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Essential oil composition and biological activities of *Hyssopus officinalis* and *Perilla frutescens*

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Abstract---This article reports on the main constituents of *Hyssopus officinalis* and *Perilla frutescens* essential oils (EOs) and their activity as antioxidant, antimicrobial, anti inflammatory and anticancer. The major component of *H. officinalis* essential oil was isopinocamphone followed by pinocamphone, *β*-pinene, *β*-phellandrene, *α*-thujene and bicyclo-germacrene. The main constituent of the essential oil of *P. frutescens* was identified as L-perillaldehyde followed by trans-caryophyllene and D-limonene. Both essential oils (hyssop and perilla) showed antioxidant activities with the used three methods and the increase of activity was concentration dependent up to 32µg/ml comparing to ascorbic acid. The values of IC_{50} for DPPH radical scavenging were in the order of *P. frutescens*< ascorbic acid< *H. officinalis*. The values of IC_{50} for FIC method were in the order of ascorbic acid< *P. frutescens*< *H. officinalis*. The IC_{50} for ABTS method was 8µg for *P. frutescence* and the values were in the order as ascorbic

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acid< *H. officinalis*< *P. frutescence*. The essential oil of both plant species showed variable activities against all the tested bacterial. The results displayed also that the essential oil of *P. frutescens* showed significant fungal inhibitions against the four tested *Candida* strains. On the contrary, *H. officinalis* essential oil was inactive against the examined fungal strains. The results indicated that *H. officinalis* and *P. frutescens* oils are promising as anti-inflammatory. *P. frutescens* essential oil represented the best choice since it has low cytotoxicity on RAW cell line. Both essential oils showed 100% cytotoxicity against colon cancer cells (HCT116). *P. frutescens* essential oil gave 100% inhibition for cancer pancreatic cancer cells (PACA2) and 81.2% inhibition for epidermoid carcinoma (A431). The study explored that both oils had low cytotoxicity on normal cell BJ1 (Normal Skin fibroblast).

Keywords---*Lamiacea*, essential oil, antioxidant, antimicrobial, anti-inflammatory, cytotoxicity, GC-MS.

Introduction

Hyssopus officinalis L. is a common plant that is most usually found in three color forms; *f. cyaneus* (blue), *f. ruber* Mill. (purple/pink) and *f. albus* Alef (white) (Baj *et al.*, 2018). The GC-MS analysis of the essential oil showed that the three forms of color have a different content of the main components. The authors found the main component of white hyssop was pinocamphone (51%) but pink hyssop contained equal amounts of pinocamphone (28.8%) and isopinocamphone (21.9%). The main constituents of *H. officinalis* essential oil were identified as pinocamphone, iso-pinocamphone, pinene, pinocarvone (Kizil *et al.*, 2010, Fatemeh and Hamedeyazdan, 2011, Zheljazkov *et al.*, 2012, Pandey *et al.*, 2014, Said-Al Ahl *et al.*, 2015, Stappen *et al.*, 2015 and Hristova *et al.*, 2015, Saeidi *et al.*, 2020, Aćimović *et al.*, 2021, Saebi *et al.*, 2021 and Jop *et al.*, 2021). Venditti *et al.*, (2015) detected linalool (35.3–51.2%) and methyl eugenol (7.3–22.7%) as the main compounds of *H. officinalis* essential. While, Jop *et al.*, (2021) identified 57 compounds in hyssop essential oil which was rich in oxygenated terpene compounds and identified the main compounds as monoterpene ketones: isopinocamphone (42.1%) and pinocamphone (10.6%). Other important constituents were mononeterpene and sesquiterpene hydrocarbons e.g., β-pinene (8.8%), germacrene-D (5.4%), bicyclogermacrene (2.7%), and (E)-β-caryophyllene (2.6%) and oxygenated constituents e.g., elemol (3.9%), myrtenyl methyl ether (3.6%).

Judžentienė (2016) reported that *H. officinalis* essential oil had many biological activities such as insecticidal, attractant, antimicrobial, antifungal and therapeutic properties. *H. officinalis* essential oil used to increase shelf life of food products and used as flavoring factors in teas, soft drinks, candies, various snacks, chewing gum, baked goods, ice creams, frozen meals. The essential oil of *H. officinalis* oil had antimicrobial properties, good antibacterial activity and weak radical scavenging activity (Kizil *et al.*, 2010). Also, antioxidant and antimicrobial against gram positive and negative bacteria, antifungal,

antiviral and anti-insecticidal activities *in vitro* were reported by Fatemeh and Hamedeyazdan, (2011). The antimicrobial activity against *S. aureus* and *C. albicans,* and antioxidant was observed by Vlase *et al.*, (2014) and anti-fungi activity against clinical isolates of five different species of genus *Candida* by Hristova *et al.*, (2015). Stappen *et al.*, (2015) found a low antimicrobial activity of *H. officinalis* essential oil against *P. aeruginosa*, but strong activities against *S. pyogenes*, *S. aureus* and *E. coli*. *H. officinalis* showed promising antifungal activity because this oil was less toxic that LC_{50} value was 58.7 ppm. Baj *et al.*, (2018) mentioned that the essential oil of hyssop was more active against positive gram bacteria (*B. subtilis*). Application with 5 and 10 µl of essential oil exhibited strong antimicrobial activity against *S. pyogenes, S. aureus*, *C. albicans* and *E. coli*, but not against *P. aeruginosa*. On the other hand, Zheljazkov *et al.*, (2012) reported that *H. officinalis* essential oil did not show significant effect as antimicrobial and anti-malarial activity against pathogens.

