PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 10(2): 20–26
https://doi.org/10.14719/pst.1809

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Free radical scavenging activity, pancreatic lipase and α-amylase inhibitory assessment of ethanolic leaf extract of *Phyllanthus amarus*

Ogundipe Adebanke[1], Adetuyi Babatunde[2], Iheagwam Franklyn[1],Adefoyeke Keleko[3], Olugbuyiro Joseph[1] & Ogunlana Olubanke[1*]

[1]Department of Biochemistry, Covenant University, Canaanland,Ota, 231119, Ogun State, Nigeria
[2]Department of Natural Sciences, Biochemistry Unit, Precious Cornerstone University, Ibadan, 200223, Oyo State, Nigeria
[3]Independent Researcher, 4 Ilupeju Street, Anisere, Sango, Ota, 231119, Ogun State, Nigeria

*Email: banke.ogunlana@covenantuniversity.edu.ng

OPEN ACCESS

ARTICLE HISTORY

Received: 07 April 2022
Accepted: 28 June 2022

Available online
Version 1.0 : 05 February 2023
Version 2.0 : 01 April 2023

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS etc.
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

CITE THIS ARTICLE

Adebanke O, Babatunde A, Franklyn I, Keleko A, Joseph O, Olubanke O. Free radical scavenging activity, pancreatic lipase and α-amylase inhibitory assessment of ethanolic leaf extract of *Phyllanthus amarus*. Plant Science Today. 2023; 10(2): 20–26. https://doi.org/10.14719/pst.1809

Abstract

Oxidative stress is associated with the pathogenesis of diabetes and obesity. The key enzymes involved in the carbohydrate and lipid metabolisms are α-amylase and pancreatic lipase respectively. The phytochemicals present in medicinal plants are known to be potent free radical scavenging agents and great inhibitors of metabolizing enzymes. *Phyllanthus amarus* leaves have antioxidant, anti-diabetic, and anti-obesity properties. This research is aimed at elucidating the carbohydrate and lipid metabolism inhibitory activities of *P. amarus* leaves through *in-vitro* screening. The phytochemical screening revealed that *P. amarus* is having high phenolic, flavonoid, tannin, and saponin content. The total protein content of *P. amarus* showed 2.720 ± 0.001 mg BSAE/g. Diphenyl-2-picrylhydrazyl (DPPH) assay showed 0.0001mg/mL, Nitric oxide assay revealed 0.452 mg/mL, Ferric Reducing Antioxidant Power (FRAP) assay showed a value of 0.132±0.010 mg RE/g, Total antioxidant capacity (TAC) indicated a value of 2.217±0.002 mg GAE/g and 2,2-azino-bis(3-ethylbenzotiazoline)-6-sulfonic acid (ABTS) inhibitory activity at the peak concentration (1.2 mg/mL) was 40.29%. The enzyme inhibitory assessment showed a decrease in α-amylase activity as the concentration increased while there was a slight decrease in α-lipase activity but a slight increase at 18.75 mg/mL.

Keywords

Diabetes, medicinal plant, obesity, oxidative stress

Introduction

The etiology of diseases is associated with instability between a generation of free radicals and the antioxidizing power of the body (1). Oxidative stress is regarded as instability between Reactive Oxygen Species and antioxidants that results in oxidative damage of nucleic acids, proteins, fats, and carbohydrates (2,3). Medicinal plants have therapeutic agents as they contain phytochemicals with vast medicinal potential which are employed in the prevention, maintenance, and treatment of diseases (4). Herbs are regarded as the major source of pharmaceuticals in the development of drugs and pharmaceuticals used in the treatment of human diseases as a result of the diversity of the phytochemicals contained in them (5).

Diabetes mellitus is reported to be a serious metabolic disorder affecting 285 million people worldwide affecting those in the age group that ranges from 20 to 79, in 2010. By 2030, it is expected to increase up to 439 million, (6). Type-2 diabetes is caused as a result of insulin resistance and it is mainly

characterized by high level of blood glucose (7). Inhibition of amylase is regarded as a major way of maintaining a reduced blood glucose level. Amylase, secreted in saliva and pancreas is responsible for catalyzing 1,4-glycosidic bonds converting polysaccharides into smaller oligosaccharides (maltose). Thus, glucose absorption rate reduction occurs as a result of inhibition of carbohydrate metabolizing enzymes (8). Some of the examples of inhibitors are miglitol, acarbose and voglibose.

