Trop J Nat Prod Res, April 2021; 5(4):753-759**ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In Vitro Inhibitory Effect and Kinetic Studies of Selected Local Plant Extracts with Anti-Obesity Potentials on Pancreatic Lipase

Hamza B. Usman*, Hassan A. Salisu, Ahmed Garba, Usman B. Idris, YakubuNdatsu, HassanaAbubakar, Fatima G. Maiyaki

Department of Biochemistry, Faculty of Natural Sciences, Ibrahim BadamasiBabangida University, P.M.B.11, Lapai, Nigeria

ARTICLE INFO

Article history:
Received 03 January 2021
Revised 15 February 2021
Accepted 26 March 2021
Published online 03 May 2021

ABSTRACT

One of the therapeutic strategies in managing obesity is the inhibition of pancreatic lipase, a key enzyme responsible for the catabolism of fats and triglycerides. In this study, the Anti-obesity efficacies of twenty-three local medicinal plant extracts (aqueous and methanol) were analyzed against porcine pancreatic lipase activity. Anti-lipase activity was determined by spectrophotometric method. The Methanol extract of *Khayasenegalensis* (Bark), *Perseaamericana* (root), *Daniela oliveri* (leaf), *Psidiumguajava* (leaf), showed more than 50 % inhibition on the enzyme activity. The aqueous extract of *Khayasenegalensis* (Bark) and *Daniela oliveri* (Bark) also showed more than 50 % inhibition on the enzyme activity. Kinetic Studies of PPL against the four effective methanol and aqueous extract showed that all the extracts exhibited a noncompetitive mode of inhibition by Michaelis-Menten non-linear regression and data by the double reciprocal plot. The Methanol extract of *Khayasenegalensis* (Bark), *Perseaamericana* (root), *Daniela oliveri* (leaf), *Psidiumguajava* (leaf), showed Km of 0.0182, 0.030, 0.0596, and 0.0398 mg/ml respectively. The aqueous extract of *Khayasenegalensis* (Bark) and *Daniela oliveri* (Bark) showed Km of 0.0204 and 0.0554 mg/ml. The result showed that Khayasenegalensis (Bark), Perseaamericana (root), *Daniela oliveri* (leaf), *Psidiumguajava* (leaf) can serve as a potential herbal alternative for the treatment of obesity. Further studies are required to elucidate the effectiveness of these active extracts and also an attempt should be explored to purify their active components to be used as safer and cheaper therapeutic agents in the future.

Keywords: Obesity, Pancreatic Lipase, Medicinal plant, inhibition, P-nitrophenylbutyrate

Introduction

Obesity has become a worldwide phenomenon that became one of the most important health issues rapidly affecting both developed and developing countries around the world.[1,2] It affects 27.5% of adults and 47.1% of children.[3] The World Health Organization (WHO) estimated that over 39% of adults are overweight; with at least 13% clinically obese in 2016 and this figure equal to about 1.9 billion overweight adults (18 years and older) and out of these, over 650 million were obese.[2–4] The disorder usually results from an imbalance between energy intake and energy expenditure. Altered lipid metabolism such as lipogenesis (a process that converts excess acids to triglyceride) and lipolysis (a process thatdegrades stored triglyceride to its monomeric units) leads to imbalanced energy intake. It is generally accompanied by an abnormally high concentration of lipids in the blood (hyperlipidemia).[5] Genetic, environmental, and nutritional factors are the three most common trigger for this disorder.Obesity has become the most prevalent disorder affecting all age groups, with its attendant comorbidities such as heart disease, type II diabetes mellitus, stroke, and cancer. [4] Therefore, prevention and treatment of these problemsare targets of health policymakers in many countries, whose aim is to reduce the obesity and overweight prevalence, and relatedcomplications over the world.[6] In the gastrointestinal tract, pancreatic lipase is the major lipid absorption enzyme that degrades triacylglycerol.[7] Management of obesity is still a challenge to the health sector. Though preventive measures in the forms of dieting and physical exercise are usually recommended, they, however, produce no sustainable weight loss in the long run. Treatment is hence required for the morbidly obese. To this end, various pharmacological agents (drugs) were developed but were unfortunately fraught with many side effects. This made this search for alternative means a necessity. In recent times, the potential of natural products for the treatment of obesity is gaining increasing emphasis. These products possess a vast source of potential compounds that can be easily developed into anti-obesity agents. The identification of such beneficial bioactive components thus became the major emphasis of modern ethnomedicinal research. It has been shown that pancreatic lipase inhibitors are useful medicines for treating hyperlipidemia and a great promise as anti-obesity agents to help limit early fat absorption.[8,9]Orlistat is the popular available anti-obesity medication that had gain acceptance as an anti-obesity drug.

