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# Inhibition of baker's yeast alpha glucosidase by extract and fractions of stem bark of *Terminalia catappa* Linn. (Combretaceae)

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**ABSTRACT**

***Background***: Paucity of clinically applicable inhibitors has limited alpha glucosidase inhibition therapeutic strategy of type 2 diabetes management. The current investigation was aimed at evaluating the anti-diabetic alpha glucosidase inhibitory potentials of *Terminalia catappa* stem bark extract and its fractions, modeling the implicated intestinal brush border membrane alpha glucosidases with the baker's yeast alpha glucosidase maltase.

***Methods***: A crude stem bark extract of *Terminalia catappa* and its n-hexane-, dichloromethane- and methanol-soluble fractions were incubated with the baker's yeast alpha glucosidase at various final assay mixture concentrations, carrying along acarbose as reference standard. $IC_{50}$ values were calculated from % inhibition vs concentration plots and analyzed using one-way ANOVA with Tukey's *post hoc* comparison.

***Results:*** The methanol, n-hexane and dichloromethane fractions inhibited alpha glucosidase with $IC_{50}$ values 173.85 ± 6.90 µg/ml, 258.56 ± 12.90 µg/ml and 329.90 ± 10.67 µg/ml respectively which are in the same order as that of acarbose (*123.26 ± 15.56 µg/ml*) and significantly smaller ($p < 0.0001$) than that of the crude extract (*527.67 ± 16.32 µg/ml*).

***Conclusion***: The three fractions potently inhibited baker's yeast alpha glucosidase, indicating presence of alpha glucosidase inhibitory molecules of diverse polarity and, hence, structures in the *T. catappa* stem bark extract. This investigation has suggested alpha glucosidase inhibition as a possible action mechanism of the anti-diabetic use of *T. catappa*, thus unmasking the stem bark extract as a repertoire of compounds with possible alpha glucosidase inhibitory activities.

**Keywords**: Type 2 Diabetes Mellitus, alpha glucosidase inhibition, *Terminalia catappa, Saccharomyces cerevisiae*

## 1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized with chronic hyperglycemia. It is a manifestation of pancreatic beta cells dysfunction of insulin secretion and/or resistance of body cells to insulin stimulation for glucose uptake and utilization [1]. DM is broadly classified into two types or forms based on the level of pancreatic dysfunction involvement. The form that is exclusively caused by the impairment of pancreatic insulin secretory function, and which is almost always autoimmune in etiology [1, 2], is classified as Type 1 or Insulin Dependent Diabetes Mellitus (IDDM). On the other hand, the form of the disease that is rather multifactorial, being a composite of some level of impaired insulin secretion, insulin resistance by all the body (except the brain) cells and a number of environmental factors like obesity and overeating, is classified as type 2 or Non-insulin Dependent Diabetes Mellitus (NIDDM) [2]. NIDDM constitutes at least 90% of all cases of diabetes mellitus [3]. Chronic hyperglycemia from uncontrolled NIDDM would ultimately result to accumulation of ketone bodies, leading to ketoacidosis and polyol metabolic pathway activation with its attendant deposition of sorbitol in critical organs like the eyes, kidneys and nervous system,

