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Contributed Paper

Innovating Botanical Extraction through Application of Microwaves for the Efficient Extraction of Ursolic Acid from *Ocimum sanctum* Leaves

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ABSTRACT

The present work is based on the optimization of a microwave based technique for the extraction of a potent bioactive compound ursolic acid from the leaves of *Ocimum sanctum*, popularly known as "Tulsi". Different extraction parameters namely, microwave power, extraction time, aqueous methanol composition and preleaching time were optimized in an organized manner using a scientific microwave extractor. Ursolic acid was quantified using HPTLC. The following optimum conditions for the maximum yield of ursolic evolved after the systematic optimization study: 40% (320 W) microwave power, 6 min extraction time (two cycles), 90% v/v methanol as the extraction solvent, 10 min preleaching time and 25:1 ml/gm as the loading ratio. A detailed comparison of the proposed technique with convention methods revealed that microwave assisted extraction (MAE) has significantly higher degree of precision and reproducibility thus making it a perfect model for complete automization. Recovery study of target analytes also ensured thermal safety.

Keywords: microwave assisted extraction (MAE), ursolic acid, *Ocimum sanctum*, HPTLC, total phenolics, antioxidant, soxhlet

1. INTRODUCTION

Plant based bioactives in the recent times have become highly valued leads in the development of potential drug candidates. With the recent technical developments in the field of separation and purification science, research in natural products has now become the mainstay in the process of drug discovery [1]. Apart from being used as drug candidates for the treatment of several ailments, many secondary metabolites like phenolics and flavonoids have also been extensively used as dietary supplements thus benefitting mankind in all possible means. In this regard "Tulsi" (*Ocimum sanctum Linn*) which is also popularly known as Holy basil due its being used for different religious purposes, presents itself as an excellent source for scientific exploration owing to its interesting array of pharmacological properties some of which are anticancer, antitussive, antibacterial, antiviral

and many more which has been vividly presented in a review article published by Singh et al [2]. The two pentacyclic triterpenoid ursolic acid and oleanolic acid serves as the biomarker for this plant with diverse pharmacological actions such as antidiabetic, anti-HIV, anti-carcinogenic, antiulcer, hepatoprotective and many more. Two excellent review articles highlighting the pharmacological significance of ursolic acid have been published by Jie Liu [3]. The immense therapeutic properties of ursolic acid makes it a highly valued bioactive entity both commercially as well therapeutically, hence its economical extraction at the industrial level becomes the need of the hour which has been the driving force behind this small project.

Eventhough, many research on ethnomedicinal properties of medicinal plants have been carried out in search of new drug candidates but large scale industrial production of these bioactives is always a challenging task. Extraction forms the basic first step in natural product research and a poorly designed extraction process is sufficient enough to jeopardize the entire objective of drug discovery. In this regard some of the conventional means of extraction often employed for large scale extraction process are reflux and Soxhlet method which poses severe thermal threat towards degradation of the target analyte due to long heating hours involved and also makes use of large volume of organic solvents whose subsequent disposal to the environment again becomes a severe threat [4]. Moreover, such methods are less precise and has problem of automation. With the growing concern on environmental issues it becomes need of the hour that industries adapt to green technologies by eliminating age old traditional heating process which ultimately increases the carbon load of the earth. These were some of the vital issues which were debated at the 2015 Paris convention for climate change. In the recent time microwave assisted extraction (MAE) has come up as an effective green alternative. MAE makes use of lesser amount of organic solvent for extraction and consumes very less energy resources as compared to conventional methods. Interaction of microwaves with plant cell, rupture of cell wall due to internal heating inside the plant cell followed by leaching of target analyte through the ruptured cell wall to the external extraction solvent forms the outline for the rapid extraction using microwave technology [4, 5]. Excellent review articles giving a vivid explanation of different phytoconstitutents which has been extracted using MAE method has been published by many authors with the first of its kind being published by Mandal et al. [6,7,8]. These articles also explain the basic concept behind the working of MAE in an illustrative manner. Extraction of ursolic acid has been attempted by many researchers in the past with varying degree of success using ultrasound assisted extraction (UAE) [9,10,11]. Extraction of ursolic acid has been attempted from different plant sources and since the yield of active constituents varies from plant to plant and also within the same plant of different geographical locations, henceforth a comparison of results regarding the yield of ursolic reported in published articles in the past is not presented in this manuscript as it can be misleading. The authors have also recently published a critical analysis review of publication trends in MAE of botanicals of the past ten years as per Scopus data [12]. The above mentioned review article comments positively on the novelty issue of such work, its economic significance and its impact on herbal industries in the near future. In this paper the applicability of microwaves on improving the extraction efficiency of

