AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *TRIPHALA* (AN AYURVEDIC FORMULATION) AND ITS CONSTITUENTS

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ABSTRACT

In the present study, methanolic extract of *Triphala* (an Ayurvedic formulation) and its constituents; *Terminalia chebula, Terminalia Belerica & Emblica officinalis* were evaluated & compared for the antioxidant activity. Antioxidant activity of the methanolic extracts were measured using ABTS & DPPH radical scavenging assays. The extracts were found to scavenge free radicals; ABTS & DPPH in a dose dependent manner. Total Phenolic content of the extracts were determined and expressed in terms of Gallic acid equivalents. On the basis of the results, it could be concluded that *triphala* and its constituents are potential source of natural antioxidants and the antioxidant activity is directly related to the phenolic compounds, suggesting direct contribution of phenolic compounds to these activities.

Key words: *Triphala, Emblica officinalis, Terminalia chebula, Terminalia belerica.*

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Received 6 January 2012, Accepted 18 January 2012

Please cite this article in press as: Sharma DK *et al.*, Total Phenolic Content and Antioxidant Activity of *Triphala* (an Ayurvedic formulation) and Its Constituents. American Journal of PharmTech Research 2012.

INTRODUCTION

Molecular oxygen is a vital component for the survival of organisms. However, under certain conditions, it may lead to the production of powerful oxidants called reactive oxygen species (ROS) such as free radicals (superoxide radicals, hydroxyl radicals, peroxy radicals, alkoxy radicals, etc.), hydrogen peroxide, lipid peroxides, singlet oxygen and hypochlorous acid[1]. Excessive production of these radicals, beyond the antioxidant capacity of the body can cause oxidative damage to DNA, proteins and carbohydrate moieties of the body[2]. They are involved directly or indirectly in various clinical disorders e.g. diabetes mellitus[3], atherosclerosis[3&4], Alzheimer's disease, Parkinson's disease, multiple sclerosis and other neurological disorders[3&5], skin disorders[3], inflammatory disorders[6,7&3], radiation injury, cataract, connective tissue disorders and toxicity produced by xenobiotics. *Triphala* is an ayurvedic formulation. It is an equiproportional mixture of fruits of three medicinal herbs, *amalaki* (*Emblica officinalis*), *haritaki* (*Terminalia chebula*) and *bibhitaki* (*Terminalia bellerica*)[8] . *Triphala* is used in Ayurvedic medicine in treating a variety of conditions and also forms part of many other Ayurvedic formulations. *Triphala* is traditionally been used as laxative in chronic constipation, colon cleansing, digestion problems and poor food assimilation. It has also been used in cardiovascular disease, high blood pressure disease, serum cholesterol reduction, poor liver function, large intestine inflammation, and ulcerative colitis[3]. *Triphala* has been found to have wound healing[9], anticancer[10], antimutagenic [11], antibacterial[12], antigout[13], hypolipidaemic[14] and antidiabetic[15] activity. The individual herbs, used in the formulation are reported to have several other health benefits. *Terminalia chebula* (*T.chebula*) possesses antibacterial[16] and antimutagenic[17] activities. *Terminalia belerica* (*T.belerica*) has antidiabetic[15] and hepato-protective[18] activity. *Emblica officinalis* is reported to posses santi-inflammatory[19], antimutagenic[11], antioxidant[20], cytoprotective[21], gastroprotective[22] and hypolipidaemic[23] activities. Very few researchers[24] have compared the antioxidant activity of constituents of *triphala*. Hence, the present study was designed to compare the antioxidant activity of *Triphala* and its ingredients.

MATERIALS & METHODS

Preperation of extracts

The fruits of amalaki (*Emblica officinalis*), *haritaki* (*Terminalia chebula*) and *bibhitaki* (*Terminalia belerica*) were collected, cleaned, shade dried and ground to obtain fine powder. *Triphala* was prepared by mixing all these ingredients in equimolar mixture. The powder was

used for preparation of extracts. The 100g fine powder was soaked in 800ml of solvent for 24 h with continuous stirring. The mixture was filtered through filter paper. The filtrate was vacuum dried in rotary vacuum evaporator at 40^0C. The extract was lyophilized and stored at 4˚c till further use. The percentage recovery of extracts was recorded. The extract was used for determining the *in vitro* antioxidant potential.

***In vitro* antioxidant potential**

Determination of total phenolic content

The total phenolic content was estimated in the *triphala* extract and its constituents using Folin-Ciocalteu reagent (FCR) based assay [25]. To a 50µl of plant extract, 950µl of water and 500µl of FCR and 2.5 ml of the 20% sodium carbonate solution were added. The mixture was then kept for 40 min at room temperature and absorbance was recorded at 725 nm. A standard curve of gallic acid was prepared. A control solution was prepared using distilled water instead of extract and the absorbance was recorded against that control. Total phenolic contents (mg/g) in the *Triphala* extract & its constituents were expressed as gallic acid equivalent (GAE).

Total phenols = Optical density (O.D.) x 938

Measurement of total antioxidant activity by ABTS method

The total antioxidant activity of plant extracts was determined by ABTS method [26]. A 2mM ABTS solution was prepared in distilled water. ABTS radical cations (ABTS•+) were produced by mixing 50ml of the ABTS stock solution with 200µl of 70mM potassium persulfate. To ensure complete oxidation of ABTS, the mixture was held at room temperature in the dark for 16 to 20 h prior to analysis. The resulting ABTS•+ solution was diluted with phosphate buffer (about four fold) to give an absorbance reading of 0.5 ± 0.05 at 734 nm. The antioxidant activity of extract of *triphala* and its constituents was evaluated in concentration ranging from 0.1 to 1.4 mg/ml. Radical scavenging analysis was performed by mixing 30 µl of the sample solution into 3.0 ml of ABTS•+ solution and reading the absorbance at 734 nm after 3 minutes. A control solution of 30 µl distilled water in 3.0 ml of ABTS•+ solution was prepared and analyzed.

