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Bioactive natural compounds from *Prosopis africana* and *Abies nobili*

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ABSTRACT

Chromatographic procedures from the aerial parts of *Abies nobilis* and stem barks of *Prosopis africana* led to the isolation of two (antimicrobial and cytotoxic) compounds. Their structures were established as 7, 3', 4'-trihydroxy-3-methoxyflavanone and dehydroabietic acid on the basis of spectroscopic techniques.

INTRODUCTION

Medicinal plants have been a rich source of useful drugs, some of which have acted as lead compounds for further development of synthetic/ semi-synthetic compounds. Initial screening of plants for probable antimicrobial actions typically begins with crude aqueous or alcoholic extract of plant followed by a range of separation and identified technologies. The analysis of plant extracts and isolated compounds for biological activity against *Mycobacterium aurum, Staphylococcus aureus* and breast cancer cells (ZR765) discovered that secondary metabolites may be useful as a resource for new active drug agents. The family Pinaceae is the second largest family in geographical range after the cupressaceae. It contains 220 species and 11 genera, found mainly in the northern but also in southern hemisphere (Stace 1992). The Leguminosae family consists of large number of trees and herbs with diverse locations and habit. The family includes approximately 700 genera and 17,000 species that are widely distribution in tropical, subtropical and temperate zones. This family is the most important in the Dicotyledonous group as they contain chemically diverse compounds such as: alkaloids, terpenoids, flavonoids and glycosides which are of interest for their biological activities (Harborne et al., 1971). The aim of this work was to isolate and characterize the active compounds against *Mycobacterium aurum* and *Staphylococcus aureus* from the aerial parts of *Abies nobilis* and the stem bark of *Prosopis africana*.

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MATERIALS AND METHODS

Aerial parts of *Abies nobilis* (AN) were purchased from Albatrees, UK in October 2003, and stem bark of *Prosopis africana* (PA) was collected by Dr. Hafsat Shittu from Albida in Nigeria during September 2002. Herbarium specimens were deposited at the Phytochemistry Laboratory at the University of Strathclyde where they were identified and given the voucher numbers ABNO1003 (*Abies nobilis*) and the *Prosopis africana* was identified at Edinburgh botanical garden and given the voucher number NIPRD/H/6385.

Extraction and isolation of compounds

The ground plant materials were extracted in a Soxhlet apparatus by using different solvent systems starting from non-polar *n*-hexane (60-80°C), semi polar chloroform or ethyl acetate and finally polar solvent as methanol for 2 to 3 days. The extracts were concentrated using a rotary evaporator (BUCHI Labortechnik AG, Switzerland) under reduced pressure at a maximum temperature of 50°C and stored at -20°C before use.

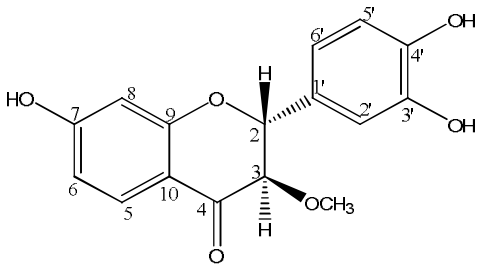
Bioassay

Alamar Blue Assay (resazurin–reduction test) was carried out and modified from[3] against *Mycobacterium aurum* (CIP 104482) and the organism was supplied by the Pasteur Institute, France. While the assay against S. *aureus* [ATCC 29213] and obtained from Fisher Scientific and breast cancer cells (ZR75) was by using the MTT assay (NCCLS 2003) and obtained from Prof Mike Barratt, University of Glasgow,UK. Cytotoxic activity was done on compound 1 as preliminary screening and the results were expressed as % of cell viability at a maximum concentration of 100µg/ml.

RESULTS AND DISCUSSION

General

Compound 1 was isolated from the ethyl acetate extract of *Prosopis africana.* The ESI-MS showed an $[M-H]^-$ at *m/z* 301.1201 established the molecular formula of the compound as $C_{16}H_{14}O_6$. The ^{1}H NMR spectrum [400MHz, CD_3OD, Table 1] showed peaks at δ 6.32 (d, *J*= 2.2Hz, H-8), δ 6.53 (dd, *J*= 2.2, 8.0Hz, H-6) and 7.69 (d, *J*= 2.2Hz, H-5) for ring-A and peaks at δ 6.78 (d, *J*= 8.0Hz, H-5'), 6.81 (dd, *J*= 2.0, 8.0 Hz, H-6') and 6.93 (d, *J*= 2.0Hz, H-2') for ring B. The spin system was confirmed from the COSY spectrum (400MHz, CD_3OD). The ^{13}C NMR data (Table 1) showed the presence of 15 carbons including a carbonyl at δ191.4, one methoxy group at δ 60.1 and quaternary carbons refers for a flavonoid type structure.



