ISSN (Online) : 2454-8405
ISSN (Print) : 0973-7200
DOI: 10.18579/jpcrkc/2018/17/2/123613

Journal of Pharmaceutical Research, Volume 17, Issue 2, April-June, 2018

Variation in the composition of the essential oils, hypericin and mineral elements in aerial parts, stem and flower of *Hypericum capitatum* (CHOISY) growing in Turkey with oxidative DNA damage protective activity

Nazim Sekeroglu[a]*, Muge Karaoglan[b], Sevgi Gezici[c], Muhittin Kulak[b], Faruk Ozkutlu[d], Oya Kacar[e], Fatih Gul[f]

[a] Department of Food Engineering, Faculty of Engineering and Architecture, Kilis 7 Aralık University, 79000, Kilis, Turkey
[b] Department of Biology, Faculty of Arts and Sciences, University of Kilis 7 Aralik, 79000, Kilis, Turkey
[c] Department of Molecular Biology and Genetics, University of Kilis 7 Aralık, 79000, Kilis, Turkey
[d] Department of Soil Science, Faculty of Agriculture, University of Ordu, 52200, Ordu, Turkey
[e] Department of Field Crops, Faculty of Agriculture, University of Uludag, 16059, Bursa, Turkey
[f] Department of Chemistry, Faculty of Science, University of Cankiri Karatekin, 18100, Cankiri, Turkey

Research Article

Received on: 2/2/2018 **Revised on:** 14/4/2018 **Accepted on:** 17/4/2018

Abstract

Background and Purpose: *Hypericum* species (*Guttiferae*) are of important for developing pharmaceutical drugs as herbal remedies, due to fact that their rich phytochemical compounds, besides, wide range of biological properties. Among the species, *Hypericum capitatum* CHOISY var. *capitatum* CHOISY, an endemic medicinal plant, naturally distributes in the Southeastern region of Turkey. Thus, we aimed to carry out a comprehensive investigation on determining phytochemical content, as well as determining essential oil composition and mineral concentration of the aerial parts, stem and flowers of the plant. Moreover, potential DNA protective capacity of the extracts from the plant was evaluated in the presented research. **Material and Methods:** Quantification of hypericin content in methanol extracts was measured by spectrophotometric method and the content of hypericin (%) calculated. Essential oil composition of the parts of the plant was performed using GC-FID and GC-MS, and concentration of major and trace elements determined by ICP-OES. DNA protective activity of the water and ethanol extracts from stem, leaves and flowers of the plant were assigned using pBR322 plasmid DNA in the presence of UV/H_2O_2. **Results:** Chromatographic analysis results were showed that nonane and α-pinene amounting to 90.72% were major components for stem, while, α-pinene, undecane and cis-ocimene by rate of 83.75% were major components for aerial parts of the plant, in addition, hypericin content in the flowers was found 0.029%. Regarding of mineral contents, concentration of sixteen mineral were determined for each parts, and most of the determined concentrations were found in accordance with tolerable limits of FAO/WHO for human consumption. As for DNA protection potential of the extracts of the plant, they exhibited powerful DNA protective potential even at the lowest concentration (25µg/ml). **Conclusion:** The results revealed that the plant has a substantial potential for developing novel drugs in pharmaceutical industry.

Keywords: *Hypericum capitatum* CHOISY var. *capitatum* CHOISY; hypericin; essential oil; DNA-protective activity; mineral content

**Author for correspondence:* Professor Nazim Sekeroglu, PhD. Department of Food Engineering, Faculty of Engineering and Architecture, Kilis 7 Aralık University, 79000, Kilis, Turkey, nsekeroglu@gmail.com

Highlights

- *Hypericum* (St. John's Wort) species, historical herbal remedy, are important medicinal plants for developing novel pharmaceutical drugs.
- *Hypericum capitatum* CHOISY var. *capitatum* CHOISY is an endemic medicinal plant that naturally grows in a small part of Turkey.
- There are limited scientific data for this endemic plant up to know, and DNA protective potential is the first report.
- To determine phytochemical composition and their biological activities are important for **pharmaceutical industry** in order to develop new drugs and contribute human health.

Abbreviations

DAC: Deutscher Arzneimittel Codex; **FAO:** The Food and Agriculture Organization of the United Nations; **GC-FID:** gas chromatography-flame ionization detector; **GC-MS:** gas chromatography-mass spectrometry; **H_2O_2:** hydrogen peroxide; **ICP-AES:** inductively coupled plasma atomic emission spectrometry; **ICP-OES:** inductively coupled plasma optical emission spectrometry; **linDNA:** linear DNA; **ocDNA:** open circular DNA; **pBR322:** plasmid DNA; **ROS:** reactive oxygen species; **scDNA:** supercoiled circular DNA; **UV:** ultraviolet.

