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Microwave assisted extraction of calcium sennosides from senna leaflets

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ABSTRACT

A microwave assisted extraction technique was developed for the extraction of calcium- sennosides from Senna leaflets. Microwave extraction at 300 W intensity and 45 minutes, 300 W intensity and 22.5 minutes and 450 W intensity and 11.25 m heating period, produced higher yields of calcium sennosides when compared to 90 minutes of conventional heating. The yield of calcium sennosides obtained by microwave was found to be 6.83%, 9.4% and 16.01% respectively, more than that obtained by conventional method .The microwave assisted extraction method reduced the amount of solvent consumed for the extraction purpose and also reduced the time of extraction drastically. This was also aided due to the increased intensity of microwave power. But there was a limitation observed on the power of microwave that can be employed and amount of solvent that can be reduced. Increasing the intensity of microwave beyond 450W for extraction of calcium sennosides for 25 g of Senna powder produced intense heating, hence not advisable to employ such intensity of power. The amount of solvent could not be reduced less than 31.25ml (sufficient for wetting the drug) for 25g of Senna powder. The products obtained by both the methods were same both physically and chemically. The method developed can be used successfully for routine laboratory scale isolation of calcium sennosides from Senna leaflets.

Key words : Microwave, Extraction, calcium sennosides, Senna, Methanolic extract.

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INTRODUCTION

Plants synthesize a tremendous number of secondary metabolites which are important from therapeutic point of view. Extraction of crude drugs for these secondary metabolites can be done by various processes depending on the physical nature of the drug and chemical properties of the constituents present in it. Various traditional methods used for the extraction of drugs include Infusion, Decoction [5], Digestion, Maceration and Percolation. Out of these Maceration and Percolation are of particular importance and most Pharmacopoeias refer to these processes for the extraction of crude drugs. The conventional extraction processes are time consuming, e.g., maceration done for 2-7 days; involve bulk amount of solvents and ultimately there might be thermal decomposition of the target molecule as in the case of Soxhlet extraction.

The demand for new extraction techniques has encouraged the development of alternative extraction techniques such as Ultrasonic Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), Supercritical Fluid Extraction (SCF) and Accelerated Solvent Extraction (ASE) [2]. These techniques have enabled automation, shortened extraction time and reduced organic solvent consumption.

Microwave-assisted extraction (MAE) is a relatively new extraction technique, which utilizes microwave energy to heat the solvent and the sample and to increase the mass transfer rate of the solutes from the sample matrix into the solvent. The usage of microwaves for extracting plant constituents is still in infancy. The microwave-assisted extraction (MAE) technique is a promising technique which is highlighted by increased extraction yield high quality products in decreased time and solvent consumption; moreover the reproducibility is better[9].

The microwave region of the electromagnetic spectrum lies between infrared and radio frequencies with frequencies between 300 MHz and 30 GHz. Besides the application of microwave for heating purposes, this region of the electromagnetic waves is used extensively for RADAR transmission and telecommunications [7]. The frequencies of 2450 MHz and 915 Hz are generally used frequencies in industry and 2450 MHz is used for most domestic microwave ovens [8].

The microwave-matter interaction is mainly caused by the interaction of the microwave with the polar molecules. In a polar molecule, due to the difference of the electro negativity of different atoms and the specific structure of the molecule, the whole molecule exhibits a partial positive charge and a partial negative charge and forms a dipole [6]. When the microwave, which consists of an alternative electric field and magnetic field, is applied on the matter with polar molecules in it, the alternative electric field causes the dipoles to oscillate fast. The friction caused by the fast oscillations results in the rapid heating of the material. Since microwave can penetrate into the materials, the heating effect happens throughout the material which is called volumetric heating. This special heating effect makes microwave a very

efficient method for heating, which also contributes to the various applications including the chemical applications [6].

Two parameters, namely dielectric constant and loss factor define the dielectric properties of materials and are important parameters for the microwave heating. The dielectric constant describes the ability of a molecule to be polarized by the electric field, while the loss factor measures the efficiency with which the microwave energy can be converted into heat. These two parameters change with the increase of microwave frequency. Molecules with different dielectric properties when exposed to microwave radiation will have different response to it. Ordinarily, the higher the dielectric constant, the more efficient the molecule absorbs the microwave energy and will be heated more efficiently. Molecules with very low dielectric constants and loss factors cannot couple with microwave oscillation efficiently and therefore will not absorb microwave energy. We call this type of molecule transparent to microwave energy.

Microwave-assisted extraction is a process of applying the microwave energy to a liquid-solid system and partition compounds of interest from the solid sample into the surrounding solvents. The special heating mechanism of microwave and the fact that different chemical substances absorb microwave to different levels make microwave-assisted extraction an efficient method for extraction and more importantly make selective extraction of target compounds possible.

