

# Phytochemical and Antidiabetic Evaluation of the Methanolic Stem Bark Extract of *Spathodea campanulata* (P. Beauv.) Bignoniaceae

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## ABSTRACT

**Background:** *Spathodea campanulata* (P. Beauv.) Bignoniaceae extract (SCE) is one of many herbal medicines used widely in Ugandan traditional medicine for various ailments. Generally most of these herbal medicines are yet to be standardized or have their phytochemical content characterized.

**Method:** This study identified the secondary metabolites in the stem bark methanolic extract and quantified them. The same extract was subjected to serial solvent fractionation, TLC characterization and antidiabetic testing.

**Results:** The secondary metabolites were found to comprise of 75% alkaloids, 10% flavonoids, 13% tannins and 17% saponins per gram of plant material. The TLC characteristics of the fractions (hexane (HX), ethylacetate (EA) and methanol (ME)) viewed under UV light revealed spots with the following Rf values; the hexane partition gave HX7EA3 (0.96 and 0.68) HX9EA1 (0.68 and 0.3), EA1ME9 (0.86 and 0.58), EA3ME7 (0.87), EA7ME3 (0.85) and EA9ME1 (0.85). The ethylacetate partition gave the following HX1EA9 (0.53, 0.34 and 0.18), HX3EA7 (0.59, 0.40 and 0.26). **Discussion:** All the fractions produced nominal reduction of hyperglycemia. Except hexane fraction at 50 mg/kg and ethylacetate fraction at 200 mg/kg all the fractions had percentage reductions of glucose greater than that of the control at the experimental doses. Although the values of percentage reductions of

hyperglycemia by the hexane fraction were apparently dose-dependent, the greatest margin of reduction of hyperglycemia was observed in the residual aqueous fraction. **Conclusion:** It was found that SCE contains valuable phytochemicals in appreciable quantities which are antidiabetic. The residual aqueous fraction is the most potent antihyperglycemic of the solvent fractions.

**Key words:** Flavonoids, *Spathodea campanulata* (P. Beauv.) Bignoniaceae, Saponins, TLC Tannins, Uganda.

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DOI : 10.5530/pj.2016.3.12

## INTRODUCTION

Phytochemicals are natural compounds or secondary metabolites synthesized by plants for the purpose of self protection from herbivorous pests. They are found in vegetables, fruits, medicinal plants, aromatic plants, leaves, flowers and roots. They may be bioactive and useful in preventing or combating human diseases or otherwise be toxic to humans.<sup>1</sup> The use of plants as herbal remedies dates as far back as the history of mankind. This practice gradually led to the search for conventional medicines from remedies used in traditional medical practices in different traditional sub-systems. Scientific and technological advancement has led to the discovery of bioactivity in many of the remedies that have been used in the historic and even since the prehistoric eras in various sub-systems of traditional medicine.<sup>2-5</sup>

Thousands of contemporary drug substances were discovered in plants and either used as such or later on synthesized. Some of them were used as starting material in semi-synthetic processes to yield drug molecules with required pharmacological properties. For instance, prior to 2007 half of the licensed drugs in the global market were either natural products or their synthetic derivatives.<sup>6</sup> The importance of these phytochemicals in the pharmaceutical and related industries is evident by the fact that phytochemical industries have been set up which extract and isolate chemicals from natural sources for various purposes. They are referred

