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Isolation and characterization of compounds in ethanolic extract of *Albizia coriaria* (Welw ex. Oliver) leaves: a further evidence of its ethnomedicinal diversity

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Abstract

Background: *Albizia coriaria* Welw ex. Oliver (*A. coriaria*) is one of the treasured medicinal plants in Africa. In continuity of our study verifying the claim of using its leaves for managing bacterial diseases and oxidative stress-mediated complications in Ugandan traditional phytomedicine, we characterized its most active (ethanolic) extract using ultraviolet–visible (UV–Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, thin layer chromatography, column chromatography and gas chromatography–mass spectrometry.

Results: UV–Vis absorption peaks occurred between 338 and 664 nm, which indicated the presence of alkaloids, flavonoids and terpenoids. FTIR spectrum of the extract indicated the presence of O–H, C=O and aromatic–C=C which confirmed the presence of alcohols, carboxylic acids and aromatics in the extract. Four known bioactive triterpenoids: lupeol (**1**), lupenone (**2**), betulinic acid (**3**), betulin (**4**) along with an aromatic alcohol: benzyl alcohol (**5**) were tentatively identified in different fractions of *A. coriaria* ethanolic leaf extract. Compound **4** was identified for the first time in this species.

Conclusions: All the compounds identified in the fractions of the *A. coriaria* ethanolic leaf extract have reported pharmacological activities, including antioxidant and antibacterial activities. This supports the traditional use of *A. coriaria* leaves in the management of oxidative stress-mediated conditions and bacterial diseases in Uganda.

Keywords: *Albizia coriaria*, Triterpenoid, Pentacyclic triterpene, Lupeol

Background

The *Albizia* genus contains important species used in traditional phytomedicine across Africa and Asia. *Albizia coriaria* (Welw ex. Oliver), a member of this genus, is one of the treasured medicinal plants in Africa. This fact is attested to by its name being available in various African languages as well as its high frequency of citation in ethnobotanical surveys (Omara et al. 2021a). The whole plant, leaves, stem and root barks, roots, seeds and flowers are used in Uganda for treatment of snake envenomation, malignancies, cardiovascular diseases, allergy, nausea, diarrhoea, malaria, tuberculosis, typhoid, anaemia, venereal diseases, post-partum haemorrhage, menorrhagia, dermatological diseases, jaundice and erectile dysfunction (Anywar et al. 2020; Bunalema et al. 2014; Katuura et al. 2016; Kyazike 2021; Namukobe et al. 2011; 2021; Nanyunja 2003; Olila et al. 2007; Omara et al. 2020a, b; Orodho et al. 2011; Schultz et al. 2020a; Tugume et al. 2016).

Across other African communities, different parts of *Albizia coriaria* (*A. coriaria*) are utilized in phytotherapeutical management of malaria, helminthiasis,

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tuberculosis, diarrhoea, breast, skin and uterine cancers, menorrhagia, hypertension, dermatological conditions, threatened abortion, venereal diseases, sore eyes, lungworms/ascaris worms (in cattle, sheep and goats), gastrointestinal infections, mosquito repellent and as toothbrush (Araya 2007; Bossard 1993; Dharani et al. 2015; Geissler et al. 2002; ICRAF 1992; Jeruto et al. 2010; Johns et al. 1995; 1990; Leiderer 1982; Obakiro et al. 2020; Ochwang'i et al. 2014; Olala 2014; Omara 2020; Orwa et al. 2007; Shiracko et al. 2016; Sirama 2014; Tsabang et al. 2017).

The molluscicidal, antimicrobial, antitumor, antigiardial, antiplasmodial, anti-inflammatory and antioxidant activities of the stem bark, leaves, flowers and roots of *A. coriaria* have been previously reported (Akanga 2008; Byamukama et al. 2015; India 2015; Johns et al. 1995; Kama-Kama et al. 2016; Luvonga 2007; Mengesha et al. 1997; Muthaura et al. 2015; Nalubega et al. 2011; Note et al. 2009; Olila et al. 2007; Omara et al. 2021b, 2022; Schultz et al. 2020b, 2021a). Despite its traditionally reported therapeutic potential, there are limited studies undertaken on the phytochemicals responsible for the various pharmacological activities of *A. coriaria* (Obakiro et al. 2020; Omara et al. 2020b; Schultz et al. 2021b). Phytochemical screening of aqueous and organic extracts of *A. coriaria* stem bark, flowers and leaves has indicated that alkaloids, flavonoids, steroids, triterpenoids, saponins, sterols, coumarins and tannins are the major secondary metabolites in this species (Agroforestry Database 2009; Akanga 2008; India 2015; Langat 2013; Mengesha et al. 1997; Omara et al. 2021b, 2022; Wanyama et al. 2011). Pure compounds: coriariosides A–E, gummiferaoside C, lupeol, lupenone, benzyl alcohol, betulinic acid, (+)-catechin and acacic acid lactone have been isolated from *A. coriaria* root and stem bark extracts (Byamukama et al. 2015; Note et al. 2010; Note et al. 2009). A recent study (Omara et al. 2021b) indicated that the ethanolic extracts of *A. coriaria* leaves had the highest antioxidant activity and were the most active against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi.* The aim of this study was therefore to characterize the most active (ethanolic) extract of *A. coriaria* leaves drawn from Mbarara district of Uganda to establish the compounds responsible for its antioxidant and antibacterial bioactivities.

Methods

Collection, authentication and preparation of leaves samples

Leaves of *A. coriaria* were sampled from wild plants in Mbarara district (0.6164° S 30.6186° E), Uganda on 28th January 2021. An authenticated voucher specimen (No. 50996) has been deposited at the herbarium of Department of Botany, Makerere University, Kampala, Uganda.

The leaf samples were transported to Chemistry laboratory of Department of Chemistry and Biochemistry (Moi University, Kenya) where they were air-dried under shade at room temperature for 3 weeks. The dry leaves were then ground into fine powder using a NutriBullet® 600 Series electric grinder (Capbran Holdings, LLC Los Angeles, CA 90025, USA). The leaf powder (500 g) was weighed using a Mettler Toledo digital analytical balance (XS204 Delta Range, Switzerland) and successively macerated in 1000 ml of ethyl acetate and ethanol at room temperature. The extracts were filtered through cotton wool and subsequently Whatman No.1 filter paper. The extracts were concentrated to dryness on a Hahnvapour HS-2005S vacuum rotary evaporator (Hahnshin S&T Limited, Korea) at 40 °C under reduced pressure. The concentrated extracts were collected in pre-weighed and labelled sample vials. The dried extracts were transferred to a desiccator containing anhydrous sodium sulphate to remove traces of water in them. Further analyses used the ethanolic extract.

Ultraviolet–visible spectrometry scanning

The crude ethanolic extract was dissolved in a ratio of 1:10 (w/v) in ethanol. It was filtered through cotton wool and then Whatman No. 1 filter paper. The extract was centrifuged at 12,000 rpm for 10 min and again filtered through Whatman No.1 filter paper, further diluted in a ratio of 1:10 with ethanol (Donkor et al. 2019) and then scanned from 200 to 800 nm on Beckham Coulter DU 720 ultraviolet–visible (UV–Vis) spectrophotometer (Beckham Coulter Inc., USA) using ethanol as the blank.

