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Chemical constituents of the roots of *Kniphofia isoetifolia* Hochst. and evaluation for antibacterial activity

[Constituyentes químicos de las raíces de *Kniphofia isoetifolia* Hocst. y evaluación de la actividad antibacteriana]

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Abstract

Context: The genus *Kniphofia* Moench, with species close to 70, is traditionally used to treat wide ranges of ailments including menstrual pains, infertility, abdominal cramps, wounds, malaria, chest complaint and hepatitis B. *Kniphofia isoetifolia,* an endemic species in Ethiopia, is known for wounds healing applications in Southern Ethiopia.

Aims: To investigate the roots of *Kniphofia isoetifolia* and evaluate the extracts and its constituents for antibacterial activity.

Methods: The roots of *Kniphofia isoetifolia* was extracted with chloroform/methanol (1:1 v/v), and the resulting crude extract was partitioned between EtOAc/H_2O. The ethyl acetate extract afforded four compounds after chromatographic purification, and their identification was based on spectroscopic analyses. The extracts and pure compounds were evaluated for *in vitro* antibacterial activity on four bacterial strains namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*.

Results: The purification of the extract of roots of *Kniphofia isoetifolia* resulted in identification of four compounds (**1** – **4**). This is the first report on the phytochemical investigation of *Kniphofia isoetifolia* and occurrence of compound **1** in the genus *Kniphofia*. The extracts and isolates demonstrated antibacterial activity. The Compound **3** in some cases demonstrated comparable zone of inhibition with gentamycin.

Conclusions: This is the first report of the occurrence of naphthoquinone derivative, 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**) in the genus *Kniphofia* which appears to have a chemotaxonomic significance to narrow down the gap between Alooideae and Asphodeloideae subfamily. The two dimeric anthraquinones (**3**, **4**) showed strong activity with highest zone of inhibition recorded for asphodeline (**3**) against *E. faecalis*.

Keywords: antibacterial; inhibition zone; *Kniphofia isoetifolia*; quinones; roots.

Resumen

Contexto: El género *Kniphofia* Moench, con cerca de 70 especies, se utiliza tradicionalmente para tratar dolores menstruales, infertilidad, calambres abdominales, heridas, malaria, dolor torácico y hepatitis B. *Kniphofia isoetifolia*, una especie endémica en Etiopía se usa en la curación de heridas en el sur de Etiopía.

Objetivos: Investigar las raíces de *Kniphofia isoetifolia* y evaluar la actividad antibacteriana sus extractos y sus componentes.

Métodos: Las raíces de *Kniphofia isoetifolia* se extrajeron con cloroformo/metanol (1: 1 v / v) y el extracto bruto resultante se repartió entre EtOAc/H_2O. Después de la purificación cromatográfica, el extracto de acetato de etilo proporcionó cuatro compuestos que se identificaron mediante análisis espectroscópicos. Se evaluó la actividad antibacteriana *in vitro* de los extractos y compuestos puros contra *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa y Enterococcus faecalis*.

Resultados: La purificación del extracto de las raíces de *Kniphofia isoetifolia* dio lugar a la identificación de cuatro compuestos (**1** - **4**). Este es el primer informe sobre la investigación fitoquímica de *Kniphofia isoetifolia* y la aparición del compuesto 1 en el género *Kniphofia*. Los extractos y compuestos aislados demostraron actividad antibacteriana. El compuesto **3**, en algunos casos, demostró una zona comparable de inhibición con gentamicina.

Conclusiones: Este es el primer reporte de la ocurrencia del derivado de naftoquinona, 3,5,8-trihidroxi-2-metilnaftaleno-1,4-diona (**1**) en el género *Kniphofia* que parece tener una significación quimiotaxonómica para reducir el espacio entre Alooideae y la subfamilia de Asphodeloideae. Las dos antraquinonas diméricas (**3**, **4**) mostraron la mayor zona de inhibición registrada para asphodeline (**3**) contra *E. faecalis*.

Palabras Clave: antibacteria; *Kniphofia isoetifolia*; quinonas; raíces; zona de inhibición.

