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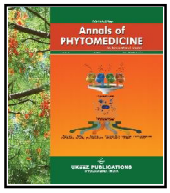

Original Article: Open Access

Optimization and comparison of different extraction techniques for colchicine from the seeds of *Gloriosa superba* L.

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Abstract

Gloriosa superba L., an important medicinal and horticultural crop, well-recognized for the commercial source of colchicine which is present in its seeds and tubers mainly. In the present study, in order to recover the key constituent, *i.e.*, colchicine from the seeds of *G. superba*, examination and comparison of five major extraction methods was done by using HPLC. The methods studied include conventional as well as modern methods. Optimization of extraction parameters like extracting solvent, extraction duration was performed for each method initially and then best condition in each was compared. Results revealed significant variation among the screened methods for colchicine content. Colchicine (%) recorded in different methods as Soxhlet, reflux, cold, SAE and MAE was 0.71%, 0.71%, 0.67%, 0.67% and 0.68%, respectively. Reflux method was found to be most attractive capable of gaining maximum total extract (18.35%) and colchicine (0.71%) with extraction duration one hour and using biosolvent, ethyl alcohol. It was noticed that using Soxhlet method, results are statistically same as in reflux but with methanol solvent (total extract (12.69%) and colchicine (0.71%)). So, we can opt either reflux or Soxhlet method of extraction depending upon our objective for extraction. The study would be useful to fulfill the increasing demand of colchicine and to evaluate germplasm with minimum resources.

1. Introduction

In medicinal plants, important constituents are secondary metabolites which are highly significant in terms of both economic and medicinal value for human beings as well as animals, as they serve as key molecules in modern medicine.One such plant is *Gloriosa superba* L., an industrially important medicinal plant and is valued due to its high colchicine content. *G. superba* belonging to family Liliaceae, commonly known as 'Kalihari' and 'Glory lily' and is native of Tropical Africa which is now growing naturally in many parts of Tropical Asia as in India, Myanmar, Malaysia and Srilanka (Jayaweera, 1982; Singh, 2006; Ade and Rai, 2009). In India, it occurs from hotter southern parts to milder mid hill zones of states like Himachal Pradesh, Jammu & Kashmir and Uttar Pradesh (Anonymous, 1982; Chopra *et al*., 1956; Chandel *et al*., 1996) The plant is declared as 'endangered' species by IUCN Red Data Book mainly due to its over exploitation from the natural habitats (Anonymous, 1997; Badola, 2002; Sivakumar and Krishnamurthy, 2002a, b). The National Medicinal Plant Board, Government of India, has also included this species in the prioritized species of medicinal plants for cultivation in India.

G. superba is mainly valued due to the presence of alkaloids which are structurally heterogeneous class of secondary biomolecules derived from basically five amino acids ornithine, lysine, phenylalanine, tyrosine and tryptophan (Thakur *et al*., 1975). The species produces an important alkaloid colchicine, which is present in seeds, stem, leaves and tubers (Sharma *et al*., 2017), while the other compounds present in plant are lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyl deacetylcolchcine (Sugandhi, 2000). A new colchicine glycoside 3-O-demethylcolchicine-3-O-alpha-D-glucopyranoside in *G. superba* seeds is reported by (Suri *et al*., 2001). Colchicine is the principal alkaloid persent in the tubers of *G. superba* (Dunuwille *et al*., 1968). Also, reported up to 90% loss of colchicine during extraction. With the discovery of colchicine in *Gloriosa*, its commercial importance has increased as it has a higher content of colchicine than *Colchicum* spp.(Yoshida *et al*., 1988). About 24 alkaloids among them, colchicine and colchicoside are the principal ones, as well as presence of 10 non-alkaloid compounds including beta-sitosterol, chelidonic acid, luteolin, stigmasterol, *etc*., in the *G. superba* (Nautiyal, 2011). Chitra and Rajamani (2009) reported colchicine, 3-demethyl colchicine and colchicoside as major alkaloids in *G. superba*.

Colchicine is a well-known an amino alkaloid derived from the amino acids phenylalanine and tyrosine, can also be stated as phenethylisoquinoline alkaloid with a tropolane ring (Kulkarni and Patel, 2010). Molecular formula of Colchicine $C_{22}H_{25}NO_6$ with IUPAC name N-[(7S) - 1,2,3,10-tetramethoxy-9-oxo-5,6,7,9- tetra hydrobenzo [*a*] heptalen-7-yl] acetamide and molecular weight is 399.44 g/mol (Maslarska and Pencheva, 2014). Chemical structure of colchicine is given in Figure 1 and photograph of studied plant is given in Figure 2.

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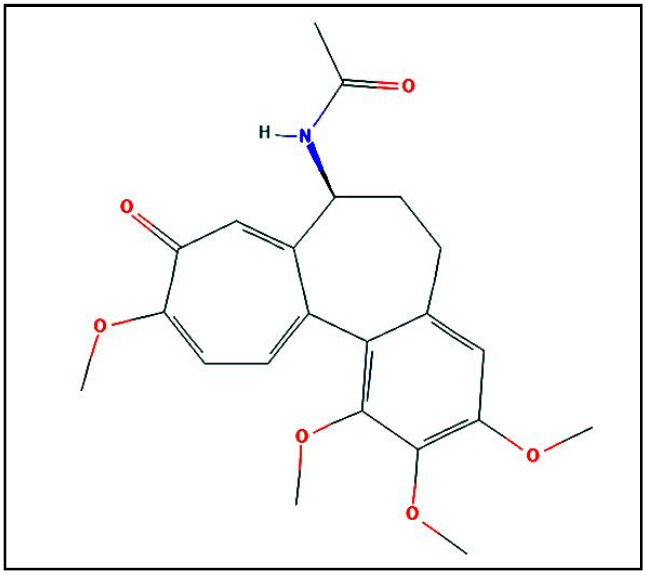


Figure 1: Chemical structure of colchicine.

