Cytotoxic flavonoids and other constituents from the stem bark of *Ochna schweinfurthiana*

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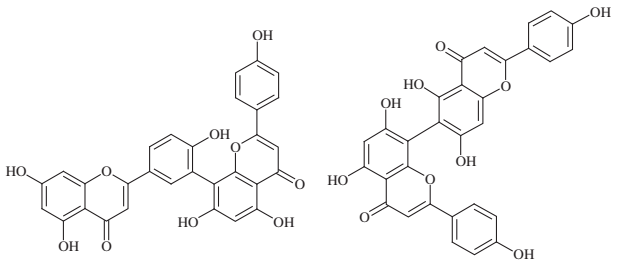

4 **6**

Seven flavonoids, hemerocallone (**1**), 6,7-dimethoxy-3′,4′-dimethoxyisoflavone (**2**), amentoflavone (**4**), agathisflavone (**6**), cupressuflavone (**8**), robustaflavone (**9**) and epicatechin (**10**), together with three other compounds, lithospermoside (**3**), β-D-fructofuranosyl-α-D-glucopyranoside (**5**) and 3β-*O*-D-glucopyranosyl-β-stigmasterol (**7**), were isolated from the ethyl acetate extract of the stem bark of *Ochna schweinfurthiana* F. Hoffm. All the compounds were characterised by spectroscopic and mass spectrometric methods, and by comparison with literature data. Cytotoxicity of the extracts and compounds against cervical adenocarcinoma (HeLa) cells was evaluated by MTT assay. Compounds **4** and **6** exhibited good cytotoxic activity, with IC_{50} values of 20.7 and 10.0 μM, respectively.

Keywords: *Ochna schweinfurthiana*; Ochnaceae; flavonoids; cytotoxic activity

1. Introduction

Ochna schweinfurthiana F. Hoffm. belongs to the Ochnaceae family and is widely distributed in African woodlands. The plant grows into a decorative shrub bearing bright-yellow flowers (Burkill 1997). In the north of Cameroon, the powdered bark is traditionally used as antimalarial and anthelmintic, and the decoction of the root, leaves or bark is used in wound dressing (Abdullahi et al. 2010). Previous phytochemical studies of the leaves and roots of this plant reported the isolation of quercetin 3-*O*-β-D-glucopyranosyl (1 → 6)-α-rhamnoside and tri-

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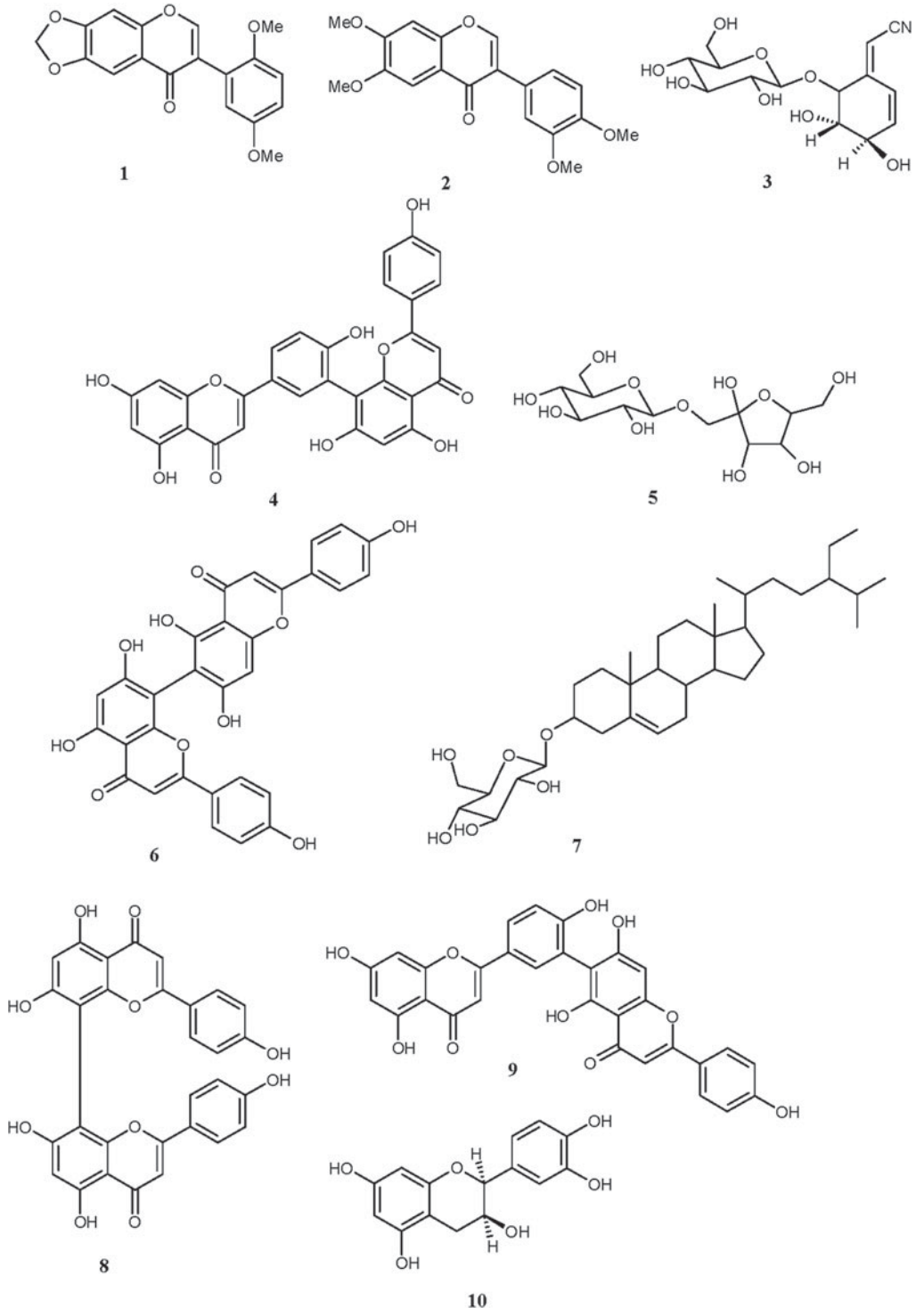

