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Quality Control and Standardization of Colchicum (*Colchicum luteum* Baker) Rhizome with the help of TLC, HPLC and HPTLC.

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Abstract

Background: Colchicum (*Colchicum luteum* Baker) is a well-known traditional medicinal plant used for therapeutic effects in Unani and Ayurvedic System of Medicine. It has been reported to have many therapeutic activities like anti-inflammatory, anti-rheumatic and anti-gout properties. It is also useful in the management of Sciatica, Headache, Wound healing, diseases of liver and spleen, Sexual debility. The present study aimed towards the evaluation of the parameters involved in the determination of the quality and purity of *Colchicum luteum* Baker**.** corm and its standardization. **Material and Methods:** Organoleptic characters, extractive values, ash values, phyto-chemical analysis, TLC, fluorescence analysis, HPLC and HPTLC profile etc. were the parameters used for the standardisation of the test drug. **Result:** Total ash values, water, alcohol, Ether soluble extractive values and volatile oil percentage was found to be 6.208%, 21.173%, 1.693%, 0.22%, and 1.60% respectively. TLC profile of *Colchicum luteum* Baker. shows 01, 03, 01, 04 and 10 spots in UV short, long wavelength and exposure in anisaldehyde-sulphuric acid at room temperature, 65°C and 95°C respectively. The HPLC of Standard Drug (Colchicine) pattern shows 10 peaks and the peak no.6 is major peak having area concentration and retention time as 99.45% at 3.19 min. While Crude Drug (*Colchicum luteum* Baker) shows 13 peaks and the peak no.2 and 1 are major peaks having area concentration and retention time as 62.28% at 3.20 min. and 33.76% at 2.85 min. respectively**.** **Conclusion:** The study will provide referential information for the good quality, purity and identification for the future batches of *Colchicum luteum* Baker.

Keywords
Phyto-chemical analysis, Quality, Standardization, Unani.

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INTRODUCTION

Nowadays the Indian herbal industry is flourishing at an admirable pace with remarkable increase in the introduction of new herbal pharmaceutical and cosmetic products in the market. But due to ignorance and awful supply chain management of herbal medicines, quality and purity of herbs and their products is not assured. As the efficacy and safety of herbal products is strongly based on their good quality therefore, determination of identity, quality and purity of the herbal medicines is unavoidable. The traditional approach towards standardization for obtaining good quality herbal medicines seems to be insufficient for the current herbal market which invites the need for more advanced techniques. Standardization of the crude drugs involves passport data of the drugs viz., botanical identification, and macroscopic, microscopic and molecular examination, identification of Phytochemical constituent by various chromatographic techniques and biological activity of the whole plant.[1] Due to the high commerce, traders have imperilled plants and their products to adulteration and substitution.

As the Corm of ***Colchicum luteum* Baker.** is often contaminated and adulterated with different plant materials such as Colchicum Sweet, hina, turbud.[2] Therefore, in this study ***Colchicum luteum* Baker**, *family* – Liliaceae was selected and standardized on their physico-chemical characteristics along with TLC, HPLC & HPTLC profile. There are about 70 species in this genus and two are native to India. It is found in hilly and valley area as Western Himalayas and Kashmir. It is also found in Afghanistan and commonly known as Suranjan.[3] It is an excellent remedy popular to the Indian medicinal system and is used from centuries for its health benefits; it can also be used for **Rheumatoid Arthritis, Gout,** Internal haemorrhoids, Sexual debility[4], Headache, Wound healing, Diseases of liver and spleen. Medicinally it possesses Carminative, Laxative, Aphrodisiac, Purgative (Aperient), Alterative, Cicatrizant etc.[5]