to as natural product or phytochemical banks. Phytochemicals are also useful in food, beverage and cosmetics industries. Huge investments are usually required in this sector which necessitates prior feasibility studies. These are done by identifying and quantifying the available chemicals in the plant material with a view to assessing its economic potentials. Worldwide annual trade in phytochemicals can be estimated in billions of dollars. A simple example is the trade in *Pygeum africanum* that reaches billions in a year.<sup>7,8</sup> *Spathodea campanulata* is an evergreen tree with reddish yellow flowers native to the equatorial rain forests of west and central Africa.<sup>9</sup> This plant is used for the treatment of various ailments in Uganda and most prominently as an antidiabetic.<sup>10</sup> Several phytochemical studies have been done on this plant and its related species most often on the vegetative parts.<sup>11</sup> It is known in Uganda as *kifabakazi* (Luganda), *omwatanshare* (Runyankole). Some common names include African tulip tree, flame of the forest, Gabon tulip, fountain tree and fireball. The plant's botanical classification and description are found elsewhere.<sup>9</sup> The use of *Spathodea campanulata* (P. Beauv.) Bignoniaceae in the Ugandan folk medicine has not yet been explored scientifically. Following this assertion it needs to be quantified for its active constituents in order to ascertain its potential usefulness in contemporary traditional medicine, pharmaceutical and related industries. This research project is part of a

program to scientifically study its medicinal use especially as an anti-diabetic agent as used in Uganda. When used to treat diabetes in traditional medicine a decoction of the stem bark is made in locally brewed banana beer. The decoction is taken as often as two or three times a day. This research project identified the chemical constituents, quantified them, separated the solvent fractions, determined their TLC profiles and explored their bioactivity.

## MATERIALS AND METHODS

### Plant material collection and extraction

The stem bark of *Spathodea campanulata* (*Bignoniaceae*) was collected locally from Rukararwe Traditional Healers Partnership (RTHP) premises in Bushenyi, Uganda. Collection was done between 9:00 and 11:00 am in the month of May 2012. Authentication was done in the Department of Science Laboratory Technology of Mbarara University of Science and Technology by Dr. Eunice Olet and a voucher (JIBI JAMES 001) deposited in the herbarium of the same department. 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. Extraction was continued till the yellow color of the extract from the plant material faded out completely. The crude extract was concentrated by distillation (for solvent recovery) and further dried in an electrical oven (Mermert®) at 40°C obtaining a yield of 2.5:1 ratio of plant material to methanolic extract.

#### Environmental considerations

Prior to beginning the collection of plant material in collaboration with the Rukararwe Traditional Healers Partnership (RTHP), two seedlings of *Spathodea campanulata* (P. Beauv.) *Bignoniaceae* 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.

### Chemicals and reagents

All the solvents used were of analytical grade, methanol, hexane and ethyl-acetate (Zayo-Sigma, Germany). Metformin (Renformin®) was from Rene Pharma, Kampala Uganda. Glucose was obtained from Excel Chemicals Ltd. Nairobi, Kenya. The glucometer (Optium™) and strips (Xceed™ and Freestyle™) were obtained from Abbott Diabetes Care Ltd, Oxon, UK.

### Laboratory animals

Healthy young adult Wister 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. These animals were divided into the control, reference and three test drug groups of six animals each (n=6).

### Phytochemical screening

Phytochemical screening was carried out in the Phytopharmacology Laboratory of the Department of Pharmacology and Toxicology of Kampala International University–Western Campus. Qualitative phytochemical tests were conducted on SCE using standard screening methods.<sup>12</sup>

**Dragendorff's test** - is used for the detection of alkaloids. An aliquot of the extract (about 100 mg) was dissolved in 1 ml of 1% hydrochloric acid. The acid-aqueous solution was then tested with 3 drops of Dragendorff's reagent. If the reactants turned opalescent it was considered low positive (+), turbid is fairly positive (++) and a precipitate is highly positive (+++).

**Resins test** - about 100 mg of the extract was reconstituted in 2 mL of 70% ethanol, 10 mL distilled water was added. A precipitate means the presence of resins.

**Fehling's test** - detects reducing sugars. The extract aliquot (about 100 mg) was dissolved in 2 mL distilled water. Two mL Fehling's reagent was added and the mixture heated in water bath for 5-10 min. The test was considered positive when the color of the solution turned red or there was a red precipitate.

Note-Fehling's solutions A and B were prepared immediately before use.