1. Introduction

Hypericum (St. John's Wort) is an important genus of the family *Guttiferae*, which includes about 484 different species of herbs, shrubs and trees on the worldwide [1] and this genus is represented by nearly a hundred of taxa in Turkey, among them, 45 taxa are endemic [2]. *Hypericum* species locally known as "Binbirdelikotu, kantaron, kanotu, kılıçotu and koyunkıran" are of the important plant species in Turkey. Those plants and their plant parts have been used for treatment of various human ailments in traditional medicine for centuries. Throughout the human history *Hypericum* species are famous for herbal remedies with their curatives properties such as mild depression, anxiety, nervous conditions as herbal infusion, and popular for both wound and burn healing properties as externally [3,4]. However, there are many scientific reports indicating phytochemical compositions of *Hypericum* species, curative properties of these species have come from their chemical compositions. These species have been reported regarding of their rich phenolic contents and various terpenoids, but these scientific studies have indicated that naphtodiantrones (pseudohypericin, hypericin, hyperforine etc.) are the main phytochemical compounds [4-6]. Hypericin is, naturally occurring red pigments, of the bioactive constituents in *Hypericum spp.* and have been reported to possess photodynamic, antidepressive and antiviral activities [7-10]. Industrial pharmaceutical preparations of *Hypericum* species have been prepared by dried herb using flower part of the plant. These medicinal preparations are aqueous extracts (herbal teas), standardized extracts, alcoholic tinctures, capsulated dry extracts or in tablets, and oil infusions [11]. It is reported that standard St. John's Wort preparations should be contain up to 0.3% hypericin, or at 2.0 - 4.5% hyperforine. According to The United States National Formulary, *Hypericum* formulations should contain not less than 0.04% hypericin. Additionally, essential oil content of *Hypericum* species ranged between 0.05 - 0.90% [12]. Thus, scientific studies focused on mainly these compounds and *Hypericum* products with high hypericin content have been demanded in the pharmaceutical market. Essential oil is of the important bioactive compounds for *Hypericum* species; however, these species are known as essential oil-poor plants (essential oil content of these species is lower than 1%, v/w) and ranging from 0.12 to 0.35% ontogenetically [13]. Higher biological activity of different *Hypericum* species among the medicinally active plants have been listed in recent published studies [6].

In addition the secondary metabolite content and composition, in commonly consumed plants, their mineral composition also has a vital importance for human health through the modulation of enzymes by affecting the biochemical and physiological processes in cellular metabolism [14]. From the view of recent scientific reports, desired quantity of mineral composition and contents in food and medicinal plants have been advised for healthy life. However, it is furthermore emphasized that excessive doses and accumulation of these elements, especially heavy metals, could cause serious health problems. Scientific studies have recently been enhanced in order to determine the mineral compositions of some food and medicinal crops. Most of the Turkish medicinal plants and products have been analyzed in point of their

mineral compositions, as well. In the recent years, we have focused on the determination of mineral contents of edible parts of some plant species [15-21].

In Turkey, some studies have been performed on determining hypericin content of *Hypericum spp*., which were found as 0.0000004% in *H. heterophyllum.* Vent [22]; 0.000003% and 0.000016% in fruit and flower of *H. pamphylicum* Robson and Davis [23], 0.00046% in aerial parts of *H. scabrum* L. [24], 0.195 and 0.008% in leaf and root of *H. triquetrifolium* Turra [25], and 0.290% in *H. perforatum* L. [26]. In this study, hypericin content in dried herba of *Hypericum capitatum* CHOISY var. *capitatum* CHOISY was found as 0.029% which was lower than the limit proposed by DAC [27], but higher than that of *Hypericum pamphylicum* Robson and Davis [23a] and *Hypericum montbretii* [28]. It is worthy to note that the accumulation level of hypericin may show changes according to the diurnal and morphogenetic variability, growing area, fresh or dried material, and methods used for extraction or quantification [26, 29, 30]. Further studies are required to determine the optimal or desired level of hypericin either using different methods or using different parts by varying harvesting times.

This study was aimed; i) to investigate the phytochemical composition of different plant parts, ii) to evaluate essential oil composition of different plant parts iii) to determine mineral concentrations of the plant parts by comparing with previous reports and risk assessment for public health and iv) to evaluate the potential DNA protective capacity of extracts of *H. capitatum* CHOISY var. *capitatum* CHOISY.

2. Experimental

2.1 Plant material

H. capitatum CHOISY var. *capitatum* CHOISY, an endemic medicinal plant, naturally distributes in the South-eastern region of Turkey. Plant material used in this work were collected from nature during full-blooming period at the end of June, in Kilis and Gaziantep, located in South-eastern part of Turkey (Fig. 1 and 2).

A voucher specimen has been deposited at Department of Biology, Faculty of Arts and Sciences, Kilis 7 Aralık University (Kilis, Turkey). Cleaned and separated flower, stem and whole-aerial parts of the plants were air dried in well-ventilated place under shade in the laboratory. Dried plant samples were kept in plastic bags until laboratory analysis. The soil samples collected where the plant grown were analyzed at Plant and Soil Analysis Laboratory of Kilis 7 Aralık University. The soil properties were alkaline, clay, lime and low salinity with pH: 8.74; lime ($CaCO_3$): 48.82%, salt: 0.048%, phosphorus: 0.0121 kg/ha, potassium: 9.75 kg/ha and organic matter: 1.34%.

