



Antihyperglycemic Activity of *Vernonia amygdalina* Leaf Extracts, *Hibiscus esculentus* Fruit Extract and *Garcinia kola* Seed Extract from Kisangani Plants

Frederick M. Katemo¹, Roland D. Marini¹ and Justin N. Kadima^{2*}

¹Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Kisangani, DR Congo.

²Department of Pharmacy, School of Medicine and Pharmacy, University of Rwanda, Rwanda.

Authors' contributions

This work was carried out in collaboration between all authors. Author FMK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors RDM and JNK managed the analyses of the study. Author JNK managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Many plants used in traditional medicine still need to be studied scientifically in order to verify their medical usefulness and standardize their pharmaceutical properties. The present study aimed at evaluating the antihyperglycemic activity of aqueous and alcoholic extracts from local species of *Vernonia amygdalina* Delile (Va), *Hibiscus esculentus* (He) and *Garcinia kola* Heckel (Gk).

Methods: The tests were done on Va-aqueous, Va-ethanolic, Va-butanolic and Va-saponin leaf extracts; He-aqueous fruit extract and Gk-aqueous seed extract. The extracts were prepared using conventional methods. The activity was evaluated in male rabbits given orally 100 mg of extracts per Kg BW and overloaded with glucose (4 g/Kg) 30 minutes later. Glibenclamide 0.2 mg/Kg was given as reference positive control. A negative control group of untreated animals was also included. Blood samples were collected on the animal ear at different times. The assay was performed using a handheld Glucometer®.

*Corresponding author: E-mail: kadima48@yahoo.com;

Results: The percentages of reduction in glycemia calculated on the basis of the negative control values were 60.5% for glibenclamide, 70.5% for Va-ethanol, 57.6% for Va-aqueous, 42.2% for Va-butanol, 54.5% for He-aqueous and 58.7% for Gk-aqueous. Va-saponins fraction was inactive; it increased the baseline glycemia instead of reducing.

Conclusion: All extracts have a relative reduction activity comparable to glibenclamide with the exception of Va-saponins. Improved tradimedcines can be prepared with ethanolic or polyphenolic dry extracts.

Keywords: *Vernonia amygdalina*; *Hibiscus esculentus*; *Garcinia kola*; antihyperglycemic activity; rabbit.

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by a chronic elevated blood sugar (hyperglycemia). DM is widespread throughout the world both in developed and in developing countries. Globally, an estimated 422 million adults are living with DM according to the latest 2016 data [1]. Despite the progress in the discovery of new therapeutic molecules, DM and its complications still constitute a challenge for the management of patients [2]. Modern drugs, including insulin and oral hypoglycemic agents are costly and have some level of toxicity and adverse drug reactions such as skin rash and low blood sugar by sulfonylureas, kidney complication and dizziness by biguanides, risk of liver disease and anemia by thiazolidinediones. In developing countries, some people don't have easy access to modern antidiabetic medicines and in place recourse to herbal therapy [3]. Recently, some clinical studies have come up with the observation that a complementary therapy with plant extracts may optimize the treatment of DM [4,5]. For example, charantine isolated from the fruit of *Momordica charantia* has been found more active than tolbutamide [6]; ginsenoside isolated from Asian ginseng root and diosgenin isolated from *Trigonella foenum-graecum* have been shown to be highly effective against experimental diabetes [7,8].

Vernonia amygdalina of Asteraceae family, *Hibiscus esculentus* of Malvaceae family and *Garcinia kola* tree of Clusiaceae family are among widespread plants used against diabetes in traditional medicine in tropical Africa. These plants are invested with many other therapeutic virtues for which they are used in folk medicine. From *Vernonia* species, a number of biologically-active compounds have been isolated including saponins, alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthenes, anthraquinones, edotides, and sesquiterpenes. As reported, Vernodalin is

antiplasmodial; Vernonioside B1 is antiplasmodial and antischistosomal and Luteolin is a powerful antioxidant and anticancer [7-11]. From *Hibiscus* species, the extracts showed many activities including antibacterial, antioxidant, nephro-hepato-protective, renal/diuretic, anti-cholesterol, and antihypertensive and antidepressant effects [12,13]. Phenolic acids (e.g. protocatechic acid), organic acid (hydroxycitric acid and hibiscus acid) and anthocyanins (delphinidin-3-sambubioside and cyanidin-3-sambubioside) are likely to contribute to the reported effects. *Garcinia* species contain mainly flavonoids of the biflavonoid type; these flavonoids are thought to be responsible for the antihyperglycemic activity of its seeds [14,15].

