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# Microwave assisted Subcritical water extraction of Berberine hydrochloride from the roots of *Berberis Aristata* using Harmony search algorithm

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**ABSTRACT**

The aim of present study is to optimize the conditions of Berberine extraction from roots of *Berberis Aristata* by Microwave assisted subcritical water extraction (MASCW).MASCW method of extraction had shown reproducibility in respect to time, solvent usage, yield and extraction repeatability. Isoquinone Berberine, is one of the wide spread representatives belonging to family of protoberberine alkaloids abundantly present in roots and stem parts of Indian barberry. The subcritical factors like time, solvent/meal ratio, extraction repeatability, size of particles and temperature were investigated and were in accordance with experimental data and predicted data. Harmony search Algorithm (HSA) was used to study the effect of five subcritical parameters on the yield of Berberine .The extraction of berberine from roots of *Berberis aristata* was carried at 110-1700C using MASCW method using different variations of five subcritical parameters. The results show that all five factors (Time, Temperature, size of particles, repeatability of extraction and sample/liquid Ratio) have statistically significant effects on the berberine concentration. The results had suggested that experimental data concentration, 223.82 µg/ml and predicted data concentration, 214.854 µg/ml of berberine are in significant correlation at subcritical parameters like temperature 1700C, particle size of 0.65 mm, time of 70 mins, solvent/meal ratio of 12 and at maximum three repetitions. Thus the extracted berberine was precipitated as berberine hydrochloride by acid and further evaluated for NMR structural studies which confirms with standard berberine hydrochloride.

**Keywords:** berberine hydrochloride, microwave assisted subcritical water extraction, Harmony search algorithm, phytochemistry, herbal remedies

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## INTRODUCTION

### BERBERINE

Among the isoquinoline alkaloids, berberine, a natural plant alkaloid is the target of increased chemical and biological interest, because it has been found to possess potent anticancer activity[1, 2] in addition to exhibiting antimalarial properties[2]. Berberine is found in herbs such as goldenseal, *Berberis Aristata* (Indian Bayberry), Oregon grape, barberry, and goldthread (Coptis chinensis), aimed at curbing bacterial, viral, fungal, yeast, and parasitic infections [2,3]

Berberis species were widely used in both Ayurveda and Traditional Chinese medicine as a preventive therapy as well as to treat diabetes, cardiovascular diseases, weight management, cancer, dementia and ageing[1, 2]. Berberine is one of the best studied plant alkaloid against Type 2 diabetes and metabolic syndrome.

*Berberis aristata* commonly known as "Daru haldi and chitra" is spinous shrub native to northern Himalaya region. The plant is widely distributed from Himalayas to Srilanka, Bhutan, and hilly areas of Nepal in Himalaya region [1, 2] It is found in Himachal Pradesh. It grows at the height of 2000-3000m especially in Kumaon and Chammba region of Himachal Pradesh. It is also found in Nilgris hills in South India [2, 3, 11], *Berberis aristata* is used in ayurvedic medicines from very long time. The plant is used traditionally in inflammation, wound healing, skin disease, menorrhagia, diarrhoea, jaundice and affection of eyes. A very valuable ayurvedic preparation 'Rashut' is prepared by this plant. The major alkaloid found in *B. aristata* is Berberine having yield of 2.23% followed by palamatine [11]. Variation of Berberine content in root and stem of B. aristata with altitude was determined. It was found that plants growing at lower altitude have more Berberine content.

### MICROWAVE ASSISTED SUBCRITICAL WATER EXTRACTION

Various extraction techniques have been applied to extract lactone alkaloids, including conventional solvent extraction[7,] Soxhlet extraction[4], high speed ultrasonic extraction[14], novel accelerated solvent extraction[13], supercritical fluid extraction[6], and pressurized liquid extraction[9]. However, all of these techniques produce only low alkaloid yields, and some techniques required high pressure, substantial energy consumption or expensive devices. Microwave-assisted subcritical water extraction (MASCW) is a fast extraction approach based on microwave irradiation, which digests the outer walls of plant tissues, leading to a quick release of effective compounds into the extraction media. Compared with other extraction methods, MASCW is faster, more convenient, safer, and more economically viable. Further, it is labor-efficient and no solvent required.

