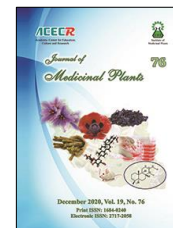




Institute of
Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Phytochemical, physicochemical and biological evaluation of *Colchicum kurdicum* (Bornm.) Stef.: a study on materia medica of Persian medicine

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ARTICLE INFO

Keywords:

Colchicum kurdicum
Tropolone alkaloid
Total flavonoid
Chromatography
Antioxidant
Anti-inflammatory activity

ABSTRACT

Background: The genus *Colchicum* (Colchicaceae) is a perennial and monocotyledon flowering plant that has more than 160 species in the world. In addition, this plant is an important medicinal plant in traditional and modern medicines. **Objective:** The aim of this study was to evaluate the phytochemical profiles and physicochemical properties, antioxidant and anti-inflammatory activities of *Colchicum kurdicum* (Bornm.) Stef. corm. **Methods:** Phytochemical profiles included total tropolone alkaloid, total phenolic/total tannin and total flavonoid contents were determined by spectrophotometric method. Moreover, tropolone alkaloid profiles was analyzed by HPLC method. Physicochemical properties including macroscopic and organoleptic properties, solubility, foreign matter, ash values and heavy metals were evaluated based on pharmacopeial protocols. In addition, in vitro anti-inflammatory and antioxidant activities of the plant were determined. **Results:** Total tropolone alkaloids, phenol, tannin and flavonoid contents of the corm were estimated to be 0.652, 0.426, 0.052 and 0.325 g/100 g corm. In HPLC analysis, N-deacetyl-N-formyl colchicine, colchifoline, colchicoside and cornigerine were calculated as highest bioactive tropolone alkaloids. Physicochemical properties were determined within an acceptable range for the pharmacopoeia. Moreover, the anti-inflammatory and antioxidant activities in 10 mg/ml of methanolic extract were estimated 84.7 % and 94.8 %, respectively. **Conclusion:** The corm of the *Colchicum kurdicum* had the main bioactive compounds. In addition, these compounds have the valuable biological activities as anti-inflammatory and antioxidant activities.

Abbreviations: HPLC, High Performance Liquid Chromatography; PVPP, Polyvinyl Polypyrrolidone; ACN, Acetonitrile; SD, Standard Deviation

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doi: 10.29252/jmp.19.76.36

Received 13 October 2019; Received in revised form 24 June 2020; Accepted 19 July 2020

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1. Introduction

The genus *Colchicum* (Colchicaceae) is a perennial and monocotyledon flowering plant with more than 160 species in the world. In addition, the bulb-like corms and underground ovaries are the individual morphological characterizations of this genus [1, 2].

Colchicum kurdicum (Bornm.) Stef. is the important medicinal plant of this family, which is named as "Sorenjân" and was used in many diseases such as osteoarthritis, gout, cancer, inflammations, jaundice and sexual impotencies in the different traditional medicines.

In addition, the main pharmaceutical organs of *C. kurdicum* are the seeds and corms, which are contained the important bioactive compounds, including alkaloids especially tropolone alkaloids and isoquinoline alkaloids, phenolic compounds, tannins, flavonoids and carbohydrates [1, 2].

Tropolone alkaloids especially colchicine as the main important bioactive compounds of this plant are used for treatment of some diseases such as gout and cancers. These alkaloids could be used in mitosis-dependent diseases by the tubulin polymerization inhibitory effects. In addition, these compounds have high potential toxicity and a narrow therapeutic index [3].

Furthermore, other bioactive compounds of *C. kurdicum* are included isoquinoline alkaloids with anticholinesterase inhibitory activities, coumaric acid, ferulic acid, caffeic acid, vanillic acid, 2-hydroxybenzoic acid, apigenin, luteolin and 4, 6-dimethoxy-3, 7-dimethylcoumarin [4].

According to the literatures, the bioactive compounds of the *C. kurdicum*, especially tropolone alkaloids, flavonoids and phenolic acids, have anti-inflammatory, anti-arthritic and anti-oxidant activity.

