



Content of alkaloids in ornamental *Papaver somniferum* L. cultivars growing in Estonia

Andres Meos, Liis Saks, and Ain Raal*

Institute of Pharmacy, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

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Abstract. The alkaloid content in industrial opium poppies (*Papaver somniferum* L.) has been studied by numerous authors, but not in ornamental cultivars. The aim of the study was to investigate the content of morphine, codeine, and papaverine in *P. somniferum* cultivars by high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). The studied poppy capsules were either grown from the ornamental poppy seeds purchased from different vendors or were unknown cultivars from home gardens in Estonia, cultivated as ornamental poppies. All dried capsules samples ($n = 34$) contained on average 362 mg/100g of morphine, 25 mg/100g of codeine, and 29 mg/100g of papaverine as determined by CE. The content of morphine determined by HPLC was 152–676 mg/100g, which is a remarkably high concentration allowing potential illegal use of ornamental poppies. Both analytical methods demonstrated similar results. Hence, CE is a suitable alternative to HPLC for quantitative analysis of opium alkaloids as it simplifies the sample preparation procedure.

Key words: pharmacognosy, opium poppy, morphine, codeine, papaverine, capsules, seeds.

1. INTRODUCTION

The opium poppy *Papaver somniferum* L. (Papaveraceae) is an important medicinal plant, which has been used for thousands of years. Its capsules and latex contain opium, which is a rich source of pharmaceutical alkaloids, such as morphine, codeine, papaverine, etc. (Prajapati et al., 2002; Frick et al., 2005; Fejer, 2007; Németh-Zámbori et al., 2011; Stranska et al., 2013). For example, in 2008 the world demand for morphine was 420 tonnes and for codeine 350 tonnes (Shukla et al., 2010). The seeds of *P. somniferum* are widely used in bakery and confectionery and are a source of good quality cooking oil. Typically, the food *P. somniferum* cultivars contains a low or moderate content of morphine (Prajapati et al., 2002; Fejer, 2007; Salamon and Fejer, 2010; Németh-Zámbori et al., 2011). European and other countries according to their traditions can also be interested in

growing *P. somniferum* of ornamental type (Németh and Bernáth, 2009).

Since ancient times *P. somniferum* has been used for medicinal purposes and in culinary or has been extensively utilized as a narcotic drug (Gümüüşü et al., 2008). Therefore, growing opium *P. somniferum* is forbidden in most countries, including Estonia (Comparini and Centini, 1985; Riigikogu, 1997). The cultivation of *P. somniferum* is strictly regulated by the International Narcotics Control Board (Frick et al., 2005).

Nevertheless, some poppy plants are grown in private gardens of different countries as ornamental flowers (Comparini and Centini, 1985; Vabariigi valitsus, 1997). There are almost 50 poppy cultivars officially registered worldwide (Németh-Zámbori et al., 2011) and according to international regulations the creation of *P. somniferum* cultivars free of alkaloids is in progress (Bernáth et al., 2003). Seeds of narcotic *P. somniferum* cultivars sometimes circulate in the market advertised as ornamental poppies for gardeners (Yoshimatsu et al., 2011).

* Corresponding author, ain.raal@ut.ee

The content of morphine and sometimes also of codeine in dried capsules or opium from commercial *P. somniferum* cultivars for pharmaceutical or food applications has been determined in several studies (Comparini and Centini, 1985; Prajapati et al., 2002; Bernáth et al., 2003; Frick et al., 2005; Yadav et al., 2006; Gümüřçü et al., 2008; Németh and Bernáth, 2009; Shukla et al., 2010; Németh-Zámbori et al., 2011); the content of morphine has also been measured in seeds (Lo and Chua, 1992; Sharma et al., 1999; Fejer, 2007; Sproll et al., 2007; Dittbrenner et al., 2008, 2009; Salamon and Fejer, 2010). Surprisingly, we could find only one publication about the content of morphine in *P. somniferum* grown in gardens for ornamental purposes (Comparini and Centini, 1985).

