



ORIGINAL ARTICLE

Monoterpenoid 5-methylcoumarins from *Centrapalus pauciflorus* with antiproliferative activity



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Abstract Thirteen undescribed monoterpene-fused 5-methylcoumarins, named centrapalus coumarins A–M, were isolated from the aerial parts of the *Centrapalus pauciflorus* together with seven known compounds. The structures were established by extensive spectroscopic analyses, including 1D NMR, 2D NMR, and HR-ESI-MS experiments. The compounds represent a wide range of chemical diversity depending on the connection of the head-to-tail coupled diisoprene unit. Centrapalus coumarins A–H and I–L are based on 6–6–6- and 6–6–7-membered tricyclic ring systems, respectively. Centrapalus coumarins D and E exhibit cyclic hemiketal structures, while centrapalus coumarins F is unique because its monoterpene part forms an additional lactone ring. Centrapalus coumarin L is the only compound containing a modified trinor-monoterpene part. Centrapalus coumarin M is unprecedented as it contains a pentacyclic heterocyclic ring system. Sixteen isolated compounds were investigated for antiproliferative activity on the human breast (MCF-7 and MDA-MB-231), cervical (HeLa and SiHa), and ovarian (A2780) cancer-cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and a few of them exhibited substantial activity. Centrapalus coumarin F demonstrated the highest potency against MCF-7, HeLa, and

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A2780 cells with IC₅₀ values of 6.59, 2.28, and 15.41 μ M, respectively. The cytotoxic activity of centrapalus coumarin F showed moderate cancer selectivity, as determined using intact fibroblast cells (NIH-3 T3). The antiproliferative activity of these 5-methylcoumarin derivatives provides evidence for the establishment of structure–activity relationships.

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1. Introduction

Coumarins represent a widely distributed family of naturally occurring oxygen-containing heterocycles, bearing a typical 2*H*-1-benzopyran-2-one framework. Many coumarins have been observed to act as phytoalexins, phototoxins, allergens, or toxic agents. Furthermore, their therapeutic effects, such as anticoagulant, anti-inflammatory, anti-cancer, antimicrobial, anti-hypertensive, anticonvulsant, anti-hyperglycemic and neuroprotective activities have been reported (Murray et al., 1982; Pal and Saha, 2020; Matos et al., 2015). Coumarins with 5-methyl substitution comprise a special group, and their occurrence in the plant kingdom is much more restricted than that of coumarins. They are mainly found in the members of tribes Mutisieae (that includes *Gerbera*), Vernoniae of the Asteraceae family, and occasionally from a few other taxa (Murray, 1997). In general, coumarins originate from the phenylpropanoid pathway and are formed from coumaric acid through hydroxylation at C-2, followed by *cis*–*trans*-isomerization and lactone formation (Bourgaud et al., 2006). Contrarily, 5-methylcoumarins have been proposed to be derived through the acetate–malonate pathway. Precursor feeding studies have suggested that 5-methylcoumarins are synthesized from acetate units, and polyketide synthase enzymes are required for the biosynthesis of 5-methylcoumarins (Pietiaenen et al., 2016; Stothers et al., 1988). These polyketide-derived coumarins are frequently substituted with one, two, or three isoprenoid units; the occurrence of such compounds represents taxonomic significance because of their highly restricted distribution. Prenyl- and geranyl-substituted coumarins are predominant in species of Mutisieae, while sesquiterpene units are naturally found in species of tribes Onoserideae and Nassauvieae (Vestena et al., 2022).

Ethuliacoumarin A from *Ethulia conyzoides* was the first discovered member of the 5-methylcoumarins. This compound exhibits powerful anthelmintic and molluscicidal activities against *Biomphalaria glabrata* and *Bulinus truncatus* (Mahmoud et al., 1983; Kady et al., 1992). Subsequently, similar compounds were isolated from the members of the *Vernonia* (Oketch-Rabah et al., 1997), *Bothriocline* (Ahmed et al., 1991), *Mutisia* (Viturro et al., 2003), *Triptilion* (Bittner et al., 1988), *Gerbera* (Liu et al., 2010), and *Gutenbergia* genera in a wide structural variety, frequently cooccurring with structurally close chromone derivatives.

The present study deals with the isolation, structure determination of coumarin constituents from *Centrapalus pauciflorus* (Willd.) H. Rob. (Asteraceae), and evaluation of the antiproliferative activity of the isolated compounds. Synonyms of *C. pauciflorus* include *Centrapalus galamensis* Cass., *Conyza pauciflora* Willd., *Vernonia galamensis* subsp. *galamensis*, *Vernonia pauciflora* (Willd.) Less., and *Vernonia afromontana* R.E.Fr., etc (The Plant List, 2013; IPNI, 2022; POWO, 2022).

The genus *Centrapalus* includes nine species, which are mainly annual or perennial herbs that occur over the whole African continent. *C. pauciflorus* is native to tropical African countries, stretching from Cape Verde and Senegal in the West to Somalia in the East and down South to Zimbabwe and Mozambique (TPD, 2022). It is a mainly unbranched, usually annual plant that can grow 3–5 m tall but is usually significantly smaller. In folklore medicine, its leaves are cooked in porridge or drunk as a tea to treat chest or stomach pains (TPD, 2022). *C. pauciflorus* is an abundance source of vernolic acid (linoleic acid 2,3-oxide); therefore, it is regarded as a potential industrial plant, which can be used to manufacture polyvinyl chloride, adhesives, petrochem-

icals, and cosmetic and pharmaceutical products. The large-scale cultivation and commercial production of *C. pauciflorus*, as an oilseed crop, has commenced in several countries (Mideksa et al., 2019). Previous phytochemical studies revealed the presence of umbelliferon, scopoletin, flavonol glycosides, phenolic acids, germacrane-type sesquiterpene lactones, sterols, and triterpenoids in *C. pauciflorus* (Maroyi, 2020). Pharmacological investigations have confirmed the analgesic, sedative, anti-dermatitis, antimicrobial, insecticidal, larvicidal, and antiulcerogenic activities and hypoglycemic potential of *C. pauciflorus* extracts and its isolated compounds (Maroyi, 2020).

In the present study, the chloroform fraction of the MeOH extract of *C. pauciflorus* was investigated for compounds with antitumor activity. First, the chloroform extract and its fractions obtained by column chromatography on polyamide were assayed on the human breast (MCF-7 and MDA-MB-231), cervical (HeLa and SiHa), and ovarian (A2780) cancer-cell lines for antiproliferative activity. The fraction eluted with 60% MeOH exhibited the highest activity, it showed 44.2 \pm 0.6%, 49.3 \pm 0.9%, 54.6 \pm 0.6% and 5.2 \pm 0.5% inhibition at 10 μ g/mL, and 70.7 \pm 0.4%, 85.3 \pm 1.0%, 63.7 \pm 1.3%, and 68.2 \pm 0.8% inhibition at 30 μ g/mL against MCF-7, MDA-MB-231, HeLa, and A2780 cells, respectively. The 60% MeOH fraction was selected for detailed phytochemical analysis. Thus, thirteen previously undescribed 5-methylcoumarins (1, 3, 4, 7, 8, 12–18, 20), named centrapalus coumarin A–M, and seven known coumarins (2, 5, 6, 9–11, 19) were described from *C. pauciflorus*. All the compounds are variably conjugated with a monoterpene structural part. The antiproliferative activity of the isolated compounds was evaluated on human gynaecological (MCF-7, MDA-MB-231, HeLa, SiHa, and A2780) cell lines.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were determined using a JASCO P-2000 polarimeter (JASCO International Co. Ltd., Hachioji, Tokyo, Japan). NMR spectra were recorded in CDCl₃ using a Bruker Avance DRX 500 spectrometer at 500 (¹H) and 125 MHz (¹³C). The signals of the deuterated solvent were taken as references. Two-dimensional (2D) experiments were performed with standard Bruker software. During the ¹H, ¹H-COSY, HSQC and HMBC experiments, gradient-enhanced versions were applied. HR-ESI-MS spectra were recorded using a Thermo Scientific Q-Exactive Plus Orbitrap mass spectrometer equipped with an ESI ion source in the positive ionization mode. The data were acquired and processed using the MassLynx software. Vacuum liquid chromatography (VLC) was performed on silica gel (15 μ m, Merck); LiChroprep RP-18 (40–63 μ m, Merck) stationary phase was used for reversed-phase VLC, and open column chromatography (OCC) was performed on polyamide (MP Biomedicals). Preparative thin-layer chromatography (PTLC) was performed on silica gel 60 F₂₅₄ plates (Merck). HPLC was conducted with Wufeng HPLC, Waters HPLC, and Agilent HPLC instruments using normal [LiChrospher Si 60 (5 μ m, 250–4 mm)], [Luna (R) 5- μ m silica (2) 100 L (250 – 21.2 mm)], reversed-phase [Kinetex

5- μm C₁₈ 100 Å (150–4.6 mm)], [Agilent ZORBAX ODS 5- μm C₁₈ 100 Å (250 × 9.4 mm)], and [Phenomenex Lux^(R) 5- μm *i*-Amylose-1] columns. The TLC plates were visualized under a UV-light at 254 nm and detected by spraying with concentrated sulfuric acid, followed by heating. All the solvents used for CC and TLC were of at least analytical grade (VWR Ltd., Hungary).

2.2. Plant material

The aerial parts of the plant were collected in August 2018 in Zaria, Nigeria (N11°7'19.758" E7°43'23.1672¹¹) and identified by Umar Gallah (National Research Institute for Chemical Technology, Zaria, Nigeria). A voucher specimen (897) has been deposited in the herbarium, Institute of Pharmacognosy, University of Szeged, Szeged, Hungary.

