

YIELD AND CHEMICAL COMPOSITION OF FLOWER HEADS OF SELECTED CULTIVARS OF POT MARIGOLD (*Calendula officinalis* L.)

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Abstract. Pot marigold (*Calendula officinalis* L.) is an annual ornamental plant which is also grown for herbal raw material used in the pharmaceutical and cosmetic industries. There are a lot of pot marigold cultivars which differ in colour and in size of inflorescences as well as participation of ligulate flowers. In the field experiment there were five cultivars of pot marigold compared: 'Orange King', 'Persimmom Beauty', 'Promyk', 'Radio' and 'Santana'. As far as morphological features are concerned 'Orange King' turned out to be the best. It produced the most numerous and shapeliest inflorescences, with the biggest number of ligulate flowers. Raw material yield of compared cultivars oscillated from 849 to 1661 kg·ha⁻¹ of flower heads, and the ligulate flowers themselves from 449 to 1141 kg·ha⁻¹. In both cases the highest yield was obtained by 'Orange King', and the lowest by 'Promyk.' The content of biologically active compounds in inflorescences of particular cultivars also varied: flower heads of 'Persimmom Beauty' contained the highest amount of essential oil (0.41 ml·100 g⁻¹), whereas 'Promyk' contained the highest amount of flavonoids (0.56% – expressed in hyperoside). In all cultivars inflorescence receptacle gathered the highest amount of essential oil and ligulate flowers gathered the least. The opposite tendency appeared in the case of flavonoids.

Key words: *Asteraceae*, raw material yield, essential oil, flavonoids

INTRODUCTION

Pot marigold belongs to medicinal plants, which had been used in the ancient times. At present it is widely used in pharmaceutical and cosmetic industry. Raw material constitute the whole flower heads *Calendulae anthodium* or ligulate flowers – *Calendulae flos* – of orange and yellow colour [Farmakopea Polska VIII 2008]. Raw material is rich in biologically active metabolites, like sesquiterpens, saponins, triterpens flavonoids, carotenoids, tannins, and essential oil. These components show antiseptic action

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and anti-inflammatory, anti-edematous, immunomodulatory activity [Ożarowski and Jaroniewski 1989, Taczanowska and Holderna-Kędzia 1998].

A lot of cultivars and forms of pot marigold are known, which differ in colour of flowers (from light yellow to orange), the size of flower heads and the participation of ligulate flowers [Rumińska 1983]. For the pharmaceutical industry as well as for farmers the most desired cultivars are those with a full flower head as well as with high yield. The usability of pot marigold as pharmaceutical material depends on chemical content of flower heads.

In available literature there are very few studies concerning the evaluation of different cultivars of pot marigold, cultivated in Poland as far as yield and the content of biologically active compounds in the inflorescences and in particular elements (ligulate flowers, tubular flowers and inflorescence receptacle) are concerned. The research carried out by Ocioszyńska et al. [1977] revealed that all parts of the flower head have similar components, however in different amounts, what causes that participation of particular elements of flower head may have a crucial role in its medicinal value.

The aim of this work was to compare yield, structure of flower heads and chemical composition of the inflorescences of selected cultivars of pot marigold.

MATERIALS AND METHODS

A field experiment was carried out in the years 2007–2009 in the Experimental Farm of the University of Life Sciences in Lublin. The study was conducted on loess soil with the granulometric composition of silt loam, characterized by a slightly acid reaction (pH 1 M KCl – 5.9), average humus content (1.8%) as well as an average content of nutrients (59.6 P; 115.1K; 67.2 Mg in mg·kg⁻¹ of soil). The experiment was set up in a randomized block design on 5 m² plots, in 4 replications. In the experiment there were 5 cultivars of pot marigold used: ‘Orange King’, ‘Persimmon Beauty’, ‘Promyk’, ‘Radio’ (characterized by orange-coloured flowers) and ‘Santana’ (characterized by yellow coloured flowers).

On the experimental plots following mineral fertilization was applied (in kg·ha⁻¹) N-60, P-22 and K-62. Seeds (dressed with the fungicide Dithane M-45) were sown in the third decade of April in the amount of 8 kg·ha⁻¹ at a row spacing of 40 cm. After emergence, thinning was done, leaving about 40 plants per m². In the later vegetation routine treatments were conducted (soil loosening, weeds control). The start and end dates of flowering were recorded as well as the flowering duration was determined for all cultivars.

