Anti-hyperglycemic and Antioxidant Potential of Water-Ethanol Extract of *Musanga cecropioides* Stem Bark

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ABSTRACT
The study investigated the effect of *Musanga cecropioides* (MC) water-ethanol stem bark extract on blood glucose level in both hyperglycemic loaded glucose rats and streptozotocin (STZ)-induced diabetic rats, and evaluated its antioxidant capacity. The *Wistar* rats were induced diabetes after fasting. Oral Glucose Tolerance Test (OGTT) was conducted on normoglycemic rats, and anti-hyperglycemic test on diabetic rats; five groups with five rats each were constituted. Group 1: negative control was treated with vehicle; Group 2, Group 3, and Group 4 were treated with increasing water-ethanol extract (200, 300 and 400 mg/kg b.w); Group 5 was the positive control, treated with glibenclamide. The antioxidant capacity of the extract was also evaluated by measuring the Ferric Reducing Antioxidant Power, Total Phenolic Content, Total Flavonoid Content, and radical scavenging activity of water-ethanol stem bark extract. In OGTT the water-ethanol extract of MC, at the dose of 300 mg/kg, significantly lowered the Area under Curve (AUC) induced by glucose. In STZ diabetic rats, the extract significantly lowered the AUC of blood glucose, at all doses. Glibenclamide was more efficient in both OGTT and anti-hyperglycemic test. The MC extract presented relevant antioxidant activity with IC_{50} = 6.23 mg/mL. Both the Total Phenolic Content and the Total Flavonoid Content increased in a dose-dependent manner. The correlation of DPPH % free radical scavenged and Total Flavonoid Content was positive and statistically significant. MC water-ethanol extract possesses a good antioxidant potential, and could be helpful to lower hyperglycemic state associated with diabetes.

Keywords: *Musanga cecropioides*, antioxidant, STZ-diabetic rats, IC_{50}, polyphenols, flavonoids.

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INTRODUCTION
Musanga cecropioides (Cecropiaceae) also known as umbrella tree or African corkwood, is an evergreen shrub, growing up to 31 m tall with an umbrella-shaped crown. Several authors have pointed out the uses of *Musanga cecropioides* in Africa for medical purposes. Some researchers have reported both the urotonic and hypotensive effect of the leaves [1-2], and

the hypotensive effect of the stem bark. [2] A recent study showed that the ethanolic leaf extract of *M. cecropioides* possesses both anti-inflammatory and anti-nociceptive effect. [3] Pulmonary disorders can be cured by decoction of bark macerate, while the bark scrapings has many properties namely, blood purification, galactogogue, analgesic and antipyretics, wounds and cough alleviation. [4-5] Phytochemistry data report that the plant contains kalaic, musangic and cecropioic acid; and host others acids. [6-13] In addition, it was recorded that leaves contain sorientin, isoorientin, vitexin, pro-cyanidins, chlorogenic acid, and catechin that were reputed to inhibit angiotensin converting enzyme. [14]

In the last decade, there was an increase in the use of antioxidants from plants, because of the worldwide trend toward the use of natural additives in food and cosmetics. The plants and spices are the most popular sources of natural antioxidants especially for their low side effects. [15-16] The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular diseases, diabetes, and diseases associated with aging. The antidiabetic activity of several plants could be attributed to their antioxidant activity. [17-18] Diabetes is a group of chronic diseases characterized by hyperglycemia. Despite a multiplicity of drugs to affect proper blood glucose control, there is a tendency among the patients to seek additional remedy in traditional antidiabetic plant extracts. [19] Herbal remedies are apparently effective, produce minimal or no side effects, and are of relatively low cost as compared to oral synthetic hypoglycemic agents. [20-21] Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research.

Despite a previous study showing the hypoglycemic and antidiabetic activities on the stem bark aqueous and ethanol extracts of *Musanga cecropioides* in normal and alloxan-induced diabetic rats [22], the effect on blood glucose of the bark sap of *M. cecropioides* related to its antioxidant potential have not been published yet. Therefore the objectives of this study were firstly to evaluate the antihyperglycemic effect of a water-ethanol extract of *M. cercropioides* stem bark in glucose loaded rats and in STZ-diabetic rats and secondly to determine the content of Total Polyphenols, Total Flavonoids, the Ferric Reducing Antioxidant Power Assay (FRAP) and DPPH free radical scavenging assay of this extract.

