

## *Gloriosa superba* roots: Content change of colchicine during *sodhana* (detoxification) process

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Ayurveda, the Science of life prescribes a variety of drugs from various sources like plant, animal or mineral origin. Some of these drugs are found to contain certain toxic components. Hence, Ayurveda recommends different *sodhana prakriya* for detoxification and/or potentiation of these drugs. The current paper investigates the fate of colchicine during the *sodhana prakriya* of *Langli* (*Gloriosa superba* L.) roots. The study aims at evaluating the underlying principle and thereafter, to find out if any alternate process can be substituted for the conventional *sodhana prakriya*.

**Keywords:** Sodhana prakriya, *Gloriosa superba*, Colchicine

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Ayurveda prescribes a variety of drugs from different sources. Some of the drugs used in Ayurveda are found to contain certain toxic constituents. These drugs undergo the *sodhana prakriya* for detoxification and/or potentiation of the drug material. *Langli* or *Gloriosa superba* Linn. is one such drug that undergoes the process of *sodhana* prior to its use in the Ayurvedic system of medicine. Based upon the composition of the individual drug, different types of processes are used for the detoxification of the toxic ayurvedic medicinal plants<sup>1</sup>.

*Langli* (*Gloriosa superba*) is also known by the name Malabar Glory Lily. It is called *karihari* or *languli* in Hindi and *indai* or *kallavi* in Marathi. The tuberous roots of *Gloriosa superba* have been used as a drug in the traditional system of medicine. The tubers are regarded as tonic, stomachic and anthelmintic when taken in doses of 5-10 grains; in larger doses they are intensely poisonous. The drug is a gastro-intestinal irritant and may cause vomiting and purging. It is sometimes used for promoting labour pains and also as abortifacient. It is considered useful in colic, chronic ulcers and piles. Externally it

is used as a local application for parasitic skin diseases and as cataplasm in neuralgic pains<sup>2</sup>.

The major component of *Gloriosa* tubers is the alkaloid colchicine (C<sub>22</sub>H<sub>25</sub>O<sub>6</sub>N) which is toxic. The content of colchicine in the tubers from different regions varies from 0.03% to 0.3%. Colchicine is used in medicine, chiefly as salicylate, in the treatment of gout and rheumatism, and in plant breeding work for inducing polyploidy.

The toxic dose in humans is around 10 mg and ingestion of doses greater than 40 mg is always fatal within three days of the ingestion of the alkaloid. After a latency of 3-5 hrs, the intoxicated patient experiences abdominal pains, gastroenteritis with haemorrhage, abundant diarrhoea leading to dehydration, hypokalemia and metabolic acidosis. Further complications include hematological alterations due to bone marrow damage, septicemia and renal insufficiency<sup>3</sup>. Colchicine being a cellular anti-mitotic agent leads to early gastrointestinal failure, myelosuppression and finally multi-organ failure. In patients with impaired renal function it causes acute pancreatitis<sup>4</sup>. A sporadic case of suicidal plant poisoning due to consumption of a plant containing colchicine has also been reported<sup>5</sup>. Hence,

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the drug is made safe for human consumption by subjecting it to the ayurvedic *sodhana prakriya*.

### Methodology

*Gloriosa superba* roots were collected from Salem in South India. Root sample was authenticated by Dr H M Pandit, Botanist, Guru Nanak Khalsa College, Mumbai.

The process of *sodhana* was carried out on the roots of *Gloriosa*. *Gloriosa* roots (10 gm) were cut into small pieces and soaked in 100 ml *Gomutra* (Cow's urine, pH between 7.8 - 8.2) for a day at room temperature. The roots were then washed with warm water and air dried<sup>6</sup>. The dried samples were then treated in a manner similar to the pre-*sodhit* samples and then subjected to the quantification process. The medium used for *sodhana* as well as the pooled washings were preserved for further analysis. With an aim to find an alternative to the traditional *sodhana prakriya*, studies were also carried out using alkaline medium (0.84% w/v sodium hydrogen carbonate; pH 8) in place of *gomutra* which is used in the conventional technique and control study using water as the medium.

Pre as well as post-*sodhit* samples were subjected to the same process. Five gm of the root sample was extracted with methanol in a Soxhlet assembly till exhaustion (24 hrs). The extract obtained was evaporated till dryness and then stored in a cool dry place. The extract so obtained was further used for the determination of colchicine content.

### Quantification studies

The High Performance Thin Layer Chromatography (HPTLC) technique was used for the quantification of the colchicine in the samples of *Gloriosa superba*.

