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Phytochemical Screening, Antioxidant Activity and Hypoglycemic Potential of Kenyan *Aloe lateritia* and *Aloe secundiflora* Extracts in Alloxan-Induced Diabetic Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author CMM designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors ENM and NWNM managed the analyses of the study. Author NWNM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the study was to investigate the phytochemical constitution, antioxidant activity, hypoglycemic potential and safety of *Aloe lateritia* and *Aloe secundiflora*. Phytochemical screening was determined using standard procedures and Gas chromatography-mass spectrometry (GC-MS) analysis. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined spectrophotometrically. Hypoglycemic studies involved daily administration of 200 mg/kg of metformin and 300 mg/kg of methanol and aqueous leaves extracts of *A. lateritia* and *A. secundiflora* to alloxan-

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induced diabetic mice for 21 days. The safety of the extracts was experimented using OECD protocol on Acute Oral Toxicity-Acute Toxic Class Method Test no. 423. The results showed the presence of hypoglycemic phytochemicals: - phenols, saponins, alkaloids, flavonoids, tannins, anthraquinones, steroids and carbohydrates in both plants. Analysis by GC-MS determined the presence of phytochemicals in *A. lateritia* and *A. secundiflora* already established in other *Aloe* species. *Aloe secundiflora* extracts were decided to have higher free radical scavenging activity than *A. lateritia* extracts. Both *A. lateritia* and *A. secundiflora* aqueous and methanol extracts showed significant decreases in FBG levels when compared to the diabetic control group while there was no significant difference between *A. secundiflora* extracts and metformin-treated group at the end of the experiment ($P<0.05$). *Aloe secundiflora* methanol extracts achieved the highest percentage glycemic change among the extracts. All the extracts were not toxic at the tested levels. The hypoglycemic and antioxidant activities established in *A. lateritia* and *A. secundiflora* can be linked to the phytochemicals present.

Keywords: Aloe lateritia; Aloe secundiflora; diabetes mellitus; medicinal plants; methanol and aqueous extracts; phytochemicals; antioxidant activity; hypoglycemic effects.

1. INTRODUCTION

Diabetes is a group of metabolic disorders affecting populations across the globe. It is caused by abnormal metabolism of carbohydrates associated with low blood insulin level or insensitivity of insulin by target organs [1]. According to WHO, This leads to high blood sugar levels over a prolonged time [2]. There were 478,000 cases of diabetes reported in Kenya in 2015 [3] with the diabetes prevalence estimate of ages between 20 and 79 years is 4.7% [2]. There are two common types of diabetes: - Diabetes type I is characterised by the shortage in the synthesis of insulin owing to loss of the beta cells and requires daily administration of insulin. Diabetes type II occurs as a result of the body's ineffective use of insulin and comprises 90% of people with diabetes around the world [4]. The estimated total economic cost of diagnosed diabetes in the world in 2012 was US$ 245 billion, which represents the substantial burden of diabetes [5]. Diabetes type II is associated with various complications like neuropathy, retinopathy, nephropathy, angiopathy, ketoacidosis and hyperlipidemia. These complications can only be minimised if there is proper maintenance of blood glucose levels [6]. Existing antidiabetic synthetic agents in use today which have been useful in controlling blood glucose include sulfonylureas, biguanides, glinides, α-glucosidase inhibitors and thiazolidinediones. Metformin is the most commonly used biguanide and works by decreasing gluconeogenesis [7]. These drugs have been shown to have adverse effects on the body [7]. Plants have played a pivotal role as a source of the currently available anti-diabetic medicines for example metformin which is derived from *Galega officinalis* [8]. About 600 *Aloe* species can be found across Africa and Asia [9]. In the last three decades, various *Aloe* species have been shown to be potent remedies in the management of diabetes. Agarwal, [10], carried out the initial studies on the hypoglycemic activity of *A. vera* in 1985. Results revealed the significant decrease in blood glucose, total cholesterol and total triglyceride levels [10]. Subsequent studies on *A. vera* have since been done [11-16]. Other studies on other *Aloe* species including *A. camperi, A. ferox* and *A. greatheadii var. davyana*have also been carried out by [17,18]. All these studies have demonstrated marked antidiabetic activity. In Kenyan traditional medicine, *Aloe* species have long been utilised therapeutically. *Aloe secundiflora* extracts are used in the treatment of malaria, diarrhoea, typhoid fever, oedema, headache and pneumonia [19] while *A. lateritia* is used in the management of colds and sickness [20]. In the present study, we have investigated the antidiabetic effects of *A. lateritia* and *A. secundiflora* which have been shown to contain phytochemicals like phenols, tannins, terpenoids, glycosides, glycoproteins, aloin and anthraquinones [21] and [22]. These phytochemicals have proven antioxidant and hypoglycemic activity [23,24].

2. MATERIALS AND METHODS

2.1 Collection of *A. secundiflora* and *A. lateritia* Plants

The *A. secundiflora* plant samples were obtained from their natural habitat in Athi River (1°27′ 25.43″S, 37°0′26.13″E) in Machakos County approximately 30 km from Nairobi while *A. lateritia* samples from Kinungi (0°-46′-12″S, 36°

30′2.67″ E) located in Nakuru County, about 78 km from Nairobi. Voucher specimens were deposited at the East African Herbarium at the National Museums of Kenya, Nairobi. Voucher specimen numbers were *C. Db 2/2015* and *C. Db 1/2014* for *A. lateritia* and *A. secundiflora* respectively.

2.2 Aqueous Extraction

Aqueous extraction process followed the methods of [25]. Mature, healthy and freshly collected leaves were washed with clean water, and the cuticula removed weighed and blended. The preparations were then soaked separately in 2 litres per every 250 gms of water for 3 hours and filtered using a Whatman No. 1 paper. The filtrate was freeze dried immediately for 72 hours to obtain crude extracts of *A. lateritia* and *A. secundiflora* which were preserved at 4°C.

