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Antioxidant and Free Radical Scavenging Activity of Triphala Determined by using Different *in vitro* Models

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ABSTRACT

Triphala is a popular polyherbal drug, which has been used to treat long list of diseases in the traditional systems from the ancient times. Acharya Charaka mentioned in his text as daily consumption of the Triphala for a period of one year, is act like Rasayana, makes a person live for hundred years. Triphala is a composite mixture of three herbs Amalaki (Emblica officinalis), Haritaki (Terminalia chebula) and Vibhitali (Terminalia belerica) also known as the 'three myrobalans'. Emblica officinalis Gaertn. belongs to Euphorbiaceae, and Terminalia chebula Retz. Terminalia bellerica belongs to Combretaceae family. The generic name 'Terminalia' comes from Latin word 'terminus' or 'terminalis' (ending), and refers to the habit of the leaves being crowded or borne on the tips of the shoots. Throughout the world, there are lot of studies carried out on this, well established the knowledge and documented. Triphala is rich in Vitamin-C, gallic acid, ellagic acid, chebulic acid, bellericanin, β-sitosterol and Flavonoids etc and a potent laxative, immumo modulator, antioxidant, antimicrobial, traditionally been used in eye diseases, stress, arthritis, colon diseases, etc. Present paper deals with antioxidant effect of individual plants and its combination (Triphala). The total phenolics content of powder as determined by Fenton reaction and was found to be good antioxidant activity as dose depended manner. The antioxidant activity of Triphala extract was carried put with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer. Triphala 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. Triphala ethanolic extract exhibited potent free radical scavenging activity. The overall antioxidant activity is attributed to its polyphenolic and other phytochemical constituents. The findings suggest that "Triphala" could be a potential source of natural antioxidant in preventing or slowing the progression of aging and age-associated oxidative stress-related degenerative diseases.

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

INTRODUCTION

The fruits of Terminalia belerica Roxb., Terminalia chebula Ret., and Emblica officinalis Gaetn. Are widely used in the Indian traditional system of medicine (Chopra et al., 1956; Kirtikar and Basu, 1991). The popular ayurvedic formulation Triphala is comprised of these three active constituents and is used as an anthelmintic and purgative (Anonymous, 1952, 1976). Triphala also forms a part of many other ayurvedic formulations.

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The half ripe fruit of T. belerica and the pericarp of T. chebula fruit were reported to be purgative (Chopra et al., 1956). The fruit of T. chebula was traditionally used to cure asthma, urinary disorders, heart disease and it has cardiotonic activity (Reddy et al., 1990). In Ayurveda, the fruit of E. officinalis is used as a cardiotonic, cerebral and intestinal tonic (Aslokar et al., 1992), and it also is reported to have anticancer properties (Rajarama Rao and Siddiqui, 1964; Aslokar et al., 1992). The fruit of E. officinalis is a rich source of vitamin C (Anonymous, 1952), a well-known antioxidant (Halliwell and Gutteridge, 1985a). The other two constituents of Triphala, namely T chebula (Anand et al., 1994) and T. belerica (Fu Naiwu, 1992), were found to prevent microsomal lipid peroxidation. The crude extract of E. officinalis was reported to counteract the hepatotoxic and renotoxic effects of metals (Roy et al., 1991) due to antioxidant properties. However, a definitive report about the free radical scavenging capacity of Triphala and its constituents is not available. Since the therapeutic activity of a drug may depend on its free radical scavenging activity (Ng and Yeung, 1987), the present article investigates the free radical scavenging properties of Triphala and its constituents.

MATERIALS AND METHODS

Plant material – Terminalia belerica Roxb., Terminalia chebula Ret and Emblica officinalis was collected from Local Herbal Garden, 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 (**Triphala)** (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 Triphala 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. 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.

