Research Article

Chemical Constituents of Root Barks of *Gnidia involucrata* and Evaluation for Antibacterial and Antioxidant Activities

Abera Kalbessa,1 Aman Dekebo,1 Hailemichael Tesso,1 Teshome Abdo,1 Negera Abdissa,2 and Yadessa Melaku1*1

1Chemistry Department, School of Applied Natural Sciences, Adama Science and Technology University, Adama, Ethiopia
2Chemistry Department, College of Natural Sciences, Jimma University, Jimma, Ethiopia

Correspondence should be addressed to Yadessa Melaku; yadessamelaku2010@gmail.com

Received 28 May 2019; Accepted 25 July 2019; Published 14 August 2019

Copyright © 2019 Abera Kalbessa et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The genus *Gnidia*, with species close to 152, is traditionally used to treat wide ranges of ailments in humans and animals. *Gnidia involucrata* is one of the species found in Ethiopia and traditionally used as a laxative, antirheumatic agent, insecticide, antibacterial agent, and antimalarial agent. In view of its traditional use, the root bark was sequentially extracted with *n*-hexane, EtOAc, and MeOH to afford 0.78%, 4%, and 6% crude extracts, respectively. The chromatographic separation of the EtOAc extract using silica gel column chromatography yielded three pure compounds: tetratriacontanyl caffeate (1), 12-O-dodeca-2,4-dien-oxyphorbol-13-acetate (2), and naringenin (3). This is the first report of the isolation of 1 and its kind from the genus and most probably from the Thymelaeaceae family. The structures of these compounds were characterized and identified by NMR and mass spectrometric analyses and comparison with literature data. The EtOAc extract and isolated compounds were assessed for their in vitro antibacterial and antioxidant activities. The EtOAc extract (1.5 mg/mL) showed significant inhibitory activity against *S. aureus*, *E. coli*, *P. mirabilis*, and *K. pneumonia* bacterial strains with the highest inhibition zone observed against *S. aureus* (23 mm), which is even greater than that of the reference drug ciprofloxacin (22 mm). However, the inhibition displayed on these bacterial strains for the three pure compounds was marginal with variable degrees of potency between the compounds. The better activity of the crude extract could be due to the synergistic interactions of several phytochemicals present in the extract, which cannot be the case when pure compounds are evaluated alone. The antioxidant activities of the extracts and isolated compounds were evaluated using DPPH and ferric thiocyanate methods. The EtOAc and MeOH extracts and compounds 1 and 2 were found to inhibit the DPPH radical by 70.7, 66.9, 85.8, and 52.8%, respectively. The EtOAc extract and compound 1 inhibited per-oxidation of lipids by 84 and 86%, respectively. The radical scavenging displayed by compound 1 was significant compared with that displayed by ascorbic acid, indicating the strong anti-oxidation potential of the extract of root barks of *G. involucrata*. Therefore, the extracts of the root bark of *G. involucrata* can be used as a remedy in combating diseases caused by bacteria and free radicals provided that further comprehensive evaluation could be recommended for the conclusive decision on potential candidacy of this plant.

1. Introduction

Infectious diseases caused by bacteria remain a serious public health problem in developing countries [1, 2]. The problem is exacerbated by the development of bacterial resistance to currently used antibacterial drugs [3, 4]. Among the major strategies to alleviate the current situation is to search for new antibacterial agents from natural sources as an alternative to synthetic antibiotics. Plants are known to produce a variety of secondary metabolites to protect themselves against a variety of their own pathogens, and therefore, those with documented traditional use appear to be a rich source of new antibacterial and antioxidant drugs. This prompts a revival in search of antibacterial and antioxidant agents from natural products.

*Gnidia* (of family Thymelaeaceae) is a widespread genus comprising about 152 species [5]. Although *Gnidia* species are being recognized for their ornamental value due to their bright and colorful flowers [6], they are also known for their use in traditional medicinal practice [7–9] and for their
economic value as the flowers of the species are employed for
dying leather [10]. *Gnidia involucrata* is one of the species
growing in Ethiopia and locally called *shuntura* in Amharic.
It is a perennial grass or subshrub that is being used in Ethiopia for the
treatment of a wide array of diseases including malaria, rheumatism,
and stomach parasite [11]. Its use as an insecticide and an
insect repellent and also for the treatment of intestinal pain,
mental problems, sexually transmitted diseases, and
tuberculosis has been documented [12, 13]. In Ethiopia, the roots
are used as a laxative and vermifuge [8]. In folk medicine, the
roots are used to reduce the size of the vaginal orifice and as
fish poison. Previous pharmacological reports indicate a
variety of therapeutic uses such as antifungal agents [14],
amylase inhibitors [15], and anti-inflammatory and gastroprotective agents [16].

