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Short communication

In vitro antimicrobial activity of extracts and an isolated compound from *Boscia albitrunca* leaves

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ABSTRACT

Boscia albitrunca is a medicinal plant widely used in the management of eye related ailments and haemorrhoid diseases. Repeated column fractionation of the n-butanol fraction of a 20% aqueous methanol leaf extract of *B. albitrunca* on silica gel and Sephadex LH-20 columns afforded a phenolic compound identified as martynoside. Structure elucidation of the isolated compound was carried out using spectroscopic techniques. The extracts and isolated compound were evaluated for antimicrobial activities using the micro dilution technique. All the extracts and compound were active against the tested Gram-positive, Gram-negative bacteria and fungi. Minimum inhibitory concentration (MIC) values for extracts ranged from 390.0 to 6250 μg/mL and martynoside isolated from the butanol fraction was the most active with the lowest MIC values of 7.81 and 31.2 μg/mL against *B. subtilis* and *K. pneumoniae* respectively. The activity demonstrated by the extracts and martynoside obtained from *B. albitrunca* against tested bacteria and fungi suggests that they could be helpful in the management of eye infections.

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1. Introduction

The search for new effective antimicrobial agents is necessary due to the increase of microbial resistance to currently available drugs. This is caused by factors such as poor compliance and inappropriate use of medication amongst others. Herbs contain copious amounts of secondary metabolites that could be exploited to prevent microbial infections (Kuete et al., 2009). It is estimated that more than 80% of health care needs in developing countries are met through traditional health care practices (WHO, 2002). Of the eight *Boscia* species occurring in southern Africa, *Boscia albitrunca* (Burch.) Gilg & Benedict (Capparaceae) is mostly used in treatment of a variety of diseases. It is commonly known as shepherd's tree (Bothma, 1982). The green fruits are used to treat epilepsy. Extracts of the leaf extracts are applied to the inflamed eye and the extracts of the roots are used for the treatment of haemorrhoids and sometimes eaten either dry or are roasted and then ground to make a substitute for coffee or pounded to obtain a white meal for porridge (Coates, 1983; Hutchings et al., 1996). In Namibia, the leaves and roots of the plant are used for the treatment of constipation and headache (Cheikhyoussef and Embashu, 2013).

In this study, we have investigated the *in vitro* antimicrobial activities of the crude extract, fractions and an isolated compound from *B. albitrunca*.

2. Materials and methods

2.1. General

Thin layer chromatographic analyses were performed at room temperature using pre-coated plates (MERCK, silica gel 60F254 0.2 thickness). Compounds were detected by viewing the TLC plates under ultraviolet light (254 and 366 nm). Open column chromatography was carried out using Sephadex LH-20 and silica gel. Nuclear Magnetic Resonance (NMR) data were obtained using a Brucker spectrometer (400 MHz). Chemical shifts are expressed in parts per million (ppm).

2.2. Sample collection

Leaves of *B. albitrunca* were collected in November 2012 from the University of KwaZulu-Natal (UKZN) Botanical Garden, Pietermaritzburg, South Africa. The plant was identified by Mrs Alison Young (Horticulturist, UKZN) and a voucher specimen was prepared (S Pendota 2) and lodged in the Bews Herbarium, (NU). The leaves were dried at 50 °C, ground into a powder using an Ultra-Centrifugal Mill (ZM 200, Retsch®, Germany) and stored at room temperature in airtight containers under dark conditions for 1 month.

2.3. Extraction

The powdered plant material (1 kg) was extracted with 8 L of 20% aqueous methanol at room temperature for 24 h and filtered. The

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filtrate was concentrated *in vacuo* at 40 °C to about a third of its original volume. This afforded a crude extract of the plant material (600 mL).

2.4. Solvent partitioning of the crude extract

The concentrated crude extract was sequentially extracted with n-hexane (3 x 800 mL), dichloromethane (3 × 800 mL), ethyl acetate (3 × 1 L) and finally n-butanol (500 mL). The solvent fractions were concentrated to dryness *in vacuo* to afford four solvent fractions: hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and n-butanol fractions.

