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Antihyperglycemic activity of the various extracts of *Costus speciosus* rhizomes

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Abstract

Costus speciosus is known to possess antidiabetic properties and used in local health traditions in India but the validation of drug is not done. The activity of various fractions of drug is also not reported. In view of its immense potential in andiabetic properties, systematic study was conducted with an objective to evaluate the anti-hyperglycemic activity of petroleum ether, chloroform, methanolic, and aqueous extracts of *C. speciosus* rhizomes on over night fasted, Streptozotocin (STZ) induced diabetic rats. Blood glucose level (BGL) monitored at 0, 30, 60, 120 and 240 minutes suggested that all extracts of *C. speciosus* resulted in reduction of BGL significantly except pet ether extract. Aqueous extract and methanolic extracts reduced initial BGL of 387 to 120 mg/dl and 303 to 161 mg/dl respectively at the end of 240 minutes. Similar studies conducted with oral glucose tolerance test (OGTT) confirmed the above findings suggesting that aqueous extract and methanolic extracts of *C. speciosus* were highly effective in bringing down the BGL from 590 to 96 mg/dl and 570 to 128 mg/dl respectively at the end of 240 minutes, which was on par with the glibenclamide. Results from multiple dose studies wherein the drug was administered for 14 days also confirmed the above findings and the serum lipid profiles high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were found to be optimum in aqueous or methanolic extracts on par with normal healthy rats or standard drug glibenclamide treated rats.

Key words: *Costus speciosus*, aqueous extract, methanolic extract, chloroform extract, petroleum ether extract, sterptozotocin, anti-hyperglycemic, diabetic, Wister rats.

1. Introduction

Since the time of Charaka and Susruta many herbal medicines in different oral formulations have been recommended for the treatment of diabetes mellitus (Madhumeha). Extracts of drugs from plant sources such as *Allium sativum* (garlic), *Azadirachta indica* (neem), *Vinca rosea* (nayantara), *Gymnema sylvestre* (meshashringe), *Trigonella foenum-graecum* (fenugreek), *Momordica charantia* (bitter gourd), *Ficus benghalensis* (banyan), *Eugenia*

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jambolina (black berry), *Ocimum sanctum* (tulsi) and *Eclipta alba* (karichalankanni) are some of the plants reported to possess antihyperglycemic activity in experimental animals [1, 3].

Many unknown and lesser known plants are used in folk and tribal medicinal practices in India. The medicinal values of these plants are not known much to the scientific world. *Costus speciosus* (Family Costaceae) is one such plant used by the locals of the regions of eastern Himalayan belt [4], Bangladesh in the treatment of diabetes and also *Costus speciosus* is mentioned in Ayurvedic literature as antidiabetic plant. *Costus speciosus* popularly known as kemuka in Sanskrit, pushpamoola in Kannada and kashmeeramu in Telugu is a rhizomatous herbaceous plant and flowers during months of July and August, the aerial parts withering away during winter months. *Costus speciosus* rhizomes are the main source of diosgenin, tigogenin and saponins. Also contain aliphatic hydroxyl ketones, triterpenes, starch mucilage, oxa-acids, fatty acids, abscisic acid, and corticosteroids [1, 2]. Medicinally it is used as anti inflammatory [3], anti arthritic, antidiabetic [4], anti cholinesterase and ecbolic agent.

Preliminary investigations carried out in laboratory showed that oral administration of freeze dried juice at dose of 200 mg/kg body weight showed better sugar reduction. Further comprehensive search of literature revealed that very limited work has been carried out to explore the effect of *C. speciosus*. Hence, the present study was carried out by extracting the dried rhizomes using various solvents like petroleum ether, chloroform, methanol and distilled water.

2. Materials and methods

***Plant material*:** Fresh rhizomes of *Costus speciosus* weighing 15 kilo grams, collected from a well grown 4 years old plant in the month of July from the herbal garden of Botany Department, Jnana Bharathi, Bangalore University, Bangalore. The rhizomes were authenticated by Prof. Balakrishna Gowda (Professor of Botany, UAS, GKVK, Bangalore) and Dr. Tejavathi (Professor of Botany, Bangalore University).

