Antioxidant and oxidative DNA damage protective properties of leaf, bark and fruit extracts of *Terminalia chebula*

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*Terminalia chebula* Retz., is an important herbal drug in Indian system of medicine. In the present study, methanol extract and fractions of leaf, bark and fruit of *T. chebula* were investigated for their antioxidant and DNA protective properties. The antioxidant activities of these extracts and fractions were evaluated by *in vitro* methods, including, DPPH radical scavenging assay, superoxide radical scavenging assay, hydroxyl radical scavenging assay, reducing power assay and metal chelating assay. DNA protective activity was also determined using hydroxyl radical induced DNA strand scission. Total phenolic content of the extracts and fractions was measured using Folin-Ciocalteu method. Among the different extracts and fractions, highest radical scavenging abilities were obtained in acetone fraction of fruit, including DPPH radical (EC50 value of 9.9 ± 0.70 μg/mL) superoxide anions (EC50 value of 27.0± 2.09 μg/mL), and hydroxyl radicals (EC50 value of 35.6 ± 1.97 μg/mL) scavenging activities. Similarly, reducing power (EC50 value of 71.4 ± 4.30 μg/mL) and percent Fe2+ chelating capacity (70.6 ± 4.84 at 500 μg/mL) was also highest in acetone fraction of fruit. Our results also revealed remarkable ability of the acetone fraction of fruit and bark in protecting the supercoiled pUC18 plasmid DNA against strand scission induced by hydroxyl radicals. In addition, our study showed significant (*P* <0.01) linear correlation between antioxidant activities and total phenolic content of extracts and fractions.

**Keywords:** Antioxidant activity, DNA protective activity, *Terminalia chebula*

Reactive oxygen species (ROS) such as superoxide anion (O2–), hydroxyl radical (·OH) and hydrogen peroxide (H2O2) are produced in the body as a result of normal metabolic activities¹. Although body can combat free radicals by deploying its internal antioxidants including glutathione, vitamin C and E, however, endogenous antioxidant defenses are not always sufficient to combat the ROS completely². A large body of evidences reported in literature has shown that free radicals have been implicated in the development of cancer in humans³. Oxidative stress also causes serious damage to vital cellular molecules such as protein and DNA and therefore, intake of diet rich in antioxidants is considered essential to boost the body’s oxidative defense response. Although, there are synthetic antioxidants such as butylated hydroxytoluene (BHT) and butyalted hydroxyanisole (BHA) available in the market, however natural antioxidants are more appropriate for consumption due to the ill side effects associated with the synthetic antioxidants⁴. Thus, recently there has been an upsurge of interest among the scientific community to find such antioxidant molecules from natural sources which can protect oxidative damage in the body without any harmful effects⁵. Phenols and flavonoids constitute such important class of compounds whose intake has been reported to protect the body from chronic diseases such as cancer, Alzheimer’s disease, cardiovascular disease, diabetes and Parkinson’s disease⁶.

*Terminalia Chebula* Retz. (Family: Combretaceae) is an important medicinal plant which is widely cultivated in India and other parts of the world⁷. The dried fruit of *T. chebula* is extensively used in Indian system of medicine (Ayurveda) for its antitussive, laxative, cardiotonic and diuretic activities⁸. It is also an important component of Triphala, a widely used ayurvedic formulation⁹. Phytochemical composition...
of *T. chebula* reported in literature shows the presence of ellagic acid, gallic acid, tannic acid, chebulic acid, chebulagic acid and many other compounds\(^\text{10}\). In addition, *T. chebula* fruit extract has also been reported to show many biological properties such as anticancer, cytoprotective, antidiabetic, antioxidant and gastro-protective\(^\text{11}\). However, the comparative antioxidant activity of leaf, bark and fruit extracts of *T. chebula* has not been reported till date. Therefore, in the present study, antioxidant activity of the different parts of *T. chebula* namely leaf, bark and fruit was assessed using standard *in vitro* assays to identify the active fraction that may be exploited for use in food and pharmaceutical industries.

