The Bioscan 10(2): 549-551, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

ESTIMATION OF CHEBULAGIC AND CHEBULINIC ACID IN *TERMINALIA CHEBULA*

T. N. RANJINI, M. A. SURYANARAYANA, K. BHANUPRAKASH* AND UMESHA
Division of Seed Science and Technology,
Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru - 560 089, INDIA
e-mail: kbp@iihr.ernet.in

KEYWORDS
RP-HPLC
PDA
Chebulagic acid
Chebulinic acid

Received on :
21.01.2015

Accepted on :
29.03.2015

***Corresponding author**

ABSTRACT
Terminalia chebula is an important medicinal tree, used in the treatment of many human ailments and it is one of the important constituent in *Triphala* ayurvedic drug along with *Emblica officinalis* and *Terminalia bellerica*. In the present investigation two important phytochemicals viz., chebulagic and chebulinic acid from dried fruits of twelve *Terminalia chebula* accessions located at Indian Institute of Horticultural Research were estimated using RP-HPLC method. Estimation of phytoconstituents was achieved by using C18 coloumn and acetonitrile - 0.05% of orthophosphoric acid in water with 0.0136% of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) as mobile phase at a flow rate of 1.5mL/min. Amongst twelve accessions, highest percentage of chebulagic and chebulinic acids were recorded in IIHRTc6 (21.10%) and IIHRTc7 (9.59%) accessions, respectively. These phytoconstituents content variation among *Terminalia chebula* accessions can be used as a maker in identification of best accessions for crop improvement programme.

INTRODUCTION

The fruit of *Terminalia chebula* Retz. (Combrataceae) commonly known in India as Harad (Sanskrit: Haritaki) is found throughout India and Southeast Asia in deciduous forest and areas of light rainfall. In Tibet, *Terminalia chebula* is called as the "King of Medicine" and is always listed first in *Ayurvedic Meteria medica* because of its extraordinary power of healing. In ayurveda, it is considered to cure all diseases and eliminate all waste from the body (Gupta, 2012). It is used for the treatment of number of diseases like cancer, paralysis, cardiovascular diseases, ulcers, gout, epilepsy etc. It is one of the important constituent in *Triphala* ayurvedic drug along with *Emblica officinalis* and *Terminalia bellerica* (Naik *et al.,* 2003).

T. chebula contains several phytoconstituents like tannins, flavonoids, sterols, amino acids, fructose, resin, fixed oils etc., however, it is fairly rich in different tannins (approximately 32%). There are about 14 hydrolysable tannins (gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulegic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl-b-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose and terchebulin) which have been isolated from fruits of *T. chebula* (Bag *et al.,* 2013).

Annual consumption of *Terminalia chebula* from herbal industry is 8158 MT during 2005-2006 (Ved and Goraya, 2008) and annual collection of *Terminalia chebula* from forest in Karnataka during 2011-2012 is 268.91 T. (Annon., 2013). The demand for this crop is growing day by day from pharmaceutical industry and the peoples associated with the herbal drug preparations. In order to meet the ever increasing demand it is important to develop genetically superior planting material through crop improvement programme. In medicinal plants breeding programme, quantification of active chemical constituents is helpful for correct identification of genotypes or accessions with higher secondary metabolite contents to develop good varieties or hybrids. Gupta *et al.* (2005) estimated anticancerous drugs vincristine, vinblastine and vindoline in ten accessions of *Catharanthus roseus* using RP-HPLC method to identify best accessions with higher secondary metabolite contents, Recently Yoirentomba and Shantibala (2011) studied seasonal variation of bioactive alkaloid content in *Aconitum Spp.* by HPLC method and Vijayalakshmidevi *et al.* (2011) screened *Catharanthus roseus* alkaloids using HPLC technique. Hence the present study was undertaken to characterize the phytochemical diversity (chebulagic and chebulinic acid) in twelve *Terminalia chebula* accessions in order to identify best accessions with respect to phytochemical content by RP-HPLC method.

