European Journal of Medicinal Plants

33(4): 1-16, 2022; Article no.EJMP.85829
ISSN: 2231-0894, NLM ID: 101583475

Piper nigrum and *Morinda lucida* Possess Antioxidant Capacities and Regulate the Activities of Key Carbohydrate and Lipid Digestive Enzymes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2022/v33i430458

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/85829

Original Research Article

Received 29 January 2022
Accepted 09 April 2022
Published 15 April 2022

ABSTRACT

Introduction: Carbohydrate and lipid digestive enzymes are instrumental in the absorbability of nutrients associated to diabetes and obesity. This study evaluated hydroethanolic extracts of *Piper nigrum* leaf and *Morinda lucida* stem bark for antioxidant capacity and enzymes (carbohydrate and lipid digestive) inhibition.
Methods: Colorimetric assays determined enzyme (α-amylase, α-glucosidase, lipase and cholesterol esterase) inhibition and antioxidant capacity (total phenolic (TPC) and flavonoid (TFC) content, radical scavenging activity (DPPH, ABTS), and ferric reducing antioxidant power (FRAP)) of hydroethanolic ethanolic extracts, ethyl acetate and hexane fractions.
Results: At 1 mg/ml extracts of *P nigrum* and *M lucida* inhibited α- amylase (9.82±1.05 - 36.63±0.69 %) and α-glucosidase (22.47±0.34 - 67.77±0.58 %) activities. At 100 µg/ml extracts and fractions inhibited lipase (56.72±1.11 - 81.61±0.71 %) and cholesterol esterase (18.14 ±0.79 - 36.84±0.70 %) activities. IC_{50} for α- amylase (2.20±0.02 - 7.8±1.42 mg/ml), α-glucosidase

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(0.16±0.01 - 3.74±0.01 mg/ml), lipase (8.58±2.57 - 53.03±5.20 µg/ml) and cholesterol esterase (172.20±5.12 - 419.80±4.55 µg/ml) were registered. At 4 mg/ml, *P. nigrum* presented a higher TPC (153.78±8.31 - 354.63±6.33 mg/ml), TFC (21.65±1.14 -33.86±0.00 mg/ml) than *M lucida* TPC (10.21±0.11 - 169.89±6.54 mg/ml), TFC (ND - 87.32±6.14 mg/ml). *P nigrum* presented radical scavenging (DPPH and ABTS) activity with IC_{50} 0.12±0.00 - 1.27±0.01 mg/ml compared to 1.31±0.02 - 3.44±0.12 mg/ml of *M lucida*. The FRAP IC_{50} values were better for *P nigrum* (3.38±0.14- 4.48±1.05 mg/ml) than *M lucida* (3.34±1.32 - 15.4±2.03 mg/ml).
Conclusion: *P nigrum* presented better antioxidant capacity and more effective on lipid digestive enzymes while *M lucida* was more effective on carbohydrate digestive enzymes.

Keywords: Morinda lucida; Piper nigrum; α-amylase; α-glucosidase; lipase; cholesterol esterase; antioxidant activity.

ABBREVIATIONS

ALCL3 : Sodium tri chloride
ABTS : 2, 2· –bis (3-ethylbenzothiaoline-6-sulfonic acid)
CAT : Catachin
DPPH : 2, 2-Diphenyl-1-picrylhydrazyl
FRAP : Ferric Reducing Antioxidant Power
HCL : Hydrogen chloride
IC50 : Inhibitory Concentration 50
Mg/ml : Milligrams per millilitres
Na2CO3 : Sodium Carbonate
NaNo : Sodium Nitrites
NaOH : Sodium hydroxide
ND : Not Detected
NT : Not Tested
Pcase : Pancreatic cholesterol esterase
pNPG : P-nitrophenol glycopyranoside
PPL : Porcine Pancreatic Lapase
PPA : Pancreatic Alpha Amylase
ROS : Reactive Antioxidant Species
RNS : Reactive Nitrogen Species
SD : Standard Deviation
TFC : Total flavonoid content
TPC : Total Phenolic Content

1. INTRODUCTION

The imbalance between energy intake and expenditure is the major cause of obesity-related diseases, which is are chronic metabolic disorders resulting from altered lipid metabolic processes including lipogenesis and lipolysis [1,2]. A global shift in diet toward increased intake of energy-dense foods that are high in fat and carbohydrates but low in vitamins, minerals, and other micronutrients as well as the increased prevalence of a sedentary lifestyle predispose to obesity [3]. It is also known to increase the risk of breast, colon, prostate, endometrium, kidney and gall bladder cancers. According to World Health Organization (WHO), it is estimated that 2.8 million people die each year and 35.8 million global disability-adjusted life years (DALYs) are due to overweight and obesity [4]. Obesity is associate with increased blood glucose and lipid concentration which are major risk factors for developing chronic diseases such as diabetes mellitus and other complications [5,6,7]. Also, the onset of diabetes and diabetic complications is associated with oxidative stress leading to cellular damage. Additionally, consumption of carbohydrate and fat enriched diets results in a significant increase of postprandial glucose and oxidative stress by the formation of reactive oxygen species through several biochemical pathways [8]. Obese subjects generally possess low antioxidant defence with enhanced levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [9].