MATERIALS AND METHODS
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 used chemicals were of analytical grade. Spectrophotometric measurements were carried out with the equipment available at the Institute of Medical Research and Medicinal Plants Studies.

Harvest and Extraction of plant material of *M. cecropioides*
Stem barks of *M. cecropioides* were harvested from the Yaoundé Suburbs (Obobogo) in Cameroun/Africa in November 2014 and identified by Dr. Onana Jean Michel, a botanist of the National Herbarium of Cameroon. 500 g of air dried and powdered stem bark, were mixed with 3 L of water: ethanol (30:70) during 48 h, and the filtrate was concentrated using a rotary evaporator at 60°C. The same operation was repeated 3 times. Finally the syrupy mass obtained was dried in the oven at 45°C, giving an extract (yield: 5% w/w). The extract was suspended in water using dimethyl sulfoxide (2%) as a suspending agent for the purpose of oral administration.

Phytochemical screening of secondary metabolites
The water-ethanol extract of stem bark of *M. cecropioides* was also subjected to phytochemical analysis. [23-24]

Experimental Animals
Healthy adult male *Wistar* rats weighing 200-250 g were used in the study. The animals were housed in clean grill cages and maintained in a well ventilated temperature controlled, at the animal house of Institute of Medical Research and Medicinal Plants studies (IMPM), Yaoundé, Cameroon; with a constant 12 h light/dark schedule rotation. 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 (16 h) 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 500μL/200g b.w. [25] The diabetic state was confirmed 72 h after streptozotocin injection. Threshold value of fasting blood glucose was taken as ≥ 200 mg/dL. Diabetic rats were weighed, matched for body weight and divided into 5 groups consisting of 5 animals each.

Anti-hyperglycaemic effect of water-ethanol extract of *Musanga cecropioides* on diabetic rats
The diabetic rats were divided into 5 groups with five rats each: Group 1 was receiving distilled water (10 mL/kg b.w), those from Groups 2, 3, 4 were treated with water-ethanol extract of *Musanga cecropioides* at different doses of 200, 300 and 400 mg/kg b.w respectively, and those from Group 5 with glibenclamide (10 mg/kg b.w). Blood sample was collected from the tested rats before the beginning of the treatment with the extract, then at 1h, 3 h and 5 h respectively.

Oral Glucose Tolerance Test on normoglycemic rats
This experiment was conducted on male rats with normal blood glucose level, according to the method earlier described. [26] The animals were fasted for 16 h prior 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

as follows: Group I: vehicle (10 mL/kg b.w, negative control), Group II: water-ethanol extract (200 mg/kg b.w), Group III: water-ethanol extract (300 mg/kg b.w), Group IV: water-ethanol extract (400 mg/kg b.w) and Group V: glibenclamide (10 mg/kg b.w, positive control). Blood was collected from the animals before administration of the extract (-60 min), and thereafter at 0, 30, 60, 90 and 150 min.

Blood Glucose Estimation

Blood samples were obtained by tail prick and fasting blood glucose levels were estimated using a One Touch Ultra glucometer (Life Scan, Inc., Milpitas, CA, USA) from all animals. Blood glucose levels were reported in mg/dL.

Determination of Total Phenolic Content (TPC)

The ability of the extract evaluated to reduce the phosphomolybdic-tungstate chromogene in Folin Ciocalteu with maximum absorbance at 760 nm. Total Phenolic Content (TPC) was assessed like previously described. [27] 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 like previously described. [28] 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.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) of an extract measured the ability of the extract to reduce the ferric tripyridyltriazine to ferrous tripyridyltriazine, yielding a blue coloration with maximum absorbance at 593 nm. FRAP assay was performed like previously described. [29] The FRAP value was calculated and expressed as mg Catechin Equivalent/g (mg CE/g) based on a calibration curve plotted, using Catechin as standard, at a concentration ranging from 50 to 600 µmol.