Phytochemical, physicochemical and biological evaluations of the medicinal plants by

different spectrophotometric, *in vitro* and *in vivo* methods are the critical stage for validation of the quality. Moreover, the findings of the phytochemical profiles and bioactive compounds could be helped to estimate the biological activities [5, 6].

In the present study, phytochemical profiles of *C. kurdicum* were evaluated by spectrophotometric and HPLC methods. Furthermore, physicochemical properties including macroscopic and organoleptic properties, solubility, foreign matter, ash values and heavy metals were determined. In addition, biological activities included anti-inflammatory and anti-oxidant activities of *C. kurdicum* were evaluated.

2. Materials and Methods

2.1. Materials

All materials were purchased from the Sigma and Merck companies. All acids and solvents were purchased from Merck Company (Germany).

2.2. Plant preparation

The plant specimen was collected from 1500 to 2500 regions of Tang-e-Rah area of the Golestan National Park (37.366335, 55.780033) in Golestan province in October to April 2018. Dr. Masoud Azadbakht performed taxonomic identification of plant samples, and representative voucher specimens were deposited in the herbarium of the Department of Pharmacognosy in Faculty of Pharmacy of the Mazandaran University of Medical Science (E1-11312) [7].

2.3. Preparation of corm extracts

The powdered corms of the *C. kurdicum* were extracted using methanol with percolation method (24 h maceration and 1:10 plant/solvent

ratio). Then, the methanolic extract was concentrated using rota-vapour and was dried using freeze-dryer. Finally, the extract was stored at 4 °C until analysis [8].

2.4. Total assay of phytoconstituents

Total tropolone alkaloids of the corm methanolic extract was determined using the acidic potassium dichromate method. One g of corm extract was dispersed in the mixture of methanol/H₂SO₄ solution (3 %, pH = 1) and was extracted using sonication during 1 hour. The final acidic extract was decanted with chloroform and aqueous phase was collected and was alkalized using NH₄OH solution (10 %, pH = 12). The solution was extracted three times with chloroform and was dried. Then, 1 ml solution of extract (1 mg/ml) and (2.5, 5, 10, 20 and 40 mg/ml) colchicine were mixed with 1 ml acidic potassium dichromate solution (pH = 2) and were incubated at room temperature for 1 hour. Finally, UV absorbance was measured at 352 nm and the graph of calibration was drawn [9, 10].

Total phenolic and tannin contents of the corm extract were determined using the Folin-Ciocalteu method. One ml of the extract (1 mg/ml) was mixed with 1.5 ml Folin-Ciocalteu and was incubated at room temperature for 10 minutes. Then, 1.5 ml sodium carbonate solution (20 %) was added to the mixture and was incubated at room temperature for an additional 1 hour and absorptions at 725 nm were recorded. In addition, calibration curve was created using a standard concentration of gallic acid and total phenolic compounds of extract were obtained by calibration curve. Moreover, 100 mg polyvinyl polypyrrolidone (PVPP), 1 ml distilled water and 1 ml of the extracts (1 mg/ml) were added into tube and was mixed using and kept in 4 °C for 15 minutes. Then, it

was centrifuged (3000 g for 15 min) and collected the supernatant. This supernatant has only simple phenolic compounds other than tannins. The phenolic content of the supernatant was measured as mentioned above. Finally, the total tannin was obtained by differences of two above amount [6].

Furthermore, the total flavonoid content the corm extract was determined using the AlCl₃ method. One ml of the extracts (1 mg/ml) was mixed with 1 ml of AlCl₃ solution (2 %) and 1 ml potassium acetate solution (10 %) and was incubated at room temperature for 30 minutes. Finally, the absorbance of the mixture was measured at 415 nm and the total flavonoid contents was determined using a standard curve with quercetin (2.5, 5, 10, 20 and 40 mg/ml) as the standard [5].