Fourier transform infrared spectroscopy

Attenuated total reflection–Fourier transform infrared (ATR–FTIR) characterization of the dry crude extract was performed at room temperature using a Nicolet 6700 FTIR spectrophotometer (Thermo Scientific, USA). The spectral resolution was set at 4 cm^{-1} and the scanning was done from 500 to 4000 cm^{-1}. The analysis was repeated twice for spectrum confirmation.

Thin layer chromatography and column chromatography

Since thin layer chromatography (TLC) is fast and its elution patterns usually carry over to column chromatography elution, the extract was subjected to one-dimensional TLC to establish the best solvent system to be used for column elution (Chepkorir et al. 2018). The extract was dissolved in ethanol in a ratio of 1: 20 (w/v). A spot of the solution was carefully applied onto TLC plates (TLC sheets ALUGRAM® Xtra SIL G/UV_{254}, Macherey–Nagel GmbH & Co. KG, Germany) and left to dry. The plates

were developed in prepared solvent systems of varying ratios of hexane/ethyl acetate and ethyl acetate/ethanol to allow the compounds in the spot to move upwards by capillary attraction. The positions of the different compounds were observed under UV light at 254 nm and 365 nm. The starting mobile phase considered was 100% n-hexane (Byamukama et al. 2015), followed by ethyl acetate and then ethanol. Eleven different pairs of (hexane/ethyl acetate and ethyl acetate/ethanol) solvent systems (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) were tested as potential mobile phases and viewed under UV light. The solvent ratios giving the best separation of the compounds in the extract were used for column chromatography.

Using the optimized solvent ratios, the crude extract was subjected to silica gel (60–120 mesh, Griffchem™ Fine Chemicals) normal phase column chromatography (7.5 × 100 cm column). Silica gel (100 g) was mixed with hexane/ethyl acetate (9:1) solvent system to form a homogenous suspension (slurry) and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into the column. The sample to load on the column was prepared by mixing and grinding 5 g of the crude extract with 5 g of silica gel into fine green powder. The sample was then introduced into the column and eluted with the solvent system established through TLC profiling (Chepkorir et al. 2018).

The column was first eluted with hexane/ethyl acetate as the mobile phase and then after the ethyl acetate/ethanol (3:7) solvent system was run. For each eluent system, equal fractions were collected and fractions with similar TLC profiles were pooled together and concentrated to dryness by rotary evaporation.

Gas chromatography–mass spectrometry analysis

The fractions obtained were reconstituted in dichloromethane: methanol (1:1, v/v) mixture, filtered through 0.45 µm filters and transferred to 2 mL vials one at a time for gas chromatography–mass spectrometry (GC–MS) analysis.

A gas chromatograph interfaced with mass spectrometer triple-quad system (Agilent 8890A GC and Agilent 5977 GC/MSD, Agilent Technologies Inc., USA) with an Agilent 7693A automatic liquid sampler, a National Institute of Standards and Technology (NIST) library and an installed Mass Hunter Workstation software was used. An HP-5MS ultra inert column (30 m × 0.25 mm × 0.25 µm) was used with an electron ionization system of 70 eV. Helium (carrier) gas flow rate was 1 mL/minute, with a splitless injection volume of 1 µL. Injector temperature was 250 °C, while the ion source temperature was 280 °C. Oven temperature was programmed from 110 °C with an increase of 10 to 200 °C, and then 5 to 280 °C. The compounds was identified from gas chromatograms based on their elution times. The mass spectra of the compounds were matched with those of the NIST 11 spectral library and/or compared with published literature for possible identification. The molecular ion of the compounds were identified using nitrogen rule (Yetayih and Ravichandran 2020).

A search was performed in various electronic databases (Scopus, PubMed, Web of Science, Springer Link, Taylor & Francis Online, Wiley Online Library, Google Scholar and Science Direct) to retrieve pharmacological information on the reported antibacterial and antioxidant activities of the identified compounds to establish if they could be responsible for the therapeutic potential of *A. coriaria* leaves as claimed in Ugandan traditional medicine.

Results

Ultraviolet–visible spectrum of the extract

The phytochemicals in the crude extract were confirmed by spectroscopic scanning for characteristic peaks in the ultraviolet and visible light regions. The scan was to establish if compounds containing σ-bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings were present (Bashyam et al. 2015; Karpagasundari and Kulothungan 2014). The spectrum for the extract (Fig. 1) indicated that maximum absorptions were recorded at wavelengths of 338 nm, 414 nm, 443 nm, 470 nm and 663 nm.

Fourier Transform infrared spectrum of the extract

The FTIR spectrum of the extract revealed peaks at wavenumbers of 3365.73 cm^{-1}, 2912.89 cm^{-1}, 1659.42 cm^{-1}, 1631.91 cm^{-1}, 1370.12 cm^{-1}, 1319.88 cm^{-1}, 1065.87 cm^{-1} and 825.40 cm^{-1} (Fig. 2).

