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Research Article

ASSESSMENT OF ANTIOXIDANT POTENTIAL AND ANTIGENOTOXICITY ANALYSIS THROUGH HYDROGEN PEROXIDE-INDUCED OXIDATIVE STRESS IN *TERMINALIA PANICULATA* ROTH

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ABSTRACT

Objective: The present study is mainly focused on the antigenotoxicity and antioxidant potential of the fruit extract of an important dye yielding plant *Terminalia paniculata* Roth.

Methods: Genoprotective activity of the fruit extract was studied by *Allium cepa* root tip assay. Three modes of treatment were used to perform the antigenotoxicity, that is, pre-treatment, post-treatment, and simultaneous treatment. For the study of antioxidant potential, four different assays were performed.

Results: Antigenotoxicity studies revealed the protective role of the extract in chromosomal aberrations induced by 2% hydrogen peroxide (H_2O_2) on *A. cepa* root tip meristem. The fruit extract showed a significant modulatory effect by means of an inhibition percentage and also it showed a characteristic reversing of chromosomal aberrations induced by H_2O_2. While in the case of antioxidant activity, the plant extract showed an appreciable antioxidant potential. Four different assays were used to determine the antioxidant potential of *T. paniculata*. Of these 2,2-diphenyl-1-picryl-hydrazyl-hydrate radical scavenging activity revealed almost equal effect to that of the standard.

Conclusions: In the present investigation, it has been found that the important dye yielding plant *T. paniculata* has a significant role in various commercial industries such as food, cosmetics, clothes, and pharmaceutics due to its efficient protective role.

Keywords: Genotoxicity, Oxidative stress, *Terminalia paniculata*, Antioxidant potential.

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INTRODUCTION

Natural products have been of immense importance to humans from ancient times. The main sources among them are the plants, without them the mankind is worthless. *Terminalia paniculata*, an arborescent tree which seems to be used as timber and shelter by our ancestors, is gaining attention toward pharmaceutical products nowadays.

Natural dyes have a massive utility in textile, drug, and cosmetic industries as a coloring agent. It is widely used in foodstuff coloring, also due to their negligible toxicity. Hence instead of synthetic colorants, natural ones can promote a perfect and healthy life. Earlier studies on the induction of giant cells [1] and apoptotic activity and DNA damage as well as metabolic inactivation [2] had proved the cytotoxicity of some common synthetic food dyes. *T. paniculata* is an important dye yielding plant belonging to Combretaceae. Its fruits are used as the major source of the dye.

Biological systems possess destructive free radicals as a result of metabolic processes. Antioxidant activity can determine the ability of a drug to scavenge these free radicals. Oxidation is a significant process in living organisms for the indispensable performing of catabolism. However, oxygen centered free radicals and several reactive oxygen species were produced inside the body may cause severe diseases such as atherosclerosis, diabetes, cancer, and cirrhosis [3]. Environmental pollutants such as automobile exhaust, radiation, air pollution, cigarette smoke, and pesticides can play a key role to generate free radicals [4]. Normally, there is a balance between the quantity of free radicals generated in the body and the antioxidant defense systems, which protect the body from the deleterious effects by scavenging these destructive free radicals [5].

Hydrogen peroxide (H_2O_2) is one of the severe mutagens and it has a great effect on the DNA directly, causing DNA strand breakage and base modification. It can modulate the transcription and suppress genomic repair pathways [6]. H_2O_2 has the ability to generate reactive oxygen species on cells and it may lead to oxidative-stress-mediated cytotoxicity by various chemical reactions. For this reason, it may cause severe oxidative damage to membrane lipids, DNA molecules, and proteins [7,8].