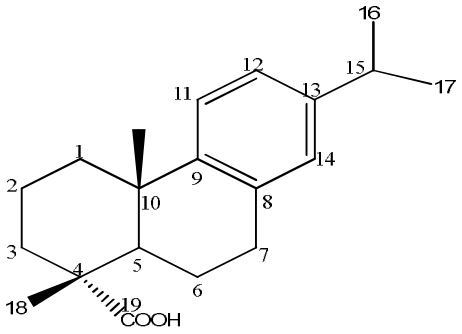
Compound 1

The HMBC experiment [400MHz, CD_3OD, Table 1] played a key role in assigning all protons and carbons as well as the position of the methoxy group in the molecule. The H-2 proton showed ^3J correlation to C-4 of ring-C, C-2' and C-6' of ring B. whereas the proton at position 3 showed ^2J correlation to C-4 and C-2 of ring C while the methoxy protons showed ^3J correlation to C-3, indicating that the methoxy is attached to C-3. In ring A, the proton at position 5 showed ^3J correlations to the carbonyl carbon (C-4) of ring C & hydroxylated carbon (C-7) and also showed a 1, 2, 4-substitutions pattern indicating that hydroxyl group at the position 7. While H-8 and H-6 showed ^3J correlations to each other's carbon. Long range ^4J 'W' coupling was also observed between H-8 and C-4. Ring-B exhibited an ABX substitution pattern with hydroxyl groups at C-3' and C-4'. In the first report of this compound[8], only the proton shifts were expressed in ϯ scale. This is the first report of the ^{1}H and ^{13}C NMR data for compound 1 that is expressed in ppm scale. The H-2 and H-3 of ring-C showed a large coupling constant (10.5Hz) indicating that they follow the *trans* diaxial configuration. Based on the spectral data and by the comparison with literature, the structure of the compound 1 was identified as 7,3',4'-trihydroxy-3-methoxyflavanone (1) reported for the first time in this plant.

Table. 1: ^{1}H (400MHz), ^{13}C and HMBC NMR data of compound 1 in CD_3OD.

Position	^{1}H	^{13}C	HMBC (H→C)
1			
2	δ 5.07 (d , J= 10.5Hz)	83.4	C-2', C-6', C-3
3	δ 4.16 (d, J= 10.5Hz)	81.7	C-2
4		191.4	C-5, C-2, C-3
5	δ 7.69 (d, J= 2.2Hz)	129.1	C-4, C-7
6	δ 6.53 (dd, J= 2.2, 8.0Hz)	113.5	C-8
7		165.8	C-5, C-8
8	δ 6.32 (d , J= 2.2Hz)	102.7	C-6
9		164.3	C-8, C-5, C-2
10		102.5	C-6, C-8
1'		128.6	C-5', C-2, C-3
2'	δ 6.93 (d., J= 2.0Hz)	116.3	C-2
3'		146.7	C-2', C-5'
4'		146.7	C-2', C-5'
5'	δ 6.78 (d, J= 8.0Hz)	115.9	
6'	δ 6.81 (dd, J= 2.0, 8.0Hz)	120.1	C-2, C-2'
3-OMe	3.28 (s)	60.1	C-3

