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Polyphenolic compounds with anti-tumour potential from *Corchorus olitorious* (L) Tiliaceae, a Nigerian leafy vegetable.

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Abstract

Chromatographic fractionation of the methanolic extract of *Corchorus olitorious* (L.) (Tiliaceae), on silica gel yielded two polyphenolic compounds. The structures of the compounds were elucidated as Methyl-1,4,5-tri-*O*-caffeoyl quinate and *Trans*-3-(4-Hydroxy-3-methoxyphenyl) acrylic anhydride, based on extensive use of spectroscopic techniques such as ^{1}H and ^{13}C NMR, DEPT and 2D NMR experiments (COSY, HSQC, HMBC), IR and MS. To establish an initial proof-of-concept for the biological relevance of these compounds, their cytotoxicity in the HeLa cell line was assessed as an index of their anti-tumour potential. The compounds when tested at a range of concentrations up to 1.6 mM were found to possess mild cytotoxic activity. The *trans*-3-(4-hydroxy-3-methoxyl phenyl) acrylic anhydride was found to be related to curcumin, a compound known to have anti-cancer activity. The plant therefore represents a source of natural 'lead' compounds with anti-tumour potential.

Key words: *Corchorus olitorius, Tiliaceae, caffeoyl quinate, ferrulic anhydride, cytotoxicity*

The largely synonymous terms `new uses for old drug', 'drug repositioning', 'drug repurposing', 'drug re-profiling', 'therapeutic switching' or 'indication switching` have gained considerable attention over the past decade in efforts to accelerate the development of drugs for novel indications and also to reduce associated prohibitive costs of bringing drugs from the bench to the market.[1,2,3] As coined in Western medicine, the terms generally refer to the development of existing or known drugs for novel indications. One of the unique attractions of this approach is the readily-available knowledge about the safety and tolerability profiles of such existing drugs, which translates to faster pace and cheaper cost of development, as most of the safety studies usually required before approval is granted by drug regulatory bodies had been carried out when the drug was developed for its maiden indication.

It is anticipated that this paradigm shift with respect to finding novel indications for drugs already in use might also be relevant to herbal medicines and vegetables currently in use, especially in many traditional societies. Focus on this area will represent further beneficial exploitation of plant biodiversity that has been recognized as a primary source of food, medicines, shelter, feed and other products and means harnessed by human beings.[4,5] While a lot of plants have been exploited for their potential as safe sources of food, only a relatively smaller proportion of these are being evaluated for their potential as therapeutic agents. This utility gap further makes attempts to examine food crops, especially vegetables, for their potential development as therapeutic agents a reasonable and welcome idea.

In this context, the Country Nigeria has a very rich plant biodiversity, with many of the species not yet receiving enough research attention to make local sourcing of pharmaceutical products a reality.[6] There is currently a strong awareness about neglected and underutilized crops all over the world, a move spearheaded by international bodies like the International Genetic Resources Institute, Consultative Group on International Agriculture Research, the Global Forum on Agriculture Research and the International Centre for Underutilized Crops.

This study has therefore been carried out in response to the need to evaluate the medicinal potential of some of Nigeria's underutilized and neglected vegetables, one of which is *Corchorus olitorious* (L.).

Corchorus olitorious (L.) (Tiliaceae) has been used for decades in South-Western Nigeria as a leafy vegetable mainly for making soup. By IGRI definition, *C. olitorious* occupy special niche as a source of earning livelihood for the peasant farmers and passes for neglected crop in the local ecology, production and consumption systems.[7] Locally, it remains inadequately characterized and neglected by research and conservation. Traditionally, *C. olitorius* has been used to treat gonorrhea, chronic cystitis, pain, fever and tumours[8] while in Southwestern Nigeria, it is popularly consumed as a leafy vegetable. Some studies have proven that *C. olitorius* possesses antinociceptive, anti-inflammatory, antipyretic activities[9], hypoglyceamic activity[10, 11] and antihypertensive activity[11]. Cardiac glycosides[12, 13, 14] ionones[15] flavonoids[16, 17] and chlorogenic acid[16] have been reported from various vegetative part of the plant. Our interest in the plant stemmed from the fact that it is one of the commonest items in the recipe for treatment of cancer locally and we desire to justify this ethnobotanical use of the plant. Furumoto *et al*[18] demonstrated phytol and monogalactosyldiacyl glycerol from the leaf extract of *C. olitorius* to have antitumour promoting properties. Also, Salawu *et al*.[19] demonstrated the in vitro cytotoxic activity of the leaf using the MTT assay and indicated isoquercetrin, hyperoside and 1,5 dicaffeoyl quinic acid to be present in the leaf extract. In this report, we report the isolation and characterization of Methyl-1,3,4-tri-O-caffeoyl quinate and Ferrulic anhydride from the whole plant of *C. olitorius* and their potential cytotoxic activity against Hela cell lines.

Whole plant of *C. olitorious* was bought in October 2013 at the vegetable section of the Sabo Market, in Ile-Ife, Osun State, Nigeria. The plant material was identified by Mr. Ogunlowo I.I. of the Department of Pharmacognosy, Obafemi Awolowo University, Nigeria, by