Some species in the genus *Gnidia* were reported to
contain secondary metabolites including marnillavanone,
kaempferol-3-O-glucoside, *Gnidia* biflavonoid, vitexin,
isorvitexin, isoorientin, mangiferin, 2,3′,5,6-pentahydroxy-
ybenzophenone-4-C-glucoside, 2,4′,6-trihydroxy-4-
methoxybenzophenone-2-O-glucoside, mhakoside A, and
yuankanin [17, 18]. Despite the enormous traditional use of
this species against various diseases, there is no information
on the chemistry and antibacterial and antioxidant studies of
the root barks of *G. involucrata*. Hence, in this study, we
report for the first time the isolation of three compounds
from the barks of *G. involucrata*. The antibacterial and
antioxidant activities of the root bark extracts and isolated compounds are also reported herein for the first time.

2. Materials and Methods

2.1. Plant Material. The root barks of *G. involucrata* were
collected in December 2016 from Kunde Gerasu Kebele,
Meta Wolkite Woreda, West Shewa, Oromia, Ethiopia.
Identification and authentication of the plant specimen was
done at the National Herbarium of Addis Ababa University
by Prof. Legesse Negash, and the voucher specimen (AB-
002/2016) has been deposited.

2.2. Extraction and Isolation. The air-dried root barks
(300 g) of *G. involucrata* were milled into powder and then
sequentially extracted with 1.5 L of *n*-hexane, EtOAc, and
methanol each for 72 hours with occasional shaking at room
temperature. The extracts were filtered and concentrated
under reduced pressure using a rotary evaporator at 40°C to
yield 2.4 g (0.8%), 12 g (4%), and 18 g (6%), respectively. The
ethyl acetate extract (10 g) was adsorbed and fractionated
over silica gel (200 g) column chromatography with in-
creasing polarity of EtOAc in *n*-hexane to afford ca. 200 mL
of each of 22 major fractions. The first four fractions were
collected with 100% *n*-hexane. Fr5-6, Fr7, Fr8-9, Fr10-11,
Fr12, Fr15-16, Fr17-18, and Fr19-20 were eluted with 9:1, 4:
1, 7:3, 3:2, 1:1, 2:3, 1:4, and 1:9 *n*-hexane:EtOAc, re-
spectively. The last fractions, Fr21-22, were collected with
100% EtOAc. Fractions 12 (875 mg) after silica gel column
chromatography with 4:1 *n*-hexane:EtOAc as an eluent
furnished compound 1 (46 mg). Fractions 17-18 (433 mg)
showed three spots on TLC, which were combined and
subjected to column chromatography on silica gel with 85:
15 CH3Cl2:MeOH as an eluent to give compound 2 (66 mg)
and compound 3 (23 mg).

2.3. Antioxidant Activities

2.3.1. DPPH Radical-Scavenging Assay. The radical-scav-
enging activities of the extracts and isolated compounds were
determined using the DPPH method [19, 20]. Four
different concentrations of the methanol extract were mixed
with 0.04% DPPH in MeOH to furnish 100, 50, 25, and
12 μg/mL. The resulting solutions were placed in an oven at
37°C for 30 minutes and subjected to a UV-Vis spectro-
photometer to record absorbance at 517 nm. Likewise, the
radical-scavenging activities of the EtOAc extract and iso-
lated compounds were evaluated following the same pro-
tocol. Ascorbic acid was used as a positive control. The
percentage inhibition was calculated using the following
formula:

\[
\text{\% inhibition} = \left( \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \right) \times 100, \quad (1)
\]

where \(A_{\text{control}}\) is the absorbance of the DPPH solution and
\(A_{\text{extract}}\) is the absorbance of the test sample (DPPH solution
plus sample).

2.3.2. Ferric Thiocyanate Method. The antilipid peroxidation
potential of the extracts and isolated compounds was
evaluated according to the method of Nagatsu [21]. 0.1 mg of
each of the extracts and pure compounds of *G. involucrata*,
100 μL of linoleic acid, EtOH (5 mL), and phosphate buffer
(5 mL, 0.05 M, pH = 7) in water were separately added into a
vial and incubated at 40°C in an oven. After 24 h, 0.1 mL
from each was taken and added into a vial containing 75% of
aqueous EtOH (7 mL), 30% of NH4SCN (0.15 mL), and
0.15 mL of 0.02 M FeCl3 in 3.5% HCl. Each was then sub-
jected to UV-Vis spectrophotometry to record the absorb-
bance at 500 nm. Absorbance of the blank and ascorbic acid
was recorded in the same fashion. The percentage inhibition
using the ferric thiocyanate method is calculated according to
the following formula:

\[
\text{Percentage inhibition} = 100 - \left( \frac{A_b \times 100}{A_b - A_i} \right) \%, \quad (2)
\]

where \(A_i\) is absorbance of the sample and \(A_b\) is absorbance of
the blank [22].

2.4. Antibacterial Activities. The antibacterial activities of the ethyl acetate extract and isolated compounds from the
barks of *G. involucrata* were tested against four bacterial
strains using the disc diffusion method [23]. The American
Type Culture Collection (ATCC) bacterial strains were
obtained from Oromia Public Health Research, Capacity
Building and Quality Assurance Laboratory Center, Adama,
Ethiopia. The antibacterial activities of all samples were
tested against three Gram-negative (Escherichia coli (ATCC 25922), Proteus mirabilis (ATCC 55659), and Klebsiella pneumonia (ATCC 700603)) and one Gram-positive (Staphylococcus aureus (ATCC 25923)) bacterial strains using the Mueller–Hinton agar (MHA) medium.