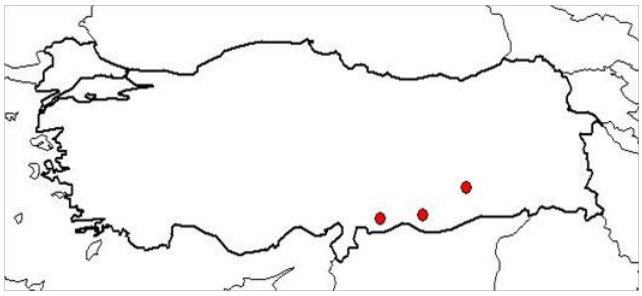

Figure 1. Geographical distribution of Hypericum capitatum CHOISY var. capitatum CHOISY (TUBIVES, 2017)

Figure 2. Hypericum capitatum CHOISY var. capitatum CHOISY during flowering vegetation

2.2 Quantification of Hypericin Content

Hypericin content was determined by spectrophotometric method by German drug Codex [27]. Briefly, one-gram of powdered samples was extracted with chloroform (Merck, Darmstadt, Germany) using Soxhlet Apparatus and then the extract was evaporated to dryness and the dried powder was then extracted with methanol. The hypericin content in methanol extracts was determined by a Spectrophotometer (Perkin-Elmer, USA) at 590 nm. Hypericin (%) content was measured according to the following formula [27], and the result was expressed as a mean of three replicates:

C= E590 x (ε × e)-1 x100

Where C: Hypericin content; E590: The spectrophotometer value at 590 nm; ε: Extraction coefficient (718); e: Amount of sample.

2.3 Chromatographic analysis by GC-FID and GC-MS

Essential oil analysis was performed by using a Hewlett Packard 6890 N GC equipped with HP-5 MS capillary column (30 m x 0.25 µm x 250 µm) and 5975C (Agilent Technologies) with mass selective detector 7890A (Agilent Technologies) model GC-FID and GC-MS. An electron ionization system with ionization energy of 70 eV was used for GC-MS detection. Helium was a carrier gas at a flow rate of 1 mL/min. Injector and MS transfer line temperature were set at 250°C. Column temperature was initially kept at 60°C for 5 min, then gradually increased to 200°C, held for 10 minutes and finally raised to 250°C at 10°C/min. Diluted samples (1/100 in acetone, v/v) of 1.0µL were injected automatically with split ratio 50:1. Individual components were identified by electronic libraries (W8N08, Wiley7n and Flavor2).

2.4 Preparation of plant samples for mineral content

First of all, the plant samples were cleaned and washed by deionized water, and then air dried. Pre-dried samples were de-moisturized at 70°C for 48 h in an oven and ground for chemical analysis. 0.2 g of ground samples were placed into burning cup, 5 mL HNO_3 65% (Merck, Darmstadt, Germany) and 2 mL H_2O_2 30%, (Merck, Darmstadt, Germany) were added immediately. After incinerating in a HP-500 CEM MARS 5 microwave (crop. Mathews NC, USA) at 200°C, the solution was cooled at room temperature for 45 min. The extracts were passed through a Whatman 42 filter paper and the filtrates were collected by high-deionized water in a 20 mL of polyethylene bottles and kept at 4°C in laboratory for ICP-AES analysis. Each sample was analyzed in triplicate. Distilled-deionized water was used for all the analytical works. Glassware and polyethylene bottles were attentively leached with 2-4% HCl and rinsed through deionized water for three times. Merck standards (R1 and R2 groups) were used as analytical reagent grade chemicals. Standard solutions of Cd, Cu, Fe, Mn and Zn were prepared in 1% HNO_3 immediately before the analysis by serial dilution of 1000 mg/L stock solution stored in polyethylene bottles. Corn Bran (standard reference material, 8433) and Peach leaves (standard reference material, 1547) were used as reference materials [32].

2.5 Instrumentation and analytical procedures

ICP-OES (Varian Vista-Pro, Australia) was used to determine the minerals. The wavelengths of the method were Al (396.152), B (208.889), Ca (370.602), Cd (214.439), Co (230.786), Cr (205.560), Cu (324.754), Fe (238.204), K (404.721), Mg (383.829), Mn (257.610), Mo (203.846), Na (588.995), Ni (216.555), P (213.618), Pb (220.353), S (181.972) and Zn (213.857).

2.6 Determination of DNA-protective activity

2.6.1 Chemicals

pBR322 plasmid DNA (Vivantis), hydrogen peroxide (H_2O_2) from Merck, agarose, ethidium bromide (EtBr), Tris-Borate-EDTA gel buffer (45 Mm Tris-borate, 1 mM EDTA), loading buffer (10 mMTris-HCl, 0.15% orange G, 0.3% xylenecyanol, 60% glycerol, 60mM EDTA) from Sigma-Aldrich, UV transilluminator gel documentation system (Vilber-Lourmat).

