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Research Article

## Isolation of Terflavin B from fruits of *Terminalia chebula* retz

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### ABSTRACT

*Terminalia chebula* Retz is known as “Haritaki” belong to the family Combretaceae. It is also known as Mother of all healings. Literature survey reveals that it have antifungal, antibacterial, anticarcinogenic, antidiabetic, antiproliferative, antiarthritic, anticaries and also multiprotective effect such as hepato-protective, pulmonary-protective, renal-protective, radio-protective, cytoprotective and cardio-protective effects. The present study investigates the phytochemical studies and structural elucidation of the medicinally active constituents in methanolic extract of fruits of *Terminalia chebula* Retz. The phytochemical analysis of *Terminalia chebula* Retz shows the presence of tannins, alkaloids, proteins, carbohydrates and phyto sterols were identified. Thin layer chromatography and High-performance thin layer chromatography analysis were confirmed the presence of tannins in methanolic extract of *Terminalia chebula* Retz. Column chromatography was used to isolate the compound and the structure was elucidated with spectroscopic methods. The Infrared spectroscopy,  $^1\text{H}$  &  $^{13}\text{C}$  Nuclear magnetic resonance spectroscopy and Mass spectroscopy analysis confirms the isolated compound was Terflavin B, A type of ellagi tannin which belongs to the hydrolysable tannins. The isolated compound was confirmed as Terflavin B. Terflavin B was reported an effective drug for the treatment of cancer and HIV disease. Thus the isolated compound may be synthesized in laboratory and can be applied for the treatment of the cancer diseases and prevention of HIV replication.

**Keywords:** High-performance thin layer chromatography; Infrared spectroscopy; *Terminalia chebula* Retz; Thin layer chromatography;  $^1\text{H}$  &  $^{13}\text{C}$  Nuclear magnetic resonance spectroscopy; Tannins; Terflavin B.

### INTRODUCTION

Plants have served mankind in different ways as food, fodder, fuel, medicine, clothing, shelter, etc., among these *T.chebula* Retz is one of the most important medicinal plant used in the medicines of Ayurveda, sidha, Unani and homeopathy because of a number of pharmacological properties. Fruits of *T.chebula* Retz are yellowish- brown, ovoid, generally 20-35 mm long and 13-25 mm wide, wrinkled and ribbed longitudinally. The pericarp of the dried ripe fruits of *T.chebula* is fibrous, 3-4 mm thick, non-adherent to the seed with astringent taste used traditionally in preparation of many Ayurvedic formulations (Chattopadhyay RR, 2007). Several recent reviews have documented extensive information on morphological characteristics, phytochemical, ethno botanical, biochemical, Ayurvedic, pharmacological activities and the medicinal uses of *Terminalia chebula* Retz (Dar Pervaiz A, 2012)

The literature documents that the fruits of *T.chebula* possesses more number of pharmacological activities. Phytochemical analysis of *T.chebula* shows the

presence of gallic acid, ellagic acid, tannic acid, ethyl gallate, chebulic acid, chebulagic acid, corilagin, mannitol, ascorbic acid and other compounds (I.S.Grover, 1992). One source lists *T. chebula* as having 32% tannin content.

However, no report documents for the structural elucidation of active principles in the successive extracts of the pericarp of the fruits of *T.chebula* Retz. The present study aimed to elucidate the structure of medicinally active constituent present in *T.chebula* Retz fruits.

### MATERIALS AND METHODS

#### Collection of plant material

The fruits of *T.chebula* Retz were purchased commercially from Tirupati, Chittoor District, Andhra Pradesh. The species were identified and authenticated by Dr. K. Madhava chetty, Departments of Botany, Sri Venkateswara University, Tirupati, Chittoor Dist, Andhra Pradesh.