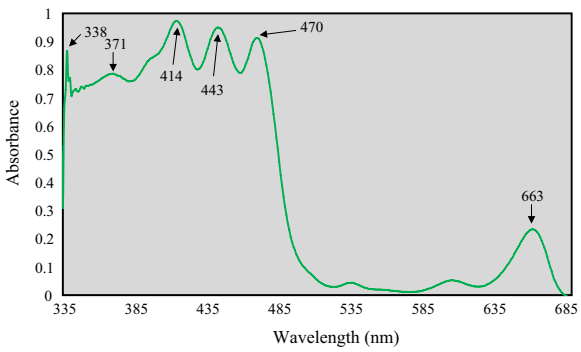


Fig. 1 Ultraviolet–visible spectrum of ethanolic extract of *A. coriaria* leaves

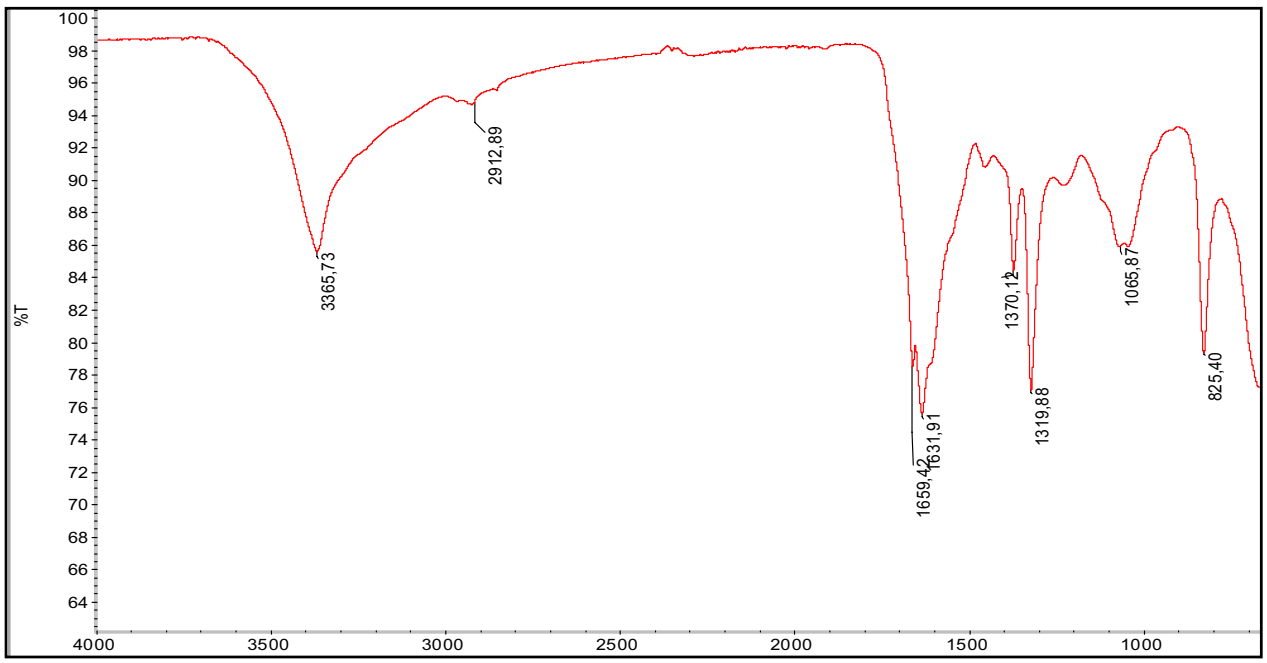
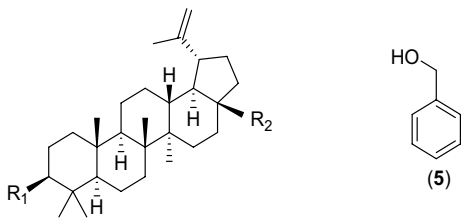


Fig. 2 FTIR spectrum of ethanolic extract of *A. coriaria* leaves



(**1**) R_1 = OH, R_2 = CH_3
(**2**) R_1 = O, R_2 = CH_3
(**3**) R_1 = OH, R_2 = COOH
(**4**) R_1 = OH, R_2 = CH_2OH

Fig. 3 Structures of compounds identified in fractions of ethanolic extract of *A. coriaria* leaves from Uganda

Compounds isolated and identified in fractions of ethanolic extract of *A. coriaria* leaves

Fractions 4–9 eluted with hexane/ethyl acetate (9:1) afforded colourless crystals, which were analysed by GC–MS and found to contain lupeol (**1**). Fractions 10–13 gave colourless needle-shaped crystals, which on analysis were found to contain lupenone (**2**). Further elution of the column with 30% ethyl acetate in ethanol gave fractions 2–6 which had betulinic acid (**3**), while fractions 7–13 and 14–20 yielded betulin (**4**) and benzyl alcohol (**5**), respectively (Fig. 3).

The compounds were tentatively identified basing on their mass spectra analysis and matching with those in NIST 11 spectral library, and comparison with published spectroscopic data. Compound **4** was identified for the first time in this species.

Discussion

UV–Vis and FTIR spectra of the extract

The UV–Vis spectrum of the extract had maximum absorptions between 383 and 664 nm. The absorption peaks at 338 nm is due to the presence of alkaloids (Sangster and Stuart 1965; Zahari et al. 2016), while the absorption at 371 nm indicated the presence of terpenoids (Malik et al. 2018). The absorption at 664 nm indicated the presence of flavonoids (Dhivya and Kalaichelvi 2017; Donkor et al. 2019; Rani et al. 2016).

In the FTIR spectrum, the stretch of 3365.73 cm^{-1} could be assigned to an O–H group (Das et al. 2016). The peaks observed at 2912.89 cm^{-1}, 1659.42 cm^{-1}, 1631.91 cm^{-1}, 1370.12 cm^{-1}, 1319.88 cm^{-1} and 1065.87 cm^{-1} are due to C–H stretching (Ashokkumar and Ramaswamy 2014), C=O bond (Nandiyanto et al. 2019), C=C bond (Oliveira et al. 2016), C–O stretching (Netala et al. 2015), C–H, N=O (Kavitha et al. 2019) and C–N stretch (Janakiraman and Johnson 2015), respectively. The presence of O–H, C=O and aromatic–C=C indicated the presence of alcohols, carboxylic acids and aromatics in the extract. These groups of compounds identified by UV–Vis and FTIR spectroscopy agreed with the secondary metabolites previously identified by classical phytochemical screening (Omara et al. 2021b).

Compounds identified in fractions of *A. coriaria* leaves extract

Compound 1 (Lupeol)

Compound **1** had a GC retention time of 36.943 min. It had a molecular ion at *m/z* 426 which suggested a molecular formula: $C_{30}H_{50}O$ (Fig. 4). Fragmentation of the molecular ion by removal of a methyl group gave the fragment peak at *m/z* 411 (Fig. 5). This peak is characteristic of a pentacyclic triterpene with an isopropenyl group (Pereira et al. 1996). When it fragments by losing ethene (CH_2=CH_2) gas, the fragment at *m/z* 383 is produced (Carvalho et al. 2010). Fragment *m/z* 383 further loses -$C_{13}H_{22}$ and -H_2O (or -$C_{13}H_{24}O$), which gives fragments *m/z* 207 (allocates the hydroxyl group at C_3 position) and *m/z* 189 (El Sayed 2016). These fragments may also arise from the cleavage between C-8/C-14 and C-12/C-13 bonds (with proton transfer) and is usually confirmatory that such a compound possess a lupane or hopane skeleton (Budzikiewicz et al. 1963; Ogunkoya 1981).

The fragments at *m/z* 189 and *m/z* 218 indicate that compound **1** is a pentacyclic triterpene. The other fragments at *m/z* 43, 55, 59, 81, 85, 91, 95, 107, 121, 135, 147, 161, 175, 218, 234, 257 and 315 are often associated with lupeol (Baek et al. 2010; Byamukama et al. 2015; Cîntă-Pînzaru et al. 2012; Doshi et al. 2015; Kiria 2018; Leite et al. 2020; Wahyuono 1985). By comparison with the foregoing literature and following NIST 11 library matching, compound **1** was tentatively suggested to be lup-20(29)-en-3β-ol (lupeol).

Byamukama et al. (2015) previously reported the presence of lupeol in ethyl acetate extract of *A. coriaria* stem bark. Abd El-Ghany et al. (2015) and Tamokou et al. (2012) also identified lupeol in the stem bark extract of other *Albizia* species (*A. lebbeck* and *A. adianthifolia*) using GC–MS. Lupeol was also reported in the root bark extract of *Albizia glaberrima* (Fotso et al. 2017), wood extract of *Albizia myriophylla* (Thammavong 2012) and stem bark extracts of *Albizia zygia* (Oloyede et al. 2019), *Albizia julibrissin* (Baek et al. 2010), *Albizia gummifera* (Rukunga and Waterman 2001a), *Albizia versicolor*, *Albizia schimperana* (Rukunga and Waterman 2001b) and *Albizia lebeckioides* (Arifnuryadin and Affandi 1998).