2.5. HPLC analysis

The HPLC analysis of the corm extract of the *C. kurdicum* was done by HPLC-UV. The separation, detection and assay of tropolone alkaloids including colchicine, demecolcine, 2-demethyl colchicine, 3-demethyl colchicine, colchicoside, colchifoline, cornigerine and N-deacetyl-N-formyl colchicinewere performed by a HPLC Smartline Manager 5000 (Knauer, Germany) with Smartline pump 1000 and EC Nucleodur C₁₈ column (4.6 mm × 250 mm, 5 µm particle size) at 25°C with UV detector 2500 basic model in 245 nm. Moreover, data acquisition and integration were performed with EZchrom Elite 3.2.0 software. The confidence of the accuracy of the individuality of the peaks was obtained by standard addition. The extract obtained from the mentioned above method (1 mg/ml) and the HPLC grade solutions of the compounds (125, 250, 500, 1000 and 2000 ppm) were injected into the HPLC with 20 µl volume and flow rate of the mobile phase was maintained at 1 ml/min. The mobile phases

were HPLC grade acetonitrile (ACN) and water. Moreover, the gradient condition were: 0-5 min, ACN 10 %; 5-20 min, ACN 100 %; 20-25 min, ACN 10 %; 25-30 min, ACN 10 %. Finally, the calibration curves of all compounds were obtained by plotting the peak region against the concentration of each sample and the amount of the compounds was calculated by the obtained formulas. Moreover, $R^2 > 0.9$ representing the linear measurements [2, 11].

2.6. Physicochemical properties of the corm

All physicochemical properties of the *C. kurdicum*, including macroscopic and organoleptic properties, solubility, foreign matter, ash values and heavy metals were separately done based on pharmacopeial protocols [12, 13].

2.7. In vitro anti-inflammatory activity

In this study, anti-denaturation activities (as a parameter for determination of anti-inflammatory activity) of the *C. kurdicum* extract were evaluated. Five ml of the extract (10 mg/ml) was mixed with 1 ml of 2 % bovine serum albumin and 1 ml of distilled water and was incubated for 1 hour at 37°C. Then, the mixture was heated at 60 °C for 10 min and was cooled. In addition, 3 ml of phosphate buffer saline (pH = 7.4) was added to the final mixture and was evaluated by UV spectrophotometric method in 600 nm. The anti-denaturation activities were calculated using the following formula:

$$\text{Percentage of anti-denaturation activities} = 100 \times (\text{Absorbance of control} - \text{Absorbance of treated sample}) / \text{Absorbance of control}$$

The control solution was contained the distilled water instead of extract. Moreover, the diclofenac sodium was used as standard drug [14].

2.8. Antioxidant activity

The radical scavenging activity of methanolic extract of *C. kurdicum* was evaluated by stable DPPH free radical. A solution of DPPH (250 µM) was prepared by dissolving DPPH (5 ml) in the methanol (2 ml) and the solution was kept in the dark at room temperature. Furthermore, different concentrations of the extract were prepared and mixed with the same volume of DPPH solution and incubated and analysed at 517 nm. The percentage of inhibition of the extract was determined by comparison with the methanol control group. In addition, ascorbic acid was used as positive control [15].

2.9. Statistical analysis

All analysis procedures were done triplet and the data were analysed by SPSS and Excel softwares and were characterized with mean ± SD.

3. Results

3.1. Phytochemical characteristics

Table 1 shows the results of the total assay of the main bioactive phytoconstituents in the corm extract of the *C. kurdicum*. The amounts of total alkaloid, total phenol, total tannin and total flavonoid contents were estimated 0.05 to 0.7%.

3.2. HPLC analysis

Fig. 1 and Table 2 show the chromatograms and HPLC results of the *C. kurdicum*. The amounts of the compounds were calculated by HPLC. According to the obtained chromatograms, N-deacetyl-N-formyl colchicine, colchifoline, colchicoside and cornigerine were the main compounds in *C. kurdicum*.

[DOI: 10.29252/jmp.19.76.36] [DOR: 20.1001.1.2717204.2020.19.76.4.6] [Downloaded from mail.jmp.ir on 2026-09-29]

Table 1. Total tropolone alkaloid, phenol, tannin and flavonoid contents of the *C. kurdicum*

Compounds	Total tropolone alkaloid (g colchicine/100 g corm)	Total phenol contents (g gallic acid/100 g corm)	Total tannin contents (g gallic acid/100 g corm)	Total flavonoid contents (g quercetin/100 g corm)
<i>C. kurdicum</i>	0.692 ± 0.021	0.426 ± 0.034	0.052 ± 0.005	0.325 ± 0.028