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INTRODUCTION

Infectious diseases are a serious health and economic problem to human being throughout the world, ultimately causing millions of mortalities, many of which are a premature death of children under five (Gahlaut and Chhillar, 2013; Minale et al., 2014; Wong et al., 2015). The rapid drive of mortality, morbidity and also the emerging of new infectious diseases is mainly due to antimicrobial resistance against currently available antibiotics (Hemaiswarya et al., 2008). The use of plants and plant-based products for healing human diseases is an old experience and practice (Petrovska, 2012). In addition to the traditional application, plants and their products have also laid a great foundation for the modern drug, for example, the majority of anticancer drugs and infectious diseases have been derived from plants and other nature-based sources and, still continue to serve as a potential source (Dias et al., 2012; Newman and Cragg, 2016).

The genus *Kniphofia* Moench (family Asphodelacea) with the species close to 70 are widely distributed in Africa with most of the species being confined to the Southern part of the region (Codd, 1968; Bosch, 2008). Traditionally, it is used to treat different ailments including menstrual pains, infertility, abdominal cramps, wounds, malaria, chest complaint, gonorrhea and hepatitis B (Matsiliza and Barker, 2001; Yineger and Yewhalaw, 2007; Mothana et al., 2009; Mahach, 2013). Owing to its colorful flower, the plant of this genus is also widely recognized for its ornamental values. Phytochemically, it is well known for its elaboration of anthraquinones including monomeric and dimeric anthraquinones and phenylanthraquinones, which have been reported to possess antimalarial and cytotoxic activities (Bringmann et al., 1999; Wube et al., 2005; Habtemariam, 2010).

K. isoetifolia (Fig. 1) is one of the endemic species growing in Ethiopia and is commonly visited by traditional healers, especially for the treatment of wounds healing in the Southern part of country. Other plants under the genus *Kniphofia* have been subjected to phytochemical and biological investigation; but this is only limited to HPLC profiling when it comes to *K. isoetifolia* (Berhanu et al., 1986) and to the best of our knowledge, there is no recorded biological activity pertaining to this plant. Thus, herein we report the isolation of four compounds (Fig. 2, one napthoquinone and three anthraquinones) from the roots of *K. isoetifolia* (Fig. 1). The antibacterial activities of these compounds and crude extracts are also reported.

Figure 1. *Kniphofia isoetifolia* (captured by MM in February 2016).

MATERIAL AND METHODS

Chemicals and equipment

Analytical grade hexane, chloroform, acetone, methanol and ethyl acetate were used for extraction and isolation (Loba Chemie Pvt Ltd, Mumbai, India). Silica gel 60-120 mm mesh size (Merck, Darmstadt, Germany), oxalic acid for deactivation of silica gel was used. Analytical TLC was performed on pre-coated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were obtained on a Bruker Avance 500 spectrometer (Bruker, Rheinstetten, Germany) using the residual solvent peak as indirect chemical shift reference. The spectra were processed using standard Bruker software (Bruker, Rheinstetten, Germany). Deuterated chloroform (98% of atom D; Sigma-Aldrich Chemie Gmbh Munich, Germany) was used for recording NMR spectra and DMSO (Sigma-Aldrich Chemie Gmbh Munich, Germany)

to dissolve sample for antibacterial susceptibility test, nutrient agar and nutrient broth for bacteria cultivation.

Plant material

The roots of *K. isoetifolia* were collected from Southern part of Ethiopia, Dawuro, Mareka district (geographic coordinates: 7°05'05.0"N 37°06'26.9"E) in February 2016 and identified by professional botanist (Dr. Balcha Abera) at Jimma University Herbarium; where the plant specimen (voucher MM-01-16) was deposited.

