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Research Article

Antimicrobial Evaluation of Anthraquinones and Preanthraquinone from the Root Extract of *Aloe kefaensis*

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The aim of this study was to isolate antimicrobial compounds from the roots of *Aloe kefaensis*, a plant endemic to Ethiopia and used to treat various microbial infections by traditional healers. The air-dried and powdered plant material was sequentially extracted with petroleum ether, dichloromethane, acetone, and methanol. Then, each solvent extract was evaluated for its *in vitro* antimicrobial activity against four bacterial (*Escherichia coli*, *Bacillus cereus*, *Salmonella typhimurium*, and *Staphylococcus aureus*) and one fungal (*Candida albicans*) strains using the agar disk diffusion method. Superior antimicrobial activity was exhibited against all the strains by dichloromethane extract, with the highest activity observed against *S. typhi* (inhibition zone diameter of 23.0 mm at 200 mg/mL). Due to similarity in their TLC profile, the acetone and dichloromethane extracts were combined and subjected to silica gel column chromatography for fractionation and isolation of the compounds. Separation of these extracts using silica gel column chromatography resulted in four anthraquinones: deoxyerythrolaccin (**1**), chrysophanol (**2**), laccaic acid D-methyl ester (**3**), and 3, 8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**4**) and one preanthraquinone, aloesaponol II (**5**). The structure of these compounds was established using NMR (1D and 2D) spectroscopic analysis and comparison with reported literature data. The isolated compounds were evaluated for antimicrobial activity and showed varying degrees of potency. Compounds **2** and **4** showed the highest activity against *Salmonella typhimurium* at 10 mg/mL, with a zone of inhibition of 28.5 and 25.0 mm, respectively, in comparison to gentamicin (26.0 mm inhibition zone diameter at 10 mg/mL). Therefore, this strong antimicrobial activity of the extracts and isolates supports the traditional usage of *Aloe kefaensis* to treat microbial diseases.

1. Introduction

As per the WHO definition, traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness [1]. Africa has an immensely rich biodiversity and knowledge of using plants to treat various ailments, including infectious diseases. It is estimated that 80% of the population in sub-Saharan Africa depends solely on traditional medicine for their primary healthcare needs because of its accessibility, cheapness, and sociocultural background [2].

Infectious diseases are a serious cause of death worldwide, accounting for about 50% of all deaths in tropical countries [3]. To combat these diseases, huge resources have been exhausted in the last four decades with the help of various strategies such as high-throughput screening, genomics, and vaccine development, but it remains a challenging public health problem because of the emerging of resistant pathogens [4]. In a period when antimicrobial resistance is increasing and limiting the usage of currently available drugs, the search for novel antimicrobial from

potential sources is crucial in order to prevent antimicrobial resistance from becoming the next pandemic [5].

Plants in the genus *Aloe* have been utilized since early times to treat a range of ailments, such as microbial infections, gastrointestinal diseases, and inflammatory conditions [6]. In line with these traditional claims, crude extracts from *A. vera*, *A. volkensii*, and *A. secundiflora* showed good antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Candida albicans*, and *Fusarium oxysporum* [7]. *A. kefaensis* is one of plants in in the genus *Aloe*, which is endemic to Ethiopia; it naturally grows in the Kaffa region as an old boundary demarcation. The local community in this region uses the plant for treating wounds, fire burns, and gastrointestinal problems [8, 9]. However, no studies have been conducted on the antimicrobial activity and phytochemical information of this plant. Therefore, anticipated by its medicinal claims, we reported here the isolation of five (**1**–**5**) compounds from roots of *A. kefaensis*. The antimicrobial activities of the isolates and the extract were also reported. Given their promising and broad spectrum activity against microbes used in this investigation, these compounds may serve as lead compounds in antimicrobial drug development strategies to address limitations associated with currently available drugs, such as side effects, narrow spectrum, and resistance to commonly used antimicrobial drugs in the market, provided that *in vivo* studies should be carried out [10, 11].

