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A coniferyl alcohol derivative from the roots of *Zanthoxylum chalybeum*

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PEER REVIEW

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Comments

This is a valuable research work in which the authors have investigated the presence of different secondary metabolites in the plant *Z. chalybeum*. In addition, they were able to isolate two pure compounds, a new coniferyl alcohol derivative and a known alkaloid.

(Details on Page 933)

ABSTRACT

Objective: To isolate and characterize chemical constituents of the roots of *Zanthoxylum chalybeum* (*Z. chalybeum*).

Methods: A number of phytochemical tests were applied to identify the class of compounds present in the CH₂Cl₂/CH₃OH (1:1) root extract. Column chromatographic separation technique was applied to separate the constituents of the CH₂Cl₂/CH₃OH (1:1) root extract and various spectroscopic techniques [UV-vis, infrared radiation, nuclear magnetic resonance (NMR) (¹H-NMR, ¹³C NMR, DEPT-135, COSY, gHSQC and gHMBC)] were used to determine the structures of pure compounds.

Results: Phytochemical screening of the CH₂Cl₂/CH₃OH (1:1) root extract of *Z. chalybeum* revealed the presence of alkaloids, flavonoids, terpenoids, tannins and anthraquinones. Column chromatographic separation of the extract yielded a new coniferyl alcohol derivative, 2, 3-epoxy-6,7-methylenedioxyconiferyl alcohol (1) together with the known alkaloid, dihydrocheleerythrine (2).

Conclusions: The present work conducted on the CH₂Cl₂:CH₃OH (1:1) root extract of *Z. chalybeum* identified various class of compounds present in the root extract. Complete characterization of two compounds were done using spectroscopic techniques of which a coniferyl alcohol derivative (1) was identified for the first time.

KEYWORDS

Z. chalybeum, Rutaceae, Alkaloid, Coniferyl alcohol derivative

1. Introduction

Zanthoxylum belongs to Rutaceae family, and means 'yellow wood' which is derived from the Greek words *xanthos* (yellow) and *xylon* (wood). The family Rutaceae consists of 250 genera and nearly 900 species, many of which are sources of essential oils used in perfumery and medicine. Among the plants belonging to this family, the genus *Zanthoxylum* is the largest and most widespread plant and native to tropical and subtropical

areas[1,2]. In Africa, the genus is reported to have close to 35 species[3]. Species of this genus are of economic importance as sources of edible fruits, oils, wood, raw materials for industries, medicinal plants, ornamentals, culinary applications, and are characterized by a satin wood commonly used in woodworking[4,5]. *Zanthoxylum chalybeum* (*Z. chalybeum*) is a deciduous tree that grows in medium altitudes up to 1500 m above sea level (Figure 1) in dry woodland, bush land or grassland of south and south western Ethiopia. In the southern Ethiopia the plant

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is used as a traditional medicine to treat both human and livestock ailments^[6]. The vernacular name of *Z. chalybeum* in Sidamigna is known as *Ga'da*.



Figure 1. *Z. chalybeum* (*Ga'da*) (Tabor Mountain, Hawassa, Dec. 12th 2014).

Previous phytochemical analysis of the genus revealed the presence of different kinds of alkaloids such as chelerythrine^[7], skimmianine, arnottianamide^[7,8], nitidine, N-methylflindersine^[7], zanthomurolanines and dihydrochelerythrine^[3], rutaecarpine and lignans^[9,10]. In continuation of the ongoing project to study the chemical constituents of medicinal plants of Ethiopian flora, we hereby present the isolation and characterization of a new coniferyl alcohol derivative from the roots of *Z. chalybeum*.

2. Materials and methods

2.1. General experimental materials

Melting point was determined with Mettler Toledo Model FP62 machine. UV spectrum was measured with GENESY'S spectrometer (200–400 nm) in chloroform at room temperature. Infrared (KBr pellet) spectrum was recorded on Perk–Elmer BX infrared spectrometer in the range 4000–400 cm^{-1} . Nuclear magnetic resonance (NMR) analysis (^1H NMR, ^{13}C NMR, DEPT–135, COSY, HMQC and HMBC) spectra were as recorded on a Bruker avance 400 MHz spectrometer with tetramethylsilane as internal standard^[11]. Structural assignments were done on the basis of gCOSY, gHMBC and gHMBC spectra^[12,13]. Thin-layer chromatography (TLC) was done using silica gel 60 F254. Column chromatography was performed on silica gel 60 (60–100 mesh).