2.3. Extraction and isolation

The air-dried powdered plant material (548 g) was extracted by percolation with MeOH (45 L) at room temperature. The MeOH extract was concentrated *in vacuo* and yielded 133 g of extract. This extract was dissolved in MeOH–H₂O (1:1) and subjected to solvent–solvent partitioning with CHCl₃ (3 × 1000 mL) to afford the organic component. After concentration, the organic phase (65.81 g) was chromatographed by OCC on polyamide (100 g) with MeOH–H₂O (1:4, 2:3, 3:2, 4:1, and 5:0) mixtures as eluents to afford 20%, 40%, 60%, 80%, and 100% MeOH fractions, respectively. These fractions were evaluated for antiproliferative activity, and the 60% MeOH fraction exhibited the highest activity and was selected for further chromatographic separations. Vacuum-liquid chromatography (VLC) was conducted using the 60% MeOH fraction (14 g) on silica gel with a gradient system of cyclohexane–EtOAc–EtOH (9:1:0, 8:2:0, 7:3:0, 50:20:1.5, 50:20:3, 50:20:6, 50:20:9, 50:20:12, 50:20:15, 50:20:20, 50:20:40, 50:20:60, and 50:20:80). The fractions collected were monitored by TLC, and those with similar profiles were combined, thereby affording combined fractions A–I. Fractions A–C obtained with cyclohexane–EtOAc–EtOH (8:2:0, 50:20:1.5, and 50:20:3) were rechromatographed by normal phase (NP)-VLC with cyclohexane–EtOAc–EtOH to afford fractions A/I and A/II. Fraction A/II was separated by RP-VLC with MeOH–H₂O gradient systems to yield fractions A/II/1–5. The NP-HPLC purification of A/II/5 with *n*-hexane–EtOAc, as a mobile phase, led to the isolation of compounds **1** and **2** (1.8 and 1.3 mg, respectively).

The RP-VLC separation of fraction B with MeOH–H₂O mixtures as eluents afforded subfractions B/I–III. Fraction B/II was subjected to NP-VLC using *n*-hexane–CHCl₃ mixtures to yield fractions B/II/1–2. A further chromatography of B/II/2 by RP-VLC with MeOH–H₂O as an eluent resulted in four subfractions B/II/2a–d. After a two-step HPLC purification (NP-HPLC using *n*-hexane–EtOAc–MeOH and RP-HPLC using MeOH–H₂O mixtures as a mobile phase), compound **17** (2.4 mg) was isolated.

NP-VLC was performed on fraction B/III using a cyclohexane–EtOAc solvent system to afford fractions B/III/1 and 2. Through NP-HPLC with the mobile phase of *n*-hexane–EtOAc–MeOH, the latter resulted in four subfractions, B/III/2a–d. RP- and NP-NPLC separations of B/III/2a with mobile

phases of MeOH–H₂O and *n*-hexane–EtOAc–MeOH, respectively, resulted in the isolation of compound **20** (4.2 mg).

Fraction C was subjected to reverse phase flash column chromatography (RP-FCC) with MeOH–H₂O mixtures as eluents, and seven fractions, C/I–VII, were obtained. White crystals were crystallized from certain fractions and separated and analyzed by TLC. The crystals were a mixture of two compounds with slightly different R_f values. The NP-HPLC separation of the crystals with *n*-hexane–EtOAc–MeOH (80:19:1), as a mobile phase, resulted in compounds **9** (20.6 mg) and **10** (15.6 mg) in pure form. Fraction C/III was subjected to NP-VLC with *n*-hexane–CHCl₃ solvent system to yield fractions C/III/1–5. Fraction C/III/1 was chromatographed by NP-VLC using cyclohexane–EtOAc, as a mobile phase, to afford subfractions C/III/1a–g. The NP-HPLC purification of C/III/1b and C/III/1c using *n*-hexane–EtOAc–MeOH (80:19:1) and (98:1:1) yielded fractions C/III/1b/1–2 and C/III/1c/1–3, respectively. The RP-HPLC separation of fractions C/III/1b/1 and 2 with MeOH–H₂O (7:3), as a mobile phase led to the isolation of **18** (1.1 mg) and **11** (156.7 mg), respectively. Normal phase preparative thin layer chromatography (NP-PTLC) was conducted on C/III/1c/2 with CHCl₃–acetone (100:2), as mobile phase; further HPLC purification was performed using MeCN–H₂O (7:3), which led to the isolation of compounds **15** (15.2 mg) and **16** (9.6 mg). NP-HPLC was conducted on fraction C/III/1e using *n*-hexane–EtOAc–MeOH (90:8:2), as a mobile phase. The fraction obtained in this separation was further purified by RP-HPLC with MeOH–H₂O (9:1), as a mobile phase, followed by RP-HPLC with MeCN–H₂O (47:53), leading to the isolation of a mixture of two stereoisomers, **5** + **6** (44.8 mg). Efforts were devoted to separating **5** + **6** by HPLC using a chiral column, but after separation, both compounds transformed into a mixture of the stereoisomers again. Fraction C/III/1f was subjected to NP-HPLC using the mobile phase of *n*-hexane–EtOAc–MeOH (80:19:1), and four subfractions (C/III/1f/1–4) were obtained. The RP-HPLC of fraction C/III/1f/3 with the mobile phase of MeOH–H₂O (75:25) resulted in the isolation of compound **19** (17.4 mg). The three-step chromatographic separation of C/III/1f/4 by RP-HPLC [mobile phases MeOH–H₂O (75:25), then MeCN–H₂O (47:53)] and PTLC [mobile phase of CHCl₃–acetone (200:7)] yielded the pure compound, **12** (3.4 mg). Fraction C/III/1g was subjected to NP-HPLC using *n*-hexane–EtOAc–MeOH (80:19:1), as a mobile phase, to yield four subfractions (C/III/1g/1–4). RP- and NP-HPLC separations were conducted on C/III/1g/3 using mobile phases of MeOH–H₂O (75:25) and *n*-hexane–EtOAc (78:22), respectively, to isolate the mixture **13** + **14** (1.6 mg). Fraction C/III/3 was subjected to NP-HPLC using *n*-hexane–EtOAc–MeOH (95:4:1) and RP-HPLC using MeOH–H₂O (85:15) to furnish compounds **7** (42.1 mg) and **8** (11.4 mg), respectively. These two stereoisomeric compounds, although well separated by HPLC, rapidly converted into a mixture of both isomers again, but at a ratio of 2:1, as observed from the NMR spectra. Fraction C/III/5 was chromatographed by NP-HPLC with *n*-hexane–EtOAc–MeOH (90:9:1) and afforded five fractions (C/III/5/a–e). The RP-HPLC separation of C/III/5/c with MeOH–H₂O (74:26) led to the isolation of compound **4** (0.7 mg).

NP-VLC was conducted on fraction C/V using *n*-hexane–CHCl₃ as an eluent, yielding three fractions (C/V/1–3). The NP-VLC purification of fraction C/V/1 with a cyclohexane–EtOAc solvent system afforded subfractions C/V/1/a–d. The

NP-HPLC separation of fraction C/V/1/c using *n*-hexane–EtOAc–MeOH (80:19:1) and the final purification by RP-HPLC with MeOH–H₂O (68:32) resulted in the pure compound, **3** (4.8 mg).

Centrapalus coumarin A (**1**), colorless oily material; $[\alpha]_{\text{D}}^{28} + 68.0$ (*c* 0.1, CHCl₃); ¹H and ¹³C NMR data (Tables 1 and 2); HR-ESI-MS positive *m/z* 311.1634 [M + H]⁺ (calcd for C₂₀H₂₃O₃ 311.1642).

Centrapalus coumarin B (**3**), colorless oily material; $[\alpha]_{\text{D}}^{28} + 160.7$ (*c* 0.1, CHCl₃); ¹H and ¹³C NMR data (Tables 1 and 2); HR-ESI-MS positive *m/z* 327.1592 [M + H]⁺ (calcd for C₂₀H₂₃O₄ 327.1591).

Centrapalus coumarin C (**4**), colorless oily material; $[\alpha]_{\text{D}}^{25} + 29.6$ (*c* 0.05, CHCl₃); ¹H and ¹³C NMR data (Tables 1 and 2); HR-ESI-MS positive *m/z* 373.1642 [M + H]⁺ (calcd for C₂₁H₂₅O₆ 373.1646).

Centrapalus coumarins D (**7**) and E (**8**), colorless oily materials; $[\alpha]_{\text{D}}^{25} + 14.9$ (*c* 0.1, CHCl₃); ¹H and ¹³C NMR data (Tables 1 and 2); HR-ESI-MS positive *m/z* 357.1329 [M + H]⁺ (calcd for C₂₀H₂₁O₆ 357.1333).

Centrapalus coumarin F (**12**), white amorphous powder; $[\alpha]_{\text{D}}^{27} -70.1$ (*c* 0.1, CHCl₃); ¹H and ¹³C NMR data (Tables 1 and 2); HR-ESI-MS positive *m/z* 341.1381 [M + H]⁺ (calcd for C₂₀H₂₁O₅ 341.1384), 363.1198 [M + Na]⁺ (calcd for C₂₀H₂₀O₅Na 363.1203).

Centrapalus coumarin G + H (**13** + **14**), colorless oily material; $[\alpha]_{\text{D}}^{27} + 24.0$ (*c* 0.1, CHCl₃); ¹H and ¹³C NMR data (Tables 3 and 4); HR-ESI-MS positive *m/z* 343.1537 [M + H]⁺ (calcd for C₂₀H₂₃O₅ 343.1540), 365.1356 [M + Na]⁺ (calcd for C₂₀H₂₂O₅Na 365.1359).

Centrapalus coumarin I (**15**), white amorphous powder; $[\alpha]_{\text{D}}^{27} + 111.8$ (*c* 0.05, CHCl₃); ¹H and ¹³C NMR data (Tables

3 and 4); HR-ESI-MS positive *m/z* 343.1540 [M + H]⁺ (calcd for C₂₀H₂₃O₅ 343.1540).

