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ANTI-OBESITY POTENTIALS OF METHANOL EXTRACTS OF *PHRAGMANTHERA INCANA* LEAVES HEMI-PARASITIC ON GUAVA, CASHEW, KOLANUT AND MANGO TREES IN HIGH-FAT DIET-INDUCED OBESE RATS

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ABSTRACT: *The anti-obesity potential of methanol extracts of Phragmanthera incana leaves hemi-parasitic on guava Psidium guajava (PIPG), cashew Anacardium occidentale (PIAO), mango Mangifera indica (PIMI) and kolanut Cola acuminata (PICA) trees were evaluated. Thirty high-fat diet-induced rats were grouped into six; four experimental, negative control and positive controls were orally administered lipid emulsion (5 mL/kg). Experimental received 400 mg/kg body weight from each of the four methanol extracts in addition to the lipid emulsion, positive control received 120 mg/kg bw Orlistat in addition to lipid emulsion while negative control received lipid emulsion alone. Blood samples were collected from ophthalmic venous plexus at 0, 90, and 180 minutes to determine plasma pancreatic lipase (PL) activity, alpha amylase activity and lipid profiles. PL inhibitory activity of the four methanol extracts showed that methanol extracts of PICA and PIAO had greater than 50% inhibition at 400 µg/mL. The α-amylase inhibitory activity of PICA was significantly higher ($p < 0.05$) when compared with PIAO, PIPG and PIMI. A significant decrease ($p < 0.05$) in total cholesterol, low density Lipoprotein-cholesterol levels and atherogenic index of plasma of PICA when compared with other treatment groups after 180 minutes of extracts administration was observed. Methanol extract of PICA was found to exhibit higher inhibitory pancreatic lipase and α-amylase activities and higher hypocholesterolemic activity when compared with those of guava (PIPG), cashew (PIAO) and mango (PIMI). This indicates that methanol extract of P. incana leaves could serve as a source of phyto-compounds that could be developed as antiobesity drugs.*

KEYWORDS: Anti-obesity, High-fat-diet, Lipid lowering, Pancreatic lipase, *Phragmanthera incana*

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INTRODUCTION

Phragmanthera incana leaves are species of mistletoes of the family *Loranthecaea* found in South-Western part of Nigeria. *P. incana* leaves are rich sources of dietary elements essential for biochemical processes and body metabolism (Adeyemi & Osilesi 2022). It is referred to as 'Afomo Onishana' and used ethnomedicinally to treat diseases such as diabetes, hypertension, and reduction of body weight (Adeyemi et al., 2022). *P. incana* leaves contains chemical constituents with different medicinal properties which could validates their ethnomedicinal claims as 'all heals' (Adeyemi, 2023). The leaves have immense potential to be used as a prospective target for drug formation because of its diverse arrays of bioactive compounds present in them (Adeyemi et al., 2023; Ogunmefun et al., 2013). The antioxidant profile of methanol extracts of *P. incana* leaves showed that the extracts are rich source of antioxidant which can potentiate its antioxidant potential *in vivo* as well as serve nutritional and therapeutic purposes (Adeyemi & Osilesi, 2023). The antidiabetic (Sanni et al., 2019), antimicrobial (Ogunmefun et al., 2015), antihypertensive (Adedapo et al., 2020) activities have been scientifically validated but the anti-obesity effects of the leaves is yet to be scientifically validated. Therefore this study evaluates the anti-obesogenic potential of methanol extracts of *P. incana* leaves hemi-parasitic on guava (*Psidium guajava*), cashew (*Anacadium occidentale*), mango (*Mangifera indica*) and kolanut (*Cola acuminata*) trees.

Figure 1: *Phragmanthera incana* leaves

METHODOLOGY

Collection and Preparation of Plant Materials

Fresh leaves of *Phragmanthera incana* from four host trees, guava, cashew, mango and kola nut trees, were collected from their natural habitat in a forest at Imota, Ikorodu Local Government Area of Lagos State Nigeria. It was identified and authenticated at Forest Herbarium of Forest Research Institute (FRIN) Ibadan. The leaves were washed under running water to remove debris and contaminants and air dried separately for one week. The dried leaves were pulverized using a mechanical grinder and stored in an airtight container until further use. The pulverized samples were extracted for 48 h by cold maceration using 70% methanol at 1:6 w/v. The mixture was filtered using Whatman filter paper no. 1, and then concentrated in a rotary evaporator at 40°C. The extracts obtained were stored below 4^{o}C until further use.

