November 2006 Volume 2 Issue 3-4

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 2(3-4), 2006 [100-104]

Effect Of Geographical Variation On Contents Of Tannic Acid, Gallic Acid, Chebulinic Acid And Ethyl Gallate In *Terminalia Chebula* Fruits

Corresponding Author

K.Jayaram Kumar
Department of Pharmaceutical Sciences,
Birla Institute of Technology,
Mesra, Ranchi 835215 (INDIA)
E-mail: kumarjay_2000@yahoo.com

Received: 19th August, 2006
Accepted: 24th August, 2006

Web Publication Date : 14th November, 2006

ABSTRACT

Terminalia chebula has been mentioned in traditional system of medicine for many diseases. This paper presents a scientific method for establishing its uses based on composition of various chemical constituents. H.P.L.C method has been developed for simultaneous estimation of tannic acid, gallic acid, chebulinic acid and ethyl gallate in different samples of Terminalia chebula. The results have shown a considerable change in these phyto nutrients in different samples of fruits of Terminalia chebula, which gives a new direction into scientific basis of its traditional uses.

INTRODUCTION

Accuracy in recording or observing the medicinal use of a plant, determining whether the ethno medical use can be demonstrated under scientific conditions in laboratory, and/or chemical characterization of compounds and role of placebo effect are important issues that need to be verified in the development of drugs of plant origin.

Terminalia chebula is a moderate size to large deciduous tree attaining a height of 15-24 m with leaves ovate, elliptic or obovate, glabrous to villous beneath. Flowers are yellowish white, in axillary and terminal often-paniculate spikes. Fruits are glabrous, shiny, ellipsoidal, obovoid or ovoid drupes, yellow to orange brown in color hard when ripe, stone very thick, bony and rough. Earlier studies on Terminalia chebula have established the presence of 2α-hydroxy micromeric acid, chebuloside-I and chebuloside-II (Singh et al., 1990 and Kundu et al., 1992) galloyl

esters of glucose, corilagin chebulinic, chebulagic acid and many other important constituents.

MATERIAL AND METHODS

Samples of fruits of Terminalia chebula Retz were obtained from different regions of India and Nepal. Each sample was given specific code for identification purposes. The fruits were properly dried before analysis and stored in airtight container as used in the normal process of handling.

H.P.L.C assay of tannic acid, Gallic acid, Cheb ulinic acid and Ethyl gallate (Kumar. J and Bhat nagar, 2005)

An inertsil ODS-3V had been used. The mobile phase consisted of Acetonitrile: Methanol: Phosphate buffer (40 mM, pH 3.0) (10:5:85). The flow rate of the eluent was 1.2 ml/min. The oven temperature had been kept at 40ºC. Purging had been done for 2.0 min. with solvent system after each run. The wavelength used for detection is 264nm.Tannic acid, gallic acid, chebulinic acid and ethyl gallate were eluted at 25.976, 3.410, 27.755 and 3.787 minutes respectively.

Optimization of extraction procedure

Four samples each of 100.0g accurately weighed T. chebula fruit powders were refluxed with methanol (50 ml) for 30, 60, 90, 120 minutes respectively. The resulting solutions were filtered concentrated under vacuum redissolved in methanol (15 ml) and their final volumes were made upto 25.0 ml. Aliquots (20 µL) of each sample were subjected to H.P.L.C. The marc obtained from 60 min. cycle was further subjected to three successive extractions of 90 min. each with methanol (50 ml). The final dilution of 1st cycle was made upto 10.0 ml while those of 2nd and 3rd cycles were made upto 5.0 ml. Aliquots (20 µL) of each dilution were subjected to H.P.L.C and contents of tannic acid, gallic acid, chebulinic acid and ethyl gallate were calculated in each case using the respective regression equations of the calibration plot and are reported in TABLE 1.

Estimation of Tannic acid, gallic acid, ethyl gallate and chebulinic acid in Terminalia chebula samples

Accurately weighed powdered samples of 2.0 g of Terminalia chebula powder had been subjected to the optimized extraction procedure. Final dilutions of all the samples had been made upto 25.0 ml in methanol. Aliquots (20 µL) of the resulting solutions had been subjected to H.P.L.C. H.P.L.C chromatogram has been represented in figure-1.

