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Ceibinin, a new positional isomer of mangiferin from the inflorescence of *Ceiba pentandra* (Bombacaceae), elicits similar antioxidant effect but no anti-inflammatory potential compared to mangiferin

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ABSTRACT

Ceiba pentandra (L.) Gaertn. (Bombacaceae) is popular for the quality of its wood. However, its leaf, stem bark and root bark have been popular in ethnomedicine and, apart from the inflorescence, have been subject of extensive phytochemical investigations. In this study, two compounds were isolated from the crude methanol extract of the inflorescence. Through data from UV, NMR, MS, electrochemical studies, differential scanning calorimetry, and thermogravimetric analysis, the structures were elucidated as 3-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (**1**) and 2-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (mangiferin, **2**). They were assessed for antioxidant efficacy (DCFDA assay) and for anti-inflammatory efficacy using the lipopolysaccharide (LPS)-induced inflammation model in the RAW 264.7 macrophages (nitrite levels quantified, using Griess Assay, as surrogate for nitric oxide (NO)). Compound **1** (named ceibinin) was established as a novel positional isomer of mangiferin (**2**). While both **1** and **2** were antioxidant against basal and hydrogen peroxide (100 μM)-induced oxidative stress (6.25 μg/ml abrogated peroxide-induced oxidative stress), ceibinin (**1**) demonstrated no anti-inflammatory potential, unlike mangiferin (**2**) which, as previously reported, showed anti-inflammatory effect. Our work reports a positional isomer of mangiferin for the first time in *C. pentandra* and demonstrates how such isomerism could underlie differences in biological activities and thus the potential for development into therapeutics.

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1. Introduction

Mangiferin (2-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone, **2**) occurs in angiosperms and ferns and was first reported from *Mangifera indica* (Anacardiaceae) [1–3]. Mangiferin has evolved to be an important molecule of interest due to the plethora of important biological activities it possesses [4–6]. For instance, it has renoprotective [7], radioprotective [8], cardioprotective [9,10], immunomodulatory [11,12], antidiabetic [13], anti-inflammatory [14], anticancer [15,16], and antiviral activities [17–19]. An isomer, isomangiferin (4-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone), was isolated from the aerial parts of *Anemarrhena asphodeloides* [20]. Another isomer (2-*C*-*β*-D-glucopyranosyl-1,3,5,6-tetrahydroxyxanthone) with a different oxidation pattern from that of mangiferin was reported from *Canscora decussate* [21]. Aritomi and Kawasaki [20] concluded that the co-existence of mangiferin with 4-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (isomangiferin), 2-*C*-*β*-D-glucopyranosyl-1,3,5,6-tetrahydroxyxanthone, 4-*C*-*β*-D-glucopyranosyl-1,3,5,6-tetrahydroxyxanthone, as well as with 3-(or 5-) *C*-glucosyl maclurin 'would not be improbable'. Therefore, in our work, we sought to identify the potential co-existence of mangiferin with another isomer by studying the inflorescence of *C. pentandra*, a part of the plant that has not been the subject of extensive phytochemical investigations, unlike, for example, the leaf, stem bark and root bark. In this study, we report the isolation of a new positional isomer of mangiferin based on the 3-*C*-*β*-D-glucopyranosyl maclurin skeleton (**1**, named ceibinin) from *Ceiba pentandra* (L.) Gaertn (syn. *Bombax pentandrum* L.). (Bombacaceae) and describe its antioxidant and anti-inflammatory effects compared to mangiferin (**2**).

C. pentandra is a deciduous tree growing up to 70 m tall, with a prickly, buttressed trunk. It flowers in December and January. In Nigeria, the tree is known for its white wood. Medicinally, in many parts of Africa, the bark decoction is used for treating stomach complaints, hernia, diarrhoea, or to relieve localised oedemas or wash sores, furuncles and leprous macules [22]. The plant's extract is also applied to treat blennorrhoea, heart trouble, asthma, and as gargle for gingivitis, aphtes (mouth ulcer) and, sometimes, toothache [22]. Previous phytochemical studies on various parts of *C. pentandra* have reported the isolation of several sesquiterpenoids from the root bark [23], isoflavones from the stem bark [24,25], and naphthoquinones from the heartwood [26]. Mangiferin (**2**) has recently been reported from the inflorescence [27]. Continuation of our phytochemical investigation of the inflorescence afforded another isomer of mangiferin, as herein reported.

2. Results and discussion

Compound **1** was isolated as a greenish-yellow amorphous powder, while compound **2** was obtained as a yellow powder. Both compounds (**1** and **2**) displayed the same λ_{max} at 240, 259, 320 and 370 nm in the UV spectra, characteristic of the presence of xanthone moieties [28–30]. HRTOFMS ES indicated that the two compounds are close isomers, with the high resolution mass spectrometry giving a signal at *m/z* 421.0759 (calculated 421.0771, $[M - H]^-$) in the negative mode and 445.0744 (calculated 445.0776) for $[M+Na]^+$ in the positive mode, for compounds **1** and **2,** respectively.

Comparison of the NMR data (Table 1) with literature values [31] indicated that the isolated compounds were xanthones. The ^{1}H NMR spectra of compounds **1** and **2** showed three singlets at δ_H: 7.24, 6.62, 6.31 and 6.91, 6.14, 6.02 for each compound, indicating the presence of a xanthone ring system. Compound **2** was isolated previously from *C. pentandra* [27] and identified as mangiferin. Therefore, compound **1** was thought to be isomangiferin. However, the ^{13}C NMR spectral data of compound **1** lacked the diagnostic

Table 1
NMR data of ceibinin, mangiferin and isomangiferin.