2.6.2 Measurement

The other aim of this study was to investigate DNA damage protection potentials of *Hypericum capitatum* CHOISY var. *capitatum* CHOISY against to DNA damage generated by hydrogen peroxide (H_2O_2) and ultraviolet (UV)-known as reactive oxygen species (ROS) generating system. DNA protective activity of the water and ethanol extracts from stem, leaves and flowers of the plant were analyzed using pBR322 plasmid DNA (Vivantis) in the presence of UV/H_2O_2 according to [33] and [34] with some modifications. For analysis, different experimental groups including control (untreated pBR322 plasmid DNA, treated with H_2O_2 and UV pBR322 plasmid DNA, treated with only H_2O_2 pBR322 plasmid DNA and treated with only UV pBR322 plasmid DNA) and treated groups with different concentration of extracts (ranging from 25µg/ml to 200 µg/ml) and H_2O_2 and UV were used. Briefly, the experiments were held a volume of 10 µl in a micro centrifuge tube, firstly pBR322 super coiled plasmid DNA (200ng) was added to each tube, after that plant extracts were added (except the control samples),

and then H_2O_2 was added to a final concentration of 2.5 mmol/L (except one of the control sample), finally all of the samples exposed to UV light for 5-8 min on a UV transilluminator at 300 nm at room temperature. At the end of the reaction, loading buffer (10 mMTris-HCl, 0.15% orange G, 0.3% xylenecyanol, 60% glycerol, 60 mM EDTA) was added in each reaction tube, analyzed in 1.5% agarose gel for electrophoresis at 100 V for approximately 1 h, in Tris-borate-EDTA gel buffer (TBE-buffer) (45 Mm Tris-Borate, 1 mM EDTA, pH 8.2), the gels were stained with ethidium bromide (EtBr) (0.5 µg/ml), and photographed under UV transilluminator gel documentation system (Vilber Lourmat), finally DNA fragmentation patterns were separated by agarose gel electrophoresis.

3. Results and Discussion

In the presented research, compositions of the essential oils, hypericin and mineral elements in different parts of *Hypericum capitatum* CHOISY var. *capitatum* CHOISY including aerial parts, stem and flower were determined using various analyses. Chemical analysis by GC-MS-Headspace revealed that the main constituents of all the analyzed plant samples were extremely variable. Essential oil compositions of different plant parts of *H. capitatum* CHOISY var. *capitatum* CHOISY were represented in Table 1.

Table 1. Essential oil components of Hypericum capitatum CHOISY var. capitatum CHOISY

Components	Stem	Aerial parts	Flower
Undecane	62.881	30.186	5.662
Nonane	17.790	1.919	-
α-**Pinene**	10.046	43.589	71.897
3-Methylnonane	2.494	-	-
2-methyloctane	1.090	-	-
cis-Ocimene	0.934	9.979	4.551
β-**pinene**	0.768	2.487	2.294
β-**Caryophyllene**	0.301	2.797	2.876
Limonene	0.273	0.805	0.643

The results are expressed as mean of three replicates (n=3).

According to results of chromatographic analysis, the main essential oil components were found as undecane, nonane, α-pinene, 3-methylnonane, 2-methyloctane, cis-ocimene, β-pinene, β-caryophyllene and limonene. The alpha-pinene, undecane and cis-ocimene were found the major essential oil constituents in flowers up to 82.11%, and the results shown in Fig.3. Although major components in the essential oil of stem were nonane and α-pinene amounting to 90.72%, aerial plant parts were characterized with α-pinene, undecane and cis-ocimene by rate of 83.75%, and the chromatographic results were presented in the Fig.4 and Fig.5.

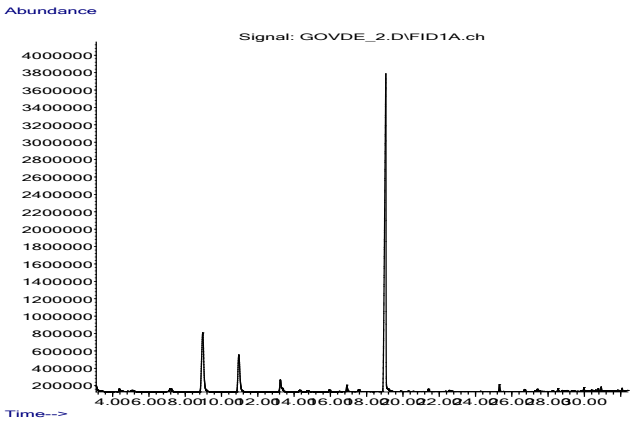

Figure 3. Flower chromatogram for Hypericum capitatum CHOISY var. capitatum CHOISY

