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ORIGINAL ARTICLE

Impact of Microwaves on the Extraction Yield of Phenolics, Flavonoids, and Triterpenoids from Centella Leaves: An Approach toward Digitized Robust Botanical Extraction

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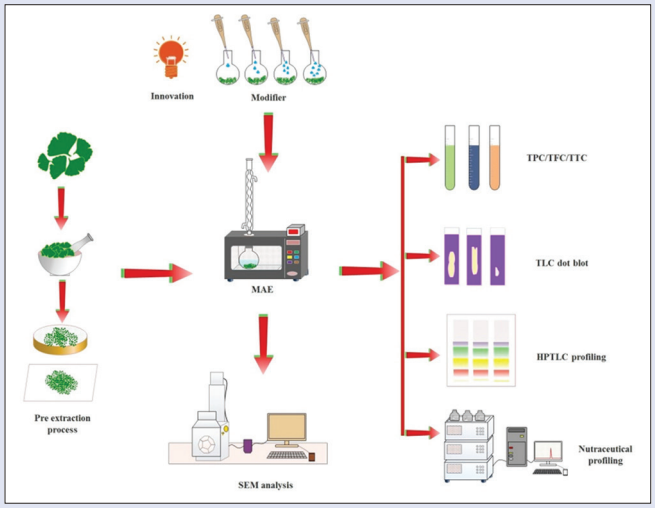
ABSTRACT

Introduction: A novel green approach of microwave-based extraction of botanicals for improved yield of bioactives has been investigated. In this regard, leaves of *Centella asiatica* which has a rich history of ethomedicinal use were chosen. **Objective:** The aim of this study is to develop a robust optimized microwave-based extraction protocol for improved yield of phenolics, flavonoids, and triterpenoids principles. **Materials and Methods:** Microwave power and extraction time were critically optimized along with the effect of moisture content through sample pretreatment. Effect of optimized operating conditions on the biological integrity of the extract and on the extraction of other nutraceutical principles was also evaluated. Results were compared to traditional extraction methods. **Results:** The final optimum extraction conditions were 50% microwave power, 6-min irradiation time, and 54% moisture content for maximum yield of phenolics and triterpenoids, whereas for flavonoids, optimum microwave power was 40% with other conditions remaining same. The proposed method was found to be three-fold better than 36 h of Soxhlet extraction and produced 200 times lesser carbon load than Soxhlet. Improved yield of other nutraceutical principles with better anti-oxidant activity was recorded for the proposed method. **Conclusion:** Switching to such greener technology is now the need of the hour. This research is a sincere effort to showcase the potential of green chemistry in the herbal drug industry.

Key words: *Centella asiatica*, flavonoids, microwave-assisted extraction, phenolics, triterpenoids

SUMMARY

- The final optimum extraction conditions were 50% microwave power, 6 min irradiation time, and 54% moisture content for maximum yield of phenolics and triterpenoids, whereas for flavonoids, optimum microwave power was 40% with other conditions remaining same.
- The proposed method was found to be three-fold better than 36 h of Soxhlet extraction and produced 200 times lesser carbon load than Soxhlet.



Abbreviation used: MAE: Microwave Assisted Extraction; HPTLC: High-Performance Thin-Layer Chromatography; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TTC: Total Triterpenoid Content; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; UAE: Ursolic Acid Equivalent; SD: Standard Deviation; DPPH: 2,2-diphenyl-1-picrylhydrazyl; AUC: Area Under the Curve; SEM: Scanning Electron Microscopy.

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INTRODUCTION

In the last few years, research in natural products with emphasis to the discovery of new biomolecules or phytopharmaceuticals have been on the rise. This surge is primarily due to innovations and developments in *in vitro* assay methods and chromatography. No matter how big the objective may be in natural product research, but the basic first step begins with "extraction." Probably, this is one of the sectors which has received less attention as most of the research input have been channelized in studying complex molecular mechanisms of extracts and isolation of pure bioactives. It is very important to understand that a poorly designed extraction strategy can cause extreme threat to the phytoconstituents in terms of thermal degradation or incomplete leaching of the desired phytoconstituents. Traditional methods are non-robust and suffers from low yield with extreme variability. Soxhlet extraction even makes the situation worse by incorporating heat threat for the thermolabile constituents,

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thus reducing the success of ending up with a new bioactive at the initial stage of research itself.

