IBEROAMERICAN JOURNAL OF MEDICINE
iberoamericanjm

Iberoamerican Journal of Medicine
ISSN: 2695-5075
ISSN-L: 2695-5075
iberoamericanjm@gmail.com
Hospital San Pedro
España

Ojah, Emmanuel Onah; Moronkola, Dorcas Olufunke; Akintunde, Adeniyi-Akee Mukaram
#-amylase and #-glucosidase antidiabetic potential of ten essential oils from Calophyllum inophyllum Linn
Iberoamerican Journal of Medicine, vol. 2, núm. 4, 2020, pp. 253-260
Hospital San Pedro
España

DOI: https://doi.org/10.5281/zenodo.3841108

IBEROAMERICAN JOURNAL OF MEDICINE
iberoamericanjm
Journal homepage: www.iberoamericanjm.tk

Original article

α-amylase and α-glucosidase antidiabetic potential of ten essential oils from *Calophyllum inophyllum* Linn

Emmanuel Onah Ojah[a,*], *Dorcas Olufunke Moronkola*[a,b], *Adeniyi-Akee Mukaram Akintunde*[c]

[a]*Medicinal Chemistry Research Group, Organic Chemistry Unit, Department of Chemistry, University of Ibadan, Ibadan, Nigeria*
[b]*Marine and Biodiversity Center, Chemistry Department, Meston Building, University of Aberdeen, Scotland, United Kingdom*
[c]*Department of Pharmaceutical Chemistry, College of Pharmacy, Igbinedion University, Okada, Edo State of Nigeria, Nigeria*

ARTICLE INFO

Article history:
Received 05 May 2020
Received in 1st revised form 25 May 2020
Received in 2nd revised form 27 May 2020
Accepted 29 May 2020

Keywords:
Diabetes mellitus
Hyperglycemia
Medicinal plants
Calophyllum inophyllum
α-amylase inhibition
α-glucosidase inhibition

ABSTRACT

Introduction: Diabetes mellitus (DM) is a multifactorial metabolic disorder which is of public health concern. Therapeutic intervention using reliable, affordable and non-toxic natural sources is crucial.
Aim of the study: This research was designed to evaluate the α-amylase and α-glucosidase inhibitory activities of ten essential oils from *Calophyllum inophyllum* Linn. The study is part of our local sourcing for natural promising leads to ameliorating diabetes mellitus globally.
Materials and methods: Essential oils from ten parts of *C. inophyllum* Linn were extracted by hydro-distillation using all-glass Clevenger-type apparatus. The percentage yields (w/v) were between 0.219 and 0.506 %. A plot of percentage inhibition versus concentration (mg/mL) of essential oils gave the IC_{50} values for each essential oil using non-linear regression analysis in reference to acarbose a standard anti-diabetic drug.
Results: The following IC_{50} values (mg/mL) were obtained in the determination of α-amylase inhibition: [(Leaf, 0.043±0.05); (Leaf-stalk, 0.044±0.02); (Flower, 0.045±0.05); (Seed, 0.042±0.03); (Pod, 0.040±0.05); (Peel, 0.047±0.09); (Stem wood, 0.047±0.02); (Stem bark, 0.049±0.05); (Root wood, 0.048±0.05) and (Root bark, 0.046±0.04)] compared to acarbose (0.034±0.02). While α-glucosidase assay gave the following IC_{50} values (mg/mL): [(Leaf, 0.044±0.02); (Leaf-stalk, 0.043±0.03); (Flower, 0.044±0.04); (Seed, 0.048±0.02); (Pod, 0.038±0.04); (Peel, 0.048±0.03); (Stem wood, 0.048±0.04); (Stem bark, 0.048±0.02); (Root wood, 0.047±0.04) and (Root bark, 0.045±0.04)] with reference to acarbose (0.032±0.04). The high α-amylase and α-glucosidase inhibitory activity of pod essential oil in comparison with the reference drug must be due to the presence of some impact bioactive phyto-contituents in it.
Conclusion: *C. inophyllum* Linn has been considered a fundamental source of potent anti-diabetic drugs which could be useful in the management of postprandial hyperglycemia.

1. INTRODUCTION

Essential oils (EO) are complex, odoriferous and naturally occurring compounds characterized by high volatility [1]. Pharmacologically, essential oils have been used as insecticide, antimicrobial, antioxidant, pesticide, deodorants and as aroma-therapeutic agents; therefore, the

* *Corresponding author.*
E-mail address: emmanuel.ojah@yahoo.com

http://doi.org/10.5281/zenodo.3841108

possibility of exploring them as anti-diabetic agent is important [2].

