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# *IN-VITRO* ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF AQUEOUS AND ETHANOLIC EXTRACT OF *VITIS VINIFERA* SEEDS

R. Gomathi *, K. Nithyasri, J. Roshika, K. Subhapathi and P. Priyadharshini

Department of Biochemistry, Holy Cross College, Tiruchirappalli - 620002, Tamil Nadu, India.

**Keywords:**

*Vitis vinifera*, Alpha-glucosidase, Free radical, 1, 1-Diphenyl-2-picryl hydrozyl, Alpha-amylase, Antidiabetic

**Correspondence to Author:**
**Dr. R. Gomathi**

Assistant Professor,
Department of Biochemistry,
Holy Cross College, Tiruchirappalli - 620002, Tamil Nadu, India.

**E-mail:** gomathi.biochemistry@gmail.com

**ABSTRACT:** In the present study, the aqueous and ethanolic extracts of *Vitis vinifera* seeds were studied for alpha-amylase and alpha-glucosidase inhibition using an *in-vitro* model. The plant extracts were also examined for their antioxidant activities by using the free radical 1, 1-diphenyl-2-picryl hydrozyl (DPPH) scavenging method under *in-vitro*. The study revealed that the different concentrations of the extract possessed a very good amount of result and exhibited potent radical scavenging activity using DPPH SA substrate. The aqueous (68.5%) and ethanolic extract (74.2%) of *Vitis vinifera* seeds showed a significant inhibitory effect on alpha-amylase activity ethanol extract (74.2%), aqueous extract (68.5%). In the alpha-glucosidase activity ethanolic extract (75.7%) and aqueous extract (74.5%) showed a significant inhibitory effect when compared with standard acarbose drugs. Therefore, it is suggested that the aqueous and ethanolic extract of *Vitis vinifera* seeds could be used as a potential source for natural anti-diabetic and antioxidant compounds and could have the potential use in the management of diabetes mellitus and hyperglycemia.

**INTRODUCTION:** Diabetes mellitus is a group of metabolic diseases distinguished by disordered metabolism and high blood sugar (hyperglycemia) resulting from defects in insulin secretion. The long-term complications include heart disease, stroke, dysfunction, and failure of various organs [1]. There are three main forms of diabetes: Type 1, Type 2 and gestational diabetes. Type 1 is autoimmune destruction. Type 2 is insulin resistance [2]. Gestational diabetes is defined AS any degree of glucose intolerance during pregnancy [3].

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The treatments are reduction of insulin, stimulation of endogenous insulin secretion, and the inhibition of the degradation of oligo and disaccharides [4, 5]. The inhibitory activity of alpha-glucosidase is used for the management of type 2 diabetes. In this inhibitory activity, the oligo and disaccharides is converted to monosaccharides due to enzymes breakdown. So, it leads to a decrease in blood glucose level which is absorbed by the mucosal border in the small intestine.

The inhibitory activity of alpha-amylase is responsible for converting starch into more simple sugars [6]. By this activity delays the glucose absorption rate and maintains the serum blood glucose level [7]. Acarbose drug inhibits the alpha-amylase and alpha-glucosidase enzymes, and the side effects are gastrointestinal tract [8]. Antioxidants are vital reducing substances that might help to

prevent oxidative damage [9]. Herbal medicines play an important role in the treatment of diabetes mellitus [10, 11]. Phytochemical constituents like terpenoids, tannin, alkaloids were studied in various plants such as Euphorbia hirta, *Linumu sitatissimum*, *Zingiber officinale* [12]. *Vitis vinifera* belongs to the family Vitaceae and the seed of the plant is used as medicine. This plant is cultivated in the Mediterranean and Central Asia [13]. The branches, leaves, fruit and seed of Vitis vinifera are used to treat skin diseases, diarrhea, throat infections, and diabetes mellitus [14, 15]. In this study, the anti-oxidant and antidiabetic activities of the aqueous and ethanolic extract from the seed of *Vitis vinifera* were carried out by alpha-amylase and alpha-glucosidase inhibitory activities.

