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IN VITRO INHIBITORY EFFECT OF METHANOL, HEXANE AND ETHYLACETATE EXTRACTS OF ALBIZIA LEBBECK (SIRIS TREE) STEM BARK ON THE ACTIVITY OF ALPHA GLUCOSIDASE

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Abstract

Alpha glucosidase is a potential target enzyme in the management of diabetes mellitus. Diabetes mellitus (DM) is a metabolic disorder caused by defect in insulin secretion, insulin action, or both. Insulin deficiency as such results to a chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. The current work studied the *in vitro* inhibitory potentials of stem bark extracts of *Albizia lebbeck* on yeast α-glucosidase activity using p-nitrophenyl-α-D-glucopyronaside (PNPG) as substrate. The Stem Bark of *Albizia lebbeck* was collected from the university premises of Usmanu Danfodiyo University Sokoto Nigeria. The stem bark was extracted with methanol and further with hexane and ethyl acetate fractions using separation funnel. Crude α-glucosidase was obtained from yeast (*Saccharomyces cereviceae*). The hexane and methanol extracts were tested for inhibitory potentials on α-glucosidase and the IC_{50} was calculated. The results indicated that at 10, 30 and 50mg/ml, the hexane extract caused 20.65, 36.52 and 61.96% reduction in the activity of α-glucosidase respectively. The methanol extract on the other hand inhibited the activity of the enzyme by 14.36, 24.30 and 31.29% respectively. The IC_{50} was calculated for the hexane and methanol extracts to be 1.68 and 2.92mg/ml respectively. The results of this study therefore concluded that hexane and methanol extracts of *Albizia lebbeck* stem bark possesses significant inhibitory effect on α-glucosidase in a dose dependant manner and may therefore have potentials as a source of lead for the development of anti-diabetic drugs.

Keywords: *In vitro, Inhibitory effect, Albizia lebbeck, Alpha glucosidase*

Introduction

Diabetes mellitus (DM) is a metabolic disorder that is caused by defect in insulin secretion, insulin action, or both (Kumar and Clark 2002). The deficiency of insulin as such results into a chronic hyperglycemia with some disturbances of carbohydrate, fat and protein metabolism as well (Beverly et al., 2004). Some complications like vascular and tissue damage occur as the disease increases that may lead to severe diabetic difficulties such as cardiovascular problems, neuropathy, ulceration, retinopathy and nephropathy (Svensson et al., 2010). Diabetes was found to be the most popular known endocrine disorder in the year 2010, it was assumed that above 200 million people globally have DM and about 300 million people are predicted to be affected by the disease in 2025 (WHO, 2013). It was also predicted that from 2010 to 2030, there will be about 69% increase in numbers of adults with diabetes in developing countries and around 20% increase from developing countries (Shaw et al., 2010). In Nigeria, the disease is increasing progressively as a result of rural to urban migrations with some inconsistent sedentary life style. The problem was also related to consumption of refine foods, fast foods and low level of exercise. In many research it had been recommended that, sugars and starch produce an un-expectable range of glycemic and insulinemic responses (Thomas et al., 2006). Other studies also reveals that some carbohydrates like those found in white bread, potatoes and in some species of white rice have the same glycemic indices as that of simple carbohydrates like sucrose (Chinedum, 2016).

A hormone known as insulin is triggered by hyperglycemia when the blood glucose level increases after meal. But the cell becomes hyperglycemic when the blood glucose is not completely absorbed resulting to a condition called diabetes mellitus (James, 2016). Nowadays a substantial proofs showed that more than 100 diseases affecting are caused by the increase of free radicals within the body and the DM is included (Zhang et al. 2015).

A postprandial hyperglycemia is lowered by the inhibition of α-glucosidase enzyme positioned at the intestinal brush boarder (Franco et al., 2002). The most popularly known α-glucosidase inhibitors include: Acarbose, Meglitol and Voglibose. Conventional treatments for the management of DM includes; reducing the demand for insulin using specific enzyme inhibitors such as Acarbose and Meglitol. But, there is a burden of unwanted side effects associated with these drugs such as diarrhea, nausea, dizziness and hypoglycemia (Prabhakar and Doble, 2008).