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causing diabetic complications manifesting as diabetic retinopathy, nephropathy and neuropathy respectively [4]. Prevention of these complications by bringing hyperglycemia under perpetual control using effective anti-diabetic agents should therefore be an ultimate goal of NIDDM management. Unlike the treatment of IDDM, designing one specific therapeutic algorithm for NIDDM is impossible because of the multifactorial nature of the latter [5-8]. Nevertheless, a number of strategies have been identified via which NIDDM could be treated, though not all have resulted into discovery of clinical anti-diabetic agents yet. The identified strategies include insulin replacement as done in IDDM; hepatic glucose output interference; intestinal glucose absorption interference; secretory functionality of pancreatic beta cells enhancement; cellular insulin sensitivity enhancement, etc. [9]. Strategies outside insulin replacement are mostly used in the control of NIDDM and have resulted in a broad group of anti-diabetics often referred to as oral hypoglycemic agents, the most deployed of which are mainly insulin secretagogues, insulin sensitizers and alpha glucosidase inhibitors [9, 10]. Alpha glucosidases are a large group of exoenzyme found widely distributed in plants, microbes and animals, cleaving terminal alpha glycosidic bonds [11]. Though in humans, there are alpha glucosidases present at varying locations performing the basic terminal glucose removal from oligosaccharide moieties for diverse biochemical ends [12-14], the ones present in the brush border membranes of intestinal epithelial cells are of utmost consideration for dietary carbohydrate absorption, as they finish up the process of carbohydrates digestion by cleaving dissacharides (e.g., maltose and sucrose), which constitute the major end products of alpha amylase activities on dietary polysaccharides, into absorbable monosaccharide units like glucose and fructose [15-18]. Inhibition of intestinal alpha glucosidases is therefore expected to greatly impair dietary carbohydrate absorption, controlling postprandial hyperglycemia, a critical complication risk factor in NIDDM [19, 20]. Inhibition of intestinal alpha glucosidases remains the only carbohydrate absorption inhibition strategy of NIDDM management in clinical medicine. However, only three clinically applicable alpha glucosidase inhibitors, namely arcabose, voglibose and miglitol, are available in medical practice till date for reasons not unconnected to adverse reactions [21], the few available being associated with a number of unbearable side effects like severe flatulence, diarrhea etc. [22]. Hence, the current spate of efforts into discovering new alpha glucosidase inhibitors for the purpose of maximally exploiting the carbohydrate absorption interference strategy of NIDDM management. And though, data from some of these discovery efforts have shown a clear difference between inhibition of mammalian intestinal alpha glucosidases and the baker's yeast (*Saccharomyces cerevisiae*) isozyme, the landscape of *in vitro* screening of compounds of diverse sources for the purpose of anti-diabetic alpha glucosidase inhibitors' discovery is still dominated by the modeling of intestinal alpha glucosidases with the baker's yeast isozyme [23]. In like manner, nature dominates the landscape of the search for new and safer anti-diabetic alpha glucosidase inhibitors, exploiting the novelties that only the structural and stereochemical intricacies of biosynthesis could inspire [24] and the general presumption of safety of natural compounds, which still holds sway despite the existence of known natural poisons [25]. *Terminalia catappa* Linn. (Combretaceae) is one natural resource used in traditional medicine, *inter alia*, for DM treatment [26, 27]. It is a perennial that grows in subtropical and tropical climates. It could grow up to a height of 35 m, standing upright with symmetrical crowns of horizontal branches with green large leaves (15-25 cm long and 10-14 cm broad). Its fruits are fleshy, firstly appearing green, turning gradually yellowish and then red, when ripe [28]. *T. catappa* has been extensively phytochemically explored and found to be rich in potentially bioactive phytochemicals including alkaloids, flavonoids and tannins [29, 30]. In the current investigation, *Terminalia catappa* stem bark extract and its various fractions were evaluated for their potential anti-diabetic alpha glucosidase inhibitory activities using the baker's yeast alpha glucosidase to model its human intestinal brush border isoforms controlling the final stage of carbohydrates digestion and, hence, absorption. Maltose, the natural substrate of the maltase glucosidase, was engaged as substrate while the accumulation of alpha glucose product of the alpha glucosidase reaction was used to monitor inhibition using a modified glucose oxidase (GOx) reaction [31].

## 2. Materials and Methods

### 2.1. Materials

*Terminalia catappa* stem bark material was collected from the University of Lagos main campus, Akoka, Lagos State, Nigeria. Identification and authentication were carried out at the Botany department of the University with deposition of herbarium sample (voucher number 7688). Baker's yeast alpha glucosidase and substrate (maltose) were from Sigma Aldrich (Germany); solvents for extraction and fractionation (methanol, dichloromethane and n-hexane) were also from Sigma Aldrich (Germany); glucose oxidase (GOx) kit for manual platform analysis of glucose product of alpha glucosidase activity was from BIOLABO (France). All chemicals, enzymes and reagents were of at least analytical standards.

