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Evaluation of Antioxidant Activity *Terminalia belerica Roxb.*, *Terminalia chebula Ret* and *Emblica officinalis* Extracts Using Fenton reaction

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ABSTRACT

Free radicals and other reactive oxygen species like hydroxyl radical, superoxide anion, singlet oxygen and hydrogen peroxide (H_2O_2) can cause oxidative damages to biological macromolecules which can lead to initiation and/or progression of various diseases. Such as cellular and metabolic injury, cancer, atherosclerosis, inflammation, aging, immune suppression, diabetes, ischemic heart disease and neurodegenerative disorder such as Alzheimer's and Parkinson's disease. In the present study the methanolic extract of Emblica officinalis, Terminalia chebula and Terminalia bellirica Extracts was evaluated for antioxidant activity by the OH free radical scavenging activity using Fenton reaction. This study was conducted to investigate the effect of Emblica officinalis, Terminalia chebula and Terminalia bellirica Extracts extract using Fenton reaction. The dried leaf of all three plants extracted with methanol using a Soxhlet extractor. The total phenolics content of bark as determined by Fenton reaction and was found to be good antioxidant activity as dose depended manner. The antioxidant activity of plant extract was carried put with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer. All the plant extracts, there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. The result suggests that the all the plant extract can be used as food antioxidant together with the improvement of food palatability. Further studies are in processing of analyzing the synergic association of extract with synthetic antioxidant and to identify compounds with antioxidant activity in cinnamon extracts. The current study demonstrates and compares the antioxidant activity of the fruit extracts of Emblica officinalis, Terminalia chebula and Terminalia bellirica extracts may be a potential source of a natural antioxidants against the different types of free radicals.

Keywords: *Emblica officinalis, Terminalia chebula and Terminalia bellirica; hydroponic; extraction; phenolic; free radicals.*

INTRODUCTION

Oxidative stress plays an important role in the pathogenesis of various diseases such as atherosclerosis, alcoholic liver cirrhosis and cancer etc. Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion (O^{-2}), perhydroxy radical (HOO-) and hydroxyl radical (HO·). These radicals are formed by a one electron reduction process of molecular oxygen (O_2). ROS can easily initiate the lipid peroxidation of the membrane lipids, causing damage of the cell membrane of phospholipids, lipoprotein by propagating a chain reaction cycle (Braca, et al., 2002; Maxwell, 1995). Thus, antioxidants defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS. Most living species have efficient defense systems to prevent themselves against oxidative stress induced

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by ROS (Niki, 1994). Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases and aging process etc (Finkel, 2000). In this respect flavonoids and other polyphenolic compounds have received the greatest attention.

The fruits of *Terminalia chebula* Retz, *Terminalia belerica* Roxb, and *Emblica officinalis* Gaertn are widely used in the Indian traditional system of medicine (Chopra, 1956). The half ripe fruit of *T. belerica* and the pericarp of *T. chebula* fruit were reported to be purgative. The fruit of *T. chebula* was traditionally used to cure asthma, urinary disorders, heart disease and it has cardiotonic activity (Reddy, 1990; Lee, 2005). In Ayurveda, the fruit of *E. officinalis* is used as a cardiotonic, cerebral and intestinal tonic (Aslokar, 19992), and it is also reported to have anticancer properties (Rajarama, 1964). The fruit of *E. officinalis* is a rich source of vitamin C, a well-known antioxidant (Halliwell B, Gutteridge, 1985). The crude extract of *E. officinalis* was reported to counteract the hepatotoxic and renotoxic effects of metals due to antioxidant properties (Roy, 1991).

This present study is aimed to assess the antioxidant capacity of the 70% methanol extracts of *T. chebula*, *T. belerica* and *E. officinalis* fruits, through their measurement of activities in scavenging of different free radicals including hydroxyl, superoxide, nitric oxide, hydrogen peroxide, peroxynitrite, singlet oxygen, hypochlorous acid, phenol, flavonoid and ascorbic acid content and total antioxidant activity with Fenton reaction.

MATERIALS AND METHODS

Plant material – Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis was collected from Local Market, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 ^{0}C temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H_2O_2, 100 lM L-ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38 ºC, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbance's were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

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The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \text{Abs: } \frac{532 \text{ nm Control Abs. - 532 nm sample Abs.} \times 100}{532 \text{ nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

RESULT

The results of the effects of the examined Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis extract as well as control solutions on OH- radical production. They show that all extract of Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis *extract* and control solutions as a DMSO inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

Table 1–Antioxidant activities of Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis extract using Fenton reaction

Concentration (µl)	% Inhibition			
	Ascorbic acid (mean±SE)	*T. belerica* (mean±SE)	*T. chebula* (mean±SE)	*E. officinalis* (mean±SE)
10	6.128±0.671	7.312±0.775	5.699±0.654	6.451±0.372
20	6.021±0.654	16.881±0.775	11.720±0.955	9.247±0.468
30	14.731±1.059	36.667±0.937	35.484±0.853	12.903±0.671
40	16.881±1.551	41.183±0.654	37.527±2.076	21.505±0.957
50	33.548±1.304	42.150±0.468	44.946±1.025	30.215 ±0.840
60	38.387±0.671	51.290±1.406	46.021±0.937	36.451±1.037
70	64.086±0.654	58.413±0.858	58.387±0.558	47.742±0.558
80	69.355±1.655	60.968±1.163	56.882±0.387	55.914±0.388
90	78.387±1.342	69.677±1.163	69.462±1.240	68.172±0.468
100	80.645±0.372	80.860±0.839	72.473±1.026	71.935±0.985

