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# Ontogenetic variation of active agent content of yarrow (*Achillea collina* Becker)

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ABSTRACT

The effect of harvesting time on the drug quality of yarrow was examined in open field experiment between 2012–2014 using the variety *Achillea collina* 'Proa'. Sampling of the plants were carried out in five phenological phases during flower development (BBCH 55–69). Essential oil content, proazulene content, total flavonoid and total phenolic content of the dried shoots were measured. Additionally, the ratio of plant organs in each phenophase was determined.

We established that the proportion of useful plant parts (flowers and leaves) in the drug increases from 56.33–59.83% (budding stage) to 65.17–73.07% (overblooming phase). The changes of chemical characteristics, however, followed these tendencies just partially. A peak accumulation level of essential oil (0.230–0.334 mg/100 g) was reached in white bud stage, after that the oil content decreased. The highest content of proazulene (0.123–0.183%) was measured in the same phenological phase. The flavonoid content of yarrow during flowering is presumably more variable than the content of other active compounds; it reached its maximum (0.900–2.837%) in green bud—early flowering phase while the total phenolic content showed two peaks: in green bud stage (178.0–233.4 mg GAE/g) and in overblown phenophase (170.9–258.5 mg GAE/g). Considerable year effect was detected in the case of each measured parameter. Based on the results, optimal drug quality could be achieved by harvesting yarrow in the first half of flowering (BBCH 61–65).



## 1. Introduction

Common yarrow (*Achillea collina* Becker) is a well-known and popular medicinal plant throughout Europe. Its flowering shoots accumulate essential oil with proazulenes as main active components. The drug contains other important ingredients like flavonoids, phenolic acids, non-volatile sesquiterpenes, as well. Yarrow is applied both in traditional medicine and in modern phytotherapy due to its spasmolytic, analgesic, anti-inflammatory and digestive effects (Németh and Bernáth, 2008).

Ontogenetic variation of active agents in medicinal plants is a well-known phenomenon (Filippini et al., 2009; Goudarzi et al., 2015; Németh, 2005; Özgüven and Tansi, 1998; Telci et al., 2009), which influences the quality of the drug. In the case of *A. collina*, several experiments dealt with the dynamics of essential oil content during ontogenesis, but the results are contradictious. According to the results of Rácz-Kotilla and Rácz (1969), the essential oil content of yarrow samples harvested in different dates reached the standards of the pharmacopoeia (0.2%) in every harvest except at the beginning (early June) and at the end of vegetation (from the middle of October). Shalaby and Verzár-Petri (1978) measured the minimum essential oil content in vegetative phase, while two maximum peaks were detected later during generative development, one in bud stage and another at the beginning of fruit ripening. Other authors declared that volatile oil content of the flowering shoots is the highest in the phase of full flowering (Berghold et al., 2006; Černaj et al., 1983). Contrary to this, Weber and Stahl (1953) and recently Carron et al. (2012) specified the early flowering stage as the one assuring maximum essential oil while in some cases budding was found as optimal (Karlová and Petříková, 2005; Kosova, 1959).

The ratio of volatile compounds may vary significantly, as well. The quantity of chamazulene increased continuously from budding until full flowering phase (Černaj et al., 1983; Rácz-Kotilla and Rácz, 1969). Different results were achieved by Usztojzsanin et al. (1989), who determined a decreasing tendency (from 82 to 15 mg/100 g DM.) from bud to full flowering stage. According to Ruminska (1970) and Carron et al. (2012) the role of ontogenesis in proazulene content was not considerable. The dynamics of the accumulation

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**Table 1**
Main soil characteristics at the experimental field of Corvinus University of Budapest.

| Soil characteristics | |
|---|---|
| pH (aqueous) | 7.78 |
| pH (KCl) | 7.36 |
| EC (μS/cm) | 90.4 |
| Salinity (mg/l) | 45.1 |
| $P_2O_5$ (*T*%) | 58.6 |
| $K_2O$ (mV) | 17.0 |
| $CaCO_3$ (%) | 18.2 |
| H (%) | 3.11 |
| Texture | Sandy |

of the main flavonoid compounds of yarrow is very similar to that of the essential oil (Karlová, 2006). Content of the main flavonoid constituents (apigenin, apigenin-7-*O*-glucoside, luteolin) increased continuously until full flowering but after that it decreased. However, the quantity of luteolin-7-*O*-glucoside showed a peak at small flower bud stage and a second maximum at full flowering stage. In another experiment the total flavonoid content followed a decreasing tendency (Karlová and Petříková, 2005). Several studies were carried out on the main phenolic compounds and the influencing factors of *Achillea* species (Giorgi et al., 2010, 2014; Vitalini et al., 2011) but their accumulation dynamics during plant development hasn't been discussed until now.