Diabetes mellitus (DM) is a chronic endocrine disorder that affects the metabolism of carbohydrates. It includes a group of metabolic diseases characterized by hyperglycemia (HG), in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to production of insulin [4-5]. Out of about 382 million people living with Diabetes world-wide it is estimated that over 20 million people are living with the disease in sub-Saharan Africa. Nigeria has the highest number of people with diabetes with an estimated 3.9 million people of adult population [6]. An effective therapeutic approach to manage diabetes is by decreasing postprandial hyperglycemia (PPHG). It can be achieved by the regulation and/or inhibition of carbohydrate hydrolyzing enzymes like α-amylase and α-glucosidase which are utilized in the digestion of carbohydrates [7]. α-amylase is involved in the breakdown of long chain carbohydrates while α-glucosidase breaks down starch and disaccharides to glucose. These inhibitors are the potential targets utilized in the development of lead compounds for the treatment of diabetes. α-glucosidase, is a very important enzyme in carbohydrates digestion [8]. It catalyzes the 1,4- α-bonds of the unabsorbed oligo- and disaccharides, and converts them into monosaccharides, which are absorbed in the upper jejunum, resulting in PPHG [9, 10]. α-amylase catalyzes the initial hydrolysis of starch and other carbohydrate polymers into shorter oligosaccharides through cleavage of α-1,4- bonds. The salivary isozyme provides an initial partial cleavage into shorter oligomers [11, 12]. Once these partially digested saccharides reach the gut, they are extensively hydrolyzed by the α-amylase synthesized in the pancreas and excreted in the lumen into simpler oligosaccharides, such as maltose, maltotriose and α-limit dextrins [13]. These sugars are eventually broken down into glucose by α-glucosidases (intestinal brush border), which is in turn absorbed from the intestinal mucosa into the portal blood, by means of the glucose transporter and sodium-glucose co-transporter, leading to postprandial hyperglycaemia [14]. Impaired regulation of PPHG constitutes a common feature in type 2 diabetes mellitus (T2DM), the most prevalent form of diabetes and accounting for about 90 % of all diabetes cases [15, 16]. After a meal, α-amylase synthesized in the pancreas and released in the duodenum, catalyzes the hydrolysis of α-1,4 glycosidic linkages in partially hydrolyzed starch (amylopectin and amylose). From this reaction, intermediate unbranched, such as maltose and maltotriose, and branched (α-limit dextrins) oligosaccharides are formed. α-glucosidase present in the brush border of the intestinal epithelium (enterocytes) is responsible for the final step of carbohydrates digestion, prior to their absorption. This enzyme converts the disaccharides and oligosaccharides into glucose, which is then transported by sodium/glucose co-transporter 1 (SGLT1) from the intestinal lumen to the cytosol of enterocytes. In turn glucose transporter 2 (GLUT2), found in the basolateral membrane of enterocytes, transports glucose from cytosol to blood via facilitated diffusion. The pancreatic α-amylase activity has been targeted for inhibition by means of the so-called starch blockers in order to mitigate PPHG [17]. Acarbose is the most widely prescribed α-amylase and α-glucosidase inhibitor, and in spite of its efficiency in the control of PPHG, the administration of this drug is accompanied by gastrointestinal adverse effects in diabetic patients such as; abdominal distention, flatulence and diarrhea [18]. Thus, the search and development of effective and safer therapeutic agents, able to control glucose levels without deleterious side effects is urgent for the management of type II Diabetes mellitus [19].

Medicinal plants have been extensively applied in the treatment of diverse disease conditions, especially in developing economies where resources, affordability and access to modern treatment is a challenge. Volatile and non-volatile phytochemicals in medicinal plants possess several pharmacological and biological properties which have been the focus of researches targeted at prospection of reliable, affordable and potent drugs [20-22]. These constituents could be found in plant extracts or essential oils with great activity for different therapeutic applications [23, 24]. Thus, plant based natural inhibitors of α-amylase and α-glucosidase could be developed as phyto-therapeutic agents for the treatment of diabetes involving the decrease in postprandial hyperglycemia by inhibiting conversion of carbohydrate into glucose and then its absorption from the intestine. This inhibition reduces glucose absorption through delayed carbohydrate digestion and extended digestion time [25].

The genus *Calophyllum* comprises of 180 to 200 species of which *C. inophyllum* Linn is the most abundant species. It is widespread in tropical areas, which tolerates varied kinds of soil such as coastal sand, clay or even degraded soil [26]. The plant possesses a wide variety of uses ranging from traditional, medicinal and industrial applications; the wood has been used in general construction and boat building, as well as for flooring, furniture, musical instruments, handicrafts, and a variety of other purposes [27]. Several species of this genus are known to be used in folk medicine [28]. The extracted oil from the fruit is used as a remedy for sciatica, shingles, neuritis, rheumatism, ulcers, and skin diseases; while seed oil is reported to have medicinal and healing properties [29]. Decoction from dried leaves is widely used in curing rheumatism, skin infections, cuts and sores [30]. Extracts from leaves and stem bark expressed antihyperglycemic and antihyperlipidemic activities [31], while leaf extract was identified to inhibit oxidative stress [32]. Its fruits are effectively utilized in the treatment of dermatitis bark is locally utilized for treating vaginal disorders after childbirth, the passing of blood, gonorrhea, and internal haemorrhages [33]. The broad spectrum of biological activities expressed by *C. inophyllum* has been associated with the chemical composition of its different parts. The root is furnished with xanthones such as brasilixanthone,