Key carbohydrate and lipid metabolizing enzymes can play a role in the management of diabetes and obesity. The enzymes of the pancreatic secretion, mainly, pancreatic alpha-amylase, pancreatic lipase, and pancreatic phospholipase A2 are involve in the hydrolysis of dietary carbohydrate and lipids [10,11,12]. Alpha-amylase catalyses the hydrolysis of the α-bonds of α-linked polysaccharides (starch and glycogen molecules) into absorbable small molecules (maltose and glucose) [13], while α-glucosidase anchored in the mucosal brush border of the small intestine catalyses the endpoint digestion of starch and disaccharide that are abundant in the human diets to maltose and maltotriose [14,15]. The end products of the effects of these two enzymes if absorbed may lead to an increase in blood sugar concentration. Hence, in diabetic condition, inhibition of these two enzymes can play a key role in controlling blood sugar level. Pancreatic lipase is the key enzyme in lipid digestion, responsible for absorption of dietary fats through the breakdown of triacylglycerols into free fatty acids and monoacylglycerols in the intestinal lumen

[12,14,16]. Pancreatic phospholipase A2 serves in the initial digestion of phospholipids to free fatty acids and lysolipids. Considerable evidence from cell and animal studies suggests the importance of pancreatic phospholipase A2 in facilitating the digestion and absorption of lipids. Given the key role these enzymes play in starch and lipid digestion, they represent attractive targets for the prevention of excessive body weight gain and obesity-related diseases including diabetes. Thus, inhibition of these enzymes is one of the therapeutic approaches for diabetes since it can cause retardation of carbohydrate digestion, which leads to the prevention of excess glucose absorption [16]. Alpha-glucosidase inhibitors (acarbose and voglibose) and pancreatic lipase inhibitor (orlistat) are clinically used for treatment of hyperglycaemia and hyperlipidemia respectively [8,17]. However, this has been associated with unpleasant gastrointestinal side effects such as abdominal pain, flatulence, meteorism, and diarrhea [18,19]. Plants or natural products seems to be an alternative source of medicine since it has been shown that some of them possess inhibitors of digestive enzymes that interfere with the hydrolysis and absorption of dietary carbohydrates and lipids. Polyphenols from teas, berries, and other plants have been associated with inhibition of these enzymes in, *In vitro* and in vivo studies. Green tea catechins inhibit the intestinal absorption of lipids in vivo [20,21] by altering the activity of pancreatic phospholipase A2. Procyanidins and cocoa extracts have also been reported to inhibit digestive enzymes with the lavado cocoa extract being the most effective [22]. Hence a possible phytomedicine that can at the same time fight against high blood glucose concentration and hyperlipidaemia may be more effective in controlling these pathologies.

Morinda lucida (*M. lucida*) and *Piper nigrum* (*P. nigrum*) are used in folk medicine for the treatment of diabetes and prevention of weight gain. In traditional medicine *M. lucida* (Benth), specie of Rubiaceae family is used in the treatment of different types of fevers, jaundice, hypertension, cerebral congestion, dysentery, diabetes and gastric ulcer [23,24]. Earlier scientific reports shown that the leaves and the roots of *M. lucida* possess antioxidant and hypoglycaemic/antidiabetic activity [25,26, 27]. Activities of *M. lucida* extracts against *Salmonella typhi* [28], isolated uterine smooth muscle contractility [29], toxicity and mutagenic studies [30,31] have all been reported. *P. nigrum*, belonging to the Piperaceae family and commonly known as black pepper, is used as a spice and in herbal medicine. Traditionally, it's used for the treatment of coughs, intestinal diseases, bronchitis, venereal diseases, colds, and rheumatism [32]. In earlier studies, oral administration of aqueous extract of *P. nigrum* leaf presented antioxidant defence and anti-atherogenic activity [33,34]. A study by Mballa et al. [7] have shown the preventive effect of the hydro-ethanolic extract of the *P. nigrum* leaf on the development of metabolic and cardiovascular changes in cafeteria diet fed rats. Also, fruits and leaves of *P. nigrum* have shown *In vitro* alpha-glucosidase, alpha-amylase and aldose reductase inhibitory activity, increased glucose consumption by adipocytes and exhibited antioxidant activity [35,36]. In vivo studies have shown that *P. nigrum* reduced hyperglycaemia and hyperlipidaemia in alloxan and streptozotocin-induced diabetic rats [36,37].

The present study was undertaken to evaluate the *In vitro* carbohydrate and lipid digesting enzyme inhibitory activities of the hydro-ethanolic extract, ethyl acetate and hexane, of *P. nigrum leaves and M. lucida* stem bark and their antioxidant properties.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *P. nigrum* and stem barks of *M. lucida* were collected in the locality of Djombé-penja, in the Littoral region and Mfou in the Centre region of Cameroon in August 2017 respectively. The identification of these plants was made at the National Herbarium of Yaoundé, Cameroon where the vouchers were kept under the numbers 25818/SFR and 2528/SRFK respectively.

2.2 Extraction and Fractionation of Plant Materials

2.2.1 Preparation of the hydro-ethanolic crude extracts

Leaves and barks collected from their natural environment were washed with tap water, shade dried for two weeks. The samples were separately ground with the help of an electric grinding mill and the powdered material obtained were macerated in a mixture of ethanol and water (70/30) (hydroethanolic) solution for 72 hours at room temperature. The resulting

solutions were then filtered through cheese cloth and Whattman filter paper N°1. The filtrate was concentrated with the aid of a rotary evaporator **(**BÜCHiRI10) at 45°C under reduced pressure and then dry (crude extract) in a hot air oven for 24 hours. The two extracts were then stored at -4°C until used. The yield of the extraction process was calculated according to the following formula:

$$Yield = \frac{\text{Mass of dry extract}}{\text{mass of powder}} \times 100$$

2.2.2 Fractionation

The fractionation was prepared from the crude extract using solvents of increased polarity as follows. The crude extract was each dissolved in 400 mL of hexane and kept for 24 hours. The upper hexane phase was collected, and extraction process was repeated twice with the lower residual phase. The hexane fractions were then pooled and stored as such. The lower phase was then mixed with subsequent solvents (ethyl acetate) and followed the same treatment as hexane fraction. The fractions were concentrated with the aid of a rotary evaporator and further to dry substances through a ventilation drying oven. The yield of each process was calculated and the fractions were stored at 4 °C until required.