Dyes obtained from the natural products have great significance both commercially as well as aesthetically. Hence, the main objective of the present study was to evaluate the antigenotoxicity and antioxidant activities of the methanolic extract of the fruits of *T. paniculata.*

METHODS

Antigenotoxicity

Three different methods are used to determine the antigenotoxicity of the fruit extract of *T. paniculata*, that is, pre-treatment, post-treatment, and simultaneous treatment [9]. In pre-treatment, at first, the roots were treated with different concentrations (0.1, 0.05, 0.01, and 0.005%) of the fruit extract for 24 h followed by H_2O_2 (2%) treatment for 1h. In post-treatment, roots were first treated in 2% H_2O_2 (1 h) and were followed by 24 h treatment with different concentrations of the fruit extract. In the case of simultaneous treatment, the roots were treated simultaneously with H_2O_2 (2%) and fruit extract of different concentrations for 1 h. H_2O_2 (2%) and distilled water were taken as positive and negative control, respectively. Then, the treated root tips were washed thoroughly with distilled water and immediately fixed in modified Carnoy's fluid (1 acetic acid:2 alcohol) for 1 h. After washing in distilled water, it was hydrolyzed in 1N HCl for 5–10 min to separate the cells during squashing and stained with 2% acetocarmine for 4 h. After staining, the root tips were destained with 45% acetic acid, squashed, and mounted on clean micro slides.

Mounted slides were observed for aberrations under a microscope (Leica DM 2000 LED) and photographs were taken.

$$\text{Abnormality percentage} = \frac{\text{Number of abnormal cells}}{\text{Total number of cells}} \times 100$$

$$\text{Inhibitory activity } (\%) = \frac{A - B}{A - C} \times 100$$

Where, A = number of aberrant cells induced by positive control, B = number of aberrant cells induced by different modes of treatment, and C = number of aberrant cells induced by negative control.

Statistical analyses

Statistical analyses were carried out using SPSS version 10. Data obtained were then subjected to Duncan's multiple range test and one-way analysis of variance to authenticate the variability of the data and accuracy of results. Mean±SE and differences between the corresponding controls and various treatments of the results are expressed and were considered statistically significant at $p<0.05$. The linear relationship between dose and the effect of aqueous extract in terms of inhibition percentage was obtained by simple regression and correlation analysis.

Antioxidant activity

Four different assays were used to detect the antioxidant activity of the methanolic extract of the fruit extract of *T. paniculata*, that is, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, nitric oxide scavenging activity, and superoxide free radical scavenging activity.

DPPH radical scavenging activity

The radical scavenging activity of different extracts was determined using DPPH assay according to Chang *et al*. [10]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10 mg/ml dimethyl sulfoxide [DMSO]) was used as a reference.

Different concentrations of sample such as 12.5 µg/mL–200 µg/mL from a stock concentration (10 mg/mL) were made up to a final volume of 20 µl with DMSO and 1.48 ml DPPH (0.1 mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture was incubated in dark at room temperature for 20 min. The absorbance of the mixture was read at 517 nm. 3 ml of DPPH was taken as control.

Hydroxyl radical scavenging activity

Different concentration of sample such as 125–2000 µg/mL from a stock solution (10 mg/mL) was mixed with 500 µl reaction mixture (2 deoxy, 2 ribose (2.8 mM), $FeCl_3$ (100 µm), ethylenediaminetetraacetic acid (EDTA) (100 µm), H_2O_2 (1.0 mM), and ascorbic acid (100 µm) in KH_2PO_4 – KOH buffer (20 mM pH 7.4) was made up to a final volume of 1 ml. A control without the test compound, but an equivalent amount of distilled water was taken. After incubation for 1 h at 37°C, add 1 ml of 2.8% trichloroacetic acid, then 1 ml 1% aqueous thiobarbituric acid was added, and the mixture was incubated at 90°C for 15 min to produce the color. After cooling, the absorbance was read at 532 nm [11].

Nitric oxide scavenging activity

Sodium nitroprusside (5 mmol/L) was mixed with different concentrations of sample such as 125 µg/mL–2000 µg/mL and incubated at 25°C for 30 min. A control without the test compound, but an equivalent amount of distilled water was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrate with sulfanilamide and subsequent coupling with N-1 naphthyl ethylenediamine dihydrochloride was measured at 546 nm [12].

