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COMPARATIVE ANTIOXIDANT EVALUATION OF ALCOHOLIC AND HYDRO-ALCOHOLIC EXTRACT OF *TRIPHALA*

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ABSTRACT
The objective of the present study was to evaluate the antioxidant potential of alcoholic and hydro-alcoholic extract of *Triphala*. The antioxidant activity was performed due to presence of phenolic compounds; flavonoids which may possess significant antioxidant potential. The results suggested that hydro-alcoholic extract (HAE) possess significant antioxidants potential as compared to alcoholic extract (AE), the antioxidants potential of extract was found to be comparable with the standard control, the activity of formulation may be attributed to its phyto-constituents. Finally study concluded that hydro-alcoholic extract of *Triphala* may be used as natural sources for scavenging free radicals.

KEYWORDS: Triphala, Free Radicals, Extract, Ayurveda.

INTRODUCTION
Free radicals are highly reactive chemical species contains unpaired electrons and can damage biological molecules. Various detoxification processes are responsible for the generation of free radicals in the human body. Ultraviolet light, metabolic processes and radiation can induce free radicals. Free radicals may reacts with various biological molecules such as; protein, lipid and DNA which leads process of tissue destruction along with various diseases. Antioxidants are the compounds either from synthetic or natural origin which reduce free radical activities and thus used for the treatment of many diseases induced by oxidative destruction of tissue. Antioxidants neutralize reactive free radical species by hydrogen donation. Various medicinal plants contain phenolic compounds; tannins, flavonoids are known to have antioxidant property and thus these medicinal plants play an important in the treatment of diseases associated with oxidative destruction such as; cancer, heart diseases and ageing.[1-6]

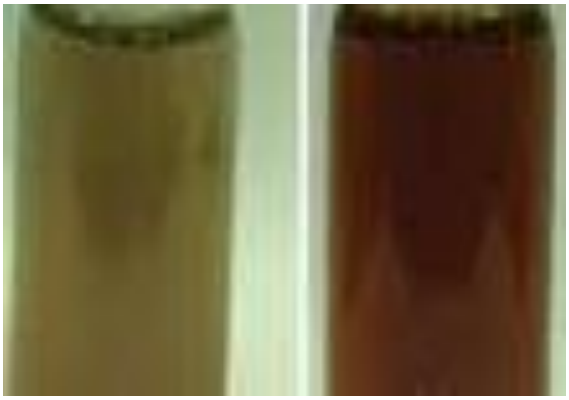
Triphala is well known ayurvedic preparation (*Churna*) used extensively for the treatment of various disease since ancient time. *Triphala* consisted of three medicinal plant *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica*. It is *Kashaya* in taste, *Ushna* in *Veerya Madhur* in *Vipak.*

Nighantu* has mentioned three types of *Triphala
- ***Swalpa Triphala**:* is containing *Draksha, Kharjura, Parushaka*
- ***Madhur Triphala**:* is composing of *Draksha, Kharjura, Kashmarya.*
- ***Sugandhi Triphala**:* is containing *Jatiphalam, Ela, Lavang*.

Traditionally *Triphala* it is used as laxative. It also used for colon cleansing & digestion problems and heart disease. Literature revealed that *triphala* possesses significant antioxidant activity due to presence of phenolic compounds. This article presented comparative antioxidant potential of alcoholic and hydro-alcoholic extract of *Triphala.*[7,8]

MATERIAL AND METHODS
Preparation of Extract
The investigation was performed at S.V.N.H.T. Ayurved College Rahuri Factory, Rahuri, Ahmednagar, Maharastra. Powdered *Triphala Churna* was extracted with methanol and methanol: water (1:1). After 24 hours

the supernatant was collected by filtration and the solvent was evaporated to make the crude extract (**Figure 1**). The residues obtained were stored in airtight bottles in a refrigerator for further use.



A **B**

Figure 1. Alcoholic (A) and hydro-alcoholic (B) extract of *triphala.*

Determination of Total Phenolic Content

Total phenolic content of the extracts were determined by standard method[9] with little modifications, using tannic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin - Ciocalteu reagent. The sample was mixed well and then allowed to stand for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then 2.5 ml of 7% sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the sample for 90 min.

