

## Research Article

# Anthraquinones from the Roots of *Kniphofia insignis* and Evaluation of Their Antimicrobial Activities

Asnakew Amare Tadesse <sup>1</sup>, Negera Abdissa Ayana,<sup>2</sup> and Dele Abdissa Keneni<sup>2</sup>

<sup>1</sup>Department of Chemistry, College of Natural Sciences, Woldia University, P.O. Box 400, Woldia, Ethiopia

<sup>2</sup>Department of Chemistry, College of Natural Sciences, Jimma University, P.O. Box 378, Jimma, Ethiopia

Correspondence should be addressed to Asnakew Amare Tadesse; [asnamare6@gmail.com](mailto:asnamare6@gmail.com)

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Sequential extraction using a cold maceration method and column chromatographic separation of the roots *Kniphofia insignis* headed to the isolation of three anthraquinones: one monomeric anthraquinone (**1**) and two dimeric anthraquinones (**2** and **3**). It was further purified by Sephadex LH-20 and recrystallized. The structures of these compounds were established based on the spectroscopic analyses including NMR (<sup>1</sup>H-NMR and <sup>13</sup>C-NMR and infrared) and comparison with reported literatures. In an *in vitro* antimicrobial assay of the crude extracts, the isolated compounds were made against four bacterial strains (*S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853) and *Fusarium* spp. fungal strain. In the crude extracts of chloroform, substantial antimicrobial activity was seen with the highest activity against *B. subtilis* (16 mm) and *E. coli* (22 mm). Meanwhile, compound **1** has a better zone of inhibition with 14 mm against *P. aeruginosa*, whereas compound **2** showed better activity (13 mm) against *Fusarium* spp. fungal strain.

## 1. Introduction

The genus *Kniphofia* Moench (family Asphodelaceae) is commonly named as “red hot poker,” containing about 71 species. About seven species of *Kniphofia* occur in Ethiopia, of which five including *K. foliosa*, *K. hildebrandtii*, *K. isoetifolia*, *K. insignis*, and *K. schimperi* are endemic [1, 2], and the genus is widely known for its ornamental value due to their colorful flowers and used in traditional medicine [3]. Its roots are used to treat abdominal cramps, wound healing, women infertility, gonorrhoea, hepatitis B, ring worm, chest pain, snake bite, and shoulder pains [1, 3–6]. The family Asphodelaceae is a rich source of mainly anthraquinones, monomeric and dimeric anthraquinones, anthrones, dimeric phenylanthraquinones, [5] and naphthoquinone [4]. It is so far only known from the Shewa and Arsi Zone. The main flowering period is from June to September, and it is commonly seen in the Sululta plains between Addis Ababa and Chanco [2]. However, it is a phytochemical investigation, and the antimicrobial activity evaluation has not been reported yet. Therefore, herein, we purpose the current project to carry out isolation and

characterization of compounds from the root of *Kniphofia insignis* and evaluate their antimicrobial activities.

## 2. Experimental Section

**2.1. General Information.** Analytical grade solvents such as *n*-hexane, chloroform, acetone, methanol, and ethyl acetate were used for extraction and column elution; silica gel 60–120 mm mesh size, oxalic acid, TLC silica gel coated plate for detection of spots, CDCl<sub>3</sub> for recording NMR spectra, and DMSO for sample preparation for antibacterial susceptibility test, standard antibiotic drug (gentamycin and chlortrimazole), Mueller–Hinton agar, nutrient agar, and saline solution were used as a culture medium during the antibacterial and antifungal test, and Sephadex LH-20 was also used for further purification. Round-bottom flask of sizes 50, 100, and 500 mL, measuring cylinder, Whatman No. 1 filter paper, pistil and mortar, weighing balance, column chromatography, and rotary evaporator were used during extraction and purification. UV chambers of 254 and 365 nm (LF-260.LS, EEC) were used for detection of spot. An

infrared (IR) spectrum was measured using the Perkin-Elmer IR spectrophotometer. The 1D ( $^1\text{H-NMR}$  (400 and 400 MHz) and  $^{13}\text{C-NMR}$  (125 MHz)) spectra were recorded using Bruker Avance NMR in deuterated solvent were used for characterization.

