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Bioactive phytochemicals from leaf extract of *Erlangea tomentosa* S. Moore (*Bothriocline longipes* (Oliv. & Hiern.) used to treat bacterial infections in Uganda

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Abstract

Background The leaves of *Erlangea tomentosa* (ET) are used to treat several bacterial infections like respiratory tract infections, skin infections, diarrhea and gastrointestinal disorders. However, there is limited information about the compounds responsible for its bacterial activities. The study aimed at isolation and characterisation of phytochemicals from the leaf extracts of ET.

Results Three compounds were isolated from leaf extracts of ET and characterised. Two compounds; eriodictyol-7-O- β -glucoside (1) and 5, 7, 3', 4'-tetrahydroxy-7-O-[6"-O-(acetyl)- β -D-glucopyranosyloxy]-flavanone (coccinoside A) (2) were identified from ethyl acetate extract while one compound; di-(2-ethylhexyl) phthalate (3) was identified from dichloromethane extract.

Conclusion Compounds; 1, 2 and 3 were isolated and identified from ET and genus *Erlangea* for the first time. The reported antibacterial properties of compound 3 in literature could be responsible for the antibacterial activities of ET and the traditional use of ET.

Keywords *Erlangea tomentosa*, Antibacterial activity, Phytochemicals

Background

Erlangea tomentosa (ET) belongs to the genera *Erlangea* and *Asteraceae* family with about 500 species. In Uganda, ET has different names depending on the region and tribe, for instance, it is called Ekyoganyanja in Rukiga/

Runyankole [3], Ekitokotoko in Rutoro [34] and Ett-watwa in Luganda [32]. ET is abundant and widely used by local communities in Uganda to treat several bacterial infections. For instance, the leaves of ET are locally used to treat skin infections, diarrhea, Syphilis and cough [4, 33, 34]. The filtrate from the pounded mixture of rock salt and leaves of ET is used to treat colic pains, stomachache and syphilis [3]. The leaves of ET are also used to treat skin infections and promote a healthy skin [38]. The decoction of ET is taken orally to treat stomach pains [39]. The leaves of ET are locally used to treat syphilis and gastro intestinal disorders [23]. ET was also reported to have a moderate level of acute toxicity [30].

Pharmacologically, the ethanol extract of ET has been reported to be active against *Staphylococcus aureus*

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(15.0 mm) [3], ethyl acetate extract was reported to be active against *Escherichia coli* (17.3 mm), *Staphylococcus aureus* (16.0 mm), *Klebsiella pneumonia* (11.7 mm) and *Salmonella typhi* (13.7 mm) while dichloromethane extract was reported active against *Escherichia coli* (17.9 mm) and *Salmonella typhi* (10.0 mm) [35]. Furthermore, the essential oil of this plant was reported to be active against *Klebsiella pneumonia* (15 mm) and *Staphylococcus aureus* (16 mm) [3].

Several phytochemicals such as flavonoids, tannins, terpenes, terpenoids, saponins, alkaloids, reducing sugars, coumarins and steroid glycosides have been reported in the aqueous and ethanol extracts of ET [3]. Some compounds have been identified as constituents of essential oils of ET such as hexadecanoic acid, myristaldehyde, phytol, indole, 2-methoxy-4-vinylphenol, linolenic acid, dihydroactinidiolide, β -loneone, eugenol and geranylacetone [3]. Anthocyanins such as erlangidin 5-O-(4''-(*E*-caffeoyl)-6''-(malonyl)- β -glucopyranoside)-3'-O-(6'''-(3II-(β -glucopyranosyl)-*E*-caffeoyl)- β -glucopyranoside) and erlangidin 5-O-(6''-(malonyl)- β -glucopyranoside)-3'-O-(6'''-(3II-(β -glucopyranosyl)-*E*-caffeoyl)- β -glucopyranoside) have been identified from the floral parts of ET [1]. 5-alkylcoumarins have also been identified from the leaf extracts of ET [40]. In this study, ET was selected based on its reported use in treating different bacterial infections and the reported bioactivities of ethyl acetate extract and dichloromethane extract. Therefore, we report that three compounds have been isolated from ethyl acetate and dichloromethane extracts of ET.

Methods

Sample collection and preparation

Fresh leaves of ET were collected, identified and prepared following the method reported by [35]. Generally, fresh samples were collected from Nyakijumba village, Rushaki, Kabale District, Uganda and identified by Mr. Rwaburindore Portase; one of the botanists at the Herbarium, Department of Plant Sciences, Microbiology and Biotechnology, School of Physical Sciences, College of Natural Sciences, Makerere University, Uganda. An account number 50892 with voucher number EY5 was archived at the Herbarium for future reference. The samples (leaves) were chopped into smaller pieces with a knife and air dried at room temperature for 30 days. The dry samples were ground into a coarse powder and stored in an air tight polythene bag.

Extraction

The coarse powder was sequentially extracted using four solvents (hexane, dichloromethane, ethyl acetate and methanol) of different polarities starting from less polar

hexane to a more polar methanol [18]. To obtain the hexane extract, the coarse powder (340 g) was macerated in hexane (950 ml) in a 5 L capacity conical flask at room temperature for 24 h. Filtration was done using cotton wool followed by Whatman No.1 filter paper. The process of extraction was repeated three times using hexane (2500 mL). The filtrate was concentrated using a Buchi Rotary vacuum evaporator (BUCHI-R 205) at 40 °C to dryness. The residue was spread on aluminium foil, air dried for some time at room temperature and weighed. The same procedures were repeated using dichloromethane, ethyl acetate and methanol to obtain four extracts; hexane extract (ETH), Dichloromethane extract (ETD), ethyl acetate extract (ETE) and methanol extract (ETM). The extracts were weighed, transferred into sample bottles and kept in a desiccator containing anhydrous sodium sulphate for three days so as to remove any water molecules from the extracts. After three days, sample bottles were removed from the desiccator, tightly stoppered and placed in a refrigerator for further experiments. The percentage yield was determined using Eq. (1).

$$\text{Extraction percentage yield} = \frac{W_1}{W_2} \times 100\% \quad (1)$$

where, W_1 = mass of crude extracts (g), W_2 = mass of powdered sample of ET (g).

