International Journal of Medicine and Pharmaceutical Research

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Research Article **Open Access**

The isolation, characterization and quantification of gallic acid from the fruit extract of *Terminalia chebula*

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A B S T R A C T

Terminalia chebula (Combretaceae) is a well-known medicinal plant commonly used in phytomedicine to cure diseases in Ayurveda, unani and homeopathic system of medicine. Chemical investigation of bioactive constituents from dried seeds of *Terminalia chebula* afforded gallic acid as one of the major constituent. Gallic acid was isolated and the structural elucidation was done using ^{1}H, ^{13}C NMR spectroscopic technique in combination with UV, IR and Mass spectral data.

Keywords: Terminalia chebula, Gallic acid, isolation, structural elucidation.

A R T I C L E I N F O

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Article History: Received 15 January 2014, **Accepted** 10 March 2015, **Published Online** 10 April 2015

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Manuscript ID: IJMPR2492

PAPER-QR CODE

Citation: Sravan Kumar Reddy Theepireddy, et al. The isolation, characterization and quantification of gallic acid from the fruit extract of *Terminalia chebula*. *Int. J. Med. Pharm, Res.*, 2015, 3(2): 983-988.

1. Introduction

Terminalia chebula (local name: haritaki, family: Combretaceae) is a moderate tree used in traditional medicines like Unani, Ayurveda and Homeopathic medicine. Ayurveda, believed to be the oldest form of health care system available on planet, gives a total approach to health, healing and longevity. *Ayur* means life

while *Veda* means science and this ancient system of natural & medical healing originated in India. The medication that will be prescribed will be plant extracts, from the plants that have medicinal value. The medicinal value of the plant in-turn depends upon some specific substances produced in the plant, which will be responsible for definite physiological action on the metabolic system of various organisms. Such substances are broadly named as Phytochemicals. One such plant that is a popular habitat of southern Asia is *Terminalia chebula* (family: Combretaceae). It is a tree tall about 50 -80 ft in height with a rounded crown and spreading branches. *Terminalia chebula* (fig 1) is a native plant in India and its dried fruit will be commonly used as a cough reliever.In Telugu, one of the Indian languages, the plant is commonly known as Karakkaya (Harad in Hindi). Generally flowers appear from April to August and fruits ripen from October to January. There has been a lot of public interest in the usage of traditional medicines for some of the diseases like cancer, HIV infection/AIDS, etc., which have often proved fatal. A detailed study was done by Hedimbi et.al.,[4] and Singh et.al.[5], to identify various traditional plants that are commonly used for treatment of HIV infection. Peltzer et. al., 2008 reported that traditional herbal therapies and Traditional Complementary and Alternative Medicine (TCAM) were commonly used for HIV naïve hospital outpatients. Langlois-Klassen et. al., in 2007 asserted that it is equally important to consider the potential for medicinal plants that have an adjuvant effect on Anti-retro viral (ARV) drug efficacy. However, they had cautioned to investigate and further identify the potential risks, benefits and drug-drug interactions between traditional medicines and anti-retroviral drugs before administering. Li et. al., had also reported a detailed study of Baicalin (BA), a flavone that is purified from the plant, Scutellaria Baicalensis Georgi, which is a traditional Chinese herbal medicine against HIV-1 infection. BA was found to be effective against HIV-1 infection without any cytotoxic and cytostatic effects. The greatest challenge in front of global research community is the treatment of HIV infections. HIV is known to be a predominantly sexually transmitted disease, while few cases are also noticed because of either blood transfusion or from an infected mother to infants [6].

Figure 1: *Terminalia chebula*plant, dried seeds & powder

The bark is dark brown with some longitudinal cracks. Leaves are 10 – 20 cm long ovate and elliptic, with two large two glands at the top of petiole. The fruit is about 1-2 inches in size. Fruit is green when unripe and the ripen fruit will yellowish grey in colour. *Terminalia chebula* is called the “king of medicines”. Many medicinal compounds have been isolated from *Terminalia chebula* and high content of phenolic compounds including, Gallic acid (GA), Ellagic acid (EA), and Chebulic acid (CA). These compounds have been known to exhibit anticancer, antimicrobial [1], anti – inflammatory [2] and HIV-1 integrase activities [3]. *Terminalia chebula* is used extensively in the preparation of many Ayurvedic formulations for infectious diseases such as chronic ulcers and fungal infections of the skin. It is used to prevent aging and impart longevity, immunity and body resistance against disease.