HPTLC was performed on Silica gel 60 F<sub>254</sub> plates (Merck, Germany) using chloroform: acetone: diethylamine, 7:2:1 (v/v) as the mobile phase. Samples were applied to the plates as 8 mm bands by means of Camag Linomat V applicator. The plates were developed in Camag twin trough chamber previously equilibrated with mobile phase for 20 min along with the plate. The development distance was 8 cm. Plates were then removed from the chamber and dried in a current of air. Densitometric scanning was done at 357 nm using Camag TLC Scanner III with winCATS software. The wavelength was selected after acquiring spectra from the standard and the samples.

Samples were prepared by dissolving appropriate quantity of the extract in methanol (Table 1). A solution of standard colchicine (S D Fine-Chem. Ltd., India) in methanol of 0.003% w/v concentration was used as the standard. All reagents used were of Analytical Grade.

A standard curve was developed between 2-12 µl of 0.003% w/v colchicine standard. Samples in duplicates were applied on the plates for quantification studies.

### Results

The roots of *Gloriosa superba* were subjected to the Ayurvedic *sodhana prakriya*. *Gloriosa superba* samples were subjected to HPTLC analysis to determine the content of colchicine in these samples. In addition to the *Gloriosa* samples the media used for *sodhana prakriya* and the subsequent water washings were also analyzed for their content of colchicine.

The various extracts obtained and their concentrations analyzed are summarized in Table 1.

A linear curve was set up in the range of 60-360ng of colchicine standard with a correlation coefficient of 0.9994 and sdv of 2.37. The R<sub>f</sub> value for standard colchicine was found to 0.39.

The amount of colchicine present in the sample was calculated from the colchicine standard curve. Quantity of colchicine in 10 gm of the *Gloriosa* roots pre-treatment, post-treatment and in the medium used as well as the washings is as stated in Table 2.

The two HPTLC plates developed for the quantification of colchicine in pre-treated as well as post-treated samples using *gomutra* and alkaline medium (pH8) are shown in Figs. 1 & 2.

### In Fig. 1,

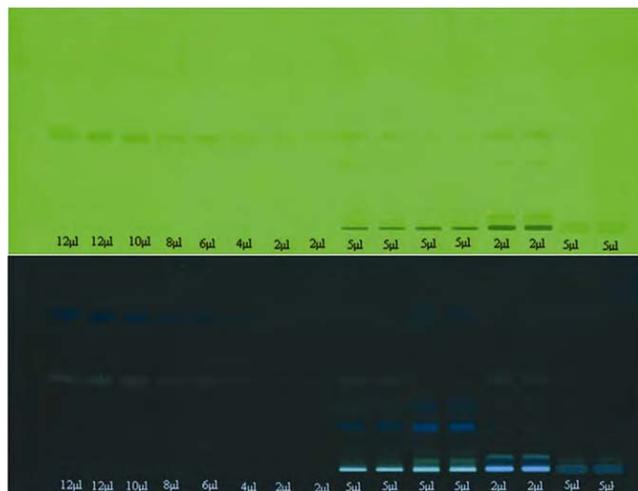
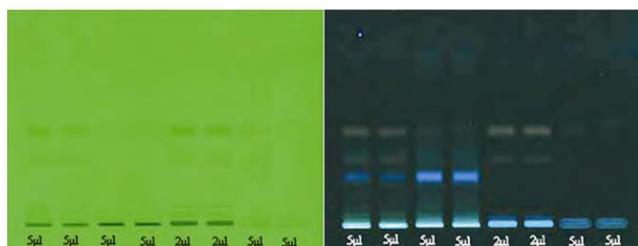
Track 1-8: Standard colchicine in decreasing order of volume (12 µl-2 µl) with a duplicate for 12 µl and 2µl.

Table 1—Weight of extract obtained from 5 gm of sample and the concentrations of the test solutions prepared.

Sample	Initial weight of sample (gm)	Weight of methanol extract obtained (mg)	Concentration of test solution (µg/µl)
Pre- <i>sodhit</i>	5	500	1.84
<i>Gomutra</i> treated	5	160	1.08
Alkaline medium treated	5	120	1.19
Water treated (control)	5	100	1.09

Table 2—Quantity of colchicine (mg per 10 gm) of the *Gloriosa superba* roots in the samples, media and the pooled washings, as quantified using HPTLC system

Quantity of colchicine (mg) in Pre-treated drug	Medium of treatment	Quantity of colchicine (mg) in		
		Post-treated drug	Medium	Washings
18.20	<i>Gomutra</i>	2.36	10.51	3.85
18.20	Alkaline medium	1.21	12.39	4.53
18.20	Water (Control)	1.30	12.01	3.90

Fig. 1—Standard curve of colchicine and samples obtained by using *gomutra* for *sodhana prakriya* of *Gloriosa superba* roots. Photo documentation of the plates at 254nm as well as at 366nm was done.Fig. 2—Samples obtained by using alkaline medium for the *sodhana prakriya* of *Gloriosa superba* roots

All the other samples were spotted in duplicates.

