STANDARDIZATION OF HOMOEOPATHIC MOTHER TINCTURE OF COLCHICUM

G. K. MUNSHI* and GOPA GHOSH*

ABSTRACT: A method of standardization of the homoeopathic mother tincture Colchicum has been prescribed. The study includes chemical identification by colour reaction, fluorescence analysis, T.L.C. separation of active ingredients and quantitative estimation of the alkaloid colchicine by spectrophotometric method.

INTRODUCTION

Colchicum, a well-reputed medicinal plant has been introduced in homoeopathic medicine from 1826 by Dr. Stapf[1] for treatment of gout and is official both in the Homoeopathic Pharmacopoeia of the United States[2] (U.S.H.P.) and Homoeopathic Pharmacopoeia of India (H.P.I.). Though the characters of the plant part used (corm) has been described in the Pharmacopoeias and the method of preparation of the mother tincture outlined, no *method of identification of the mother tincture or its active ingredients have* been prescribed. As a result it often becomes difficult to opine about its genuinity and quality. It was, therefore, thought necessary to lay out a method of identification of the tincture as well as to make a survey of the market samples available.

METHOD & MATERIAL

The source of preparation of the drug as mentioned in the Pharmacopoeias are from the fresh corms of *Colchicum autumnale* (Liliaceae), a species indigenous to various parts of Europe and England and not cultivated in India.[3] Therefore, it leads to the possibility of adulteration of the plant source to meet the market demand. *Colchicum luteum,* an allied species is now cultivated in India, in the Western Himalayas to be used as a substitute of *C. autumnale*[4]. It has the same pharmacological action and contains alkaloid colchicine but in lower concentration than *C. autumnale*[5]. Although H.P.I. mentions it as a synonym of *C. autumnale* according to Bentham and Hooker[6] it is a separate species.

The present study has been carried out with the fresh corms of *C. luteum,* purchased from the market whose botanical identity was confirmed[3] and mother tincture was prepared as per H.P.I. specification (C.D.L. reference tincture). Identification and assay was made on the basis of the active alkaloid colchicine. Mother tinctures from seven different manufacturers were purchased along with Boericke and Taffel's sample of *C. autumnale* and used for comparative study. Colour reaction and fluorescence analysis was tested according to Trease and Evans[4] and Wallis[3]. Alkaloids were

* Central Drugs Laboratory, Cal.-700 016.

separated by T.L.C. in silica gel G coated glass plates using methanol: ammonia (98:2) I and chloroform: acetone: ammonia (25:20:4) II as mobile phases. Alkaloid positive spots were detected by spraying with Dragendroff's reagent. Colchicine was identified by co-T.L.C. of the samples with authentic standard. Assay was performed by a spectrophotometric method modified from King[9]. The method is based on the acid hydrolysis of colchicine to Colchiceine and formation of a complex with ferric chloride solution and carried out as follows: 1 ml each of the nine samples and standard colchicine solution of known concentration (0.0024%) was taken in a stoppered conical flask with 4ml of 1N HCl and heated on a water bath for one hour for complete hydrolysis of the alkaloid. The solution was then cooled to room temperature, 0.1ml of 5% Ferric chloride solution added and the absorption was measured after ten minutes in a Perkin-Elmer Spectrophotometer fitted with a scanning apparatus for recording the absorption spectrum in the visible range from 400 to 550 nm wavelength. Readings were taken against a blank which was prepared as above but contained 1ml fluorescence free alcohol in place of sample.

DISCUSSION

The comparative result has been given in Table I. The colour of the mother tincture varied in different samples from colourless, pale yellow to deep brown, though according to the Pharmacopoeia the same quantity of the powdered corm should have been taken for preparation of mother tincture. The nature of fluorescence differed in samples 1, 3 and 6 from 2, 4, 5, 7, 8 and 9. The colour reaction with concentrated sulphuric acid gave a characteristic yellow colour for colchicine in samples 2, 4, 5, 7, 8 and 9 though intensities were different. Samples 1, 3 and 6 showed no change in colour. Ferric chloride solution gave a red colouration for colchicine in samples 2, 4, 5, 7 and 8 while 3 and 6 became yellow and sample 1 showed no change.