% ABTS•+ inhibition = [1 – (A_{734}nm test/ A_{734}nm control)] x 100

Free radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined by DPPH scavenging assay [27]. A 100 µM solution of DPPH in methanol was prepared. The free radical scavenging action of *Triphala* extract and its constituents was seen in concentration ranges from 10-200 µg/ml. A 0.5 ml sample solution was added to 2.0 ml of DPPH in a 20 ml test tube. A control solution was

prepared by adding 0.5 ml of methanol to 2 ml DPPH solution. Samples were vertexed for 10 to 15 seconds and held at room temperature (22 ± 3°C) in the dark for 30 minutes. The absorbance of the sample and control solutions was determined at 517 nm and the % DPPH radical scavenging activity was calculated as follows:

$$\% \text{ DPPH radical scavenging activity} = [1 - (A_{517}\text{nm test} / A_{517}\text{nm control})] \times 100$$

Statistical analysis:

Statistical analysis was carried out using ANOVA followed by Dunnet's test. A 'p' Value < 0.01 was considered to be significant. IC_{50} values from the *in vitro* data were calculated by regression analysis.

RESULTS AND DISCUSSION

The results showed maximum recovery of *T.chebula* (48%) followed by *T.belerica* (32%) and *E.officinalis* (29%). Their equimolar mixture *Triphala* showed 32% recovery.

Total phenolic content

The total phenolic content in the extracts of *triphala* and its components was determined in terms of Gallic acid equivalent**.** The results suggest that *T.chebula* has a higher % of Gallic acid equivalents (358.54±5.24) as compared with *E.officinalis* (313.0±12.51) & *T.Belerica* (301.54±2.30). Their equimolar mixture *triphala* contains (335.29± 3.07) of Gallic acid equivalents.(Table 1) The total phenolic content is an indication of strong antioxidant activity[28&29]. In one of the earlier studies it was found that *triphala* contains high level of polyphenols and that the antioxidant and radioprotecting ability of *triphala* arise from the polyphenols, which reduce oxidative stress by converting the reactive oxygen free radicals to non-reactive products[29].

Table.1 Total Phenolic content in the extracts measured as Gallic acid equivalent (mg of GAE/ gram of extract)

S.No.	Sample	mg of GAE/gm of extract
1.	*Triphala*	335.29± 3.07*
2.	*Terminalia belerica*	301.54±2.30$^{*\wedge}$
3.	*Terminalia chebula*	358.54±5.24$^{*\#}$
4.	*Emblica officinalis*	313.0±12.51$^{*\wedge}$

[n=3], Means within a column with different superscripts differ at p<0.01 using Dunnet's test as a post hoc test. GAE = Gallic acid equivalents

Total antioxidant activity:

Total antioxidant activity was determined by ABTS Radical Scavenging assay. ABTS•+ is a blue coloured chromophore which is reduced to ABTS in a conc. dependent manner upon addition of

extract. The extract showed potent radical scavenging activity in concentration dependent manner. IC_{50} of the *triphala* extract, *T.chebula* , *T.bellerica* & *E.officinalis* was found to be 0.56±0.007, 0.59±0.018, 0.60±0.015 & 0.22±0.014 mg/ml respectively.(Table 2) On the basis of IC_{50} values it could be concluded that the order of Total antioxidant activity of *triphala* and its constituents is: *E.officinalis*> *Triphala*> *T.chebula*> *T.belerica* .

Table 2: IC_{50} value of *Triphala* and its constituents in ABTS & DPPH radical scavenging assays.

Free radical scavenging assay	**IC_{50} value**			
	Triphala	***T. Chebula***	***T.Belleric***	***E.officinalis***
ABTS (mg/ml)	0.56±.007*	0.59±.018$^{*\wedge}$	0.60±.015$^{*\wedge}$	0.22±.014$^{\#}$
DPPH (µg /ml)	55.16±14.10*	42.73±14.44*	62.46±17.30*	47.73±11.7*

[n=3], Means within a row with different superscripts differ at p<0.01 using Dunnet's test as a post hoc test.

DPPH Radical Scavenging assay:

The extracts showed potent DPPH radical scavenging activity in concentration dependent manner. IC_{50} of the *Triphala* extract, *T.chebula* , *T.bellerica* and *E.officinalis* was found to be 55.16±14.10, 42.73±14.44, 62.46±17.30 , 47.73±11.7 mg/ml, respectively. On the basis of IC_{50} values it could be concluded that the DPPH scavenging activity of *triphala* and its constituents is: *T.chebula* > *E.officinalis*> *Triphala* > *T.belerica* (Table2). It was found that in the absence of *triphala*, ABTS•+ radical, DPPH did not show any decay; however, in the presence of *triphala* ABTS•+ radical decayed in milliseconds & DPPH in seconds[29].

CONCLUSION

In this study we conclude that methanolic extract of *Triphala* and its ingredients have good antioxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. The results of this study show that methanolic extract of *triphala* and its constituents can be of use as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidative activity of methanolic extract of *Triphala* are currently unclear. Therefore, it is suggested that further work could be done on the isolation and identification of the antioxidative components in *triphala* and its constituents.

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