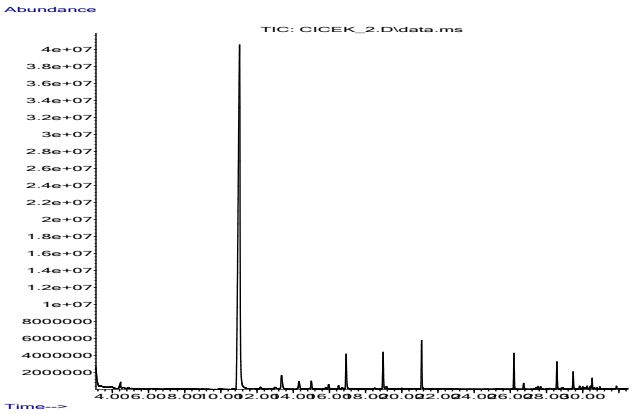

Figure 4. Stem chromatogram for Hypericum capitatum CHOISY var. capitatum CHOISY

H. capitatum var. capitatum and *H. capitatum* var. *luteum* are two varieties of *Hypericum capitatum* growing in Turkey that they had been analyzed for their essential oil constituents in the aerial plant parts by [35]. As a result of this study, α-pinene (20.3%), caryophyllene oxide (11.8%), hexadecanoic acid (8.9%), β-caryophyllene (6.5%) and undecane (3.8%) were found as major constituents for *H. capitatum* var. *capitatum*, and camphor (15.3%), germacrene D (10.5%), β-myrcene (7.4%), caryophyllene oxide (6.5%) and β-cububene (6.3%) were found

as the main components of *H. capitatum* var. *luteum*. Up to now, many studies have been performed on determining essential oil components of *Hypericum* spp. collected from different regions of Turkey [24,36-38]. The results of these studies based on main components of several *Hypericum* species including *H. hircinum, H. confertum, H. perforatum* L., *H. hyssopifolium* subsp. *elongatum* var. *microcalycinum, H. scabrum, H. uniglandosum, H. scabroies, H. kotschyanum, H. salsugineum* and *H. thymopsis* were summarized in Table 2. Major essential oil components of different *Hypericum* species grown in Turkey were α-pinene, and/or β-pinene that could be inferred from previous studies. As a result of this research, it could be easily declared that α-pinene is found the major essential oil component in the flower part of *H. capitatum* CHOISY var. *capitatum* CHOISY, which is consistent with the results of the previous studies using different *Hypericum* species.

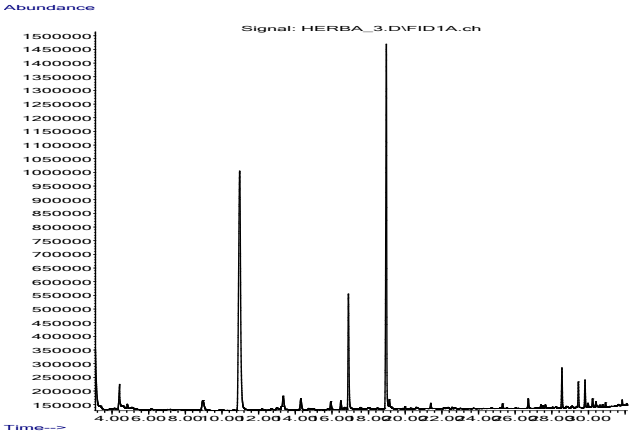

Figure 5. Aerial parts chromatogram for Hypericum capitatum CHOISY var. capitatum CHOISY

Concentrations of sixteen elements in different plant parts of *H. capitatum* CHOISY var. *capitatum* CHOISY were collectively represented in Tables 3 and 4. It was determined that each plant parts contain significant values of elements, of which content in each plant presented a wide variability. Macro and micro elements determined in varying concentrations (mg/kg level based on dry weight) were Ca, K, Na, P, S, B, Fe, Mg, Mn, Mo, Zn, Cd, Cr, Cu, Ni and Pb. Regarding with macro elements, concentrations accumulated widely variable according to the different organs of plant for each macro element, and given in Table 3.

In this study, both macro and micro element concentrations of different parts of *H. capitatum* CHOISY var. *capitatum* CHOISY (mg/kg) were summarized in the Table 4 and Table 5. According to mineral content analysis results, macro element concentrations were determined as follows: 6020 (flower) to 10390 mg/kg (aerial) for Ca; 11451 (aerial part) to 15161 mg/kg (flower) for K; 83 (aerial) to 100 mg/kg (stem) for Na; 1346 (stem) to 2880 mg/kg (flower) for P; and 1222 (stem) to 2476 mg/kg (aerial) for S, whereas micro element concentrations were found to have higher micro element concentrations ranging from 2016 mg/kg (for Mg) to 0.52 mg/kg (for Mo) in the aerial parts of the plant than that of other plant parts. Macro element concentrations determined in the present study are accordance with the previous reports, and these concentrations are suitable for human consumption considering by tolerable limits of FAO/WHO [39-42].

