Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Chemical composition, antioxidant, and cytotoxic potential of essential oils of some cultivated plants in Egypt

Mohammed I. Ali[1], Ahmed R. Hamed[2*],Emad M. Hassan[1*], Faten M. Abou elella[3], Sayed A. El-Toumy[4], Samy M. Mohamed[1] and Ahmed M. Aboul-Enein[3]

[1]*Medicinal and Aromatic Plants Research Department, National Research Centre, Dokki, Giza, 12622, Egypt*
[2]*Chemistry of Medicinal Plants Department, National Research Centre, Dokki, Giza, 12622, Egypt*
[3]*Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt*
[4]*Chemistry of Tannins Department, National Research Centre, Dokki, Giza, 12622, Egypt*

Abstract

This study aimed to identify chemical the composition of essential oils (EOs) of *Cymbopogon citratus*, *Lavandula dentata*, *Artemisia abrotanum*, and *Laurus nobilis*, and evaluate their antioxidant and cytotoxic activities. ***Methods:*** The chemical composition investigated by GC/MS, while antioxidant activity evaluated by DPPH and $ABTS^+$ assays. The cytotoxic effect evaluated by MTT technique against human cancer cells; breast (MDA-MB-231), lung (A549), colon (Caco2), liver (HepG2) and doxorubicin-resistant liver (HepG2/DOX). Flow cytometry was utilized to evaluate apoptosis and cell cycle arrest after treating Caco2 cells with EO of *C. citratus*. ***Results:*** The most prominent compound of EOs was citral in *C. citratus*, artemisia ketone in *A. abrotanum* and eucalyptol in *L. dentata* and *L. nobilis*. The EOs showed weak antioxidant activity; except the EO of *L. nobilis* which had moderate $ABTS^+$ activity. Also, the EOs exhibited a very weak cytotoxic effect; except the EO of *C. citratus* showed a high cytotoxic effect against Caco2 and MDA-MB-231, and moderate cytotoxic effect against A549, HepG2 and HepG2/DOX. Cell cycle analysis revealed that the proportion of treated cells reduced in the G0/G1 and G2/M phases, and increased in the S phase. On the other hand, the EO of *C. citratus* reduces the number of viable and necrotic cells, and increases the number of early and late apoptotic cells. ***Conclusion:*** The results revealed that EO of *L. nobilis* had moderate $ABTS^+$ activity and the EO of *C. citratus* showed high cytotoxic effect. Flow cytometry showed the EO of *C. citrate* significantly induces apoptosis and S phase arrest.

Keywords: *Essential oils; Cytotoxicity; Antioxidant; Medicinal plants; Cell cycle; Apoptosis*

1. Introduction

Over the past decades, there has been increasing interest in the investigation of natural products from different sources particularly from higher plants for the discovery of new therapeutic agents. Therefore, in the last few years, much attention has been given to the essential oils (EOs) and pharmacological activities of aromatic plants. Many studies suggested that the pharmacological activities of these plants are particularly related to the chemical composition of their EOs. Among of promising species were *Cymbopogon citratus* "Poaceae, commonly known as lemongrass", *Lavandula dentata* "Lamiaceae, commonly known as lavender", *Artemisia abrotanum* "Asteraceae, commonly known as southernwood" and *Laurus nobilis* "Lauraceae, commonly known as bay laurel".

The selected plants contain EOs along with other chemical compounds including; phenolic acids, flavonoids, sterols, tannins, sesquiterpenes and anthocyanins [1–9].

Extracts prepared from *C. citratus* showed diverse pharmacological activities including analgesic, antioxidant, anti-inflammatory, cytotoxic, antidepressant, anti-diabetic, antimicrobial and antiviral activities [10–15]. In addition, EO of lemongrass exhibited hypotension, vasorelaxation, antioxidant, cytotoxic, anxiolytic, antimicrobial, anticonvulsant, anti-diabetic, depressant, neuro-protective, anti-inflammatory, anticholinesterase activities and reduced the blood cholesterol level [16–25].

L. dentata extracts displayed many biological activities such as; antioxidant, anti-proliferative, antibacterial, anti-inflammatory, anti-diabetic, anti-asthma, hypolipidemic, hypoglycemic and apoptotic effect [26–30]. Furthermore, EO distillated from lavender showed different biological activities such as antioxidant, cytotoxic, antimicrobial, anticoagulant, analgesic and anti-diabetic activities [31–34]

A. abrotanum extracts exhibited several biological activities such as antimalarial, immunosuppressant effect, antioxidant, anti-proliferative and antimicrobial activities [2,35,36]. Additionally, the EO of Southernwood showed cytotoxic activity against the rhabdomyosarcoma cell line and antimicrobial activity [37].

Previous studies on *L. nobilis* extracts demonstrated various biological activities including anti-acetyl-cholinesterase, anti-inflammatory, anti-nociceptive, cytotoxic, antioxidant, antidiarrheal, antimicrobial and anti-

*Corresponding author e-mail: n1ragab2004@yahoo.com ; (Ahmed R. Hamed).
Received date 03 September 2024; revised date 02 October 2024; accepted date 08 October 2024
DOI: 10.21608/ejchem.2024.317876.10339
©2025 National Information and Documentation Center (NIDOC)

diabetic activities [38–43]. Also, the EO of bay laurel exhibited anticonvulsant, anti-acetyl-cholinesterase, anti-butyryl cholinesterase, cytotoxic, antimicrobial and antioxidant activities [44–47].

Cancer is a global health issue characterized by uncontrolled cell growth, leading to the formation of tumors, and developing countries are particularly affected. Conventional treatments include surgery, chemotherapy, and radiotherapy, while newer approaches encompass hormone therapy, stem cell therapy, and immunotherapy. The choice of treatment depends on the type, location, and stage of cancer. However, standard treatments can harm healthy cells and cause side effects, driving the search for new treatment methods [48,49].

Recent research highlights the potential of novel approaches in cancer treatment. Medicinal plants and their bioactive compounds show promising results as anti-cancer agents due to their anti-carcinogenic and chemo-protective properties, with several plant-derived products undergoing clinical trials [50–53].

Antioxidants play a crucial role in the biological system by neutralizing free radicals and preventing oxidative stress associated with aging and various diseases. These compounds are classified as enzymatic or non-enzymatic, both of which are used to maintain body homeostasis. Antioxidants increase the plasma antioxidant capacity by inhibiting oxidation and destroying free radicals, thereby reducing the risk of disease. Superoxide dismutase (SOD) is the first line of defense against reactive oxygen species, while non-enzymatic antioxidants, both endogenous and exogenous, provide additional protection. Maintaining optimal levels of exogenous antioxidants through diet is important to support normal biological processes. Antioxidants are used in the pharmaceutical and food industries and demonstrate therapeutic properties in treating various human diseases [54,55].

In the present study, we focused on identifying the chemical composition of the EOs of four tested plants and their biological efficacy as antioxidant activity and cytotoxic effect.

2. Experimental

2.1. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), minimum essential medium with Earle′s salts (EMEM), Trypsin-Versene (EDTA) Mix, Dulbecco's Phosphate-Buffered Saline (DPBS), L-Glutamine and penicillin/streptomycin stock (Lonza Verviers SPRL, Belgium). Fetal Bovine Serum (FBS; SeraLab, UK) and Doxorubicin (trade name: Adriamycin; Pfizer company, Australia). DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma, Germany). MTT dye (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) and Di-methyl sulfoxide (DMSO) (Serva Electrophoresis GmbH, Germany). Coulter DNA Prep reagents kit and Annexin V-FITC apoptosis detection kit (Beckman Coulter Inc., USA). Folin-Ciocalteu reagent (Sigma Chemical Co., USA), saturated solution of sodium carbonate and $ABTS^{+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); Bio Basic Inc, Canada).

2.2. Plant materials

The aerial parts of ***Lavandula dentata***, ***Artemisia abrotanum*** and ***Laurus nobilis*** were collected from the experimental farm of the National Research Centre, while ***Cymbopogon citratus*** was collected from Agricultural Research Station in Al-Qanatir Al-Khairiya. Plants kindly identified by Dr. Mohamed El-Gebally, former Researcher of Botany, National Research Centre, Dokki, Cairo, Egypt. The samples were collected in December and specimens stored in herbarium of National Research Centre under the voucher number; M277 for *A. abrotanum*, M278 for *C. citratus*, M279 for *L. nobilis* and M280 for *L. dentata*.

2.3. Extraction of EOs

One hundred grams of fresh aerial parts of collected plants were subjected to water distillation using Clevenger's apparatus. The distillation was continued for 3 h after boiling. The volatile substances were collected, dried over anhydrous sodium sulphate and stored at -20℃ until use [56].

2.4. Gas chromatography/mass spectroscopy (GC/MS) analysis for EOs

The GC/MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977B) at American University in Cairo, Egypt. Samples were diluted with hexane (1:19, v/v). The GC was equipped with an HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1 ml/min at a split ratio of 30:1, injection volume of 1 µl and the following temperature program: 50 ℃ for 1 min; rising at 4 ℃/min to 150 ℃ and held for 6 min; rising at 4 ℃/min to 210 ℃ and held for 1 min. The injector and detector were held at 280 and 220 ℃, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 50-450 and solvent delay of 3 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST mass spectral library data and compared to kovat index (KI) calculated by standard of alkane mixture C8–C40.

$$\mathrm{KIi} = (100\ x\ Pz) + 100\ (\frac{RTi - RTz}{RTn - RTz})$$

Whereas;

KIi = kovat index of compound 'i'

i = compound of essential oil that is being analysed

z = alkane which elutes before 'i'

n = alkane which elutes after 'i'
Pz = carbon number of the 'z'
RTi = retention time of 'i'
RTz = retention time of 'z'
RTn = retention time of 'n'

2.5. Estimation of total phenolics

The phenolics content (TPC) was determined by Folin–Ciocalteu according to the method described by Singleton *et al.*, [57] and Meda *et al.*, [58] with some modifications. Briefly, 20 µl of the sample were added to 96-well plate and 100 µl of Folin–Ciocalteu reagent (diluted 1:9 with deionized water) were added. After 15 min incubation, the mixture was neutralized with 80 µl of saturated Na_2CO_3. After a 2 h of reaction, the absorbance of the mixture was measured at 760 nm with a Zenyth 200rt microplate reader. Appropriate blank was prepared using the solvent only in addition to the same amount of folin-ciocalteu reagent and Na_2CO_3 to get rid of any inherent solvent activity and the negative controls were also run in parallel to correct for any non-phenolics absorbance by colored samples at the test wavelength. Gallic acid (GAC) was used as a standard curve. Concentration of TPC was determined from a standard calibration curve and expressed as milligrams of GAC equivalent per gram of sample (mg GAC/1 g sample).

2.6. Antioxidant activity

2.6.1. DPPH radical scavenging assay

The method used in the present study is based essentially on previous reports by Braca *et al.*, [59] and Nara *et al.*, [60] as modified by Hamed [61]. Briefly, samples were prepared in DMSO as 10× stock solutions (1 mg/ml). The stock solutions (20 µl/well) were dispensed in triplicates onto 96-well plates. The assay was started with the addition of a DPPH reagent (0.004% wt/v in methanol, 180 µl/well). Ascorbic acid was used as positive control. The plate was immediately shaken for 30 seconds and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm on a Zenyth 200rt microplate reader (Biochrom Ltd, Cambridge, UK). The percentage of antioxidant activity was calculated according to the following equation (1):

$$\% \text{ Scavenging Activity} = \frac{\text{Ab} - \text{As}}{\text{Ab}} \times 100 \qquad \textbf{(1)}$$

Whereas; Ab: Absorbance of blank.
As: Absorbance of sample.

