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HYPOGLYCEMIC AND ANTIDIABETIC ACTIVITIES OF AQUEOUS EXTRACT OF PSEUDOLACHNOSTYLIS MAPROUNEIFOLIA VAR. MAPROUNEIFOLIA PAX (EUPHORBIACEAE) STEM BARK IN EXPERIMENTAL WISTAR RATS

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ABSTRACT
The present scientific work was designed to investigate potential hypoglycaemic and antidiabetic activities of aqueous extract of *Pseudolachnostylis maprouneifoilia var. maprouneifolia* stem bark and its soluble fractions in experimental Wistar rats. Results indicated, in normal treated animals, at the highest oral dose of 600 mg/kg body weight, aqueous extract reached significant reduction of blood glucose to 68.5±0.1 mg/dl while its chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions reduced blood glucose level from 81.5.±0.2 to 77.2±0.1 mg/dl compared to untreated group (86.8±0.1 mg/dl, 0% reduction of blood glucose) after 180 min of observation. Glibenclamide reached until 66.6±0.3 mg/dl (23.88%) reduction of blood glucose These results indicated that samples of *P. maprouneifolia var. maprouneifolia* stem abrk and glibenclamide possess hypoglycaemic activity. On the other hand, the administration of aqueous extract and its fractions in treated diabetic Wistar rats at the highest oral dose of 600 mg/kg body weight causes significant reduction of glucose level ranging from 140.4 ±1.7 to 170.8±1.3 mg/dl compared to untreated diabetic animals with (202.7±0.3 mg/kg) after 180 min of observation. The observed concentration of glucose in diabetic rats after 180 min of observation was high and these animals were submitted to a particular treatment aiming the reduction of glucose level under 100 mg/dl. Thus, after 21 days of treatment, the aqueous extract and its fractions produced significant reduction of glucose level in treated diabetic animals ranging from 119.3±0.5 to 128.6 ±0.3 mg/dl at Day 21 and from 86.3±1.1 to 90.0±0.7 at Day 30 compared to untreated diabetic animals (248.3±0.2 and 257.6±0.3 mg/dl respectively) as a sign of their antidiabetic properties. These results demonstrated that these samples have hypoglycaemic and antidiabetic activities. In addition, aqueous extract showed good antihyperglycemic effect in treated animals. Glibenclamide used as a reference product exhibited high activities compared to the tested samples. These results constitute a scientific base supporting and justifying the use of *P. maprounefolia var. maprouneifolia* stem bark for the treatment of diabetes mellitus type 2 in traditional medicine in Democratic Republic of Congo and other African countries where it is found.

KEYWORDS: *Pseudolacnhostylis maprouneifolia var. maprouneifolia*, Euphorbiaceae, stem bark, aqueous extract, hypoglycaemic, antidiabetic and antihyperglycemic activities.

1. INTRODUCTION
Diabetes is one the most complicated metabolic human disorders characterized by a high level of glucose in body (hyperglycemia) with some disturbances of carbonate, fats and proteins metabolism caused by the defects in insulin secretion related to its action. It is a condition when excess amount of sugar is excreted out in the urine due to hyperglycemia state related to the incapacity of body to produce sufficient amount of insulin for the treatment of this condition.[1] It is also a result of a relative or an absolute lack of the hormone insulin or its activity on target tissues or both.[2] Some phenomenon's such as lipidaemia, hyperglycemia and oxidative stress affected people on some vital organs such as kidneys, nerves, eyes and blood vessels.[3,4] According to some studies, diabetes may cause myocardial infarction, cardiovascular disorders and terminal nephritis, complications reported to be the most important causes of mortality and irreversible blindness.[1]

The disease is also characterized by persistent hyperglycemia producing high amount of blood sugar with long-term complications such as cardiovascular, retinopathy, poor blood flow and renal disorders. Its development can be prevented or delayed in people with impaired glucose tolerance by implementing life-style changes of life or the use of appropriate therapeutic agents.[5] The disease rapidly increases worldwide since it is estimated that its prevalence will be 5.4% by the year 2025 with the global diabetic population reaching to 300 million.[6] Current statistic suggests that about 382 million people are living with diabetes around the globe and this number is estimated to increase to 471 million in 2035. The prevalence of prediabetes in other studies was found to be 15.9%. This is higher than the estimated Ethiopian national prevalence compared to other parts of the country (9.7%). This suggests that the prevalence of DM in the study area may increase in the social conditions to diabetic state.[7]

Diabetes disorders are classified into two following major groups: type 1 or insulin-dependent diabetes mellitus (IDDM) and type 2 or non-insulin-dependent diabetes (NIDDM) which becomes a growing health problem as it is the most common form and patient population with it rises every year.[8,9,10] Type 2 diabetes mellitus (T2DM) is the most encountered form of diabetes.[11] About 90% of diabetic patients are diagnosed with type 2 diabetes in the worldwide (T2DM) and more cases are found in developing countries,[12] where people turn to traditional medicine using different aqueous preparations based medicinal plant parts (leaves, stem bark or bark, root bark or root, and fruits) to treat the disease and find in general some alleviations.

Despite considerable progress in the treatment of the disease with known synthetic drugs such as glibenclamide, chlorpropamide, metformin insulin, these drugs are expensive and have more side effects leading to several limitations of use. Hence, several studies are conducted for finding more efficient, safer, tolerable and less expensive antidiabetic drugs from natural source. In addition, picnogenol, galegine, acarbose, quercetin, oleanolic acid, kaempferol, ursolic acid, rutin, miglitol, voglibose, pygnogenol among many others are antidiabetic drugs of natural origin found with defined mechanisms of action, but, they are not extensively used in therapeutic.[5,9] It is well known that medicinal plants have been used since ancient times to prevent conditions associated with diabetes and to treat the disease. Some of them are now currently investigated and reported to possess hypoglycaemic, antidiabetic and antihyperglycemic activities at different extents *in vivo* in animal model or *in vitro* tests.[6,13-18] In some studies, active hypoglycaemic and antidiabetic constituents belonging to different phytochemical groups such as alkaloids, anthocyanins, xanthones, organic acids, sugars, coumarins, polysaccharides, pectins, flavonoids, steroids, terpenoids and tannins were isolated from some medicinal plants and reported.[1,5,9-11,19-25] Unfortunately, more of them are not used in actual therapy for the treatment of diabetes perhaps because of the poor quality with unclear methods of randomization, treats to blinding and lack of baseline demographics in clinical trials. But they constitute lead compounds for the discovery of new hypoglycaemic and antidiabetic products from natural source with less side effects and no harmful. Anyway, several medicinal plants such as of *Aloe vera* (L.) Burm.f. whole plant (Aloaceae); leaf of Banaba; fruit, seeds, pulp leaf of *Momordica charantia* L. (Cucurbitaceae), flower buds of *Capparis spinosa* L. (Capparaceae), seeds of *Coffea arabica* L. (Rubiaceae), *Allium sativum* L. whole plant (Alliaceae), leaf of *Psidium guajava* L. (Myrtaceae); leaf of of *Urtica dioca* L. (Urticaeae) leaf of *Salvia officinalis* L. (Labiatae/Lamiaceae) and many others.[9] as well as some isolated compounds from different medicinal plants were submitted to clinical trials in patients with diabetes. Results from these studies indicated that all extracts and isolated compounds were able to significantly decrease glycaemia level in patients with diabetes. Their effects were interesting and encouraging although they were weaker compared to known standard antidiabetic drugs.[9,20]

