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Standardization of drying and extraction techniques for better colchicine recovery from *Gloriosa superba*

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Key words

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ABSTRACT

Gloriosa superba is an important medicinal plant that is used in the treatment of diseases like gout, arthritis, cancer and act as anthelmintic, antimitotic and antiabortive agent. It's tuber is a major source of colchicine for the pharmaceutical industries and requires drying before extraction. The accelerated artificial drying technique using microwave vacuum oven was evaluated with other methods of extraction & purification for colchicine by performing High Performance Liquid Chromatography (HPLC). It was observed that the recovery of colchicine from extracts prepared by Soxhlet method were greater than that of sonication. Likewise, colchicine content in extract of microwave vacuum dried tuber was greater as compared to the hot air oven dried tuber. This study is useful for pharmaceutical industries for extracting colchicine using accelerated drying techniques economically and in less time consuming manner.

INTRODUCTION

Gloriosa superba which is also known as Flame lily is a tuberous herb, is spread widely in tropical Asia, Southern Africa and Tropical regions. The medicinal value of *Gloriosa superba* lies in its possesion of Colchicine [2, 7]. Colchicine, present in the seeds and tuberous root of *Gloriosa superba* is used for the remedial actions of sprains, bruises, colic, hemorrhoids, chronic ulcers, cancer, nocturnal seminal emission, leprosy and impotence [6]. This plant has gained importance in medicine in recent years for the production of colchicine in large scale [4]. Hence, primary processing of medicinal plant parts especially tubers requires drying to enhance shelf life and recovery. Accelerated drying techniques by using artificial heat source provide early drying.

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MATERIALS AND METHODS

Processing of material

Large tubers of the plant *Gloriosa superba* were collected in the month of June, 2014 from the College of Forestry, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli (Dist. Ratnagiri), Maharashtra, India. The freshly collected tuber which was brought to laboratory and washed thoroughly with tap water to remove dirt, soil and other removable impurities followed by the distilled water wash. The washed samples were cut into small pieces and dried in two methods. In first Method the tubers were kept in Hot Air Oven to dry for 48 Hrs. at a temperature

of 60°C until constant weight was achieved. The second set was placed in Microwave Vacuum Dryer for 80 mins. at 110°C temp. After complete drying, tubers were powdered by mechanical grinder to get fine-coarse powder and stored in airtight boxes for successive extraction.

Preparation of extracts

Soxhlet extraction: Both the samples of dried and grinded tubers of *Gloriosa superba* were added separately to the solvent according to the 1:10 ratio of Solute: solvent, 10 grams of powdered tuber of each sample was placed in separate Soxhlet's thimble with 100 ml of Methanol (CH_3OH) as solvent. Both the Soxhlet units were kept on for 6 Hrs or ~18-19 Cycles without adding of solvent during process. Temperature of Soxhlet Units was maintained to around 45-50°C. After the completion of extraction process both the extracts were placed in a dry, cold place for further analysis.

Sonicator extract: The dried powdered tubers of *Gloriosa superba* following the solute: solvent (1:10) ratio, 10 g of powdered tuber was added to 100 ml Methanol in beaker. The beaker was covered by aluminum foil to avoid evaporation of methanol. Placed the container of Sonicator for 32 Mins or 4 Cycles of 480 sec each at 65°C temp.

Purification

The extracts were diluted with distilled water in equal proportion (v/v). Same volume of Petroleum Ether as of undiluted Methanolic extract was added to the diluted Methanolic extract. Then by using separation funnel, aqueous phase was taken separately into another beaker. Chloroform (volume equal to undiluted Methanolic Extract) was added to aqueous phase. Again with separation funnel the organic phase was separated for further process. From organic phase, chloroform was aerated and residues were re-dissolved in 10 ml Methanol followed by filtration. After filtering the extracts were kept in dry and cold place for further use in study.

Preparation of standard solutions

To make the standard solutions 1.0 mg precisely weighed mass of standard Colchicine (Molychem,Thane, India) was added to 10 ml Methanol in a volumetric flask to get 100 ppm solution. The standard solutions of 5, 10, 20, 40 and 80 ppm were prepared by further diluting the stock standard solution with the specified mobile phase.

HPLC analysis

The entire detection and separation process was performed on Thermo Scientific Dionex Ultimate 3000 Standard Systems. Fitted with Thermo Scientific Dionex Ultimate 3000 auto sampler, Analytical column Acclaim C18. 150×4.6 mm, Chromeleon software. The mobile phase used was mixture of Methanol: Acetonitrile: Water: 0.1% Ortho-Phosphoric Acid (OPA) (50:30:15:5) [5]. The mobile phase and sample solutions were filtered through 0.45 mm membrane filter. The sample solutions were also filtered using 0.45 m membrane filters. The mobile phase was delivered by isocratic flow at the flow rate of 1 ml min^{-1}. All determinations were achieved at ambient temperature, with injection volume was 20 ml and detection at 257.0 nm. Dionex Ultimate 3000 Diode Array Spectrophotometer was used to determine the wavelength of maximum absorbance.

RESULTS AND DISCUSSION

The concentration of colchicine in extracted samples was measured using standard calibration curve fitted with r^2 value (Fig. 1). The retention time of colchicine in standard extract was 2.03 min (Figure 2 & 3). The sample extracted with Soxhlet provided better recovery of colchicine from tubers of *G. superba* averaging 256.44 ppm compared to that of 156.50 ppm in sonication method. Among the accelerated drying technique the high recovery of colchicine was observed in microwave vacuum oven drying technique (278.98 ppm) compared to hot air oven drying (233.94 ppm) under Soxhlet extraction technique (Figure 4).

