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Original Article

In vitro Antioxidant and Antidiabetic Potential of Crude Extracts from the Seed Coat and Fruit Pulp of *Strychnos madagascariensis*

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ABSTRACT

Diabetes mellitus remains a global health issue despite the advance in orthodox medicine. This study investigated the *in vitro* antioxidant and antidiabetic potential of crude extracts from the seed coat and pulp of *Strychnos madagascariensis*. The phytochemical screening was carried out using standard protocols. Different extracts were prepared from the fruit parts by maceration using methanol, *n*-hexane, ethyl acetate, and water for antioxidant and antidiabetic assays, and their percentage yield was calculated. The antioxidant potential of the extracts was determined using 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2′2- diphenyl-1-picrylhydrazyl (DPPH). Antidiabetic activities of the extracts were investigated using α-amylase, α-glucosidase, and pancreatic lipase assays. Terpenoids, alkaloids and cardiac glycosides were present in both the fruit parts. However, saponin present in the fruit pulp was absent in the seed coat (testa). The percentage yields are as follows; water > ethyl acetate > hexane > methanol (seed coat) and methanol > water > ethyl acetate > hexane (fruit pulp), respectively. The crude extracts scavenged ABTS and DPPH radicals in different degrees. The aqueous extract of the pulp and seed coat (testa) showed significant ($P < 0.05$) higher scavenging activity against ABTS (IC_{50}; 0.012 and 0.006 mg/ml) and DPPH (IC_{50}; 0.06 mg/ml and 0.064 mg/ml) radicals than other extracts. The crude extracts inhibited α-amylase, α-glucosidase, and pancreatic lipase. The aqueous and methanol extracts of the fruit parts showed better amylase inhibitory activity than other extracts. The aqueous extract of the seed coat (IC_{50}; 0.0785 mg/ml) showed the highest glucosidase inhibitory activity. In addition, methanol extract of the seed coat (IC_{50}; 0.069 mg/ml) exhibited the highest inhibitory activity on pancreatic lipase compared to the extracts in other solvents. Hence, the aqueous and methanol crude extracts of *Strychnos madagascariensis* seed coat and fruit pulp could be used in the preparation of nutraceutical products for managing diabetic mellitus.

Key words: Alkaloids, Diabetes, Hyperglycaemia, Hyperlipidaemia, Phytochemicals.

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INTRODUCTION

Diabetes mellitus (DM) is a collection of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both.[1] Poorly managed hyperglycaemia often leads to long-term damage and failure of different organs, including the heart, nerves, blood vessels, kidneys and eyes.[1] In the diabetic state, glucose produced by the hydrolytic action of α-amylase and α-glucosidase is not utilized by the cells, thus leading to hyperglycaemia. In addition, diabetes enhances high levels of lipids and fats in the blood, which further intensify diabetic complication.[2] The cumulative effects of these processes aggravate the production of free radicals due to compromised antioxidant defense systems.[3] Despite the potency of antidiabetic drugs such as voglibose, miglitol, acarbose, their safety remains burdensome.[4] The drugs are associated with unwanted side effects such as abnormal weight gain, headache, nausea, diarrhea and dizziness.[5] Hence, the search for safer and cheaper alternative antidiabetic agents from plant origin.

Medicinal plants are potential sources of novel therapeutics against several chronic diseases.[6] They possess naturally occurring bioactive chemicals that have medicinal properties, and hence, extensive exploration of medicinal plants in the management of diseases. The remedies derived from medicinal plants are acceptable based on its safety, accessibility, and affordability.[7] *Strychnos madagascariensis* belongs to the Loganiaceae family. The plant is endemic to tropical and subtropical Africa including South Africa, Lesotho, Zimbabwe, and Swaziland.[8] They are generally found in woodlands, rocky places, riverine fringes and coastal forest of southern Africa.[9] *Strychnos madagascariensis* fruit is used traditionally as a food in the north coastal region of Kwazulu Natal in South Africa and the Southern part of Zimbabwe where it is claimed to curb diabetes and hypertension.

However, there is still a paucity of scientific evidence regarding the antioxidant and antidiabetic activities of this plant, despite its usage in folklore medicine. Hence, this study is the first report of the in *vitro* antioxidant and antidiabetic potential of *Strychnos madagascariensis* fruit.

