

## Research Article

### Antidiabetic Properties of an Aqueous-Methanolic Stem Bark Extract of *Spathodea campanulata* (Bignoniaceae) P. Beauv

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**Abstract:** *Spathodea campanulata* (Bignoniaceae) P. Beauv. is a common medicinal plant in the central and south-western regions of Uganda. It is popular for its use primarily in the treatment of diabetes and some other ailments. In this study the antidiabetic properties of an aqueous methanolic extract (SCE) of the stem bark were explored using experimental rat models. In normoglycemic rats, the extract reduced blood glucose levels with a significant effect ( $p < 0.05$ ) after 2 h at the 800 mg/kg dose. The standard chlorpropamide also produced significant lowering of normal blood glucose at all the time intervals studied (0.5, 1, 2, 4 h.). In the oral glucose tolerance test (OGTT), SCE reduced glucose-induced glycemia in a moderate manner. SCE 200, 400, 800 mg/kg bodyweight caused reduction in glycemia by 62, 63 and 35% respectively. SCE (200, 400 and 800 mg/kg) caused reduction in hyperglycemia by 10, 29 and 4% respectively, in the alloxan-induced hyperglycemia. The standard drug Chlorpropamide (400 mg/kg) significantly ( $p < 0.05$ ) reduced hyperglycemia by 85%. Prolonged treatment (daily dose for 17days) with SCE reduced alloxan diabetes as well; 33% by the 200 mg/kg dose, 66% by the 400 mg/kg dose and 42.9% by the 800 mg/kg dose. Both single dose and multiple dose effects were not significant ( $p < 0.05$ ). Therefore, SCE has hypoglycemic and antihyperglycemic effects in the experimental diabetic models used and at the doses applied. Further purification of the crude extract may improve on the potency. Toxicity studies are also required to standardize its safety.

**Keywords:** Alloxan, antihyperglycemic, diabetes, hypoglycemic, spathodea

## INTRODUCTION

Diabetes mellitus is a complicated chronic metabolic disease characterised by either a total or partial insulin deficiency or by cellular refractoriness to insulin activity. The disease affects the metabolism of carbohydrates, fats and proteins. A common intersection point in the manifestation of all types of diabetes mellitus is hyperglycaemia which causes micro- and macro-vascular complications that result in cardiovascular disease, neuropathy, retinopathy and nephropathy (Gokce and Haznedaroglu, 2008). Currently available orthodox therapies for diabetes

mellitus include insulin and the oral hypoglycaemic agents like the sulphonylureas, biguanides,  $\alpha$ -glucosidase inhibitors, dipeptidyl peptidase IV (DPP-4) inhibitors and glinides. The key parameter for observing the efficacy of these agents is the eventual reduction of the disturbing hyperglycaemia. Diabetes mellitus whose global prevalence is projected at 5% (Ndiaye *et al.*, 2008) is known to have existed from historic time. Consequently various folk medicines have remedies which have been used for its treatment in some circumstances for ages. In central and south-western Uganda one of the most commonly used medicinal plants for this purpose is *Spathodea*

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*campanulata*, (Hamill *et al.*, 2003). The plant is known in some local languages as *omwatanshare* (Runyankole) and *kifabakazi* (Ruganda, Kiswahili). It is a tree that grows both in forests and around homesteads most often as live fencing or demarcation for grazing lands. It is readily available, affordable and accepted by the locals.

In folk medicine of different parts of the world, the stem bark of *Spathodea campanulata* also commonly known as African tulip tree, flame of the forest, fountain tree, fireball, Gabon tulip tree and fire tree is of the family Bignoniaceae and is used in the treatment of various diseases. The plant has two IPNI identifications. The IPNI identification number 110661-1 is attributed to the nomenclature done by Ambroise Marie Françoise Joseph Palisot de Beauvois in 1805. That done by Francis Buchanan-Hamilton in 1845 has the IPNI identification number 110660-1. Both authors gave the same name *Spathodea campanulata* (Bignoniaceae), [www.ipni.org](http://www.ipni.org). This African tulip tree comes from the rainforests of Equatorial Africa, especially West Africa where it is Native. It is widely planted throughout tropical and subtropical regions of the world, notably Indonesia, South America, French Polynesia, Fiji, Hawaii and Samoa where it is noted as an invasive ornamental and street tree. It invades abandoned farmland and mature forests (Chin, 1989).