The microwave-assisted extraction process is a combination of different effects. When a polar solvent with a relatively high dielectric constant and loss factor is used the solvent will be heated by the microwave energy through dipole rotation. The heated solvents will accelerate the process of desorption of matrix-solvent interface and the diffusion of the target compounds into the solvent (Hawthorne *et al.*, 1995). In this case the microwave serves mainly as an energy supplier to heat the system. The special extraction mechanism of microwave-assisted extraction can be better interpreted when a non-polar solvent is used in extracting fresh plant materials.

Paré and Belanger (1994) studied the extraction of fresh mint leaf with a non-polar solvent under microwave irradiation. Fresh plant materials such as mint leaves are made of a multitude of pocket-like cavities that are defined by the cells, glands, vascular vessels, and the like, all of which contain different chemical species and more importantly different levels of water content. When the system of fresh mint leaf and a non-polar solvent is exposed to microwave radiation, microwave will travel freely through the solvent which is transparent to microwave energy and reach the sample. A significant fraction of microwave rays is absorbed by the sample, mainly the water in the glandular and vascular systems, which results in a sudden rise in temperature inside the sample. The rise of temperature causes gasification of the water in the glandular and vascular systems. The gasification causes a dramatic expansion in volume and thus creates an explosion at the cell level. The substances located in the cells are then free to flow out of the cell to the surrounding solvent. The scanning electron micrographs

of the extracted fresh pepper mint reveals that after 40s of irradiation of the pepper mint leaf it was totally disrupted, while a two-hour distillation caused only the shrinkage of them. Even though not all samples have the same micro structure as the fresh leaves, selective and localized fast heating effects play an important role in the high efficiency of extracting target components into the solvents.

From the above description it is quite understandable that microwave assisted extraction is a very efficient method for extraction of key components from plant materials. Paré (1995), in his patent listed the application of microwave-assisted extraction on various plant materials and showed that 40s to around two minutes microwave irradiation treatment to sea parsley, pepper mint leaves, cedar, garlic obtained extracts of interest compared to that obtained by 90 min to several hrs of steam distillation or similar standard methods. It is suggested by Paré (1995b) that the microwave is better in extracting heat sensitive component than distillation method.

MATERIALS AND METHODS



SENNA DRIED LEAFLETS



SENNA POWDER

Conventional Extraction of Calcium Sennosides from Senna Leaflets [10]

Procedure

25g of powdered senna leaflets was extracted with 75ml benzene for 15min on electric shaker, filtered in vacuum and solvent distilled off. The left over marc was dried at room temperature and extracted with 75ml of 70% methanol for 30min on electric shaker, filtered under vacuum. The marc was re-extracted with 50ml of 70% methanol for 15min, filtered and the methanolic extracts combined. The methanolic extract was concentrated to $1/8^{\text{th}}$ volume, acidified to pH 3.2 by adding HCl with constant stirring. It was set aside for 15min at 5°C, filtered under vacuum and 1g of anhydrous calcium chloride in 12.5ml of denatured spirit was added with constant stirring. The pH of the solution was adjusted to 8 by addition of ammonia and set aside for 15min. The precipitate obtained was dried.



Conventional extraction Product

Microwave Extraction of Calcium Sennosides from Senna leaflets

Calcium Sennoside from senna leaflets was extracted by varying the parameters of amount of solvent used, temperature and time. The extraction was performed thrice with varying results.



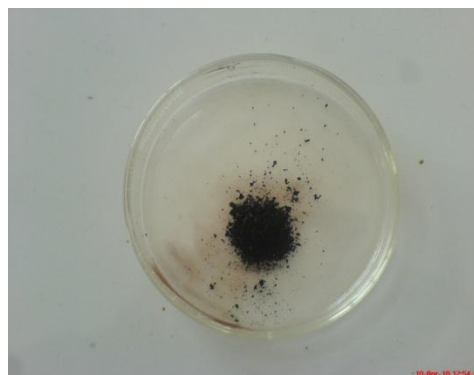
MICROWAVE EXTRACTION OF CALCIUM SENNOSIDES

25g of powdered Senna leaflets were extracted with varying amounts of benzene for different time intervals and varied power of Microwave thrice, filtered in vacuum and the solvent distilled off. The left over marc was dried at room temperature and again extracted with varying amounts of 70% methanol for different time intervals and varied power of Microwave thrice. The marc was re-extracted with varying amounts of 70% methanol for different time intervals and varied power of Microwave thrice, filtered and the methanolic extracts combined. The methanolic extracts were concentrated to $1/8^{\text{th}}$ volume, acidified to pH 3.2 by adding HCL with constant stirring. The mixtures were set aside at different time intervals in all the three cases at 5°C , filtered under vacuum and 1g of anhydrous calcium chloride in 12.5ml of denatured spirit was added with constant stirring. The pH of the solution was adjusted to 8 by

addition of ammonia and was set aside at different time intervals in all the three procedures. The precipitate obtained in all the three cases were dried and weighed. The percentage yield was also calculated.