ursolic acid from the leaves of *Ocimum sanctum* has been evaluated with a view that this research can become the basis for its large scale industrial extraction in the near future. Several factors which were thought best to influence the extraction process have been studied in an organized fashion to derive the optimum conditions for maximum yield of ursolic acid.

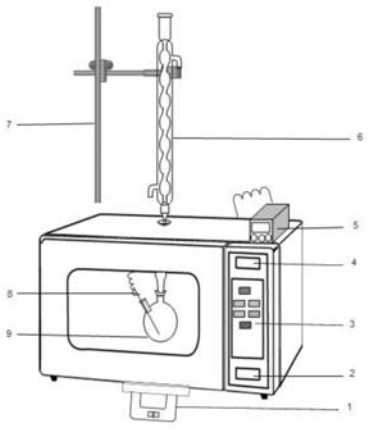
2. MATERIALS AND METHODS

2.1 Reagents

Solvents which were used in the current experiment were procured from S.d Fine Chemicals (Mumbai, India) and for HPTLC purpose, HPLC grade solvents of the same company were used. Ursolic acid was purchased from Sigma (St. Louis, MO).

2.2 Extraction System

The extraction setup consisted of a microwave extractor (CATA R) manufactured by Catalyst Systems (Pune, India) powered with a magnetron of 2450MHz and having varying power levels (5 levels) with a maximum power of 800W (Figure 1). The instrument is also equipped with a reflux unit, timer, temperature controller, exhaust unit, beam reflector and a stirring device.



1: Magnetics tirrer, 2: Door switch, 3: Control panel, 4: Display, 5: Temperature controller, 6: Condenser, 7: Stand, 8: Probe, 9: Extraction flask

Figure 1. Schematic diagram of the extraction unit.

2.3 Conventional Extraction Techniques

Leaves of *Ocimum sanctum* were collected locally inside the University campus, authenticated by a taxonomist and the voucher specimen (RT/Pcog/8/2014) was deposited in the herbarium of Department of Rural Technology. Drying of the leaves was carried out under shade, the dried leaves was then milled to 40 mesh powder just before the experiment. Three conventional extraction techniques namely, Soxhlet, maceration and stirring extraction of 24 h duration were used for comparison with MAE technique. Methanol was used as the extraction solvent. 1 g of powdered plant material was used for all extraction techniques. Exhaustive Soxhlet extraction was performed using a classical Soxhlet apparatus. The extraction was conducted for 8 h each day for 3 days, with fresh solvent being used each day. The completion of extraction after 24 h of Soxhlet extraction was ascertained by collecting 5 ml of the siphoning liquid from the Soxhlet siphon tube and examining for presence of any dissolved residue. No residue was detected upon evaporation of the collected liquid after approximately 24 h of soxhlet extraction. After the completion of extraction all the extracts were pooled together and washed with petroleum ether and finally evaporated under reduced pressure. The dried residue was reconstituted in methanol for analysis. Maceration was carried out in a close conical flask. Stirring extraction was similar to that of maceration carried out at room temperature but with an added advantage of continuous stirring through a magnetic stirrer. Percentage extraction yield (w/w) for ursolic acid was obtained by using the formula

$$\text{Percentage extraction yield (w/w) for ursolic acid} = \frac{\text{Mass of ursolic acid (in extracted solution)} \times 100}{\text{Mass of leaf powder taken}}$$

