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Full Length Research Paper

Evaluation of anti-inflammatory effect of *Zosima absinthifolia* and deltoin

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In this study, n-Hexane extracts prepared from aerial part of *Zosima absinthifolia* (ZAA) and the root of *Z. absinthifolia* (ZAR) (Umbelliferae) as well as deltoin were evaluated for their anti-inflammatory activity using the carrageenan-induced rat paw oedema test. The anti-inflammatory activity of *Z. absinthifolia* was not found to be significantly different at doses of 25 and 50 mg/kg. However, the aerial part of ZAA at 75 mg/kg, i.p., and Deltoin at 10 mg/kg, i.p. showed a significant reduction with 86.56 and 71.05% respectively in rat paw oedema induced by carrageenan against the reference anti-inflammatory drug indomethacin (3 mg/kg, i.p.) (87%). The ED_{50} of deltoin, ZAA and ZAR were determined as 5.08, 69.82 and 38.51 mg/kg, respectively. Furthermore, content of deltoin and columbianadin in both ZAA and ZAR were also analyzed by HPLC. Deltoin content of plant was found higher than the columbianadin content in ZAA and ZAR. The present study reveals that the aerial part of *Z. absinthifolia* possesses promising anti-inflammatory activity in rats.

Key words: Anti-inflammatory activity, deltoin, umbelliferae, *Zosima*.

INTRODUCTION

Natural products have an enormous importance as medicines in treating and preventing various human diseases for thousand of years. Plants in general and medicinal plants in particular represent excellent sources of biologically active natural compounds that not only have therapeutic uses in themselves, but that may also be used in the development of semi-synthetic and synthetic medicines (Verpoorte, 1998; McCurdy and Scully, 2005; Chin et al., 2006; Ji et al., 2009). Different approaches to drug discovery from plants, can be distinguished such that random selection which is followed by chemical screening or followed by biological assays, follow-up biological activity reports and follow-up ethnomedical selection (Pieters and Vlietinck, 2005). In recent years, the ethnomedical studies have become of more interest in medicinal plants and other natural products to discovery of new active compounds (McCurdy and Scully, 2005). Around the world, at least 35,000 plant species are known to be used for medicinal purposes (Özkan and Arıhan, 2005). In Turkey many plants are also known for their medicinal properties in Turkish folk medicine to treat a wide range of diseases. The ethnomedical approach is considered to be more useful for discovering new active natural compounds and to prove the usefulness of medicinal plants in folk medicine by modern scientific methods (Şener, 1994).

Zosima absinthifolia (Vent.) Link (Syn. *Z. orientalis* Hoffm. (ZA) is one of the member of Umbelliferae family is mainly distributed from Central to Southwest Asia (Chamberlain, 1972). This plant is known as *ayi eli* or *peynir otu* in Turkey and added to a traditional cheese made in East Anatolia (Özçelik, 1994) and aerial parts of the plant are edible after cooked in the same region (Aksakal and Kaya, 2008). Plant fruits used as a food spice in Turkey and Iran (Razavi et al., 2009) as well as used as a digestive, carminative and anti-inflammatory

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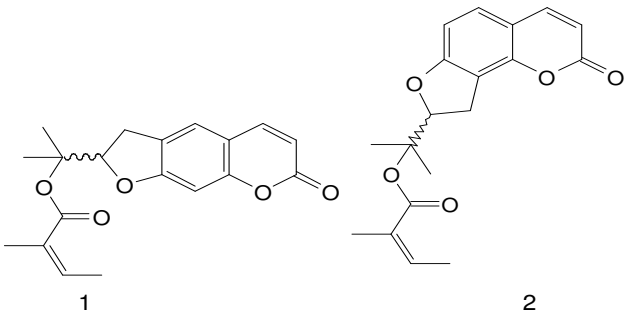

Figure 1. Chemical structures of the isolated coumarins; columbianadin (1), deltoin (2).

agent in Turkish folk medicine (Doğal Tedavi, 2008). Aerial parts of the plant have also some medicinal usage in Pakistan folk medicine to relieve indigestion, stomach gas and to treat cough and bowel disorders (Goodman and Ghafoor, 1992).

