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Effect of seasonal variations on essential oil production and composition of *Plectranthus amboinicus* (Lour.) grow in Egypt

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Abstract

Essential oils extracted from aerial parts of *Plectranthus amboinicus* (Lour.) and its chemical constituents as affected by four seasonal, namely summer, winter, spring and autumn growing variation were investigated. The hydro - distilled essential oil content ranged from tr (< 0.1%) to 0.5%. The highest essential oil contents (0.2% or 0.5 ml plant^{-1}) were recorded in summer. The lowest essential oil contents (tr % or 0.1 ml plant^{-1}) were recorded in winter. The essential oils consists of carvacrol as the most abundant component, followed by thymol. The highest amount of carvacrol (15.9%) resulted from the essential oil extracted from herbs collected in autumn. On the other hand the highest amount of thymol (12.7%) produced from the essential oil extracted from herbs collected in spring. The chemical classes of essential oil such as oxygenated monoterpenes and oxygenated sesquiterpenes increased in summer and were recorded values of 62.3 and 11.5%, respectively compared with other seasons. On the other hand monoterpene hydrocarbons and sesquiterpene hydrocarbons were increased in winter and autumn seasons which recorded the values of 31.4 and 22%, respectively. Alcohols are the major constituents of the oxygenated monoterpenes of *Plectranthus amboinicus* (Lour.) essential oil. Carvacrol and thymol represented the highest concentrations among the alcohols. This indicates that *Plectranthus amboinicus* (Lour.) essential oil grown in Egypt belongs to the carvacrol and thymol chemo - type.

Introduction

Plectranthus amboinicus (Lour.) belongs to the family *Lamiaceae* (*Labiatae*) and it is a perennial with 3 – 10 years of life span. It grows naturally and distributed in tropical Africa and Asia. Previously, the antifungal activity of *P. amboinicus* (Lour.) essential oil has been reported by Murthy *et al.* (2009). Senthilkumar and Venkatesalu (2010) showed that the essential oil of *P. amboinicus* (Lour.) is one of the expensive and ecofriendly sources of natural mosquito larvicidal agent to control/reduce the population of malarial vector mosquito.

The variation in chemical composition of the essential oils is known to vary with the seasonal variation (Senthilkumar and Venkatesalu, 2010). Rao *et al.* (1996) indicated that the lowest values of essential oil content, linalool as well as the maximum values of citronellol for rose-scented geranium (*Pelargonium* species) resulted in summer months. On the other hand the percentage of geraniol was the highest during cool winter season months followed by rainy and autumn season months; isomenthone and γ-eudesmol did not exhibit any definite seasonal trends. *Santolina rosmarinifolia* essential oil yields increased in the months of March, April, May and June (Pala-Paula *et al.*, 2000). Also Pala-Paula *et al.*, 2000 found that there is a significant correlation between *Santolina rosmarinifolia* essential oil and temperature. The composition of essential oil obtained from *Ocimum gratissimum* leaves in different seasons of the year was reported by Murbach Freire *et al.* (2006), the amounts of phenylpropanoid eugenol and the monoterpene 1, 8-cineole were the maximum in all four seasons. In the essential oil extracted in the spring the sesquiterpene components appear in the highest relative percentage, whereas monoterpene linalool was not detected in autumn. Chemical composition of the essential oils from aerial parts of basil (*Ocimum basilicum* L.) as affected by four seasonal, namely summer, autumn, winter and spring growing variation were investigated. The maximum amounts were observed in winter while minimum in summer, samples collected in winter were found to be richer in oxygenated monoterpenes, while those of summer were higher in sesquiterpene hydrocarbons. The contents of most of the chemical constituents varied significantly with different seasons (Hussain, 2008). The maximum amount of essential oil extracted from *Tetradenia riparia* (Hochst.) Codd was resulted in winter and the minimum was in spring (Gazim *et al.*, 2010). Independent of season, among which thymol was the main component of *Origanum syriacum* essential oil in the summer and *p*-cymene was the

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main component in the early spring. From the other compounds occurred in lower amounts, γ–Terpinene ranged from 0.6 - 19.1% and its maximum level was observed in summer, α–terpinene varied between 0.7 - 4.9% and the maximum level detected in summer season, carvacrol values ranged from 0.7 - 8.9% with the maximal value spring. To obtain favorable phenol content of the oil, *O. syriacum* should be harvested in summer months (Toncer *et al.*, 2010). The chemical composition of the volatile oils of *L. flavescens* and *L. petersonii* did not show any significant seasonal variation in the major components, while for *Leptospermum madidum* subsp. sativum the levels of major constituents of the volatile oils varied with the harvest season (Demuner *et al.*, 2011). The composition of the *Pimenta pseudocaryophyllus* (Gomes) essential oil changed month by month. The best yield of oil was obtained in November, and the major component chavibetol was present in all samples. The chavibetol content showed significant seasonal variation, with the maximum percentages of 69.1% and 70.9% measured in January and November, respectively (Barata *et al.*, 2011).

