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In Vitro study on α-amylase inhibitory activities of *Digitaria exilis, Pentadiplandra brazzeana (Baill)* and *Monodora myristica.*

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ABSTRACT

In the present study, the hot and cold aqueous extracts of *Digitaria exilis* and *Pentadiplandra brazzeana* (Baill) as well as the ethanolic extract of *Monodora myristica* were screened for their anti diabetic activity via inhibition of α-amylase. The root of *Pentadiplandra brazzeana* Baill and the grains of Acha (*Digitaria exilis*) were extracted by Soaking in hot and cold water while the seed of *Monodora myristica* was extracted using 70% ethanol. α-amylase was significantly inhibited by *Digitaria exilis, Pentadiplandra brazzeana* Baill and *Monodora myristica.* Results showed that the three plants can act as potent α-amylase inhibitor. The cold aqueous extract of *Pentadiplandra brazzeana* Baill showed the highest inhibition against pancreatic α-amylase among the plants studied with IC_{50} value of 197.63±1.450 µg/ml while the ethanolic extract of *Monodora myristica* showed the least with IC_{50} value of 408.17±2.945. α-amylase inhibitors from herbal sources offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia by decreasing glucose release from starch and may have potential for use in the treatment/management of diabetes mellitus and obesity.
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Keywords: α-amylase inhibition, diabetes mellitus, postprandial hyperglycemia.

INTRODUCTION

Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity, as well as, dental caries and periodontal diseases (Sales et al., 2012).

Diabetes Mellitus is a common metabolic and a multi-system disorder comprising of metabolic and vascular abnormalities resulting from insulin deficiency (Ankush and Nikhil, 2009). This

disorder affects millions of people worldwide; its occurrence continues to increase throughout the world (Ng et al., 2014). The study of metabolic enzymes associated with this disease is of special interest because of its application in clinical biochemistry (Gupta and Phatak, 2003).

Diverse therapeutic strategies are available for the treatment of Type II diabetes, one of such strategies is the inhibition of degradation of dietary starch by glucosidases such as α-amylase and α -glucosidase (Rang et al., 2003).

The use of herbs in the management of diabetes is not new as herbs were known for a long time and had been used by many people to treat a variety of diseases. Plants are important source of chemical constituents with potential for inhibition of α-amylase and can be used as therapeutic or functional food sources as such many plants have been used to treat/manage diabetes. Plants may be a valuable source of novel anti-diabetic agents as they may contain new pharmacologically active agents which could be specific inhibitors for α-amylase (Tarling et al., 2008).

Inhibition of all or some of the intestinal disaccharidases and pancreatic α-amylase by inhibitors could regulate the absorption of carbohydrate. These inhibitors offer an effective strategy to lower the levels of post-prandial hyperglycemia via control of starch breakdown (P et al., 2011).

Acha, *Digitaria exilis* (fonio or hungry man's rice) is an indigenous cereal widely cultivated in the middle belt region of Nigeria. It's one of the most nutritious of the cereals known to man, its rich in methionine and cysteine the limiting amino acids of most cereals. The low carbohydrate content of acha has made it to be a complement in diabetes' diets (Balami et al., 2009). For this reason acha has been used to manage and treat diabetes in some parts of northern Nigeria with focus on its low sugar content. However, studies on the effect of the plant against carbohydrate metabolizing enzymes have not been reported.

Pentadiplandra brazzeana Baill is the sole species in the genus Pentadiplandra (family Pentadiplandraceae). It has many medicinal uses such as: abortifacients, ecbolics; arthritis, rheumatism, subcutaneous parasitic infection, diarrhoea, dysentery, general healing; genital stimulants/ depressants, diuretics; pain-killers; pulmonary troubles; venereal diseases; vermifuges (Burkill, 1985).

Monodora myristica (Annonaceae) is a flowering plant commonly known as 'Ariwo' in South Western Nigeria. It is widely distributed from Africa to Asia, South America and Australia (Omobuwajo et al., 2003). It's a tropical tree that grows wild in many African countries including Nigeria (Okafor, 1987). Nutritional value of *M. myristica* centres on its usefulness as a seasoning because of its aromatic flavor (Ekeanyanwu et al., 2010). There are several reports of its medicinal use; the bark is used in the treatments of stomach-aches, febrile pains, eye diseases and hemorrhoids (Burkill, 1985; Ekeanyawu et al., 2010). Its phytochemical constituent indicates that the plant could be useful in the management of free radical related diseases of which diabetes is one.

This study sorts to investigate the *in vitro* inhibitory effects of *Digitaria exile, Pentadiplandra brazzeana* (Baill) and *Monodora myristica* on the activities of α–amylase, a diabetic related carbohydrate metabolizing enzyme.

