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Isolation and estimation of Colchicine- a valuable phytochemical in the plant parts of *Iphigenia stellata* Blatt.using High Performance Liquid Chromatography

ABSTRACT

An attempt has been made to isolate and estimate the quantity of colchicine in the plant parts of *Iphigenia stellata* blatt. by using solvent extraction followed by high performance liquid chromatography. Colchicine is a drug of choice for management of gout. It is also used in the treatment of familial mediterranean fever. Colchicine has recently been approved by the US FDA for reducing the risk of myocardial infarction (MI), stroke, coronary revascularization, and cardiovascular death in adult patients with established atherosclerotic disease or with multiple risk factors for cardiovascular disease. Colchicine also induces polyploidy in plants. Pure Colchicine in powder form was isolated from the plant parts namely seeds, corms and capsules of *Iphigenia stellata* blatt. Our research project study was mainly conducted to estimate the quantity of colchicine present in the plant parts of *Iphigenia stellata* blatt. and to isolate the same in powder form. In our findings colchicine quantity was 10.6 mg/g in the seeds, 1.3 mg/g in the corms and 1.0 mg /1gm in the capsule walls .We have isolated dry pure Colchicine powder from the *Iphigenia stellata* blatt. plant parts using solvent extraction followed by concentration, purification, crystallisation and drying in vacuum oven. The yield obtained is 410 mg/50 g from seeds, 25 mg/50 g from corms and 22 mg /50 g from the capsule walls. We have reported highest amount of colchicine in the *Iphigenia stellata* seeds.

KEYWORDS: Capsules, Chloroform extract, Corms, Ethyl acetate, HPLC, *Iphigenia stellata* Blatt., Methanol extract, Seeds, Vacuum oven.

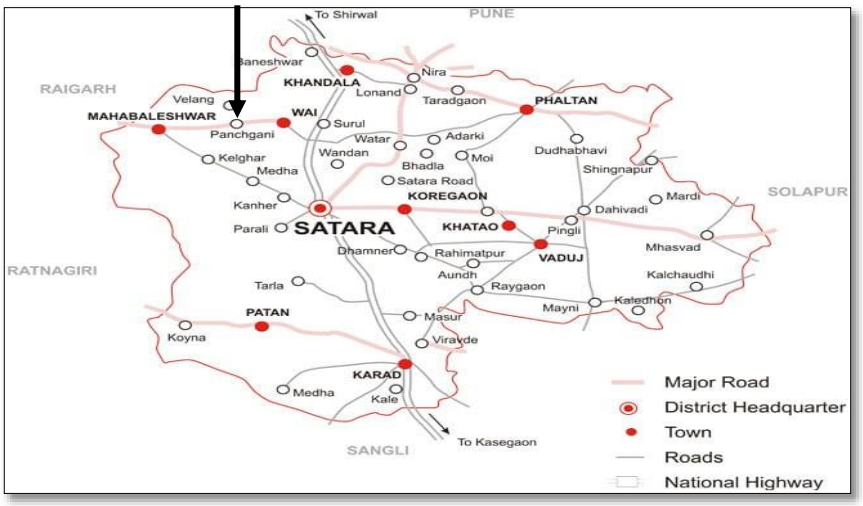
INTRODUCTION

Iphigenia stellata Blatt.is a slender perennial herbaceous plant belonging to the family Colchicaceae grows along with other species of *Iphigenia* in discrete patches in Western Ghats of Maharashtra at Panchgani, Pune, Gaganbawada, Panhala and Radhanagari. (20) The main purpose of the present investigation is to quantitative estimation of colchicine powder of the Bhuicharkra plant parts. It is commonly known as ranlasun, gulabibhuichakra, and star grass lily (12; 15; Flicker, 2011). Plants consists of corm, stem, leaves, capsule, root, seeds and reproduce mainly by tubers. There are six species of *Iphigenia* in India viz *I. indica*, *I.*

pallida, *I. stellata*, *I. mysorensis*, *I. magnifica* and *I. sahyadrica* (1, 2, 3, 5, 10). Crystalline colchicines alkaloid has occupied financial position due to its exploitation for the treatment of goat, fever ,tumor, cancer, skin infection, scrofula , snake bite, rheumatism, polyploidy and plant breeding (13, 8, 21, Shrivastav *et.al.*, 2002, Bharti*et.al*, 2006, 14, 16, Murkytiy*et.al.*, 2009). Quantitative estimation of colchicine powder was done using HPLC technique. Colchicine was isolated from the plant parts by solvent extraction followed by purification and drying.