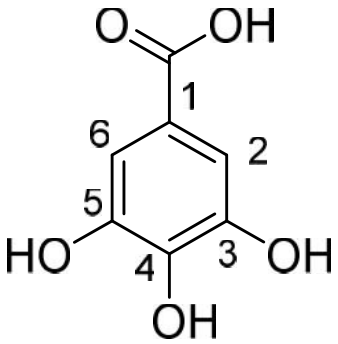

Figure 2: Chemical structure of Gallic acid

HIV is a causative agent for AIDS (acquired immuno-deficiency syndrome) and the suppression of HIV infection through viral replication for the longest possible period, has become the major focus of anti-retro viral therapy (ART). Though current antiretroviral drugs are improving the quality of life in HIV/AIDS patients [7], owing to the rapid emergence of drug resistant viral strains as well as the thrust for new anti-viral drugs that act by new mechanism, much attention was focused by research community over exploration of new anti-HIV agents [8]. During the process of exploring various methods for treating this deadly infection, researchers have identified HIV-1 protease (PR), reverse transcriptase (RT) and integrase (IN) enzymes as prime targets [9]. Raltegravir, Elvitegravir as well as Dolutegravir are now available HIV-1 IN inhibitors in the market. In continuation to these, search for plant derived HIV-1 IN inhibitors are gaining lot of importance [3,10]. In this work we’ve detailed the ^{1}H-NMR, ^{13}C-NMR, HPLC and LC-Mass processes of the Gallic acid as well as crude extract of *Terminalia chebula*. Gallic acid (fig. 2) seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal haemorrhage. Gallic acid is also used to treat albuminuria and diabetes [11].

2. Materials and Methods

Plant Material: The fresh mature fruits of *Terminalia chebula* were purchased from Ayurvedic shop, Habsiguda, Hyderabad. The fruits were selected according to the uniformity of the shape.

Extraction and fractionation:

The dried fruits of *Terminalia chebula* are powdered in an electrical grinder. 250 gm of the powdered fruit was taken in hexane (1000 mL) and stirred for 24 h at room temperature and the hexane layer was decanted to remove fatty acids, chlorophylls and the lipids. Remaining crude residue was extracted with 95% ethanol (1000 mL) for 48 h at room temperature. The solution was filtered and the residue was extracted two more times each with 95% ethanol (500 mL) for 48 hr at RT and the extracts were filtered under suction pump.

The combined extracts were concentrated under reduced pressure using a rotary evaporator at 50-55°C to obtain crude residue is 148 gm. This residue was tested for organic compounds by using high performance liquid chromatography (HPLC) and Liquid chromatography–mass spectrometry (LCMS). Analysis of LCMS and HPLC confirmed the fruit extract of *Terminalia chebula* contains phenolic compounds as major constituents. The crude extract of *Terminalia chebula* shows 18 % of gallic acid by HPLC and the molecular weight was confirmed by LC-MS. The pure gallic acid was isolated following column chromatographic technique from the crude extract of *Terminalia chebula* by using 100-200 mesh silica gel.

Method of HPLC analysis: High Performance Liquid Chromatographic (HPLC) analysis was performed using Purosphase RP-18 (250 x 4.0 mm, 5 µm). Two different mobile phase were used for the investigation among which one was Buffer: Methanol (85:15), while the other was Acetonitrile: Buffer: Methanol (50:20:30). The buffer solution used was prepared by dissolving 27.2 grams of sodium perchlorate and 3.08 grams of ammonium acetate in 1000 ml of water, whose P^H was adjusted to 3.5 using phosphoric acid. The flow rate was adjusted to 0.7 ml/min with an injection volume was 10 μL. The detector wavelength was 277 nm. The column temperature was 40°C with a run time of 65 min.

