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Anti-inflammatory activity and chemical composition of *Pycnocycla bashagardiana* fruit's essential oil in animal models

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ABSTRACT

Objective(s): *Pycnocycla bashagardiana* is an endemic species found only in Iran. Due to the presence of myristicin as the major component of the fruit's oil we were prompted to assess the antinociceptive and anti-inflammatory properties of *P. bashagardiana* fruit's essential oil (PBFEO).

Materials and Methods: The analgesic activities of PBFEO (100, 200, and 400 mg/kg, IP) were studied by hot-plate and formalin tests in mice. Control and standard groups received vehicle and morphine (5 mg/kg, IP), respectively. The acute anti-inflammatory effect of PBFEO (200 and 400 mg/kg, IP) were assessed by carrageenan-induced paw edema method in 30 min, 1, 2, 3, and 4 hr after carrageenan injection and the chronic anti-inflammatory effect of PBFEO (50 and 100 mg/kg, IP) were assessed by the cotton pellet-induced granuloma method in rats.

Results: In hot-plate and formalin tests, the studied doses of PBFEO were not effective. However, in carrageenan test, all studied doses of PBFEO significantly reduced the paw edema in comparison to the control animals ($P<0.05$). Anti-inflammatory activity of PBFEO (200 and 400 mg/kg, $P<0.05$) was found to be more than mefenamic acid (30 mg/kg). In cotton pellet-induced granuloma, PBFEO was also effective regarding the transudate and granuloma formation amount. PBFEO was analyzed by gas chromatography-mass spectrometry and 12 constituents, representing 96.0% of the oil, were identified. The major component of the oil was characterized as myristicin which might be responsible for the anti-inflammatory activity.

Conclusion: The results suggest that PBFEO possesses biologically active constituents that have significant peripheral anti-inflammatory effects.

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Introduction

Plants belonging to the Apiaceae family are rich in secondary metabolites and embody numerous genera of high economic and medicinal value, yielding flavonoids, coumarins, acetylenes, terpenes, and essential oils (1). It is well known that the existence of essential oils and oleoresin is a characteristic feature of this family (2). *Pycnocycla* is a genus belonging to the Apiaceae family, subfamily Apioideae, tribe Echinophoreae, and comprises approximately 20 species of herbaceous perennial, multicaulis, and spinous plants widely distributed in subtropical and tropical regions (3). *Pycnocycla* is characterized by eight species in Iran, all of which are native or endemic (4). *Pycnocycla bashagardiana* Mozaff. is an endemic species found only in the south of Iran. It is commonly distributed in the Jask County, Hormozgan Province (4). Due to the widespread use of *P. bashagardiana* fruits in Iranian traditional medicine for relief and treatment of pain and inflammation-based disorders such as rheumatoid arthritis, we were prompted to assess the analgesic and anti-inflammatory activities of the fruit's essential oil and examine the pharmacological basis for the folkloric use of it as an antinociceptive and anti-inflammation agent. As the fruits of *P. bashagardiana* contain a high amount of essential oils (1.6%, v/w) and possess a strong smell, we were prompted to evaluate the mentioned effects of its essential oil for the first time. The *P. bashagardiana* fruit's essential oil (PBFEO) was also analyzed by Gas chromatography and GC-MS in order to detect the potentially responsible compounds for observed activities.

Materials and Methods

Plant material and preparation of essential oil

Fresh fruits of *P. bashagardiana* were collected in September 2014 from Bashagard village, Jask County, Hormozgan Province, Iran: (25°38′38″N, 57°46′28″E, 900 m). Specimens were identified by N Kazemivash and the voucher was deposited in the Herbarium of

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Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran, under code numbers 5067- AUPF. Fruits were subjected to hydrodistillation in a Clevenger-type apparatus for 3 hr. At the end of distillation, the oil was collected, dried with anhydrous Na_2SO_4, measured, and transferred to a clean glass vial and kept (−18 °C) for biological and analytical tests.

Animals

Male Wistar rats weighing 150–200 g and male NMRI mice (20–30 g) were used in present study. Animals were kept in groups of six per standard cage, on 12 hr light/dark cycle, and the air temperature was maintained at 22±2 °C. Experiments reported in this study were carried out in accordance with local guidelines for the care of laboratory animals of Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS).

