Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 6, Issue 1, 2013 ISSN - 0974-2441

Research Article

COMPARISON OF CONVENTIONAL AND SOUND ASSISTED METHODS FOR EXTRACTION OF *EICHHORNIA CRASSIPES* (MART.) SOLMS

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Received: 5 November 2012, Revised and Accepted: 16 December 2012

ABSTRACT

Eichhornia crassipes (Mart.) Solms is an aquatic weed mostly noted for its rapid growth and environmental problems. This plant in recent years has been exploited for its phytochemical constituents, pharmaceutical properties and adsorption characteristics. This study was carried out to find the methods that best suit the extraction of fresh and dried *E.crassipes*. The conventional reflux method, ultra sound assisted extraction viz sonic bath and homogenizer were compared in term of its yield of extract. The results showed that the reflux method gave better yield compared to other methods for dried *E.crassipes* whereas for fresh *E.crassipes*, the yield of the extract obtained by ultrasonic homogenizer was high. Compound factors like rapid evaporation, condensation, increased temperature in reflux method, and floating nature of the plant have been attributed to the increased yield in reflux method for dried *E.crassipes* and the mechanism of action of the ultrasonic homogenizer in the disruption of cell walls for fresh *E.crassipes*.

Keywords: *Eichhornia crassipes*; hyacinth; extraction; reflux; sonic bath; homogenizer

INTRODUCTION

Extraction is the most crucial step in the isolation of compounds from natural sources. A small change in the extraction condition leads to a dramatic change in the nature of the compound being extracted. Extraction method being adopted should be able to extract the metabolites to the maximum extent thereby resulting in good yield and should not change the nature of the metabolites extracted. Resurgence in plant derived chemicals in the past few decades entails the need for optimizing a pertinent extraction method. Classical extraction techniques like extraction under reflux has been widely used in the laboratories for the extraction of active constituents from the plants. However, recent years have witnessed the growth of new methods like ultra sound assisted extraction for extracting the constituents. In this study, the extraction yield of different methods like classical reflux method, ultra sound assisted extraction viz sonic bath and homogenizer was compared for *Eichhornia crassipes*.

Eichhornia crassipes (Mart.) Solms is an aquatic perennial herb that belongs to the family Pontederiaceae. The English common names of *Eichhornia crassipes* are waterhyacinth, water hyacinth and water-hyacinth. Waterhyacinth is the standardized spelling adopted by the Weed Science Society of America to denote that it is not an aquatic relative of true "hyacinth" (*Hyacinthus* spp.), as the two-word spelling suggests (Lalitha et al., 2012). Waterhyacinth contains many phytochemicals (Nyananyo et al., 2007; Ndubuisi et al., 2007 Lata and Dubey, 2010; Jayanthi et al., 2011). Many phenalene compounds have been isolated from waterhyacinth (Greca et al., 1992; Hölscher and Schneider, 2005; Greca et al., 2009; Wang et al., 2011). The plant has been reported to show antimicrobial activity (Fareed et al., 2008; Bobbarala et al., 2009; Zhou et al., 2009; Baral et al., 2010; Shanab et al., 2010), antioxidant activity (Bodo et al., 2004; Liu et al., 2010; Jayanthi et al., 2011), wound healing activity (Ali et al., 2010; Jayanthi and Lalitha, 2012), antitumour activity (Ali et al., 2009) and larvicidal activity (Jayanthi et al., 2012). This plant as it has been proven to be a medicinal herb, a suitable extraction method for extracting the metabolites may be studied. Hence this study was aimed at finding the suitable extraction method for extracting the metabolites from dried and fresh *E.crassipes*.

MATERIAL AND METHODS

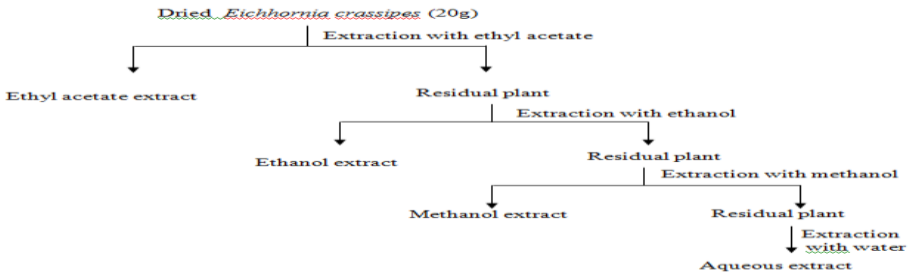
Plant Material

Eichhornia crassipes (Mart.) Solms (Waterhyacinth) was collected from Singanallur boat house, Coimbatore. The plant was authenticated by Dr.G.V.S.Murthy, Scientist F & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore-641 002 with the number BSI/SRC/5/23/2011-12/Tech. The shoot and leaves of *E.crassipes* was used for the present study.