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MATERIALS AND METHODS

Chemicals

Butylated Hydroxyl anisole (BHA), ascorbic acid (AA), $FeCl_2$, $FeCl_3$, NaOH, potassium sodium tartrate, sodium carbonate, Tris-HCl buffer, phosphate buffer, 3,5-dinitrosalicylic acid, potassium persulfate, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6- sulphonic acid) (ABTS) were all purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Other reagents used are 4-nitrophenyl-a-D-glucopyranoside, *p*-nitrophenyl palmitate, orlistat, methanol, hexane, ethyl acetate, and water. All chemicals used were of analytical standard.

Plant collection and identification

Strychnos madagascariensis's fruits were harvested in August 2019 at Mbazwana (27.4937° S, 32.5882° E) KwaZulu-Natal, South Africa. The fruit was identified by Dr. N.T. Ntuli at the Department of Botany, University of Zululand. The plant's sample (voucher number VH10) was deposited at the University's herbarium.

Plant extraction

The seed coat and pulp of *Strychnos madagascariensis* ripe fruit were air-dried separately and pulverized using laboratory blender (Clarkson BB25EP). Different quantity of the pulverized samples was used to prepare different crude extracts by maceration using methanol, *n*-hexane, ethyl acetate and water (200 ml) on an automatic shaker (150 rpm; 25°C) for 72 hr. The crude extracts were filtered using Whatman filter paper 1. The extracts containing organic solvents were concentrated using Heidolph rotary evaporator (45 rpm, 40°C), whereas the aqueous extract was freeze-dried (Virtis Benchtop K). Some of the extracts were sticky, oily, while others were solid, and the percentage yield of each crude extracts was calculated using the formula below:

% yield = $(W_2)/ (W_1) \times 100$

Where,

W1 represents the weight of the initial sample and

W2 represents the final weight of the extract.

Subsequent to the extraction process using different quantities (25 g to 60 g) of the fruit parts, the weights of the different crude extracts were used to determine their percentage yield (Table 1).

Phytochemicals screening

Phytochemical screening was conducted using standard qualitative methods with slight modifications as described by Ahumuza and Kirimuhuzya, (2011) and Bibi et al., (2012).[10-11] The following phytochemicals were tested for; Terpenoids, alkaloids, saponins, tannins, flavonoids and cardiac glycosides, steroids.

Antioxidant studies

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity

The determination of ABTS scavenging activity of the extracts was carried out with the method of Re *et al.*[12] ABTS solution (0.003 g/ml) was mixed in the ratio 1:1 (v/v) with different concentrations of the crude extracts (0-5 mg/ml) respectively. The mixture was left to stand for 10 min at 25°C. The absorbance was read at 734 nm using a spectrophotometer (Biotek plate reader). Ascorbic acid and butylated hydroxyanisole (BHA) served as positive controls. The percentage inhibition was calculated using this formula:

% Inhibition = $(A_0 - A1)/A_0) \times 100$

Where,

A_0 = Absorbance of control

A_1 = Absorbance of sample

2,2 -Diphenyl-1-Picryl Hydrazyl (DPPH) scavenging activity

The DPPH scavenging activity of the seed coat (testa) and pulp extracts was determined by the method of Brand (1995).[13] The DPPH (0.02 mg/ml) was mixed (1:1 v/v) with various concentration of crude extracts (0-5 mg/ml) which were dissolved in methanol. Thereafter, the mixture was made to stand for 60 minutes at 25 °C, and the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid and Butylated Hydroxyl anisole (BHA) served as the positive controls. The percentage inhibition was calculated using this formula :

% Inhibition = $(A_0 - A1)/A_0) \times 100$

Where,

A_0 = Absorbance of control

A_1 = Absorbance of sample

α-Amylase inhibitory assay

The inhibitory effect of the seed coat (testa) and pulp crude extracts of *S. madagascariensis* on α-amylase was determined using the method described by Adisakwattana *et al* (2011).[14] Starch (200 µl) and plant extracts (300 µl) at different concentrations (0-5mg/ml) were made to stand for 5 minutes at 25 ^{0}C for binding to take place. Thereafter, 200 µl of α-amylase (1 mg/ml) was added to each mixture and was further incubated for 15 minutes at 37 ^{0}C. The reaction was terminated by the addition of 250 µl of 3,5-dinitrosalicylic acid (1 % in 2 M NaOH) and heated for about 10 minutes at 100 ^{0}C then cooled on ice. Thereafter, potassium Sodium Tartrate (205 µl; 40 %) was added and absorbance was read at 450 nm using a spectrophotometer. The percentage inhibition was calculated using this formula:

% Inhibition = $(A_0 - A1)/A_0) \times 100$.