### Materials and Methods

#### Plant material

*T. chebula* plant material, namely leaves, bark, and fruit were collected from Herbal Garden and Herbarium Research Institute in Indian System of Medicine, Joginder Nagar, Himanchal Pradesh, India.

#### Preparation of crude extracts

The leaf, bark, and fruit parts of *T. chebula* were dried in shade and ground to fine powder in a grinder (Model: Twister, Bajaj Electricals Ltd., Mumbai, India). Total of 100 g of powdered plant material was extracted with 500 mL of methanol at 40°C by continuous stirring for 6h. This was repeated thrice; extracts were pooled, filtered, and lyophilized\(^\text{4}\).

#### Fractionation of crude extracts

The crude leaf, bark and fruit extract was extracted successively with chloroform, ethyl acetate, acetone, and methanol. All fractions were evaporated to dryness and dissolved in 100 mg/mL of dimethyl sulfoxide (DMSO) and diluted in methanol to obtain various working concentrations.

#### Determination of total phenolic content (TPC)

Total phenol content was determined according to the Folin–Ciocalteu method\(^\text{2}\) using gallic acid (5-50 μg) as the standard [standard curve: Absorbance = 0.0301 gallic acid (μg) – 0.2342 (\(R^2 = 0.9999\))]. In brief, 0.5 mL of extract/fraction solution was mixed with 0.5 mL of 1N Folin–Ciocalteu reagent. Then 1 mL of 20% Na₂CO₃ were added and the reaction tubes were incubated for 10 min at room temperature. The absorbance was measured at 730 nm using double beam UV-VIS spectrophotometer (Model: UVD2960, Labomed, USA). The results were expressed as mg of gallic acid equivalents per gram dry weight.

### DPPH radical scavenging assay

In order to determine DPPH radical-scavenging activity a method described by Abe *et al.*\(^\text{13}\) was used with modifications. An aliquot of 1.0 mL of 0.5 mM DPPH radical in methanol was mixed with 2.0 mL of the sample and to this 2 mL of 0.1M sodium acetate buffer (pH 5.5) was added. After 30 min of incubation at room temperature in the dark, the absorbance of the sample was measured at 517 nm in a UV/Vis spectrophotometer. Methanol was used as negative control. The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical discoloration, using the equation:

\[
\text{%RSA} = \left(\frac{A_0 - As}{A_0}\right) \times 100
\]

where, \(A_0\) is the absorbance of the control (containing all reagents except the test compound) and \(As\) is the absorbance of test compound. The extract/fraction concentration providing 50% of radical scavenging activity (EC\(_{50}\)) was calculated from the graph of percent RSA against extract/fraction concentration.

### Superoxide radical scavenging activity

The superoxide scavenging ability of the extract/fraction was assessed by the method of Nishikimi *et al.*\(^\text{14}\). The reaction mixture, containing PMS (120 μM), NADH (936 μM) and NBT (312 μM) in phosphate buffer (0.1M, pH 7.4) and different concentrations of extract/fraction was incubated at room temperature for 5 min and the colour was read at 560 nm against a blank. The capability of scavenging the superoxide radical was expressed as inhibitory concentration of test compound required to produce 50% inhibition (EC\(_{50}\)) of superoxide anions.

### Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by the deoxyribose method\(^\text{15}\). The reaction mixture, containing different concentrations of extract/fraction, was incubated with 200 μL of deoxyribose (2.8 mM), 200 μL of H₂O₂ (1 mM), 400 μL of FeCl₃ (200 mM), 1.04 mM of EDTA (1:1, v/v) and 200 μL of ascorbic acid (1 mM) in phosphate buffer (pH 7.4) for 30 min at 37°C. The reaction was terminated by adding 0.5 mL of TBA (1% w/v) and 0.5 mL of TCA (5% w/v) and then heating the tubes in a boiling water bath for 30 min. The contents were cooled and the absorbance of the mixture was measured at 532 nm against reagent blank. Scavenging activity was expressed as inhibitory concentration of test compound required to produce 50% inhibition of hydroxyl radicals (EC\(_{50}\) value).
Reducing power assay

The reducing power of extract and fractions was determined by the method of Oyaizu. Different concentrations of extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide \([K_3Fe(CN)_6]\) (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and \(\text{FeCl}_3\) (2.5 mL, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. The extract/fraction concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance against extract/fraction concentration.

