

## Flavonoids of *Steganotaenia araliacea*

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### Abstract

Phytochemical investigation of the stem and bark of *Steganotaenia araliacea* led to the isolation of two flavonoids. The structures of these compounds were identified as apigenin 4'-glucoside 1 and sophoraflavone B 2 using spectroscopic analysis including 1D (<sup>1</sup>H, <sup>13</sup>C and DEPT), 2D (COSY, HMQC and HMBC), Mass Spectrometer (EI-MS and HRMS), Infrared (IR) and Specific rotation. These compounds have not been previously reported from *Steganotaenia* species and are reported from this plant for the first time. *Steganotaenia araliacea* is one of four medicinal plants for more than twenty years used by traditional healers to treat various ailments including HIV in Tanga, Tanzania.

**Keywords:** *Steganotaenia araliacea*, apiaceae, flavonoids, apigenin 4'-glucoside, sophoraflavone B.

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## Introduction

The Tanga Aids Working Group (TAWG) in collaboration with both traditional healers and medical doctors are using a mixture of four medicinal plants to treat people living with HIV/AIDS. The group has treated over 5000 AIDS patients with herbs prescribed by local healers and has reportedly helped to improve the quality of life of their patients by boosting their immune system, reducing suffering and prolonging life, in some cases, by 5-10 years (Scheinman, 2004; Kayombo et al., 2007). As part of our research programme we have been investigating the medicinal plants used by TAWG for novel organic compounds that may be responsible for the reported anti-HIV activity. Our previous results on the investigation of the plant *Pyrenacantha kaurabassana* have recently been reported (Omolo et al., 2012). One of the four other plants used by traditional healers in the Tanga region of Tanzania for the treatment of HIV is *S. araliacea* Hochst (family Apiaceae). The family comprises of about 453 genera and more than 3,750 species mainly found in temperate regions, of which 28 genera and 137 species are indigenous in southern Africa (Van Wyk, 2000; ICBN, 2006). Eriksen (2001) has reported that *S. araliacea*, is overexploited in Tanga compared to the other three species used for HIV treatment due to over harvesting for medicinal purposes and agricultural expansion.

Apart from being used by TAWG for the treatments of people suffering from HIV, the bark of this plant is used in Tanga, Tanzania to treat oedema and gastric reflux, whereas the leaves and fruits are known to treat dysentery and diarrhoea. The stems and bark are powerful remedies for sore throats and colds (Hayman, 1998). In Kenya, the plant is used as an antivenin; treating snakebites (Owuor and Kisangau, 2006). *S. araliacea* stem bark has been employed in other diverse situations in man and animal conditions. The extract has found use as a rodent poison, insect repellent, asthma and as a diuretic agent in man (Agunu, 1997; Abubakar et al., 2001). The root is used for the treatment of headaches in South Africa. The leaves are used as vermifuge in Sierra Leone, as an ophthalmic lotion in the DRC and as an anticonvulsant in Gambia (Agunu, 1997). Saponins and lignans have been isolated from this plant that have been used as a rodent poison, insect repellent, diuretic agent, antileukemic, antimitotic and as an anti HIV agent (Lavaud et al., 1992; Wickramaratne et al., 1993; Agunu, 1997; Abubakar et al., 2001; Meragelman et al., 2001; Li et al., 2009). In this paper we report on our research conducted on *S.*

*araliacea*. The structural elucidation of two flavonoids from the stems and barks of this plant is outlined.

## Materials and Methods

### *General experimental procedure*

The 1D ( $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT) and 2D (COSY, HMQC, HMBC) spectra were acquired on 600 MHz Varian VnmrJ 600 spectrometers and referenced to residual solvent signals. Mass spectra were obtained on Waters API Q-TOF Ultima (HRMS) and EI-MS Mass Spectrometer instruments. Infrared (IR) absorptions were measured on a Perkin Elmer System 2000 FT-IR Spectrophotometer using KBr pellets. The samples were dissolved in dichloromethane and analyzed on a NaCl window. Melting points were determined on a Kofler micro-hot stage melting point apparatus and are uncorrected. Specific rotations  $[\alpha]_D$  were determined using Jasco Polarimeter DIP-370 (Digital Polarimeter) at 16 °C in dichloromethane. A quartz Microcell with a tube length of 100 mm and a volume of 1.0 ml was used. TLC experiments were run on ready made 0.25 mm thick layer of Merck silica gel 60 F<sub>254</sub> coated aluminium foil and glass plates. Spots on the TLCs were detected by observing under UV light (254 & 366 nm) and spraying with vanillin sulphuric acid. Normal column chromatography was conducted using different sizes of columns packed with Merck silica gel 60 (size 0.040- 0.063 mm). Preparative thin layer chromatography were run on 0.5 mm thick layer Merck silica gel 60 HF<sub>254</sub> containing gypsum (CaSO<sub>4</sub> binder) coated on 20 × 20 cm glass plates.

