



**ISOLATION, CHARACTERIZATION AND BIOLOGICAL
EVALUATION OF FATTY ACID METHYL ESTERS (FAMES)
FROM *VINCA ROSEA* SEEDS**

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ABSTRACT

Vinca rosea is a perennial shrub of the Apocynaceae family used as an ornamental shrub as well as medicinal herb. One of the therapeutic approaches for preventing diabetes mellitus type 2 is to retard the absorption of glucose via inhibition of α -glucosidases viz. glucoamylase and α -amylase. In the present study, fatty acids methyl esters (FAMES) are isolated from the seeds of *Vinca rosea* ethanol

extract. Their structures were elucidated on the basis of GC-MS data, as methyl stearate, methyl oleate and methyl palmitate. FAMES exhibited potent inhibitory activity against glucoamylase and α -amylase with IC_{50} value 67.98 μ g/mL and 73.89 μ g/mL respectively. The antioxidant activity of FAMES was evaluated by DPPH method. The results indicate that FAMES possesses considerable antioxidant activity. The highest radical activity was detected (IC_{50} =137.30 μ g/mL). This investigation systematically supports the use of FAMES to study the role of antioxidant therapy in the management of Diabetes Mellitus Type 2.

KEYWORDS: *Vinca rosea*, FAMES, glucoamylase, α -amylase, antioxidant activity.

INTRODUCTION

Vinca rosea (*Catharanthus roseus*) Linn. apocynaceae is a herbaceous subshrub also known as Madagascar Periwinkle, worldwide. The two classes of active compounds in *Vinca* are alkaloids and tannins. The leaves and stems are the sources of dimeric alkaloids, vincristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine.^[1] The leaves are used traditionally in various regions of the world

including India, West Indies etc. to control diabetes. Significant antihyperglycemic activity of the leaf extract has been reported in laboratory animals.^[2] Many reports are available on antidiabetic activity of this plant using crude extracts of leaves, flowers, and seeds.^[3]

Diabetes Mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with a disturbance of carbohydrate, fat metabolism and protein metabolism resulting from defects in insulin secretion, insulin action or both.^[4] Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is named as hyperglycemia. In particular, type 2 diabetes mellitus is the most encountered form of diabetes. One of the therapeutic approach of controlling blood glucose levels in individual with type 2 diabetes is to target α -amylase and intestinal glucoamylase controlling postprandial hyperglycemia by retarding the absorption of glucose.^[5,6] α -glucosidases inhibitors include acarbose and miglitol which show tremendous side effects such as bloating, flatulence and diarrhoea.^[7]

Recently, it has been demonstrated that hyperglycemia may lead to glycation of antioxidant enzyme, which could alter the structure and function of antioxidant enzymes such that they are unable to detoxify the free radicals. The free radicals produced are involved in oxidative stress destroying the pancreatic β -cells and leading to diabetic complications such as retinopathy, neuropathy, nephropathy and cardiopathy. Thus, antioxidants play an important role to prevent diabetic complications as they destroy free radicals and also enhance the insulin secretion. Hence search for natural compound from medicinal plants possessing both antioxidant and α -glucosidase inhibiting property has become an important aspect.

Fatty acids methyl esters (FAMES) are known to have antibacterial, antifungal^[8] and antidiabetic properties.^[9,10] However, little is known about antidiabetic properties of fatty acids of methyl esters isolated from *Vinca rosea*. The present work was under taken to explore the effect of FAMES in the management of Type 2 Diabetes Mellitus by using antioxidant therapy.

MATERIALS AND METHODS

Plant extraction

The seeds of *Vinca rosea* were collected from the University Campus of Mumbai, Kalina, Santacruz, Mumbai-98. The plant material was identified and authenticated at Blatter Herbarium, St. Xavier's College, Mumbai-01. The voucher specimen no. R4144 was reserved

for further reference. They were air dried for 6-7 days and were ground to a coarse powder in a grinder. The powdered seeds (10g) were soaked in ethanol at room temperature for 7 days, replacing the solvent after every 24 hrs. The total extract (6g) obtained was stored in the refrigerator which was used for further isolation.