2.3 Methanolic Extraction

Organic extraction was done using the methods of [26]. *Aloe lateritia* and *A. secundiflora* freshly obtained leaves were dissected to remove the cuticula to obtain *Aloe* latex which was blended and extracted with methanol. The extracts were then filtered by Whatman No.1 paper and the filtrates concentrated in a vacuum at 50°C in a rotary evaporator to obtain *A. lateritia* and *A. secundiflora* concentrated extracts which were stored in airtight containers at 4°C.

2.4 Qualitative Phytochemical Analysis of *A. secundiflora* and *A. lateritia*

Phytochemical investigations were carried out both the aqueous and methanol extracts of *A. lateritia* and *A. secundiflora*.

2.4.1 Test for alkaloids

The presence of alkaloids was determined by first dissolving 0.02 gms of extract in 1 ml methanol, filtering the mixture, followed by boiling the extract with 2 ml of 1% hydrochloric acid for 5 min. Five drops of Dragendorff's reagent was then added to the extract. Formation of an orange precipitate indicated the presence of alkaloids [27].

2.4.2 Test for saponins

Frothing test determined the presence of saponins. Half a gram (0.5 gms) of the plant extract was shaken in 5 ml of distilled water and allowed to stand for 10 minutes. The stable froth of more than 1.5 cm and persisting for at least 30 min indicated the presence of saponins [28].

2.4.3 Test for flavonoids and flavones

One gram of extract was dissolved in 10 ml distilled water and then filtered using Whatman filter No.1. 10 mg magnesium turnings were added into 1 ml of the filtrate, followed by the addition of 0.05 ml concentrated sulphuric acid. The presence of magenta red observed within three minutes confirmed the presence of flavonoids [29].

2.4.4 Test for steroids

Two millilitres of acetic anhydride will be added to 0.5 gmsmethanolic extract of each sample with 2 ml of 0.1 M H_2SO_4. The colour change from violet to blue or green indicated the presence of steroids [29].

2.4.5 Test for tannins

Half a gram (0.5 gms) of the water extract was dissolved in 2 ml of distilled water and filtered. Two drops of 2% (w/v) ferric chloride was added to the filtrate. A blue, black precipitate indicated the presence of tannins [30].

2.4.6 Test for carbohydrates

Presence of carbohydrates was assayed using Molisch's test. Ten milligrams of the plant extracts were dissolved in 2 ml of water, and 0.5 ml of the polish reagent added. Two millilitres of concentrated sulphuric acid from a dropper was carefully poured down the side of the tube so that the acid formed a layer beneath the aqueous solution without mixing with it. A red colouration changing to dark purple forms at the interface determined the presence of carbohydrates [31].

2.4.7 Test for anthraquinones

The Bontrager test was used in which 2 mg of the test sample was shaken with 4 ml of hexane. The upper lipophilic layer was separated and treated with 4 ml dilute ammonia. If the lower layer changed from violet to pink, it indicated the presence of anthraquinones [32].

2.5 Quantitative Phytochemical Analysis of *A. secundiflora* and *A. lateritia*

The phytochemical investigation was carried out both the aqueous and methanol extracts of *A. lateritia* and *A. secundiflora*.

2.5.1 Quantification of saponins

Five grammes of the processed herbal powders were weighed into a conical flask and 100 $cm^3$of 20% aqueous ethanol added and heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was then filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over the water bath at about 90°C. The concentrate was transferred into a 250 ml separation funnel, and 20 ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the diethyl ether layer discarded. The purification process was then repeated. Sixty millilitres of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content calculated as a percentage [29].

2.5.2 Quantification of alkaloids

Five grammes of the herbal drug was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol added and covered, and then allowed to stand for 4 hrs. This was filtered, and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed [33,34].

2.5.3 Estimation of total flavonoids

Total flavonoid content was determined. A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of the sample solution. After one h at room temperature, the absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. Plant extracts were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as mg/g of quercetin using the following equation based on the calibration curve: $Y = 0.1001 \times +0.0076$, $R^2 = 0.975$, where x is the absorbance and Y is the quercetin equivalent [35].

2.5.4 Determination of total phenols

Total phenolic content extracts were determined spectrophotometrically using Folin-Ciocalteu reagent. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water at a concentration of 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and left to stand for 30 min at 40°C for colour development. Absorbance was then measured at 760 nm wavelength. Results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y = 0.1042 \times -0.005$, $R^2 = 0.9649$, where x is the absorbance and Y is the tannic acid equivalent [34,35].

2.6 Gas Chromatography-Mass Spectrometry Analysis of *A. lateritia* and *A. secundiflora* Methanol Extracts

Gas chromatography-mass spectrometry analysis was done according to GC-MS and qualitative analysis protocol prepared by [36]. Agilent 6890 GC ported to a 5973 MS detector (California, USA) was used for identification and quantification of individual phytochemicals, using MS libraries previously compiled from purchased standards. Ion source temperature of 200 °C and head pressure of 9 kPa were utilised. The interface temperature was 250°C. Mass range of 50-300 µ was used with injection mode of split, 100; 1 and injection volume of 1 ml. The GC was outfitted with a DB-5 MS 15 M; 0.25mm ID; 0.25 µm; 0.25 µm df capillary column, a split injection piece (250°C) and direct GC-MS coupling (260°C). Helium 99.999% (1 ml/min) was used as the carrier gas. The crude methanol extracts were extracted using ethyl acetate followed by diethyl ether and centrifuged to obtain supernatants which were derived using bis (trimethylsilyl), trifluoroacetamide (BSTFA, 100 µL), trimethylchlorosilane (TMCS, 20 µL) and pyridine (20 µL) for 30 min at 70°C. The preparations were then injected for analysis. The oven temperature program for analyzing the methanolic extracts of *A. secundiflora* and *A. lateritia* utilized a preliminary oven temperature of 70°C, maintained for 1 min, followed by a steady climb to 150°C at a rate of 5°C/min and then increased to 250°C at the rate of 28°C/min for 3 min and maintained for 25 min. Compounds were identified from their mass spectra through matching with corresponding compounds available in National Institute of Standards and Technology (NIST) 2011 database.