Analgesic activity of PBFEO

Thermal method

Hot-plate test in mice

The hot-plate procedure was employed for the purpose of preferential assessment of the possible centrally mediated analgesic effect of PBFEO (5). Animals were individually placed on a controlled hot-plate maintained at 55±0.2 °C. Briefly, the animals were placed on the hot-plate apparatus and latency to licking or shaking of the paws or jumping was recorded. PBFEO (50, 100, 200, and 400 mg/kg, IP) was given to the separate group by intraperitoneal injection. Morphine (5 mg/kg) and vehicle (sweet almond oil, 10 ml/kg) were also administered by the same route. The latency was recorded before and 15, 30, 45, and 60 min following intraperitoneal administration of the agents and is expressed as the percentage of maximal possible effect (MPE). MPE% = 100% × (postdrug latency − predrug latency)÷(cutoff − predrug latency). A 15 sec cutoff time was used to prevent tissue damage.

Chemical method

Formalin test in mice

The analgesic effects of PBFEO were investigated by formalin test. 30 min after separate injection of different doses of the essential oil (100, 200, and 400 mg/kg), morphine (5 mg/kg, positive control) and the vehicle, formalin (50 µl of 2.5%) was injected into the hind paw of the mice. Scoring of nociceptive behaviors began after formalin injection and was continued for 60 min. A nociceptive score was recorded for each five-minute time block by measuring the amount of time spent in each of the following behavioral types: 3, the injected paw was licked, bitten, or shaken; 2, the injected paw was elevated; 1, the injected paw had little or no weight placed on it; and 0, the injected paw was not favored. Pain rating ranging from zero to three, was calculated (6). Individual time course determinations in the formalin test were changed to area-under-the-curve values, zero to ten min after formalin injection (AUC phase I) and 10–60 min after formalin injection (AUC phase II) (6).

Anti-inflammatory activity of PBFEO

Carrageenan-induced paw edema in rats

Acute anti-inflammatory activity was evaluated on the basis of paw edema inhibition induced by the injection of carrageenan (0.1 ml 2%) into the sub-plantar region of the right hind paw of the rats (7, 8). Male rats were divided into four different groups of six animals each that separately received PBFEO (200 and 400 mg/kg), mefenamic acid (30 mg/kg), and the sweet almond oil as a vehicle (10 ml/kg, IP) 1 hr before the injection of carrageenan. The paw volume was measured 0.5, 1, 2, 3, and 4 hr after the carrageenan administration using a plethysmometer (model PM 4500, Borj Sanat Co, Iran). Anti-inflammatory activity was revealed as the inhibition percent of the edema when compared with the control group. The percentage inhibition of edema was measured by the following equation: % inhibition of edema= 100 [(Vcontrol −Vtest)/Vcontrol].

Cotton pellet-induced granuloma

The chronic anti-inflammatory activity of PBFEO was measured on the basis of cotton pellet-induced granuloma according to the method of Winter and Porter (9). Four groups of six rats were used. Pellets weighing just about 60 mg each were made with 5 mm of dental cotton tampons. The pellets were sterilized in an autoclave for 30 min at 120 °C under 15 lb pressure. Rats were anesthetized and pellets were subcutaneously implanted in the axilla region of each rat through a single needle incision. Each group was treated daily, for 7 consecutive days with PBFEO (50 and 100 mg/kg), indomethacin (5 mg/kg), and vehicle (sweet almond oil, 10 ml/kg), IP. On the eighth day, rats were anesthetized over again; the cotton pellets together with the granuloma tissues were separated surgically and made free from extraneous tissues. The wet pellets were weighed for the purpose of the wet weight, and then dried in an incubator at 60 °C for 18 hr until a constant weight was obtained; after that, the dried pellets were weighed for a second time. The exudates' quantity (mg) was calculated by subtracting the constant dry weight of the pellet from the immediate wet weight of the pellet. Dry weight of granuloma was calculated after deducting the weight of the cotton pellet from the constant dry weight of the pellet and taken as an amount of granuloma tissue formation. The percent inhibitions of exudates and granuloma tissue formation were considered.

Statistical analysis

Comparisons between groups were made by one-way ANOVA analysis followed by the *post hoc* Tukey's test and $P<0.05$ was considered as significant difference of means. The data were analyzed using the Graphpad Prism 5 statistical software.

