CHARACTERIZATION OF ESSENTIAL OILS ISOLATED FROM RANUNCULACEAE SPECIES FROM ROMANIA

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Abstract: Nowadays, the interest to essential oil-bearing plants has increased significantly due to their therapeutic and industrial potential. Therefore, the main aim of this study was to examine and confirm the presence of essential oils in seven different Ranunculaceae species from Romania by hydrodistillation. The results show that five (*Aconitum toxicum*, *Aconitum variegatum, Hepatica nobilis*, *Ranunculus carpaticus,* and *Ranunculus polyanthemos)* out of the seven investigated species contain volatile oils isolated for the first time from aerial parts. The hydrodistillation of the above-mentioned species resulted in a yellow to cloudy white colored essential oil with a waxy texture and yields ranging from 0.013 to 0.245 %. Our results are in accordance with some previous studies that have revealed similar quantities of essential oils in other taxa belonging to the Ranunculaceae family.

Keywords: buttercup, volatile oils, hydrodistillation, color, yield

INTRODUCTION

In the recent years, an increasing demand was observed for essential oil-bearing plants due to their diverse bioactive properties including antimicrobial, antiparasitic, insecticidal, antiviral, antifungal and antioxidant effects (Hyldgaard *et al.*, 2012). Furthermore, essential oils have been known and used for centuries with different purposes such as cosmetic, medicine, perfumery and some with commercial purposes as well for flavoring, fragrance and food industry (Baser and Buchbauer, 2010). Despite the therapeutic and industrial potential of essential oils, their presence in many plant species is still unknown.

Ranunculaceae Juss. (buttercup family) is one of the largest flowering plant families including approximately 60 genera and 2,500 species distributed worldwide, but most commonly spread in temperate and cold areas of the northern hemisphere (Heywood *et al.*, 2007). Although many Ranunculaceae species are well-known as being highly poisonous, a great number of this plants have been used for a long time as traditional medicines, spices, and vegetables on the condition that their toxicity to be reduced after a cautious processing (Hao *et al.*, 2015). In Romania, the buttercup family is represented by approx. 23 genera and 110 species, distributed in all regions of the country (Cristea, 2014). Indigenous species have been known and used for food (e.g. *Caltha palustris*) or as ornamentals, while some of them were highly appreciated in traditional medicine showing beneficial effects on various ailments such as bronchitis, diarrhea, fever, gout, and rheumatism (Tămaş, 2005; Alexan *et al.*, 1991).

Beside the above-mentioned uses of the Ranunculaceae species, essential oils that have been found and determined only in a few of these species may add a value to their therapeutic and healing properties. The first phytochemical investigation revealing the presence of essential oils in this plant family was reported by Aboutabl *et al.* (1989), who

isolated and identified the volatiles in *Nigella sativa* seeds. Similar studies show that essential oils extracted from *Nigella* species have several beneficial effects including antioxidant, antimicrobial, anticancer and hepatoprotective activities (Baser and Buchbauer, 2010; Edris, 2007; Fico et al., 2004; Kokoska *et al.*, 2008). Other reports, from the scientific literature also describe the occurrence of volatile oils determined by hydrodistillation from different genera of buttercup family such as *Aconitum* (Zhang *et al.*, 2009), *Adonis* (Mohadjerani *et al*., 2013), *Anemone* (Shi *et al*., 2012), *Aquilegia* (Radulovic *et al.,* 2007), *Consolida* (Kokoska *et al.,* 2012), *Delphinium* (Gulec *et al*., 2007), *Ranunculus* (Terzioglu *et al.*, 2008; Erdogan *et al*., 2014) and *Trollius* (Witkowska-Banaszczak, 2013).

In contrast to these reports, others from the taxonomical literature still refer to the Ranunculaceae family as a taxon without essential oil content in their chemical composition (Watson and Dallwitz, 1992). Therefore, the main aim of this study was to investigate and determine the essential oil content from seven different Ranunculaceae species in order to confirm or reject previous hypotheses.

