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1, 1-diphenyl, 2-picryl hydrazyl radical scavenging activity of aqueous extract of *Terminalia belerica* bark

AKHLESH SINGHAL[1]*, GOVIND NAYAK[1], ASHOK BUDHWANI[2] and AHSISH SINGHAI[3]

[1]Lakshmi Narain College of Pharmacy, Bhopal (India).
[2]Daksh Institute of Pharmacy, Chhatarpur (India).
[3]VNS Institute of Pharmacy, Bhopal (India).

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ABSTRACT

In the present investigation we had attempted to investigate the in vitro antioxidant potential of aqueous extract of Terminalia Billerica bark (TBB). The 1, 1-Diphenyl, 2-picryl hydrazyl assay method had been performed at different doses (100-500 mg). The total phenolic and total flavonoids contents had also been determined. The results of the present study shows that the aqueous extract of TBB possess antioxidant activity through the DPPH free radical scavenging activity. The preliminary phytochemical investigations indicates the presence of flavonoids and polyphones. The results are found to be significant then compared with the standard Ascorbic acid. Further studies are required to determine the mechanism and isolation of active constituents involved in the antioxidant activity.

Key words: Terminalia Billerica; DPPH; flavonoids, phenolic contents.

INTRODUCTION

Free radicals had been implicated in several human diseases e.g. atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, ageing, inflammatory response syndrome, respiratory diseases, liver diseases, cancer and AIDS[1,2]. Many herbal plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite[3]. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity[4]. Therefore, many researchers are in search for antioxidants of natural origin.

Terminalia belerica is a large deciduous tree with characteristic bark traditionally called as Baheda or Bibitaki. It is grown throughout India. Its principal constituents are Gallic acid, ellagic acid, β-sitosterol, chebulagic acid[5]. It is astringent, tonic, expectorant and laxative. Its pulp is used in dropsy, piles and diarrhea. It is also useful in leprosy, fever and hair care. It is also used in oxalic acid and preparation of ink. It is used in case of rheumatism. Seed oil is applied in skin disease and premature graying of hair[6]. In the present investigation we have attempted to investigate the antioxidant potential of the barks ot *Terminalia belerica*.

MATERIAL AND METHODS

Plant Material and Extraction

The barks of Terminalia Billerica were

collected from Bhopal, cut into small pieces, dried in shade for 5 days, then grinded. This material was macerated in water for 72 hours with occasional shaking in dark. Macerate was decanted, filtered and the marc was pressed. The macerates were concentrated to give aqueous extract [22.13%w/w].

Chemicals

Various chemicals used were DPPH (1, 21-dphyenyl-2-picryl-hydrazyl) (Sigma Chemicals, USA) and aluminum chloride. Ascorbic acid obtained from Sisco Research Laboratories Pvt. Ltd. India. Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All chemicals and solvents were of analytical grade.

Preliminary phytochemical investigations

The preliminary phytochemical screening of the extract was carried out to know the different constituents present in aqueous fractions of Terminalia Billerica as per the standard procedures. The extract were tested for alkaloids[7,8], sterols[9], triterpenes[10], saponins[11], flavonoids[12], tannins[8,13], carbohydrates[8], glycosides and amino acids[8], Shinoda test and thin layer chromatography was also carried out to confirm the presence of flavonoids[12].

DPPH free radical scavenging method

Free radical scavenging potentials of *Terminalia belerica* was tested against a ethanolic solution of DPPH (13). 0.1 mM solution of DPPH in ethanol was prepared and 0.1 mL of this solution was added to 3.0 mL of different concentration (100-500 µg/mL) of *Terminalia belerica* bark (TBB) prepare in water. It was incubated at room temperature for 30 minutes and the absorbance was measured at 517 nm against the corresponding balnk solution using Shimadzu-1600 UV-spectrophotometer. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contained DPPH and distilled water without any extract using the following equation:

$$\% \text{ Scavenging Activity} + [(AC - As / AC] \times 100.$$

Where, Ac is the absorbance of the control reaction and As is the absorbance in the presence of the sample of the extracts.

The antioxidant activity of the extract was expressed as IC50, defined as the concentration (in µg/mL) of extracts that inhibits the formation of DPPH radicals by 50%. The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. (Table 1).

Determination of total phenolics

Total phenolic contents (C) in the extracts were determined by the modified Folin-Ciocalteu method (14,15). An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed top stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using Shimadzu-1600 UV-spectrophotometer. Samples of extract were evaluated at different concentration. Total phenolic content was expressed as mg/g Gallic acid equivalent. The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. (Table-2).

$$C = c. V/m$$

Where, C = Total phenolic content mg/g plant extract; c = conc. of gallic acid established via calibration curve; V = Volume of extract/ m = Wt. of plant extract (gm).

