



Chemical composition and antimicrobial activity of the essential oils of *Thevetia peruviana* and *Plumeria rubra* cultivated in Egypt

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Abstract: *Thevetia peruviana* and *Plumeria rubra* (family Apocyanaceae) are shrubs cultivated in Egypt for ornamental purposes. This study was done to compare the volatile oils of *T. peruviana* and *P. rubra* which were prepared by using the hydrodistillation method from fresh flowers and leaves of each plant. Identification and quantification of the oil components were carried out by GC/MS analysis. The major components of *T. peruviana* flowers oil were Nonadecane (37.69 %), followed by 1-Nonylcycloheptane (18.39%), while Perilla alcohol (20.49%), β -ylangene (10.62%) and β -Elemene (7.19%) were predominant in the leaves oil. On the other hand, the major constituents of *P. rubra* flowers oil were Methyl dihydroepi-jasmonate (35.41%), Linalool (14.31%) and Methyl jasmonate (11.99%), while 1-Nonylcycloheptane (32.16%) and Menthol (17.89%) were of highest abundance in the leaves oil. Also, the antimicrobial activity was investigated against *Staphylococcus aureus* ATCC 4175, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* CNCM A21, and *Candida albicans* ATCC 60193. The minimum inhibitory concentrations (MIC) were determined and compared with those of standard antibiotics (Ofloxacin and Amphotericin B), the tested oils showed a good inhibitory effect against the investigated microbial strains with a minimum inhibitory concentration (MIC) range of 25 to 300 μ g/mL except for the *P. rubra* leaves oil, which showed no activity against *Pseudomonas aeruginosa*.

Keywords: Apocyanaceae; *Plumeria rubra*; *Thevetia peruviana*; Volatile oils; Antimicrobial activity.

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1. INTRODUCTION

Essential oils (EOs) are defined as volatile secondary metabolites derived from plants that give them a distinct aroma, flavor, or all of them. They are produced by around 17,500 plant species from distinct angiosperm families e.g., Apocyanaceae, Asteraceae, Lamiaceae, Myrtaceae, Rutaceae and Zingiberaceae ¹. EOs are complicated combinations of different hydrocarbons and oxygenated compounds. Hydrodistillation, steam distillation, dry distillation, or mechanical cold pressing of plants are the most common methods for obtaining essential oils ². Examples of the most promising type of EOs are lavender volatile oil which possesses antimicrobial effects versus clinical strains of bacteria, that cause respiratory tract infections ³. Also, thyme volatile oil has potential activity against *S. aureus* and *K. pneumonia* besides cinnamon and clove oils which restricted the progression of

Salmonella typhimurium and *Listeria monocytogenes* which constitute a serious public health problem ⁴⁻⁶. *Thevetia peruviana* and *Plumeria rubra* belong to the family Apocyanaceae which is one of the most medicinally diverse families in the plant kingdom that contain essential oils in different plant parts ⁷. Considering the published data about these two plants, we found that volatile oils of the flowers and the leaves of *Thevetia peruviana* and *Plumeria rubra* cultivated in Egypt have not been investigated, and the antimicrobial activity was not assessed against the specific microorganisms in our study. Therefore, this study aims to compare the chemical components of the essential oils extracted from vegetative organs (the leaves and the flowers) of the Egyptian *Thevetia peruviana* and *Plumeria rubra* also, the EOs antimicrobial activities were investigated towards *S.aureus*, *E.coli*, *P.aeruginosa* and *Candida albicans*.

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2. METHODS

2.1. Plant material

Flowers and leaves of *Thevetia peruviana* and *Plumeria rubra* were collected in June 2019 from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt. Their identities were confirmed by Prof. Dr. Wafaa Mohamed Amer, Professor of Taxonomy, Faculty of Science, Cairo University. Voucher specimens (No. Th 2019-178) and (No. Pl 2019-179) for *T. peruviana* and *P. rubra* respectively, were deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, October 6 University.

