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Superoxide radical scavenging activity and reducing capacity assessment of some important medicinal plants of Gujarat, India

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Abstract: *Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of many ailments. They are responsible for creating oxidative stress and stress related diseases and disorders. Antioxidants especially natural antioxidants could be the best way to cure them. In the present work, leaf and stem of Aerva lanata, Terminalia bellirica, Terminalia chebula, Terminalia catappa, Zea mays hair, Tribulis terrestris* fruit *and Boerhaavia diffusa* L. root *were evaluated for their antioxidant efficacies by two in vitro antioxidant assays viz. superoxide anion radical scavenging activity and reducing capacity assessment. The extraction was done by decoction extraction method. Terminalia species showed best super oxide anion radical scavenging activity and reducing capacity assessment inspite of using decoction extract. Hence, they could be a good source of antioxidants and can be used therapeutically. Further studies could be done in T. chebula extracts which might lead to compounds with good antioxidant activity singly or in combination with other drugs.*

Key words*: Terminalia, antioxidant activity; super oxide anion radical scavenging activity; reducing capacity assessment; decoction extracts*

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I. Introduction

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological condition like metabolic activity and atmospheric pollutants and radicals like cigarette smoke, pesticide, UV radiation, smog etc. they become harmful when not being eliminated by the endogenous antioxidant systems (Yazdanparast *et al*., 2008) and have been implicated in more than 100 diseases including cancer (Paz-Elizur *et al*., 2008), Alzheimer's disease (Moreira *et al*., 2005), ageing (Liu and Mori, 2006), arthritis (Colak 2008), inflammation (Mukherjee *et al*., 2007), diabetes, (Naito *et al*., 2006; Jain, 2006), AIDS (Sepulveda and Watson, 2002), etc.

Free radicals are highly reactive oxygen species that are capable of damaging molecules such as DNA, protein and carbohydrates. The body is under constant attack from these free radicals formed as a consequence of the body's normal metabolic activity (Surai, 2002). Some free radicals likesinglet oxygen (O_2)superoxide ($O_2^{\cdot}$), hydroxyl ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$), Hydrogen peroxide (H_2O_2) peroxinitrite ($^{\cdot}ONOO$), nitric oxide ($NO^{\cdot}$) and cyanide (CN) produced through oxidative process within the mammalian body. The human body possesses many defense mechanisms against oxidative stress including antioxidant enzymes and non-enzymatic compounds (El-Saleh and Hameed, 2009).

The implication of oxidative stress in the etiology and progression of several acute and chronic clinical disorders led to considerable interest in search for antioxidants that can scavenge free radicals and elevate the defense system. Although several synthetic antioxidants have been suggested for the prevention and treatment of diseases, the various side effects and toxicity have become an issue (Ghafar *et al*., 2010) Therefore, natural antioxidants from food and herbal products have attracted much attention and great efforts have been made to search for effective therapeutic agents for oxidative stress related diseases (Piao *et al*., 2008). The beneficial effect of antioxidants on promoting health is believed to be achieved through several possible mechanisms, such as directly reacting with and quenching free radicals, chelating transition metals, reducing peroxides, and stimulating the antioxidative defense enzyme system (Zhou *et al*., 2004).

In the recent years, the quest for natural food additives has become an increasing concern. Consumer's demand for healthier foods has been the initiative for many researchers seeking for natural alternatives (El-Chaghaby *et al*., 2014). Various studies have focused on natural antioxidantsin plant kingdom and their applications in food systems to prevent oxidation. The most widely used synthetic antioxidants in foods are very effective in their role as antioxidants. Synthetic antioxidants such as butylatedhydroxytoluene (BHT),

butylatedhydroxyanisole (BHA) and propyl gallate (PG) have been widely used around the world for decades (Almey *et al*., 2010).