Track 9, 10: Pre-*sodhit* sample 5µl  
Track 11, 12: Post-*sodhit* sample 5µl  
Track 13, 14: Medium (*gomutra*) 2µl  
Track 15, 16: Pooled washings 5µl

#### In Fig. 2,

Track 1, 2: Pre-*sodhit* sample, 5µl  
Track 3, 4: Post-*sodhit* sample, 5µl  
Track 5, 6: Medium (alkaline), 2µl  
Track 7, 8: Pooled Washings, 5µl

## Discussion

The roots of *Gloriosa superba* have been used in Ayurveda since many years, as an emetic. But the roots contain a toxic principle, colchicine which is alkaloidal in nature and in high doses is not recommended for internal use<sup>7</sup>. Various complications ranging from diarrhoea to bone marrow damage, post ingestion of high doses of colchicine have been reported. Hence, the roots have been subjected to the Ayurvedic *sodhana prakriya* so as to reduce the concentration of colchicine to tolerable limits.

The screening of the pre-*sodhit* samples of *Gloriosa superba* has indicated the presence of colchicine in the samples to an extent of 0.18% w/w on dry weight basis. The post-*sodhit* samples have shown to contain significantly reduced amounts of colchicine. The percentage of colchicine in the *gomutra* treated sample was found to be 0.024% and that in the sample treated with alkaline medium was found to be 0.012%. The results obtained from the HPTLC quantification studies have indicated that both the processes using the different media for the *sodhana prakriya* have led to a significant decrease in the content of colchicine in the sample. The control study carried out with water as the medium has also yielded results similar to those obtained by using alkaline medium.

The *sodhana prakriya* prescribed for *Gloriosa* involves soaking of the sample in *gomutra* for 24 hrs and then washing with warm water and subsequent drying. Colchicine has high solubility in water (1 gm dissolves in 22 ml of water)<sup>8</sup>. The use of *gomutra* in *sodhana prakriya* exposes the sample to an aqueous environment which readily dissolves colchicine. The analysis of the medium used for *sodhana prakriya* as well as the subsequent water washings show presence of colchicine in them. Also the post-*sodhit* samples of *Gloriosa* show small quantities of the colchicine. Hence, it can be concluded that the decrease in the toxicity of the *Gloriosa* roots post- *sodhana* treatment is due to the significant reduction in the concentration of colchicine because of its solubilization in the medium. With this

possible mechanism involved, an attempt was made to put forth an alternate medium for *gomutra* and hence the sample was treated with the alkaline medium. The results obtained from the alternate medium are comparable to the conventional method used. Furthermore a control study was undertaken with water as the medium and results obtained thereof compared with that of the alkaline medium. It was found that both the alkaline medium as well as water showed comparable results and hence it can be concluded that water alone could be used as an alternate medium as the pH does not play a significant role in the reduction of the colchicine level from the drug.

### Conclusion

The experimental results suggest that the *sodhana prakriya* for *Gloriosa superba* roots leads to the decrease in the concentration of the toxic component, colchicine due to solubilization in the treatment medium. Further the results also indicate that the proposed method with a change in the treatment medium is at par with the conventionally used Ayurvedic *sodhana prakriya* with respect to the detoxification process as far as the content of colchicine is concerned.

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### References

- 1 Nabar M, Pimpalgaonkar P B & Laddha K S, Studies on *sodhana prakriya* of *Gunja* (*Abrus precatorius* Linn.) seeds, *Indian J Traditi Knowle*, 10(4) (2011) 693-696
- 2 Anonymous, *The Wealth of India*, revised edn, Vol. IV, (NISCOM, New Delhi), 2005, 139-140.
- 3 Bruneton J, *Pharmacognosy: Phytochemistry, Medicinal Plants*, (Lavoisier Publishing, Paris), 1999, 954.
- 4 Ting J Y S, Acute pancreatitis related to therapeutic dosing with colchicine: a case report, *J Med Case Rep*, (1) (2007) 64.
- 5 Nagesh K R, Menezes R G, Rastogi P, NaikN R, Rasguinha J M , Senthikumar S &Fazil A, Suicidal plant poisoning with *Colchicum autumnale*, *J Forensic Leg Med*, 18(6) (2011) 285-7.
- 6 Deshpande A P, Javalgekar R R, Ranade S, *Dravyagunavigyan*, (Anmol Prakashan, Pune), 1994, 315.
- 7 Bharathi P, Philomina D, Chakkaravarthi S, Estimation of colchicine in six different species of *Gloriosa* grown in vivo, *Indian J Pharm Sci*, 69 (2006) 806-9.
- 8 Anonymous, *The Merck Index*, 14<sup>th</sup> edn, (Merck & Co, Inc., U S A), 2006, 415.