Many evidences were reported that the medicinal plants have been used as an alternative means of treating several diseases for a very long period of time (Zhang et al. 2015). A study reported that some of the medicinal plants could be used as a remedy for the treatment of diabetes mellitus. These medicinal plants have anti-hyperglycemic activities which play a significant role in bringing back the pancreases resulting to an increase in insulin production or prevents the rate of glucose absorption (Patel et al. 2012).

It was discovered that polyphenols or carbohydrates hydrolyzing enzymes like the green tea polyphenols have, the ability to inhibit the activities of α-glucosidase and sucrose (Hara and Honda, 1993). The berry polyphenols also is capable to inhibit the activity of α-glucosidase and α-amylase (Dougall et al. 2005). Many studies reported that some anti-diabetic plants contains anti-diabetic agents which could be extracted from different parts of the plants (Saidu et al., 2013). Some studies also reported that flavonoids, saponin, amino acids, and glycosides are the anti-diabetic agents present in the plants (Patel et al. 2012).

Albizia lebbeck is widely spread in tropical and subtropical regions of Africa (Ali, 2010). It is popularly known as; flea tree, fry wood, koko and woman's tongue tree. It is also called Siris, though this name may refer to any locally common member of the genus (Ali, 2010). The barks and seeds of this plant are also used by some local people or traditional healers in the treatment of illness such as boils, cough, flu, conjunctivitis, lung problems, pectoral problems, abdominal tumors, inflammation and many more illness (Lipika and Ravindra, 2016). In addition to all these it was reported that the ethanolic stem bark extract of the plant have hypoglycemic and anticancer properties (Ashraf et

al. 2015). This study was aimed to study the in vitro inhibitory potentials of stem bark extracts of Albizia lebbeck on yeast α-glucosidase activity using p-nitrophenyl-α-D-glucopyronaside (PNPG) as substrate.

Methods

Study area/Sampling site

The study was conducted within the premises of Usmanu Danfodiyo University in Sokoto State, North-Western part of Nigeria for a period of July to November in 2017.

Collection of plant material

The Stem Bark of Albizia lebbeck was collected from the university premises of Usmanu Danfodiyo University Sokoto, Nigeria. Identification of the plant was done in herbarium section of the Department of Biological science Usmanu Danfodiyo University Sokoto. The stem bark were air dried in the Undergraduate IV laboratory of the department of Biochemistry Usmanu Danfodiyo University Sokoto, the leaves were pulverized to fine powder using wooden Pestle and Mortar.

Preparation of the plant

Crude methanol extract of the stem bark was prepared by soaking 40g of the powdered sample in 400ml of 100% cold methanol for 24hours with a frequent shaking using a laboratory shaker. The mixture was then filtered through a clean white cotton cloth followed by Whatman filter paper to obtain the filtrate after which it was evaporated using rotary evaporator and further concentrated using water bath to obtain the residue. The corresponding percentage yield was calculated (Ref.).

%yield= (weight of extract + container) – weight of container/Initial weight of sample x 100

Solvent extraction of the crude methanol extract

4g of the dried methanol extract was soaked in 20ml of methanol followed by addition of 20ml of hexane in a separation funnel. The mixture was shaken vigorously for 30minutes and the two formed layers were separated in a separate beaker. After the separation, 20ml of ethyl acetate was added to the methanol residue and mixed vigorously for 30minutes. The ethyl acetate extract was obtained in the process described in hexane. All the extracts (hexane and ethyl acetate) were concentrated by drying

Concentration Preparation of extract

After drying, 1.5g, 1g, and 0.5g each of the residues (methanol, hexane, ethyl acetate extracts) respectively were dissolved in 2ml of 10% Dimethyl sulphoxide (DMSO) to obtain the stock solution from which different concentrations (10, 30 and 50mg/ml) of all the extracts were prepared in sodium phosphate buffer.

Alpha-glucosidase

Crude α-glucosidase was obtained from yeast (*Saccharomyces cereviceae*), the yeast was homogenized using pestle and mortar, 10gram of the homogenate was then mixed with 200ml of 0.1M phosphate buffer (pH 6.8) and stirred for 30minutes. It was allowed to settle for 10minutes after which the solution was filtered with Whitman's filter paper into a conical flask surrounded with ice. The filtrate (i.e. the crude extract) was obtained and kept in a refrigerator and the debris was discarded.

