CHEMICAL COMPOSITION OF *Elsholtzia ciliata* (Thunb.) Hyland ESSENTIAL OIL IN VIETNAM WITH MULTIPLE BIOLOGICAL UTILITIES: A SURVEY ON ANTIOXIDANT, ANTIMICROBIAL, ANTICANCER ACTIVITIES

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ABSTRACT

Elsholtzia ciliata (Thunb.) Hyland is commonly known as Vietnamese balm - a spicy, lemonscented culinary herb in Asian cuisine, especially in Vietnam. The biological activities of *E. ciliata* essential oils (Ec EOs) in Vietnam have yet to be thoroughly studied and have received less attention than other species of genus *Elsholtzia* in the world. In this study, we evaluated the antioxidant, antimicrobial, and anticancer of Ec EOs and examined their chemical compositions. Fresh leaves of *E. ciliata* were hydro-distilled to yield essential oil of 0.82% dry weight, respectively. Gas chromatography-mass spectrometry (GC-MS) analyses revealed that Ec EOs principally possessed complex mixtures of monoterpenes and sesquiterpenes. (Z)-β-Farnesene (22.72%), neral (15.66%), geranial (15.62%), and β-ocimene (13.30%) were the major components of Ec EOs. In the antioxidant assay, the radical scavenging capacities of Ec EOs against DPPH were 26.55 g/L (IC₅₀). In the antimicrobial assay, the evaluation of antimicrobial activity using the agar wells diffusion method showed that Ec EOs in all concentrations was active against the Gram-positive bacteria (*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus*), Gram-negative bacteria (*Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae*). In the anticancer assay, EcE Os can be toxic to Hep G2 cells with IC₅₀ reaching 0.00204%.

Keywords: Anticancer, antimicrobial, antioxidant, Elsholtzia ciliata, essential oil.

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INTRODUCTION

Elsholtzia (Lamiaceae family) has comprised over 30 species in Asia, Africa, North America, and European countries for centuries (Guo et al., 2012). Elsholtzia plants are mostly aromatic due to their abundant volatile oils. These plants have been used in traditional medicine to treat ailments such as rheumatic pain, nephritis, night blindness, pharyngitis, fever, diarrhea, and digestive disorders (Wu & Li, 1977). The biological actions of volatile components are of interest to many phytochemistry and pharmacology researchers (Thanaseelungkoon et al., 2018; Sripahco et al., 2022). According to Wang et al. (2022), modern pharmacological studies show that the genus Elsholtzia has multiple biological utilities such as antioxidant, antiinflammatory. antimicrobial. insecticidal. antiviral, hypolipidemic, hypoglycemic, analgesic, antiarrhythmic, antitumor, antiacetylcholinesterase, and immunoregulator activities. Using the hydro-distillation and GC-MS method, a total of 572 volatile compounds were identified from 21 species of Elsholtzia. them. α -pinene. Among β -pinene, acetophenone, caryophyllene oxide, carvacrol, benzaldehyde,... widely exist (Guo et al., 2012). Compared to other species of the Elsholtzia genus around the world, Ec EOs in Vietnam have received less attention and have not been well documented yet.

Elsholtzia ciliata, also known as Vietnamese balm, is a widely used herb in Asian cuisine. It is an annual plant that grows to a height of 40 cm to 60 cm, with hairy, green, square stems and ovate to ellipticlanceolate leaves. The species is known for its purple flowers that bloom from July to October and its wide use in traditional medicine to treat various illnesses (Nguyen et al., 2021). The essential oil's composition was disclosed by phytochemical studies (Pingzhao et al., 2016; Wang et al., 2017; Pudziuvelyte et al., 2018) along with the existence of flavonoids (Nugroho et al., 2017; Pudziuvelyte et al., 2018), phenolic acids, steroids (Pudziuvelyte et al., 2018), and triterpenes (Liu et al., 2007), the most prevalent non-volatile component class being phenolics (Pudziuvelyte et al., 2018). The essential oil has been found to have antiacetylcholinesterase activity, anti-oxidative properties, antibacterial activity, vasorelaxant activity, anti-leishmanial activity, cytotoxicity, anti-inflammatory activity (Pudziuvelyte et al., 2017), and insecticidal activities (Pingzhao et al., 2016), primarily refers to total extracts or the essential oil (Liu et al., 2012; Ma et al., 2018; Pudziuvelyte et al., 2020). Due to a warm disposition and a spicy flavor, E. ciliata has the effect of causing diuresis to reduce edema and induce diaphoresis to relieve superficies. It also removes moisture to regulate the stomach (Nguyen et al., 2021).

