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Antidiabetic activity of methanol extract and fractions of *Costus speciosus* leaves in normal and alloxan induced diabetic rats

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Abstract

Introduction: *Costus speciosus* is a common plant used for the treatment of diabetes mellitus and various other ailments in traditional medicine in Sri Lanka. The present study was conducted to investigate the hypoglycemic effect of the leaves of *C. speciosus* in both normal and alloxan induced male Wistar diabetic rat models. **Methods:** A methanolic extract of the leaves was produced and used for the preliminary phytochemicals screening. Methanol extract was further partitioned with different solvents (n-hexane, chloroform, ethyl acetate and n-butanol) according to their polarity. Male Wistar rats weighing 150-220 g were randomly divided during the test. *In vivo* antidiabetic activity of partitioned fractions of *C. speciosus* leaves was performed in normal rats and alloxan induced NIDDM rats. Standard t-test was used to determine statistical significance. **Results:** A glucose tolerance test with normal rats indicated that peak levels of blood glucose were reached in 90 minutes after the glucose load. It is noteworthy that the test group recorded a significantly ($p < 0.05$) lower blood glucose level at 90 minutes, indicating that the 80 % methanol extract exerted an overall hypoglycemic effect with normal rats at 90 minutes, despite being challenged with glucose load. The same dose showed an improvement in the glucose tolerance of alloxan-induced diabetic rats, reducing the blood glucose at 90 minutes by 60 % compared with control. These effects were found to be comparable with the effect of the synthetic drug glipizide at a dose of 20 mg/kg. In alloxan-induced diabetic rats, long term administration of the 80% methanolic extract of *C. speciosus* leaves daily for 6 weeks resulted in a significant lowering of fasting and postprandial serum glucose when compared to diabetic control rats. **Conclusion:** Diabetic rats group treated with *C. speciosus* extract displayed significantly ($p<0.05$) decreased blood glucose level compared to the control group.

Keywords: Alloxan, *Costus speciosus,* diabetes mellitus, hypoglycemia, anti-hyperglycemia

INTRODUCTION

Diabetes mellitus is a carbohydrate-metabolic disease associated with elevated blood glucose levels. The disease is distinguished by either a defect in insulin action or, in some cases, a lack of insulin in the body. Additionally, it exhibits deficiencies in total carbohydrate metabolism that interfere with homeostasis and the metabolism of fat and protein [1]. Due to insulin deficiency, the cells and tissues of the body are unable to absorb sufficient glucose from the blood, resulting in higher glucose levels in the plasma than its normal plasma glucose values (hyperglycemia). Organs include the kidneys, liver, eyes, nerves, heart, and blood vessels may suffer long-term damage if the hyperglycemia lasts for a long time [2].

Asia shows the greatest prevalence of diabetes mellitus (DM), while the incidence is increasing drastically. Recent estimates indicated that 171 million people in the world were suffering from diabetes in the year 2000, and this is projected to be 366 million by 2030 [3]. When the prevalence of diabetes in Sri Lanka is concerned, one-quarter of the affected population lives mostly in urban areas [4]. Between 2005 and 2006, a nationwide cross-sectional study was conducted to assess the province- and ethnic-specific prevalence of diabetes among Sri Lankan adults. This study was shown that the provincial and ethnic distribution of diabetes closely resembled that of obesity (waist circumference more than the basal metabolic index-BMI) and the income level in the respective provinces and ethnic groups. It also shown the physical activity level had an inverse relationship in Sri Lanka [5,6].

Investigators have reported that an estimated 80–85% of the population in both developing and some developed countries rely on traditional medicine [7]. Plant secondary metabolites are frequently associated with the bioactivity of plants that have been investigated by previous investigators. The isolation of chemical compounds or fractions with optimum therapeutic activity with less toxicity is the intent of modern research on phytochemistry and ethanopharmacology. Diabetes mellitus is one of the diseases in which medicinal plants have been widely used, particularly in the Asian region. There are many side effects of insulin therapy and other oral hypoglycaemic agents that necessitate the use of more effective and safer anti-diabetic drugs. For example, long-term use of Metformin causes diarrhoea, nausea, weakness, indigestion, abdominal discomfort, and headache [8]. Plant-based drugs are considered to be less toxic and free from side effects than synthetic ones. As a result, they serve an important role in alternative medicine.

