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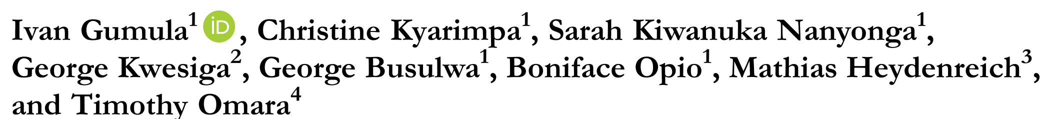
Antibacterial Properties of Phytochemicals Isolated from Leaves of *Alstonia boonei* and Aerial Parts of *Ipomoea cairica*



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Abstract

Objective: The leaves of *Alstonia boonei* and aerial parts of *Ipomoea cairica* are used for treatment of microbial infections among other ailments in African traditional medicine. The aim of this study was to investigate the antimicrobial phytochemicals in *A. boonei* leaves and *Ipomoea cairica* aerial parts to validate their traditional use in Ugandan herbal medicine. **Methods:** The plant materials were separately extracted using a dichloromethane/methanol (1:1) solvent system and subjected to repeated chromatographic separation to isolate pure compounds. The chemical structures of the isolated compounds were determined through ^{1}H NMR, ^{13}C NMR and 2D NMR (COSY, HSQC and HMBC). The antibacterial activity of the extracts and pure compounds were assessed using the agar well diffusion method. **Results:** Chromatographic fractionation of the extracts yielded *trans*-fagaramide and a pentacyclic lupane-type triterpenoid, lupeol, from *A. boonei*, and friedelin from *I. cairica*. Trans-fagaramide was identified for the first time in the *Alstonia* genus while friedelin was identified for the first time in *I. cairica*. The isolated compounds demonstrated antibacterial activity, with *trans*-fagaramide showing a minimum inhibitory concentration (MIC) of 125 μg/mL against *Pseudomonas aeruginosa* and 250 μg/mL against *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. Friedelin exhibited a MIC of 125 μg/mL against *Escherichia coli* and 250 μg/mL against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. **Conclusion:** The antibacterial activities observed in this study support the traditional use of *A. boonei* and *I. cairica* by indigenous communities in Uganda for treating microbial infections.

Keywords

Alstonia boonei, antibacterial, friedelin, *Ipomoea cairica*, lupeol, trans-fagaramide

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Introduction

Despite the significant progress made in the treatment of emerging and known microbial infections, pathogenic diseases have remained among the major causes of morbidity and mortality. For example, it accounted for at least 13.5% (13.7 million) deaths reported globally in 2019.[1] The underlying causes of this disease burden is partly due to continuous development of resistance to the current-use conventional drugs. In addition, infringement on the pristine environment and domestication of wildlife have spiraled human–animal interactions, opening new windows for transmission of zoonoses and unique pathogens to humans.[2] A case in point is the COVID-19 pandemic that has, by and large, been considered the worst infectious disease witnessed in the twenty-first century.[3]

The burden of pathogenic diseases is disproportionately high in developing countries and this has prompted communities to embrace traditional medicine for healthcare.[4–6] In Africa, traditional medicine has long been a cornerstone of healthcare for communities where plant-based remedies are frequently used to treat a wide range of ailments, including microbial infections. The preference for medicinal plants over conventional

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medicine is largely driven by cultural perspicacity and beliefs, both of which varies across countries.[7–9] In East Africa, the use of herbs for primary healthcare has been widely documented.[10] In Uganda, the use of herbal remedies is an integral part of the healthcare systems.[11,12] This study focused on two species (*Alstonia boonei* and *Ipomoea cairica*) used in the treatment of microbial infections in Uganda.