DPPH free radical scavenging assay

The ability of the extract to scavenge free radicals by converting 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (purple in color) into diphenyl hydrazine (yellow in color), was measured at 517 nm as previously described. [30] The percentage (%) of radical scavenging effect of extract was calculated as follows:

% radical scavenging effect = [(Abs1-Abs2)/Abs1] × 100

Where Abs1 is the absorbance of the control (containing all reagents except for the extract), and Abs2 is the absorbance of plant extract (optical density in the presence of the extract). IC_{50} value (concentration at which the DPPH radicals were scavenged by 50%) (mg/mL) was estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± SD for triplicate measurements.

Statistical Analysis

Results were expressed as mean ± SD. Statistical analysis was carried out by using one way ANOVA, followed by Dunnet test, for comparison with vehicle control or followed by Newman-Keuls Multiple Comparison Test, for antioxidant parameters, using GraphPad Prism 5.03 Software. A value of *$p \leq 0.05$ was considered to be significant.

Table 1: Preliminary phytochemical analysis of stem bark water-ethanol extract of *Musanga cecropioides*.

Phytochemical constituents tested	Result
Alkaloids	+
Phenolic compounds	+++
Catechic tanins	++
Flavonoids (or bioflavonoids)	+++
Triterpenes	++
Quinones	-
Saponins	-
Oils	-

(+) = indicates presence of phytochemicals and (-) = indicates absence of phytochemicals. +++ = shows high concentration; ++ = shows moderate concentration; + = shows small concentration.
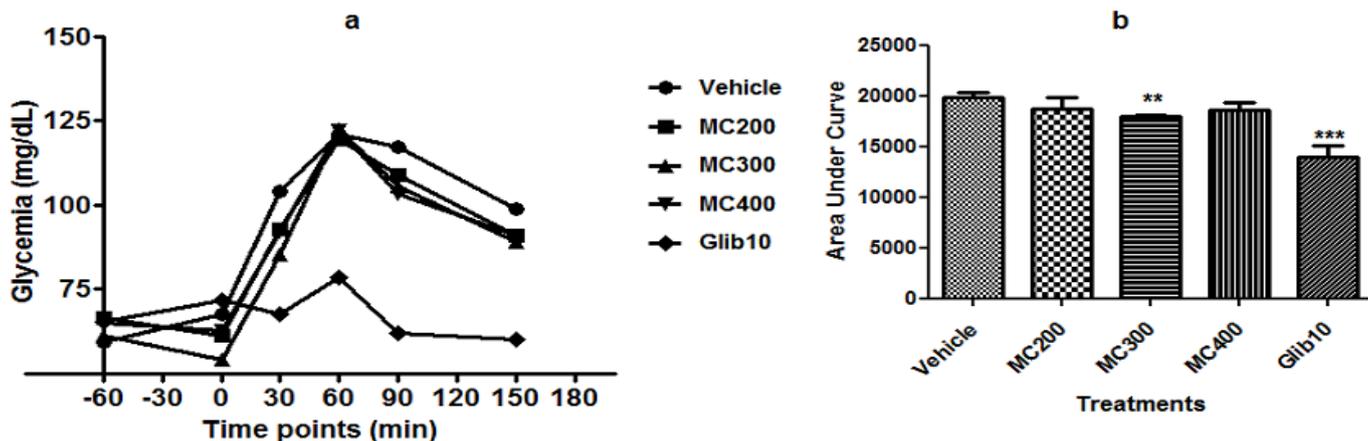