2.6.2. ABTS decolorization assay

The ABTS assay was carried out as previously described by Re *et al.*, [62] with some modifications. Briefly, the $ABTS^+$ was produced by reacting equal volume from 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for at least 12h before use. The $ABTS^+$ solution was then diluted and measured on spectrophotometer to obtain an absorbance of 0.75 at 734nm. Samples were prepared in DMSO as 10× stock solutions (1 mg/ml). The stock solutions (20 µl/well) were dispensed in triplicates onto 96-well plates. The assay was started with the addition of $ABTS^+$ reagent (180 µl/well). Ascorbic acid was used as positive control. The plate was immediately shaken for 30 seconds and incubated in the dark for 5 minutes at room temperature. The absorbance was measured at 734 nm on a Zenyth 200rt microplate reader (Biochrom Ltd, Cambridge, UK). The percentage of antioxidant activity was calculated according to equation (1).

Samples producing radical scavenging activities >50% inhibition of free radicals at 100 µg/ml in a preliminary screen were further tested using five serial 2-fold dilutions (100, 50, 25, 12.5 and 6.25 µg/ml). EC_{50} was determined using nonlinear-regression analysis of the dose and % antioxidant activity relationship by using GraphPad Prism® V6.0 software (GraphPad Inc., San Diego, USA).

2.7. Cytotoxic activity

2.7.1. Cell culture

Human triple negative breast cancer cells (MDA-MB-231), human lung carcinoma cells (A549), human colon adenocarcinoma cells (Caco2) and resistance human hepatocellular carcinoma cells (HepG2/DOX) were maintained as a monolayer culture in DMEM medium. While, human hepatocellular carcinoma cells (HepG2) maintained as a monolayer culture in an EMEM medium contains essential amino acids. Both media supplemented with 10% (v/v) heat-inactivated FBS, 4 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin sulphate. All cells were maintained at humidified CO_2 incubator with 5% CO_2 at 37°C and the monolayer was passage at 70-90% confluence using trypsin-EDTA solution.

2.7.2. Cell viability assay (MTT assay)

The cytotoxic activity in the present study is based essentially on the previous report of Mosmann [63] with some modification. Briefly, HepG2 (25×10^3 cells/well), A549, Caco2 (5×10^3 cells/well), MDA-MB-231 (8×10^3 cells/well) or HepG2/DOX (10^4 cells/well) cells were seeded onto 96-well plates in a total volume of 200 µl and left overnight to form a semi-confluent monolayer. Cell monolayers were treated in quadruplicate with vehicle (DMSO, 0.1% v/v) or test sample (100 µg/ml) for an exposure time of 48 h. Doxorubicin hydrochloride was used as a reference cytotoxic drug. At the end of exposure, 30 µl/well of MTT solution in DPBS (5 mg/ml) were added

to all wells and left to incubate for 90 min. The formation of formazan crystals was visually confirmed using phase contract inverted microscope (Olympus CK2, Japan). DMSO (100 µl/well) was added to dissolve the formazan crystals with shaking for 10 min after which the absorbance was read at 492 nm against blank (no cells) on a TriStar2 LB 942 microplate reader (Berthold Technologies, Bad Wildbad, Germany). The percentage of cell death was calculated using the following formula:

$$\% \text{ cell death} = 100 - [\frac{At-Ab}{Ac-Ab} \times 100]$$

Whereas,

At: Absorbance of treated cells

Ab: Absorbance of blank (medium only without cells)

Ac: Absorbance of control

Dose-response experiments were performed on samples producing >50% loss of cell viability using five serial 2-fold dilutions (100, 50, 25, 12.5 and 6.25 µg/ml). IC_{50} values were calculated using the dose response curve fit to non-linear regression correlation using GraphPad Prism® V6.0 software (GraphPad Inc., San Diego, USA).

2.8. Study on the mechanism of action of EO *of C. citratus* in Caco2 cells

2.8.1. Treatment of Caco2 cells

Caco2 cells (0.1×10^6) were seeded in 6-well plates, after 24h of cell plating, cells exposed to IC_{50} dose of EO of *C. citratus* (≈35 µg/ml) or doxorubicin (0.5 µg/ml) which used as a positive control for 48 h. After treatment, only the adherent cells underwent trypsinization, followed by centrifugation at 1000 rpm for 3 min to remove the trypsin.

2.8.2. Cell cycle analysis

Cell cycle assay was performed using a Coulter DNA Prep reagents kit according to the manufacturer's instructions (Beckman Coulter Inc., USA). Briefly, the cell pellet was re-suspended in 0.5 ml PBS and fixed by transfer into centrifuge tubes containing 4.5 ml of pre-chilled 70% ethanol and kept at 4℃ for at least 2 h, then centrifugation at 1000 rpm for 5 min and decanted ethanol thoroughly. Cells were washed by suspending in 5 ml PBS, then centrifugation at 1000 rpm for 5 min. Subsequently, fixed cells were re-suspended in 1 ml of propidium iodide (PI) staining solution and incubated in the dark at room temperature for 30 min. Cell cycle analysis was measured by CytoFLEX flow cytometer (Beckman Coulter Inc., USA). The percentage of cells in the G0/G1, S, and G2/M phases was calculated using Cell Lab Quanta SC software.

2.8.3. Apoptosis assay

Apoptosis assessment was performed using an Annexin V-FITC apoptosis detection kit according to the instructions provided by the manufacturer (Beckman Coulter Inc., USA). Briefly, the cell pellet was washed three times with PBS, followed by centrifugation at 1000 rpm at 4℃ for 5 min, and subsequent removal of the supernatant. Cells were then re-suspended in ice-cold 1X binding buffer, which was prepared by diluting the binding buffer 10X. Subsequently, 1 µl of annexin V-FITC solution and 5 µl of PI solution (250 µg/ml) were added to 100 µl of cell suspension. The tube was placed on ice and incubated in the dark for 15 min, followed by the addition of 400 µl of ice-cold 1X binding buffer and gentle mixing. The percentage of apoptotic cells was determined by CytoFLEX flow cytometer (Beckman Coulter Inc., USA).

2.9. Statistical Analysis

Statistical analysis of the data was conducted utilizing one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons test *via* GraphPad Prism® V6.0 software (GraphPad Inc., San Diego, USA). The results are expressed as mean ± standard mean error (SME). The statistical significance was established at $P<0.05$.

3. Results

3.1. Chemical composition of EOs

The chemical compositions of EOs were determined by GC/MS and the chromatograms of analysis were display in **Figure 1**. The mass spectra of the constituents were comparing with the NIST library and compounds were identified, characterized and then recognized as listed in **Table 1**.

The yields of EOs of *C. citratus*, *L. dentata* and *L. nobilis* from fresh aerial parts were 1.3, 1.4 and 1.3%, respectively (v/w). The high percentage of identified compounds was belonging to oxygenated monoterpenes (79.1, 68.2 and 49.5%, respectively) and monoterpene hydrocarbons (10.6, 18.9 and 27.6%, respectively).

Twenty-six compounds of EO of *C. citratus* were identified, and *E*-citral (36.7%) and *Z*-citral (32.2%) were the majority of the composition, followed by *β*-myrcene (8.8%). Whereas, the chromatographic profile performed on the EO of *L. dentata* led to identify fifty-seven compounds and eucalyptol (31.2%), camphor (19.7%) and *α*-pinene (5.5%) were the most prominent constituents. On the other hand, the EO of *L. nobilis* has forty-three compounds, and the most prominent compounds were eucalyptol (35.5%), *α*-terpinyl acetate (16.3%) and sabinene (10.6%). The percentage of EO of fresh aerial parts of *A. abrotanum* was 0.5% (v/w). Thirty-four compounds were identified and belong to different groups of terpenes with a high percentage of oxygenated sesquiterpenes (39.5%) and oxygenated monoterpenes (39.2%). The most prominent constituents were artemisia ketone (31.8%), *α*-eudesmol (20.6%) and *γ*-eudesmol (5.1%).

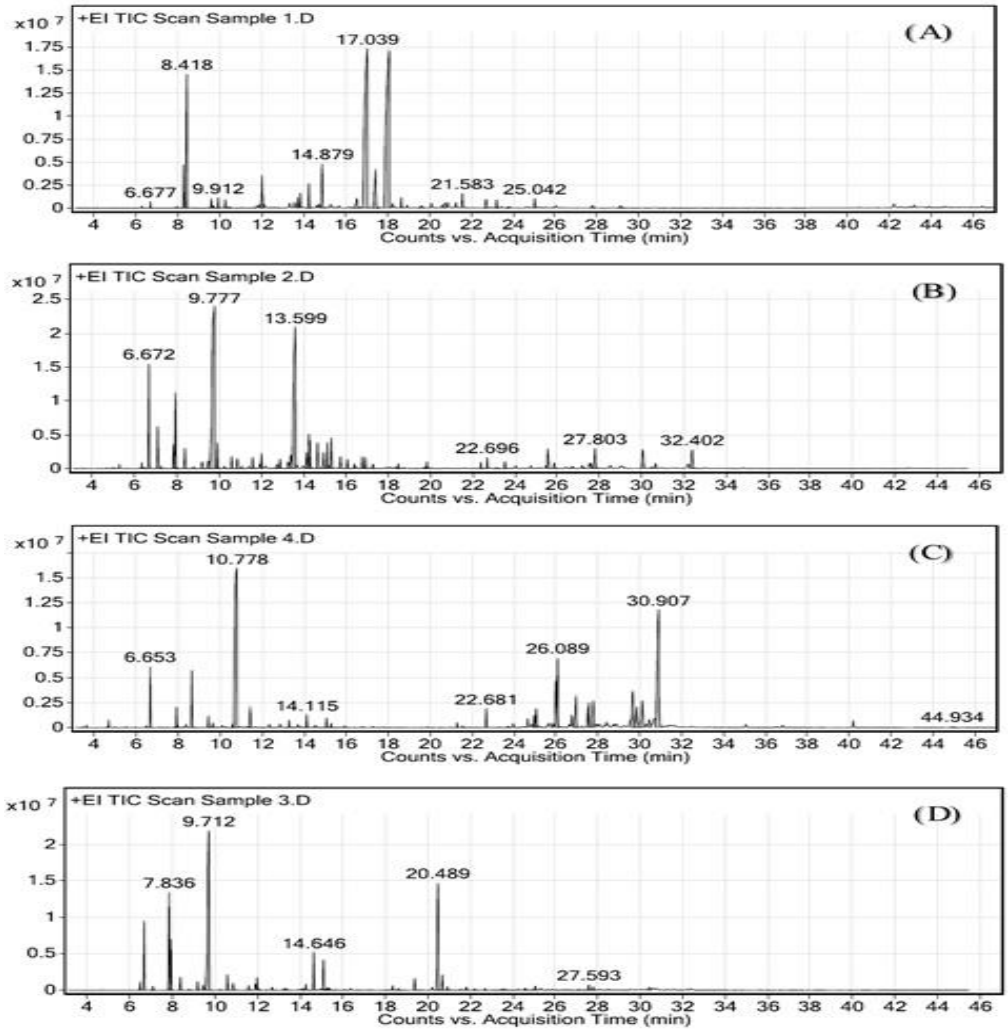

Fig. 1. GC/MS chromatograms of essential oils of (A) *C. citratus*, (B) *L. dentate*, (c) *A. abrotanum* and (D) *L. nobilis*

Table 1. Chemical composition of essential oils of aerial parts of selected plants by GC/MS

No.	RT[a]	Name	%				KI[b]	KI[c]
			C. citratus	*L. dentata*	*A. abrotanum*	*L. nobilis*		
1	3.608	Hexanal	-----	-----	0.1	-----	800	801
2	4.628	2-Hexenal, (*E*)	-----	-----	0.07	-----	847	846
3	4.973	1-Hexanol	-----	0.06	-----	-----	873	863
4	6.332	Tricyclene	-----	0.26	-----	-----	920	921
5	6.457	Artemisia triene	-----	-----	0.12	-----	924	923
6	6.474	*α*-Thujene	-----	0.07	-----	0.64	925	924
7	6.666	*α*-Pinene	0.28	5.54	3.53	6.33	931	932
8	7.082	Camphene	-----	2.07	-----	0.38	945	946
9	7.249	2,4(10)-thujadien	-----	0.13	-----	-----	951	953
10	**7.828**	**Sabinene**	-----	1.27	0.06	**10.63**	971	969
11	7.922	*β*-Pinene	0.07	4.15	1.26	4.69	974	974
12	8.008	1-Octen-3-ol	-----	0.06	0.11	-----	977	974
13	8.226	3-Octanone	-----	-----	0.06	-----	984	979
14	8.259	Sulcatone	2.33	-----	-----	-----	986	981
15	**8.387**	***β*-Myrcene**	**8.84**	1.14	-----	1.23	990	988
16	8.636	Yomogi alcohol	-----	-----	3.83	-----	998	999
17	8.778	*α*- Phellandrene	-----	0.14	-----	0.13	1003	1002
18	9.181	*α*-Terpinene	-----	0.43	-----	0.81	1015	1014