Iron (Fe) is an essential nutrient for all organisms and required for both the hemoglobin formation and transfer of oxygen and electron [43]. However, Fe concentrations were found to range from 108.9 (stem) to 171.7 (aerial) mg/kg in the presented study, average Fe concentrations of the plants have been reported in the ranges of 31-98 mg/kg [41] and 20 mg/kg for edible plants [39] previously. These values are higher than the limit proposed by FAO/WHO, 1984, but lower than the previous reports [40,42]. Nevertheless, higher iron concentrations are recommended for healthy human diets, the water-soluble amount of this metal is important to take dietary and daily consumption. Different plant species and their parts have wide range of manganese (Mn) concentrations that manganese limits have been set for edible plant as 2 mg/kg [39]. Herein, Mn concentration was in the ranges of 8.6 mg/kg (stem) and 23.4 (aerial) mg/kg which values were much lower than the previous reports [40,42,44] but higher than reported by [45]. It should be taken into account that the amounts of daily-use of this kind of plants, especially herbal infusions, at most 10g in dry weight bases, because the concentration is still higher than the daily consumption limits were determined by FAO/WHO for edible plants. In the present study, molybdenum (Mo) contents of *H. capitatum* CHOISY var. *capitatum* CHOISY were found to vary from 0.12 (stem) to 0.73 (flower) mg/kg. The results of presented work had no differences regarding of Mo contents compared with previous findings performed by [41] and [46]. In terms of Zinc (Zn) concentration of this plant parts, the Zn values were shown changes from 20.12 (stem) to 48.12 (aerial part) mg/kg (Table 4). Previous studies performed by different plant species indicated that the concentrations

Table 2. Main components determined in different *Hypericum* species from Turkey

***Hypericum* Species/ Components**	**α-pinene**	**β-pinene**	**Undecane**	**Myrcene**	**2,6 Dimetyl-3,5 heptadien-2-one**	**Hexadecanoic acid**	**Spathulenol**	**Nonacosane**	**Baeckeol**
H. hircinum (Kıyan *et al.*, 2014)	88.3	2.8	-	3.0					
H. confertum (Kıyan *et al.*, 2014)	21.5	2.7	1.0	0.5					
H. perforatum L. (Kıyan *et al.*, 2014)	33.3	12.5	0.8	0.7					
H. hyssopifolium subsp. *elongatum* var. *microcalycinum* (Kıyan *et al.*, 2014)	57.8	4.8	2.8	2.6					
H. scabrum (Serbetci, 2002)	2.9	71.6	-	3.8					
H. uniglandosum (Ozkan *et al.*, 2013)	2.7	-	1.9	-	40.7	2.7	1.5	3.2	0.9
H. scabroies (Ozkan *et al.*, 2013)	3.1	-	0.3	-	-	17.7	5.3	4.4	4.1
H. kotschyanum (Ozkan *et al.*, 2013)	14.4	8.7	0.1	-	-	9.2	6.3	11.1	2.4
H. salsugineum (Ozkan *et al.*, 2013)	-	-	-	-	-	23.2	-	42.7	6.1
H. thymopsis (Ozkan *et al.*, 2013)	44.0	1.7	-	-	-	-	8.0	-	32.9

Table 3. Mineral contents of different parts of *Hypericum capitatum* CHOISY *var. capitatum* CHOISY (mg/kg)

Parts	Minerals														
	Ca			K			Na			P			S		
Aerial	10390	±	346	11451	±	1121	83	±	6	1925	±	125	2476	±	198
Flower	6020	±	248	15161	±	1245	93	±	9	2880	±	187	2306	±	101
Stem	6873	±	176	14641	±	986	100	±	11	1346	±	201	1222	±	142

Data are shown as means ± SD from three independent experiments (n=3).

Table 4. Mineral contents of different parts of *Hypericum capitatum* CHOISY *var. capitatum* CHOISY (mg/kg)

Parts	Minerals																	
	B			Fe			Mg			Mn			Mo			Zn		
Aerial	25.1	±	2	171.7	±	12	2016	±	99	23.4	±	3	0.52	±	0.05	48.12	±	2.1
Flower	20.9	±	5	170.6	±	21	1744	±	114	22.1	±	8	0.73	±	0.10	42.08	±	5.4
Stem	20.0	±	4	108.9	±	17	790	±	101	8.6	±	2	0.12	±	0.08	20.12	±	1.8

Data are shown as means ± SD from three independent experiments (n=3).

