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Profiling of The Essential Oil of *Murraya Paniculata* Cultivated in Egypt over Four Different Seasons Using Gas Chromatography-Mass Spectrometry and Screening for Antimicrobial and Anticancer Activities

Hala H. Zaatout [1], Hend A. Al-koriety [2 *], Gamal A. Omran [3], Amira M. Beltagy [2]

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria, 21521, Egypt*
[2] *Department of Pharmacognosy, Faculty of Pharmacy, Damanhour University, Damanhour,22111, Egypt*
[3] *Department of Biochemistry, Faculty of Pharmacy, Damanhour University, Damanhour, 22111, Egypt*

Abstract

This study aims to analyze the composition of essential oils of *Murraya paniculata* L. leaves over four-season cycles using GC-MS to investigate the effect of climate variation on the chemical composition as well as on their activities. The yield of the oil obtained by hydrodistillation ranged from 0.02% to 0.1% v/w; being the highest in spring and the lowest in summer. GC-MS identified a total of thirty-five components in the oil samples collected during the four seasons with molecular weight ranging from 202 to 296 m/z. In all samples, sesquiterpene hydrocarbons were predominating (51.28-90.89%). The antimicrobial screening showed that the essential oils were active on *Bacillus Subtilis* ATCC 19659 and *Staphylococcus aureus* ATCC 6538P using the disc diffusion method. The *in vitro* anticancer activity was investigated against human breast cancer (MCF7), liver cancer (Huh7) and prostate cancer (PC3) cell lines by MTT assay. Results showed that the oil behave as a very potent anticancer agent with the IC_{50} values of 1.67, 5.78, 6.5 µg ml^{-1}, respectively compared with Staurosporine with IC_{50} values of 5.75, 8.31 and 4.02 µg ml^{-1}, respectively. It was concluded that the best time for collecting the plant and preparing the oil with higher yield and promising biological activities is during spring and the oil can be used as a candidate for antimicrobial and/or anticancer therapy.

Keywords: *Murraya paniculata*; seasonal variation; essential oil; antimicrobial; anticancer activity

1. Introduction

Murraya paniculata L. (Orange jasmine), also known as *M. exotica* Linn [1], is a highly valuable plant due to its distinctive aroma and medicinal properties. It is a highly variable evergreen shrubby plant with small white flowers, small oblong fruits and hard wood [2] that grows in the tropical and subtropical climates [3]. It is not native to Egypt [4] but it is propagated as an ornamental plant. It contains many phytochemicals such as alkaloids, flavonoids, tannins and phenolic compounds which are responsible for their antinociceptive [5] , antioxidant [6, 7], anti-diabetic [8], antimicrobial [9] and analgesic properties [10]. It is commonly used in traditional medicine for the treatment of abdominal pain, headache, edema, thrombosis and blood stasis. It has been used as a detoxifier, anti-convulsant, local anesthetic, expectorant [3] and a source of perfume and flavor [11]. Leaves are stimulant and astringent and they are used to treat diarrhea, dysentery, dental and gum diseases as well as rheumatism, coughs and hysteria [12]. It is also traditionally used for management of respiratory and cardiovascular problems [13]. The essential oils of *Murraya paniculata* L. have been shown to possess anti-inflammatory, analgesic [14] and antiamoebic properties [15] as well as antioxidant activity [16]. Previous studies showed that the essential oils exhibited antimicrobial effect against gram-positive and gram-negative bacteria as well as antifungal activities [11]. Furthermore, they showed anticancer properties against hepatic and breast cells [17, 18]. *Murraya paniculata* extracts exhibited cytotoxic activity against human colon cancer [19-21] and breast cancer [22]. Studies have been conducted on the essential oil profiles of *Murraya paniculata* L. collected from various regions around the world [7, 11, 23-28]. Essential oils are produced by plants in an attempt to adapt the environmental stressors, such as water shortages, intense radiation, high temperatures, and heavy metal contents [29]. As a result, constituents and yield of essential oils are changed qualitatively and quantitatively in response to a variety of factors such as seasonal changes (temperature, humidity and rainfall) [30, 31]. The impact of seasonal variation on essential oils has been studied and monitored in many

*Corresponding author e-mail: hendabdelsalam36@gmail.com.
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plants [31-36], where it is important to determine the best time of harvesting and collecting the plants to obtain the highest yield of active constituents and achieve the highest efficacy [37]. The purpose of this work is to profile the composition of essential oils obtained from *Murraya paniculata* L. leaves over the four-season cycles from July 2019 to May 2020 to investigate the effect of seasonal climate conditions on the chemical composition as well as the antimicrobial and anticancer activities of the collected oils.

