INTRODUCTION
The genus Vitex (Verbenaceae) is constituted by 250 species of small trees and shrubs which occur in tropical and subtropical regions. Vitex thyrsiflora Baker (Verbenaceae) is a glabrous under shrub that is widely distributed in Cameroon. This plant is reported to be useful in the treatment of orchitis since they have shown promising antioxidant activity. Among all secondary metabolites, phenolic antioxidants appear to be the most important since they have shown promising antioxidant activity in both in vivo and in vitro investigations. Plant phenolics are mainly classified into five major groups, other Vitex species have resulted in the isolation of iridoids, diterpenes, steroids, flavonoids and triterpenoids. Several of previous compounds isolated from Vitex genus have shown antioxidants activities. Among all secondary metabolites, phenolic antioxidants appear to be the most important since they have shown promising antioxidant activity in both in vivo and in vitro investigations. Plant phenolics are mainly classified into five major groups, phenolic acids, flavonoids, lignans, stilbenes and tannins. The antioxidant activities of these compounds are mediated through various mechanisms including metal chelation, free radical scavenging, and inhibition of lipid peroxidation. The objective of the current study was to estimate the antihyperglycemic and anti-oxidant potential of ethanol extract of Vitex thyrsiflora leaves on diabetic rats.

METHODS: The antihyperglycemic effect of an ethanol extract of Vitex thyrsiflora leaves was investigated in normal male rats and streptozotocin induced diabetic male rats and its antioxidant potential was evaluated. After preparation of the extract, it was subjected to a phytochemistry screening, and tested on male rats made hyperglycemic in the Oral Glucose Tolerance Test (OGTT) and in streptozotocin diabetic rats. Glibenclamide (10 mg/Kg) was served as a positive control in both experiments. The evaluation of the antioxidant potential was done through the determination of the total polyphenols and total flavonoids contents, and by using the ferric reducing antioxidant power assay and the free radical scavenging activity on DPPH method.

RESULTS: The results show that the extract contains alkaloids, flavonoids, steroids, saponins and phenolic compounds. The test on hyperglycemic rats in OGTT showed that, the extract was effective (at a dose of 200 mg/Kg) to significantly decreased glucose levels. In streptozotocin diabetic rats, the IC50 value was found to be 65.97, based on the log (inhibitor) vs. normalized response-Variable slope.

CONCLUSION: FRAP appears to be significantly highly correlated with total polyphenols content and total flavonoids content. This shows that the ethanol extract of V. thyrsiflora leaves could be served to prevent acute hyperglycemia, but not a chronic hyperglycemic state.

Keywords: Antihyperglycemic, antioxidant, diabetic rats, Vitex thyrsiflora.
compounds have been attributed to various mechanisms which have been established by various assay procedures. Antioxidant activity has a fundamental role in cellular protection during an inflammation process. The identification of phytochemical compounds in plant species has been exploited in recent years, due to the growing popularity of herbal medicines and consumers growing enthusiasm for foods with bioactive characteristics that aid in preventing and fighting disease. Diabetes is characterized by chronic hyperglycemia, a source of increased oxidative stress and tissue oxidative damage. In particular, hyperglycemia promotes the glycation of proteins resulting in the formation of advanced glycation products (AGE). Thus antioxidant molecules appear as an opportunity for a strategy to fight not only against hyperglycemia, but also against complications related to diabetes mellitus. Then, the objectives of this study based on these findings, was first to evaluate the antihyperglycemic effect of an ethanol extract of V. thyrsiflora leaves in glucose loaded rats and in STZ-diabetic rats and second to determine the Total Polyphenols Content (TPC), Total Flavonoids Content (TFC), the Ferric Reducing Antioxidant Power Assay (FRAP) and the DPPH free radical scavenging assay of this extract.

MATERIALS AND METHODS

Experimental

Drugs and Chemicals
Streptozotocin, quercetin and catechin were obtained from Sigma Chemicals (St. Louis, MO). Glibenclamide (Glycomin®) was obtained from Strides Arcolat Ltd. Bangalore, India. All other chemicals used were of analytical grade. Spectrophotometric measurements were done using the equipment available at Institute of Medical Research and Medicinal Plant Studies.

