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NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *TERMINALIA BELERICA* BARK

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ABSTRACT

In the present investigation, am attempt has been made to investigate the *in vitro* antioxidant potential of aqueous extract of *Terminalia belerica* bark (TBB). The nitric oxide assay method has been performed at different doses (100-500 mg). The total phenolic contents and total flavonoid contents have 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 investigation indicates the presence of flavonoids and polyphenols. The results are found to be significant when 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 belerica*, DPPH, Flavonoid, 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[1-4]. 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[5, 6]. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity[7]. Therefore, many researchers are in search for antioxidants of natural origin.

Terminalia belerica is a large deciduous tree with characteristic bark traditionally

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called as Baheda or Bibitaki. It is grown throughout India. Its principal constituents are gallic acid, ellagic acid, β-sitosterol and chebulagic acid[8]. It is astringent, tonic, expectorant and laxative. Its pulp is used in dropsy, piles and diaorrhea. 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 diseases and premature graying of hair[9, 10]. In the present investigation, we have attempted to investigate the antioxidant potential of the barks of *Terminalia belerica*.

EXPERIMENTAL

Material and methods

Plant material and extraction

The barks of *Terminalia belerica* were collected from Bhopal, cut into small pieces and dried in shade for 5 days. The dried barks were then grinded. This material was macerated in water for 72 hours with occasional shaking in dark. Macerate was decanted and filtered. The marc was pressed and filtration was done 2-3 times. The macerates were concentrated to give aqueous extract (22.13% w/w).

Chemicals

Various chemicals used were DPPH (1, 1-diphenyl-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 investigation

The preliminary phytochemical screening of the extract was carried out to know the different constituents present in aqueous fractions of *Terminalia belerica* as per the standard procedures. The extract were tested for alkaloids[11, 12], sterols[13], triterpenes[14], saponins[15], flavonoids[16], tannins[12,17], carbohydrates[12], glycosides and amino acids[12]. Shinoda test and thin layer chromatography was also carried out to confirm the presence of flavonoids[16].

Nitric oxide scavenging method

Sodium nitroprusside (final concentration 5 mM) in phosphate buffer saline was incubated with 0.5 mL of different concentrations of drug at 25^{o}C for 5 hours. Control experiment was done without test compounds, but with equivalent amounts of buffer in an

identical manner. After 5 hours of incubation, Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthyl ethylene diamine dihydrochloride) was added to 0.5 mL of the incubated solution and absorbance was immediately measured at 546 nm using the JASCO V530 UV-VIS spectrophotometer. Percentage inhibition of nitric oxide free radical was calculated by using the following equation:

$$\% \text{ Inhibition} = \{1 - (\text{Abs. of sample} / \text{Abs. of blank})\} \times 100 \quad ...(1)$$

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates[18] (Table 1).

Table 1: Nitric oxide (NO) scavenging activity of aqueous extract of *terminalia belerica* bark (tbb)

S. No.	Conc. (µg/mL)	% Scavenging	
		Ascorbic acid	Aq. TBB
1.	100	48.82	29.52 ± 0.079
2.	200	53.64	31.17 ± 0.112
3.	300	58.98	38.86 ± 0.115
4.	400	82.28	58.65 ± 0.021
5.	500	92.23	65.10 ± 0.047
(n = 3, X ± SEM)			

Determination of total phenolics

Total phenolic contents (C) in the extracts were determined by the modified Folin-Ciocalteu method[18, 19]. 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 to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the JASCO V530 UV-VIS spectrophotometer. Samples of extract were evaluated at different concentrations. Total phenolic contents 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).

$$C = c.V/m \quad ...(2)$$

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 (g)

Table 2: Total phenolic and total flavanoid contents in *terminalia belerica* bark (tbb)

S. No.	Sample	Aqueous TBB (Aq. TBB)
1.	Total phenolic content [a]	0.1226 ± 0.77
2.	Total flavanoid 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 quercetin/g of dry plant material

Determination of total flavonoids

Total flavonoid contents (TFC) were determined using the method of Ordon et al.[20], 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).

$$TFC = (Abs. \times Dilution\ factor \times 100)/ E^{1\%} \times Wt.\ of\ extract\ (g) \quad ...(3)$$

RESULTS AND DISCUSSION

Preliminary photochemical investigations of *Terminalia belerica* 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 use of spraying reagent ferric chloride solution confirmed its presence.

