CHEMICAL CONSTITUENTS OF THE ESSENTIAL OILS OF *NEPETA NUDA* L. SSP. *NUDA* (*LAMIACEAE*) FROM ROMANIA

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The essential oils of *Nepeta nuda* L. ssp. *nuda* obtained by hydrodistillation using neo-Clevenger extractor have been analysed and characterized by capillary GC-FT-IR systems. Eucalyptol was the most abundant component of *N. nuda* oils in different percentages: chemotype *Basarabi* (34.8%), *Frăsinei* (44.87%), and *Herculane* (31.9%). Nepetalactone isomers were found in all ecotypes in various amounts due to ecological conditions, analytical method and plant phenophase.

Key words: *Nepeta nuda*, essential oil composition, GT-FT-IR systems, chemotypes.

INTRODUCTION

The genus *Nepeta* L. (*Lamiaceae, Nepetoideae*) includes 280 species around the world and some of them have been used for medicinal purposes (Pooter *et al.* 1988, Aydin *et al.* 1998). Most oils of *Nepeta* species contain nepetalactones as the main constituents, but different oil compositions have been detected in several *Nepeta* species: *N. racemosa* (Baser *et al.* 1993), *N. discolor* (Mathela *et al.* 1994), *N. italica* and *N. sulfuriflora* (Kökdil *et al.* 1997a) and *N. cilicia* (Kökdil *et al.* 1997b).

N. nuda (subfam. *Nepetoideae*, sect. *Orthonepeta*) is a perennial plant with stems of 50–120 cm, glabrous or puberulent. Leaves 2–5(8) cm, ovate-oblong, more or less cordate at base, crenate, the lower sessile or shortly petiolate, the upper sessile. Inflorescence branched, rarely spike-like. Bracteoles 2–3 mm, linear-lanceolate; calyx 4–6 mm, teeth 1–2 mm; corolla 6–8(10) mm, pale violet or white. The species consists of two subspecies with the following characteristic differences (Turner 1972, Jarvic *et al.* 2001): (a) ssp. *nuda* (incl. *N. nuda* ssp. *pannonica* (L.) Gams, *N. pannonica* L.): lower leaves petiolate, inflorescence lax, calyx-teeth linear-lanceolate, pale green or blue-tinged, corolla pallid violet; (b) ssp. *albiflora* Gams: lower leaves sessile or subsessile, inflorescence compact, calyx-teeth lanceolate, white-tinged, corolla white (in S Yugoslavia, N Greece).

N. nuda ssp. *nuda* is widespread in S. E., E. C. Europe (including Romania) and it extends both northwards to C. Russia and southwards to S. W. Asia. This

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taxon differs from the related species of the *Nepeta* genus grown up in Romania in the following respects (orig., I. Pădure, 2004):

1a Calyx-teeth shorter than the tube; flowers hermaphrodite; leaves subsessile or petiolate; nutlets smooth or tuberculate 2

1b Calyx-teeth longer than the tube; flowers unisexuate (rarely dioeciuos); central flowers of each cyme female, the stamens represented by staminodes; outer flowers male, with a rudimentary pistil; all leaves petiolate; nutlets tuberculate ... 3

2a Leaves ovate, cordate at the base, all petiolate; corolla white with small purple spots; nutlets smooth ..... *N. cataria*

2b Leaves ovate-oblong, the middle and the upper ones sessile; corolla pale violet or white; nutlets tuberculate ..... *N. nuda*

3a Plant glabrescent, leaves elongated ovate-lanceolate, crenate-serrate; cymes 3- to 5- flowered, lax ..... *N. ucranica*

3b Stem and leaves dense tomentose to lanate, leaves triangular ovate, serrate, with larger teeth; cymes dense..... *N. parviflora*