Pseudolachnostylis maprouneifolia var. maprounrifolia Pax (Euphorbiaceae) named with the common name Kudu berry is a genus in the Phyllanthaceae (Euphorbiaceae) familly, first described as a genus in 1899.[26-28] It is a small tree that is normally found on rocky outcrops.[29] Sometimes as a many-setmmed shrub, it more commonly becomes a tree with a crow that can be compact and rounded, or lax with dropping branches that can reach down almost to ground. It usually grows from 3-10 metres tall, though specimens up to 18 metres have been recorded. The straight bole can be unbranched for up to 3.5 meters and up to 25 cm in diameter. The tree is harversted from the wild for local use as a medicine, food and source of wood. It is often grown as an ornamental, shade-providing tree, valued especially for the red of its foliage before the leaves fall from the tree [30]. *P. maprouneifolia var. maprouneifolia* is native to central and Southern Africa. It bears small greenish white flowers. The small yellow-green flowers are followed by yellow berries that are relished by birds and apparently by kudus as well. Leaves are alternate, broadly ovate to rounded, margin entire. They display bright autumn colours of yellow or red. Flowers in few-flowered axillary clusters, small and inconspicuous, pale, green, unisexual on separate trees. Fruit ovoid to subspherical, slightly grooved, consisting of a 3-locular woody capsule enclosed in leathery test, also slightly ribbed as if about to split into six segments and yellow when ripe. During the flowering season, a variety of insects (wasp and bees) can pollinate the flowers.[28] *P. maprouneifolia var. maprounrifolia* is a larval food plant for the butterflies *Abantis paradisea* and *Deudorix dinochares*. It is a variable species with at least three varieties recorded in different regions. All varieties are widespread and hardly differ in their ecology. The

flowers are very attractive to honeybees. During the flowering season, a variety of insects such as wasps and bees pollinate the flowers. Kudu berry is at best in autumn when it changes colour to the most beautiful red.[27,28,31,32] It can make a beautiful shade tree in parks and other public open spaces, especially in frost-free areas. Propagate this tree from seed in nature, the seeds germinate after they have gone through the digestive system of browsers. This plant is rare in cultivation, but has a high horticultural potential. It exists four varieties

- *P. maprouneifolia var. dekindtii* (Pax) Radcl.-Sm.- found in Katanga (Zaire actual Democratic Republic of Congo : DR-Congo), Tanzania, Angola, Malawi, Mozambique, Zambia, Botswana, Namibia, Limpopo and Mpumalanga.
- *P. maprouneifolia var. glabra* (Px) Brenan found in Burundi, Zaire (actual DR-Congo), Tanzania, Angola, Malawi, Mozambique, Zqmbia, Zimbabwe, Botswana, Namibia, Limpopo, Mpumalanga.
- *P. maproneifolia var. maprouneifolia* found in Katanga (DR-Congo), Burundi, Tanzania, Angola, Malawi, Mozambique, Zabia, Zimbabwe, Botswana and Caprivi Strip.
- *P. maprouneifolia var. polygyna* (Pax & K. Hoffm.) Radcl.-Sm.- found in Tanzania, Zambia and Malawi.

Figure 1: *Pseudolanchostylis maprouneifolia var. maprouneifolia* Pax tree, leaves and fruits (Euphorbiaceae).

Table 1: Traditional uses of the plant parts of *P. maprouneifolia.*

Pant part	Traditional uses
Leaves	In Zimbabwe, they are boiled and the strained liquid is rubbed into incisions on the side to treat pains. A leaf decoction is taken to treat cough and fever. An infusion of leaves is given to cattle to treat haematuria. They are used as a folder. In East Africa, the leaves pounded together with leaves, bark and roots of pigeon pea (*Cajanus cajan* (L.) Millsp. in water are used as ear drops to treat earaches.
Stem bark	In East Africa, the stem bark and root mixed with poisonous insects are burned and the resulting ashes applied to incisions as a cure for tumours. Bark extract is drunk mixed in porridge to treat diarrhoea, dysentery and used as aphrodisiac. In Democratic Republic of Congo, an aqueous decoction of the stem bark is used to treat diarrhoea, dysentery and diabetes. In Zimbabwe, a bark infusion is employed to treat dizziness and vomiting. In Southern Africa, the pulverized bark mixed in porridge is taken to treat pneumonia, tuberculosis and anaemia.
Root	In East Africa, the roots mixed with poisonous insects are burned and the ashes applied to incisions as a cure for tumours. A root decoction is taken as purgative to treat stomach-ashes and abdominal problems. Dried powdered root mixed in porridge is taken to treat diarrhoea, dysentery and used as aphrodisiac. In Tanzania, roots and bark mixed with poisonous insects are burned and the ashes applied to incisions as a cure for tumours. In Southern Africa, the smoke of burning roots is inhaled to treat pneumonia. In Zimbabwe, a root infusion is employed to treat abdominal pains, gonorrhoea and female sterility.
Pulpa	In Zambia, the pulp of peeled roots is applied to leprous scores.
Fruit	The fruit is edible and used as a folder.
Wood	In Zimbabwe, it is used to make toys, joinery, tiernery and hamiticrafts, and is also used as firewood and to make charcoal.