The test bacterial species were transferred from the stock cultures, and microbial suspensions were prepared in a nutrient broth for 24 h at 37°C until the turbidity of bacterial suspensions reached 1.5 × 10⁸ CFU·mL⁻¹ by comparison with the 0.5 McFarland Standard. The disc diffusion assay was carried out by swabbing each test strain on the Mueller–Hinton (MH) agar plate using the 1/10 dilution of the microbial suspensions. Sterling paper discs (Whatman No.1 filter paper) impregnated with sterile 20 μL (1.5 mg/mL) concentrations of the sample were placed onto the surface of the agar plate with equal distance from each other. A disc impregnated with sterile solvent (water) was taken as a negative control. Ciprofloxacin was used as the standard drug (positive control). After overnight incubation at 37°C, zones of inhibition around the disc were observed. Anti-microbial activity (x) was then characterized and classified based on the inhibition growth zone diameters and described as slight (x = 4–8 mm), high (x = 8–12 mm), and very high (x > 12 mm) [24].

3. Results and Discussion

3.1. Characterization of Isolated Compounds. The root bark of G. involucrata was sequentially extracted with n-hexane, EtOAc, and MeOH. The EtOAc extract was subjected to column chromatography for further purification following its better radical-scavenging activity and peroxide formation inhibition and afforded three compounds 1–3 (Figure 1). The characterization of the spectroscopic data for the identification of the structure of these compounds are detailed below.

Compound 1 was isolated as a white crystal from the EtOAc extract. Its TLC showed a spot at Rf 0.42 with n-hexane: EtOAc (7: 3) as a mobile phase which was visualized after dipping in vanillin/H2SO4. The ESI-MS provided a molecular ion peak at m/z 553 for [M + Na]⁺ and m/z 1083 for [2 M + Na]⁺, both corresponding to the molecular formula of C34H48O8, indicating eleven indices of hydrogen deficiency. The IR spectrum showed the stretching band due to the presence of hydroxyl at 3415 cm⁻¹. The strong absorption bands at 2925 cm⁻¹ and 1714 cm⁻¹ were evident for the presence of the C-H stretching of alkyl groups and α,β-conjugated carbonyl, respectively, whereas the bands at 1641 and 1259 cm⁻¹ were apparent for C=C and C-O stretching, respectively.

The 1H-NMR spectrum (Table 1) showed signals for four olefinic protons at δH 5.76 (1H, d, J = 15.2 Hz, H-2), 6.16 (1H, m, H-5'), 5.69 (1H, br.s, H-4'), and 7.21 (1H, dd, J = 10 Hz, H-3'). The downfield-shifted olefinic proton signal at δH 7.58 was assigned to proton at C-1, beta to the carbonyl group. Signals in the 1H-NMR spectrum at δH 4.02 and 3.95 were due to the presence of oxygenated methylene protons. The signals at δH 2.47 and 2.59 also accounted for the presence of methylene protons in different environments.

The 1H-NMR spectrum (Table 1) indicated the presence of three protons in the aromatic region at δH 6.88 (1H, d, J = 8.0 Hz), 7.04 (1H, dd, J = 1.8 and 8.0 Hz), and 7.13 (1H, pseudosinglet), which were assigned to H-5, H-6, and H-2, respectively, based on their coupling constants and mutual couplings. It also exhibited signals corresponding to a pair of doublets at δH 6.25 and 7.57 with the vicinal coupling constant JHH = 15.6 Hz. This indicated a trans geometry, and the signals were assigned to H-8 and H-7, respectively. These spectral features observed in the aromatic region in combination with the trans double-bond protons, which are peri to the carbonyl group (from the chemical shift value), were a clear indication of the presence of the caffeic acid moiety. The signal at δH 4.19 (2H, t) was apparent for the existence of protons on oxygenated methylene carbon. The spectrum also showed signals accounting for 34 hydrogen atoms centered at δH 1.27, indicating the presence of protons on many methylene groups. The signal at δH 1.73 (4H) and δH 0.90 (10H) revealed the presence of hydrogen atoms on two and five methylene groups, respectively. The presence of terminal methyl protons was evident at δH 0.88.

The proton-decoupled 13C-NMR spectrum (Table 1) of compound 1 with the aid of DEPT-135 showed signals corresponding to 34 carbon atoms including five methine groups (two olefinic and three aromatic), twenty-four methylene groups (one oxygenated), four quaternary carbon atoms (one carbonyl carbon), and a methyl carbon atom. The highly downfield-shifted carbon signal at δC 168.0 and the two olefinic carbon atoms at δC 143.9 and 115.5 were evident for the presence of the α,β-unsaturated carbonyl group of the caffeic acid moiety. The signal at δC 64.9 for the oxygenated methylene carbon and long aliphatic chain revealed the presence of caffeic acid ester. Based on these spectroscopic data and the literature information, the structure of compound 1 was concluded to be pentacontanyl caffeate, previously reported from the leaves of Artemisia argyi except for the long chain alcohol component [25].