MATERIAL AND METHODS

Materials : Site of Collection
Panchgani in Satara district of Maharashtra.



Individuals of *Iphigenia stellata* with corms and capsules containing seeds were collected from Panchgani in Satara district of Maharashtra. Dense patches of *I. stellata* grow in the peripheral region of tableland in this region during monsoon. Panchgani is located in the middle of five hills in the Sahyadri mountain ranges. Geographical co-oridnates for Panchgani city are 17°55'0" North and 73°49'0" East. Elevation of Panchgani is 1,293 m /4,242 ft. (11). Fully developed plants of *I. stellata* along with the corms were collected in 19th August, 2023 and brought to the R.B. Madkholkar Mahavidyalaya, Chandgad, botany research laboratory. Different parts of the plant were separated and air dried to avoid fungal infection. Seeds were cleaned and stored in plastic bottles whereas corms were stored in thick plastic bags at room temperature in a cool and dry place. After one month the material was

transported to Alkaloids Pvt Ltd, Medchal, Telangana for quantification and isolation of Colchicine.

Methods :

Cleaning and preservation of collected plant parts

Full grown, healthy, dark brown to black coloured and mature seeds were with 2 mm in diameter, matured light brown coloured large sized corms were with 1.29 to 2.31g fresh weight per single corm, 1.6 to 1.9 cm equatorial diameter and 2.3 to 3. 2 cm polar diameter, matured brownish black capsules were with 0. 21 to 0.24 gm fresh weight per single capsule, 0.4 to 0.8 mm equatorial diameter and 0.8 mm to 1. 0 cm polar diameter. The plant parts were washed with distilled water for fifteen minutes and then this material was immersed in 50 ml of 0.1% mercuric chloride for five minutes to remove all the traces of disinfection from surface of the plant material. After one month the dried plant parts were transported to the laboratory of Alkaloids Private Limited, Medchal, Telangana, India for further experimental work related to the quantification and isolation of Colchicine.

Isolation of Colchicine

Plant materials were oven dried at 20 ^{0}C for 9 days. 50g of seeds were powdered and extracted successively with 90% methanol. Four extractions were performed with 250mL, 250 mL, 200 mL and 200 mL of 90% methanol respectively. All extractions were conducted at 50°C temperature under stirring. The four extracts were combined and concentrated to 50 ml. It was then partitioned five times with 50 mL chloroform each time. The chloroform layers were separated in a separating funnel and the combined extracts measuring about 250 mL were concentrated to a thick paste under vacuum in a rotary evaporator. The resulting solid mass was dissolved in 5 mL of ethyl acetate and transferred to a beaker. Colchicine crystallised upon cooling for 24 hours in a refrigerator. The crystallised colchicine was filtered through a Buchner funnel and dried in a vacuum oven at 60°C for 10 hours to obtain 410 mg of an off-white powder. The colchicine powder thus obtained was subjected to HPLC analysis. The above described extraction process was repeated for corms and capsule walls.

Quantification of Colchicine

Quantification of Colchicine in the plant parts was carried out by HPLC technique. The plant parts were ground to a fine powder. They were then extracted with methanol. The methanol extract was filtered and the volume made up to the mark in volumetric flasks. The samples prepared were suitably diluted and then injected in the HPLC system. Reversed phase HPLC analysis was conducted and the results calculated.

