South African Journal of Botany 122 (2019) 518–521

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Antimicrobial lupenol triterpenes and a polyphenol from *Elaeodendron transvaalense*, a popular southern African medicinal bark

G.P. Khumalo [a], N.J. Sadgrove [a], S.F. Van Vuuren [b], B.-E. Van Wyk [a,*]

[a] Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa
[b] Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Road, Park Town 2193, South Africa

ARTICLE INFO

Article history:
Received 5 February 2018
Received in revised form 9 July 2018
Accepted 12 July 2018
Available online 5 October 2018

Edited by J Van Staden

Keywords:
Cassine transvaalensis
Gastrointestinal infections
Lupane triterpene
Skin infections
MIC
Traditional medicine

ABSTRACT

Elaeodendron transvaalense stem bark is widely used in southern African traditional medicine, mainly for gastrointestinal tract disorders and skin ailments. The aim of the study was to investigate the possible scientific rationale for the popularity and ethnobotanical uses of *E.transvaalense*. To achieve this aim, target pathogens related to infections were tested with crude bark extracts and also the main active antimicrobial compounds. Stem bark was purchased from traditional medicine (muthi) markets, powdered and extracted with either methanol or dichloromethane. For the antimicrobial testing, the micro-titre plate broth two-fold serial dilution assay in 96 well plates was undertaken. Bioassay guided fractionation and polar stationary phase column chromatography was used to isolate the active compounds. Crude bark extracts demonstrated moderate activity against Gram-negative(*Escherichia coli*ATCC 8739, *Pseudomonas aeruginosa*ATCC 27853, *Salmonella typhimurium*ATCC 9290, *Shigella sonnei*ATCC 14028) and Gram-positive(*Staphylococcus aureus*ATCC 25923, *Staphylococcus epidermidis*ATCC 12228) organisms. A dichloromethane extract yielded three major triterpenes; lup-20(30)-ene-3α,29-diol(**1**), 6β-hydroxylup-20(29)-ene-3-one(**2**) and 30-hydroxylup-20(29)-ene-3-one(**3**) and one major polyphenol, 4′-O-methylepigallocatechin (**4**). Compounds **1**, **3** and **4** were previously reported from the stem bark of *E. transvaalense*, but this is the first report of **2** in the stem bark of this species, although it was previously reported from the root bark. The presence of **1**–**3** was confirmed to be also in the aqueous extract but a much higher relative abundance of 4′-O-methyl epigallocatechin (**4**) was found. The four compounds demonstrated moderate antimicrobial activity with the lowest MIC value of 0.093 mg/mL against *E.coli* and *S.typhimurium* from **4**, the polyphenol. The antimicrobial activity of *E.transvaalense* stem bark is therefore at least partly due to the presence of lupenol triterpenes and 4′-O-methyl epigallocatechin. The presence of all four compounds in the dichloromethane and aqueous extracts was confirmed, but the overwhelmingly major component in the aqueous extract was **4**. The results support the traditional uses of the bark against gastrointestinal tract and skin infections.

1. Introduction

Elaeodendron transvaalense (Burtt Davy) R.H. Archer (Celastraceae) is the source of one of the most popular barks sold in the traditional medicinal (muthi) markets in southern Africa(Archer and Van Wyk, 1998; Williams etal., 2000; Van Wyk etal., 2009). As a consequence of the unsustainable harvesting of stem bark for the muthi market trade, *E. transvaalense* has the conservation status of Near Threatened in the South African Red Data list (SANBI, 2012).

