Year: 2014; Volume: 1; Issue: 1
Article ID: BT14 04; Pages: 1-5

BioMed Research

The Open Access Publisher

www.bmrjournals.com

BMR Biotechnology

Research Article

Effect of Process Parameters on the Extraction of Colchicine from *Colchicum autumnale* L Seeds

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Received 30 April 2014; Accepted 23 May 2014; Published 23 May 2014

Abstract

Phytocompounds play a vital role in our lives since nowadays people rely more on herbal sources of drugs to be used for curing various ailments or to be used prophylactically in the form of neutraceutical.One such plant used to cure certain ailments is *Colchicum autumnale* L., whose medicinal benefit is attributed to the phytocompound colchicine present in it. The present study focused on studying the effect of two process parameters viz solvents of varying polarity and mode of extraction on the extraction of colchicine from *Colchicum autumnale* L. seeds. Solvents used included ethanol, methanol, chloroform and acetone whereas three methods of extraction were opted included maceration, reflux and soxhelation. Maceration extracted 0.939,1.072, 0.714 and 0.352 %w/w of colchicine in methanol, ethanol, chloroform and acetone respectively. For reflux, the results were- 2.6, 3.34, 1.91 and 0.483%w/w respectively for ethanol, methanol, and chloroform and acetone.In case of soxhelation the results were 2.63% w/w in ethanol, 3.49% w/w in methanol, 2.34% w/w in chloroform and 0.774% w/w in acetone. Thus, it was concluded from the study work that soxhelation proved to the best mode of extraction and methanol was identified as the most effective solvent for extracting colchicine. Furthurmore, since our study revolves around conventional modes of extraction, the work might be useful for areas where modern extraction techniques still are unavailable.

Keywords: Colchicine, gout, *Colchicim autumnale* L., Suranjan

1. Introduction

Colchicine is a phenethylisoquinoline alkaloid (amino alkaloid) which occurs in seeds of *Colchicum autumnale* L., *Colchicum luteum* L. (Liliaceae) as well as in tubers of *Gloriosa superba* L. (Liliaceae) . It was known since the time of Dioscorides but was not utilized for its medicinal purpose due to its poisonous nature. However, literature shows that it was used to control flares of gout by Arabs. Colchicum corm appeared in *London Pharmacopoeias* of 1618, 1627, 1632 and 1639. After deletion it was reintroduced in the *Pharmacopeia* of 1820.However, later in 1824 use of Colchicum seeds was introduced in the *Pharmacopeia*. The credit of isolation of colchicine

goes to Pelletier and Caventou which was achieved in 1820 [1, 2].

Colchicine is not only a potent anti-gout agent but is also used for the treatment of other ailments like hepatic cirrhosis ,primary biliary , pericarditis;,Sweet syndrome; amyloidosis, sarcoid arthritis, acute inflammatory calcific tendonitis, leukemia, adenocarcinoma of the gastrointestinal tract, mycosis fungoides and topically to treat intraurethral condyloma acuminata in men. All the uses of colchicine however are not approved by FDA since colchicine was developed prior to federal regulations requiring FDA review [3]. Apart for medicinal actions, colchicine is also well known for bringing about polyploidy i.e. increase in number of chromosomes [4]. As stated earlier, two different species of Colchicum contains colchicine viz *C. autumnale* L. (Suranjan Shirin) and *C.luteum* L. (Suranjan Talakh).The genus derives its name from Colchis which is one of the places where the plant is found.

In the present study *C. autumnale* L. was used. Effect of two process parameters including solvent and mode of extraction was studied upon the extraction of colchicine from *C. autumnale* L seeds. The objective of the study was to best mode of extraction and the most effective solvent for extraction of colchicine so as to extract maximum colchicine in a single go.

2. Materials & Methods

2.1 Materials

C.autumnale L. (Suranjan Shirin) seeds were purchased from a unani medical store (Shamsi Dawakhana) situated in Old Delhi, India and were authenticated by a taxonomist. Standard colchicine was purchased from S.D.Fine Chemicals, India.Chemicals used were of analytical grade and obtained from S.D.Fine Chemicals, India.

