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ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTI-DIABETIC POTENTIAL OF VITIS VINIFERA LINN LEAF EXTRACTS

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ABSTRACT

Inflammation is considered as an overwhelming burden to the healthcare status and is associated with significant number of diseases such as cancer, chronic lower respiratory disease, inflammatory bowel diseases, diabetes, asthma, osteoporosis, nephritis etc. Diabetes mellitus is accompanied with long-lasting damage, dysfunction, and failure of different organs, especially the kidneys, heart, nerves, eyes, and blood vessels. The aim of this study was to evaluate various leaf extract fractions of *Vitis vinifera*for antioxidant, anti-inflammatory and antidiabetic potential. Membrane stabilization, proteinase inhibition and albumin denaturation methods were used to assess anti-inflammatory activity. The antidiabetic activity was evaluated by non-enzymatic glycosylation of hemoglobin and alpha amylase inhibition methods. The antioxidant activity was evaluated by ferric reducing/antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and radical mediated DNA damage methods. The hydroalcoholic extract showed overall significant antioxidant, anti-inflammatory and antidiabetic activity in a concentration dependent manner compared to ethyl acetate and petroleum ether extracts. This might be due to the presence of high phenolic and flavonoid content in the hydroalcoholic extract. The results are suggestive of potential *in-vitro* anti-inflammatory, antidiabetic and protective activity of hydroalcoholic extract of *Vitis vinifera* leaf.

KEYWORDS: *Vitis vinifera,* DNA damage, Anti-inflammatory, Antidiabetic, Gel electrophoresis.

INTRODUCTION

Inflammation is a protective response to tissue injury caused due to response to stress, trauma, noxious stimuli by heat, chemical agents, antigen-antibody reactions, and microbial effect. The signs and symptoms of inflammation characterized by redness, pain, heat and swelling and loss of function.[1] Inflammation is associated to significant number of diseases such as cancer, respiratory tract disease, inflammatory bowel diseases, stroke, Alzheimer's, diabetes, osteoporosis, multiple nephritis etc. Diabetes mellitus is a metabolic disorder characterized by elevated levels of blood sugar, with disturbances in fat, carbohydrate, and protein metabolism due to impaired secretion of insulin, defect in insulin action, or both.[2] The chronic hyperglycemia leads to the development of various vascular complications like stroke, coronary heart disease, neuropathy, and retinopathy.[3] The prevalence of diabetes among adults (aged 20-79 years) is 6.4%, affecting 285 million adults all over the world and is increasing with time and might affect 439 million adults by 2030 globally. A study conducted by Shaw and colleges[4] reported that there will be a 69% increase in numbers of adults with diabetes in developing countries and 20% increase in developed countries between 2010 and 2030. The Reactive Oxygen Species (ROS) play a significant role in the pathogenesis of serious ailments, such as atherosclerosis, cataract, cardiovascular disorders (CVD), diabetes, neurodegenerative disorders, and other inflammatory diseases. Antioxidants have the capability to scavenge free radicals produced due to various metabolic reactions in human body which can lead to serious conditions like cancer, diabetes, and CVD.[5]Living cells possess two major groups of natural antioxidant defense mechanism viz enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants include catalase, ascorbate, glutathione, guaiacol peroxidase, glutathione reductase, dehydroascorbate reductase, and superoxide dismutase. Non-enzymatic defense systems include vitamin A, E, and C, minerals, tocopherols, phenolic acids, enzyme cofactors, and some nitrogenous compounds.[6] Since these natural antioxidant defense mechanisms are inadequate on their own, therefore, it is imperative to include antioxidants in our diets.[7] Synthetic antioxidants are reported to have carcinogenic potential, hence nowadays they are being replaced by natural antioxidants that are capable of stabilizing or eliminating the ill effects of ROS.[8] The natural antioxidants also protect human body from deleterious effects of free radicals generated from the metabolic reactions and can delay the progression of diseases like stroke, inflammation, and cancer. They also have the ability to improve antioxidant capacity of plasma and prevent lipid oxidative rancidity in foods.[9,10] Study conducted by Shebis and colleagues[11] reported that these antioxidant components have a potential antimutagenic, antitumor, antibacterial,

anticancer, and anti-inflammatory activities. Plant tissues are the major biological systems that are associated with the synthesis of important compounds like carotenoids, α-tocopherol, ascorbic acid, and phenolic compounds like flavonoids and phenolic acids that have been reported to exhibit several therapeutic effects. Most of these therapeutic effects are mainly attributed due to the presence of phenolic acids and flavonoids, that have received considerable attention in the research field[12] Grapes (*Vitis vinifera* L.) a species of *Vitis*, possess significant amount of phenolic compounds, especially flavonoids, which offer health benefits via their vital antioxidant activity to neutralize free radicals in biological systems.[13,14] Grapes act as rich source of phenolic acids, flavonoids which exhibits many biological activities that can prevent the progression of diseases and also prove beneficial for health which makes it a good candidate in the field of pharmaceutical and food industry. [15] Previous studies have shown several biological activities of *Vitis vinifera* as antifungal, antibacterial, anticancer, antiviral, hepatoprotective, hypoglycemic, spasmolytic, vasorelaxant, renal protective, and cardioprotective.[12,16–22] Keeping in view its traditional medicinal profile, the present study was designed to evaluate *Vitis vinifera* Linn. Leaf extract fractions for *in vitro* antidiabetic, anti-inflammatory, and antioxidant potential.