Costus speciosus (Koenig), commonly known as "crepe ginger" or "spiral flag" in English, "thebu" in Sinhala, and "kostum" in Tamil, is a plant widely used in complementary medicine [9]. The plant is widely distributed in Asia and other tropical countries like Sri Lanka, India, Nepal, Pakistan, Taiwan, Malaysia, and China. In Sri Lanka, it occurs mostly in the wet zone covering the Western, Southern, and Sabaragamuwa provinces. *C. speciosus* (Koenig) is a common starting material in the commercial manufacture of steroidal hormones. Burning sensation, flatulence, constipation, helminthiasis, leprosy, skin diseases, fever, hiccup, asthma, bronchitis, inflammation, and anaemia are some of the Ayurvedic conditions that the rhizomes can help with. It is also used to make sexual hormones and contraceptives [10]. According to Eliza et al. 2009 [11], eramanthin extracted from *C. specious* acts primarily by stimulating insulin release from pancreatic β cells.

Animal experimental models are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of type 2 diabetes and for testing various therapeutic agents. The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals, dietary or surgical manipulations, or by a combination thereof. In recent years, a large number of new genetically modified animals, including transgenic, generalized, and tissue-specific modified mice, have been engineered for the study of diabetes [12]. Animals exhibiting a syndrome of insulin resistance and type 2 diabetes with characteristics similar to humans comprise a wide range of species with genetic, experimental, or nutritional causation [13].

The primary purpose of this research is to evaluate the hypoglycemic efficacy of methanolic extract of *C. speciosus* plant leaves and to use fractions utilising biochemical and enzymatic parameters of several rat models and/or in-vitro bioassays for the treatment of hyperglycemia. Additionally, a detailed search of the activity profile of substances performed by applying *in-vitro* bioassays, insulin indexes for the correction of hyperglycemia, and biochemical and enzymatic parameters of several rat models.

MATERIALS AND METHODS

Materials

Sodium citrate, sodium chloride, methanol, n-hexane, ethyl acetate, n-butanol, acetone, conc. hydrochloric acid, conc. sulphuric acid, basic bismuth sub nitrate, glacial acetic acid, chloroform, calcium hydroxide, acetic anhydride, ferric chloride, ferric sulphate, lead acetate, sodium hydroxide, formaldehyde, anesthetic ether, potassium hydroxide, benzene, ammonia solution, sodium bicarbonate, bromocresol green, sodium phosphate, citric acid, anhydrous sodium sulphate and p-anisaldehyde were purchased from BDH Laboratory Supplies (India).

Male Wistar rats, aged 8-10 weeks (150-220 g), were obtained from the laboratory animal centre, Medical Research Institute, Ministry of Health, Colombo, Sri Lanka. The rats were housed two per cage in a room with a 12-h light and 12-h dark and an ambient temperature of 25-28 ºC in an animal room at Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka, with lighting from 0600 to 1800 h and maintained on standard pelleted diet, with water and libitum.

Extraction of *C. speciosus* leaves in 80 % methanol

The leaves of *C. speciosus* were air dried for 48 hours and ground using a mechanical grinder to a coarse powder before extraction. The extracts for the preliminary investigation were prepared by the reflux extraction method. For one hour, 200 g of the powdered plant material were refluxed in 80% methanol. Later, methanol extract were filtered and dried under vacuum (Büchi rotary evaporator, West Germany) at 40 °C, and the yield was recorded. The percentage yields were reported, and the dried extract was labelled and preserved under nitrogen in a refrigerator at 4 °C till use in the subsequent experiments.