The ***Colchicum*** is a mixture of constituents, consisting of Colchicine is the main alkaloid isolated from all species of the genera, *Colchicum.* The major phenolic compounds obtained from the genus, *Colchicum,* are 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-hydroxybenzoic acid (vanillic acid), 3-(3-hydroxyphenyl)-2-propanoic acid (coumaric acid), 3-(3,4-dihydroxyphenyl)-2-propanoic acid (caffecic acid), and 3,4,5,7 tetrahydroxyflavone (luteolin). (7.5%).[6]

The antiarthritic activity of hydroalcoholic extract of Colchicum luteum was due to its modulatory effect on the expression of pro-inflammatory cytokine in the synovium. It produced a significant inhibition of joint swelling in both, formaldehyde and CFA (Complete Freund's adjuvant) induced arthritis. Serum TNF-α level was also reduced significantly. The expression of proinflammatory mediators (TNF-R1, IL-6 and IL-1β) was also found to be less in the CLHE treated group as compared to control.[7]

The colchicum preparations have a specific clinical effect in the treatment of acute gout. It acts against the inflammatory response to the urate crystals and has no effect on the concentration of uric acid in blood or on uric acid excretion. It prevents attacks of Familial Mediterranean fever 8 and also useful in Falciparum malaria. Colchicine has a specific effect in gouty arthritis and can be used both to prevent and to relieve acute attacks. It prevents migration of neutrophils in to the joint, apparently by binding to tubulin, resulting in the depolymerisation of the microtubules and reduced cell motility. Colchicine-treated neutrophils develop a 'drunken walk'. Colchicines may also prevent the production of a putative inflammatory glycoprotein by neutrophils that have phagocytosed urate crystals, and other mechanism may also be important in bringing about its effects.[9]

The present study was carried out in order to standardise the rhizome of ***Colchicum luteum* Baker**. with a view to develop its quality parameters, and also to deliver referential information for the identification of the crude drug so as to check the substitution and adulteration and to ensure the effectiveness of a drug in treating different body ailments. Parameters include macroscopy, powder analysis, physicochemical parameters and preliminary phyto-chemical screening along with HPLC & HPTLC profile.

MATERIAL and METHOD

Collection of sample

Dried Corm of ***Colchicum luteum* Baker**. was procured from Khari Bawli, New Delhi and was properly recognized from the accessible literature and authenticated by Prof. Abdul Latif. The sample with specimen voucher no. SC-0227/17 was deposited in the museum of Department of Pharmacy, Faculty of Unani medicine, Aligarh Muslim University, Aligarh, for future reference. It was crushed and sieved to coarse powder mechanically and stored in air tight container for study.

Macroscopy and organoleptic characters

The organoleptic characters of the crude drug were observed with sensory organs and was analysed for

its colour, odour and taste, size, shape, fracture and surface.[10,11,12]

Physicochemical parameters

Ash values, alcohol and water soluble extractive values, volatile oil estimation and loss on drying of the test drug was determined as per the methods recommended by Ayurvedic Pharmacopeia of India (API).[13] and British Pharmacopeia.[14]

The fluorescence analysis of the rhizome powder was done by treating with the different chemical reagents and observed under Ultra violet light and day light.

TLC

Thin layer chromatographic analysis of the methanolic extract of ***Colchicum luteum* Baker**. was carried out via Chloroform: Methanol (9:1) as mobile phase in percolated silica gel 60F254 TLC plates. Spotted TLC plates were sprayed by Anisaldehyde-sulfuric acid and were also visualized in day light at room temperature, 65°C, 95°C and UV short and long wavelength. The Rf value of spots was determined by the given formulae.[15]

R_f value=Distance travelled by the Spot / Distance travelled by the solvent

Preliminary phyto-chemical screening

The extracts were introduced to preliminary phyto-chemical analysis and investigated for the presence of various phyto-constituents like alkaloids, carbohydrates, glycosides, flavonoids, proteins, steroids, saponins, etc. with following parameters.[16,17]

HPLC profile of *Colchicum luteum* Baker.