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Determination of in vitro pancreatic lipase inhibitory activity of methanol extracts of *P. incana* leaves

In vitro pancreatic lipase activity was determined by measuring the hydrolysis of *p*-Nitrophenyl Butyrate (*p*-NPB) to *p*-nitrophenol using a method previously reported by Jeong et al. (2014) and Jo et al. (2017). Orlistat was used as a positive control.

Determination of in vivo Pancreatic Lipase, α-Amylase Activities and Lipid Profiles

Thirty HFD fed rats were grouped into six; four experimental, negative control and positive control. The high-fat diet given to the animals were compounded according to Kim et al. (2005) with slight modification, with 40% fat. They were deprived of food overnight and orally administered lipid emulsion (5 mL/ kg). The experimental received 400 mg/kg bw from each of the four extracts in addition to the lipid emulsion, the positive control received 120 mg/kg bw Orlistat in addition to lipid emulsion while the negative control received lipid emulsion alone. The lipid emulsion was prepared with 7 mL of olive oil, 93 mg of cholic acid, and 7 mL of deionized water as described by Jo et al (2017). Food was withheld during the test. Blood samples were collected from the ophthalmic venous plexus at 0, 90, and 180 minutes using heparinized capillary tube and centrifuged at 6300 rpm for ten minutes and used to determine plasma pancreatic lipase activity, alpha amylase activity and lipid profiles using Fortress Diagnostic Kits United Kingdom. All experimental procedures were performed according to the guidelines of the National Institute of Health (NIH, 2010). Ethical protocols were strictly adhered to as approved by Babcock University Health and Research ethics committee (BUHREC 771/18).

Determination of Total Cholesterol, triglyceride and atherogenic index of plasma

Plasma total cholesterol and Triglyceride (TG) were determined following the procedure reported by Richmond, (1973). High Density Lipoprotein-Cholesterol (HDL-Cholesterol) was determined following the procedure reported by Friedewald et al., (1972) using Fortress diagnostic kits while LDL-C was calculated using de Cordova formula;

LDL Cholesterol (mg/dl) = 3/4{Total Cholesterol – HDL-Cholesterol} (de Cordova & de Cordova, 2013). The atherogenic index of plasma (AIP), defined as Logarithm [log] of the ratio of plasma concentration of TG to HDL-C was estimated according to Khazal, (2014).

AIP = Log {TG/HDL-C}

Statistical Analysis

The results were expressed as the Mean ± Standard Deviation (SD) and analyzed using repeated measures analysis of variance (ANOVA) with Dunnett's multiple comparison. Regression analysis was performed to calculate IC_{50} values, $p < 0.05$ were considered as statistically significant using GRAPHPAD PRISM® version 9.0, GraphPad Software Inc., San Diego, CA, USA.

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RESULTS

In vitro Pancreatic Lipase Inhibitory Activity of Methanol Extracts of *P. incana* leaves

Pancreatic Lipase (PL) inhibitory activity of the four methanol extract showed that methanol extracts of *P. incana* leaves from kolanut (PICA) and *P. incana* leaves from Cashew (PIAO) had greater than 50% inhibition at 400 µgm/mL. Methanol extract of PICA exhibited the most potent inhibition among the extracts with an EC_{50} = 76.90 followed by methanol extract of PIAO with an EC_{50} = 86.01. (Figure 1)