**Foam assay** - Permits to recognize the presence of saponins, either of steroidal type or triterpenoids. The volume of an ethanol aliquot was diluted 5 times its volume in water and shaken strongly for 5–10 min. The test is positive if there is a 2 mm high foam on the surface of the solution and it persists for 5–10 min.

**Ferric chloride assay**- detects the presence of phenolic compounds and/or tannins in the plant extract. The assay would detect phenols and tannins. Three drops of the 5% ferric chloride solution in physiologic saline were added to the extract aliquot (about 100 mg in 2 ml distilled water).

A positive assay is revealed by the following observations:

- Red-wine color, phenolic compounds in general.
- Dark green color, pirocatecolic type tannins.
- Blue color, pirogalotanic type tannins.

**Ninhydrine assay**- It recognizes the presence of free amino acids or amines in plant extracts. An aliquot (about 100 mg in 2 ml distilled water) from the extract was mixed with 2 mL 2% ninhydrine solution in water. The mixture was heated for 5-10 min in the water bath. This assay was considered positive when it developed a blue-purple color.

**Antocyanidine assay**- recognizes the C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> sequence structure characteristic of the flavonoid group. A 2 mL aliquot of the ethanolic extract was warmed with 1 mL concentrated hydrochloric acid for 10 min. It was allowed to cool and 1 mL of water and 2 mL amyl alcohol were added. The mixture was shaken to mix the phases and after, allowed to rest and separate. A positive assay was indicated by a red to brown color of the amyl phase.

**Shinoda Assay** - detects the presence of flavonoids in the plant extract. An aliquot was reconstituted in methanol, diluted with 1 mL concentrated hydrochloric acid and a small strip of metal magnesium. After 5 min of reaction, 1 mL amyl alcohol was added; the phases mixed and allowed to rest until they separated again.

The assay was considered positive for the presence of flavonoids, if the amyl alcohol turned dark yellow, orange, red or brown.

**Borntreger's test** - permits the identification of quinines. About 100 ml of the extract was dissolved in 2 mL chloroform. 1 mL of 5% sodium hydroxide, potassium hydroxide or ammonium hydroxide in water was added. It was shaken to mix the phases and allowed to rest until phases separated. If the upper alkaline aqueous phase turned pink (++) or red (+++), it was considered positive.

### Phytochemical quantification

The quantities of the phytochemical components present in the crude herbal material (powdered stem bark) of *Spathodea campanulata* were determined using the corresponding standard methods. For the alkaloids,<sup>13</sup> flavonoids,<sup>14</sup> saponins<sup>15</sup> and tannins<sup>16</sup> the corresponding methods were used.

**Alkaloid determination using Harborne (1998) method:** An aqueous extract of an equivalent of 10 g of the powdered herbal sample in a 500 ml beaker was concentrated on a water bath to one-quarter the original volume. Concentrated ammonium hydroxide was then added drop-wise until precipitation was formed and completed. The whole solution was allowed to settle, precipitate and was collected by decanting. The precipitate was further washed with dilute ammonium hydroxide and then filtered. The residue which is the alkaloid was dried and weighed. This was expressed as a percentage of the powdered plant material.

**Saponin determination Using Obadoni and Ochuko (2001) Method:** The aqueous extract from 10 g of herbal sample was reduced to 80 ml over water bath at about 90°C and the concentrate transferred into a 250 ml separating funnel. 40 ml of diethyl ether was added and shaken vigorously, the aqueous layer was recovered and the ether layer discarded. This purification process was repeated and then, 60 ml of n-butanol added to extract repeatedly. The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride; the remaining solution heated in a water-bath to evaporation. The residue (saponin) was dried in the oven to a constant weight which was calculated and expressed as a percentage of the plant material.

