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Evaluation of pancreatic lipase inhibition activity and antioxidant potential of *Vinca rosea* leaves

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Abstract

The high cost, side effects and withdrawal of synthetic drugs from market have opened avenues for plants with high pancreatic lipase inhibitory activities as alternate to address the obesity. The present study was aimed to measure the pancreatic lipase inhibitory activity and associated antioxidant activities of leaves from *Vinca rosea* a commonly growing ornamental plant of garden. Water and ethanolic extracts of leaves from *Vinca rosea* were prepared and evaluated for porcine pancreatic lipase inhibitory effect using DNPB (2,4-dinitrophenyl butyrate) and Triolein as substrate. The total phenolic contents of extracts, saponin content of leaves, DPPH radical scavenging activity and Ferric reducing power assays of both types of extracts were also measured. The lipase inhibitory activities of water and ethanolic extract of *Vinca rosea* leaves were 32.39% and 40.81% respectively using DNPB (2,4-dinitrophenyl butyrate) as substrate. Using Triolein as substrate, values were 28.55% and 40.78% respectively. The total phenolic, saponin (%), DPPH free radical scavenging activity (%) and ferric reducing power assay (absorbance) were measured as 11-12 mgGAE/g, 1.34-1.81, 32.24-41.48 and 0.69-0.71 respectively. Both the extracts, water and ethanolic were same except the difference in pancreatic lipase inhibitory activity and DPPH radical scavenging activities which were higher in ethanolic. The study suggested that both water and ethanol extracts are capable as anti pancreatic lipase and antioxidant with slight edge for more polar ethanol and can act as promising natural alternates of the established lipase inhibitory molecules i.e. orlistat and antioxidant BHT.

Keywords: *Vinca rosea*, Inhibition of pancreatic lipase, antioxidant activity, total phenolics, DPPH, FRPA

Introduction

The increase in prevalence of obesity or excess body weight among the stressed population of today's developing world demands for strategic changes in their food habits. High energy diet, diet with substantial amount of fat or other preliminary causes identified as reasons for obesity or extra weight are most of the time inescapable because of fast life style. Out of the several strategies applied for, the play with normal physiology of digestion using some safe food grade agents appears novel. Digestion of lipids or fat has been reported to be reduced by using inhibition of Pancreatic-lipase activities. It is anticipated that inhibition of lipid digestion may lead to poor absorption of energy dense lipids and therefore its availability in body system [1]. The role of antioxidants is well established to resist the stress, another leading cause of obesity. Thus a natural food supplement with enhanced pancreatic lipase inhibition activity and anti-oxidant activities is a promising alternate to deal with the diet related obesity problems.

Vinca rosea is a herbaceous subshrub belonging to family *Apocynaceae* commonly known as 'Madagascar periwinkle', synonyms as *Lchnera rosea*, *Catharanthus roseus* Linn, *Ammocallis rosea*. Although, originated from Madagascar (Island in South African region), the plant have made their presence in different agro climatic zone [2] because of very high survival rate. Right from ancient age this plant has a long history to be used as traditional medicine in many countries like China, India, Mexico Malaysia and South Africa [3-5]. Alkaloids and tannins are classes of active compounds in *Vinca* and the major alkaloid vincamine and its closely related semi-synthetic derivative, known as ethyl-apovincaminatate or vinpocetine are widely used as a medicinal agent as vasodilators, blood thinning, hypoglycemic and memory-enhancing agents [6]. It is cultivated worldwide for their phytochemical constituents which are having well known anticancer activities [7] also. *Vinca rosea* leaf juice was reported possessing hypolipidemic activity [8]. The lipid-lowering action of phytochemicals appears to be mainly related to an inhibitory activity on the human pancreatic lipase (HPL), involved in the lipid

absorption [9-10, 11]. Found that Majoridine and Akuammine were anti-lipase fraction present in the flowers of *V. major* possessed high PPL inhibition activity and the identified compounds had significant binding interactions with active site residues of PPL.