Superoxide free radical scavenging activity

Different concentration of sample from 125 to 2000 µg/ml, 0.05 ml of riboflavin solution (0.12 mM), 0.2 ml of EDTA solution (0.1M), and 0.1 ml nitroblue tetrazolium solution (1.5 mM) was mixed in test tube and reaction mixture was diluted up to 2.64 ml with phosphate buffer (0.067M). A control without the test compound, but an equivalent amount of distilled water was taken. The absorbance was read at 560 nm after illumination for 5 min incubation in fluorescent light and also measured after 30 min at 560 nm [13].

In all four different antioxidant assays, the percentage of inhibition was calculated using the following formula.

$$\text{Percentage of inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

RESULTS

Antigenotoxicity

The present is study mainly focused on the protective effect of *T. paniculata* fruit extract on H_2O_2-induced oxidative damage. H_2O_2 is an important exogenous oxidant and it can induce oxidative stress on the *Allium cepa* root tip cells. Furthermore, it may cause a wide range of aberrations. However, the present study investigated the potential activity of extract to modulate the intensity of aberrations due to the exogenous oxidant. Oxygen centered free radicals and several reactive oxygen species produced inside the body may cause severe diseases, so the studies regarding the oxidative damages and its cure are very much informative and effective. The modulatory activity of *T. paniculata* on *A. cepa* treated with H_2O_2 was clearly mentioned in Table 1. The result revealed that there is a dose-dependent decrease of chromosomal aberrations. The H_2O_2-treated root tips showed a variety of chromosomal aberrations, particularly nuclear lesions. Cytoplasmic shrinkage, nuclear disintegration, bizarre nucleus, etc., were also found. The H_2O_2 root tips treated when supplemented with plant extract can cause a drastic change back to the normalcy (Fig. 1). It meant that the plant extract has an effective correlation on the inhibitory activity at different levels of concentrations. All three treatments (pre, post, and simultaneous) showed an almost equal effect on the various concentrations. In pre-treatment, the percentage

Table 1: Modulatory activity of *T. paniculata* on *A. cepa* root tip meristem treated with 2% H_2O_2. Means with the same superscript letter do not differ statistically at the level of 0.05 ($p<0.05$; DMRT test)

Concentration	Chromosomal abnormal percentage±SE	Percentage of inhibition±SE
Negative control	5.69±0.05[a,b]	0000[a]
Positive control	95.13±0.33[a]	0000[a]
Pre-treatment (%)		
0.005	60.0000±3.65923[a]	74.5±0.5[a]
0.01	54.1333±2.17128[a]	77.5±0.32[b]
0.05	52.43333±1.08985[b]	75.7±0.39[a]
0.1	46.6333±1.44491[b]	78.5±0.26[b]
Post-treatment		
0.005	61.7000±1.37961[a]	70.7±0.46[a]
0.01	57.2000±1.50997[a]	75.4±0.72[a]
0.05	52.1667±1.67564[b]	77.7±0.45[b]
0.1	47.3000±1.05987[a,b]	78.3±0.26[d]
Simultaneous treatment		
0.005	64.6667±1.07445[a]	61.23±0.14[a]
0.01	59.6333±1.63333[a]	72.73±0.24[b]
0.05	54.9333±1.98522[a]	76.5±0.25[c]
0.1	48.4333±0.88380[a,b]	79.2±0.8[b]

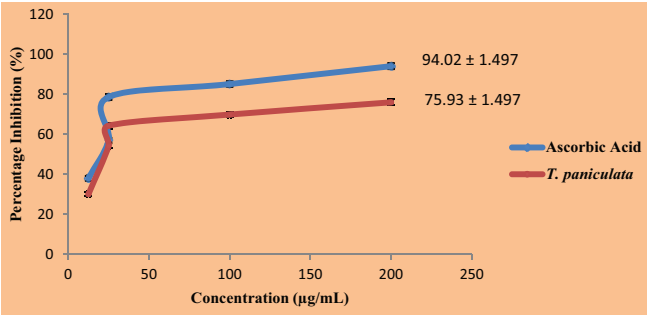
T. paniculata: Terminalia paniculata, A. cepa: Allium cepa, H_2O_2: Hydrogen peroxide, DMRT: Duncan's multiple range test