MATERIALS AND METHODS

Collection of plant material

Fully grown, green, healthy leaves of *Vitis vinifera* were collected in Laar area of District Ganderbal, Jammu & Kashmir and authenticated by Akhtar H. Malik the curator, Centre for Biodiversity and Taxonomy, University of Kashmir, India (under Voucher Specimen No. 2691-KASH University of Kashmir).

Preparation of the extracts

The leaves were collected, thoroughly washed with tap water, afterwards air dried at room temperature under shade for 2 weeks. The leaf petioles were carefully manually separated and were pulverized using electric grinder to obtain a coarse powder. The hydroalcoholic (ethanol/water 80/20, v/v; 2L) ethyl acetate, and petroleum ether extracts were prepared by maceration (125 g/2 L) with respective solvents at room temperature for 3 days with occasional shaking. The extracts were filtered with Whatman No.1 filter paper and the filtrates were dried under a vacuum using a rotary evaporator. The extracts were concentrated and stored at 4°C in airtight glass bottles before use.

Characterization of prepared extracts

Preliminary phytochemical screening

The leaf extracts of *Vitis Vinifera* were screened for the presence of bioactive components according to standard methods.[23,24]

Estimation of total phenolic content (TPC)

Test sample 100 μL was diluted with 6.0 mL of water in a 10.0 mL volumetric flask, to which was added 500 ml of Folin-Ciocalteu reagent. After 1 min, 1.5 mL of 20% (w/v) sodium carbonate (Na_2CO_3) was added, and the volume was made up to 10.0 mL with water. Sample was incubated for 2 hours at 25 °C, the absorbance was measured at 765 nm, which was compared to Gallic acid calibration curve. Total phenols were estimated as Gallic acid equivalents, expressed as $mg_{gallic\ acid}/g_{extract}$.The tests were performed in triplicates and consequently average of triplicates was used to present the data, which was calculated by using the following equation.[25]

$$\mathrm{TPC} = \mathrm{C} \times \frac{V}{M}$$

Where, C is the concentration of gallic acid (mg/ml) established from the calibration curve; V is the volume of the extract solution in ml; M is the weight of extract g.

Estimation of total flavonoid content (TFC)

1 ml of each extract in methanolic stock solution (10 g/L) was mixed with 1 ml of aluminium trichloride in ethanol (20 g/L) and was diluted with 25 ml of ethanol. Afterwards absorption was measured at 415 nm after the mixture was kept at 20°C for 40 minutes. Blank samples were prepared by mixing 1 ml of extract with few drops of acetic acid and diluted with ethanol to 25 ml. The rutin calibration curve was also prepared in the same way in alcoholic medium and TFC of extracts were calculated as rutin equivalents, expressed as $mg_{rutin}/g_{extract}$. All determinations were carried out in triplicated, and the mean values were calculated by the following equation.[26]

$$\mathrm{TFC} = \mathrm{C} \times \frac{V}{M}$$

Where C is the concentration of rutin(mg/ml) established from the calibration curve; V is the volume of the solution in ml: M is the weight of extract in g.

2, 2-Dipheny1-1-Picrylhydrazyl (DPPH) assay

DPPH solution (0.004% w/v) was freshly prepared in 95% methanol. Stock solutions of 10 mg/m1 from extracts were prepared by using methanol as a solvent. Respective aliquots of 10 µL, 20 µL, 30 µL, 40 µL and 50 µL representing concentrations of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL and 500 µg/mL of the stock solutions were added to test tubes (5 ml capacity). The various concentrations of respective extract were mixed with DPPH solution to make up the volume to 3 ml. The reaction mixture was subjected to incubation for 30 minutes in dark and absorbance was recorded at 517 nm. Ascorbic acid was used as a standard (1mg/m1). The scavenging capacity of ascorbic acid with distilled water was also measured. Control was prepared in the same way as for the test group except the addition of the test sample. All the tests were performed in triplicate. DPPH scavenging effect was calculated as the percentage of DPPH discoloration using the following equation.[27]

$$\% \text{ scavengingeffect} = \frac{\text{Absorbancecontrol} - \text{Absorbanceofsample}}{\text{Absorbanceofcontrol}} \times 100$$