## MATERIALS AND METHODS:

**Collection of Plant Material:** The seed of *Vitis vinifera* was collected in February from the farm, Dindigul, Tamil Nadu, India. The plant was authenticated by Amlanandan, Director, Rapinat herbarium, St. Joseph College, Tiruchirappalli, Tamil Nadu. The voucher specimen number: K.S.001 (11:02:2021).

**Chemicals and Reagents:** Alpha (α)-Glucosidase, porcine pancreas alpha (α)-amylase, p-nitrophenyl-α-D-glucopyranose (p-NPG), 3, 5- dinitrosalicylic acid (DNS), 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, and acarbose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Soluble starch, sodium potassium tartarate, sodium dihydrogen phosphate ($NaH_2PO_4$), disodium hydrogen phosphate ($Na_2HPO_4$), sodium chloride, sodium hydroxide, potassium ferricyanide, ferric chloride (FeCl3) were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used, including the solvents, were of analytical grade.

**Preparation of Aqueous and Ethanolic Extract:** The *Vitis vinifera* seeds were washed in running water, cut into small pieces and then shade dried for a week at 35-40 ºC. After which, it was grinded to a uniform powder of 40 mesh size. The ethanol and aqueous extracts were prepared separately by soaking 100 g each of the dried powder plant materials in 100 L of ethanol and aqueous separately using a Soxhlet extractor continuously for 10 h. The extracts were filtered through Whatmann filter paper. No. 42 (125 mm) to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -20 ºC.

**Antioxidant Activity (DPPH Free Radical Scavenging Activity) Determination:** The antioxidant activity of the aqueous and ethanolic extract was examined on the basis of the scavenging effect on the stable DPPH free radical activity [16]. Ethanolic solution of DPPH (0.05mM) (300 µl) was added to 40 µl of an aqueous and ethanolic extract with different concentrations (100-500 µg/ml). DPPH solution was freshly prepared and kept in the dark at 4 °C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min, and absorbance was measured spectrophotometrically at 540 nm. Ethanol was used to set the absorbance to zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as a percentage of inhibition, were calculated according to the following equation.

$$\text{Percent (\%) inhibition of DPPH activity} = [(A - B) / A] \times 100$$

Where B and A are the absorbance values of the test and the blank sample, respectively. A percent inhibition versus concentration curve was plotted, and the concentration of sample required for 50% inhibition was determined and represented as $IC^{50}$ value for each of the test solutions.

**Alpha-amylase Inhibitory Assay:** This assay was carried out using a modified procedure [17]. A total of 250 µL of ethanolic extract (20-100 µg/ml) was placed in a tube and 250 µL of 0.02M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was pre-incubated at 25 ºC for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at for 25 ºC for 10 min. The reaction was terminated by adding 500

µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5mL distilled water, and the absorbance was measured at 540 nm using a spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition:

% Inhibition = [(Abs control – Abs ethanol extract) / Abs control] × 100

Concentrations of extracts resulting in 50% inhibition of enzyme activity ($IC_{50}$) were determined graphically.

**Alpha-Glucosidase Inhibitory Assay:** The effect of the ethanolic extract on α-glucosidase activity was determined according to the method described by using α-glucosidase from Saccharomyces cerevisiae [18]. The substrate solution p-nitrophenyl glucopyranoside (p-NPG) was prepared in a 20mM phosphate buffer and pH 6.9. 100 µL of α-glucosidase (1.0 U/mL) was pre-incubated with 50 µL of the different concentrations (20-100 µg/ml) of the ethanolic extract for 10 min. Then50 µL of 3.0 mM (PNPG) as a substrate dissolved in 20 Mm phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37 ºC for 20 min and stopped by adding 2mL of 0.1 M $Na_2CO_3$. The α-glucosidase activity was determined by measuring the yellow-colored para nitrophenol released from PNPG at 405 nm. The results were expressed as a percentage of the blank control. The α-glucosidase inhibitory activity was calculated as percentage inhibition

% Inhibition = [(Abs control - Abs ethanol) / Abs control] × 100.

Concentrations of extracts resulting in 50% inhibition of enzyme activity ($IC_{50}$) were determined graphically.