The spectral data of compound 1 are in agreement with the structure shown in Figure 1.
quaternary carbons. The downfield-shifted signal observed at δC 209.3 was evident for the presence of the α,β-unsaturated carbonyl group. In fact, the high chemical shift value for this conjugated carbonyl (δC 209.3) could be due to the deshielding and steric effects of the di-alpha substituent, which could not allow the planarity of the carbonyl group with the double bond. The signals at δ 173.9 and 167.2 were apparent for the presence of two ester moieties. The signals observed at δC 118.8, 145.3, 128.3, and 145.7 were evident for the olefinic groups. The other signals due to quaternary carbons were observed at δC 118.8, 145.3, 128.3, and 145.7 were evident for the olefinic groups. The other signals due to quaternary carbons were observed at δC 118.8, 145.3, 128.3, and 145.7 were evident for the olefinic groups. The other signals due to quaternary carbons were observed at δC 118.8, 145.3, 128.3, and 145.7 were evident for the olefinic groups.

Therefore, based on this spectroscopic evidence, compound 2 was identified as prostratin (12-O-phorbol-13-acetate). Prostratin (12-O-phorbol-13-acetate), a nontumor-promoting phorbol ester, reported to inhibit HIV-1 cell entry and replication, blocks completion of reverse transcription of the HIV-1 genome in lymphoid tissue and infection of CD4+ T lymphocytes and at the same time reactivates virus from latency, restricts primary resting of CD4+ T-cell susceptibility to HIV-1 infection in primary blood mononuclear cells (PBMCs) and in lymphoid tissue [28, 29], has antireplicative and anticytopathic activities against HIV [30], and inhibits ornithine decarboxylase induction, edema, and hyperplasia [31]. 12-O-Dodeca-2,4-dienylphorbol-13-acetate isolated in this work from this plant may exhibit activities displayed by those compounds containing the prostratin nucleus. Therefore, the presence of this compound adds one positive attribute to the root barks of this plant.

Compound 3 was obtained as pale yellow needles. Its TLC profile showed a spot at Rf value 0.60 in 15% CH2Cl2 in MeOH as a mobile phase which was visualized after dipping in iodine. The IR spectrum of compound 3 showed the presence of hydroxyl and carbonyl stretching at 3328 and 1641 cm⁻¹, respectively. The 13C-NMR spectral data with the aid of DEPT-135 indicated a total of fifteen carbon atoms including seven methine groups, one methylene group, and one methyne group. 

**Table 1: **1H- and 13C-NMR (CDCl3) spectral data of compound 1 and data of closely related compounds in the literature [20].

| No. | 1H-NMR data of compound 1 | 1H-NMR spectral data reported in the literature [25] | 13C-NMR data of compound 1 | 13C-NMR spectral data reported in the literature [25] |
|-----|--------------------------|---------------------------------|---------------------------|---------------------------------|
| 1   | —                        | 127.4                           | 127.6                     |                                  |
| 2   | 7.13 (1H, br.s)          | 7.16 (1H, d, J = 1.8)           | 114.4                     | 115.2                             |
| 3   | —                        | —                               | 145.0                     | 146.2                             |
| 4   | —                        | —                               | 146.5                     | 148.6                             |
| 5   | 6.88 (1H, d, J = 8.0 Hz) | 6.87 (1H, d, J = 8.0)           | 115.4                     | 116.3                             |
| 6   | 7.04 (1H, dd, J = 1.8 and 8.0 Hz) | 7.04 (1H, dd, J = 8.0 and 1.8) | 122.3                     | 122.4                             |
| 7   | 7.57 (1H, d, J = 15.6)   | 7.53 (1H, d, J = 15.9)          | 143.9                     | 145.5                             |
| 8   | 6.25 (1H, d, J = 15.6)   | 6.28 (1H, d, J = 15.9)          | 115.5                     | 115.8                             |
| 9   | —                        | —                               | 168.0                     | 167.4                             |
| 1′   | 4.19 (2H, t)             | 4.14 (2H, t, J = 6.7)           | 64.9                      | 64.6                              |
| 2′   | 1.73 (2H, m)             | 1.68 (m)                        | 31.9                      | 32.6                              |
| 3′   | 1.73 (2H, m)             | —                               | 29.7                      | NR                                |
| 4′-24′ | 1.27–0.88 (42H)       |                                  | 29.7–22.6                 | NR                                |
| 25′  | 0.88 (3H, t)             | —                               | 14.1                      | 14.3                              |

NR: not reported.
seven quaternary carbon atoms. The downfield signal observed at $\delta_C$ 197.2 was evident for the presence of the carbonyl group. The signal at $\delta_C$ 47.9 was indicative of the presence of aliphatic carbon in the ring. Other signals were observed at $\delta_C$ 83.4, 164.5, 96.1, 166.1, 94.8, 163.3, 101.9, 128.5, 129, 114.9, and 157.6. Of these, the signals situated at $\delta_C$ 197.2, 164.5, 166.1, 163.3, 101.9, 128.5, and 157.6 accounted for quaternary carbons. These spectral data are consistent with the spectral data reported for naringenin, previously reported from *Nyctanthes arbortristis* [32].