Bioactivity guided solvent partitioning of 80% methanol extract of *C. speciosus*

Dried extracts of 80% methanol from *C. speciosus* leaves (1 g each) were dissolved in 100 mL of distilled water. The solvents for partitioning were chosen on the basis of their polarity, boiling points, and ability to evaporate, and represented a polarity range from non-polar to polar. The solvents selected were n-hexane, chloroform, ethyl acetate and n-butanol. 80% methanol extract of the respective plant was suspended in distilled water and partitioned successively (5 x 20 mL) with each solvent. The resultant extracts were labelled as HF, CF, EF, and BF fractions, respectively, and dried under vacuum at 40 °C. The yield was determined, placed in a desiccator overnight, and preserved under nitrogen in a refrigerator at 4 °C in tightly sealed, dark glass containers (Figure 1).

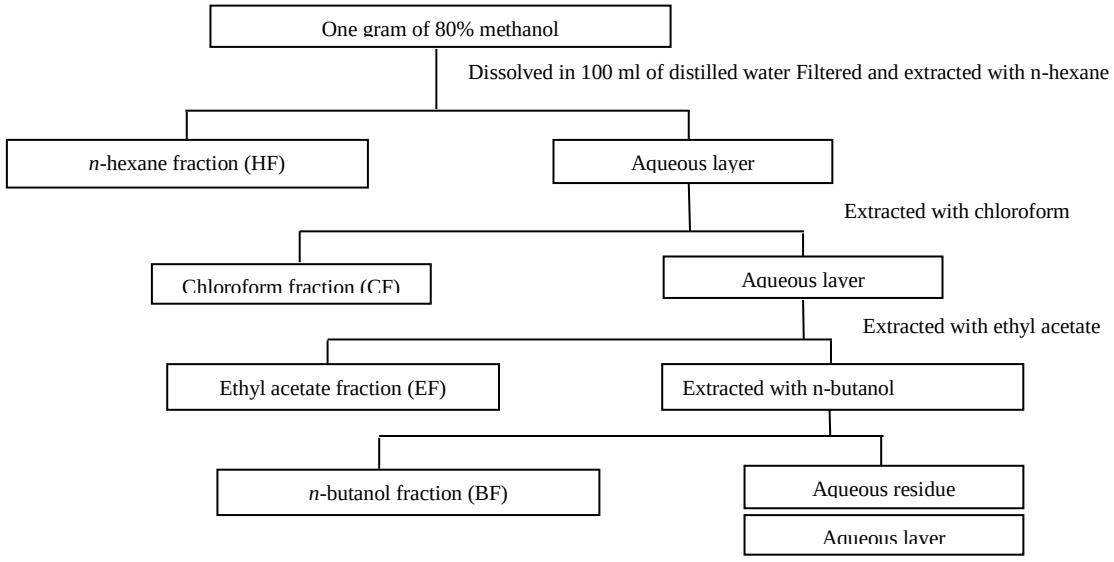

Figure 1: Procedure of the solvent partitioning of 80% methanol extract of *C. speciosus* leaves

Preliminary phytochemical screening of 80% methanolic extract of *C. speciosus* leaves

A standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones using standard procedures [14-17].

Test for alkaloids

Preliminary test

A 100 mg of an alcoholic extract was dissolved in a dilute hydrochloric acid solution and clarified by filtration. The filtrate was tested with Dragendroff's and Mayer's reagents. The treated solutions were observed for precipitation.

Confirmatory test

Five grams of the alcoholic extract were treated with a 40% calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10.0 mL portions of chloroform. Chloroform extracts were combined and concentrated in vacuum to about 5.0 mL. Chloroform extract was then spotted on thin-layer plates. The solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatograms, which were detected by spraying the chromatograms with freshly prepared Dragendroff's spray reagent.

Test for steroidal compounds

Salkowski's test

Chloroform 2.0 mL was used to dissolve 0.5 g of alcoholic extract in a test tube. Concentrated sulfuric acid was carefully added to the wall of the test tube to form a lower layer.

