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Comparative Hypoglycemic Activity of Flavonoids and Tannins Fractions of *Stachytarpheta indica* (L.) Vahl Leaves Extracts in Guinea-Pigs and Rabbits

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ABSTRACT

The study assessed whether the reported anti-hyperglycemic effect of *Stachytarpheta indica* (L.) Vahl is related to the activity by tannins or flavonoids fractions using Guinea-pigs and Rabbits as experimental animals. Total leaf aqueous extract, tannins and flavonoids fractions were prepared following conventional procedures. Hyperglycemia was induced by Oral Glucose Tolerance Test and blood glucose levels were measured with Glucometer (One Touch Ultra®). Measurements were performed in groups of animals treated with extracts (100 mg or 200 mg/kg single dose) in the same way as for negative untreated groups and positive glibenclamide (0.25 mg/kg) treated groups. Phytochemical fractionation yielded 6.4% of tannins and 2.5% of flavonoids. The extracts were active in both the guinea-pigs and rabbits. Flavonoids exhibited superior activity compared to tannins ($p<0.05$). The findings confirmed the hypoglycemic effect reported for *Stachytarpheta indica* (L.) Vahl in other animal species, and that could be related more likely to flavonoids than to tannins.

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1. INTRODUCTION

Diabetes is a chronic metabolic affection listed among life-threatening or deadly non-communicable diseases in the global world. According to WHO report in 2014, 9% of adults 18 years and older worldwide had diabetes [1]. In 2012 diabetes was the direct cause of 1.5 million deaths worldwide and more than 80% of those deaths occurred in low- and middle-income countries [2]. WHO projects that diabetes will be the 7th leading cause of death in 2030 [3]. Statistics also show the disease will affect more than the current 347 million people with diabetes by 2030 [4,5].

As a chronic disease with multiple complications, diabetes has captured attention of many researchers striving to scrutinize pathophysiological mechanisms and develop more efficient medicines. A new class of biotech research is promising with unfortunately a related higher cost expected. In poor developing countries where people in the majority can barely afford such costs, a number of plants have been used to manage diabetic patients in folk medicines [6-10], and the innovation will stem from isolating plant active secondary metabolites that can be cost-effective and safer than synthetic products.

Stachytarpheta indica (L.) Vahl (Figure 1) is an invasive herb species of Verbenaceae family found in many continents. Studies have been reported in the literature referring to many of its biological activities, e.g. wound healing [11], antimicrobial activity [12-15], hepatoprotection [16], hypoglycemic activity [17] and cardiovascular effects [18,19].

In the Eastern part of the Democratic Republic of Congo (DRC), the extracts of *Stachytarpheta indica* are used in folk medicine for many purposes, particularly as antidiabetic, febrifuge, tonic, hepatoprotective, diuretic, antihypertensive, anti-dyspeptic, and anti-inflammatory, according to traditional healers' claims. This paper assessed the antidiabetic potential of tannins and flavonoids fractions on diabetic animal model to look at the fraction endowed with that activity.



(a) **(b)** **(c)**

Figure 1. Photos of *Stachytarpheta indica*

(a) From Wikipedia; (b) and (c) species growing in Bukavu

2. MATERIALS AND METHODS

2.1. Plant material

The plant growing in Bukavu city was authenticated in the herbarium of Centre de Recherche en Sciences Naturelles (CRSN) de Lwiro [20] and in the Laboratory of Ecology and Plants Resource Management at the Faculty of Sciences and Applied Sciences of the Official University Official of Bukavu (UOB). Fresh leaves of *S. indica* were collected, shed-dried out of moisture and insects in the Laboratory of pharmacognosy; then ground in a mortar and sieved.

2.2. Preparation of plant extracts

The first step consisted of identification of different groups of secondary metabolites in chloroform, methanol and aqueous extracts using usual color and precipitation reactions specific to alkaloids, quinones, saponins, terpenoids, tannins, flavonoids and anthocyanins [21-23]. The second step engaged the fractionation and separation of tannins and flavonoids using common procedures described elsewhere [24].