Anecdotes recorded at the Faraday and KwaMai-Mai muthi markets in Johannesburg indicate that aqueous bark infusions or decoctions canbe used as an enema or administered orally for the treatment ofstomach complaints, internal wounds or as an emetic for treating a congested chest. In Namibia, Zimbabwe and South Africa(Neuwinger, 2000), infusions and decoctions are used to treat diarrhoea, stomach ailments, intestinal cramps (Gelfand etal., 1985; Pujol, 1990), fever (Hutchings etal., 1996; Ndawonde etal., 2007), herpes, coughs (Mabogo, 1990), skin ailments, rashes, swellings, inflammation, stomach cramps (Von Koenen, 2001), sexually transmitted diseases, HIV/AIDS, arthritis and cancer (Tshikalange and Hussein, 2010). Powdered bark is either boiled in water or it may be licked from the palm of the hand and washed down with water (Pujol, 1990; Hutchings etal., 1996). According to Palmer and Pitman (1972), it is the best available medicine for treating stomach-ache and fevers. A study of the cytotoxicity against cancer cell lines has been previously conducted on the species and isolated compounds (Tshikalange and Hussein, 2010). Of the five compounds isolated, lupane triterpenes and β-sitosterol demonstrated the most pronounced activity against Vero and breast cancer cells, with the lowest concentration demonstrated by the 3-oxo-lupenol isolate.

* Corresponding author.
E-mail addresses: Sandy.vanvuuren@wits.ac.za (S.F. Van Vuuren), bevanwyk@uja.c.za (B.-E. Van Wyk).

https://doi.org/10.1016/j.sajb.2018.07.020
0254-6299/

Powdered bark or bark extracts are traditionally the most used in therapeutic applications consistent with antimicrobial outcomes, but extracts or isolated compounds have not yet been tested for possible antimicrobial effects against gastrointestinal or skin pathogens. Thus, the objective of the current study was to determine the antimicrobial effects of the crude extracts against skin and gastrointestinal pathogens and to identify the main chemical compounds responsible for the antimicrobial activity.

2. Materials and methods

2.1. Materials

Stem bark of *Elaeodendron transvaalense* [previously known as *Cassine transvaalensis* (Burtt Davy) Codd (The Plant List, 2017)] was purchased from traders at the Faraday and KwaMai-Mai muthi markets in Johannesburg. The five samples purchased were identified by Gugulethu Khumalo, Ben-Erik Van Wyk and Ekaterina Kotina, as part of a wider survey of the morphology and anatomy of medicinal barks [photographic voucher number 23]. The tree is endemic to southern tropical Africa, with the distribution range extending from the north-eastern parts of South Africa and Swaziland to Botswana, Namibia, Angola, MozambiqueZimbabwe and Zambia. It is commonly known as bushveld saffronwood or as *bosveldsaffraan* in Afrikaans, *omupya* in Herero, *sōhais* in Nama, *mudangwa* in Shona, *ngcotfo* in Swati, *ximapana* in Tsonga, *monomane* in Tswana, *mulumaŋamana* in Venda and *ingwavuma* in Zulu (Van Wyk etal., 2011). The external bark appearance is light grey to strong brown underneath; the texture is smooth with vertical fissures in young branches and relatively coarse in older trunks with irregular fissures. Lenticels are absent. The inner bark is pink in colour and smooth.

2.2. Extraction for minimum inhibitory concentration testing

Dried and powdered bark of the five samples was mixed and soaked in either methanol or dichloromethane for 48 h. The extracts were filtered through Whatman 1 filter paper and the solvent allowed to evaporate completely. Sample dry weights of 0.327 g (3.3% g/g) (methanol) and 0.069 g (0.7% g/g) (dichloromethane) were obtained.

2.3. Antimicrobial studies

2.3.1. Culture preparation

The bacterial strains and other test organisms for antimicrobial screening were selected based on the traditional uses as recorded by traders on the Johannesburg muthi markets (intestinal complaints and infections). These include Gram-negative strains; *Escherichia coli*ATCC 8739, *Pseudomonas aeruginosa*ATCC 27853, *Salmonella typhimurium*ATCC 9290, *Shigella sonnei*ATCC 14028 and Gram-positive strains; *Staphylococcus aureus*ATCC 25923 and *Staphylococcus epidermidis*ATCC 12228.

