

**Isolation of 3-demethylcolchicine from *Gloriosa superba* sludge and coupling with  $\alpha$ -acetobromoglucose to yield colchicoside and thiocolchicoside**

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**ABSTRACT**

Post purification of colchicoside from *Gloriosa superba* extract over the batches, a sludge was found to analyzed containing 3-demethylcolchicine very prominent for synthesizing colchicoside which obviously biosynthesized in the plant of *G. Superba*. It was isolated in 0.35% yield with 97% of chromatographic purity. Subsequently it was reacted to couple with prepared  $\alpha$ -acetobromoglucose to yield colchicoside. It was analyzed to found of the assay of naturally recovered colchicoside.

**Keywords:** *Gloriosa superba* sludge; 3-demethylcolchicine; Isolation; Coupling

**INTRODUCTION**

One of the very important exported medicinal plants of India that has become endangered within a very short span of the last 50 years is *Gloriosa. Superba L.* (Family-*Colchicaceae*). The root is used as a germicide, to cure ulcers, piles, haemorrhoids, inflammation, scrofula, leprosy, dyspepsia, worm's infestation, flatulence, intermittent fevers, and debility arthritis and against snake poison.(Wealth of India,1948-76) The corm (or tuber) which looks like a hoe, It has been the most used in indigenous medical systems of India as well as in Africa. The medicinal importance of the plant is due to the presence of alkaloids (nearly 24 of them) of which colchicines and colchicoside are the principal ones, as well as to the presence of 10 non-alkaloid medicinal compounds including B-sitosterol, chelidonic acid, luteolin, stigmasterol etc. Colchicine is used as a mitosis-arrest agent and in cancer therapy and diabetics, in addition to promote polyploidy in agriculturally important crop plants. Colchicoside is used against acute inflammation like gout and other forms of arthritis.

Recently, there has been an increased demand for the plant, as a source of colchicine and colchicoside, but the supplies from the conventional sources has not been sufficient.

The export of *Gloriosa* seeds started from 1984, although earlier only the tuberous corms were exported. Indiscriminate collection of tubers for commerce has resulted in the scarcity of the plant in the Indian scrub jungles. So the corms were

prohibited from export. This has resulted in large scale exploitation of the plant from the wild, as well as promoted its extensive cultivation in several parts of Peninsular India. The former source even today accounts for 95% of the total marketed quantity of the plant and out of this 70% comes from destructive collections.

Our previous study (Krishnamurthy, 2008-2009) shows plants from large scale cultivation, tissue culture sources, do not contain the required percentage of the alkaloids, and hence the importers abroad often reject or price poorly the exported *Gloriosa superba* in recent years. The seeds from wild sources, depending on the provenance, on an average yield 0.9% (range 0.6 to 2.0%) colchicine and 0.82% colchicoside (range 0.5 to 1.8%) but on repeated cultivation or in tissue culture the yield has come down to nearly 0.2% (in cultivation) or even 0% (in tissue culture plants). Again, as we suggested in earlier study, elicitation methodologies have been developed to enhance the alkaloid levels both under cultivation and under tissue culture conditions. It is for the exporters of *Gloriosa superba* to take advantage of this technology.

As it is known, colchicine is a pseudo alkaloid that has been widespread used for some time for the treatment of gout. The use of 3-demethyl-thiocolchicine glucoside, thiocolchicoside, is also widespread in therapy for treating contractures and inflammatory conditions that affect the muscular system (Calogero, 1992).

#### MATERIALS AND METHODS

*Gloriosa superba* sludge (Bombardelli, 2009) was obtained from the production section of the Alchem International Limited to R&D. Silica gel 60-120 mesh acidic, chloroform, methanol were obtained from the Indian manufacturer. Sludge was analyzed by the HPLC method in quality control department of the company and was found to contain 0.15% of presence of 3-demethylcolchicine of 20% purity. Qualitative determination was done by preparing a mobile phase comprising of EtOAc: AcOH: H<sub>2</sub>O, 7:2:1. The TLC of Merck make was spotted with the sludge diluting in methanol and was run in the mobile phase. On attaining the maximum R<sub>f</sub> values of the compounds, the TLC plate was taken out and observed under UV chamber. 30% lower and 40% higher impurities were found.

