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Bioassay Guided Fractionation of Anti-Hyperglycemic Compounds Extracted from *Cheilocostus speciosus*, and Model Development for Sustained Release of Anti-Hyperglycemic Compounds Using Acarbose

L.L. Edirisinghe [a *], S.I. Rathnayake [b], A.G.A.W. Alakolanga [a], K.G.C. Senarathna [c], K.B. Wijesekara [c]

[a] Department of Export Agriculture, Uva Wellassa University, Passara road, Badulla, Sri Lanka, 90000

[b] Department of Science and Technology, Uva Wellassa University, Passara road, Badulla, Sri Lanka, 90000

[c] Department of Biosystems Technology, Uva Wellassa University, Passara road, Badulla, Sri Lanka, 90000

*** Corresponding author email address**: lakmini@uwu.ac.lk

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Abstract

Cheilocostus speciosus (COS) leaves are traditionally used for managing diabetes, though prolonged consumption risks severe hypoglycemia. This study explored anti-hyperglycemic compounds from ethanol extracts of COS leaves and developed a sustained drug delivery system using montmorillonite (MMT). Ethanol extracts showed strong α-amylase and α-glucosidase inhibition (IC_{50}: 14.62 ppm and 21.20 ppm), comparable to acarbose, a standard diabetic drug. Active fractions were isolated via gravity column, size exclusion, and thin-layer chromatography, confirming COS leaves' anti-hyperglycemic potential. To enhance therapeutic applicability, acarbose was intercalated into MMT as a nanocarrier for sustained release. X-ray diffraction confirmed increased interlayer spacing (1.185 nm to 1.403 nm) when increased acarbose concentration from 50-100 ppm, while intensified OH stretching peak in FTIR indicated acarbose integration into the clay matrix. Intercalation efficiency improved with higher acarbose concentrations (43.77% to 52.27%). *In-vitro* studies revealed controlled acarbose release over 8 hours (45.66%) and sustained slow release, following pseudo-second-order kinetics ($r^2 = 0.9767$). These findings suggest COS as a promising source of anti-diabetic compounds and highlight MMT's potential as a nanocarrier for safer, sustained therapies. Future research will focus on large-scale isolation, structural elucidation, and integration of active compounds into MMT for enhanced diabetic treatments.

Keywords: *Cheilocostus speciosus*, Ethanol extract, α–amylase inhibitor, α–glucosidase inhibitor, montmorillonite, sustained drug release

1. Introduction

Diabetes mellitus (DM) is a prevalent metabolic disorder characterized by elevated blood glucose levels due to insufficient or absent insulin secretion by the pancreas. More than 425 million people worldwide are currently affected by diabetes mellitus (DM), with this number projected to rise to 629 million by 2045 [1]. The two most prominent general diabetes types are called type I and type II. Type I diabetes accounts for 5% - 10% of diabetes while Most of the population (90 %-95%) in the world has Type II diabetes [2]. In Type II diabetes, the secretion of insulin is resisted. α-amylase and α-glucosidase enzymes cannot be inhibited by insulin with the lower level of insulin produced. Thus, blood glucose level rises than the range of a healthy human being [2].

In ayurvedic medicine, extracts of many plants were identified as therapeutic agents for type II DM. Among these medicinal plants, leaves of *Cheilocostus speciosus* (COS) are popular among Sri Lankans which are included in the main meals as a salad [3]. COS has various medicinal properties and is used particularly in the treatment of asthma, fungal diseases, rheumatism, diabetes, hepatoprotective disorders and is used as an ornamental plant [4]. There are only handful of literature (Table 1) available related to the antidiabetic therapeutic effects of COS plant parts [5-12].

Table 1
Antihyperglycemic activity of different parts of *C. speciosus*

Plant part	Research findings so far
Rhizome	COS rhizome increases the insulin secretion and peripheral utilization of glucose. Most of these studies also have shown cholesterol lowering effects of COS. The ethanol extract of COS rhizome showed a significant reduction in blood glucose, glycosylated hemoglobin and increase in liver glycogen and insulin in alloxan induced diabetic rats treated for 60 days [5].
	Petroleum ether, chloroform, methanol and aqueous extracts of COS rhizome were studied in streptozotocin induced diabetic rats on the oral glucose tolerance after a single dose of extracts and the hypoglycemic effects after multiple doses of extracts for 14 days. Hypoglycemic effects observed were highest with methanol and water extracts of COS which were in parallel with glibenclamide [6].

