

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

3
4 **ABSTRACT**

5 An attempt has been made to isolate and estimate the quantity of colchicine in the plant
6 parts of *Iphigenia stellata* blatt. by using solvent extraction followed by high performance
7 liquid chromatography. Colchicine is a drug of choice for management of gout. It is also used
8 in the treatment of familial mediterranean fever. Colchicine has recently been approved by
9 the US FDA for reducing the risk of myocardial infarction (MI), stroke, coronary
10 revascularization, and cardiovascular death in adult patients with established atherosclerotic
11 disease or with multiple risk factors for cardiovascular disease. Colchicine also induces
12 polyploidy in plants. Pure Colchicine in powder form was isolated from the plant parts
13 namely seeds, corms and capsules of *Iphigenia stellata* blatt. Our research project study was
14 mainly conducted to estimate the quantity of colchicine present in the plant parts of *Iphigenia*
15 *stellata* blatt. and to isolate the same in powder form. In our findings colchicine quantity was
16 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
17 isolated dry pure Colchicine powder from the *Iphigenia stellata* blatt. plant parts using
18 solvent extraction followed by concentration, purification, crystallisation and drying in
19 vacuum oven. The yield obtained is 410 mg/50 g from seeds, 25 mg/50 g from corms and
20 22 mg /50 g from the capsule walls. We have reported highest amount of colchicine in the
21 *Iphigenia stellata* seeds.

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

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26 **INTRODUCTION**

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

34 *pallida*, *I. stellata*, *I. mysorensis*, *I. magnifica* and *I. sahyadrica* (1, 2, 3, 5, 10). Crystalline
35 colchicines alkaloid has occupied financial position due to its exploitation for the treatment of
36 goat, fever ,tumor, cancer, skin infection, scrofula , snake bite, rheumatism, polyploidy and
37 plant breeding (13, 8, 21, Shrivastav *et.al.*, 2002, Bharti*et.al.*, 2006, 14, 16, Murkytiy*et.al.*,
38 2009). Quantitative estimation of colchicine powder was done using HPLC technique.
39 Colchicine was isolated from the plant parts by solvent extraction followed by purification
40 and drying.

41 MATERIAL AND METHODS

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43 Materials : Site of Collection

44 Panchgani in Satara district of Maharashtra.

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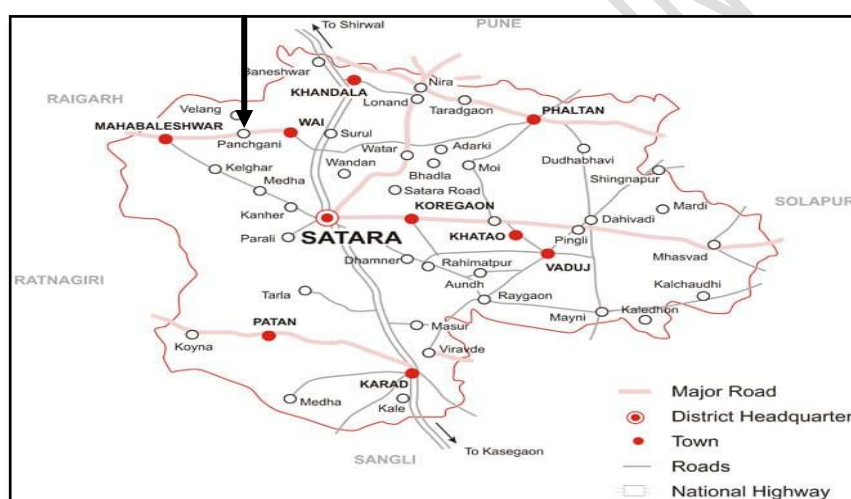
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63 Individuals of *Iphigenia stellata* with corms and capsules containing seeds were
64 collected from Panchgani in Satara district of Maharashtra. Dense patches of *I. stellata* grow
65 in the peripheral region of tableland in this region during monsoon. Panchgani is located in
66 the middle of five hills in the Sahyadri mountain ranges. Geographical co-ordinates for
67 Panchgani city are 17°55'0" North and 73°49'0" East. Elevation of Panchgani is 1,293 m
68 /4,242 ft. (11). Fully developed plants of *I. stellata* along with the corms were collected in
69 19th August, 2023 and brought to the R.B. Madkholkar Mahavidyalaya, Chandgad, botany
70 research laboratory. Different parts of the plant were separated and air dried to avoid fungal
71 infection. Seeds were cleaned and stored in plastic bottles whereas corms were stored in thick
72 plastic bags at room temperature in a cool and dry place. After one month the material was

