An *In vitro* Study to Assess Alpha Amylase Inhibition and Antioxidant Activities of The Ethanol and Acetone Extracts of *Anacardium occidentale* Linn (Cashew) Stem Bark

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Abstract

Diabetes Mellitus (DM) is an endocrine disease due to glucose intolerance. The journey to fghting against DM is limited by the inability of some diabetic patients to have access to oral antidiabetic drugs. Alpha-amylase signif cantly contributes to the handling of hyperglycemia. This study investigated the DPPH free radical scavenging activity, the total phenolic content and the alpha-amylase inhibition activity of *Anacardium occidentale* Linn (cashew) stem bark *in vitro*. The total phenolic content of the extracts was found to be 3.572 ± 0.39 and 3.145 ± 0.28 mgg⁻¹. The DPPH free radical scavenging potency (IC₅₀) of acetone and ethanol extracts were 6.743 ± 1.16 µgmL⁻¹ and 9.186 ± 1.06 µgmL⁻¹ respectively while ascorbic acid was 2.796 ± 1.06 µgmL⁻¹.

The preliminary phytochemical screening showed that terpenes, phenols, glycosides, tannins, favonoids, alkaloids and saponins were present in all the extracts. On the contrary, sterols were tested negative in the extracts. The alpha amylase inhibitory potency (IC_{50}) of the acetone and ethanol extracts were 46.07 µgmL⁻¹ and 51.31 µgmL⁻¹ respectively and acarbose (the standard drug) was 24.97 µgmL⁻¹. The inhibition potency on -amylase as observed together with its potential antioxidant capacity in this study proposes an efficient function of the stem bark of *A. occidentale* in management of DM specifically the type II and its related complications associated with oxidative stress.

Keywords: Diabetes mellitus, IC_{50} , -amylase inhibition, DPPH, Antioxidant, Total phenolic content, Phytochemical screening, Acarbose, Gallic acid, Ascorbic acid

Introduction

Diabetes Mellitus (DM) is an endocrine disease due to glucose intolerance. Diabetes can cause hyperglycemia, oxidative stress, polyuria, nephropathy, polyphagia, polydipsia, ketosis and disorders of the cardiac system [1].

e journey to ghting against DM is limited by the inability of some victims of DM to have access to oral antidiabetic drugs [2]. Reported that, over three hundred million people will be diabetic by 2025.

Currently, the treatment procedures for DM employ the application of oral hypoglycemic and antihyperglycemic drugs, insulin therapy, physical activity, life style, diet therapy, and xenotransplantation. Present antidiabetic drugs don't give signi cant control of blood glucose [3]. For a long time, medicinal plants have performed a signi cant function in the treatment of human diseases which includes diabetes too. Many reported medicinal plants show anti-diabetic e ect and can serve as a supplement for synthetic drugs. Examples of such drugs for management of diabetes are acarbose, miglitol, Nateglinide, Repaglinide and voglibose [4]. However, these drugs have some gastrointestinal side e ects including abdominal pain, atulence and diarrhea and other side e ects such as pharyngitis, headache and nausea. erefore, it is the need of time to identify antidiabetic substances from natural sources having fewer side e ects but more e cient pharmacological response [5].

is research seeks to assess the alpha-amylase inhibitory and the antioxidant activities of ethanol and acetone extracts of *Anacardium occidentale* stem bark in vitro.

Materials and Method

Materials

Apparatus and equipment: Electronic balance, water bath, stirrer, mortar and pestle were obtained from University Development Studies, Navrongo campus. Whatmann No1 lter papers were purchased from Alpha chemical shop, Navrongo, spectrophotometer (Biotek Synergy H1 Hybrid Reader, USA), UV-Vis spectrophotometer (Genway 7B Series, USA), test tubes, 96 well microplate, micropipettes and the pipette tips were supplied by the faculty of pharmacy and pharmaceutical sciences of the Kwame Nkrumah University of Science and Technology, Ghana.