Alstonia boonei De Wild. (Apocynaceae) is a deciduous tree locally known as *mubajangalabi* (Luganda), *nsiwa* (Lusoga) and *mujwa* (Runyoro) in Uganda.[13] It is used in the treatment of malaria and microbial infections.[14] In other parts of Africa (where it is called pattern wood or stoolwood), this species is used in the treatment of (cerebral) malaria, snake envenomation, insomnia, arrow poison, hypertension, microbial infections, cancer, painful micturition, diarrhoea and inflammations.[15–17] In some African countries, *A. boonei* is a sacred tree that is cherished and therefore not usually eaten.[18] The stem bark, leaves and roots of this species has antiplasmodial,[19–23] analgesic, anti-inflammatory,[24] antipyretic,[25] antimicrobial,[26] antidiarrheal,[27] anticancer,[28] antioxidant, anti-ulcer,[29] hepatoprotective and neuroprotective activities.[16,30] Plants are known to produce a vast array of secondary metabolites that serve protective roles against pathogens and environmental stressors. These bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolics, are often responsible for the antimicrobial properties observed in medicinal plants. Although the antimicrobial and antidiarrheal activities of *A. boonei* has been studied,[26,27] complete characterization of the compounds associated with its bioactivities is far from accomplished. Marini-Bettolo et al[31] first isolated boonein, a monoterpenoid δ-lactone from the bark of *A. boonei*. Omitola[27] reported the presence of boonein and β-amyrin in the leaf extracts while Olanlokun et al found an antimalarial compound: tetrahydro-4-((E)-7-hydroxy-10-methoxy-6,14-dimethyl-15-m-tolylpentadec-13-enyl) pyran-2-one in the stem bark extract of this species.[32] Kiganda[33] found that *A. boonei* stem and root barks had cycloeucalenol, lupeol, phenanthridine-6(5H)-one, lupeol acetate, stigmasterol, lichexanthone, β-sitosterol and echitamine. Acylated anthocyanins and flavonoids have also been characterized in this species.[34] Recently, methanolic stem bark extract of *A. boonei* was found to contain loganic acid, along with two new compounds: secoxyloganin and sweroside.[35]

On the other hand, *Ipomoea cairica* (L.) Sweet is an invasive vine in family Convolvulaceae.[36] It is a twining perennial herb that persists for more than two years. With showy white to lavender-colored flowers (hence the name five-fingered morning glory), *Ipomoea cairica* (*I. cairica* hereafter) is native to tropical Africa and Asia.[34] In Uganda, it is known as *akarandarugo*, and its leaves and stems are used in the treatment of microbial infections,[37] cervical cancer, uterine diseases and colic pain.[38] In Kenya, powdered leaves and roots are applied topically for breast, cervical and skin cancer treatment.[39] In Brazilian traditional medicine, *I. cairica* is used in the treatment of rheumatism and inflammations.[40] To date, the antinociceptive,[40] larvicidal,[41–43] anticancer[44] and antidiabetic[45] activities of *I. cairica* extracts and some coumarins (scopoletin, umbelliferone), lignans (arctigenin, matairesinol, (+)-pinoresinol and trachelogenin) and acylated anthocyanins in it has been reported.[34] This species is also rich in pentasaccharide resin glycosides (cairicosides A-F), mainly in its aerial parts.[44–46]

Research into the chemical composition and biological activities of compounds from *A. boonei* and *I. cairica* are still inadequate to understand the compounds associated with their uses claimed in traditional medicine. Such studies are crucial for understanding the pharmacological basis of their traditional uses and for potentially developing novel plant-derived antimicrobial agents that could be used to counter the increasing antimicrobial resistance crisis.[26] In continuity of our search for bioactive natural products with antimicrobial activities in Uganda,[47–50] the objective of the present study was to isolate and characterize the phytochemicals in *A. boonei* leaves and *I. cairica* aerial parts and to assess their antibacterial activities. By identifying and testing these compounds against common bacterial pathogens, this study aimed to provide scientific validation for the traditional use of these plants in treating microbial infections and to contribute to the growing body of knowledge on plant-based antibacterial agents.

Materials and Methods

Plant Organs and Extraction of Phytochemicals

Leaves of *A. boonei* and aerial parts of *I. cairica* were sampled from Nakawuka village, Wakiso District and Nakawa Division, Kampala District, Uganda, respectively. Both samples were identified and authenticated by a taxonomist at Makerere University Herbarium where voucher samples (IG005 and IG006) were deposited. The samples were dried under shade for 3 weeks.

The pulverized samples (1 kg of *A. boonei* leaf powder and 620 g of *I. cairica* aerial parts) were exhaustively extracted by cold percolation at room temperature using a mixture of dichloromethane and methanol (DCM/MeOH, 1:1, v/v) for 48 h, with occasional stirring. The choice of this solvent system is because DCM and MeOH have different polarities and their mixture is able to solubilize both non-polar and polar compounds. Thus, we targeted this solvent system because it would extract a wide variety of phytochemicals, including alkaloids, flavonoids, terpenoids, and steroids. The extracts obtained were then filtered, and the filtrates were concentrated by rotary evaporation at 40 °C. The crude extracts obtained were stored at 4 °C until further analysis.