***Preparation of the extracts*:** The rhizomes were cleaned under tap water, excised in to small pieces and dried under shade for about 10 days. After drying the excised rhizomes weighed 1285 grams, grounded using mixer grinder. Further the grounded powder of the rhizomes were extracted using following solvents in order based on their polarity, petroleum ether, chloroform, methanol and distilled water. Among this aqueous extract was freeze dried at IISc, Bangalore.

Experimental animals: Albino Wister rats (150 -225 g) of either sex bred in the animal house of Drug testing laboratory, Palace road, Bangalore were procured and used in this study. The animals were fed on a standard pellet diet (AMRUT, Laboratory Animal Feed, Sangli) and had free access to ozonised filter water *ad libitum*. The animals were maintained in their respective groups under controlled conditions of temperature and humidity. All the studies were conducted in accordance with CPCSEA guidelines and the experiments were carried out as per the approval of Institutional Ethics Committee.

Induction of diabetes: Adult albino Wister rats of either sex were made diabetic with an intraperitoneal injection of 65 mg/kg body weight of Streptozotocin (Sigma Aldrich chemical company) dissolved in 0.1 M cold citrate buffer, pH 4.5, immediately before use. Streptozotocin injected [6] animals exhibited massive glucosuria and hyperglycemia within few days.

Table 1. Antihyperglycemic effects of various extracts of *C. speciosus* in STZ induced diabetic rats.

			Blood Glucose Levels mg/dl				
Groups	Treatments	Dose	0 min	30 min	60 min	120 min	240 min
Group 1	Vehicle, 5% Tween 80	5 ml/kg	65.5 ± 4.8	58.5 ± 4.1	65.75 ± 1.6	58.75 ± 2.6	61.25 ± 1.7
Group 2	Pet Ether extract	200 mg/kg	355.0 ± 46.4	347.5 ± 39.7	335.3 ± 26.6	351.0 ± 31.9	357.0 ± 32.4
Group 3	Chloroform extract	200 mg/kg	348.0 ± 46.1	310.5 ± 36.4	290.5 ± 17.7*	306.5 ± 29.6*	210.3 ± 41.5*
Group 4	Methanolic extract	200 mg/kg	303.0 ± 33.1	244.5 ± 34.8*	247.5 ± 30.4*	207.3 ± 34.6*	160.5 ± 31.9**
Group 5	Aqueous extract	200 mg/kg	387.3 ± 43.7	220.3 ± 40.9*	181.3 ± 39.9**	155.3 ± 38.3**	119.5 ± 44.9**
Group 6	Glibenclamide	2 mg/kg	336.8 ± 50.9	221.0 ± 43.5*	193.5 ± 29.7*	138.3 ± 16.4**	125.3 ± 38.5**
Group 7	Diabetic control	5 ml/kg	470.0 ± 32.1	488.3 ± 24.0	491.0 ± 20.6	476.0 ± 4.4	479.3 ± 7.6

Values are Mean ± S.E; n=5, ONE-Way: ANOVA **p<0.01, *p<0.05, NS; Not significant
Post test: Dunnet's compared all readings to diabetic control, *significant p<0.05, **significant p<0.01,

Table 2. Antihyperglycemic effects of various extracts of *C. speciosus* in oral glucose tolerance test of STZ induced diabetic rats.