comparison of the sample with voucher specimen NHCO9 at the Forest Research Institute of Nigeria, Ibadan, Oyo State, Nigeria. The fresh plant materials were chopped into smaller pieces and subsequently milled in a grinder using methanol as solvent. The crude methanolic extract was filtered and concentrated *in vacuo* to yield 16 g. The crude extract was run on an open column (internal diameter 35 x 4 cm) of silica gel (230-400 mesh size) with 300 ml each of solvents of increasing polarity, from 100% ethyl acetate (EtOAc) to 100% methanol (MeOH), collecting eluate of about 15 ml in each test tube. The eluates were analysed on thin layer chromatographic plates using EtOAc : MeOH (9:1, solvent system 1), EtOAc : MeOH : H_2O (water) : AcOH (Acetic acid) (10:2:1:0.1, solvent system 2) and butanol (BuOH) : methanol : water (6.5:3.5:1, solvent system 3). The chromatograms were viewed under the Ultra Violet (UV) light at both 254 and 366 nm and were subsequently sprayed with 10% sulphuric acid in methanol. Test tubes with similar TLC profiles were bulked together to give three fractions CO-1 (6.5 g, eluted with 100% EtOAc - 20% MeOH in EtOAc), CO-2 (4.2 g, eluted with 10-30% MeOH in EtOAc) and CO-3 (3.9 g, eluted with 40% MeOH in EtOAc). CO-2 was subjected to repeat column chromatography as already described to give two fractions CO-2A (2.1g) and CO-2B (1.6 g). CO-2A was injected into a Lobar$^{(R)}$ RP-18 column and eluted with the following solvent mixtures: H_2O (100%), H_2O : MeOH (8:2, 7:3, 6:4, 5:5, 4:6). Eluates were collected in test tubes and analysed on TLC, using solvent system 2. Test tubes with similar TLC profiles were bulked to give seven fractions CO-2Ai-vii. CO-2Av (0.056 g, eluted with H_2O : MeOH (1:1)) was further purified on silica gel by isocratic elution using 300 ml of EtOAc : MeOH (8:2) to give Compound **2** (0.025 g). CO-2B was subjected to reversed-phase chromatography on Lobar$^{(R)}$ RP-18 column using as mobile phase 100 ml each of H_2O: MeOH in the following proportions (7:3, 6:4, 5:5, 4:6, 3:7 and 100% MeOH). The eluates were analysed on TLC silica gel plates as described before and bulked to give five fractions CO-2Ba to CO-2Be. CO-2Be (0.180 g, eluted with 50% MeOH in water)

was purified further on silica gel using 400 ml of isocratic EtOAc : MeOH (7:3) to give Compound **1 (**0.027 g**)**. To assess cytotoxicity, a cell viability assay was carried out as previously reported.[20] Briefly, HeLa cells were grown in Minimum Essential Medium (MEM), supplemented with 10% Foetal Bovine Serum (FBS), 1% non-essential amino acid (NEAA), glutamine (2 mM) and 1% antibiotic-antimycotic solution, and were plated into black, flat-bottomed 96-well plates at a density of 1 x 10^5 cells/ml (100 µl/well). The cells were incubated for 24 h at 37^{o}C in a humidified atmosphere of 5% CO_2. On the day of treatment with compounds, the medium was aspirated and each of serially-diluted concentrations of each compound, prepared using the growth medium, was added to separate wells in duplicate and a further incubation was done for 48 h. To evaluate cell viability, the viability reagent Alamar Blue (AB) was added to the wells and the plates were incubated for 3 h. They were then removed from the incubator and allowed to stand at room temperature for 30 min, following which they were loaded onto Flexstation 3 (Molecular Devices). Fluorescence was read at an excitation of 530 nm (544 nm used) and an emission of 590 nm. Averages of fluorescence values for each set of treatments were calculated. Each average was then converted to a percentage, with the average for the control wells (which only received DMSO) taken as 100% and every other average (in each of the wells treated with different concentrations of each compound) expressed as a proportion of this percentage value. In order to assess the corresponding effects of the treatments on the morphology of the cells, bright field images were acquired, following treatments, on a Nikon Eclipse TS100 inverted microscope, for control cultures treated with growth medium only and also for cultures treated with increasing concentrations of the two compounds. Statistical significance of treatments was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (each treatment compared to the control). Differences between means were considered statistically significant at $P<0.05$.

Two compounds (Compound 1 and Compound 2) were identified from this work (Figure 1), and their spectroscopic data are as shown in chart 1. Compound **1** was obtained as a deep-brown, amorphous powder. The molecular formula $C_{35}H_{32}O_{16}$ was indicated by the $(M+K)^+$ ion peak at m/z 731 in the ESI MS spectrum. The ^1H NMR spectrum of compound 1 displayed 11 signals at δH: 1.79 (2H, m, H-6),(1.96 (2H, m, H-2), 3.57 (s), 3.58 (d, J = 2.6 Hz), 3.40 (quartet), 5.09 (quartet), 6.16 (d, J = 15.9 Hz), 6.78 (d, J = 8.2 Hz), 6.99 (dd, J = 8.2, 2.0 Hz), 7.05 (d, J = 2.0 Hz) and 7.43 (d, J = 15.9 Hz). The aromatic signals indicated an ABX coupling pattern, with the signals at δH 7.50 and 6.26 each having a coupling constant of 15.9 Hz, characteristic of a *trans*-olefinic system. This coupling pattern suggested a caffeoyl moiety. In the aliphatic region, the signal at δH, 5.09 was observed in the ^1H-^1H COSY spectrum to correlate with signals at 3.58 and 2.01, while the signal at δH 3.94 coupled only with the signal at δH 1.79. No correlation was observed, however, between the signals at δH 3.57 and 3.95, suggesting a different orientation in space. In the ^{13}C NMR, 7 signals were displayed in the aliphatic region at δc: 37.2, 37.6, 52.6, 71.1, 71.4, 68.7 and 174.9 for a carboxylic acid group. A signal was observed at δc 73.7 for quaternary carbon, for which was observed a long-range correlation between the signals at δH 5.09, 3.94, 2.01, 1.79 and the methoxy signal at 3.57. This is suggestive of a quinic acid moiety. The remaining resonances were assignable to the caffeoyl moiety. The point of attachment of the caffeoyl group to the quinic skeleton was deduced mostly from the long range HMBC correlation. A correlation was observed between the methoxy proton signals at δH 3.58 and the carbonyl carbon signal at δc 174.9, indicating the esterification of the quinic acid at C-1. Each of the proton signals at δH 5.09 and 3.57 was observed in the HMBC spectrum to correlate with the carbonyl carbon signal at δH 166.9, indicating esterification with caffeoyl groups occurring at C-3 and C-4 of the quinate moiety. The remaining oxygenated methine proton at H-5 on the quinate moiety was observed not to correlate with any carbonyl carbon. Also, the proton signals of H-3 and