Isolation and Spectroscopic Analysis of Compounds

Chromatographic separation was done using 15 g and 100% of *n*-hexane (to remove fats and oils for two days) and then serially with solvent systems of increasing polarity at 5% increments upto 100% ethyl acetate and methanol. The fractions obtained were concentrated on a rotary evaporator and spotted on analytical TLC plates (TLC sheets ALUGRAM® Xtra SIL G/UV254, Macherey-Nagel GmbH & Co. KG, Germany).

Fractions with similar TLC profiles were combined.[51] For each eluent system, two litre solvent volumes were used and 250 ml fractions were collected. The fractions were concentrated and repeatedly fractionated using DCM/MeOH (1:1) as the eluent system to obtain the pure compounds.[52]

The structure of each compound isolated was established from NMR data obtained on a Bruker AV-500 spectrometer (MC-Murry) ie, 1D (^{1}H and ^{13}C) and 2D (H-H COSY, HSQC and HMBC) spectra of the samples dissolved in deuterated chloroform. The spectra in FID format were processed using MestReNova (version 8.1.1). NMR results with residual chloroform peaks were used as the references. The results were compared with published spectroscopic data.

Antibacterial Activity Assessment

The antibacterial assay followed the agar well diffusion method. Briefly, the bacterial strains were American Type Culture Collection (ATCC) of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella typhi* (*S. typhi*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). The choice of the bacteria was informed by the bacterial diseases for which the plants are traditionally used to treat, the WHO priority list of bacteria as well as the availability of the test strains.[49] The bacteria were all obtained from Department of Biological Sciences, Kyambogo University, Kampala, Uganda where the assays were performed.

The bacterial cultures were maintained on nutrient agar slants for a day after incubation at 37 °C. They were stored at 4 °C as stock cultures for antibacterial assays. Using sterile 6-mm glass cork borers, wells were carefully made on the agar plates without distorting the media. Tetracycline (0.5 mg/mL) and dimethyl sulfoxide (DMSO) were used as the positive and negative controls, respectively. In the bioassays, exactly 50 μL of the prepared extracts (0.5 mg/mL in DMSO) and the controls were pipetted into the wells, left on the bench for 60 min and thereafter incubated at 37 °C for 24 h. The antibacterial activities were determined by measuring the inhibition zone diameter (ZOI).

Utilizing the broth-dilution method, bacteria with the highest sensitivity to the extracts and isolated compounds (**1** and **3**) were used for minimum inhibitory concentration (MIC).[49] Compound **2** was obtained in very little quantities, and was therefore, not tested. The test sample was serially diluted in sterile Mueller Hinton broth by a factor of 2 to 4 in a volume of 300 mg/mL for crude extract and 100 μg/mL for compounds **1** and **3**. In sequence of increasing dilution, the test organism was aseptically added to each of the tubes containing the sample. The dilutions were in series of 500, 250, 125, 62.5, 31.25, 15.265, 7.8125, 3.90625 mg/mL for the extracts and the corresponding values in μg/mL for the compounds. The tubes were then incubated for 24 h at 37 °C and turbidity was used to look for growths.

Results

Isolated Compounds from A. boonei *Leaf Extracts*

Two compounds: *trans*-fagaramide (**1**) and lupeol (**2**) were isolated from extract of *A. boonei* leaves (Figure 1). Compound **1** was obtained as a white powder (29.1 mg), soluble in DCM and its NMR spectral data and spectra are given in Table S1 and Figures S1-S7. The ^{13}C NMR showed 13 peaks, including that of an amide carbonyl (δ 166.3). The HSQC revealed the presence of a methylenedioxy group with a cross-peak coordinate at δ_H 5.69 (2H), (δ_C 101.4). From ^{1}H NMR and ^{1}H-^{1}H COSY, the presence of a trans-double bond was deduced (δ_H 7.54 (1H, d, $J = 15.6$ Hz); δ_H 6.32 (1H, d, $J = 15.6$ Hz)). Furthermore, the HSQC spectra revealed a cross peak at δ_H 7.54, δ_C 140.1, implying that the double bond was adjacent to an electron-withdrawing atom. From ^{1}H NMR and ^{1}H-^{1}H COSY spectra, it was also deduced that 3 aromatic protons were present (δ_H 6.98 (brs, 1H); δ_H 6.94 (d, 1H, $J = 8.0$) and δ_H 6.76 (dd, 1H, $J = 8.0$, 1.7 Hz), two of which were *ortho* to each other. The ^{1}H NMR and ^{1}H-^{1}H COSY spectra also revealed the presence of an isobutyl moiety [δ_H 3.21 (2 Hz, multiplet H–11, H–13/14); δ_H 1.85 (1H, a septet of doublets, $J =$

