Two New Pentacyclic Triterpenoids, an Alkaloid and a Long-chain Fatty Acid from *Albizia Coriaria* (Welw ex. Oliver)

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Herein, we report the occurrence of four new compounds in ethanolic extract of *Albizia coriaria* Welw ex. Oliver leaves along with other compounds previously reported in this species. The compounds were isolated and characterized using column chromatography, Fourier Transform Infrared (FTIR) and gas chromatography/mass spectrometry. FTIR spectrum of the extract showed phenolic O−H stretching (3362.30 cm^{-1}), C=O (1660.08 cm^{-1}), C−O stretching (1369.46 cm^{-1} and 1319.00 cm^{-1}) and C−N stretch (1072.44 cm^{-1}) which confirmed the presence of alcohols, carboxylic acids and nitrogen-containing compounds. Oleanolic acid (**1**), oleanolic acid acetate (**2**), pterin-6-carboxylic acid (**3**), undecanol (**4**), betulinic acid (**5**), betulin (**6**) and benzyl alcohol (**7**) were tentatively identified in the extract. Compounds **1-4** are being reported for the first time in *Albizia coriaria*.

Introduction

Many lead compounds and therapeutic molecules have been discovered from natural products. Plants represent a huge proportion of natural sources of drugs currently used in both human and veterinary medicine [1]. Species emanating from the genus *Albizia* are widely used in traditional medicine, owing to their inherent possession of bioactive compounds [2]. *Albizia coriaria* Welw ex. Oliver (*A. coriaria*) is a representative species of this genus that is widely utilized in African herbal medicine [3]. Its organs and the whole plant are used for managing various ailments and conditions including dermatological diseases, gastrointestinal diseases and infections, ophidian bites, malignancies, malaria, cough (tuberculosis), cardiovascular diseases, anaemia, venereal diseases and reproductive conditions [4-30]. It is also used in the treatment of lungworms/ascaris worms in

cattle, sheep and goats, as a mosquito repellent and toothbrush [18, 25, 31-33].

Various bioactivities of the stem bark, leaves, flowers and roots of this species have been validated *in vitro*. These include molluscicidal [34], antimicrobial [7, 35-42], antiproliferative [43], anti-giardial [23], antiplasmodial [44], anti-inflammatory [45], antimycobacterial [46, 47] and antioxidant activities [36, 37, 40].

Investigation of the chemical composition of *A. coriaria* stem bark has led to the identification of lupeol, lupenone, benzyl alcohol, betulinic acid, (+)-catechin and acacic acid lactone [39], and 43 other compounds including hexanal, (2,4-dinitrophenyl)hydrazine; hydroquinone and 7-hydroxy-3′-methoxyflavone, trimethylsilyl ether [46]. In the root bark extracts, Gummiferaoside C and Coriariosides A-E were earlier characterized [43, 48]. Recently, we reported the presence of lupenone, lupeol, betulinic acid, betulin and benzyl alcohol in the leaves of this species sampled from Mbarara district of Western Uganda [49]. The current study reports for the first time, the occurrence of two pentacyclic triterpenoids (oleanolic acid and oleanolic acid acetate), an alkaloid (pterin-6-carboxylic acid), a long-chain fatty acid (undecanol), along with betulinic acid, betulin and benzyl alcohol in ethanolic extract of *A. coriaria* leaves from Kole district of Northern Uganda, East Africa.

Experimental part

Sample collection and preparation

A. coriaria leaves were sampled from Otangula village, Kole district, Uganda (2º17’35.0’’N 32º46’31.2’’E) on Wednesday 20th January 2021. A specimen voucher (no. 50995) was authenticated by a taxonomist at Natural Chemotherapeutics Research Institute (NCRI), Kampala, Uganda. It was deposited at Department of Botany, Makerere University herbarium, Kampala, Uganda for future reference.

Owing to the hot weather in Uganda in January 2021, the laboratory sample was washed under running tap water to remove dusts and then shade-dried for two days in Otino Waa Secondary School Biology Laboratory, Kole, Uganda. This was done to avoid sample deterioration prior to transportation for further drying. The sample was then packaged in sterilized polyethylene bags and transported to School of Sciences and Aerospace Studies, Chemistry Laboratory, Moi University, Eldoret, Kenya where they were dried under shade at room temperature for 3 weeks.