There are several antioxidant assays to evaluate the antioxidant potential of natural plant extracts. It is imperative to evaluate more than one antioxidant assay when screening the antioxidant efficacy of plant extracts because different extract possess different phytoconstituents in different concentrations and it will scavenge which radicals is not known. The various antioxidant assays generally used are DPPH radical scavenging activity, Super oxide anion radical scavenging activity, ABTS cation radical scavenging activity, FRAP activity, Reducing capacity assessment, Hydroxyl radical scavenging activity, Hydrogen peroxide scavenging activity, Nitric oxide radical scavenging activity, Oxygen radical absorbance capacity, α-amylase inhibitory activity, β-carotene bleaching by linoleic acid activity, Metal chelating activity, Linoleic acid peroxidation, FTC (Ferric thiocyanate) activity, Ferrous ions chelating activity, etc (Chanda and Dave, 2009).

Determination of antioxidant activity provides useful information with regard to health promotion and functional quality of raw material. It can be used as a parameter to characterize nutritional health food or plants and their bioactive components (Dalamu *et al*. 2010). Different methods evaluate different aspects of antioxidant properties and hence the compounds involved in the particular reaction also differ.

In the present work, we focused on two antioxidant assays i.e. super oxide anion radical scavenging activity and reducing capacity assessment. Considering the above, an attempt has been made to evaluate the superoxide anion free radical scavenging ability and reducing capacity assessment of 11 plant extracts belonging to five different families. The plants and parts selected were *Aerva lanata* Linn. leaf and stem (Amaranthaceae), *Terminalia bellirica* (Gaertn.) Roxb. leaf and stem (Combretaceae), *Terminalia chebula* Retz. leaf and stem (Combretaceae), *Terminalia catappa* L. leaf (Combretaceae), *Zea mays* Corn hair young and old (Poaceae), *Tribulis terrestris* L. fruit (Zygophyllaceae) and *Boerhaavia diffusa* L. root (Nyctaginaceae) (Fig.1). The traditional uses and reported activities of the selected plants is reported earlier (Ram *et al*., 2015).

II. Material and methods

Plant collection

A. lanata was collected from sea-shore region of Veraval; *Terminalia* species were collected from Jamnagar; *Z. mays* corn hair was collected from the local market of Rajkot, Gujarat, India. *T. terrestris* and *B. diffusa* were purchased from Rajkot, Gujarat, India. They were thoroughly washed, separated, and dried under shade. The dried plant parts were homogenized to fine powder and stored in air tight bottles which were later used for extraction.

Decoction method

Five grams of dried powder was extracted with 100 ml of ultra pure distilled water at 100 ^{o}C for 30 min in water bath (Li *et al.,* 2007; Chanda *et al.,* 2013). It was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm in centrifuge (Remi centrifuge, India) for 10 min. The supernatant was collected and solvent was evaporated using a rotary vacuum evaporated (Equitron, India) to dryness. The residue was weighted to obtain extractive yield and it was stored in airtight bottle at 4^{o}C.

Determination of superoxide (SO) anion radical scavenging assay

The antioxidant activities of eleven parts of seven plants were measured by the method described by (Robak and Gryglewski, 1988). Superoxide radicals were generated by oxidation of NADH and assayed by the reduction of NBT. The reaction mixture 3.0 ml consisted of 1.0 ml of the solvent extract of different concentrations diluted by distilled water, 0.5 ml Tris HCL buffer (16mH, pH 7), 0.5 ml NBT (0.3 mM), 0.5 ml NADH (0.936 mM) and 0.5 ml PMS (0.12 mM). The superoxide radical generating reaction was started by the addition of PMS solution to the recation mixture. The reaction mixture was incubated at 25^{o}C for min and then the absorbance was measured at 560 nm using UV-VIS Spectrophotometer (Shimadzu, Japan), against a blank sample. Percentage of inhibition was calculated using the formula described above.

Reducing capacity assessment

The reducing capacity of different plant extract was determine using the modified method of (Athukorala *et al*., 2006). 1.0 ml of different concentration (20 to180 mg ml^{-1}) of different plant extracts and fraction diluted by distilled water, was mixed with 2.5 ml of phosphate buffer (200 mM pH 6.6) and 2.5 ml $K_3Fe(CN)_6$ (30 mM). The mixture was then incubated at 50^{o}C for 20 min. Thereafter, 2.5 ml of TCA (600mM) was added to the reaction mixture, and then centrifuged for 10 min. at 3,000 rpm. The upper layer of solution (2.5ml) was mixed with 2.5ml distilled water and 0.5ml $FeCl_3$(6nm), and the absorbance was measured at 700 nm using a UV- VIS spectrophotometer (Shimadzu, Japan) against a blank sample.