MATERIALS AND METHODS
Materials
Plant materials
The roots of *Pentadiplandra brazzeana* was obtained from Warri Area of Delta State, Nigeria. Acha (*Digitaria exiles)* was obtained from Jos main market in Jos North Area of Plateau State, while *Monodora myristica* seeds were obtained from a local market in Ile-Ife Osun State, Nigeria. The plants were identified and authenticated at the Department of Botany, Obafemi Awolowo University Ile-Ife, Nigeria.

Chemicals and reagents

Pancreatic α-amylase, ethanol, 3,5-dinitrosalicylic acid (DNSA) and starch soluble were products of Sigma Aldrich Co., St Louis, USA, Other chemicals and reagents used were of analytical grade.

Preparation of plant extracts

The roots of *Pentadiplandra brazzeana Baill* was shade dried and chopped into small pieces and pulverized into a fine powder while the grains of Acha (*Digitaria exilis)* were pulverized to fine powder. The plant materials were extracted by Soaking in hot and cold sterile distilled water for 4 hours with continuous stirring after which they were left overnight in a refrigerator. The extracts were then filtered using whatman filter paper. The filtrates were used as the aqueous plant extracts for the experiment. *Monodora myristica* was extracted using 70% ethanol as solvent and concentrated using rotary evaporator.

α-Amylase inhibition assay

The inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method as reported by Jain et al. (2013). The total assay mixture composed of 1000 μl of 0.02 M Sodium phosphate buffer (pH 6.9 containing 6 mM Sodium chloride), 1000 μl (0.04 units of pancreatic α-Amylase solution) and 400 μl extracts at various concentration ranging from 100-500 µg/ml (w/v). After pre-incubation at 37 ^{0}C for 10 min, 1000 μl of 1% (w/v) starch solution was added to each tube and incubated at 37 °C for additional 15 min. The reaction was terminated with 1.0 ml DNSA reagent, and placed in boiling water for 5 min after which it was cooled to room temperature, and the absorbance was measured at 540 nm using vis spectrumlab S23A. The control did not contain any plant extract and represented 100% enzyme activity.

The % inhibition of alpha amylase was calculated as follows:

$$\text{Inhibitory activity (\%)} = \frac{OD_{control} - OD_{test}}{OD_{control}}$$

The IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined by performing the assay as above with varying concentrations of the plant extracts.

RESULTS

The hot and cold water extracts of *Digitaria exile, and Pentadiplandra brazzeana* (Baill) as well as the ethanolic extract of *Monodora myristica* were obtained and used for the inhibition studies. α-amylase inhibition by cold water extracts of *Pentadiplandra brazzeana and digitalis exilis* showed a dose dependent increase inhibition of the enzyme activity. *Pentadiplandra brazzeana* Baill cold water extract showed the highest inhibition of 89.58% at concentration of 500 µg/ml (IC_{50} value 197.63±1.450 µg/ml) while the *Digitaria exile* showed an inhibition of 57.88% at the same concentration with IC_{50} value of 368.58±2.404 µg/ml (Figure 1). α-amylase inhibition by the hot aqueous extracts of *Pentadiplandra brazzeana and Digitalis exilis* showed inhibition of 95.09% (IC_{50} value 257.99±13.490 µg/ml) and 87.44% (IC_{50} value 231.49±1.618) respectively (Figure 2). *Monodora myristrica* showed maximum inhibition of 96.58% at 1000 µg/ml (IC_{50} value 408.17±2.945 µg/ml), the plant showed a dose dependent increase in its inhibition (Figure 3).
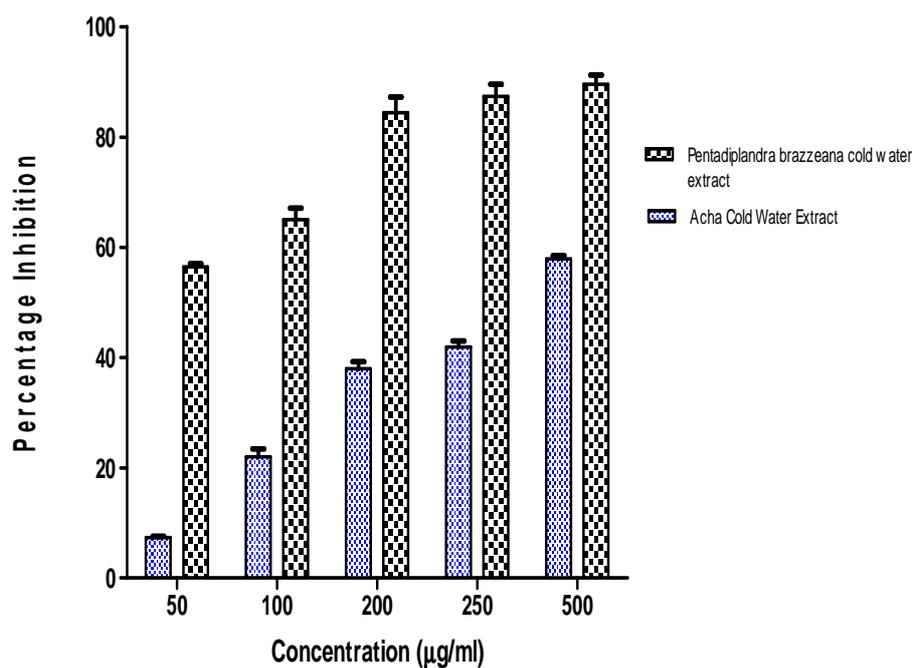
Figure 1: α-amylase inhibition by the cold aqueous extracts of *Pentadiplandra brazzeana* and *Digitalis exilis.*
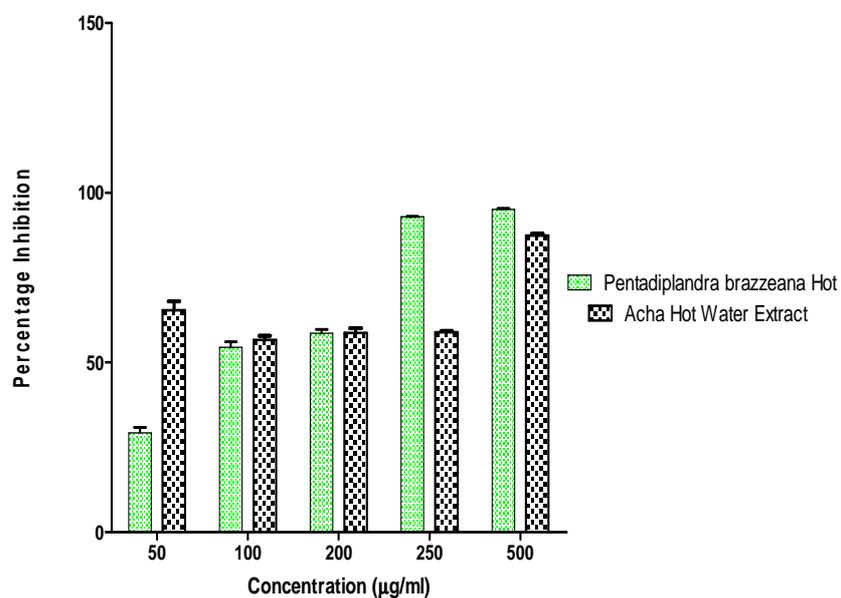