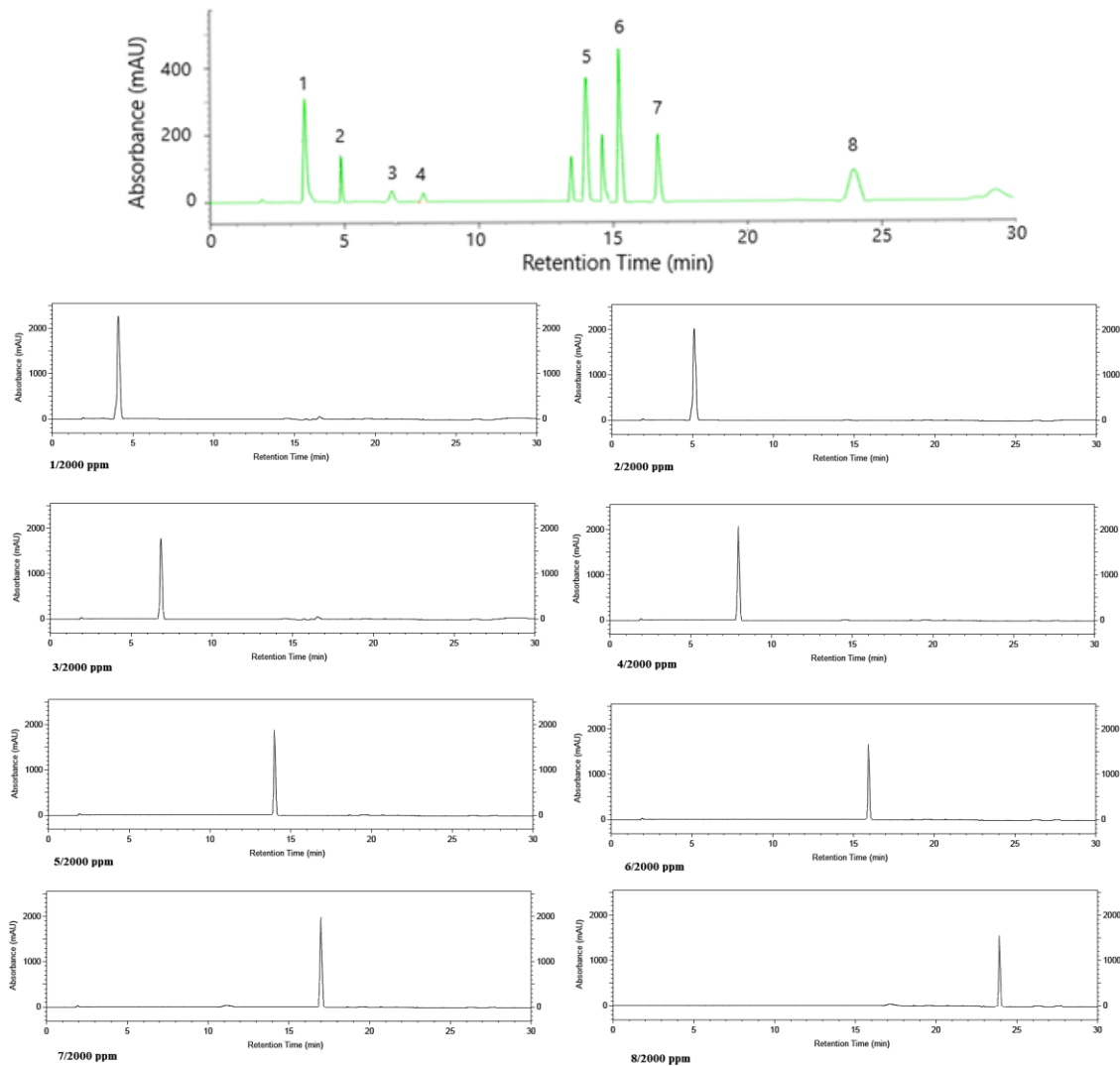


Fig. 1. The HPLC curves of the tropolone alkaloid-rich extract of the *C. kurdicum* and the standard curves of the compounds (1: Colchicoside, 2: 2-demethyl colchicine, 3: 3-demethyl colchicine, 4: Demecolcine, 5: Colchifoline, 6: N-Deacetyl-N-formyl Colchicine, 7: Colchicine, 8: Cornigerine)