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RESULT

The results of the effects of the examined *Triphala* extract as well as control solutions on OH- radical production. They show that all extract of *Triphala extract* and control solutions as a DMSO inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Triphala* 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 Triphala extract using Fenton reaction

Constrictions (in µl)	% of Inhibition	
	Ascorbic Acid	Triphala *extract*
10	22.71	15.18
20	38.13	23.13
30	46.12	32.30
40	52.30	44.40
50	61.35	50.10
60	73.10	62.63
70	79.52	66.41
80	82.28	71.21
90	86.17	76.28
100	90.42	87.85

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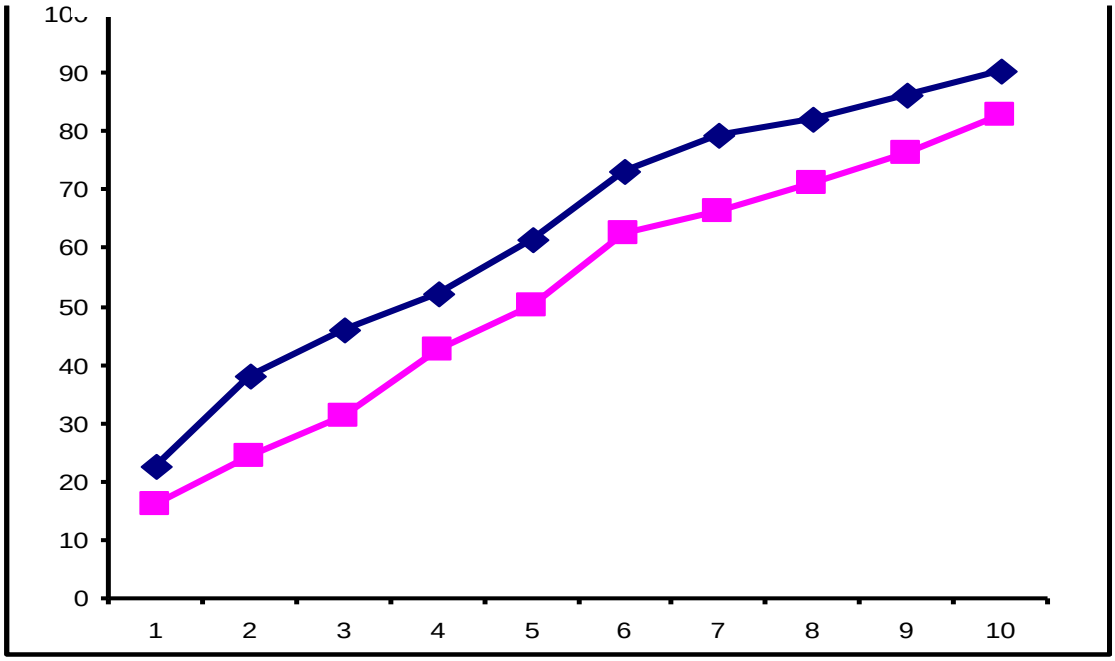

Fig. 1 Antioxidant Activity of Triphala extract

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DISCUSSION

The body's innate mechanism has many enzymes and nonprotein compounds that protect it from the free radicals and reactive oxygen species produced inside the body during normal metabolism and also due to external stimuli. Major compounds include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione, which also play a major role in detoxification and coordinate the body's antioxidant defense processes. The superoxide dismutase is a metalloprotein that scavenges superoxide anions. Catalase is a heme protein, localized in the peroxisome or the microperoxisome, which catalyzes the decomposition of H2O2 to water and oxygen and thus protects the cell from oxidative damage produced by H_2O_2. The glutathione peroxidase catalyzes the reaction of hydroperoxides, which reduces glutathione to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxide. Glutathione reductase is involved in the regeneration of glutathione that has been converted to GSSG by oxidation and thiol transfer reactions. Glutathione, a major nonprotein thiol, is mainly involved in detoxification (Halliwell & Gutterridge, 1985).

The current study indicates that the effects of the examined *Triphala* extract as well as control solutions on OH- radical production. They show that all extract of *Triphala extract* and control solutions as a DMSO inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Triphala* presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