Ceibinin NMR data (400 MHz (DMSO-d_6))						
S/No	a	b	c	^{1}H NMR	^{13}C NMR	HMBC Correlations
1	161.6	161.6	161.8	–	161.8	
2	107.3	106.6	97.6	–	163.7	
3	163.6	168.0	163.7	–	107.3	
4	93.3	93.1	104.5	6.62 ppm (1H, s)	93.2	109.0, 145.1, 152.1, 161.8 and 178.4
4a	156.1	155.7	156.5	–	156.1	
10a	150.7	154.6	151.4	–	152.1	
5	102.5	103.0	103.0	7.24 ppm (1H, s)	101.0	145.1, 152.1, 161.8 and 178.4
6	153.6	155.7	155.0	–	156.1	
7	143.7	147.3	144.3	–	145.1	
8	108.1	106.6	107.9	6.31 ppm (1H, s)	107.3	101.0, 156.1, 163.7 and 178.4
8a	111.7	109.4	111.7	–	109.0	
9	179.0	176.5	179.6	–	178.4	
9a	101.2	100.6	102.2	–	101.0	
1′	73.0	73.6	73.2		73.2	
2′	70.3	70.4	71.2		70.3	
3′	78.5	79.1	79.1		79.1	
4′	70.0	69.8	71.4		70.6	
5′	81.3	81.5	81.9		81.5	
6′	61.4	61.5	62.1		61.5	

[a] Mangiferin [32].
[b] Mangiferin [27].
[c] Isomangiferin [20].

signals of isomangiferin at δ_C 97.6 and 104.5 for C-2 and C-4, respectively. Instead, the proton signal at δ_H 6.31 was observed to have a cross peak in the HSQC with the carbon signal at δ_C 93.2 resembling C-4 of mangiferin. In compound **1**, the HMBC spectrum showed $^{1}H–^{13}C$ long-range correlation between the anomeric proton (δ_H 4.57) and the signal at δ_C 107.5 (C-3), instead of 104.5 (C-4) for isomangiferin (Table 1), allowing the placement of the glycoside unit on C-3. It was observed that the aryl protons for compound **1** and mangiferin (**2**) were similar, suggesting that in both compounds, carbons 4, 5 and 8 were aromatic methine carbons but with differing oxygenation pattern. In compound **1,** the proton signal at δ_H 7.24 was observed to have long-range correlation, with the signal at δ_C: 145.0, 152.1, 161.7 and 178.4 in the HMBC spectrum, while the signal at δ_H 6.62 correlated with the same set of carbon signals in addition to the signal at δ_C 109.0, indicating that the proton signals at δ_H 7.24 and 6.62 were in similar chemical environment and were thus assigned H-5 and H-8, respectively. The signal at δ_H 6.31 correlated with the signals at δ_C:101.0, 107.5, 156.1, 163.7 and 178.3 and was assigned to H-4. We reasoned that the attachment of the glucose unit at carbon 3 makes the signal of H-4 to be the most upfield of the aryl protons in compound **1**, unlike in mangiferin where the most upfield signal is H-8. Therefore, supported by careful consideration of the chemical shift values, compound **1** was identified as 3-*C*-β-D-glucopyranosyl-1,2,6,7-tetrahydroxyxanthone (named **ceibinin,** Fig. 1).

In establishing the structures, we proposed different oxidative reactions for the isomers, underpinned by the presence of two catechol groups on compound **1,** compared with only one in mangiferin (see Fig. 2). To further support this proposition, electrochemical measurements on the isomers were conducted using cyclic voltammetry (CV). Fig. 3A displays cyclic voltammograms recorded for 10 μM mangiferin isomers (compound **1** and mangiferin) in 0.1 M $HClO_4$, showing well-defined, irreversible, anodic peaks at 0.42 V at the screen-print carbon electrode surface for both isomers, which agreed with previous measurements of mangiferin [33, 34]. Thus, it was concluded that the redox potentials for the two isomers are similar.

The voltammograms behaviour (Fig. 3B and C) when the scan rate was increased from 50 to 300 mV s^{-1} is shown below. A linear relationship was observed between peak current intensity and the square root of the scan rate, illustrated in Fig. 3D, which indicated that the oxidation processes were diffusion controlled. Overall, compounds **1** and **2** showed similar CV behaviour and electrochemistry.

For further evidence to demonstrate the difference between compound **1** and mangiferin, they were subjected to Differential Scanning Calorimetry (DSC) (Fig. 4A). Mangiferin had an onset melting temperature of 266.62 °C, which ended at 279.10 °C, with the peak temperature being 275.30 °C. For compound **1,** the onset melting temperature was 263.40 °C, the end temperature was 273.14 °C, with the peak temperature being 268.55 °C. It was observed that, for compound **1,** the DSC curve showed a perturbation of the curve before 150 °C, unlike in mangiferin. Therefore, Thermogravimetric Analysis (TGA) was carried out to understand the observed DSC profiles. It was observed that, compound **1,** unlike mangiferin, melted with decomposition, losing 24.26 % w/w of the mass of the compound (Fig. 4 – B and C).