Hyssop essential oil showed a moderate cytotoxic activity against the human cancer cells examined and induced a concentration-dependent inhibitory effect (Venditti *et al.*, 2015). The antioxidant activity of *H. officinalis* essential oil was lower compared to butylated hydroxytoluene (BHT) and ascorbic acid (Baj, *et al.*, 2018). *Perilla frutescens* is an annual herb of *Perilla* genus in the *Lamiaceae* family and has been commonly used as a kind of traditional Chinese medicine in China for more than 1000 years then spread out to the Asian countries and reached Japan. It is an edible plant, with its fresh leaves usually used as vegetables, and commonly used for seasoning pickles or as garnish for raw fish dishes in Japan. It is a popular leafy vegetable in Korea, which is generally consumed as a pickle or wrapping with roasted meats, in addition to its uses in the folk healthcare systems in several Asian Countries.

Omer *et al.*, (1998) cultivated two varieties (the green and red color) of *P. frutescens* for the first time in Egypt and reported that the essential oil of both varieties mainly consists of perillaldehyde, caryophyllene oxide, limonene and caryophyllene with some significant differences in the oil constituents of the two varieties. The main components of *P. frutescens* essential oil were 2-acetylfuran, perillaldehyde, caryophyllene, laurolene, 2-hexanoylfuran, 2-butylamine, asarone, farnesene, caryophyllene, and (Z, E) --farnesene (Tian *et al.*, 2014). You *et al.*, (2014) analyzed the chemical composition of purple *Perilla* and the major components were carvone (32.55%), perillaldehyde (20.52%), caryophyllene (9.85%), 2-furyl methyl ketone (7.53%), 2,6 dimethyl-6-(4-methyl-3-pentenyl)-2-norpinene (5.17%), and -terpinyl acetate (3.41%). Ahmed and Al-Zubaidy (2020) showed that the main components of *P. frutescens* were perillaldehyde, ß-dehydro-elsholtzia ketone, perilla ketone, limonene, ß caryophyllene, (Z, E) alpha-Farnesene, shisofuran and trans-shisool. Erhunmwunsee *et al.*, (2021) showed that perillaldehyde is the main compound of *P. frutescens* essential oil.

Lin *et al.*, (2016) showed perillaldehyde (54.35 %), limonene (23.81 %), trans-caryophyllene (7.2 %), cis,trans-α-farnescene (7.02 %) and linalool (2.40 %) as the major componen0074s of the essential oil of red *P. frutescens* and perillaldehyde (65.26 %), limonene (12.49 %), cis, trans-α-farnescene (7.31 %), trans-caryophyllene (5.91 %), and linalool (2.75 %) for green *P. frutescens*. Omer *et al.*, (1998) established in the first report of cultivation of *P. frutescens* in Egypt that

both oils showed good inhibitory activity against *A. niger*, *C. albicans*, *B. subtilis* and *E. coli*. The activity against *B. subtilis* was generally stronger than against other species. This may be attributed to its high content of perillaldehyde and caryophyllene oxide. Kang *et al.*, (1992) reported that perillaldehyde was the major compound responsible for the activity of the essential oil of *P. frutescens*. They also reported that perillaldehyde showed moderate and broad-spectra activity against several Gram-positive and Gram-negative bacterial strains as well as fungi. It showed an MIC between 125 and 100 mg/ml, close to that of the essential oil. The activity of perillaldehyde may be due to synergisity with polygodial. *P. frutescens* has significant anti-allergic, anti-inflammatory, and strong antitumor-promoting activities (Banno *et al.*, 2004).

Ahmed, (2019) reported that perilla essential oil had antibacterial activity against *S. aureus* and *E. coli* and the MIC for them were 500 µg/mL and 1250 µg/mL, respectively. Perilla essential oil also had antifungal activity against *T. mentagrophytes* and the doses of perilla reduced the production of α-toxin, enterotoxins A and B, and toxic shock syndrome toxin 1 (TSST-1) in both methicillin-sensitive *S. aureus* and methicillin-resistant *S. aureus*. Chen *et al.*, (2020) showed that *perilla* essential oil had high antioxidant activity with IC_{50} = 2.60 µl/ml, and had antifungal activity against *C. gloeosporioides* and *F. fujikuroi* and also showed higher cytotoxicity against MGC-803 and A549. Erhunmwunsee *et al.*, (2021) indicated that perilla essential oil performed strong anticancer, anti-depressant, and anti-inflammatory effects *in vivo* study. Tian *et al.*, (2014) reported good results for perilla essential oil as antioxidant and antifungal.

Since the antiseptic properties of medicinal plants have also been well-documented and recorded for a very long time, a re-investigation of traditional medicines to discover new drugs for the treatment of cancer, inflammation, and infectious disease is an appealing vision. In addition, there has been a recent resurgence of interest in herbal remedies as a result of knowledge that plant-based preparations are less likely to cause adverse reactions than synthetic pharmaceuticals. Natural antimicrobial products with high antioxidant concentrations are especially desirable because they can treat inflammation symptoms as well as obstruct the microbial trigger, having pluripotent effects. The goal of the current study was to investigate the main chemical components of *H. officinalis* and *P. frutescens* essential oils using GC-MS and their antioxidant, anti-inflammatory, anticancer, and antimicrobial activities *in vitro*.

Materials and Methods

Hyssopus officinalis L. seeds were imported from PHARMASAAT-SEEDS and PLANTS Company (PHARMASAAT-SEEDS AND PLANTS OF MEDICINAL AND SPICE HERBS). Seeds were cultivated at SEKEM Experimental Farm, SEKEM Company, El-Adlya Belbeis, EL-Sharkiya Governorate, and an herbarium specimen was kept in the NRC Herbarium under the No. M524. Aerial parts (10 kg) of *H. officinalis* were collected in May, 2021. The same steps in cultivation the plants and extracting the volatile oil from its aerial parts were performed as mentioned in the published article of Eldeghedy *et al.*, (2022). The essential oil of *Perilla frutescens* was imported from Oshadhi Company, London, England.