RESULTS AND DISCUSSIONS

Sabale and Kalebere (2000) and Yadav and Sardesai (2002) reported morphological features and microclimate of *Iphigenia* Species from Western Ghats of Maharashtra which includes morphology of *Iphigenia stellata* Blatt.We have studied dark brown to black coloured and mature seeds were with 2 mm in diameter, matured brown coloured, large sized corms were with 1.29 to 2.31 g fresh weight per single corm, 1.6 to 1.9 cm equatorial diameter and 2.3 to 3. 2 cm polar diameter, matured brownish black capsules with 0. 21 to 0.24 g fresh weight per single capsule, 0.4 to 0.8 mm equatorial diameter and 0.8 mm to 1. 0 cm polar diameter, We have reported Colchicine content upon analysis of the samples prepared from the plant parts such as seeds, corms and capsules of *Iphigenia stellata* blatt. by using HPLC technique. The HPLC chromatograms obtained for the standard and samples are represented in Figures 1, 2, 3, and 4. The results obtained are tabulated in Table 1. The colchicine content was found to be 10.6 mg/1 g in the seeds, 1.3 mg/1g in the corms and 1.0 mg /1g in the capsule walls.

We have reported highest amount of colchicine in the *Iphigenia stellata* seeds. In India, the colchicine yielding species include *Colchicum leutum*, *Gloriosa superba* and *Iphigenia stellata* (6). Using HPLC method Sabale and Mane (2011) reported 47 mg/100 g dry weight of colchicine in the corms and 71 mg/100 g dry weight of colchicine in the seeds of *Iphigenia stellata* Blatt.

We have obtained maximum yield by selecting suitable solvent and optimum extraction conditions. The concentration of extracts under controlled conditions, crystallisation of Colchicine using appropriate solvent and suitable conditions has resulted in high recovery of the compound from the seeds of *Iphigenia stellata* blatt. The yield obtained is 410 mg/50 g from seeds, 25 mg/50 g from corms and 22 mg /50 g from capsule walls.

