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PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIOXIDANT STUDIES OF *TERMINALIA BELLIRICA* HYDROALCOHOLIC EXTRACT

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ABSTRACT

The aim of this research is to screen phytochemicals, estimate the phenolic and flavonoid compound content and determine the antioxidant capacity of the extract of *Terminalia bellirica* leaves. The well-known test protocol available in the literature was used to determine the qualitative analysis of different phytochemical constituents and the quantitative analysis of total phenols and flavonoids. In various in vitro models, namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assays, the hydro alcoholic extract of Terminalia bellirica leaves was studied for antioxidant activity. In the tested models, the extract exhibited dose-dependent free radical scavenging properties. For the DPPH method, the *Terminalia bellirica* extract showed an IC50 value of 58.29µg/ml, comparable to that of ascorbic acid (IC50=14.33µg/ml). The present study describes *Terminalia bellirica*'s phytochemical profile and antioxidant activity, which will be used for further medicinal applications.

Keywords: *Terminalia bellirica*, Phytochemical test, Flavonoids content, Antioxidant activity.

INTRODUCTION

Exogenous chemicals or endogenous metabolic processes in the human body are formed by free radicals or highly reactive oxygen species. These are capable of oxidising nucleic acids, proteins, lipids and DNA bio-molecules and can trigger various degenerative diseases such as neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis, etc[1,2]. Antioxidants are the compounds that stop free radicals from attacking and thus reduce the risk of these disorders [3]**.** Almost all organisms, with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds, are protected to some

extent by free radical damage. Ascorbic acid, tocopherol, phenolic acids, glutathione, polyphenols and flavonoids. Prior and CaO_4 have reported that they protect antioxidant supplements or dietary antioxidants from the harmful effects of free radicals. Much attention has now been paid to the use of natural antioxidants to protect the human body, particularly brain tissue, from oxidative damage caused by free radicals. Several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacolacolgy in the last two decades[5]. Keeping this in view, the present study has been conducted to evaluate the antioxidant activity of *Terminalia bellirica* which are traditionally well known for their various activities.

MATERIALS AND METHODS

Material and method

Plant material

The leaves of *Terminalia bellirica* were collected from Bhimbetka Bhojpur, Bhopal (M.P.). Plant material leaves selected for the study were thoroughly washed under running tap water and then rinsed in distilled water; at room temperature, they were allowed to dry for some time. The plant material was then shade-dried for about 3 to 4 weeks without any contamination. The electronic grinder was used to grind dried plant material. The colour, odour, taste and texture of the powdered plant material were observed. The dried plant material was packed and stored for phytochemical and biological studies in air-tight containers.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Extraction by maceration process

100gm of dried plant material were exhaustively extracted with Hydroalcoholic solvent (methanol: water: 80:20) using maceration method. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening of the extract

The extract of *Terminalia bellirica* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, proteins, amino acids and flavonoids[6].

Total flavonoids determination

The total flavonoid content was determined using $AlCl_3$ method[7]. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

DPPH free radical scavenging assay

DPPH scavenging activity was measured by the spectrophotometer with slightly modification of method[8]. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

RESULTS AND DISCUSSION

The percentage yields of Pet ether and hydroalcoholic extract obtained from *Terminalia bellirica* are depicted in the Table 1. Preliminary phytochemical studies of the extract were done according to the published standard methods. Phytochemical analysis revealed the presence flavonoids Table 2. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.022X + 0.005$, $R^2=0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. TFC of hydroalcoholic extract of *Terminalia bellirica* showed the content values of 0.741mg/100mg quercetin equivalent. DPPH radical scavenging assay measured hydrogen donating nature of extracts[9]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *Terminalia bellirica* hydroalcoholic bark extract was found to be 58.29µg/ml as compared to that of ascorbic acid (14.33µg/ml).

Table 1 % Yield of barks of *Terminalia bellirica*

S. No.	Solvents	% Yield
1.	Hydroalcoholic	7.54

Table 2 Phytochemical screening of extract of *Terminalia bellirica*

S. No.	Constituents	Hydroalcoholic extract of barks of *Terminalia bellirica*
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	-Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	-Ve
4.	Saponins	
	A) Froth Test:	+Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins and Amino Acids	
	A) Xanthoproteic Test:	-Ve
7.	Carbohydrate	
	A) Fehling's Test:	-Ve
8.	Diterpenes	
	A) Copper acetate Test:	-Ve

Table 3 Total total flavonoid content of *Terminalia bellirica* extract

S. No.	Extracts	Total flavonoids content (mg/ 100 mg of dried extract)
1.	**Hydroalcoholic**	0.741

Table 4 % Inhibition of ascorbic acid and *Terminalia bellirica* hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	41.25	30.21
2	20	53.32	42.12
3	40	68.78	48.45
4	60	72.23	52.23
5	80	78.45	55.65
6	100	83.32	59.98
	IC 50	14.33	58.29

CONCLUSION

In this study, the multiple antioxidant activity of the extract shown clearly indicates the potential application value of the two plants. However, prior to its possible application as an antioxidant ingredient, either in animal feed or in human health foods, the in vivo safety of both plants needs to be thoroughly investigated in experimental rodent models. The above results showed that the hydroalcoholic extract of *Terminalia bellirica* leaves could exhibit antioxidant properties. Potential natural antioxidants may be provided by further studies on the use of the above plants for their antioxidant role in different systems.

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