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ABSTRACT

Phytochemical study of the leaves and the stem bark of *Pseudospondias microcarpa* (A. Rich.) Engl. afforded eight phenolic compounds: scopoletin (**1**), ferulic acid (**2**), isovitexin (**3**), rhoifolin (**4**), quercetin 3-*O*-α-L-rhamnopyranoside (**5**), justicialoside A (**6**), granduloside A (**7**) and pithecellobiumol B (**8**). The structures of the isolated compounds were elucidated by spectroscopic means including 1D and 2D NMR and MS, and by comparison with previously reported data. This is the first report on the isolation of these compounds from the genus *Pseudospondias*. The chemotaxonomic significance of the isolated compounds within the family Anacardiaceae is discussed.

Keywords: Anacardiaceae*; Pseudospondias microcarpa* (A. Rich.) Engl.; chemotaxonomy; HPLC-DAD; 2D NMR

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1. Subject and source

The genus *Pseudospondias* Engl., distributed throughout the sub-Sahara tropical Africa, belongs to the cashew and sumac family Anacardiaceae (Aubreville, 1950). There are only two recognised species in the genus: *P. microcarpa* (A. Rich.) Engl. and *P. longifolia* Engl. (The Plant List, 2013). *P. microcarpa* (A. Rich.) Engl. (synonyms: *Spondias microcarpa* A. Rich. and *Spondias zanzee* G. Don) is a spreading tree of up to 20 m high. The trunk is often irregularly twisted with branches growing near the base. The leaves are oblong-ovate to elliptic, odd pinnate on stalks of 30 cm, with 2-8 pairs leaflets (Aubreville, 1950; Burkill, 1985). *P. microcarpa* produces edible grape shape fruits measuring about 2.5 cm, red or blue-black when ripe and commonly called African grape (Adongo et al., 2015). The plant is used in ethnomedicine across Africa. In Cameroon, the stem bark macerate is mixed with *Coster afer* and palm wine for the treatment of helminthiasis and constipation (Noumi and Yomi, 2001). In Congo, the whole plant is used for the treatment of malaria, dyspepsia, diarrhoea and opportunistic infections (Mbatchi et al., 2006; Bruno, 2013) and the decoction of leaves is drunk in Tanzania to relieve chronic cough and malaria (Kisangau et al., 2007). The fresh stem bark and leaves of *P. microcarpa* (A.Rich.) Engl. were collected from Mount Eloundem (3º49′1.794″N 11º25′59.412″E at 800 m altitude), Centre Region, Cameroon, in June 2015 and identified by Mr Victor Nana (botanist). A voucher specimen (41437/SFR) was deposited at the Cameroon National Herbarium.

2. Previous work

Previous pharmacological reports on *P. microcarpa* showed that the hydro-ethanolic extracts of the leaves and the ethyl acetate extracts of the stem bark and the roots had moderate antimicrobial and antioxidant properties, while the ethanolic extracts of the roots and stem bark exhibited good antiplasmodial activity, with IC_{50} of 1.13 μg/mL and 4.33 μg/mL, respectively (Yondo et al., 2009; Malebo et al., 2010). The hydro-ethanolic extract of the leaves displayed analgesic and anticonvulsant activities, and an anxiolytic-like activity similar to that of diazepam (Adongo et al., 2015; 2016). The presence of alkaloids, coumarins, flavonoids, polyphenols, quinones, saponins, tannins, and triterpenes were reported on the basis of qualitative phytochemical screening of the stem bark and leaves of *P. microcarpa*, but no phytochemicals were previously isolated and identified (Yondo et al., 2009; Sidjui et al., 2014).

