

## Hypoglycemic Activity of Aqueous and Ethylacetate Leaf and Stem Bark Extracts of *Pappea capensis* in Alloxan-Induced Diabetic BALB/c Mice

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**Abstract:** The present study was performed to determine the optimal dose of alloxan monohydrate required to induce diabetes in male BALB/c mice and investigate *in vivo* hypoglycemic activity of aqueous and ethylacetate leaf and stem bark extracts of *Pappea capensis* L in alloxanized diabetic BALB/c mice. In addition, the proximate composition of *P. capensis* powder was investigated. The seven groups used in determining the optimal alloxan dose to induce diabetes included the normal mice intraperitoneally administered with a single dose of 0.1 mL physiological saline and doses of 50.0, 77.6, 120.4, 186.9, 290.0 and 480 mg/kg body weight in 0.1 mL of physiological saline. Blood glucose levels were determined at 0, 24 and 48 h using a glucometer. The hypoglycemic activity of aqueous and ethylacetate extracts was studied in the normal and diabetic mice orally administered with 0.1 mL physiological saline; diabetic mice orally administered with 0.075 mg glibenclamide, 1.25, 2.5 and 5 mg extract all in 0.1 mL physiological saline. Blood glucose levels were determined at 0, 2, 4, 6 and 24 h, respectively. The proximate composition of *P. capensis* powder was estimated using standard procedures. Results show that a single dose of alloxan at 186.9 mg/kg body weight administered to 3-5 weeks old mice induced stable diabetes in 48 h; oral administration of ethylacetate leaf and stem bark extracts at 100 and 200 mg/kg body weight induced hypoglycemic activity in a dose independent manner which was similar to that of glibenclamide at 3 mg/kg body weight from the second to the 24<sup>th</sup> h. Total ash and lipid were higher while the crude protein and carbohydrate were lower in leaves compared to the stem barks. In conclusion, *P. capensis* is a nutritious plant whose ethylacetate extracts possess *in vivo* hypoglycemic activity.

**Keywords:** Alloxanized BALB/c mice, diabetes mellitus, ethylacetate extracts, hypoglycemic activity, *Pappea capensis*

### INTRODUCTION

Diabetes Mellitus (DM) is the most severe and challenging metabolic pandemic of the 21<sup>st</sup> century, because it affects essential biochemical activities in almost every cell in the body and increases the risk of cardiac and renal disorders. This pandemic is characterized by excessive blood glucose due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced to control blood glucose (Davis and Granner, 1996). This disorder affects carbohydrate, protein and fat metabolism (Davis and Granner, 1996) and chronic hyperglycemia causes glycation of body proteins that in turn leads to secondary complications that affects eyes, kidneys, nerves and arteries (Sharma, 1993). The worldwide survey reported that DM is affecting nearly 10% of the

population (Siddharth, 2001). The treatment of DM is based on oral hypoglycemic agents and insulin injections. The hypoglycemic agents currently used in clinical practice have characteristic profiles of adverse side effects (Williams and Pickup, 1991). However, DM is also managed in Kenyan communities using hypoglycemic medicinal plants that are affordable to the rural communities of the underdeveloped and developing countries and are thought to be safe. Jacket plum or wild plum, *Pappea capensis* (L.) is one of the medicinal plants used in the management of diabetes.

*Pappea capensis* (L.) is a deciduous or evergreen tree that grows up to 3.9 m tall and belongs to the Litchi family Sapindaceae (Van Wyk and Gericke, 2000). This plant is fairly adapted to a wide range of ecological areas and it is known to be drought-tolerant, thus able to grow in marginal lands. The leaves are

simple and oblong, hard-textured and wavy. New leaves are an attractive pinky-bronze when they emerge in spring and this contrasts well with the dark green colour of the old leaves (Mng'omba *et al.*, 2007). *P. capensis* is widespread in southern Africa from the Northern Cape through the drier Karoo, Eastern Cape, KwaZulu-Natal, to the Northern provinces, as well as Mozambique, Zimbabwe and northwards into eastern and southern tropical Africa (Fivaz and Robbertse, 1993; Van Wyk and Gericke, 2000; Mng'omba *et al.*, 2007). In Kenya, it is distributed in Lukenya hills, Ngong hills, northern Kapenguria and semi-arid regions of southern part of Embu County such as Siakago.

