INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 2, p. 161-171, 2019

RESEARCH PAPER OPEN ACCESS

Antimicrobial and Immunomodulatory Properties of Crude Extract and Compounds from *Boswellia dalzielii* Hutch

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Key words: *Boswellia dalzielii*; antimicrobials; immunomodulatory; oxidative burst.

http://dx.doi.org/10.12692/ijb/14.2.161-171 Article published on February 12, 20199

Abstract

In the North of Cameroon, *Boswellia dalzielii* is a medicinal plant used in traditional medicine to treat infectious diseases (dysentery, typhoid, cholera and malaria). The aim of this study is to identify and characterized the bioactive compounds from the roots of this plant. The methanol crude extract from the root of *Boswellia dalzielii* Hutch was obtained by maceration in methanol. Partition of part of this crude extract using solvent-solvent extraction in n-hexane, ethyl acetate, n-butanol yield hexane, yield hexane, ethyl acetate, n-butanol and residual fractions. Ethyl acetate fraction showed the highest antibacterial activity and was subjected to column chromatography. Crude extract, fractions and pure compounds were tested for their antibacterial properties using microdilution method with Alamar Blue as dye. Immunomodulatory activities were determined using chemiluminescence immunoassay. Ethyl acetate fraction exhibited highest antimicrobial activities and its fractionation afforded 3 known compounds and a mixture: trans-desoxyrhapontigenin-3-O-rutinoside (1), α-amyrin (3) and trans-desoxyrhaponticin (4) and a mixture of *β*-amyrin and lupeol (2) in a ratio (1:2). Mixture (2) as far as compounds (1) and (3) showed no antimicrobial activity against all bacteria strains tested while desoxyrhapontigenin-3-O-rutincompound (4) (MIC=125µg/mL) was relatively active. Ethyl acetate fraction exhibited lowest immunomodulator activity while compound (3) showed highest immunomodulating activities towards polymorphoneutrophils (IC_{50} < 7 µg/mL). These results showed that *Boswellia dalzielii* possess both antibacterial and immunomodulatory effects due to different comounds and may justify it use in traditional medicine to fight infectious diseases.

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Introduction

Infectious diseases are major causes of morbidity and about 50% of mortality in the developing countries (Ahmad *et al*., 2015). Mortality burden attributable to infectious diseases exceeds 17 million deaths (OMS, 2012). The antimicrobial resistance to antibiotics, uncontrolled urbanization, the unprecedented increase in population movements, and poverty in developing countries constitute fertile ground upon which new infections have flourished, while others, thought to have been eradicated, have reappeared (Usha *et al*., 2010, O'Brien & Stelling, 2011). The pharmacological and phytochemical study of plants from traditional could lead not only to the discovery of new antimicrobial compounds, but also to the valorisation of local plant species through the investigation on their efficacy and safety.

On other hand, many clinical disorders are associated with the dysfunction of the immune system. Suppression of the immune system is required in the management and treatment of inflammation and allergic diseases, while stimulation is highly desirable for the treatment of immunossuppressive diseases (Mahomoodally *et al*., 2012). During an innate immune response first line defense is mediated by professional phagocytes including polymorphonuclear neutrophils (PMNs) and macrophages (Sharma *et al*., 2015). Upon activation, phagocytes migrate to the site of inflammation, and produce various mediators, such as chemokines, cytokines, and ROS to eradicate the infectious agents. Phagocytosis is the elimination of pathogens through a process called oxidative burst. Although normally it has a protective role, the over expression of inflammatory mediators, such as ROS, NO and proinflammatory cytokines due to an inappropriée immune response can cause damage to body tissues, and leads to various chronic illnesses. Inhibitors of ROS, TNF-α, IL-1β, etc. are being used for the treatment of various clinical conditions and compounds having capability to modulate immune response and target the mediators of inflammation with less toxicity have great therapeutic value (Ahmed *et al*., 2012). In this context, the pharmacological and phytochemical study of plants from traditional pharmacopoeias could lead not only to the discovery of new antimicrobial compounds, but also to the valorisation of local vegetal species whose efficacy and safety would have been demonstrated in laboratory investigations.