Centrapalus coumarin J (**16**), white amorphous powder; $[\alpha]_{\text{D}}^{27} -186.0$ (*c* 0.05, CHCl₃); ¹H and ¹³C NMR data (Tables 3 and 4); HR-ESI-MS positive *m/z* 343.1540 [M + H]⁺ (calcd for C₂₀H₂₃O₅ 343.1540), 365.1361 [M + Na]⁺ (calcd for C₂₀H₂₂O₅Na 365.1360).

Centrapalus coumarin K (**17**), colorless oily material; $[\alpha]_{\text{D}}^{25} + 31.7$ (*c* 0.1, CHCl₃); ¹H and ¹³C NMR data (Tables 3 and 4); HR-ESI-MS positive *m/z* 325.1434 [M + H]⁺ (calcd for C₂₀H₂₁O₄ 325.1440).

Centrapalus coumarin L (**18**), white amorphous powder; $[\alpha]_{\text{D}}^{27} + 45.4$ (*c* 0.05, CHCl₃); ¹H and ¹³C NMR data (Tables 3 and 4); HR-ESI-MS positive *m/z* 285.1114 [M + H]⁺ (calcd for C₁₇H₁₇O₄ 285.1121).

Centrapalus coumarin M (**20**), white crystalline material; $[\alpha]_{\text{D}}^{27} + 76.6$ (*c* 0.05, CHCl₃); ¹H and ¹³C NMR data (Table 5); HR-ESI-MS positive *m/z* 325.1431 [M + H]⁺ (calcd for C₂₀H₂₁O₅ 325.1434).

2.4. Determination of antiproliferative properties

The effects of the isolated compounds on the growth of a panel of human adherent cancer-cell lines were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Cell lines isolated from cervical (HeLa and SiHa), breast (MCF-7 and MDA-MB-231), and ovarian cancers (A2780) and intact murine fibroblasts (NIH-3 T3), were purchased from the European Collection of Cell Cultures (Salisbury, UK). The SiHa cell line was obtained from the American Tissue Culture Collection (Manassas, VA, USA). All the cells were maintained in minimal essential

Table 1 ¹H NMR spectroscopic data of compounds **1–4**, **7**, **8** and **12** [500 MHz, CDCl₃, δ ppm (*J* = Hz)].

Position	1	2	3	4	7	8	12
6	7.00, d (8.1)	6.99, d (8.2)	–	–	7.02, d (7.6)	7.03, d (7.6)	7.04, d (8.0)
7	7.32, t (8.1)	7.31, t (8.2)	6.99, d (8.8)	7.07, d (8.7)	7.34, t (7.6)	7.36, t (7.6)	7.40, t (8.0)
8	7.14, d (8.1)	7.12, d (8.2)	7.03, d (8.8)	7.05, d (8.7)	7.13, d (7.6)	7.16, d (7.6)	7.20, d (8.0)
9	2.64, s	2.64, s	2.55, s	2.77, s	2.73, s	2.64, s	2.66, s
1'a	4.95, d (17.2)	5.13, d (17.6)	4.94, d (17.4)	5.11, d (16.9)	5.16, d (9.5)	5.16, d (9.5)	1.43, d (6.5)
1'b	5.18, d (11.3)	5.10, d (10.9)	5.18, d (10.5)	5.08, d (10.1)	5.11, d (17.7)	5.11, d (17.7)	–
2'	6.00, dd (17.2, 11.3)	6.18, dd (17.6, 10.9)	6.02, dd (10.5, 17.4)	6.15, dd (16.9, 10.1)	6.16, dd (17.7, 9.5)	6.16, dd (17.7, 9.5)	4.80, q (6.5)
4'α	1.78, dd (14.1, 11.5)	1.95, dd (13.9, 11.7)	1.78, dd (14.1, 10.6)	1.83, dd (14.1, 12.2)	2.08, d (14.0)	2.14, d (14.2)	2.54 ^a dd (15.2, 2.5)
4'β	1.88, dd (14.1, 1.7)	1.76, d (13.9)	1.89, dd (14.1, 1.6)	1.68, br d (14.1)	2.31, d (14.0)	2.37, d (14.2)	1.78 ^a dd (15.2, 9.2)
5'	4.83, ddd (11.5, 8.3, 1.7)	4.92, dd (11.7, 8.1)	4.83, ddd (10.6, 8.0, 1.6)	4.36–4.43, m	–	–	5.27, br d (9.2)
6'	5.36, d (8.3)	5.39, d (8.1)	5.38, d (8.0)	1.71–1.78, m, 2.23–2.30 m	3.69, s	3.68, s	–
7'	–	–	–	2.78–2.84 m	–	–	7.00, br s
8'	1.82, s	1.84, s	1.82, s	1.28, d (7.1)	5.39, d (4.5)	5.31, d (12.2)	1.84, s
9'	1.71, s	1.78, s	1.72, s	–	1.65, s	1.63, s	–
10'	1.58, s	1.61, s	1.58, s	1.56, s	1.62, s	1.61, s	1.58, s
11'	–	–	–	3.74, s	–	–	–
6(OH)	–	–	5.05, s	4.85, s	–	–	–
8'(OH)	–	–	–	–	3.19, d (4.5)	3.02, d (12.2)	–

^a Signals may be reversed.

Table 2 ^{13}C NMR data of compounds **1–4**, **7**, **8**, and **12** (125 MHz, CDCl_3 , δ ppm).

Position	1	2	3	4	7	8	12
2	160.8	160.9	164.2	n.d.*	160.5	160.3	160.9
3	105.1	107.5	105.1	103.4	107.2	106.9	108.9
4	164.2	163.5	161.1	n.d.*	160.1	160.0	167.4
4a	114.8	114.8	115.6	n.d.*	114.3	114.2	112.1
5	137.2	137.2	121.4	123.8	137.3	136.5	136.7
6	127.4	127.4	150.3	150.7	127.7	127.8	126.4
7	130.8	130.7	115.0	115.4	131.2	131.3	131.9
8	114.9	114.9	119.2	120.2	115.0	115.3	114.8
8a	154.1	153.9	148.5	148.7	154.1	154.2	156.1
9	23.8	23.7	13.8	12.6	24.0	23.8	21.3
1'	114.3	112.2	114.2	111.6	113.1	113.3	15.6
2'	144.6	145.2	144.7	145.8	144.5	144.1	93.0
3'	38.0	36.7	38.1	36.6	36.3	36.6	45.4
4'	42.4	42.8	42.5	43.5	39.3	39.3	36.3
5'	72.0	72.2	72.1	74.5	105.1	103.6	78.4
6'	122.7	122.8	122.8	38.7	62.7	63.6	129.6
7'	138.5	138.7	138.6	35.9	64.0	63.5	149.3
8'	25.8	25.9	25.8	17.3	98.4	98.3	10.5
9'	18.6	18.6	18.6	176.7	12.0	12.4	174.0
10'	26.1	25.1	26.2	24.8	25.3	25.8	23.2
11'	–	–	–	52.0	–	–	–

* not detected.

Table 3 ^1H NMR spectroscopic data of compounds **13–18** [500 MHz, CDCl_3 , δ ppm ($J = \text{Hz}$)].

Position	13 + 14	15	16	17	18
6	7.01, d (8.0)/7.02 d (8.0)	7.10, d (7.8)	7.10, d (8.0)	7.11, d (7.9)	7.08, d (8.0)
7	7.33, br t (8.0)	7.39, t (7.8)	7.38, t (8.0)	7.38, t (7.9)	7.38, t (8.0)
8	7.17, d (8.0)	7.19, d (7.8)	7.17, d (8.0)	7.18, d (7.9)	7.18, d (8.0)
9	2.68, s	2.72, s	2.74, s	2.74, s	2.67, s
1'a	2.50–2.66, m (2H)	5.22, d (17.5)	5.06, d (17.3)	4.96, d (17.4)	5.06, d (17.4)
1'b	1.98, dd (13.4, 6.2)/2.03, dd (13.4, 6.2)	5.10, d (10.3)	5.09, d (10.5)	5.09, d (10.8)	5.14, d (10.6)
2'	2.10, dd (13.4, 6.2)/2.13, dd (13.4, 6.2)	6.19, dd (17.5, 10.3)	6.18, dd (17.3, 10.5)	6.02, dd (17.4, 10.8)	6.11, dd (17.4, 10.6)
4' α	3.05, d (16.0)/2.99, d (16.6)	3.45, d (15.3)	3.61, d (14.0)	2.91 d (13.7) ^{a,b}	3.19, d (13.2) ^a
4' β	2.91, d (16.0)/2.94, d (16.6)	2.68, d (15.3)	2.41, d (14.0)	2.74 d (13.7) ^{a,b}	3.03, d (13.2) ^a
6'	3.36, s/3.38, s	4.30, s	4.29, s	–	4.55, d (17.3) ^a 4.51, d (17.3) ^a
8'	1.41, s/1.43 s	1.41, s	1.44, s	2.06, s	–
9'	1.27, s	1.27, s	1.31, s	1.70, s	–
10'	1.60, s/1.57, s	1.66, s	1.65, s	1.57, s	1.66, s
7'-OH	–	3.54, s	3.86, br s	–	–

^a signals can be reversed.^b data obtained from ^1H NMR recorded in benzene d_6 at 328 K.

medium supplemented with fetal bovine serum (10%), nonessential amino acids (1%), and penicillin–streptomycin (1%) at 37 °C in a humidified atmosphere containing 5% CO_2 . All the media and supplements were purchased from Lonza Group Ltd. (Basel, Switzerland). Cells were seeded into 96-well plates (5000 cells/well). After overnight incubation, the isolated compounds were added in two final concentrations (10 and 30 μM). After incubation for 72 h under cell-culture conditions, MTT solution (20 μL , 5 mg/mL) was added to each well and incubated further for 4 h. Finally, the medium was discarded, and the precipitated formazan was dissolved in dimethyl sulfoxide (DMSO) during 60 min of shaking at 37 °C. The absorbance was determined at 545 nm using a microplate reader (SpectoStarNano, BMG Labtech, Ortenberg, Germany). A clinically used anticancer agent, cisplatin

(Ebewe GmbH, Unterach, Austria), was included as a reference compound. For the compounds with substantial activities (exceeding 50% cell growth inhibition at 30 μM), the assays were repeated with a set of dilutions (0.1–30 μM) to calculate the IC_{50} values. Calculations were performed using the GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, CA, USA).

