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Research Article

Indole Alkaloids with Antifungal Potential from the Stem Bark of *Tabernaemontana stapfiana*

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ABSTRACT
Fungal infections are a very common health problem that is often treated by traditional medicine. *Tabernaemontana stapfiana* Britten (Apocynaceae) is used in the traditional medicine of Kenya against several ailments including fungal infections. The monoterpenoid indole alkaloids coronaridine (1) and pericyclivine (2), together with lup-20(29)en-3β-yl-acetate (3) and curcuphenol (4) were isolated from the stem bark of this species. In addition, a series of indole alkaloids including trimeric compounds were characterized by ESI-MS investigations. The alkaloid containing methanol and ethyl acetate crude extracts exhibited cytotoxic activity against HT29 and PC3 cancer cell lines as well as antifungal activity against the phytopathogens *Septoria tritici* and *Botrytis cinerea*.

Keywords: *Tabernaemontana stapfiana*, Apocynaceae, indole alkaloids, curcuphenol, antifungal activity, cytotoxic activity.

INTRODUCTION

Fungal infections are more common today than ever before. There is also an increase in opportunistic infections, due to diseases like HIV/AIDS[1]. Although *Candida albicans* remains the major species isolated from clinical samples in HIV patients, other infections such as those caused by *Cryptococcus neoformans, Microsporum gypseum* and *Trichophyton menthagrophytes* are emerging[2,3]. Over the years, intensive efforts have been made to discover new clinically useful antifungal drugs. However, some fungi develop resistance to specific drugs. Plants have an almost limitless ability to synthesize antifungal substances. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and other animals.

Traditional medicine is widely practiced in Kenya, whereby this has been documented by ethnobotanical surveys[2,4-8]. The high cost of imported conventional drugs and/or inaccessibility to Western health care facilities has led to overreliance on traditional medicine since it is affordable and available to rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and acceptable system from a cultural perspective[5,8]. Infections associated with fungi are among some of the indications treated using traditional remedies in Kenya. Natural products of higher plants may provide a new source of antifungal agents with possibly novel mechanisms of action[9].

The alkaloid rich genus *Tabernaemontana* (Apocynaceae) contains approximately 100 species with pan-tropical distribution, which are used as timber, ornamental and traditional medicine[10,11]. *Tabernaemonatana stapfiana* Britten, an evergreen medium-sized tree growing in secondary forests in Eastern Africa, is used as traditional medicinal plant in most parts of Kenya against abdominal problems and diverse ailments, including fungal infections[12,13]. *Tabernaemontana* species are well known for their monoterpenoid indole alkaloid contents with various skeletal types described[14]. The indole alkaloids tubotaiwin and tubotaiwin-*N*-oxide were isolated from the root bark of *T. stapfiana* during early investigations[15]. In addition, further indole alkaloids of different structural types were isolated from the stem bark[16]: the monomeric compounds pericyclivine, perivine, ibogamine, isovoacangine, and the bisalkaloids conodurine, conoduramine, 12,20-epoxyconoduramine, gabunine, gabunamine and tabernamine. Recent investigations indicated significant antifungal and antibacterial activity for the organic extracts from root and stem bark of *T. stapfiana* Britten[2,12], however, the responsible compound were not characterized. These results prompted us to investigate the stem bark of *T. stapfiana* Britten collected in Kenya by detailed ESI-MS experiments combined with isolation and determination of biological activities.

