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

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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.

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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 2 α -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, Chebulinic acid and Ethyl gallate (Kumar. J and Bhatnagar, 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 40°C. 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

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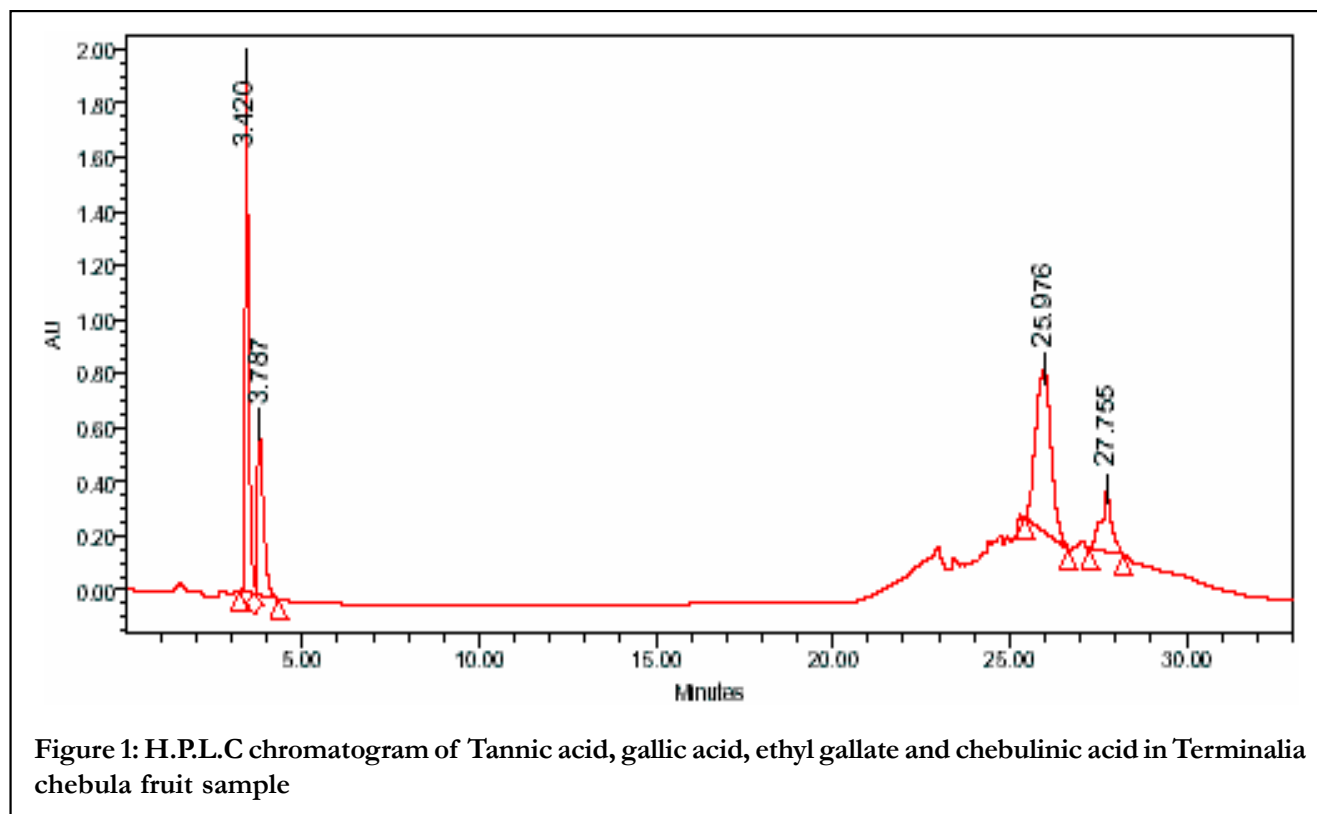


TABLE 2: Influence of Geographical Distribution on Tannic acid (%w/w) of dried fruits of Terminalia chebula.

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

tional system of medicine. These findings give scientific basis for establishing the traditional uses of Terminalia chebula and a new direction further re-

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				

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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*.

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