Therefore, the aim of our study was to determine the content of morphine and other opium alkaloids in

known and unknown *P. somniferum* cultivars grown in Estonian gardens as ornamental plants. For this purpose both high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) were used. Data obtained by CE coincided with some minor differences with HPLC data for morphine and codeine. In addition, papaverine and apomorphine were determined by CE.

2. MATERIALS AND METHODS

2.1. Plant material

The seeds of samples Nos 1–15 (Table 1) were obtained from the United Kingdom using an internet page (<http://chilternseeds.co.uk/>); the seeds of samples Nos 16–22 were purchased from the mall Prisma in Tartu, Estonia. All seeds were sown into three home gardens,

Table 1. Content of morphine with standard deviation and number of samples in dried capsules of different *Papaver somniferum* cultivars grown in private gardens of Estonia determined by HPLC

No.	Cultivar of <i>P. somniferum</i>	Content of morphine, mg/100g	Place and year of cultivation
1	'Queens Poppy'	676 ± 72; n = 4	Lääne-Viru county, Vinni municipality, Võhu village (2012)
2	var. <i>paeoniflorum</i> 'Scarlet Peony'	335 ± 29; n = 4	same
3	var. <i>paeoniflorum</i> 'Black Peony'	332 ± 38; n = 4	same
4	'White Cloud'	344 ± 37; n = 4	same
5	var. <i>paeoniflorum</i> 'Flemish Antique'	416 ± 91; n = 4	same
6	'Giganteum'	323 ± 57; n = 4	same
7	'Pink Chiffon'	277 ± 66; n = 4	same
8	var. <i>paeoniflorum</i> 'Black Dragon'	396 ± 71; n = 4	same
9	'Lauren's Grape'	627 ± 70; n = 4	same
10	var. <i>paeoniflorum</i> 'Yellow Peony'	415 ± 80; n = 4	same
11	'Danish Flag'	193 ± 66; n = 4	same
12	'Hungarian Blue Breadseed Poppy'	152 ± 29; n = 4	same
13	'Hen and Chickens'	234 ± 99; n = 4	same
14	'Giganteum'	202 ± 54; n = 4	same
15	'Lilac Pompom'	385 ± 32; n = 4	Harju county, Kuusalu municipality, Pudisoo village, Kirsi farm (2010)
16	'Hen & Chiken'	427 ± 131; n = 4	same
17	'Mák setý plnokvětý'	377 ± 83; n = 3	same
18	var. <i>paeoniflorum</i> , pink flowers, unknown	438 ± 85; n = 4	Tartu (2011)
19	var. <i>paeoniflorum</i> , pink flowers, unknown	570 ± 40; n = 3	Tartu (2012)
20	var. <i>paeoniflorum</i> , violet flowers, unknown	309 ± 137; n = 4	Tartu (2011)
21	var. <i>paeoniflorum</i> , violet flowers, unknown	526 ± 25; n = 3	Tartu (2012)
22	var. <i>paeoniflorum</i> , white flowers, unknown	212 ± 27; n = 3	Tartu (2011)
23	Unknown	57 ± 47; n = 4	Tartu (2012)
24	Unknown	387 ± 41; n = 4	Tartu (2012)
25	Unknown	417 ± 59; n = 3	Harju county, Kuusalu municipality, Pudisoo village, Kirsi farm (2011)
26	Unknown	539 ± 36; n = 3	same
27	Unknown	538 ± 127; n = 4	same (2010)
28	Unknown	384 ± 86; n = 4	same (2012)
29	Unknown	355 ± 33; n = 4	same (2011)
30	Unknown	362 ± 79; n = 3	same (2010)
31	Unknown	403 ± 74; n = 4	Harju county, Kuusalu municipality, Pudisoo village, Punsu farm (2011)
32	Unknown	523 ± 60; n = 4	same (2012)
33	Unknown	571 ± 42; n = 4	Tartu county, Tähtvere municipality, Tüki village (2012)
34	Unknown	525 ± 141; n = 4	Jõgeva county, Jõgeva municipality, Laiuse (2012)

where the *P. somniferum* cultivar samples were cultivated in 2010–2012. Samples Nos 23–34 are unknown cultivars from different home gardens in Estonia (Table 1); the gardeners cultivated them as ornamental poppies during several years and they did not know the names of these cultivars.

