

Antioxidant Activity of Leaves of *Terminalia Bellirica* (Combretace)

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Abstract

Terminalia bellirica is one of the very popularly used plant in traditional medicine for the treatment of various diseases. Fruits of *T. bellirica* is having several applications as medicine and used in various household activities, however leaves remains relative less explored part. Therefore during this study solvent extract of *T. bellirica* was studied for the presence of phytochemicals and antioxidant activity. *T. bellirica* exhibited the occurrence of oil and fats, glycosides, protein and amino acids, carbohydrates, tannins, phenolic compounds, alkaloids and flavonoids as major photochemical groups. Extract also exhibited high dose dependent antioxidant activity in DPPH radical scavenging assay (IC₅₀: 62.51 µg/mL), reducing power assay (OD value of 1.07±0.18 at 1000 µg/ml), hydrogen peroxide scavenging assay (IC₅₀: 183.64µg/ml) and total antioxidant assay (OD value of 1.167±0.13 at 1000 µg/ml). Quantitative polyphenolic analysis of methanol extract of *T. bellirica* displayed the availability of high amount of phenolic (171.08 mg GAE/g plant extract) and flavonoids (47.66mg QE/g plant extract) content. Above findings emphasizes on the high phytochemicals mediated antioxidant potential of *T. bellirica* and emphasizes on its possible use in medicine.

Keywords: *Terminalia bellirica*; Antioxidant activity; Phytochemical analysis

Introduction

Free radicals are the atoms or group of atoms with one or multiple unpaired electrons in its outermost orbit. Free radicals are commonly classified in to two broad groups, Reactive oxygen and Nitrogen species (ROS and RNS respectively). Free radicals are

produced in the cells as the byproducts of metabolism and their accumulation can cause Aging, Cardiac problems, Cancer, arthritis, diabetes and neurodegenerative disorders. In nonliving system, presence of free radicals can cause the corrosion of plastics, diminishing in paints, and deprivation of work of art [1]. Free radicals are known for both beneficial and harmful effects in the cell, however much emphasis is given to the harmful effects of free radicals. They are capable of initiating and/or increasing the diseases. As of now around 100 different type of diseases are known to be associated with free radicals [2].

Antioxidants are the molecules with the potential of neutralize free radicals; thus protect the cell from free radical mediated oxidative stress. Medicinal plants represent a very popular source of antioxidant compounds, as they are reported to synthesize several phytoconstituents with antioxidant potential. Plants synthesized these phytochemicals to protect themselves from oxidative stress caused by sunlight, oxygen and other stress factors. These phytochemical are distributed throughout the plant tissues such as, leaves, fruits, stem, roots, and flowers etc., however their distribution may differ. The parts of plants exposed most to the stress factors contains maximum amount of antioxidant in it. Plant phenolics, flavonoids, tannins, carotenoids, and minerals are the principle antioxidant compounds present in plants [3].

The genus *Terminalia* is the second largest genus in the family *Combretaceae*. *Terminalia bellirica* is commonly known as Bahera and possess several uses in traditional healthcare system. *T. bellirica* fruits are traditionally used as purgative and antipyretic material/ it is also used for the treatment of diarrhoea, heartburn, nausea, respiratory problems, worm infections and allergic problems [4]. In recent years various parts of *T. bellirica* have been scientifically evaluated and reported to exhibit analgesic, antimicrobial, antidiarrhoeal, wound healing, antihypertensive, antioxidant, antiulcer, hepatoprotective and antimutagenic activities [5]. It's important to mention here that although all the parts of this plants been widely studied however studies on leaves is relatively limited, therefore the main focus of this research work is to estimate the phytochemical composition and antioxidant potential of methanol extract of *T. bellirica*.

Material and Method**Plant material collection**

T. bellirica leaves were harvested from the plant growing in Jaipur, Rajasthan, India, during January 2014.

Processing of plant sample

The leaves of *T. bellirica* were properly washed in distilled water to remove any surface impurities. The leaves were then oven dried at 50°C. Dried leaves were consistently ground to make uniform powder. Ten gram powder of *T. bellirica* leaf was mixed with methanol (10% w/v) in a conical flask. Further the flask was sealed and kept in an orbital shaker at a speed of 120rpm for a period of 24 hour at room temperature. Later resulting blend was filtered dried and transferred in an air tight receptacle and stored in refrigerator.

Phytochemical screening

Preliminary phytochemical analysis of *T. bellirica* leaves was performed by using pre reported methods [6].

Antioxidant activity

Antioxidant activity of *T. bellirica* leaves was carried out by using DPPH radical scavenging, reducing power, hydrogen peroxide scavenging and total antioxidant activity [7-10]. Antioxidant activity was evaluated at multiple concentrations and each experiment was executed in three replicates.

