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Comparative study of Antioxidant Activity of Methanolic and Ethanolic Extracts of *Stevia rebaudiana* Leaves

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ABSTRACT

The antioxidant activity of *Stevia rebaudiana* (L.) leaves belonging to the family Asteraceae was investigated. Methanolic and ethanolic extracts are used to estimate antioxidant activity. The antioxidant activity was studied using reducing power of *Stevia rebaudiana* extracts by Oyaizu method, the content of total phenols is estimated by a standard method using the Folin ciocalteau reagent and total flavonoid was determined by aluminium chloride colorimetric method. Different concentrations of methanolic extract, ethanolic extract and standard butylated hydroxyl toluene solutions absorbance was measured at 700 nm and was compared. The extract has shown more or less similar reducing power capacity at different concentrations ranging from 100 - 1000µg /ml. The total phenolic content of methanolic and ethanolic extracts *Stevia rebaudiana* leaves was found to be 280mg TAE/g and 810mg TAE/g respectively. The phenolic compounds are known to have direct antioxidant property. The total flavanoids content of methanolic and ethanolic extracts was found to be 12mg CE/g and 15mg CE/g respectively. The tannins and flavonoids present in the leaves extract may be responsible for antioxidant activity.

Keywords: TAE (Tannic acid equivalent), CE (Curcumin equivalent).

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INTRODUCTION

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions [1]. Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease [2, 3]. The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumers concern about the safety of synthetic antioxidants in foods [4, 5]. The antioxidants may be enzymatic or non – enzymatic, super oxide dismutase, glutathione peroxidases, catalase and peroxidases are some examples which come under enzymatically potential antioxidants. In the non-enzymatic category some of the known and documented antioxidants are vitamin C, vitamin E, vitamin A, carotenoids, uric acid6. *Stevia rebaudiana (*Asteraceae*)* is mostly found in semi-humid subtropical region [7]. It is used as zero calorie natural sweetener, hypoglycemic, cardiotonic, vasodilator, antimicrobial, digestive tonic, diuretic, antihypertensive, appetite stimulant, anti-inflammatory, antipyretic [8]. Hence in the present investigation to evaluate the *in-vitro* antioxidant activity of methanol and ethanol leaf extracts of *Stevia rebaudiana* by reducing power and scavenging of hydrogen peroxide method.

MATERIALS AND METHODS

Plant Materials and Preparation of Freeze Dried Extract

The selected medicinal plant was *Stevia rebaudiana* was collected. Materials dried at room temperature and coarsely ground before extraction. Each part was extracted by maceration method using methanol and ethanol as solvent. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Determination of Total Phenolic Compounds

Total phenolic compound contents were determined by the Folin-Ciocalteau method [9]. The extract samples (0.5 ml) were mixed with 5 ml of 10% Folin-Ciocalteau reagent and 4ml of 1M sodium carbonate. The mixture was kept aside for 15 min and the absorbance were determined against a solvent blank (methanol) at 765nm. The total phenolic concentrations were determined from the standard and the results were expressed in terms of Tannic acid equivalents (TAE).

Determination of Flavonoid Content

Total flavonoids were estimated using aluminium chloride colorimetric method [10]. 1ml of sample is mixed with 3ml of extract solvent (methanol or ethanol), 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water. Allowed to remain at

room temperature for 30 min. The absorbance of the reaction mixture is measured at 415nm. The flavonoids are expressed in terms of curcumin equivalent.

Determination of Reducing Power

Fe (III) reduction is often used as an indicator of electron- donating activity, which is an important mechanism of phenolic antioxidant action [11]. Reducing power of *Stevia rebaudiana* methanolic and ethanolic extracts was estimated by Oyaizu method [12]. Different concentrations of organic extract and standard solutions of butylated hydroxyl toluene (BHT, 100 - 1000µg /ml) were prepared. 1ml of the prepared concentrations, 2.5ml of potassium ferricyanide, 2.5 ml of phosphate buffer (p^H6.6, 0.2M) were added and this mixture incubated at 50ºC for 20 minutes. A portion (2.5ml) of tichloroacetic acid (10%) was added to the mixture. Then, it was centrifuges for 10 min at 300 rpm. The upper layer of the solution (2.5ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated hydroxyl toluene was used as standard.

Scavenging of Hydrogen Peroxide

The Hydrogen peroxide-scavenging activity of extract was determined by the method of Ruch et al [13]. Hydrogen peroxide (40 mM) was prepared in phosphate buffered (pH 7.4). Standard (ascorbic acid) and extract solutions were prepared at concentrations of 20, 40, 60, 80, 100µg/ml in distilled water. To 3.4 ml of standard or extract solutions 0.6 ml of hydrogen peroxide solution was added. The reaction mixture was incubated at room temperature for 10 min, and the absorbance was determined at 230 nm. The percentage of scavenging was calculated as follows: % H_2O_2 Scavenging = $[(A_0-A_1)/A_0]$ x 100 where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extracts and standard.

RESULTS AND DISCUSSION

Total Phenol Contents

Total phenol compounds are reported as tannic acid equivalents by reference to standard curve for methanolic extract (0.0031x+0.9101, r^2=0.9806) and ethanolic extract (y=0.0013x+0.7028, r^2=0.972). The total phenolic contents for methanolic extract and ethanolic extract of *Stevia rebaudiana* leaves were 280 and 810 mg tannic acid equivalent/g respectively. Ethanolic extract of *Stevia rebaudiana* leaves has higher total phenols than methanolic extract.