### 2.2. METHODS

2.2.1. *Extraction and fractionation*

*Terminalia catappa* stem bark material was dried and pulverized into a coarse powder. The pulverized material (1.0 kg) was macerated in methanol (3 liters) for 3 days. The ensuing extract was decanted and the mac subjected to further maceration in methanol (3 liters) for additional 2 days, followed by decanting. Decanted extracts were bulked, filtered and concentrated to dryness *in vacuo* at 40ºC using a rotary evaporator. The dried methanol crude extract (100 g) was triturated in 100 ml each of n-hexane, dichloromethane and methanol successively in that order and each concentrated to dryness to obtain n-hexane-, dichloromethane- and methanol-soluble fractions (13 g, 35g and 48 g) respectively.

2.2.2. *Evaluation of Alpha Glucosidase Inhibitory Activities*

Alpha glucosidase final assay mixtures containing 50-250 µg/ml concentrations of each of test samples (crude extract, n-hexane, dichloromethane, methanol fractions, and the reference standard, acarbose) were prepared by pre-incubating 2 ml of sample stock prepared in 5% DMSO/0.15 M Phosphate buffer (pH 7.0) with 0.1 ml of baker's yeast alpha glucosidase (1U/ml) at 37ºC for 15 min. 0.9 ml of the substrate (0.5 mM maltose prepared in 5% DMSO/0.15 M Phosphate buffer) was added to each mixture and incubated at 37ºC for 30 min. A control experiment was set up with 2 ml 5% DMSO/0.15 M Phosphate buffer in place of test substance. The reaction was immediately stopped after the incubation period by immersing assay tubes in boiling water for 10 min and afterwards allowed to stand for two hours before subjection to the glucose oxidase (GOx) test using a slightly modified BIOLABO GOx assay kit procedure [32]. The kit consisted essentially of three reagents as follows: Reagent R1, made up of GOx (20,000 IU/l), Phosphate buffer (0.15 M), Peroxidase (1000 IU/l), 4-aminoantipyrine (0.8 mM/l); Reagent R2, containing 4-chlorophenol and Reagent R3, containing 5.55 mM glucose standard. 50 µl of the alpha glucosidase reaction mixture was added to 2.5 ml of R1/R2 mixture. The ensuing mixture was incubated for 20 min at 25ºC and its absorbance taken at 500 nm before and after incubation using a UV/Vis Spectrophotometer, using a micro-cuvette. % inhibition was calculated using the following formula and $IC_{50}$ calculated afterwards from % inhibition vs concentration plots for each of the extract, fractions and the standard, arcabose.

$$\%\ Inhibition = \frac{\Delta ABScontrol\ -\ \Delta ABStest}{\Delta ABScontrol}$$

Where $\Delta ABScontrol$ = change in absorbance of the mixture containing no test substance and $\Delta ABStest$ = change in absorbance of the mixture containing a test substance (crude extract, fraction or arcabose). $IC_{50}$ values were subjected to one-way ANOVA statistics using Tukey's post hoc comparison protocols of graphpad prism 9.4.0.

## 3. RESULTS

The crude extract and all its fractions demonstrated alpha glucosidase inhibitory activities. A summarized comparison of these activities with one another and with that of the reference standard is as presented in the % inhibition-concentration stack plot (Figure 1). A more detailed and informative comparison could however be gleaned from the $IC_{50}$ values (Table 1) extracted from individual % inhibition-concentration plot for each sample. The $IC_{50}$ values of the fractions, methanol (173.85 ± 6.90 µg/ml), n- hexane ( 258.56 ± 12.90 µg/ml) and dichlorometane (329.90 ± 10.67 µg/ml), are in the same order as that of acarbose (123.26 ± 15.56 µg/ml). As a matter of fact the methanol fraction and acarbose $IC_{50}$ values are not statistically different ($p > 0.05$). Moreover, the IC50 values of the fractions are significantly smaller ($p < 0.0001$) than that of the crude extract (527.67 ± 16.32 µg/ml) (Figure 2).