The Jacket plum leaves can be processed into vinegar, jelly and jam (Palmer and Pitman, 1972). Seeds are rich in edible, non-drying and fairly viscous oil which constitutes about 74% and is used for making soap and oiling guns (Van Wyk and Gericke, 2000). It is a good fodder for livestock and produces edible fruits. Among Kenyan communities the boiled stem barks are used traditionally to treat whooping cough and sparingly the leaves are used in the management of diabetes mellitus.

Phytochemical studies on *P. capensis* revealed that it contains polyphenols, saponins, flavonoids, tannins, carotenoids, retinol, tocopherol, ascorbic acid, riboflavin and thiamin and mineral elements among them selenium, zinc and magnesium in substantial amounts (Karau *et al.*, 2012). In the forkloric set up, hot water extract of *P. capensis* has been reported to manage DM, however, no documented scientific investigation has been conducted to ascertain the validity of this claim. Therefore, this study was carried out to investigate the optimum dose for induction of diabetes mellitus in male BALB/c mice and determine the *in vivo* hypoglycemic activity of aqueous and ethylacetate leaf and stem bark extracts of *P. capensis* in alloxanized diabetic BALB/c mice. In addition, the proximate composition of *P. capensis* leaf and stem bark powders were determined.

## MATERIALS AND METHODS

**Study site:** This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University in January 2012. Kenyatta University is 23 km from Nairobi off Thika Road.

**Collection of plant materials:** Green leaves and stem barks of *P. capensis* were collected in March 2011 from Kambara village, Siakago in Embu County of Kenya. The plant was authenticated by a taxonomist at the Department of Plant and Microbial Sciences, Kenyatta

University, Kenya and a voucher specimen deposited at the Kenyatta University Herbarium for future reference.

**Preparation of the plant powder:** The stem barks were peeled off while still fresh and cut into small pieces and then dried under shade at room temperature for one month. The leaves were collected while green and also dried under shade for one month. The dried stem barks and the leaves were separately ground using an electric mill. The powdered plant materials were kept at room temperature away from direct sunlight in closed, dry plastic bags.

One hundred grams of each powdered plant material was extracted in 1 L of distilled water at 60°C in a metabolic shaker for 6 h. After extraction, the extract was decanted into a clean dry conical flask and then filtered through folded cotton gauze into another clean dry conical flask. The filtrate was then freeze dried in 200 mL portions using a Modulyo Freeze Dryer (Edward, England) for 48 h. The freeze-dried powder was then weighed and stored in an airtight container at -20°C until used for bioassay.

**Proximate composition:** Proximate composition includes moisture, crude protein, ether extract (fat content), crude fiber, ash and Nitrogen Free Extract (NFE). The composite dried samples were prepared and weighed for proximate analysis. Moisture was determined by oven dehydration method at 105°C until a constant weight of dry matter was achieved. Crude protein was determined by estimating the nitrogen content of the plant materials, using Kjeldhal method. Crude fat was determined by ether extraction method using soxhlet technique. Crude fiber was determined by acid digestion and alkali digestion and by using Foss fibertech. Ash content was determined in muffle furnace at 550°C for 6 h. For all these determinations, powdered samples were used in triplicate in accordance with AOAC (2010). NFE was calculated by difference to represent the soluble carbohydrate in the extract. Gross energy calculation for the powder was derived by multiplying the percentage of crude protein by 5.65, soluble carbohydrate by 4 and crude fat by 9.4 (Charrondiere *et al.*, 2004).

**Experimental animals:** The study used male BALB/c mice (3-5 weeks old) that weighed 20-30 g with a mean weight of 25 g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. The mice were housed at a temperature of 25°C with 12 h light/12 h darkness photoperiod and fed on rodent pellets (Unga Feeds Limited, Kenya) and water *Ad libitum*. The experimental protocols and procedures used in this

study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University.

