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Phytochemical Screening, Polyphenolic Content and Alpha-Glucosidase Inhibitory Potential of *Leptadenia hastata* (Pers.) Decne

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ABSTRACT: *Leptadenia hastata* (Pers.) Decne. (Asclepiadaceae) is widely used as vegetable and traditionally in the management of diabetes mellitus and in the treatment of wounds and stomach ache. In this study, phytochemical screening, total phenolic contents and alpha-glucosidase activity of *L. hastata* leaf extracts were evaluated with the view to validating its antidiabetic potentials. Acetone, methanol and water extracts were screened for the polyphenolic contents while methanol and water extract were used for the evaluation of alpha-glucosidase activity. Phytochemical screening of *L. hastata* leaf indicated the presence of phenolic glycosides, tannins, flavonoids, proanthocyanidins, alkaloids and saponins. The total phenolics, total flavonoids and proanthocyanidins contents were in the ranges of 17-38, 10-16 and 4-10 mg/g respectively depending on the extraction solvent. The methanol and water extracts had 69.81 and 37.02 % inhibitory effect on alpha-glucosidase activity respectively. The results indicated that *L. hastata* leaf is rich in polyphenols and possess significant alpha-glucosidase inhibition potential and may therefore be a source of lead compounds in the management of diabetes mellitus and/or other diseases that may be caused by oxidative stress.
Keywords: *Leptadenia hastata,* phytochemicals, polyphenols, alpha-glucosidase, effect, inhibition.

INTRODUCTION

Considerable evidence shows that more than 100 diseases affecting human beings are caused or aggravated by accumulation of free radicals or reactive oxygen species and the associated lipid peroxidation in the body (Chen and Yen, 2005). Some of these diseases include malaria, atherosclerosis, cancer, diabetes, acquired immunodeficiency syndrome (AIDS) and heart diseases (Luximon-Ramma *et al*., 2002; Li *et al.,* 2010).

Experimental studies indicate a significant reduction in the risks for a variety of diseases upon feeding on polyphenol-rich foods, vegetables and beverages (Kris-Etherton *et al*., 2002). Polyphenols in plants are considered to be important ingredients in human diet. They are reported to exert a lot of biological effects such as antioxidant activity and inhibitory effects on carbohydrates hydrolyzing enzymes due to their ability to binds with protein (Griffiths and Moseley, 1980). The decrease in the cases of oxidative-stress associated diseases like cancer, diabetes (Gerber *et al.,* 2002*;* Serafini *et al.*, 2002) and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (Di Matteo and Esposito, 2003) as well as inflammation and problems caused by cell and cutaneous aging (Ame, 1993) have been associated with the consumption of foods and vegetables that are rich in polyphenol antioxidant compounds.

Several studies reported the inhibitory effects of polyphenols on carbohydrates hydrolyzing enzymes. These include the green tea polyphenols that inhibit the activities of α-glucosidase and sucrase (Hara and Honda, 1992), berry polyphenols that inhibit the activity of α-glucosidase and α-amylase (McDougall and Stewart, 2005) and sweet potato polyphenols which inhibit the activity of α-glucosidase (Matsui *et al.,* 2001).

It has now been adopted that an effective anti-diabetic compound should have both hypoglycemic and antioxidant properties, with minimal or no side effects. Several African medicinal plants were reported to have both hypoglycemic and antioxidant activities (Atawodi,

2005). The edible tropical plants are considered to be good in qualities and quantities of polyphenols and their biological function due to strong exposure to sunlight (Hanamura *et al.,* 2005).

Leptadenia hastata belongs to the family asclepiadaceae widely used in tropical Africa as vegetable (Burkil, 1985). The plant is medicinally important in the treatment of many ailments (Kerharo and Adams, 1974; Burkil, 1985; Oliver-Boyer, 1986; Aliero *et al.,* 2001). Ethnobotanical information obtained from traditional medical practitioners in Northern Nigeria revealed that *L. hastata* is used for the treatment of diabetes mellitus. The antibacterial and antimicrobial effects of *L. hastata* have been reported (Aliero and Wara, 2009) and result of its toxicity studies showed that the plant is safe to use (Tambuora *et al*., 2005). This study evaluates the polyphenolic content and alpha-glucosidase inhibitory potential of *L. hastata* with a view to validating its potential in the management of diabetes mellitus.

MATERIALS AND METHODS

Chemicals and Reagents

All the reagents used for the study were of analytical grade. They included: tannic acid, quercetin, $FeCl_3$, Folin-Ciocalteu phenol reagent and sodium carbonate purchased from Sigma Chemical Co. (St. Louis, MO, USA), vanillin, $AlCl_3$ ethanol, sodium acetate solution, hydrochloric acid and ascorbic acid from BDH, Other materials were maltose and Glucose GOD/PAP assay kit from Randox Laboratories LTD (Admore, Diamond Road, United Kingdom).