Determination of α-glucosidase enzyme inhibition

Alpha-glucosidase inhibition was determined using the method of (Adams *et al.,* 2010; Andrew *et al.,* 2013) with modifications. The reaction mixture contained 1.5ml of 0.1M sodium phosphate buffer at (pH 6.8), and 0.5ml α-glucosidase from beaker yeast (saccharomyces cereviceae). The mixture was incubated at 37°C for 5 minutes. The reaction was activated by the addition of 0.5ml of 5mM p-nitrophenyl-α-D-glucopyranosidase (PNPG) in the presence or absence of 0.5ml of different concentrations of plant extracts (10, 30 and 50mg/ml), then incubated for 20min at 37°C. 1ml of 0.2M Na_2CO_3 was added into the mixture to terminate the reaction. The change in absorbance at 405nm was determined spectrophotometrically.

The % inhibition was calculated as:

$$\frac{ABScontol - ABSsample}{ABScontrol} \mathbf{100}$$

Acarbose 10mg/ml was used as positive control.

Statistical analysis

The IC_{50} value of each extract was determined through a linear regression analysis of the dose response curve using Microsoft Excel.

Results

The % yield of Albizia lebbeck stem bark extracts was presented in table 1. The result indicated that the methanol extract had the highest % yield. The result of inhibitory effect of Albizia lebbeck extracts against α-glucosidase at concentrations of 10, 30 and 50mg/ml are presented in table 2. The result indicated that the effect of the plant extract was dose dependent. Result of IC50 of hexane extract of Albizia lebbeck stem bark against α-glucosidase was presented in figure 1. The result indicated that the IC50 is 1.68mg/ml and the inhibitory effect was dose dependent. Result of IC50 of ethyl acetate extract of Albizia lebbeck stem bark against α-glucosidase was presented in figure 3. The result indicated that the IC50 is 2.30mg/ml and the inhibitory effect was dose dependent. Result of IC50 of methanol extract of Albizia lebbeck stem bark against α-glucosidase was presented in figure 2. The result indicated that the IC50 is 2.92mg/ml and the inhibitory effect was dose dependent.

Discussion

In this study, the inhibitory effect of methanol, hexane and ethyl acetate extracts of Albizia lebbeck stem bark on α-glucosidase was studied. The methanol and hexane extracts were found to possess inhibitory effects with (Table 2).

The methanol extract was observed to have low inhibitory effect (Fig 2) compared to the hexane extract (Fig 1) which shows high inhibitory effect both in a dose dependant manner. The inhibitory effect was in consistent with the result reported by Saidu et al. (2013) on similar plant; Albizia chevaleiri leaf with an inhibition of 82.2% and IC50 of 28µg/ml which was compared with standard anti-diabetic drug; Acarbose and reported that it has higher inhibition than the drug.

The ethyl acetate extract (Fig 3) was observed to be enhancing the activity of the enzyme rather than inhibition following a decrease in inhibitory effect with increase in concentration of the plant extract. The inhibitory effect decreases from 85.63% to 4.30% at 10 and 50mg/ml respectively. This effect may be as a result of reversible inhibition of the enzyme by the substrate product or as a result of presence of some phenolic compounds that stimulate the activity of the enzyme (ref.). Previous reports by Marugan et al. (2010) indicated that isolated pulicarside from Pulicaria undulate have a strong α-glucosidase promoter activity. The activation of α-glucosidase enzyme is related to prolongation of its stability, this may be either shelf stability or operational stability (Marugan et al., 2010). This observation however, does not contradict the results that Albizia lebbeck has inhibitory potential on alpha glucosidase since it may possess other inhibitory mechanisms.

Conclusion

Albizia lebbeck stem bark has inhibitory potential on alpha-glucosidase activity. The hexane extract is the most active solvent extract among the solvents, and the inhibitory effect was concentration dependant. These extracts may therefore be potential sources of α-glucosidase inhibitors which may serve as anti-diabetic agents. At the end of this study, there is possibility to suggest that there are some bioactive compounds present in stem bark of Albizia lebbeck extracts which may be responsible for their α-glucosidase inhibitory activity. However further studies would be required to isolate the bioactive compounds and determine their individual IC50.

