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QUALITATIVE AND QUANTITATIVE PROFILE OF TANNIC ACID ISOLATED FROM *TERMINALIA CHEBULA*

Himesh Soni*, Sarvesh Sharma, Sita Sharan Patel, Kaushelendra Mishra, A.K. Singhai

*Lakshmi Narain College of Pharmacy, Raisen Road, Bhopal (M.P.) - 462021.

ABSTRACT

Terminalia chebula (Combretaceae) is called the "King of Medicines". The fruit of *Terminalia chebula* is being used for the treatment of different types of diseases and disorders since antiquity. During the last five decades, apart from the chemistry of *T. chebula* compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of *T.chebula*. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. In the present work we have investigated the qualitative and quantitative determination of tannic acid isolated from the *T.Chebula*. Qualitative estimation was carried out by treating the sample with 5% $FeCl_3$ and thin layer chromatographic (TLC) method. The total phenolic content was found to be 20.01 as mg GAE/g. The simultaneous determination of the % condensed tannins was carried out by HPLC techniques. HPLC separation was performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ) using Water[0.1 % orthophosphoric acid and acetonitrile in the ratio 90 : 10 (v/v) at flow rate of 1.0 mL/min. Detection of tannic acid were performed at 280 nm.

Keywords: Tannic acid, *T.chebula,* HPLC, TLC & isolation.

INTRODUCTION

Terminalia Chebula has been extensively used in ayurveda, unani & homoeopathic medicine and has become "King of Medicines". *T.chebula* is a tropical tree 15-20m high, which has yellow-white flowers, odoriferous. The fruit is a dry drupe. It is found in India, Indochina, Burma, Cambodia, Thailand, Laos, Vietnam and Malaysia. Mature myrobalan fruits are drupes which are ovate and longitudinally wrinkled. The fruits are 2-3.5 cm long, and 1.3 - 2.5 cm broad. It is hard and stony. The pulp is 3 to 4 mm thick, which is not, attached to seeds. It has slight odour and slightly bitter, astringent taste. The small immature fruits are known as 'Himaj'. These fruits are pale brownish to black, compressed, ovate and tapering on both ends. Fruits show a scar of pedicel at one side. Myrobalan contains about 30 % of the hydrolysable tannins, which consists of chebulinic acid, chubulagic acid and D- galloyl glucose. It contains free tannic acid, gallic acid, ellagic acid and resin myrobalanin and anthraquinone glycosides have been reported in myrobalen [1]. The Sanskrit name 'Haritaki' is rich with meaning, referring to the yellowish dye (harita) that contains the god Siva (Hari, i.e. the Himalayas) and that it cures (harayet) all the diseases. Its other commonly used Sanskrit name, Abhaya, refers to the 'fearlessness' it provides in the face of the disease. According to Indian mythology, this plant originated from the drops of ambrosa (Amrita) which fell on the earth when Indra was drinking it [2]. *T. Chebula* possesses a wide variety of activities like antimicrobial [3], antioxidant [4], antiviral[5], anticarcinogenic[6], hypocholesterolemic [7], radioprotective [8], antispasmodic & antipurgative [9].

The tannic acid is the important chemical constituent of *T. Chebula.* The other names Penta-(m-digalloyl)-glucose; tannin; gallotannin having Formula $C_{76}H_{52}O_{46}$ and molecular weight 1701.20 g/mol with melting point begins to decompose at 210°C (410°F)[10].

MATERIALS AND METHODS

Plant Material

The Fruits of *T. Chebula* was obtained from the Pharmacognosy research lab. L.N.C.P. Bhopal (M.P.). A voucher specimen was deposited.

Isolation of tannic acid

The seedless myrobalen fruit were extracted with acetone under reflux. After complete extraction solvent was recovered under conventional distillation unit. A paste was obtained. This was purified with ethyl acetate by refluxing under slow stirring (25-30rpm). The ethyl acetate fraction was filtered and again solvent was recovered.

***Corresponding Author Himesh Soni E mail:** himeshsoni@rediffmail.com

A paste thus obtained was dissolved in 3-4 times D.M. water with stirring at 30rpm.The solution was passed through a hyflo bed in a filter press to obtained clear solution & stand the above solution for 30 min. Again the solution was filtered and spray dried. The powder tannic acid was obtained [11].

Qualitative analysis by Thin layer chromatography

For thin layer chromatographic studies of tannic acid, precoated Silca gel F_{254} aluminum plates (20 X 20cm) were used[12].The tannic acid was separated using $CHCl_3$:ethyl acetate:formic acid [25:2:0.8]. The colour and R_f values were recorded using UV_{254}.

Determination of total phenolic contents

The Folin-Ciocalteu reagent assay was used to determine the total phenolic contents. The acetone extract 1ml(10mg/ml)was mixed with 0.5ml Folin-Ciocalteu reagent previously diluted with 7ml deionized water. The solution was allowed to stand for 3min. at 25^0C before adding 0.2ml of saturated sodium carbonate solution. The mixture was allowed to stand for another 120 min and absorbance was measured at 725nm.Gallic acid was used as standard for the calibration curve. The total phenolic contents of the extract were calculated in terms of Gallic acid equivalent (GAE) [13].

$$C = c \times V/m$$

Where, C= total phenolic compound in mg/gm of the extract

c = concentration of gallic acid (established via calibration curve)

V = volume of the extract in ml

m = wt. of extract in gm

Quantitative estimation of tannic acid by HPLC

The HPLC analysis was performed using a LC-100, Cyberlab™, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of solvent A:B[90:10]. Solvent A is water with o-phosphoric acid[pH= 2.4-2.6] and solvent B is acetonitrile. The separation was performed using isocratic elution (0-15 min) with a flow rate of 1.0 ml/min and a column temperature of 25°C. The injection volume was 25µl, and UV detection was effected at 280 nm. HPLC grade solvents were obtained from Shyam brothers, 27-sindhi market, Bhopal. After isolation the tannic acid (10µg/ml) were subjected to HPLC column and the obtained record were superimposed on the retention time values of the standard tannic acid.