Estimation of polyphenolic compounds

Total phenolic and flavonoid content of *T. bellirica* leaf was determined using the Folin–Ciocalteu and aluminum chloride method respectively [11]. Each experiment was conducted at multiple concentrations in triplicates.

Statistical analysis

The results of antioxidant activities and polyphenolic content of methanolic extract of *T. bellirica* leaves are represented in the form of mean \pm standard deviation (n=3).

Calculations were performed by using Microsoft Excel 2013 while the graphs were plotted by using GraphPad Prism 5 software.

Result and Discussion

Medicinal plants exemplifies a very important, viable and diverse source of products that can be used for the treatment of various diseases. Before the development of modern medicine system, medicinal plants served as a very important source of medicine. Even today a large number of people around the world are dependent on plants for curing various diseases. Adoring to WHO about 80% of global population depends on medicinal plants for the medicine in direct or indirect form. A large number of medicinal compounds available in market were actually derived from medicinal plants. Other than potential medicinal properties, drugs prepared from plants are having great acceptability among people. Keeping these things in context *T. bellirica* leaves were selected and studied. The discoveries of the research are as following:

Extract yield

Dried powder of leaves of *T. bellirica* (10 gm) was extracted by cold extraction process with methanol. After drying, 10 gm of *T. bellirica* powder yielded 0.53gm of extract which is approximately 5.3% of the dried plant powder.

Preliminary phytochemical analysis

Phytochemical compounds are ubiquitously distributed in all parts of plants. They are non-nutritional metabolites synthesized by plants in self-defense for sunlight and other stress factors. These phytochemicals are very useful for humans prospective, as they possess several pharmaceutical properties and are being used as medicine. In this study, methanol extract of *T. bellirica* exhibited the occurrence of oil and fats, glycosides, protein and amino acids, carbohydrates, tannins, phenolic compounds, alkaloids and flavonoids as major photochemical groups (Table 1). Results of the phytochemical analysis are in coherence with earlier study where ethanol extract of *T. bellirica* leaves revealed the availability of saponins, tannin, amino acids, proteins, alkaloids, flavonoids and glycosides as major phytochemical groups [12].

Table 1: Phytochemical analysis of *T. bellirica* leaves

Phytochemicals tested	Methods used	Results
Tannins	Ferric chloride method	+ + +
Phenolics	Ferric chloride method	+ + + +
Oil & fats	Spot method	+
Alkaloids	Wagner’s reagent	+ + +
Flavanoids	Aluminum chloride test	+
Saponins	Foam method	-
Carbohydrate	Molish’s method	+ + +
	Benedict’s method	+ + +
Glycosides	Brontrager’s method	+
Protein and amino acids	Ninhydrin method	+

Here, +: present and -: Absent

Antioxidant activity

Medicinal plants are a very potential source of antioxidant compounds. Phytochemicals such as phenolic compounds, flavonoids, vitamins, pigments are known to possess antioxidant properties. A potential antioxidant can effectively protect the cell from oxidative damage by neutralizing the free radicals. Thus help the plant to withstand in stress conditions. Similarly the use of herbal antioxidants in animal cell systems can shield the cellular biomolecules from getting damaged by oxidative stress and other toxic material which are synthesized in the cell. *T. bellirica* leaves exhibited very high antioxidant potential in various *in vitro* methods including DPPH radical scavenging assay (IC50: 62.51 µg/mL), reducing power assay (OD value of 1.07±0.18 at 1000 µg/ml), hydrogen peroxide scavenging assay (IC50: 183.64µg/ml) and total antioxidant assay (OD value of 1.167±0.13 at 1000 µg/ml) (Figure 1). In previous studies, methanol extract of *T. bellirica* leaves was described to show significant antioxidant activity *in vitro* and *in vivo*. During the study, antioxidant potential of the plant extract was going up with increase in doses of the extract [13].