Having established phytochemically, electrochemically and thermogravimetrically that ceibinin (**1**) and mangiferin (**2**) are different but related, we extended our curiosity to the potential differences in their biological activities. As the known compound mangiferin has been shown to have antioxidant properties [4,35,36], we assessed and compared the abilities of ceibinin and mangiferin to alter basal levels of intracellular reactive oxygen species (ROS), as well as their abilities to modulate increased ROS induced by hydrogen peroxide, an oxidative stressor. Ceibinin and mangiferin each up to 100 μM for 24 h did not have any significant effect on cell viability. We show that both compounds significantly and concentration-dependently reduced basal levels of intracellular oxidative stress (Fig. 5 – A, B), as well as peroxide-induced intracellular oxidative stress (Fig. 5 – C, D) in cultured HeLa cells. Their inhibitory effects on basal oxidative stress were more pronounced at 24 h than at 3 h (Fig. 5 – A, B). They were largely equipotent in their reduction and eventual abrogation of peroxide-induced oxidative stress, whether at 3 h or 24 h (Fig. 5 – C, D). They demonstrated high antioxidant potency, such that, at 24 h, the effect of peroxide (100 μM) was abolished by 6.25 μg/ml (14.8 μM) of each compound. We consider the potent antioxidant effects of the two compounds to be attributable, at least, in part, to the presence of the phenolic groups.

Mangiferin has also been reported to have anti-inflammatory activity [4,14,37,38]. We therefore assessed this activity for both mangiferin and ceibinin, using the LPS-induced nitric oxide (NO) model in the RAW264.7 cells, in which the Griess Assay has been used to quantify nitrite release, which is a surrogate for the production of NO, an inflammatory mediator [39] Neither compound was cytotoxic to the RAW 264.7 cells. Our study demonstrated that mangiferin (CP2) inhibited LPS-induced nitrite release concentration-dependently, albeit with moderate-to-low potency (Fig. 6A), an effect similar to that of the positive control parthenolide, an anti-inflammatory natural compound [40], which also but very potently inhibited LPS-induced nitrite release in a

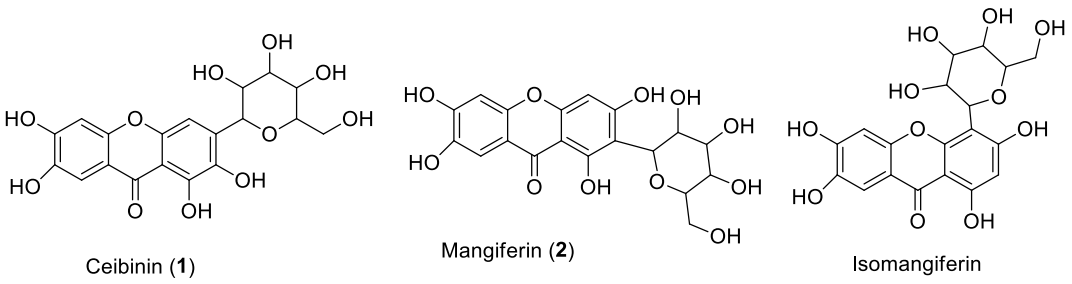

Fig. 1. Structures of ceibinin (**1**), mangiferin (**2**) and isomangiferin.

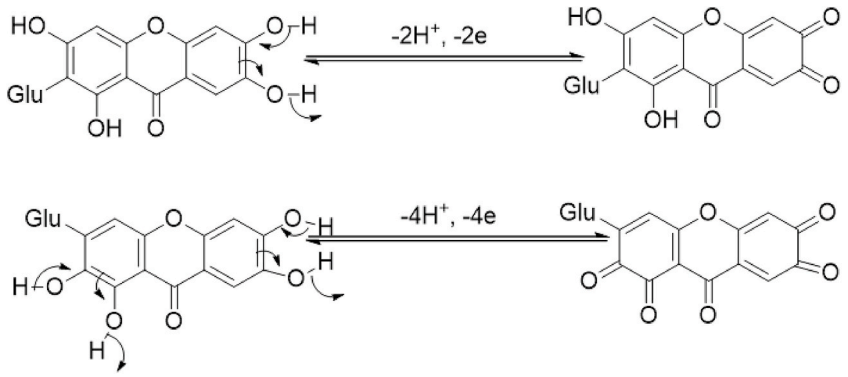

Fig. 2. Proposed reducing pattern for mangiferin (above) and compound 1 (ceibinin, below).