Determination of ferric reducing/antioxidant power (FRAP)

1 mL of different concentrations ranging from 100 - 500 µg/ml of sample extracts were mixed with 2.5 mL of sodium phosphatebuffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was shaken vigorously and subjected to incubation for 20 min at 50 °C. After incubation, 2.5 mL of 10% trichloroacetic acid (w/v) was added and mixture was centrifuged at 1000 rpm in a refrigerated centrifuge for 8 min. 2.5 ml from the upper layer was taken out and mixed with 2.5 ml of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using spectrophotometer. Ascorbic acid was taken as positive control and was tested for the reducing power assay in the same way as described earlier. All tests were performed in triplicate.[28]

DNA protective effect of extracts on hydroxyl radicals mediated DNA damage

Calf thymus DNA was subjected to oxidation with Fenton reagent and the measurement of extent of DNA oxidation was investigated using agarose gel method. The reaction mixture contained 5µg of calf thymus DNA, Fenton's reagent (H_2O_2 30mM, Ascorbic acid 50µM, $FeCl_3$ 80µM) in TAE (Tris acetate EDTA) buffer and extracts in a final volume of 30µl. The mixture was subjected to incubation at 37°C for half an hour. After incubation 20µl of the mixture was loaded in 1% agarose gel with ethidium bromide as staining agent and

electrophorised. Gallic acid 100µg was used as the standard. The electrophoresis was carried out at 100 V for 15 min and results were recorded and photographed with gel documentation system (Alpha Digidoc (RT2) digital gel documentation system).[29]

Pharmacological characterization

Evaluation of Anti-inflammatory activity

Inhibition of albumin denaturation (Bovine Serum Albumin (BSA) assay)

BSA solution (0.2%, w/v) was prepared in Tris Buffered saline and pH was adjusted to 6.8 using glacial acetic acid. Different concentrations (100 - 500 µg/ml) of respective extract were prepared using methanol as a solvent and were added to test tubes. 5 m1 of 0.2% w/v BSA was added to each of the above test tubes. The control contains 5 ml of 0.2% w/v BSA solution with 50 µl methanol. The standard consists of 100 µg/ml of aspirin in methanol with 5 ml 0.2% w/v BSA solution. The solutions were heated on water bath at 72°C for 10 minutes, and subsequently cooled for 20 minutes. The turbidity of the solutions (measure of protein precipitation) was measured at 600 nm by using a spectrophotometer. All the experiments were conducted in triplicates and the mean values of the absorbances were taken using the following equation.[30]

$$\%\ \text{Anti} - \text{Denaturation Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Membrane stabilization

*Preparation of Human Red Blood Cells (HRBC) Suspension***:**blood was collected from the batch mates who had not taken any non-steroidal anti-inflammatory drugs (NSAIDs) for at least two weeks before the experiment and transferred to centrifuge tubes. The tubes were subjected to centrifugation at 300 rpm for 10 minutes and then washed with equal volume of normal saline.[31]

The membrane stabilization was done by heat induced hemolysis. The reaction mixture (2 m1) containing 1 ml of test sample of different concentrations (100 - 500 µg/m1) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. Afterwards the centrifuge tubes were incubated on water bath at 56°C for 30 minutes. After incubation tubes were cooled and mixture was subjected to centrifugation at 2500 rpm for 5 minutes. Absorbance of the supernatant that contain released

hemoglobin was measured at 560 nm. The % inhibition of hemolysiswas determined, as was the BSA assay.[32]

Proteinase inhibitory action

Test sample 1 ml of concentrations (100 – 500 µg/ml) was mixed with 0.06 mg of trypsin and 1 ml of 20 mM Tris HCl buffer (pH 7.4). The mixture was subjected to incubation at 37°C for 5 minutes. After incubation 1 ml of casein (0.8% w/v) was added ad then the mixture was again incubated for 20 minutes. The reaction was ended by adding 2 ml of 70% perchloric acid. Afterwards the reaction mixture was centrifuged, and absorbance of supernatant was measured at 210 nm against buffer as blank. The experiment was performed in triplicate and mean values are taken. The % inhibition of proteinase inhibitory activity was calculated as was the BSA assay.[31]

Evaluation of antidiabetic activity

Preparation of VitisVinifera stock solution: Stock solution (10 mg/ml) was prepared by dissolving 100 mg of the extract in 10ml of Dimethylsulfoxide (DMSO). From this stock solution, respective aliquots of 10 µL, 20 µL, 30 µL, 40 µL and 50 µL representing concentrations of 100 - 500 µg/mL respectively were prepared using DMSO solution.