GC-MS analysis

The GC-MS analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument in the National Research Centre with the specifications mentioned by Said-Al Ahl *et al.*, (2014) and Eldeghedy *et al.*, (2022).

The Biological activities

The approval from Ethical Committee of The Medical Research in the National Research Centre regarding this research was taken under the No. of (1342032022) for 2022.

Antioxidant capacity determination

Samples from both essential oils were subjected for antioxidant activity with DPPH according to Brand-Williams *et al.*, (1995), Ferrous Ion Chelating (FIC) Ability according to the method of Singh and Rajini (2004) and ABTS radical scavenging activity as described by Floegel *et al.*, (2011).

Antimicrobial assays of EOs (Bacterial and fungal strains)

The reference bacterial strains including *Proteus vulgaris* (ATTC 13315), *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923) and *Salmonella enterica* were obtained from the culture collection of the Department of Microbiology and Immunology, National Research Centre, Cairo, Egypt. In addition, a resistant strain of *S. aureus* to most of antibiotics including vancomycin 30 µg, oxacillin 5 µg, amoxycillin 10 µg, erythromycin 15 µg, streptomycin 10 µg, cefuroxime sodium 30µg, trimethoprim/sulphamethozole 25 µg, gentamycin 120 µg, and rifampicin 5 µg were also obtained. The chemicals, Cefoxitin 30 µg, cefotaxime 30 µg, and colistin sulphate 10 µg which had a bacteriostatic effect against this strain were also purchased.

Candida strains (*C. albicans, C. glabrata, C. tropicalis and C. krusei*) were isolated primarily using sabouraud dextrose agar (SDA, Oxoid, UK) and single pure colonies were identified by culturing on rice agar medium with 1% tween 80, germ-tube test was used for *C. albicans* (Marinho *et al.*, 2010). Accurate identification and differentiation of Candida isolates were achieved with CHROM agar medium, API 20 C Aux and multiplex-PCR (Gündeş *et al.*, 2001, Hermansyah et al., 2017, Ozcan *et al.,* 2010). All the used fungal strains were isolated according to Khalaf *et al.*, (2021) from the culture collection of the Department of Microbiology and Immunology, National Research Centre, Cairo, Egypt.

Agar disc diffusion assay

Both EOs were evaluated against bacterial and fungal strains by using agar disc diffusion assay according to Lorian (2005). Discs were dipped into EO diluted with DMSO (bacteria) and 10% tween 80 (fungi) with a concentration of 20%. Vancomycin 30 µg was used as a positive control for *S. aureus* and ciprofloxacin 5

µg for other bacteria and fluconazole 25 µg for *C. albicans* and DMSO as a negative control. The plates were incubated at 37°C for 24 h (bacteria) and 28 °C for 48-72 h (fungi) then the diameter of inhibition zone was reported in mm.

Anti-inflammatory activity (nitric oxide assay)
Cell line and cell culture

The American Type Culture Collection was the source of the murine macrophage cell line (RAW 264.7) was purchased. USA-sourced cells (ATCC) were grown in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 g/ml streptomycin, and 250 g/mL amphotericin B at 37°C in a 5% CO_2 incubator.

Anti-inflammatory assay

To study the anti-inflammatory activity of the essential oil of both plant species, the nitric oxide (NO) generation in LPS-stimulated RAW 264.7 cells was tested. In order to determine nitric oxide (NO), RAW 246.7 cells were planted in 96-well plates at a density of 10×10^3 cells/well and allowed to develop for 24 hours to measure adherence. The test samples were applied to the cells for one hour, and they were subsequently cultured for 24 hours in new DMEM with 1µg/ml LPS. According to the Griess reaction, the amount of nitrite in the culture media was assessed as a sign of NO production (Wu *et al.*, 2008). In a 96-well plate, 100µl of cell culture supernatant were combined with 100 µl of Griess reagent for the reaction, and the absorbance at 540nm was recorded using an ELISA reader.

Cell culture (seeding and treatment)

The cells of RAW 264.7 macrophage cell line were grown in RPMI1640 media (Roswell Park Memorial Institute) supplemented with 10% heat-inactivated fetal bovine serum and 1% pen/strep. The cells underwent two subcultures in a humidified incubator with a 5% CO_2 environment at 37°C before the experiment.

Procedure

The following processes were all performed in a bio-safety class II level Laminar Flow Cabinet in a sterile environment (Baker, SG403INT, and Sanford, ME, USA). RAW 264.7 cells were suspended in RPMI media and 1×10^5 cells were seeded into each well of 96-well plates and then allowed to grow for 24 hours before being used in studies. The EOs samples were added to the cells at concentrations of 100, 50, 25 and 12.5 µg/ml, and left to react for an hour. They were then stimulated for an additional 24 hours with 10 µg/ml of LPS. The supernatant was smoothly transferred to fresh 96-well plates in order to determine NO.

Nitric oxide assay

By measuring nitrite in the supernatants of cultivated RAW 264.7 cells, nitric oxide production was evaluated. The assay was performed mostly in accordance with the prior description (Yoon *et al.*, 2009). Using the Griess reagent, the amount of nitrite, a stable metabolite of NO used as an indication of NO generation, in the culture medium was determined after pre-incubating RAW

264.7 cells (1 × 10^5 cells/ml) with LPS (10 µg/ml) for 24 hours (1 percent sulfanilamide and 0.1 percent naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid). 50 µl of the Griess reagent were combined with 50 µl of the cell culture medium. The mixture was then let to sit for 15 min at room temperature, and then the absorbance at 540nm was determined using a micro plate reader. In each experiment, the fresh culture medium was used as a blank. The amount of nitrite was calculated from a sodium nitrite standard curve as expressed in equation:

$$\text{Nitric Oxide inhibition (\%)} = (\text{Control} - \text{Test}) \times 100 / \text{Control}$$

Cytotoxic effect on six human cell lines

Cell viability was evaluated as mentioned by to Mosmann (1983) by converting yellow MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan in a mitochondrial-dependent reaction.