1,3,5-trihydroxy-2-methoxy-xanthone, caloxanthone A, pyranojacareubin, caloxanthone B and tovopyrifolin [34]. The genus *Calophyllum* has been reported to be rich in coumarins [35], triterpenoids [36], and flavonoids [37]. Several coumarins isolated from two *Calophyllum* species were found to inhibit HIV-1 replication and cytophaticity activities. Xanthone derivative obtained from the root bark of *C. inophyllum* Linn has been identified as antimicrobial and cytotoxic agent [38]. Five bioactive compounds isolated from *C. inophyllum* Linn leaves namely; mixture of calophyllic and isocalophyllic acids, 3-oxo-friedelin-28-oic acid, canophyllic acid, amentoflavone, and shikimic acid showed dose-dependent lipid-lowering activity in *in-vivo* experiments [39]. Calophyllolide a complex coumarin from *C. inophyllum* Linn was reported as an anticoagulant and anti-inflammatory agent [40]. The plant has also been identified as a good anticancer agent [41]. Ojah et al, identified the chemical constituents of ten essential oils extracted from *C. inophyllum* Linn by GC-MS analysis. The study revealed that the plant is furnished with monoterpenes, sesquiterpenes and their oxygenated analogs [42]. Although some phytochemical constituents from the plant have been reported and volatile chemical constituents in the plant have been characterized in previous studies, no study has been performed on the α-amylase and α-glucosidase inhibitory potential of essential oils from different parts of the plant.

The aim of this study is to evaluate the α-amylase and α-glucosidase inhibitory activities of ten essential oils from *C. inophyllum* Linn.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Fresh samples of *C. inophyllum* Linn were collected from the Botanical garden, University of Ibadan, Ibadan, Oyo State, Nigeria. The samples were authenticated in the Herbarium, Department of Botany, University of Ibadan, Nigeria, where voucher samples were deposited with specimen voucher number UIH - 22659. The collection of the samples was done during the daytime. The plant was sorted into ten parts: leaf, stalk, flower, seed, pod, peel, stem wood, stem bark, root wood, and root bark.

2.2. EXTRACTION OF ESSENTIAL OILS BY HYDRODISTILLATION

Each separated part (leaf, stalk, flower, seed, pod, peel, stem wood, stem bark, root wood, and root bark) of *C. inophyllum* Linn was air-dried, pulverized and hydro-distilled for 3 hours in an all-glass Clevenger-type apparatus designed to British Pharmacopeia (BP) specifications. Essential oils were procured in 0.219 to 0.560% yields. Each of the oils had a distinct characteristic pleasant smell. The essential oils were refrigerated until the assay was carried out.

2.3. EXTRACTION OF WHEAT ALPHA-AMYLASE

500 g of malted whole wheat flour was added slowly with mild stirring to 1 L of 0.2 % calcium acetate solution at room temperature and continuously stirred for 2 hours on a stirrer. The suspension was then centrifuged at 40 °C at 12000 g for 10 minutes. The resultant clear brown supernatant was stored at 2 °C to 3 °C prior to heat treatment. Since β-amylase interferes with the enzymatic determination of α-amylase, it was inactivated by heating the extract at 70 °C for 15 minutes. α-amylase is resistant to inactivation by this treatment at pH between 6.5 and 8.0. The pH of the extract was first adjusted to 6.6 with cold ammonium hydroxide (4%). Heat treatment was carried out at 85 °C to 90 °C and other at 72 °C to 74 °C using a water bath with continuous stirring. The extract was then cooled to 2 °C to 3 °C until use [43].

2.4. DETERMINATION OF ALPHA-AMYLASE INHIBITION ACTIVITY

The assay mixture containing 200 µL of sodium phosphate buffer (0.02 M), 20 µL of enzyme (0.25 mg/mL) and the essential oils over a concentration range 20-100 µg/mL were incubated for 10 minutes at room temperature followed by addition of 200 µL of starch (5 mg/mL) in all test tubes. The reaction was terminated with the addition of 400 µL DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 mL of distilled water and absorbance was measured at 540 nm using a Shimadzu UV-2000i double beam spectrophotometer. The control samples were prepared without the essential oils. The inhibition (%) was calculated according to the following formula (Eqn. 1)

$$\alpha - \text{amylase inhibition } (\%) = \frac{\textbf{Abs } 540 \text{ (control)} - \textbf{Abs } 540 \text{ (EO)}}{\textbf{Abs } 540 \text{(control)}} * 100$$

Where:

Abs_{540} (control) is the absorbance of the control at wavelength 540 nm.

Abs_{540} (EO) is the absorbance of essential oils at wavelength 540 nm.

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference α-amylase inhibitor. All tests were performed in triplicate [44].

2.5. DETERMINATION OF YEAST ALPHA-GLUCOSIDASE INHIBITION ACTIVITY

p-Nitrophenyl-α-D-glucopyranoside, acarbose, baker's yeast alpha glucosidase was dissolved in 100 mM phosphate buffer (pH 6.8) and used as the enzyme extract. *p*-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Essential oils were used in the concentration

ranging from 0.02 to 0.1 mg/mL. Different concentrations of plant extracts were mixed with 320 µL of 100 mM phosphate buffer (pH = 6.8) at 30 °C for 5 minutes. 3 mL of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm using a Shimadzu UV-2000i double beam spectrophotometer. The control samples were prepared without any essential oil. The inhibition (%) was calculated according to the formula below (Eqn. 2):

$$\alpha - \text{glucosidase inhibition } (\%) = \frac{\text{Abs 410 (control)} - \text{Abs 410 (EO)}}{\text{Abs 410(control)}} * 100$$

Abs_{410} (control) is the absorbance of the control at wavelength 410 nm.
Abs_{410} (EO) is the absorbance of essential oils at wavelength 410 nm.
The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the positive control and all tests were performed in triplicate [45].

