ASIAN JOURNAL OF PHARMACY & LIFE SCIENCE

MICROWAVE-ASSISTED EXTRACTION OF FLAVONOIDS FROM *ZANTHOXYLUM BUDRUNGA* W. OPTIMIZATION OF EXTRACTION PROCESS

UPENDRA BHADORIYA*[1], SWETA TIWARI[2], MAHENDRA MOURYA[3], SANTOSH GHULE[1].

1. College of Pharmacy, IPS Academy, Rajendra Nagar, Indore, India.
2. Amruta Herbals Pvt. Ltd., Sanwer Road Indore, India.
3. Alpa Lab. Ltd., Pithampur, Indore, India.

Abstract:

Microwave-assisted extraction (MAE) technique was developed & optimized for the extraction of different phytoconstituents i.e; flavonoids from Zanthoxylum budrunga W. (ZBW). Several influential parameters of the MAE procedure (solvent concentration, solvent volume, microwave power and extraction time) were studied through single factor experiments and orthogonal experiment for the optimization of the extraction protocol. The optimal conditions of MAE were; ethanol concentration 65%, solvent volume 25 ml microwave power 250W and extraction time 6.0 min, while extraction yield of ZBW was 8.32 mg/g. Process optimized by altering various parameters & % of flavonoids obtained was calculated. The developed MAE method provided a good alternative for the extraction of flavonoids & other constituents from Zanthoxylum budrunga.

Key words: Microwave-assisted extraction (MAE), flavonoids, Zanthoxylum budrunga.

Corresponding Author's E-mail: - ***bhadoriyaupendra@yahoo.co.in***

Received: 10/02/11 Accepted: 03/03/11

Introduction:

Zanthoxylum budrunga, is an aromatic plant from rutaceae family commonly known as budrung, found very useful in many diseases. The constituent's of bark, leaves, fruits and seeds having medicinal importance, since study on different part reveal's its medicinal value. Morphologically *Zanthoxylum budrunga* (ZBW) is a small or moderate sized tree, pericarp of unripe fruits is pleasantly aromatic & taste like the rind of a fresh orange. The fruit are hot and bitter, used as digestive appetizer, also used in asthma, acute pain, heart diseases, dyspepsia & in diarrhoea. The distribution of plant mainly in Konkan, Deccan Mysore, Malabar, Annamalais and Orissa, also found in Assam and Meghalaya[1,2]. Study on different parts reveals presence of flavones i.e. 5-methoxy- 7-hydroxy flavol, essential oils, resins, alkaloids, lignans & many others important phytoconstituents. Presences of these components have been proved its medicinal value in many diseases[3-5].

Conventional techniques of extractions like; heating, boiling or refluxing can be used to extract phytoconstituents i.e; flavonoids, however, the disadvantages are the loss of flavonoids due to ionization, hydrolysis and oxidation during extraction[6,7]. Other techniques which include supercritical carbondioxide extraction, subcritical water extraction, ultrasonic assisted extraction (UAE) and microwaveassisted extraction (MAE) have also become of interest as alternatives for the conventional methods. Among these, MAE is the simplest and the most economical technique for extraction of many plant derived compounds[8,9]. The mechanism involve in microwave heating generally the dipole rotation of the solvent in the microwave field which causes the solvent temperature to rise and increases the solubility of the compound of interest. In microwave heating molecules of the polar solvent could not align themselves quickly enough to the high frequency electric field of microwave; this causes the solvent molecules to dissipate the absorbed energy in the form of heat[8]. Nevertheless, no reports on MAE of flavones from *Zanthoxylum budrunga* W. (ZBW) have been published. In the present study, MAE for the flavones from *Zanthoxylum budrunga* W. was investigated and the operational parameters were optimized. The objective of the work was to establish the optimised condition of MAE for *Zanthoxylum budrunga* for the development and application of the medicinal plant resource.

Materials and methods:

Plant material

Collection of fruits of *Zanthoxylum budrunga* W. was done from the Mulsi village, Pune, India, in the month of Jan-Feb, 2008. Taxonomic identification of the plant was done by the University of Pune (Department of Botany), Ganeshkhind, Pune. Samples were pulverized in a knife mill and were passed through a 20-mesh sieve. Microwaves-assisted extraction (MAE) was performed with a MSP-100E multimode microwave extraction system. In all of the experiments, the pressure was set under 300 kPa to prevent the dissolution of the target compound[10,11]. A 15 ml ethanol solution was added to 0.5 g of dried sample powder placed in an inner vessel. The extraction was carried out with different extraction conditions. The extract was filtered and the filtrate was collected and freeze-dried for determination of flavonoids.

Determination of flavonoids:

The UCARY-100 spectrophotometer (Varian) was used to determine the content of flavonoids in the above isolated product at 510 nm[12,13]. The flavonoid content was calculated using the following linear equation based on the calibration curve prepared by rutin, range from 8.0 to 40 µg ml^{-1}.