Where,

A_0 = Absorbance of control

A_1 = Absorbance of sample

α-Glucosidase inhibitory assay

The inhibitory effect of the crude extracts of *S. madagascariensis* on α-glucosidase was determined using the method described by Rayar and Manivannan.[15] Phosphate buffer (10 mM, pH 6.8; 50 µl), 20 µl of plant extract at different concentrations (0-5 mg/ml) and the enzyme (0.5 unit/ml; 10 µl) were pre-incubated for 15 minutes at 37 °C. Then, 4-nitrophenyl-a-D-glucopyranoside) (2.5 mM; 20 µl) was added to begin the reaction. The mixture was further incubated for another 20 minutes at 37 °C before the addition of sodium carbonate (0.1 M; 50 µl) to reduce the reaction. The absorbance was read using a spectrophotometer at 405 nm. However, only methanol and aqueous crude extracts were investigated. Distill water served as a negative control.

Pancreatic lipase inhibitory activity

The inhibitory activity of the crude extracts on pancreatic lipase was investigated using the method described by Slanc *et al.*[16] The mixture of Tris-HCl buffer (75 mM, pH 8; 125 µl), various concentrations of the extract (0-5 mg/ml; 75 µl) and 50 µl of pancreatic lipase (10 mg/ml; 50 µl of) in various cells of 96 well plate was incubated for 15 minutes at 37 °C. The reaction was initiated by adding 3,3 mM *p*-nitrophenyl palmitate (25 µl) followed by a 30-minute incubation. Absorbance was

measured at 405 nm using a spectrophotometer. Orlistat served as the positive control while methanol and water (1:1) served as the blank.

Data analysis

All treatments were in triplicate, and the values were expressed as mean ± standard deviation. One-way Analysis of Variance (ANOVA) was used to analyze the data. The IC_{50} (the concentration at which 50 % of the biological component was inhibited or scavenged) values were determined using the graph pad prism 6.01. The significant values of the percentage (%) inhibition of the extracts against the measured parameters were considered as $P < 0.05$.

RESULTS

Percentage yield

The hexane, ethyl acetate, and aqueous extracts from the seed coat had comparable percentage yield ranging from 25.92-30.26 % while the hexane and ethyl acetate extracts from the fruit pulp had low percentage yields (Table 1).

Phytochemical screening

Both the seed coat and pulp of ripe *S. madagascariensis* fruits tested positive for terpenoids, alkaloids, and cardiac glycosides but negative for tannins and flavonoids. The seed coat differed from the pulp in that it tested negative for saponins while the pulp was positive for those groups of phytochemicals (Table 2).

Antioxidant properties

ABTS scavenging activity

Based on the ABTS assay, the extracts, except aqueous extracts of both samples, had concentration-dependent radical scavenging activities (Figure 1). The methanol crude extract of both samples and the fruit pulp's hexane and ethyl acetate extracts showed good free radical scavenging activity (Figure 1). The aqueous crude extracts from the seed coat (IC_{50}: 0.012 mg/ml) and fruit pulp (IC_{50}: 0.006 mg/ml) had the highest scavenging activities, while the seed coat's ethyl acetate and hexane extracts showed poor antioxidant activity (Table 3).

DPPH scavenging activity

The crude extracts from both fruit parts had concentration-dependent scavenging activity except for the seed coat's aqueous extract (Figure 2). The methanolic extract from the seed coat showed the highest antioxidant activity while the seed coat's hexane extract had the lowest activity. The seed coat's aqueous crude extract had the highest scavenging activity (IC_{50} values: 0.0195 mg/ml) against DPPH. However, all the extracts from the fruit pulp exhibited good scavenging activity (Table 3).

α-Amylase activity

The methanol crude extracts of both fruit parts exhibited moderate α-amylase inhibitory activity (Figure 4). The aqueous, ethyl acetate and hexane extracts from both fruit parts showed weak inhibitory potential.

Table 1: Percentage yield from the seed coat and fruit extracts.

Solvent	Seed coat (%)	Fruit pulp (%)
Methanol	9.4	34.25
Hexane	25.92	0.42
Ethyl acetate	29.96	1.2
Water	30.26	23.3

Table 2: Phytochemical components of *S. madagascariensis*.