Chelating power on ferrous (\(\text{Fe}^{2+}\)) ions

The ferrous ion-chelating potential of the extract/fraction was investigated according to the method of Dinis et al. Briefly, the reaction mixture, containing 200 µL of different concentrations of extract/fraction, 740 µL of methanol, 20 µL of 2 mM \(\text{FeCl}_2\) and 40 µL of 5 mM ferrozine, shaken well and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank. The ability of the extract to chelate ferrous ion was calculated using the following equation: Chelating effect (%) = \[\frac{(\text{absorbance of control} - \text{absorbance of test sample})}{\text{absorbance of control}}\] × 100

DNA damage protective activity assay

Induction of DNA scission by Fenton’s reagent was measured on pUC18 plasmid DNA, according to the procedure described by Kitts et al. with modifications. Briefly, 2 µL of test compound (1 mg/mL) was mixed with \(\text{KH}_2\text{PO}_4\) buffer (0.05 mol/L, pH 7.4), 2 µL of EDTA-Na\(_2\) (10.09 g/L), \(\text{FeSO}_4\) (2.42 g/L), \(\text{H}_2\text{O}_2\) (1.02 g/L) and 0.1 µg/mL of pUC18 plasmid DNA in a micro-centrifuge tube. The molar ratio of \(\text{FeSO}_4/\text{EDTA}\) was kept at 0.53. The final volume of the reaction mixture was brought to 12 µL with de-ionized distilled water and incubated for 1h at 37°C. Following incubation, 3 µL of autoclaved distilled water and 3 µL of 6× DNA loading dye (Fermentas) was added and loaded onto a 1.2 g/100 mL of agarose gel. Electrophoresis was performed at 80 V in a Tris-acetate-EDTA.\(\text{Na}_2\) (TAE) buffer (7.25 g/l of tris acetate and 0.29 g/l of EDTA, pH 7.4) using a horizontal gel electrophoresis system (Atto Corp., Yushima, Bunkyo, Tokyo, Japan). The gel was then stained with ethidium bromide (0.5 µg/mL in deionized distilled water) for 10-15 min with gentle shaking followed by destaining with distilled water. Finally, DNA bands were visualized with Gel Doc system (Ultra-Violet Products [UVP] Ltd., Cambridge, U.K.).

Statistical analysis

All experiments were carried out in triplicate. Data are expressed as mean ± standard deviation. The differences between the treatments (solvent) in each parameter were analyzed using one-way analysis of variance (ANOVA) followed by Turkey’s HSD test. This treatment was carried out using SPSS version 16.0 programme (SPSS Inc., Chicago, IL, U.S.A.).

Results and Discussion

Phenolic compounds have been reported to possess various chemical and biological effects and act as antioxidants at different levels in antioxidant sequence. According to Harborne, the extraction of phenolic acids from plant materials is influenced by their chemical properties like acidity, polarity, and hydrogen-bonding ability of the hydroxyl groups in the aromatic ring. As depicted in Table 1, the total phenols were present in the order: acetone fraction of fruit (671.1±7.98 mg GAEs/g dry weight)>