Collection of Plant Material and Preparation of Extracts

Fresh leaves of *L. hastata* were collected from the Biological garden on the main Campus of the Usmanu Danfodiyo University Sokoto, and authenticated at the Herbarium of Botany Unit of the same Institution where voucher specimen No. UDUH/AB/ 0011060 was prepared and deposited. The leaves were air dried ground into powder and one hundred (100) gramms of the sample were separately extracted with acetone, methanol and water. This was followed by mixing and agitation for about 6 hours and was allowed to stand overnight, filtered and concentrated under reduced pressure to dryness at 40°C. The percentage yield for each of the extract was calculated used directly for quantitative determination of polyphenols and evaluation of alpha-glucosidase inhibitory effect.

Phytochemical Screening

The presence of phenolics glycosides, tannins, flavonoids, flavonols, proanthocyanidins, alkaloids, anthraquinones and saponins were screened according to the method of Trease and Evans (1989) and El Olemy *et al.* (1994).

Determination of Total Phenolics

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe *et al.*, 2003). An aliquot of the extract (0.5 ml of 1:10 g/l) was mixed with 5 ml Folin-Ciocalteu reagent (diluted previously with 1:10, v/v water) and 4 ml (75 g/l) of sodium carbonate. The tubes were votexed for 15s and allowed to stand for 30 min at 40°C for colour development. Absorbance was measured at 765 nm using Jenway 6100 digital spectrophotometer. Samples of extract were evaluated at final concentration of 1.0 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent.

Determination of Total Flavonoids

Total flavonoids were estimated using Ordonez *et al*. (2006) method. Thus: to 0.5 ml (0.5 ml of 1:10 g/l) of sample, 0.5 ml of 2 % $AlCl_3$ ethanol solution was added. After 60 min at room temperature, the absorbance was measured at 420 nm. A yellow colour indicates the presence of flavonoids. Samples of extracts ware evaluated at 0.1 mg/ml. Total flavonoids contents was calculated as quercetin (mg/g).

Determination of Total Proanthocyanidins

Determination of proanthocyanidins was based on the Sun *et al.* (1998) procedure. Exactly 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3 ml of 4 % vanillin-methanol solution and 1.5 ml hydrochloric acid. The mixture was allowed to stand for 15 min. The absorbance was read at 500 nm. Total proanthocyanidins contents were calculated as tannic acid equivalents (mg/g).

Assay for Alpha-glucosidase Inhibitory Activity

Alpha glucosidase activity was assayed using 0.1 M acetate buffer pH 6.0 and 0.56 M maltose (0.56 M. 1 H_2O in 100 ml redistilled water) was used as

a substrate (Calzyme, 2004). To the test tubes containing 0.5 ml of plant extract, 1.5 ml acetate buffer followed by 0.5 ml alpha glucosidase (1:50) suspension were added and incubated at 25°C for 5 minutes. The same was repeated for the control experiment using 0.5 ml redistilled water to replace the plant extract. 0.5 ml maltose was added to the above set up and also re-incubated for 5 minutes at the same temperature. 0.1 ml of the resultant assay mixture was pipetted into separate test tubes, each containing 1 ml of glucose oxidase. Each test tube was replicated three times. These were then incubated for 10 minutes at 37°C. The enzyme activity was quantified by measuring the absorbance at 500 nm against the reaction blank. 0.5 ml and 1 ml distilled water were used to replace alpha glucosidase in the blank treatment for the test and control experiments respectively. One unit of alpha glucosidase is defined as the amount of enzyme which catalyzes the release of one micromole of glucose per minute at 25°C, pH 6.0. Percentage inhibition of alpha glucosidase by the extract was calculated.

Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Waller Duncan's multiple range test using the Statistical Analysis System (SAS) 2003 version. Difference at $p< 0.05$ was regarded as significant.

RESULTS

Percentage Yield and Phytochemical Analysis of *L. hastata* Leaf Extracts

The percentage yield of acetone, methanol and water extracts of *L. hastata* leaf were: 9.2, 11.4 and 31.5 % respectively. The results of qualitative phytochemical constituents of *L. hastata* leaf are presented in Table 1. The results indicate that with the exception of flavonols and anthraquinones, all the phytochemical parameters tested were present.