For flavonoids isolation, aliquots of 40 g of plant powders were soaked in 400 ml of aqueous methanol (85%) for 24 hours and the mixture was filtered (Whatman filter no-1) thereafter. The marc was further extracted twice with 400 ml methanol 85% and 400 ml methanol 50% aqueous solutions. Filtered macerates were combined and evaporated at 65°C to remove methanol. The resulting aqueous phases were degreased with 50 ml of toluene. The water was thereafter

evaporated under vacuum with Rotavapor at 45°C. The flavonoids residue obtained was weighed and stored until *in vivo* experiments.

For tannins, aliquots of 25 g of the plant powder were macerated in 250 ml of aqueous acetone 70% for 30 min. The operation was repeated three times. The three fractions were filtered and distilled to remove acetone. The aqueous solutions were washed with toluene for degreasing. After evaporation of water to dryness under vacuum, the residue obtained was weighed and also conserved for *in vivo* tests.

2.3. Animals

Guinea-pigs aged 3 to 4 months old and weighing 350 to 450 g and rabbits weighing 1.5 kg to 2.5 kg BW were chosen for this experiment. These animals were kept in the animal boundary of the Faculty of Medicine and Pharmacy, prepared and used according to the standards required for experiment on laboratory animals [25].

2.4. Hypoglycemic activity test

The anti-hyperglycemic effect was evaluated on the glucose tolerance test (GTT) as previously described [8, 26]. In brief, the animals were randomly assigned into 7 groups:

(1) (GCG) guinea-pig control group given only 1ml saline/100g BW;
(2) (GRG) guinea-pig reference group given glibenclamide 0.25mg/kg BW;
(3) (GFG) guinea-pig flavonoids group given flavonoids 100mg/kg BW;
(4) (GTG) guinea-pig tannins group given tannin fraction 100mg/kg BW,
(5) (RCG) rabbit control group given only physiological saline;
(6) (RRG) rabbit reference group given glibenclamide 0.2mg/kg BW,
(7) (REG) rabbit extract group given total extract 200mg/kg.

Animals were fasted fourteen hours before an experiment to enable stable baseline of glucose levels and avoid food interference on the absorption of the plant extracts. The first blood drop samples were collected just before administration of respective medications given by force-feeding (T0). Glucose 50% solution (w/v) was administered as 4g/kg to each animal 30 minutes later after medications. Then a series of second blood drop samples were collected at T30, T60,

T90, and T120 minutes after glucose loading. One touch electronic Glucometer (One Touch Ultra®) was used for glucose concentration measurement in all collected blood samples.

2.4. Data analysis

Mean glucose levels (MGL) in blood samples were calculated for each group of 3 animals at different times. Mean percentages of reduction (MPR) in glucose levels from control values were also calculated at each time. The t-student test was used to compare means of glycemia in different groups at $p<0.05$ significance level.

3. RESULTS

3.1. Phytochemical groups identified

The identification tests were positive for flavonoids, tannins, terpenoids, quinones, anthocyanins and alkaloids. For our extracts, only flavonoids and tannins fractions were prepared, that yielded 6.4% as total tannins and 2.5% as total flavonoids residues.

3.2. Anti-hyperglycemic activity measured

Table 1 presents the MGLs values obtained under the experimental conditions described in methods. The baseline values before treatment were close to 100 mg/dL and not significantly different between groups ($p>0.05$). The glucose peak-concentrations were observed 30 minutes after glucose load varying from 313±9 mg/dL in GRG to 426±2 mg/dL in RCG. From there, the rates of MGLs were significantly different between groups. In guinea-pigs, the reference glibenclamide exhibited the highest reduction rate from 313 mg/dL (T30) to 115 mg/dL (T120), followed by flavonoids extract from 382 mg/dL (T30) to 105 mg/dL (T120), and then by tannins from 341 mg/dL (T30) to 236 mg/dl (T120). The t-test comparing MGLs globally indicated significant difference ($p=0.001$) between groups at T90 and T120.

Table 2 shows that flavonoids had the greater activity between T90 and T120 than tannins. MPRs at T120 were about 45% for the reference glibenclamide, 30% for flavonoids extract and 20% for tannic extract. The t-test comparing MPRs globally also indicated significant difference ($p<0.05$) between groups at T90 and T120.

Definitely, as shown in Figure 2, the administration of 100 mg/kg of flavonoids and tannins extracts in guinea-pigs showed the hypoglycemic potential as compared to untreated control animals and to reference glibenclamide. In rabbits given a total leaf-extract, the profile of the evolution of MGLs is similar to that observed in guinea-pigs.

