Quantitative phytochemical analysis of traditionally used medicinal plant terminilia chebula

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Abstract

The present study was conducted for the photochemical analysis of *Terminilia chebula* plant extracts of leaves, fruits, seed, stem and roots. The formation of yellow colour indicated the presence of flavonoids while the brown colour formation indicated the presence alkaloids and terpenoids. The phenol content was maximum in roots (72.46mg/gdw) followed by seed, leaves, stem and fruits. The sugar content was highest in leaves (7.12mg/gdw) followed by fruits, stem, root and seed. The protein content was maximum in fruits (44.40mg/dgw) followed by seed, leaves, stem and root.

Keywords: Phenol, terpenoids, flavonoids, alkaloid, protein, secondary metabolites.

INTRODUCTION

Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates bioactive principles and lead compounds in synthetic drugs (Ncube., 2008; Parveen et al., 2010). One of the most important medicinal plants, which are widely used in the traditional system of medicine, is *Terminalia chebula* (Anithia et al., 2010). This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable and safe (Ramakrishina et al., 2011). Natural products play an important role in the field of new drugs research and development because of their low toxicity, easy availability and cost effective (Chessbrough, 2000). The primary metabolite like chlorophyll, amino acids, nucleotides, simple carbohydrates or membrane lipids, play recognized roles in photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation (Taiz and Zeiger., 2006).Secondary metabolites are synthesized by the plants as part of the defense system of the plant (Phan et al., 2001). The plant contains chebulinc acid, tannic acid, gallic acid, resin, anthroquinone and sennoside. It also contains glycosides, sugar, triterpenoids ,steroids and small quantity of phosphoric acid these compounds were proven to exhibit antibacterial, anti fungal, anti viral and anticarcinogenic (Neamsuvan et al., 2012; Prashith et al., 2010). The *Terminilia chebula* shows antioxidant, adaptogenic and anti-anaphylactic, hypolipidemic, hepato protective, cardio protective, anti-diabetic, wound healing, immuno- modulatory and chemo preventive (Chattopadhyay et al., 2009; Kim et al*.,* 2008; Sahu and Mahato 1994; Souza et al., 2010). *Terminalia chebula* is rich in tannin, which is hydrolysable to pyrogallal was found in fruits (Ghosh et al., 2008).

MATERIALS AND METHODS

Collection of plant

Herbal Plant *Terminalia chebula* was collected from the dense forest at Bengali, Karnataka state, India, based on its ethno medical importance.

Preparation of powder

The leaves, fruits, seed, stem and root powders were prepared by (Ncube et al., 2008) washing with distilled water, surface sterilized with 10% sodium hypochlorite solution, rinsed with sterile distilled water and air dried at room temperature under shadow and then milled to fine powder.

Extraction of plant material

Methanolic extract

The methanol extract was prepared by (Chessbrough, 2000) taking 10grams of powdered sample, were soaked in 50ml of methanol and it was kept in Soxhled apparatus at 80 degree Celsius for 48 hours. This extraction was taken and allowed for evaporation and it was concentrated with Dimethyl Sulfoxide (4.64g).

Phytochemical activity

The phytochemical analysis of Terminilia chebula was carried out by following Yankanchi and Koli (2010).

Test for alkaloids

The *Terminalia chebula* extracts of leaves, fruits, seed, stem and root were filtrated then treated with Potassium mercuric iodide (Dragendroffs reagent) and observed for the colour change in the test tubes.

Test for flavonoids

0.2 grams of plant extracts such as leaves, fruits, stem and root was added into test tube containing 2ml of diluted sodium hydroxide and mixed well. After mixing 2ml of diluted hydrochloride was added into the test tubes and observed for colour change.

Test for terpenoids

0.5grams of plant extracts such as leaves, fruits, seed, stem and root was added to test tubes containing 2ml of chloroform and content was mixed well. Then 2ml of concentrated sulphuric acid was added carefully and observe for presence of reddish brown colour.

Quantities assay for tannin

The quantities assay for tannin acids was carried out by following Phan et al. (2001). The 500mg finely dried plant extract was added into a glass beaker containing 5ml of 70% aqueous acetone. The content solution was uniformly mixed and gently boiled in a water bath for 30 minutes. The solution was centrifuged at 3000 rpm for 10 minutes at 4^0C and supernatants were collected and stored in freezing condition. The pallet were dissolved in 5ml of 70% aqueous acetone and recentrifuged at 3000rpm for 10 minutes at 4^0C. The supernatants were collected and mixed with freezing stored supernatants. To this supernatants 1 ml of Folin-denis reagent, 3ml of Sodium carbonate solution was added and solution was diluted to 20 ml by using distilled water. The solution was mixed well and incubated at room temperature for 30 minutes. The absorbance was measured in a spectrophotometer at 700nm.