2.7 Antioxidant Activity of *A. secundiflora* and *A. lateritia* Methanol Extracts

Antioxidant activity was done according to a method described by [37]. Free radical

scavenging activity using DPPH was by 0.1 mM solution of DPPH prepared in solvent ethanol and 1 ml added to 3 ml of the methanol extracts in different concentration (5, 10, 15, 20, 25, 30 µg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Absorbance was then measured at 517 nm using a spectrophotometer. Ascorbic acid was used as the reference standard compound being used and the test done in triplicates.

The scavenging activity was calculated using the following equation:

DPPH scavenging activity (%) = {A0 - A1 / A0} × 100.

Where A0 was the Absorbance of control response and A1 was the Absorbance in the presence of test or standard sample.

2.8 Experimental Animals

Male Swiss Albino mice at least six weeks old with a weight of 22 ± g inbred at the Kenya Medical Research Institute (KEMRI) animal house were used. The experiments were done at the same facility. Mice were housed in ventilated cages under standard environmental conditions of temperature and humidity. Dark/light cycle was observed at all time. The wood shavings in the cages were changed on a daily basis. The continuous supply of pellets (Mice Pellets UNGA feeds) and water *ad libitum* were observed. The independent Animal Care granted approval for the use of laboratory animals and protocols and Use Committee (ACUC) and scientific ethics and review unit (SERU) of Kenya Medical Research Institute (KEMRI), with approval number *SERU CTMRD 0183133*.

2.9 Oral Glucose Tolerance Test (OGTT)

The test was done as described by [38]. Overnight fasted normal mice were divided into six groups of 5 mice each. Metformin (5 mg/kg) and the four extracts (300 mg/kg) were orally administered as shown in Table 1. After 30 min post the administration, 2 g/kg per body weight of glucose solution was loaded via oral gavage to all the groups. Blood glucose was then determined using a glucometer and recorded after 30 min of treatment (considered as 0 min) and after 30, 60, and 120 min of glucose loading.

2.10 Induction of Experimental Diabetes

Mice were fasted overnight received a single injection of alloxan monohydrate (Sigma Aldrich, USA) at a dose of 150 mg/kg body weight intraperitoneally. The induction of diabetes was confirmed by measuring blood glucose levels of each mouse daily after five days. Mice with glucose levels above 200 mg/dl were considered diabetic based on the method of [39].

2.11 Hypoglycemic Studies

After induction of diabetes, the hypoglycemic experiment was carried on seven groups of five mice each. The animals were grouped the same way as OGTT experiment except for the diabetic control group. Metformin and the extracts were orally administered in their respective dosages daily at 0900 hrs for 21 days using gavage. The FBG level was measured in the 4th, 7^{th}, 14^{th} and 21^{st} days. The relationship between the FBG of the extracts groups versus the metformin, reasonable control and diabetic control groups was then investigated.

Table 1. Grouping of animals forgotten and hypoglycemic studies

Group	Drug	Oral administration in 0.1 ml physiological saline
1	Normal control	This was the usual control group
2	Positive control (Metformin)	Alloxan induced diabetic mice orally administered with 100 mg/kg body weight
3	Diabetic control	This was the diabetic control group
4	*A. lateritia* methanol extract	Alloxan induced diabetic mice received 300 mg/kg body weight
5	*A. lateritia* aqueous extract	Alloxan induced diabetic mice administered with 300 mg/kg body
6	*A. secundiflora* methanol extract	Alloxan induced diabetic mice treated with 300 mg/kg body weight
7	*A. secundiflora* aqueous extracts	Alloxan induced diabetic mice administered with 300 mg/kg body weight

2.12 Blood Glucose Determination

A prick obtained blood on the lateral tail vein and blood glucose levels determined using a glucometer (GlucoPlus^{TM}-Canada) based on the glucose oxidase method.

Percentage change in glycaemia was calculated as follows:-

%BG ={Glucose level at time (0) - Glucose level at time (t)/Glucose level at time zero (0)}X 100

2.13 Biochemical Parameters

Blood was collected at euthanasia through cardiac puncture. The blood was allowed to clot and left for 10 min at room temperature for serum formation. The serum was then separated by centrifugation at 6000 rpm for 10 min and stored at−20°C. Biochemical parameters were determined on the serum specimen using a Reflotron^{R} plus/Sprint chemistry analyser at Biochemistry department of Jomo Kenyatta University of Agriculture and Technology. Liver enzymes- Aspartate aminotransferase (AST), Alanine transferase (ALT) and blood urea were assessed using commercial kits.

2.14 Acute Oral Toxicity of *Aloe lateritia* and *Aloe secundiflora*

Three female mice were used in each step. The mice fasted for 4 hours before the administration. Four fixed doses of 5, 50, 300 and 2000 mg/kg body weight of methanol and aqueous *A. lateritia* and *A. secundiflora* extracts were administered stepwise. Animals were observed individually after dosing: - once during the first 30 minutes and periodically every day for 14 days. Behavioural patterns and signs of toxicity, discharges, diarrhoea and salivation were monitored. Individual change in weight of the mice was determined and recorded weekly. All mice were subjected to gross necropsy [40].

2.15 Statistical Analysis

All data was first stored using Microsoft Excel and expressed as mean +/- 1.96*SEM. Statistical analysis was done by SPSS version 21.0 using Analysis of Variance (ANOVA) and post-ANOVA to compare the means between different treatment groups. Tukey's t-test was then used to determine the significant difference between the groups. All test results were considered significance statistically at P≤ 0.05.

3. RESULTS

3.1 Extraction Yield

The extraction yield of the aqueous extraction was 14 gms and 20 gms for *A. lateritia* and *A. secundiflora* respectively whereas the methanol extraction produced 4.2 gms and five gms of *A. lateritia* and *A. secundiflora* respectively for every removal.

3.2 Phytochemical Screening of *A. secundiflora* and *A. lateritia* Aqueous Extracts

The qualitative investigation of *A. secundiflora* and *A. lateritia* extracts established the presence of various hypoglycemic phytochemicals (Table 2).

A further quantitative analysis determined total phenols (mg GAE/g, flavonoids (mg QE/g) alkaloids (%) and saponins (%) as shown in (Table 3).