Previous researches have revealed that ethanolic extract of the aerial parts of *Z. absinthifolia* showed antimycobacterial activity (Al-Shamma and Mitscher, 1979)and methanolic extract of the fruits exhibited significant phytotoxic, antioxidant and antiproliferative activities (Razavi et al., 2009). Moreover, recently antibacterial activity of the fruits essential oil has been reported by Razavi and Nejad-Ebrahimi (2009) as well as three coumarin derivatives. These derivatives that are imperatorin, auraptene and 7-prenyloxycoumarine with allelopatic effect were isolated from the n-hexane extract of the plant fruits (Razavi et al., 2010). Coumarine derivatives (bergapten, deltoin, columbianadin, isobergapten, isopimpinellin, imperatorin, pimpinellin, sophodin, and umbelliferone), flavonoids (quercetin, kaempferol) and alkaloids were isolated from *Z. absinthifolia* In previous studies (Nikonov and Baranauskaite, 1965; Crowden et al., 1969; Malikov and Saidkhodzhaev, 1998; Başer et al., 2000). In addition, the essential oil obtained from the fruits of this plant was analysed by GC-MS and sixteen components were determined constituting 95.8% of the oil (Başer et al., 2000). The aim of this work was to evaluate the anti-inflammatory effect of *Z. absinthifolia* and deltoin as one of the major constituent of this plant. Columbianadin and deltoin content were also analyzed by HPLC qualitatively as well as quantitatively in aerial part and root of the plant. No data is available with respect to anti-inflammatory activity of *Z. absinthifolia* and deltoin in literature that prompted us to produce this study.

MATERIALS AND METHODS

General experimental procedures

Thin layer chromatography assays were done on precoated silica gel sheets 60 F_{254} (Merck 1. 05554) under 254 nm and 366 nm UV light. Column chromatography was carried out on silica gel (0.2 – 0.5 mm, 30 - 70 mesh Merck 1.07733). Melting point was measured with electrothermal model 9100 apparatus. Mass spectra was recorded using a Waters 2695 Alliance Micromass ZQ, LC/MS. Varian Mercury 400, 400 MHz High Performance Digital FT-NMR Spectrometer was used for ^{1}H NMR and ^{13}C NMR (in $CDCl_3$).

Plant material

Z. absinthifolia (Vent.) Link was collected in 2005 from flowering plants near Bolu (Turkey). Taxonomic identity of the plant was confirmed by Doúan et al. (2003), a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University. Voucher specimens were kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF No: 23847)

Extraction and isolation

Air-dried and powdered aerial parts of the plant (600 g) were extracted with n-hexane under reflux for 5 h. The extract was evaporated to dryness under vacuum to yield 10.3416 g (1.72%). Separation of the coumarine derivatives was performed on Silica gel (30 - 70 mesh) by eluting with n-hexane in increasing amounts of EtOAc until to obtain 68 fractions. Fractions 19 - 21 gave columbianadin (1) (21.1 mg) and 30 - 35 gave deltoin (2) (Figure 1) (48.9 mg) which were eluted with n-hexane: EtOAc (80:20). The structures of the isolated compounds were elucidated on the basis of their MS, ^{1}H NMR and ^{13}C NMR data and chemical correlations with the data that have been mentioned in the literature (Tosun et al., 2006; Razavi et al., 2008).

HPLC analysis

Chromatography was performed on Agilent LC 1100 (Agilent Technologies, California, USA) consisting of an automated gradient controller and a diode array detector (DAD). Separation was carried out using a Waters Spherisorb S5W column (25 cm x 4 mm x 5 µm) (Hichrom). The mobile phase was made up of n-hexane and EtOAc (75:25 v/v) applied at a flow rate of 1 ml/min and column temperature was 25°C and 10 µL portions were injected into the liquid chromatograph.

Sample and standard solutions preparation for HPLC analysis

Air-dried and powdered aerial parts and roots of the plant (1 g) were extracted with n-hexane under reflux for 5 h. The extract was filtered and concentrated to dryness *in vacuo* to afford a residue and then dissolved in ethyl acetate and adjusted to the 25 ml. The sample solution was filtered through 0.45 µm micropore membrane prior to HPLC analysis.

The reference standards; columbianadin (10 mg) and deltoin (10 mg) were accurately weighed in 10 ml volumetric flask and dissolved in ethyl acetate then diluted to the final volume separately. Five different concentrations (0.025, 0.05, 0.1, 0.2 and 0.4 mg/ml) were prepared by diluting the stock solutions.

Animals

Male and female *Sprague dawley* rats (200 - 250 g) were used in this experiment. The animals were obtained from the Animal House, Yüzüncü Yıl University. All animals were housed in standard cages (48 × 35 × 22 cm) at room temperature (20 ± 2°C), with artificial light from 7.00 am to 7.00 pm, and provided with pelleted food (Van

Animal Feed Factory, Van, Turkey) and water *ad libitum*. Prior to administration of the drugs, the rats of the anti-inflammatory activity groups were fasted for 12 h with free access to tap water. The protocol for the study was approved by the Ethical Committee of Yüzüncü Yıl University Faculty of Medicine, Animal Breeding and Research.