This study was conducted for the purpose of clarifying the chemical composition and biological activities of *E. ciliata* in Vietnam, creating a scientific basis for the use of this medicinal plant resource, and at the same time, creating a premise for further research in creating products with high biological activity for the protection and care of public health.

MATERIALS AND METHODS

Plant material

Leaves of *E. ciliata* were collected in the morning of July (temperature range, 25–28 °C) from fresh plant growth in Long An province (10°36'21.4"N-106°36'49.4"E). The samples were air-dried for 5 days and weighed for extracting the essential oils.

Isolation of essential oils

Essential oils were extracted from the airdried plants (300 g each) using steam distillation for three hours in a Clevenger-type apparatus as recommended by European Pharmacopoeia (ver. 8.2, Monograph 2.8.12). The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4 °C in the dark until further analysis. Measurements were carried out in three replications. The measurement was conducted in triplicate. Essential oil yields (%) were determined using the equation as follows: Essential oil (%) = Oil weight (g)/100 g of dried arieal parts (% w/w) (Zhang et al., 2015).

GC-MS analysis

The GC-MS analysis was run on an Agilent 6890 N gas chromatograph connected to an Agilent 5973 N mass selective detector. They were equipped with а gas chromatography-flame ionization detector (GC-FID) and a HP-5MS (30 cm \times 0.25 mm $\times 0.25 \,\mu$ m) capillary column. The essential oil sample was diluted in n-hexane to obtain a 1% solution. The injector temperature was maintained at 250 °C with the volume injected being 1.0 µL. The flow rate of carrier gas (helium) was 1.0 mL/min, with the mass spectra scanned from 50 m/z to 550 m/z (Liang et al., 2020).

The response intensity (RI)were determined from gas chromatograms using a series of n-alkanes (C_5 – C_{36}) under the same operating conditions. Based on RI, the chemical constituents were identified by comparing them with n-alkanes as a reference. The components of the essential oil were identified by matching their mass spectra with various computer libraries (Wiley 275 libraries, NIST 05, and RI from other literature) (Adam, 2005). The measurement was conducted in triplicate.

Antioxidant activities

The DPPH radical-scavenging capacity was quantified according to Boulanouar et al. (2013): Ec EOs (1.0 mL) was added to 2 mL of a 60 µM DPPH methanol solution, the reaction mixture was kept at room temperature and away from light for 30 min before the absorbance was recorded at 517 nm. The scavenging activity was estimated by scavenging effect (%) = [100*(Ac - As/Ac)], (Ac: control sample absorbance; As test sample absorbance). The measurement was conducted in triplicate. The concentration of oil at which 50% of the DPPH radicals (IC₅₀) were scavenged was calculated. Vitamin C was employed as a reference compound.

Antimicrobial activities

The antibacterial activity of Ec EOs was quantified according to (Nguyen & Hoang,

2022) with some modifications: The agar diffusion method was used in three Gram positive bacteria (Bacillus cereus, Bacillus subtilis, Staphylococcus aureus) and three Gram negative bacteria (Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae). All the microbial inoculums were provided by the Biotechnology Center of Ho Chi Minh City and prepared per protocol as described by (Abers et al., 2021). Bacteria were initially grown on Tryptic Soy Agar (TSA) slants at 37 °C for 24 hours, then stored at 4 °C and transferred to a fresh TSA slant on a monthly basis. Approximately 10⁶–10⁸ CFU/mL of the microorganisms were inoculated by the spread plate method in Nutrient agar (NA) (tryptone 5 g/L; meat extract 1 g/L; yeast extract 2 g/L; sodium chloride 5 g/L; agar 12 g/L). Wells of 6 mm diameter were created in the center of the disk, and 40 µL of essential oil solution, dimethyl sulphoxide (DMSO), and chloramphenicol (30 µg) were pipetted into the middle of each disk. The concentration of essential was diluted Ec EOs was diluted into five levels (12.5, 25, 50, 75, and 100%) by using sterile DMSO. Incubating at 37 °C for 24 hours, all the plates were observed for zones of growth inhibition, and the diameters in millimeters of these zones were measured. The measurement was conducted in triplicate. Ciprofloxacin was used in parallel experiments as a positive control.