Centella asiatica (L.) Urban commonly known as "Gotu kola" belongs to the family Apiaceae. The genus *Centella* was reported having >50 species and *C. asiatica* is one of the most ubiquitous among them. This is a creeper perennial plant, stem divided into nodes and internodes. The size and shape of *C. asiatica* can differ due to geographical and ecological conditions. The plant is extremely rich in phenolics and triterpenoids which are the two main categories of phytoconstituents associated in the combat with many diseases.[1] The scope of research in phenolics has been beautifully reviewed by Kala *et al.*[2] Triterpenoids are one of the largest group of secondary metabolites present in plants.[3] They have received extensive attention due to their broad-spectrum pharmacological activities associated with a low toxicity profile. They are known mostly for antiangiogenic, antipruritic, antiallergic, antitumor, antiviral, antimicrobial, antioxidant, and spasmolytic activities.[4] The various biological markers reported for the herb are asiaticoside, madecoside, madecassic acid, madecassoside, madasiaticoside, brahmic acid, and madasiatic acid.[5] *C. asiatica* popularly is used as a memory enhancer, furthermore, the plant is used to treat depression, wound healing, gastric ulcer, leprosy, skin problem, and many more other ailments.[6]

Microwave-assisted extraction (MAE) in recent times have shown tremendous potential as an efficient green alternative to traditional extraction methods in natural product extraction.[7] The scope of MAE in natural product extraction and its working mechanism has been critically reviewed by Kala *et al.*[8] The issues such as thermal degradation, low yield with poor reproducibility and precision, long extraction hours, use of large volume of solvent, and increasing carbon footprints have been successfully addressed by MAE technology. In today's technological era, sustainability is a critical issue and a particular developed technology can be made sustainable only when it is in tandem with the environment.[9] In this regard, traditional methods such as Soxhlet, reflux, maceration even though require low capital investment but may not be sustainable in the current context of growing concerns on the relation between technology and environmental/climate issues. The need of the hour is automatized, precise, energy saving, eco-friendly technologies. No herbal industry is devoid of extraction setup and having said on the sustainability issue of any technology, it becomes important that industries adopt "greener methods" sooner or later. In light of the fact that there exists huge commercial scope with *C. asictica* owing to its richness in terpenoids and phenolic content, it was thought worthful to develop a robust standardized microwave-based extraction protocol for phenolics/flavonoids and terpenoids from the said plant. The data so presented can act as a platform for the scale-up of such technologies in the near future and extrapolation of such technology for the extraction of other class of phytoconstituents.

MATERIALS AND METHODS

Plant sample collection

The fresh leaves of *C. asiatica* used in the current study were collected from its natural habitat (latitude: 20°17'41"N; longitude: 81°27'37"E) of Guru Ghasidas University campus, Bilaspur (C.G), India. The plant material was identified by a taxonomist and the voucher specimen (Voucher specimen no. 23012) is deposited at Janki Ammal Herbarium, CSIR-Indian Institute of Integrative Medicine (Government of India), Jammu and Kashmir. Leaves were shade dried and size reduction was carried out to obtain uniform plant matrix passed through 60 mesh size sieve. Uniform powder thus obtained was stored in zip lock pouches for extraction protected from light and moisture.

Chemical and reagents

All solvents used in extraction and chromatography process were purchased from Fischer Scientific (India). Standards and reagents such as ursolic acid (purity ≥90%), gallic acid, alpha-tocopherol (purity ≥96%) quercetin, Folin-Ciocalteu's phenol reagent and aluminium chloride were purchased from Sigma-Aldrich Co., (St. Louis, MO, USA). Vanillin (4-hydroxy-3-methoxybenzaldehyde) of AR grade was purchased from Sisco Research Laboratories Pvt. Ltd., (India). Ascorbic acid (purity 99%) was purchased from HIMEDIA. Asiaticoside (≥95% w/w) was purchased from Natural Remedies Pvt. Ltd., Bengaluru, India.

Apparatus

Microwave extraction was performed with a commercially available microwave extractor CATA-R manufactured by Catalyst Systems (Pune, Maharashtra, India). The extraction system comprised of a microwave extractor cavity, equipped with a magnetron of 2450 MHz with a nominal maximum power of 850 W, temperature controller, reflux unit, 10 power levels, time controller, powerful exhaust system, beam reflector and a stirring device. The whole system was run at atmospheric pressure. HPTLC system (Camag, Muttenz, Switzerland) with Linomat5 injector, scanner3 was used with WinCATS version 1.4.4 (Camag, Muttenz, Switzerland) for quantification. A double-beam spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used. High-performance liquid chromatography (HPLC) was done using Binary Gradient HPLC system P3000 (Analytical Technologies, Baroda, India) equipped with UV detector and autosampler using COSMOSIL 5C18-MS-II column (4.6 I. D × 250 mm).