### 3.2. Antioxidant Activities

#### 3.2.1. DPPH Radical-Scavenging Assay.

The extracts and isolated compounds were assessed for their radical-scavenging activities using DPPH. The results showed that the ethyl acetate and methanol extracts and compound 1 and compound 2 inhibited the DPPH radical by 70.7, 66.9, 85.8, and 52.8% at 100 $\mu$g/mL, respectively (Table 3). The IC$_{50}$ parameter is also applied to express the antioxidant activity of compounds or mixtures by the DPPH [33]. In view of this, the radical-scavenging activity of the samples analyzed in this work was also expressed in terms of IC$_{50}$ value. The results showed that the EtOAc extract, the methanol extract, and compounds 1 and 2 displayed IC$_{50}$ values of 7.9, 17.7, 73.0, and 84.9, respectively. The best activity was shown by compound 1 which likely accounts for the activity displayed by the EtOAc extract. The radical-scavenging activity of compound 1 was found comparable with that of ascorbic acid which inhibits the radical by 90%. The activity displayed by compound 1 is likely due to the presence of phenolic hydroxyl groups. Therefore, ethyl acetate extract and compound 1 may be used for the treatment of various life-threatening diseases caused by free radicals.

#### 3.2.2. Ferric Thiocyanate Methods.

The degree of lipid peroxidation which was evaluated using the ferric thiocyanate method can be used to measure the antioxidant potential of compounds or extracts. Table 3 shows the results of the antilipid peroxide formation of the root bark extracts and constituents of *G. involucrata*.

As depicted in Table 3, the EtOAc extract and compound 1 inhibit peroxide formation by 84 and 86%, respectively.

### Table 2: $^1$H- and $^{13}$C-NMR (CDCl$_3$) spectral data of compound 2 and literature reported for closely related compounds [26].

| No. | $^1$H-NMR spectral data of compound 2 | $^1$H-NMR literature data [26] | $^{13}$C-NMR spectral data of compound 2 | $^{13}$C-NMR literature data [26] |
|-----|--------------------------------------|---------------------------------|----------------------------------------|---------------------------------|
| 1   | 7.58 (1H, s)                         | 7.57 (1H, s)                    | 160.8                                  | 161 |
| 2   | —                                    | —                               | 132.8                                  | 133 |
| 3   | —                                    | —                               | 209.3                                  | 209.1 |
| 4   | —                                    | —                               | 73.7                                   | 74 |
| 5(a)| 2.47 (1H, d)                         | 2.46 (1H, d)                    | 38.4                                   | 38.9 |
| 5(b)| 2.59 (1H, d)                         | 2.53 (1H, d)                    |                                        |      |
| 6   | —                                    | —                               | 140.7                                  | 140.7 |
| 7   | 5.69 (1H, br.s)                      | 5.66 (1H, d)                    | 129.2                                  | 129.5 |
| 8   | 3.29 (1H, m)                         | 3.22 (1H, dd)                   | 38.9                                   | 39.3 |
| 9   | —                                    | —                               | 78.4                                   | 78.4 |
| 10  | 3.24 (1H, br.s)                      | 3.23 (1H, s)                    | 56.0                                   | 56.4 |
| 11  | 2.16 (1H, m)                         | 2.15 (1H, m)                    | 43.0                                   | 43.4 |
| 12  | 5.43 (1H, d)                         | 5.44 (1H, d)                    | 76.6                                   | 76.8 |
| 13  | —                                    | —                               | 65.7                                   | 66 |
| 14  | 1.09 (1H, d)                         | 1.08 (1H, d)                    | 36.3                                   | 36.6 |
| 15  | —                                    | —                               | 25.7                                   | 26 |
| 16  | 1.25 (3H, s)                         | 1.24 (3H, s)                    | 23.8                                   | 24 |
| 17  | 1.2 (3H, s)                          | 1.19 (3H, s)                    | 16.8                                   | 17 |
| 18  | 0.88 (3H, d)                         | 0.87 (3H, d)                    | 14.4                                   | 14.6 |
| 19  | 1.74 (3H, s)                         | 1.75 (3H, d)                    | 10.1                                   | 10.3 |
| 20(a)| 3.95 (1H, d)                        | 3.97 (1H, d)                    | 68.0                                   | 68.3 |
| 20(b)| 4.02 (1H, d)                        | 4.02 (1H, d)                    |                                        |      |
| 1'  | —                                    | —                               | 167.2                                  | 167.3 |
| 2'  | 5.76 (1H, d)                         | 5.76 (1H, d)                    | 118.8                                  | 119.1 |
| 3'  | 7.21 (1H, dd)                        | 7.21 (1H, dd)                   | 145.7                                  | 145.8 |
| 4'  | 5.69 (1H, br.s)                      | 6.16 (1H, dd)                   | 128.3                                  | 128.5 |
| 5'  | 6.16 (1H, m)                         | 6.13 (1H, m)                    | 145.3                                  | 145.5 |
| 6'  | 2.14 (2H, m)                         | 2.13 (2H, m)                    | 33.0                                   | 33.3 |
| 7'  | 1.40 (2H, m)                         | 1.41 (2H, m)                    | 32.9                                   | 28.6 |
| 8'-11' | 1.27 (8H, m)                      | 1.28 (4H, m)                    | 31.8–22.6                              | 31.6 |
| 12' | 0.88 (3H, t)                         | 0.86 (3H, t)                    | 14.1                                   | 14.2 |
| 1"  | —                                    | —                               | 173.9                                  | 174 |
| 2"  | 2.10 (3H, s)                         | 2.08 (3H, s)                    | 21.1                                   | 21.3 |
respectively, demonstrating their potential in preventing the formation of lipid peroxides. The results turned out to be comparable with those of ascorbic acid which inhibited the DPPH radical by 87%. On the contrary, the MeOH extract and compound 2 were shown to have low ability of inhibiting peroxide formation compared with the natural antioxidant. This indicates that the antioxidant compound of the root bark extracts of *G. involucrata* resides in the EtOAc extract, with the main active ingredient found to be compound 1.