Boswellia dalzielii Hutch belonging to the family *Burseraceae* commonly known as Frankincense tree, is a specie of wooded and shrubby savannas plants. It's about 20 m of height and 70 cm in diameter for stem. The plant is found in North and Far North of Cameroon where it is used for traditional medicine and known as « andekeehi » in foulfoulde (local language). A decoction prepared from the stem bark of *B. dalzielii* is used in malaria, influenza; rheumatism, septic sores, venereal diseases and gastrointestinal ailments treatments and its infusion is used in treatments of some skin deseases (shingles, scabies, ringworm) (Jansen *et al*., 2010, Kémeuzé *et al*., 2012). Ethanol extract of *B. dalzielii* was active against *Trypanosoma rhodesiense* and *Leishmania donovani* with percentage growth inhibition of 100 and 95.6%, respectively (Lagnika *et al*., 2013); The decoction of the stem bark is used to treat rheumatism, septic sores, veneral diseases and gastrointestinal ailments (Olukemi *et al*., 2005).

However, in spite of its numerous uses in traditional medicine in Africa, the Central African species is less known and studied compared to its more popular congeners.

Boswellic acid known ascytotoxic compound are the main bioactive components of frankincense from genus *Boswellia* (Csuk *et al*., 2015). Some antimicrobial and antispasmodic compounds mainly protocatechuic acid and incensole have been identified in this plant (Alemika *et al*., 2004, Kakkar & Bais, 2014).

In the present study, we examine the antibacterial and immunomodulatory activities of isolated molecules as well as the MeOH extract of *B. Dalzielii*. These compounds are isolated here for the first time.

Material and methods

Plant material

The roots of *B. Dalzielli* were collected in North Region of Cameroon (9° 37' 60" North latitude and 13 ° 55' 60" East longitude) in November 2013. The plant was identified by Professor Mapongmetsem, a Botanist at the University of Ngaoundéré and then confirmed at the Cameroon National Herbarium in Yaoundé according to the voucher specimen N° 4359/SRFK. The collected roots were washed and dried at room temperature (22 ± 2°C). When completely dried, the roots bark were ground into powder and kept in airtight containers until use.

Extraction

Root bark powder (2000 g) was macerated into 8 L of methanol (MeOH) at room temperature for 72 h, with occasional shaking and then filtered through Whatman filter paper No.1. The crude methanol extract (196.34) was obtained after solvent separation under reduced pressure using a rotary evaporator at 40°C. Part of crude extract (150 g) was disolved in water and successively extracted with dichloromethane (DCM), ethyl acetate (EtOAc), and n-butanol (buOH) to yield after solvent evaporation in the same conditions as above DCM (25.2 g), EtOAc (12.2 g), BuOH (39.6 g) and residue (18.7 g) fractions.

Isolation and identification of compounds from B. Dalzielii

A part of EtOAc fraction (9.2 g) was subjected to column chromatography using silica gel (Merck) type 100 (70-230 Mesh ASTM) and eluted with gradient system (Hex-EtOAc, CH_2Cl_2, and CH_2Cl_2-CH_3OH) to afford 559 fractions of 150 mL each. These fractions were combined based on their thin layer chromatography (TLC) profiles into 13 major fractions labeled 1 to 13. Fraction 3 was chromatographed on a silica gel column, eluting with n-hexane-EtOAc (90–10) to obtain compound 2 and 3. Fraction 6 was purified with a CH_2Cl_2/ CH_3OH (99.5:0.5) as solvent system to obtain compound 4. Fraction 9 was chromatographed on a silica gel column, eluting with increase polarity hexane-EtOAc (99:1 to 85:15) then gradually with CH_2Cl_2/ CH_3OH (99.5:0.5 to 90:10) to obtain compound 1. All the solvents used were distilled commercial grade. The purity was checked by TLC. Pre-coated plates of silica gel GF_{254} (Merck) were used for this propose; the spots were detected with UV lamp at 254 and 366 nm and by spraying with 50% H_2SO_4 or ceric sulphate following by heating. Structures (Figure 1) of these compounds have been assigned on the basis of spectroscopic data (^1H and ^{13}C NMR, ^1H-^1H COSY, HSQC, HMBC), mass spectra, and by comparison of those compounds to compounds described in the literature.

Antibacterial assays

Tested microorganisms comprise *Pseudomonas aeruginosa* NCTC 10662, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 14028 (Gram-negative bacteria), *Bacillus subtilis* (clinical isolate) and *Staphylococcus aureus* (NCTC 6571) (Gram-positive bacteria) obtained from International Center for chemical and Biological Sciences of University of Karachi, Pakistan. These organisms were maintained on Muller Hinton Agar and prior to the antimicrobial assay, the microbial strains were subculture on Muller Hinton Broth.