2.3.2. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) method (Eloff, 1998) was used to determine the susceptibility of test pathogens to plant extracts and isolated compounds. A starting concentration of 8 mg/mL was used for crude extracts while the starting concentration for isolated compounds was 1.25 mg/mL. Acetone was used as a primary solvent for dissolving crude extracts and **4**, while dimethyl sulfoxide (DMSO) at no higher than 6.25% in highest concentration was used for the triterpenes. These solvents were included as negative controls and ciprofloxacin at a starting concentration of 0.01 mg/mL was used as the positive control. Using aseptic technique, 100 μL of Müller Hinton broth was introduced in each well of a 96 well micro-titre plate. Crude extracts as well as isolated compounds (100 μL) were transferred to the first row of the micro-titre plate. Serial dilutions were performed, followed by the addition of 100 μL of the culture (at 1×10^6 CFU/mL) to all the wells. Each plate was thereafter sealed with a sterile adhesive sealing film. The test method was done in duplicate and repeated thrice to confirm accuracy of results. The plates were then incubated overnight at 37 °C. Thereafter, 40 μL of *p*-iodonitrotetrazolium chloride (INT) was added as a growth indicator to each well of the micro-titre plates after incubation. The red colour of the MeOH extracts was very dilute at 1–2 mg/mL, so that after addition of INT, there was a clear difference between the colours. In cases where there was the slightest doubt, we streaked out each well on the agar plate and left it on the desk overnight to double-check or confirm the presence of pathogens. The MIC values were recorded as the lowest concentration of the tested compounds and crude extracts by the appearance of the typical red colour indicating visible microbial growth.

2.4. Compound isolation and structural elucidation

2.4.1. Isolation from dichloromethane (DCM) extracts

Dried and powdered bark (427 g) was soaked in DCM for 48 h. The solvent was then filtered through celite (simply to remove all solids) and evaporated completely. Isolates **1**, **2** and **3**: 400 mg of extract were subjected to flash chromatography over silica gel using 20% ethyl acetate in cyclohexane (80% v/v) as the mobile phase. Combinations ofisolates were guided using TLC and the same mobile phase. Three compounds were isolated and identified as lup-20(30)-ene-3α,29-diol (**1**) (23.3 mg), 6β-hydroxylup-20(29)-ene-3-one(**2**) (67.1 mg) and 30-hydroxylup-20(29)-ene-3-one(**3**) (94.4 mg). The NMR spectra for the three compounds isolated were generated on a 500 MHz Bruker Avance (Bruker, Germany) and the ^{13}C spectra were matched exactly to published values; **1** was matched to spectra reported by Ullah etal. (1999), **2** was matched to spectra by Hisham etal. (1996), and **3** to spectra by Tinto etal. (1992).

Isolation of **4** from DCM extract: A further 400 mg of extract was subjected to flash chromatography over silica gel using 80% ethyl acetate in20% cyclohexane (v/v) as the mobile phase. TLC and the same mobile phase was used to guide fraction collection, which afforded **4**(150 mg), identified by comparing NMR spectra to that provided by Drewes and Mashimbye (1993). The ^{13}C spectra of the compounds **1**–**4** are provided in Appendix A.

2.4.2. Isolation of *1*, *2*, *3* and *4* from aqueous extracts

Dried and powdered bark (327 g) was soaked in 2 L of double-distilled H_2O for 48 h. The aqueous phase was separated by filtration and the more lipophilic fraction was partitioned into ethyl acetate using three consecutive washes of 100 mL. The organic phase was separated and evaporated to dryness and triterpenes were partitioned into DCM from the residue and confirmed by TLC and NMR. Isolate **4** remained in the residue at >90% purity. The residue was subjected to column chromatography using a 80:20 ratio of ethyl acetate:hexane to afford 1.1 g of pure **4**.

3. Results and discussion

The dichloromethane crude extract of *E.transvaalense*(Table 1) exhibited higher activity against the screened bacterial strains compared to the methanolic extract, with values equal to or above 1 mg/mL for the MeOH extract and values ranging from 0.042 to 1 mg/mL for the DCM extract. According to the stricter criteria proposed by Van Vuuren and Holl (2017) this activity may be described as moderate. Due to the more pronounced activity from the DCM extract it is conceivable that the active compounds are of moderate polarity.