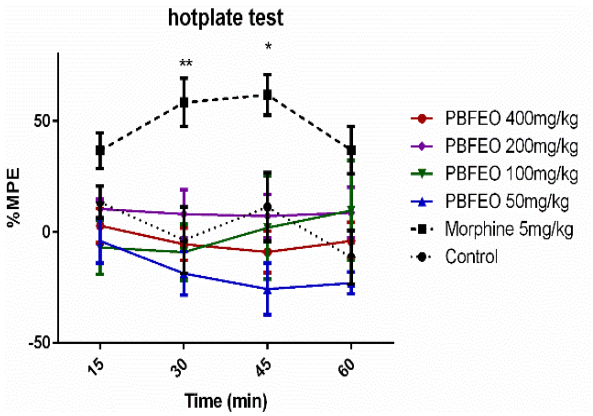

Figure 1. Antinociceptive activity of *Pycnocycla bashagardiana* fruit's essential oil (PBFEO) in the hot-plate Test

The vehicle, morphine (5 mg/kg; IP) or PBFEO (50, 100, 200, and 400 mg/kg, IP) was administered 15 min prior to the placement of the animal on the hot-plate and reaction time of mice was measured at 15 min intervals for one hour. Data represent mean± SEM of six animals in each group. **P*<0.05, ***P*<0.01 compared with the control group.

Analysis of the essential oil

Oil sample analysis was achieved on an Hp-6890 gas chromatograph equipped with an FID and a DB-5 capillary column, 30 m × 0.25 mm, 0.25 µm film thickness, temperature was programmed as follows: 60 °C –240 °C at 4 °C/ min. The carrier gas was N_2 at a flow of 2.0 ml/min; injector port and detector temperature were 250 °C and 300 °C, respectively. The sample was injected by splitting and the split ratio was 1:10.

GC/MS analysis was done 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. Scan mass range 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 known by their retention time, retention indices, relative to C_9-C_{28} n-alkanes, computer matching with the WILEY275.L library, as well as by comparison of their mass spectra with data already available in the literature (10). The percentage of composition of the identified compounds was computed from the GC peaks areas without any correction factors and was calculated relatively.

Results

Antinociceptive activity of PBFEO

Hot plate test

PBFEO with low and high doses of 50 and 400 mg/kg did not increase the reaction time in tested animals; even the lowest tested dose of PBFEO induced hyperalgesia 30 min after treatment (Figure 1). Morphine significantly indicated antinociceptive activity in the hot-plate test.

Formalin test

The effect of systemic intraperitoneal administration of different doses of the PBFEO (100, 200, and 400 mg/kg) on the behavioral responses during the first (phase I) and the second phases (phase II) of the formalin test were calculated. In formalin test, morphine revealed antinociceptive effects in both phases I and phase II (Figure 2) but PBFEO did not produce any antinociception effect compared with the control group.

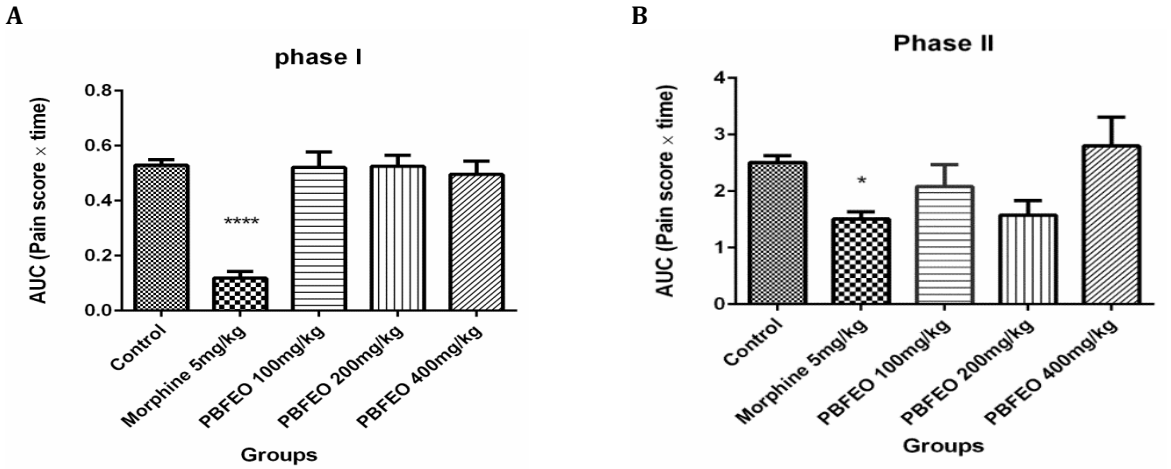

Figure 2. Effects of *Pycnocycla bashagardiana* fruit's essential oil (PBFEO) on nociceptive response in phases I (A) and II (B) of the formalin test. Values indicate mean±SEM (n=6–8). **P*<0.05, *****P*<0.0001: Significant difference compared with the control