2. Materials and Methods

2.1. General Information. Solvents such as petroleum ether, ethyl acetate, chloroform, acetone, and methanol (Loba-Chemie Pvt. Ltd., Mumbai, India) were used for extraction and isolation purposes. A rotary evaporator (Labo Rota 4000, Heidolph Instrument) was used for solvent evaporation. A UV chamber (UV-Tec 254 nm and 365 nm) was used for the detection of spots on the TLC plate. Column chromatography separation was performed on silica gel 60–120 mm mesh size (Merck, Darmstadt, Germany). Further purification of the fractions obtained from column chromatography was made using sephadex LH-20. Thin-layer chromatographic (TLC) analyses were made on precoated silica gel 60 F254 plates. Deuterated acetone and chloroform were used for recording the NMR spectra of the isolated compounds. The NMR spectra of compounds **2–5** were recorded on the Bruker Avance 500 spectrometer at 500 MHz (1H) and 125 MHz (^{13}C), but the NMR spectra of compound **1** were recorded on the Bruker Avance 400 at 400 MHz (1H) and 100 MHz (^{13}C). Mueller–Hinton agar, nutrient broth, DMSO, gentamicin, and chlotrimazole have been used during antimicrobial evaluation.

2.2. Collection and Preparation of Plant Materials. The roots of *A. kefaensis*, whose vernacular name is "Hargisa" (Afaan Oromo), were collected from the Jimma zone's Agaro district, which is located at 7°51′N 36°35′E and at a height of 1560 meters above sea level in Ethiopia's Oromia regional state. The collection of the plant has been done by approaching traditional healer living in that district. Identification of the plant has been made by Dr. Dereje Denu, a botanist from the Biology Department at Jimma University, Ethiopia, and the voucher specimen CH8 has been deposited.

2.3. Extraction and Isolation. The air-dried and ground roots (0.8 kg) of *A. kefaensis* were sequentially extracted with petroleum ether, dichloromethane, acetone, and methanol using cold maceration for 48 hours each and resulted in 3.2 g, 7.5 g, 10 g, and 7 g, respectively. Based on the TLC profile, acetone (8 g) and dichloro methane (5.5 g) extracts were combined, and a total of 13.5 g was subjected to column chromatography packed with silica gel, which was eluted with petroleum ether containing an increasing amount of ethyl acetate (EtOAc) to afford 69 fractions ~200 mL each. The fractions eluted with 35% EtOAc in petroleum ether gave compound **1** (19 mg) after being purified on Sephadex LH-20 (eluting with $CHCl_3$/MeOH; 1 : 1). Fractions eluted with 65–100% of ethyl acetate in petroleum ether were combined and applied to small-size column chromatography, followed by further purification of the fractions on Sephadex LH-20 (eluting with $CHCl_3$/MeOH; 1 : 1), and yielded four compounds: **2** (23 mg), **3** (24 mg), **4** (35 mg), and **5** (30 mg). The purity of the isolated compounds was monitored by thin-layer chromatographic (TLC) analyses, which was visualized under ultraviolet light (254 and 365).

2.4. Antimicrobial Assay. The extracts and obtained compounds were screened for *in vitro* antibacterial activities against four bacteria strains (*Escherichia coli* (ATCC25922), *Bacillus cereus*, *Salmonella typhimurium* (ATCC13311), and *Staphylococcus aureus* (ATCC25923)) by agar disc diffusion method [12, 13] at the Microbiology Laboratory, Jimma University. The test solution with concentrations of 200 mg/mL and 10 mg/mL was prepared by dissolving the crude extract and compounds, respectively, in DMSO. The inoculums with 0.5 McFarland turbidities were then uniformly swabbed over the Müeller–Hinton agar medium using a sterile swab. Then, the filter paper discs (6 mm in diameter, made from Whatman No. 1 filter paper and sterilized by an autoclave at 121°C for 20 min) were impregnated with the test samples and placed on the surface of the medium at appropriate distance from one another to avoid overlap of zones of growth inhibitions. Inhibition zones were measured in mm after 24 hours' incubation at 37°C and compared with the standard drugs. 1% DMSO was used as a negative control, and gentamycin was used as a standard antibacterial drug. The antifungal activity of the extract and compounds was evaluated against *Candida albicans* (ATCC14053) by following the same method as the antibacterial activity test, except the Petri dishes were incubated at room temperature for 48 hours and clotrimazole was used as the standard drug.