The dry leaves were powdered using a NutriBullet® 600 Series electric grinder (Capbran Holdings, USA) and then 0.5 kg of it was serially macerated in 1000 ml of ethyl acetate and ethanol at room temperature. The extracts were separately filtered through cotton wool and then Whatman No.1 filter paper. They were eventually concentrated to dryness by

rotary evaporation at 40 ºC. The crude extracts were transferred to a desiccator of anhydrous sodium sulphate to remove any traces of water.

Fourier transform infrared scanning of the crude extract

The ethanolic extract was subjected to Attenuated Total Reflection-Fourier transform infrared (ATR-FTIR) characterization. The scan was done on a Nicolet 6700 FTIR spectrophotometer (Thermo Scientific, USA) at a spectral resolution of 4 cm^{-1} and scan range of 500 cm^{-1} to 4000 cm^{-1}.

Thin layer chromatography and column chromatography of the crude extract

Thin layer chromatography (TLC) was used to optimize the solvent ratios for column chromatography [50]. Briefly, the ethanolic extract was reconstituted in ethanol (1: 20, w/v), spotted onto TLC plates, dried and developed in solvent systems of different hexane: ethyl acetate and ethyl acetate: ethanol ratios. The number of compounds and their retention factors (Rf) were calculated after visualization under UV light (254 nm and 365 nm).

The extract was subjected to silica gel (60-120 mesh) column chromatography (7.5 × 100 cm column) using the optimized solvent ratios. Silica gel (100 g) was mixed with hexane: ethyl acetate solvent system to form a slurry before it was poured into the column. The sample was introduced into the column and then eluted with the established solvent systems [49]. For every elution, equal fractions were collected and those with similar TLC profiles were combined and concentrated to dryness by rotary evaporation.

Characterization of compounds in the fractions

Seven fractions from chromatographic isolation and TLC profiling were re-dissolved in dichloromethane/methanol (1:1). They were separately filtered through 0.45 µm filters and then transferred to 2 mL vials for gas chromatography-mass chromatography (GC-MS) characterization as previously described [49].

Briefly, the characterization was performed in a gas chromatograph interfaced with mass spectrometer triple-quad system (Agilent 8890A GC and Agilent 5977 GC/MSD, Agilent Technologies) supplied with an Agilent 7693A automatic liquid sampler, a National Institute of Standards and Technology (NIST) library, an installed Mass Hunter Workstation software and an HP-5MS ultra inert column (30 m × 0.25 mm × 0.25 µm).

Compound identification was based on the gas chromatogram elution times, mass spectra matching with those of the compounds in NIST 11 spectral library and/or compared with published spectroscopic data. Compound molecular ions were established using Nitrogen rule [51]. Further, the identified molecular ions were individually analyzed for their capacity to

yield important ions in the high mass region by logical neutral losses [52].

Results and discussion

Fourier Transform infrared spectrum of the crude extract

The FTIR spectrum of ethanolic extract of *A. coriaria* leaves (**Figure 1**) had a stretch at 3362.30 cm^{-1} which could be assigned to O-H stretching of phenolics [53]. The peaks observed at 2358.93 cm^{-1}, 1660.08 cm^{-1}, 1369.46 cm^{-1} and 1319.00 cm^{-1}, 1370.12 cm^{-1} and 1072.44 cm^{-1} are due to O =C=O stretching [54], conjugated or aromatic C=O bond [55], C−O stretching [56] and C−N stretching [57], respectively. This confirmed the presence of alcohols, carboxylic acids and nitrogenous compounds in the extract.

