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α-Amylase inhibitory effect of 3β-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from *Spondias mombin* leaf

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ABSTRACT

Spondias mombin is a traditional herb used in the treatment of diabetes mellitus by traditional healers in southwest Nigeria. In this study, we investigated the antidiabetic activity using α-amylase inhibitory assay and isolated an active compound. The bioactivity assay-guided study demonstrated the presence of an α-amylase inhibitory fraction from *S. mombin* leaf. An active compound, 3β-olean-12-en-3-yl (9Z)-hexadec-9-enoate was also studied. This is reported, from this plant, for the first time. The methanol extract, diethyl ether fraction and the isolated compound exhibited significant enzyme inhibitory activity against *Aspergillus oryzae* α-amylase. Our study revealed, for the first time, the isolation and α-amylase inhibitory activity of 3β-olean-12-en-3-yl (9Z)-hexadec-9-enoate from *S. mombin* leaf.

1. Introduction

Non-insulin-dependent diabetes mellitus is a heterogeneous disease with both genetic and environmental causative factors. It is characterised by hyperglycaemia caused by decrease in the secretion of insulin from the pancreatic Langerhans β-cells. A new alternative therapeutic approach of treating non-insulin diabetes mellitus involves inhibiting α-amylase and α-glucosidase. These are the key enzymes involved in starch breakdown and intestinal absorption. The inhibition of these enzymes significantly decreases the digestion and uptake of carbohydrate, thereby decreasing the postprandial blood glucose level in the non-insulin-dependent diabetes mellitus patients (Puls, Keup, Krause, Thomas, & Hoffmeister, 1977).

Acarbose and voglibose are currently used as α-amylase and α-glucosidase inhibitors and have exhibited side effects such as abdominal distension, bloating, flatulence and diarrhoea (Chakrabarti & Rajagopalan, 2002; Kimmel & Inzucchi, 2005). These side effects are possibly caused by excessive inhibition of the pancreatic α-amylase, leading to bacterial fermentation of undigested carbohydrate in the colon (Bischoff, 1994).

Several studies have reported the isolation of α-amylase and α-glucosidase inhibitors from dietary plants. These isolated inhibitors have been reported to be effective in decreasing postprandial hyperglycaemia with minimal side effects (Kwon, Vattem, & Shetty, 2006). Hence a search has ensued for α-amylase inhibitors from plants used by traditional healers in southwest Nigeria for the treatment of diabetes mellitus.

Spondias mombin Linn (Anacardiaceae) is a deciduous tree found in forest and Savannah regions (Hutchinson, 1959). It is used traditionally as food and for its medicinal values. The young leaves are cooked as greens and the green fruits pickled in vinegar and eaten like olives with salt and chilli. The aromatic fruit of *S. mombin,* rich in vitamins B1 and C, are eaten as a means of alleviating thirst, to flavour drinks and ice cream and mainly to produce wine, sold as "Vinho de Taperida" (Moran, 1987). In traditional folklore medicine, *S. mombin* is used to treat diabetes, intestinal disorders, typhoid fever and as an abortifacient.

Various parts of *S. mombin* have been reported to exhibit various biological effects, such as antidiarrhoeal activity (Iwu, 1993), antimicrobial and astringent effect (Abo, Ogunleye, & Ashidi, 1999; Kokwaro, 1976; Oliver-Bever, 1960), antipsychotic effect (Ayoka, Akomolafe, Iwalewa, Akanmu, & Upkonmwan, 2006), antiinflammatory activity (Villegas et al., 1997), as an abortifacient (Offiah & Anyanwu, 1989), for antiviral activity (Corthout, Pieters, Claeys, Vanden Berghe, & Vlietinck, 1991, 1992) and beta lactamase inhibitory activity (Coates et al., 1994). There is, however, no report of the antidiabetic activity of *S. mombin*.

A bioactivity-guided study of the leaf of *S. mombin* was carried out to investigate the α-amylase inhibitory effect of *S. mombin* fractions and to isolate the compound responsible for the inhibitory activity.

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2. Materials and methods

2.1. Materials

S. mombin leaf was collected from Sagamu Ogun State, Nigeria. A voucher specimen (FHI 106132) was deposited at the Herbarium of the Forestry Research Institute of Nigeria, Ibadan. All chemicals used were of reagent grade, purchased from Sigma Co., UK and VWR International.

2.2. General procedure

EIMS and IR were obtained with Micromass Autospec M and Perkin Elmer 1725 instruments.

Column chromatography was performed with Silica gel G (70–230 mesh, Machenerey Nagel).