2.2. Plant material

The root of *Z. chalybeum* were collected in December, 2014 from Sidama Zone, Hawassa Zuria Woreda from Tabor Mountain, located around Hawassa city, the capital of Southern Nations Nationalities and Peoples Regional state, located 275 km from Addis Ababa, capital of Ethiopia (Figure 1). The plant material was identified by botanist Reta Regasa and specimen was deposited at the herbarium of Hawassa College of Teacher Education, Hawassa, Ethiopia.

2.3. Extraction and isolation

The ground roots of *Z. chalybeum* (500 g) were extracted

by cold percolation with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1) three times for 24 h while shaking at speed of 230 r/min and temperature controlled at 28.4 °C. The marc left was further extracted with MeOH as above. The extract was concentrated using a rotary evaporator to yield a brownish crude extract (50 g, 10% yield). A 49 g portion of the crude extract was subjected to column chromatography (40 cm length and 40 mm diameter, 200 g of silica gel) and eluted with increasing gradient of ethyl acetate in *n*-hexane.

A total of 23 fractions were collected each 100 mL. Fractions 12–13 showed three spots on TLC (30% ethyl acetate in *n*-hexane as eluent). These fractions were combined and further purified by small column (increasing gradient of ethyl acetate in *n*-hexane as eluent). Fraction 5 was eluted 8% ethyl acetate in *n*-hexane showed a yellow precipitate (single spot on TLC) which was further washed by 100% *n*-hexane using suction filtration to give 40 mg white crystalline solid identified as compound 1.

2.4. Phytochemical screening

Phytochemical screening tests were done to determine the class of compounds present in the crude extract following the standard protocols^[14,15]. The results were reported as (+Ve) for presence and (–Ve) for absence.

2.4.1. Test for alkaloids

About 0.5 g of extract was defatted with 5% diethyl ether for 15 min and dissolved in 5 mL of 1% hydrochloric acid. The resulting mixture was centrifuged for 10 min at 300 r/min. To the acid solution, a drop of Dragendroff's reagent was added and a white to buff precipitate was observed which proves the presence of alkaloids^[14,15].

2.4.2. Test for flavonoids

About 5 mL of dilute aqueous ammonia solution was added to a 0.2 g of the aqueous filtrate of the plant extract, followed by addition of concentrated H_2SO_4 . The instant disappearance of yellow coloration indicated the presence of flavonoids in the crude extract^[14,15].

2.4.3. Test for terpenoids (Salkowski test)

About 0.2 g of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated H_2SO_4 . A reddish brown coloration at the interface confirmed the presence of terpenoids^[14,15].

2.4.4. Test for saponins

About 0.2 g of powdered sample extract was boiled in 2 mL of distilled water on a water bath and then filtered. A fraction of aqueous filtrate (1 mL) was mixed with 2 mL of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with about three drops of olive oil and shaken vigorously. Formation of an emulsion confirmed the presence of saponins^[15].

2.4.5. Test for tannins

About 0.2 g of the dried powdered samples was boiled in 10 mL of distilled water in a test tube and then filtered.

Addition of 0.1% FeCl₃ solution resulted in a characteristic blue–black color^[15].

2.4.6. Test for free anthraquinones

About 0.5 g of the extract was boiled with 10% HCl for few minutes in water bath and filtered. The filtrate was allowed to cool and equal volume of CHCl₃ was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. The formation of rose–pink colour was taken as an indication for the presence of anthraquinones^[15].

