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To cite this article: Mainen Moshi, Cosam Joseph, Esther Innocent & Mayunga Nkunya (2004) *In Vitro* Antibacterial and Antifungal Activities of Extracts and Compounds from *Uvaria scheffleri*, *Pharmaceutical Biology*, 42:4-5, 269-273, DOI: [10.1080/13880200490511035](https://doi.org/10.1080/13880200490511035)

To link to this article: <https://doi.org/10.1080/13880200490511035>



Published online: 29 Sep 2008.



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In Vitro Antibacterial and Antifungal Activities of Extracts and Compounds from *Uvaria scheffleri*

Mainen J. Moshi¹, Cosam C. Joseph², Esther Innocent¹ and Mayunga H.H. Nkunya²

¹Department of Pharmacology and Toxicology, Institute of Traditional Medicine, MUCHS, Dar Es Salaam, Tanzania;

²Department of Chemistry, Faculty of Science, University of Dar es Salaam, Dar es Salaam, Tanzania

Abstract

Petroleum ether, dichloromethane, and ethanolic extracts of the stem bark and leaves of *Uvaria scheffleri* Diels (Annonaceae) exhibited antifungal activity against *Aspergillus niger* (wild strain), *Aspergillus fumigatus* (wild strain), and a *Penicillium* species (wild strain). The ethanol extract of the stem bark was also active against *Candida albicans* (Strain H6392). The dichloromethane extract of the leaves showed the highest antifungal activity and in addition it showed antibacterial activity against *Staphylococcus aureus* (NCTC 6571). Fractionation of the dichloromethane extract of the leaves yielded nine known compounds. They included a 1:1 mixture of stigmasterol (**1**) and β -sitosterol (**2**), which showed antifungal activity against *Candida albicans*. Others were 3-farnesylindole (**3**), 2',6'-dihydroxy-3',4'-dimethoxychalcone (**4**), 2'-hydroxy-3',4',6'-trimethoxychalcone (**5**), 5-hydroxy-7,8-dimethoxyflavanone (**6**), 5,7,8-trimethoxyflavanone (**7**), and a 3:2 mixture of 2',6'-dihydroxy-4'-methoxychalcone (**8**) and 5,7-dihydroxyflavone (**9**). Compound **7** and the mixture of compounds **8** and **9** showed antibacterial activity against *Escherichia coli* (NCTC 10418, MIC 125 μ g/ml) and *Staphylococcus aureus* (MIC 125 μ g/ml), respectively. The mixture of compounds **8** and **9** was also active against *Candida albicans* (MIC 31.25 μ g/ml), *Aspergillus niger*, *Aspergillus fumigatus*, and the *Penicillium* species (MIC 1000 μ g/ml). We conclude that *Uvaria scheffleri* extracts contain compounds with antifungal and antibacterial activity. The activities observed in this study are weak. Based on previous studies, it is being speculated that, possibly, the most active compounds were lost during fractionation. Further work to isolate more antifungal and antibacterial compounds is suggested.

Keywords: Antibacterial, antifungal, chalcones, extracts, flavanones, flavones, sterols, *Uvaria scheffleri*.

Introduction

Uvaria scheffleri Diels (Annonaceae) is one of 16 *Uvaria* species occurring in Tanzania and consists of small trees, scrambling shrubs, or lianas, 1.2 m to several meters tall or long. It is widely distributed in East Africa, growing in the coastal evergreen and mountain forests upcountry (Verdcourt, 1971). In Tanzania, it is found in the Kilimanjaro, Arusha, and Tanga regions (Verdcourt, 1971), where it is traditionally widely used for the treatment of fever (Kokwaro, 1976), a symptom that may be associated with malaria, or bacterial or even viral infection. Indeed, extracts of dried leaves, root bark, and stem bark showed positive antimalarial activity against *Plasmodium falciparum* with IC₅₀ values ranging from 5 to 100 μ g/ml (Nkunya et al., 1991). Eight other *Uvaria* species are also reported to have antimalarial activity (Verdcourt, 1971; Vollesen, 1980; Nkunya et al., 1991). Previous phytochemical investigations of the stem bark extracts yielded 3-farnesylindole as the antimalarial constituent (Nkunya et al., 1990). In the same study, two prenylated chalcones, schefflerin and isoschefflerin, were isolated (Nkunya et al., 1990). Other compounds that have previously been isolated from the plant are D:B-friedoolean-5-en-3 β -ol, 2'-hydroxy-3',4',6'-trimethoxychalcone, 2',6'-dihydroxy-3',4'-dimethoxychalcone, β -sitosterol, and benzyl benzoate (Nkunya et al., 1990). Uvaretin, diuvaretin, and (8',9'-