Procedures

All next operations were carried out in a biosafety class II level Laminar Flow Cabinet under a sterile environment (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium (DMEM for PACA2, A549, PC3, and BJ1), 1% L-glutamine, and 1% antibiotic antimycotic mixture (10,000U/ml potassium penicillin, 10,000 g streptomycin sulfate, and 25 µg amphotericin B). Cells were batch grown for 10 days before being planted at a concentration of $10x10^3$ cells per well in original complete growth media in 96-well microtiter plastic plates for 24 h at 37 ºC under 5% CO_2 using a water jacketed carbon dioxide incubator, (Sheldon, TC2323, Cornelius, OR, USA). Cells were incubated either alone (negative control) or with various sample concentrations to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/ml). Media was aspirated after 48 hours of incubation, 40 µl of MTT salt (2.5 g/ml) were added to each well, and the plate then incubated for a further four hours at 37°C with 5 % CO_2.

A volume of 200 µl of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C to stop the reaction and dissolve the crystals that had formed. A known cytotoxic natural reagent at a concentration of 100 µg/ml was utilized as a positive control since it causes 100% mortality under the identical circumstances (Thabrew *et al.*, 1997 and El-Menshawi *et al.*, 2010). The absorbance was then measured at 595nm with a reference wavelength of 620nm using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). The statistical significances were calculated between samples and negative control (cells with vehicle) using independent t-test with SPSS 11 program. For dissolution of plant extracts, DMSO was used and its final concentration on the cells was not exceeding than 0.2%. The percentage of change in viability was calculated according to the formula:

(Reading of extract / Reading of negative control) -1) x 100
Analysis of probability was carried for IC_{50} determination using SPSS 11 program.

The concentration of the sample ranged between (0.78 to 100 µg/ml)

Results and Discussion

Essential Oil Constituents

Twenty-six compounds that represent 99.83% of the total compounds of the essential oil of *H. officinalis* have been separated and identified by GC-MS as illustrated in Table (1). The total of oxygenated compounds reached 58.77%, while nonoxygenated compounds represented 41.03% from the total identified compounds. From another chemical point of view, monoterpene compounds represented 88.76%, while sesquiterpenes correspond to 11.07% from the identified compounds. The main constituent was isopinocamphone that accounted for 34.00%, followed by pinocamphone (21.27%), β-Pinene (13.19 %), β-Phellandrene (13.10), α-Thujene (3.89 %) and Bicyclo-germacrene (3.47%). These results are well-matched with those of Jop *et al.*, (2021), Aćimović *et al.*, (2021), Hristova *et al.*, (2015), Said-Al Ahl *et al.*, (2015) and Stappen *et al.*, (2015). They showed that isopinocamphone was the main component and followed by pinocamphone, *β*-pinene (8.8%), D-germacrene, bicyclo-germacrene, (E)-*β*-caryophyllene and *α*-elemol. On the contrary Venditti *et al.*, (2015) reported that the main constituents were linalool and methyl eugenol of the hyssop essential oil.

Table 1
The relative percentages of the main constituents in the essential oil of *Hyssopus officinalis* and *Perilla frutescens* as analyzed with GC-MS

Compound	RT	KI_{Cal}	KI_{Lit}	*H. officinalis* %	*P. frutescens* %
α-Thujene	3.89	932	931	0.20 ± 0.01	-
α-Pinene	4.04	940	939	0.81 ± 0.07	1.30 ± 0.03
Camphene	4.43	959	953	0.13 ± 0.01	0.09 ± 0.01
Sabinene	4.97	982	976	1.84 ± 0.05	0.14 ± 0.01
β-Pinene	5.10	987	980	13.19 ± 1.06	1.21 ± 0.04
β-Myrcene	5.48	1002	991	0.67 ± 0.04	-
Pseudolimonene	5.84	1016	1004	-	0.12 ± 0.01
D-Limonene	6.55	1041	1024	-	14.21 ± 0.67
β-phellandrene	6.64	1044	1029	13.15 ± 0.11	-
L-linalool	9.16	1119	1095	-	0.99 ± 0.03
3-Methoxymethoxy-6,6-dimethyl-2-methylene-bicyclo [3.1.1] heptane	11.03	1157	1160	3.19 ± 0.08	-
trans-pinocamphone	11.44	1165	1178	21.27 ± 0.15	-
Iso-Pinocamphone	12.10	1177	1180	34.00 ± 0.67	-
Myrtenol	13.05	1215	1184	0.29 ± 0.05	-
α-Terpineol	13.12	1221	1189	-	0.18 ± 0.01
trans-Shisool	16.25	1296		-	1.47 ± 0.13
L-Perillaldehyde	16.42	1299	1290	-	50.64 ± 0.48
β-Elemene	18.2	1345	1375	-	0.10 ± 0.01

perillartine	18.88	1362	1374	-	0.08 ± 0.05
β-Copaene	19.89	1385	1374	-	0.32 ± 0.01
β-Bourbonene	20.26	1373	1377	0.28 ± 0.03	0.12 ± 0.00
γ-elemene	20.53	1399	1430	-	0.23 ±0.01
α-Gurjunene	21.13	1412	1410	0.18 ± 0.02	-
trans-Caryophyllene	21.7	1424	1417	1.77 ± 0.13	20.58 ± 0.56
α-Humulene	23.22	1454	1455	0.23 ± 0.03	1.49 ± 0.03
Alloaromadendrene	23.37	1457	1460	0.64 ± 0.07	-