Plant material
The leaves of V. thyrsiflora Baker (Verbenaceae) were collected at Melong, in the Littoral Region of Cameroon in November 2009, and authenticated was performed by Mr. Nana who compared it with a Voucher Specimen (No 34861 HNC), in the Cameroon National Herbarium, Yaoundé.

Extraction
The extract was obtained by maceration of air-dried and powdered leaves of V. thyrsiflora (3.0 Kg) with EtOH (3x15 L, 72 h) at room temperature, to obtain a crude extract (150 g).

Phytochemical screening of secondary metabolites
The V. thyrsiflora leaves ethanol extract was also subjected to phytochemical analysis according to the methods of Harborne and Evans.

Experimental Animal
Healthy adult male Wistar rats weighing 200-250 g were used in the present study. The animals were housed in clean grill cages and maintained in a well ventilated temperature controlled room at the animal house of Institute of Medical Research and Medicinal Plants studies, Yaoundé, Cameroon, with a constant 12h light/dark schedule. The animals were fed with standard rat pellet diet and clean drinking water was made available ad libitum.

Induction of diabetes
Rats were fasted overnight (16h) before inducing diabetes with streptozotocin. Streptozotocin was prepared in freshly prepared sodium chloride solution 0.9% and was injected intraperitoneally at a concentration of 55 mg/Kg body weight in a volume of saline of about 500µl/200g body weight by applying the protocol of Szكدelski. Control rats were injected with saline solution only. The diabetic state was confirmed 72h after streptozotocin injection. Threshold value of fasting blood glucose was taken as ≥ 200mg/dl. Diabetic rats were weighed, matched for body weight and divided into 5 groups consisting of 5 animals each.

Antihyperglycaemic effect of ethanol extract of V. thyrsiflora leaves on diabetic rats
The approval of the Institutional Animal Ethics Committee was obtained before starting the study. An International protocol for conducting experiments on animals were followed.

Experimental design
The Diabetic rats were divided into 5 groups with five rats each: (A, B, C and D). Group A rats received DMSO 3%, those of group B and C were treated with ethanol extract of V. thyrsiflora at the doses of 200 and 300 mg/Kg body weight respectively, and Group D received glibenclamide (10 mg/Kg body weight). Blood samples were collected before the commencement of treatment with the extract and then after, at 1h, 3h and 5h intervals.

Oral Glucose Tolerance Test on normoglycemic rats
This study was carried out on normal male rats with normal blood glucose level, according to the method of Schoenefelder et al. The animals were fasted for 16 h prior to the study. Five groups with 5 animals each were constituted and the animals received a dose of 3 g/Kg of glucose by oral route 60 min after haven been treated with the extract as follows:

Group I: vehicle (DMSO 3%; 10 ml/Kg b. w, negative control),
Group II: water-ethanol extract (200 mg/Kg b.w),
Group III: ethanol extract (300 mg/Kg b. w),
Group IV: ethanol extract (400 mg/Kg b. w)
Group V: glibenclamide (10 mg/Kg b. w), was served as positive control.

Blood was collected from the animals before administration of the extract (-60 min), and then after at 0, 30, 60, 90 and 150 min post administration.

Blood Glucose Estimation
Blood samples were obtained by tail prick and fasting blood glucose levels were estimated with a One Touch Ultra glucometer (Life Scan, Inc., Milpitas, CA, USA) in all animals. Blood glucose levels were expressed in mg/dl.

Dosage of phenolic compounds and antioxidants evaluation

Determination of Total phenolic content (TPC)
The ability of the extracts to reduce the phosphomolybdic-tungstate chromogene in Folin Ciocalteu with maximum absorbance at 760 nm (Total phenolic content, TPC) was assessed as earlier
Described by Vinson et al., data were reported as mean±SD for triplicate measurements. Catechin was used as control and the results were expressed as mg Catechin equivalent/g (mg CE/g).