### *Plant Materials*

The stems and barks of *S. araliacea* were collected by TAWG in collaboration with traditional healers from Muheza district in Tanga (Tanzania) on 22 March 2009. A voucher specimen number 4226 HOS verified by Prof. HVM Lyaruu, a botanist from the Department of Botany, University of Dar es Salaam (Tanzania) was deposited in the University Herbarium, University of Dar es Salaam, Tanzania.

## Results and Discussion

### *Results: Extraction and Isolation*

The air-dried powder of stems and barks of *S. araliacea* (1.8 kg) was extracted sequentially starting from CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, MeOH and lastly, with water to obtain four crude residues of masses 26.70 g, 6.36 g, 106.24 g and 30.36 g respectively. A portion of the methanol extract was subjected to column chromatography using silica gel (47 cm, Ø: 2.5 cm; Si gel: 20 g, 70-230 mesh; Merck 9385) as the stationary phase and a solvent step gradient (2-50%) of MeOH: CH<sub>2</sub>Cl<sub>2</sub> as the mobile phase. A total of 60 fractions was collected and pooled as three groups (fractions 3-7), (fractions 20-28) and (fractions 40-54) based on TLC analysis. The combined fractions 20-28 were subjected to silica gel chromatography (29.5 cm, Ø: 1.5 cm; Si gel: 1 g, 70-230 mesh; Merck 9385) using 10% MeOH-CH<sub>2</sub>Cl<sub>2</sub> yielding a yellow crystalline material, **1** (12 mg). Fractions 40-54 were also subjected to silica gel chromatography (26 cm, Ø: 1.0 cm; Si gel: 0.5 g, 70-230 mesh; Merck 9385) that led to the purification of a yellow crystalline material, **2** (5 mg) after eluting with 10-15% MeOH-CH<sub>2</sub>Cl<sub>2</sub>.

**Apigenin 4'-glucoside (1)** Yellow crystalline material (MeOH); m.p 230-234 °C;  $[\alpha]^{16}_D -10$  (c 0.8, MeOH); IR (KBr)  $\nu_{\max}$ : 3350, 1793 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz)  $\delta$  7.77 (2H, d,  $J = 8.4$  Hz, H-2' & H-6'), 6.81 (2H, d,  $J = 8.4$  Hz, H-3' & H-5'), 6.71 (1H, s, H-8), 6.54 (1H, s, H-3), 6.39 (1H, s, H-6), 4.98 (1H, d,  $J = 6.6$  Hz, H-1''), 4.28 (1H, t,  $J = 9.0$  Hz, H-2''), 3.80 (2H, m, H-6''), 3.55 (1H, t,  $J = 10.2$  Hz, H-4''), 3.47 (1H, m, H-5''), 3.41 (1H, m, H-3''); 3.42 (1H, m, H-2''), <sup>13</sup>C NMR (CD<sub>3</sub>OD, 150 MHz)  $\delta$  182.6 (C, C-4), 165.5 (C, C-2), 163.2 (C, C-5), 162.5 (C, C-7), 161.3 (C, C-4'), 157.5 (C, C-8a), 128.2 (CH, C-2' & C-6'), 121.0 (C, C-1'), 115.9 (CH, C-3' & C-5'), 105.6 (C, C-4a), 102.4 (CH, C-3), 100.8 (CH, C-1''), 99.7 (CH, C-6), 94.6 (CH, C-8), 79.0 (CH, C-5''), 71.5 (CH, C-3''), 70.8 (CH, C-2''), 70.0 (CH, C-4''), 60.9 (CH<sub>2</sub>, C-6''); EIMS  $m/z$  432.2 (calcd for C<sub>21</sub>H<sub>20</sub>O<sub>10</sub> [M<sup>+</sup>] 432.1) (Li *et al.*, 2009).