H-4 only were observed to be shifted downfield by about 1.00 and 0.19 ppm respectively while the signal of H-5 was relatively unaffected compare with the free quinic acid.[21] A review of the ^{1}H NMR signals for some quinic acid derivatives revealed that those spectra run in CD_3OD had a higher resonance value than in the DMSO which was used in our own experiment[21,22,23,24] so we relied on the HMBC correlation in lieu of comparative analysis with the ^{1}H NMR chemical shifts of the proton in free quinic acid. The third caffeoyl moiety was therefore placed on the hydroxyl group on carbon 1. In the literature, reports had been made for the occurrence in nature, of 3,4,5-tri-*O*-,[22] 1,3,5-tri-*O*-,[24] and 1,4,5-tri-*O*-,[21] derivatives of the quinic esters. Thus, compound 2 is identified as Methyl-1,4,5-tri-*O*-(3′,4′-dihyroxy cinnamoyl) quinic ester. It is supposed that compound **2** exists as the free quinic acid but the process of extraction must have led to the formation of the methyl ester.[21]

The ^{1}H NMR spectrum of compound 1 displayed six signals at δH: 3.16(s), 6.26 (d, J = 15.9 Hz), 6.83 (d, J = 8 Hz), 7.04 (dd, J = 8,2 Hz), 7.11 (d, J = 2 Hz) and 7.50 (d, J = 15.9 Hz) indicating an ABX ring system conjugated with a *trans* olefinic system. The COSY spectrum showed correlation between the signal at δH 7.50 and the signal at δH 6.26 with a coupling constant of 15.9 Hz is characteristic of a *trans* olefinic system. The coupling constant of 8 Hz between the signal at δH 6.83 and 7.04 indicates ortho coupled protons. The ^{13}C NMR displayed 10 signals, indicated in the DEPT spectrum as; four quaternary carbon signals, five methine signals and one methoxy signal. The resonances of the carbon were assigned fully from the long range proton to carbon bond correlations (Chart 1). However, the ESI MS displayed a molecular ion of m/z of 393 $(M + Na)^{+}$ a mass twice the expected mass for a ferrulic acid unit. This compound was deduced therefore as an anhydride of the ferrulic acid. The NMR spectrum of the compound was closely related to that of curcumin. Compound 2 is a bioisostere of curcumin obtained by the bioisostercic replacement of the methylene bond in curcumin with –*O*-. Caffeic anhydrides were recently reported from *Nothopanax delavayi*[25,26]

In order to explore the anti-tumour potential of both compounds, they were examined for cytotoxic activity using the HeLa cell line, which is a widely used cell culture model of (cervical) cancer. The effects of 48 h incubation of the cultures with concentrations of the compounds up to 1600 µM were examined (molar concentrations prepared based on the molecular weight calculated from the derived molecular formula of each compound – 692 g/mole for Compound 1 and 370 g/mole for Compound 2). However, only at 800 µM or higher were the compounds found to elicit significant toxicity, with values of 71.0% ± 8.3 and 19.6% ± 11.4 for Compound 1, and 22.3% ± 10.9 and 5.3% ± 0.2 for Compound 2, each compared to control, for 800 µM and 1600 µM of each compound, respectively, as shown in Figure 2. The data revealed compound 2 as more than thrice as toxic as compound 1 at comparable concentrations showing evidence of significant toxicity. Outcomes of microscopic examination of the treated cultures were in agreement with the viability data, as they showed evidence of loss of cells as well as rounding up of cells in wells that were treated with the concentrations of compounds revealed by the Alamar Blue viability assay as cytotoxic, whereas control cultures did not show any such loss or rounding up of cells. Compound **2** is closely related to curcumin, *i.e.*, it is an analogue of curcumin, with bioisosteric replacement of the methylene bond in curcumin with –*O*- (Figure 1). Curcumin has been popular as a chemopreventive compound in cancer therapy.[27, 28, 29, 30] While the result of our cytotoxicity experiments do not indicate potent cytotoxic activity for either compound, it does raise the possibility that through further structure-activity relationship (SAR) studies, analogues of the compounds exhibiting significantly increased potency might be obtained, and these can serve as useful leads for the development of novel chemotherapeutics. They can also be part of a collection of natural small molecules that can be developed into a unique compound library for high-throughput screening to identify those

targeting established molecular pathways, with the ultimate goal of discovering novel therapeutics for various disease conditions.

To gain insight into the likely molecular basis for the observed cytotoxic properties the isolated compounds, literature search was conducted to identify molecular targets that have been implicated in tumorigenesis. The 3D structures, solved by either of x-ray crystallography or solution NMR, of seven human protein targets were downloaded from the RCSB protein databank (www.rcsb.org). These include metalloproteinase-9 (1gkc.pdb)[31], aryl sulfotransferase (1ls6.pdb)[32], fibroblast growth factor receptor 2 (4j96.pdb)[33], epidermal growth factor receptor (4zau.pdb)[34], serine/threonine-protein kinase b-raf (5fd2.pdb)[35], mitogen-activated protein kinase kinase mlt (5hes.pdb)[36], and ras binding domain (rbd) of b-raf (5j18.pdb)[37]. All seven protein structures were in complex with inhibitors. To ascertain the structural integrity of the downloaded protein structures, visual inspections were performed using Visual Molecular Dynamics[38] software. The complexed ligands were first stripped from each protein and saved as separate structure files. Three dimensional structures were generated for Compound 1, Compound 2 and curcumin. Gasteiger charges were added to all the ligands using the AutoDock4 software[39]. To map the binding site residues for each protein system, AutoDock4 was employed and the topological boundaries for docking were defined as grid dimensions. Using AutoDock Vina[40], each of the ligands (from the present work and the reference inhibitors in the PDB structures) was docked into binding site of the molecular targets using a complex generation scheme that treated the ligands as fully flexible while the receptor coordinates were fixed. The best conformation was saved for post docking analysis in each case.