MATERIAL AND METHODS

Plant Material

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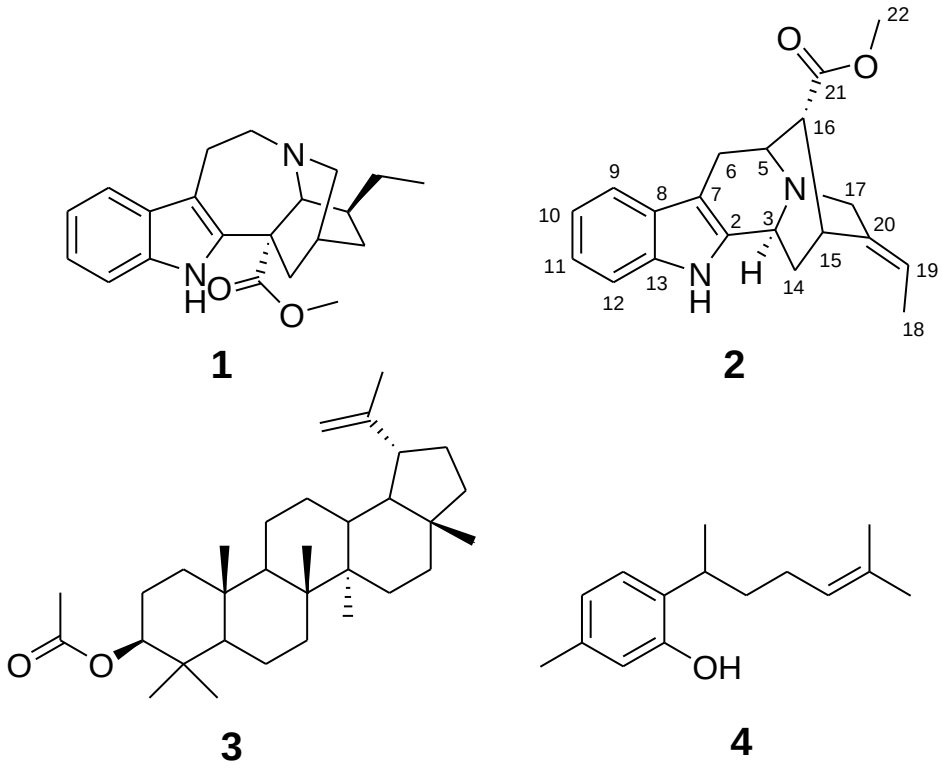

Figure 1: Structures of coronaridine (**1**), pericyclivine (**2**), lup-20(29)en-3β-yl-acetate (**3**) and curcuphenol (**4**) isolated from the stem bark of *Tabernaemonatana stapfiana*