Inoculum of each bacterial strain was prepared from a 24 hours Muller Hinton agar culture. For this purpose, some colonies of each bacterial strain were dispersed in 0.9% NaCl solution and diluted to match the 0.5 McFarland standard turbidity scale, corresponding to about $1.5x10^8$ CFU. This microbial suspensions were diluted to match the optical density of 0.1 at 600 nm (Jenway 6105UV/Vis spectrophotometer, 50Hz/60Hz) corresponding to about 10^6 CFU/ml. This final dilution was used as inoculum for antibacterial testing.

Bacterial sensitivity test

The susceptibility of bacteria to tested samples was determined using microplate Alamar blue assay (MABA) under aseptic conditions (Jimenez-Arellanes *et al., 2003)*. Each bacterial strains was cultured at 37°C in MHA. Into each well of a 96-weel plate, 183 µL of MHB was introduced. Then 10 µL of test

substance (15 mg/ml for crude extract or different fractions diluted in a 2,5% DMSO solution) were added followed by 7 µL of inoculum (1,5×10^6 CFU/mL) for final concentration of 5×10^5 CFU/mL. Each sample was assayed in triplicates and incubated at 37°C for 24 hrs, time after which 20 µl of freshly prepared Alamar Blue solution were added in each well and the mixtures incubated again at 37°C for 2 h. and the change in color (bleu to pink) was read using a plate reader. The absorbance of each well at 570 and 600 nm were recorded and bacterial growth inhibition percentage was calculated using the following equation:

$$I\% = 100 - \frac{(\varepsilon ox)\lambda 2\, A\lambda 1 - (\varepsilon ox)\lambda 1\, A\lambda 1}{(\varepsilon ox)\lambda 2\, A°\lambda 1 - (\varepsilon ox)\lambda 1\, A°\lambda 2}$$

ε_{ox}=molar extinction coefficient of alamar Blue oxidized form (BLUE)

A=absorbance of test wells

A°= absorbance of positive growth control well

λ1= 570 nm

λ2= 600 nm

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of the compound or extract that inhibits the visible growth of microorganisms was determined by broth microdilution method in 96- well microtiter plates by the serial dilution method in Mueller Hinton Broth as described by Collins & Franzblau (1997) with some modifications. A volume of 100 µL of test material in 10% (v/v) DMSO in sterile distilled water was pipetted into the first row of the plate containing 100 µL of MHB. Serial dilutions were performed. The concentration varied from 3750 µg/mL to 117.2 µg/mL for extract/fractions and from 1000 µg/mL to 31.25 µg/mL for pure compounds. The final concentration of DMSO was lower than 2.5% and does not affect the microbial growth. Then, 7 µL of inoculum 1.5 × 10^6 CFU/mL was added to each well to obtain a concentration of 5×10^5 cfu/mL after completing each well with appropriate medium. The plates were covered with sterile plate sealers and incubated at 37°C for 18 to 24 h. The samples were assayed in triplicates. After incubation, 20 µL of freshly prepared Alamar Blue solution were added to one of the three wells with the same concentration. The microplates were incubated again at 37°C for 2 h. The colour shift from blue to pink was observed. The MIC was the lowest concentration of the extract that did not permit any visible growth compared to the control well. The MBC was determined by sub-culturing on Mueller Hinton Agar, the content of the well without alamar blue colour, that is, which had no colour change after incubation. The plates were incubated at 37°C for 18 to 24 hours. The lowest concentration of the extract, which did not produce any bacterial colony, was regarded to be the MBC.

Immunomodulatory assays

Isolation of human polymorphoneutrophils (PMNs)

Heparinised blood was obtained by vein puncture aseptically from healthy volunteers (25–38 years' age). The buffy coat containing PMNs was collected by dextran sedimentation and the cells were isolated after the LSM density gradient centrifugation. PMNS were collected from the tube base. Cells were washed twice and suspended in Hank's Balance Salt Solution [Ca and Mg free] (HBSS--), pH 7.4. Neutrophils were purified from RBCs contamination using hypotonic solution. Cells were adjusted to their required concentration using Hank's Balance Salt Solution containing Ca^{2+} and Mg^{2+} ($HBSS^{++}$).

Chemiluminescence assay

Luminol chemiluminescence assay was performed as described by Helfand *et al.* (1982) modified by Haklar *et al.* (2001). Briefly, 25µL of diluted whole blood (1:50 dilution in sterile HBSS++) or 25µL of PMNCs (10^6) cells were incubated with 25µL of serially diluted plant extract with concentration ranges between 6.25 and 100µg/mL. Control wells contained HBSS++ and cells without plant extract. Tests were performed in white 96 wells plates, which were incubated at 37°C for 30 min in the luminometer. 25µL of opsonized zymosan-A, followed by 25µL of luminol (7×105M) along with HBSS++ were added to each well to obtain a total volume 200µL in a well. Luminol is used as a probe in this assay, as having a

low molecular weight it can cross the cell membrane, and hence can detect both intra and extra cellular ROS, produced by the cells (Dahlgren and Briheim, 1985; Allen, 1986).

