

Induction of Nrf2 activity by *Daucus carota* subsp. *maximus*: bioassay-guided isolation of chrysoeriol, luteolin 7-O-glucoside and eugenin as active compounds

Waed Alsheikh¹, Lutfun Nahar^{1,2,*}, Kenneth J. Ritchie¹, Satyajit D. Sarker^{1,*}

¹Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom

²Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, Olomouc 78371, Czech Republic

*Corresponding authors. Lutfun Nahar, Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom. E-mail: L.Nahar@ljmu.ac.uk; Satyajit D. Sarker, Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, United Kingdom. E-mail: S.Sarker@ljmu.ac.uk.

Abstract

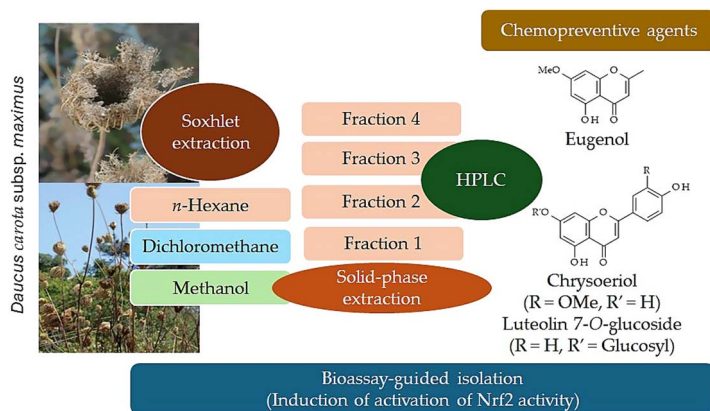
Objectives: To investigate the Nrf2 activity-inducing property of *Daucus carota* subsp. *maximus* and to isolate the bioactive compounds responsible for this.

Methods: The cell viability (MTT) assay was performed using the AREc32 cell line to determine non-cytotoxic concentrations of the test samples. Subsequently, a luciferase reporter gene assay was conducted with these non-toxic concentrations in AREc32 cells to evaluate the induction of the cellular antioxidant defence system through activation of the Nrf2 transcription factor. Bioactive compounds were then isolated from the active fractions by HPLC, and their structures elucidated using spectroscopic techniques.

Key findings: The active methanolic extract was subjected to bioassay-guided fractionation, yielding five compounds: apigenin (1), chrysoeriol (2), luteolin (3), luteolin 7-O-glucoside (4), and eugenin (5). Among these, compounds 2, 4, and 5 exhibited more than a two-fold induction of Nrf2 activity, indicating their potential as cancer chemopreventive agents. Notably, eugenin (5) demonstrated the highest potency, inducing Nrf2 activity by 8.1-fold.

Conclusions: The high level of Nrf2 induction and simple chemical structure suggested that eugenin (5) could be a suitable candidate for generating synthetic analogues to develop new cancer chemopreventive agents.

Graphical abstract



Keywords *D. carota*, Apiaceae, chemoprevention, Nrf2, AREc32 cells, eugenin

Received: 3 July 2026. Revised: 12 August 2026. Accepted: 20 September 2026

© The Author(s) 2026. Published by Oxford University Press on behalf of the Royal College of Pharmacy.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

Introduction

Cancer remains one of the leading causes of mortality worldwide, accounting for ~10 million deaths in 2020 [1–3]. The global incidence of cancer is projected to rise markedly, reaching over 28 million cases by 2040 and 35 million by 2050, compared with an estimated 20 million cases in 2022 [4–7]. Current therapeutic strategies include surgical tumour removal, chemotherapy, radiotherapy, targeted therapies, and immunotherapies [8]. However, concerns regarding toxicity, adverse effects, and the high economic burden associated with these treatments have intensified interest in cancer chemoprevention, a strategy involving the use of natural or synthetic chemicals to prevent, delay, or reverse carcinogenesis [9].

Phytochemicals, particularly dietary phytochemicals such as curcumin, lycopene, and resveratrol, have demonstrated chemopreventive potential by modulating multiple cell-signalling pathways, including JAK/STAT, PI3K/Akt, Wnt, NF- κ B, TGF- β , and MAPK [10–12], and by activating endogenous defence mechanisms through Nrf2 induction [9]. Cancer chemopreventive agents are broadly categorised into two principal classes: tumour-blocking agents and tumour-suppressing agents (Fig. 1) [13].

As part of our ongoing investigations into the cancer chemopreventive potential of both edible and non-edible plants from diverse geographical regions, and the bioassay-guided isolation of their active constituents [14–16], *Daucus carota* subsp. *maximus* (Desf.) Ball (family: Apiaceae), collected from the Syrian Coastal Mountain Range, was selected for study owing to its documented use in Syrian traditional medicine [17–20]. Although the essential oil of this species has been examined for its chemical composition and selected bioactivities [17–20], no phytochemical or bioactivity studies of the extracts from the aerial parts, particularly with respect to cancer chemoprevention, have been reported to date. Therefore, we present here, for the first time, an evaluation of the cancer chemopreventive activity of extracts of *D. carota* subsp. *maximus*, using induction of Nrf2 activity as an indicator of chemopreventive potential [16], together with the bioassay-guided isolation [21] of active compounds employing preparative high-performance liquid chromatography (HPLC).

It is worth noting that the present work uncovers a previously unreported Nrf2-inducing capacity in *D. carota* subsp. *maximus*, considerably expanding the chemopreventive potential of the genus and positioning this subspecies as a promising reservoir of antioxidant defence modulators. The combination of AREc32-based luciferase screening with HPLC-assisted purification provides a methodological innovation, enabling precise attribution of Nrf2 activation to discrete phytochemical entities. Identification of apigenin, chrysoeriol, luteolin, luteolin 7-O-glucoside, and eugenin as active constituents advances the phytochemistry of *Daucus* species and reveals new functional roles for these compounds in redox-regulated signalling. The exceptional potency of eugenin, coupled with its structural simplicity, offers a strategic lead compound for synthetic optimisation, revealing a translational pathway toward next-generation chemopreventive agents. Overall, the study provides mechanistic clarity, novel chemical leads, and a robust discovery pipeline, collectively strengthening the scientific basis for Nrf2-targeted chemoprevention.