In this part of the world (West Africa), the use of medicinal plants to treat health ailments has a long history and is still a useful means. More important is its ease of accessibility and relatively low side effects to the convenience of many. Some of these plants are used for the treatment of obesity, even though the mechanisms are not clear. This study would hence be based on verifying the anti-obesity potential of some selected tropical plants. Also, since dietary fat absorption is the major cause of fat build-up, interrupting this medium would provide a ready means for exploitation. Pancreatic lipase (PL) is known to play a key role in the efficient digestion of triglycerides.[7] It is secreted into the duodenum through the duct system of the pancreas and is responsible for the hydrolysis of dietary fats.[10] This

*Corresponding author. E mail: ubhamzat@ibbu.edu.ng,
Tel: +2348161211483

Citation: Usman HB, Salisu HA, Garba A, Idris UB, Ndatsu Y, Abubakar H, Maiyaki FG. *In vitro* inhibitory effect and kinetic studies of selected local plant extracts with anti-obesity potentials on Pancreatic Lipase. Trop J Nat Prod Res. 2021; 5(4):753-759. doi.org/10.26538/tjnpr/v5i4.26

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

enzyme has been widely used for the determination of the potential efficacy of natural products as anti-obesity agents.[11] Thus, the anti-obesity efficacy of the selected plants would be verified through the kinetic mechanism of pancreatic lipase inhibition.To the best of our knowledge, this study had not been reported in any scientific literature**.**

Materials and Methods

The part of plants of interest were collected between the months of May-July 2018 within the local environment, mostly within the premises of Ibrahim BadamasiBabangida University Main campus in Lapai, Niger state North Central Nigeria while some were purchased at the local market where they are obtainable. The plants were identified and confirmed by a botanist at the biological science department of the institution and assigned voucher numbers as follows: African Mahogany (00008), Ilorin Balsam (00057), Avocado (00117), Guava (00046), Neem (00037), Bitter leaf (00004), Pawpaw (00025), Moringa (00107), Mango (00002), plantain (00113), Bitter Orange (00213), Black cumin (00135), Watermelon (00072), Nutmeg (00212), Tamarind (00114), Roselle (00088), Ginger (00096), and Apple (00099). Orlistat (Ecoslim, MicronovaPharmaInd Ltd, Nigeria), p-nitrophenyl butyrate (Lab Tech Chemicals), and Pancreatin (porcine pancreatic lipase) (Guangzhou JHD Chemicals ltd) were purchased. All other reagents and chemicals were of analytical grade and used as received.

Plant extractions:
all plant materials were washed thoroughly with clean water. The stem barks, roots, and leaves were cut into small pieces and air-dried at room temperature (25-30^{o}C) for 5-10 days (depending on the sample type). The dried plant materials were grounded into a fine powder using a laboratory blender. Twenty (20) g of the powdered materials were added in 200 mL of 80% (v/v) methanol each, macerated for 3 days at ambient temperature (25^{0}C), and then filtered using a Whatman filter paper (No.1). The filtrates were evaporated at 45^{o}C in a rotary vacuum evaporator. The dried crude extracts were then collected and stored at 0^{0}C until further analysis. The sample bottles containing the extract were covered with aluminum foil paper for protection against light.

In vitro Pancreatic Lipase Inhibition Assay:
Different concentrations of the plant extracts were prepared with 50 mM of phosphate buffer ab initio as stock. PPL activity was assayed by the method previously described by Kim, *et al*.,[12] with slight modification. In brief, PPL (20 mg/ml) and substrates were fully vortexed in advance at 1:2 ratio (v/v), and onto the solution aliquot, 500 µL of 0, 2.0, 4.0, 6.0 mg/mL each plants extract was added and made up to 2 mL with buffer, mildly vortexed and incubated for 5 min, then the reaction was stopped by adding 500 µL of 1:3 ratio (v/v) of distilled water/methanol. The Appearance of p-Nitrophenol was measured by a change in absorbance at 400 nm using a UV-Visible spectrophotometer. The percentage activity inhibition was calculated through the equation:

$$\text{Inhibition (\%)} = \frac{\Delta\text{Abs of control} - \Delta\text{Abs of extract}}{\Delta\text{Abs of control}} \times 100$$

Kinetics study and statistical analysis
To measure the inhibition mode of both methanol and aqueous extract of plant material, inhibition mode was determined according to Michaelis-Menten kinetics nonlinear regression equation and data plotted by double-reciprocal Lineweaver-bulk analysis. Statistical analysis was done using GraphPad Prism version 8.0.4 software.

Results and Discussion

Series of experiments were performed to obtain the optimum conditions for the PPL Activity. The result indicated an optimum enzyme concentration of 20 mg/ml and a pH of 7.4, hence these conditions were used for further inhibitory studies. Aqueous and Methanol extract of twenty-three (23) different plant parts were evaluated for their inhibitory effects on PPL enzyme using in vitro assay (Tables 1 and 2).

Table 1: Percentage Inhibitory effect of Methanol Plant Extracts on PPL

Scientific Name	Common Name	Part Used	% Inhibition
KhayasenegalensisA.Juss	African Mahogany	Stem bark	73.8
Perseaamericana Mill.	Avocado	Root	72.55
Daniela oliveri	Ilorin Balsam	Stem bark	68.7
Psidiumguajava L.	Guava	Leaf	65.0
AzadirachtaindicaA.Juss	Neem	Leaf	43.38
VernoniaamygdalinaDelile	Bitter leaf	Leaf	31.8
KhayasenegalensisA.Juss	African Mahogany	Leaf	29.30
Carica papaya L.	Pawpaw	Leaf	27.04
Perseaamericana Mill.	Avocado	Seed	24.43
MalusdomesticaBorkh.	Apple	Fruit	23.89
Moringaoleifera Lam.	Moringa	Leaf	23.70
Mangiferaindica L.	Mango	Leaf	23.60
Daniela oliveri	Ilorin Balsam	Leaf	16.32
Musa acuminataColla	Plantain	leaf	15.45
Moringaoleifera Lam.	Moringa	Seed	9.89
Citrus aurantium L.	Bitter Orange	Leaf	8.02
CitrulluslanatusThunb.	Water melon	Seed	7.73
MyristicafragransHoutt.	Nutmeg	Seed	7.68
Perseaamericana L.	Avocado	Fruit	6.21
Tamarindusindica L.	Tamarind	Fruit	5.30
Hibiscus sabdariffa L. Calyx	Roselle	Fruit	0
Nigella sativa L.	Black cumin	Seed	-
GingiberofficinaleWilld.	Ginger	fruit	-