***IN VITRO* ANTIOXIDANT ACTIVITY**

DPPH Radical Scavenging Assay

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The antioxidant activity of sample was expressed in terms of IC_{50}.[10] 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations (10 to 500 µg/ml) of extracts. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solvents used for extract preparations along with DPPH without any extract were used as control. Inhibition of DPPH free radical in percentage was calculated by the formula:

Inhibition (%) = $[(A_{control}-A_{test})/A_{control}] \times 100$

Where;* $A_{control}$ *is the absorbance of the control (L-Ascorbic acid)

A_{test} is the absorbance of reaction mixture sample (in the presence of sample).

RESULTS AND DISCUSSION

The antioxidant potential of alcoholic and hydro-alcoholic extracts of *Triphala* was measured using DPPH radical scavenging assay. DPPH scavenging activity increased with increasing phenolic components such as flavonoids, phenolic acids and phenolic diterpenes, the presence of hydroxyl groups in phenolic compounds is considered responsible for their radical scavenging effect and antioxidant power. The dose response curve of DPPH radical scavenging activity of extracts were observed when compared with standard ascorbic acid and presented in **Figure 2**. Potent antioxidant activity was observed in dose dependent manner as shown in **Figure 3**; and presence of polyphenolic groups can be attributed to the radical scavenging activity. Reducing power assay was also performed to establish prominent antioxidant activity of *Triphala*. In this assay, the yellow colour of the test solution changes to green and blue. Increased absorbance of the reaction mixture indicated increased reducing power of the extract. The reducing power of extract increased with its concentration. The results suggested that hydro-alcoholic extract (HAE) possess significant antioxidants potential as compared to alcoholic extract (AE), the antioxidants potential of extract was found to be comparable with the standard control, the activity of formulation may be attributed to its phyto-constituents.

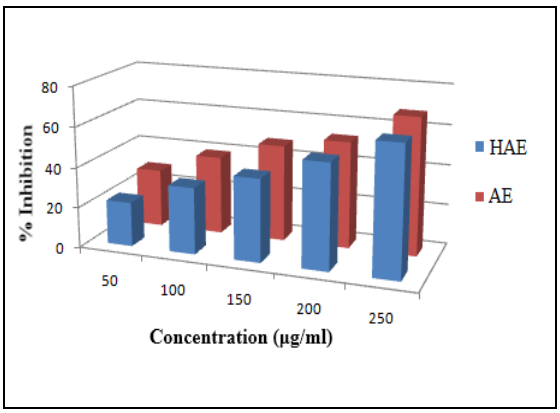


Figure 2. Comparison of DPPH Assay of Alcoholic and Hydro-Alcoholic Extracts of *Triphala.*

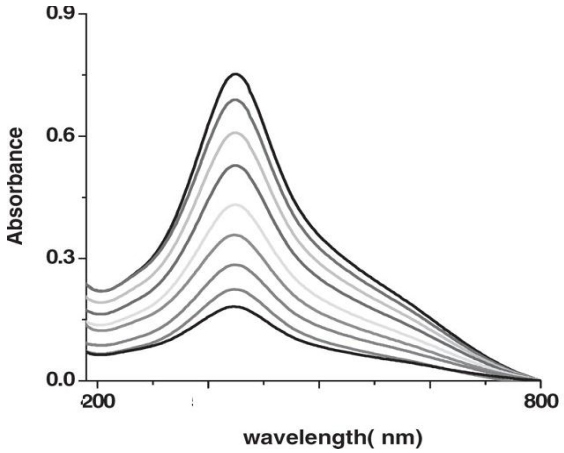


Figure 3. Concentration dependent antioxidant potential of extract.

CONCLUSION

Study concluded that hydro-alcoholic extract of *Triphala* may be used as natural sources for scavenging free radicals; to treat degenerative diseases related to oxidative stress. The finding also suggests that *Triphala* can be used as potential source of natural antioxidant.

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