The various plant parts of *S. campanulata* (leaves, stem bark, flowers and roots) have been widely utilized in traditional medicine by various cultures worldwide, although applications vary by region. Its use in ethno-medicine for the treatment of diseases include ulcers, dysentery, oedemas, skin eruptions, scabies, wound healing, urethral discharge diuretic and anti-inflammatory, while the leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons and veterinary application among others have been attributed to the plant in different cultures (Burkill, 1985; Hutchinson and Dalziel, 1954). In Ghanaian traditional medicine, the stem bark is used for wound healing, where it is applied as a paste to the wound (Mensah *et al.*, 2003, 2006), treatment of dyspepsia and peptic ulcer (stem bark and leaf); arthritis and fracture (leaf, root bark and fruit); toothache and stomach ache (stem bark); and stomach ulcer (root bark and seed) (Agbovie *et al.*, 2002). In Cameroon, the leaves and stem bark of *Spathodea campanulata* are widely used as antimalarial remedy (Titanji *et al.*, 2008). The aqueous, chloroform and hexane extracts of stem bark were investigated by Makinde *et al.* (1988) based on its use as an antimalarial agent in south-western Nigeria. Ilodigwe *et al.* (2010) studied the anticonvulsant activity of the leaf extracts of this plant based on its traditional use to control epilepsy in Eastern Nigeria (Iboland). In India, Ayurvedic system of medicine has existed for over four thousand years. From ancient literature it is evidenced that the various parts of the plants were used in Siddha, Ayurveda and Unani medicine for the treatment of diseases of human beings and animals (Palaniswamy

*et al.*, 2008). In South America, the stem bark preparations are employed against fungal skin diseases, herpes, stomach aches, diarrhoea and as enemas (Jardim *et al.*, 2003).

In neighbouring Rwanda *Spathodea campanulata* is similarly used as treatment for diabetes mellitus and some clinical investigation was carried out on its use by Niyonzima *et al.* (1999). This necessitates a thorough scientific screening and standardisation, which to the best of our knowledge has yet to be done in Uganda. Research work on this plant therefore marked the start of a broad-based program on screening and standardising local traditional antidiabetic remedies. It will be on the basis of such findings that recommendations will be directed to the appropriate authorities to encourage domestication and conservation of such valuable plants if necessary.

## MATERIALS AND METHODS

**Research approval and authorisation:** This research project was approved and authorised by the following; Mbarara University of Science and Technology (MUST); the Faculty of medicine Research and Ethics Committee (FREC) (Ref: DMS 6), Institutional Review Committee (IRC) (Ref: MUIRC 1/7), Uganda National Council for Science and Technology (UNCST) (Ref: HS 1206) and finally the Office of the President of the Republic of Uganda (Ref: ADM 154/212/01).

**Environmental considerations:** Prior to beginning the collection of plant material in collaboration with the Rukararwe Traditional Healers Partnership (RTHP), two seedlings of *Spathodea campanulata* were obtained from the RTHP medicinal plants nursery and planted near the KIU-WC staff residential area. This was in compensation for any eventual adverse environmental consequences of collecting the medicinal plant material. The trees are presently mature.

**Plant material collection and extraction:** The stem bark of *Spathodea campanulata* was collected locally from Rukararwe Traditional Healers Partnership premises in Bushenyi, Uganda. Collection was done between 9 and 11 am in the month of May. Collection was done sustainably with the aid of tree nursery and conservation staff from RTHP. Authentication was done in the department of Science Laboratory Technology of Mbarara University of Science and Technology by Dr. Eunice Olet and a voucher deposited in the herbarium of the same department. The voucher was tagged, JIBI JAMES 001. The stem bark was shade-dried, powdered and taken for extraction. The plant material was extracted in 50% methanol using a soxhlet extractor (Quickfit™) at the department of Pharmacology and Therapeutics of Mbarara University of Science and Technology. The crude extract was concentrated by distillation and further dried in an electrical oven (Mermmet™, Germany) at 40°C.

**Chemicals and reagents:** Glucometer and strips (Optium™; Xceed™ and Freestyle) were obtained from Abbott Diabetes Care Ltd, Oxon, UK. The solvent used was of analytical grade; methanol Zayo-Sigma Ltd. Germany. Chlorpropamide was from Sigma (St. Louis, MO, USA).