Column chromatography of the dichloromethane crude extract afforded four main compounds (**1**–**4**), as shown in Fig. 1. Compound **1** demonstrated the most pronounced activity from the three triterpenes by inhibiting all test organism strains selected, with the lowest MIC value of 0.10 mg/mL against *Pseudomonas aeruginosa* and *Salmonella*

Table 1
Minimum inhibitory concentration (MIC) values (mg/mL) of isolated compounds and crude bark extracts of *Elaeodendron transvaalense* against selected test organisms (pathogens). The compounds are 1, lup-20(30)-ene-3α,29-diol; 2, 6β-hydroxylup-20(29)-ene-3-one; 3, 30-hydroxylup-20(29)-ene-3-one and 4, 4′-*O*-methylepigallocatechin.

	Organism					
	Escherichia coli ATCC 9739	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923	*Staphylococcus epidermidis* ATCC 12228	*Shigella sonnei* ATCC 14028	*Salmonella typhimurium* ATCC 9290
E.transvaalense MeOH extract	1.33	1.00	1.33	1.67	1.00	1.33
E.transvaalense DCM extract	0.67	0.42	0.50	0.50	0.75	1.00
Compound **1**	0.23	0.10	0.16	0.21	0.31	0.10
Compound **2**	0.26	0.26	0.52	0.52	0.83	0.16
Compound **3**	0.52	1.04	0.31	0.31	0.63	0.31
Compound **4**	0.09	0.19	0.19	0.19	0.19	0.09
Ciprofloxacin (μg/mL)	0.16	0.08	0.10	0.16	0.10	0.02
Acetone	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00
DMSO	8.00	8.00	4.00	8.00	5.33	4.00

typhimurium. Compounds **1** and **2** exhibited moderate activity against *Escherichia coli* with MIC values of 0.23 mg/mL and 0.26 mg/mL, respectively. The lowest MIC values from compounds **1** and **2** were against *Salmonella typhimurium* at 0.10 mg/mL and 0.15 mg/mL, respectively.

Compounds **1**–**3** were isolated from the DCM extract but their presence in the aqueous extract, together with **4**, was confirmed by TLC and NMR. Only small concentrations of **1**–**3** were detected using aqueous extraction at room temperature. However, a high relative abundance of compound **4** (0.34% g/g of dried bark) was confirmed. This isolate demonstrated the most pronounced antimicrobial activity, with values ranging from 0.09 to 0.19 mg/mL. This has implications for ethnobotanical uses that employ aqueous extraction but it should be noted that decoctions are likely to yield higher levels of the non-polar compounds, especially when saponins are present. Powdered bark of *E.transvaalensis* is known to be ingested directly by licking it from the palm of the hand and washing it down with water (Pujol, 1990; Hutchings etal., 1996). This is a traditional method of administration known as *khotha*, the Zulu word for 'lick', 'lick up' or 'lick clean' (Doke and Vilakazi, 1972). The bark has been reported to be toxic (Von Koenen, 2001) so that the dosage need to be carefully controlled.

The identity of isolates **1**–**3** were assigned as follows; lup-20(30)-ene-3α,29-diol(**1**), 6β-hydroxylup-20(29)-ene-3-one(**2**) and 30-hydroxylup-20(29)-ene-3-one(**3**). Compounds **1** and **3** were previously identified from *E.transvaalense*, but the current study is the first report of **2** in the stem bark *E.transvaalense*, although it was previously isolated from the root bark (Drewes etal., 1991). All three isolates are lupenol derivatives but **1** is a diol. Isolates **1** and **3** are 3-oxo-lupenol derivatives and so may be of relevance in the anticancer activity that is described anecdotally in traditional medicine (Tshikalange and Hussein, 2010). The greatest cytotoxicity was ascribed to the 3-oxo derivatives (Sheng-Ding etal., 1984). These had the lowest antimicrobial activities in the current study. Conversely, in the previous study, the diol **1** was the least cytotoxic, but the same diol **1** demonstrated the most pronounced antimicrobial activity in the current study. Thus, structure activity relationships suggest that reduction of the 3-oxo group on **2** and **3** enhances antimicrobial activity and reduces cytotoxicity.

