European Journal of Medicinal Plants

32(12): 46-53, 2021; Article no.EJMP.78527
ISSN: 2231-0894, NLM ID: 101583475

Microwave Assisted Extraction of Berberine and Preparation of Berberine Hydrochloride from Berberis Aristata Variety of Nepal, and Quantification using RP-HPLC and HPTLC Methods

Hemanth Kumar Manikyam [a,b*≡], Prathibha Tripathi [cϖ], Jyoti Joshi [d#], Jayaram Balasubramanian [e†], Sandeep Balvant Patil [f‡], Trishna Lamichhane [f¥] and Janardan Lamichhane [gŀ]

[a] Faculty of Chemistry, North East Frontier Technical University, Aalo, Arunachal Pradesh, India.
[b] Larke Himal Jadibuti Udhyog, Kathmandu, Nepal.
[c] Ayurveda Campus, Tribhuvan University, Nepal.
[d] International Agribusiness-Coimbatore, India.
[e] Department of Pharmacology, Dr. Shivajirao kadam College of Pharmacy, Sangli, Maharashtra, India.
[f] Bio-aesthetics Nepal Pvt Ltd, Kathmandu, Nepal.
[g] Department of Biotechnology, Director-Organic farming and Natural Product Research Centre, Kathmandu University, Kavre, Nepal.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2021/v32i1230434
Editor(s):
(1) Dr. Prem K. Ramasamy, Brandeis University, USA.
(2) Prof. Marcello Iriti, Milan State University, Italy.
Reviewers:
(1) Karen Cordovil, Brazil.
(2) Marcelo Barcellos da Rosa, Federal University of Santa Maria, Brazil.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/78527

Original Research Article

Received 06 October 2021
Accepted 13 December 2021
Published 14 December 2021

≡ Research Head;
ϖ Assistant Professor;
 # Laboratory Chief;
† Head-Horticulture;
‡ Associate Professor and Head;
¥ Director Research;
ŀ Director-Organic;
**Corresponding author: E-mail: phytochem2@gmail.com;*

ABSTRACT

Berberis aristata a Himalayan woody spiny shrub with yellow flowers and red berries commonly called as Daruharidra in Sanskrit and locally in Nepal is called as Chutro or Chitra. The root and stem are the two parts widely used in traditional medicines of India and China. Berberine is the key active ingredient present in stem and root parts. Berberine hydrochloride is the derivative of berberine. The present study aimed to study the microwave assisted extraction of berberine and its conversion into berberine hydrochloride and quantifying by RP-HPLC and HPTLC methods.In the present paper we have mentioned microwave assisted extraction of berberine and preparation of berberine hydrochloride in detail. Berberine extracted from roots of Berberis aristata using microwave assisted extraction in 80% ethanol to obtain 20% pure berberine crude by HPTLC densitometry at 350 nm absorption. The crude berberine was further purified to berberine hydrochloride by adding 10% Hcl in aqueous solution of berberine and allowed to crystallize at 5 ^{0}C over 24 hours. The crystals were further purified and recrystallized in ethanol and subjected to RP-HPLC. Reverse phase HPLC was carried out on Shimadzu UV detector at wave length of 265 nm using Acetonitrile-0.1% phosphoric acid solution (50:50) (add 0.1g sodium dodecyl sulfonate per 100ml) as the mobile phase; Phenomenex RP-column (250 mm × 4.6 mm, 5 µm), With flow rate 1.0 mL/minute, 5 µL injection volume, column temperature 25 ^{0}C for run time of 35 minutes, and retention time of berberine hydrochloride was 12.008 minutes with purity of 82% and recovery of 90% yield obtained.

Keywords: Berberis aristata; root; microwave assisted extraction; RP-HPLC; HPTLC; berberine; berberine hydrochloride.