MATERIALS AND METHODS

Present investigation comprises of twelve *Terminalia chebula* accessions, viz., IIHRTc1, IIHRTc2, IIHRTc3, IIHRTc4, IIHRTc5, IIHRTc6, IIHRTc7, IIHRTc8, IIHRTc9, IIHRTc10, IIHRTc11 and IIHRTc12 located at herbal garden, section of medicinal crops, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru. HPLC analysis was carried out in the Natural Remedies Pvt.Ltd.Co., Bengaluru by following their standardized methodology to estimate chebulagic and chebulinic acid.

Sample preparation

Chebula fruits were collected from different accessions and sun dried for a week to obtain constant weight. They were dehusked and endocarps were separated. Dehusked fruits were powdered in grinding mill and sieved in 60 mesh sieve. 0.250g was transferred into 100 mL volumetric flask, dissolved in 50mL of hot water and sonicated for 10 minutes and made up to 100 mL with water, after cooling. The crude extract was filtered through 0.45 microns (PES filter papers only) membrane filter paper prior to inject into the HPLC system.

Chromatographic condition

HPLC analysis was performed using Shimadzu High Performance Liquid Chromatographic system and separation was achieved by a reverse-phase column (Phenomenex- Luna 5μC-18(2) Size: 250x4.60 mm) using mobile phase with buffer A (0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 900 mL of HPLC grade water with 0.5ml of orthophosphoric acid. The final volume was made upto 1000 mL with water, filtered through 0.45μ membrane, degassed and sonicated for 3 minutes) and buffer B (Acetonitrile) in different gradient programme (Table 1). Flow rate was adjusted to 1.5mL/min and sample volume of 20μL was injected. Readings were taken at 270 nm using SPD-M 10Avp Photo diode array detector in combination with Class LC 10A software.

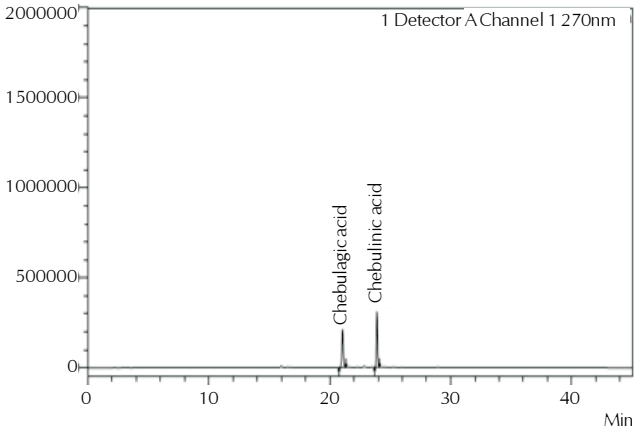

Figure 1: HPLC chromatogram for standards of chebulagic acid (RT value 21) and chebulinic acid (RT value 23.8) in *Terminalia chebula* fruits