Isolation of compound 1 and 2 from ETE

Isolation of compounds from ETE (2.56 g) was done using column chromatography. The column was packed with silica gel as a stationary phase and eluted with n-hexane—ethyl acetate gradient solvent system and later ethyl acetate—methanol gradient solvent systems. The gradient solvent system was achieved by adding 5% increments of a more polar solvent. Fractions were collected at different time intervals and monitored by thin layer chromatography (TLC) and spots were visualized using ultra violet (UV) lamp and later by iodine vapor as a staining agent. All fractions collected were concentrated to dryness at 40 °C using a Buchi Rotary vacuum evaporator (BUCHI-R 205). A total of twenty-seven (27) fractions were obtained after pooling together sub-fractions of same retention factor. Fractions that were relatively pure and of reasonable quantities were each further fractionated using a smaller silica gel column of smaller diameter to obtain pure compounds.

Fraction 16 (12.50 mg) that was eluted by the solvent system of hexane: ethyl acetate (20:80 v/v) was reloaded on a smaller silica gel column and eluted using ethyl acetate (5:95 v/v) solvent system to obtain compound 1 (1.65 mg). Fraction 19 (29.90 mg) that was eluted from the bigger column using ethyl acetate- methanol (90: 10

v/v) solvent system was reloaded on a smaller silica gel column and purified using ethyl acetate: methanol (85:15 v/v) system to obtain compound 2 (17.20 mg). The dry compounds were weighed and taken for spectroscopic experiments to elucidate their structures.

Isolation of compound 3 from ETD

ETD (9.02 g) was loaded on the silica gel column and eluted with n-hexane–ethyl acetate gradient solvent system made by adding 5% increments of ethyl acetate. Fraction 2 that was relatively pure and of reasonable quantity (60 mg) was further fractionated using a smaller silica gel column of smaller diameter and seven different fractions obtained. Fraction 2 (D2-2) of reasonable quantity (21.31 mg) was again loaded on the column of smaller diameter and five different fractions were collected. Fraction 1 (D2-2-1) showed one spot on TLC plate and was therefore considered pure. It was concentrated to dryness using Buchi Rotary vacuum evaporator (BUCHI-R 205) to give a yellow viscous oil of quantity (9.03 mg) and noted as compound 3, stored in a tightly stoppered bottle and considered for several spectroscopic experiments. The percentage yield for compounds isolated from both ETE and ETD was determined using Eq. (2).

$$\text{Isolation percentage yield} = \frac{M_1}{M_2} \times 100\% \quad (2)$$

where, M_1 = mass of compound isolated (mg), M_2 = mass of crude extracts (g) loaded on the column.

Characterisation of compounds; 1, 2 and 3

Characterisation was done using different spectroscopic techniques such as Ultraviolet and Visible (UV–Vis) Spectroscopy, Fourier Transformer Infrared Spectroscopy (FT-IR), Mass Spectroscopy (MS) and Nuclear magnetic resonance (NMR) Spectroscopy [7]. Each of the samples was dissolved in methanol for UV–Vis spectroscopy. For Mass Spectroscopy experiments, each compound (0.5 mg) was put in a well labeled sample vial, dissolved in a suitable solvent to make 1 μ M solution. The sample solution (2 ml) was put into the MS machine and analysed.

For NMR analysis, compound 1 and 2 was dissolved in deuterated methanol (CD_3OD) while compound 3 was dissolved in chloroform (CDCl_3). The dissolved sample was transferred into an NMR tube through a Pasteur pipette plunged with glass wool to filter out any undissolved particles. The sample was loaded onto the NMR auto-sampler spectrometer and the spectrometer commanded to run both 1D and 2D experiments on Bruker AV-500 MHz NMR instrument. Both one dimensional (1D) ^1H -NMR and ^{13}C -NMR and two dimensional (2D) experiments including COSY, HSQC and HMBC were

employed to elucidate the structures of the compounds. The spectra obtained were carefully analysed and the results compared with the literature from different publications to characterise the compounds.

Eriodictyol-7-O- β -glucoside (1); White-pale yellowish solid; UV λ_{max} (MeOH) nm: 285, 338; IR ν_{max} (NaCl) cm^{-1} : 3462, 1647, 1547, 1518; ^1H -NMR spectral data (500 MHz, CD_3OD): aglycone δ_{H} 5.22 (1H, dd, $J=13.0, 3.4$ Hz, H-2), 3.02 (1H, dd, $J=17.2, 13.0$ Hz, H-3_a), 2.65 (1H, ddd, $J=17.2, 4.7, 3.4$ Hz H_b-3), 6.08 (1H, d, $J=2.0$ Hz, H-6), 6.11 (1H, d, $J=2.0$ Hz, H-8), 6.85 (1H, s H-2'), 6.71 (2H, s, H-5' & H-6'), glucosyl moiety 4.88 (1H, d, $J=7.0$ Hz, H-1''), 3.31 (1H, m, H-2''), 3.37 (1H, H-3'' & H-5''), 3.27 (1H, dd, H-4''), 3.61 (1H, dd, $J=12.1, 5.5$ Hz, H_a-6''), 3.88 (1H, br d, $J=12.1, 2.3$ Hz H_b-6''); ^{13}C -NMR spectral data (500 Hz, CDO_3D): δ_{C} 80.94 (C-2), 44.64 (C-3), 198.84 (C-4), 165.22 (C-5), 98.33 (H-6), 167.32 (H-7), 93.29 (C-8), 164.83 (C-9), 105.28 (C-10), 131.77 (C-1'), 115.12 (C-2'), 146.81 (C-3'), 116.62 (C-4'), 119.65 (C-6'), 101.59 (C-1''), 74.98 (C-2''), 78.56 (C-3''), 71.52 (C-4''), 78.14 (C-5''), 62.70 (C-6''); positive ESI–MS m/z 451 $[\text{M} + \text{H}]^+$, 289 $[\text{Aglycon} + \text{H}]^+$, 473 $[\text{M} + \text{H}]^+$, 901 $[2\text{M} + \text{H}]^+$, 923 $[2\text{M} + \text{Na}]^+$, calculated for $\text{C}_{21}\text{H}_{22}\text{O}_{11}$.