$A = 11.237$, $C = 0.0141$, $R^2 = 0.9997$

Results and discussion:

The factors concerning MAE include ethanol concentration, solvent volume, microwave power and extraction time. The influence of each factor was studied. The mixtures of ethanol–water were tested under the same conditions. The results were summarized in Figure 1. It can be observed that the extraction yield of ZBW was greatly influenced by the ethanol concentration. The extraction yield of ZBW increased sharply with the increase of ethanol concentration up to 65 %. When ethanol concentration increased from 65 to 85 %, however, extraction yield slightly decreased. It was in accordance with existed studies[14,15]. From these results, it is clear that the addition of some amount of water enhances the extraction efficiency might be due to the increase in swelling of plant material by water, which increased the contact surface area between the plant matrix and the solvent, therefore, further studies were conducted with 65 % ethanol.

Solvent volume also affect extraction yield, in MAE higher solvent volume may give lower recoveries[16,17]. To investigate the influence of solvent volume on extraction yield of ZBW, experiments were performed by increasing the solvent volume from 5 to 50 ml under the experimental conditions described above. It is seen in Figure 2. that the extraction yield of ZBW increased with the increase of solvent volume and reached its maximum at 25 ml/g. It decreased as solvent volume was above 25 ml/g. This was probably due to the larger volume of 65 % ethanol causing excessive swelling of the material by water and absorbing the effective constituent of plant material. Therefore, a volume of 25 ml was enough for extraction.

Microwave power also greatly influenced extraction yield. In order to evaluate the effect of microwave power MAE, the different microwave powers were controlled for 6 min, e.g., 80, 165, 250, 335 and 420 W. The results are shown in Figure 3. The experimental results demonstrate that the extraction yield of ZBW increase with the enhancement of the microwave forward power when the processing forward power is in the range of 80 – 250 W, and then the extraction yield of ZBW are not significantly changed from 250 to 420 W. More electromagnetic energy was transferred to the extraction system quickly and improved the extraction efficiency when the microwave power increased from 80 to 250 W. Therefore, it was decided to use 250 W microwave powers in the following experiments.

Where; A is the absorbance, C is the flavonoid content in $\mu g\ ml^{-1}$.

Extraction time also affect extraction yield in MAE. Studies were carried out at different times, e.g., 3, 4, 5, 6, 7 and 8 min. The results are shown in Figure 4.

The experimental results demonstrates that the extraction yield of ZBW with the increase of the extraction time from 3 to 8 min, do not change from 6 to 8 min. The significance of the extraction time can be presumed to be related to the time required for the desorption process to take place[18] Therefore, 6 min was considered as the appropriate extraction time.

Optimization of MAE conditions of ZBW:

Since various parameters potentially affect the MAE process. In this work, four parameters were evaluated: ethanol concentration, solvent volume, microwave power and extraction time. Microwave power was found to be the most important determinant of extraction yield of ZBW. The best combination shown was $A_2B_3C_2D_3$ which is in specific, ethanol concentration was 65%, solvent volume was 25 ml, microwave power was 250 W.

and extraction time was 6 min, these were all the optimal extraction condition, while extraction yield of ZBW was 8.32 mg/g.

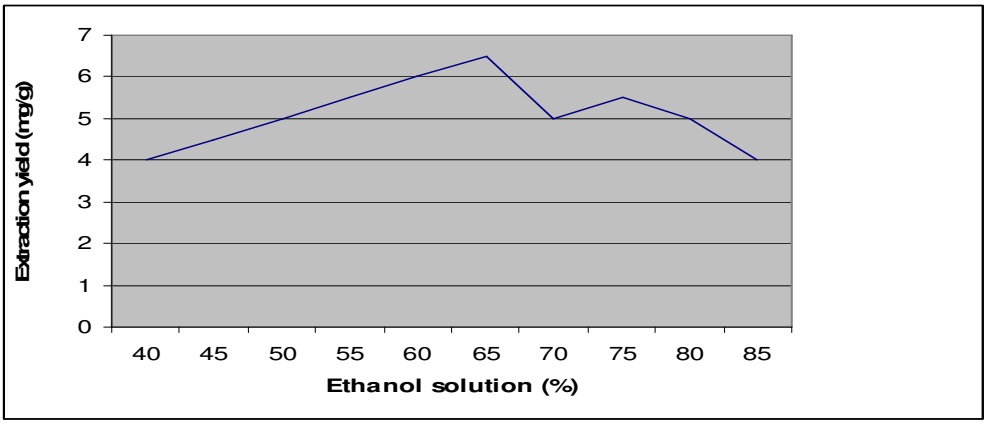

Figure 1. Effect of ethanol concentration on the extraction yield of ZBW. (Ethanol concentration Vs results)

