



Qualitative and Quantitative Estimation of Tannins in Leaves of *Terminalia chebula* Retz. (Combretaceae)

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ABSTRACT

Terminalia is a well-known genus belonging to family Combretaceae. Most of the plant species coming under the genus were used as traditional medicines to cure lot many diseases. Especially the fruits of *Terminalia chebula* were found to have profound pharmacological activities. In the current work, leaves of *Terminalia chebula* were extracted by ultrasonic assisted extraction using different solvents in increasing order of polarity namely hexane, chloroform, ethyl acetate, acetone, ethanol, ethanol:distilled water (40:10, 30:20, 20:30, 10:40) and distilled water. The percentage yield was determined for each solvent. Several reports have suggested that tannins in the fruits of *Terminalia chebula* imparted medicinal properties to the fruits. Therefore, quantitative determination of total tannins was done in the leaves of *Terminalia chebula* and results were expressed as mg of tannic acid equivalent /g of extract. Polar solvents such as ethyl acetate, acetone, ethanol, ethanol: distilled water and distilled water extracts yielded better tannin content than non-polar solvents ranging from 470.61 ± 0.01 to 698.13 ± 0.008 mg/g. Pearson's correlation analysis showed that a statistically significant positive correlation existed between percentage yield of the extracts and total tannin content (0.687^{**} , $p < 0.01$). **Key words:** *Terminalia chebula* leaves, ultrasonic assisted extraction, percentage yield, tannins.

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INTRODUCTION

Plants have been proved handy in wide variety of fields and were used for various purposes from ancient time itself. Among the wider applications, its utility in medical field were found to be magnificent [1,2]. Attentiveness towards medicinal plants has hence increased from time to time. Many of the plants which were included under medicinal category essentially possessed different pharmacological activities [3,4]. Several studies have suggested that the presence of numerous phytochemicals imparted these medicinal properties to plants [5]. Medicinal plants therefore can contribute a major role in therapeutic healing [5,6]. However, better understanding and evaluation of medicinal plants are hence required.

In the present study medicinally potential deciduous tree, *Terminalia chebula* Retz. was evaluated. *Terminalia chebula* Retz. (Haritaki), is a wild as well as cultivated tree coming under the family Combretaceae [7,8,9]. The fruits of Haritaki were proven therapeutically active and constituted an important part of Triphala, an ayurvedic mixture [10]. The fruits of *Terminalia chebula* were reported to have antioxidant [11], antibacterial [12], anti-inflammatory [13], antidiabetic [14] and immunomodulatory activities [15]. Multitude of phytochemicals contributed to the pharmacological action of *Terminalia chebula* fruits [16].

Among the different phytochemicals identified in *Terminalia chebula*, tannins were of the majority which in turn suggested that tannins were the major phytoconstituent responsible for different pharmacological actions of *Terminalia chebula* fruits [9]. Therefore the current work mainly focused on the qualitative and quantitative estimation of tannins in the leaves of *Terminalia chebula* Retz. which in turn will prove it as medicinally useful as fruits.

MATERIAL AND METHODS

Collection of plant material: Leaves of *Terminalia chebula* were collected from Napier Museum Thiruvananthapuram, Kerala. The plant material was identified and the voucher specimen bearing

collection number 95980 was deposited at Jawaharlal Nehru Tropical Botanic Garden, Palode, Thiruvananthapuram.

Plant material preparation: The leaves of *Terminalia chebula* were dried under shade and ground to powder using blender according to standard procedures [17,18]. Dried and blended leaf powder were subjected to ultrasonic assisted extraction. Different solvent systems in increasing order of polarity were used for the purpose namely; Hexane < Chloroform < Ethyl acetate < Acetone < Ethanol < Ethanol: Distilled water (40:10, 30:20, 20:30, 10:40) < Distilled water.

Ultrasonic assisted extraction: About 25gm of powdered leaves were kept immersed in 50ml solvent and subjected to ultrasonic power for 10 minutes. Later the mixture was filtered using whatman's filter paper. The collected filtrate was then dried in oven at 40°C. Dried extracts were then stored in air tight vessels for future use [19,20].