2.2. Extraction, Identification, and Analysis of the essential oils

Specimens of fresh flowers and leaves of *T. peruviana* and *P. rubra* (1kg, each) were subjected to Hydro-distillation using Clevenger apparatus⁸, for further analysis, the essential oils were kept with anhydrous sodium sulfate and preserved in dark ampoules in the refrigerator. According to the Egyptian Pharmacopoeia (2005), the specific gravity and refractive index of each of the examined oil specimens were measured.

GC-MS analysis of volatile oil specimens from the leaves and the flowers of both plants was performed by experimental conditions described by utilizing an Agilent Technologies GC-MS system with a (7890B) gas chromatograph and mass spectrometer detector (5977A). Electron ionization (EI) at 70 eV was used to obtain mass spectra, with a spectral range of m/z 50-550 and a solvent delay of 5 minutes. Various components of the oils were identified by comparing their mass fragmentation pattern with standards reported in Adams library⁹.

The retention indices (Kovats indices) of the components of the EOs were estimated using GLC analysis of a sequence of authentic n-alkanes under identical experimental circumstances.

2.3. Antimicrobial activity

2.3.1. Microorganisms and culture media

A series of bacterial and fungal strains (available in the stock culture at the Microbiology Department, Faculty of Pharmacy, October 6 University) were used for susceptibility testing comprising of *Staphylococcus aureus* (No. ATCC 4175) as representative gram-positive bacterium while *Escherichia coli* (No. ATCC 10536) and *Pseudomonas aeruginosa* (No. CNCM A21) as

gram-negative ones. The yeast *Candida albicans* (No. ATCC 60193) was the tested fungal strain. The tested bacteria and yeast were grown on the solid culture medium Trypticase soy agar (Antibiotic Medium No. 2, Difco lab. USA) prepared by solubilization in distilled water (pH 6.5 ± 0.1 at 25°C) followed by autoclaving at 121°C for 20 min for sterilization¹⁰.

2.3.2. Antimicrobial activity testing

The antimicrobial activity of both EOs of flowers and leaves of the two plants under study was evaluated using the disc diffusion method, using Whatman filter paper discs of 0.5 cm diameter¹¹. Solutions of the investigated oil samples were prepared in DMSO (20 % v/v). Aliquots of 20 µL of each of the tested samples (corresponding to 4 µL of oil) were, separately, aseptically added on filter paper discs. The stock solution of various tested oils was 3 mg/mL and the measured volume of stock solutions was dispensed in the conical flask to prepare serial dilutions¹². A control disc containing 20 µL of DMSO was used. Ofloxacin (5 µg/disc) and Amphotericin B (5µg/disc), were used as standards. Zones of inhibition were measured for all the tested oil samples.

Minimum inhibitory concentration (MIC) was estimated using the dilution technique¹³, which involved performing a series of double-fold serial dilutions on 100-well microtiter plates to produce concentrations ranging between 0.5 to 500 µg/mL. The tested essential oils (100 µL) were added to the first well and homogenized. After performing a sequence of double-fold dilutions until the last well of the plate, 100 µL of bacterial suspension was poured into the respective wells and control wells. The plates were sealed and placed in plastic bags before being incubated for 24 hours at 37°C. The MIC is the lowest concentration of oil in which no growth may be seen by visual inspection.

3. RESULTS

3.1. Essential oil yield

On a fresh weight basis, the yields of the volatile oil obtained by hydro-distillation from the leaves and the flowers of *T. peruviana* were 0.57% w/w and 0.85% w/w, respectively, whereas the yields of essential oils obtained from the leaves and the flowers of *P. rubra* were 1.17% w/w and 1.4% w/w, sequentially.