Obesity, as defined by World Health Organization (WHO), is the accumulation of abnormal or excessive fat (9). It tends to affect the physiological state of the body. It poses the risk of developing disorders such as cancer (10), poor mental health (11), cardiovascular diseases (12,13), diabetes (14), and musculo-skeletal disorders (15). Natural products constitute inhibitors of pancreatic lipase developed into clinical products as a remedy for obesity. The major phytochemicals such as polyphenols, flavonoids, saponins, and caffeine are contributed to the inhibition of pancreatic lipase. For instance, anthocyanin (phenolic compound) inhibits pancreatic lipase leading to the reduction in body weight as a result of restriction in calorie intake (16, 17, 18, 19).

In developing countries, plants play a vital role in diabetes treatment because there are limited resources. There is a high demand for the use of alternative medicine in diabetes treatment due to the side effects related to the usage of hypoglycaemic agents (20). WHO exerted a expert group on diabetes which further suggested folklore methods of treatment in managing the disease, due to the increased death rate resulting from the problems caused by the adoption of commonly used anti-diabetic therapeutic agents (citation needed). Various natural products are used in the management of diabetes traditionally but it is vital to validate their mechanism of action (21).

One of the medicinal plants used in the treatment of diabetes is *Phyllanthus amarus* which belongs to the family Euphorbiaceae. It is an annual herb (10-15cm in height) and having green smooth tiny seeds. Its medicinal properties are traditionally attributed to the treatment of diabetes, jaundice and obesity. The common names of *P. amarus* are *Iyin-Olobein* in South-Western part of Nigeria. *P. amarus* contain proteins (6.10%), carbohydrates (45.52%), fiber (24.50%) and mineral elements (22). It has high carbohydrate content, particularly polysaccharide which may be responsible for its anti-diabetic activity. Carbohydrate diet causes a reduced level in blood glucose concentration as an aftermath of increased concentration of blood glucose (23). *P. amarus* is reported to have anti-hypertensive, anti-diabetic, anti-inflammatory, anti-microbial, and many more properties including that of being hepatoprotective. The use of ethanolic extract of *P. amarus* leaf for the prevention and treatment of diabetes and obesity need to be scientifically validated (24). Considering the wide range of medicinal importance of *P. amarus* and the increased morbidity and mortality rate among diabetes patients and the prevalence of obesity in growing countries like Nigeria, it is crucial to validate the therapeutic effect of *P. amarus* as a cheap and accessible medicine over conventional drugs.

Several *in-vitro* and *in-vivo* studies have been carried out on the leaves of this plant but there is still a resurgence of interest due to the carbohydrate and lipase inhibitory agents contained in it. The present study is aimed to assess the amylase and lipase inhibitory action of the phytochemical constituents in the ethanolic leaf extract of *P. amarus*.

Materials and Methods

P. amarus leaves as shown in fig 1. were sourced from specific locations in Covenant University (N6^0 39’53.32644” E3^{0}9’35.9496”), Ogun State, Nigeria. The leaves were identified by a botanist in the Department of Biological Sciences, Covenant University. The voucher numbers were deposited at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria.

Fig. 1. *P. amarus* (source: Internet).

Chemicals and Reagent

Chemicals of analytical grade and salts of high purity were bought from reliable sources. The list of chemicals include; acetonitrile, sodium phosphate buffer, pancreatic α-amylase, gallic acid, starch solution, dinitrosalicyclic acid, porcine pancreatic lipase, 4-nitrophenylbutyrate, p-nitrophenyl-α-Dglucopyranoside, porcine pancreatic α-amylase, ascorbic acid, BHA (butylated hydroxyanisole), (2,2-diphenyl-1-picryl-hydrazyl) DPPH reagent, potassium ferricyanide and ferric chloride and acarbose.

Plant Extracts preparation

Fresh leaves of the selected plant were dried in a cool place, an electric blender was used in pulverizing the dried leaves into powdery form. The pulverized form of *P. amarus* (150g) was steeped into 90% ethanol in the ratio of 1:5 (v/v) for 72 hours, followed by filtration. A rotary evaporator was used to concentrate obtained filtrate at 50°C to achieve the solvent-soluble fractions (25).

Assessment of total alkaloid content

Ten percent of 200cm^3 acetic acid was mixed with 2.5g of powdered sample. After a duration of 4 hours, the extract was concentrated to a quarter of its initial volume. Thereafter, 15 drops of concentrated ammonium hydroxide were added to the filtrate until precipitation was finalized. The oven-dried residue was weighed (26).

$$Percentage\ of\ alkaloid\ (\%) = \frac{weight\ of residue}{weight\ o\ sample} * 100$$

……………. (1).