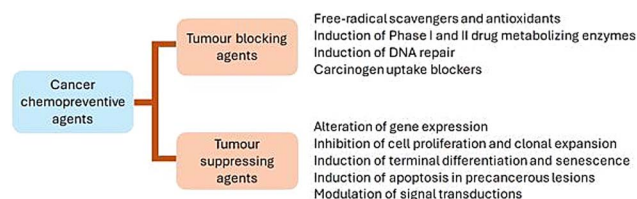


Figure 1 Classes of cancer chemopreventive agents.

Materials and methods

General

All chemicals and reagents were obtained from Sigma-Aldrich (Dorset, UK), and solvents were sourced from Fisher Scientific (Loughborough, UK). Deuterated NMR solvents were purchased from GOSS Scientific (Crewe, UK). 0.25% trypsin–EDTA, penicillin–streptomycin, and phosphate-buffered saline (PBS) were obtained from Thermo Fisher Scientific (Paisley, UK). DMEM basal medium, foetal bovine serum (FBS), and geneticin (G418) were purchased from Biosera (Neuville, France). The luciferase assay reagent was obtained from Promega (Wisconsin, USA). White 96-well flat-bottom plates were sourced from Greiner Bio-One (Frickenhäusen, Germany), and clear 96-well flat-bottom plates from Falcon (North Carolina, USA).

A Dionex Ultimate 3000 UHPLC (Thermo Fisher Scientific, Massachusetts, USA), hyphenated with a diode array detector (DAD), was used for analytical HPLC analyses. A reversed-phase (RP) Phenomenex column (Luna 5 μ m C₁₈(2) 100 Å, 150 $\times$ 4.6 mm, Phenomenex, California, USA) was used for analytical HPLC runs. A preparative Dionex Ultimate 3000 UHPLC (Thermo Fisher Scientific, Massachusetts, USA) or a Jasco LC-4000 Series (Jasco, Tokyo, Japan) and a reversed-phase Phenomenex preparative column (Luna 10 μ m C₁₈(2) 100 Å, 150 $\times$ 21.2 mm, Phenomenex, California, USA) were used for isolating compounds from active fractions. A RP Phenomenex semi-preparative column (Luna 5 μ m C₁₈, 150 $\times$ 10 mm, Phenomenex, California, USA) was used for further purification of impure compounds. A flow rate of 1 mL/min, 2 mL/min, or 10 mL/min was used for analytical, semi-preparative, and preparative HPLC, respectively. Variable UV–vis wavelengths, 220, 254, 280, and 320 nm, were used to monitor the chromatograms. The column thermostat temperature was maintained at 25°C for the column chamber. The NMR experiments were performed in CD₃OD or DMSO-*d*₆ on a Bruker AMX-600 NMR spectrometer (600 MHz for ¹H, and 150 MHz for ¹³C) (Bruker, Massachusetts, USA). Chemical shifts δ were in ppm, and the coupling constants *J* in Hz. ESI-MS data were recorded through a Micromass LCT time-of-flight (TOF) mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionisation (ESI) source.

Plant materials

Aerial parts of wild-grown *D. carota* subsp. *maximus* (Desf.) Ball were collected from the Syrian Coastal Mountain Range in August 2020. The plant material was taxonomically identified by Professor Ahmad Jadou (Faculty of Agriculture Engineering, University of Aleppo, Syria), and a voucher specimen (PAJ-UA-200001) was deposited in the Herbarium of the University of Aleppo, Syria. The

collected aerial parts were shade-dried and subsequently ground using a grain grinder before extraction.

Extraction

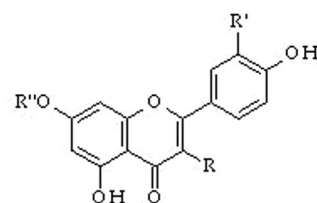
Ground aerial parts (850 g) were extracted with a Soxhlet (1 L), successively, using *n*-hexane, dichloromethane (DCM), and methanol (MeOH), 900 mL each. The temperature was set close to the boiling point of each solvent, and 10 cycles were used for the extraction with each solvent. The resulting extracts were filtered through standard laboratory filter paper, and the filtrates were evaporated to dryness using a rotary evaporator (Buchi, Flawil, Switzerland) using the water bath temperature maintained at 35–45°C, depending on the solvent (for *n*-hexane and DCM 35°C, and for MeOH 45°C). Dried extracts were weighed, transferred to clean glass vials, and kept at 4°C for further use.

Fractionation

As the MeOH extract was the most active extract among the three extracts, a portion (2 g) of the MeOH extract was fractionated by solid-phase extraction (SPE) [21] on a Strata C₁₈ E (20 g) RP cartridge (Phenomenex, California, USA) using a step gradient to obtain four fractions as follows: 20% MeOH in water, 40% MeOH in water, 80% MeOH in water and 100% MeOH, each 200 mL, yielding, respectively, fractions F1–F4). Before fractionation, the cartridge was cleaned with MeOH (50 mL) and equilibrated with water (100 mL) under vacuum. All fractions were concentrated to dryness using a rotary evaporator, maintaining the water bath temperature at 45–50°C, and were further dried with a nitrogen blowdown concentrator and maintained at 4°C for further analyses.