3. Results and discussion

3.1. Isolation and structure determination of the compounds

Twenty compounds (**1–20**) were isolated from the CHCl_3 fraction of the MeOH extract prepared from the aerial parts of *C.*

Table 4 ^{13}C NMR data of compounds **13–17** [125 MHz, CDCl_3 , δ ppm].

Position	13 + 14	15	16	17	18
2	162.8	161.1	161.1	161.1	160.9
3	161.2	114.2	116.4	109.6	116.6
4	99.98/99.95	168.9	168.2	166.1	167.0
4a	114.6/114.7	116.6	116.6	116.4	114.
5	136.59/136.61	135.4	135.3	135.9	137.1
6	127.6/127.7	128.1	128.1	128.3	128.3
7	130.7/130.8	131.2	131.1	131.2	131.6
8	115.3	114.8	114.8	114.9	115.0
8a	153.8	152.6	152.4	152.9	153.5
9	23.7/23.8	23.8	23.5	23.9	23.9
1'	17.4	115.1	113.4	113.6	113.9
2'	29.7/29.8	141.0	145.7	143.4	144.0
3'	78.8/78.9	44.2	42.4	42.9	42.8
4'	48.3/48.5	53.4	53.7	53.5	53.3
5'	203.2/203.0	208.4	210.6	196.3	207.4
6'	65.9/66.0	93.7	94.9	147.8	78.3
7'	61.6/61.7	72.0	72.5	137.5	–
8'	24.8/24.9	24.9	24.8	19.6	–
9'	18.5/18.7	26.9	27.1	20.3	–
10'	24.7/25.0	30.2	23.2	23.9	27.0

Table 5 ^1H and ^{13}C NMR data of compound **20** [500 MHz (^1H), and 125 MHz (^{13}C), CDCl_3 , δ ppm ($J = \text{Hz}$)].

Position	^1H NMR	^{13}C NMR	Key HMBC correlations
2	–	161.0	
3	–	103.8	H-6'
4	–	167.6	H-6'
4a	–	111.2	H-6, H-8, H-9
5	–	142.2	H-7, H-9, H-1'
6	7.00, d (7.8)	124.9	H-9
7	7.38, t (7.8)	132.2	
8	7.20, d (7.8)	115.2	
8a	–	157.2	H-7, H-8
9	2.76–2.85, m	34.2	H-6
	3.31, dd (13.2, 7.5)		
1'	1.65–1.75, m	33.1	
	2.28–2.38, m		
2'	5.70, dd (12.4, 5.1)	128.8	H-10'
3'	–	130.4	H-10'
4'	2.17, d (13.5)	37.6	H-2', H-10'
	3.03, d (13.5)		
5'	–	129.9	H-4', H-8'
6'	3.50, d (10.1)	51.1	
7'	2.61–2.72, m	37.3	
8' β	3.54, d (10.5)	76.3	
8' α	4.27, d (8.3)		
9'	1.14, d (7.0)	12.2	H-8'
10'	1.98, s	26.9	H-2', H-4'

pauciflorus by a combination of chromatographic methods, such as OCC, VLC, preparative TLC and HPLC. The structure elucidation was conducted by spectroscopic analysis, including 1D (^1H and ^{13}C JMOD) and 2D NMR (COSY, HSQC, HMBC and NOESY) and HR-ESI-MS experiments.

Compound **1** (centrapalus coumarin A), was isolated as a colorless oily material with the optical rotation of $[\alpha]_{\text{D}}^{28} + 68.0$ (c 0.1, CHCl_3). The molecular formula of **1** was

observed to be $\text{C}_{20}\text{H}_{22}\text{O}_3$ based on the HR-ESI-MS peak at m/z 311.1634 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{23}\text{O}_3$ 311.1642). The ^1H NMR and ^{13}C JMOD spectra of **1** indicated the presence of one vinyl [δ_{H} 4.95 d (17.2 Hz), 5.18 d (11.3 Hz), 6.00 dd (17.2, 11.3 Hz); δ_{C} 114.3, 144.6]; one 2-methyl-1-propenyl [δ_{H} 5.36 d (8.3 Hz), 1.82 s, 1.71 s; δ_{C} 122.7, 138.5, 25.8, 18.6, 26.1], two methyl groups (δ_{H} 2.64 s, 1.58 s; δ_{C} 23.8, 26.1]; a skeleton comprising seven quaternary carbons (δ_{C} 164.2, 160.8, 154.1, 137.2, 114.8, 105.1, and 38.0); four methines [δ_{H} 7.32 t (8.1 Hz), 7.14 d (8.1 Hz), 7.00 d (8.1 Hz), and 4.83 ddd (11.5, 8.3, 1.7 Hz); δ_{C} 127.4, 130.8, 114.9, 72.0]; and one methylene group [δ_{H} 1.78 dd (14.1, 11.5 Hz), 1.88 ddd (14.1, 1.7 Hz); δ_{C} 42.4] (Tables 1 and 2). The NMR spectroscopic data were very similar to those of preethulia coumarin (**2**) (Bohlmann and Zdero, 1982; Appendino et al., 2001) (Fig. 1), which was isolated in our experiment and showed that compound **1** is a 5-methylcoumarin derivative connected with a monoterpene unit. The monoterpene part was elucidated by COSY and HMBC correlations. The ^1H – ^1H COSY spectrum afforded sequences of correlated protons: $-\text{CH}_2-\text{CH}(\text{OR})-\text{CH} = (\delta_{\text{H}}$ 1.78 dd, 1.88 dd, 4.83 ddd, and 5.36 d) and a vinyl group $\text{CH}_2 = \text{CH}-$ (δ_{H} 4.95 d, 5.18 d, and 6.00 dd). The connectivities of these structural fragments were determined based on the HMBC correlations of C-3' (δ_{C} 38.0) with H-10' (δ_{H} 1.58), H-2' (δ_{H} 6.00), and H-4' (δ_{H} 1.78, 1.88); and C-7' (δ_{C} 138.5) with H-6' (δ_{H} 5.36), H-8' (δ_{H} 1.82), and H-9' (δ_{H} 1.71) (Fig. 2). These data showed that **1** and preethulia coumarin (**2**) are stereoisomers. The configuration of the compounds was solved using the NOESY spectra. Key NOESY correlations of **1** were observed between H-6' and H-4' at δ_{H} 1.78 (α) and between H-4' at δ_{H} 1.88 (β) and H-5', confirming the opposite orientation of the vinyl and the 2-methyl-1-propenyl groups, as that of preethulia coumarin (**2**). The same configuration of C-3' was indicated by the Overhauser effects between H-4' β /H-2' for **1** and H-5'/H₃-10' for preethulia coumarin (**2**). The three-dimensional (3D) molecular model of **2** indicates that H-5' and H₃-10' probably have the most probably axial

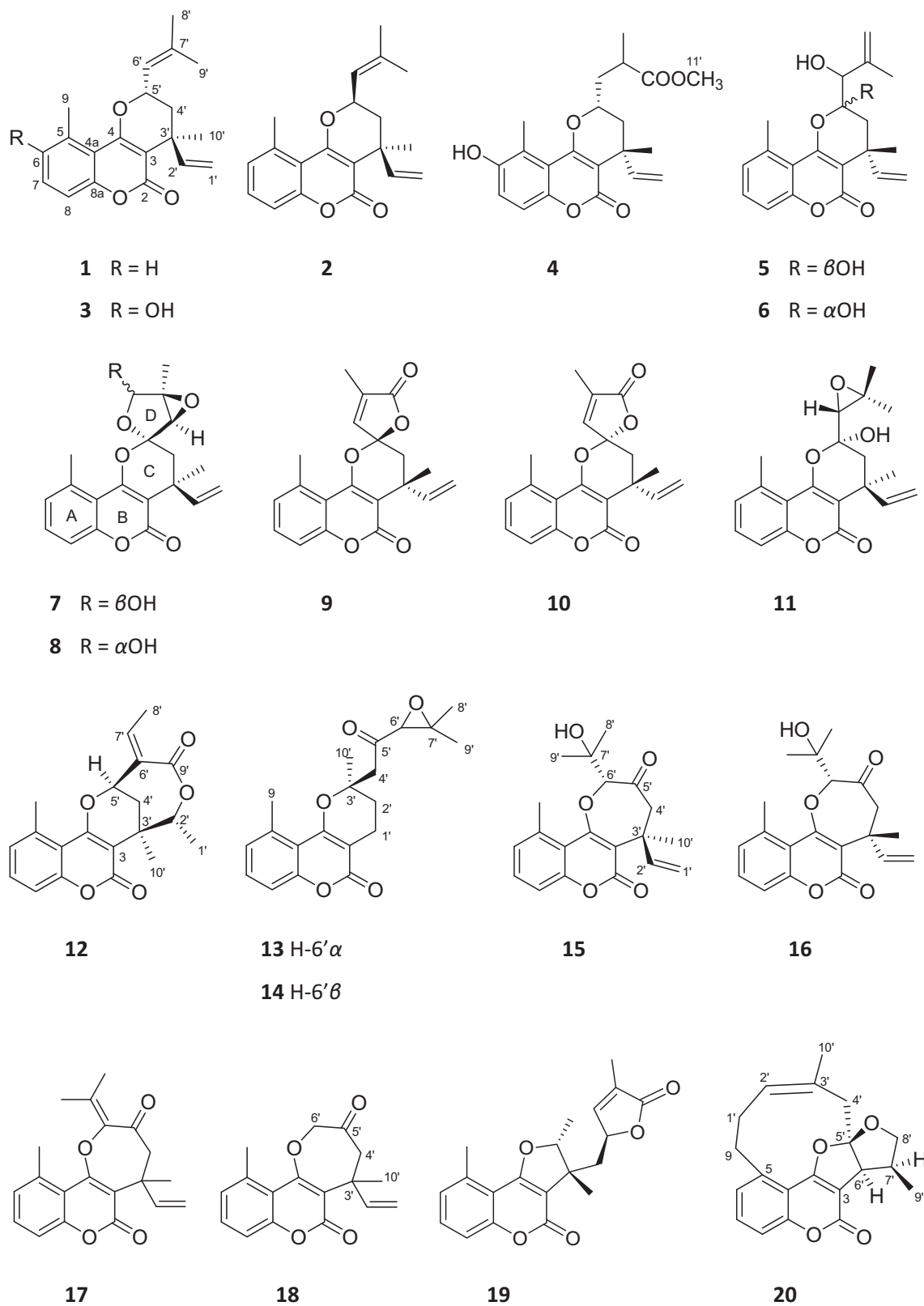


Fig. 1 Structure of compounds 1–20 isolated from *C. pauciflorus*.