### 3.3. Antibacterial Activities

The antibacterial activity of the EtOAc extract and isolated compounds 1–3 of the root barks of *G. involucrata* was investigated using the agar well diffusion method, against some selected human pathogens, with the results being presented in Table 4.

The extract displayed a broad range of antibacterial activities against all tested pathogens used in this study including *S. aureus*, *E. coli*, *P. mirabilis*, and *Klebsiella pneumonia* with inhibition zones of 23, 14, 12, and 12 mm, respectively. The activity was pronounceable against *S. aureus* compared to ciprofloxacin used as the standard antibiotic. The wide zone of inhibition of the EtOAc extract showed that it had great potential as a remedy for infectious diseases caused by bacterial pathogens. Among the isolated compounds, compound 2 was found active against *S. aureus* and *E. coli* with inhibition zones of about 8 and 11 mm, respectively. On the contrary, compound 3 displayed modest activities against *P. mirabilis*, *E. coli*, and *K. pneumonia* with inhibition zones of 7, 12, and 8 mm, respectively. The better activity of the crude extract could be due to the synergistic interactions of several phytochemicals present in the extract, which cannot be the case when pure compounds are evaluated alone.

### Table 3: DPPH radical-scavenging and antilipid peroxidation activities of extracts (EtOAc and MeOH) and isolated compounds (1 and 2).

| Samples        | % DPPH inhibition | Antilipid peroxidation inhibition (%) |
|----------------|-------------------|--------------------------------------|
|                | 100 μg/mL         | 50 μg/mL | 25 μg/mL | 12.5 μg/mL |                  |
| EtOAc extract  | 70.7 ± 0.02       | 64.1 ± 0.04 | 57.5 ± 0.03 | 46.2 ± 0.05 | 84 ± 0.04        |
| MeOH extract   | 66.9 ± 0.01       | 61.3 ± 0.02 | 54.7 ± 0.01 | 41.5 ± 0.10 | 45 ± 0.06        |
| Compound 1     | 85.8 ± 0.03       | 79.2 ± 0.05 | 73.4 ± 0.01 | 65.0 ± 0.02 | 86 ± 0.01        |
| Compound 2     | 52.8 ± 0.01       | 41.5 ± 0.01 | 34.9 ± 0.02 | 16.9 ± 0.03 | 33 ± 0.09        |
| Ascorbic acid  | 90 ± 0.02         |          |          |            | 87 ± 0.02        |

Data are reported as mean ± SEM; ascorbic acid was used as a positive control.

### Table 4: Zone of bacterial growth inhibition (mm) of the EtOAc extract and isolated compounds from the root barks of *G. involucrata* at 1.5 mg/mL.

| Samples        | Gram-positive bacteria | Zone of bacterial growth inhibition in mm | Gram-negative bacteria |
|----------------|------------------------|----------------------------------------|------------------------|
|                |                        | *S. aureus* | *P. mirabilis* | *E. coli* | *Klebsiella pneumonia* |
| EtOAc extract  |                        | 23 ± 0.02  | 12 ± 0.01    | 14 ± 0.04  | 12 ± 0.03  |
| Compound 1     |                        | 6 ± 0.01   | 6 ± 0.01     | 6 ± 0.02   | 6 ± 0.04   |
| Compound 2     |                        | 8 ± 0.02   | 6 ± 0.02     | 11 ± 0.02  | 6 ± 0.02   |
| Compound 3     |                        | 6 ± 0.01   | 7 ± 0.02     | 12 ± 0.03  | 8 ± 0.01   |
| Ciprofloxacin  |                        | 22 ± 0.03  | 24 ± 0.03    | 21 ± 0.02  | 19 ± 0.02  |