Isolation and identification of compounds

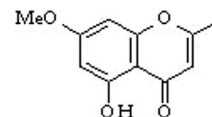
As fractions F2 and F3 emerged as the active fractions, they were subjected, first, to method development for optimum separation of compounds using the analytical HPLC and various solvent systems (not described in this article), and then to preparative HPLC for isolating individual compounds using the optimised mobile phase. The purity of the isolated compounds was assessed by analytical HPLC, and impure compounds were subjected to semi-preparative HPLC purification. A shallow gradient of 25%–55% MeOH in water (both solvents contained 0.1% trifluoroacetic acid, TFA) over 30 min, at a flow rate of 10 mL/min, using the prep-HPLC separation, luteolin 7-*O*-glucoside (**4**, *t_R* = 24.2 min) (Fig. 2) from the SPE fraction F2. Similarly, F3 was subjected to prep-HPLC separation, using a shallow gradient of 30%–45% acetonitrile (ACN) in water, both containing 0.1% TFA, over 40 min at a flow rate of 10 mL/min to obtain luteolin (**3**, *t_R* = 15.4 min), and a mixture of apigenin and chrysoeriol (**1** and **2**, *t_R* = 24.5–16.1 min), and eugenin (**5**, *t_R* = 38.9 min) (Fig. 2). The mixture of apigenin (**1**) and chrysoeriol (**2**) was further purified by semi-preparative HPLC using an isocratic mobile phase (2 mL/min), comprising 27% ACN in water (both contained 0.1% TFA), affording pure apigenin (**1**, *t_R* = 20.1 min) and chrysoeriol (**2**, *t_R* = 22.1 min). The structures of all isolated compounds were elucidated conclusively by comprehensive NMR and MS analyses, and by comparison with respective published data.



Apigenin (**1**) R = R' = R'' = OH; Chrysoeriol (**2**) R = R'' = H; R' = OMe

Luteolin (**3**) R = R'' = H; R' = OH

Luteolin 7-*O*-glucoside (**4**) R = H; R' = OH; R'' = Glucosyl



Eugenin (**5**)

Figure 2 Chemical structures of compounds (**1**–**5**) isolated from the active fractions of the MeOH extract of *D. carota* subsp. *maximus*.

Apigenin (1, 2.3 mg): $\lambda_{\max}$ (MeOH) 254 and 348 nm; ^1H NMR (CD₃OD, 600 MHz) δ 7.86 (2H, d, J = 8.4, H-2' and H-6'), 6.96 (2H, d, J = 8.4, H-3' and H-5'), 6.60 (1H, s, H-3), 6.46 (1H, d, J = 2.1, H-8) and 6.21 (1H, d, J = 2.1, H-6); ^{13}C NMR (CD₃OD, 150 MHz) δ 182.3 (C-4), 165.9 (C-7), 164.6 (C-2), 161.9 (C-5), 158.0 (C-9), 149.6 (C-4'), 129.1 (C-2' and C-6'), 121.6 (C-1'), 116.5 (C-3' and C-5'), 103.9 (C-10), 103.3 (C-3), 99.3 (C-6) and 94.5 (C-8); ESI-MS (negative ion mode) $[\text{M-H}]^-$ m/z 269.1050 corresponding to the molecular formula C₁₅H₁₀O₅ [22, 23].

Chrysoeriol (2, 2.9 mg): $\lambda_{\max}$ (MeOH) 252 and 346 nm; ^1H NMR (DMSO-*d*₆, 600 MHz) δ 7.54 (1H, dd, J = 2.2, 8.5, H-6'), 7.53 (1H, d, J = 2.2 H-2'), 6.92 (1H, d, J = 8.5, H-5'), 6.88 (1H, s, H-3), 6.51 (1H, d, J = 2.0, H-8), 6.17 (1H, d, J = 2.0, H-6) and 3.87 (3H, s, 3'-OMe); ^{13}C NMR (DMSO-*d*₆, 150 MHz) δ 182.3 (C-4), 164.6 (C-7), 164.2 (C-2), 161.8 (C-5), 157.8 (C-9), 151.2 (C-4'), 148.5 (C-3'), 122.0 (C-1'), 120.8 (C-6'), 116.4 (C-5'), 110.7 (C-2'), 104.2 (C-10), 103.7 (C-3), 99.3 (C-6), 94.5 (C-8) and 56.4 (3'-OMe); ESI-MS (negative ion mode) $[\text{M-H}]^-$ m/z 299.0556 corresponding to the molecular formula C₁₆H₁₂O₆ [24, 25].

Luteolin (3, 6.8 mg): $\lambda_{\max}$ (MeOH) 254 and 348 nm; ^1H NMR (CD₃OD, 600 MHz) δ 7.38 (2H, overlapped peaks, H-2' and H-6'), 6.89 (1H, d, J = 8.1, H-5'), 6.54 (1H, s, H-3), 6.43 (1H, d, J = 1.4, H-8) and 6.20 (1H, d, J = 1.4, H-6); ^{13}C NMR (CD₃OD, 150 MHz) δ 182.5 (C-4), 164.9 (C-7), 164.6 (C-2), 161.8 (C-5), 158.0 (C-9), 149.6 (C-4'), 145.6 (C-3'), 122.3 (C-1'), 118.9 (C-6'), 115.5 (C-5'), 112.8 (C-2'), 103.9 (C-10), 102.5 (C-3), 98.7 (C-6) and 93.6 (C-8); ESI-MS (negative ion mode) $[\text{M-H}]^-$ m/z 285.0397 corresponding to the molecular formula C₁₅H₁₀O₆ [26, 27].

