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Bioactive compounds from *Hagenia abyssinica* with activity against bean pathogenic bacteria

Isabel Nyokabi Wagara[1], Waudo Walyambillah[2], Beatrice Were[3] David Muthama[4], Hellen Gathiru[1] and Josphat Clement Matasyoh[4*]

[1]Department of Biological Sciences, Egerton University, P. O. Box 536-20115, Egerton, Kenya.
[2]Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya.
[3]Department of Biological Sciences, University of Eldoret, P. O. Box 1125-30100, Eldoret, Kenya.
[4]Department of Chemistry, Egerton University, P. O. Box 536-20115, Egerton, Kenya.

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Common bean (*Phaseolus vulgaris* L.) is a major food crop in Africa and its production is plagued by diseases that reduce the potential yield, thus threatening food security. This study evaluated compounds from *Hagenia abyssinica* for bioactivity against bean bacterial pathogens *Xanthomonus axonopodis* pv*. phaseoli* and *Pseudomonus savastanoi* pv*. phaseolicola*. One triterpenoid, 1,3,19-trihydroxy-2-oxo-12-ursen-28-oic acid (1) and two flavans namely, 3,3',4',5'-tetrahydroxyflavan (2) and 3,3',4',5,7-pentahydroxyflavan (3) were isolated from the bark of *H. abyssinica*. Structures of the compounds were elucidated based on nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectra (HRESIMS) data analysis. These compounds and the ethyl acetate extract were evaluated against the bean pathogens using agar disc diffusion method. The ethyl acetate crude extract showed activity against the two pathogens with minimum inhibitory concentrations of 1.25 mg/mL. Compound 1 showed good activity against *X. axonopodis* pv*. phaseoli* and *P. savastanoi* pv*. phaseolicola* with minimum inhibitory concentrations of 5 and 1.25 mg/mL, respectively, whereas compounds 2 and 3 showed modest activity. This study demonstrated that compound 1 and the ethyl acetate crude extract from *H. abyssinica* have good activity against the two bean pathogens and can be used in the development of biopesticides to control bean diseases.

Key words: Bean pathogens, bioactive compounds, *Hagenia abyssinica*, *Xanthomonus axonopodis* pv*. phaseoli, Pseudomonus savastanoi* pv*. phaseolicola*.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major legume crop that is largely consumed among various communities in sub-Saharan Africa. It provides a cheaper alternative source of protein and household food security to the low-income earners in towns and the rural poor population (Gichangi et al., 2012). Often referred to as "the meat of

*Corresponding author. E-mail: jmatasyoh@egerton.ac.ke.

the poor," beans provide a highly nutritious food, containing protein, fiber, complex carbohydrates, vitamins, and micronutrients (CIAT, 2019). Beans also provide income for millions of people, particularly in Africa. However, its productivity is constrained by bacterial infections such as common bacterial blight caused by *Xanthomonas axonopodis* pv*. phaseoli* and halo blight caused by *Pseudomonas savastanoi* pv*. phaseolicola*. There are no effective, economical and environmentally friendly strategies of managing these diseases, especially for resource-poor farmers. According to literature (Macharia et al., 2009), approximately 263 tons of synthetic pesticides are applied at an average rate of 0.82 kg/ha and of these pesticides, 8% were classified as highly hazardous compounds by the World Health Organization, 25% as carcinogens, while 43% are said to be possible carcinogens. These synthetic pesticides are used by farmers across the region. Moreover, some of these pesticides cause "collateral damage" to other flora or to fauna, and may persist in the environment for years. Therefore, there is need to find alternative ways of controlling these pathogens using extracts and compounds from natural sources. The main objective of this study was to improve food security through the production of biopesticides for the control of bean diseases. Due to climate change, emerging, re-emerging and endemic plant diseases continue to be a major challenge in food production leading to food insecurity especially in sub-Saharan Africa. The study identified the medicinal plant *Hagenia abyssinica* as the source of bioactive compounds with activity against pathogenic bacteria that affect beans. This plant has been reported to show antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* (Karumi et al., 2013; Wolde et al., 2016). The bioactive compounds can be used for standardization in the development of biopesticides formulations from the plant materials. In our continued search for molecules with activity against bean phytopathogens, we report here the isolation and elucidation of three antibacterial compounds from *H. abyssinica*.