The essential oils of *N. nuda* ssp. *nuda* were chemically investigated in Turkey by Kökdil *et al.* (1998) and Baser *et al.* (2000) from the different populations in the north and central Anatolia. Nepetalactone isomers were not present in the oil of *N. nuda* (Kökdil *et al.* 1998). Morphological and chemical differences between the two subspecies exist and are well-known (Kokkini & Babalonas 1982). In the essential oil of *N. nuda* ssp. *albiflora* 4aα-7α-7aα-nepetalactone and 4aα-7α-7aβ-nepetalactone were found as the main constituents (Sarer *et al.* 1996, Takeda *et al.* 1996). De Pooter *et al.* (1987, 1988) reported the presence of varying amounts of 1,8-cineol, a mixture of nepetalactones and germacrene-D as chief components in different *N. nuda* oils. Handjieva *et al.* (1996) showed that a nepetalactone isomer was present in high concentrations in the oil from Bulgarian populations of *N. nuda*.

In Romania, the essential oil of *N. nuda* was investigated for the first time from the chemical point of view (Pădure, 2005). In the essential oil of *N. nuda* ssp. *nuda* the eucalyptol was found as the main constituent and a mixture of nepetalactones is present.

MATERIAL AND METHODS

Plant material: The leaves and flowering tops of *N. nuda* ssp. *nuda* were collected in May 2003 near Basarabi in Dobrudja, in June 2002 from Frăsinei (on Oltului Valley) and in July 2003 near Herculane in S.W. of Romania. Voucher specimens are kept at the Herbarium of USAMV Bucharest (BUAG 23778). The material was harvested in vegetative or full flowering stages, between 11–13 AM.

The herbal was dried at room temperature and stored 2–7 days in paper bags and preserved in dark and dry places.

Isolation of the essential oil: Air-dried herbal parts of the collected plant were subjected to hydrodistillation for 3h using a neo-Clevenger system to produce oil.

Gas chromatography: FISIONS GC chromatograph with DB 5 column 25 m length and 0.25 mm internal diameter. Carrier gas has been nitrogen, initial ramp 40° C, isothermal for 5 minutes, final temperature 280° C and 4° C/min gradient. Using the Nicolet GC-FR-IR transfer line with MCT high sensitive nitrogen cooled detector, all peaks from GC system can be identified by infrared spectra using specific infrared gas phase flavours library. The FT-IR parameters have been: 4 000–750 cm^{-1} spectral range, 8 cm^{-1} resolution and 7 scan/sec.; acquisition speed; transfer line and cell temperature 250° C. Due to the non-destructive IR analysis, the sample was conducted in a classic FID detector for quantification after the IR transfer line.

Identification of the components: Compound identification was performed using a chemical library and Kovats indices as a confirmation for the chromatographic peak position.

RESULTS AND DISCUSSION

GC and GC-MS analyses of the oils were carried out according to a procedure that has been described above. The chemical compounds of *N. nuda* chemotypes are listed in Table 1. The chromatograms with GC traces and an interesting comparison between FT-IR nepetalactone spectra are presented in Figures 1, 2, 3. The yield and composition of the essential oils of *N. nuda* chemotypes can be seen in Table 2.

The population of *N. nuda* differs in the following ecological and pedological respects: *"Basarabi" ecotypes*: present oligotrophic and calcareous soil with a superficial rocky texture, sunny place, on slope's base, moderate draining, unpolluted zone, natural habitat (natural reserve); *"Frăsinei" ecotype* presents eutrophic soil, fertile soil with moderate draining, unpolluted zone, sunny place, natural habitat (meadow); *"Herculane" ecotype* presents eutrophic soil, skeleton-like texture, no slope, poor draining, sunny and ruderal place near housekeeping, polluted zone, natural habitat.

The chemical constituents and percentage composition of three essential oils of *N. nuda* ssp. *nuda* were listed in Table 1. Sixteen compounds were identified using library searched and Kovats indices representing up to 95.11% of the total oil amount.