Total Flavonoid Contents

Total flavonoid contents are reported as curcumin equivalents by reference to standard curve for methanolic extract (0.0151x, r^2=0.9947) and ethanolic extract (y=0.0131x, r^2 = 0.994). The total flavonoid contents for methanolic extract and ethanolic extract of *Stevia rebaudiana*

leaves were 12 and 15 mg curcumin equivalent/g respectively. Ethanolic extract of *Stevia rebaudiana* leaves has higher total phenols than methanolic extract.

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [14].

Reducing Power

In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Pert's Prussian blue at 700 nm [15]. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure-1 & 2 shows the dose - response curves for the reducing powers of the methanolic and ethanolic extracts. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. The reducing power of methanolic extract ($p<0.00002$) and ethanolic extract ($p>0.02$) was found. Hence the reducing power of ethanolic extract is greater than methanolic extract.

Hydrogen Peroxide Scavenging

Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. No extract showed good scavenging activity. The % H_2O_2 Scavenging activity at 20 µg/ml for standard, methanol and ethanol was found to be 78.6%, 87% and 67.7% respectively. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems. The plants extracts exhibited different levels of antioxidant activity in all the models studied. Further investigation of individual compounds, their *in vivo* antioxidant activities and in different antioxidant mechanisms is needed.

Figure-1: REDUCTIVE ABILITY OF *Stevia rebaudina* METHANOLIC EXTRACT

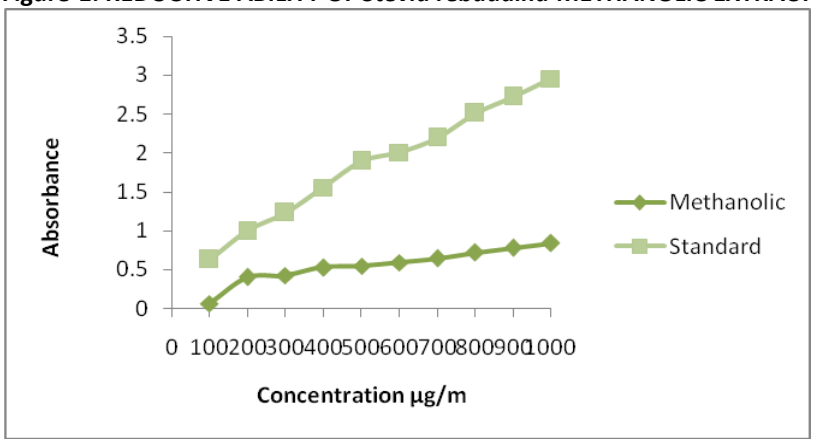

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Figure-2: REDUCTIVE ABILITY OF *Stevia rebaudina* ETHANOLIC EXTRACT

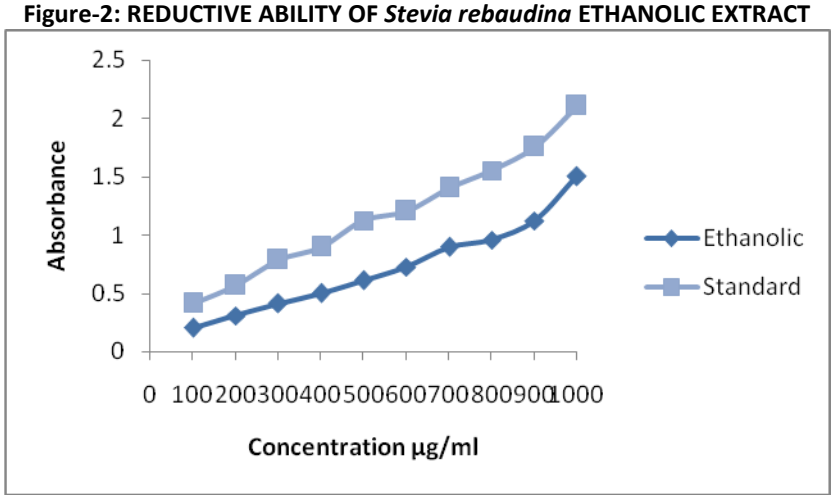
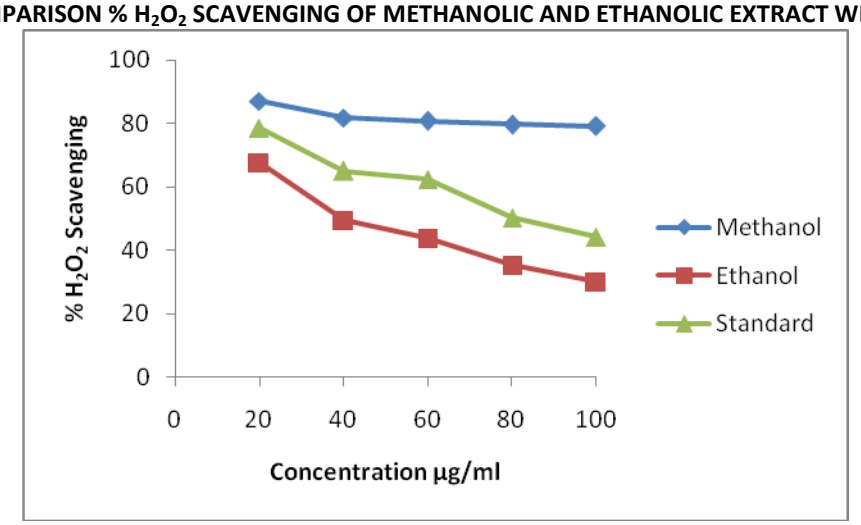


Figure-3: COMPARISON % H_2O_2 SCAVENGING OF METHANOLIC AND ETHANOLIC EXTRACT WITH STANDARD



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