3. Present study

Dried and ground leaves (437.0 g) of *P. microcarpa* were successively extracted with *n*-hexane, DCM and MeOH using a Soxhlet extractor to yield 13.4 g, 2.7 g and 12.5 g of *n*-hexane, DCM and MeOH extracts, respectively. The *n*-hexane and DCM extracts of the leaves were found to mainly contain chlorophyll and were not purified. The MeOH extract of the leaves was fractionated using solid-phase extraction and purified as follows. A portion of the dried MeOH extract (2.0 g) was suspended in 10 mL of 10% MeOH-water and loaded on to a Strata C-18-E cartridge (Phenomenex, USA) (20 g), previously washed with MeOH (50 mL), followed by equilibration with water (100 mL). The cartridge was eluted with MeOH-water mixture of decreasing polarity to obtain four fractions: 20, 50, 80 and 100% MeOH in water (200 mL each) (F1-F4, respectively). The process was repeated twice. All fractions were concentrated to dryness using a combination of rotary evaporator and freeze-dryer. F1 and F4 were not purified as preliminary ^{1}H NMR of both extracts revealed a rich contents in tannins and chlorophyll, respectively. F2 (198.8 mg) and F3 (328.6 mg) were found to have similar analytical HPLC profiles and were mixed together for separation of their constituents. Separation of phenolic compounds was performed on an Agilent 1260 infinity series equipped with a binary pump, an autosampler, a column chamber, a degasser and a UV/DAD detector (Agilent, UK) using an ACE prep-column (150 × 21.2 mm, 10 μm, Hichrom Ltd, UK), injection volume 120 μL, sample concentration of 75 mg/mL, volume flow rate 10 mL/min, mobile phase gradient of water (A) and methanol (B) both containing 0.1% trifluoro acetic acid (TFA): 30-75% B, 0-20 min, monitored at 230 nm to yield compounds **3** (6.1 mg), **4** (2.4 mg), **5** (1.6 mg), **6** (2.1 mg) and **7** (1.9 mg) having retention times 11.20, 14.69, 16.31, 18.67 and 19.23 min, respectively.

The dried and ground stem bark (276.0 g) was subjected to extraction as described above to yield 2.2 g, 1.0 g and 5.9 g of *n*-hexane, DCM and MeOH extracts, respectively. The DCM extracts (4.0 g) was suspended in 10 mL of 30% MeOH in water solution and cleaned up using a Strata C-18-E cartridge eluted with 80% MeOH in water. The obtained extract was evaporated to dryness and combined with the MeOH extract as they showed similar analytical HPLC profile. The combined extract (2.0 g) was fractionated using solid-phase extraction as previously described. Fractions F2 (597.6 mg) and F3 (194.3 mg) were combined and purified by preparative HPLC-DAD analysis as outlined above, and using a mobile phase gradient comprising water (A) and methanol (B) both containing 0.1% TFA: 30-65% B, 0-30 min, monitored at 230 nm to yield compounds **1** (3.1 mg), **2** (3.6 mg) and **8** (4.3 mg) having retention

times 13.28, 13.00, and 13.33 min, respectively. F1 and F4 were not purified as their preliminary ^{1}H NMR revealed a high contents in tannins and chlorophyll, respectively.

Structures of the isolated compounds were elucidated by extensive spectroscopic analyses including 1D and 2D NMR and HRESIMS and by comparison with previously reported data. NMR analyses were performed on Bruker AMX 600 instrument (Bruker, Germany) and MS analyses on a Waters LCT Premier™ ESI-TOF Mass Spectrometer (Waters, UK). The compounds were identified as scopoletin (**1**) (Darmawan et al., 2012), ferulic acid (**2**) (Baldé et al., 1991), isovitexin (**3**) (Krafczyk et al., 2008), rhoifolin (**4**) (Osterdahl, 1979), quercetin 3-*O*-α-L-rhamnopyranoside (**5**) (Jiang et al., 2015), justicialoside A (**6**) (Guetchueng et al., 2019), granduloside A (**7**) (Tagousop et al., 2017), and pithecellobiumol B (**8**) (Wang et al., 2017) (Figure 1).