of inhibition ranged from 74% to 79%, similarly in post and simultaneous treatments, it was from 70% to 79% and 61% to 80%, respectively. The linear relationship between percentage inhibition and concentrations (0.005%, 0.01%, 0.05%, and 0.1%) of aqueous extract of *T. paniculata* was obtained by regression and correlation analyses (Fig. 2).

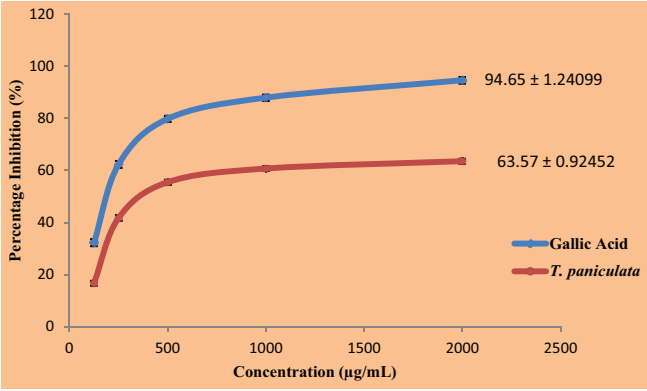
Antioxidant activity

Various metabolic actions in the body will generate multiple destructive free radicals. The present study revealed that the fruit extract of *T. paniculata* has an appreciable ability to scavenge these free radicals. All the four different antioxidant assays gave significant results about the scavenging effect of the plant. Dose-dependent scavenging activity was observed in all assays. In DPPH radical scavenging assay, the IC_{50} was obtained as 23.068 µg/mL, which was almost similar to that of ascorbic acid (20.399 µg/mL**)**. In the case of hydroxyl radical scavenging activity, the maximum effect was found at the highest concentration, that is, 63.57±0.92. Its IC_{50} value was 419.64 µg/mL. Here, gallic acid was used as the standard with IC_{50} value, 199.23 µg/mL. For nitric oxide scavenging assay, the concentrations tested were 150, 250, 500, 1000, and 2000 µg/mL. Here also dose-dependent scavenging activity was observed. A similar condition was noted in the superoxide free radical scavenging activity. That means *T. paniculata* showed significant antioxidant potential.