**Tannin determination:** Tannins are known to form colloidal solution in water and are soluble in alcohol, glycerine and dilute alkalis but practically insoluble in most organic solvents except acetone, and are precipitated from solutions by strong potassium dichromate or chromic acid solution.<sup>16</sup> Thus, aqueous extract of 10 g of the herbal sample in a 250 ml beaker was extracted repeatedly with an organic solvent (chloroform or petroleum ether), the aqueous layer recovered and chloroform extract discarded. Using strong potassium dichromate or chromic acid tannins are precipitated, washed with organic solvent (chloroform, hexane or petroleum ether) and dried to a constant weight. The outcome was expressed as a percentage of the crude plant material.

**Flavonoid determination by the method of Bohm and Kocipai-Abyazan (1994)** 10 g of the plant sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The mixture was shaken continuously for 12 h on a laboratory rotator at a speed of 100 rates per minute and then left to stand for 24 h. The mixture was filtered using Whatman No.1 filter paper and then dried in the hot air oven for 20 h. The weight of the extract was then determined. The extract was extracted twice with 20 ml portions of ethyl acetate and thereafter extracted with 10 ml of amyl alcohol. The ethyl acetate mixture was dried in a hot air oven at 70°C and weighed to determine the amount of flavonoids present.

## TLC characterization

The phytochemical characterization of SCE was done through solvent separation, thin layer chromatography and spectrophotometric quantification of the solvent fractions. Briefly, a 100 g dried methanolic extract (SCE) was reconstituted in distilled water and serially extracted in a one liter separating funnel between the solvents hexane, ethyl acetate and chloroform in this order according to increasing polarity. This solvent extraction was done with each solvent repeatedly until the color of the fraction was completely extracted. The aqueous fraction was retained in the funnel. The solvent fractions were concentrated by drying in an electrical oven (Mermert\*) at 40°C and further subjected to TLC and the retardation factor ( $R_f$ ) values of the separated phases determined. Briefly, the mobile phases were prepared by mixing the solvents in increasing ratios to obtain graded changes in polarity. Such were for example, hexane 9: ethylacetate 1 (HX9:EA1). The concentrated fractions were spotted on the ready-made TLC plates (Merck, Germany). The plates were placed in the mobile phase in TLC jar. The migration distance was predetermined at 8 cm. Movement was stopped when the mobile phase

reached the 8 cm limit. The plate was removed dried, the distance moved by the spot measured and the retardation factor ( $R_f$ ) value calculated. The relation used to calculate the  $R_f$  value was  $R_f = \text{distance moved by spot (cm)} / \text{distance moved by solvent front (cm)}$

## Antidiabetic activity of fractions

The solvent fractions were separately tested for antidiabetic activity using a modification of the oral glucose tolerance test (OGTT) applied earlier.<sup>9</sup> Animals (healthy albino rats) were fasted over night and divided in to five groups of six rats each. Control animals were given 1 ml of distilled water orally via a canula. The positive control was given an oral dose (500 mg/kg) of metformin. *Spathodea campanulata* extract was given by oral canula in three doses 200, 400 and 800 mg/kg to test groups. 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.

### Statistical analysis

In the bioactivity test, the data were expressed as mean  $\pm$  SEM. Results obtained from these tests were analyzed using the statistical package for social sciences (SPSS) version 17. A one-way analysis of variance (ANOVA) and the Dunnett's post hoc test were used as the statistical tool for comparing the means difference between the control and experimental groups. The 95% confidence limit ( $p < 0.05$ ) was applied. The percentage reduction of glucose-induced hyperglycemia by the treatment and control groups was determined from the relation; percentage reduction =  $(\text{maximum glucose-induced hyperglycemia} - \text{glycemia at 4 h}) / \text{maximum glucose-induced hyperglycemia} \times 100$

## RESULTS

Phytochemical screening of SCE gave positive results for flavonoids, tannins, alkaloids, reducing sugars, saponins and amino acids, (Table 1). Phytochemical quantification of SCE yielded the following percentage values for the phytochemicals; alkaloids (7.5), flavonoids (10), saponins (17.8) and tannins (13.9). (Table 2). The  $R_f$  values were obtained for the different fractions according to the following observed spots; the hexane partition gave HX7EA3 (0.96 and 0.68) HX9EA1 (0.68 and 0.3), EA1ME9 (0.86 and 0.58), EA3ME7 (0.87), EA7ME3 (0.85) and EA9ME1 (0.85). The ethylacetate partition gave the following HX1EA9 (0.53, 0.34 and 0.18), HX3EA7 (0.59, 0.40 and 0.26). (Table 3). The aqueous partition did not give any visible spot.