Figure 2: *Gloriosa superba* L. plant.

The seeds are the best source of colchicine as colchicine content is 2-5 times higher than in tubers (Jana and Shekhawat, 2011). Colchicine is used in treatment of gout, arthritis (Nadkarni, 2002), rheumatism (Gupta, 1982), cancer (Chopra *et al*., 1956) and in plant breeding for inducing polyploidy (Jana and Shekhawat, 2011). Plant is also documented to be used for treating cholera, typhus, Bright's disease, piles, skin diseases, leprosy, gonorrhea and chronic ulcers by different authors (Chopra *et al*., 1956; Lakshmi and Swathi, 2015). Seeds are reported to be used for rheumatic pain and as a muscle relaxant (Gupta, 1982).

Keeping in view the nature of targeted compound (*i.e.*, alkaloid), two polar solvents, *viz*., methanol and ethyl alcohol were used for extraction by five different methods, *viz*., soxhlet, reflux, cold extraction, sonication assisted extraction and microwave assisted extraction. To the best of our knowledge, comparative studies of different extraction methods for the extraction of colchicine from *G. superba* have not been documented earlier. The purpose of the study is to optimize five different extraction methods for *G. superba* and compare them with their best conditions. This study helps us to select an appropriate method for the isolation of colchicine for different purposes, in addition to this also in conservation of our valuable plant resources.

2. Material and Methods

2.1 Plant material and chemicals

The seeds of *G. superba* were procured from Department of Medicinal Plants, TNAU, Coimbatore, Tamil Nadu, India.The standard compound, *i.e.*, Colchicine was purchased from Sigma Aldrich (Catalogue No. C9754). Solvents, *i.e.*, methanol and ethyl alcohol used were of analytical grade. HPLC solvents acetonitrile, glacial acetic acid and water were of Merck brand (Darmstadt, Germany) manufactured by Merck, India.

2.2 HPLC system

The system used is of Waters binary HPLC unit with Waters HPLC pump 515, dual λ absorbance detector 2487 and program used for data analysis was Empower II software. HPLC method was used as developed by Sharma *et al.*, (2020).

2.3 Sample preparation for extraction

The procured seed material was properly cleaned, air dried for two days in dark place and then dried in oven (35-40°C) for 30 min. The dried material was ground with pestle and mortar and sieved to form uniform particle size of powdered material. For optimization and comparison of extraction technique,this material was used.

2.4 Extraction experiments

The powdered plant material was extracted using five different extraction methods, *viz*., soxhlet extraction, reflux extraction, cold extraction, Sonication assisted extraction and Microwave assisted extraction. In each method, extraction duration was decided by previous experiments with longer time duration, for instance, in Sonication assisted extraction trials had done upto 40 min but results shows stabilized colchicine (%) from 5 to 40 min extraction period, similar the case for microwave assisted extraction.

(i) Soxhlet, reflux and cold extraction: Seed sample was 2 g with 100 ml solvent. Each experiment was conducted with two solvents, *viz*., methanol and ethyl alcohol and for five extraction durations, *viz*., 0.5 h, 1 h, 1.5 h, 2 h and 4 h with four replications in each.

(ii) Sonication assisted extraction (SAE) and microwave assisted extraction (MAE): Seed sample was 2 g with 50 ml solvent. The experiment was conducted with two solvents, *viz*., methanol and ethyl alcohol and for six extraction durations, *viz*., 1 min, 2 min, 3 min, 4 min and 5 min and 10 min with four replications in each.

In each method, after extraction, filtration (except soxhlet), and distillation off the solvent from each sample was done and the residue was air dried upto a constant weight. Total extract (%) was noted and then each sample was further analysed for quantification of colchicine by using HPLC.

2.5 HPLC sample preparation

The well dried extracted samples were diluted with mobile phase (acetonitrile : water :: 60:40, v/v), centrifuged at 4000-4500 rpm then filtered through 0.2 µm membrane prior to injection in the HPLC system. HPLC method developed by Sharma *et al*. (2019) was followed for the HPLC sample preparation and their qualitative and quantitative estimation using same Waters HPLC unit.

2.6 Comparison of different extraction methods

To find out the best extraction method for *G. superba* in terms of total extract (%) and colchicine content (%) studied extraction methods were compared with their best conditions. This experiment was conducted under completely randomized design (CRD) with five treatments, *i.e.*, extraction methods and five replications.

2.7 Statistical analysis

The entirestudy was divided into five experiment sets of extraction based on experiment designs, factors considered and factor level. These experiments set were further compared in terms of total extract (%) and colchicine content (%) in the extract. Analysis was performed with OP-STAT software. The factors examined are the extraction durations and solvents type. CRD factorial statistical design was applied in each experimental set. Comparison of each method with their best conditions was statistically analyzed by one-way analysis in order to conclude the best extraction method both in terms of total extract and colchicine content.