#### Extraction Procedure

The Fruits of *T.chebula* Retz were shade dried and mechanically reduced to a coarse powder. The weight of the coarse powder was around 100 g. The powder was subjected to hot continuous successive extraction in a Soxhlet apparatus with solvents in the increasing order of polarity using petroleum ether, benzene, ethyl acetate, methanol and water. Extractives were concentrated below 50°C and further drying was carried out

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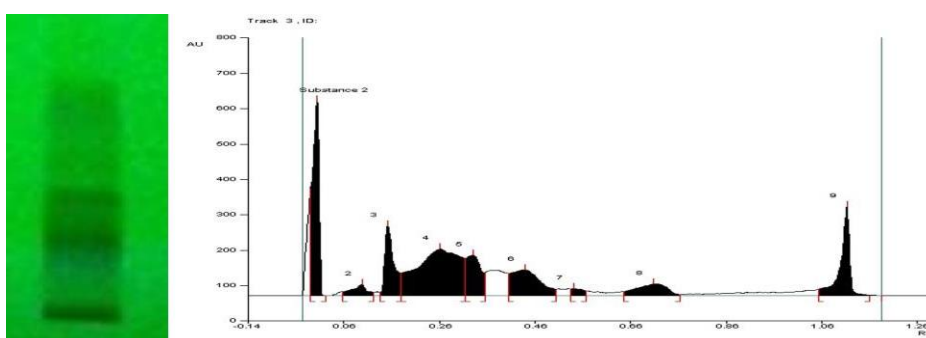
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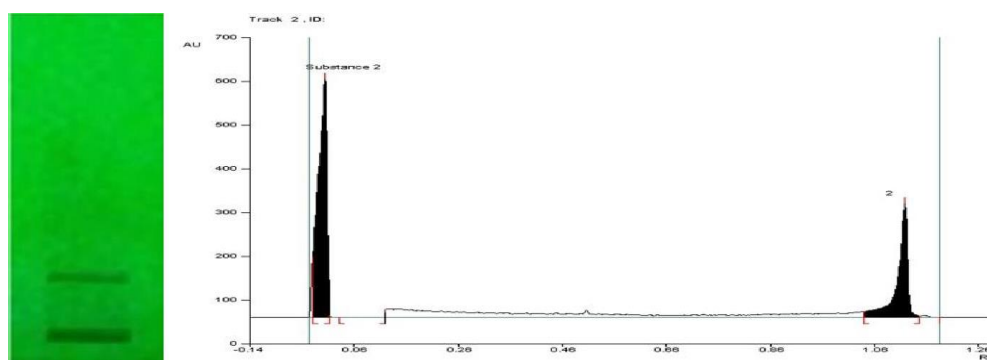
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**Table 1: Phytochemical tests of different extracts**

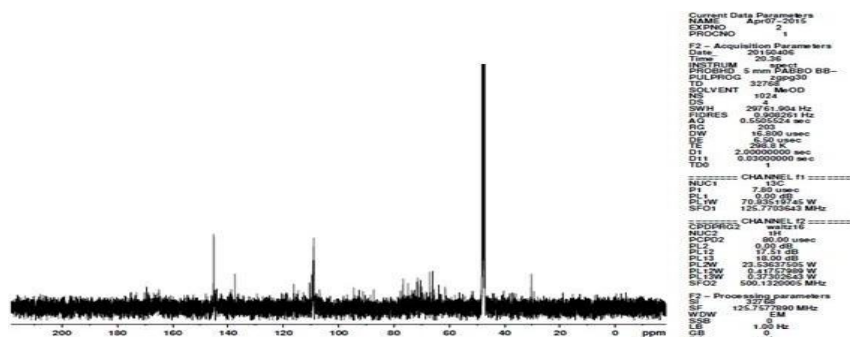
S.no	Test	Pet. ether extract	Benzene extract	E.acetate extract	Methanol extract	Water extract
1	Tannins	+ve	-ve	+ve	+ve	+ve
2	Alkaloids	-ve	-ve	+ve	+ve	+ve
3	Phyto sterols	-ve	-ve	+ve	+ve	-ve
4	Flavonoids	-ve	-ve	-ve	-ve	-ve
6	Cardiac glycosides	-ve	-ve	-ve	-ve	-ve
7	Anthraquinone glycosides	-ve	-ve	-ve	-ve	-ve
8	Carbohydrates	-ve	-ve	+ve	+ve	+ve
9	Proteins	-ve	-ve	-ve	+ve	+ve
10	amino acids	-ve	-ve	-ve	-ve	-ve
11	Fats and fixed oils	-ve	-ve	-ve	-ve	-ve