MATERIALS AND METHODS

Plant

The bark of *H. abyssinica* was collected from Mt. Elgon Forest (1.1493° N, 34.5418° E), Kenya in March 2019. Prof. S. T. Kariuki, a taxonomist of the Herbarium at Egerton University, identified the plants. Voucher specimens were deposited at the Department of Biological Sciences, Egerton University, Kenya.

General experimental procedures

Nuclear magnetic resonance (NMR) spectroscopy experiments were performed with a Bruker Avance III spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C). High-resolution electrospray ionization mass spectra (HRESIMS) were carried out using LTQ Orbitrap spectrometer (Thermo Scientific, USA) equipped with a HESI-II ion source. For column chromatography, Silica gel 60 (0.063 – 0.2 mm, Macherey-Nagel) and Sephadex LH–20 (18 – 111 µm, GE Healthcare) were used. Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 plates (0.20 mm, Macherey-Nagel). Semi-preparative high-performance liquid chromatography (HPLC) was done on Shimadzu LC-20AP pump equipped with DGU-20A5R degassing unit, Shimadzu SPD-M20A detector, Shimadzu SIL-20ACHT auto-sampler using LabSolutions software.

Extraction and isolation of the bioactive compounds

The bark material of *H. abyssinica* was air dried under shade to constant weight and ground to a fine powder using a Thomas-Wiley mill model 4. Two kilograms of the ground material were soaked in methanol at room temperature and the contents were then filtered through Whatman no. 1 filter paper. The filtrate was concentrated in vacuum at 60°C using Buchi Rotavapor R-205.

The concentrated crude methanol extract was suspended in distilled water and extracted sequentially with hexane followed by ethyl acetate. The ethyl acetate crude extract (200 g) was subjected to column chromatography using the solvent mixture of ethyl acetate, hexane, methanol (6:3:1) to yield four fractions after pooling together those with similar TLC profiles. Fraction 2 and 3 were purified on a reverse preparative HPLC using Gemini C18 column (10 × 250 mm, 10 µm particle size, Phenomenex). The mobile phase used was double distilled water (with 0.1% formic acid) (A) and HPLC grade methanol (B). Fraction 2 yielded compounds 1 (10.2 mg) and 2 (11.8 mg) while fraction 3 yielded compound 3 (12.1 mg).

Antibacterial assay

The test organisms used in this study were *X. axonopodis* pv. *phaseoli* and *P. savastanoi* pv. *phaseolicola*. The antibacterial assays of the extracts and pure compounds were performed using agar disc diffusion method as described by Kajaria et al. (2012) with a slight modification. The media used in this assay was nutrient agar (28 g/1000 mL of distilled water). A 24-h bacterial population of 1.5×10^{8} CFU/mL (1.0×10^{8} – 2.0×10^{8} CFU/mL) was spread on the plate containing media and left to dry. All extracts were weighed and a 20 mg/mL concentration of the extracts and compounds were made using dimethyl sulfoxide (DMSO). Blank sterile disc of Whatman filter paper No. 1, of 6 mm in diameter was impregnated with 100 µL of the different extracts and plated against the test organisms. Chloramphenicol was used as the reference standard whereas DMSO was the negative control. The plates were incubated at 32°C overnight and zone of inhibition measured in millimeters.