2.3 Enzyme Inhibitory Activities of Plant Extracts

2.3.1 *In vitro* α-amylase inhibition assay

The method earlier described by Xiao et al. [38] was used in this assay with slight modification. Into each test tube, 125 µL of 0.02 M sodium phosphate buffer, 500 µL of porcine pancreatic alpha-amylase solution (PPA**)** and 500 µL of plant extracts/fractions at different concentrations (0.05 – 4 mg/mL w/v) were added. The mixture was incubated at 37 °C for 10 min after which 500 µL of starch soluble (1% w/v) was added to each test tube and further incubated at 37 °C for 15 min. Then 200 µL of iodine solution (coloured reagent (5mM I and 5mM KI)) was added for colour development. The reaction was stopped by the addition of 40 µL of HCL to the mixture. The absorbance was read at 620 nm using u/v spectrophotometer. A control test was prepared in same way except that it did not contain the plant extract and its enzyme activity represents 100% activity. The α-amylase inhibitory activity was calculated using the following equation:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

2.3.2 *In vitro* α-Glucosidase inhibition activity

The effect of the plant extracts on α-glucosidase activity was determined according to the chromogenic method described by Kim et al. [39] with slight modifications. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in distilled water. Then, phosphate buffer (20 mM, pH 6.9), 3 Mm of glutathione reduced solution, and sodium carbonate (100 mM) were also prepared while 0.15 units of α-glucosidase (from *Bacillus stearothermophilus*) were pre-incubated with each extract/fraction at different concentrations for 5 minutes. 10 mM of substrate (pNPG) was then added to start the reaction. The reaction mixture was incubated at 37 °C for 20 minutes and stopped by adding 2 mL of 100 mM Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow-coloured p-nitrophenol released from pNPG at 400 nm. Acarbose was used as the reference drug while phosphate buffer was used as control. The α-glucosidase inhibitory activity was calculated using the following equation:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

2.4 Inhibiting Activity of Lipids Digestive Enzymes

2.4.1 Pancreatic lipase inhibition activity

The inhibition of pancreatic lipase was carried out according to the method described by Sharma et al. [40]. Briefly, 80 µL of each extract at different concentrations (3-100 µg/mL) were mixed with 20 µL of porcine pancreatic lipase (PPL, 4 mg/mL) and 90 µL of phosphate buffer, then incubated at 37 °C for 37 min. After incubation, 10 µL of substrate (10 mM p-nitrophenyl butyrate) was added to the mixture and further incubated at 37 °C for 30 min and the absorbance was read at 405 nm using an ELISA microplate reader. Orlistat was used as the reference drug. Percentage of inhibition was calculated using the formula bellow:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Where test = absorbance of sample (plant extract or orlistat), control = absorbance of enzyme + substrate.

2.4.2 Cholesterol esterase inhibition activity

The inhibition of pancreatic cholesterol esterase (PCase) was carried out according to the method described by Adisakwattana et al. [41]. Briefly, 50 µL of each extract at different concentrations (6 - 200 µg/mL) was mixed with 50 µL of 5.16 Mm taurocholic acid and 50 µL of an aqueous solution of cholesterol esterase and incubated for 10 min at 25 °C. Then 50 µL of 0.2 mM p-Nitrophenyl Butyrate solution was added to the mixture and further incubated for 5 min at 25 °C. The absorbance was read at 405 nm using an ELISA microplate reader. Orlistat was used as the reference drug. Percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Where test = absorbance of sample (plant extract or orlistat), control = absorbance of enzyme + substrate.

2.5 *In vitro* Antioxidant Activities of Crude Extracts and Fractions

2.5.1 Total phenolic content

Total phenolic content (TPC) of *P. nigrum leaf and M. lucida* stem bark extract and fractions were analysed using the Folin– Ciocalteu colorimetric assay method described by Li et al. [42] with some modifications. 200 µL of each sample (extracts/fraction/catechin) was added to 1 mL Folin–Ciocalteu phenol reagent (2 N, 10 times diluted). After 4 min, 800 µL of a sodium carbonate solution (1 mM Na_2CO_3, 75 mg/mL) was added in the reaction. The mixture was homogenized and incubated for 2 hours at room temperature. The absorbance was then measured at 765 nm. Standard curve for catechin in the range of 10-100 µg/mL was prepared in the same manner and results were expressed as mg catechin equivalent per gram of extract/fraction (mgCAT/g).

2.5.2 Total flavonoid content

Total flavonoid content (TFC) was determined using the aluminium colorimetric method described by Chang et al. [43] using quercetin as the standard. A calibration curve of quercetin was prepared in the range concentration of 10-100 µg/mL. Briefly 500 µL of extracts, and fractions were placed in different test tubes and to each 1500 µL of distilled water and 150 µL of sodium nitrite ($NaNO_2$) (5%) were added and incubated for 5 minutes at room temperature. After which 150 µL of $AlCl_3$ (10%) was added to the mixture and 6 minutes later 500 µL of 1 M NaOH (4%) was added in the reaction medium. The mixture was homogenized and the absorbance of the solution (pink color) was read at 510 nm. The total flavonoid content was expressed as mg quercetin equivalent per gram of extracts (mgQE/g).

2.5.3 Ferric Reducing Antioxidant Power (FRAP) assay

The quantification of antioxidant compounds reducing ferrous iron was carried out using the method described by Benzie and Strain [44]. The reaction medium consisting of 75 µL sample (extract/ gallic acid) and 2 mL freshly prepared FRAP reagent was mixed and incubated for 15 minutes at room temperature. The absorption of the reaction mixture was measured with spectrophotometer at 793 nm with gallic acid as standard. The result was expressed in mg gallic acid equivalent per gram of extract (mg gallic acid equivalent per g of extract).