Total phenolic content

An extract (1mg/mL) of 0.5 mL and 2.5 mL Folin–Ciocalteu (FC) Assy reagent (1:10 v/v) were mixed together. A 2 mL volume of sodium carbonate (7.5%) was added after 5 minutes of incubation. Itt was further incubated at 40°C for 30 minutes. Reading was taken using an ultraviolet spectrophotometer at 765 nm. The varying concentration (0.02 - 0.1 mg/mL) of gallic acid and the sample's phenolic content were calculated using the standard curve as mg of gallic acid equivalent (mg GAE/g extract) (25).

Total flavonoid content

An extract (1 mg/mL) of 0.15 mL was added to 2mL of distilled water. After thorough mixing, the same amount of sodium nitrite (5%) was added and was set to incubate for 6 minutes. Further incubation was set up for 5minutes after adding 0.15mL of $AlCl_3$ (10%) . To the resulting incubated mixture 1 mL of Sodium hydroxide (4%) was added and 1.2 mL of distilled water to make up to a final volume of 5 mL. The absorption rate of the mixture was taken at 420 nm. Rutin was used as standard and dH_2O as blank. The total flavonoid content was illustrated as rutin equivalent mg RE/g extract using the standard curve (27).

Total tannin content

This assay was carried out according to the principle of the Folin Ciocalteu method. A sample (1mg/mL) extract of 0.5 mL, 3.75 mL of dH_2O and 0.25 mL of FC reagent (1:10v/v) were mixed together. Finally, 0.5 mL of Na_2CO_3 (35%) was mixed and the absorbance was read at 725nm. Different concentrations (0.1-0.00625 mg/mL) of tannic acid was used and the assay was conducted in triplicate. The plant extract's tannin content was calculated as tannic acid equivalent mg TAE/g extract (25).

Total saponin content

A sample of 0.05 mL and 0.25 mL of dH_2O were added together. A volume of 0.25 mL vanillin reagent of exactly 800 mg was dissolved in 10 mL ethanol and 2.5 mL of sulphuric acid (72%) added to the initial mixture. The absorbance was measured at 544nm after incubation at 60°C for 10 minutes. The standard used was diosgenin and the assay was conducted in triplicate and the values were calculated as diosgenin equivalents (mg DE/g extract) (28).

Total protein content

To a 0.2mL sample 2mL of alkaline copper sulphate ($AlKCuSO_4$) was added and then incubated for 10 minutes. A further incubation was set for 30 minutes after adding 0.2 mL of Folin Ciocalteu reagent. The mixture was measured at 660 nm in a spectrophotometer. BSA (Bovine Serum Albumin) of varying concentrations of 0.05-1 mg/mL was set as a standard for the assay. The values were illustrated as BSA equivalents (mg BSAE/g extract) (29).

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH assay was done following the method according to Iheagwam *et al.* (2020) (citation needed). 0.5 mL DPPH (0.1 mM) was added to 0.5mL of sample and further incubated in dark for 30 minutes. Different concentrations (0.00125-0.000019 mg/mL) of ascorbic acid were used.

$$\%\ inhibition\ of\ DPPH\ scavenging\ activity = \frac{Ac-As}{Ac} * 100$$

…………….(2).

Ferric reducing antioxidant power (FRAP) assay

One mL sample was evenly mixed with a volume of 2.5 mL [$K_3Fe\ (CN)^6$, 1%] and was put in for incubation for 20 minutes at 50°C. Trichloroacetic acid of 2.5mL was pipetted into the resulting mixture, the exact volume was taken off, and later added 2.5mL of dH_2O. The resulting mixture reading was measured at 700 nm in an ultraviolet spectrophotometer after adding 0.5 mL of $FeCl_3$. Ascorbic acid was used as the assay standard (25).

2,2-azino-bis(3-ethylbenzotiazoline)-6-sulfonic acid (ABTS) assay

ABTS radical solution was prepared by a thorough mixing of potassium persulfate (2.45 mmol/L) and $ABTS^*$ (7 mmol/L) in the same proportion. The resulting mixture was kept in the dark for 12-16 hours. One mL of the radical solution was diluted with a required amount of ethanol/methanol (1:89 v/v) to achieve an 0.700 ± 0.200 absorbance value just immediately before the use at 745 nm. Thereafter, 0.1 mL of sample/standard was added to a volume of 3.9 mL ABTS solution and was set to incubate at 25°C for 6 minutes. The absorbance of the final mixture was measured at 745 nm in an ultraviolet spectrophotometer and read against methanol as blank, ABTS solution was used as control, and butylated hydroxytoluene (0.0375-1.2 mg/mL) as standard (30).

$$\%\ inhibition\ of\ ABTS\ scavenging\ activity = \frac{Ac-As}{Ac} * 100$$

…………....(3)

Where Ac implies control's absorbance and As is the absorbance of the plant extract.

