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Comparative Study on the Phytochemical Compositions and Antihyperglycemic Potentials of the Leaves Extracts of *Combretum paniculatum* and *Morinda morindoides*

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Authors' contributions

This work was carried out in collaboration with all authors. Authors ALO and IAO designed the study and supervised the whole research. Authors MMA and JEE performed the extraction and participated in all the experimental works. Author MIK carried out the hypoglycemic analysis, performed the statistical analysis and wrote initial draft of the manuscript. Author IAO managed the literature searches and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The phytocheimcal components and antihyperglycemic potential of methanol and ethanol leaves extracts of *Combretum paniculatum* Vent (*Combretaceae*) and *Morinda morindoides* (Baker) Milne-Redh (*Rubiaceae*) grown in Nigeria have been studied. The phytochemical composition was determined by established methods while the *in vitro* hypoglycemic effect was performed by determining the inhibitory potentials of the extracts on α-amylase and α-glucosidase. Results

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showed that the ethanol extract of *C. paniculatum* displayed the most potent inhibition of both α-amylase (IC_{50}: 5.06 mg/mL) and α-glucosidase (IC_{50}: 1.96 mg/mL). The ethanol extract of *C. paniculatum* inhibited α-amylase and α-glucosidase in a non-competitive and mixed non-competitive manner. The presence of phytochemicals such as phenols, steroids, flavonoids and athraquinones were confirmed in the extracts.

Aims: To determine the phytochemical compositions and hypoglycemic potentials of the methanol and ethanol leaves extracts of *Combretum paniculatum* and *Morinda morindoides*.

Study Design: Extraction of the air-dried and pulverized leaves of *C. paniculatum* and *M. morindoides* with both methanol and ethanol, and testing the various extracts for the phytochemical composition and hypoglycemic potentials.

Place and Duration of Study: The leaves of *C. paniculatum* were collected from Ibefun, Oyo State, in May 2013 while those of *M. morindoides* were collected from Etegbin Area, Shibiri, Lagos, State, Nigeria, in June 2013.

Methodology: The pulverized leaves were extracted separately with ethanol and methanol for 24 h. The resulting infusions were decanted, filtered and evaporated in a rotary evaporator. The dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared. Phytochemical compositions of the extracts were determined using the methods described previously. Moreover, the hypoglycemic potentials were evaluated as described previously.

Results: The ethanol extract of *C. paniculatum* possessed mild inhibition of α-amylase and strong inhibition of α-glucosidase compared to other extracts.

Conclusion: The present results justify the use of *C. paniculatum* in the treatment of sugar related disorders in Nigeria.

Keywords: Combretum paniculatum; Morinda morindoides; hyperglycemia; phytochemical composition; hypoglycemic activity.

1. INTRODUCTION

Type 2 diabetes mellitus is a disorder of the endocrine system, majorly characterized by glycemic imbalance, which stimulates several metabolic errors and finally results into oxidative stress and chronic complications [1]. Current statistics suggests that about 382 million people are living with diabetes around the globe and this number is projected to increase to 471 million in 2035. South Africa tops the list of diabetics in Africa with prevalence of 8.27% followed by Nigeria with 4.99% of the population [2]. In fact, this disease is associated with a reduced quality of life and increased risk factors for mortality and morbidity among its sufferers, who are mostly poor and socially disadvantaged [2].

Glycemic control is the most important goal in diabetes care as its impairment leads to several complications such as nephropathy, neuropathy and cardiovascular disease in diabetic patients [3]. Different classes of drugs such as biguanides, insulin secretagogues, thiazolidinediones and α-glucosidase inhibitors have been used widely to manage this condition. However, these antidiabetic drugs produced undesirable side effects such as hypoglycaemia, weight gain and gastro-intestinal disturbances [4]. Due to these, the use of herbal agents in the management of diabetes mellitus has gained prominence in all parts of the world, and some of the plants used include *Combretum paniculatum* and *Morinda morindoides*.

