Research Article

Phytochemical Screening and *In vitro* Free Radical Scavenging of *Terminalia chebula*

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ABSTRACT

The aim of the study is to analyze the phytochemicals and in vitro free radical scavenging of *Terminalia chebula*. Phytochemical screening and *in vitro* free radical scavenging of *Terminalia chebula* to be explored for the treatment of various diseases. *Terminalia chebula* is a native plant in India and Southeast Asia. It has potential healing benefits and traditionally used for the treatment of various diseases such as renal failure and diabetes mellitus. *Terminalia chebula* is considered to be a useful herbal medicine with natural origin, highly safe to be used and lesser or no side effects. The present study targets the potentiallity of *Terminalia chebula* as a traditional medicine that can be used to treat various diseases and ailments. Phytochemical analysis and *in vitro* free radical scavenging were done done on *Terminalia chebula*.

Keywords: *Terminalia chebula*, plant medicine, extract.

INTRODUCTION

Medicinal plants with therapeutic properties are considered as important sources for the treatment of various diseases in human beings. The World Health Organization (WHO) stated that plant extracts or their active components are acquired by approximately 80% of the whole world through traditional medicines.[1] Plants are used in traditional herbal medicines, which are being acknowledged in today's society as an important source of health due to their wide range of benefits including low cost of production, higher safety margins, non-toxic property and minimal risk of side effects.[2,3] Plants are the basic foundation of traditional medication, which is involved in the production of drugs that are used by human beings today.[4,5]

It was proven that herbal and medicinal plants such as *Terminalia chebula* (T. chebula) have been used by both western and eastern countries for about 60,000 years.[6] Terminal chebula, also called as the "King of Medicine" in Tibet is one of the valuable medicinal plants that has a powerful ability of healing and it is ranked the highest in the list of "Ayurverdic Meteria Medica" due to this property. The entire parts of the plant have medicinal uses due to the presence of various phytochemicals in different parts of the plant.[7] The components of T. chebula are widely used to produce new drugs that are very active in overcoming numerous diseases. The use of T. chebula in curing various diseases is getting a lot of attention in modern researches due to its numerous advantages.

Terminalia chebula belonging to the family Combretaceae contain phytoconstituents, which allow it to carry out a wide range of activities including antifungal, antibacterial, antiviral, antidiabetic, antimutagenic, antocarcinogenic[8], hypocholectrolemic[9], antioxidant, radio-protective[10], antipasmodic and antiulcer. *Terminalia chebula* helps in preventing cardiac injury and kidney diseases. It is also known to be an active consitutent of Triphala, which is a herbal medicine used in curing painful eyes, stomach problems and enlarged liver.

Terminalia chebula can be largely found in India, Burma and Sri Lanka. The dried ripe fruit of the plant called black myrobalan has a great healing property, in which it can be used in the treatment of various diseases such as asthma, gout, heart and bladder diseases, diabetes mellitus[11], sore throat, diarrhea and vomiting.[12] Antioxidant and free radical scavenging activities are found in black myrobalan.[13] It is activated in the presence of cancer cells and helicobactor pyroli bacteria.[14,15] The fruit is used as an anticaries agent and also used in wound healing, enhancing gastrointestinal movement and anaphylactic shock, which is an extreme allergic response.[16-19]

In wound healing treatment, the leaves and fruits of T. chebula reduce the epithelization rate and increase contraction rate during skin healing.[20,21] The fruit also contains a large amount of phenolic compounds and flavonol glycosides and other phytochemical compounds, which contribute to its healing and therapeutic properties.[22] T. chebula plays an important role as an ayuverdic traditional medicine that is capable of treating a lot of diseases due to the presence of various secondary metabolites, which contribute to the healing and pharmacological properties of the plant used in ayuverda, siddha, unani and homeopathy medicines.[23]

MATERIALS AND METHODS

Preparation of *Terminalia chebula* Extract[24]

Terminalia chebula (T. Chebula) extract was prepared by conventional organic solvent extraction. 50g of dried and

powdered plant material of T. chebula was added to 500ml of methanol and left overnight. The extract was then filtered using Whatman No. 1 filter paper.

The T. chebula extract was then used for the experiment after evaporating the solvent under vacuum using a rotatory evaporator. The residues were dissolved in DMSO and stored at 4°C.

Phytochemical Test

The phytochemical tests was carried out as described by Harborne et al (1998).[25]

Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for glycosides

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for cardiac glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

Test for Terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for phenols

To 1ml of the extract, a few drops of Phenol Ciocalteau reagent was added followed by few drops of 15% Sodium carbonate solution. Formation of blue or green color indicates presence of phenols.

Test for coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Steroids and phytosteroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Phlobatannins

To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of Phlobatannins.

Anthraquinones

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of Anthraquinones.

Estimation of Total Phenolic Content

Total phenolic content of extracts was assessed according to the Folin–Ciocalteau method (Slinkard & Singleton, 1977) with some modifications. Briefly, Different concentrations of extracts (200, 400 and 600 µg), made to 2 ml with distilled water and 1 ml of Folin–Ciocalteau's reagent were seeded in a tube, and then 1 ml of 100 g/l sodium carbonate was added.