Some authors have demonstrated the antidiabetic activity of crude extracts and polyphenols of these plants in rats and mice. Few studies have investigated the hypoglycemic activity in rabbits. Rabbits are commonly used for screening prior to testing in a larger animal model. Many plants used in traditional medicine still need to be studied scientifically in order to verify their efficacy and standardize their pharmaceutical quality according to the actual content in bioactive compounds that can vary from soil to soil of cultivation. The aim of this study was to evaluate the antihyperglycemic activity of polyphenols and saponins extracts from the local species of these three plants.

2. MATERIALS AND METHODS

2.1 Plant Materials

Leaves of *Vernonia amygdalina* Delile (Va) and fruits of *Hibiscus esculentus* (He) and seeds of *Garcinia kola* were used (Fig. 1). Va-leaves and He-fruits were freshly harvested in Kisangani city; while Gk-seeds were bought at the big market of Kisangani, in the municipality of Makiso.



Fig. 1. Vernonia amygdalina (top), Hibiscus esculentus (middle) and Garcinia kola (bottom)

The botanical identity of the species harvested was confirmed by a botanist at the herbarium of the Faculty of Science of University of Kisangani.

V. amygdalina is a shrub 3 to 5 m high; the leaf is the edible part and used against diabetes in traditional medicine [16,17]. *H. esculentus* also called *Hibiscus hispidissimus* A. Chev or *Abelmoschus esculentus* (L.) Moench is a large annual cosmopolitan grass with erect stems up to 0.8 to 2.5 m high; the fruit is angular capsule, elongated, conical and pointed, 8 to 15 cm long; the green fruit is edible and used against diabetes [18]. *G. kola* also known as *Garcinia dinklagei* Engel., *Garcinia courauana* Engel., *Garcinia nitidula* Engel., *Garcinia gjadidi* De Wild , or in vulgar names of Small kola, Bitter-kola, False kolatier, can reach 20 m in height, giving yellow or orange globular or ovoid fruits containing 3 to 4 ellipsoid seeds; the seed is edible and used against diabetes in traditional medicine [19].

2.2 Preparation of Plant Extracts

The parts removed were shade-dried at room temperature in the laboratory before being transformed into powders and sieved on 1 mm mesh cloth. The extracts were prepared using adapted conventional methods [20-24].

Va-saponins fraction: Mix 20 g of the powder of leaves with 50 ml hot methanol/water (30:70)

solution. After cooling and decantation, filter and evaporate methanol. The aqueous phase is then mixed with equivalent volume of n-butanol, stirred and separated. The addition of diethyl ether into the n-butanol precipitates saponins. The precipitate is taken up with 40 ml of distilled water and completed to 100 ml.

Va-butanol fraction: Boil (15 minutes) 20 g of the powder of the leaves in 100 ml of water and filter on Whatman paper n°3; subject the filtrate to liquid-liquid extraction with n-butanol; evaporate the n-butanol extract to dryness, take up the residue with distilled water and bring the volume to 100 ml.

Va-ethanol fraction: Boil (15 minutes) 20 g of the powder of the leaves in 100 ml of ethanol and filter on Whatman paper n°3; evaporate the ethanol extract to dryness, take up the residue with distilled water and bring the volume to 100 ml.

Va-aqueous decoction 20%: Boil (15 minutes) 20 g of the powder of the leaves in 100 ml of water and filter on Whatman paper n°3; place the filtrate in a 100 ml volumetric flask and bring to volume for immediate use.

He-decoction 20%: Boil during 15 minutes 20 g of each finely ground fruits in 100 ml of water and filter; place the filtrate in a 100 ml volumetric flask and bring to volume for immediate use.

Gk-decoction 20%: Boil for 15 minutes 20 g of each finely ground seeds in 100 ml of water and filter; place the filtrate in a 100 ml volumetric flask and bring to volume for immediate use.