Thus, the present study aimed at employing a fast microwave-assisted subcritical water method for the extraction of lactone alkaloids like berberine from Berberis Aristata. It also investigated the effects of process variables, including microwave power, temperature, time, and solvent meal ratio, on berberine extraction. Extraction process was simulated using a Harmony search algorithm with five variables, and the best combination of process variables was determined.

### Harmony search algorithm HARMONY SEARCH ALGORITHM

Harmony Search (HS) is a musicians behaviour inspired evolutionary algorithm developed in 2001 by Geem et al.[5], though it is a relatively new meta heuristic algorithm, its effectiveness and advantages have been demonstrated in various applications like traffic routing, multi objective optimization, design of municipal water distribution networks, load dispatch problem in electrical engineering, rostering problems[12], clustering, structural design, classification and feature selection to name a few[10].

## MATERIALS AND METHODS

### PREPARATION OF PLANT MATERIAL:

The stems and roots of Berberis Aristata were collected from Kathmandu Nepal at the end of the harvest season, subsequently dried in oven at 30°C overnight and ground into fine powder using different mesh sizes.

## MICROWAVE ASSISTED SUBCRITICAL WATER EXTRACTION INSTRUMENTATION:

Microwave assisted Subcritical water extraction processes were performed using Microdigest ETHOS EX with a focused irradiation process under atmospheric pressure conditions was used for the MAE. The emission frequency of the extractor was 2,450 MHz and deliver upto 1200 watts power and the microwave power was linear and adjustable between 60 W and 300 W with 30 W intervals. The extractor was equipped with one vessel that could access a 270 mL quartz tube and a cool water circulation system using a graham-type refrigerant column (400 mm length). Precisely, a 2.0 g sample was placed into the quartz tube and blended with 50 mL of the extraction solvent. Upon completion of the extraction, the extract tube was removed from the vessel and filtered using No. 1 filter paper under vacuum. The filter was transferred into a volumetric flask and the final volume was diluted up to 100 mL for quantitative analysis. The temperature values were measured by a digital thermometer. Pressure was controlled by pressure gauge manometer (with an accuracy of 2 bars).Thermal supply was an electrical element fed with 220V alternative current (AC). The glycerol was an intermediate to transfer stable heat.

## METHOD OF EXTRACTION

Dried roots were grinded to proper mesh size as mentioned in Table 1. The grinded Berberis Aristata roots are placed in extraction vessels of microwave extraction unit with 2gm each sample and subcritical conditions like mesh size, time, temperature, pressure and solvent meal ratio were maintained as mentioned in Table 1. Extraction is carried out at optimized subcritical conditions using water as solvent. The process is repeated with same sample number of times as mentioned in Table 1. The extracted solution is collected and concentrated with 80% of water v/w. The concentrated solution is then acidified with con.HCL adjusting ph to 5.8 and the solution is left overnight to precipitate the berberine as berberine hydrochloride. The precipitated berberine hydrochloride was then dried and subjected to further studies.

## NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPIC ANALYSIS

Nuclear magnetic resonance (NMR) studies were conducted to investigate the electronic interactions between berberine hydrochloride. The pure samples along with extracted samples were prepared by mixing (25 mM each, previously dissolved in deuterated dimethyl sulfoxide; DMSO-d6). Samples of 600 µL each were taken in 3-mm NMR glass tubes, and their proton NMR (1 H NMR) spectra in DMSO-d6 were recorded at 21°C on a 400-MHz Bruker AV spectrometer (Bruker Ultra shield 400 PLUS, Germany) equipped with a 3-mm nalorac dual proton-optimized probe. The obtained spectra were analyzed for proton chemical shifts utilizing Bruker TOPSPIN 2.0 software. The difference in the chemical shifts of protons in berberine hydrochloride. The chemical shifts were reported in parts per million (ppm) and are referenced by the residual solvent peak at 2.503 ppm

## DESIGN OF EXPERIMENT

The objective of DOE is the selection of the points where the response should be evaluated. The choice of the design of experiments can have a large influence on the accuracy of the approximation). In this study, a five factors central composite design (CCD) was employed for MASCW modelling and optimization.