Combretum paniculatum is a shrub with vivid scarlet flowers attaining 15 m length and is widespread in tropical Africa. A high degree of antiviral activity against HIV-2 was achieved with the acetone extract of *C. paniculatum* [5]. The aqueous extract of inflorescences of the plant has anti-tumor activity against carcinoma of the lung [6]. The antimicrobial, anti-inflammatory, antishistosomal, anti-HIV and central nervous system stimulation activities of *C. paniculatum* have been documented [7]. The cytotoxic activity of pheophorbide-a and pheophorbide a-methyl ester isolated from the leaves of *C. paniculatum* have been reported [8]. Other compounds such as cyanidin 3,5-O-*β*-D-diglu-copyranoside and pelargonidin 3,5-O-*β*-D-diglucopyranoside [9], as well as cholest-5-en-3-ol, 2-phyten-1-ol, isoquercitrin, p-coumaric acid, 2, 3, 8-tri-O-methylellagic acid, beta-sitosterol, gallocatechin, apigenin and apigenin-7-glucoside [10] were characterised from the plant. Till moment, the authors are unaware of any analysis on the hypoglycemic activity of this plant.

Extracts and compounds of *M. morindoides* are known to possess antimicrobial [11-13], antidiarrheal [14], anticomplimentary [15], xanthine oxidase inhibiting and superoxide scavenging activity [16], antimalarial [17-20], antispermatogenic [21,22], cytotoxic effects [23] and possesses biochemical effects on lipid profile, bilirubin and some marker enzymes level in the plasma of male albino rats [24]. The plant contains antimalarial iridoids [19], quercetin, quercetin-7,4′-dimethylether, luteolin-7-glucoside, apigenin-7-glucoside, quercetin-3-rhamnoside, kaempferol-3-rhamnoside, quercetin-3-rutinoside, kaempferol-3-rutinoside, chrysoeriol-7-neohesperidoside and kaempferol-7-rhamnosylsophoroside [25,26]. Though studies have been performed on the antidiabetic efficacy of the root [27] and leaf extracts [28] of this plant confirming the claim of the traditional healers, no work could be found on the mechanism by which the extract elicit this potential.

Despite the usage of these plants in the management of sugar-related disorders in Nigeria, there is dearth of information on their efficacy and possible mechanism of antidiabetic action. Therefore, this study aimed to determine the phytochemical and hypoglycemic potential of *C. paniculatum* and *M. morindoides* leaf extracts and the mechanism by which they elicit this action. This is in continuation of our previous studies on the hypoglycaemic potentials of some Nigerian medicinal plants [29,30].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Porcine pancreatic α-amylase, rat intestinal α-glucosidase and paranitrophenyl-glucopyranoside were products of Sigma-Adrich Co., St Louis, USA while starch soluble (extra pure) was obtained from J. T. Baker Inc., Phillipsburg, USA. Other chemicals and reagents were of analytical grade and the water used was glass-distilled.

2.2. Plants Collection

The leaves of *C. paniculatum* were collected in Ibefun, Oluyole Local Government, Oyo State, Nigeria. The plant was identified and authenticated by Dr. S. O. Shosanya, a taxonomist at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen FHI 109950 was deposited in the Institute's herbarium. The leaves of *M. morindoides* were obtained from Etegbin area, Shibiri, Ojo Local Government area, Lagos State. Botanical authentication was achieved at the Herbarium, Department of Botany, University of Lagos, Nigeria, where a voucher specimen LUH 5618, was also deposited. The leaves were air-dried, pulverized and kept in airtight plastic bags till moment of analysis.

2.3 Preparation of Plant Extracts

The pulverized leaves were divided into two portions of 10 g each and extracted separately with ethanol and methanol for 24 h. The flasks were shaken and kept still to allow the plant material settle at the bottom of the flask. The resulting infusions were decanted, filtered and evaporated in a rotary evaporator (Cole Parmer SB 1100, Shangai, China). The dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to yield a stock solution from which lower concentrations were prepared.

2.4 Phytochemical Screening

Phytochemical compositions of the leaf extracts were determined using the methods described previously [31,32].