5, 7, 3', 4'-tetrahydroxy-7-O-[6''-O-(acetyl)- β -D-glucopyrancsyloxy]-flavanone (coccinoside A) (2); Yellow powder; UV λ_{max} (MeOH) nm: 284, 335 (sh); IR ν_{max} (NaCl) cm^{-1} : 3334, 1714, 1654; ^1H -NMR spectral data (500 MHz, CD_3OD): aglycone δ_{H} 5.26 (1H, dd, $J=11.8$ and 3.3 Hz, H-2), 3.06 (1H, dd, $J=17.5$ and 3.4 Hz, H_b-3), 2.68 (1H, dd, $J=3.4$ and 17.5 Hz, H_a-3), 6.12 (1H, d, $J=2.3$ Hz, H-6), 6.14 (1H, d, $J=2.3$ Hz, H-8), 6.85 (1H, d, $J=2.1$ Hz, H-2'), 6.72 (2H, s, H-5' & H-6'), glucosyl moiety 4.90 (1H, $J=7.0$ Hz, H-1''), 3.35 (1H, m, H-2''), 3.38 (1H, m, H-3'' & H-5''), 3.34 (1H, q, H-4''), 3.81 (1H, br d, $J=12.2$ Hz, H_a-6'') and 3.62 (1H, dd, $J=9.2, 12.2$ Hz, H_b-6''), acetyl moiety 1.94 (s, H-2''). ^{13}C -NMR spectral data (500 Hz, CDO_3D): δ_{C} 80.86 (C-2), 44.33 (C-3), 198.64 (C-4), 165.09 (C-5), 97.13 (C-6), 167.19 (C-7), 98.18 (C-8), 164.71 (C-9), 105.12 (C-10), 131.73 (C-1'), 114.96 (C-2'), 147.11 (C-3'), 146.68 (C-4'), 116.46 (C-5'), 119.48 (C-6'), 101.47 (C-1''), 74.86 (C-2''), 78.45 (C-3''), 71.39 (C-4''), 78.02 (C-5''), 61.67 (C-6''), 173.17 (C-1'''), 20.97 (C-2'''); negative ESI–MS m/z 491 $[\text{M} - \text{H}]^-$, calculated for $\text{C}_{23}\text{H}_{24}\text{O}_{12}$.

Di-(2-ethylhexyl) phthalate (3); yellow viscous oil; ^1H -NMR spectral data (500 MHz, CDCl_3): δ_{H} 7.69 (2H, dd, $J=5.7$ and 3.3 Hz, H-2 & H-5), 7.50 (2H, dd, $J=5.7, 3.3$ Hz, H-3 & H-4), 4.22 (4H, dd, $J=10.9, 5.9$ Hz, H-3' & H-3''), 1.68 (2H, m, H-4' & H-4''), 1.33 (4H, m, H-5' & H-5''), 1.35 (4H, m, H-6' & H-6''), 1.27 (4H, m, H-7' & H-7''), 0.86 (6H, t, $J=7.8$ Hz, H-8' & H-8''), 1.39 (H-9' & H-9''), 0.89 (6H, t, $J=6.84$ Hz, H-10' & H-10''). ^{13}C -NMR spectral data (500 MHz, CDCl_3): δ_{C} 132.22 (C-1 & C-6),

128.79(C-2 & C-5), 130.79(C-3&C-4), 167.67(C-1'&C-1''), 68.17(C-3'& C-3''), 38.90(C-4'&C-4''), 30.44(C-5'&C-5''), C-28.90(C-6'&C-6''), 22.90(C-7'&C-7''), C-14.00(C-8'&C-8''), 23.90(C-9'&C-9''), 10.95(C-10'&C-10''); positive ESI-MS m/z 391 $[M+H]^+$, calculated for $C_{24}H_{38}O_4$.

Results

Percentage yields of extracts and compounds

The sequential extraction process using various solvents yielded the four extracts such as ETH (8.60 g), ETD (11.44 g), ETE (3.36 g) and ETM (10.61 g). Dichloromethane had the highest yield followed by methanol, hexane and finally ethyl acetate. A summary of masses of extracts and isolated compounds and their percentage yields is presented in Table 1.

Characterisation of compound 1, 2 and 3

Compound 1: The UV-Vis spectrum of compound 1 (Fig. 1a) showed two bands; band I which is a shoulder peak between 320 to 390 nm with its maximum at 338 nm and band II between 260 to 310 nm with its maximum at 285 nm. These two bands are characteristic to flavonoids therefore the UV-Vis spectrum gave a clue that compound 1 could be a flavonoid [28]. The presence of a shoulder peak of band I [17] [28]) indicated that compound 1 could be a flavanone [29]. Band I represents a B-ring absorption due to the cinnamoyl system and band II represents the absorption that can be assigned to the A-ring benzoyl system [6]. The FT-IR spectrum (Fig. 1b) helped in identification of several functional groups of compounds 1. The characteristic broad peak at 3462 cm^{-1} was due to the O-H stretch of compound 1. The prominent peak at 1654 cm^{-1} indicated the C=O stretch of carbonyl (ketone) group of the compound. The peak at 952 cm^{-1} was due to out-of-plane C-H stretch of the benzene ring (s) that indicated the presence benzene ring (s) of compound 1.