Accordingly the sludge weighing 10g was added to silica gel in methanol and the admixture was concentrated under vacuum to get dry admixed. It was then loaded on to the column containing 500g of silica gel conditioned with chloroform. It was first run with chloroform and then with marginal increase of polarity. The fractions were collected and analyzed by TLC until the lower impurity was being discarded. At 5% polarity the 3-demethyl fraction was collected of the volume of 2000ml. It was then concentrated on rotary evaporator under vacuum to get light brown colour compound and was further crystallized in ethanol and dried to get 135mg of 3-demethylcolchicine of the purity of 95%. Table 1 may be referred.

**Table-1: Description of sludge and 3-demethylcolchicine and isolation by column chromatography.**

Weight of sludge	Sludge: adsorbent	Initial purity	Final purity	Weight of 3-demethylcolchicine
10g	1:10	20%	95%	0.135g
10g	1:10	20%	95%	0.139g
10g	1:10	20%	95%	0.134g

**Pilot isolation of 3-demethylcolchicine on Prototype Column:** 500g of sludge was processed as mentioned earlier and was loaded on to the column containing 5kg of the silica gel. 200L of chloroform and methanol was employed for the isolation of 3-demethylcolchicine. It was run uninterrupted. It took five days to get the compound isolated. It was also analyzed for its purity and was found to be 95% pure. All the solvents was recovered by distillation method and made re employable for further R&D work. Total 3-demethylcolchicine obtained was 1.35g.

**Confirmation of 3-demethylcolchicine:** Since no standard 3-demethylcolchicine was available for the comparison studies so it was decided to couple with  $\alpha$ -acetobromoglucose as prepared by disclosed method mentioned in Vogel's Practical Handbook of Organic Chemistry. 3-demethylcolchicine was dissolved in water-dioxane mixture under alkaline condition while maintaining the temperature of 0-5°C. At this temperature the base was added and the reaction mass was become red blood in colour. After this  $\alpha$ -acetobromoglucose was dissolved in dioxane, was added without any delays into the reaction mixture, and was allowed to run for 24-48h. Nitrogen purge was introduced throughout the reaction as it takes 24-48h for completion.

On completion of the reaction the mass was washed with chloroform to get rid of any reaction impurities. Then the mass was exchanged with MeOH: CHCl<sub>3</sub> mixture to leach the coupled tetraacetatecolchicoside. On successful washes the volume of the mixture was concentrated under reduced pressure on a rota evaporator. Thick viscous light orange coloured syrup was obtained. Which was made to dissolve in MeOH, that was then in fume hood was hydrolyzed with an alkali. An exothermic reaction takes place and was allowed until the hydrolysis completed.

The reaction mass was washed with chloroform to get rid of any impurities. Thereafter it was extracted with MeOH: CHCl<sub>3</sub> to get the colchicoside. After exhaustive exchange the solution was concentrated under reduced pressure on rotary evaporator to recover the white crystalline solids. It was further crystallized with MeOH under refrigerating condition over 16 hours to get the pure product. On analyzing the purity of the product was found to be 97%.

**Synthesis of colchicoside and thiocolchicoside:** 01g of 3-demethylcolchicine was dissolved in water and dioxane mixture and added to three neck round bottom flask in basic medium of TEA under nitrogen atmosphere. Subsequently 06g of  $\alpha$ -acetobromoglucose was dissolved in dioxane and added to reaction mixture. This mass was kept agitated under the identical conditions over 24-48h. The temperature of the reaction was maintained at 0±5°C. Monitoring of the progress of the reaction was done by TLC using the mobile phase as mentioned earlier. Post reaction the mass was washed with sodium bicarbonate solutions and then with chloroform. The converted product was exchanged with methanolic chloroform. It was then dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure in rotary evaporator. As a result the brownish syrupy mass was obtained. It was then dissolved in methanol and deprotected, using 1% sodium hydroxide solution as an exothermic reaction. Finally the product was recovered with 10% methanolic chloroform. Resulting mass was dried under reduced pressure and the off white colchicoside was thus recovered was 02g. On crystallization it yielded 1.5g of pure colchicoside of 99% assay.