Table 1. Effect of *Pycnocycla bashagardiana* fruit's essential oil **(PBFEO)** on the inflammation induced by carrageenan

Groups	Dose (mg/kg)	0.5 hr	1 hr	2 hr	3 hr	4 hr
Control	10	0.31±0.45	0.50±0.03	0.61±0.04	0.8±0.2	0.65±0.03
Mefenamic Acid	30	0.13 ± 0.02** (56.91)	0.24±0.03**** (51.34)	0.35±0.03** (42.39)	0.6±0.04* (25.51)	0.50±0.04* (23.52)
PBFEO	200	0.16 ± 0.03* (48.42)	0.21±0.03**** (57.34)	0.43±0.06* (29.88)	0.47±0.07*** (41.91)	0.31±0.03****,# (52.68)
PBFEO	400	0.15± 0.03* (50.01)	0.19±0.04**** (62.68)	0.27±0.04**** (55.71)	0.34±0.05****, ## (58.09)	0.24±0.05****;## (62.92)

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; #*P*<0.05, ##*P*<0.01, Significant difference compared with the mefenamic acid group

Table 2. Effect of *Pycnocycla bashagardiana* fruit's essential oil (PBFEO) on the cotton pellet-induced granuloma pouch in rats

Group	Doses (mg/kg)	Mean weight of granuloma (mg)	Inhibition (%)
Control	10	74.90 ± 4.65	-
Indomethacin	5	62.10 ± 1.98 *	17.09
PBFEO	50	82.92±6.01	-10.71
PBFEO	100	67.14±6.94*	10.36

Each value represents the mean ± SEM of 6 rats. **P*<0.05, Significant difference compared with the control group

Anti-inflammatory activity of PBFEO

Carrageenan-induced inflammation test

The acute anti-inflammatory effect of PBFEO in rat paw edema induced by carrageenan was established using the essential oil administered IP (Table 1). PBFEO (200 and 400 mg/kg), as well as mefenamic acid (30 mg/kg), significantly inhibited (*P*<0.05) the carrageenan paw edema formation, which was determined at the third hour of the experiment (peak of edema formation) by 41.91, 58.09, and 25.51%, respectively. At the fourth hour of the experiment, this edema inhibition reached 23.52%, 52.68%, and 62.92%.

Cotton pellet-induced inflammation test

In the cotton pellet-induced inflammation experiment, a reduction was observed just for the group treated with PBFEO (100 mg/kg, IP) daily for 7 days. The reduction of inflammation was 10.36% in comparison with the standard drug indomethacin (17.09%) (Table 2).

Analysis of the essential oil

PBFEO was analyzed by GC and GC/MS to determine the possible compounds responsible for the observed analgesic and anti-inflammatory activities. The hydrodistillation of the fruit of *P. bashagardiana* gave pale yellow oil with pleasant odor and yield of 1.6% (v/w).

As shown in Table 3, twelve components were identified in this oil, which represented about 96% of the total chromatographical material. The studied essential oil was dominated by the presence of phenylpropanoids constituting 76.9% of the total oil composition. Monoterpene hydrocarbons comprised 10.9% while sesquiterpenoids constituted only 8.2% of which 4.5% were hydrocarbons and 3.7% were oxygenated ones. The major constituent of fruit essential oil was characterized as myristicin (76.1%).

Table 3. GC/MS analysis of the essential oil from the fruits of *Pycnocycla bashagardiana*

Compound[a]	KI[b]	KI[c]	Percentage
Sabinene	973	975	1.6
β-Pinene	981	979	1.4
Z. β. Ocimene	1041	1037	3.8
E. β. Ocimene	1052	1050	4.1
β-Cubebene	1387	1391	1.6
Methyl eugenol	1400	1401	1.0
α-Guaiene	1443	1440	1.1
δ-Guaiene	1507	1508	0.8
Myristicin	1523	1520	76.1
Caryophyllene oxide	1589	1583	0.8
Isomyristicin	1619	1624	0.8
β-Eudesmol	1658	1651	2.9
Total			96.0

[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 conditions listed in the Materials and Methods section
[c] KI, (Kovats index) from literature

Discussion

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 many side effects, while plants still hold their own unique place, by the way of having the least side effects. Therefore, a systematic method should be used to find out the efficacy of plants against inflammation and pain so as to use them as herbal anti-inflammatory drugs.