Percentage yield: Percentage yield of all the solvents were calculated [19]

Percentage yield = [(Weight of the petri+ extract) – Weight of petri] / Weight of powdered sample taken * 100 (where 'petri' denotes petridish).

Phytochemical screening: The extracts obtained were screened for the presence of various phytochemicals such as alkaloids, flavonoids, phenols, tannins, coumarins, terpenoids and glycosides according to the standard procedures [21,22,23].

Determination of total tannins (Polyphenols): Total tannin or polyphenolic contents were determined by Folin Ciocalteu method [23,24]. Absorbance was measured at 725nm using a UV-visible spectrophotometer (version 4.1.27-2.9.4). Quantitative determination of tannins was carried out in triplicate. Tannic acid was used as standard for the measurement of tannins in samples. The results obtained were expressed as mg of Tannic Acid Equivalent (TAE)/g of dried sample (mg TAE/g of sample).

Statistical analysis: The results (all the triplicate values, n=3) were statistically analyzed using Microsoft excel and the data obtained were represented as mean $\pm$ standard error. Correlation analysis were done using SPSS (version 22) to determine the relation between percentage yield and tannin content with a level of significance set as $p < 0.01$.

RESULTS AND DISCUSSIONS

Drugs from natural sources have been proved useful since past time itself [1,2]. Many phytochemicals present in the plants imparted medicinal properties to plants [8]. The deteriorative side effects of allopathic medicine have greatly attracted people more towards medicinal plants. Therefore, study of medicinal plants, which was called as phytochemical studies became highly relevant. In the present study the leaves of *Terminalia chebula* were shade dried and extracted using different solvents in increasing order of polarity by ultrasonic assisted extraction and percentage yield for the solvents were calculated. Many studies have reported that selection of appropriate solvents and extraction techniques were essential [25]. In the current study almost all solvents produced good yield (6.03 ± 0.01 to $20.44 \pm 0.02\%$) (Table 1, Figure 1). However polar solvents produced better yield than non- polar solvents like hexane and chloroform. Percentage yield of Eth:Dw (Ethanol:Distilled water) extracts were much better ranging from 16.65 ± 0.01 to $20.44 \pm 0.02\%$.

Preliminary phytochemical screening was done according to the standard procedures. Results obtained were tabulated and represented as follows (Table 3). Hexane and chloroform extracts of leaves of *Terminalia chebula* mainly showed the presence of terpenoids, steroids and flavonoids. However, polar solvent extracts indicated the presence of alkaloids, phenols, flavonoids, tannins, coumarins and glycosides. All these phytochemicals such as simple phenols, flavonoids, tannins, coumarins and glycosides were categorized under phenolics [26]. According to many reports phenolic compounds exhibited many pharmacological actions especially, polyphenolic compounds such as tannins were of greater importance among them [26].

The genus *Terminalia* Linn belonging to family Combretaceae included many plant species with wide range of pharmacological activities [27]. For example, *Terminalia bellerica* were reported to have many biological activities due to the presence of several phytochemicals such as bellericanin, gallic acid, ellagic acid, ethyl gallate and lignans such as termilignan and thannilignan [28]. Similarly, *Terminalia arjuna* were reported to possess arjunin, arjunic acid, terminic acid, arjunolone, baicalein and luteolin, [29]. Several reports on the fruits of *Terminalia chebula* had also shown the presence of phytochemicals such as gallic acid, ellagic acid, chebulinic acid, chebulagic acid, terchebulin corilagin, punicalgin, flavonol, and chebulin [7,9,30] which imparted medicinal properties to the fruits. Hence the leaves of *Terminalia chebula* could also be considered medicinal due to the presence of different phytochemicals.