3. RESULTS AND DISCUSSION

Several medicinal plants have been reported to possess anti-diabetic activity and hence, the use of herbal drugs as complementary and alternative therapy to existing medications for the treatment of diabetes mellitus. Development of α-amylase and α-glucosidase inhibitor from natural products such as medicinal plants has been considered as a unique opportunity for a more economic management of diabetes. In recent years the popularity of alternative medicine has increased geometrically for various reasons ranging from potency to affordability [46]. *Pterocarpus soyauxii* a unique Nigerian medicinal plant has been identified as a potent anti-diabetic agent [47]. Aqueous extract of *Salacia oblonga* has been also identified as a good α-amylase and α-glucosidase inhibitor [48]. Hence the need to explore more plants with good anti-diabetic activity.

3.1. PERCENTAGE YIELD OF ESSENTIAL OILS FROM *C.INOPHYLLUM* LINN

Essential oils obtained from ten (10) parts of *C. inophyllum* Linn gave characteristic odours and colours. The oils were procured in 0.219 to 0.506 % yields (Table 1), with the highest yield from the peel, which gave 0.560 %, while the root had the lowest yield (0.219%), which may be due to its high fiber content.

3.2. ALPHA-AMYLASE INHIBITION OF *C.INOPHYLLUM* LINN

The percentage inhibition obtained for the standard anti-diabetic drug acarbose was relatively high for the concentration range used (0.1-0.02 mg/mL) as indicated on Table 2. Maximum percentage inhibition of 95.7 % was obtained for acarbose at 0.1 mg/mL and decreased slightly to 59.65 % at 0.02 mg/mL. The ten oils (Leaf, Leaf-stalk, Flower, Seed, Pod, Peel, Stem wood, Stem bark, Root wood, and Root bark) exhibited concentration-dependent inhibition similar to acarbose the standard anti-diabetic drug used. Percentage inhibition of the standard drug (95.68 %) was in close range with pod essential oil with inhibition efficiency of 80.32% at 0.1 mg/mL (Table 2). A graph of percentage inhibition versus concentration (mg/mL) of essential oils was plotted from which the IC_{50} values were obtained for each fraction using linear regression analysis in reference to the central standard. An inverse relationship exists between the percentage inhibition efficiency and the IC_{50} values. The higher the IC_{50} value the lower the activity of the essential oils and vice versa. The following IC_{50} values were obtained in the determination of α-amylase inhibition: [(Leaf, 0.043±0.05 mg/mL); (Leaf-stalk, 0.044±0.02 mg/mL); (Flower, 0.045±0.05 mg/mL); (Seed, 0.042±0.03 mg/mL); (Pod, 0.040±0.051 mg/mL); (Peel, 0.047±0.04 mg/mL); (Stem

Table 1. Percentage (%) yield of ten essential oils from *C. Inophyllum* Linn

Plant parts	% yield (weight/volume)	Color	Odor
Leaf	0.333	Pale Yellow	Leafy
Leaf-stalk	0.313	Colorless	Herbal
Flower	0.288	Colorless	Floral
Seed	0.305	Cloudy white	Pleasant
Pod	0.506	Pale Red	Nut-like
Peel	0.560	Pale Yellow	Fruity
Stem wood	0.341	Pale Yellow	Woody
Stem bark	0.307	Colorless	Nut-like
Root wood	0.219	Pale Red	Woody
Root bark	0.279	Pale Red	Nut-like

Table 2. Percentage α-amylase inhibition of essential oils from *C. Inophyllum* Linn

Plant parts	Concentration (mg/mL)				
	0.1	0.08	0.06	0.04	0.02
Acarbose (standard)	95.68	83.54	72.34	65.87	59.65
Leaf	76.33	71.96	65.04	58.69	51.76
Leaf-stalk	72.80	68.74	64.78	58.57	52.34
Flower	74.98	70.81	65.90	61.96	54.69
Seed	73.86	65.98	64.80	56.97	49.87
Pod	80.32	73.33	69.40	60.73	55.22
Peel	72.16	67.74	61.77	56.63	51.94
Stem wood	70.58	66.81	63.99	58.39	50.26
Stem bark	73.46	65.11	61.31	54.54	50.21
Root wood	72.99	66.76	60.95	55.17	52.72
Root bark	71.86	67.91	62.45	59.13	53.22

wood, 0.047±0.02 mg/mL); (Stem bark, 0.049±0.05 mg/mL); (Root wood, 0.048±0.05 mg/mL) and (Root bark, 0.046±0.04 mg/mL)] compared to the standard anti-diabetic drug acarbose (0.034±0.02 mg/mL). The standard anti-diabetic drug with the lowest IC_{50} value of 0.034±0.05 mg/mL exhibited the highest α-amylase inhibition activity followed closely by Pod essential oil (0.040±0.05 mg/mL) as indicated on the bar chart in Figure 1. The least activity was expressed by the highly fibrous stem bark essential oil 0.049±0.01 mg/mL. The high α-amylase inhibition of the pod essential oil in comparison with the standard anti-diabetic drug must be due to the presence of some important bioactive phyto-contituents.