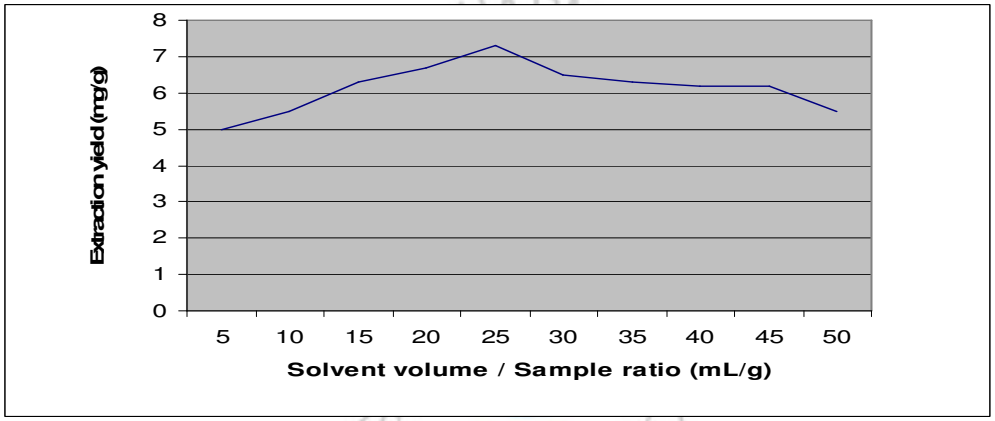

Figure 2. Effect of solvent volume on the extraction yield of ZBW. (Solvent volume Vs results)

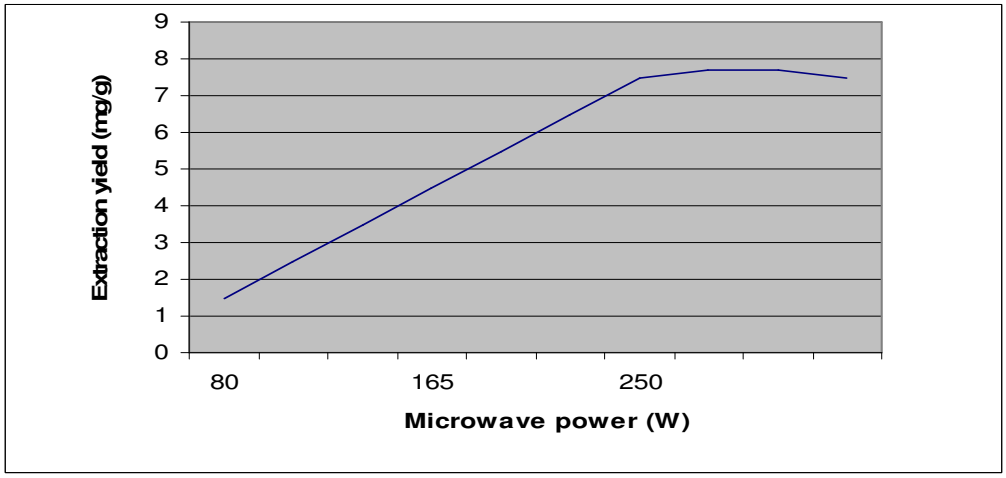

Figure 3. Effect of microwave power on the extraction yield of ZBW. (Microwave power Vs results)

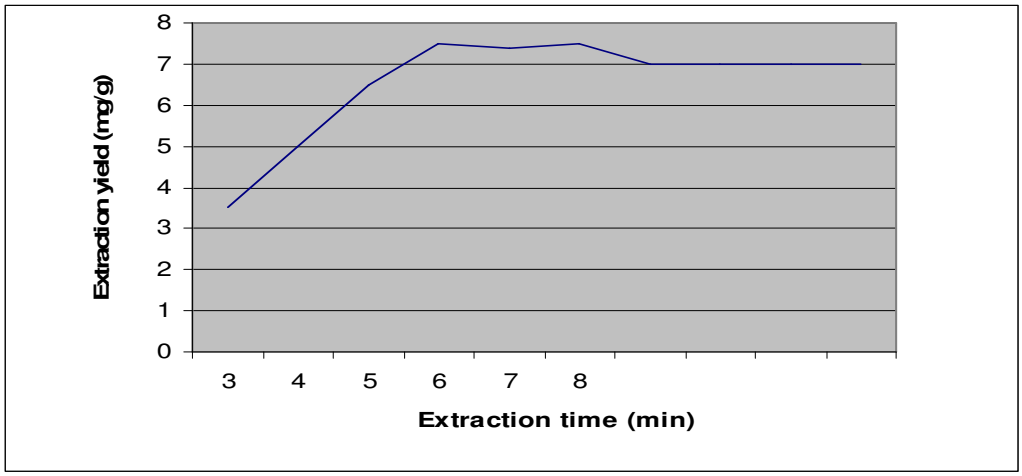

Figure 4. Effect of extraction time on the extraction yield of ZBW. (Extraction time Vs results)

Table 1. The factors and levels for the orthogonal design.