Luteolin 7-*O*-glucoside (4, 4.6 mg): $\lambda_{\max}$ (MeOH) 254, 267 (sh) and 348 nm; ^1H NMR (DMSO-*d*₆, 600 MHz) δ 12.94 (1H, s, 5-OH), 10.09 (1H, br s, 4'-OH), 9.58 (1H, br s, 3'-OH), 7.43 (2H, overlapped peaks, H-2' and H-6'), 6.92 (1H, d, J = 8.0, H-5'), 6.80 (1H, d, J = 1.2, H-8), 6.74 (1H, s, H-3), 6.45 (1H, d, J = 1.2, H-6), 5.05 (1H, br d, J = 7.8, glucose anomeric proton) and 3.38–4.20 (unresolved peaks, masked by the huge solvent peak, the rest of the protons of the glucose unit, detected and confirmed by COSY, HSQC and HMBC); ^{13}C NMR (DMSO-*d*₆, 600 MHz) δ 182.4 (C-4), 165.0 (C-2), 163.4 (C-7), 161.5 (C-5), 158.0 (C-9), 150.4 (C-4'), 146.2 (C-3'), 121.8 (C-1'), 119.7 (C-6'), 116.5 (C-5'), 113.9 (C-2'), 105.8 (C-10), 103.5 (C-3), 100.4 (glucose anomeric carbon), 100.2 (C-6), 95.2 (C-8), 77.5 (C-5 of glucose), 76.8

(C-3 of glucose), 73.5 (C-2 of glucose), 70.0 (C-4 of glucose) and 61.1 (C-6 of glucose); ESI-MS (negative ion mode) $[M-H]^-$ m/z 447.4141 corresponding to the molecular formula $C_{21}H_{20}O_{11}$ [28–30].

Eugenin (**5**, 9.1 mg): $\lambda_{\max}$ (MeOH) 230, 250, 292 and 325 (sh) nm; ^1H NMR (CD_3OD , 600 MHz) δ 6.49 (1H, br s, H-8), 6.31 (1H, br s, H-6), 6.09 (1H, s, H-3), 3.86 (3H, s, 7-OMe) and 2.37 (3H, s, 2-Me); ^{13}C NMR (CD_3OD , 150 MHz) δ 182.7 (C-4), 168.3 (C-2), 165.9 (C-7), 161.7 (C-5), 158.4 (C-9), 107.8 (C-3), 104.5 (C-10), 97.7 (C-6), 92.0 (C-8), 55.0 (7-OMe) and 18.9 (2-Me); ESI-MS (positive ion mode) $[M+H]^+$ m/z 207.1875 corresponding to the molecular formula $C_{11}H_{10}O_4$ [31, 32].

Cell culture and cell viability assay (MTT assay)

The modified MCF7 human breast cancer cell line, AREc32, was kindly provided by Professor Roland Wolf (University of Dundee, UK) [33]. AREc32 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), high glucose, supplemented with 10% foetal bovine serum (FBS), 1% penicillin–streptomycin, and 0.8 mg/mL geneticin (G418), in T75 cell culture flasks maintained at 37°C in a humidified atmosphere containing 5% CO_2 (Binder, Tuttlingen, Germany).

The cell viability assay (MTT assay) [34] was performed using AREc32 cells to determine non-cytotoxic concentrations of the test samples for subsequent use in the luciferase reporter assay. The procedure followed was that described previously [14]. Cell viability (%) was calculated using the following equation:

$$\text{Cell viability} = (A_1/A_0) \times 100$$

Where A_0 is the mean absorbance of untreated wells, and A_1 is the mean absorbance of treated cells.

Luciferase reporter gene assay

The luciferase reporter gene assay using the AREc32 cell line was performed to evaluate the cancer chemopreventive potential of the crude extracts, fractions, and isolated compounds, as indicated by the induction of the cellular antioxidant defence system via activation of the Nrf2 transcription factor [33]. The assay protocol followed the method previously described [14].

Briefly, AREc32 cells were seeded into 96-well plates at a density of 1.2×10^4 cells/well and incubated for 24 h. Cells were then treated with a non-cytotoxic concentration of each test sample (crude extract, fraction, or isolated compound) and incubated for a further 24 h. After incubation, the culture medium was removed, and the cells were washed twice with phosphate-buffered saline (PBS, 100 μ L). $1 \times$ luciferase reporter lysis buffer (20 μ L) was added to each well, followed by a freeze–thaw cycle at $-20^\circ C$ for 24 h to ensure complete cell lysis. The thawed lysate was transferred to a white opaque 96-well plate and immediately mixed with freshly prepared luciferase assay reagent (100 μ L). Luminescence was measured using a TECAN microplate reader (Männedorf, Switzerland). Luciferase activity was compared with the basal activity in untreated control cells and expressed as fold induction relative to control. *Tert*-Butylhydroquinone (tBHQ, 10 μ M) served as the positive control. All experiments were performed in three independent runs, each comprising five replicates.

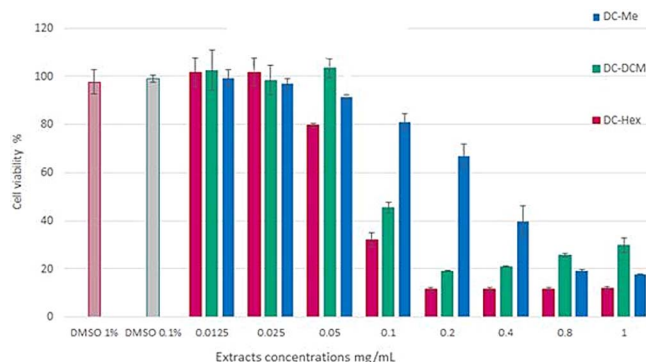


Figure 3 Cell viability assay (MTT assay) of *n*-hexane, DCM, and MeOH extracts of *D. carota* subsp. *maximus* using AREc32 cells [24 h, DMSO represents the vehicle control, results are plotted as means $\pm$ SEM of 3 independent experiments (5 replicates/experiment)].