2.5.4 DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay

Radical scavenging assay was carried out using DPPH free radical test, employing the method of Blois [45] with some modifications. The initial absorbance of DPPH (0.1 mM) in methanol was measured using spectrophotometer at 517 nm until the absorbance reading stabilized and gallic acid was used as positive control. Briefly in 2 mL of DPPH methanolic solution, 500 µL of extract/fraction or standard was added. After agitation the samples were incubated in a dark room for 30 minutes and the change in absorbance was measured at 517 nm. The percentage inhibition was calculated according to the formula:

$$\% \text{ DPPH Inhibition} = \frac{\text{Absorbance DPPH control} - \text{Absorbance DPPH test}}{\text{Absorbance DPPH control}} \times 100$$

The values of the IC_{50} (The concentration with 50% of inhibition) were calculated from the equation from the linear regression of concentration against percentage inhibition.

2.5.5 ABTS radical scavenging assay

$ABTS^{\bullet}+$- scavenging activity was assessed according to the method described by Arnoe et al. [46] with some modifications. A mixture of ABTS (7 mM) and Potassium persulfate (4.9 mM) in methanol was prepared and stored at room temperature for 24 h in a dark room to produce $ABTS^{\bullet}+$. The solution of $ABTS^{\bullet}+$ (8mL) was diluted with 72 mL of methanol and the

absorbance adjusted to 1.50 at 734 nm. Into 1800 µL of ABTS˙+ solution, 200 µL of extracts/fractions or standard (catechin) solution at different concentrations (0.025 to 2mg/mL a multiple of two increments) were added to the diluted solution. After incubation at room temperature for 15 minutes, the absorbance was read at 734 nm. The radical scavenging activity was calculated as the percentage of inhibition according to the formula:

$$Percentage\ inhibition\ (\%) = \frac{\text{ABTS control} - \text{ABTS sample}}{\text{ABTS control}} \text{X } 100.$$

The IC50 was obtained graphically from the linear regression curve.

2.6. Statistical Analysis

The results are expressed as the mean ± SEM for triplicates using Microsoft Excel 2013, IC_{50} was calculated from linear regression with Graphpad Prism 5 software. Significant difference between groups was evaluated using analysis of variance (ANOVA) followed by Waller Duncan multiple comparison tests. Difference of $p<0.05$ was considered statistically significant.

3. RESULTS

3.1 Inhibitory Activity on Carbohydrate Digestive Enzymes

The inhibitory activities of *P. nigrum* and *M. lucida* crude extracts/fractions against α-glucosidase and α-amylase are presented in Table 1. For the *P. nigrum*, the hydro-ethanol extract showed the best inhibitory activity against α-glucosidase with high percentage inhibition (55.03±0.44) and a low IC_{50} (0.95 mg/mL). Against α-amylase it was the hexane fraction that had a better inhibitory effect as indicated by the IC_{50} (2.20 mg/mL). For the *M. lucida* stem bark, the ethyl acetate fraction had the best inhibitory activity against α-glucosidase with IC_{50} value of 0.16 mg/mL closely followed by the hydro-ethanolic extract. Meanwhile the *M. lucida* stem bark extracts and fractions had a weak inhibitory activity against α-amylase.

Generally, the enzymes inhibitory capacity of the two plants extracts was concentration dependent with the highest concentration portraying the best activity in both enzymes inhibition. The *P. nigrum,* was average in inhibiting both enzymes with the ethyl acetate fractions showed almost the same activities on both enzymes, while the hexane fractions as more effective on the α-amylase. The *M. lucida* stem bark had a better inhibitory activity against α-glucosidase than α-amylase at all tested fractions as indicated by the lower IC_{50} values (0.16 - 0.99 mg/mL) for α-glucosidase compared to α-amylase (7.02 - 26.63 mg/mL).

3.2 Inhibitory Activity on Lipid Digestive Enzymes

The inhibitory activities towards pancreatic lipase and cholesterol esterase are reported in Table 2. All the plant extracts and fractions exhibited anti-lipase activity in a concentration dependent manner with maximum percentage inhibition above 50% obtained at 100µg/mL. However, these anti-lipase activities of *P. nigrum* (hydro-ethanol, IC_{50}, 15.95 µg/mL and ethyl acetate $IC_{50,}$ 15.75µg/mL) were not comparable to orlistat (IC_{50}, 7.48µg/mL). On the other hand, the ethyl acetate fraction of *M. lucida* (IC_{50}, 8.58µg/mL) showed close value to orlistat (IC_{50}, 7.48µg/mL).

At 100 µg/mL orlistat significantly inhibited the cholesterol esterase activity up to 68.64 % with an IC_{50} of 88.30 µg/mL meanwhile for the extracts, the inhibitory activity was weak with very high IC_{50} values. The ethyl acetate fraction of *P. nigrum* (IC_{50}, 172.2µg/mL) showed the best anti-cholesterol esterase activity amongst the rest of the extracts.

3.3 Antioxidant Potential of Plants Extracts

3.3.1 Total phenolic content and total flavonoid content

The total phenolic and flavonoid content of *P. nigrum* extracts/fractions are presented in Table 3. The TPC was higher in the hydro-ethanolic crude extracts (326.1±0.74 mg CAT/g) compared to ethyl acetate (57.85±2.41 mg CAT/g). The TFC was higher in hydro-ethanol crude extracts than ethyl acetate showed.

For *M. lucida*, the TPC and TFC of the tested extracts increase with concentrations (Table 3). The TPC was higher in the hydro-ethanolic crude extracts (169.89±6 mg CAT/g) and decreases with the fractionation. The TFC was only consistent in the hydro-ethanol and ethyl acetate fraction. And hydro-ethanolic extract had the best TFC.