	Eremanthin isolated from COS rhizome has significantly reduced blood glucose level in a dose dependent manner and glycosylated hemoglobin HbA1c in streptozotocin induced diabetic rats treated for 60 days. Eremanthin has also increased plasma insulin and tissue glycogen while showing hypolipidemic effects [7].
Roots	Ethanol extract of COS root significantly reduced blood glucose concentration, increased glycogenesis and decreased gluconeogenesis in alloxan induced rats treated for 4 weeks. Improvement of lipid parameters and hepatic antioxidant enzyme activities were also observed in their study [8].
	Costunolide (20 mg/kg) isolated from COS root has significantly decreased glycosylated hemoglobin (HbA1c), total cholesterol, triglyceride, LDL cholesterol, markedly increased plasma insulin, tissue glycogen, HDL cholesterol and serum protein and restored the altered liver enzymes in plasma in streptozotocin induced diabetic rats treated for 30 days [9].
Leaves	one study reported the effect of COS leaf methanol extract and water extract in reversing the insulin resistance induced by a high fat diet in male Wistar rats treated for 4 weeks [10].
	Another study reported the glucose binding capacity and the reduction of glucose diffusion rate with COS leaf extracts *in vitro*. They also have stated an amylase inhibitory effect of 18 % which was significantly lower than that of Acarbose with 2 % COS leaf using a slightly different method [11].
	Alpha glucosidase inhibition activity of methanolic extract of cos leaves was observed with IC50 67.5 µg/mL and for alpha amylase inhibition it was 5.88 mg/mL [12].

Currently, Acarbose and miglitol are considered as competitive inhibitors of intestinal -glucosidases and reduce the postprandial digestion and absorption of starch and disaccharides [13]. Therefore, these synthetic drugs often used for Type II diabetic patients. One of the major drawbacks of current synthetic drugs is the need to use them multiple doses per day. However, on the other hand, direct consumption of COS leaves for a longer period also poses risks of severe hypoglycemia [6]. Therefore, the current study aimed to scientifically evaluate the antidiabetic properties of COS leaf extract, perform bioassay-guided fractionation of antihyperglycemic compounds from the extract, and develop a model for the sustained drug release of these compounds using Acarbose.

2. Materials and Methods

2.1 Materials

Chromatography grade solvents were used for the extractions and assays and purchased from Sigma Aldrich unless otherwise stated. Extraction of plant materials was performed using an ultrasound sonicator (ROCKER Ultrasonic cleaner, model – SONER 206). UV absorption of the solutions was measured using a UV-1601, Shimadzu spectrophotometer. Gravity column chromatography was performed using Fluka 60741 silica gel (70-230 mesh) and size exclusion chromatographic separations were performed with Sephadex LH-20 column using 100% Chloroform) Thin Layer Chromatography (TLC) was performed on 0.2 mm thick Silica gel (60F) precoated Aluminium sheets.

2.2 Collection of plant materials

The leaves of COS, collected from Badulla area during the period of January- March 2020, Sri Lanka which was authenticated by the Deputy Director/National Herbarium and the voucher sample (Voucher No. NH/BOT/14/2019-31) was deposited at the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka.

2.3 Preparation of ethanol extract of COS leaves

The collected fresh and healthy leaves without any defects were cleaned under tap water to remove any dirt and then air-dried for about 4 days. After drying, the leaves were oven-dried at 40°C for 48 hours. Dry leaves were grounded using an electric grinder and 220 g of COS leaves powder was obtained by a 500 µm pore size laboratory test sieve.
Dry powder of COS leaves (20 g) was extracted three times with absolute ethanol (200 mL × 3) using the sonicator. The extract was filtered and then ethanol was evaporated using a rotary evaporator at 40°C to get the ethanol crude extract (11.5 g). This ethanol extract of the COS leaves was screened for α–amylase and α–glucosidase inhibition activities and bioactivity guided fractionation was carried out to isolate pure active compounds.

2.4 Determination of the α–amylase inhibition activity of the ethanol extract of COS leaves

The DNSA reagent using 96 mM 3,5-dinitrosalicylic acid in 20 mL deionized water, 5.31mM sodium potassium tartrate in 12 mL distilled water and 8 mL 2M NaOH, and (1 % w/v) potato starch in distilled water were prepared. Porcine pancreatic α-amylase (24 unit/mL) was dissolved in 5ml of 20 mM phosphate buffer which contains 6.7 mM NaCl (pH 6.9)

Ethanol extract of the COS leaves was dissolved in deionized water with 10% DMSO (10-25 ppm extracts). For 100 µL of each concentration, 100 µL of α-amylase was mixed with starch solution and incubated 30 min at ambient temperature. Then it was again incubated at 85°C in a water bath for 15 min and allowed to cool to room temperature and was diluted with 900 µL distilled water. Blanks were prepared by adding DNSA reagent and incubated 10 min at 37°C prior to the addition of starch solution to inactivate the enzyme, it was incubated then at 85°C for 15 min in a water bath and soon after cooling down to room temperature it was diluted with 900 µL distilled water. Negative controls were conducted in the same manner replacing plant extract with 10% DMSO (100 µL) in deionized water. The absorbance was measured at 540 nm and the IC_{50} was calculated by plotting percentage inhibition against the concentration. Acarbose (0-100 ppm) was used as the positive control [14].