Statistical analysis

All experiments were performed in triplicate, with a minimum of three replicates per tested concentration. Data are presented as means $\pm$ SEM (standard error of the mean). Graphs were generated using Microsoft Excel for Microsoft 365 MSO Version 2502, with SEM displayed as error bars.

Results and discussion

Soxhlet extraction of the shed-dried and ground aerial parts of *D. carota* subsp. *maximus* (Desf.) Ball, successively, using three different solvents, *n*-hexane, DCM, and MeOH, in the sequential order of polarity, afforded three different extracts with 3.4%, 0.8%, and 5.1% yields, respectively. The MeOH extract exhibited AREc32 cell viability of $>80\%$ and $>95\%$, respectively, at concentrations of 0.05 and 0.025 mg/mL, in the MTT assay (Fig. 3). Overall, the *n*-hexane extract was found to be toxic at these concentrations, and $>80\%$ cell viability was only achieved with lower concentrations (0.025 and 0.0125 mg/mL). More than 80% cell viability was observed at the concentrations of 0.05, 0.025, and 0.0125 mg/mL of the DCM extract. Therefore, 0.025 mg/mL could be used as the non-toxic starting concentration for *n*-hexane extract, while 0.05 mg/mL concentration was suitable for DCM and MeOH extracts for the luciferase assay. In the luciferase assay, the MeOH extract displayed 2.98-fold induction (data not shown) of Nrf2 activity and was selected for further chromatographic analysis for isolating active compounds.

The active MeOH extract was fractionated by SPE to obtain four fractions, Fr1 (67.8% yield), Fr2 (12.0% yield), Fr3 (5.6% yield), and Fr4 (2.1% yield), sequentially eluted, respectively, with 20% MeOH in water, 40% MeOH in water, 80% MeOH in water, and 100% MeOH. All four SPE fractions (Fr1–Fr4) were first subjected to the MTT assay (data not shown) to determine their non-cytotoxic concentrations, followed by evaluation in the luciferase reporter assay. Fractions Fr2 and Fr3 were identified as the most active, inducing 4.3-fold and 7.3-fold activation of Nrf2 activity, respectively (Fig. 4).

A combination of RP prep- and semi-prep-HPLC purification of the SPE fractions Fr2 and Fr3, using the method as described in Section 2.5, resulted in the isolation of four flavones, including apigenin (**1**), chrysoeriol (**2**), luteolin (**3**), and luteolin 7-*O*-glucoside

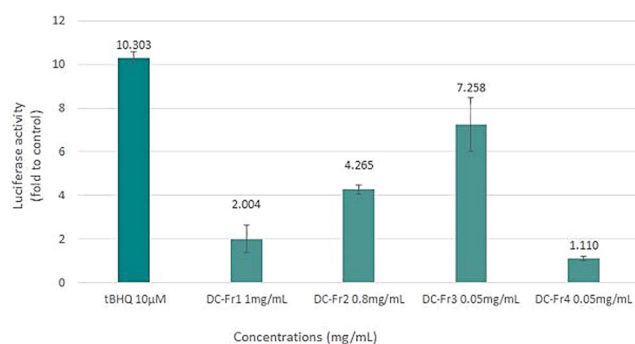


Figure 4 Luciferase activity of the SPE fractions (Fr1-Fr4) of the active MeOH extract.

(4), and a chromone, eugenin (5). Compounds 1–4 displayed UV-Vis absorptions at 300–380 nm and 240–295 nm, characteristic of flavonoids [35, 36], and the UV-Vis spectrum of 5 (λ_{max} 230, 250, 292, and 325 nm) supported the presence of chromone skeleton in 5 [32]. 1D and 2D NMR (nuclear magnetic resonance) spectroscopic analyses, complemented by mass spectroscopic (MS) analyses, confirmed the identity of these compounds, and all experimental spectroscopic data corresponded well with respective published data as outlined in the Materials and Methods section earlier.

It is noteworthy that apigenin (1) and luteolin (3) are widely distributed throughout the plant kingdom and have previously been reported from other subspecies of *D. carota*, though not from subsp. *maximus* [37]. The Apiaceae family appears to be a particularly rich source of eugenin (5) [31], and although eugenin (5) has been reported from *D. carota*, it has not been documented in subsp. *maximus*. Luteolin 7-*O*-glucoside (4), also known as cynaroside, is recognised as the predominant flavone in carrots [30]. While several chrysoeriol glycosides have been isolated from *D. carota* [30, 38], the aglycone chrysoeriol (2) has not previously been reported from any subspecies. Thus, this study provides the first report of chrysoeriol (2) isolated from *D. carota*.

All compounds [1–5], isolated from the active SPE fractions (Fr2 and Fr3), were first subjected to MTT assay using AREC32 cells as described earlier to determine non-toxic concentrations ($\geq 90\%$ cell viability), and then to luciferase assay. The non-toxic concentration of luteolin 7-*O*-glucoside (4, 250 μM) was higher than that of the aglycone flavones (1–3, 100 μM) but was the same as that of eugenin (5, 250 μM) (data not shown). The luciferase assay showed that chrysoeriol (2), luteolin 7-*O*-glucoside (4), and eugenin (5) induced the activation of Nrf2 activity, respectively, 5.3, 4.6, and 8.1-fold, whereas the induction with the positive control *t*-butylhydroquinone (tBHQ) was 16.1 and 5.8-fold at 20 μM and 10 μM concentrations (Fig. 5). An induction of activation of Nrf2 activity by 2-fold or more is considered an indication for any test samples to possess cancer chemopreventive potential [14, 16, 33].