Table 2. The amounts of the tropolone alkaloids in the *C. kurdicum* based on HPLC data (mg/100 g corm)

Compounds	Colchicoside	2-demethyl colchicine	3-demethyl colchicine	Demecolcine	Colchifoline	N-deacetyl-N-formyl colchicine	Colchicine	Cornigerine
<i>C. kurdicum</i>	135.56 ± 1.34	48.12 ± 0.98	22.04 ± 0.92	18.76 ± 0.91	136.93 ± 0.82	177.65 ± 0.81	66.34 ± 0.23	129.95 ± 0.92

3.3. Physicochemical characteristics

Table 4 shows the physicochemical properties of the corm of the *C. kurdicum*. According to obtained data, the shape of the corms has been varied from oblong to oval. The colour of the powdered corms was grey and odour and taste of them were spicy and bitter, respectively. In addition, solubility of dry powder of *C. kurdicum* corms was estimated sparingly soluble in different solvents. Other physicochemical properties of the corm were illustrated in table 3.

3.4. Anti-inflammatory and anti-oxidant activities

The results of the anti-denaturation and radical scavenging activities of the *C. kurdicum* are shown in the table 4. According to this study, the anti-denaturation and radical scavenging activities of the methanolic extract of *C. kurdicum* were estimated $84.7 \pm 1.2\%$ and $94.8 \pm 1.8\%$.

Table 3. Physicochemical data of the *C. kurdicum*

Characteristics		<i>C. kurdicum</i>
Organoleptic Properties	Shape	Oblong to Oval
	Powder Colour	White to Gray
	Odour	Mild Spicy
	Taste	Bitter
Solubility	Water	Sparingly Soluble
	KOH 10%	Sparingly Soluble
	H ₂ SO ₄ 5%	Freely Soluble
	Methanol	Sparingly Soluble
	Ethanol 96%	Soluble
	Chloroform	Freely Soluble
	Hexane	Freely Soluble
Foreign Matter (%)		1.8 ± 0.16
Moisture Content (%)		5.4 ± 1.53
Ash Content (%)	Total Ash	7.8 ± 1.54
	Acid Insoluble Ash	4.3 ± 1.23
	Sulphated Ash	25.5 ± 0.89
	Water Insoluble Ash	0.96 ± 0.05
	Water Soluble Ash	3.2 ± 0.23
Heavy Metals (µg/kg)	Hg	0.5 ± 0.01
	Cu	0.3 ± 0.02
	Cd	0.1 ± 0.07
	Pb	0.1 ± 0.06

Table 4. The anti-inflammatory and antioxidant activities of the *C. kurdicum* methanolic extract.

Compounds	<i>C. kurdicum</i> (10 mg/ml)	Diclofenac Sodium (10 mg/ml)	Ascorbic Acid (10 mg/ml)
Anti-denaturation activity (%)	84.7 ± 1.2	94.4 ± 1.5	-
Radical scavenging activity (%)	94.8 ± 1.8	-	81.8 ± 1.4

3. Discussion

In this study, phytochemical and physicochemical properties and anti-inflammatory and anti-oxidant activities of the corm of *C. kurdicum* was evaluated. The preliminary phytochemical studies could be helped to estimate the constituents of the plants and potential and valuable biological activities. In the different studies, the bioactive compounds of the plants have been evaluated. Researchers determined the phytochemical profiles of *C. luteum* Baker. In addition, this study reported the alkaloids, phenolic compounds, flavonoids, sterols, tannins and saponins in the methanolic extracts of the plant corms [4]. Moreover, other researchers detected the similar compounds in the corm extract of *Gloriosa superba* Linn [16].

In the different studies phytoconstituents of the plants and other bioresources were assayed. In a study, the chemical constituents of different parts of the *C. baytopiorum* C.D.Brickell that total alkaloids of the plant were determined by the spectrophotometric method [17]. In this study, total alkaloids were estimated 5.27%, 2.96% and 1.96% in the perigon, perigon tube and the leaves, respectively. In addition, researchers determined the total phenolic compounds, total tannins and total flavonoids of the *C. autumnale*. In this study, the estimated amounts of total phenolic compounds, tannins and flavonoids were 138.7 mg/l, 82 mg/l and 66.3 mg/L [18]. One study reported the spectrophotometric method for alkaloid assay based on the backbone structures. In this study, the alkaloid compounds were determined by complex with metal [19].