$$\% \text{ condensed tannins (tannic acid)} = \frac{AXWo}{Ao \times W}$$

Where

A = peak area of the sample (condensed tannins)

Ao = peak area of the reference standard (tannic acid)

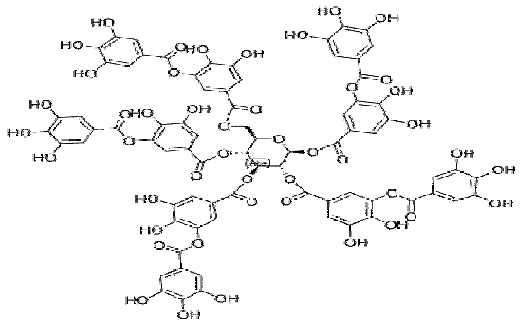
W = weight in mg of the sample taken

Wo = weight in mg of the reference standard taken

RESULT AND DISCUSSION

Keeping in view of the ethno-pharmacological importance of fruits *T.chebula* the isolation of important phytoconstituent tannic acid was undertaken for standardization. Organoleptic evaluation (Table1) showed the following characters: colour- yellowish to light brown and odour - odourless. The organoleptic studies indicated important characteristics such as typical tongue sensitizing sour taste, aromatic odour, etc, which are useful diagnostic characters. The solubility study and loss on drying of sample were determined (Table 1). The isolated sample was subjected for qualitative estimation by using 5% $FeCl_3$ (Table 1) and TLC analysis (Table 2). The Quantative determination of phenolic content & % condensed tannin were found to be 20.01 as mg GAE/g and 55 % (Table 3) respectively. The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. HPLC separation of isolated sample with reference to standard were performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ) (Table 4 & 5). Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally considering multiple constituents present in the herbal medicines [14].

Fruits of *T. Chebula*



Structure of tannic acid

Table 1: Physiochemical Evaluation of Tannic acid

S.No.	Characteristics	Observation	Test method
1.	Identification	Dark bluish ppt & light purple spot	5% $Fecl_3$ & TLC
2.	Appearance	glistening scales or spongy mass	Visual
3.	Colour	Yellowish to light brown	Visual
4.	Odour	Odourless	Organoleptic
5.	Taste	Characteristics	Organoleptic
6.	LOD	6.52	I.P 1996
7.	Solubility in water	(+)ve	1.P 1996
8.	Solubility in ethyl alcohol	(+)ve	1.P 1996

[+]ve = Present

Table 2 : TLC Profile of Tannic acid

S.No.	Solvent System	R_f of Sample	Inference
1.	$CHCl_3$:ethyl acetate: Formic acid [25:2:0.8]	0.67, 0.4, 0.31,0.2	0.31[dark purple spot] Gallic acid
2.	Water: acetonitrile[90:10]	0.49	Light purple spot

Table : 3 Quantative estimation of Phenolic content & % condensed tannin from *T.chebula*

S.No.	Quantitative analysis	Inference
1.	Total Phenolic content	20.01 as mg GAE/g
2.	% Condensed tannins	55%

Table 4: HPLC analysis of tannic acid [Standard]

ID	NAME	RT(min.)	Height	Area	Conc.	Theo.Plate	Tail.factor
1.		1.250	17	146.2	0.107	421	1. 0
2.		2.80	396	8283.8	6.043	8336	1.16
3.		4.077	4	21.9	0.016	18226	0.82
5.	Tannic acid	5.800	8031	127747.1	93.193	2650	1.40
6.		6.820	27	373.6	0.273	1.06	4840

Table 5: HPLC analysis of tannic acid [Sample]

ID	NAME	RT(min.)	Height	Area	Conc.	Theo.Plate	Tail.factor
1.		0.349	20	130.2	0.094	57	1.06
2.		1.269	35	529.9	0.383	140	0.98
3.		2.759	276	1510.6	1.093	5065	0.70
4.	Gallic acid	4.304	40	896.1	0.648	736	0.82
5.		4.941	542	6533.8	4.728	3348	1.15
6.	Tannic acid	5.483	4979	71088.5	51.437	6103	0.94
7.		7.24	1603	24784.9	17.933	4369	0.80
8.		7.901	257	4387.2	3.174	4817	1.24
9.		8.39	532	9742.8	7.050	4694	1.22
10		8.89	37	302.2	0.219	29084	1.50
11		9.471	1493	15660.0	11.331	16249	1.17

Fig:1 HPLC chromatogram of Standard tannic acid

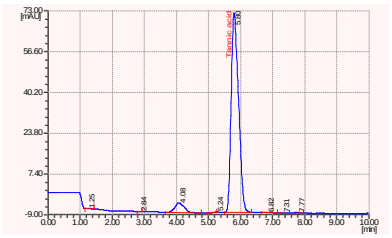
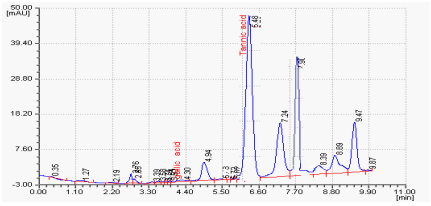


Fig:2 HPLC chromatogram of Sample



CONCULSION

The tannic acid was isolated from *T.chebula* and identified by TLC and HPLC methods. Tannic acid is therapeutically used in treatment of infections, allergy, cough, spam disease & wound healing, hence this plant and their phytoconstituents are useful for the above disease affecting the mankind. Further studies required to proof the potential of this plant.

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