2.3 Test of Antihyperglycemic Effect in Rabbits

The protocol has been described elsewhere [22-24]. Male rabbits aged 5 to 8 months weighing 1 to 1.8 Kg were used. The animals lived at normal controlled temperature and photoperiod environment during 10-day acclimation period and the entire experimental period. Two days before the experimentation, they were divided into 8 groups of 5 rabbits each including: (1) Control, (2) Reference, (3) Va-ethanol, (4) Va-butanol, (5) Va-aqueous, (6) Va-saponin, (7) He-aqueous and (8) Gk-aqueous. The day of experiment, blood samples were taken by transverse incision of the marginal vein of the earlobe before the administration of the extracts (baseline T0). Then after, the control group received 1 ml of water orally; the reference group received 1 ml of glibenclamide 0.2 mg/Kg; the test groups received orally 1 ml of extract or 100 mg/100 g body weight. Thirty minutes later, a second series of blood samples were taken before the administration of glucose (baseline T30); then after, all animals received glucose 50% w/v solution overload as 4 g per Kg of body weight. Other series of blood samples were collected hourly at T90, T150 and T210. The blood was collected directly on a test strip and the blood glucose read on the handheld Glucometer®.

2.4 Data Analysis

The activity was expressed as percentage of change in glycemia (PRG) on the basis of negative control according to the following formula:

$$PRG = 100 \cdot \frac{(G_{control} - G_{extract})}{G_{control}}$$

The values were calculated as mean \pm ESM (Standard Error on Mean). The treatment of data was carried out with Excel Windows software using 'ANOVA' and Tukey's post hoc test for significance at 95% confidence limit.

3. RESULTS

The extraction yielded $6.95 \pm 0.89\%$ of Va-ethanolic extract, $6.8 \pm 0.6\%$ of Va-aqueous

extract, $2.85 \pm 0.08\%$ of n-butanolic extract and 4 ± 0.5 of Va-saponin extract. Fig. 2 presents the evolution of glycemia in time for different experimental groups.

As shown, the baseline values before the extracts were administered were slightly reduced or almost unchanged 30 minutes after in Va-ethanol, Va-aqueous, Va-butanol, Gk-aqueous and He-aqueous groups while the glycemia was however significantly ($p < 0.05$) increased by Va-saponins from 98 to 244 mg/dL. After glucose overloading, the peak of glycemia appeared at 1 hour (T90) reaching the values of 308.5 ± 1.2 for negative control, 292.0 ± 5.1 Va-butanol, 161.2 ± 3.6 for Va-aqueous, 158.2 ± 5.1 for Va-ethanol, 201.3 ± 9.4 for He-aqueous, 276.2 ± 30.4 for Gk-aqueous, and 105.5 ± 6.7 for glibenclamide.

Table 1 shows the AHGA values as PRG at T210 time. They varied from 42.5% for Va-butanol to 70.5% for Va-ethanol. The mean value of glycemia at T210 observed in Va-saponins group was superior to that produced in the control group (287 vs. 268). The effect of Va-ethanol extract was relatively 17% higher than the effect of the reference drug ($R = 1.17$).

4. DISCUSSION

The findings from the current study have shown the capacity of tested extracts to significantly ($p < 0.01$) reduce induced hyperglycemia in rabbits, at the exception of the saponins extract. At equivalent doses, Va-ethanol extract was the most active (70%) compared to V-aqueous and Va-butanol. The ethanolic extract induced an intense and prolonged activity superior to that of glibenclamide.

At the equivalent doses used, GK-aqueous activity is comparable to Va-butanol and less than the activity of He-aqueous. They take longer to lower blood glucose compared to Va-ethanol; this may relate to the speed of absorption.