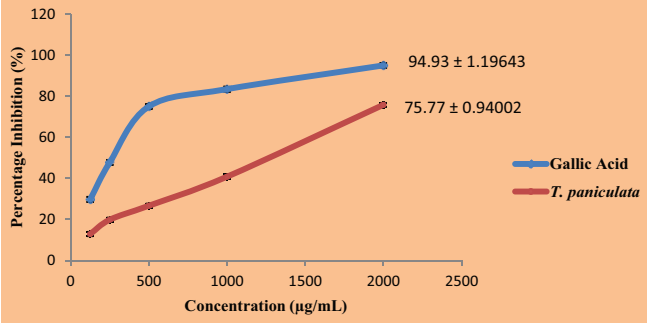
DPPH free radical scavenging assay



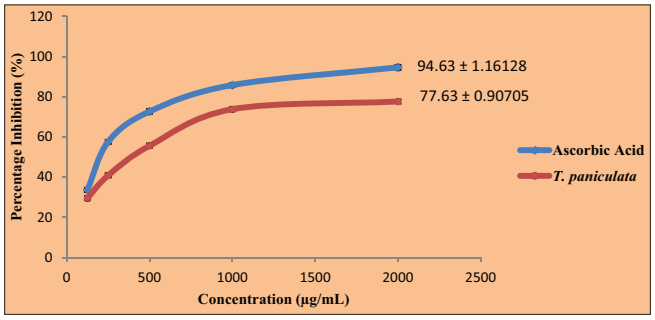
Hydroxyl radical scavenging activity



Nitric oxide scavenging activity



Superoxide free radical scavenging activity



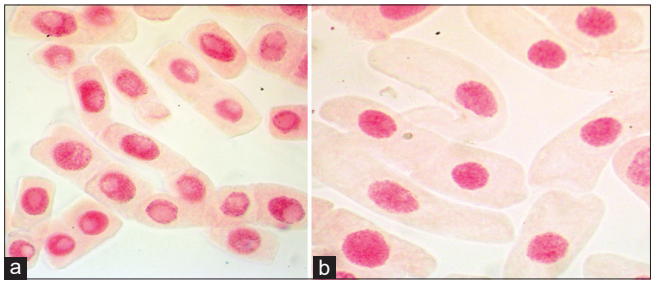


Fig. 1: (a) Nuclear lesions induced after treatment with 2% hydrogen peroxide; (b) modulatory action of *Terminalia paniculata* extract showing return of cells to normalcy after treatment

DISCUSSION

H_2O_2 was already proved that it is an important exogenous oxidant and makes damages to the cells and tissues. The addition of H_2O_2 may cause cancer associated to several inflammation risks [14]. Hence, the modulatory action of the plant extract was screened using H_2O_2. The results regarding the percentage of inhibition of *T. paniculata* can be explained that in the qualitative phytochemical analysis when the phenolic content was tested, it revealed an intense color. Moreover, previous studies [15,16] have revealed that the combined action between the activity of phenolic contents and H_2O_2 can scavenge ROS generated by H_2O_2. The present study revealed that *T. paniculata* fruit extract has an appreciable antioxidant potential associated with anti-genotoxicity.

DNA is a biologically important target for reactive oxygen species, free oxygen is relatively unreactive with DNA. H_2O_2 and free oxygen have a major role to participate in the production of singlet oxygen and peroxynitrite. The production of these species may be synchronized with reactions involving iron, and under some other conditions, they might be important contributors to H_2O_2 toxicity.

The protective effects could be co-related to cellular adaptive responses. This may be due to the ability of flavonoids to scavenge free radicals and induce the expression of genes coding for the antioxidant enzymes [17]. The antioxidant modulatory effect of the plant extract might be due to the presence of phenolic compounds [16]. In some flavonoids, the phytochelatins chelate the exogenous compounds and detoxify the reactive oxygen species [18]. Hence, the present results gave an idea about the genoprotective effect of the fruit extract of *T. paniculata* which may be due to the combined or individual actions of the phytochemicals present in it.

Four different assays were used to test the antioxidant potential of the fruit extract of the plant, *T. paniculata*. Widely used methods can generate free radical species but which are neutralized by antioxidant compounds [19]. IC_{50} values revealed that the concentration of extracts of *Terminalia arjuna* caused a 50% neutralization of DPPH radicals [20]. From the present study the IC50 value of *Terminalia paniculata* was recorded as 23.068µg/ml whereas the IC50 value for ascorbic acid (standard) was recorded as