2. Experimental

2.1. Plant material

Fresh leaves of *Murraya paniculata* L. were collected from ornamental plant garden at Shbeen El Koom, Menoufia, Egypt (Summer in July 2019, Autumn in November 2019, Winter in February 2020 and Spring in May 2020). Plant identification was confirmed by Prof. Dr. Sliem Zeidan Heneidy and Prof. Dr. Sania Ahmed Kamal at Botany Department, Faculty of Science, Alexandria university, Egypt. The voucher specimen was kept in the herbarium there, under number 10802.

2.2. Essential oils extraction

Fresh leaves, one kilogram each, were subjected to hydrodistillation using a Clevenger- type apparatus for 4 hours. After oil samples collection, any water traces were removed using anhydrous Na_2SO_4. The volumes of the obtained oil were measured in the apparatus's graduated tube as 0.2, 0.5, 0.3, 1 ml for summer, autumn, winter and spring seasons, respectively. Then the oil yields were expressed as percentages relative to the amount of fresh plant material used in the extraction (yield 0.02, 0.05, 0.03 and 0.1 % v/w for summer, autumn, winter and spring seasons, respectively). The essential oil samples were kept in a sealed vial under refrigeration for gas chromatography mass spectrometry (GC-MS) analysis.

2.3. GC-MS analysis conditions

The GC–MS analyses were done using a Thermo Scientific Gas Chromatograph GC Trace 1300 coupled with an EI Mass spectrometer ISQ 7000 model (Thermo Scientific USA) equipped with Thermo TR-50 MS capillary column (30 m in length × 250 μm in diameter × 0.25 μm in thickness of film). GC-MS is a simple, rapid technique used for determination of molecular weight as well as fragmentation pattern of compounds. For the purpose of identifying compounds, the fragment ions with various relative abundances were compared with library spectra. Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV), MS transfer line temperature 300 °C and ion source temperature 300 °C. Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 ml/min. The temperature was set at 60°C for 2 min, then increased to 100 °C at a rate of 10 °C/min for 5 min, then to 150 °C for 5 min, then to 200 °C for 5 min, then to 250 °C for 20 min. One microliter of the prepared oil was injected in a splitless mode. Identification of the peaks was done by comparing their mass spectra with those present in the REPLIB and MAINLIB libraries in addition to data published in the literature [38]. Percentage composition was calculated using GC peak areas.

2.4. Determination of the antimicrobial activity

2.4.1. Microorganisms and growth conditions

The used organisms were provided by the Department of Microbiology and Immunology, Faculty of pharmacy, Alexandria University. Tested strains were *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus Subtilis* (ATCC 19659), *Staphylococcus aureus* (ATCC 6538P) and *Candida albicans* (ATCC 2091). The reference strains were obtained from American Type Culture Collection. The bacterial and fungal strains were grown at 37°C and 25°C, respectively and maintained on Mueller-Hinton agar for bacteria and Sabouraud agar for fungi.

2.4.2. Agar diffusion test

The disc diffusion method [39] was employed for the determination of antimicrobial properties of essential oil. 0.1 ml of suspension of the test microorganisms comprising 10^8 CFU/ml was distributed on the solid media plates. Filter paper discs were soaked with 10 μl of the different concentrations of each oil sample (10%, 1%, 0.1%v/v), and placed on the seeded plates. Dimethyl sulphoxide (DMSO) was used as negative control to dissolve the oil. Standard discs of Ciprofloxacin (200 μg/ml), Clotrimazole (5%) were used as positive control for comparison. For 24 hours, the plates were incubated at 37°C.The diameters of inhibition zones were measured to assess antimicrobial activity.