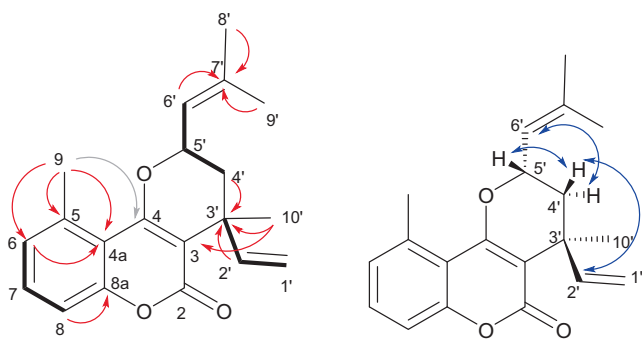


Fig. 2 Key COSY (–), HMBC (→), and NOESY (H ↔ H) correlations of centrapalus coumarin A (**1**).

position on the ring with a chair conformation. Previously unpublished ^{13}C NMR data of **2** are listed in Table 2.

Compound **3** (centrapalus coumarin B) was isolated as a colorless oily compound with the optical rotation of $[\alpha]_{\text{D}}^{28} + 160.7$ (c 0.1, CHCl_3). The molecular formula of **3** was observed to be $\text{C}_{20}\text{H}_{22}\text{O}_4$ based on the HR-ESI-MS peak at m/z 327.1592 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{23}\text{O}_4$ 327.1591). Comparing the ^1H NMR and ^{13}C NMR JMOD spectral data of **3** with those of **1** revealed that both compounds are based on a 5-methylcoumarin structure substituted with a monoterpene unit (Tables 1 and 2). The monoterpene parts of **1** and **3** were the same, as indicated by the good agreement of the ^1H and ^{13}C NMR data. The difference between the two compounds lies in the aromatic ring, in that **3** contains an additional hydroxyl group at C-6 and NOE correlations of 6-OH with protons at δ_{H} 2.55 (H₃-9) and 6.99 (H-7). This was demonstrated by the *ortho*-coupled doublet protons at δ_{H} 6.99 d (8.8) (H-7), and 7.03 d (8.8) (H-8), carbon resonance of C-6 at δ_{C} 150.3 (for **1** δ_{C} 127.4), and the OH singlet at δ_{H} 5.05 (6-OH), which exhibited an HMBC correlation with C-6. The stereochemistry of **3** was analyzed by NOESY spectroscopy and found that the configuration of C-3' and C-5' were identical to those of **1**, with regard to the H-2'/H-4' β , H-4' α /H-6', and H-5'/H-4' β correlations.

Compound **4** (centrapalus coumarin C) was isolated as a colorless oily compound with the optical rotation of $[\alpha]_{\text{D}}^{25} + 29.6$ (c 0.05, CHCl_3). It was shown by HR-ESI-MS to have the molecular formula of $\text{C}_{21}\text{H}_{24}\text{O}_6$, according to the quasi-molecular ion peak at m/z 373.1642 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{25}\text{O}_6$ 373.1646). The ^1H NMR and ^{13}C JMOD NMR spectral data of **4** showed a similar 6-hydroxy-5-methylcoumarin core as that of compound **3** (Tables 1 and 2). For the monoterpene part, the ^1H - ^1H COSY spectrum suggested structural fragments with correlated protons: $-\text{CH}_2-\text{CH}(\text{OR})-\text{CH}_2-\text{CH}(\text{CH}_3)-$ (δ_{H} 1.83 d, 1.68 d, 4.36–4.43 m, 2.23–2.30 m, 1.71–1.78 m, and 1.28 d) (C4'–C8') and $-\text{CH}=\text{CH}_2$ (δ_{H} 6.15 dd, 5.11 d, and 5.08 d) (vinyl group). These structural parts, together with a quaternary carbon connecting methyl (δ_{H} 1.56 s) and quaternary carbons at δ_{C} 36.6, and carbonyl at 176.7 were connected by the inspection of the long-range C–H correlations observed in the HMBC spectrum between C3' and H-1', H-2', and H-10'; C-10' and H-4' α , and H-2', and C-9' and H-6', H-7', H-8', and OCH₃ group. These data established the constitution of this compound as depicted by the structural formula of **4**. The relative configurations of

chiral carbons C3' and C-5' were defined by the NOESY correlation between H-5' and H-10'.

Compounds **7** and **8** (centrapalus coumarins D and E, respectively) were isolated as a mixture of two stereoisomers in ratio 2:1 whose components rapidly transform into each other when separated by HPLC. Fortunately, it was possible to solve the structure of both compounds as a mixture. The protonated molecular ion of compounds **7** and **8** at m/z 357.1329 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{21}\text{O}_6$ 357.1333) in the HR-ESI-MS spectrum offered the molecular formula, $\text{C}_{36}\text{H}_{56}\text{O}_8$. The ^1H NMR and ^{13}C NMR JMOD spectral data indicated a similar 3'-methyl-3'-vinyl substituted A/B/C ring system as that of **1** with a difference of C-5', which is a quaternary carbon for **7** and **8** (δ_{C} 105.1 and 103.6), and a methine for **1** (δ_{C} 72.0, δ_{H} 4.83) (Tables 1 and 2). The HMBC correlations between C-5' and the proton signals at δ_{H} 5.39 (**7**) and 5.31 (**8**) (H-8') suggested that the C-6'–C-9' part of the molecule was cyclized connecting to C-5' through an oxygen linkage, thereby forming ring D and resulting in a tetracyclic monoterpene-coumarin structure. Ring D was substituted with a hydroxy and an epoxy group, as confirmed by the HMBC correlations of C-6' (**7**: δ_{C} 62.7, **8**: δ_{C} 63.6); C-7' (**7**: δ_{C} 64.0, **8**: δ_{C} 63.5); and C-8' (**7**: δ_{C} 98.4, **8**: δ_{C} 98.3) with 8'-OH (**7**: δ_{H} 3.19 d, **8**: δ_{H} 3.02 d); C-6' with H-9' (**7**: δ_{H} 1.65 s, **8**: δ_{H} 1.63 s); and C-5' and C-7' with H-8' (**7**: δ_{H} 5.39 d, **8**: δ_{H} 5.31 d). The coupling constant of H-8'/8'-OH was different for compounds **7** and **8** (**7**: J = 4.5 Hz, **8**: J = 12.2), which demonstrates the opposite configuration of **7** and **8** in this position. NOESY correlations provided evidence of the stereochemistry of these compounds. The key Overhauser effect of **7** was observed between the 8'-OH group and H-9, which was possible only when the spiro structure and 8' β -OH exist as in the depicted structure of **7**. For the other isomers, the distance between the H-9 and the hydroxyl group is greater than 3 Å (Fig. 3). The NOE correlation of H-8' with H-9' indicated their α orientations. The Overhauser effect between H-6' and H-2' indicated that the vinyl group is β -oriented, and the 10'-

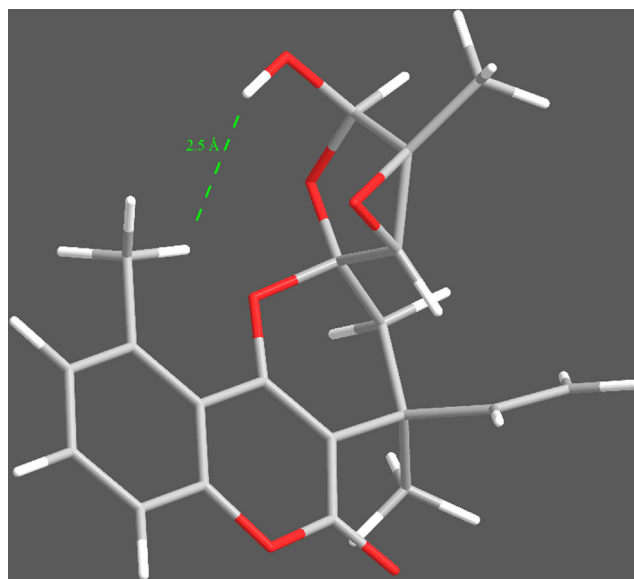


Fig. 3 3D model of centrapalus coumarin D (**7**) with indication of the key NOESY correlation.

methyl group exhibits the α orientation. Accordingly, NOESY cross-peaks between 10'-methyl/H-4' α and H-4' β /H-6' were detected. For compound **8**, a strong NOESY correlation was observed between H-9 and H-8', confirming that the C-8' configuration is reversed in this compound. Other NOE correlations corresponded to the NOE correlations of the major isomer.