The $^1\text{H-NMR}$ spectrum (Fig. 1c) identified aromatic protons of A-ring and B-ring. The three protons observed at δ 6.83 (1H, br s, H-2'), 6.71 ppm (1H, d, H-5') and 6.70 ppm (1H, d, H-6') were assigned to the AMX pattern of the B-ring. The two protons observed at δ

6.12 ppm and 6.09 ppm and were assigned to AX system of A-ring. The two protons; H_a-3 (δ 5.31 ppm) and H_b-3 (δ 2.70 ppm) that are on the same carbon atom with a coupling constant, $J=3.4\text{ Hz}$ because of the stereochemistry between the protons and they are attached on carbon atom next to a chiral center; and this is characteristic of protons of a flavanone [16, 17]. The peak at δ 4.89 ppm (1H, d, $J=7.0\text{ Hz}$) was assigned to an anomeric proton and this indicated the presence of a sugar substituent. The coupling constant, $J=7.0\text{ Hz}$ identified the sugar substituent as β -glucose [17]. Starting from the anomeric proton at δ 4.89, other protons in the sugar were identified by COSY spectrum at δ 3.38 ppm (H-2''), 3.60 ppm (H-3''), 3.36 ppm (H-4''), 3.58 ppm (H-5''), 3.88 ppm (H-6''a) and 3.61 ppm (H-6''b). The two protons at position 6'' (H_a-6'' and H_b-6'') of a glycoside molecule with δ 3.67 and 3.88 ppm respectively and with $J=12.1\text{ Hz}$ implying that the protons were next to a chiral center [11]. There were also seven aromatic carbon atoms (δ 165.21 ppm, 167.31 ppm, 164.84 ppm, 105.28 ppm, 131.90 ppm, 146.82 ppm, 147.24 ppm, δ 98.33 ppm, 93.0 ppm, 115.12 ppm, 116.62 ppm and 119.65 ppm) and a peak of a carbonyl carbon (ketone) at δ 198.10 ppm. The peaks of carbon atoms of C-ring in positions 2, 3 and 4 at δ 80.92 ppm, 44.20 ppm and 198.10 ppm respectively are characteristic to a flavanone skeleton [16, 17]. Using HSQC experiments, five methine carbon atoms (δ 98.33 ppm, 93.0 ppm, 115.12 ppm, 116.62 ppm and 119.65 ppm) of aromatic rings, six methine and two methylene saturated carbon atoms were identified. The HMBC spectrum (Fig. 1 f) identified quaternary carbon atoms, the connectivity of rings A, B, C and the glycoside. Ring B was connected to ring C by cross peak H-2' (δ 6.94 ppm)/ C-2 (δ 80.92 ppm) through a $^3J_{CH}$ and cross peak H-2 (δ 5.32 ppm)/C-2' (δ 115.12 ppm) through a $^3J_{CH}$. Proton H-3 of ring C also correlated with carbon C-1' of ring B through a $^3J_{CH}$. The connectivity of the glycoside to ring A was through the anomeric proton H-1'' at δ 4.49 ppm that correlated with carbon C-7 at δ 167.31 ppm of ring A through a $^3J_{CH}$. Using data from UV-Vis, FT-IR and NMR spectra, the molecular formula; $C_{21}H_{22}O_{11}$ was assigned to compound 1. The

Table 1 The percentage yield of each leaf extract of ET

Extract	Mass of the powder (g)	Mass of the extract (g)	Yield (%)	Mass loaded on the column (g)	Mass of the compound (mg)	Yield (%)
ETH	340.00	8.60	2.53	ND	ND	ND
ETD	331.00	11.44	3.45	9.02	9.03	0.10
ETE	318.30	3.36	1.06	2.56	1.65	0.06
					17.20	0.67
ETM	314.00	10.61	3.38	ND	ND	ND

ETH=hexane extract, ETD=dichloromethane extract, ETE=ethyl acetate extract, ETM=methanol extract, ND=isolation not done



mass spectrum of compound **1** exhibited a molecular ion $[M+H]^+$ peak at m/z 451.1 that was observed from ESI-MS (Fig. 1h). Also the pseudo-molecular fragment ions $[2 M+Na]^+$ peaks at m/z 923.2, $[2 M+H]^+$ at m/z 901.2 and $[M+Na]^+$ at m/z 473.1 were identified on the mass spectrum. These pseudo-molecular fragment ions are characteristic to flavonoids and are observed commonly when a sample is concentrated [17]. The fragment ion peak at m/z 289.1 was assigned to a resultant protonated aglycone ion $[M-Glu+H]^+$. The MS data confirmed compound **1** as a flavanone with a glycoside substituent [17] and was therefore identified as eriodictyol-7-*O*- β -glucoside.

Compound 2: The 1H -NMR spectra of compound **2** was similar to that of compound **1** indicated by the similarity in chemical shifts and coupling constants. The only difference was noticed at the peak at δ 1.95 ppm that appeared as a singlet integrating into 3 protons (methyl group) and that indicated that these protons do not couple with any other protons of compound **2**. The presence of peaks on ^{13}C -NMR spectra (Fig. 2d) of compound **2** at δ 173.17 (C-1'') ppm and 20.97 (C-2'') that were absent on ^{13}C -NMR spectrum (Fig. 1d) of compound **1** also brought out the differences between compound **1** and **2**. The HMBC correlation of H-2'' δ 1.94 ppm and C-1'' at δ 173.17 ppm through a $^2J_{CH}$ indicated compound **2** was acetylated while compound **1** was not. The mass spectrum of compound **2** had a molecular ion $[M-H]^-$ peak at m/z 491.0 on negative mode of ESI-MS that corresponded to its molecular ion [5]. Compound **1** and **2** were similar compounds Fig. 4, the only difference was on the glycoside carbon (C-6'') where compound **2** was acetylated while compound **1** was not. Generally, spectroscopic experimental results of compound **2** were similar to the spectra data for 5,7,3',4'-tetrahydroxy-7-*O*-[6''-*O*-(acetyl)- β -D-glucopyransyloxy]flavanone (coccinoside A) that has been isolated from *Pyracantha coccinea* leaves [5] and therefore, were considered as the same compounds.