Extraction and isolation

The powdered plant material (1 kg) was extracted with chloroform/methanol (1:1) three times, each for 24 h to give 98 g of a reddish-brown crude extract (CE-1) after removal of the solvent. The resulting crude extract (90 g) was then subjected to differential extraction between ethyl acetate and water in (9:1) three times to afford 36 g of ethyl acetate extract (CE-2) after concentration of rotary evaporator. The ethyl acetate extract (25 g) was adsorbed on silica gel (60-120 mesh) and subjected to column chromatography (500 mm diameter) on 3% oxalic acid impregnated silica gel (195 g) eluting with *n*-hexane with increasing amounts of ethyl acetate. The fractions collected with 1% EtOAc in *n*-hexane were reduced to two sub fractions as "*sf1*" (4.4 g) and "*sf2*" (3.3 g) based on TLC analyses. Chromatographic separation of "*sf1*" with *n*-hexane in increasing amount of ethyl acetate gave a monomeric anthraquinone, chrysophanol (**2**, 24 mg), and asphodeline (**3**, 18 mg) at 0.5% and 5% EtOAc in *n*-hexane respectively. Similarly, sub-fraction, "*sf2*" was subjected to column chromatography on silica gel and the fraction eluted with 2% ethyl acetate in *n*-hexane afforded 3,5,8-trihydroxy-3-methylnaphthalene-1,4-dione (**1**, 17 mg). The combined fractions at 30% EtOAc in *n*-hexane gave another dimeric anthraquinone, 10-hydroxy-10,7′(chrysophanolanthrone)chrysophanol (**4**, 12 mg) after purified on column chromatography on silica gel using ethyl acetate in *n*-hexane.

Antibacterial activity

Test strains

Two Gram-positive, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and two-Gram negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa (*ATCC 27853) were used for *in vitro* evaluation of the antibacterial activity. The standard strains were obtained from Medical Microbiology Research Laboratory, Jimma University Specialized Hospital previously donated from Ethiopia Public Health Institute, Addis Ababa, Ethiopia on February 2016. The bacteria strains were reactivated by sub-culturing in nutrient broth at 37°C and maintained on nutrient agar slant at 4°C for further use in Microbiology Laboratory, Department of Biology Jimma University.

Standard inoculums preparation

The standard inoculum for antibacterial susceptibility test was prepared based on the standard protocol (Stephen et al., 2005). In the criteria of National Committee for Clinical Laboratory, a standardized suspension of bacteria isolates was prepared and the turbidity of the inoculum was matched with the turbidity standard of 0.5 McFarland. A McFarland is a barium sulfate standard against which the turbidity of the test and control inoculum was compared. This standard was prepared by mixing two solutions of a 0.5-mL aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) was added to 99.5 mL of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension. A 0.5 McFarland standard concentration, equivalent to cell density correspond to 1.5 x 10^8 CFU/mL, is a mixture of 0.5 mL of 1% ($BaCl_2.2H_2O$) in 99.5 mL of 1% H_2SO_4 solution. A small volume of turbid solution was transferred to a screw-cup bottle of the same types as used for preparing test and control inoculum. Culture containing test tube with approximately equal concentration or density with 0.5 McFarland standards was used for inoculation of media. The standard was used after shaking immediately before use; and stored in a well-sealed con-

tainer in a dark place at room temperature of 25°C when not used to prevent evaporation. When matched with the standard, the inoculate were confluent growth. For each bacteria strain, the standard gentamicin was taken as positive control and DMSO as the negative control.

Antibacterial activity test (agar disk diffusion method)

An agar disk diffusion method was used to evaluate the antibacterial activity of both crude extract and isolated compounds on nutrient agar. Briefly, the bacteria stock cultures were maintained on the nutrient agar slants, which were stored at 40°C. Agar cultures of the test microorganisms were prepared according to manufacturer's instruction. The test solutions were prepared by dissolving 50 mg of the test samples to achieve final stock concentrations of 50 mg/mL in DMSO. Freshly grown liquid culture of the test pathogens solution of having similar turbidity with 0.5 McFarland were seeded over the Müeller-Hinton Agar medium with sterile swab. Sterile Whatman filter paper discs were soaked with 30 µL of the above stock solution concentration of the samples and air dried to evaporate the solvent and then applied over the seeded plates at equidistance. The plates were then inverted and incubated at 37°C for 24 hr. After the incubation period, the plates were observed for a clearance zone around the disks. The clear zones formed around each disk were measured in millimeter. Each experiment was carried out in triplicates. The mean of the inhibition zone of each test sample was taken for evaluating the antibacterial activity.

Spectroscopic data of isolated compounds

3,5,8-Trihydroxy-2-methylnaphthalene-1,4-dione (1). Red crystals. ^{1}H NMR δ_H (500 MHz, $CDCl_3$) and ^{13}C NMR δ_C (125 MHz, ppm, $CDCl_3$), see Table 1 and Supporting Information.