Phytochemicals	Seed coat	Fruit pulp
Terpenoids	+	+
Alkaloids	+	+
Saponins	-	+
Cardiac glycosides	+	+
Tannins	-	-
Flavonoids	-	-

Sign notations: + present, - absent

Table 3: IC_{50} values (mg/ml) of the extracts of *S. madagascariensis* on ABTS and DPPH.

Extract	ABTS	DPPH
SCME	0.0665 ± 0.0013	0.0735 ± 0.0018
SCHE	ND	ND
SCEAE	ND	ND
SCAE	0.0120 ± 0.0005	0.0195 ± 0.0016
FPME	0.0650 ± 0.0066	0.0505 ± 0.0010
FPHE	0.0475 ± 0.0076	ND
FPEAE	0.0280 ± 0.0209	0.0925 ± 0.00425
FPAE	0.0060 ± 0.0005	0.0640 ± 0.00476
AA	0.0720 ± 0.0343	0.0210 ± 0.00001
BHA	0.0525 ± 0.0107	0.0665 ± 0.0017

AA and BHA were used as the standard. Values are expressed as mean ± SD.
SCME- seed coat methanolic extract, **SCHE-** seed coat hexane extract, **SCEAE-** seed coat ethyl acetate extract, **SCAE-** seed coat aqueous extract, **FPME-** fruit pulp methanolic extract, **FPHE-** fruit pulp hexane extract, **FPEAE-** fruit pulp ethyl acetate, **FPAE-** fruit pulp aqueous extract, **AA-** Ascorbic acid, **BHA-** Butylated hydroxyanisole and **ND** represents not determined. Values are expressed as mean ± SD.

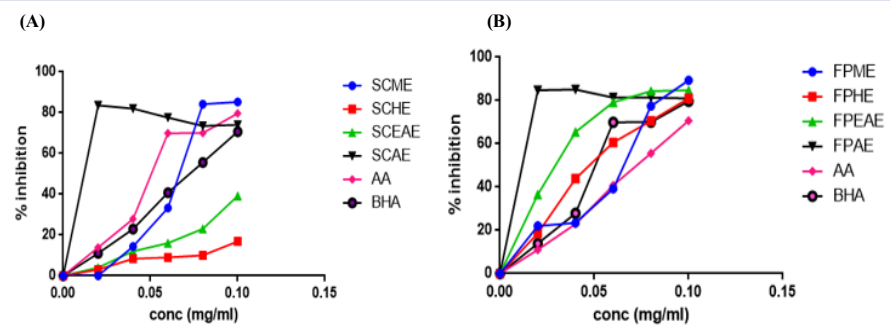

Figure 1: Scavenging activity of *S. madagascariensis* seed coat (A) and fruit pulp (B) extracts against ABTS radical. **SCME-** seed coat methanolic extract, **SCHE-** seed coat hexane extract, **SCEAE-** seed coat ethyl acetate extract, **SCAE-** seed coat aqueous extract, **FPME-** fruit pulp methanolic extract, **FPHE-** fruit pulp hexane extract, **FPEAE-** fruit pulp ethyl acetate, **FPAE-** fruit pulp aqueous extract, **AA-** Ascorbic acid, **BHA-** Butylated hydroxyanisole.

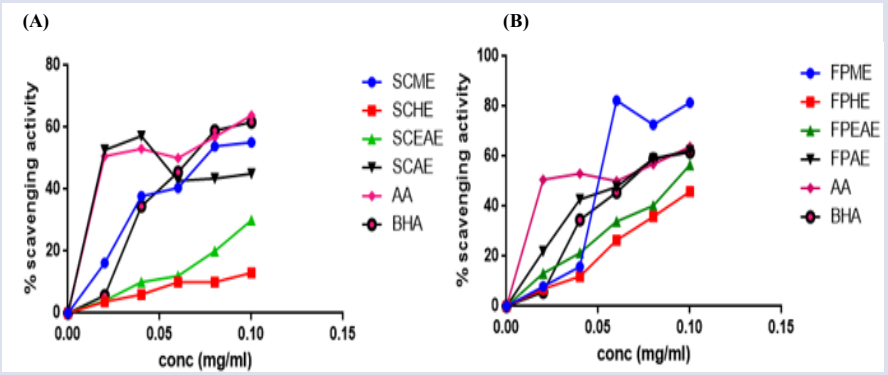

Figure 2: Scavenging activity and of *S. madagascariensis* seed coat (A) and fruit pulp (B) extracts against DPPH radical. Values are expressed as mean ± SD. **SCME-** seed coat methanolic extract, **SCHE-** seed coat hexane extract, **SCEAE-** seed coat ethyl acetate extract, **SCAE-** seed coat aqueous extract, **FPME-** fruit pulp methanolic extract, **FPHE-** fruit pulp hexane extract, **FPEAE-** fruit pulp ethyl acetate, **FPAE-** fruit pulp aqueous extract, **AA-** Ascorbic acid, **BHA-** Butylated hydroxyanisole.