**Laboratory animals:** Healthy young adult Wistar rats (*Rattus norvegicus*) of both sexes weighing 150-200 g bred and maintained at the laboratory animal facility of the department of Pharmacology and Toxicology, Kampala International University-Western Campus were used according to the NIH guidelines for the Care and Use of Laboratory Animals in Teaching and Research (NIH Publication No. 83-27, 1985). The animals were kept and maintained under ambient laboratory conditions of temperature, humidity, 12 h light/12 h darkness cycle. The animals were allowed access to standard rodent feed (Nuvita®, Jinja Uganda) and tap water *ad libitum*. Prior to the experiments the animals were fasted overnight while maintaining their free access to tap water. The animals were divided into the control, reference and three test drug groups of six animals each (n = 6).

**Effect of SCE on normoglycaemic rats:** Animals (normoglycaemic Wistar rats) were fasted over night and divided in to five groups of six rats each. The distribution of the animals into the different treatment groups was stratified based on the basal glycaemia (blood glucose level at zero hour). Control animals were administered 10 mL/kg bodyweight of distilled water orally (Group I). The positive control (Group II) was given an oral dose (400 mg/kg) of chlorpropamide. *Spathodea campanulata* extract was administered by oral canula in three doses 200, 400 and 800 mg/kg (Groups III, IV and V, respectively). Blood samples were collected from the tail vein just prior to and 0.5, 1, 2 and 4 h after the treatment administration. Serum glucose was measured using the Optium® glucometer and strips manufactured by Abbot Laboratories UK. The process was repeated. Data was expressed as mean±SEM. Results obtained from this test were analyzed using the statistical package for social sciences (SPSS) version 17. A one-way Analysis of Variance (ANOVA) and the Dunnet's post hoc test were used as a statistical tool for comparing the means difference between the control and experimental groups. The 95% confidence limit (p<0.05) was applied. This procedure is a modification of that previously done by Ajikumaran *et al.* (2006).

**Effect of SCE on the Oral Glucose Tolerance Test (OGTT):** Animals (normal albino rats) were fasted over night and divided in to five groups of six rats each. Control animals were given 1ml of distilled water orally via a canula (Group A). The positive control (Group E) was given an oral dose (400 mg/kg) of chlorpropamide.

*Spathodea campanulata* extract was given by oral canula in three doses 200, 400 and 800 mg/kg (Groups B, C and D, respectively). Sixty minutes after the extract administration all the animals were given a glucose loading, 5 g/kg bodyweight orally. Blood samples were collected from the tail vein just prior to and 30, 60, 120 and 240 min after the glucose loading. Serum glucose was measured using the Optium® glucometer and Optium® Freestyle strips. The process was done repeatedly. This procedure was adopted and modified from that previously used by Goksel and Mehmet (2008), Cunha *et al.* (2008) and Ajikumaran *et al.* (2006).

**The effect of SCE on alloxan-diabetic rats:** Male Wistar rats weighing between 150 and 200 g were given a single dose of alloxan monohydrate in distilled water, 200 mg/kg body weight intra-peritoneally (Cooperstien and Walkins, 1981). After 3 days the animals that had blood glucose levels above 10 mmol/l were considered diabetic and included in the experiment. Animals were divided into five groups including six rats each (n = 6). Group I (control) was given 1ml distilled water and Groups II-IV diabetic rats were given a reconstituted aqueous solution of *Spathodea campanulata* Extract (SCE) at the dose levels 200, 400 and 800 mg/kg bodyweight. Group V was given 40 0mg/kg bodyweight of chlorpropamide as the standard treatment. The rats were fasted over night and blood samples were collected prior to and 0.5, 1, 2 and 4 h intervals after administration of the extract. The blood glucose level was estimated with the same glucometer used above. The same rats were given the same treatment daily as per their groups for 17 days in a repeated-dose study. Blood was collected from the tail vein of non fasted rats on the 7<sup>th</sup>, 14<sup>th</sup> and 18<sup>th</sup> days after the single-dose study and serum glucose levels measured as above. The study was done repeatedly. The data obtained was expressed as mean±SEM analysed using SPSS version 17. A comparison of mean difference was done through the one-way Analysis of Variance (ANOVA) with the Dunnet's post hoc test. The 95% confidence limits (p<0.05) was applied. Percentage reduction of glycaemia was calculated for the different treatment groups as a means of comparing the effect of individual treatment groups to that of the control.