Table 2: Percentage Inhibitory effect of Aqueous plant Extracts on PPL

Scientific Name	Common Name	Part Used	% Inhibition
KhayasenegalensisA.Juss	African Mahogany	Stem bark	64.7
Daniela oliveri	Ilorin Balsam	Stem bark	69.4
Moringaoleifera Lam.	Moringa	Seed	33.78
Perseaamericana Mill.	Avocado	Root	33.0
Carica papaya L.	Pawpaw	Leaf	32.49
Psidiumguajava L.	Guava	Leaf	32.0
AzadirachtaindicaA.Juss	Neem	Leaf	31.91

Vernoniaamygdylina	Bitter leaf	Leaf	30.59
Musa acuminataColla	Plantain	leaf	30.34
Nigella sativa L.	Black (cumin)	Seed	28.12
Tamarindusindica L.	Tamarind	Fruit	25.6
KhayasenegalensisA.Jus s	African Mahogany	Leaf	19.2
CitrulluslanatusThunb.	Water melon	Seed	17.61
Moringaoleifera Lam.	Moringa	Leaf	17.0
Perseaamericana Mill.	Avocado	Seed	15.62
GingiberofficinaleWilld.	Ginger	fruit	9.88
Mangiferaindica L.	Mango	Leaf	9.45
Perseaamericana Mill.	Avocado	Fruit	9.22
MyristicafragransHoutt.	Nutmeg	Seed	7.30
Daniela oliveri	Ilorin Balsam	Leaf	7.08
Citrus aurantium L.	Bitter Orange	Leaf	5.59
MalusdomesticaBorkh.	Apple	Fruit	4.29
Hibiscus Sabdariffa L. Carlx	Roselle	Fruit	0

Among them; methanol extracts of *KhayaSenegalensis* (stem bark), *Perseaamericana* (root), *Daniela oliveri* (stem bark), and *Psidiumguajava* (leaf) showed 73.4, 72.6, 68.9, and 55% inhibitory effect on PPL, respectively. While the aqueous extracts of *KhayaSenegalensis* (stem bark), and *Daniela oliveri* (stem bark) showed 64.7 and 69.4% inhibitory effect on PPL, respectively. Therefore, these extracts were utilized for further kinetic studies. The remaining extracts obtained from different parts of the plants used showed <50% inhibition on PPL, therefore were excluded. The specific concentration at which the most active extracts exhibited the highest % inhibition on the enzyme was also determined to serve as the IC_{50}. Figures 1 and 2 indicatedthe results obtained for the methanol and aqueous extracts respectively.The result showed that the optimum inhibition for the extracts was obtained at extract concentration 10 mg/ml for all the active plant extracts considered.

Kinetic mode of action of *Khayasenegalensis* (stem bark), *Perseaamericana* (root), *Daniela oliveri* (stem bark), and *Psidiumguajava* (leaf) on Porcine Pancreatic Lipase (PPL).

Irreversible and reversible inhibitors are the two-common classification of enzyme inhibitors. An irreversible inhibitor normally binds covalently to an enzyme by preventing enzyme-substrate complexes formation. Since this bonding occurs at the active site, it induced conformational changes on the enzyme. In such instance, if a plot of relative enzyme activity Vs the enzyme concentration (molar) is extrapolated, a group of straight lines with the same slope will be obtained. An increased inhibitor concentration results in a rightward line. The reversible inhibitor binds non-covalently to an enzyme and can be renewed by simple methods (such as dialysis). If a plot of relative enzyme activity Vs enzyme concentration (molar) is extrapolated, a group of straight lines will be obtained. The slopes of the lines decrease relative to an increased inhibitor concentration. Reversible inhibition can be grouped into competitive, non-competitive, and uncompetitive. In competitive inhibition, the molecules are competing with the normal substrate for the active site of the enzyme. In this type of inhibition, the Michaelis constant (Km) increases, while the maximum reaction rate (Vmax) remains the same.In non-competitive inhibition the molecule has no structural resemblance with a normal substrate; therefore, the inhibitor usually binds to a different domain other than the catalytic site of the enzyme. In this type of inhibition, Km does not change, while Vmax decreases. Uncompetitive inhibition, the molecule binds only to the enzyme-substrate complex but not the free enzyme. They are characterized byboth Km and Vmax decrease. The measure of how potent an inhibitor is (Ki) is also considered. It is defined as the concentration requires in producing half-maximum inhibition. The smaller the Ki the greater the binding affinity of the inhibitor and the smaller the concentration of the extract required to inhibit the enzyme activity.

