Rapid and high yield Extraction method for Saponins from *Safed musli*

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ABSTRACT

Objectives: We aimed to develop, compare and optimise rapid and high yield extraction method for saponins of *Safed musli* using conventional extraction techniques and as well as modern microwave assisted solvent extraction method. **Materials and methods:** Roots of *Safed musli* (*Chlorophytum borivilianum*) are extracted by maceration, soxhlet, sonication and microwave methods. Extract further fractionated to obtain total saponins. Microwave assisted solvent extraction (MASE) method is optimised using Taguchi L9 orthogonal array design. Total saponins are estimated by High Performance Thin Layer chromatography (HPTLC) from all extracts obtained by different methods. **Results:** Factors namely temperature, irradiation time, irradiation power and powder size which potentially affects extraction efficiency are considered while optimizing MASE by statistical orthogonal array design procedure and saponins are quantified using HPTLC. Under developed optimum conditions, MASE showed significantly higher yield (5.11%) and drastic reduction in extraction time (4 min) than conventional extraction methods. **Conclusion:** Saponins of *Safed musli* shown highest yield in MASE and then maceration, soxhlet and sonication followed. The developed and optimised method of saponin extraction by MASE can have huge industrial applications after scale up.

Key words: HPTLC, Microwave assisted solvent extraction, Maceration Saponins, Orthogonal test L9 (34) Sonication, Taguchi Design.

INTRODUCTION

In Ayurveda, Siddha, Unani, *Safed musli* roots are very popular and well known for its aphrodisiac as well as immune-modulatory activity and hence it is important ingredient of 50 Ayurvedic and Unani preparations. *Safed musli* is also one of the important ingredients of very popular and useful Ayurvedic formula-Chyawanprash. Thirteen species of *Chlorophytum*, reported from India, sold as '*Safed musli*' in the Indian drug market. From research it is confirmed that the therapeutic effects of *Safed musli* are due to the presence of large amount of saponins. Among all species, *Chlorophytum borivilianum* produces the highest yield and highest saponin content. Its International drug market value is more than 300-700 tons per year. But factors like poor seed germination and dormancy are affecting uniform supply of this musli in market.[1] A solution to overcome such situation is the development of Rapid and high yield extraction method in order to obtain valuable metabolites. Traditionally the very common method for extraction of this saponin has been Soxhlet extraction. But the Soxhlet extraction method requires long heating time, bulk amount of organic solvents which again involves high risk of thermal decomposition of drug substances and pollution.[2] Despite of large preference to this method, researchers needs new fast and reliable methods of extraction. Microwave assisted Solvent extraction[3] offers simultaneous heating of sample material and solvent to obtain improved yield. The principle of MASE depends on dielectric properties of the solvent as well as of matrix where cell bursting is caused due to localized internal superheating followed by penetration of solvent into matrix and thus dissolution of the active components.[2] This surely enables improved and selective extraction of active phytochemicals with less time.[4] Hence the present work is reporting a new MASE method for fast and efficient extraction of Saponins from the roots of *Chlorophytum borivilianum* and comparison with conventional extraction techniques and optimization using Taguchi L9 orthogonal array design.[5]