Method of LC-MS analysis:

Liquid chromatography–mass spectrometry (LCMS) analysis was performed using Waters-Quattromicro Mass in which Symmetry Shield RP-18 (150x4.6 mm, 3.5 µm) column was used for the analysis and the flow rate was adjusted to 0.8 ml/min.

The UV detector used was at UV 254 nm. Methanol was used a diluent with a sample concentration of 1 mg/ml. The column temperature was 25°C with a run time of 65 min, while the injection volume was 20 μL. 0.1 % TFA in water was used as buffer solution. The buffer stock was used to prepare two sets of solutions as follows; Solution A preparation: Buffer: Acetonitrile (950:50 v/v); Solution B preparation: Buffer: Acetonitrile (125:125 v/v).

Method of IR, UV, NMR and Mass analysis:

Melting point was recorded on a Polmon digital melting point apparatus (Model No. MP-96), FT-IR characterization was done on a Perkin Elmer Spectrum 100-USA using KBr pellet and UV-VIS spectra were recorded on a Shimadzu UV-VIS 1601 spectrophotometer. The ^{1}H NMR and ^{13}C NMR were recorded on a BRUKER AV-300 MHz spectrometer in DMSO-d6 using TMS as internal standard. Mass spectra were recorded on a VG AUTOSPEC Electro spray mass spectrometer.

3. Results and Discussion

The phytochemicals are bioactive substances most prominent being alkaloids, flavonoids, tannins and phenolic compounds initiating positive physiological activity in the human body are attributed to the medicinal value of plants. The crude extract of *Terminalia chebula* had various bioactive components possessing various activities. Gallic acid is one of the major important compound of *Terminalia chebula* extract and this has been previously identified by other researchers [12-15]. Our results were similar to these researchers where gallic acid was identified as one of the compound in the 95% ethanolic extract.

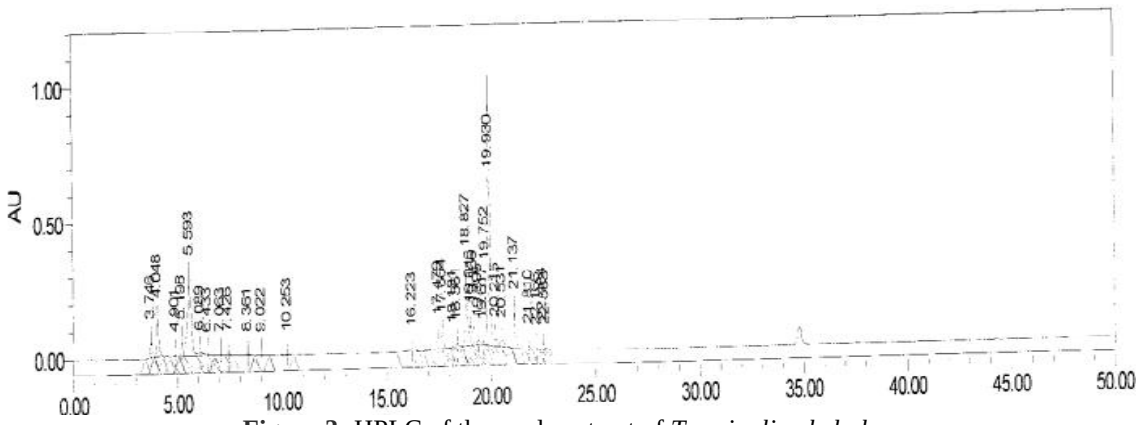

Figure 3: HPLC of the crude extract of *Terminalia chebula*

HPLC and LC-MS profile of the crude extract (Figure 3 and 4, Table1) shows the presence of a range of phytochemicals like Gallic acid, Ellagic acid, Corilagin, Chebulinic acid and Chebulagic acid or Chebulaginic acid. As per the LC-MS analysis of crude fruits extract of *Terminalia chebula* shows a retention time (RT) at 4.23 is showing gallic acid with molecular ion mass at m/z 169.22 $[M-H^+]^-$, retention time at 10.57 shows Corilagin with molecular ion mass at m/z 633.24 $[M-H^+]^-$, RT at 13.35 shows Chebulaginic acid or Chebulagic acid with molecular ion mass at m/z 953.30 $[M-H^+]^-$, RT at 16.20 shows Ellagic acid with molecular ion mass at m/z 301.18 $[M-H^+]^-$ and RT at 19.30 shows Chebulinic acid with molecular ion mass at m/z 955.42 $[M-H^+]^-$. The content of gallic acid is 18.25% at retention time 5.59 by HPLC (figure-3). The puregallic acid obtained by silica gel (100-200 mesh) column chromatography was 22.6 grams from 148 gm of 95% ethanolic extract from *Terminaliachebula*.