Table 1. continued

Germacrene-D	24.33	1475	1485	1.28 ± 0.22	2.11 ± 0.04
Bicyclo-germacrene	24.85	1506	1500	3.47 ± 0.26	0.44 ± 0.12
α-farmesene	24.95	1510	1508	-	2.74 ± 0.37
zingibrene	25.82	1534	1495	-	0.21 ± 0.01
Hedycaryol	27.25	1558	1548	1.05 ± 0.23	-
cis-Farnesol	27.37	1574	1485	-	0.08 ± 0.01
Spathulenol	28.29	1579	1578	0.13 ± 0.04	-
Caryophyllene oxide	28.38	1581	1583	0.08 ± 0.01	1.09 ± 0.03
Globulol	28.61	1605	1585	0.09 ± 0.02	-
Veridiflorol	28.9	1612	1593	0.05 ± 0.01	-
Epiglobulol	29.29	1621	1597	0.13 ± 0.02	-
8-epi-gama-eudesmol	30.51	1649		0.17±0.02	-
Elmol	31.94	1680	1547	1.51±0.37	-
Oxygenated compounds	--	--	--	58.77	54.5
Non-Oxygenated compounds	--	--	--	41.03	45.41
Monoterpenes	--	--	--	88.76	70.45
sesquiterpenes	--	--	--	11.07	29.51
Total of identified compounds	--	--	--	99.83	99.96

Twenty-seven compounds that represent 99.95% of the total compounds have been identified as the main constituents of the essential oil of *P. frutescens* as separated and identified by GC-MS as shown in Table (1). The oxygenated compounds accounted for 54.5%, while non-oxygenated compounds corresponded to 45.41% from the identified compounds. In another chemical way, monoterpene compounds represented 70.45%, while sesquiterpenes correspond to 29.51% from the identified compounds. The foremost constituent was identified as L-perillaldehyde which reached (50.64%) followed by trans-caryophyllene (20.58%) and D-limonene (14.21%). These results coincide with those of Omer *et al.,* (1998) who reported perillaldehyde (34.2% and 32.9%) as the main compound followed by caryophyllene oxide (23.3% and 6.0%), β-caryophyllene (10.5% and 10.4%), limonene (13.1% and 4.5%), p-mentha-2,8-dione (4.6% and 6.6%) and a-farnesene (3.4% and 4.5%) were found to be the main constituents of green- and red variety oils, respectively. Farnesol was found at 7.4% in the oil of the red-variety and absent in the green-variety. These findings are in agreement also with Ahmed and Al-Zubaidy (2020) and Erhunmwunsee *et al.,* (2021) who reported that the main component of *P. frutescens* essential oil was perillaldehyde, while You *et al.*, (2014) and Tian *et al.*, (2014) mentioned that the major components of *P. frutescens* essential oil were carvone and 2-acetylfuran, respectively.

Antioxidant Activity

It is very challenging to assess a product's antioxidant activity using just one technique. The antioxidant properties of the investigated natural product are described in detail by a combination of methods than by a single method alone (Ciz *et al.*, 2010). Due to the vast differences in sample preparation, essential oil extraction conditions (solvent, temperature, etc.), and result expression, antioxidant activity assessment may necessitate the use of a combination of different techniques (Viuda-martos *et al.*, 2010; Perez-Jimenez *et al.*, 2008**)**. The oxidative burst that occurs in various cells (monocytes, neutrophils, eosinophils, and macrophages) as mentioned earlier (Huang *et al.*, 2005) is one of the inflammatory responses, so if essential oils can remove some free radicals, they can also act as anti-inflammatory agents. The antioxidant activity of both EOs under investigation had been determined by different methods which work by different mechanisms. The antioxidant activities of both essential oils were determined with three recommended methods; DPPH, FIC and ABTS and the results are shown in Table (2). As can be observed, both EOs demonstrated varying levels of scavenging prowess.

Table 2
Antioxidant activities of *Hyssopus officinalis* and *Perilla frutescens* essential oils measured by DPPH, metal-chelating (Ferrozine) and ABTS Assay

Essential oil	DPPH % inhibition				IC_{50}[a]
	4µg/ml	8µg/ml	16µg/ml	32µg/ml	
H. officinalis	21.36 ± 0.22	27.84 ± 3.31	44.39 ± 2.98	64.23 ± 1.60	21.90
P. frutescens	32.50±1.29	48.34±1.53	68.41±4.04	83.40±1.08	10.20
Ascorbic acid	8.562±2.090	25.372±0.847	58.105±1.794	89.846±0.296	16.6
	FIC % inhibition				
H. officinalis	21.36 ± 0.50	25.15± 0.50	27.11± 0.52	38.99 ± 0.95	103.03
P. frutescens	22.36±0.97	27.03±1.20	29.41±0.62	42.47±0.50	55.74
Ascorbic acid	44.86±3.17	50.50±1.12	52.01±0.75	54.00±0.28	8.04
	ABTS % inhibition				
H. officinalis	60.76 ±1.53	65.14±1.59	67.29±0.14	69.43±0.65	0.21
P. frutescens	41.29 ±1.93	47.14 ±2.81	52.57 ±1.55	59.14 ±1.59	8.0
Ascorbic acid	85.95±1.94	94.86±0.15	95.00±0.25	95.38±0.08	0.1653

[a]Concentration (µg/ml) for 50% inhibition for DPPH (µg/ml) for a 50% chelating effect for FIC and (µg/ml) for 50% inhibition for ABTS

Both the studied EOs showed antioxidant activities with the used three methods in which increasing EOs concentrations increased the antioxidant capability up to 32µg/ml comparing to ascorbic acid. In the DPPH method, IC_{50} that expressed as the concentration capable of scavenging 50% of the DPPH radical was 10.20 for *P. frutescens* oil. *P. frutescens* EO showed strong radical-scavenging effect (83.40 ±1.08) at 32µg/ml, which is lower than those recorded for ascorbic acid (IC_{50} was 16.6 and scavenging effect was 89.846±0.296). This activity was followed by the

H. officinalis EO (64.23 ± 1.60) at 32µg/ml. The values of IC_{50} for DPPH radical scavenging were in the order of *P. frutescens*< ascorbic acid< *H. officinalis*.