## RESULTS AND DISCUSSION:

**Comparative Analysis of Aqueous And Ethanolic Extract of *Vitis Viniferaseed* Sample:** The result of phytochemical screening of aqueous and ethanolic extract of *Vitis vinifera* seeds shown in **Table 1.** The aqueous extract of *Vitis vinifera* seeds showed the presence of tannin, saponin, flavonoids, Steroids, Cardiac glycoside, anthraquinones, coumarin, Phenol, xanthoprotein, alkaloids, emodin, and the absence of carbohydrates, terpenoids, phlobatannins, anthocyanin, leucoanthocyanin. The ethanolic extract of *Vitisvinifera* seeds indicated the presence of tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, anthocyanin, anthroquinone, coumarin, glycosides, phenol, xanthoprotein, alkaloids, emodin, carbohydrate, and the absence of leucoanthocyan in. *Indigenous communities* that have widely used *Vitis vinifera* in Sri Lanka, India, and Bangladesh were conducted to evaluate the phytochemicals revealed presence of alkaloid, steroid, and anthraquinones with considerable amounts of phenolic compounds [19, 20].

**TABLE 1: QUALITATIVE ANALYSIS OF AQUEOUS AND ETHANOLIC EXTRACT *VITIS VINIFERA* SEEDS**

| Test no. | Phytochemical Constituents | Aqueous extract | Ethanolic Extract |
|---|---|---|---|
| 1 | Tannin | +++ | +++ |
| 2 | Phlobatannins | A | ++ |
| 3 | Saponins | +++ | +++ |
| 4 | Flavonoids | + | +++ |
| 5 | Steroids | ++ | +++ |
| 6 | Terpenoids | A | +++ |
| 7 | Cardiac glycosides | +++ | +++ |
| 8 | Leucoanthocyanin | A | A |
| 9 | Anthocyanin | A | ++ |
| 10 | Anthraquinone | +++ | +++ |
| 11 | Coumarin | +++ | +++ |
| 12 | Protein | +++ | +++ |
| 13 | Glycosides | ++ | +++ |
| 14 | Phenol | +++ | +++ |
| 15 | Xanthoprotein | +++ | +++ |
| 16 | Alkaloids | + | ++ |
| 17 | Emodin | +++ | +++ |
| 18 | Carbohydrates | A | ++ |

+ - Trace ++ - Moderate +++ - Strong A- Absence

**Antioxidant Activity of Aqueous And Ethanolic Extract of *Vitis Vinifera* Seed Sample By Dpph Assay:** The result showed that the aqueous and ethanolic extract of *Vitis vinifera* seeds showed maximum potent antioxidant activities at high concentrations when compared with ascorbic acid **Table 2.** The antioxidant activity showed 42.96% to 52.34% for aqueous extract and 57.03% to 79.68% for ethanolic extract at concentration (100-500) µg/ml while ascorbic acid gave 71.87% to 83.59 % at the same concentration **Fig. 1.** According to Delgado *et al.* 2011, the aqueous extract of *Vitis vinifera* seeds showed a higher amount of antioxidant and free radical scavenging activity [21].

**TABLE 2: *IN-VITRO* ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *VITIS VINIFERA* SEEDS BY DPPH AND COMPARISON WITH STANDARD DRUG ASCORBIC ACID**

| S. no. | Concentration | Antioxidant Activity by Dpph (%) | | |
|---|---|---|---|---|
| | | Aqueous | Ethanolic Extract | Ascorbic Acid |
| 1 | 100 (µg/ml) | 42.96 | 57.03 | 71.87 |
| 2 | 200 (µg/ml) | 44.53 | 63.28 | 76.56 |
| 3 | 300 (µg/ml) | 46.87 | 68.75 | 78.90 |
| 4 | 400 (µg/ml) | 48.43 | 77.34 | 81.25 |
| 5 | 500 (µg/ml) | 52.34 | 79.68 | 83.59 |

Each value was obtained by calculating the average of three experiments and data are presented as mean ± SEM