Drugs that contain radical scavengers are well known for therapeutic activity (Yan et al., 1992; Liu and Xiao, 1994). Certain plants exhibit efficient antioxidant properties due to their phenolic constituents (Toda et al., 1991). Phytochemical analysis of Triphala (Table 1) supports some previous observations for the presence of tannins. Tannin content was reported to be 21% in Terminalia belerica, 30-32% in Terminalia chebula and 28% in Emblica officinalis (Anonymous, 1952, 1976). Tannins (Hong et al., 1995), phenols (Toda et al., 1991), lignans and flavonoids (Faure et al., 1991) are reported to have significant antioxidant properties. The results from various free radical scavenging systems revealed that Triphala as a whole and its constituents were individually strong antioxidants. Prevention of haemolysis and mitochondrial lipid peroxidation further confirmed that it is active against the effects offree radicals on biological membranes (Tables 5 and 6). The antioxidant properties of Triphala can therefore be attributed to the presence of tannins. The ECso values observed by DPPH reduction showed that the antioxidant activity of triphala exhibited synergism. It is known that crude extracts from plants are more active pharmacologically than their isolated active principles due to the synergistic effects of the various compounds present in the extracts (Hamburger and Hostettman, 1991). All the extracts oftriphala (TB, TC, EO and TR) were equally efficient in scavenging superoxide and peroxide radicals. Free radicals may cause damage in biological systems. They in turn, induce cellular damage (Halliwell and Gutteridge, 1985b) that may lead to cancer, rheumatism, liver injury, ischemic heart disease as well as ageing. Some therapeutic effects, such as antiinflammatory (Summanen et al., 1993), antimutagenic (Grover and Saroj Bala, 1992) and antihepatotoxic (Anand et al., 1994) effects of E. officinalis, T. chebula and T. belerica, respectively, used in the present study, could be due to their antioxidant properties. Use of plant extracts for disease treatment could be relatively harmless (Valenzuela et al., 1986) whereas certain synthetic antioxidants may induce toxic side effects (Williamson et al., 1978). Triphala can be considered a model herbal

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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

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners form one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. Triphala has been used for centuries against various ailments in the Indian traditional medicine system. Studies in the recent past have indicated that Triphala has immense potential in the reduction of oxidative damage as well as in the prevention and treatment of cancer. Few studies indicated that antioxidants from dietary supplements may promote tumor growth and metastasis. However, it is noteworthy that Triphala acts as an anticancer agent by exhibiting prooxidant effects in cancer cells. The dual nature of Triphala, acting as an antioxidant in normal cells and prooxidant in cancer cells, facilitates its function as both a chemopreventive and chemotherapeutic agent. Interestingly, Triphala has shown high efficacy and safety in humans as well as in experimental studies. Thus our results were congruent with the findings of others. Further studies can be designed to prove the antioxidant activity of Triphala in experimental animal models and also an attempt can be made to analyze the phenolic antioxidants present in it.

REFERENCES

1) Anand K.K., Singh E., Saxena A.K., Chandan B.K. and Gupta V.N. (1994) Hepatoprotective studies of a fraction from the fruits of Terminalia belerica Roxb. on experimental liver injury in rodents. Phytother. Res. 8: 287-292.
2) Anonymous (1952) The Wealth of India. Raw Materials, Vol. 3, 168 pp., CSIR, New Delhi, India. Anonymous (1976) The Wealth of India. Raw Materials, Vol. 10, pp. 168-170, CSIR, New Delhi, India.
3) Aslokar L., Kakkar K.K. and Chakre O.J. (1992) Supplement to Glossary ofIndian Medicinal Plants with Active Principles pp. 291-293. Directorate CSIR, New Delhi, India.
4) Beauchamp C. and Fridovich I. (1971) Superoxide dismutase: Improved assays and an assay applicable to aerylamide gels. Anal. Biochem. 44: 276-287.
5) Campos R., Garrido A., Guerra R. and Valenzuela A. (1989) Silybin dihemisuccinate protects against glutathione depletion and lipid peroxidation induced by acetaminophen on rat liver. Planta Med. 55: 417--419.
6) Chopra R.N., Nayar S.L. and Chopra I.C. (1956) Glossary of Indian Medicinal Plants, p. 106, 241, 242. CSIR, New Delhi, India. Faure M., Lissi E., Torres R. and Videla L.A. (1991) Antioxidant activities of lignans and flavanoids. Phytochemistry 29: 3773-3775.
7) Fu Naiwu, Quan Lamping, Huang Lei, Zhang Ruyi and Chen Yayan (1992) Antioxidant action of extract of Terminalia chebula and its preventive effect on DNA breaks in human white cells induced by TPA. Chinese Traditional and Herbal Drugs 23: 26-29.
8) Grover I.S. and Saroj Bala (1992) Antimutagenic activity of Terminalia chebula in Salmonella typhimurium, Indian J. Exp. Biol. 30: 339-341. Halliwell B. and Gutteridge J.M.C. (1985a) Free radicals, ageing and disease. In: Free Radicals in Biology and Medicine. pp. 279-315, Clarendon, Oxford.
9) Halliwell B, Gutterridge JMC (1985): Free Radicals in Biology and Medicine. Oxford, Clarendon Press, pp. 107–170, 627–630.