Accepted: July 5, 2004

Address correspondence to: Mainen J. Moshi, Department of Pharmacology and Toxicology, Institute of Traditional Medicine, MUCHS, Box 65001, Dar es Salaam, Tanzania. E-mail: mmoshi@much.ac.tz

dihydroxy)-3-farnesylindole were isolated from the root bark (Nkunya et al., 1991). Uvaretin has been shown in different studies to have antibacterial (Hufford & Lasswell, 1978; Nkunya et al., 1993), cytotoxic (Cole et al., 1976; Hufford & Lasswell, 1976), and antimalarial activity (Nkunya et al., 1991; Weenen et al., 1991). Similarly, diuvaretin has antibacterial (Anam, 1993), antimalarial (Weenen et al., 1991), and cytotoxic activity (Lasswell & Hufford, 1977).

Extracts of the plant were assayed for antibacterial activity as an attempt to further follow up on the traditional uses of the plant for the treatment of fevers. The extracts were also tested for antifungal activity, which has not been reported before. Results from these assays are herein discussed.

Materials and Methods

Materials

Petroleum ether, dichloromethane, and ethanol were purchased from Fisher Scientific UK Ltd (Loughborough, Leicestershire, UK). Sabouraud's dextrose agar (SDA) and broth and Mueller Hinton agar and broth were purchased from Oxoid Ltd (Basingstoke, Hampshire, England), and dimethylsulfoxide (DMSO) was purchased from Sigma (Poole, Dorset, England).

Collection and preparation of plant material

The stem bark and leaves were collected from Kwamgongo village, 5 km from Mombo off the road to Lushoto, Tanga region (Tanzania), and the voucher specimen (voucher no. LBM 12913) was deposited at the Herbarium of the Department of Botany, University of Dar es Salaam. The plant materials were air-dried and then extracted sequentially with petroleum ether, dichloromethane, and ethanol. The crude extracts of both the stem bark and leaves were tested for antibacterial and antifungal activity. The dichloromethane extract of the leaves, which showed the best activity, was chosen for bioassay guided fractionation. It was partitioned to simpler fractions on silica gel, 400 mesh (BDH Chemicals Ltd, Poole, England), by vacuum liquid chromatography (VLC), and the fractions obtained were again subjected to the same tests. The active fractions were further fractionated using silica gel, 400 mesh (BDH Chemicals Ltd) and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala Sweden). The subsequently isolated pure compounds were also tested for antibacterial and antifungal activity. Details of the isolation of compounds, spectral data, and structure elucidation will be published elsewhere.

Antibacterial and antifungal tests

The organisms used in this study, which include *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 0031), *Candida albicans* (strain HG 392), local strains of *Aspergillus niger*,

Aspergillus fumigatus, and a *Penicillium* species were generously supplied by the Department of Microbiology and Immunology, Muhimbili University College of Health Sciences. The hole plate method was used (Malele et al., 1998). Holes made with a cork borer were filled with 10 µl of extracts (1 g/ml), pure compounds (100 mg/ml), standard drug (ampicillin 10 µg/ml, gentamicin 10 µg/ml, ketoconazole 20 µg/ml), or solvent (DMSO). The inhibition zones were measured in millimeters. Minimum inhibitory concentrations (MICs) for bacteria were determined using the same method, whereas for fungi the disk diffusion method was used.

