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Research article

Chemical constituents of the roots of *Kniphofia schimperi* and evaluation of their antimicrobial activity

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ABSTRACT

Kniphofia schimperi is one of the endemic plants of Ethiopia and is widely used for the treatment of microbial infections. However, the biological and phytochemical information pertaining to this plant has not been reported so far. Anticipated by these claims, the chromatographic isolation of the $CHCl_3/CH_3OH$ (1:1 v/v) extract of the roots of *K. schimperi* afforded five compounds, *viz.*, knipholone (**1**), asphodeline (**2**), *β*-sitosterol (**3**), 9-pentacosenoic acid (**4**), and nonacosanoic acid (**5**). The structures of the isolated compounds were identified based on their NMR (1D and 2D) spectral data analysis and comparison with reported literature data. The crude extract and isolated compounds were evaluated for their *in vitro* antimicrobial activity against four bacterial strains (*Escherichia coli*, *Salmonella typhimurium, Staphylococcus aureus*, and *Bacillus cereus*) and a fungal strain (*Candida albicans*) using the agar disk diffusion method. The test samples showed moderate antimicrobial activity, with the highest activity observed for compound **3** (with a zone of growth inhibition of 15.5 ± 0.71 mm) against *S. typhimurium*.

1. Introduction

Medicinal plants have long been used as a key strategy in combating infectious diseases [1]. The search for bioactive agents from medicinal plants has recently yielded effective solutions to certain diseases that the synthetic drug industry has so far been unable to afford [2,3]. Herein, our project on *K. schimperi* was chosen based on the traditional medicinal application of the plant in the local community. So far, a number of compounds have been reported from the genus *Kniphofia* [4–12]. Yet, little is known about the phytochemical constituents of *K. schimperi*. Therefore, as part of our exploration of Ethiopian medicinal plants for antimicrobial secondary metabolites, we report the isolation of five compounds (**1**–**5**) (Fig. 1) from the roots of *K. schimperi*. The antimicrobial activity of the extract and isolated compounds is also reported.

2. Materials and methods

2.1. General information

Analytical-grade chloroform, methanol, petroleum ether, and ethyl acetate (Loba Chemie Pvt. Ltd., Mumbai, India) were used for extraction and column elution. A rotary evaporator (Labo Rota 4000, Heidolph Instrument) was used for extract concentration.

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Column chromatographic separation was done on silica gel 60–120 mm (Merck, Darmstadt, Germany). Further purification was carried out on Sephadex LH-20. TLC analysis was performed on pre-coated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany) using petroleum ether and ethyl acetate as mobile phase. Visualization of spots on the TLC plate was achieved by using UV lamps (UV-Tec 254 nm and 365 nm) and iodine vapor. ^{1}H NMR (400 MHz and 500 MHz) and ^{13}C NMR (100 MHz and 125 MHz) spectra were recorded on the Bruker Avance 400 and 500 Spectrometers, respectively, using $CDCl_3$ as a solvent. Distilled water, Mueller-Hinton agar, nutrient broth, DMSO, gentamicin, and clotrimazole were used for antibacterial and antifungal activity tests.

2.2. Plant sample collection

The roots of *K. schimperi* were collected from Inxoxo, Addis Ababa (Finfinnee), Ethiopia, in 2019. The collected roots were brought to Organic Chemistry Research Laboratory of the Chemistry Department at Jimma University, washed with tap water, air-dried, crushed, and subjected to extraction.

2.3. Extraction and isolation

The air-dried and powdered roots of *K. schimperi* (1 Kg) were exhaustively extracted with $CHCl_3/CH_3OH$ (1:1 v/v) for 24 h at room temperature using maceration method. The crude extract was concentrated using a rotary evaporator, and 40 g of reddish-brown crude extract was obtained.

Thirty five grams of the crude extract were adsorbed on 25 g of silica gel, powdered with a mortar and pestle, and subjected to silica gel column chromatography. The column was eluted using petroleum ether with an increasing gradient of ethyl acetate. Meanwhile, 66 fractions were collected, and those with similar TLC profiles were combined for further purification. Accordingly, the fractions eluted with 12% ethyl acetate in petroleum ether gave a mixture of two compounds, and further purified on Sephadex LH-20 using $CHCl_3/CH_3OH$ (1:1 v/v) as eluent to afford compounds **3** (35 mg) and **4** (40 mg).

The fractions eluted with 14% ethyl acetate in petroleum ether yielded a mixture of three compounds, which afforded compound **5** (30 mg) after repetitive washing with acetone.