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ISSN: 2582-8401

10) Halliwell B. and Gutteridge J.M.C. (1985b) Protection against oxygen radicals in biological systems; the superoxide theory of oxygen toxicity. In: Free Radicals in Biology and Medicine. pp. 100-103, Clarendon Press, Oxford.
11) Hamburger M. and Hostettmann K. (1991) Bioactivity in plants. The link between phytochemistry and medicine. Phytochemistry. 30: 3864-3874.
12) Hong C.Y., Wang C.P, Huang S.S. and Hsu F.e. (1995) The inhibitory effect of tannins on lipid. peroxidation of heart mitochondria. 1. Pharm. Pharmacal. 47: 138-142.
13) Kimura Y., Okuda H., Okuda T., Hatono T., Agata I. and Shigeru A. (1984) Studieson the activities of tannins and related compounds; VI Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. Planta Med. 50: 473--476.
14) Kirtikar K.R. and Basu B.D. (1991) Indian Medicinal Plants Vol. II, p. 1017, 2220. Lalit Mohan Basu, Allahabad, India. Liu 1. and Xiao P.G. (1994) Recent advances in the study of antioxidative effects of Chinese medicinal plants. Phytother. Res. 8: 445--451.
15) Navarro M.C., Montilla M.P., Martin A., Jimenez J. and Utrilla M.P. (1993) Free radical scavenger and antihepatotoxic activity of Rosmarinus tomentosus. Planta Med. 59: 312-314.
16) Ng T.B. and Yeung H.W. (1987) Scientific basis of the therapeutic effects of Ginseng. In: Folk Medicine. The Art and the Science, Steiner R.P., ed., pp. 139-152., American Chern. Soc. Publ. USA.
17) Rajarama Rao M.R. and Siddiqui H.H. (1964) Pharmacological studies on Emblica officinalis Gaertn. Indian 1. Exp. BioI. 2: 29-31.
18) Reddy V.R.e., Kumari S.V.R., Reddy B.M., Azeem M.A., Prabhakar M.e. and Appa Rao A.V.N. (1990) Cardiotonic activity of the fruit of Terminalia chebula. Fitoterapia LXI: 517-525.
19) Roy A.K., Dhir H. and Talukdar G. (1991) Phyllanthus emblica fruit extract and ascorbic acid modify hepatotoxic and renotoxic effects of metals in mice. Int. J. Pharmacog. 29: 117-126.
20) Sreej ayan and Rao M.N.A. (1994) Curcuminoids as potent inhibitors of lipid peroxidation. J. Pharm. Pharmcol. 46: 1013-1016.
21) Summanen J., Kankaanranta H., Moilanen E., Asmawi M.Z., Vapaatalo H., Vuorela H. and Hiltunen R. (1993) Antiinflammatory activity of leaf extracts of Emblica officinalis. Planta Med. 59 (Supp1.): A 666.
22) Toda S., Kumura M. and Ohinishi M. (1991) Effect of phenolcarboxylic acids on superoxide anion and lipid peroxidation induced by superoxide anion. Planta Med. 57: 8-10.
23) Uchiyama M. and Mihara M. (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86: 271-278.
24) Valenzuela A., Guerra R. and Videla L.A. (1986) Antioxidant properties of the flavonoids silybin and (+)-cyanidanol: Comparision with butylated hydroxyanisol and butylated. hydroxytoluene. Planta Med. 52: 438-440.
25) Williamson D., Esterez P. and Witschi H.P. (1978) Studies on the pathogenesis of butylated hydroxytoulene induced lung damage in mice. Toxicol. Appl. Pharmacal. 43: 557-587.
26) Yan J., Gyogan Z., Long C. and Ma Xingynan (1992) Influence of ginsenosides Rb 1, Rb 2 and Rb 3 on electric and contractile activities of normal and damaged cultured myocytes. Acta Pharmacolog. Sin. 13: 403--406.