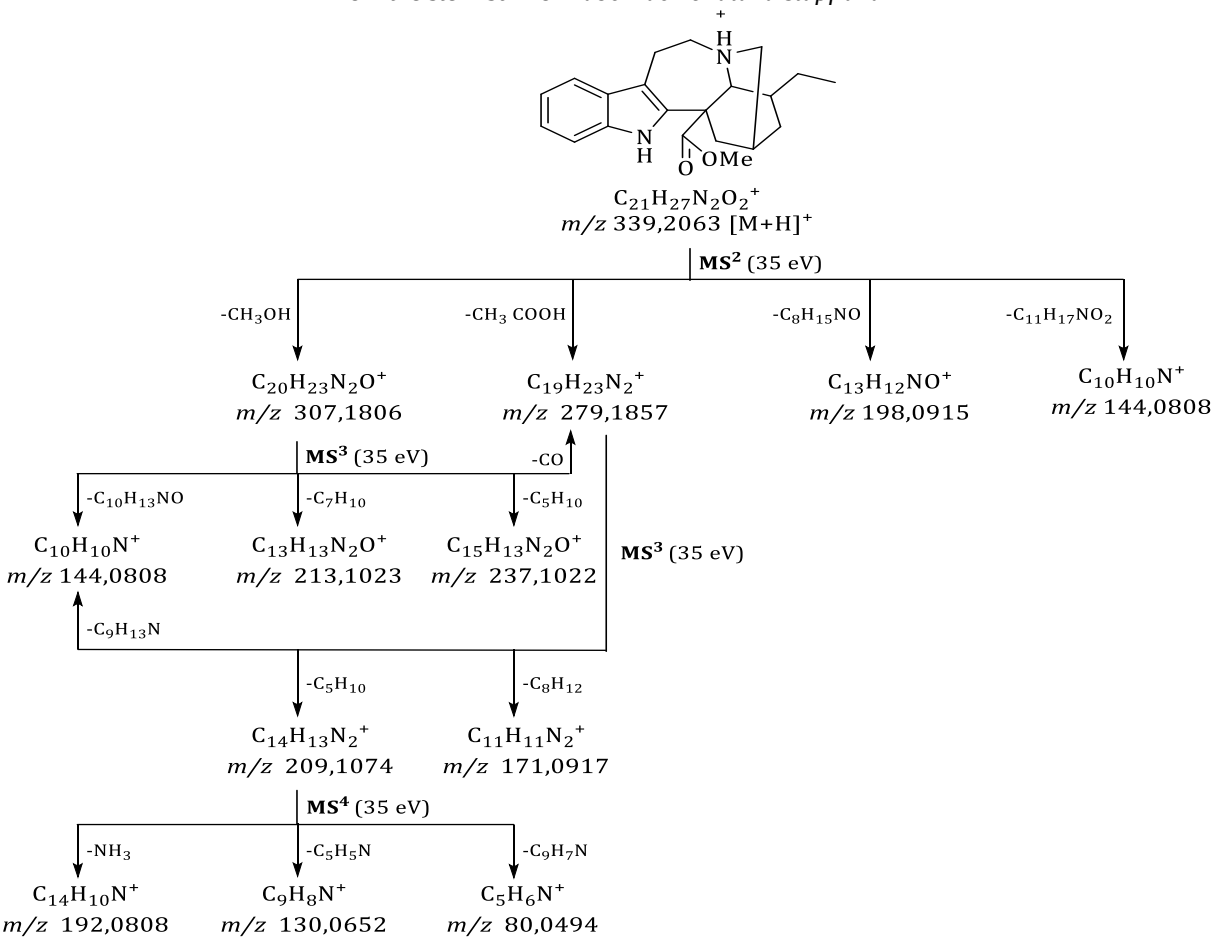

Figure 2: Fragmentation scheme of (-)-coronaridine (**1**), CID (normalized collision energy for all MS/MS measurements), ESI, positive mode

Table 1: 2D NMR data of pericyclivine (2), $CDCl_3$

C	13C □	1H □, multiplicity, *J* (Hz)	HMBC	COSY	ROESY
2	136.7				
3	50.4	4.30 brd (9.9)	C-2, C-5, C-7, C-14	H-14b	H-14b, H-17
5	53.2	3.73 dd (3.1, 10.8)	C-2, C-3, C-6, C-7, C-17	H-6b, H-16	H-6a, H-6b, H-16, H-17
6a	23.9	3.26 dd (15.8, 1.5)	C-2, C-5, C-7, C-8, C-16, C-21	H-6b	H-6b
6b		2.98 s	C-2, C-7		
7	105.4				
8	126.9				
9	117.8	7.40 br d (8.1)	C-7, C-8, C-11, C-13	H-10	H-10
10	119.3	7.04 t (7.7, 8.7)	C-8, C-12		
11	121.5	7.10 t (8.7, 8.4)	C-9, C-12, C-13	H-10	
12	111.0	7.29 d (8.1)	C-8, C-10	H-11	H-11
13	136.6				
14a	26.8	2.61 m	C-2, C-3 C-20, C-15		H-14b
14b		1.79 tr (11.7, 11.4)			
15	27.1	2.99 brs	C-2, C-5, C-7, C-16	H-14a	H-14a, H-14b, H-16. H-18
16	43.6	2.84 brdd (11.0, 2.6)	C-5, C-15, C-21		
17	55.7	3.67 s	C-3, C-19, C-20,		
18	12.9	1.62 dt (6.7, 2.0)	C-19, C-20		
19	115.2	5.27 brq (6.7)	C-15, C-17, C-18	H-18	H-17, H-18
20	138.0				
21	172.4				
22	50.9	3.06 (s)	172.4		
NH	-	8.06 brs			