RESULTS AND DISCUSSION

Compound 1 was obtained as a dark brown substance whose mass spectral data gave a molecular ion peak of m/z = 503.3362 that calculated for the molecular formula $C_{30}H_{47}O_{6}$, [M + H] (Figure 1). The 1D and 2D NMR spectral data of compound 1 are summarized in Table 1. Its ^{1}H-NMR spectrum showed characteristics signals of 7 methyl groups at δH 0.58 (H-24), 1.08 (H-25), 0.70 (H-26), 1.36 (H-27), 1.09 (H-29), 0.85 (H-30), and one

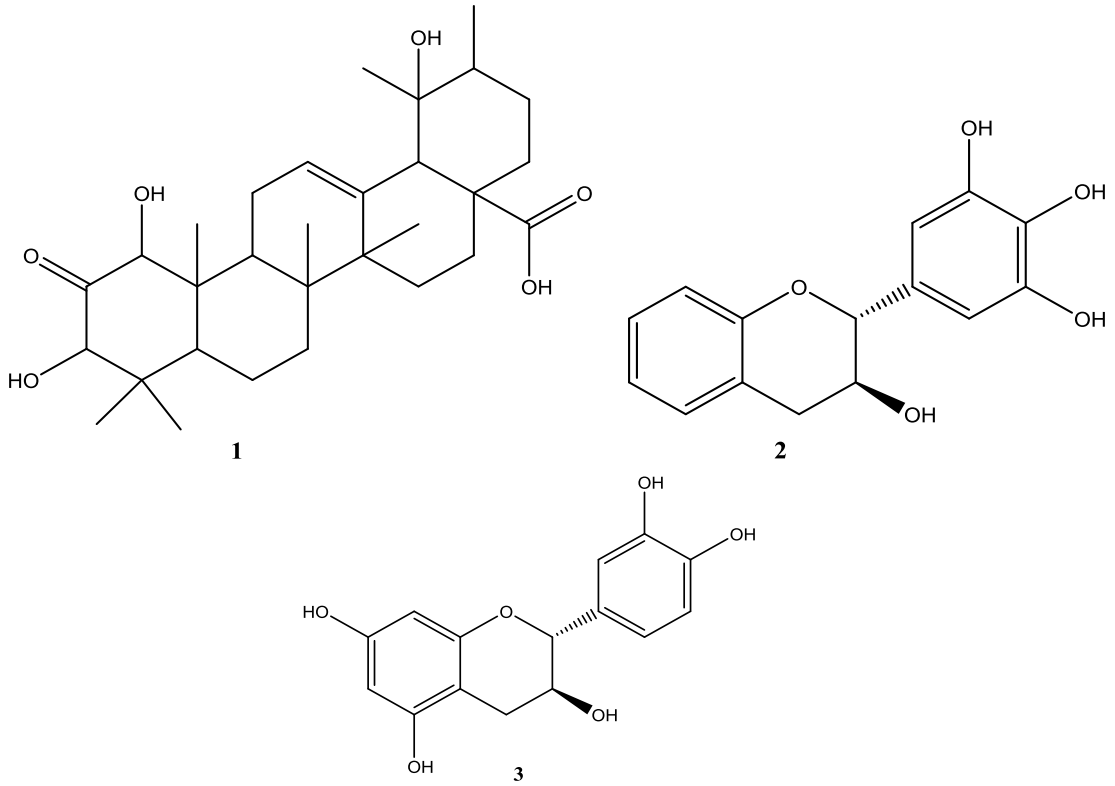

Figure 1. Structure of compounds 1, 2 and 3.
Source: Authors

olefinic proton at δH 5.16 (H-12) indicating that it is an ursen-12-en skeleton. Two oxymethine protons are observed at 4.08 (H-1), 4.00 (H-3) and an oxygenated quaternary carbon at C – 19 indicating the presence of hydroxyl groups in those positions.