The choice of the molecular targets for docking was informed by the potential significance of the proteins in tumorigenesis and tumour survival. While there is a chance that there are other interaction partners within the cells, the choice of the macromolecules used in this work was

informed by their intrinsic significance and the availability of good experimental data. The co-crystallized ligands in the crystal structure serve as benchmark, not just for adjudging the minimum required affinities but to put the affinities computed for the compounds in proper perspective. The energetics of each ligand-target interaction are presented in Table 1. In most of the examined cases, compounds **1** and **2** and curcumin demonstrated thermodynamically spontaneous coupling with the receptors (i.e. binding free energy < 0). All three compounds demonstrated binding affinities superior to that obtained for the co-crystallized inhibitors of metalloproteinase-9, FGFR2, and EGFR. For all other proteins (with the exception of the ras binding protein), compound **2** proved a more avid binder compared with curcumin. Molecular weight of compound **1** appears to be a crucial factor in frustrating binding interaction with aryl sulfotransferase, while its ability to exploit the amphipathic nature (figures 4 and 5) of metalloproteinase-9, FGFR2, and EGFR binding sitest allows it to form stronger ligand-receptor complex compared with compound **2** and curcumin. Thus, at the molecular level, both curcumin derivatives and curcumin possess sufficient physicochemical attributes to spontaneously interact with the examined tumorigenic proteins and in energetically favourable fashion. This provides atomistic support for the observed cytotoxic properties of the compounds **1** and **2**, at least at the level of interaction with molecular targets implicated in tumorigenesis.

Compound 1:

FT IR υ_{max} cm-^{1}: 3421-3187, 2829, 1687, 1771, 1579, 1354, 772 ; UV nm : 192, 207, 327; ^{1}H-NMR (600 MHz, DMSO-d_6). δ: 1.789 (2H, m, H-6), 2.01(2H, m, H-2), 3.57 (3H, s, H-8), 3.58 (^{1}H, d, J = 2.58 Hz, H-4), 3.94 (1H, m , H-5), 5.09(1H, m, H-3), 7.43 (1H, d, J = 15.9 Hz H-3′) 7.05 (d, J = 2 Hz).6.78 (d, J = 8. Hz), 6.99 (dd, J = 8, 2 Hz), 6.16 (1H, d, J = 15.9 Hz, H-

2′). ^{13}C-NMR (150 MHz, DMSO-d6), δ: 174.9 (Cq, C-7), 165.7 (Cq; C-1′,C-1″, C-1‴), 148.3 (Cq, C-7′,C-7″, C-7‴), 145.5 (Cq, C-6′,C-6″, C-6‴), 144.9 (t, C-3′,C-3″, C-3‴), 125.6 (Cq, C-4′,C-4″, C-4‴), 121.3 (t, C-5′,C-5″, C-5‴), 115.7 (t, C-3′,C-3″, C-3‴), 114. 7 (t, C-2′, C-2″, C-2‴), 114.3 (t, C-9′,C-9″, C-9‴), 74.0 (Cq, C-1), 70.9 (t, C-4), 70.5 (t, C-3), 68.1 (t, C-5), 37.2 (d, C-2) and 36.3 (d, C-6). ESI MS m/z 731 $(M + K)^+$ (requires C; 60.69%, H; 4.66% and O; 34.65%, found C; 60.73%, H; 4.66% and O; 34.67%).

Compound 2:

FT IR υ_{max} cm-1: 3281, 2826, 1687, 1579, 1355, 772; UV nm: 193, 208, 320, ^{1}H-NMR (600 MHz, DMSO-d_6), δ:3.16 (3H, s, H-10, 10′), 6.26 (2H, d, J = 15.9 Hz, H-2, 2′), 6.83 (2H, d, J = 8 Hz, H-8, 8′), 7.04 (2H, dd, J = 8, 2 Hz, H-9, 9′), 7.11 (2H, d, J = 2 Hz, H-5) and 7.50 (2H, d, J = 15.9 Hz, H-3, 3′). ^{13}C-NMR (150 MHz, DMSO-d6), δ:166.2 (q, C-1, 1′), (d, C-2, 2′), 114.6 (d, C-3, 3′), 125.9 (C-4,4′), 115.77 (d, C-5, 5′), 146.7 (q, C-6, 6′), 148.6 (q, C-7, 7′), 116.57 (d, C-8,8′) and 121.21 (q, C-9, 9′) and 56.5 (OCH3). ESI MS m/z 393 $(M + Na)^+$ (requires C; 64.86%, H; 4.90% and O; 30.24%, found C; 64.92%, H; 4.90% and O; 30.27%).

FIGURE LEGENDS

Figure 1: Charts showing the proposed structures of Compound **1,** Compound **2** and curcumin.

Figure 2: Effects of Compound **1** and Compound **2** on the viability of HeLa cells. Cultured HeLa cells were incubated for 48 h with the indicated concentrations of the compounds before viability was quantified using the alamar blue dye. Plates were read on Molecular Devices Flexstation 3 using an excitation wavelength of 530 nm (actual wavelength used was 544 nm) and an emission wavelength of 590 nm. Values shown are Means ± SEM (Standard error of

the mean). One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was used to assess significance of effects ($P<0.05$ taken as significant). *$P<0.05$ and ***$P<0.0001$ compared to control.