2.5 Hypoglycemic Potentials of the Extracts

2.5.1 α-Amylase inhibitory assay

This assay was carried out using a modified procedure of McCue and Shetty [33]. A total of 250 µL of extract was placed in a test tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/mL) was added. This solution was pre-incubated at 25°C for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then incubated at 25°C for 10 min. The reaction was terminated by adding 500 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm using a spectrophotometer (Spectrumlab S23A, Globe Medical England). The control and blank were prepared using the same procedure replacing the extract with DMSO and distilled water

respectively. The α-amylase inhibitory activity was calculated as percentage inhibition, thus;

$$\% \text{ Inhibition} = [(\Delta A_{control} - \Delta A_{extract})/A\Delta_{control}] \times 100$$

where $\Delta A_{control} = A_{control} - A_{blank}$ and $\Delta A_{extract} = A_{extract} - A_{blank}$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.5.1.1 Mode of α-amylase inhibition

The mode of inhibition of α-amylase by the leaf extract was conducted using the most potent extract according to the modified procedure previously described [34]. Briefly, 250 µL of the (5 mg/mL) extract was pre-incubated with 250 µL of α-amylase solution (0.5 mg/mL) for 10 min at 25°C in one set of tubes. In another set of tubes α-amylase was pre-incubated with 250 µL of phosphate buffer (pH 6.9). 250 µL of starch solution at increasing concentrations (0.3–5.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25°C, and then boiled for 5 min after addition of 500 µL of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted to determine the mode of inhibition.

2.5.2. α-Glucosidase inhibitory assay

The effect of the plant extracts on α-glucosidase activity was determined according to an established procedure [35]. The substrate solution, p-nitropheynyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. 100 µL of α-glucosidase (E.C. 3.2.1.20) (0.5 mg/mL) was pre-incubated with 50 µL of the different concentrations of the extracts for 10 min. Then 50 µL of 3.0 mM pNPG dissolved in 20 mM phosphate buffer (pH 6.9) was added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow coloured para-nitrophenol released from pNPG at 405 nm. The control and blank were prepared using the same procedure by replacing the extract with DMSO and distilled water respectively. Percentage inhibition was calculated thus;

$$\% \text{ Inhibition} = [(\Delta A_{control} - \Delta A_{extract})/A\Delta_{control}] \times 100$$

where $\Delta A_{control} = A_{control} - A_{blank}$ and $\Delta A_{extract} = A_{extract} - A_{blank}$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

2.5.2.1 Mode of α-glucosidase inhibition

The mode of inhibition of α-glucosidase by the extracts was determined using the extract with the lowest IC_{50} according to the modified method described above [34]. Briefly, 50 µL of the (5 mg/mL) extract was pre-incubated with 100 µL of α-glucosidase solution (0.5 mg/mL) ofor 10 min at 25°C in one set of tubes. In another set of tubes, α-glucosidase was pre-incubated with 50 µL of phosphate buffer (pH 6.9). 50 µL of pNPG at increasing concentrations (0.63 - 2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 min at 25°C and 500 µL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a para-nitrophenol standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted to determine the mode of inhibition.

2.6 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 statistical package (GraphPad Software, USA). The data were analysed by one way analysis of variance (ANOVA) followed by Bonferroni test. All the results were expressed as mean ± SEM for triplicate determinations.

3. RESULTS AND DISCUSSION

The management of hyperglycemia is the hallmark of treatment in diabetes and one of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard the digestion and absorption of carbohydrates by the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract [36]. Though, synthetic α-glucosidase inhibitors such as acarbose and voglibose are presently in use but are bedeviled by undesirable

side effects such as nausea, diarrhoea and liver failure [37], which necessitated this study.

Table 1 showed the phytochemical composition of different extracts of *C. paniculatum* and *M. morindoides*. Steroid was detected in all the tested extracts while flavonoid was conspicuously absent in all the extracts. Phenolic compounds and tannins were detected in both the methanol and ethanol extracts of *C. paniculatum* while saponins and anthraquinones were detected in all the extracts except ethanol extract of *C. paniculatum*.