The triterpene lupeol has excellent bioactivities, including antioxidant and antibacterial activities. Its antioxidant properties is mediated through scavenging of free radicals, decreasing lipid peroxidation and increasing endogenous blood antioxidant enzyme levels (Tchimene et al. 2016). As an antibacterial agent, it has been reported to be effective against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi*, *Bacillus cereus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus lutea*, *Shigella boydi*, *Shigella dysentriae* and *Vibrio mimicus* (Siddique and Saleem 2011). These reports support the use of *A. coriaria* leaves in Uganda for treating cough, diarrhoea and typhoid. This indicates that the leaves could also be used instead of the stem bark and roots, to encourage sustainable utilization of this medicinal plant.

Compound 2 (Lupenone)

Compound **2** had a GC retention time of 36.550 min. Its mass spectrum (Fig. 6) had a molecular ion peak at *m/z* 424, which suggested a molecular formula $C_{30}H_{48}O$. Compound **2** was closely related to

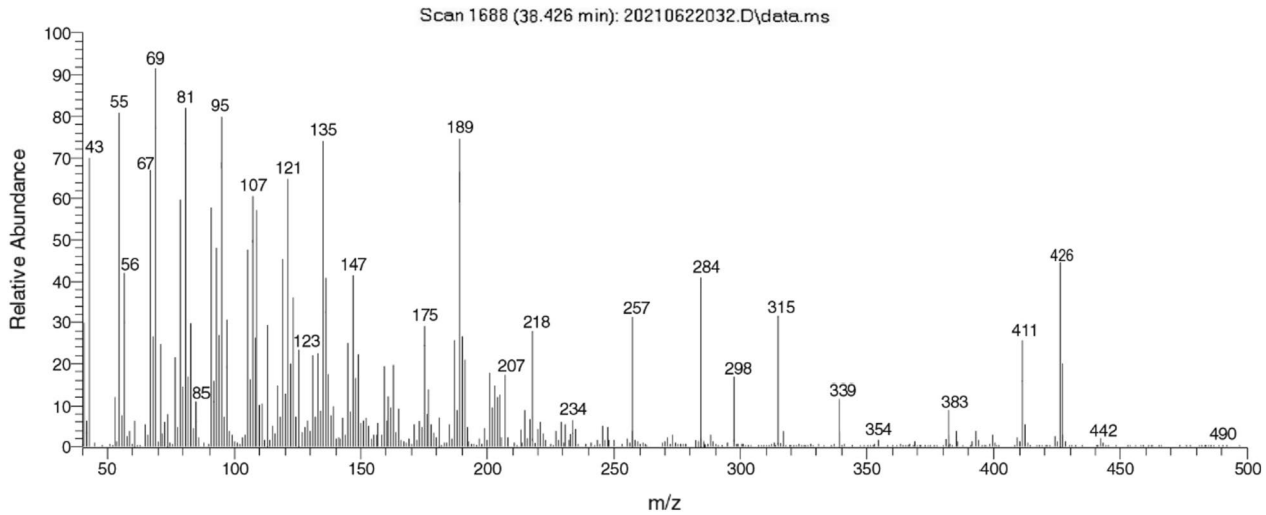


Fig. 4 Expanded mass spectrum of compound **1** (Lupeol)

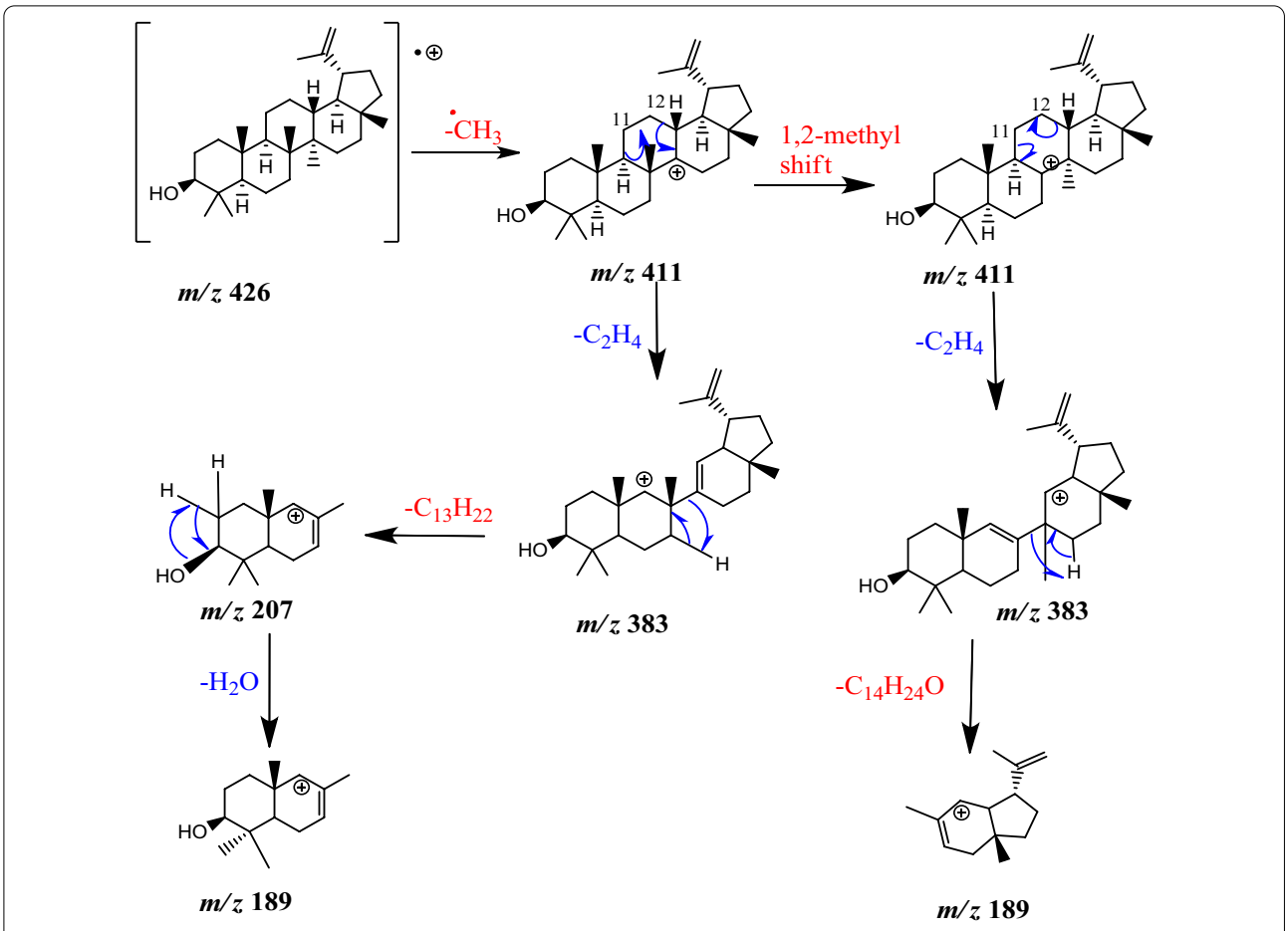


Fig. 5 Fragmentation pattern of compound **1** (Lupeol)