**2.2. Collection and Preparation of Plant Material.** The roots of *Kniphofia insignis* were collected from Ethiopia, North Shewa Zone Oromia Regional State, Jida District, which is about 116 km away from Addis Ababa. It was collected, air-dried, and powdered so as to allow the penetration of the solvents during the extraction and stored in an appropriate container in Jimma University Organic research laboratory. The plant material was identified by Jimma University Botanist Dr. Dereje Denu, and the voucher specimen (voucher number CH1) has been deposited in Jimma University Herbarium.

**2.3. Extraction and Isolation.** The shaded dried roots (1 kg) of powdered *K. insignis* were subjected to sequential extraction with *n*-hexane, chloroform, acetone, and methanol using the cold maceration technique three times for 24 h each with 2.5 L at room temperature. Then, the crude extracts were concentrated using a rotary evaporator so as to remove the solvent to yield 10 g (1%), 27.5 g (27.5%), 17 g (1.7%), and 45 g (4.5%) *n*-hexane, chloroform, acetone, and methanol extracts, respectively. The crude extracts were subjected to antimicrobial activities against four bacterial strains (*S. aureus* (ATCC 25923), *E. coli* (ATCC 35218), *P. aeruginosa* (ATCC 27853), and *B. subtilis* (ATCC 6633)) and fungal strain (*Fusarium* spp.).

Based on its bacterial activities test and TLC profile, 23 g of chloroform extract was adsorbed on silica gel (60–120 mm mesh size) and subjected to 500 mm diameter column chromatography on silica gel (165 g) eluting with *n*-hexane with increasing amounts of ethyl acetate gradient. The following ratios of solvent combinations were used in the elution process in *n*-hexane to ethyl acetate ratio of 100 : 0 to 0 : 100. The main fractions which yield one pure compound at 1% of ethyl acetate in *n*-hexane were followed by further purification using Sephadex LH-20 in provided compound **1** (32 mg). The other fraction eluted with 5% of ethyl acetate in *n*-hexane yields two dimeric anthraquinones: asphodeline (**2**, 4.3 mg) and 10-hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone (**3**, 3 mg) up on further purification using small column chromatography. The isolated compounds were characterized by the spectroscopic techniques: FT-IR,  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  for compound **1**, and  $^1\text{H-NMR}$  and using reported  $^{13}\text{C-NMR}$  for compounds **2** and **3** at Addis Ababa University.

**2.4. Antimicrobial Assay.** The crude extracts and isolated compounds were evaluated for *in vitro* antimicrobial activities against four bacteria strains (*E. coli* ATCC 35218, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, and *P. aeruginosa* ATCC 27853) and one fungus strain (*Fusarium* spp.) by the agar disc diffusion method. The

antimicrobial activity test was done using the agar disc diffusion method following the standard procedures [7–9]. The stock solution was prepared by dissolving 100 mg of the crude extract in 1 mL DMSO, 25 mg of the compound **1** in 0.5 mL, 4 mg of compound **2** in 0.2 mL to get 100 mgmL<sup>-1</sup> of crude extracts, and 50 and 20 mgmL<sup>-1</sup> final stock solutions of **1** and **2** pure compounds, respectively. Also, the inhibition zone was measured in mm after 24 h of incubation for bacteria and 48 h for fungus at 37°C, and compared with the standard drug, gentamycin for bacteria and clotrimazole for fungus, the inhibition diameter was measured.