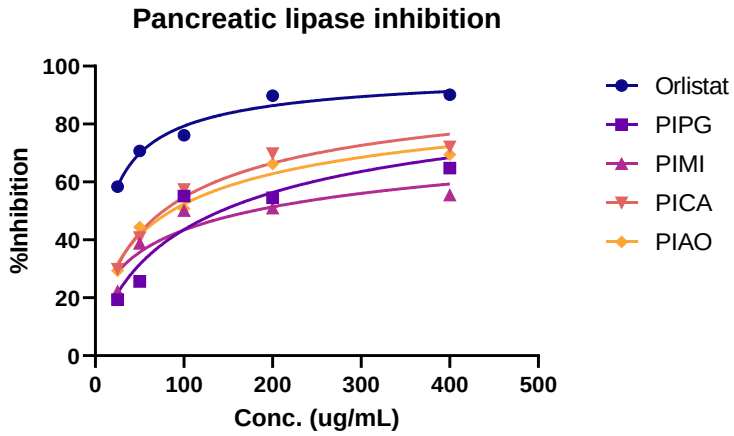

Figure 1: *In vitro* pancreatic lipase inhibition of Orlistat and methanol extracts of *P. incana* leaves

Conc – Concentration

PIPG – extract of *P. incana* leaves from Guava

PICA – extract of *P. incana* leaves from Kolanut

PIAO – extract of *P. incana* leaves from Cashew

PIMI – extract of *P. incana* leaves from Mango

In Vivo Percentage Inhibition of Pancreatic Lipase (PL) Activity of Methanol Extracts of *P. incana* Leaves in HFD-fed Rats

There was a significant increase ($p < 0.05$) in percentage inhibitory activity of methanol extracts of *P. incana* leaves on high-fat fed rats at different time intervals. Methanol extracts of *P. incana* leaves from kolanut (47.37, 69.76, 72.16% at 0, 90 and 180 minutes respectively) and cashew (46.77%, 66.17%, 69.46% at 0, 90 and 180 minutes respectively) exhibited a significantly higher ($p < 0.05$) percentage inhibition when compared with other methanol extracts.

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Inhibition of α -Amylase Activity by Methanol Extracts of *P. incana* Leaves

The standard, orlistat had the highest percentage inhibitory α-amylase activity ($p < 0.05$), (IC_{50} = 451.8) followed by *P. incana* (C. *acuminata*) (IC_{50} = 315.6) < *P. incana* (*A. occidentale*) (IC_{50} = 229.9) < *P. incana* (*P. guajava*) (IC_{50} = 193.9) and *P. incana* (*M. indica*) (IC_{50} = 143.6) (Table 1). The α-amylase inhibitory activity of methanol extract of PICA was significantly higher ($p < 0.05$) when compared with methanol extracts of PIAO, PIPG and PIMI.

Table 1: IC_{50} α-amylase inhibitory activity of HFD-fed rats treated with Orlistat and methanol extracts of *P. incana* leaves

Assay	Extract	IC_{50}	Concentration (mg/kg)
Alpha-Amylase inhibitory activity	Control	88.21	400
	Orlistat	451.8[a]	
	PIPG	193.9[d]	
	PICA	315.6[b]	
	PIAO	229.9[c]	
	PIMI	143.6[d]	

Values on the same column with different superscript are significantly different from each other at $p < 0.05$

IC_{50} - half maximal inhibitory concentration

PIPG – extract of *P. incana* leaves from *P. guajava*

PICA – extract of *P. incana* leaves from *C. acuminata*

PIAO – extract of *P. incana* leaves from *A. occidentale*

PIMI – extract of *P. incana* leaves from *M. indica*

Lipid Profiles Levels of HFD-Fed rats Treated with Methanol Extracts of *P. incana* Leaves

The levels of triglycerides of rats treated with Orlistat and methanol extracts increased after 90 minutes and decreased after 180 minutes (Figure 3). After 180 minutes of extracts administration, there was a significant decrease ($p < 0.05$) in total cholesterol in the PICA group when compared with other treatment groups. An increase in the level of HDL-C after 180 minutes of extracts administration. A significant increase ($p < 0.05$) in HDL-C levels was observed in PIAO after 90 and 180 minutes. At 180 minutes there was a significant increase ($p < 0.05$) in HDL-C levels in PICA when compared with standard drug and other treatment groups (PIPG, PIMI and PIAO). A decrease in the level of LDL-C after 180 minutes of extracts administration (figure 3). A significant increase ($p < 0.05$) in LDL-C levels was observed after 90 minutes in the four treatment groups. At 180 minutes there was a significant decrease ($p < 0.05$) in LDL-C levels in methanol extracts of PICA when compared with Orlistat and other treatment groups (methanol extracts of PIPG, PIMI and PIAO) (Figure 3). The Atherogenic Index of Plasma (AIP) of Orlistat and methanol extracts of *P. incana* leaves decreases after 180

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minutes of extracts administration. There was a significant decrease ($p < 0.05$) in the AIP values for Orlistat and all methanol extract of PICA leaves (Figure 3).