Figure 1. Structures of compounds **1**–**10**.

methoxy lophirone A (Abdullahi et al. 2011, 2014). In this study, the chemical constituents of an ethyl acetate extract of the stem bark of *O. schweinfurthiana* were examined and 10 known compounds were obtained. The methanolic and ethyl acetate extracts displayed a moderate cytotoxic activity on cervical adenocarcinoma (HeLa) cells, while compounds **4** and **6** exhibited good cytotoxic activity on the same cell line.

2. Results and discussion

The ethyl acetate extract, obtained from partition of methanolic crude extract of the stem bark of *O. schweinfurthiana* resuspended in water, was separated using various chromatographic techniques to afford hemerocallone (**1**), 6,7-dimethoxy-3′,4′-dimethoxyisoflavone (**2**), lithospermoside (**3**), amentoflavone (**4**), β-D-fructofuranosyl-α-D-glucopyranoside (**5**), agathisflavone (**6**), 3-β-*O*-D-glucopyranosyl-β-stigmasterol (**7**), cupressuflavone (**8**), robustaflavone (**9**) and epicatechin (**10**). The chemical structure of each isolated compound (Figure 1) was elucidated on the basis of spectroscopic methods, including 2D NMR experiments, and confirmed by comparison with the literature. To the best of our knowledge, there are no published reports on these compounds in the plant. The extracts and compounds **3**–**6**, **8** and **9** were evaluated for their cytotoxicity against HeLa cells using the MTT assay.

The measurement of cell viability showed that both the methanolic and ethyl acetate crude extracts had a moderate cytotoxic activity on the tested human cancer cell line, and amentoflavone (**4**) and agathisflavone (**6**) exhibited a good activity (Table 1). However, compounds **3**, **5**, **8** and **9** were inactive. Compounds **1**, **2**, **7** and **10** were obtained in too low quantity and could not be tested.

Amentoflavone and agathisflavone were previously isolated from *Selaginella tamariscina* (Selaginellaceae) and *Rhus parviflora* (Anacardiaceae), respectively. Both compounds showed good cytotoxicity against HeLa cancer cell lines with IC_{50} values of 20.0 and 15.2 μM, respectively (Ying et al. 2010; Shrestha et al. 2012). Amentoflavone has previously shown antiviral activity (Lin et al. 1999). It also induced apoptosis in MCF-7 breast cancer cells (Pei et al. 2012), and inhibited the proliferation of human promyelocytic leucaemia (HL-60), MCF-7, HeLa and human hepatocellular carcinoma BEL-7402 cells with IC_{50} values between 47 and 77 μM (Ying et al. 2010). Agathisflavone inhibited β-secretase at a 10 μM concentration (Shrestha et al. 2012).