**Optimization of the alloxan dose to induce diabetes:**

The identification of the intraperitoneal optimum dose of alloxan to induce diabetes was performed using a logarithmic scale (Thomson, 1985) running from 50 to 480 mg/kg body weight with 6 dose levels at 50, 77.6, 120.4, 186.9, 290.0 and 480.0 mg/kg body weight, respectively. The doses were administered once intraperitoneally at each level to five BALB/c mice. The animals were monitored for changes in blood sugar within 24 h and after 48 h. Within this period any fatality observed in any dose level was recorded. After 48 h, the diabetic animals were examined for suitability in the bioassays by determining the consistency of blood glucose within 24 h. This was done by measuring blood glucose levels after every 2 h.

**Induction of hyperglycemia:**

Hyperglycemia was induced experimentally by a single intraperitoneal administration of 186.9 mg/kg body weight of a freshly prepared 10% alloxan-monohydrate (2, 4, 5, 6 tetraoxypyrimidine; 5-6-dioxuracil) obtained from Sigma (Sigma Chemical, St. Louis, OH). Forty-eight h after alloxan administration, blood glucose level was measured using a glucometer (Contour® TS, Bayer Pty. Ltd; Healthcare Division, Japan). Mice with blood glucose levels above 2000 mg/L (>11.1 mMol/L), were considered diabetic and were used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 h (Szkudelski, 2001), but allowed free access to water until the end of the experiment.

**Experimental design:**

The mice were randomly divided into six experimental groups of five mice each. These groups included: the normal unmanipulated mice (the reference group of the experiment) orally administered with 0.1 mL physiological saline; the alloxan-induced diabetic control mice (the negative control group) orally administered with 0.1 mL physiological saline, alloxan-induced diabetic control mice, orally administered with 0.1 mL of the reference drug glibenclamide (3 mg per kg body weight, positive control group) and alloxan induced diabetic experimental mice treated with 0.1 mL physiological saline containing 1.25, 2.50 and 5.00 mg of plant extracts, respectively (50, 100 and 200 mg of plant extracts per kg body weight, respectively).

**Preparation of extracts for injection in mice:**

The appropriate doses of freeze-dried plant extracts were made by dissolving 125 mg (to inject at 50 mg/kg body

weight), 250 mg (to inject at 100 mg/kg body weight) and 500 mg (to inject at 200 mg/kg body weight), in 10 mL physiological saline, respectively. Glibenclamide, a sulfonyl urea was prepared by dissolving 7.5 mg (to inject at 3 mg/kg body weight) in 10 mL of physiological saline. (0.1 mL) of the plant extract and reference drug solution was orally administered to each mouse according to the experimental design.

**Blood sampling:**

Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at the start of the experiment and this was repeated after 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup> and 24<sup>th</sup> h, respectively. Every time the blood glucose levels were determined with a glucometer (Contour® TS, Bayer Pty, Ltd; Healthcare Division, Japan).

**Data management and statistical analysis:**

Data was entered in the Microsoft® Excel spread sheet, cleaned and then exported to Statistical Package for Social Sciences (SPSS Version 17.0) software for analysis. Results were expressed as Mean±Standard Deviation (S.D.) of the blood glucose levels per the number of animals used in every study point. One-way ANOVA and post-ANOVA (Bonferroni-Holm) test was used to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drug and diabetic mice treated with plant extracts at doses of 50, 100 and 200 mg/kg body weight. Student's t-test was used to compare the means of proximate composition between the leaf and stem bark powders.  $p \leq 0.05$  was considered statistically significant.

## RESULTS

The yield of the lyophilized aqueous leaf and stem bark extracts were 17.69 g/100 g and 16 g/100 g, respectively, while the yield of the lyophilized ethylacetate leaf and stem bark extracts were 3.28 g/100 g and 2.81 g/100 g, respectively. Table 1 shows the results of optimization of an intraperitoneal dose of alloxan-monohydrate required to induce diabetes in male BALB/c mice. Results shows that the optimal and safe intraperitoneal dose of alloxan-monohydrate required to induce diabetes is 186.9 mg/kg body weight after 48 h. Doses below 186.9 mg/kg body weight had no effect on the blood glucose levels even after 48 h; the blood glucose levels of the experimental mice were similar to those of the control mice for all the tested doses. Intraperitoneal alloxan doses of 290.0 and 480 mg/kg body weight induced blood glucose levels outside the determination range of the glucometer; this was also accompanied by the death of one mice at the

Table 1: Optimization of an intraperitoneal dose of alloxan-monohydrate required to induce diabetes in BALB/c mice