3. Results and Discussion

3.1. Characterization of the Isolated Compounds. The chromatographic separation of the combined acetone-dichloromethane extracts of the roots of *A. kefaensis* has resulted in the isolation of five compounds (**1**–**5**, Figure 1). Compound **1** was obtained as a yellow amorphous solid with an Rf value of 0.41 in a petroleum ether/EtOAc (7 : 3) solvent system. The ^{1}H NMR spectrum (Table 1) showed the presence highly downfield-shifted proton signal at δ_H 13.32 indicating the presence of a hydroxyl group involved in hydrogen bonding. It also showed four aromatic protons at δ_H 6.64 (d, $J = 2.5$ Hz), 7.09 (d, $J = 2.7$ Hz), 7.18 (d, 2.5 Hz), and 7.57 (d, $J = 2.7$ Hz) ppm, two sets of metacoupling aromatic protons. This could only be possible when every two aromatic rings are present. Downfield-shifted proton signal at δ_H 2.76 integrated for three protons is also indicating the presence of aromatic attached methyl group *peri* to carbonyl group, as evidenced from its chemical shift. The ^{13}C NMR spectrum (Table 1) showed signals for protonated carbons at δ_C 125.5, 113.1, 109.2, and 107.9, one aromatic CH_3 group at δ_C 24.1, and only two quaternary carbons at δ_C 162.7 and 146.3, which may be due to the small amount of the sample. Based on these spectroscopic data and in comparison with the reported literature, compound **1** was identified as deoxyerythrolaccin which has previously been reported from the roots of *A. vera and A. dawei*. Antimicrobial activity of this compound has been studied against *Xanthomonas campestris*, *Pectobacterium carotovorum* subsp., and *Pseudomonas syringae*, showing more activity against *Xanthomonas campestris* [14–16].

Compound **2** was isolated as a yellow amorphous solid with an Rf value of 0.46 in the petroleum ether/EtOAc (6 : 4) solvent system. The two highly downfield-shifted signals at δ_H 12.07 and 11.96 in the ^{1}H NMR spectrum indicated the presence of hydroxyl protons involved in hydrogen bonding. It further showed three mutually coupled aromatic protons at δ_H 7.79 (dd, $J_1 = 7.2$, $J_2 = 2.4$), 7.64 (d, $J = 7.2$), and 7.25 (1H, dd, $J_1 = 7.2$, $J_2 = 2.4$) which were assigned to H-5, H-6, and H-7, respectively. The other two remaining protons resonating at the 7.06 (*d*, *J = 2.5*) and 7.60 (*s*) were assigned to H-2 and H-4, respectively. This spectrum also showed presence of aromatic CH_3 group at δ_H 2.44. The ^{13}C NMR spectrum (Table 1) showed 15 carbon signals ascribed five aromatic methines (124.5 (C-2), 120.0 (C-4), 121.5 (C-5), 137.0 (C-6), and 124.7 (C-7)), seven aromatic quaternary carbons (δ_C 162.8 (C-1), 149.5 (C-3), 162.5 (C-8), 113.8 (C-1a), 133.4 (C-4a), 133.7 (C-5a), and 116.0 (C-8a)), two carbonyl carbons (δ_C 192.6 (C-9) and 182.0 (C-10)), and one methyl group (δ_C 22.4) group. Presence of two chelated hydroxyl protons signals at δ_H 12.07 and 11.96 and the downfield-shifted carbonyl signals at δ_C 192.6 and 182.0 showed that the compound is 1,8-dihydroxyanthraquinone. Based on these spectroscopic data and comparison with the data in the literature, compound **2** was identified as 1,8-dihydroxy-3-methylanthracene-9,10-dione, trivial name chrysophanol. According to several scientific studies, this compound has a wide range of biological activities, including anticancer, antimicrobial, antidiabetic, anti-inflammatory, antiprotozoal, hypolipidemic, hepatoprotective, neuroprotective, antiulcer, and antiobesity. It is known to be present in a number of plants, primarily those belonging to the Polygonaceae, Rhamnaceae, Fabaceae, Liliaceae, Asphodelaceae, Buphorbiaceae, Meliaceae, Podocarpaceae, Picramniaceae, and Hemerocallidaceae family [17–19].