2.5. Spectral data of compound 1

White powder (40 mg), melting point 124–125 °C, UV–vis λ_{\max} (CHCl₃) 286; IR (KBr) ν_{\max} cm⁻¹: 3441, 3000, 1600, 1150, ¹H NMR (400 MHz, CDCl₃) δ in ppm: 6.86 (1H, *dd*, H-8), 6.82 (*s*, 1HH-5), 6.80 (*dd*, 1H, H-9), 6.02 (*s*, 2H, H-7'), 4.73 (*d*, 1H, H-3), 4.25 (*dd*, 1H, H-1), 3.89 (*dd*, 1H, H-1'), 3.10 (*m*, 1H, H-2); ¹³C NMR (400 MHz, CDCl₃) δ in ppm 147.9 (C-6), 147.1 (C-7), 135.1 (C-4), 119.4 (C-5), 108.2 (C-8), 106.5 (C-9), 101.1 (C-7), 85.9 (C-3), 71.7 (C-1), 54.3 (C-2).

3. Results

The preliminary screening tests of the crude extract (CH₂Cl₂/CH₃OH, 1:1) confirmed the presence of alkaloids, flavonoids, terpenoids, tannins and anthraquinones, and absence of saponins as indicated in Table 1. Column chromatography separation of the CH₂Cl₂/CH₃OH (1:1) extract yielded a new coniferyl alcohol derivative, 2,3–epoxy–6,7–methylenedioxy coniferyl alcohol compound 1 together with the known alkaloid, dihydrochelerythrine compound 2, previously isolated from *Zanthoxylum nitidum*^[5,7].

Table 1

Phytochemical screening of the root of *Z. chalybeum*.

Chemical component	Reagent used	Result
Alkaloids	Dragendroff's reagent	+
Flavonoids	Dilute ammonia solution	+
Terpenoids	Chloroform and concentrate H ₂ SO ₄	+
Saponins	Warming in water bath	-
Tannins	FeCl ₃	+
Free anthraquinones	10% Ammonia solution	+

+: Presence; -: Absence.

Table 2

Complete NMR spectral data of compound 1.

Position	¹³ C–NMR (δ in ppm)	¹ H–NMR (δ in ppm)	HMQC	COSY	HMBC
1	71.7	3.89 (<i>d</i> , 1H, H-1), 4.25 (<i>dd</i> , 1H, H-1')	H _{1,1'} →C ₁	H _{1,1'} ↔H ₂	H _{1,1'} →C _{2,3}
2	54.3	3.1 (<i>m</i> , H-2)	H ₂ →C ₂	H ₂ ↔H _{1,3}	H ₂ →C _{1,3,4}
3	85.7	4.73 (<i>d</i> , <i>J</i> =4.4, H-3)	H ₃ →C ₃	H ₃ ↔H ₂	H ₃ →C _{1,2,4,5,9}
4	135.0	–	–	–	–
5	119.3	6.82 (<i>dd</i> , <i>J</i> =0.8, 1.2, H-5)	H ₅ →C ₅	–	H ₅ →C _{3,4,6,9}
6	147.8	–	–	–	–
7	147.1	–	–	–	–
7'	101.0	6.02 (<i>s</i> , 2H, H-7')	H _{7'} →C _{7'}	–	H _{7'} →C _{6,7}
8	108.1	6.86 (<i>dd</i> , <i>J</i> =8.0, 0.8, H-8)	H ₈ →C ₈	H ₈ ↔H ₉	H ₈ →C _{4,6,9}
9	106.5	6.80 (<i>dd</i> , <i>J</i> =8.0, 1.2, H-9)	H ₉ →C ₉	H ₈ ↔H ₉	H ₉ →C _{3,4,5,7}

Compound 1 was isolated as a white powder (melting point: 124–125 °C) with *R_f* value of 0.63 (30% ethyl acetate/*n*-hexane). The UV spectrum indicated absorption maxima (λ_{\max} , CH₃OH) at 286 nm attributed to a conjugated aromatic ring. The IR spectrum revealed a broad vibration at 3441 cm⁻¹ for hydroxyl group, around 3000 cm⁻¹ vibration due to sp³ C–H functional group and stretching vibrations at 1600 cm⁻¹ suggesting the presence of benzene ring and 1100 cm⁻¹ for C–O stretching vibration. The ¹H NMR spectrum (CDCl₃, Table 2) showed peaks at δ 3.10 (*m*, 1H) were attributed to a methine linking to a heteroatom. A doublet of doublet signals at δ 3.89 (*dd*, 1H) and δ 4.25 (*dd*, 1H) connected to the same carbon at δ 71.7 from gHMOC spectrum revealed the presence of diastereotopic methylene protons adjacent to a chiral center (to a methine at δ 3.10, based on gCOSY and gHMBC spectral evidence). A doublet signal at δ 4.73 (*d*, 1H) showed the presence of methine proton attached to a heteroatom and following the gCOSY and gHMBC evidence this methine was also connected to a methine at δ 3.10 (*m*, 1H) forming an oxirane ring (Figure 2).