### 4. Conclusion

The ethyl acetate extract after silica gel column chromatography furnished three compounds identified as pentacosyl caffeate (1), 12-O-dodeca-2,4-dienoylphorbol-13-acetate (2), and naringenin (3). This is the first report of caffeic acid ester derivative (1) and its kind from the genus *GNidia*. The work presented herein has also demonstrated that the EtOAc extract of the root bark of *G. involucrata* had strong antibacterial activity compared to ciprofloxacin used as a positive control. The antioxidant activities displayed by the EtOAc extract and compound 1 were significant compared with those displayed by ascorbic acid, indicating the potential of the root barks of this species as natural antioxidants. Therefore, the biological activities displayed by the EtOAc extract and isolated compounds from the root bark of *G. involucrata* corroborate the traditional use of this plant against various ailments caused by bacteria. Further comprehensive evaluations including in vivo antibacterial activity tests are recommended for conclusive decision on potential candidacy of the plant for formulation and medicinal uses.

### Data Availability

The NMR spectra used for the interpretation of compounds in this study are included as supplementary information files. Other data used to support the findings of this study are available from the corresponding author upon request.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.
Acknowledgments
The authors are grateful to Adama Science and Technology University for the fund.

Supplementary Materials
Supporting File 1: 1H-NMR spectrum of compound 1.
Supporting File 2: 13C-NMR spectrum of compound 1.
Supporting File 3: DEPT-135 spectrum of compound 1.
Supporting File 4: 1H-NMR spectrum of compound 2.
Supporting File 5: 13C-NMR spectrum of compound 2.
Supporting File 6: DEPT-135 spectrum of compound 2.

References
[1] M. A. A. Al-Bari, M. A. Sayeed, M. S. Rahman, and M. A. Mossadik, “Characterization and antimicrobial activities of phenolic acid derivative produced by Streptomyces bangladeshensis, a novel species collected in Bangladesh,” Research Journal of Medicine and Medical Sciences, vol. 1, no. 2, pp. 77–81, 2006.
[2] U. A. Khan, H. Rahman, Z. Niaz et al., “Antibacterial activity of some medicinal plants against selected human pathogenic bacteria,” European Journal of Microbiology and Immunology, vol. 3, no. 4, pp. 272–274, 2013.
[3] G. G. F. Nascimento, L. Juliana, C. F. Paulo, and G. L. Silva, “Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria,” Brazilian Journal of Microbiology, vol. 31, no. 4, pp. 247–256, 2000.
[4] E. Hofer, B. R. Quintaes, E. M. F. d. Reis et al., “Emergência da múltipla resistência a antimicrobianos em Vibrio cholerae isolados de pacientes com gastroenterite no Ceará, Brasil,” Revista da Sociedade Brasileira de Medicina Tropical, vol. 32, no. 2, pp. 151–156, 1999.
[5] P. Bhandurge, N. Rajarajeshwari, S. Ganapaty, and S. Pattanshetti, “The Gnidia genus: a review,” Asian Journal of Biomedical and Pharmaceutical Sciences, vol. 3, no. 19, pp. 1–31, 2013.
[6] B. Peterson, Flora of Tropical East Africa. Thymelaeaceae, Crown Agents for Oversea Governments and Administrations, London, UK, 1978.
[7] Y. R. Sohni, T. Mutangadura-Mhlanga, and P. G. Kale, “Bacterial mutagenicity of eight medicinal herbs from Zimbabwe,” Mutation Research/Genetic Toxicology, vol. 322, no. 2, pp. 133–140, 1994.
[8] R. P. Borris, G. Blaskó, and G. A. Cordell, “Ethno-pharmacologic and phytochemical studies of the thymelaeaceae,” Journal of Ethnopharmacology, vol. 24, no. 1, pp. 41–91, 1988.
[9] N. M. Munkcombe, P. Galebotswe, K. Modibesane, and N. Morebodi, “Phenylpropanoid glycosides of Gnidia polycephala,” Phytochemistry, vol. 64, no. 8, pp. 1401–1404, 2003.
[10] C. M. Van-Wyk and N. Gericke, People’s Plants, Briza Publications, Pretoria, South Africa, 2000.
[11] G. Getaneh, “Ethnobotanical study of traditional medicinal plants and their conservation status in Ethiopia,” M. S. thesis, Addis Ababa University, Addis Ababa, Ethiopia, 2011.
[12] A. Getachew, U. Befikadu, and W. Amha, “Systematic review on traditional medicinal plants used for the treatment of malaria in Ethiopia: trends and perspectives,” Malaria Journal, vol. 16, no. 1, p. 307, 2017.
[13] A. Berhanu, Z. Asfaw, and E. Kelbessa, “Ethnobotany of plants used as insecticides, repellents and antimalarial agents in Jabiethman district, West Gojam,” SINET: Ethiopian Journal of Science, vol. 29, no. 1, pp. 87–92, 2006.
[14] S. K. Singh, V. K. Sharma, Y. Kumar, S. S. Kumar, and S. K. Sinha, “Phytochemical and pharmacological investigations on mangiferin,” Herbapolonica, vol. 55, no. 1, pp. 126–139, 2009.
[15] S. Kumar, V. Kumar, M. Rana, and D. Kumar, “Enzymes inhibitors from plants: an alternate approach to treat diabetes,” Pharmacogony Communications, vol. 2, no. 2, pp. 18–33, 2012.
[16] E. Küpeli, M. Aslan, İ. Gürbüz, and E. Yesilada, “Evaluation of in vivo biological activity profile of isoorientin,” Zietschrift für Naturforschung C, vol. 59, no. 11–12, pp. 787–790, 2004.
[17] J. Ferrari, C. Terreaux, S. Sahpaz, J. D. Msonthi, J.-L. Wolfener, and K. Hostettmann, “Benzophenone glycosides from Gnidia involucrata,“ Phytochemistry, vol. 54, no. 8, pp. 883–889, 2000.
[18] J. Ferrari, C. Terreaux, T. Kúrtán et al., “Isolation and on-line LC/CD analysis of 3, 8′-linked biflavonoids from Gnidia involucrata,” Helvetica Chimica Acta, vol. 86, no. 8, pp. 2768–2778, 2003.
[19] W. Brand-Williams, M. E. Cuvelier, and C. Berset, “Use of a free radical method to evaluate antioxidant activity,” LWT—Food Science and Technology, vol. 28, no. 1, pp. 25–30, 1995.
[20] H. Wang, X. D. Gao, G. C. Zhou, L. Cai, and W. B. Yao, “In vitro and in vivo antioxidant activity of aqueous extract from Choeropsis axillaris fruit,” Food Chemistry, vol. 106, no. 3, pp. 888–895, 2008.
[21] A. Nagatsu, “Investigation of anti-oxidative compounds from oil plant seed,” FABAD Journal of Pharmaceutical Sciences, vol. 29, pp. 203–210, 2004.
[22] İ. Gürçin, Z. Huyut, M. Elmaş, and H. Y. Aboul-Enein, “Radical scavenging and antioxidant activity of tannic acid,” Arabian Journal of Chemistry, vol. 3, no. 1, pp. 43–53, 2010.
[23] P. A. Wayne, Performance Standards for Antimicrobial Disc Susceptibility Test, Approved Standard: M2-A6, National Committee for Clinical Laboratory Standards (NCCLS), New York, USA, 5th edition, 1997.
[24] A. Obdak, D. Zielinska, A. Rzepkowska, and D. Koblonsk-Krajewska, “Comparison of antibacterial activity of Lactobacillus plantarum strains isolated from two different kinds of regional cheeses from Poland: Oscypek and Korycinski cheese,” BioMed Research International, vol. 2017, Article ID 6820369, 10 pages, 2017.
[25] J. Degenhardt, J. Gershenson, I. T. Baldwin, and A. Kessler, “Attracting friends to feast on foes: engineering terpene emission to make crop plant more attractive to herbivore enemies,” Current Opinion in Biotechnology, vol. 14, no. 2, pp. 169–176, 2003.
[26] G. Jieru, Z. Jinwen, S. Penghua et al., “Two new diterpenoids from the buds of Wikstroemiaichangaeaphne,” Molecules, vol. 17, no. 6, pp. 6424–6433, 2012.
[27] K. Michal, W. Vitthal, L. I-Wen et al., “Allantergic phorbol ester from the seeds of Aquilaria malaccensis,” International Journal of Molecular Sciences, vol. 17, no. 3, p. 398, 2016.
[28] A. Biancotto, J. C. Grivel, F. Gondois-Rey et al., “Dual role of prostratin in inhibition of infection and reactivation of human immunodeficiency virus from latency in primary
blood lymphocytes and lymphoid tissue,” *Journal of Virology*, vol. 78, no. 19, pp. 10507–10515, 2004.