Figure 3: Photomicrographs showing the effects of the different concentrations of Compound **1** and Compound **2** on the morphology of HeLa cells. The toxic effects of the compounds caused concentration-dependent cell loss and/or rounding up of remaining cells, compared to control cultures not treated with the compounds which were confluent and remained intact. Images were acquired, following 48 h treatments, on a Nikon Eclipse TS100 inverted microscope (x10 magnification). Scale bar = 20 µm.

Figure 4: Figure showing metalloproteinase-9 in purple ribbon with bound ligands in liquorice representing (A) N-2-[(2r)-2-{[formyl(hydroxy)amino]methyl}-4-methylpentanoyl]-N,3-dimethyl-l-valinamide, (B) Compound **1**, (C) Compound **2**, and (D) curcumin. Curcumin and Compounds 1 and 2 demonstrated strong binding to most molecular targets. Docking simulations were performed using Autodock Vina while VMD was employed as the graphics tool.

Figure 5: Figure showing aryl sulfotransferase in purple ribbon with bound ligands in liquorice representing (A) adenosine-3'-5'-diphosphate, (B) compound **1**, (C) compound **2**, and (D) curcumin. Curcumin and Compounds 1 and 2 demonstrated strong binding to most molecular targets. Docking simulations were performed using Autodock Vina while VMD was employed as the graphics tool.

Table 1: Table showing calculated binding free energies for co-crystallized ligands, curcumin and Compounds 1 and 2. Curcumin and Compounds 1 and 2 demonstrated strong binding to most molecular targets. Docking simulations were performed using Autodock Vina while VMD was employed as the graphics tool.

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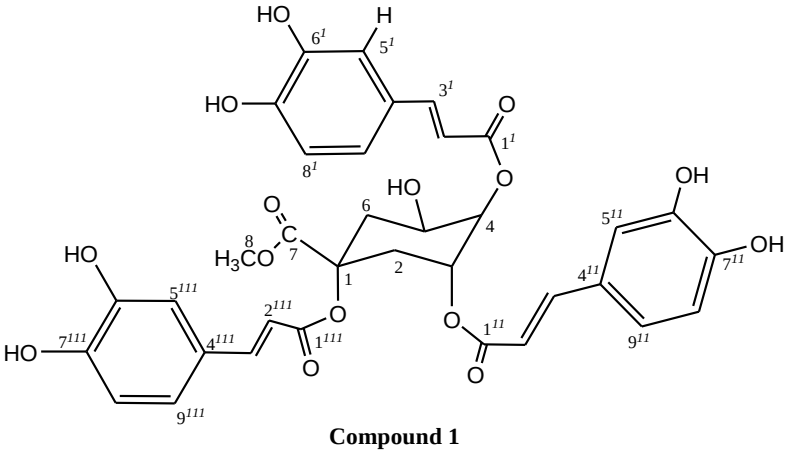
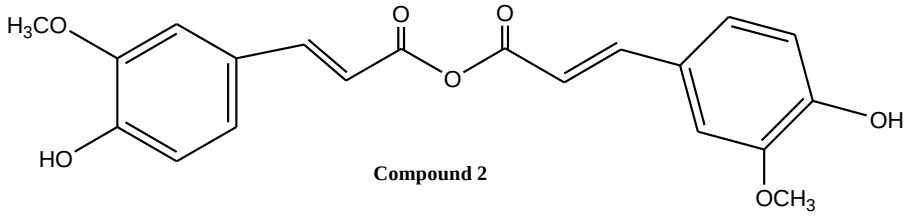
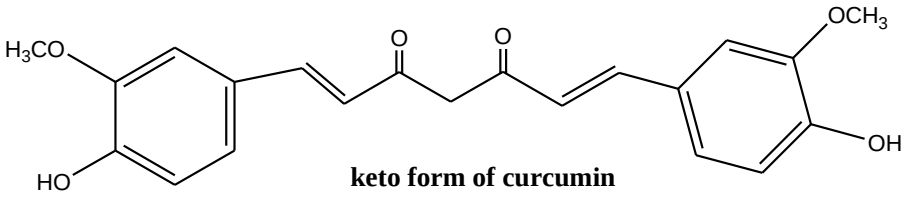
Figure 1: Charts showing the proposed structures of Compound **1,** Compound **2** and curcumin.



