

In-vitro Study of Triphala on Antioxidant Activity

ABSTRACT : *Triphala (combination of aqueous extract of Amlaki, Haritaki & Bahera) is extensively used as household remedy in India and South East Asia from ancient time. Triphala used for biliary disorders, diarrhea, dyspepsia, cough, renal problems, and hepatic disorders. For the last few decades it's anti-inflammatory, anticancer and antioxidant roles may be clinically established in rheumatism, carcinogenesis and oxidative stress-related pathogenesis. In-vitro study on the Total antioxidant activity of triphala & its constituent gave very good results.*

Indian system of medicine has rich history of using plants in medicinal purpose. Triphala is the combination of three medicinal plants extensively used in ayurveda from ancient time. These three plants are Amlaki *Phyllanthus emblica* (syn. *Emblica officinalis*) Phyllanthaceae family, Haritaki (*Terminalia chebula*) Combretaceae family & Bahera (*Terminalia bellirica*) Combretaceae family. Triphala is an important ayurvedic formulation used in the treatment of liver & kidney dysfunction.

One of the best medicinal plants acknowledged by mankind is Amlaki. It maintains a glowing state of skin and reduces wrinkle formations. It is a very useful tool for improving the body's immunity as it readily promotes the body's ability to form antibodies in order to fight any invasion of antigens. It is large deciduous tree with grey or red bark; leaves are pinnate, linear oblong- obtuse. Fruits depressed-globose, succulent, yellow or pink when ripe ⁽¹⁾.

Therapeutic Uses : Fruits are used as astringent, anti-diarrhoeal, beneficial for urinary infection, prescribed in anaemia, jaundice & dyspepsia ⁽¹⁾.

Chemical Composition of Amlaki : It is an excellent source for vitamin C & also contains carotene, nicotinic acid, D-glucose, D-fructose, riboflavin, embicol, mucic & phyllemblic acids. Phyllembin & some fatty acid. ⁽²⁾

Another important plant is Haritaki contains chebulinic acid; tannic acid & ellagic acid are the most growth inhibitory factors ⁽³⁾ which inhibits the rate of cell proliferation & cell death in cancer cell line. Antioxidant activity of the extract was indicated by reduced lipid peroxide levels in treated wounds. The extract was active against *Staphylococcus aureus* & *Klebsiella sp.* microorganism ⁽⁴⁾. 10% aqueous solution of that extract

inhibited glycolysis of salivary bacteria for upto 90 minutes post rinsing ⁽⁵⁾. It is a large tree; young branchlets, leaf buds & leaves with long, soft shining, rust- coloured, sometimes silvery hairs. Fruits ovoid or ellipsoidal from a broad base, more or less 5-ribbed when dry.

Therapeutic Uses : Fruits are used as astringents, laxative, stomachic tonic. It is also used in stomatitis, chronic ulcers; asthma, chronic diarrhea, dysentery & flatulence. ⁽⁶⁾

Chemical Composition of Haritaki : Anthraquinone glycoside, chebulinic acid, tannic acid, terchebin, vitamin C, arachidinic, linoleic, oleic, palmitic, & stearic acid etc. ⁽⁷⁾

The rest medicinal plant in Triphala is Bahera. It is a large tree with slight soft, rust coloured pubescence on young branchlets. Leaves are clustered at the end of the branchlets, broad elliptic or obviate- elliptic. Fruits ovoid grey velvety with more or less 5 indistinct furrows; it is thick & hard.

Therapeutic Uses : Fruits are useful in diarrhea, dyspepsia, cough, headache, hoarseness & eye disease. It is also used as antipyretic & antileprotic agent. ⁽⁸⁾

Chemical Composition of Bahera : It contains chebulagic acid, ellagic acid & its ethyl ester, gallic acid, fructose, galactose, glucose, mannitol & rhamnose etc. ^(9,10)

Triphala has been used in traditional medicine as a household remedy for various diseases, including biliary disorders, diarrhea, dyspepsia, cough, renal problems, and hepatic disorders. For the last few decades, extensive work has been done to establish the biological activities and pharmacological actions of triphala and its aqueous extracts. The bioactive components of triphala has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, antioxidant, anticarcinogenic, antibacterial, antiulcer activities. It's anti-inflammatory, anticancer and antioxidant roles may be clinically exploited to control rheumatism, carcinogenesis and oxidative stress-related pathogenesis.

