



International Journal of ChemTech Research CODEN(USA): IJCRGG ISSN : 0974-4290 Vol.5, No.5, pp 2173-2176, July-Sept 2013

Evaluation of Different Extraction Methods for Optimization of Extraction of Aerial Roots of Rhaphidophora aurea Entwined over Two Diverse Host Trees

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Abstract: Different methods of extraction have a tendency to produce a noticeable difference in the yield. In order to maximize the yield, the aerial roots of *Rhaphidophora aurea* entwined over *Azadirachta indica* (MN) and *Lawsonia inermis* (MM) were extracted via refluxing, sonic bath, ultrasonic homogenizer and microwave methods. The influencing parameters like volume of solvent, contact time, temperature and the effectiveness of the method were analyzed in this study. The ultrasonic homogenizer and microwave assisted extraction method seems to maximize the efficiency, minimize the cost, drastically reducing the time, solvent volume and gave a good yield compared to other methods.

Key words: Sonic bath, Ultra sonic homogenizer, Microwave assisted extraction, *Rhaphidophora aurea*.

Introduction

Historically "natural plant extraction" is almost equivalent to "phyto-extraction"¹. Hence the word extraction, seeing that the term is exploited biologically and pharmaceutically, involves the partition of therapeutically active fractions of plant via suitable solvents with typical extraction methods. The main intention of extraction is to achieve the therapeutically desired portion and to eradicate the inert fraction from plant.

The crude extracts directly obtained from plants are used as a remedial agent or otherwise the crude part can be further fractionated and purified by chemicals and solvents. Overall the crude extracts finally contribute herbal drugs, which are all having traditional medicinal value. So the standardization of extraction and its methods are taking important attention in the field of phytochemistry.

The plant *Rhaphidophora aurea* is a foliage plant, commonly known as *Pothos aurea* and widely distributed in our plant kingdom. In the present study the aerial roots of *Rhaphidophora aurea* entwined over *Azadirachta indica* (MN) and *Lawsonia inermis* (MM) were extracted with ethyl acetate and ethanol by three different extraction procedures (refluxing, sonic bath, ultrasonic homogenizer) and the aqueous extraction additionally by microwave assisted extraction method. The extraction method was standardized based on the comparison of the yield and time of extraction.

Materials and method

Appliances

The appliances used in this study are LG microwave (1200W), Biologics inc ultrasonic homogenizer, Pci sonic bath, Ajay Tm heating mantle (300W), SHIMADZU electronic balance and Equitron rotary evaporator.

Plant material

The aerial roots of *Rhaphidophora aurea* intertwined over *Lawsonia inermis* (MM) were collected from Palakkad district, Kerala and *Azadirachta indica* (MN) were collected from Coimbatore, Tamil Nadu. Both the plant materials were sequentially extracted with suitable volumes of solvent. After extractions, the contents were filtered and the filtrate distilled using rotary evaporator. The extracts obtained were weighted and preserved for further use.

Extraction methods

Conventional method (refluxing)

The defatted MM and MN plant residue (30g) were extracted with 120 ml ethyl acetate in a heating mantle for three hours, after which it was filtered and the filtrate distilled to yield extract. The same procedure was adopted for ethanol and aqueous extraction.

Extraction using Ultra sonic bath

The defatted MM and MN plant residue (30g) was sonicated with 120 ml ethyl acetate in a sonic bath at intervals of 15 mins for three hours at room temperature, after which it was filtered and the filtrate distilled to yield extract. The same procedure was adopted for ethanol and aqueous extraction.

Extraction using Ultrasonic homogenizer

The defatted MM and MN plant residue (30g) was extracted with 100 ml ethyl acetate in a Ultrasonic homogenizer at intervals of 10 mins for three hours at room temperature. During the homogenization, rate the pulse of sonic waves were maintained at ten. The combined filtered solvents were then distilled to yield extract. The same procedure was adopted for ethanol and aqueous extraction.

Microwave assisted extraction

The defatted MM and MN plant residue (20g) was extracted with 90 ml of distilled water in a microwave oven for 90 seconds at 160° C. The filtered solvents were distilled and the residue was weighed.

Results and discussion

The main endeavour of this study is to maximize the extraction efficiency with minimum quantity of plant material, time, solvents and cost. The defatted plant residue MM and MN were extracted by four different extraction procedures and the results are shown in table 1 and 2.