Table 2. Qualitative analysis of phytochemicals present in *A. secundiflora* and *A. lateritia* extracts

Phytochemical	*A. secundiflora* methanol	*A. secundiflora* aqueous	*A. lateritia* methanol	*A. lateritia* aqueous
Alkaloids	++	++	++	++
Tannins	+	+	+	+
Carbohydrates	+++	++	++	++
Phenols	+++	++	++	++
Saponins	++	++	++	+
Steroids	++	+	+	+
Anthraquinones	+	+	+	+
Flavonoids and Flavones	++	+	+	+
Aloin	+++	++	+++	+++

+++: Highly present, ++: moderately present, +: low presence

Table 3. Summary of selected phytochemicals in *A. secundiflora* and *A. lateritia* extracts

Phytochemical	*A. lateritia* methanol	*A. lateritia* aqueous	*A. secundiflora* methanol	*A. secundiflora* aqueous
Phenols (mg GAE/g)	15.45 ±0.09	12.11±0.06	54.70±0.05	49.56±0.21
Flavonoids (mg QE/g)	0.21 ±0.19	0.21 0.14	0.43±0.3719	0.32±0.07
Alkaloids (%)	7.78±0.67	9.23±0.78	10.37± 0.64	13.45±0.38
Saponins (%)	5.96±0.1	3.72±0.25	11.10± 0.5	12.69±0.02

The application of GC-MS led to the identification of phytochemical compounds present in *A. lateritia* and *A. secundiflora* established on their peak zones, molecular weight and formula (Tables 4 and 5 respectively).

3.3 Antioxidant Activity of *A. lateritia* and *A. secundiflora* Methanol Extracts

Antioxidant activity determined by DPPH assay showed that *Aloe secundiflora* methanol extracts had a higher maximum percentage inhibition than*A. Patricia*. The increase of the percentage inhibition with the rise in the concentration of both extracts was determined by the growth the scavenging of free radicals created by DPPH (Fig. 1).

3.4 Effects of *Aloe lateritia* and *A. secundiflora* Extracts on Oral Glucose Tolerance Test

Post metformin and extracts administration, there was no significant difference in blood glucose levels in all the groups ($P > 0.05$). However, there was a significant difference in blood glucose in all the groups after glucose loading ($P > 0.05$). This confirmed the induction of hyperglycemia. Analysis between the groups showed *Aloe lateritia* and *A. secundiflora* methanol, and aqueous extracts groups were not significantly different to metformin group at 30 min 60 min and 120 min ($P < 0.05$). A significant difference was however observed at 60 min between the metformin and the healthy control groups ($P < 0.05$). Comparison between 120 min and peak hyperglycemia level (30 min) revealed a significant difference ($P < 0.05$) was achieved in all the treatment groups (Table 6 and Fig. 2).

3.5 Hypoglycemic Effects of *A. lateritia* and *A. secundiflora* Extracts

Aqueous and methanol extracts of these plants proved highly efficient in causing significant hypoglycemic reaction in the experimental diabetic mice.

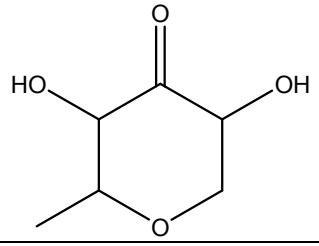
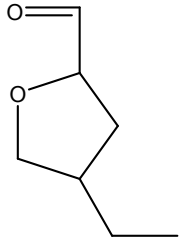
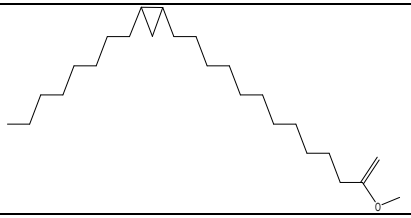
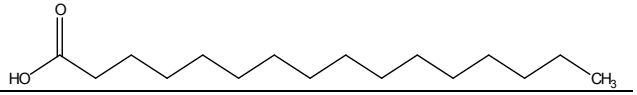
3.5.1 Aloe lateritia

Daily oral administration of *Aloe lateritia* methanol extracts had better blood glucose reducing ability than the aqueous extracts. At day 7, day 14 and day 21 days reduction in blood glucose which was significantly different to the diabetic control ($P<0.05$). Blood glucose changes in day 14 were significantly different from day 0 and day 7 for both extracts. Additionally day 21 changes were significantly different from day 0, day 7 and day 14. At the end of the experiment (Day 21), *A. lateritia* methanol extracts showed no significant difference ($P<0.05$) relative to metformin. The percentage glycemic change in *A. lateritia* methanol and *A. lateritia* aqueous was 37.75% and 35.53% while metformin brought about a reduction of 55.46% (Table 7 and Fig. 3).

3.5.2 Aloe secundiflora

A significant reduction ($P < 0.05$) in the blood glucose levels was realised upon daily oral administration of *A. secundiflora* aqueous and methanol extracts for 21 days. Day 7, day 14 and day 21 days had blood glucose reduction which was significantly different to the diabetic control group ($P<0.05$). Further comparison of the two extracts established that blood glucose changes on day 14 were significantly different from day 0 and day 7. Moreover Day 0, day 7 and day 14 changes were substantially different from Day 21 for both extracts. A corresponding significant ($P<0.05$) reduction in blood glucose was also observed in the treatment groups compared to the diabetic control. No significance difference ($P<0.05$) between the extracts when compared to metformin on day 21. The percentage glycemic change in *A. lateritia* methanol and *A. secundiflora* aqueous was 42.97% and 47.60%. The *A. secundiflora* extracts had a better blood reduction profile than *A. lateritia* extracts. The *A. secundiflora* methanol extracts led to the highest blood glucose reduction among the extracts (Table 7 and Fig. 4).