Extraction of reducing sugar

Extraction of sugars from the sample was usually carried out by (Souza et al., 2010) using 80 per cent ethanol. The crushed material is refluxed in a Soxhlet's apparatus for about 30 minutes. After refluxing, alcohol was wiped out from the extract with the addition of distilled water. The extract was centrifuged at 3000 rpm .To the supernatant, 1 ml of saturated lead acetate solution and 1 ml of saturated Di-sodium hydrogen phosphate solution was added. After centrifugation, the clear supernatant was collected and made up to a known volume with distilled water. To make standard, 1ml aliquots are taken from the supernatant, respectively in hard-glass test tubes. The 5ml of Somogyi's reagent and distilled water are added so that final volume of the solution in each test tube comes to 15ml. The tubes covered with lids are heated in a boiling water bath for about 15 minutes. These are then cooled to room temperature. The tubes, in which the solutions have reprecipitated, are discarded. 1ml of 2.5 per cent potassium iodide solution was added to each test tube. Then 3ml of 1.5N Sulphuric acid was added to this and shaken well till the golden yellow colour is formed. A burette was filled with 0.005 N sodium thiosulphate solutions. It was titrated with the sample on addition of 1-2 drops of starch solution to the latter. The end point was determined by the complete disappearance of the colour from the solution. A blank was also prepared by mixing 5ml of Somogyi's reagent and 10ml of distilled water. It was heated in a boiling water bath for about 15 minutes. After cooled and 1 ml of 2.5 per cent potassium iodide solution and 3ml of 1.5 N sulphuric acid are added. Titration was done using 0.001 N sodium thiosulphate solution containing starch solution and colorless end-point was determined.

Identification of amino acid, phenolic and aromatic compounds by using TLC method

A sample of 500mg/ml concentration of plant extracts were prepared by following Kiyota (2006). From this solution, 4µl of the sample prepared was taken and spotted on the silica coated TLC plate. It was then kept at solvent position with the solvent to run under capillary pressure. Here ethanol, methanol and acetic acid (5ml, 5ml, and 100µl) were used as a solvent. The spots were then identified in the iodine chamber for phenolic compounds, ferric chloride for aromatic compounds and ninhydrin for amino acids.

Column chromatography

The crude aqueous methanol extract of *Terminalia chebula* was subjected to column chromatography

Figure 1. The formation of reddish brown colour in the plant extracts of *Terminilia chebula* when treated with Potassium Bismuth solution indicated the presence of Alkaloids. Note where L--Leaf, F--Fruit, Se--Seed, St--Stem, R--Root

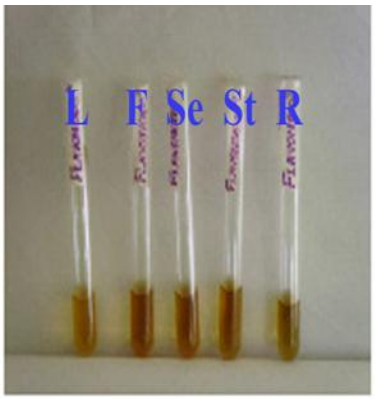

Figure 2. The formation of yellow colour in the plant extracts of *Terminilia chebula* when treated with 5% Sodium Hydroxide solution containing diluted Hydrochloride solution thus indicated the presenc of Flavoniods. Note where L--Leaf, F--Fruit, Se--Seed, St--Seed, R--Root

(Parveen et al., 2010) over silica gel 100-200 mesh. The column was eluted with solvents of increasing order of polarity. The fractions were collected in 10ml each and allowed to evaporate to get the residue. Each fraction was tested for the presence of various constituents by Thin Layer Chromatography.

RESULT

Terminalia chebula

Kingdom-Plantae, Subkingdom-Tracheobionta, Super division-Spermatophyta, Division-Magnoliophyta, Class-Magnoliopsida, Subclass-Rosidae, Order-Myrtales, Family-Combretaceae, Genus-Terminalia, Species-*Terminalia chebula*

Phytochemical activity:

Alkaloid

There was a reddish brown colour formation in the test tubes after treating with Potassium Bismuth iodide solution thus indicates the presence of alkaloid in the plant extract (Figure 1).

Flavonids

The yellow colour was formed in the test tubes after treating with 5% Sodium hydroxide and diluted hydrochloride acid thus indicated the presence of flavonoids (Figure 2).