MATERIALS AND METHODS

Plant material. Seven species belonging to the five genera of the Ranunculaceae family, namely *Aconitum toxicum* Rchb, *Aconitum variegatum* L., *Caltha palustris* L., *Hepatica nobilis* Mill., *Ranunculus carpaticus* Herbich, *Ranunculus polyanthemos* L. and *Trollius europaeus* L. have been used to carry out this experiment. Herbarium specimens were used as being considered essential in the pharmaceutical community for documenting the source material used for drug discovery (Eisenman *et al*., 2012). The plants were harvested from their natural habitat from five different mountain areas from Romania (Mt. Almajului, Mt. Intorsurii, Mt. Piatra Craiului, Mt. Postavaru and Mt. Stamba) during the summer periods of 2016 and 2017. Plant identification was carried out in the field. The voucher specimens were prepared and (Figure 1) have been stored in the Herbarium collection (CLA) at the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania. The voucher specimen numbers and the origin of plant materials are given in Table 1.

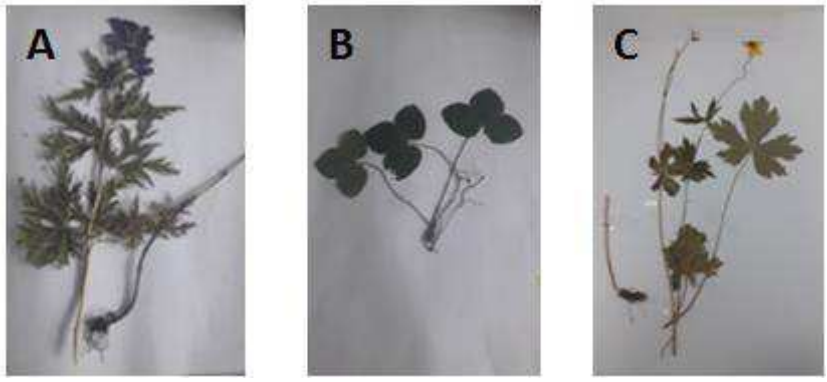

Figure 1. Voucher specimen of *A. toxicum* (A), *Hepatica nobilis* (B) and *R. carpaticus* (C)

Table 1

Voucher specimen number and origin

Plant species	HSV[a)]	Collected site
Aconitum toxicum Rchb.	CLA30063	45°33′12.6′′N, 25°25′27.0′′E
Aconitum variegatum L.	CLA30048	46°12′50.8′′N, 22°51′35.4′′E
Caltha palustris L.	CLA30064	45°37′53.4′′N, 26°08′36.7′′E
Hepatica nobilis Mill.	CLA30065	44°40′46.1′′N, 21°42′28.4′′E
Ranunculus carpaticus Herbich	CLA30044	45°34′56.1′′N, 25°33′21.7′′E
Ranunculus polyanthemos L.	CLA30051	46°12′50.8′′N, 22°51′35.4′′E
Trollius europaeus L.	CLA30066	45°34′56.1′′N, 25°33′21.7′′E

Note [a]) Herbarium specimen number.

Oil isolation

Aerial parts of the plant species were air-dried and ground into powder using a GM100 Retsch electric mill (Retsch, Rheinische, Germany) (Figure 2-A). The samples were prepared (Figure 2-B), weighted (weight ranging from 8.8 to 97.8 g) and then subjected to hydrodistillation in 1 L of distilled water for 3-4 hour (timing set from the moment when the content of the flask started to boil) using a Clevenger-type apparatus (Merci, Brno, Czech Republic) (Figure 2-C). The heating intensity was regulated in such a way to make about 3 ml of liquid to flow to the receiver every 30 minutes. The color of the oily layers was determined based on visual color evaluation (Figure 2-D). After distillation, the oils were quantified by micropipette aspiration and stored in a sealed vial at +4ºC for future analyses. The yield was calculated according to the following formula:

$$\text{EOs yield (\%)} = \frac{v}{w} \; x \; 100$$

Figure 2. Hydrodistillation process of plant material using a Clevenger-type apparatus

RESULTS AND DISCUSSION

Our results show that only five out of the seven investigated species contained essential oils, while the other two had a very low or insignificant amount.