Lieberman's test

Acetic anhydride, 2.0 mL was used to dissolve 0.5 g of alcoholic extract in a test tube and cooled well in an ice-bath. Concentrated sulfuric acid was then carefully added.

Test for phenolic compounds

Test A

Three drops of a freshly prepared mixture of 1.0 mL of 1% ferric chloride and 1.0 mL of potassium ferrocyanide were added to a filtered solution of 2.0 mL aqueous macerate.

Teat B

The dried alcoholic extract (100 mg) was dissolved in water. A few crystals of ferric sulfate were added to the mixture.

Flavonoids

Test for free flavonoids

Ethyl acetate, 5.0 mL was added to a solution of 0.5 g of the extract in water. The mixture was shaken, allowed to settle and then inspected.

Lead acetate test

To a solution of 0.5 g of the extract in water about 1.0 mL of a 10% lead acetate solution was added.

Reaction with sodium hydroxide

A diluted sodium hydroxide solution was added to a solution of 0.5 g of the extract in water. The colour change was examined.

Test for saponins

Froth test

Distilled water 10.0 mL were used to dissolve 0.5 g of the alcoholic extract in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds, and allowed to stand in a vertical position, and observed over a 30 minutes period of time.

Test for tannins

Ferric chloride test

A portion of the alcoholic extract was dissolved in water. The solution was clarified by filtration. 10% ferric chloride solution was added to the clear filtrate.

Formaldehyde test

Formaldehyde 3 drops and diluted hydrochloric acid 6 drops were added to a solution of 0.5 g of the extract in 5.0 mL water. The resulting mixture was heated to boiling for 1 minute and cooled. The precipitate that formed (if any) was washed successively with hot water, warm alcohol, and warm 5% potassium hydroxide.

Test for phlobatannins

An aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid. The color change was examined.

Test for anthraquinones

Test for free anthraquinones (Borntrager's test)

The hydro-alcoholic extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 mL of benzene and filtered. To the filtrate 5.0 mL of a 10% ammonia solution was added and the mixture was shaken.

Test for O-anthraquinone glycosides (Modified Borntrager's test)

Plant extract weighing 5.0 g was boiled with 10 mL of 5% sulphuric acid for 1 hour and filtered while hot. The filtrate was shaken with 5.0 mL of benzene. The benzene layer was separated and half its own volume of 10% ammonia solution was added.

Evaluation of hypoglycemic activity of 80% methanol extract of *C. speciosus* leaves in glucose loaded normal Wistar rats

Eighteen rats were used in this study and were divided into three groups. The 80% methanol extract of *C. speciosus* was investigated for their hypoglycemic activity on normoglycemic rats. The dried extracts were dissolved in distilled water. A glucose load 2.5 g/kg was given and the extracts were administered after 30 minutes at a dose of 20 mg/kg. Distilled water 1.0 mL/kg and glipizide 20 mg/kg were administered to group I and group III respectively, after the first 30 minutes of the glucose load. The rats were grouped and labelled as below.

Group I – Control (distilled water, 1.0 mL/kg)

Group II – Rats treated 20 mg/kg of *C. speciosus* (80% methanol)

Group III – Rats treated with 20 mg/kg of glipizide

Diabetes induction in Wistar rats

Male albino Wistar rats (10 weeks old) were injected with a single intraperitoneal dose of 150 mg/kg b.w. of alloxan monohydrate (ALX) freshly prepared in normal saline after a 24-hour fast and baseline plasma glucose level check. Thirty minutes after the injection, the animals were given 10% glucose solution, and then 5% glucose solution till the next day. The plasma glucose level was measured 72 hours after

injection using the Accu-Check active glucometer. Diabetic rats with plasma glucose levels of 200 mg/dL or above were crosschecked two weeks later before being included in the research.