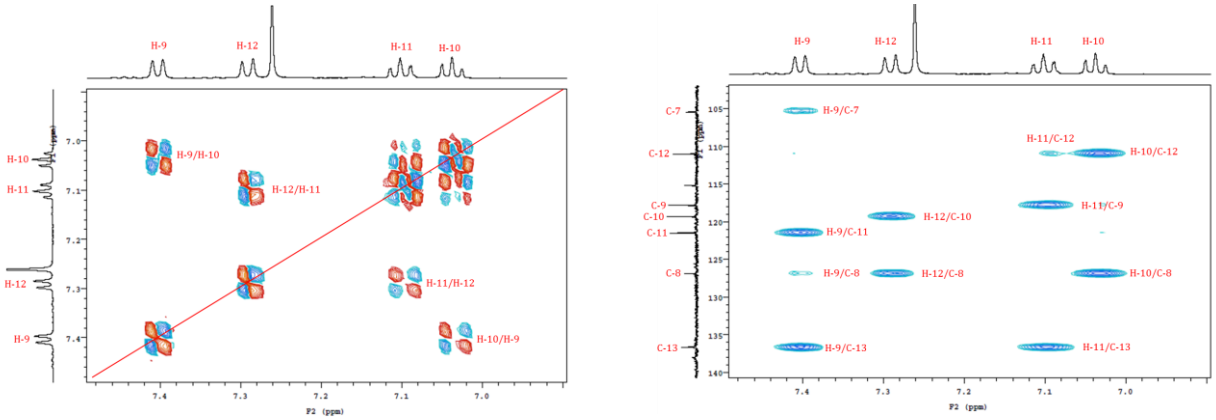

Figure 3: Expansion of COSY and HMBC (600 MHz, $CDCl_3$) spectra of pericyclivine (**2**).

Tabernaemonatana stapfiana Britten was collected from Kaptagat Forest in the Keiyo District located in the Rift Valley Province of Kenya, about 40 km east of Eldoret Town in March 2006 and authenticated by the taxonomist Mr. Kirimi of Kenyatta University. Re-collection of this plant was done in March 2012 from the same place. A voucher (reference number ER/001/06) is deposited at the Kenyatta University herbarium. The species is also treated with the synonyms *Conopharyngia bequaertii* De Wild., *Conopharyngia johnstonii* Stapf, *Conopharyngia stapfiana* (Britten) Stapf, *Sarcopharyngia stapfiana* (Britten) Boiteau, and *Tabernaemontana johnstonii* (Stapf) Pichon.

Extraction and Isolation

Dried pulverized stem bark was sequentially extracted with *n*-hexane, ethyl acetate and methanol. The ethyl acetate extract (13 g) was further separated by repeated column chromatography on silica gel using *n*-hexane/ethyl acetate and chloroform/methanol gradients to isolate coronaridine (**1**, 30 mg, Fig. 1)[17], pericyclivine (**2**, 23.1 mg)[11] and lup-20(29)en-3β-yl-acetate (**3**, 12.5 mg)[18].

The methanol extract (19 g) was fractionated using Diaion HP20 eluted successively with water, methanol, acetone and ethyl acetate. Therefrom the methanol fraction (12 g) was purified on silica gel (*n*-hexane/ethyl acetate gradient) followed by Sephadex LH20 to obtain curcuphenol (**4**, 4.8 mg)[19] and more coronaridine (**1**, 4.8 mg).

Coronaridine (**1**): □□□$_D^{25}$ = –32 (*c* = 0.17, $CHCl_3$). ESI-FTMS/MS, CID 35%, *m/z* (rel. intensity [%]): 339.2073 $[M+H]^+$ (70) calc. for $C_{21}H_{27}N_2O_2^+$ 339.2067; 307.1806 $[M+H-CH_3OH]^+$ (100) calc. for $C_{20}H_{23}N_2O^+$ 307.1805; 279.1857 $[M+H-CH_3COOH]^+$ (30) calc. for $C_{19}H_{23}N_2^+$

279.1856; 198.0915 (19) calc. for $C_{13}H_{12}NO^+$ 198.0913; 144.0808 (57) calc. for $C_{10}H_{10}N^+$ 144.0808.

Pericyclivine (**2**): ESI-FTMS/MS, CID 35%, *m/z* (rel. intensity [%]): 323.1750 $[M+H]^+$ (3) calc. for $C_{20}H_{23}O_2N_2^+$ 323.1754; 306.1484 $[M+H\text{-}NH_3]^+$ (54) calc. for $C_{20}H_{20}NO_2^+$ 306.1489; 291.1489 $[M+H\text{-}CH_3OH]^+$ (99) calc. for $C_{19}H_{19}N_2O^+$ 291.1492; 263.1540 $[M+H\text{-}CH_3COOH]^+$ (25) calc. for $C_{18}H_{19}N_2^+$ 263.1543; 222.1276 (40) calc. for $C_{16}H_{16}N^+$ 222.1277; 180.1018 (60) calc. for $C_{10}H_{14}NO_2^+$ 180.1019, 166.0862 (100) calc. for $C_9H_{12}NO_2^+$ 166.0863; 158.0964 (49) calc. for $C_{11}H_{12}N^+$ 158.0964, 144.0807 (48) calc. for $C_{10}H_{10}N^+$ 144.0808.