A study evaluated the total phenolic compounds and flavonoids of some medicinal plants by the Folin-Ciocalteu and aluminium chloride methods [20].

In this study, tropolone alkaloid profiles of the *C. kurdicum* were reported. In one study the amounts of colchicine-like compounds of some *Colchicum* species have been determined by HPLC method [11]. According to this study, *C. speciosum* and *C. macrophyllum* had higher amounts of colchicine.

In the literature, physicochemical properties of the plants have been determined. In this study, shape, powder colour, odour and taste of corms, solubility in the several solvents, percent of foreign matter, moisture contents, ashes and the amount of heavy metals of the *C. kurdicum* were evaluated.

In different studies, the physicochemical properties of some plants were evaluated. Organoleptic properties of the corm of *Colchicum luteum* have been explained ovoid shape and bitter taste. In addition, moisture content of *C. luteum* have been estimated 6% and total Ash, acid insoluble ash, water soluble ash and water insoluble ash were 1.33%, 6%, 1.24% and 4.28%, respectively. In this study, the moisture contents of *C. kurdicum* were calculated $5.4 \pm 1.53\%$.

Various ash values, which are used for determining the total properties of the compounds of medicinal plant, have been evaluated in the powdered corms of *C. kurdicum*.

Amount of heavy metals in the medicinal plants are the important parameter, which are determining the quality control. Heavy metals especially Hg, Cu, Cd and Pb have the standard amounts in plants and functional foods. In addition, high amounts of them induce the several complications such as cerebral and cardiovascular disorders. Based on standardization references, maximum amount of Hg, Cu, Cd and Pb were estimated 4, 6, 0.1 and 0.6 µg/kg, respectively [21-26].

One study evaluated a novel method for rapid determining anti-inflammatory activities of the compounds. According to this study, anti-denaturation compounds are effective for improving the inflammatory conditions such as rheumatoid arthritis and osteoarthritis [27].

Researchers evaluated the *in vitro* anti-arthritic activities of the *Oryza sativa* var. Joha rice [14]. According to the study, the anti-inflammatory and anti-arthritic activity with anti-denaturation effects of the plant was 40 % to 60 %. Other researchers reported the anti-denaturation effects of the *Ziziphus oenoplia* between 50 % to 98 % for aqueous, ethanolic and ethyl acetate extracts [28]. In the present study, the anti-denaturation activity of the methanolic extract of *C. kurdicum* was estimated 84.7 ± 1.2 % and was significant against the negative control. In addition, the anti-oxidant activity of the extract was calculated 94.8 ± 1.8 %.

5. Conclusion

According to this study, the corm of the *C. kurdicum* had the main bioactive compounds and the appropriated physicochemical parameters. In addition, these compounds have the valuable biological activities especially anti-inflammatory and antioxidant activities, which could be used for treatment of inflammatory and oxidative diseases such as gout, rheumatoid arthritis, cardiovascular diseases and premature aging.

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So, *C. kurdicum* is the appropriated natural source for this valuable compounds and played the important role for drug discovery and design studies.

Author contributions

Mohammad Azadbakht was the main study investigator, contributed to the collection of the data, and critically revised the manuscript; Ali Davoodi the study investigator, contributed to the collection of the data, and critically revised the manuscript; Seyed Jalal Hosseinimehr participated in the data interpretation and revised the manuscript; Saeed Emami, Masoud Azadbakht, Fatemeh Mirzaee and Hossein Bakhshi Joubari participated in the data interpretation.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This article was a part of PhD thesis of Dr. Ali Davoodi, PharmD, supervised by Professor Mohammad Azadbakht. We thank all pharmacy school personnel especially pharmacognosy laboratory personals. This study was supported by a grant from Mazandaran University of Medical Sciences, Sari, Iran (Funding code: 1396-1102).