Fig. 1: (a) Effect of *Musanga cecropioides* water-ethanol extract on glycaemia during Oral Glucose Tolerance Test (OGTT) and (b) Area Under Curve associated with this effect of *Musanga cecropioides* water-ethanol extract.
Data are expressed as means ± S.D (n = 5). **$p \leq 0.01$; ***$p \leq 0.001$ compared with the corresponding value for vehicle control rats. MC200: *Musanga cecropioides* (200 mg/kg); MC300: *Musanga cecropioides* (300 mg/kg); MC400: *Musanga cecropioides* (400 mg/kg); Glib10: Glibenclamide (10 mg/kg).
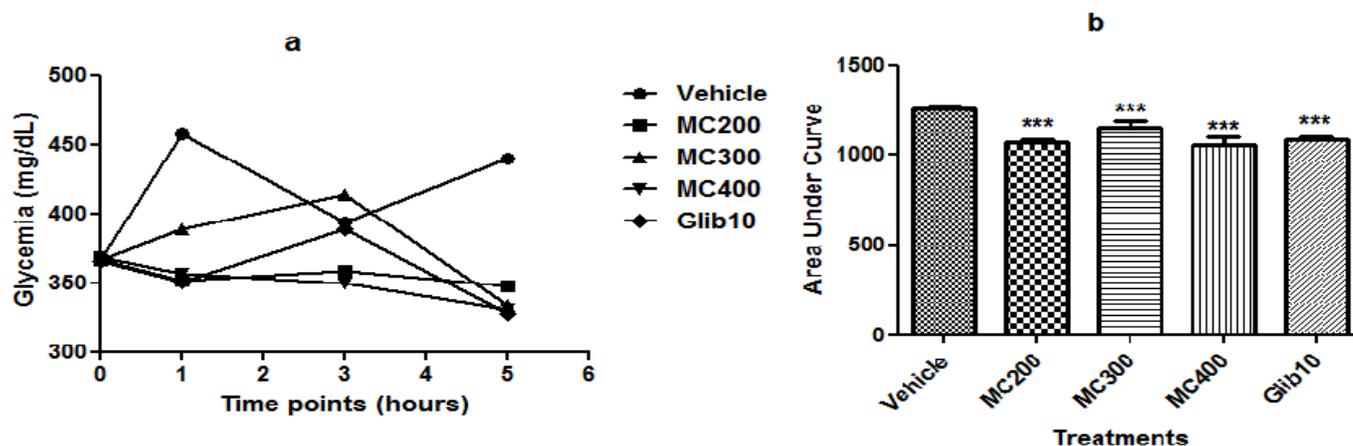
Fig. 2: (a) Effect of *Musanga cecropioides* water-ethanol extract on glycemia of STZ-induced diabetic rats; (b) Area Under Curve associated with this effect of *Musanga cecropioides* water-ethanol extract.

Data are expressed as means ± S.D (n = 5). $p \leq 0.001$ compared with the corresponding value for vehicle control rats. MC200: *Musanga cecropioides* (200 mg/kg); MC300: *Musanga cecropioides* (300 mg/kg); MC400: *Musanga cecropioides* (400 mg/kg); Glib10: Glibenclamide (10 mg/kg).

RESULTS

Phytochemical screening of stem bark water-ethanol extract of *Musanga cecropioides*

Phytochemical screening revealed that the stem bark water-ethanol extract of *Musanga cecropioides* contained alkaloids, phenolic compounds, catechic tannins, triterpenes and saponins.

Effects of *Musanga cecropioides* water-ethanol extract on Oral Glucose Tolerance Test (OGTT) in normoglycemic male rats

The Area Under the Curve associated with the effect of *M. cecropioides* on OGTT showed that only the dose of 300 mg/kg of the extract significantly reduced blood sugar levels [Figure 1], when compared with the control group (19839.00 ± 513.80 to 17936.30 ± 104.70; $p \leq 0.001$); but glibenclamide also significantly reduced the Area Under the Curve observed in the control group (19839.00 ± 513.80 to 14165.00 ± 1158.00; $p \leq 0.001$).

Effects of *Musanga cecropioides* water-ethanol extract on blood glucose of STZ-induced diabetic rats.

The Area Under the Curve obtained from each group illustrates the significant decline observed in blood glucose level of the diabetic rats treated with the doses of 200 mg/kg, 300 mg/kg and 400 mg/kg (1253.00 ± 20.60 to 1068.20 ± 14.80, 1152.00 ± 40.10 and 1055.70 ± 40.70 respectively; $p \leq 0.001$), when compared to that of vehicle control [Figure 2].