2.5 Determination of the α–glucosidase inhibition activity of the ethanol extract of COS leaves

A 2 units/mL solution of α-glucosidase (100 U/mg of protein) and 5 mM p-nitrophenyl-α-D-glucopyranoside solution (substrate) were prepared in 20 mM phosphate buffer (pH 6.0). Plant extracts (15-40 ppm) were dissolved in 10 % DMSO. The reaction was carried out at 37 ℃ using 50 µL enzyme, 250 µL substrate and 50 µL plant extract for 7 min. The blank was carried out at 37 °C using 50 µL 20 mM phosphate buffer, 250 µL substrate and 50 µL plant extract for 7 min. Absorption was measured at 415 nm after the addition of 400 µL 0.4 M glycine (pH 10.4). The negative control reaction was performed by replacing plant extract from 50 µL of aqueous 10% DMSO and the positive control was performed by replacing the plant extract from Acarbose (0 -100 ppm). The absorption of controls was recorded in the same manner as described above. The IC_{50} value was calculated by plotting percentage inhibition of enzyme activity against log10 plant extract concentration [14].

2.6 Calculation of % inhibition and IC_{50}

% Inhibition of α-amylase and α-glucosidase activity was calculated using the following formula

$$\% \text{ inhibition} = \frac{\{\text{Absorbance(control)} - \text{Absorbance Sample (Test-Blank)}\} \times 100\%}{\text{Absorbance (control)}}$$

The concentration of extract inhibits 50% of enzyme activity (IC_{50}) was measured using a series of ethanolic extract concentrations of COS leaves. IC_{50} was detected by plotting % inhibition Vs. log10 plant extract concentration and calculated by logarithmic regression analysis from mean inhibition values.

2.7 Bioassay guided fractionation of antihyperglycemic compounds

Ethanolic extract of COS leaves identified as a potential candidate with anti-hyperglycemic properties and isolation of compounds responsible for the anti-hyperglycemic effect was done with bioassay guided fractionation (Fig. 1)

Gravity column chromatography using Fluka 60741 silica gel upon dry loading was done with the hexane: ethyl acetate: methanol solvent system followed by Thin Layer chromatography (TLC) to find the best fraction with antihyperglycemic properties in terms of both α–amylase inhibitory activity and α–glucosidase inhibitory activity.

Similar patterns with TLC were pooled and further purified Size Exclusion Chromatography (SEC) with Sephadex LH-20 column as the stationary phase and chloroform as the mobile phase. For the fractions collected from SEC, TLC was done to identify the purified fractions. According to the TLC observations the fractions with only single spot in TLC was used for the further characterization. The isolated compounds containing fractions were dried and FTIR was done at the university. The remaining samples were sent to the SLINTEC for the ^{13}C NMR and ^{1}H NMR analysis.

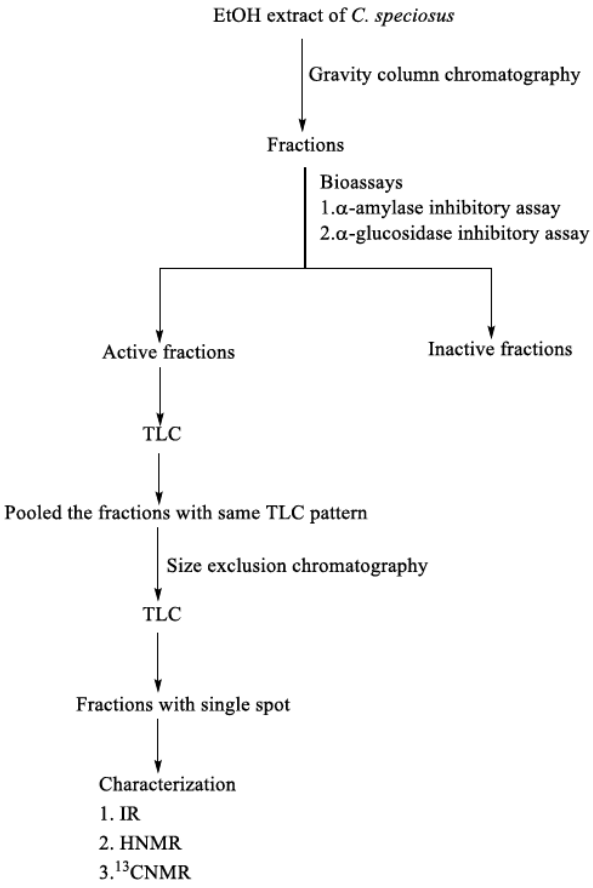

Fig. 1. Flow chart for the activity guided fractionation

2.8 Model development for the sustained drug release of antihyperglycemic compounds by using Acarbose.

2.8.1 Acarbose intercalation into MMT at different pH levels

The concentration of 50 ppm acarbose solution was prepared and Initial Acarbose concentration was determined using UV method by plotting calibration curve. Then a series of acarbose solution was prepared by changing the pH from 4-9 using 0.1 M HCl solution and 0.1 M NaOH solution.