Extraction protocol

Soxhlet extraction

A total of 1 g of the powdered sample was extracted using classical Soxhlet apparatus for 36 h to ensure exhaustive extraction; simultaneously, maceration was also carried out for the same time period using 1 g of the plant sample. Ethanol was used as the solvent for all types of extraction.

Microwave assisted extraction (MAE)

Accurately weighed 1 g of leaf powder powder was extracted with 25 mL ethanol, after allowing an initial preleaching time of 5 min.[10] Extraction was performed by irradiating the suspension inside the extraction vessel with microwaves at different predetermined experimental conditions. Mandal *et al.* had described microwave power and irradiation time as one of the most critical factors for the optimization of MAE.[11] Henceforth, the initial primary focus was to optimize the said parameters as it is often said "well begun is half the job done." To fulfil the said task, five levels of microwave power were optimized against three different irradiation time. Microwave extractor was operated in an intermittent way, i.e., irradiation-cooling-irradiation. Ice cold water was circulated through the condenser during extraction. After extraction, the extract was filtered through No. 1 Whatman filter paper and then centrifuged at 4000 rpm (3520 ×*g*), the supernatant was evaporated to obtain the weight of the dried residue. The residue was then reconstituted in suitable solvent for different quantitative analysis.

Performance indicative parameters

Three performance indicative parameters, namely total phenolic content (TPC), total flavonoid content (TFC), and total triterpenoid content (TTC) was chosen for the optimization study. Conditions resulting in maximum yield of total phenolics, flavonoids, and triterpenoids would be considered as optimum for MAE. TPC, TFC, and TTC for Soxhlet and maceration were also determined and used for comparison with MAE.

TPC was determined spectrophotometrically using the Folin-Ciocalteu method by reconstituting the dried extract residue in ethanol. The results

were expressed as µg gallic acid equivalent (GAE) per gram of dried extract using the equation based on calibration curve: y = 0.0953x + 0.0177, R^2 = 0.9982 where "x" is GAE and "y" is the absorbance of the sample.[12]

TFC of *C. asiatica* was estimated by aluminum chloride colorimetric assay as described by Mandal *et al.*[13] Quercetin was used as standard and the following equation based on calibration curve of quercetin was used; y = 0.00258x + 0.00491, R^2 = 0.9967, where "x" is Quercetin equivalent and "y" is the absorbance of the sample. Results were expressed as µg quercetin equivalent/g dried extract.

The determination of TTC of *C. asiatica* was performed as per the standard protocol described by Fan and He[14] with some minor modification. Ursolic acid was used as standard and the following equation based on the calibration curve of ursolic acid was used; y = 0.0215x – 0.3687 (R^2 = 0.9957). Results were expressed as µg ursolic acid equivalent/g of dry sample.

2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay

The free-radical scavenging activity of the extract was done against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as described by Alara *et al.*[15] Briefly, 2 mL of 0.1 mM DPPH stock solution in methanol was mixed with 0.2 mL of the extract (100 µg/mL). Incubation of the reaction mixture was carried out in dark for 30 min followed by recording of absorbance at 517 nm using a ultraviolet–visible spectrophotometer.[16] % scavenging activity was reported on comparison with control.

Sample pretreatment

Sample pretreatment was performed by adding 1, 3, 5, and 7 mL of distilled water (modifier) to 1 g of the sample to carry out moistening of the sample before exposure to microwaves. 10 min of hold time was allowed for moistening of the sample upon addition of modifier. The moisture content was determined after addition of modifier and before MAE.

Nutraceutical analysis

Ascorbic acid estimation was carried out as described by Kala *et al.*[10] Estimation of alpha-tocopherol was carried out according to the Association of Official Analytical Chemists Method 992.03 (AOAC, 2012).[17] Ascorbic acid was quantified by HPLC using a mobile phase composition of 0.2% metaphosphoric acid:methanol (90:10) with detection being carried out at 254 nm. The compounds were identified by chromatographic comparisons with authentic standards.

Chromatography analysis (high-performance thin layer chromatography)

A phenolic fingerprint of the extract obtained from MAE, Soxhlet and maceration was produced using high-performance thin-layer chromatography (HPTLC) as per the method of Kala *et al.*[18] The mobile phase used was toluene: ethyl acetate: formic acid: methanol in the ratio of 6:6:1.6:0.4 (v/v). Quantification was carried out at 366 nm, and the results were analyzed using WinCATS software (Camag, Muttenz, Switzerland).