Nitric oxide exhibits numerous physiological properties and it is also implicated in several pathological states. The interaction of nitric oxide with other radicals leads to the formation of more hazardous radical such as peroxy nitrile anion and hydroxyl radical. The absorption maximum of a stable NO radical in methanol was at 546 nm. The IC_{50} values were found to be 363 µg/mL and 144 µg/mL for aqueous *Terminalia belerica* (Aq.TBB) and ascorbic acid, respectively. The extract had shown the activity in dose dependent manner (Fig. 2).

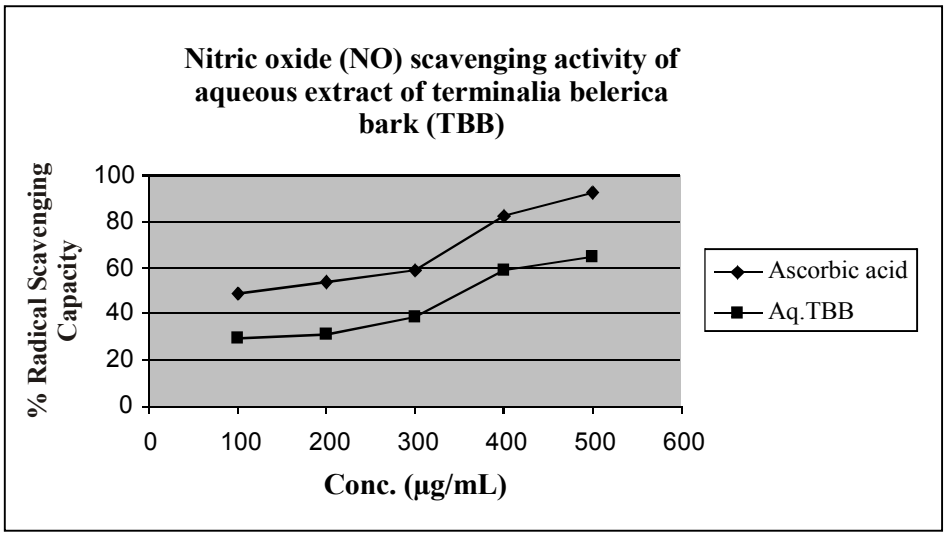

Fig. 1: A significant decrease in the concentration of NO radical due to the scavenging ability of both *Terminalia belerica* and ascorbic acid

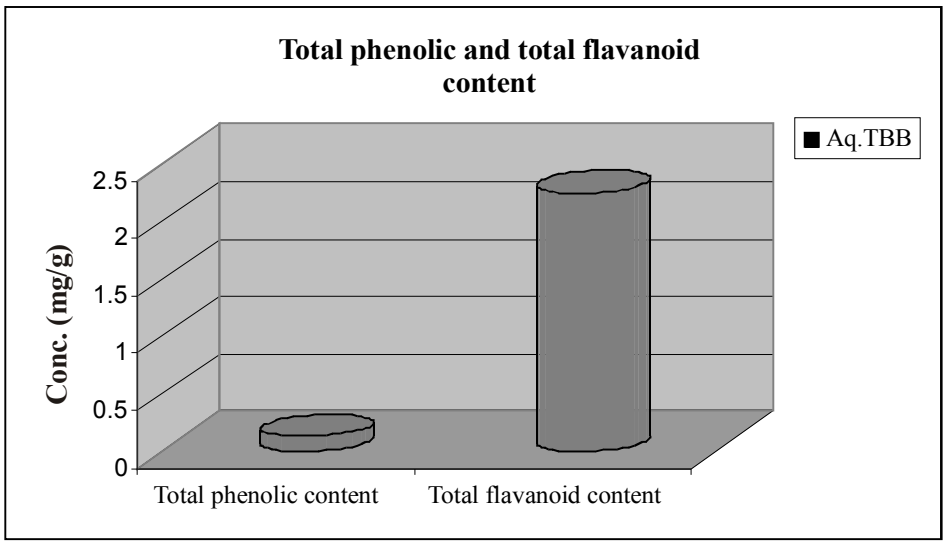

Fig. 2: Total phenolic and flavanoid contents of *Terminalia belerica* bark

Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties[21], which play an important role in adsorbing 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 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 flavanoid content in mg/g quercetin quivalent was found to be 2.244 in aqueous extract of TBB (Fig. 2).

CONCLUSION

The results of the present study shows that the aqueous extract of the barks of *Terminalia belerica* possess antioxidant activity (based on the DPPH free radical scavenging activity). The preliminary phytochemical investigation indicates the presence 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 used in this study; the present results indicate clearly that the aqueous extract of *Terminalia belerica* bark possess antioxidant properties 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*.

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