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Antioxidant Effect of *Triphala* - Critical Review

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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*).

Key words: *Triphala, three myrobalans, antioxidant.*

INTRODUCTION

Oxidative stress is an imbalance between oxidant level i.e., pro-oxidants and antioxidants within the body. It results when harmful free radicals are generated in excess and body's natural antioxidants ability to detoxify them is perturbed.[1] This leads to inordinate formation and/or scanty removal of these free radicals which are mainly classified as Reactive oxygen species (ROS) and Reactive nitrogen species (RNS).[2] When an excess of free radicals is formed, they can overwhelm protective enzymes such as superoxide dismutase, catalase and peroxidase and cause destructive and lethal cellular effects (e.g., apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration. Further more, reactive oxygen species seem to influence cell signalling pathways in ways that are only now being unravelled.[3],[4] Oxidative stress plays a causal role in several pathological conditions in the body and targeting them with antioxidants has shown to reduce the deleterious effects of ROS.[5] This makes different molecules having antioxidant activity, as the savior in such stressed situation. Such molecules have been found to counteract the harmful effect that occurs due to increased ROS in various disorders like heart disease, Alzheimer disease, aging and many others by removing free radical intermediates and inhibiting oxidation of other molecules.[6],[7],[8] An antioxidant may be defined as 'any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate.[9] Antioxidants have been traditionally divided into two classes, primary or chainbreaking antioxidants and secondary or preventative antioxidants.[10]

Human body possesses natural antioxidants to control the level of ROS by scavenging them both

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enzymatically and nonenzymatically.[11] Enzymatic pathways include a platter of enzymes namely SOD, CAT, GPx, GR etc. Different nonenzymatic antioxidants include various vitamins (like vitamin A, C, E, carotenoids, α-lipoic acid, etc.), trace elements [like coenzyme Q10 (CoQ10), copper, selenium, zinc, etc.] and cofactors (like uric acid, folic acid, albumin, etc.). Antioxidant enzymes either readily converts the reactive free radical O2 - into H2O2 or activates intracellular pathways to regenerate other antioxidative metabolites.[12] These metabolites then react with the reactive radicals and change them into the stabilized form. Other than this metabolites or enzymes, certain genes (e.g., nuclear factor erythroid 2-related factor 2 (Nrf-2) and heme oxygenase-1 (HO-1)) and their end products are also capable in maintaining cellular homeostasis in oxidative stress related organ pathophysiological conditions.[13] In addition to the ROS scavenging properties, exogenous antioxidant molecules are also capable of modulating different gene expression to improve the oxidative stress.[14],[15],[16]

Table 1 - Regional names of ingredients of *Triphala.*

***Emblica officinalis*[17]**	***Terminalia chebula*[18]**	***Terminalia bellerica*[19]**
Assamese: *Amlakhi, Amlakhu, Amlaku.*	Assamese: *Shilikha*	Assamese: *Bhomora, Bhomra, Bhaira.*
Bengali : *Amla, Dhatri.*	Bengali: *Haritaki*	Bengali: *Bayada, Baheda*
English: Emblic Myrobalan	English: Myrobalan	English: Beleric Myrobalan
Gujrati: *Ambala, Amala*	Gujrati: *Hirdo, Himaja, Pulo-harda*	Gujrati: *Bahedan*
Hindi: *Amla, Aonla*	Hindi: *Harre, Harad, Harar*	Hindi: *Bahera*
Kannada: *Nellikayi, Bela nelli, pottadenollikayi.*	Kannada: *Alalekai*	Kannad: *Tare kai, Shanti Kayi*
Kashmiri: *Amli, Embali*	Kashmiri: *Halela*	Kashmiri: *Babelo, Balali*
Malayalam: *Nellikka*	Malayalam: *Katukka*	Malayalam: *Tannikka*
Marathi: *Anvala, Avalkathi*	Marathi: *Hirda, Haritaki, Harda, Hireda*	Marathi: *Baheda*
Oriya: *Ainla, Anala*	Oriya: *Harida*	Oriya: *Baheda*
Punjabi: *Aula, amla*	Punjabi: *Halela, Harar*	Punjabi: *Bahera*
Tamil: *Nellikkai, nelli*	Tamil: *Kadukkai*	Tamil: *Thanrikkai*
Telugu: *Usirika*	Telugu: *Karaka, Karakkaya*	Telugu: *Thanikkaya*
Urdu: *Amla, Amlaj*	Urdu: *Halela*	Urdu: *Bahera*

Chemical constituents[20]

Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in the plant extracts. HPLC analysis and Folin-Ciocalteau and Foli Denis method showed that *Triphala* contains 38±3% polyphenols and 35±3% tannins. *Triphala* contains sufficient gallic acid and hence can be used as a marker compound for in-vivo studies. HPLC studies reveal the presence of four phenolics gallic acid (0.026% w/w), tannic acid (0.024% w/w), syringic acid (0.016% w/w) and epicatechin (0.013% w/w) along with ascorbic acid (0.036% w/w) in *Triphala*. E.officinalis contained ascorbic acid (0.026%), gallic acid (0.081%), T.bellirica contained gallic acid (0.005% w/w), tannic acid (0.004% w/w) and ascorbic acid

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(0.023%), while T.chebula contained gallic acid (0.024% w/w), tannic acid (0.011% w/w), syringic acid (0.009% w/w) and epicatechin (0.0060% w/w) together with ascorbic acid (0.02%).*Triphala* contains numerous other phenols.

