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IN VITRO ANTIOXIDANT ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF DEVA VATI TABLETS: AN AYURVEDIC FORMULATION

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ABSTRACT

The generation of free radical is responsible for the development of several aging-related diseases including cancer, cardiovascular disorder, diabetes, neurodegenerative disorders. The objective of the present study was to evaluate the in *vitro* antioxidant potential of a hydro-alcoholic extract of deva vati tablet (HEDV). The formulation was standardized pharmaceutically and microbiologically. *In vitro* antioxidant study such as total phenolic content, DPPH assay and ABTS assay were performed. Quercetin was taken as the standard for antioxidant activity. The total phenolic content of HEDV was found to be 8.41 µg. The antioxidant activity was estimated by IC_{50} value and the values are (1.97 µg/ml (DPPH radical scavenging assay) and 0.660µg/ml.(ABTS radical scavenging assay) Hence, the extract showed significant *in vitro* antioxidant activity by terminating the actions of free radicals.

KeyWords: Antioxidant activity, Ayurvedic Formulation, Deva Vati, Free radicals

INTRODUCTION

Oxidative stress is imposed on the cell as a result of decreased levels of antioxidants. This can be caused intrinsically, for example, by DNA mutations that have altered the cellular antioxidant defense system activity, or extrinsically by a deficiency in dietary minerals (cofactors), or by toxins and other factors which deplete the antioxidant defences[1]. An increased level of oxidants in the cell can also result in oxidative stress. Oxidative stress may result in adaptation of the cell or organism by triggering up-regulation of the immune defense system; however, this can also result in cell injury and cell death [2].Cellular interaction with ROS results in damage to DNA molecules, indicating that oxidative stress likely to play an important role in increasing the risk of cancer through enhanced mutagenesis, carcinogenesis, and aging[3]. Excessive production of reactive oxygen species (ROS) plays an important role in the pathogenesis and progression of various diseases involving different organs[4]. Antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by the interruption of ROS attack, by scavenging the reactive metabolites or by converting them to less reactive molecules[5].

Deva vati is a judicious combination of highly documented hepatoprotective herbs providing perfect protection to the liver in health, infection and inflammation. Deva vati is a polyherbal formulation consisting of eight medicinal plants derived from the traditional system of medicine in India, the Ayurveda. The plants used in this formulation are *Zingiber officinale, Coriandrum sativum, Eclipta alba, Swertia chirata, Picrorhiza kurroa, Embelia ribes, Plumbago zeylanica ,Holarrhena antidysentrica* and *Asphaltum punjabinum.* Since there is a lack of scientific data regarding the pharmacological evaluation of Deva vati, the present study was aimed to screen hydroalcoholic extract of Deva vati tablets for its antioxidant activity.

MATERIALS & METHODS

Collection of drugs.

Deva vati tablet (Batch no: DUK-114, Manufacturing date- June 2013, Expiry date- May 2016) was procured from Sri Sri Ayurveda Trust, Thrissur.

Extraction of deva vati tablets.

The collected tablets were powdered coarsely using a clean mortar and pestle. Powder (170gms) was extracted with hydroalcoholic mixture (ethanol and water in 50/50 proportion) at room temperature for 72 hrs by cold maceration method. The extract was concentrated under vacuum for the removal of ethanol. The aqueous portion was later lyophilized to obtain a solid mass.

Estimation of total phenols

The total phenolic content of hydroalcoholic extract of deva vati tablets(HEDV) was determined by the Folin-Ciocalteau assay method. An aliquot (100µl) of extract (10mg/ml) or standard solution of Gallic acid (1mg/ml) was mixed with 500µl of Folin-ciocalteau reagent and incubated for 5min at room temperature. Then 400µl of 7.5% sodium carbonate was added to the above mixture and kept in dark for 30 min to complete the reaction. After incubation, the absorbance of the mixture was measured against the blank at 760 nm. Distilled water was used as the reagent blank. All the tests were performed in triplicate in order to get mean the values. The total phenol content in the extract was derived from the gallic acid standard curve and was expressed as Gallic acid equivalents (GAE) per gram of extract [6].