Compound **3** was obtained as orange crystals with an Rf of 0.54 in a PE : EtOAc (7 : 3) solvent system. The ^{1}H NMR spectrum (Table 1) showed the presence for chelated hydroxyl group at a signal at δ_H 13.17, and three-singlet proton at δ_H 3.96 (δ_C 51.9) for a methoxy group and downfield-shifted methyl signal (at δ_H 2.74 and δ_C 19.4). In the ^{1}H NMR spectrum, the presence of three downfield-shifted *singlet* aromatic protons at δ_H 7.74, 7.22, and 6.69 is, respectively, ascribed to protons at H-5, H-4, and H-2. The ^{13}C NMR spectrum (Table 1) showed methyl ester (δ_H 3.96, δ_C 51.9, and δ_C 167.2 for the ester carbonyl). Comparing ^{13}C NMR and ^{1}H NMR spectral data with spectral data reported in literature, the structure of compound **3** was identified as laccaic acid D methyl ester, which had previously been isolated from *Aloe turkanensis* and studied for its anticancer activity against the human extra hepatic bile duct carcinoma (TFK-1) and liver (HuH7) cancer cell lines [20].

Compound **4** was isolated as a yellow powder (Rf value 0.70) in a petroleum ether/EtOAc (5 : 5) solvent system. In the ^{1}H NMR spectrum (Table 1), a downfield-shifted signal at δ_H 13.3 was due to a hydroxyl proton at C-8 involved in hydrogen bonding. In ring C, three mutually coupled aromatic protons at δ_H 7.59 (dd, $J_1 = 7.2$, $J_2 = 2.5$), 7.11 (d, $J = 7.5$), and 6.66 (1H, dd, $J_1 = 7.4$, $J_2 = 2.5$) were assigned to H-5, H-6, and H-7, respectively. The other singlet proton at δ_H 7.20 was assigned to H-4. This spectrum also indicates the presence of an aromatic CH_3 group at δ_H 2.79. The ^{13}C NMR spectrum showed sixteen carbon signals accounting for four methines (δ_C 124.6, 123.4, 135.0, and 112.2), one methyl (δ_C, 23.2), eight quaternary carbon atoms (δ_C 145.5, 137.4, 110.8, 108.2, 107.0, 106.9, 163.2, and 161.7), and three carbonyl signals (δ_C 188.8, 182.3, and 165.5). The structure elucidation of compound **4** was finally achieved by comparing these spectral data with the data reported in the literature. Thus, this compound was identified to be 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid, previously isolated from *Emex spinosus* and studied for potential antibiofilm agents against methicillin-resistant *Staphylococcus aureus* [21, 22].

Compound **5** was isolated as a colorless solid with an Rf value of 0.41 in a petroleum ether/EtOAc (6 : 4) solvent system. In the ^{1}H NMR spectrum (Table 1), a highly downfield-shifted proton signal at δ_H 15.17 indicates presence of chelated hydroxyl group in the β-position to a carbonyl. It also showed three aromatic protons along with HMQC experiment at δ_H 6.83, 6.88, and 6.89. In addition, the spectral data revealed signals for an oxymethine at C-3 (δ_H 4.34) and two methylenes at C-2 (δ_H 2.73 and 2.94) and C-4 (δ_H 2.85 and 3.19) adjacent to chiral carbon. This ^{1}H spectrum was in agreement with the presence of a preanthraquinone skeleton when supported with ^{13}C NMR and DEPT-135 signals indicating carbonyl at C-1 (δ_C 203.1), an

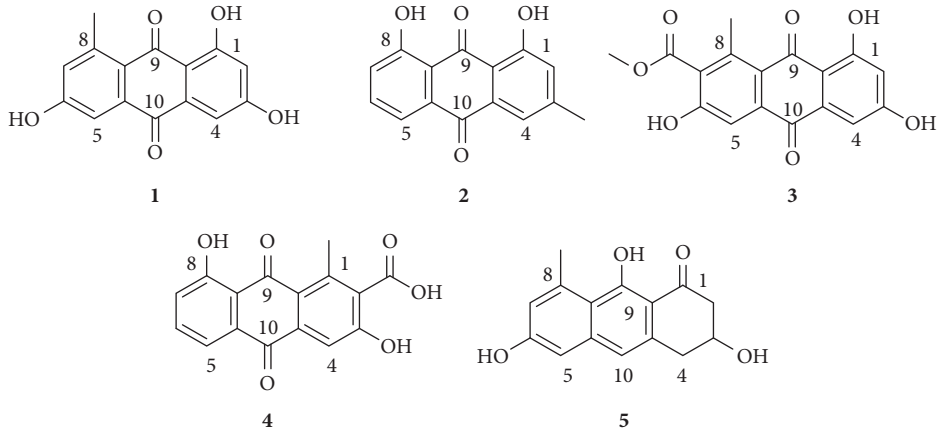

Figure 1: Structure of isolated compounds from roots of *A. kefaensis*.