T.L.C. separation in solvent I showed a major Dragendroff positive spot at Rf 0.90 and a minor spot at Rf 0.1 in samples 2, 4, 5, 7, 8 and 9 samples; 3 and 6 showed a single spot at Rf 0.1 while sample 1 showed no separation. Rf 0.90 was identified as colchicine. In solvent II, three spots at Rf 0.52, 0.63 and 0.95 were visible in sample 2 and two spots at Rf 0.53 and 0.95 in samples 4, 5, 7, 8 and 9. Samples 1 and 3 showed no Dragendroff positive spots while sample 6 showed a single spot at Rf 0.95. Spot at 0.52-0.53 was identified as colchicine.

Absorption spectrum of the standard colchicine solution and samples 2, 4, 5, 7, 8 and 9 after colour reaction showed a maxima at 474 nm. Sample 3 showed a maxima at 464 nm and 6 at 484 nm. Sample 1 showed no absorption peak in the said wavelength. Quantitative estimation showed the presence of 0.0106% of colchicine in COL reference standard (8), 0.0302% in sample 2, 0.007% in sample 4, 0.0104% in sample 5, 0.003% in sample 7

TABLE—I

Preparations	Response in different tests						Assay of colchicine by $FeCl_3$ method (%)	Absorp. maxima of colour complex followed in $FeCl_3$ method
	Fluorescence		Colour reaction		TLC No. of spots and Rf values			
	Day light	UV light	Conc. H_2SO_4	$FeCl_3$	Solvent I	Solvent II		
Std.	White	Yellowish green	Yellow (deep)	Red	one Rf. 0.90	one Rf. 0.53	0.0024	474 nm
T-1	Pale yellow	Greenish yellow	No response	No response	Nil	Nil	—	No absorption
T-2	Brown	Yellowish green	Yellow	Red	one Rf. 0.90	three Rf. 0.52, 0.63, 0.95	0.0302	474 nm
T-3	Deep brown	Greenish yellow	No change	Yellow	one Rf. 0.1	Nil	—	464 nm
T-4	Pale yellow	Yellow green	Yellow (faint)	Red	two 0.90, 0.10	two 0.53, 0.95	0.007	474 nm
T-5	Yellow	Yellowish green	Yellow	Light red	two 0.90, 0.1	two 0.53, 0.95	0.0104	474 nm
T-6	Light brown	Green yellow	No change	Yellow	one 0.1	one 0.95	—	484 nm
T-7	Pale yellow	Yellow green	Yellow (faint)	Light red	two 0.90, 0.10	two 0.53, 0.95	0.003	474 nm
T-8	Yellow	Yellow green	Yellow	Red	two 0.90, 0.10	two 0.53, 0.95	0.0106	474 nm
T-9	Deep brown	Yellow green	Yellow	Red	two 0.90, 0.10	two 0.53, 0.95	0.321	474 nm

Std. = Standard Colchicine solution; T-1 to T-7 = Market samples; T-8 = Mother tincture prepared from authentic *C. luteum;* T-9 = B. & T. sample purchased from market.

and 0.0312% in sample 9, absorption being recorded at 474 nm wavelength.

From the present study it may be concluded that mother tincture from *C. luteum* and B. & T. sample prepared from *C. autumnale* are similar in nature though content of colchicine is high in B. & T. sample. Sample Nos. 4, 5 and 7 have been prepared from the same plant source though the concentration is different in the mother tincture. Sample 2 showed a difference in physical colour, T.L.C. separation and high concentration of colchicine content because it may have been prepared from *C. autumnale* or an increased quantity has been used for preparation. Samples 3 and 6 showed the presence of the minor alkaloidal spot and difference in absorption maxima due to its preparation from old or exhausted corms or some other species of *Colchicum*. Sample 1 however showed no presence of active ingredient and so may be concluded as spurious.

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