Table 2: High-resolution ESI-FTMS and UHPLC-ESI-MS/MS data of alkaloids from the methanol extract of *Tabernaemontana stapfiana*.

MW	$[M+H]^+$ (*m/z*)	Elemental composition	Error (ppm)	MS/MS ESI-CID (*m/z*, Relative Intensity)[b]	Rt (min)
264	265.1699	$C_{18}H_{21}N_2^+$	0.02	265 (70), 251 (10), 250 (53), 248 (35), 236 (55), 233 (30), 222 (45), 208 (15), 193 (10), 170 (12), 158 (25), 143 (10), 134 (100), 116 (10), 108 (22), 107 (10).	8.25
278	279.1855	$C_{19}H_{23}N_2^+$	0.1		
280	281.2011	$C_{19}H_{25}N_2^+$	0.4	281 (98), 264 (5), 252 (100), 235 (10), 206 (13), 194 (15), 182 (15), 168 (10), 157 (25), 144 (100), 130 (16), 122 (80), 120 (10), 93 (5), 93 (25), 79 (27).	8.46
294	295.1804	$C_{19}H_{23}N_2O^+$	0.1		
296	297.1964	$C_{19}H_{25}N_2O^+$	0.8		
308	309.1961	$C_{20}H_{25}N_2O^+$	0.1	309 (20), 307 (98), 293 (100), 277 (45), 263 (98), 248 (22), 230 (17), 211 (15), 205 (20), 194 (10), 181 (15), 144 (10), 130 (19), 122 (5).	8.24
322	323.1753	$C_{20}H_{23}N_2O_2^+$	0.3	323 (30), 322 (10), 305 (100), 279 (13), 277 (33), 263 (5), 252 (10), 224 (8), 221 (13), 220 (20), 213 (25), 206 (7), 195 (37), 172 (10), 168 (80), 144 (20), 122 (20).	6.29
				323 (100), 305 (10), 289 (5), 276 (7), 250 (13), 235 (5), 223(13), 207 (13), 181 (10), 181 (8), 180 (27), 170 (55), 166 (20), 148 (9), 144 (74), 134 (20), 122 (16).	7.07
				323 (80), 306 (30), 291 (43), 280 (15), 262 (25), 246 (30), 235 (12), 222 (42), 193 (14), 192 (20), 169 (23), 168 (28), 166 (100), 157 (24), 144 (82), 132 (11), 107 (10).	8.48
336	337.1910	$C_{21}H_{25}N_2O_2^+$	0.6	337 (70), 304 (95), 276 (25), 239 (10), 228 (15), 227 (65), 195 (41), 168 (92), 167 (100), 143 (17), 122 (20), 121(40).	8.79
				337 (44), 305 (66), 277 (23), 250 (5), 234 (6), 228 (43), 221 (23), 206 (7), 196 (33), 169 (6), 168 (100), 167 (7), 143 (13), 122 (28).	8.88
338	339.1704	$C_{20}H_{23}N_2O_3^+$	0.2		
338	339.2066	$C_{21}H_{27}N_2O_2^+$	0.5		
352	353.1863	$C_{21}H_{25}N_2O_3^+$	0.8	353 (75), 352 (100), 321 (75), 289 (38), 262 (57), 248 (38), 234 (25), 193 (13), 138 (27), 137 (36), 135 (15).	5.93
354	355.2015	$C_{21}H_{27}N_2O_3^+$	0.2		
366	367.2015	$C_{22}H_{27}N_2O_3$	0.4		
368	369.2171	$C_{22}H_{29}N_2O_3^+$	0.4	369 (10), 352 (5), 307 (12), 294 (5), 277 (10), 265 (25), 251 (20), 239 (30), 209 (10), 200 (24), 175 (15), 174 (100), 162 (30), 158 (20), 122 (13), 121 (34) , 106 (19), 91 (25), 78 (5).	9.16
382	383.1970	$C_{22}H_{27}N_2O_4^+$	0.5		
384	385.2117	$C_{22}H_{29}N_2O_4^+$	1.2		
510	511.2075	$C_{27}H_{31}N_2O_8^+$	0.3	511 (62), 350 (24) 349 (100), 317 (10).	7.65
568	569.3276	$C_{38}H_{41}N_4O^+$	0.2	569 (15), 552 (10), 459 (100), 457 (45), 438 (35), 421 (35), 395 (16), 372 (15), 336 (13), 331 (65), 305 (67), 295 (43), 273 (70), 245 (95), 224 (15), 193 (20), 164 (32), 163 (37), 157 (52), 132 (12), 108 (22).	13.48
616	617.3855	$C_{40}H_{49}N_4O_2^+$	0.8		
658	659.3598	$C_{41}H_{47}N_4O_4^+$	0.9		
670	671.3615	$C_{42}H_{47}N_4O_4^+$	3.4		
672	673.3753	$C_{42}H_{49}N_4O_4^+$	0.7		
674	675.3926	$C_{42}H_{51}N_4O_4+$	3.1		
688	689.3703	$C_{42}H_{49}N_4O_5^+$	0.8		
690	691.3857	$C_{42}H_{51}N_4O_5^+$	0.3		