**Figure 1: HPTLC Plate and Chromatogram of *Terminalia chebula* Retz methanolic extract**



**Figure 2: HPTLC Plate and Chromatogram of Isolated compound**



**Figure 3: <sup>13</sup>C NMR Spectra of isolated compound**

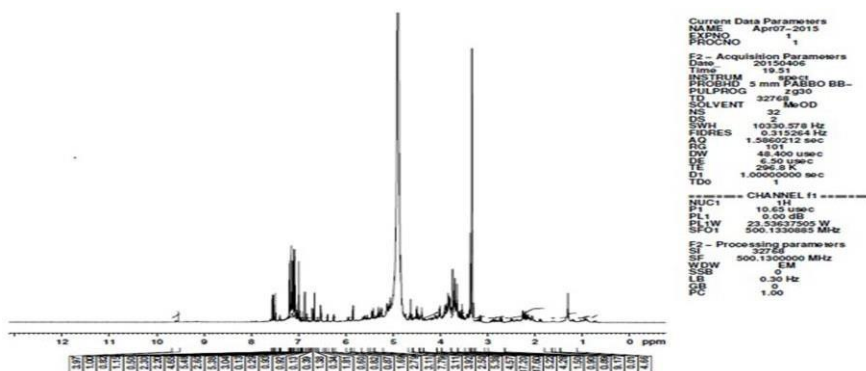


Figure 4:  $^1\text{H}$  NMR Spectra of isolated compound

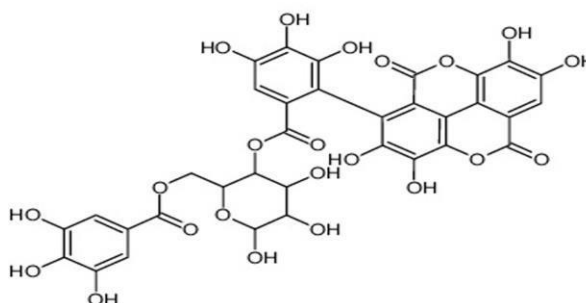


Figure 5: Structure of Terflavin B

under reduced pressure. The five dried extractives were stored in a dessicator for further evaluation.

The plant extracts were screened for the presence of biologically active compounds like tannins, alkaloids, steroids, tri terpenoids, flavonoids, Saponins, glycosides, carbohydrates, proteins, amino acids, fats and fixed oils (Anwesa Bag, 2012 & Roopalatha U C, 2013)

#### Thin Layer Chromatography

TLC of crude extract was carried out on TLC glass plates coated with silica gel G with 0.2mm thickness. Plates were activated at  $105^\circ\text{C}$  for 30 min in a hot air oven;  $10\mu\text{l}$  of crude extract was applied on the glass plates at equal distance with the help of micropipette. Chloroform: Glacial acetic acid: Methanol: Water (16:8:3:2) used as a mobile phase. The chromatogram was developed. TLC plate was observed under UV chamber at 365 nm. The  $R_f$  values were calculated and tabulated. Same procedure was followed to analysis sample by HPLC.

#### Isolation of active compound by Column Chromatography

The column was packed up to 2/3 portions with silica gel by wet packing procedure. The extract was mixed well with activated silica gel and was used for performing column chromatography. The methanolic extract of *T.chebula* Retz was defatted using petroleum ether for 6 h. The column was then eluted with a mixture of benzene and methanol in different ratios (10, 9:1, 8:2 and 7:3). Every 50 ml fractions were collected in different conical flasks and distilled to reduce the capacity of

fractions by using mobile phase ratio. After analyzing with TLC, single compound fractions were clubbed together and evaporated to get residue. The isolated compound purity was confirmed by thin layer chromatography and High performance thin layer chromatography using chloroform: glacial acetic acid: methanol: water (16:8:3:2). It confirmed that isolated compound was tannin (Mukherjee PK, 2006).