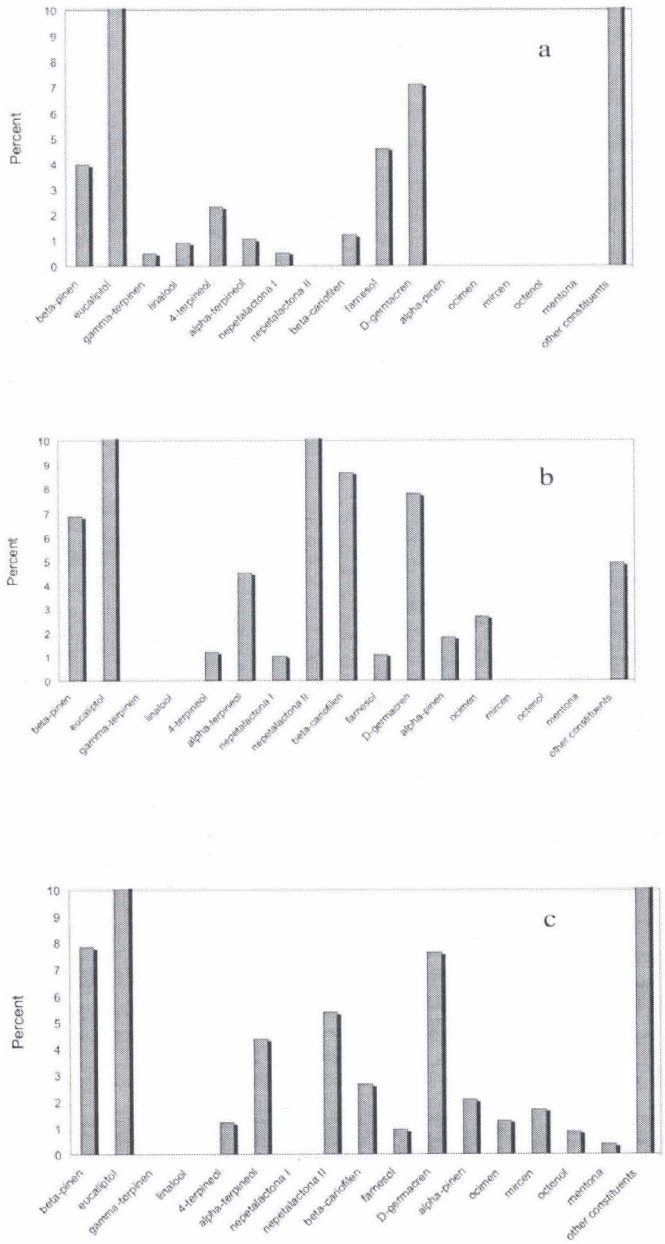

Fig. 1. Essential oils of *Nepeta nuda* L. subsp. *nuda* chemotypes a) "Basarabi", b) "Frăsinei", and c) "Herculane".