702	703.3867	$C_{43}H_{51}N_4O_5$+	0.2		
704	705.4005	$C_{43}H_{53}N_4O_5^+$	0.8	705 (100), 688 (15), 656 (5), 448 (5), 395 (5), 322 (23), 280 (7), 225 (5), 165 (15).	13.00
				705 (40), 688 (10), 448 (5), 395 (5), 322 (23), 296 (40), 280 (7), 225 (5), 165 (15).	13.19
718	719.3816	$C_{43}H_{51}N_4O_6^+$	1.8	719 (35), 687 (12), 656 (10), 556 (10), 527 (9), 461 (10), 409 (14), 395 (100), 383 (20), 337 (10), 310 (40), 279 (70), 273 (24), 254 (10), 221 (10), 193 (25), 179 (28), 155 (10).	13.30
				719 (10), 656 (5), 628 (12), 596 (12), 556 (10), 530 (15), 409 (20), 395 (100), 337 (65), 279 (27), 247 (8), 220 (5), 179 (11).	13.49
720	721.3958	$C_{43}H_{53}N_4O_6^+$	0.3		
734	735.4111	$C_{44}H_{55}N_4O_6^+$	0.7		
704	353.2041[a]	$C_{43}H_{54}N_4O_5^{2+}$	0.2	544 (15), 523 (20), 522 (10), 353 (100), 337 (80), 336 (55), 307 (85), 279 (47), 248 (40), 194 (28), 190 (40), 181 (50), 179 (38), 144 (18), 129 (36), 123 (18).	8.57
				545 (20), 524 (25), 353 (60), 337 (90), 307 (65), 292 (24), 247 (20), 220 (15), 180 (23), 129 (24).	8.78
				544 (20), 523 (15), 353 (20), 338 (54), 307 (53), 306 (30), 281 (20), 191 (20), 189 (23), 130 (30).	9.00
				546 (10), 545 (15), 524 (15), 353 (10), 352 (15), 338 (45), 337 (100), 307 (50), 279 (20), 262 (15), 233 (10), 190 (16), 182 (12), 129 (12).	9.19
1010	506.2734[a]	$C_{62}H_{72}N_6O_7^{2+}$	0.5		
1024	513.2809[a]	$C_{63}H_{74}N_6O_7^{2+}$	0.9		
1036	519.2628[a]	$C_{63}H_{70}N_6O_8^{2+}$	0.1		
1050	526.2703[a]	$C_{64}H_{72}N_6O_8^{2+}$	0.5		
1056	529.2935[a]	$C_{64}H_{78}N_6O_8^{2+}$	0.3		
1070	536.3012[a]	$C_{65}H_{80}N_6O_8^{2+}$	0.3		

[a]$[M+2H]^{2+}$

[b]Ions selected for MS/MS are bold.

General procedures

Optical rotations were measured using a JASCO P-2000 digital polarimeter. UV spectra were obtained on a JASCO V-560 UV/VIS spectrophotometer. ^1H, ^{13}C NMR and 2D spectra were recorded on an Agilent DD2 400 NMR spectrometer at 399.915 and 100.569 MHz and on an Agilent VNMRS 600 NMR spectrometer at 600 and 150 MHz, respectively. The ^1H NMR chemical shifts are referenced to internal TMS (δ_H 0.0); ^{13}C NMR chemical shifts are referenced to internal $CDCl_3$ (δ_C 77.0). Analytical TLC was performed on silica gel plates 60 F254 (Merck). Spots were visualized using UV light at 254 and 366 nm or by spraying with vanillin-H_2SO_4 or Dragendorff reagent.