			Blood Glucose Levels mg/dl				
Groups	Treatments	Dose	0 min	30 min	60 min	120 min	240 min
1	Vehicle, 5% Tween 80 + Glucose (2 g/kg)	5 ml/kg	65.5 ± 4.8	58.5 ± 4.1	65.7 ± 1.6	58.75 ± 2.6	61.25 ± 1.7
2	Pet Ether extract + Glucose (2 g/kg)	200 mg/kg	398.5 ± 27.8	576.0 ± 4.5	524.8 ± 14.4	468.0 ± 36.17	367.5 ± 52.5
3	Chloroform extract + Glucose (2 g/kg)	200 mg/kg	407.0 ± 42.6	580.3 ± 3.6	431.0 ± 20.5	317.5 ± 8.3*	288.8 ± 10.6*
4	Methanolic extract + Glucose (2 g/kg)	200 mg/kg	403.3 ± 31.8	570.3 ± 15.6	262.0 ± 9.7**	217.8 ± 19.5**	127.5 ± 26.2**
5	Aqueous extract + Glucose (2 g/kg)	200 mg/kg	219.5 ± 20.1	589.8 ± 1.2**	269.0 ± 27.1**	192.5 ± 14.4*	95.75 ± 10.7**
6	Glibenclamide + Glucose (2 g/kg)	2 mg/kg	284.0 ± 14.6	588.0 ± 1.3	247.0 ± 20.9**	180.5 ± 14.1**	75.75 ± 4.9**
7	Diabetic control + Glucose (2 g/kg)	5 ml/kg	412.5 ± 3.2	574.0 ± 3.7	534.3 ± 16.7	379.8 ± 20.0	333.8 ± 10.27

Values are Mean ± S.E; n=6, ONE-Way: ANOVA **p<0.01, *p<0.05, NS; Not significant vs. group 7.
Post test: Dunnet's compared all readings to diabetic control, *significant p<0.05, **significant p<0.01.

Table 3. Antihyperglycemic effects of various extracts of *C. speciosus* administered for two weeks in STZ induced diabetic rats

	Treatment	Blood glucose level mg/dl			Body weight		
		Before treatment	After treatment	% changes	Before treatment	After treatment	% changes
Group 1	Vehicle, 5% Tween 80	75.8 ± 2.3	71.75 ± 2.0	5.4↓	202.0 ± 4.0	204.5 ± 5.1	1.2 % ↑
Group 2	Chloroform extract	278.4 ± 15.3	162.0 ± 16.3*	42.5↓	133.2 ± 2.1	107.6 ± 4.3**	19.5 %↓
Group 3	Methanolic extract	408.6 ± 18.6	240.0 ± 19.8*	41.2↓	143.5 ± 1.9	103.3 ± 3.1**	27.7 %↓
Group 4	Aqueous extract	342.0 ± 26.4	126.0 ± 31.2**	63.26↓	155.2 ± 1.27	108.9 ± 1.1**	29.8 %↓
Group 5	Glibenclamide	336.7 ± 16.8	91.0 ± 26.8**	72.9↓	135.2 ± 4.1	115.1 ± 3.9**	14.7 %↓
Group 6	Diabetic control	447.5 ± 15.7	330.5 ± 18.9	27.5↓	160.5 ± 3.1	116.5 ± 3.4**	27.4 %↓

Values are Mean ± S.E; n=5, Statistics: ONE-Way- ANOVA followed by Dunnet's test.
**p<0.01, *p<0.05, NS; Not significant, Compared all readings to Diabetic Control for BGL comparisons.
Means compared with normal healthy rats for body weight comparisons.

Table 4. Effects of various extracts of *C. speciosus* administered for two weeks on serum lipid profiles.

Groups	Treatment	Serum lipid profiles				
		TG	TC	LDL	HDL	VLDL
Group 1	Vehicle, 5% Tween 80	66.75 ± 4.4	62.25 ± 6.1	34.9 ± 5.39	11.5 ± 1.2	13.35 ± 0.9
Group 2	Chloroform extract	55.5 ± 1.3*	27.75 ± 1.1**	11.08 ± 0.6**	5.25 ± 0.3**	11.05 ± 0.2*
Group 3	Methanolic extract	50.75 ± 0.8**	54.25 ± 0.9*	35.28 ± 0.5	10.25 ± 0.3	14.38 ± 0.3
Group 4	Aqueous extract	58.75 ± 5.2*	54.25 ± 5.3*	36.23 ± 3.8	10.4 ± 1.0	15.5 ± 0.7
Group 5	Glibenclamide	73.0 ± 1.3*	51.5 ± 14.5*	27.1 ± 11.9*	9.8 ± 2.9*	12.6 ± 0.3
Group 6	Diabetic control	61.2 ± 1.5	34.25 ± 4.9**	15.58 ± 3.7**	6.32 ± 0.9*	12.25 ± 0.3