1. INTRODUCTION

Kingdom: Plantae
Phylum: Angiosperms
Order: Ranunculales
Family: Berberidaceae
Genus: Berberis
Species: B. aristata

Berberis aristata a Himalayan woody spinous shrub with beautiful yellow flowers and dark purple berries, known for its medicinal properties used from ancient times mentioned in Ayurveda, Charaka samhita and Susruta samhita [1]. The Berberis species in Ayurveda is known as Daruharidra see Fig.1. The yellow bark and root are used to prepare concoctions to treat different diseases as per Ayurveda. Because of yellowish pigmentation of root and bark the Berberis is also called as tree turmeric. There are near about 21 species of Berberis in Nepal among which Berberis aristata, Berberis asiatica, Berberis vulgaris, Berberis lycium and Berberis nepalensis are popular species for trade [2]. Locally in Nepal Berberis species are called as Chutro, Rasanjan, Marpyashi or Chitra and in Sanskrit it is called as Daruharidra. The extracts of Berberis root and bark has antibacterial, anti-inflammatory, antidiabetic, antipyretic, diaphoretic and antiseptic properties [3]. The roots are used to treat amenorrhea. The extract is bitter, astringent and pungent taste containing alkaloids. Daruharidra alleviates kapha and pitta doshas and has hot potency. The fruit is sour and sweet with cold potency. Since ancient times the concoctions are prepared from root and bark to treat hepatic and cardiac problems. Extracts were used to treat eye diseases, jaundice and other fevers. A preparation like “Rashut” is famously used in Ayurveda for eye disorders, contains Berberis as major constituent. American Indians used Berberis aristata to treat neuralgia and menorrhagia. Recent studies have shown extracts of Berberis aristata has powerful anticancer activity and potency to treat Type 2 diabetes. In many Traditional Indian and Chinese medicine it’s considered that every part of Berberis can be used as medicine. Many Ayurvedic preparations contains Daruharidra as main ingredient used in eye care, skin diseases, jaundice and diabetes like Darvyadikvatha, Darvyadileha, Darvyaditaila, Rasanjana, Dasangalepa [4, 5].

The main constituent of Root and bark of Berberis aristata is berberine a benzyl isoquinoline alkaloid with different pharmacological activities. Berberine also present in other plants like Goldenseal, Coptis and other species of Berberis. Berberine has broad medicinal values as Anthelmintic, antiviral, antibacterial, antifungal and immunomodulatory

agent [6]. Many research articles published reported that berberine content in root part is from 1.5-4% in most of the Berberis species depending on the altitude and seasons the berberine content changes, the berberine content at low altitudes reported to be upto higher when compared to higher altitude plants. Berberine content is reported to be higher in B.asiatica 4.3%, B.lycium 4.0% and Berberis aristata 3.8% [3].

Quaternary protoberberine alkaloids (QPA) [3,7] can be extracted by several methods like Solid-Liquid extractions (SLE) by percolation and maceration, Microwave assisted extraction (MAE),Ultra high pressure extraction (UPE), supercritical fluid extractions (SFE), pressurized liquid extraction (PLE) and Ultra sound assisted extraction (UAE) [7]. The key principle behind these extraction methods is the interconversion reaction of QPA salts and the base. The berberine salts are water soluble, stable in acidic and neutral media and basic salts soluble in solvents. While extracting the QPA salts are converted into their specific bases and later extracted in different solvents. Berberine being QPA salt different classical extraction techniques like maceration, percolation, are used either hold or cold continuous system of extraction using different solvents like methanol, chloroform, and ethanol and in acidic or basic aqueous extraction. Berberine photosensitive and thermo sensitive and degrades at high temperature and exposure to light, its extraction and recovery is quiet challenging. Out of which Microwave assisted extraction of berberine proven to produce high yields [8,9].