### 3. Results and Discussion

**3.1. Characterization of Isolated Compounds.** Compound **1** (32 mg) was isolated as red amorphous solid. It was isolated with 1% of ethyl acetate in *n*-hexane, and its *Rf* value was 0.87 in 95% *n*-hexane in ethyl acetate. Its FT-IR spectral analysis showed a strong band in the region of 3426 and 2917 cm<sup>-1</sup> for the hydroxyl (OH) stretching and aromatic (C–H) stretching vibrations, respectively. Other characteristic signals for carbonyl (C=O) bond vibrations and strong C–O bond stretching were also observed at 1616 and 1268 cm<sup>-1</sup>, respectively.

$^1\text{H-NMR}$  spectrum (400 MHz, CDCl<sub>3</sub>) (Table 1) displayed three –OH protons at  $\delta_{\text{H}}$  13.04, 12.36, and 12.17 involved in hydrogen bonding and was assigned to –OH groups at C-5, C-8, and C-1, respectively, of an anthraquinone skeleton. In ring A, two *meta*-coupled aromatic protons at  $\delta_{\text{H}}$  7.14 (1H, *d*, *J*=1.8, H-2) and 7.73 (1H, *d*, *J*=4 Hz, H-4) were observed in addition to the biosynthetically expected methyl group ( $\delta_{\text{H}}$  2.51;  $\delta_{\text{C}}$  22.3 at C-3;  $\delta_{\text{C}}$  149.1), whereas, in ring C, there were two *ortho*-coupled equivalent aromatic protons resonating at 7.32 (2H, *d*, *J*=8.0 Hz) which were assigned to H-6 and H-7, confirming the presence of chelated –OH group at  $\delta_{\text{H}}$  13.04 being at C-5 ( $\delta_{\text{C}}$  158.3).

The  $^{13}\text{C-NMR}$  spectral data (Table 1) revealed the presence of fifteen carbon signals including two carbonyl carbons ( $\delta_{\text{C}}$  190.6 and 186.6), four aromatic methine carbons ( $\delta_{\text{C}}$  129.6, 129.5, 124.6, and 120.8), three oxygenated aromatic quaternary carbons ( $\delta_{\text{C}}$  162.9, 158.3, and 157.6), five aromatic quaternary carbons ( $\delta_{\text{C}}$  149.1, 133.2, 114, 112.8, and 112.5), and a methyl carbon ( $\delta_{\text{C}}$  22.3). Therefore, compound **1** was identified as 1,5,8-trihydroxy-3-methyl-anthraquinone, trivial name helminthosporin (**1**) (Figure 1), which has been previously reported from *Aloe dawei* and *Aloe lateritia* subspecies *graminicola* [12], but it was reported for the first time from the genus *Kniphofia*.

Compound **2** (4.3 mg) was isolated as a red crystal from 5% ethyl acetate in *n*-hexane. The  $^1\text{H-NMR}$  (Table 1) spectrum showed the presence of two aromatic methyl groups resonating at  $\delta_{\text{H}}$  2.33(3H, *s*, 3-CH<sub>3</sub>) and 2.53(3H, *s*, 3'-CH<sub>3</sub>), eight aromatic protons, and four chelated hydroxyl groups at  $\delta_{\text{H}}$  (12.00, 12.09, 12.45, and 12.53), which confirmed that this compound is dimeric anthraquinone. In one half of the molecule, the  $^1\text{H-NMR}$  spectrum showed three mutually coupled aromatic protons in the ABX spin system

TABLE 1:  $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C-NMR}$  (125 MHz) spectral data of compounds **1**, **2**, and **3**.