Determination of total flavonoids

Total flavonoids contents (TFC) were determined using the method of Ordon ez dt al., (16) of sample solution. A volume of 1.5 ml of 2% $AlCl_3$ ethanol solution was added to 1.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Total flavonoid content was expressed as mg/g quercetin equivalent. The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. (Table-2).

$$TFC = (Abs. X Dilution factor X 100)/E1\% X Wt. of extract (gm).$$

RESULTS AND DISCUSSION

Preliminary phytochemical investigation of Terminalia Billerica bark showed the presence of carbohydrates, tannins and flavonoids. Shinoda test and thin layer chromatography for flavonoids using mobile phase n-butanol: water: glacial acetic acid (40:50:10) and spraying reagent as ferric chloride solution again confirmed its presence radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (19). The absorbance maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPh radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, resulting in scavenging of the radical by hydrogen donati.

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the in-vitro antioxidant activity of crude plant extracts[17,18] is stable free on[20]. Although the DPPH radical scavenging abilities of the extracts were lower than those of ascorbic acid, it was evident that the extracts did show the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The IC_{50} values were found to be 353 µg/mL and 132 µg/mL for Aqueous Terminalia Billerica (Aq. TBB) and ascorbic acid respectively. The extract has shown the activity in dose dependent manner. (Fig. 1).

Table 1: DPPH Free radical scavenging activity of aqueous extract of Terminalia Billerica bark (TBB)

S. No.	Conc. (µgm/ml)	% Scavenging	
		Ascorbic Acid	Aq. TBB
1.	100	48.82	25.46 ± 0.122
2	200	58.79	35.29± 0.160
3	300	68.46	43.87 ± 0.101
4	400	82.28	54.76 ± 0.056
5	500	92.23	62.51 ± 0.060

(n= 3, X ± SEM).

Tbale 2: Total Phenolic and total flavonoid content in Terminalia Billerica Bark (TBB)

S. No.	Sample	Aqueous TBB (Aq. TBB)
1.	Total phenolic content [a]	0.1226 ± 0.77
2	Total flavonoid content [b]	2.244 ± 0.02

(n = 3, X ± SEM).

[a]Expressed as mg Gallic acid/g of dry plant material.

[b]expressed as mg querctin/g of dry plant material.

Polyphenols are the major plant compounds with antioxidant activity. This activity is

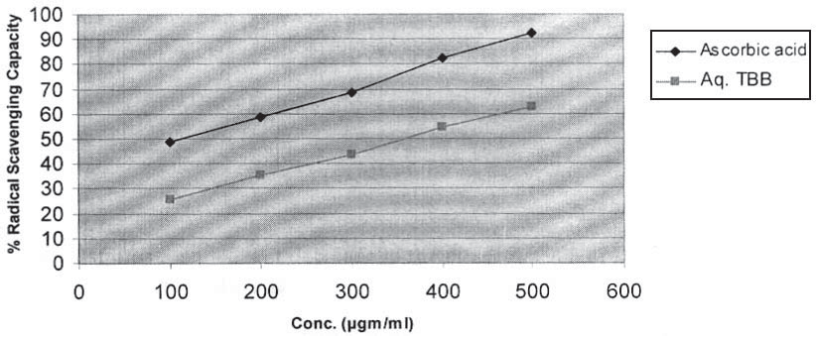

Fig. 1: Illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of both *Terminalia belerica* and ascorbic acid

believed to be mainly due to their rebox properties[21], which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The results from this study strongly suggest that phenolics are important components of these plants, and some of their pharmacological effects could be attributed to the presence of these valuable humorous constituents. The total phenolic content in mg/g gallic acid equivalent (GAE) was found to be 0.1226 in aqueous extract of TBB. The total flavonoid in mg/g querceting equivalent was found to be 2.244 in aqueous extract of TBB.

CONCLUSION

The results of ht present study shows that the aqueous extract of the barks of *Terminalia belerica* posses antioxidant activity through the DPPh free radical scavenging activity. The preliminary phytochemical investigation indicates the present of flavonoids in *Terminalia belerica* bark. Polyphenols like flavonoids and tannins are the well known natural antioxidants. So, the antioxidant potential of *Terminalia belerica* may be due to the presence of flavonoids and phenolic contents. Although in most cases, the biological activities of the extracts from the barks of *Terminalia belerica* are not as high as those of the standard compounds use in this study; the present results indicate clearly that the aqueous extract of *Terminalia belerica*bark possess antioxidant prosperities and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This study has to some extent validated the medicinal potential of the *Terminalia belerica* fruit.

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