III. Results and discussion

Super oxide anion radical scavenging activity

The biological toxicity of superoxide is due to its capacity to inactive iron-sulphur cluster-containing enzymes, which are critical in a wide variety of metabolic pathways, thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radicals. In the PMS/NADH-NBT system, superoxide anion generated by the oxidation of NADH reduces NBT. Antioxidants consume superoxide anions and decrease the absorbance of reduced NBT at 560 nm (Zhang *et al*., 2009). The reducing power assay demonstrates the electron donor properties of the different plant extracts by neutralizing free radicals and forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging (Gulcin et al., 2010).

In the present work, seven different plants and their parts were evaluated for their super oxide anion radical scavenging activity and reducing capacity assessment. Different parts and different plants showed different levels of super oxide anion radical scavenging activity and reducing capacity. The super oxide anion radical scavenging activity of *A. lanata* leaf and stem extracts and *T. bellirica* leaf and stem extracts is given in Fig. 2. The concentration range of *A. lanata* leaf and stem extracts ranged from 30-180 µg/ml and 40-240 µg/ml and IC_{50} value was 85.5 µg/mL and 80 µg/mL respectively. Both the extracts showed almost similar super oxide anion radical scavenging activity (Fig. 2A and Fig. 2B). Both these extracts showed good super oxide anion radical scavenging activity which was very much better than that of standard gallic acid (IC_{50} value = 185 µg/mL). The concentration range of *T. bellirica* leaf and stem extracts was 10-60 µg/ml and 30-180 µg/ml and the IC_{50} value was 41µg/mL and 85.5µg/mL respectively (**Fig.2C and Fig. 2D**). *T. bellirica* leaf extract showed better super oxide anion radical scavenging activity than stem extract, though both the extracts had very much lower IC_{50} value than that of standard gallic acid.

The super oxide anion radical scavenging activity of *T. chebula* leaf and stem extracts and *Z. mays* corn hair young and old extract is given in Fig. 3. The concentration range of *T. chebula* leaf and stem extract ranged from 10-60 µg/ml and 8-48 µg/ml and IC_{50} value was 25.5 µg/mL and 20.5 µg/mL respectively. *T. chebula* stem extract showed better super oxide anion radical scavenging activity than that of *T. chebula* leaf extract as indicated by its lower IC_{50}value (Fig.3A and Fig. 3B). The concentration range of *Z. mays* corn young and old extract ranged from 40-240 µg/ml and 20-120 µg/ml and IC_{50}value was 134 µg/mL and 56 µg/mL respectively (Fig. 3C and Fig. 3D). *Z. mays* old corn hair showed better super oxide anion radical scavenging activity as compared to young corn hair.

The super oxide anion radical scavenging activity of *T. catappa* leaf, *T. terrestris* fruit and *B. diffusa* root extract is given in Fig. 4. The concentration range of *T. catappa* leaf, *T. terrestris* fruit and *B. diffusa* root extract ranged from 15-90 µg/mL, 60-360 µg/mL and 50-300 µg/mL and IC_{50} value was 42.5 µg/mL, 115µg/mL and **84** µg/mL respectively (Fig. 4A, Fig. 4B and Fig. 4C). Out of three extracts, *T. catappa* leaf extract showed better super oxide anion radical scavenging activity as compared to *T. terrestris* fruit and *B. diffusa* root extract.

All the plant extracts showed good super oxide anion radical scavenging activity which was better than standard gallic acid. The best activity was shown by *T. chebula* stem extract followed by *T. chebula* leaf extract. This antioxidant activity also showed a direct correlation with total phenol content as also reported by other researchers (Kaneria and Chanda, 2013, Zhao *et al*., 2014, Rakholiya *et al*., 2015).