Table 4. Characterization of *A.lateritia* methanol extracts using GC-MS

Phytochemical	Formula	Molecular weight g/mol	peak	Structure
4H-pyran-4-one, 2-3-dihydro-3,5-dihydroxy-6-methyl (Phenol)	$C_6H_8O_4$	144.13	5.117	
2-Furancarboxaldehyde, 5-(hydroxymethyl)-2-Furaldehyde (Aldehyde)	$C_6H_6O_3$	126.11	8.234	
Cyclopropanedecanoic acid 2-octyl- methyl ester (Fatty acid)	$C_{20}H_{38}O_2$	310.52	14.484	
Palmitic acid (Fatty acid)	$C_{16}H_{32}O_2$	257.42	15.268	

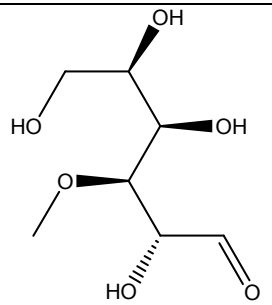
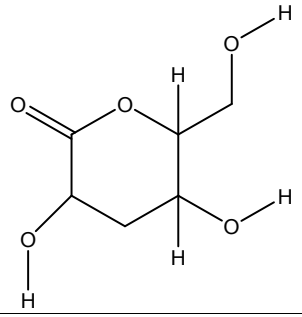
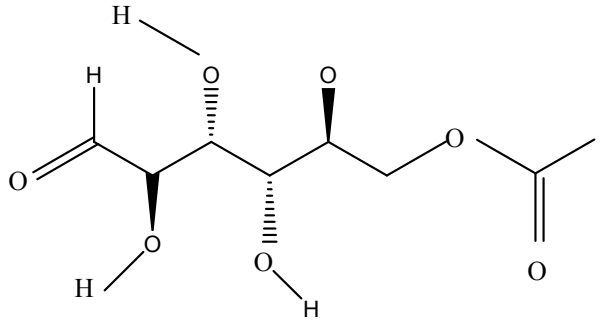
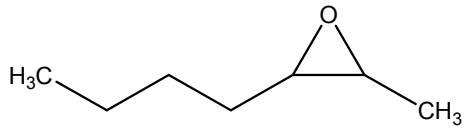
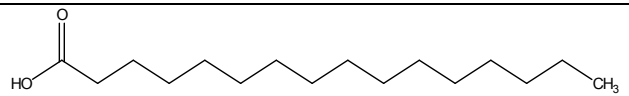
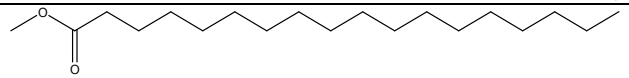
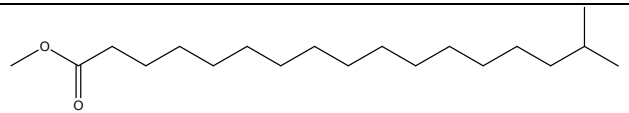
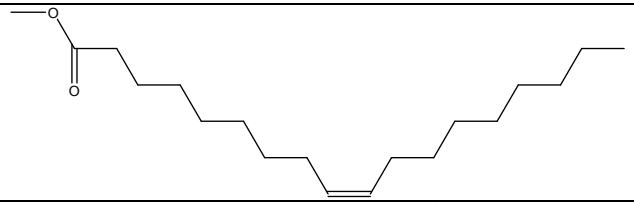
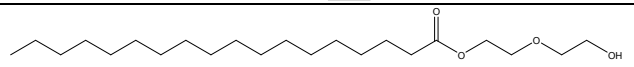
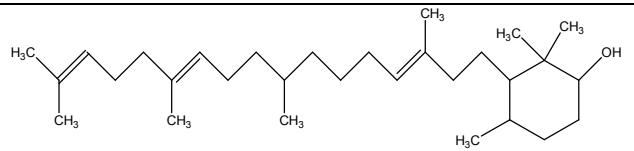
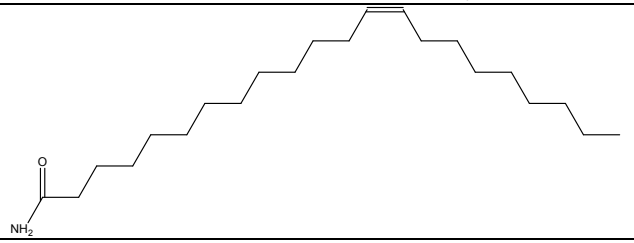
Phytochemical	Formula	Molecular weight g/mol	peak	Structure
3-O-methyl-d-glucose (Carbohydrate)	$C_7H_{14}O_6$	194.18	15.534	
3-Deoxy-d-manoic lactone (Ester)	$C_6H_{10}O_5$	162.14	16.101	

Table 5. Characterization of *A.secundiflora* methanol extracts using GC-MS

Phytochemical	Formula	Molecular Weight/mol	Peak	Structure
6-O-Acetyl -d-mannose (Carbohydrate)	$C_8H_{14}O_7$	222.19	8.284	
Oxirane -2-butyl-3-methyl-cis (Alkane)	$C_7H_{14}O$	114.19	6.150	
Hexadecanoic acid (Fatty acid)	$C_{16}H_{32}O_2$	256.42	14.518	
octadecanoic acid, methyl ester (Fatty acid)			15.234	
heptadecanoic acid:16-methyl-methyl ester (Fatty acid)	$C_{19}H_{38}O_2$	298.50	15.534	

Phytochemical	Formula	Molecular Weight/mol	Peak	Structure
9-octadecanoic acid(z)-9-octadenyl ester, z) oleic acid (Fatty acid)	$C_{19}H_{36}O_2$	296.49	16.101	
Octadecanoic acid, 2(2-hydroxyethoxy)ethyl ester (Fatty acid)	$C_{22}H_{44}O_4$	372.58	16.284	
2,2,4-trimethyl -3-(3,8,12,16) tetramethyl-heptadeca,3,7,11,15-tetraenyl)- cyclohexanol (Isoprenoid)	$C_{30}H_{52}O$	428.73	21.285	
Erucylamide, 13-docosenamide (Fatty acid)	$C_{22}H_{43}NO$	337.58	21.418	