## RESULTS

**Effect of SCE on normoglycaemia in rats:** In the study on the effect of SCE on normoglycaemia in rats, it was observed that the extract caused generally a decrease in blood glucose levels compared to the negative control (distilled water treated) group. This effect culminated in a significant reduction (p<0.05) at the highest dose (800 mg/kg bodyweight) after the

Table 1: The effect of SCE on Normoglycaemic rats  $p < 0.05$  Mean  $\pm$  SEM

Treatment group	Glycaemia at 0 hr	Glycaemia at 0.5 hr	Glycaemia at 1 hr	Glycaemia at 2 hr	Glycaemia at 4 hr
Control	3.1 $\pm$ 0.230	3.07 $\pm$ 0.23	3.37 $\pm$ 0.16	2.7 $\pm$ 0.140	2.53 $\pm$ 0.31
SCE 200 mg/kg	3.07 $\pm$ 0.22	3.33 $\pm$ 0.28	3.03 $\pm$ 0.32	2.72 $\pm$ 0.21	2.15 $\pm$ 0.33
SCE 400 mg/kg	3.28 $\pm$ 0.24	3.17 $\pm$ 0.11	2.85 $\pm$ 0.23	2.48 $\pm$ 0.26	2.23 $\pm$ 0.26
SCE 800 mg/kg	3.08 $\pm$ 0.24	3.22 $\pm$ 0.26	3.32 $\pm$ 0.23	1.42 $\pm$ 0.30*	2.47 $\pm$ 0.39
Chlorpropamide	3.0 $\pm$ 0.170	0.71 $\pm$ 0.00*	0.84 $\pm$ 0.08*	0.78 $\pm$ 0.07*	0.71 $\pm$ 0.00*

\*Values significantly low at  $p < 0.05$  compared to the control (distilled water treated group), n = 6

Table 2: The Effect of SCE single dose on alloxan-induced diabetes mellitus

Treatment group	Glycaemia at 0 hr	Glycaemia at 0.5 hr	Glycaemia at 1 hr	Glycaemia at 2 hr	Glycaemia at 4 hr	%-tage reduction
Control	22.4 $\pm$ 2.7	22.8 $\pm$ 2.90	21.2 $\pm$ 3.6	21.7 $\pm$ 4.3	20.9 $\pm$ 4.6	6.70
SCE 200 mg/kg	27.0 $\pm$ 2.6	29.0 $\pm$ 2.00	27.6 $\pm$ 2.3	26.2 $\pm$ 2.5	24.3 $\pm$ 3.5	10.0
SCE 400 mg/kg	25.9 $\pm$ 1.7	27.2 $\pm$ 2.44	24.7 $\pm$ 3.2	24.2 $\pm$ 4.6	18.4 $\pm$ 3.1	29.0
SCE 800 mg/kg	17.7 $\pm$ 2.1	17.1 $\pm$ 1.30	19.0 $\pm$ 1.4	17.5 $\pm$ 2.9	17 $\pm$ 1.90	4.00
Chlorpropamide	21.3 $\pm$ 2.2	3.50 $\pm$ 2.40*	3.9 $\pm$ 2.9*	4.6 $\pm$ 3.1*	3.2 $\pm$ 2.5*	85.0

\*Values significant at  $p < 0.05$ , n = 6 compared to the control

Table 3: The effect of SCE multiple doses on alloxan hyperglycaemia

Treatment	Day 1 Glycaemia	Day 7 Glycaemia	Day 14 Glycaemia	Day 18 Glycaemia	%-tage reduction
Control	19.6 $\pm$ 2.25	15.8 $\pm$ 2.67	14.9 $\pm$ 3.5	14.7 $\pm$ 4.1	25.0
SCE 200 mg/kg	17.4 $\pm$ 1.41	8.5 $\pm$ 1.30	10.8 $\pm$ 10	11.6 $\pm$ 1.4	33.4
SCE 400 mg/kg	20.6 $\pm$ 2.10	10.1 $\pm$ 2.6	12.3 $\pm$ 2.3	7.0 $\pm$ 1.40	66.0
SCE 800 mg/kg	16.8 $\pm$ 1.97	13.9 $\pm$ 1.1	14 $\pm$ 2.10	9.60 $\pm$ 3.0	42.9
Chlorpropamide	19.1 $\pm$ 2.4 0	12.6 $\pm$ 3.5	12.1 $\pm$ 2.5	14.4 $\pm$ 2.9	24.6