According to earlier studies tannins were the crucial compounds (about 32- 34%) which imparted medicinal value to the fruits of *Terminalia chebula* [9]. Therefore, total tannins in leaf extracts were also determined and the results were expressed as mg tannic acid equivalent /g of extract. Tannin content was

found to be greater in polar solvents. Non polar solvents like hexane and chloroform extracts produced negligible results. The results obtained showed that leaves of *Terminalia chebula* contained tannins ranging from 470.61 ± 0.01 to 698.13 ± 0.008 mg/g (Table 1, Figure 2). Hence similar to fruits, leaves of *Terminalia chebula* also possessed greater tannin content. Tannins such as condensed tannins and hydrolysable tannins were known to have a wide range of applications such as usage in leather industry, in wine processing unit, as adhesive and fungicide in wood industry, as an anticorrosive and also in 3D printing [31]. Since leaves of *Terminalia chebula* contained considerable amount of tannin content, we could infer that it has high economic value also.

Correlation analysis was done to determine the relationships between percentage yield of extracts obtained from ultrasonic assisted extraction and tannin content. Correlation coefficient was determined using Pearson's correlation test. According to the correlation analysis, tannin content and percentage yield of the extracts showed positive correlation of (0.687) with a level of significance of $p < 0.01$ (Table 2). The positive correlation indicated that as the percentage yield increased tannin content also increased.

Table 1: Percentage yield and tannin content in leaves of *Terminalia chebula*

Solvents	UAE Percentage yield (%)	Total tannin (mg/g)
Hexane	6.03 ± 0.01	Negligible
Chloroform	9.53 ± 0.02	Negligible
Ethyl acetate	9.05 ± 0.02	605.13 ± 0.003
Acetone	11.60 ± 0.1	626.73 ± 0.006
Ethanol	12.05 ± 0.01	671.56 ± 0.01
Ethanol:distilled water (40:10)	16.65 ± 0.01	656.59 ± 0.01
Ethanol:distilled water (30:20)	20.44 ± 0.02	668.22 ± 0.01
Ethanol:distilled water (20:30)	18.06 ± 0.01	698.13 ± 0.008
Ethanol:distilled water (10:40)	17.53 ± 0.01	638.32 ± 0.001
Distilled water	15.05 ± 0.02	470.61 ± 0.01

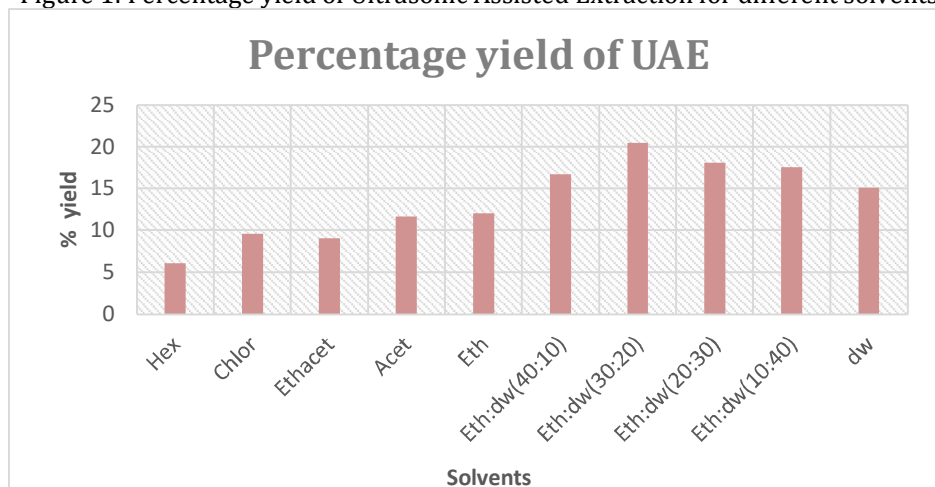
Values were represented as Mean $\pm$ Standard Error (n=3)

Table 2: Correlation analysis between percentage yield of UAE and tannin content

		UAE yield	Tannin
UAE yield	Pearson Correlation	1	.687**
	Sig.(2-tailed)		.000
	N		30
Tannin	Pearson Correlation	.687**	1
	Sig.(2tailed)	.000	
	N	30	

