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Original article

In vivo analgesic and anti-inflammatory effects of the essential oil from *Artemisia sieberi* fruit

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Abstract

Background and objectives: Drugs with analgesic properties such as opioids and NSAIDs have not been effective in all cases, because of their low potency and side effects. As a result, looking for other alternatives is necessary. Plants are important sources of new phytochemicals that possess significant therapeutic effects. Regarding the traditional use of *Artemisia sieberi* fruit as a natural painkiller and anti-inflammatory agent and the high content of essential oil in the fruits, we were prompted to investigate the anti-inflammatory and analgesic activities of *A. sieberi* fruits oil. **Methods**: *Artemisia sieberi* fruits essential oil was extracted by hydrodistillation method. The analgesic and anti-inflammatory activities of the oil were studied by formalin and carrageenan tests, respectively at the doses of 0.2, 0.4 and 0.8 mg/kg for the experimental animals. Control group received sweet almond oil as the vehicle and standard groups received morphine (2 mg/kg) and indomethacin (5 mg/kg) for the formalin and carrageenan tests respectively. **Results:** All doses of *A. sieberi* fruits essential oil induced antinociceptive activity during the second phase of the formalin test but the maximum effect was observed at the dose of 0.8 mg/kg. In carrageenan test all the experimental doses of the oil significantly reduced the inflammation (p<0.05). Anti-inflammatory activity of *A. sieberi* oil (0.2, 0.4 and 0.8 mg/kg) was found to be as considerable as the standard drug indomethacin (5 mg/kg). **Conclusion:** *Artemisia sieberi* fruit essential oil showed analgesic and anti-inflammatory effects which might be attributed to the major components of the studied oil, camphor and 1,8-cineole.

Keywords: analgesic, animal model, anti-inflammatory, *Artemisia sieberi*, fruit

Introduction

Pain is an unpleasant emotional feeling associated with actual or potential tissue damage and is usually treated with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and opioids, although continuous usage of these drugs may have adverse effects. NSAIDs are frequently associated with gastrointestinal disorders like gastric or duodenal ulceration and renal damage.

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Opioids cause seizure and respiratory depression, and also cause addiction and tolerance. Regarding the adverse effects of chemical drugs it is much safer to use herbal medicines as good sources of effective pain killers with lower or no side effects [1].

The genus *Artemisia* is one of the widely distributed genus of Asteraceae family, 34 species have been reported in Iran. *A. sieberi* Besser. is native to Iran and is locally called "*Dermaneh*". It grows wild in deserts and semi-desert regions. It has nutritional value for animals and medicinal usage for humans [2]. *Artemisia sieberi* is one of the plants used in folk medicine as a painkiller.

The previous studies showed that *A. sieberi* had antimicrobial [3], insecticidal [4], anticandidal [5], vermicidal, anti-parasitic [6], anti-fungal [5], anti-diabetic [7], spasmolytic and antipyretic effects.

The anti-inflammatory and anti-nociceptive activities of the other *Artemisia* species such as *A. drancunculus* [8], *A. compestris* [9], *A. annua* [10], *A. herba-alba* [11], *A. copa* [12], *A. vulgaris* [13], *A. absinthium* [14] and *A. scoparia* [15] have been reported.

It was previously shown that the main components of *A. sieberi* essential oil from the aerial parts are camphor, 1,8-cineole, and thujone [16]. There are some investigations that have shown anti-inflammatory and analgesic activities of camphor and 1,8-cineole [17]. The high content of essential oil in *A. sieberi* fruit encouraged us to investigate the analgesic and anti-inflammatory activities of *A. sieberi* fruits oil.

Experimental

Plant material

Fresh fruits of *A. sieberi* were collected from Bandar Abbas, Hormozgan Province in June 2016. The Fruits were identified by R. Asadpour and the voucher specimen was deposited at the Herbarium of the Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran Under code number of 219_PMP/A.

Chemicals

Formalin and morphine sulfate were purchased from Merck chemical company (Germany) while indomethacin and carrageenan were prepared from Temad Pharmaceutical Co. (Iran) and Sigma (U.S.A), respectively.