<table>
<thead>
<tr>
<th>Extract/ fractions</th>
<th>Leaf (mg GAEs/g dry wt)</th>
<th>Bark (mg GAEs/g dry wt)</th>
<th>Fruit (mg GAEs/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>448.2 ± 6.26(^c)</td>
<td>526.2 ± 7.16(^d)</td>
<td>581.5 ± 6.91(^d)</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>106.6 ± 4.83(^a)</td>
<td>77.8 ± 5.86(^a)</td>
<td>171.4 ± 7.88(^a)</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>263.7 ± 3.90(^b)</td>
<td>153.4 ± 4.54(^c)</td>
<td>494.3 ± 7.95(^c)</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>538.2 ± 6.89(^d)</td>
<td>463.9 ± 4.99(^c)</td>
<td>671.1 ± 7.98(^c)</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>278.4 ± 5.14(^b)</td>
<td>558.1 ± 11.98(^e)</td>
<td>336.2 ± 6.67(^b)</td>
</tr>
</tbody>
</table>

GAEs= Gallic acid equivalents
\(^a\)Data presented as mean ± standard deviation of three parallel measurements. Different letters within a column for a particular treatment represent significance at \(P<0.05\) applying Turkey’s HSD test.
crude extract of fruit (581.5±6.91 mg GAEs/g dry weight)> methanol fraction of bark (558.1±11.98 mg GAEs/g dry weight)> acetone fraction of leaf (538.2±6.89 mg GAEs/g dry weight). Lowest level of phenols was observed in chloroform fraction of bark (77.8±5.86 mg GAEs/g). Phenolic compounds have various therapeutic applications against the different diseases caused by oxidative stress. It has been reported that polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity.

Free radicals are considered to play an important role in numerous age related diseases such as cardiovascular diseases, hyperglycemia and cancer. DPPH a stable free radical is used to measure the free radical scavenging capacity of antioxidant compounds. DPPH radical scavenging activity is followed as change in color as a result of reduction of DPPH stable free radical by antioxidant compounds. In the present study, DPPH radical scavenging activity of leaf, bark and fruit crude extracts and fractions from *T. chebula* was investigated. As shown in Table 2, the highest DPPH radical scavenging activity was observed in acetone fraction of fruit (EC50 of 9.9±0.70 μg/mL). Crude fruit extract (EC50 of 12.9±1.23 μg/mL), methanol fraction of bark (EC50 of 16.2±2.04μg/mL), and acetone fraction of leaf (EC50 of 16.8±0.52μg/mL) also showed good DPPH radical scavenging activity. The lowest DPPH radical scavenging activity was observed in chloroform fraction of bark (EC50 of 157.0±7.75 μg/mL). Further more, the comparison of DPPH radical scavenging activity of extracts and fractions derived from leaf, bark, and fruit of *T. chebula* revealed that acetone fraction of fruit and crude fruit extract has better antioxidant potential than bark and leaf, which was also supported by their phenolic content. Many studies in literature have revealed direct correlation between phenolic compounds and antioxidant properties of plant extracts.

Presence of reductants in plant extracts result in reduction of ferric ion/ ferricyanide complex to its ferrous form and the amount of ferrous ion complex can then be followed by measuring the formation of Prussian blue at 700 nm. The antioxidant action of reductones depends upon the breaking of free radical chain by donating a hydrogen atom, or by reacting with certain precursors of peroxide to prevent its formation. The reducing power assay is generally used to determine the electron or hydrogen donating capacity of natural antioxidants which is an important mechanism of phenolic antioxidant action. The reducing power of methanolic extract of leaf, bark and fruit of *T. chebula* ranged from EC50 of 104.0±4.42 μg/mL to EC50 of 158.2±5.99 μg/mL (Table 2). Out of all the extracts/fractions of leaf, bark and fruit, highest reducing power was observed in acetone fraction of fruit (EC50 of 71.4± 4.30μg/mL) which may be associated with higher level of phenolic compounds.

Superoxide radical scavenging activity of leaf, bark and fruit extracts/fractions of *T. chebula* was evaluated by the PMS-NADH-NBT reduction system. It has been reported that antioxidant molecules inhibit formation of blue NBT by O2− derived from dissolved oxygen by the PMS-NADH coupling reaction. The results presented in Table 3, showed significant inhibition of NBT reduction by acetone fraction of fruit (EC50 of 27.0± 2.09 μg/mL) which was similar to the DPPH radical scavenging and reducing power results. The other extracts and fractions which showed better superoxide radical scavenging activity were crude methanolic extract of fruit (EC50 of 34.8±3.72 μg/mL), methanolic fraction of bark (EC50 of 37.5± 3.41 μg/mL) and acetone fraction of leaf (EC50 of 36.7± 2.45 μg/mL).