Before the advent of nonlinear regression, it was common to use the Lineweaver-Burk plot to transform curved data into straight lines so that linear regression could be used to evaluate them. Lineweaver-Burk plots; plots the reciprocal of substrate concentration vs. the reciprocal of enzyme velocity. But due to distortion of the experimental error by reciprocals (transformations), it should be used cautiously because it does not obey the assumptions of linear regression.[13]Lineweaver-Burk plot was used in this work to display data obtained. Km and Vmax were determined by nonlinear regression to obtain the most accurate values, as the use of slope and intercept of a linear regression line to determine values for Vmax and Km is misleading.The inhibition mode of PPL by Khayasenegalensis methanol and aqueous extract at (2, 4, and 6) mg/mL were analyzed by Michaelis-Menten nonlinear regression and data plotted by the double-reciprocal plot as shown in Figure 3. The kinetic parameters of PPLcatalyzed reactions were obtained by nonlinear regression of the Michaelis-Menten plot. The inhibition type was estimated by comparing the changes in the kinetics parameters, Ki, Km, and Vmax. The estimated parameters showed a change in the Vmax of enzyme-substrate reaction and the Km were not affected by the extract concentration, hence indicating a noncompetitive mode inhibition. Noncompetitive inhibitor possesses a non-competitive inhibitory effect on PPL, which indicated that the K.S component can combine not only with free enzyme but also with enzyme-substrate complex, and the binding constants are the same. The Ki values 67.34 and 43.41 were found for the methanol leave and the aqueous bark of K.S respectively. This study further substantiated the findings that KS bark and leaves inhibition mode that had previously been documented to have an effect of anti-porcine pancreatic lipase. The kinetic data from our finding showed reversibly non-competitive inhibition. This corroborated the studies by Marche et al., (2015) that drink made from the plant's bark extract was tested against porcine pancreatic lipase activity in a variant sample of Khaya tea. The result showed that at the yielding concentration (3 mg/ml) the extract inhibits the enzyme activity by up to 70 percent though competitively. Phytochemicals studies of KS showed the presence of alkaloids, saponins, glycosides terpenoids in both methanol and aqueous extract, which may explain the reported inhibitory activity from this and many others.[15] This study further substantiated the findings that had previously been documented, that KS has an anti-hyperlipidemia effect and weight reduction effect.[16]The inhibition mode of PPL by methanolic root extract of Perseaamericana (Avocado) at (2, 4, and 6) mg/mL was also investigated by Michaelis-Menten nonlinear regression and data plotted by the double-reciprocal plot as shown in Figure 4. Kinetic parameters calculated showed a change in the Vmax of enzyme-substrate reaction and the Km (0.030) was not affected by the extract concentration, hence indicating a noncompetitive mode inhibition. The inhibitor possesses a non-competitive inhibitory effect on PPL, which indicated that active metabolites in Avocado can bind allosterically not only to the free enzyme but also to the enzyme-substrate complex, and the binding constants are the same. The Ki value at all extract concentrations was found to be 30.98. Phytochemicals screenings of Avocado showed the presence of saponins, phytosterols tannins, polyphenols, alkaloidscarotenoids, tocopherols, and terpenoids as themain bioactive components in the extract of leaves, fruit, pulp, and roots, which may explain the reported inhibitory activity from this work and many other similar works.[17–19] This result provided a kinetic basis on the findings that Avocado contains nutrients and bioactive compounds that showed an anti-obesity effect, therefore, helps in reducing the risk of becoming overweight/obese.[20,21] It also serves as nutraceuticals against obesity.[22]

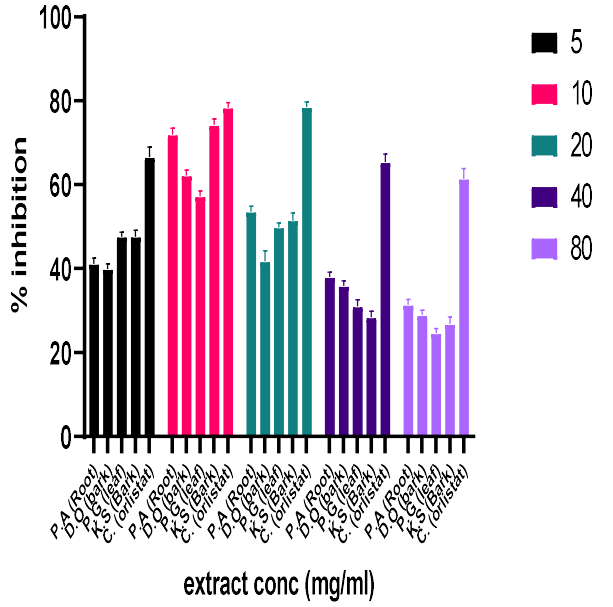

Figure 1: % inhibitory effect of Methanol plant extracts at a specified concentration.
P.S =*Perseaamericana*, D.O = *Daniela oliveri,* P.G = *Psidiumguajava,* K.S = *Khayasenegalensis,* C.= Control