Table 1: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **1** (in acetone-d6) and ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **2** (in $CDCl_3$), **3**, **4**, and **5** (in acetone-d6).

Position	1		2		3		4		5	
	δ_H, m (J in Hz)	δ_C	δ_H, m (J in Hz)	δ_C	δ_H, m (J in Hz)	δ_C	δ_H, m (J in Hz)	δ_C	δ_H, m (J in Hz)	δ_C
1	—	162.7	—	162.8	—	165.4	—	145.5	—	203.1
1a	—	122.4	—	113.8	—	111.0	—	123.4	—	110.1
2	6.64, d (2.5)	109.2	7.06, d (2.5)	124.5	6.69, s	108.5	—	134.9	2.73 and 2.94, m	46.7
3	—	161.8	—	149.5	—	164.4	—	161.6	4.31, m	65.4
4	7.18, d (2.5)	107.9	7.60, d (2.5)	120.0	7.72, s	107.0	7.20, s	107.0	2.85 and 3.19, m	38.2
4a		136.7	—	133.4	—	134.7	—	137.4	—	136.2
5	7.57, d (2.7)	113.1	7.79, dd (7.2, 2.4)	121.5	7.74, s	112.2	7.59, dd (7.2, 2.5)	112.2	6.83, s	107.6
5a	—	134.3	—	133.7	—	137.2	—	110.8	—	142.2
6	—	164.1	7.64, d (7.2)	137.0	—	158.2	7.11, d (7.5)	124.6	—	158.7
7	7.09, d (2.7)	125.5	7.25, dd (7.2, 2.4)	124.7	—	123.6	6.66, dd (7.4, 2.5)	108.3	6.88, d (2.5)	119.6
8	—	146.3	—	162.5	—	141.0		163.9	—	141.3
8a	—	110.0	—	116.0	—	130.0		106.9	—	117.0
9	—	188.0	—	192.6	—	188.5		188.8	—	166.4
10	—	182.4	—	182.0	—	181.3		182.3	6.89, d (2.5)	119.6
1-OH	13.32, s	—	12.07, s	—	13.17, s	—	—	—	—	—
8-OH	—	—	11.96, s	—		—	—	—	—	—
9-OH	—	—	—	—		—	13.3, s	—	15.17, s	—
Ar-CH_3	2.76, s	24.1	2.44, s	22.4	2.74, s	19.4	2.79, s	23.2	2.76, s	24.0
-OCH_3	—		—	—	3.96, s	51.9	—	—	—	
-COO-	—	—	—	—	—	167.2	—	165.4	—	—

oxymethine at C-3 (δ_C 65.4), and two methylenes at C-2 (δ_C 46.7) and C-4 (δ_C 38.2). Both spectral data showed downfield-shifted methyl signal (at δ_H 2.76 and δ_C 24.0) due to the deshielding effect of the neighboring carbonyl group, the HMBC (Heteronuclear Multiple Bond Correlation) experiment gives correlations between methyl signal and one aromatic proton H-7, confirming that the methyl group is at C-8. The ^{13}C NMR and DEPT-135 spectrum further showed three aromatic and one aliphatic methines (119.6, 107.6, 117.0, and 65.4), two oxygenated aromatic quaternary carbons (158.7 and 166.4), and five quaternary aromatic carbons (110.1, 142.2, 136.2, 116.8, and 141.3). Based on this spectral data along with 2D experiments (COSY, HMQC, and HMBC) and comparison with structurally related compound (aloesaponol I) in the literature, compound **5** has been identified as aloesaponol II. Aloesaponol I, which resembles compound **5** structurally, has been isolated from *Aloe megalacantha* and reported to show good cytotoxic activity against a human cervix carcinoma cell line KB-3-1 [23, 24].