### Table 2 — DPPH radical scavenging activity and reducing power of crude extracts and fractions of leaf, bark and fruit of *Terminalia chebula*.

<table>
<thead>
<tr>
<th>Extract/fractions</th>
<th>DPPH radical scavenging activityEC50 (μg/mL)</th>
<th>Reducing powerEC50 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Bark</td>
</tr>
<tr>
<td>Crude extract</td>
<td>17.9 ± 0.95a</td>
<td>17.2 ± 0.96a</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>113.0 ± 9.64c</td>
<td>157.0 ± 7.75c</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>45.0 ± 1.99b</td>
<td>87.5 ± 2.01b</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>16.8 ± 0.52a</td>
<td>19.6 ± 1.73a</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>40.5±1.92b</td>
<td>16.2±2.04a</td>
</tr>
</tbody>
</table>

aData presented as mean ± standard deviation of three parallel measurements. Different letters within a column for a particular treatment represent significance at *P*< 0.05 applying Turkey’s HSD test.
It has been reported that superoxide radicals give rise to toxic hydroxyl radicals, damaging bio-macromolecules with severe consequences\textsuperscript{24}. The superoxide anion scavenging activity observed in the present study could be due to the action of free hydroxyl groups of phenolic compounds present in the extracts/fractions of \textit{T. chebula}\textsuperscript{35,36}. Hydroxyl radical is highly reactive that can inflict damage on all types of biological molecules and causes severe damage to cells and therefore its removal is essential\textsuperscript{29}. Phenolic compounds are also known to exert scavenging activity against hydroxyl radicals forming oxidation products aldehydes and dimers\textsuperscript{37}. As depicted in Table 3, \textit{T. chebula} fruit crude extract showed better hydroxyl radical scavenging activity (EC\textsubscript{50} of 42.9±2.55 \(\mu\)g/mL) as compared to bark (EC\textsubscript{50} of 50.2±1.95 \(\mu\)g/mL) and leaf (EC\textsubscript{50} of 51.2±3.10 \(\mu\)g/mL) among all the fractions derived from leaf, bark, and fruit, the acetone fraction of fruit inhibited the degradation of deoxyribose by Fe\textsuperscript{3+}-ascorbic acid-EDTA-H\textsubscript{2}O\textsubscript{2} system at low concentrations with EC\textsubscript{50} value of 35.6±1.97 \(\mu\)g/mL as it contains high amount of phenolic compounds. Hydroxyl radical scavenging is an important antioxidant activity because of very high reactivity of hydroxyl radicals which enables them to react with a wide range of molecules found in living cells such as sugars, amino acids, lipids and nucleotides\textsuperscript{38}. Comparison of these results indicates that crude extract of fruit and its acetone fraction would be a rich source of antioxidants and merits further investigation.

Metal chelating activity is one of the important mechanisms of antioxidant activity. The order of percent chelating activity among the crude extracts of leaf, bark, and fruit of \textit{T. chebula} is: fruit (67.4±4.40\%)>bark (51.1±1.65\%)>leaf (47.8±3.38\%) at 500 \(\mu\)g/mL concentration (Table 4).

<table>
<thead>
<tr>
<th>Extract/fractions\textsuperscript{a}</th>
<th>Superoxide radical scavenging activityEC\textsubscript{50} ((\mu)g/mL)</th>
<th>Hydroxyl radical scavenging activityEC\textsubscript{50} ((\mu)g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Bark</td>
<td>Fruit</td>
</tr>
<tr>
<td>Crude extract</td>
<td>41.8±2.75\textsuperscript{a}</td>
<td>39.1±3.44\textsuperscript{a}</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>155.0±2.79\textsuperscript{b}</td>
<td>300.0±13.04\textsuperscript{c}</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>83.3±2.46</td>
<td>140.0±6.58</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>36.7±2.45</td>
<td>45.1±5.27</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>79.6±3.67</td>
<td>37.5±3.41</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data presented as mean ± standard deviation of three parallel measurements. Different letters within a column for a particular treatment represent significance at \(P <0.05\) applying Turkey’s HSD test.