The contents of tannic acid, gallic acid, ethyl gallate and chebulinic acid have been calculated using respective regression equations and have been reported in TABLE-2, TABLE-3, TABLE-4 and TABLE- 5 respectively.

RESULTS AND DISCUSSION

There is significant variation in the contents of tannic acid, gallic acid ethyl gallate and chebulinic acid. H.P.L.C method is very effective to trace out

TABLE 1: Contents of tannic acid, gallic acid, chebulinic acid and ethyl gallate in different cycles of extraction

Reflux period min	% (w/w) of constituents Mean ± S.D			
	Tannic acid	Gallic acid	Chebulinic acid	Ethyl gallate
30	0.874±0.0026	0.1132±0.00125	0.0182±0.00326	0.0142±0.00423
60	0.892±0.00123	0.1148±0.00145	0.0191±0.000286	0.0144±0.00125
90 x 1	0.928±0.00235	0.1056±0.00164	0.0194±0.000243	0.0147±0.00184
90 x 2	0.056±0.00117	0.0046±0.00086	0.0095±0.000125	0.0011±0.00094
90 x 3	0.024±0.00065	0.0024±0.00043	ND^f	ND^f
90 x 4	ND^f	ND^f	ND^f	ND^f
120	0.934±0.00624	0.1149±0.00156	0.0195±0.0045	0.0148±0.00156

ND^f = not detected

Full Paper

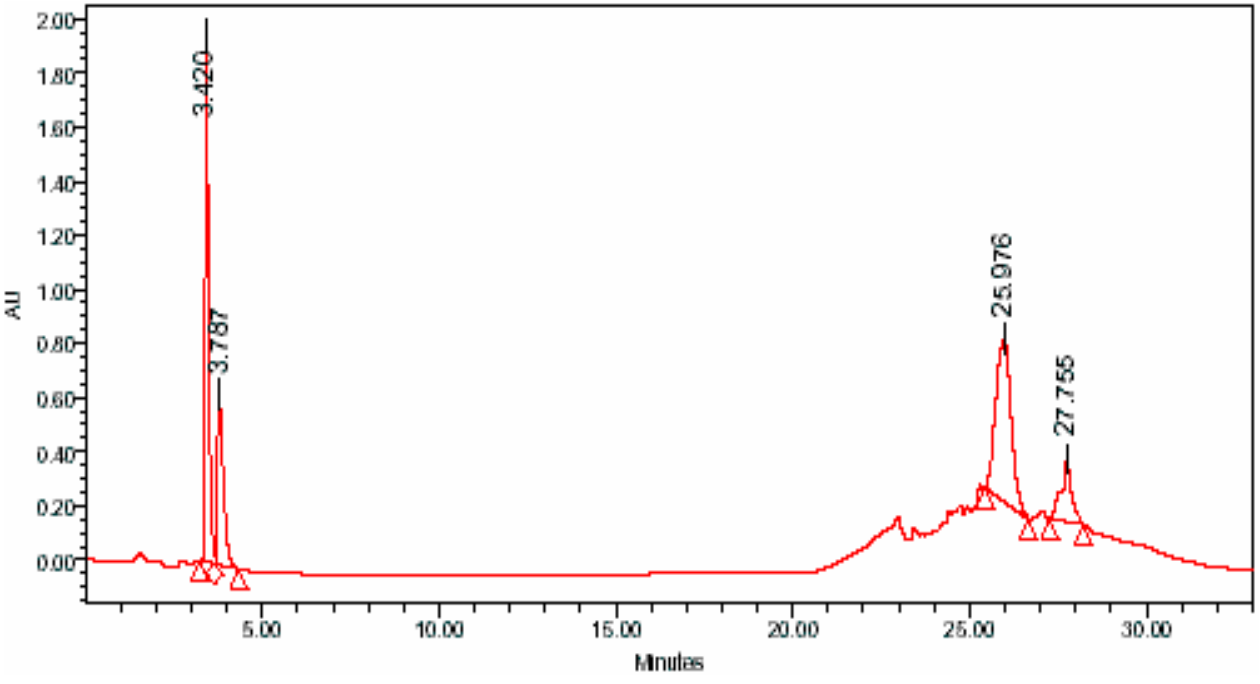

Figure 1: H.P.L.C chromatogram of Tannic acid, gallic acid, ethyl gallate and chebulinic acid in Terminalia chebula fruit sample

TABLE 2: Influence of Geographical Distribution on Tannic acid (%w/w) of dried fruits of Terminalia chebula.