Non-enzymatic glycosylation of hemoglobin method

Hemoglobin 60mg in 100 ml of 0.01M phosphate buffer (pH 7.4) was incubated in presence of glucose 2 g/ 100 ml concentration for 72 h. the assay was performed by adding 1 ml of glucose solution, 1 ml of gentamycin (20 mg/ 100ml), in 0.01 M phosphate buffer (pH 7.4). Afterwards the mixture was incubated in at room temperature udder dark conditions for 72 h. The degree of glycosylation of hemoglobin was measure colorimetrically at 520 nm. α-Tocopherol (Trolox) was used as a standard. The % inhibition of non-enzymatic hemoglobin glycosylation activity was determined, as was the BSA assay.[33]

Inhibition of alpha amylase enzyme

Test samples (0.5 ml) and standard drug (100 – 500 µg/ml) were added to 05 ml of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (0.5 mg/ml) and the mixture was incubated at 25˚C for 10 minutes. After incubation, 500µl of starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each test tube and the reaction mixture was then incubated at 25˚C for 10 minutes. After that the reaction was terminated with 1 ml of 3,5-dinitrosalicylic acid color

reagent. The tubes were then incubated in a boiling water bath for 5 minutes, then cooled at room temperature and absorbance was measured at 540 nm using acarbose as a standard drug. The % inhibition of alpha amylase enzyme was determined as was the BSA assay.[34,35]

Statistical analysis

The obtained findings were presented as mean ± standard error mean (SEM) and were analyzed by GraphPad Prism (8) using linear regression. The differences were considered statistically significant when $p<0.05$

RESULTS AND DISCUSSION

The percentage yield of hydroalcoholic extract (HAE), ethyl acetate extract (EAE), and petroleum ether extract (PEE) of *VitisVinifera* leaves was found to be 12.88%, 8.57% and 6.2% respectively. The phytochemical screening of the extracts revealed the presence of different phytoconstituents like flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. Flavonoids, phenols, tannins were found to be present in all the extracts but were predominantly found in HAE as compared to EAE and PEE. PEE as revealed from the results contain phytoconstituents in small quantities as compared to other extracts **(Table 1).** TPC of different extracts was determined from the Gallic Acid calibration curve $y = 0.0024x + 0.2438$ with $R^2 = 0.9979$. TFC of different extracts was determined from the Rutin calibration curve $y = 0.0077x + 0.195$ with $R^2 = 0.998$.The HAE showed highest phenolic and flavonoid content as compared EAE and PEE. TPC expressed as $mg_{gallicacid}/g_{extract}$ and TFC expressed as $mg_{rutin} /g_{extract}$of the extracts and are shown in **(Table 2).**

Table 1: List of Phytoconstituents present in the prepared extracts from leaves of VV.

Phytoconstituents	***Extracts of leaves of VV***		
	EAE	**PEE**	**HAE**
Tannins	++	+	+++
Flavonoids	++	+	+++
Terpenoids	-	-	-
Saponins	-	-	+
Steroids	+	+	-
Carbohydrates	+	-	+
Glycosides	-	-	+
Alkaloids	+	+	-
Proteins	-	-	-
Fixed oils	+	+	-

The sign (+) indicates the presence and (−) indicates absence of phytoconstituents.

Table 2: Total Phenolic and Flavonoid content of the prepared VV leaf extracts.

Extracts	TPC	TFC
HAE	239	17.6
EAE	193.3	10.5
PEE	149.5	5.6

Reactive oxygen species (ROS) induce oxidative stress due to presence of unpaired electron and are involved in a variety of biological processes, such as mutations, carcinogenesis, degenerative and other diseases, inflammation, aging and development.[36] The use of natural antioxidants, mainly phenolic and flavonoid compounds act as promising alternative to synthetic antioxidants in respect of low cost, high compatibility with dietary intake and harmful or side effects.

Various methods have been employed by different researchers to determine antioxidant activity. In the present study, the extracts of *VitisVinifera* leaves were evaluated for antioxidant activity by DPPH radical scavenging, FRAP, and DNA damage inhibition. The DPPH method is usually used in evaluating free radical scavenging activity because it is fast, easy and reproducibility of results is good. Also, DPPH is a stable, synthetic radical and does not react with solvents like water, methanol, or ethanol. When DPPH reacts with an antioxidant, the purple color fades or disappears due to its conversion to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in absorbance. Discoloration occurs due to the decreasing quantity of DPPH radicals in the solution which reflects the radical scavenging activity of the extracts.[37] The FRAP assay is simple, quick, inexpensive and the reaction is reproducible and direct method to determine the "total antioxidant power" of antioxidants.[38] Oxidative DNA damage has been implicated to be involved in various degenerative diseases.[39] The DPPH scavenging activity of all prepared extractswas found to be concentration-dependent **(Fig. 1A).** The HAE, EAE, and PEE showed maximum activity of 97.01%, 88.90% and 80.40% respectively at 500µg/ml, whereas ascorbic acid at the same concentration exhibited 93.10% inhibition. HAE, EAE and PEE and Ascorbic acid were found to have IC_{50}values of 98.3µg/ml, 133.7µg/ml, 250.1µg/ml, and 117.1µg/ml respectively. The reducing power ability of the extracts was also concentration dependent. Reducing power of HAE was found to be comparable to standard (Ascorbic acid) **(Fig.1B).**

