IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
July 2017 Vol.:9, Issue: 4

Comparative Phytochemical Composition and Hypoglycemic Activity of Some Plants Used by Traditional Healers to Treat Diabetes in Kisangani

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Submission: 5 July 2017
Accepted: 10 July 2017
Published: 25 July 2017

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Antidiabetic plant extracts, flavonoids, tannins, saponins, hypoglycemic bioactivity, rabbits.

ABSTRACT

Background: Phytochemical and Biological studies are always needed to define chemical composition, bioactivity and toxicity of plants from folk medicines before integrating them into conventional medicines. Here we compared phytochemical composition and antihyperglycemic activity of some plants used in Kisangani to treat diabetes. Methods: The plants tested are *Aloe vera (AV), Bidens pilosa (BP), Cassia alata (CA), Cassia occidentalis (CO), Catharanthus roseus pink flower (CRp), Catharanthus roseus white flower or alba (CRw), Mangifera indica (MI), Morinda lucida (ML), Morinda morindoides (MM), Panda oleosa (PO), Terminalia catappa (TC), and Vernonia amygdalina (VA)*. Their content in polyphenols, saponins, alkaloids and mineral ash were compared. Hyperglycemia was induced in rabbits by oral glucose tolerance test with glibenclamide 0.2 mg/kg as reference. Blood glucose level was assayed by Folin-Wu photometric method. The mean percentage in glucose level reduction (MPR) was calculated from control untreated animals. The relative potency of each extract (RP) was calculated from glibenclamide MPR taken as 100%. Results: Flavonoids, tannins and saponins, were the main components; alkaloids were found only in CRp, CRw, ML and MM. The water content varied from 67% to 88%; Total ashes content was lower in roots (9%) than other parts (11-16%). Glibenclamide gave MPR=56.8% and RP=100%. MPR and RP for plant extracts were, ML(29.8%; 52.4%), CA(31.9%; 56.2%), MI(46.6%; 81.9%), MM(46.6%; 81.9%), TC(47.2%; 83.1%), VA(49.4%; 86.9%), CO(54.4%; 95.8%), CRw(57.4%; 101.0%), BP(60.8%; 107.0%), CRp(63.2%; 111.1%), AV(64.5%; 113.4%), PO(83.2%; 146.3%).Conclusion: All plants but Panda oleosa have been studied by others; the main phytochemical groups reported have been confirmed in the local species. All plants exhibited some antihyperglycemic activity, differing however by their relative potency.

INTRODUCTION

Diabetes mellitus is defined as a heterogeneous group of metabolic dysfunctions mainly characterized by chronic hyperglycemia and resulting from pancreatic failure to secrete sufficient insulin or from the combination of resistance to insulin action and excessive or inappropriate glucagon action [1]. It is a complex disease by its pathophysiological mechanisms, its genetic determinism, as well as the genesis of its complications [1]. In 2013, the WHO estimates showed a global prevalence of 382 million people with diabetes and expected to rise to 592 million by 2035 [2]. The impact of this pathology on health systems is very heavy through human losses, disability and cost associated with treatment [3]. In the poorest countries, modern medicines for the management of diabetes are less available or cost unaffordable in remote rural area. Worldwide, a particular attention has focused on the use of medicinal plants in the treatment and control of this disease in accordance with WHO recommendations. That is, the use of medicinal plants in the treatment of diabetes has increased in many developing countries. Currently, diabetes is one of the most diseases targeted in phytotherapy researches. Phytochemical and Biological studies are always needed to define the chemical composition, the bioactivity and the toxicity of plants from folk medicines before integrating them into conventional medicines. In order to make a contribution to the study of plants used in traditional medicine in Democratic Republic of Congo (DRC), ethnopharmacological surveys have been conducted to list the antidiabetic plants used [4, 5]. The current study aimed at evaluating the chemical composition and the antidiabetic potentials of 12 plants listed in Kisangani city to verify and compare the chemical content and the antidiabetic potential of local species with other species.