3. Results

In the present study, out of the two solvents, the mean total extract and mean colchicine content were higher when extraction was done with methanol under all extraction methods. From Figure 3, it can be stated that reflux method is the best among all others. The mean colchicine content was obtained maximum (0.717%) under reflux extraction with ethyl alcohol as solvent (Figure 4).

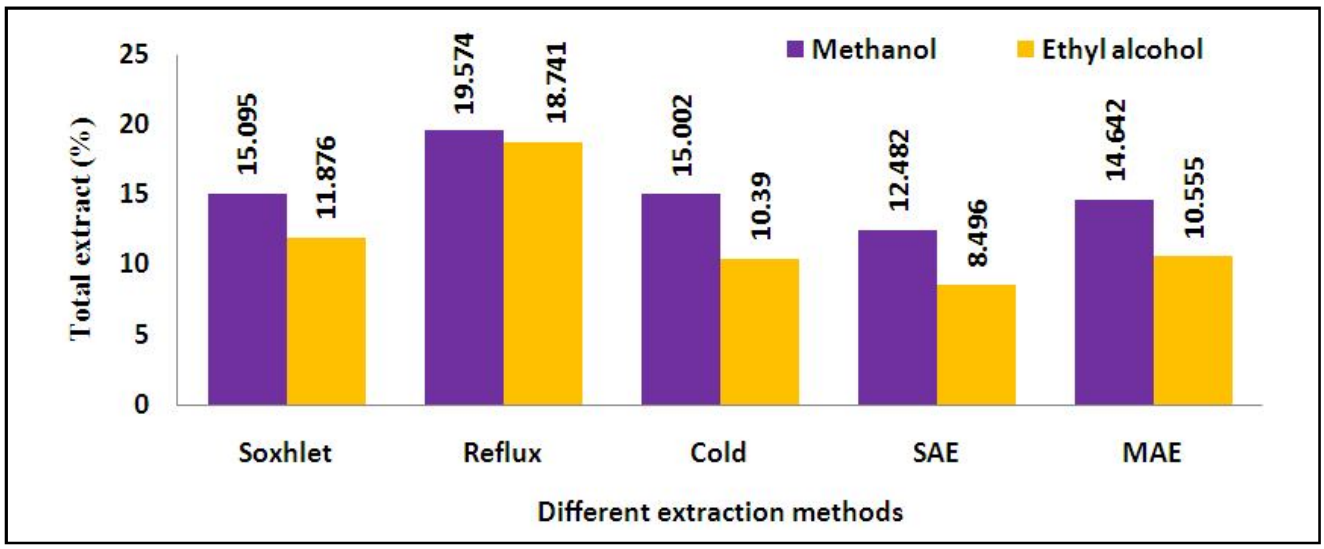


Figure 3: Mean total extract from *G. superba* under different extraction methods.

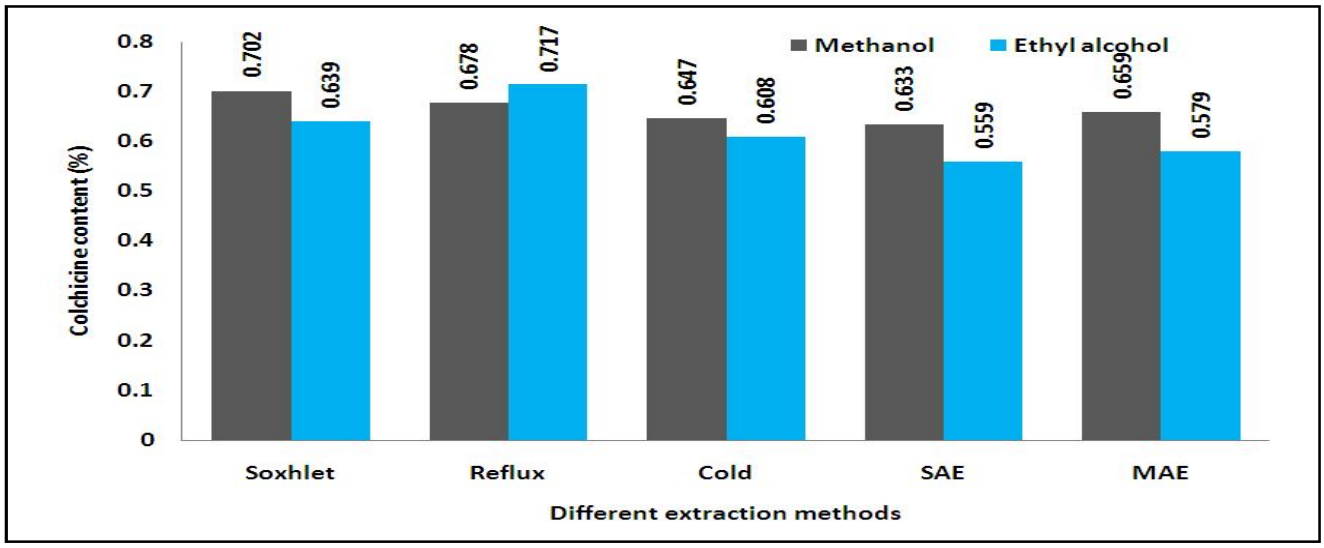


Figure 4: Mean colchicine content in *G. superba* under different extractions method.