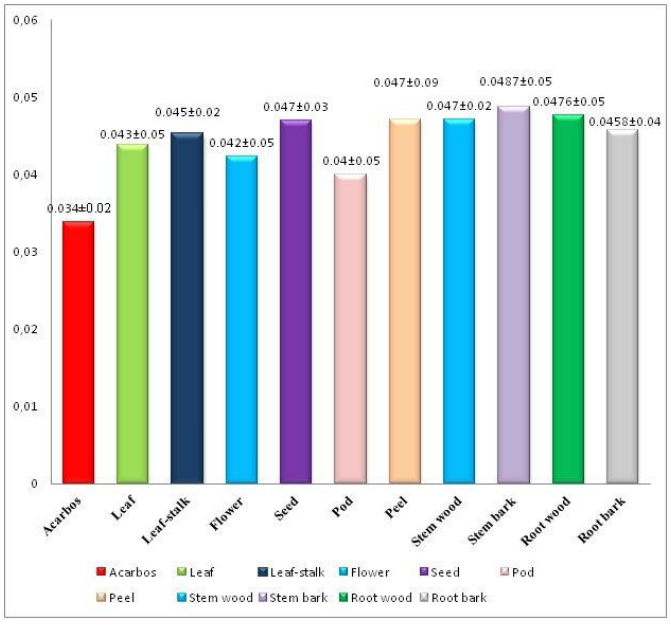

Figure 1: *IC_{50} (mg/mL) values from α-amylase inhibition of Calophyllum inophyllum Linn. Data were analyzed by non-linear regression analysis on MS Excel from the mean inhibitory data. IC_{50} - Concentration of essential oil that scavenged 50% of acarbose.*

3.3. ALPHA-GLUCOSIDASE INHIBITION OF *C.INOPHYLLUM* LINN

The percentage inhibition obtained for the standard anti-diabetic drug acarbose was relatively high for the concentration range used (0.1-0.02 mg/mL). Optimum percentage inhibition of 96.34% was obtained for acarbose at 0.1 mg/mL and decreased slightly to 60.28 % at 0.02 mg/mL. This trend indicates that the percentage inhibition of the standard used in the study was concentration-dependent. The ten oils (Leaf, Leaf-stalk, Flower, Seed, Pod, Peel, Stem wood, Stem bark, Root wood, and Root bark) exhibited concentration-dependent inhibition similar to acarbose the standard anti-diabetic drug used. Percentage inhibition of the standard was in close range with the pod essential oil with inhibition efficiency of 80.32 % at 0.1 mg/mL as indicated in Table 3. A graph of percentage inhibition versus concentration (mg/mL) of essential oils was plotted from which the IC_{50} values were obtained for each essential oil using linear regression analysis in reference to the central standard. An inverse relationship exists between the percentage inhibition efficiency and the IC_{50} values. The higher the IC_{50} value the lower the activity of the essential oils and vice versa. The following IC_{50} values were obtained in the determination of α-amylase inhibition: [(Leaf, 0.044±0.02 mg/mL); (Leaf-stalk, 0.043±0.03 mg/mL); (Flower, 0.044±0.04 mg/mL); (Seed, 0.048±0.02 mg/mL); (pod, 0.038±0.04 mg/mL); (Peel, 0.048±0.03 mg/mL); (Stem wood, 0.048±0.04 mg/mL); (Stem bark, 0.048±0.02 mg/mL); (Root wood, 0.047±0.04 mg/mL) and (Root bark, 0.045±0.04 mg/mL)] compared to the standard anti-diabetic drug acarbose (0.032±0.04 mg/mL). The standard anti-diabetic drug with the lowest IC_{50} value of 0.032±0.04 mg/mL exhibited the highest α-amylase inhibition activity followed closely by the pod essential oil (0.038±0.01 mg/mL) as indicated on Figure 2. The least activity was expressed by the Seed oil (0.048±0.02 mg/mL).

