

A Comparative Phytochemical Analysis of Various Biotypes of *Terminalia chebula* Retz. Fruits of Western Ghats

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Abstract: *Terminalia chebula* Retz. is one of the most important medicinal plants used for various ailments in traditional medicines. This plant is used by the pharma industry to prepare many medicinal formulations to cure different diseases. In this current study five biotypes of *Terminalia chebula* Retz. were collected from Western Ghats and studied to prove that this plant has important phytoconstituents with high pharmacological value. The biotypes taken for study were compared by analysing the morphological features and the presence of different types of secondary metabolites such as flavonoids, phenols, terpenoids and tannins. Thin Layer Chromatography analysis done to identify the presence of important compound Gallic acid in all the types taken for study. This plant can be a source of useful drugs but further studies are required to isolate the active components for proper drug development.

Keywords: Flavonoids, Phenols, Terpenoids, Tannins, *Terminalia chebula* and TLC

I. Introduction

Most of the plants and their parts were used in traditional medicines like Ayurvedic or Unani medicines because they do not show any side effects compared to allopathic medicines. About 80% of the world population use traditional medicines, which are predominantly based on plant materials [1].

Terminalia chebula Retz. is a moderate tree used in traditional medicines. It belongs to the family Combretaceae. It is commonly called as Black myrobalan, Ink tree (or) Chebulic myrobalan. It is extensively used in Unani, Ayurveda and Homeopathic medicine. *Terminalia chebula* Retz. is a popular traditional medicine not only used in India but also in other countries of Asia and Africa. This is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active chemicals present in this plant. *Terminalia chebula* Retz. is used for the treatment of number of diseases like cancer, paralysis, cardiovascular diseases, ulcers, leprosy, arthritis, gout, epilepsy etc. It has been reported as antioxidant [2], antibacterial [3], antiviral [4], antidiabetic [5], antifungal, anticancerous, antiulcer, anti-mutagenic, wound healing activities etc.[6]. The ethanolic extract of *Terminalia chebula* Retz. induce cytotoxicity in cancer cells of MCF-7 [7] proved that this plant is a promising drug for cancer treatment. Phytoconstituents of *Terminalia chebula* Retz. have high pharmacological properties and used in traditional medicines [8]. Phytochemical study of this plant was not much recorded scientifically. Hence this work is a step towards phytochemical characterization of *Terminalia chebula* Retz.

II. Habitat

Terminalia chebula Retz. grow in India, Myanmar, Bangladesh, Iran, Egypt, Turkey, China etc. It is a moderate sized (or) large tree found throughout India chiefly in deciduous forests and areas of light rain fall but occasionally also in slightly moist forests up to about 1500 meter elevation throughout India, flowers appear from April-August and fruits ripen from October-January. *Terminalia chebula* Retz. is also called as Haritaki, Harad, Hirada, Alalekaayi, Kadukkai, Horitoky, Hilikha, Karakkaya in India, Aralu in Srilanka, Zhang-Qin-Ge, Hezi in China, Harra, Harro in Tibet, Myrobalane in Germany, Myrobalan in France.

Macroscopic characteristics: It is a deciduous tree, younger stems glabrescent, woody. Leaves are 10-20 cm long, sub-opposite, simple, exstipulate, petiolate, at the base of the lamina on the petiole two prominent gland were present, lamina broadly elliptic to elliptic – oblong, rarely ovate, the bases obtuse, the margins entire, the tips acute, glabrescent.

III. Material And Methods

Collection of Materials from Western Ghats

Terminalia chebula Retz. identified with the help of Floras [9],[10] and collected from different geographical location of Western Ghats at the outskirts of Sagar, Shimoga district of Karnataka. Five biotypes were collected separately for analysis.

Phytochemical Analysis

Preparation of the Plant Extract

Extracts were prepared by hot extraction process using Soxhelt apparatus. The methanolic extracts were concentrated in Roto flash evaporator and dried and stored in freezer for further biochemical analysis. The extracts were used for the preliminary phytochemical screening using the following tests [11].

Phytochemical Screening

Test for Alkaloids

Iodine Test: Mix 3 ml test solution and added few drops of dilute iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling [12].

Wagner's Test: To 2-3 ml extract add few drops Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids [13].

Dragendorff's Tests: To 2-3 ml extract, add few drops Dragendorff's reagent Formation of orange brown precipitate.

Test For Carbohydrates (Molisch's Test)

Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of con. H_2SO_4 down along the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet colour at the inter phase of the two layers was a positive test.

Test For Flavonoids

Pew's Tests: To 2-3 ml extract, added zinc powder in a test tube, followed by drop wise addition of con. HCl. Formation of purple red or cherry colour indicates the presence of flavonoids [14].