2.4 Microwave Assisted Extraction (MAE)

MAE was performed as per our earlier reported methods [4, 6]. Briefly, 1 g of the plant powder was extracted using 25 ml of methanol after allowing a pre-leaching time of 5 min. The instrument was operated at different conditions as per the experimental protocol for obtaining the optimized extraction conditions. The sample was treated under microwave irradiation in an intermittent way, i.e. irradiation-cooling-irradiation. The irradiation time was kept for 1min and 1min was taken to cool the sample solution between two irradiations. The temperature was set at 80 degrees. The extraction system would go into an "auto cut-off" mode if the temperature increased beyond the pre set limit. The temperature probe was immersed in the extraction vessel through a side tube. To prepare the extract sample for further analysis, the irradiated suspension after extraction was centrifuged at 4000 rpm (3520×*g*), the clear supernatant was collected, washed twice with 10 ml petroleum ether and finally evaporated under reduced pressure. The dried residue was then reconstituted with 10 ml methanol in a volumetric flask.

2.5 Determinations of Total Phenolics and Flavonoids Content

Total phenolics content (TPC) was determined by the Folin-Ciocalteau colorimetric method using a UV/VIS spectrophotometer as described by Sutivisedsak et al. [13]. Absorbance's were measured at 750 nm. By using the calibration curve prepared with standard solutions of gallic acid, the content of total phenols in the samples was expressed as gallic acid equivalents (µg GAE/g sample)

Total flavonoids content (TFC) was determined as reported by Jaroslava et al. [5] using $AlCl_3$ reagent. Absorbance's were taken at 446 nm. Flavonoids content was expressed as quercetin equivalent (µg quercetin/g sample).

2.6 Comparison of Antioxidant Activity for Different Extraction Methods

The DPPH radical scavenging assay was used to determine the antioxidant activity of the extract following previously described method by Lee et al [14]. The reaction mixture comprised 2 ml of freshly prepared DPPH (1.0×10^{-4} M in 50% methanol) and 1 ml of sample solution containing various concentrations. The mixture was shaken vigorously and incubated at 25°C for 15 min in the dark; the absorbance of the mixture was then determined at 517 nm. Inhibition of DPPH radical was calculated as a percentage (%) using the following equation: inhibition (%) = $\{(A_{control} - A_{sample}) / A_{control}\} \times 100$ where A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

2.7 HPTLC Analysis

A well established HPTLC method for the detection of ursolic acid as reported by Velmani et al was followed [15]. Briefly, sample volume: 10 µl, band width: 8 mm, HPTLC plate: precoated silica gel aluminum plate $60F_{254}$ (20 cm×10 cm), mobile phase: petroleum ether: chloroform: ethylacetate: methanol (4:1:0.1:0.1), solvent front: 80 mm, quantification wavelength: 366 nm (Camag TLC scanner III). A 5-point calibration curve (100-250 ng) was prepared using a standard solution of concentration 0.1 mg/ml in methanol.

2.8 Statistical Analysis

The one way ANOVA test was used to calculate the significance of the differences of the yield of ursolic acid. All experimental results are expressed as means of yield ± SD,

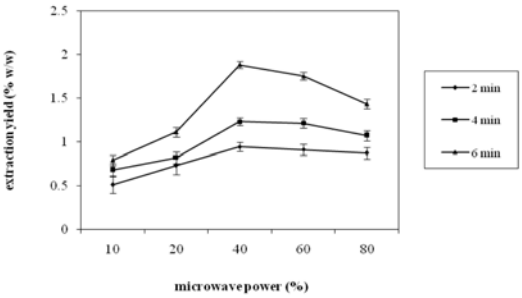
and the means were compared using Student's t test. The p values < 0.05 are considered significant. Individual comparison in between extraction methods and antioxidant activity was done by unpaired t-test

3. RESULTS AND DISCUSSION

This work basically comprises of an organized study of several influential extraction parameters namely, microwave power, irradiation time, solvent composition, loading ratio and preleaching time to obtain the maximum yield of ursolic acid.