Biomarker estimation

Asiaticoside was estimated in the extracts using HPLC as per the method described by Gupta *et al.*[19] Mobile phase of acetonitrile:methanol: water (26:24:50) with a flow rate of 1 mL/min was used and the detection was carried out at 204 nm.

Scanning electron micrographs

All the specimens were examined with a JEOL JSM-6700F (Akishima, Tokyo, Japan) scanning electron microscope under high vacuum condition and at an accelerating voltage of 5.0 kV.

Statistical analysis

All experiments were carried out in triplicate and the means were compared using Student's *t*-test and the Duncan multiple range test. Values of $P < 0.05$ are considered statistically significant. Results were expressed as a mean ± standard deviation. All statistical analyses were performed using the free online statistical software Graph Pad Prism version 7.0. (GraphPad Software, San Diego, California)

RESULTS AND DISCUSSION

Effect of microwave power and time

Figures 1-3 illustrate the yield of total phenolics, flavonoids, and triterpenoids content when microwave power was varied with respect to time. The range of operating microwave power and irradiation time was selected based on preliminary studies (data not shown). Preleaching time (time of contact between solute and solvent before irradiation) of 10 min and solvent volume 25 mL was kept constant throughout the experiment.[20] Results provided conclusive evidence of a significant rise in extraction yield of phenolics content between 20% (170 W) and 50% (425 W) microwave power for all irradiation time selected, with drastic rise between 30% and 50% microwave power. Increase in microwave power results in rapid delivery of electromagnetic energy to the plant matrix resulting in volumetric heating of solvent and simultaneous heating of plant matrix as well. This results in the development of internal heat stress leading to degradation of cellulose content of cell wall producing fractures which cause easy leaching out of analytes.[21] Increase in irradiation time also had a positive impact on the yield with increase in microwave power but up to a certain limit. Too less irradiation time even at higher microwave power is insufficient to cause thermal stress inside the plant matrix, and thus, the impact on cell wall integrity is not severe enough to cause quick and easy leaching of analytes.[22] However, longer extraction time accompanied by higher microwave power shall cause excessive heat stress both within the solvent and inside the plant matrix as well, resulting in degradation of analytes. The fact is evident from the decreasing trend observed in the graph after a certain microwave power level when operated at the maximum irradiation time. Phenolics, flavonoids, and triterpenoids show the different intensity of increasing trend with increase in microwave power as a function of time

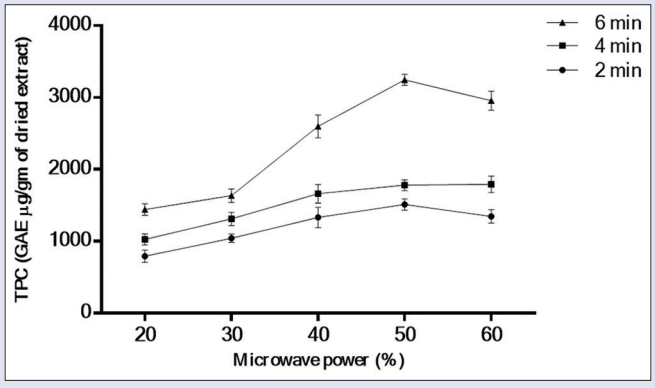

Figure 1: Effect of microwave power and time on the yield of total phenolic content. Extraction conditions: 1 g plant material, 25 mL ethanol, 10 min preleaching time

due to different extent of sensitization of individual principles toward microwaves. The decreasing trend after a certain microwave power at the highest irradiation time also can be explained similarly as well. The results clearly state that striking the right balance between microwave power and the irradiation time is extremely important for optimum yield. Lower microwave power may be too less intense and higher microwave power may cause excessive heat stress which can make the analytes vulnerable to thermal stress. However, any decision on the use of the right microwave power has to be guided by irradiation time and both factors need to be evaluated simultaneously. The above results indicate 50% microwave power (425 W) and 40% (340 W) power with the highest selected extraction time (6 min) to be optimum for TPC/TTC and TFC, respectively.