The Va-saponins extract seemed inactive or capable of elevating blood sugar instead of reducing. However, the study by Okodowa et al. [25] showed antidiabetic effect of *Vernonia amygdalina* leaf n-butanol extract containing mainly saponins in fortified diet-fed streptozotocin-treated rat model of type-2 diabetes. Some saponins like ginsenoside isolated from the roots of Panax ginseng, and diosgenin isolated from *Trigonella foenum-*

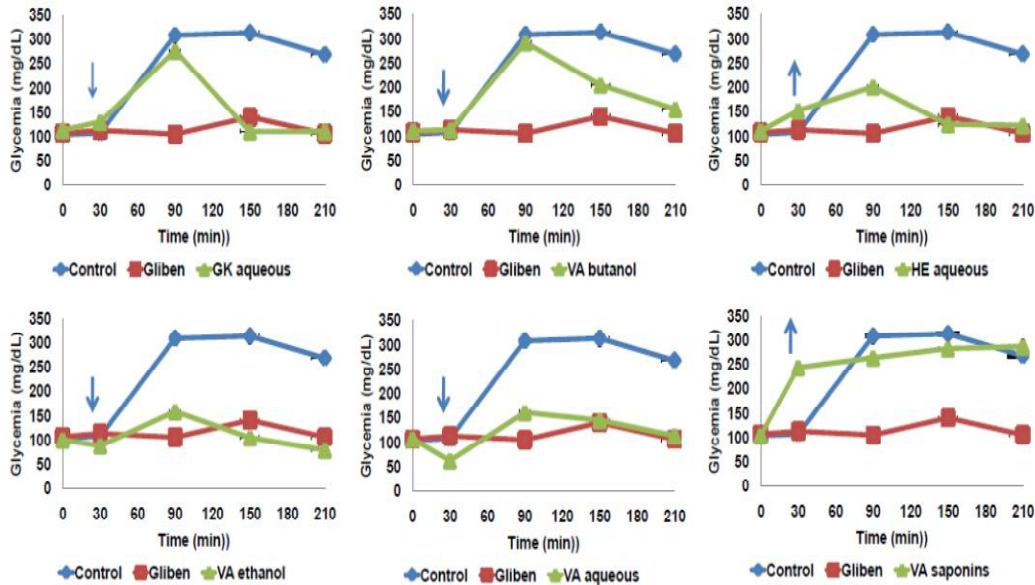


Fig. 2. Effect of various extracts on induced hyperglycemia compared to control and gliben
 Control =untreated group; Gliben=glibenclamide group; GK=garcinia kola group; HE=hibiscus esculentus group;
 VA= vernonia amygdalina group. The values are means of 5 animals. The extracts (100 mg/Kg) and
 glibenclamide (0.2 mg/Kg) were administered at T0 and glucose (4 g/Kg) at T30

Table 1. Mean antihyperglycemic activity

	T210	PRG(T210)	Ratio
Control (negative)	268.2±10.3	0	
Glibenclamide	106.0±2.5	60.5	1.00
Va-ethanol	79.2±2.0	70.5	1.17
Va-aqueous	113.6±3.5	57.6	0.95
Va-butanol	155.0±3.7	42.2	0.70
Va-saponins	287.0±2.0*	-7.0*	*
He-aqueous	122.0±8.6	54.5	0.90
Gk-aqueous	110.7±7.1	58.7	0.97

Percentage of change in glycemia (PRG) *Value > control

Graecum have been found endowed of antihyperglycemic activity [26-28] contrary to our findings. Even though saponins do not influence blood sugar levels in glucose tolerance protocol, their property of reducing the level of triglycerides and cholesterol in the blood is recognized by virtue of their surfactant property. Some studies have found a significant decrease in weight gain induced by raw saponins of *V. amygdalina* leaves in rat [29].

The chemical compounds holding this activity were not determined in this study but may be similar to those described elsewhere in the literature [Fig. 3].

For *Vernonia* species, a number of biologically-active compounds have been found in Va-leaves

[10,11,30-33]. Vernodalinal (sesquiterpene lactone), vernonioside B1 (saponoside) and luteolin (flavonoids) are among the active compounds. As already mentioned, vernodalinal is antiplasmodial; vernonioside B1 is antiplasmodial and antischistosomal, and Luteolin is a powerful antioxidant and anticancer [30-33]. The precise active ingredient responsible for the antidiabetic is still unknown. Furthermore, the Va-leaves contain about ten amino acids including arginine [34]. It is also possible that these amino acids have a direct influence on blood glucose levels. Arginine has been found hypoglycemic in mice lacking GLP-1 receptors by improving the sensitivity of cells to insulin [35]. It is also known that the regular consumption of dietary fibers is linked to the increase in the synthesis of GLP-1, a hormone made in the