It is known that climatic conditions can change the vegetal secondary metabolism and consequently, alter the composition of essential oils, throughout the seasons of the year. Thus, the aims of the present study were to investigate whether seasonal variations in composition of essential oil of *Plectranthus amboinicus* (Lour.) and acclimate this plant under Egyptian climatic conditions to use as a natural source of essential oil.

Materials and Methods

Plant material

Uniform seedlings of *Plectranthus amboinicus* (Lour.) were transplanted into plastic pots (30 cm diameter and 50 cm height) in open field of the experimental farm of National Research Centre (NRC) during the end of February 2011. Herb of *Plectranthus amboinicus* (Lour.) was collected from a cultivated plants by cutting the plants 5 cm above the soil surface. The harvests were accomplished in the middle of each season [summer (July), winter (February), spring (April) and autumn (October)] during the year of 2012. Total fresh and dry mass (g plant^{-1}) were recorded.

Essential oil isolation

Fresh mass (aerial part) was collected from each seasons then 300 g from each replicate of all seasons was subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oil content was calculated as a relative percentage (v/w). In addition, total essential oils (ml plant^{-1}) were calculated by using the dry mass. The essential oils extracted from *Plectranthus amboinicus* (Lour.) were collected from each season and dried over anhydrous sodium sulphate to identify the chemical constituents. Each extraction of essential oil is identified by the name of the season in which it was harvested. Meteorological data at Giza, Egypt during the growing seasons are presented in Table I.

GC/MS

The essential oil was analyzed on a VG analytical 70 - 250S sector field mass spectrometer, 70 eV, using a SPsil5, 25 m x 30 m, 0.25 μm coating thickness, fused silica capillary column, injector 222°C, detector 240°C, linear temperature 80–270°C at 10°C/min. Diluted samples (1/100, v/v, in n - pentane) of 1 ml were injected, at 250°C, manually and in the split less mode flame ionization detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same temperature program.

Qualitative and quantitative analyses

Identifications were made by library searches (Adams, 2007) combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature (Adams, 2007) or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature (Adams, 2007). Component relative concentrations were calculated based on GC peak areas without using correction factors.

Statistical analysis

In this experiment, one factor was considered: seasonal variations. For each season there were 4 replicates, each of which had 10 pots; in each pot 3 individual plants were planted. The experimental design followed a complete random block design. According to Snedecor and Cochran (1990). The averages of data were statistically analyzed using one-way analysis of variance (ANOVA-1). Significant values determined according to P values (P “0.05 = significant, P < 0.01 = more significant and P < 0.001 = highly significant). The applications of that technique were according to the STAT-ITCF program

(Foucart, 1982).

Results and Discussion

Effect of seasonal variations on essential oil and its constituents

Essential oil content (% and ml plant^{-1}) affected with various seasons. The highest essential oil contents (0.2 % or 0.5 ml plant^{-1}) were recorded in summer (Table II). The lowest essential oil contents (tr % or 0.1 ml plant^{-1}) were recorded in winter. ANOVA indicated that both essential oil percentage and yield (ml plant^{-1}) were significant in seasonal variations (Table II).

Eighteen constituents were identified in essential oil extracted from *Plectranthus amboinicus* (Lour.) aerial parts, accounting for 95.4 – 99.9% of total constituents, and belong to four chemical main classes. Oxygenated monoterpenes [OM] (44.7 - 62.3%) class was the major one, the remaining fractions as monoterpene hydrocarbons [MH] (8.3 - 31.4%), sesquiterpene hydrocarbons [SH] (11.6 - 22.0%) and oxygenated sesquiterpenes [OS] (2.8 - 11.5%) formed the minor classes (Table III). The main constituents of *Plectranthus amboinicus* (Lour.) essential oil as detected by GC/MS were carvacrol and thymol. The highest amount of carvacrol (15.9%) resulted from the essential oil extracted from herbs collected in autumn. On the other hand the highest amount of thymol (12.7%) produced from the essential oil extracted from herbs collected in spring. Alcohols are the major constituents of the OM of *Plectranthus amboinicus* (Lour.) essential oil. Carvacrol and thymol represented the highest concentrations among the alcohols. This indicates that *Plectranthus amboinicus* (Lour.) essential oil grown in Egypt belongs to the carvacrol and thymol chemo - type. The chemical classes of essential oil such as OM and OS increased in summer and were recorded values of 62.3 and 11.5%, respectively compared with other seasons. On the other hand MH and SH were increased in winter and autumn seasons which recorded the values of 31.4 and 22%, respectively. ANOVA indicated that the changes in all constituents were highly significant for seasonal variations except the constituents of myrcene and trans-Sabinene hydrate were insignificant. As well as the changes in all essential oil classes were highly significant (Table III). Similar constituents were found by Senthilkumar and Venkatesalu (2010) of *Plectranthus amboinicus* (Lour.) under Indian conditions. Our results are in agreement with the findings of Otan *et al.* (1994) and Kokkini (1996) stated that, the season of collecting may strongly affect the essential oil yield of the plants

Table 1. Meteorological data at Giza, Egypt during the growing season (2012).

