Comparison of Conventional and Novel Extraction Techniques for the Extraction of Scopoletin from *Convolvulus Pluricaulis*

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ABSTRACT

Conventional extraction techniques like soxhlet extraction and reflux extraction and novel extraction techniques like Supercritical Fluid extraction (SFE), Ultrasonic Assisted Extraction (UAE) and microwave assisted extraction method (MAE) were used for the extraction of *Convolvulus Pluricaulis*. The scopoletin content in the extracts obtained using different extraction techniques was estimated using pre validated HPTLC method. The conventional and novel extraction techniques were compared on the basis of various parameters like extraction time, organic solvent consumption and extraction yield. The novel extraction techniques were found to be better than conventional methods as they require less time, consume less solvent and give higher yields of scopoletin. From amongst the novel extraction techniques used Microwave assisted extraction was found to give highest yield of scopoletin.

Keywords: Scopoletin, Ultrasound assisted extraction, Supercritical fluid extraction, Microwave assisted extraction.

INTRODUCTION

Convolvulus pluricaulis is a highly valued Medhya rasayana drug of the Ayurvedic system of medicine. It is described in Ayurveda as Shankpushpi.[1,2] It is reported to have anxiolytic,[3] antidepressant,[4,5] antiulcer,[6] neuroprotective[7] and nootropic[8] activities. It is one of the best and prominent natural medicines that help in improving memory.[9] Scopoletin (7-Hydroxy-6-methoxy coumarin) (Fig. 1) is one of the major coumarins in *Convolvulus pluricaulis* which is responsible for its biological activity.[10]

Conventional methods of extraction such as soxhlet and reflux which have been used for many decades are very time consuming and require relatively large quantities of solvent. The large amount of solvent used not only increases operating costs but also causes additional environmental problems. Therefore there is an increasing demand for new extraction techniques with shortened extraction time, reduced organic solvent consumption and increased pollution prevention. Recently several novel extraction methods such as Ultrasound assisted extraction (UAE), Microwave assisted extraction (MAE) and Supercritical fluid extraction (SFE) have been developed.[11] These methods can shorten the extraction time, decrease the solvent consumption and increase the extraction yield. A comparative study was made to estimate the extraction efficiency of scopoletin from *Convolvulus pluricaulis* using conventional and novel extraction techniques.

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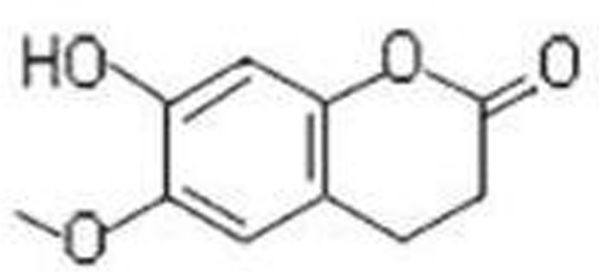

Figure 1: Structure of scopoletin.

MATERIALS AND METHODS

Plant material

The authenticated dried powdered plant of *Convolvulus pluricaulis* was obtained as a gift sample Amsar Pvt Ltd, Indore.

Chemicals and reagents

The solvents used for extraction were of Analytical Grade (AR) and for chromatographic studies were of HPLC grade (S. D. Fine chemicals). The standard scopoletin was purchased from Sigma-Aldrich.

Soxhlet extraction

Soxhlet extraction was carried out using dried and powdered plant of *Convolvulus Pluricaulis* using ethanol as a solvent. Ethanol was used as a solvent because it can extract both polar as well as non polar constituents. 10 grams of plant powder was placed in a thimble which was attached to soxhlet apparatus. Different drug: solvent ratios were tried i.e. 1:3, 1:5 and 1:7. The extraction was carried out for 12 hours. This was performed in three replicates. The extracts obtained were analysed for scopoletin content by HPTLC.

Reflux extraction

Dried and powdered plant of *Convolvulus pluricaulis* was refluxed with ethanol at 60°C. 10 gram of plant material was added to the round bottom flask (RBF) and kept on a heating mantle with constant temperature (60°C). The evaporated solvent collected back due to condensation through the condenser which was connected to the same RBF. The Reflux extraction was carried out at different time intervals i.e. 4, 6 and 8 hrs. The drug: solvent ratio (1:5) was kept case of constant as it was optimised during Soxhlet extraction. The extracts obtained were analysed for scopoletin content by HPTLC.