Mice groups	Body weight (g)	Blood glucose (mmol/L) per day		
		0	1	2
Control group	24.20±4.66	7.83±1.00	7.91±0.25	7.57±0.40
50 mg/kg bw	19.8±1.92	7.22±0.73	6.30±0.67	7.13±2.21
77.6 mg/kg bw	21.2±2.59	8.34±1.28	8.38±1.11	8.00±1.70
120.4 mg/kg bw	16.67±3.21	7.41±0.37	6.97±0.91	6.46±1.50
186.9 mg/kg bw	15.00±3.24	7.42±1.26	8.38±0.93	32.54±6.25 <sup>Abc</sup>
290.0 mg/kg bw	17.00±1.87	7.51±1.20	7.66±0.14	32.98±12.73 <sup>Abc</sup>
480.0 mg/kg bw	17.20±1.64	7.42±1.26	9.62±1.94	30.68±13.55 <sup>Abc</sup>

Results are expressed as Mean±Standard Deviation (S.D.) for five animals per dose; Means followed by similar upper case letters in the same column are not significantly different at p≤0.05 by ANOVA and post ANOVA (Bonferroni-Holm) test

Table 2: Hypoglycemic effects of oral administration of ethylacetate leaf extracts of *Pappea capensis* in alloxan-induced diabetic BALB/c mice

Mice group	Treatment	Blood glucose levels (mmol/L)				
		0 h	2 h	4 h	6 h	24 h
Normal control	Saline	5.5±0.3	6.3±0.3	6.2±0.1	5.8±0.3	5.1±0.1 <sup>B</sup>
Diabetic control	Saline	24.8±3.1 <sup>A</sup>	26.6±3.2 <sup>A</sup>	27.0±2.6 <sup>A</sup>	27.7±2.8 <sup>A</sup>	29.3±2.6 <sup>C</sup>
Diabetic control	Glibenclamide (3 mg/kg body weight)	28.3±1.4 <sup>B</sup>	24.8±2.0 <sup>A</sup>	18.3±3.4	11.3±2.0 <sup>cf</sup>	6.2±0.8 <sup>dgi</sup>
Diabetic treated	50 mg/kg body weight	28.0±1.9 <sup>B</sup>	26.6±1.6 <sup>A</sup>	25.7±1.3 <sup>A</sup>	24.3±1.3 <sup>A</sup>	22.0±1.3 <sup>B</sup>
	100 mg/kg body weight	32.7±0.4 <sup>B</sup>	31.0±0.6 <sup>A</sup>	28.3±0.9 <sup>Ab</sup>	24.7±1.4 <sup>Acf</sup>	18.1±0.7 <sup>Bdgi</sup>
	200 mg/kg body weight	28.4±1.4 <sup>B</sup>	22.7±2.3	19.4±1.9 <sup>b</sup>	15.0±2.0 <sup>c</sup>	9.8±0.6 <sup>Adgi</sup>

Results were expressed as Mean±Standard Error of Mean (SEM) of five mice per group; Means followed by similar upper case letters in the same column are not significantly different at p≤0.05 by ANOVA and post ANOVA (Bonferroni-Holm) test; <sup>a</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 2<sup>nd</sup> h; <sup>b</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 4<sup>th</sup> h; <sup>c</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 6<sup>th</sup> h; <sup>d</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 24<sup>th</sup> h; <sup>e</sup>: p≤0.05 when blood glucose levels at 2 h is compared to blood glucose at 4<sup>th</sup> h; <sup>f</sup>: p≤0.05 when blood glucose levels at 2<sup>nd</sup> h is compared to blood glucose at the 6<sup>th</sup> h; <sup>g</sup>: p≤0.05 when blood glucose levels at 2<sup>nd</sup> h is compared to blood glucose at the 24<sup>th</sup> h; <sup>i</sup>: p≤0.05 when blood glucose levels at the 4<sup>th</sup> h is compared to blood glucose at the 24<sup>th</sup> h; <sup>j</sup>: p≤0.05 when blood glucose levels at the 6<sup>th</sup> h is compared to blood glucose at the 24<sup>th</sup> h by ANOVA and post ANOVA (Bonferroni-Holm) test

Table 3: Hypoglycemic effects of oral administration of ethylacetate stem bark extracts of *Pappea capensis* in alloxan-induced diabetic BALB/c mice

