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Research Article

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Benzoylbetulin from Roots of *Teclea nobilis*

Tesfaye Nuru, Solomon Girmay, Yadessa Melaku, Milkyas Endale*

Department of Applied Chemistry, Adama Science and Technology University, P.O.Box 1888, Adama, Ethiopia

Abstract *Teclea nobilis* is widely distributed in tropical Africa and used as the remedy for gonorrhea, fever and analgesics. The roots of *Teclea nobilis* were exhaustively extracted using dichloromethane/methanol (1:1) and methanol to give 2.36% and 2.12% yield, respectively. Phytochemical screening analysis conducted on roots extracts of *Teclea nobilis* revealed the presence of alkaloid, tannins, flavonoids, steroids and saponins. Silica gel column chromatographic separation of the crude extract afforded benzoylbetulin (3), isolated for the first time from natural source, along with a known furoquinoline alkaloid maculine (1) and a known steroid lupane (2). The structures of compounds were characterized using spectroscopic methods (^{1}H NMR, ^{13}C NMR and DEPT-135). The extracts and isolated compounds were evaluated *in vitro* for antibacterial activities using disc diffusion method against *E coli, S. aureus, P. aeruginosa* and *K. Pneumonia*. Moderate inhibition zone (9 mm) was observed for maculine (1) against *S. aureus* compared to standard Ceftriaxone (20 mm).

Keywords Steroid, furoquinoline Alkaloid, Teclea nobilis, phytochemical screening, antibacterial activity

Introduction

The majority of the rural population in developing countries still uses traditional medicine to meet some of the primary health care needs owing to the high cost of modern pharmaceuticals and health care practices [1]. The indigenous peoples of different localities have developed their own indigenous knowledge of plant resource uses, management and conservation [1,2]. Teclea nobilis belonging to the genus Teclea and family Rutaceae is locally termed as 'atesa' in Amharic. Previous studies have shown that the genus Teclea used as analgesic, anti-inflammatory and antipyretic activities [3]. Despite its wide traditional use, there is limited report on the chemical constituents and antibacterial studies of the roots extract of this plant from Ethiopian flora. In an ongoing effort to study the chemical constituents of medicinal plants of Ethiopia, we hereby report a comprehensive phytochemical analysis of the roots of Teclea nobilis.

Materials and Methods

Collection and Identification of Plant Material

The roots of Teclea nobilis were collected in February 2018 from Sidama zone around Yirgalem town in manche kebele, Godimo place 60 km away from Hawassa city. The plant was identified by Mr. Shambel and a voucher specimen (H001-1004) was deposited at the National Herbarium, Addis Ababa University, Ethiopia.

Extraction and Isolation

The air-dried grounded roots (500 g) of Teclea nobilis was soaked in dichloromethane/methanol (1:1, 2 L) for 72 hr at room temperature. The mixture was filtered and concentrated under reduced pressure at a temperature of 40°C

using rotary evaporator to give 11.8 g (2.36%) crude extract. The marc left was further extracted with methanol (2 L) for 72 hr, filtered and concentrated in rotary evaporator to furnish 10.6 g (2.12%) crude extract. The dichloromethane/methanol (1:1) extract (6 g) was subjected to silica gel column chromatography and eluted with increasing gradient of ethyl acetate in n-hexane. A total of 51 fractions were collected. Fraction 6 (eluted with 20 % EtOAc in n-hexane) afforded compound 3 (13 mg). Similarly, the methanol extract (5 g) was subjected to silica gel column chromatography and eluted with increasing gradient of ethyl acetate in n-hexane. A total of 35 fractions were collected. Fraction 6 of methanol extract (eluted with 20% EtOAc in n-hexane) yielded compound 2 (8 mg). Fractions 16-17(eluted with 50% EtOAc in n-hexane) were combined and further purified by silica gel chromatography using increasing gradient of ethyl acetate in n-hexane and afforded compound 1 (10 mg).

Phytochemical Analysis of Extracts

The crude extracts obtained were subjected to phytochemical analysis to determine the presence of secondary metabolites such as alkaloid, tannins, flavonoids, steroids, and saponins using standard protocols [4, 5].

Antibacterial Activity

The methanol and dicloromethane:methanol (1:1) crude extracts and isolated compounds were evaluated in vitro for antibacterial activity using disc diffusion method against one gram positive bacteria Staphylococcus aureus and three gram negative bacteria *Escherichia coli, Klebsiella pneumania* and *Pseudomonas aeruginosa*. The bacterial cultures were inoculated into the Muller Hinton Agar (MHA). Ceftraxone was used as positive control. Approximately, 20 mL of sterile MHA were poured into sterile culture plates and allowed to set wells of about 6 mm in diameter which were punched on the plates. Standard solutions of 1.5 mg/mL concentration of the extracts and isolated compounds were prepared and 5 µL solutions from the concentration were loaded to the discs in different replications. The plates were incubated at 37 °C. The antibacterial activity of the plant extracts and compounds were evaluated by measuring the zone of inhibition against the test organism after 24 hr.