Isolation of Fatty Acids of Methyl esters (FAMES)

The ethanol extract (6g) was dissolved in water and extracted with petroleum ether by solvent extraction method. The petroleum ether was removed under reduced pressure and the residue (2.5g) was dissolved in absolute methanol (20 mL) containing conc. H₂SO₄ (0.1 mL) and refluxed for two hours. After cooling the reaction mixture, the methanol was removed under reduced pressure and the residue was dissolved in water, extracted with CH₂Cl₂ (2 X 30 mL), neutralized with saturated sodium bicarbonate solution (2 X 20 mL) and dried over anhydrous sodium sulphate. After filtration, the solvent was removed and residue (126 mg) was analysed using GC-MS. GC-MS spectra was obtained on Thermo-Fischer Trace GC ultra and Polaris Q column used was DB-Wax (0.25 X 0.25 X 30). Sample was injected in splitless mode. The injection volume was 1 µL, and the injector temperature was 250⁰C. The carrier gas (He) flow rate was 1 mL/min, whereas the column temperature was programmed linearly in a range of 60-220⁰C at a rate of 5⁰C/min. the transfer line temperature was 220⁰C. The ion source temperature was 200⁰C. EI mass spectra (70 eV) were acquired in the m/z range of 50-1000.

Glucoamylase assay

0.5mL of the reaction mixture containing 0.1mL modulator, 0.3mL of 100mM acetate buffer (pH 4.5), and 0.1mL of glucoamylase were incubated at 37⁰C for 30 min. Then, add 0.5mL of starch solution (5mg/mL prepared in 100mM acetate buffer pH 4.5) and incubated further at 37⁰C for 30 min. The reaction was terminated by keeping the test-tubes in boiling water bath for 1-2 minutes, cooled under running tap water; add 2 mL of DNS (3,5-dinitrosalicylic acid) and the test-tubes were kept in boiling water bath for 15 minutes. The test-tubes were cooled and diluted with 7mL of distilled water. The absorbance was recorded at 530nm using spectrophotometer and liberated glucose was estimated. The % inhibition (I) was calculated as,

$$\% I = \{(Ac - As)/Ac\} \times 100$$

Where, Ac and As are the absorbance of the control and sample, respectively.

α - amylase assay

0.5 mL of the reaction mixture containing 0.1 mL modulator, 0.3 mL of 20mM phosphate buffer (pH 4.5), and 0.1 mL of α -amylase were incubated at 37⁰ C for 30 minutes. Then added 0.5 mL of Starch solution (10mg/mL prepared in 20mM phosphate buffer pH 7.0) and incubated further at 37⁰ C for 30 minutes. The reaction was then terminated by keeping the test tubes in boiling water bath for 1-2 minutes, cooled under running tap water. 1mL of DNS (3,5-dinitrosalicylic acid) was added and the test-tubes were kept in boiling water bath for 15 minutes. The test-tubes were cooled and diluted with 7mL distilled water. The absorbance was recorded at 530nm using spectrophotometer and liberated glucose was estimated. Acarbose was used as a standard for both the assays. The maximum inhibition was determined from plots of percent inhibition (% I) versus modulator and calculated as below.

$$\% I = \{(Ac - As)/Ac\} \times 100$$

Where, Ac and As are the absorbance of the control and sample, respectively.

IC₅₀ is defined as the concentration of sample required to inhibit 50% of digestive enzyme activity. IC₅₀ values of Acarbose and FAMES were determined from plots of percentage inhibition versus concentration (μ g/mL).