Table 5. Mineral contents of different parts of *Hypericum capitatum* CHOISY *var. capitatum* CHOISY (mg/kg)

Parts	**Minerals**														
	Cd			**Cr**			**Cu**			**Ni**			**Pb**		
Aerial	0.0278	±	0.0080	4.1228	±	0.25	10.0	±	1.2	1.481	±	0.12	4.807	±	0.11
Flower	0.0385	±	0.004	3.5678	±	0.16	14.2	±	0.9	1.765	±	0.18	5.783	±	0.12
Stem	0.0405	±	0.005	7.4067	±	0.33	6.7	±	0.6	1.525	±	0.19	11.015	±	0.25

Data are shown as means ± SD from three independent experiments (n=3).

of Zn can differ from species to species. Zn concentration of *L. semisanguifluus* were reported as 74.3 mg/kg by [47], but [40] found zinc concentrations in different plants were ranging from 0.26 to 4.80 mg/kg. Although, the daily consumption limits for this metal has not yet been published, it has significance for human health due to play special roles in regulation of human physiology.

Cd, Cr, Cu, Ni and Pb are microelement that determined in the different parts of *H. capitatum* CHOISY var. *capitatum* CHOISY, and their contents were given in Table 5. Among which, cadmium (Cd) reveals serious health risks in the environment, because of its relatively easy bioavaibility properties. Therefore, the determination of the contents of Cd in plant species is a serious issue for human health. In this study, Cd concentrations in analyzed different plant parts were ranged from 0.0278 (aerial) to 0.0405 (stem) mg/kg (Table 5), which values are lower than the limits established by WHO, 1999, and the recommended consumption limit of Cd is 0.3 mg/kg as the upper limit for safe human consumption [48]. Furthermore, there are also limits proposed as sufficient or normal (0.01-0.2 mg/kg), excessive or toxic (5-30 mg/kg), and tolerable in crop plants (0.05-0.5 mg/kg) [41].

Chromium (Cr) is considered as an essential micronutrient in human metabolic processes, but excessive amounts of this heavy metal could result in carcinogenesis. For this reason, intake of Cr is critical for human metabolism. Contents of Cr either varies from 0.01 to 0.35 mg/kg [49], or from 0.07 to 0.41 mg/kg [50], or the permissible levels (0.02 mg/kg), which has been established as the upper limit for safe human consumption that recommended for medicinal plants [39]. In the present study, Cr values were in the range of 3.5678 (flower) and 7.4067 (stem) mg/kg. Those values are much higher than the acceptable range for human consumption. Regarding daily using amounts St. John's Wort, these amounts could not be reached for short-term use as herbal remedy. Copper (Cu) contents of different plant parts of *H. capitatum CHOISY var. capitatum CHOISY* ranged from 6.7 (stem) to 10.0 (aerial) mg/kg. In general, Cu content varies from 3.8 to 6.7 mg/kg for wheat grains, 3-8 mg/kg for leafy vegetables [41] and limited as 3.0 mg/kg for edible plants by authorities [39]. Considering daily uses amount of the plant, measured concentrations could be evaluated in the acceptable ranges for human health. Sufficient or normal and tolerable concentrations of Nickel (Ni) were proposed to range within 0.1–5 and 1-10 mg/kg, but 10-100 mg/kg concentration is regarded as excessive or toxic [41,49]. In the present study, Ni concentrations were found between 1.481 (aerial) and 1.765 (flower) mg/kg, which were in the acceptable range for human consumption. Although established permissible levels for lead (Pb) is 0.43 mg/kg [39], Pb concentration of *H. capitatum CHOISY var. capitatum CHOISY* was determined as ranging from 4.807 (aerial) to 11.015 (stem) mg/kg in this study (Table 5). These determining values were much more than permissible limit in medicinal plants; however, it should be considered that medicinal plants are used in low quantities apart from food crops. Thus, determining Pb values could be regarded as tolerable for human health.

Apart from determining main and essential oil components, and mineral contents of different parts of *H. capitatum* CHOISY var. *capitatum* CHOISY, DNA protective potential of the plant were analyzed with aqueous and ethanol extracts. To identify DNA protective effect of the extracts plasmid DNA derived from pBR322 was used in the presence of ultraviolet and hydrogen peroxide. pBR322 plasmid DNA, isolated from Escherichia coli, has 4361 base pairs (bp) and on agarose gel electrophoresis shows two bands including scDNA (supercoiled circular DNA) and ocDNA (open circular DNA). scDNA is the native form of DNA derived from pBR322 and moves faster than ocDNA on gels. When DNA is exposed to UV light, in the presence of H_2O_2, this situation leads to production of free hydroxyl radicals, and also change native formation of DNA (scDNA), produced ocDNA and linear DNA (linDNA) [33,34,51]. The addition of the water and ethanol extracts of the plant at 25µg/ml-200µg/ml concentration to the reaction mixture prevent to change the formation of linDNA, and help to protect the native formation of DNA.