Although the methanolic and ethyl acetate extracts showed a moderate cytotoxic activity on HeLa cells, this study indicated that *O. schweinfurthiana* can be considered as a possible source of secondary metabolites exhibiting a cytotoxic activity on HeLa cells. It would be worthwhile to

Table 1. Cytotoxic activity of the extracts and compounds **3**–**6**, **8** and **9** in HeLa cells[a].

Extracts and compounds	% Viability at 20 μg/mL	IC_{50} (μM)[b]
MeOH extract	13.9	
EtOAc extract	9.4	
Lithospermoside **(3)**	70.5	
Amentoflavone **(4)**	1.9	20.7 ± 1.58
Saccharose **(5)**	75.5	
Agathisflavone **(6)**	1.5	10.0 ± 0.88
Cupressuflavone **(8)**	67.6	
Robustaflavone **(9)**	69.0	
Mitoxantrone[c]	0.0	0.2 ± 0.05
Vehicle	100.0	

[a] Extracts and compounds were evaluated for cytotoxicity by standard procedures as described in the Experimental section. HeLa (cervical cancer) human cell lines were used. Results are shown as % viability and IC_{50} values (μM).
[b] Results are the means of at least three independent determinations ± SD.
[c] Positive control.

establish structure–activity relationships in order to highlight the functional groups important for the activity of the isolated compounds.

3. Conclusions

We report the phytochemical study of *O. schweinfurthiana* and demonstrated the cytotoxic activity of the methanolic and ethyl acetate extracts, as well as of some isolated compounds. Flavonoids, cyanoglycoside, saccharose and sterol glycoside were identified from the ethyl acetate extract for the first time. The results of the bioassay confirmed the effects of amentoflavone and agathisflavone in HeLa cells as demonstrated by Ying et al. (2010) and Shrestha et al. (2012).

Supplementary material

Experimental details relating to this article are available online.

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References

Abdullahi MI, Iliya I, Haruna AK, Sule MI, Musa AM, Abdullahi MS. 2010. Preliminary phytochemical and antimicrobial investigations of leaf extracts of *Ochna schweinfurthiana* (Ochnaceae). Afr J Pharm Pharmacol. 4:83–86.

Abdullahi MI, Musa AM, Haruna AK, Pateh UU, Sule MI, Abdulmalik IA, Abdullahi MS, Abimiku AG, Iliya I. 2014. Isolation and characterization of an anti-microbial biflavonoid from the chloroform-soluble fraction of methanolic root extract of *Ochna schweinfurthiana* (Ochnaceae). Afr J Pharm Pharmacol. 8:93–99.

Abdullahi MI, Musa AM, Haruna AK, Sule MI, Abdullahi MS, Abdulmalik MI, Akinwande Y, Abimiku AG, Iliya I. 2011. Antimicrobial flavonoid diglycoside from the leaves of *Ochna schweinfurthiana* F. Hoffm. (Ochnaceae). Niger J Pharm Sci. 10:1–7.

Burkill HM. 1997. The useful plants of West Tropical Africa. Families M–R. Vol. 4 2nd ed. Kew: Royal Botanic Gardens.

Lin YM, Flavin MT, Schure R, Chen FC, Sidwell R, Barnard DL, Huffman JH, Kern ER. 1999. Antiviral activities of biflavonoids. Planta Med. 65:120–125.

Pei JS, Liu CC, Hsu YN, Lin LL, Wang SC, Chung JG, Bau DT, Lin SS. 2012. Amentoflavone induces cell-cycle arrest and apoptosis in MCF-7 human breast cancer cells via mitochondria-dependent pathway. In Vivo. 26:963–970.

Shrestha S, Park JH, Lee DY, Cho JG, Seo WD, Kang HC, Yoo KH, Chung IS, Jeon YJ, Yeon SW, Baek NI. 2012. Cytotoxic and neuroprotective biflavonoids from the fruit of *Rhus parviflora*. J Korean Soc Appl Biol Chem. 55:557–562.

Ying J, Guogang Z, Enlong M, Hongmei Z, Jin G, Jie H. 2010. Amentoflavone and the extracts from *Selaginella tamariscina* and their anticancer activity. Asian J Tradit Med. 5:226–229.