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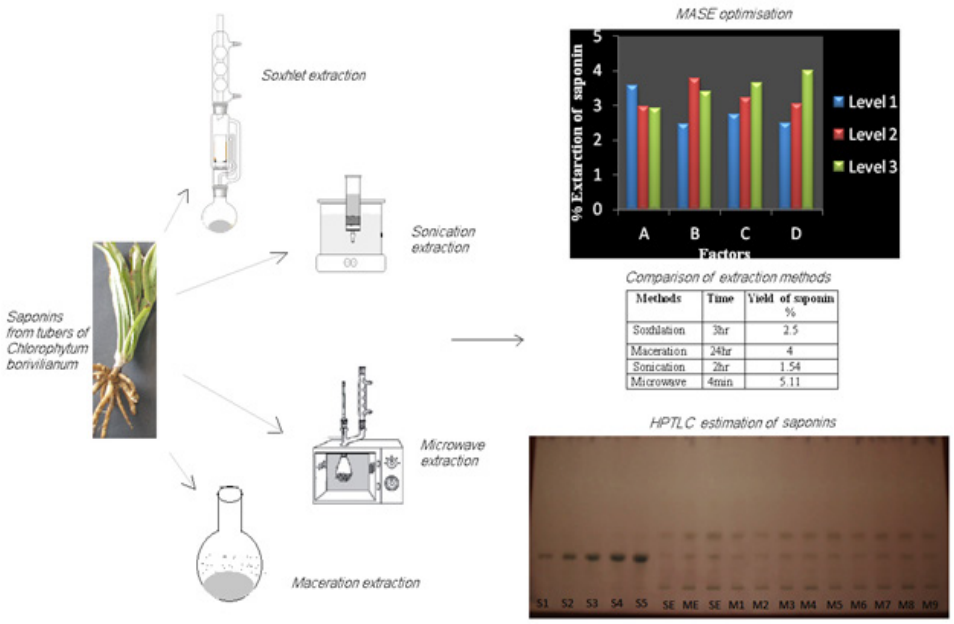

Graphical Abstract

MATERIALS AND METHODS

Plant materials

Chlorophytum borivilianum roots were purchased from local cultivator and authenticated by Head, Botany Department, Government Vidarbha Institute of Science and Humanities, Amravati). The roots were dried, powdered and defatted by petroleum ether and sieved through mesh size #22, 44, 60.

Reagents and Apparatus

All solvents used were of analytical grade. Percolated silica gel 60F254 plates for HPTLC analysis were obtained from E. Merck. Standard Saponin was isolated from tubers and confirmed in previous publication.[6] The microwave extractor was from Catalyst Systems (Pune, India). A HPTLC system was from Camag. A normal 500 ml capacity glass Soxhlet extractor was used Soxhlet extraction. Maceration was performed in 500 ml capacity glass maceration apparatus. Sonication extractor was from Spectralab Pvt. Ltd.

Extraction of Saponins

Soxhlet extraction

Soxhlet extraction was performed using a classical glass Soxhlet apparatus of capacity 500 ml. The tubers were washed, dried, powered and defatted with petroleum ether. 15 g powder (screened through mesh 22) was extracted for 3 hr with 300 ml. Finally extract was evaporated to dryness under vacuum.

Maceration extraction

Cold maceration extraction of 3 g sample (# 22) with 60 ml methanol was carried out in a closed glass conical flask for 24 hr. The suspension was filtered and filtered extract were evaporated to dryness under vacuum and processed further.

Sonication extraction

Sonication extraction was carried out in sonicator for 2hr. In this method, sound waves of high frequency pulses of 20 kHz are generated in an ultrasonic bath. The waves generated from transmitter easily penetrate the cell membrane by inducing a mechanical stress on the cell. This increases cell wall permeability causes solubilisation of maximum amount of active constituents.[7] Heat was not applied in this method. 3 g powdered drug sample (screened through sieve 22) was extracted with 60 ml methanol. The suspension was filtered and filtered extract were evaporated to dryness under vacuum and processed further.

Microwave Assisted Extraction

3 g of powder was placed in MASE vessel along with 60 ml of methanol solvent. Then vessel placed inside the microwave cavity and MASE was carried out at different power levels for different irradiation time, irradiation temperature, and different particle sizes. After extraction,

Table 1: Results of the orthogonal design (A-D are code)

Level	Microwave power (%) A	Irradiation temperature B	Irradiation time, (min) C	Sieve Number D
1	20	60	1	22
2	60	50	2	44
3	100	40	4	60

Table 2: Results of orthogonal test L9 (3^4)

Tests	A	B	C	D	Saponin (mg)	% Saponin
1	1	1	1	1	50.1	1.67
2	1	2	2	2	120.1	4.00
3	1	3	3	3	153.3	5.11
4	2	1	2	3	94	3.13
5	2	2	3	1	98.1	3.27
6	2	3	1	2	76	2.53
7	3	1	3	2	76.5	2.54
8	3	2	1	3	112.5	3.75
9	3	3	2	1	75.2	2.50

extracts were filtered and evaporated under vacuum to dryness.