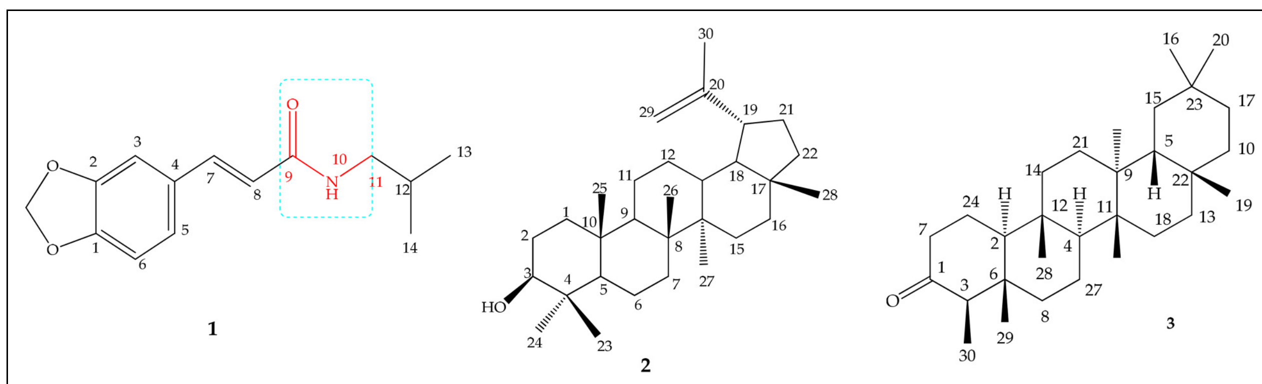

Figure 1. Structure of bioactive compounds characterized in dichloromethane/methanol extracts of *A. boonei* leaves (**1** and **2**) and *I. cairica* aerial parts (**3**). In the structure of trans-fagaramide (**1**), the secondary amide linker pharmacophore believed to contribute to its antimicrobial activity is highlighted.

6.5. 1.7 Hz) and δ_H 0.95 (6H, d d, J = 6.7, 1.7 Hz). In addition, the HMBC indicated a correlation between the methylene proton at δ_H 3.21 H–11a and a carbonyl carbon at δ_C 166.3 (C–9) thus suggesting a connection with the amide group. The linkage between the *trans*-double bond with aromatic ring system at position 4 was deduced from the HMBC interaction between a proton at δ7.54 (H–7) with aromatic carbons at δc 106.4 (C–3) and δc 123.7 (C–5).

Compound **2,** obtained as a brown solid, was eluted with ethyl acetate/hexane (3:7) from the column. The ^{1}H NMR spectrum of **1** (Table S2**;** Figure S8) revealed the presence of 7 methyl protons at δ 0.76, 0.79, 0.83, 0.94, 0.97, 1.03 and 1.68 (integrated for 3H-each). A sextet of one proton at δ 2.38 ascribable to H-19, is characteristic of lupeol.[53,54] The H-3 proton showed a multiplet at δ 3.2 while a pair of broad singlets at δ 4.57 and δ 4.69 (1H, each) was indicative of olefinic protons at H-29 a & b.[55,56] The structure was further substantiated by the ^{13}C NMR experiments which showed seven methyl groups at δ 28.1 (C-23), δ 18.1 (C-28), δ 16.2 (C-25), δ 16.0 (C-26), δ 15.4 (C- 24), δ 14.6 (C-27) and δ 19.4 (C-30); there is an exomethylene group at δ 109.5 (C-29) and δ 150.6 (C-20). A signal at δ_C 79.1 was attributed to C-3 with a hydroxyl group attached to it (Table S2**;** Figure S9).