The shift from synthetic to natural source of antioxidant has led to screening of more and more plants [12]. The antioxidant activities of plants are attributed mainly to phenolic and polyphenol constituents that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Besides neutralizing free radicals and inhibiting lipid oxidation, these compounds also inhibit the formation of toxins such as malondialdehyde (MDA). [13] Reported that vindolicine an alkaloids from leaves extract of *Vinca rosea* has very good anti-oxidant potential in oxygen radical absorbance capacity and DPPH assays and it also alleviated H₂O₂-induced oxidative damage in β-TC6 cells at 12.5 μg/mL and 25.0 μg/mL.

Keeping in view the reported promising pancreatic lipase inhibition and antioxidant activities and sporadic report on the antioxidant potential and pancreatic lipase inhibition effect of extracts from *Vinca rosea* leaves, the present study was made.

Material and Methods

Preparation of *Vinca rosea* leaves extracts

The leaves of *Vinca rosea* were freshly collected, cleaned and washed thoroughly to remove extraneous dirt. The excess water was drained out and the leaves were dried in a hot air oven at 50 ± 2°C for 8 hours. Dried leaves were ground using laboratory grinder to get fine particles of powder. Extracts were prepared by dissolving 10 g of powder in 100 mL aqueous/ ethyl alcohol (95%) as solvent with intermittent stirring at a hot water bath (55°C) for 4 hr. It was further allowed for overnight extraction at room temperature. It was centrifuged at 5000rpm for 10 min twice. The mixtures were filtered twice with whatman filter paper-1 to remove the residual contents. The obtained water and ethanolic extracts of *Vinca rosea* leaves were used for analysis of pancreatic lipase inhibition and antioxidant activities.

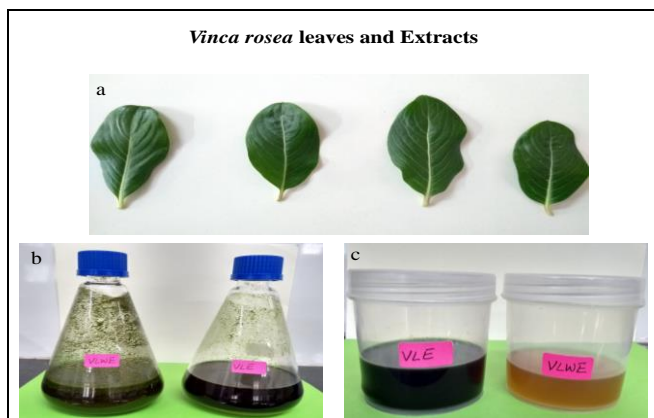


Fig 1a): *Vinca rosea* leaves. **b)** Water (VLWE) and Ethanolic Crude extracts (VLE) of *Vinca rosea* leaves. **c)** Water (VLWE) and Ethanolic filtered extracts (VLE) of *Vinca rosea* leaves.

Assay of Porcine Pancreatic Lipase inhibition activities

The extracts were evaluated for their porcine pancreatic lipase inhibiting activity by using:

a) Inhibition assay using DNPB as substrate: Lipase activity of plant extracts was measured by using 2,4-dinitrophenyl butyrate (DNPB) as substrate as per method of [14]. Reaction mixture was prepared with 0.1mL of

porcine pancreatic lipase (200unit/mL) in 0.1M potassium phosphate buffer with pH 6.8 followed by 0.1mL of inhibitor solution (Plant Extracts) was added. For initiation of reaction 0.5mL of 25mM DNPB was added. The final volume of reaction mixture was 0.7mL. Whole reaction mixture was kept at 37 °C in incubator. After 1 min of incubation the 2,4-dinitrophenol was released from 2,4-dinitrophenyl butyrate (DNPB) in this reaction by the porcine pancreatic lipase. Free 2,4-dinitrophenol was measured as absorbance at 360 nm. The orlistat (1mg/mL) was taken as positive control. Lipase inhibitory activity (%) was calculated by using equation as:

$$\text{The lipase inhibitory activity (\%)} = [1 - (A_c - A_s) / A_0] \times 100$$

Whereas

A_c is the absorbance of the substrate of the porcine lipase enzyme in the presence of the sample

A_s is the absorbance of the porcine lipase enzyme with the sample without substrate