***In vivo* detail activity profile of partitioned fractions of *C. speciosus* leaves in normal rats and alloxan induced NIDDM rats**

The effect of single dose on hypoglycemic activity of HF, EF, CF and BF fractions of 80% methanolic extract on normal Wistar rats

Thirty rats, divided into five groups (n = 6), were used in this study. The dried partitioned n-hexane (HF), chloroform (CF), ethyl acetate (EF) and n-butanol (BF) fractions were dissolved in distilled water. A glucose load 2.5 g/kg was given and the fractions were administered after 30 minutes at a dose of 20 mg/kg. Distilled water (1.0 mL/kg) was administered to group I, after the first 30 minutes of the glucose load. This served as the control group. The rats were grouped and labelled as below.

Group I – Control (distilled water, 1.0 ml/kg)

Group II – Rats treated with 20 mg/kg of HF fraction

Group III – Rats treated with 20 mg/kg of CF fraction

Group IV– Rats treated with 20 mg/kg of EF fraction

Group V – Rats treated with 20 mg/kg of BF fraction

The effect of antidiabetic effect of HF, EF, CF and BF fractions of 80% methanolic extract in alloxan induced NIDDM Wistar rats after 42 days

In the experiment, a total of thirty alloxan-treated diabetic rats were used. The rats were divided into five groups (n=6). The rats were grouped and labelled as below.

Group I consisted of alloxan-treated diabetic rats administered with distilled water (1.0 mL/kg)

Group II – Alloxan-treated diabetic rats administered with 20 mg/kg of HF fraction

Group III – Alloxan-treated diabetic rats administered with 20 mg/kg of CF fraction

Group IV– Alloxan-treated diabetic rats administered with 20 mg/kg of EF fraction

Group V– Alloxan-treated diabetic rats administered with 20 mg/kg of BF fraction

A single dose of solution was administered every day orally using an intragastric tube for 42 days. After 42 days of administration, animals were decapitated, blood was collected, and serum was separated immediately.

Collection of blood and determination of blood glucose levels

Blood samples were collected from the tip of the tail of the rats before and after 90 minutes of the administration of extracts/ fractions/ drugs. A Bionime glucometer, model GM300 (Bionime Corporation, Bionime GmbH, Switzerland), was used to measure blood glucose levels.

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

NIDDM [18] was induced in overnight fasted adult male rats weighing 150–200 g by a single intraperitoneal injection of 150 mg/kg alloxan monohydrate (Sigma Aldrich, UK) dissolved in normal saline administered to each rat according to the body weight. The elevated blood glucose levels determined at 72 hours confirmed hyperglycemia. Animals with a blood glucose level greater than 250 mg/dl were considered diabetic. Rats found with permanent NIDDM were used for the antidiabetic study. This model has been used in earlier studies to induce type II diabetes in rats [19]. Glipizide (20 mg/kg) was used as the standard drug.

Statistical analysis

The results are shown as means ± SEM. The statistical methods used to analyse the data in this study were unpaired. Student's t-test (two-tailed) and two-way analysis of variance (ANOVA) using the MS-Excel software program. P values less than 0.05 were considered statistically significant.

RESULTS

Extraction of *C. speciosus* in 80% methanol

The percentage yield of *C. speciosus* leaves extracted using 80 % methanol is 0.038 %. *C. speciosus* has the highest extractable value for methanol-soluble material.

Preliminary phytochemical screening of an 80% methanol extract of *C. speciosus* leaves

Hydro-alcoholic solvents are being scientifically proven by previous investigators as the best solvent to extract maximum phytochemicals. The 80% methanolic extract used in this study should contain both polar and non-polar phytochemicals. Table 1 shows the presence and absence of the phytochemicals in the 80% methanolic extracts [20].