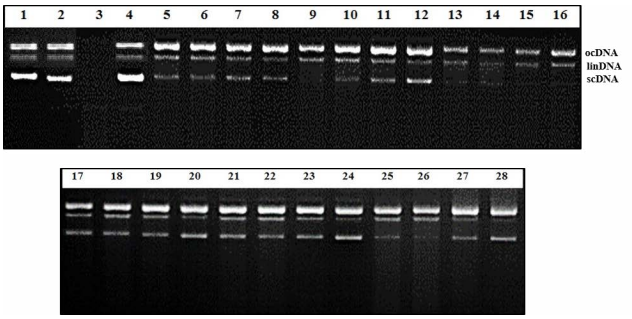

Figure 6. DNA protective activity of Hypericum capitatum CHOISY var. capitatum CHOISY extracts

Control Samples: Line 1, DNA + UV; Line 2, DNA; Line 3, DNA + H_2O_2 + UV; Line 4, DNA + H_2O_2. Line 5, DNA + UV+ H_2O_2 + the water extracts from leaves of H. *capitatum*

var. *capitatum* at 25µg/ml concentration; Line 6, DNA + UV + H_2O_2 + the water extracts from leaves of *H. capitatum var. capitatum* at 50µg/ml concentration; Line 7, DNA + UV + H_2O_2 + the water extracts from leaves of *H. capitatum var. capitatum* at 100µg/ml concentration; Line 8, DNA + UV + H_2O_2 + the water extracts from leaves of *H. capitatum var. capitatum* at 200µg/ml concentration; Line 9, DNA + UV + H_2O_2 + the water extracts from flowers of *H. capitatum var. capitatum* at 25µg/ml concentration; Line 10, DNA + UV + H_2O_2 + the water extracts from flowers of *H. capitatum var. capitatum* at 50µg/ml concentration; Line 11, DNA + UV + H_2O_2 + the water extracts from flowers of *H. capitatum var. capitatum* at 100µg/ml concentration; Line 12, DNA + UV + H_2O_2 + the water extracts from flowers of *H. capitatum var. capitatum* at 200µg/ml concentration; Line 13, DNA + UV + H_2O_2 + the water extracts from stem of *H. capitatum var. capitatum* at 25µg/ml concentration; Line 14, DNA + UV + H_2O_2 + the water extracts from stem of *H. capitatum var. capitatum* at 50µg/ml concentration; Line 15, DNA + UV + H_2O_2 + the water extracts from stem of *H. capitatum var. capitatum* at 100µg/ml concentration; Line 16, DNA + UV + H_2O_2 + the water extracts from stem of *H. capitatum var. capitatum* at 200µg/ml concentration; Line 17, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. capitatum var. capitatum* at 25µg/ml concentration; Line 18, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. capitatum var. capitatum* at 50µg/ml concentration; Line 19, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. capitatum var. capitatum* at 100µg/ml concentration; Line 20, DNA + UV + H_2O_2 + the ethanol extracts from leaves of *H. capitatum var. capitatum* at 200µg/ml concentration; Line 21, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. capitatum var. capitatum* at 25µg/ml concentration; Line 22, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. capitatum var. capitatum* at 50µg/ml concentration; Line 23, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. capitatum var. capitatum* at 100µg/ml concentration; Line 24, DNA + UV + H_2O_2 + the ethanol extracts from flowers of *H. capitatum var. capitatum* at 200µg/ml concentration; Line 25, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. capitatum var. capitatum* at 25µg/ml concentration; Line 26, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. capitatum var. capitatum* at 50µg/ml concentration; Line 27, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. capitatum var. capitatum* at 100µg/ml concentration; Line 28, DNA + UV + H_2O_2 + the ethanol extracts from stem of *H. capitatum var. capitatum* at 200µg/ml concentration.

The results of DNA fragmentation patterns showed that the water and ethanol extracts from stem, leaves and flowers have protective activity with all concentration, and protective activity increases depending on the extract concentration, the extracts at 25µg/ml concentration have minimum DNA protective potential. As can be seen from Fig. 6, among the extracts from stem, leaves and flowers, the extracts from flowers have maximum protective potential, whereas the extracts from stem have minimum protective activity both the water and the ethanol extracts. In addition to these results, the ethanol extracts from stem have less DNA protective activity than the other extracts from other parts of the plant. When compared with the water extracts and the ethanol extracts each other, it was observed that all the water extracts possess more protective potential than all the ethanol extracts.

4. Conclusions

Hypericum species are of the most important medicinal plants throughout the world since historical times. According to scientific studies, their therapeutic bioactive compounds are mainly hypericin and several derivatives of hypericin. In this research, an endemic *Hypericum* species, *H. capitatum* CHOISY var. *capitatum* CHOISY, was analyzed in terms of their phytochemical compositions and biological properties. It was revealed that this endemic plant contains highly active phytochemical compounds and/or products which can have potential uses in pharmaceutical industries. In addition, this study is the first scientific report to evaluate DNA protection potentials of the extracts against UV radiation in the presence of H_2O_2. Consequently, it would be possible for development novel herbal drugs containing compounds isolated from *H. capitatum* CHOISY var. *capitatum* CHOISY in the near future, when confirmed with further studies.

5. Conflict of interest

The authors declare that they have no conflict of interests.

6. Acknowledgements

This work was financially supported by Kilis 7 Aralik University, Scientific Research Projects Unit (Project number: 2013/01/LTP/01).

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