Nitric oxide (NO) radical scavenging activity

A 10 mM solution of sodium nitroprusside was prepared and a volume of 2mL was dissolved in 0.5 mL of a pH 7.4 phosphate buffer saline and 0.5 mL of varying concentrations of the sample was added. The mixture was set for incubation for 150 minutes at 25°C. An equal volume of 0.5 mL of the resulting mixture and Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% napthylethylenediamine dichloride were mixed and proceeded for an another incubation period of 30 minutes. The absorbance was measured at 546 nm against a blank (phosphate buffer) and the Griess reagent was set as the control (31).

$$\%inhibition\ of\ \ NO\ scavenging\ activity = \frac{Ac-As}{Ac} * 100$$

…………....(4)

Where Ac implies control's absorbance and As is plant extract's absorbance.

Total antioxidant capacity (TAC)

A volume of 1 mL of phosphomolybdate reagent made up of 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were added to 0.1 mL of extract. After incubating for 90 minutes, at 95°C, the incubated mixture was measured at 695 nm against blank. The control used was gallic acid and distilled water replaced the sample/standard (25).

Carbohydrate Inhibitory assessment

α-amylase solution (2 U/µL in phosphate buffer, 6.8) of 500 µL was mixed with 250 µL of acarbose/sample and was set to incubate at 37°C for 20 minutes. A volume of 250 µL starch solution (1% in pH 6.8 phosphate buffer) was mixed and incubated for 1 hour at 37°C. The mixture was measured in the spectrophotometer at 540 nm against buffer as control and DNSA as blank after 1mL of dinitrosalicyclic acid (DNSA) reagent was mixed and boiled for 10 minutes (32).

$$\%inhibition\ of\ \alpha - \text{amylase}\ activity = \frac{Ac - As}{Ac} * 100 \ldots.(5)$$

Where Ac implies control's absorbance and As is plant extract's absorbance.

Lipase inhibitory assessment

An assay buffer of 164 mL and 6 mL solution of pancreatic lipase were mixed in a 96-well plate. An extract of 20 mL was added and incubated at 37°C for 10 minutes. A 10 mL of the substrate was mixed and further put for incubation at 37°C 15 minutes. The reading of the final mixture was taken at 405 nm (33).

$$\%inhibition\ of\ \text{alpha lipase} = \frac{Ac - As}{Ac} * 100 \ldots \ldots (6)$$

Statistical analysis

The data were analyzed using the GraphPad Instat software (GraphPad Prism Inc., San Diego, CA, USA). All columns of the extracts were compared to the control in an ANOVA with the Dunnet post-test to evaluate levels of significance. The mean SEM ± was used to express all of the data. Values that are significantly different from each other were indicated by P ≤ 0.05 values.

Results

Voucher referencing and extraction yield of the selected plants

This study was conducted with aqueous 90% ethanol extract of the *P. amarus* leaves. The percentage yield of this selected plant (Pa/Bio/H841) was calculated at 23.53%.

Phytochemical Screening and Total Protein Content of P. amarus leaf extract

The ethanolic extract of the leaf showed the presence of phytochemicals stated in Table 1. These phytochemicals include; phenols, flavonoids, tannins, alkaloids and saponins. The leaves of *P. amarus* are reported to have high nutritive value. This study reported the presence of high protein content (2.720 ± 0.001 mg BSAE/g) in the leaf extract.

Table 1. Phytochemical screening and total protein content of *P. amarus*

Phytochemicals and Protein content	*P. amarus*
TPC (mg GAE/g)	0.212 ± 0.001
TFC (mg RE/g)	0.199 ± 0.020
TSC (mg DE/g)	2.335 ± 0.007
TTC (mg TAE/g)	0.143 ± 0.065
TAC (%)	10.00 ± 0.003
TPC (mg BSAE/g)	2.720 ± 0.001

Values are illustrated as means ± standard error of the mean. Different letters in superscripts vary significantly from each other (p>0.05). GAE, RE, DE, TAE, BSAE, TPC, TFC, TSC, TTC, and TAC represent Gallic acid equivalent, Rutin equivalent, Diosgenin equivalent, Tannic acid equivalent, Bovine serum albumin equivalent, Total phenolic content, Total Flavonoid Content, Total Saponin Content, Total Tannin Content, Total Alkaloid Content.