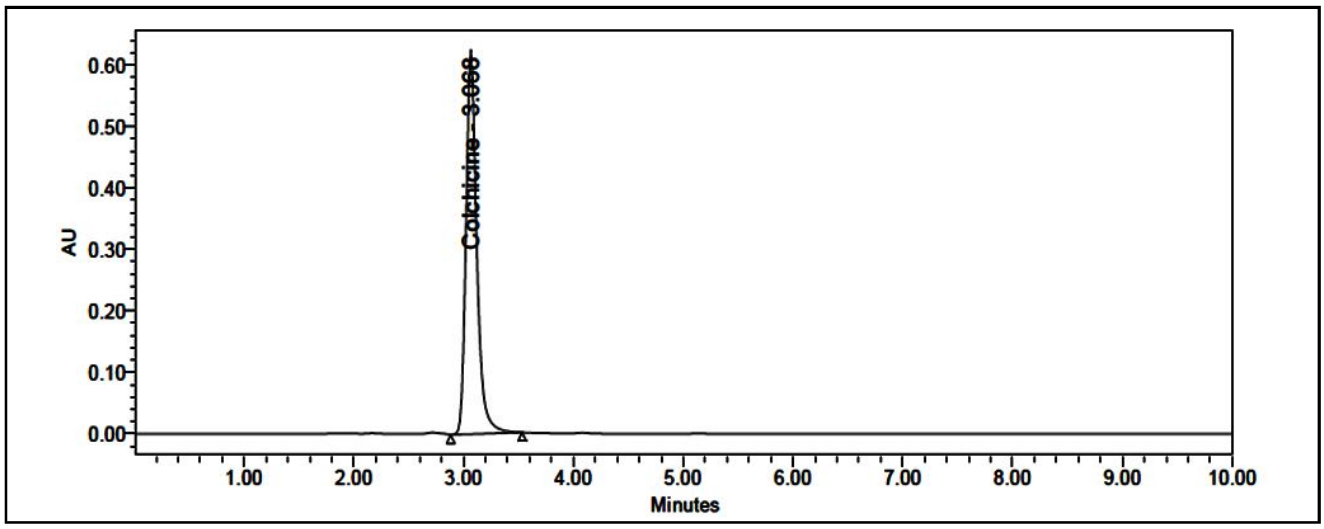
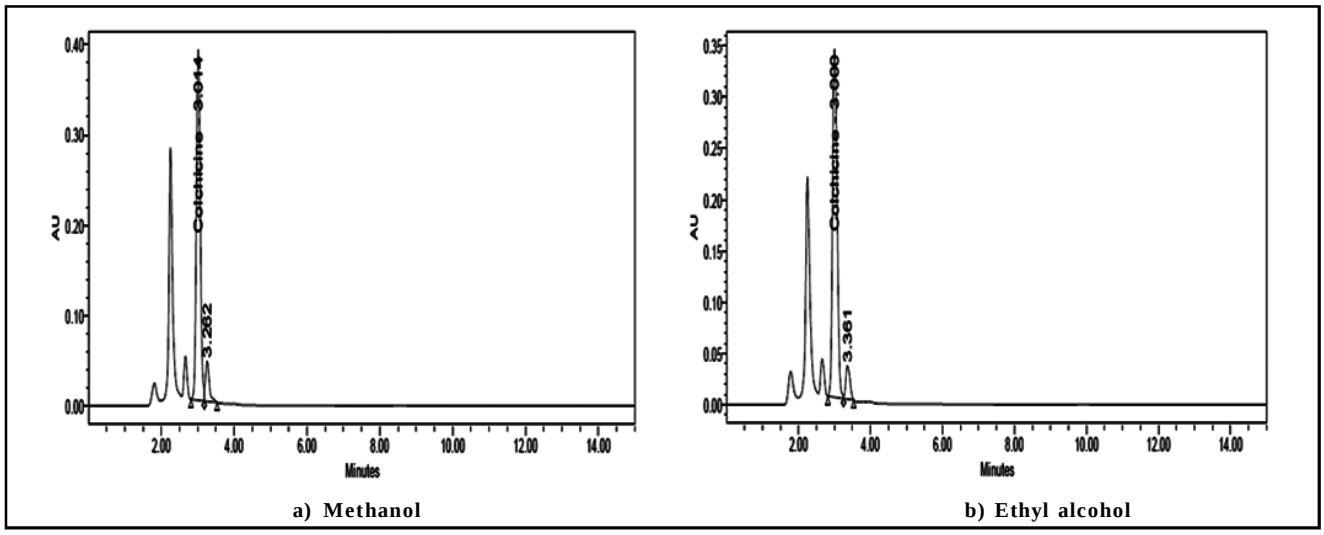


Figure 5: Chromatogram of colchicine (reference compound).



a) Methanol **b) Ethyl alcohol**

Figure 6: Chromatogram of *G. superba* samples extracted by (a) methanol and (b) ethyl alcohol.

The HPLC chromatogram of colchicine (reference compound) is given in Figure 5 and HPLC chromatogram of *G. superba* sample is given in Figure 6.