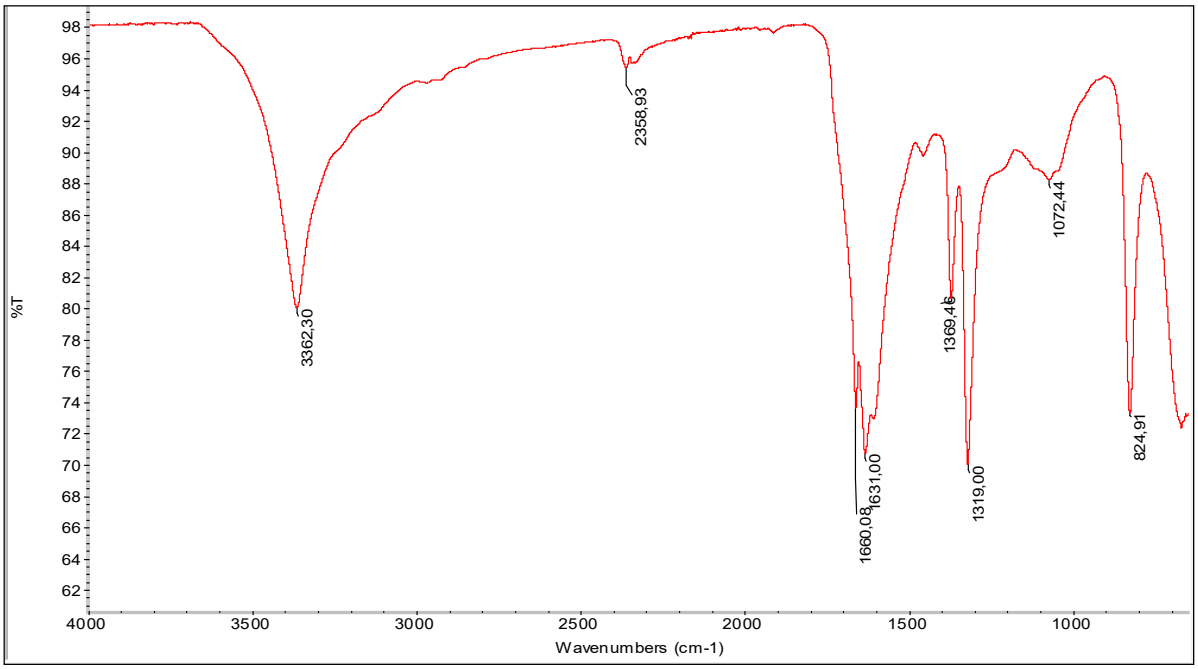

Figure 1. FTIR spectrum of ethanolic extract of *A. coriaria* leaves from Kole district, Northern Uganda.

Compounds tentatively identified in ethanolic extract of A. coriaria leaves

Chromatographic fractions 6–19 eluted with hexane: ethyl acetate (4:6) afforded a white solid which was analyzed and found to contain oleanolic acid (**1**, Rf = 0.570). Fractions 22–30 gave crystals which contained Oleanolic acid acetate (**2**, Rf = 0.533). Further elution of the column yielded fractions 35–48 and 52–66 which contained Pterin-6-carboxylic acid (**3**, Rf = 0.490) and Undecanol (**4**, Rf = 0.440), respectively. Further chromatographic elution using 30% ethyl acetate in ethanol gave fractions 2–17, 20–33 and 37–46 which afforded betulinic acid (**5**, Rf = 0.596), betulin (**6**, Rf = 0.500) and benzyl alcohol (**7**, Rf = 0.480), respectively (**Figure 2**). Of the seven compounds identified, **1-4** are reported for the first time in *A. coriaria*.

Compound 1 (Oleanolic acid)

Compound **1** was obtained as a white solid, soluble in ethanol. Its GC retention time was 31.875 minutes, with a molecular ion at *m/z* 456 which suggested a molecular formula $C_{30}H_{48}O_3$. The mass spectrum of compound **1**

(**Figure 3**) had a molecular ion peak at *m/z* 455, which corresponded to a deprotonated oleanolic acid molecule [58-60]. The spectral data also showed presence of a carboxylic acid group at *m/z* 407, that strongly suggested a C-12 unsaturated pentacyclic triterpene containing a carboxylic acid group in either ring D or E [61]. Another peak was observed at *m/z* 391 due to loss of -CH_2 group from the *m/z* 407 fragment (**Figure 4**)[62, 63]. The other peaks observed at *m/z* 255, 269, 283 and 297 are fragments of the enhanced products from the molecular ion [64]. Thus, compound **1** was tentatively deduced to be oleanic acid/oleanolic acid.