Position	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta_{\text{H}}$ ( <i>m</i> , <i>J</i> in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ ( <i>m</i> , <i>J</i> in Hz)	$\delta_{\text{C}}$ [10]	$\delta_{\text{H}}$ ( <i>m</i> , <i>J</i> in Hz)	$\delta_{\text{C}}$ [11]
1	—	162.9	—	162.9	—	161.8
1'	—	—	—	162.2	—	161.9
1a	—	114.0	—	114.8	—	112.6
1'a	—	—	—	113.9	—	114
2	7.14 (1H, <i>s</i> )	124.6	7.73 (1H, <i>s</i> )	125.6	6.83 (1H, <i>d</i> , <i>J</i> =4)	117.2
2'	—	—	7.16 (1H, <i>s</i> )	121.4	7.06 (1H, <i>d</i> , <i>J</i> =4)	124.3
3	—	149.1	—	149.0	—	148.9
3'	—	—	—	149.2	—	149.6
3'-CH <sub>3</sub>	—	—	2.53 (3H, <i>s</i> )	149.2	2.47 (3H, <i>s</i> )	22.0
4	7.73 (1H, <i>d</i> , 4)	120.8	—	137.3	6.63 (1H, <i>brs</i> )	120.9
4a	—	133.2	—	132.8	—	148.4
4'a	—	—	—	134.0	—	133.3
4'	—	—	7.87 (1H, <i>s</i> )	120.4	7.66 (1H, <i>d</i> , <i>J</i> =4)	120.9
5	—	158.3	7.88 (1H, <i>d</i> , <i>J</i> =8)	124.3	6.80 (1H, <i>d</i> , <i>J</i> =4)	119.9
5a	—	112.5	—	130.4	—	148.3
5'a	—	—	—	131.1	—	132.8
5'	—	—	8.0 (1H, <i>d</i> , <i>J</i> =8)	120.0	8.03 (1H, <i>d</i> , <i>J</i> =8)	119.3
6	7.32 (1H, <i>d</i> , 8.0)	129.5	7.73 (1H, <i>s</i> )	137.2	7.43 (1H, <i>t</i> , <i>J</i> =8)	137.3
6'	—	—	7.65 (1H, <i>d</i> , <i>J</i> =8)	135.0	8.67 (1H, <i>d</i> , <i>J</i> =8)	132.2
7	7.32 (1H, <i>d</i> , 8.0)	129.6	7.34 (1H, <i>d</i> , <i>J</i> =8)	124.2	6.98 (1H, <i>d</i> , <i>J</i> =8)	116.9
7'	—	—	—	133.5	—	142.2
8	—	157.6	—	162.8	—	161.5
8'	—	—	—	159.7	—	159.1
8a	—	112.8	—	115.7	—	114.7
8'a	—	—	—	115.5	—	115.9
9	—	190.6	—	192.8	—	193.0
9'	—	—	—	192.8	—	192.1
10	—	186.6	—	182.4	—	70
10'	—	—	—	181.9	—	181.5
3-CH <sub>3</sub>	2.51 (3H, <i>s</i> )	22.3	2.33 (3H, <i>s</i> )	22.3	—	22.2
1-OH	12.17 (1H, <i>s</i> )	—	12.53 (1H, <i>s</i> )	—	11.81 (1H, <i>s</i> )	—
1'-OH	—	—	12.45 (1H, <i>s</i> )	—	12.38 (1H, <i>s</i> )	—
5-OH	13.04 (1H, <i>s</i> )	—	—	—	—	—
8-OH	12.36 (1H, <i>s</i> )	—	12.09 (1H, <i>s</i> )	—	12.18 (1H, <i>s</i> )	—
8'-OH	—	—	12.00 (1H, <i>s</i> )	—	12.48 (1H, <i>s</i> )	—