The results obtained in soxhlet method were found statistically significant and are presented in Figures 7 and 8. The extraction durations had positive effect on mean total extract and minimum (8.460%) was obtained for 1/2 h extraction, which increased to maximum (16.861%) at 4 h extraction which was however, found statistically at par with 2 h (16.326%) extraction. Under methanol extraction, the total extract was minimum at 1/2 h extraction (10.800%) and maximum (19.155%) at 4 h extraction which was however, found statistically at par with 2 h (18.770%) extraction. Under ethyl alcohol extraction, the minimum total extract (6.121%) was obtained in 1/2 h which kept increasing with increase in extraction duration and reached maximum (14.568%) at 4 h extraction. The values of total extract obtained at 1.5 h (13.780%), 2 h (13.881%) and 4 h (14.568%) extraction with ethyl alcohol were statistically at par (Figure 7). The mean content of colchicine was found higher (0.702%), under methanol extraction than ethyl alcohol (0.639%). Under different extraction durations, the mean colchicine content was minimum (0.581%) under 1/2 h extraction and maximum (0.699%) at 2 h and 4 h extraction, which was however, statistically at par with 1.5 h (0.690%) extraction. Among different solvents, in methanol solvent, the colchicine content was minimum (0.631%) under 1/2 h extraction and maximum (0.720%) under 2 h and 4 h extraction which was however, found statistically at par with 1 h (0.717%), one and 1/2 h (0.719%) extraction. Under ethyl alcohol solvent, the colchicine content was minimum (0.531%) under 1/2 h extraction and maximum (0.679%) under 2 h and 4 h extraction. The values of colchicine content obtained at 1.5 h (0.661%), 2 h (0.679%) and 4 h (0.679%) extraction with ethyl alcohol were statistically at par (Figure 8). On the basis of results obtained, it is concluded that extraction of samples with methanol for 1 h (0.717%), 1.5 h (0.719%), 2 h (0.720%) and 4 h (0.720%) duration, extracted approximately same colchicine content under soxhlet extraction method.

Under reflux extraction method, the mean total extract was higher in methanol extraction (19.574%) than the ethyl alcohol extraction (18.741%). The different extraction durations had positive effect on mean total extract and minimum (17.888%) was obtained for 1/2 hextraction, which increased to maximum (20.320%) at 4 h extraction. The results obtained were found statistically significant and are presented in Figures 7 and 8.

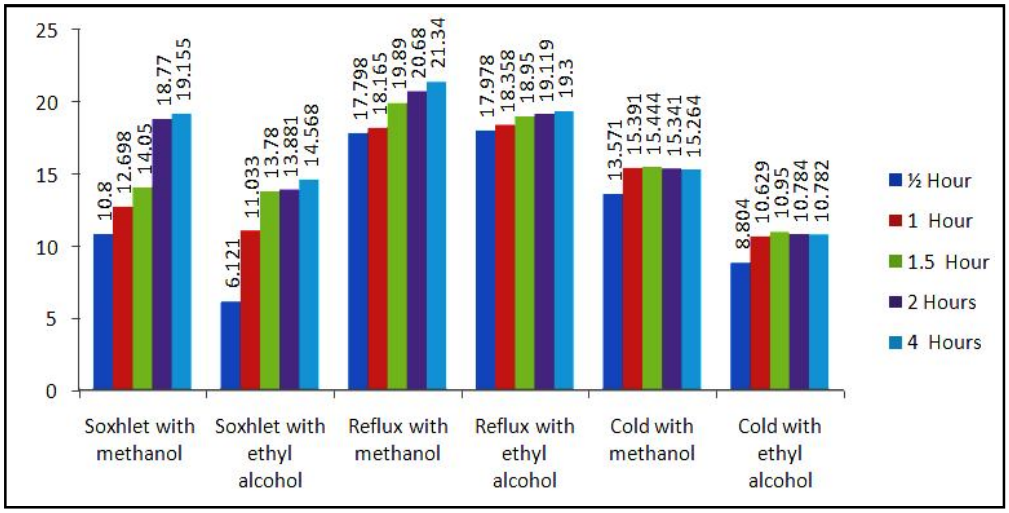


Figure 7: Effect of extraction duration and solvent on total extract (%) of *G. superba* by using Soxhlet, Reflux and Cold extraction method.

Among different extracting solvents, in methanol extraction, the total extract was recorded minimum at 1/2 h extraction (17.798%) which kept increasing with increase in extraction duration and reached maximum (21.340%) under 4 h extraction. Under ethyl alcohol extraction, the minimum total extract (17.978%) was obtained in 1/2 h which kept increasing with increase in extraction duration and reached maximum (19.300%) at 4 h extraction. The values of total extract obtained at 1.5 h (18.950%), 2 h (19.119%) and 4 h (19.300%) extraction with ethyl alcohol were statistically at par (Figure 7). The mean content of colchicine was found higher (0.717%) under ethyl alcohol extraction than methanol (0.678%). Under extraction durations, the mean colchicine content was minimum (0.665%) under 1/2 h extraction and maximum (0.729%) at 4 h extraction, which was however, statistically at par with 2 h (0.720%) extraction (Figure 8). Under ethyl alcohol, the colchicine content was minimum (0.693%) under 1/2 h extraction and maximum (0.732%) under 2 h extraction. In methanol, the colchicine content was minimum (0.637%) under 1/2 h extraction and maximum (0.727%) under 4 h. The values of colchicine content obtained at 2 h (0.712%) and 4 h (0.727%) extraction with methanol were statistically at par with 1 h (0.716%), 1.5 h (0.718%), 2 h (0.728%) and 4 h (0.732%) extraction with ethyl alcohol solvent. On critical examination of results, it is concluded that colchicine content was found approximately same when extraction was done with ethyl alcohol for 1 h (0.716%), 1.5 h (0.718%), 2 h (0.728%) and with methanol for 2 h (0.712%) and 4 h (0.727%) extraction duration under reflux extraction method.