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

75 **Methods :**

76 **Cleaning and preservation of collected plant parts**

77 Full grown, healthy, dark brown to black coloured and mature seeds were with 2 mm
78 in diameter, matured light brown coloured large sized corms were with 1.29 to 2.31g fresh
79 weight per single corm, 1.6 to 1.9 cm equatorial diameter and 2.3 to 3.2 cm polar diameter,
80 matured brownish black capsules were with 0.21 to 0.24 gm fresh weight per single capsule,
81 0.4 to 0.8 mm equatorial diameter and 0.8 mm to 1.0 cm polar diameter. The plant parts were
82 washed with distilled water for fifteen minutes and then this material was immersed in 50 ml
83 of 0.1% mercuric chloride for five minutes to remove all the traces of disinfection from
84 surface of the plant material. After one month the dried plant parts were transported to the
85 laboratory of Alkaloids Private Limited, Medchal, Telangana, India for further experimental
86 work related to the quantification and isolation of Colchicine.

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88 **Isolation of Colchicine**

89 Plant materials were oven dried at 20 °C for 9 days. 50g of seeds were powdered and
90 extracted successively with 90% methanol. Four extractions were performed with 250mL,
91 250 mL, 200 mL and 200 mL of 90% methanol respectively. All extractions were conducted
92 at 50°C temperature under stirring. The four extracts were combined and concentrated to 50
93 ml. It was then partitioned five times with 50 mL chloroform each time. The chloroform
94 layers were separated in a separating funnel and the combined extracts measuring about 250
95 mL were concentrated to a thick paste under vacuum in a rotary evaporator. The resulting
96 solid mass was dissolved in 5 mL of ethyl acetate and transferred to a beaker. Colchicine
97 crystallised upon cooling for 24 hours in a refrigerator. The crystallised colchicine was
98 filtered through a Buchner funnel and dried in a vacuum oven at 60°C for 10 hours to obtain
99 410 mg of an off-white powder. The colchicine powder thus obtained was subjected to HPLC
100 analysis. The above described extraction process was repeated for corms and capsule walls.

101 **Quantification of Colchicine**

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

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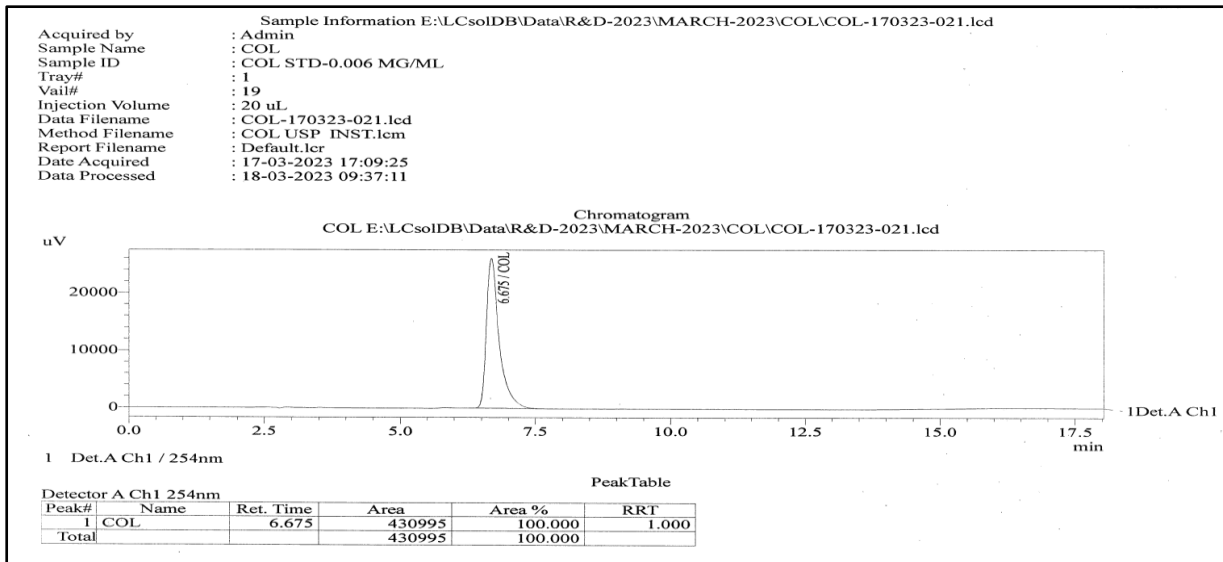
108 **RESULTS AND DISCUSSIONS**