2.5. Determination of the anticancer activity on human cancer cell lines

2.5.1. Cancer cell lines and culture

The human cancer cell lines PC3 (prostate cancer), MCF7 (breast cancer) and Huh7 (hepatocellular carcinoma) were obtained from ATCC. PC3, MCF7 and Huh7 cells were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Lonza®, Basel, Switzerland). The medium was supplemented with 10% fetal bovine serum, 10 μg/ml of insulin and 1% penicillin-streptomycin **(**Sigma–Aldrich, St. Louis, USA).

2.5.2. *In vitro* cytotoxic activity by MTT test

The cytotoxic activity of the essential oil was evaluated on cell viability using MTT-assay. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Serva, Heidelberg, Germany) was employed in this test to detect metabolically active living cells using mitochondrial succinate

dehydrogenase which converts the MTT into a dark purple insoluble formazan crystals [40, 41] with some modifications. Cells were seeded (1×10^4 cells/well) in growth medium (DMEM) into 96-well flat bottom microdilution plates and incubated at 37 °C for 24h in a 5% CO_2 incubator. Each essential oil sample was added at different concentrations to each well (0.4, 1.6, 6.3, 25 and 100 µg/ml) whereas the medium in control wells was substituted by serum free medium (SFM) containing an equivalent volume of dimethyl sulfoxide (DMSO) and incubated for another 24h. SFM was then removed and 100 µL of MTT was added to each well and incubated at 37 °C for further 3h to allow MTT to react. MTT solution was removed and 100 µL isopropanol was added to each well to dissolve the produced purple formazan crystals (MTT byproduct) with shaking for 1h at room temperature. At the end, the plates were read at a wavelength of 570 nm using a microplate reader (Bio-Rad, USA). The treated samples and the control were compared. The percent viabilities of cells were calculated using the following equation: (AT/AC) ×100

Where, AT stands for the average absorbance of cells treated with various oil concentrations and AC stands for the average absorbance of untreated cells in culture medium (control). Cytotoxicity was expressed as IC_{50}-value which was calculated as the concentration of the essential oil inhibiting cell viability by 50%. Staurosporine was utilized as a positive control. All measurements were performed in triplicate. The means and standard deviations were calculated. Data are presented as mean ± standard deviation (SD) (MS Excel 2010).

3. Results

3.1. Chemical composition of oils using GC-MS analysis

Thirty-five compounds were identified in the oils samples collected during the four seasons with molecular weight ranging from 202 to 296 m/z as shown in (Table1). Among the seasonal cycles, the major chemical components of the samples were *α*-cubebene (43.19 %), germacrene D (27.69 %), *δ*-elemene (20.41%), *α*-curcumene (16.04 %) and (Z)-caryophyllene (12.49 %). The results shown in (Figure1) revealed a variation in oil yields according to collection season, with oil yields ranging from a maximum of 0.1% (Spring season) to a minimum of 0.02% (Summer season). Sesquiterpene hydrocarbons were predominating (51.28 - 90.89%) in all the examined samples (Figure 2).

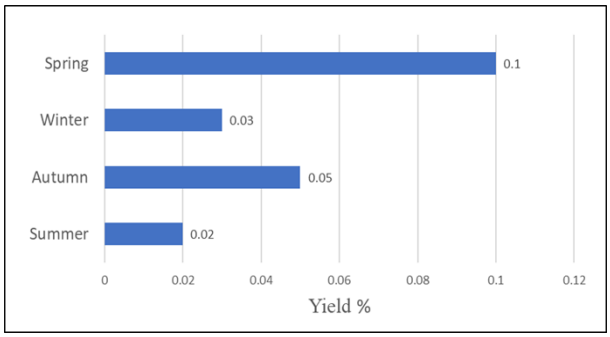


Figure 1. Essential oil yields during the different seasons

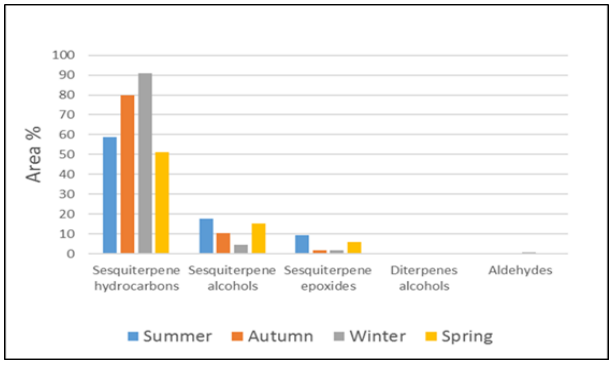


Figure 2. Essential oil composition during the different seasons

Table 1: Chemical composition of the essential oils from *M. paniculata* L. leaves collected during the four seasons