HPLC profile of the methanolic extract of the ***Colchicum luteum* Baker**. was done. For this Shimadzu Prominence Isocratic HPLC System equipped with LC-20 AD Solvent delivery unit, Rheodyne Injector, SPD-20A prominence Uv-vis detector system along with C18 column, 250 x 4.6 mm 5U with guard column was used. The methanolic extract of coarsely powdered drug was obtained with the help of soxhlet's extraction method, extract was filtered and allowed to evaporate on water bath. This dried alcoholic extract was dissolved in HPLC grade methanol and used for study. The chromatographic analyses were carried out at room temperature using reversed phase and software driven peaks were obtained (Figure 2). The pressure and flow rate was 127 kgf and 1.0 ml/min, respectively. Detector for HPLC was UV and the wavelength was 254 nm. Mobile phase for HPLC profile of extract consisted of HPLC grade methanol (Merk life science Pvt. Ltd.) only.[18,19]

RESULTS AND DISCUSSION

Modern system of medicine relies on sound experimental data, toxicity studies and human clinical studies. But there is a lack of pharmacopoeial standards on raw material / finished products. The insufficient quality standards have led to the occurrence of mild to serious adverse effects. Hence, the standardization of herbal ingredients is the basic requirement in order to establish the identity, purity and quality.[20] Herbals are traditionally considered safe and are remarkably consumed by people without prescription. However, it is advocated that some can cause health problems, some are not effective and some may interact with other medicines. Standardization is crucial for the assessment of the quality, purity and authenticity of the drugs, based on the physicochemical parameters, TLC, HPLC, HPTLC and on the presence of active principles.[21] A standardized and good quality drug is the assurance of its therapeutic effectiveness and global acceptance. ***Colchicum luteum* Baker**. is a well-known drug of Unani System of Medicine used to treat various body ailments such as inflammatory conditions. Therefore, for this study ***Colchicum luteum* Baker**. was selected and standardized on their physicochemical parameters such as organoleptic characters, ash values, extractive values, volatile oil estimation, fluorescence analysis, qualitative estimation, TLC along with HPLC profile.

Organoleptic characters of *Colchicum luteum* Baker.

Organoleptic properties are the critical parameter for the rapid identification and consumer acceptance. Sensory evaluation-visual macroscopy, colour, odour, taste, fracture are the common features helped in identification of the crude drug. The organoleptic properties of rhizome of ***Colchicum luteum* Baker**. have been mentioned in **Table 1**.

Physicochemical analysis of *Colchicum luteum* Baker.

Ash values, alcohol and water soluble extractive values, loss of weight in powdered drug after drying at 105^{0}C and moisture contents are the indicators of the purity, quality and authenticity of any crude drug. Therefore, to standardise an herbal drug these parameters have basic importance and unavoidable. Total ash values, acid insoluble and water soluble ash values reveals the information related to the adulteration of crude drug with inorganic matter. The water and alcohol soluble extractive values indicate the amount of the extract that the drug yields in a solvent.[13,14,17] Less or more extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying, or storage of plant products.[22] Low or high moisture

contents may affect the quality of the drug and hence, its efficacy. The excessive moisture is an ideal medium for the growth of the different types of microorganisms which subsequently damages the drug.[23] This drug is also well known for its oil contents. The inappropriate method of extraction of oil or distillation and storage may spoil the quality of the drug and hence the oil.[23] Therefore, to assess the quality of ***Colchicum luteum* Baker**. it is also necessary to determine volatile oil percentage of the drug. All the values were determined in triplet and the results are depicted in **Table 2**. Successive extractive values of powdered drug in different solvents viz. petroleum ether, diethyl ether, chloroform, benzene, alcohol, methanol and distilled water were also determined with Soxhlet's apparatus. The extractive values were expressed in percentage and depicted in **Table 3**.

Fluorescence analysis of *Colchicum luteum* Baker.

