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Evaluation of *Narcissus tazetta* Under Different Habitats

AMIRA A. HABIB, NAHLA S. ABDEL-AZIM*, WEGDAN L. ABDEL-KHALEK†, KHALED A. SHAMS and NAHED M. HASSAN
Phytochemistry Deptartment, National Research Centre, 12311 Dokki, Cairo, Egypt
Fax: (2)(2)3370931; Tel: (2)(2)3371211; E-mail: nahlaabdelazim@yahoo.com

In this work, the bulbs of *Narcissus tazetta* were cultivated in three different localities under different habitats representing the loamy soil, the new reclaimed sandy soil and the sandy soil. Evaluation of the cultivated plant samples from each locality, including essential oil content and the total alkaloids; was done. The loamy soil showed to be the most suitable habitat for the cultivation of the plant. Investigation of the essential oil revealed that the highest oil content was recorded in the plants grown in the loamy soil (0.13 %). The plants cultivated at sandy soil gave the lowest yield of the oil (0.10 %). GC/MS of the essential oil for all samples showed that the main constituents of the oil are the same, but only differ in their percentages. The maximum terpenoid percentage (65.1 %) was determined in the plants cultivated in the loamy soil followed by sandy soil samples (52 %) and then new reclaimed sandy soil samples (47.65 %). The main constituents were α-pinene, limonine, linelool, methyl and ethyl cinnamate in all samples.The total alkaloids were extracted from the collected leaves of the cultivated samples. The highest alkaloid percent was found in the loamy soil leave samples (0.28 %) while the lowest one was found at the sandy soil samples (0.12). TLC-densitometry analysis indicated that the main spot of alkaloids might be identified as narcessine.

Key Words: *Narcissus tazetta*, Essential oil, Alkaloids, TLC densitometry analysis.

INTRODUCTION

Narcissus tazetta belongs to the family Amaryllidaceae[1]. In Egypt, it is commercially grown outdoors for cut flowers and essential oil extraction that used in perfume industry. The plant is mostly winter-blooming, usually blooms in December and January. Moreover, it is used in landscape gardening in a wide range of locations, perhaps more than other bulbs. It may be planted in groups in front of shrubs, as colonies beneath tree, or as individual gardens or even in a neutralized meadow.

†American University, Cairo, Egypt.

Narcissus tazetta has been reported to elaborate a variety of alkaloids[2]. Most of these alkaloids are known for their medicinal properties. Several isolated alkaloids have shown a wide range of biological activities. The methanol extract from several Amaryllidaceous plants was also found to posses antibacterial and antifungal activities[3]. Recently, the plant is also used as a medicinal plant, since the leaves and bulbs of *Narcissus* contain anticancer compound[4].

In this study the bulbs were cultivated in three different localities: the loamy soil, the new reclaimed sandy soil and the sandy soil. Phytochemical studies included the investigation of the essential oil content of *Narcissus tazetta* samples collected from the three localities as well as the alkaloid contents.

EXPERIMENTAL

The gas chromatography instrument was Hewlett Packard model 6890. The conditions were as follows:

Capillary column : Model Hp Carbwax, length, thickness 0.3 mm.
Temperature program : Oven temperature 40ºC rose to 220ºC (4ºC/min).
Injection temperature : 270ºC.
Detector temperature : 300ºC
Carrier gas : Helium at 0.8 cm/min.

Mass spectrometer was Hewlett Packard model 5973 mass selective detector. The automatic TLC sampler and TLC Scanner3 with CATS Software were used for alkaloids scanning.

Plant material: Bulbs of *Narcissus tazetta* were obtained from the Botanic Garden, Ain Shams University, Cairo, Egypt and identified by Prof. Dr. Sayed Farag Khalifa, Professor of Plant Taxonomy & Flora, Botany Department, Ain-Shams University, Cairo, Egypt.

Extraction of the essential oils: About 50 g of fresh flowers were collected from the three different localities and extracted separately with hexane (250 mL × 3 times). The combined hexane extract was decolourized with Fuller's earth and evaporated under reduced pressure to give yellowish semisolid residue. The oil percentage in each sample was recorded.

Extraction of the total alkaloids: All samples were dried in the electrical oven at 40ºC and ground to a fine powder. 100 g of each sample was separately macerated in 90 % aqueous methanol (three times). The combined methanol extract was evaporated *in vacuo* at 45ºC. The obtained dark brown residue was then dissolved in 10 % HCl solution and filtered. The filtrate (acidic solution) was partitioned with chloroform (3 times × 150 mL). The chloroform layer was rejected and the acidic solution was rendered alkaline (pH-10) with ammonia solution (25 %). The alkaloids were extracted from the alkaline solution by partition with chloroform.

The combined chloroform layer was washed with distilled water till free from alkalinity, dried over anhydrous sodium sulphate and evaporated *in vacuo* at 40ºC, then dried by passing a stream of nitrogen. The weight of the total alkaloids was recorded and kept in a closed vial for each sample.