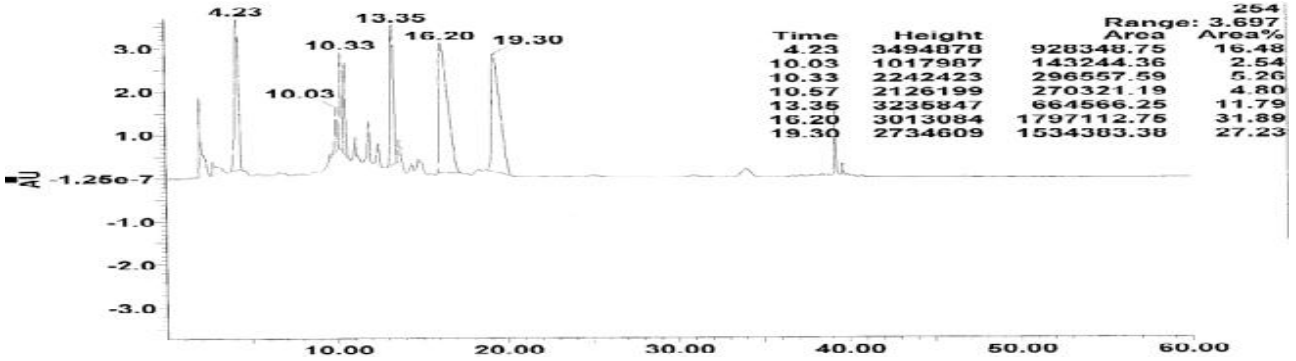

Figure 4: LC-MS of the crude extract of *Terminalia chebula*

Table 1: HPLC and LC-MS profile of the crude extract

S.No	HPLC RT (min)	LC-MS RT (min)	Mass Number
1	5.59	4.23	169.22 [M-H]-
2	17.58	10.33	541.10 [M-H]-
3	17.66	10.57	633.24 [M-H]-
4	18.83	13.35	953.30 [M-H]-
5	19.75	16.20	301.18 [M-H]-
6	21.13	19.30	955.42 [M-H]-

Isolation and characterization of Gallic acid:

The crude extract was purified with the column chromatographed by using 100-200 mesh silica gel eluted with solvent system dichloromethane and methanol. Seven fractions of each 500 mL were collected using 100% dichloromethane, 2.5% methanol in DCM, 5% methanol in DCM, 7.5% methanol in DCM, 10 % methanol in DCM, 12.5 % methanol in DCM and 15% methanol in DCM.As per TLC analysis, pure gallic acid was found in4th, 5th& 6thfractions(7.5%, 10%, 12.5% methanol in DCM), which upon distillation over rotary evaporator resulted in gallic acid with 99.6% purity by HPLC with a melting point of 257-261°C. Characterization of compound thus obtained using IR, UV, ^{1}HNMR, ^{13}C NMR and mass spectroscopic technique further confirmed the compound to be gallic acid. The UV (MeOH) spectra show that λ_{max} at 271.2 nm and 218 nm. The IR spectrum showed intense absorption bands at 3281 cm^{-1} and 3495 cm^{-1} corresponding to hydroxyl group of carboxylic acid and phenolic –OH group respectively with an absorption band corresponding to aromatic –C-H at 3062 cm^{-1}. Absorption bands at 1667 cm^{-1}and 1611 cm^{-1}correspond to carbonyl and aromatic C=C functional groups. The molecular formula of the compound was determined to be $C_7H_6O_5$ based on the ^{13}C-NMR spectral data and EIMS [*m/z* 168.97, $(M-H)^+$]. The structure of isolated gallic acid under investigation was further supported by ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6) spectral measurements (fig 5& 6). The ^{1}H- and ^{13}C-NMR spectral data of the isolated compound is recorded and assigned in the table 2 and 3 respectively. From this study, it was concluded that the isolated compound is gallic acid.