Reducing capacity assessment

The reducing capacity of plant extracts is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity (Roginsky and Lissi, 2005). In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power of the test sample. The reducing power method reflects the electron ability of antioxidants present in the extracts to convert Fe^{3+}in to Fe^{2+}complex which can be followed by measuring the formation of Perls' Prussian blue at the absorbance of 700 nm (Amarowicz *et al*., 2010). In fact, the increase in the absorbance indicates an increase in the reducing power activity. The extracts reveal that they were electron donors and they were capable of reducing Fe^{3+}ions in a linear concentration- dependent manner.

Reducing capacity assessment of ascorbic acid and *A. lanata* leaf and stem and *T. bellirica* leaf and stem are shown in Fig. 5A and 5B respectively. In *A. lanata* there was concentration dependent increase in absorbance of reaction mixture of both leaf and stem decoction extracts and standard ascorbic acid (Fig. 5A). The absorbance of stem was more than that of leaf and it was as good as that of standard ascorbic acid. It can be stated that the reducing capacity of stem was as good as that of standard ascorbic acid.

The reducing capacity of leaf and stem of *T. bellirica* also showed a steady increase in absorbance of the reaction mixture with increase in the concentration (Fig. 5B). However, the reducing capacity of both parts of this plant was much better than that of standard ascorbic acid. The reducing capacity of leaf was slightly

better than that of stem as depicted by its higher level of absorbance. The reducing capacity assessment of ascorbic acid and *T. chebula* leaf and stem and *Z. mays* young and old corn hair is given in Fig. 6A and 6B respectively. Irrespective of the part or plant, the decoction extracts showed a steady increase in absorbance of the reaction mixture. In *T. chebula*, both leaf and stem showed a considerably higher absorbance of the reaction mixture than that of standard ascorbic acid, similar to that of *T. bellirica* but unlike *T. bellirica* leaf and stem, in *T. chebula* reducing capacity of both parts (leaf and stem) was same (Fig. 6A). Young and old corn hair showed entirely different trend (Fig. 6B).Though there was a slight increase in absorbance of reaction mixture of both the parts, it was considerable less than that of standard ascorbic acid (Fig. 6B).

The reducing capacity assessment of ascorbic acid and *T. catappa* leaf, *T. terrestris* fruit and *B. diffusa* root are given in Fig. 7. All the three plants showed different level of reducing capacity. Like other *Terminalia* parts, *T. catappa* leaf showed maximum absorbance which was considerably more than that of standard ascorbic acid (Fig. 7). While the other two plant parts showed poor absorbance like *Z. mays* corn hair indicating poor reducing ability of these plant parts.

These results indicate that the different plant extracts are able to act as electron donors and therefore, react with free radicals, converting them to more stable products and thereby terminating radical chain reactions. The presence of reducers (i.e. antioxidants) cause the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form (Isabel *et al*., 2007), thus terminating the radical chain reaction that may otherwise be very damaging. Antioxidant can be explained as reductants and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of other (Gulchin *et al*., 2010). Out of 11 extracts, plants belonging to *Terminalia* species showed maximum absorbance and hence it has maximum reducing capacity, which was better than that of standard. Out of three *Terminalia* species, *T. chebula* leaf and stem showed best activity (Fig. 6A). The results of the present work suggests that *Terminalia* species especially *T. chebula* leaf and stem possess higher level of reductones which can react with free radicals and block radical chain reactions. Further reducing redox potential could decrease proton motive force, which is linked to a decrease of intracellular pH and thus a deactivation of the growth of microorganisms in food (Juneja, 2003). Sarkar *et al*., (2012) reported that plant extracts possesses reducing power are potent in reducing the toxic iron level and attenuating oxidative stress and fibrosis status in the liver of mice. Sarikurkcu *et al*., (2008) reported the reducing power of species belongs to the *Marrubium* genus.

IV. Conclusion

In the present study 2 important antioxidant assays were performed with 11 plant extracts. Amongst all the plants evaluated, *T. chebula* leaf and stem showed best super oxide anion radical scavenging activity and best reducing capacity assessment. Hence *T. chebula* leaf and stem can be used as a natural source of antioxidant. They can be used individually or in combination with other extracts since they possess good antioxidant activity.