DPPH radical scavenging assay

1mL of various concentrations of the extracts in methanol was added to a 1mL of DPPH radical solution in methanol (final concentration of DPPH was 0.2mM). The mixture was shaken vigorously and allowed standing for 30min; the absorbance of the resulting solution was measured at 517nm with a spectrophotometer. Inhibition of free radical DPPH in percent (%I) was calculated in following way:

$$\% I = \{(Ac - As)/Ac\} \times 100$$

Where A_c is the absorbance of the control reaction (containing all reagents except the test compound) and A_s is the absorbance of the test compound. Ascorbic Acid was used as a control and Methanol as Blank.

Results are presented as mean \pm standard error of the mean.

RESULTS AND DISCUSSION

In order to characterize the constituent profiles of the FAMES, it was subjected to GC-MS. Total ion chromatogram of FAMES revealed three major peaks at retention times (Rt) 38.33 min, 42.79 min and 43.19 min. (Fig 1). The mixture was made up of methyl palmitate (mol. wt. 270), methyl oleate (mol. wt. 296) and methyl stearate (mol. wt. 298).

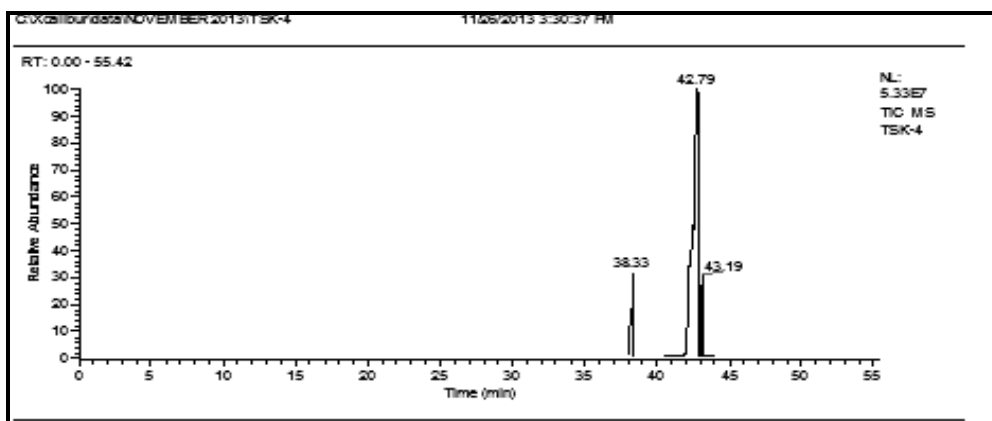
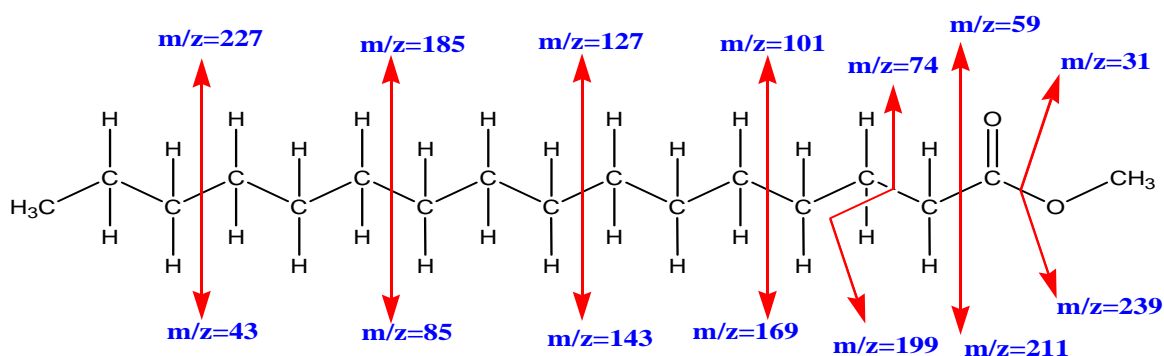
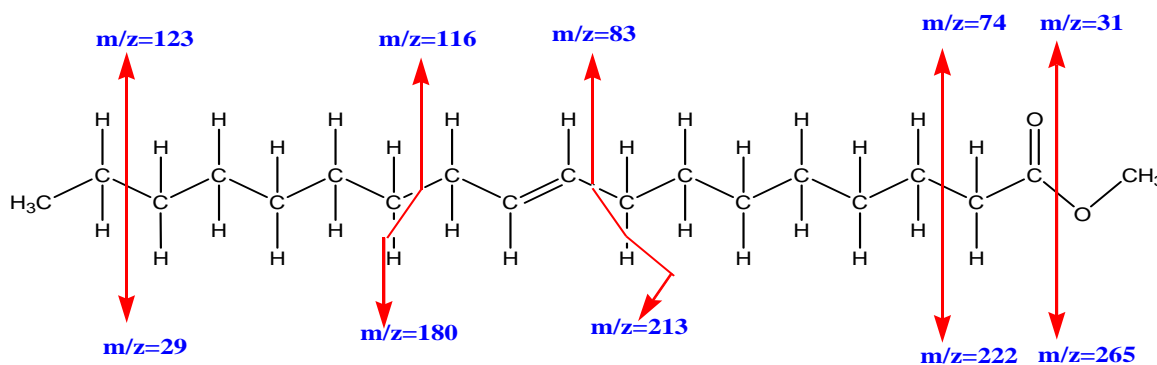