Table 1. Evolution of Mean Glucose Levels (MGLs ± SD) at different times

Time	GCG	GRG	GFG	GTG	RCG	RRG	REG
T0	112 ±6	109 ±7	112 ±5.7	107 ±4.7	98±3	96±4	97±3
T30	410 ±6	313 ±9	382 ±10	341 ±14	430±28	419±16	426±2
T60	396 ±5	262 ±16**	365 ±11	336 ±13	398±6	382±8	278±3**
T90	391 ±10	195 ±13**	312 ±14**	328 ±14	402±2	277±6+*	222±3++
T120	248 ±13	115 ±14**	105 ±8**	236 ±15	322±9	101±2**	142±2**

Legend: MGLs for 3 animals; **Significant difference compared to control. Control untreated animals (GCG and RCG); animals treated with glibenclamide (GRG and RRG); animals treated with flavonoids extract (GFG); animals treated with tannins extract (GTG) and animals treated with total extract (REG) of *Stachytarpheta indica*.

Table 2. Mean Percentages of reduction (MPRs) in glucose levels at different times

Time	GRG		GFG		GTG		RRG		REG	
	MPR	±SD	MPR	± SD	MPR	± SD	MPR	± SD	MPR	± SD
T30	23.7	1.2	6.8	1.1	16.9	2.3	2.2	9.6	0.5	6.9
T60	34.0	3.5	7.9	1.7	15.2	2.3	4.0	3.1	30.2	0.6
T90	50.0	2.2	20.1	2.0	16.1	1.3	31.1	2.0	44.9	0.5
T120	53.7	3.6	57.6	1.4	4.9	1.3	68.7	0.3	55.8	1.7

Legend: MPRs (mean percentage of reduction in glucose level from control values). Control untreated animals (GCG and RCG); animals treated with glibenclamide (GRG and RRG); animals treated with flavonoids extract (GFG); animals treated with tannins extract (GTG) and animals treated with total extract (REG) of *Stachytarpheta indica*.

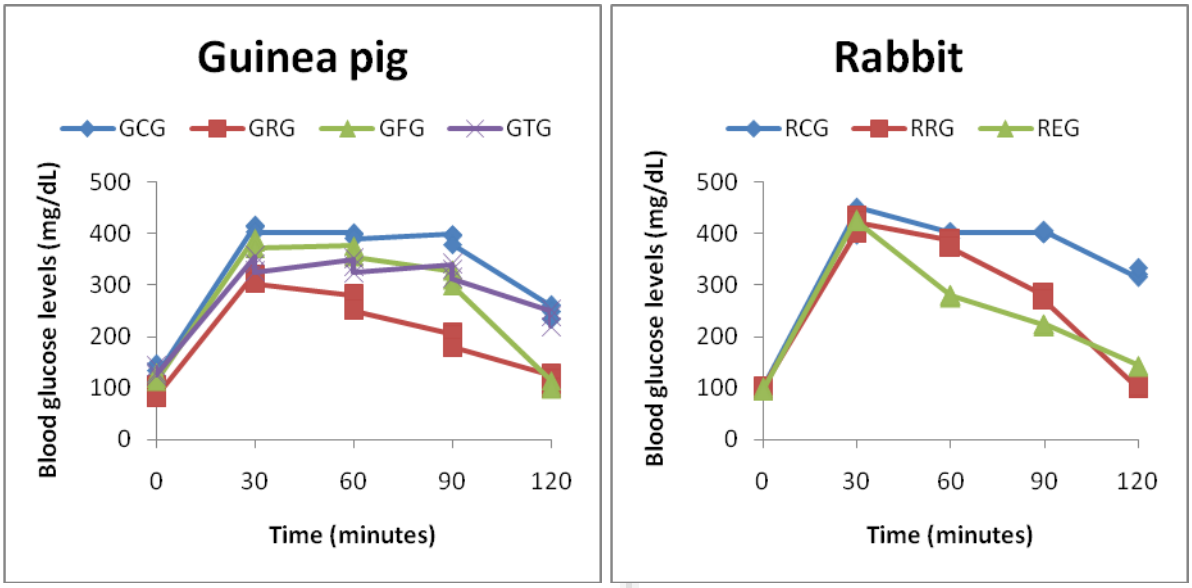

Figure 1. Evolution of Glycemia in 3 animals at each measurement time.