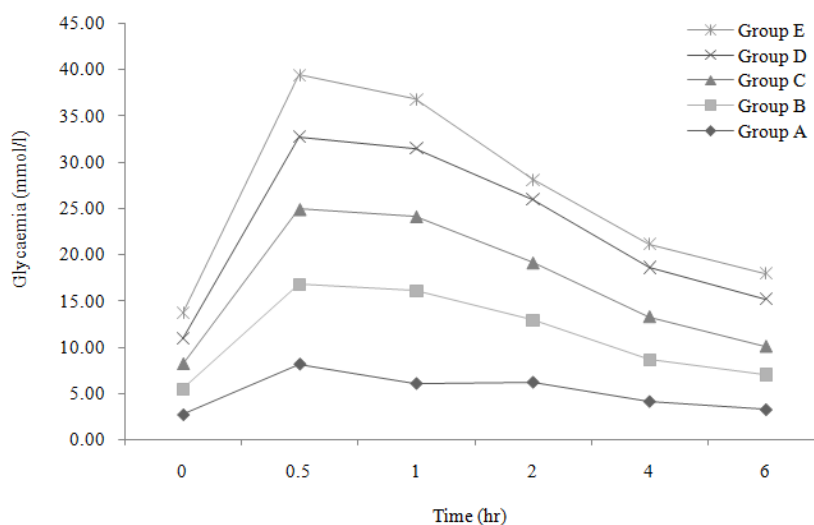


Fig. 1: The effect of SCE on oral glucose tolerance. Groups: A is control, B is SCE 200 mg/kg, C is SCE 400 mg/kg, D is SCE 800 mg/kg and E is Chlorpropamide 400 mg/kg

second hour (Table 1). Meanwhile, the positive control (chlorpropamide 400 mg/kg treated) group caused a significant reduction ( $p < 0.05$ ) of blood glucose (compared to the negative control) at all the time intervals that glycaemia was measured (0.5, 1, 2 and 4 h).

**Effect of SCE on glucose tolerance:** The Oral Glucose Tolerance Test (OGTT), showed that the SCE reduced glucose-induced rise in glycaemia. A significant reduction was not observed with all treatments at any level but the highest reduction of glycaemia (63%) was obtained with the SCE 400 mg/kg treatment followed by the SCE 200 mg/kg treatment (62%). When compared to the control value (60%) there was a

tendency for a dose-dependent reduction which was interrupted by the drop in the value of the SCE 800 mg/kg treatment (35%). However the positive control (chlorpropamide 400 mg/kg) was almost equivalent to the control with a value of 58% (Fig. 1).

**Effect of SCE on alloxan-induced diabetes:** Alloxan-induced diabetes mellitus in rats was reduced by treatment with SCE though not significantly ( $p < 0.05$ ) the greatest reduction (29%) in the acute phase was observed with the 400 mg/kg dose. The 200 mg/kg dose produced a reduction of 10% while the 800 mg/kg dose of SCE produced a reduction of 4%. The glycaemia reduction by SCE in this acute phase had the tendency to be dose-dependent. The chlorpropamide (400 mg/kg)

treatment group produced a glycaemia reduction of 85%. At all the time intervals during which glycaemia was measured, chlorpropamide produced a significant reduction in glycaemia ( $p < 0.05$ ) Table 2.

**Effect of SCE repeated treatment on alloxan-induced diabetes:** The multiple dose treatment with SCE resulted in a reduction of alloxan-induced hyperglycaemia at the end of the eighteen day period as presented in Table 3. The reduction by SCE 200 mg/kg treatment was 33.4%, 400 mg/kg was 66% while 800 mg/kg reduced glycaemia by 42.9%. Chlorpropamide treatment reduced glycaemia by 24.6%.

## DISCUSSION

The reduction of glycemia observed with SCE is similarly observed with other plants used in folk medicine in various settings both in Africa and other parts of the world where alternative medicine relies on herbal medicines for the treatment of diabetes mellitus. Ndiaye *et al.* (2008) observed this hypoglycemia effect in *Parinari excelsa* an antidiabetic herbal remedy among the Senegalese people. Cunha *et al.* (2008) observed hypoglycemia activity in *Leandra lacunosa* which is used in Brazilian folkloric medicine to treat diabetes mellitus.