The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. A calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration curve and the total phenolic content of extracts was expressed as mg of catechol equivalents per gram of extract.

Antioxidant Assay

DPPH Assay

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams with slight modifications.

1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of extract solution of varying concentrations (200, 400 and 600µg). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control.

The decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-V is spectrophotometer. The inhibition % was calculated using the following formula.

$$\%\ of\ Inhibition = \frac{(A\ of\ control - A\ of\ Test)}{A\ of\ control} \times 10$$

Nitric Oxide Radical Inhibition Assay

Sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide; it interacts with oxygen to produce nitrite ions, which can be estimated by the use of GriessIllosvoy reaction (Garrat, 1964). In the present investigation, GriessIllosvoy reagent was modified using naphthylethylenediaminedihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentration of extract (200–600 µg) or standard solution (0.5 ml) were incubated at 25 °C for 150 min.

After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylenediaminedihydrochloride (1%) was added, mixed and allowed to stand for 30 min. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Ascorbic acid was used as positive control. The scavenging activity was calculated using the formula.

$$\%\ of\ Inhibition = \frac{(A\ of\ control - A\ of\ Test)}{A\ of\ control} \times 100$$

Hydrogen Peroxide Scavenging Assay

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer (8500 II, Bio- Crom GmbH, Zurich, Switzerland). Extracts (200–600 µg) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after ten minute against a blank solution containing in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of extract and standard was calculated using the following equation:

$$\%\ of\ Inhibition = \frac{(A\ of\ control - A\ of\ Test)}{A\ of\ control} \times 100$$

RESULTS AND DISCUSSION

Phytochemical Test

Based on the phytochemical test, it was concluded that the T. chebula extract contains carbohydrates, tannins, quinones, cardiac glycosides, terpenoids and coumarins. Flavonoids and phenols are weakly present in the extract. Saponins, alkaloids, glycosides, phlobatannins and anthraquinones are absent in the extract. From the test for steroids and phytosteroids, only steroids are present in the extract while phytosteroids are absent.

Table 1: Phytochemical Tests of T. chebula

S. No	Phytochemical Tests	*Terminalia chebula*
1	Carbohydrates test	+
2	Tannins test	+
3	Saponins test	-
4	Flavonoids test	Weakly +
5	Alkaloid test	-
6	Quinones test	+
7	Glycosides test	-
8	Cardiac glycosides test	+
9	Terpenoids test	+
10	Phenols test	Weakly +
11	Coumarins test	+
12	Steroids &Phytosteroids	Steroids
13	Phlobatannins test	-
14	Anthraquinones test	-

+ Present; - Absent

Table 2: Total Phenolic Content of *Terminalia chebula*

Concentration (µg)	Terminalia	Catechol	Phenol Content of Terminalia
200	0.0112	1.844	1.214750542
400	0.0207	3.565	2.322580645
600	0.0421	5.552	4.549711816

Table 3: DPPH Scavenging Assay

Concentration (µg)	Control	% of Inhibition			
		Terminalia	% of Inhibition	Ascorbic Acid	% of Inhibition
200	1.068	0.621	41.853933	0.53	50.374532
400	1.068	0.424	60.299625	0.31	70.973783
600	1.068	0.225	78.932584	0.13	87.827715

Table 4: Nitric Oxide Inhibition Assay

Concentration (µg)	Control	Terminalia	Ascorbic acid	Terminalia	% of Ascorbic acid
200	0.784	0.598	0.405	23.7244898	48.34183673
400	0.784	0.399	0.246	49.10714286	68.62244898
600	0.784	0.261	0.118	66.70918367	84.94897959

Table 5: Hydrogen Peroxide Scavenging Assay

Concentration (µg)	Control	Terminalia	Ascorbic acid	%Terminalia	% of Ascorbic acid
200	0.582	0.385	0.405	33.84879725	30.41237113
400	0.582	0.274	0.246	52.9209622	57.73195876
600	0.582	0.203	0.118	65.12027491	79.72508591

Estimation of Total Phenolic Content

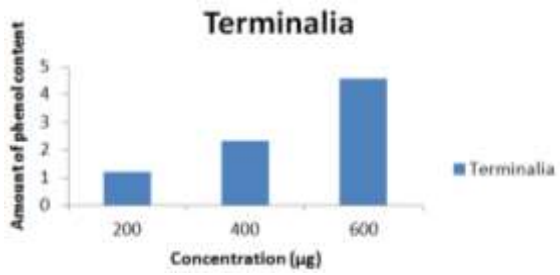

Figure 1: Total phenolic content of T. chebula against its concentration

The total phenolic content of the extract was estimated as described by Folin-Ciocalteu method. From the results the total phenolic content of T. chebula gradually increased corresponding to the concentration of the extract. The phenolic content of T. chebula extract is 1.21µg at a concentration of 200µg and reaches a value of 4.55µg at a concentration of 600µg of the extract.