Table 1. Percentage inhibition and inhibitory concentrations of *P. nigrum* and *M. lucida* extracts and fractions on α-amylase and α-glucosidase activities

Concentration (mg/mL)	α-glucosidase (% inhibition)	IC_{50} (mg/mL)	α-amylase (% inhibition)	IC_{50} (mg/mL)	α-glucosidase (% inhibition)	IC_{50} (mg/mL)	α-amylase (% inhibition)	IC_{50} (mg/mL)
	Piper nigrum				***Morinda lucida***			
Hydroethanolic crude extract								
0.031	6.25±0.34	0.95±0.11	4.02±0.45	5.98±0.14	$9.89±0.89^{cγ}$	0.99±0.02	ND	7.02±1.32
0.062	7.01±1.12		7.10±2.10		$11.38±1.95^{cγ}$		$0.80±1.07^{dα}$	
0.125	9.21±2.14		10.32±2.03		$14.16±0.50^{cγ}$		$2.05±0.19^{dα}$	
0.250	$20.62±3.97^{cα}$		$12.18±5.83^{cβ}$		$33.09±3.35^{bγ}$		$3.14±0.55^{dα}$	
0.500	$31.80±4.76^{bα}$		$15.42±1.72^{bβ}$		$34.91±0.62^{bγ}$		$5.63±0.67^{cα}$	
1.000	$50.72±1.54^{aα}$		$19.81±0.86^{bβ}$		$46,61±4.38^{aγ}$		$9.82±1.05^{bα}$	
2.000	$53.07±0.77^{aα}$		$29.88±0.81^{aβ}$		-		$14.33±0.23^{aα}$	
4.000	$55.03±0.44^{aα}$		$32.13±0.51^{aβ}$		-		-	
Ethyl acetate fraction								
0.031	ND	3.74±0.01	ND	3.30 ± 0.02	$09.43±0.58^{eγ}$	0.16±0.01	ND	7.8±1.42
0.062	ND		ND		$33.41±1.08^{dβ}$		$0.67±0.45^{dβ}$	
0.125	ND		ND		$56.72±2.32^{cα}$		$1.16±0.28^{dβ}$	
0.250	$8.49±2.20^{dβ}$		$31.37±7.25^{eα}$		$59.50±1.28^{cα}$		$1.66±0.38^{dβ}$	
0.500	$24.33±4.71^{cβ}$		$33.64±1.97^{cα}$		$63.57±0.70^{bα}$		$5.43±1.60^{cα}$	
1.000	$36.94±2.05^{bβ}$		$36.63±0.69^{bα}$		$67.77±0.58^{aα}$		$8.78±1.93^{bα}$	
2.000	$45.06±2.64^{aβ}$		$47.31±2.33^{aα}$		-		$12.39±1.95^{aα}$	
4.000	$50.33±0.34^{aα}$		$52.06±3.61^{aα}$		-		-	
Hexane fraction								
0.250	$11.37±0.39^{cγ}$	2.77±0.02	$07.43±0.61^{dγ}$	2.20±0.02	$10.34±0.08^{eγ}$	2.97±0.04	ND	
0.500	$14.56±0.44^{cγ}$		$14.89±2.71^{dβ}$		$23.01±1.04^{dβ}$		ND	
1.000	$22.47±0.34^{bγ}$		$23.48±0.82^{cβ}$		$36.62±2.34^{cα}$		ND	
2.000	$33.63±16.76^{aβ}$		$44.03±4.42^{bα}$		$44.50±1.48^{cα}$		ND	
4.000	$38.18±03.76^{aβ}$		$55.45±6.73^{aα}$		$57.10±1.24^{cα}$		ND	

^{a,b,c,d}: concentration effect on the inhibitory capacity of plant extracts and fractions. Means with the same letters (a, b, c, and d) within a column are not significantly different from each other at p>0.05; $\alpha\beta\delta$: compares the enzyme inhibitory capacity of plant extracted with the same solvent. Means designated with the same symbols are not significantly different from one another at p>0.05. ND = not detected

Table 2. Percentage inhibition and inhibitory concentrations of *P. nigrum* and *M. lucida* extracts and fractions on lipase and cholesterol esterase activities

Concentration (µg/mL)	Lipase (% inhibition)	IC_{50} (µg/mL)	Cholesterol esterase (%)	IC_{50} (µg/mL)	Lipase (%inhibition)	IC_{50} (µg/mL)	Cholesterol esterase (%inhibition)	IC_{50} (µg/mL)
	Piper nigrum				***Morinda lucida***			
	Hydro-ethanol crude extract							
3.13	$46.32 \pm 1.36^{c\beta}$	15.95±2.32	ND	419.80±4.55	$35.72 \pm .070^{b\beta}$	53.03±5.20	ND	296.60±4.69
6.23	$49.86 \pm 0.11^{c\beta}$		$6.96 \pm 0.79^{c\gamma}$		$41.05 \pm 0.90^{a\alpha}$		$15.90 \pm 1.86^{d\delta}$	
12.5	$56.15 \pm 1.76^{b\beta}$		$10.29 \pm 0.20^{c\gamma}$		$45.49 \pm 0.90^{a\alpha}$		$21.22 \pm 5.25^{c\delta}$	
25.00	$58.39 \pm 2.51^{b\beta}$		$12.65 \pm 0.14^{c\gamma}$		$49.43 \pm 0.93^{a\alpha}$		$17.45 \pm 3.93^{d\delta}$	
50.00	$59.65 \pm 2.51^{b\beta}$		$14.66 \pm 0.26^{b\gamma}$		$52.74 \pm 0.63^{a\alpha}$		$24.23 \pm 3.25^{c\delta}$	
100.00	$76.56 \pm 3.30^{a\alpha}$		$18.14 \pm 0.79^{b\gamma}$		$56.72 \pm 1.11^{a\alpha}$		$31.29 \pm 1.84^{a\beta}$	
200.00	-		$26.83 \pm 0.69^{a\gamma}$		-		$35.21 \pm 0.02^{a\beta}$	
Ethyl acetate fraction								
3.13	$37.11 \pm 0.88^{c\beta}$	15.75±3.12	ND	172.20±5.12	$43.84 \pm 0.68^{c\beta}$	8.58±2.57	ND	333.20±5.68
6.23	$47.43 \pm 1.16^{b\beta}$		$4.62 \pm 0.71^{d\gamma}$		$53.78 \pm 0.63^{c\beta}$		$12.20 \pm 0.02^{b\gamma}$	
12.5	$55.21 \pm 0.93^{b\alpha}$		$7.72 \pm 0.10^{d\gamma}$		$60.21 \pm 1.09^{b\beta}$		$16.14 \pm 0.16^{b\gamma}$	
25.00	$60.99 \pm 1.00^{b\alpha}$		$10.97 \pm 0.08^{d\gamma}$		$63.00 \pm 1.45^{b\beta}$		$17.99 \pm 0.40^{b\gamma}$	
50.00	$75.49 \pm 0.72^{a\alpha}$		$18.54 \pm 1.07^{c\gamma}$		$64.10 \pm 1.05^{b\beta}$		$24.15 \pm 0.67^{a\beta}$	
100.00	$81.61 \pm 0.71^{a\alpha}$		$36.84 \pm 0.70^{b\beta}$		$73.71 \pm 0.23^{a\alpha}$		$29.68 \pm 0.54^{a\beta}$	
200.00			$54.17\ 0.85^{a\alpha}$				$32.20 \pm 0.93^{a\beta}$	
Orlistat								
3.13	$40.55 \pm 0.07^{c\beta}$	7.48±1.02	ND	88.30±4.21				
6.23	$47.19 \pm 0.70^{b\beta}$		$28.47 \pm 0.30^{c\beta}$					
12.5	$54.52 \pm 0.27^{b\beta}$		$31.25 \pm 0.08^{c\beta}$					
25.00	$78.54 \pm 0.43^{a\alpha}$		$36.18 \pm 0.42^{c\beta}$					
50.00	$91.05 \pm 0.23^{a\alpha}$		$47.33 \pm 0.44^{b\beta}$					
100.00	$97.84 \pm 0.82^{a\alpha}$		$62.83 \pm 0.16^{a\alpha}$					
200.00	-		68.64 ± 2.38^{a}					