Fig 1. GC of FAMES

The structures of the methyl esters of these three fatty acids were confirmed by IR, GC-MS, and their MS fragmentation pattern. These fatty acids had satisfactory MS and IR data and were compared with the fragmentation pattern of those standard mass spectra from literature data. The molecular formula of the fatty acid with R_t 38.33 was deduced to be $C_{17}H_{34}O_2$ from the molecular ion peak which appeared at m/z 270, 239, 227, 199, 185, 171, 157, 143, 129, 101, 87, 74, 57, 59, 55 (100%). The possible fragmentation pattern may be as follows.



The molecular formula of the fatty acid with R_t 42.79 was deduced to be $C_{19}H_{36}O_2$ from the molecular ion peak which appeared at m/z 296, 265, 222, 180, 166, 152, 123, 97, 83, 74, 69, 55 (100). The possible fragmentation pattern may be as follows.



The distinct molecular ion with prominent ion for the loss of methanol $m/z=32$ from the ester moiety and another distinct fragment at $m/z=74$ and $m/z=222$ from a rearrangement involving the ester moiety confirm that it is methyl ester.

The molecular formula of the fatty acid with R_t 43.19 was deduced to be $C_{19}H_{38}O_2$ from the molecular ion peak which appeared at m/z 298, 267, 255, 241, 227, 213, 199, 185, 171, 157, 143, 129, 111, 97, 87, 74 (100), 69, 55. The possible fragmentation pattern may be as follows.