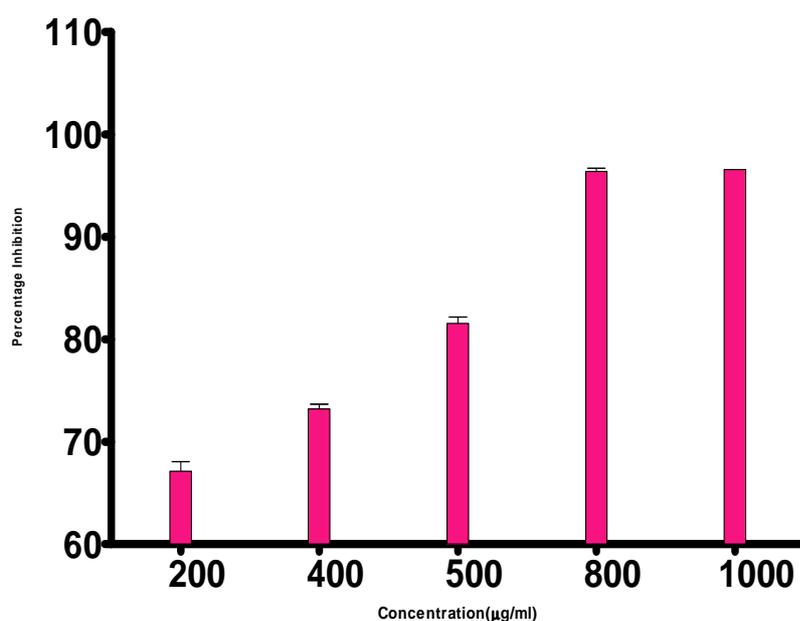**Figure 2**: α-amylase inhibition by the hot aqueous extracts of *Pentadiplandra brazzeana* and *Digitalis exiles.*

Figure 3: α-amylase inhibition by the ethanolic extract of *Monodora myristrica*.

Table 1: *Pentadiplandra brazzeana* (hot and Cold), *Digitalis exilis* (hot and cold) and *Monodora myristica* (Ethanolic extract) and their corresponding IC_{50} Values.