The essential oils obtained from the aerial parts of *Aconitum*, *Hepatica* and *Ranunculus* species due to hydrodistillation, exhibited a yellow to cloudy white color with waxy texture. The volatiles' content was ranging from 0.013 to 0.245 % as presented in Table 2. While the highest essential oil content was recorded in *R. carpaticus* with a yield value of 0.245 %, the lowest oil content was registered in *A. variegatum (*0.013 %). In the other species intermediate values were recorded as follow: *A. toxicum* (0.020 %), *H. nobilis* (0.205 %) and *R. polyanthemos* (0.052 %) respectively.

Table 2

Essential Oil characteristics (EOs) obtained from the seven Ranunculaceae species

Plant species	Plant part used	Weight of sample (g)	EOs volume (µl)	EOs yield% (v/w)	EOs color
A. toxicum	Aerial part	97.8	20	0.020	waxy yellow
A. variegatum	Aerial part	32.2	4	0.013	waxy yellow
C. palustris	Aerial part	20	-	-	-
H. nobilis	Leaves	39	80	0.205	waxy yellow
R. carpaticus	Aerial part	14.3	35	0.245	waxy yellow
R. polyanthemos	Aerial part	28.6	15	0.052	cloudy white
T. europaeus	Aerial part	8.8	-	-	-

In accordance with our results, similar essential oil yield values have previously been reported by Terzioglu *et al.* (2008) and Erdogan *et al*. (2014) for *Ranunculus arvensis* (0.25 %), *R. constantinopolitanus* (0.18 %), and *R. marginatus* var. *trachycarpus* (0.6 %). Furthermore, our results can also be supported by the findings of Kokoska *et al.* (2012), who determined a relatively low essential oil content in *Consolida regalis* (0.019 %), *Delphinium elatum* (0.003 %), *Nigella hispanica* (0.011 %) and *N. nigellastrum* (0.028 %) seeds. In addition, the amount of essential oils isolated from *Aconitum tanguticum* (Zhang *et al.*, 2009), *Delphinium formosum* (Gulec *et al*., 2007) and *Ficaria kochii* (Tavakoli *et al*., 2012) have shown similar values such as 0.7, 0.22 and 0.3 %, respectively.

On the contrary, no essential oil content was detected in *C. palustris* and *T. europaeus* by analyzing the aerial parts of the plant. It is noteworthy that Witkowska-Banaszczak (2013) in her study detected essential oils in the flowers of *T. europaeus* (0.3 %). It is also important to mention that the results may differ depending on the part of the plant used for essential oil quantification. Therefore, the examination of the Ranunculaceae family from the volatiles point of view remains unexplored and needs further investigations.

The chemical profile of the essential oils obtained in this study will be analyzed by GC-MS to reveal their chemical composition. Similar studies have already been carried out by others investigating the chemical profile of essential oils in different species such as *A. wolgensis*, *C. regalis*, *D. elatum*, *F. kochii*, *N. hispanica*. These results show that volatiles were mostly characterized by a high amount of fatty acids represented especially by hexadecanoic, octadecadienoic and octadecatrienoic acids. Besides, Zhang *et al*. (2009) and Erdogan *et al*. (2014), reported the presence of relatively high amounts of monoterpenoids and sesquiterpenoids in essential oil from herbal parts of *A. tanguticum* and *R. marginatus* var. *trachycarpus*.

CONCLUSION

As a conclusion, we isolated for the first time the essential oils from the aerial parts of five species belonging to Ranunculaceae family, namely *Aconitum toxicum*, *Aconitum variegatum, Hepatica nobilis*, *Ranunculus carpaticus,* and *Ranunculus polyanthemos* and obtained new, valuable results. In addition, these results also confirm once again the existence of essential oils in the buttercup family that needs to be investigated in the near future also in other species of the taxa.

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