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Research Article

Chrysophanol from the Roots of Kniphofia Insignis and Evaluation of Its Antibacterial Activities

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Sequential extraction of the roots of *Kniphofia insignis* using cold maceration techniques and column chromatographic separation leads to the isolation of one monomeric anthraquinone, chrysophanol (1). The structure of the compound was established using spectroscopic analyses including NMR (1H and 13C-NMR, infrared) and comparison with reported literature. The in vitro antibacterial activities of the crude extracts and the isolated compounds were evaluated against four bacterial strains (S. *aureus* ATCC 25923, *B. subtilis* ATCC 6633, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853). Among them, the crude extract, acetone extract, has shown substantial antibacterial activity with the highest activity against *E. coli* (22.3 mm). However, compound 1 has a better zone of inhibition with 19.3 mm against *P. aeruginosa.*

1. Introduction

Kniphofia Moench (family *Asphodelaceae*), which is commonly named as "Red Hot Pokers," contains about 71 species. Among those, seven of them are found in Ethiopia and five of them, including *K. insignis*, *K. schimperi, K. hildebrandtii*, *K. isoetifolia*, and *K. foliosa*, are endemic [1–3]. *Kniphofia* is widely known for its ornamental value because of its colorful flowers and is used in traditional medicine [4]. Its roots are used to heal gonorrhea, abdominal cramps, hepatitis B, wound healing, ringworm, women infertility, chest pain, snake bite, and shoulder pains [2, 4–7]. *Asphodelaceae* is the main source of quinones, mainly, naphthoquinone [5], monomeric and dimeric anthraquinones, dimeric phenylanthraquinones, and anthrones [7]. It was 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 Chancho [3]. *K. insignis* which is clearly distinguished from the other species by its white perianth, which is unusual in the genus, and fusiform roots (Figure 1). The species often grow in water-logged or flooded meadows between 2500 and 3100 m. It is so far only known from the Shoa and Arsi Zone. The main flowering period is from June to September. The plant occurs scattered, and it is commonly seen in the "Sululta" plains between "Addis Ababa" and "Chancho" during its flowering period [1]. However, since it is rich in a compound, further phytochemical investigation and antibacterial activities evaluation have been required. Therefore, the current project was purposed to isolate and characterize compounds from the root of *Kniphofia insignis* and to evaluate its antibacterial activities.

2. Experimental Section

2.1. General Information. Petroleum ether, chloroform, acetone, methanol, and ethyl acetate, all analytical grade solvents, were used for extraction and column elution; Silica gel 60–120 mm mesh size, TLC silica gel coated plate for detection of spots, $CDCl_3$ was used for recording NMR spectra and, DMSO for sample preparation for antibacterial susceptibility test, Standard antibiotic drug (gentamycin, positive control), Mueller Hinton agar and nutrient agar solution were used for culture media for an antibacterial test. Round bottom flask of size 50, 100, and 500 mL, measuring cylinder, Whatman No 1 filter paper, pistil, and mortar,

Figure 1: Picture of the study plant (photo taken by Asnakew, September 2017).

weighing balance, column chromatography, and rotary evaporator were used during extraction and purification. UV chamber 254 and 365 nm (LF-260. LS, EEC) for detection of a spot. An infrared (IR) spectrum was measured on a Perkin–Elmer IR spectrophotometer. The 1D (^{1}H-NMR 400 and 400 MHz, ^{13}C-NMR (100 MHz)) spectra were recorded on Bruker Avance NMR in a deuterated solvent and were used for characterization.

2.2. Collection and Preparation of Plant Material. *Kniphofia insignis* roots were collected from Ethiopia, North Shewa Zone Amhara Regional State, Debre Birhan district which is about 130 km away from Addis Ababa on January 17, 2019. The roots were collected, washed with tap water, air dried, powdered to allow penetration by the solvents during the extraction, and stored in an appropriate container in Jimma University Organic laboratory. The plant material was identified by Jimma University Botanist Dr. Dereje Denu and gave the voucher specimen (voucher number CH1) has been deposited in Jimma University Herbarium.