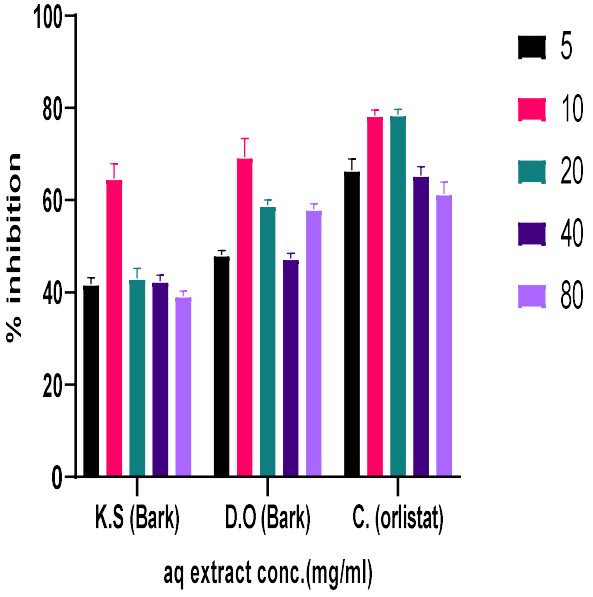

Figure 2: % inhibitory effect of aqueous plant extracts at a specified concentration.

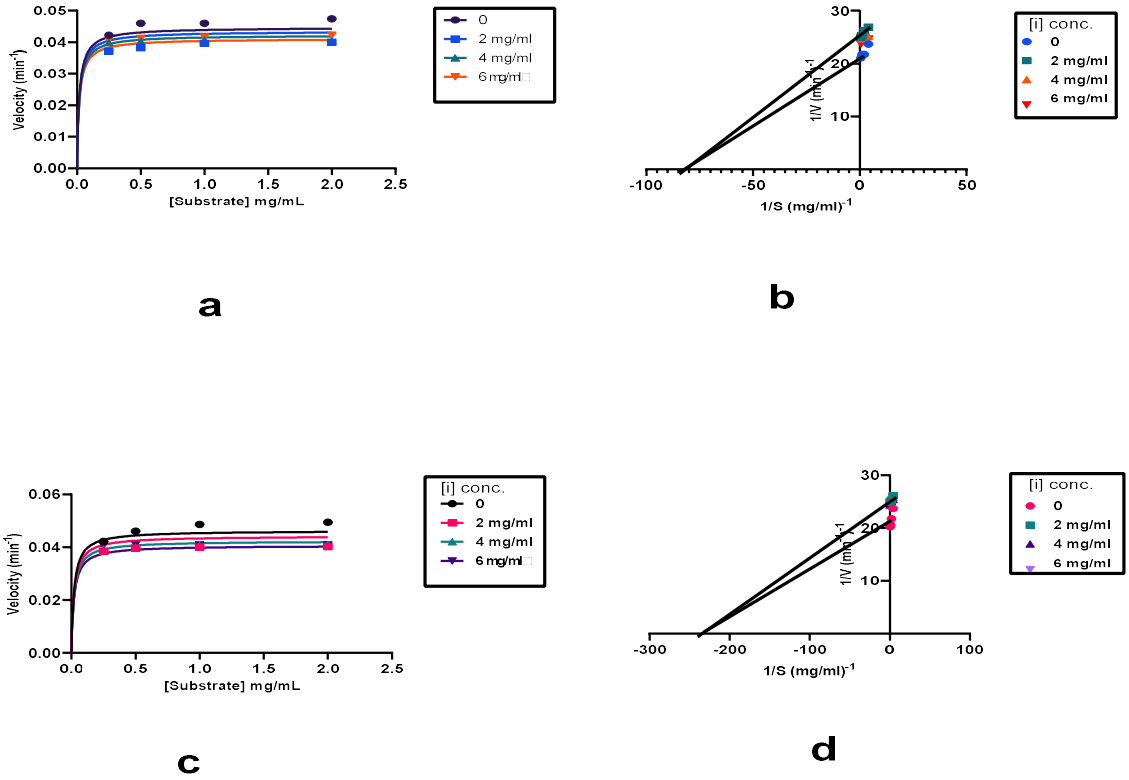

Figure 3: (a) MichaelisMenten**(b)** the Lineweaver-Burk plot of kinetic analysis for PPL at varied concentrations of methanol leaf extract of *Khayasenegalensis***(c)**MichaelisMenten**(d)** the Lineweaver-Burk plot of kinetic analysis for PPL at varied concentrations of aqueous leaf extract of *Khayasenegalensis.*

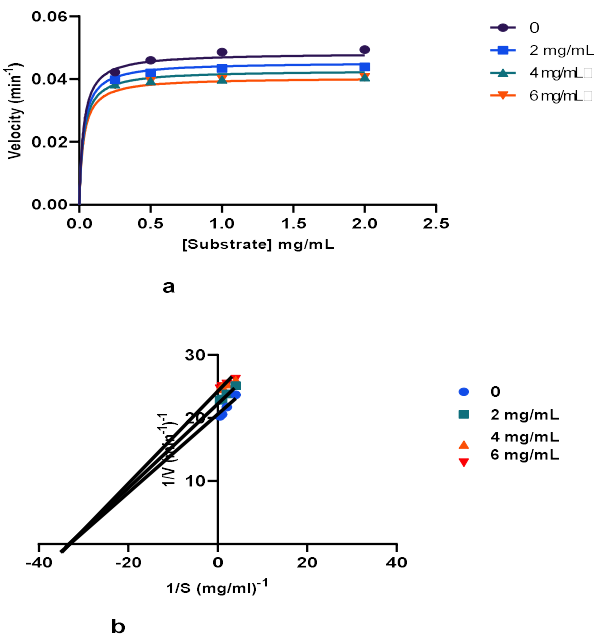

Figure 4:(a) MichaelisMenten**(b)** the Lineweaver-Burk plot of kinetic analysis for PPL at varied concentrations of methanol root extract of *Perseaamericana*.