Mice group	Treatment	Blood glucose levels (mmol/L)				
		0 h	2 h	4 h	6 h	24 h
Normal control	Saline	5.1±0.6	6.3±0.3	6.1±0.6	4.7±0.3	5.7±0.5
Diabetic control	Saline	16.5±0.3 <sup>A</sup>	21.2±1.9 <sup>B</sup>	23.8±1.7 <sup>Abc</sup>	19.3±2.5 <sup>A</sup>	30.2±1.1 <sup>Adgi</sup>
Diabetic control	Glibenclamide (3 mg/kg body weight)	20.6±2.9 <sup>A</sup>	15.3±2.4 <sup>B</sup>	4.6±0.7 <sup>bc</sup>	4.5±0.4 <sup>cf</sup>	4.0±0.4 <sup>dg</sup>
Diabetic treated	50 mg/kg body weight	17.4±0.5 <sup>A</sup>	14.5±1.7 <sup>B</sup>	14.5±4.9	11.7±4.4	13.9±4.9
	100 mg/kg body weight	16.8±0.5 <sup>A</sup>	7.6±0.2 <sup>aa</sup>	7.3±0.5 <sup>b</sup>	6.5±0.5 <sup>c</sup>	6.2±0.3 <sup>dg</sup>
	200 mg/kg body weight	18.1±2.2 <sup>A</sup>	11.6±0.8 <sup>B</sup>	6.4±0.6 <sup>bc</sup>	6.1±0.4 <sup>cf</sup>	6.9±0.3 <sup>dg</sup>

Results were expressed as Mean±Standard Error of Mean (SEM) of five mice per group; Means followed by similar upper case letters in the same column are not significantly different at p≤0.05 by One-Way ANOVA and post ANOVA (Bonferroni-Holm) test; <sup>a</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 2<sup>nd</sup> h; <sup>b</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 4<sup>th</sup> h; <sup>c</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 6<sup>th</sup> h; <sup>d</sup>: p≤0.05 when blood glucose levels at 0 h is compared to blood glucose at the 24<sup>th</sup> h; <sup>e</sup>: p≤0.05 when blood glucose levels at the 2<sup>nd</sup> h is compared to blood glucose at the 4<sup>th</sup> h; <sup>f</sup>: p≤0.05 when blood glucose levels at the 2<sup>nd</sup> h is compared to blood glucose at the 6<sup>th</sup> h; <sup>g</sup>: p≤0.05 when blood glucose levels at the 2<sup>nd</sup> h is compared to blood glucose at the 24<sup>th</sup> h; <sup>i</sup>: p≤0.05 when blood glucose levels at the 4<sup>th</sup> h is compared to blood glucose at the 24<sup>th</sup> h by ANOVA and post ANOVA (Bonferroni-Holm) test

480 mg/kg body weight on the second day post alloxan administration.

As depicted in Table 2 and 3, the ethylacetate leaf and stem bark extracts lowered blood glucose levels in a dose independent manner; however, the 100 and 200 mg/kg body weight ethylacetate leaf extracts doses significantly lowered blood glucose levels from the second to the twenty-fourth h. Similarly, the 100 and 200 mg/kg body weight ethylacetate stem bark extracts

doses significantly lowered blood glucose levels from the 2<sup>nd</sup> to the 24<sup>th</sup> h (Fig. 1). In the 2<sup>nd</sup> h, the 100 and 200 mg/kg body weight doses of the ethylacetate leaf extracts lowered the blood glucose levels by 5.28 and 20.58%, respectively, while the reference drug, glibenclamide lowered blood glucose levels by 13.12%. At the 24<sup>th</sup> h, the 100 and 200 mg/kg body weight doses of the ethylacetate leaf extracts lowered the blood glucose levels by 44.58 and 66.96%, respectively, while