Anticancer activities

MTT assay in two cell lines (mesenchymal stem cells (UC-MSCs), Hep G2) were provided by Stem Cell Institute-Unversity of Science and performed as previously reported by Navarra et al. (2015): Those cells were seeded onto 96-well plates at a density of 5×10^3 cells/well. For the cell count assay, the cytofluorimetric analyses, and the comet assay, the cells were seeded onto six-well plates (1×10^5 cells/well). The next day, the media were replaced with fresh medium (untreated cultures) or with medium containing increased concentrations of Ec EOs ranging from 0.01–0.03% (0.1 - $0.3 \ \mu L/mL$), and the cells were incubated for 24, 48, and 72 hours (proliferation assays). Ec EOs was diluted 1:1 in a 1:9 water/DMSO solution and then further diluted in culture media to obtain the final concentrations. The same DMSO concentrations used to dissolve Ec EOs to the final concentration of 0.01, 0.02, and 0.03% served as vehicle controls (0.009, 0.018, and 0.027% DMSO in culture media, respectively). Paclitaxel (Taxol) diluted in DMSO was used as a positive control. The OD was measured at 570 nm using a fluorescence multi-detection reader (Thermo Fisher Scientific, USA). Untreated cells were utilized as a control. GraphPad Prism 9 software was used to determine the IC₅₀ values based on nonlinear regression. The measurement was conducted in triplicate. Cell viability (%) = $OD_{test}/OD_{control} \times 100$ (Ganot et al., 2013).

Data analysis

The data of biological activities were analyzed by SPSS 26.0, GraphPad Prism 9. All data were presented as means \pm standard deviation (SD) values. The statistical analysis was conducted in the one-way ANOVA using SPSS 26.0. The significant difference among treatments, controls, and standards were determined by using one-way ANOVA with the confidence level of 95% (p < 0.05).

RESULTS AND DISCUSSION

Essential oil yields

EOs yields of *E. ciliata* was presented in Table 1. The yields of *E. ciliata* EOs was 0.82% (w/w). The EOs yields of *E. ciliata* grown in Vietnam in this study was higher than the study of Pingzhao et al. (2016) (0.03%).

Table 1. Yields of essential oils extracted from Esholtzia ciliata leaves

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Samples	Fresh weight (g)	EOs (g)	Yields (%)						
Elsholtzia ciliata	300.00 ± 0.00	2.45 ± 0.23	0.82 ± 0.08						

Note: Data were presented in means ± standard deviations (SD).

Chemical composition of *Esholtzia ciliata* essential oils

The chemical components of EOs from *E. ciliata* by GC-MS was indicated in Table 2. Ec EOs contained a complex mixture consisting mainly of monoterpenes and sesquiterpenes. The essential oil contained higher amounts of monoterpenoids (59.05%) than sesquiternoids (37.09%; Table 2). The major compounds of *E. ciliata* EOs were (Z)- β -Farnesene (22.72%), followed by neral (15.66%), geranial (15.62%) and β -ocimene (13.30%).

This set of components is quite similar to those found by Nguyen et al. (2011) found the main constituents were neral (15.2–20.5%), geranial (19.5–26.5%), limonene (10.9–14.2%), and (Z)- β -farnesene (10.8–11.7%). Similarly, Dang et al. (2017) reported that Ec Eos in Hue contained a total of 34 compounds, with 26 of them identified, accounting for 97.5% of the oil's chemical composition. The predominant components were found to be geranial (28.4%), (Z)- β -ocimene (23.0%), and neral (21.7%). It differed from the essential oil of E. ciliata aerial parts measured in the previous reports. For example, Kim & Jung (2003) found that (62.7%), dihydrotagetone b-caryophellene (5.0%), and germacrene D (4.0%) were the main components. Rosefuran (84.8%) 1,8-cineole (4.6%), dehydroelsholtzia ketone (65.2%), elsholtzia ketone (7.6%), p-cymene (4.3%), and camphor (3.6%) were the main components of the essential oil of E. ciliata that was gathered from the La Chung Valley in the Himalayan region of India (Thappa et al., 2011). In comparison with Ec EOs collected from China, Tian (2013) determined that linalool (12.1%), caryophyllene eugenol (11.0%), (9.7%),caryophyllene oxide (9.6%), 1-octen-3-ol (8.9%), verbenone (6.9%), and spathulenol (7.2%) were main compounds of the essential oil of E. ciliata leaves collected from China. In addition, Coltun et al. (2021) identified 17 compounds in E. ciliata essential oil growth in