Similarly, studies conducted on Male Sprague Dawley rats indicated that Avocado fruits showed modulating effect on the level of expression of lipoprotein lipase.[23]

The inhibition mode of PPL by Daniela oliveri (stem bark) methanolic and aqueous extract at (2, 4, and 6) mg/mL was also investigated by Michaelis-Menten nonlinear regression and data plotted by the double-reciprocal plot as shown in Figure 5. Kinetic parameters calculated showed a change in the *V*max of enzyme-substrate reaction and the *K*m (0.0596 and 0.0554) was not affected by the extract concentration, hence indicating a noncompetitive mode inhibition. Noncompetitive inhibitor possesses a non-competitive inhibitory effect on PPL, which indicated that the Daniela oliveri component may have allosterically bounded not only to the free enzyme but also to the enzyme-substrate complex, and the binding constants are the same. The Ki values 60.07 and 73.4 were found for the methanol leave and the aqueous bark of K.S respectively. Within the limitation of this finding, no studies supported our findings that methanol and aqueous bark extract *Daniela oliveri* had anti-obesity potentials. But the relationship between obesity and type II two diabetes has been established.[24,25]

The inhibition mode of PPL by methanolic leaf extract of *P. guajava* at (2, 4, and 6) mg/mL was also investigated by Michaelis-Menten nonlinear regression and data plotted by the double-reciprocal plot as shown in Figure 6. Kinetic parameters calculated showed a change in the *V*max of enzyme-substrate reaction and the *K*m (0.0398) was not affected by the extract concentration, hence indicating a noncompetitive mode inhibition.

.

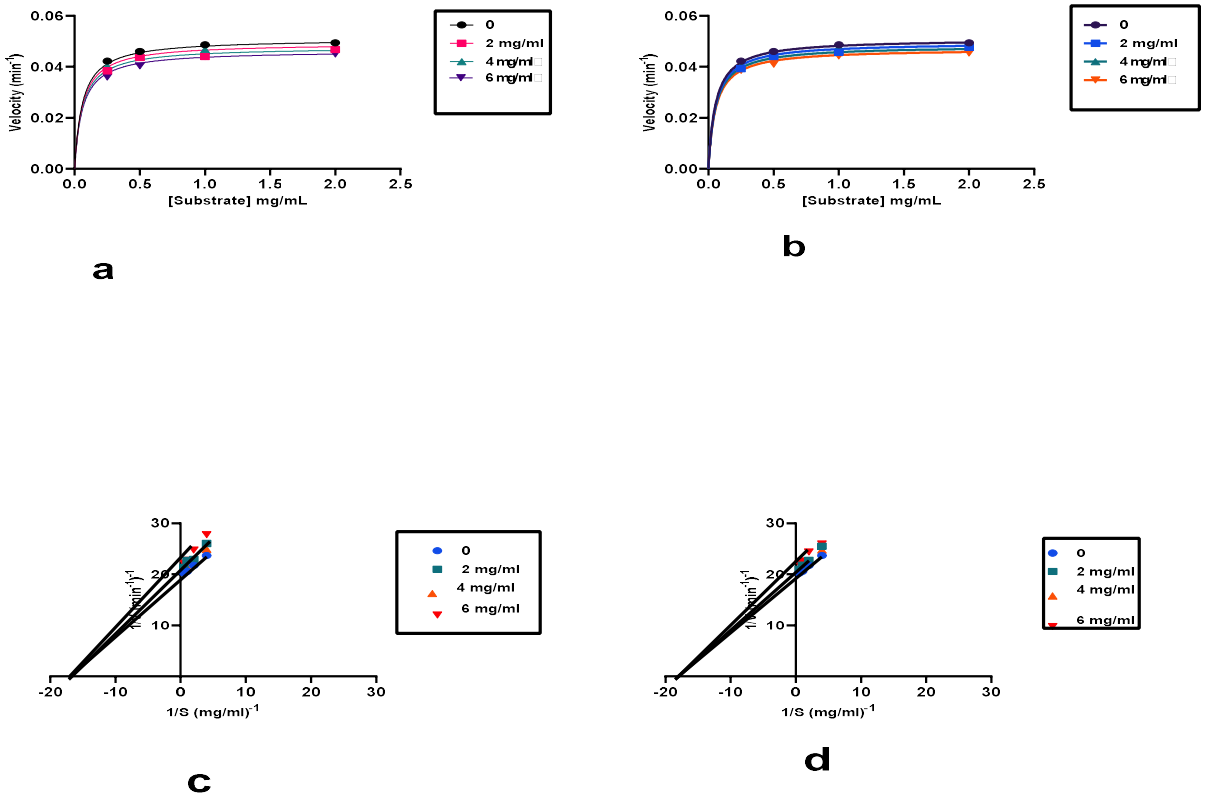

Figure 5:(a) MichaelisMenten**(b)** the Lineweaver-Burk plot of kinetic analysis for PPL at varied concentrations of methanol bark extract of Daniela oliveri**(c)**MichaelisMenten**(d)** the Lineweaver-Burk plot of kinetic analysis for PPL at varied concentrations of aqueous bark extract of *Daniela oliveri*