2.5. Isolation of compound from B. albitrunca leaf extracts

The n-butanol fraction (4.0 g) was fractionated on a silica gel column using DCM/EtOAc (9:1) followed by an increasing gradient of EtOAc up to 100% and then up to 10% MeOH in EtOAc. Four fractions (A_1–A_4) were obtained based on TLC analysis. Further column fractionation of fraction A_2 on Sephadex LH-20 using EtOAc/MeOH (9:1) followed by an increasing gradient of methanol up to 20% and subsequent TLC analysis using DCM/MeOH (9:1) resulted in the isolation of compound **1** (37 mg).

2.6. Antibacterial activity

The minimum inhibitory concentration (MIC) of the crude extract, fractions and isolated compound of *B. albitrunca* was determined using the micro-dilution assay in 96-well micro-plates (Eloff, 1998). One hundred microlitres of each resuspended sample (50 mg/mL for fractions and 1.0 mg/mL for compound) in 80% ethanol were two-fold serially diluted with sterile distilled water, in duplicate in a 96-well micro-plate for each of the four bacteria. A similar two-fold serial dilution of neomycin (Sigma) (0.1 mg/mL) was used as a positive control against each bacterium. Water, 80% ethanol and bacteria-free broth were separately included as negative controls. Overnight cultures (incubated at 37 °C in a water bath with shaking) of four bacterial strains: two Gram-positive (*Bacillus subtilis* ATCC 6051 and *Staphylococcus aureus* ATCC 12600) and two Gram-negative (*Escherichia coli* ATCC 11775 and *Klebsiella pneumoniae* ATCC 13883) were diluted with sterile Mueller–Hinton (MH) broth (1 mL bacteria per 50 mL MH, approximately 10^6 CFU/mL of microorganisms). One hundred microlitres of each bacterial culture were added to each well. The plates were covered with parafilm and incubated overnight at 37 °C. Bacterial growth was tested by adding 50 μL of 0.2 mg/mL *p*-iodonitrotetrazolium chloride (INT) to each well and the plates incubated at 37 °C for 1 h. Bacterial growth in the wells was indicated by a red–pink colour, whereas clear wells indicated inhibition of growth by the tested sample. MIC values were recorded as the lowest concentration of the sample showing a clear well. Each assay was repeated twice with two replicates.

2.7. Antifungal activity

The antifungal activity of the crude extract, fractions and isolated compound was evaluated against *Candida albicans* (ATCC 10231) using the micro-dilution assay (Eloff, 1998) which was modified for fungi (Masoko et al., 2007). An overnight fungal culture was prepared in Yeast Malt (YM) broth. Four millilitres of sterile saline were added to 400 μL of a 24-h-old *C. albicans* culture to give approximately 10^6 CFU/mL of microorganisms. The absorbance was read at 530 nm and adjusted with sterile saline solution to match that of a 0.5 M McFarland standard solution. From this prepared stock, a 1:1000 dilution with sterile YM broth was prepared. The assay was repeated twice with three replicates each.

3. Results and discussion

Structure elucidation of the isolated compound was carried out using NMR spectroscopic techniques: ^1H (400 MHz) and ^{13}C NMR (100 MHz), and DEPT together with 2D experiments (**g**COSY, gHSQC and gHMBC). Comparison of the ^1H and ^{13}C NMR along with the DEPT spectrum indicated the presence of feruloly group from the ABX pattern of the aromatic protons and trans olefinic protons. There were two anomeric protons at δ 5.31 and 4.45 belonging to rhamnosyl and glucosyl respectively. There was another ABX system assigned to 3,4-dihydroxyphenylethyl group. The positions of the substituents were assigned based on the HMBC correlations. The compound (Fig. 1) was identified as:

Martynoside, 3-Hydroxy-4-methoxyphenethyl 3-O-α-L-rhamnopyranosyl-4-O-(3-methoxy-4-hydroxy-trans-cinnamoyl)-β-D-glucopyranoside (1): ^1H NMR (MeOD, 400 MHz): Aglycone, δ 6.77 (1H, d, J = 2.0 Hz, H-2), 6.85 (1H, d, J = 8.2 Hz, H-5), 6.70 (1H, dd, J = 8.1, 2.0 Hz, H-6), 2.82 (2H,t, J = 7.4, H-7), 4.04 (1H, dd, J = 17.0 and 7.2 Hz, H-8), 3.72 (1H, dd, J = 17.4 and 7.7 Hz, H-8), 3.80 (3H, s, OCH_3), glucosyl Hs, δ 4.45 (1H, d, J = 7.8 Hz, H-1′), 3.45 (1H, t, J = 8.4 Hz, H-2′), 3.89* (1H, m), 4.93 (1H, t, 9.5 Hz, H-4′), 3.56 (1H, m, H-5′), 3.57 (1H, m, H-6′), rhamnosyl Hs, δ 5.31 (1H, d, J = 1.3 Hz, H-1″), 3.54 (1H,d, J = 3.2 Hz, H-2″), 3.87* (1H, m, H-3″), 3.33 (1H, t, J = 9.3 Hz, H-4″), 3.65 (1H, m, H-5″), 1.13 (3H, d, J = 6.2 Hz, H-6″), feruloly, δ 7.35 (1H, d, J = 1.9 Hz, H-2‴), 6.88 (1H, d, J = 8.2 Hz, H-5‴), 7.16 (1H, dd, J = 8.2, 1.9 Hz, H-6‴), 7.66 (1H, d, J = 15.9 Hz, H-7‴), 6.43 (1H, d, J = 15.9 Hz, H-8‴), 3.92 (3H, s, OCH_3). *overlapping of signals

^{13}C NMR (MeOD, 100 MHz): Aglycone, δ 132.7 (C-1), 116.8 (CH, C-2), 146.9 (C-3), 147.4 (C-4), 112.6 (CH, C-5), 120.8 (CH, C-6), 36.3 (CH_2, C-7), 71.5(CH_2, C-8), 56.4 (OCH_3), glucosyl Cs, δ 103.9 (CH, C-1′), 76.3 (CH, C-2′), 79.6 (CH, C-3′), 70.3 (CH, C-4′), 76.0(CH, C-5′),62.4 (CH_2, C-6′), rhamnosyl Cs, δ 101.9(CH, C-1″), 72.3 (CH, C-2″), 72.1 (CH, C-3″),73.7 (CH, C-4″), 69.5 (CH, C-5″), 18.6 (CH_3, C-6″), feruloly, δ 127.5 (C-1‴), 111.4 (CH, C-2‴), 148.8 (C-3‴), 150.3 (C-4‴), 116.1 (CH, C-5‴), 124.3 (CH, C-6‴), 146.8 (CH, C-7‴), 115.4 (CH, C-8‴), 167.2 (C = O, C-9‴), 56.4 (OCH_3). The spectra data are in good agreement with that of the

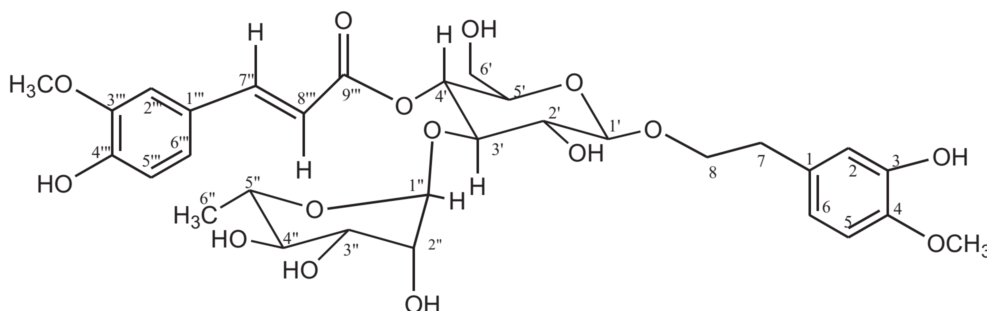

Fig. 1. Structure of active compound isolated (martynoside) from *Boscia albitrunca*.

Table 1
Antimicrobial activity of *B. albitrunca* extracts and an isolated compound (martynoside) expressed as (MIC) against the different Gram-positive and Gram-negative bacteria. Values in bold were considered as noteworthy antimicrobial activity.