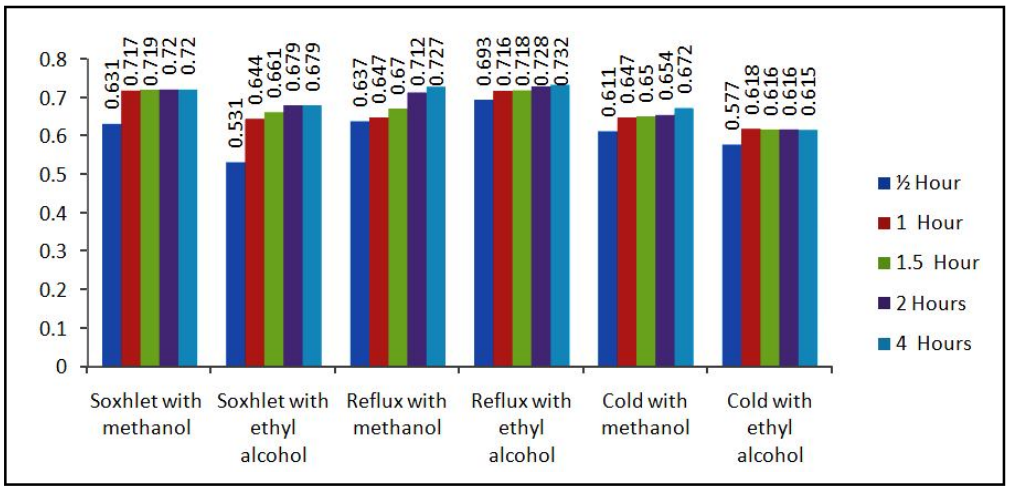


Figure 8: Effect of extraction duration and solvent on colchicine (%) of *G. superba* by using Soxhlet, Reflux and Cold Extraction method.

The extraction of samples undercold extraction method was done as given in experimental section. The results obtained were found statistically significant and are presented in Figures 7 and 8. The mean total extract was higher in methanol extraction (15.002%) than the ethyl alcohol extraction (10.390%). The extraction duration employed had very little effect on mean total extract and minimum (11.187%) was obtained for 1/2 h extraction which increased to maximum (13.197%) at 1.5 h extraction and thereafter stabilized. The values of mean total extract obtained under 1 h (13.010%), 1.5 h (13.197%), 2 h (13.062%) and 4 h (13.023%) extraction were statistically at par. Under methanol extraction, the minimum total extract (13.571%) was obtained in 1/2 h extraction and maximum (15.444%) at 1.5 h extraction. The values of total extract obtained at 1 h (15.391%), 1.5 h (15.444%), 2 h (15.341%) and 4 h (15.264%) extraction with methanol were statistically at par. Under ethyl alcohol extraction, the minimum total extract (8.804%) was obtained in 1/2 h and maximum (10.950%) at 1.5 h extraction. The values of total extract obtained at 1.5 h (10.950%), 2 h (10.784%) and 4 h (10.782%) extraction with ethyl alcohol were statistically at par. The mean content of colchicine was found higher (0.647%) under methanol extraction than ethyl alcohol (0.608%). Under extraction durations, the mean colchicine content was minimum (0.594%) under 1/2h extraction and maximum (0.643%) at 4 h extraction.

Under methanol solvent, the colchicine content was minimum (0.611%) under 1/2h extraction which kept increasing slightly with increase in extraction duration and reached maximum (0.672%) under 4 h. Under ethyl alcohol solvent, the colchicine content was minimum (0.577%) under 1/2h extraction and maximum (0.618%) under 1 h extraction. The values of colchicine content obtained at 1 h (0.618%), 1.5h (0.616%), 2 h (0.616%) and 4 h (0.615%) extraction with ethyl alcohol were statistically at par. On the basis of the results presented in Figure 8, it is concluded that extraction with methanol for 4 h under cold extraction method gave maximum colchicine content (0.672%).

The results obtained for sonication assisted extraction of this experiment were found statistically significant and are presented in Figures 7 and 8. The mean total extract was higher in methanol extraction (12.482%) than the ethyl alcohol extraction (8.496%). The extraction duration had positive effect on mean total extract and minimum (8.593%) was obtained under 1 min extraction and maximum (12.007%) at 5 min extraction which was found statistically at par with 10 min extraction duration.

Under methanol extraction, the minimum total extract (10.923%) was obtained in 1 min extraction and maximum (14.194%) at 5 min extraction which was however, found statistically at par with 10 min (14.183%) extraction. In ethyl alcohol extraction, the minimum total extract (6.262%) was obtained in 1 min extraction and reached maximum (9.856%) at 4 min extraction. The values of total extract obtained at 4 min (9.856%), 5 min (9.821%) and 10 min (9.825%) were statistically at par.