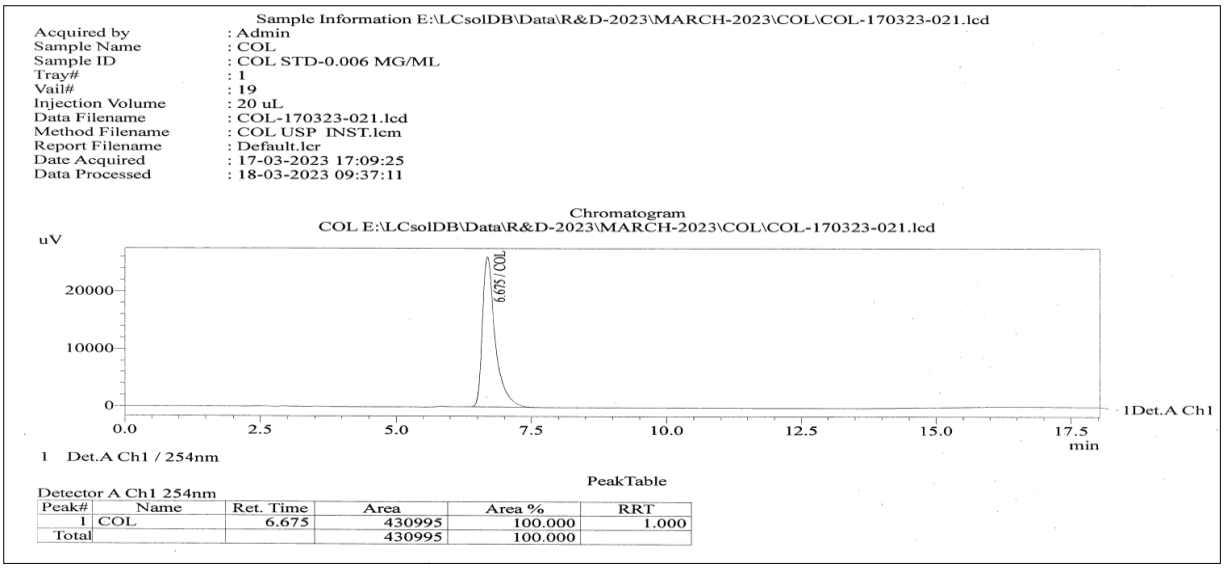


Figure 1- Colchicine Standard HPLC Chromatogram

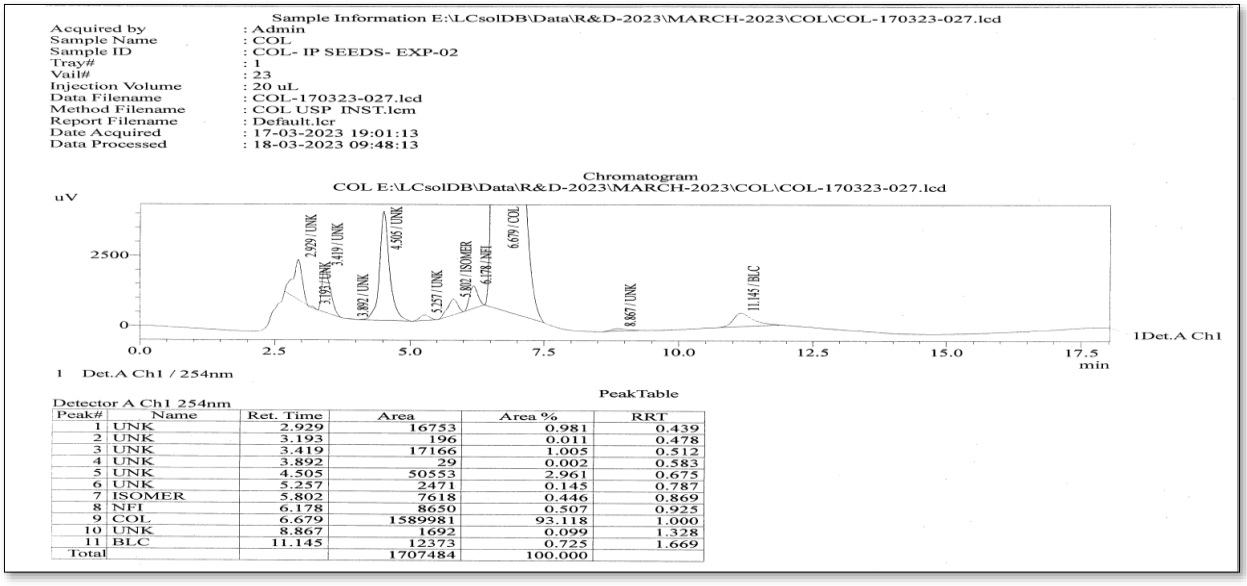
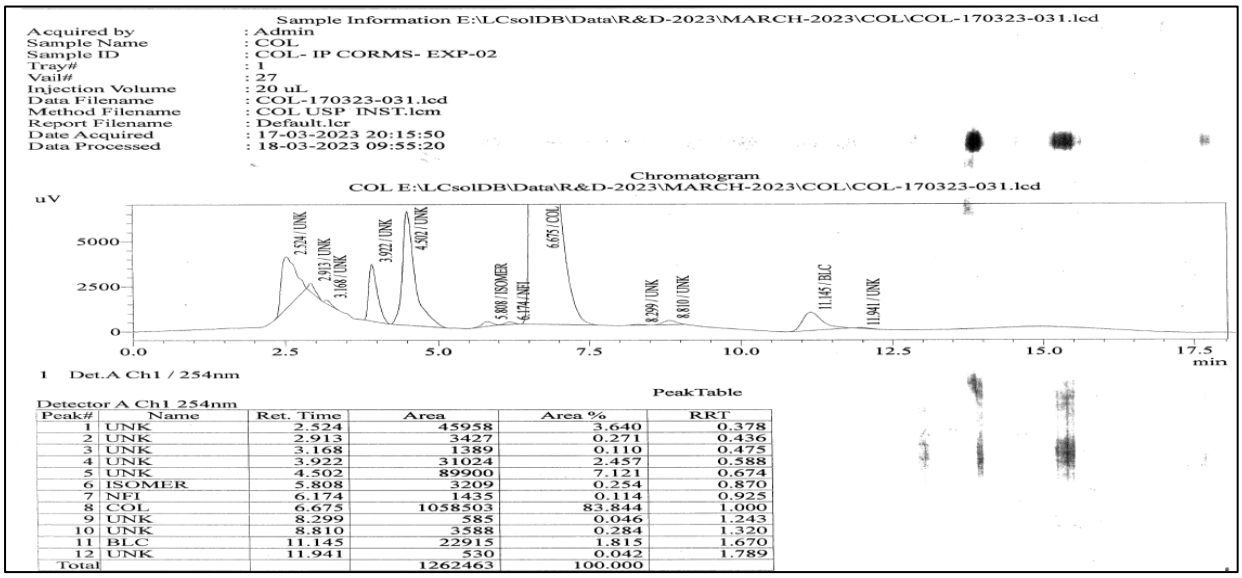