Glucose loading is another means of inducing diabetes in experimental animals. However this type of experimental diabetes is temporal though still lasting long enough for the experimental purpose. Reducing oral glucose-induced hyperglycemia had also been observed in plant extracts by Goksel and Mehmet (2008) and Ndiaye *et al.* (2008). Improving glucose tolerance as observed here with the administration of SCE could be a result of either inhibiting glucose absorption from the gastrointestinal tract or by improving on glucose utilization at cellular level. There is thus the need for *in vitro* studies to clearly account for the observation in this study.

Alloxan causes diabetes by the excessive production of Reactive Oxygen Species (ROS) which are cytotoxic to pancreatic  $\beta$ -cells of Langerhans. Consequently this significantly reduces the synthesis and release of insulin by the pancreas (Sakurai *et al.*, 2001). The resultant effect is inability of the system to extract and convert blood glucose to the stored form glycogen hence the observed hyperglycemia. Excessive production of ROS also affects other organs like the liver, kidney and the haemopoietic system (Sabu *et al.*, 2005). It is also documented that there is a decrease in antioxidant enzyme levels and enhanced lipid peroxidation in alloxan-induced diabetes (Roy *et al.*, 2005; Sepici *et al.*, 2007). In this study SCE reduced blood glucose apparently in a dose-dependent manner except for a drop in the trend of the effect at the highest dose of study (800 mg/kg bodyweight). Various mechanisms could be involved in producing the observed effect independently or in combination. Either

the extract could have the cytoprotective potentials against the cytotoxic oxidative stress from the ROS produced by alloxan, enhancement of glucose extraction and consumption by cells or an effect related to improvement of insulin secretion and sensitivity of the insulin receptor. Work is in progress in this laboratory to narrow down to the actual mechanism by which SCE produces the effect or establish if it is a multifunctional agent. Furthermore, in the multiple dose study the performance of the extract in reducing alloxan-induced diabetes after 17 days seemed to indicate that the extract may have a cumulative advantage since in this test its ultimate reduction of glycaemia *vis-a-vis* the standard chlorpropamide is greater at the 200 and 400 mg/kg doses. It has been established that the phytochemical components of this plant include glycosides, reducing sugars (Iloigwe *et al.*, 2010) so it is likely that the highest dose of study (800 mg/kg bodyweight) may possess a significant amount of sugars to affect the extent of its hypoglycemia potentials in the models used here. This is apparently evident by the fact that at 800 mg/kg bodyweight, the blood glucose reducing effect is lower than that of the 400 mg/kg dose hence the highest dose is not the optimal one.

The observation that this extract has marginal hypoglycemia and antihyperglycaemic effects may put it at an advantage since an accidental overdose may not cause the hypoglycaemic shock experienced with some contemporary antidiabetic agents like insulin and the sulphonylureas. Severe hypoglycemia is usually the most worrisome effect of therapy with such agents (Ferner, 1988). This further confirms its constant and extensive use in traditional medicine in this region in the treatment of diabetes mellitus and other ailments. Conversely of the fact that diabetes treatment is long lasting, compliant users may sometimes take large quantities being uninformed of the existence of dose-related adverse effects. Actually there is the general belief locally just like elsewhere in the world that herbal medicines are free of adverse effects and several mouthfuls are even preferred for optimum therapy.

It is hypothesised that while crude the SCE hypoglycaemic effect could be diluted by other components present in the extract. This could be the reason why the effect is marginal or not statistically significant ( $p < 0.05$ ) at any time with the lower doses. Further purification and separation of the constituents of the extract may yield a more potent fraction. The separation process with the aim of isolating the active fraction (s) is ongoing in these laboratories.

## CONCLUSION

The present study on *Spathodea campanulata* stem bark methanolic extract was aimed at probing into the claimed antidiabetic activity of the plant in traditional medicine in central and south western Uganda. After using the four models above it is concluded that SCE

has a marginal antidiabetic activity which could be both hypoglycemic and antihyperglycemic as well at the doses of the study. Further, repeated doses could yield a more beneficial antihyperglycemic effect. This justifies its use in traditional medicine for treatment of diabetes mellitus. The results obtained further justify the use of this plant in various other ailments round the world without the danger of severe hypoglycemic side effects.

It is recommended that repeated dose (chronic or subchronic) toxicity studies be carried out to completely ascertain the toxicity/safety profile of this medicinal plant at organ or cellular level.

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