Pseudolanchnostylis maprouneifolia var. maprouneifolia is a medicinal plant largely used in some provinces in Democratic Republic of Congo (DR-Congo) in traditional medicine to treat various ailments among

which diabetes. Recently,[33] were carried out the first investigation to report the phytochemical screening and antidiarhoeal activity of *P. maprouneifolia* as the first scientific study conducted on this medicinal plant without specification the variety. Taking account of its frequent use to treat diabetes mellitus type 2 in Democratic Republic of Congo (DR-Congo), this medicinal plant can be a potential new source for the cure of diabetes and thus, the objective of the present study is to investigate aqueous extract (decoction), which is the typical traditional preparation taken by people and its fractions for its *in vivo* hypoglycemic, antidiabetic and antihyperglycemic activities in animal model.

2. MATERIALS AND METHODS

2.1. Vegetal material

Stem barks of ***Pseudolachonostylis maprouneifolia*** *var.* ***maprouneifolia*** Pax (Euphorbiaceae) were collected in Katanga (Lubumbashi) one province of DR-Congo on 05/05/2015. The plant was authentified in the Institut National d`Etudes et Recherches en Agronomie (INERA), Department of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of the plant N° 05052015PMSB was deposited in the herbarium of this institute. Plant materials were dried at room temperature and reduced to powder using an electronic blender and the powder was kept in brown bottle.

2.2. Preparation of aqueous extract and its fractionation

50 g of powdered plant material were mixed with 300 ml distilled water and boiled on a hotplate for 15 min. After cooling and filtration on paper filter Watman N° 1, the filtrate was evaporated *in vacuo* yelding dried extract denoted as Pm-1 (20.54 g). 10 g of Pm-1 extract were dissolved in 200 ml distilled water and filtered. The filtrate was submitted to successive and exhaustive extraction with solvents of different polarities chloroform, ethylacetate and *n*-butanol. All fractions and aqueous residual phase were treated as described above giving corresponding dried extracts denoted as Pm –l.1 : 2.06 g for chloroform, Pm-1.2 : 2.18 g for ethylacetate, Pm -1.3: 2.22 g for *n*-butanol and Pm -1.4 : 3.18 g for residual aqueous phase.

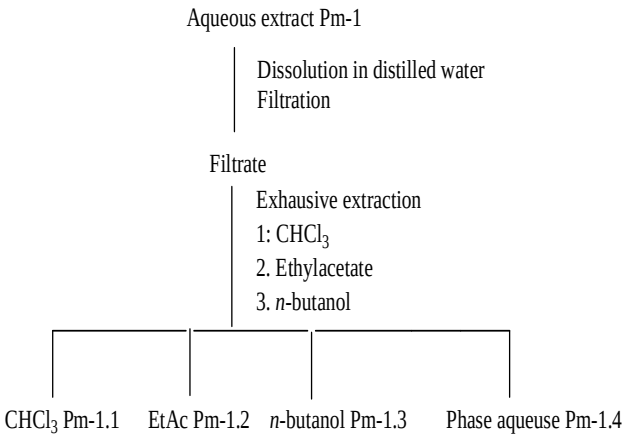

Figure 1: Fractionation of aqueous extract Pm-1.

2.2. Qualitative phytochemical screening

The identification of major phytochemical groups such as alkaloids, flavonoids, steroids terpenoids, reducing sugars, aminated compounds, anthraquinones, anthocyanins, saponins, cardiotonic glycosides, coumarins and tannins (proanthocyanidins) was performed by TLC (plates thichness layer 0.25 mm, Merck, Germany) using different mobiles phases and reagents described in the literature. Reagent Stiasny (formol + HCl conc), froth test and HCl 0.2N + isoamylc alcohol after heating and extraction of the red color with organic solvent were used to detect catechic and gallic tannins, saponins and anthocyanins.[34,35]

2.3. Assessment of hypoglycemic activity in normal Wistar rats

The evaluation of hypoglycemic activity in normal Wistar rats (140-150 g body weight) was carried out using procedures previously described by.[36,37] Selected normal animals with mean glucose level of 85.0±0.3 mg/dl were separately and orally administered 200, 400 and 600 mg/kg body weight of aqueous extract and its soluble fractions. They were observed for 180 min. At each 30 min after administration of glibeclamide, aqueous extract Pm-1 and its soluble fractions until to 180 min of observation, glucose levels in treated animals were recorded using a glucosemeter and compared to untreated group.

2.4. Induction of Diabetes Mellitus and treatment

Diabetes mellitus was induced in Wistar rats by intraperitoneal injection of streptozocin (STZ) dissolved in citrate buffer 0.1M adjusted at pH 4.5 at a dose of 60 mg/kg after 6 h according to procedures previously described by.[38,39] Animals were left in contact with STZ for three days and the glycaemia was measured at Day 4. Animals with blood glucose level over 185 mg/dl were considered as diabetic and selected for the antidiabetic test. Animals were grouped as followed: goup I and II orally received 5 ml distilled water and 2.5 mg/kg bw of glibenclamide as negative and positive groups respectively. Group III to V were treated orally with aqueous extract and groups VI to XVII treated with fractions at respective oral doses of 200, 400 and 600 mg/kg body weight for 180 min and 21 days respectively.

2.5. Effects of crude extracts on oral glucose tolerance

The hyperglycemia was provoked in normal rats by oral administration of 4g/kg of glucose by oral route and animals were left for three days. Blood samples were collected just on Day 4 and the glucose level was measured and animals with blood glucose level over 130 mg/dl at 180 min were considerd as hyperglycaemic and selected for this study.[40-42] These animals were grouped in four groups as followed: group I received 5 ml distlled water and group II glibencamide (2.5 mg/kg bw) as negative and positive group respectively. Groups II and III received orally the doses of aqueous extract Pm-1 (200 and 400 mg/kg bw). After administration tested

samples in hyperglycemic Wistar rats, they were observed for 180 min.

2.5. Determination of blood glucose level

All blood samples were collected from the tail artery of all rats at determinated intervals according to the experimental time. The determination of the blood glucose levels was carried out with a glucosemeter instrument (ONE TOUCH Vita LIFESCAN, Inc, Milpitas, CA 95035, USA) soaked with glucose oxidase reactive with strips and results were expressed in mg/dl and in percentage.[38,43]

% decrease of glucose level: $\frac{GLNC - GLTA}{GLNC} \times 100$

Where GLNC is the glucose level of negative control group and GLTA the glucoe level of treated animals

2.6. Determination of insulin concentration

Immediately after experiments, all the rats were fasted for 8-12 hours. They were sacrificed by cervical dislocation technique. Then, blood samples were drawn from the rat's heart. The blood samples were centrifuged at 3500 rpm for 20 min to get serum. The serum samples were analyzed for insulin content by using immune radio assay kit (MP Bio chemicals Orange burgusing a commercial available DSL-1800, Diagnostic System Laboratories, Inc, USA). Insulin values were expressed in µg/ml.[37]

2.8. Statistical analysis

The experimental results were expressed as mean ± standard error of the mean (S.E.M). Data were statistically analyzed by analysis of variance (ANOVA). T-student's test was used to analyse significant differences between the groups and p values < 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical screening

Results from the qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins (gallic, cathechic and proanthocyanidins), aminated cmpounds, reducing sugars, anthaquinones, terpenoids, steroids and saponins. Anthocyanins, coumarins and cardiotonic glycosides were not detected in aqueous extract of this medicinal plant in our experimental conditions (Table 1). These chemical groups were also detected in fractions according to their respective solubility. Our results are in good agrrement with.[33] using *P. maprouneifolia* stem bark without specification its variety.