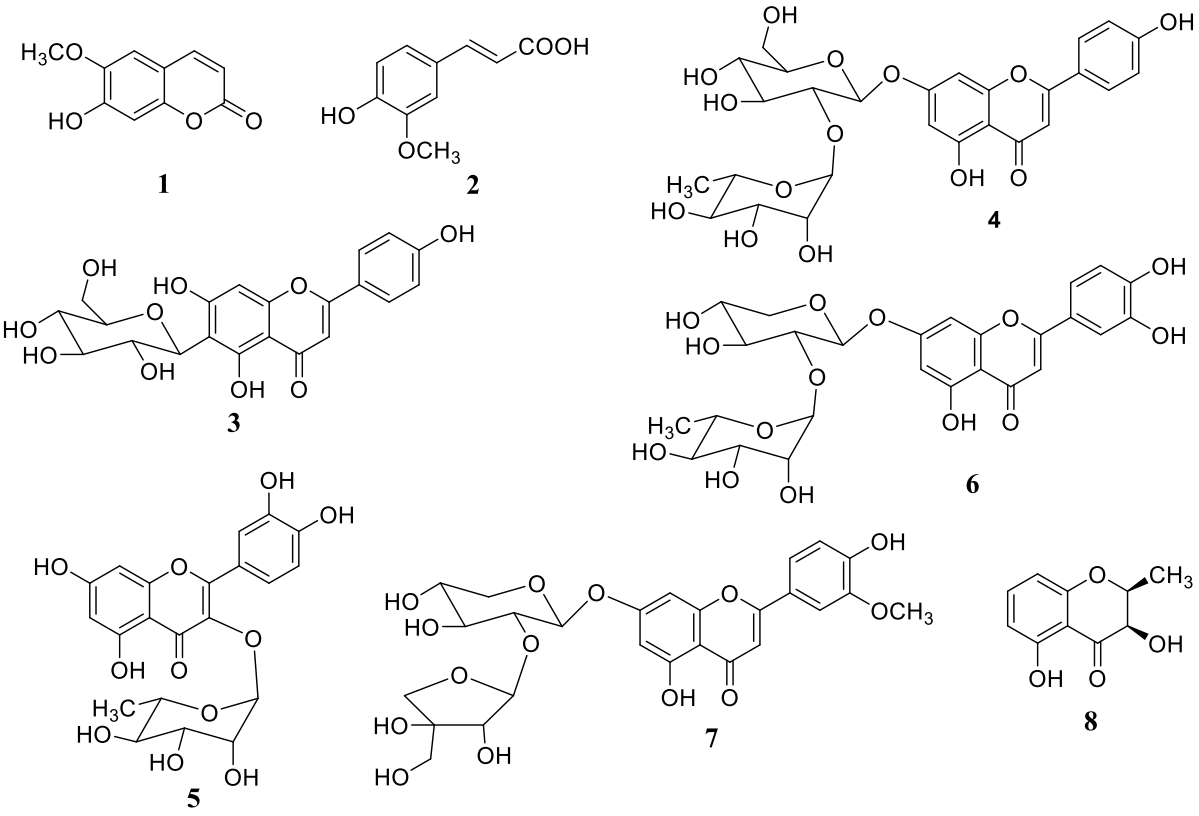

Figure 1. Structures of phenolic compounds **1**-**8** isolated from *P. microcarpa*

4. Chemotaxonomic significance

In the present study, eight phenolic compounds (**1**-**8**) were isolated and identified from the leaves and stem bark of *P. microcarpa*. The composition of the leaves appeared to be different from that of the stem bark as no identical compound was isolated from both organs.