Two grams of the fractions eluted by 20–27% ethyl acetate in petroleum ether were combined, adsorbed on 2 g of silica gel, and loaded to small column chromatography, which was eluted using petroleum ether in an increasing gradient of ethyl acetate. Sub-fractions eluted by 35–60% ethyl acetate in petroleum ether were further purified on Sephadex LH-20 using $CHCl_3/CH_3OH$ (1:1 v/v) as mobile phase and resulted in compounds **1** (15 mg) and **2** (25 mg).

2.4. Antimicrobial assay

The crude extract and isolated compounds (**2, 3,** and **4**) were evaluated for *in vitro* antibacterial and antifungal activities using agar disk diffusion method following the standard procedures [13,14]. Four bacterial strains, *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 13311), *S. aureus* (ATCC 25923), *B. cereus,* and a fungal strain, *C. albicans* (ATCC 14053), were used for antimicrobial assay. The microbial strains were obtained from the Microbiology Research Laboratory, Biology Department, Jimma University.

The strains were initially activated and kept at 4 ^{O}C on nutrient agar slants. With a sterile cotton swab, freshly grown cultures of

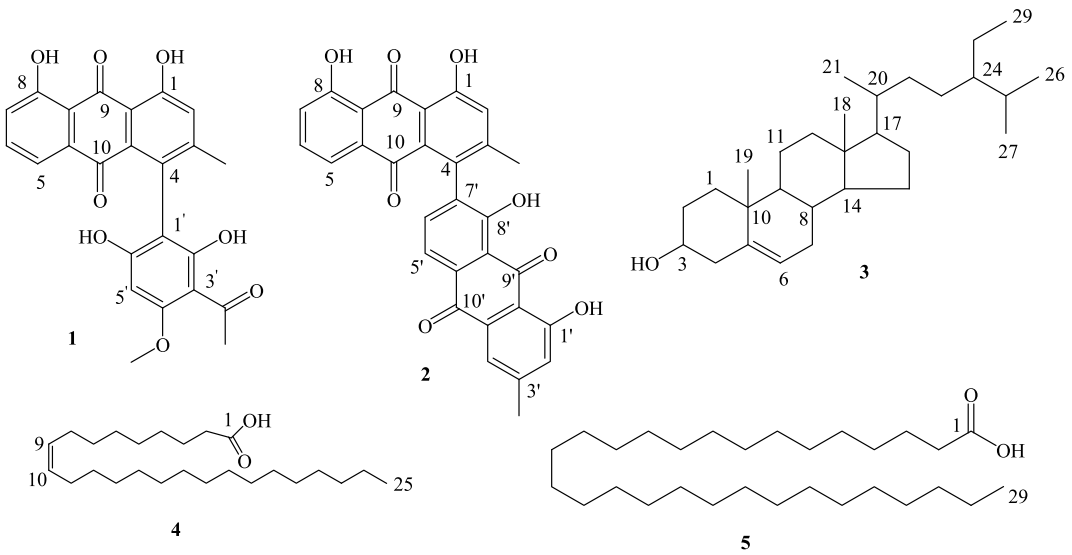

Fig. 1. Chemical structures of compounds isolated from the roots of *K. schimperi*: knipholone (**1**), asphodeline (**2**), *β*-sitosterol (**3**), 9-pentacosenoic acid (**4**), and nonacosanoic acid (**5**).

strains having similar turbidity with 0.5 McFarland were spread over the Mueller-Hinton agar medium on a Petri dish. The paper discs with a diameter of 6 mm were made from Whatman No. 1 filter paper and sterilized for 2 h while wrapped in aluminum foil. 100 mg of crude extract and 10 mg of isolated compounds were dissolved in 1 mL and 0.5 mL of DMSO, respectively, to make the stock solutions. The discs were then immersed in each stock solution. The discs impregnated with the test solutions were air dried and placed on a culture plate at equidistant positions. For the antibacterial assay, gentamicin and DMSO were employed as positive and negative controls, respectively. The plates were incubated for 24 h at 37 ^{O}C, and the diameter of the growth inhibition zone was measured with a transparent ruler in millimeters. The antifungal properties of the crude extract and isolated compounds were evaluated using a similar approach, with the exception of using clotrimazole as a positive control and incubating the plates for 72 h. All experiments were done in duplicate, and the results were expressed as mean ± standard deviation. Data were analyzed statistically using one way ANOVA and Tukey HSD test at a significance level of 5%.