Table 1: Qualitative phytochemical screening.

Chemical groups	Results	Chemical groups	Results
Alcaloids	+++	Reducing sugars	++
Flavonoids	++	Anthocyanins	-
Tannins	+++	Coumarins	-
Gallic tannins	+++	Cardiotonic glycosides	-
Cathechic tannins	+++	Anthraquinones	+
Proanthocyanidins	++	Terpenoids and steroids	++
Aminated compounds	++	Saponins	++

2.2. Hypoglycemic activity of aqueous extract Pm-1 of *P. maprouneifolia var. maprouneifolia* stem bark and its fractions after180 min d'observation

In the present investigation, the hypoglycemic activity of aqueous extract Pm-1 of *P. maprouneafolia var. maprounrifolia* stem bark (Pm-1) and its soluble fractions was evaluated in normal Wistar rats. The hypoglycemic change in blood glucose levels of normal animals at different time intervals after oral administration of test samples, at oral doses of 200, 400 and 600mg/kg body weight (bw) is presented in Tables 1 and 2 respectively. Results in Table 1 showed that the oral administration aqueous extract Pm-1 at all oral doses exhibited hypoglycemic activity in dose-dependent manner. It gradually deacrease the glucose level of normal animals from 30 to 180 min of observation (Table 2). After 180 min of observation, it induced significant reduction ($p < 0.05$) of blood glucose level to 68.5±1.1 mg/dl of treated rats with the highest oral dose of 600 mg/kg bw, corresponding to 21.17% reduction in blood glucose level (BGL) The administration of glibenclamide as the reference hypoglycemic product reached significant decrease of BGL in treated animals to 66.6±0.3 mg/dl (23.88% reduction) compared to untreated rats with BGL of 85.0 ±0.5 mg/dl (0% reduction).

Table 2: Effects of aqueous extract Pm-1 of *P. maprouneafolia var.maprouneifolia* stem bark on fasting plasma glucose level (mg/dl) and reduction % in normal rats after 180 min of observation.

Groups	Treatment (mg/kg bw)	0 min	30 min	60 min	120 min	180 min
I	NR + 5 ml DW	85.1±0.5	84.5±0.1	85.1±0.6	85.7±0.2	87.5 ± 0.5
II	NR + GLb (2.5)	85.2±0.8	80.6±0.2	75.9±0.1	71.3±0.4	66. 6 ± 0.3
			4.61%	10.81%	16.80%	23.88%
Pm-1						
III	NR + 200	84.8±0.1	82.3±0.4	79.8±0.4	76.1±0.6	74.6± 0.4
			2.60%	6.22%	11.20%	14.74%
IV	NR + 400	84.6±0.3	80.9±0.2	77.2±0.5	74.3±0.6	71.0± 0.3
			4.26%	9.28%	13.30%	18.85%
V	NR + 600	83.7±0.2	77.7±0.4	75.2±0.5	71.6±0.01	68.5± 0.1
			8.04%	11.63%	16.45	21.17%

Pm-1: aqueous extract, DW; distilled water, NR: normal rats, GLb: glibenclamide

Figure 1 show the production of insulin by normal rats (negative control) and the effect of glibenclamide (Glb) on the production of insulin level in treated normal animals at oral doses of 1 to 2.5 mg/kg bw. This product caused significant increase of insulin level in dose-dependent manner after 180 min of observation. The highest insulin level of 32 µg/ml was obtained with the administration of the highest oral dose of 2.5 mg/kg bw in treated normal Wistar rats (Fig.1).

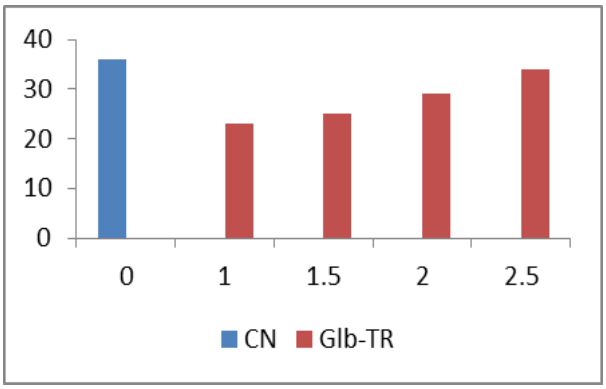

Figure 1: Production of insulin by negative control group and glinbecamide (Glb) in treated normal Wistar rats (GLb-TR) after 180 min of observation

The same results were also obtained with aqueous extract Pm-1 (Fig.2) given at oral doses of 200, 400 and 600 mg/kg respectively in normal treated animals. The highest level of insulin (28 µg/ml) was obtained with the administration of the highest oral dose of 600 mg/kg bw by aqueous extract Pm-1 after the same time of observation.

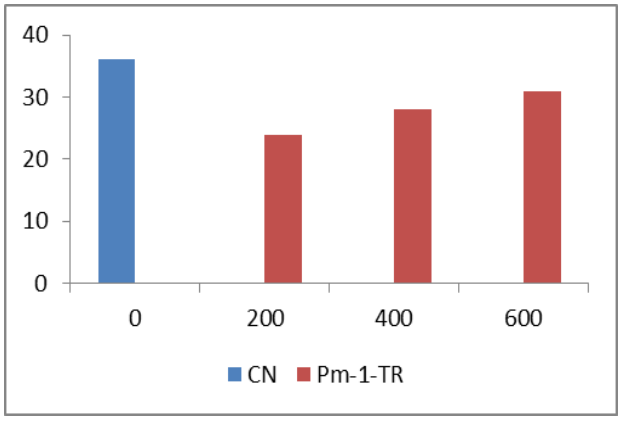

Figure 2. Production of insulin by negative control group and by aqueous extract Pm-1 in treated normal Wistar rats (Pm-1-TR) after 180 min observation.

