Optimization of Microwave Assisted Extraction of *Annona squamosa* Seeds

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This paper focuses on the optimization of various parameters for microwave assisted extraction of *Annona squamosa* seeds. Two parameters namely, sample to solvent ratio and the total extraction time were optimized. Extraction efficiency was found to be affected significantly by both these parameters. A decrease in the extraction efficiency was noted if the total extraction time was extended beyond a certain limit. Non-polar solvents proved better than the polar ones with respect to extraction efficiency.

Keywords: Microwave, Extraction, Optimization, Extraction efficiency

Introduction

Extraction as a pharmaceutically used term can be defined as the technique used for the separation of therapeutically desired active constituent(s) and elimination of unwanted insoluble material by treatment with selective solvents (South-East Asian (SEA) Regional Workshop on Extraction Technologies for Medicinal and Aromatic Plants, 2006). Extraction mainly involves the release of complex plant constituents and solubilization of secondary metabolites from the matrix, followed by separation of soluble target compounds from the crude extract through selective use of solvents (Yrjönen, 2004).

The various procedures that can be used for the extraction of medicinal plants include: Maceration, infusion, percolation, decoction, Soxhlet extraction, counter current extraction, aqueous-alcoholic extraction by fermentation, sonication, supercritical fluid extraction, thermal desorption, phytonic extraction, steam distillation and enfleurage method. The head space trapping technique, Microwave Assisted Extraction (MAE), solid phase microextraction, protoplast extraction technique, microdistillation and molecular distillation are some of the newer methods of extraction (Armstrong, 1999 and SEA Regional Workshop on Extraction Technologies for Medicinal and Aromatic Plants, 2006).

The MAE was first used in 1975 for acid digestion applications under atmospheric conditions (Christen and Veuthey, 2001). The principle of MAE is based on two processes, i.e., ionic conduction and dipole rotation. When the moisture within plant cells gets heated

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due to microwave effect, it evaporates and generates tremendous pressure on the cell wall ultimately rupturing it and leaching out the active constituents to the surrounding solvent, thus improving the yield of phytoconstituents. Various physical parameters such as solubility, dielectric constant, dissipation factor, solvent nature, extraction time, microwave power, matrix characteristics affect the efficiency of MAE (Mandal *et al.*, 2007). The advantages of MAE include short extraction times compared to the traditional extraction methods and minimal solvent consumption (Hao *et al.*, 2002; and Ahuja and Diehl, 2006). This method is suitable for heat labile components (Bart, 2005). The MAE has been reported for fast extraction of plant phenolics (Proestos and Komaitis, 2007), extraction of flavonoids from *Herba epimedii* (Chen *et al.*, 2007), extraction of bioactive saponins from chickpea (Kerem *et al.*, 2005), and extraction of tea polyphenols and tea caffeine from green tea leaves (Pan *et al.*, 2003).

To have a complete idea of the bioactivity of crude extracts, it becomes necessary to optimize the extraction methodology to achieve the maximum possible extraction efficiency.

A. squamosa, commonly known as custard apple or sweet apple, is a small tree reaching 6 m in height; partially deciduous; with 15-20 cm long oval leaves, sub-spherical greyish beige fruit, approximately 10 cm in diameter; contains a number of black seeds surrounded by white, sweet and juicy flesh. An ether extract of the seeds has been found to be moderately toxic against adult *Tetropium castaneum*. Petroleum ether extract of seeds exhibited insecticidal activity against *Corcyra cephalonica*. Constituents of the bark, roots, seeds and stems include; aporphine alkaloids, anonaine, roemerine, norcorydine, corydine, norisocorydine and glaucine. Carvone, linalool, limonene have also been reported. Anonaine has been shown to have antimicrobial properties against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis* and *Candida albicans* (Golob and Gudrups, 1999).

This study aims to optimize the extraction time and seed:solvent ratio for MAE of *A. squamosa* seeds to extract as many compounds from the seeds as possible.

Materials and Methods

The seeds of *A. squamosa* were collected between October 2007 and February 2008. Fresh seeds, free from any visible contamination were collected. These were thoroughly washed with distilled water, air-dried, and stored in the dark in air-tight containers. The dried material was crushed to coarse powder. All the solvents (Merck) used for extraction were of analytical grade. The solvents employed were methanol, ethanol (50%), hexane, acetone, chloroform, chloroform:methanol (2:1), and water.