DPPH Radical Scavenging Assay

DPPH radical scavenging activity was measured according to the method described by Blois *et al.*, The antioxidant activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical. A 1ml solution of 0.3mM DPPH in methanol was added to 500µl of various concentrations of sample and the reference compound, mixed and left to stand in the dark at room temperature for 30 min and then absorbance was measured at 517 nm against a blank. A control reaction was carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Antiradical activity was expressed as percentage inhibition (I %) and calculated using the following equation[7].

$$\text{Percentage inhibition (I \%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where '$Abs_{control}$' was the absorbance of the control reaction and 'A_{test}' was the absorbance in the presence of the sample/ standard. The antioxidant activity of the extract was expressed as $IC_{50.}$ (IC_{50} - concentration required to obtain a 50% radical scavenging activity).

ABTS* Radical Cation Assay

ABTS radical scavenging activity of HEDV was measured according to the method described by Rei *et al,*. ABTS radical cation ($ABTS^{*+}$) was produced by reacting equal volumes of 7mM ABTS salt and 2.45 mM potassium per sulfate and the mixture was allowed to stand in the dark for 16 h at room temperature. The resultant solution was diluted with ethanol until an absorbance of 0.70 ± 0.02 at 734 nm. Varying concentration of the sample was allowed to react with 900µl of $ABTS^{*+}$ solution and the reaction mixture was vortexed for 10 sec. After minutes, the absorbance reading was recorded at 734 nm. A control reaction was carried out without the sample. Ascorbic acid was used as reference standard. All the tests were performed in triplicate in order to get the mean values [8]. The percentage inhibition of $ABTS^{+}$ by the sample was calculated according to the formula:

$$\text{Percentage inhibition (I \%)} = \frac{\text{Abs}_{\text{control 1}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where '$Abs_{control}$' was the absorbance of the control reaction and 'A_{test}' was the absorbance in the presence of the sample/ standard. The antioxidant activity of the extract was expressed as $IC_{50.}$ (IC_{50} - concentration required to obtain a 50% radical scavenging activity).

RESULTS AND DISCUSSION

Phenolic compounds and flavonoids are known to contribute to the quality and nutritional values of foods in terms of color, aroma and flavor and also in providing health-beneficial effects. The phenolic compounds have been recognized as antioxidant agents which act as free radical terminators. The antioxidant activity of phenolics is mainly due to the redox properties, which allows it to act as reducing agent, hydrogen donators, and singlet oxygen quenchers. The Folin-Ciocalteu method is a routine assay for studying phenolic antioxidants as it is rapid, convenient, simple and reproducible[9]. A calibration curve was plotted. (**Table no. 1**and **Figure no.1)** The total phenolic content

of the extract was expressed as µg GAE/g extract. The total phenolic content of HEDV was found to be 8.41 µg GAE/g extract.

DPPH is the most extensively used assay to detect the antioxidant activity of plants. Its violet color disappears in the presence of a substance which can donate a hydrogen depending on the antioxidant activity [10]. This assay is being used widely as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517nm (violet color) and when it is quenched by the extract, there is a decrease in absorbance[11]. DPPH scavenging capacity of HEDV at the concentrations of 0.2 to 5µg/ml was compared with quercetin. The DPPH radical scavenging activity of HEDV and quercetin is presented in **Table no. 2, Figure no. 2 and 3**. The IC_{50} values of HEDV and quercetin were found to be 1.97 µg/ml and 0.06 µg/ml respectively. Though the DPPH radical scavenging ability of the HEDV was less than that of quercetin, the study proved that the extract has the hydrogen-donating ability and HEDV serve as free radical scavenger/inhibitor, acting possibly as primary antioxidants.

The decolourization of ABTS radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods [12]. In this assay, ABTS is converted to its radical cation by the addition of sodium persulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical reactions involve electron transfer and take place at a much faster rate compared to DPPH radicals [13]. The ABTS radical scavenging activity of HEDV and quercetin is presented in **Table no. 3, Figure no. 4 and 5**. ABTS scavenging capacity of HEDV at the concentrations of 0.2 to 2 µg/ml was compared with quercetin. The IC_{50} values of HEDV and quercetin were found to be 0.660µg/ml and 0.047µg/ml respectively. HEDV showed potential activity in $ABTS^{+}$ decolourization. Decolourization of ABTS radical in the present study reflects the antioxidant capacity of HEDV to donate electrons or hydrogen atoms to inactivate this radical cation.