The luminometer results were monitored as chemiluminescence RLU with peak and total integral values set with repeated scans at 30 s intervals and 1 s points measuring time(Sharma *et al.*, 2015).

Result

Structure of compound 1 (BDR-M315)

Compound 1, a chestnut solid, displayed a molecular ion peak $[M]^+$ at *m/z* 550 according to its EI-MS mass spectrum corresponding to the molecular formula $C_{20}H_{22}O_8$. NMR data of compound 1 were similar to those of (Choi *et al.*, 2005), but with additional signal for a rhamnose unit. Indeed, the ^1H NMR spectrum of compound **1** spectrum showed the presence of two independent aromatic rings. An AA′BB′ system at δ_H 7.45 (*d*, *J* = 8.8 Hz, H-2′ et H-6′) and 6.90 (*d*, *J* = 8.8 Hz, H-3′ et H-5′) corresponding to a *p*-disubstituted phenyl ring, and signals of an ABX-type aromatic system of three aromatic protons at δ_H 6.72 (*s*, H-2), 6.45 (*s*, H-4) and 6.65 (*s*, H-6) suggested the presence of a 1,3,5-trisubstituted aromatic ring. Moreover, the spectrum exhibited a pair of desoxyrhapontigenin doublets at δ_H 7.02 (1H, d, *J* = 16.4 Hz, H-*β*) and 6.88 (1H, d, *J* = 16.8 Hz, H-*α*). The vicinal coupling constant $^3J_{HH}$ around 16 Hz was indicative of their *trans* configuration. The signal of three singlet protons at δ_H 3.79 was attributed to the methoxy group at C-4′. The aglycone was identified as desoxyrhapontigenin (Choi *et al.*, 2005) based on ^{13}C NMR, DEPT, COSY, HSQC, and HMBC spectra.

Table 1. ^1H NMR data of compound 1 in MeOD (400 MHz).

Position	δ_C	δ_H (*m*, *J* (Hz))	HMBC	COSY
1	141.2	-		
2	107.8	6.72 (*s*)	3; 4; 6; *α*	H-4; H-6
3	160.4	-		
4	104.3	6.45 (*s*)	2; 5; 6	H-2; H-6
5	159.7	-		
6	108.2	6.65 (*s*)	2; 4; 5; *α*	H-2; H-4
1′	131.4	-		
2′	128.9	7.45 (*d*, 8.8)	3′; 4′; 5′; *β*	H-3′
3′	115.2	6.90 (*d*, 8.8)	1′; 3′; 4′; 5′	H-2′
4′	160.9	-		
5′	115.2	6.90 (*d*, 8.8)	1′; 3′; 4′; 5′	H-6′
6′	128.9	7.45 (*d*, 8.8)	4′; *β*	H-5′
α	127.4	6.88 (*d*, 16.8)	1; 1′; 2′; 6′, 2; 6; *β*	H-*α*
β	129.7	7.02 (*d*, 16.4)	1; 1′; 2′; 6′;*α*	H-*β*
4′-OCH_3	55.7	3.79 (*s*)	4′	
		Glucopyranoside unit		
1″	102.4	4.86 (*d*, 9.0)	3; 3″	H-2″
2″	74.9	3.43 (*m*)	1″	H-1″
3″	77.9	3.46 (*m*)		
4″	71.4	3.38 (*m*)		
5″	76.9	3.55 (*m*)		
6″	67.6	3.66 (*m*) ;	1‴	H-6″a
		4.00 (*dd*, 1.5; 13.5)	1‴	H-6″b
		Rhamnopyranoside unit		
1‴	102.2	4.72 (*d*, 0.8)	6″; 2‴; 3‴	H-2‴
2‴	72.1	3.86 (*dd*, 1.6; 3.2)		H-1‴ ; H-3‴
3‴	72.4	3.68 (*dd*, 3.2; 9.2)		H-2‴; H-4‴
4‴	74.1	3.34 (*dd*, 3.2; 9.2)	6‴	H-3‴; H-5‴
5‴	69.8	3.65 (*m*)		H-4‴; H-2‴
6‴	17.9	1.18 (*d*, 7.5)	4‴; 5‴	H-5‴

The NMR spectrum of compound 1 also exhibited two sugar units' signals. The signals at δ_H 4.86 (*d*, *J* = 9.0 Hz, H-1″) together with those at δ_C 102.4, 74.9, 77.9, 71.4, 76.9 and 67.6 indicated the presence of a glucosyl moiety, while the signal at δ_H 4.72 (*d*, *J* = 0.8 Hz, H-1‴) together with those at δ_C 102.2, 72.1, 72.4, 74.1, 69.8 and 17.9 were characteristic of the presence of a rhamnosyl moiety.