TLC densitometry analysis: 1 % stock solutions of the alkaloid samples were prepared. Quantitative amounts of each sample were applied on TLC silica gel ready-made plate (Merck 60 F 254, 20 × 20 cm). Samples were applied in band wise using CAMAG Automatic TLC sampler III, distance from lower edge was 10 cm, band length was 8 mm, track distance was 15 mm and 12 bands represented all samples were applied. Optimization trials to achieve the best conditions for the separation as well as the right wavelength for TLC densitometry evaluation was done by multi-wavelength scan (200, 250, 300, 350 and 400 nm). The best conditions were found to be as follows: wavelength scans: 200 nm and scanning speed: 20 mm/s. The plate developed with $CHCl_3$: MeOH (9:1). Multilevel calibrations *via* peak area by linear regression were done to evaluate the results.

RESULTS AND DISCUSSION

The phytochemical studies included the investigation of the essential oil content of *Narcissus tazetta* samples collected from the three different localities. The results obtained revealed that the highest oil content was recorded in the plants grown in the loamy soil (0.13 %). The plants cultivated in sandy soil gave the lowest yield of the oil (0.10 %).

GC/MS analysis of the essential oil for all samples revealed that the main constituents of the oil are the same, but only differ in their percentages (Tables 1-3). The percentage of the terpenoid compounds varied obviously. The maximum terpenoid percentage (65.1 %) was determined in the plants cultivated in the loamy soil followed by sandy soil samples (52%) and then the new reclaimed sandy soil samples (47.65 %). The oil was devoted to the terpenoid fraction. This conclusion is in agreement with that reported[5].

The percentage of aromatic compounds varied from 29.67 to 46.38 % in samples from the laoamy and new reclaimed soils, respectively. The main constituents were α-pinene, limonine, linelool, methyl and ethyl cinnamate in all samples. The obtained data were in agreement with previous results[6]. The authors recorded ∝-pinene, limonene, linalool, methyl- and ethyl-cinnamate as main constituents.

According to the reports[7,8], the Amaryllidaceous plants are known to contain alkaloids. They studied 150 species belonging to 36 genera and over 100 compounds were isolated and identified. In this work, the total alkaloids were extracted from leaves and bulbs that were collected from

TABLE-1
GC/MS DATA OF ESSENTIAL OIL OF FLOWERS OF
Narcissus tazetta (LOAMY SOIL)

No.	Rt.	Rel. (%)	M^+	b.p. (ºC)	Mass fragments	Compounds
1	3.89	22.24	136	93	41 (19.23), 79 (33.2), 105 (17.4)	α-Pinene
2	21.68	1.84	128	57	43 (18.6), 78 (29.5), 107 (89.5)	1-Ethoxy-4-methyl-pent-2-ene
3	22.29	5.96	136	67	41 (23.15), 53 (20.5), 93 (67.3)	Limonene
4	23.01	1.07	152	81	55 (14.5), 83 (65.5), 123 (15.9).	2,4-Decadienal
5	24.35	0.85	134	119	65 (13.2), 91 (72.6), 103 (8.42)	*p*-Cymene
6	24.6	0.82	154	43	55 (32.5), 71 (29.6), 111 (48.62), 125 (9.5)	1,4-Cineol
7	25.0	13.42	139	43	55 (59.0), 67 (19.5), 93 (25.7), 121 (9.5)	Linnalool
8	25.73	2.38	156	57	67 (21.2), 85 (41.5), 128 (72.4)	Undecane
9	27.21	14.86	154	59	43 (95.5), 81 (65.5), 167 (15), 121 (32.5)	α-Terpineol
10	27.64	7.80	136	93	41 (34.9), 51 (19.4), 77 (45.6), 121 (29.4)	γ-Terpinene
11	28.37	3.03	162	131	51 (39.5), 103 (92.5)	Methyl cinnamate
12	29.97	15.98	176	131	51 (25.4), 77 (37.5), 103 (79.2), 147 (12.4)	Ethyl cinnamate
13	32.12	0.63	176	91	85 (15), 105 (55), 147 (12.5)	1,1-Diethyl-propyl benzene
14	37.27	2.24	232	91	115 (29.5), 187 (74), 189 (5)	1-Propyloctyl benzene
15	41.07	6.94	284	88	7 (1.25), 91 (15), 101 (60), 157 (11.25)	2-Phenyl-tridecane

TABLE-2
GC/MS DATA OF ESSENTIAL OIL OF FLOWERS OF *Narcissus tazetta* (NEW RECLAIMED SANDY SOIL)