Shinoda Tests: To 2-3 ml extract, few fragments of magnesium metal were added in a test tube, followed by dropwise addition of concentrate HCl. Formation of magenta colour indicated the presence of flavonoids [13].

Alkaline reagent test: 2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids [12].

Test For Phenols (Ferric Chloride Test)

Ferric Chloride Test: A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour indicates the presence of phenols [15].

Ellagic Acid Test: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or niger brown precipitate occurred in the extract indicated the presence of phenols [15].

Test For Saponins (Foam Test)

To 2ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins [13].

Test For Terpenoids

Salkowki's test: 1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test For Sterols

Liebermann-Burchard Test: Mix 2ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops concentrated H_2SO_4 from the side of the test tube. First red then blue and finally green colour indicates the presence of sterols [13].

Salkowski's Test: To 2 ml of extract, add 2ml chloroform and 2 ml concentrated H_2SO_4 and was shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols [13].

Test For Tannins

Gelatin Test: To the extract, gelatin (gelatin dissolves in warm water immediately) solution was added. Formation of white precipitate indicated the presence of tannins [16].

Lead acetate test: To 5 ml of extract, add few drops of 10% lead acetate solution were added. Formation of yellow or red precipitate indicates the presence of tannins [16].

Thin Layer Chromatography

TLC was carried out on Silica gel plates. Starting line was marked at a distance of 1.5cm from the bottom of the plate and finishing line was marked at a distance of 2cm from the top of the plate.

Adsorbent used- Silica gel for TLC

Preparation of plates: - Silica gel with a mean pore width of preferably 6 to 10 nm is used as a base material. As smaller the particles better the separation efficiency. Silica gel plates of 0.2 mm thickness were prepared by spreading method with the help of applicator. And final spot taken on a pre coated silica gel and develop it in the solvent system to a distance of 2cm.

Activation plates: - Plates were activated at 105°C for 45 min in an electric oven.

Standard Solution: - prepare 1mg/ml solution of gallic acid in water.

Solvent System: - Chloroform: Ethyl acetate: Formic acid (2.0: 2.0: 0.8).

Chamber Preparation: A clean and dry chamber was taken. The solvent was introduced to a height of 0.5cm - 1cm from the chamber in order to moisten the TLC plate and to equilibrate the chamber with solvent vapour. The closed chamber was allowed to saturate with solvent vapour. The TLC was then introduced in the chamber in such way that the solvent system just wet the lower edge of the TLC plate and not to wet where the spots applied. Avoid any contact between the sides of the plates. Spots of the sample were applied with the help of micro capillaries.

Development of Chromatogram: The solvent migrates up the plate through the capillary action. The substance was separated as a result of interaction between the samples, mobile and stationary phase into individual component. Migration behaviour of the separated substance is given in the form of RF value (relative to front).

Distance travelled by solute (solute front)

$$RF = \frac{\text{Distance travelled by solute (solute front)}}{\text{Distance travelled by solvent (solvent front)}}$$

Ascending development of chromatogram was done. The plate was removed from the chamber, when the solvent front had reached the predetermined height and the solvent front was marked precisely with pencil. Then the plate was dried and observed under UV light.

Visualization: Scan the plate under UV at 254 nm and 366 nm and finger print profile. Spray the plate with 5% ferric chloride in methanol. Note the Rf of the band separated.

IV. Results

Phytochemical analysis revealed the methanolic extract of fruit of *Terminalia chebula* Retz. to contain tannins, saponins, terpenoids and phenols in greater proportions, while flavonoids in smaller concentrations.

Table- 1: Macroscopic evaluation of *Terminalia chebula* Retz. fruit Parameters

Parameters	Standard value	Biotype 1	Biotype 2	Biotype 3	Biotype 4	Biotype 5
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Taste	Astringent	Slightly bitter	Bitter	Sour and Bitter	Bitter	Bitter
Colour	Greenish Red	Green	Lightly Reddish	Reddish Green	Green	Lightly reddish
Size (Average Mean value)	L-3.55 cm B- 2.2 cm	L- 3.54 cm B- 2.71cm	L-3.21cm B-2.03cm	L-4.18 cm B-2.68 cm	L-3.31 cm B- 2 cm	L-3.12cm B-2.29 cm
Shape	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid
Tips	Both edge tapering	Tip round Base tapering	Both side tapering	Tip with dimple Base tapering	Both side tapering	Both

Table- 2: Results of photochemical screenings of methanolic extracts of fruits of *Terminalia chebula* Retz.