DISCUSSION

The results suggest that methanol extract of *P. incana* leaves possess pancreatic lipase inhibitory effects *in vitro* and *in vivo*. The Pancreatic Lipase (PL) activity of methanol extracts of *P. incana* leaves against triglycerides *in vivo* showed that there was a significant increase in percentage inhibitory activity at different time intervals. It has been established that agents with more than 50% PL inhibitory activity are shown to be potent anti-obesity agents (Jo et al., 2017). The greater than 50% inhibition shown by methanol extracts of PICA, PIPG, PIAO and PIMI indicates that these extracts contain bioactive compounds with anti-obesity effects. The percentage inhibition of the *P. incana* methanol extracts which were higher than 50% shows that the extracts could prevent the hydrolysis of total dietary fats being secreted into the duodenum via the duct system of the pancreas as pancreatic lipase, which is responsible for the hydrolysis of 50-70% of dietary fat (Birari & Bhutani, 2007; Sharma & Karmar, 2018). Methanol extract of *P. incana* leaves from kolanut exhibited the most potent inhibition followed by *P. incana* leaves from cashew when compared with the other test extracts.

A putative link between complex carbohydrate metabolism in the gut as well as its association in early onset of obesity through the inhibition of α-amylase have been previously reported (Marcovecchio et al., 2016; Viljakainen et al., 2015). The α-amylase inhibitory effect shown by the methanol extracts of *P. incana* leaves indicates that methanol extract of *P. incana* leaves from kolanut is the most potent alpha-amylase inhibitor. Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lowering postprandial glucose levels. Alpha-Amylases hydrolyzes complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α-glycosidase to monosaccharide which are absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels (El-kaissi & Sherbeeni, 2011).

The changes observed in triglycerides, total cholesterol, High Density Lipoprotein-Cholesterol (HDL-C) and Low Density Lipoprotein-Cholesterol (LDL-C) of HFD-fed rats treated with methanol extracts of *P. incana* leaves were generally moderate. The ability of the extracts to bind and block the activity of endogenous lipases released by the pancreas in response to fat intake thereby inhibiting the breakdown of fat molecules and a reduced secretion of chylomicrons which eventually decreased the absorption of triglycerides as previously reported (Punit & Kashyap, 2015) for the binding activity of the standard drug Orlistat. In contrast to LDL-C, the changes observed in HDL-C, total cholesterol were minor and do not point to a clear direction regarding their associations to cardiovascular outcome, which might be due to the relatively short duration of the study. Significant reduction of total cholesterol, LDL-cholesterol, triglyceride has been demonstrated and associated with improvements in cardiovascular risks and related outcomes. Low density lipoprotein-Cholesterol is the primary target of therapy for preventing artherosclerotic cardiovascular disease (ASCVD). Triglycerides are esters of the trihydric alcohol glycerol with three long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food. The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

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Atherogenic index of plasma (AIP) is a critical index that can be used as a stand-alone index for cardiac risk estimation and can act as an adjunct over individual lipid profiles (Khazal, 2014). Atherogenic index of plasma was found to be one of the strongest markers in predicting the cardiovascular disease (CVD) risk (Myat et al., 2018), the best determinant for fractionated esterification rate of HDL-C and more useful than routine lipid parameters. It can be used as a diagnostic indicator when the other atherogenic risk parameters appear normal. All the methanol extracts showed a low atherogenic index over the period examined.

CONCLUSION

Methanol extracts of *P. incana* leaves from cashew, guava, mango and kolanut exhibited abilities to inhibit pancreatic lipase and α-amylase activities as well as a significant reduction in the level of blood lipids. Methanol extracts of *P. incana* leaves from kolanut (PICA) was found to exhibit higher inhibitory of pancreatic lipase and α-amylase activities as well as higher hypocholesterolemic activity when compared with those of guava, cashew and mango. This indicates that methanol extract of *P. incana* leaves could serve as a source of phyto-compounds that could be developed as antiobesity drugs. However, further studies should be carried out on various fractions of methanol extracts of *P. incana* leaves from kolanut to identify the fractions with the highest anti-obesity potential.