2. METHODS

2.1. Plant materials

The 12 plants tested were *Aloe vera* L (AV) of Aloeaceae, *Bidens pilosa* (BP) of Asteraceae, *Cassia alata* (CA) of Caesalpiniaceae, *Cassia occidentalis* L (CO) of Caesalpiniaceae, *Catharanthus roseus* while flower (CRw) of Apocynaceae, *Catharanthus roseus* L. G. Don pink flower (CRp), of Apocynaceae, *Mangifera indica* L (MI) of Anacardiaceae, *Morinda lucida* Benth (ML) of Rubiaceae, *Morinda morindoides* Milne-Redh (MM) of Rubiaceae, *Panda oleosa* Pierre (PO) of Pandaceae, *Terminalia catappa* L(TC) of Combretaceae, and *Vernonia amygdalina* Del (VA) of Asteraceae species. The samples consisting of young leaves, roots or thick stem barks were harvested in the morning 7 to 9 am in the area around Kisangani. Species were identified by a botanist at IFYA (Institut Facultaire Agronomique de Yangambi) where their herbarium specimens were recorded. The fresh plant organs were shade-dried at room temperature, then ground with mortar and stored away from moisture until analysis at the Faculty of Sciences of the University of Kisangani. Some tests were processed on fresh materials.

2.2. Phytochemical screening

Common available reactions and procedures were used [6, 7] with little adaptations. A given aliquot of plant powder was either infused for 30 minutes in distillate water or macerated for 24 hrs in a specific medium. Then a specific reagent was added. The intensity of a specific color or precipitate indicated very a possible presence of the compound. In summary, Dragendorff and Mayer reagents were used for alkaloids; Lieberman Burchard and $FeCl_3$ reagents for saponosides; Shinoda test (cyanidine reaction) and $FeCl_2$ for flavonoids; $FeCl_3$ for tannins and Borntrager reaction for quinones.

Testing for saponins

5 g of raw powder was soaked in 50 mL distillated water in a beaker; 10 mL of the filtrate was placed in a test tube of 16 mm in diameter and 160 mm in height and stirred. Foaming which persisted on warming for 10 minutes was taken as an evidence for the presence of saponins.

Testing for tannins and phenolics

0.5 g of the powder was stirred with 10 mL of distilled water and then filtered. A few drops of 1% $FeCl_3$ reagent was added to the filtrate. Blue-black or blue-green coloration or precipitation was taken as an indication of the presence of phenolics and tannins.

Testing for alkaloids

1 g of the powder was soaked in 10 mL of 5% HCl for 24 hours. The solution obtained was filtered and 3 mL of the filtrate was treated with a few drops of Dragendorff-Mayer's reagents. The turbidity or orange precipitate of the filtrate on addition of the reagent was taken as evidence of the presence of alkaloids.

Detection of quinones

5 g of the powder was wetted with a few drops of HCl 1/5 N and then soaked in 30 mL of ether-chloroform mixture (1:1, v/v) for 24 hours in a conical flask. Then, 2 mL of the filtrate was mixed with the same volume of NaOH 1/10 N. Red to purple coloration was taken as an indication of the presence of quinones.

Detection of flavonoids

5 g of the powder was heated with 50 mL of distillated water for 30 minutes in a hot bath. After cooling and filtration, 5 mL of the filtrate was mixed with 5 mL of ethyl alcohol 95%, 2 g of magnesium turnings, and a few drops of isoamyl alcohol. The appearance of a pink, red or orange color in the isoamyl alcohol supernatant layer indicates the presence of flavonoids.

Detection of terpenes-sterols

1 g of plant material, coarsely crushed, was macerated for 24 hours in 20 mL of ether. About 5 drops of the macerate is evaporated on a watch glass. The residue is mixed with 2 drops of acetic anhydride. The addition of a drop of 32% sulfuric acid turns the solution to mauve-green coloration in the presence of sterols and terpenes.