Pharmacological effects

Numerous experimental evidences have shown that *Amla* fruit possess antioxidant, hepato-protective, hypocholesterolemic and antiinflammatory activities,[21] gastroprotective.[22] *Terminalia chebula* is potent antibacterial, anticaries, anticancer, antimutagenic agent and also inhibits occurrence of local anaphylaxis. *Terminalia bellerica* is laxative, antihelmenthic. These compounds are believed to be responsible for the pharmacological activities such as antimicrobial, antioxidant, antisalmonella, hepatoprotective, antispasmodic and bronchodilatory activities.[23]

DISCUSSION

Antioxidants are the class of compounds which can potentially inhibit the oxidation of other intracellular molecules, protecting our body from the detrimental effects of free radicals.[24] Free radicals are the electron deficient, highly reactive molecules that can oxidize the cellular macromolecules causing severe damage to subcellular organelles.[25],[26] Antioxidants act through different mechanisms at various sites exerting a number of biochemical effects.

There are a wide range of antioxidants are available in nature, few of them are included enzymes (Superoxide dismutase, catalase, glutathione peroxide etc), minerals (Zinc, selenium, copper, manganese), Vitamins (Vitamin A, C and E) high molecular weight compounds (albumin, transfererin, ceruplasmin), low molecular weight compounds (lipid soluble antioxidants – tocopherol, quinines, bilirubin, water soluble antioxidants – ascorbic acid, uric acid etc). Plant origin antioxidants include *Amalaki*, *Guduchi*, *Yastimadhu*, *Bakuchi*, *Katuki* etc. Plants having vitamins, flavonoids, polyphenols etc possess remarkable antioxidant activity. In the recent past, there has been growing interest in exploiting the biological activities of different Ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects.[27],[28] In Ayurveda formulation of some *Rasayanas* with well defined antioxidant properties has been done.[29],[30] *Rasayanas* are a group of non toxic polyherbal drug preparations, which are immunostimulatory and thereby prevent the causation of disease[31] and promote health and longevity.[32] *Triphala* has been tested as an antioxidant and also as a radioprotector in mice.[33],[34] *Triphala* is gentle for people of all ages, and recommended for everybody.[35]

Antioxidant effect of *Emblica officinalis*

Vasudha shukla *et. al.,* demostrated Aqueous and alcoholic extracts of amalki (Emblica officinalis), spirulina and wheatgrass were prepared and analyzed for antioxidant vitamin content (vitamin C and E), total phenolic compounds. Antioxidant status, reducing power and effect on glutathione S-transferase (GST) activity were evaluated in vitro. Vitamin C content of crude amalaki powder was found to be 5.38 mg/g, while very less amount 0.22 mg/g was detected in wheat grass. Amalki was rich in vitamin E like activity, total phenolic content, reducing power and antioxidant activity. Total antioxidant activity of aqueous extract of amalki, spirulina and wheat grass at 1mg/ml concentration were 7.78, 1.33 and 0.278 mmol/l respectively. At similar concentrations the total antioxidant activity of alcoholic extract of amalaki, spirulina and wheat grass was 6.67, 1.73 and 0.380 mmol/l respectively.[36]

Reddy *et. al.,* suggested that the amelioration of alcohol-induced oxidative stress might be due to the combined effect of phytophenols such as tannins, flavonoid compounds and Vitamin-C.[37] Shivananjappa et. al., demonstrated that E. officinalis increased the levels of GSH, antioxidant capacity and activities of antioxidant enzymes (SOD; CAT; GSH peroxidase; GSH reductase; and GSH S-transferase).[38] Additionally, when administered once daily for 7 days the active tannoids of E. officinalis induced a rise in both frontal cortical as well as striatal SOD, CAT and GSH peroxidase (GPX) activity, with associated reduction in lipid peroxidation in these brain areas. The results also specify that the antioxidant activity of

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E. officinalis may reside in the tannoids of the fruits of the plant, which have vitamin C-like properties, rather than vitamin C itself.[30]

Antioxidant effect of *Terminalia bellerica*

Ramesh Kumar *et. al*., (2011) postulated that the crude aqueous extract of the fruits of Terminalia belerica Roxb have antioxidant properties since these contains enzymatic and non – enzymatic antioxidants, these can be very effective against microbes causing various diseases. In vitro assessment of the antioxidant activity of ethanolic fractions of both these plants to scavenge 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) and highly reactive hydroxyl radicals showed that the semi pure compounds present in the fractions are useful potential source of antioxidants and can be used in the therapy of diseases like cancer, coronary heart disease, ageing and any other disease related to oxidative stress. These fractions being non-toxic showed significant antioxidant activity at scavenging free radicals. They also significantly scavenge hydroxyl radical which is known to cause cellular damage.[39]