The interpretation ^{13}C NMR spectra together with Heteronuclear Single Quantum Coherence (HSQC) showed that compound 1 had a total of 30 carbons which consisted of two carbons of a trisubstituted double bond, seven methyl groups, seven methylene groups, seven methine groups and nine quaternary carbons. The presence of a signal of a carbonyl group at δ 179.4 (C-28) suggested the compound as ursolic acid (Seebacher et al., 2003). Additionally, another carbonyl group is observed δ 211.7 (C-2). The HSQC spectrum was used to assign protons directly attached to carbon atoms shown in Table 1.

The Heteronuclear Multiple Bond Correlation (HMBC) between δH 0.69 (H-25) and δc 84.1 (C-1) placed the hydroxy group in position one. Similar correlations of the protons of the methyl groups in positions 23 and 24 with δc 80.6 (C-3) placed the other hydroxyl group at position 3. The third hydroxyl group was assigned position 19 through HMBC of δH 2.38 (H-18) and δH 1.09 (H-29) with C-19. There were also HMBC between two oxymethine protons δH 4.08 (H-1) and δH 4.00 (H-3) and the carbonyl carbon C-2 placing it at position 2. The HMBC spectrum also showed correlations of δH 2.38 (H-18) with C-12 and C-13 confirming the position of the double bond. These HMBC confirmed that compound 1 is 1,3,19-Trihydroxy-2-oxo-12-ursen-28-oic acid.

Compound 2 was obtained as yellowish powder. Its molecular formular of $C_{15}H_{14}O_5$ was determined from its HRESIMS m/z 275.075 [M + H] (Figure 1). In the proton NMR spectrum, the aromatic signals at δ 6.50 (H, d, 2.17 Hz), δ 6.71 (1H, d, 2.17 Hz), δ 7.40 (1H, d, 1.90 Hz) and δ 7.49 (1H, d, 8.11 Hz) is indicative of a 1',3',4',5' tetra-substitution in ring B. Correlation spectroscopy (COSY) showed that the two oxygenated methine protons δ 4.63 (H-3) and δ 5.28 (H-2) are adjacent to each other with a vicinal coupling constant of 7.52 Hz indicating they are *trans* to each other. The HMBC of δ 5.28 (H-2) with carbon atoms in ring B place it position 2 and also confirmed the positions 2' and 6'. The analysis of the NMR data suggested compound 2 to 3,3',4',5'-Tetrahydroxflavan. This was the first reported isolation of this compound.

Compound 3 was also obtained as a yellowish powder and its molecular formular of $C_{15}H_{14}O_6$ was determined from HREIMS at m/z 291.0824 [M+H] (Figure 1). The aromatic methine proton signals at δ 5.69 (1H, d, 2.13 Hz) and δ 5.89 (1H, d, 2.13 Hz) suggest *meta* coupled

Table 1. ^{1}H NMR (700 MHz) and ^{13}C NMR (175 MHz) spectral data of compounds **1** – **3.**

S/N	1 (DMSO)		2 (DMSO)		3 (DMSO)	
	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)
1	84.1, CH	4.08	-	-	-	-
2	211.7, Cq	-	82.3, CH	5.28	81.5, CH	4.47
3	80.6, CH	4.00	67.6, CH	4.63	66.8, CH	3.81
4	45.2, Cq	-	29.2, CH_2	3.16, 4.47	28.6, CH_2	2.35, 2.65
5	51.0, CH	1.44	100.3, CH	7.53	156.6, Cq	-
6	18.3, CH_2	1.44, 1.62	119.7, CH	7.40	95.6, CH	5.89
7	33.0, CH_2	1.28	95.1, CH	6.50	156.9, Cq	-
8	41.2, Cq	-	96.4, CH	6.71	94.3, CH	5.69
9	47.4, CH	2.12	156.6, Cq	-	155.8, Cq	-
10	48.7, Cq	-	115.0, Cq	-	99.5, Cq	-
11	26.9, CH_2	1.95				
12	128.3, CH	5.16				
13	138.0, Cq	-				
14	41.6, Cq	-				
15	28.7, CH_2	1.69				
16	25.7, CH_2	1.40				
17	47.4, Cq	-				
18	53.6, CH	2.38				
19	72.1, Cq	-				
20	41.9, CH	1.26				
21	26.4, CH_2	1.61				
22	37.7, CH_2	1.49, 1.60				
23	17.0, CH_3	0.58				
24	29.2, CH_3	1.08				
25	12.3, CH_3	0.69				
26	16.9, CH_3	0.70				
27	24.4, CH_3	1.36				
28	179.4, Cq	-				
29	26.8, CH_3	1.09				
30	16.8, CH_3	0.85				
1’			131.8, Cq	-	131.0, Cq	-
2’			119.7, CH	-	115.0, Cq	6.72
3’			157.8, Cq	-	148.1, Cq	-
4’			146.2, Cq	-	145.3, Cq	-
5’			157.5, Cq	-	115.5, CH	6.68
6’			116.5, CH	7.49	118.9, CH	6.59