**Determination of Total flavonoid content (TFC)**

The Total Flavonoid Content (TFC) was measured as earlier described. Total Flavonoid Content of the extract was expressed as mg Quercetin Equivalent/g (mg QE/g) through the calibration curve with quercetin. Data were reported as mean±SD for triplicate measurements.

**RESULTS**

Phytochemical screening revealed that the ethanol extract of *V. thyrsiflora* leaves contained alkaloids, flavonoids, steroids, phenolics compound, saponosids, catechic tannins and anthraquinones (Table 1). The Area Under Curve (AUC) associated with the effect of ethanol extract of *V. thyrsiflora* leaves on Oral Glucose Tolerance Test (OGTT) in normoglycemic male rats was significantly decreased (**p ≤ 0.01) at 200mg/Kg, when compared to vehicle control group (474±26.93 to 388.60±16.05), as well as in the group receiving glibenclamide at 10mg/Kg, where the AUC was decreased from 474±26.93 to 392.50±19.45 (Figure 1b).

**Table 1: Phytochemical screening of ethanol extract of *V. thyrsiflora* leaves**

<table>
<thead>
<tr>
<th>Phytochemical constituents tested</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Tri Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+++</td>
</tr>
<tr>
<td>Phenolics compound</td>
<td>+++</td>
</tr>
<tr>
<td>Saponosids</td>
<td>+</td>
</tr>
<tr>
<td>Catechic tannins</td>
<td>+++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>++</td>
</tr>
<tr>
<td>Glucosides</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) = indicates presence of phytochemicals and (+) = indicates absence of phytochemicals. +++ = shows high concentration; ++ = shows moderate concentration; + = shows small concentration.
The administration of ethanol extract of *V. thyrsiflora* leaves at 200mg/Kg or 300mg/Kg to diabetic rats didn’t show any difference in the blood glucose level, according to the AUC associated with each group tested. Even glibenclamide didn’t show a significant effect on lowering the high blood glucose level state induces by streptozotocin (Figure 2).

![Figure 2](image)

**Figure 2:** a. Effect of *V. thyrsiflora* ethanol extract on glycemia of STZ-induced diabetic rats; b. Area under curve associated with the effect of ethanol extract of *V. thyrsiflora* leaves on glycemia of STZ-induced diabetic.

Data are expressed as means ± S.D (n = 5); p<0.001 compared with the corresponding value for vehicle control rats. VT200: *V. thyrsiflora* 200 mg/Kg; VT300: *V. thyrsiflora* 300 mg/Kg; Glib10: Glibenclamide (10 mg/Kg).

The Total Polyphenol Content (TPC) appears to be significantly dose dependent on the concentrations of the extract, until 40 mg/Kg (79.36±0.3717 mg Catechin/g), where the TPC became stable and not different to that of 50 mg/Kg (Figure 3). The Total Flavonoid Content (TFC) evaluated in *V. thyrsiflora* ethanol extract showed a significant dose dependent increase when compared to 2.5mg/Kg. The content was the same at doses of 5mg/Kg and 10 mg/Kg (Figure 4). The correlation between Ferric Reducing Antioxidant Power (FRAP) and Total Flavonoid Content (TFC) showed a very strong positive correlation (Pearson r = 0.9223 with R²=0.8507, p** = 0.0088) (Figure 5). The correlation between FRAP and TPC was significantly high (p**=0.0010) (Figure 6).

![Figure 3](image)

**Figure 3:** Total Polyphenol Content of ethanol extract of *V. thyrsiflora*.

Data are expressed as means ± S.D (n = 3); a significantly different from 2.5 mg/ml, b significantly different from 2.5 mg/ml and 5 mg/ml, c significantly different from 2.5 mg/ml, 5 mg/ml and 10 mg/ml, d significantly different from 2.5 mg/ml, 5 mg/ml, 10 mg/ml and 20 mg/ml.

![Figure 4](image)

**Figure 4:** Total Flavonoid Content (TFC) of ethanol extract of *V. thyrsiflora*.

Data are expressed as means ± S.D (n = 3); a significantly different from 2.5 mg/ml, b significantly different from 2.5 mg/ml, 5 mg/ml and 10 mg/ml, c significantly different from 2.5 mg/ml, 5 mg/ml, 10 mg/ml and 20 mg/ml, d significantly different from 2.5 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml and 40 mg/ml.

