1688, 1637, 1512, 1461, 1160, 1024; ^{1}H NMR (MeOD, 600 MHz): aglycone: 3.16 (1H, dd; 11.9, 3.8, H-3), 5.23 (1H, brs, H-12), 1.05 (3H, s, H-23), 0.84 (3H, s, H-24), 0.94 (3H, s, H-25), 0.81 (3H, s, H-26), 1.15 (3H, s, H-27), 0.90 (3H, s, H-29), 0.93 (3H, s, H-30), sugar moiety: 4.34 (1H, d, 7.7, H-1′), 3.23 (d, 4.7, H-2′), 3.45 (1H, d, 8.9, H-3′), 3.50 (1H, d, 8.9, H-4′), 3.38 (1H, d, 9.2, H-5′), 3.84 (2H, brs, H-6′), 4.34 (1H, d, 7.7, H-1″), 3.20 (1H, d, 8.5, H-2″), 3.32 (1H, d, 9.2, H-3″), 3.48 (1H, m, H-4″), 3.90 (1H, dd; 11.3, 5.2, H-5″eq), 3.26 (1, d, 10.4, H-5″ax); ^{13}C NMR (MeOD, 150 MHz): aglycone: 38.4 (C-1), 25.6 (C-2), 89.5 (C-3), 38.7 (C-4), 55.6 (C-5), 17.9 (C-6), 32.6 (C-7), 41.3 (C-8), 47.6 (C-9), 36.5 (C-10), 23.1 (C-11), 122.2 (C-12), 143.8 (C-13), 41.5 (C-14), 27.4 (C-15), 22.7 (C-16), 46.2 (C-17), 39.2 (C-18), 45.8 (C-19), 30.2 (C-20), 33.5 (C-21), 32.4 (C-22), 27.1 (C-23), 15.6 (C-24), 14.5 (C-25), 16.3 (C-26), 25.0 (C-27), 180.5 (C-28), 32.2(C-29), 22.6 (C-30), sugar moiety: 105.1 (C-1′), 74.0 (C-2′), 74.9 (C-3′), 79.5 (C-4′), 74.6 (C-5′), 60.6 (C-6′), 104.0 (C-1″), 73.5 (C-2″), 76.4 (C-3″), 69.5 (C-4″), 65.7 (C-5″); HRESIMS m/z: 773.4395 $[M + Na]^+$ (calcd 773,4452 for $C_{41}H_{66}O_{12}Na^+$), m/z 575 $[M- CO_2-$ xylose $+ 2H]^{++}$, m/z 409 $[M - CO_2-$ xylose$-$ galactose $+ H]^+$.

4. Conclusion

This work reports the chemical investigation of the leaves and stem bark of *Acacia polyacantha* from which a new oleanane-type triterpenoid saponin named polyacanthoside A was isolated. The cytotoxicity of polyacanthoside A was evaluated in a panel of 9 cancer cell lines including sensitive and drug resistant phenotypes. This compound showed significant cytotoxic activity especially on leukemia cells with a hypersensitivity on some resistant cancer cell lines demonstrating the interesting anticancer potential.

Supplementary material

Experimental section, NMR, MS and cytotoxicity data of compound **1** and all the isolated compounds are available alongside Figures S1–S10, Table S1-S2.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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