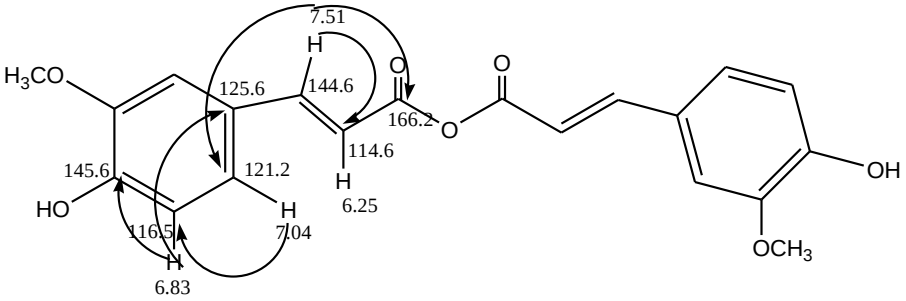
Compound 1



Compound 2

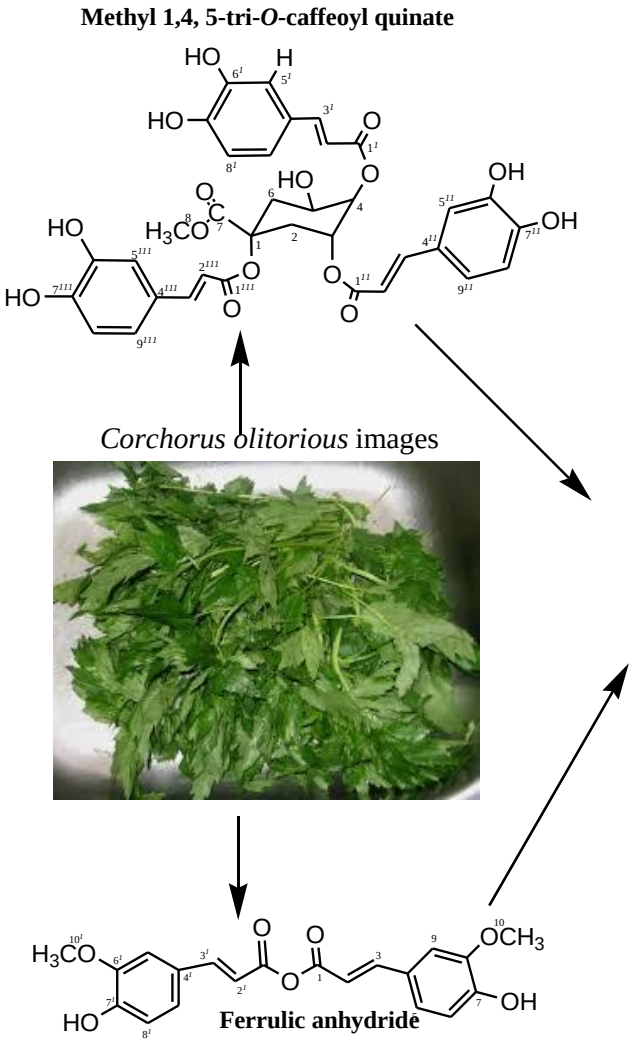


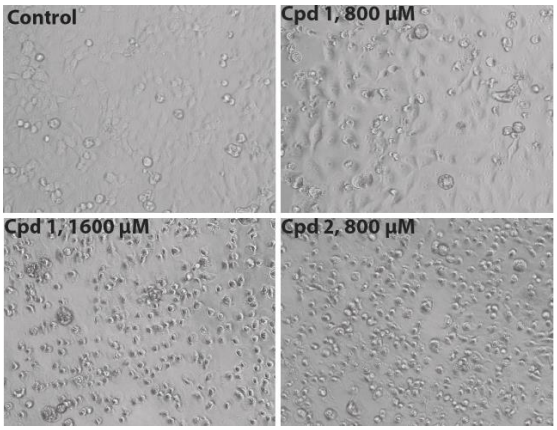
keto form of curcumin



HMBC correlations for half of compound 2

Graphical abstract





Effects of Compound 1 and Compound 2 on the viability of HeLa cells.

Figure 3: Photomicrographs showing the effects of the different concentrations of Compound **1** and Compound **2** on the morphology of HeLa cells.

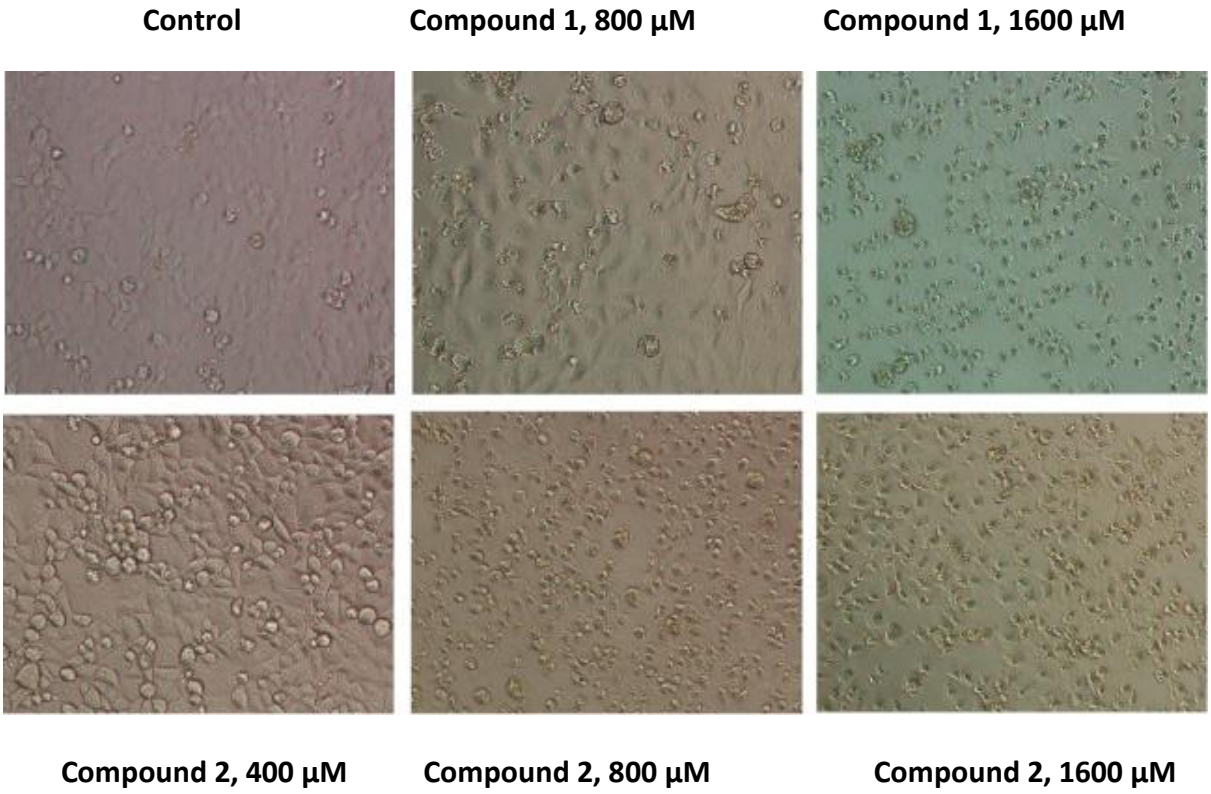


Figure 2: Effects of Compound **1** and Compound **2** on the viability of Hela cells