In this study, we assessed the antinociception and anti-inflammatory activity of the essential oil from the fruits of *P. bashagardiana*. It is the first report describing the anti-inflammatory activities of *P. bashagardiana* fruits in acute and chronic inflammation. Carrageenan-induced edema has been usually presented as an acute inflammation model in the experimental animal. It is well known that carrageenan-induced paw edema is regarded by biphasic episode with the involvement of inflammatory mediators. In the first phase (for the duration of the first 2hr after carrageenan injection),

chemical mediators such as histamine and serotonin play a role, while in the second phase (3– 4 hr after carrageenan injection) kinins and prostaglandins are implicated (11).

Our results revealed that administration of PBFEO inhibited edema starting after half an hour and during all phases of inflammation, which is possibly inhibition of different aspects and chemical mediators of inflammation such as prostaglandins. The inhibitory activity shown by PBFEO during a period of 4 hr in carrageenan-induced inflammation was in some way more efficient than that revealed by the group treated with mefenamic acid as standard drug.

The cotton-pellet granuloma is a widely used manner for the calculation of chronic anti-inflammatory substances (12). The dry weight of the pellet correlates with the amount of granulomatous tissues, the moist weight of the pellets correlates with transuda. Chronic inflammation happens by means of the development of proliferating cells. These cells can be either spread or in granuloma form. PBFEO (100 mg/kg) indicated significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which shows its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

Heat-induced and formalin-induced pain models were used in the present study in order to assess the anti-nociceptive effect of *P. bashagardiana* fruit essential oil in experimental mice. Formalin test consists of two phases; the first phase (neurogenic pain) is caused by direct chemical stimulation of nociceptive afferent fibers, predominantly C fibers, which can be suppressed by opiates like morphine (13). The second phase (inflammatory pain) results from the action of such inflammatory mediators as prostaglandins, serotonin, and bradykinin in peripheral tissues and also from functional changes in the spinal dorsal horn (14). The associated effects, observed after using different doses of PBFEO, did not show any significant anti-nociception properties in both phases, compared to the control group. The hot-plate test was also used as a thermal nociception model to define the central anti-nociceptive activity of PBFEO. It did not practically increase the reaction time of animals against the thermal stimulus; even the lowest tested dose induced hyperalgesia. Furthermore, in agreement with the results of the first phase of the formalin test, the essential oil did not display an analgesic effect in contrast to morphine. Thus, it could be concluded that the essential oil does not have anti-nociceptive properties.

The second phase of the formalin test indicates the effect of the drug on the inflammation process. In the present study, no reduction in pain behaviors was observed. It is assumed that the essential oil of *P. bashagardiana* fruit contains a substance that directly stimulates pain receptors in addition to formalin-induced pain. However, despite the reduction of inflammation in animals, there are painful irritations that make them show different pain behaviors.

The phytochemical results indicated that the anti-inflammatory effects of PBFEO may be due to its myristicin content. The major constituent of the oil was myristicin (76.1%). It was observed that myristicin comprised more than three-fourths of the oil composition. The anti-inflammatory activity of myristicin has been previously examined on RAW 264.7 macrophages stimulated with polyinosinic-polycytidylic acid in mice (13), and since the results have shown marked anti-inflammatory activity, it could be concluded that the observed activities of the studied oil were related to its high content of myristicin. Lee and Park (13) demonstrated that myristicin could inhibit the production of several inflammatory mediators such as nitric oxide (NO), interleukin 6 (IL-6), and IL-10. Since NO is believed to be a major pro-inflammatory mediator related to the bacterial and viral infections, obtained results suggest that the studied myristicin rich essential oil might have anti-inflammatory activity against the pathologic and excessive production of NO in virus-stimulated macrophages and monocytes (13). Excessive production of IL-6 often correlates with some inflammatory autoimmune diseases including Crohn's disease, psoriasis, and rheumatoid arthritis (14). IL-10 has also been implicated in promoting the pathobiology of autoimmune diseases such as lupus and encephalomyelitis (15). These results justified the use of *P. bashagardiana* fruits in traditional medicine. Therefore, PBFEO could be a potential candidate as an anti-inflammatory agent in the management of inflammation-based disorders.

Also, we used formalin and a heat-induced pain model for assessing the anti-nociceptive effect of *P. bashagardiana* essential oil in experimental mice. Our data demonstrated that *PBFEO* did not produce an antinociceptive effect in mice subjected to both the acute thermal (hot plate) and chronic (persistent) formalin pain stimuli.

Conclusion

P. bashagardiana essential oil has an inflammatory activity against acute and chronic inflammation in rats. This effect could be related to the high content of myristicin in this essential oil.

Conflicts of interest

The authors declare that no conflict of interest exists.

Acknowledgment

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