3.2. Antimicrobial Activities of Extracts from Roots of Aloe kefaensis. Sequentially extracted samples from roots of *A. kefaensis* were tested for their antimicrobial activity against four bacterial strains (*E. coli*, *S. aureus*, *B. cereus*, and *S. typhi*) and one fungal strain (*C. albicans*) by the agar disk diffusion method. The activities of the all crude extracts were assessed by the diameter of the zone of inhibition in millimeters by taking the extracts at concentration of 200 mg/mL. The zones of inhibition for different extracts fall between 9.0 and 23.0 mm as compared to gentamicin

Table 2: *In vitro* antimicrobial activities of the extracts (diameter of zone of inhibition (mm)).

Sample type		Strains				
		S. aureus	*B. cereus*	*E. coli*	*S. typhi*	*C. albicans*
Extracts (200 mg/ml)	PE	9.0	NI	8.0	13.0	10.0
	DCM	20.5	17.0	18.5	23.0	20.5
	AC	18.0	16.0	15.5	22.0	18.5
	ME	NI	NI	NI	14.0	9.0
Serially diluted DCM extract	100 mg/mL	16.0	11.0	17.5	18.0	15.0
	50 mg/mL	13.0	10.0	16.0	15.5	15.0
	25 mg/mL	7.0	8.0	11.0	11.0	11.0
	12.5 mg/mL	7.0	7.5	8.0	10.0	9.0
Controls	G 10 mg/mL	23.5	23.0	20.0	25.5	NC
	C 25 mg/mL	NC	NC	NC	NC	19.5
	D 1%	NI	NI	NI	NI	NI

Note. These results are average results of two separate experiments: NI: no inhibition; NC: not conducted; PE: petroleum ether; DCM: dichloromethane; AC: acetone, ME: methanol; G: gentamicin; C: clotrimazole; D: DMSO.

ranging between 20.0 and 25.5 mm as indicated in Table 2. The generated data were taken from two separate experiments in duplicate. As we might learn from Table 2, the antimicrobial activity result showed that the dichloromethane and acetone extracts have showed better activity against all tested strains. The superior antimicrobial activity was exhibited by dichloromethane extract with the zone of inhibition of 17.0, 18.5, 20.5, 20.5, and 23.0 mm against *B. cereus*, *E. coli*, *S. aureus*, *C. albicans*, *and S. typhi*, respectively. Following its good activity, the dichloromethane extract's concentration-dependent effects were tested against all strains using serially diluted extract. Different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml of the extract have shown varying activities against the strains.

The results of this study have shown higher concentrations (200 mg/ml) potent than diluted concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml) on all microbes. The highest dose (100 mg/ml) of the diluted extract revealed the maximum activity (18.0 mm inhibition zone) against *S. typhi*, followed by inhibition effect (17.5 mm) against *E.coli*). According to Biniam et al. 2019 [25], the antimicrobial activity of the leaf extracts of *Aloe* species such as *A. camperi*, *A. elegansi*, and *A. eumassawana* improved against *E.coli* and *S. aureus* when the concentration of the extracts was increased. In addition to this, Kedarnath et al. 2013 [26] have reported the chloroform extract of *A. vera* leaf to show highest inhibition zone at higher concentration. In this finding, the DCM extract showed more antibacterial activity against *S. typhi* the Gram-negative bacteria than the Gram-positive *B. cereus*. These results are in contrast to other researchers' findings who reported that most plant extracts have more activity against Gram-positive bacteria, but it is in agreement with the *in vitro* effects of *A. vera* extract on the inhibition of these bacteria as reported by Redda et al. [27].

3.3. Antimicrobial Activities of Isolates from Roots of Aloe kefaensis. Following the promising results of the antimicrobial effects of the extracts, antimicrobial screening was also carried out for the compounds (deoxyerythrolaccin, chrysophanol, laccaic acid D methyl ester, 3, 8-dihydroxy-1-methylanthraquinone-2-carboxylic acid, and aloesaponol II) against all strains as indicated in Table 3. These compounds showed antibacterial activity with varying degrees of potency. Almost all compounds have displayed stronger activity against *S. typhi* the Gram-negative bacteria than other strains used in this study. The superior antimicrobial activity by dichloromethane extract against S. *typhi* (23.0 mm) might pertain to these compounds. The presence of a nonpolar methyl moiety as part of their functional group (Figure 1) might help the compounds to pass the outer lipid membrane of this Gram-negative bacterium [4]. The isolated compounds also showed good antifungal activity against *C. albicans* with superior activity exhibited by chrysophanol (21.0 mm inhibition zone at 10 mg/mL) and 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (19.0 mm inhibition zone at 10 mg/mL) which is even greater than that of the reference drug, clotrimazole (16.0 mm inhibition zone at 25 mg/mL). Therefore, this investigation has shown the potential of the obtained monomeric anthraquinones and preanthraquinone as a lead structure for the development of antimicrobial drugs, provided that further *in vivo* taste should be carried out. This might help us to tackle challenges associated with fighting infectious diseases which is becoming worsened than ever before because of the fast development of resistance to the antimicrobial agents that are currently in market, the emergence of resistant strains of a number of microbes, and the changing nature of the infections observed in the elderly and other immune-compromised patients [28]. The strong antimicrobial activity of the crude extracts and isolated compounds is in line with the traditional usage of the plant for the treatments of microbial infections.