Some constituents in many natural products exhibit fluorescence in the daylight ultra violet light and if the substance itself is not fluorescent, it may often be converted into fluorescent through the application of different reagents. Hence, the qualitative assessment of the test drug is carried out in this manner also which serves as an important parameter for pharmacognostic evaluation of crude drugs.[24] (**Table 4**)

Phytochemical analysis of *Colchicum luteum* Baker.

The efficacy and pharmacological therapeutic effects of any herbal medicine is depends on their secondary metabolites i.e. phytoconstituents such as alkaloids and glycosides etc. The presence or absence of these phytoconstituents also indicates the quality of the crude drug.[16,17,24] Therefore, it is also necessary to determine the presence of active secondary metabolites in the test drug, the results are shown in **Table 5**.

TLC of *Colchicum luteum* Baker.

TLC is one of the important parameter equips with the qualitative and semi-quantitative information of the drug. If the drug is adulterated or exhausted which in turn may increase or decreases the number of spots and change in the R*f* values.[15] The TLC profile along with images of TLC are illustrated in **Table 6** and **Figure 1** respectively

HPLC profile of methanolic extract of *Colchicum luteum* Baker.

The preparative and analytical HPLC has been widely employed for the analysis of herbal medicines in lieu of its high separation capacity. It can also be utilized to analyse almost all constituents of herbal products provided that an optimized procedure is developed which involves optimization of mobile phase and stationary phase along with other chromatographic parameters.[25] The adulteration and impurities can also be determined by this technique. If there is any change in number of peaks or retention time or area of peaks from standard it indicates adulteration or deterioration in the drug. The HPLC of Standard Drug (Colchicine) pattern shows 10 peaks and the peak no.6 is major peak having area concentration and retention time as 99.45% at 3.19 min. While Crude Drug **(*Colchicum luteum* Baker)** shows 13 peaks and the peak no.2 and 1 are major peaks having area concentration and retention time as 62.28% at 3.20 min. and 33.76% at 2.85 min. respectively. The HPLC profile of the Standard and test drug was obtained and recorded for future reference. The details are depicted in **Figure 2 & 3** and **Table 7 & 8**.

HPTLC profile of methanolic extract of *Colchicum luteum* Baker.

HPTLC is also an accurate method of qualitative as well as quantitative estimation of constituent in a sample. By this method we can determine the amount of marker (colchicine) in Crude sample (Colchicum Luteum Baker.) which is ensure that our sample will show how many therapeutic effect during pharmacological study.[26]

HPTLC Procedure

The HPTLC plates GF $_{254}$ (20cm × 10cm) (E.Merck) were used without any pre-treatment. All chemicals and solvents used were of analytical and HPLC grade (E.Merck).

A Camag HPTLC System equipped with Linomate IV sample applicator (Camag, Muttenz, Switzerland), TLC Scanner 3 (Wincats version 1.4.3), UV cabinet and twin trough glass tank was used for the analysis. The sample was applied using automated TLC sampler in 4mm bands at 15mm from the bottom, both side 5mm space between the two bands.

Marker Preparation

Standard solution prepared by dissolving 1mg Colchicine in 1 ml methanol.

Sample solution Preparation

Sample solution prepare by dissolving 30mg sample in 1ml methanol.

Detection of colchicine

The HPTLC plates were developed in a Camag twin-trough glass tank pre saturated with the developing solvents. The composition of the developing solvent of varying polarities was based up on clear separation of the compounds on the HPTLC plate. The plates were developed to a height of about 7.5cm from the base in Toluene: Ethyl Acetate: Diehylamine : Methanol (7:2:1:0.3 v/v). After development, the plate was removed, dried and spots were visualized under UV light. Quantitative

evaluation of the plate was performed in the reflectance/ absorbance mode at 254nm slit width 5mm× 0.4mm, scanning speed 20mm/s and data resolution 100 m/step.