Mass spectrometry

The main alkaloid-containing extracts from the stem bark of *Tabernaemontana stapfiana* Britten were dissolved in methanol and investigated by direct-infusion ESI-FT-ICR mass spectrometry. The positive ion high resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (BrukerDaltonics, Billerica, USA) equipped with an InfinityÔ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent, off axis spray). Nitrogen was used as drying gas at 150 °C. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 ml/h. The data was acquired with 512k data points, zero filled to 2048k by averaging 16 scans and evaluated using the Bruker XMASS software (Version 7.0.8).

The methanol extract which had high alkaloid content (as indicated by HRMS) was subjected to liquid chromatography/electrospray tandem mass spectrometry (LC-ESI-MS/MS). The positive ion ESI mass spectra and the MS/MS experiments of the selected samples were obtained from a TSQ Quantum Ultra AM system (ThermoFinnigan) equipped with a hot ESI source (HESI, electrospray voltage 3.0 kV, sheath gas: nitrogen; vaporizer temperature: 50 °C; capillary temperature: 250 °C; collision gas: argon; collision pressure: 1.5 mTorr). The MS is coupled with an Accela UHPLC system equipped with a RP-18 column (1.7 mm particle size, 50x2.1 mm, Syncronis C18, Thermo Scientific). For the HPLC a gradient system was used starting from H_2O:CH_3CN 95:5 (v/v, each of them containing 0.2 % formic acid) to 0:100 within 20 min; flow rate 150 ml/min. The CID measurements were performed during the HPLC run by using collision energies of 30 or 40 eV. All spectra are averaged and background subtracted.

The high resolution ESI mass spectra of compounds **1** and **2** as well as the corresponding Collision-Induced-Dissociation (CID) MS^n measurements were obtained from an Orbitrap Elite mass spectrometer (Thermofisher Scientific, Bremen, Germany) equipped with an HESI

Table 3: Antifungal activity (growth inhibition [%]) of extracts and compounds of *Tabernaemontana stapfiana* against *S. tritici and B. cinereal.*

	Septoria tritici		*Botrytis cinerea*	
extract/compound	250[a]/ 42[b] µg/ml	28[a]/ 14[b] µg/ml	250[a]/ 42[b] µg/ml	28[a]/ 14[b] µg/ml
methanol	92.8 ± 0.7	14.9 ± 5.1	97.7 ± 0.5	70.3 ± 2.0
ethyl acetate	37.8 ± 6.8	11.5 ± 11.5	100.7 ± 1.3	31.4 ± 2.2
n-hexane	27.1 ± 7.4	14.9 ± 5.1	62.8 ± 2.5	2.1 ± 3.8
1	79.0 ± 5.3	17.6 ± 15.1	64.7 ± 1.9	51.9 ± 0.9
4	99.0 ± 5.4	60.0 ± 12.6	101.0 ± 0.8	57.1 ± 2.0

[a]test concentration of extracts
[b]test concentration of compounds

electrospray ion source (spray voltage 4 kV; capillary temperature 275 °C, source heater temperature 40 °C; FTMS resolution 60.000). Nitrogen was used as sheath gas. The sample solutions were introduced continuously via a 500 µl Hamilton syringe pump with a flow rate of 5 µl/min. The data were evaluated by the Xcalibur software 2.7 SP1.

Biological Assays

The crude extracts of *Tabernaemontana stapfiana* were tested for their cytotoxic activity[20] against the colon cancer cell line HT29 and the prostate cancer cell line PC3 and for antifungal activity[21] against the phytopathogens *Septoria tritici* Desm. and *Botrytis cinerea* Pers. according to the methods described in the mentioned references.

Briefly, for the cytotoxicity assay the human prostate cancer cell line PC-3 and the colon cancer cell line HT-29 were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-alanyl-L-glutamine (200 mM) and 1.6% hepes (1 M). 5 × 10^2 PC-3 cells and 1.5 × 10^3 HT-29 cells were seeded overnight into 96-well plates and exposed to a serial dilution of each compound (10 µM and 10 nM) and extract (50 and 0.50 µg/ml) for three days. Cytotoxicity was determined utilizing a modified XTT method (0.25 mg/ml XTT, 6.5 µM PMS).