Extraction kinetics

Having said about the optimum microwave power, the obvious question that arises is what is the fate of extraction after 6 min? To investigate the fate of extraction of TPC, TFC and TTC, extraction was performed at their respective optimum power level as a function of time [Figure 4]. The extraction was performed at an interval of 2 min till 14 min. The extraction pattern of the three principles (phenolics, flavonoids, and triterpenoids) witnessed three different extraction phases.[10,12] The first phase (2–4 min) indicates desorption of the analyte from the matrix and extraction of analytes located at the surface of the matrix. The second phase (4–8 min) represents bulk extraction as it signifies interim diffusion of analytes from the interior of the complex cellular channels to the outside organic solvent. Maximum extraction takes place in this phase. During MAE the diffusion of analytes from inside of the matrix is accelerated owing to rupture/perforations created in the cell wall, thus easing out the diffusion process. The same is not admissible in Soxhlet or maceration as there occurs no rupture in the cell wall and the analytes traverses through the tough rigid cell wall through simple diffusion process making it time-consuming and cumbersome. Henceforth, any acceleration provided to the interim diffusion of the analytes through the complex cellular channels to the outside extraction solvent shall accelerate the process of extraction.[10] The last phase beyond 8 min symbolizes the end of extraction which is either characterized by a plateau phase or decline due to thermal degradation. It shall be judicious to stop the extraction process before the beginning of this stage. All the three principles (phenolics, flavonoids, and triterpenoids) exhibited the three-phase extraction pattern as described above. However, their increasing or decreasing intensity may vary based on their level of sensitization to microwave and their actual content present in the matrix. For phenolics, flavonoids, and triterpenoids, the percent rise in extraction yield for the first 2 min (Phase-I) was 17.8%, 28%, and 20%, respectively, clearly validating the above theory of Phase-I extraction pattern. The extraction yield escalated drastically in the next 2 min slot (Phase-II). However, the later part of Phase-II had reached a plateau stage indicating the end of the process. Significant degradation of the three principles resulted in Phase-III. Henceforth, the optimum extraction time for phenolics, flavonoids, and triterpenoids was found to be 6 min, beyond which significant degradation was evident.

Anti-oxidant activity

Anti-oxidant activity was basically performed to abolish any fear that the application of electromagnetic waves could jeopardize the biological activity. The plant has already been reported to possess anti-oxidant activity, and it can be anticipated that if the anti-oxidant activity of the extract prepared from MAE is retained, the biological integrity shall be deemed to be considered intact. More is the % free radical scavenging, better is the anti-oxidant activity. The anti-oxidant activity of the extracts at 100 µg/mL prepared from MAE, Soxhlet, and maceration are given in

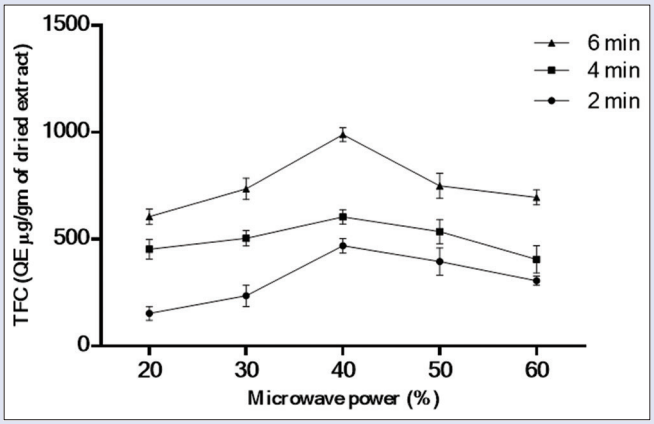

Figure 2: Effect of microwave power and time on the yield of total flavonoid content. Extraction conditions: 1 g plant material, 25 mL ethanol, 10 min preleaching time

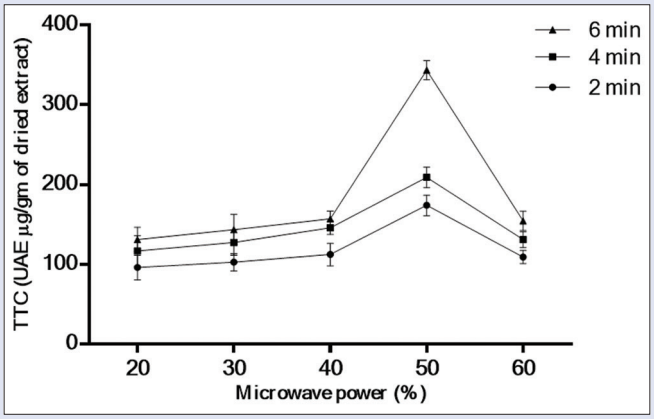

Figure 3: Effect of microwave power and time on the yield of total triterpenoid content. Extraction conditions: 1 g plant material, 25 mL ethanol, 10 min preleaching time