2.3. Aspergillus oryzae α-amylase inhibitory activity determination

The inhibitory activity of *S. mombin* leaf fractions and the isolated compound on α-amylase from *A. oryzae* was assayed, using a method adopted from Bernfeld's reports with modification (Bernfeld, 1951, 1955). Soluble starch (1 g), used as substrate, was suspended in phosphate buffer (100 ml, pH 7.4).

The soluble starch (1 ml) was incubated with the test sample (1 ml) and *A. oryzae* α-amylase solution (1 ml, E.C.3.2.1.1., 10 U/ml) for 10 min at 25 °C. The reaction was stopped by adding 3,5-dinitrosalicylic acid and boiled for 5 min at 90 °C.

The mixture was cooled and diluted with deionised water. The absorbance of the mixture was measured at 540 nm and α-amylase inhibition was expressed as percentage of inhibition.

% Inhibition = Go − Gi/Go

2.4. Extraction and isolation of inhibitor from S. mombin leaf

The air-dried, powdered leaf of *S. mombin* were macerated with 80% methanol at room temperature and filtered. Evaporation of the solvent under reduced pressure yielded the methanol extract which was suspended in water and re-extracted with chloroform, chloroform/methanol and diethyl ether, successively.

The diethyl ether fraction was applied to a flash column, using Silica gel G and varying polarities of chloroform and methanol as mobile phase to give six fractions.

The major fraction was further fractionated by column chromatography (on Silica gel G, using varying polarities of petroleum ether, ethyl acetate and methanol as mobile phase) to yield five subfractions. Subfraction 1 was the most active and was further purified by preparative TLC (Silica gel GF_{254}, 1000 μg, 20 × 20 cm), using hexane/diethyl ether/acetic acid (7:3:0.2) as mobile phase to yield the isolated compound.

3. Results

3.1. Extraction

The dried leaf extract of *S. mombin* was extracted with 80% methanol and an activity-guided assay of the fractions was carried out using the α-amylase inhibitory assay.

At a concentration of 250 mg/ml, the methanol extract inhibited 39% of the α-amylase activity. The methanol extract was evaporated, reconstituted in water and, successively partitioned with chloroform, chloroform/methanol and diethyl ether. The highest inhibitory activity was observed in the diethyl ether fraction (73% inhibition at 70 mg/ml) (Fig. 1). This fraction was subjected to flash chromatography on silica gel and the most active fraction was further fractionated into five subfractions (Fig. 2).

Subfraction 1, at a dose of 70 mg/ml, showed a significant 67% α-amylase inhibition (Fig. 2) and it was further purified by preparative TLC to yield 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate as active compound (Analytical TLC, on Silica gel GF_{254} and mobile phase at toluene/diethyl ether/acetic acid, 8:2:0.1, Rf = 0.20, and it reacted positively with Lieberman–Burchard and anisaldehyde–sulphuric acid reagents, and at a dose of 20 mg/ml 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate, the isolated compound, exhibited 57% α-amylase inhibition.

3.2. Identification of the chemical structure of the isolated compound

EIMS of the isolated compound showed a mass ion peak at *m*/*z* 662 from which a molecular formula of $C_{46}H_{78}O_2$ was assigned.

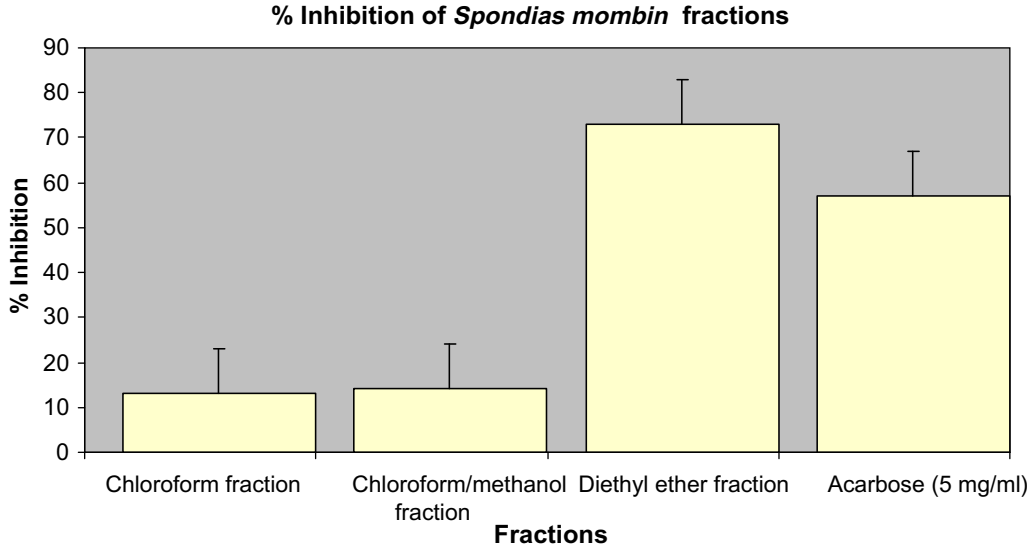

Fig. 1. α-Amylase inhibitory activity of fractions of *S. mombin* leaf.