Results and Discussion

Phytochemical screening

The phytochemical screening test conducted on roots extracts of *Teclea nobilis* revealed the presence of alkaloid, tannins, flavonoids, steroids and saponins (Table 1).

Table 1: Phytochemical screening test on the roots extracts of *Teclea nobilis*

Secondary metabolites	Observation
Alkaloid	+
Tannins	+
Saponins	+
Steroid	+
Flavonoid	+

+ shows presence

Characterization of Compounds

Compound **1** was isolated as white solid with R_f value of 0.6 (50% EtOAc in *n*-hexane as eluent). The spot turned orange when sprayed with dragendorff reagent, which is an indication of an alkaloid. The ^{1}H NMR spectrum showed the presence of a pair of mutually coupled doublets with AB multiplicity pattern appearing at δ_H 7.56 (*d*, H-2, *J*=2.8) and 7.01 (*d, J*=2.8 Hz, H-3) which are characteristic of a furan ring in furoquinoline alkaloids. A downfield methoxy signal was observed resonating at δ_H 4.44 (δ_C 58.9). The ^{1}H NMR spectrum further revealed two aromatic signals at δ_H 7.48 (*s*, H-5) and 7.29 (*s*, H-8) suggesting *para*-oriented aromatic protons (H-5 and H-8). A downfield singlet signal observed at δ_H 6.09 (2H, δ_C 101.6) is characteristic of a methylenedioxy substituent (also confirmed from DEPT-135 spectrum pointing down). The above spectral data pattern suggest that the compound have furoquinoline alkaloid skeleton.

The ^{13}C NMR spectrum showed signals at δc 155.9 (C-4), 163.1 (C-9a) and 143.8 (C-8a). In agreement with furoquinoline alkaloid skeleton, the chemical shift positions observed for C-8a and C-9a suggest sp^2 aromatic quaternary carbons attached to nitrogen atom [6]. Olefinic carbons of the furan moiety were observed at δ_C 150.8 (C-6) and δ_C 146.1 (C-7). Due to the presence of two para oriented singlets resonating at δ_H 7.48 and 7.29, assigned to H-5 and H-8, respectively, the only possible position of the methylenedioxy moiety is at C-6/C-7 position. The corresponding carbon signals for C-5 and C-8 were observed at δ_C 102.5 and δ_C 98.0, respectively, whereas that of two vicinal oxygenated sp^2 aromatic quaternary carbons, attached to methylenedioxy moiety, appeared at δ_C 150.8 (C-6) and δ_C 146.1 (C-7). Thus, based on the above spectra feature this compound was found to be identical with furoquinoline alkaloid reported in literature with trivial name maculine (**1**), previously isolated from the leaves of *Teclea nobilis* [3]. However, this is the first report from the roots of the plant.

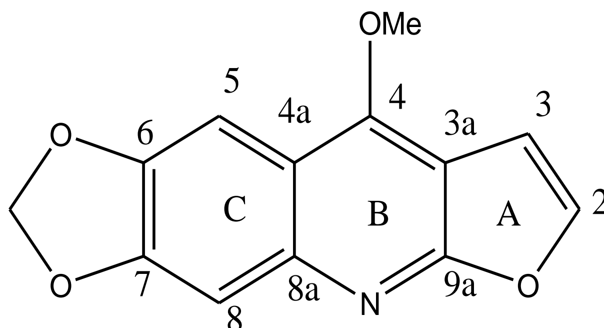

Table 2: ^{1}H ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **1**

Position	δ_H (δ in ppm, *J* in Hz)	δ_C	Maculine (1) [3]	
			δ_H	δ_C
2	7.56 (1H, *d*, *J*=2.8 Hz)	142.6	7.55 (1H, *d*, *J*=3 Hz)	142.8
3	7.01 (1H, *d*, *J*=2.8 Hz)	104.5	7.01 (1H, *d*, *J*=3 Hz)	104.7
3a	-	102.5	-	102.7
4	-	155.9	-	156.2
4a	-	114.3	-	114.5
5	7.48 (1H, *s*)	104.5	7.50 (1H, *s*)	104.7
6	-	150.8	-	150.9
7	-	146.1	-	146.3
8	7.29 (1H, s)	98.0	7.23 (1H, *s*)	98.2
8a	-	143.8	-	144.1
9a	-	163.1	-	163.4
MeO-4	4.44 (3H, s)	58.9	4.39 (3H, *s*)	59.2
$-OCH_2O-$	6.09 (2H, s)	101.6	6.07(2H, *s*)	101.8