1.1 Microwave Assisted Extraction

Microwave assisted extraction technique combines microwaves with solvent extraction. The selective heating of the solvent and compounds by microwaves in the process of extraction increases kinetics of extraction and lead to increase of yields. MAE has several advantages over traditional methods such as less tome of extraction, less solvent consumption, higher yields and low process cost. Electromagnetic light spectrum of microwaves ranges from 300 MHz to 300 GHz with wavelengths of 1cm to 1m. By application of microwaves specific material interactions that can absorb electromagnetic energy and then convert into heat. The dipole rotation of organic molecules induced by microwaves causes breaking of hydrogen bonding. This phenomenon makes the microwave assisted extraction process an efficient process of extracting phytochemicals with increased kinetics and ionization resulting in high speed extraction process with high yields. Breaking of hydrogen increases the penetration of solvents more efficiently into plant matrix [15].

Quantification of berberine is carried out after extraction and purification using chromatographic methods. As per literature available UV spectroscopic method of analysis of berberine is used to quantify with RP-HPLC and HPTLC methods. Maximum absorption of berberine can be achieved at 348 nm [3]. HPLC method of analysis is the simple, robust and widely used method for qualitative and quantitative analysis of berberine content. In this study we had used both RP-HPLC and HPTLC method of quantification of berberine hydrochloride after MAE extraction.

2. MATERIALS AND METHODS

Plant material: Dried roots of B. aristata are obtained from local market.

Berberine Hydrochloride: 97% pure berberine hydrochloride was purchased from HJHERB BIOTECHNOLOGY CO.LTD, China.

Chemicals and reagents: HPLC grade and analytical grade solvents methanol, ethanol, water Hydrochloric acid, sodium dodecyl sulfonate, n-Propanol: Formic acid are purchased from Finar chemicals distributor Kathmandu.

2.1 Method of Extraction

2.1.1 Extraction of berberine and preparation of berberine hydrochloride

100 grams of dried roots Fig..4 of B.aristata were powdered to coarse powder. Microwave assisted Soxhlet apparatus used to extract berberine using 80% ethanol as solvent at temperature 65^0C for about 8 hours, three cycles in the ratio of 1:10. The three extractions were collected and concentrated in Rotary evaporator. The crude was weighed. Crude berberine thus extracted is added to reaction flask and solubilized in methanol in 1:10 ratio, activated charcoal of 1% was added to the weight of crude and stirred at 5^0C for 30 minutes and then filtered. The methanol extract is concentrated and dissolved in water and 10% Hydrochloric acid was added slowly by 2 times of crude berberine at 5 ^{0}C by

stirring till yellow crystals of berberine hydrochloride are formed. The crystals are further washed with ethanol. The crystals are filtered and dried in oven for further analysis.

2.2 RP-HPLC Method

2.2.1 Preparation of standard and sample solutions

4.8 mg of standard stock solution of berberine hydrochloride (equivalent to 3.96 mg of berberine) was prepared in 10 ml mobile phase mentioned in HPLC method and make made up to 99 µg/ml of berberine from stock. 10 mg of berberine hydrochloride sample dissolved in 10 ml of mobile phase for sample analysis.

2.2.2 RP-HPLC Chromatographic conditions

Reverse phase HPLC assay carried out by Acetonitrile-0.1% phosphoric acid solution (50:50) (add 0.1g sodium dodecyl sulfonate per 100ml) as the mobile phase; Phenomenex RP-column (250 mm × 4.6 mm, 5 µm), the detection wavelength is 265 nm Shimadzu UV detector. With flow rate 1.0 mL/minute, 5 µL injection volume, column temperature 25 ^{0}C for run time of 35 minutes and analysis was carried out on Shimadzu UV detector, auto sampler, and degasser as per General rule 0512 of Part 4 in Chinese pharmacopoeia (2015 edition) [10,11].

2.3 Assay

Assay carried out per below standard calculation:

Assay%=

[(AT/AS)*(WS/DS)*(DT/WT)*(P/100)]*100
Where: AT: Peak Area of berberine obtained with test preparation.
AS: Peak Area of berberine obtained with standard preparation.
WS: Weight of working standard taken in mg
WT: Weight of sample taken in mg
DS: Dilution of Standard solution
DT: Dilution of sample solution
P: Percentage purity of working standard

2.4 HPTLC Method

2.4.1 Preparation of standard

240 mg of standard berberine was dissolved in 100 ml of methanol (AR grade). 500 mg of the dried methanol extracts of powdered root samples were dissolved in 5 ml of methanol and 5 µL of each sample were used.