3. Results and discussions

3.1. Structure elucidation of isolated compounds

Compound **1** was isolated as a brown, amorphous solid. The ^{1}H NMR spectrum (Table 1) showed proton signals assignable to a CH_3 group attached to aromatic ring (3H, s, at δ_H 2.18), CH_3 group attached to carbonyl (3H, s, at δ_H 2.69), OCH_3 group (3H, s, at δ_H 3.98), phenolic OH groups (1H, s, at δ_H 5.21, 11.97, 12.62 and 14.20), and aromatic protons (1H, s, at δ_H 6.18; 1H, dd, at δ_H 7.25; 1H, s, at δ_H 7.29; and 2H, dd, at δ_H 7.63). The two downfield shifted signals at δ_H 12.62 and 11.97 are caused by hydroxyl protons at C-l and C-8 peri to carbonyl group and involved in intramolecular hydrogen bonding. The signals observed at δ_H 7.63 (2H, m) and 7.25 (1H, d, J = 7.5) are due to aromatic protons located on ring C of the anthraquinone nucleus. The singlet proton signals observed at δ_H 7.29 and 2.18 were attributed to H-2 and the methyl group attached to C-3 on ring A of the anthraquinone nucleus, respectively. This implies

Table 1
^{1}H NMR (500 MHz),^{13}C NMR (125 MHz) and HMBC spectral data of compound **1** and **2** in $CDCl_3$.

H/C	δ_C	1	HMBC	δ_C	2	HMBC
		δ_H, *m* (J in Hz)			δ_H, *m* (J in Hz)	
1	161.9			160.2		
1a	115.3			114.3		
2	125.0	7.29, *s*	C-1a,C-4, 3-Me, C-8a,C-6′,C-2′	122.0	7.71, *d* (1.2)	C-3,C-1a,C-10
3	152.4			150.1		
4	125.8			131.4		
4a	132.9			133.7		
5	120.1	7.63,*dd* (7.5,1.5)	C-5a, C-8a,C-7, C-10, C-8	120.5	7.87, *dd* (10, 2.5)	C-1a,C-8a,C-7, C-4,C-6,C-10
5a	134.4			134.0		
6	137.2	7.63, *t* (7.5)	C-7, C-8, C-5a, C-8a,C-10	137.6	7.71, *t* (7.5)	C-8a′,C-5,C-7,C-5a,C-8, C-10
7	123.9	7.25, *d* (7.5)	C-8a,C-5, C-1a	125.2	7.32, *dd* (10, 2.5)	C-8a,C-5
8	159.3			162.9		
8a	115.4			116.3		
9	192.8			193.1		
10	182.8			182.2		
1′	106.2			160.8		
1′a				116.6		
2′	163.3			125.0	7.14, *brs*	C-8a′,C-4′
3′	107.2			148.6		
4′	163.2			122.1	7.84, *s*	C-8a′,C-1a′,C-2′,C-10′
4′a				133.2		
5′	91.1	6.18, *s*	C-6′,C-4′, C-1′,C-3′, C-2′, *C*OMe	120.0	7.98, *d* (7.5)	C-1a′,C-4′,C-4,C-7′,C-6′, C-10′
5′a				134.2		
6′	162.9			138.9	7.63, *d* (10)	C-4,C-7′,C-5a′
7′				131.6		
8′				163.3		
8′a				114.2		
9′				193.0		
10′				182.2		
*C*OMe	203.5					
3-Me	21.0	2.18, *s*	C-3, C-2,C-4	22.7	2.50, *s*	C-2,C-3
3′-Me				21.5	2.31, *s*	C-4′,C-3′
CO*Me*	33.2	2.69, *s*	*C*OMe, C-3, C-1			
4′-OMe	55.6	3.98, *s*	C-4′, C-2′,C-6′			
1-OH		11.97, *s*	C-1, C-1a, C-2, C-8a		12.50, *s*	C-8a,C-4,C-1
1′-OH					12.42, *s*	C-8a′,C-1′
8-OH		12.62, *s*			12.06, *s*	C-8a,C-7,C-8
8′-OH					11.97, *s*	C-8a′,C-8′
2′-OH		14.20, *s*				
6′-OH		5.21, *s*	C-5′, C-1′			