Drugs and chemicals

Lambda-carrageenan (Type IV) and indomethacin were obtained from Sigma (Steinheim, Germany).

Anti-inflammatory activity

The method of Winter *et al*. (1962) with slight modification was used. Sixty-six rats of either sex were divided into eleven groups of six animals each. Group I (control I) received 0.2 ml isotonic saline solution (ISS) per os (p.o.). Group II, (standard reference drug-II) received 3 mg/kg indomethacin i.p. and Group III received 25 mg/kg *Z. absinthifolia* aerial part extract i.p., Group IV received 50 mg/kg *Z. absinthifolia* aerial part extract i.p., Group V received 75 mg/kg *Z. absinthifolia* aerial part extract i.p., Group VI received 25 mg/kg *Z. absinthifolia* root extract i.p., Group VII received 50 mg/kg *Z. absinthifolia* root extract i.p., Group VIII received 75 mg/kg *Z. absinthifolia* root extract i.p., Group IX received 5 mg/kg deltoin i.p., Group X received 10 mg/kg deltoin i.p., Group XI received 15 mg/kg deltoin i.p. The drugs were administered 1 h before injection of 0.05 ml of 1% suspension of carrageenan into the subcutaneous tissues of the right hind paw. Since, the hydration state of the animals can modify the intensity of swelling, the rats were fasted 12 h before the experiment with water *ad libitum*. The degree of oedema was measured 30 min before and 3 h after injection of carrageenan. Difference in the paw volume, determined before and after injection of the oedema-provoking agent indicated the severity of oedema. One control group and reference group were used in this study. Volumes of right hind paw of controls and treated animals were measured with a plethysmometer (model 7140, Ugo Basile, Italy). The rats were kept under the same experimental conditions. The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with control and calculated by the following formula where d*t* is the difference in paw volume in the drug-treated group and d*c* the difference in paw volume in the control group (Kouadio et al., 2000).

$$1\% = 1 - (dt/dc) \times 100$$

Statistical analysis

Results are reported as mean ± S.E.M. (standard error of mean) or as percentages. The total variation was analysed by performing one way analysis of variance (ANOVA). Tukey's HSD (Honestly significant difference) test was used for determining significance (Sümbüloğlu and Sümbüloğlu, 1998). Probability levels of less than 0.05 were considered significant.

RESULTS

HPLC analysis

HPLC analysis that was performed on ZAA and ZAR revealed that deltoin and columbianadin are the major constituents of both extracts. The calibration curves were established as $Y = 21.349X - 89.216$ ($r^2 = 0.9998$) for columbianadin and $Y = 25.067X - 159.827$ ($r^2 = 0.9993$) for deltoin by plotting the ratio of peak areas to the concentration of each substance.

Columbianadin and deltoin content were determined in the aerial part extract as 0.0806, 0.1701% (w/w) and in the root extract 0.6427, 2.0395% (w/w) respectively. Deltoin content of plant was found higher than the columbianadin content in both parts. Furthermore, the root extract were detected to contain columbianadin and deltoin in higher percentage when we compare with the aerial part (Figure 2).

Antiinflammatory activity

Table 1 shows the results of antioedematous effect of intraperitoneally administered *Z. absinthifolia* on carrageenan paw oedema in rats. The greatest anti-inflammatory activity was observed in the reference group receiving indomethacin with 87.44 % inhibition of the inflammation. *Z. absinthifolia* aerial part extract and root extract showed moderate activities at 25 and 50 mg/kg doses compared to indomethacin group. However 75 mg/kg dose of *Z. absinthifolia* aerial part extract exhibited significant anti-inflammatory activity while the root extract showed moderate activity at the same dosage. The inhibition percentage of *Z. absinthifolia* aerial part extract (86.56%) was determined as much as indomethacin (87.44%) at 75 m/kg dose. The ED_{50} values of the aerial part and root extracts were found as 69.82 mg/kg and 38.51 mg/kg respectively. On the other hand deltoin induced more inhibition against carrageenan paw oedema (71.05%) at 10 mg/kg dose than the 5 and 15 mg/kg doses as given in Table 2. Deltoin ED_{50} value was also determined as 5.08 mg/kg.