In conclusion,The study showed significant *in vitro* antioxidant activity by terminating the actions of free radicals. Phytoconstituents like glycosides, phenolics and flavones can scavenge a wide range of reactive oxygen species and hence have been recommended as antioxidants. In the present study, the phytochemical screening of plant extract showed the presence of phytoconstituents like flavonoid, phenolics, tannins and glycosides which may be responsible for its antioxidant effects.Thus, the present study reveals that Deva vati, an ayurvedic formulations possess antioxidant activity.

Table No.1: Extraction yield analysis

Method	Solvent	Yield in Grams	Percentage yield (%w/w)
Cold maceration	50% ethanol	21.48	12.64

Table No.2: Estimation of total phenolic content

Sample.	**Concentration (µg/ml)**	**Absorbance.**
Standard. (Quercetin)	0.05	0.072±0.0014
	0.1	0.205±0.018
	0.25	0.536±0.038
	0.5	1.129±0.059
	1	1.977±0.038
HEDV	0.5	0.578±0.056

Figure No.1: The standard graph for gallic acid for the estimation total phenolic content.

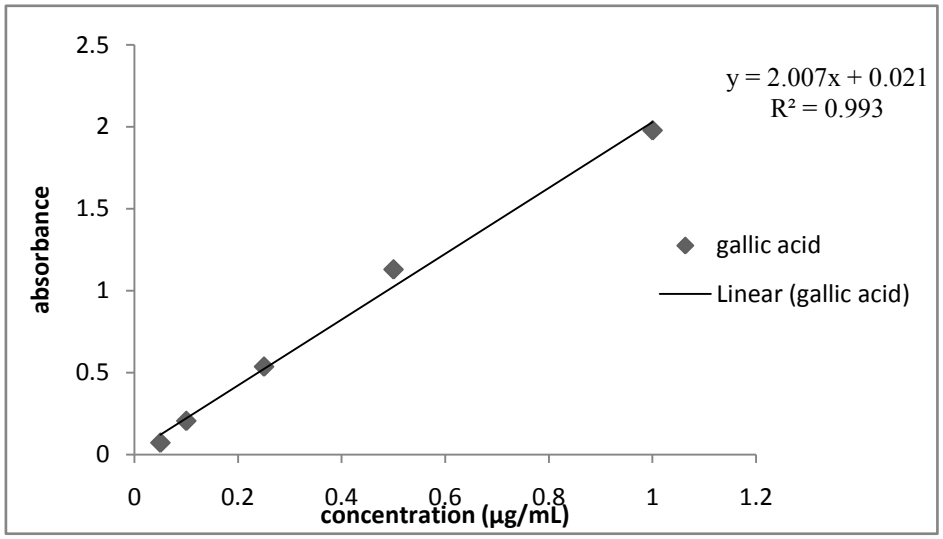
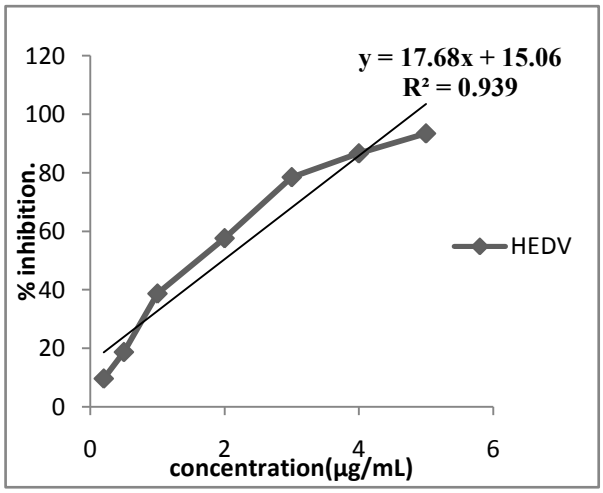