that C-4 (δ_C 125.8) is the point of attachment of the aryl substituent (acetylphloroglucinol methyl ether), the presence of which is evident from the proton signals observed at δ_H 14.20 due to a chelated hydroxyl proton at C-2′, an acetyl CH_3 group (δ_H 2.69) at C-3′, and a methoxy group (δ_H 3.98). The ^{13}C NMR spectra (Table 1) showed carbon signals assignable to a CH_3 group (δ_C 21.0), an acetyl CH_3 group (δ_C 33.2), OCH_3 group (δ_C 55.6), aromatic carbons (δ_C 106.2, 107.1, 115.3, 115.4, 120.1, 123.9, 125.0, 125.8, 132.9, 134.4, 137.2, 152.4, 159.3, 161.9, 162.9, 163.2, and 163.3), conjugated ketone carbonyl groups (δ_C 182.8, 192.8), and a non-conjugated ketone carbonyl group (δ_C 203.5). Furthermore, the NOESY spectrum of compound **1** revealed through space coupling for H-5′ and OCH_3 protons, supporting the placement of the OCH_3 group at C-4'. HMBC spectrum showed correlation for H-2 to C-1a, C-4, Ar$\underline{C}H_3$, C-8a, C-6′, and C-2'; H-5 and H-6 to C-5a, C-8a, C-7, C-10, and C-8; H-7 to C-8a, C-5, and C-1a; H-5′ to C-6′, C-4′, C-1′, C-3′, C-2′, and $\underline{C}OCH_3$; $ArCH_3$ protons to C-3, C-2, and C-4; OCH_3 protons to C-4', C-2′, and C-6'; $COCH_3$ protons to $\underline{C}OCH_3$, C-3′, and C-1'; OH-1 to C-1, C-1a, C-2, and C-8a; OH-6′ to C-5′and C-1'. These spectroscopic evidences show that compound **1** is knipholone. The spectral data of compound **1** are also in good agreement with the data reported for knipholone from *K. foliosa* [7].

Compound **2** was isolated as a reddish-yellow, amorphous solid. The ^{1}H NMR spectrum (Table 1) showed proton signals assignable to methyl groups at δ_H 2.31 (3H, s, 3′-CH_3) and δ_H 2.50 (3H, s, 3-CH_3), signals attributed to aromatic protons at δ_H 7.14 (1H, *brs*, H-2′), δ_H 7.32 (1H, dd, H-7), δ_H 7.63 (1H, d, H-6′), δ_H 7.71 (1H, t, H-6 and 1H, d, H-2), δ_H 7.84 (1H, s, H-4′), δ_H 7.87 (1H, dd, H-5), and at δ_H 7.98 (1H, d, H-5′). Furthermore, ^{1}H NMR spectral data of compound **2** showed four deshielded signals at δ_H 11.97 (1H, s), 12.06 (1H, s), 12.42 (1H, s), and 12.50 (1H, s), which are characteristic for chelated hydroxyl protons. The ^{13}C NMR spectra of compound **2** (Table 1) showed 30 carbon signals assignable to benzylic methyl groups (δ_C 21.5 and 22.7), aromatic carbons (δ_C 114.2, 114.3, 116.3, 116.6, 120.0, 120.5, 122.0, 122.1, 125.0, 125.2, 131.4, 131.6, 133.2, 133.7, 134.0, 134.2, 137.6, 138.9, 148.6, 150.1, 160.2, 160.8, 162.9, 163.3), and conjugated ketone carbonyl carbons (δ_C 182.2, 182.2, 193.0, 193.1) in accordance with ^{1}H NMR spectrum. COSY spectrum of compound **2** showed coupling for H-5 and H-6, H-6 and H-7, H-5′ and H-6′, supporting the placement of these protons adjacent to each other. HMBC spectrum revealed correlations for H-2 to C-3, C-1a and C-10; H-2′ to C-8a′ and C-4'; H-4′ to C-8a′,C-1a′,C-2′ and C-10'; H-5 to C-1a, C-8a, C-7,C-4,C-6, and C-10; H-5′ to C-1a′,C-4′,C-4,C-7′,C-6′ and C-10′, H-6 to C-8a′,C-5,C-7,C-5a, C-8 and C-10; H-6′ to C-4, C-7′ and C-5a'; H-7 to C-8a and C-5; 3-CH_3 to C-2 and C-3; 3′-CH_3 to C-4′, and C-3'; OH-1 to C-8a, C-4 and C-1; OH-1′ to C-8a′and C-1'; OH-8 to C-8a,C-7 and C-8; OH-8′ to C-8a′ and C-8′ in corroboration of the proposed structure. The spectral data suggest that compound **2** is asphodeline. This compound was previously isolated from *K. isoetifolia* [5] and *K. insignis* [15].