Total Polyphenol Content (TPC)

Figure 3 shows the Total Polyphenol Content, which is dose-dependent of the extract concentration. 40 mg/mL is the dose with the highest Total Polyphenol Content (53.01 ± 1.13 mg CE/g) amongst all doses used. However from 5 mg/mL to 10 mg/mL, there is no variation in the amount of polyphenols.

Total Flavonoid Content (TFC)

The amount of flavonoids content increased significantly from 5 mg/mL, reaching its maximum at 40 mg/mL (4267.00 ± 309.80 mg QE/g). It is substantially identical from 5 mg/mL to 20 mg/mL concentrations of the extract [Figure 4].
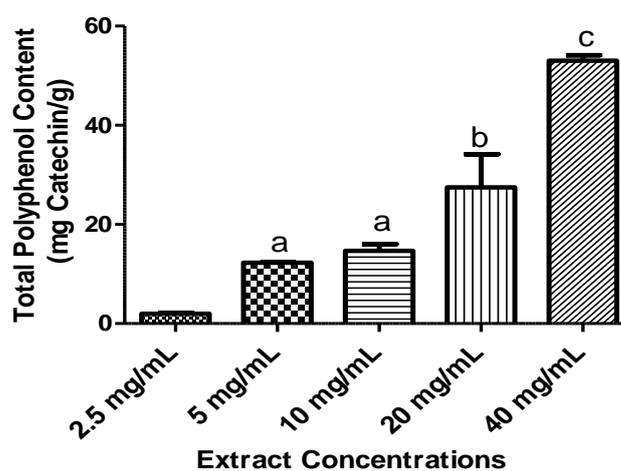

Fig. 3: Total Polyphenol Content of *Musanga cecropioides* water-ethanol extract.

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.
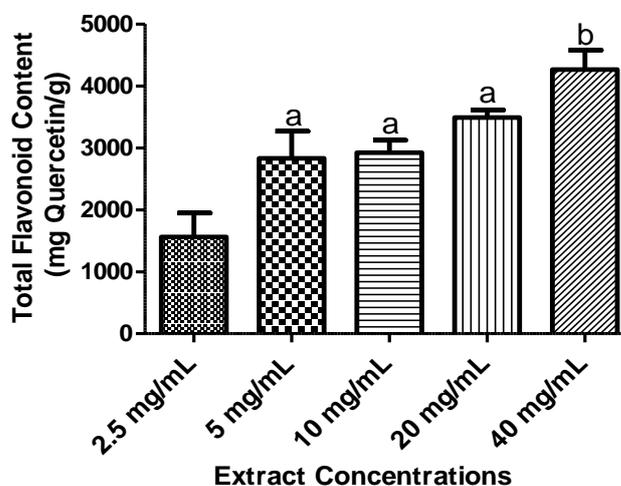

Fig. 4: Total Flavonoid Content (TFC) of *Musanga cecropioides* water-ethanol extract.