3.2. Physical characters of oil samples

The four oil samples showed noticeable variation in their physical characters. The volatile oils obtained from *T. peruviana* leaves and flowers were colourless, miscible with 70% ethanol, and have a slightly characteristic odor. At 20°C, the refractive indices were 1.461 and 1.508, respectively, while at 25°C, the specific gravity was 0.85 and 0.87, respectively. Essential oils derived from the leaves and flowers of *P. rubra* were yellow in color, have a pleasant aromatic odor and are miscible with 70% ethanol. At 20°C, the relative refractive indices were

1.493 and 1.532, respectively, and the specific gravity of was 0.89 and 0.92 at 25°C.

3.3. Chemical composition of hydro-distilled oils

GC/MS analyses of examined oils were displayed in Tables (1 – 5) & Figure 1. The total number of constituents identified in the flowers and the leaves of *T. peruviana* under the adopted operating conditions were (20 and 23) compounds respectively, while that identified in the flowers and the leaves of *P. rubra* were (15 and 19) compounds sequentially.

Table 1. Identified components in the hydro-distilled essential oil of the flowers of *Thevetia peruviana* cultivated in Egypt

Peak No.	KI (Calculated)	KI (Reported)	Identified Compound	Formula	%
1	997	997	Heptane, 2,2,4,6,6-pentamethyl-	C ₁₂ H ₂₆	1.42
2	1128	1126	p-mentha-1(7),8-dien-2-ol	C ₁₀ H ₁₆ O	2.08
3	1224	1227	Methyl nonanoate	C ₁₀ H ₂₀ O ₂	1.57
4	1370	1370	α-ylangene	C ₁₅ H ₂₄	1.61
5	1378	1384	α-Copaene	C ₁₅ H ₂₄	1.46
6	1422	1420	α-Caryophyllene	C ₁₅ H ₂₄	1.81
7	1457	1460	β- Caryophyllene	C ₁₅ H ₂₄	0.39
8	1534	1537	Butylated Hydroxytoluene	C ₁₅ H ₂₄ O	5.91
9	1661	1661	α-Cadinol	C ₁₂ H ₁₈ O	0.52
10	1743	1745	4-t-Butyl-2-(1-methyl-2-nitroethyl) cyclohexanone	C ₁₃ H ₂₃ NO ₃	4.01
11	1899	1903	n-Heptadecanal	C ₁₇ H ₃₄ O	2.05
12	1910	1910	Nonadecane	C ₁₉ H ₄₀	37.69
13	1935	1940	1,2-15,16-Diepoxyhexadecane	C ₁₆ H ₃₀ O ₂	8.4
14	1969	1971	1-Nonylcycloheptane	C ₁₆ H ₃₂	18.39
15	2100	2110	Methyl linolenate	C ₁₉ H ₃₂ O ₂	0.54
16	2145	2146	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	5.74
17	2159	2160	2-Dodecen-1-yl(-)dihydro-2,5-furandion	C ₁₆ H ₂₆ O ₃	3.04
18	2241	2245	1,3,12-Nonadecatriene-5,14-diol	C ₁₉ H ₃₄ O ₂	0.87
19	2481	2482	Methyl 4,7,10,13-hexadecatetraenoate	C ₁₇ H ₂₆ O ₂	1.22
20	2632	2630	Ergostenal	C ₂₈ H ₄₈ O	1.28
Total identified percentage					100

Table 2. Identified components in the hydro-distilled essential oil of the leaves of *Thevetia peruviana* cultivated in Egypt.