Compound **3** was isolated as an amorphous solid. The ^{1}H NMR spectrum showed singlet signals at δ_H 0.70 and 1.03 ppm, each integrated for three protons, indicating the two angular methyl groups (H-18 and H-19, respectively). The multiplet signals at δ_H 0.84–0.94 ppm integrated for 12 protons and are assignable to the remaining four methyl groups (H-21, H-26, H-27 and H-29), which are almost in an equivalent chemical environment. A multitude of multiplet signals at δ_H 1.27–2.35 ppm reveal the existence of several methylene and methine protons. The multiplet signal at δ_H 3.66 ppm integrated for one proton corresponds to oxymethine proton (H-3). The doublet of doublet signal at δ_H 5.37 ppm is a characteristic for a vinylic proton (H-6), which is congruent with the vinylic carbon signals exhibited at δ_C 122.6 and 130.0 ppm in ^{13}C NMR spectra. It is worth noting that H-6 couples differently with each of the two allylic protons (H-7) since the latter protons are diastereotopic and hence not chemically equivalent. The appearance of the signal for H-6 as a doublet of doublet rather than a triplet is due to this reason. In addition to vinylic carbons, ^{13}C NMR spectrum also revealed the presence of various aliphatic carbon signals, assignable to methyl groups (δ_C 11.9–19.8 ppm), methylene, methine, and quaternary carbons (δ_C 21.0–56.7 ppm), as well as oxymethine carbon (δ_C 73.7 ppm) as judged by DEPT-135. The spectral data of compound **3** point to β-sitosterol, which was reported from *Ficus abutilifolia* [16].

Compound **4** was isolated as an amorphous solid. The ^{1}H NMR spectrum showed a triplet proton signal integrated for three protons at δ_H 0.90 ppm ($J = 8$ Hz), which indicates the methyl group attached to a methylene group. The multiplet proton signals exhibited at δ_H 5.32–5.40 ppm suggest the presence of olefinic protons, which is also evident from carbon signals observed at δ_C 129.8 and 130.1 ppm in ^{13}C NMR spectrum. The ^{1}H NMR spectrum further showed multiplet signals at δ_H 1.98–2.09 ppm for the two methylene groups at allylic positions (H-8 and H-11) and a triplet signal at δ_H 2.36 ppm ($J = 8$ Hz) for a methylene group attached to electron withdrawing group (H-2). A highly downfield-shifted broad signal at δ_H 10.91 ppm for hydroxyl proton supports the presence of carboxyl functional group. This is also evident from the carbon signal exhibited at δ_C 180.7 ppm for carbonyl carbon in ^{13}C NMR spectra. The multiplet proton signals at δ_H 1.16–1.68 ppm in ^{1}H NMR spectrum are indicative of a lengthy aliphatic chain. Moreover, ^{13}C NMR spectrum demonstrated 25 carbon signals, assignable to two olefinic (δ_C 129.8 and 130.1 ppm), one carbonyl (δ_C 180.7 ppm), twenty one methylene (δ_C 22.9–34.3 ppm), and one methyl (δ_C 14.3 ppm) carbons in accordance with DEPT-135. The spectral data show that compound **4** is 9-pentacosenoic acid and these data also agree with previous report for the synthetic analogue of the same compound [17].

Compound **5** was isolated as a white, amorphous solid. The ^{1}H NMR spectrum showed proton signals assignable to CH_3 group (3H, t, H-29) at δ_H 0.88, extended chains of CH_2 groups (50H, brs, H-4 to H-28) at δ_H 1.25, CH_2 group located beta to carboxyl group (2H, t, H-3) at δ_H 1.63, and CH_2 group alpha to carboxyl group (2H, t, H-2) at δ_H 2.35. ^{13}C NMR spectra of compound 3 revealed carbon peaks assignable to CH_3 groups (δ_C 14.1, C-29), CH_2 groups (δ_C 22.7–33.9, C-2 to C-28), and COOH group (δ_C 179.3, C-1). DEPT-135 also revealed negative signals for all carbons except for the most shielded peak at 14.1 (positive on DEPT-135) and the most deshielded peak at 179.3 (absent on DEPT-135). Based on these spectroscopic data and their comparison with literature data of the same compound reported from the leaves of *Vernonia glaberrima* [18], compound **5** has been elucidated as nonacosanoic acid.

3.2. Antimicrobial activity

The antimicrobial activity test (Table 2) showed that the crude extract was only effective against two bacterial strains, while *β*-sitosterol (**3**) and 9-pentacosenoic acid (**4**) were effective against all tested strains except *E. coli*. Asphodeline (**2**), was only effective

against *S. aureus*. Compound **3** was the most potent, with the highest zone of growth inhibition against *S. typhimurium*. The crude extract was less effective than the isolated compounds, possibly due to antagonistic interactions between the various phytochemicals present in the extract. The results suggest that some of the isolated compounds have potential as antimicrobial agents.