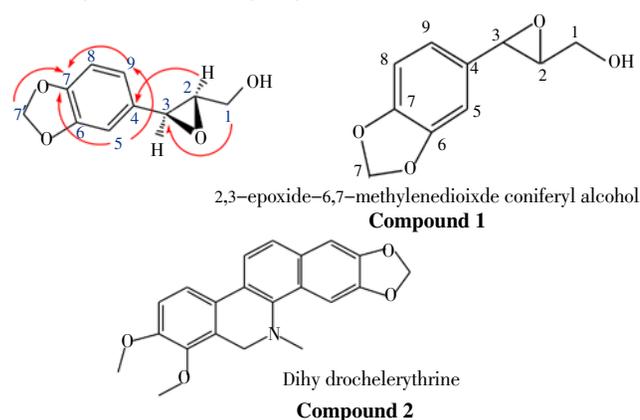


Figure 2. Important HMBC correlations of compound 1.

The presence of a singlet at δ 6.02 integrated for two protons is a characteristic of methylenedioxy group. This is further supported by the correlation of methylenedioxy protons with the oxygenated quaternary carbons at δ 147.8 and δ 147.1 of the phenyl ring.

The presence of doublet of doublet signal at δ 6.80 (*d*, *J*=8.0, 1.2, 1H, H-9) and doublet of doublet at δ 6.86, (*dd*, *J*=8.0, 0.8, 1H, H-8) coupled with a doublet of doublet signal at δ 6.82 (*dd*, *J*=1.0, 0.8, 1H, H-5) suggested the presence of an ABX spin system on the aromatic ring. The

^{13}C NMR spectrum (CDCl_3 , Table 2) coupled with the aid of DEPT-135 (Table 2) revealed a total of ten carbons; one quaternary carbon at $\delta 135.0$, two oxygenated quaternary carbons $\delta 147.1$ and $\delta 147.8$, three sp^2 methine carbons at $\delta 106.5$, $\delta 108.1$, $\delta 119.3$ ppm, two sp^3 methine carbons at $\delta 54.3$ and $\delta 85.7$, two methylene carbon signals at $\delta 101.1$ and $\delta 71.7$ which stand for methylenedioxy (C-7') and methylene (C-1) respectively.

The ^1H - ^1H COSY experiment (Table 2) revealed correlation between proton at $\delta 3.10$ (H-2) and protons at $\delta 3.89$ (H-1), $\delta 4.25$ (H-1') and $\delta 4.73$ (H-3). The ^1J connectivity of methine proton at $\delta 3.10$ (H-3) with $\delta 54.30$, methine proton at $\delta 4.73$ (H-4) with $\delta 85.70$, aromatic proton at $\delta 6.80$ (H-9) with $\delta 119.3$, aromatic proton at $\delta 6.82$ (H-5) with $\delta 106.5$, aromatic proton at $\delta 6.86$ (H-8) with $\delta 108.1$, methylene protons at $\delta 3.89$ and $\delta 4.25$ with $\delta 71.7$ (C-1) and oxymethylene protons at $\delta 6.02$ with $\delta 101.0$ are all established following the observed ^1J gHSQC correlations. Moreover, the gHMBC spectrum (Table 2) revealed methylene protons $\delta 3.89$ (H-1), $\delta 4.25$ (H-1') showed correlation with methine carbons $\delta 54.3$ (C-2) and $\delta 85.7$ (C-3), and methine proton $\delta 4.73$ (H-3) showed correlation with carbons signals at $\delta 54.3$ and $\delta 71.7$ coupled with the correlation of methine proton at $\delta 3.10$ (H-2) with carbons signals at $\delta 71.7$ (C-1) and $\delta 85.7$ (C-3) supported the connectivity of C-1 to C-4.