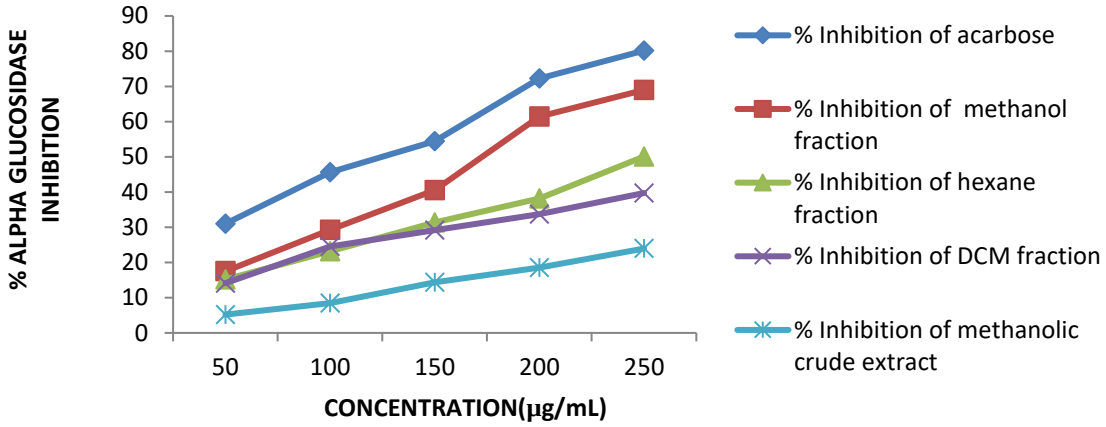


**Figure 1**: % Inhibition-concentration Stack plot for *T. catappa* stem bark extract and its (n- hexane, DCM and methanol) fractions

**Table 1:** $IC_{50}$ values (µg/ml) for the extract and fraction sample tests

| S/N | Test Sample | IC50 ± SEM |
|---|---|---|
| 1 | Acarbose | 123.26 ± 15.56 |
| 2 | MeOH | 173.85 ± 6.90 |
| 3 | n-Hexane | 258.557 ± 12.90 |
| 4 | Dichloromethane | 329.9 ± 10.67 |
| 5 | Crude Extract | 527.67 ± 16.32 |

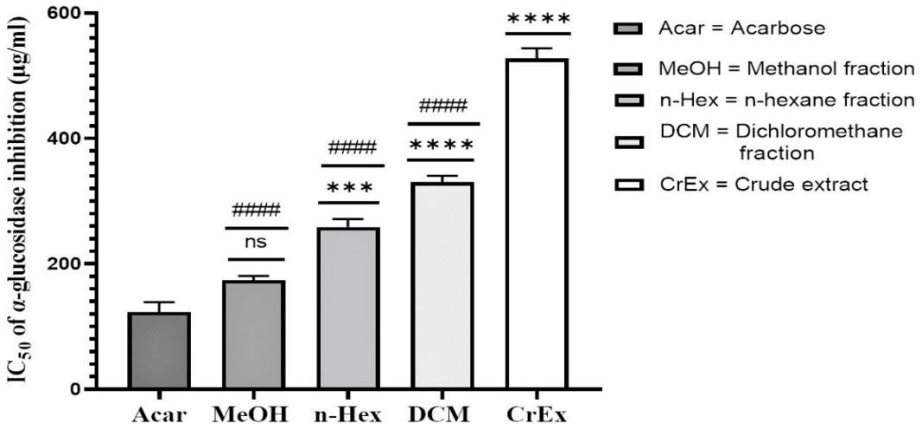


**Figure 2**:One-way ANOVA comparison of the $IC_{50}$ values of alpha glucosidase inhibition of *T. catappa* stem bark extract and its n-hexane, dichloromethane and methanol fractions with that of the reference standard, acarbose. *** significantly higher $IC_{50}$ compared to acarbose ($p < 0.001$); **** significantly higher $IC_{50}$ compared to acarbose ($P < 0.0001$); $^{ns}$ no significant difference compared to acarbose; $^{####}$ significantly smaller $IC_{50}$ compared to crude extract ($p < 0.0001$).