Soluble fractions from the partition of aqueous extract Pm-1 were also evaluated for their potential hypoglycemic activity in normal Wistar rats. Results in Table 3 indicated that all fractions displayed hypoglycemic activity in dose-dependent manner. With the highest oral dose of 600 mg/kg bw, Pm-1.1, Pm-1.2, Pm-1.3 and Pm-1.4 soluble fractions rich in terpenoids an steroids, flavonoids, saponins and phenolic compounds respectively, reached significant decrease of blood glucose level to 81.2±0.2, 77.2±0.2, 82.9±0.1 and 80.2±0.8 mg/dl corresponding to 6.45, 8.90, 4.50 and 7.60% reduction of glucose level respectively compared to untreated group (86.8±0.1 mg/dl, 0% reduction).

The production of insulin by different soluble fractions in treated normal Wistar rats is shown in Figure 3. It was also observed that they significantly increase the amount of insulin level in treated normal Wistar rats in dose-dependent manner. At highest oral dose of 600 mg/kg bw, this level was 20, 24, 18 and 22 µg/ml for Pm-1.1, Pm-1.2, Pm-1.3 and P1.4.

Table 3: Effects of fractions from Pm-1 extract on fasting plasma glucose level (mg/dl) and reduction % in normal rats after 180 min of observation.

Fractions	Treatment (mg/kg pc)	0 min	30 min	60 min	120 min	180 min
I **NC**	5 ml DW	85.3±0.3	85.6±0.3	87.2±0.4	87.9±0.8	86.8±0.1
Pm-1.1						
VI	200	85.0±0.2	85.6±0.2	85.1±0.1	84.2±0.3	83.1±0.1
VII	400	83.2±0.3	84.8±0.1	84.8±0.4	83.8±0.4	82.4±0.3
VIII	600	82.6±0.3	81.9±0.2	82.4±0.3	81.5±0.2	81.2±0.2
Pm-1.2						
IX	200	82.5±0.1	81.9±0.4	81.6±0.2	80.7±0.3	80.1±0.2
X	400	80.6±0.4	81.9±0.2	81.6±0.4	80.3±0.3	79.1±0.3
XI	600	80.1±0.3	81.3±0.3	80.9±0.3	77.2±0.1	77.2±0.1
Pm-1.3						
XII	200	84.1±0.3	82.6±0.1	82.9±0.1	83.5±0.3	84.2±0.2
XIII	400	82.3±0.1	81.8±0.2	81.3±0.3	83.9±0.4	82.5±0.3
XIV	600	81.6±0.3	82.6±0.4	82.1±0.2	81.5±0.3	82.9±0.1
Pm-1.4						
XV	200	84.3±0.4	83.6 ±0.2	83.0 ±0.1	82.6 ±0.2	83.4±0.3
XVI	400	83.1±0.3	81.6±0.2	81.9±0.2	82.3±0.1	82.0±0.1
XVII	600	82.6±0.2	82.1±0.6	83.2±0.3	83.9±0.3	80.2±0.2

NC: negative conrol, DW; distilled water, Pm -1.1 to Pm-14: chloroform, ethylacetate, *n*-butanol and residual aqueous phase respectively from the partition of the lyophilized aqueous extract Pm-1. The positive control in Table 2 is also valid for Table 3 soluble fraction respectively, with Pm-1.2 soluble fraction rich in flavonoids as the most active productor fraction compared to others after 180 min of observation.

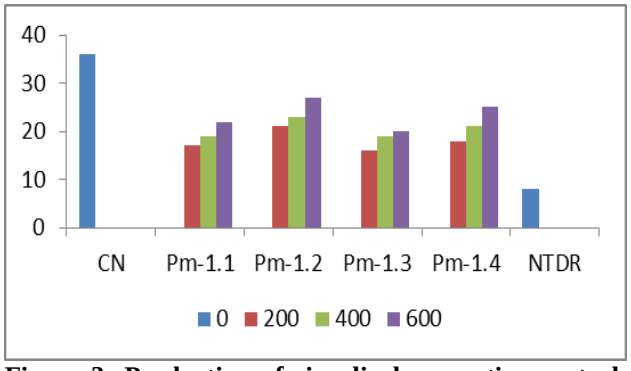

Figure 3: Production of insulin by negative control group and by fractions Pm-1.1 to Pm-1.4 in treated normal Wistar rats (GLb-TR) after 180 min of observation.

However, it was observed that the level of insulin in normal Wistar rats (NC: negative control) (36 µg/ml) remained higher compared to treated animals treated with Glb, aqueous extract and its soluble fractions (Fig. 1, 2 and 3) due probably to the high amount and good organization of β-pancreatic cells secreted in normal Wistar rats state after 180 min of observation. Increased level of insulin in normal treated animals in hypoglycemic and antidiabetic tests is a common phenomenon occurring in various medicinal plants such as *Allium sativum,*[44] *Ricinus communis,*[45] *Pseuderanthemum palatiferum,*[46] and other.

From these reported results, it has been concluded that the administered aqueous extract Pm-1 and its soluble fractions Pm-1.1 to Pm-1.4 from P. *maprouneifolia var. maprouneifolia* stem bark had a hypoglycemic property comparable to glibenclamide used as reference product since they produced significant reduction of BGL in treated normal animals compared to untreated group after 180 min of observation. In addition, they possess glibenclamide like-effect. These effects may be due to the potentiation of receptors or inhibition of glucose reabsorption in the proximal tubules of the kidney as also

previously reported for other medicinal plant extracts.[40,47]

2.3. Antidiabetic activity of aqueous extract Pm-1 and its fractions on treated diabetic Wistar rats

The STZ-induction in adult animals produces a type 2 diabetes mellitus due to the increase of glucose level and is a useful experimental model used to study the activity of antidiabetic agents from natural and synthetic sources.[48] Also, it is well known that STZ selectively and intensively destroys β-pancreatic cells resulting in significant increase of blood glucose as already mentioned above.[38,39]

In the present study, the administration of STZ provoked significant elevated levels of blood glucose to 188.4 ± 0.5 mg/dl at 30 min and gradually continued to increase to 202.7 ± 0.3 mg/dl after 180 min of observation in treated animals, effect which may be attributed to the selective cytotoxic effect of STZ on β-pancreatic cells [37-39] These animals were considered as diabetic and selected for the experience.