Table 1 continued…

19	9.456	*p*-Cymene	-----	0.91	0.68	0.67	1023	1020
20	9.582	D-Limonene	0.49	-----	-----	-----	1027	1024
21	**9.699**	**Eucalyptol**	0.13	**31.11**	0.36	**35.49**	1030	1026
22	9.912	cis-*β*-Ocimene	0.5	1.25	-----	-----	1037	1032
23	10.244	trans-*β*-Ocimene	0.41	0.13	-----	0.1	1046	1044
24	10.438	Bergamal	0.08	-----	-----	-----	1052	1051
25	10.579	*γ*-Terpinene	-----	0.61	0.2	1.51	1056	1054
26	**10.778**	**Artemisia ketone**	-----	-----	**31.78**	-----	1062	1056
27	10.850	cis-Sabinene hydrate	-----	0.56	-----	0.7	1064	1065
28	11.053	Linalool oxide 2	-----	0.15	-----	-----	1071	1067
29	11.420	Artemisia alcohol	-----	-----	1.3	-----	1081	1080
30	11.5785	*α*-Terpinolene	-----	0.8	-----	0.5	1086	1086
31	11.996	Linalool	2.45	0.95	-----	1.28	1099	1095
32	12.712	Cis-p-menth-2-en-1-ol	-----	0.46	-----	0.46	1119	1118
33	12.882	*α*-Campholenal	-----	0.61	-----	-----	1124	1122
34	13.266	(+)-Nopinone	-----	0.56	-----	-----	1135	1135
35	13.324	trans-Pinocarveol	-----	1.24	0.57	0.33	1137	1135
36	**13.546**	**Camphor**	-----	**19.66**	0.07	-----	1143	1141
37	13.711	trans-Chrysanthemal	0.58	-----	-----	-----	1148	-----
38	13.846	Citronellal	0.82	-----	-----	-----	1152	1148
39	14.130	Pinocarvone	-----	0.94	0.93	0.2	1160	1160
40	14.246	Borneol	-----	2.2	-----	-----	1164	1165
41	14.288	*δ*-Terpineol	-----	1.37	-----	0.72	1165	1162
42	14.651	Terpinen-4-ol	-----	1.55	-----	4.24	1175	1174
43	14.944	Cryptone	-----	1.31	-----	-----	1184	1183
44	15.110	*α*-Terpineol	-----	1.56	-----	3.46	1189	1186
45	15.210	Myrtenol	-----		-----	0.11	1192	1194
46	15.293	Myrtenal	-----	1.9	0.36	0.35	1194	1195
47	15.377	Estragole	-----	-----	-----	0.27	1196	1195
48	15.745	Verbenone	-----	0.81	-----	-----	1207	1204
49	16.071	trans-Carveol	-----	0.52	-----	-----	1217	1215
50	16.470	Citronellol	1.13	0.34	-----	-----	1229	1223
51	16.774	Cuminal	-----	0.77	-----	-----	1238	1238
52	16.918	Carvone	-----	0.68	-----	-----	1242	1239
53	**17.039**	***Z*-Citral "Neral"**	**32.15**	-----	-----	-----	1245	1235
54	17.281	Piperitone	-----	0.33	-----	-----	1253	1249
55	17.430	Geraniol	4.21	-----	-----	-----	1257	1249
56	**18.092**	***E*-Citral "Geranial"**	**36.66**	-----	-----	-----	1277	1264
57	18.359	Bornyl acetate	-----	0.09	-----	0.55	1284	1284
58	18.487	*p*-Cymen-7-ol	-----	0.32	-----	-----	1288	1289
59	18.639	2-Undecanone	0.59	-----	-----	0.24	1293	1293
60	18.752	Perilla alcohol	-----	0.07	-----	-----	1296	1294
61	19.395	*δ*-Terpinyl acetate	-----	-----	-----	1.28	1316	1316
62	19.642	Neric acid	0.26	-----	-----	-----	1323	-----
63	19.749	*p*-Mentha-1,4-dien-7-ol	-----	0.1	-----	-----	1327	1325
64	19.842	Hexyl tiglate	-----	0.38	-----	-----	1329	1330
65	**20.489**	***α*-Terpinyl acetate**	-----	-----	-----	**16.3**	1349	1346
66	20.694	Eugenol	-----	-----	-----	1.71	1356	1356
67	20.759	Geranic acid	0.69	-----	-----	-----	1358	-----
68	20.940	Neryl acetate	-----	-----	-----	0.37	1363	1359
69	21.299	*α*-Copaene	-----	-----	0.38	-----	1374	1374
70	21.564	Geranyl acetate	0.89	-----	0.16	0.17	1383	1379
71	21.834	*β*-Elemene	-----	-----	-----	0.39	1391	1389

Table 1 continued…

72	22.216	Methyleugenol	-----	-----	-----	0.15	1403	1403
73	22.393	*α*-Gurjunene	-----	0.44	-----	-----	1408	1409
74	22.696	trans-Caryophyllene	0.62	0.68	1.43	0.23	1418	1417
75	23.133	Coumarin		0.1	-----	-----	1432	1432
76	23.222	trans-*α*-Bergamotene	0.48	-----	-----	-----	1435	1432
77	23.445	trans-Cinnamyl acetate	-----	-----	-----	0.11	1443	1443
78	23.529	cis-muurola-3,5-diene	-----	0.44	-----	-----	1445	1448
79	23.585	trans-Isoeugenol	-----	-----	-----	0.21	1447	1448
80	23.752	*α*-Humulene	0.07	-----	0.12	0.06	1452	1452
81	24.599	Germacrene D	-----	-----	-----	0.26	1480	1484
82	24.967	*β*-Selinene	-----	-----	1.01	-----	1492	1489
83	25.042	2-Tridecanone	0.56	-----	-----	-----	1494	1495
84	25.070	Bicyclogermacrene	-----	-----	1.58	0.65	1495	1500
85	25.563	*α*-Chamigrene	-----	1.64	-----	-----	1511	1503
86	25.868	*δ*-Cadinene	-----	0.46	0.31	-----	1520	1522
87	26.964	2,5,9-Trimethylcycloundeca-4,8-dienone	-----		3.54	-----	1553	-----
88	27.113	Nerolidol	-----	-----	-----	0.11	1558	1561
89	27.258	Palustrol	-----	0.07	-----	-----	1562	1567
90	27.600	Spathulenol	-----	0.4	2.47	0.88	1573	1577
91	27.803	Caryophyllene oxide	0.23	1.69	2.73	0.52	1579	1582
92	29.148	Selina-6-en-4-ol	0.23	-----	-----	-----	1614	
93	**29.674**	***γ*-Eudesmol**	-----	-----	**5.11**	-----	1626	1630
94	29.865	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl	-----	-----	3.05	-----	1630	-----
95	30.060	tau.-Cadinol	-----	1.89	-----	-----	1634	1638
96	30.130	tau.-Muurolol	-----	-----	4.28	-----	1635	1640
97	30.454	*β*-Eudesmol	-----	0.1	1.27	0.58	1642	1649
98	30.666	*α*-Cadinol	-----	0.45	-----	-----	1647	1652
99	**30.907**	***α*-Eudesmol**	-----	-----	**20.59**	-----	1652	1652
100	32.402	Shyobunol	-----	2.08	-----	-----	1685	1688
101	44.934	13-Epimanool	-----	-----	0.12	-----	2048	2059
		Hydrocarbons compounds	**11.76**	**22.56**	**10.68**	**29.21**		
		Monoterpene	10.59	18.9	5.85	27.62		
		Sesquiterpene	1.17	3.66	4.83	1.59		
		Oxygenated compunds	**83.99**	**77.44**	**82.86**	**70.79**		
		Monoterpenes	79.08	68.2	39.2	49.53		
		Sesquiterpenes	0.46	6.68	39.5	2.09		
		Diterpenes	-----	-----	0.12	-----		
		Esters	0.89	0.09	0.16	18.78		
		Others	3.56	2.47	3.88	0.39		
		ToTal	**95.75**	**100**	**93.54**	**100**		

[a] Retention time by minute
[b] Kovats index determined by co-injection of n-alkanes C8–C40 on present instrument using HP-5MS column
[c] Kovats index according to Adams book [64] for comparison

3.2. Total phenolics of EOs

The analysis revealed that EO of *C. citratus* has a high amount of phenolic content (22.2±0.9 mg GAC/g EO), followed by EO of *L. nobilis* (10.3±0.5 mg GAC/g EO), then EOs of *L. dentata* (4.1±0.5 mg GAC/g EO) and *A. abrotanum* (3.6±0.2 mg GAC/g EO).

3.3. Antioxidant activity of EOs

Pre-screening of antioxidant activity using DPPH-radical scavenging and $ABTS^+$-decolorization assays revealed that all EOs have weak antioxidant activity from 1.4±1.1 to 18.5±0.8% at 100 µg/ml, except for EO of *L. nobilis* which showed 61.2±0.5% activity by using $ABTS^+$-decolorization assay as showed in **Figure 2**. A dose-response experiment using $ABTS^+$ for EO of *L. nobilis* revealed that the EC_{50} value was 81.2±5.3 µg/ml as shown

in **Figure 3**. In contrast, EC_{50} values for other EOs were considered greater than 100 µg/ml. Additionally, ascorbic acid was used as a positive control and gave an EC_{50} value of 2.3±0.1 µg/ml.

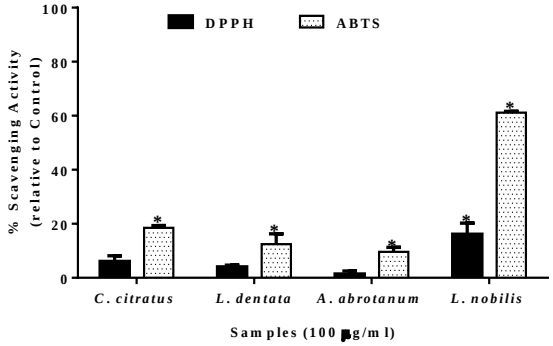

Fig. 2. Pre-screen of antioxidant activity of essential oils at 100 µg/ml. DPPH and ABTS was performed as mentioned in the Materials and Methods section. Data are means ± SEM of triplicate assays. * denotes significance level of P<0.05 (one-way ANOVA and Dunnett's test).

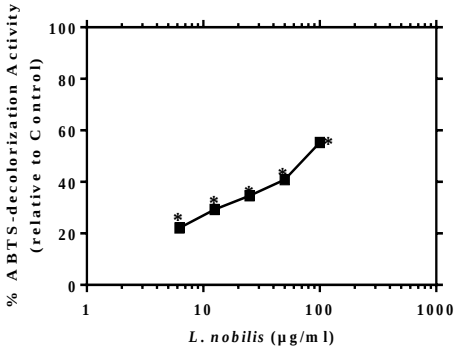

Fig. 3. Concentration-response curve for ABTS-decolorization activity of EO of *L. nobilis*. ABTS was performed as mentioned in the Materials and Methods section. Data are means ± SEM of triplicate assays. * denotes significance level of P<0.05 (one-way ANOVA and Dunnett's test).

3.4. Cytotoxic effect of EOs against various cancer cell lines

Evaluation of the cytotoxic effect of different EOs towards the proliferation of different human cancer cell lines at a 100 µg/ml is depicted in **Figure 4**. The pre-screen revealed that the EO of *C. citratus* has a strong cytotoxic effect against all cell types with a loss of cell viability from 77.0±0.9 to 100% relative to vehicle control. In contrast, the other EOs exhibited weaker cytotoxic effects at 100 µg/ml with a loss of cell viability from zero to 35.6±3.0% relative to vehicle control.