The bioactivity studies indicated that the various fractions reduced glucose-induced hyperglycemia though there was no statistically significant reduction ( $p < 0.05$ ) (Table 4). Considering the percentage reduction of hyperglycemia, the control had a reduction of 44.7%. The hexane fraction had a seemingly dose-dependent reduction with 44.4% at 50 mg/kg, 48.2% at 100 mg/kg and 49% at 200 mg/kg. The ethylacetate fraction reduced hyperglycemia by 48.5% at 50 mg/kg, 46.5% at 100 mg/kg and 42.3% at 200 mg/kg. The residual aqueous fraction reduced hyperglycemia by 67.3% at 50 mg/kg, 53.4% at 100 mg/kg and 74.7% at 200 mg/kg. Metformin the standard euglycemic agent reduced the hyperglycemia by 48% at the dose of 500 mg/kg (Table 5). A graphical representation of the effects of the fractions on glucose-induced hyperglycemia is in Figure 1 (a-c). The relationships between the hexane, ethylacetate and aqueous fractions at the doses studied vis-à-vis the controls are depicted here.

## DISCUSSION

Traditional herbal medicines form an enormous source of drug discovery containing a diverse array of chemical substances.<sup>17</sup> They either consti-

**Table 1: Results of Phytochemical screening of the methanolic stem bark extract of *Spathodea campanulata***

Phytochemical	Presence Status
Flavonoids	+
Alkaloids	+
Tannins	++
Saponins	++
Reducing sugars	+
Amino Acids	+

**Table 2: Phytochemical quantification of the stem bark powder of *Spathodea campanulata***

Phytochemical	Amount (g)	Percentage
Alkaloids	0.75	7.5
Flavonoids	1.0	10
Saponins	1.78	17.8
Tannins	1.39	13.9

**Table 3: Thin layer chromatography results of *Spathodea campanulata* methanolic stem bark extract partitions**

Partition	Mobile Phase	Rf value	
Hexane	HX7EA3	0.96	
		0.68	
		0.68	
	HX9EA1	0.30	
		0.86	
		0.58	
	EA3ME7	0.87	
		0.85	
		0.85	
EA9ME1	0.85		
	Ethylacetate	HX1EA9	0.53
		0.34	
HX3EA7		0.59	
		0.40	
		0.26	

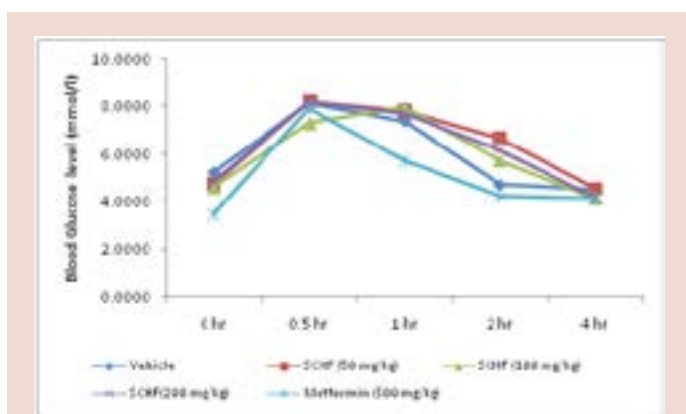
**Table 4: Antidiabetic activity of SCE fractions (Mean  $\pm$  SEM) n=6**