Essential oil extraction

Freshly collected fruits were immediately submitted for hydrodistillation in a Clevenger-type apparatus for 2 h. At the end of the distillation, the essential oil was collected, dried with anhydrous Na_2SO_4 and kept in glass vials at -18 °C for further analysis.

Animals

The experiments were performed on Wistar male rats weighting (200-250 g) and male Swiss albino mice (20-25 g). They were maintained on normal diet in the animal house at the temperature of 24±2° °C and light/dark cycle of 12/12 h.

Formalin test

Male albino mice (20-25 g) were divided to five groups (six animals in each group). Control animals received sweet almond oil (10 mL/kg, *i.p.*), while the standard group received morphine (2 mg/kg, *ip*) and the test groups received *A. sieberi* fruit essential oil (0.2, 0.4 and 0.8 mg/kg, *i.p.*). Formalin test was used to investigate the analgesic effect of the *A. sieberi* essential oil. Fifteen minutes after injection of different doses of the essential oil, morphine or vehicle, formalin (2.5%) were injected into the right hind paw of mice and the animal was immediately placed in the formalin test container. Scoring of nociceptive behaviors began immediately after formalin injection and was continued for 60 min. Nociceptive scores recorded based on limping (score 1), lifting (score 2), licking and biting (score 3). A weighted average nociceptive score (pain rating), ranging from zero to three, was calculated by multiplying the time spent in each category, by the category weight, swimming the products, and then dividing by the total time for each five-minute time block. Individual time course determinations in the formalin test were converted to area-under-the-curve values, zero to

five minutes after formalin injection (AUC phase I) and 15 - 60 minutes after formalin injection (AUC phase II) [18,19].

Carrageenan-induced paw edema test

Acute anti-inflammatory activity was evaluated on the basis of paw edema inhibition induced by the injection of 0.1 mL carrageenan 2% into the sub-plantar region of the right hind paw of the rat [20,21]. Male rats were divided into five different groups of six animals each that separately received *A. sieberi* essential oil (0.2, 0.4 and 0.8mg/kg, *i.p.*), Indomethacin (5 mg/kg, *i.p.*), and the vehicle, sweet almond oil (10 mL/kg, *i.p.*) thirty minute before the injection of carrageenan. The paw volume was measured after 0.5, 1, 2, 3, 4 and 5 h after the carrageenan administration using a Plethysmometer (model PM 4500, Borj Sanat Co., Iran) [22].

Anti-inflammatory activity was revealed as the inhibition percent of the edema when compared with the control group. The percentage inhibition of edema was calculated by the following equation:

$$\% \text{ inhibition of edema} = 100\ (1 - Vt/Vc)$$

Vc is the edema volume in the control group and Vt is the edema volume in tested groups.

Analysis of the essential oil

Analysis of the oil sample was performed on a HP-6890 gas chromatograph (GC) equipped with a FID and a DB-5 capillary column, 30 m×0.25 mm, 0.25 μm film thickness, temperature programmed as follows: 60-240 °C at 4 °C/min. The carrier gas N_2 was at a flow of 2.0 mL/min; the temperatures of injector port and detector were 250 °C and 300 °C, respectively. Samples were injected by splitting and the split ratio was 1:10. The analysis of GC/MS was performed on a Hewlett-Packard 6890/5972 system with a DB-5 capillary column (30 m×0.25 mm; 0.25 μm film thickness. The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. The range of scan mass was from 40-400 m/z at a sampling rate of 1.0 scan/s. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oil were identified by their retention time, retention indices, relative to C_9-C_{28} *n*-alkanes, computer matching with the WILEY275.L library and by comparison of their mass spectra with those of authentic samples or with data already available in the literature as well [23,24]. The percentage of composition of the identified compounds was computed from the GC peaks areas without any correction factors and was calculated relatively.