To assess its purity it was then taken as 01g, dissolved in 04g of water, and added to three necked round bottom flask at 20±30°C. 0.5g of NaSCH<sub>3</sub> was added over the period of 15-20 minutes. Reaction was monitored with the mobile phase of CHCl<sub>3</sub>: AcOH: H<sub>2</sub>O (7:2:1). On completion of the reaction it was exchanged with 10% methanolic chloroform until the entire product was recovered. The solution was dried

in rotary evaporator under reduced pressure to get syrupy brownish residue. It was then made to solubilise with methanol and kept in the freezer for crystallization overnight. Recovered crystallized product was 1.5g of fast yellow color. Its specification was as per USP. Figure 1 and 2 may be referred as synthetic schemes.

**Analysis:** The drug was analyzed by the polarimeter for its SOR, purity by HPLC and confirmation of the structure by 300 MHz <sup>1</sup>HNMR. All were within the specifications of the invention.

## RESULTS AND DISCUSSION

Though the content of 3-demethylcolchicine in the sludge was low but the finding of presence of the compound was very much exciting as it is very important from economy point of view. As and when there is scare city of the colchicoside one has the synthetic and isolation tools in hand. Colchicoside is largely used for making thiocolchicoside one of the prominent anti muscular drugs required only in few mg of dosages to combat any spasmodic ailments.

3-demethylcolchicine was also converted to 3-demethylthiocolchicine by reacting with sodium methyl mercaptan and was also coupled with  $\alpha$ -acetobromoglucose (2, 3, 4, 6-Tetra-O-acetyl- $\alpha$ -glucopyranosyl bromide) to get directly thiocolchicoside synthesized. All the compounds synthesized were on analysis was found to follow the specification as mentioned in FP/KP. (Bomardelli, 2009) while preparing at lower scale hurdles were faced with, specifically with regards to it solidification and recovering it for further purification. However it was recovered and the big challenge was to stabilize it, as storing in the desiccators didn't stabilize, the product next day rather was found to be degraded.

It was stabilized while admixing with the stabilizer (CaCO<sub>3</sub>), stored it in the deep freezer, and was found to be stable over the month until we finished our research on synthesis of drug with the complete specifications of FP/KP.

As a matter of fact the purity of the colchicoside is only 95% when it is extracted and isolated from *Glorisa superba* seeds. Over all the isolation of the colchicoside form the extracted mass takes more than 48 hrs to get 95% pure colchicoside. It was established that synthetic colchicoside and thiocolchicoside could be as potent as naturally occurred on the basis of their examination of specification.

Its isolation followed by the synthesis of colchicoside may decrease the severity of lack of less availability of colchicoside required for the synthesis of prominent drug thiocolchicoside. Column chromatography at laboratory and pilot scale was found to be a very compatible commercial technology as it is practiced worldwide for isolation and purification of the natural products of complex nature. Post synthesis the drug was completely assessed for its assay and physical properties to ascertain whether it meets the commercial specifications. Table 2 results complies the specifications.

Scheme 2 may be referred for the synthesis of thiocolchicoside from the prepared colchicoside (Bomardelli, 2007).

Relaxant drugs reduce muscle tone, are used in therapy for the treatment of contractures, and muscle spasm. Muscle spasm is one of the main factors responsible for chronic pain; it characterises several pathologies of the loco motor apparatus as well as inflammatory-rheumatic and degenerative orthopaedic pathologies; when it affects articulations, further to pain, it causes rigidity, which reduces joint mobility and flexibility in the affected part. For these reasons, the study of molecules endowed

with muscle relaxant and antispasmodic properties still raises remarkable clinical interest (Calogero, 1992).