Flower heads were harvested gradually as the plants bloomed, from the beginning of July until the end of August. There were 8 harvests, usually at 6-day intervals. After each harvest, the number of flower heads were determined and based on that the rate of blooming of particular cultivars was calculated. During the harvest, the diameter of flower heads (n = 30) and the number of ligulate and tubular flowers were determined (n = 10). The overall average value of these features was calculated for particular cultivars from all harvests. During the blooming, the number of flower heads per particular branch of the plant was determined based on 20 randomly selected plants from each

treatment. After the end of harvest, the total yield of flower heads, as well as ligulate flowers (calculated in $\text{kg}\cdot\text{ha}^{-1}$) were determined. Remaining plant material was qualified as a vegetative parts and separately weighted.

Directly after harvest, flower heads were dried in a drying room at a temperature of 35°C . Then essential oil content was determined, by steam distillation (method 2.8.12 Polish Pharmacopoeia VIII 2008). The flavonoids content was determined by use of spectrophotometer (method 2.2.25, Polish Pharmacopoeia VIII 2008) and expressed in hyperoside equivalents.

Table 1. Mean monthly air temperatures and amount of precipitation at ES Felin in the years 2007–2009

Tabela 1. Średnie miesięczne temperatury i sumy opadów w GD Felin w latach 2007–2009

Month – Miesiąc	Temperature – Temperatura $^{\circ}\text{C}$				Precipitation – Opady mm			
	2007	2008	2009	mean for średnio dla 1951–2000	2007	2008	2009	mean for średnio dla 1951–2000
IV	8.7	9.3	11.4	7.5	17	56	3	41
V	15.0	12.8	13.6	13.0	81	102	71	58
VI	18.1	17.7	16.4	16.5	88	26	125	66
VII	19.2	18.3	19.9	17.9	87	77	57	78
VIII	18.4	19.3	19.0	17.3	38	55	58	70
Mean – Średnio	15,9	15,5	16,0	14,4	-	-	-	-
Amount – Suma	-	-	-	-	311	316	314	313

The obtained results were statistically elaborated by means of variance analysis method. The significance of differences was determined using Tukey's test, for the significance level $\alpha = 0.05$.

The weather conditions during period of pot marigold cultivation are shown in table 1.

RESULTS AND DISCUSSION

Plants of all cultivars started blooming in the first decade of July (the earliest was cv. 'Promyk'). Cultivars 'Radio' and 'Orange King' started blooming the latest and it was longest (until the end of August). Rumińska [1983] reports that the period of pot marigold blooming extends in time (it starts in June and can last up to late autumn) and its length depends mainly on weather conditions during the vegetation period and the cultivar.

The number of inflorescences gathered during each harvest varied within examined cultivars as far as the rate of blooming was concerned. The highest number of inflorescences was obtained from the cultivar 'Promyk' in the second, third and fourth harvest (55% in relation to all gathered flower heads). During subsequent harvests, the number of inflorescences decreased systematically. In case of cv. 'Orange King' the flowering

rate was slower. Only 20% of inflorescences were gathered from the first to the fourth harvest. Next bloomings rapidly increased and gained their maximum values at the turn of July and August (over 60% of all flower heads derived from the fifth and sixth harvest). Cultivars ‘Persimmon Beauty’, ‘Radio’ and ‘Santana’ characterised the most systematical rate of flowering. Plants bloomed the most intensely in the third decade of July (40% of flower heads were obtained from the fourth and fifth harvest).

The number of inflorescences is a vital feature differentiating the cultivars of pot marigold. In own experiment the highest number of inflorescences was produced by cv. ‘Orange King’ (67.9 pieces·plant⁻¹, while ‘Promyk’ and ‘Radio’ produced significantly less (respectively 41.1 and 53.0 pieces·plant⁻¹) (tab. 2). While comparing the distribution of inflorescences on particular branches, it was revealed that significant differences existed only in case of branches of the second rank. No variation of the discussed feature in particular cultivars was noted on branches of first and third rank (tab. 2).