<table>
<thead>
<tr>
<th>Extract/fractions\textsuperscript{a}</th>
<th>Percent chelating effect at 500 (\mu)g/mL concentration</th>
</tr>
</thead>
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<tr>
<td>Leaf</td>
<td>Bark</td>
</tr>
<tr>
<td>Crude extract</td>
<td>47.8±3.38\textsuperscript{d}</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>14.1±0.85\textsuperscript{a}</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>26.8±1.76 \textsuperscript{b}</td>
</tr>
<tr>
<td>Acetone fraction</td>
<td>54.2±3.55 \textsuperscript{d}</td>
</tr>
<tr>
<td>Methanol fraction</td>
<td>35.4±1.74 \textsuperscript{d}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Data presented as mean ± standard deviation of three parallel measurements. Different letters within a column for a particular treatment represent significance at \(P <0.05\) applying Turkey’s HSD test.

The effectiveness of \textit{T. chebula} leaf, bark and fruit extracts in preventing oxidative damage of plasmid pUC18 DNA by OH radical was also evaluated.
Damage of DNA by hydroxyl radicals results in cleavage of one of the phosphodiester chains of the supercoiled plasmid DNA thereby producing relaxed form. In this assay, hydroxyl radical is generated by the Fenton reaction in the presence of transition metal such as Fe$^{3+}$ and H$_2$O$_2$. Hydroxyl radicals from the Fenton’s reagent can react with the nucleotides in DNA and cause strand breakage which may cause carcinogenesis, mutagenesis and cytotoxicity. The results obtained through gel electrophoresis (Fig. 1) showed that acetone fraction of fruit significantly protected the pUC18 plasmid DNA from damage by hydroxyl radicals at 30.0 µg/mL concentration. Similarly, ethyl acetate and methanolic fractions of fruit and acetone, methanolic fractions of bark also showed good protection of plasmid DNA from damage by hydroxyl radicals at the same concentration.

In addition, we also studied the effect of different concentrations (5.0, 10.0, 20.0 and 30 µg/mL) of acetone fraction of leaf, bark and fruit on protection of plasmid DNA from damage by hydroxyl radicals. The acetone fraction of fruit protected the plasmid DNA from damage by hydroxyl radicals at low concentration as 5 µg/mL (Fig. 2). The protection of DNA from hydroxyl radicals by these fractions from leaf, bark and fruit of T. chebula may be attributed to the phenolic compounds present in them which might prevent the reaction of Fe$^{3+}$ ions with H$_2$O$_2$ or quench OH radical by donating hydrogen atom or electron. To our knowledge, this is first report to date on the comparative efficiency of leaf, bark and fruit extracts of T. chebula in protecting oxidative DNA damage.

**Conclusion**

*T. chebula* is an important component of several herbal preparations used in Indian system of medicine for curing variety of diseases. To our knowledge this is the first report demonstrating the comparative antioxidant and DNA protective properties of three different parts namely leaf, bark, and fruit of *T. chebula* growing in north-western Himalaya. The present investigation suggests that the three parts of *T. chebula* differ significantly in their antioxidant potential as seen in the DPPH radical scavenging assay, reducing power assay, metal ion chelating assay, superoxide radical scavenging assay, hydroxyl radical scavenging and DNA protective activity assays. In addition, our results showed significant linear correlation between phenolic content of leaf, bark, fruit and their antioxidant activities. Further, it is suggested that acetone and ethyl acetate fractions of fruit and bark might have a good potential as a source for natural health products due to their antioxidant and DNA protective activities and merits further investigation for *in vivo* antioxidant activity.

**Acknowledgement**

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References