Extract (μg/mL)	Antibacterial activity MIC (μg/mL)				Antifungal activity (*C.a.*)	
	B.s.	*S.a.*	*E.c.*	*K.p.*	MIC μg/mL	MFC (μg/mL)
Crude extract	3125.0	1560.0	6250.0	6250.0	3125.0	3125.0
Hex	3125.0	3125.0	**780.0**	1560.0	6250.0	6250.0
DCM	3125.0	6250.0	1560.0	1560.0	1560.0	3125.0
EtOAc	**3125.0**	**780.0**	1560.0	3125.0	**390.0**	**780.0**
BuOH	**390.0**	**780.0**	1560.0	**780.0**	1560.0	6250.0
Compound (μg/mL)						
1	7.81	125.0	125.0	31.2	62.5	250.0
Neomycin (μg/mL)[a]	1.56	1.56	0.39	0.78		
Amphotericin B (μg/mL)[b]					0.15	9.80

MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration; *B.s., Bacillus subtilis; S.a., Staphylococcus aureus; E.c., Escherichia coli; K.p., Klebsiella pneumoniae. C.a., Candida albicans* Hex, hexane; DCM, dichloromethane; EtOAc, ethyl acetate; BuOH, butanol.

[a] Positive control for the antibacterial assay.

[b] Positive control for the antifungal assay.

phenylpropanoid glycoside martynoside isolated from *Clerodendron trichotomum* (Kim et al., 2001).

The MIC and minimum fungicidal concentration (MFC) values of *B. albitrunca* extracts and the isolated compound against the tested bacteria and *C. albicans* are presented in Table 1. According to Ríos and Recio (2005) an MIC value less than 1 mg/mL for crude extracts or 0.1 mg/mL for isolated compounds should be considered effective and proposed that activity would be very interesting in MICs of 0.1 mg/mL and 0.01 mg/mL for extracts and isolated compounds respectively. On the other hand, Fabry et al. (1998) described active crude extracts as those having MIC values <8 mg/mL, whilst Gibbons (2005) suggests that isolated phytochemicals should have MIC values <1 mg/mL. In this study, however, MIC and MFC values of less than 1 mg/mL (1000 μg/mL) were considered to be of good activity. All the extracts showed a broad spectrum of activity against the selected bacteria with MIC values ranging from 390.0 to 6250.0 μg/mL. The MIC values of the fractions are lower than that of the standard antibacterial agent (neomycin) used in this study. The butanol fraction demonstrated the best antibacterial activity (Table 1) with an MIC value of 390.0 to 1560.0 μg/mL. The traditional uses of the plant for eye infections are related to bacterial infections. This prompted selection of the butanol fraction of *B. albitrunca* which demonstrated the best antibacterial activity for phytochemical investigation in order to target its bioactive constituent and provide rationale for the ethnomedicinal uses of the plant. Fractionation of the butanol fraction to target and isolate its only major compound detected by viewing the TLC profile of the fraction under ultraviolet lamp afforded a phenylpropanoid glycoside identified as martynoside.

The isolated compound (martynoside) showed good antibacterial activity with MIC values <250 μg/mL. Of all the samples tested, it was the most active with the lowest MIC values (7.81 and 31.2 μg/mL) against *B. subtilis* and *K. pneumonia* respectively. Phenylethanoid glycosides like martynoside have previously been reported to have antimicrobial activity (Dembitsky, 2005). The activity of martynoside on *B. subtilis* and *K. pneumoniae* is noteworthy though lower than the activity of the standard antibacterial agent (neomycin). Contamination of eye drops with the genus *Bacillus* may cause severe eye infection and could develop into complete panophthalmitis or endophthalmitis (Kotiranta et al., 2000; Gerri and Hall, 2006). *K. pneumoniae* can cause endophthalmitis, an infection that occurs as a result of seeding of organisms into the interior of the eye following surgery (postoperative), trauma (post-traumatic) or an infection (Billy and Michelle, 2010). As indicated in Table 1, the extracts exhibited a broad spectrum of activity against *C. albicans* with MIC and MFC values ranging from 390.0 to 6250.0 μg/mL. The ethyl acetate fraction showed good activity with MIC and MFC values ranging from 390.0 to 780.0 μg/mL. Its MIC and MFC values are lower than that of the standards (neomycin and amphotericin B) used in this study. Martynoside showed moderate activity against *C. albicans* with MIC and MFC values ranging from 62.5 to 250.0 μg/mL compared to standard antifungal agent amphotericin B. According to the WHO (1979) *Candida* spp. are the most common causes of endogenous endophthalmitis, leading to scarring of the chorioretina and blindness.

To the best of our knowledge this is the first report on the extracts and a compound from *B. albitrunca* and the results observed correspond with the therapeutic use of this plant in the traditional medicine.

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