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, 10 mg/mL and 20 mg/mL.
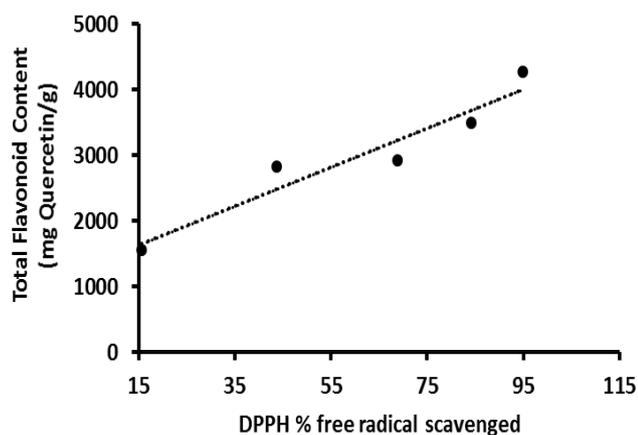
Fig. 5: Correlation between DPPH % free radical scavenging activity and the Total Flavonoid Content (mg QE/g), (Pearson r = 0.09570; R^2 = 0.9158, p = 0.0107 (*p* < 0.05).
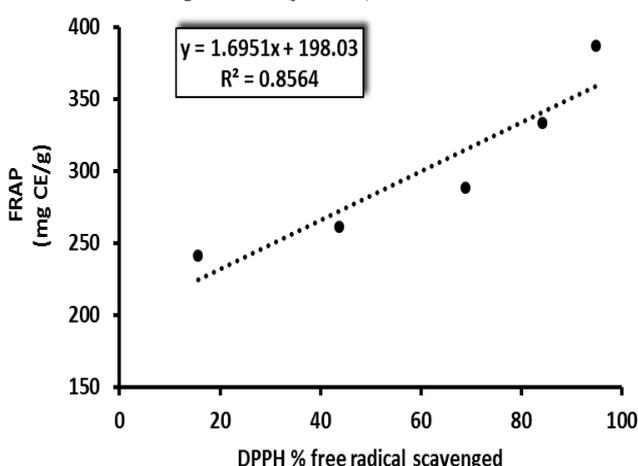

Fig. 6: Correlation between DPPH % free radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) (mg QE/g), (Pearson r = 0.9254; R^2 = 0.8564, p = 0.0242 (*p* < 0.05).

2, 2-diphenylpicrylhydrazyl (DPPH) % free radical scavenging activity and Correlation with TPC, and with TFC

An examination on the DPPH scavenging activity showed that an increase in concentration of the extract leads to a proportionate increase in scavenging capacity. The DPPH scavenging activity has highest inhibitory activity at 40 mg/mL. IC_{50} value was found to be 6.23 (CI95%: 5.399 to 7.197, R^2 = 0.9945) for the water-ethanol extract, based on the log (inhibitor) vs. normalized response-Variable slope**.**

Correlation between DPPH % free radical scavenging activity and TPC gave R^2 = 0.762 with p = 0.0533 (*p* > 0.05). Correlation of DPPH % free radical scavenging activity with TFC gave R^2 equal to 0.9158 with p = 0.0107 (*p* ≤ 0.05), which is a positive significant correlation [Figure 5].

Ferric Reducing Antioxidant Power (FRAP), and Correlation with DPPH % free radical scavenged activity

The Ferric Reducing Antioxidant Power is dose dependent of the concentration of the extract. The Ferric Reducing Antioxidant Power reached its maximum (387.00 ± 15.40) at a concentration of 40

mg/mL of extract and was significantly elevated, compared to the other values obtained with lower concentrations of the extract.

Correlation between DPPH % free radical scavenged activity and Ferric Reducing Antioxidant Power (FRAP) gave R^2 = 0.8564 with p = 0.0242 (*p* < 0.05), this means that DPPH and FRAP are positively and highly correlated at a significance level of p = 0.05. Its means also that 85.64% of the antioxidant power is shared between FRAP and DPPH [Figure 6].

DISCUSSION

In the present study the acute antihyperglycemic activity of water-ethanol extract of *M. cecropioides* stem bark was evaluated in normoglycemic rats during OGTT and in STZ-diabetic rats. OGTT data over 2 h indicates that *M. cecropioides* stem bark extract reduced plasma glucose concentration in the oral glucose induced hyperglycemic rats. The extract at a dose of 300 mg/kg significantly lowered AUC, when compared with vehicle control group. The decline is more pronounced with glibenclamide. From the study, it is suggested that the possible mechanism by which the plant extract decreased the blood glucose level could be by potentiating insulin effect by increasing either peripheral glucose uptake or pancreatic secretion of insulin from ß cells of islets of Langerhans. Those results are in line with previous results [22], demonstrating that subacute oral administration, for 14 days of the aqueous and ethanolic extracts of *M. cecropioides* stem bark in normal and diabetic rats, significantly lowered the fasting plasma glucose levels in normal and alloxan-induced diabetic rats in dose-dependent manner.