A₀ is the absorbance at 100% enzyme activity on the substrate (without sample)

b) Inhibition assay using triolein as substrate: Pancreatic lipase inhibitory activity of different plant extracts were determined by measuring rate of oleic acid liberated from triolein as substrate as per method of [15]. The suspension mixture was prepared by adding 120 mg of triolein as substrate, 90 mg of gum arabic, 10.16 mg taurocholic acid in 9mL of 0.1N TES buffer (pH 7.0) having 0.1M NaCl. The whole mixture was sonicated for 5 min. In this suspension 50 μL of porcine pancreatic lipase (500 U/mL), 50 μL of extracts were added and incubated for 30 min at 37°C. A blank without plant extract were also prepared. After this incubation, incubation mixture (400 μL) was added to 3 mL of chloroform/hexane (1: 1) containing 2% (v/v) ethanol solution and mixture was consistently shaken for 10 min followed by centrifugation of mixture at 5000rpm for 10 min. The upper aqueous layer was pipetted out. The remaining lower organic layer was mixed with 1mL of copper reagent and vortexed for 10 min. The mixture was again centrifuged for 10 min at 5000 rpm. 1 mL of upper organic layer containing copper salts of oleic acid was taken and added with 0.5 mL of 0.1% (w/v) bathocuproine-chloroform solution containing 0.05% (w/v) 3-tert-butyl-4-hydroxyanisole. The mixture components were allowed to react for 10 min. The absorbance of reaction mixture was measured at 480nm. The lipase inhibition (%) was calculated using the formula:

$$\text{Pancreatic Lipase Inhibitory activity (\%)} = (A - B) / A \times 100$$

Whereas

A is lipase activity in the reaction solution without extracts (Blank).

B is lipase activity in the reaction solution containing plant extracts or orlistat

Measurement of Phytoingredients

Vinca rosea leaves were measured for saponin and total phenolic contents as below:

a) Determination of total phenolics: The total phenolic content in extracts was measured by the method suggested by [16]. Briefly, extract from leaves of *Vinca rosea* (100 μL) was

mixed with 0.75ml of Folin-Ciocalteu reagent, the volume was made up ten times (8.5mL) with distilled water and kept it to stand for 5 min at room temperature. Further, 0.75 mL of 6% sodium bicarbonate was added to mixture and incubated at room temperature for 90min in dark. Absorbance was measured against a blank at 765nm. A standard curve was plotted using different concentrations of gallic acid, and the amount of total phenolics was calculated as gallic acid equivalents (GAE) in mg/g of dried leaves powder.

b) Determination of Saponin: The quantitative measurement of saponin percentage was carried out using the method reported by [17]. 5 g sample of ground leaves powder was added in 100mL of 20% aqueous ethanol in 250mL conical volumetric flask. This mixture was heated in hot water bath for 4 hours at a constant temperature of 55°C with constant stirring and is filtered out. Re-extraction of the residue mixture was done by same protocol. The combined extract was heated at 90°C hot water bath to get evaporated and concentrated till volume remained 40mL. Using firstly 20mL of diethyl ether, followed by 60 mL n-butanol and finally 10 mL of 5% sodium chloride, aqueous layer were extracted by a separator funnel of volume 250 mL. The process of mixing, agitation, fractionation and collection of upper aqueous layer was repeated twice with each of the solvents. The recovered solution after 2nd stage of 10 mL of 5% sodium chloride was heated in water bath for 30 minutes. This solution was dried in an oven in crucible to a constant weight. The saponin percentage was calculated as:

$$\text{Saponin \%} = \text{Weight of saponin (g)} / \text{Weight of sample (g)} \times 100$$

Measurement of Antioxidant activities

Antioxidant activities of extracts from *Vinca rosea* leaves were measured as below:

a) Radical Scavenging activity using DPPH assay: The DPPH radical scavenging activities of extracts were measured as per [18]. 1mL of extract was taken in test tube and its volume was made up to 4 mL with distilled water. 1mL of 1mM DPPH methanolic solution was prepared and was added (the DPPH solution was kept protected from light with aluminum foil wrap). All test tubes were shaken well and allowed to stand for 30 min at room temperature. Control was prepared by adding 1 mL DPPH solution and 4 mL of DW. Absorbance (Eppendorf BioSpectrometer basic) was taken at 517 nm (DW used as blank). Free radical scavenging activity (FRSA) was calculated using the following formula –