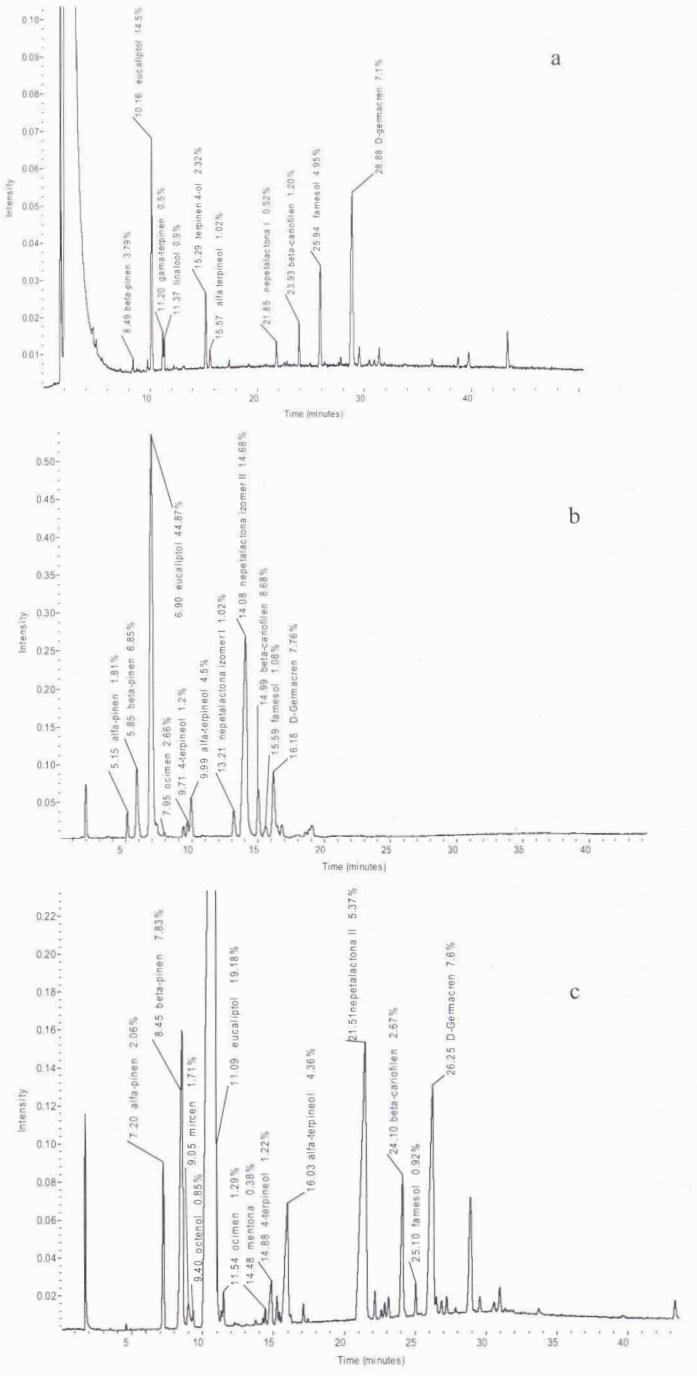

Fig. 2. Essential oils GC traces of several *Nepeta nuda* L. subsp. *nuda* chemotypes a) "Basarabi", b) "Frăsinei", and c) "Herculane".