Compound **2** was obtained as colorless amorphous solid with R_f value of 0.62 (20% EtOAc in *n*-hexane as eluent) from methanol extract. The ^{1}H NMR spectrum (Table 3) revealed the presence of seven methyl singlet signals at δ_H 0.98 (Me-23), 0.77(Me-24), 0.83 (Me-25), 1.04 (Me-26), 0.95(Me-27), 0.80 (Me-28) and 1.69 (Me- 30). The presence of terminal olefinic protons was observed at δ 4.57 (H-29a) and 4.70 (H-29b). Oxygenated sp^3 methine proton was observed at δ 3.18 (m, 1H, H-3) which is a characteristic of most steroids with hydroxyl group at C-3 position.

The ^{13}C NMR spectrum (Table 3) revealed the presence of thirty carbon signals which is a characteristic feature of triterpenes. The ^{13}C NMR and DEPT-135 spectra displayed the presence of seven methyl carbons signals at δ_C 14.6, 16.0, 15.4, 18.0, 16.2, 19.4 and 28.0.

The ^{13}C and DEPT-135 spectrum also showed ten methylene carbon signals at δ_C 18.4, 20.9, 25.2, 27.5, 29.7, 29.9, 34.3, 35.6, 38.7 and 40.0. Presence of five methine carbons (δ_C 48.0, 48.3, 50.5, 55.3 and 38.1), two olefinic carbons (δ_C 150.9 and 109.4), of which one is quaternary, and additional quaternary carbon peaks (at δ_C 38.9, 37.2, 40.9, 42.9 and 43.0) were also confirmed. Oxygenated sp^3 methine at C-3 was observed at δ_C 78.9. Based on the above

spectral data and comparison with literature, compound **2** was identified as lupeol (**2**), a compound widely occurs in plants [7, 8].

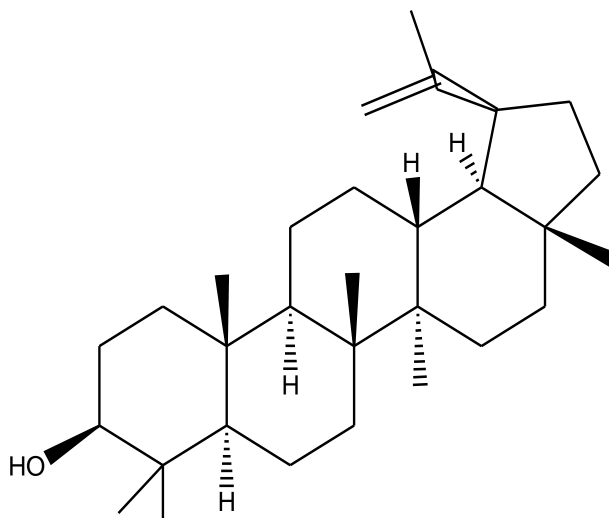

Table 3: ^{1}H ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **2**

Position	δ_H (δ in ppm, *J* in Hz)	δ_C	Lupane [9]	
			δ_H	δ_C
1		38.7		38.9
2		27.5		27.6
3	3.18(m,1H)	78.9	3.17(m,1H)	79.2
4		38.9		39.1
5		55.3		55.5
6		18.4		18.5
7		34.3		34.5
8		40.9		41.0
9		50.5		50.6
10		37.2		37.4
11		20.9		21.1
12		25.2		25.3
13		38.1		38.3
14		42.9		43.0
15		27.5		27.6
16		35.6		35.8
17		43.0		43.2
18		48.3		48.5
19		48.0		48.2
20		150.9		151.2
21		29.9		29.9
22		40.0		40.0
23	0.98	28.0	0.95	28.2
24	0.77	15.4	0.77	15.6
25	0.83	16.2	0.81	16.3
26	1.04	16.0	1.01	16.1
27	0.95	14.6	0.93	14.7
28	0.80	18.0	0.77	18.2
29a	4.57(m)	109.4	4.55(m)	109.5
29b	4.70(d,2.2)		4.68(d,2.2)	
30		19.4		19.5

Compound **3** was isolated as colorless amorphous solid with R_f value of 0.7 (20% EtOAc in *n*-hexane as eluent). The ^{1}H NMR spectrum (400 MHz, CDCl3, Table 4) revealed the presence of seven methyl singlet signals at δ_H 0.99 (Me-23), 0.76(Me-24), 0.83 (Me-25), 1.03 (Me-26), 0.95 (Me-27), 0.79 (Me-28) and 1.68 (Me-30). The presence of terminal olefinic protons was observed at δ 4.56 (H-29a) and 4.68 (H-29b). Oxygenated sp^3 methine proton was observed at δ 3.17 (m, 1H, H-3) which is a characteristic of most steroids with hydroxyl group at C-3 position.