In this study the effects of five independent variables X1 (solvent/meal ratio), X2 (number of extractions), X3 (temperature), X4 (particle size) and X5 (time) on berberine extraction is studied. Table 1 shows the independent variables and their levels used for central composite design. Thirty two combinations of the independent variables along with their experimental and predicted yield are shown in Table 2

Data pertaining to five independent, and one response, variable were analyzed to get a quadratic regression equation of the form of Equation 1 and the regression equation is given as Equation 2.

Where Y is the berberine hydrochloride yield (µg/ml), b0 is the value for the fixed response at the central point of the experiment.bn, bm, bnm are the linear, quadratic and cross product coefficients, respectively.

**EQUATION 1**

$$Y = b_0 + \sum_{n=1}^{5} b_n X_n + \sum_{n=1}^{5} b_{mn} X_n^2 + \sum_{n<m}^{5} b_{mn} X_n X_m$$

**EQUATION 2**

$$Y = 449.862 - 1.081 * Time - 5.007 * Temperature - 20609.0 * Ratio + 0.001 * Time * Time + 0.016 * Temperature * Temperature + 109134 * Ratio * Ratio + 0.001 * Time * Temperature + 51.01 * Time * Ratio + 127.38 * Temperature * Ratio$$

The predicted values of berberine hydrochloride content were calculated using the regression model and compared with experimental values the value for the coefficient of determination (R2) was 0.99 which indicates the adequacy of the applied model. The statistical analysis of data revealed that linear, quadratic and interaction coefficients were significant. In this study we used music inspired Harmony search metaheuristic algorithm to optimize (maximize) the regression equation (Equation 2), depicting the relationship between the independent variables and the berberine hydrochloride yield. The optimal berberine hydrochloride yield predicted is 214.854 µg/ml at parameter setting 16:1 v/w solvent/meal ratio, five extractions, 170 $^{0}$C temperature, 0.65 mm particle size and 70 min extraction time. In order to verify the claim experiential yield of berberine hydrochloride obtained at this parameter setting was 223.82 µg/ml.

**RESULTS**

Table 2 shows the experimental data of MASCW berberine extraction in different Subcritical conditions. The second order regression equation was developed which is an empirical relationship between the concentration values (Y) of the extracted berberine and the test variables in uncoded unit. All five factors (Time, Temperature, particle size, number extractions and sample/liquid Ratio) have statistically significant effects on the berberine concentration. Optimal conditions were investigated by using different operational methods. The table 2 indicate that increasing of temperature (110 $^{o}$C to 170 $^{o}$C), increases the berberine concentration. Increasing of ratio (sample/solvent) has also an increasing effect at higher temperatures. The results had suggested that experimental data concentration, 223.82 µg/ml and predicted data concentration, 214.854 µg/ml of berberine are in significant correlation The maximum

**TABLE 1** Independent variables and their levels used for central composite design.

| Independent variables | Symbol | Coded variable levels | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | -3 | -2 | -1 | 0 | 1 | 2 | 3 |
| Solvent/meal ratio (v/w) | $X_1$ | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| Number of extractions | $X_2$ | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Temperature ( C) | $X_3$ | 110 | 120 | 130 | 140 | 150 | 160 | 170 |
| Particle Size (mm) | $X_4$ | 0.05 | 0.15 | 0.25 | 0.35 | 0.45 | 0.55 | 0.65 |
| Time (min) | $X_5$ | 5 | 15 | 25 | 35 | 45 | 55 | 65 |