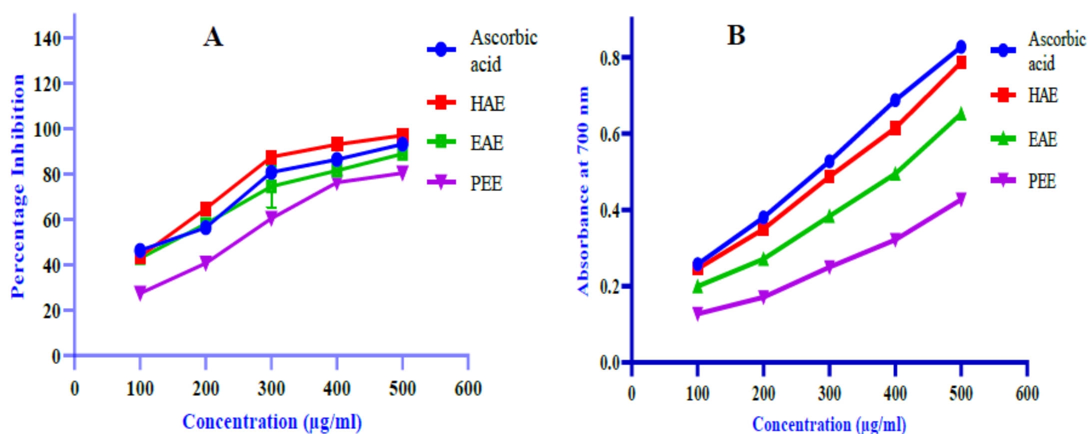
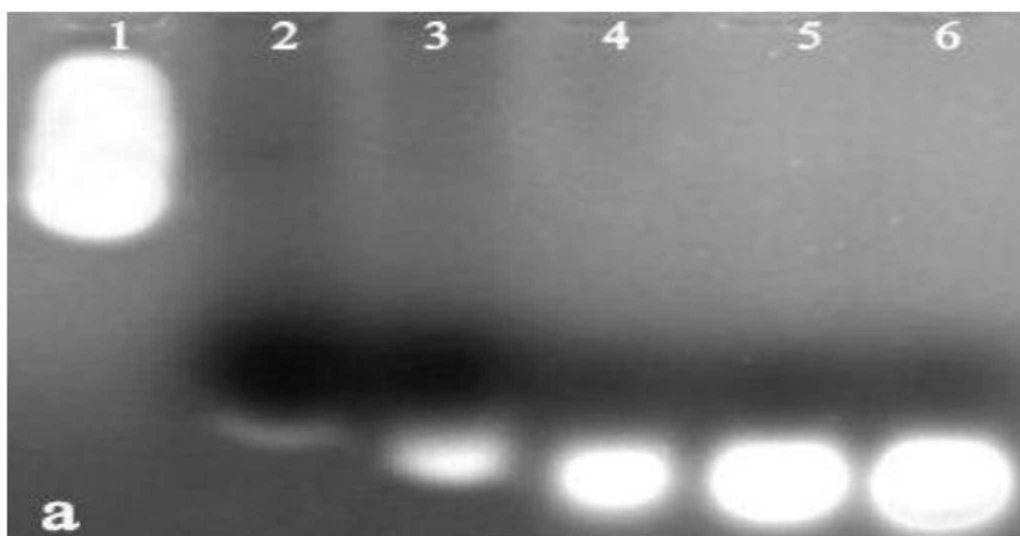


Fig. 1: Free radical scavenging activity of extracts: (A) Scavenging activity by DPPH assay. (B) Reducing power activity. The values are presented as mean ± SEM, (n = 3).