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Aerva lanata **Linn.**

***Terminalia bellirica* (Gaertn.) Roxb.**

***Terminalia chebula* Retz.**

***Terminalia catappa* L.**

***Zea mays* L.**

***Tribulus terrestris* L.**

***Boerhaavia diffusa* L.**

Fig. 1. Images of selected plants

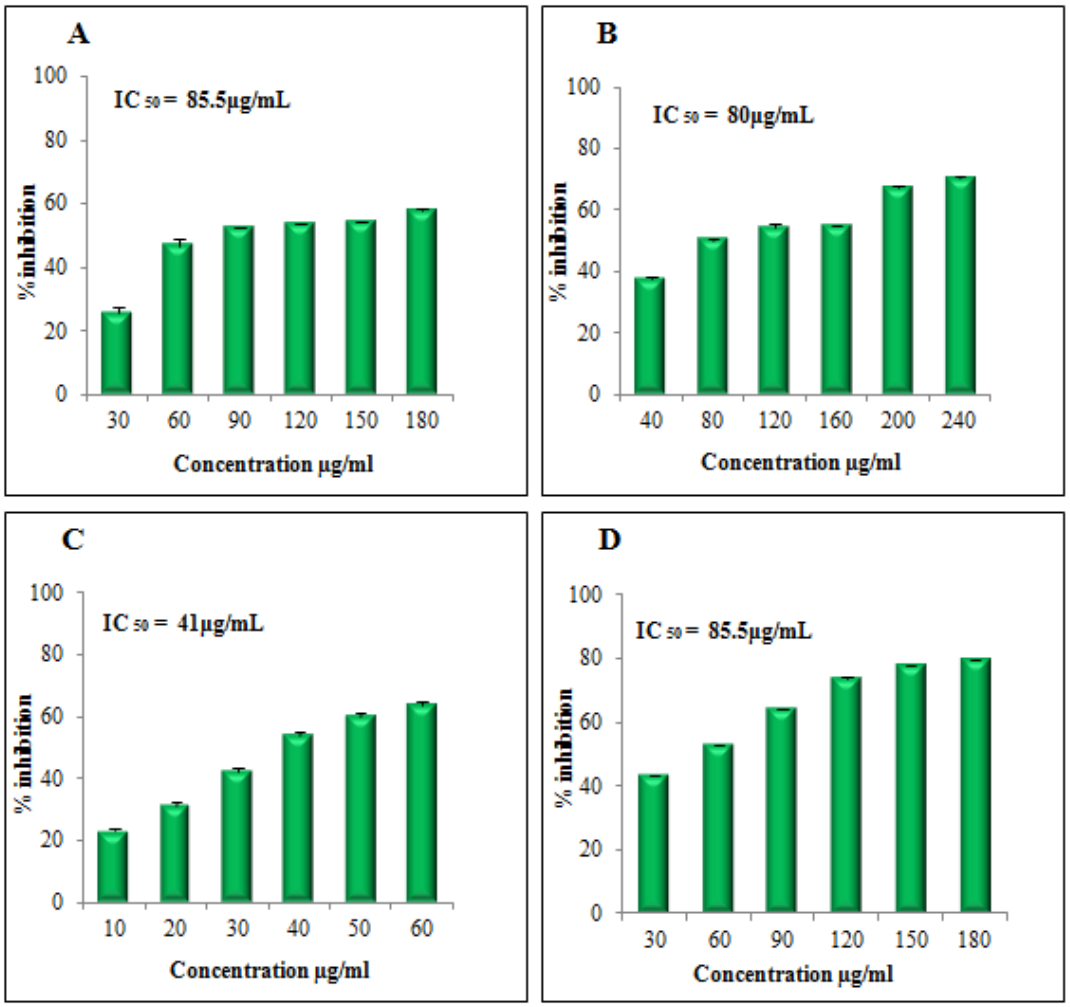


Fig. 2. Superoxide anion radical scavenging activity of *A. lanata* leaf (A) and stem (B), *T. bellirica* leaf (C) and stem (D).