3.1 Effect of Microwave Power

Figure.2 shows the effect of microwave power on extraction yield of ursolic acid. It was observed that with the increase in time there was significant improvement in extraction yield when the microwave power was increased from 10% to 40%, with the rise in extraction yield being sharp between 20% and 40% microwave power range. The acceleration observed in the extraction can be due to the direct effects of microwave energy on the plant matrix resulting in heating of the plant matrix as well as the extraction solvent through ionic conduction and dipole rotation causing dissipation of heat in a volumetric fashion. [16]. Microwaves are absorbed both by the plant matrix as well as the solvent and both the components gets heated simultaneously resulting in a synergistic heating effect. This synergistic heating when achieved at the right combination (in this case between 20% - 40% microwave power) gave favourable results in terms of improved product yield. However, the same phenomenon can cause excessive heating resulting in thermal degradation of analytes which is very well evident from the decrease in extraction yield as seen from the effect of microwave power range between 40% - 80%. These results are in accordance with the pattern observed in the MAE of polyphenols from *Myrtus communis* leaves. [17]

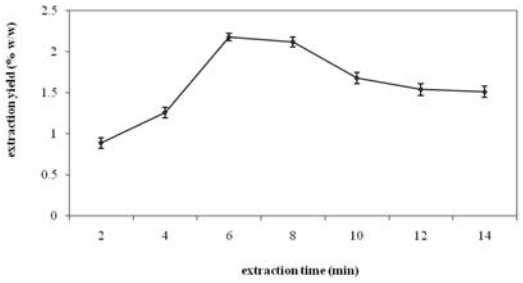


Extraction conditions: 25 ml methanol as extraction solvent and 5 min of preleaching time.

Figure 2. Effect of microwave power on the yield of ursolic acid.

3.2 Effect of Irradiation Time

Duration of microwave radiation of 2, 4, 6, 8, 10, 12 and 14 min at 40% microwave power on the extraction yield of ursolic acid were studied (Figure 3). The extraction kinetics as obtained can be explained by segregating the total duration into 3 phases as explained below.



Extraction conditions: 40% power level, 25 mL methanol as extraction solvent, and 5 min of preleaching time.

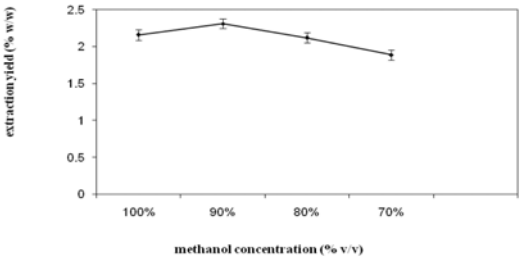
Figure 3. Effect of extraction time (irradiation time) on the yield of ursolic acid.

The first phase is represented by a short rise in extraction yield between 2 min and 4 min which characterizes the extraction of analytes located at the surface of plant matrix. This is followed by the second phase

characterized by the sharp rise in extraction yield between 4 min - 6 min representing the diffusion of target analyte from inside of the complex celluar channels towards the external extraction solvent. These complex channels tends to get disrupted due to the internal thermal stress developed inside the plant matrix upon absorption of microwaves [18]. This disruption facilitates rapid diffusion of the analytes which can be clearly seen in the kinetic graph pattern (Figure 3) between 4 - 6 min extraction time. The third phase which begins after 6 min signifies that extraction has reached a steady state with a decline observed after 8 min. Henceforth, extraction was assumed to have reached the end stage at 6 min itself. The decline in extraction yiled observed after 8 min can be accounted for the increase in thermal stress inside the plant matrix resulting in degradation of the analytes because of over exposure to microwaves [19]. Based on the above observations 6 min was considered optimum for maximum extraction yield. Similar pattern was also reported in the extraction of total phenols from *Ipomoea batatas* leaves [20]