MAE 1 Product



MAE 2 Product



MAE 3 Product

General conformation tests for calcium sennosides

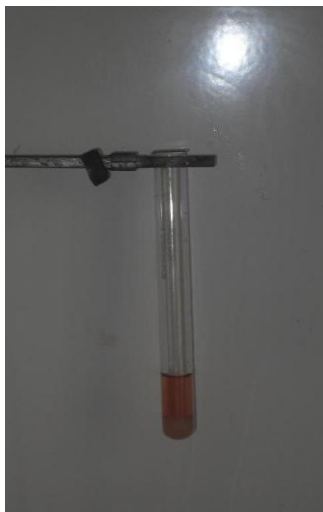
Borntager's Test

To 3ml sample, dilute sulphuric acid was added, boiled and filtered. To the cold filtrate, equal volume of benzene was added and shaken. The organic solvent was separated and ammonia was added. It was observed that the ammoniacal layer turned pink confirming the presence of anthraquinone glycoside presence.

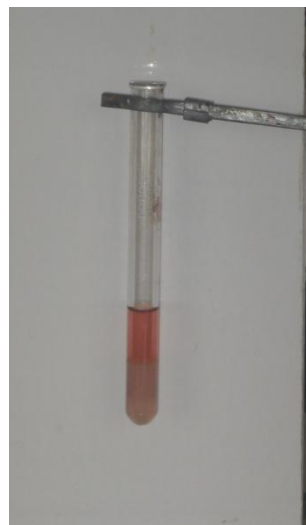
Modified Borntager's Test

To 5ml sample, 5ml Ferric chloride and 5ml dilute hydrochloric acid was added. It was then heated for 5min in boiling water bath. Cooled and benzene was added. It was shaken

well, separated and equal volume of dilute ammonia was added. It was observed that the ammoniacal layer turned pink confirming the presence of anthraquinone glycoside.



Borntager's Test



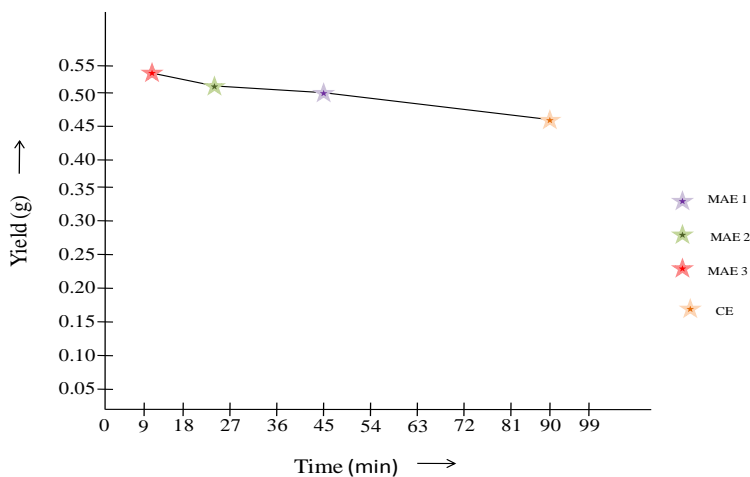
Modified Borntager's Test

RESULTS AND DISCUSSION

S.No	Conventional Extraction			Microwave Extraction				
	Duration (m)	Yield (g)	Amount of Solvent used (ml)	Intensity (W)	Duration (m)	Yield (g)	Amount of Solvent used (ml)	Percent increase in yield*
1	90	0.468	125	300	45	0.5	62.5	6.8
2	-	-	-	300	22.5	0.512	62.5	9.4
3	-	-	-	450	11.25	0.543	31.25	16

Graph: The parameters selected for comparison were time required and percent yield.

COMPARISON OF CONVENTIONAL EXTRACTION AND MICROWAVE EXTRACTION METHODS



CONCLUSION

The objective of the project was to replace conventional extraction by the Microwave assisted extraction. Microwave assisted extraction required shorter time, lesser quantity of solvents, higher extraction rate and better product with lower loss. There is also less risk of decomposition and oxidation of phytoconstituents. It was also found that increase in the power of microwave increased the yield by 6.8%-16% along with reduced solvent consumption and time. However, there was a limiting factor on the reduction in the quantity of solvent. Reduction of solvent quantity beyond a certain value is not recommended as a certain minimum quantity of solvent is required to wet the drug for extraction purpose. The results were found to be encouraging and therefore it is recommended that extraction of calcium sennosides from senna leaflets be carried out by microwave at higher power using less solvent and reduced time.

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