Table 2. Percentage α-glucosidase inhibition of essential oils from *C. Inophyllum* Linn					
Plant parts	**Concentration (mg/mL)**				
	0.1	**0.08**	**0.06**	**0.04**	**0.02**
Acarbose (standard)	96.34	85.80	74.11	68.50	60.28
Leaf	74.18	72.44	65.09	59.67	52.46
Leaf-stalk	74.48	69.45	65.94	59.08	55.14
Flower	73.05	71.11	64.02	60.94	53.63
Seed	72.42	64.06	62.34	55.92	50.82
Pod	82.05	75.73	70.90	62.74	56.42
Peel	73.46	67.43	62.79	56.69	52.90
Stem wood	72.50	66.81	61.99	57.33	50.45
Stem bark	74.09	65.18	62.39	55.57	51.71
Root wood	72.16	65.56	61.45	56.66	53.04
Root bark	73.85	68.96	63.48	58.84	53.08

3.4. ALPHA-AMYLASE AND GLUCOSIDASE INHIBITION OF *C.INOPHYLLUM* LINN

The pod essential oils showed peak α-amylase and α-alpha glucosidase activity while essential oils from other parts showed appreciable inhibition activity. The high inhibition efficiency expressed by pod essential oils in both the α-amylase and α-glucosidase inhibition studies must be triggered by the presence of bioactive constituents. Generally, the alpha amylase and alpha glucosidase inhibition of all parts of *C. inophyllum* Linn were concentration dependent. The high α-amylase and α-glucosidase inhibition of the pod essential oils in comparison with the standard anti-diabetic drug must be due to the presence of some impact bioactive phyto-contituents in the plant. Results obtained for α-amylase and α-glucosidase inhibition are consistent with values reported earlier in literature [49, 50].

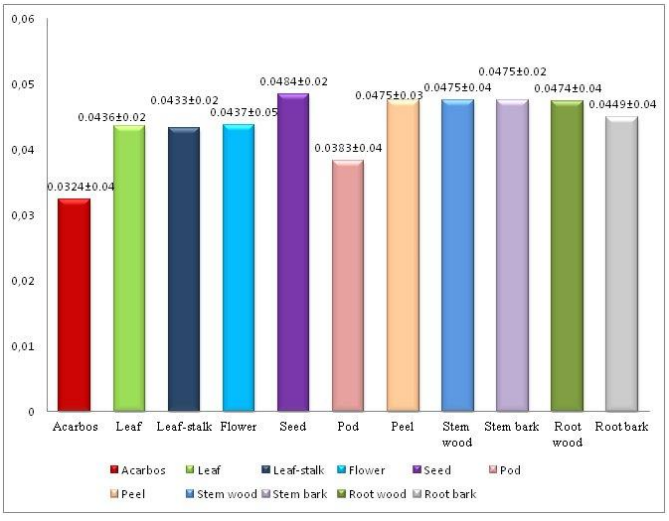

Figure 2: *IC_{50} (mg/mL) values from α-glucosidase inhibition of Calophyllum inophyllum Linn. Data were analyzed by non-linear regression analysis on MS Excel from the mean inhibitory data. IC_{50} - Concentration of essential oil that scavenged 50% of acarbose.*

4. CONCLUDING REMARKS

The α-amylase and α-glucosidase inhibitory activities of *C. inophyllum* Linn revealed that the plant could be an interesting component against postprandial hyperglycemia and other diseases associated with diabetes mellitus. Future researches are necessary to confirm this observational study.

5. RECOMMENDATIONS

- It is highly recommended that bio-assay guided isolation and characterization of non-volatile components from various parts of *C. inophyllum* Linn should be carried out to identify specific compounds responsible for the activities observed in this study.
- *In vivo* study should be carried out on volatile and non-volatile components of this plant prior to clinical study.

6. ACKNOWLEDGEMENTS

We acknowledge the use of J-Organic Chemistry research laboratory facilities, Department of Chemistry, University of Ibadan, Ibadan, Nigeria in plant extractions, preparation and *in-vitro* antidiabetic studies.

7. REFERENCES

1. Maedeh M, Hamzeh I, Hossein D, Majid A, Reza RK. Bioactivity of Essential Oil from Satureja hortensis (Laminaceae) against Three Stored-Product Insect Species. African J. Biotech. 2011;10(34):6620-7. doi: 10.5897/AJB11.469.

2. Buchbauer G. The Detailed Analysis of Essential Oils Leads to the Understanding of Their Properties. Perfum Flavor. 2000;25:64-7. doi: 10.3923/rjphyto.2011.66.69.

3. Ayat A, Mohamad EA, Mohamad JK, Foroogh N. In vitro α-amylase and α-glucosidases inhibitory effects of some plant extracts. Int J. Pharmacogn Phytochem Res. 2015;7(2):315-8. doi: 10.4103/0973-1296.166018.

4. Laoufi H, Benariba N, Adjdir S, Djaziri R. In vitro α-amylase and α-glucosidase inhibitory activity of Ononis angustissima extracts. J. App. Pharm. Sci. 2007;7(2):191-8. doi: 10.7324/JAPS.2017.70227.

5. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010;87(1):4-14. doi: 10.1016/j.diabres.2009.10.007.

6. Dahiru T, Alhaji A, Aliyu A, Shehu A. A review of Population based Studies on Diabetes Mellitus in Nigeria. Sub-saharan Afr J Med. 2016;3(2):59-61.

7. Xiao JB, Hogger P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. Curr Med Chem. 2015;22(1):23-38. doi: 10.2174/0929867321666140706130807.

8. Li YQ, Zhou FC, Gao F. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. J Agric Food Chem. 2009;57(24):11463-8. doi: 10.1021/jf903083h.

9. Proença C, Freitas M, Ribeiro D, Oliveira EFT, Sousac JLC, Tomé SM et al. α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. J Enzyme Inhib Med Chem. 2017;32(1):1216-28. doi: 10.1080/14756366.2017.1368503.