Antioxidant effect of *Terminalia chebula*

The antioxidant activity of an aqueous extract of the crude drug, as estimated by thiobarbituric acid reactive substances, was tested by studying the inhibition of radiation-induced lipid peroxidation in rat liver microsomes at different doses in the range of 100–600 µg/ml. The IC50 in this assay was 14.5 µg/ml. The extract was also found to restore the antioxidant enzyme superoxide dismutase following radiation-induced damage. The median inhibitory activity of the extract was 11.5 µg/ml in the 1, 1- diphenyl-2-picrylhydrazyl radical scavenging assay.[32]

Antioxidant activity of *Triphala*

Methanolic extract of *Triphala* (70%) has shown high antioxidant activity in the in-vitro studies. Some reports have shown the radio- protective activity of *Triphala* in mice exposed to gamma radiation.[34] Two studies G.H. Naik *et. al.*, suggest that *Triphala* exhibits antioxidant activity under γ-irradiation conditions. Under these conditions, damage to biomolecules is initiated by the free radicals produced by the radiolysis of water. Hence it is appropriate to study their free radical scavenging ability. The antioxidant and radioprotecting ability of *Triphala* arises from the polyphenols, which reduce oxidative stress by converting the reactive oxygen free radicals to non-reactive products. The radical scavenging experiments were carried out by using fast reaction kinetic tools like pulse radiolysis and stopped flow technique and the reactivity of *Triphala* towards different radicals such as hydroxyl radicals, superoxide radicals, DPPH and ABTS− were determined. *Triphala* was found to be an effective scavenger of DPPH and superoxide radicals.[40] The ferric-reducing antioxidant power (FRAP) assay measures the antioxidant potentials of "antioxidants" to reduce the Fe^{3+}/ 2, 4, 6-tripyidyl-s-triazine (TPTZ) complex present in stoichiometric excess to the blue colored Fe^{2+}-TPTZ form.[41]

Triphala is effective in inhibiting ϒ-radiation induced damage in microsomal lipids and plasmid pBR 322 DNA. *Triphala* is rich in polyphenols (38±3%) and tannins (35±3%). Polyphenolic contents in *Triphala* are responsible for the antioxidant and radioprotecting ability, reduce the oxidative stress by converting reactive oxygen free radicals to non-reactive products.[20] *Triphala* significantly prevents cold-stress induced oxidative stress. Cold stress induced oxidative stress is measured by Lipid Peroxidation (LPO), enzymatic Superoxide Dismutase (SOD), Catalase (CAT), non-enzymatic (Vitamin C) antioxidation status. Administration of *Triphala* (1g/Kg/body weight/48 days) prevents Cold Stress induced oxidative stress and elevation in LPO and Corticosterone levels. The antioxidant property can be correlated to prevention of cold stree induced oxidative stress.[42] *Triphala* and the individual ingredients of *Triphala* effectively inhibit ϒ-radiation induced strand break formation in plasmid DNA. They inhibit radiation induced lipid peroxidation and possess ability to scavenge free radicals like DPPH and superoxide. *Triphala* mixture is more effective as it possess combined activity of all the three ingredients.[43] Superoxide radical scavenging activity of *Triphala* using xanthine and xanthine oxidase activity showed that in addition to reacting with superoxide radical, *Triphala* also

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inhibited uric acid formation. *Triphala* is rich in phenols/polyphenols (38±3%), tannins (35±3%), flavonoids were absent. HPLC analysis revealed that gallic acid content was 73±5 mg/g and increased to 150±5mg/g upon acid hydrolysis.[44],[45]

Work of Painuli *et. al.* Shows that Gallic acid either in free form or conjugated form together with other unidentified compounds possessed antibacterial activity (extracts being bactericidal in nature), being a potent antioxidant (scavenging free-radicals) and also cytoprotective in nature. The genetically and enzymatically in-built phytosignatures of *Triphala* have been found as free radical scavengers and bactericides that viably suites to manage and contain the oxidative stress and bacteria that are directing ophthalmic disorder(s) invitro and are safely extendable invivo. It further opens the arena for exploring the active phytosignatures present in herbal plants to be used as a growth rejenuvator or for obtaining the growth regulatory compounds. Antimicrobial, antioxidant activity, viability and better proliferation of cells in the presence *Triphala* extract, provides the concentration that could be used in formulation of new herbal eye drug. These plant-based formulations with zero side effects are safer clinically than the steroid and penicillin based eye drop formulation with various side effects.[21]

CONCLUSION

Antioxidants are the compounds which can potentially inhibit the oxidation of the intracellular molecules, protecting the body from the effects of free radicals. Three ingredients of *Triphala* is having antioxidant property and become more potent when it used a compound. Because of multi action of *Triphala* it is used in so many diseases. Methanolic extract of *Triphala* has shown high antioxidant activity in the in-vitro studies.

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