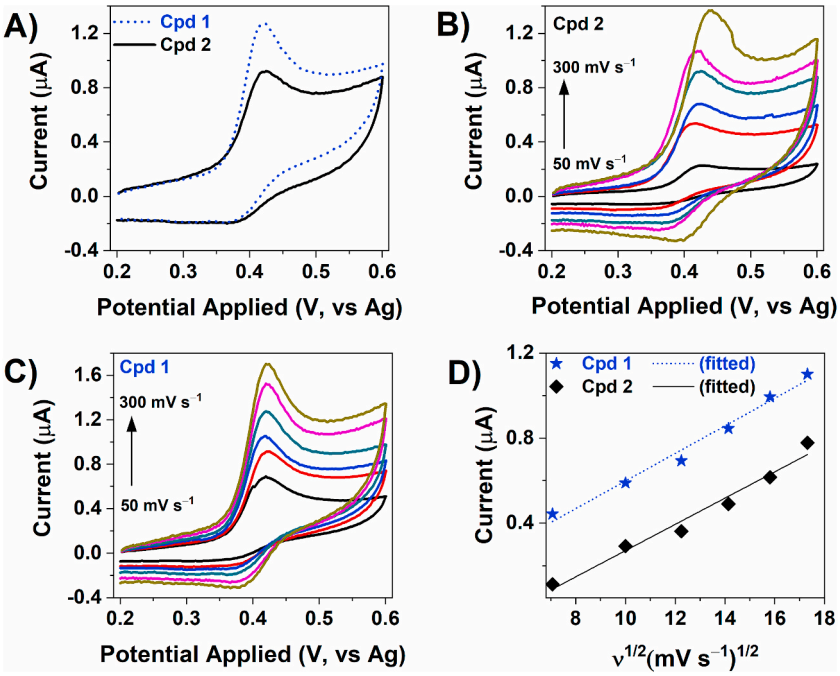

Fig. 3. A. Cyclic voltammograms of 10 μM mangiferin (**2**) (a) and ceibinin (**1**) (b) in 0.1 M $HClO_4$. $\nu = 200$ mV s^{-1}. **B.** Cyclic voltammograms of 10 μM mangiferin in 0.1 M $HClO_4$ at different scan rates: (—) $\nu = 50$ mV s^{-1}, (—) $\nu = 100$ mV s^{-1}, (—) $\nu = 150$ mV s^{-1}, (—) $\nu = 200$ mV s^{-1}, (—) $\nu = 250$ mV s^{-1}, (—) $\nu = 300$ mV s^{-1}. **C.** Cyclic voltammograms of 10 μM compound **1** in 0.1 M $HClO_4$ at different scan rates: (—) $\nu = 50$ mV s^{-1}, (—) $\nu = 100$ mV s^{-1}, (—) $\nu = 150$ mV s^{-1}, (—) $\nu = 200$ mV s^{-1}, (—) $\nu = 250$ mV s^{-1}, (—) $\nu = 300$ mV s^{-1}. **D.** Variation of anodic peak current vs. square root of the scan rate for mangiferin and compound **1** samples. Peak currents were extracted from the voltammograms shown in Fig. 3 (B, C).

concentration-dependent manner (Fig. 6B), thus confirming the anti-inflammatory effect of mangiferin. In contrast, however, our work revealed that ceibinin (CP1), unlike mangiferin, had little to no effect on LPS-induced nitrite release and could therefore not be considered anti-inflammatory (Fig. 6A). Our work therefore establishes that, while the positional isomers ceibinin and mangiferin share antioxidant efficacy and potency, ceibinin does not share the anti-inflammatory efficacy of mangiferin. This observation is a notable example from nature of how differential biological efficacies could be underpinned and explained by positional isomerism within a chemical structure. Such knowledge could be usefully exploited in therapeutics development, in this case for diseases where oxidative stress and inflammation contribute to pathology. It could also be relevant to the development and standardisation of relevant chemical and biological assays for quality control and related purposes.

Some limitations of the current work include the use of a limited range of antioxidant and anti-inflammatory assays and the lack of profiling of ceibinin for other biological activities that have been attributed to mangiferin, as doing that could have afforded a further