The unripe capsules were collected before the colour change of the seeds, sliced into at least six parts, and dried immediately after harvesting during 10 days in a dark room at room temperature. The samples were preserved in well-closed minigrip bags at room temperature in the absence of light. Before analyses, the dried plant samples were powdered in a mortar and the powder was sieved through a 3 mm sieve.

2.2. Determination of alkaloids

The HPLC analyses of morphine and codeine were performed according to the monograph *Opium, Raw* of the *European Pharmacopoeia* (2010). About 0.5 g of dried and ground herbal material was suspended in 20 mL of ethanol (50% V/V), mixed with the aid of ultrasound for 30 min, allowed to cool, and diluted to 25 mL with the same solvent. To 5.0 mL of the supernatant liquid 2.5 mL of ammonium chloride buffer solution pH 9.5 and 5.0 mL of water were added and mixed. Of this solution 1.0 mL was transferred to the solid phase extraction column about 8 cm long and about 10 mm in internal diameter containing 0.75 g of kieselguhr for chromatography and allowed to stand 15 min. The column was eluted with 2 quantities, 2 mL and 1 mL, of a mixture of 15 volumes of 2-propanol and 85 volumes of methylene chloride. The mixed eluent was used for HPLC analysis. Chromatography standards were treated in the same way. HPLC analysis was carried out immediately after sample preparation. Column: Phenomenex Luna C8(2), 250 mm × 4.0 mm, particle size 5 µm. Eluent: 1.0 g of sodium heptanesulphonate monohydrate dissolved in 420 mL of water, pH adjusted to 3.2 with dilute phosphoric acid, and 180 mL of acetonitrile added. Injection volume 20 µL, flow rate 1 mL/min, detection wavelength 280 nm, column temperature 40 °C.

For CE the extract of herbal material with 50% alcohol prepared for HPLC analysis was used without additional purification. The extract (0.75 mL) filtered through a 0.45 µm nylon membrane filter was mixed with the background electrolyte (0.25 mL). A modified method (Walker et al., 1996) was used to perform CE. Quartz capillary 50 cm × 50 µm, temperature 20 °C, injection by pressure 30 mbar 5 s, voltage 25 kV, detection wavelength 280 nm. Background electrolyte: 1 + 9 mix (V/V) of methanol and 200 mM phosphate buffer, pH 4.5. Between runs the capillary was washed

2 min with 0.1 M sodium hydroxide solution, 2 min with water, and 2 min with the background electrolyte. Standards were prepared using substances that conformed to the corresponding *European Pharmacopoeia* monographs.

3. RESULTS AND DISCUSSION

The content of morphine in different known *P. somniferum* cultivars varied more than four times (152–676 mg/100g, Table 1). The average morphine level in all *P. somniferum* cultivars studied ($n = 34$), as well as the average concentration in known, unknown, and *P. somniferum* var. *paeoniflorum* cultivars was similar (389, 359, 395, and 395 mg/100g, respectively). The cultivars richest in morphine content were ‘Queen’s Poppy’ (676 mg/100g) and ‘Lauren’s Grape’ (627 mg/100g), other varieties contained morphine less than 600 mg/100g (Table 1). The variability of morphine content in different cultivars is illustrated in Fig. 1.

Ornamental poppy capsules from Italy contain 227 mg/100g of morphine (Comparini and Centini, 1985), but the content is much higher (110–1140 mg/100g, average 475 mg/100g) in 99 poppy lines from Turkey (Gümüüşcü et al., 2008) as well as in capsules from Hungary (760–1150 mg/100g; Németh-Zámbori et al., 2011). In plant material from India the content varies from 20 to 1050 mg/100g (Prajapati et al., 2002). According to Bernáth et al. (2003), alkaloid-free cultivars for seed production are accumulating less than 0.2 mg/100g of morphine in capsules and cultivars with especially high alkaloid level contain 15–25 mg/100g of morphine. In the straw of poppy cultivars the highest morphine content of 10 mg/100g was fixed by the German Federal Health Agency (Sharma et al., 1999). By Stranska et al. (2013) the high-morphine cultivar ‘Buddha’ contains 1640 mg/100g of morphine, but there is also a medium content (790 mg/100g) and a low content (610 mg/100g) of morphine in capsules of *P. somniferum* cultivated in the Czech Republic. There is no clear definition of morphine-rich *P. somniferum* cultivars, but a cultivar is considered industrial if its ripe capsule walls contain at least 800 mg/100g of morphine (Matyasova et al., 2011).