Ultrasound assisted extraction

The Ultrasound assisted extraction was carried out using both probe ultrasonicator and bath ultrasonicator. Ethanol was used as a solvent. In case of probe ultrasonicator the plant material is in direct contact with ultrasonic waves whereas in bath ultrasonicator the ultrasonic waves have to pass through the glass material in order to come in contact with the plant material. Probe Ultrasonicator was from Dakshin Pvt Ltd with power of 30 watts and frequency 20 KHz. Bath Ultrasonicator was from Supersonics with power of 30 watts. Here both instruments were used in order to study the effect of direct and indirect contact of ultrasonic waves on extraction of scopoletin from *Convolvulus pluricaulis*. The time of extraction reported was different for similar phytoconstituents from different plant varieties.[12–15] This experiment was carried out at different time intervals in order to determine the optimum time required for extraction of scopoletin from *Convolvulus pluricaulis*. The extracts obtained were analysed for scopoletin content by HPTLC.

Supercritical fluid extraction

The absence of both light and air during the extraction process along with relatively mild operating temperature can reduce the tendency of degradations, while the use of CO_2 as a solvent allows working in an environmentally clean media. SFE was performed using Supercritical fluid extractor from Supercritical fluid Technologies. Inc SFT-XW-100 by static extraction method. 100 g powder of *Convolvulus pluricaulis* was packed in a thick muslin cloth and loaded into an extraction vessel. It was equipped with temperature controllers and pressure valves. CO_2 gas was pressurized with a pump and allowed to pass through plant powder. The fluid CO_2 and the dissolved compounds were transported to separators, where the solvation power of the fluid is increased by increasing the pressure of the CO_2 gas. The product is then collected via valve located in the lower part of the separators. Supercritical fluid extraction was carried out at constant temperature and time and the pressure of extraction was varied. The pressure of extraction of scopoletin from different plant varieties reported in the literature was with ethanol as a modifier.[16–18] In this experiment only pure carbon dioxide was used for extraction so that resultant extract will be free from ethanol residues. The effect of different pressures on extraction efficiency was estimated by using carrying out the extraction at different pressures. Pure carbon dioxide was used. HPTLC method was used for analysis of the extracts.

Microwave assisted extraction

Microwave Assisted Extraction was carried out at constant temperature and time and the extraction power of microwave was varied. Ethanol was used as a solvent.

The instrument used was scientific microwave system from Catalyst Systems CATA-4R. The extraction power of microwave reported was different for extraction of similar phytoconstituents from different plant varieties.[19–22] This experiment was performed using different extraction power of microwave in order to estimate the optimum microwave power required for maximum extraction of scopoletin from *Convolvulus pluricaulis*. The extracts obtained were analysed for scopoletin content by HPTLC.

HPTLC method

All the ethanolic extracts of *Convolvulus pluricaulis* prepared by different extraction methods were analysed using a pre-validated HPTLC method.[23] Silica Gel 60 GF_{254} plates (20 × 10 cm) were used as stationary phase. Sample and standard solutions were applied on the plate as 8 mm wide band with an automated Camag TLC applicator, Linomat 5 positioned 15 mm from the bottom of the plate and 20 mm from the side of the plate. The mobile phase used was toluene: ethyl acetate: methanol (6:6:0.5).[23] After development, the plate was removed and dried and spots were visualized in Camag UV chamber at 366 nm. The quantification was done using Wincats software.

RESULTS AND DISCUSSION

Optimisation of ultrasound assisted extraction

The extraction was carried out at constant temperature (60°C) and power (30 watts) and the extraction time was varied. In case of probe ultrasonicator, the extraction was carried out at different time intervals such as 5, 10, 15 and 20 minutes. When extraction was carried out using probe ultrasonicator, the yield of extract increased with increase in time of extraction. Maximum yield was obtained at 20 minutes. The time was not extended beyond 20 minutes as there was no significant increase in yield after 20 minutes. Hence the optimum time was chosen as 20 minutes with probe ultrasonicator.

For bath ultrasonication different time intervals such as 15, 30, 45 and 60 minutes were used. When extraction was carried out using bath ultrasonicator, the yield of extract increased with increase in time of extraction. Maximum yield was obtained at 60 minutes. The time was not extended beyond 60 minutes as there was no significant increase in yield after 60 minutes. Thereby 60 minutes was the optimized time.

Optimisation of supercritical fluid extraction

Supercritical fluid extraction was performed at varying pressures i.e. 4500 psig, 5000 psig, 6000 psig and 6500 psig. The temperature (50°C) and extraction time (60 minutes) were kept constant. In case of SFE, the yield of extract increased with increase in pressure of extraction. Maximum yield was obtained at pressure 6000 psig and temperature 50°C after 60 minutes. Hence the optimum pressure for Supercritical fluid extraction was chosen as 6000 psig.