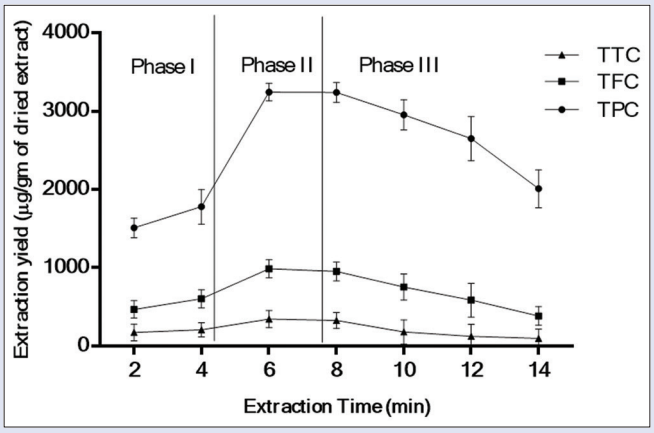

Figure 4: Extraction kinetics depicting three different phases of extraction with passage of time. Extraction conditions: 1 g plant material, 25 mL ethanol, 10 min preleaching time, 50% microwave power for phenolics and triterpenoids, and 40% microwave power for flavonoids

Table 1. The biological activity of an extract depends on the synergistic activity of all the phytoconstituents present in the extract. MAE method

resulted in increased extraction of phytoconstituents whose cumulative impact resulted in improved biological activity.

High-performance thin layer chromatography analysis

Similar phenolic fingerprint pattern [Figure 5] for extract produced from MAE, Soxhlet, and maceration was observed which indicated that no new compounds were detected from extract produced from MAE. Thus, the possibility of formation of any undesirable toxic compound due to exposure to electromagnetic waves can be ruled out. The total cumulative area under the curve (AUC) obtained for extract produced from MAE, Soxhlet, and maceration are given in Table 1. Results indicate significant dominance of MAE. Higher AUC indicates increased extraction of phytoconstituents.

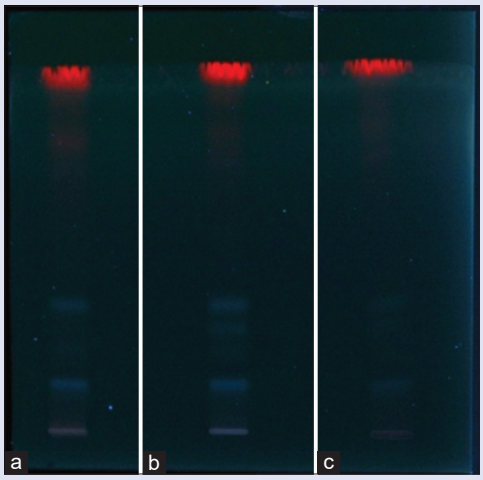

Figure 5: HPTLC phenolic fingerprint. (a) Extract produced from 36 h of Soxhlet extraction; (b) Extract produced from 6 min of microwave-assisted extraction (without modifier); (c) extract produced from 36 h of maceration

Nutraceutical analysis

This study was carried out to check whether the optimized MAE conditions can be extrapolated for the extraction of other nutraceutical principles present in the plant. In this regard, the effect of MAE condition on the extraction yield of ascorbic acid and tocopherol was evaluated. The yield of ascorbic acid and α-tocopherol from the extract obtained through MAE, Soxhlet, and maceration are given in Table 1. Extracts produced from MAE significantly produced more yield for both the nutraceutical principles. Thus, results clearly validate that MAE extraction conditions can be very well be extrapolated for the extraction of other nutraceutical principles as well.

Estimation of biomarker

The major biomarker present in *C. asiatica* is asiaticoside, and the plant owes most of its biological integrity because of the presence of this particular biomarker. Amount of asiaticoside in the extract produced from optimized MAE conditions, Soxhlet extraction and maceration was estimated [Table 1]. Results were in support of MAE whose yield was found to be significantly more than that of the other two methods. This fact is a clear indicative that the optimized MAE extraction conditions so derived is safe not only for the common class of phytoconstituents (such as phenolics and triterpenoids) but also for biomarkers and nutraceutical principles as well.