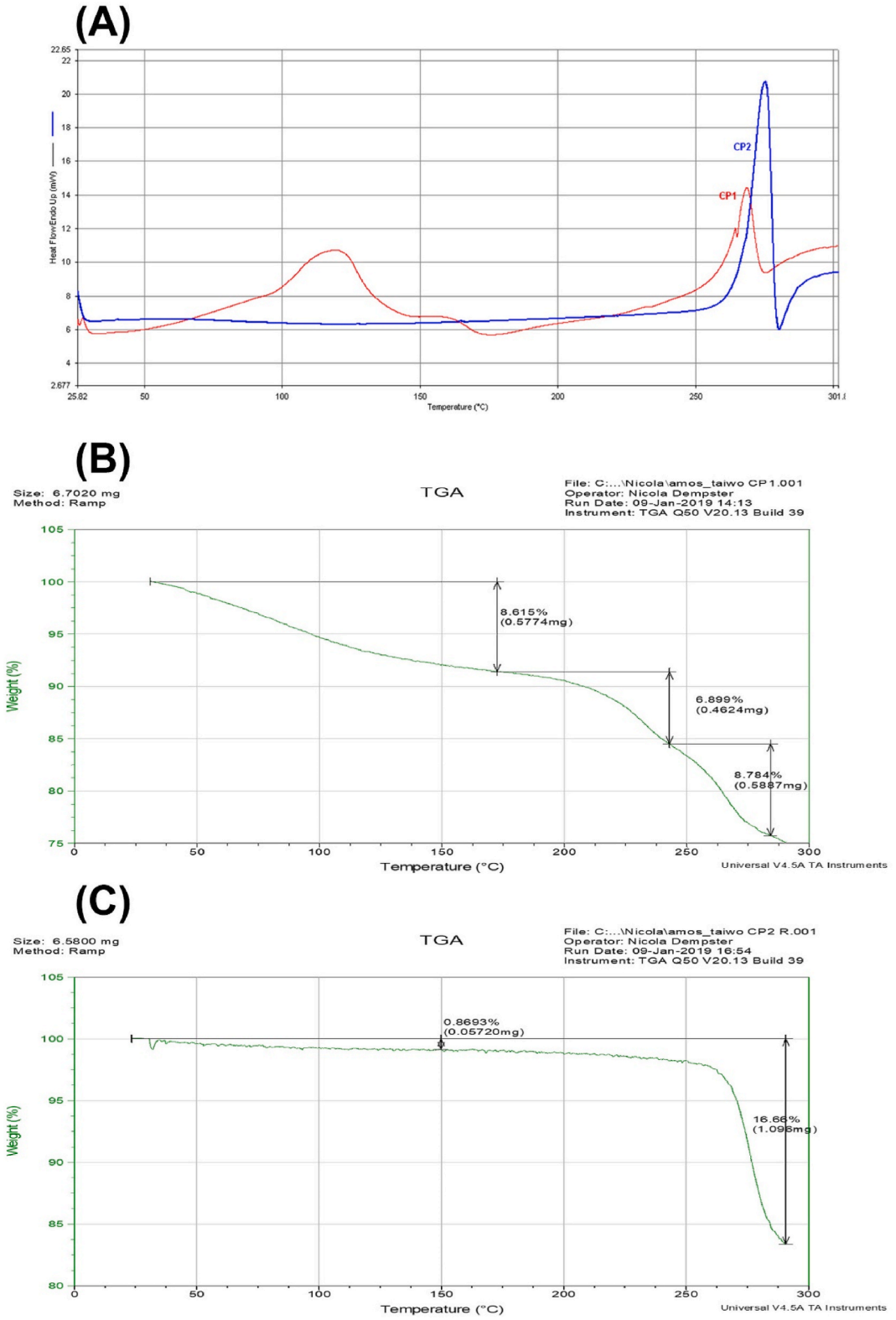

Fig. 4. A. DSC profiles of Compound 1 (CP1-red colour) and Mangiferin (CP2- blue colour). **B.** Thermogravimetric analysis of Compound **1**. **C.** Thermogravimetric analysis of mangiferin.

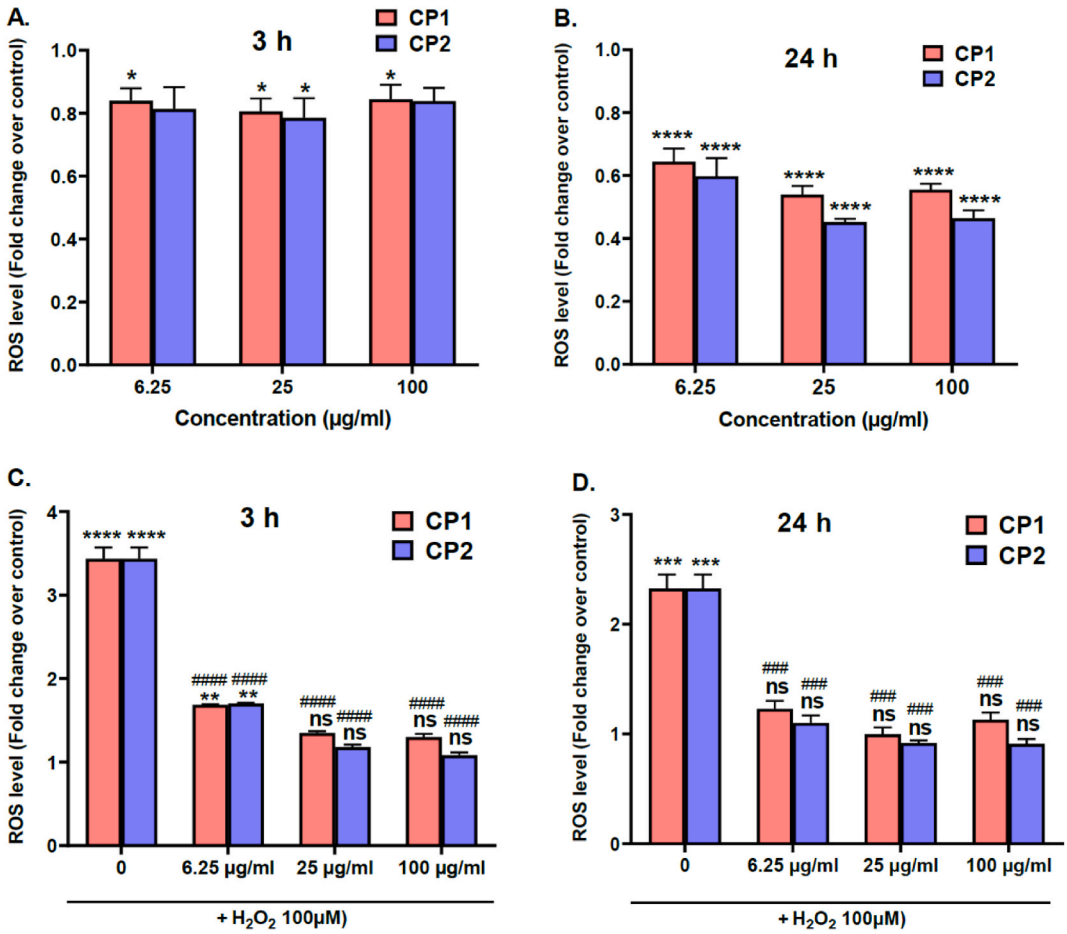

Fig. 5. Effects of Compound 1 (ceibinin, CP1) and mangiferin (CP2) on basal intracellular oxidative stress measured at **(A)** 3 h and **(B)** 24 h after treatment; and on hydrogen peroxide-induced intracellular oxidative stress measured at **(C)** 3 h and **(D)** 24 h after treatment. Each bar represents the Mean ± SEM of three independent experiments (A, B) or triplicate values of an experiment that was run three independent times with similar results (C, D). In A and B, *P < 0.05 and ****P < 0.0001 with respect to the negative control (i.e., cells not treated with CP1 or CP2), while in C and D, **P < 0.01, ***P < 0.001, ****P < 0.0001 and 'ns' (not significant)with respect to the negative control (i.e., cells without peroxide and without CP1 or CP2); ###P < 0.001 and ####P < 0.0001 with respect to hydrogen peroxide alone (i.e., cells treated with hydrogen peroxide but without CP1 or CP2).

differentiation, or otherwise, of the two isomers. We hope to do that in the future. For example, mangiferin has been demonstrated in many research reports to possess antioxidant, anti-inflammatory, antidiabetic, anticancer, antiviral, antimicrobial, analgesic, and immunomodulatory properties [4,5,18,19,41,42].