A1	A2	A3	A4	A5	A6	C1	C2	C3	C4
27.28± 0.0021	26.13± 0.0062	32.80± 0.0051	28.26± 0.0041	29.36± 0.0051	21.26± 0.0061	39.13± 0.0041	42.12± 0.0053	41.26± 0.0046	40.36± 0.004
G1	**G2**	**G3**	**G4**	**HP1**	**HP2**	**HP3**	**HP4**	**UL1**	**UL2**
36.70± 0.0051	37.86± 0.0041	40.12± 0.0026	39.26± 0.0037	26.23± 0.0046	27.34± 0.0039	25.12± 0.0046	26.28± 0.0054	27.28± 0.0046	26.34± 0.003
UL3	**UL4**	**UL5**	**UL6**	**MP1**	**MP2**	**MP3**	**MP4**	**MP5**	**KA1**
26.75± 0.0051	27.32± 0.0061	27.43± 0.0071	25.16± 0.0061	40.86± 0.0071	42.12± 0.0061	41.39± 0.0071	41.25± 0.0061	41.16± 0.0021	27.68± 0.003
KA2	**KA3**	**KA4**	**KA5**	**OR1**	**OR2**	**OR3**	**OR4**	**OR5**	**WB1**
32.80± 0.0031	31.92± 0.0045	32.63± 0.0056	27.63± 0.0061	27.78± 0.0071	28.68± 0.0081	32.62± 0.0073	31.82± 0.0063	30.26± 0.0065	27.58± 0.007
WB2	**WB3**	**WB4**	**WB5**	**WB6**	**JH1**	**JH2**	**JH3**	**JH4**	**JH5**
27.46± 0.0078	26.18± 0.0076	24.23± 0.0074	27.28± 0.0065	28.63± 0.0054	31.26± 0.0056	33.10± 0.0059	38.16± 0.0061	32.18± 0.0072	31.23± 0.006
JH6	**KL1**	**KL2**	**KL3**	**KL4**	**TN1**	**TN2**	**TN3**	**TN4**	**NP1**
31.68± 0.0065	32.12± 0.0061	27.63± 0.0071	28.42± 0.0068	29.63± 0.0061	27.63± 0.0062	32.81± 0.0071	31.83± 0.0081	27.86± 0.0069	25.26± 0.006
NP2	**UP1**	**UP2**	**UP3**	**UP4**	**UP5**				
27.28± 0.0052	31.23± 0.0061	32.64± 0.0071	32.84± 0.0078	33.12± 0.0074	33.09± 0.0075				

the changes compared to other methods. This variation is one of the main factors for the diversified uses of Terminalia chebula as mentioned in traditional system of medicine. These findings give scientific basis for establishing the traditional uses of Terminalia chebula and a new direction further re-

Full Paper

TABLE 3: Estimation of % w/w of Gallic acid (by H.P.L.C) in different coded samples of fruits of Terminalia chebula