Table 2. Inhibition Percentage (at 1.5 mg/mL) of bacterial growth and MIC/MBC (mg/mL) by crude methanol extract of the root bark of *B. dalzielii* and its fractions and compounds.

		Bacterial strains				
Extract	Inhibition parameters	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*	*B. subtilis*
MeOH Extract	% Growth inhibition	22.00 ± 0.57^c	0.00 ± 0.00^a	0.00 ± 0.00^a	18.67 ± 2.03^b	40.33 ± 0.67^d
	MIC/MBC	3.75/>3.75	3.75/>3.75	3.75/>3.75	3.75/>3.75	3.75/>3.75
Cl_2CH_2 fraction	% Growth inhibition	49.67 ± 0.67^d	17.00 ± 0.57^a	36.67 ± 2.03^c	25.67 ± 0.88^b	33.00 ± 1.73^c
	MIC	<3.75/ND	<3.75/ND	3.75/ND	<3.75/ND	<3.75/ND
EA fraction	% Growth inhibition	56.00 ±2.00^b	10.33 ± 4.91^a	57.33 ± 0.33^b	60.67 ± 2.85^b	69.67 ± 1.45^c
	MIC	1.875/3.75	3.75>3.75	1.87/3.75	3.75/W3.75	1.875/3.75
n-butanol fraction	% Growth inhibition	44.67 ± 4.33^c	0.00 ± 0.00^a	0.00 ± 0.00^a	26.33± 0.88^b	49.00 ± 00^c
	MIC	>3.75/ND	>3.75/ND	>3.75/ND	>3.75/ND	>3.75/ND
Residual fraction	% Growth inhibition	8.00 ±1.15^b	00± 00^a	00± 00^a	00± 00^a	00± 00^a
	MIC	>3.75/ND	>3.75/ND	>3.75/ND	>3.75/ND	>3.75/ND
1	MIC	125/250	ND	500/1000	ND	250/500
2	MIC	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND
4	MIC	ND	ND	ND	ND	ND
Ofloxacin	Growth inhibition (%)	94.99 ± 0.24	94.89 ± 0.14	90.8± 0.83	93.7 ± 0.33	90.56 ± 2.33
	MIC	15.62*31.25	31.25/.125	31.25/62.50	31.25/62.50	31.25/62.50

ND: not determined; MIC: Minimum Inhibitory Concentration; MBC Minimum Bactericidal Concentration; EA: ethyl acetate; 1, 2, 3 et 4 compounds.

The configuration of the glucopyranoside unit was found to be *β* due to its high coupling constant *J* = 9.0 Hz while the rhamnopyranoside unit had *α* configuration due to its small coupling constant *J* = 0.8 Hz. All these signals along with the signals between δ_H 3.00 and 4.00 belonging to the other protons of the sugar moiety revealed a rutinoside moiety (González-Cortazar *et al.*, 2013). The presence of rutinoside moiety was supported by the EI-MS fragmentation. Indeed, the sugar type on the aglycone was determined by the loss from the molecular ion $[M]^+$ of a fragment corresponding to a hexose or pentose (respectively -162 uma, -132 uma). The loss of 146 uma and 176 uma corresponding to a deoxyhexose, in the view of the ^{13}C NMR data mentioned above and the literature was attributed to the loss of a rhamnose (Cuyckens *et al.*, 2002 Cuyckens and Claeys, 2004). Thus, the fragment at *m/z* 404 $[M-146]^+$*m/z* in the EI mass spectrum was characteristic of the loss of a rhamnose unit, another fragment at *m/z*. 242 $[M-146-162]^+$ indicated the additional loss of a glucoside unit. This last fragment at *m/z* 242 $[M-308]^+$ was characteristic of the loss of a rutinoside unit (rhamnopyranosyl- (*α*1-6) - glucopyranoside). In addition, the absence in the mass spectrum of the peak at *m/z* 388 $[M-162]^+$ corresponding to the loss of a hexose (glucose) from the molecular ion, suggested that the two sugars are contiguous. Moreover, the HMBC correlations observed between the anomeric proton of the rhamnosyl moiety at δ_H 4.72 (d, *J* = 0.8 Hz, H-1‴) and the carbon at δ_C 67.6 corresponding to the C-6"