#### STRUCTURAL ELUCIDATION

The isolated compound structure was elucidated by UV, IR, NMR and mass spectroscopy.

#### RESULTS AND DISCUSSION

The fruits of *T.chebula* Retz were collected and extracted with various solvents (petroleum ether, benzene, ethyl acetate, methanol and water) and the percentage yield of different extracts was calculated. The high percentage yield were observed in methanolic extracts.

The phytochemical investigation showed the presence of tannins in petroleum ether extract, ethyl acetate extract, methanol extract and water extract. Alkaloids, carbohydrates and phyto sterols were present in ethyl acetate extracts. The maximum amount of tannins, alkaloids, carbohydrates, Phyto sterols and proteins were present in methanolic extracts. So the Methanolic extract was selected for isolation of active constituents. The phytochemical investigation data were shown in Table: 1.

The thin layer chromatogram and HPTLC report showed that the targeted compound  $R_F$  was found to be 0.56 and 0.9 respectively. The extracts chromatogram was shown in Figure no 1.

Isolation of targeted compound was done by column chromatography using benzene and methanol as a mobile phase. The fractions which are eluted from the 9:1 and 8:2 mobile phases are showed different components. Fractions 5<sup>th</sup>-11<sup>th</sup> in the ratio of 7:3 mobile phase were exhibited similar bands and similar  $R_F$  values of 0.90-0.91. The targeted compound was confirmed as tannins by ferric chloride test.

As per the HPTLC (Figure no 2) result shows that the isolated compound was single and  $R_F$  value was 0.9. The isolated compound was hygroscopic in nature. So it was stored in dry temperature in air tight containers.  $\lambda_{max}$  of isolated compound was determined as 365nm using methanol as a blank.

The IR spectral data helped in determining the type of functional group. The wave number at 2078  $cm^{-1}$  (C-H str), 3275 $cm^{-1}$ (O-H str), 629  $cm^{-1}$ (C-H bend) and 1701  $cm^{-1}$ (Carbonyl group) may confirm that the compound was poly hydroxyl containing hetero aromatic compound.

The  $^{13}C$  NMR spectrum shows total number of carbon and also confirms the substitution group avails near to that carbon (Figure no 3). The data proved that aromatic hydroxyl group observed at  $\delta$ 145, 144 ppm and the aldehyde or carboxylic C=O group was confirmed at  $\delta$  166 ppm. The aliphatic O-CH<sub>3</sub> was confirmed at  $\delta$  73ppm. The aromatic -CH group can be confirmed at  $\delta$ 116 and fused aromatic carbon at  $\delta$  137ppm. The data concluded that the structure consists of 34 carbons and the structure was fused hetero aromatic consisting of poly hydroxyl group. The  $^1H$  NMR spectra indicated the number of hydrogen and the substituted functional groups (Figure no 4). As per the data it proved that poly hydroxyl hetero aromatic compound.

The molecular weight of the isolated compound was found to be 784 g/mol. It was confirmed as Terflavin-B. The base peak was obtained at 711.9 which indicated that the cyclic ring cleave to get the fragment of C<sub>29</sub>H<sub>19</sub>O<sub>17</sub>. The fragmented ions were found at the ratio of m/z values 727, 629, 555, 385, 217, 203. The daughter ions were found at m/e of 97 and 65. The molecular weight was confirmed by mass spectroscopy as Terflavin B (Figure no: 5).

## CONCLUSION

The major active constituent was identified in Methanolic extract of fruits of *T.chebula Retz* and also isolated. The isolated compound was confirmed as Terflavin-B a type of hydrolyzed ellagitannin. They are having Anti mutagenic, radio protective, chemo preventive and preventing HIV replication activities. It was concluded that the isolated compound may be applied for the treatment of the cancer diseases and HIV. Future

perspective in this study may be extended to the synthesis the same drug for the application of clinical trial studies to produce an effective drug for the treatment of cancer and HIV disease in the market.

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