Compound **12** (centrapalus coumarin F) was obtained as a white amorphous powder with the optical rotation of $[\alpha]_D^{27} -70.1$ (c 0.1, CHCl₃). Its molecular formula was deduced to be C₂₀H₂₀O₅ from the protonated molecular ion at m/z 341.1381 [M + H]⁺ (calcd for C₂₀H₂₁O₅ 341.1384) detected in the HR-ESI-MS spectrum. The ¹H NMR and ¹³C NMR JMOD spectral data of **12** showed similar A, B, and C rings as those of **1** (Tables 1 and 2). The linkage of the C-10' methyl group at C-3' in **12** was evident with regard to its cross-peaks with C-3, C-3', and C-4' in the HMBC spectrum. However, further parts of the monoterpene unit were observed to be different. The connected structural parts were CH₃-CH < (δ_H 1.43 d and 4.80 q; δ_C 15.6 and 93.0) (C-1'-C-2' fragment) and CH₃-CH = C-C(O)- (δ_H 1.84 s, 7.00 brs; δ_C 10.5, 149.3, 129.6, and 174.0) (C-8'-C-7'-C-6'-C-9' fragment). The first one is linked to C-3' because the proton at δ_H 4.80 (H-2') exhibited HMBC correlations with C-3, C-4', and C-10', while the second fragment bound to C-5' according to the HMBC cross-peak between H-7' (δ_H 7.00) and C-5'. The chemical shift value of C-9' (δ_C 174.0) indicated the presence of a lactone ring. Thus, the elucidated structure of **12** corresponds to the molecular composition. NOESY correlations between H-1'/H-10' and H-10'/H-4' revealed the α orientations of 10'- and 1'-methyls and H-5'.

Compounds **13** and **14** (centrapalus coumarins G and H, respectively) were isolated as a mixture of two isomers. The molecular formulas of compounds **13** and **14** were shown to be C₂₀H₂₂O₅ based on the HR-ESI-MS peak at m/z 343.1537 [M + H]⁺ (calcd for C₂₀H₂₃O₅ 343.1540). The ¹H NMR and ¹³C NMR JMOD spectra of **13** and **14** showed two very close sets of ¹H and ¹³C signals, suggesting the presence of a stereoisomer pair at a ratio of approximately 1:1 (Tables 3 and 4). The aromatic range of the spectra confirmed the presence of a 5-methylcoumarin structure for **13** and **14**. However, an interesting arrangement of the monoterpene component that substantially differed from those previously described was observed. The vinyl and methyl groups previously attached to ring C at C-3' was absent, but the COSY spectrum of the monoterpene part showed the structural fragment of -CH₂-CH₂- (δ_H 2.60 m, 1.98 dd/2.03 dd and 2.10 dd/2.13 dd; δ_C 17.4, 29.7/29.8). The HMBC correlation of the monoterpene component revealed the -CH₂-CH₂-C(CH₃)(OR)-CH₂-CO-CH(O)C(CH₃)₂ structure (δ_H 2.60 m, 1.98 dd/2.03 dd, 2.10 dd/2.13 dd, 2.91 d/2.94 d, 3.05 d/2.99 d, 3.36 s/3.38 s, 1.41 s/1.43 s, 1.27 s, and 1.60/1.57 s; δ_C 7.4, 29.7/29.8, 78.8/78.9, 48.3/48.5, 203.2/203.0, 65.9/66.0, 61.6/61.7, 24.8/24.9, 18.5/18.7, and 24.7/25.0) (C-1'-C-9'). This unusual monoterpene segment was corroborated by HMBC correlations observed between H-1'/C-3, C-4, C-3', H-10'/C-2', C-3', C-4', H-4'/C-5', H-6'/C-5', C-7', C-8', and H-8'/C-9'. The stereochemistry of the two stereoisomers was investigated by NOESY spectroscopy. H-10' correlated with H-9, H-2'a, and H-2'b for both compounds, which suggested that they have the same configuration at C-3', and the difference lies in the configuration of C-6'.

Compound **15** (centrapalus coumarin I) was isolated as a white amorphous powder with the optical rotation of $[\alpha]_D^{27} + 111.8$ (c 0.05, CHCl₃). The molecular formula of compound **15** was determined to be C₂₀H₂₂O₅ by analyzing the prominent ion peak at m/z 343.1540 [M + H]⁺ (calcd for C₂₀H₂₃O₅ 343.1540) through HR-ESI-MS. The ¹H NMR and ¹³C NMR JMOD spectral data of **15** revealed similar pattern characteristics to 5-methylcoumarin derivatives, such as previously discussed compounds. The same methyl and vinyl groups were identified at position C-3' as in the case of **1-11**, as shown by the NMR signals at δ_H 1.66 s and δ_C 30.2 (C-10'), and δ_H 5.22 d, 5.10 d (H-1'), and 6.19 dd (H-2') and δ_C 115.1 (C-1'), and 141.0 (C-2'), and HMBC cross-peaks between C-3'/H-1', H-2', and H-10' (Tables 3 and 4). However, a substantial difference was observed in its ring C, which was a seven-membered ring in contrast to the six-membered ring C in compounds **1-14**. The C-4'-C-9' part of the molecule comprised a keto group (δ_C 208.4); an O-substituted quaternary carbon (δ_C 72.0); an O-substituted methine (δ_H 4.30 s, δ_C 93.7); a methylene (δ_H 3.45 d, 2.68 d; δ_C 53.4); and two tertiary methyls (δ_H 1.41 s and 1.27 s; δ_C 24.9 and 26.9). The long-range heteronuclear correlations between C-3'/H₂-4', C-5'/H₂-4', H-6', C-6'/H₂-4', H-8' and C-7'/H-6', H₃-8', and H₃-9' enabled to elucidate an oxepane ring C substituted with a keto group at C-5' and a 1-hydroxyisopropyl group at C-6'. The stereochemical assignment was determined by a set of NOESY cross-peaks between H-9/H-8', OH/H-10', H-10'/H-4' α , H-4' β /H-2', H-4' β /H-1', and H₂'/H-6', indicating the α -orientation of a 1-hydroxyisopropyl group, H₃-10', and H-4' α (δ_H 3.45 d) and the β -orientation of H-4' β (δ_H 2.68 d), H-2', and H-6'.

Compound **16** (centrapalus coumarin J) was obtained as a white amorphous powder with the optical rotation of $[\alpha]_D^{27} -186.0$ (c 0.05, CHCl₃). The molecular formula of **16** was calculated as C₂₀H₂₂O₅ by its quasi-molecular ion peak observed at m/z 343.1540 [M + H]⁺ (calcd for C₂₀H₂₃O₅ 343.1540) through HR-ESI-MS. The evaluation of its ¹H NMR and ¹³C NMR JMOD spectral data revealed the same planar structure as that of **15**. Difference was found in the chemical shift values of C-1'-C-3', C-10', H-1', and H-4' (Tables 3 and 4), and NOESY correlations suggested the opposite stereochemistry at C-3'. For **16**, H-6' and H₃-10' exhibited NOESY cross-peaks, from which the β position of the 10'-methyl group was confirmed.

Compound **17** (centrapalus coumarin K) was isolated as a colorless oily compound with the optical rotation of $[\alpha]_D^{27} + 31.7$ (c 0.05, CHCl₃). The molecular formula of **17** was shown to be C₂₀H₂₀O₄ based on the HR-ESI-MS peak at m/z 325.1434 [M + H]⁺ (calcd for C₂₀H₂₁O₄ 343.1440). The ¹H and ¹³C NMR JMOD spectroscopic data of compound **17** revealed a similar structural pattern as that of **15** (Tables 3 and 4). The main difference was observed in the chemical shifts of C-6' and C-7'. The deshielded signals of C-6' (**17**: δ_C 147.8; **15**: δ_C 93.7) and C-7' (**17**: δ_C 137.5; **15**: δ_C 72.0) (δ_C) suggested the presence of a C-6'/C-7' olefin bond in **17** instead of H-6' and 7'-OH in **15**. The HMBC correlations of H-8' and H-9' with C-5' (weak ⁴J), C-6', and C-7' confirmed the isopropylidene group at C-6'. The configuration of the chiral carbon, C-3', could not be determined. Interestingly, the 4'-methylene protons were not visible as two doublets in the ¹H NMR spectrum, similar to compounds **13-16**, and subsequently, **18**. The signals of this methylene group was located

in the ^1H NMR spectrum within the range of 2.80–3.25 ppm. Oppositely, the 4'-methylene carbon (δ_{C} 53.5) was visible in the JMOD spectrum. The ^1H NMR spectra were recorded under different conditions to detect the missing protons and measurement in benzene d_6 at 328 K revealed a pair of doublet at 2.91 and 2.74 ppm with a coupling constant of $J = 13.7$ Hz. These protons demonstrated mutual coupling in the ^1H - ^1H COSY spectrum recorded at 328 K; therefore, these signals were assigned to $\text{H}_{2-4'}$. Another unexpected observation during the structural determination of **17** was defining C-10'. The protons of this methyl group in the HSQC spectrum did not exhibit an HSQC correlation with the corresponding carbon. This behavior was probably related to the presence of a double bond in the C-6'-C-7' position, which was in conjugation with

the keto group, C-5', causing anisotropy around carbons C-4' and C-10'.

Compound **18** (centrapalus coumarin L) was isolated as a white amorphous powder with the optical rotation of $[\alpha]_{\text{D}}^{27} + 45.4$ (c 0.05, CHCl_3). Its molecular formula, $\text{C}_{17}\text{H}_{16}\text{O}_4$, was obtained from the quasi-molecular peak at m/z 285.1114 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{17}\text{H}_{17}\text{O}_4$ 285.1121) during HR-ESI-MS. The ^1H and ^{13}C NMR signals of **18** exhibited a similar 5-methylcoumarin structure substituted with a seven-membered ring C, as observed in compounds **15–17** (Tables 3 and 4). However, the monoterpene component of **18** comprised only seven carbons, in contrast to ten in **15–17**. The seven-membered ring was substituted with methyl and vinyl groups at C-3', as confirmed by the long-range heteronuclear correlations of C-3 and C-3' with $\text{H}_{2-1'}$, $\text{H}_{2-2'}$, and $\text{H}_{3-10'}$.