2.4.2 HPTLC method for the estimation of Berberine

The alcoholic extract of roots of B .aristata was subjected to HPTLC analysis and a method was developed and standardized to obtain the quantitative yield of marker compound berberine in the extract. The conditions of HPTLC analysis of root methanol extract are as follows:

2.5 Procedure

A number of solvent systems were tried for methanol extract. A good separation was observed in the solvent system: n-Propanol: Formic acid: Water (9: 0.1: 0.9 v/v/v). Samples were applied on precoated silica gel 60F254 GLP (Merck) (20x10 cm). Along with this varying concentration of berberine standard from 2 µL to 8µl were also applied on TLC plates from about 1 cm edge using a band length of 8 mm. The chromatogram was developed in a twin trough chamber upto a distance of 80 mm and slit dimensions was 6.0x0.45 mm [12].

3. RESULTS

3.1 Microwave Assisted Extraction of Berberine and Preparation of Berberine Hydrochloride

Microwave assisted extraction of berberine is proven as one of the best methods to get better yields .In the present experiment of 100 gram roots of B.aristata were coarsely powdered and subjected to Microwave assisted extraction using 80% ethanol in the ratio of 1:10 for three times each cycle 2.5 hour approximately. The total liquid extracts filtered and concentrated. Total 8.0 gram of dark yellow colored crude obtained which contain 1.6 gram of berberine content by HPTLC (20% pure). Crude berberine thus extracted is added to reaction flask and solubilized in methanol in 1:10 ratio, activated charcoal of 1% was added to the weight of crude and stirred at 5^{0}C for 30 minutes and then filtered. The methanol extract is concentrated and dissolved in water and 10% Hydrochloric acid was added slowly by 2 times of crude berberine at 5 ^{0}C by stirring till yellow crystals of berberine hydrochloride are formed. The crystals are further washed with ethanol and assay done

by RP-HPLC; Berberine Hydrochloride purity achieved 82% with 90% of recovery.

3.2 RP-HPLC

The concentration of 10 mg/10 mL berberine hydrochloride sample was prepared in methanol. The sample quantification was carried out against standard 92% berberine hydrochloride using Shimadzu UV detector in mobile phase Acetonitrile-0.1% phosphoric acid solution (50:50) (add 0.1g sodium dodecyl sulfonate per 100ml) ; Phenomenex RP-column (250 mm × 4.6 mm, 5 µm)the detection wavelength is 265 nm Shimadzu UV detector. With flow rate 1.0 mL/minute, 5 µL injection volume, column temperature 25 ^{0}C for run time of 35 minutes. Berberine hydrochloride retention time of 12.008 minutes obtained repeatedly see Fig.2. The calculation was carried out as per Assay and purity of berberine hydrochloride obtained as 82% with recovery percentage of 90%.

3.3 HPTLC Quantification

Standard berberine showed single peak in HPTLC chromatogram. The calibration curve of berberine (Fig. 1) was prepared by plotting the concentration of Berberine versus average area of the peak over the ranges of 2 µL to 8 µL/spot. The correlation co-efficient was found to be linear. Amount of berberine in the sample (alcoholic extract of B .aristata) was computed from calibration curve. Satisfactory resolution was found in solvent system n-propanol: formic acid: water (9: 0.1: 0.9 v/v/v). The Rf values obtained were calculated through CAMAG HPTLC software supplied with the instrument. The Rf value of marker compound (berberine) was found to be 0.26 see Fig.3. On spectral assignment we observed that all the spectra appeared at 0.26 are of same type and it shows the uniformity of compound. Amount of Berberine content in sample is 20.14 mg /100mg of crude extracted.