Antioxidant Assay

DPPH Scavenging Assay[26]

DPPH assay confirmed the presence of free-radical scavenging activity of the extract. Phenolic compounds act as metal chelators that scavenge the oxidation of metal compounds such as calcium and potassium obtained in minute amounts from our diets, which stimulate the release of free radicals.[29] 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is used to evaluate the ability of the plant extracts to act as potent antioxidants.

Discoloration of DPPH radical was observed as the concentration of the extract increased. At a concentration of 200µg, the extract showed an inhibition of 41.85%. The percentage inhibition was the maximum (78.93%) for the concentration of 600µg as compared to the standard ascorbic acid.

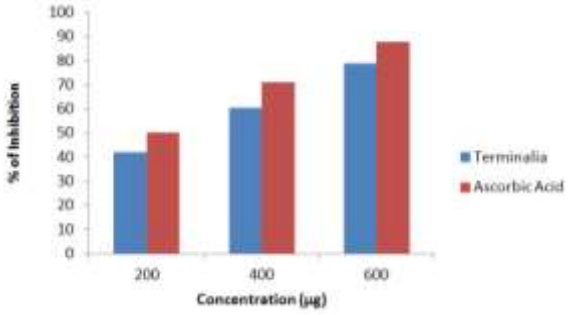

Figure 2: % of inhibition by T. chebula against its concentration

Nitric Oxide Inhibition Assay[27]

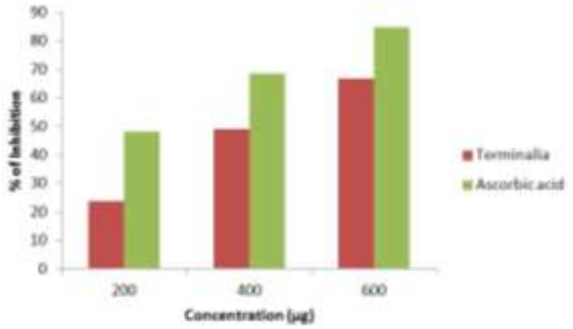

Figure 3: % of inhibition by T. chebula against its concentration

Nitric oxide inhibition assay confirmed the presence of nitric oxide scavenging activity of T. chebula extract. Nitric oxide produced spontaneously from the incubation of sodium nitroprusside in aqueous solution such as phosphate buffer saline at physiological pH reacts with oxygen to produce nitrite ions, which are reduced by the T. chebula extract due to the presence of antioxidant properties in the extract.

The antioxidant components directly compete with oxygen to react with nitric oxide, which prevents the production of nitrite.

The extract reached the maximum percentage of inhibition (66.71%) at a concentration of 600µg. The nitric oxide scavenging activity of the extract was examined from its capability to inhibit nitrite production by directly competing with oxygen and oxides of nitrogen.[30]

From the experiment, it is concluded that the antioxidant activity of T. chebula can be due to the presence of phenolic components in the extract.

Hydrogen Peroxide Scavenging Assay[28] Hydrogen peroxide scavenging assay confirmed the antioxidant activity of T. chebula extract. 200µg of T. chebula showed 33%inhibition. As the concentration of the extract increased there was a simultaneous increase in the radicle scavenging activity of the extract. 65% scavenging activity was exhibited by 600µg of the extract.

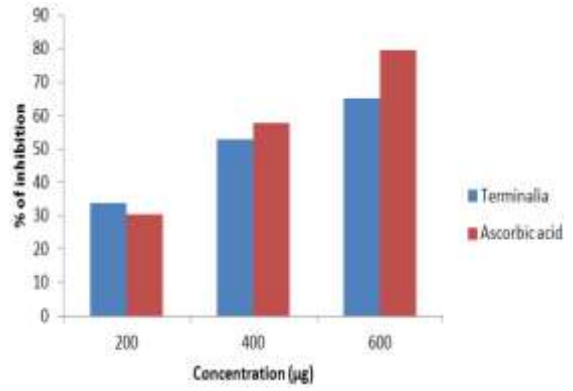

Figure 4: % of inhibition by T. chebula against its concentration

CONCLUSION

In today's world, synthetic compounds are widely used in various industries such as in the food and drugs industry. Use of synthetic compounds leads to the production of free radicals.

They are usually generated by normal cell metabolisms or external sources such as medication, pollution and radiation.

High concentration of free radicals may lead to a phenomenon called oxidative stress.[31] Free radicals react with biological molecules and alter the structure of cells, resulting in various free-radical induced diseases such as cancer, cataract and cardiovascular diseases.[32]

The harmful effects of free radicals can be overcome by the presence of natural antioxidants.

A balance between antioxidants and free radicals is important to maintain physiological functions.[33]

Herbal products protects the body from oxidative stress due to the presence of numerous phytochemicals that exist in the form of polyphenols.[34]

In this study the antioxidant activity of T. chebula was proved. Now a days properties of herbal products are explored for it to be used in drug formulations.

In future T.chebula can also be used for the treatment of various diseases such as cancer, cataract and arthritis.

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