[29] Y. D. Korin, D. G. Brooks, S. Brown, A. Korotzer, and J. A. Zack, “Effects of prostratin on T-cell activation and human immunodeficiency virus latency,” *Journal of Virology*, vol. 76, no. 16, pp. 8118–8123, 2002.

[30] R. Gulakowski, J. McMahon, R. Buckheit, K. Gustafson, and M. Boyd, “Antireplicative and anticytopathic activities of prostratin, a non-tumor-promoting phorbol ester, against human immunodeficiency virus (HIV)1,” *Antiviral Research*, vol. 33, no. 2, pp. 87–97, 1997.

[31] Z. Szallasi and P. M. Blumberg, “Prostratin, a nonpromoting phorbol ester, inhibits induction by phorbol 12-myristate 13-acetate of ornithine decarboxylase, edema, and hyperplasia in CD-1 mouse skin,” *Cancer Research*, vol. 51, no. 19, pp. 5355–5360, 1991.

[32] R. Jain and M. Mittal, “Naringenin, a flavanone from the stem of *Nyctanthes arbor-tristis*,” *International Journal of Biology, Pharmacy, and Applied Sciences*, vol. 1, no. 7, pp. 964–972, 2012.

[33] M. Olszowy and A. L. Dawidowicz, “Is it possible to use the DPPH and ABTS methods for reliable estimation of antioxidant power of colored compounds?,” *Chemical Papers*, vol. 72, no. 2, pp. 393–400, 2018.