The results (Table 1) obviously show that extraction using ultrasonic Homogenizer method gave a maximum yield compared to yields obtained from sonic bath and refluxing technique. Ethyl acetate extract using Homogenizer method gave one fourth better yield compared to sonic bath and two times better yield compared to refluxing for MM, In the case of MN Homogenizer method gave two fourth better yield compared to refluxing and sonic bath. Ethanol extraction, Homogenizer gave two fourth better yield for MM and five times better yield for MN compared to refluxing. Aqueous extraction (Table 2) Homogenizer gave one fourth better yield for MM and five times better yield for MM and two fourth better yield for MN compared to refluxing and one fourth better yield for MN compared to refluxing and one fourth better yield for MN mand one and half times better yield for MN compared to sonic bath. The solvent recovery percentage of Homogenizer was more, compared to refluxing and sonic bath.

Method	Time (Hours)	Volume of solvents	Ethyl acetate (mg)		Ethanol (mg)		Solvent recovery
		(ml)	MM	MN	MM	MN	(%)
Refluxing	3	120	288	97	335	81	30
Sonic bath	3	120	470	98	450	51	35
Ultrasonic Homogenizer	3	100	580	156	550	464	55

Table 1: Yield of ethyl acetate and ethanol extract of MM and MN obtained in different extraction methods

Table 2: Yield of aqueous extract of MM and MN

Method	Time	Volume of	Aqueous (mg)		Colour
	(Hours)	solvents (ml)	MM	MN	of the extract
Refluxing	3	90	1043	773	Brown
Sonic bath	3	90	1113	1137	Brown
Ultrasonic Homogenizer	3	90	1342	2056	Pale Brown
Microwave	90 Sec	90	779	517	Dark brown

Pulsed electric field² entails the sound waves with high frequencies; this raises the permeability of cell walls and generates cavitations³ to maximize the yield efficiency. In ultrasonic extraction, the frozen and liquid elements are accelerated and vibrated, because the solute rapidly diffuses away from frozen phase to solvent ^{4, 5}. The enhancements of the sonic extraction are interrupted enhanced diffusion, engorgement, capillary consequence and hydration progression^{4, 6}. This ultrasonic method has a latent to increase extraction effectiveness, reducing time and maximum extraction yield⁷. Ultrasound extraction was accounted as an uncomplicated and more efficient substitute to conventional extraction technique⁸. The advantages of the ultrasounds are, reduce the experimental time, condense the usage of material and least expenditure on solvents; increase the product yield and useful for segregation and cleansing of the bioactive principles⁹.

The results from table 2 showed, that microwave extraction yield maximum with minimum time (90 sec), which yield was equal to almost half of the yield of other three extraction methods. Microwave extraction method yield of MM was equal to three fourth of the yield of refluxing and half of the yield of Homogenizer and sonic bath, the yield of MN was equal to three fourth of the yield of refluxing and half of the yield of sonic bath and one third of the yield of Homogenizer. During the microwave extraction, the plant residue color become decolorized (dark brown to pale brown), in the case of others extraction method, the plant residue colour did not decolorize. Organic solvents at relatively high temperature and pressure may corrode the equipment, especially the extraction vessel¹⁰. Hence, in microwave extraction in the present study only water was chosen as a extractant to find out the extraction efficiency.

Polar solvents like water are generally thought to be better than non- polar ones^{11, 12} and this universal solvent may increase extraction / product yield¹³ compared to ethyl acetate, ethanol and other non polar solvent. Microwave assisted extraction method are reported to produce maximum yield compared to ultrasonic Homogenizer¹⁴⁻¹⁸.

In microwave extraction, even heating is the interference of feeble hydrogen boundaries generated by the dipole rotation of the atom or molecules ¹⁴. The Comparison of refluxing, sonic bath and ultrasonic Homogenizer, microwave assisted extraction method yielded maximum because of its effectual heating, quicker energy transfer, decreased thermal gradients, specific heating and condensed apparatus size, faster start up and process heating control. Inside the microwave the liquid phase takes up the microwaves, the kinetic energy of its molecules enhances and consequently, the distribution rate increases too^{14, 19}.

The aqueous extract of the aerial roots of *Rhaphidophora aurea* entwined over *Azadirachta indica* (MN) and *Lawsonia inermis* (MM) in microwave assisted extraction method yielded maximum with minimum (time) when compared to ultrasonic Homogenizer, sonic bath and refluxing method. To conclude, from the comparison of all the four extraction methods, the ultrasonic Homogenizer extraction method may be considered as a standard method for extraction with ethyl acetate and ethanol and microwave assisted extraction method for aqueous extraction.

Acknowledgement

The authors thank the authorities of the Avinashilingam Institute for Home Science and Higher Education for Women University (Etd. u/s 3 of UGC Act 1956), Coimbatore-43 for having providing the facilities to carry out this research work.

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