Results

Antibacterial and antifungal activities

Antifungal activity was detected against *Aspergillus niger*, *Aspergillus fumigatus*, and the *Penicillium* species in all the three extracts of the leaves and stem bark, respectively (Table 1). The highest activity was detected in the dichloromethane extract of the leaves, with an MIC of 625 µg/ml. The ethanol extract of the stem bark was the only extract that showed activity against *Candida albicans*. The petroleum ether and ethanol extracts of the leaves were inactive against the *Penicillium* species. The dichloromethane extract of the leaves showed activity against *Staphylococcus aureus*; all the other extracts were inactive against the three bacteria used (Table 1). Fractionation by VLC and chromatography on silica gel and Sephadex LH-20 of this extract yielded nine compounds, which, based on their spectral properties (UV, MS, ¹H and ¹³C NMR), were determined to be already reported compounds (Panichpol & Watermann, 1978; Bohlmann & Abraham, 1979a, 1979b; Bohlmann et al., 1980; Nkunya et al., 1987, 1990; Nkunya & Weenen, 1989). These included a 1:1 mixture of stigmaterol (**1**) [0.0019%] and β-sitosterol (**2**) [0.0019%], 3-farnesylindole (**3**) [0.005%], 2',6'-dihydroxy-3',4'-dimethoxychalcone (**4**) [0.146%], 2'-hydroxy-3',4',6'-trimethoxychalcone (**5**) [0.0057%], 5-hydroxy-7,8-dimethoxyflavanone (**6**) [0.0013%], 5,7,8-trimethoxyflavanone (**7**) [0.0086%], and a 3:2 mixture of 2',6'-dihydroxy-4'-methoxychalcone (**8**) [0.0195%] and 5,7-dihydroxyflavone (**9**) [0.013%] (Fig. 1). The results in Table 1 show that the mixture of stigmaterol (**1**) and β-sitosterol (**2**) had activity against *Candida albicans* only. Compounds **3**, **4**, **5**, **6**, and **7** were inactive against *Candida albicans*, but there was not enough material to test on the other fungi. Compound **7** showed antibacterial activity only against *Escherichia coli*, while the mixture of compounds **8** and **9** had activity against *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus fumigatus*, the *Penicillium* species, and *Candida albicans*. The MIC values of the leaf dichloromethane extract, isolated compounds, and the standard drugs are shown in Table 2. The activity of compounds **8** and **9** on *Candida albicans* was the best, MIC 31.25 µg/ml, but much lower than that of ketoconazole (0.125 µg/ml).

Table 1. Antibacterial and antifungal activities of extracts and compounds from the stem bark and leaf of *Uvaria Scheffleri* Diels. The results are reported as mean $\pm$ SD (n = 3).

Organisms	Standard drugs				Crude extract fractions					Isolated compounds								
	Amp	Genta	Keto	PEL	PES	DCML	DCMS	ETL	ETS	1 & 2	3	4	5	6	7	8 & 9		
<i>E. coli</i>	27.2 $\pm$ 1.3	30.3 $\pm$ 1.5	NT	-	-	-	-	-	-	-	-	-	-	-	7.8 $\pm$ 0.6	-		
<i>S. aureus</i>	29.0 $\pm$ 1.0	NT	NT	-	-	8.4 $\pm$ 0.9	-	-	-	-	-	-	-	-	-	8.2 $\pm$ 1.0		
<i>P. aeruginosa</i>	NT	26.2 $\pm$ 2.2	NT	-	-	-	-	-	-	-	-	-	-	-	-	-		
<i>A. niger</i>	NT	NT	35 $\pm$ 0.5	14.0 $\pm$ 2.0	NT	29.2 $\pm$ 0.8	16.2 $\pm$ 2.2	15.2 $\pm$ 2.8	15.7 $\pm$ 1.5	-	NT	NT	NT	NT	NT	28.0 $\pm$ 1.0		
<i>A. fumigatus</i>	NT	NT	30 $\pm$ 0.3	17.2 $\pm$ 1.0	NT	27.2 $\pm$ 1.3	18.2 $\pm$ 1.3	16.5 $\pm$ 1.3	17.4 $\pm$ 1.8	-	NT	NT	NT	NT	NT	27.7 $\pm$ 1.6		
<i>C. albicans</i>	NT	NT	36 $\pm$ 0.2	-	NT	-	-	-	8.8 $\pm$ 24.2	1.6 $\pm$ 2.0	-	-	-	-	-	15.0 $\pm$ 2.6		
<i>Penicillium</i> spp.	NT	NT	29 $\pm$ 0.4	-	NT	28.3 $\pm$ 1.5	17.5 $\pm$ 1.5	-	17.0 $\pm$ 2.6	-	NT	NT	NT	NT	NT	29.2 $\pm$ 0.8		

Amp, ampicillin; Keto, ketoconazole; Genta, gentamicin; PES, petroleum ether extract of the stem bark; DCML, dichloromethane extract of the leaves; DCMS, dichloromethane extract of the stem bark; ETL, ethanol extract of the leaves; ETS, ethanol extract of the stem bark; numbers 1–9 represent the isolated compounds; NT, not tested.