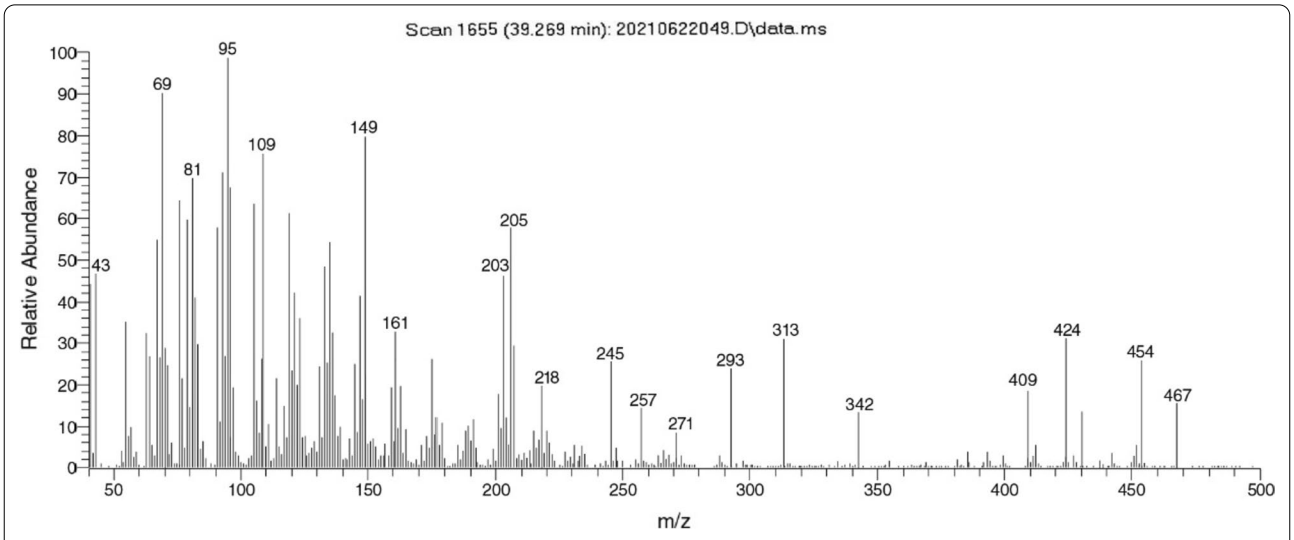


Fig. 6 Expanded mass spectrum of compound **2** (Lupenone)

compound **1** as its spectrum gave a molecular ion at *m/z* 424, corresponding to two fewer hydrogen atoms than the latter (Arifnuryadin and Affandi 1998).

Fragmentation of the molecular ion (*m/z* 424) by removal of a methyl (CH_3) and C_6H_{10}, respectively, yields the fragment ions at *m/z* 409 and *m/z* 342 (Fig. 7). The fragment at *m/z* 409 further loses ethene (CH_2=CH_2) gas to yield fragment *m/z* 381 (Suttiarporn et al. 2015). The fragment *m/z* 342 loses -C_4H_{10}, forming fragment *m/z* 271. This fragmentation pattern agrees with the one suggested for lupenone in open literature (Ahmad et al. 2015; Branco et al. 2004; Budzikiewicz et al. 1963; Cordeiro et al. 1999; Heinzen et al. 1996; Suttiarporn et al. 2015). Other fragments occurred at *m/z* 203, *m/z* 205 and *m/z* 218, which are indicative that compound **2** possess a lupane-triterpene skeleton (Pereira et al. 1996; Prashant and Krupadanam 1993; Yam-Puc et al. 2019). The mass spectral data also suggested the presence of a carboxylic acid group (at *m/z* 205), with the fragment at *m/z* 409, suggesting that it is either attached to ring A or B of the pentacyclic ring (Pereira et al. 1996). The other fragments (*m/z* 43, 81, 95, 105, 120 and 149) are usually observed in the mass spectrum of lupenone (Byamukama et al. 2015; Kiria 2018; Ogukwe et al. 2018). This literature supported the NIST 11 spectral library matching that compound **2** is lup-20(29)-en-3-one (lupenone).

Lupenone was earlier identified in ethyl acetate extract of *A. coriaria* stem bark (Byamukama et al. 2015). It was also isolated from petroleum ether leaf extract of *Albizia inundata* (Andrade et al. 2020) as well as stem bark extracts of *Albizia julibrissin* (Baek et al. 2010), *Albizia gummifera* (Rukunga and Waterman 2001a), *Albizia versicolor*, *Albizia schimperana* (Rukunga and Waterman 2001b) and *Albizia falcataria* (Arifnuryadin and Affandi 1998). Lupenone is a lupane-type triterpenoid with appreciable antioxidant activity (Çulhaoğlu et al. 2015; Jeong et al. 2013) and antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *Bacillus subtilis* (Prachayasittikul et al. 2010). The identification of compounds **1** and **2** in the same extract in this study aligns well with previous reports which indicated that lupeol and lupenone often co-occur in plants (Gutierrez-Lugo et al. 2004; Rukunga and Waterman 2001a, b; Xu et al. 2018), including *A. coriaria* (Byamukama et al. 2015).

Compound **3** (Betulinic acid)

Compound **3** had a GC retention time of 31.259 min. It showed a molecular ion at *m/z* 456, with a molecular formula: $C_{30}H_{48}O_3$. Its mass spectrum (Fig. 8) showed prominent peaks at *m/z* 254 and *m/z* 283, which are

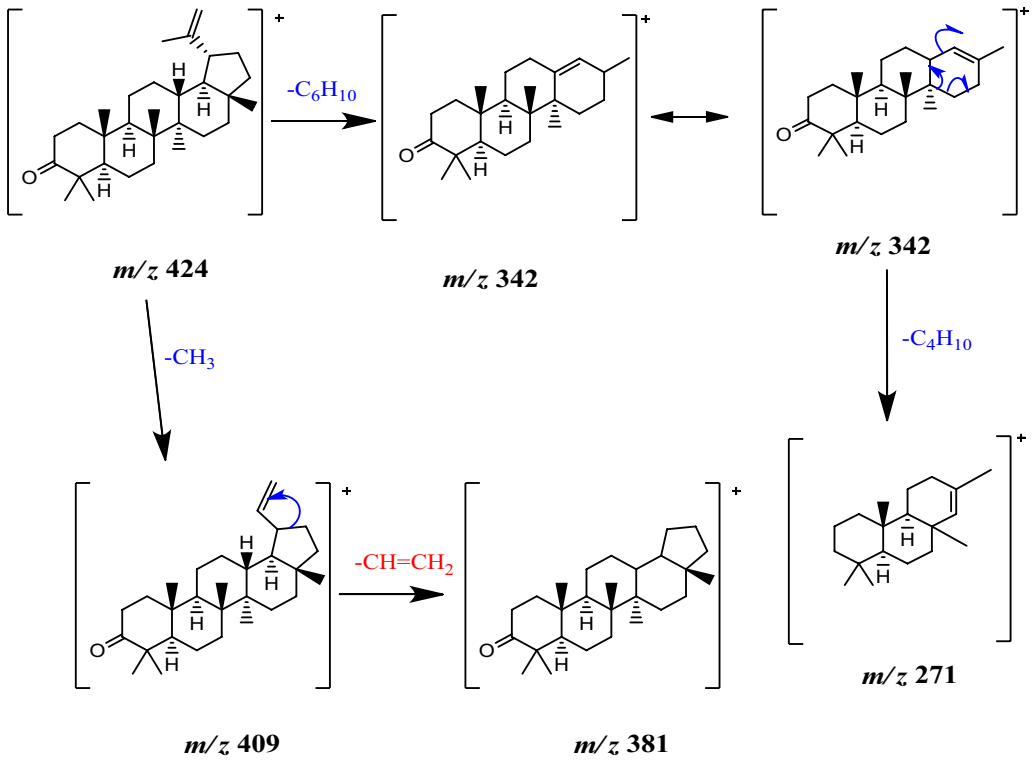


Fig. 7 Fragmentation pattern for major ions of compound **2** (Lupenone)