Sample pretreatment

Sample pretreatment was carried out with the intention to bring innovation in MAE operations. Results are shown in Tables 1 and 2. The results are indicative of the fact that increase in moisture content from 3.96% (control) to 54% indicated a significant rise in extraction yield of all the three principles approximately by threefold along with a significant rise in AUC and biomarker content as well [Tables 1 and 2]. However, sample pretreatment did not result in any significant increase of nutraceutical principles indicating early exhaustive extraction of such principles. The biological integrity of the extract produced from pretreated matrix was also intact as evident from improved

Table 1: Consolidated data table

Parameters	Extraction methods			
	Soxhlet	MAC	MAE	MAE (1 mL modifier)
Time	36 h	36 h	6 min	6 min
Moisture content (%)	3.96 ± 0.1^{a}	3.96 ± 0.1^{a}	3.96 ± 0.2^{a}	54.2 ± 0.2^{b}
TPC (GAE µg/g of dried extract)	2797 ± 188.1^{c}	1423 ± 115^{d}	3245 ± 142^{e}	9611 ± 350^{f}
TFC (QE µg/g of dried extract)	866 ± 52.3^{g}	655 ± 51^{h}	989 ± 42^{i}	2571 ± 90^{j}
TTC (UAE µg/g of dried extract)	255 ± 16.8^{k}	189 ± 15.5^{l}	344 ± 12^{m}	777 ± 30^{n}
Biomarker profiling				
Asiaticoside (mg/g of dried extract)	38.5 ± 2.5^{a1}	25.5 ± 2.7^{b1}	47.6 ± 1.3^{c1}	58.5 ± 1.6^{d1}
HPTLC profiling				
AUC	6408 ± 132^{a2}	5784 ± 140^{b2}	7548 ± 88.3^{c2}	9459 ± 128.2^{d2}
Bioactivity profiling				
Antioxidant (percentage inhibition at 100 µg/mL)	70 ± 2.4^{a3}	58 ± 2.7^{b3}	82 ± 1.4^{c3}	89 ± 1.4^{d3}
Nutraceutical profiling				
Ascorbic acid (µg/g of dried extract)	595 ± 26.2^{a4}	842 ± 33.1^{b4}	868 ± 17.1^{c4}	871 ± 17.1^{c4}
α-tocopherol (µg/g of dried extract)	5.28 ± 0.8^{a5}	4.85 ± 0.9^{a5}	7.09 ± 0.5^{b5}	7.1 ± 0.5^{b5}
Reproducibility				
RSD	6.4	7.9	3.9	3.6
Carbon load				
g CO_2/g of extract	24,000	NA	106.2	106.25

Data marked with different letters in each row are significantly different at $P<0.05$. Results are expressed as mean±SD ($n=3$). SD: Standard deviation; MAC: Maceration; MAE: Microwave-assisted extraction; TPC: Total phenolic content; GAE: Gallic acid equivalent; TFC: Total flavonoid content; TTC: Total triterpenoid content; RSD: Relative SD; QE: Quercetin equivalent; HPTLC: High-performance thin-layer chromatography; AUC: Area under the curve; NA: Not applicable; UAE: Ursolic acid equivalent

anti-oxidant activity [Table 1]. Further increase in moisture content to 76% yielded no significant difference probably indicating that exhaustive extraction has occurred. Increase in moisture content beyond 76% resulted in a significant decrease in extraction yield of all three principles [Table 2].

Results are in agreement with the principles of microwave heating. Moisture present in the matrix absorbs microwaves and generates heat stress within the sample matrix resulting in degradation of cellulose content of cell wall causing effective exhaustive leaching of analytes through the compromised cell wall.[23] However, the increase in moisture content can result in excessive heat stress which can degrade the phytoconstituents as well. Obtaining a balanced moisture content which is only favorable for MAE is very important.

Scanning Electron Microscopy (SEM) analysis

Scanning electron micrographs for control (macerated sample), MAE and Soxhlet treated samples were studied at two different magnifications [Figure 6]. At ×500 magnification the surface morphology was studied and at ×30000 magnification the complex cellular view was captured. Results were supportive of the fact that MAE causes cell wall rupture resulting in leaching of the analytes as explained above. The surface morphology and inside cellular view of the control and Soxhlet treated sample appeared intact and unaltered. Whereas, the surface morphology of microwave-treated sample appeared distorted and disoriented which is evident of the impact of localized thermal stress. Further to state that at ×30000 wider cellular pores were observed which could have been easy exit points for analytes resulting in exhaustive extraction. In case of maceration and Soxhlet extraction, no such phenomenon was observed and in such process leaching of the analytes occurs through a lengthy process of permeation of solvent through the rigid cell wall followed by solubilization of the analytes. Similar observations were also reported by Das *et al.*[24] and Inamdar *et al.*[25]

Comparison with other conventional extraction methods

Table 1 shows a comparative profile of MAE with that of Soxhlet extraction and maceration. Results are self-explanatory and indicative of the dominance of the proposed method in terms of yield, time, energy consumption, and reproducibility. Energy calculations were based on measuring the total consumption of energy resources by the process, and then by using standard conversion data, the amount of CO_2 can be calculated which is supposed to be liberated by the burning of coal to generate the required power to run the said extraction process in terms of electricity.[10]