Treatment	Dose (mg/kg)	Blood Glucose levels (mmol/l)				
		0 hr	0.5 hr	1 hr	2 hr	4 hr
Control		5.22 $\pm$ 0.44	8.14 $\pm$ 0.39	7.38 $\pm$ 0.46	4.70 $\pm$ 0.52	4.50 $\pm$ 0.18
Hexane fraction	50	4.76 $\pm$ 0.31	8.20 $\pm$ 0.41	7.80 $\pm$ 0.11	6.68 $\pm$ 0.69	4.56 $\pm$ 0.50
	100	4.60 $\pm$ 0.33	7.30 $\pm$ 0.74	7.96 $\pm$ 0.50	5.72 $\pm$ 0.45	4.12 $\pm$ 0.35
	200	4.84 $\pm$ 0.25	8.20 $\pm$ 0.78	7.70 $\pm$ 0.37	6.16 $\pm$ 0.28	4.18 $\pm$ 0.25
Ethylacetate fraction	50	4.64 $\pm$ 0.38	8.58 $\pm$ 0.32	7.04 $\pm$ 0.34	4.54 $\pm$ 0.24	4.42 $\pm$ 0.16
	100	4.66 $\pm$ 0.41	8.78 $\pm$ 0.59	7.32 $\pm$ 0.19	5.94 $\pm$ 0.45	4.70 $\pm$ 0.21
	200	4.72 $\pm$ 0.40	8.26 $\pm$ 0.77	8.42 $\pm$ 0.76	6.40 $\pm$ 0.76	4.86 $\pm$ 0.34
Residual	50	12.84 $\pm$ 0.53	10.66 $\pm$ 0.89	6.40 $\pm$ 0.36	4.06 $\pm$ 0.43	3.48 $\pm$ 0.67
Aqueous fraction	100	9.90 $\pm$ 0.85	8.46 $\pm$ 0.37	6.16 $\pm$ 0.34	4.10 $\pm$ 0.25	3.94 $\pm$ 0.41
	200	4.48 $\pm$ 0.20	15.00 $\pm$ 0.20	6.32 $\pm$ 0.41	5.38 $\pm$ 0.39	3.80 $\pm$ 0.19
Metformin	500	3.48 $\pm$ 0.28	7.92 $\pm$ 0.31	5.72 $\pm$ 0.93	4.24 $\pm$ 0.59	4.12 $\pm$ 0.16

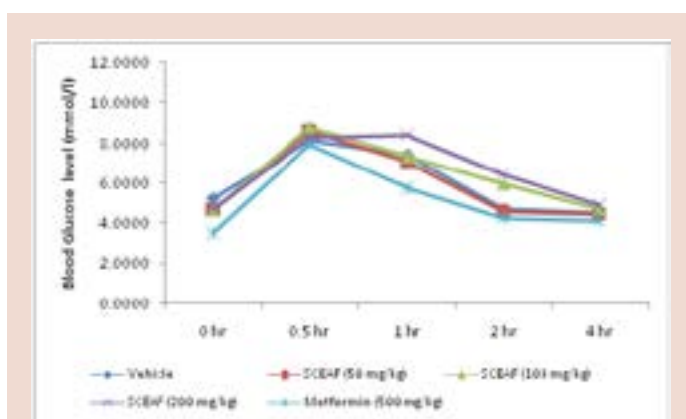
**Table 5: Percentage reduction of glucose-induced hyperglycemia by SCE fractions**

Treatment	Dose (mg/kg)	Percentage reduction of hyperglycemia
Control		44.7
Hexane fraction	50	44.4
	100	48.2
	200	49.0
Ethylacetate	50	48.5
	100	46.5
	200	42.3
Residual Aqueous	50	67.3
	100	53.4
	200	74.7
Metformin	500	48.0