Compound 3: The 1H NMR spectrum (Fig. 3 a) of compound **3** showed downfield peak at δ 7.68 ppm that is a doublet of a doublet (dd) with coupling constant $J=5.7, 3.3$ Hz and this was assigned to protons H-2 and H-5 (Fig. 3 and Fig. 4). Another peak in the downfield region observed at δ 7.5 ppm was a doublet of a doublet (dd) with coupling constant $J=5.7, 3.3$ Hz was assigned to H-3 and H-4. These two peaks in the downfield region indicated the presence of aromatic protons of the benzene ring. The presence of two doublet of doublet (with the same coupling constants), each representing two equivalent aromatic protons suggested the presence of an ortho di-substituted benzene ring bearing the same substituent in both positions. This indicated that compound **3** is a

symmetrical molecule. Peaks observed at δ 4.22 ppm that was doublet of a quartet (dq) with coupling constants $J=10.9, 5.9$ Hz were assigned to H-3' and H-3'' that were on a carbon atom bonded to an ester (C-O linkage). The peak at δ 1.68 ppm that was a triplet of a pentet indicated the presence of methine proton that was assigned to H-4' and H-4'' that is attached to carbon atom adjacent to the carbon atom that was bonded to an ester (C-O linkage). Finally, the peaks with chemical shifts in the up-field region between δ 1.39–0.86 ppm that are multiplets were attributed to a number of methylene and methyl protons. The information obtained from 1H NMR spectrum indicated that compound **3** has a total of 38 protons. On the COSY spectrum, a cross peak at δ 7.69 ppm (H-2/H-5) and 7.50 ppm (H-3/H-4) implied that proton H-2/H-5 and H-3/H-4 were attached to adjacent carbon atoms. A cross peak at δ 4.22 ppm (H-3'/H-3'') and 1.68 ppm (H-4'/H-4'') implied that proton H-3'/H-3'' and H-4'/H-4'' are attached to adjacent carbon atoms. In the rest of the downfield region, complex correlations were observed indicating correlations between aliphatic methyl and methylene protons of compound **3**. A number of cross peaks were identified by HSQC spectrum (Fig. 3 d). In the downfield region, methine cross peaks at δ 7.69 ppm/ δ 128.79 ppm was assigned to H-2/H-5 correlating to C-2/C-5 whereas δ 7.50 ppm/30.79 was assigned to H-3/H-4 correlating to C-3/C-4. A proton peak at δ 4.22 ppm was observed correlating the carbon peak at δ 68.17 ppm indicating a methylene group. In the up-field region, a proton peak at δ 1.68 ppm correlated with a carbon peak at δ 38.90 ppm indicating the methine group. In the rest of the up-field region several proton and carbon correlations were observed at δ 1.33 and 30.44 ppm for a methylene of H-5'/H-5'' and C-5''/C-5'', δ 1.35 ppm and 28.90 ppm for a methylene of H-6'/H-6'' and C-6'/C-6'', δ 1.27 and 22.90 ppm for a methylene of H-7'/H-7'' and C-7'/C-7'', δ 0.86 and 14.00 ppm for a methyl of H-8'/H-8'' and C-8'/C-8'', δ 1.39 and 23.90 ppm for a methylene of H-9'/H-9'' and C-9'/C-9'' and finally δ 0.89 and 10.95 ppm for a methyl group of H-10'/H-10'' and C-10'/C-10'' respectively. The general connectivity in the molecule was identified by HMBC spectrum (Fig. 3e) H-2 at δ 7.69 ppm showed a correlation with carbon atom at δ 132.40 ppm through a $^2J_{CH}$, C-3 at δ 130.79 ppm through a $^2J_{CH}$ and C-1' at δ 167.67 ppm through a $^3J_{CH}$. These identified the attachment positions of the two identical aliphatic parts of the molecule to the aromatic ring. H-3 at δ 7.50 ppm showed correlation with C-2 at δ 128.8 ppm through $^2J_{CH}$ and C-1 at δ 132.22 ppm through a $^3J_{CH}$. H-3' at δ 4.22 ppm showed correlation with C-4' at δ 38.90 ppm, C-5' at δ 30.44 ppm and C-1' at δ 167.67 ppm. Using data from UV-Vis, FT-IR and NMR