CONCLUSION

The optimum MAE conditions for extraction of phenolics and triterpenoids was found to be 50% microwave power, 6 min extraction

Table 2: Effect of sample pretreatment on extraction yield

Amount of modifier	Moisture content (%)	TPC (GAE µg/gm of dried extract)	TFC (QE µg/gm of dried extract)	TTC (UAE µg/gm of dried extract)
1 mL	54.2	9611^{a}±350	2571^{e}±90	777^{i}±30
3 mL	76.5	9580^{a}±374	2562^{e}±92	988^{i}±36
5 mL	84.17	6940^{b}±345	1791^{f}±84	711^{j}±35
7 mL	88.21	5110^{c}±328	1302^{g}±85	580^{k}±32
Control	3.96	3245^{d}±142	989^{h}±42	344^{l}±12

Data marked with different letters (column wise) are significantly different at $P<0.05$. Results are expressed as mean±SD ($n=3$). Control: MAE without modifier. TPC: Total phenolic content; GAE: Gallic acid equivalent; TFC: Total flavonoid content; TTC: Total triterpenoid content; QE: Quercetin equivalent; UAE: Ursolic acid equivalent; SD: Standard deviation

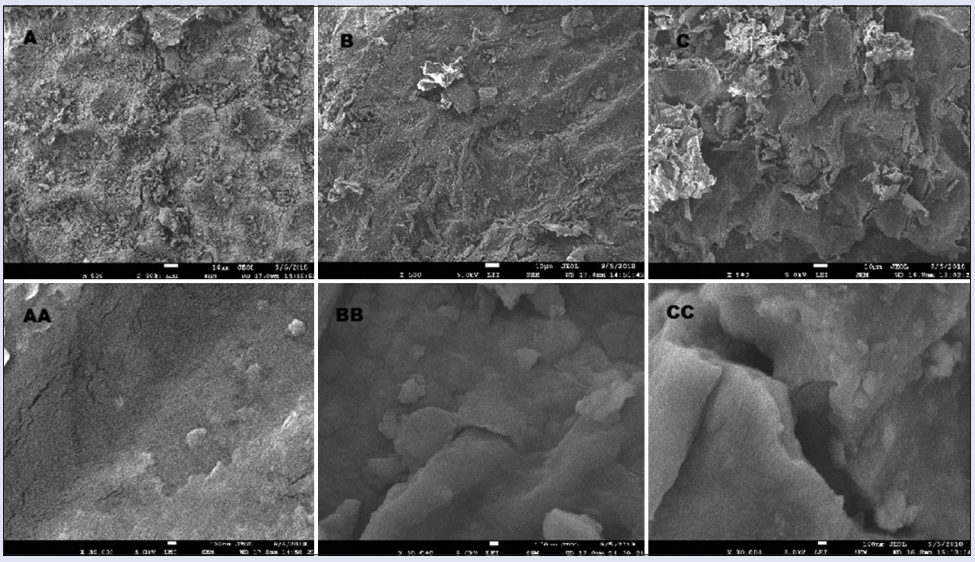

Figure 6: SEM images of marc for a better understanding of impact of microwaves. (A) surface morphology of marc obtained from maceration (×500); (AA) cellular morphology of marc obtained from maceration (×30000). (B) surface morphology of marc obtained after Soxhlet extraction (×500); (BB) cellular morphology of marc obtained after Soxhlet extraction (×30000). (C) surface morphology of marc obtained after MAE (×500); (CC) cellular morphology of marc obtained after MAE (×30000)

time and moisture content 50%–70%. For flavonoids, the optimum conditions of MAE differed slightly with microwave power 40% yielding best results with other conditions being the same. The yield obtained from 6 min of MAE at optimal conditions (sample pretreatment applied) was found to be threefold better than 36 h of soxhlet extraction for the extraction of all the three phyto-principles and produced 200 times lesser carbon load than Soxhlet. Comparison with Soxhlet is more valuable because both the operations involve application of heat, whereas maceration is devoid of heat. At the optimum extraction conditions, increased yield also resulted in better biological activity as well, thus making extracts produced from MAE superior in all respect. Green chemistry research is the need of the hour as environment and issues related to climate change has a strong impact on our economic growth. Innovations should be directed in making technology greener and sustainable for ensuring overall growth. In this regard, switching to such greener technology becomes a mandatory requirement. This research is a sincere effort to showcase the potential of green technology in the herbal drug industry.

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Conflicts of interest

There are no conflicts of interest.

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