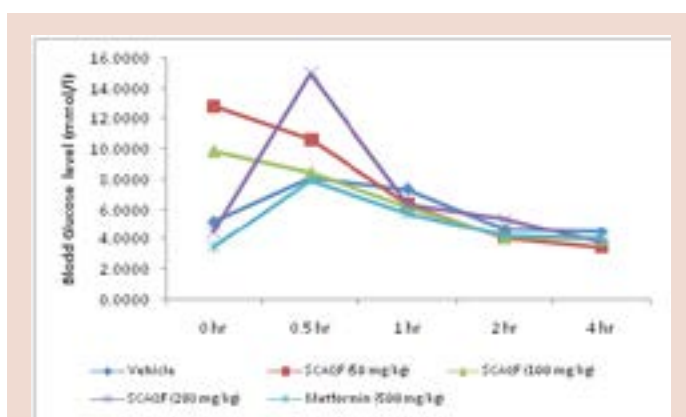




**Figure 1a:** Effect of *Spathodea campanulata* hexane fraction (SCHF) on glucose-induced hyperglycemia (mmol/l).



**Figure 1b:** Effect of *Spathodea campanulata* ethylacetate fraction (SCEAF) on glucose-induced hyperglycemia (mmol/l).



**Figure 1c:** Effect of *Spathodea campanulata* residual aqueous fraction (SCAQF) on glucose-induced hyperglycemia (mmol/l).

tute or are the basis of most of the lead compounds and candidate drugs that go through to approval even in recent times worldwide,<sup>6,18</sup> The preliminary phytochemical screening has revealed that *Spathodea campanulata* contains some secondary metabolites that have been

observed in other circumstances to possess various forms of biological activity. Such as flavonoids, tannins, saponins, alkaloids which are observed to possess antioxidant, antidiarrhoea, antimalarial and antibacterial effects.<sup>19,20</sup> Although these phytochemicals are found in the methanolic stem bark extract they are similar to those in other parts like the leaves and flowers. While the presence of these secondary metabolites may be indicative of potential biological activity, for the purpose of assessing the value of these potentials it is necessary to quantify them. The quantification study which was done on the stem bark revealed the predominant presence of saponins followed by tannins, then flavonoids and alkaloids in this order. The quantities of the phytochemicals obtained per unit weight of crude extract were indicative of their high potential economic values since the values ranged between 7.5 and 17 percent per gram of dry extract. Quantity is part of a three star assurance in traditional medicine standardization including safety and efficacy.<sup>21</sup> It is therefore very necessary to establish the existing quantities of various phytochemicals in order to note the reliability of the active ingredients as well as determine optimal use strategies for the medicine. This is the first time the phytochemical quantities were established in the stem bark of *Spathodea campanulata*.

Retention factor ( $R_f$ ) values have been determined for various spots that indicate separated components of the solvent fractions. This procedure will further lead to identifying as close as possible the chemical(s) responsible for the observed antidiabetic effect in a recent study.<sup>9</sup> Elsewhere GC-MS technique has been used to identify the chemicals in the extracts from the flowers and leaves<sup>21</sup> but this study explored the content of the stem bark as it is used in folk medicine in Uganda. When the standardization is done it will lead to proper use and ensure the safety and efficacy of the medicinal plant as well as gain recognition and acceptance worldwide.<sup>22</sup>

When the antidiabetic study was carried out on the fractions at a quarter of the doses of the crude extract the percentage reduction of glycaemia appeared to be better though the activity is shared in the different fractions. This was in line with earlier speculations that purification of the crude extract could improve on the antidiabetic potency of *Spathodea campanulata* in a recent related study.<sup>9</sup> In comparing the activity of the extract fractions, it was evident that the hexane fraction reduced hyperglycemia in a dose-dependent manner which is in consonance with fundamental pharmacological principles. The aqueous fraction produced an activity with a tendency to be dose-dependent also. The activity of the ethylacetate fraction seemed to be inversely dose-dependent. The standard metformin performed better than the control though at some doses the test drug fractions were more potent. This could be explained from the basic knowledge that metformin is referred to as a euglycemic agent returning hyperglycemia to normal and not capable of causing hypoglycemic shock. The experimental duration was limited to four hours in consonance with local laboratory experience. Since the animals were starved overnight longer experimental durations left them hypoglycemic because even during the experiment they were not fed. Earlier studies with similar durations proved successful<sup>9</sup> though elsewhere longer periods up to six hours were practiced.<sup>23-25</sup>