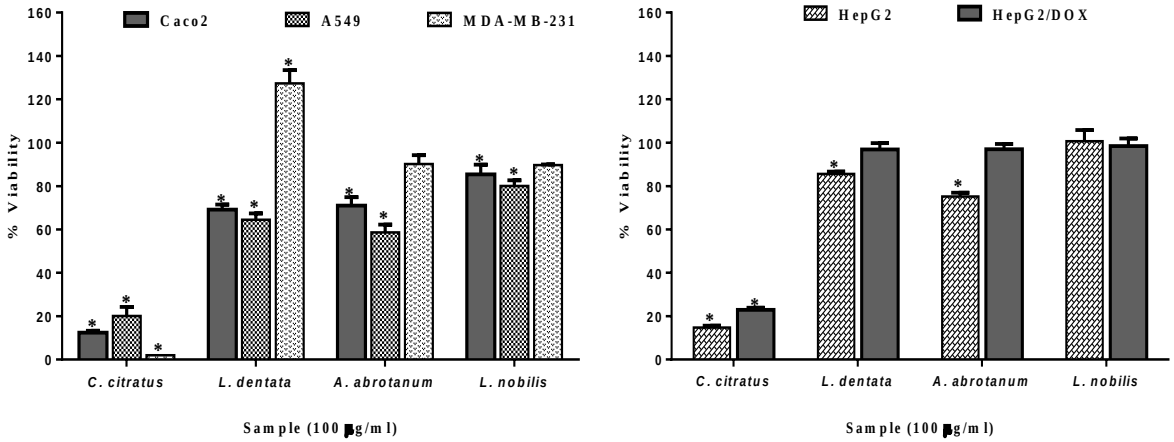

Fig. 4. Pre-screening of essential oils against the proliferation of different cancer cell lines at 100 µg/ml. Indicated cells were cultured as monolayers and treated as mentioned at the Materials and Methods sections. Data are means ± SEM at least duplicate assays. * denotes significance level of P<0.05 (one-way ANOVA and Dunnett's test).

Based on the above findings, the EO of *C. citratus* was selected for further testing in a dose-response experiment to determine its IC_{50}. Therefore, the IC_{50} values for other EOs were considered greater than 100 µg/ml.

Figure 5 displays the dose-dependent effect of the EO of *C. citratus* on cells tested for IC_{50} detection. As presented, the IC_{50} values was 33.7±0.4, 34.6±4.2, 56.1±2.3, 61.8±4.6 and 85.3±1.4 µg/ml against Caco2, MDA-MB-231, A549, HepG2 and HepG2/DOX, respectively as listed in **Table 2**.

Table 2. IC_{50} values were statistically calculated using graphpad prism "Non-linear regration curve fit based on three dose-response experiments".

Cell lines	IC_{50} (µg/ml)	
	C. citratus	**Doxorubicin**
Caco2	33.7 ± 0.35	0.25 ± 0.014
A549	56.1 ± 2.32	0.16 ± 0.006
MDA-MB-231	34.6 ± 4.16	0.045 ± 0.017
HepG2	61.8 ± 4.6	0.06 ± 0.003
HepG2/DOX	85.3 ± 1.36	>20

Doxorubicin was also tested as a positive control, and its IC_{50} values were 0.25±0.014, 0.045±0.017, 0.16±0.006, 0.06±0.003 and >20 µg/ml for Caco2, MDA-MB-231, A549, HepG2 and HepG2/DOX, respectively.

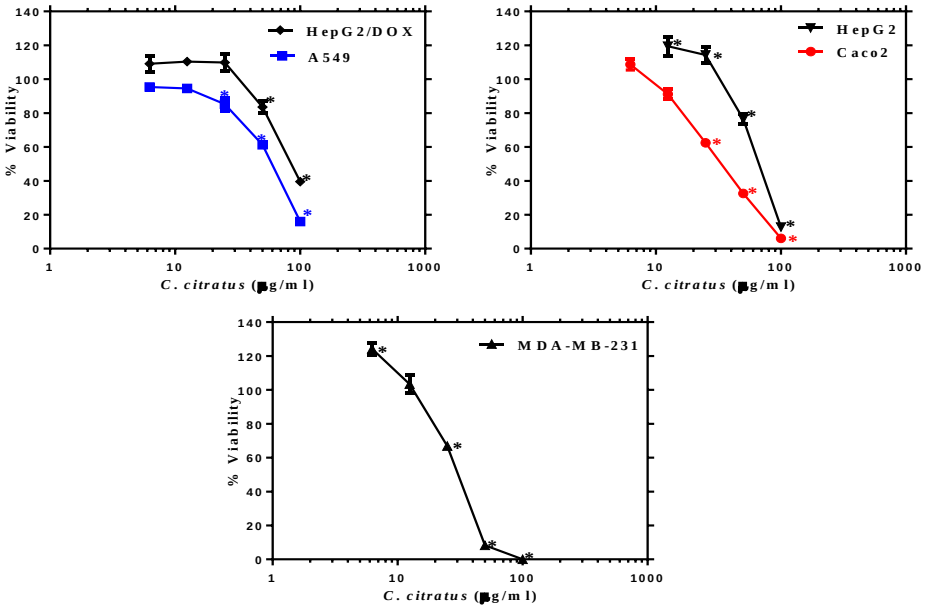

Fig. 5. Dose-response curves showing the effect of EO of *C. citratus* against the proliferation of different cell lines. Indicated cells were cultured as monolayers and treated as mentioned at the Materials and Methods sections. Data are means ± SEM at least duplicate assays. * denotes significance level of P<0.05 (one-way ANOVA and Dunnett's test).

3.5. Effect of EO of *C. citratus* on the cell cycle

Flow cytometric analysis of the treatment of the Caco2 cells with EO of *C. citratus* led to S phase arrest. Meanwhile, the percentage of cells in the G0/G1 phase was reduced from 80.1% to 77.3% and the G2/M phase was reduced from 0.39% to 0.36% in the treated cells compared to untreated cells. Moreover, the percentage of cells in the S phase increases from 19.5% to 22.3% in the treated cells compared to untreated cells (**Figure 6**).

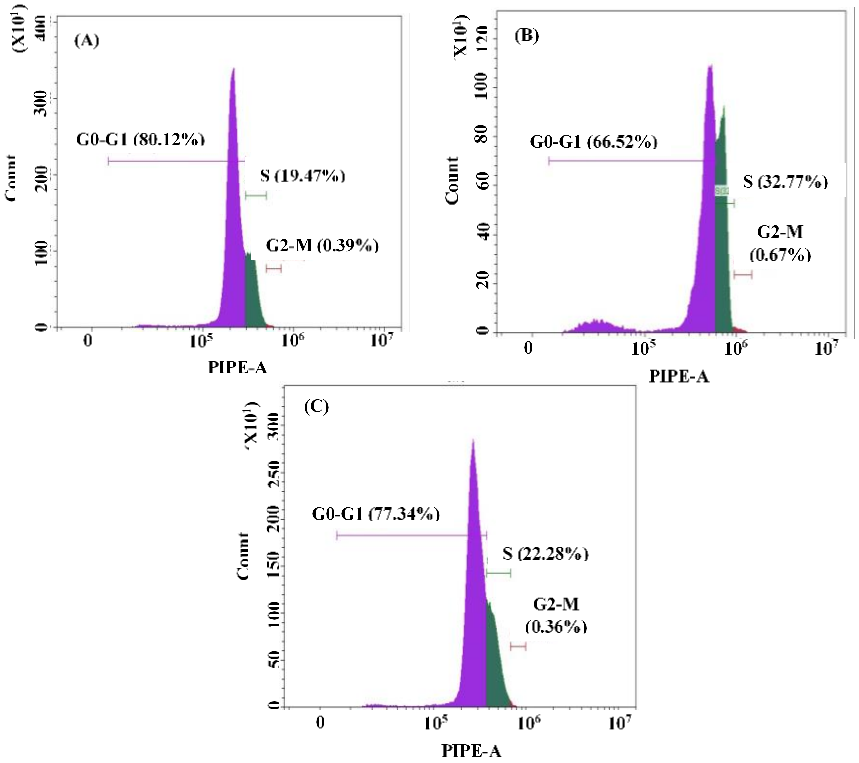

Fig. 6. Flow cytometric cell cycle analysis in Caco2 cells. Cells were culture, treated and prepared as mentioned at the Materials and Methods sections. Graphs (A) Control, (B) EO of *C. citratus* at 35 µg/ml and (C) positive control at 0.5 µg/ml.

Meanwhile, the percentages of cells in different phases in positive control were 66.5% in the G0/G1 phase, 32.8% in the S phase and 0.67% in the G2/M phase.

3.6. The apoptotic effect of EO on *C. citratus*

Flow cytometry results showed that the treatment of Caco2 cells with the EO of *C. citratus* significantly induced apoptosis in treated cells compared to untreated cells. Notably, the percentage of viable cells decreased significantly from 96.9% to 87.4% and necrotic cells decreased from 1.3% to 0.9% in treated cells compared to untreated cells.

In addition, early apoptotic cells increased significantly from 1.5% to 11.3% and late apoptotic cells increased from 0.23 % to 0.42% in treated cells compared to untreated cells (**Figure 7**).

On the other hand, the percentages of different cells in positive control were 0.9% viable cells, 0.2% in early apoptotic cells, 18.5% late apoptotic cells and 80.5% necrotic cells.

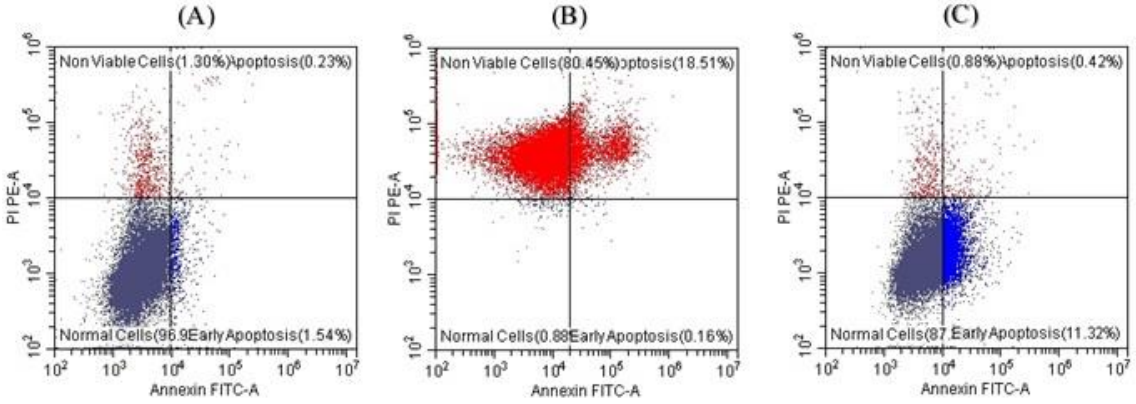

Fig. 7. Apoptotic effect on Caco2 cells analyzed with Annexin V-FITC apoptosis detection kit. Graphs (A) Control, (B) EO of *C. citratus* at 35 µg/ml and (C) positive control at 0.5 µg/ml.

4. Discussion

The results of the chemical composition of EO obtained from *C. citratus* in the present study agree with previous studies conducted in Egypt for example; Soliman et al., [65] demonstrated that geranial was most abundant component (42.86%) followed by neral (36.83%) and *β*-myrcene (8.05%). Furthermore, analysis of EOs obtained during different seasons indicates the major chemical compositions were geranial (from 33.09% to 46.38%), neral (from 26.92% to 35.91%) and *β*-myrcene (from 1.90% to 9.84%) [24].