Literature data (Shuljgin, 1969; Duke, 1983) and our experience show that the average yield of capsules may be 37–145 g from 7–15 plants per square metre. If the average content of morphine in capsules is 362 mg/100g, the theoretical yield of morphine is 134–525 mg per square metre. In case of a home garden opium poppy bed of 10 m × 10 m, the yield of morphine from one bed of ornamental *P. somniferum* cultivars could be about 13–50 g. Thus, the content of morphine in ornamental poppies cultivated in Estonia is not

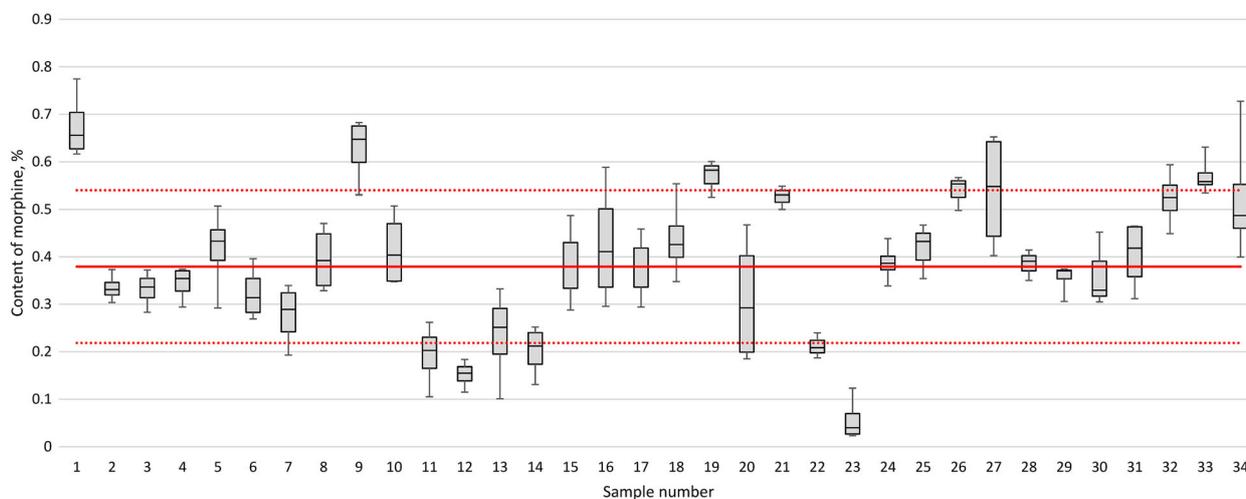


Fig. 1. Boxplot of morphine content in dried capsules of 34 different poppy cultivars determined by HPLC. Boxes demonstrate the first quartile, median, and the third quartile; whiskers show minimum and maximum results for each sample measured. The solid line marks average and the dashed lines standard deviation limits of all samples. Sample numbers refer to Table 1.

especially high, but absolutely remarkable from the point of view of potential use for narcotic purposes. It was reported that in 2007, injectable poppy liquid was the most common substance of abuse among injecting drug users in Kohtla-Järve, Estonia (Lõhmus et al., 2008). At the same time, in the capital city Tallinn one out of 301 injecting drug users injected poppy liquid (Vorobjov et al., 2012). In Ukraine 89% (Booth et al., 2004) and in Moldova 92% of such addicts (Rhodes et al., 2011) were reported to use poppy liquid, making it the most commonly injected drug in these countries. Our findings indicate that ornamental poppies grown in home gardens could possibly be abused in a similar manner and unsuspecting gardeners could provide a source of crude material for abuse.