Table 2. The number of inflorescences (piece·plant⁻¹) of the examined cultivars of pot marigold – mean from years 2007–2009

Tabela 2. Liczba koszyczków kwiatowych (sztuka·roślina⁻¹) wybranych odmian nagietka lekarskiego – średnio z lat 2007–2009

Cultivar Odmiana	Total number of inflorescences Ogólna liczba kwiatostanów	Including inflorescences on branches W tym kwiatostanów z odgałęzień		
		first rank I rzędu	second rank II rzędu	third rank III rzędu
Orange King	67.9	11.8	41.6	14.5
Persimmon Beauty	62.3	11.0	41.0	10.3
Promyk	41.1	12.8	30.0	8.3
Radio	53.0	11.0	31.2	10.8
Santana	61.1	13.1	39.5	10.5
LSD _{0,05} – NIR _{0,05}	10.6	n.s.	9.8	n.s.

n.s. – no significant differences – różnice nieistotne statystycznie

Rumińska [1983] maintains that there can be up to 50–60 inflorescences on a single pot marigold plant. Similar results were obtained in the experiment of Król [2011a] and Ganjali et al. [2010]. On the other hand, in the experiments carried out in Serbia [Crno-barac et al. 2009], pot marigold produced about 30 flower heads, whereas in the study of Khalid et al. [2006] this number ranged from 70 to 140 per plant. Such big discrepancy of this value results from the differences in climatic conditions and experimental factors.

The examined cultivars varied significantly in the size of inflorescences. The cultivar ‘Promyk’ produced the smallest flower heads (58.5 mm) while cv. ‘Orange King’ produced the biggest (80.7 mm) (tab. 3). The number of ligulate flowers in flower head

ranged from 210 (cv. 'Persimmon Beauty') to 299 (cv. 'Orange King') and only these extreme values proved to be statistically different. The number of tubular flowers was 2–3 time smaller and in this case there were no major differences among the examined cultivars (tab. 3).

Table 3. Diameter of flower head (mm), number of ligulate and tubular flowers and the contribution on particular parts (%) in total mass of flower heads – mean from years 2007–2009
Tabela 3. Średnica koszyczka kwiatowego (mm), liczba kwiatów języczkowych i rurkowych oraz udział poszczególnych części (%) w całkowitej masie kwiatostanu – średnio z lat 2007–2009

Cultivar Odmiana	Diameter of flower heads Średnica koszyczka	Number of flowers per head Liczba kwiatów w koszyczku		Contribution in flower head Udział w masie koszyczka		
		ligulate języczkowe	tubular rurkowe	ligulate flowers kwiaty języczkowe	tubular flowers kwiaty rurkowe	receptacle osadnik
Orange King	80.7	299	92	68.0	10.0	22.0
Persimmon Beauty	75.7	210	147	51.1	16.0	32.9
Promyk	58.4	242	115	61.4	13.0	25.5
Radio	73.2	228	136	58.6	14.7	26.7
Santana	74.9	234	131	59.7	15.4	24.9
LSD _{0,05} – NIR _{0,05}	8.46	85	n.s.			

n.s. – no significant differences – różnice nieistotne statystycznie

According to Tyszyńska-Kownacka [1973] the size of pot marigold flowers as well as the contribution of ligulate flowers depends not only on the cultivar but also on climatic, soil and agronomic conditions. In our experiment ligulate flowers had the biggest contribution in the total mass of inflorescence (from 51.1% in case of cv. 'Persimmon Beauty' to 68% in cv. 'Orange King') (tab. 3). The participation of tubular flowers was much smaller (10–16%), and mass of inflorescence receptacle oscillated from 22% to 32%. Rumińska [1983] reports that the contribution of ligulate flowers in cultivars grown in Poland is about 50%. In others experiments the participation of ligulate flowers in the mass of inflorescence oscillated from 40% [Piccaglia et al. 1997] to 65% [Martin and Deo 2000].