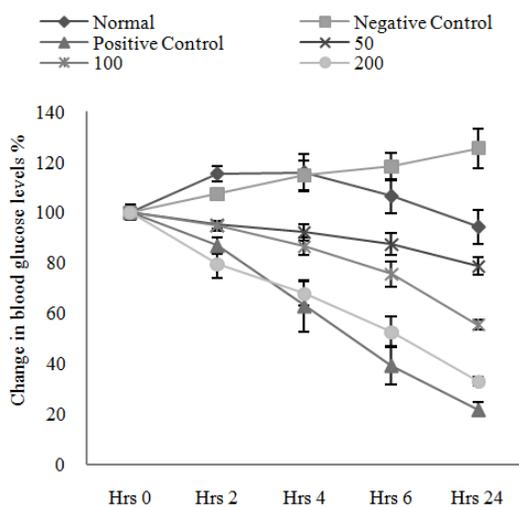


Fig. 1: Mean percentage change in blood glucose levels after oral administration of ethyl acetate leaf extracts of *P. capensis* in alloxan-induced diabetic male BALB/c mice. Values are expressed as % Means±SEM for five animals at each time point

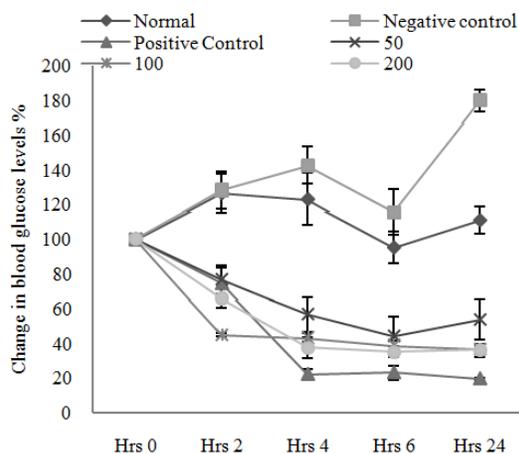


Fig. 2: Mean percentage change in blood glucose levels after oral administration of ethyl acetate stem bark extracts of *P. capensis* to alloxan-induced diabetic male BALB/c mice. Values are expressed as % means±SEM for five animals at each time point

the reference drug, glibenclamide lowered blood glucose levels by 78.13%.

In the 2<sup>nd</sup> h, the 100 and 200 mg/kg body weight doses of the ethyl acetate stem bark extracts lowered the blood glucose levels by 54.94 and 33.88%, respectively, while the reference drug, glibenclamide lowered blood glucose levels by 22.99%. At the 24<sup>th</sup>, the 100 and 200 mg/kg body weight doses of the

Table 4: Proximate composition of leaf and stem bark powder of *P. capensis*

Proximate composition	Leaf	Stem bark
Dry matter (g/100 g)	91.54±0.24	91.38±0.02
Moisture content (g/100 g)	8.46±0.42	8.61±0.01
Total ash (g/100 g)	5.33±0.15	3.33±0.07*
Crude protein (g/100 g)	8.66±0.53	9.72±0.41*
Lipid content (g/100 g)	1.13±0.12	0.67±0.06*
Crude fiber (g/100 g)	19.11±0.89	18.17±0.52
Nitrogen Free Extract (NFE) (g/100 g)	56.27±1.04	58.42±1.08*
Gross energy (kJ/100 g dry matter)	28877±513	29920±391

Results are expressed as Mean±Standard Deviation (S.D.) for three determinations; \*: p≤0.05 is considered statistically significantly different by student's t-test

ethyl acetate stem bark extracts lowered the blood glucose levels by 63.28 and 63.83%, respectively, while the reference drug, glibenclamide lowered blood glucose levels by 80.23% (Fig. 2). Oral administration of both the aqueous leaf and stem bark extracts of *P. capensis* at 50, 100 and 200 mg/kg body weight doses to alloxan-induced diabetic BALB/c mice had no significant effect on the blood glucose levels (data not shown).

As depicted in Table 4, the leaf powder of *P. capensis* contained significantly a higher total ash and lipid content and a significantly lower total protein and nitrogen free extract compared to the stem bark powders. The dry matter, moisture content, crude fiber and gross energy content were comparable for both the leaf and stem bark powders of the plant.