Optimisation of microwave assisted extraction

Microwave Assisted Extraction was carried out at constant temperature (60°C) and time (1minute). The power of microwave was varied as 140, 280, 420 and 560 watts i.e. 20%, 40%, 60% and 80% respectively. It was observed that the yield of extract increased with increase in power of microwave used. Maximum yield was obtained when extraction was carried out using power of 560 watts at 60°C for 1 minute. When the power of extraction was increased beyond 560 watts it led to charring and complete evaporation of solvent, hence the power of microwave used for extraction was restricted to 560 watts. Hence the optimum microwave power for Microwave extraction was chosen as 560 watts.

Comparison of extraction yield obtained by different extraction techniques

The peak area obtained for standard scopoletin and that for different extracts were compared using HPTLC chromatograms (Figure 2) and the content of scopoletin in each extract was calculated. The temperature was maintained between 50–60°C for all the methods. Hence the same effect will be seen for all the extraction methods. The scopoletin content obtained with conventional extraction methods is about 10% whereas with novel extraction methods the scopoletin content obtained was about 20–40% (Table No. 1). There is 2–3 times increase in scopoletin content extraction yield with novel extraction methods. In case of Supercritical fluid extraction, the yield was slightly lesser than conventional methods because carbon dioxide being non polar, could not efficiently extract the semi- polar scopoletin. In this method there was no organic solvent consumption, hence the extract obtained was free from ethanol residues. In general there is prominent increase in scopoletin content with novel extraction methods as compared to conventional extraction methods.

Comparison of extraction time required for different extraction techniques

The time required for extraction for conventional and novel extraction techniques were compared. The time required for each extraction technique is given in the Table 2. The extraction time required for conventional extraction methods i.e. soxhlet and reflux is about

