Novel influenza virus inhibitors from the bark of *Burkea africana*

Christina E. Mair[1], Ulrike Grienke[1], Anke Wilhelm[2], Ernst Urban[3], Martin Zehl[1,3,4], Judith M. Rollinger[1], and Michaela Schmidtke[5]

[1] Department of Pharmacognosy, Faculty of Life Sciences, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
[2] Department of Chemistry, University of the Free State, PO Box 339, 9300 Bloemfontein, South Africa
[3] Department of Pharmaceutical Chemistry, Faculty of Life Sciences, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
[4] Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Währinger Straße 38, 1090 Vienna, Austria
[5] Institute of Medical Microbiology, Section Experimental Virology, Jena University Hospital, Hans-Knöll-Straße 2, 07745 Jena, Germany

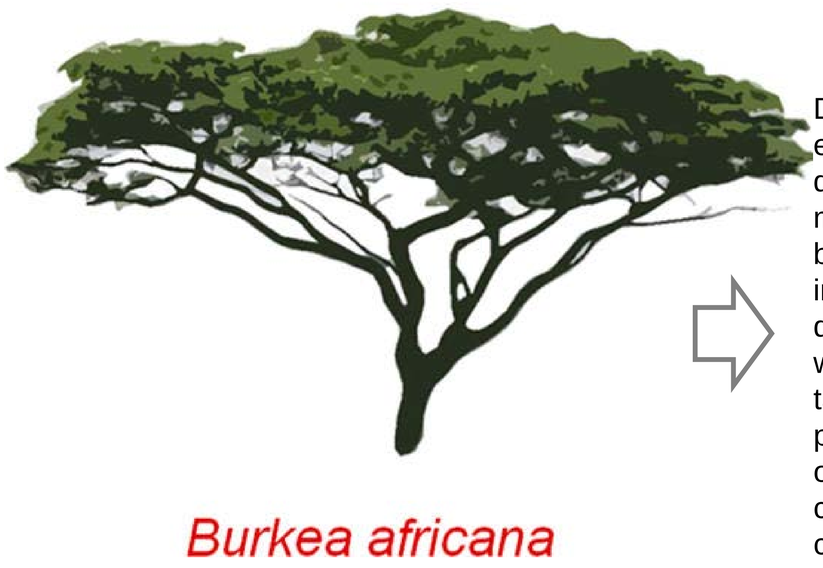
Thüringer Aufbaubank
ESF EUROPA FÜR THÜRINGEN EUROPÄISCHER SOZIALFONDS

BACKGROUND & AIM

Burkea africana **Hook is a flat-topped tree belonging to the family of Leguminosae and widely distributed in the tropical and subtropical African regions south to South Africa. Its bark decoctions are used e.g. for the treatment of colds and coughs. In the literature, little can be found on the phytochemical composition of *B. africana*.**

Therefore, bark extracts of *B. africana* were included in a study aiming to identify novel anti-influenza natural lead compounds.

Isolation and characterization of eight new triterpene saponins (1-8) with four so far undescribed aglycone structures from *Burkea africana*



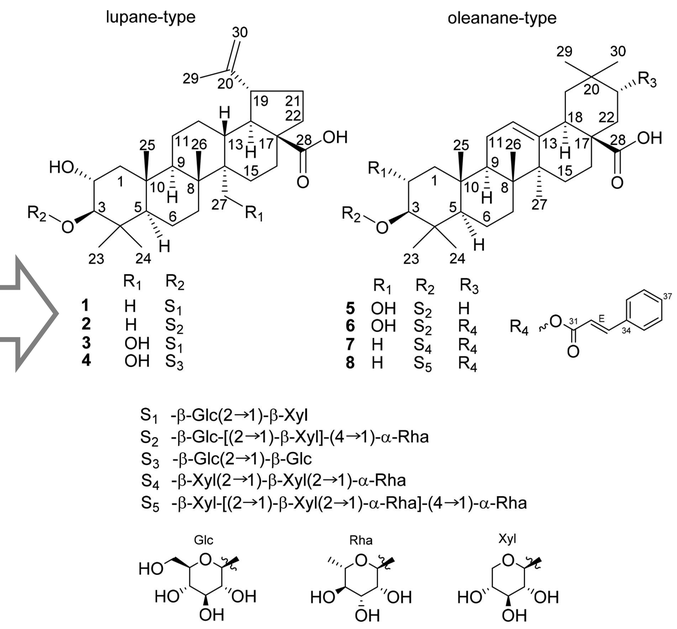
Fractions: A1 and A2

Defatted materials were extracted successively with dichloromethane and methanol. The two resulting bark extracts were combined in order to cover a wide and drug-like range of polarity within one extract. Finally, tannin depletion via polyamide gel was carried out in order to remove common assay interfering compounds.
A1 and A2 represent tannin-depleted fractions.