3.3 Effect of Aqueous Methanol Concentration

Figure 4 shows that the yield of ursolic acid was greatly influenced by aqueous methanol concentration. Highest yield was obtained with 90% v/v methanol concentration. Further increase in water content resulted in significant fall in extraction yield. Presence of some amount of water can increase the mass transfer process by increasing the relative polarity of the solvent [18, 19]. Effective swelling of the plant matrix in presence of water increases the surface area for solute solvent interaction resulting in better extraction efficiency. At higher water concentration beyond 90% v/v methanol, probably the degree of polarity was no more favorable for the solubilizing of ursolic acid. From the above results 90% v/v methanol was chosen to be the optimum extraction solvent. Similar graph pattern was also reported for the MAE of polyphenols from *Myrtus communis* leaves. [17]

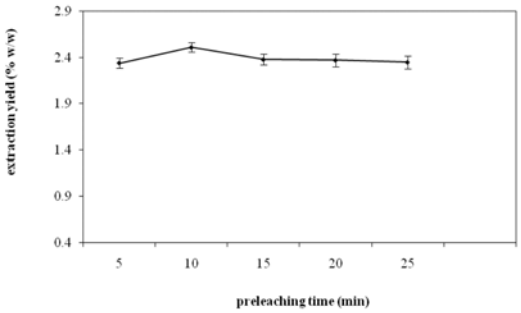


Extraction conditions: 40% power level, 25 mL methanol as extraction solvent, 6 min extraction time and 5 min preleaching time.

Figure 4. Effect of methanol concentration on the yield of ursolic acid.

3.4 Effect of Preleaching Time

Preleaching time can be defined as the time of contact between plant matrix and extraction solvent before the beginning of actual extraction process. Figure 5 shows that a preleaching time of 10 min is favorable for enhancing the extraction yield. However, further increase in preleaching time did not show any promising effect on the extraction performance. Preleaching time of 10 min probably allowed sufficient swelling of the plant matrix. Improved hydration status results in better absorption of microwave by the plant matrix leading to thermal stress and ultimately cell bursting causing release of target analyte to the surrounding solvent [16]. Results were in agreement with our previously published reports [16,19]

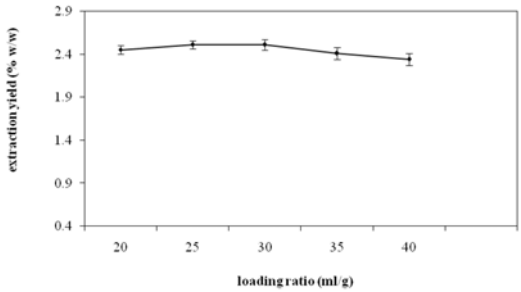


Extraction conditions: 40% power level, 6 min extraction time and 90% v/v 25 ml of methanol.

Figure 5. Effect of preleaching time on the yield of ursolic acid.

3.5 Effect of Solvent to Material Ratio (Loading Ratio)

In conventional extraction process it is believed that large amount of solvent may enhance the extraction efficiency particularly in maceration and percolation. But in case of MAE the matrix to be extracted should always be immersed in extraction solvent. To investigate the influence of solvent to material ratio on the yield of ursolic acid, several loading ratio (20:1, 25:1, 30:1, 35:1, 40:1, ml/g) were examined. It can be seen in Figure 6 that the yield of triterpenoid increased with the increase in the amount of solvent, before the ratio of solvent to material reached 30, at which the yield reached its highest value, and then dropped slightly. This can be accounted to the fact that larger use of solvent volume will cause more absorption of microwave energy and thus sufficient microwave energy may not be available for direct interaction with the plant matrix for facilitating internal thermal stress followed by cell fracture for effective leaching out of the target analyte [18]. Such observation was in parallel to our earlier published reports and similar reports also were stated for the extraction of anthraquinones [21] where increase in extraction solvent volume had decreased the yield of the target analyte due to dilution effect as explained above. Interestingly exact similar result of 25:1 ml/g of loading ratio was reported in the extraction of *Ipomoea batatas* leaves. [20]