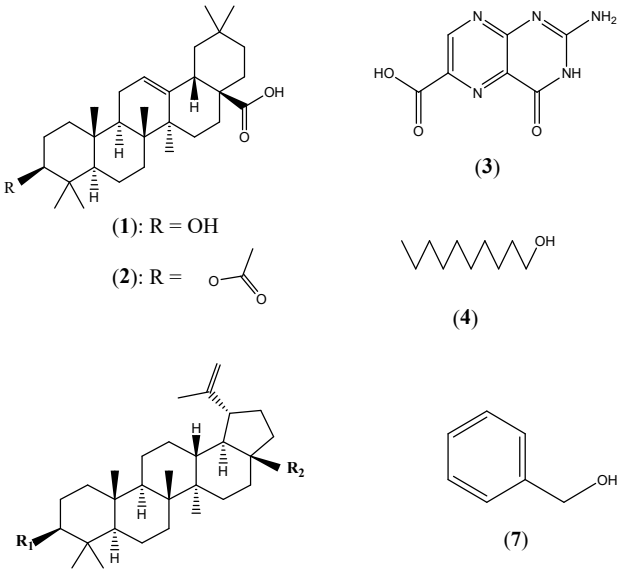

Figure 2. Structure of compounds identified in ethanolic extract of *A. coriaria* leaves from Kole district, Uganda.

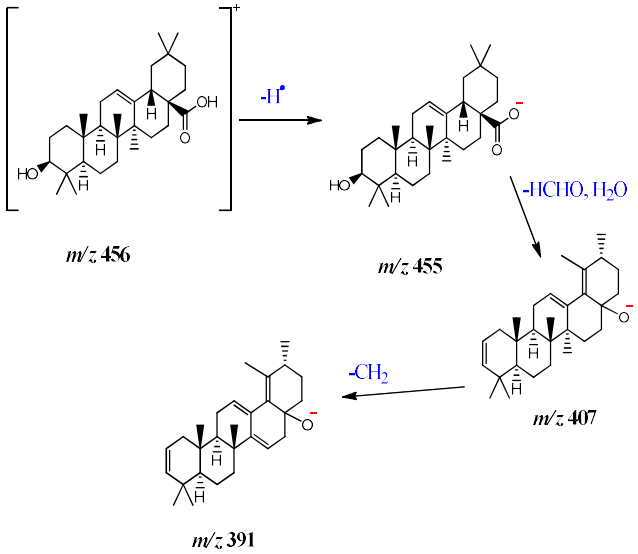

Figure 4. Fragmentation pattern of compound **1** (Oleanolic acid)

Free oleanolic acid is being reported for the first time in *A. coriaria*. However, it has been previously reported in the ethanolic extracts of *Albizia julibrissin* stem bark [65], a sister species in the *Albizia* genus. Oleanolic acid is one of the best known pentacyclic triterpenoids with widespread occurrence throughout Kingdom plantae in the form of free acid or aglycones for triterpenoid saponins [58, 66].

Compound 2 (Oleanolic acid acetate)

Compound **2**, eluted with ethyl acetate: ethanol (3:7) had a GC retention time of 30.992 minutes. Its mass spectrum (**Figure 5**) had a molecular ion at *m/z* 497, with a molecular formula $C_{32}H_{50}O_4$. Fragments *m/z* 269 and *m/z* 423, characteristic of pentacyclic triterpenes possessing carboxylic acid groups were present [67, 68]. The spectrum further suggested the presence of a carboxylic acid group, [M-COOH]$^+$ at *m/z* 301 [67, 68]. The ion peak observed at *m/z* 469 could have been due to loss

of HCHO from the molecular ion (**Figure 6**). Further loss of HCHO and H_2O molecules as in oleanolic acid could have given the peak observed at *m/z* 423 [67, 68]. These spectral characteristics supported the NIST library suggestion that compound **2** is 3-*O*-acetyloleanolic acid (oleanolic acid acetate)[69].