No.	R_t	Class	Constituents	Formula and molecular weight	Area %			
				$C_{15}H_{24}$	Summer	Autumn	Winter	Spring
1	15.38	STHC	***δ*-Elemene**	204	18.64	14.88	**20.41**	17.24
2	16.67	STHC	*β*-Bourbonene	204	1.49	1.28	0.89	2.35
3	16.87	STHC	*β*-Elemene	204	6.92	3.61	2.60	7.88
4	17.87	STHC	(Z)-caryophyllene	204	7.39	**12.49**	10.96	-
5	18.04	STHC	germacrene B	204	-	-	0.28	-
6	18.08	STHC	*γ*-Elemene	204	1.20	7.55	2.82	-
7	18.49	STHC	(z)-*β*-farnesene	204	-	1.17	-	-
8	19.06	STHC	*α*-Caryophyllene	204	2.81	3.03	1.83	-
9	19.31	STHC	*γ*-Muurolene	204	-	0.65	0.34	-
10	19.63	STHC	*α*-Amorphene	204	-	0.46	-	-
11	19.91	STHC	***α*-Cubebene**	204	-	-	**43.19**	-
12	19.96	STHC	**Germacrene D**	204	-	**27.69**	0.20	-
13	20.24	STHC	Aromadendrene	204	1.76	-	-	-
14	20.46	STHC	***α*-Curcumene / (Ar-curcumene)**	$C_{15}H_{22}$ 202	**16.04**	3.86	2.26	12.64

15	20.73	STHC	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-(Elixene)	$C_{15}H_{24}$ 204	-	-	2.29	-
16	21.04	STHC	*β*-Sesquiphellandrene	204	-	2.11	2.36	-
17	21.41	STHC	*δ*-Cadinene	204	-	0.73	0.46	-
18	22.02	STHC	*β*-Vatirenene	$C_{15}H_{22}$ 202	1.47	-	-	-
19	22.56	STA	*trans*-Nerolidol	$C_{15}H_{26}O$ 222	-	0.68	0.33	-
20	22.69	STA	Lanceol, *cis*	$C_{15}H_{24}O$ 220	-	0.67	-	-
21	23.86	STE	Isoaromadendrene epoxide	220	3.17	-	-	1.73
22	24.19	STA	Spathulenol	220	4.32	1.25	0.88	4.47
23	24.43	STE	Ledene oxide-(II)	220	0.99	1.37	0.68	0.54
24	24.69	STHC	*β*-Guaiene	$C_{15}H_{24}$ 204	0.97	0.30	-	-
25	25.05	STE	Aromadendrene oxide-(1)	$C_{15}H_{24}O$ 220	1.78	-	-	0.99
26	25.27	STHC	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylene bicyclo [4.1.0] heptane	$C_{15}H_{22}$ 202	-	-	-	11.17
27	25.37	STA	(-)-spathulenol	$C_{15}H_{24}O$ 220	11.53	7.28	2.89	-
28	25.81	STA	*α*-Cadinol	$C_{15}H_{26}O$ 222	0.84	0.52	0.31	-
29	25.93	STE	Caryophyllene oxide	$C_{15}H_{24}O$ 220	1.62	0.50	-	2.49
30	26.00	STE	Aromadendrene oxide-(2)	220	1.65	-	-	-
31	26.32	STA	6-(*p*-Tolyl)-2-methyl-2- heptenol [(+,-)-E-Nuciferol]	$C_{15}H_{22}O$ 218	-	-	-	3.2
32	26.63	STE	*Trans*-Z-*α*-Bisabolene epoxide	$C_{15}H_{24}O$ 220	-	-	0.97	-
33	26.74	STA	Tricyclo [5.2.2.0(1,6)] undecan-3-ol, 2-methylene-6,8,8-trimethyl-	220	0.84	-	-	7.62
34	30.14	A	*cis*, *cis*, *cis*-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$ 234	-	-	0.84	-
35	33.19	DTA	Phytol	$C_{20}H_{40}O$ 296	-	0.47	-	-
Constituents' classes								
Sesquiterpenes								
Sesquiterpene hydrocarbons					**58.69**	**79.81**	**90.89**	**51.28**
Oxygenated sesquiterpenes					**26.74**	**12.27**	**6.06**	**21.04**
Sesquiterpene alcohols					**17.53**	**10.4**	**4.41**	**15.29**
Sesquiterpene epoxides					**9.21**	**1.87**	**1.65**	**5.75**
Diterpenes								
Oxygenated diterpenes (alcohols)					-	**0.47**	-	-
Aldehydes					-	-	**0.84**	-
Total identified (%)					**85.43**	**92.55**	**97.79**	**72.32**
Extraction yields v/w (%)					**0.02**	**0.05**	**0.03**	**0.1**