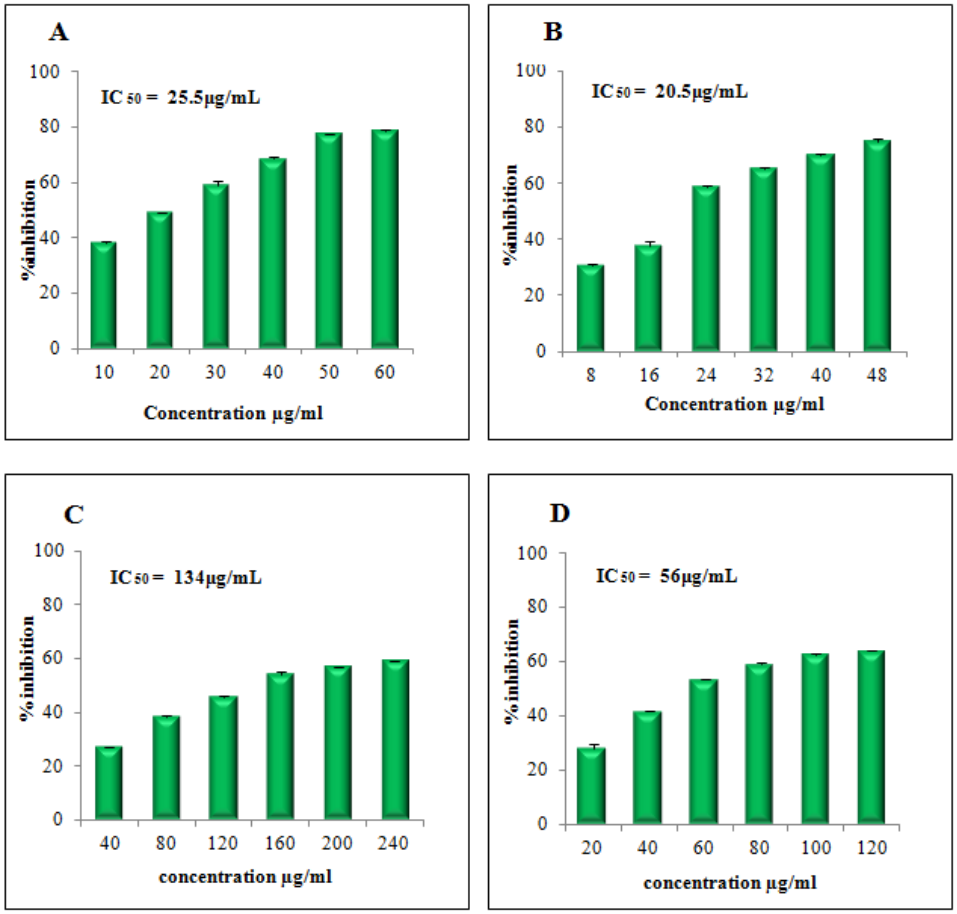


Fig. 3. Super oxide anion radical scavenging activity *T. chebula* leaf (A) and stem (B) and *Z. mays* hair young (C) and old (D).