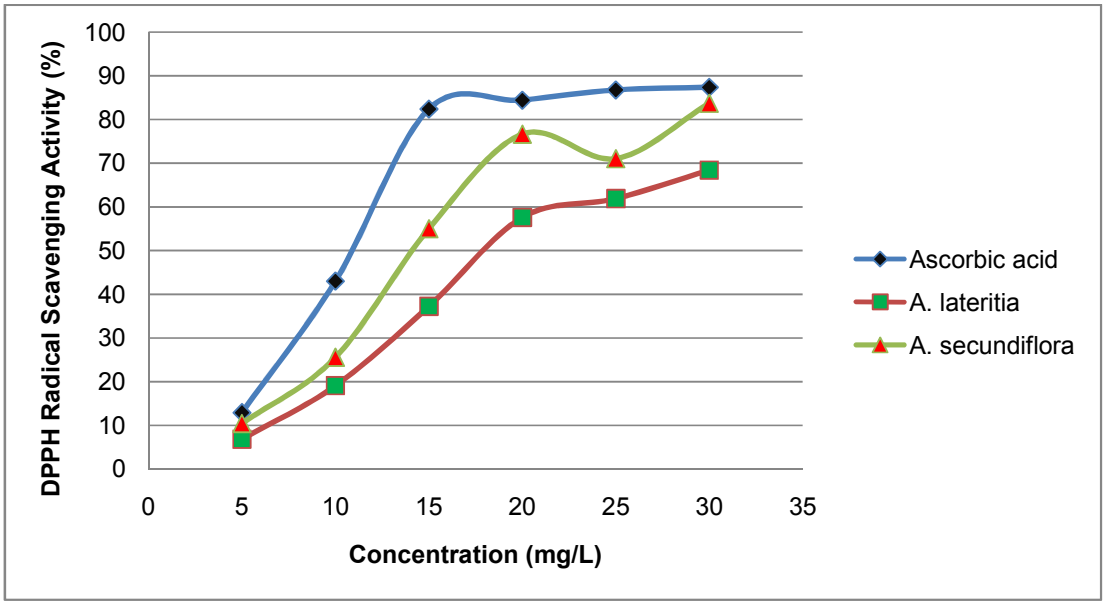


Fig. 1. Antioxidant activity of *A. lateritia* and *A. secundiflora* methanol extracts at different concentrations

Table 6. Effects of *A. lateritia* and *A. secundiflora* extracts on oral glucose tolerance test in mice

Group	Blood glucose level (mg/dl)			
	0 Min	30 Min	60 Min	120 Min
Normal control	101.53±2.37	128.43±4.68^{c3}	119.61±5.90^{c3}	108.01±2.55^{c4}
Metformin	101.25±3.90	110.43±0.72^{d2}	106.17±0.94^{b2}	88.69±0.68^{a4}
AL methanol	98.30±0.77	115.19±0.54^{c4}	110.33±0.68^{c4}	97.37±1.42^{b5}
AL aqueous	101.23±1.51	117.28±1.50^{c1}	112.25±0.49^{c1}	98.32±4.32^{a4}
AS methanol	100.19±0.70	114.20±3.52^{d2}	109.44±2.75^{c2}	91.33±2.53^{a4}
AS aqueous	99.21±3.90	114.36±1.57^{d2}	109.07±2.63^{c2}	95.65±1.62^{a3}

Data expressed as mean ± SEM; n = 5 mice in each group. ANOVA and Tukey's T-test did statistical significance (P ≤ 0.05). Means in respective columns followed by a similar letter are not significantly different; implies in individual rows that do not share a similar number are significantly different. AL: Aloe lateritia, AS: Aloe secundiflora

Table 7. Effects of *A. secundiflora* and *A. lateritia* extracts on fasting blood glucose in alloxan induced Swiss albino mice

Group	Blood glucose level (mg/dl)			
	0 Day	7 Day	14 Day	21 Day
Normal control	96.68±3.58	96.07±5.13	96.20±1.51	96.12±2.76
Metformin	228.29±5.58^{b3}	142.06±9.16^{c2}	124.66±3.81^{b2}	107.56±4.00^{b2}
Diabetic control	217.00±10.29^{a2}	220.62±17.70^{a4}	221.18±9.68^{a5}	225.91±2.35^{a5}
AL methanol	216.38±10.47^{b4}	174.82±7.87^{b3}	148.03±6.13^{c2}	134.31±3.89^{b5}
AL aqueous	208.35±17.65^{b4}	179.06±9.11^{b3}	145.30±4.251^{c2}	133.52±4.53^{c5}
AS methanol	233.15±8.95^{c3}	159.95±8.51^{b2}	129.51±3.58^{b25}	124.89±2.58^{b4}
AS aqueous	224.16±4.76^{c4}	170.34±13.37^{b3}	137.34±4.52^{c2}	128.13±3.06^{b2}

Data expressed as mean ± SEM; n = 5 mice in each group. ANOVA and Tukey's T-test did statistical significance (P < 0.05). Means in respective columns followed by a similar number are not significantly different; implies in individual rows that do not share a similar letter are significantly different. AL: Aloe lateritiaAS: Aloe secundiflora

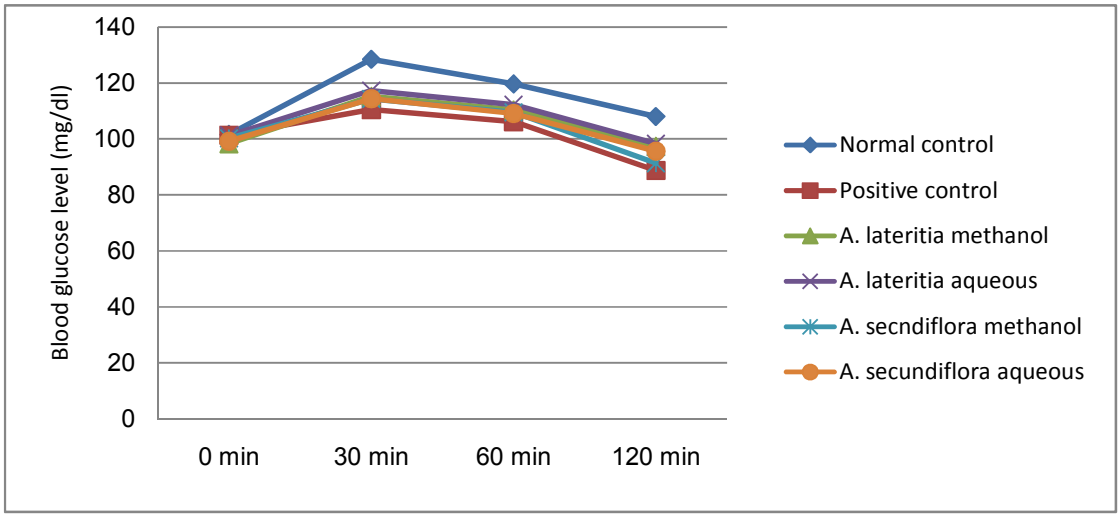


Fig. 2. Effects of metformin and extracts on oral glucose tolerance test in alloxan induced diabetic mice. The arrow shows the point of drug administration.