Fig. 1. Berberis aristata, with red berries

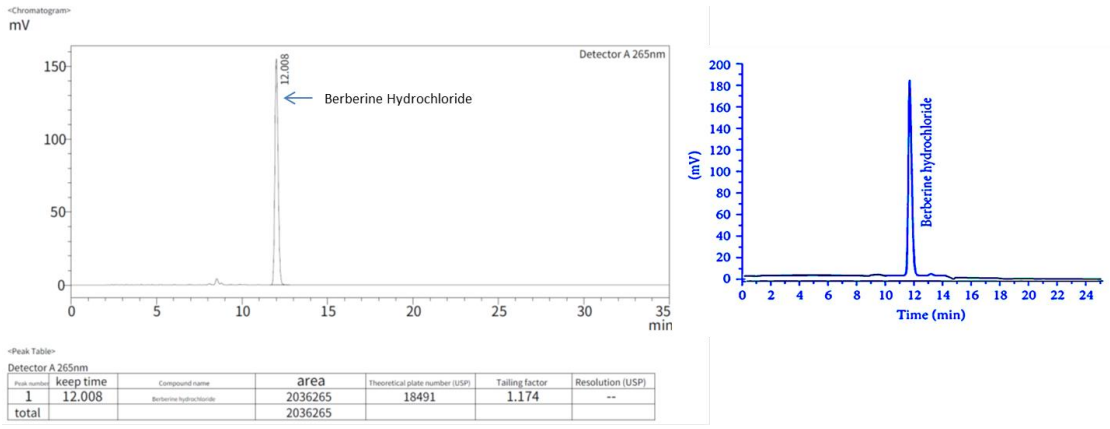

Fig.2. Left side- RP-HPLC chromatogram of sample berberine hydrochloride with retention time 12.008 minute and STD 97% chromatogram on right side with retention time of 11.98 minute

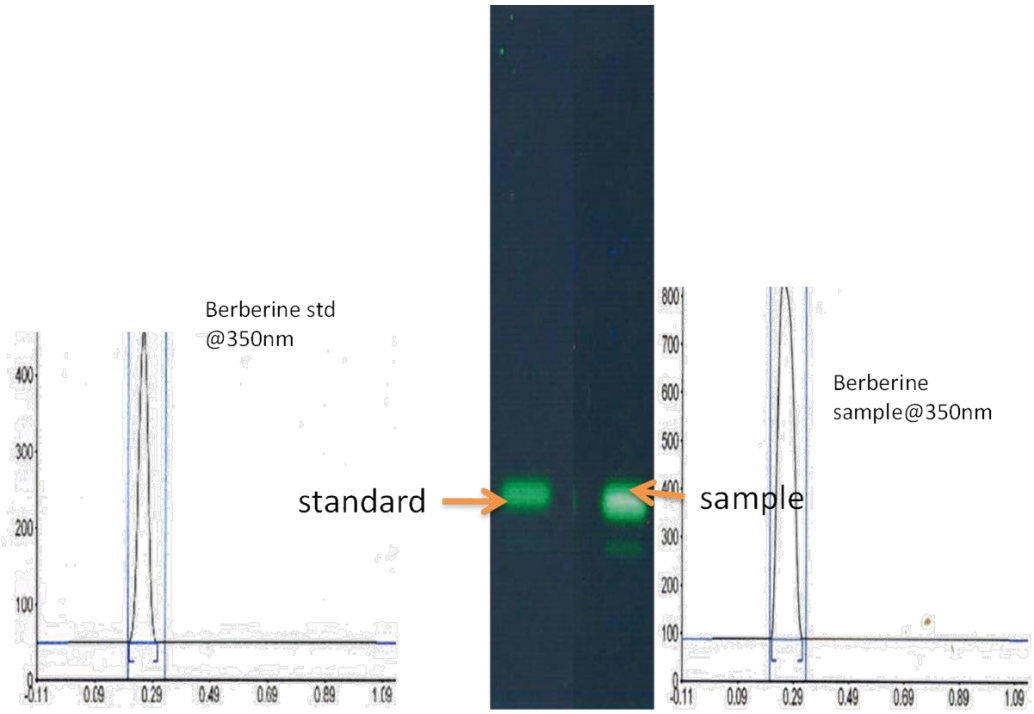

Fig.3. HPTLC quantification of berberine crude with absorption at 350 nm wavelength

