Ultra performance liquid chromatography-tandem mass spectrometric analysis of ethyl acetate fraction from saudi Lavandula coronopifolia Poir and evaluation of its cytotoxic and antioxidant activities

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**ABSTRACT**

**Introduction:** The ethyl acetate fraction of the Saudi Lavandula coronopifolia Poir has been previously reported to have hepatoprotective activity against ethanol-induced oxidative stress. The aim of the current study was to investigate the chemical composition, cytotoxic effect, and antioxidant activities of ethyl acetate fraction of the aerial parts of Saudi L. coronopifolia Poir.

**Methods:** Air dried aerial parts of L. coronopifolia were extracted using 90% ethyl alcohol. The dried extract was suspended in water, defatted with light petroleum and then fractionated with ethyl acetate. The ethyl acetate fraction was subjected to ultra performance liquid chromatography-tandem mass spectrometric (UPLC-ESI/MS/MS) analysis in a negative ionization mode. The antioxidant activity of the fraction was determined using free radical 2,2-diphyenyl-picrylhydrazyl (DPPH) scavenging assay and its cytotoxic effect against HepG-2 (human hepatocarcinoma) and MCF-7 (human breast carcinoma) cells were determined using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) cell viability assay.

**Results:** The major components of the ethyl acetate fraction included carvacrol-O-diglucoside, (34.98%) and trihydroxy ursolic acid (12.07%). Moreover, the DPPH radical scavenging activity of ethyl acetate fraction was measured. The ethyl acetate fraction revealed an antioxidant potential with EC50 = 17.8 ± 1.3 µg/mL. Additionally, the ethyl acetate fraction showed cytotoxic activity against HepG-2 and MCF-7 cells with IC50 = 29.3 ± 0.9 µg/mL and 14.6 ± 0.3 µg/mL, respectively.

**Conclusion:** The ethyl acetate fraction of the Saudi L. coronopifolia has antioxidant activity and also cytotoxic activity against breast and liver cancer cells.

**Implication for health policy/practice/research/medical education:**
The ethyl acetate fraction of Lavandula coronopifolia is an affordable source for compounds which have antioxidant activity and also cytotoxic activity against heaptic and breast cancers.

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**Introduction**
Genus Lavandula comprises 47 species belonging to the mint family (Lamiaceae). Lavandula species are very rich in volatile oils which make the Lavandula genus one of the most valuable group of aromatic and medicinal plants with immense economic value for pharmaceutical, cosmetics, perfumery, food and flavor industries and aromatherapy. L. coronopifolia is a woody perennial
herb with a pleasant aroma. It is widely distributed across North Africa, Saudi Arabia and eastern Iran (1). Moreover, *Lavandula* contains phenolics, flavonoids, anthocyanins, sterols and tannins (2). *Lavandula* species have many pharmacological actions including antioxidant (3), antimicrobial (4), antidepressant (5, 6) and anticancer (7) activities. A previous study using the plant growing in Saudi Arabia, showed that its volatile oil component was rich in phenol-2-amino-4,6-bis (1,1-dimethylethyl), carvacrol, n-hexadecanoic acid, trans-2-carene-4-ol, 17-pentatetracontane, caryophyllene oxide and 1-hexacosanol (8). Six major phenolic compounds were identified in the methanolic extract of the Jordanian plant via high-performance liquid chromatography (HPLC) tandem mass which included caffeic acid, rosmarinic acid, rutin, querceatin, luteolin, and hesperidin (9). *L. coronopifolia* has antioxidant and antimicrobial activities (8,10). Our previous findings revealed that ethyl acetate fraction of *L. coronopifolia* had hepatoprotective effect against ethanol-induced oxidative stress and subsequent cell death in HepG-2 cells (11). However, the chemical composition of the ethyl acetate fraction is unknown. Therefore, in this study, the chemical composition of the ethyl acetate fraction of *L. coronopifolia* was analyzed for the first time using UPLC-ESI-MS/MS to identify its bioactive constituents. In addition, we tested the DPPH radical scavenging activity of this fraction as a measure for its antioxidant activity. The anticancer activity of *L. coronopifolia* against breast and hepatic cancer cell lines was also investigated.