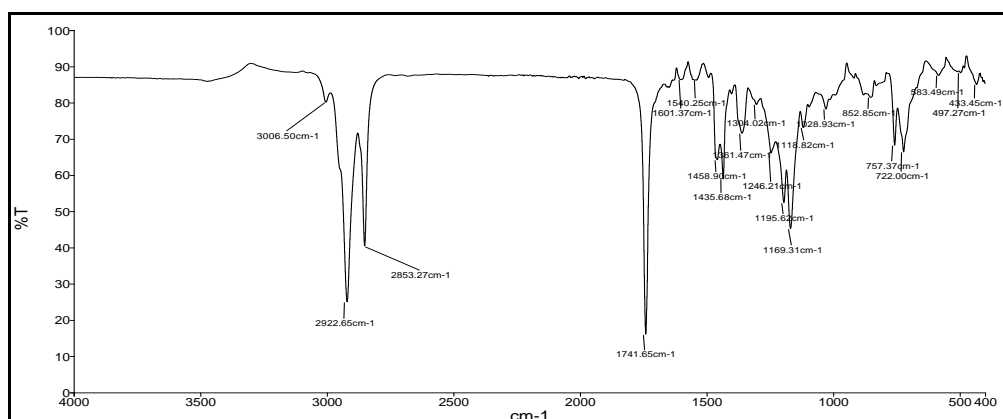
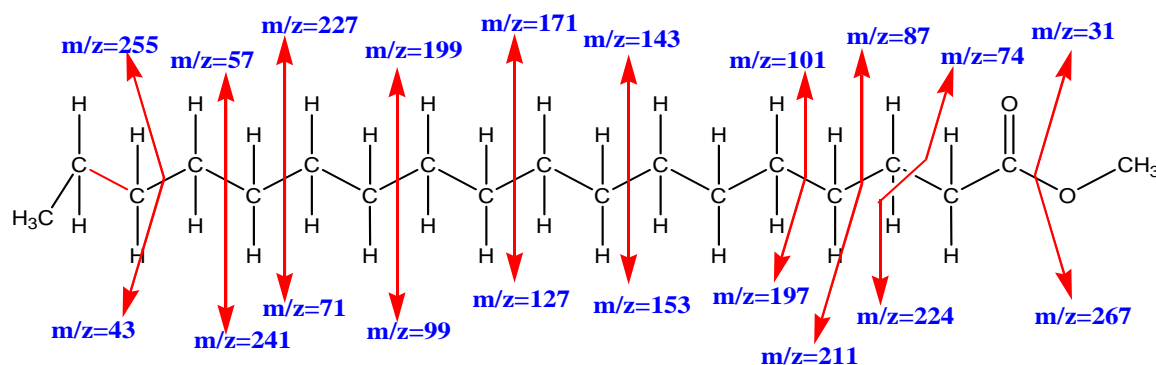


Fig.2 IR of FAME

IR spectrum displayed strong carbonyl ($-C=O$) absorption at 1741 cm^{-1} . Absorption at 3006 cm^{-1} are characteristic to olefinic ($=C-H$) stretching and the absorption peak at 1601 cm^{-1} corresponding to $C=C$ stretching. Absorption at 2922 and 2853 cm^{-1} marks $-C-H$ stretching vibrations of $-CH_3$ and $-CH_2$ groups. This mixture of FAME was studied against the effect on glucoamylase and α -amylase, digestive enzymes related to diabetes. Though FAMEs have been isolated from *Vinca rosea* earlier,^[11] its inhibitory action on glucoamylase studied for the first time.

Table 1. Effect of the FAMEs on glucoamylase as compared to Acarbose

Concentration ($\mu\text{g/mL}$)	Acarbose		FAMEs	
	% inhibition	IC ₅₀ value ($\mu\text{g/mL}$)	% inhibition	IC ₅₀ value ($\mu\text{g/mL}$)
20	23.92	75.13	24.88	67.98
40	34.44		36.84	
60	43.54		52.15	
80	55.02		56.45	
100	58.85		61.24	

Table 2. Effect of the FAMEs on α -amylase as compared to Acarbose

Concentration ($\mu\text{g/mL}$)	Acarbose		FAMEs	
	% inhibition	IC ₅₀ value ($\mu\text{g/mL}$)	% inhibition	IC ₅₀ value ($\mu\text{g/mL}$)
20	22.22	84.68	35.97	73.89
40	29.10		39.68	
60	37.56		41.26	
80	46.56		52.38	
100	58.73		59.78	

The percentage inhibition at 20, 40, 60, 80 and 100 $\mu\text{g/mL}$ concentrations of FAME showed a concentration dependent decrease in percentage inhibition. Thus, the highest concentration of 100 $\mu\text{g/mL}$ showed a maximum inhibition of 59.78% and the standard drug acarbose showed inhibition of 58.73% with IC₅₀ values of 73.89 and 84.68 $\mu\text{g/mL}$ respectively, in the case for α -amylase (Table 2). In the case of glucoamylase the percentage inhibition of 61.24% was observed at a concentration of 100 $\mu\text{g/mL}$ and that of acarbose was 58.85% with IC₅₀ values of 67.98 and 75.98 $\mu\text{g/mL}$ respectively (Table 1).