*Data are expressed as mean ±SD, n=3. ns=non-significant. Significantly different at *p < 0.05, **p < 0.01, ***p < 0.001 when compared to Control in Dunnett's Multiple Comparison.* ^{a,b,c,d}*: concentration effect on the inhibitory capacity of plant extracts and fractions. Means with the same letters (a, b, c, and d) within a column are not significantly different from each other;* $\alpha\beta\delta$*: compares the enzyme inhibitory capacity of plant extracted with the same solvent. Means designated with the same symbols are not significantly different from one another, ND = not detected, NT: not tested*

Table 3. Total phenolic and total flavonoid content of *Piper nigrum* and *Morinda lucida* extracts and fractions

Conc:(mg/mL)	TPC (mg CAT/g.)	TFC (mg QE/g)	TPC (mg CAT/g)	TFC (mg QE/g)
	Piper nigrum		***Morinda lucida***	
	Hydro-ethanol crude extract			
1.00	108.1±8.49$^{c\beta}$	11.41±0.27$^{c\beta}$	ND	15.03±0.67$^{c\alpha}$
2.00	254.4±9.58$^{b\beta}$	22.23±0.57$^{b\beta}$	8.22±1.66$^{b\alpha}$	42.24±5.45$^{b\alpha}$
4.00	326.1±0.74$^{a\beta}$	33.86±0.00$^{a\beta}$	169.89±6.54$^{a\alpha}$	87.32±6.14$^{a\alpha}$
Ethyl acetate fraction				
1.00	ND	01.09±0.12$^{c\gamma}$	ND	7.74±0.94$^{c\beta}$
2.00	57.85±02.41$^{b\gamma}$	06.98±0.39$^{b\gamma}$	ND	26.53±3.53$^{b\beta}$
4.00	153.78±8.31$^{a\gamma}$	21.65±1.14$^{a\gamma}$	124.61±0.39$^{a\beta}$	61.83 ±1.74$^{a\beta}$
Hexane fraction				
1.00	142.88±3.44$^{c\alpha}$	8.03±3.63$^{c\beta}$	ND	ND
2.00	277.73±3.72$^{b\alpha}$	18.45±7.30$^{b\beta}$	ND	ND
4.00	354.63±6.33$^{a\alpha}$	30.38±6.02$^{a\beta}$	10.21±0.11$^{a\gamma}$	ND

Values are presented as Means ± Standard deviation. Means with the same superscripts (a, b, c, and d) within a column are not significantly different from each other at p>0.05. αβδ: compares the antioxidant capacity of plant extracted with the same solvent. Means designated with the same symbols are not significantly different from one another at p>0.05; TPC: Total phenolic contents; TFC: Total flavonoid contents; ND = Not detected

Table 4. Free radical scavenging activities and iron reducing power of extracts/fractions

Concentration (mg/mL)	DPPH (%)	IC_{50} (mg/mL)	ABTS (%)	IC_{50} (mg/mL)	FRAP (mgGAE/g)	IC_{50} (mg/mL)	DPPH (%)	IC_{50} (mg/mL)	ABTS (%)	IC_{50} (mg/mL)	FRAP (mgGAE/g)	IC_{50} (mg/mL)
				Piper nigrum						***Morinda lucida***		
					Hydro-ethanol crude extract							
0.25	33.10 ±0.73$^{e\gamma}$	1.12±0.02	46.51 ±1.35$^{d\beta}$	0.28±0.01	09.85±0.87$^{e\gamma}$	4.18±0.22		1.80±0.01		1.31±0.02		13.9±2.01
0.50	43.60 ±0.95$^{d\gamma}$		63.53 ±2.01$^{c\beta}$		12.49±0.26$^{d\gamma}$		33.80 ±0.92$^{d\alpha}$		36.64 ±1.93$^{d\alpha}$		7.57±10.05$^{c\alpha}$	
1.00	50.40 ±1.94$^{c\gamma}$		92.75 ±0.47$^{b\beta}$		18.45±0.17$^{c\gamma}$		43.99 ±3.14$^{c\alpha}$		52.53 ±0.72$^{c\alpha}$		9.28±0.35$^{c\alpha}$	
2.00	57.20 ±0.83$^{b\gamma}$		95.80 ±0.40$^{a\beta}$		29.20 ±0.18$^{b\gamma}$		65.04 ±0.56$^{b\alpha}$		68.25 ±3.33$^{b\alpha}$		4.13±1.85$^{b\alpha}$	
4.00	59.7 ±0.36$^{a\beta}$		95.92 ±0.33$^{a\beta}$		47.62 ±0.51$^{a\beta}$		79.09 ±0.16$^{a\alpha}$		88.58 ±0.76$^{a\alpha}$		21.35±2.40$^{a\alpha}$	
Ethyl acetate fraction												
0.25	39.07 ±0.10$^{e\beta}$	0.45±0.11	18.47 ±0.11$^{e\gamma}$	0.96±0.15	9.02±0.44$^{e\gamma}$	4.48±1.05		2.56±0.10		2.23±0.11		15.4±2.03