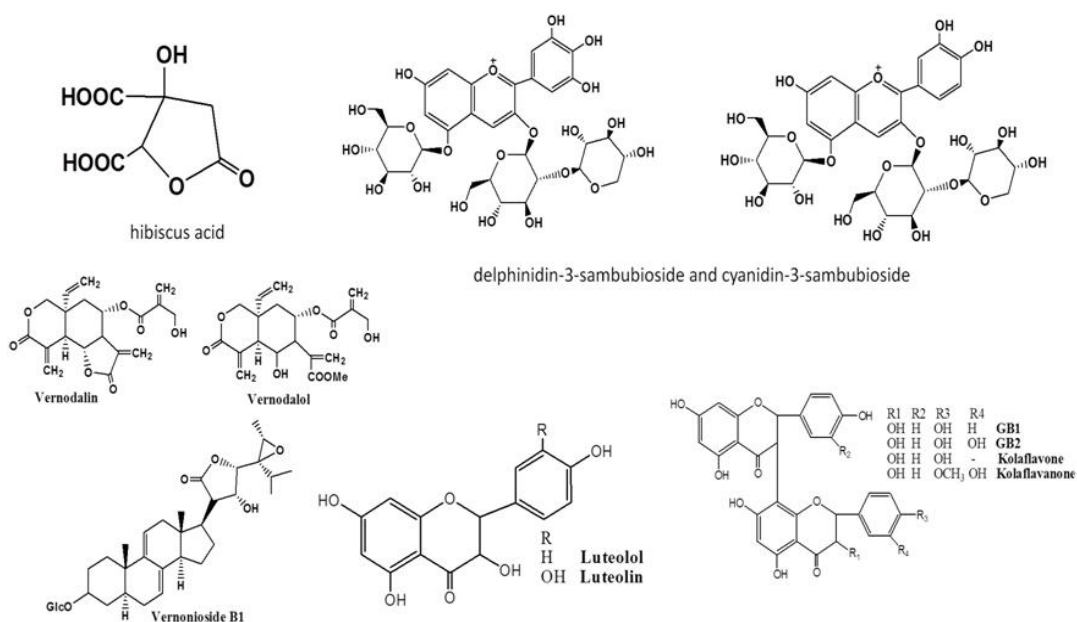


Fig. 3. Some secondary metabolites from *V. amygdalina*, *H. esculentus*, *G. kola* species

intestine that induces 70% of the insulin released after ingestion of dietary carbohydrates [35,36]. In addition, an amino acid, 4-hydroxyisoleucine extracted from Fenugreek seeds stimulates the secretion of glucose-dependent insulin, decreases insulin resistance and inhibits hepatic glucose release [28].

For Hibiscus species, the activity might be linked to strong antioxidant activity, inhibition of alpha-glucosidase and alpha-amylase, inhibition of angiotensin-converting enzyme (ACE), and direct vaso-relaxant effect or calcium channel modulation. The species contains mucilage as the major active ingredient [12,13]. This soluble fiber is a very hydrophilic heterogeneous polysaccharide which would be responsible for antihyperglycemic activity by reducing the rate of absorption of carbohydrates in the intestine. Phenolic acids (e.g. protocatechic acid), organic acid (hydroxycitric acid and hibiscus acid) and anthocyanins (delphinidin-3-sambubioside and cyanidin-3-sambubioside) are likely to contribute to the reported effects [12,13].

Studies on Gk report that aqueous decoction of Gk contains mainly flavonoids of the biflavonoid type. These flavonoids are thought to be responsible for the antihyperglycemic activity of Gk seeds [10,14,15].

Finally, the findings concern total extracts and should not extrapolated to the potential pure

compounds. Improved tradimedcines that can be prepared with these extracts shall be standardized and validated according to pharmaceutical requirements.

5. CONCLUSION

The purpose of this study was to compare the antihyperglycemic activity of different extracts of three plants, identify the most active plant and the phytochemical group responsible for this activity, in light of the chemical composition of each extract. All extracts have a relative activity comparable to glibenclamide with the exception of Va-saponins. Improved tradimedcines can be prepared with ethanolic or total polyphenolic dry extracts.

CONSENT

It is not applicable.

ETHICAL ISSUES

The study protocol was approved by the ethical committee of the University FMP 130/2016 and fulfilled the requirements of EEC Directive applicable to animal experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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