Optimization method

MASE of Saponins from *Chlorophytum borivilianum* has been optimized using Taguchi-based optimization technique.[8] In this method minimum number of experiments required for accurate optimization reduces time and cost of extraction. All the results are mean of triplicate experiments. In the present work, three levels i.e. K1, K2, K3 are allotted for each of the factors as shown in Table 1. A L9 orthogonal array scheme which requires 9 experiments for optimization process was performed and extraction results are summarized in Table 2. The optimum level for each factor was determined from the graphical representation of the analysis of mean values from each level for a particular factor.

RESULTS AND DISCUSSION

Extraction

Extraction dried root powder was extracted by different conventional extraction techniques (soxhlet, sonication and maceration) and MASE as per our previously reported method where Methanolic extract fractionated by diethyl ether to give solid precipitate of saponins. Further extraction efficiency was reported by comparing yield of all methods.

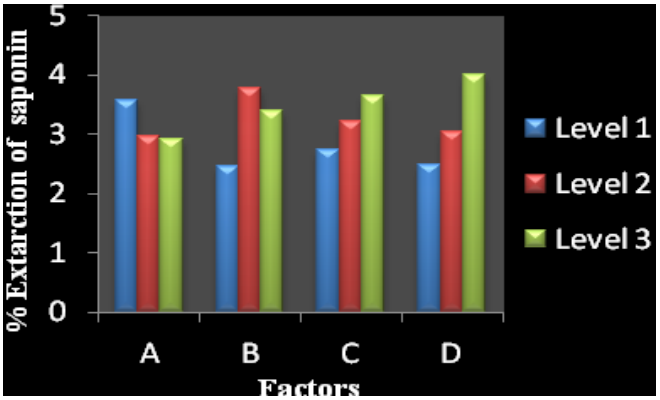

Figure 1: Graph showing Percentage extraction of saponin, obtained under orthogonal condition of MASE

Percentage extraction of Saponin = mass of saponin in crude extract ×100/ mass of raw material. A=Microwave power [level1=20%; level2=60%; level3=100%]; B=Irradiation temperature [level1=60°C; level2=50°C; level3=40°C] C=Irradiation time [level1=1min; level2=2min; level3=4min]; D=Sieve number [level1=22; level2=44; level3=60].

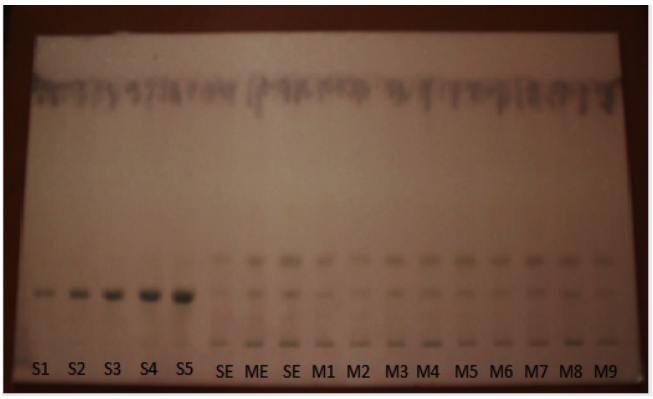

Figure 2: HPTLC chromatogram obtained by different extraction methods

S1-S5 = Standard compound spots; SE= Soxhlet extraction; ME= Maceration extraction; SE=Sonication extraction; M1= MASE Test1; M2= MASE Test2; M3= MASE Test3; M4= MASE Test4; M5= MASE Test5; M6= MASE Test6; M7= MASE Test7; M8= MASE Test8; M9= MASE Test9.