The analysis of the EO of *C. citratus* gave a similar pattern to those published in other geographical locations. Several studies in different countries identified citral as the most abundant compound. In Algeria and Angola, the most abundant components were geranial (42.2 and 40.55 %, respectively) and neral (31.5 and 28.26%, respectively), followed by *β*-myrcene (7.5 and 10.5%, respectively) [20,66]. In Brazil, the major constituent was geranial (43.08%) followed by neral (32.19%) and *β*-myrcene (17.58%) [16]. In India, the most abundant compounds were geranial (42.4%), neral (29.8%) and *β*-myrcene (8.9%) [23].

The GC/MS analysis of EO of *L. dentata* corroborates the chemical constituents performed previously in Tunisia by Dammak et al., [67] who obtained eucalyptol (35±1.9%) as a major component followed by camphor (32±0.2%), but, *β*-pinene (5.8±0.9%) was the fourth most abundant compound after fenchone (9.3±1%). Also, the EO of *L. dentata* obtained from Brazil contains eucalyptol (52.23%), camphor (19.51%) and fenchone (14.73%) as major compounds [68].

Chromatographic profiles performed on EO of *L. dentata* harvested from different countries showed eucalyptol was the most abundant compound for instance, in Mexico and Italy, the majority was eucalyptol (68.59 and 69.08, respectively) and *β*-pinene (11.53 and 4.84%, respectively) [69,70]. Also, in Morocco, the major compounds were eucalyptol (48.82%), camphor (17.01%), borneol (9.58%) and *p*-cymene (6.62%) [71].

Previous studies on the composition of EO of *L. nobilis* grown in Egypt under the same weather conditions demonstrated the same results as our study. For example, El-Sawi et al., [72] demonstrated that the major constitutions were eucalyptol (50.38%), *α*-terpinenyl acetate (19.97%) and 4-terpineol (6.84%). Also, Nagah et al., [73] exhibited the yield of EO of fresh leaves was 1.9% (v/w) and the main compositions were eucalyptol (27.41%), linalool (19.37%) and *α*-terpinyl acetate (14.65%).

On the other hand, the analysis of EO of *L. nobilis* gave the same results to those from different countries and identified eucalyptol as the most abundant compound. In Portugal, the main constitution were eucalyptol (27.2%), *α*-terpinyl acetate (10.2%) and linalool (8.4%) [74]. In Morocco and Palestinian, the major compounds were eucalyptol (36.58 and 40.39%, respectively), *α*-terpinyl acetate (15.42 and 15.07%, respectively) and sabinene (12.08 and 10.35%, respectively) [47,75]. In addition, the main composition of EOs obtained from leaves purchased from different cities in Turkey was eucalyptol (41.2-64.4%), *α*-terpinyl acetate (1.5-15%) and sabinene (1.5-15.9%) [76].

Although, the majority of compounds listed in **Table 1** were already identified as *A. abrotanum* volatiles, and their identity didn't match with the volatile profile of any previously analyzed EO of Southernwood. For example, the EO distilled from the Egyptian sample had a high percentage of 2-hydroxy-eucalyptol (38.7%), *β*-eudesmol (12.4%) and camphor (12.1%) [77]. Khodakov et al., [78] demonstrated that the EO profiles defer based on the vegetative phases of *A. abrotanum* grown in Crimea. The predominant components were eucalyptol and camphor in all phases. Eucalyptol reaches the highest levels at the start and end of vegetation (33.19% and 26.31%) and the lowest level was during budding (15.46%) and mass flowering (19.54%). In contrast, the level of camphor was low at the start of vegetation (20.33%) and then increased during following vegetative phases (budding 44.15% and mass flowering 44.63%) and remained stable at a high level up to the end of vegetation (43.76%).

The above data are consistent with the assertion that the observed differences in the EO profiles depends on several factors, such as climate (temperature, humidity level and sunshine duration), season, collection site, soil composition and genetic factors. Therefore, there may be differences in the proportion of its components.

The TPC of EO of lemongrass was 156.29±4.94 mg GAC/ml EO [79]. Also, Alencar et al., [80] found that TPC of EO of *C. citratus* was 1.6 mg GAC/g EO. On the other hand, the TPC of EO of *L. nobilis* was 112.3±0.3 mg GAC/g EO [81], which is higher than that found by Dammak et al., [67] (15.15±1.64 mg GAC/g EO). While, the amount of TPC of EO of *L. dentata* was 4.09±1.54 mg GAC/g EO [67].

We commenced the biological evaluation with testing the antioxidant activity of EOs. Our data demonstrated that the EO of lemongrass had weak antioxidant activity and was consistent with data previous report of Hacke et al., [4] who found that the antioxidant activity via ABTS and DPPH radicals scavenging assays exhibited weak activity with IC_{50} values >100 µg/ml. In addition, a DPPH-radical scavenging assay for EO showed very low activity with an IC_{50} value of 1.8 mg/ml [82]. On the contrary, another study exhibited that the EO of *C. citratus* had moderate antioxidant activity by DPPH-radical scavenging assay with an IC_{50} value of 41.7 µg/ml [66].

The results of the present study demonstrated that the EO of *L. nobilis* had weak antioxidant activity; this result is in agreement with previous study that has shown that the antioxidant activity evaluated by ABTS and DPPH radical scavenging assays gave IC_{50} values of 152.60±0.98 and 599.32±1.95 µg/ml, respectively [45]. The antioxidant activity was also examined by DPPH radical scavenging activity and exhibited activity with an IC_{50} value of 176±46 µg/ml [83]. On contrast, other previous studies on the EO of *L. nobilis* showed high activity, e.g., Mssillou et al., [84] demonstrated that the EO had moderate DPPH-radical scavenging activity with an IC_{50} value of 82.01 µg/ml. Also, Ovidi et al., [46] found that the EO had high antioxidant activity as observed by DPPH and ABTS assays that gave IC_{50} values of 0.18±0.04 and 2.58±0.08 µg/ml, respectively.

The EO of *L. dantata* showed very low antioxidant activity with IC_{50} values of 113.29±0.012 mg/ml and 53.029±0.02 mg/ml determined by DPPH and ABTS, respectively [85]. In addition, antioxidant activity confirmed by DPPH and FRAP assays and showed weak activity with IC_{50} values of 12.95±1.3 mg/ml and 11.88±0.23 mg/ml, respectively [33]. In contrast, Imelouane et al., [86] showed that the EO of aerial parts and flowers of *L. dantata* had moderate DPPH-radical scavenging activity with IC_{50} values of 32.12±0.57 and 41.29±1.21 µl/ml, respectively.

The survey on the EO of *A. abrotanum* revealed a lack of prior research on its potential antioxidant properties. However, various other species of *Artemisia* have been studied extensively. For example; the EOs of *A. annua* collected from different regions have very weak antioxidant activity as assessed by DPPH and ABTS with IC_{50} values of 5-6.5 and 2.4-3.5 mg/ml, respectively [87]. Also, the EOs of *A. campestris* and *A. herba-alba* showed very low DPPH-radical scavenging activity with IC_{50} values of 7.80±0.05 and 2.61±0.01 mg/ml, respectively [88]. In contrast, another study on the EO of *A. annua* exhibited significant antioxidant activity with IC_{50} values of 29±5.3 µg/ml for DPPH assay and 9.22±0.3 µg/ml for FRAP assay [89], The EO of *A. jordanica* showed significant DPPH-radical scavenging activity with IC_{50} value of 2.18±0.24 µg/ml [90].

The results of the cytotoxic effect of EO of *C. citratus* obtained in the present study are consistent with those of Najar et al., [91], Abdulhasan [92] and Abdel-Gwad et al., [79], who found a moderate cytotoxic activity toward different human cancer cells with IC_{50} values of 38.4 µg/ml for MDA-MB-231, 29.13 µg/ml for A549 and 77.413 µg/ml for HCT116 (colon cancer). Furthermore, A549 cells treated with EO of *C. citratus* (IC_{50}= 29.13 µg/ml) exhibited a significant increase in the expression of the p53 gene by 4.47±0.7 fold compared with untreated cells. This confirmed that the cytotoxic effect due to the up-regulation of the p53 gene induced apoptosis. In addition, Trang et al., [93] demonstrated that the EO of lemongrass exhibited a high cytotoxic effect against A549, NCI-H1975 and NCI- H1650 (lung adenocarcinoma) and NCI-H1299 (lung large cell carcinoma) with IC_{50} values ranged from 1.73±0.37 to 8.93±0.50 µg/ml. This induced apoptosis and cycle arrest in A549 cells and western blot analysis indicated that the apoptotic effect was induced by altering the regulating proteins of the apoptosis process such as caspase-3, Bcl-2, and Bax. On the other way, the study by Mukhtar et al., [19] showed that the EO of *C. citratus* had a weak effect on HepG2 and HepG2/DOX with IC_{50} values reaching 129.7±11.4 µg/ml and 281.8±16.1 µg/ml, respectively.

Biological screening of the EO of *L. nobilis* for cytotoxic activity indicated that the EO has low activity and this study was in agreement with previous studies. The EO has a very low cytotoxic effect against various cancer cell lines including: HepG2, Caco-2, MCF-7 (breast cancer) and HeLa (cervical adenocarcinoma) with IC_{50} values

from 673.82±1.07 to 873.13±2.88 µg/ml [47]. Conversely, other studies showed a highly cytotoxic effect of EO against HepG2, MCF7, HeLa, U251 (brain tumor) and H460 (lung carcimona) with IC_{50} values between 0.6 and 1.8 µg/ml [72]. Also, Çöven et al., [43] demonstrated that the EO of *L. nobilis* has anti-proliferation activity against Caco-2, A549, MDA-MB-231, HepG2, HeLa, PC-3 (prostate adenocarcinoma), HEK293 (embryonic kidney), PANC-1 (pancreatic carcinoma) and CCD-34Lu (healthy lung fibroblasts) cells with IC_{50} values between 7.33±0.82 and 46.08±5.21 µg/ml.

The EO of *L. dentata* showed a low cytotoxic effect against non-small lung cancer cell (Calu-3) with IC_{50} value of 388.84 µg/ml and the cell cycle analysis indicated an increase cells in G0/G1 phase and a decrease cells in S phase compared to untreated cells, which explains the cytotoxic effect of EO of *L. dentate* against Calu-3 cells via apoptosis [31]. On the contrary, Imelouane et al. [86] showed that the EO of aerial parts and flowers showed moderate cytotoxicity against other cell types such as murine macrophage (P388Dl), prostate (PC3), fibrobasts (V79), human Caucasian (U-373 MG) and MCF-7 with IC_{50} values between 46±1.57 and 101±4.40 µg/ml.

To the best of our knowledge there are no previous studies that showed the anti-proliferative activity of *A. abrotanum*. On the other hand, other species of *Artemisia* had anti-proliferative activity e.g.; the EO of *A. jordanica* showed very low cytotoxic effect with IC_{50} values of 15.4 mg/ml for HeLa, 2.55 mg/ml for MCF-7, 0.44 mg/ml for HepG2 and 0.38 mg/ml for Caco2 [90]. Also, the EO of *A. campestris* exhibited weak activity with IC_{50} values of 0.76±0.09 mg/ml for Caco2, 0.28±0.06 mg/ml for MCF-7 and 0.43±0.04 mg/ml for T47D (human breast adenocarcinoma) [88]. Conversely, the EO of *A. herba-alba* showed moderate cytotoxic effect with IC_{50} values 16±5 µg/ml for MCF-7 and 80±5 µg/ml for T47D [88]. Additionally, Russo et al., [94] demonstrated that the EOs of flowers and leaves of *A. arborescens* collected from different locations in Italy exhibited a cytotoxic effect against human malignant melanoma cells (A375) with IC_{50} values between 4.5±0.18 and 23.4±0.15 µg/ml. They also conducted western blot analysis to confirm the apoptotic effect of EOs against A375 cells, and the EOs led to repressed the expression of the anti-apoptotic protein Bcl-2 and increased pro-apoptotic protein Bax, caspase-3, caspase-9 and increased the level of tumor suppressor gene (PTEN).

The dual effect of these EOs in this study compared to previous studies shows very high and very low or no cytotoxic effects depending on cell type, concentration, exposure time and plant collection areas.