The confirmation of the structure was accomplished through 2D NMR experiments (COSY, HSQC and HMBC) (Figures S10–S12). The H-H COSY spectrum of the compound exhibited some cross peaks such as between δ 2.38, H-19 and one methylene proton δ1.33, H-21 and between methine proton δ3.18, H-3 and methylene s (δ 1.57, H-2). In the HMBC spectrum, the methine proton at δ3.18 (H-3) showed cross peaks with a methyl carbon δ28.1, C-23) by J_2 correlation and a methyl carbon δ 18.4, C-6) by J_3 correlation. The methine proton at δ 2.38 (H-19) showed cross peaks with two methylene carbon δ29.9 (C-21) and δ109.5 (C-29), a methine carbon 48.4 (C-18), a methyl carbon δ 19.4 (C-30)] and a quaternary carbon δ 150.6 (C-20). The pair of broad singlets of proton at δ 4.57 and 4.69 showed cross peaks with a methylene carbon δ 48.1(C-19), δ150.6 (C-20) and δ 109.5 (C-29)] by J_3 correlation.

Isolated Compound from I. cairica Aerial Part Extract

One compound (**3**) weighing 58.4 mg was isolated from the extract of *I. cairica* aerial parts on re-chromatography (Figure 1). It was obtained as a white amorphous solid with 1% ethyl acetate in *n*-hexane. The ^{13}C NMR displayed 30 significant peaks implying the compound had 30 non-equivalent carbon atoms (Table S3; Figure S13). The spectrum had the following peaks: carbon at δ 167.7 (C-1) assigned to carbonyl carbon, carbons at δ 59.6 (C-2), δ 58.4 (C-3), δ 53.2 (C-4) and δ 42.9 (C-5) assigned to CH, carbons at δ 42.3 (C-6), δ 39.8 (C-9), δ 38.4 (C-11), δ 37.6 (C-12), δ 22.7 (C-22) and δ 22.4 (C-23) assigned to quaternary carbons, carbons at δ 41.7 (C-7), δ 41.4 (C-8), δ 39.1 (C-10), δ37.3 (C-13), δ 35.8 (C-14), δ 35.5 (C-15), δ 32.2 (C-17), δ 31.9 (C-18), δ 22.9 (C-21), δ 22.3 (C-24) and δ 18.1 (C-27) assigned to CH_2 carbons, carbons at δ 35.2 (C-16), δ 29.8 (C-19), δ 23.7 (C-20), δ 20.4 (C-25), δ 18.8 (C-26), δ 14.8 (C-28), δ 14.3 (C-29) and δ 6.9 (C-30) assigned to CH_3 carbons.