Microfractions: B1 to B13

A time-based microfractionation via flash chromatography was performed with 250 mg of pooled sample A1+A2. Among the 13 obtained microfractions, B7, B8, and B9 displayed anti-influenza virus activity (Table 1). Their calculated selectivity indices suggest a virus-specific activity of the contained compounds. To identify the compounds present in these fractions, a high-resolution electrospray ionization mass spectrometry as well as 1D and 2D NMR methods were used.

New triterpene saponins: 1-8



Cytotoxicity and anti-influenza A virus activity in MDCK cells

Cytotoxicity and cytopathic effect (CPE) inhibition assays

Cytotoxicity was studied on two-day-old confluent MDCK cell monolayer in 96-well-microtiter plates. Serial three-fold dilutions of the extract (up to 100 µg/mL) or compounds (up to 100 µM) were prepared in test medium. Cell viability was analyzed after 72 h of incubation as described previously [Schmidtke et al., J Virol Methods. 2001 Jun;95(1-2):133-43].
CPE assays were performed with influenza virus A/Hong Kong/68 (HK/68, H3N2) and A/Jena/8178/09 (Jena/8178; A(H1N1)pdm09). The inhibition of virus-induced CPE by the tested extracts and compounds (same concentration as in cytotoxicity assay) was scored 48 h post infection as described (Schmidtke et al., J Virol Methods. 2001 Jun;95(1-2):133-43]. The 50% cytotoxic and the 50% inhibitory concentration (CC_{50} and IC_{50} values) were determined from the mean dose response curves of 3 separate experiments by linear regression. The selectivity index (SI) was represents the ratio of both values ($SI = CC_{50}/IC_{50}$).

Table 1: Cytotoxicity and anti-influenza A virus activity of A1, A2, and B1 to B13.

code	CC_{50} [µg/mL] in MDCK cells	IC_{50} [µg/mL] of CPE of IAV[b] HK/68	selectivity index [CC_{50}/IC_{50}]
A1	49.2 ± 22.5	4.0 ± 0.29	12
A2	33.3 ± 7.38	3.0 ± 2.02	11
B1	> 100	n.a.c	-
B2	> 100	n.a.c	-
B3	> 100	n.a.c	-
B4	> 100	n.a.c	-
B5	> 100	n.a.c	-
B6	26.6 ± 6.00	5.8 ± 0.59	5
B7	1.4 ± 0.27	0.17 ± 0.04	8
B8	0.76 ± 0.41	0.13 ± 0.03	6
B9	2.4 ± 1.25	0.17 ± 0.05	14
B10	> 100	n.a.c	-
B11	18.6 ± 9.87	n.a.c	-
B12	> 100	31.6 ± 13.5	-
B13	> 100	n.a.c	-

Table 2: Cytotoxicity and anti-influenza A virus activity of compounds 1 to 8 and their aglycones.

code	CC_{50} [µM] in MDCK cells	IC_{50} [µM] of CPE IAV[a] HK/68	IAV[b] Jena/8178	selectivity index IAV[a] HK/68 [CC_{50}/IC_{50}]	selectivity index IAV[a] Jena/8178 [CC_{50}/IC_{50}]
1	> 100	n.a.[c]	38.6 ± 15.7	-	-
2	10.4 ± 0.52	1.05 ± 0.44	1.89 ± 0.08	11	6
3	> 100	n.a.[c]	n.a.[c]	-	-
4	> 100	n.a.[c]	43.0 ± 11.8	-	> 2
5	15.5 ± 1.69	1.7 ± 0.07	n.a.[c]	9	-
6	5.8 ± 0.69	1.8 ± 0.23	1.8 ± 0.03	3	3
7	1.5 ± 0.80	0.05 ± 0.02	0.27 ± 0.13	31	6
8	1.2 ± 0.10	0.17 ± 0.18	0.16 ± 0.07	7	7
alphitolic acid	49.9 ± 6.68	n.a.[c]	n.a.[c]	-	-
27-hydroxyalphitolic acid	> 100	n.a.[c]	26.5 ± 4.71	-	> 4
maslinic acid	23.3 ± 2.93	n.a.[c]	n.a.[c]	-	-
21-cinnamoyloxy-maslinic acid	> 100	11.3 ± 7.35	6.9 ± 2.97	> 9	> 14
21-cinnamoyloxy-oleanolic acid	> 100	8.9 ± 3.95	6.8 ± 4.12	> 11	> 15
oseltamivir	-	0.003 ± 0.001	0.064	-	-

[a] Their 50% mean cytotoxic concentration (CC_{50}) in MDCK cells and their 50% inhibition concentration (IC_{50}) determined against HK/68 and Jena/8178 in the CPE inhibition assay in MDCK cells are presented ($n = 3$). [b] Influenza A virus (IAV). [c] No activity (n.a.).

SUMMARY

The study has given access to eight so far undiscovered triterpene saponins and four so far undescribed aglycone structures from *B. africana* bark. The antiviral results might help to explain the rationale behind the traditional use of this herbal remedy in Southern Africa and warrant further studies on the antiviral mechanism of action.