Statistical analysis

Comparisons between AUC phase I and phase II of the formalin test in groups were made by one-way ANOVA analysis followed by the post-hoc Tukey's test. Data obtained in the carrageenan test were also scrutinized using one way analysis of variance (ANOVA) followed by the post-hoc Tukey's test. The results were expressed as mean ± standard error and $p<0.05$ was considered as significant difference of the means. The data were analyzed using the GraphpadPrism6 statistical software.

Results and Discussion

The effect of systemic intraperitoneal (*i.p.*) administration of different doses of the *A. sieberi* essential oil on the behavioral responses during the first and second phase of the formalin test was observed. As mentioned in figure 1, in the first phase of the formalin test 0.2, 0.4 and 0.8 mg/kg doses of the essential oil did not reduce the behavioral noxious response, but morphine, as a standard analgesic drug, significantly ($p<0.01$) reduced pain behavior.

In the second phase of the formalin test, as indicated by figure 1, all of the studied doses of the essential oil reduced the behavioral noxious response (doses of 0.2 ($p<0.05$), 0.4 ($p<0.01$), and 0.8 mg/kg ($p<0.001$) compared to the vehicle), and morphine, as a standard analgesic drug, significantly ($p<0.01$) reduced pain

behavior.

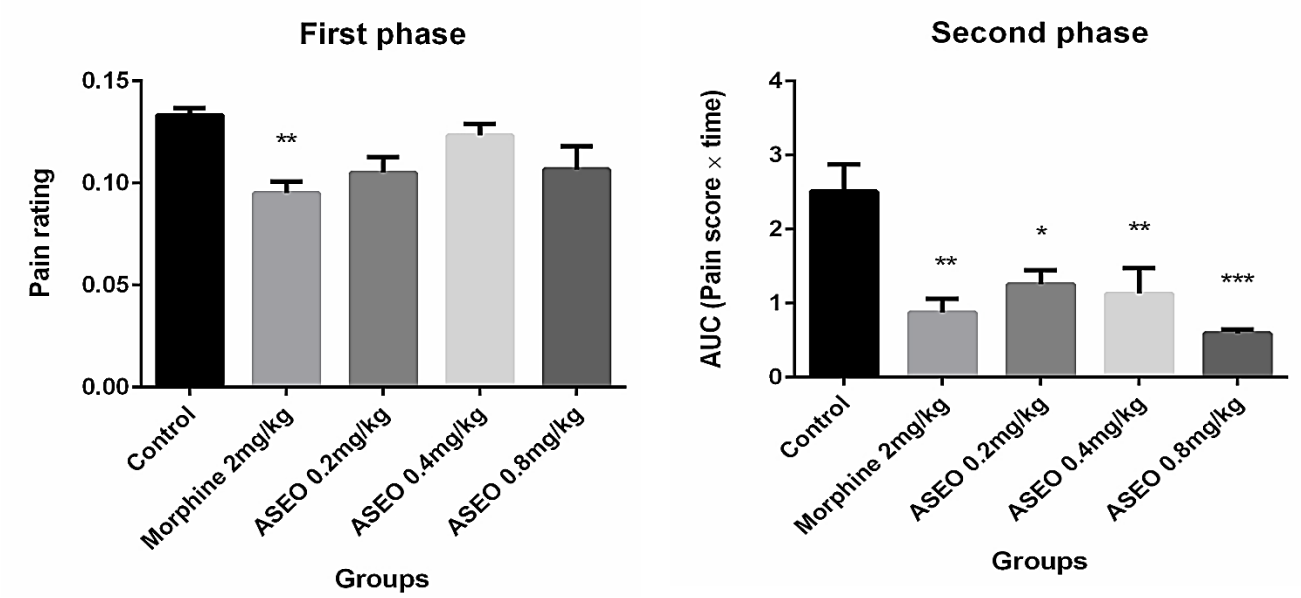

Figure 1. Effects of *Artemisia sieberi* essential oil (ASEO) on nociceptive response in the first and second phases of the formalin test. Values represent mean±SEM (n = 6). *p < 0.05, **p<0.01, ***p<0.001: Significant difference compared with the control group; (one way ANOVA, followed by Tukey's test)

The acute anti-inflammatory effect of *A. sieberi* essential oil in rat paw edema induced by carrageenan was established using the essential oil administered intraperitoneally (figure 2).