Peak No.	KI (Calculated)	KI (Reported)	Identified Compound	Formula	%
1	1023	1024	p-Cymene	C ₁₀ H ₁₄	1.41
2	1127	1129	Cyclopentanol, 1-(1-methylene-2-propenyl)	C ₉ H ₁₄ O	0.67
3	1149	1150	Isopulegol	C ₁₀ H ₁₈ O	5.25
4	1174	1174	Isomenthol	C ₁₀ H ₂₀ O	4.50
5	1261	1261	Perilla alcohol	C ₁₀ H ₁₆ O	20.49
6	1369	1370	α-Longipinene	C ₁₅ H ₂₄	2.78
7	1376	1384	α-Copaene	C ₁₅ H ₂₄	5.36
8	1392	1395	β-Elementene	C ₁₅ H ₂₄	7.19
9	1406	1405	β-ylangene	C ₁₅ H ₂₄	10.62
10	1422	1420	α-Caryophyllene	C ₁₅ H ₂₄	0.24
11	1434	1434	β-copaene	C ₁₅ H ₂₄	6.54
12	1456	1460	β- Caryophyllene	C ₁₅ H ₂₄	3.46
13	1466	1470	Aromandendrene	C ₁₅ H ₂₄	2.26
14	1469	1471	Aristolochene	C ₁₅ H ₂₄	3.99
15	1478	1479	β-Acoradiene	C ₁₅ H ₂₄	2.86
16	1520	1520	γ-Cadinene	C ₁₅ H ₂₄	3.78
17	1534	1534	Butylated Hydroxytoluene	C ₁₅ H ₂₄ O	1.46
18	1562	1560	Germacrene-B	C ₁₅ H ₂₄	3.19
19	1743	1740	4-t-Butyl-2-(1-methyl-2-nitroethyl) cyclohexanone	C ₁₃ H ₂₃ NO ₃	1.14
20	1935	1935	1,2-15,16-Diepoxyhexadecane	C ₁₆ H ₃₀ O ₂	0.56
21	1969	1971	1-Nonylcycloheptane	C ₁₆ H ₃₂	6.92
22	2103	2110	Methyl linolenate	C ₁₉ H ₃₂ O ₂	1.46
23	2144	2146	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	3.85
Total identified percentage					99.98

KI: Kovats index

Table 3. Identified components in the hydro-distilled essential oil of the flowers of *Plumeria rubra* cultivated in Egypt.

Peak No.	KI (Calculated)	KI (Reported)	Identified Compound	Formula	%
1	1008	1008	Δ-Carene	C ₁₀ H ₁₆	0.81
2	1100	1100	Linalool	C ₁₀ H ₁₈ O	14.31
3	1152	1150	Isopulegol	C ₁₀ H ₁₈ O	1.54
4	1165	1165	α-Acetoxytoluene	C ₉ H ₁₀ O ₂	1.86
5	1186	1188	α-Terpineol	C ₁₀ H ₁₈ O	4.45
6	1228	1230	Citronellol	C ₁₀ H ₂₀ O	1.49
7	1452	1450	δ-Patchoulene	C ₁₆ H ₂₈	4.93
8	1532	1532	Lilial	C ₁₄ H ₂₀ O	6.89
9	1655	1655	Methyl jasmonate	C ₁₃ H ₂₀ O ₃	11.99
10	1683	1680	n-Hexyl salicylate	C ₁₃ H ₁₈ O ₃	0.53
11	1853	1855	Polygodial	C ₁₅ H ₂₂ O ₂	1.11
12	1904	1904	Phenylethyl Alcohol	C ₈ H ₁₀ O	0.84
13	2145	2146	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	3.03
14	2260	2260	Methyl dihydroepi-jasmonate	C ₁₃ H ₂₂ O ₃	35.41
15	2627	2630	Ergostenal	C ₂₈ H ₄₈ O	10.23
Total identified percentage					100

KI: Kovats index

Table 4. Identified components in the hydro-distilled essential oil of the leaves of *Plumeria rubra* cultivated in Egypt.