Then 100 mL of pH adjusted solutions were taken to separate beakers and 1.0 g of MMT was added to each beaker. Then each beaker was stirred for 24 hrs at room temperature using magnetic stirrer. Then 5 mL of supernatant was taken to another container and 5 mL of 0.001 M $KMnO_4$/0.1 M NaOH solution was added to the same container. After proper mixing of mixture, it was left aside for 15 minutes for the color development and then each mixture was scanned by UV -Vis Spectrophotometer within 300 nm-750 nm to find the Acarbose concentration after intercalation. The same procedure was followed for the distilled water samples at different pH levels. For each pH of acarbose and distilled water, this experiment was done 3 times. pH with optimum intercalation was identified and was used for further studies.

2.8.2 Determination the effect of initial concentration of acarbose for the intercalation

Above mentioned intercalation procedure was carried out (three trials) with 50 ppm and 100 ppm initial acarbose solution at pH 6 (Optimum pH). Control experiments were conducted in triplicate using distilled water. The supernatants of the clay solutions were separated via centrifugation (4000 rpm, 30 min) and the isolated acarbose-intercalated MMT were dried at 40 ^{0}C for 24 hrs and characterized using FTIR and XRD.

2.8.3 Characterization

UV-Visible Spectrometry analysis was carried out to determine the intercalation percentage of acarbose into montmorillonite. The measurements were carried out with a 1 cm quartz cuvette in an absorbance mode in a wavelength range of 300-750 nm. Pure Na-MMT, Dried acarbose intercalated clay (MMT) at different pH levels and at different initial concentrations were characterized by FTIR and XRD

2.8.4 *In-vitro* drug release testing

Accurately 8.00 g of NaCl, 2.00 g of KCl, 1.44 g of Na_2HPO4 and 0.24 g of KH_2PO_4 were measured and dissolved in 800 mL of distilled water to prepare the Phosphate Buffer Saline (PBS) solution. After adjusting pH to 7.4, the solution was volumed up to 1 l. The dialysis cell system was prepared and the intercalated acarbose MMT drug was inserted into the dialysis cell. The dialysis cell and receiver compartment were initially filled with pH 7.4 PBS (250 ml PBS solution). The receiver chamber was stirred at a speed of 80 rpm for a homogeneous distribution. Then the dialysis cell was placed in the receiver compartment with PBS solution. 10 mL samples from the receptor solution at pre-determined time periods were removed continuously (from 0 to 24 h) and effective sink conditions were achieved by refilling the receptor solution with 10 mL of fresh PBS solution. The removed 10 mL PBS solutions were used to determine the drug concentration that passed through the dialysis tube using Spectroscopic analysis.

3. Results and Discussion

3.1 Selection of solvent and the yield of plant extract

Cold and hot water extracts are commonly used in the preparation of recipes according to the ayurveda. But when using low polar solvents in this process, there is a tendency to loss some important bioactive compounds which are responsible for the enzyme inhibitory activities. According to the literature, high polar solvents like ethanol, water, ethyl acetate yield the highest inhibitory activity compounds. Hence, ethanol, high polar solvent was used for the extraction procedure in this study to obtain high inhibitory activities. The percent yield of ethanolic crude extract of COS leaves was calculated as 5.68 %.

3.2 α–amylase and α–glucosidase inhibition activity of the ethanol extract of COS leaves

Active fractions obtained from the chromatographic separation of the ethanol extract of COS showed positive results for both α–amylase and α–glucosidase enzyme inhibitory activities. IC_{50} values of ethanol extract of COS against alpha amylase and alpha glucosidase were 14.62 ppm, 21.20 ppm respectively. The observed inhibitory activity of ethanol extract against porcine pancreatic α–amylase is comparatively higher than α–glucosidase inhibitory activity. The inhibitory activities of ethanol extract of COS leaves always compared with standard drug, Acarbose to verify the accuracy of biochemical assays as well as to check the possibility of substituting acarbose. According to the results, even though there was amylase inhibitory activity with ethanol extract of COS leaves, the IC_{50} for amylase inhibition (14.62 ppm) was significantly higher than that of standard clinical Acarbose drug (0.065 ppm) for porcine pancreatic amylase ($p < 0.01$). The percent α-amylase inhibitions (%) of COS leaves extract at varying concentrations (10-25 ppm) and Acarbose at varying concentrations (0.025-0.100 ppm) are shown in Fig. 2 and Fig. 3 respectively. It also showed that the inhibition of amylase activity is also dose dependent.