One Way ANOVA analysis of the inhibition zones of the crude extract, compound **2**, **3**, and **4** revealed that the differences between and within groups are statistically significant (p-values: 0.000003866, 0.00000791, 0.000002728, and 0.00001692 respectively, $p < 0.05$).

Tukey HSD test for the inhibition zones of the crude extract showed that the difference is statistically significant between *E. coli* and *S. typhimurium* (0.00002509, $p < 0.05$); *E. coli* and *S. aureus* (0.00001782, $p < 0.05$); *S. typhimurium* and *B. cereus* (0.00002509, $p < 0.05$); and *S. aureus* and *B. cereus* (0.00001782, $p < 0.05$). However, there is no statistically significant difference between *E. coli* and *B. cereus* (1, $p > 0.05$) as well as *S. typhimurium* and *S. aureus* (0.2664, $p > 0.05$). Tukey test for the inhibition zones of compound **2** revealed that there is statistically significant difference between *E. coli* and *S. aureus, S. typhimurium* and *S. aureus* as well as *S. aureus* and *B. cereus* (p-values: 0.0000173, $p < 0.05$). There is no statistically significant difference between *E. coli* and *S. typhimurium, E. coli* and *B. cereus,* as well as *S. typhimurium* and *B. cereus* (p-values: 1, $p > 0.05$). For compound **3** and **4,** the differences are statistically significant between all pairs of bacterial strains ($p < 0.05$) except *S. typhimurium* and *S. aureus* (p-value: 0.2143, $p > 0.05$) for compound **3**; and *S. typhimurium* and *B. cereus* (p-value: 0.3709, $p > 0.05$) as well as *S. aureus* and *B. cereus* (0.1304, $p > 0.05$) for compound **4**.

The antimicrobial activity observed for the $CHCl_3/CH_3OH$ (1:1 v/v) crude extract of *K. schimperi* in the present study is lower than the antimicrobial activity reported for $CHCl_3/CH_3OH$ (1:1 v/v) and acetone crude extracts of other *Kniphophia species* tested against the same strains in the previous investigations. For instance, the $CHCl_3/CH_3OH$ (1:1 v/v) crude extract of the roots of *K. isoetifolia* showed inhibition zones of 23 ± 0.81 and 28 ± 0.51 mm against *S. aureus* and *E. coli,* respectively, in the previous study [5]. However, the $CHCl_3/CH_3OH$ (1:1 v/v) crude extract of the roots of *K. schimperi* demonstrated reduced efficacy against *S. aureus* and *E. coli* in the present study. This may be attributed to the species differences. In another report, the acetone crude extract of *K. pumila* showed growth inhibition zones of 12.6 ± 0.39, 10.7 ± 0.32, and 9.7 ± 0.28 mm against *E. coli, S. aureus,* and *S. typhimurium*, respectively [19], which are also higher than those of the present study for the same strains. This implies that the difference in extraction solvent, in addition to the species difference, has an impact on the potential of the extract to inhibit microbial growth.

When compared to the isolated compounds, the $CHCl_3/CH_3OH$ (1:1 v/v) crude extract of *K. schimperi* has lower activity, which may be attributed to the antagonistic effects of the constituents in the crude extract. The literature demonstrating the possibility of antagonistic interactions between secondary metabolites in plants further supports this observation [20].

Comparison of the antimicrobial activity of compounds isolated in the present study with the same or analogous compounds isolated from other plants in the previous investigations revealed that the same or analogous compounds can have different antimicrobial potential against the same strains depending on concentration and other factors. For instance, asphodeline (**2**), which was isolated from *K. isoetifolia,* showed inhibition zones of 22 ± 0.32 and 20 ± 0.05 mm at 50 mg/mL against *S. aureus* and *E. coli*, respectively [5]. In the present study, asphodeline (**2**) demonstrated smaller inhibition zones (9.0 ± 0.84 mm and 0 mm) at 20 mg/mL against *S. aureus* and *E. coli*, respectively. The concentration difference is undoubtedly the cause of this variation in the outcome. On the other hand, β-sitosterol (**3**), which was isolated from *Sida rhombifolia,* showed inhibition zones of 12 mm and 11 mm at 50 mg/mL against *S. typhimurium* and *S. aureus*, respectively [21]. In the present study, β-sitosterol (**3**) showed higher inhibition zones of 15.5 ± 0.71 mm and 14.3 ± 0.14 mm even at a lower concentration (20 mg/mL) against *S. typhimurium* and *S. aureus*, respectively. This may be caused by the non-uniformity of the depth of the media (agar) and the swabbing of the inoculum, which are inevitable. In the present study, 9-pentacosenoic acid (**4**) exhibited inhibition zones of 9.0 ± 0.56 mm and 11.5 ± 0.71 mm at 20 mg/mL against *S. typhimurium* and *S. aureus,* respectively. However, its analogous fatty acid (11-hexacosenoic acid), which was isolated from *Sida rhombifolia,* showed higher inhibition zones of 11 mm and 12 mm at 50 mg/mL against *S. typhimurium* and *S. aureus,* respectively [21]. This may be due to the difference in chain length and position of the double bond, as these factors can affect the bioactivity of fatty acids [22,23], in addition to the concentration difference.