**Materials and Methods**

**Plant material**

The plant was collected in March 2009, from Shaza Mountains in Saudi Arabia. Plant identity was proved by Professor Jakob Thomas from the College of Science, King Saud University. A voucher specimen (#15799) was prepared and deposited at the herbarium unit of Pharmacognosy Department, College of Pharmacy, King Saud University. The aerial parts of *L. coronopifolia* were ground after air-drying, into coarse particles till use.

**Extraction of plant material**

Air dried aerial parts (300 g) of *L. coronopifolia* were extracted by 90% ethyl alcohol till complete exhaustion to afford (30 g) of dry extract. The dried extract was suspended in water, defatted with light petroleum and then fractionated with ethyl acetate to afford 3.5 g of ethyl acetate fraction.

**UPLC-ESI-MS/MS analysis**

The ethyl acetate fraction of *L. coronopifolia* was prepared as solution of 100 μg/mL using HPLC grade methanol, filtered using a membrane disc filter (0.2 μm) then subjected to LC-ESI-MS analysis as described by (12).

**Results**

**Characterization of the components of the ethyl acetate fraction of aerial parts of *L. coronopifolia***

Sixty compounds were tentatively identified by UPLC-ESI-MS/MS (negative ionization mode) from the ethyl acetate fraction of *L. coronopifolia* (Figure 1). Tables 1 and 2 show the list of the identified compounds along with their retention time (Rt), detected mass (M-H) and MS/MS fragment ions. UPLC-ESI-MS/MS chromatograms of some identified compounds are shown in Figure 2. Eleven phenolic acids were identified (2, 3, 4, 5, 6, 7, 8, 28, 34, 39 and 41). Most of the identified phenolic acids belong to the group of hydroxycinnamate derivatives, namely caftaric acid and its isomers, chichoric acid, caffeic, rosmarinic acid, and different salvianolic acids. Some of these compounds were previously reported in *Lavandula* species as salvianolic acid B, rosmarinic acid and caffeic acid (15,16). Six compounds (4, 25, 35, 38, 40 and 42) were identified as phenolic acid derivatives. Twenty-four triterpenoids were detected as shown in Tables 1 and 2. All of the detected compounds were ursoic acid derivatives. These compounds include dihydroxy, trihydroxy, tetrahydroxy, carboxy, monomethoxy, monomethoxy

**Antioxidant assay**

The antioxidant activity of the ethyl acetate fraction of *L. coronopifolia* was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University using the free radical 2,2-diphyenyl-pirclyhydrazyl (DPPH) scavenging assay, as described by (13).

**Cell culture and cytotoxicity assay**

HepG-2 (Human hepatocarcinoma) and MCF-7 (human breast carcinoma) cells were obtained from VACSERA Tissue Culture Unit and maintained in DMEM supplemented with 10% FBS and 100 µg/mL penicillin-streptomycin-amphotericin B solution. The cytotoxic effects of the ethyl acetate fraction of *L. coronopifolia* against HepG-2 and MCF-7 cells were determined using the MTT cell viability assay as described by (14).
Table 1. Major compounds identified in ethyl acetate (EA) fraction of Lavandula coronopifolia Poir using UPLC-ESI-MS/MS