In comparison with other molecules, mangiferin has been known to be potent as an antioxidant [43]. Sato et al. [44] found mangiferin to be comparably as active an antioxidant as rutin, a standard flavonoid commonly used as an antioxidant for medical purposes. Also, Stoilova et al. [45] found the antioxidant activity of mangiferin to be comparable to that of caffeic acid and rosmarinic acid. These properties make it to be a promising molecule in therapeutics development for a wide range of pathological conditions. None of mangiferin's structural isomers so far reported (isomangiferin and, of course, the new isomer reported here, ceibinin) has been as extensively investigated. This study, however, establishes ceibinin as a new isomer of mangiferin and demonstrates ceibinin's capacity as an antioxidant equipotent with mangiferin, although with poor anti-inflammatory effect, unlike mangiferin. An extensive investigation of ceibinin, vis-à-vis mangiferin, as we plan to do in the future, will confirm its bioactivity profiles and reveal how much similar or different to mangiferin it is, which can guide their deployment in therapeutics development or chemical biology.

It should be noted that, whereas mangiferin demonstrated antioxidant potency similar to or lower than what has been previously reported (see Ref. [44]), its anti-inflammatory potency in this study was lower than what has been generally reported (see Ref. [46]), although certain reports have also tested it up to 400 µM (approximately 170 µg/ml) [47]. Nevertheless, in this study there was evidence that its anti-inflammatory effect was significantly higher than that of ceibinin. The anti-inflammatory assay used in this study can be considered reliable, as the positive control parthenolide demonstrated an anti-inflammatory effect with a potency consistent with previous reports [40].

Mechanistically, mangiferin is known to elicit its antioxidant and anti-inflammatory effects by acting on a wide range of targets,

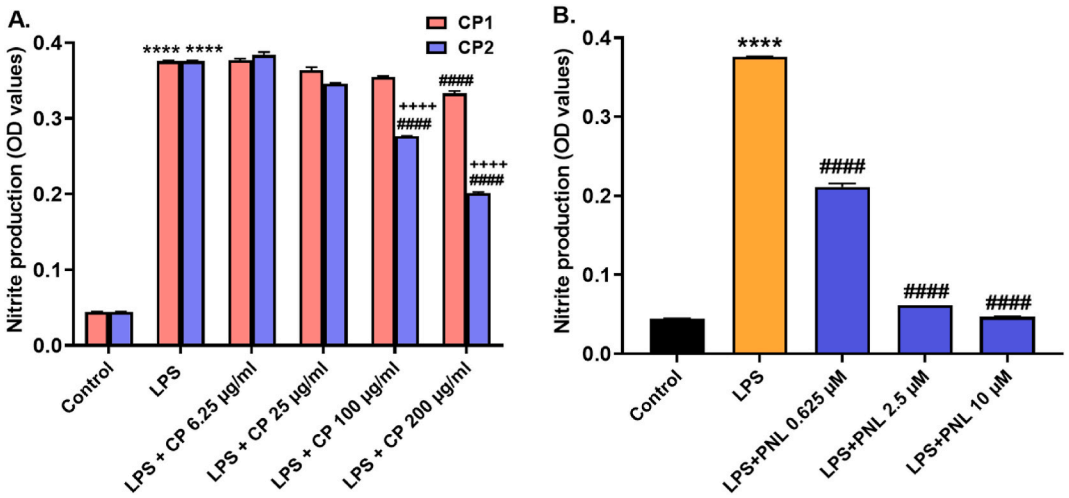

Fig. 6. Assessment of anti-inflammatory effects using RAW264.7 cells. Effects on LPS-induced nitrite production (indicative of nitric oxide (NO) production and, thus, inflammation) of **(A)** Compound 1 (ceibinin, CP1) and mangiferin (CP2); and **(B)** the anti-inflammatory compound parthenolide (PNL), following 24 h treatment. The 6.25 µg/ml, 25 µg/ml, 100 µg/ml and 200 µg/ml of CP1/CP2 correspond to 14.8 µM, 59.2 µM, 235.8 µM and 473.6 µM, respectively. Nitrite levels were quantified using the Griess Assay. Each bar represents the Mean ± SEM of duplicate values of an experiment that was repeated twice with similar results. ****P < 0.0001 with respect to the negative control ('Control'); ####P < 0.0001 with respect to LPS alone; ++++ for comparison of the effects of the same concentrations of CP1 and CP2 on LPS-induced nitrite production.

including the nuclear factor erythroid 2-related factor (Nrf)2/heme oxygenase (HO)-1, peroxisome proliferator-activated receptor (PPAR)-γ/nuclear factor (NF)-κB, p38 mitogen-activated protein kinase (MAPK), *c*-Jun *N*-terminal kinase (JNK), AMP-activated protein kinase (AMPK), and NLR family pyrin domain containing 3 (NLRP3) inflammasome signalling pathways [48,49]. We postulate that these targets were engaged by mangiferin in this study and the isomer ceibinin could also have engaged some or all of these targets, although this remains to be determined.