Chrysophanol (2). Yellow amorphous solid. ^{1}H NMR δ_H (500 MHz, $CDCl_3$) 2.47 (3H, $3CH_3$), 7.11 (1H, *brs*, H-2), 7.30 (1H, *dd, J* = 10, 5 Hz, H-7), 7.66 (1H, *t*, H-6), 7.69 (1H, *s*, H-4), 12.03 (1H, *s*, 1-OH), 12.13 (1H, s, 8-OH). ^{13}C NMR δ_C (125 MHz, ppm, $CDCl_3$,) 22.7 (C-11), 114.2 (C-1a), 116.2 (C-8a), 120.3 (C-5), 121.8 (C-6), 124.7 (C-2), 124.9 (C-7), 133.6 (C-4a), 134.0 (C-5a), 137.3 (C-4), 149.7 (C-3), 162.7 (C-8), 163.0 (C-1), 182.3 (C-10), 192.9 (C-9).

Asphodeline (3). Red crystals. ^{1}H NMR δ_H (500 MHz, $CDCl_3$) 2.31 (3H, s, 3'-CH_3), 2.50 (3H, *s*, 3-CH_3) 7.14 (1H, *brs*, H-2'),7.32 (1H, *dd, J* = 10, 5 Hz, H-7), 7.63 (1H, *d, J* = 10, H-6'), 7.71 (2H, *t, J* = 7.5, H-6, (*s*, H-2), 7.84 (1H, *s*, H-4'), 7.88 (1H, *dd, J* = 10, 5 Hz, H-5), 7.98 (1H, *dd, J* = 10 Hz, H-5'). ^{13}C NMR δ_C (125 MHz, ppm, $CDCl_3$), 21.5 (3'-CH_3) 22.7 (3-CH_3), 114.2 (C-8a'), 114.3 (C-1a), 116.3 (C-8a), 116.6 (C-1a'), 120.0 (C-5'), 120.5 (C-5), 122.0 (C-2), 122.1 (C-4'), 125.0 (C-2'), 125.2 (C-7), 131.4 (C-4), 131.6 (C-7'), 133.2 (C-4a'), 133.7 (C-4a), 134.0 (C5a), 134.2 (C-5a'), 137.6 C-), 138.9 (C-6'), 148.6 (C-3'), 150.0 (C-3), 160.2 (C-1), 160.8 (C-1'), 162.9 (C-8), 163.3 (C-8'), 182.2 (C-10') 182.2 (C-10), 193.0 (C-9'), 193.1 (C-9).

10-Hydroxy-10,7'-(chrysophanolanthrone)chrysophanol (4). Red crystals. ^{1}H NMR δ_H (500 MHz, ppm, $CDCl_3$) 2.26 (3H, *s*, 3-CH_3), 2.44 (3H, *s*, 3'-CH_3),6.61 (1H, *d*, J = 2.5 Hz, H-2), 6.78 (1H, *d, J* = 2.5 Hz, H-2'), 6.95 (1H, *dd, J* = 10, 5 Hz, H-7), 7.04 (1H, *d, J* = 2.5 Hz, H4),7.41(1H, *t, J* = 7.5, H-6), 7.63 (1H, *d, J* = 2.5 Hz, H-4), 8.01 (1H, *d, J* = 10 Hz, H-5'), 8.64 (1H, *d, J*= 10 Hz, H-6'), 2.42 (3H, s, 3-CH_3). ^{13}C NMR δ_C (125 MHz, ppm, $CDCl_3$), 22.4 (3'-CH_3), 71.2 (C-10), 112.3 (C-1a'), 113.9 (C-1a), 114.5 (C-8a), 116.0 (C-8a'), 118.0 (C-7), 118.2 (C-5), 118.9 (C-5'), 119.5 (C-4'), 120.2 (C-2), 121.5 (C-C-2'), 124.6 (C-4), 132.6 (C-7'), 133.1 (C-4a'), 133.3 (C-3'), 136.8 (C-6'), 138.0 (C-6), 141.2 (C-4a),146.3 (C-5a), 149.0 (C-3), 149.6 (C-3'), 158.9 (C-8'), 162.5 (C-1'), 162.7 (C-8), 182.0 (C-9'), 192.7 (C-9), 192.7 (C-10').