Peak No.	KI (Calculated)	KI (Reported)	Identified Compound	Formula	%
1	1023	1024	p-Cymene	C ₁₀ H ₁₄	0.86
2	1148	1150	Isopulegol	C ₁₀ H ₁₈ O	3.09
3	1218	1216	p-Mentha-1,8-dien-7-ol	C ₁₀ H ₁₆ O	9.05
4	1372	1370	α -Ylangene	C ₁₅ H ₂₄	0.38
5	1452	1450	δ -Patchoulene	C ₁₆ H ₂₈	2.44
6	1457	1460	β - Caryophyllene	C ₁₅ H ₂₄	1.16
7	1469	2146	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	5.29
8	1536	1534	Butylated hydroxytoluene	C ₁₅ H ₂₄ O	8.74
9	1639	1640	Menthol	C ₁₀ H ₂₀ O	17.89
10	1724	1725	5,7-Dodecadien-1,12-diol	C ₁₂ H ₁₈ O ₂	0.46
11	1743	1740	4-t-Butyl-2-(1-methyl-2-nitroethyl)	C ₁₃ H ₂₃ NO ₃	4.25
12	1810	1810	7,11-Hexadecadienal	C ₁₆ H ₂₈ O	3.17
13	1933	1935	1,2-15,16-Diepoxyhexadecane	C ₁₆ H ₃₀ O ₂	2.54
14	1970	1971	1-Nonylcycloheptane	C ₁₆ H ₃₂	32.16
15	2110	2110	2,5-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	1.19
16	2160	2160	2-Dodecen-1-yl(-)dihydro-2,5-furandion	C ₁₆ H ₂₆ O ₃	3.9
17	2240	2245	1,3,12-Nonadecatriene-5,14-diol	C ₁₉ H ₃₄ O ₂	1.14
18	2481	2482	Methyl 4,7,10,13-hexadecatetraenoate	C ₁₇ H ₂₆ O ₂	1.45
19	2626	2630	Ergostenal	C ₂₈ H ₄₈ O	0.84
Total identified percentage					100

KI: Kovats index

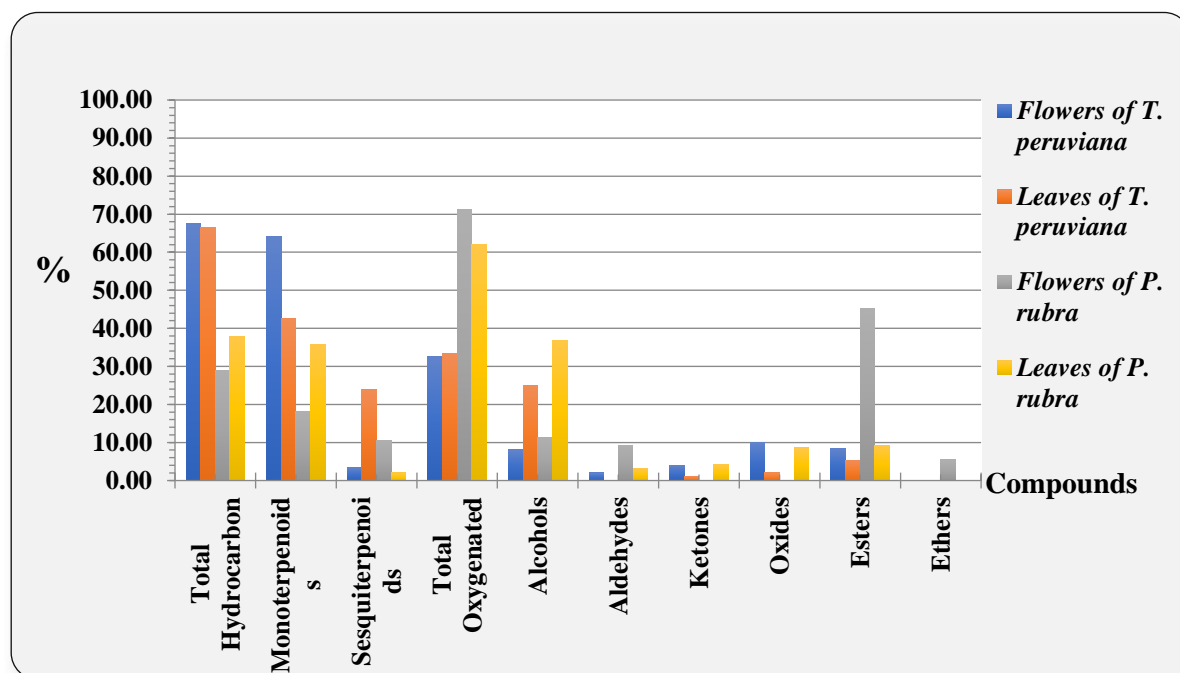
**Figure 1.** Histogram representing the percentage of the different volatile oil constituents present in the flowers and the leaves of *T. peruviana* & *P. rubra*