the Republic of Moldova. The main ones are (Z)-cicerone (50.8%) and rosefuran epoxide (20.6%). Other important compounds are eucalyptol (6.3%), β -caryophyllene (6.2%), and acetophenone (2.2%). In contrast, we detected (Z)- β -Farnesene (22.72%), neral (15.66%), geranial (15.62%) and β -ocimene (13.30%) was the major components of Ec EOs. Environmental (climatic, regional, or seasonal)

and genetic variations, among other things, may have contributed to changes in the chemical composition of EOs. Besides that, the large climate difference areas may be one of the reasons for the differences in essential oil composition. Moreover, the difference in harvesting time and growth years may also cause differences in essential oil components (Liang et al., 2020).

Table 2. Identification of chemical components of Esholtzia ciliata essential oils by GC-MS

No.	Compounds	RI	Chemical formula	MW (g/mol)	Chemical class	Area (%)
1	1-Octen-3-ol		C ₈ H ₁₆ O	128.21	Monoterpenes	4.15 ± 0.04
2	2 6-Methyl-5-hepten-2-one		C ₈ H ₁₄ O	126.20	Monoterpenes	0.96 ± 0.04
3	β-Myrcene	991	$C_{10}H_{16}$	136.23	Monoterpenes	0.22 ± 0.01
4	3-Octanol	994	C ₈ H ₁₈ O	130.23	Monoterpenes	0.26 ± 0.01
5	D-Limonene	1034	$C_{10}H_{16}$	136.23	Monoterpenes	1.68 ± 0.46
6	β-ocimene	1049	C10H16	136.23	Monoterpenes	13.30 ± 0.32
7	Acetophenone	1071	C ₈ H ₈ O	120.15	Monoterpenes	1.88 ± 0.23
8	Rosefuran	1095	$C_{10}H_{14}O$	150.22	Monoterpenes	0.29 ± 0.01
9	Linalool	1100	C ₁₀ H ₁₈ O	154.25	Monoterpenes	0.26 ± 0.04
10	(Z)-Geranic acid. methyl ester	1170	$C_{11}H_{18}O_2$	182.26	Monoterpenes	0.17 ± 0.03
11	(E)-Geranic acid. methyl ester	1175	$C_{11}H_{18}O_2$	182.26	Monoterpenes	0.32 ± 0.01
12	Isogeranial	1184	$C_{10}H_{16}O$	152.23	Monoterpenes	0.88 ± 0.06
13	Nerol	1231	$C_{10}H_{18}O$	154.25	Monoterpenes	1.37 ± 0.25
14	Neral	1246	C ₁₀ H ₁₆ O	152.23	Monoterpenes	15.66 ± 0.56
15	Geraniol	1256	$C_{10}H_{18}O$	154.25	Monoterpenes	0.67 ± 0.16
16	Geranial	1275	C ₁₀ H ₁₆ O	152.23	Monoterpenes	15.62 ± 0.60
17	(Z)-Geranyl acetate	1384	$C_{12}H_{20}O_2$	196.29	Monoterpenes	1.36 ± 0.04
18	(E)-α-Bergamotene	1420	$C_{15}H_{24}$	204.35	Sesquiterpene	0.25 ± 0.02
19	Caryophyllene	1456	$C_{15}H_{24}$	204.35	Sesquiterpene	$\textbf{9.57} \pm \textbf{0.02}$
20	(Z)-β-Farnesene	1462	$C_{15}H_{24}$	204.35	Sesquiterpene	22.72 ± 1.30
21	Humulene	1472	$C_{15}H_{24}$	204.35	Sesquiterpene	1.25 ± 0.08
22	Germacrene D	1498	$C_{15}H_{24}$	204.35	Sesquiterpene	1.96 ± 0.17
23	δ-Cadinene	1525	$C_{15}H_{24}$	204.35	Sesquiterpene	0.13 ± 0.01
24	Nerolidol	1569	$C_{15}H_{26}O$	222.37	Sesquiterpene	0.73 ± 0.04
25	Caryophyllene oxide	1605	$C_{15}H_{24}O$	220.35	Sesquiterpene	0.28 ± 0.02
26	α-Cadinol	1654	$C_{15}H_{26}O$	222.37	Sesquiterpene	0.22 ± 0.02
Total						

Notes: MW: Molecular weight. Data were presented in means ± standard deviations (SD).