Cultivar 'Orange King' produced well-developed inflorescences and gave the highest yield of flower heads (1661 kg·ha⁻¹). The lowest yield was obtained from dwarf cultivar 'Promyk' (849 kg·ha⁻¹), which had the smallest flower heads. Other cultivars ('Santana', 'Radio' and 'Persimmon Beauty') yielded on the similar level (1287–1335 kg·ha⁻¹) (tab. 4). Cultivar 'Orange King' produced the highest yield of ligulate flowers (1141 kg·ha⁻¹), while cv. 'Promyk' – the lowest (449 kg·ha⁻¹). Also cv. 'Persimmon Beauty' produced relatively small yield of ligulate flowers, what was the consequence of low participation of these flowers in the total mass of flower head.

Table 4. The yield of air-dry flower heads, ligulate flowers and vegetative parts ($\text{kg}\cdot\text{ha}^{-1}$) depending on the cultivar – mean from years 2007–2009

Tabela 4. Plon suchych koszyczków, kwiatów języczkowych oraz części wegetatywnych ($\text{kg}\cdot\text{ha}^{-1}$) w zależności od odmiany – średnio z lat 2007–2009

Cultivar Odmiana	Yield of flower heads Plon koszyczków	Yield of ligulate flowers Plon kwiatów języczkowych	Yield of vegetative parts Plon części wegetatywnych
Orange King	1661	1141	2908
Persimmon Beauty	1287	649	2585
Promyk	849	449	1962
Radio	1323	772	2827
Santana	1335	787	3113
LSD _{0,05} –NIR _{0,05}	260	121	290.7

The yield of pot marigold raw material (flower heads) depends mainly on climatical conditions and the cultivar characteristic. In the native research [Rumińska 1983, Dedio et al. 1986, Hojden et al. 1990, Biesiada et al. 2006, Król 2011a] the yield of air dry flower heads ranged from 1000 to 2000 $\text{kg}\cdot\text{ha}^{-1}$. Similar results were obtained in Serbia [Crnobarac 2009] and in India [Gantait and Chattopadhyay 2004]. In the research carried out in Romania [Georgeta 2005] and in Brasil [Gomes et al. 2007] the yields of marigold were lower and did not exceed 1000 $\text{kg}\cdot\text{ha}^{-1}$. In Chile they gained the yield up to 4000 $\text{kg}\cdot\text{ha}^{-1}$ [Berti et al. 2003].

The vegetative mass of plants (leaves and stems) is largely interdependent with the mass of flower heads. In the discussed experiment the following cultivars: ‘Santana’, ‘Orange King’, ‘Radio’ produced much higher vegetative mass than ‘Persimmon Beauty’ and ‘Promyk’ (tab. 4).

The content of biologically active components is the evidence of the quality of herbal raw material. In case of pot marigold, the content of essential oil and flavonoids (expressed in hyperoside equivalents) was analysed. In our experiment most of the examined cultivars characterized similar content of the essential oil (0.24 $\text{ml}\cdot 100\text{ g}^{-1}$ to 0.30 $\text{ml}\cdot 100\text{ g}^{-1}$), with the exception of flower heads of cv ‘Persimmon Beauty’, in which 0.41 $\text{ml}\cdot 100\text{ g}^{-1}$ of essential oil was recorded. Probably, this was due to the fact of high participation of inflorescence receptacle in the mass of flower head in this cultivar. According to Ocioszyńska et al. [1977] the well-developed floral inflorescence receptacle increases content of essential oil in raw material. According to Paim et al. [2010] the content of essential oil in pot marigold is between 0.1 to 0.4 $\text{ml}\cdot 100\text{ g}^{-1}$ depending on the region of cultivation and cultivar. In research carried out in Romania [Radulescu et al. 2000] – 0.2 $\text{ml}\cdot 100\text{ g}^{-1}$ of essential oil was obtained, in France [Chalchat et al. 1991] – 0.3 $\text{ml}\cdot 100\text{ g}^{-1}$, while in regions of warm climate (Brasil, Egipt, South Africa) – 0.1 $\text{ml}\cdot 100\text{ g}^{-1}$ [Gazim et al. 2008, Khalid and Teixeira de Silva 2010, Okoh et

al. 2008]. In the native research pot marigold contained 0.2–0.3 ml·100 g⁻¹ of essential oil. [Dedio et al. 1986, Taczanowska and Hołderna-Kędzia 1998, Król 2011b].