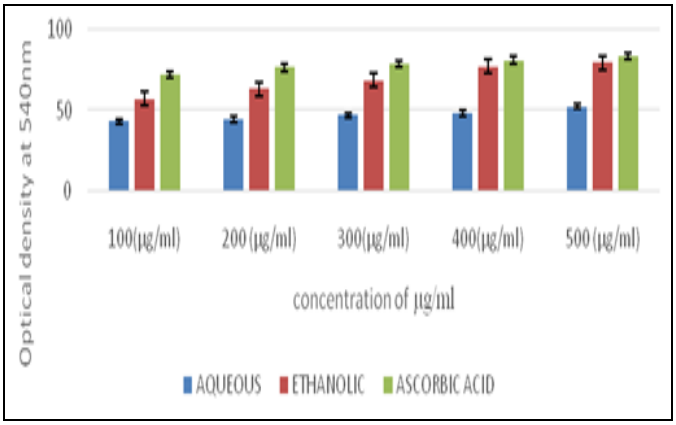


**FIG. 1: *IN-VITRO* ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *VITIS VINIFERA* SEEDS BY DPPH AND COMPARISON WITH STANDARD DRUG ASCORBIC ACID**

***In-vitro* Alpha-Amylase Inhibitory Assay:** In this study, the *in-vitro* alpha-amylase inhibitory activities of the aqueous and ethanolic extract was investigated. The experiment's result showed a dose-dependent increase in percentage inhibitory activity against the alpha amylase enzyme. The synthesized (100-500 µg/ml) of the various concentrations exhibited potent α-amylase inhibitory activity in a dose-dependent manner. The alpha-amylase inhibitory activity from 43.97% to 53.19% for aqueous extract and 55.31% to 77.30% for ethanolic extract at concentration (100-500 µg/ml) **Table 3**. Acarbose was a standard drug for α-amylase inhibitor. Acarbose at a concentration of (100-500 µg/ml) showed α-amylase inhibitory activity from 67.37% to 82.97% at the same concentrations **Fig. 2.** The previous studies suggested that the antidiabetic activity of grape pomace showed significant inhibition of alpha-glucosidase activity and also magnified the glucose uptake in rats [22].

**TABLE 3: *IN-VITRO* ANTIDIABETIC ACTIVITY OF THE SYNTHESIZED AQUEOUS AND ETHANOLIC EXTRACT USING ALPHA-AMYLASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE**

| S. no. | Concentration | Alpha-Amylase (%) | | |
|---|---|---|---|---|
| | | Aqueous | Ethanolic | Acarbose |
| 1 | 100 (µg/ml) | 43.97 | 55.31 | 67.37 |
| 2 | 200 (µg/ml) | 46.09 | 59.57 | 73.04 |
| 3 | 300 (µg/ml) | 49.64 | 65.95 | 77.30 |
| 4 | 400 (µg/ml) | 51.06 | 72.34 | 81.56 |
| 5 | 500 (µg/ml) | 53.19 | 77.30 | 82.97 |

Each value was obtained by calculating the average of three experiments and data are presented as mean ± SEM

**TABLE 3: *IN-VITRO* ANTIDIABETIC ACTIVITY OF THE ALPHA-GLUCOSIDASE METHOD AND COMPARISON OF AQUEOUS AND ETHANOL EXTRACT OF *VITIS VINIFERA* SEEDS WITH STANDARD DRUG ACARBOSE**

| S. no. | Concentration | Alpha Glucosidase ( %) | | |
|---|---|---|---|---|
| | | Aqueous | Ethanol | Acarbose |
| 1 | 100 (µg/ml) | 36.11 | 52.77 | 74.07 |
| 2 | 200 (µg/ml) | 38.88 | 59.25 | 76.85 |
| 3 | 300 (µg/ml) | 40.74 | 71.29 | 81.48 |
| 4 | 400 (µg/ml) | 46.29 | 73.14 | 82.40 |
| 5 | 500 (µg/ml) | 50 | 77.77 | 85.18 |

Each value was obtained by calculating the average of three experiments and data are presented as Mean± SEM