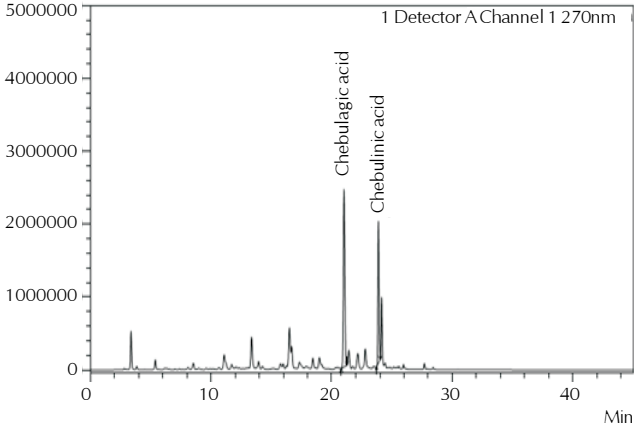

Figure 2: HPLC chromatogram of chebulagic acid in IIHRTc6 accession

Standard curve for chebulagic acid and chebulinic acid

Stock solution was prepared by weighing accurately 5 mg each of chebulagic acid and chebulinic acid into 100ml volumetric flask, dissolved in 50mL of hot water by sonication, cooled and made up to 100mL with water. From the stock solution, different dilutions/ concentrations were made. Different concentrations of stock solutions were injected into injection port with 20 microlitre loop being attached to the HPLC unit. HPLC analysis was done by maintaining above mentioned chromatographic conditions. A sequence of injections were made from higher concentration to lower concentration of standards. A particular peak with retention time of 21 and 23.8 minutes were identified as the standard concentration peaks for chebulagic acid and chebulinic acid respectively (Fig.1). By the repeated injections of the standards standardized the peak at a particular retention time. Then, the standard curve was prepared by entering component name, concentration and units.

Extract preparation protocol for purity check

Weighed about 0.100g of *Terminalia chebula* extract into a 100 ml volumetric flask, dissolved in 50ml of hot water and sonicated for 10 minutes, cooled and made up to 100 mL with water. Filtered through 0.45microns (PES filter papers only) membrane filter paper.

Procedure for estimation of chebulagic and chebulinic acid

Injected three times the standard preparation and calculated the mean area and the relative standard deviation (RSD). Injected 20μL of sample preparation and record the chromatogram at 270 nm.

Calculated chebulagic acid and chebulinic acid using following formula.

$$\frac{\text{Area of the sample} \times \text{Weight of standard in mg} \times \text{Sample dilution} \times \text{Purity of standard}}{\text{Area of the standard} \times \text{Standard dilution} \times \text{Sample weight in mg}}$$

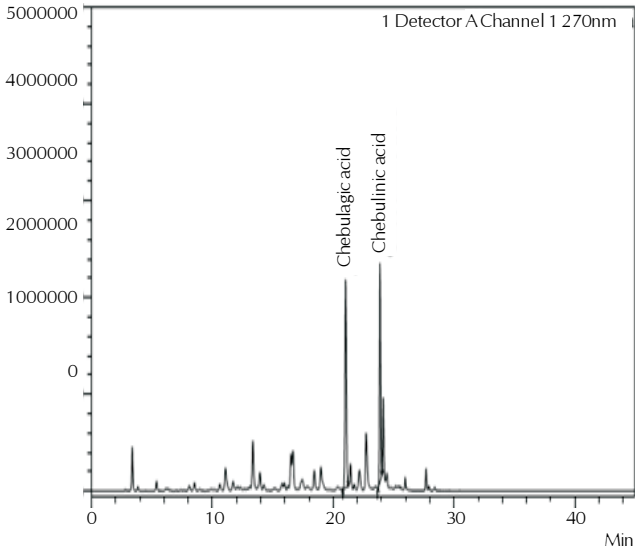

Figure 3: HPLC chromatogram of chebulinic acid in IIHRTc7 accession

RESULTS AND DISCUSSION

Data pertaining to chebulagic acid and chebulinic acid contents obtained for twelve accessions of *Terminalia chebula* is presented in Table 2. Highest chebulagic acid and chebulinic acid were recorded in the IIHRTc6 (21.10%) with an area of 61.84% (Fig. 2) and IIHRTc7 (9.59%) with an area of 44.78% (Fig. 3), respectively. Least chebulagic acid content of 12.11% (43.387% area) was recorded in the IIHRTc1 and least amount of chebulinic acid (2.8%) was recorded in IIHRTc12 with 21.4% of area. Accessions named IIHRTc6 and IIHRTc7 with highest chebulagic acid and chebulinic acids can be successively utilized in the crop improvement programme. These results were in support with variation noticed in forskolin content (ranges from 0.025% to 0.798%) among 13 IIHR accessions of *Coleus forskolin* using HPLC method (Hegde *et al.*, 2005). Kumar *et al.* (2010) reported maximum reserpine content in *Rauwolfia serpentine* plants collected from Coimbatore (Tamil Nadu) than the plants collected from other states such as Tirupathi (Andra Pradesh), Trissur (Kerala), Shimoga (Karnataka). These results clearly showed that phytoconstituent contents in medicinal plants vary with respect to genetics, geographical and environment conditions. Hence, quantification of phytoconstituents is essential in medicinal plant breeding study.