All the extracts effectively preventedthe DNA damage due to the oxidative stress of the hydroxyl radicals. This effectiveness of the extracts to prevent oxidative DNA damage may be due to its hydroxyl radical scavenging activity. This suggests that these extracts may combat free radical (like hydroxyl radical) mediated oxidative DNA damage. Hydroalcoholic fraction was found to inhibit the degradation induced by Fenton's reagent more effectively than other extracts and the activity was almost comparable to that ofstandard Gallic acid. The results of HAE, EAE, and PEE were photographed and presented in the **Fig. 2.**



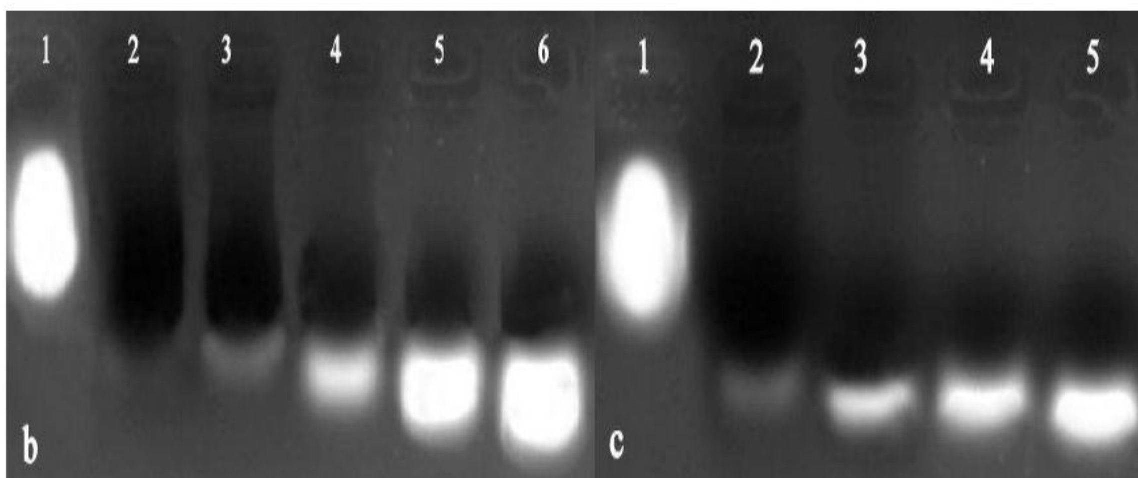


Fig. *2:* Gels showing the inhibition of hydroxyl radicals-mediated DNA degradation by leaf extracts of VV. (a=Hydroalcoholic extract, b=Ethyl Acetate extract, c=Pet ether extract) In (a), (b), and (c) Lane 1: DNA untreated and Lane 2: DNA + Fenton's reagent In (a), Lanes 3-5: DNA + Fenton's reagent + extract 20µg, 50µg, and 100µg respectively In (b), Lanes 3-5: DNA + Fenton's reagent + extract 50µg, 100µg, and 150µg respectively In (c), Lanes 3-5: DNA + Fenton's reagent + extract 100µg, 150µg, and 200µg respectively Lane 6 in (a) and (b): DNA + Fenton's reagent + Gallic acid (100µg) as standard.

Protein denaturation defined as a pathological condition which includes the loss of shape is considered as one of the causes of inflammation. Agents that are able to prevent this condition are therefore considered beneficial and are therefore implicated in anti-inflammatory drug development process[40] Adoption of the BSA protein denaturation assay for evaluation of anti-inflammatory potential of extracts has circumvented the ethical issues associated with the use of animals, especially in the early stages of screening of new drugs or biomolecules. During inflammatory response, lyses of lysosomes results in the release of enzymes that produce a variety of disorders like tissue damage. NSAIDs exhibit their anti-inflammatory potential either by inhibiting the release and stabilizing the lysosomal enzymes and lysosomal membranes respectively.[41] Exposure of red blood cells to harmful elements like phenlyhydrazine, hypotonic medium, methyl salicylate, and heat results in the rupture of membranes, hemolysis and oxidation of hemoglobin[42] Since RBCs membrane is similar to that of lysosomal membrane[43] hence lysis of red blood cell membrane due to the heat was taken as the degree of the anti-inflammatory potential of extracts. Interleukin-1 (IL-1) which exists in two isoforms, IL-1α and IL-1β is considered as one of the principal mediators in the pathogenesis of acute and chronic inflammation. Enzyme caspase-1 (IL-1β converting

enzyme, ICE) a cysteine protease plays significant role in the pathogenesis of various ailments like stroke, inflammatory bowel syndrome, brain damage, rheumatoid arthritis, and unscheduled programed cell death. Inhibiting caspase-1 could be a new possible therapeutic approach for the treatment of such diseases.[44,45] The anti-inflammatory activity of the extracts was determined by inhibition of albumin denaturation, membrane stabilization, and proteinase inhibition. All extracts displayed concentrationdependent % inhibition of albumin denaturation, membrane stabilization, and proteinase inhibition **(Fig. 3).** Maximum % inhibition of albumin denaturation as 82.44%, 75.37%, 72.83%, and 85.60% was observed at 500 µg/ml from HAE, EAE, PEE, and ascorbic acid respectively. Maximuminhibition of membrane lysis in RBCs was 70.2% observed in HAE, followed by63.1% in EAE and58.4% in PEE at 500µg/ml. Maximum proteinase inhibition of 79.49%, 76. 4%, 64.2% and 84.8% was shown by EAE,HAE, PEE and ascorbic acid respectively atsame dose level. The IC_{50} values are presented in **Table 3.**

