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Der Pharmacia Sinica, 2011, 2 (3): 207-210

Der Pharmacia Sinica

ISSN: 0976-8688
CODEN (USA): PSHIBD

Chemical Composition of the Essential Oil of *Nepeta Sintenisii* leaves Growing wild in Darkesh Protected Area(North Khorassan Province Iran)

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ABSTRACT

Nepeta is a one of the most important genera of the Lamiaceae family with regard to the number of species. Some species of this genus are important medicinal plants and their extracts have been used for medicinal purposes. In this investigation aerial parts of Nepeta sintenisii . was subjected to hydrodistillation and the chemical composition of the isolated essential oil was analyzed by GC/MS method for first time. twenty one constituents (97.82% of the total oil) were identified of which alpha-terpinolene (47.86), E-β-farnesene (22.67%), nepetalactone (4.93%), β- bisabolene (3.65%) and germacrene-D (2.27%) were the main components. The constituents of the volatile oil of N. sintenisii is similar to the composition of the other Nepeta genus.

Keywords: Nepeta sintenisii, Lamiaceae, Essential oil composition, alpha-terpinolene, E-β-farnesene.

INTRODUCTION

Nepeta (Lamiaceae) is a genus of perennial or annual herbs which is found in Asia, Europe and North Africa. About 250 species of Nepeta are reported (1) of which, 67 species are present in Iran (2). Nepeta species are widely used in folk medicine because of their antispasmodic, expectorant, diuretic, antiseptic, antitussive, antiasthmatic and febrifuge activities (3-5). Nepeta cataria (Catnip) is the most famous Nepeta species which has a long history of use as a tea in Europe before real tea was imported from the orient. The flowering tops of plant have also been used as a sedative drug (6).Many reports on the essential oils of Nepeta species show that the main constituents of the oil are diastereomeric nepetalactones. These compounds are responsible for their feline attractant or insect repellant properties (7). Nepeta sintenisii is a herbaceous wild plant endemic to Iran (2, 8) and no phytochemical studies about this plant has been reported. In continuation of investigations on chemical composition of the essential oils of various Nepeta species which are grown in Iran.

MATERIALS AND METHODS

2.1- Plant material

Leaves of *Nepeta Sintenisii* were collected at the flowering stage from the Darkesh Protected Area, Bojnourd (North Khorassan Province Iran) In Jun 2010,and identified at the Research center for plant of Department of environment of North Khorassan,Iran. A voucher specimen has been deposited in the Herbarium of research center for plant .

2.2- Isolation of the essential oil

leaves of *Nepeta Sintenisii* were air-dried for 4 days before isolation of essential oil. The plant material (100gr) was cut into small pieces and The essential oil was obtained by the hydrodistillation method, using a Clevenger apparatus. The temperature and pressure of hydrodistillation were 120ºC and 560 mmHg respectively. The distillation time was six hours. The resulting pale yellow oil was then dried over anhydrous sodium sulphate and 30 µL were solubilized in 1 mL of dichloromethane before the GC injection. 1 µL of this solution was directly used for analysis.

2.3-Gas chromatography and mass spectrometry

Gas chromatographic analysis was performed on an Hewlett-Packard(HP)6890A instrument equipped with a flame ionization detector and Rtx-5MS (15 m × 0.25 mm × 0.25 µm) capillary column, while the essential oil components were identified on an Agilent Technologies 5973N mass spectrometer. The GC settings were as follows: the initial oven temperature was held at 35 ºC for 6 min and ramped at 5 ºC min−1 to 150 ºC for 0 min, and then ramped at 10 ºC min−1 to 280 ºC for 3 min. The injector temperature was maintained at 250 ºC. The samples (1 µL) were injected neat, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0 mL min−1. Spectra were scanned from 20 to 550 m/z at 2 scans s-1. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes under the same operating onditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature [9-14]. Component relative percentages were calculated based on GC peak areas without using correction factors.

RESULTS AND DISCUSSION

The average yield of essential oil obtained after hydrodistillation of the leaves of *Nepeta Sintenisii* was about 0.35%. Table 1 reports the chemical composition of the essential oil under study. Thirty components were identified ,accounting for %97.82 of the total oil.

The various compounds were identified by comparison of their Kováts retention indexes, determined utilizing a non-logarithmic scale on non-polar (Rtx-5MS) columns, and by comparison of the mass spectra of each GC component with those of standards and with reported data[15].

High resolution gas chromatography-mass spectrometric (HP GC-MS) analysis and Kováts Index values showed that its principal components are the alpha-terpinolene (47.86), E-β-farnesene (22.67%), nepetalactone (4.93%), β- bisabolene (3.65%) and germacrene-D (2.27%).

Table 1. Percentage composition of the essential oil isolated from aerial parts of *Nepeta Sintenisii*

NO	compound	Experimentally determined KI[a]	HP GC-MS Peak area [%]	Method of identification
1	p-xylene	888	0.39	GC-MS,Ms
2	Decane	1000	1.12	GC-MS,Ms
3	1,8-cineole	1093	0.3	GC-MS,Ms
4	Trans-β-Ocimene	1129	0.59	GC-MS,Ms
5	1,4-dimethyl benzene	1212	2.87	GC-MS,Ms
6	2,4-dimethyl-2-pentene	1216	0.34	GC-MS,Ms
7	Nepetalactone	1357	4.93	GC-MS,Ms
8	β -Bourbonene	1378	0.45	GC-MS,Ms
9	α -terpinolene	1387	47.86	GC-MS,Ms
10	5,6,7,7a-tetrahydro-cyclopenta[c]pyran-1(4aH)-one	1392	1.28	GC-MS,Ms
11	β -caryophyllene	1412	1.91	GC-MS,Ms
12	α- caryophyllene	1446	0.37	GC-MS,Ms
13	E- β -farnesene	1458	22.67	GC-MS,Ms
14	Germacrene-D	1475	2.27	GC-MS,Ms
15	β-bisabolene	1507	3.65	GC-MS,Ms
16	β - sesquiphellandrene	1521	1.98	GC-MS,Ms
17	(+)-spathulenol	1572	1.23	GC-MS,Ms
18	(-)-caryophyllene oxide	1577	1.32	GC-MS,Ms
19	Bis(2-methyl propyl)-1,2-benzendicarboxylic acid	1870	0.38	GC-MS,Ms
20	Dibuthyl phthalate	1966	0.7	GC-MS,Ms
21	1,2-benzendicarboxyl	2552	1.21	GC-MS,Ms
			Total: 97.82%	

Acknowledgment

The authors are grateful to Mr. Joharchi and Mr .Memariani for identification of the plant material and to the Payame Noor University (PNU) of iran for the financial support.

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