References

1. Adam Z., Khamis S., Ismail A., Harmid M. Inhibitory Properties of Ficus deltoidea on α-Glucosidase Activity. Res. J. Med. Plant 2010; 4(2):61-75
2. Ali SI. Flora of West Pakistan, Mimosaceae. Shamim Printing Press. Karachi, Pakistan: 2010; 1–41.
3. Andrew O., Saidu Y., Landan J., Bilbis L., Adam A. α-glucosidase inhibitory potentials of selected anti-diabetics plants used in North-western Nigeria. Journal of Medicinal Plants Research, 2013; 7(12): 2010-18.
4. Ashraf MA, Imam MIW, Naznin AK., Bytul MR., Ranjan KB., Rafiqul MI. Anti-diabetic and antioxidant activities of ethanolic

extract of Semecarpus anacardium (Linn.) bark: BMC Complement Altern Med. 2015; 15: 138. doi: 10.1186/s12906-015-0662-z
5. Beverley B., Eschwège E. Diagnosis and classification of diabetes and impaired glucose tolerance. Textbook of Diabetes. Ed: John C Pickup and Gareth Williams Third edition, 2004; 2: 2.1-2.11.
6. Chinedum OE. The concept of low glycemic index and glycemic load foods as panacea for type 2 diabetes mellitus; prospects, challenges and solutions. Afr Health Sci. 2016; 16(2): 468–479. doi: 10.4314/ahs.v16i2.15
7. Dougall DG., Athaide I., Bottazzo GF., Islet cell antibodies are not specifically associated with insulin dependent diabetes in rural Tanzanian Africans. Diabetes Res Clin Pract; 2005; 9: 219-224.
8. Franco OL., Rigden DJ, Melo FR., Grossi MF. Plant α-amylase inhibitors and their interaction with insect α-amylases structure, function and potential for crop protection. Eur. J. Biochem, 2002; 269,397-412.
9. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and alpha-glucosidase activities by tea polyphenols. Biosci Biotechnol Biochem. 1993; 57:123–124
10. James Norman. Normal Regulation of Blood Glucose: The Important Roles of Insulin and Glucagon: Diabetes and Hypoglycemia. Available from: https://www.endocrineweb.com/conditions/diabetes/normal-regulation-blood-glucose
11. Kumar PJ, Clark M. Textbook of Clinical Medicine. Pub: Saunders (London), 2002; 1099-1121.
12. Lipika DB, Ravindra S. An ethno-botanical study of medicinal plants in Chitrakoot area District Satna, Madhya Pradesh India. International Journal of Botany Studies; 2016; 1(5): 14-18
13. Marugan JJ., Zheng W., Motabar O., Southall N., Goldin E., Sidransky E., et al. Evaluation of 2-thioxo-2,3,5,6,7,8-hexahydropyrimido[4,5] pyrimidin-4(1H)-one analogues as GAA activators. Eur. J. Med. Chem, 2010; 45(5),1880-1897.
14. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on anti-diabetic medicinal plants having insulin mimetic property Asian Pac J Trop Biomed. 2012; 2(4): 320–330.
15. Prabhakar PK, Doble M. (2008). A target based therapeutic approach towards diabetes mellitus using medicinal plants. Cur. Diab. Rev, 2008; 4:291-308.
16. Saidu Y, Nwanchukwu FC, Bilbis LS, Faruk UZ, Abbas AY. Hypoglycemic and Hypolipidemic Effects of Root Extracts of Albizzia Chevalieri in Alloxan Induced Diabetic Rats. Niger. J. Basic App. Sci, 2010: 18(1): 72-78
17. Shaw JE., Zimmet P., Cowie C., Ekoe JM. Classification of Diabetes mellitus and other categories of Glucose intolerance. International Textbook of Diabetes Mellitus, 2010: chapter 1, 3rd Ed., pg 3-14.
18. Svensson M., Eriksson JW, Dahlquisz G. Early glycemic control, age at onset, and development of Microvascular Complications in Childhood-onset type 1 diabetes: a population-based study in northern Sweden. Diabetes Care, 2010; 27:955-962.
19. Thomas J., Nzerue CM, Volcy J, Edeki T. Use of Octreotide to treat prolonged Sulfonylurea-induced Diabetes Mellitus and its treatment of hypoglycaemia in a patient with chronic renal failure. Int J Artif Organs, 2006; 26:86-89.
20. World Health Organization Consultation. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications, Part 1: Diagnosis and classification of Diabetes Mell. Geneva: World Health Organization, 2013.
21. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, et al. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases Molecules 2015, 20, 21138–21156; doi:10.3390/molecules20121975

Table 1: % yield of Albizia lebbeck stem bark extracts.