Among the isolated compounds, flavones apigenin (1) and luteolin (3) were not considered active, as the extent of induction was less than 2-fold (Fig. 5). Eugenin (5) was found to be the most active compound in the luciferase assay, and considering the structural simplicity and feasibility for conducting laboratory synthesis and easy structural modifications [39], this compound and its synthetic analogues could be ideal candidates for further studies aiming at developing new cancer chemopreventive agents. In

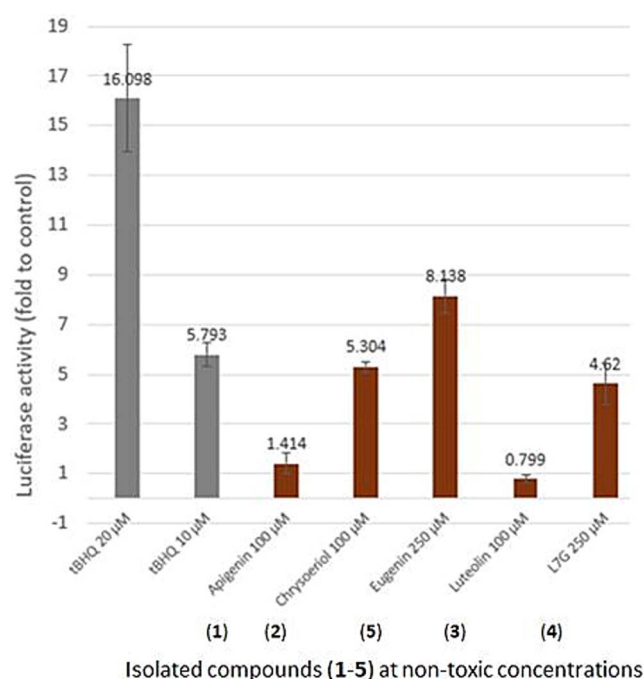


Figure 5 Luciferase activity of compounds 1–5 (at respective non-toxic concentration) isolated from the active SPE fractions.

a recent study, the influence of an α,β -unsaturated carbonyl, sulfonyl, or sulfinyl moiety towards Nrf2 activation was demonstrated [40], albeit it was mainly focused on α,β -unsaturated sulfonyl functionality. An α,β -unsaturated carbonyl functionality makes any compound suitable for undergoing Michael addition reaction [40, 41] with the nucleophilic thiols of cysteine residues in KEAP1 (Kelch-like ECH-associated protein 1), a negative regulator for Nrf2 involved in the activation of the Nrf2 signalling pathway. In compounds 1–5, an α,β -unsaturated carbonyl moiety is present (C-2/C-3-unsaturation, and a carbonyl functionality at C-4), but other structural features, e.g. a methoxyl group in 2, a glucosyl unit in 4, and the absence of the second aromatic ring in 5, might have contributed to the observed differences in the induction ability for activation of Nrf2 activity.

Chrysoeriol (2) previously showed an inhibitory effect on DNA adduct formation with benzo[a]pyrene in MCF-7 breast cancer cells, and it was suggested that this compound could offer chemoprevention against environmental pro-carcinogens such as benzo[a]pyrene [42]. Cancer chemopreventive potential of 2 was further demonstrated by its ability to selectively inhibit the formation of a carcinogenic estrogen metabolite, 2-hydroxy-17 β -estradiol *via* inhibiting CYP1B1 (cytochrome P450 1B1) activity, in MCF-7 breast cancer cells [43]. Yoon and Patk [44] reported that chrysoeriol (2) could offer cancer chemoprevention through its ameliorating effect on COX-2 expression through NF- κ B, AP-1 (activator protein 1), and MAPK regulation via the TLR4/MyD88 signalling pathway in lipopolysaccharide-stimulated murine macrophages. Similarly, the cancer chemopreventive potential of luteolin 7-*O*-glucoside (4) was reported previously [45]. However, none of the previous reports looked at the ability of these compounds (2 and 4) to induce the activation of Nrf2 activity. Eugenin (5) is considered an emerging cancer chemopreventive agent that has previously been found to mediate its chemopreventive activity *via* Nrf2 pathway induction, turning on Nrf2-driven antioxidant

and anti-inflammatory cascades in the body, which protect cells from DNA damage and oxidative stress that can lead to cancer [46], and this has been further substantiated by the findings from the studies described in this paper.

Conclusion

This study provides the first report on the cancer chemopreventive potential of extracts of *D. carota* subsp. *maximus*, evaluated through the induction of Nrf2 activity as an indicator of chemopreventive activity, together with the bioassay-guided isolation of five compounds: apigenin (1), chrysoeriol (2), luteolin (3), luteolin 7-O-glucoside (4), and eugenin (5). Among these isolates, eugenin (5) exhibited the highest chemopreventive potency, demonstrating an 8.1-fold induction of Nrf2 activity. Given its strong activity and structurally simple scaffold, eugenin (5) represents a promising lead candidate for the development of new synthetic analogues with enhanced cancer chemopreventive potential.

Acknowledgements

W.A. thanks the LJMU international scholarship in collaboration with the Council for At-Risk Academics (CARA) for funding her PhD scholarship.

Author contributions

Conceptualization and methodology, K.J.R., L.N., and S.D.S.; formal analysis and investigation, W.A.; resources, K.J.R., L.N., and S.D.S.; writing—original draft preparation, W.A., K.J.R., L.N., and S.D.S.; writing—review and editing, K.J.R., L.N., and S.D.S.; supervision, K.J.R., L.N., and S.D.S.; project administration, K.J.R., L.N., and S.D.S.; funding acquisition, W.A. All authors have read and agreed to the published version of the manuscript.

Conflicts of 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.