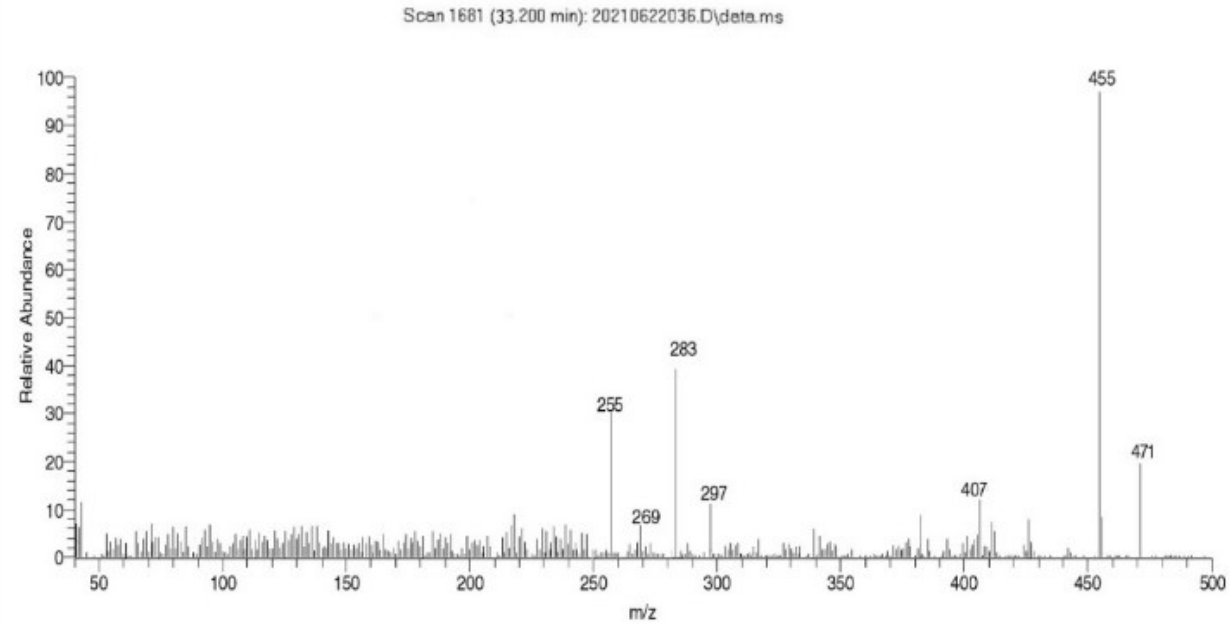

Figure 3. Expanded mass spectrum of compound **1** (Oleanolic acid)