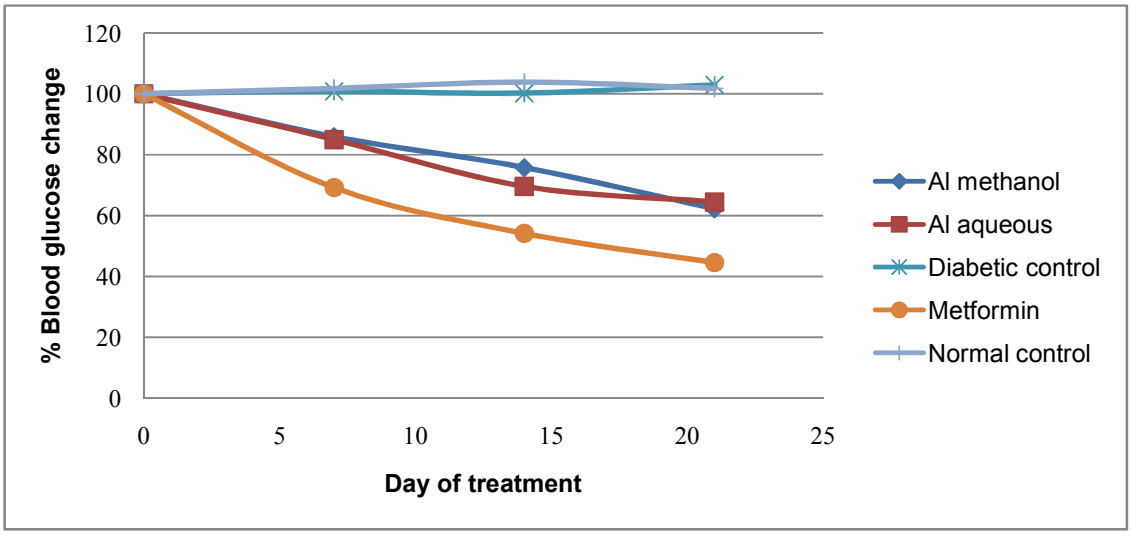


Fig. 3. Mean percentage change in blood glucose levels of methanol and aqueous extracts of *A. lateritia* administered to alloxan induced diabetic Swiss Albino mice. Al: *A. lateritia*

Table 8. Effects of *A. lateritia* and *A. secundiflora* extracts on AST, ALT and Serum urea in serum in diabetic induced mice

Treatment	Biochemical parameter		
	AST	ALT	Serum urea
Normal control	144.58±4.55	36.08±1.00	5.47±032
Metformin	181.16±2.03*	36.56±1.20	5.74±0.57
Diabetic control	200.21±3.62*	36.58±1.28	8.11±0.26
A.L methanol	226.24±3.31*	47.02±1.44*	7.44±0.32
A.L aqueous	206.60±3.86*	36.56±1.97	7.24±0.38
A.S methanol	198.98±2.64*	40.72±2.27*	7.22±0.38
A.S aqueous	192.49±2.31*	57.22±1.38*	7.30±0.41

*Data expressed as mean ± SEM; n = 5 mice in each group. AL: Aloe lateritia AS: Aloe secundiflora. *P < 0.05 is considered significant when the mean of the test group is significantly different from the standard control*

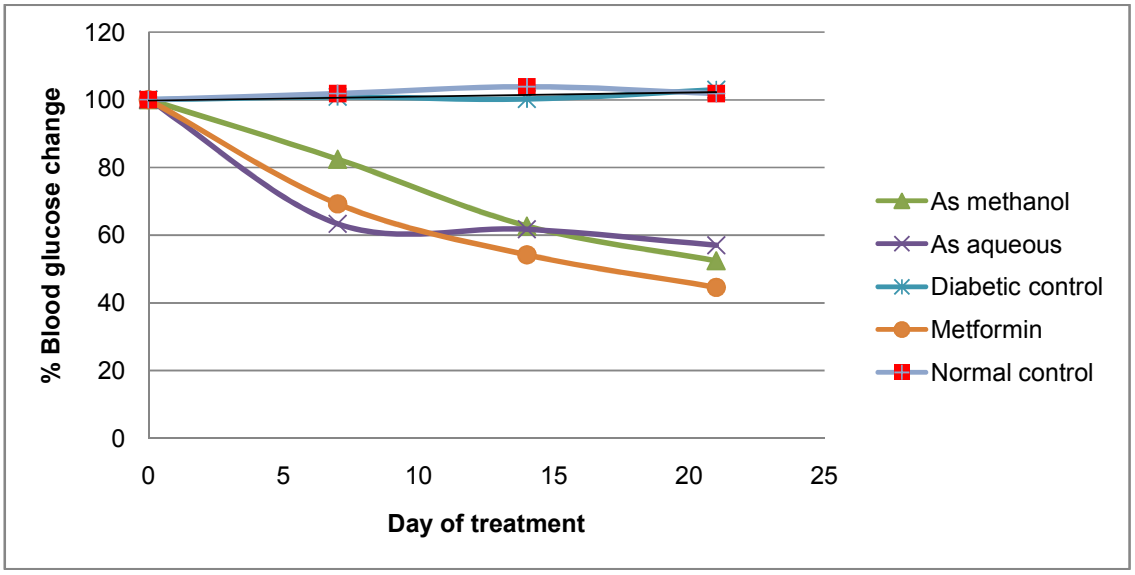


Fig. 4. Mean percentage change in blood glucose levels of methanol and aqueous extracts of *A. secundiflora* administered in alloxan-induced diabetic Swiss Albino mice. As: *A. secundiflora*

3.6 Effects of *A. lateritia* and *A. secundiflora* on Biochemical Parameters in Alloxan-Induced Diabetic Mice

Administration of 300 mg/kg per body weight of *A. lateritia* and *A. secundiflora* to mice for 21 days led to increase in ALT and AST levels when compared to the healthy control group although these values remained within the normal range of ALT (17-77) and AST (54-298) in mice according to Normal clinical chemistry values by Research Animal Resource, University of Minnesota [41]. Levels of Urea across the treatment groups were comparable relative to the standard control (Table 8).