Discussion

Uvaria scheffleri was reported earlier to have antimalarial activity (Nkunya et al., 1991), and subsequently, 3-farnesylindole (Nkunya et al., 1990), uvaretin (Nkunya et al., 1991; Weenen et al., 1991), and diuvaretin (Weenen et al., 1991), which have antimalarial activity, were isolated from the plant. In addition, uvaretin (Hufford & Lasswell, 1978; Nkunya et al., 1993) and diuvaretin (Anam, 1993) have antibacterial and cytotoxic activity (Hufford & Lasswell, 1976; Lasswell & Hufford, 1977). In this study, the antifungal properties of this plant are being reported for the first time. Unfortunately, although the dichloromethane extract showed good activity, especially, against the non-*Candida* fungi, following chromatographic separation none of the identified pure compounds showed antifungal activity. Antifungal activity was detected from the mixtures of compounds 1 and 2 and of 8 and 9. Previous studies show that both stigmaterol (1) (Ragasar et al., 1997; Jain & Singh, 1999; Gonzalez et al., 2000; Tip Pyang & Miles, 2000; Singh & Dubey, 2001) and β -sitosterol (2) (Banerjee et al., 1984; Jain & Singh, 1999; Kiprono et al., 2000) have antifungal activity, in agreement with the current results. Antifungal activity was also exhibited by the mixture of compounds 8 and 9 against *Candida albicans*. To the best of our knowledge, it is the first time the antifungal activity of the mixture of 2',6'-dihydroxy-4'-methoxychalcone (8) and 5,7-dihydroxyflavone (9) has been reported. It is difficult to tell whether both or just one of them is responsible for the antifungal activity. However, two of the studies done on flavones reported positive activity against different types of fungi (Weidenborner & Jha, 1997; Silva et al., 1998), suggesting the possibility that 5,7-dihydroxyflavone (9) may be playing a role in the antifungal activity of the mixture.

The dichloromethane extract of the leaves also showed a weak antibacterial activity, which did not improve substantially through fractionation. Compound 7 and the mixture of compounds 8 and 9, which were subsequently isolated, showed a weak antibacterial activity. Compound 1 (Srivastava et al., 1997; Jain & Singh, 1999) and compound 2 (Sharma, 1993; Jain & Singh, 1999) are reported to have antibacterial activity, but their mixture that was used in this study was inactive on the bacteria used. Because neither uvaretin (Hufford & Lasswell, 1978; Nkunya et al., 1993) nor diuvaretin (Anam, 1993), which are known to have good antibacterial activity, were isolated, it is difficult to make any conclusive remarks regarding the antibacterial activity of the leaf extracts. The two were previously isolated from this plant (Weenen et al., 1991).

We conclude that *Uvaria scheffleri* extracts contain compounds with antifungal and antibacterial activity. The activities observed in this study are weak. Based on previous studies, it is speculated that, possibly, the most active compounds were lost during fractionation. Further work to isolate more antifungal and antibacterial compounds is suggested.