Concentration (mg/mL)	DPPH (%)	IC_{50} (mg/mL)	ABTS (%)	IC_{50} (mg/mL)	FRAP (mgGAE/g)	IC_{50} (mg/mL)	DPPH (%)	IC_{50} (mg/mL)	ABTS (%)	IC_{50} (mg/mL)	FRAP (mgGAE/g)	IC_{50} (mg/mL)
				Piper nigrum						***Morinda lucida***		
Hydro-ethanol crude extract												
0.50	51.20 ±0.55$^{d\beta}$		35.49 ±1.90$^{d\gamma}$		11.47±0.46$^{d\gamma}$		25.67 ±0.51$^{e\beta}$		27.13 ±0.61$^{d\beta}$		7.87±0.04$^{d\alpha}$	
1.00	62.17 ±0.16$^{c\beta}$		58.88 ±1.94$^{c\gamma}$		17.32±0.48$^{c\gamma}$		37.87 ±1.07$^{c\beta}$		39.36 ±0.95$^{c\beta}$		9.98±0.16$^{c\alpha}$	
2.00	77.34 ±0.61$^{b\beta}$		84.60 ±2.02$^{b\gamma}$		27.74±2.04$^{b\gamma}$		55.83 ±2.95$^{b\beta}$		58.52 ±1.65$^{b\beta}$		13.03±0.14$^{b\alpha}$	
4.00	83.87 ±0.90$^{a\alpha}$		95.61 ±10.16$^{a\beta}$		44.73±0.45$^{a\gamma}$		71.77 ±0.51$^{a\beta}$		80.69 ±0.13$^{a\beta}$		20.49±0.31$^{a\alpha}$	
Hexane fraction												
0.25	33.13 ±0.73$^{e\gamma}$	1.27 ±0.01	53.76 ±1.07$^{e\alpha}$	0.12±0.00	11.18±0.29$^{e\beta}$	3.38±0.14	3.44±0.12		3.29±0.16			3.34±1.32
0.50	43.01 ±0.95$^{d\gamma}$		73.43 ±1.22$^{d\alpha}$		15.27±0.47$^{d\beta}$		22.54 ±0.99$^{d\gamma}$		22.95 ±1.15$^{d\gamma}$		21.05±1.11$^{d\gamma}$	
1.00	50.42 ±1.94$^{c\gamma}$		92.85 ±0.29$^{c\beta}$		22.44±0.86$^{c\beta}$		31.44 ±1.89$^{c\gamma}$		30.79 ±1.19$^{c\gamma}$		28.97±1.14$^{c\gamma}$	
2.00	57.16 ±0.83$^{b\gamma}$		94.40 ±0.30$^{b\beta}$		33.70±3.96$^{b\beta}$		45.41 ±1.10$^{b\gamma}$		43.26 ±3.05$^{b\gamma}$		40.24±3.01$^{b\gamma}$	
4.00	59.70 ±0.36$^{a\beta}$		95.99 ±0.04$^{a\beta}$		57.04±1.76$^{a\beta}$		61.87 ±2.66$^{a\gamma}$		66.11 ±1.45$^{a\gamma}$		66.12±1.15$^{a\gamma}$	

a,b,c,d: concentration effect on antioxidant capacity of plant extracts. Means with the same letters (a, b, c, and d) within a column are not significantly different from each other at p>0.05. αβδ: compares the antioxidant capacity of plant extracted with the same solvent. Means designated with the same symbols are not significantly different from one another at p>0.05. nt: not tested

3.3.2 Free radical scavenging activities and iron reducing power of extracts/fractions

The free radical scavenging activities and iron reducing power of extracts/fractions were recorded in Table 4. The free radical scavenging activities of *P. nigrum* extract/fractions with respect to DPPH is lower compared to ABTS (Table 4). This is confirmed by their IC_{50} values that vary from 0.45 to 1.27 mg/mL for DPPH and from 0.01 to 0.96 mg/mL for ABTS. The exception was in the ethyl acetate fraction whose IC_{50} for DPPH (0.45 mg/mL) was better than its IC_{50} for ABTS (0.96 mg/mL). The FRAP did not vary much between extract and fractions of *P. nigrum* though the hexane fraction had the best activity.

In *M. lucida* the IC_{50} values for DPPH and ABTS did not differ much although it varied between extract and fractions. The DPPH and ABTS IC_{50} values for the hydro-ethanolic crude extract (1.8 mg/mL and 1.31 mg/mL), ethyl acetate (2.56 mg/mL and 2.23 mg/mL) and hexane fractions (3.44 mg/mL and 3.29 mg/mL) were obtained respectively. The hydro-ethanolic extract of *M. lucida* had the best radical scavenging activities while the hexane extract had the least activity. The hexane fraction had the best FRAP presenting an inversely proportional relationship between FRAP and the free radical scavenging activity.