Optimization of microwave extraction conditions

As microwave extraction has been carried out for the first time it requires optimization for its different parameters which was done using an orthogonal array design. The factors were microwave power (A), irradiation temperature (B), irradiation time (C) and particle size (D). For each variable, the influence on the yield of saponin was considered from three levels. Figure 1 has been constructed based on the mean values obtained for each level from a particular factor to know influence of each variable on the extraction result. Figure 1 show when microwave power level was increased from 20% to 100% there was 0.66% decrease in amount of saponin. It has been also observed that the highest yield of saponin (5.11%) obtained when the sample was extracted with methanol at 50°C and yield was decreased at 60°C. Graphical representation of the analysis of means Figure 2 indicates that 20% microwave power

Table 3: Average yield of Saponin at different levels for each factors

Levels	A	B	C	D
K1	3.59	2.44	2.65	2.48
K2	2.97	3.67	3.21	3.02
K3	2.93	3.38	3.64	3.99

Table 4: Comparison of MASE with conventional extraction methods

Extraction methods	Extraction time	Solvent consumption (ml/g)	Yield of saponin (%)
Soxhlet	3 hr	20	2.5±0.1%
Maceration	24 hr	20	4±0.2 %
Sonication	2 hr	20	1.54±0.1%
MASE	4 min	20	5.11±0.3%

and irradiation temperature of 50°C were ideal to obtain maximum saponin content. Because it has been observed that with high microwave power and high irradiation temperature there might have been intense internal superheating of the plant matrix resulting in degradation of the active constituents which because of polar nature is more prone to damage due to intense microwave heating.[8]

Maximum amount of saponin was obtained with irradiation time of 4 min and yield of was dropped saponin slightly when irradiation time was increased to 2 min (Figure 2) This indicates that a irradiation time of 4 min is sufficient to bring about the extraction of saponin. Powder mesh size # 60 gave higher amount of saponin followed by # 44 and #22 Figure 2. This indicates that powder of fine mesh size 60 is suitable for extraction. With the use of fine particles, microwaves will be facilitated with deep penetration ability resulting in thermal degradation of active constituents.

From Table 3, the optimized experimental conditions are: microwave power- 20%, irradiation temperature - 50˚C, irradiation time- 4 min and powder of mesh size-60. It is possible to enhance the yield of Saponin using a different levels combination of factors found at optimum conditions.

Comparison of MASE and other conventional techniques

The selection of extraction methods mainly depends on the advantages and disadvantages of the processes such as extraction yield, complexity, production costs, environmental friendliness and safety.[9,10] In general soxhlet, maceration and sonication are the most commonly used extraction methods. The drawbacks of soxhlet, maceration and sonication are the large amount of solvent and long extraction time needed.[11] Compared with conventional extraction methods MASE method is selective, rapid, efficient, and economic. The results of comparison of MASE with conventional extraction methods for extraction of saponins from *Chlorophytum borivilianum* roots are shown in Table 4 in terms of amount of saponin. Highest yield of Saponins in less time was obtained by MASE which confirms reliability of this method.

Saponin Quantification by HPTLC

In HPTLC analysis, samples were spotted (2 µl) in the form of bands of width 6 mm, positioned 10 mm from the bottom of the plate, with a Camag microlitre syringe (100 ul) on precoated Silica gel F^{254} (20 cm×10 cm). The mobile phase used is chloroform: glacial acetic acid: methanol: water (16:8:3:2, v/v/ v). Linear ascending development for 30 min at room temperature in a twin trough glass chamber up to the height of the solvent front 80 mm was carried out. Quantification was done by Camag TLC scanner III at 264 nm. Standard solution (10 µg/µl in methanol) volumes of 1–5 µl were used corresponding