Antioxidant activity of FAME was tested against DPPH radical at 200, 400, 600, 800, and 1000 $\mu\text{g/mL}$. Results obtained in the antioxidant activity are shown in the Table 3. The activity of the standard was much higher than the FAMEs but it exhibited its best DPPH scavenging activity at 137.30 $\mu\text{g/mL}$ of IC₅₀ value.

Table 3. DPPH radical scavenging activity of FAMEs

Sample	IC ₅₀ value ($\mu\text{g/mL}$)
FAME	137.30
Ascorbic acid	5.30

CONCLUSION

The aim of the study was to analyse the activities of FAMEs relevant to hypoglycemic conditions. Our present investigation confirms mild antioxidant and antidiabetic potency of FAMEs. FAMEs isolated from *vinca rosea* seeds exhibited remarkable inhibitory activity against glucoamylase and α -amylase, compared to acarbose. The antioxidant activity of FAMEs may also help to control free radicals, which scavenge the free radicals generated during hyperglycemia. To the best of our knowledge, this is the first report of FAMEs isolated from *vinca rosea* seeds with potent inhibitory activity against glucoamylase.

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REFERENCES

1. Edwin JE, Sheeja E, Motwani S, Dutt KR and Goel RK. Comparative evaluation of Antihyperglycemic and hypoglycemic activity of various parts of *Catharanthus roseus* Linn. Res. J. Med. Plant, 2008; 2: 10-15.
2. Nammi S, Boini MK, Lodagala SD, Behara RBS. The juice of fresh leaves of *Catharanthus roseus* Linn. reduces blood glucose in normal and alloxan diabetic rabbits. BMC Compliment. Alter. Med., 2003; 3: 4.
3. Sen MK, Mamun-Or-Rashid ANM, and Md. Towfique N. Ethnomedical profiles of different plant parts of *Catharanthus roseus* L. –A Review, J. of Drug Discovery and therapeutics, 2003; 1(4): 1-7.
4. Barcelo A, Rajpathak S. Incidence and prevalence of Diabetes Mellitus in the Americas. American Journal of Public Health, 2001; 10: 300-308.
5. Krentz AJ and Bailey CJ. Oral antidiabetic agents: Current role in type II Diabetes Mellitus. Drugs, 2005; 65: 385-411.
6. Sheen AJ. Is there a role for α -glucosidase inhibitors in the prevention of type II Diabetes Mellitus. Drugs, 2003; 63(10): 933-951.
7. Hanefeld M. The role of acarbose in the treatment of non-insulin dependent Diabetes Mellitus. J. Diabetes Complications, 1998; 12: 228-237.

8. Agarmoorthy G, Chandrashekhran M, Venkatesalu V. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your eye mangrove from India. *Brazilian Journal of Microbiology*, 2007; 38: 739-742.
9. Artanti N, Tachibana S, Kardono LBS, Sukiman H. Isolation of α -glucosidase Inhibitors produced by an Endophytic Fungus, *Colletotrichum* sp. TSC13 from *Taxus sumatrana*. *Pakistan Journal of Biological Sciences*, 2012; 15(14): 673-679.
10. Nguyen TH, Um BH and Kim SM. Two unsaturated fatty acids with potent α -glucosidase inhibitory activity purified from the body wall of sea cucumber (*Stichopus japonicas*) J. *Food Sci.*, 2011; 76(9): 208- 214.
11. Satyan RS, Malarvannan S, Eganathan P, Rajalakshmi S, Parida A. Growth inhibitory activity of fatty acid methyl esters in the whole seed oil of madagascar periwinkle (Apocyanaceae) against *Helicoverpaarmigera* (Lepidoptera: Noctuidae). *J Econ Entomol.*, 2009; 102(3): 1197-1202.