Fig.4 . B.aristata root

4. DISCUSSION

Berberis aristata locally in Nepal known as Chutro or Chitra had been widely used in traditional medicines of India and China in order to treat many illnesses like diabetes, skin diseases, eye diseases, live disorders. The key compound B.aristata root or stem is Berberine which is having anticancer, anti-inflammatory, anthelminthic, anti-arthritis, antidiabetic activities. Many scientific researchers had proven berberine has COX-2 inhibitory activity [13,14]. Some recent scientific articles had shown anti-cataract activity of Berberine. Berberine had attracted many drug researchers to treat cancer. In our present research we had mentioned detailed microwave assisted extraction method berberine and preparation of berberine hydrochloride. 100 gram root powder of Berberis aristata is extracted by microwave method with 80% ethanol in 3 cycles for 8 hours total time in 1:10 ratio of meal to solvent.8.0 gram crude obtained which contain 1.6 gram of pure berberine by HPTLC (equivalent to 20%) i.e. 20.14mg/100 mg. The crude berberine was further dissolved in methanol in 1:10 ratio and activated charcoal was added and filtered. The filtrate was concentrated and dissolved in water.

To the aqueous solution 10% Hcl was added drop wise and kept at 5 ^{0}C for 24 hours for crystallization of berberine hydrochloride. The yellow needle like crystals of berberine hydrochloride were further washed with ice cold ethanol and dried in oven. The RP-HPLC assay (as per Chinese Pharmacopoeia) was conducted on Shimadzu UV detector in mobile phase Acetonitrile-0.1% phosphoric acid solution (50:50) (add 0.1g sodium dodecyl sulfonate per 100ml) ; Phenomenex RP-column (250 mm × 4.6 mm, 5 µm)the detection wavelength is 265 nm Shimadzu UV detector. With flow rate 1.0 mL/minute, 5 µL injection volume, column temperature 25 ^{0}C for run time of 35 minutes. Berberine hydrochloride retention time of 12.008 minutes obtained repeatedly. The calculation was carried out as per Assay and purity of berberine hydrochloride obtained as 82% with recovery percentage of 90%.

5. CONCLUSION

Hydrogen bond destruction by microwaves enhances the penetration of solvents into plant matrix leading to high extractive values of phytochemicals. Electromagnetic waves thoroughly increases dielectric heating and coalescence of cell matrix and facilitate the solubility of phytochemicals into the solvents leading faster extraction of Berberine. The process of extraction was optimized at 20% power level giving 90% Berberine extraction with 20% purity by HPTLC and Crude Berberine thus extracted is added to reaction flask and solubilized in methanol in 1:10 ratio, activated charcoal of 1% was added to the weight of crude and stirred at 5^{0}C for 30 minutes and then filtered. The methanol extract is concentrated and dissolved in water and 10% Hydrochloric acid was added slowly by 2 times of crude Berberine at 5 ^{0}C by stirring till yellow crystals of Berberine hydrochloride are formed. The crystals are further washed with ethanol and assay done by RP-HPLC; Berberine Hydrochloride purity achieved 82% with 90% of recovery. Thus microwave assisted extraction of Berberine is fast and effective method. Thus further comparative studies are recommended with different methods of extraction.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rathi B, Sahu J, Koul S, Kosha RL. Detailed pharmacognostical studies on Berberis aristata DC plant. Anc Sci Life. 2013Apr;32(4):234-40.
DOI: 10.4103/0257-7941.131981. PMID: 24991073; PMCID: PMC4078475.
2. Adhikari, Bhaskar, Pendry, Colin, Pennington R, Milne, Richard. A revision of berberis S.S. (Berberidaceae) in Nepal. Edinburgh Journal of Botany. 2012;69.
DOI:10.1017/S0960428612000261.
3. Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Cris¸ an G and Buzoianu AD. Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders. Front. Pharmacol. 2018;9:557.
DOI: 10.3389/fphar.2018.00557
4. Shailja Choudhary, Hemlata Kaurav, Madhusudan S, Gitika Chaudhary. Daruharidra (Berberis aristata): Review based upon its Ayurvedic Properties. International Journal for Research in Applied Sciences and Biotechnology. 2021;8(2):98-106.
Available:
https://doi.org/10.31033/ijrasb.8.2.12
5. Aswal J, Dobhal, Rajendra, Uniyal, Devi Prasad, Chander, Vikas & Nautiyal, Vikas. A review on Pharmacological potential of Berberine; an active component of Himalayan Berberis aristata. The Journal of Phytopharmacology. 2017;6:53-58.
DOI:10.31254/phyto.2017.6108.