Quantification of moisture and mineral ashes

The fresh material of known weight is dried in an oven at 105°C until constant weight. By the difference in weight between the fresh material and the dry matter, the moisture is deduced there from. The crude ash was obtained after ignition at high temperature (550^{o}C) of a dry material.

2.3. Measurement of antihyperglycemic activity in rabbits

Animals' treatment

The hypoglycemic activity was realized with a filtrate of 20% w/v aqueous extract of each ground plant organ obtained by infusing 20 g of material in 80 ml distilled water for 15 minutes and then completed to 100 ml. The test was processed as previously described [8] using male rabbits aged 5-8 months weighing 1.5 kg to 2.5 kg BW. These animals were kept, prepared and used according to the standards required for experiment on laboratory animals [9]. During the 10-day acclimation period and throughout the experimental period, the animals were kept in a normal temperature and photoperiod environment. Two days before the experiment, the animals were distributed in 3 groups of 5 rabbits each: (i) a control untreated group (CG) to which only physiological water was administered; (ii) a reference group (RG) treated with glibenclamide (0.2 mg/kg) and t (iii) group treated with different extracts (100 mg/kg). Thirty minutes later, all animals were subjected to 50% w/v glucose solution overload (4 g/kg). Blood samples were taken by transverse incision of the marginal vein of the lobule of the ear at T30, T60, T90, T120, T150 and T180 minutes. The blood was aspirated with a 2 ml syringe containing previously sodium fluoride solution as anticoagulant and glycolysis inhibitor.

Laboratory glucose test

Each stabilized blood sample was poured into a hemolytic tube. After defecation and centrifugation, the supernatants were used for the quantitative determination of blood glucose by a modified photometric method of Folin-Wu [10].

2.4. Statistical analysis

Quantitative values are expressed in mean ± SD. Significance of difference was estimated with ANOVA test at 0.05% Confidence limit.

3. RESULTS AND DISCUSSION

3.1. Comparative phytochemical composition

The comparative content in phytochemical groups is shown in **Table.** The major components were tannins in all extracts but CRw, ML. Flavonoids were found in CA, CO, MM, PO and VA. Saponins were not detected in BP, CRp and MI. Alkaloids were found in 4 extracts, CRp, CRw, ML and MM. Sterols and terpenes were found in BP, ML and TC extracts. Quinones were present only in AV extract. The water content varied from 67% to 88%; Total ash content was lower in roots (9%) than other parts (11-16%).

Sometimes the result may be inconsistent with data reported in the literature. The differences would be related to the parts used, the origin of the plant, or the technical procedures. The absence means the test was not positive in the experimental conditions rather than absolute absence.

A number of bioactive molecules have been isolated from those plants and tested for their various biological activities like anticancer, anti-inflammatory, antimicrobial, antifungal, anti-parasites, antiviral effects. *A. vera* contains 75 potentially active constituents including vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids [11]. *B. pilosa* contains 201 compounds comprising 70 aliphatics, 60 flavonoids, 25 terpenoids, 19 phenylpropanoids, 13 aromatics, 8 porphyrins, and 6 other compounds [12]. From *C. alata*, 12 compounds have been isolated such chrysoeriol, kaempferol, quercetin, and derivatives [13]. *C. occidentalis* is rich in flavonoids, alkaloids, lignin, tannins, and phenols [14] while *C. roseus* possesses flavonoids, saponins and alkaloids [15]. *M. indica* contains polyphenols, flavonoids and triterpenoids; its stem bark contains protocatechuic acid, catechin, mangiferin, and the tetracyclic triterpenoids [16]. *M. morindoides* contains saponosides, flavonoids such as quercetin, luteolin, apigenin, kaempferol, rutinoside; terpenes; tannins and anthraquinones[17]. *T. capatta* is rich in ellagitannins (punicalin and punicalagin), gallic acid and C-flavonoid glycosides [18]. *From V. amygdalina*, saponins, alkaloids, tannins, steroids, flavonoids and glycosides were found in very high, high, or low concentrations [19].