Table 5. Comparison between the percentage of the chemical composition of the hydro-distilled essential oils of both plants

Chemical Constituents	Percentage of the volatile oil obtained from:			
	<i>Thevetia peruviana</i>		<i>Plumeria rubra</i>	
	Flowers	Leaves	Flowers	Leaves
	%	%	%	%
Total Hydrocarbon:	67.51	66.58	28.82	37.84
Monoterpenoids	64.09	42.71	18.17	35.84
Sesquiterpenoid	3.42	23.87	10.65	2.00
Total Oxygenated constituents:	32.49	33.4	71.18	62.16
Alcohols	8.06	24.86	11.19	36.87
Aldehydes	2.05	(-)	9.34	3.17
Ketones	4.01	1.14	(-)	4.25
Oxides	9.97	2.09	(-)	8.57
Esters	8.4	5.31	45.15	9.3
Ethers	(-)	(-)	5.5	(-)

Table 6. Antimicrobial effect of the four tested volatile oils samples obtained from the flowers and the leaves of *Thevetia peruviana* and *Plumeria rubra* cultivated in Egypt.

Tested solutions	Diameter of zone of inhibition in mm (Relative potency %)*			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
Oil from the flowers of <i>T.peruviana</i>	8 (29%)	6 (18%)	8 (35%)	17 (71%)
Oil from the leaves of <i>T.peruviana</i>	15 (54%)	5 (15%)	7 (30%)	3 (13%)
Oil of the flowers of <i>P. rubra</i>	17 (61%)	9 (27%)	20 (87%)	4 (17%)
Oil of the leaves of <i>P. rubra</i>	24 (86%)	20 (61%)	0	20 (83%)
Ofloxacin (5µg/disc)	28 (100%)	33 (100%)	23 (100%)	0
Amphotericin B (5µg/disc)	0	0	0	24 (100%)

*Percentage of Relative Potency as compared to standard.

Table 7. The minimum inhibitory concentration (MIC) values of the four tested volatile oils samples from the flowers and the leaves of *Thevetia peruviana* and *Plumeria rubra* cultivated in Egypt.

Tested solutions	Minimum inhibitory concentration (MIC; µg/mL)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
Oil from the flowers of <i>T.peruviana</i>	100	150	100	50
Oil from the leaves of <i>T.peruviana</i>	100	300	150	300
Oil of the flowers of <i>P. rubra</i>	100	150	50	300
Oil of the leaves of <i>P. rubra</i>	25	50	(-)	50
Ofloxacin	1.562	3.125	3.125	NT
Amphotericin B	NT	NT	NT	12.5

Values are the mean of two replicate; (-): not active; NT: not tested

4. DISCUSSION

The hydrocarbon substances were revealed to be dominant in the whole chromatographic profiles of *T. peruviana* oil samples in both flowers and leaves (Tables 1,2 and 5) represented by; nonadecane (37.69%) and 1-nonylcycloheptane (18.39%) followed by 1,2-15,16-Diepoxyhexadecane (8.4%), Butylated Hydroxytoluene (5.91%) and Linoleic acid ethyl ester (5.74%) in the flowers and were dominated by β -ylangene (10.62%) and β -Elemene (7.19%) in the oil leaves followed by 1-nonylcycloheptane (6.92%), β -copaene (6.54%) and α -Copaene (5.36%). Total oxygenated compound (33.4%) represented by Perilla alcohol which was the major constituent (20.49%) in *T. peruviana* leaves followed by Isopulegol (5.25%) and Isomenthol (4.50%). From the previous results, we can figure out that monoterpenoids constitute (64.09%) in the flowers while in the leaves they constitute only (42.71%). Also, the percentage of sesquiterpenoids in flowers and leaves were (3.42% and 23.87%) respectively.