**TABLE 2** Central composite arrangement for independent variables X1 (solvent/meal ratio, v/w), X2 (number of extractions), X3 (temperature, C), X4 (particle size, mm) and X5 (time, min) and their response berberine concentration µg/ml

| Runs | Temperature | Time (min) | Concentration Experimental data (µg/ml | Concentration Predicted data (µg/ml | Variables levels (encoded) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 170 | 60 | 269.59 | 255.86 | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ |
| 2 | 140 | 40 | 226.54 | 219.52 | 1 | 1 | -1 | -1 | 1 |
| 3 | 170 | 20 | 190.99 | 179.86 | 1 | 1 | 1 | -1 | -1 |
| 4 | 170 | 40 | 246.94 | 238.32 | -1 | 1 | -1 | 1 | 1 |
| 5 | 140 | 40 | 230.19 | 225.75 | 1 | -1 | 1 | 1 | -1 |
| 6 | 120 | 20 | 175.24 | 169.45 | -2 | 0 | 0 | 0 | 0 |
| 7 | 170 | 20 | 248.97 | 239.43 | 0 | 2 | 0 | 0 | 0 |
| 8 | 140 | 40 | 219.85 | 213.68 | -1 | -1 | -1 | -1 | 1 |
| 9 | 120 | 60 | 218.64 | 209.63 | -1 | -1 | 1 | -1 | -1 |
| 10 | 120 | 40 | 189.32 | 175.45 | 1 | -1 | 1 | -1 | 1 |
| 11 | 140 | 40 | 228.65 | 216.46 | -1 | -1 | -1 | 1 | -1 |
| 12 | 140 | 40 | 219.82 | 211.32 | 1 | -1 | -1 | 1 | 1 |
| 13 | 140 | 60 | 236.79 | 229.91 | -1 | 1 | 1 | 1 | -1 |
| 14 | 120 | 60 | 201.32 | 198.32 | 1 | 1 | 1 | 1 | 1 |
| 15 | 140 | 40 | 240.16 | 229.57 | 0 | 0 | 0 | 0 | 0 |
| 16 | 140 | 40 | 235.6 | 225.91 | -1 | 1 | -1 | -1 | -1 |
| 17 | 170 | 60 | 271.48 | 254.65 | 1 | 1 | -1 | 1 | -1 |
| 18 | 140 | 20 | 199.32 | 179.32 | -1 | -1 | 1 | 1 | 1 |
| 19 | 120 | 20 | 180.2 | 175.63 | -1 | 1 | 1 | -1 | 1 |
| 20 | 140 | 40 | 246.79 | 249.04 | 0 | 0 | -2 | 0 | 0 |

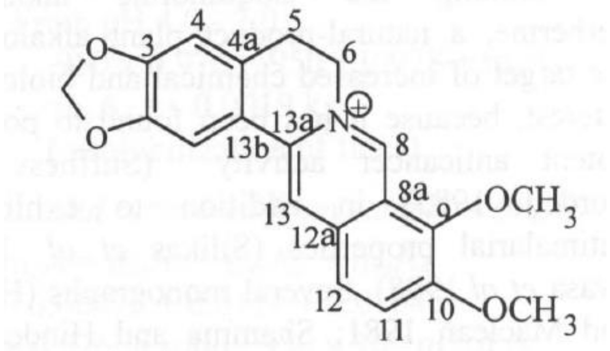


**FIGURE 1** structure of berberine

**Table 3** proton NMR shifts of standard berberine hydrochloride

| S.No | PPM | Structure | |
|---|---|---|---|
| 1 | 9.906 | H | A |
| 2 | 8.959 | H | B |
| 3 | 8.210 | H | C |
| 4 | 8.017 | H | D |
| 5 | 7.806 | H | E |
| 6 | 7.096 | H | F |
| 7 | 6.179 | C-H2 | G |
| 8 | 4.953 | C-H2 | J |
| 9 | 4.095 | C-H3 | K |
| 10 | 4.073 | C-H3 | L |
| 11 | 3.354 | C-H2 | M |