Results presented in Table 4 indicated the administration of the reference product glibenclamide in diabetic Wistar rats, produced significant decrease of glycaemia from 30 min of observation at 153.2 ± 0.5 mg/dl compared to untreated group (188.4 ± 0.5 mg/dl). This effect gradually continued to decrease until 180 min of observation. At this time, the glycaemia in treated animals was 96.8±0.2 mg/dl compared to negative control group with glycaemia of 202.7±0.3 mg/dl and its effect was significant ($p < 0.01$). On the other hand, the administration of aqueous extract Pm-1 also induced significant reduction ($p < 0.05$) of glycaemia at all administered oral doses in diabetic treated animals compared to untreated groups (Table 1). At the highest oral dose of 600 mg/kg bw, the extract reached significant ($p < 0.05$) reduction of glycaemia to 140.4 ± 1.7 mg/dl compared to negative control group with a glycaemia of 202.7 ± 0.3 mg/dl after180 min of observation.

Tableau 4: Effects of fractions from aqueous extract Pm-1 of *P. maprouneifolia var. Maprouneifolia* stem bark on fasting glucose level (mg/dl) in streptozocin-induced diabetic Wistar rats after 180 min of observation

Groups	Treatment (mg/kg bw)	0 min	30 min	60 min	120 min	180 min
I	5 ml DW	85.3±0.3	85.6±0.5	87.2±0.4	87.9±0.8	86.8±0.8
II	NC, STZ: 60	187.1±3.4	188.4±0.5	195.6±0.7	198.3±0.2	202.7±0.3
III	Glibenclamide 2.5 mg/kg	182.2±0.4	153.2±0.5	141.7±0.3	110.8±0.6	96.8±0.2
Pm-1						
IV	DR + 200	196.2±0.4	184.2±0.5	180.7±0.3	176.8±0.6	148.8±0.2
	DR + 400	195.3±0.5	183.5±0.7	176.5±0.5	173.2±1.2	144.6±0.5
	DR + 600	195.8±0.1	180.3±0.3	172.3±0.9	168.3±1.2	140.4±0.7
Pm-1.1						
V	DR + 200	197.3 ±0.4	163.95±0.2	158.3±0.4	154.6±1.2	150.6±1.1
VI	DR +400	196.3±0.8	160.3±0.7	154.6±0.5	151.3±0.9	148.3±0.7
VII	DR +600	195. 5±0.2	157.3±0.8	155.6±1.4	151.3±1.3	146.3±1.7
Pm-1.2						
VIII	DR +200	199.5±0.4	160.2±0.5	156.3±1.6	152.6±1.2	147.3±1.1
IX	DR + 400	191.3±0.8	158.6±1.8	152.6±0.8	149.6±0.3	145.3±1.3
X	DR + 600	193.6±0.8	155.3±0.2	152.3±1.4	148.3±0.7	144.3±0.7
Pm-1.3						
XI	DR + 200	195.6±0.3	186.5±0.5	183.6±1.5	180.6±1.2	178.3±0.3
XII	DR + 400	194.6±0.5	182.3±0.7	180.3±0.9	177.3±1.4	174.8±1.5
XIII	DR + 600	196.3±0.8	179.3±0.3	177.5±1.2	173.2±0.8	170.8±1.3
Pm-1.4						
XIV	DR + 200	198.6±0.2	160.3	156.3	153.6	151.5±1.2
XV	DR + 400	197.3±0.5	157.3	152.3	150.3	148.6±0.4
XVI	DR + 600	196.3±0.4	155.3	150.3	147.3	146.9±1.5

See Tables 2 and 3, DR: diabetic rats

The same effects were also observed with the administration of different soluble fractions at all different oral administered doses. At the highest oral dose of 600 mg/kg bw, all soluble fractions reached significant ($p < 0.05$) reduction of glycaemia ranging from 144.3 ± 1.7 to 170.8 ±1.3 mg/dl, with soluble fraction Pm-1.2 as the most active, compared to negative diabetic rats (202.7±0.3 md/dl). The rate decrease of glycaemia by aqueous extract Pm-1 and its soluble fractions was comparable to glibenclamide. This observation lend to suggest that these samples form *P. maprouneifolia var. maprouneifolia* stem bark possess glibenclamide like-effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibition hepatic glycogenesis.[33] Because they deacrease fasting plasma glucose (FPG) in STZ-induced diabetic rats after 180 min of observation after administration, it was assumed that, it might be due at first to their cumulative effects during the period of treatment.

After the evaluation of antidiabetic activity of theses samples form *P. maprouneifolia var. maprouneifolia* stem bark after 180 min of observation, obtained results were interesting and promising, but it was observed that the glycaemia in treated Wistar diabetic rats remained higher than 100 mg/dl. Thus, it was decided to immediately submit these diabetic rats to a special treatment aiming to reduce as soon as possible the glycaemia near or under 100 mg/dl. For this treatment, it was decided to give orally the same oral doses twice a day (morning at 9 h00 and afternoon at 16h00). Results from this investigation for 21 days of treatment are presented in Table 5. Results showed that the standard drug glibenclamide (2.5 mg/kg) was found to reach significantly ($p < 0.05$) reduction of glycaemia from 92.4 mg/dl at J-7 and gradually continue to decrease until J-21with glycaemia of 84.6 ± 0.8 mg/dl, and at the last day J-30 of measurement of glycaemia with glycaemia of 78.4 ± 0.3 mg/dl corresponding to 59.54, 62.24 and 69.56% reduction of glycaemia after these respective times of treatment compared to negatve diabetic control 228.4, 248.3 and 257.6 mg/dl at J-7, 21 and 30 respectively.

Tableau 5. Effets of aqueous extract Pm-1 from *P. maprouneifolia* and its fractions on plasma glucose level in STZ-induced diabetic Wistar rats after 21 days of treatment.