## CONCLUSION

SCE is rich in phytochemicals such as tannins, flavonoids, glycosides, reducing sugars, alkaloids, proteins, and saponins. The rank order of the extract content in increasing quantities is alkaloids, flavonoids, tannins and saponins. The hexane and ethylacetate fractions contain multiple chemical entities which are responsible for its antidiabetic activity. All the three fractions tested possess antihyperglycemic activity with the hexane fraction exhibiting a clearer dose-dependent activity.

## LIMITATIONS AND PROSPECTS

The separation of the constituents by TLC and the determination of their  $R_f$  values needs to be taken further towards a vivid identification of the chemical entities. This could be done by use of standard charts. There is the need to proceed to proper purification, Identification and structure elucidation using modern technological tools like high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS), Fourier Transform infra red or MS (FT-IR/MS). It is worth noting that the TLC visualization was done with UV light and some phytochemicals may not be fluorescent under UV light and could be missed out.

## ACKNOWLEDGEMENTS

Rukararwe Traditional Healers Partnership in Bushenyi, Uganda; for providing the plant material.

Kyumugasho Syson (Ms.) was a good initiator of the collaboration arrangement with the Rukararwe Traditional Healers Partnership.

Kampala International University–Western Campus authorities; for allowing us free access to the Department of Pharmacology and Toxicology Laboratories.

Kiiza Ronald of the Pharmacology and Toxicology laboratories KIU Western campus; for his untiring assistance. Claude Kirimuhuzya, Kiplagat Magoi, Komakeich Aboda-KIU Western Campus; Prof. Armando Cuellar Cuellar–Faculty of Pharmacy, University of Havana, Cuba for their assistance in phytochemical studies.

Ntare Moses of the department of Public Health, Mabarara University of Science and Technology (MUST) for his guide on statistical analysis.

Kiven Oliver, Kumbo Cameroon for providing the much needed logistics for the manuscript preparation.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest, financial or otherwise. The research project is facilitated by the authors supported by VLIR-UOS via the HEFS Platform (ZIUS2013VOA0902). It is part of the PhD research project to be presented for its award by Tanayen Julius Kihdze of Mbarara University of Science and Technology (MUST), Uganda.

## ABBREVIATION USED

**TLC:** Thin layer chromatography, **SCE:** *Spathodea campanulata* stem bark methanolic extract, **GC-MS:** Gas chromatography-mass spectrometry, **HX:** Hexane, **EA:** Ethylacetate, **ME:** Methanol, **OGTT:** Oral glucose tolerance test, **R<sub>f</sub>:** Retardation factor, **HPLC:** High performance liquid chromatography, **GC:** Gas chromatography, **MS:** Mass spectrometry, **FT-IR/MS:** Fourier Transform infra red or Mass Spectrometry, **UV:** ultra violet.

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## SUMMARY

- Spathodea campanulata* methanolic stem bark methanolic extract contains secondary metabolites comprising of 7.5% alkaloids, 10% flavonoids, 13% tannins and 17% saponins per gram of plant material.
- The TLC characteristics of the fractions (hexane (HX), ethylacetate (EA) and methanol (ME)) viewed under UV light revealed spots with the following  $R_f$  values; the hexane partition gave HX7EA3 (0.96 and 0.68) HX9EA1 (0.68 and 0.3), EA1ME9 (0.86 and 0.58), EA3ME7 (0.87), EA7ME3 (0.85) and EA9ME1 (0.85).
- The ethylacetate partition gave the following HX1EA9 (0.53, 0.34 and 0.18), HX3EA7 (0.59, 0.40 and 0.26).

### PICTORIAL ABSTRACT



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