In the antifungal bioassay the crude extracts and pure compounds were tested in 96-well microtiter plate assays against the phytopathogenic ascomycetes *Botrytis cinerea* Pers. and *Septoria tritici* Desm. according to the monitoring methods approved by the fungicide resistance action committee (FRAC) with minor modifications. Crude extracts were examined at 250 and 28 µg/ml, while pure compounds were tested at 42 and 14 µg/ml. The solvent DMSO was used as negative control (max. concentration 2.5 %), while the commercial fungicide pyraclostrobin (Sigma Aldrich, Germany) served as positive control (100% inhibition at 83.3 µM). Five to seven days after inoculation, pathogen growth was evaluated by measurement of the optical density (OD) at λ 405 nm with a TecanGENios Pro microplate reader (5 measurements per well using multiple reads in a 3 x 3 square). Each experiment was carried out in triplicates.

RESULTS AND DISCUSSION

The phytochemical investigation of the stem bark from *Tabernaemontana stapfiana* resulted in the isolation of the monoterpenoid indole alkaloids coronaridine (**1**)[17] and pericyclivine (**2**)[11] from the ethyl acetate extract. Furthermore, lup-20(29)en-3β-yl-acetate (**3** mg)[18] and curcuphenol (**4**)[19] were obtained from the ethyl acetate and methanol extract, respectively.

To our knowledge, this is the first record for the isolation of curcuphenol from this genus. However, the occurrence of lupeol or the acetate thereof is common in the genus and the family[11].

The structures of isolated compounds (Fig. 1) were elucidated by analysis of ESI-HRMS, ESI-MS/MS (Fig. 2) and NMR spectroscopic data as well as by comparison of spectral data with published data[17,18,19]. The structures of **1** and **2** were verified by 2D NMR experiments. Based on 2D NMR (Tab.1, Fig. 3) the previous assignment of carbon atoms of pericyclivine (**2**) was corrected.

In addition, in the methanol extract, a series of monoterpenoid indole alkaloids were detected by ESI-HRMS investigations (Table 2). The results indicate the presence of monomeric (*m/z* 265 to 385 $[M+H]^+$), dimeric (*m/z* 569 to 735 $[M+H]^+$) and even trimeric (*m/z* 506 to 536 $[M+2H]^{2+}$) indole alkaloids (Table 2). To our knowledge, trimeric indole alkaloids were not reported from the genus before. So far, only one tetrameric monoterpene indole alkaloid with the molecular weight of 1404 g/mol named alasmontamine A was isolated from *Tabernaemontana elegans*[22]. The majority of alkaloids were characterized by ESI-MS/MS experiments. The loss of a 162 unit in the compound with $[M+H]^+$ at *m/z* 511.2075 indicates the occurence of an indole alkaloid glycoside.

Biological activity: Since *Tabernaemontana stapfiana* is used in the folk medicine against fungal infections and the anticancer activity of indole alkaloids is known, the crude extracts were tested for their cytotoxic and antifungal activity. The alkaloid containing MeOH and ethyl acetate extracts of the stem bark significantly inhibited at a concentration of 50 µg/ml the growth of the colon cancer cell line HT29 (92.8 ± 1.8% and 85.0 ± 3.7%, respectively) as well as of the prostate cancer cell line PC3 (93.6 ±1.2% and 87.9 ± 3.0%, respectively). In contrast, the *n*-hexane extract was not active in the same concentration. Also the isolated monomeric indole alkaloids **1** and **2** did not induce a growth inhibition of the tested cell lines up to a concentration of 10 µM. This is in accordance with previous investigations, where cytotoxic activity was mainly observed for bisindole alkaloids like vincristine and related compounds, whereas monomeric compounds exhibited little or no activity[11,23].

The antifungal activities of extracts and compounds against the phytopathogens *Septoria tritici* and *Botrytis cinerea* are summarized in Table 3. The methanolic crude extract exhibited significant activity against both test organisms and also the isolated indole alkaloid coronaridine and the sesquiterpene curcupenol were both active. These results support the ethnobotanic use of *T. stapfiana* against several fungal infections. Not only indole alkaloids but also non alkaloidal compounds contribute to the biological activity. Furthermore, the ESI-MS investigations indicate that the stem bark of *T. stapfiana* is a rich source of bioactive indole alkaloids of different structural types, which are not fully elucidated yet.

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