However, further studies such as HPLC profiling might be necessary to compare the variation of the composition of the two organs in details. To the best of our knowledge, this is the first report on the isolation and identification of any phytochemical from the genus *Pseudospondias*. The genus *Pseudospondias* belongs to the Spondioidae tribe of the Anarcadiaceae family (Mitchel and Daly, 2015). This family is a rich source of phenolic compounds including flavonoids, phenolic acid derivatives and tannins (Sameh et al., 2018). Apart from scopoletin (**1**), ferulic acid (**2**), isovitexin (**3**) and quercetin 3-*O*-α-L-rhamnopyranoside (**5**), which were previously reported in some other Anarcadiaceae species, compounds **4**, **6-8** are reported here for the first time from this family. Scopoletin (**1**) was reported from *Mangifera reba* Pierre and *Pleiogynium solandri* (Benth.) Engl. (El-Fiki and Ahmed, 1999; Duong et al., 2017), ferulic acid (**2**) from *Mangifera indica* L. (Gentile et al., 2019), isovitexin (**3**) from *Lannea microcarpa* Engl. (Picerno *et al.*, 2006) and quercetin 3-O-α-L-rhamnopyranoside (**5**) from *Rhus parviflora* Roxb. and *Sclerocarya birrea* hochst. (Shrestha et al., 2012; Cadiz-Gurrea et al., 2019). Though, compounds **4**, **6-7** are new findings within the family, their aglycones as well as several glycosylated flavones and flavonols have been identified and isolated from numerous species belonging to the Anacardiaceae family. For example, apigenin 7-*O*-β-D-glucopyranoside was isolated from *Mangifera indica* L. (Abdullah et al., 2014), chryoseriol 7-*O*-β-D-glucopyranoside, luteolin 7-*O*-β-D-glucopyranoside and luteolin 7-*O*-β-D-glucuronide from *Rhus parviflora* Roxb. and *Rhus typhina* L. (Shrestha et al., 2012; Cadiz-Gurrea et al., 2019; Wang et al., 2019), quercetin 3-*O*-β-D-galactopyranoside from *Mosquitoxylon jamaicense* Krug & Urb. and *Pleigynium solandri* (Benth.) Engl. (El-Fiki and Ahmed, 1999; Montenegro et al., 2007) and quercetin 3-*O*-rutinoside from *Pistacia vera* L. and *Pleigynium solandri* (Benth.) Engl. (Montenegro et al., 2007; Tomaino et al., 2010).

The co-occurrence of some of these phenolic compounds within the species of the family Anacardiaceae might have some chemotaxonomic implications, at least at the family level. However, the chromanone, pithecellobiumol B (**8**), is a new reported skeleton within the Anacardiaceae. It has previously been reported from the leaves and twigs of *Pithecellobium clypearia* Benth. (Fabaceae) (Wang et al., 2017).

Appendix 1. Supplementary data

^{1}H NMR and MS spectra of isolated compounds are provided as supplemental information.

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References

Abdullah, A. S. H., Mohammed, A. S., Abdullah, R., Mirghani, M. E. S., Al-qubaisi, M., 2014. BMC Complementary and Alternative Medicine 14, 199.

Adongo, D. W., Kukuia, K. K. E., Mante, P. K., Ameyaw, E. O., Woode, E., 2015. BioMed Research International 2015, 397943.

Adongo, D. W., Mante, P. K., Edem Kukuia, K. K., Ameyaw, E. O., Woode, E., Azi, I. H., 2016. Journal of Basic and Clinical Physiology and Pharmacology 27(5), 533.

Aubréville, A., 1950. Flore forestière soudano-guinéenne: AOF, Cameroun, AEF.

Baldé, A. M., Claeys, M., Pieters, L. A., Wray, V., Vlietinck, A. J., 1991. Phytochemistry 30(3), 1024.

Burkill, H. M., 1985. The useful plants of west tropical Africa. Edition 2. Vol. 1: families AD. Kew, Royal Botanic Gardens.

Bruno, E. 2013. Phytopharmacology 4(2), 149.

Cadiz-Gurrea, D. L. L. M., Lozano-Sanchez, J., Fernandez-Ochoa, A., Segura-Carretero, A., 2019. Molecules 24(5), article number: 966.

Darmawan, A., Kosela, S., Kardono, L. B., Syah, Y. M., 2012. Journal of Applied Pharmaceutical Science 2(12), 175.

Duong, T. T. T., Do, T. N. V., Nguyen, H. X., Le, T. H., Dang, P. H., Nguyen, N. T., Nguyen, T. N., Nguyen T. D., Nguyen, M. T. T., 2017. Tetrahedron Letters 58(23), 2280.