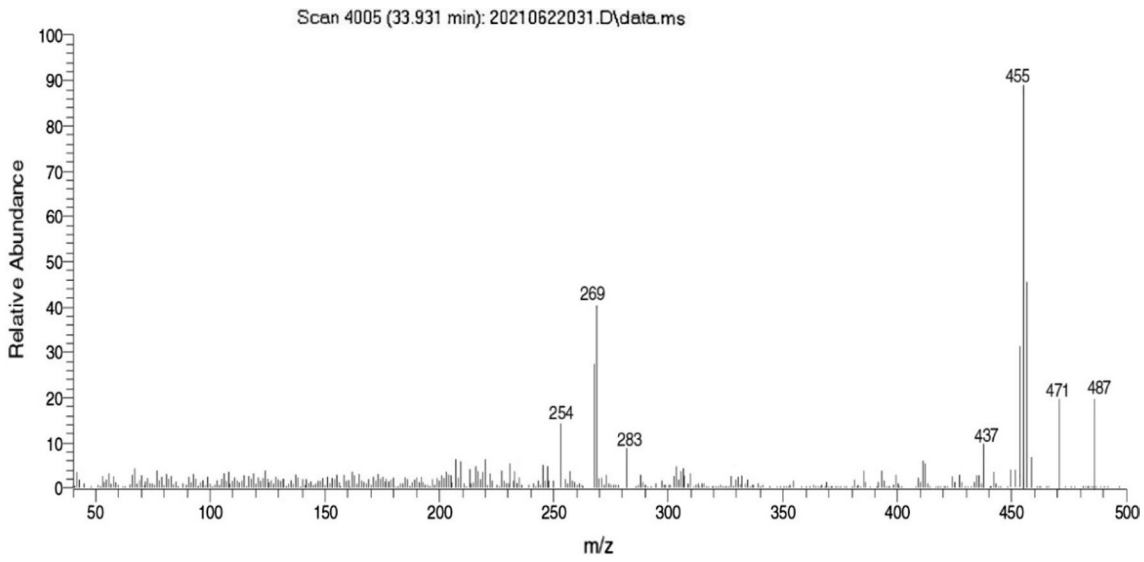


Fig. 8 Expanded mass spectrum of compound **3** (Betulinic acid)

associated with pentacyclic triterpenoids of the lupane series (Joshi et al. 2013; Koma and Sani 2014; Nambooze 2019). The other prominent fragment peaks were at *m/z* 455 (base peak) and *m/z* 457, which likely corresponds to the deprotonated (pseudo-molecular negative) ion, $[M\text{-}H]^-$ and protonated molecular ion $[M+H]^+$, respectively, for betulinic acid (Cichewicz and Kouzi 2004; Koma and Sani 2014; Räsänen et al. 2019; Shin et al. 1999). Fragments *m/z* 437 $[M^+\text{-}H_2O]$ and *m/z* 411 $[M^+\text{-}COOH]$ were also observed, indicating the presence of a carboxylic acid group (Ayatollahia et al. 2011).

Other fragments occurred at *m/z* 207 and 220, which are characteristic for betulinic acid (Baek et al. 2010; Choi et al. 2006; Lee et al. 2009; Ogunmoye et al. 2018). Prominent fragment peaks were also observed at *m/z* 437 $[M-CH_2]^+$ and 471 $[M-O]^+$, which are typical of a pentacyclic triterpene with an isopropenyl group (Nambooze 2019). The other fragments (*m/z* 254, 269 and 283) are due to loss of H_2O, $-CH_3$ or $-CH_3OH$ from the enhanced ions and are usually observed in the mass spectrum of betulinic acid (Peng et al. 2017; Srivastava and Chaturvedi 2010). These spectral characteristics supported that compound **3** is 3β-hydroxy-lup-20(29)-en-28-oic acid (betulinic acid).

Betulinic acid was previously isolated from *A. coriaria* stem bark extract (Byamukama et al. 2015). It was also identified in ethanolic extract of *Albizia julibrissin* stem bark (Baek et al. 2010) as well as stem bark extract of *Albizia lebbeck* (Thube et al. 2014). Betulinic acid is a widely distributed phenolic compound (pentacyclic lupane-type triterpene) in kingdom plantae and has been indicated to elicit intriguing pharmacological activities, including antioxidant and antibacterial activities (Karan et al. 2019; Sulaiman et al. 2020). Specifically, it exhibited antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae* and *Bacillus subtilis* (Koma and Sani 2014; Nambooze 2019; Shai et al. 2008).

Compound **4** (Betulin)

Compound **4** was obtained as colourless crystals, with a GC retention time of 24.69 min. The mass spectrum of compound **4** (Fig. 9) revealed the presence of prominent fragments at *m/z* 442 and *m/z* 464, suggesting that it is a lupeol-type triterpene (Cîntă-Pînzaru et al. 2012; Tijjani et al. 2012). The molecular ion peak occurred at *m/z* 442, corresponding with the molecular formula $C_{30}H_{50}O_2$. Another fragment occurred at *m/z* 428, formed from the loss of a methyl (CH_3) from the protonated molecular ion, *m/z* 443 (Zhang et al. 2019). Other fragments (*m/z* 411, 407, 395 and 393) are due to loss of H_2O, $-CH_3$ or $-CH_3OH$ from *m/z* 425 or *m/z* 410 (Fig. 10). The peaks observed at *m/z* 217 and *m/z* 189 are due to ring opening of the protonated molecular ion, followed by loss of water (Zhang et al. 2019). These spectroscopic data supported that compound **4** is the known compound (lup-20(29)-ene-3β,28-diol), commonly called betulin (Heinzen et al. 1996).