A1	A2	A3	A4	A5	A6	C1	C2	C3	C4
1.28± 0.0043	1.27± 0.051	1.292± 0.0543	1.32± 0.0412	1.31± 0.0124	1.276± 0.0412	1.396± 0.0056	1.343± 0.0061	1.386± 0.0043	1.446± 0.0039
G1	**G2**	**G3**	**G4**	**HP1**	**HP2**	**HP3**	**HP4**	**UL1**	**UL2**
1.293± 0.0041	1.282± 0.0015	1.276± 0.005	1.34± 0.0056	0.82± 0.0496	0.883± 0.0031	0.946± 0.0563	0.983± 0.0471	0.973± 0.0052	0.943± 0.0054
UL3	**UL4**	**UL5**	**UL6**	**MP1**	**MP2**	**MP3**	**MP4**	**MP5**	**KA1**
0.873± 0.0031	0.863± 0.0024	0.853± 0.024	0.825± 0.0016	1.352± 0.0014	1.386± 0.0064	1.348± 0.0064	1.376± 0.0035	1.393± 0.0034	1.442± 0.0065
KA2	**KA3**	**KA4**	**KA5**	**OR1**	**OR2**	**OR3**	**OR4**	**OR5**	**WB1**
1.13± 0.0041	1.18± 0.0049	1.24± 0.0041	1.25± 0.0036	1.196± 0.0021	1.136± 0.0039	1.25± 0.0049	1.248± 0.0056	1.246± 0.0049	1.239± 0.0056
WB2	**WB3**	**WB4**	**WB5**	**WB6**	**JH1**	**JH2**	**JH3**	**JH4**	**JH5**
1.235± 0.0029	1.256± 0.0014	1.248± 0.0013	1.245± 0.0014	1.249± 0.0024	1.23± 0.0027	1.246± 0.0028	1.25± 0.0026	1.248± 0.0039	1.284± 0.0037
JH6	**KL1**	**KL2**	**KL3**	**KL4**	**TN1**	**TN2**	**TN3**	**TN4**	**NP1**
1.225± 0.0042	1.132± 0.0043	1.212± 0.0042	1.285± 0.0052	1.256± 0.0041	1.18± 0.0054	1.135± 0.0041	1.284± 0.0044	1.264± 0.0043	0.82± 0.0045
NP2	**UP1**	**UP2**	**UP3**	**UP4**	**UP5**				
0.97± 0.0061	1.25± 0.0065	1.248± 0.00418	1.264± 0.0043	1.278± 0.0068	1.284± 0.0062				

TABLE 4: Estimation of % w/w of Ethyl gallate (by H.P.L.C) in different coded samples of fruits of Terminalia chebula

A1	A2	A3	A4	A5	A6	C1	C2	C3	C4
0.7532±0.024	0.684± 0.0016	0.778± 0.0014	0.8614±0.013	0.834± 0.0012	0.284± 0.0014	0.91± 0.0009	0.98± 0.0012	1.06± 0.0013	1.07± 0.001
G1	**G2**	**G3**	**G4**	**HP1**	**HP2**	**HP3**	**HP4**	**UL1**	**UL2**
0.58± 0.0017	0.55± 0.0012	0.79± 0.0012	0.81± 0.0013	1.014± 0.0013	1.012± 0.0014	1.253± 0.0013	1.183± 0.0014	1.252± 0.0015	1.749± 0.001
UL3	**UL4**	**UL5**	**UL6**	**MP1**	**MP2**	**MP3**	**MP4**	**MP5**	**KA1**
1.024± 0.0011	1.029± 0.0012	1.028± 0.0015	1.018± 0.0012	1.06± 0.0014	1.04± 0.0012	0.92± 0.0013	1.08± 0.0014	0.95± 0.0016	0.754± 0.001
KA2	**KA3**	**KA4**	**KA5**	**OR1**	**OR2**	**OR3**	**OR4**	**OR5**	**WB1**
0.7683±0.019	0.78± 0.0071	0.892± 0.0013	0.851±0.014	0.861± 0.0015	0.852± 0.0016	0.762± 0.0015	0.858 ±0.014	0.861±0.013	0.856± 0.001
WB2	**WB3**	**WB4**	**WB5**	**WB6**	**JH1**	**JH2**	**JH3**	**JH4**	**JH5**
0.757± 0.0016	0.786± 0.0012	0.861± 0.0014	0.8542±0.013	0.796± 0.0015	0.932± 0.0012	1.012± 0.0012	1.12± 0.0016	1.09± 0.0015	0.953± 0.001
JH6	**KL1**	**KL2**	**KL3**	**KL4**	**TN1**	**TN2**	**TN3**	**TN4**	**NP1**
0.832± 0.0014	0.753± 0.005	0.786± 0.0015	0.868± 0.0015	0.872± 0.009	0.763± 0.0012	0.778± 0.0012	0.852± 0.009	0.860± 0.0015	0.863± 0.001
NP2	**UP1**	**UP2**	**UP3**	**UP4**	**UP5**				
1.126± 0.0013	1.173± 0.0012	1.098± 0.0013	1.124± 0.0012	0.967± 0.0011	0.835± 0.0016				

Full Paper

TABLE 5: Estimation of % w/w of chebulinic acid (by H.P.L.C) in different coded samples of fruits of Terminalia chebula