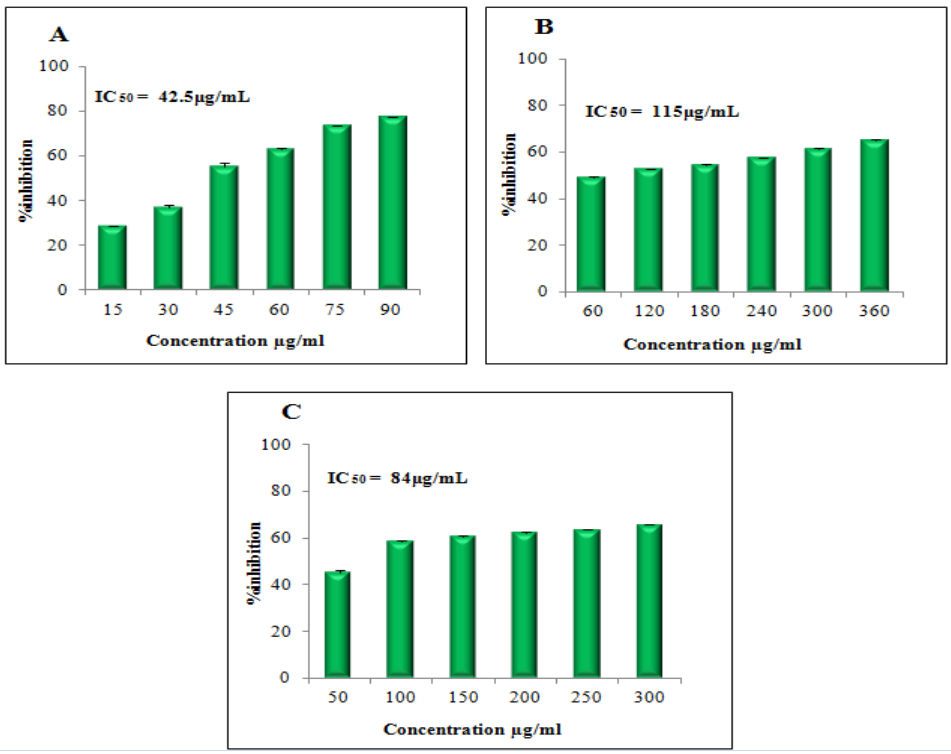


Fig. 4. Superoxide anion radical scavenging activity of *T. catappa* leaf (A), *T. terrestris* fruit (B) and *B. diffusa* root (C).

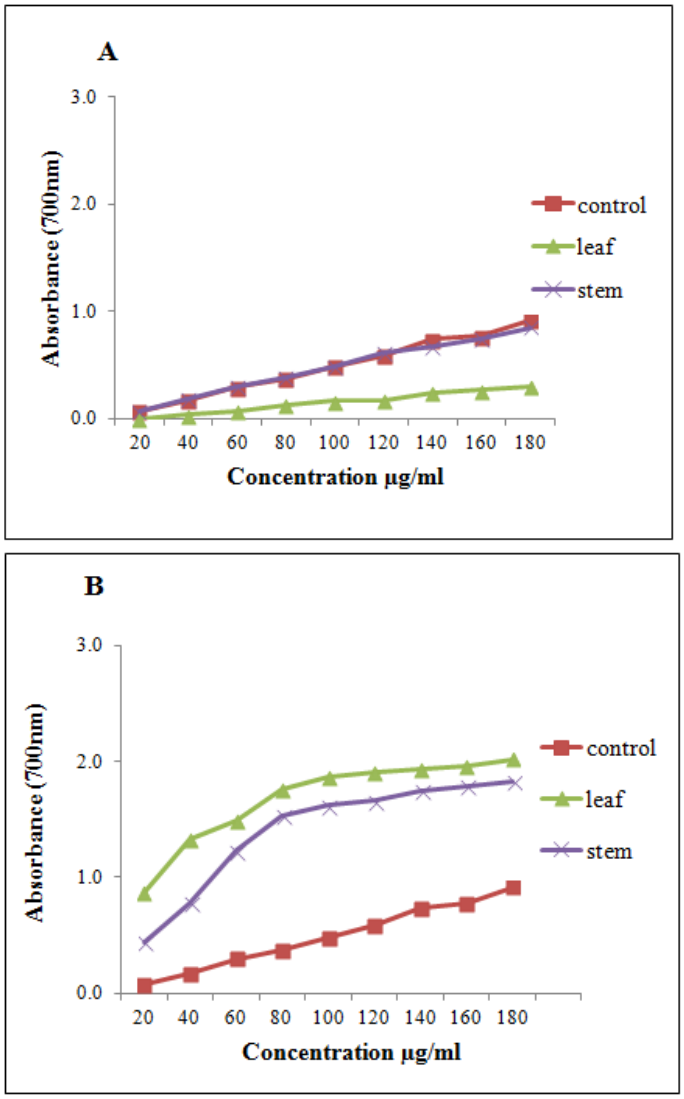
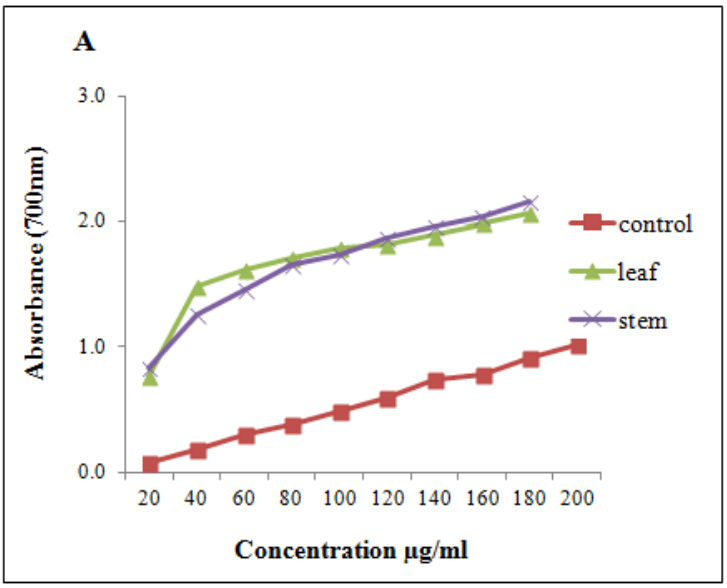