Regarding, oxygenated constituents in the leaves of *T. peruviana* represented by alcohols reaching (24.86%) while in flowers, only represented by Diepoxyhexadecane (8.4%).

On the other hand, the chromatographic profiles for both the flowers and the leaves of both oil samples of *P. rubra* appeared to be dominated by oxygenated compounds (71.18% in flowers and 62.16% in leaves). Methyl dihydroepi-jasmonate (35.41%) and methyl jasmonate (11.99%) were found to be major constituents in the flowers in addition to Ergostenal (10.23%), Lillial (6.89%) and α -Terpineol (4.45%). While menthol (17.89%), p-mentha-1,8-dien-7-ol (9.05%), Butylated

hydroxytoluene (8.74%) and Linoleic acid ethyl ester (5.29%) were the major constituents in the leaves. Alcohols were the major constituent in the leaves sample (36.87%) of the total oxygenated compounds in addition to esters (9.3%) in contrast to the flowers oil sample, esters were the major constituent represented by (45.15%), while alcohols represented only by (11.19%). Moreover, total hydrocarbon constitutes (37.84%) in the leaves of *P. rubra* while in the flowers were (28.82%), results are shown in (Tables 3-5).

Our study was in accordance with the study conducted by Khang et. al., [2020] ¹⁴ as it proved that the compositions of the essential oils of different parts of *T. peruviana* composed of monoterpenes and sesquiterpenes as major constituents.

On the other hand, a study done on the Chinese *Plumeria rubra* flowers figured out that it is rich in hydrocarbons (38.6%) ¹⁵ which is higher than Egyptian *P. rubra* (28.82%) and the major constituent was the oxygenated compounds (71.18%) represented by esters (45.15%). These variations may be attributed due to the chemical composition of volatile oils can be altered by cultivation circumstances, geographical origin, weather, genotype, and harvesting season ¹⁶.

5. CONCLUSIONS

Our findings showed that GC/MS profiles of the volatile oils extracted from both plants revealed consistent quantitative and qualitative variations in their chemical composition. EOs of *T. peruviana* flowers exhibit an excellent antifungal activity while the leaves display a good activity against *S. aureus*.

Moreover, *Plumeria rubra* flowers have good activity against *P. aeruginosa* but leaves showed potent activities against *S. aureus*, some Gram-negative bacteria in addition to *Candida albicans*. This will motivate us to use these oils in the therapy of infectious diseases as well as alternative antimicrobial agents for the preservation of foods and pharmaceutical industries.

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Conflicts of Interest: The authors declare no conflict of interest is associated with this publication.

Author Contribution: Shaimaa A. El Zanaty performed the hydrodistillation to obtain essential oils, participated in GC/MS analysis, and wrote the paper. Noha A. Seif Eldein revised and finalized the paper. Eman A. El Gebaly shared in the antimicrobial study. Elsayed M. El Ghaly revised and finalized the paper. Heba A. El Gizawy participated in the interpretation of GC/MS charts and revised the paper and conceived the project.

List of Abbreviations: MIC: Minimum Inhibitory Concentration; EOs: Essential oils; Kg: Kilogram; GC: Gas Chromatography; MS: Mass Spectrometry; GLC: Gas Liquid Chromatography; KI: Kovats Index; NT: Not Tested.