DISCUSSION

In present study anti-inflammatory activity of *Z. absinthifolia* and deltoin which was isolated from the aerial part of this plant were evaluated by using carrageenan paw oedema test model on rats. No reports have been found about the anti-inflammatory activity of this plant to date. Current study results revealed that both root and aerial part extracts showed similar inhibitory effect in rat paw oedema induced by carrageenan which were not significant when compared with the indomethacin group at 25 and 50 mg/kg doses. However, 75 mg/kg dose of *Z. absinthifolia* aerial part extract (86.56%) exhibited anti-inflammatory activity as much as indomethacin (87.44%) while the root extract (57.04%) showed moderate activity at the same dosage.

Furthermore, deltoin caused a significant inhibition against carrageenan paw oedema at 10 mg/kg dose by 71.05% as one of the constituent of this plant.

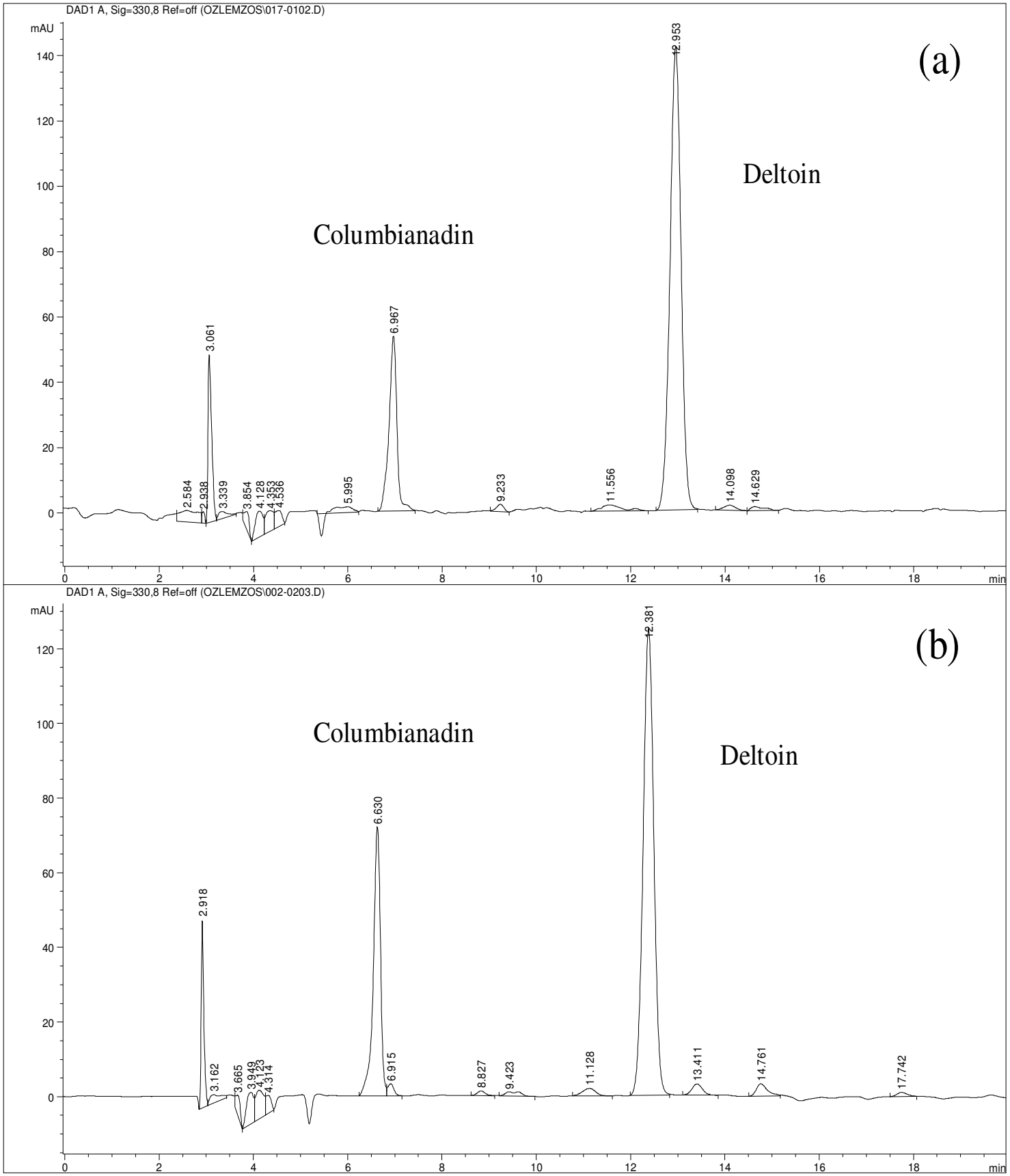

Figure 2. Chromatogram of the root extract (a) and aerial part (b) extract.