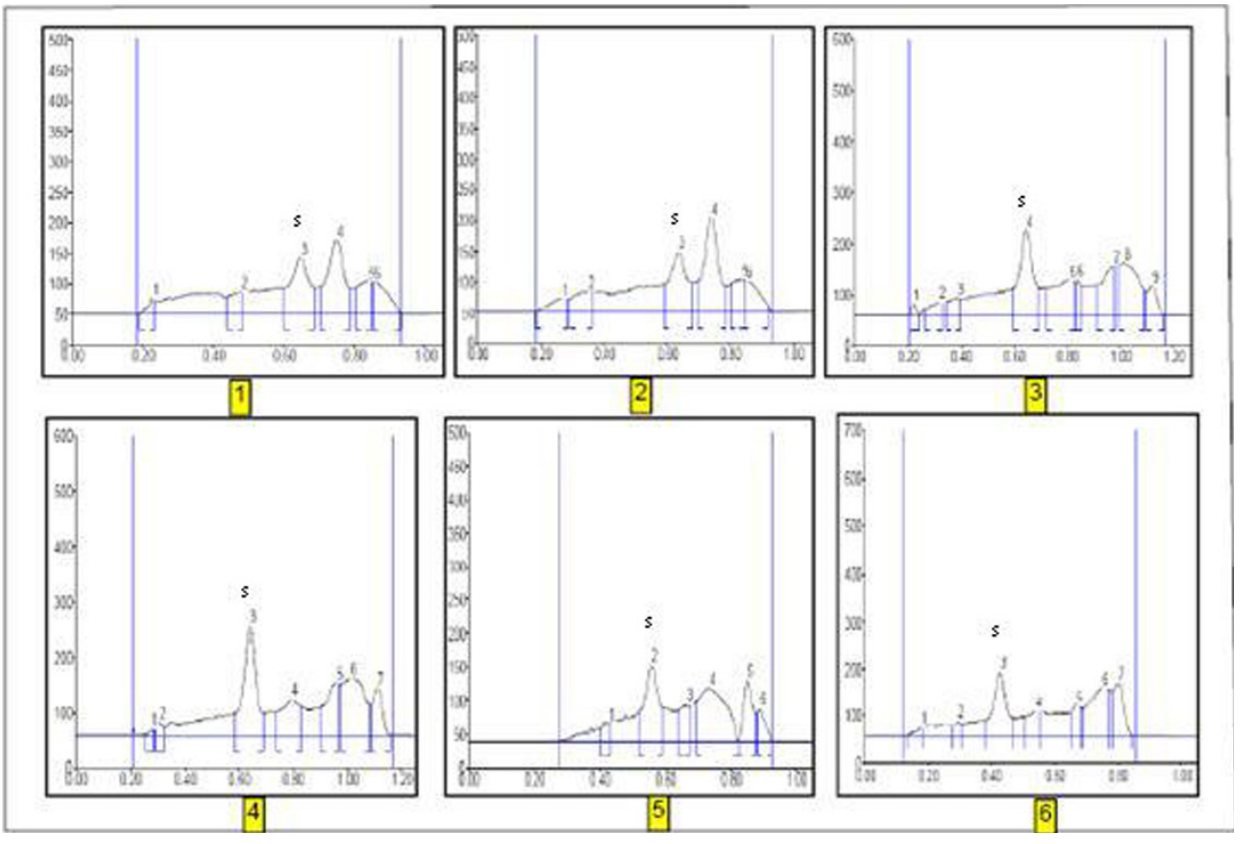

Figure 2: HPTLC chromatograms obtained by:1- Soxhlet extraction, 2- Reflux extraction, 3- Probe ultrasonication, 4- Bath ultrasonication, 5- SFE, 6- MAE. S- Peak of scopoletin.

Table 1: Percentage Yield of Scopoletin Obtained by Different Extraction Techniques

Extraction method	Time (minutes)	Pressure/ Power	% yield of scopoletin
Soxhlet	720	–	9.58
Reflux	240	–	8.57
	360	–	9.43
	480	–	10.95
UAE (Probe ultrasonicator)	5	60 watts	14
	10	60 watts	16.39
	15	60 watts	22.61
	20	60 watts	23.55
UAE (Bath ultrasonicator)	15	60 watts	11.59
	30	60 watts	18.26
	45	60 watts	22.23
	60	60 watts	31.87
MAE	1	140 watts	18.12
	1	280 watts	20.16
	1	420 watts	36.33
	1	560 watts	45.10
SFE	60	4500 psig	6.73
	60	5000 psig	7.07
	60	6000 psig	7.99
	60	6500 psig	7.51

Table 2: Time Consumed for Different Extraction Techniques

Sr. No.	Extraction method	Extraction time (min.)
1.	Soxhlet extraction	720
2.	Reflux extraction	480
3.	Ultrasound assisted extraction	20
i)	Probe ultrasonication	20
ii)	Bath ultrasonication	60
4.	Supercritical fluid extraction	60
5.	Microwave assisted extraction	1

Table 3: Solvent Consumption for Different Extraction Techniques

Sr. No.	Extraction method	Solvent consumption (ml)
1.	Soxhlet extraction	200
2.	Reflux extraction	100
3.	Ultrasound assisted extraction	
i)	Probe ultrasonication	50
ii)	Bath ultrasonication	50
4.	Supercritical fluid extraction	–
5.	Microwave assisted extraction	50

500–700 minutes and for novel extraction methods like UAE, SFE and MAE, it is about 1–60 minutes (Table No. 2). There is 10 times decrease in extraction time with novel extraction methods. Thus with the novel extraction methods there is remarkable reduction in the time required for extraction.

Comparison of solvent consumption for different extraction techniques

The consumption of organic solvent for conventional extraction methods is about 100–200 ml whereas novel extraction methods consume about 50 ml of solvent (Table No. 3). There is 4 times decrease in solvent consumption with novel extraction methods. In case of Supercritical fluid extraction no organic solvent was consumed as the extraction was carried out using carbon dioxide. Thus with novel extraction methods the organic solvent consumption can be significantly reduced, thereby environmental pollution caused by organic solvents can also be reduced. The novel extraction methods are environment friendly methods.

CONCLUSION

Thus, based on this study, it can be concluded that novel extraction methods give better yield as compared to conventional extraction methods in shorter period of time with less solvent consumption. These methods are superior to conventional extraction methods with respect to extraction time, solvent consumption and extraction efficiency.

From amongst the novel extraction methods used, microwave assisted extraction was found to be the best one for extraction of scopoletin from *Convolvulus pluricaulis*, as it gave the highest yield in shorter period of time. In spite of all these advantages the novel extraction techniques have limited field of application. The equipments used for these process have high capital cost. More research is needed to improve the understanding of extraction mechanism, remove technical barriers, improve the design and scale up of the novel extraction systems for their industrial applications.