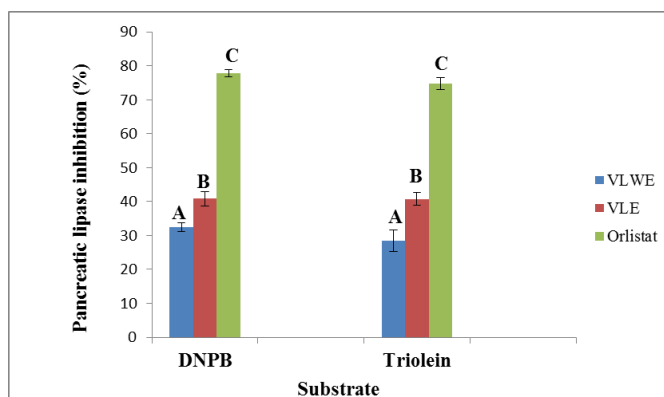
$$\text{FRPA (\%)} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}} \times 100$$

b) Ferric reducing antioxidant power assay: The reducing power of the extracts was determined according to the method of [19]. 1 mL of leaves extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide in a 10mL test tube. Then this mixture was incubated for 20 min at 50°C followed by the addition of 2.5 mL of 10% trichloroacetic acid and then centrifuged at 7000rpm for 10 min. After that 2.5 mL supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1% w/v), and absorbance was measured at 700 nm (Eppendorf Bio Spectrometer basic) against blank without any extracts and 0.1% ferric chloride. An increase in the OD

values (absorbance) of the reaction mixture indicated the reducing power of the sample.

Results and Discussion

The porcine pancreatic lipase inhibition activity of water and ethanolic extract from *Vinca rosea* leaves were 32.39% and 40.81% respectively using DNPB (2,4-dinitrophenyl butyrate) as substrate. The lipase inhibition activities using triolein substrate were 28.55% and 40.78% respectively. The lipase inhibitory activities of orlistat, a well known pancreatic lipase inhibitor were 77.81% and 74.75% using DNPB (2,4-dinitrophenyl butyrate) and Triolein as substrate respectively (Fig 1). Pancreatic lipase inhibition activity shown by *Vinca* leaves water extract (VLWE) was significantly ($P < 0.05$) less than the inhibition activity of *Vinca* leaves ethanolic extracts (VLE). Either of the extracts VLWE or VLE has significantly lower ($P < 0.05$) Pancreatic lipase inhibition activity than shown by commercial Orlistat. Method employed for measurement of Pancreatic lipase inhibition activity viz: DNPB (2,4-dinitrophenyl butyrate) and Triolein as substrate has insignificant effect and percentage of inhibition shown by either methods were almost same. [11] Reported that the anti-lipase activity of methanolic extract of *Vinca* flowers (VFE) were significantly higher (34%) than water extracts (19%). The ether and chloroform extracts had less than 20% inhibitions. Thus it appeared that leaves which are more easily available than flower had considerable porcine pancreatic lipase inhibition activity.



Whereas VLWE-Vinca Leaves Water Extract, VLE-Vinca Leaves Ethanolic Extracts, DNPB- 2,4-dinitrophenyl butyrate Mean±SE with different superscripts differ significantly ($P < 0.05$), $n=6$

Fig 2: Porcine Pancreatic Lipase inhibition activities of extracts from *Vinca rosea* leaves

The total phenolic content of VLWE and VLE are given in Table-1. The total phenolic content estimated from either of the extracts water/ethanolic and expressed as mg GAE/ g of dried powder of plant were almost same. The saponin content in *Vinca rosea* leaves were found in the range of 1.34-1.81% respectively. A great variability 8.18 ± 1.61 to 21.46 ± 2.39 mg GAE/g in total phenolic content of *Catharanthus roseus* leaves was reported by [20]. Use of phenolic content as an indicator of antioxidant properties as also cited by [21]. Saponins have been cited as naturally occurring phytochemicals similar to the hormones found in the human body. The biological properties of saponins are determined by the polarity, hydrophobicity, and nature of the reactive groups on it [22]. Qualitative analysis of phytochemicals in methanolic *Vinca* flower extracts showed positive tests for phenols and saponin contents [11].