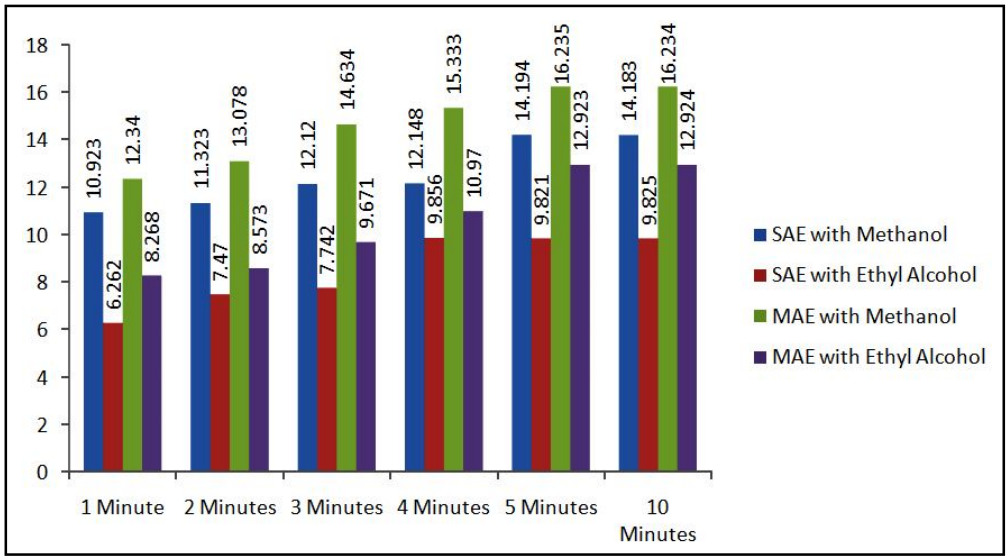


Figure 9: Effect of extraction duration and solvent on total extract (%) of *G. superba* by using sonication assisted extraction (SAE) and microwave assisted extraction (MAE).

The mean content of colchicine was found higher (0.633%), under methanol extraction than ethyl alcohol (0.559%). Under different extraction durations, the mean colchicine content was minimum (0.555%) under 1 min extraction and maximum (0.630%) at 10 min extraction. The values of total extract obtained at 5 min (0.629%) and 10 min (0.629%) extraction were statistically at par. Under methanol solvent, the colchicine content was minimum (0.600%) under 1 min extraction which slightly increased with increase in extraction duration and reached maximum (0.671%) under 10 min which was however, found statistically at par with 5 min (0.670%) extraction. Under ethyl alcohol solvent, the colchicine content was minimum (0.509%) under 1 min extraction and maximum (0.589%) under 10 min. The values of colchicine content obtained at 5 min (0.588%) and 10 min (0.589%) extraction with ethyl alcohol were statistically at par. On the basis of results presented in Figure 10, it is concluded that extraction with methanol for 5 min and 10 min under sonication assisted extraction, extracted almost same colchicine content.

The results obtained for microwave assisted extraction of this experiment were found statistically significant and are presented in Figures 9 and 10. The mean total extract was higher in methanol extraction (14.642%) than ethyl alcohol extraction (10.555%). The extraction duration had positive effect on mean total extract and minimum (10.304%) was obtained under 1 min extraction and maximum (14.579%) at 5 min and 10 min extraction. Among solvents, in methanol extraction, the minimum total extract (12.340%) was obtained in 1 min extraction and maximum (16.235%) at 5 min extraction which thereafter stabilized with further increase in extraction duration. Under ethyl alcohol extraction, the minimum total extract (8.268%) was obtained in 1 min which kept increasing with increase in extraction duration and reached maximum (12.924%) at 10 min extraction. The values of total extract obtained at 5 min (12.923%) and 10 min (12.924%) extraction with ethyl alcohol were statistically at par.

The mean content of colchicine was found higher (0.659%), under methanol extraction than ethyl alcohol (0.579%). Under extraction durations, the mean colchicine content was minimum (0.605%) under 1 min extraction and maximum (0.629%) at 4 min extraction. The values of total extract obtained at 3 min (0.620%), 4 min (0.629%), 5 min (0.626%) and 10 min (0.626%) extraction were found statistically at par.

Under methanol solvent, the colchicine content was minimum (0.633%) under 1 min extraction which kept increasing slightly with increase in extraction duration and reached maximum (0.680%) at 4 min extraction which was however, found statistically at par with 5 min (0.679%) and 10 min (0.679%) extraction. Under ethyl alcohol solvent, the colchicine content was minimum (0.576%) under 1 min extraction and maximum (0.594%) under 3 min. The values of colchicine content obtained at 1 min (0.576%), 2 min (0.578%) and 4 min (0.578%) extraction with ethyl alcohol were found statistically at par (Figure 10). On critical examination of results, it is concluded that colchicine content was found maximum (0.680%) when extraction was done with methanol for 4 min under microwave assisted extraction.