CONCLUSION

Present study shows that the methods of standardization and identification of ***Colchicum luteum* Baker**. *i.e.* organoleptic characters along with physico-chemical analysis are the basic and useful parameters to analyse the originality of the test drug. A good quality of drug is the assurance of its efficacy. The results of phytochemical analysis and HPLC fingerprinting also play a key role in identification and authentication of ***Colchicum luteum* Baker**. Further these analytical parameters for quality assurance also indicating effectiveness of ***Colchicum luteum* Baker**. for treating various body ailments. The data obtained in the present work will be useful in identification, standardisation and quality assurance of different samples of ***Colchicum luteum* Baker**. and will also be useful in the preparation of the drug's monograph for inclusion in various pharmacopoeias.

SUMMARY

The present study aims towards the evaluation of the parameters involved in the determination of the quality and purity of ***Colchicum luteum* Baker**. rhizome and its standardization. The parameters used for the standardisation of the test drug includes organoleptic characters, extractive values, ash values, phyto-chemical analysis, TLC, fluorescence analysis and HPLC profile etc. The study will provide referential information for the good quality, purity and identification for the future batches of ***Colchicum luteum* Baker**.

Table 1: Organoleptic characters of Colchicum luteum Baker

Rhizome of *Colchicum luteum* Baker.	Characters
Shape	Translucent or opaque and gibbously ovoid with tapering apex and prominent longitudinal groove on one side
Size	2.5-5 cm long and 1.5-2.5 cm broad
Colour	Pale yellow to deep brown horny corms
Fracture	Horny
Surface	convex side is a prominent scar
Odour	Odourless
Taste	bitter and acrid taste

Table-2. Physico-Chemical Characters of Colchicum luteum Baker

S. No.	Physico-Chemical Parameters	Result (Mean±SEM)
1.	Water soluble matter (%)	21.173±0.1775
2.	Alcohol soluble matter (%)	1.693±0.1041
3.	Ether soluble matter (%)	0.22±0.0115
4.	Total Ash (%)	6.208±0.1121
5.	Acid Insoluble Ash (%)	1.66±0.4045
6.	Water Soluble Ash (%)	4.066±0.2028
7.	Volatile Oil (%)	1.60±0.1155
8.	Total Alkaloid (%)	4.42±0.1039
9.	pH (1% solution)	7.20±0.2309
10.	pH (10% solution)	7.20±0.2309

Table-3. Successive Extractive Values of Colchicum Luteum Baker

S. No.	Solvent	Extractive values in % (Mean±SEM)
1.	Petroleum Ether	0.22±0.0115
2.	Toluene	0.26±0.0057
3.	Chloroform	0.16±0.0057
4.	Acetone	1.0±0.0115

5.	Methanol	3.86±0.2656
6.	Acetonitrile	0.20±0.0173
7.	Distilled water	17.08±0.2596

Table 4: Fluorescence analysis of Colchicum luteum Baker

Reagents	Visible light	UV light	
		Short 254nm	Long 366nm
Powder as such	Ivory White	Light Greenish	Light Indigo
Powder+1N HCl	Ivory White	Whitish	Light Indigo
Powder+50% H_2SO_4	Pale yellow	Light green	Brown
Powder+50% HNO_3	Light Greenish	Light Greenish	Indigo
Powder+1N NaOH in water	Light Yellowish	Greenish	Light Greenish
Powder+1N NaOH in methanol	Ivory White	Light Greenish	Light Brown
Powder+Wagner's reagent	Brown	Brown	Indigo
Powder+Drangendorff reagent	Yellow	Green	Indigo
Powder+Benedict's reagent	Brown	Brown	Indigo
Powder+Fehling reagent	Yellowish	Brown	Indigo
Powder+Lead Acetate (1%)	Brown	Brown	Indigo