4. DISCUSSION

The present data discussed the effect of extracts and fractions from *P. nigrum* and *M. lucida* on inhibition of α-amylase, α-glucosidase, lipase and cholesterol esterase as an attempt in the management of diabetes and obesity. Carbohydrate and lipid rich diets are directly related to increase in blood glucose and lipid profiles. Hence, inhibition of major carbohydrate and lipid metabolizing enzymes prevents increase in blood glucose and lipid concentrations and regulates their concentration to standard for smooth functioning of the physiological system [16]. It was in this regard that *M lucida* and *P nigrum* played a great role in maintaining the blood glucose and lipid profiles towards normal.

In an earlier study, aqueous and ethanol extract of the stem bark of *M. lucida* demonstrated hypoglycemic properties on diabetic rat model [25,47]. Similarly, Odutuga et al. [48] reported the anti-hyperglycemic of ethanol stem/bark extract of *M. lucida* in alloxan-induced diabetic rats. In addition to anti-hyperglycemic activity, Adeneye *et al* [47,49] reported anti-hyperlipidemic effects in experimental rats and suggested that these properties may probably mediate enhanced peripheral glucose utilization and/or enhanced insulin secretion. The present study revealed the anti-hyperglycemic activity of *M. lucida* which may be possible by regulating the activity of main carbohydrate digestion enzymes (alpha amylase and alpha glucosidase). This is one of the major ways in delaying the breaking down of carbohydrate in the small intestine and hence reducing the postprandial blood glucose increase in persons suffering from diabetes [16,47]. Similarly, *P. nigrum* leaf extract showed *In vitro* α-glucosidase inhibitory activity with the hydroethanolic extracts being the most effective (IC_{50}, 0.98). Magaña-Barajas et al. and Pereira et al. [35,36] earlier reported the α-amylase and α-glucosidase inhibitory activities of *P. nigrum*.

Adipose tissues remain the major site of energy storage and homeostasis in the body. The accumulation of fat in the adipose tissues is one of the aetiologies of obesity. Delaying dietary fat digestion and absorption may play a role in the prevention of hyperlipidemia and hence obesity [8]. Pancreatic lipase is the most important enzyme responsible for the digestion of dietary fat. Hence, inhibition of this enzyme will slow down the deposition of fat into adipose tissue and suppression of weight gain which is of beneficial effects to overweight and obesity [12]. The present study demonstrated that all extract concentration inhibited pancreatic lipase and cholesterol esterase substantially. This inhibition may have an effect on hyperlipidemia due to delayed hydrolysis of dietary triglyceride and cholesterol esters. Irrespective of the concentration tested the hydroethanolic extract and fractions of *P. nigrum* and *M. lucida* were found to inhibit lipase and cholesterol esterase. This may also explain earlier results [50,51] that *P. nigrum* reduced obesity markers in high fat diet fed rats and have hypolipidemic activity. The effects of *M. lucida* on lipase showed that there is no significant different between the ethyl acetate fraction and the reference drug orlistat. Similarly, earlier studies did not show any difference between *M lucida* extracts and referenced drug (Orlistat), which was used as positive control [52].

The antioxidant potentials of *P. nigrum* and *M lucida* were equally evaluated in this study. This

may be useful to fight against oxidative stress related to diabetes and obesity complications. Several reports have shown that there is a positive correlation between the effects of polyphenols and flavonoids content on inhibitory potentials of α-amylase and α-glucosidase [12,53]. The results of the quantification of bioactive compounds present in *M. lucida* and *P. nigrum* extracts showed that they contain high amount of total phenolic and flavonoids compounds [32,35,54], Phenolic compounds and flavonoids have been reported to show antioxidant activity in biological systems and acting as scavengers of singlet oxygen, free radicals [55] and have the ability to inhibit pancreatic α-amylase, α-glucosidase, pancreatic lipase activity and pancreatic cholesterol esterase [8,56]. Following the analysis of the *In vitro* antioxidant results, it's found that, extracts from *P. nigrum* leaf and *M. lucida* bark showed antioxidant activity. The ethyl acetate fraction from *P. nigrum* shows DPPH radical inhibition these results agreed with the earlier report of Asimi et al. [57], where ethyl acetate and water extract of black pepper showed a concentration-dependent increase in DPPH radical scavenging ability. Mariutti et al. [58], also reported that ethanol extract of black pepper scavenged DPPH with an IC_{50} value of 110±2 g/kg. Other species of Piper equally possess antioxidant activity as reported by Ramesh et al [59]. The linearity between total phenol of *P. nigrum* extracts and their antioxidant activity has earlier been reported [60,61]. All plants extracts and fractions from *M. lucida* showed antioxidant capacity. The highest antioxidant capacity was marked by the hydro-ethanol crude extract. These results are like those reported earlier by other researcher [53] that the antioxidant activity of the ethanol extract of *M. lucida* is linked to its richness in total polyphenols and flavonoids content. The polyphenolic compounds have an antioxidant activity linked to the redox properties which enable them to act as reducing agents, metal chelators and as free radical scavengers due to the presence of many hydroxide groups that can react with free radicals [62]. *M. lucida* is reported to contain high levels of flavonoids, alkaloids, tannins and saponin and the presence of these compounds has been linked to hypoglycaemic and anti-diabetic activity of the plant [63].

5. CONCLUSION

Morinda lucida and *Piper nigrum* possess inhibitory activity against major carbohydrate and lipid digestive enzymes, which may automatically regulate hyperglycaemia and hyperlipidemia. Also, the study revealed the antioxidant capacity of these plants which may respond to postprandial oxidative stress related to consumption of lipid and carbohydrate rich meal. The enzyme inhibitory activity and antioxidant properties exhibited by *M. lucida and P. nigrum* is attributed to the phenolic compounds and flavonoids present in the different extracts. The remarkable inhibition of these plants extracts, and fractions indicate their potential use as important and convenient sources of anti-obesity and anti-diabetic agents.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analysed during the study are included in the manuscript without any restriction.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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