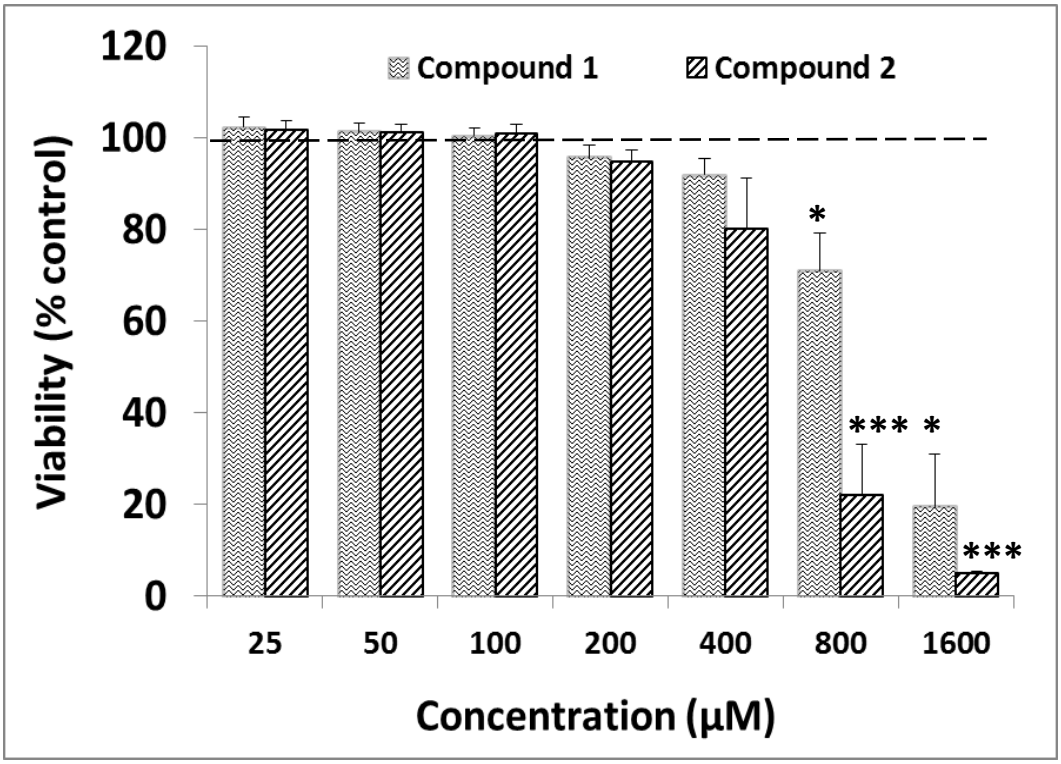


Figure 4: Figure showing metalloproteinase-9 in purple ribbon with bound ligands in liquorice representing (A) N-2-[(2r)-2-{[formyl(hydroxy)amino]methyl}-4-methylpentanoyl]-N,3-dimethyl-l-valinamide, (B) Compound **1**, (C) Compound **2**, and (D) curcumin.

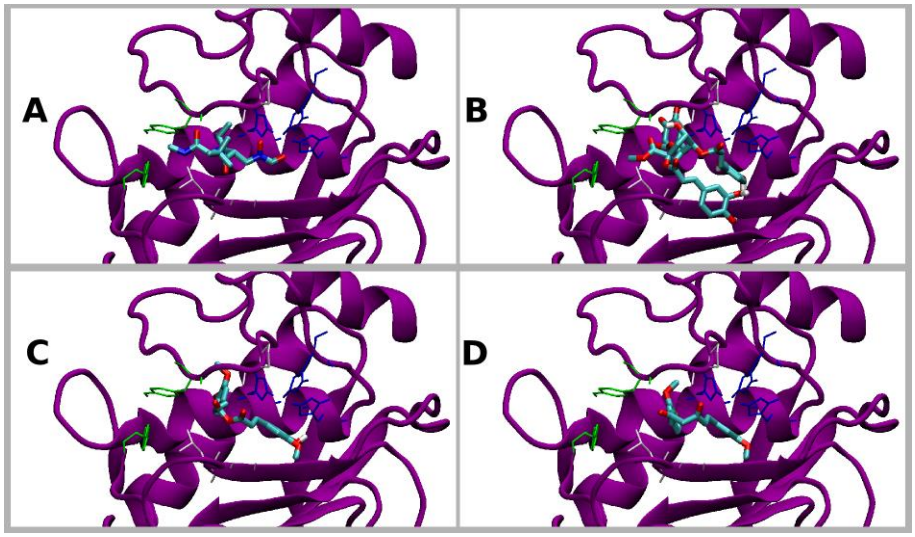


Figure 5: Figure showing aryl sulfotransferase in purple ribbon with bound ligands in liquorice representing (A) adenosine-3'-5'-diphosphate, (B) compound **1**, (C) compound **2**, and (D) curcumin.

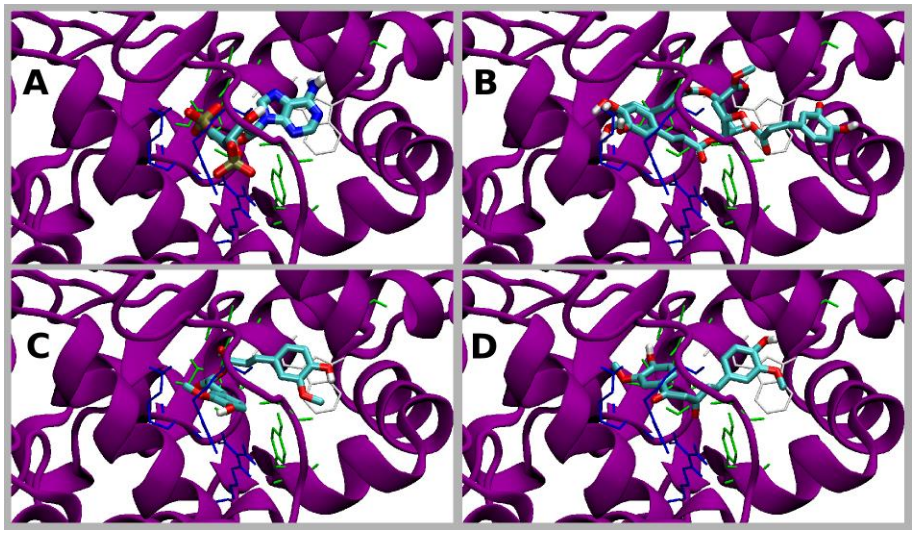


Table 1: Table showing calculated binding free energies for co-crystallized ligands, curcumin and Compounds 1 and 2.