Table 1 Detection of phytochemical groups and moisture and total ashes in the tested plants

Plants	Sample	ALK	FLAV	TAN	SAP	QUIN	TERS	% H_2O	% Ashes
A. vera	Leaf			+	+	+		88.3±2.2	16.2±0.8
B. pilosa L.	Leaf		+	+			+	84.2±2.5	13.9±0.9
C. alata	Leaf		+	+	+			84.3±3.3	14.3±1.3
C. occidentalis	Leaf		+	+	+		+	84.3±3.2	10.6±0.6
C. roseus (pink)	Root	+		+				83.5±2.7	9.7±0.8
C. roseus (white)	Root	+			+			83.3±2.7	9.7±0.5
M. indica	Back			+				67.2±0.2	19.3±0.6
M. lucida	Leaf	+			+		+	80.6±1.9	14.8±1.7
M. morindoides	Leaf	+	+	+	+			79.9±1.3	11.9±1.7
P. oleosa	Back		+	+	+			86.1±2.1	16.8±1.6
T. catappa	Leaf			+	+		+	76.2±1.0	17.3±0.5
V. amygdalina	Leaf		+	+	+			85.0±2.6	10.9±0.4

Flavonoids (FLAV); Tannins (TAN); Saponins (SAP); Alkaloids (ALK); Quinones (QUIN) and terpenes-sterols (TERS).

3.2. Comparative hypoglycemic activity

Table-2 presents MGL values measured at different times. The baseline of blood glucose levels in our animals was between 72 and 110 mg%, consistent with normal values range (70 to 120 mg %) reported for rabbits. For the control group, the maximum MGL was 333±6 mg% at T120 while for reference group (glibenclamide) it was 140±2 at the same time. Among the extracts, the lowest value was given by *P. oleosa* extract (48±17) and the highest by *T. catappa* extract (240±1). As described in Methods section, the glycemia of each extract at T120, T150 and T180 were divided by the corresponding values observed in control saline group and then averaged to calculate the mean percentage of reduction (MPR). Also, each MPR was divided by MPR of the reference glibenclamide group to calculate the relative potency (RP).

Fig.1 shows MPR and RP values. For the reference glibenclamide, MPR was 56.8% which was taken as RP100%. The MPR and RP% for the extracts were as follow from smallest to highest: ML(29.8%;52.4%), CA(31.9%;56.2%), MI(46.6%;81.9%), MM(46.6%;81.9%), TC(47.2%;83.1%), VA(49.4%;86.9%), CO(54.4%;95.8%), CRw(57.4%;101.0%), BP(60.8%;107.0%), CRp(63.2%;111.1%), AV(64.5%;113.4%), PO(83.2%;146.3%). Thus, PO was the most potent (1.46) and ML the last potent (0.52) compared to glibenclamide.

All plants but *P. oleosa* have intensively been studied elsewhere for their antidiabetic potentials [14-24]. The species use locally also exhibited some capacity to reduce hyperglycemia induced in rabbits. This is consistent with some studies done in other animal models (rats or mice) concerning *A. vera* [20], *B. pilosa* [21], *C. alata* [22], *C. occidentalis*[23], *C. roseus*[25], *M. indica*[25], *M. lucida*[26, 27], *M. morindoides* [28], *T. catappa* [29], and *V. amygdalina* [30, 31]. The antidiabetic activity would be related to saponins, flavonoids or tannins, substances known for their anti-radical activity.

Table 2 Blood glycemia in rabbits treated with plant extracts after glucose overload