REFERENCES

1. Bocquet L, Sahnaz S, Rivière C. Sustainable Development and Biodiversity: An overview of the antimicrobial properties of hop. 2018. 31–57 p.
2. Stringaro A, Colone M, Angiolella L. Antioxidant, Antifungal, Antibiofilm, and Cytotoxic Activities of *Mentha* spp. Essential Oils. *Medicines*. 2018 Oct 21;5(4):112.
3. Fabio A, Cermelli C, Fabio G, Nicoletti P, Quaglio P. Screening of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infections. *Phyther Res*. 2007 Apr;21(4):374–7.
4. Salehi B, Abu-Darwish MS, Tarawneh AH, Cabral C, Gadetskaya A V., Salgueiro L, et al. *Thymus* spp. plants - Food applications and phytopharmacy properties. *Trends Food Sci Technol*. 2019;85(January):287–306.
5. Brnawi WI, Hettiarachchy NS, Horax R, Kumar-Phillips G, Ricke S. Antimicrobial activity of leaf and bark Cinnamon essential oils against *Listeria monocytogenes* and *Salmonella typhimurium* in broth system and on celery. *J Food Process Preserv*. 2019;43(3):1–8.
6. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li H Bin. Antibacterial and antifungal activities of spices. *Int J Mol Sci*. 2017;18(6):1–62
7. Islam MS, Lucky RA. a Study on Different Plants of Apocynaceae Family and Their Medicinal Uses. *Univers J Pharm Res*. 2019 Mar 7;4(1):42–6.
8. Cassel E, Vargas RMF, Martinez N, Lorenzo D, Dellacassa E. Steam distillation modeling for essential oil extraction process. *Ind Crops Prod*. 2009 Jan 1;29(1):171–6.
9. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry No.Ed.4. 2007.
10. Bridson EY, Brecker A. Design and Formulation of Microbial Culture Media. *Methods Microbiol*. 1970 Jan 1;3:229–95.
11. Zaidan MR, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop Biomed*. 2005;22(2):165–70.
12. Dickert H, Machka K, Braveny I. The uses and limitations of disc diffusion in the antibiotic sensitivity testing of bacteria. *Infection*. 1981 Jan;9(1):18–24.
13. El-Nashar HAS, Mostafa NM, El-Badry MA, Eldahshan OA, Singab ANB. Chemical composition, antimicrobial and cytotoxic activities of essential oils from *Schinus polygamus* (Cav.) *cabrera* leaf and bark grown in Egypt. *Nat Prod Res*. 2020;0(0):1–4.
14. Khang P Van, Mai XT, Hoang Phu H, Thuong SD, Sida S. Extraction, chemical compositions, and cytotoxic activities of essential oils of *Thevetia peruviana*. *Toxicol Environ Chem*. 2020;102(1–4):124–31.

15. Liu Y, Wang H, Wei S, Yan Z. Chemical Composition and Antimicrobial Activity of the Essential Oils Extracted by Microwave-Assisted Hydrodistillation from the Flowers of Two *Plumeria* Species. *Anal Lett.* 2012;45(16):2389–97.
16. Moghaddam M, Mehdizadeh L. Chemistry of Essential Oils and Factors Influencing Their Constituents. *Soft Chemistry and Food Fermentation.* 2017. 379–419 p.
17. El-Sakhawy FS, El-Tantawy ME, Ross SA, El-Sohly MA. Composition and antimicrobial activity of the essential oil of *Murraya exotica* L. *Flavour Fragr J.* 1998 Jan;13(1):59–62.
18. He W, Shi C, Long X, Liu X, Zhao X. Antimicrobial activity and mechanism of action of Perilla essential oil against *Staphylococcus aureus*. *E3S Web Conf.* 2020;145:3–8.
19. Herman A, Tambor K, Herman A. Linalool Affects the Antimicrobial Efficacy of Essential Oils. *Curr Microbiol.* 2016 Feb 1;72(2):165–72.
20. Al-Bayati FA. Isolation and identification of antimicrobial compound from *Mentha longifolia* L. leaves grown wild in Iraq. *Ann Clin Microbiol Antimicrob.* 2009 Jun 12;8(1):1–6.