The ^{13}C NMR spectrum (Table 4) revealed the presence of thirty carbon signals which is a characteristic feature of triterpenes and five additional carbons attributed to a benzoyl moiety. The ^{13}C NMR and DEPT-135 spectra displayed the presence of seven methyl carbons which resonated at δ_C 14.6, 16.0, 15.4, 18.0, 16.2, 19.4 and 28.0. Ten methylene carbon signals were observed resonating at δ_C 18.4, 21.0, 25.2, 27.5, 29.7, 29.9, 34.3, 35.6, 38.8 and 40.0. Presence of five methine carbons (δ_C 48.0, 48.30, 50.5, 55.3 and 38.1), two olefinic carbons (δ_C 150.8 and 109.4), of which one is quaternary, and additional quaternary carbon peaks (at δ_C 38.9, 37.2, 40.8, 42.8 and 43.0) were also confirmed. Oxygenated sp^3 methine at C-3 was observed at δ_C 78.9. The methyl signal which appeared at δ_C 18.0 (C-28), in case of compound **2**, appeared at δ_C 71.8 (C-28) in case of compound **3** suggesting the methyl (C-28) is oxidized to oxymethylene, also confirmed from DEPT-135 spectrum. This suggests that the main skeleton is a known steroid betulin. However, In addition to betulin skeleton, the ^{1}H NMR spectrum revealed a benzoyl moiety with characteristic peaks of a monosubstituted aromatic ring resonating at δ_H 7.7 (2H, H-2',6'), 7.2 (2H, H-3',5') and 7.5 (H-4') coupled with the observation in ^{13}C NMR spectrum and DEPT-135 spectra with characteristic peaks resonating at δ_C 167.6, 128.8 (attributed to C-2',6'), 130.9 (C-3',5'), 132.4 (C-1') and 128.6 (C-4'). There are two possible positions in which the benzoyl moiety can be attached to main betulin skeleton (C-3 and C-28) forming ester linkage. On the basis of spectral feature, the benzoyl moiety is suggested to be attached to oxymethylelne (C-28) forming ester linkage considering the observation of downfield chemical shift value of C-28 and no change in the chemical shift value of C-3 compared to compound **2**,. Thus, based on the above spectral data and comparison with literature, the structure of the compound was identified as benzoylbetulin (**3**).

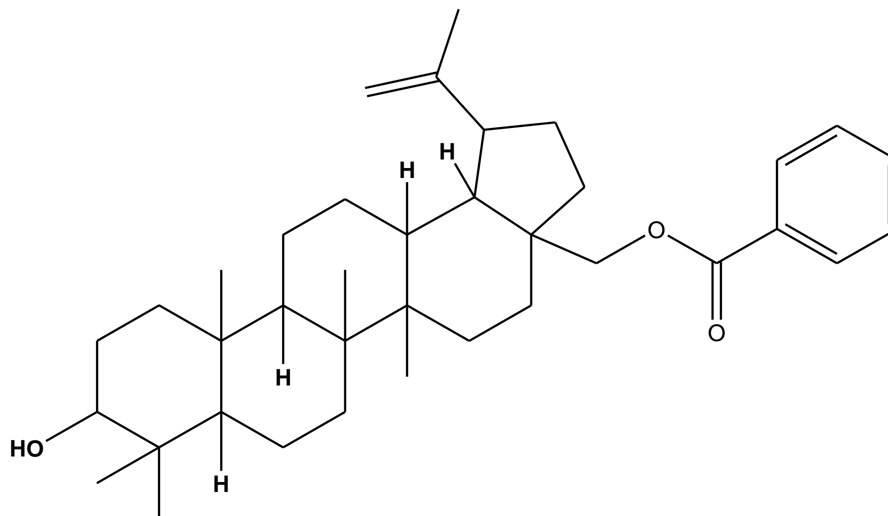

Table 4: ^{1}H ($CDCl_3$, 400MHz), ^{13}C NMR ($CDCl_3$, 150MHz) and DEPT-135 spectral data of benzoylbetulin (**3**)