Compound 2 was isolated from VLC fraction of n-hexane extract of *Abies nobilis.* The HRESI-MS indicated a molecular ion at *m/z* 300.2087 which was analyzed for the molecular formula $C_{20}H_{28}O_2$. IR (KBr) absorption at 1464, 2649, 2927cm^{-1} were indicative of the presence of C=C, O-H and C-H groups stretching respectively. The ^{1}H NMR spectrum [400MHz, $CDCl_3$, Table 2] showed aromatic, aliphatic protons and 4 methyls; two appearing as equivalent doublets at δ 1.21 ppm (each d, *J*= 7.0 Hz, Me-16, Me-17) and two other methyl groups attached to quaternary carbons and appearing as singlets at δ 1.27ppm (Me-18) and δ 1.20 ppm (Me-20). In the aromatic region of the spectrum it showed a doublet at δ 7.16 (*J*= 8.0 Hz, H-11), doublet at δ 6.88 (*J*=1.2Hz, H-14) and doublet of doublets at δ 6.99 (*J*= 1.8Hz, 8.0Hz, H-12) indicating an ABX substitution pattern. A septet proton at δ 2.88ppm (H-15) showed COSY correlation to two methyl groups (Me-16 and Me-17) whereas these methyls also showed COSY correlation to each other indicating the presence of an isopropyl group. In ^{13}C NMR data [100MHz, $CDCl_3$, Table 2] showed 20 carbon atoms including one at δ184.5ppm due to a carbonyl function. This number of carbon atoms corresponds to diterpene moiety which contains a carboxylic acid group and 4 methyls. Two methyls at positions 16 and 17 showed HMBC correlations to the aromatic ring and another methyl (δ 1.27ppm) gave a correlation to the carboxylic carbon. Some methylens (δ 1.55, 1.87, 2.87 and 2.93) exhibited HMBC correlation to the aromatic ring whereas some other methylens (δ 1.68 and 1.83) showed correlation to the carboxylic acid and one methyl (δ 1.27). The optical rotation of compound 2 was found to be $[\alpha]_D$ = +57. Further comparisons of the NMR data to those of the literature identified that the compound is dehydroabietic acid (2). This is the first report of this compound from this plant. The experimental data were in agreement with the published data (Gigante et al., 1995).



Compound 2

Table. 2: ^{1}H and ^{13}C NMR data of compound **2** in $CDCl_3$

Position	^{1}H	^{13}C	^{3}J HMBC
1	1.49, 2.34m	37.9	
2	1.69, 1.90m	18.6	
3	1.68, 1.83m	36.7	C-19, Me-18
4		47.4	
5	2.23 dd, *J*= 1.8, 12.3Hz	44.6	
6	1.55, 1.87m	21.8	C-8
7	2.87, 2.93m	30.0	C-9, C-14
8		134.7	
9		146.8	
10		36.9	
11	7.16 d, *J*= 8.0Hz	124.2	
12	6.99 dd, *J*= 1.8, 8.0Hz	123.9	
13		145.8	
14	6.88 d, *J*= 1.8Hz	127.0	
15	2.88septet	33.5	C-12, C-14
16	1.21 d, *J*= 7.0Hz	24.0	C13, C17
17	1.21 d, *J*= 7.0Hz	24.0	
18	1.27 (s)	16.3	C3, C5, C19
19		184.5	
20	1.20 (s)	25.2	C1, C5, C9

Broth micro dilution method was carried out as described previously. Compound 1 and compound 2 both showed activity against *Mycobacterium aurum* (CIP 104482) with an MIC of 413.63µM for compound 1 and an MIC of 51.96 for compound 2 as compared to standard drug (ethambutol at 29.36 µM) as a positive control (Figure 1) whereas the activity against S.aureus were measured as MIC for compounds 1 and 2 as 827.81 and 416.38 µM compared to gentamicin as a standard at 0.31 µM as shown in Figure 2. The activity of compound (1) was observed and that supported the antimycobacterial activity of flavonoids which puplished by (Begum et al., 2008). Compound 2 showed good activity against *M. aurum* and this supported the previous studies that the C-13 isopropyl group of abietic-type resin acids is a structural requirement for the inhibition of mycobacterium (Copp 2003) and the activity of compound 2 against *S. aureus* is famous for abietane type compounds as mentioned in the previous publications while the activity of compound 1 against *S. aureus* was observed as reported for flavanones type compounds that could be attributed to the lipophilicity of such type of compounds which helping in their penetration of the mycobacterial cell wall (Gibbons 2004). While the cytotoxicity against ZR75 (Breast cancer cell) was observed for compound 1 with 40% cell viability against ZR75 cell line with low toxicity against human cell lines while compound 2 showed only 1% cell viability as compared to Triton X as a positive control and showed 76.4% cell viability against human foreskin (HS27) and 10% cell viability against mouse, CH3/ a connective tissue, aerolar and adipose (L929) (Figure 3,4,5). These results indicate that diterpene compound 2 was more cytotoxic and supported the cytotoxic effect of an abietic acid mixture (37% abietic acid, 6% dehydroabietic acid, and a reminder of unknown compounds) against breast cancer lines (Mellanen et al., 1996) and this is the first time of cytotoxic effect of dehydroabietic acid alone.