Legend: Control untreated animals (GCG and RCG); animals treated with glibenclamide (GRG and RRG); animals treated with flavonoids extract (GFG); animals treated with tannins extract (GTG) and animals treated with total extract (REG) of *Stachytarpheta indica.*

DISCUSSION

Stachytarpheta indica species collected for this study corresponded to the botanical description as an annual herb, 0.3-0.9 m high; stems erect, dichotomously branches; young branches nearly quadrangular, leaves 5-10 cm long, elliptic, obtuse or acute, coarsely serrate, glabrous or nearly so, base much tapering and decurrent into the petioles, with flowers deep blue, about 1 cm across, sessile in long, slender, nearly continuous glabrous spikes, reaching 30 cm long [20, 27].

The presence of flavonoids and tannins among other secondary metabolites is consistent with some studies from other species growing in different part of the world [12-15]. However, some discrepancies have been found. Some Indian authors did not detect alkaloids [17, 27], while others did [28]. Some detected flavonoids while others did not. From studies in Bangladesh [29], the plant contains Ipolamide, C29-C35 hydrocarbons, α-spinasterol, a saturated aliphatic ketone, a saturated aliphatic carboxylic acid and an unsaturated hydroxycarboxylic acid. Stems and leaves contain iridoid glycoside, tarphetalin. Leaves also contain traces of choline, an iridoid,

phenolic acids, chlorogenic acid, catechuic tannins, flavonols, luteolol and apigenol glucuronides, friedelin, stigmasterol, ursolic acid, hispidulin, scuttelarein and ipolamide [30].

In the Eastern part of the Democratic Republic of Congo (DRC), the extracts of *Stachytarpheta indica* are used in folk medicine as antidiabetic, febrifuge, tonic, hepatoprotective, diuretic, antihypertensive, anti-dyspeptic, and anti-inflammatory, according to traditional healers' claims [20]. In Bangladesh, the plant is abortifacient; used to treat intestinal worms, venereal diseases, ulcers, dropsy, stomach ailments, purulent ulcers, fevers and rheumatic inflammations; juice of the plant is used against cataract and open sores; infusion of the bark is used against diarrhea and dysentery; leaves are used in cardiac troubles and rubbed in sprains and bruises [27]. This confirms multipurpose use of this plant as claimed in DR Congo folk medicine.

Zeroing on the antidiabetic potential, only one study was found in the available literature conducted with *S. indica* in streptozocin induced diabetic wistar strain rats [17]. Another study reported on the activity of ethanolic leaves extract of *Stachytarpheta jamaicensis* on Alloxan-Induced Diabetic Sprague Dawley Rats [31] and the authors suggested that genipin and linolenic acid present in the extract might be the contributing properties for hypoglycemic and antioxidant activities. The results obtained in the current study conducted in guinea-pig and rabbits confirmed the antidiabetic potential as much more related to flavonoids fraction than tannins even though the last also showed the potential.

Herbal medicines for diabetes have been classified into four categories according to their mode of action: (1) drugs acting by modifying glucose utilization, (2) drugs acting by inhibition α-glucosidase the enzyme implicated in hydrolytic cleavage of oligosaccharide in the brush border of small intestine mucosa, (3) drugs acting like insulin, (4) drugs acting on insulin secreting beta cells, and (5) drugs acting by miscellaneous mechanisms. Several mechanisms are attributed to flavonoids for antidiabetic activity [32-38]. Flavonoids prevent diabetes most by their antioxidant effect.

CONCLUSION

The identification tests of secondary metabolites applied on *Stachytarpheta indica* L. Vahl species growing in DRC revealed the presence of alkaloids, flavonoids, quinones, polyphenols, saponins, terpenoids, tannins and anthocyanins in leaf extracts. The findings confirm the

hypoglycemic effect reported for *Stachytarpheta indica* (L.) Vahl in other animal species, and that could be related more likely to flavonoids than to tannins.

Ethical issues

Authorization has been obtained from UOB Ethical Research Committee

Conflict of interest

No conflict

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