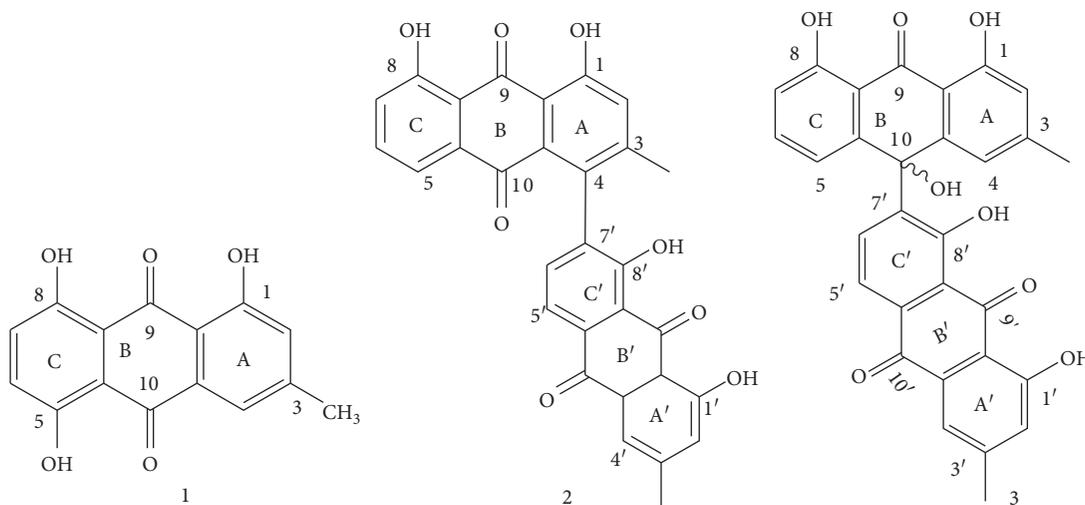
FIGURE 1: The structures of helminthosporin (**1**), asphodeline (**2**), and 10-hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone (**3**) isolated from root of *K. insignis*.

TABLE 2: Zone of inhibition of the extracts and compound **1** and compound **2** in mm.

Test organism	Crude		Extract			Pure			DMSO
	Hexane	Chl	Ac	Meth	1	2	G	C	
<i>E. coli</i>	16	22	18	16	14	13	32	—	NI
<i>P. aeruginosa</i>	12	15	14	12	15	12	22	—	NI
<i>S. aureus</i>	13	13	15	14	12	14	33	—	NI
<i>B. subtilis</i>	13	16	18	15	13	11	31	—	NI
<i>Fusarium</i> spp.	8	12	18	14	12	13	—	20	

Key. Chl = chloroform; Ac = acetone; Meth = methanol; G = gentamycin; NI = no inhibition; C = clotrimazole CH<sub>3</sub>Cl.

at  $\delta_{\text{H}}$  7.88 (1H, *d*,  $J = 8$  Hz), 7.73 (1H, *s*), and 7.34 (1H, *d*,  $J = 8$  Hz) assigned for H-5, H-6, and H-7, respectively, of the ring C and singlet proton resonated at  $\delta_{\text{H}}$  7.73 (1H, *s*, H-2) (Table 1).

Similarly, in the other half of the molecule (ring A'B'C'), the <sup>1</sup>H-NMR spectrum displayed that the broad singlet proton at  $\delta_{\text{H}}$  7.16 (1H, *s*) and  $\delta_{\text{H}}$  7.87 (1H, *s*) in ring C' was assigned to H-2' and H-4', respectively, with biosynthetically expected methyl group being at C-3'. In ring A', two *ortho*-coupled aromatic protons with AX spin system were resonating at  $\delta_{\text{H}}$  8.0 (1H, *d*,  $J = 8$  Hz) and 7.65 (1H, *d*,  $J = 8$ ) for H-5' and H-6', respectively, leaving C-7' for point of attachment to the first chrysophanol moiety (ring ABC). Thus, the structure of compound (**2**) was found to be 4,7'-bichrysophanol, trivial name asphodeline (**2**). As reported so far, it is the common metabolite from the genus *Kniphofia* including *K. albescens*, *K. linearifolia*, *K. ensifolia*, *K. evansii*, *K. northiae*, *K. foliosa*, *K. tysonii*, and *K. isoetifolia* [4, 13].