Extraction of dried *Eichhornia crassipes* (Mart.) solms

The plant material was washed several times to remove soil and other debris, roots cut off and shade dried for 20 days. It was then, chopped and powdered in grinders.

The suitability of the extraction method for the extraction of dried *E.crassipes* was analyzed using three different methods viz reflux, sonic bath and homogenizer. The dried plant material (20g) was extracted thrice with ethyl acetate for 1h. The residual plant material was then extracted thrice with ethanol, methanol and water successively. Equal volume of the solvent was used in all the three extraction methods. After each hour of extraction, extractant was replaced with fresh solvent. The flow chart indicating the extraction of dried *E.crassipes* in all the three extraction methods is shown in scheme 1.



Scheme 1:Flowchart for the extraction of dried *E.crassipes*

Extraction of fresh *E. crassipes*

The chopped fresh *E.crassipes* (350g) was first defatted with petroleum ether. The defatted *E.crassipes* (100g) was extracted with ethyl acetate by reflux, sonic bath and homogenizer for 1 h and the residual plant material was then combined and refluxed with ethyl acetate for 6 h. This was then divided equally and extracted by three different methods with ethanol and the residual plant material was then combined and extracted by reflux method with the same solvent for 6 h. This was again divided in to three portions and extracted by different methods with water for 1 h. The residual plant material from the extraction methods were combined and extracted by reflux method for 6 h.

Extraction of fresh *E. crassipes* with ethyl acetate

Fresh *E.crassipes* was extracted by ten different methods viz reflux, ultrasonic bath, ultrasonic homogenizer, hot continuous extraction (soxhlet), microwave assisted extraction, percolation, maceration, infusion, digestion, hot aqueous extraction (decoction). The time of extraction and the volume of the solvent used for extraction are given in table 1.

Table 1:Time of extraction and the volume of the solvent used for extraction

S.No	Method	Time (h)	Volume of Solvent used for Extraction (mL)
1	Reflux	6	1000
2	Ultrasonic bath	5	520
3	Ultrasonic Homogeniser	2	400
4	Hot Continuous extraction (Soxhlet)	1	100
5	Microwave assisted extraction	1.40	890
6	Percolation	600 600	540 (hot condition) 500 (cold condition)
7	Maceration	72	280
8	Infusion	0.5	100
9	Digestion	1	150
10	Hot aqueous extraction (Decoction)	1	140

RESULTS AND DISCUSSION

The extraction of metabolites from dried material is typically a two step process involving steeping the plant material in solvent to facilitate swelling and hydration processes and the mass transfer of soluble constituents from the material to solvent by diffusion and osmotic processes (Vinatoru, 2001).

Extraction of dried *E.crassipes*

The yield of the crude extract obtained in various solvents by different methods from dried *E.crassipes* is given in table 2. Reflux method gave a higher yield of the extract compared to the other methods irrespective of the solvent used in case of dried *E.crassipes*. A unique feature noted during extraction was, the dried plant material floated over the solvent in the extracting vessel. This may be due to the presence of large number of wax like compounds as noted in certain other plants (Amaral et al., 1990). The observed increase in extraction yield by the reflux method for dried *E.crassipes* is believed to be the result of the rapid evaporation and condensation of the solvent at its boiling point, over the floating plant material thus catalyzing the extraction. Furthermore, temperature affects many physical properties including viscosity, diffusivity, solubility and surface tension (Yang et al., 2008; Boonkird et al., 2008). In reflux method, an increased temperature is obvious which allows the solvent to have higher capacity to solubilize analytes, while surface tension and solvent viscosity decreases with temperature, which improves sample wetting and matrix penetration, respectively (Amirah et al., 2012).