3.3. Physical and spectroscopic data of isolated compounds

knipholone (1): Brown amorphous solid (15 mg); *Rf* value: 0.68 in 30% ethyl acetate in petroleum ether; ^{1}H NMR (500 MHz,

Table 2
Diameter of growth inhibition zone (mm) of crude extract (100 mg/mL) and isolated compounds (20 mg/mL).

	Samples	Strains				
		E. coli	*S. typhimurium*	*S. aureus*	*B.cereus*	*C. albicans*
Growth inhibition	Extract	NI	7.2 ± 0.35	8.0 ± 0.71	NI	NI
	2	NI	NI	9.0 ± 0.84	NI	NI
	3	NI	15.5 ± 0.71	14.3 ± 0.14	10.5 ± 0.71	12.0 ± 0.28
	4	NI	9.0 ± 0.56	11.5 ± 0.71	10.0 ± 0.56	8.0 ± 0.28
	Gentamicin	25.5 ± 0.71	26.0 ± 0.71	25.0 ± 0.28	23.8 ± 0.35	NA
	Clotrimazole	NA	NA	NA	NA	28.0 ± 0.56
	DMSO	NI	NI	NI	NI	NI

'NI' = no inhibition, 'NA' = not applicable, the given results are average of two replicate tests.

$CDCl_3$, Table 1, Figs. S1a and S1b); ^{13}C NMR (125 MHz, $CDCl_3$, Table 1, Figs. S1c and S1d).

asphodeline (2): Reddish-yellow amorphous solid (25 mg); *Rf* value: 0.63 in 10% ethyl acetate in petroleum ether; ^1H NMR (500 MHz, $CDCl_3$, Table 1, Figs. S2a and S2b); ^{13}C NMR (125 MHz, $CDCl_3$, Table 1, Figs. S2c, S2d and S2e).

***β*-sitosterol (3):** Amorphous solid (35 mg); *Rf* value: 0.74 in 3% ethyl acetate in petroleum ether; ^1H NMR (400 MHz, $CDCl_3$, Figs. S3a and S3b) δ: 1.27 and 1.43 (2H, m, H-1), 1.62 and 1.27 (2H, m, H-2), 3.66 (1H, m, H-3), 2.28 and 2.35 (2H, m, H-4), 5.37 (1H, dd, H-6), 1.69 and 2.03 (2H, m, H-7), 1.31 (1H, m, H-8), 1.27 (1H, m, H-9), 1.43 (2H, m, H-11), 1.62 and 1.31 (2H, m, H-12), 1.27 (1H, m, H-14), 1.31 and 2.03 (2H, m, H-15), 1.27 and 1.86 (2H, m, H-16), 1.31 (1H, m, H-17), 0.70 (3H, s, H-18), 1.03 (3H, s, H-19), 1.43 (1H, m, H-20), 0.94 (3H, m, H-21), 1.27 (2H, m, H-22), 1.31 (2H, m, H-23), 1.43 (1H, m, H-24), 1.43 (1H, m, H-25), 0.84 (3H,m, H-26), 0.86 (3H, m, H-27), 1.31 (2H, m, H-28), 0.88 (3H, m, H-29); ^{13}C NMR (100 MHz, $CDCl_3$, Figs. S3c and S3d) δ: 37.0 (C-1), 32.0 (C-2), 73.7 (C-3), 42.3 (C-4), 130.0 (C-5), 122.6 (C-6), 32.0 (C-7), 31.6 (C-8), 50.0 (C-9), 36.6 (C-10), 21.0 (C-11), 39.7 (C-12), 42.3 (C-13), 56.7 (C-14), 22.7 (C-15), 27.2 (C-16), 56.0 (C-17), 14.1 (C-18), 19.0 (C-19), 36.2 (C-20), 18.8 (C-21), 34.1 (C-22), 25.1 (C-23), 45.8 (C-24), 23.5 (C-25), 19.8 (C-26), 19.3 (C-27), 22.7 (C-28), 11.9 (C-29).