Table 1: Preliminary screening of phytochemical constituents of 80% methanol extract of *C. speciosus* leaves

SR	Plant metabolite	Presence/absence of phytochemicals	Observations
I	Alkaloids		
	Preliminary test	+	Precipitation observed
	Confirmatory TLC	+	An orange or dark colored spots against a pale yellow background was confirmatory evidence for presence of alkaloids.
II	Test for steroidal compounds		
	Salkowski's test	++	A reddish brown colour at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).
	Lieberman's test	++	A colour change from purple to blue to green indicated the presence of a steroid nucleus.
III	Test for phenolic compounds		
	Ferric chloride test	-	No colour change observed
	Ferric sulfate test	-	No colour change observed
IV	Flavonoids		
	Test for free flavonoids	-	No yellow colour observed in the organic layer
	Lead acetate test	-	No precipitate observed
	Reaction with sodium hydroxide		No colour change observed
V	Test for saponins		
	Froth test	++	"honey comb" froth above the surface of liquid persisted after 30 min.
V	Test for tannins		
	Ferric chloride test	-	No colour change observed
	Formaldehyde test	-	No precipitate observed
VII	Test for Anthraquinones		
	Test for free anthraquinones (Borntrager's test)	++	Red colour observed in ammonia layer
	Test for O-anthraquinone glycosides (Modified Borntrager's test)	-	No colour change observed

– Not detected + Present in low concentration ++ Present in moderate concentration +++ Present in high concentration

Effect of hypoglycemic activity of 80% methanolic extracts of *C. speciosus* leaves in glucose loaded normal Wistar rats

C. speciosus leaves were investigated to assess the hypoglycemic activity of methanol-soluble extracts. Firstly, an 80% methanol extracts was assayed on glucose loaded (2.5 g/kg) fasted, nomoglycemic rats. Plant extract was tested for its hypoglycemic activity at a dose of 20 mg/kg body weight. Hypoglycemic activity produced by 80% methanol extract of *C. speciosus* produced 61.1% hypoglycemia.

Table 2: Hypoglycemic activity of methanol soluble extracts of *C. speciosus* leaves in normal Wistar rats

Group	*Blood glucose levels mg/dl*	
	Control/standard	*C. speciosus*
Distilled water	150.6 ± 3.2	-
80% methanol extract (20 mg/kg)	-	58.6 ± 2.7 [b] (61.1% [a])
Glipizide (20 mg/kg)	100.1 ± 15.1 [b] (29.6% [a])	

Number of rats per group = 6 each value is Mean ± SEM for six rats
[a] % reduction when compared to normal control at different time intervals
[b] P < 0.05 by comparison with normal control

Glipizide (29.6%) showed lesser activity than the plant extracts under investigation. However, glipizide (20 mg/kg) significantly (P<0.05) reduced blood glucose levels after 90 minutes of administration when compared to control rats (Table 2). The results of the study showed that methanol-soluble extracts of all plants have greater hypoglycemic activity compared to glipizide.

In vivo detail activity profile of partitioned fractions of *C. speciosus* leaves in normal rats and alloxan induced NIDDM rats

The selected *C. spesiosus* on further investigation have shown 80% methanolic extracts are more hypoglycemic. On solvent partitioning fractions of 80% methanolic extracts, the detailed activity profile of *C. speciosus* was studied. This was done to provide scientific evidence of the activity of methanol-soluble constituents in plants, which are consumed as aqueous infusions or decoctions. At a dose of 20 mg/kg, all fractions of 80% methanolic extracts were tested for hypoglycemic activity on initially normoglycemic rats, followed by an alloxan-induced antidiabetic rat model with the evaluation of enzymatic parameters on the correlation of hypoglycemia.

The effect of single dose on hypoglycemic activity of HF, EF, CF and BF fractions of 80% methanolic extract on normal Wistar rats

The results obtained from the *in vivo* assay on hypoglycemic activity indicated that the *n*-butanol fractions of *C. speciosus* (40.5%) had a greater potential for lowering blood glucose levels in normal rats. Consequently *n*-hexane and ethyl acetate were showed 30.0% and 37.5% activity at a dose of 20 mg/kg (Table 3).