Values are Mean ± S.E; n=5, Statistics: ONE-Way- ANOVA followed by Dunnet's test.
**p<0.01, *p<0.05, NS; Not significant. Compared all readings to normal control.
TG = Triglycerides, TC = Total cholesterol

Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, on 4th day after the injection with STZ. Adult albino Wister rats with blood glucose levels more than 200 mg/dl were considered to be diabetic and were used in this experiment. The extracts at the dose of 200 mg/kg body weight were administered orally after suspending in 5% Tween-80 solution. The blood samples were collected from retro orbital plexus and the blood glucose levels were determined using Glucometer [8].

Experiment 1. Evaluation of various extracts of *Costus speciosus* for antihyperglycemic properties in STZ induced diadetic rats.

After induction of diabetes the rats were divided into seven groups of six animals each and screened for antihyperglycemic activity of the various extracts of *Costus speciosus* in overnight fasted diabetic rats. The blood samples were collected from retro orbital plexus and the blood glucose levels were determined using Glucometer.

Group 1: Healthy rats, 5% Tween 80 (5 ml/kg body weight) orally.

Group 2: Extract of *C. speciosus*, 200 mg/kg body weight.

Group 3: Chloroform extract of *C. speciosus*, 200 mg/kg body weight.

Group 4: Methanolic extract of *C. speciosus*, 200 mg/kg body weight.

Group 5: Aqueous extract of *C.speciosus* at the dose of 200 mg/kg body weight.

Group 6: Glibenclamide, 2 mg/kg body weight.

Group 7: Diabetic rats, 5% Tween 80 (5 ml/kg body weight) orally.

Experiment 2. Evaluation of various extracts of costus sprciosus for antihyperglycemic properties in STZ induced rats in presence of glucose load (oral glucose tolerance test).

Overnight fasted diabetic rats were divided in to seven groups of six animals each as mentioned above and received the respective treatments. After 30 min of drug administration the rats of all the groups were orally administered with 2 g/kg of glucose. Blood samples were collected from retro orbital plexus just prior to drug administration and at 30, 60, 120 and 240 min after glucose loading. Blood glucose levels were measured immediately using Glucometer. The extracts used in Experiment 1 were used for confirmation in this experiment.

Experiment 3. Evaluation of various extracts of *Costus speciosus* for antihyperglycemic properties in STZ induced diabetic rats under multiple dose studies.

In multiple dose studies, the extracts at the dose of 200 mg/kg body weight twice daily was given for 14 days and BGL was monitored only at the end of the experiment. Blood samples were collected from retro orbital plexus just prior to and on the last day of the treatment. Blood glucose levels were determined using Glucometer, on the last day of the experiment. The animals were sacrificed with excess dose of ether anesthesia and blood was collected for estimation of lipid profiles, piece of liver was extracted for glycogen estimation. The body weights of all the animals of all the groups were recorded before starting the treatment and at the end of the treatment period.

Statistical analysis: Data obtained was analyzed using prism software and the results were expressed as Mean ± SEM, n=6. Statistical significance was determined by using One-way analysis of variance (ANOVA) followed by Dunnet's test.