3.4. Potential Mode of Action of the Isolated Compounds. Possible modes of action of isolated compounds against bacteria could be through changing the permeability of the cell membrane, blocking DNA replication, inhibiting the growth of biofilms, breaking down cell walls, inhibiting

Table 3: *In vitro* antimicrobial activities of the isolated compounds.

Strains	Compounds					Controls		
	1	2	3	4	5	G	C	D
S. aureus	13.0	29.0	13.0	24.5	16.0	24.0	NC	NI
B. cereus	13.0	26.0	13.0	24.0	12.5	25.0	NC	NI
E. coli	19.0	25.0	13.0	19.0	16.0	25.0	NC	NI
S. typhi	16.5	28.5	17.0	25.0	17.0	26.0	NC	NI
C. albicans	17.5	21.0	10.0	19.0	14.0	—	16.0	NI

Note. These results are average results of two separate experiments; compound's concentration: 10 mg/mL; NI: no inhibition; NC: not conducted; G: gentamicin; C: clotrimazole; D: DMSO.

endotoxins, blocking respiratory metabolism, and inhibiting the synthesis of proteins and nucleic acids [29–31]. Mechanisms responsible for antifungal activity include interfering with cell wall synthesis, efflux pump inhibitory activity, and inhibiting biofilm formation or disrupting biofilm [29]. The exact process of action may differ based on the target microorganism, but certain possible mechanisms may be linked to structural features, including the presence of substituent groups such as $COOCH_3$, CH_3, COOH, and position of OH groups [29]. In line with this, all isolated compounds from the roots of *Aloe kefaensis* have a methyl group attached to the anthraquinone core that can affect the compound's lipophilicity and membrane permeability so as to enhance antimicrobial activity, possibly by facilitating the penetration of the compound into microbial cells [4, 32].

4. Conclusions

The bioassay and traditional utilization-based isolation of compounds from roots of *Aloe kefaensis* have resulted in five anthraquinones. All compounds have demonstrated significant antibacterial activities with superior activity of compound **2** and **4** against *Salmonella typhimurium*. The isolated compounds have also showed good antifungal activity against *C. albicans*, with **2** (21.0 mm) and **4** (19.0 mm) demonstrating very potent activity which was even greater than that of the reference drug, clotrimazole, showing an inhibition zone of 16.0 mm. Therefore, this work has shown the promise of the obtained compounds as a lead structure for the development of antimicrobial drugs, yet further *in vivo* testing has to be done.

Data Availability

Antimicrobial discs, NMR data, and TLC analysis have been provided in supplementary information.

Additional Points

Highlights. (i) Compounds isolated from roots of *Aloe kefaensis* showed promising antimicrobial activity. (ii) Deoxyerythrolaccin, chrysophanol, laccaic acid D methyl ester, 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid and aloesaponol II were isolated from root extracts of *Aloe kefaensis*. (iii) The compounds could serve as lead structure for the development of antimicrobial drugs, provided further *in vivo* testing should be done.

Disclosure

The findings presented in this manuscript are available in M.Sc. thesis format in the Jimma University Open Access Institutional Repository.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

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Supplementary Materials

The Supplementary Materials include information on the Zone of Growth Inhibition of Crude Extract, the Zone of Growth Inhibition of Dichloromethane (DCM) Extract at Various Concentrations, TLC of Isolated Compounds, the Zone of Growth Inhibition of Isolated Compounds, and NMR data of compounds. (*Supplementary Materials*)

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