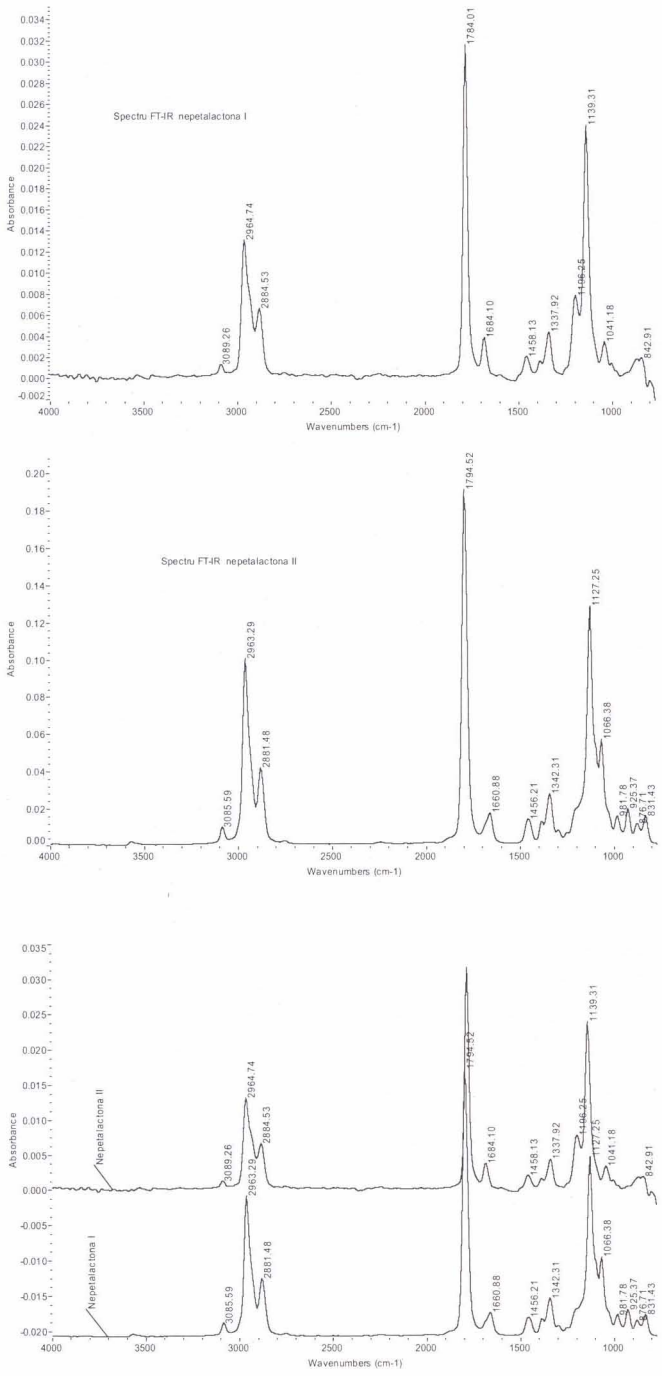

Fig. 3. Comparative analysis of nepetalactone FT-IR spectra of *Nepeta nuda* L. oil.

Table 1

The percentage composition of the essential oils of *N. nuda* ssp. *nuda* compounds

Chemical constituents	"Basarabi" chemotype	"Frăsinei" chemotype	"Herculane" chemotype
β–pinen	9.09	6.85	13.1
eucaliptol	34.8	44.87	31.9
γ–terpinen	1.20	–	–
linalool	2.20	–	–
4–terpineol	5.57	1.2	2.03
α–terpineol	2.44	4.5	7.26
nepetalactona I	1.24	1.02	traces
nepetalactona II	traces	14.68	8.94
β–cariofilen	2.90	8.68	4.44
farnesol	11.02	1.08	1.53
D–germacren	17.04	7.76	12.65
α–pinen	–	1.81	3.43
ocimen	–	2.66	2.14
mircen	traces	–	2.84
octenol	–	–	1.41
mentona	traces	–	0.63

The plants were collected in different stages from several ecotypes listed in Table 2. In "Frăsinei" and "Herculane" chemotypes the percentage of full blooming stage are representative to result a good yield of percentage of the oil.

Table 2

Origin of chemotypes and plant used parts in compound identification of *N. nuda* ssp. *nuda*

Chemo type	Used parts	Phenophase	Dry material (g)	Oil amount (ml)	Yield (%)	Time of harvesting (hour)
Basarabi	SL	SS	64,1	0,25	0,4	13
Frăsinei	SL	50%FB+40%BB+FB, 10%EB	137,84	4,47	3,2	11
Herculane	SIL	40%FB+50%BB+FB, 10%EB	104,6	0,70	0,66	12

Legend: stem with leaves (SL), stem with inflorescence and leaves (SIL), *phenophase and blooming percent*: floral bud (FB), beginning of blooming (BB), full blooming (FB), end of blooming (EB), sterile shoots (SS).

CONCLUSION

In Romania, the chemical composition of *N. nuda* L. ssp. *nuda* essential oil was investigated for the first time. Nepetalactone isomers were found in all studied populations. In the essential oil of *N. nuda* ssp. *nuda* the eucalyptol (31.9–44.87%)

was found as the main constituent and a mixture of nepetalactone isomers are present in different amounts: nepetalactone I (traces to 1.24%), and nepetalactone II (traces to 14.68). Other constituents like β-pinnene, α-terpineol, β-caryophyllene, farnesol or germacrene-D were found in all *N. nuda* chemotypes. Its percentage depends on ecological and climatic conditions, soil type and texture, the moment of harvesting, plant phenophase or analytical method.

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