Table 1: Total Phenolic content (mg GAE/g) and Saponin (%) of Water (VLWE) and Ethanolic (VLE) Extracts of *Vinca rosea* Leaves

Parameters	Treatments		P value of t-test
	VLWE	VLE	
Total Phenolic content (mg GAE/g)	11.00±0.94	12.22±.081	0.782
Saponin (%)	1.58±0.10		

Whereas VLWE- *Vinca* Leaves Water Extract, VLE- *Vinca* Leaves Ethanolic Extracts, GAE- Gallic acid Equivalent.

Mean±SE with different superscripts in a row differ significantly (P<0.05), n=6.

The Antioxidant activity of water (VLWE) and ethanolic extracts (VLE) of *Vinca rosea* leaves were evaluated for DPPH free radical scavenging (FRSA) activity and ferric reducing power assay (FRPA). The results in Table-2 shows the DPPH free radical scavenging (FRSA) activity of water, ethanolic extracts and synthetic antioxidant BHT (100ppm) were 32.24%, 41.78% and 66.33% respectively. The DPPH free radical scavenging (FRSA) activity of ethanolic extracts was significantly (P<0.05) higher than water extracts but it was significantly (P<0.05) lower than BHT. It is anticipated that the antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1, 1-diphenyl-2-picryl hydrazine with discoloration. The method is based upon the reduction of methanol DPPH solution in the presence of hydrogen donating antioxidant due to formation of the non radical form DPPH-H by the reaction [23]. [24] Also reported the value for DPPH (inhibition %) in crude aqueous extracts and crude ethanolic extracts as 47.16±4.15 and 25.58±1.56 respectively. A good antioxidant potential measured as DPPH inhibitory activity of *Catharanthus roseus* (L.) / *Vinca rosea* (L.) leaves extracts were also reported by [13].

Ferric reducing power assay measures the electron donating capacity of antioxidant molecules. Measuring the absorbance at 700nm of resultant blue-green color of solution as the reduction of the ferric ion (Fe³⁺) to ferrous ion (Fe²⁺) occurs and an increased absorbance is indicative of higher reducing power [25]. The ferric reducing power assay (FRPA) of water and ethanolic extracts of *Vinca rosea* leaves shown in Table-2 reflects that the absorbance at 700nm for ethanolic extracts had slightly higher value than water extract but there was no significant (P>0.05) difference in between these. The ferric reducing power assay (FRPA) of BHT was significantly (P<0.05) higher than ethanolic and water extracts which were due to higher reducing power and antioxidant activity. [26] Suggested that Fe (III) reducing is can be used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. Amount of Fe²⁺ complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The values for DPPH and FRPA in present study further showed that both water and ethanol extracts were capable of electron donation with slight edge for more polar ethanol.

Table 2: Antioxidant activity of Water (VLWE) and Ethanolic (VLE) Extracts of *Vinca rosea* Leaves as DPPH free radical scavenging activity and FRPA ferric reducing power assay

Parameters	Treatments		
	VLWE	VLE	BHT
DPPH (FRSA %)	32.24±0.96 ^A	41.78±0.98 ^B	66.33±2.41 ^C
FRPA (Absorbance at 700nm)	0.69±0.10 ^A	0.71±0.10 ^A	2.74±0.25 ^B

Whereas VLWE-*Vinca* Leaves Water Extract, VLE-*Vinca* Leaves Ethanolic Extracts, BHT- Butylated hydroxytoluene, DPPH -2, 2-diphenyl-2-picrylhydrazyl hydrate, FRSA- free radical scavenging activity, FRPA- ferric reducing power assay.

Mean±SE with different superscripts in a row differ significantly (P<0.05), n=6.

Conclusion

Extracts of *Vinca rosea* leaves showed good pancreatic lipase inhibition and antioxidant activities. Although, both anti-lipase and anti-oxidant activities were comparatively lesser than the established lipase inhibitory molecules i.e. orlistat and antioxidant BHT, but looking for natural alternate it appears promising. The identified plant parts could be used for comprehensive and safer therapeutic strategy for the management of obesity and other related diseases.

Conflicts of interest

The publication of this research paper the authors declare that there is no conflict of interest prevails.

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