10. Zeng L, Zhang G, Liao Y, Gong D. Inhibitory mechanism of morin on α-glucosidase and its anti-glycation properties. Food Funct 2016;7: 3953-63. doi: 10.1039/c6fo00680a.

11. Janeček Š, Svensson B, MacGregor EA. α-Amylase: an enzyme specificity found in various families of glycoside hydrolases. Cell Mol Life Sci. 2014;71(7):1149-70. doi: 10.1007/s00018-013-1388-z.

12. Sales PM, Souza PM, Simeoni LA. A-amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci. 2012;15(1):141-83. doi: 10.18433/j35s3k.

13. Brayer GD, Sidhu G, Maurus R, Rydberg EH, Braun C, Wang Y, et al. Subsite mapping of the human pancreatic α-amylase active site through structural, kinetic, and mutagenesis techniques. Biochemistry. 2000;39(16):4778-91. doi: 10.1021/bi9921182.

14. Patel H, Royall PG, Gaisford S, Williams GR, Edwards CH, Warren FJ, et al. Structural and enzyme kinetic studies of retrograded starch: inhibition of α-amylase and consequences for intestinal digestion of starch. Carbohydr Polym. 2017;164:154-61. doi: 10.1016/j.carbpol.2017.01.040.

15. International Diabetes Federation. IDF Diabetes Atlas. 8th ed. 2017.

16. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes. Diabetes Care. 2018;41(Suppl 1):S13-S27. doi: 10.2337/dc18-S002.

17. Butterworth PJ, Warren FJ, Ellis PR. Human α-amylase and starch digestion: an interesting marriage. Starch-Stärke. 2011;63:395-405. doi: https://doi.org/10.1002/star.201000150.

18. Etxeberria U, de la Garza AL, Campion J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opin Ther Targets. 2012;16(3):269-97 doi: 10.1517/14728222.2012.664134.

19. Kahn SE, Cooper ME, Del-Prato S. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet. 2014;383(9922):1068-83. doi: 10.1016/S0140-6736(13)62154-6.

20. Mohammadhosseini M, Akbarzadeh A, Hashemi-Moghaddam H, Mohammadi Nafchi A, Mashayekhi HA, Aryanpour A. Chemical composition of the essential oils from the aerial parts of Artemisia sieberi by using conventional hydrodistillation and microwave assisted hydrodistillation: A comparative study. J Essent. Oil-Bear Plants 2016;19(1):32-45. doi: https://doi.org/10.1080/0972060X.2015.1119067.

21. Mohammadhosseini M, Sarker SD, Akbarzadeh A. Chemical composition of the essential oils and extracts of Achillea species and their biological activities: A review. J. Ethnopharmacol. 2017;199;257-315 doi: 10.1016/j.jep.2017.02.010.

22. Aidi-Wannes W, Mhamdi B, Saidani-Tounsi M, Marzouk B. Lipid and volatile composition of borage (Borago officinalis L.) leaf. Trends Phytochem Res. 2017;1(3):143-8.

23. Camilo CJ, Alves Nonato CDF, Galvão-Rodrigues FF, Costa WD, Clemente GG, Sobreira Macedo MAC, et al. Acaricidal activity of essential oils: a review. Trends in Phytochem Res. 2017;1(4):183-98.

24. Ganesan K, Xu B. Ethnobotanical studies on folkloric medicinal plants in Nainamalai, Namakkal District, Tamil Nadu, India. Trends Phytochem Res. 2017;1(3):153-68.

25. Shimabukuro M. Higa N, Chinen I, Yamakawa K, Takasu N. Effects of a single administration of acarbose on postprandial glucose excursion and endothelial dysfunction in type 2 diabetic patients: a randomized crossover study. J Clin Endocrinol Metab. 2006;91(3):837-42. doi: 10.1210/jc.2005-1566.

26. Dweck AC, Meadowst T. Tamanu (Calophyllum inophyllum) – The African, Asian, Polynesian and Pacific Panacea. Int J Cosmetic Sci. 2002;24(6):341-8. doi: 10.1046/j.1467-2494.2002.00160.x.

27. Al-Jeboury FS, Locksley HD. Xanthones in the heartwood of Calophyllum inophyllum; A geographical survey. Phytochemistry. 1971;10(3):603-6. doi: https://doi.org/10.1016/S0031-9422(00)94704-6.

28. Dharmaratne HRW Napagoda MT, Tennakoon SBP. Xanthones from root of Calophyllum thwaitesii and their bioactivity. Nat Prod Res. 2009;23(6):539-45. doi: 10.1080/14786410600899118.

29. Burkhil HM. The useful plants of west tropical Africa. Families E-I. 2nd ed. XX Royal Botanic Gardens Kew. 1994;2:522.

30. Uma SM., Murthy PN, Sahoo KS, Sahu KC. Formulation and evaluation of herbal tablet containing methanolic extract of Calophyllum inophyllum. Int J Pharm. 2012;2(1):181-6.