Our work further underpins the relevance and primacy of natural products as sources of novel scaffolds for the development of chemical tools or drug leads.

3. Conclusions

Overall, our work reveals an entirely new isomer of mangiferin – ceibinin – from *C. petandra*, which shares mangiferin's antioxidant activity but not its anti-inflammatory activity. This further supports the consideration of plants and other natural products as ever-credible and abundant sources of new chemical entities with the potential to reveal novel activities or combinations thereof that could be useful in optimising leads for the development of novel therapeutics.

4. Materials and methods

4.1. General

^{1}H and ^{13}C NMR spectra (for both 1D and 2D experiments) were obtained on the Bruker AV400 (IconNMR) Spectrometer on both 400 and 100 MHz, while MS analyses were carried out on an Agilent LCMS comprising a 1100 series LC/MSD Trap SL at the School of Chemistry and Physics of the University of Kwazulu-Natal in Pietermaritzburg, South Africa. Differential Scanning Calorimetry (DSC) was carried out on a PerkinElmer DSC 8000 coupled to an Intracooler 2 Chiller supplied with Pyris Software 10.1., while the Thermogravimetric Analysis (TGA) was carried out on TGA Q50 equipped with TA instrument explorer, QTA version 5.5.3 at the School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK. The Solid Phase Extraction (SPE) was carried out on Phenomenex C_{18} cartridges (20 g), while column chromatography was carried out on Silica gel (ASTM 230–400 mesh, Merck). Size exclusion chromatography was achieved on Sephadex LH-20 (Pharmacia). Column eluates were analyzed by Thin Layer Chromatography (TLC) performed at room temperature on analytical silica gel 60 GF_{254} pre-coated aluminium-backed plates (Merck, 0.25 mm thick) using ethyl acetate:methanol:water:acetic acid (10:2:1:0.2, solvent 1) as the mobile phase. The resulting spots on TLC plates were visualized under UV light (254 nm) and detected by the use of 1 % vanillin/H_2SO_4. The electrochemical experiments were performed with an Autolab potentiostat/galvanostat (PGSTAT101) controlled by Nova 2.1.3 software. Measurements were carried out in a methacrylate cell (DRP-CELL 70614) employing disposable screen-printed carbon electrodes – (SPCE, 6.1208.110). All instruments and software for the electrochemical experiments were from Metrohm AG.

4.2. Isolation of compounds

The inflorescence of *C. Pentandra* was collected during the flowering season in December 2015 and was identified by Mr I.I. Ogunlowo of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, by comparison with a voucher specimen deposited in IFE Herbarium (Voucher number: FP1-2355). The vegetative part was air-dried and milled. The powdered inflorescence (2.4 kg) was extracted with 100 % methanol (2.5 L x 3) and the crude extract was concentrated *in vacuo* to give 384.7 g of the crude extract. The crude extract (20 g) was dissolved in 100 ml of water and subjected to solvent partitioning with *n*-hexane. The aqueous fraction (2 g) was subjected to SPE with MeOH:H_2O (20:80, CP1; 50:50, CP2, 80:20, CP3 and 0:100). Fraction CP3 was subjected to gel permeation on Sephadex LH-20 using 10–30 % MeOH in ethyl acetate (EtOAc) as the mobile phase. The eluate (5 ml) was collected in each test tube. Analysis of the eluate gave fractions CP3a-CP3e. Fraction CP3c 0.318 g (eluted with 20 % methanol) gave a mixture of two spots, which were subjected to repeated purification on silica gel to give a greenish-yellow powder compound **1** [0.054 g, R_f 0.54 (EtOAc:MeOH:H_2O AcOH, 10:2:1:0.2 solvent 1)] and a dull-yellow powder compound **2** (0.085 g, R_f 0.50) (solvent 1).

4.3. Differential scanning calorimetry (DSC)

Each of the compounds **1** and **2** was prepared as a pellet and introduced into the PerkinElmer DSC 8000. The sample was run isothermally at 25 °C for 1 min, followed by gradient temperature control from 25-300 °C at the rate of 20 °C/min. The N_2 purge rate was set at 50 ml/min. For the TGA, the temperature was set at 25–300 °C at the rate of 20 °C/min, with balanced purge flow rate being 40 ml/min with nitrogen gas.

4.4. Electrochemical studies

The electrochemical experiments were performed with an Autolab potentiostat/galvanostat (PGSTAT101, Metrohm AG) controlled by Nova 2.1.3 software. Measurements were carried out in a methacrylatel cell (DRP-CELL 70614) employing disposable screen-printed carbon electrodes (SPCE 6.1208.110). Electrochemical measurements were carried out using procedures adapted from Ref. [50]. CV experiments were performed scanning the potential from 0.2 V to 0.6 V (vs Ag, SPCE pseudo-reference electrode) using 0.1 M perchloric acid ($HClO_4$) as the supporting electrolyte. Scan rates (ν) varied from 50 mV s^{-1} to 300 mV s^{-1}. In all measurements, 8 ml of ~10 μM mangiferin in 0.1 M $HClO_4$ was transferred to the cell, followed by 5 min nitrogen gas purging. A start potential of 0.2 V was kept for 10 s, and data collected immediately after. New SPCEs were used for each performance.