The essential oil (0.2, 0.4 and 0.8 mg/kg) significantly inhibited (p<0.05), similar to the indomethacin (5 mg/kg), the carrageenan-induced rat paw edema formation which was determined at the third hour of the experiment (peak of edema formation) by 66.6, 64.2, and 74.1 %, respectively. At the fifth hour of experiment, this edema inhibition was reached to 72%, 60% and 76% (table 1).

The essential oil found in the fruits of *A. sieberi* was analyzed by GC and GC/MS to determine the possible compounds responsible for the observed analgesic and anti-inflammatory activities. The hydrodistillation of *A. sieberi* gave a yellow oil with a pleasant odor and a yield of 2.1% (v/w). As shown in table 2, thirty one components were identified in this oil which presented about 90.9% of the total chromatographical material. The major constituents of the oil were camphor (47.0%) and 1,8-cineol (20.9%).

The pain management is undoubtedly one of the most common and yet most difficult aspects in medicine. In spite of important development in the field of synthetic drugs during recent years, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having the least side effects.

Therefore, a systematic method should be made to find out the efficacy of plants against pain and inflammation. One of these plants which have been introduced in folk medicin of Iran is *A. sieberi*. A few researches have been done for evaluating its chemical composition and anti-fungal, anti-bacterial, insecticidal and verimicidal activities, but there has been no study on *A. sieberi* fruit essential oil for anti-inflammatory and analgesic effect in published literature. The present study used formalin-induced pain and Carrageenan-induced paw edema models for determination of the analgesic and anti-inflammatory effects of the essential oil from the

fruits of *A. sieberi* in experimental animals.