Groups	Treatment (mg/kg bw)	J-1	J-7	J-14	J-21	J-30
I	5 ml distilled water	85.3±0.3	85.6±0.5	87.2±0.4	87.9±0.8	86.8±0.8
II	NC, STZ: 60	202.7±0.3	228.4±0.5	235.6±0.7	248.3±0.2	257.6±0.3
III	Glibenclamide: 2.5	96.8±0.2	92.3±0.5	87.3±0.1	84.6±0.8	81.8±0.2
Pm-1						
IV	DR + 200	148.8±0.2	143.2±0.3	132.3±1.3	125.5±1.4	93.5±0.8
V	DR + 400	144.6±1.45	140.3±1.2	128.2±1.5	121.3±0.7	90.3±1.7
VI	DR + 600	140.4±1.7	136.6±0.5	130.2±0.7	119.3±0.5	86.3±1.1
Pm-1.1						
VII	DR +200	150.6±1.1	148.3±0.2	145.3±0.2	136.6±1.4	98.3±0.8
VIII	DR + 400	148.3±0.7	144.3±0.5	140.2±1.5	130.6±1.2	94.5±1.4
IX	DR + 600	146.3±1.7	140.6±1.2	137.3±1.7	132.3±1.8	91.3±1.1
Pm-1.2						
X	DR + 200	147.3±1.1	146.3±0.3	136.2±0.7	127.3±0.5	94..2±0.1
XI	DR + 400	145.3±1.3	143.2±0.2	132.5±0.5	124.6±1.0	90.5±1.4
XII	DR + 600	144.3±0.7	140.3±1.0	128.6±0.5	122.3±0.7	89.3±0.9
Pm-1.3						
XIII	DR + 200	178.3±0.3	162.3±1.5	158.3±0.3	148.5±1.2	103.2±1.5
XIV	DR + 400	174.8±1.5	159.3±0.1	155.3±0.6	132.5±0.4	99.5±1.3
XV	DR + 600	170.8±1.3	152.3±1.3	148.2±0.3	139.6±1.7	96.5±1.5
Pm-1.4						
XVI	DR + 200	151.5±1.2	147.3±0.5	144.2±0.8	141.3±1.4	96.5±1.1
XVII	DR + 400	148.6±0.4	145.3±1.3	140.3±1.7	138.6±1.1	92.5±1.4
,XVIII	DR + 600	146.9±1.5	136.2±0.5	132.5±0.8	128.6±0.3	90.0±0.7

See Tables 1, 2 and 4.

Aqueous extract Pm-1 also caused significant ($p < 0.05$) reduction of glycaemia level in this special treatment in dose-dependent manner (Table 3). At the highest oral dose of 600 mg/dl, it significantly ($p < 0.05$) brought back the glycaemia to 136.6±0.5 at J-7 (40.19% reduction), 119.3 ± 0.3 (51.95% reduction) at J-21 and

86.3±1.1 mg/dl at J-30 (66.50% reduction), all both compared to untreated diabetic Wistar rats (228.4 ±0.5, 248.3 ± 0.2 and 257.6 ± 0.3 mg/dl at J-7, 21 and 30 respectively.

Different soluble fractions acted in the same way as aqueous extract Pm-1. They induced significant reduction of glycaemia of treated diabetic Wistar rats ranging from 152.3 ±1.3 mg/dl (33.31% reduction) to 136.6 ± 0.5 (40.19% reduction) at J-7 and from 132.5 ±0.5 (46.63% reduction) to 148.2 ± 0.3 mg/dl (40.31% reduction) at J-21 at the highest oral dose of 600 mg/kg bw. At the last day J-30 of measurement of glycaemia, the blood glucose levels induced by all soluble fractions in treated Wistar diabetic rats was lower than 100 mg/dl compared to untreated diabetic Wistar rats (228.4 ±0.5, 248.3 ± 0.2 and 257.6 ± 0.3 mg/dl at J-7, 21 and 30 respectively (Table 3) . This reduction was very significant when 600 mg/kg bw were administered producing reduction of glycaemia from 96.5±15 (62.53%) to 90.0±0.7 mg/dl (65.06%) with Pm-1.2 soluble fraction rich in flavonoids as the most active. This antidiabetic effect of the reference product and *P. maprouneifolia var. maprounrifolia* samples is probably due to their capacity to regenerate β-pancreatic cells destroyed by STZ, which in turn, stimulate the secretion of insulin indispensable to the decrease of blood glucose in treated diabetic animals as also previously observed for many other antidiabetic medicinal plant extracts.[13,15,17,20,24,44,46]

Figures 4, 5 and 6 showed the production of the hormone insulin by normal Wistar rats (negative normal control), (negative non treated diabetic animals (NTDR) and by glibenclamide, aqueous extract Pm-1 and its soluble fractions respectively (Fig. 4, 5 and6). In general, it was observed that, during this time of treatment, all samples exerted a strong production of the hormone in treated diabetic Wistar rats compared to untreated diabetic.

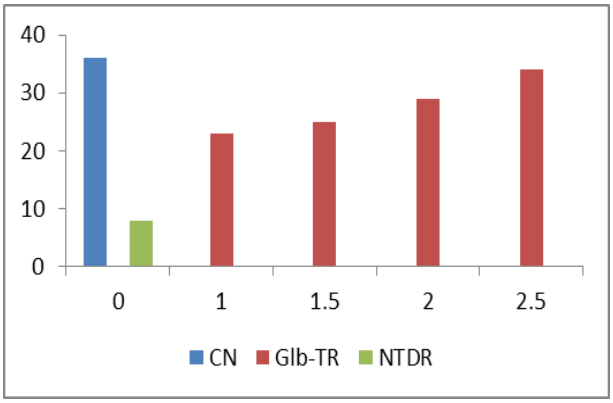

Figure 4: Production of insulin by normal Wistar rats (CN), non treated diabetic rats (NTDR) and by glibenclamide in treated diabetic Wistar rats after 21 days of treatment.

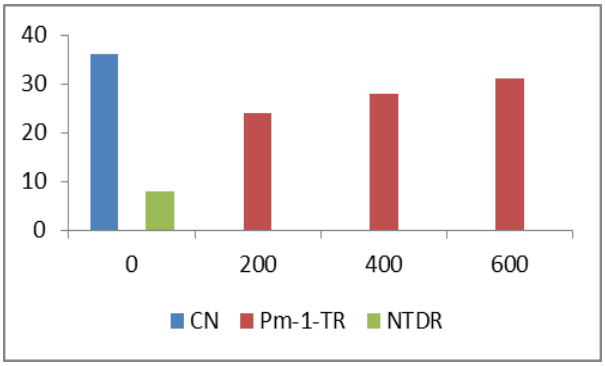

Figure 5: Production of insulin by normal Wistar rats (CN), non treated diabetic rats (NTDR) and by aqueous extract Pm-1in treated Wistar rats after 21 days of treatment.