31. Silpa S, Fathima A, Shakeel I. Phytochemical screening and evaluation of anti-hyperglycemic and anti-hyperlipidimic activity of methanolic extracts of Calophyllum inophyllum on Albino Wistar Rats Int J Adv Res. 2014;2(8):743-52.

32. Varsha G, Uma MB, Ramasamy M, Karunanithi M. Effect of Ethanolic Extract of Calophyllum inophyllum Leaves on Oxidative stress Complications in Mouse Model. Asian J Pharm Clin Res. 2016;9(3):250-2.

33. Um YL Jo YW. Inhibitory effects of Calophyllum inophyllum extract on atopic dermatitis induced by DNCB in mouse. Am J Phytomed Clin Therap. 2016;4(6):165-73.

34. Kijjoa A, Gonzalez TG, Pinto MM, Silva AM, Anantachoke C, Herz W. Xanthones from Calophyllum teysimannii var. inophylloide. Phytochemistry. 2000;55(7):833-6. doi: 10.1016/s0031-9422(00)00289-2.

35. Ee GC, Jong VYM, Sukari MA, Rahmani M, Kua ASM. Xanthones from Calophyllum inophyllum. Pertanika J Sci Technol. 2009;17(2):307-12.

36. Govindachari TR, Vishwanathan N, Pai BR, Ramadas Rao U, Srinivasan M. Triterpenes of Calophyllum inophyllum linn. Tetrahedron. 1967;23(4):1901-10. doi: https://doi.org/10.1016/S0040-4020(01)82592-8.

37. Hang NTM, Chien NQ, Van Hung N. Triterpenes from the leaves of the Vietnamese plant Calophyllum inophyllum L. Tap chi hoa hoc. 2006;44(1):115-8.

38. Patil AD, Freyer AJ, Eggleston DS, Haltiwanger RC, Bean MF, Taylor PB, et al. The inophyllum's novel inhibitors of HIV-1 reverse transcriptase isolated from the Malaysian tree, C. inophyllum Linn. J Med Chem 1993;36(26):4131-8. doi: 10.1021/jm00078a001.

39. Janki P, Atul S, Khanna AK, Bhatia G, Awasthi SK, Narender T. Antidyslipidemic and antioxidant activity of the constituents isolated from the leaves of Calophyllum inophyllum. Phytomedicine. 2012;19(14):1245-9. doi: 10.1016/j.phymed.2012.09.001.

40. Arora RB, Mathur CN, Seth SD. Calophyllolide, a complex coumarin anticoagulant from C. inophyllum Linn. J Pharm Pharmacol 1962;14:534-5. doi: 10.1111/j.2042-7158.1962.tb11133.x.

41. Itoigawa M, Ito C, Tan HT, Kuchide M, Tokuda H, Nishino H, et al. Cancer chemo-preventive agents, 4-phenylcoumarins from Calophyllum. inophyllum. Cancer Lett. 2001;169(1):15-9. doi: 10.1016/s0304-3835(01)00521-3.

42. Ojah EO, Moronkola DO, Pettrelli R, Nzekoue FK, Cappellacci L, Giordani C, et al. Chemical Composition of ten Essential Oils from Calophyllum Inophyllum Linn And their Toxicity against Artemia Salina Eur J Pharm Med Res. 2019;6(12):185-94.

43. Kneen E, Sandsted RM, Hollenbeck CM. Amylase and diastatic activity. Cereal Chem. 1943;20(4):399.

44. McCue PP, Shetty K. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase. Asia Pac J Clin Nutr. 2004;13(1):101-6.

45. Kim YM, Jeong MH, Wang WY, Lee HI, Rhee HI. Inhibitory effect of pine extract on alpha- glucosidase activity and postprandial hyperglycemia. Nutrition. 2005;21(6):756-61. doi: 10.1016/j.nut.2004.10.014.

46. Sulistiyani, Safithri M, Sari YP. Inhibition of α-glucosidase activity by ethanolic extract of Melia azedarach L. leaves. IOP Conf Ser: Earth Environ Sci. 2016;31:12-25. doi: 10.1088/1755-1315/31/1/012025.

47. Ojah EO, Moronkola DO. In-vitro alpha-amylase and glucosidase inhibitory potential of leaf hexane, ethyl acetate and methanol fractions from Pterocarpus soyauxii Taub Trends Phytochem Res. 2020; 4(1):37-44.

48. Chelladurai GRM, Cinnachamy C. Alpha amylase and Alpha glucosidase inhibitory effects of aqueous stem extract of Salacia oblonga and its GC-MS analysis. Braz J Pharm Sci. 2018;54(1):1-10. doi: https://doi.org/10.1590/s2175-97902018000117151.

49. Kazeem MI, Dansu TV, Adeola SA. Inhibitory Effect of Azadirachta indica A. Juss Leaf Extract on the Activities of α-amylase and α-glucosidase. Pak J Biol Sci. 2013;16(21):1358-62. doi: 10.3923/pjbs.2013.1358.1362.

50. Mohamed EA, Siddiqui MJ, Ang LF, Sadikun A, Chan SH, Tan SC, et al. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from Orthosiphon stamineus Benth as anti-diabetic mechanism. BMC Conplement Altern Med. 2012;12:176 doi: 10.1186/1472-6882-12-176.