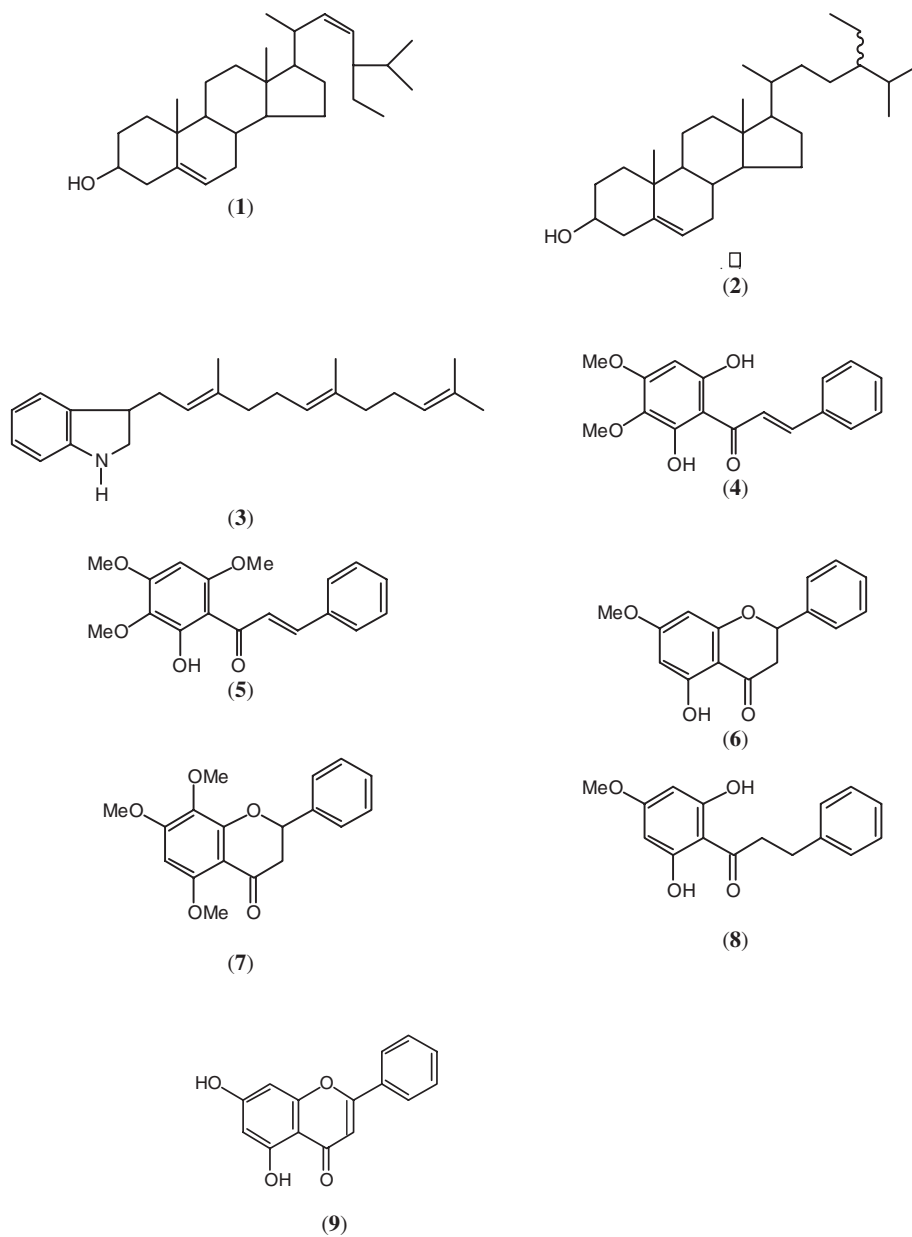


Figure 1. Compounds isolated and tested. Compounds 1 and 2 (1 & 2), and 8 and 9 (8 & 9), were tested as a mixture.

Table 2. Minimum inhibitory concentrations of the leaf dichloromethane extract, isolated compounds and standard drugs.

Organism	Minimum inhibitory concentrations (MICs), µg/ml					
	DMCL	7	8 & 9	Ampicillin	Gentamicin	Ketoconazole
<i>A. niger</i>	625	NT	1000	NT	NT	0.125
<i>A. fumigatus</i>	625	NT	1000	NT	NT	0.125
<i>Penicillium</i> spp.	625	NT	1000	NT	NT	0.25
<i>C. albicans</i>	NT	NT	31.2	NT	NT	0.125
<i>S. aureus</i>	NT	NT	125	0.01	NT	NT
<i>E. coli</i>	NT	125	NT	0.25	NT	NT
<i>P. aeruginosa</i>	NT	NT	NT	NT	1.0	NT

DMCL, dichloromethane extract of the leaves; NT, not tested. The numbers stand for the tested compounds.

- ## Acknowledgments
- We thank Mr. Daniel Kamala for his technical support while testing for antifungal and antibacterial activities and are grateful for access to the NAPRALERT database of the University of Illinois at Chicago.
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