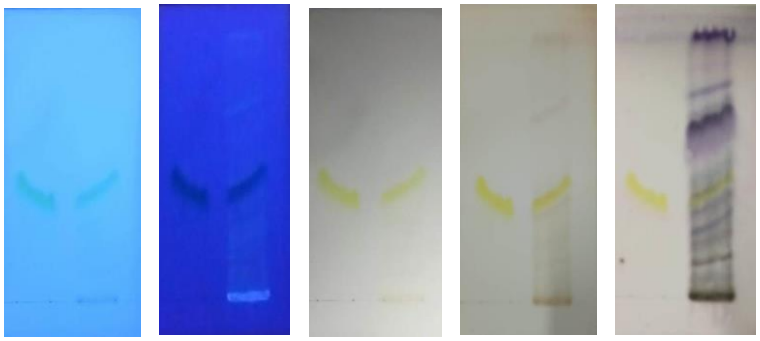
Table 5: Phytochemical analysis of Colchicum luteum Baker

Chemical Constituents	Test / Reagents	Extract	Inference
Alkaloids	Mayer's reagent	Alcoholic	Present
	Wagner's reagent	Aqueous	Present
	Hager's reagent	Aqueous	Present
Carbohydrates	Molish's test	Aqueous	Present
Proteins	Xanthoproteic test	Alcoholic	Present
Glycosides	General Test	Aqueous	Present
Fats and fixed oils	Copper sulphate / Sodium hydroxide	Petroleum	Present
	Sodium bisulphate test	Petroleum	Present
Terpenoids	Sulphur powder sink test	Aqueous & Alcoholic	Present
	Salkowski test	Alcoholic	Present
Starch		Aqueous	Present

Table-6.TLC Profile of Methanolic Extract of Colchicum luteum Baker

Spray / Light treatment	Chloroform : Methanol (9:1)	
	No. of Spots	Rf. Values and Colour of spots
UV Short wave length	1	0.32 (Y)
UV long wave length	3	0.32 (Y), 0.55(P), 0.91(P)
Anisaldehyde- sulphuric acid Sprayed (at RT)	1	0.32 (Y)
Anisaldehyde- sulphuric acid Sprayed (at 65°C)	4	0.27(P), 0.32 (Y), 0.55(P), 0.91(P)
Anisaldehyde- sulphuric acid Sprayed (at 95°C)	10	0.09(Br), 0.17(LB), 0.24(LV), 0.27(P), 0.32 (Y), ,0.38(G), 0.43(I), 0.55(P), 0.62(B), 0.70(V), 0.91(P)

Note: V=Violet, LV=Light Violet, P=Purple, Br=Brown, LB=Light Brown B=Black, G=Green, Y=yellow, I=Ivory



1 2 3 4 5

1. UV Short wave length 2. UV long wave length 3. Sprayed TLC in Day Light
4. Sprayed TLC at 65° C 5. Sprayed TLC at 95° C
Figure- 1 TLC
Mobile Phase: Chloroform: Methanol (9:1)

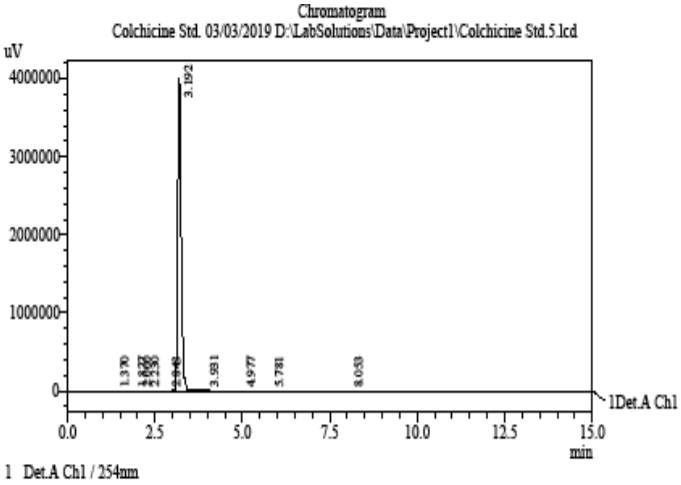


Figure-2 Standard Colchicine HPLC Chromatograph

"Table-7. HPLC Profile of Methanolic Extract of Colchicine Marker".