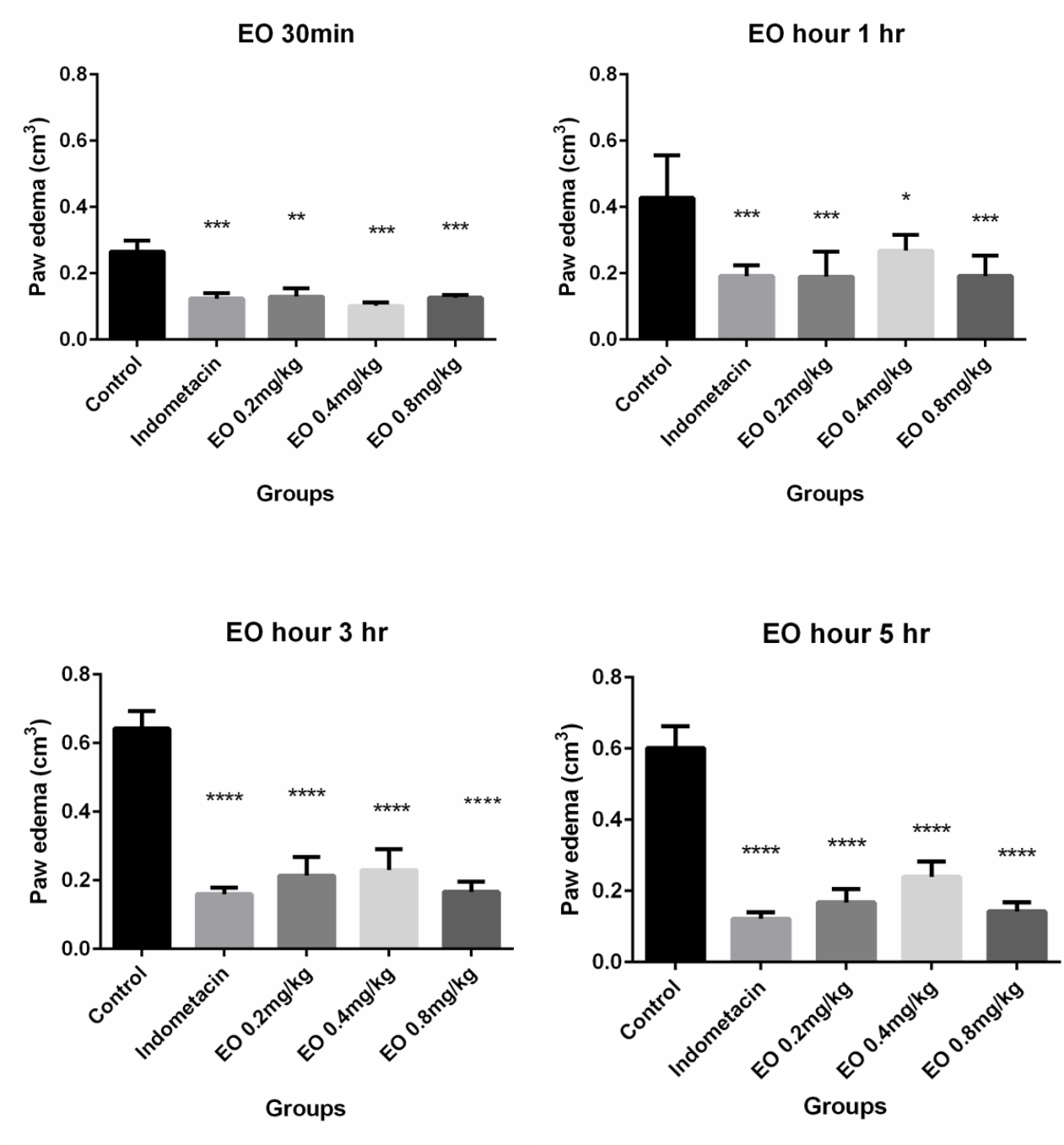

Figure 2. Acute anti-inflammatory activity of *Artemisia sieberi* essential oil (EO) in Carrageenan induced inflammation test. Values represent mean±SEM (n = 6). **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001: Significant difference compared with the control group; (one way ANOVA, followed by Tukey's test)

Table 1. Effect of *Artemisia sieberi* essential oil on the inflammation induced by carrageenan

Groups	Thickness variation ± SEM (inhibition%)					
	30min	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control	0.266±0.03	0.428±0.06	0.588±0.06	0.642±0.05	0.482±0.05	0.600±0.06
Indomethacin (5 mg/kg)	0.124±0.016*** (53.38%)	0.192±0.01*** (55.14%)	0.196±0.03**** (66.6%)	0.160±0.02**** (75.0%)	0.164±0.025**** (65.97%)	0.122±0.01**** (79.66%)
A. sieberi essential oil 0.2 mg/kg	0.100±0.00*** (62.4%0)	0.190±0.03*** (55.6%)	0.118±0.03**** (79.9%)	0.214±0.05**** (66.6%)	0.208±0.022*** (56.84%)	0.168±0.04**** (72.0%)
0.4 mg/kg	0.126±0.00** (52.63%)	0.268±0.02* (37.38%)	0.292±0.04*** (50.34%)	0.230±0.06**** (64.17%)	0.220±0.04*** (54.35%)	0.240±0.04**** (60.0%)
0.8 mg/kg	0.130±0.02*** (51.13%)	0.192±0.03*** (55.14%)	0.226±0.03**** (61.56%)	0.166±0.03**** (74.14%)	0.168±0.04**** (63.14%)	0.142±0.025**** (76.33%)

Each value represents the mean±SEM (%inhibition) of 6 rats. *p<0.05, **p<0.01, ***p< 0.001, ****p<0.0001, Significant difference compared with the control group

Table 2. GC/MS analysis of *Artemisia sieberi* fruits essential oil.