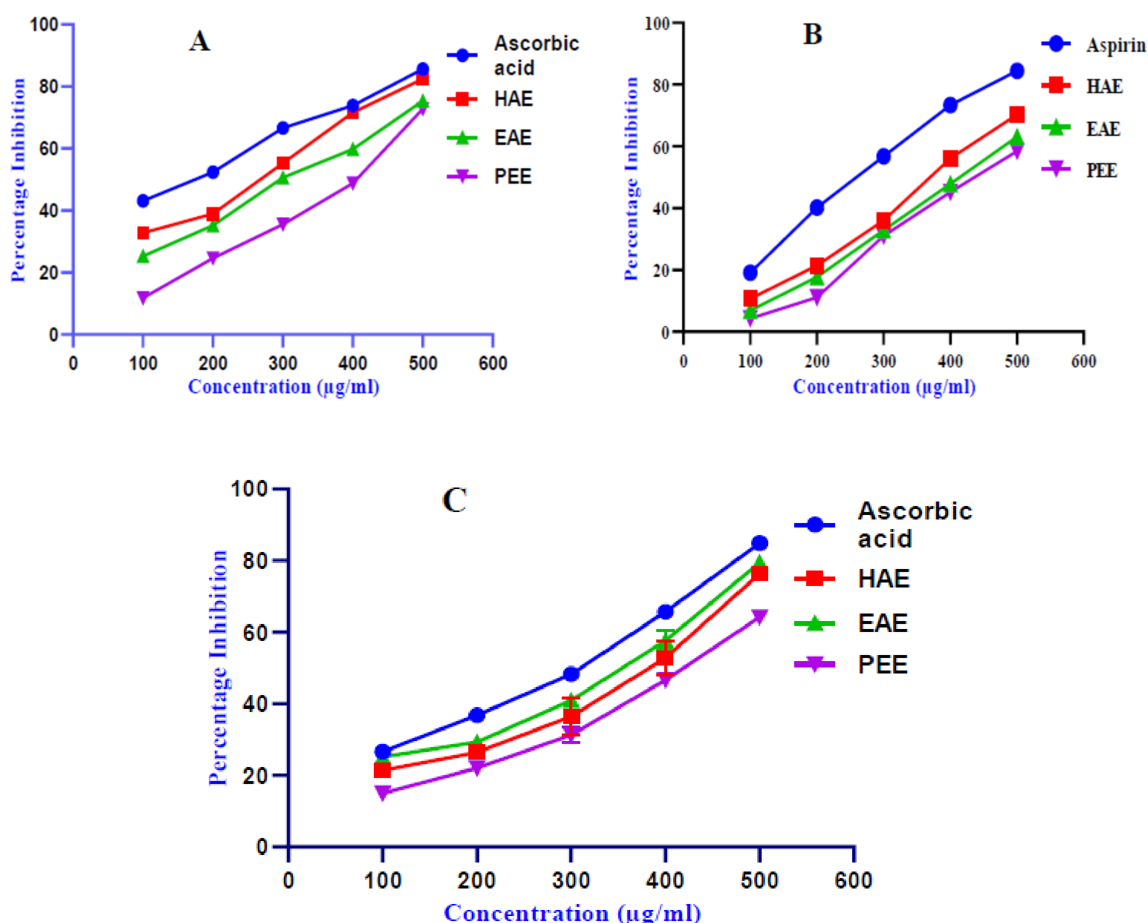


Fig. 3: Anti-inflammatory activity of VVleaf extracts: (A) % inhibition of albumin denaturation; (B) % membrane stabilization; (C) % proteinase inhibition. The values are presented as mean ± SEM, (n = 3).

Table 3: IC_{50} values of the prepared leaf extracts and standard for anti-inflammatory methods.

Extracts	IC_{50} (µg/ml) anti-inflammatory methods		
	Albumin denaturation	**Membrane stabilization**	**Proteinase inhibition**
HAE	253.0	372.0	353.6
EAE	305.8	414.4	325.5
PEE	377.0	440.3	415.0
Standard	Ascorbic acid (165.3)	Aspirin (270.7)	Ascorbic acid (283.2)

In addition to the major hemoglobin (hemoglobin A), erythrocyte of an adult has, several minor hemoglobins (hemoglobin A_{1a}, hemoglobin A_{1b}, and hemoglobin A_{1c})[46] Glucose has a tendency to react nonenzymatically with the NH2-terminal amino acid of the beta chain of human hemoglobin through ketoamine linkage, resulting in the formation of hemoglobin A_{1c}. Among the different anti-diabetic therapeutic approaches one major strategy is to decrease the postprandial hyperglycemia. This is achieved by retarding the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes (α-amylase, α-glucosidase and sucrase)[47] Acarbose and miglitol are competitive inhibitor of α-glucosidases and reduce absorption of starch and disaccharides.