Peak	Retention Time	Area	Height	Area %	Height %
1	1.370	1176	43	0.004	0.001
2	1.877	4883	923	0.017	0.023
3	2.000	2025	295	0.007	0.007
4	2.230	4540	283	0.016	0.007
5	2.843	27080	2462	0.097	0.061
6	3.192	27874	39999	99.459	99.535
7	3.931	104648	14097	0.373	0.351
8	4.977	3425	379	0.012	0.009
9	5.781	2388	145	0.009	0.004
10	8.053	1319	73	0.005	0.002
	Total	28026	40187	100.00	100.00

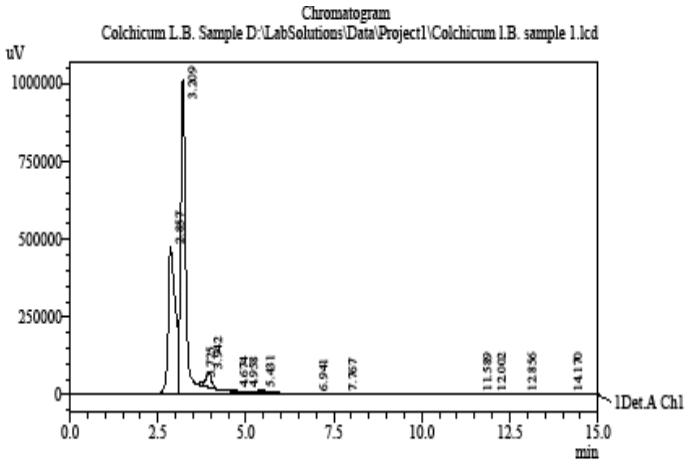


Figure- 3 Sample (Colchicum Luteum Baker) HPLC Chromatograph

"Table-8. HPLC Profile of Methanolic Extract of Colchicum luteum Baker".

Peak	Retention Time	Area	Height	Area %	Height %
1	2.857	65368	475033	33.763	30.451
2	3.209	12058	10128	62.283	64.927
3	3.725	79866	12678	0.413	0.813
4	3.942	512873	48456	2.649	3.106
5	4.674	19637	1344	0.101	0.086
6	4.958	7920	1036	0.041	0.066
7	5.431	117696	7441	0.608	0.477
8	6.941	3516	161	0.018	0.010
9	7.767	1360	76	0.007	0.005
10	11.589	1486	100	0.008	0.006
11	12.002	1930	107	0.010	0.007
12	12.856	17630	635	0.091	0.041
13	14.170	1685	78	0.009	0.005
	Total	19361	15599	100.00	100.00

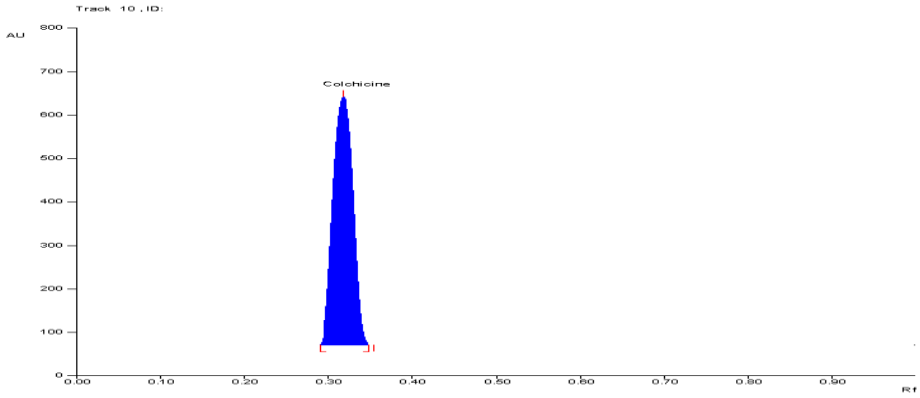


Figure 4 Standard Colchicine HPTLC Chromatograph

"Table-9. HPTLC Profile of Colchicine Standard in Methanol".