The result of percentage inhibition of α-amylase by methanol and ethanol extracts of *C. paniculatum* and *M. morindoides* leaves is shown in Figs. 1(a) and 1(b). With the exception of 0.32 mg/mL, methanol extract of *C. paniculatum* possessed significantly higher percentage inhibition (P = .05) of the enzyme than ethanol extract. However, for *M. morindoides*, at all concentrations tested, there was no significant difference between the two extracts. Table 2 showed the IC_{50} values for the inhibition of α-amylase and α-glucosidase by *C. paniculatum* and *M. morindoides*. Among the extracts tested, methanol extract of *C. paniculatum* had the lowest IC_{50} for α-amylase inhibition and this is lower than the standard, acarbose.

Figs. 2(a) and 2(b) shows the percentage inhibition of α-glucosidase by the extracts of *C. paniculatum* and *M. morindoides* leaves. At lower concentrations (0.32 - 0.63 mg/mL), there was no significant difference between the percentage inhibition of the ethanol and methanol extracts of *C. paniculatum*. At higher concentrations, there was significant difference (P= .05) between the inhibitions of the enzyme by ethanol and methanol extracts. With regards to *M. morindoides* extracts, there was no significant difference between the ethanol and methanol extracts at all concentrations tested. Ethanol extract possessed the lowest IC_{50} for α-glucosidase inhibition but it is higher than that of acarbose (Table 2).

Table 1. Phytochemical composition of *C. paniculatum* and *M. morindoides* leaves

Phytochemicals	*C. paniculatum*		*M. morindoides*	
	Methanol	**Ethanol**	**Methanol**	**Ethanol**
Tannins	+	+	-	-
Steroids	+	+	+	+
Phenolics	+	+	+	-
Saponins	+	-	+	+
Anthraquinones	+	-	+	+
Flavonoids	-	-	-	-

***+ High concentration**; + Low concentration; - Absent*

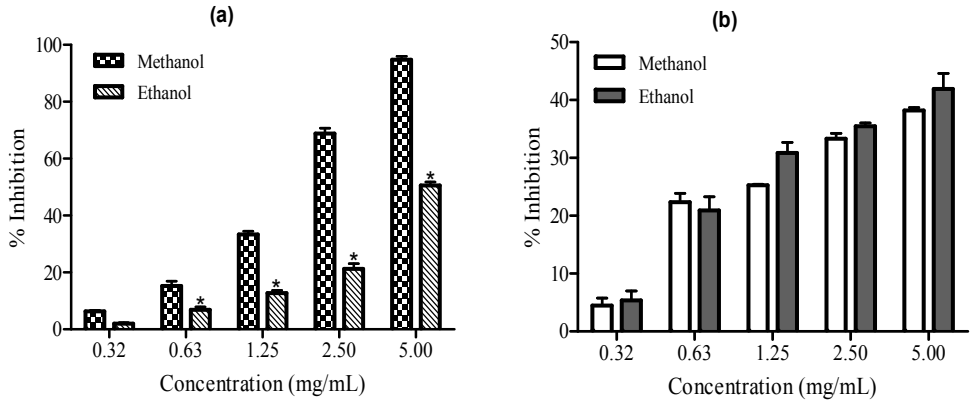

Fig. 1. Inhibitory potency of (a) *C. paniculatum* and (b) *M. morindoides* leaves extracts against α-amylase activity. The values are expressed as means ± SEM of triplicate determinations
**Significantly different at the same concentration (P= .05)*

Table 2. IC_{50} values of α-amylase and α-glucosidase inhibition by *C. paniculatum M. morindoides* leaf extracts

Extracts	IC50 (mg/mL)	
	α-Amylase	α-Glucosidase
C. paniculatum methanol	2.27±0.02[a]	2.50±0.02[a]
C. paniculatum ethanol	5.06±0.03[b]	1.96±0.01[b]
M. morindoides methanol	6.43±0.01[c]	2.05±0.01[b]
M. morindoides ethanol	5.63±0.02[b]	2.68±0.02[a]
Acarbose	2.60±0.01[a]	0.63±0.00[c]