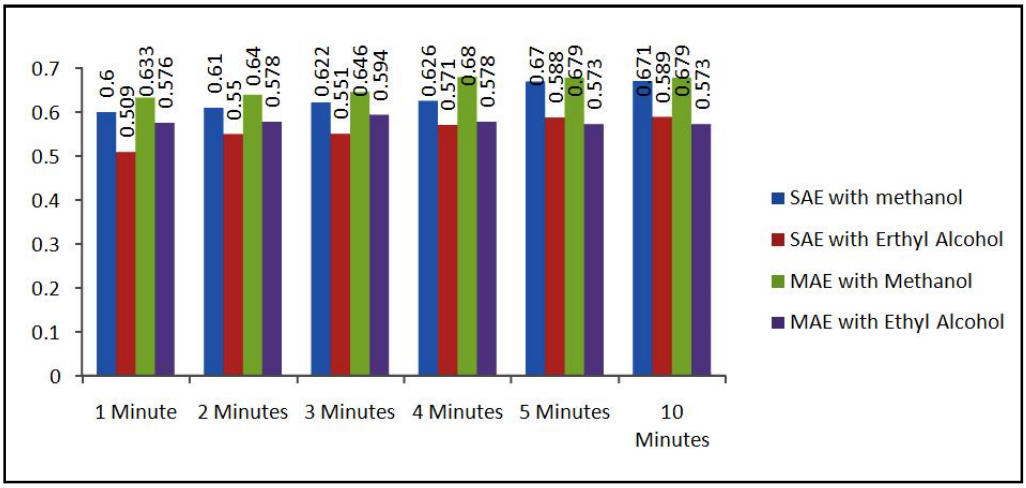


Figure 10: Effect of extraction duration and solvent on colchicine (%) of *G. superba* by using sonication assisted extraction (SAE) and microwave assisted extraction (MAE).

3.1 Comparison of different extraction methods

In this experiment, the best extraction condition under individual extraction method was selected for comparision of different extraction methods so as to find out the best extraction method for extraction of colchicine from *G. superba* seeds (Table 1). The total extract was recorded maximum (18.358%) under reflux method when extraction was done with ethyl acohol for 1 h and minimum total extract (12.698%) was recorded when the extraction was done soxhlet extraction for 1 h with methanol solvent.

Table 1: Comparison of different extraction methods in *G. superba*

Extraction method	Extracting solvent	Extraction duration	Total extract (%)	Colchicine (%)
Soxhlet extraction	Methanol	1hr	12.698 (3.563)	0.717 (0.847)
Reflux extraction	Ethyl alcohol	1 hr	18.358 (4.285)	0.716 (0.846)
Cold extraction	Methanol	4 hr	15.264 (3.907)	0.672 (0.820)
Sonication assisted extraction	Methanol	5 min	14.194 (3.767)	0.670 (0.818)
Microwave assisted extraction	Methanol	4 min	15.333 (3.916)	0.680 (0.825)
$CD_{0.05}$		0.046	0.006	
SE(m)		0.015	0.002	

Values in the parentheses are transformed values using square root transformation.

The colchicine content was recorded maximum under soxhlet extraction method when extraction was done with methanol for 1 h (0.717%) duration and this value was found statistically at par when the extraction was done with ethyl alcohol for 1 h under reflux extraction method (0.716%). This suggests that for extraction of colchicine from seeds Soxhlet and reflux methods are suitable in terms of maximum colchicine content.

4. Discussion

Effect of various extraction parameters on extract quality has been accounted globally. Also, the best use of any medicinal plant, it is very important that effective method for extraction of their bio-active constituents must be developed, so that maximum yield can be obtained by using our resources (energy, time, solvent, apparatus, *etc*.) economically. The availability of standardized plant extracts is very much important for commercial production. For extract preparation, selection of appropriate extraction method is a key consideration. Extraction of alkaloids mainly colchicine from the seeds of *G. superba* has been done by using different solvents and different extraction methods for different durations by various workers in different research experiments. Different solvents like methanol (Chitra and Rajamani, 2009; Lakshmi and Swathi, 2015; Senthilkumar, 2013) and methanol : water :: 50 : 50 (Kannan *et al*., 2007) has been used for extraction of phytoconstituents from the seeds of *G. superba*. Different extraction methods such as soxhlet extraction (Lakshmi and Swathi, 2015; Senthilkumar, 2013), sonication (Jason *et al*., 2014; Chitra and Rajamani, 2009) and cold extraction (Lakshmi and Swathi, 2015), percolation (Kannan, 2007) and freeze drying extraction (Jason *et al*., 2014) has been used for extraction of phytoconstituents from seeds of *G. superba*.

4.1 Optimization of extraction solvent

In the present study, it is revealed that both solvents methanol and ethyl alcohol were statistically at par for colchicine content in 1 h extraction duration, but both solvents were found best in different methods. Methanol extracted high colchicine content at 1 h extraction duration in Soxhlet extraction method and ethyl alcohol gives high colchicine content at 1 h extraction duration in reflux extraction method.

4.2 Optimization of extraction duration

As seen in Figures 7-10, there was a certain correlation between the increasing extraction duration, total extract (%) and colchicine content (%). Based on the information obtained from tables 1 h extraction is sufficient for maximum colchicine content (%), after that content is stabilized and statistically at par. But, the total extracts (%) shows increasing trend up to 4 h extraction duration which is statistically at par with 2 h extraction duration. In the present studies, we conclude the optimal extraction duration for maximum colchicine (%) is 1 h.

5. Conclusion

In the present investigation, results revealed that extraction method, solvent and extraction duration had a significant effect on the colchicine content of *G. superba* seed samples. Major outcome of the present investigation revealed that the reflux method with 1 h extraction duration using ethyl alcohol solvent was favourable method for extraction of total extract (%) and colchicine (%) from *G. superba*. It is advantageous as it uses ethyl alcohol which is a biosolvent and so, is preferred in pharmaceutical industries. Colchicine content obtained by Soxhlet method with 1 h extraction duration and methanol solvent is also statistically same as by reflux method with 1 h extraction duration and ethyl alcohol solvent. Keeping this in view, Soxhlet method can be the best extraction method where our aim is to get colchicine only, for use in analytical work, plant breeding experiments, *etc*. This study will also be helpful in designs and decisions related to such extraction works.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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