Compound[a]	KI[b]	RKI[c]	Percentage
1. Tricyclene	925	927	0.3
2. Artemisia triene	931	929	0.1
3. Camphene	950	954	4.4
4. Yomogi alcohol	996	999	2.0
5. α-Terpinene	1011	1017	0.3
6. ρ-Cymene	1028	1026	1.9
7. 1,8-Cineol	1036	1033	20.9
8. γ-Terpinene	1060	1062	0.7
9. Camphenilone	1080	1083	0.1
10. Artemisia alcohol	1089	1084	0.8
11. Hotrienol	1100	1101	0.4
12. β-Thujone	1119	1114	2.9
13. Camphor	1143	1146	47.0
14. Sabina ketone	1162	1159	0.2
15. Borneol	1166	1169	0.4
16. Terpinene-4-ol	1179	1177	1.4
17. Piperitol	1194	1193	0.2
18. Carvone	1241	1243	0.8
19. *cis*-Chrysanthenyl acetate	1259	1262	0.2
20. Bornyl acetate	1291	1289	0.4
21. ρ-Cymene-7-ol	1290	1287	0.1
22. Carvacrol	1300	1298	0.4
23. Hydrocinnamic acid, ethyl ester	1331	1335	0.2
24. Cinnamic acid, methyl ester	1366	1370	1.7
25. Jasmone	1399	1394	0.6
26. β-Caryophyllene	1432	1428	0.1
27. Bicyclogermacrene	1501	1494	0.3
28. α-Calacorene	1552	1548	0.1
29. Spathulenol	1581	1577	1.3
30. Caryophyllene oxide	1587	1583	0.6
31. 6,10,14-trimethyl-2-pentadecanone	1844	1840	0.1
Total			90.9

[a]Compounds listed in order of elution. [b]KI (Kovats index) measured relative to *n*-alkanes (C_9-C_{28}) on the non-polar DB-5 column under condition listed in the Materials and Methods section. [c]KI, (Kovats index) from literature.

The formalin test is an *in vivo* model which is composed of two distinct phases. The first phase known as neurogenic pain is caused by direct chemical stimulation of analgesic afferent fibers, predominantly C fibers, which could be suppressed by opioids like morphine [25]. The

second phase known as inflammatory pain results from the action of inflammatory mediators such as prostaglandins, serotonin and bradykinin in peripheral tissues and from functional changes in the spinal dorsal horn [26]. The associated effects, observed using different doses of *A. sieberi* essential oil, showed analgesic features in second phase, significantly attenuating the pain response similar to morphine.

It is the first report describing the anti-inflammatory activities of *A. sieberi* fruits in acute inflammation. Carrageenan-induced edema has been used as an experimental animal model for acute inflammation. It is well known that carrageenan induced paw edema is characterized by biphasic episode with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play the role, while in the second phase (3-4 h after carrageenan injection) kinins and prostaglandins are complicated [27].

Our results revealed that administration of the essential oil from the fruits of *A. sieberi* inhibited the edema starting from the half hour and during all phases of inflammation, which is probably due to inhibition of different aspects and chemical mediators of inflammation such as prostaglandins. The inhibitory activity shown by *A. sieberi* during a period of 5 h in carrageenan-induced paw inflammation was as efficient as the exhibited results by the group treated with indomethacin as the standard drug; so it has shown remarkable anti-inflammatory activity. Inhibition of cyclooxygenase, prostaglandins and leukotriens are possible.

Researches on chemical composition of *A. sieberi* have shown the main constituents of oil are: 1,8-cineole, camphor and thujone. Camphor is a terpenoid compound and has been used topically to relive pain. Several studies that have been performed on plants containing camphor have shown anti-inflammatory, anti-oxidative and antimicrobial effects [28].

Previous reports have shown that 1,8-cineole, a terpenoid oxide present in several plants essential oils, has anti-inflammatory and anti-nociceptive effects [29]. It is concluded that camphor and 1,8-cineole might have important roles in the anti-inflammatory effect of *A. sieberi* fruit and this plant has an inhibitory effect against acute inflammation and chronic phase of pain; therefore, *A. sieberi* essential oil could be a potential candidate as an anti-inflammatory agent in management of inflammation based disorders.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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