carbon of the glucose unit;the correlations between the two protons at δ_H 3.66 (*m*, H-6"a) and 4.00 (dd, *J* = 1.5, 13.5 Hz, H-6"b) and the anomeric carbon of the rhamnose unit at δ_C 102.2 have shown that the two sugars are 1→6 bounded and therefore confirmed the presence of a rutinoside unit.

Table 4. Effect of crude extract (methanol) and fraction on luminol enhanced oxidative burst using zymosan-activated whole blood.

Extract and fractions (25µg/mL)	%Inhibition
CH_3OH Extract	34,8±4,0
Cl_2CH_2 fraction	24,7±5,9
EA fraction	18,9±6,3
n-butanol fraction	32,2±5,1
Residual fraction	27,8±13,2
Ibuprofen	73.2±1.4

Data are expressed as means S.E.M of three determinations; EA: ethyl acetate.

The position of the sugar residue was defined unambiguously to be at C-3 due to the long-range correlation between the anomeric proton of the glucoside unit at δ_H 4.86 (*d*, *J* = 9.0 Hz, H-1″) and the carbon C-3 (δ_C 162) of the aglycone (Figure 1). All the substitution pattern of the aromatic rings of compound 1 was found to be similar to those of Desoxyrhapontigenin (Choi *et al.*, 2005). These assignments were confirmed by the COSY, HSQC, and HMBC experiments (Table 6). On the basis of all the above informations, the natural product was identified as 5-hydroxy-4'-methoxystilben-3-O-*β*-[rhamnopyranosyl-(*α*1→6)-glucopyranoside] or desoxyrhapontigenin-3-O-rutinoside (1).

The others compounds, 2, 3 and 4 (figure 2) were known and were identified by comparing their NMR spectroscopic data with those reported in the literature, *α*-amyrin (Bdr-203), *trans*-desoxyrhaponticin (Bdr-342) and mixture of *β*-amyrin and lupeol (Bdr-207) in ratio (1:2) (Bdr-207).

Antimicrobial activity of tested subtances

The MeOH, hex, EtOAc, buOH and residue extracts as well as isolated compounds showed variable antibacterial activities (Table1). The most resistant bacteria species was *Escherichia coli* (all I %< 50%). Other microorganisms tested showed good sensitivity only to one extract or fraction with inhibition percentage more than 50%. Gram positive strains (*Staphylococcus aureus* and *Bacillus subtilis*) were in general, more sensitive among the bacteria strains tested with at least three extracts with inhibition percentage more than 40%. The partition increased the antibacterial activities of the crude extract.

The residue fraction showed no activity. The results of the MIC determinations (Table 2 &3) showed that the AE fraction was the most active amongst the crude extract and fractions and exhibited the best activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (MIC=1.87mg/mL) than crude methanol extract, dichloromethane and n-butanol fractions (MIC=3.75 mg/mL). Compound 1 isolated from AE fraction was more active (MIC=250 µg/mL) than others tested compounds but less than positive control Ofloxacin (MIC < 31.5 µg/mL).

Immunomodulatory activity

The preliminary screening results on human whole blood phagocytes showed that none of the extracts possess significant inhibitory activity at 25 µg/mL (Table 4). The partition decreases the immunomodulatory activity of crude methanolic extract. Furthermore, AE fraction of *B. dalzielii* and the compound from this fraction showed dose-dependent significant inhibition of chemiluminescence. The fraction and compound tested inhibited the reactive oxygen species production.

Table 5. Effect of fraction and compounds on the PMNs oxidative burst.

Code	Concentration	% Inhibition	IC_{50}
EA fraction	50µg/ml	97,7 ± 0,3	6,7 ± 0,7
	5µg/ml	42,9 ± 2,8	
	0,5µg/ml	12,8 ± 7,3	
4	50µg/ml	93,78 ± 0,40	6,43 ± 0,78
	5µg/ml	44,62 ± 2,96	
	0,5µg/ml	0	
1	ND	ND	
2	ND	ND	
3	ND	ND	
Ibuprofen			2.5 ± 0.6

ND: not determined; EA: ethyl acetate*;* 1, 2, 3 et 4 compounds; each percentage represents a mean of triplicate.