Antioxidant Analysis

To determine the radical scavenging ability of *P. amarus* leaf extract, antioxidant assays such as DPPH, Nitric oxide, FRAP, TAC, and ABTS were carried out. The result of FRAP and TAC showed 0.132 ± 0.01 mg RE/g and 2.217 ± 0.002mg GAE/g respectively as shown in table 2. The inhibitory percentage of ABTS at the highest concentration (1.2 mg/mL) is 40.29%, as shown in fig 2 below. Table 3 shows the 50% inhibitory concentrations (IC_{50}) value DPPH to be0.0001 mg/mL and Nitric oxide value of *P. amarus* as 0.0452 mg/mL. The DPPH IC_{50} value of *P. amarus* showed to have more potent antioxidant power than ascorbic acid (0.0003 mg/mL).

Enzyme Inhibitory assessment

Carbohydrate and lipid inhibitory enzyme assessment illustrated in fig 3 and fig 4 showed that *P. amarus* inhibited α-amylase and pancreatic lipase respectively.

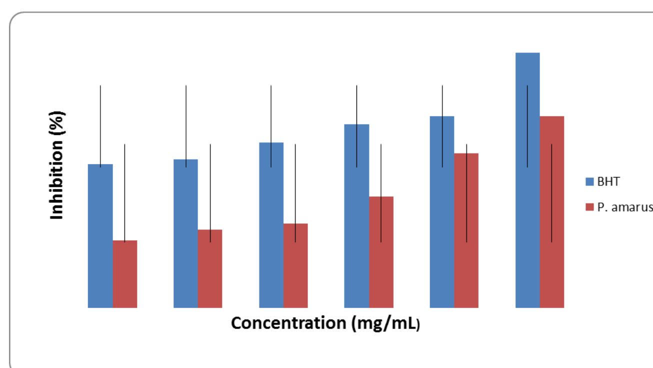

Fig. 2. *P. amarus* scavenging activity on ABTS radical .

Table 2. Antioxidant activity of *P. amarus*

Antioxidant Assays	*P. amarus*
FRAP(mg RE/g)	0.132 ± 0.010
TAC (mg GAE/g)	2.217 ± 0.002

FRAP = Ferric reducing antioxidant power, TAC = Total antioxidant capacity
RE = Rutin equivalent, GAE= Gallic acid equivalent

Table 3. DPPH and Nitric Oxide IC_{50} values of *P. amarus*

	DPPH (mg/mL)	NITRIC OXIDE (mg/mL)
Ascorbic acid	0.0003	0.0198
P. amarus	0.0001	0.0452

Discussion

Medicinal plants are adopted worldwide for the prevention and therapeutic purpose of diseases. A remarkable number of people all over the world are dependent on herbs as drugs (34). *P. amarus* is a common plant and it is used in traditional medicine as a result of its wide-range of medicinal potentialities. *P. amarus* is reported to have various pharmacological activities in both *in vitro* and *in vivo* studies (35).

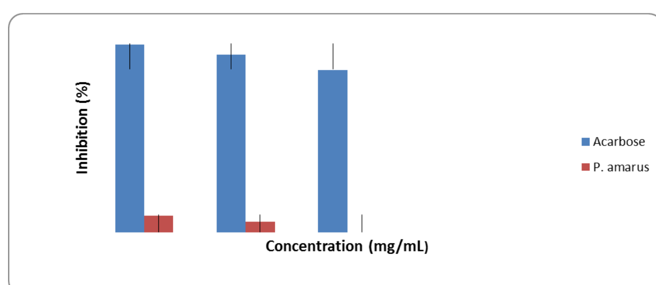

Fig. 3. Inhibitory activity *P. amarus* on α-amylase.

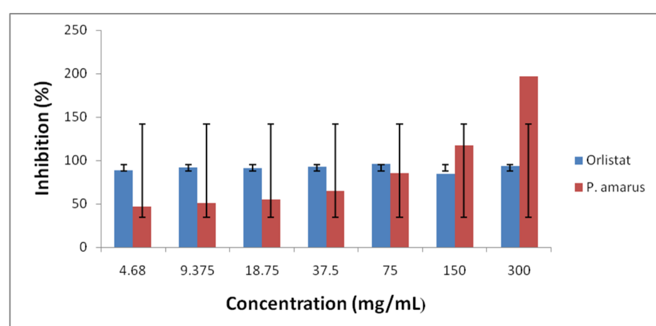

Fig. 4. Inhibitory activity of *P. amarus* on alpha lipase.