Position	δ_H (δ in ppm, *J* in Hz)	δ_C	DEPT-135 δ_C
1		38.8	38.8
2		27.5	27.5
3	3.17(m,1H)	78.9	78.9
4		38.9	
5		55.3	55.3
6		18.4	18.4
7		34.3	34.3
8		40.8	
9		50. 5	50.5

10		37.2	
11		21.0	21.0
12		25.2	25.2
13		38.1	38.1
14		42.8	
15		27.5	27.5
16		35.6	35.6
17		43.0	
18		48.3	48.3
19		48.0	48.0
20		150.8	
21		29. 9	29.9
22		40.0	40.0
23	0.99	28.0	28.0
24	0.76	15.4	15.4
25	0.83	16.2	16.2
26	1.03	16.0	16.0
27	0.95	14.6	14.6
28	4.0	71.8	71.8
29a	4.56(m)	109.4	109.4
29b	4.68(d,2.2)		
30		19.4	19.4
1’		132.4	
2’	7.7	128.8	128.8
3’	7.2	130.9	130.9
4’	7.5	128.6	
5’	7.2	130.9	130.9
6’	7.7	128.8	128.8
7’	-	167.6	

Zone of bacterial growth inhibition

The antibacterial tests showed considerable antibacterial activity against the bacterial species used in the study. Methanol extract showed promising activity against the tested strains except for *S. aureus* and *P. aeruginosa*. The methanol extract was found to inhibit *E. coli* and *K. pneumonia* but extract of 1:1 ratio of dichloromethane with methanol have not any activity against bacterial species used in study. From the compounds isolated compound **1** showed promising activity except for *P. aeruginosa* while compound **3** has no any activity against bacterial species used in the study.

Table 5: Zone of bacterial growth inhibition diameter (mm)

Sample	**Inhibition diameter (mm)**			
	S. aureus	***E. coli***	***K. pneumonia***	***P. aeruginosa***
Methanol extract	-	8	7	-
Methanol/dichloromethane (1:1) extract	-	-	-	-
Compound **1**	9	8	8	-
Compound **3**	6	6	6	6

Conclusion

For decades, medicinal plants has been used and continues to be an alternative approach on treatment for various diseases caused by protozoan, bacteria, fungi, viruses and helminthes. *Teclea nobilis* is one of these medicinal plants used traditionally to heal various infectious diseases. Phytochemical screening analysis conducted on roots extract of *Teclea nobilis* revealed the presence of alkaloid, tannins, flavonoids, steroids and saponins. Silica gel column chromatographic separation of the crude extract afforded a benzoylbetulin (**3**), isolated for the first time from natural source, along with a known furoquinoline alkaloid maculine (**1**) and a known steroid lupane (**2**). The extracts and

isolated compounds were evaluated *in vitro* for antibacterial activities by using the disc diffusion method against *E coli, S. aureus, P. aeruginosa* and *K. Pneumonia.* Moderate inhibition zone (9 mm) was observed for maculine (**1**) against *S. aureus* compared to standard Ceftraxone (20 mm). The finding of these pharmacologically important secondary metabolites from root extracts brings the attention of experts to look more on the medicinal importance of the plant.

Acknowledgement
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References

1. WHO (2002). Traditional Medicine: Growing Needs and Potential. WHO Policy Perspectives on Medicines. World Health Organization, Geneva pp. 1-6.
2. Dawit, A. (2001). Plants as primary source of drugs in the traditional health care practices of Ethiopia. Plant genetic resource of Ethiopia, 6,101-11.
3. Yenesew, A., Dagne, E. (1988). Alkaloids of Teclea nobilis. Phytochemistry 27, 651-653.
4. Yadav, R.N.S., Agarwala, M. (2011). Phytochemical Analysis of Some Medicinal Plants. Journal of Phytology, 3, 10-14.
5. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H. (2011). Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sci-encia. 1, 98-106.
6. Wondimu, A., Dagne, E., Waterman, P.G. (1988). Quinoline alkaloids from the leaves of *Teclea simplicifolia*. J. Phytochemistry, 27(3), 959-960.
7. Furukawa, S., Takagi, N., Ikeda, T., Ono, M., Nafady, A.M., Nohara, T., Sugimoto, H., Doi, S., Yamada, H. (2002). Two novel long-chain alkanoic acid esters of lupeol from Alecrim- Propolis. Chem Pharm Bull. 50. 439-440.
8. Chepkirui, C. (2012). Phytochemical investigation of three *Erythrina* species and *Teclea nobilis*. University of Nairobi, Kenya.
9. Daisy Nyawira Njeru (2015). Phytochemical investigation of the stem bark and the leaves of *Teclea simplicifolia* for analgesic activity. University of Nairobi, Kenya.