EXTRACTS	IC_{50}
Pentadiplandra brazzeana (Hot)	257.99 ± 13.490 µg/ml
Pentadiplandra brazzeana (Cold)	197.63 ± 1.450 µg/ml
Digitalis exilis (Hot)	231.49 ± 1.618 µg/ml
Digitalis exilis (Cold)	368.58 ± 2.404 µg/ml
Monodora myristica (Ethanolic extract)	408.17 ± 2.945 µg/ml

DISCUSSION

α-amylase is involved in a number of important biological processes, such as digestion of carbohydrate into glucose or processing of the oligosaccharide moieties of glycoprotein. There is now a great deal of interest in amylase inhibitors (Kim et al., 2000). Inhibition of α-amylase could result in delayed carbohydrate digestion and glucose absorption with attenuation of post prandial hyperglycemic excursions. It has been reported that inhibitors usually do not alter the total amount of carbohydrate absorbed and therefore do not cause any net nutritional caloric loss although they slow down carbohydrate digestion (Girish, et al., 2010). In most diets, carbohydrates are the greatest source of calories. Before being absorbed by the body, carbohydrates must be broken down into monosaccharides. This breakdown occurs due to two major enzymes: amylase and glucosidase (Obiro et al., 2008).

Investigations have shown that the use of herbs by diabetic patients is a common practice worldwide and many herbal extracts have been reported for their anti-diabetic activities (Modak et al., 2007; Afolayan and Sunmonu, 2010). Alpha-amylase inhibitors

with activity against mammalian forms of the enzyme are present in many plants and it is suggested that they were developed by plants in order to strengthen their defense against predators. (Tundis et al., 2010). The aflavins and catechins present in green and black teas have been reported to inhibit alpha-amylase and alpha-glucosidase activity as well as retard starch digestion in an *in vitro* model (Koh et al., 2010). Alpha-amylase inhibitors are also present in grains, including wheat and rice (Tundis et al., 2010).

The present work was carried out with a view to exploring some traditional food plants, commonly used in daily life, for their α-amylase inhibitory potential *in vitro*. All the three plants selected namely *Pentadiplandra brazzeana, Digitalis exile* and *Monodora myristica* are used locally as food plants and also are known for possessing many medicinal values. These plants have been under-utilized as they are consumed mainly as spices and not for their medicinal use. However, these plants have a long history of medicinal uses.

The hot and cold aqueous extracts of *Digitaria exilis* and *Pentadiplandra brazzeana* Baill as well as the ethanolic extract of *Monodora myristrica* were screened for anti diabetic activity via inhibition of α-amylase. α-Amylase was significantly inhibited by *Digitaria exilis* and *Pentadiplandra brazzeana* Baill. *Pentadiplandra brazzeana* Baill showed a dose dependent inhibition with the hot and cold water extract. The highest inhibition of 95.09% at concentration of 500µg/ml (IC$_{50}$ value 257.99±1.390 µg/ml) was obtained with the hot water extract for *Pentadiplandra brazzeana Baill;* and 87.44% (IC$_{50}$ value 231.49±1.618 µg/ml) for the hot water extract of *Digitaria exilis*. The hot water extract of *Digitaria exilis* also exhibited a dose dependent inhibition (inhibition from 50 µg/ml to 250 µg/ml) but remained almost constant at about 60% inhibition above these concentrations likewise, its cold water extract exhibited a dose dependent inhibition. Results

of present investigation agree with the reports of medicinal plant with high *in vitro* inhibition against amylase obtained by Prabhakar, et al. (2013). Their report showed that the aqueous extract of *Withania somnifera* leaf showed 92.7% and the methanolic extract of *Ocimum Sanctum* showed 92.6% inhibition against porcine pancreatic α-amylase among the medicinal plants studied.

The literature on phytochemical analysis of these plants (*Digitaria exilis, Pentadiplandra brazzeana* Baill and *Monodora myristica*) indicates that the plants possess a large number of vital compounds that might form a part of healthy diet and the rich fiber content of the plant suggests that it might decrease the starch intake and may reduce the incidence of metabolic disorders like diabetes.

The ethanolic extract of *Monodora myristica* showed a dose dependent increase in its inhibition with maximum inhibition of 96.85% at 1000 µg/ml (IC$_{50}$ value 408.12±2.945). Aqueous extract of *M. myristica* have been reported to possess moderate potential (50-70%) inhibitory effect on pancreatic alpha amylase (Oba et al., 2010). The anti α-amylase activity of *M. myristica* seed may be due to the presence of phenolic compounds which have been shown to interact with and inhibit enzyme (Arts et al., 2002; Rhon et al., 2002). The reactive mechanism involved in the inhibition of alpha amylase by this spice may also be due to the presence of flavinoids which have been reported to inhibit α-amylase (Kim et al., 2000). The seed of *M. myristica* has proven antioxidant properties (Akinwunmi and Oyedapo, 2013). Its use could be beneficial in the management of degenerative processes and diseases caused by reactive species including type 2 diabetes.

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