Additionally, correlation of oxymethylene protons at $\delta 6.02$ (H-7') with oxygenated quaternary carbons at $\delta 147.8$ (C-6) and $\delta 147.1$ (C-7), correlation of aromatic proton at $\delta 6.86$ with quaternary carbon at $\delta 135.1$, correlation of aromatic proton at $\delta 6.82$ with carbons at $\delta 135.0$, $\delta 147.1$ and $\delta 106.5$ coupled with the correlation of the methine at $\delta 4.73$ with the quaternary carbon at $\delta 135.0$, gave a complete evidence for the ABX substitution pattern of the phenyl ring. Thus, based on the above spectroscopic evidence compound 1 was identified to be a new coniferyl alcohol derivative and 2, 3-epoxy-6,7-methylenedioxy coniferyl alcohol, shown below (Figure 2).

4. Discussion

In order to promote Ethiopian herbal drugs and traditional use of medicinal plants, there is an urgent need to evaluate the therapeutic potentials of the drugs as per the WHO guidelines^[16]. Bioactive extracts should be validated and standardized on the basis of phytochemical constituents^[17]. *Z. chalybeum* is the plant belonging to Rutaceae family. About 35 species are distributed in Africa only. The leaf and roots of the plant are claimed to have various medicinal uses including antimalarial and abdominal pain in southern parts of Ethiopia.

The traditional use of the plant may be attributed to its high contents of alkaloid, tannins, anthraquinones and terpenes constituents. In spite of the rich biodiversity of the genus and its use traditionally, there are limited studies on the phytochemistry and biological activity of the genus. The present work conducted on the $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (1:1) root extract of *Z. chalybeum* succeeded in identification of a new coniferyl alcohol derivative (1) and known alkaloid, dihydrochelerythrine (2), from the roots of the plant. To the best of our knowledge, compound 1, coniferyl alcohol derivative (2, 3-epoxy-6,7-methylenedioxy coniferyl alcohol), was isolated for the first time from the genus *Zanthoxylum*. A phenylpropanoid ester, (E)-O-geranylconiferyl alcohol (9Z, 12Z)-linoleate, and derivatives of coniferyl alcohol were previously isolated from the bark of *Zanthoxylum scandens*^[19], and roots of *Ligularia duciformis*^[16], respectively.

As this work is one of the few attempts to phytochemically analyze the polar extracts of the plant, further work is recommended on polar fractions and extracts of the root and leaf of the plant so as to identify more novel and bioactive compounds in support of its traditional use.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The plant *Z. chalybeum* (Family: Rutaceae) has shown different biological activities (antimalarial, trypanocidal, cytotoxic, anti-inflammatory, antiviral activities and others). Secondary metabolites isolated from *Z. chalybeum* include alkaloids (chelerythrine, berberines, nitidine, etc.) and lignans. Phytochemical examination of the *Z. chalybeum* is

very important to discover the metabolites responsible for the plant's biological effects.

Research frontiers

The current work is focusing on the isolation and structure elucidation of the secondary metabolites found in the CH₂Cl₂/CH₃OH (50%) root extract. Different chromatographic, spectrometric, and spectroscopic techniques were employed to achieve the task.

Related reports

Various plant organs were examined for the presence of secondary metabolites, including leaves, root and stem barks. Different secondary metabolites have been isolated from the plant *Z. chalybeum* (e.g. alkaloids, lignans, and volatile oils). The authors are trying to examine the CH₂Cl₂/CH₃OH (50%) root extract of the plant.

Innovations and breakthroughs

The present research work is reporting the isolation and structure elucidation of a new coniferyl alcohol derivative. This new compound is considered as an addition to the natural products library. In addition, a known alkaloid was isolated, namely dihydrochelerythrine.

Applications

Reviewing current literature, the plant *Z. chalybeum* has reported many biological activities. Different metabolites were isolated. Characterization of the plant's secondary metabolites will help finding the compounds responsible for such activities. Isolation and structure elucidation of new secondary metabolites is a very important step in the process of drug discovery.

Peer review

This is a valuable research work in which the authors have investigated the presence of different secondary metabolites in the plant *Z. chalybeum*. In addition, they were able to isolate two pure compounds, a new coniferyl alcohol derivative and a known alkaloid.

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