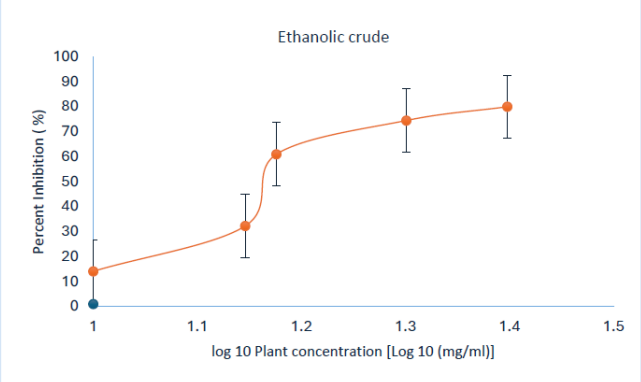

Fig. 2. % α–amylase inhibition of ethanol extract of COS leaves

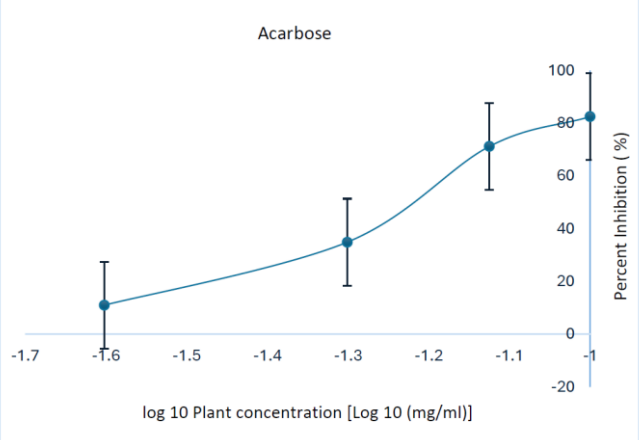

Fig. 3. % α–amylase inhibition of standard acarbose

α-glucosidase inhibitory effect of ethanolic leaf extract of COS was also compared with that of the control diabetic drug, acarbose. α-glucosidase inhibition percentages of

ethanol extract of COS leaves at varying concentrations (15-40 ppm) and Acarbose at varying concentrations (0.025-0.100 ppm) are shown in Fig. 4 and Fig. 5 respectively. It is indicated that the alpha glucosidase inhibitory activity is also dose dependent.

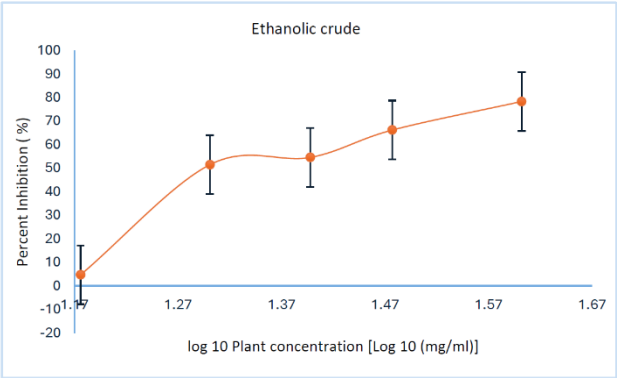

Fig. 4. % α–glucosidase inhibition of ethanol extract of COS leaves

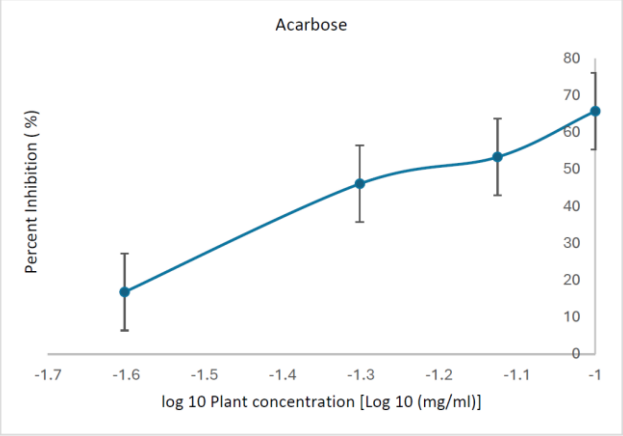

Fig. 5. % α–glucosidase inhibition of standard acarbose

Even though there was α-glucosidase inhibitory activity with ethanol extract of COS leaves, the IC_{50} for glucosidase inhibition was 21.20 ppm which was significantly higher than the IC_{50} value of standard clinical Acarbose drug (0.068 ppm) ($p < 0.01$). When comparing α- amylase and α-glucosidase assay of Acarbose, the α-amylase inhibitory activity (0.065 ppm) was greater than α-glucosidase inhibitory activity (0.068 ppm) and the similar pattern was observed for ethanol extract of COS. This may be due to the inhibition of α-glucosidase through hydroxylating C-3 of the flavanol ring carbon by flavonoids present in the extract which is considered as one of the important phytochemical compounds [15].