109 Sabale and Kalebere (2000) and Yadav and Sardesai (2002) reported morphological
110 features and microclimate of *Iphigenia* Species from Western Ghats of Maharashtra which
111 includes morphology of *Iphigenia stellata* Blatt. We have studied dark brown to black
112 coloured and mature seeds were with 2 mm in diameter, matured brown coloured, large sized
113 corms were with 1.29 to 2.31 g fresh weight per single corm, 1.6 to 1.9 cm equatorial
114 diameter and 2.3 to 3.2 cm polar diameter, matured brownish black capsules with 0.21 to
115 0.24 g fresh weight per single capsule, 0.4 to 0.8 mm equatorial diameter and 0.8 mm to 1.0
116 cm polar diameter, We have reported Colchicine content upon analysis of the samples
117 prepared from the plant parts such as seeds, corms and capsules of *Iphigenia stellata* Blatt. by
118 using HPLC technique. The HPLC chromatograms obtained for the standard and samples are
119 represented in Figures 1, 2, 3, and 4. The results obtained are tabulated in Table 1. The
120 colchicine content was found to be 10.6 mg/1 g in the seeds, 1.3 mg/1g in the corms and 1.0
121 mg/1g in the capsule walls.

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

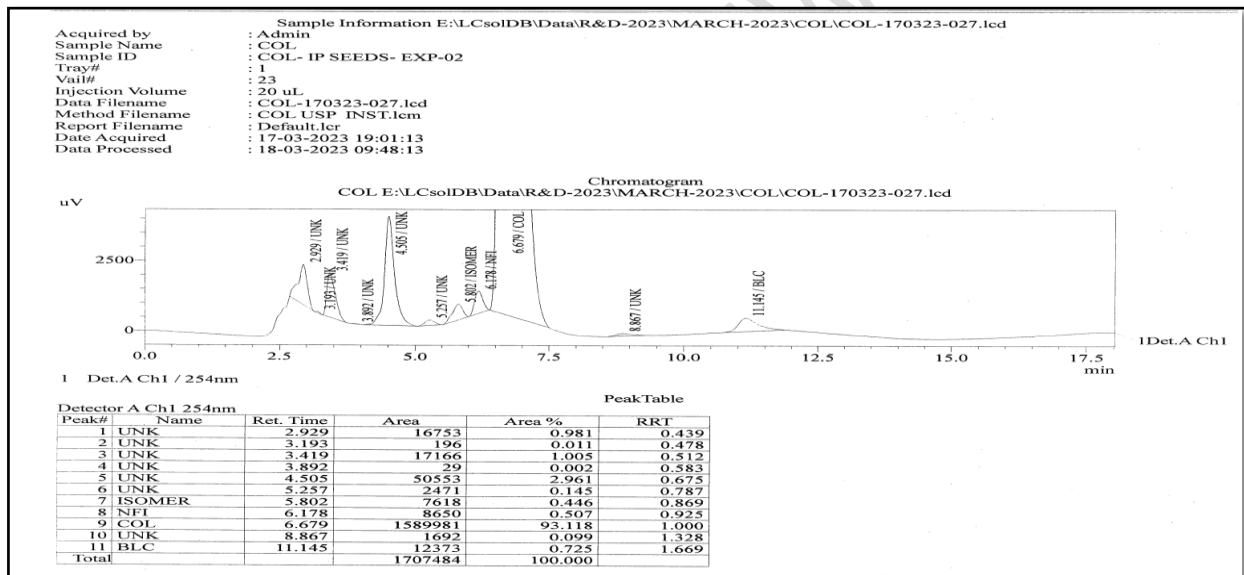
127 We have obtained maximum yield by selecting suitable solvent and optimum extraction
128 conditions. The concentration of extracts under controlled conditions, crystallisation of
129 Colchicine using appropriate solvent and suitable conditions has resulted in high recovery of
130 the compound from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

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Figure 1- Colchicine Standard HPLC Chromatogram