Antidiabetic activity of leaf extracts of *Vitis Vinifera* were investigated by estimating degree of inhibition of non-enzymatic hemoglobin glycosylation and alpha amylase enzyme. The different *Vitis Vinifera* leaf extracts and tocopherol displayed concentration dependent inhibition of hemoglobin glycosylation and alpha amylase **(Fig. 4**). Maximum hemoglobin glycosylation inhibition of 76.8%, 68.6%, 64.0%, and 87.9% was observed inHAE, EAE, PEE, and Trolox respectively. In case of alpha amylase, the maximum % inhibition of 77.5% was seen inHAE followed by72.64%, 61.5% and 84.8 % in EAE, PEE and acarbose respectively. The IC_{50} values are presented in **Table 4.**

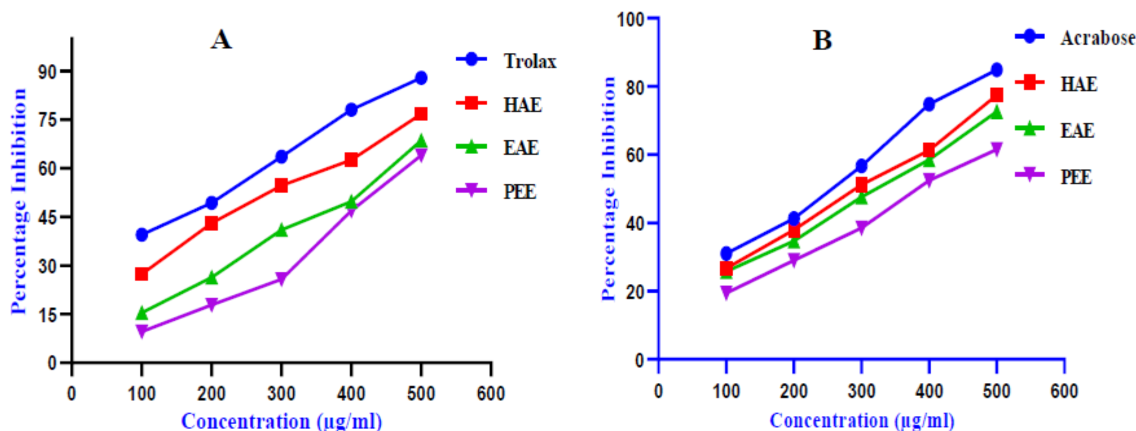


Fig. 4: Anti-diabetic activity of extracts from VV: (A) %inhibition of hemoglobin glycosylation;(B) % alpha amylase inhibition. The values are presented as mean ± SEM, (n = 3).

Table 4: IC_{50} values of the prepared leaf Extracts and Standard for antidiabetic methods.

Extracts	IC_{50} (µg/ml) antidiabetic methods	
	Non-enzymatic glycosylation	**Alpha amylase inhibition**
HAE	275.2	292.7
EAE	375.0	318.2
PEE	424.1	391.2
Standard	Trolox (190.7)	Acarbose (245.3)

CONCLUSION

The preliminary phytochemical analysis of *Vitis vinifera* leaf shows the presence of important bioactive constituents that possess anti-inflammatory, antidiabetic and antioxidant potential. Overall, the hydroalcoholic extractshoweda significant free radical scavenging, anti-inflammatory and antidiabetic activity whereas ethyl acetate extract was found to be more potent proteinase inhibitor. Thus, it can be concluded that *Vitis vinifera* leaf extract consists of important bioactive molecules, including a high percentage of phenolic and flavonoid content. These biomolecules alone or in combination can be employed in the management of diabetes and inflammation, which makes *Vitis vinifera* a prospective candidate for further exploration for its medicinal potential.

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List of abbreviations

BSA: Bovine serum albumin;CVD: Cardiovascular diseases; DMSO: Dimethyl sulfoxide; DNA: Deoxyribonucleic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EAE: Ethyl acetate extract;EDTA: Ethylenediaminetetraacetic acid; H_2O_2: Hydrogen peroxide; HAE: Hydroalcoholicextract; HCL: Hydrochloric acid;IC_{50}: Half maximal inhibitory concentration;PEE: Petroleum ether extract;ROS: Reactive oxygen species:TFC: Total flavonoid contentTPC: Total phenol content;UV: Ultraviolet; VV: *Vitis vinifera*

CONFLICT OF INTEREST

The authors have no conflict of interest.

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