Statistical analysis

Data were measured as mean ± SD. Statistical analysis was performed using SPSS Version 20 Software. The global comparison was done using two-way ANOVA followed by Least Significant Difference (LSD) multiple comparison test. Pair wise testing was done with the help of unpaired post hoc test. $P<0.05$, implying that it is statistically significant.

RESULTS AND DISCUSSION

Chromatographic purification of extract of the roots of *K. isoetifolia* lead to the isolation of a naphthoquinone derivative; 3,5,8-trihydroxy-2-methylnaphthalen-1,4-dione (**1**), one monomeric anthraquinone; chrysophanol (**2**), and two dimeric anthraquinones; asphodeline (**3**) and 10-hydroxy-10,7'-(chrysophanolanthrone)chrysophanol (**4**) (Fig. 2).

Compounds **2**, **3** and **4** are common denominators in the genus and their structures were identified through analyses of their NMR spectral data and comparing with already reported literature for chrysophanol (Berhanu et al., 1986), asphodelin (Van Wyk et al., 1995) and 10hydroxy-10,7'-(chrysophanolanthrone)chrysophanol (Wube et al., 2005). The co-occurrence of these compounds in the root of *K. isoetifolia* is then possible that the phenol-oxidative coupling leading to the dimeric anthraquinones have occurred between two chrysophanol moieties to form compound **3**; and chrysophanol and its corresponding chrysophanol anthrone in case of **4**. However, this is the first report of compound **1** and its kind from the genus *Kniphofia* having been previously reported from *Aloe dawei* (Abdissa et al., 2014). Comparative studies on the roots of fourteen *Kniphofia* species showed that phenylanthraquinones particularly, knipholone to be the major pigment in these taxa and was suggested that it might be a taxonomic marker for the genus *Kniphofia* (Berhanu et al., 1986; Yenesew et al., 1988). In this study; however, none of phenylantharquinone derivatives including knipholone have been detected.

Compound **1** was obtained as a red crystal. The ^{1}H NMR spectrum (Table 1) displayed two *ortho*-coupled aromatic protons at δ_H 7.11 (1H, *d, J* = 10 Hz, H-6) and δ_H 7.22 (1H, *d, J* = 10 Hz, H-7), two downfield shifted proton singlets at δ_H 12.75 and 11.42 assignable to two hydroxyl protons involving in hydrogen bonding, and further proton singlet at δ_H 2.04 (3H, *s*) for a methyl group. The ^{13}C NMR spectrum (Table 1) also revealed signals for eleven non-equivalent carbon atoms, out of which the signals at 188.9 and 182.0 correspond to two carbonyl carbon atoms of quinoid functionality. This indicates that compound **1** is a 1,4-naphthoquinone derivative.

Furthermore, three downfield shifted carbon signals at δ_C 153.8, 157.1, 157.6 in the ^{13}C NMR spectrum confirmed the presence of three oxygenated substitution. One of the hydroxyl groups (δ_H 11.42) showed long range HMBC cross couplings with its neighboring carbons; C-5, C-6 and C-10 and hence placed at C-5 (Table 1). The second hydroxyl group appearing at δ_H 12.75 was then placed at C-8 (δ_C 157.1), which was further confirmed by its HMBC correlation to C-7, C-8 and C-9 carbon atoms (Table 1). The upfield shifted resonance (δ_C 8.3) of the methyl group (δ_H 2.04; δ_C 8.3), clearly indicated its attachment to a quinonoid ring (Abdissa et al., 2014), which otherwise showed HMBC cross-coupling with C-1 (δ_C 188.9) C-2 (δ_C 121.6) and C-3 (δ_C 153.8) revealed its placement being at C-2 as expected biogenetically (Bringmann et al., 2008). Thus, compound **1** was characterized and identified as 3,5,8-tri-hydroxy-2-methylnaphthalen-1,4-dione, trivial name hydroxydeoserone.