## DISCUSSION

The current study was undertaken to determine the *in vivo* hypoglycemic activity of aqueous and ethyl acetate leaf and stem bark extracts of *P. capensis* in alloxan-induced diabetic male BALB/c mice. The optimal and safe intraperitoneal dose of alloxan-monohydrate of 186.9 mg/kg body weight that induced diabetes on 8-12 h fasted mice in 48 h post administration observed in this study, differs from the optimal and safe dose of 160 mg/kg body weight required to intraperitoneally induce diabetes in overnight fasted male albino rats in 96 to 120 h as reported by Ashok *et al.* (2007). This could be explained by the fact that induction of diabetes depends on the animal species, route of administration and nutritional status. Animals kept on overnight fast are more susceptible to alloxan (Kastumata *et al.*, 1992), while glucose is known to protect the beta cells (Szkudelski, 2001). High glucose level prevents the superoxide derivative, that causes the cell damage (Martens *et al.*, 2005). However, the frequently used intravenous dose of alloxan to induce diabetes in rats is 65 mg/kg body weight (Boylan *et al.*, 1992). Additionally, when alloxan is given intraperitoneally or

subcutaneously, its effective dose must be 2-3 times higher than the dose of 65 mg/kg body weight (Ashok *et al.*, 2007). This dose range for rats agrees with the optimal and safe alloxan dose observed in this study of 186.9 mg/kg body weight. The death of one mouse (20%) observed on administration of alloxan at a dose of 480 mg/kg body weight could be associated with the fact that the range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic causing the loss of many animals (Szkudelski, 2001). This loss is suggested to be due to kidney tubular cell necrotic toxicity at high doses of alloxan (Lenzen *et al.*, 1996). Induction of diabetes using alloxan-monohydrate to male BALB/c mice avails a functional and physiological model for the study of hypoglycemic agents that could be used in the management of diabetes mellitus.

The observation that both the leaf and stem bark ethylacetate extracts at doses of 100 and 200 mg/kg body weight lowered blood glucose from the second to the 24<sup>th</sup> h indicates that these extracts have hypoglycemic constituents. These hypoglycemic constituents could be the polyphenols, alkaloids, flavonoids, saponins, tannins and steroids reported to be present in *P. capensis* (Karau *et al.*, 2012). Phenols, flavonoids, alkaloids, tannins, terpenoids and sterols have been associated with hypoglycemic activity (Elliot *et al.*, 2000). As reported by Glauce *et al.* (2004), flavonoids like myricetin, a polyhydroxylated flavonol has insulinomimetic properties and stimulates lipogenesis and glucose transport in the adipocytes, hence lowering blood sugar (Elliot *et al.*, 2000). Similar studies on *Pterocarpus marsupium* found epicatechin and catechin flavonoids to have anti-diabetic properties (Subramanian, 1981). The alkaloid 1-ephedrine promotes the regeneration of pancreas islets following destruction of the beta cells, hence restoring the secretion of insulin and thus corrects hyperglycemia (Elliot *et al.*, 2000). The tannin epigallo-catechin-3-gallate exhibits anti-diabetic activity as demonstrated by Broadhurst *et al.* (2000). Terpenoids are used by patients with high blood pressure and diabetes because they help to reduce diastolic blood pressure and lower the sugar level in blood (Hawkins and Ehrlich, 2006). Due to the presence of terpenoids, the leaves and seeds of *E. officinalis* are used in the treatment of diabetes (Treadway, 1994). The lowering of blood glucose levels by *P. capensis* in the same manner regardless of the dosage might suggest that the extracts may have been absorbed in the cell system through active transport where a particular concentration saturation of the extracts occurred resulting to the rest being excreted. The observation that the three tested doses of aqueous leaf and stem bark extracts of *P. capensis*

could not significantly affect the blood glucose levels in alloxan induced diabetic male BALB/c mice indicates that these extracts lacked constituents with hypoglycemic activity. This observation could be associated with differences in the ratios of the phytoconstituents in the aqueous extracts compared to the ethylacetate extracts. Aqueous extracts have phytochemical constituents' phenols, flavonoids, tannins, saponins, alkaloids, steroids and cardiac glycosides which have been reported to demonstrate hypoglycemic activity.