Funding

Liverpool John Moores University (LJMU) international scholarship, in collaboration with The Council for At-Risk Academics (CARA), supported the PhD studentship of W.A.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

- World Health Organization (WHO). Cancer. 2026. <https://www.who.int/news-room/fact-sheets/detail/cancer> (30 May 2026, date last accessed).
- J. Ferlay; M. Ervik; F. Lam; M. Colombet; L. Mery; M. Piñeros. *et al.* *Global Cancer Observatory: Cancer Today*. Lyon: International Agency for Research on Cancer, 2020. <https://gco.iarc.fr/today> (30 May 2026, date last accessed).
- Fink H, Langselius O, Vignat J. *et al.* Global and regional cancer burden attributable to modifiable risk factors to inform prevention. *Nat Med* 2026;32:1306–15. <https://doi.org/10.1038/s41591-026-04219-7>
- World Health Organization (WHO). *Global Cancer Burden Growing Amidst Mounting Need for Services*, 2024. <https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing-amidst-mounting-need-for-services> (30 May 2026, date last accessed).
- Cancer Research UK. *Worldwide Cancer Incidence Statistics*, 2026. <https://www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer/incidence> (30 May 2026, date last accessed).
- Bray F, Laversanne M, Sung H. *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer J Clinicians* 2024;74:229–63. <https://doi.org/10.3322/caac.21834>
- Luo, Q., Smith, D.P. Global cancer burden: Progress, projections, and challenges. *Lancet* 2025; 406: P1536–7. [https://doi.org/10.1016/S0140-6736\(25\)01570-3](https://doi.org/10.1016/S0140-6736(25)01570-3)
- Liu B, Zhou H, Tan L. *et al.* Exploring treatment options in cancer: tumor treatment strategies. *Signal Transduction and Targeted Therap* 2024;9:175. <https://doi.org/10.1038/s41392-024-01856-7>
- Ritchie KJ, Sarker SD. In “Medicinal natural products – A disease-focused approach” (Editors: Sarker, S. D. and Nahar, L.), *Cancer Chemopreventive Natural Products*. *Ann Rep Med Chem* 2020; 55: 273–95. <https://doi.org/10.1016/bs.armc.2020.02.004>
- Lekhakh N, Bhattarai HK. Phytochemicals in cancer chemoprevention: preclinical and clinical studies. *Cancer Control* 2024;31:10732748241302902. <https://doi.org/10.1177/10732748241302902>
- Russo GL, Spagnuolo C, Russo M. Reassessing the role of phytochemicals in cancer chemoprevention. *Biochem Pharmacol* 2024;228:116165. <https://doi.org/10.1016/j.bcp.2024.116165>
- Ramos S. Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. *Mol Nutr Food Res* 2008;52:507–5026. <https://doi.org/10.1002/mnfr.200700326>
- Sur Y-J. Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 2003;3:768–80. <https://doi.org/10.1038/nrc1189>
- Khuniad C, Nahar L, Talukdar AD. *et al.* Cancer chemopreventive potential of *Claoxylon longifolium* grown in southern Thailand: a bioassay-guided isolation of vicenin 1 as the active compound and *in silico* studies on related C-glycosyl flavones. *Molecules* 2025;30:3173. <https://doi.org/10.3390/molecules30153173>
- Jasim HA, Nahar L, Jasim MA. *et al.* Chalcones: synthetic chemistry follows where nature leads. *Biomolecules* 2021;11:1203. <https://doi.org/10.3390/biom11081203>
- Basar N, Nahar L, Oridupa OA. *et al.* Utilization of the ability to induce activation of the nuclear factor (erythroid-derived 2)-like factor 2 (Nrf2) to assess potential cancer chemopreventive activity of liquorice samples. *Phytochem Anal* 2016;27:233–8. <https://doi.org/10.1002/pca.2616>