**Sophoraflavone B (2)** Yellow crystalline material (MeOH); m.p 212-214 °C;  $[\alpha]^{16}_D -48$  (c 0.5, MeOH); IR (KBr)  $\nu_{\max}$  3480, 1792 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz)  $\delta$  7.90 (2H, d,  $J = 8.7$  Hz, H-2' & H-6'), 7.84 (1H, d,  $J = 7.8$  Hz, H-5), 6.93 (2H, d,  $J = 8.7$  Hz, H-3' & H-5'), 6.92 (1H, s, H-8), 6.90 (1H, d,  $J = 8.7$  Hz, H-6), 6.70 (1H, s, H-3), 4.98 (1H, d,  $J = 6.6$  Hz, H-1''), 4.28 (1H, t,  $J = 9.0$  Hz, H-2''), 3.80 (2H, m, H-6''), 3.55 (1H, t,  $J = 10.2$  Hz, H-4''), 3.47 (1H, t,  $J = 8.4$  Hz,

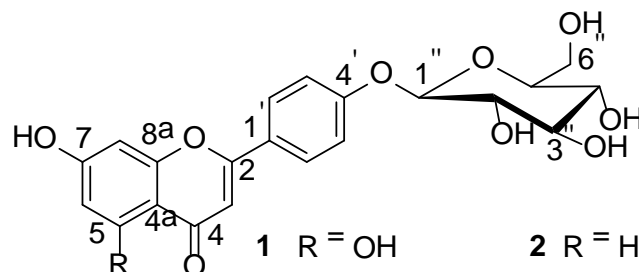
H-5''), 3.41 (1H, m, H-3'');  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 150 MHz)  $\delta$  176.1 (C, C-4), 163.0 (C, C-2), 162.2 (C, C-7), 160.5 (C, C-4'), 157.8 (C, C-8a), 126.2 (CH, C-5), 127.9 (CH, C-2' & C-6'), 121.4 (C, C-1'), 115.8 (C, C-4a), 115.8 (C, C-3' & C-5'), 114.9 (CH, C-6), 104.3 (CH, C-3), 102.3 (CH, C-8), 100.8 (CH, C-1''), 79.0 (CH, C-5''), 71.5 (CH, C-3''), 70.8 (CH, C-2''), 70.0 (CH, C-4''), 60.9 ( $\text{CH}_2$ , C-6''); HRMS  $m/z$  416.1109 (calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_9$  [ $\text{M}^+$ ] 416.1107 (Shirataki *et al.*, 1986 and Yoo *et al.*, 2004).

## Discussion

Phytochemical investigation of *S. araliacea* led to the isolation of two flavonoids **1** and **2** from the MeOH extract using column chromatography. The  $^1\text{H}$  NMR spectrum of compound **1** showed the presence of five aromatic and six aliphatic proton peaks that were later confirmed to be attributed to a flavone glycoside showing a 5, 7-dihydroxy-8-C-substituted ring A. The anomeric sugar proton at  $\delta$  4.98 (1H, d,  $J = 6.6$  Hz, H-1'') revealed that, compound **1** had one glucose unit at C-4'. The most downfield signal was due to the quaternary carbon C-4 ( $\delta$  182.6) due to hydrogen bonding with hydroxy group at C-5. Two doublet signals at  $\delta_{\text{H}}$  7.77 (2H, d,  $J = 8.4$  Hz) and  $\delta_{\text{H}}$  6.81 (2H, d,  $J = 7.8$  Hz) indicated the presence of a *para*-disubstituted aromatic ring. While two other singlets at  $\delta_{\text{H}}$  6.71 (1H, s),  $\delta_{\text{H}}$  6.39 (1H, s) and results from COSY, HSQC and HMBC correlations revealed the presence of a second tetrasubstituted aromatic ring. Another singlet in the aromatic region at  $\delta_{\text{H}}$  6.54 (1H, s) suggested the presence of a 2-substituted chromen-4-one which was found to be attached to the carbon at C-3 (Figure 1).