We found that methanol extract of *C. paniculatum* displayed the highest inhibition of α-amylase while both extracts of *M. morindoides* possessed less than 50% inhibition of the enzyme. The result culminated in the low IC_{50} (2.27 mg/mL) obtained for the methanol extract of *C. paniculatum*. The possession of a lower IC_{50} similar to the standard, acarbose suggests that the extract provides similar physiological as well as side effects, arising from the excessive inhibition of α-amylase [38]. Therefore, ethanol extract of *C. paniculatum* was selected for further study because a good antidiabetic agent should necessarily be a mild inhibitor of this enzyme so as to prevent the side effect of synthetic agents like acarbose [39]. Ethanol extract of *C. paniculatum* also displayed the best inhibition of α-glucosidase and this resulted in its lowest IC_{50}. This is because it is desirable of a potent antidiabetic drug to be a strong inhibitor of α-glucosidase.

Figs. 3(a) and 3(b) showed the mode(s) of inhibition of both α-amylase and α-glucosidase by the ethanol extract of *C. paniculatum*. These show that ethanol extract of *C. paniculatum* inhibited α-amylase non-competitively while α-glucosidase was inhibited in a mixed non-competitive manner.

The pure non-competitive inhibition of α-amylase by the ethanol extract of *C. paniculatum* indicated that the active components in the extract also binds to a site other than the active site of the enzyme and combines with either free enzyme or the enzyme-substrate complex, possibly interfering with the action of both [40]. However, the inhibitor had equal affinity for both the free enzyme and enzyme-substrate complex. Similarly, the mixed non-competitive inhibition of α-glucosidase by the ethanol extract also suggests that the inhibitory components in the extract also bind to a site other than the active site of the enzyme but has different affinities for the free enzyme and enzyme-substrate complex [41].

The effect of oral administration of ethanol extract of *C. paniculatum* on starch-loaded postprandial hyperglycemia is shown in Fig. 4. At all durations tested, the extract-treated group had significantly lower (*P*= .05) blood glucose level compared to the control animals.

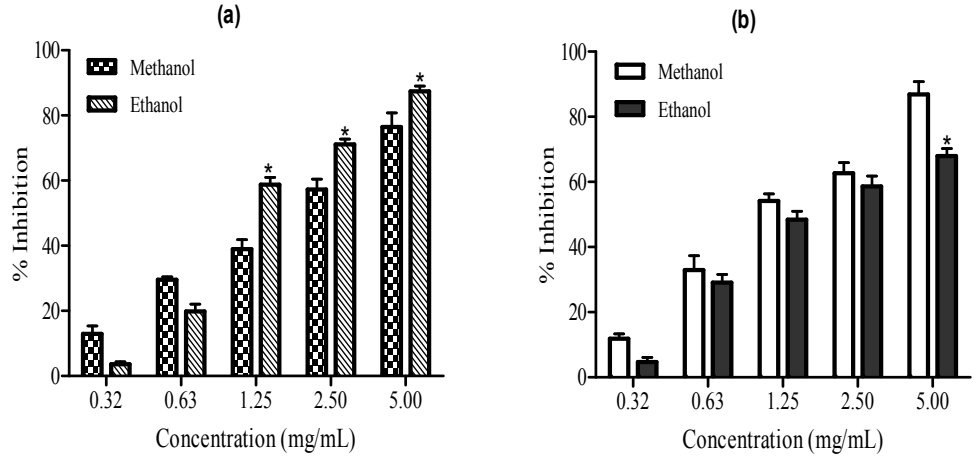

Fig. 2. Inhibitory potency of (a) *C. paniculatum* and (b) *M. morindoides* leaves extracts against α-glucosidase activity. The values are expressed as means ± SEM of triplicate determinations