Table 6 Antiproliferative properties of the isolated compounds.

Compound	Conc. or IC_{50}	Cell growth inhibition (%) \pm SEM and calculated IC_{50} values (μM)				
		MCF-7	MDA-MB-231	HeLa	SiHa	A2780
1	10 μM	–*	–	47.94 ± 0.77	–	22.11 ± 2.65
	30 μM	26.42 ± 2.11	–	76.85 ± 3.23	30.23 ± 1.63	62.14 ± 0.86
	IC_{50}	n.d.**	n.d.	9.21	n.d.	19.65
2	10 μM	21.77 ± 3.14	–	44.80 ± 1.24	–	22.17 ± 1.32
	30 μM	33.82 ± 3.50	25.76 ± 2.63	75.91 ± 1.49	25.63 ± 2.18	54.83 ± 0.84
	IC_{50}	n.d.	n.d.	9.58	n.d.	26.50
3	10 μM	22.22 ± 2.72	–	42.97 ± 0.02	22.40 ± 2.60	28.94 ± 1.16
	30 μM	45.91 ± 1.20	21.98 ± 1.52	75.33 ± 0.44	24.28 ± 1.70	57.20 ± 0.66
	IC_{50}	n.d.	n.d.	8.44	n.d.	27.29
5	10 μM	–	–	–	–	–
	30 μM	26.74 ± 3.18	–	22.75 ± 0.87	22.61 ± 1.08	20.62 ± 0.22
7 + 8	10 μM	–	–	–	–	–
	30 μM	–	–	22.60 ± 1.93	–	–
9	10 μM	29.23 ± 1.08	–	26.18 ± 1.01	22.64 ± 1.98	26.43 ± 1.51
	30 μM	70.66 ± 0.85	47.65 ± 0.90	74.97 ± 0.27	66.12 ± 1.34	57.79 ± 1.13
	IC_{50}	15.30	n.d.	14.59	18.94	26.94
10	10 μM	20.16 ± 1.86	–	–	24.26 ± 1.71	–
	30 μM	43.78 ± 1.60	–	24.90 ± 2.86	35.90 ± 0.89	–
11	10 μM	23.83 ± 1.01	–	–	24.76 ± 0.60	–
	30 μM	40.62 ± 1.50	–	21.45 ± 2.48	27.70 ± 0.54	–
12	10 μM	57.26 ± 1.05	–	69.48 ± 1.30	20.83 ± 1.68	37.22 ± 3.02
	30 μM	69.71 ± 1.01	26.23 ± 1.38	94.60 ± 0.55	40.84 ± 1.49	67.96 ± 2.01
	IC_{50}	6.59	n.d.	2.28	n.d.	15.41
13 + 14	10 μM	–	–	–	28.95 ± 0.82	–
	30 μM	37.77 ± 1.72	20.72 ± 1.02	40.58 ± 3.72	47.92 ± 1.89	32.28 ± 1.06
15	10 μM	–	–	–	–	–
	30 μM	20.28 ± 2.44	–	–	–	–
16	10 μM	–	–	–	–	–
	30 μM	23.16 ± 3.06	–	22.93 ± 2.51	–	–
19	10 μM	–	–	–	–	–
	30 μM	26.42 ± 2.63	32.79 ± 3.25	38.02 ± 2.48	22.22 ± 0.82	27.74 ± 1.29
20	10 μM	21.33 ± 3.47	–	38.39 ± 2.02	–	21.82 ± 2.54
	30 μM	28.38 ± 1.66	21.39 ± 2.03	42.00 ± 1.36	21.12 ± 1.29	30.27 ± 2.97
cisplatin	10 μM	66.91 ± 1.81	42.72 ± 2.68	42.61 ± 2.33	60.98 ± 0.92	83.57 ± 2.21
	30 μM	96.80 ± 0.35	86.44 ± 0.42	99.93 ± 0.26	88.95 ± 0.53	95.02 ± 0.28
	IC_{50}	5.78	10.17	12.43	4.29	1.30

* Cell growth inhibition values less than 20% were considered negligible and are not given numerically.

** : not determined.

The presence of a 5'-keto group (δ_C 207.4) was suggested by the isolated 4'- and 6'-methylenes ($\delta_{H-4'}$ 3.19 d, 3.03 d, each 13.2 Hz; $\delta_{H-6'}$ 4.55 d, 4.51 d, each 17.3 Hz), and further confirmed by the HMBC correlations between C-5', H₂-4', and H₂-6'.

Compound **20** (centrapalus coumarin M), was isolated as a white crystalline powder with the optical rotation of $[\alpha]_D + 76.6$ (c 0.05, CHCl₃). Its molecular formula was shown to be C₂₀H₂₀O₄ based on the HR-ESI-MS peak at m/z 325.1431 [M + H]⁺ (calcd for C₂₀H₂₁O₅ 325.1434). The ¹H and ¹³C NMR data of **20** exhibited an unusually complex pentacyclic coumarin structure. Although rings A and B were similar to those of previously described compounds, the methyl group at C-5 was replaced by a methylene group (δ_H 2.82 m, 3.31 dd; δ_C 34.2) (Table 5). Rings C and D were elucidated as five-membered rings, while ring E formed a ten-membered macrocycle. The COSY spectrum revealed the presence of structural parts A >CH-CH(CH₃)-CH₂- (δ_H 3.50 d, 2.66–2.50 m, 3.54 d, 4.27 d, and 1.14 d; δ_C 51.1, 37.3, 76.3 and 12.2) [C-6'-C-7'(C-9')-C-8' fragment] and B -CH₂-CH₂-CH= (δ_H 2.76–2.85 m, 3.31 dd, 1.65–1.75 m, 2.28–2.38 m, 5.70 dd; δ_C 34.2, 33.1, 128.8) (C-9-C-1'-C-2' fragment). The HMBC correlations between H-6' (δ_H 3.50 d), C-3 (δ_C 103.8), C-4 (δ_C 167.6), H-8' (δ_H 4.27 d), and C-5' (δ_C 129.9) revealed the existence of five-membered rings C and D. A macrocyclic ring was formed between C-5 and C-5' with the participation of structural part B and quaternary carbon C-3' (δ_C 130.4), methylene C-4' (δ_H 2.17 d, 3.03 d, δ_C 37.6), and methyl group C-10' (δ_H 1.98 s, δ_C 26.9). Their connection was verified by HMBC correlations of C-5/H-9, C-4'/H-2', C-4'/H-10', C-5'/H-4', and C-10'/H-2'. The NOESY spectrum showed that H-6', H-7', and C-4' exhibited an α -orientation, while C-9' was β -oriented. This was confirmed by Overhauser effects between H-4'/H-6', H-6'/H-8' α , H-8' β /H-9', and H-8' α /H-7'. In compound **20**, the 5-methylcoumarin part is usually condensed with a head-to-tail coupled monoterpene unit. Instead of a C-3/C-3' connection, C-3/C-6' and C-9/C-1' were formed, resulting in a new macrocyclic carbon skeleton.

Other known monoterpene-coupled coumarin derivatives (**2**, **5**, **6**, **9–11**, **19**) were isolated and identified from the *C. pauciflorus* extract. They were identified based on a comparison between their spectroscopic and reported data. Preethulia coumarin (**2**) was isolated from *Vernonia cinarescens* (Bohlmann et al., 1982). Isoethuliacoumarin A (**5**) and 5'-epi-isoethuliacoumarin A (**6**) were isolated from *Ethulia conyzoides* (Mahmoud et al., 1998). Compounds **9** and **10** were previously reported as mixtures of an oxidation product prepared from natural coumarins of *Ethulia conyzoides* (Mahmoud et al., 1998). Ethuliacoumarin (**11**) has been obtained from *Ethulia conyzoides* (Mahmoud et al., 1983), *Conyza bovei* (Metwally, 1996), *Vernonia brachycalyx* (Keige et al., 1998), *Bothriocline amplifolia* (Ahmed et al., 1991), *B. ripensis* (Ahmed et al., 1991) and exhibited molluscicidal activity. Hoehnelia coumarin (**19**) was isolated from *Ethulia vernonioides* (Schuster et al., 1993).

3.2. Discussion of antiproliferative activities

The antiproliferative properties of 16 of the 19 isolated compounds were investigated against a panel of human adherent cell lines of gynecological origin (Table 6). The amounts of

six compounds were insufficient for the *in vitro* assay; therefore, only a limited conclusion was obtained concerning their structure–activity relationships. Generally, HeLa cells appeared to be the most sensitive, while MDA-MB-231 cells from triple-negative breast cancer exhibited a minor growth-inhibiting action. The 5-methylcoumarin derivatives with a 2-methyl-1-propenyl group on ring C (**1–3**) exhibited similar antiproliferative effects; their activity against HeLa cells was comparable to that of cisplatin, but the other cell lines were less sensitive. The structurally closely related **5** was ineffective, which may have been due to the changed orientation of the substituents at C-3', and the increased polarity because of the presence of two hydroxy groups. The presence of a five-membered ring D (**7 + 8**, **9**, and **10**) was not advantageous, particularly when it bears an epoxy group (**7 + 8**). The 5-methylcoumarin coupled with a seven-membered ring (**12**) was confirmed to be the most potent compound; its action on MCF-7 cells (IC₅₀, 6.59 μ M) was similar to that of cisplatin (IC₅₀, 5.78 μ M), while it was substantially more effective against HeLa cells (IC₅₀, 2.28 μ M). The cancer selectivity of centrapalus coumarin F (**12**) was determined using the same assay against intact murine fibroblasts (NIH-3 T3). Treatment with **12** resulted in an inhibition of 30.72 \pm 1.29% and 52.5 \pm 0.92% at 10 and 30 μ M, respectively, while the reference agent, cisplatin caused 73.88 \pm 1.63% and 97.10 \pm 0.15%, at the same concentrations. The calculated IC₅₀ values against the NIH-3 T3 cells were 25.42 and 5.19 μ M for **12** and cisplatin, respectively, indicating an improved cancer selectivity for **12**. A mixture of **13** and **14** demonstrated the most pronounced activity against SiHa cells, but the growth inhibition was less than 50% at 30 μ M. Compounds with a seven-membered ring C (**15** and **16**) elicited no relevant actions, and 5-methylcoumarin derivatives containing a five-membered ring C (**19** and **20**) exhibited only modest activities.