Table 2: Observation Table for ^{1}H-NMR spectra (DMSO-d_6) of Isolated Gallic acid

Groups	Chemical Shift (¢) ppm	Multiplicity
Aromatic 2H	6.91	s
-OH; 1H	8.83	bs
-OH; 2H	9.18	bs
-COOH; 1H	12.22	bs

Table 3: Observation Table for ^{13}C-NMR spectra (DMSO-d_6) of Isolated Gallic acid

Groups	Chemical Shift (¢) ppm
C-2; C-3	108.7
C-1	120.4
C-4	138.0
C-3; C-5	145.4
Carbonyl C	167.5

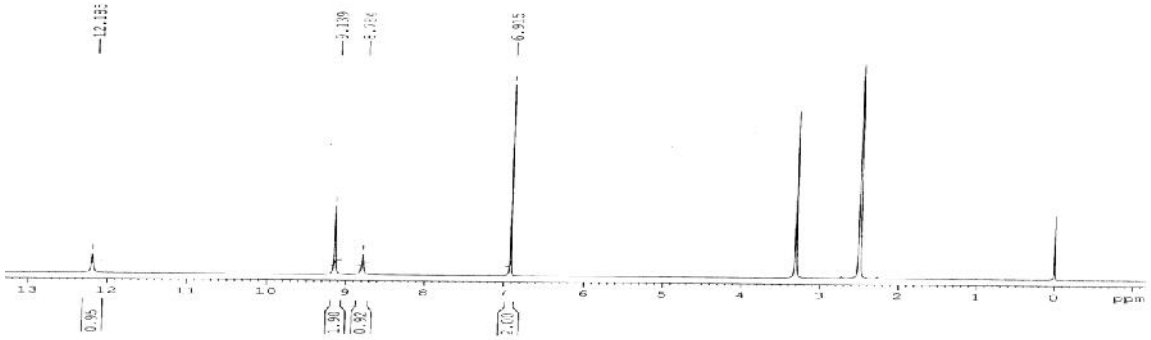

Figure 5: ^{1}H-NMR of the gallic acid extracted from *Terminalia chebula*

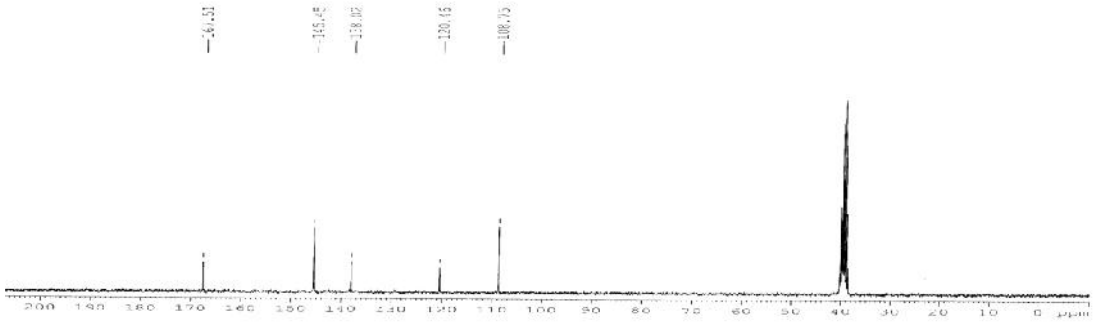

Figure 6: ^{13}C-NMR of the gallic acid extracted from *Terminalia chebula*

In conclusion, around 18% of gallic acid was found in crude extract of *Terminalia chebula*, which was isolated with 99.6% HPLC purity. The compound thus isolated was well characterized using UV, IR, Mass, ^{1}H-NMR as well as ^{13}C-NMR spectral studies and the data is presented.

4. Acknowledgement

Authors acknowledge the generous support received from Dr. B.P.S. Reddy Chairman & Managing Director Hetero Research Foundation, Hyderabad 500018, India for this research work. Authors also extend their acknowledgements to Dr. S. S. Reddy, Dr. N. Sudhakar and analytical department for their support.

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