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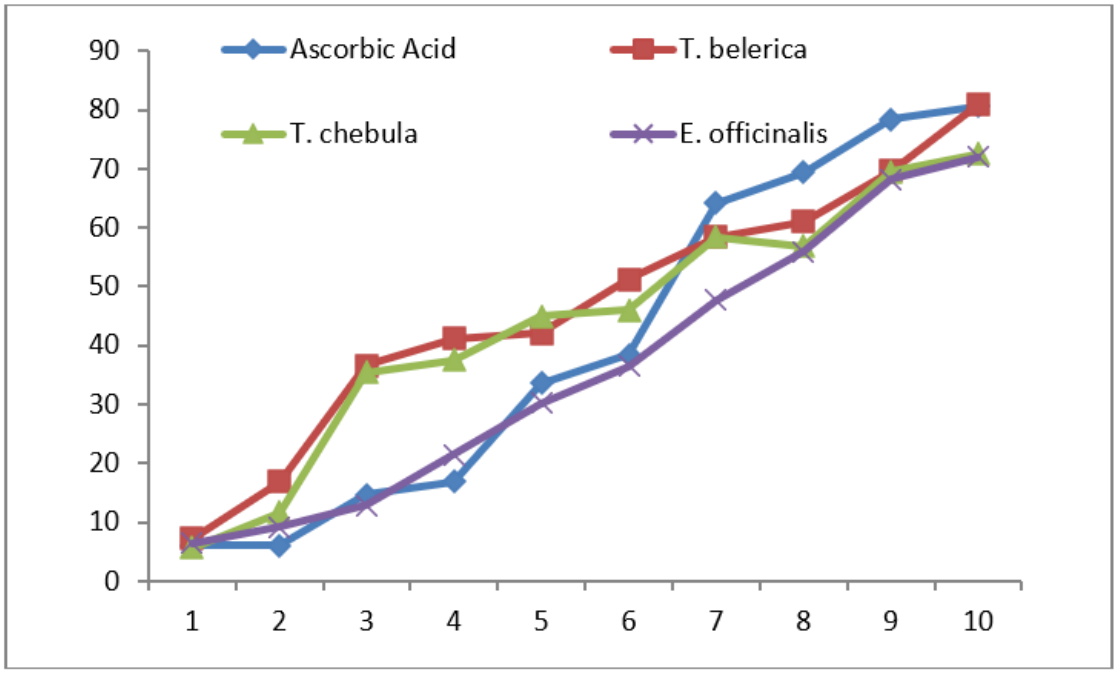

Fig. 1 Antioxidant Activity of Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis extract

DISCUSSION

Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. Keeping in mind the adverse effects of synthetic antioxidants, researchers have channelled their interest in isolating natural antioxidants (Kuo, et al., 2005) which are very effective to control the oxidative stress and hence prevent the initiation of disease propagation. Interestingly, quite a few studies on the antioxidant properties of the three plant materials, viz., *T. chebula* (Cheng, et al., 2003; Chattopadhyay, 2007), *T. belerica* (Sabu, et al., 2000 and 2009) and *E. officinalis (*Bhattacharya, 1999; Liu, 2008) have been done earlier. However, this study provides a definitive report about the free radical scavenging capacity of *T. chebula*, *T. belerica* and *E. officinalis*, since the antioxidant activity of a drug may depend on the free radical scavenging activity (Liu, 2008). The most detrimental of the free radicals formed in biological systems is the hydroxyl radical that causes enormous damage on biomolecules of the living cells (Ng, et al., 1987). As the extracts or standard mannitol is added to the Fenton reaction mixture the hydroxyl radicals are scavenged and thereby sugar damage can be blocked. The results, as can be found from Figure 1 and Table 1, indicate that the fruit extracts are better hydroxyl radical scavengers than standard mannitol, with *T. chebula* being the best in comparison to *T. belerica* and *E. officinalis*. Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis can be considered a model herbal drug for experimental studies. The material may also be useful for free radical induced disorders such as paracetamol toxicity, heavy metal and radiation toxicity.

CONCLUSION

The results from various free radical scavenging systems revealed that all the fruit extracts were individually strong antioxidants, with some varying scavenging activities for different ROS at different magnitudes of potency. Furthermore, evaluation of in vivo antioxidant activity of these fruit extracts has also provided interesting results that might be beneficial for

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the pharmacological use of these plants in clinical trials. The wide use of these fruits in the Indian indigenous system of medicine as anti-inflammatory and antihepatotoxic may be in part due to their antioxidant potency. Further, the isolation of the compounds responsible for the antioxidant activity has to be taken up which may result in modern drugs from these plants. Also the studies on antioxidant activity of the well known Ayurvedic formulation, Triphala, a mixture of these fruits, should be carried out and that is in progress. Thus our results were congruent with the findings of others. Further studies can be designed to prove the antioxidant activity of Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis in experimental animal models and also an attempt can be made to analyze the phenolic antioxidants present in it.

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