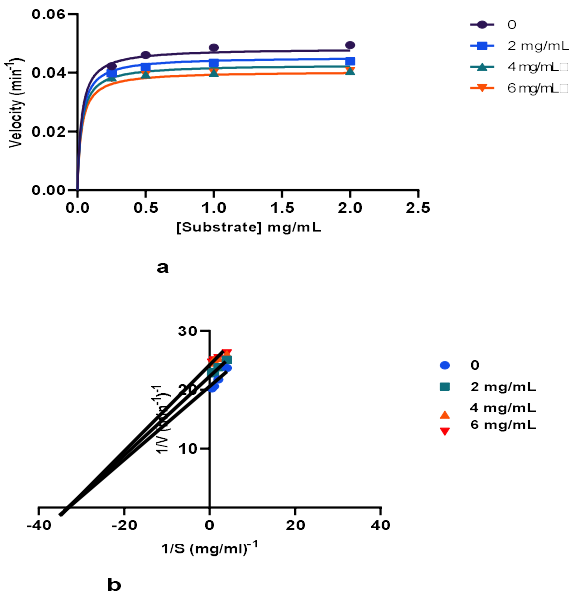

Figure 6:(a)*MichaelisMenten***(b)** the Lineweaver-Burk plot of kinetic analysis for PPL at varied concentrations of methanol bark extract of *Psidiumguajava*.

Comparatively, the potency of the inhibitors (Ki) was also considered, the following was the trend observed from these findings: extract of *Psidiumguajava* (2.644) > extract of *Perseaamericana* (30.98) > aqueous extract of *Khayasenegalensis* (43.41) > methanol extract of *Daniela oliveri* (60.07) > methanol extract of *Khayasenegalensis* (67.34) > aqueous extract of *Daniela oliveri* (73.4). Conversely, Orlistat (positive control), showed a Ki of 4.697, which is way below the Ki of *Psidiumguajava*. This might be due to the presence of phenolic functionalities in *P. guajava* extract, which was reported to be a potent inhibitor of PPL.[29]

Also, phytochemical screening of the plant revealed that it contains polyphenols, saponins, tannins, phytate, oxalate, saponin, steroid, alkaloids, and steroids, and it is used as an antidiabetic medicine.[26,27] This study suggested that phytochemicals soluble in methanol, exhibit some structural feature that resulted in binding and inhibition of PPL, and that phenolic compounds may be responsible for this inhibition The inhibitor possesses a non-competitive inhibitory effect on PPL, which indicated that active metabolites in *P. guajava*may have reacted not only to free enzyme but also with the enzyme-substrate complex, and the binding constants are the same with a Ki value at all extract concentration at 2.644. This result indicated a kinetic basis for the finding that indicates that *P. guajava* leaves aqueous extracts to possess potential anti-obesity effects thus, can be used as an adjuvant in the treatment of obesity and other dyslipidemias.[11,28]