No.	Rt.	Rel. (%)	M^+	b.p. (ºC)	Mass fragments	Compounds
1	3.69	15.38	136	93	41 (19.23), 79 (33.2), 105 (17.4)	α-Pinnene
2	16.11	0.62	128	55	41 (95), 73 (68), 100 (85).	Cyclohexanoic acid
3	21.74	6.91	136	67	41 (23.15), 53 (20.5), 93 (67.2).	Limonine
4	22.45	12.73	150	43	51 (25.4), 79 (52.3), 91 (98.4), 108 (76. 9)	Benzyl acetate
5	23.63	2.29	108	91	51 (22.7), 77 (35. 8), 89 (5), 107 (43.1).	Benzyl alcohol
6	23.92	11.6	13.9	43	55 (59.0), 67 (19.5), 93 (25.7), 121 (9.5)	Linnalool
7	24.11	15.27	13.6	93	41 (34.9), 51 (19.4), 77 (45.6), 121 (29.4)	α-Terpinene
8	25.05	5.62	246	105	41 (3.75), 91 (8.75), 151 (19), 214 (42).	1-Methyl undecyl benzene
9	26.57	8.81	162	131	51 (39.2), 103 (92.5).	Methyl cinnamate
10	26.91	13.68	176	131	51 (25.4), 77 (37.5), 103 (79.2)	Ethyl cinnamate
11	34.19	2.91	204	93	41 (56.7), 107 (45), 121 (86.4) 161 (19.2).	Germacrane-A
12	37.51	0.77	260	105	77 (3.75), 91 (13.75), 181 (27.8).	1-Methyl dodecyl benzene
13	38.23	3.48	212	105	51 (21.7), 77 (28.4), 91 (71.3), 194 (15.1)	Benzyl benzoate

the three different localities. The highest percent 0.25 and 0.28 % of the total alkaloids was found to be in the leaf samples at the flowering stage. The per cent of the total alkaloids of the bulbs was found to be 0.12 %. The results also showed that the maximum alkaloid content of both leaves and bulbs was found in the samples collected from the loamy soil, where by the lowest value was estimated in those from the sandy soil.

TABLE-3
GC/MS DATA OF ESSENTIAL OIL OF FLOWERS OF
Narcissus tazetta (SANDY SOIL)

No.	Rt.	Rel. (%)	M^+	b.p. (ºC)	Mass fragments	Compounds
1	03.54	2.52	136	93	41 (19.23), 79 (33.2), 105 (17.4)	α-Pinene
2	03.7	6.00	136	93	41 (25.1), 79 (52.4), 121 (19.5)	Δ^3-Carene
3	16.45	8.31	136	67	41 (23.15), 53 (20.5), 93 (67.3)	Limonine
4	17.44	4.68	154	41	69 (45.2), 81 (21.9), 121 (5), 139 (2.5)	Nerol
5	18.93	19.43	139	43	55 (59.0), 67 (19.5), 93 (25.7), 121 (9.5)	Linalool
6	19.96	7.8	154	43	55 (32.5), 71 (29.6), 111 (48.62),125 (9.5)	1,4-Cineol
7	21.1	13.47	134	91	51 (39.5), 78 (42.7), 105 (31.7), 115 (50.3),	Cinnamic alcohol
8	21.42	11.91	162	131	51 (39.2), 103 (92.5)	Methyl cinnamate
9	21.58	4.91	154	59	43 (95.5), 81 (65.5), 107 (15), 121 (53.5)	α-Terpineol
10	21.8	6.83	260	91	105 (25), 133 (55), 232 (15)	2-Phenyl tridecane
11	31.32	10.91	176	131	51 (25.4), 77 (37.5), 103 (79.2), 147 (12.4)	Ethyl cinnamate
12	35.31	3.18	194	120	65 (21.4), 92 (31.9), 138 (19.2)	Isobutyl salicylate

TLC-densitometry analysis indicated that the main spot of alkaloids might be identified as narcessine, depending on the UV data. Evaluations as well as comparison of the total alkaloid contents in the tested samples were done using TLC densitometry relative to an authentic narcessine sample of known concentration[9].

The results of thin layer densitometric analysis revealed that the highest per cent of narcessine was found to be in the flowering stage of all samples from the three localities. The samples collected from the loamy

soil also had the highest percent of narcessine 49.0 %, followed by new reclaimed sandy soil 44.995 % and the lowest percentage was found in sandy soil samples 39.21 %.

In addition, Table-4 summarized the presence or absence of the same oil constituents in the different localities and their percentages.

TABLE-4
EFFECT OF DIFFERENT LOCALITIES ON THE OIL PERCENTAGES AND THE MAIN CONSTITUENTS OF *Narcissus tazetta*

Compounds	Localities		
	Loamy soil	New reclaimed sandy soil	Sandy soil
α-Pinene	22.24	15.38	2.52
Limonine	05.96	6.91	8.31
p-Cymene	00.85	-	-
Cineol	00.82	-	7.8
Linalool	13.42	11.60	19.43
α-Terpineol	14.86	-	4.91
γ-Terpinene	07.80	15.27	-
Methyl cinnamate	03.03	8.81	11.91
Ethyl cinnamate	15.89	13.68	10.61
2-Phenyl tridecane	06.94	-	6.83
Other compounds	08.01	28.39	27.68
Percentages of the total oil	00.13	00.11	00.10

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