17. Badalamenti N, Modica A, Ilardi V. *et al.* *Daucus carota* subsp. *maximus* (Desf.) ball from Pantelleria, Sicily (Italy): isolation of essential oils and evaluation of their bioactivity. *Nat Prod Res* 2022;36:5842–7. <https://doi.org/10.1080/14786419.2021.2018588>
18. Khoury M, El Beyrouthy M, Eparvier V. *et al.* Chemical diversity and antimicrobial activity of the essential oils of four Apiaceae species growing wild in Lebanon. *J Essential Oil Res* 2018;30:25–31. <https://doi.org/10.1080/10412905.2017.1372314>
19. Gaglio R, Barbera M, Aleo A. *et al.* Inhibitory activity and chemical characterization of *Daucus carota* subsp. *maximus* essential oils. *Chem Biodivers* 2017;14:e1600477. <https://doi.org/10.1002/cbdv.201600477>
20. Valente J, Resende R, Zuxarte M. *et al.* Bioactivity and safety profile of *Daucus carota* subsp. *maximus* essential oil. *Indust Crops Prods* 2015;77:218–24. <https://doi.org/10.1016/j.indcrop.2015.08.037>
21. Sarker SD, Nahar L. *Natural Products Isolation* 3rd edn. New Jersey, USA: Humana Press – Springer-Verlag, 2012.
22. Ersöz T, Harput ÜŞ, Saracoglu I. *et al.* Phenolic compounds from *Scutellaria pontica*. *Turk J Chem* 2002;26:581–8. <https://journals.tubitak.gov.tr/chem/vol26/iss4/16>
23. Karaoglan ES, Ozgen U, Calis I. *et al.* Isolation of major compounds of *Origanum micranthum* and *Origanum minutiflorum*. *FABAD J Pharm Sci* 2020;45:135–43.
24. Park Y, Moon B-H, Yang H. *et al.* Complete assignments of NMR data of 13 hydroxymethoxyflavones. *Magn Reson Chem* 2007;45:1072. <https://doi.org/10.1002/mrc.2063>
25. Bashyal P, Parajuli P, Pandey RP. *et al.* Microbial biosynthesis of antibacterial chrysoeriolin recombinant *Escherichia coli* and bioactivity assessment. *Catalysts* 2019;9:112. <https://doi.org/10.3390/catal9020113>
26. Zheng Z-P, Cheng K-W, Chao J. *et al.* Tyrosinase inhibitors from paper mulberry (*Broussonetia Papyrifera*). *Food Chem* 2008;106:529–35. <https://doi.org/10.1016/j.foodchem.2007.06.037>
27. Iqbal K, Iqbal J, Staerk D. *et al.* Characterization of Antileishmanial compounds from *Lawsonia inermis* L. leaves using semi-high resolution antileishmanial profiling combined with HPLC-HRMS-SPE-NMR. *Front Pharmacol* 2017;31:337. <https://doi.org/10.3389/fphar.2017.00337>
28. Chiruvella K, Mohammed A, Dampuri G. *et al.* Phytochemical and antimicrobial studies of methyl angolensate and luteolin-7-O-glucoside isolated from callus cultures of *Soymida febrifuga*. *Int J Biomed Sci* 2007;3:269–78.
29. Xu T, Wang C, Jiang S. *et al.* Glycosylation of luteolin in hydrophilic organic solvents and structure-antioxidant relationships of luteolin glycosides. *RSC Adv* 2022;12:18232–7. <https://doi.org/10.1039/D2RA03300C>
30. Hwang JT, Kim HJ, Ryuk JA. *et al.* Efficiency of the enzymatic conversion of flavone glycosides isolated from carrot leaves and anti-inflammatory effects of enzyme-treated carrot leaves. *Molecules* 2023;28:4291. <https://doi.org/10.3390/molecules28114291>
31. Nahar L, Alsheikh W, Ritchie KJ. *et al.* Naturally occurring eugenin: biosynthesis, distribution, bioactivity, and therapeutic potential. *Phytochem Lett* 2024;61:191–7. <https://doi.org/10.1016/j.phytol.2024.04.017>
32. Griffiths PJF, Ellis GP. Benzopyrones—VI¹: the ultraviolet absorption spectra of chromone and 2-substituted chromones. *Spectrochimica Acta Part A: Mol Spectros* 1972;28:707–13. [https://doi.org/10.1016/0584-8539\(72\)80039-4](https://doi.org/10.1016/0584-8539(72)80039-4)
33. Wang XJ, Hayes JD, Wolf CR. Generation of a stable antioxidant response element-driven reporter gene cell line and its use to show redox-dependent activation of Nrf2 by cancer chemotherapeutic agents. *Cancer Res* 2006;66:10983–94. <https://doi.org/10.1158/0008-5472.CAN-06-2298>
34. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Meth* 1983;65:55–63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4)
35. Taniguchi M, LaRocca CA, Bernat JD. *et al.* Digital database of absorption spectra of diverse flavonoids enables structural comparisons and quantitative evaluations. *J Nat Prod* 2023;86:1087–119. <https://doi.org/10.1021/acs.jnatprod.2c00720>
36. Mabry TJ, Markham KR, Thomas MB. The ultraviolet spectra of flavones and flavonols. “*Systematic Identification of Flavonoids*.” USA: Springer, 1970, 41–164.
37. Ismail J, Shebaby WN, Daher J. *et al.* The wild carrot (*Daucus carota*): a phytochemical and pharmacological review. *Plants* 2024;13:93. <https://doi.org/10.3390/plants13010093>
38. Cheng L, Liu G-Y, Pan Y-C. *et al.* Chemical constituents of the aerial parts of *Daucus carota*. *Chem Nat Compds* 2019;55:665–8. <https://doi.org/10.1007/s10600-019-02773-w>
39. Maurya AK, Agarwal K, Gupta AC. *et al.* Synthesis of eugenol derivatives and its anti-inflammatory activity against skin inflammation. *Nat Prod Res* 2020;34:251–60. <https://doi.org/10.1080/14786419.2018.1528585>
40. Egbujor MC, Buttari B, Profumo E. *et al.* An overview of NRF2-activating compounds bearing α,β -unsaturated moiety and their antioxidant effect. *Int J Mol Sci* 2022;23:8466. <https://doi.org/10.3390/ijms23158466>
41. Nair DP, Podgorski M, Chatani S. *et al.* The thiol-Michael addition click reaction: a powerful and widely used tool in materials chemistry. *Chem Materials* 2013;26:724–44. [dx.doi.org/10.1021/cm402180t](https://doi.org/10.1021/cm402180t)
42. Takemura H, Nagayoshi H, Matsuda T. *et al.* Inhibitory effects of chrysoeriol on DNA adduct formation with benzo[a]pyrene in MCF-7 breast cancer cells. *Toxicol* 2010;274:42–8. <https://doi.org/10.1016/j.tox.2010.05.009>
43. Takemura H, Uchiyama H, Ohura T. *et al.* A methoxyflavonoid, chrysoeriol, selectively inhibits the formation of a carcinogenic estrogen metabolite in MCF-7 breast cancer cells. *The J Steroid Biochem Mol Biol* 2010;118:70–6. <https://doi.org/10.1016/j.jsbmb.2009.10.002>
44. Yoon H-S, Park CM. Chrysoeriol ameliorates COX-2 expression through NF- κ B, AP-1 and MAPK regulation via the TLR4/MyD88 signaling pathway in LPS-stimulated murine macrophages. *Exp Ther Med* 2021;22:718. <https://doi.org/10.3892/etm.2021.10150>
45. Baskar AA, Ignacimuthu S, Michael GP. *et al.* Cancer chemopreventive potential of luteolin-7-O-glucoside isolated from *Ophiorrhiza mungos* Linn. *Nutr Cancer* 2011;63:130–8. <https://doi.org/10.1080/01635581.2010.516869>
46. Jeong W-S, Jun M, Kong A-NT. Nrf2: a potential molecular target for cancer chemoprevention by natural compounds. *Antioxid Redox Signal* 2006;8:99–106.