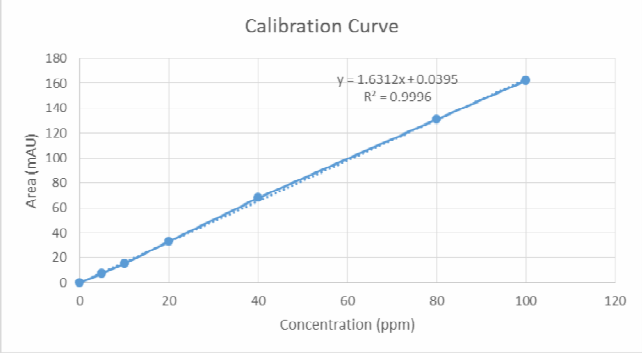

Figure 1: Calibration Curve of Colchicine

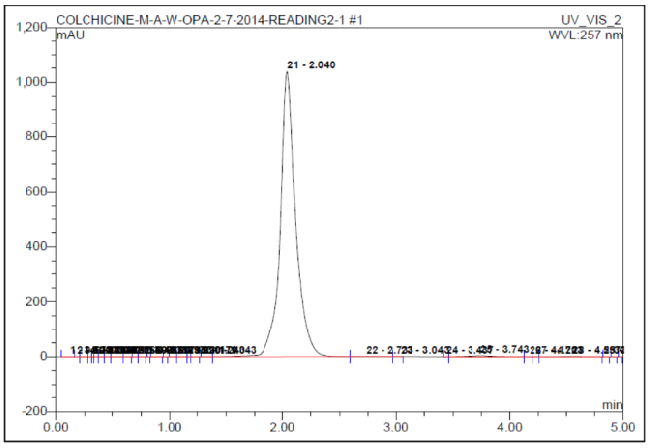

Figure 2: Chromatogram of Standard Colchicine

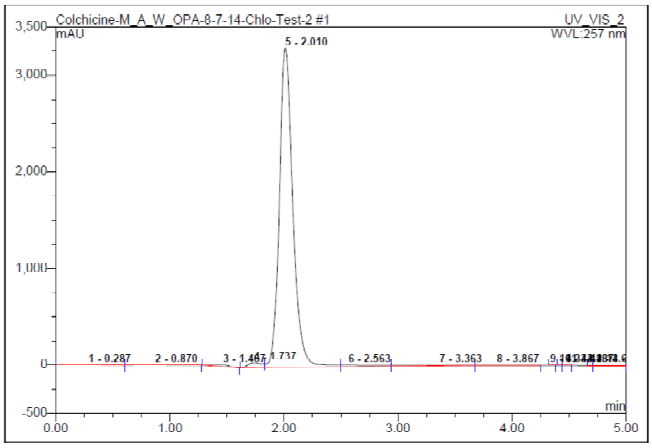

Figure 3: Chromatogram of extract showing peak at same retention time.

The colchicine content in the tuber of *Gloriosa superba* is different in all the methods (Figure 3, 4, 5 and 6). Concentration of Colchicine is varying in different sample due to difference in extraction and drying method. There are studies which have shown samples of extracts shows variations in

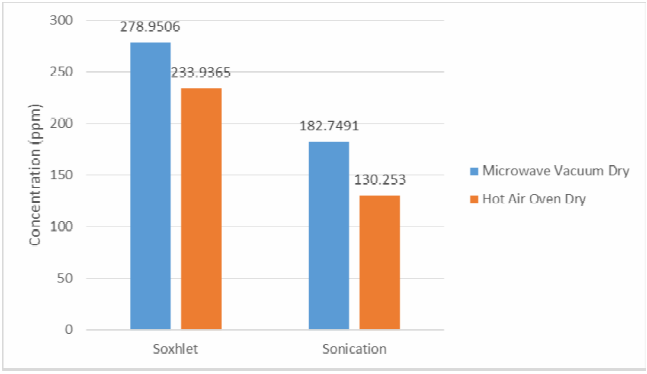

Fig. 4: Comparison of colchicine content obtained through different drying method

alkaloids recovery under different drying [1, 3] and extraction methods [8]. This finding indicates that the process of drying, extraction and purification positively governs the colchicine recovery in *G. superba.*

CONCLUSION

In the current scenario of market where demand of herbal medicines are high, time in processing chemicals and production of medicine should be as low as possible to achieve that demand. This study is done to identify the better and faster techniques by which can recover more Colchicine as compare to others so that increasing demand of the chemical can be fulfilled. We have taken 2 different techniques for extraction and same for drying tuber of *Gloriosa superba* in this study for comparing the combination of drying techniques with extraction techniques to get maximum recovery of Colchicine content in tuber.

Throughout this study we found that when the extract the tuber dried in Microwave Vacuum Dryer is taken by Soxhlet apparatus Colchicine content was maximum in it. We have also examined other extract which was taken by Sonicator of tuber dried in same condition but the level of Colchicine in the extract was ~36% less than the former. Likewise the Sonicator extract of Hot Air Oven Dried tuber is also ~42% less than the Soxhlet extract. This finding is going to save the time and money of Industries as well as the consumers because for recovery of more Colchicine in lesser time, Industries will be benefited and for consumers price of the medicine having colchicine will be lesser.

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