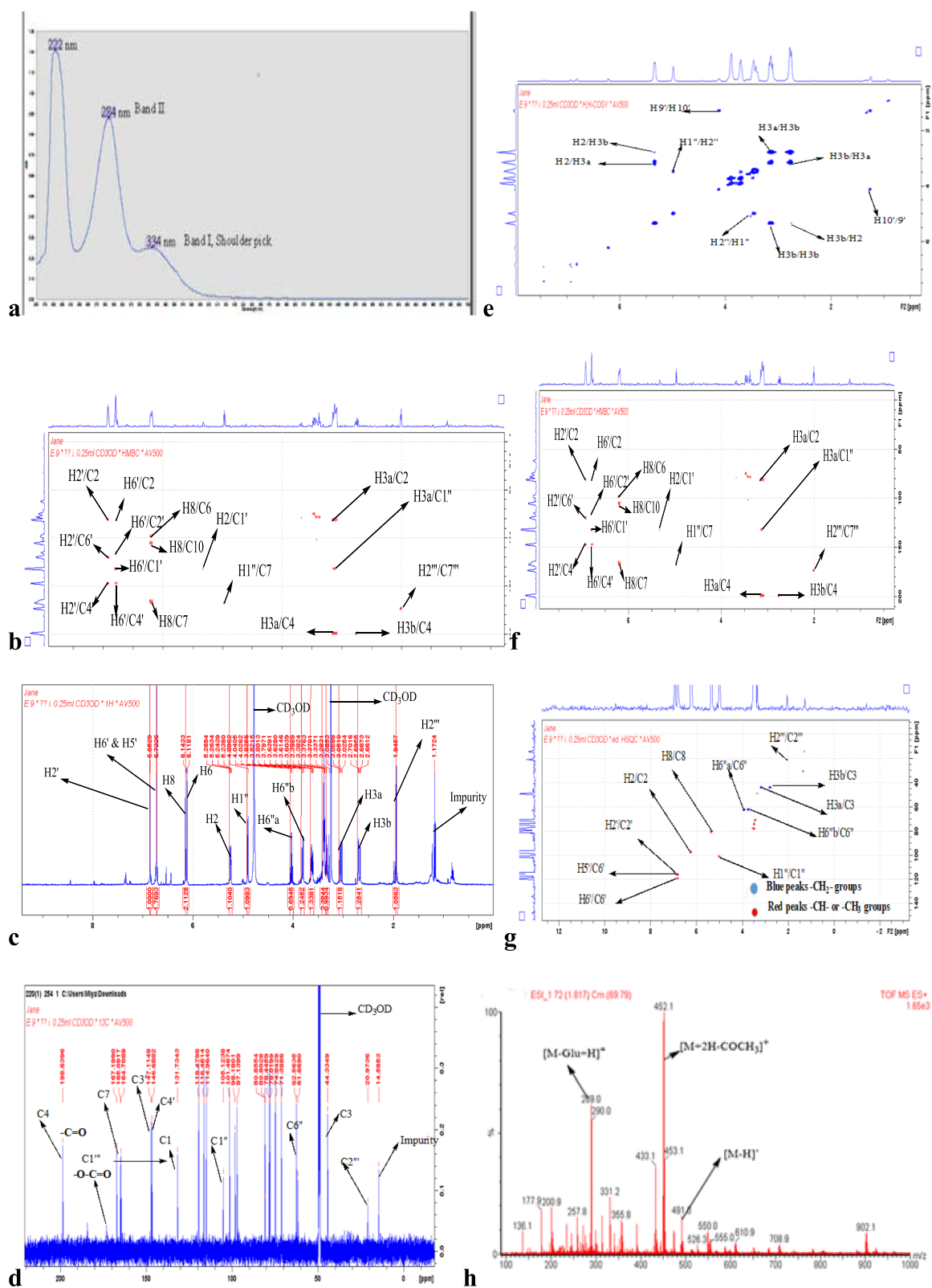


Fig. 2 Spectra data for compound **2**: **a** UV-Vis, **b** FT-IR, **c** ^1H NMR, **d** ^{13}C NMR, **e** COSY, **f** HMBC, **g** HSQC, **h** MS

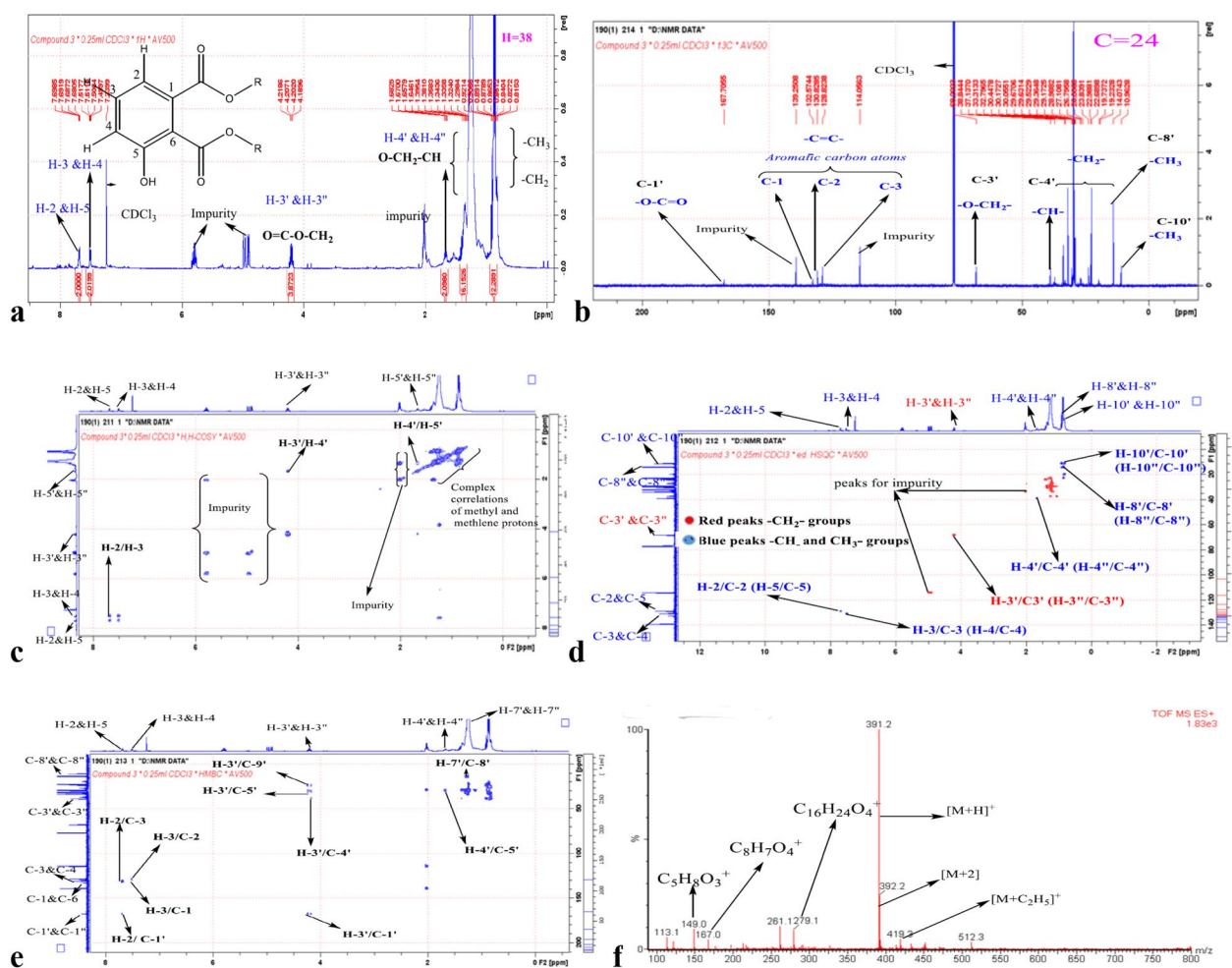


Fig. 3 Spectra data for compound **3**: **a** ^1H NMR, **b** ^{13}C NMR, **c** COSY, **d** HSQC, **e** HMBC **f** MS

spectra, the molecular formula; $C_{24}H_{38}O_4$ was assigned to compound 3.