Table 1. Effect of intraperitoneal treatment with the extract of *Zosima absinthifolia* and indomethacin on carrageenan induced hind paw oedema in rats.

Groups	Dose	Paw oedema (ml %)	Inhibition (%)
Control (ISS)**	0.1 ml	1.043 ± 0.127	-
Indomethacin	3 mg/kg	0.024 ± 0.061[a]	87.44
Zosima aerial part extract	25 mg/kg	0.135 ± 0.016[ab]	39.83
Zosima aerial part extract	50 mg/kg	0.596 ± 0.077[ab]	40.40
Zosima aerial part extract	75 mg/kg	0.602 ± 0.080[acd]	86.56
Zosima root extract	25 mg/kg	0.646 ± 0.100[ab]	35.39
Zosima root extract	50 mg/kg	0.483 ± 0.042[ab]	51.66
Zosima root extract	75 mg/kg	0.430 ± 0.078[ab]	57.04
F value		18.010	
P value		0.000	

Data were presented as mean ± standard error of the mean (n = 6).
*S.E.M.: Standard error mean **ISS: Isotonic saline solution.
Post-hoc Tukey's HSD test: [a] :P < 0.05 compared to control (ISS) group.
[b] :P < 0.05 compared to indomethacin group, [c] :P < 0.001 compared to *Zosima* aerial part 25 mg/kg, [d] :P < 0.001 compared to *Zosima* aerial part 50 mg/kg.

Table 2. Effects of deltoin on rat paw edema (mean ± SEM*).

Groups	Dose	Paw edema (ml %)	Inhibition (%)
Control (ISS)**	0.1 ml	1.003 ± 0.112	-
Indomethacin	3 mg/kg	0.024 ± 0.061[a]	87.44
Deltoin	5 mg/kg	0.533 ± 0.041[ab]	46.69
Deltoin	10 mg/kg	0.290 ± 0.118[a]	71.05
Deltoin	15 mg/kg	0.436 ± 0.040[ab]	56.38
F value		21.755	
p value		0.000	

Data were presented as mean ± standard error of the mean (n = 6).
*SEM: Standard error of the mean. ** ISS: Isotonic saline solution.
Post-hoc Tukey's HSD test: a: p < 0.05 compared to control (ISS) group
b: p < 0.05 compared to indomethacin group.

Z. absinthifolia which belongs to Umbelliferae family is known to contain coumarine derivatives (Nikonov and Baranauskaite, 1965; Crowden et al., 1969; Malikov and Saidkhodzhaev). Deltoin as well as columbianadin which are furanocoumarins were determined by us as the major constituents of this plant. Significant differences were found in the amount of these two coumarins between the content of aerial part and root extracts but there is only remarkable difference between the anti-inflammatory activity of ZAA and ZAR at 75 mg/kg dose. On the other hand, as against the higher deltoin and columbianadin content of root extract, more inhibitory effect against carrageenan paw oedema was determined in aerial part extract.

Our findings revealed that deltoin could be probably responsible from the anti-inflammatory activity of *Z. absinthifolia* in part. Inhibitory activity of deltoin on NO which and its derivatives play a role in inflammation was also determined in previous research in macrophage cell line (Wang et al., 1999). In addition, columbianadin that was isolated previously from *Angelica pubescens* by bioassay directed fractionation as one of the constituent responsible for the anti-inflammatory and analgesic activities of this plant (Chen et al., 1995) could be considered as another anti-inflammatory active compound. However there was not found any relationship between the anti-inflammatory activity and deltoin as well as columbianadin content of the *Z. absinthifolia.* Thus, it can be suggested that more compounds could be responsible from the potential anti-inflammatory activity of *Z. absinthifolia*.

In conclusion the n-hexane extract of *Z. absinthifolia* possesses promising anti-inflammatory activity in rats and the results of current study suggested that anti-inflammatory activity is probably due to the synergic interaction of the compounds including deltoin,

columbianadin as well as other compounds which were identified as constituents of this plant. Moreover there is no doubt deltoin is responsible partly from the anti-inflammatory activity of *Z. absinthifolia* but further analysis with the isolated compounds are needed to elucidate the compound(s) which are responsible from the anti-inflammatory activity of *Z. absinthifolia*.

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