2.3. Extraction and Isolation. About 0.5 kg shade-dried roots of K. *insignis* were subjected to sequential extraction with petroleum ether, chloroform, acetone, and methanol using the cold maceration technique three times for 24 h each with 1.5 L at room temperature and the crude extracts were concentrated using a rotary evaporator to remove solvents to obtained 6.5 g (1.3%), 16.75 g (3.35%), 19.25 g (3.85%) and 29.88 g (5.98%) of petroleum ether, chloroform, acetone and methanol extracts respectively. The crude extracts were subjected to an antibacterial test against four bacteria strains (S. *aureus* (ATCC 25923), E. *coli* (ATCC 35218), P. *aeruginosa* (ATCC 27853) and B. *subtilis* (ATCC 6633) to.

Due to its antibacterial activities test and TLC profile, 15 g acetone extract was adsorbed on silica gel (60–120 mm mesh size) and subjected to 500 mm diameter column chromatography on silica gel (115 g) eluting by petroleum ether with increasing amounts of ethyl acetate gradient elution in the ratio of 100 : 0 to 0 : 100 petroleum ether to ethyl acetate. The main fractions which yield one pure compound at 3% of ethyl acetate in petroleum ether followed by further purification and recrystallization yielded compound 1 (18 mg). The isolated compound was characterized by spectroscopic techniques: FT-IR, ^{1}H-NMR and ^{13}C-NMR at Addis Ababa University.

Table 1: ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (125 MHz) spectral data of compound 1.

Position	1		[5]	
	δ_H (*m*, *J* in hz)	δ_C	δ_H (*m*, *J* in hz)	δ_C
1	—	163.0	—	163.0
1a	—	113.7	—	114.2
2	6.97 (1H, *s*)	122.8	7.11 (1H, *s*)	124.7
3	—	148.1	—	149.7
4	7.43 (1H, *s*)	135.5	7.45 (1H, *s*)	137.3
4a	—	133.3	—	133.6
5	7.79 (1H, *dd*, 10, 5)	119.2	—	120.3
5a	—	133.8	—	134.0
6	7.65 (1H, *t*, 7.5)	120.5	7.66 (1H, *t*)	121.8
7	7.27 (1H, *dd*, 10, 5)	123.0	7.30 (1H, *dd*, 10, 5)	124.9
8	—	162.3	—	162.7
8a	—	115.7	—	116.2
9	—	188.9	—	192.9
10	—	181.5	—	182.3
3-CH_3	2.45 (3H, *s*)	21.83	2.47 (3H, *s*)	22.7
1-OH	11.87 (1H, *s*)	—	12.03 (1H, *s*)	—
8-OH	12.17 (1H, *s*)	—	12.13 (1H, *s*)	—

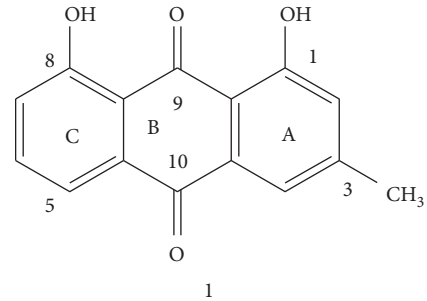

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Figure 2: The structures of chrysophanol (1) are isolated from the root of *K. insignis*.

2.4. Antibacterial Assay. The *in vitro* antibacterial activities of crude extracts and isolated compounds were evaluated against four bacteria strains (E. *coli* ATCC 35218, S. *aureus* ATCC 25923, B. *subtilis* ATCC 6633, and P. *aeruginosa* ATCC 27853) by agar disc diffusion method following the standard procedures [8–10]. The stock solution was prepared by dissolving 100 mg of the crude extract in 1 mL DMSO, 30 mg of the compound 1 in 0.6 mL 100 $mgmL^{-1}$ of crude extracts, and 50 $mgmL^{-1}$ final stock solution of compound 1, respectively. An inhibition zone was measured in mm after 24 h incubation at 37°C and compared with the standard drug, gentamycin, the inhibition zone was measured three times, and the average diameter was recorded.

3. Results and Discussion

3.1. Characterization of Isolated Compound. Compound 1 (18 mg) was isolated as a red crystalline solid. It was isolated

Table 2: Zone of inhibition of the extracts, compound 1 in mm.