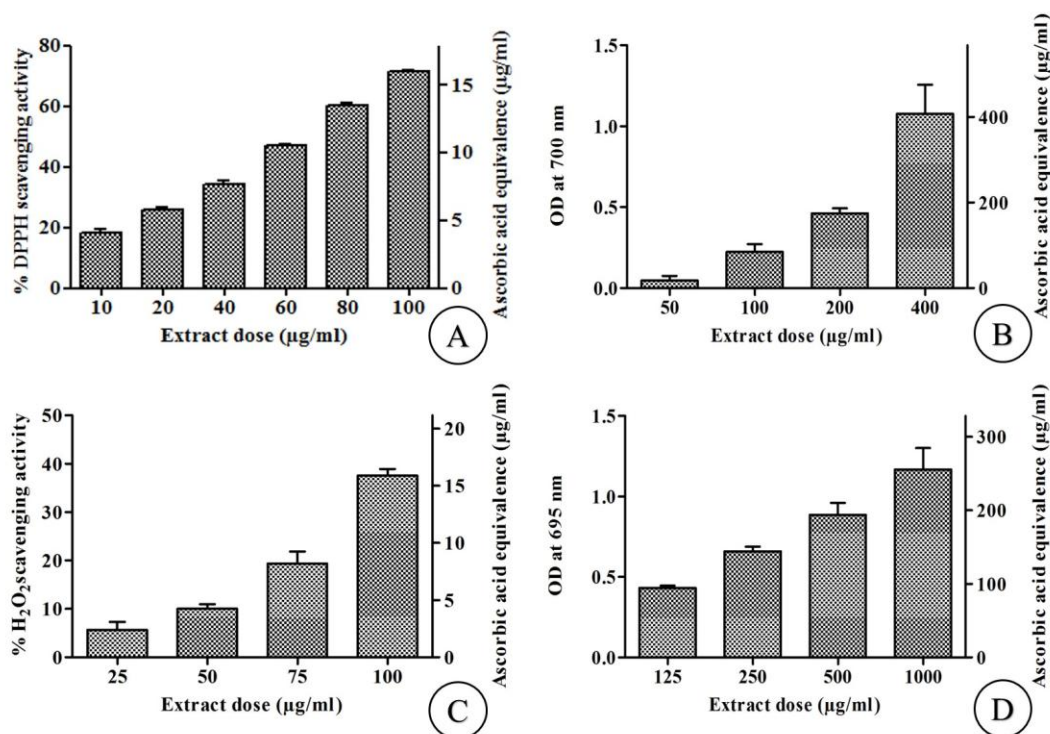


Figure 1: Antioxidant activity of *T. bellirica* in comparison to ascorbic acid. Here, A: DPPH radical scavenging assay, B: Reducing power potential, C: Hydrogen peroxide scavenging activity and D: Total antioxidant activity.

Estimation of polyphenolic compounds

Plant derived antioxidants are usually polyphenolic class of compounds. These compounds possess several medicinal properties and are extensively use in pharmaceutical, food and cosmetic industries as lead molecules, preservatives or natural additives. Polyphenols are among most common groups of phytochemical compounds present in the plant and vegetables, as of now about 8000 chemically different polyphenols have been identified and being explored for various medicinal properties. Phenolic compounds and flavonoids are the most important type of polyphenols and in past, many phenolic and flavonoid compounds have been reported to possess high antioxidant activity [14-15]. In the current study, methanol extract of *T. bellirica* leaves showed the presence of high amount of phenolic (171.08 mg GAE/g extract) and flavonoids (47.66mg QE/g extract) content (Figure 2). Earlier also researchers have documented the total phenolic content and total flavonoid content of *T. bellirica* leaf

extracts [13], however it is important to estimate it because the geographical location and the extraction procedures changes in the composition of these compounds.

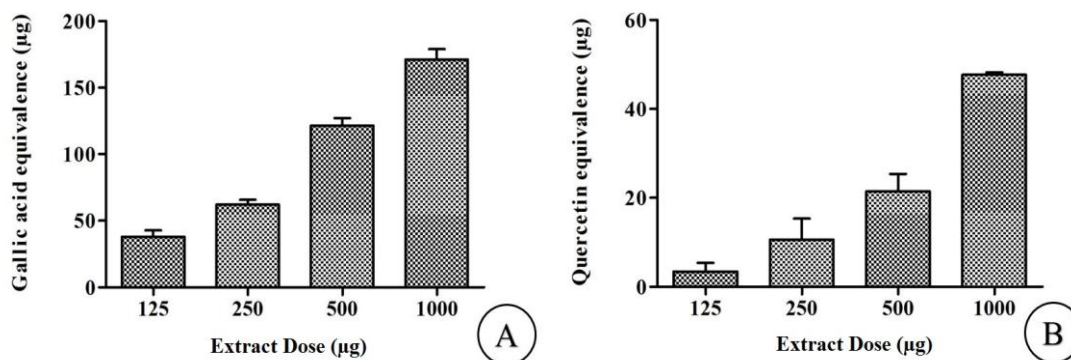


Figure 2: Polyphenolic content of *T. bellirica* leaves. Here, A: Total Phenolic content, and B: Total flavonoid content

Conclusion

In the presented study, *T. bellirica* leaves was screened for the antioxidant potential by various *in vitro* methods. The finding of this study emphasizes on the strong antioxidant potential of *T. bellirica* and provides valuable information about the pharmacological potential of this plant. Further the *T. bellirica* displayed the presence of high amount of polyphenolic compounds, which are perhaps responsible group of phytochemical present in this plant for the antioxidant potential.

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