In own experiment in all examined cultivars, the highest content of essential oil was found in inflorescence receptacle (0.44 ml·100 g⁻¹ – 0.49 ml·100 g⁻¹) and the lowest in ligulate flowers (0.15 ml·100 g⁻¹ – 0.21 ml·100 g⁻¹). The content of essential oil in tubular flowers oscillated between 0.29 ml·100 g⁻¹ – 0.35 ml·100 g⁻¹ (tab. 5). The results gained by the author in this experiment confirm most of the results of other scientists. Similar differences in the content of essential oil in particular elements of flower head were reported also in other research [Ocioszyńska et al. 1977] and in foreign ones [Marcal et al. 1987, Lastra and Piquet 1999, Korakhashvili et al. 2007]. The only exception was the research carried out by Petrovic et al. [2010], who recorded higher content of essential oil in ligulate flowers than in tubular.

Table 5. Content of essential oil (ml·100 g⁻¹) in inflorescences as well as in particular parts of flower heads of the examined cultivars – mean from years 2007–2009

Tabela 5. Zawartość olejku eterycznego (ml·100 g⁻¹) w kwiatostanach oraz poszczególnych częściach koszyczków badanych odmian – średnio z lat 2007–2009

Cultivar Odmiana	Fower head Koszyczek kwiatowy	Part of flower head Części koszyczka		
		ligulate flowers kwiaty języczkowe	tubular flowers kwiaty rurkowe	receptacle dno kwiatowe
Orange King	0.24	0.20	0.30	0.44
Persimmom Beauty	0.41	0.21	0.35	0.48
Promyk'	0.27	0.19	0.29	0.45
Radio	0.30	0.19	0.29	0.49
Santana	0.29	0.15	0.29	0.47
Mean – Średnio	0.30	0.19	0.30	0.47
LSD _{0.05} –NIR _{0.05}	0.039	0.042	0.041	n.s.

n.s. – no significant differences – różnice nieistotne statystycznie

Some authors [Marcal et al. 1987, Korakhashvili et al. 2007] claim that cultivars with yellow flowers contain more essential oil than the ones with orange flowers. In our experiment this was proved only in case of cv. 'Orange King', which had significantly less essential oil in comparison with the cultivar 'Santana' with yellow flowers (tab. 5).

The content of flavonoids in flower heads of compared cultivars ranged from 0.40% (cv. 'Persimmon Beauty') to 0.56% (cv. 'Promyk') (tab. 6). According to Polish Pharmacopoeia VIII [2008] pot marigold raw material should contain not less than 0.4% of flavonoids, expressed in hyperoside. Hence, all the examined cultivars fulfilled the requirements of Polish Pharmacopoeia.

Kurkin and Sharova [2007] and Vidal-Ollivier et al. [1989] report that the content of flavonoids in flower heads ranged from (0.2% to 0.7%). Similar contents were obtained in research in Poland [Ocioszyńska et al. 1977, Król 2011a, 2011b]. However in the research conducted by Branzila [2004] the content of flavonoids exceeded 1%. According to Raal and Kirsipuu [2011] such big diversity comes from cultivar features as well as climatic conditions (in his experiment the differences between examined cultivars were 0.4%).

Ocioszyńska et al. [1977] emphasises that more flavonoid compounds are to be found in ligulate flowers. This is confirmed by the research carried by Branzila [2004] and Lastra and Piquet [1999] who presented a positive correlation between participation of ligulate flowers and flavonoids content in flower heads of pot marigold. This attitude is not shared by Raal and Kirsipuu [2011] who claim that there are no simple dependences between the content of these compounds and the structure of flower head. The research of Paim et al. [2010] revealed even higher content of flavonoids in inflorescences, with a high participation of tubular flowers. In our experiment in all examined cultivars the highest amount of flavonoids was found in ligulate flowers (0.57–0.71%). Tubular flowers had slightly less flavonoids, while the smallest content was found in inflorescence receptacle (0.14–0.21%). Analysing the obtained results it is hard to define precisely the impact of particular elements of flower heads on the flavonoids level. Nevertheless it was noted that cv. ‘Persimmom Beauty’, having the highest percentage of inflorescence receptacle had the smallest amount of these compounds. Also Ocioszyńska et al. [1977] show that bigger participation of inflorescence receptacle decreases the content of flavonoids in pot marigold raw material.