Figure 2- HPLC chromatogram of sample prepared by extraction of *Iphigenia stellata* Seeds with Methanol

Colchicine content	=	Standard peak area / Sample	X	Standard dilution / Sample	X	Purity of standard

		peak area		dilution		

Colchicine content	=	1589981 / 429810	X	20.1 / 20	X	0.3 / 50	X	100 / 5074	X	25 / 1	X	94.58	=	1.06%



Peak#	Name	Ret. Time	Area	Area %	RRT
1	UNK	2.524	45958	3.640	0.378
2	UNK	2.913	3427	0.271	0.436
3	UNK	3.168	1389	0.110	0.475
4	UNK	3.922	31024	2.457	0.588
5	UNK	4.502	89900	7.121	0.674
6	ISOMER	5.808	3209	0.254	0.870
7	NFI	6.174	1435	0.114	0.925
8	COL	6.675	1058503	83.844	1.000
9	UNK	8.299	585	0.046	1.243
10	UNK	8.810	3588	0.284	1.320
11	BLC	11.145	22915	1.815	1.670
12	UNK	11.941	530	0.042	1.789
Total			1262463	100.000	

Figure 3 - Figure 2- HPLC chromatogram of sample prepared by extraction of *Iphigenia stellata* corms with Methanol

Colchicine content	=	1075315 / 429810	X	20.1 / 20	X	0.3 / 50	X	100 / 5372.5	X	10 / 2	X	94.58	=	0.13%

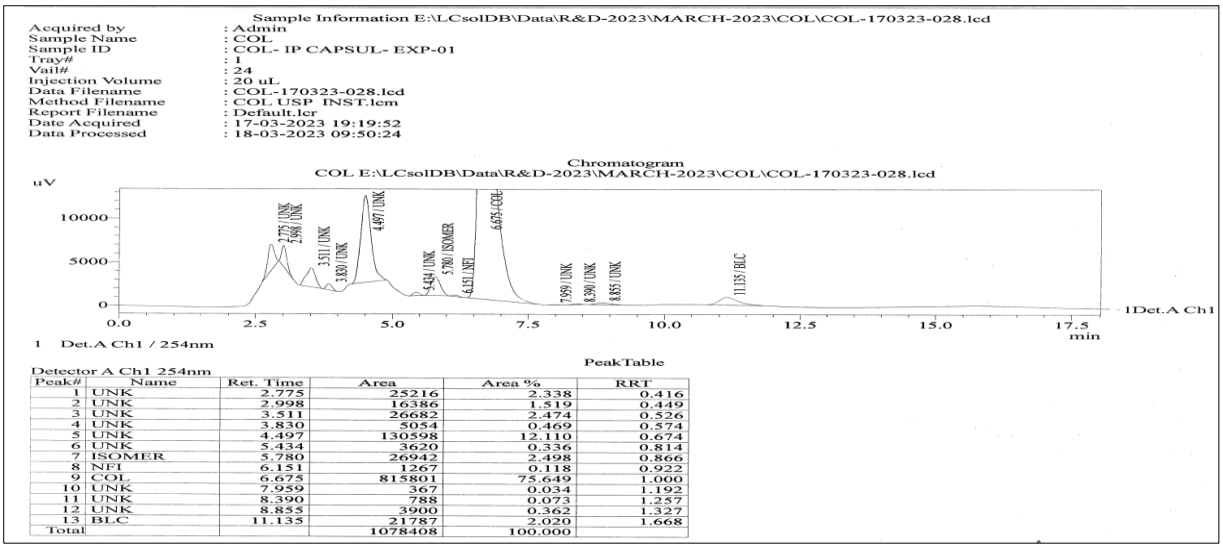
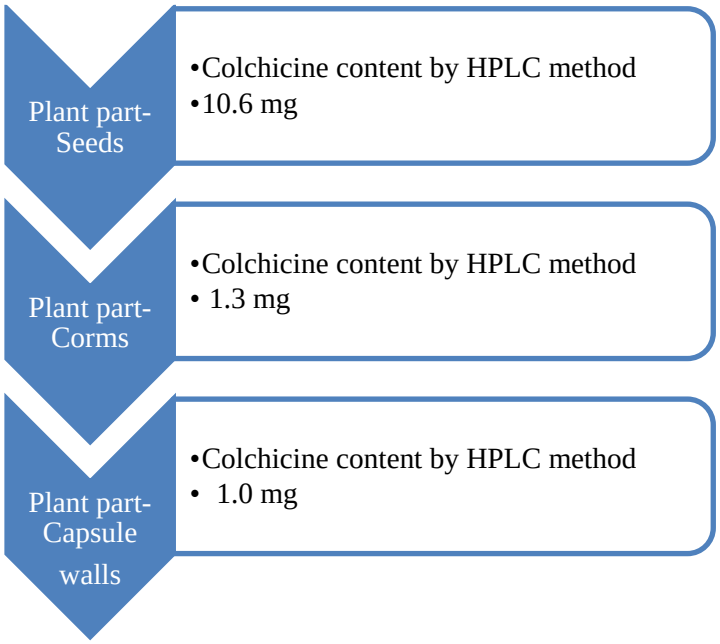