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

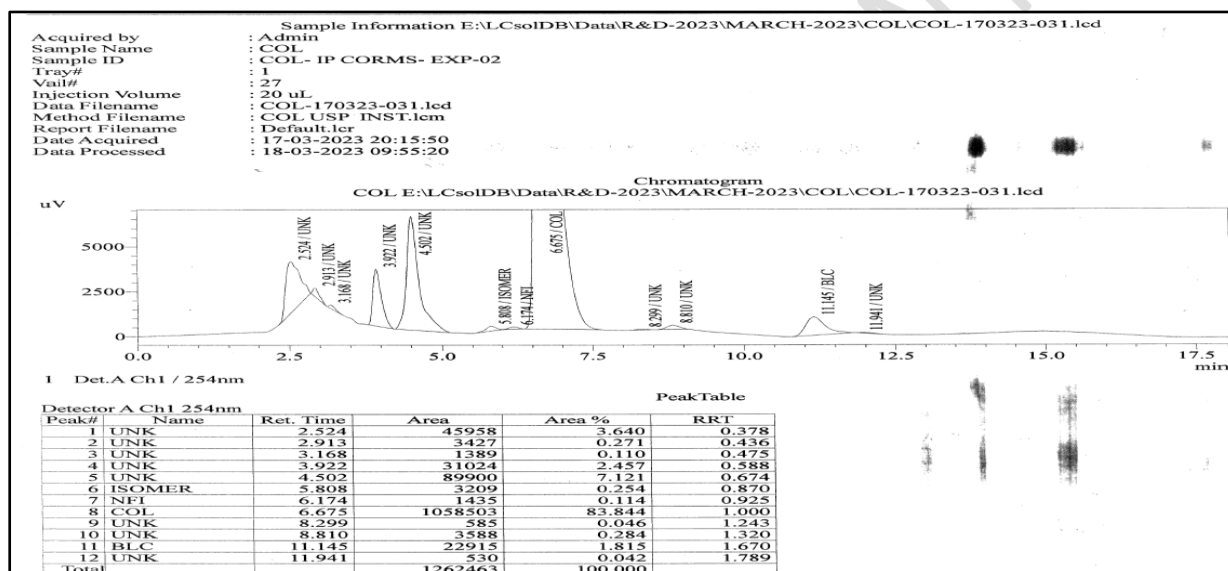
Colchicine content	=	Standard peak area	X	Standard dilution	X	Purity of standard
		Sample		Sample		

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		peak area		dilution		
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Colchicine content	=	$\frac{1589981}{429810}$	X	$\frac{20.1}{20}$	X	$\frac{0.3}{50}$	X	$\frac{100}{5074}$	X	$\frac{25}{1}$	X	94.58	=	1.06%
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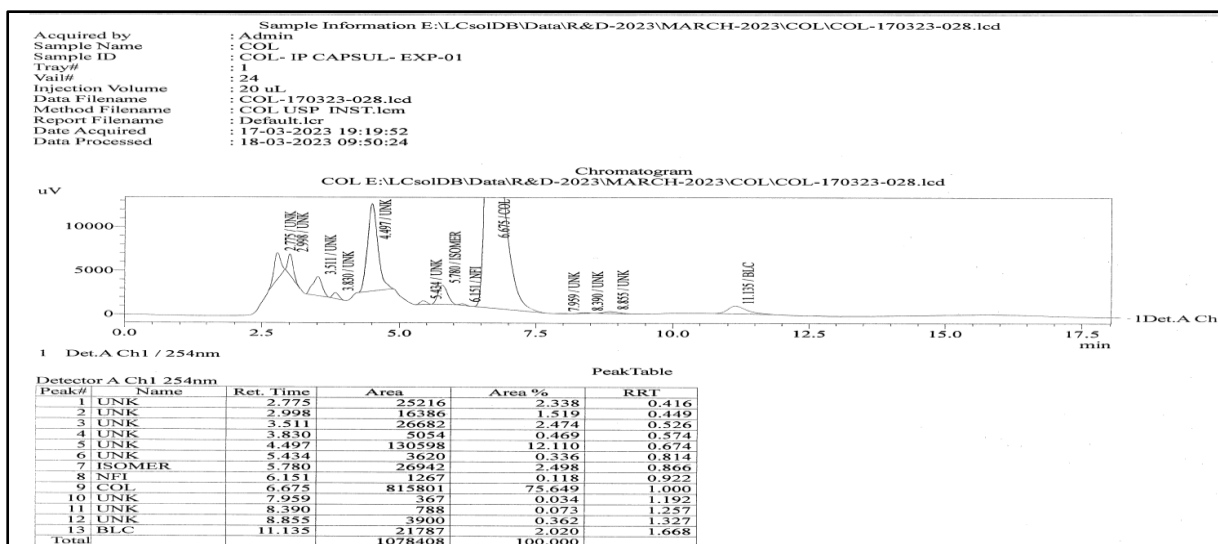