The findings from this study indicate that short-term administration of the stem bark extract of *M. cecropioides* is associated with a significant decrease in blood glucose levels in STZ-diabetic rats; a process likely to have a positive impact on glucose homeostasis in diabetic patients. Since streptozotocin selectively destroys ß-cells of the pancreas by necrosis [31], we would expect the extract to exert no effect on plasma glucose concentrations in STZ diabetic rats, if the mode of action is mediated through insulin production. Therefore, the results from the study indicate that the *M. cecropioides* hypoglycaemic effect in diabetic rats, observed with stem bark extract appeared to involve mechanisms that do not involve insulin. The above experimental model is quite sensitive and relatively specific to all major classes of oral hypoglycemic drugs including sulfonylureas, biguanides, meglitinide analogues, thiazolidinediones and α-glucosidase inhibitors. [32] Insulin and others antidiabetic drugs are well known to stimulate glucose uptake by peripheral cells and tissues. [33] We suggest that the extract may also promote glucose entry into cells, like it has been observed for herbal extracts containing corosolic acid. [34]

Alkaloids, phenolic compounds, catechin tannin, flavonoids or triterpenes found in this extract might be responsible for those activities in both OGTT and anti-hyperglycemic test. Many studies reported the hypoglycaemic activities of those compounds. [35-38]

The TPC in the *M. cecropioides* (MC) extract was in the range of 1.99 ± 0.19 mg CE/g to 53.01 ± 1.13 mg CE/g. A non-significant relationship was found between TPC and DPPH value (p = 0.0533), so the high content of TPC is not necessarily related to good antioxidant capacities. The antioxidant activity from plants owing, to phenolic compounds is well documented. [39] However, high TPC values did not linearly correspond to a high antioxidant activity, because Folin-Ciocalteu reagent is not specific to just polyphenols but to any other substances that could be oxidized by the reagent. [40]

TFC of the *M. cecropioides* extract was also determined. In this study, TFC were increased with TPC (R^2 = 0.8756, data not shown).

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_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. [41] A smaller IC_{50} value corresponds to a higher antioxidant activity of plant extract. [42] In the DPPH test of the present study, the IC_{50} value of *M. cecropioides* extract was 6.23 mg/mL, which can be considered to be smaller when compared with previous studies on DPPH of plant extracts [43], and represent a higher radical scavenging activity for *M. cecropioides* extract.

Specialized experimental studies have reported that, the phenols and flavonoids can be responsible for the antioxidant activity of medicinal plants. [44] Therefore, in order to determine the relative importance, the DPPH free radical scavenging activity was correlated with the content of these compounds.

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 our study, the R^2 value of the correlation between DPPH % free radical scavenged and the Total Phenolic Content was equal to 0.762 with p = 0.0533 and R^2 = 0.9158 with p = 0.0107 for Total Flavonoid Content respectively. This correlation of DPPH % free radical scavenged and Total Flavonoid Content is statistically significant at *p* < 0.05. These results suggested that a great part of the antioxidant capacity of the *M. cecropioides* extract is attributed to the Total Flavonoid Content in the extract, which have the hydrogen-donor ability to scavenge the free radicals. Similar studies suggested a linear relationship between antioxidant capacity and flavonoid contents of the plant extract. [44-45]

The Ferric Reducing Antioxidant Power (FRAP) results followed the same tendency as those obtained using the DPPH method. The correlation between the data obtained by the DPPH and FRAP methods was extremely significant (r = 0.9354); this has previously been reported by Katalinić *et al.* [46], who analyzed the polyphenolic profile and antioxidant properties of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). Both methods are based on the same mechanism of action, the single-electron transfer, which explains the observed correlation. [47]

The present study confirmed that the water-ethanol stem bark extract of MC is a potential source of natural antioxidants, with some potent anti-hyperglycemic phytoconstituents. It possesses significant hypoglycemic potential as it reversed the fasting blood sugar of diabetic rats to near normalcy. Future studies are needed to investigate the properties of *M. cecropioides* extracts, and studies are in progress to isolate and identify the active compounds from *M. cecropioides* extracts responsible for the hypoglycemic effect and antioxidant activity.

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