Table 2:Yield (g) of extracts obtained in different extraction methods for dried *Eichhornia crassipes* (3h)

S.No	Solvent used for extraction	Method		
		Reflux	Ultrasonic homogenizer	Sonic bath
		Yield (g)		
1	Ethyl acetate	0.405	0.369	0.410
2	Ethanol	1.649	1.116	1.012
3	Methanol	0.719	0.643	0.394
4	Water	1.503	0.818	0.714

A comparison of the yield obtained in various solvents for dried *E.crassipes* revealed that the yield of ethyl acetate extract obtained in sonic bath was comparable with that of the reflux method and homogenizer method. The higher yield of ethyl acetate extract can be due to the agitation in the sonic bath that might have caused efficient steeping of ethyl acetate due to its less viscosity compared to the other solvents used for extraction (Pires et al., 2007). On agitation, the solvent penetrates through the dried plant material providing an efficient extraction. Wu et al., 2001 has observed similar findings when the extraction of ginseng was carried out using sonic probe and sonic bath where sonic bath gave a slightly better extraction yield than the probe. The authors have attributed the higher yield by sonic bath to the agitation and higher temperature in the sample container.

Extraction of the dried *E.crassipes* using water has given comparable yield with ethanol. This may be due to the relative polarity of the compounds present in the plant. Polysaccharides like D-xylose, L-galactose and L-arabinose (Anjaneyalu et al., 1983), galactomannan composing D-galactose and D-mannose and a branched (1→3)-β-D-glucan (Issa 1988), water soluble sugars like galactose, glucose, xylose and arabinose (Arifkhodzhaev and Shoyakubov, 1995) have been isolated from waterhyacinth. These compounds are highly soluble in water and such compounds would have been extracted during aqueous extraction. Also, other compounds of less polarity that have not been extracted by solvents previously used for extraction would also have been extracted furthering the yield of aqueous extract.

Moreover, in ultra sound extraction, the phenomenon of cavitation in the solvent mixture is affected by surface tension, viscosity and medium vapour pressure. In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture increases as the surface tension increases while the viscosity and vapour pressure decreases. Water has a higher surface tension than ethanol, which need higher energy to produce cavitation bubbles that collapsed at a high intensity produces a shock wave that passes through the solvent enhancing mass transfer within the plant material (Ou et al., 1997; Mason et al., 1991; Roldan- Gutierrez et al., 2008; Amirah et al., 2012) which might have resulted in higher yield of aqueous extract compared to other solvent extracts.

Extraction of fresh *E. crassipes*

In case of fresh *E.crassipes*, the yield of the extract obtained by conventional method was found to be very less than from sonication. The yield of ethyl acetate extract obtained was approximately 4 times more in ultrasonic bath and 13 times more with ultrasonic homogenizer than by reflux and similar trend has been noted with other two solvents. Hence, it is obvious that extraction by ultrasonic homogeniser best suits extraction of fresh *E.crassipes*.

Table 3:Yield of the extract obtained by conventional and sound assisted extractions for fresh *E.crassipes*

S.No	Solvent used for extraction	Method and Yield of the extracts (mg)		
		Reflux	Ultrasonic homogeniser	Ultrasonic bath
1	Ethyl acetate	80	360	121
2	Ethanol	1490	4630	1539
3	Water	590	1330	770

Extraction of fresh *E. crassipes* with ethyl acetate

Extraction of fresh *E.crassipes* with ethyl acetate by ten different methods viz reflux, ultrasonic bath, ultrasonic homogenizer, hot continuous extraction (soxhlet), microwave assisted extraction, percolation, maceration, infusion, digestion, hot aqueous extraction (decoction) showed that the yield of the extract obtained from infusion was more (750 mg) than extraction with other techniques. The solvent and time taken in ultrasonic bath, ultrasonic homogenizer, decoction, maceration, soxhlet and microwave assisted extraction was half, compared to the reflux method. Ultrasonic homogeniser has given 525 g yield in 2 h with a lesser solvent (400 ml) whereas ultrasonic bath gave a yield of 130 mg in 5 h with 520 ml ethyl acetate. Moreover, reflux method gave only 170 g yield in 6 h with 1000 ml solvent. The time of extraction and the yield obtained in each extraction is given in table 4. Thus, the fresh *E.crassipes* gave better yield of ethyl acetate extract in ultrasonic homogeniser with less solvent compared to the reflux and sonic bath extraction.