(-) dash indicates absence of the compound, STHC: Sesquiterpene hydrocarbons, STA: Oxygenated sesquiterpenes (alcohols), STE: Oxygenated sesquiterpenes (epoxides), A: Aldehydes, DTA: Oxygenated diterpenes (alcohols)

3.2. Antimicrobial activity

Antimicrobial activities of the essential oil samples from *M. paniculata* L. leaves are shown in Table 2. The applied oil displayed varying degrees of efficacy against different examined microbes. Spring oil showed the highest activity, followed by summer oil, winter oil and autumn oil respectively (Figure 3). *S. aureus* and *B. subtilis* were the most susceptible microorganisms. Generally, only the gram-positive bacteria were sensitive to the tested oil.

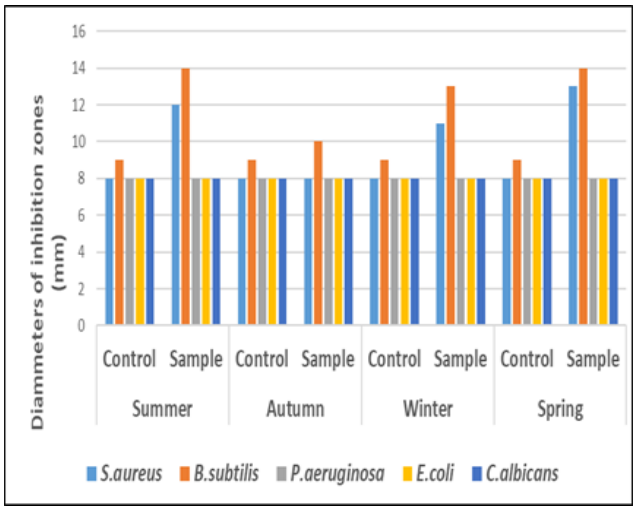


Figure 3: Seasonal effect on the antimicrobial activity of the essential oil samples

3.3. Cytotoxic activity

The effect of *M. paniculata* L. spring essential oil on the viability of PC3, MCF7 and Huh7 cells are shown in (Figure 4 a, b and c) respectively. It showed a concentration-dependent cytotoxic effects, where treating cells with concentrations of 0.4, 1.6, 6.3, 25 and 100 µg/ml inhibited the viability of PC3, MCF7 and Huh7 cells with IC_{50} values of 6.5, 1.67 and 5.78 µg ml^{-1}, respectively compared with Staurosporine with IC_{50} values of 4.02, 5.75 and 8.31 µg ml^{-1}, respectively as shown in (Figure 5).