| No. | Tentative identification                        | R_t (min) | MS<sup>1</sup> | MS<sup>2</sup> | EA (%) | Ref. |
|-----|-----------------------------------------------|-----------|---------------|---------------|--------|------|
| 1   | Carvacrol-O-diglucoside                        | 0.80      | 473           | 149 (100%)    | 34.98  | (17) |
| 2   | Chicoric acid                                  | 2.42      | 473           | 295, 179.0 (100%), 149 | 0.53  | (18) |
| 3   | Caffeic acid                                   | 6.28      | 179           | 134.9 (100%) [M-H COOH] | 0.50  | (15) |
| 4   | Hydroxy benzoic acid derivative                | 8.78      | 355           | 137 (100%), 78 | 0.60  | (19) |
| 5   | Rosmarinic acid                                | 10.24     | 359           | 196.9, 160.1 (100%) | 2.88  | (15, 20) |
| 6   | Azelaic acid                                   | 10.79     | 187           | 170, 125 (100%) | 0.58  | (21) |
| 7   | Azelaic acid isomer                            | 10.94     | 187           | 171, 125 (100%) | 2.42  | (21) |
| 8   | Salvianolic acid C                             | 12.36     | 491           | 293 (100%)     | 0.20  | (22, 23) |
| 9   | Salvianolic acid A isomer                      | 12.56     | 493           | 295.1, 197.1, 178.9, 134.9 (100%) | 1.02  | (20) |
| 10  | Tetrahydroxy urosolic acid                     | 13.76     | 519           | 501.0 (100%) [M-H-18], 455, 426, 407, 379, 325, 289, 223, 159 | 0.70  | (24) |
| 11  | Tetrahydroxy urosolic acid isomer              | 14.10     | 519           | 501.0 (100%) [M-H-18], 455, 426, 407, 379, 289, 223, 159 | 4.38  | (24) |
| 12  | Trihydroxy urosolic acid                       | 15.18     | 503           | 485 (100%)     | 0.60  | (26) |
| 13  | Umbelliferone rutinoside                       | 15.29     | 469           | 161 (100%)     | 0.53  | (25) |
| 14  | Pimarane diterpenes                            | 15.82     | 329           | 293.3, 229.1, 210.9, 193.3, 183.1, 171.4, 167.3, 154.8, 139.3 (100%), 127.4, 98.7, 71.3, 56.9, 44.8, 43.1 | 0.65  | (26, 27) |
| 15  | Luteolin derivative                            | 15.88     | 519           | 285 (100%)     | 0.50  | (28) |
| 16  | 2α, 3β, 19α, 2β trihydroxy urosolic acid isomer | 16.60    | 503           | 485 [M-H-HCOOH], 441, 409, 393 (100%), 375, 359 | 3.42  | (24) |
| 17  | 2α, 3β, 19α, 2β trihydroxy urosolic acid      | 16.85     | 503           | 485 [M-H-HCOOH], 441, 435, 393.2(100%) | 3.79  | (24) |
| 18  | 2α, 3β, 19α, 2β trihydroxy urosolic acid isomer | 17.00    | 503           | 455.6 (100%), 436.8, 419.8, 395.0, 377.0 | 0.55  | (24) |
| 19  | Monohydroxylymono-methoxy urosolic acid        | 17.11     | 501           | 501 (100%), 455 (100%), 421, 388, 337, 305, 225, 59 | 3.19  | (29) |
| 20  | Urosolic acid derivative                       | 17.17     | 517           | 455, 437, 419, 395, 377 | 0.70  | (29) |
| 21  | Urosolic acid derivative                       | 17.25     | 547           | 501 (100%) [M-H-COOH], 455 | 0.80  | (29) |
| 22  | Methoxy urosolic acid                          | 17.72     | 485           | 485 (100%), 439 (100%) [M-HCOOH], 391[M-H,O-OCH3] | 4.13  | (29) |
| 23  | Caftaric acid derivative                       | 18.54     | 487           | 311, 179 | 2.10  | (19) |
| 24  | Asiatic acid dihydroxy urosolic acid           | 20.70     | 487           | 487 (100%), 409 [M-H-HCOOH, HCH2OH], 373 | 0.56  | (24, 29) |
| 25  | Urosolic acid methyl ester                    | 20.92     | 469           | 451 (100%) [M-H,H2O], 407, 373, 309 | 2.69  | - |
| 26  | Salvianolic acid G                             | 23.86     | 339           | 295 (100%)     | 2.87  | (23) |
| 27  | 9-Hydroxy palmitic acid                        | 23.92     | 271           | 225.2 (100%) [M-H 46], 197.5 [-C,H4], 127.0 [-C,H3], 97.2 [-CHOH] | 3.43  | (30) |
| 28  | Asiatic acid dihydroxy urosolic acid isomer    | 24.66     | 487           | 487 (100%), 409 [M-H-HCOOH, HCH2OH], 373 | 0.98  | (24, 29) |
| 29  | Palmitic acid 1                                | 25.95     | 255           | 140(100%)     | 1.11  | (4) |
| 30  | Oleic acid                                    | 26.41     | 281           | 111(100%)     | 0.93  | (4, 31) |
| 31  | Long chain fatty acid                          | 29.80     | 383           | 337 [M-H-COOH], 309 [M-H-COOH-C2H4] | 2.89  | - |