Chemicals and reagents

3, 5-dinitrosalicylic acid (DNS), 40% 5.31 M sodium potassium Tartrate, Folin Ciocalteau phenol reagent, 2, 2-diphenyl-1picrylhadrazyl (DPPH), alpha amylase from porcine pancreas and potato starch were supplied by Sigma-Aldrich (St. Louis, MO, USA). Acetone and 90% ethanol were also purchased from Alpha Chemical Shop, Navrongo. Distilled water was purchased from Navrongo senior high school (Upper East Region, Ghana), absolute methanol, 6.7 mM sodium chloride, 0.02 M sodium phosphate bu er (pH 6.9), 1% sodium hydroxide, 7.5% sodium carbonate, acarbose, ascorbic acid and gallic acid were also obtained from Kwame Nkrumah University of Science and Technology (Kumasi, Ghana).

Method

Sample collection

Matured stem bark of *A. occidentale* was harvested from Nyoja Jembo Cashew Farm at Kpassa Jumbo No1 in the Volta region of Ghana in the month of January 2018. It was then authenticated by

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Received December 18, 2020; Accepted January 10, 2021; Published January 10, 2021

Citation: Mborige B (2021) An *In vitro* Study to Assess Alpha Amylase Inhibition and Antioxidant Activities of The Ethanol and Acetone Extracts of *Anacardium occidentale* Linn (Cashew) Stem Bark. Biochem Physiol 10: 296.

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Dr. Imoro Wahab, a botanist in the Department of Applied Biology, Faculty of Applied Sciences, Navrongo campus of the University for Development Studies, Ghana.

Sample preparation

e matured stem bark of *A. occidentale* was chopped into piece, washed under running water to remove any contaminant and then shade dried. e dried pieces were pounded using mortar and pestle. It was then sieved and ne uniform powdered sample obtained.

Extraction

e extraction was carried out by maceration using a protocol previously used by Sahira & Cathrine (2015).

Phytochemical screening

e phytochemical screening was carried out using a method previously used [6].

Total phenolic content

e gallic acid solution was prepared by dissolving 10 mg (0.01g) of Gallic in 50 mL of distilled water in volumetric ask (200 μ gmL⁻¹). e total phenolic contents present in the stem bark extracts of *A. occidentale* L. were determined using the Folin Ciocalteau phenol reagent colorimetric method based on redox reaction described by Waterhouse (2002) and Rodolfo et al.

- 1. To 100 μL of the extract in a test tube, 0.5 mL of Folin Ciocalteu phenol reagent and 1 mL of 7.5% sodium carbonate (Na $_2CO_3$) were added.
- e content was mixed and allowed to stand for 30 min at room temperature in the dark and the absorbance measured at 700 nm in03 Tw 478-1.189 TD(using ts)0.5(pdded.ext in].)Ted an0.6gram]TJmj0.% s acid s

Total phenolic content

e content of phenols present in the extracts were estimated as gallic acid equivalents in milligram per gram of extract (mgg

e -amylase enzyme catalyzes the hydrolysis of -1, 4 glycosidic linkages of polysaccharides to yield maltose units which are in turn acted upon by other glucosidases down the GIT to produce glucose residues [28]. In the -amylase inhibition assay, acetone extract (IC₅₀ of 46.07 µgmL⁻¹) was more potent compared to that of ethanolic extract (IC₅₀ of 51.31 µgmL⁻¹). Percentage alpha amylase inhibition of the two plant extracts was plotted as function of concentration in comparison with acarbose as shown in Figure 3. For the two extracts of *A. occidentale* stem bark, it was found that acetone extract shows a better -amylase inhibition compared to the ethanolic extract. A previous study by Dineshkumar et al., proposed that, the inhibition of -amylase activity by plant extracts may be due to the presence of potential -amylase inhibitors such as avonoids, alkaloids, terpenes and glycosides.

Conclusion

e study shows that extracts obtained from the stem bark of *A. occidentale* are rich in bioactive secondary metabolites, exerting previotion

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