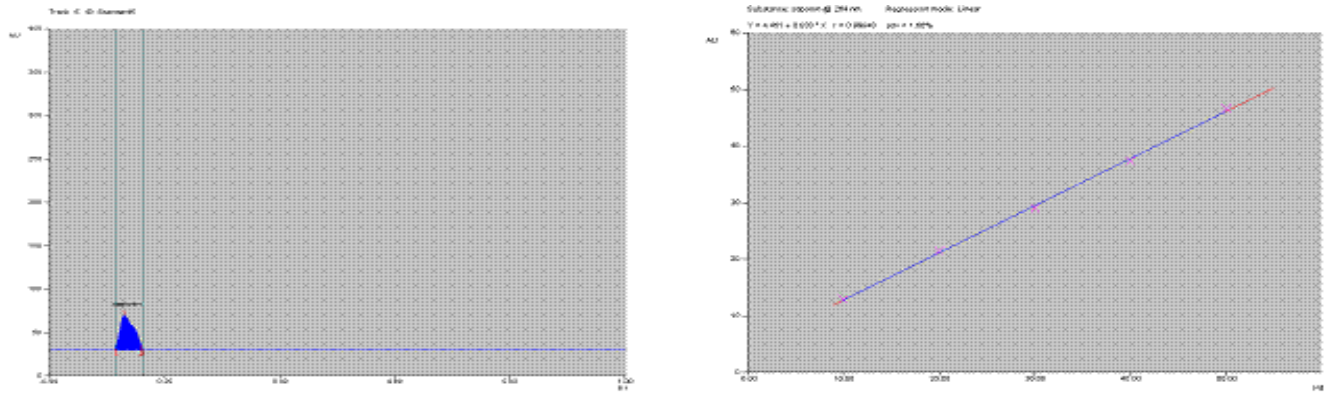

Figure 3: Chromatogram of standard Saponin and Calibration curve for total saponin content determination

to an amount of 1000–5000 ng. The different extracts obtained from extraction methods were subjected to HPTLC fingerprinting studies where, the concentration of standard solution was (10 µg/µl in methanol). The fingerprinting pattern of different extracts of *Chlorophtum borivilianum* roots are shown in Figure. 2 and 3. All extract are compared qualitatively as well as quantitatively by HPTLC fingerprinting in respect to the number and quantity of phytoconstituents present in each extracts, It has been found that maximum amount of saponins content is present in extract obtained by microwave assisted extraction. Track 9 and track 10 shows maximum area and it has shown higher quantity of saponin. Other parameters like percent yield, extraction time and solvent quantity are also compared. It has been found that, microwave assisted extraction method is effective in terms of time, quality and quantity of the saponins.

CONCLUSION

From this study it is explored that microwave extraction is most suitable extraction method for fast and exhaustive isolation of maximum amount of medicinally important saponins from tubers of *Chlorophtum borivilianum* as compared to maceration, soxhlet and ultrasound methods. Optimization method of orthogonal array design in case of MASE shows that the amount of saponins extracted is highly dependent on microwave power, irradiation temperature, irradiation time and matrix characteristics. Comparative extraction time and percentage of saponin fraction also confirms that MASE is the fastest and efficient extraction method among all extraction methods. Future scope involves industrial scale-up and necessary commercialisation of this novel method.

CONFLICTS OF INTEREST

Authors do not have any conflict of interest.

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Highlights of Paper

- *Safed musli* roots are very popular and important ingredient of more than 50 Ayurvedic and Unani preparations.
- Saponins are active constituents responsible for therapeutic efficacy of roots.
- Most of species of *Safed musli* are under endangered or rare list of biodiversity survey.
- Developed microwave assisted extraction method is rapid and allows exhaustive isolation of total saponins.

Author Profile

- **Dr. S. L Deore** is GOLD MEDALIST in B.PHARM from North Maharashtra University in 2004. She has completed M.Pharm from Govt. College of pharmacy, Amravati in 2006. She has awarded PhD in pharmaceutical sciences by SGB Amravati University in May 2011. She has isolated two saponins in her PhD work. She has published 35 research papers in various national- international journals about the medicinal plants and herbal drugs. She has authored two books "Plant biosynthesis" and "Experimental Phytopharmacognsoy" for Nirali Prakashan, Pune. Recently she has published Textbook of Pharmacognosy by PharmMed Press, Hyderabad. Her areas of research are isolation and structural elucidation of phytochemicals, neutraceutical development, and traditional medicine screening, chromatographic and phytochemical analysis of extracts.

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