The  $^{13}\text{C}$  NMR spectrum showed the presence of a carbonyl carbon signal as revealed by a peak at  $\delta_{\text{C}}$  182.6 (C-4); five oxygenated aromatic carbon signals at  $\delta_{\text{C}}$  165.5 (C-2),  $\delta_{\text{C}}$  163.2 (C-5),  $\delta_{\text{C}}$  162.5 (C-7),  $\delta_{\text{C}}$  161.3 (C-4') and  $\delta_{\text{C}}$  157.5 (C-8a); and five aromatic carbon signals each bearing a hydrogen atom at  $\delta_{\text{C}}$  128.2 (C-2' & C-6'),  $\delta_{\text{C}}$  115.9 (C-3' & C-5'),  $\delta_{\text{C}}$  102.4 (C-3),  $\delta_{\text{C}}$  99.7 (C-6) and  $\delta_{\text{C}}$  94.6 (C-8). The carbon signals at  $\delta_{\text{C}}$  100.8, 79.0, 71.5, 70.8, 70.0 and at  $\delta_{\text{C}}$  60.9 confirmed the type of carbohydrate moiety attached to the natural product. The  $^{13}\text{C}$  NMR and DEPT spectra further showed an oxymethylene carbon (C-6'') peak at  $\delta_{\text{C}}$  61.0. On this basis the carbohydrate

was corroborated as D-glucose by direct comparison with authentic sugar. The *para* disubstituted system with two sets of equivalent carbon peaks confirmed the presence of symmetry in the one aromatic ring. While for the second aromatic ring the H-8 proton signal showed HMBC correlation to C-6 ( $\delta_C$  99.7), C-7 ( $\delta_C$  162.5) and C-8a ( $\delta_C$  157.5); and H-6 proton signal showed correlations to C-5 ( $\delta_C$  163.2) and C-7 indicating that the two hydroxy groups were attached to C-5 and C-7 respectively.



**Fig. 1 Chemical structures of isolated compounds (1) and (2) from *S. araliacea*.**

The EIMS spectrum of compound **1** showed a molecular ion  $[M^+]$  peak at  $m/z$  432.1 corresponding tentatively to the molecular formula  $C_{21}H_{20}O_{10}$ . The IR spectrum of compound **1** showed characteristic absorption bands for hydroxy ( $3350\text{ cm}^{-1}$ ) and carbonyl ( $1793\text{ cm}^{-1}$ ) functional groups. Compound **1** was hence identified as apigenin 4'-glucoside. This natural product, apigenin 4'-glucoside **1** although known, has now been isolated for the first time from the genus *Steganotaenia*. The compound was previously synthesized by Oyama and Kondo (2004) and later isolated from the ethanolic extract of the leaves of *Machilus japonica* var. *kusanoi* (Lee et al., 2009).

Inspection of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound **2** showed them to be similar to those of compound **1**. The only difference was, the oxygenated carbon peak (C-5) at  $\delta_C$  163.2 in the  $^{13}\text{C}$  NMR spectrum of compound **1** was absent in the  $^{13}\text{C}$  NMR spectrum of compound **2** and an extra carbon peak (C-5) was present at  $\delta_C$  126.2 in the  $^{13}\text{C}$  NMR spectrum of compound **2**. A proton peak at  $\delta_H$  4.98 indicated anomeric peak with other peaks in the range of  $\delta_H$  3.41-4.28 for the carbohydrate moiety. The correlation between the anomeric proton ( $\delta_H$  4.98) and C-4' ( $\delta_C$  160.5) corroborated the position of sugar at C-4'. All assignments were confirmed by  $^1\text{H}$ - $^{13}\text{C}$

correlations in the HMBC and HSQC spectra. The HRMS spectrum of compound **2** showed a molecular ion  $[M^+]$  peak at  $m/z$  416.1109 corresponding to the molecular formula  $C_{21}H_{20}O_9$ .

The IR spectrum of compound **2** showed an O-H stretching band at  $3480\text{ cm}^{-1}$  and C=O stretching band at  $1792\text{ cm}^{-1}$ . From the spectroscopic data the identity of the compound was confirmed as sophoraflavone B. The spectral data of **2** were in good agreement with the literature values reported by Yoo *et al.*, (2004). The group (Yoo *et al.*, 2004) reported similar spectroscopic data of the flavonoid part of the molecule **2**. However, the compound was later reported to be isolated from the roots of *Sophora subprostrata* (Shirataki *et al.*, 1996).

### Conclusion

We report the isolation of two flavonoids from the plant *S. araliacea*. Two compounds are reported for the first time from *Steganotaenia* species. The structure–activity relationships for the isolated compounds **1** and **2** need be done to obtain clarity on the reported medicinal uses of this species. In particular, the activity against a HIV models need to be conducted.

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