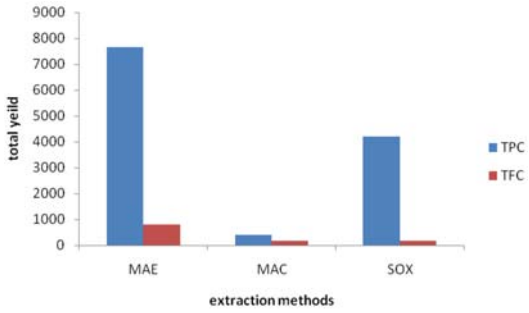
Extraction conditions: 40% power level, 6 min extraction time, 90% v/v methanol and 10 min preleaching time.

Figure 6. Effect of solvent to solid loading ratio on extraction yield of ursolic acid.

3.6 Effect of Extraction Cycle

The effect of repeated and successive extractions of the residue (extraction cycle) was also investigated in this experiment. The extraction conditions were set at the optimum parameters obtained so far in the study. A second successive extraction of the residue yielded further 0.19% w/w ursolic acid taking the final extraction yield to 2.70% w/w. The above data reflects that 92.91% of the extraction was over in the first extraction cycle itself. A successive third extraction did not show any presence of ursolic acid.

Hence the final optimum extraction conditions can be depicted as: 40% (320 W) microwave power, 6 min extraction time (two cycles), 90% v/v methanol as the extraction solvent, 10 min preleaching time and 25:1 ml/gm as the loading ratio.



Total yield represents yield of total phenolics (TPC) as gallic acid equivalent (µg GAE/g sample) and yield of total flavonoids (TFC) as quercetin equivalent (µg quercetin/g sample). MAC: maceration, SOX: Soxhlet. All data represents mean of three experiments (n=3).

Figure 7. Comparison of extraction techniques on the yield of total phenolics and flavonoids.

3.7 Effect of Extraction Techniques on the Extraction Yield of Phenolics/ Flavonoids and Antioxidant Activity

Figure 7 depicts a comparative study of MAE, maceration and Soxhlet on the yield of total phenolics and flavonoids. MAE extraction was carried out under the optimum conditions so derived whereas maceration and Soxhlet extraction duration was of 24 h. TPC and TFC of extracts obtained from different extraction method was basically done to check if microwaves had the same positive effect (in terms of production of increased yield) on phenolics too as it had shown for ursolic acid. In this case also MAE proved its extreme supremacy in terms of significant higher yield of total phenolics and flavonoids content over the other two conventional techniques. For total phenolics the yield in 6 min of MAE was 1.8 times and 19 times higher than Soxhlet and maceration process respectively. Henceforth, MAE can prove to be highly effective not only for extraction of ursolic acid but also for the extraction of phenolics and flavonoids. Such findings form a strong ground for the fact that MAE process can be extrapolated to the extraction of several other phytoconstituents. These results could definitely be interesting for industries manufacturing nutraceutical supplements from plant sources.

For comparison of antioxidant activity in extract obtained from various extraction methods, the antioxidant activity was represented by IC_{50} index which is the concentration of the sample solution producing a 50% reduction in the radical absorbance, higher IC_{50} value indicate lower antioxidant activity. The IC_{50} value of extract obtained from MAE was found to be significantly lower thus indicating maximum antioxidant potency (Figure 8). This could be due to the fact that more amount of antioxidant principles were probably extracted out with MAE which obviously did not happen with conventional techniques. Similar results were also reported by Laddha et al. [21]. Lower IC_{50} value of extract obtained from MAE also indicated that microwaves did not affect or alter the inherent biological property of the plant matrix. It can be assumed that if antioxidant property was retained with microwave treatment so other biological properties of ursolic acid may also have been intact. Henceforth, from these results it can be concluded that MAE not only offers higher yield but also retains the biological activity.