A1	A2	A3	A4	A5	A6	C1	C2	C3	C4
0.482± 0.0012	0.495± 0.0014	0.511± 0.0013	0.513± 0.0018	0.509± 0.0017	0.488± 0.0016	0.488± 0.0013	0.476± 0.0012	0.438± 0.0013	0.428± 0.0012
G1	**G2**	**G3**	**G4**	**HP1**	**HP2**	**HP3**	**HP4**	**UL1**	**UL2**
0.042± 0.0016	0.045± 0.0016	0.0428±0.014	0.0464±0.015	0.553± 0.0016	0.562± 0.0015	0.549± 0.0012	0.620± 0.0016	0.561± 0.0014	0.623± 0.0011
UL3	**UL4**	**UL5**	**UL6**	**MP1**	**MP2**	**MP3**	**MP4**	**MP5**	**KA1**
0.550± 0.0017	0.582± 0.0009	0.575± 0.0012	0.568± 0.0012	0.432± 0.0015	0.428± 0.0011	0.425± 0.0014	0.422± 0.0012	0.431± 0.0015	0.482± 0.0012
KA2	**KA3**	**KA4**	**KA5**	**OR1**	**OR2**	**OR3**	**OR4**	**OR5**	**WB1**
0.488± 0.0012	0.512± 0.0012	0.499± 0.0013	0.512± 0.0016	0.498± 0.0012	0.501± 0.0012	0.508± 0.0015	0.513± 0.0013	0.512± 0.0012	0.486± 0.0012
WB2	**WB3**	**WB4**	**WB5**	**WB6**	**JH1**	**JH2**	**JH3**	**JH4**	**JH5**
0.495± 0.0013	0.502± 0.0014	0.509± 0.0016	0.508± 0.0012	0.492± 0.0019	0.483± 0.0013	0.488± 0.0015	0.492± 0.0012	0.494± 0.0013	0.449± 0.0012
JH6	**KL1**	**KL2**	**KL3**	**KL4**	**TN1**	**TN2**	**TN3**	**TN4**	**NP1**
0.485± 0.0013	0.489± 0.0014	0.510± 0.0017	0.508± 0.0018	0.488± 0.0015	0.498± 0.0016	0.495± 0.0015	0.502± 0.0014	0.496± 0.0015	0.549± 0.0016
NP2	**UP1**	**UP2**	**UP3**	**UP4**	**UP5**				
0.623± 0.009	0.484± 0.0012	0.492± 0.0016	0.496± 0.0017	0.488± 0.0018	0.486± 0.0015				

search work to establish traditional uses with respect to specific constituent of Terminalia chebula.

ACKNOWLEDGEMENTS

The authors are thankful to the Vice chancellor of Birla Institute of technology for providing the funds for the project. The authors wish to thank the Dr. D.Sasmal, Head of the department of biotechnology for allowing to use various instruments of the department.

REFERENCES

[1] J.G.Bruhn, B.Holmstedt; Natural products as medicinal agents. J.Beal, E.Reinhard, (Eds.), Hippokrates, Stuttgart, 224-235 **(1981)**.

[2] G.A.Cordell; Changing strategies in natural product chemistry.Phytoche., **40**, 1585–1612 **(1995)**.

[3] A.Jossang, M.Seuleiman, E.Maidou, B.Bodo; Penta cyclic triterpenes from Combretum nigricans.Phyto chemistry, **41(2)**, 591-594 **(1996)**.

[4] A.P.Kundu, S.B.Mahato; Triterpenoids and their glycosides from Terminalia chebula.Phytochemistry, **32 (4)**, 999-1002 **(1993)**.

[5] J.Kumar, S.P.Bhatnagar; H.P.L.C method for simultaneous estimation of tannic acid, gallic acid, chebuli nic acid and ethyl gallate in Terminalia chebula fruits **3(15)**, 141-143 **(2005)**.

[6] P.P.Lu, X.Liu, X.G.Li, J.Q.Wen; Chemical constituents of fruits of Terminalia chebula Retz.Shang hai.Yike.Daxue.Xuebao., **18(3)**, 233-235 **(1991)**.

[7] C.Singh; 2-a-Hydroxymicromeric acid, a pentacyclic triterpene from Terminalia chebula.Phytochem., **29(7)**, 2348-2350 **(1990)**.