Conclusion

The inhibition mechanism of *Khayasenegalensis*, *Perseaamericana*, *Daniela oliveri*, and *Psidiumguajava* extract were elucidated in theory and this study may provide an insight into the use of these plants extract as an alternative potential anti-obesity medication and/or nutraceuticals. Therefore, a new study in vivo is desirous to authenticate these findings using an animal model. An attempt would be made to identify, isolate, characterize and purify these active phytochemicals, for studies on the biochemistry of their lipid-lowering nature for broad application prospects and high economic potential as safe therapeutic agents in the management of obesity and other related complications.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Bustanji Y, Al-masri IM, Mohammad M, Hudaib M, Tawaha K, Tarazi H, Alkhatib HS. Pancreatic lipase inhibition activity of trilactoneterpenes of *Ginkgo biloba* Pancreatic lipase inhibition activity of trilactoneterpenes of *Ginkgo biloba*. J Enzyme Inhib Med Chem. 2011;26(4):453-459.
2. Rahman HA, Saari N, Abas F, Ismail A, Mumtaz MW, Hamid AA. Anti-obesity and antioxidant activities of selected medicinal plants and phytochemical profiling of bioactive compounds. Int J Food Prop. 2017;20(11):2616-2629.
3. Cercato LM, White PAS, Nampo FK, Santos MR V. A systematic review of medicinal plants used for weight loss in Brazil : Is there potential for obesity treatment ? J Ethnopharmacol. 2016;176:286-296.
4. Ahm MR andRahman M. Medicinal plants having anti-obesity potentiality available in Bangladesh : A review. Biol Med Case Rep. 2018;2(1):11.
5. Patra S. Journals Review of Medicinal Plants for Anti-Obesity Activity Abstract. *Transl Biomed*. 2015;6(3):21.
6. Hasani-ranjbar S, Jouyandeh Z, Abdollahi M. A systematic review of anti-obesity medicinal plants - an update. J DibetesMetabDisord. 2013;12:28.
7. Liu TT, Liu XT, Chen QX, Shi Y. Lipase Inhibitors for Obesity: A Review. Biomed Pharmacother. 2020;128:110314.
8. Gholamhoseinian A andShahouzehi B. Inhibitory Effect of Some Plant Extracts on Pancreatic Lipase. Int J Pharmacol. 2010;6(1):18-24.
9. Sharma N, Sharma VK, Seo S. Screening of some medicinal plants for anti-lipase activity. J Ethnopharmacol. 2005;97:453-456.
10. Birari R andBhutani K. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today. 2007; 12:879-889.
11. Simão AA, Marques TR, Marcussi S, Corrêa AD. Aqueous extract of psidiumguajava leaves: Phenolic compounds and inhibitory potential on digestive enzymes. An Acad Bras Cienc. 2017;89(3):2155-2165.
12. Kim J, Kim H, Park H, Youn S, Choi D, Shin C. Development of Inhibitors Against Lipase and a-glucositesfom Derivatives of Monascus Pigment. FEMS MicrobiolLett. 2007;276(1):93-98.
13. Motulsky HJ. *Equation: Michaelis-Menten Model, Curve Fitting Guide*. Accessed December 21, 2019. https://www.graphpad.com/guides/prism/7/curve-fitting/reg_michaelis_menten_enzyme.htm
14. Marche AG, Guiama VD, Carl Moses F. Mbofung. Anti-hyperlipidemic and anti-weight gain effects of khaya tea on high-fat-diet rats. Int J InnovSci Res. 2015; 18(2):224-231.
15. Ugoh SC, Oluwabunmi O,Agarry SAG. Studies on the antibacterial activity of Khayasenegalensis [(Desr.) A. Juss)] stem bark extract on Salmonella enterica subsp. entericaserovarTyphi [(ex Kauffmann and Edwards) Le Minor and Popoff]. Asian Pac J Trop Biomed. 2014;4(Suppl 1):2-6.
16. Yasir M, Das S, Kharya MD. The phytochemical and pharmacological pro fi le of Perseaamericana Mill. Pharmacogn *Rev*. 2010; 4(7):77-85.

17. Ajayi OE, Awala SI, Olalekan OT, Alabi OA. Evaluation of Antimicrobial Potency and Phytochemical Screening of Perseaamericana Leaf Extracts against Selected Bacterial and Fungal Isolates of Clinical Importance. Microbiol Res J Int. 2017;20(1):1-11.
18. Jimenez P, Garcia P, Quitral V, Vasquez K, Parra-Ruiz C, Reyes-Farias M, Garcia-Diaz DF, Robert P, Encina C, Soto-Covasich J. Pulp, Leaf, Peel and Seed of Avocado Fruit: A Review of Bioactive Compounds and Healthy Benefits. Food Rev Int. 2020;00(00):1-37.
19. Idris A, Ndukwe GI, Gimba CE. Preliminary phytochemical screening and Antimicrobial activity of seed extracts of Perseaamericana (avocado pear). Bayero J Appl Sci. 2009;2(1):173-176.
20. Haddad E, Wien M, Oda K, Sabaté J. Postprandial gut hormone responses to Hass avocado meals and their association with visual analog scores in overweight adults: A randomized 3 × 3 crossover trial. *Eat Behav*. 2018;31:35-40.
21. Heskey C, Oda K, Sabaté J. Avocado Intake, and Longitudinal Weight and Body Mass Index Changes in an Adult Cohort. *Nutrients*. 2019;11(3):691.
22. Tramontin NDS, Luciano TF, Marques SDO, de Souza CT, Muller AP. Ginger and avocado as nutraceuticals for obesity and its comorbidities. *Phyther Res*. 2020;34(6):1282-1290.
23. Monika P andGeetha A. The modulating effect of Perseaamericana fruit extract on the level of expression of fatty acid synthase complex, lipoprotein lipase, fibroblast growth factor-21 and leptin - A biochemical study in rats subjected to experimental hyperlipidemia and obesity. *Phytomedicine*. 2015;22(10):939-945.
24. Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. *Diabetes, MetabSyndrObes Targets Ther*. 2014;7:587-591.
25. Siddiqui S. Obesity and diabetes: interrelationship. *Adv Obesity, Weight Manag Control*. 2018; 8(2):155-158.
26. Daniels A, Famurewa O, Olu M, Ea A. Screening of DaniellaOliveri against Three Bacteria and One Fungus. *Res J Pharm BiolChem Sci*. 2013;4(1):939-945.
27. Temitope O, Fasusi O, Ogunmodede A, Thonda AO, Oladejo BO, Yusuf-Babatunde AM, Ige OO. Phytochemical Composition and Antimicrobial Activity of Daniellaoliveri Extracts on Selected Clinical Microorganisms. Int J Biochem Res Rev. 2016;14(1):1-13.
28. Naseer S, Hussain S, Naeem N, Pervaiz M, Rahman M. The phytochemistry and medicinal value of Psidiumguajava (guava). *ClinPhytoscience*. 2018;4(1):32.
29. Lunagariya NA, Patel NK, Jagtap SC, Bhutani KK. Inhibitors of Pancreatic Lipase: State of the Art and Clinical Perspectives. EXCLI J. 2014;13:897-921.