Table 1: Different gradient program followed during HPLC analysis

Time (min)	Buffer A concentration(%)	Buffer B concentration(%)
0.01	95	5
18	80	20
25	65	35
28	65	35
35	80	20
40	95	5
45	95	5

Table 2: Chebulagic acid and Chebulinic acid content (%) in *Terminalia chebula* accessions

Accessions	Chebulagic acid (%)	Chebulinic acid (%)
IIHRTc1	12.11	9.49
IIHRTc2	18.88	7.34
IIHRTc3	19.71	8.63
IIHRTc4	18.12	9.17
IIHRTc5	16.84	9.20
IIHRTc6	21.10	8.10
IIHRTc7	18.96	9.59
IIHRTc8	17.12	6.46
IIHRTc9	15.08	5.89
IIHRTc10	20.09	4.17
IIHRTc11	16.42	7.36
IIHRTc12	15.96	2.80
Mean	17.53	7.35
F- test	*	*
S.E.m ±	1.68	0.63
C.D.	3.96	2.42

REFERENCES

Annonymous. 2013. Proceedings of seminar on "conservation, collection and sustainable management of medicinal plants", KaMPA, Bangalore.

Bag, A., Bhattacharyya, S. K. and Chattopadhyay, R. R. 2013. Therapeutic potential of *Terminalia chebula* Retz. (*Combretaceae*): The Ayurvedic wonder. **3(3):** 244-252.

Gupta, M. M., Singh, D. V., Tripathi, A. K., Pandey, R., Verma, R. K., Singh, S., Shasany, A. K. and Khanuja, S. P. S. 2005. Simultaneous determination of Vincristine, Vinblastine, Catharanthine, and Vindoline in leaves of *Catharanthus roseus* by High-Performance Liquid Chromatography. *J. Chromatographic Science.* **43:** 450-453.

Gupta, P. C. 2012. Biological and pharmacological properties of *Terminalia chebula* Retz. (haritaki). *Int. J. Pharmacy and Pharmaceutical Sci.* **4(3):** 62-67.

Hegde, L., Kumar, T. V. and Himabindu, K. 2005. Evaluation of *Coleus forskohlii* accessions for tuber and forskolin yield. *Acta. Hort.* **1:** 217-219.

Kumar, V. H., Nirmala, Shashidhara, S. and Rajendra, C. E. 2010. Reserpine content of *Rauwolfia serpentina* in response to geographical variation. *Inter. J. Pharma. and Bio. Sci.* **1(4):** 429-434.

Naik, G. H., Priyadarsini, K. I., Naik, D. B., Gangabhagirathi, R. and Mohan, H. 2003. Studies on the aqueous extract of *Terminalia chebula* as a potent antioxidant and a probable radio protector. *Phytomedicine.* **11:** 530-538.

Ved, D. K. and Goraya, G. S. 2008. Demand and supply of medicinal plants in India, NMPB, New Delhi, pp. 27-30.

Vijayalakshmidevi, S. R., Anju, S. and Melchias, G. 2011. In vitro screening of *C. roseus* alkaloids for antibacterial activity. *The Bioscan.* **6(2):** 245-248.

Yoirentomba Meetei Sinam and Shantibala Devi, G. A. 2011. Seasonal variation of bioactive alkaloid content in *Aconitum Spp.* from Manipur, India. *The Bioscan.* **6(3):** 439-442.