2.2 Methods

2.2.1 Preparation of Plant Material and Extraction of Colchicine

The seeds were cleaned manually to remove adhering foreign matter and were powdered with the help of a grinder (Usha Lexus , India).The powdered material was then kept in an air-tight container until use. Three modes of extraction viz maceration (MAC), soxhelation (SOX) and reflux (REF) and four solvents of varying polarities including ethanol (Eth), methanol (Meth), chloroform (Chl) and acetone (Ace) were chosen for the extraction of colchicine from *Colchicum autumnale* L. seeds.

2.2.1.1 Extraction through MAC

5 g of powdered material was soaked in 50 ml of solvent (Solvent: drug ratio=10:1 ml/g) for 72 hours at room temperature. The extracts were evaporated to dryness using rotary vacuum evaporator (HAHN SHIN, HS-2005 V-N) at 40°C under inert atmosphere to obtain buff colored sticky mass. The extract was weighed and was stored for further analysis.

2.2.1.2 Extraction through REF

Hot solvent extraction was done using a reflux apparatus (Rama Scirntific, India) for 2 hours at 50°C using solvent:drug ratio of 10:1ml/g. Furthur processing of the extracts were done in the same manner as in case of maceration.

2.2.1.3 Extraction through SOX

Hot solvent extraction was done using soxhlet assembly (Rama Scientific, India) for 2 hours at 50°C using solvent:drug ratio of 10:1ml/g. Before packing the powdered material in the column, it was imbibed with the respective solvents for about a minute.

2.2.2 Quantitative Estimation of Colchicine Spectrophotometrically

Quanatitative analysis of colchicine in different extracts of *Colchicim autumnale* L. was done by UV spectrophotometric method, where standard colchicine was used as reference compound.

2.2.2.1 Determination of Absorbance Maxima/Lambda Max (λ_{max})

A known amount of colchicine was dissolved in methanol and was checked for its λ_{max} value with the help of a UV spectrophotometer (Shimadzu, Japan).

2.2.2.2 Preparation of Standard and Sample

Different dilutions of colchicine (25, 12.5, 6.25, 3.125 and 1.56 µg/ml) were prepared in methanol. Absorbance of all the dilutions was then taken at the λ_{max} value and a calibration curve (Absorbance versus Concentration) was plotted for the same.

In case of sample, weighed amount of the extract was dissolved in methanol (Final concentration of the

sample solution- 1gm/ml). Absorbance of the sample dilution was then taken at the same λ_{max} value of the standard and the amount of colchicine was then calculated through the standard calibration plot of colchicine.

3. Result

λ_{max} value of standard colchicine was found out to be 352.8 nm. Calibration plot was thus made at the same λ_{max} value.The calibration plot showed a good linearity (R^2= 0.993).

Amount of colchicine in each extract was estimated with the help of calibration plot (Table 1, figure1) of standard colchicine. Percentage yield of colchicine in different extracts has been given in table 2.

It was observed that of all the solvents used, Meth was found to be most effective. Eth also proved to be a good solvent for the extraction but not as good as Meth. Chl and Ace proved to be poor solvents for extracting colchicine. Moreover, among the different modes of extraction employed, SOX proved to be the best technique. Although, REF also yielded good results. MAC was only able to extract minute quantities.

Table 1- Absorbance for Different Concentrations of Colchicine

Concentration (µg/ml)	Absorbance
25	0.778
12.5	0.381
6.25	0.236
3.125	0.076
1.56	0.044