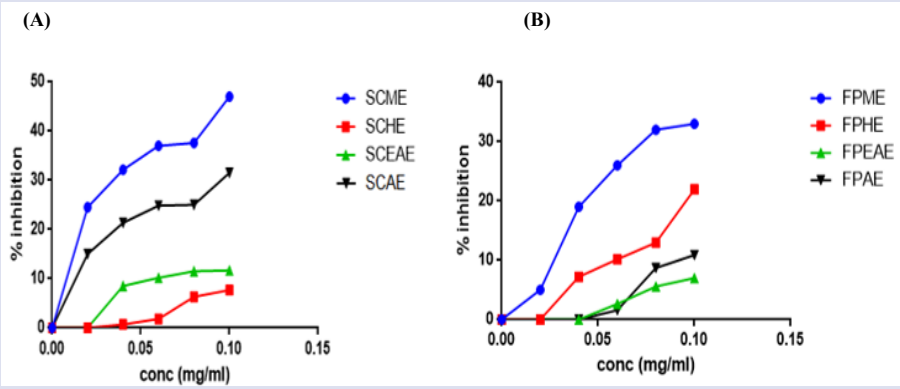

Figure 3: Inhibitory effects and IC_{50} values of *S. madagascariensis* seed coat (A) and fruit pulp (B) extracts against α-amylase.

However, all the extracts from both fruit parts displayed concentration-dependent inhibitory activity.

α-Glucosidase activity

The methanol and aqueous extracts from both fruit parts showed concentration-dependent inhibitory activity on α-glucosidase (Figure 4). The seed coat's aqueous extract showed higher inhibitory activity on α-glucosidase when compared to the seed coat's methanol extract. By contrast, the fruit pulp's methanol extract displayed higher inhibitory activity when compared to the fruit pulp's aqueous extract. All the extracts had concentration-dependent inhibitory activity except for the fruit pulp's methanol extract (Figure 4). The hexane and ethyl acetate extracts of both fruit parts were not tested.

Pancreatic lipase activity

The *in vitro* inhibitory effect of the extracts on pancreatic lipase is shown in Figure 5. The seed coat and fruit pulp methanol extracts showed concentration-dependent inhibitory activity. However, the methanol extract from the seed coat (IC_{50}: 0.069 mg/ml) and fruit pulp (IC_{50}: 0.071 mg/ml) had the highest activity when compared to the other extracts (Table 4).

DISCUSSION

Many plants are known to possess naturally occurring bioactive chemicals which may have ameliorative effects on several ailments, including diabetes.[17] The present study aimed to investigate the *in vitro* free radical scavenging and anti-diabetic activities of fruit parts (seed coat and pulp) of *S. madagascariensis*. This was to confirm the alleged anti-diabetic claim of ripe *S. madagascariensis* fruits by locals