**Total % of area under peak** 89.52%

monohydroxy ursolic acid and other derivatives. One pimarane diterpene was detected in the ethyl acetate fraction. Moreover, six compounds were identified as flavonoids including 17, 43, 44, 45, 46 and 54. Two coumarins (peaks 14 and 37), palmitic acid and oleic acid (Peaks 6 and 7), and carvacrol-O-diglucoside (Peaks 1) were also detected.

Antioxidant activity and anticancer activity
In this study, the antioxidant activity of the ethyl acetate
fraction of *L. coronopifolia* was compared to ascorbic acid, a well-known antioxidant. The fraction showed concentration-dependent antioxidant activity as demonstrated by increase in its DPPH radical scavenging activity (Figure 3). The concentration of the fraction which resulted in 50% DPPH scavenging activity (EC50 =17.8 ± 0.8 µg/mL) was similar to ascorbic acid (EC50 =14.2 ± 0.5 µg/mL).

We also investigated the anticancer activity of the fraction of *L. coronopifolia* against HepG-2 (hepatocellular carcinoma) and MCF-7 (breast carcinoma) in comparison to cisplatin, a well-known anticancer agent. As shown in Figure 4, the fraction showed dose-dependent cytotoxic activity against HepG-2 and MCF-7 cells. In addition, the IC50 values (the concentration which inhibits 50% of the cell viability) of the fraction in HepG-2 and MCF-7 cells were 14.6 ± 0.3 µg/mL and 29.3 ± 0.9 µg/mL, respectively (Table 3).

**Discussion**

In this study, we analyzed the chemical composition of the ethyl acetate fraction of *L. coronopifolia* for the first time using UPLC-ESI-MS/MS to identify its bioactive constituents responsible for their cytotoxic and antioxidant activities. Some of the identified compounds as caffeic acid, rosmarinic acid (9), and Pimarane diterpene derivatives (26,27) were reported before in *L. coronopifolia*.

In ethyl acetate fraction of *L. coronopifolia*, phenolic acids and their derivatives were identified based on their MS fragmentation and previous studies. The MS/MS

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**Table 2. Minor compounds identified in ethyl acetate (EA) fraction of Lavandula coronopifolia Poir using UPLC-ESI-MS/MS**