Metals chelation may render important antioxidative effects by retarding metal-catalyzed oxidation. Using FIC method, analysis of metal ion-chelating properties showed that all of the EOs studied were capable of chelating iron (II) and did so in a concentration-dependent manner (Table 2). The ascorbic acid was higher in chelating of iron (II) than *H. officinalis* and *P. frutescens* essential oils. The highest concentration of *H. officinalis* EO showed antioxidant activity with 38.99% at 32µg/ml and with 42.47±0.50 at 32µg/ml for *P. frutescens* EO. IC_{50} that expressed as the concentration capable of scavenging 50% of the FIC radical for the different EOs were 103.03 and 55.74 for *H. officinalis* and *P. frutescens* EOs, respectively. The values of IC_{50} were in the order of ascorbic acid< *P. frutescens*< *H. officinalis*.

The equilibrium between Fe^{+2} ions and ferrozine is disturbed when they interact in the presence of a rival contestant agent, as shown by a decrease in color intensity, which demonstrates the antioxidant activity of the compound. With ABTS method, *H. officinalis* EO showed the higher antioxidant inhibition (69.43±0.65% at 32µg) comparing to the *P. frutescence* EO which showed the lower percentage of inhibition values (59.14% at 32µg/ml) as shown in Table (2) but was lower than ascorbic acid (95.38% at 32µg/ml). The IC_{50} values ranged from 0.16 for ascorbic acid to 8µg for *P. frutescens* and were in the order as ascorbic acid< *H. officinalis*< *P. frutescens*. Since essential oils can be used to protect food from oxidant hazards, their antioxidant capacity is a biological trait of great concern (Maestri *et al.*, 2006). The ability of the essential oils to scavenge free radicals may help in the prevention of certain diseases like cancer, heart disease, cancer and deterioration of the immune system. More evidence suggests that the cellular harm caused by free radicals may be the root cause of these diseases (Aruoma, 1998 and Kamatou and Viljoen, 2010). These findings are in line with other studies on the antioxidant power of essential oils. Saleh *et al.* (2010) used the spectrophotometer method of DPPH along with DPPH/TLC to compare the antioxidant qualities of 248 essential oil-bearing plants. The essential oil of 17 species mostly belong to *Lamiaceae* family had significant antioxidant activity. Phenols, non-phenols, oxygenated, and non-oxygenated compounds were among the active substances discovered by the DPPH/TLC method.

Antimicrobial Activity

The antibacterial potentialities of the EOs of *H. officinalis* and *P. frutescens* were assayed by using agar disc diffusion protocol and the results are summarized in Table (3) as inhibition zones (IZ) and minimum inhibition concentration (MIC). The antibacterial properties are the resultant of the major and minor components in the essential oils (Jianu *et al.*, 2013). The data showed that all EOs inhibited all screened bacteria with variable efficacy except *S. enterica* that did not show any inhibition zones with *H. officinalis*. *H. officinalis* EO showed a moderate antibacterial activity against *E. coli* (ATCC 35218) with IZ: 11mm, and *S. aureus* (ATCC 25923) with IZ: 8mm, while it showed a weak antibacterial effect against *P. vulgaris* (ATCC 13315) with IZ: 5mm and *S. aureus* (resis.) with IZ: 4 mm. The EO

of *P. frutescens* showed a remarkable antibacterial activity against *P. vulgaris* (ATCC 13315) (IZ: 17mm) and weak effect against *E. coli* (ATCC 35218) (IZ: 10mm), *S. aureus* (ATCC 25923) (IZ: 10 mm), S. *aureus* (resis.) (IZ: 12 mm) and *S. enterica* (IZ: 8mm).

Table 3
Inhibition zone (IZ mm) and MIC µg/ml of *Hyssopus officinalis* and *Perilla frutescens* essential oils against the different strains of bacteria

Essential oils / strains	*H. officinalis*		*P. frutescens*		VA 30 µg (IZ, mm)	CIP 5 µg (IZ, mm)	Am 10 µg (IZ, mm)
	IZ (mm)	MIC µg/ml	IZ (mm)	MIC µg/ml			
P. vulgaris (ATCC 13315) -	5±0.2	20	17±0.24	5	NA	30	13
E. coli (ATCC 35218) +	11±0.3	80	10±0.20	5	NA	30	NA
S. aureus (ATCC 25923) -	8±0.24	80	10±0.20	10	15	NA	NA

Table 3. continued

S. aureus (resis.) -	4±0.23	30	12±0.24	10	Resis. *	NA	NA
S. enterica +	NA	NA	8±0.10	30	NA	NA	10

*Resis.: resistant, ** Values are the average (n = 3) of the inhibition zone diameter (mm) ± standard deviation, b minimum inhibitory concentrations, MIC: maximum inhibition (no growth at all), VA: Vancomycin, CIP: Ciprofloxacin, AM: amoxycillin, NA: no activity.