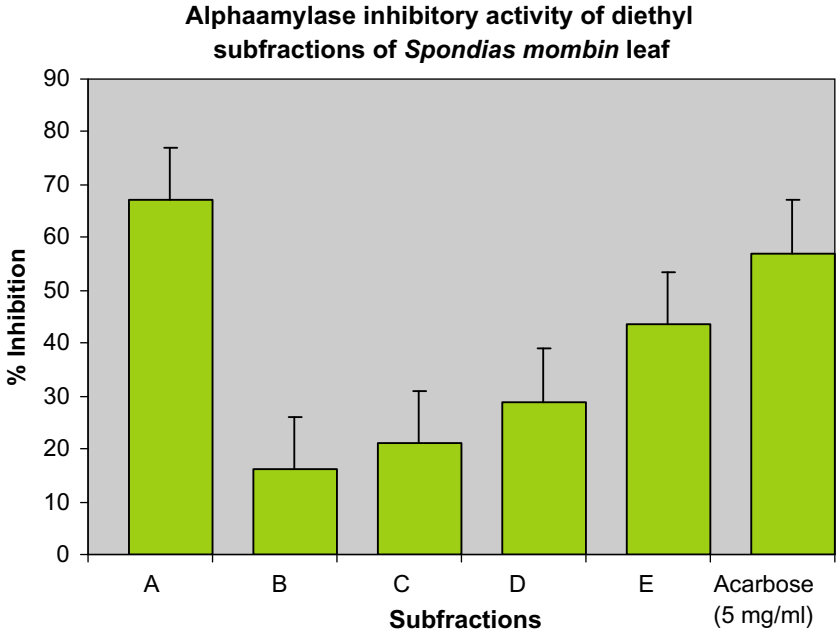

Fig. 2. α-Amylase inhibitory activity of diethyl ether subfractions of *S. mombin* leaf.

Table 1
α-Amylase inhibitory activity of isolated compound (3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate) from *S. mombin* leaf.

TLC bands	Absorbnce (Au)$^{+}$			Mean ± SEM	% Inhibition
Compound 1 20 mg	0.399	0.484	0.381	0.421 ± 0.22	57.0**
Acarbose 5 mg/ml	0.370	0.405	0.402	0.392 ± 0.016	60.0**
Control	0.909	1.109	0.891	0.970 ± 0.540	

** $P<0.05$.

The MS and IR data, when compared with those in the literature (Faizi & Naz, 2004), indicated that the isolated compound was 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate. 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate (1): gum, EIMS *m*/*z* 662; calc. for $C_{46}H_{78}O_2$, 215(16), 185(18), 57(100, C_4H_9). IR (KBr) 2954, 2849, 1733, 1163.

3.3. Enzyme activity

The methanol extract of the leaf of *S. mombin* showed α-amylase inhibitory activity of 39% while the isolated compound, at 20 mg/ml, exhibited a significant α-amylase inhibitory activity of 57%. The activity of the isolated compound was not significantly different from the activity of Acarbose (60% at 5 mg/ml) (a known α-glucosidase inhibitor) (Table 1).

4. Discussion and conclusion

The diethyl ether fraction of *S. mombin* leaf showed the highest α-amylase inhibitory activity. This is the first report of the α-amylase inhibitory activity of 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate, the isolated compound from *S. mombin* leaf, though previous reports have shown the α-amylase inhibitory activity of ursolic acid and oleanolic acid (Ali, Houghton, & Soumyanath, 2006).

Also, previous studies have reported the isolation of ellagitannins, anacardic acid and galloylgeraniin from *S. mombin*, but the isolation of 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate from the plant has not previously been reported.

Finally, the α-amylase bioassay-guided study of the methanol extract from the leaf of *S. mombin* led to the isolation of 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate.

This study shows the possibility of using *S. mombin* leaf extract and 3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate to decrease post-prandial hyperglycaemia.

Also, the study justifies the use of *S. mombin* in the management of diabetes mellitus by traditional healers in southwest Nigeria.

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