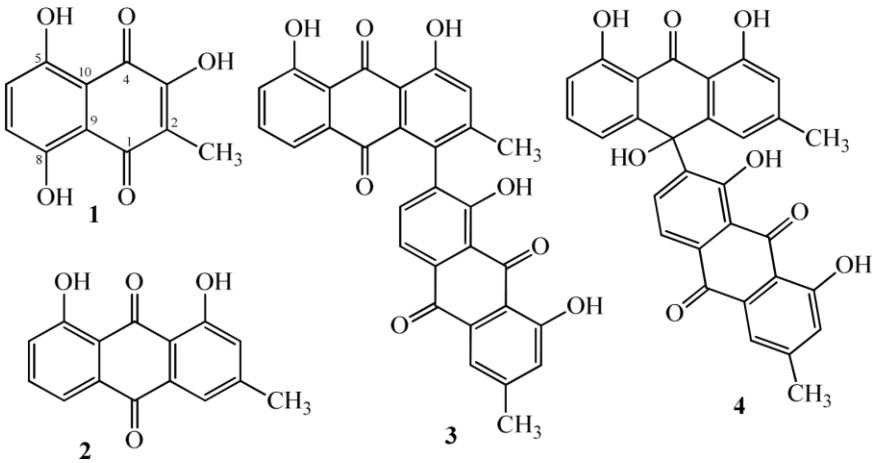

Figure 2. Compounds isolated from the roots of *K. isoetifolia*

Table 1. NMR data for 3, 5, 8-trihydroxy-2-methylanphthalene-1,4-dione (1) (in $CDCl_3$, 500 MHz).

Position	δ_H (m, *J* in Hz)	δ_C	HMBC
1		188.9	
2		121.6	
3		153.8	
4		182.0	
5		157.6	
6	7.11 (1H, *d, J* = 10 Hz)	127.5	C-8, C-10
7	7.22 (1H, *d, J* = 10 Hz)	131.5	C-5, C-8
8		157.1	
9		110.2	
10		110.8	
11	2.04 (3H, *s*)	8.3	C-1, C-2, C-3
5-OH	11.42 (1H, *s*)		C-5, C-6, C-10
8-OH	12.75 (1H, *s*)		C-7, C-8, C-9

Antibacterial activity

In this study, four bacterial strains namely; *S. aureus*, *E. faecalis*, *P. aeruginosa* and *E. coli* were used to evaluate the antibacterial activities of the extracts and isolated compounds. These strains are among the drug resistant pathogenic bacterial strains to most of the first line drugs (Mehta et al., 2012; Rasheed et al., 2014) and any substance toxic to these strains are also believed to be active on other bacterial strains. With this regard, two crude extracts and four isolated compounds were evaluated for *in vitro* antibacterial activity using disk diffusion method (Table 2), which showed varying degree of responses against the bacterial strains. The two crude extracts showed considerable activity on both Gram-positive and Gram-negative bacterial strains with zone of inhibition ranging from 21-28 mm with closely related potency observed between the two crude extracts. However, the crude extract, CE-1 exhibited highest zone of growth inhibition (28 mm) on Gram-negative bacteria, *E. coli* while the highest growth inhibition (28 mm) was recorded for CE-2 ($p<0.05$) on Gram-positive bacteria, *E. faecalis* (Table 2). This variation of inhibition may be related to the synergetic effects of the various kinds of compounds in the crude extracts.

It was also observed that there is resemblance zone of growth inhibition for some compounds but it varies for others from strain to strain. Chrysophanol (**2**) showed comparable zone of growth inhibition against the four tested bacteria strains and it is the least active among the tested samples. The activity induced by compound **1** resembles with that of chrysophanol (**2**) except that the former showed better activity (23 mm) on *P. aeruginosa* strain (Table 2). The number and sites of phenolic hydroxyl groups positively influence the antimicrobial activity of anthraquinones and other phenolic compounds that increased hydroxylation enhances cellular toxicity (Cowan, 1999). Emodin with one more extra phenolic hydroxyl group over chrysophanol showed stronger antibacterial activity than chryso-

phanol against the *S. aureus* and *P. aeruginosa* strains even at lower concentration of 0.5 mg/mL (Basu et al., 2005). In line with this, compounds **3** and **4** displayed strong activity against the test bacterial strains (inhibition zone diameter ranging between 18-30 mm) with the highest inhibitory potential recorded for asphodeline (**3**) against *E. faecalis* (30 mm) compared to their monomer; chrysophanol (**2**). This indicates that dimerization, which introduces more hydroxylation may have positive synergetic effect in inhibiting the growth of bacteria.