Levels	Ethanol concentration (%)	Solvent volume (ml)	Microwave power (W)	Extraction time (Min.)
	A	B	C	D
1.	60	15	165	4
2.	65	20	250	5
3.	70	25	335	6

Conclusion:

Conventional techniques of extractions like; heating, boiling or refluxing may offer some disadvantages i.e. loss of active constituents due to ionization, hydrolysis and oxidation during extraction, to overcome these problems new techniques like microwave-assisted extraction came in practice. In present study microwave-assisted extraction was found to be an effective method for extracting flavonoids from *Zanthoxylum budrunga* W. Extraction yield of ZBW were affected by ethanol concentration, solvent volume microwave power and extraction time. The optimal condition through single factor experiments was determined as followings: ethanol concentration 65%, solvent volume 25 ml, microwave power 250 W and extraction time 6.0 min. This showed great potential of microwave-assisted extraction for industrial application in the near future.

References:

1. Kritikar K. R., Basu B. D., Indian Medicinal Plants Text, 1993; 1: 464-465.
2. http://www.henriettesherbal.com/plants/zanthoxylum-budrunga. Accessed on 06/01/08.
3. Ahmad M. U., Rahman M. A., Hug E., Chowdhry R., Alkaloid of *Zanthoxylum budrunga,* Fitoterapia, 2003; 74: 191-193.
4. Joshi B., Moore M. K., William S. & Puar M. S., Alkaloids of *Zanthoxylum budrunga* wall. nmr assignments of dihydrochelerythrine, (±)-evodiamine and zanthobungeanine, Phytochemical Analysis, 2007; 20: 25.
5. Yao Xue Xue Bao, Pharmacognostic study on the root of fourteen medicinal species in the genus *zanthoxylum*, PubMed, 1985; 20: 598-605.
6. Huang W, Xue A, Niu H, Jia Z, Wang JW (). Optimised ultrasonicassisted extraction of flavonoids from *Folium eucommiae* and evaluation of antioxidant activity in multi-test systems *in vitro.* Food Chem. 2009; 114: 1147–1154.
7. Li H, Chen B, Yao SZ. Application of ultrasonic technique for extracting chlorogenic acid from *Eucommia ulmodies* Oliv. Ultrasonics Sonochemistry, 2005; 12: 295–300.
8. Hemwimon S, Pavasant P, Shotipru A. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. Separat. Purif. Technol. 2007; 54: 44–50.
9. Zhang F, Chen B, Xiao S, Yao S Optimization and comparison of different extraction techniques for sanguinarine and chelerythrine in fruits of *Macleaya cordata* (Willd) R.Br. Separat Purif. Technol. 2005; 42: 283–290
10. Liu XYU, Zhang J, Wang RX. Study on Extracting Process of Pueraria Flavone and Evaluation of Anti-oxidant Activity. Food Sci. 2007; 28: 232-237.
11. Zhang F, Yang Y, Su P, Guo ZK Microwave-assisted Extraction of Rutin and Quercetin from the Stalks of *Euonymus alatus* (Thunb.) Sieb Microwave-assisted Extraction of Rutin and Quercetin. Phytochem. Anal. 2009; 20: 33–37.
12. Zhuang XP, Lu YY, Yang GS. Extraction and determination of flavonoid in ginkgo. Chin. Herb. Med. 1992; 23: 122-124.
13. Chen JJ, Li XG. Hypolipidemic effect of flavonoids from mulberry leaves in triton WR-1339 induced hyperlipidemic mice. Asia Pacific J. Clin. Nutr. 2007; 16: 290-294.
14. Duan R, Wang B, Shi HX. A Study on the Flavonoid-extracting Technology by Microwave from Ginkgo Biloba Leaves. J. Huaihai Institute Technol. 2001; 10: 46–48.
15. Ma HL, Wang C, Liu WM. Microwave-assisted extracting of flavonoids from Radix Pueraria. J. Jiangsu Univ. (National Science Edition). 2005; 26: 98–100.
16. Eskilsson CS, Bjorklund E. Analytical-scale microwave-assisted extraction. J. Chromatogr. A, 2000; 902: 227–250.
17. Guo Z, Fan G, Duan Y, Qin C, Wen M. Microwave-assisted extraction of effective constituents from a Chinese herbal medicine Radix puerariae. Anal. Chim. Acta 2001; 436: 41–47.
18. Chen LG, Jin HY, Zhang HR, Li J, Qu CL, Zhang HQ. Dynamic microwave assisted extraction of flavonoids from Herba Epimedii. Separation Purif. Technol. 2008; 59: 50–57.