3.7 Acute Oral Toxicity Test of *A. lateritia* and *A. secundiflora* Extracts

There was no behavioural changes, mortality or toxicity signs observed after oral administration of *A. lateritia* and *A. secundiflora* methanol and aqueous extracts up to the dose level of 2000 mg/kg body weight in mice during the 14 day assessment period. Necropsy analysis revealed that no treatment-related pathological changes occurred in the major organs. At the end of the experiment, the skin and the orifices, serosal surfaces, lungs, livers, spleens, kidneys and uterine regions of the mice in all the dosages had standard size, color and state of hydration.

4. DISCUSSION

Screening of *A. lateritia* and *A. secundiflora* extracts showed that the methanol extracts were richer in phenols, saponins, alkaloids, flavonoids, tannins and carbohydrates than the aqueous extracts. These phytochemicals have been shown to be excellent antidiabetic metabolites [42]. The radical scavenging activity by DPPH assay indicates that the extracts of both plants contain antioxidant compounds. Antioxidant activity has β-cell protective effects which leads to improved insulin secretion and lowering of blood glucose hence the correction of hyperglycaemia [43]. Various synthetic drugs acting as antioxidants to protect against oxidative damage have adverse side effects. Consumption of traditional medicines and food supplements with natural antioxidants can be a better alternative [44]. Furthermore, studies have established that the majority of phytochemicals possessing antidiabetic activity such as phenolic acids and indoles have proven antioxidant capacity [21]. The hypoglycemic action of *A. lateritia* and *A. secundiflora* can, therefore, be associated with their antioxidant activity. Oral glucose tolerance test usually checks the maintenance of blood glucose homeostasis after glucose overload. A critical application of this test is in detection of more delicate changes in insulin resistance development during management of diabetes [45].

The resulting lowering effect of blood glucose levels by crude aqueous and methanol extracts of *A. lateritia* and *A. secundiflora* in the experimental Swiss albino mice demonstrates that these plants have hypoglycemic activity. *Aloe secundiflora* extracts at a dose of 300 mg/kg body weight was the most active extract. However, their percentage glycemic change was lower than standard drug metformin. The ability of these plants to lower blood glucose is in agreement with other findings on other *Aloe* species. A dosage of 300 mg/kg body weight of *A. vera, A. ferox* and *A. greatheadii* led to significant clinical improvement of diabetes in experimental models which was comparable to current conventional drugs [43] and [17]. Studies on *A. vera* established that administration of the *Aloe* gel extracts and glibenclamide significantly reduced changes caused by streptozotocin in the kidney tissues of diabetic-induced rats treated with the *Aloe* leaf extracts. This combination brought about a decrease in the levels of serum urea and creatinine levels when compared to the diabetic control [46]. Beppu et al. [15] revealed that the administration of basal diets supplemented with components of *A. arborescens* Miller whole leaf to streptozotocin-induced diabetic mice led to a significant decrease in blood glucose levels. Kareru et al. [47] determined that oral administration of crude methanol extracts of *A. camper* exhibited blood glucose lowering effects in experimental diabetic rats. This was attributed to the presence of hypoglycemic bioactive molecules like flavonoids, alkaloids, and saponins present in the extracts. *Aloe vera* and *A. arborescent* were also determined to have antidiabetic activity in *in vivo* and in human trials by increasing insulin secretion of the β cells of the pancreas and by preventing oxidative stress and inhibiting the related pancreatic β-cell destruction [43]. The results of organ function test revealed that the plant extracts of *A. lateritia* and *A. secundiflora* did not significantly alter the liver and kidney biomarkers. The increased levels of serum AST and ALT above normal levels is a shared pointer of liver dysfunction which occurs as a consequence of deposition of lipids in the cytoplasm of hepatocytes and also due to necrosis in hepatocellular and skeletal muscles during diabetes [48]. The renal function test for blood urea showed that there was no significant change in the levels in the *Aloe* extracts and vehicle treated groups when compared to the healthy control group levels. Damage at subcellular level in various organs like liver, kidney, spleen, heart and pancreas usually leads to the varying degrees of ALT and AST [49]. The results of acute oral toxicity test of *A. secundiflora* and *A. lateritia* extracts indicate that a single oral dosage of extracts resulted in an LD_{50} of greater than 2000 mg/kg per body weight. Female mice were used in this study because females are usually more sensitive than males in LD_{50} analysis [40]. This outcome is similar to that observed in *A. ferox* resin which was shown not to cause signs of acute toxicity or mortality, and its LD_{50} predicted to be potentially higher than 5.0 g/kg [50].

5. CONCLUSION AND RECOMMENDATION

The present study present determined that *A. lateritia* and *A. secundiflora* extracts exhibited hypoglycemic activity on experimental alloxan induced diabetic Swiss Albino mice when orally administered orally with therapeutic dose of 300 mg/kg. This outcome may be owed to the availability of phytochemicals like phenols, saponins, alkaloids, anthraquinones, flavonoids, sterols, aloin and tannins which have been shown to act separately or synergistically to ameliorate hyperglycemic. Nevertheless, all-inclusive phytochemical and pharmacological analysis is warranted to identify specific bioactive compounds responsible for hypoglycemic activity and potentially deduce their mode of mechanism.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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