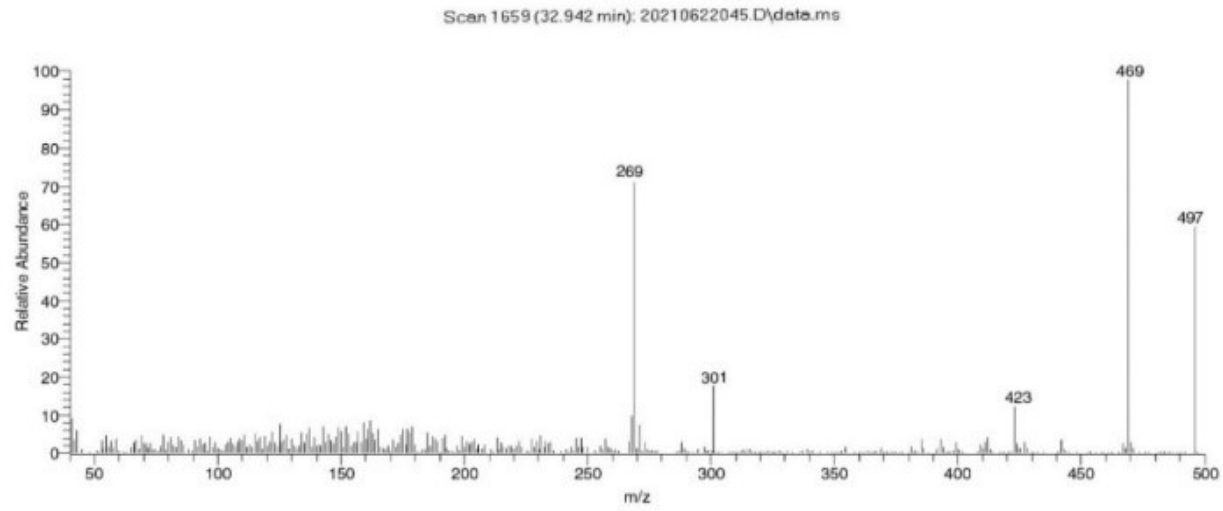

Figure 5. Mass spectrum of compound **2** (Oleanolic acid acetate)

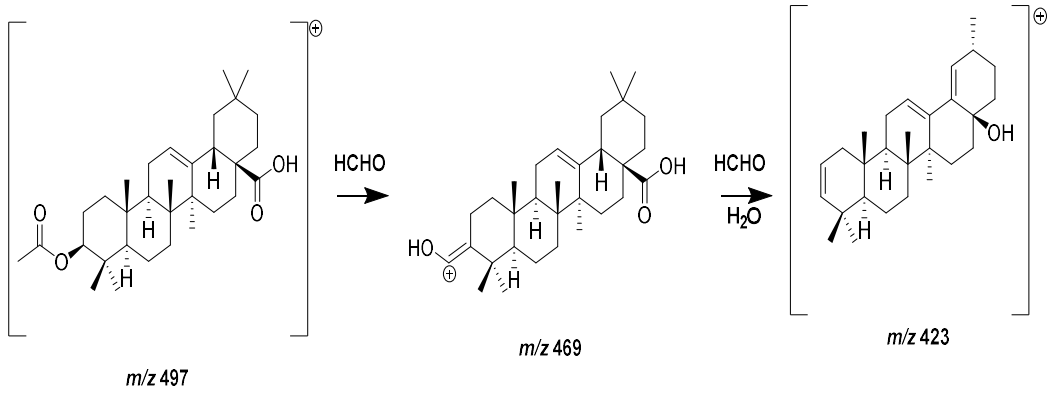

Figure 6. Fragmentation pattern for major ions of compound **2** (Oleanolic acid acetate)

Compound 3 (Pterin-6-carboxylic acid)

Compound **3** was eluted with hexane: ethyl acetate (4:6) from the column. It had a GC retention time of 21.579 minutes. The molecular ion of compound **3** occurred at *m/z* 207, suggesting a molecular formula $C_7H_5N_5O_3$ (**Figure 7**).