Figure 1. GC/MS chromatogram of Ec EOs

Antioxidant activities

In this study, Ec EOs exhibited considerable antioxidant activity, based on the DPPH method, compared with that of standard antioxidant vitamin C. Ec EOs had an IC₅₀ value of 26.55 g/L for DPPH radical scavenging activity, while the IC₅₀ value of vitamin C was 27.23 mg/L (Fig. 2).

The antioxidant activity of Ec EOs could be attributed to the effect of its essential compounds, geranial, and neral (two isomers of citral). Geranial and neral have been found in some essential oils to show substantial antioxidant activity (Lim et al., 2022). Furthermore, minor compounds (monoterpene hydrocarbons and sesquiterpene hydrocarbons) in Ec EOs, can enhance antioxidant activity. These chemical compounds of EO might act individually or synergistically as antioxidants. Barbieri et al. (2017) indicated that enhanced antioxidant activity was found in other EOs, which were rich in oxygenated monoterpenes.



Figure 2. The free radical-scavenging activity of *Esholtzia ciliata* essential oil and vitamin C. Scavenging effect (%) and linear regression of Ec EOs (C) and vitamin C (D) in the antioxidant test

Antimicrobial activities

So far, few studies have addressed the antimicrobial activity of Ec EOs with microbial strains. In this investigation, the *in vitro* antimicrobial activity of Ec EOs against selected microorganisms was tested. Antimicrobial activities of Ec EOs in three Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and three Gram-negative bacteria (*Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*) presented in Figure 3.

As shown in Figure. 3, each concentration of EOs displayed antibacterial effects to

different degrees against *B. cereus*, *B. subtilis*, *S. aureus*, *P. mirabilis*, *E. coli*, and *K. pneumoniae*. EcEOs inhibited *B. cereus*, *B. subtilis*,

S. aureus, P. mirabilis, E. coli, K. pneumoniae, with a maximum inhibition zone ranging from 12.83 ± 0.76 mm to 21.93 ± 0.40 , respectively (Fig. 3). In contrast, Ec EOs at a concentration of 12.5% in DMSO inhibited all microorganisms tested with the lowest inhibition zone ranged from 3.50 ± 0.50 mm to 8.53 ± 0.55 mm, respectively (Fig. 3). The inhibition zone of the Ec EOs in all concentration was lower than that of ciprofloxacin and had significant differences. Besides, the antimicrobial activity decreased following the decrease in the concentration of Ec EOs (Fig. 3).



Figure 3. Inhibition zone diameters (mm) of *Esholtzia ciliata* essential oil concentrations (% of essential oil in DMSO) in three Gram positive bacteria (A) and three Gram negative bacteria (B). Antimicrobial activities of Ec EOs (75%, 100%) against *Escherichia coli* (C); (-) DMSO; (+) Chloramphenicol (30 µg) (C)

The activity of EOs depended on their chemical compounds (Bellik et al., 2019). The analysis of compounds in Ec EOs showed that all found components have antibacterial properties. Thus, the anti-microorganism activity of the essential oil of *E. ciliata* is related to its major components. Several studies indicated (Z)- β -Farnesene, neral, geranial, and β -ocimene have significant antimicrobial activity (Bui et al., 2022;

Dangol et al., 2023). In addition, 1-Octen-3ol, humulene, acetophenone, and germacrene D are well-known chemicals exhibiting solid antimicrobial activity (Nafis et al., 2019; Badalamenti et al., 2022; Cai et al., 2022; Mukhammadjon et al., 2022) and these compounds also appeared with a relatively high ratio in the essential oil of *E. ciliata*. Nazzaro et al. (2017) reported that the antibacterial mechanisms of action of EOs has been postulated that the hydrophobic constituents either disrupt cytoplasmic membranes via a cascade of different reactions leading to cytoplasmic leakage, cell lysis, and ultimate death, or via the inhibition of sporulation.