The apoptotic process is often confirmed through various techniques such as flow cytometry, DNA ladder and microscope. At the molecular level, the essential oils modulate the expression of apoptosis-related genes such as Bcl-2 and Bax and activate the apoptotic pathway through caspases enzymes. These suggest that essential oils may have potential as natural anti-cancer agents [93].

One of the cell death mechanisms is cell cycle arrest and the initiation of apoptosis in abnormal cells [95]. Our results indicated that the EO of *C. citratus* triggered a notable increase in the number of cells in the S phase, coupled with a reduction in the population of cells in the G0/G1 and G2/M phases. Consequently, the progression of the cell cycle in Caco2 cells was significantly blocked during S phase causing S phase arrest.

The cell cycle is intricately linked to the process of apoptosis, whereby cell cycle arrest results in apoptosis through modulation of different signaling molecules and regulatory proteins.

Both apoptosis and necrosis are involved in cell death, but they display distinct morphological features. Flow cytometry was utilized to evaluate apoptosis by staining with Annexin V-FITC and propidium iodide after treatment with EO of *C. citratus* in Caco2 cells and demonstrated that apoptotic cells increased in treated cells compared to untreated cells. These results are in agreement with Trang et al., [93] who found that EO of *C. citratus* had a significant apoptosis and cell cycle arrest effect on A549 as assessed by fluorescent nuclear staining assays and flow cytometric analysis. Also, western blot analysis indicating that the EO of *C. citratus* induces apoptotic effect by altering apoptosis-regulating proteins such as Bax, Bcl-2 and caspase-3 suggesting the involvement of the intrinsic apoptotic pathway.

In general, essential oils derived from various botanical sources have exhibited anticancer effects by promoting apoptosis and inducing cell cycle arrest across diverse cancer cell lines. The essential oil extracted from rosemary significantly diminished Bcl-2 expression while enhancing Bax expression in HepG2 [96]. Essential oils obtained from three distinct *Salvia* species facilitated apoptosis in DU-145 cells (prostate cancer) by altering the levels of Bcl-2 and Bax proteins and augmenting the generation of reactive oxygen species [95]. A synergistic application of fennel and geranium essential oils led to the induction of cell cycle arrest and apoptosis in MCF-7 cells through the modulation of p53, caspase-3, mir-21, mir-92a, Bcl-2, and ki-67 expression levels [97]. Collectively, these investigations imply that essential oils possess considerable potential as anticancer agents through their capacity to influence apoptosis-related proteins and regulate cell cycle dynamics.

5. Conclusion

The chromatographic profile performed on EOs revealed that the most abundant compounds of EOs of *C. citratus*, *L. dentata*, *A. abrotanum* and *L. nobilis* were citral, eucalyptol, artemisia ketone and eucalyptol, respectively. All EOs had very weak antioxidant activity and very low cytotoxic effect, except for the EO of *L. nobilis* which had moderate $ABTS^{+}$-decolorization activity and the EO of *C. citratus* showed a strong cytotoxic effect against Caco2 and MDA-MB-231 cell lines, and moderate effect against A549, HepG2 and HepG2/DOX

cell lines. Flow cytometry analysis showed that the EO of *C. citratus* significantly induces apoptosis and S phase arrest in Caco2 cells.

6. Conflicts of interest

Authors declare no conflict of interest.

7. Acknowledgement

The authors sincerely thanks to Dr. Ahmed E. El-Gohary (Medicinal and Aromatic Plants Research Department, National Research Centre, Egypt) for his support and collaboration in collecting plant materials.

8. References

[1] S. V. Serkerov, S.I. Ibragimova, New steroidal compound from *Artemisia abrotanum*, Chem. Nat. Compd. 50 (2014) 478–479. https://doi.org/10.1007/s10600-014-0991-z.

[2] H.O. Elansary, A. Szopa, P. Kubica, H. Ekiert, D.O. El-Ansary, F.A. Al-Mana, E.A. Mahmoud, Polyphenol content and biological activities of *Ruta graveolens* l. and *Artemisia abrotanum* l. in northern Saudi Arabia, Processes 8 (2020) 531. https://doi.org/10.3390/PR8050531.

[3] G. Costa, S. González-Manzano, A. González-Paramás, I.V. Figueiredo, C. Santos-Buelga, M.T. Batista, Flavan hetero-dimers in the *Cymbopogon citratus* infusion tannin fraction and their contribution to the antioxidant activity, Food Funct. 6 (2015) 932–937. https://doi.org/10.1039/c5fo00042d.

[4] A.C.M. Hacke, J.A. Marques, J.C.R. Vellosa, A.A. Boligon, F.D. da Silva, D. de Souza, J.S. Bonini, J.B.T. Rocha, R.P. Pereira, Ethyl acetate fraction of *Cymbopogon citratus* as a potential source of antioxidant compounds, New J. Chem. 42 (2018) 3642–3652. https://doi.org/10.1039/c7nj04352j.

[5] L. Longo, G. Vasapollo, Anthocyanins from bay (*Laurus nobilis* L.) berries, J. Agric. Food Chem. 53 (2005) 8063–8067. https://doi.org/10.1021/jf051400e.

[6] Z. Petkova, G. Stefanova, T. Girova, G. Antova, M. Stoyanova, S. Damianova, V. Gochev, A. Stoyanova, V.D. Zheljazkov, Phytochemical investigations of laurel fruits (*Laurus nobilis*), Nat. Prod. Commun. 14 (2019) 1–10. https://doi.org/10.1177/1934578X19868876.

[7] A. Turk, J.H. Ahn, Y.H. Jo, J.Y. Song, H.K. Khalife, H. Gali-Muhtasib, Y. Kim, B.Y. Hwang, M.K. Lee, NF-κB inhibitory sesquiterpene lactones from Lebanese *Laurus nobilis*, Phytochem. Lett. 30 (2019) 120–123. https://doi.org/10.1016/j.phytol.2019.02.003.

[8] M. Berendika, S.D. Drozdek, D. Odeh, N. Oršolić, P. Dragičević, M. Sokolović, I.E. Garofulić, D. Đikić, I.L. Jurčević, Beneficial effects of laurel (Laurus nobilis L.) and myrtle (Myrtus communis L.) extract on rat health, Molecules 27 (2022) 581. https://doi.org/10.3390/molecules27020581.

[9] M. del M. Contreras, F. Algieri, A. Rodriguez-Nogales, J. Gálvez, A. Segura-Carretero, Phytochemical profiling of anti-inflammatory *Lavandula* extracts via RP–HPLC–DAD–QTOF–MS and –MS/MS: Assessment of their qualitative and quantitative differences, Electrophoresis 39 (2018) 1284–1293. https://doi.org/10.1002/elps.201700393.

[10] I.A. Chowdury, M. Debnath, F. Ahmad, M.N. Alam, A.M. Saleh, S. Chowdhury, R. Barua, M.M.U. Mazumdar, A.H.M. Kamal, Potential phytochemical , analgesic and anticancerous activities of *Cymbopogon citratus* Leaf, Am. J. Biomed. Res. 3 (2015) 66–70. https://doi.org/10.12691/ajbr-3-4-2.

[11] F. Tavares, G. Costa, V. Francisco, J. Liberal, A. Figueirinha, M.C. Lopes, M.T. Cruz, M.T. Batista, *Cymbopogon citratus* industrial waste as a potential source of bioactive compounds, J. Sci. Food Agric. 95 (2015) 2652–2659. https://doi.org/10.1002/jsfa.6999.

[12] S. Umukoro, S.I. Ogboh, O. Omorogbe, A.-L.A. Adekeye, M.O. Olatunde, Evidence for the involvement of monoaminergic pathways in the antidepressant-like activity of *Cymbopogon citratus* in mice, Drug Res. (Stuttg). 67 (2017) 419–424. https://doi.org/10.1055/s-0043-106586.

[13] H.A. Garba, A. Mohammed, M.A. Ibrahim, M.N. Shuaibu, Effect of lemongrass (*Cymbopogon citratus* Stapf) tea in a type 2 diabetes rat model, Clin. Phytoscience 6 (2020) 19. https://doi.org/10.1186/s40816-020-00167-y.

[14] A.H. Salih, R.H. Salih, H.Y. Ahmed, Bioactivity of *Cymbopogon Citratus* aqueous extract against measles virus and some bacterial isolates, Casp. J. Environ. Sci. 20 (2022) 585–592. https://doi.org/10.22124/cjes.2022.5702.

[15] B. Rahhal, M. Qneibi, N. Jaradat, M. Hawash, M. Qadi, L. Issa, S. Bdir, Multi-biological activity assessment and phytochemical characterization of an aqueous extract of the *Cymbopogon citratus* grown in Palestine, BMC Complement. Med. Ther. 24 (2024) 27. https://doi.org/10.1186/s12906-024-04338-z.

[16] F. V. Moreira, J.F.A. Bastos, A.F. Blank, P.B. Alves, M.R. V. Santos, Chemical composition and cardiovascular effects induced by the essential oil of *Cymbopogon citratus* DC. Stapf, Poaceae, in rats, Brazilian J. Pharmacogn. 20 (2010) 904–909. https://doi.org/10.1590/S0102-695X2010005000012.

[17] C.A.R. de A. Costa, D.O. Kohn, V.M. de Lima, A.C. Gargano, J.C. Flório, M. Costa, The GABAergic system contributes to the anxiolytic-like effect of essential oil from *Cymbopogon citratus* (lemongrass), J. Ethnopharmacol. 137 (2011) 828–836. https://doi.org/10.1016/j.jep.2011.07.003.