| No. | Tentative identification        | Rt (min) | MS1 | MS2              | EA (%) | Reference |
|-----|---------------------------------|----------|-----|------------------|--------|-----------|
| 34  | Salvianolic acid B isomer       | 0.89     | 717 | 519 (100%), 339, 313, 179, | ✔      | (20)      |
| 35  | Caffeic acid diglucoside        | 1.32     | 503 | 341 (100%) [M-H-162], 179 | ✔      |           |
| 36  | Syringic acid derivative        | 6.01     | 501 | 197 (100%), 135   | ✔      | -         |
| 37  | Esculetin -O- glycoside         | 6.38     | 339 | 177 (100%)       | ✔      | (32)      |
| 38  | Homovanillic acid rhamnoside    | 6.79     | 327 | 181 [M-H-146]    | ✔      | -         |
| 39  | Chicoric acid isomer            | 7.90     | 473 | 295, 179 (100%), 115 | ✔      | (18)      |
| 40  | Hydroxy caffeic acid rutinoside | 9.35     | 503 | 195 [M-H-308]    | ✔      | -         |
| 41  | Caffeic acid isomer             | 9.82     | 311 | 265.0 (100%) [M - H - COOH], 179 [M - H - tartaric] , 149.0 [M-H- caffeoyl] | ✔      | (18)      |
| 42  | Ferulic acid derivative         | 10.55    | 383 | 193 (100%), 137   | ✔      | -         |
| 43  | Naringenin derivative           | 10.66    | 519 | 271.2 (100%)     | ✔      | (33)      |
| 44  | Apigenin acetyl glucoside       | 11.58    | 473 | 177              | ✔      | (34)      |
| 45  | Chrysoeriol acetyl hexoside     | 12.15    | 503 | 299, 285 (100%)  | ✔      | (35)      |
| 46  | Quercetin derivative            | 14.90    | 503 | 301.0 (100%)     | ✔      | (24)      |
| 47  | Urticosidic acid derivative     | 15.51    | 503 | 455 (100%)       | ✔      | (29)      |
| 48  | Carboxy urosolic acid           | 16.33    | 485 | 467.3, 425.3, 375.1, 359.5, 259.8 | ✔      | (24)      |
| 49  | Dihydroxy monomethoxy urosolic acid | 16.87   | 517 | 351 (100%), 255.3 | ✔      | -         |
| 50  | Dihydroxy monomethoxy urosolic acid isomer | 17.34   | 499 | 455 (100%), 472.3 [M-H-COOH], 453.2, 441.8, 423.2 | ✔      | (29)      |
| 51  | Urticosidic acid derivative     | 17.34    | 517 | 455 (100%)       | ✔      | (36)      |
| 52  | Urticosidic acid derivative     | 17.65    | 503 | 485.0 (100%), 455.6, 436.8, 419.8, 395.0, 377.0 | ✔      | (24)      |
| 53  | Dihydroxy monomethoxy urosolic acid isomer | 18.11   | 487 | 455.5 (100%), 468.8, 467.1, 421.0, 409, 392.9, 374.8, 325.2, 270.8, 59.0, 57.6 | ✔      | (29)      |
| 54  | Tormentic acid                 | 21.13    | 503 | 455 (100%)       | ✔      | (24)      |
fragmentation of phenolic acids showed the presence of caffeic hydroxycinnamic acid or tartaric hydroxycinnamic acid moieties (37). Peak 3 with molecular ion peak at m/z 179 [M-H] was identified as caffeic acid (20). Peaks 2 and 39 showed M-H at m/z 473 and were identified as chicoric acid isomers (Figure 2A) (18). Peak 4 was identified as rosmarinic acid. It was found as a major component in the ethyl acetate fraction (2.88%) and was previously reported in L. pedunculata (20). Peak 34 showed an [M-H] ion at m/z 717 as well as fragments ion at m/z 519, 339 (100%), 197, 179 which were characteristic for salvianolic acid B (Figure 2C). Peak 41 showed [M-H] molecular ion at m/z 311 and fragment ions at m/z 265.0 (100%) [M-H -COOH], 179 [M-H-tartaric], 149.0 [M-H-caffeoyl] which coincided with the loss of mass of caffeic acid and tartaric acid, and was thus identified as caftaric acid isomers (Figure 2D) (18). Phenolic acid derivatives such as compounds 4 and 40 with [M-H] at m/z 355 and 503 and MS² base peak fragments at m/z 137 and 195 consequently were characterized as hydroxyl benzoic acid derivative (38) and hydroxy caffeic acid rutinoside.

Triterpenes, hydroxylated ursolic acid, and ursolic acid derivatives were also identified in ethyl acetate fraction of L. coronopifolia. Dihydroxy, trihydroxy, tetrahydroxy, carboxy, monomethoxy, monomethoxy monohydroxy

![Figure 2. Ultra performance liquid chromatography-tandem mass spectrometeric (UPLC-ESI-MS) chromatograms of some identified compounds from Lavandula coronopifolia.](image)

Figure 2: Ultra performance liquid chromatography-tandem mass spectrometeric (UPLC-ESI-MS) chromatograms of some identified compounds from Lavandula coronopifolia.