This study also predicts that the low inhibitory activity of COS leaf ethanol extract is due to phytochemical compounds contained in the extract.

Considering the summarized results in the table 2, this study suggests that ethanolic extract of COS leaves is a potential candidate for the development of anti-hyperglycemic formulation which can substitute the standard clinical drug, Acarbose with further studies.

Table 2: IC_{50} values for α-amylase and α-glucosidase of the ethanol extract of COS leaves

Extract	IC_{50} (α-amylase inhibition)	IC_{50} (α-glucosidase inhibition)
a.Ethanol extract of COS leaves (Slightly high inhibition performance for α-amylase compared to α-glucosidase)	(14.62 ppm) (extract has very low inhibition performance compared to acarbose)	(21.20 ppm) (extract has very low inhibition performance compared to acarbose)
b. Acarbose (slightly high inhibition performance for α-amylase compared to α-glucosidase)	(65 ppb) (High inhibition performance)	(68 ppb) (High inhibition performance)

a- IC_{50} - Concentration (ppm) of extract required for 50% inhibition of enzyme activity.

b- Acarbose clinically used inhibitor of α-amylase and α-glucosidase.

3.3 Bioassay guided fractionation of antihyperglycemic compounds

The activity-guided fractionation process began with dry loading of the ethanolic plant extract onto a column using chloroform. Initially, the gravity column chromatography was performed to isolate fractions with higher activity in both α-amylase inhibition and α-glucosidase inhibition. The fractions with low enzyme activity were neglected due to absence of responsible active compound while fractions with higher enzyme activity were selected for further studies. Fractions were eluted with a hexane:ethyl acetate gradient (100:0 to 0:100) and collected in 2 mL aliquots. Each fraction was screened for α-amylase inhibition, and those showing significant activity were further tested for α-glucosidase inhibition.

Table 3: Summary of the inhibition activity in both α-amylase and α-glucosidase

Fraction	α-amylase inhibitory percentage (%)	α-glucosidase inhibitory percentage (%)
6	32.01	98.40
18	40.57	No activity
21	41.80	69.97
23	44.69	95.75
25	49.51	93.76
27	44.42	82.61

Fractions with higher enzyme activity were selected for further investigation due to the presence of active compounds. Based on the results, two highly pure and enzymatically active fractions (fraction 6 and 23), highlighted in table 3, were identified.

Further purification was performed for the above fractions using size exclusion chromatography (SEC). Fractions displaying a single spot on TLC were dried and sent for ^{1}H NMR and ^{13}C NMR analysis. Those fractions were also subjected to characterization using IR to see the similarity of the functional groups. Some fractions were

contained two active compounds, suggesting that the observed antihyperglycemic activity might result from a combination of compounds rather than individual components. The quantities of isolated compounds were insufficient for HPLC analysis. Moreover, efforts to structurally identify the active compounds were hindered by their limited quantities and the sensitivity limitations of the NMR instrument. Therefore, re-isolation of the active compounds using a larger amount of crude extract is necessary for comprehensive structural elucidation and characterization.

3.4. Model development for the sustained drug release of antihyperglycemic compounds by using Acarbose.

3.4.1. Determine the effect of pH on intercalation of drug into MMT

According to the results, it was observed that pH 6 – 6.8 gave the best result (Fig. 6) with highest amount of acarbose intercalation. Therefore, further studies were carried out at pH 6.

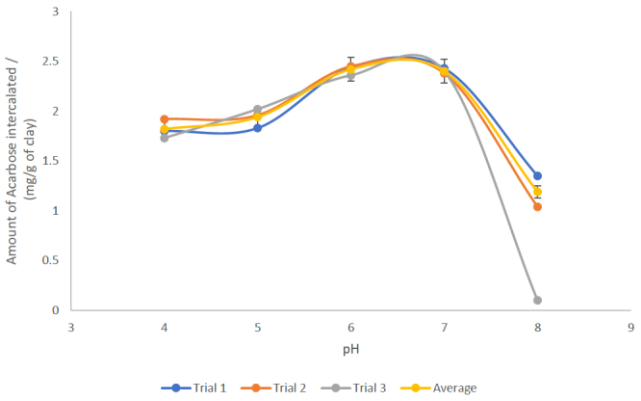

Fig. 6. The effect of pH on intercalation of acarbose into MMT

3.4.2. Determination of the effect of initial acarbose concentration for intercalation

The effects of acarbose initial concentrations were studied at pH 6 and two different initial concentrations of acarbose and the intercalation percentages were calculated. The results suggested that the intercalation of acarbose has been increased from 2.18 (47.77%) mg g^{-1} to 5.14 (52.57%) mg g^{-1} with the increment initial concentration of acarbose from 50 ppm to 100 ppm.