The structural similarity of **1** to lupeol, a dietary 3-oxo lupenol triterpene, is evident. Lupeol is well known for its powerful anti-inflammatory and anti-cancer activities (Saleem, 2009) and it will be of interest to investigate possible anti-inflammatory effects of the lupenol triterpenes in *E.transvaalense*.

The identity of isolate **4** was assigned as 4′-*O*-methyl epigallocatechin, which was identified in the extracts of *E.transvaalense* in an earlier study by Tshikalange and Hussein (2010) and demonstrated to have

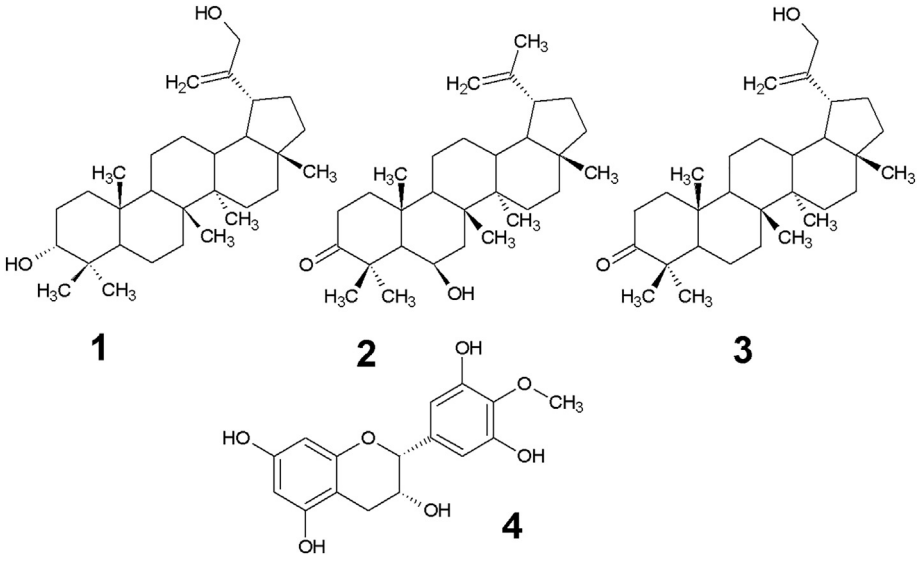

Fig. 1. Compounds isolated from the bark of *Elaeodendron transvaalense*: **1**, lup-20(30)-ene-3α,29-diol; **2**, 6β-hydroxylup-20(29)-ene-3-one; **3**, 30-hydroxylup-20(29)-ene-3-one and **4**, 4′-*O*-methylepigallocatechin.

low cytotoxicity against cancer cell lines. The higher yield in aqueous extracts and the generally lower MIC values (Table1) provides a clear indication that this isolate is of importance in possible antimicrobial effects in traditional practice, especially in cases where aqueous extracts are used, rather than direct ingestion of bark powder. However, combination studies of the triterpenes and **4** should be conducted to provide a clearer picture of such antimicrobial outcomes in practice.

Thus, our results show that the stem bark of *E.transvaalense* contains three main lupenol triterpenes and one polyphenol with moderate antimicrobial activity against the selected pathogens. They also provide a plausible scientific rationale for the popularity of the bark in African traditional medicine.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sajb.2018.07.020.

Acknowledgements

Funding from the National Research Foundation to the Chair in Indigenous Plant Use at the University of Johannesburg (NRF Grant Number 8442) is gratefully acknowledged.

References

Archer, R.H., Van Wyk, A.E., 1998. A taxonomic revision of *Elaeodendron* Jacq. (Cassinoideae: Celastraceae) in Africa. South African Journal of Botany 64, 93–109.