PDB code, name	Co-crystallized compound	Binding energy/ kcal			
		Inhibitor	Compound1	Compound2	Curcumin
1LS6, Aryl sulfotransferase	Adenosine-3'-5'-diphosphate	-12.4	2.5	-9.0	-8.3
1LS6	*p*-nitrophenol	-6.0	3.9	-8.2	-8.2
1GKC (metalloproteinase-9)	N~2~-[(2r)-2-{[formyl(hydroxy)amino]methyl}-4-methylpentanoyl]-n,3-dimethyl-l-valinamide	-6.9	-8.6	-7.5	-7.2
4J96, fibroblast growth factor receptor 2	Phosphomethylphosphonic acid adenylate ester	-7.6	-9.4	-7.8	-7.7
4ZAU, epidermal growth factor receptor	N-(2-{[2-(dimethylamino)ethyl](methyl)amino}-4-methoxy-5-{[4-(1-methyl-1h-indol-3-yl)pyrimidin-2-yl]amino}phenyl)prop-2-enamide	-7.2	-8.2	-7.5	-7.3
5FD2, serine/threonine-protein kinase b-raf	6-[2-[[3-(dimethylsulfamoylamino)-2,6-bis(fluoranyl) phenyl]amino]pyridin-3-yl]-7~{h}-purine	-10.9	-9.6	-9.1	-8.5
5HES, mitogen-activated protein kinase kinase kinase mlt	N-(3-{[5-(4-chlorophenyl)-1h-pyrrolo[2,3-b]pyridin-3- yl]carbonyl}-2,4-difluorophenyl)propane-1-sulfonamide	-12.7	-7.7	-9.1	-8.9
5J18, ras binding domain	N-[2-methoxy-5-({[(e)-2-(2,4,6-trimethoxyphenyl) ethenyl]sulfonyl}methyl)phenyl]glycine	-5.0	-5.4	-4.7	-4.9

Cover letter

Dear Editor,
Bioorganic and Medicinal Chemistry Letters (BMCL),

We would like to submit for consideration for publication in Bioorganic and Medicinal Chemistry Letters our manuscript titled 'Polyphenolic compounds with anti-tumour potential from *Corchorus olitorious* L. (Tiliaceae), a Nigerian leafy vegetable,' which showcases the potential for plants used indigenously to yield useful leads for drug design and development. It also shows the reasonability and possibility of profiling indigenous plants to discover whether or not there exists any scientific rationale for their ancient and/or current local uses.

Corchorus olitorious, the focus of this report, is one of the underutilized crops that have been eaten in Nigeria as leafy vegetables for many years. While there are many folkloric uses of the plant species reported in the literature and evidence from biological studies that it has anti-tumour activity, the compounds so far isolated from the plant are yet to fully account for the reported biological properties.

Our manuscript therefore advances knowledge in this regard by revealing the isolation and characterization from the plant species, as well as some biological profiling, of two polyphenolic compounds, one of which is a bioisosteric analogue of curcumin, a compound variously reported in the literature to have great chemopreventive and chemotherapeutic efficacy against cancer.

We should be grateful to have this manuscript considered for publication in your Journal, as the work reported therein stands at the chemistry-biology interface. We consider it appropriate, helpful and timely to report our findings through your esteemed Journal, in order to make them accessible to the scientific community as well as any other users of the literature.

Regards and best wishes.

Bamigboye J. Taiwo, PhD

Corresponding Author (on behalf of all authors)

Response to the reviewers comments.

Point 1. We have revised the conclusion to state that the plant species is a source of 'lead' compounds with antitumour potential**.**

Pont 2 The addresses of the authors have been denoted as advised.

Point 3 The editing of the errors pointed out in the text had been carried out.

Reviewer 1

In the revision, we clearly state that the two isolated compounds are being reported for the first time in the plant species. Compound 2 had been reported before as the free quinic acid (Merfort, I. Phytochemistry 1992, 31, 2111). We obtained it as a methyl ester, which we proposed was formed during extraction from the methanol used for the process. This occurrence was also pointed out by Mefort in the cited article. So in nature, the compound is not new and we rely on the characterization of Mefort, especially, the absolute configuration and high resolution mass determination.

For compound 1, high resolution mass was recommended. We initially reported the results of the elemental analysis on the isolated compounds. We believe this method has been used by many before the advent of high resolution mass spectrometer and is still being accepted in lieu of HRMS because of the few centers that provide the service and the high cost. After re-isolation of the compounds, we made effort to obtain the service at Swanse but still cold not yet get the service done because of the processes of registration. So we want to request the kind permission of the editor to use the result of the elemental analysis.

Reviewer 2

The editing errors pointed at by the reviewer had been made and we are grateful for it.

Overall

Shortly after the review of the manuscript, we initiated a review of the local, common and botanical names of the some vegetables consumed in our community. This was done in collaboration with the Botany Department, Pharmacognosy Department and The Forest Research Institute of Nigeria, Ibadan, Oyo state Nigeria. One of the vegetables was the one we worked with and it was properly identified as *Corchorus olitorious* L. (Tiliaceae). This informed the change of the name of the plant in our manuscript from *Strobilanthes crispus* to *Corchorus olitorious* L. We have reviewed and included in the write up the work done so far on the plant species.

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