***In-vitro* A-Glucosidase Inhibitory Assay:** The results of antidiabetic activity were using α-glucosidase inhibitory assay of aqueous and ethanolic extract of *Vitis vinifera* shown in **Table 3.** The extracts revealed a significant inhibitory action of an α-glucosidase enzyme. The percentage inhibition at 100-500 µg/ml concentrations of extracts showed a dose-dependent increase in

percentage inhibition. The alpha-glucosidase inhibitory activity from 36.11% to 50% for aqueous extract and 52.77% to 77.77% for ethanol extract at concentration (100-500 µg/ml) **Table 2.** Acarbose is a standard drug for α-amylase inhibitors. Acarbose at a concentration of (100-500 µg/ml) showed α-amylase inhibitory activity from 74.07% to 85.18% at the same concentrations 100 µg/ml **Fig. 3.**

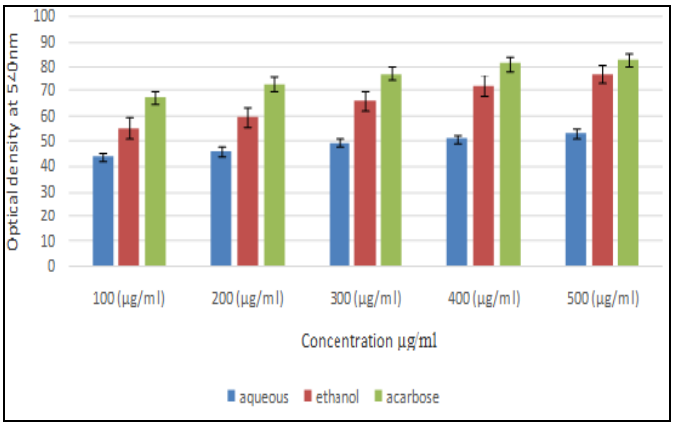


**FIG. 2: *IN-VITRO* ANTIDIABETIC ACTIVITY OF THE SYNTHESIZED AQUEOUS AND ETHANOLIC EXTRACT USING ALPHA-AMYLASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE**

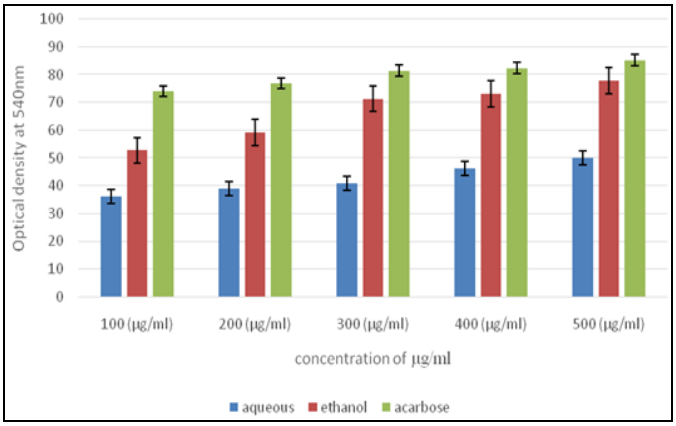


**FIG. 3: *IN-VITRO* ANTIDIABETIC ACTIVITY OF THE SYNTHESIZED AQUEOUS AND ETHANOLIC EXTRACT USING ALPHA-GLUCOSIDASE METHOD AND COMPARISON WITH STANDARD DRUG ACARBOSE**

**CONCLUSION:** Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Therefore alternative treatments are of high-interest means by using medicinal plants or phytotherapy. The result revealed that the aqueous and ethanolic extract of *Vitis vinifera* seeds showed a significant effect on DPPH ethanol (76.3%) and aqueous (59.6%) are compared with standard ascorbic acid. The *Vitis vinifera* seeds (100-500 µg/ml) of the various concentrations exhibited potent α-amylase inhibitory activity in ethanol (74.2%) and aqueous (68.5%) and compared with standard acarbose (88.5%) at a concentration of (100-500 µg/ml). The extracts revealed a significant inhibitory action of α-glucosidase activity in ethanol (75.7%) and aqueous (74.5%) and compared with the standard acarbose (92.9%) at a concentration (100-500µg/ ml). The plant *Vitis vinifera* seeds showed significant enzyme inhibitory activity, so the compound responsible for inhibiting activity has to be done for the usage of the antidiabetic agent.

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**CONFLICTS OF INTEREST:** The authors declare that there are no conflicts of interest regarding this review.

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