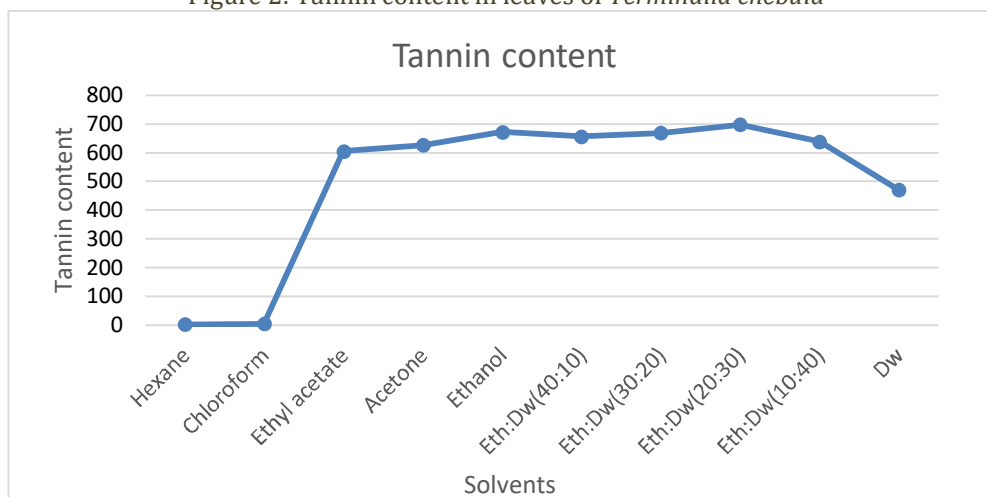
** Correlation is significant at 0.01 level (2 tailed)

Figure 1: Percentage yield of Ultrasonic Assisted Extraction for different solvents



Where 'Hex' stands for Hexane, 'Chlor' stands for Chloroform, 'Eth acet' stands for Ethyl acetate, 'Eth' stands for Ethanol, 'Eth:dw' stands for Ethanol:distilled water and 'dw' stands for Distilled water

Figure 2: Tannin content in leaves of *Terminalia chebula*



Where 'Eth:Dw' stands for Ethanol:Distilled water and 'Dw' stands for Distilled water

Table 3: Qualitative analysis of leaves of *Terminalia chebula*

Solvents	Phytochemicals						
	Alkaloids	Flavonoids	Phenols	Tannins	Coumarins	Terpenoids	Glycosides
Hexane	(-)	(-)	(-)	(-)	(-)	(+)	(-)
Chloroform	(-)	(+)	(-)	(-)	(-)	(+)	(-)
Ethyl acetate	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Acetone	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Ethanol	(-)	(+)	(+)	(+)	(+)	(-)	(+)
Eth:dw (40:10)	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Eth:dw (30:20)	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Eth:dw (20:30)	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Eth:dw (10:40)	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Dw	(-)	(+)	(+)	(+)	(+)	(-)	(-)

Where (+) stands for 'presence', (-) stands for 'absence', 'Eth:dw' stands for Ethanol: distilled water and 'Dw' stands for Distilled water.

CONCLUSIONS

Terminalia genus coming under the Combretaceae family were reported to be highly medicinal by scientific community. It was because of the presence of vast majority of phytochemicals such as phenols, alkaloids, flavonoids, terpenoids, tannins, glycosides and coumarins. These phytochemicals imparted antioxidant, antibacterial, anti-tumorigenic, anti-inflammatory, wound healing, antiviral, anti-mutagenic, anti-diarrhoeal and many more pharmacological potentialities to the plant. Studies on the fruits of *Terminalia chebula* also revealed the presence of many phytochemicals, especially tannins were the major components. The present work showed that similar to fruits, leaves of *Terminalia chebula* also contained greater tannin content. Since leaves of *Terminalia chebula* contained many of these phytochemicals we could also conclude that leaves of the plant *Terminalia chebula* may also be medicinally valuable.

Future Scope: A detailed phytochemical, pharmacological and molecular level studies on the leaves of *Terminalia chebula* should be done to establish whether the leaves of the plant could be replaced for the fruits in its medicinal activities.

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