3. Results and Discussion

Hypoglycemic effects in STZ induced diabetic rats: Acute effects of various extracts of *C. speciosus* in overnight fasted diabetic rats are

presented in Table 1. Blood glucose level (BGL) of rats of Group 1 was compared with BGL of other rats to confirm that the drug STZ has induced diabetes in experimental animals ($p< 0.01$) at all intervals of sampling. It was noticed that all the extracts of *C. speciosus* resulted in reduction of BGL significantly except pet ether extract. Aqueous extract and methanolic extracts were significantly ($p<0.01$) effective in reducing initial BGL of 387 to 120 mg/dl and 303 to 161 mg/dl respectively, which was on par with glibenclamide that reduced BGL from 337 to 125 mg/dl at the end of 240 minutes. Chloroform extract was found to be better than pet ether extract but not superior to methanolic or aqueous extract in antihyperglycemic properties.

***Oral glucose tolerance test*:** Results of OGTT are presented in Table 2. An overdose of glucose was fed to diabetic and normal rats to evaluate the efficacy of various drug extracts on antihyperglycemic properties. Results from this study showed that aqueous extract and methanolic extracts of *C. speciosus* were highly effective in bringing down the BGL from 590 to 96 mg/dl and 570 to 128 mg/dl at the end of 240 minutes, which was on par with the glibenclamide that reduced BGL from 588 to 76 mg/dl. The pet ether extract was less effective (576 to 368 mg/dl) than chloroform extract (58 to 289 mg/dl) in reducing BGL, however all the extracts have reduced BGL to varying degrees. These results are in consonance with the earlier experiments suggesting that all extracts of *C. speciosus* are antihyperglycemic.

Multiple dose studies: The changes in BGL and body weight are reported in Table 3 and changes in serum lipid profile are reported in Table 4. There was a significant ($p<0.01$) reduction in body weight in all diabetic rats within 14 days ranging from 14.7 to 27.4%. Significant ($p<0.01$) decrease in BGL was observed in rats treated with aqueous extract of *C. speciosus* which was on par with glibenclamide in reducing the BGL from 342 to 126 mg/dl and 337 to 91 mg/dl respectively. The choloroform extract and methanolic extracts also lowered BGL significantly ($p<0.05$) compared to diabetic control by bringing down from 278 to 182 and 409 to 240 mg/dl respectively (Table 3). The triglyceride levels of the animals treated with all the extracts have come down significantly compared to the normal control group and glibenclamide treated group which is not a desired effect. Further the concentration of high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) decreased in chloroform extract but in methanolic extract and aqueous extracts it was on par with healthy rats (Table 4). These results suggested that aqueous extract and methanolic extract of *C. speciosus* are better than other extracts and equivalent to the control drug glibenclamide.

The antihyperglycemic activity of a drug is the ability of the drug to lower very high blood sugar levels to acceptable lower levels. In literature very less work has been reported, however available reports are cited here. Hypoglycemic properties (reducing blood sugar levels from moderately high levels to normal levels) in many herbs such as *Momordica charantia*, Fenugreek, *Gymnema sylvestre*, *Ficus benghalensis*, *Ocimum sanctum*, *Eugenia jambolina* and *Eclipta alba* have been reported [4]. Studies conducted in *Costus speciosus* have revealed that alkaloids of the herb have anticholinesterase properties [5]. Ethanolic extract of *Ocimum sanctum* leaves have been studied for its lowering effects of glycogen content and carbohydrate metabolism in streptozotocin induced rats [6]. The

antidiabetic activity of ethanol extract of *Cassia kleinii* leaf has been reported [7]. Antihyperglycemic and hypoglycemic properties of *Aporosa lindleyana* in normal and alloxan induced diabetic rats have been reported [8]. Antihyperglycemic properties of alcoholic extract of *Aralia cachemirica* roots has been reported [9]. In this study we report that results from three different independent experiments suggest that all the four extracts of petroleum ether, chloroform, methanolic, and aqueous extracts of *C. speciosus* were antihyper-glycemic. Aqueous and methanolic extracts were superior to other extracts in bringing down the BGL from very high levels to acceptable levels within 240 minutes and the same was verified for its reproducibility of results in long duration multiple dose studies. It was confirmed that aqueous and methanolic extracts were on par with standard drug in maintaining serum lipid profiles.

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