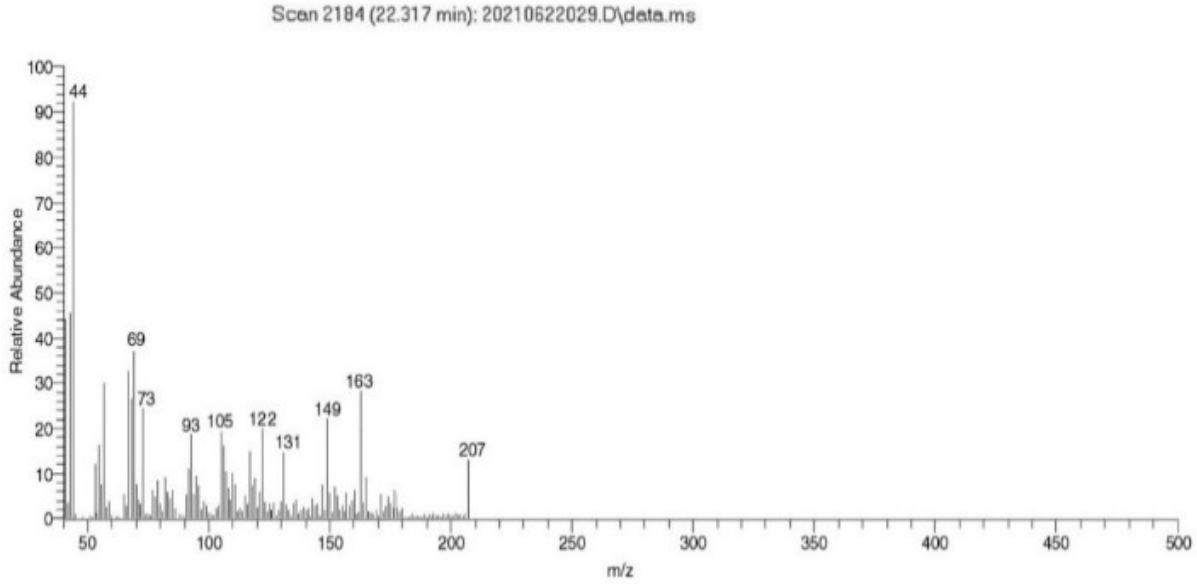

Figure 7. Mass spectrum of compound **3** (pterin-6-carboxylic acid)

The fragment observed at *m/z* 163 is due to loss of carbon dioxide (CO_2, mass = 44) from the molecular ion [70]. Loss of N_2HCHO (mass = 58) from *m/z* 163 (**Figure 8**) could have yielded the fragment at *m/z* 105 as observed in the fragmentation of pterins [71]. The fragment at *m/z* 149 is due to loss of glycolaldehyde gas ($HOCH_2CHO$, mass = 59) from the molecular ion [71]. Loss of H_2O from *m/z* 149 gives the fragment at *m/z* 131. The other peaks at *m/z* 57, 69, 105 and 122 are usually observed in the spectrum of pterin-6-carboxylic acid [72-75]. Thus, compound **3** was suggested to be 2-amino-4-hydroxy-6-pteridine carboxylic acid (pterin-6-carboxylic acid) as initially indicated by NIST 11 library matching. Compound **3**, a typical

alkaloid, was previously characterized in *Albizia lebbeck* leaf extracts [75].

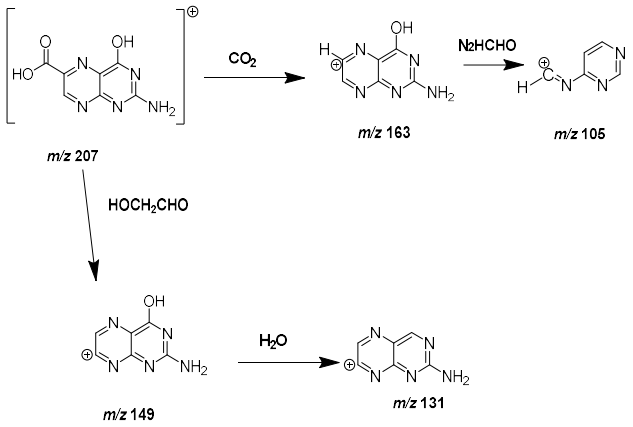

Figure 8. Fragmentation pattern of compound **3** (pterin-6-carboxylic acid)

Compound 4 (Undecanol)

Compound **4** was obtained as a pale greenish liquid with mild odor, soluble in ethanol. Its GC retention time was 15.307 minutes. The mass spectrum of compound **4** (**Figure 9**) had a base peak at *m/z* 55 and a molecular ion at *m/z* 172, which suggested a molecular formula $C_{11}H_{24}O$. The fragment observed at *m/z* 154 is due to loss of water from the molecular ion [76, 77]. The fragment at *m/z* 125 is due to loss of water and vinyl ($CH_2 = CH$) group from the molecular ion [76, 78]. Loss of ethene ($CH_2=CH_2$) molecule from *m/z* 125 gave the fragment at *m/z* 97 (**Figure 10**).

The other fragments observed at *m/z* at 83, 69, 55, 41 and 27 are due to loss of -CH_2 from the preceding fragments (*m/z* 97, 83, 69, 55 and 41, respectively)[79]. These spectral patterns confirmed that compound **4** is Undecan-1-ol (undecanol or 1-undecanol). Though it has never been reported before in *A. coriaria*, undecanol was earlier reported in *A. zygia* stem bark fixed oil analyzed by GC-MS [80].

The other compounds: betulinic acid (**5**), betulin (**6**) and benzyl alcohol (**7**) were identified as reported previously [49].