Extraction

Extraction was carried out with a domestic microwave oven (Electrolux EM30EC90SS). Extraction was done at a constant power of 720 W. About 1 g of powdered seed material was soaked in 50 mL of the respective solvent while frequently shaking before heating. This was followed by centrifugation at 10,000 rpm for 15 min after which the extracts were filtered

using Whatman # 1 filter paper (Whatman International Ltd., England) and were allowed to evaporate. Once the constant dry weight was achieved, the extraction efficiency was calculated as percent dry weight of the initial quantity (1 g).

Optimization of Seed-to-Solvent Ratio

The seed-to-solvent ratio was optimized using different volumes of water (20-50 mL) as a solvent while keeping the extraction time and quantity of seed material (1 g) constant.

Optimization of Total Extraction Time

For optimization of extraction time the sample-to-solvent ratio was kept 1:50 (g/mL). The extraction time selected was on the basis of the boiling point and nature of the solvent. In case of hexane and water, a relatively longer extraction time was tried because the former is microwave transparent and the latter has high boiling point. Intermittent cooling was employed to avoid vigorous boiling, which may lead to thermal degradation of labile phytochemicals. Duration of heating and cooling cycles for each solvent is presented in Table 1. First heating cycle was kept long enough for the solvent to start boiling. Several cooling cycles of 40 sec duration were provided after every heating until the total extraction time was completed.

Table 1: Heating and Cooling Cycles for Different Solvents			
Solvent	First Heating Cycle(s)	Cooling Cycle(s)	Reheating Time(s)
Acetone	25	40	10
Methanol	10	40	10
Ethanol (50%)	40	40	10
Chloroform	60	40	30
Water	60	40	30
Hexane	40	40	30
Chloroform: Methanol (2:1)	20	40	10

Results and Discussion

Optimization of Seed-to-Solvent Ratio

As evident from Table 2, the highest extraction efficiency was obtained by keeping the seed:solvent ratio at 1:50 (g/mL). Results indicate that 'solvent volume', is one of the significant parameters to be considered during extraction procedures.

Table 2: Optimization of Sample-to-Solvent Ratio		
Volume of Solvent (mL)	Weight of Dried Extract (g)	Extraction Efficiency (%)
20	0.11	11
30	0.09	9
40	0.12	12
50	0.14	14

Optimization of Total Extraction Time

It can be seen from Table 3 that maximum extraction efficiency was achieved with a mixture of chloroform and methanol (in just 50 sec), which is higher than the efficiency obtained when either of the solvents is used alone. Moreover, the extraction time is also significantly reduced when the mixed solvent system is used. This solvent mixture has earlier also been applied successfully by other workers (Quiros and Costa, 2006) for extraction purposes.

Methanol, due to its high dissipation factor, provides better overall heating efficiency. Chloroform, being low in polarity, remains transparent to microwave (Mandal *et al.*, 2007). Dielectric properties of the solvent towards microwave heating play an important role in microwave extraction. Mixtures of high and low microwave absorbing solvent composition has been found to produce optimum results (Zhou and Liu, 2006). Though the polar solvents are usually believed to be better than the non-polar ones for MAE, according to the 'broken cell-wall theory', microwave-transparent solvents are better than microwave absorbing

Table 3: Optimization for Time Duration		
Solvent	Time (s)	Extraction Efficiency (%)
Water	90	9.0
	180	10.0
	300	6.0
	540	8.0
Methanol	90	8.0
	180	9.0
	300	4.0
	310	5.0
Chloroform	180	16
	300	16
	420	16
	480	15
Acetone	120	11
	180	10
	215	6
Ethanol (50%)	70	6
	140	6
Hexane	240	10
	300	11
	420	10
	540	9
Chloroform: Methanol (2:1)	50	17
	70	15

ones (Proestos and Komaitis, 2007). The latter theory may explain the better extraction efficiency obtained with chloroform: methanol mixture, as well as with chloroform alone.

Conclusion

The MAE of *A. squamosa* seeds give maximum efficiency with different solvents in the time range of 50 sec to 5 min. which is much lower than time required in the conventional Soxhlet

system. Sample-to-solvent ratio of 1:50 (g/mL) was found to be appropriate for achieving higher extraction efficiency. It is clear from the results that once the maximum extraction efficiency has been attained, a further increase in the total extraction time proves of no advantage. Higher extraction efficiency, obtained with chloroform:methanol mixture, chloroform, and hexane than that obtained with water, methanol and ethanol, indicates that the microwave-transparent solvents has proved to be better than the more polar solvents with high dielectric constants. Results obtained are in line with the 'broken cell-wall theory', as mentioned in the discussion above. This study may prove useful where various bioactivities of *A. squamosa* seeds, such as antioxidant and antimicrobial are to be evaluated. ✳

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