The ^{1}H NMR spectrum of **3** (Figure S14) displayed the following peaks; multiplet at δ 1.53 (3H, H-2, H-5, H^a-10), δ 2.27 (1H, H-3), δ 1.40 (1H, H-4), δ 2.38 (1H, H^a-7), δ 2.31(1H, H^b-7), δ 1.28(3H, H^a-8, H^a-14, H^a-17), δ 1.76 (1H, H^b-8), δ 0.93 (1H, H^b-10), δ 1.38 (1H, H^a-13), δ 1.57 (1H, H^b-13), δ 1.46 (1H, H^b-14), δ 1.39 (1H, H^a-15), δ 1.20 (1H, H^b-15), δ 1.43 (1H, H^b-17), δ 1.31 (1H, H^a-18), δ 1.50 (1H, H^b-18,), δ 1.38 (2H, H-21), δ 1.95 (1H, H^a-24), δ 1.68 (1H, H^b-24), δ 1.46 (1H, H^a-27) and δ 1.36 (1H, H^b-27), singlets at δ 1.00 (3H, H-25), δ 1.18 (3H, H-19), δ 0.99 (3H, H-16), δ 1.05 (3H, H-26), δ 0.72 (3H,H-28) and δ 0.91 (3H, H-29) and a doublet at δ 0.88 (3H, J = 6.8, H-30). Protons attached to particular carbons was established using HSQC spectrum (Figure S15) which revealed the that carbons; C-1, C-6, C-9, C-11, C-12, C-22 and C-23 had no protons, C-2 was bonded to H at δ 1.53, C-3 bonded to H at δ 2.27, C-4 bonded to H at δ 1.40, C-5 bonded to H at δ 1.53, C-7 bonded to H at δ 2.38 and 2.31, C-8 bonded to H at δ 1.28 and 1.76, C-10 bonded to H at δ 1.53 and 0.93, C-13 bonded to H at δ 1.38 and 1.57, C-14 bonded to H at δ 1.28 and 1.46, C-15 bonded to H at δ 1.39 and 1.20, C-16 bonded to H at δ 0.99, C-17 bonded to H at δ 1.28 and 1.43, C-18 bonded to H at δ 1.31 and 1.50, C-19 bonded to H at δ 1.18, C-20 bonded to H at δ 1.50, C-21 bonded to H at δ 1.38, C-24 bonded to H at δ 1.95 and 1.68, C-25 bonded to H at δ 1.00, C-26 bonded to H at δ 1.05, C-27 bonded to H at δ 1.46 and 1.36, C-28 bonded to H at δ 0.72, C-29 bonded to H at δ 0.91 and C-30 bonded to H at δ 0.88. Further connectivity was established using H-H COSY, HSQC and HMBC spectra (Figures S15-S17). H-H COSY revealed the coupling of the following protons; H-2 and H-24, H-3 and H-30, H-4 and H-27, H-7 and H-24, H-8 and H-27, H-10 and H-17, H-13 and H-18, H-14 and H-21, H-15 and H-5. This implied that C-24 is bonded to C-2 and C-7, C-3 is bonded to C-30, C-27 is bonded to C- 8 and C-4, C-10 is bonded to C-17, C-13 is bonded to C- 18, C-14 is bonded to C-21, C-5 is bonded to C-15. From HMBC; C-1 coupled with H-7 and H-3, C-2 coupled with H-3, H-4, H-7, H-8 and H-24, C-3 coupled with H-7, H-8 and H-29, C-4 coupled with H-2, H-8, H-14, H-25 and H-28, C-5 coupled with H-10, H-13, H-19 and H-26, C-6 coupled with H-2, H-3, H-24, H-27 and H-29, C-7 coupled with H-2 and H-3, C-8 coupled with H-4 and H-29, C-9 coupled with H-4, H-5, H-21, H-25, and H-26, C-10 coupled with H-5 and H-19, C-11 coupled with H-4,H-13, H-18, H-25 and H-26, C-12 coupled with H-2, H-4, H-14, H-21 and H-28, C-13 coupled with H-5, H-10 and H-19, C-14 coupled with H-2, H-4 and H-28, C-15 coupled with H-16, H-17 and H-20, C-16 coupled with H-15, H-17 and H-20, C-17 coupled with H-15, H-16 and H-20, C-18 coupled with H-4 and H-25, C-19 coupled with H-5, H-10 and H-13, C-20 coupled with H-15, H-16 and H-17, C-21 coupled with H-5 and H-26, C-22 coupled with H-5, H-10, H-13, H-17, H-18 and H-19, C-23 coupled with H-5, H-10, H-15, H-16, H-17 and H-20, C-24 coupled with H-7, C-25 coupled with H-4 and H-18, C-26

coupled with H-5 and H-21, C-27 coupled with H-4, C-28 coupled with H-14, H-2, H-4 and H-24, C-29 coupled with H-2, H-3 and H-8 and C-30 coupled with H-3 (Table S3). These NMR data indicated that compound **3** has 50 protons, giving its molecular formula as $C_{30}H_{50}O$.

Antibacterial Activity of the Crude Extracts and Isolated Compounds

The antimicrobial activity of crude extracts and isolated compounds (**1** and **3**) were investigated. In the screening assay, extracts of *A. boonei* leaves had mean ZOI of 9.0 ± 1.0, 20.0 ± 2.5 and 10.0 ± 0.7 mm against *P. aeruginosa*, *E. coli* and *S. aureus*. The extract had no inhibitory activity against *S. typhi*. Similarly, DMSO had no inhibitory effects against the tested bacterial strains. Tetracycline (positive control) had average inhibition diameters of 50.0 ± 0.0, 50.0 ± 0.2, 50.0 ± 0.0 and 60.0 ± 0.9 mm, respectively. Extracts of aerial parts of *I. cairica* had mean ZOI of 23.0 ± 1.0, 26.0 ± 2.0, 13.0 ± 0.5 and 20.0 ± 0.8 mm against *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi*, respectively.

Upon testing for MIC, compounds **1** and **3** exhibited antibacterial activity with the most susceptible bacteria being *P. aeruginosa* and *E. coli*. The MIC were 125 μg/mL against *P. aeruginosa* and 250 μg/mL against *S. aureus*, *S. typhi* and *E. coli* for **1**, 125 μg/mL against *E. coli* and 250 μg/mL against the other bacteria for **3** (Table 1).