Ontogenetic variability is in tight connection with the changing organic constitution of the plant and the different accumulation level of the active compounds in different organs. The essential oil accumulation is most abundant in the inflorescences of *Achillea* spp. (Gudaityté and Venskutonis, 2007; Németh et al., 2007; Rácz-Kotilla and Rácz, 1969; Ruminska, 1970). Similarly, the quantity of other active agents, like chamazulene, differs with the plant organs. The chamazulene content of the leaves and stems is less than half of the value of the flowers (Rácz-Kotilla and Rácz, 1969; Ruminska, 1970; Usztojzsanin et al., 1989). There seems to be no significant difference in the flavonoid content among the different harvested plant parts (Karlová, 2006; Karlová and Petříková, 2005). According to Giorgi et al. (2010), the quantity of total phenolics of *A. collina* is higher in the leaves than in the inflorescences both in infusions and methanolic extracts while the content of chlorogenic acid, caffeic acid and 4,5-di-*O*-caffeoylquinic acid is various (Giorgi et al., 2009, 2010).

In this study our goal was to define the optimal harvesting time of yarrow assuring potentially the best drug quality based on four different quality parameters under Hungarian climatic conditions.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

*A. collina* Becker cultivar 'Proa' (origin: Pharmaplant, Gemany) was used in the experiment. The trial was carried out in 2012–2014 at the Research Station of the Corvinus University in Budapest (47°54′N, 19°14′E). Soil samples from the upper layer (0–30 cm) were taken at the experimental field and analyzed for their main characteristics (Table 1). The typical climate at the region of Budapest is moderate, continental with significant annual variation in temperature. Usually the precipitation is well distributed during spring while the summer period is the driest pending the year. The daily mean temperatures and amount of precipitation during the sampling period of each year are presented in Figs. 1 and 2. The temperature values proved to be typical for the season with greater and lesser fluctuations in the same year and between years. The quantity and distribution of precipitation during the examined period fluctuated significantly, as well. Three-fold difference in total precipitation was observed during the trial from the 1st of May to 31st of July, with 183 mm, 102 mm and 313 mm total precipitation values in consequitive years, respectively. While in 2012 and 2014 the precipitation was well distributed and its amount was relatively high, in 2013 the plants suffered from the lack of rainfall during flower development.

Annual plants were measured in each year. Seedlings were raised in hydroculture until planting them to the open field. The planting was made in the first decade of May in each experimental year to a spacing of 50 × 25 cm. The plants did not receive irrigation, only the natural precipitation after planting them to the open-field.

### 2.2. Treatments

The plants were harvested in five different phenological stages (Table 2 and Fig. 3) according to the international Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie scale, also known as BBCH scale (Hack et al., 1992). Bulk samples representing the studied plant stands in 3 replications were taken with a maximum of 30 cm stem length from a 30 $m^2$ big plot area. In one bulk sample approximately 50 individual plants were harvested. The determination of phenological stages was performed on every each harvested plant. The fresh plant material was dried in a storehouse at room temperature. The determination of plant organ ratio and active agent content was performed on these bulk samples. For the determination of active agent content of the plant material, the dried samples were cut, mixed and homogenized.

### 2.3. Ratio of plant organs

The dried shoots were separated into inflorescenses, leaves and stems. The mass of these fractions was measured in each sample. According to this, the ratio of the mentioned plant organs was calculated in percentage.

### 2.4. Essential oil extraction

The essential oil content was determined by hydrodistillation of 20 g cut herb in a Clevenger-type apparatus (500 ml) for 2 h according to the method described in VII. Hungarian Pharmacopoea (*Achilleae herba*) (1986). At the end of the distillation the essential oil was rinsed from the graduated tube of the apparatus with hexane, and then the solvent was evaporated. One extraction was carried out for each of the three field replicates. The essential oil content was calculated to dry matter content.

### 2.5. Proazulene content

The proazulene content of the essential oils was determined by the method recommended in the VIII. Hungarian Pharmacopoea (*Millefolii herba*) (2004). The distilled oil was diluted with xylene to 50 ml, and then the absorption of the mixture was measured in a Thermo Evolution 201 spectrophotometer at 608 nm. Liquid xylene was used as compensation. Proazulene content was expressed as chamazulene percentages using the following formula: $(2.1 \times A)/m$ where $A$ is the absorbance and $m$ is the mass of the sample in grams. One analysis was carried out for each of the three field replicates.

### 2.6. Total flavonoid content

Determination of total flavonoid content of the samples was carried out by the method recommended in the VIII. Hungarian Pharmacopoea (*Crataegi folium cum flore*) (2004). Shortly, 0.4 g powdered plant material was extracted with 40 ml ethanol (60 v/v%) at 60 °C for 10 min in two runs and the combined, filtered extract was diluted to 100 ml with ethanol. From this extract