[18] C.A.R.A. Costa, L.T. Bidinotto, R.K. Takahira, D.M.F. Salvadori, L.F. Barbisan, M. Costa, Cholesterol reduction and lack of genotoxic or toxic effects in mice after repeated 21-day oral intake of lemongrass (*Cymbopogon citratus*) essential oil, Food Chem. Toxicol. 49 (2011) 2268–2272. https://doi.org/10.1016/j.fct.2011.06.025.
[19] M.H. Mukhtar, M.Z. El-Readi, M.E. Elzubier, S.H. Fatani, B. Refaat, U. Shaheen, E.B. Adam Khidir, H.H. Taha, S.Y. Eid, *Cymbopogon citratus* and citral overcome doxorubicin resistance in cancer cells via modulating the drug's metabolism, toxicity, and multidrug transporters, Molecules 28 (2023) 3415. https://doi.org/10.3390/molecules28083415.
[20] M.N. Boukhatem, M.A. Ferhat, A. Kameli, F. Saidi, H.T. Kebir, Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs, Libyan J. Med. 9 (2014) 25431. https://doi.org/10.3402/ljm.v9.25431.
[21] M.R. Silva, R.M. Ximenes, J.G.M. da Costa, L.K.A.M. Leal, A.A. de Lopes, G.S. de Barros Viana, Comparative anticonvulsant activities of the essential oils (EOs) from *Cymbopogon winterianus* Jowitt and *Cymbopogon citratus* (DC) Stapf. in mice, Naunyn. Schmiedebergs. Arch. Pharmacol. 381 (2010) 415–426. https://doi.org/10.1007/s00210-010-0494-9.
[22] G.S. Tayeboon, F. Tavakoli, S. Hassani, M. Khanavi, O. Sabzevari, S.N. Ostad, Effects of *Cymbopogon citratus* and Ferula assa-foetida extracts on glutamate-induced neurotoxicity, Vitr. Cell. Dev. Biol. - Anim. 49 (2013) 706–715. https://doi.org/10.1007/s11626-013-9656-7.
[23] S.K. Bharti, A. Kumar, O. Prakash, S. Krishnan, A.K. Gupta, Essential oil of *Cymbopogon citratus* against diabetes: Validation by *In vivo* experiments and computational studies, J. Bioanal. Biomed. 5 (2013) 194–203. https://doi.org/10.4172/1948-593X.1000098.
[24] Y.F. Madi, M.A. Choucry, M.R. Meselhy, E.S.A. El-Kashoury, Essential oil of *Cymbopogon citratus* cultivated in Egypt: seasonal variation in chemical composition and anticholinesterase activity, Nat. Prod. Res. 35 (2021) 4063–4067. https://doi.org/10.1080/14786419.2020.1713125.
[25] P. Pallavi, P.P. Sahoo, S.K. Sen, S. Raut, Comparative evaluation of anti-biofilm and anti-adherence potential of plant extracts against *Streptococcus mutans*: A therapeutic approach for oral health, Microb. Pathog. 188 (2024) 106514. https://doi.org/10.1016/j.micpath.2023.106514
[26] R.A.A. Mothana, R. Gruenert, P.J. Bednarski, U. Lindequist, Evaluation of the *in vitro* anticancer, antimicrobial and antioxidant activities of some Yemeni plants used in folk medicine, Pharmazie 64 (2009) 260–268. https://doi.org/10.1691/ph.2009.8789.
[27] M.A. Ali, M. Abul Farah, F.M. Al-Hemaid, F.M. Abou-Tarboush, *In vitro* cytotoxicity screening of wild plant extracts from Saudi Arabia on human breast adenocarcinoma cells, Genet. Mol. Res. 13 (2014) 3981–3990. https://doi.org/10.4238/2014.May.23.9.
[28] F. Algieri, A. Rodriguez-Nogales, T. Vezza, J. Garrido-Mesa, N. Garrido-Mesa, M.P. Utrilla, M.R. González-Tejero, M. Casares-Porcel, J. Molero-Mesa, M. Del Mar Contreras, A. Segura-Carretero, J. Pérez-Palacio, C. Diaz, N. Vergara, F. Vicente, M.E. Rodriguez-Cabezas, J. Galvez, Anti-inflammatory activity of hydroalcoholic extracts of *Lavandula dentata* L. and *Lavandula stoechas* L., J. Ethnopharmacol. 190 (2016) 142–158. https://doi.org/10.1016/j.jep.2016.05.063.
[29] M.R. Bashm, B. Rahmati, M. Poorgholam, The effect of *Lavandula dentata* aerial parts hydroalcoholic extract on learning and memory in male streptozotocin-induced diabetic rat, Daneshvar Med. 27 (2019) 1–8.
[30] Z.N. Almohawes, H.S. Alruhaimi, Effect of *Lavandula dentata* extract on ovalbumin-induced asthma in male Guinea pigs, Brazilian J. Biol. 80 (2020) 87–96. https://doi.org/10.1590/1519-6984.191485.
[31] B. Justus, C.C. Kanunfre, J.M. Budel, M.F. de Faria, V. Raman, J.P. de Paula, P.V. Farago, New insights into the mechanisms of French lavender essential oil on non-small-cell lung cancer cell growth, Ind. Crops Prod. 136 (2019) 28–36. https://doi.org/10.1016/j.indcrop.2019.04.051.
[32] C. Benbrahim, M.S. Barka, A. Basile, V. Maresca, G. Flamini, S. Sorbo, F. Carraturo, R. Notariale, M. Piscopo, A. Khadir, A. Zatout, S. Mechaala, Chemical composition and biological activities of oregano and lavender essential oils, Appl. Sci. 11 (2021) 5688. https://doi.org/10.3390/app11125688.
[33] Y. El Abdali, A. Agour, A. Allali, M. Bourhia, A. El Moussaoui, N. Eloutassi, A.M. Salamatullah, A. Alzahrani, L. Ouahmane, M.A.M. Aboul-Soud, J.P. Giesy, A. Bouia, *Lavandula dentata* L.: Phytochemical analysis, antioxidant, antifungal and insecticidal activities of its essential oil, Plants 11 (2022) 311. https://doi.org/10.3390/plants11030311.
[34] M.K. Ali, W.K. Ali, A.A. Kaki, B. Hadil, R. Aicha, N.K. Chaouche, *In vitro* characterization of the essential oil extracted from *Lavandula dentata* and its application in the field of biotherapy, Pakistan J. Biol. Sci. 26 (2023) 300–310. https://doi.org/10.3923/pjbs.2023.300.310.
[35] B. Cubukcu, D.H. Bray, D.C. Warhurst, A.H. Mericli, N. Ozhatay, G. Sariyar, *In vitro* antimalarial activity of crude extracts and compounds from *Artemisia abrotanum* L, Phyther. Res. 4 (1990) 203–204.
[36] S. Joghee, Nagamani, Immunomodulatory activity of ethanolic extract of *Artemisia abrotanum*, Int. J. Pharmacogn. Phytochem. Res. 7 (2015) 390–394.
[37] S.S. Almahdawy, A.M. Said, I.S. Abbas, A.H. Dawood, The evaluation of antimicrobial and cytotoxic activity of the essential oil extracted from the aerial parts of southernwood herb (*Artemisia abrotanum* l.) that recently

grown in Iraq, Asian J. Pharm. Clin. Res. 10 (2017) 384–387. https://doi.org/10.22159/ajpcr.2017.v10i10.21725.
[38] E.Y. Qnais, F.A. Abdulla, E.G. Kaddumi, S.S. Abdalla, Antidiarrheal activity of *Laurus nobilis* L. leaf extract in rats, J. Med. Food 15 (2012) 51–57. https://doi.org/10.1089/jmf.2011.1707.
[39] R.R. Mohammed, A.K. Omer, Z. Yener, A. Uyar, A.K. Ahmed, Biomedical effects of *Laurus nobilis* L. leaf extract on vital organs in streptozotocin-induced diabetic rats: Experimental research, Ann. Med. Surg. 61 (2021) 188–197. https://doi.org/10.1016/j.amsu.2020.11.051.
[40] A. Ferreira, C. Proença, M.L.M. Serralheiro, M.E.M. Araújo, The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal, J. Ethnopharmacol. 108 (2006) 31–37. https://doi.org/10.1016/j.jep.2006.04.010.
[41] E. Kupeli, I. Orhan, E. Yesilada, Evaluation of some plants used in Turkish folk medicine for their anti-inflammatory and antinociceptive activities, Pharm. Biol. 45 (2007) 547–555. https://doi.org/10.1080/13880200701498895.
[42] H. Rizwana, N. Al Kubaisi, N.N. Al-Meghailaith, N.M. Moubayed, G. Albasher, Evaluation of chemical composition, antibacterial, antifungal, and cytotoxic activity of *Laurus nobilis* L grown in Saudi Arabia, J. Pure Appl. Microbiol. 13 (2019) 2073–2085. https://doi.org/10.22207/JPAM.13.4.19.
[43] F.O. Çöven, S. Gür, E. Uyar, K.A.M.H. Alsakini, F. Karabey, F. Çöven, İ. Çaliş, A. Nalbantsoy, Assessment of *in-vitro* cytotoxicity and *in-ovo* virucidal antiviral efficacy of various plant extracts and bioactive molecules, Kafkas Univ. Vet. Fak. Derg. 30 (2024) 171–178. https://doi.org/10.9775/kvfd.2023.30306.
[44] M. Sayyah, J. Valizadeh, M. Kamalinejad, Anticonvulsant activity of the leaf essential oil of *Laurus nobilis* against pentylenetetrazole- and maximal electroshock-induced seizures, Phytomedicine 9 (2002) 212–216. https://doi.org/10.1078/0944-7113-00113.
[45] R. Yakoubi, S. Megateli, T. Hadj Sadok, C. Bensouici, E. Bağci, A synergistic interactions of Algerian essential oils of *Laurus nobilis* L., *Lavandula stoechas* L. and *Mentha pulegium* L. on anticholinesterase and antioxidant activities, Biocatal. Agric. Biotechnol. 31 (2021) 101891. https://doi.org/10.1016/j.bcab.2020.101891.
[46] E. Ovidi, V.L. Masci, M. Zambelli, A. Tiezzi, S. Vitalini, S. Garzoli, *Laurus nobilis*, *Salvia sclarea* and *Salvia officinalis* essential oils and hydrolates: Evaluation of liquid and vapor phase chemical composition and biological activities, Plants 10 (2021) 707. https://doi.org/10.3390/plants10040707.
[47] N. Jaradat, M. Abualhasan, M. Hawash, M. Qadi, N. Al-Maharik, S. Abdallah, A. Mousa, A. Zarour, M. Arar, S. Sobuh, F. Hussein, L. Issa, A. Jaber, H. Hamduni, S. Alshahatit, Chromatography analysis, in light of *vitro* antioxidant, antidiabetic, antiobesity, anti-inflammatory, antimicrobial, anticancer, and three-dimensional cancer spheroids' formation blocking activities of *Laurus nobilis* aromatic oil from Palest, Chem. Biol. Technol. Agric. 10 (2023) 25. https://doi.org/10.1186/s40538-023-00396-6.
[48] Z. Abbas, S. Rehman, An overview of cancer treatment modalities, in: H. Shahzad (Ed.), Neoplasm, IntechOpen, London, United Kingdom, 2018: p. Pp: 139-157. https://doi.org/10.5772/intechopen.76558.
[49] M. Tewari, Cancer and its treatment: an overview, Int. J. Adv. Res. 10 (2022) 950–956. https://doi.org/10.21474/ijar01/14476.
[50] S. Gezici, N. Şekeroğlu, Current perspectives in the application of medicinal plants against cancer: novel therapeutic agents, Anticancer. Agents Med. Chem. 19 (2019) 101–111. https://doi.org/10.2174/1871520619666181224121004.
[51] M. Mahmoud, E.M. Mohamed, A.M. Aboul-Enein, A.A. Diab, E.A. Shalaby, Anticancer and antioxidant activities of ethanolic extract and semi-purified fractions from guava and mango seeds, Biomass Convers. Biorefinery (2023). https://doi.org/10.1007/s13399-023-04216-7.
[52] A.M.A.-Z. Mona, A.A. Farghaly, E.M. Hassan, N.S. Abdel-Samie, Phenolic compounds of *Codiaeum variegatum* spirale lessened cytotoxic and genotoxic effects of mitomycin C in mice somatic and germ cells, Cytol. Genet. 53 (2019) 494–501. https://doi.org/10.3103/S0095452719060057.
[53] R.A. Hassan, E.M. Hassan, N.A. Ibrahim, N.M. Nazif, Triterpenes and cytotoxic activity of *Acokanthera oblongifolia* Hochst. growing in Egypt, Res. J. Pharm. Biol. Chem. Sci. 6 (2015) 1677–1686.
[54] M.S. Malik, M. Azam, M.A.R. Basra, Impact of natural antioxidants on biological systems, Lahore Garrison Univ. J. Life Sci. 4 (2020) 139–162. https://doi.org/10.54692/lgujls.2019.0402105.
[55] C.J. Mbah, I. Orabueze, N.H. Okorie, Antioxidants properties of aatural and synthetic chemical compounds: Therapeutic effects on biological system, Acta Sci. Pharm. Sci. 3 (2019) 28–42. https://doi.org/10.31080/asps.2019.03.0273.
[56] E. Guenther, The Essential Oils, D.Van Nostrand Company, Inc., New York. p.427, 1948.
[57] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Methods Enzymol. 299 (1999) 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.
[58] A. Meda, C.E. Lamien, M. Romito, J. Millogo, O.G. Nacoulma, Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity, Food Chem. 91