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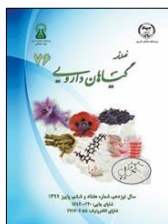
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How to cite this article: Azadbakht M, Davoodi A, Hosseinimehr SJ, Emami S, Azadbakht M, Mirzaee F and Bakhshi Jouybari H. Phytochemical, physicochemical and biological evaluation of *Colchicum kurdicum* (Bornm.) Stef.: a study on materia medica of Persian medicine. ***Journal of Medicinal Plants*** 2020; 19(76): 36-45.
doi: 10.29252/jmp.19.76.36



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مقاله تحقیقاتی

ارزیابی فیتوشیمیایی، فیزیکوشیمیایی و بیولوژیکی گل حسرت کردی: یک مطالعه بر روی مفرده پزشکی در طب ایرانی

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چکیده

اطلاعات مقاله

گل‌واژگان:

گل حسرت کردی

آلکالوئیدهای تروپولونی

فلاونوئید تام

کروماتوگرافی

آنتی اکسیدان

فعالیت ضد التهابی

مقدمه: گیاهان جنس سورنجان (*Colchicum*)، گیاهانی تک لپه‌ای، گل‌دار و چند ساله بوده که بیش از ۱۶۰ گونه در جهان دارد. همچنین، این گیاه در طب سنتی و مدرن کاربرد زیادی دارد. **هدف:** هدف از این مطالعه ارزیابی فیتوشیمیایی و فیزیکوشیمیایی، فعالیت‌های آنتی‌اکسیدانی و ضد التهابی بنه گل حسرت کردی می‌باشد. **روش بررسی:** مقادیر تام آلکالوئیدهای تروپولونی، فنول، تانن و فلاونوئید با روش اسپکتروفتومتری تعیین شد. همچنین، آلکالوئیدهای تروپولونی با روش HPLC آنالیز شدند. خصوصیات فیزیکوشیمیایی شامل خواص ماکروسکوپی و ارگانولپتیک، حالیت، ماده خارجی، مقادیر خاکستر و فلزات سنگین بر اساس پروتکل‌های فارماکوپه‌ای مورد بررسی قرار گرفتند. فعالیت‌های ضد التهابی و آنتی اکسیدانی گیاه نیز تعیین شدند. **نتایج:** آلکالوئیدها، ترکیبات فنلی، فلاونوئیدها، تانن‌ها، ترپنوئیدها و استروئیدها، کومارین‌ها، ساپونین‌ها و گلیکوزیدها در بنه سورنجان تشخیص داده شدند. علاوه بر این، میزان تام آلکالوئیدهای تروپولونی، فنل، تانن و محتوای کل فلاونوئید بنه به ترتیب 0.005 ± 0.002 ، 0.052 ± 0.002 و 0.325 ± 0.028 گرم در ۱۰۰ گرم محاسبه شد. در آنالیز HPLC، N-داستیل-N-فرمیل کلشی سین، کلشی فولین، کلشی کوزید و کورنی‌ترین بیشترین میزان محاسبه شدند. خصوصیات فیزیکوشیمیایی در محدوده مناسبی تعیین شد. علاوه بر این، فعالیت‌های ضد التهابی و آنتی اکسیدانی در ۱۰ میلی گرم در میلی‌لیتر عصاره متانولی به ترتیب $1/2 \pm 84/7$ و $1/8 \pm 94/8$ درصد تخمین زده شد. **نتیجه‌گیری:** بنه گل حسرت کردی حاوی ترکیبات فعال زیستی بود. همچنین، این ترکیبات دارای فعالیت‌های بیولوژیکی با ارزش بویژه فعالیت‌های ضد التهابی و آنتی اکسیدانی بودند.

مخفف‌ها: HPLC، کروماتوگرافی مایع با کارایی بالا؛ PVPP، پلی‌وینیل پیلیرولیدون؛ ACN، استونیتریل؛ SD، انحراف استاندارد

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تاریخ دریافت: ۲۱ مهر ۱۳۹۸؛ تاریخ دریافت اصلاحات: ۴ تیر ۱۳۹۹؛ تاریخ پذیرش: ۲۹ تیر ۱۳۹۹

doi: 10.29252/jmp.19.76.36

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