3.4.3. Characterization of Acarbose intercalated MMT

The intercalation of Acarbose into MMT was confirmed by using the FTIR and XRD techniques. Fig. 7 shows the FTIR spectra of acarbose, pure MMT and the effect of initial concentration at the intercalation of acarbose into MMT. The intensities of characteristic bands for acarbose increased with increasing the initial concentration of the reaction solution. The peak corresponds to combination O-H groups of acarbose and MMT has been more broaden in intercalated MMT at 100 ppm than 50 ppm.

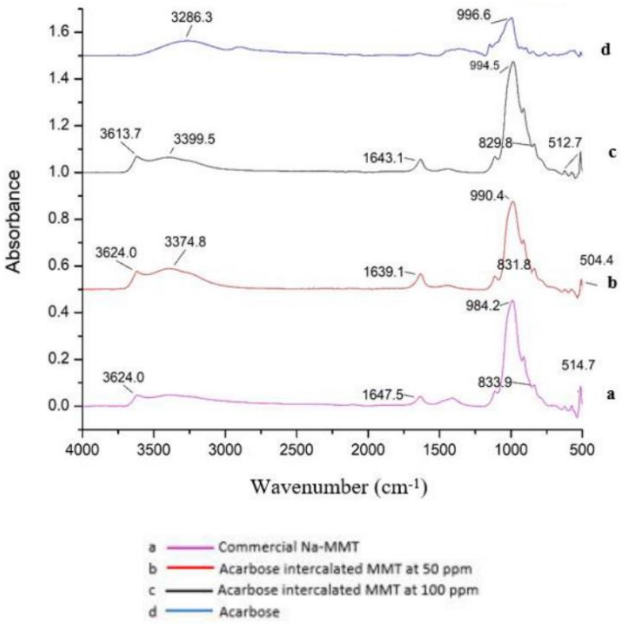

Fig. 7. Comparison of FTIR spectra of acarbose and MMR with acarbose intercalated MMT

The shifting of the peak at 984.2 cm^{-1} in MMT and peak at 990.4 cm^{-1} in acarbose confirms the intercalation of acarbose into MMT because of shifting the peaks into the shorter wavelength (blue shifting), when MMT layers are charged with intercalant molecules [16]. Increment of concentration has shown further shifting in the corresponding peak.

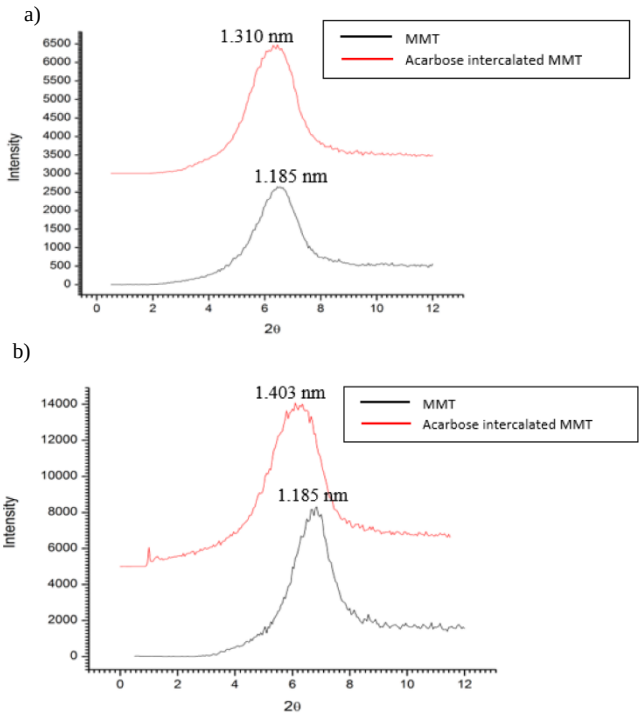

Fig 8. a) XRD patterns of intercalated Na-MMT at initial concentration 50 ppm, b) XRD patterns of intercalated Na-MMT at initial concentration 100 ppm

Fig. 8 illustrates the 001 basal phase reflection peak of unmodified MMT d-spacing value considerably increased to from 1.185 nm to 1.310 nm for acarbose (50 ppm/MMT). The increment of d spacing, which is observed in XRD spectra, confirms the successful intercalation of acarbose into MMT. This is because occupying more space within interlayers due to the presence of acarbose. The interlayer space/d spacing of unmodified montmorillonite has been increased from 1.185 to 1.310 nm and 1.403 nm, when the acarbose initial concentration increased from 50 ppm to 100 ppm accordingly. Therefore, it is suggesting that when the initial concentration of acarbose increases, the amount of intercalated acarbose also can be increased because of high initial acarbose concentration.