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Figure 3 - Figure 2- HPLC chromatogram of sample prepared by extraction of *Iphigenia stellata* corms with Methanol

Colchicine content	=	$\frac{1075315}{429810}$	X	$\frac{20.1}{20}$	X	$\frac{0.3}{50}$	X	$\frac{100}{5372.5}$	X	$\frac{10}{2}$	X	94.58	=	0.13%
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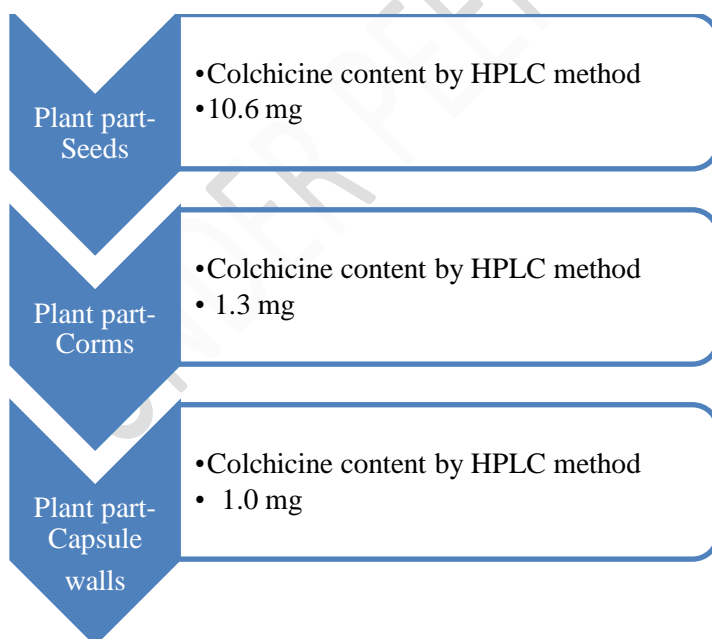
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Figure 4 - HPLC chromatogram of sample prepared by extraction of *Iphigenia stellata* capsule walls with Methanol HPLC profile

$$\text{Colchicine content} = \frac{815883}{429810} \times \frac{20.1}{20} \times \frac{0.3}{50} \times \frac{100}{5004.7} \times \frac{10}{2} \times 94.58 = 0.10\%$$

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



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Photoplate-1

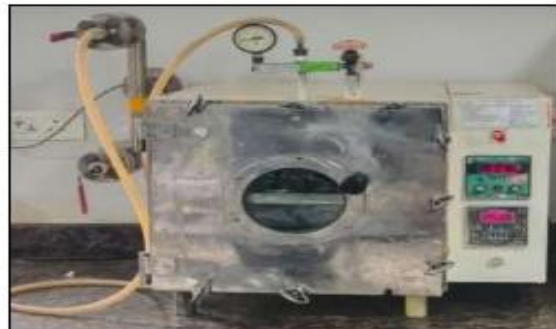
**1. Instrument utilized for the evaluation of colchicine –
High performance liquid chromatography method**



**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**



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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



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198 **Table -2-** Data for the extraction of Colchicine from *Iphigenia stellata* Blatt. plant parts using
 199 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

200

201 **ACKNOWLEDGEMENT**

202 Authors are thankful to Dr. S. D. Goral, Principal of R.B. Madkholkar Mahavidyalaya,
 203 Chandgad, District- Kolhapur for Internet, library and laboratory facilities.

204 **CONFLICTS OF INTEREST**

205 The authors declare no conflicts of interest.

206 **FUNDING STATEMENT**

207 We are thankful to the Alkaloids Bioactives Private Limited (formerly Alkaloids Private
 208 Limited), Medchal, Telangana for financial support and for carrying out the experimental
 209 work related to the isolation & quantification of colchicine.

210

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