Conflict of Interest

The authors declared that there is no conflict of interest

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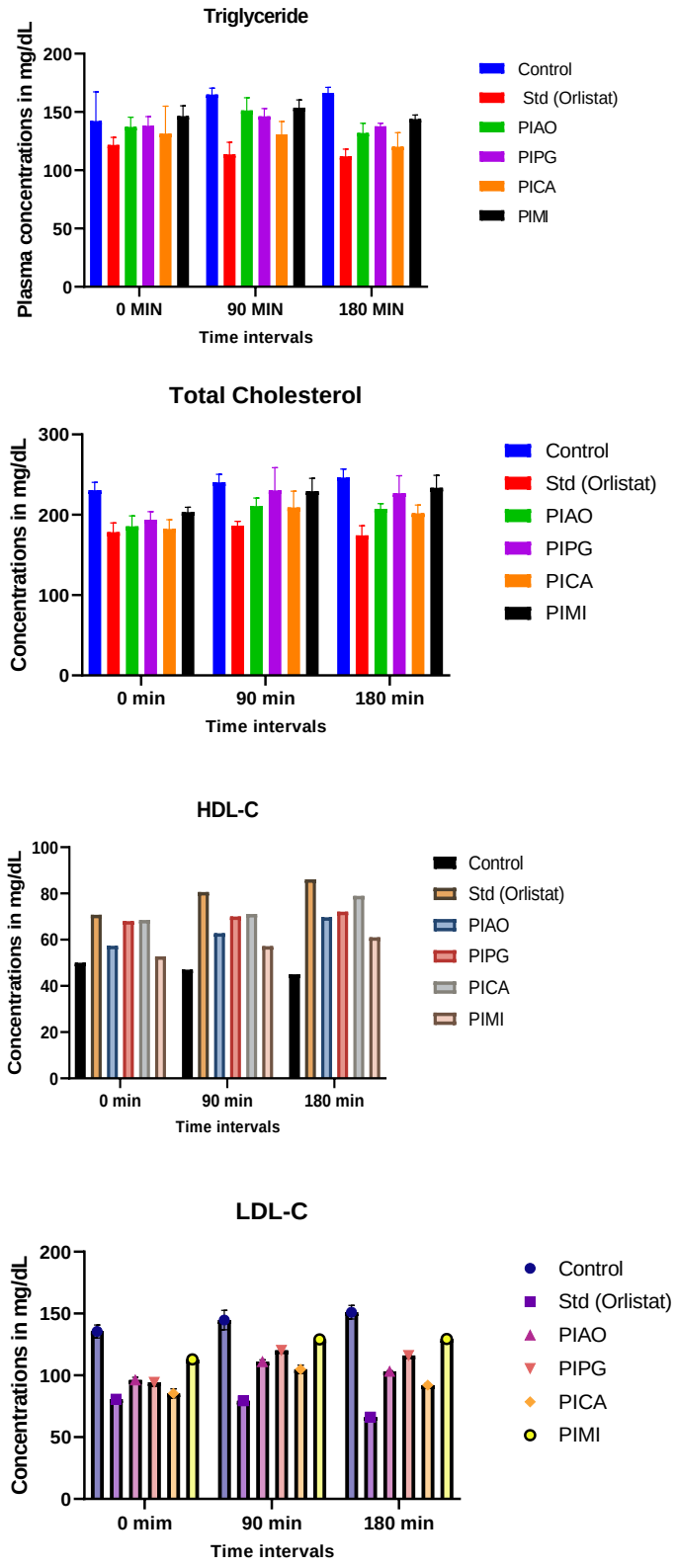

Figure 3: Lipid profiles levels of HFD-fed rats treated with Methanol extracts of *P. incana* leaves

PIPG – extract of *P. incana* leaves from *P. guajava*

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PICA – extract of *P. incana* leaves from *C. acuminata*

PIAO – extract of *P. incana* leaves from *A. occidentale*

PIMI – extract of *P. incana* leaves from *M. indica*

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