![Figure 3. 2,2-diphenyl-picrylhydrazyl (DPPH) radical scavenging activity of Lavandula coronopifolia ethyl acetate fraction (LV E). Data are presented as averages ± standard deviations from three experiments.](image)

Figure 3: 2,2-diphenyl-picrylhydrazyl (DPPH) radical scavenging activity of Lavandula coronopifolia ethyl acetate fraction (LV E). Data are presented as averages ± standard deviations from three experiments.
of these peaks showed the same molecular ion peak at m/z 271 and 273 
[\text{CHOH}] were identified as ions at m/z 293.3, 139.3 (100%), indicates the characteristic fragmentation of dihydroxy ursolic acid which is known as asiatic acid (29). Dihydroxy ursolic acid was previously reported in L. canariensis (24). Peaks 13, 15, 18, 19, 20 and 58 were identified as trihydroxy ursolic acid isomers (Figure 2E). MS1 of these peaks showed the same molecular ion peak at m/z 503 [M-H] and MS2 fragment ions at m/z 485 [M-H-H2O], 441 [M-H-H2O-COOH], 409, 393 (100%), 375, 359 which are characteristic fragments for polyhydroxylated ursolic acid isomers (29). The peaks 10, 11, 12 were identified as tetrahydroxursolic acid isomers from their MS1 which showed the same molecular ion peak m/z 519 [M-H] and MS2 fragment ions at m/z 501.0 [M-H-H2O], 455 [ursolic acid], 426, 407, 379, 325, 289, 223 and 159 (29). Polyhydroxy ursolic acid derivatives were previously reported in Lavandula species (24). Peak 27 with MS1 [M-H] at m/z 469 and MS2 fragment ions at m/z 451 (100%) [M-H-H2O], 407, 373, 309 was characteristic fragmentation of ursolic acid methyl ester. Peaks 47, 49, 50 and 52 showed the same [M-H] at m/z 517 and MS2 fragment ion at m/z 455 was identified as ursolic acid derivatives (may be dihydroxy monomethoxy ursolic acid isomers) (29). Peak 55 with deprotonated molecular ion peak at m/z 485 with MS1 fragment ions at m/z 455 (100%) [M-H-OMe] is characteristic for methyl ursolic acid. In addition, peaks 48 and 56 with the same deprotonated molecular ion peaks at m/z 499 [M-H] with MS2 fragment ions at m/z 455.3 (100%) [M-H-COOH], were identified as carboxy ursolic acid (29). Peaks 21-24 with MS1 [M-H] at m/z 501 517, 547 and 485 respectively were identified as mono-hydroxy mono-methoxy ursolic acid, ursolic acid derivative, an ursolic acid derivative and methoxy ursolic acid, respectively. Fragmentation of peak 60 gave molecular ion peak at m/z 501 and main base peak fragment ion at m/z 455 (ursolic acid), by loss of 46 Da; it was tentatively characterized as ursolic acid derivative (29).

In the present study, one pimarane diterpene was detected in the ethyl acetate fraction. Peak 16 with deprotonated molecular ion peak at m/z 329 and MS2 fragment ions at m/z 293.3, 139.3 (100%), indicates the presence of pimarane diterpene derivative related to the previously reported in L. multifida (26, 27). Mass fragmentations obtained by ESI MS/MS at m/z 285, 269, 301, 317, 315 and 299 are characteristic for the aglycones of kaempferol, apigenin, quercetin, myricetin, isorhamnetin and chrysoeriol respectively. The loss of 204, 189 and 176 allow the identification of acetyl hexoside, acetyl rhamnoside, and glucuronide, respectively. Peaks 17 and 18, 19, 20 and 58 were identified as trihydroxy ursolic acid isomers (Figure 2E). MS1 of these peaks showed the same molecular ion peak at m/z 503 [M-H] and MS2 fragment ions at m/z 485 [M-H-H2O], 441 [M-H-H2O-COOH], 409, 393 (100%), 375, 359 which are characteristic fragments for polyhydroxylated ursolic acid isomers (29). The peaks 10, 11, 12 were identified as tetrahydroxursolic acid isomers from their MS1 which showed the same molecular ion peak m/z 519 [M-H] and MS2 fragment ions at m/z 501.0 [M-H-H2O], 455 [ursolic acid], 426, 407, 379, 325, 289, 223 and 159 (29). Polyhydroxy ursolic acid derivatives were previously reported in Lavandula species (24). Peak 27 with MS1 [M-H] at m/z 469 and MS2 fragment ions at m/z 451 (100%) [M-H-H2O], 407, 373, 309 was characteristic fragmentation of ursolic acid methyl ester. Peaks 47, 49, 50 and 52 showed the same [M-H] at m/z 517 and MS2 fragment ion at m/z 455 was identified as ursolic acid derivatives (may be dihydroxy monomethoxy ursolic acid isomers) (29). Peak 55 with deprotonated molecular ion peak at m/z 485 with MS1 fragment ions at m/z 455 (100%) [M-H-OMe] is characteristic for methyl