Anticancer activities

An MTT assay was performed to measure cytotoxicity against Hep G2 and UC-MSC cells. Ec EOs inhibited the proliferation of Hep G2 and UC-MSC cells. The results of the MTT assay are shown in Figure 4, *E. ciliata* produces time (24 hours) and dose (0.001953 to 0.5%) dependent inhibition of cell proliferation towards Hep G2 and UC-MSCs. At 24 hou

rs, the IC_{50} value of Ec EOs was 0.007799% for UC-MSC and 0.00204% for Hep G2 cell lines.



Ec EOs concentations (%)

Figure 4. Cell viability of HepG2 and UC-MSCs cells when treat with increasing concentration of Ec EOs (0.001 to 0.5%) and Paclitaxel for 24 hours

MTT is water-soluble that is taken up by the viable cell. The reduction product of MTT is a water-insoluble blue formazan, which must be dissolved for calorimetric measurement (Ahamad et al., 2020). The untreated Hep G2 and UC-MSC cells in control experiments maintained their original morphology after 24 hours. In contrast, Hep G2 and UC-MSC cells lost their original shape at 24 hours of Ec EOs treatment. The presence of sesquiterpenes has mainly explained the activity of essential oil on anticancer (Pudziuvelyte et al., 2017). Previous studies have shown that (Z)- β -farnesene has a good correlation between compound contents and anticancer activity against MCF-7 cells. Afoulous et al. (2013) investigated the cytotoxicity of MCF-7 cells

and found that (Z)- β -farmesene had an R² value of 0.73, indicating a strong correlation. Tan et al. (2018) also found that (Z)- β farnesene acted synergistically with tamoxifen to kill MCF-7 cells. Zeng et al. (2015) recently demonstrated that niral and geranial can induce caspase 3 activity, activate p53, and decrease Bcl-2 expression, resulting in apoptosis in two endometrial cancer cell lines (ECC-1 and Ishikawa) at an IC₅₀ of 15-25 μ M. Sousa et al. (2023) found that β -ocimene alters plasma membrane permeability, interacts with internal cell targets, disrupts important metabolic pathways, and decreases mitochondrial membrane potential to promote both cell necrosis and apoptosis.

(2014)found Kim et al. that α -Caryophyllene and caryophyllene, which are found in the essential oil of E. ciliata as the major constituents, also could contribute to the anticancer activity against tested cell lines as there is some data published about its in vitro and in vivo anticancer effect. In another experiment, Choi et al. (2015) reported that the rat pheochromocytoma (PC12) cell lines were exposed to 10, 25, and 50 g/mL of the essential oil from E. ciliata without any actual harm. Through the NF-B and p38 MAPK pathways, it was discovered that the aqueous extract of E. ciliata may block the production of cytokines (Kim et al., 2011). p38 MAPK is involved in the control of cell survival and death, is necessary for cell invasion and migration, and its levels are often linked to poor prognoses in cancer patients (Koul et al., 2013).

CONCLUSION

Ec EOs are volatile oils containing rich bioactive sources and nutritional values. Fresh leaves of E. ciliata were hydro-distilled to yield essential oil of 0.82% dry weight, respectively. This study found that these EOs possessed antioxidant, antimicrobial, and anticancer activities. GC-MS analyses revealed that Ec EOs had a complex mixture of compounds that consisted of monoterpenes and sesquiterpenes. (Z)- β -Farnesene (22.72%), followed by neral (15.66%), geranial (15.62%), and β -ocimene (13.30%)

were accounted as the major components of Ec EOs. In the antioxidant assay, the radical scavenging capacities of Ec EOs against DPPH were 26.55 g/L (IC₅₀). In the antimicrobial assay, Ec EOs in all concentrations was active against the Grampositive bacteria (B. cereus, B. subtilis, aureus), Gram-negative bacteria (P. S. mirabilis, E. coli, K. pneumoniae). In the anticancer assay, Ec EOs can be toxic to UC-MSC cells with IC_{50} reaching 0.007799%. However, the cytotoxicity of Ec EOs on Hep G2 was higher than that of UC-MSCs (IC_{50} reached 0.00204%). The findings of this study suggested that Ec EOs are a promising source to treat oxidative stress and antimicrobial as well as a natural medicine against cancer.

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