3.4.4. *In-vitro* drug releasing study

Acarbose was gradually released in the first 8 h up to 45.66 % then slow release was observed afterwards in the simulated intestinal fluid (PBS 7.4). The maximum release of acarbose from MMT in intestinal fluids was observed to be 60.58 % and 3.09 mg/g of clay after 24 hrs. (Fig. 9). This is in accordance with the other reported drug delivery systems where 60 – 75% release was observed [17, 18]. The releasing of the drug from the matrix can be explained as an ion-exchange process between the intercalated drug and the alkali metal ions of the buffer [19].

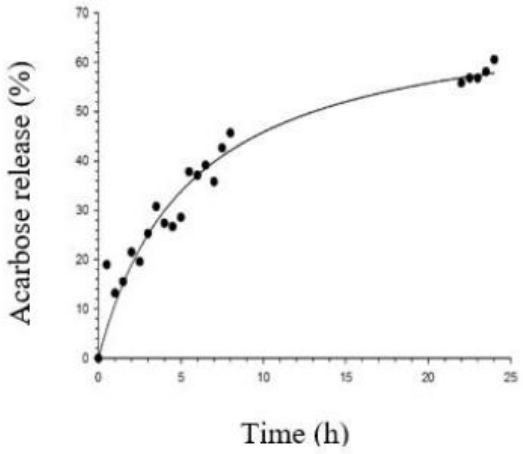

Fig. 9. Percentage release of acarbose for 24 hours' time

3.4.5. Drug releasing kinetics

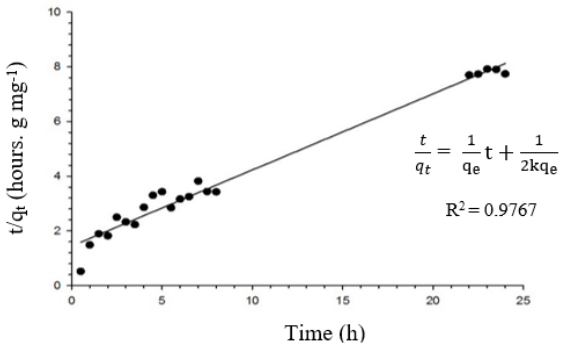

Fig. 10. Pseudo second order kinetic model for the acarbose release study for 24 hours of time

A correlation coefficient (R^2) of 0.9767 was obtained for the Pseudo second order kinetics model (Fig. 10). The acarbose releasing at equilibrium shows a good fit for with pseudo second order model. Results suggest that the rate of the release process appears to be controlled by the chemical process according to the pseudo second order reaction mechanism [65] and the rate limiting step may be a chemical adsorption involving valence forces through sharing or exchange of electrons between acarbose molecules and MMT [20, 21].

4. Conclusion

The ethanol extract of *Cheilocostus speciosus* (COS) leaves demonstrates significant α-amylase and α-glucosidase inhibitory activities (IC_{50}: 14.62 ppm and 21.20 ppm, respectively), though lower than the commercial drug acarbose. However, the extract may offer the advantage of reduced side effects. A key challenge with Thebu leaves is their rapid release in the stomach, potentially causing a sharp decline in blood glucose levels. To mitigate this, active compounds from the extract were isolated and intercalated into a montmorillonite (MMT) matrix to develop a controlled-release formulation.

Fractions obtained from column chromatography and size exclusion chromatography of the ethanol extract of COS leaves were screened for enzyme inhibitory activity. Several isolated fractions, each containing 2–3 compounds, exhibited potent inhibition, with α-amylase and α-glucosidase inhibitory activities reaching up to 98%. These findings highlight the potential of COS as a natural source of bioactive compounds for enzyme inhibition.

As a preliminary step prior to intercalation of ethanolic plant extract into MMT, acarbose intercalation into MMT was studied. UV-Vis, FTIR, and XRD analyses confirmed successful intercalation, with optimal conditions at pH 6. *In-vitro* release studies under simulated intestinal conditions (pH 7.4) using dialysis tube method showed a gradual increase in the first 8 h up to 45.66% and observed a slow release afterwards. This study emphasized the use of montmorillonite as a matrix material for sustained release drug formulations for future pharmaceutical studies.

Further large-scale studies are necessary to isolate and elucidate the structure of the active compound responsible in the EtOH extract of COS leaves. Additionally, the intercalation of this active compound into the MMT matrix and its sustained drug release properties should be investigated in the future to assess the potential of the extract as a safe alternative to synthetic antidiabetic drugs and to ensure the safe use of traditional and Ayurvedic medicines.

Conflicts of Interest

No conflicts of interest are disclosed by the authors.

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