9-pentacosenoic acid (4): Amorphous solid (40 mg); *Rf* value: 0.70 in 3% ethyl acetate in petroleum ether; ^1H NMR (400 MHz, $CDCl_3$, Figs. S4a and S4b) δ: 2.36 (2H, t, $J = 8.0$ Hz, H-2), 1.61–1.68 (2H, m, H-3), 1.16–1.33 (8H, m, H-4 to H-7), 1.98–2.09 (2H, m, H-8), 5.32–5.40 (2H, m, H-9 and H-10), 1.98–2.09 (2H, m, H-11), 1.16–1.33 (26H, m, H-12 to H-24), 0.90 (3H, t, $J = 8.0$ Hz, H-25), 10.91 (1H, s, COOH); ^{13}C NMR (100 MHz, $CDCl_3$, Figs. S4c and S4d) δ: 180.7 (C-1), 34.3 (C-2), 24.8 (C-3), 29.5 (C-4), 29.7 (C-5), 29.8 (C-6), 29.9 (C-7), 32.1 (C-8), 129.8 (C-9), 130.1 (C-10), 32.1 (C-11), 29.8 (C-12), 29.8 (C-13), 29.6 (C-14), 29.5 (C-15), 29.4 (C-16), 29.3 (C-17), 29.2 (C-18), 29.2(C-19), 27.4 (C-20), 27.3 (C-21), 24.8 (C-22), 29.9 (C-23), 22.9 (C-24), 14.3 (C-25).

nonacosanoic acid (5): White amorphous solid (30 mg); *Rf* value: 0.54 in 15% ethyl acetate in petroleum ether; ^1H NMR (500 MHz, $CDCl_3$, Figs. S5a and S5b) δ: 2.35 (2H, t, $J = 7.5$ Hz, H-2), 1.63 (2H, t, $J = 7.5$ Hz, H-3), 1.25 (50H, *br*s, H-4 to H-28), 0.88 (3H, t, $J = 7.5$ Hz, H-29); ^{13}C NMR (125 MHz, $CDCl_3$, Figs. S5c and S5d) δ: 179.3 (C-1), 33.9 (C-2), 24.7 (C-3), 29.1 (C-4), 29.3 (C-5), 29.4 (C-6), 29.5 (C-7), 29.6 (C-8), 29.7 (C-9), 29.7 (C-10), 29.7 (C-11 to C-26), 31.9 (C-27), 22.7 (C-28), 14.1 (C-29).

4. Conclusion

The roots of *K. schimperi* were analyzed, and five compounds were isolated through chromatographic separation. These compounds were knipholone (**1**), asphodeline (**2**), *β*-sitosterol (**3**), 9-pentacosenoic acid (**4**), and nonacosanoic acid (**5**). Antimicrobial activity tests were conducted on some of these compounds, and it was found that compounds **3** and **4** were effective against all tested strains except *E. coli*, while compound **2** was effective against only *S. aureus*. Compound **3** showed the highest level of activity, with the greatest zone of growth inhibition against *S. typhimurium*. Among the isolated compounds, knipholone (**1**) and asphodeline (**2**) are the common denominators in the genus Kniphofia. Furthermore, knipholone (**1**) is known as the chemical marker for the genus Kniphofia, as previous reports indicate. On the other hand, the isolation of *β*-sitosterol (**3**), 9-pentacosenoic acid (**4**), and nonacosanoic acid (**5**) from the genus Kniphofia is the first of its kind. Additionally, the isolation of all compounds from *K. schimperi* is for the first time. As this study is the first attempt to isolate bioactive compounds from *K. schimperi*, it is not without limitations. In this study, a $CHCl_3/CH_3OH$ (1:1 v/v) solvent system was used for the extraction of active principles from *K. schimperi*. This extraction technique leads to crude extracts containing complex mixtures of secondary metabolites with very close polarity, which in turn leads to poor separation. Because of this, other constituents that may have potent bioactivity were missed in this study. Therefore, partitioning of the crude extract between immiscible solvents and/or the use of other techniques, such as sequential extraction with a single solvent at a time, are recommended to avoid this problem.

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Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Ebisa Mirete Deresa: Writing – original draft, Writing - review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Negera Abdissa:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization, Data curation. **Dele Abdissa:** Writing – review & editing, Methodology, Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28927.

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