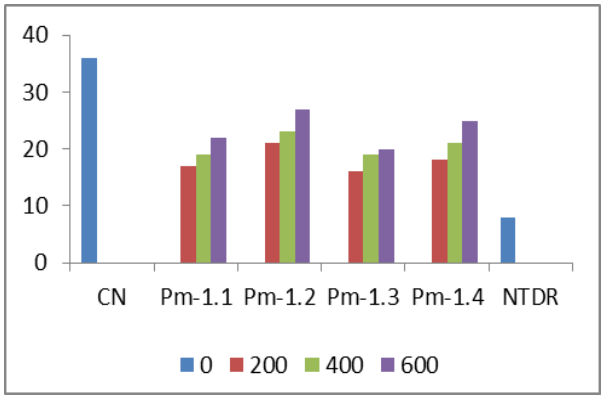

Figure 6: Production of insulin by normal Wistar rats (CN), non treated diabetic rats (NTDR) and by soluble fractions in treated Wistar rats after 21 days of treatment.

Animals after 180 min of observation. This production had largely contributed to the strong reduction of glycaemia in treated diabetic animals after 21 days of treatment until Day 30 of observation.

By comparison, the hypoglycemic activity in normal Wistar rats and antidiabetic activity in diabetic Wistar rats of the aqueous extract Pm-1 and its fractions can be ranked as Pm-1 > Pm-1.2 > Pm-1.4 > Pm-1.1 > Pm-1.3. This indicated these both activities are located in Pm-1.2 soluble fractions rich in flavonoids and suggested in further the isolation of active principles in this most active soluble fraction. Moreover, activies of fractions are weaker compared to the parent aqueous extract Pm-1 suggesting that compounds in these fractions acted in synergistic manner for the manifestation of high activity as seen in Pm-1 extract.

2.4. Effects aqueous extract Pm-1of *P. maprouneifolia var. maprouneifolia* stem bark on hyperglycemic Wistar rats in oral glucose tolerance test (OGTT)

The effects of various doses of the aqueous extract Pm-1 of *P. maprouneifolia var. maprouneifolia* stem bark on oral glucose tolerance test (OGTT) are shown in Table 6. The blood glucose concentration in negative control group gradually and significantly increased from 30 min

to 180 min of observation from 137.1 ±0.3 to 151.3 ± 0.7 mg/dl after oral administration of 4 g glucose indicating that these treated animals are in hyperglycemic state and were selected for the present experiment.

Glibenclamide administered to hypeglycemic animals caused gradually significant decraease of blood glucose level form 92.3±0.2 at 30 min to 78.2±0.5 mg/dl (32.67 to 42.30%) after 180 min of observation. The same effect was also observed with the administration of aqueous extract Pm-1 of *P. maprouneifolia* stem bark reaching gradually significant deacrease of blood glucose level in 30 to 180 min ranging from 111.7±0.1 (18.52% reduction) to 88.7 ±0.3 mg/dl (35.30% reduction) from 30 to 180 min of observation. This decrease was dose-dependent (Table 3). At the highest oral dose of 400 mg/kg bw, the blood glucose level in treated hyperglycemic animals was reduced to 88.7 ± 0.3 mg/dl (35.75% reduction) by aqueous extract Pm-1. However, of the 3 test doses, 600 mg/kg of extracts was found to be the most effective dose compared to that seen with others ($p < 0.005$). These results clearly showed that aqueous extract form *P. mapronrifolia var. maprouneifolia* is able to significantly reduce blood glucose levels in hyperglycemic rats and more suggested that rats are also able to tolerate glucose in their organism.

Table 6: Effect of different doses of the lyophilized aqueous extract of *P. maprouneifolia var. maprouneifolia* stem bark and glibenclamide on serum glucose level (mg/dl) in oral glucose tolerance test (OGTT).

Time (minutes)	Group I HR + glucose	Group II HR + Glb (2.5 mg/kg bw)	Group III HR+ 200 mg/kg bw Pm-1	Group IV HR+ 400 mg/kg bw Pm-1
0	133.2 ± 0.4	132.3 ± 0.4	131.2 ± 0.2	133.3 ± 0.2
30	137.1 ± 0.3	92.3 ± 0.2	124.4 ± 0.4	111.7 ± 0.1
		30.23%	**6.02%**	**8.70%**
60	144.6± 0.6	86.7 ± 0.4	120.6 ± 0.3	107.4 ± 0.3
		34.46%	**8.07%**	**11.93%**
90	148.8 ± 1.3	82.3 ± 0.4	116.3 ± 0.1	101.8 ± 0.3
		37.79%	**11.43%**	**14.62%**
180	161.3 ± 0.7	78.2 ± 0.5	98.2 ± 0.3	88.7 ± 0.3
		40.90%	**25.15%**	**33.46%**

HR: hyperglcemic rats, Glb: glibenclamide

Our results are in good agreement with other studies previously reporting results for other antidiabetic medicinal plant species for the tolerance of animals to glucose,[40,47,49] but in other studies, the increasing of blood glucose levels in OGTT was also previously reported,[50,51] indicating the intolerance of animals to this sugar.

In general, during this experimental study, repeated oral dose administration in treated normal, diabetic and hyperglycemic Wistar rats in separate specific groups in a determined interval, had progressively promoted the reduction of blood glucose levels in dose-dependent manner over a period of treatment in different treated animals according to the case. Very good and encouraging results were obtained after a long period of observation or treatment comfirming that aqueous extract and its solube frcations possess hypoglycemic, antidiabetic and antihyperglycemic activities in animal model according to the case. This is also the first report of hypoglycemic, antidiabetic and antihyperglycaemic activities of *P. maprouneifolia var. maprouneiflia* stem bark.

For drugs controlling diabetes mellitus and their mechanism of action, the common strategy for treatment is focused mainly on regulation and decrease of blood sugar to fall within the normal level by using different medicinal plants suspected traditionally with hypoglycemic, antidiabetic and antihyperglycemic activities. The main mechanisms in both traditional and modern medicines involve reduction of blood sugar through stimulating and regeneration of pancreatic β-cells, inhibiting other hormones or substances elevating blood sugar; increasing the affinity and sensitivity of insulin receptors. On the other hand, other mechanisms such as lowering glycogen release, enhancement glucose utilization within many tissues and organs, clearing free radicals resisting lipid peroxidation, correction of lipid and protein metabolic disorders and improving human blood circulation are also involved.[48]

3. CONCLUSION

In conclusion, the present study had revealed that aqueous extract from *P. maprouneifolia var. maprouneifolia* stem bark and its soluble fractions possess remarkable and interesting hypoglycemic, antidiabetic and antihyperglcemic activities in animal model. The traditional medicines from readily available medicinal plant species offer great potential for the discovery new hypoglycemic, antidiabetic and antihyperglycemic agents. This finding can support and justify the traditional used of this plant part as a start plant material for the preparation of remedies for the treatment of diabetes type 2 in Democratic Republic of Congo and other African countries where it is used for the same medical purpose.

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