Compound **3** (3 mg) was isolated as red crystal. The <sup>1</sup>H-NMR data of **3** (Table 1) confirmed that this compound is also dimeric anthraquinone. However, its spectral data were similar to compound **2** with one additional aromatic proton due to the difference position of dimerization. Two aromatic methyl signals at  $\delta_{\text{H}}$  2.28 (3H, *s*, 3-CH<sub>3</sub>) and 2.47(3H, *s*, 3'-CH<sub>3</sub>) were observed for the two aromatic methyl groups and four chelated -OH groups at  $\delta_{\text{H}}$  (12.48, 12.38, 12.18, and 11.81). Three mutually coupled aromatic protons in the ABX spin system at  $\delta_{\text{H}}$  6.80 (1H, *d*,  $J = 4$  Hz, H-5),  $\delta_{\text{H}}$  7.43 (1H, *t*,  $J = 8$  Hz, H-6), and  $\delta_{\text{H}}$  6.98 (1H, *d*,  $J = 8$  Hz, H-7) were observed. Two *meta*-coupled aromatic protons at  $\delta_{\text{H}}$  6.83(1H, *d*,  $J = 4$  Hz, H-2) and 6.63 (1H, *brs*, H-4) were observed. The remaining spectral data for the other half of the molecule ring A'B'C' system of **3** are similar to that of **2**; two *meta*-coupled proton signals at  $\delta_{\text{H}}$  7.06 (1H, *d*,  $J = 4$  Hz, H-2') and  $\delta_{\text{H}}$  7.66 (1H, *d*,  $J = 4$  Hz, H-4') and two *ortho*-coupled aromatic protons with AX spin system at  $\delta_{\text{H}}$  8.03 (1H, *d*,  $J = 8$  Hz, H-5') and  $\delta_{\text{H}}$  8.67 (1H, *d*,  $J = 10$  Hz, H-6') were observed.

The first chrysophanol moiety showed an ABX spin system for H-5, H-6, and H-7; the second chrysophanol moiety showed an AX spin system for H-5' and H-6' and leaves H-7' for point of attachment to the first chrysophanol moiety. Deshielded H-4' ( $\delta_{\text{H}}$  7.66) and H-5' ( $\delta_{\text{H}}$  8.03) (ring A'B'C') in contrast to H-4 ( $\delta_{\text{H}}$  6.63) and H-5 ( $\delta_{\text{H}}$  6.80) (ring ABC) indicates the second moiety has a carbonyl group

located at C-10'. Therefore, compound **3** was characterized and identified as 10-hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone, which was previously reported from other *Kniphofia* species such as *K. isoetifolia* [4], *K. ensifolia* [14, 15], *K. foliosa*, and *K. thomsonis* [10].

**3.2. Evaluation of Antimicrobial Activity.** The antimicrobial activity of the extracts (100 mg·mL<sup>-1</sup>) and the isolated compounds **1** (50 mg·mL<sup>-1</sup>) and **2** (25 mg·mL<sup>-1</sup>) is shown in Table 2.

The crude extracts showed considerable activity on both Gram-positive and Gram-negative bacterial strains with zone of inhibition ranging from 12 to 22 mm, with the highest activity (22 mm) observed for chloroform extract against *E. coli*. However, the inhibitions displayed on both Gram-negative and Gram-positive bacteria for the isolated compounds that have been tested were good with variable degree of potency. The better activity of the crude extracts over the isolated compound could be accounted to the synergistic interactions of several compounds present in the extract, which cannot be the case when single compounds are evaluated. Acetone extract showed the highest inhibition (18 mm) against the fungal strain, *Fusarium* spp., and lowest by hexane extract (8 mm), whereas compound **2** showed a higher inhibition (13 mm) as compared to compound **1** against the same strain.

## 4. Conclusion

Phytochemical investigation of the roots of *Kniphofia insignis* has resulted in identification of three anthraquinones, namely, helminthosporin (**1**), asphodeline (**2**), and 10-hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone (**3**). From its sequential extraction, 10, 28, 17, and 45 g of *n*-hexane, chloroform, acetone, and methanol crude extracts were obtained, respectively. Its antimicrobial evaluation has revealed that the extracts have almost a better activity against the strains. The antimicrobial analysis showed that the chloroform extract showed the highest inhibition against *E. coli*.

## Data Availability

All data used to support the findings of this study are available in the manuscript.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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