Source: Authors

pattern due to tetra-substitution in ring A. The other aromatic proton signals of δ 6.59 (1H, dd, 1.75, 8.15 Hz), δ 6.68 (1H, d, 8.15 Hz), and δ 6.75 (1H, d, 1.75 Hz) show both meta and para coupling patterns suggesting trisubstitution in ring B. The COSY and HMBC of the oxymethine protons δ 3.81 (H-3) and δ 4.47 (H-2) showed a similar pattern as in compound 2. Just like in compound 2, the vicinal coupling constant of 7.58 Hz between H-2 and H-3 indicates they are *trans* to each other. Therefore, compound 3 was deduced as 3,3’4’,5,7-Pentahydroxyflavan.

Ursane triterpenoids related to 1 have been reported to show antibacterial activity against human pathogenic bacteria (Jian-Jun et al., 2008) but to the best of our knowledge this is the first report on bean pathogens. It is also known that the introduction of functionalities into ring A like 1 improves biological activities (Li-Rong et al., 2016). Flavans like 2 and 3 have been reported to show antibacterial activities against a wide range of bacterial pathogens (Babe et al., 2018). The antibacterial activities of compounds 1 to 3 were tested against *X. axonopodis* pv. *phaseoli* and *P. savastanoi* pv. *phaseolicola*. The

Table 2. Inhibition zones (mm) and minimum inhibitory concentrations of compounds **1** – **3** and ethyl acetate crude extract.

Compound	Inhibition zone (mm)		Minimum Inhibitory Concentration (mg/mL)	
	X. axonopodis	***P. phaseolicola***	***X. axonopodis***	***P. phaseolicola***
1	16.33 ± 3.06	16.33 ± 1.15	5.00	1.25
2	6.33 ± 0.58	6	6	6
3	7.33 ± 0.58	6	6	6
*ETAOC extract	11.00 ± 1.00	10.67 ± 0.58	1.25	1.25
Chloramphenicol	20.00	40.00	-	-

*ETAOC = Ethyl acetate.
Source: Authors

results (Table 2) indicated that compound 1 was the most active with an inhibition diameter of 16.33 ± 3.06 mm for *X. axonopodis* pv. *phaseoli* compared to 20 mm for the reference standard chloramphenicol. Similar results were observed for *P. savastanoi* pv. *phaseolicola*. This compound had a minimum inhibition concentration of 5.0 and 1.25 mg/mL for *X. axonopodis* pv. *phaseoli* and *P. savastanoi* pv. *phaseolicola,* respectively. Compounds 2 and 3 showed modest activity.

Conclusion

This study demonstrated that the compounds isolated from *H. abyssinica* have activity against the bean pathogens *X. axonopodis* pv*. phaseoli* and *P. savastanoi* pv*. phaseolicola*. The results indicated that these compounds can be used as leads in the development of biopesticides to control bean diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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