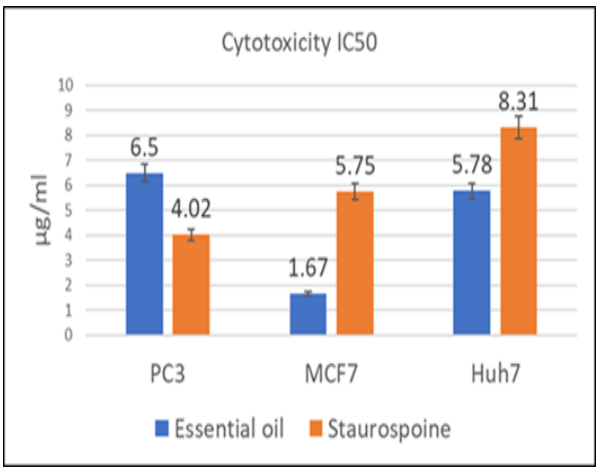
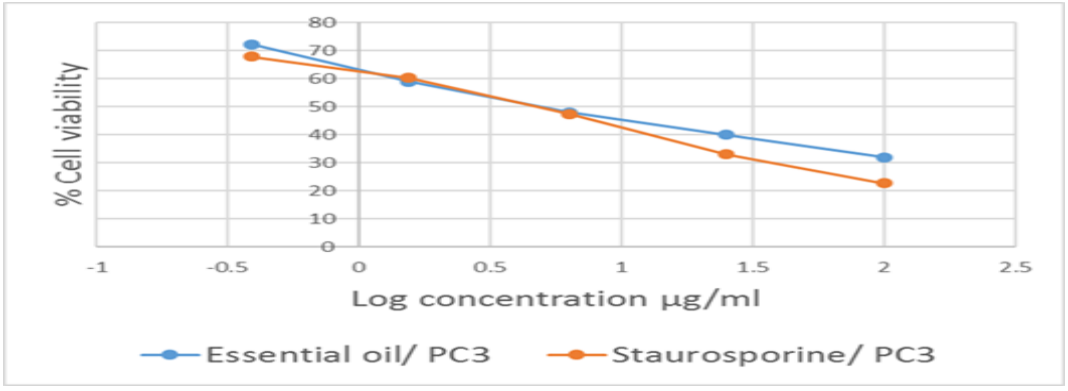


Figure 5. IC_{50} values of essential oil of *Murraya paniculata* L. against PC3, MCF7 and Huh7 compared to Staurosporine. Data are expressed as the mean± SD of three experiments each conducted in triplicate.

Table 2: The antimicrobial activity of essential oils from *Murraya paniculata* L. leaves collected during the four seasons

		Diameters of zones of inhibition (mm)									
		Bacteria								Fungi	
		Gram-positive				Gram-negative					
Tested Samples	Season	*S. aureus*		*B. subtilis*		*P. aeruginosa*		*E. Coli*		*C. albicans*	
		C	**S**	**C**	**S**	**C**	**S**	**C**	**S**	**C**	**S**
1	Summer	8	12	9	**14**	8	8	8	8	8	8
2	Autumn	8	8	9	10	8	8	8	8	8	8
3	Winter	8	11	9	13	8	8	8	8	8	8
4	Spring	8	**13**	9	**14**	8	8	8	8	8	8
Ciprofloxacin		8	30	9	30	8	30	8	30	-	-
Clotrimazole		-	-	-	-	-	-	-	-	9	18