Table 3 Half maximum inhibitory concentration (IC50) of of ethyl acetate (LV E) fraction of Lavandula coronopifolia Poir against HepG-2 and MCF-7 cells after the treatment for 48 hours, as measured by MTT assay

| Cell line          | Tested fraction | IC50 (µg/mL) ± SD | LV E     | Cisplatin |
|--------------------|-----------------|-------------------|----------|-----------|
| HepG-2 (Hepatocellular carcinoma) | 14.6 ± 0.3      | 3.67 ± 3.8        |          |           |
| MCF-7 (Breast carcinoma)       | 29 ± 0.9        | 5.71 ± 8.1        |          |           |

The data are presented as µg/mL. These are the mean of three determinations.

Cytotoxic and antioxidant activities of Lavandula coronopifolia

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Lavandula angustifolia – Lavandula coronopifolia

Lavandula coronopifolia molecular ion peaks at m/z 473 and ESI-MS/MS base peak fragment ion at m/z 149. Thus, this compound was identified as carvacrol-O-diglucoside (17).

An antioxidant agent can reduce or inhibit oxidative damage by scavenging free radicals. These radicals and other reactive oxygen species play a significant role in the pathogenesis of many diseases (42,43). Numerous plant extracts including those belonging to the Lamiaceae family have been reported to have antioxidant activity. In this study, the ethyl acetate fraction of L. coronopifolia showed concentration-dependent antioxidant activity as demonstrated by increase in its DPPH radical scavenging activity. These results support the previous literature about the antioxidant potential of this plant and many other Lavandula species (3,9). The antioxidant activity of ethyl acetate fraction can be due to carvacrol and ursolic acid. These compounds have been reported to have antioxidant activity in previous studies (44,45).

Cancer is the second leading cause of death all over the world. Conventional cancer therapies are associated with serious side effects. Hence, there is an increasing demand to utilize alternative approaches to treat cancer. Plant-derived compounds have been reported to have activity against different types of cancer (46,47). Importantly, these compounds are relatively not associated with toxic side effects (48). In this study, the ethyl acetate fraction of L. coronopifolia showed dose-dependent cytotoxic activity against HepG-2 (hepatocellular carcinoma) and MCF-7 (breast carcinoma) cells. Consistent with our results, previous studies demonstrated the anticancer potential of Lavandula species (49,50). The cytotoxic activity of ethyl acetate fraction can be attributed to the presence of carvacrol and ursolic acid. In accordance with our results, previous studies have shown that these compounds have antitumor activity against different types of cancer including breast and liver cancers (51-53).

Conclusion
Our findings revealed that the ethyl acetate fraction of L. coronopifolia has antioxidant and cytotoxic activities. These activities may be attributed to the high percentage of phenolic compounds and polyhydroxylated ursolic acid derivatives.

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Authors’ contributions
All authors made considerable contributions to the manuscript. SA and WHBH designed the study. SA, WHBH, HMA, MAE and RA performed the experiments. SA, WHBH and AEME interpreted the results. SA and AEME wrote the manuscript. All authors revised the manuscript and confirmed it for publication.

Conflict of interests
The authors declare that there are no conflicts of interest.

Ethical considerations
This study was only performed on commercially available cell lines and no human specimens or animal models were examined. All ethical issues have been approved by the research ethics committee of Faculty of Pharmacy, Zagazig University (P-1-12-2016).

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