The observed hypoglycemic activity could also be associated with minerals such as iron, chromium, manganese, vanadium, molybdenum, zinc and magnesium which have been reported to be present in this plant (Karau *et al.*, 2012). Iron influences glucose metabolism and reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver (Niederer *et al.*, 1984). Chromium functions as a cofactor in insulin-regulating activities. It facilitates insulin binding and subsequent uptake of glucose into the cell and therefore decreases fasting glucose levels, improves glucose tolerance, lowers insulin levels and decreases total cholesterol in type II diabetic subjects (Mooradian *et al.*, 1994; Baker, 1996). Manganese is an activator and constituent of several enzymes like kinases and enzymes of oxidative phosphorylation (Friedman, 1987). Magnesium is a cofactor of the glycolytic enzyme hexokinase and pyruvate kinase. It also modulates glucose transport across cell membranes (Mooradian *et al.*, 1994; O'Connell, 2001). Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells (Song *et al.*, 1998). Zinc also influences glyceraldehyde-3-phosphate dehydrogenase, the enzyme involved in glycolysis (Manuel *et al.*, 2002). Molybdate is an effective antihyperglycemic agent in diabetics with severe insulin resistance. It is associated with substantial reduction of hyperinsulinaemia and an increase in pancreatic insulin stores. The glucose-lowering effect of molybdenum may be partly related to attenuation of hepatic glucose production and possibly also to increased glucose usage. Hence, molybdenum proves to be an effective blood glucose-lowering agent in severely diabetic patients (Reul *et al.*, 1997).

The presence of dry matter, crude fiber, moisture, total ash, total protein, lipids and soluble carbohydrate in *P. capensis* powder is an indication of the holistic nature of herbal medicine. The dry matter is an indication of total solids which include carbohydrates, fats, proteins, vitamins, minerals and antioxidants. The lipid content composed of triacylglycerols, phospholipids, cholesterol and cholesterolesters is

hydrolysed to fatty acids, lysophospholipids and 2-monoacylglycerols in the lumen of the intestine, absorbed and metabolized to yield energy. Cholesterol serves as a stabilizing component of cell membranes and as a precursor of bile salts and steroid hormones. The ash content is an indication of the mineral content; minerals are used by the human body for the proper composition of bone and blood and maintenance of normal cell function. They function along with vitamins as essential components in enzymes and coenzymes. The proteins present in this plant powder are used to synthesize the numerous body proteins and their catabolism results in the supply of energy (Robinson, 1978). The crude fiber composed principally of indigestible cellulose, hemicellulose and lignins and pectins, gums and mucilages is nutritionally beneficial in the small intestines since it aids in the absorption of trace elements in the gut and reduces the absorption of cholesterol (Le Veille and Sanberlich, 1966), reduces the risk of coronary heart disease, obesity and diabetes mellitus (Monago and Uwakwe, 2009); digestion of soluble fiber, undigested starch and sugars by bacterial flora in the normal human gut yields short chain fatty acids which are absorbed by the colonic epithelial cells of the gut and some travel to the liver through the hepatic portal vein where they are metabolized to yield energy. Carbohydrates composed principally of starch after digestion to monosaccharides are absorbed through the small intestines and catabolised to yield energy. Carbohydrates also provide the amino acids serine, glycine, alanine, glutamate, glutamine, aspartate and asparagine in addition to contributing to the sweetness, appearance and textural characteristics of many foods (Muhammad *et al.*, 2009). The low moisture content of *P. capensis* powder indicates the decreased perishability of this plant material since a high moisture content promotes susceptibility to microbial growth and enzyme activity which accelerates spoilage (Monago and Uwakwe, 2009). The caloric value (leaf powder, 28877kJ/100 g; stem bark powder, 29920 kJ/100 g) of *P. capensis* shows that this plant could be a reliable source of energy and can provide a large portion of the daily requirement of 10,460 to 12,552kJ for adults if large quantities are consumed.

### CONCLUSION

In conclusion, the findings of this study indicate that, the optimum alloxan-monohydrate dose that induced diabetes in 3-5 weeks old male BALB/c mice was 186.9 mg/kg body weight. Oral administration of ethylacetate leaf and stem bark extracts of *P. capensis* at doses of 100 and 200 mg/kg body weight

demonstrated hypoglycemic activity in a dose-independent manner comparable to that demonstrated by glibenclamide at 3 mg/kg body weight. The total ash and lipid content were higher while the crude protein and carbohydrate were lower in leaves compared to the stem barks of *P. capensis* powder. The demonstrated hypoglycemic activity could be associated with the phytonutrients present in *P. capensis*. The study recommends continued use of *P. capensis* in the management of diabetes.

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