	Crude extract								Compound		Controls	
Test strains	P.E	[11]	Chl	[11]	Ac	[11]	Met	[11]	Cpd 1	[5]	G	DMSO
E. coli	17.3	16	17.2	22	22.3	18	18	16	14.5	17	32	NI
P. aeruginosa	14.1	12	14.7	15	13.7	14	15	12	13.7	23	22	NI
S. aureus	14.3	13	14.1	13	16.2	15	16.3	14	19.3	16	33	NI
B. subtilis	12.5	13	15.4	16	17.9	18	16.7	15	15.4	—	31	NI

Note: *P.E* = petroleum ether; Chl = chloroform; Ac = acetone; Met = methanol; *G* = gentamycin; NI = no inhibition; cpd = compound; DMSO = dimethyl sulphoxide.

at 3% of ethyl acetate in petroleum ether and its *Rf* value was 0.91 in 95% petroleum ether in ethyl acetate. Its FT-IR spectral analysis, showed a strong band in the region of 3421 and 2919 cm^{-1} 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 1617 and 1271 cm^{-1}, respectively.

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) (Table 1) displayed two –OH proton at δ_H 11.87, and 12.17 involved in hydrogen bonding and were assigned to -OH groups at C-1 and C-8 respectively, of an anthraquinone skeleton. In ring A, two *meta*-coupled aromatic protons at δ_H 6.97 (1H, *s*, H-2) and 7.43 (1H, *s*, H-4) were observed in addition to the biosynthetically expected methyl group (δ_H 2.45 (3H, *s* 3-CH_3); δ_C (21.83) at C-3 (δ_C 148.13), whereas, in ring C, three mutually coupled aromatic protons in the ABX spin system resonating at 7.79 (1H, *dd*, $J = 10$, 5 Hz), 7.65 (1H, *t*, $J = 7.5$ Hz), and 7.27 (1H, *dd*, $J = 10$, 5 Hz) which were assigned to H-5, H-6, and H-7, respectively.

The ^{13}C-NMR spectral data (Table 1) revealed the presence of fifteen carbon signals including two carbonyl carbon ($\delta_{C,}$ 188.9 and 181.5) for C-9 and C-10; five aromatic methine carbons ($\delta_{C,}$ 122.8, 135.5, 119.2, 120.5, and 123.1) for C-2, C-4, C-5, C-6, C-7, two oxygenated aromatic quaternary carbons ($\delta_{C,}$ 163, and 162.3) for C-1 and C-8, five aromatic quaternary carbons ($\delta_{C,}$ 148.13, 113.7, 133.3, 133.8, and 115.7) for C-3, C-1a, C-4a, C-5a, and C-8a and a methyl carbon (δ_C 21.83) at C-3, respectively. Therefore, compound 1 was identified as 1,8-dihydroxy-3-methyl-anthraquinone, trivial name chrysophanol (1) (Figure 2) which has been reported from *Kniphofia* species, *K. foliosa* [3, 7], and *K. isoetifolia* [5] and reported here for the first time.

3.2. Evaluation of Antibacterial Activity. The antibacterial activity of extracts (100 $mgmL^{-1}$) and the compound 1 (50 $mgmL^{-1}$) (Table 2).

The crude extracts showed considerable activity on both Gram-positive and Gram-negative bacterial strains with a zone of inhibition ranging from 12.5–22.3 mm, with the highest activity (22.3 mm) observed for acetone extract against E. *coli*. However, the inhibitions displayed on both Gram-negative and Gram-positive bacteria for compound 1 were good with variable degrees of potency. The highest activity of the crude extracts than compound 1 could be due to the synthetic interactions of several compounds present in the extract, which cannot be observed for a single compound, and lowest by petroleum ether extract (12.5 mm), whereas compound 1 showed higher inhibition (19.3 mm) for *S. aureus* and minimum inhibition (13.7 mm) for *P. aeruginosa*. Generally, both the crude extract and compound 1 show better activities than the reported one.

4. Conclusion

Sequential extraction of roots of *Kniphofia insignis* has yielded isolation of one monomeric anthraquinone chrysophanol (1). From sequential extraction, 6.5, 16.75, 19.25, and 29.88 g of petroleum ether, chloroform, acetone, and methanol crude extracts were obtained, respectively. Its antibacterial activities have shown that the extracts have almost better activity against the strains with the highest for acetone extract against *E. coli*.

Both the crude extract and compound 1 show better activities than the reported one which will be due to the difference in polarity of compounds as the polarity of solvent increases.

Data Availability

All data are available in this article.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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