Figure 4 - HPLC chromatogram of sample prepared by extraction of *Iphigenia stellata* capsule walls with Methanol HPLC profile

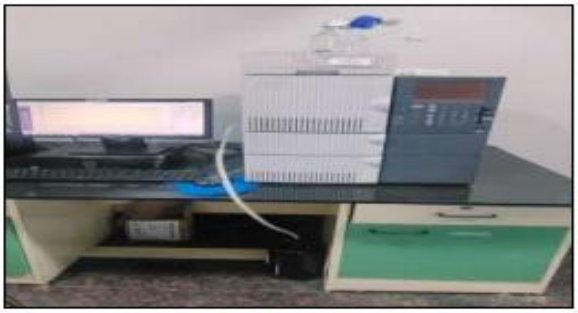
Colchicine content	=	815883 / 429810	X	20.1 / 20	X	0.3 / 50	X	100 / 5004.7	X	10 / 2	X	94.58	=	0.10%

Table 1- Colchicine content (mg / 1 gm dry weight) in seeds, corms and capsule walls of *Iphigenia stellata* Blatt. by HPLC method



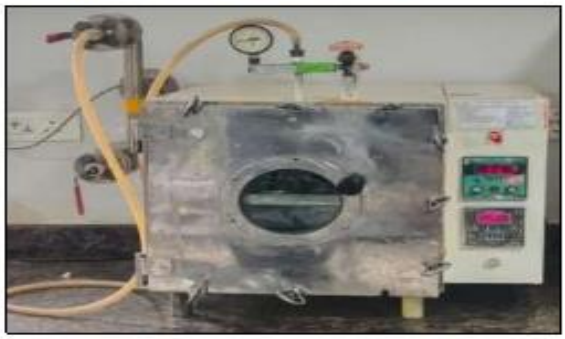
Photoplate-1

1. Instrument utilized for the evaluation of colchicine – High performance liquid chromatography method



2. Instrument utilized for the samples extracts Filtration procedure- Buchner's funnel

3. Colchicine crystals separated from the Plant parts with the help of sophisticated Experimental instrument vacuum oven



Photoplate-2

Iphigenia stellata Blatt. a. Flowers; b. & c. Entire Plants; d. Corms; e. Capsule walls; f. Seeds; g. & h. Plant Collections

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Photoplate 3
Our expected final product -Colchicine powder content separated from the seeds ,corms and capsule walls of *Iphigenia stellata* Blatt. with the help of sophisticated experimental instrument vacuum oven method