Though being reported for the first time in *A. coriaria*, betulin was identified by GC–MS in the pericarp extract of a sister species (*Albizia lebbeck*) in Egypt (El-Hawary et al. 2011). Oloyede et al. (2019) also identified betulin in the stem bark extract of *Albizia zygia*. Betulin possesses a range of biological activities, including

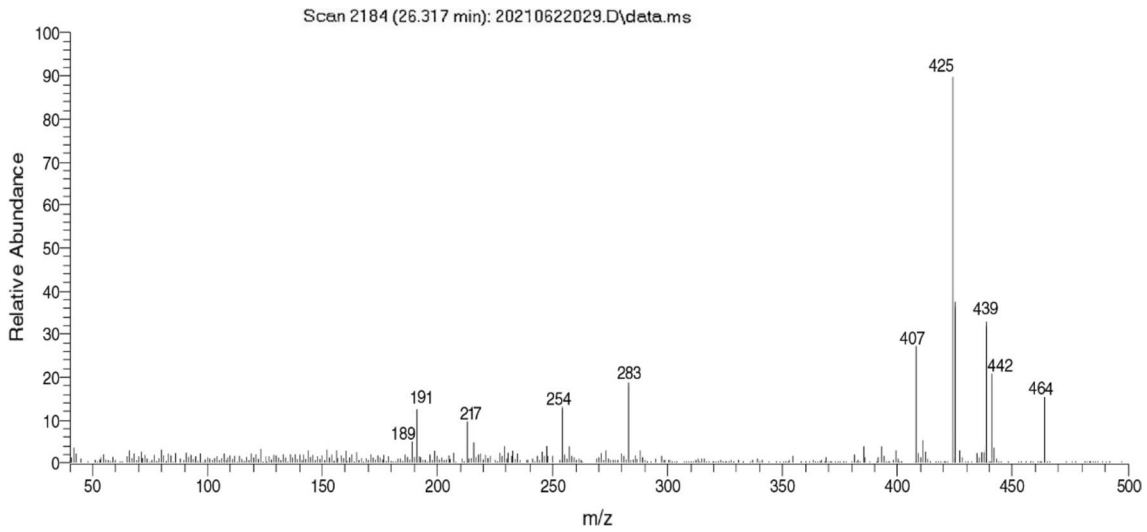


Fig. 9 Expanded mass spectrum of compound **4** (Betulin)

antioxidant and antibacterial activities (Prachayasittikul et al. 2010; Šiman et al. 2016).

Compound **5** (Benzyl alcohol)

Compound **5** was obtained as a brown powder with a GC retention time of 9.686 min. Its mass spectrum (Fig. 11) showed distinct peaks at *m/z* 108, *m/z* 91 and *m/z* 77, which are characteristic of benzylic alcohols (Dasgupta and Steinagel 1997; Tedankara 2021). The molecular ion occurred at *m/z* 108, confirming the molecular formula C_7H_8O. Loss of a proton from the molecular ion (*m/z* 108) gives the fragment at *m/z* 107. Rearrangement and loss of carbon monoxide (CO) gas gives the peak observed at *m/z* 79 (Fig. 12), which may lose two protons successively to yield the fragment ion at *m/z* 77 (Yetayih and Ravichandran 2020). The fragment ion *m/z* 77 (phenyl cation, $C_6H_5^+$) may also be formed through alpha cleavage, i.e. direct loss of $-CH_2OH$ (Tedankara 2021). Loss of acetylene (C_2H_2) from *m/z* 77 leads to the formation of *m/z* 51 (cyclobutenyl cation) (Nicolescu 2017).

Fragmentation (inductive cleavage) of the molecular ion through loss of a hydroxyl (mass = 17) yields the common benzyl cation fragment (intense ion) for alkylbenzenes at *m/z* 91 due to benzylic cleavage of the highly stable aromatic ring (Müller and Volmer 2017; Yetayih and Ravichandran 2020). This ion, called the tropylium ion or cycloheptatrienyl cation ($C_7H_7^+$), may undergo further fragmentation through successive loss of acetylene molecule to give the cyclopentadienyl cation observed at *m/z* 65 (Müller and Volmer 2017; Nicolescu 2017; Yetayih and Ravichandran 2020). The other small fragments (*m/z* 57 and *m/z* 80) are usually observed in the mass spectrum of benzyl alcohol (Yetayih and Ravichandran 2020) due to the presence of small amounts of carbon-13 in the sample (Tedankara 2021). Thus, compound **5** was deduced to be benzyl alcohol as suggested by NIST 11 spectral library matching.

Benzyl alcohol was previously identified in ethyl acetate extract of *A. coriaria* stem bark (Byamukama et al. 2015). It has been previously reported to possess various bioactivities including antioxidant, bacteriostatic and bactericidal activities (Lucchini et al. 1990; Seung-Joo et al. 2005).

Overall, the compounds identified agreed with results of UV–Vis and FTIR spectroscopy which indicated the presence of alcohols, terpenoids and aromatics in the ethanolic extract of *A. coriaria* leaves. Compounds **1**–**4** are terpenoids, while **5** is an aromatic alcohol. The results of this study indicate that most of the compounds found in *A. coriaria* leaves are also present in its stem bark as previously reported (Byamukama et al. 2015). This study, therefore, by extension could also explain the ethnomedicinal diversity of this species because of its inherent possession of bioactive triterpenes.

Conclusions

Phytochemical investigation of ethanolic extract of *A. coriaria* leaves led to the identification of known bioactive pentacyclic triterpenes (**1**–**4**) and an aromatic alcohol (**5**). This is the first time betulin (**4**) is being reported in this species. All the compounds identified have been previously reported to possess antioxidant and antibacterial activities, among other bioactivities. This study, therefore, for the first time indicated that *A. coriaria* leaves possess therapeutic compounds with antioxidant and antibacterial activities which lends credence to its use in