Fig. 5. Reducing capacity assessment of *A. lanata* (A) and *T. bellirica* (B)



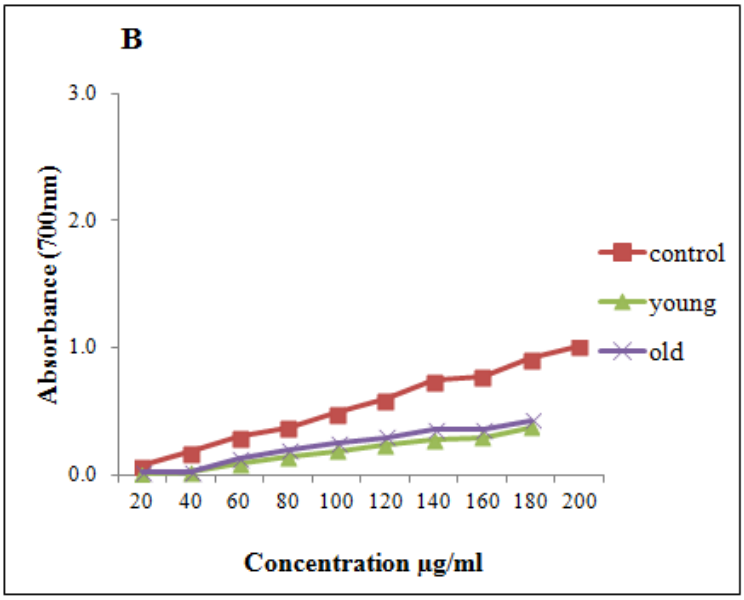


Fig. 6. Reducing capacity assessment of *T. chebula* (A), *Z. mays* hair (B)

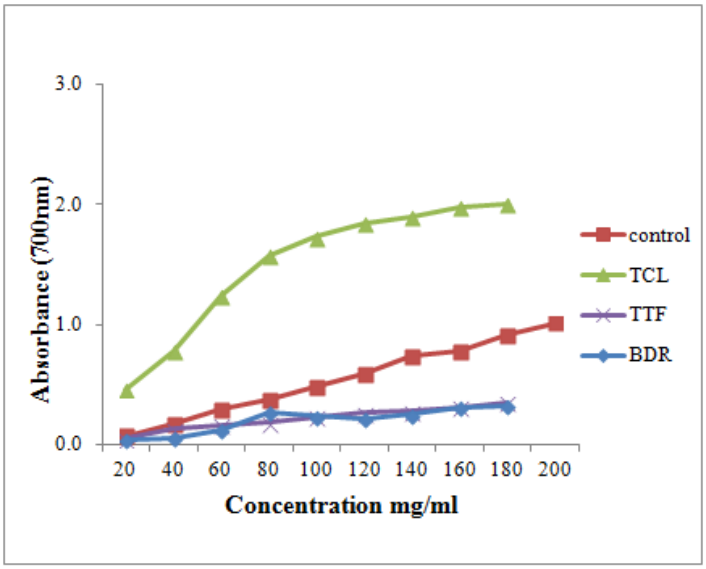


Fig. 7. Reducing capacity assessment of *T. catappa* leaf, *T. terresris* fruit and *B. diffusa* root (BDR).

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