Doke, C.M., Vilakazi, B.W., 1972. Zulu-English Dictionary. 2nd ed. Witwatersrand University Press, Johannesburg.

Drewes, S.E., Mashimbye, M.J., 1993. Flavonoids and triterpenoids from *Cassine papillosa*and the absolute configuration of 11,11-Dimethyl-1,3,8,10-tetra-hydroxy-9-methoxypeltogynan. Phytochemistry 32, 1041–1044.

Drewes, S.E., Mashimbye, M.J., Field, J.S., Ramesar, N., 1991. 11,11-Dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogyan and three pentacyclic triterpenes from *Cassine transvaalensis*. Phytochemistry 30, 3490–3493.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.

Gelfand, M., Mavi, S., Drummond, R.B., Ndemera, B., 1985. The Traditional Practitioner inZimbabwe: His Principles of Practise and Pharmacopoeia. Mambo Press, Gweru, Zimbabwe.

Hisham, A., Kumar, J.G., Fujimoto, Y., Hara, N., 1996. 20,29-Epoxysalacianone and 6β-hydroxysalacianone, two lupane triterpenes from *Salacia beddomei*. Phytochemistry 42, 789–794.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg, South Africa.

Mabogo, D.E.N., 1990. Ethnobotany of the Vhavenda. (M.Sc. Thesis). University of Pretoria.

Ndawonde, B.G., Zobolo, A.M., Dlamini, E.T., Siebert, S.J., 2007. A survey of plants sold by traders at Zululand muthi markets, with a view to selecting popular plant species for propagation in communal gardens. African Journal of Range and Forage Science 24, 103–107.

Neuwinger, H.D., 2000. African Traditional Medicine. Medpharm Scientific Publishers, Stuttgart.

Palmer, E., Pitman, N., 1972. Trees of Southern Africa. 2. Balkema, Cape Town.

Pujol, J., 1990. Naturafrica – The Herbalist Handbook. Jean Pujol Natural Healers' Foundation, Durban.

Saleem, M., 2009. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Letters 285, 109–115.

SANBI, 2012. Red List of South African Plants. South African National Biodiversity Institute redlist.sanbi.org, Accessed date: 25 January 2018.

Sheng-Ding, F., Berry, D.E., Lynn, D.G., Hecht, S.M., Campbell, J., Lynn, W.S., 1984. The chemistry of toxic principles from *Maytenus nemerosa*. Phytochemistry 23, 631–633.

The Plant List, 2017. http://www.theplantlist.org, Accessed date: 7 September 2017.

Tinto, W.F., Blair, L.C., Alli, A., Reynolds, W.F., McLean, S., 1992. Lupane triterpenoids of *Salacia cordata*. Journal of Natural Products 55, 395–398.

Tshikalange, T.E., Hussein, A., 2010. Cytotoxicity activity of isolated compounds from *Elaeodendron transvaalense* ethanol extract. Journal of Medicinal Plant Research 4, 1695–1697.

Ullah, N., Ahmed, Z., Ahmed, S., Muhammad, P., Malik, A., 1999. A pentacyclic triterpene from *Daphne oleoides*. Phytochemistry 50, 839–841.

Van Vuuren, S., Holl, D., 2017. Antimicrobial natural product research: a review from a South African perspective for the years 2009–2016. Journal of Ethnopharmacology 208, 236–252.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 2009. Medicinal Plants of South Africa. 2nd ed. Briza Publications, Pretoria.

Van Wyk, B., Van den Berg, E., Coates Palgrave, M., Jordaan, M., 2011. Dictionary of Names for Southern African Trees. Briza Academic Books, Pretoria.

Von Koenen, E., 2001. Medicinal, Poisonous and Edible Plants in Namibia. Klaus Hess Publishers, Windhoek.

Williams, V.L., Balkwill, K., Witkowski, E.T.F., 2000. Unravelling the commercial market formedicinal plants and plant parts on the Witwatersrand, South Africa. Economic Botany 54, 310–327.