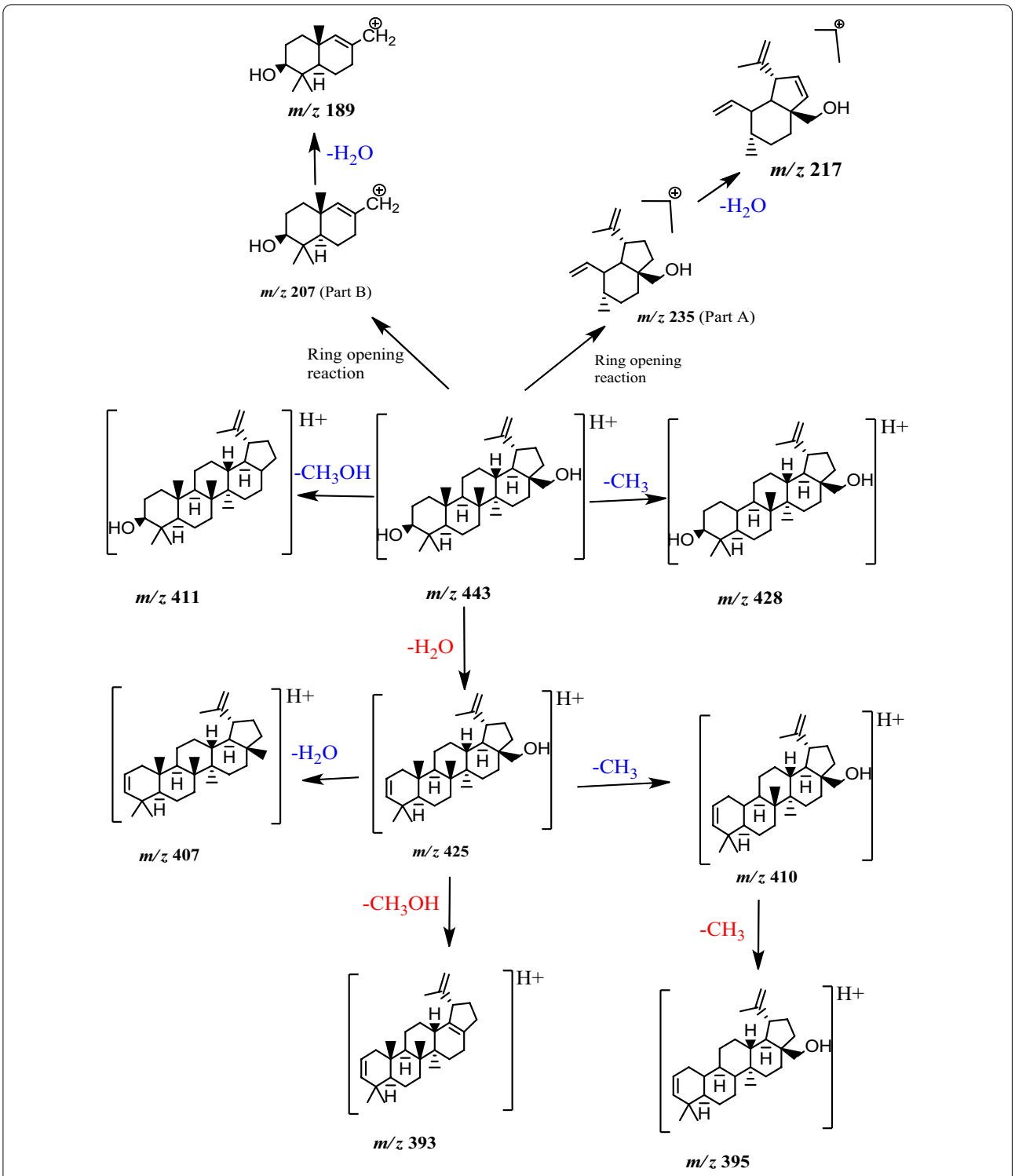


Fig. 10 Major peaks from fragmentation of compound **4** (Betulin)

traditional management of oxidative stress-induced conditions and bacterial diseases in Uganda. Further studies should establish the toxicity of the identified compounds and the leaves extract. In vivo studies and clinical trials using the identified compounds and the extract should be explored. The compounds present in other parts of

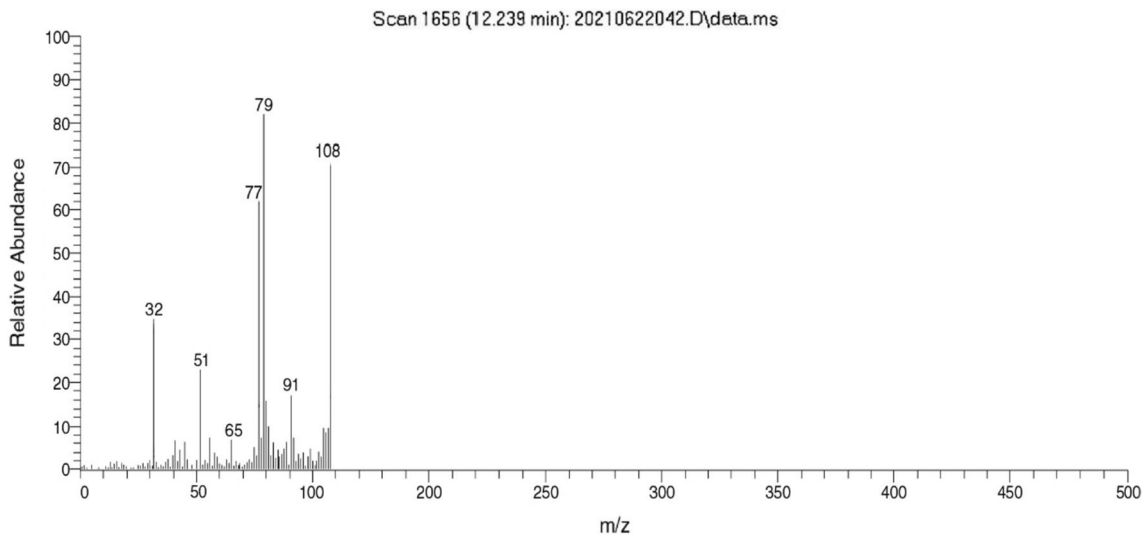


Fig. 11 Expanded mass spectrum of compound **5** (Benzyl alcohol)

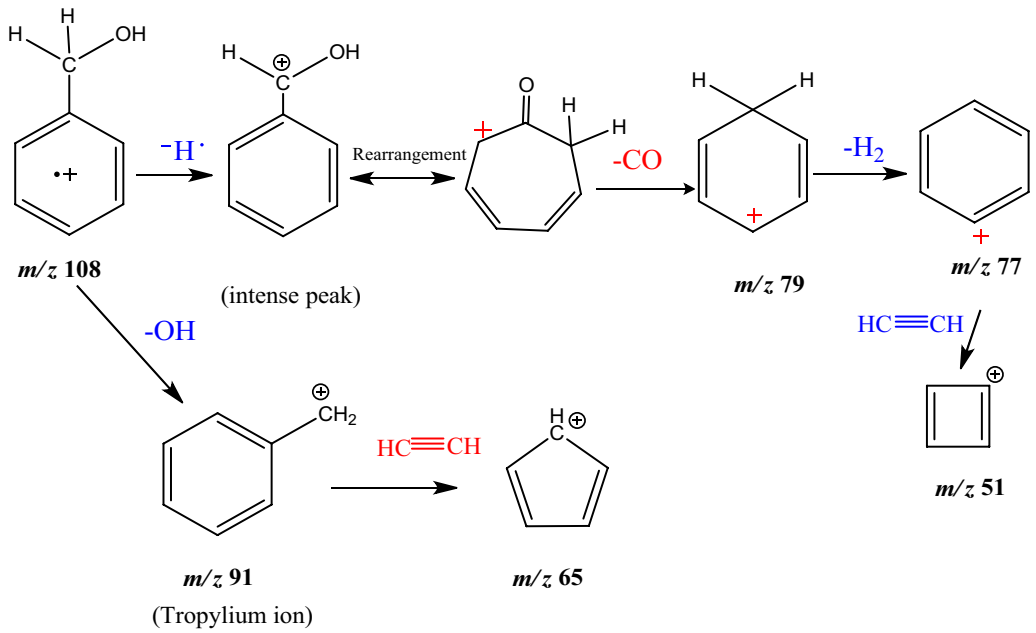


Fig. 12 Fragmentation pattern for compound **5** (Benzyl alcohol)

A. coriaria such as flowers and seeds traditionally used in Uganda for treatment of malaria and cancer should be investigated to establish if they contain the same compounds as the stem bark, roots and the leaves.

Abbreviations

A. coriaria: *Albizia coriaria* (Welw ex. Oliver); *E. coli*: *Escherichia coli*; GC–MS: Gas chromatography–mass spectrometry; FTIR: Fourier transform infrared; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; UV–Vis: Ultraviolet–visible.

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Authors' contributions
TO, AKK and VJK designed the study. TO collected samples and performed the analytical work. AKK and VJK supervised the study. TO analysed the collected data and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The raw data supporting the conclusions of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
This study was approved by the Department of Chemistry and Biochemistry, Moi University, Kenya (Approval No. MS/ACH/4316/20). The species used in this study has a stable population trend and is of ***Least Concern*** according to IUCN Red List Categories.

Consent for publication
Not applicable.

Competing interests
The authors declare that there is no conflict of interest regarding the publication of this paper.

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