Table 3: Hypoglycemic activity of partitioning fractions of *C. speciosus* in normal rats

Group/treatment	Blood glucose levels mg/dl	
	Control	*C. speciosus*
Distilled water	142.2 ± 2.1	-
n-hexane fraction	-	99.5 ± 3.1 [b] (30.0% [a])
Chloroform fraction	-	113.6 ± 4.6 [b] (20.1% [a])
Ethyl acetate fraction	-	88.9 ± 2.7 [b] (37.5% [a])
n-butanol fraction	-	77.1 ± 2.6 [b] (45.4% [a])

Number of rats per group = 6 each value is Mean ± SEM for six rats
[a] % reduction when compared to 20 mg/kg dose
[b] P < 0.05 by comparison with the control (distilled water)

Observation of the hypoglycemic activity of solvent partitioned fractions indicated that the different fractions had varied hypoglycemic activity. There were, however, approximate similarities observed in the activities of non-polar and polar fractions of an 80% methanolic soluble extract.[20] This study also revealed that chloroform fractions of all plants produced significantly (p<0.5) lower activity when compared to control rats fed with distilled water. However, all partitioning fractionation produced lower hypoglycemic activates than that of original 80% methanol extracts of respected plants under investigation.

Antidiabetic effect of HF, EF, CF and BF fractions of 80% methanolic extract in alloxan induced NIDDM Wistar rats after 42 days

Seven weeks after alloxan induction of diabetes in male Witar rats, the fasting blood glucose levels were measured. On day zero, the hyperglycemic rats (blood glucose >250 mg/dl) were divided into three groups (each with 6 rats). The initial fasting blood glucose level and the blood glucose level at the end of the 42 day treatment were measured. Distilled water and glipizide (20 mg/kg) were administered orally once a day to the control group for seven weeks, serving as positive control groups. The treatment groups were administered *n*-hexane, ethyl acetate and n-butanol fractions orally, once a day, for seven weeks.

As shown in Table 4 , the daily pre-treatment of fractions (20 mg/kg) once a day for 42 days in alloxan -diabetic rats showed a significant reduction in blood glucose level when compared with the diabetic control (P<0.05) in a dose dependent manner. When compared to the initial value, similar anti-diabetic activity was observed (P 0.05).

At the 0^{th} and 42^{nd} days of administration, plasma glucose levels were measured in experimental rats. alloxan -treated diabetic rats showed a significant increase in the levels of blood glucose (> 250 mg/dl) when compared to normal rats. All plant fraction studies showed antidiabetic activity in dose dependent manner. It was observed that *C. speciosus*, (21.7%) with the ethyl acetate fraction and the highest activity (45.7%) *n*-butanol fraction at a dose of 20 mg/kg when compared with the diabetic control rats and the initial value. Oral administration of the *n*-butanol fraction of the plant at 20 mg/kg showed a highly significant antidiabetic effect (Table 4).

Table 4: The effect of pretreatment fractions of *n*-hexane, ethyl acetate and *n*-butanol fractions of *C.speciosus* on alloxan-induced NIDDM rats

Pretreatment	Control/standard		*C.speciosus*
	Initial	42-day treated	42-day treated
Diabetic + distilled water 1.0 mL/kg	76.1 ± 3.9	257.1 ± 3.5	
Diabetic + n-hexane	261.6 ± 5.2		91.0 ± 2.0
20 mg/kg		-	(31.2%)
Diabetic + chloroform	249.3 ± 7.8		68.6 ± 1.2
20 mg/kg		-	(33.4 %)
Diabetic + Ethyl acetate	255.4 ± 9.2		87.1 ± 1.2
20 mg/kg		-	(41.3%)
Diabetic + n-butanol	249.4 ± 11.4		76.6 ± 1.6
20 mg/kg		-	(45.7%)

Number of rats per group = 6 each value is Mean ± SEM for six rats
[a] % reduction when compared to control groups (normal/diabetic)
[b] P < 0.05 by comparison with the control (distilled water)

After 42 days of administration, the effect of n-butanol fraction at a dose of 20 mg/kg showed increased hypoglycemic activity compared to other fractions. Therefore, it's recommended as the most active fraction of the plant *C. speciosus* from all tested partitioned extractions.