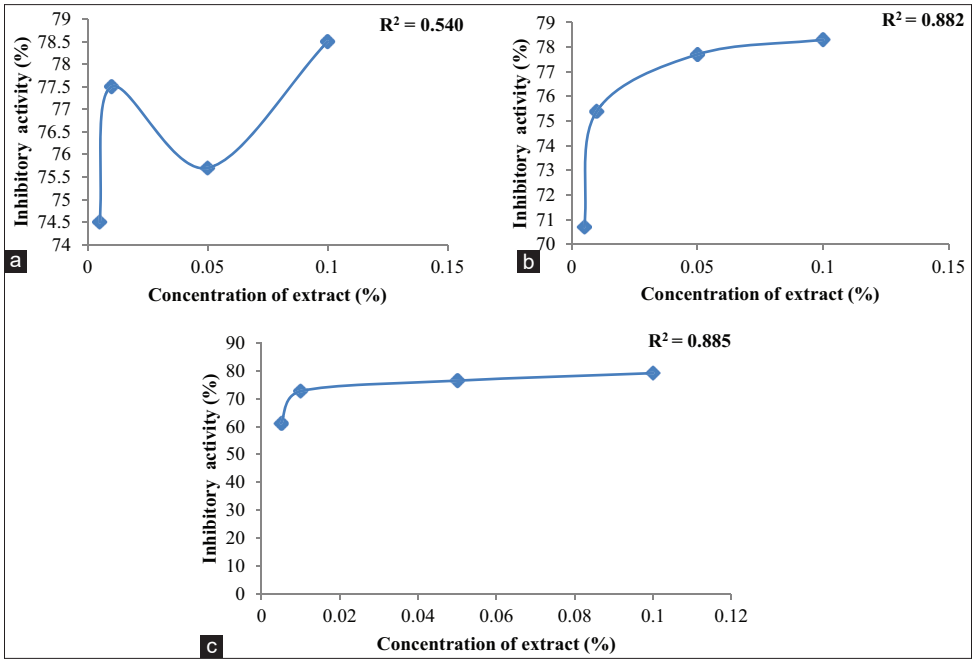


Fig. 2: Relationship between different concentrations of aqueous extract of *Terminalia paniculata* and percentage inhibition induced by hydrogen peroxide (2%) in *Allium cepa* root. (a) Pre-treatment; (b) post-treatment; (c) simultaneous treatment

20.399µg/ml. Thus, it can be inferred that the *Terminalia paniculata* possess high antioxidant activity at par with the standard. DNA can be damaged by most reactive free radical species like hydroxyl free radicals. Some of the dietary phytochemicals can prevent DNA damage by scavenging HO• and Fe2+ ions. An antioxidant donates hydrogen to the hydroxyl free radical and makes them inactive from being involved in further reactions [21]. In the present study, the hydroxyl radical scavenging activity revealed a considerable antioxidant potential of the plant extract.

Stem bark extracts of *T. arjuna* were found to be rich in antioxidant compounds [20]. Similarly, the methanolic fruit extract of *T. paniculata* showed significant antioxidant potential. Moreover, the antioxidant activity of *T. arjuna* L. extracts showed variations depending upon the nature of extracting solvents as well as the nature of raw materials to be extracted.

Plants, especially fruits, vegetables, medicinal herbs, etc., have a vivid variety of free radical scavenging molecules such as phenolic acids, flavonoids, quinones, coumarins, and tannins. Furthermore, they may contain some other endogenous metabolites with high antioxidant activity [22,23]. Similarly, the preliminary phytochemical studies of the fruit extract of *T. paniculata* revealed significant occurrence of several phytochemical compounds, which are of great therapeutic value.

So many reports have been published earlier to evaluate the genotoxicity of plant extracts using *A. cepa* root meristem. Several important chromosome aberrations such as C-metaphase, vagrant chromosomes, laggards in metaphase, and anaphases were obtained. C-metaphase was found in lower concentrations of plant extracts. Plant-derived compounds are being used in anticancer studies [24]. Plant extract is the main source of potential bioactive compounds that might have generated the aberrations in *Allium* root tip meristem [25]. Apoptosis is an important developmental and regulated process which may occur due to the cytogenetic changes in the cell [26]. The results obtained from the present study with the modulatory action of the fruit extract of *T. paniculata* gave strong findings regarding their antigenotoxicity. Furthermore, it has an appreciable antioxidant potential.

CONCLUSIONS

The present study is mainly focused on the antigenotoxicity and antioxidant potential of the fruit extract of an important dye yielding plant *T. paniculata* Roth. From the investigation, antigenotoxicity studies revealed the protective role of the fruit extract induced by H_2O_2. The fruit extract showed a substantial modulatory effect by means of an inhibition percentage and while in the case of antioxidant activity, the fruit extract showed significant antioxidant potential.

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AUTHORS' CONTRIBUTIONS

All authors contribute equally.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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