A free radical may be defined as any species capable of independent existence that one or more unpaired electrons (Halliwell 1991). In recent years the term Reactive Oxygen Species (ROS) has adopted to include molecules such as hydrogen peroxide (H₂O₂), hypochlorous acid

TABLE 1: Percentage of Inhibition calculation of different doses of Triphala

| | % of inhibition | 1mg/ml | 2 mg/ml | 3 mg/ml | 4 mg/ml | 5 mg/ml | 6 mg/ml |
|----------|-----------------|--------|---------|---------|---------|---------|---------|
| Amlaki | (%) | 32 | 49 | 61 | 69.2 | 74.3 | 74.9 |
| Haritaki | (%) | 35 | 53 | 64.6 | 72.3 | 76.3 | 77 |
| Bahera | (%) | 20 | 29.4 | 56.2 | 65.8 | 69.2 | 69.8 |
| Triphala | (%) | 45.3 | 53.4 | 64.2 | 70 | 78.2 | 78.6 |

(HOCl) & singlet oxygen (O₂). ROS cause tissue damage by variety of different mechanisms, such as DNA damage, Lipid peroxidation, Protein damage, Oxidation of important enzymes e.g. anti protease enzyme. ⁽¹¹⁾

The difference types of toxic effects of the free radicals can be blocked by the antioxidants which either scavenge the free radicals or block their synthesis.⁽¹²⁾In-vitro study of Amlaki, Haritaki & Bahera individually & collectively in Triphala shows potent antioxidant activity either by scavenging or by inhibiting the free radicals.⁽¹³⁾

Materials & methods: Materials: Aqueous extract of the dry, grinded fruits of Amlaki, Haritaki, Bahera & Triphala (4fruits Amlaki: 2 fruits Haritaki: 1 fruit Bahera; dried & grinded) ⁽¹⁴⁾ as a whole are taken in different concentration. Then the test was performed taking the extract in a dose depending manner.

Method : Estimation of antioxidant activity of plant extract has been done by DPPH radical cation decolorisation assay (Molyneux P 2004). ⁽¹⁵⁾

The basic principle of this test is based on DPPH (Diphenylpicrylhydrazyl) scavenging activity on spectrophotometer. 100 µl of aqueous solution of sample is taken & add 1.9 ml of Ethanol & then add 1 ml DPPH (300mili Molar) solution. Total mixture incubated at room temperature for 20 minutes. Then the absorbance noted 517 nm (including Blank). Blank is prepared by above said method but without sample, instead of sample distilled water is used.

Calculation: % of inhibition (%)

$$= \frac{\text{O.D. (BLANK)} - \text{O.D. (SAMPLE)}}{\text{O.D. (BLANK)}} \times 100$$

O.D. = Optical Density.

Triplicate in each condition, and then take the mean value for % of inhibition DPPH scavenging activity.

Table No. 1 shows the antioxidant activity was expressed in inhibition of percentage(%) of DPPH

scavenging activity of Amlaki, Haritaki, Bahera & Triphala as a whole. It has been calculated by the Microsoft Exel Software, version 2007 for graph diagram. Fig.1, Fig2, Fig3 & Fig4 shows Amlaki, Haritaki, Bahera & Triphala as a

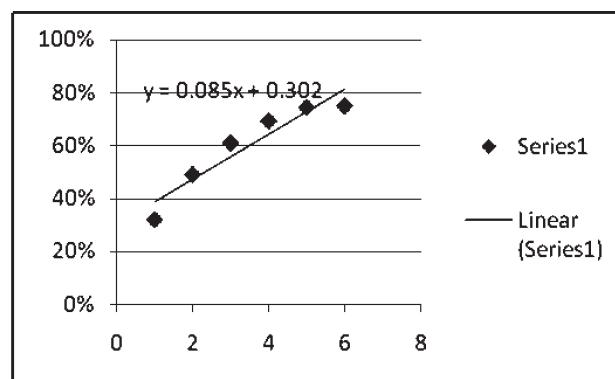


Fig. 1. Antioxidant activity of Amlaki.