DISCUSSION

The hypoglycemic potential of methanol-soluble extracts from *C. speciosus* leaves was examined. First, rats that had been fasted and given a 2.5 g/kg dose of glucose were tested with an extract of 80% methanol. At a dose of 20 mg/kg body weight, plant extract was examined for its hypoglycemic potential. Hypoglycemic activity produced by 80% methanol extract of *C. speciosus* produced 61.1% hypoglycemia. Glipizide (29.6%) showed lesser activity than the plant extracts under investigation. However, glipizide (20 mg/kg) significantly (P<0.05) reduced blood glucose levels after 90 minutes of administration when compared to control rats (Table 2). The results of the study showed that methanol-soluble extracts of all plants have greater hypoglycemic activity compared to glipizide.

The hypoglycemic activity of the solvent-partitioned fractions was observed, and the results showed that the hypoglycemic activity of the various fractions varied. However, approximate commonalities between the polar and non-polar fraction of an 80% methanolic soluble extract's activities were found [20]. This study also revealed that chloroform fractions of all plants produced significantly (p<0.5) lower activity when compared to control rats fed with distilled water. However, all partitioning and fractionation produced lower hypoglycemic activations than the initial 80% methanol extracts of the studied respectable plants. As shown in Table 4, the daily pre-treatment with fractions (20 mg/kg) given once daily for 42 days in alloxan-diabetic rats considerably decreased blood glucose levels as compared to the diabetic control group (P<0.05).

At the 0th and 42nd days of administration, plasma glucose levels were measured in experimental rats. When compared to control rats, diabetic rats treated with alloxan had blood glucose levels that were much higher (> 250 mg/dl). All plant fraction experiments revealed dose-dependent antidiabetic efficacy. When compared to the diabetic control rats and the initial value, it was found that C. speciosus (21.7%) with the ethyl acetate fraction had the highest activity (45.7%) in the n-butanol fraction at a dose of 20 mg/kg. Oral administration of the *n*-butanol fraction of the plant at 20 mg/kg showed a highly significant antidiabetic effect (Table 4). After 42 days of administration, the effect of the n-butanol fraction at a dose of 20 mg/kg showed increased hypoglycemic activity compared to other fractions. As a result, it is recommended as the most active fraction of the plant *C. speciosus* of all partitioned extractions tested.

CONCLUSION

For Sri Lankan people and society as a whole, diabetes mellitus has emerged as a significant metabolic syndrome. In addition to insulin therapy and oral hypoglycemic medications, phytotherapy offers a variety of natural resources with hypoglycemic effects. *Costus speciosus*, one of the most popular medicinal plants with hypoglycemia properties, was shown to have hypoglycemic activity in the studied animal groups. According to analysis of crude fractions, the active ingredients in *C. speciosus* include alkaloids, flavonoids, polyphenolic acids, and saponin aglycones, all of which have exceptional therapeutic potential. Additionally, an 80% methanolic extract of *C. speciosus* leaves that was tested on rats shown hypoglycemic and antidiabetic action. Experimental hyperglycemia was produced on by administering ALX intraperitoneally to normal rats as part of the preliminary investigation into the effects of hypoglycemia on plant species. In comparison to controls, *C. speciosus* hypoglycemic activity reduced hyperglycemia in rats, favouring a restoration of glucose levels close to those of the reference medication glipizide. The outcomes in ALX-induced diabetic rats supported any possibility for insiulinogenic activity with the plant fractions under investigation. For the first time, the impact of oral administration of partitioned fractions of *C. speciosus* on diabetic rats caused by ALX was investigated. The findings indicated the presence of an antidiabetic action.

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CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

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