6. Salehi B, Selamoglu Z, Sener B, Kilic M, Kumar Jugran A, de Tommasi N, Sinisgalli C, Milella L, Rajkovic J, Flaviana B, Morais-Braga M, F. Bezerra C.; Rocha EJ, Coutinho DM, Oluwaseun H, Ademiluyi A, Khan Shinwari Z, Ahmad Jan S, Erol E, Ali Z, Adrian Ostrander E, Sharifi-Rad J, de la Luz Cádiz-Gurrea M, Taheri Y, Martorell M, Segura-Carretero A, Cho C, Berberis Plants W. Drifting from Farm to Food Applications, Phytotherapy, and Phytopharmacology. Foods. 2019;8: 522.
Available:https://doi.org/10.3390/foods8100522
7. Grycova L, Dostal J, Marek R. Quaternary protoberberine alkaloids. Phytochemistry. 2007 Jan;68(2):150-75.
DOI: 10.1016/j.phytochem.2006.10.004. Epub 2006 Nov 15. PMID: 17109902.
8. Belwal T, Pandey A, Bhatt ID. et al. Optimized microwave assisted extraction (MAE) of alkaloids and polyphenols from Berberis roots using multiple-component analysis. Sci Rep. 2020;10:917. Avaialable: https://doi.org/10.1038/s41598-020-57585-8
9. Manikyam, Hemanth Kumar & Ramesh C. & Poluri, Krishna Mohan & Assad, Assif. Microwave assisted Subcritical water extraction of Berberine hydrochloride from the roots of Berberis Aristata using Harmony search algorithm. Journal of Herbal Medicine Research. 2017;1-9.
DOI:10.28933/jhmr-2017-11-0101.
10. Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction of curcumin by sample-solvent dual heating mechanism using Taguchi L9 orthogonal design. J Pharm Biomed Anal. 2008 Jan 22;46(2):322-7.
DOI: 10.1016/j.jpba.2007.10.020. PMID: 18309573.
11. Compendium of HPLC application for Traditional Chinese Medicine and Chemical drugs in China Pharmacopoeia
12. Chinese Pharmacopoeia | USP; 2015.
13. Sehrawat, Rashmi & Pant, Jagdish & Rajasekaran, Arunachalam. HPTLC Fingerprinting Profile of Marker Compound (Berberine) in Roots of Berberis aristata DC. Pharmacognosy Journal. 2011;3: 41–44.
DOI:10.5530/pj.2011.19.8.
14. Fukuda K, Hibiya Y, Mutoh M, Koshiji M, Akao S, Fujiwara H. Inhibition by berberine of cyclooxygenase-2 transcriptional activity in human colon cancer cells. J Ethnopharmacol. 1999 Aug;66(2):227-33.
DOI:10.1016/s0378-8741(98)00162-7.
PMID: 10433483.
15. Manikyam HK, Ramesh C, Yadav P and Kenttar A: Berberine chloride induced apoptosis in HepG2 cancer cell line via PPAR gamma activation and COX2 inhibition by regulating prostaglandin E2 (PGE2) signalling pathway. Int J Pharm Sci & Res. 2018; 9(11):4723-28.
DOI:10.13040/IJPSR.0975-8232.9(11).4723-28.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/78527