These 34 samples of poppy cultivars we studied contained also notable amounts of codeine, papaverine, and apomorphine (Table 2). The average concentration of morphine was about 14 times higher than the content of codeine in our samples. This agrees with literature

Table 2. Minimum, maximum, and average content (mg/100g) of morphine, codeine, papaverine, and apomorphine with standard errors in dried capsules of 34 different poppy cultivars determined by CE

Alkaloid	Min	Max	Avg	SE
Morphine	48	616	362	21
Codeine	0	83	25	5
Papaverine	0	138	29	5
Apomorphine	1	59	12	2

data: the codeine content was found to be 4–22 times less than that of morphine by several authors (Prajapati et al., 2002; Gümüşçü et al., 2008; Németh-Zámbori et al., 2011). In 99 *P. somniferum* lines from Turkey the content of codeine was found to be 5–270 mg/100g and that of papaverine 1–440 mg/100g (Gümüşçü et al., 2008). Apomorphine is not a native opium alkaloid, probably it was detectable as the morphine derivative formed in the process of sample preparation.

Both HPLC and CE methods showed similar results. This was confirmed by two-tailed *t*-test, which demonstrated no statistically significant differences between the results obtained with these two methods. Nevertheless, CE seems to be a more suitable method for the determination of opium alkaloids in moderate concentrations.

The average content of morphine in seeds of ornamental poppy cultivars ($n = 15$) was 18 mg/100g, ranging from 0 to 47 mg/100g between different cultivars. The content of codeine was 5 mg/100g (0–19 mg/100g). Lo and Chua (1992) found three white poppy seed samples to contain 58–62 mg/100g of free and bound morphine. Poppy seeds with a morphine content of more than 1 mg/100g are regarded as not safe if consumed in usual quantities and poppy seeds for decorative purposes could contain up to 10 mg/100g of morphine (Sproll et al., 2007).

Although HPLC is the mainstay of quantitative analysis, CE offers a strong alternative in measuring complex mixtures containing substances with different chemical structures and properties. The most prominent advantage of CE in the analysis of opium alkaloids is the simplicity of sample preparation. Also, the possibility of

measuring other alkaloids in addition to morphine and codeine in a single run is a considerable benefit. On the other hand, a drawback of this method is its minor sensitivity compared with HPLC. Hence, it was possible to measure low levels of alkaloids in the seeds only with the latter method. As the separation of substances occurs due to different principles, these two methods with their advantages and disadvantages are complementary. Obtaining similar results with two entirely different separation techniques enables to place greater confidence in the validity of the results.

It can be concluded that capsules of *P. somniferum* contain morphine in remarkable concentrations, which makes their illegal use a considerable risk. Our results show CE to be a suitable alternative to HPLC analysis as it allows excluding the extra sample purification step by solid phase extraction.

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Alkaloidisisaldus Eestis kasvavate unimagunate (*Papaver somniferum* L.) ilusortides

Andres Meos, Liis Saks ja Ain Raal

Unimaguna (*Papaver somniferum* L.) tööstuslike sortide alkaloidisisaldust on uuritud mitmete autorite poolt, dekoratiivsortide oma aga mitte. Käesoleva töö eesmärk oli analüüsida morfiini-, kodeiini- ja papaveriinisaldust erinevates unimagunasortides, kasutades kõrgefektiivse vedelikkromatograafia (HPLC) ning kapillaarelektroforeesi (CE) meetodeid. Uuritud taimne materjal kasvatati kaubandusvõrgus saadaolevatest seemnetest või koguti mitmetest Eesti aedadest, kus neid kasvatati tundmatute ilusortidena. CE põhjal oli kõikide kuivatatud kuparde proovide ($n = 34$) keskmine morfiinisisaldus 362 mg/100 g, kodeiinisisaldus 25 mg/100 g ja papaveriinisisaldus 29 mg/100 g. HPLC järgi jäi morfiinisisaldus erinevates sortides vahemikku 152–676 mg/100 g. Nii suur morfiinisisaldus on märkimisväärne ja võimaldab potentsiaalselt unimaguna ilusortide illegaalset kasutamist. Tulemused olid mõlema analüütilise meetodiga sarnased. CE pakub HPLC-le tugevat alternatiivi, kuna proovide ettevalmistamine analüüsiks on viimasest lihtsam.