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HPLC profiling conclusively distinguished two important Unani drugs, namely, Suranjan Shirin (*Colchicum autumnale*) and Suranjan Talkh (*Colchicum luteum*)

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Suranjan (colchicum) is one of the prime drugs used for arthritis in Unani System of Medicine. Two varieties of the drug are available in the market under the name of Suranjan; one is Suranjan Shirin (*Colchicum autumnale*) and the other is Suranjan Talkh (*Colchicum luteum)*. The two varieties are often confused with each other due to morphological resemblance. So there is a need to set a distinction between these two varieties of Suranjan. For this purpose the marker compound (Total Alkaloid Content) were estimated quantitatively and the High Performance Liquid Chromatography (HPLC) was conducted on both the drugs. 3 g of the powdered drug was extracted in petroleum ether and dissolved in 6 mL of 75% ethanol to yield test sample. Methanol at a flow rate of 1 mL/min was used as a standard. The peaks eluted were detected at 254 nm and compared with the authentic standard at 3.2 min of retention time. The colchicine concentration was found to be higher in Suranjan Talkh (0.21%) as compared to the Suranjan Shirin (0.15%). Therefore the present study offers a phytochemical concentration criterion, namely, colchicine content to distinguish between Suranjan Shirin (*Colchicum autumnale*) and Suranjan Talkh (*Colchicum luteum*).

Keywords: Colchicine, *Colchicum autumnale, Colchicum luteum*, HPLC, Suranjan Shirin, Suranjan Talkh

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Suranjan (Colchicum) is a drug of great repute and is considered as the first line agent for the management of arthritis by the physicians of Unani medicine. It has been mentioned by almost all renowned Unani authors in their books including Unani Pharmacopoeia. The medicinal properties of this plant were well known to the Arabs. Masihi and Hussain have described that white variety (Shirin) is better than the black (Talkh) variety because the latter one has element of toxicity (Dymock 1890; Baitar 1999). Suranjan Shirin (*Colchicum autumnale* Linn.) and Suranjan Talkh (*Colchicum luteum* Baker) are perennial and annual herbs, respectively (Bhattacharjee, 2004). They belong to family Colchicaceae (previously Liliaceae) (Toplan, 2016). There are about 70 species in this genus and among them 02 species are deployed for making anti-arthritic single or compound formulations in alternative system of medicine (Bhattacharjee, 2004). The fresh corms of Suranjan Shirin is 4 cm long, 3 cm broad, 2 cm thick, bluntly conical in shape, flattened on one side, convex on other side and enveloped in an outer brown and inner yellow membranous coat. Internally the corm is firm, white and fleshy, exudes when cut, a bitter juice that is white and milky due to the presence of numerous starch grains in it (Anonymous, 2008). The fresh corm of Suranjan Talkh are 30-45 mm long, 10-16 mm broad, 7-12 mm thick, oval, planoconvex with a slight contraction at the convex surface. Suranjan Shirin is inodorous and tasteless while Suranjan Talkh has bitter taste. Colchicine is the main therapeutically active alkaloid isolated from all the species of the genera *Colchicum* (Evans, 2009). *Colchicum luteum* contains a higher concentration of alkaloid as compared to *Colchicum autumnale* (Basu, 1996). Suranjan Talkh is distinguished from the Suranjan Shirin by its bitter taste, smaller size, darker colour and a reticulated appearance of the corms. The corms of *C. luteum* are occasionally adulterated with corms of *C. autumnale* (Chopra *et al*., 1958). These two varieties which are sold in Indian market are often mutually confused with each

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other and have significantly different therapeutic behavior. Therefore, there is a need to distinguish the 02 varieties of Suranjan. Some studies have been undertaken to determine the comparative pharmacology but their botanical and phytochemical identification has not been attempted by an accurate method. To resolve the problem present study was designed to estimate the colchicine content by High Performance Liquid Chromatography (HPLC). Quantitative estimation of marker compound (Total Alkaloid Content) was also conducted to make a distinction between the two varieties (Paech & Tracey, 1955).

Materials and methods

Collection and authentication of plant material

The test drugs, namely Suranjan Talkh (*Colchicum luteum*) and Suranjan Shirin (*Colchicum autumnale*) were procured from the local market of Aligarh. The identity was confirmed by Professor S H Afaq, Pharmacognosy Section, Department of Ilmul Advia, A K Tibbiya College, Aligarh Muslim University, Aligarh. The samples were further authenticated by National Institute of Science Communication and Information Resources, New Delhi (CSIR-NISCAIR /RHMD/ Consult/ 2015/ 2844/ 37-1 & 2). The specimen of the test drugs were submitted to Mawalid-e-Salasa (Museum) of the Department of Ilmul Advia, AMU, Aligarh for future reference with the voucher number SC-0171/15.