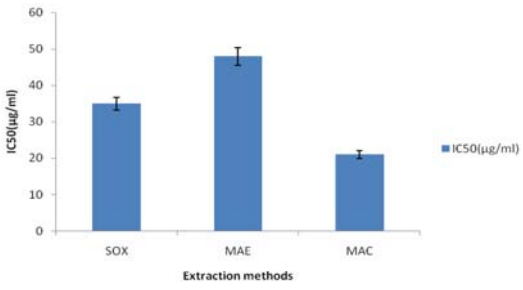


Figure 8. Comparison of antioxidant activity (IC_{50} index) of the extract obtained from different extraction methods.

3.8 Extraction Mechanism

During the MAE, microwaves rapidly deliver energy to the solvent and plant matrix, causing the rupture of the cell wall, and enhance the extraction due to the improved penetration of the solvent into the plant matrix. Microwave treatment greatly affects the structure of a cell as reported in our earlier publications [18,19]. The higher temperature attained by the cell wall, during MAE, causes dehydration of cellulose and thus greatly affects the integrity of the cell wall. Whereas, in case of heat reflux extraction, mechanism depends upon a series of permeation and solubilization processes to bring the analytes out of the matrix [7]. Such a process is time consuming as the cellular channels are complex and the solvent takes a long time to penetrate through it. On the other hand in case of MAE, direct leaching of the analytes from the ruptured or perforated cell wall takes place into the extracting solvent.

3.9 Stability Studies

Stability at the optimum conditions so derived was performed by subjecting standard ursolic acid (at two concentration level, 0.50 µg/µl and 1.00 µg/µl dissolved in 90% v/v methanol) to microwave treatment for 6 min at 40% microwave power. The recovery of ursolic acid was taken as the indicative marker for the stability of ursolic acid at the derived operating extraction conditions. Results (Table 1) showed that average complete recovery at the operating extraction conditions varied from 96% to 98% with no change in R_f value, thus nullifying any fear of thermal degradation at the optimum conditions.

Table 1. Recovery studies of standard ursolic acid under optimum MAE conditions.

Compound	Initial concentration of ursolic acid µg/µl	Recovered concentration after MAE, µg/µl	RSD %, n=3	Average (%)	Recovery
Ursolic acid	0.5	0.48	1.21	96	
	1.0	0.98	1.19	98	

3.10 Repeatability

For reproducibility check of the proposed extraction method, five samples of the same weight (1 g) were processed under the optimum extraction conditions as obtained from the systematic study of different extraction parameters. The mean percentage extraction of ursolic acid (w/w) obtained under the optimized conditions was found to be 2.70% w/w (Table 2), which clearly shows the supremacy of proposed technique over commonly used traditional methods. The relative standard deviation (R.S.D) value was found to be 3.81%, which definitely indicates that the proposed microwave extraction technique has an appreciable degree of precision. In order to determine the repeatability of the chromatographic process, 1 g of the sample was extracted under the optimal MAE conditions as obtained. The R.S.D. of the chromatographic analysis (HPTLC) was calculated to be 0.88% obtained by quantifying the sample repeatedly for five times under the same HPTLC conditions.

Table 2. Profiling of MAE with other conventional techniques.

Extraction method	Extraction time	Solvent volume	R.S.D (%)	Response
MAE	6 min	25 ml	3.81 (n=5)	2.7
Maceration	24 h	100 ml	14.72 (n=5)	0.9
Soxhlet extraction	24 h	100 ml	8.61 (n=5)	1.9
Stirring extraction	24 h	100 ml	11.23 (n=5)	1.1

Extraction conditions: 40% power level, 25 mL methanol as extraction solvent, 6 min extraction time, 90% v/v methanol as the extracting solvent and 10 min of preleaching time. Response= % extraction of ursolic acid, w/w. Weight of the powdered leaf sample = 1 g. MAE was performed at the optimum conditions as obtained in the study