**Table-2: Specification of synthesized thiocolchicoside as per FP.**

Parameters	Results
Assay (HPLC) Purity 102-99.36%	NLT98%
<b>Residual substance (other impurities) by HPLC</b>	
Colchicoside NMT 0.50%	0.15%
N-deacetyl-N-formylthiocolchicoside NMT 0.50%	0.25%
Total other impurities NMT 0.50%	0.10%
<b>Physical characteristics</b>	
Appearance yellow crystalline powder	complies
LOD NMT 4.0%	1.58%
Sulphated ash NMT 0.1%	0.07%
SOR (-) 550 to (-) 580	-565
<b>Residual solvents</b>	
Chloroform NMT60ppm	20ppm
Methanol NMT 3000ppm	265 ppm
Ethanol5000ppm	890 ppm
<b>Solubility</b>	sol in H <sub>2</sub> O/EtOH
<b>pH</b> 6.0-7.5	6.72

- QC results of prepared thiocolchicoside as per FP

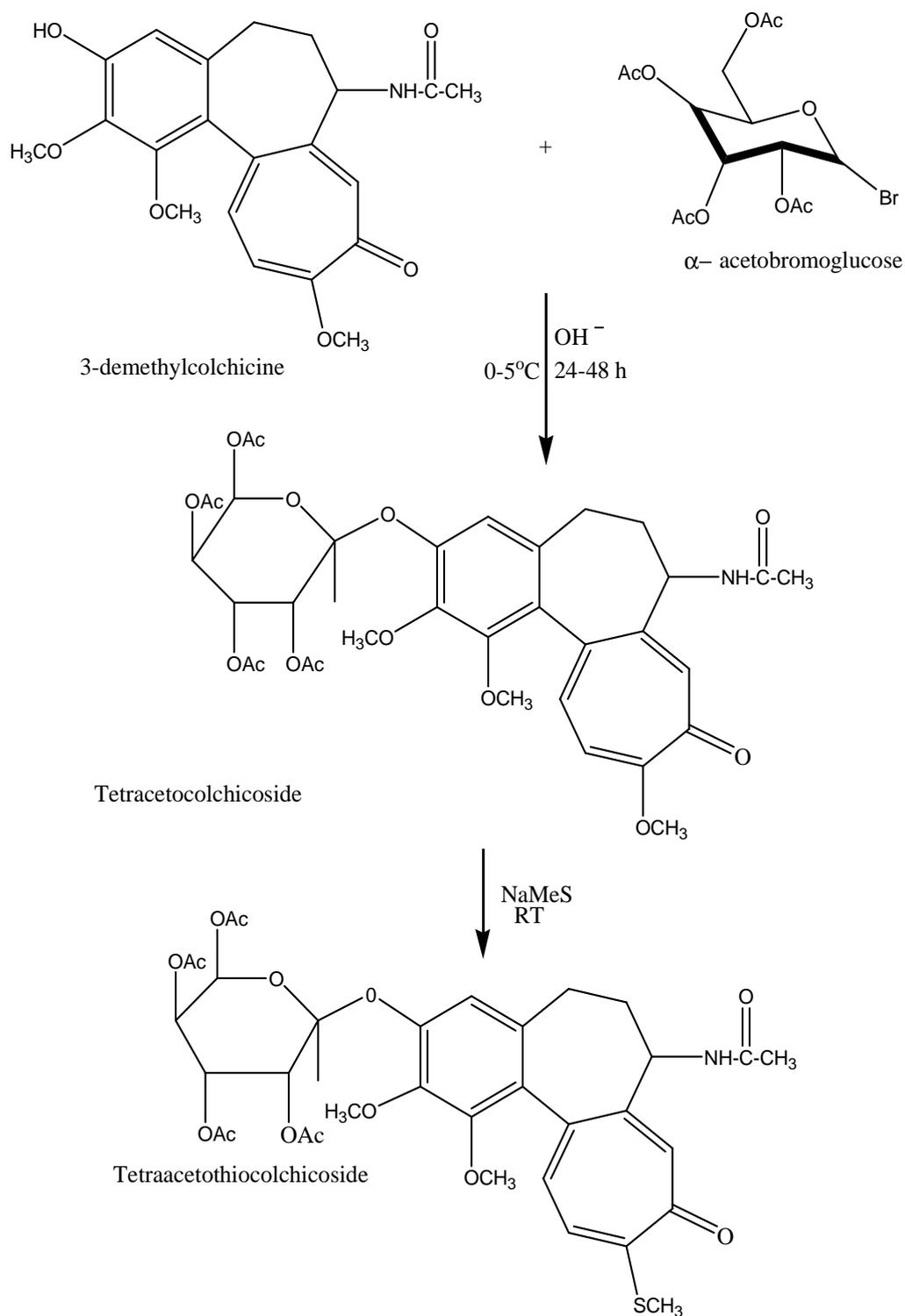