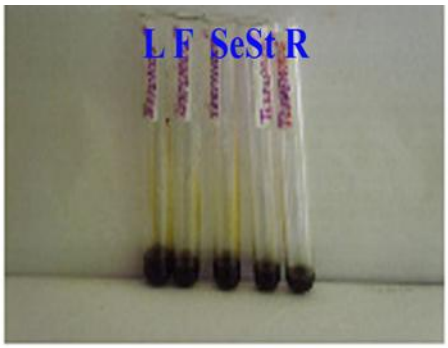

Figure 3. The formation of reddish brown colour in the plant extracts of *Terminilia chebula* when treated with chloroform containing concertrated Sulphuric acid solutin thus indicated the presence of Terpenoids. Note where L--Leaf, F--Fruit, Se--Seed, St--Stem, R--Root

Table 1. Purification of methanol extracts compounds of *Terminalia chebula* by using column chromatography

No of fractions	Eluent	Ratio	Colours of fraction	Rf value
F1-F2	Acetone	100	Brown	1.52
F3-F5	Acetone, Ethanol	75:25, 50:50, 25:75	Dark Brown	1.6
F6-F7	Acetone, Ethanol	75:25, 50:50, 25:75	Dark Brown	1.25, 1.18
F8-F13	Ethanol	100	Light Brown	1.51
F14-F18	Ethanol, Methanol	75:25, 50:50, 25:75	Colourless	-
F19-F40	Methanol	100	Colourless	-

Terpenoid

The Terpenoid was present in the plant extract as there was reddish brown colour formation in the test tubes after treating with chloroform and concentrated Sulphuric acid (Figure 3).

Quantity of Tannic acids

The phenol content was found maximum in root (85.36mg/gdw) followed by seed (78.30mg/gdw), stem (72.46mg/gdw), roots (65.30mg/gdw) and leaves (21.39mg/gdw).

Reducing sugar

The maximum content of sugar was in leaves (7.12mg/gdw) followed by fruit (5.70mg/gdw), stem (4.80mg/gdw), root (4.10mg/gdw) and seed (3.7mg/gdw).

Thin layer Chromatography

In thin layer chromatography the amino acids showed pink colour spots were observed after treating with ninhydrin. The sugars showed the purple and black spots after treating with ferric chloride. The phenolic compounds showed blue spots after treating with iodine solution

Column chromatography

Higher content of protein was observed in fruits of *T. Chebula* (44.40mg/gdw) followed by seed (42.10mg/gdw), roots (40.60mg/gdw), leaves (36.10mg/gdw) and stem (29.40mg/gdw). The Purification of compounds in various extracts was performed by using column chromatography was presented in (Table 1).

DISCUSION

Terminalia chebula is called the 'king of medicine' in Tibet and is always listed at the top of the listed in Ayurvedic medicine (Kannan al., 2009). A diverse arsenal of new antibacterial agents is urgently needed to combat the diminishing efficacy of existing antibiotics (Ahmad et al., 1989). In earlier studies, lipids were reported to be higher in leaves (46.0mg/gdw) of *M. oleifera* (Souza et al., 2010) and the roots (39.0mg/gdw) of *C. quadrangularis* (Shariff

et al., 2006). In modern therapeutic treatments, nanoparticles and liposomes are being used to develop delivery systems that are convenient and effective for tackling problems in disease treatments (Phan et al., 2001). Proteins are complex nitrogenous organic substances that are one of the most important plant products to man. A part from this, the protein hydrolytes from various sources are reported to possess antioxidant activity (Luziatelli et al., 2011; Shah et al., 2006). Plant phenols are groups of natural products with variable structure that are well known for their beneficial effects on health possess significant antimicrobial and antioxidant activities (Prashith et al., 2010; Sahu and Mahato 1994). *Terminalia chebula* have been noted to possess shikimic acid, gallic acid, B –sitosterol, tannic acid, triethyl eser of chebulic acid, ethylester of gallic acid and ellagic ethaedioic acid (Ates and Erdourul, 2003). *Terminalia* plant contains several constituents like tannins, flavonoids, sterols, amino acids, fructose, resin, and fixed oils. It is also found to contain compounds like anthraquinones, 4, 2, 4 chebulyl-dglucopyranose, terpinenes and terpinenols (Archana *et al.,* 2010). Primary metabolite also acts as precursors for bioactive compounds used as therapeutic drugs (Neamsuvan et al., 2012; Prashith et al., 2010). Phytochemical from medicinal plants serve as lead compounds in drug discovery and design (Ebi and Ofoefuse., 2000). Aellagitanninterchebulin along with punicalagin, terflavin-A, shikimic, tricontanoic, palmitic acids, beta-sitosterol, daucosterol, triterpene chebupentol were found in fruits (Kim et al., 2006). The compounds phloroglucinol and pyrogallol, isolated along with ferulic, vanillic, p-coumaric and caffeic acids constitutes for the antioxidant activity of the plant (Mazumder et al., 2003). The aqueous extract of *Terminalia chebula* was tested for its potential antioxidant activity by means of its ability to inhibit gamma-radiation-induced lipid peroxidation in the tested rat liver microsomes and the damage to superoxide dismutase enzyme in rat liver mitochondria (Ashish et al., 2010).

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