**Table 4** proton NMR shifts of MASCW extracted berberine hydrochloride

| S.No | PPM | Structure | |
|---|---|---|---|
| 1 | 9.912 | H | A |
| 2 | 8.968 | H | B |
| 3 | 8.210 | H | C |
| 4 | 8.020 | H | D |
| 5 | 7.804 | H | E |
| 6 | 7.094 | H | F |
| 7 | 6.178 | C-H2 | G |
| 8 | 4.944 | C-H2 | J |
| 9 | 4.096 | C-H3 | K |
| 10 | 4.072 | C-H3 | L |
| 11 | 3.357 | C-H2 | M |

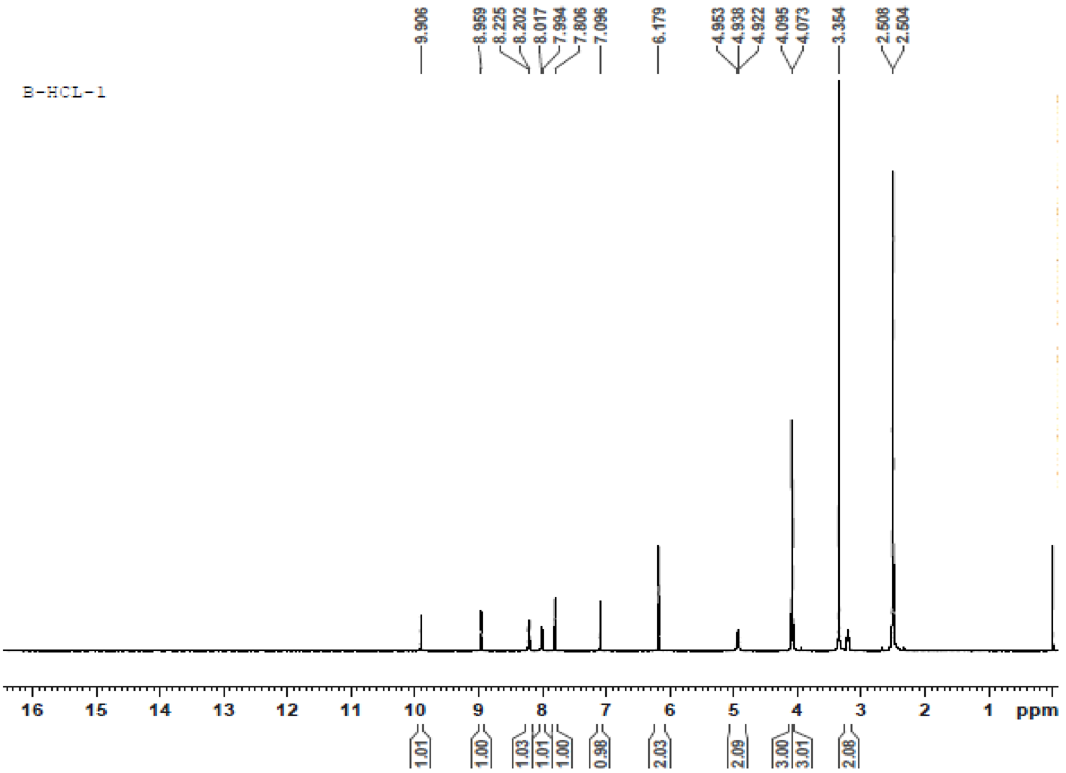


**FIGURE 2** HNMR of berberine hydrochloride standard

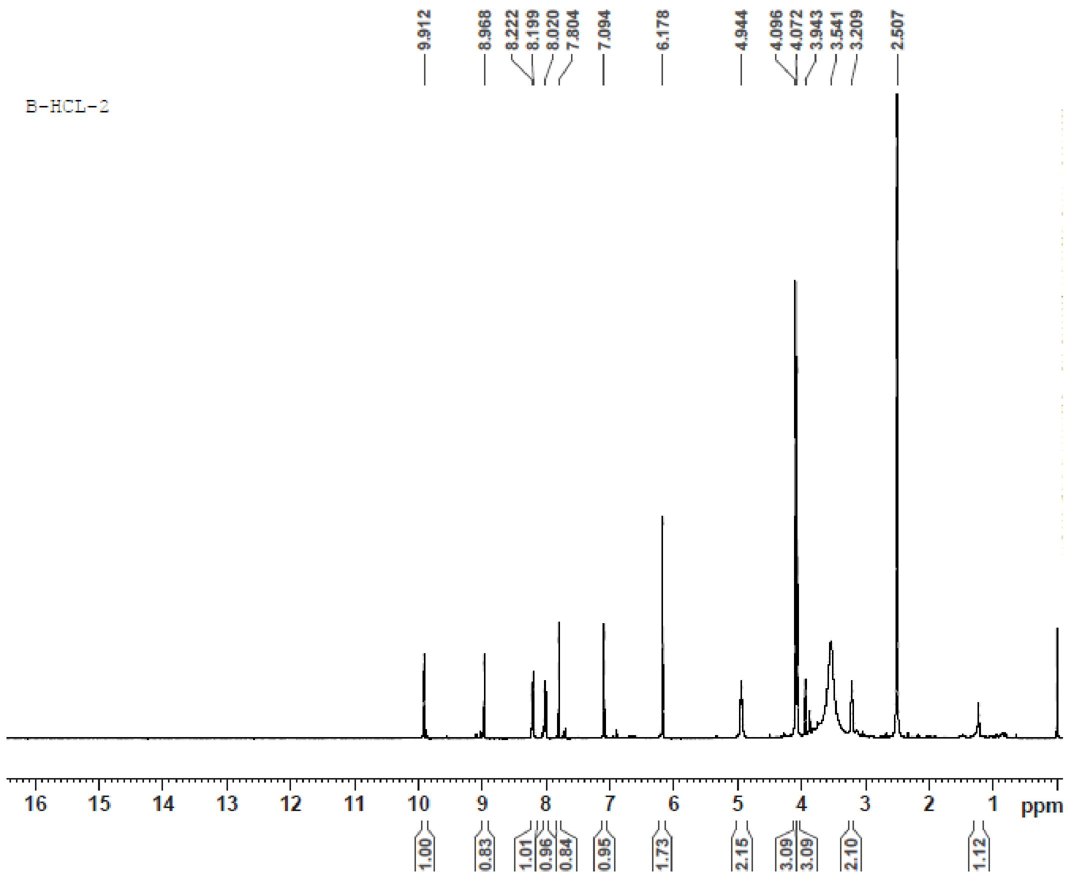


**FIGURE 3** HNMR of extracted berberine hydrochloride

concentrations of berberine was obtained more than 250 µg/ml at temperature 170$^0$C, by time interval of 70 mins as evidenced from table 2.

## NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPIC ANALYSIS

The proton signals for berberine hydrochloride (Fig. 1) were assigned based on a report published by Tripathi et al. Figure 2 demonstrates the 1HNMR spectra of standard berberine hydrochloride and figure 3 demonstrates the 1HNMR spectra of extracted berberine hydrochloride. Table 3 and 4 gives proton shifts of standard berberine hydrochloride and extracted berberine hydrochloride by MASCW method

## DISCUSSION

The response table 2 indicate that the concentration of the berberine increases with time. Temperature had direct effect on the yield of berberine[15]. Parameters like Sample/ liquid ratio decreased the yield by increasing the subcritical parameter[16]. Solubility and diffusibility of berberine increases by increase of temperature. Proton NMR studies play an important role in displaying the chemical shift of the protons in structure prediction[8]. The results obtained from the NMR spectroscopic study shows the purity of structure of berberine hydrochloride extracted by MASCW method matched with standard berberine hydrochloride.

## CONCLUSION

In this work, we examined the feasibility of MASCW extraction as a potential method for extraction of berberine from barberry root which represent a low-cost solvent free source of extracting bio-active compounds[4]. Extraction of berberine from Berberis Aristata root was studied using MASCW at various temperatures, times and sample/solvent ratios through Harmony search algorithm[10] and response surface methods.

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