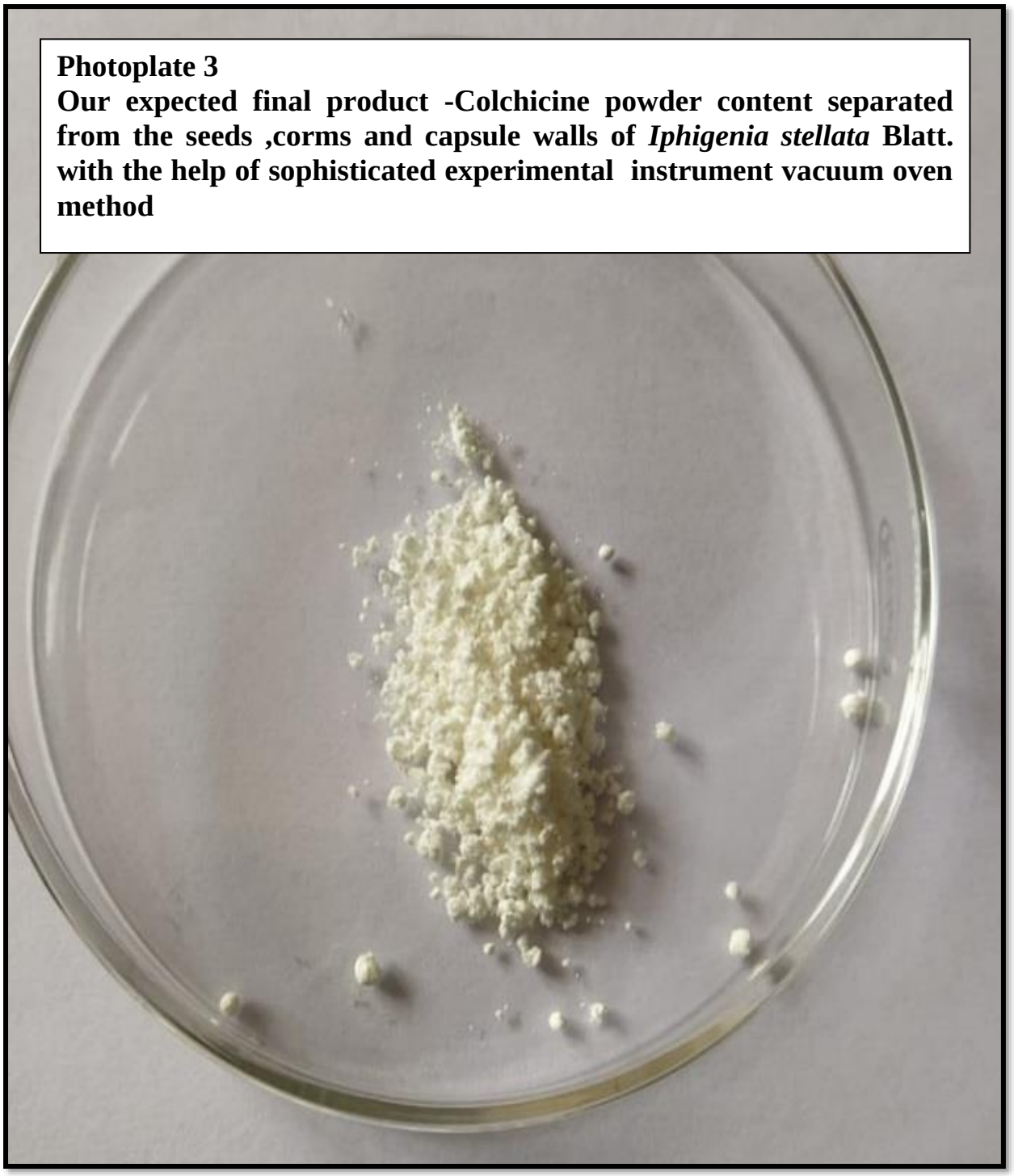

Table -2- Data for the extraction of Colchicine from *Iphigenia stellata* Blatt. plant parts using 90% Methanol :

Sr. No.	Plant Part	Weight of the Plant part (gm)	Input volume (ml) of solvent	Output volume (ml) of extract	Total solids %	Quantity of extractive matter in grams
1.	Seeds	50	250	190	2.01	3.819
			200	185	1.19	2.205
			200	195	0.42	0.826
			200	190	0.12	0.228
				760		7.078
2	Corms	50	250	165	1.89	3.118
			200	192	0.99	1.900
			200	189	0.72	1.360
			200	189	0.06	0.113
				735		6.491
3.	Capsule walls	50	250	170	1.99	3.383
			200	183	1.09	1.994
			200	194	0.61	1.183
			200	196	0.09	0.176
				743		6.736

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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