The evaluation of the effect of *V. thyrsiflora* ethanol extracts leave on hyperglycemic normal rats in OGTT showed that, the 200mg/Kg dose was the most active to lower the AUC induced by glucose loaded in normal rats male (**p ≤ 0.01). Glibenclamide (10 mg/Kg), a widely used antidiabetic drug was effective to decrease the AUC observed in the vehicle group (**p ≤ 0.01). The results of OGTT in normal rats could be correlated with the ability of the extract to probably enhance the secretion of insulin in the likely manner of sulfonylureas and inhibit α-glucosidases present in the border brush of the small intestine. Enhanced tissue uptake of blood glucose induced by *V. thyrsiflora* might also be taken into consideration as an alternative possibility. In current study, the decrease in blood glucose at 200 mg/Kg and not at 300 mg/Kg could be
explained by the presence of hyperglycemic compounds which have become the majority at this dose.

The Pearson correlation coefficient ($R^2$) is often used for measuring and describing the degree of linear regression between two continuous quantitative variables that are normally distributed. In current study, the $R^2$ value of the correlation between the iron reduction technique "FRAP" (Ferric Reducing Antioxidant Power) and the Total Phenolic Content was equal to 0.9477 with $p^{**}=0.0010$ and $R^2=0.8507$ with $p^{**}=0.0088$ for Total Flavonoid Content respectively. These results suggested that a great part of the antioxidant capacity of the ethanol extract of V. thyrsiflora leaves is attributed to the Total Polyphenol Content, mainly to flavonoid content in the extract, which have the hydrogen-donor ability to reduce iron. Similar studies suggested a linear relationship between antioxidant capacity and flavonoid contents of the plant extract.\textsuperscript{33,34}

The DPPH assay is often used to evaluate the ability of antioxidant to donate hydrogen or to scavenge free radicals. However, DPPH scavenging activity is best represented by IC\textsubscript{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution.\textsuperscript{35} In current study IC\textsubscript{50} was 65.97 mg/ml, $R^2=0.9945$ for the V. thyrsiflora leaves ethanol extract based on the log (inhibitor) vs normalized response - variable slope, which can be considered to be low antioxidant activity (IC\textsubscript{50} from 50.62 to 110.46 mg/ml) according to the scale of Surinut et al.,\textsuperscript{36} this might suggest that the antioxidant activity of this extract would not be mediated by donating hydrogen to scavenge free radicals but by the reduction of iron.

**CONCLUSION**

Based on the results of the present study, we conclude that the plant extract possesses antioxidant potential. The findings of the present study also suggest that V. thyrsiflora ethanol leaf extract could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. However, this work is the first report which evaluates antihyperglycemic effect of ethanol extract of V. thyrsiflora leaves and its antioxidant potential. Further studies should be carried out to evaluate $\alpha$-amylase inhibitory and beta-glucosidase inhibitory activities isolate and identify active compounds, to understand the mechanism of action against hyperglycemia.

**AUTHOR'S CONTRIBUTION**

The manuscript was carried out, written, and approved in collaboration with all authors.

**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**REFERENCES**


https://doi.org/10.1016/j.biopha.2017.10.102

https://doi.org/10.1007/978-94-009-5570-7


https://doi.org/10.1016/j.jep.2006.07.027

https://doi.org/10.1021/jf0009293

https://doi.org/10.1016/S0308-8146(02)00119-X

https://doi.org/10.1006/abio.1996.0292

https://doi.org/10.1038/1811199a0

https://doi.org/10.1016/j.jep.2006.04.019

https://doi.org/10.1007/s00125-007-0886-7


https://doi.org/10.1076/phbi.35.2.1301276

https://doi.org/10.1351/PAC-REP-12-07-15

https://doi.org/10.2500/IJPSDR.2016.080107

https://doi.org/10.1016/j.foodchem.2008.03.089