There is also observed difference in sensitivity based on Gram stain (+ve or –ve) as clearly demonstrated by 10-hydroxy-10,7'-(chrysophanolanthrone)chrysophanol (**4**), which showed higher growth inhibition for the Gram-positive bacteria; *S. aureus* (25 mm) and *E. faecalis* (28 mm) in contrast to the Gram-negative bacteria; *P. aeruginosa* (17 mm) and *E. coli* (18 mm).Moreover, the slight activity variation observed between the two dimeric anthraquinones **3** and **4** could be partly due to site of dimerization, which took place between C-10/C-7' in compound **4**, resulted in sacrification of one of the carbonyl groups at C-10. It is also worth to mention that the position of dimerization in the anthraquinones have already been indicated to influence the antiplasmodial activities of the compounds. Compound **4** with C-10/C-7' position of dimerization has been reported to possess highest inhibition of growth of malaria parasite, *P. falciparum* (IC_{50} 0.26 µg/mL) and weak cytotoxic activity against human epidermoid carcinoma (KB) cell line (IC_{50}, 104 µg/mL) in comparison to compound **3**, with C-4/C-7' dimerization (Wube et al., 2005; Induli et al., 2013). The antibacterial activity results of the crude extracts and isolated compounds are in line with the traditional uses of this plant for wound healing and clearly indicating its antibacterial potential.

CONCLUSIONS

Chromatographic separation of the extract of roots of *K. isoetifolia* lead to the isolation of four compounds. It is the first time to report 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (**1**) from the genus *Kniphofia*, which appears to be narrowing the gaps between the two subfamily Alooideae and Asphodeloideae distinctness chemotaxonomically. The crude extracts and the isolated compounds showed considerable zone of inhibition against the four tested bacteria strains. The dimeric anthraquinones showed strong activities with highest zone of inhibition recorded for asphodeline (**3**) against *E. faecalis*, the value close to that of the reference drug, gentamycin. Thus, the observed antibacterial activities of the extracts and pure compounds could give insight about potential of traditional medicinal plants as good source for lead compounds in development of antibacterial drugs.

Table 2. Diameter of zone of growth inhibition (in mm) of crude extracts and isolated compounds from the roots of *K. isoetifolia*

Bacterial strains	Gram strains	Crude extracts and compounds						Controls	
		CE-1	CE-2	1	2	3	4	(+)	(-)
S. aureus	+ve	23 ± 0.81*	22 ± 0.10*	16 ± 0.15*	10 ± 0.26*	22 ± 0.32*	25 ± 0.01*	31 ± 0.06	NA
E. faecalis	+ve	23 ± 0.89*	28 ± 0.29*	19 ± 0.15*	18 ± 0.06*	30 ± 0.12*	28 ± 0.15*	31 ± 0.56	NA
P. aeruginosa	-ve	22 ± 0.30*	21 ± 0.00*	23 ± 0.06*	17 ± 0.25*	22 ± 0.06*	17 ± 0.15*	43 ± 0.01	NA
E. coli	-ve	28 ± 0.51*	21 ± 0.10*	17 ± 0.06*	16 ± 0.30*	20 ± 0.05*	18 ± 0.06*	39 ± 0.06	NA

Key: NA: Not active, CE-1: methanol/chloroform (1:1 v/v) extract, CE-2: ethyl acetate extract, +ve: Gram positive, -ve: Gram negative, (+): Gentamycin and (-): DMSO. Each group represents the mean ± SD for the bacteria. Two-way ANOVA followed by Least Significant Difference (LSD) multiple comparison test. *p<0.05 for all test groups among the bacterial strains.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Meshesha M	Deyou T	Tedla A	Abdissa N
Concepts or ideas		X		X
Design		X		X
Definition of intellectual content	X			
Literature search	X			
Experimental studies	X			
Data acquisition	X			
Data analysis	X	X	X	X
Statistical analysis	X			
Manuscript preparation	X	X	X	X
Manuscript editing		X	X	X
Manuscript review		X	X	X

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