3.11 Comparison of MAE with other Conventional Extraction Techniques

Industrial extraction of potent bioactives for mass production is always a challenging issue. In India traditionally heat reflux extraction is used for large scale extraction of these bioactives which is neither ecofriendly nor does it provide any assurance of thermal safety to the target analytes and hence these extraction methods are not favorable from a commercial perspective. MAE is relatively a new technique, which is rapidly emerging as an efficient green alternative. The mechanism of heating during MAE is based on the direct effect of microwaves with the plant matrix. The natural moisture present inside the plant cell heats up instantly upon absorbing microwaves and produces a thermal stress from inside resulting in the destruction of the cell wall [18]. Moreover, the solvent also absorbs microwaves and gets heated up separately. Both this phenomenon's, heating of the plant matrix and heating up of the solvent occurs separately and simultaneously which ultimately improves the overall heating efficiency [4, 21]. Upon comparison with traditional extraction methods, MAE method was clearly superior in terms of extraction efficiency, extraction time, usage of organic solvents and accuracy (Table 2). These factors are the most critical as they are only responsible for adding up to the industrial cost of production of extracts or bioactive(s). Such significant advantages in these critical factors can definitely help in reducing the industrial cost of production of such bioactive(s) with improved product quality. Moreover, with MAE complete automation of botanical extraction with higher degree of accuracy and precision can be achieved.

On extraction time, MAE was the fastest extraction method with only 6 min of extraction time and preleaching time of 10 min. Maceration, Soxhlet and Stirring extraction all yielded poorer results compared to MAE with reduced reproducibility. If performance achieved through MAE was considered to be 100%, then maceration, Soxhlet and stirring extraction could exhibit only 33.3%, 70.3% and 40.7% extraction efficiency respectively with respect to the yield of ursolic acid. These features along with an ease of operation, higher degree of reproducibility and suitability for complete automation would position MAE as a valuable and cost effective technology suitable for today's highly competitive industries with growing demand for increased productivity, improved efficiency and reduced cycle time.

3.12 Environmental Impact

The total energy consumed for extraction of ursolic acid under optimum conditions of MAE was calculated to be 0.06 KWh and that for 24 h Soxhlet extraction was 8.40 KWh.

The power consumption was measured with the help of a wattmeter at the microwave generator entrance and the electrical heater power supply. The quantity of CO_2 released was calculated based on the fact that to obtain 1 kWh energy from coal or fuel will release 800 g CO_2 to the atmosphere during combustion of fossil fuel [19]. Based on the above equation the quantity of carbon dioxide released for MAE process was 51.22 g of CO_2 to that of 6720 g of CO_2 released out of 24 h Soxhlet extraction. To sequester the CO_2 released from Soxhlet extraction 0.15 tree seedlings has to be grown for 10 years as compared to 0.001 tree seedlings for CO_2 released for MAE [22].

4. CONCLUSION

The optimal conditions thus derived from this systemic optimization study is as follows; 40% (320 W) microwave power, 6 min extraction time (two cycles), 90% v/v ethanol as the extraction solvent, 10 min preleaching time and 25:1 ml/gm as the loading ratio. The results were in agreement with some of the recent published reports based on the MAE of bioactive principles from leaf samples. Different plant parts react differently with microwaves because of the varying nature of hardness and hence the results of this current work were compared to MAE of leaf samples only and were found to be in agreement. The findings of this research can be an excellent platform for adapting green techniques at the industrial level provided necessary investment and involvement of industry with academic research takes place. All the experiments have been done using a laboratory scale commercially purchased microwave extractor which has its own sample loading capacity. Technology transfer (from 1 g to several kilograms) to industries and further scale up is a separate aspect and can only be done with industrial collaborations. Nevertheless, all inventions born through research occur at the laboratory bench top and its scaling up to industrial level is vital for making the invention sustainable and beneficial to mankind. Conventional extraction techniques like Soxhlet involves long heating hours which in turn burns excess energy and that's where we should make way for greener technology which makes use of lesser amount of energy resources and thus protects the environment. In the recent era it is highly important that technology and environment should go hand in hand. In this regard green technologies shall be the key for sustainable growth in the near future. However, all laboratory research cannot be directly scaled up at the industrial level as industries have their own scale up issues driven by cost-benefit analysis. Such green technologies shall play a pivotal role in the sustainable development of industries in the near future.

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