El-Fiki, N. M., Ahmed, F. I., 1999. Al-Azhar Journal of Pharmaceutical Sciences 24, 38.

Gentile, C., Di Gregorio, E., Di Stefano, V., Mannino, G., Perrone, A., Avellone, G., Sortino, G., Inglese, P., Farina, V., 2019. Food Chemistry 277, 471.

Guetchueng, T. S., Nahar, L., Ritchie, K. J., Ismail, F. M. D., Dempster, N. M., Sarker, S. D., 2019. Phytochemistry Letters 31, 101.

Jiang, C.-L., Tsai, S.-F., Lee, S.-S., 2015. Natural Product Communication 10(1), 63.

Kisangau, D. P., Lyaruu, H. V., Hosea, K. M., Joseph, C. C., 2007. Journal of Ethnobiology and Ethnomedicine 3(1), 29.

Krafczyk, N., Kötke, M., Lehnert, N., Glomb, M. A., 2008. European Food Research and Technology 228(2), 187.

Malebo, H. M., Tanja, W., Cal, M., Swaleh, S. A. M., Omolo, M. O., Hassanali, A., Sequin, U., Hamburger, M., Brun, R., Ndiege, I. O., 2010. Tanzania Journal of Health Research 11(4), 39.

Mbatchi, S., Mbatchi, B., Banzouzi, J., Bansimba, T., Ntandou, G. N., Ouamba, J.-M., Berry, A., Benoit-Vical, F., 2006. Journal of Ethnopharmacology 104(1-2), 168.

Mitchell, J. D., Daly, D. C., 2015. PhytoKeys 55, 1.

Montenegro, H., Gonzalez, J., Ortega-Barria, E., Cubilla-Rios, L., 2007. Pharmaceutical Biology 45(5), 376.

Noumi, E., Yomi, A., 2001. Fitoterapia 72(3), 246.

Osterdahl, B. G., 1979. Acta Chemica Scandinavica B33, 400.

Picerno P., Mencherini, T., Loggia, R., Della Meloni, M., Sanogo, R., Aquino, R. P., 2006. Journal of Pharmacy and Pharmacology 58(7), 981.

Sameh, S., Al-Sayed, E., Labib, R. M., Singab, A. N., 2018. Evidence-Based Complementary and Alternative Medicine, article number: Article ID 5382904.

Shrestha, S., Lee, D.-Y., Park, J.-H., Cho, J.-G., Seo, W.-D., Kang, H. C., Jeon, Y.-J., Yeon, S.-W., Bang, M.-H., Baek, M.-I., 2012. Journal of the Korean Society for Applied Biological Chemistry 55(5), 689.

Sidjui, S. L., Melong, R., Nnanga, N., Toghueo, K. R. M., Zeuko'o, M. E., Sikadeu, S., Guedje, N. M. and Folefoc, N. G., 2014. International Journal of Pharmacy and Pharmacology 3(3), 160.

Tagousop, C. N., Ngnokam, D., Harakat, D., Voutquenne-Nazabadioko, L., 2017. Phytochemistry Letters 19, 172.

The Plant List – A Working List of All Plant Species (2013). Version 1.1, http://www.theplantlist.org/1.1/browse/A/Anacardiaceae/Pseudospondias/ (accessed on 08.02.2020).

Tomaino, A., Martonara, M., Arcoraci, T., Monteleone, D., Giovinazzo, C., Saija, A., 2010. Biochemie 92(9), 1115.

Yondo, J., Fomekong, G. I. D., Kontangui, M. C., Wabo, J. P., Tankoua, O. F., Kulate, J. R., Mpoame, B. M., 2009. Annals of Nutrition and Metabolism 55, 205.

Wang, L., Xu, M.L., Xin, L., Ma, C., Yu, G., Saravanakumar, K., Wang, M.-Y., 2019. Medicinal Chemistry Research 28(6), 917.

Wang, Y. X., Ren, Q., Yan, Z. Y., Wang, W., Zhao, L., Bai, M., Song, S. J., 2017. Bioorganic and Medicinal Chemistry Letters 27(21), 4823.