Total Alkaloid estimation

Alkaloid was extracted and estimated in *Colchicum luteum and Colchicum autumnale* separately by the method described by Paech & Tracey (1955). For extraction of alkaloids, 10 g powder of each drug was extracted by Soxhlet's apparatus with chloroform and few drops of ammonia. After extraction, the solvents were evaporated and then 100 mL of distilled water was added to the dried extracts. For the conversion of alkaloids into salt, extracts of both the test drugs were acidified (pH-2) with dilute hydrochloric acid. The chloroform soluble portion was separated with the help of separating funnel. The water portion was neutralized with ammonium hydroxide to release the alkaloid and this fraction was again extracted with chloroform to obtain the free alkaloids. The chloroform was evaporated and the content was weighed. The percentage of alkaloid was calculated with reference to the powder of both the drugs.

Extraction and quantitative estimation of colchicine

Colchicine was extracted from both the test drugs separately and the yield or percentage of colchicine was calculated and compared in respect of the weight of the powdered test drugs taken for extraction. 3 g powder each of *Colchicum luteum and Colchicum autumnale* was taken and extracted two times with 150 mL of petroleum ether by regular shaking for 60 min, followed each time by filtration. The solid residues were dried in shade and then extracted with 60 mL of dichloromethane at room temperature for 30 min with recurrent shaking. The 10% solution of ammonia (3 mL) was added to the mixture with vigorous shaking for 10 min; the mixture was left untouched for 30 min and then filtered. The residue was washed twice with 60 mL of dichloromethane and then combined with the filtrate. The organic phase was evaporated to dryness and the extract was weighed, the percentage of colchicines was calculated with reference to the powder the *C. luteum* and *C. autumnale*. The identity and purity of the colchicines extract was confirmed by dissolving it in 6 mL of 70% ethanol and comparing with the standard colchicines (10 mg/mL) by HPLC (Bharathi *et al*., 2006; Rahman *et al*., 2011).

HPLC of extracted drugs

Qualitative estimation of colchicine in extracted test samples was done by comparing the retention time of the test drug with that of the standard obtained from Otto, Mumbai. All HPLC measurements were made on a Shimadzu Corporation system (Analytical instruments Division, Kyoto, Japan). The system consists of an LC 20AD isocratic solvent pump, an UV/VIS SPD-20A detector and a rheodyne injector with position sensing switch and 20 µL sample loop along with porous silica with 5 µm diameter C_{18} 250 x 4.6 mm column. The mobile phase consisted of acetonitrile: 3% acetic acid (60:40), at a flow rate of 1 mL/min. The peaks eluted were detected at 245 nm and identified with authentic standards. All determinations were performed at ambient temperature. The injection volume was 20 µL and the total run time was 15 min. All chemicals used were of HPLC grade (Bharathi *et al*., 2006, Rahman *et al*., 2011).

Results and discussion

Alkaloid and colchicine estimation

Active phytochemical constituents in *C. luteum* and *C. autumnale* are alkaloids, such as colchicine,

2-desmethylcolchicine, lumicolchicine, N-desacetyl N-formylcolchicine, and luteidine (Chommadov *et al*., 1970; Koul 1977). Colchicine is considered to be the most important pharmacologically active compound obtained from both the test drugs. It shows activity against inflammatory conditions which is mainly due to the inhibition of microtubules in pro-inflammatory cells including macrophages (Rao *et al*., 1997, Nair *et al*., 2011). That is why it is used mainly in arthritis and other inflammatory conditions. Since colchicine has a narrow therapeutic index with no clear cut distinction between non-toxic, toxic and lethal dose therefore it is used with care so as to avoid the possible toxic effect. Ingestion of more than 0.5 mg/kg of colchicine causes serious side effects and can even be fatal. Lethal intoxication cases have been reported after ingestion of only 7 mg of colchicine (Shimi *et al*., 2015). To ensure the efficacy and safety of test drugs and to forefend the possibility of toxic effect because of the confounding of the 02 drugs it is necessary to analyze these drugs on physicochemical basis specially the qualitative and quantitative assessment of pharmacologically and therapeutically active constituents. The mean percentage of alkaloids and colchicine as determined in the present study in Suranjan Talkh and Suranjan Shirin (Table. 1) will help to select the genuine sample for therapeutic application and its accurate dose determination.

High performance liquid chromatography is a powerful tool of analysis. It is a technique that can be used to separate the compounds of a mixture and to identify, quantify and purify the individual components of plant materials and their medicinal preparations.

Since, colchicine is of primary importance, therefore, it was considered useful to confirm the identity of colchicine extracted from both the test drugs of colchicum variety by HPLC and the presence of colchicine in test drugs was confirmed by comparing the retention time with that of the standard. The retention time or the peaks of colchicine in both the samples of test drugs and standard colchicine were eluted at 3.2 min indicating authenticity and presence of colchicine in both of the test drugs (Fig. 1 & Fig. 2).