Table 4:Time and yield of ten different methods of extraction of fresh *E.crassipes* with ethyl acetate

Method of extraction	Time of extraction	Volume of Solvent (ml)	Yield (mg)
Reflux	6 h	1000	170
Sonic bath	5 h	520	130
Ultrasonic Homogenizer	2 h	400	525
Hot Continuous Extraction (Soxhlet)	1 h	100	540
Microwave extraction	1.40 h	890	400
Percolation	25 days	540 (h.c)	500
	25 days	500 (c.c)	400
Maceration	3 days	280	200
Infusion	30 min	100	750
Digestion	1 h	150	250
Hot Aqueous Extraction (Decoction)	1 h	140	200

Several researchers have reported the advantage of the use of ultrasonic homogeniser (Paniwnyk et al., 2001; Sheu et al., 2009; Alupului et al., 2009; Yang et al., 2009) and ultrasonic bath (Boonkird et al., 2008; Zhang et al., 2009) for extracting the plant metabolites. Sonic extraction in most cases has proved to improve the extraction yield (Vintoru, 2001, Wang and Weller, 2006) and this holds good for the extraction of fresh *E.crassipes*. The enhancement of extraction efficiency by ultrasound is partially due to its efficacy in breaking down cell walls by the mechanical waves. These waves formed by the ultrasound enable generation locally of micro-cavitations in the solvent surrounding the plant material and therefore, a heating of this plant material, enhancing the release of the extract (Alupuli et al, 2009). Cavitation bubbles produced, collapse at or near walls or interfaces thus improving the mass transfer across the solid- liquid interface thus introducing a kinetic energy in the whole volume (Alupului et al., 2009). The solvent diffuses through the cell wall and washes out the cell contents once the cell wall is broken and this phenomenon is greatly affected by ultrasonic irradiation. Ultrasound can facilitate swelling and hydration and so cause an enlargement in the pores of the cell wall. This involves the diffusion process and therefore enhances mass transfer (Vinatoru, 2001).

The results obtained in the present study also demonstrate that among the two sonic assisted methods, the homogeniser gave higher yield compared to the sonic bath for both dried and fresh *E.crassipes*. The difference between the two techniques may be partly attributed to the different mode of action of the machines used in the extractions. Multiple factors contribute to the variability and inefficiency of ultrasonic bath compared to the homogeniser (Torti et al., 1995).

Ultrasonic waves are effective at emulsifying lipids and are typically employed to break up the lipid membrane surrounding bacteria and animal cells. However, the lipid membrane of a plant cell is encased within a rigid, fibrous cell wall that is probably more resilient to ultrasonic waves. Thus, the effectiveness of sonication depends on the extent of cell wall damage during grinding. Moreover, the ultrasonic waves in a sonic bath are typically not focused or uniform; thus some samples may receive more sonication than others. Sonicating probes (homogenisers), are employed to disrupt cells, as probes better focus the sound waves within the sample, while sonicating baths are typically used for cleaning purposes. Hence, sonication is not rather appropriate for the purpose of rupturing the cell wall. The superiority of the homogeniser is partly due to its efficiency on breaking down cell walls (Torti et al., 1995).

The joint action of the cavitations and the efficient breaking of the cell walls (Torti et al., 1995), higher ultrasound energy provided by the direct sonication using ultrasonic homogenizer than indirect sonication by the ultrasonic bath (Wu et., 2001) may contribute to the higher yield of the homogeniser compared to the sonic bath. There are several reports on the higher yield in ultrasonic homogenizer extraction (probe) compared to sonic bath extraction (Torti et al., 1995; Salgado et al., 2006; Pereira et al., 2010).

CONCLUSION

Reflux method gave good yield for dried *E.crassipes* and ultrasonic homogenizer gave better yield of extracts in case of fresh *E.crassipes*. Thus, dried *E.crassipes* can be efficiently extracted by reflux method whereas ultrasonic homogenizer can be successfully employed for extraction of fresh plant. The extraction of both dried and fresh *E.crassipes* can be carried out by feasible method giving better yield. *E.crassipes*, a plant which requires instant consideration can be exploited for various applications with the methods available at hand.

ACKNOWLEDGEMENT

The authors thank the authorities of Avinashilingam Institute for Home Science and Higher Education for Women for providing necessary facilities to carry out this work.

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