REFERENCES

1. Sethiya NK, Mishra SH. Review on ethnomedicinal uses and phytopharmacology of memory boosting herb Convolvulus pluricaulis Choisy. Aust. J. Med. Herbal. 2010; 22(1), 19–25.
2. Velishala H. Shankapushpi: A short review. International Research Journal of Pharmacy 2012; 3(1), 81–83.
3. Nahata A, Patil UK, Dixit VK. Anxiolytic activity of *Evolvulus alsinoides* and *Convolvulus pluricaulis* in rodents. Pharm. Biol. 2009; 47(5), 444–451.
4. Dinesh D, Rekha V. Evaluation of the antidepressant-like activity of *Convolvulus pluricaulis* choisy in the mouse forced swim and tail suspension tests. Med. Sci. Monit. 2007; 13(7), 155–61.
5. Pawar AS, Dhuley JN, Naik SR. Neuropharmacology of an Extract derived from Convolvulus microphyllus. Pharm. Biol. 2001; 39(4), 253–258.
6. Sairam K, Rao CV, Goel RK. Anti ulcer Effect of *Convolvulus pluricaulis* Chois. on gastric ulceration and secretion in rats. Indian. J. Exp. Biol. 2001; 39(4), 350–354.
7. Bihaqia SW, Sharma M, Singh AP, Tiwari M. Neuroprotective role of *Convolvulus pluricaulis* on aluminium induced neurotoxicity in rat brain. J. Ethnopharmacol. 2009; 124(3), 409–415.
8. Malik J, Karan M, Vasisht K. Nootropic, anxiolytic and CNS-depressant studies on different plant sources of shankhpushpi. Pharm. Biol. 2011; 49(12), 1234–42.
9. Sharma K, Bhatnagar M, Kulkarni SK. Effect of *Convolvulus pluricaulis* Choisy and Asparagus racemosus Wild on learning and memory in young and old mice: a comparative evaluation. Indian. J. Exp. Biol. 2010; 48(5), 475–85.
10. Aulakh GS, Narayanan S, Mahadevan G. Phyto-Chemistry and Pharmacology of Shankapushpi – Four Varieties. Anc. Sci. Life. 1988; 7(3), 149–56.
11. Wang L, Weller CL. Recent advances in extraction of neutraceuticals from plants. Trends Food. Sci. Technol. 2006; 17, 300–312.
12. Devgun V, Nanda A, Ansari SH. Comparison of Conventional and non Conventional methods of extraction of Heartwood of *Pterocarpus Marsupium* Roxb. Acta. Pol. Pharm. 2012; 69(3), 475–485.
13. Mircea V. An Overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason. Sonochem. 2001; 8, 303–313.
14. Hromadkova Z, Kostalova Z, Ebringerova A. Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. Ultrason. Sonochem. 2008; 15, 1062–1068.
15. Wang J, Zhao YM, Guo CY, Zhang SM, Liu CL, Zhang DS et al. Ultrasound assisted extraction of total flavonoids from *Inula helenium*. Pharmacogn. Mag. 2012; 8(30), 166–170.
16. Tzeng TC, Lin YL, Jong TT, Chang CM. Ethanol modified supercritical fluids extraction of scopoletin and artemisinin from *Artemisia annua* L. Sep.Purif. Technol. 2007; 56, 18–24.
17. Cheah ELC, Chan LW, Heng PWS. Supercritical carbon dioxide and its application in the extraction of active principles from plant materials. Asian.J.Pharm.Sci. 2006; 1, 59–71.
18. Teng WY, Chen CC, Chung RS. HPLC Comparison of Supercritical Fluid Extraction and Solvent Extraction of Coumarins from the Peel of *Citrus maxima* Fruit. Phytochem.Anal. 2005; 16, 459–462.
19. Jain T, Jain V, Pandey R, Vyas, Shukla SS. Microwave assisted extraction for phytoconstituents-An Overview. Asian. J. Research. Chem. 2009; 2(1), 19–25.
20. Shailajan S, Yeragi M. Optimisation of Microwave assisted extraction of Luteolin from leaves of *Vitex negundo* Linn and its comparison with conventional extraction method. International Journal of Pharmaceutical Research and Development 2011; 3(5), 128–134.
21. Jaiswal Y, Tatke PA. An Overview of Microwave Assisted Extraction and its Applications in Herbal Drug Research. J.Med.Plants.Res. 2011; 5(1), 21–31.
22. Mandal V, Mohan Y, Hemalatha. Microwave Assisted Extraction — An Innovative and Promising Extraction Tool for Medicinal Plant Research. Pharmacogn.Rev.2007; 1(1), 7–18.
23. Dhruv N, Tatke PA. Isolation of scopoletin and standardization of formulation containing Shankpushpi. Thesis for MPharm submitted to C. U. Shah College of pharmacy, S. N. D. T. Women's University.