Discussion

Chromatographic fractionation of the extracts followed by detailed spectroscopic analysis in this study yielded *trans*-fagaramide (**1**) and a pentacyclic (lupane-type) triterpenoid–lupeol (**2**) from *A. boonei*, and friedelin (**3**) from *I. cairica*. Compound **1** was identified by complete analysis of its HMBC and comparison of its spectral data with published literature.[50,54,55] Finally, the partial structures were joined to give the final structure as (2E)-3-(2H-1,3-benzodioxol-5-yl)-N-(2-methylpropyl)prop-2-enamide, a lignan also known as *trans*-fagaramide (Figure 1). This lignan has been previously reported in plants such as *Zanthoxylum leprieurii*,[50] *Zanthoxylum zanthoxyloides*[55] and *Fagara heitzii*.[56] It is the first time *trans*-fagaramide is being reported in this species and genus.

Similarly, the analysis and comparison of spectral data of **2** with preceding reports[48,50,57] led to its identification as lupeol, a well-known pentacyclic triterpenoid. The lupane series triterpenoid (**2**) and its ester (lupeol acetate) has been previously characterized with other functional triterpenoids (α-amyrin, β-amyrin, cycloeucalenol, friedelan-3-one and ursolic acid) from *A. boonei* stem and root barks.[28,33,58,59] It is also widely reported in species in genera such as *Albizia*,[48,60] *Bryophyllum*,[61] *Combretum*[62] and *Zanthoxylum*.[50] Through comparison of the spectral data of **3** with published literature,[63–65] it was deduced to be friedelin. In *I. cairica*, only pentasaccharide resin glycosides (cairicosides A-F), β-sitosterol 3-O-glycopyranoside and tracheloside have been previously isolated from the aerial parts,[44–46,66] and friedelin (**3**) is hereby identified for the first time in this species. Friedelin was initially isolated from the root extracts of *Ipomoea* species (*I. batatas*).[64] Nevertheless, it is a common pentacyclic triterpenoid found in several plants such as *Azima tetracantha*,[67] *Diospyros glandulosa*,[63] *Maytenus forsskaoliana*,[65] *Polygonum bistorta*,[68] and *Quercus stenophylla*.[69]

The antibacterial activity of extracts and compounds **1** and **3** were investigated. Extracts of *A. boonei* leaves had inhibitory effects on the growth of *P. aeruginosa*, *E. coli* and *S. aureus* but no activity against *S. typhi*. The DCM/MeOH extracts obtained from aerial parts of *I. cairica* also inhibited the growth of *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi*, respectively. These results suggested that these plant extracts elicit antibacterial effect against pathogenic bacteria, corroborating a previous report on *A. boonei*.[26] In addition, the inhibition zones recorded in this study for *A. boonei* leaf extracts were higher than 5-10 mm previously reported for the ethanolic and methanolic root extracts of this species from Ghana.[26] Organic (hexanic, chloroform and ethyl acetate) fractions of methanolic extract of *A. boonei* leaves were found inhibit a panel of pathogenic *S. typhi*, *Shigella dysentriae*, *Enterococcus faecalis*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *S. aureus*, *Proteus mirabilis* with MIC ranging from 1.33 to 25.0 mg/mL.[70] A previous report on the antibacterial activity of methanolic extracts of *I. cairica* leaves and flowers found that they had inhibitory activity against *E. coli* (ZOI = 22 mm and 11 mm), *Klebsiella pneumonia* (ZOI = 11 mm and 10 mm), *S. typhi* (ZOI = 13 mm and 11 mm), *Bacillus subtilis* (ZOI = 10 mm and 15 mm), *S. aureus* (ZOI = 8 mm and 13 mm) which are lower than the inhibition diameters we found in the current study.[71]

Assessment of MIC of **1** and **3** suggested that they had antibacterial activity against the tested panel of bacteria. The MIC values span from 125 μg/mL to 250 μg/mL (Table 1). In comparison with previous studies, *trans*-fagaramide (**1**) has been previously demonstrated to have antimicrobial activity against *Mycobacterium tuberculosis* (H37Rv strain, MIC = 6 μg/mL and multidrug resistant strain, MIC = 12.2 μg/mL).[50] Trans-fagaramide is an amide alkaloid with various biological and chemical properties beyond its antimicrobial efficacy that could contribute to its overall therapeutic potential. Firstly, trans-fagaramide exhibit antioxidant properties which could protect tissues from oxidative stress and improve the host's defense mechanisms against microbial pathogens, thus enhancing its therapeutic efficacy against infections.[56] Secondly, it possesses a secondary amide linker in its structure (Figure 1). This linker

Table 1. Minimum Inhibitory Concentrations of the Extracts (mg/mL) and Compounds (μg/mL) from *A. boonei* and *I. cairica*.