Constituents	Biotype 1	Biotype 2	Biotype 3	Biotype 4	Biotype 5
Alkaloids	+	+	+	+	+
Tannins	+++	+++	+++	+++	+++
Carbohydrates	++	++	++	++	++
Flavonoids	++	++	++	++	++
Phenols	+++	+++	+++	+++	+++
Sapponins	-	-	-	-	-
Sterols	-	-	-	-	-
Terpenoids	+++	++	+++	+++	+++

+++ Highly present

+ Very less quantity present

++ Moderately present

_ Completely absent

Table- 3: T.L.C. Identification

Sample No	Biotype 1	Biotype 2	Biotype 3	Biotype 4	Biotype 5	Std Gallic acid
Rf value	0.75	0.074	0.075	0.75	0.75	0.75

V. Conclusion

Different types of secondary metabolites are reported from *Terminalia chebula* Retz. that have effective functions on many diseases. Especially the Terpenoids, Tannins and Phenols are present in more quantity which make this *Terminalia chebula* Retz. (haritaki) with high pharmacological properties. Hence it is considered as most important medicinal plants used in medicines of Ayurveda, Siddha, Unani and Homeopathy. It is also the source of a variety of biologically active phytoconstituents such as chebulic acid, chebulinic acid, chebulagic acid, gallic acid, corilaginellagic acid and other related compounds which are responsible for antimicrobial, antioxidant, antihyperglycemic, anticancer and protective effects on various vital organs such as nerves, heart, kidney and liver. There is an urgent need to investigate the biological activity of its phytoconstituents for development of effective, safe and cheap herbal drugs.

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Bibliography

- [1]. WHO, Regional Office for Western Pacific, Research guidelines for evaluating the safety and efficacy of herbal medicines. Manila. (1993).
- [2]. S. Suchalatha, and C.S.Devi, Antioxidant activity of ethanolic extract of *Terminalia chebula* fruit against iso proterenol – induced oxidative stress in rats. Indian Journal of Biochemistry and Biophysics, 42, 2005, 246-249.
- [3]. P. Kannan, S.R. Ramadevi, and Waheeta Hopper, Antibacterial activity of *Terminalia chebula* fruit extract, African Journal of Microbiology Research, 3(4), 2009, 180-184.
- [4]. T.G. Kim, S.Y. Kang, K.K. Jung, J.H. Kang, E. Lee, H.M. Han and S.H. Kim, Antiviral activities of extracts isolated from *Terminalia chebula* Retz., *Sanguisorba officinalis* L., *Rubus coreanus* Miq and *Rheum palmatum* L. against hepatitis B virus, Phytotherapy research, 15(8), 2001, 718-720.
- [5]. N. K. Rao and S. Nammi, Antidiabetic and renoprotective effects of the chloroform extract of *Terminalia chebula* Retz. seeds in streptozotocin-induced diabetic rats, BMC Complement Altern Med., 6: 17, 2006.
- [6]. D. V. Surya Prakash, N. Sree Satya, Sumanjali AvaniGadda and Meena Vangalapati, Pharmacological Review on *Terminalia chebula*. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3 (2), 2012, 679-683.
- [7]. Deena Priscilla and Jasmine, Assessing the potential of the ethanolic extracts of *Terminalia chebula* against breast cancer. World Journal of Pharmacy and Pharmaceutical Sciences, 2 (6), 2013, 5702-5708.
- [8]. Prakash Chandra Gupta, Biological and pharmacological properties of *Terminalia chebula* Retz. (haritaki)- An overview. International Journal of Pharmacy and Pharmaceutical Sciences, 4, (3), 2012, 62-68.
- [9]. J.S. Gamble, The Flora of the Presidency of Madras, (Botanical Survey of India, Calcutta, India, 1957).
- [10]. Saldanha, J. Cecil, Flora of Karnataka, (Oxford & IBH Publishing & Co., New Delhi, 1984).
- [11]. O. Victor Njoku and Chidi Obi, Phytochemical constituents of some selected medicinal plants, African Journal of Pure and Applied Chemistry, 3 (11), 2009, 228-233.
- [12]. K.R. Khandelwal, Practical pharmacognosy, (19th Edition, Nirali Prakashan, Pune, 2008).
- [13]. C. K. Kokate, Practical Pharmacognosy, (4 Ed. Vallabh Prakashan, New Delhi, 2008).
- [14]. K. Peach, M.V. Tracey, Modern methods of plant analysis, (Vol. 3, Springer Verlag, Berlin, 1956).
- [15]. R.D. Gibbs, Chemotaxonomy of Flowering Plants, (Vol.1, McGill Queen's University Press, Montreal and London, 1974).
- [16]. G.E. Treare and WC Evans, Pharmacognosy, (17th ed., Bahiv Tinal, London, 1985).