Seasons	T (°C)		Rs	RH	ETp
	Max.	Min.	(MJm^{-2}d^{-1})	(%)	(mmd^{-1})
Summer	35.4	20.5	35.8	90.3	49.6
winter	18.0	10.9	24.9	48.3	28.3
Spring	27.5	14.3	26.7	70.6	30.4
Autumn	22.6	10.5	13.0	48.2	23.1

Monthly average. T: temperature; Rs: solar radiation; RH: relative humidity; ETp: potential evapotranspiration.

Table 2. Effect of seasonal variations on essential oil content

Seasons	Essential oil content	
	%	ml plant^{-1}
Summer	0.2	0.5
Winter	tr	0.1
Spring	0.1	0.2
Autumn	0.1	0.2
F Ratio	6.9*	6.5*

*P ≤ 0.05 according to F-values of the 1-way analysis of variance (ANOVA-1).
**P < 0.01 according to F-values of the 2-way analysis of variance (ANOVA-1).
***P < 0.001 according to F-values of the 1-way analysis of variance (ANOVA-1)
tr = < 0.1% or < 0.1 g.

Table 3. Effect of seasonal variations on essential oil constituents

No.	Components	RI [a]	RI [b]	Seasonal variations				F Ratio
				Summer	Winter	Spring	Autumn	
1	1-Octene	788	792	1.6	8.8	1.0	3.2	277.4***
2	Myrcene	988	991	0.8	0.8	0.9	0.8	0.2
3	α-Terpinene	1014	1018	1.4	6.0	5.0	5.2	22.8***
4	*ρ*-Cymene	1020	1082	1.4	6.9	0.8	7.3	967.0***
5	β-Phellandrene	1025	1031	1.5	6.4	1.1	7.3	245.4***
6	γ-Terpinene	1054	1062	1.6	2.5	0.8	2.1	23.0***
7	trans-Sabinene hydrate	1098	1068	0.6	0.6	0.6	0.5	0.8
8	Methyl octanoate	1123	1107	1.9	2.1	1.5	5.6	618.7***
9	Borneol	1165	1160	9.5	5.9	1.3	0.4	551.9***
10	α-Terpineol	1186	1189	4.3	0.5	5.3	5.9	335.1***
11	Dihydrocarveol	1192	1192	5.6	4.7	6.8	0.5	900.0***
12	Estragole	1195	1195	6.3	3.9	7.2	4.4	97.2***
13	Thymol	1289	1290	11.7	9.3	12.7	9.1	211.6***
14	Carvacrol	1298	1298	13.4	15.2	13.2	15.9	53.3***
15	Undecanal	1305	1156	9.0	6.5	11.3	2.4	348.3***
16	α-Humulene	1452	1450	11.5	8.2	10.0	11.1	20.1***
17	β-Selinene	1489	1485	6.0	3.4	10.7	10.9	134.3***
18	Caryophyllene oxide	1582	1581	11.5	8.2	8.2	2.8	458.2***
MH = Monoterpene Hydrocarbons				8.3	31.4	9.6	25.9	282.7***
OM = Oxygenated Monoterpenes				62.3	48.7	59.9	44.7	5819.7***
SH = Sesquiterpene Hydrocarbons				17.5	11.6	20.7	22.0	198.4***
OS = Oxygenated Sesquiterpenes				11.5	8.2	8.2	2.8	458.2***
Total identified				99.6	99.9	98.4	95.4	

RI[a] = retention index on SPB-5 column.
RI[b] = retention index on SPB-5 column [from literature (Senthilkumar and Venkatesalu, 2010)].
*P ≤ 0.05 according to F-values of the one-way analysis of variance (ANOVA-1).
**P < 0.01 according to F-values of the one-way analysis of variance (ANOVA-1).
***P < 0.001 according to F-values of the one-way analysis of variance (ANOVA-1).

and the concentration of its main components. The variations in essential oil content and composition could be due to its effect of different seasons on enzymes activity and metabolism improvements (Burbott and Loomis, 1969). Studies conducted by Lima *et al.* (2003) showed that the amount of special metabolites produced during the development of the plant can be affected by radiation (high or low), temperature (high or low), precipitation (high, low, and total dry matter), winds, altitude, soil, and time of harvest, among other factors. Temperature, relative humidity, the total duration of exposure to sun, and wind patterns have a direct influence, especially in species that have histological structures for the storage of essential oil on the leaf surface (Valmorbida *et al*., 2006). The variation in chemical composition of the essential oil is known to vary with the seasonal variation (Senthilkumar and Venkatesalu, 2010). Hussain *et al.* (2008) observed that the temperature influenced the decrease in oil yield of *O. Basilicum*, on the other hand they showed that the highest yield of essential oil was in the winter (0.8%) and decreased in summer to 0.5%.

Conclusion

In general, the growing season affected the essential oil content and its chemical composition. The production of essential oils and their utilization as potential natural sources for new phytomedicines could be of economic value. This species can acclimated in Egypt but no flower or fruits can produced under climatic conditions in Egypt, and this is the first assessment of seasonal variations in the essential oil from its herb.

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