Table 6. Content of flavonoids (expressed in hyperoside equivalents) in inflorescences and in particular parts of flower heads of the examined cultivars – mean from years 2007–2009 (%)

Tabela 6. Zawartość flawonoidów (w przeliczeniu na hiperozyd) w kwiatostanach oraz poszczególnych częściach koszyczków badanych odmian (%) – średnio z lat 2007–2009

Cultivar Odmiana	Fower head Koszyzek kwiatowy	Part of flower head Części koszyczka		
		ligulate flowers kwiaty języczkowe	tubular flowers kwiaty rurkowe	receptacle dno kwiatowe
Orange King	0.54	0.71	0.50	0.14
Persimmom Beauty	0.40	0.57	0.44	0.14
Promyk	0.56	0.70	0.57	0.21
Radio	0.46	0.58	0.51	0.20
Santana	0.53	0.70	0.64	0.18
Mean – Średnio	0.50	0.65	0.53	0.17
LSD _{0.05} – NIR _{0.05}	0.092	0.056	0.075	0.049

Korakhashvili et al. [2007] report that more flavonoids are to be found in orange inflorescences. It was not proved in our experiment, where the cultivar with yellow flowers (cv. 'Santana') had similar or slightly higher content of flavonoids in flower heads, than other cultivars of orange flowers (tab. 6) Also Raal and Kirsipuu [2011] no dependence was found between the colour of flowers and the content of flavonoids.

CONCLUSIONS

1. The compared cultivars of pot marigold varied in the length of period of flowering, the rate of flowering and morphological characteristics of inflorescences. Cultivar 'Orange King' was distinguished by useful features such as yield and size of flower heads.

2. The morphological features influenced the yield of whole flower heads as well as ligulate flowers. The highest yield was obtained by cv. 'Orange King', while the lowest by cv. 'Promyk'.

3. Chemical composition of flower heads varied between cultivars. The highest content of volatile oil was found in cv. 'Persimmom Beauty' while flavonoids in cv. 'Promyk' and 'Santana'.

4. In all cultivars inflorescence receptacle gathered the highest amount of essential oil, while the least was found in ligulate flowers. The opposite dependency was observed in case of flavonoids.

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PLONOWANIE ORAZ SKŁAD CHEMICZNY KOSZYCZKÓW WYBRANYCH ODMIAN NAGIETKA LEKARSKIEGO (*Calendula officinalis* L.)

Streszczenie. Nagietek lekarski (*Calendula officinalis* L.) jest jednoroczną rośliną ozdobną, którą uprawia się również na surowiec zielarski dla przemysłu farmaceutycznego i kosmetycznego. Znanych jest wiele odmian nagietka różniących się barwą i wielkością kwiatostanów oraz udziałem kwiatów języczkowych. W doświadczeniu polowym porównywano 5 odmian uprawnych nagietka: ‘Orange King’, ‘Persimmom Beauty’, ‘Promyk’, ‘Radio’ i ‘Santana’. Pod względem cech morfologicznych najkorzystniej prezentowała się odmiana ‘Orange King’, która miała najliczniejsze i najdorodniejsze kwiatostany charakteryzujące się największą liczbą i masą kwiatów języczkowych. Plony surowca badanych odmian wahały się w granicach: od 849 do 1661 kg·ha⁻¹ koszyczków kwiatowych, a samych kwiatów języczkowych od 449 do 1141 kg·ha⁻¹. W obydwu przypadkach najwyższe plony wydała odmiana ‘Orange King’, a najniższe – ‘Promyk’. Zawartość związków biologicznie czynnych w kwiatostanach poszczególnych odmian również była zróżnicowana: najwięcej olejku eterycznego zawierały koszyczki odmiany ‘Persimmom Beauty (0,41 ml·100 g⁻¹)’ zaś flawonoidów – ‘Promyk’ (0,56% – w przeliczeniu na hiperozyd). U wszystkich odmian najwięcej olejku gromadziły osadniki kwiatostanowe, a najmniej – kwiaty języczkowe. Odwrotna tendencja wystąpiła w przypadku flawonoidów.

Słowa kluczowe: *Asteraceae*, plon surowca, olejek eteryczny, flawonoidy

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