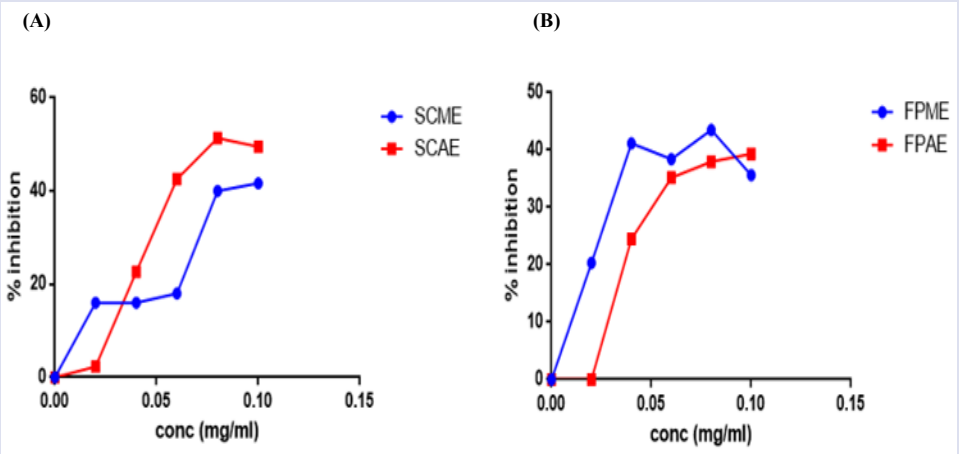

Figure 4: Inhibitory effects of *S. madagascariensis* seed coat (A) and fruit pulp (B) methanol and aqueous extracts respectively against α-glucosidase.

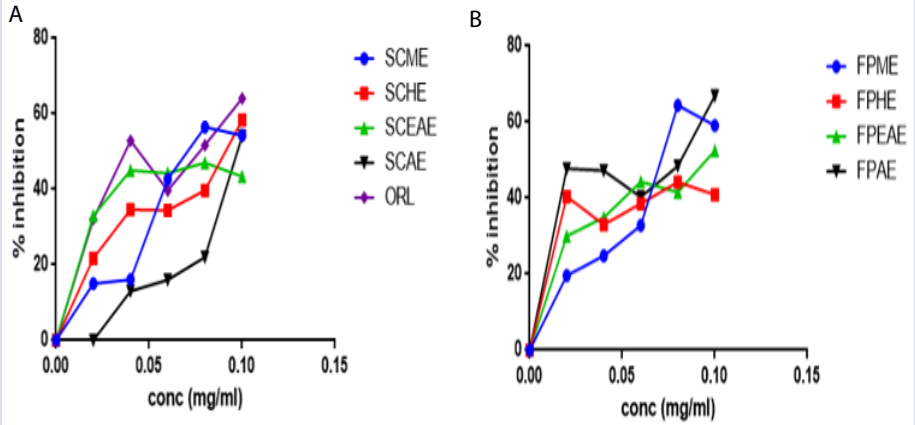

Figure 5: Inhibitory effects and IC_{50} values of *S. madagascariensis* seed coat (A) and fruit pulp (B) extracts against pancreatic lipase.

Table 4: IC_{50} values (mg/ml) of the extracts of *S. madagascariensis* on α-glucosidase and pancreatic lipase inhibition.

Extract	α-glucosidase	Pancreatic lipase
SCME	ND	0.0690 ± 0.0210
SCHE	ND	0.0895 ± 0.0021
SCEAE	ND	ND
SCAE	0.0785 ± 0.02100	0.0975 ± 0.0007
FPME	ND	0.0705 ± 0.0277
FPHE	ND	ND
FPEAE	ND	0.0950 ± 0.0293
FPAE	ND	0.0805 ± 0.3360
ORL	ND	0.0550 ± 0.0070

SCME- seed coat methanolic extract, **SCHE-** seed coat hexane extract, **SCEAE-** seed coat ethyl acetate extract, **SCAE-** seed coat aqueous extract, **FPME-** fruit pulp methanolic extract, **FPHE-** fruit pulp hexane extract, **FPEAE-** fruit pulp ethyl acetate extract, **FPAE-** fruit pulp aqueous extract, **ORL-** Orlistat and **ND** represents not determined. Values are expressed as mean ± SD.

in the northern coastal region of KwaZulu Natal. The medicinal efficacy of plant extracts is determined by the method of extraction[18] and the solubility of the concerned phytochemicals in the solvent used during the extraction. The high extraction yield of methanol and water suggest that the compounds in the seed coat (testa) and fruit pulp are highly soluble in these solvents since the extractability of a solvent is generally due to the solubility of the compounds in the solvent used .[19] Phytochemical screening of the seed coat and fruit pulp indicated that alkaloids, cardiac glycosides, and terpenoids were present in the seed coat and fruit pulp of *S. madagascariensis* (Table 2), which might impart antioxidant and anti-diabetic activities to these fruit parts. Indeed, in support of this, the aqueous extracts of both fruit parts, the seed coat's methanol, and the fruit pulp's hexane and ethyl acetate extracts showed high antioxidant activities when compared to other extracts from the fruit parts. Apart from the methanol and aqueous extracts which showed anti-diabetic activities by reducing the activities of carbohydrate digestive enzymes (Figure 3 and 4), the seed coat's hexane and fruit pulp's ethyl acetate extracts showed lipase inhibitory activity (Figure 5) suggest that these extracts could prevent hyperlipidemia which is one of the contributory factors to diabetes mellitus.[20] This shows the potential of the seed testa and pulp of the ripe fruit of *S. madagascariensis* to reduce post-prandial free fatty acid and subsequent control on diabetes mellitus which is linked to oxidative stress due to the damaging effects of hyperlipidemia.[21] The inhibition of these enzymes delays the release of glucose, causing a decrease in blood sugar levels.[22] Several relevant phytochemicals such as alkaloids, terpenoid, cardiac glycosides, saponins, steroids have shown amelioration of hyperglycemia through the inhibition of α-amylase and α-glucosidase.[23-24] Hence, the high antioxidant and antidiabetic activities of the fruit parts' extracts observed for *S. madagascariensis* in this study may contribute to the inhibition of the manifestation of diabetes-related symptoms.