**Significantly different at the same concentration (P= .05)*

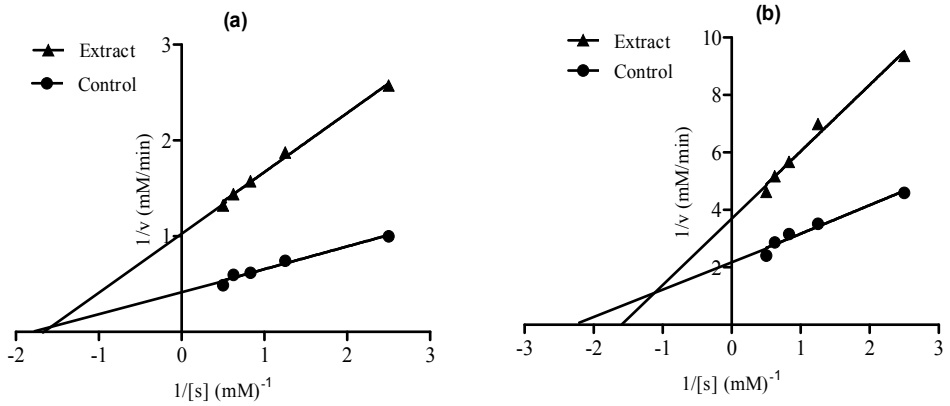

Fig. 3. Mode of inhibition of (a) α-amylase and (b) α-glucosidase by ethanol extract of *C. paniculatum* leaves

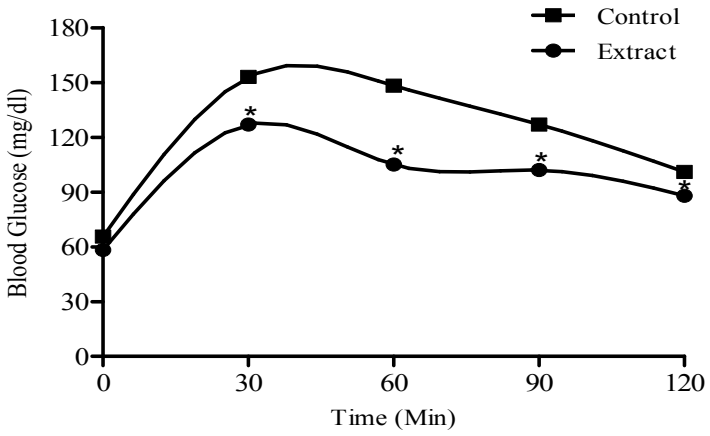

Fig. 4. Effect of administration of ethanol extract of *C. paniculatum* on blood glucose level of starch - loaded rats

** Values are significantly different from the control*

In order to ascertain the antihyperglycemic effect of *C. paniculatum*, ethanol extract of the plant was orally administered to starch-loaded rats. The significant reduction in the postprandial blood glucose level of the extract-treated rats compared to the control suggests the plant possesses antihyperglycemic potential [38]. Therefore, we inferred that the antihyperglycaemic effect of the extract may be due to the inhibition of the pancreatic α-amylase and intestinal α-glucosidase in the rats, thereby lowering their blood glucose levels.

The α-amylase and α-glucosidase inihibitory effect as well as the antihyperglycemic potential of the ethanol extract of *C. paniculatum* may be due to the presence of phytochemicals such as tannins, phenolics and steroids in the plant. Tannins have been found to induce phosphorylation of insulin receptors and translocation of glucose transporter, thereby helping in the reduction of blood glucose level [42] while phenolics have been found to possess antioxidant, hypoglycemic and antiglycation potentials [43]. Steroids on the other hand, are involved in the stimulation of pancreatic β-cells and subsequent secretion of insulin [44]. It can therefore be concluded that the antihyperglycemic potential of the ethanol extract of *C. paniculatum* may be due to the presence of these phytochemicals present in it.

4. CONCLUSION

This study revealed that out of all the extracts of plants tested, ethanol extract of *C. paniculatum* displayed mild and strong inhibition of α-amylase and glucosidase respectively. *M. morindoides* extracts did not exhibited potent inhibition of both enzymes. Ethanol extract of *C. paniculatum* also inhibited both enzymes in a non-competitive manner and reduces postprandial blood glucose level of starch-loaded rats. It can be concluded that ethanol extract of *C. paniculatum* possesses hypoglycemic potential and its mode of antidiabetic action may be due to inhibition of pancreatic α-amylase and intestinal glucosidase.

CONSENT

It is not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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