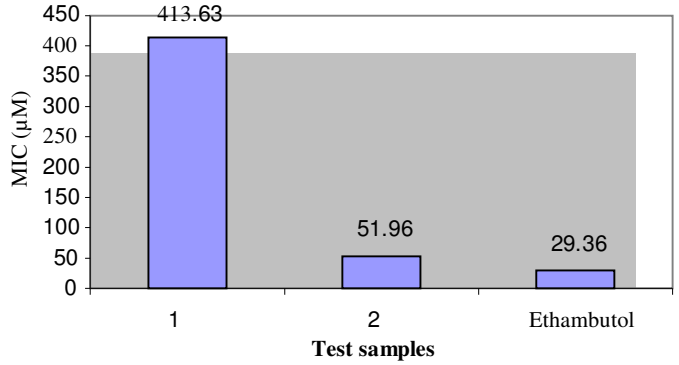


Fig. 1: MICs of isolated compounds against *M. aurum*Test substances: 7,3',4'-trihydroxy-3-methoxyflavanone (1), dehydroabietic acid (2), Ethambutol = Standard drug as a positive control.

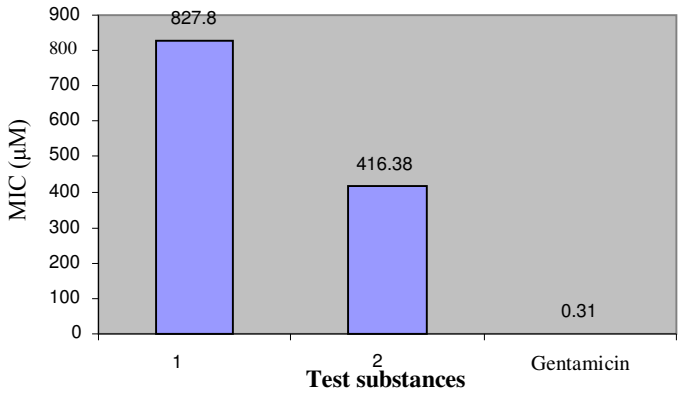


Figure 2: MICs of isolated compounds against *S. aureus* Test substances: 7,3´,4´-trihydroxy-3-methoxyflavanone (1), dehydroabietic acid (2), gentamicin= Standard antibiotic as a positive control.

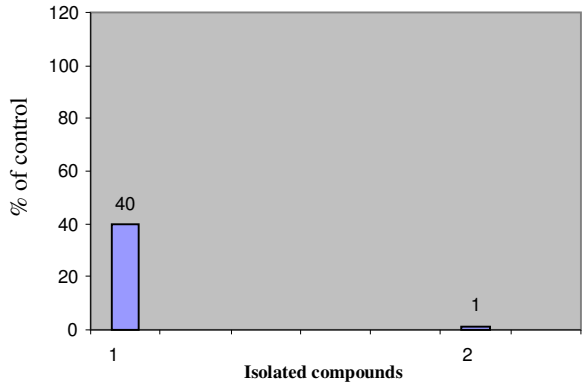


Fig. 3: Cytotoxicity effects of isolated compounds against ZR75 Isolated compounds; 7,3',4'-trihydroxy-3-methoxyflavanone (1) and dehydroabietic acid (2)

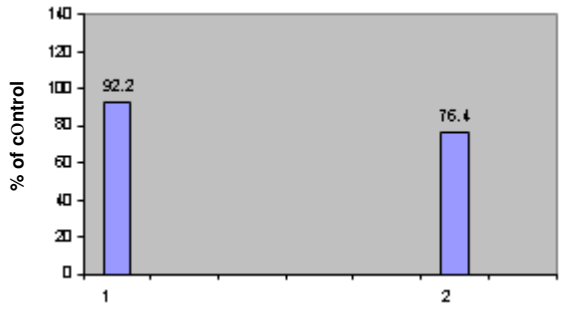


Fig. 4: Cytotoxicity effects of isolated compounds against HS27.

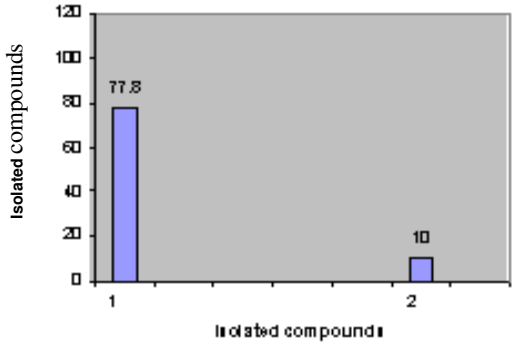


Fig. 5: Cytotoxicity effects of isolated compounds 1 and 2 against L929.

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