DMSO was used as a control, S: sample, C: control



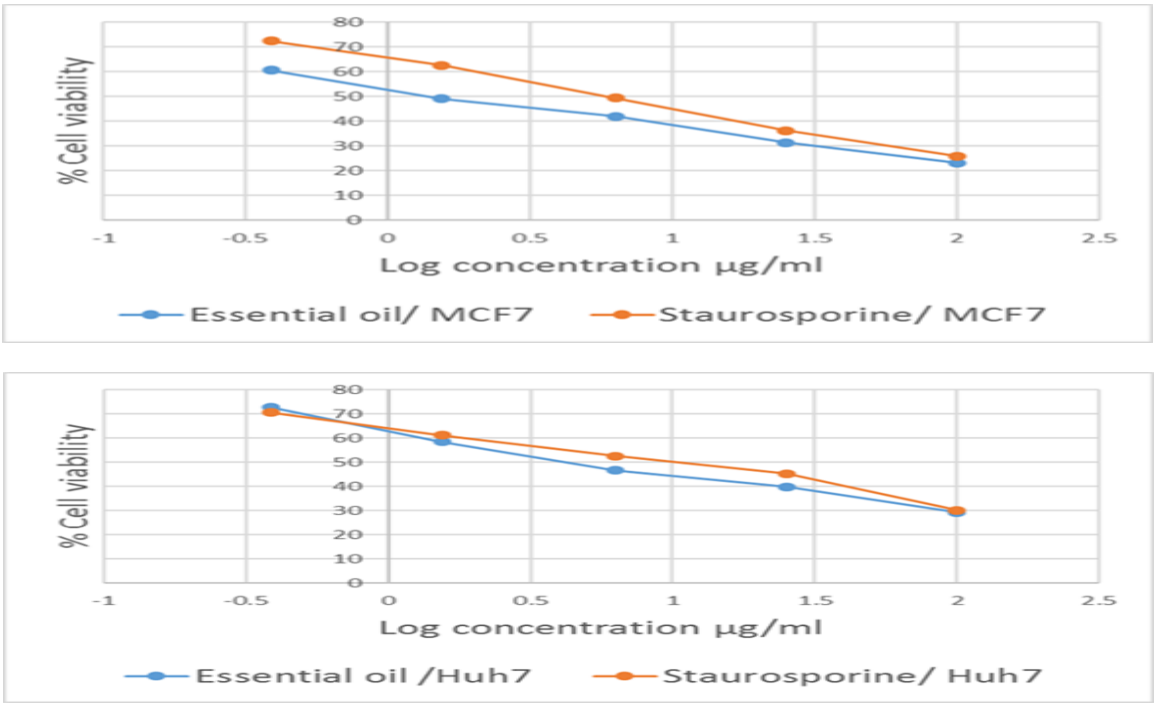


Figure 4: Cytotoxic effects of essential oil (0.4-100 µg/ml) on PC3 (a), MCF7 (b) and Huh7 (c) cell lines compared to Staurosporine

4. Discussion

Egypt climate data represent four distinct seasons; spring, summer, autumn, and winter. Each season has its characteristic conditions (Temperature, humidity, and the presence or absence of rainfall) [42].To investigate the effect of seasonal variation on the essential oils' composition and yield, the essential oils of *M. paniculata* L. leaves over the period from July 2019 to May 2020 (four different seasons) were analyzed by GC-MS for identifying the oil components. The main chemical classes identified in the oil samples during different seasons were sesquiterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated diterpenes and aldehydes. The most common class of compounds were the sesquiterpene hydrocarbons with highest percentage (90.89%) in winter followed by oxygenated sesquiterpenes with highest percentage (26.74%) in summer. Aldehydes were represented as *cis*, *cis*, *cis*-7,10,13-Hexadecatrienal which was identified only in the winter sample with a percentage of 0.84% and not identified in the rest of the studied oils samples. Diterpene alcohol was represented as phytol which was identified only in autumn sample with a percentage of 0.47% and not identified in the rest of the studied oils. The highest oil yield was observed during spring and autumn, indicating that the plant was well adapted to these climatic conditions. On the other hand, Summer and Winter samples had the lowest yield, which could be attributed to the extreme environmental and climatic conditions observed in both seasons [43]. The plant's production of essential oils is thought to be a defense mechanism and a response to various stress factors [44]. Water content is thought to be an important factor in controlling sesquiterpene content. The presence of water in leaves promotes the production of sesquiterpenes [45].This can explain the high content of sesquiterpene hydrocarbons in winter leaves oil (wet season) and a significant decrease in the percentage of that in summer and spring leaves oils (dry seasons) but this accumulation is light and temperature-dependent, which could explain why oxygenated sesquiterpenes comprised the lowest percentages among the oils studied [46]. The major chemical components of the samples among the seasonal cycles were α-cubebene (43.19 %), germacrene D (27.69 %), δ-elemene (20.41%), α-curcumene (16.04 %) and (Z)-caryophyllene (12.49%). It is possible to conclude that these components represented the typical strong odour components of *M. paniculata* L. oil. The findings of the current study in Egypt agreed with those of a previous study on the chemical composition of essential oil of *M. paniculata* L. leaves from the mountains of central Cuba, which revealed that α-cubebene, germacrene D and δ-elemene were the predominant components of the studied plant [7]. Moreover, another study on the essential oil