Table No. 3: Percentage inhibition and IC_{50} values of DPPH radical method

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
HEDV	0.2	9.6±0.090	1.97
	0.5	18.67±0.015	
	1	38.67±0.106	
	2	57.61±0.029	
	3	78.49±0.012	
	4	86.63±0.065	
	5	93.45±0.030	
Standard (Quercetin)	0.005	9.69±0.090	0.06
	0.01	19.54±0.038	
	0.02	35.84±0.004	
	0.05	58.62±0.014	
	0.1	80.79±0.012	
	0.2	96.06±0.007	

Figure No. 2 and 3: DPPH radical scavenging activity of HEDV and Quercetin



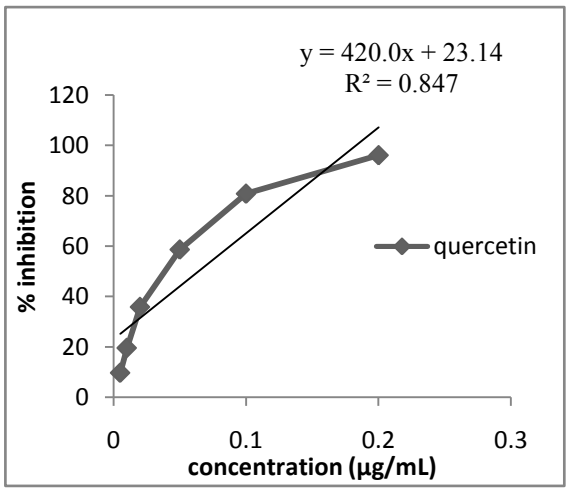
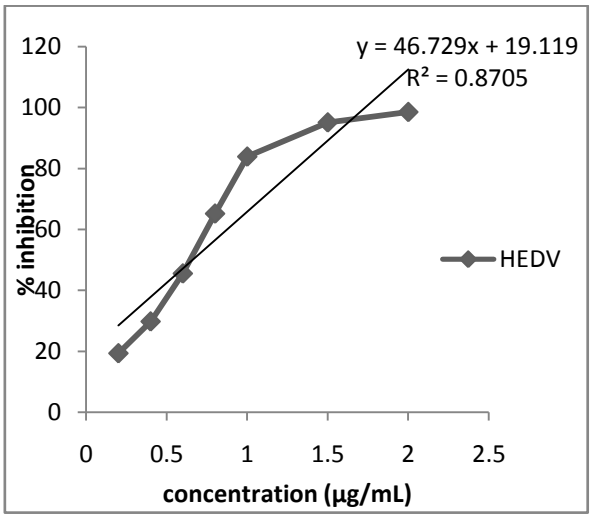
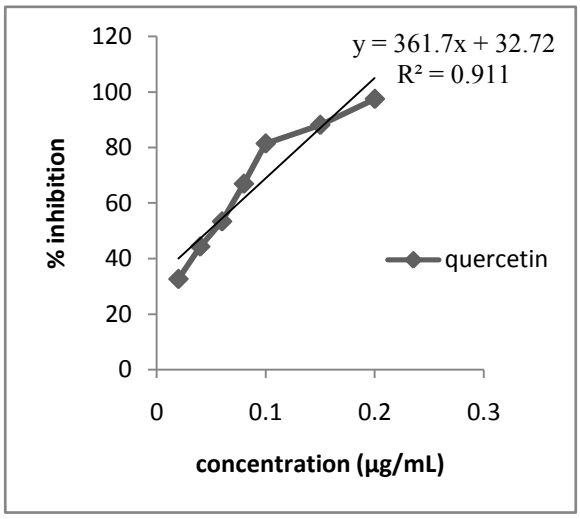


Table No. 4 Percentage inhibition and IC_{50} values of ABTS radical by HEDV and Quercetin

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
HEDV	0.2	19.34±0.007	0.660
	0.4	29.79±0.048	
	0.6	45.55±0.047	
	0.8	65.21±0.014	
	1	83.93±0.006	
	1.5	95.16±0.004	
	2	98.59±0.001	
Standard. (quercetin)	0.02	32.60±0.012	0.047
	0.04	44.30±0.055	
	0.06	53.35±0.002	
	0.08	66.92±0.033	
	0.1	81.43±0.023	
	0.15	88.14±0.004	
	0.2	97.50±0.004	

Figure No. 4 and 5: ABTS radical scavenging activity of HEDV and Quercetin





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