The ESI–MS spectrum (Fig. 3f) of compound **3** showed various characteristic fragmentation patterns at m/z 391.2, 279.1, 261.1, 167.0, 149.0 and 113.1. The ion peak at m/z = 391 was assigned to the molecular ion $(M + H)^+$ while the fragment ion m/z = 167.0 was due to the McLafferty rearrangement and it was assigned to the fragment $C_8H_7O_4^+$ [22, 42]. The fragment ion peak at m/z = 149.0 was assigned to the cleavage of esters with shift of two hydrogen atoms and followed by elimination of water molecules and this was assigned to fragment $C_8H_5O_3^+$. The fragment ion at m/z = 113.1 was due to the retention of a positive charge by the alkyl group and this was assigned to fragment $C_8H_{17}^+$. The fragmentation pattern of m/z 149.0, 167.0 and 279.1 is characteristic for phthalates [22]. The MS spectra of compound **3** confirmed a phthalate with a characteristic molecular ion $[M + H]^+$

peak at $m/z = 391$ for molecular weight 390 and was identified as di-(2-ethylhexyl)phthalate (DEHP).

Discussion

The varying yields of extracts obtained using solvents of different polarities indicate the presence of phytochemicals with a wide range of polarities in the leaves of ET. The extraction yields were influenced by the polarity of the solvents and the solubility of the chemical constituents in the plant powder [43]. This suggests that ET contains chemical compounds with diverse polarities, as evidenced by the ability of each solvent to yield a significant mass of extract.

Eriodictyol-7-O-glucoside (**1**) has been isolated from *Juelia Subterranea* (*Balanophoraceae*) (Desalbres, Duteil, Lallemand, De Groot-Pfleiderer, & Verloza, 1977; [31]). All the spectroscopic experimental results were close to spectroscopic data from literature of eriodictyol-7-O- β -glucoside [36]. Therefore, all

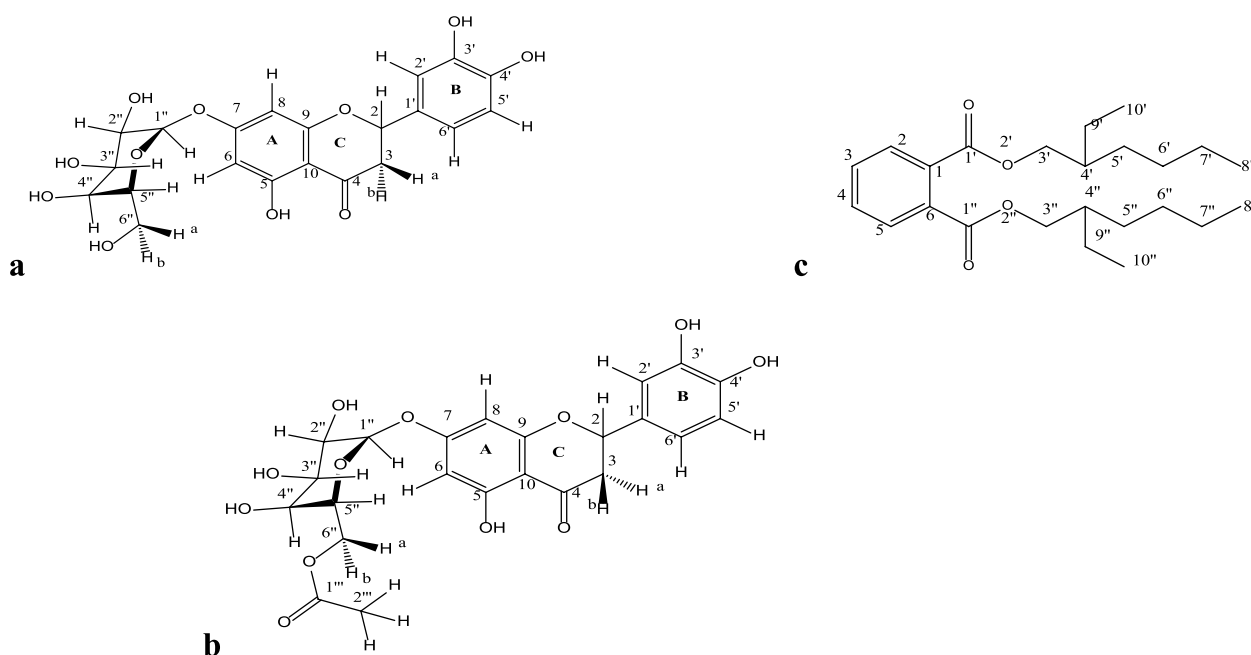


Fig. 4 Structures of isolated compounds: **a** Eriodictyol-7-O-glycoside (**1**), **b** 5,7,3',4'-tetrahydroxy-7-O-[6''-O-(acetyl)-β-D-glucopyranosyloxy]-flavanone (**2**) and **c** di-(2-ethylhexyl) phthalate (**3**)