Table 1: Phytochemical Analysis of *L. hastata* Leaf

Phytochemical	Results
Phenolic glycosides	Positive
Tannins	Positive
Flavonoids	Positive
Flavonols	Negative
Proanthocyanidins	Positive
Alkaloids	Positive
Anthraquinones	Negative
Saponins	Positive

Polyphenolic Contents of *L. hastata* Leaf Extracts

The results of total phenol, total flavonoids and total proanthocyanidins contents of acetone, methanol and water extracts of *L. hastata* leaf are presented in Table 2. The results show that acetone extract (35.77 mg/g) had highest content of total phenol than methanol (23.0 mg/g) and water (17.58 mg/g). The flavonoids content of methanol (15.85 mg/g) is higher than that of acetone (10.5 mg/g) and water (12.15 mg/g). The methanol extract (9.69 mg/g) had highest content of proanthocyanidins compared to water and acetone with 6.06 and 4.24 mg/g respectively.

Alpha-glucosidase Inhibitory Effect of Methanol and Water Extracts of *L. hastata* Leaf *in vitro*

The amount of glucose liberated from maltose by the action of alpha glucosidase enzyme in the presence of methanol and water extract of *L. hastata* leaf is presented in Table 3. The result show that both the methanol and water extracts of *L. hastata* leaf significantly ($p<0.05$) inhibited the activity of *A. niger* α-glucosidase.

Table 2: Polyphenol Contents of the Acetone, Methanol and Water Extracts (mg/g) of *L. hastata* Leaf

Polyphenols	Acetone	Methanol	Water
Total phenolics *	37.77 ± 1.12^a	23.00 ± 0.42^b	17.58 ± 1.55^c
Total Flavonoids **	10.50 ± 0.34^b	15.85 ± 0.23^a	12.15 ± 0.48^b
Proanthocyanidins *	4.24 ± 0.86^b	9.69 ± 0.85^a	6.06 ± 0.86^a

* Expressed as mg tannic acid/g of dry plant material.
** Expressed as mg quercetin/g of dry plant material.
Values are mean ± Standard deviation of 3 replicates.
Mean followed by the same superscript across the rows indicates that the value is not significantly different from the other at p<0.05.

Table 3: *In vitro* Alpha Glucosidase Inhibitory Effect of Methanol and Water Extracts of *L. hastata* Leaf

Treatments	α-glucosidase activity (μg glucose/ml/minute)	Inhibition (%)
Control	92.14 ± 10.67^a	-
Methanol Extract	27.81 ± 2.31^c	69.81
Water Extract	58.03 ± 4.93^b	37.02

Values are mean ± standard deviation of 3 replicates. Mean followed by the same superscript in each column are not significantly different ($p<0.05$)

DISCUSSION

Phytochemicals especially polyphenols have received increasing attention because of interesting new discoveries considering their biological activities (Cho *et al.,* 2003). In this study, a number of phytochemical are present in the extract of this species and they constitute a major group of compounds that act as primary antioxidants with high redox potentials and singlet oxygen quenchers (Kahkonen *et al.,* 1999). Patients with diabetes mellitus are characterized with elevated level of oxidative damage, decreased level of antioxidant defenses and are prone to lipid abnormalities due to lipid peroxidation (Asayama *et al.,* 1993). The current work revealed that *L. hastata* leaf contain substantial amount of polyphenols although, the percentage composition differs depending on the extraction solvent, probably due to differences in polarity. Several studies indicated that some African and Chinese medicinal plants possess more potent antioxidant activity than common fruits and vegetables (Redmond, 2009) and phenolic compounds were linked with antioxidant activity of the plants (Atawodi, 2005; Li *et al.,* 2010). Thus improved health and nutrition can be achieved not only from the consumption of fruits and vegetables with high antioxidant capacities, but also from medicinal herbs and plants (Atawodi, 2005). Reasonable amount of the polyphenols observed in this study, is an indication of the potential of *L. hastata* in the management of diseases related to the accumulation of free radicals in the body including diabetes mellitus. According to Li (2010), phenolic compounds possess multiple biological properties such as antitumor, antimutagenic and antibacterial properties and these activities might be related to their antioxidant activity.

Digestion of starch can be delayed by feeding on foods containing compounds that inhibit intestinal carbohydrates hydrolyzing enzymes (Lehmann and Robin, 2007). Drugs such as arcabose, miglitol and voglibose also perform this task. Several plants were known to have hypoglyceamic property via the inhibition of carbohydrates hydrolyzing enzymes. Studies indicated that other members of the family asclepiadaceae such as *Mondia whtei*, *Gymnema sylvestre*, and *G. montanum*, possess potent antioxidant and antidiabetic potentials due to their possession of polyphenolic compounds and their ability to inhibit the activity of α- glucosidase, α-amylase and lipases (Oben *et al*., 2010; Kunga *et al*., 2010; Anon, 2011). The result of this study indicates that the extracts may possess significant inhibitors of α-glucosidase and may thus be effective in reducing postprandial hyperglyce-amia. The significance of this in the management of diabetes mellitus cannot be over emphasized. In addition, the potential of *L. hastata* as a source of lead compound (s) with significant α-glucosidase inhibitory activity is also apparent and thus isolation of these compounds may be of interest.

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