(2005) 571–577. https://doi.org/10.1016/j.foodchem.2004.10.006.
[59] A. Braca, N. De Tommasi, L. Di Bari, C. Pizza, M. Politi, I. Morelli, Antioxidant principles from *Bauhinia tarapotensis*, J. Nat. Prod. 64 (2001) 892–895. https://doi.org/10.1021/np0100845.
[60] K. Nara, T. Miyoshi, T. Honma, H. Koga, Antioxidative activity of bound-form phenolics in potato peel., Biosci. Biotechnol. Biochem. 70 (2006) 1489–1491. https://doi.org/10.1271/bbb.50552.
[61] A.R. Hamed, Investigation of multiple cytoprotective actions of some individual phytochemicals and plant extracts. Chapter 7: Development of cell-free and intracellular screening systems for testing antioxidant properties of plant extracts, (PhD); The University of Nottingham, United Kingdom. Pp: 162-163, 2009.
[62] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
[63] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays., J. Immunol. Methods 65 (1983) 55–63. https://doi.org/10.1016/0022-1759(83)90303-4.
[64] R.P. Adams, Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed., Allured Pub Corp, USA, 2007.
[65] W.S. Soliman, S. Salaheldin, H.M. Amer, Chemical composition evaluation of Egyptian lemongrass, *Cymbopogon citratus*, essential oil, Int. J. Sci. Eng. Res. 8 (2017) 630–634.
[66] M.O. Soares, A.F. Vinha, S.V.P. Barreira, F. Coutinho, S. Aires-Gonçalves, M.B.P.P. Oliveira, P.C. Pires, A. Castro, Evaluation of antioxidant and antimicrobial properties of the Angolan *Cymbopogon citratus* essential oil with a view to its utilization as food biopreservative, J. Agric. Sci. 5 (2013) 36–45. https://doi.org/10.5539/jas.v5n7p36.
[67] I. Dammak, Z. Hamdi, S.K. El-Euch, H. Zemni, A. Mliki, M. Hassouna, S. Lasram, Evaluation of antifungal and anti-ochratoxigenic activities of *Salvia officinalis*, *Lavandula dentata* and *Laurus nobilis* essential oils and a major monoterpene constituent 1,8-cineole against Aspergillus carbonarius, Ind. Crops Prod. 128 (2019) 85–93. https://doi.org/10.1016/j.indcrop.2018.11.006.
[68] C.M. de Oliveira Xavier, E.H.A. Silva, I.V.M. de Siqueira, L.O. de Macedo, V.B. Bernardo, H.F. Goulart, A.E.G. Santana, R.A.N. Ramos, P.G.V. Aquino, G.A. de Carvalho, Chemical composition and acaricidal activity of essential oil of *Lavandula dentata* L. on engorged females of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae), Acta Parasitol. 69 (2024) 1141–1147. https://doi.org/10.1007/s11686-024-00835-w.
[69] P.G. Silva-Flores, L.A. Pérez-López, V.M. Rivas-Galindo, D. Paniagua-Vega, S.A. Galindo-Rodríguez, R. Álvarez-Román, Simultaneous GC-FID quantification of main components of *Rosmarinus officinalis* L. and *Lavandula dentata* essential oils in polymeric nanocapsules for antioxidant application, J. Anal. Methods Chem. 2019 (2019) 2837406. https://doi.org/10.1155/2019/2837406.
[70] C. Giuliani, M. Bottoni, R. Ascrizzi, F. Milani, A. Papini, G. Flamini, G. Fico, *Lavandula dentata* from Italy: analysis of trichomes and volatiles, Chem. Biodivers. 17 (2020) e2000532. https://doi.org/10.1002/cbdv.202000532.
[71] H. Belcadi, A. Aknouch, S. El Amrani, A. Chraka, J. Kassout, M. Lachkar, M. Mouhib, A. Ibn Mansour, Moroccan *Lavandula dentata* L. essential oil: γ-irradiation effect on the chemical composition and antibacterial activity, Sci. African 23 (2024) e02087. https://doi.org/10.1016/j.sciaf.2024.e02087.
[72] S.A. El-Sawi, M.E. Ibrahim, A.M. Ali, *In vitro* cytotoxic, antioxidant and antimicrobial activities of essential oil of leaves of *Laurus nobilis* L. grown in Egypt and its chemical composition, Med. Aromat. Plant Sci. Biotechnol. 3 (2009) 16–23.
[73] N. Nagah, I. Mostafa, G. Dora, Z. El-Sayed, A.-M. Ateya, Essential oil composition, cytotoxicity against hepatocellular carcinoma, and macro and micro-morphological fingerprint of *Laurus nobilis* cultivated in Egypt, Egypt. J. Bot. 61 (2021) 521–540. https://doi.org/10.21608/ejbo.2021.56342.1603.
[74] C. Ramos, B. Teixeira, I. Batista, O. Matos, C. Serrano, N.R. Neng, J.M.F. Nogueira, M.L. Nunes, A. Marques, Antioxidant and antibacterial activity of essential oil and extracts of bay laurel *Laurus nobilis* Linnaeus (Lauraceae) from Portugal, Nat. Prod. Res. 26 (2012) 518–529. https://doi.org/10.1080/14786419.2010.531478.
[75] A. Ailli, N. Handaq, H. Touijer, A.A. Gourich, A. Drioiche, K. Zibouh, B. Eddamsyry, F. El Makhoukhi, A. Mouradi, Y.A. Bin Jardan, M. Bourhia, A. Elomri, T. Zair, Phytochemistry and biological activities of essential oils from six aromatic medicinal plants with cosmetic properties, Antibiotics 12 (2023) 721. https://doi.org/10.3390/antibiotics12040721.
[76] G. Kendir, G. Özek, A. Köroğlu, T. Özek, The quality evaluation of the laurel leaf samples sold in the market under the name of "Defne Yaprağı," Protoplasma 261 (2024) 303–316. https://doi.org/10.1007/s00709-023-01897-9.
[77] K.A. Khalid, A.E. El-Gohary, Productivity of wormwood (*Artemisia abrotanum*) enhanced by trace elements, Bull. Natl. Res. Cent. 44 (2020) 120. https://doi.org/10.1186/s42269-020-00381-1.
[78] G. V. Khodakov, I. V. Kotikov, V.N. Pankovetskii, Component composition of essential oil from *Artemisia abrotanum* and *A. dracunculus*, Chem. Nat. Compd. 45 (2009) 905–908.

[79] N.M. Abdel-Gwad, E.A.-M. Mahmoud, S.A. Al-Askalany, E.A. Hanafy, Antioxidant, antibacterial and cytotoxic effect of *Cymbopogon citratus*, *Mentha longifolia*, and *Artemisia absinthium* essential oils, Egypt. J. Chem. 65 (2022) 287–296. https://doi.org/10.21608/EJCHEM.2021.86171.4174.
[80] D.D. de O. Alencar, E.L. de Souza, E.T. da C. Almeida, A.L. da Silva, H.M.L. Oliveira, M.T. Cavalcanti, Microencapsulation of *Cymbopogon citratus* D.C. stapf essential oil with spray drying: development, characterization, and antioxidant and antibacterial activities, Foods 11 (2022) 1111. https://doi.org/10.3390/foods11081111.
[81] E.S. Yilmaz, M. Timur, B. Aslim, Antimicrobial, antioxidant activity of the essential oil of bay laurel from Hatay, Turkey, J. Essent. Oil-Bearing Plants 16 (2013) 108–116. https://doi.org/10.1080/0972060X.2013.764158.
[82] J. Marinković, B. Nikolić, T. Marković, M. Radunović, J. Ilić, M. Bošković, A. Ćirić, D. Marković, *Cymbopogon citratus* essential oil: An active principle of nanoemulsion against *Enterococcus faecalis* root canal biofilm, Future Microbiol. 16 (2021) 907–918. https://doi.org/10.2217/fmb-2021-0081.
[83] M.A. Smach, J. Hafsa, J. Ben abdallah, B. Charfeddine, K. Limem, Neuroprotective and anti-amnesic effects of *Laurus nobilis* essential oil against scopolamine-induced memory deficits in mice brain, J. Ethnopharmacol. 319 (2024) 117151. https://doi.org/10.1016/j.jep.2023.117151.
[84] I. Mssillou, A. Agour, A. El Ghouizi, N. Hamamouch, B. Lyoussi, E. Derwich, Chemical composition, antioxidantactivity, and antifungal effects of essential oil from *Laurus nobilis* L. flowers growing in Morocco, J. Food Qual. 2020 (2020) 8819311. https://doi.org/10.1155/2020/8819311.
[85] D. Imen, H.-H. Soumaya, C. Imed, M.B.J. Jouda, L. Ahmed, C. Rym, Essential oil from flowering tops of *Lavandula dentata* (L): Chemical composition, aantimicrobial, antioxidant and insecticidal activities, J. Essent. Oil-Bearing Plants 24 (2021) 632–647. https://doi.org/10.1080/0972060X.2021.1944326.
[86] B. Imelouane, A. Elbachiri, J.-P. Wathelet, J. Dubois, H. Amhamdi, Chemical composition, cytotoxic and antioxidant activity of the essential oil *Lavandula dentata*, World J. Chem. 5 (2010) 103–110.
[87] F.S. Sharopov, A. Salimov, S. Numonov, A. Safomuddin, M. Bakri, T. Salimov, W.N. Setzer, M. Habasi, Chemical composition, antioxidant, and antimicrobial activities of the essential oils from *Artemisia annua* L. growing wild in Tajikistan, Nat. Prod. Commun. 15 (2020) 1–7. https://doi.org/10.1177/1934578X20927814.
[88] K. Cheraif, B. Bakchiche, A. Gherib, S.K. Bardaweel, M.Ç. Ayvaz, G. Flamini, R. Ascrizzi, M.A. Ghareeb, Chemical composition, antioxidant, anti-tyrosinase, anti-cholinesterase and cytotoxic activities of essential oils of six Algerian plants, Molecules 25 (2020) 1710. https://doi.org/10.3390/molecules25071710.
[89] K. Chebbac, Z. Benziane Ouaritini, A. El Moussaoui, M. Chalkha, S. Lafraxo, Y.A. Bin Jardan, H.A. Nafidi, M. Bourhia, R. Guemmouh, Antimicrobial and antioxidant properties of chemically analyzed essential oil of *Artemisia annua* L. (Asteraceae) native to Mediterranean area, Life 13 (2023) 807. https://doi.org/10.3390/life13030807.
[90] N. Jaradat, Phytochemical profile and *In vitro* antioxidant, antimicrobial, vital physiological enzymes inhibitory and cytotoxic effects of *Artemisia jordanica* leaves essential oil from Palestine, Molecules 26 (2021) 2831. https://doi.org/10.3390/molecules26092831.
[91] B. Najar, J.E. Shortrede, L. Pistelli, J. Buhagiar, Chemical composition and *in vitro* cytotoxic screening of sixteen commercial essential oils on five cancer cell lines, Chem. Biodivers. 17 (2020) e1900478. https://doi.org/10.1002/cbdv.201900478.
[92] G.A. Abdulhasan, Lemongrass essential oil shows an antiproliferative effect on lung cancer cells and its role in expression of P53 gene, Iraqi J. Agric. Sci. 52 (2021) 359–366. https://doi.org/10.36103/ijas.v52i2.1297.
[93] D.T. Trang, T.K. Van Hoang, T.T.M. Nguyen, P. Van Cuong, N.H. Dang, H.D. Dang, T.N. Quang, N.T. Dat, Essential oils of lemongrass (*Cymbopogon citratus* Stapf) induces apoptosis and cell cycle arrest in A549 lung cancer cells, Biomed Res. Int. 2020 (2020) 5924856. https://doi.org/10.1155/2020/5924856.
[94] A. Russo, M. Bruno, R. Avola, V. Cardile, D. Rigano, Chamazulene-rich *Artemisia arborescens* essential oils affect the cell growth of human melanoma cells, Plants 9 (2020) 100. https://doi.org/10.3390/plants9081000.
[95] A. Russo, V. Cardile, A.C.E. Graziano, R. Avola, M. Bruno, D. Rigano, Involvement of Bax and Bcl-2 in induction of apoptosis by essential oils of three Lebanese *Salvia* species in human prostate cancer cells, Int. J. Mol. Sci. 19 (2018) 292. https://doi.org/10.3390/ijms19010292.
[96] W. Feng-Xiang, L. Jun-Xing, W. Lin, L. Hong-Zhi, L. Jia-Bin, Expression of Bcl-2 and Bax genes in the liver cancer cell line HepG2 after apoptosis induced by essential oils from *Rosmarinus officinalis*, J. Chinese Med. Mater. 31 (2008) 877–879.
[97] I. El-Garawani, S.H. El Nabi, E. Nafie, S. Almeldin, *Foeniculum vulgare* and *Pelargonium graveolens* essential oil mixture triggers the cell cycle arrest and apoptosis in MCF-7 cells, Anti-Cancer Agents Med. Chem. (Formerly Curr. Med. Chem. - Anti-Cancer Agents) 19 (2019) 1103–1113. https://doi.org/10.2174/1573399815666190326115116.