Extract	Yield (%)
Methanol	3.95
Hexane	2.67
Ethyl acetate	1.50

Table 2: Inhibition of *Albizia lebbeck* stem bark extracts against α-glucosidase

Concentration (mg/ml)	Methanol extract (%)	Hexane extract (%)	Ethyl acetate extract (%)
10	14.36	20.65	85.63
30	24.30	36.52	25.62
50	31.29	61.96	4.30

Figure 1: Plot of inhibitory effect of hexane extract of Albizia lebbeck against alpha glucosidase at concentration of 10, 30 and 50mg/ml showing the IC50.

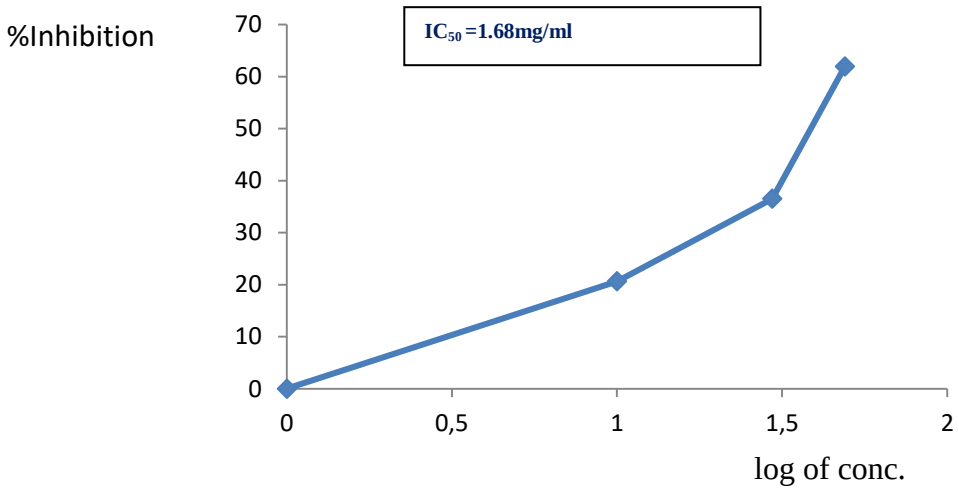

Figure 2: Plot of inhibitory effect of methanol extract of Albizia lebbeck against alpha glucosidase at concentration of 10, 30 and 50mg/ml showing the IC50.

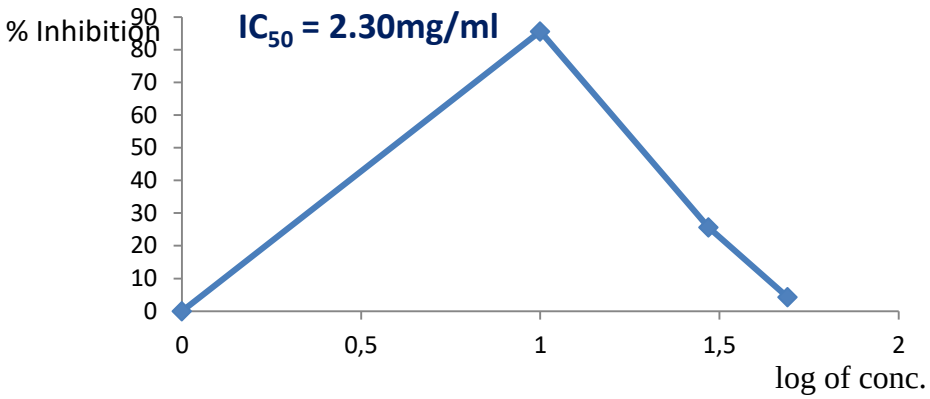

Figure 3: Plot of inhibitory effect of ethyl acetate extract of Albizia lebbeck stem bark against alpha glucosidase at concentration of 10, 30 and 50mg/ml showing the IC50.