Discussion

In the present study, crude extracts, fractions and compounds of *B. dalzielli* were tested for their antibacterial activity and their capacity to trigger phagocyte myeloperoxidase (MPO) -dependent as measured by Luminol -amplified chemiluminescence

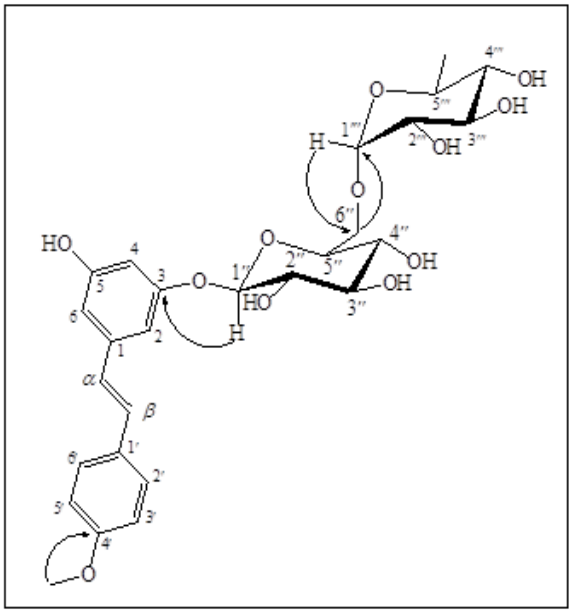

Fig. 1. Important HMBC correlations.

The result of the present study showed differences between the antimicrobial activities of crude MeOH extract and fractions (table 1). This suggests that *B. dalzielli* contains several antibacterial principles with different polarities. The partition of the MeOH extract enhanced its antimicrobial activity in EtOAc fraction, and reduced that of other fractions. This indicates that the active principles might be more concentrated in EtOAc fraction and more diluted in another fraction.

Natural products that produce minimum inhibitory concentrations (MIC) in the range 100–1000 mg mL^{-1}*in vitro* susceptibility tests can be classified as antimicrobials (Abreu *et al.*, 2012). Also, the activity of compounds is considered to be significant when the MIC is below 10 µg/mL, and moderate when such values vary between 10 and 100 µg/mL (Kuete, 2010; Ntie-Kang *et al.*, 2013). The results of the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) determinations (Table 2) showed that the EtOAc fraction was the most active but moderate, its CMI are 1,875 mg/mL for Gram positive strains and *Pseudomonas aeruginosa* strains. Previous studies also reported that Gram positive bacteria were most susceptible showing lower MIC and MBC than gram negative strains due to the difference of the nature of cell wall which makes diffusion of drugs easily for the gram positive (Popova *et al.*, 2013). Compound Bdr-315 showed antibacterial activity (MIC =125 mg/mL) against *S. aureus* but, less than standard compound (MIC=15.62 µg/mL. *P. aeruginosa* was the least sensitive (MIC ≥ 500 mg/mL) microorganism against compounds (Tables 1 and 2). *S. aureus* is the major cause of bacteraemia, associated with higher morbidity and mortality compared to other bacteraemia-causing pathogens (Sama *et al.*, 2015) and about 2% of patients in Cameroon are infected by

staphylococcus spp (Joubouhi *et al.*, 2017). The present study showed that *B. dalzielii* possess interesting inhibitory properties against *S. aureus* species.The results of screening on human whole blood phagocytes showed (table 4) no significant inhibitory activity at the tested concentration (25 µg/mL). The ethyl acetate fraction and compounds from this fraction showed dose-dependent significant inhibition of chemiluminescence with significant activity (IC_{50}< 10 µg/mL).