4. Conclusions

Thirteen previously unreported compounds, named centrapalus coumarins A–M (**1**, **3**, **4**, **7**, **8**, **12–18**, **20**) based on the 5-methylcoumarin skeleton, and seven known coumarins (**2**, **5**, **6**, **9–11**, **19**), from the aerial parts of *C. pauciflorus* were isolated and described. The compounds are hybrid molecules of a monoterpene and a 4-hydroxy-5-methylcoumarin unit. The diversity of the compounds originates from the connection of the monoterpene part. Centrapalus coumarins F (**12**) and M (**20**) are based on new heterocyclic ring systems. The monoterpene part of centrapalus coumarin L (**18**) was derived through the loss of a C₃ unit.

Most of the tested molecules exhibited negligible or modest antiproliferative actions against human cancer cells of gynecological origin. However, centrapalus coumarin F (**12**) was more active against HeLa cells than the reference agent, cisplatin. Based on the *in vitro* results, its cancer selectivity is considerable.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2023.104777>.

References

- Ahmed, M., Jakupovic, J., Bohlmann, F.T., Mungai, M.G., 1991. A 5-methylcoumarin and glaucolides from *Bothriocline amplifolia*. *Phytochemistry* 30, 2807–2808. [https://doi.org/10.1016/0031-9422\(91\)85154-R](https://doi.org/10.1016/0031-9422(91)85154-R).
- Appendino, G., Cravotto, G., Minassi, A., Palmisani, G., 2001. Three-component tandem Knoevenagel/hetero Diels-Alder reactions total synthesis of (±)-preethulia coumarin. *Eur. J. Org. Chem.*, 3711–3717 [https://doi.org/10.1002/1099-0690\(200110\)2001:19 < 3711::AID-EJOC3711 > 3.0.CO;2-6](https://doi.org/10.1002/1099-0690(200110)2001:19 < 3711::AID-EJOC3711 > 3.0.CO;2-6).
- Bittner, M., Jakupovic, J., Bohlmann, F., Grenz, M., Silva, M., 1988. 5-Methylcoumarins and chromones from *Triptilion* species. *Phytochemistry* 27, 3263–3266. [https://doi.org/10.1016/0031-9422\(88\)80039-6](https://doi.org/10.1016/0031-9422(88)80039-6).
- Bohlmann, F., Zdero, C., 1982. Glaucolides and other constituents from South African *Vernonia* species. *Phytochemistry* 21, 2263–2267. [https://doi.org/10.1016/0031-9422\(82\)85189-3](https://doi.org/10.1016/0031-9422(82)85189-3).
- Bourgaud, F., Hehn, A., Doerper, S., Gontier, E., Kellner, S., Matern, U., 2006. Biosynthesis of coumarins in plants: a major pathway still to be unravelled for cytochrome P450 enzymes. *Phytochem. Rev.* 5, 293–308. <https://doi.org/10.1007/s11101-006-9040-2>.
- International Plant Names Index (IPNI) 2022. Published on the Internet <https://www.ipni.org>, The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Botanic Gardens. (Retrieved 08 September 2022).
- Kady, M.M., Brimer, L., Furu, P., Lemmich, E., Nielsen, H.M., Thilborg, S.T., Thastrup, O., Christensen, S.B., 1992. The molluscicidal activity of coumarins from *Ethulia conyzoides* and of dicumarol. *Planta Med.* 58, 334–337. <https://doi.org/10.1055/s-2006-961479>.
- Keige, A., Maxwell, S.R.J., Vogler, B., Klaiber, I., Kraus, W., 1998. Novel coumarins and sesquiterpene lactones from *Vernonia brachycalyx*. *Pharm. Pharmacol. Lett.* 8, 43–45.
- Liu, S.Z., Feng, J.Q., Wu, J., Zhao, W.M., 2010. A new monoterpene-coumarin and a new monoterpene-chromone from *Gerbera delavayi*. *Helv. Chim. Acta* 93, 2026–2029. <https://doi.org/10.1002/hlca.201000017>.
- Mahmoud, A.A., Ahmed, A.A., Iinuma, M., Tanaka, T., 1998. Further monoterpene 5-methylcoumarins and an acetophenone derivative from *Ethulia conyzoides*. *Phytochemistry* 48, 543–546. [https://doi.org/10.1016/S0031-9422\(97\)00907-2](https://doi.org/10.1016/S0031-9422(97)00907-2).
- Mahmoud, Z.F., Sarg, T.M., Amer, M.E., Khafagy, S.M., 1983. Anthelmintic coumarin from *Ethulia conyzoides* var. *gracilis* Asch. & Schweinf. *Die Pharmazie* 38, 486–487. PMID: 6634918.
- Maroyi, A., 2020. *Centrapalus pauciflorus*: Review of its medicinal uses, phytochemistry and pharmacological properties. *J. Pharm. Nutr. Sci.* 10, 287–294. <https://doi.org/10.29169/1927-5951.2020.10.05.15>.
- Matos, M.J., Santana, L., Uriarte, E., Abreu, O.A., Molina, E., Yordi, E.G., 2015. Coumarins – an important class of phytochemicals. In A. Rao A.V., Rao, L.G. (Eds.) *Phytochemicals – Isolation, Characterisation and Role in Human Health*, 25, pp. 533–538. <https://dx.doi.org/10.5772/59982>.
- Metwally, M.A., 1996. Coumarin derivative from *Conyza bovei*. *Boll. Chim. Farm.* 135, 223–224.
- Mideksa, A., Tesfaye, K., Dagne, K., 2019. *Centrapalus pauciflorus* (Willd.) H. Rob. neglected potential oil crop of Ethiopia, agromorphological characterization. *Genet. Resour. Crop Evol.* 66, 545–554. <https://doi.org/10.1007/s10722-018-0719-1>.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 55–63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4).
- Murray, R.D.H., Méndez, J., Brown, S.A., 1982. *The Natural Coumarins, Occurrence, Chemistry and Biochemistry*. John Wiley and Sons Ltd., Chichester.
- Murray, R.D.H., 1997. Naturally occurring plant coumarins. In Herz, W., Kirby, G.W., Moore, R.E., Steglich, W., Tamm, C.H., (Eds.), *Progress in the Chemistry of Organic Natural Products*, New York, Springer, pp. 1–199. https://doi.org/10.1007/978-3-7091-6527-0_1.
- Oketch-Rabah, H.A., Lemmich, E., Dossaji, S.F., Theander, T.G., Olsen, C.E., Cornett, C., Kharazmi, A., Christensen, S.B., 1997. Two new antiprotozoal 5-methylcoumarins from *Vernonia brachycalyx*. *J. Nat. Prod.* 1997 (60), 458–461. <https://doi.org/10.1021/np970030o>.
- Pal, D., Saha, S., 2020. Coumarins: An Important Phytochemical with Therapeutic Potential. In: Swamy, M.K. (Ed.), *Plant-Derived Bioactives, Chemistry and Mode of Action*. New York City, USA, Springer, pp. 205–222.
- Pietiaenen, M., Kontturi, J., Paasela, T., Deng, X., Ainasoja, M., Nyberg, P., Hotti, H., Teeri, T.H., 2016. Two polyketide synthases are necessary for 4-hydroxy-5-methylcoumarin biosynthesis in *Gerbera hybrida*. *Plant J.* 87, 548–558. <https://doi.org/10.1111/tbj.13216>.
- Plants of the World Online (POWO) 2022. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; <http://www.plantsoftheworldonline.org/> (Retrieved 08 September 2022).
- Schuster, N., Christiansen, C., Jakupovic, J., Mungai, M., 1993. An unusual [2+2] cycloadduct of terpenoid coumarin from *Ethulia vernonioides*. *Phytochemistry* 34, 1179–1181. [https://doi.org/10.1016/S0031-9422\(00\)90741-6](https://doi.org/10.1016/S0031-9422(00)90741-6).
- Stothers, J.B., Stoessl, A., 1988. Carbon-13 NMR studies. Part 137. Confirmation of the polyketide origin of kotonin by incorporation of [1,2-¹³C]acetate. *Can. J. Chem.* 66, 2816–2818. <https://doi.org/10.1139/v88-435>.
- The Plant List (2013). Version 1.1. Published on the Internet; <http://www.theplantlist.org/> (accessed 01 January 2022).
- Tropical Plants Database, Ken Fern. tropical.theferns.info/viewtropical.php?id=Centrapalus+pauciflorus > PROTA (Plant Resource of Tropical Africa). <http://www.prota.org>.
- Vestena, A.S., Meirelles, G.D.C., Zuanazzi, J.A., von Poser, G.L., 2022. Taxonomic significance of coumarins in species from the subfamily Mutisioidae. *Asteraceae*. *Phytochem. Rev.* <https://doi.org/10.1007/s11101-022-09828-x>.
- Vituro, C.I., de la Fuente, J.R., Maier, M.S., 2003. Antifungal methylphenone derivatives and 5-methylcoumarins from *Mutisia friesiana*. *Z. Naturforsch. C* 58, 533–540. <https://doi.org/10.1515/znc-2003-7-815>.