P. amarus is reported to be rich in phytochemicals such as flavonoids, alkaloids, lignans, tannins and phenols (36,37,38). The potent radical scavenging capacity of *P. amarus* is due to the high phenolic content and the presence of other phytochemicals such as flavonoids, tannins, and saponins, as reported in the present study.

Saponins are anti-nutritional factors that reduce glucose and lipid uptake particularly the gut cholesterol *via* physicochemical interaction of intra-lumen. Nwankpa*et al*. (39) reported the presence of saponins in an aqueous extract of *P. amarus*. These phytochemicals have been shown to interact with the key enzymes. Therefore, it can be concluded that high saponin and other phytochemical contents in the plant may be acting as great inhibitors of α--amylase and pancreatic lipase.

Conclusion

The result in this present study confirmed *P. amarus* to have anti-diabetic and anti-obesity activities. The phytochemicals such as saponin, flavonoids and tannin present in plant leaves are haing a great inhibitory effect on α-amylase and α-lipase. This finding can be further validated through clinical trials.

Acknowledgements

The Researchers acknowledge Covenant University, Ogun State, Nigeria for equipping the laboratory with sophisticated equipment for effective delivery of this research project.

Authors contributions

AO: Resources investigation, data curation, analysis, methodology, original draft, writing, and analysis; **AB:** Supervision, review, editing; **FI:** methodology, supervision, data curation and analysis; **KA:** review and editing; **OOG:** analysis, methodology, review and editing; **JO:** methodology, supervision, review and editing. **OO:** Conceptualization, analysis, methodology, supervision, review and editing.

Compliance with ethical standards

Conflict of interest: All the authors declared that they have no competing interest related to this article.

Ethical issues: None.

References

1. Temidayo O, Mutiyat A, Solomon I, Oreoluwa, D. *Phyllanthusamarus* extract restored deranged biochemical parameters in rat model of hepatotoxicity and nephrotoxicity, Heliyon, 2020; 6. https://doi.org/10.1016/j.heliyon.2020.e05670
2. Prior R, Cao G. Antioxidant capacity and polyphone compounds of teas. Proceedings of the Society for Experimental Biology and Medicine. 1999; 220:255–261. https://doi.org/10.1046/j.1525-1373.1999.d01-44.x
3. Azab AE, Albasha MO, Elsayed ASI. Prevention of nephropathy by some natural sources of antioxidants. Yangtze Medicine. 2017; 1: 235– 266.https://doi.org/10.4236/ym.2017.14023
4. Sanaberg F, Corrigan D. Natural Remedies. Their origin and uses. Taylor and Francis, Abingdon. 2001. ISBN 9780415272025. https://doi.org/10.4324/9780203302996
5. Ogunlana OO, Adetuyi BO, Esalomi EF, Rotimi MI, Popoola JO, Ogunlana OE, Adetuyi OA. Antidiabetic and Antioxidant Activities of the Twigs of Andrograhispaniculata on Streptozotocin-Induced Diabetic Male Rats. BioChem. 2021 1(3):238-249. https://doi.org/10.3390/biochem1030017
6. 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. https://doi.org/10.1016/j.diabres.2009.10.007

7. International Diabetes Federation 2013. IDF Diabetes Atlas 6th ed. IDF: Brussels, ISBN 2-930229-85-3 (online version at www.idf.org/diabetesatlas (accessed on 24.07.14.)

8. Kumar V, Makkar H, ; Becker K. Detoxified *Jatropha curcas* kernel meal as a dietary protein source: Growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* L.) fingerlings. Aquacult. Nutr. 2011; 17 (3): 313-326. https://doi.org/10.1111/j.1365-2095.2010.00777.x/pdf

9. World Health Organization. Obesity and overweight. 2015

10. Ogunlana OO, Ogunlana OE, Adekunbi TS, Adetuyi BO, Adegboye BE, and Iheagwam FN. Anti-inflammatory Mechanism of Ruzu Bitters on Diet-Induced Nonalcoholic Fatty Liver Disease in Male Wistar Rats. Evidence-Based Complementary and Alternative Medicine. 2020; https://doi.org/10.1155/2020/5246725

11. Anstey KJ, Cherbuin N, Budge M, Young J. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obesity Reviews, 2011; 12 (5): e426–37. https://doi.org/10.1111/j.1467-789X.2010.00825.x

12. Singh M, Tiwari N, Shanker K, Verma R, Grupta A, Grupta. Two new lignans from *P. amarus*. Journal of Asian Natural Products Research. 2009;11:562-568 https://doi.org/10.1016/j.jep.2011.09.040