## 4. DISCUSSION

In this study, the baker's yeast (*Saccharomyces cerevisiae*) alpha glucosidase (maltase) served as target screen for the evaluation of potential alpha glucosidase inhibitory activities of *Terminalia catappa* stem bark crude extract and fractions using a modified version of glucose oxidase reaction as inhibition monitor. The baker's yeast is a unicellular eukaryote that has enjoyed unparalleled patronage as a modeling tool in the study of mammalian biochemistry in both health and disease, for phylogenetic reasons [33]. It is therefore not uncommon to see the baker's yeast alpha glucosidase modeling the human intestinal brush border alpha glucosidases in alpha glucosidase inhibitor discovery researches. As a matter of fact, most of such alpha glucosidase inhibition-related researches till date have utilized the baker's yeast alpha glucosidase despite the non-perfect alignment of its inhibition data with those of the mammalian intestinal brush border isoforms directly implicated in the disease under consideration [23]. The utility of the baker's yeast enzyme in this alpha glucosidase reaction was therefore not out-of-place. Notwithstanding, its amenability to glucose oxidase (GOx) determination is not as obvious: While the alpha glucosidase reaction product is alpha glucose, the substrate of the GOx reaction is beta glucose [34, 35]. This warranted the two-hour pre-GOx-reaction intervening period in the experimental design, enabling enough mutarotation conversion of alpha to beta glucose, in whose favour the equilibrium was expected to completely shift, courtesy of Le Chatelier's principle [36]. By and large, the stack splot of % inhibition vs concentration (Figure 1) revealed a face-value higher potency of acarbose over the extract and its fractions. A more careful analysis using the $IC_{50}$ values however revealed that the alpha glucosidase inhibitory activity of acarbose was in the same order as those of the extract and fractions. Moreover, statistical analysis of the $IC_{50}$ values (Figure 2) made clearer the relative alpha glucosidase inhibitory activities of the extract and the fractions compared to the standard, revealing, the existence of no statistical difference between the potencies of the methanol fraction and acarbose ($p > 0.05$). On another hand, comparative analysis of the $IC_{50}$ values of the fractions with that of the crude extract revealed significantly smaller $IC_{50}$ value for each of the fractions compared to the extract ($p < 0.0001$) and hence much more potent alpha glucosidase inhibitory activities by each of the fractions than by the parent extract. The fact that the three fractions showed significantly higher activity than the crude extract showed the extract as harbouring alpha glucosidase inhibitory compounds of diverse polarity and, hence, structures. This implies diverse inhibition mode of the enzyme by the structurally diverse molecules, suggesting that the molecular components of each fraction, and by extension, the crude extract components, would most likely have varying ligand-enzyme binding modes responsible for their activities. For instance, while some components might act by binding at the substrate's binding site at the enzyme's active site, some might be binding at a different location and different orientation at the active site, and hence with different amino acid residues. However, others might just be allosteric binders, binding at locations completely different from but with consequential conformational changes at the active site [37]. This conjecture is further alluded to by the fact that the order of increase in alpha glucosidase inhibition seen in the fractions did not synchronize with their order of polarity. Though, the highest-polar methanol fraction was the most potent of the three fractions ($IC_{50}$ $173.85 \pm 6.90$ µg/ml), the least-polar n-hexane fraction was not the least-potent: The n-hexane fraction ($258.56 \pm 12.90$ µg/ml) demonstrated stronger alpha glucosidase inhibition than the dichloromethane fraction ($IC_{50}$ $329.90 \pm 10.67$ µg/ml). In a nutshell, fractionation of the extract did not lead to confining or streamlining of its alpha glucosidase inhibitory activity into one fraction, Instead, it led to separation of the alpha glucosidase inhibitory principles in the extract into three (n-hexane, dichloromethane and methanol) fractions, each of which could be explored further for the discovery and development of potential anti-diabetic alpha glucosidase inhibitors.

## 5. CONCLUSION

Methanol, dichloromethane and n-hexane fractions of *Terminalia catappa* stem bark extract have shown potent inhibition against the baker's yeast alpha glucosidase. This, in a way, has provided a molecular basis for the traditional anti-diabetic use of the plant. It has also unmasked the stem bark extract of *Terminalia catappa* as a veritable source of molecular species of diverse structures that could be developed into clinically deployable anti-diabetic alpha glucosidase inhibitors.

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**Conflicts of interest**

There are no conflicts of interests associated with either this article or the investigation reported therein.

**Authors' contributions**

OSA designed the research and participated in data collection and manuscript writing; AVM participated in data collection and manuscript writing; CAO participated in material collection and manuscript writing. All authors proofread and approved the manuscript.

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