	Extracts		Isolated compounds	
Bacteria	*A. boonei*	*I. cairica*	1	3
P. aeruginosa	125	250	125	250
S. typhi	250	125	250	250
E. coli	250	125	250	125
S. aureus	250	250	250	250

pharmacophore is present in the structures of rifampicin, amikacin, capreomycin, isoniazid and pyrazinamide which are drugs used in the treatment for tuberculosis, a microbial infection.[50,72]

Although lupeol (**2**) was not tested in this study, it has been isolated from plant extracts and shown to elicit antibacterial activity against *Shigella dysentriae, E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *Bacillus cereus*, *Shigella boydi*, *Enterobacter aerogenes*, *Listeria monocytogenes*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus lutea*, and *Vibrio mimicus*.[73–76] In addition to this, lupeol possess anti-inflammatory and immunomodulatory effects which can improve the healing process during infections and reduce tissue damage caused by inflammatory responses.[77] Like trans-fagaramide, lupeol has significant antioxidant activity which can protect cells from oxidative damage that occurs during infections.[78] Further, lupeol promotes wound healing by enhancing the deposition of collagen and promoting the proliferation of keratinocytes and fibroblasts.[79] This is mediated through activation of PI3k/Akt and p38 MAPK, suppression of NF-κB signaling and Keratin 16 and the cytoprotective effects of MMP-2 and Tie-2.[80] These effects are particularly relevant in infections where skin integrity is compromised. Lupeol is also known to possess antibiofilm activity which can make bacteria more susceptible to antibiotics and the immune system.[81]

On the other hand, friedelin (**3**) isolates from plants have also shown antibacterial activity against *B. subtilis*, *S. aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.[82–84] Friedelin is a pentacyclic triterpenoid just like lupeol, specifically a perhydropicene derivative with an oxo group at position 3 and methyl groups at positions 4, 4a, 6b, 8a, 11, 12b, and 14a. Its antimicrobial activity is thus influenced by its ability to exert anti-inflammatory, antioxidant, and immunomodulatory effects.[85]

Taken together, these reports suggests that the isolated compounds could be responsible for the claimed antibacterial effect of these compounds. Such inhibitory activities may also be related to the interaction of the compounds with the bacterial membrane proteins or lipids leading to bacterial membrane stabilization and disruption, inhibition of membrane-bound enzymes, modulation of immune response and lipid raft disruption. For example, trans-fagaramide being an amide compound with potential amphipathic properties may insert itself into the bacterial membrane. This can disrupt the membrane's lipid bilayer, increasing its permeability and leading to leakage of cellular contents. The disruption of membrane integrity can cause osmotic imbalance, leading to cell death.[86]

The observed differences in ZOI and MIC in the antimicrobial assays could be due to factors such as physicochemical properties of the compounds (solubility, molecular size, stability and lipophilicity), diffusion rate in the agar medium, microbial strain differences and compound interactions (in the case of extracts).[87] The higher MIC values obtained suggests that higher doses of the plant extracts are needed to effectively treat microbial infections using the plant parts.[49]

Conclusion

This study was undertaken to verify the traditional claims of using *A. boonei* leaves and *I. cairica* aerial parts in ethnomedicinal treatment of microbial infections in Uganda. Isolation and characterization of compounds from their DCM/MeOH extracts afforded antibacterial compounds (trans-fagaramide and lupeol from *A. boonei*, and friedelin from *I. cairica*). These results lend credence to the use of these species in herbal treatment of bacterial diseases in Uganda. *In vivo* studies using animal models to evaluate the efficacy and safety of the extracts or isolated compounds from *A. boonei* and *I. cairica* could provide stronger evidence of their pharmacological effects, dosage safety, and potential toxicity, complementing the *in vitro* antibacterial results. Further studies should consider examining the polyphenolic content and antioxidant potential of the extracts and isolated compounds.

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Data Availability

Data supporting the conclusions of this study are available within this article and its supplementary files.

Declaration of Conflicting Interests

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