13. Czernichow S, Kengne AP, Stamatakis E, Hamer M, Batty G. Body mass index, waist circumference and waist-hip ratio: which is the better discriminator of cardiovascular disease mortality risk?: evidence from an individual-participant meta-analysis of 82 864 participants from nine cohort studies. Obesity Reviews, 2011; 12(9): 680–7. https://doi.org/10.1111/j.1467-789X.2011.00879.x. Epub 2011

14. Adetuyi BO, Okeowo TO, Adetuyi OA, Adebisi OA, Ogunlana O, Oretade OJ, Marraiki N, Beshbishy AM, Welson NN, Batiha GE. Ganoderma lucidum from red mushroom attenuates formaldehyde- induced liver damage in experimental male rat model. Biology2020;*9*(10):313. https://doi.org/10.3390/biology9100313

15. Anandacoomarasamy A, Caterson I, Sambrook P, Fransen M, March L. The impact of obesity on the musculoskeletal system. The International Journal of Obesity. 2008; 32(22):11–22. https://doi.org/10.1038/sj.ijo.0803715

16. Kim H, Kang M. Screening of Korean medicinal plants for lipase inhibitory activity. Phytotherapy Research. 2005; 19: 359-61. https://doi.org/10.1002/ptr.1592

17. Ogunlana OO, Adetuyi BO, Adekunbi TS, Adegboye BE, Iheagwam FN, Ogunlana OE. Ruzu bitters ameliorates high–fat diet induced non-alcoholic fatty liver disease in male Wistar rats. Journal of Pharmacy and Pharmacognosy Research 2021;9 (3):251-26 https://jppres.com/jppres/pdf/vol9/jppres20.868_9.3.251.pdf

18. Moreno D, Ilic N, Poulev A,Raskin A. Effects of *Arachis hypogaea* nutshell extract on lipid metabolic enzymes and obesity parameters. Life Sciences. 2006; 78:2797-803. https://doi.org/10.1016/j.lfs.2005.11.012

19. Shimoda H, Seki E, Aitani M. Inhibitory effect of green coffee bean extract on fat accumulation and body weight gain in mice. BMC Complement Alternative Medicine. 2006; 6: 9. https://doi.org/10.1186/1472-6882-6-9

20. Adetuyi BO, Dairo JO, Didunyemi MO. Anti-Hyperglycemic Potency of Jatropha Gossypiifolia in Alloxan Induced Diabetes. BiochemPharmacol (Los Angel). 2015; 4 (5):193.doi:10.4172/2167-0501.1000193

21. Ogunlana OO, Adetuyi BO, Rotimi M, Esalomi I, Adeyemi A, Akinyemi J, Ogunlana O, Adetuyi O, Adebisi O, Okpata E, Baty R, Batiha G. Hypoglycemic Activities of Ethanol Seed Extract of *Hunteria umbellate* (Hallier F.) on Streptozotocin-induced Diabetic Rats. Clinical Phytoscience 2021;7 (1): 1-9. https://doi.org/10.1186/s40816-021-00285-1

22. Pius O, Babawale O, Sola E, Comfort, O. A comparative study of nutritional and phytochemical composition of *Phyllanthus amarus* leaf and seed'. American Journal of Pharmacology and Toxicology. 2015; 7: 321–327. https://doi.org/10.5829/idosi.aejts.2015.7.4.9699

23. Champe P, Harvey R, Ferrier D. Biochemistry. Lippincott Williams and Wilkins. 2005, ISBN 978-1-60831-412-6

24. Adetuyi BO, Adebisi OA, Awoyelu EH, Adetuyi OA,Ogunlana OO. Phytochemical and Toxicological effect of Ethanol extract of Heliotropium indicum on Liver of Male Albino Rats. Letters in Applied NanoBioscience (LIANB) 2020; 10(2):2085-2095 https://doi.org/10.33263/LIANBS102.20852095

25. Iheagwam F, Nsedu E, Kazeem O, DeCampos O, Ogunlana, O, Chinedu, S. *Nauclea latifolia Sm.* leaf extracts extenuate free radicals, inflammation, and diabetes-linked enzymes. Oxidative Medicine and Cellular Longevity. 2020;1: 13. https://doi.org/10.1155/2020/5612486

26. Adetuyi BO, Omolabi FK, Olajide PA, Oloke JK. Pharmacological, Biochemical and Therapeutic Potential of Milk Thistle (Silymarin): A Review World News of Natural Sciences 2021;37:75-91