4.5. Cell culture and cell viability assay

Two cell lines were grown and used as adherent monolayer cultures: the human cervical adenocarcinoma cell line, HeLa, and the transformed human lung fibroblast cell line, MRC-5 SV2 (which was derived from the parental cell line MRC5, such that it does not senesce). Each cell line was grown in a 75 cm^2 tissue culture flask using Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % Foetal Calf Serum, 2 mM L-glutamine and 1 % antibiotic-antimycotic solution (containing penicillin, streptomycin and amphotericin B). The resulting cultures in flasks or plates were incubated at 37 °C in a humidified atmosphere of 5 % CO_2. Suspensions of cells were prepared by trypsinisation and seeded into micro-clear, opaque, flat-bottom, 96-well plates at a density of 7.5×10^4 cells per ml (100 μl/well, i.e., 7500 cells per well) and exposed after 24 h of incubation to a range of concentrations of each compound prepared in growth medium (stock concentrations were prepared in DMSO, but final DMSO concentration that cells were exposed to was not more than 0.1 %v/v), with treatments lasting for up to 48 h. Following treatment, viability was assessed as we previously reported using the MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay [51]. In addition, changes to the morphology of the cells caused by the treatments were monitored and captured on an Olympus CKX41 microscope fitted with an Olympus DP71 U-TVIX-2 camera, as previously reported [51].

4.6. Assessment of effects of compounds on reactive oxygen species (ROS) levels

Assessment of reactive oxygen species (ROS) was carried out using a DCFDA Cellular ROS Detection Assay Kit (Abcam, Cat. No. 113851), as previously reported [52]. DCFDA (or H2DCFDA or DCFH-DA) is a fluorogenic dye that measures ROS activity within the cell. When DCFDA diffuses into the cell, it becomes deacetylated by cellular esterases to a non-fluorescent compound, which then gets oxidised by ROS into 2′,7′-dichlorofluorescein (DCF), a highly fluorescent compound that can be detected by fluorescence spectroscopy. HeLa cells were used for this experiment, grown as reported in an earlier section and seeded into dark, clear bottom, 96-well plates at a density of 25,000 cells per well (100μl/well). The plates were incubated overnight (18–24 h) at 37 °C in a humidified atmosphere of 5 % CO_2. The growth medium was then aspirated from each well and the cultured cells were rinsed with 100 μl of the supplied buffer (1*X*). The buffer was aspirated and the cells were stained by incubation with 25 μM DCFDA (prepared from a 20 mM stock) in the dark at 37 °C for 45 min at 100 μl/well, after which the DCFDA solution was removed and the cultures washed with buffer. They were then exposed to the test compounds (prepared in full growth medium without phenol red) in the absence and presence of hydrogen peroxide, in order to determine how each compound affected basal levels of ROS as well as how it modified peroxide-induced ROS. Following addition of compounds, fluorescence was read on a CLARIOstar microplate reader (BMG Labtech, UK) (Ex/Em = 485/535 nm) at various time points up to 24 h. Background wells (untreated or diluent-treated, stained cells) as well as blank wells

(medium only) were included in each experiment. Blank readings were subtracted from all measurements and fold changes determined with respect to negative controls.

4.7. Assessment of anti-inflammatory effects of compounds

Murine macrophage RAW264.7 cells were grown in DMEM supplemented with 10 % FBS, 2 mM glutamine, 1 mM sodium pyruvate and 1 % antibiotic-antimycotic solution (sodium pyruvate and antibiotic-antimycotic solution not included during treatments). They were seeded into 96-well plates at 1×10^6 cells/ml (100 μl/well) and incubated for 24 h. The medium was then discarded and the cultures were pre-treated for 1 h with the test compounds, after which they were treated for 24 h with 1 μg/ml lipopolysaccharide (LPS) (from *S. typhimurium* (*S*-form), Caltag Medsystems Ltd.), in the continued presence of the test compounds. Parthenolide, a potent anti-inflammatory compound [40], was used as a positive control. Nitric oxide (NO) production was then assessed, as previously reported [53], by quantifying nitrite levels in the well supernatants using the Griess Reagent System (Promega, G2930), which involved sequential addition of sulphanilamide and *N*-1-naphthylethylenediamine dihydrochloride (NED) to each sample and each standard, followed by a 10-min incubation (room temperature, protected from light) each time, before absorbance reading at 550 nm. Nitrite standards were included to generate a standard reference graph. The MTT assay was used to confirm that each treatment was not toxic to cells, in order to exclude the possibility of reduction in NO levels occurring as a result of cell death.

4.8. Data presentation and analysis

Where relevant, values are shown as mean ± SEM (standard error of the mean). Each treatment was done in duplicate or triplicate and the average of the readings was taken. Statistical analyses were done using the GraphPad Prism software (Version 9.3.1) (GraphPad Software, Inc., CA, USA). Analysis of variance (ANOVA) followed by a post-hoc test for multiple comparisons was used to determine statistically significant differences between means, with a *P*-value of less than 0.05 considered statistically significant.

Additional information

No additional information is available for this paper.

Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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CRediT authorship contribution statement

Bamigboye J. Taiwo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alex H. Miller:** Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing. **Alistair J. Fielding:** Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Satyajit D. Sarker:** Formal analysis, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Fannie R. van Heerden:** Resources, Supervision. **Amos A. Fatokun:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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