## CONCLUSIONS

Though the content of 3-demethylcolchicine in the *Glorisa superba* sludge was low but it was efficiently isolated and purified. Post purification it was coupled with  $\alpha$ -acetobromoglucose to prepare semi synthetic colchicoside which is only biosynthesized in the plant of *Glorisa superba* that is being extracted, isolated and purified to get 95% of pure colchicoside and then reacted with methylating agent to get commercialized contracturent. We have successfully established a semi synthetic route for synthesizing colchicoside and thiocolchicoside.

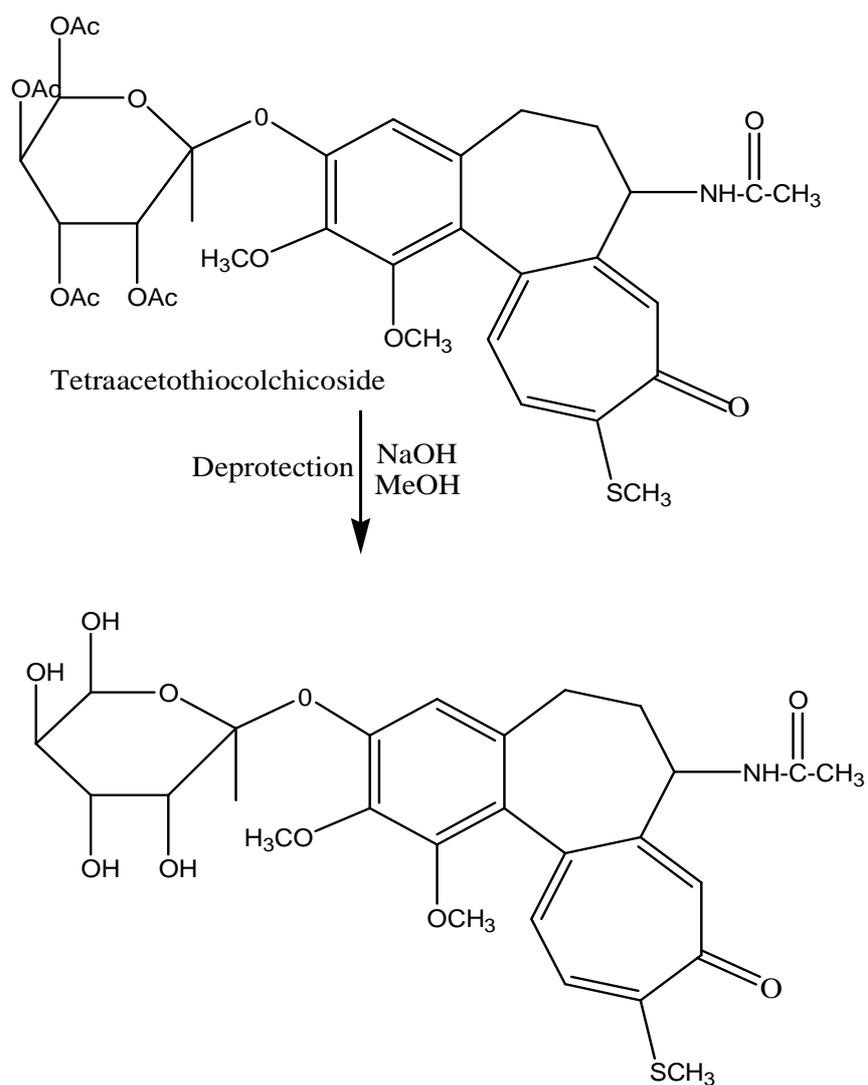
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**Scheme-1: Semi synthesis of colchicoside from 3-demethylcolchicine.**



**Thiocolchicoside FP/KP**

**Scheme-2: Synthesis of thiocolchicoside.**