27. Jamuna S, Paulsamy S, Karthika, K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herbs, *Hypochaerisradicata* for *in vitro* antioxidant activities. Asian Pacific Journal of Tropical Biomedicine. 2014; 4(1): 359-367. https://doi.org/10.12980/APJTB.4.2014C1030

28. Jaiswal D, Rai P, Kumar A, Mehta S, Watal, G. Effect of *Moringa oleifera* lam. Leaves aqueous extract therapy on hyperglycemic rats. Journal of Etnopharmacology. 2009; 123: 392–6.http://dx.doi.org/10.1016/j.jep.2009.03.036

29. Ogunlana OO, Ogunlana OE, Popoola JO, Adetuyi BO, Adekunbi TS, David OL, Adeleye OJ, Udeogu SA, Adeyemi AO. Twigs of Andrographis paniculata (Burn. F) Nees attenuates Carbon Tetrachloride (CCl4) Induced Liver Damage in Wistar Albino Rats. RASAYAN Journal of Chemistry 2021;14(4): 2598-2603 https://doi.org/10.31788/RJC.2021.1445987

30. Ebrahimzadeh M, Nabavi S, Nabavi F,Bekhradnia, A. Antioxidant and free radical scavenging activity of *H. officinalis L. var. angustifolius, V. odorata, B. hyrcana* and *C. speciosum*. Pakistan Journal of Pharmaceutical Sciences. 2010; 23(1): 29–34.

31. Adetuyi BO, Toloyai TY, Ojugbeli ET, Oyebanjo OT, Adetuyi OA, Uche OZ, Olisah MC, Adumanya OC, Jude C, Chikwendu JK, Akram M, Awuchi CG, Egbuna C. Neurorestorative Roles of Microgliosis and Astrogliosis in Neuroinflammation and Neurodegeneration. *Scicom Journal of Medical and Applied Medical Sciences.* 2021;1(1):1-5 https://doi.org/10.54117/sjmams.v1i1.1

32. Iheagwam F, Nsedu E, Kazeem O, DeCampos O, OgunlanaO, Chinedu S. GC-MS Analysis and Inhibitory Evaluation of *Terminalia catappa* Leaf Extracts on Major Enzymes Linked to Diabetes Evidence-Based Complementary and Alternative Medicine. 2019: 14. https://doi.org/10.1155/2019/6316231

33. Anyanwu G, Hafiz M,Jamshed I, Khalid Rauf.Ethylacetate Fraction of *Anthocleista vogelii* Planch Demonstrates Anti-obesity Activities In Preclinical Models. Tropical Journal of Pharmaceutical Research. 2015;18(3):547-554 https://doi.org/10.4314/Tjpr.V18i3.15

34. Ogbera A, Dada O, Adeyeye F, Jewo P. Complementary and alternative medicine use in diabetes mellitus. West African Journal of Medicine. 2010; 29(3):158-62. https://doi.org/10.4314/wajm.v29i3.68213

35. Hasenah Ali , Houghton P, Amala S. alpha-Amylase inhibitory

activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. Journal of Ethnopharmacology. 2006;107(3):449-55 http://dx.doi.org/10.1016/j.jep.2006.04.004

36. Didunyemi MO, Adetuyi BO, Oyewale IA. Inhibition of lipid peroxidation and in-vitro antioxidant capacity of aqueous, acetone and methanol leaf extracts of green and red *Acalypha wilkesiana* Muell. Arg**.** International Journal of Biological & Medical Research. 2020; 11(3):7089- 7094

37. Kussuya C, Daniella V, Lucilia G, Jao B. Antiorganic solvents provides a higher efficiency in extracting inflammatory properties of extract, fractions and compounds for antimicrobial activities compared to water lignans isolated from *Phyllanthus amarus*. Planta Med. 2005; 71(8):721-6. http://dx.doi.org/10.1055/s-2005-871258.

38. James-Okoro PO, Iheagwam FN, Sholeye MI, Umoren IA, Adetuyi BO, Ogundipe AE, Braimah AA, Adekunbi TS, Ogunlana OE, Ogunlana OO. Phytochemical and in vitro antioxidant assessment of Yoyo bitters. World News of Natural Sciences .2021;37:1-17.

39. Nwankpa P, Chuwuemeka O, Uloneme G, Etteh C, Ugwuwzumba P, Nwosu D. Phyto-nutrient composition and antioxidative potential of ethanolic leaf extract of sida acuta in Wistar Albino Rats. African Journal of Biotechnology. 14 (49):3264-3269. http://dx.doi.org/10.5897/AJB2015.14897