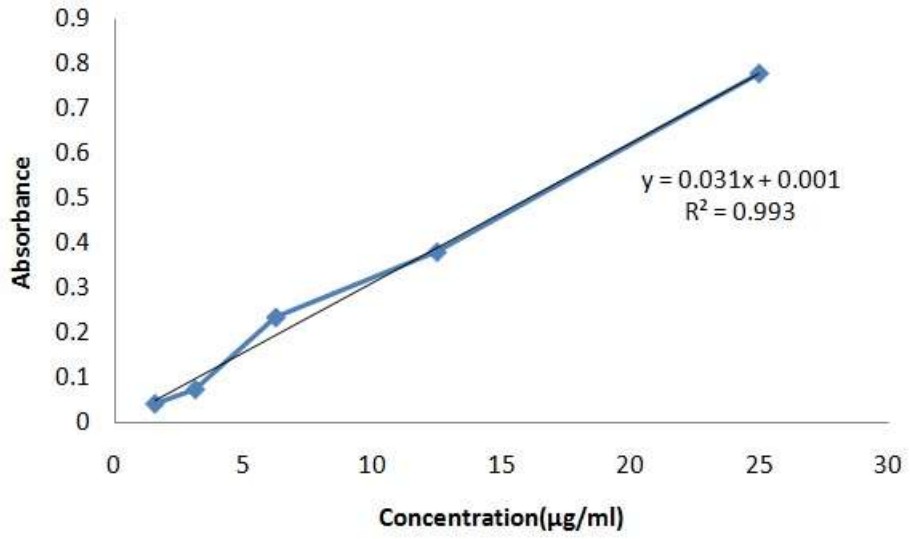

FIGURE: 1

Table 2- Percentage Yield (%w/w) of Colchicine in Different Extracts of *Colchicum autumnale* L. Seed

Method	Solvent	Percentage Yeild (%w/w) of Colchicine
Maceration	Ethanol	0.939
	Methanol	1.072
	Chloroform	0.714
	Acetone	0.352
Reflux	Ethanol	2.60
	Methanol	3.34
	Chloroform	1.91
	Acetone	0.483
Soxhelation	Ethanol	2.63
	Methanol	3.49
	Chloroform	2.34
	Acetone	0.774

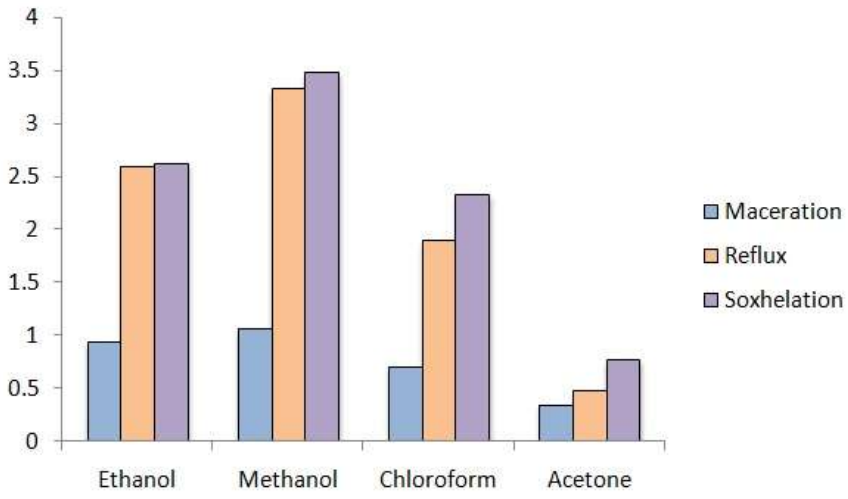

FIGURE: 2

4. Discussion

As seen from the results, SOX was identified as the best extraction technique for colchicine. This might be due to the reason that in this mode of extraction, the solvent comes in contact with the plant material again and again extraction the phytocompound in each go. Furthur, Meth was found out to be the most effective solvent. This might be due to the reason that maximum solubility of colchicine is seen in Meth and Eth.

5. Conclusion

Various areas including pharmacy colleges and other small scale research institutes are still lacking modern techniques of extraction like microwave assisted extraction, ultrasound assisted extraction, supercritical fluid extraction etc. Our research would be beneficial for such areas which rely on conventional modes of extraction. The objective of the present study was to reveal the most effective solvent and the best mode of extraction of colchicine from *Colchicum autumnale* L. seeds in order to extract maximum phytocompound in a single run. This will be able to save time, solvents and energy. Our results concluded that soxhelation was the best extraction mode among all and methanol was found to be the most effective solvent for the same, for the extraction of colchicine from *Colchicum autumnale* L. seeds.

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6. Conflict of Interest- None

7. References

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