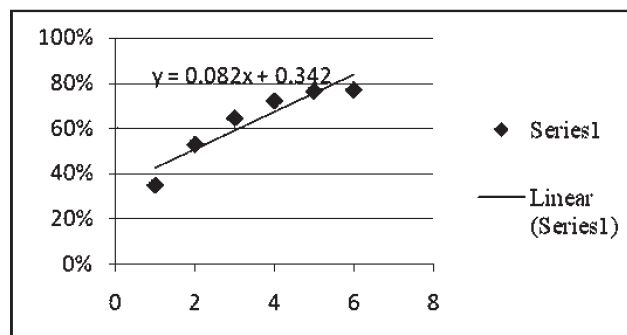


Fig. 2. Antioxidant activity of Haritaki .

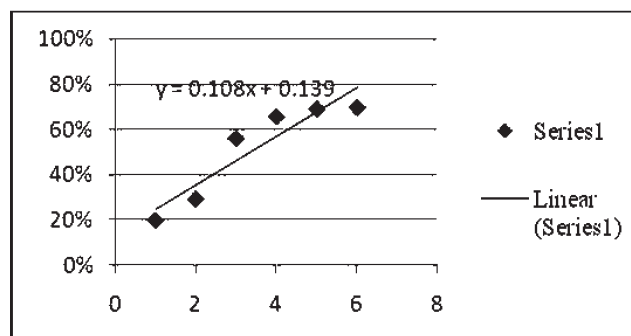


Fig. 3. Antioxidant activity of Bahera.

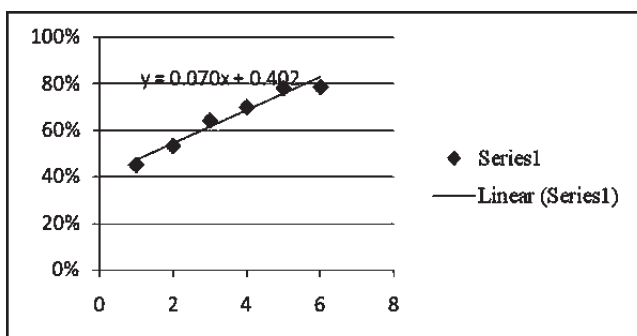


Fig.4 Antioxidant activity of Triphala

whole show good amount of antioxidant activity& this activity increases with the increasing concentration.

Discussion

Antioxidants slow down the process of excess oxidation and protect cells from the damage caused by free radicals. When cells are attacked by free radicals, excess oxidation causes damage and destroys cells. Antioxidants stop this process. The cellular damage caused by free radicals can be responsible for causing and/or accelerating many diseases. Triphala is rich in antioxidants and is recommended to guard against free radicals and protect from damaging excess oxidation.

The dry fruits of above mentioned three plants & triphala as a whole easily available in the market & is affordable for all socio- economic structure. Triphala used for biliary disorders, diarrhea, dyspepsia, cough, renal problems, and hepatic disorders from ancient time ⁽¹⁶⁾. This proven activity of antioxidant in this study will be very much helpful in modern medicine oxidative stress related diseases specially precancerous/premalignant conditions & thus helps in prevention of cancers.

Acknowledgement

Authors are thankful to the executive members of the

Antioxidant Society of India for their kind support to perform this study. □

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Received : 1 March, 2011

Revised 8 June, 2011

Re Revised 25 July, 2011

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