the information obtained from UV-Vis, FT-IR, NMR and ESI-MS data and literature confirmed compound **1** as eriodictyol-7-O-β-glucoside. Compound **2**, that was identified as 5,7,3',4'-tetrahydroxy-7-O-[6''-O-(acetyl)-β-D-glucopyranosyloxy]-flavanone (coccinoside A). The spectra and structures of compound **1** and **2** were similar. The only difference was noticed on the acetyl moiety present at position 6'' of the sugar moiety (Fig. 4). 5,7,3',4'-tetrahydroxy-7-O-[6''-O-(acetyl)-β-D-glucopyranosyloxy]-flavanone (coccinoside A) has been isolated and identified from *Pyracantha coccinea* leaves [5]. Despite the fact that there is no reported antibacterial activity of eriodictyol-7-O-glycoside (**1**) and coccinoside A (**2**), similar compounds such as eriodictyol, luteolin and quercetin are reported to exhibit antibacterial activity [2, 8, 27]. This implies that compound **1** and **2** could also be having antibacterial properties. Eriodictyol-7-O-glycoside is reported to have some other biological activities such as treatment of Meniere's disease [41] and antioxidant activities. It was reported to have a protective effect on cisplatin-induced toxicity in the renal mesangial cell line of the human body [21]. Eriodictyol-7-O-glycoside activates the nuclear erythroid 2-related factor 2-antioxidant responsive element that is directly associated with its neuro-protection against ischemia [21]. The reported activity of eriodictyol-7-O-glycoside was attributed to the presence of hydroxyls at position 5 of ring A and position 3' and 4' of ring B. For instance, the presence of hydroxyl groups at the 3' and 4' positions of ring B (a pyrogallol

group) could be responsible for the antioxidant activity compound **1**. The presence of hydroxyl group in compound **1** and **2** (Fig. 4) indicated that, these compounds could also have a wide range of biological activities like other flavonoids.

DEHP (**3**) has been isolated from *Ambrosia maritima* [10], *Calotropis gigantea* flower [20], white shrimp muscle (*Litopenaeus vannamei*) [25], ripened fruits of *Nauclea latifolia* [15], *Aloe vera* Linne [24], *Calotropis gigantea* flower [20], *Penicillium janthinellum* (O. H. [13]) and *Aspergillus awamori* [26]. DEHP is reported to have a wide range of antibacterial properties for example, it was reported to be active against *Staphylococcus aureus* (1.47 µg/ml), *Bacillus subtilis* (3.5 µg/ml), *Streptococcus equosemens* (2.37 µg/ml), *Pseudomonas aeruginosa* (6.2 µg/ml) and *Escherichia coli* (5.4 µg/ml) (M. H. [12]). Another study reported DEHP with abroad spectrum antibacterial activity against several organisms such as *Bacillus subtilis* (37.5 µg/ml), *Staphylococcus aureus* (18.75 µg/ml), *Staphylococcus epidermidis* (9.37 µg/ml), *Escherichia coli* (37.5 µg/ml), *Pseudomonas aeruginosa* (75 µg/ml) and *Klebsiella pneumonia* (75 µg/ml) [37]. DEHP was reported to be moderately active against *Bacillus subtilis* and *Staphylococcus aureus* at 100 µg/ml and had activity similar to tetracycline at 1 mg/ml against the same organisms [15]. DEHP was also reported to be a bioactive principle against anti-leukaemic and anti-mutagenic effect in-vitro [24]. It is also reported to have antitumour

activity against Ehrlich ascites carcinoma cells in-vitro [20], and as a pro inflammatory agent in human blood by different studies [19]. DEHP was reported to have broad spectrum mosquito larvicidal activity and the activity was attributed to inhibition of acetyl cholinesterase enzyme activity and therefore a good antimalarial agent [37]. According to reported bioactivities of DEHP, it contributes to several medicinal properties of ET and therefore supports the use of the plant species by the local community.

Conclusion

Eriodictyol-7-*O*- β -glucoside (1) and 5,7,3',4'-tetrahydroxy-7-*O*-[6"-*O*-(acetyl)- β -D-glucopyranosyloxy]-flavanone (coccinoside A) (2) were isolated and identified from ETE while DEHP (3) was isolated and identified from ETD. These compounds are being reported for the first time as constituents of *Erlangea* genus and the Asteraceae family, with the exception of DEHP, which was previously isolated from *Ambrosia maritima*, also within the Asteraceae family. The identification of DEHP in ET suggests that this compound may play a key role in the plant's antibacterial activity, thereby validating its traditional use by local communities as a remedy for bacterial infections. The identification and characterization of bioactive compounds, such as DEHP, in ET leaves lay the groundwork for standardizing herbal medicinal products derived from this plant. The characterized compounds, particularly DEHP, can serve as marker compounds for standardizing ET-based herbal products. Future studies may focus on evaluating the antibacterial activity of the isolated compounds and exploring the methanol extract for further compound isolation and characterization.

Abbreviations

μ g	Micrograms
^{13}C -NMR	Carbon-13, nuclear magnetic resonance spectroscopy
1D	One Dimensional
^1H -NMR	Proton Nuclear magnetic resonance spectroscopy
2D	Two Dimensional
CD_3OD	Deuterated methanol
CDCl_3	Deuterated chloroform
COSY	Correlation spectroscopy
ET	<i>Erlangea tomentosa</i>
ETD	Ethyl acetate extract
ETE	Dichloromethane extract
ETH	Hexane extract
ETM	Methanol extract
DEHP	Di-(2-ethylhexyl)phthalate
UV-Vis	Ultra-violet and visible spectroscopy
FT-IR	Fourier transform-infra red spectroscopy
HMBC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
m/z	Mass to charge ratio
mg	Milligrams

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Author contributions

Jane Namukobe and Yeremiah Ekyibetenga conceptualized the research study, collected data, analyzed it and wrote the main manuscript text, analysis of NMR data during the structural elucidation of compounds, MS analysis of isolated compounds. Robert Byamukama, Adebola Omowunmi Oyedele and Mathias Heydenreich: data analysis. All authors reviewed the manuscript.

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Availability of data and material

Not applicable. They can be available if they are requested by anyone.

Declarations

Ethics approval and consent to participate

All experimental research and field studies were performed in accordance to the WHO guidelines (2003) on good agricultural and collection practices for medicinal plants.

Consent for publication

Not applicable.

Plant authentication

The plant was identified and authenticated by Mr. Rwaburindore Portase, Makerere University, Herbarium, Department of Plant Science, Microbiology and Biotechnology, College of Natural Sciences.

Competing interests

The author declare no competing interests.

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