Based on the data of the minimum inhibitory concentration (MIC), the *H. officinalis* EO showed low activities against *E. coli* (ATCC 35218) and *S. aureus* (ATCC 25923) (MIC: 80µg/ml), while it showed a moderate activity against *P. vulgaris* (ATCC 13315) (MIC: 20 µg/ml) and *S. aureus* (resis.) (MIC:30µg/ml). *P. frutescens* EO showed strong antibacterial activities with MIC at 5µg/ml against *P. vulgaris* (ATCC 13315) and *E. coli* (ATCC 35218). While, it showed moderate activity against *S. aureus* (ATCC 25923) and *S. aureus* (resis.) (MIC 10µg/ml). The selected antibiotics showed a varied activity against the bacterial strains, vancomycin with 30µg showed an activity against *S. aureus* (ATCC 25923) (ZI: 15mm), while it was inactive against *S. aureus* (resis.). The ciprofloxacin at 5µg exhibited the maximum inhibition on *S. entrica*, but did not show any activity against *P. vulgaris* (ATCC 13315) and *E. coli* (ATCC 35218) (IZ 30 mm). On the other hand, amoxycillin 10µg was active against *S. aureus*, *P. vulgaris* (ATCC 13315) (IZ: 13mm) and *S. entrica* (IZ: 10 mm). The antifungal activities of *H. officinalis and P. frutescens* EOs were examined and the results as inhibition zone (IZ mm) and MIC µg/ml are listed in Table (4).

Table 4
Inhibition zone (IZ mm) and MIC µg/ml of the EOs of *Hyssopus officinalis* and *Perilla frutescens* against some *Candida strains*

Essential oils / strains	*H. officinalis*		*P. frutescens*		fluconazole 25 µg
	IZ mm	MIC µg/ml	IZ mm	MIC µg/ml	
C. glabrata	NA	NA	15	5	21
C. albicans	NA	NA	NA	5	20
C. tropicalis	NA	NA	9	10	18
C. krusei	NA	NA	11	10	26
C. albicans (ATCC 10231)	NA	NA	13	10	23

P. frutescens EO showed a significant fungal inhibition against the four tested *Candida* strains with IZs ranged from 9-15 mm but didn't show any effect against *C. albicans*. *P. frutescens* EO inhibited the growth of *C. glabrata* (IZ: 15mm), *C. albicans* (ATCC 10231) (IZ: 13 mm), *C. krusei* (IZ: 11mm) and *C. tropicalis* (IZ: 9 mm). *H. officinalis* EO didn't show any effect against all investigated fungal strains. All these data were evaluated with the respecting of the reference drug (fluconazole 25 mcg) with inhibition zones ranging from 18 to 26µg/ml.

According to You *et al.*, (2014) the results generally showed that the bioactivity properties of the essential oils are related to the synergistic effects of their various major and minor components. The mechanism of the essential oil's anti-candida action may involve increasing yeast membrane permeability and interfering with normal membrane transport by altering membrane ATPase (Hristova *et al.*, 2015). On the other hand, to our results, Hristova *et al.* (2015) reported that the essential oil of hyssop had inhibitory effects on a number of candida strains. They attributed this activity to the oil's complex chemical makeup and the synergistic effects of constituents like cis- and trans-pinocamphone and *α*- and *β*-pinene. *H. officinalis* displayed activity against *S. aureus*, *E. coli* and *C. albicans* with no activity against *E. faecalis* and *P. aeruginosa* (Venditti *et al.*, 2015).

Anti-inflammatory Activity

To ascertain whether EOs have anti-inflammatory properties, the effects of EOs on NO production in RAW 264.7 cells were quantified. NO is a small molecule that, as is well known, participates in signaling in a variety of pathophysiological processes, particularly a number of processes connected to inflammation (Kim *et al.*, 2013). The production of NO is increasing when an inflammatory stimulus begins, which blocks the pro-inflammatory effect. A rise in NO in the cells, however, can be harmful and cause a number of inflammatory diseases (Sharma *et al.,* 2007). In order to confirm its capacity to control inflammation, research is being done on the impact on NO production. A common parameter that is used as a marker for macrophages is nitric oxide. Application of the different essential oils at the rate of 100, 50µg/ml after the application of LPS resulted in inhibition percentage but these concentrations were toxic so we treated the RAW264.7 macrophage cells with essential oils of *H. officinalis* and *P. frutescens* essential oils to result in 42.3 and 41.6%, respectively (Table 5).

Table 5
Anti-inflammatory, Nitric oxide inhibition and Cell viability of *H.officinalis* and *P. frutescens* essential oil

Essential Oil	% Nitric oxide inhibition at 25µg/ml	% Cell viability of RAW cell line at 25µg/ml
H. officinalis (hyssop)	42.3±0.47	79.6±1.6
P. frutescens (perilla)	41.6±0.82	96.1±0.97
LPS	------	97.3±0.89
Dexamethasone (+ve)	94.1±0.78	79.3±0.51

The cell viability of *H. officinalis* and *P. frutescens* EOs at 25µg/ml was 79.6 and 96.1%, respectively (Table 5). In the same pattern, LPS and dexamethasone gave 97.3 and 79.3% cell viability, respectively. The results displayed in Table 5 explore that *H. officinalis* and *P. frutescens* oils was represented the most promising as anti-inflammatory. This finding is agreed with the results of Xu *et al.*, (2022) who mentioned that the low concentration of *P. frutescens* essential oil was effective at psoriasis-like skin lesions. It reduced the expression of lymphocyte antigen 6 complex locus G6D (Ly-6G) and deactivated inflammatory factors interleukin 1 (IL-1), interleukin 6 (IL-6), inducible nitric oxide synthase (iNOS), and cyclooxygenase $_2$ (COX_2).

Cytotoxicity Effect
Primary screening

The potentiality of the two essential oils have been determined against several human cancer cell lines at 100µg/ml, using DMSO as control, doxorubicin as the positive control and cancer cells with the used media without DMSO as the negative control. The results of the applied essential oils are shown in Table (6).