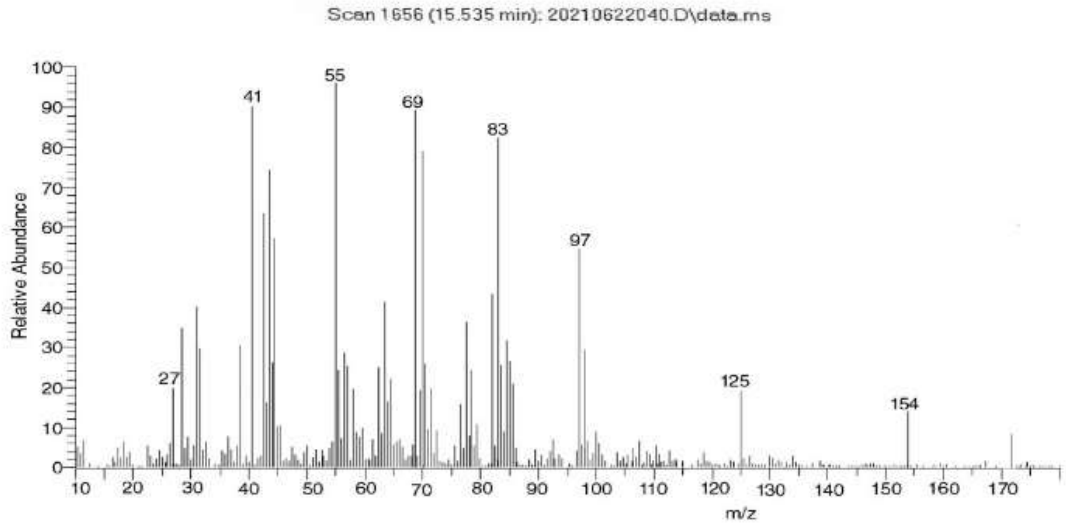

Figure 9. Mass spectrum of compound **4** (Undecanol).

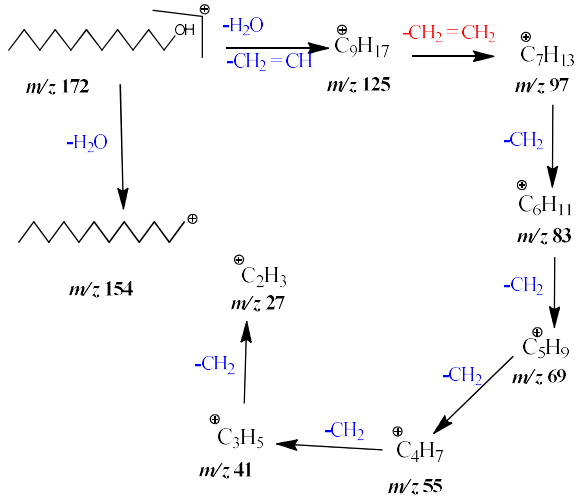

Figure 10. Fragmentation pattern of compound **4** (Undecanol)

All in all, the compounds identified in the ethanolic extract support the traditional use of this species in the management of bacterial diseases and oxidative stress-mediated complications in Northern Uganda. For example, compounds **1** and **2** have been reported to elicit antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [66, 68, 81-83]. Compounds **3** and **4** had antibacterial activity [75, 84], though their antioxidant activity has not been reported. The other compounds (**5-7**) have well documented bioactivities [49]. The occurrence of bioactive compounds **1-4** in this species, further lends credence to its wide use in traditional medicine in Uganda and across Africa. It also confirms that there is intraspecific variation in the bioactive compounds present in this species.

Conclusions

Isolation and characterization of the ethanol extract of *A. coriaria* leaves from Kole district of Uganda resulted in the identification of two triterpenes: oleanolic acid (**1**) and oleanolic acid acetate (**2**); an alkaloid (pterin-6-carboxylic acid **3**), and an organic alcohol (undecanol **4**), along with betulinic acid (**5**), betulin (**6**) and benzyl alcohol (**7**). Compounds **1-4** have been identified for the first time in this species. The isolated compounds possess an array of pharmacological activities, lending credence to the use of the leaves in Ugandan herbal medicine.

Acknowledgements

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