CONCLUSION

This study highlights the free radical scavenging and enzyme inhibitory potential of extracts from the seed coat and fruit pulp of *S. madagascariensis* and offers scientific backing to its folklore usage in the management of diabetes mellitus. The extracts displayed diverse anti-diabetic activities that could be linked to the phytochemicals such as alkaloids, terpenoids, cardiac glycosides, present in the extracts. The aqueous and methanol extracts of the fruit parts showed higher *in vitro* antioxidant and antidiabetic activity compared to other extracts by efficiently scavenging free radicals and inhibiting carbohydrates (α-amylase and α-glucosidase) and lipids (pancreatic lipase) digestive enzymes making the ripe fruit of importance in the management of diabetes mellitus. For further studies, detailed phytochemical constituents of the fruit parts (seed coat and pulp) need to ascertain using Liquid chromatography Mass Spectroscopy (LC-MS). Likewise, *in vivo* antidiabetic potential of the crude extracts needs to be carried out.

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CONFLICTS OF INTEREST

None declared.

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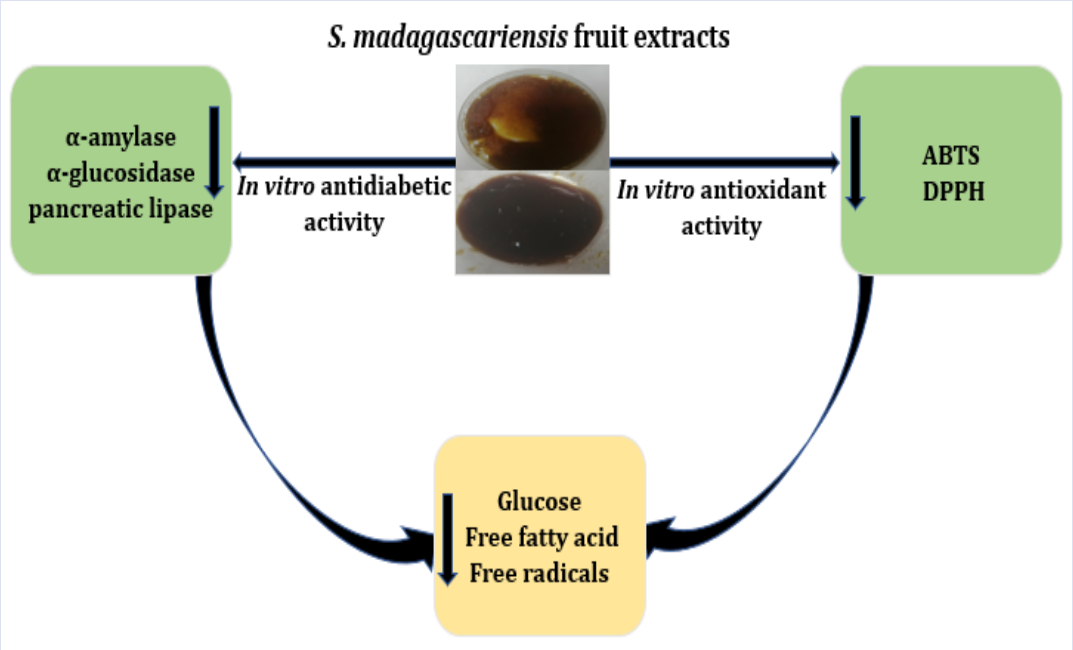
GRAPHICAL ABSTRACT



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