Table 6
Cytotoxicity percentage of *H. officinalis* and *P. frutescens* essential oils at 100 ppm

Cell line / EOs	PC3 Prostate cancer	PACA2 Pancreatic cancer	A431 Epidermoid carcinoma	A549 Lung cancer	MCF7 Breast cancer	HCT116 Colon cancer
H. officinalis	17.2±1.1	45.3±2.4	35.2±1.8	24.3±1.2	33.2±1.6	100±zero
P. frutescens	40.3±2.6	100±zero	81.2±3.1	21.5±1.9	56.4±2.9	100±zero

Table 6. continued

DMSO Control (0.5%)	1	1	1	5	3	1
Negative control	0	0	0	0	0	0
Positive Control	100	100	100	100	100	100

Negative control = Cancer Cells with the used media without DMSO

Positive Control = Doxorubicin

The ability of the investigated essential oils to inhibit the growth of the six cancer cell lines used (PC3, PACA2, A431, A549, MCF7, and HCT116) at 100 ppm (100 g/ml) was tested and on normal skin fibroblast cell line (BJ1). *P. frutescens* essential oil was higher than *H. officinalis* essential oil in the cellular growth inhibition. The essential oil of *P. frutescens* was effective against HCT116 and PACA2 with 100% inhibition, followed by A431 with 81.2% inhibition. At the same time, *H. officinalis* essential oil inhibited cellular growth of colon cancer cell by 100%, making it a powerful inhibitor of HCT116 growth. Regarding the susceptibility of the cell lines towards the used essential oils, HCT116 cell line was the most affected (100%) for both essential oils studied. On the other hand, PC3 cell line was the least affected one (17.2 for *H. officinalis* and 40.3% for *P. frutescens* oil). The effect of both essential oil on Lung Cancer cell (A549) was low (24.3 and 21.5 inhibition for *P. frutescens* and *H. officinalis* EO, respectively). Breast cancer cell line (MCF7) was also moderately affected (*P. frutescens* with 56.4%, *H. officinalis* with 33.2%). The value of IC_{50} of essential oils and doxorubicin (+ve control) against different cell lines was calculated that shown in Fig (1).

Secondary screening

The essential oils were subjected to secondary screening to calculate their IC_{50} and selectivity index. The results illustrated in Table (7) and Fig. (1) revealed that *P. frutescens* was promised essential oil with IC_{50} of 48.8, 66.5, 30.8 and 100 for PACA2, A431, HCT116 and BJ1, respectively. The essential oil of *P. frutescens* was promised as resulted from secondary screening in which to calculate their IC_{50} and selectivity index. The results in Table (7) also indicated that essential oil of *H. officinalis* showed IC_{50} of 41.6±3.2 and 74.3±4.5 for HCT116 and Bj1, respectively. While the essential oil of *P. frutescens* showed IC_{50} of 48.8, 66.5, 30.8 and 100 for PACA2, A431, HCT116 and BJ1, respectively.

Table 7
IC_{50} of cytotoxicity cancer cell lines (µg/ml)

Essential oils	IC_{50} of cytotoxicity cancer cell lines (µg/ml)				
	PACA2	A431	MCF7	HCT116	Bj1
H. officinalis	--	--	--	41.6±3.2	74.3±4.5
P. frutescens	48.8±3.4	66.5±4.2	--	30.8±2.2	100.1±5.7
Positive control	±1.226.2	31.5±2.2	±1.326.1	±2.437.6	13.5±0.6

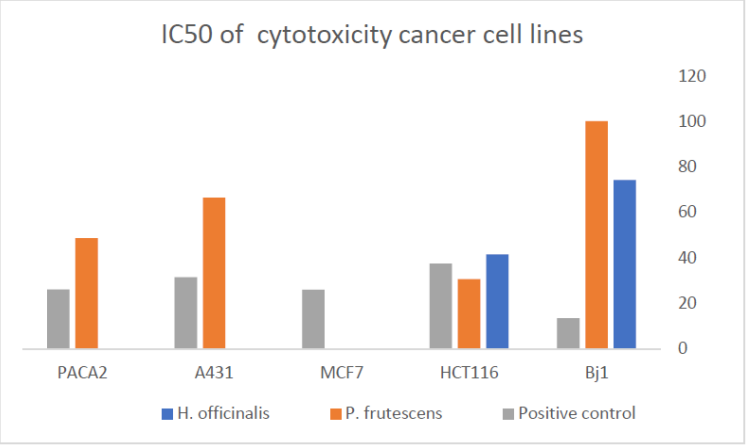

Fig 1. IC_{50} of cytotoxicity cancer cell and normal cell line BJ1 (µg/ml)

Thus, these promising essential oils were screened for their safety on the normal cell line BJ1 and their selectivity indexes were calculated. Both oils have low cytotoxicity on normal cell BJ1 as shown in Table (7) and Fig (1). Regarding selectivity index that displayed in Table (8), *P. frutescens* is promising in PACA2 cell line and the same pattern was showed in A431. While for HCT116, *P. frutescens* showed the same results. Regarding MCF7 cell line, no selectivity was observed for both *P. frutescens* and *H. officinalis* EO.

Table 8
Selectivity index of *H. officinalis* and *P. frutescens* essential oils on normal cell (BJ1) and IC_{50} of (Bj1)

Essential oils	*H. officinalis*	*P. frutescens*
IC_{50} (µg/ml)	74.3±4.5	100.1±5.7
Selectivity index for PACA2	---	2.05
Selectivity index for A431	--	1.51
Selectivity index for HCT116	1.786	3.25
Selectivity index for MCF7	----	---

Recommendation

It is possible to view the essential oil of *P. frutescens* as inspiring natural entity to be used in the investigation of natural medicines based on the results that were presented and discussed above. But before using this essential oil to develop new, effective drug entity, especially as anti-inflammatory and anti-tumor agents, it is crucial to subject it to additional biological studies on experimental animals.

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Conflicts of Interest

The authors declare that no conflict of interest.

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