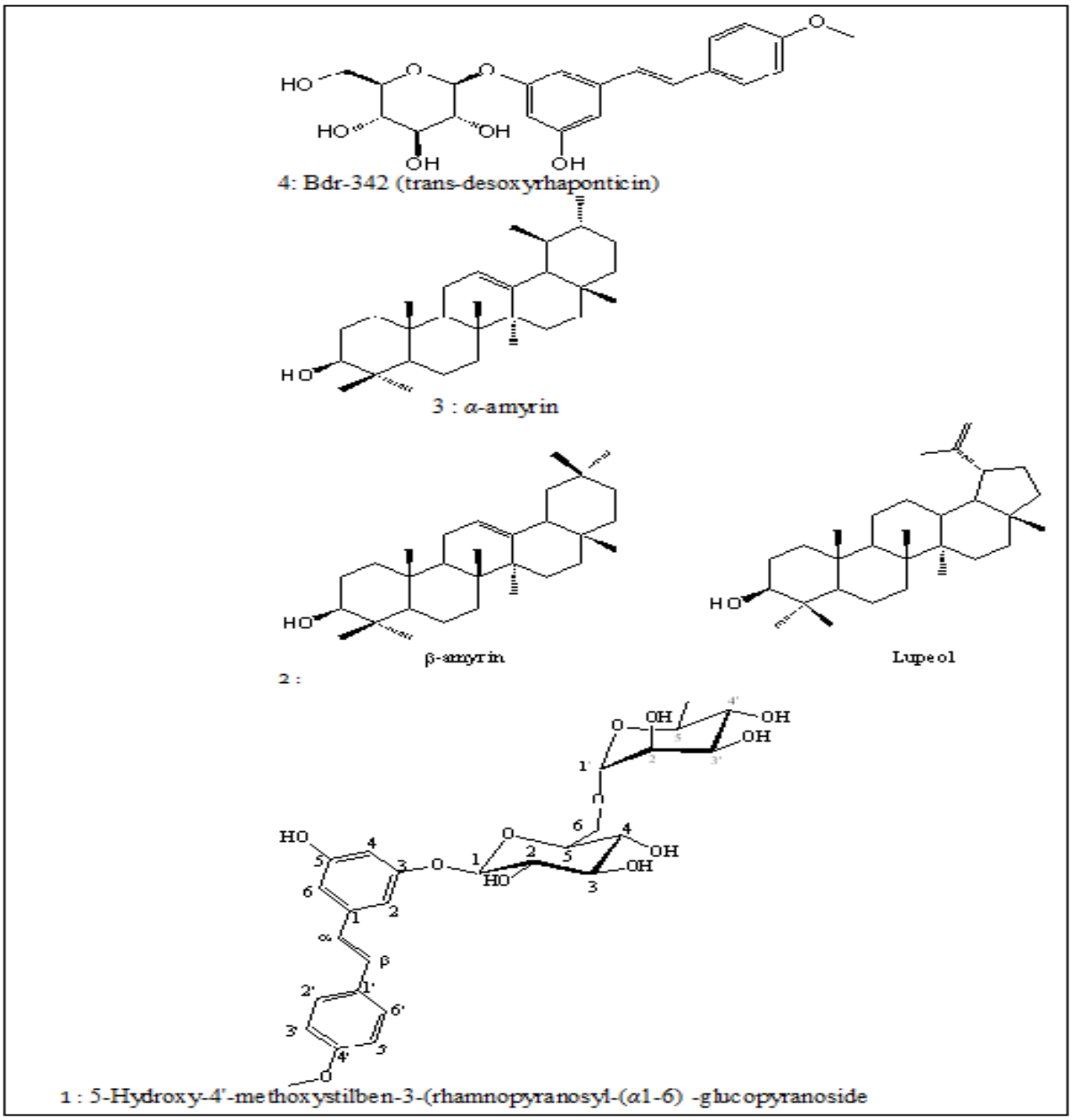

Fig. 2. Structures of compounds isolated from the plant *B. dalzielii*.

Luminal probes capable of detecting the level of reactive oxygen species (ROS) were used to study the effect of these plant extracts and compounds on oxidative burst. Luminol largely detects HOCl-, which means that luminol detection is mainly dependent on the MPO/H_2O_2 system. In stimulated PMNs, inhibition of chemiluminescence may be mediated by three main mechanisms: cell death, scavenging of ROS and inhibition of enzymes involved in the signal transduction pathways of the ROS generation process by these cells. Oumar *et al.* (2014) showed that *B. dalzeilii* has immunomodulatory effect on phagocytes. Indeed, aqueous extract and methanol extract stimulate macrophages to increase its

phagocytic and lysosomal enzyme activities, and also to produce nitric oxide.

The results suggest that this plant and compounds from them were able to modulate significantly the immune response of phagocytes, emphasizing their potential as a source of new immunomodulatory agents. However, it should be noted that a battery of *in vivo* tests as well as other pathways of activation of oxidative burst should be conducted to know biochemical mechanism and to confirm whether the *in vitro* results reported here could be translated into *in vivo* activities that might support the traditional uses of these medicinal plant in humans.

Acknowledgments

The authors gratefully acknowledge The World Academy of Sciences (TWAS), H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences ICCBS, Dr Panjwani Center for Molecular Medicines and Drug Research, University of Karachi, Pakistan, for the award of TWAS-ICCBSS and Postgraduate Fellowship

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