Treatment	Mean glycemia ± SD (mg/100ml)						
	Part	T30	T60	T90	T120	T150	T180
Saline 0.9%	-	256±6	321±4	327±6	333±6	289±4	246±3
Glibenclamide (Ref)	.	96±4	106±4	123±5	140±2	123±3	106±3
A. vera	Leaf	95±3	102±3	103±6	103±14	153±5	56±4
B. pilosa L.	Leaf	200±4	213±5	186±4	160±4	126±2	93±3
C. alata	Leaf	200±2	201±5	210±4	220±2	196±2	173±5
C. occidentalis	Leaf	124±4	150±6	170±5	190±14	154±3	118±5
C. roseus (pink)	Root	200±5	120±3	126±3	132±3	130±2	128±2
C. roseus (white)	Root	160±3	113±4	116±2	120±2	112±2	105±2
M. indica	Back	180±2	133±2	146±5	160±2	153±4	146±3
M. lucida	Leaf	200±2	213±5	206±7	200±7	200±2	200±4
M. morindoides	Leaf	120±3	133±3	146±4	160±2	153±3	146±3
P. oleosa	Back	60±10	60±7	54±6	48±17	48±4	48±2
T. catappa	Leaf	200±4	253±5	246±8	240±1	153±3	66±3
V. amygdalina	Leaf	133±2	137±4	128±3	120±2	106±2	93±3

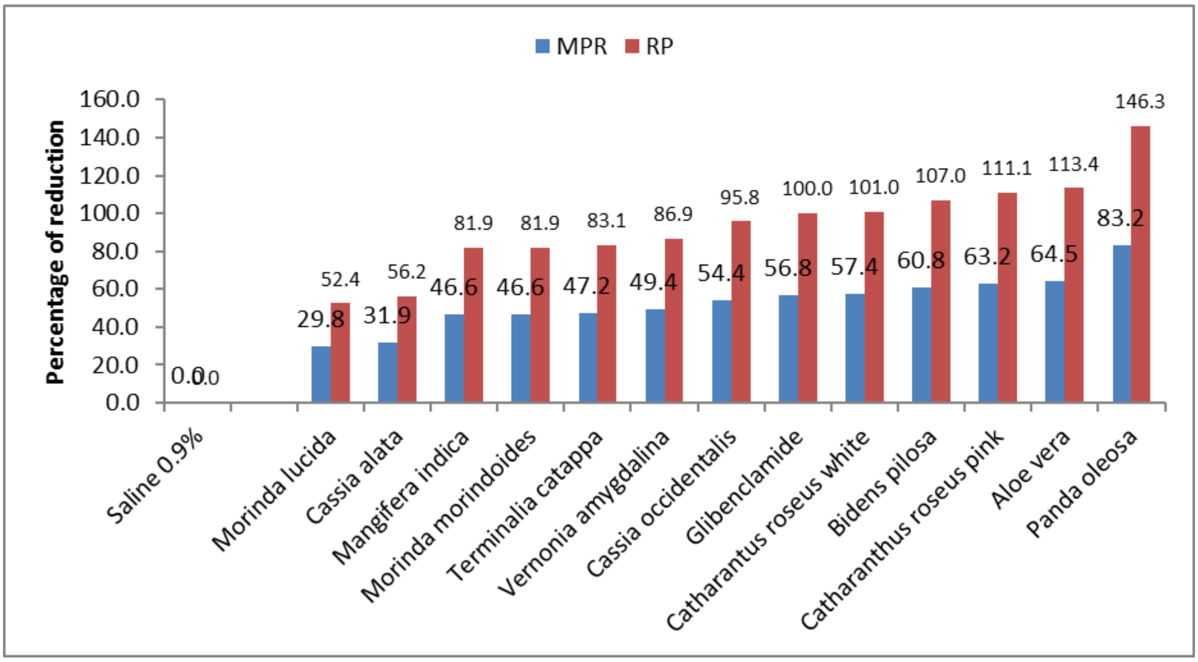

Figure 1: Comparative hypoglycemic potency of different plants and Glibenclamide reference

Mean percentage of reduction from control (MPR); Relative potency (RP) vs. Reference drug

CONCLUSION

All crude extracts from the twelve plants tested have shown some antihyperglycemic activity, differing by their relative potency. All plants but *Panda oleosa* have been studied by others. However, *Panda oleosa* showed the highest hypoglycemic activity. Thus, it is worth deeply studying its phytochemical and bioactivity.

ETHICAL ISSUES

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

CONFLICT OF INTEREST

N/A

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