Results of this study show that both the species of colchicum, namely, Suranjan Talkh (*Colchicum luteum*) and Suranjan Shirin (*Colchicum autumnale*) contain colchicine as one of the main constituents. The colchicine content in Suranjan Talkh (0.21%) is higher than Suranjan Shirin (0.15%). It suggests a more toxic/effective nature of Suranjan Talkh. Physico-chemical parameters, viz., total alkaloid content, qualitative and quantitative estimation of colchicine and HPLC analysis showed clear differences between these 02 test drugs. Thus, the

Table 1 — Estimation of marker compounds

S.NO.	Marker Compounds	Suranjan Talkh (*C. luteum*)	Suranjan Shirin (*C. autumnale*)
1.	Alkaloid (%)	0.66±0.03	0.33±0.06
2.	Colchicine (%)	0.21±0.05	0.15±0.08

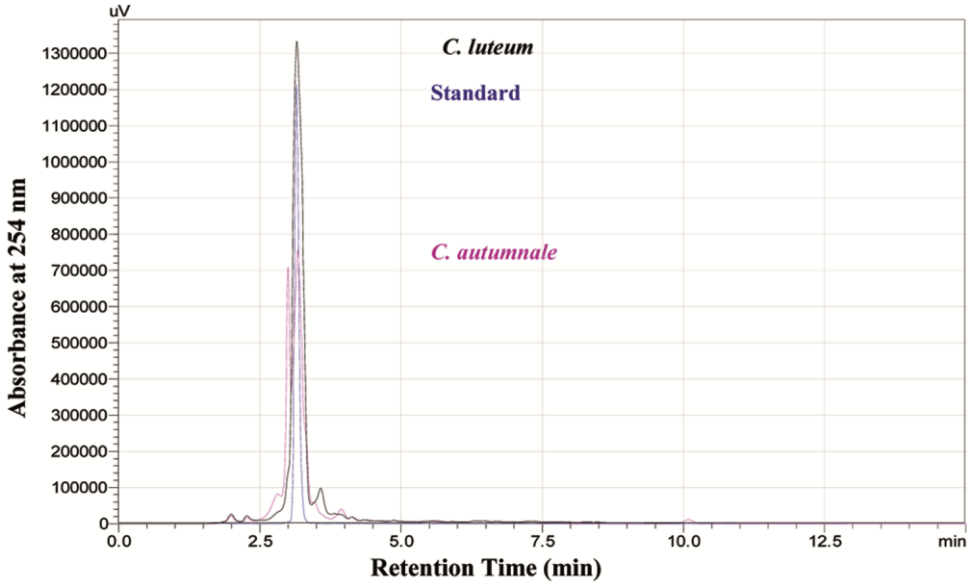

Fig. 1 — HPLC comparison of colchicines extracted from *C. luteum* and *C. autumnale* with standard colchicines

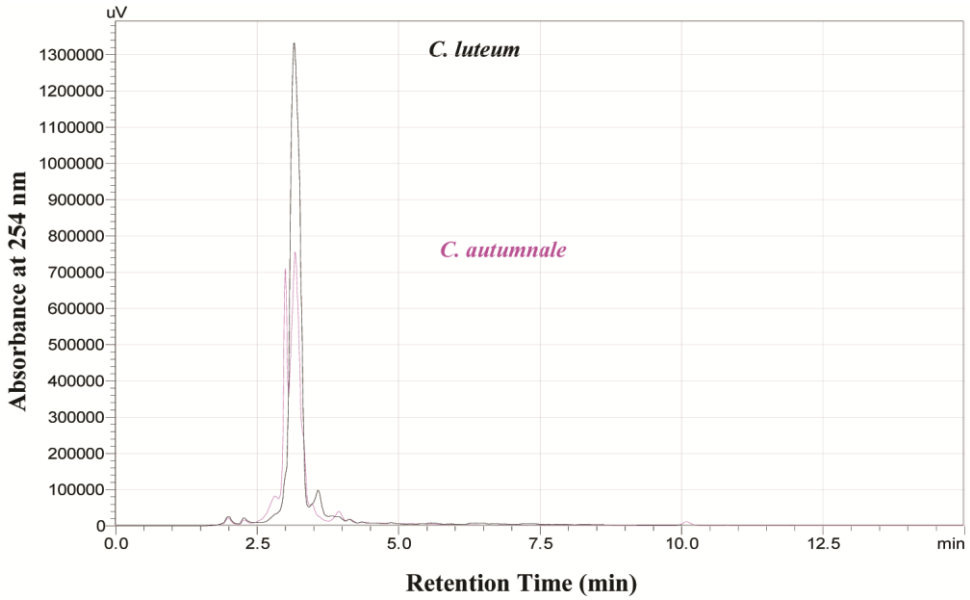

Fig. 2 — Comparative HPLC of *C. luteum* and *C. Autumnale*

study provided accurate parameters for distinguishing the two often confounded species, namely, *C. luteum* and *C. autumnale*. The study also partially validated the claim of Unani physicians that Suranjan Talkh is more effective then Suranjan Shirin which may be due to the presence of high alkaloid contents in Suranjan Talkh (0.66%) in comparison to Suranjan Shirin (0.33%). Hence, the present method could be used for routine quality control as well as to identify the drug.

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