Peaks	Rf Value	Area %
1	0.32	100

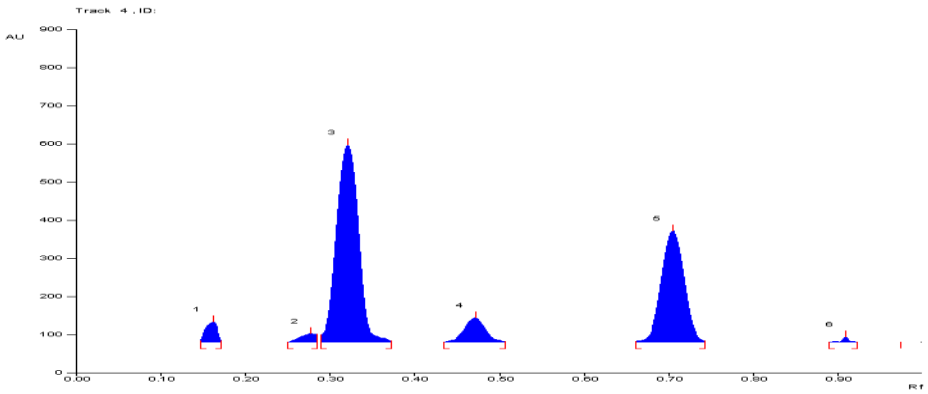


Figure-5 HPTLC Chromatograph of Hydro Alcoholic Extract of Colchicum luteum Baker

"Table10. HPTLC Profile of Hydroalcoholic Extract of Colchicum luteum Baker".

Peaks	Rf Value	Area %
1	0.16	5.48
2	0.28	2.29
3	0.32	53.80
4	0.47	6.64
5	0.70	30.38
6	0.91	1.40

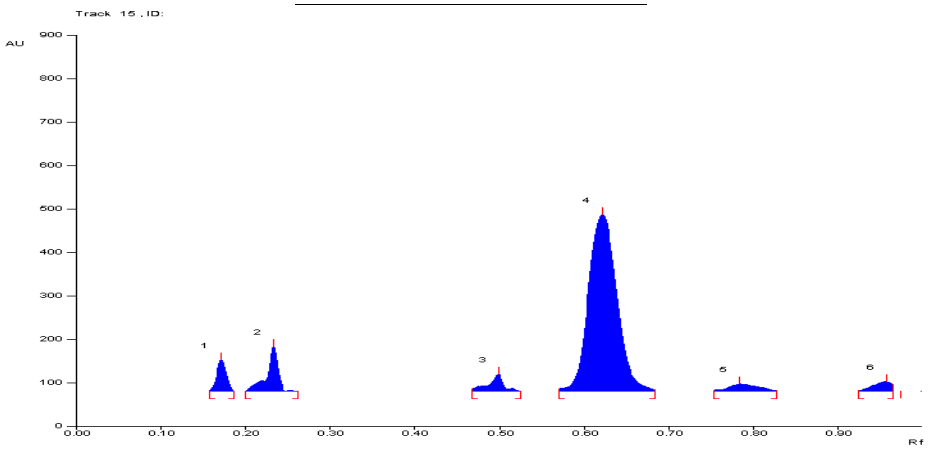


Figure 6 HPTLC Chromatogram of Hydro Alcoholic Extract of Colchicum luteum Baker (Hydrolysed Form)

"Table11. HPTLC Profile of Hydroalcoholic Extract of Colchicum luteum Baker (Hydrolysed Form)".

Peaks	Rf Value	Area %
1	0.17	10.95
2	0.23	15.70
3	0.50	5.94
4	0.62	61.49
5	0.78	2.58
6	0.96	3.34

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