Trypanocidal Activity of Oil of the Young Leaves of *Nepeta cataria* L. Obtained by Solvent Extraction

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Abstract

Trypanocidal activity was found in the volatile oil of the Young leaves of *Nepeta cataria* L. which has been extracted by diethyl ether and analyzed by GC and GC/MS. Four compounds in the oil of *N. cataria*, representing 97.53% of the oil were identified. The oil was enriched of monoterpenes with α- citral (51.95%) as the major compound. Other main compounds were β- citronellol (9.03%), geraniol (4.31%) and nerol (32.24%). Nepetalactone was not detected in that oil.

Keywords: ***Nepeta cataria,*** **Lamiaceae, trypanocidal, essential oil composition, α- citral, nerol**

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Introduction

Nepeta is a genus of Lamiaceae family, with almost 280 species, widespread in Europe, Asia and Africa [1]. Sixty seven species of which are found in Iran including *Nepeta cataria* L. [2]. These plants have been used in folk medicine as disinfectant and cure against colds [3]. The essential oil of *N. cataria*, commonly named catnip, has been evaluated as a fungistatic and bacteriostatic agent [4]. The essential oil of *N. cataria* is the most intensively studied oil and characterized by the presence of various nepetalactone isomers which have a specific opioid receptor subtype agonist activity [5, 6]. But about the *N. cataria* var citriodora Balb., Mamladze *et al*., reported no presence of nepetalactone isomers in that oil [7]. In this study, we aimed to analyze trypanocidal activity of the volatile oil of catnip extracted by diethyl ether as a solvent and detect the presence of nepetalactone. Because, there was not any report about the solvent extraction of the young leaves. Also, there is no repot in literature reviews about the biological activity of the volatile oil *N. cataria* against the epimastigotes of *Trypanosoma cruzi* the ethiological agent of Chagas disease.

Experimental

Plant Material

Young Leaves of *N. cataria* L. were collected from Medicinal Plants Garden housed at Medicinal Plants Garden at Tehran University in July 2006. Voucher specimens were deposited at the Herbarium of Medicinal Plants Research Center housed at Tehran University of Medical sciences.

Isolation Procedure, Identification and Quantification of the Oil

Fifty mg of the young leaves of the plants was put in to small glass tubes and added 3 ml of ethylic ether and kept in 4°C over night. Ether extract was eluted over silicagel small column to separate the color pigments and chlorophyll then injected to gas chromatogragh (G 5000, Hitachi co.) with PEG 6000 column (3 mm diameter×3 m lengh). FID (flame ionization detector) was used. The temperature program is followed: Injector temperature 160 °C, oven temperature 100°C for 1min, flow rate 5°C/ min increased to final temperature 200°C for 15 min. Detector temperature was 210°C. Nitrogen used as the carrier gas.

GC-MS was performed on a cross-linked 5% methyl phenyl siloxane (HP-5, 30 m × 0.25 mm i.d., 0.25 μm film thickness), carrier gas, He; split ratio, 1:15; quadruple mass spectrometer (Hewlett-Packard 6890) operating at 70 eV ionization energy. The retention indices for all the components were calculated by using retention times of n-alkanes (C8-C25) that were injected after the essential oil at the same temperature and conditions. The components were identified by comparison of retention indices (RI, DB-5) with those reported in the literatures and also by comparison of their mass spectra with the published mass spectra or Wiley library [8]. Percentage of each component was calculated on the basis of the peak area.

Evaluation of trypanocidal activity

Epimastigotes of *T. cruzi* (Tulahuen strain, prepared in Kyoto University, Japan) were kept in GIT medium (Wako Pure Chemical Industrial, Ltd, Osaka, Japan) supplemented with hemin (12.4 μM, Wako). The epimastigotes in GIT medium (10 μL) were incubated with a test sample dissolved in EtOH (5 μL) and autoclaved salin (185 μL). All samples were incubated at 27 ˚C for 24 h. The movement of epimastigotes was observed under a microscope. We assumed that

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immobilized organisms were dead. The control contained ethanol in the same proportion as used to dissolve the drugs. Each assay was performed in duplicate. Minimum lethal concentration (MLC, concentration on which, all epimastigotes were dead) of each compound was performed. Gentian violet (MLC = 6.3 μM) is used as a positive control [9].

Results and Discussion

Young Leaves of *N. cataria* collected in Iran, were extracted by diethyl ether as a solvent to obtain the volatile oil. The oil was yellow with pleasant odor, which was dominated by citral smell. The results of the biological test of the oil showed strong *in vitro* trypanocidal activity against the epimastigotes of *T. cruzi* (MLC = 6.2 μM).

Results obtained from GC and GC-MS of the essential oils of *N. cataria* is represented in Table 1. Volatile oil was pale yellow, with an intense and distinct odor.

Four compounds were identified from the oil of *N. cataria*, representing 97.53% of the total oil. The main components were α- citral (51.95%), β- citronellol (9.03%), gera niol (4.31%) and nerol (32.24%). Nepetalactone was not detected in this oil although, it was found in the oil, previously extracted from the aerial parts of catnip originated in northern parts of Iran [6]. It seems that the extraction of the young leaves with ether solvent is free of

Table 1. Percentage composition of the volatile oil of *Nepeta cataria.*

Compound	R_I [a]	Percentage in oil
β- citronellol	1228	9.03
Nerol	1228	32.24
Geraniol	1255	4.31
α- citral	1270	51.95
$C_xH_yO_z$ [b]		97.53
Total		97.53
Unidentified		2.47

[a] Retention Indices
[b] Oxigenated monotrpenes

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nepetalactone derivatives and enriched of citral as a trypanocidal monoterpene aldehyde [9]. In conclusion, the time of plant gathering and the method of oil extraction are so much important to reach the pharmacological or biological activities of the oil products. We have previously reported the trypanocidal activity of the *Dracocephalum kotschyi* and *D. subcapitatum* with presence of citral as one of active components [9, 10].

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