composition of *M. paniculata* L. leaves from China showed that *α*-cubebene, germacrene D, *δ*-elemene, *α*-curcumene and (Z)-caryophyllene were the major components of the oil [47]. *δ*-Elemene, *α*-curcumene, *β*-elemene, *β*-bourbonene, spathulenol and ledene oxide (II) were identified in all essential oils studied over all seasons, as they represent the predominant components of the studied oils. By referring to (Table 1), (Z)-caryophyllene, *γ*-elemene, *α*-caryophyllene,(-)-spathulenol and *α*-cadinol were present in all oil samples except that of spring. Germacrene B, *α*-cubebene, elixene, *trans*-Z-*α*-bisabolene epoxide and *cis*, *cis*, *cis*-7,10,13-hexadecatrienal were only identified in winter leaves while (z)-*β*-farnesene, *α*-amorphene, lanceol-*cis* and phytol were only identified in autumn oil. *γ*-Muurolene, germacrene D, *β*-sesquiphellandrene, *δ*-cadinene and *trans*-nerolidol were only identified in autumn and winter samples. Aromadendrene, *β*-vatirenene, aromadendrene oxide (2) were only present in summer oil. Isoaromadendrene epoxide, aromadendrene oxide (1) and tricyclo [5.2.2.0 (1,6)] undecan-3-ol, 2-methylene-6,8,8-trimethyl- were not identified in autumn and winter seasons. *β*-Guaiene was only present in summer and autumn samples. 4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylene bicyclo [4.1.0] heptane and 6-(*p*-tolyl)-2-methyl-2-heptenol were only identified in spring oil while caryophyllene oxide was not present in winter oil. The presence or absence of certain compounds during specific seasons reflected the significant effect of seasonal and climate conditions on the volatiles profile of the studied oils. The results revealed a great difference in the composition, percentage, and yield of the studied volatile oils, the absence or presence of some compounds, and the fluctuation of the percentages of identified compounds over the four seasons, demonstrating that the chemical profile of *M. paniculata* L. oil is affected by Egyptian seasonal and climate conditions.

This study is a part of the search for useful and promising natural compounds derived from Egyptian plants. In the current study, we discovered that essential oils of *M. paniculata* L. leaves have promising antimicrobial and cytotoxic activities. Our results revealed that summer and spring oils significantly inhibited the growth of gram-positive *B. subtilis*. It also displayed moderate activity against gram-positive *S. aureus*. On the other hand, it showed no antibacterial activity against gram-negative *P. aeruginosa* and *E. coli* and no antifungal activity. As concerns, the winter oil inhibited the growth of both S. *aureus* and *B. subtilis* with greater inhibition zone in case of *B. subtilis*. However, the other tested microbes were not susceptible to the oil. Regarding autumn oil, it inhibited the growth of *B. subtilis*. Nevertheless, no susceptibility was observed among the other tested bacterial and fungal strains. Cytotoxicity was monitored by determining the effect of the test samples on cell viability. The survival curve of the cell line after the specified time period was obtained by plotting the relationship between surviving cells and oil concentration, Their cytotoxic activity was then quantified using the survival curves in terms of IC_{50} [48]. Based on the findings, it was demonstrated that the tested oils effectively suppressed the proliferation of human prostate cancer cells (PC3), human breast cancer cells (MCF7) and human hepatocellular carcinoma (Huh7) in a dose dependent manner with different IC_{50} values and varying degrees of potency. It demonstrated the most potent antiproliferative activity against breast cancer with IC_{50} value of 1.67 μg ml^{-1} followed by liver cancer with IC_{50} of 5.78 μg ml^{-1} and prostate cancer with IC_{50} of 6.5 μg ml^{-1}, when compared to Staurosporine (positive control), which had IC_{50} values of 5.75, 8.31 and 4.02 μg ml^{-1} respectively. According to IC_{50} values, the effect of the studied oils against breast and liver cancer exceeded the effect of the standard Staurosporine. As a result, Volatiles of the *Murraya paniculata* L. leaves are promising starting materials for developing novel anticancer drugs that could be even more effective than Staurosporine. The anticancer activities observed for the oils could be attributed to the activity of volatile constituents present in the oil sample, such as (E-caryophyllene) [49].

5. Conclusions

The chemical composition and biological activities of *Murraya paniculata* L. essential oils were both influenced by the harvesting season. The seasonal variation data could be valuable in determining the best season for optimal yield. The findings of the present study indicate that *Murraya paniculata* L. essential oils cultivated in Egypt possess very good antimicrobial and anticancer properties. Further *in vivo* and clinical research investigations are recommended to elaborate the biological activities of *Murraya paniculata* L. essential oils for various medicinal applications.

Disclosure of conflict of interests

The authors declare that they have no competing interests.

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