

1 **COMPARATIVE HYPOLIPIDAEMIC EFFECT OF AQUEOUS EXTRACT OF**
2 ***PSIDIUM GUAJAVA* LEAVES AND ASCORBIC ACID IN MALE RATS**

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8 **ABSTRACT**

9 Hypolipidaemic properties of 200mg/kg of aqueous extract of *Psidium guajava* leaves (AEPGL)
10 and ascorbic acid (vitC) in male albino *Wistar* rats was carried out in this study. The AEPGL and
11 vitC were respectively administered orally to the rats in test groups for four weeks, and the
12 hypolipidaemic properties determined from their effect on serum lipid profile, using serum total
13 cholesterol(TC), triglyceride(TG), low-density lipoprotein cholesterol(LDL-C), very low-density
14 lipoprotein cholesterol(VLDL-C), high density lipoprotein cholesterol(HDL-C), TG/HDL-C and
15 atherogenic index of plasma (AIP). The results showed that AEPGL and vitC, respectively,
16 significantly increased ($P \leq 0.05$) the HDL-C level, and decreased the TC, TG, LDL-C, VLDL-
17 C, TG/HDL-C and AIP in rats. However, the percentage increase in serum HDL-C, and decrease
18 in serum TC, TG, (LDL-C), VLDL-C, TG/HDL-C and AI associated with administration of
19 AEPGL was observed to be higher than that recorded for vitamin C. The results of this study
20 therefore suggested that AEPGL and vitC possess hypolipidaemic, hence antiatherogenic,
21 properties in male rats; and that the implicated hypolipidaemic and antiatherogenic properties of
22 AEPGL is higher than that of vitamin C. The observations reported in this study support the local
23 use of *P. guajava* leaves in the management of hypertensive conditions.

24 Key words : *Psidium guajava*, ascorbic acid, hypolipidaemia, antiatherogenicity.

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29 INTRODUCTION

30 Guava (*Psidium guajava*) is a common shade tree or shrub planted in most door-yard gardens in
31 the tropics. Its' leaves are known to contain vitamin C and other phytochemicals with antioxidant
32 properties (Begum et al., 2002a,b; Suntornsuk et al., 2002; Qian and Nihorimbere, 2004).
33 However, quercetin has been reported to be the main active constituent in the plant, and that the
34 spasmolytic and antidiarrheal effects of *P. guajava* leaves decoctions utilized in the treatment of
35 various gastrointestinal disorders are associated with its quercetin-derived flavonoid glycosides
36 (Lozoya et al., 2002). Literature report shows that guava intake has a beneficial effect on serum
37 total and high-density lipoprotein cholesterol levels and on systemic blood pressure (Singh et al.,
38 1992). This beneficial effect may be related to the antioxidant properties reported for *P. Guajava*
39 fruits by Jimenez-Escrig et al. (2001).

40 The long history of guava's use in folk/traditional medicine has motivated the modern-day
41 medicinal plant researchers to study more on the ehnbobotanical properties of the extracts from
42 different guava plant parts. Our earlier studies showed that aqueous extracts of *P. guajava* leaves
43 have a beneficial effects on the haematological parameters, liver and reproductive tissue
44 functions in rats (Uboh et al., 2010a,b). The antioxidants content of the guava extracts, including
45 polyphenols, ascorbic acid and carotenoids, have been reported to cause a significant decrease in
46 the serum lipid profile (Jimenez-Escrig et al., 2001; Yamashiro et al., 2003; Qian and
47 Nihorimbere, 2004). Also, oral administration of vitamin C have been reported to cause a
48 significant decrease in the serum total cholesterol (TC), very low-density lipoprotein (VLDL)
49 and a non significant increase in high density lipoprotein (HDL) in albino Wistar rats (Hillstrom
50 et al., 2003; Eteng et al., 2006).

51 Based on the fact that vitamin C is reported to be one of the antioxidants content of guava leaves'
52 extract, this study assessed and compared the effect of vitamin C and the aqueous extract of *P.*
53 *guajava* leaves on serum lipid profile in male albino *Wistar* rat model. Moreover, this present
54 study aimed at adding validity, or otherwise, to the use of the extracts from different part of *P.*
55 *guajava* plant in the treatment of atherosclerotic conditions and other cardiovascular diseases,
56 including hypertension, in folk medicine.

MATERIALS AND METHODS

Identification and preparation of Plant Materials:

Fresh leaves of *P. guajava* were collected from local garden at the University of Calabar, Calabar, Nigeria. The sample of the plant specimen was identified and authenticated by a Botanist from the Department of Botany in the same University. The Voucher specimen was deposited in the herbarium of the same University. The leaves were sorted to eliminate any dead matter and other unwanted particles, air-dried for 2 weeks, and then ground into fine powder using an electric dry mill (Moulinex). 200g of the ground powder was soaked in 1.0 litre of distilled water for 48 hours at room temperature. The mixture was filtered into 500ml conical flask with Watman filter paper (No.1). The filtrate was dried at a temperature of 30°C for 10 hours to produce a gel-like extract, which weighed 20.5g. Appropriate concentration of the extract was subsequently made by dilution with distilled water into 200mg/kg body weight and administered to the animals.

Handling and treatment of Animals:

Eighteen male rats weighing 230 to 280g were obtained from the disease free stock of the animal house, Biochemistry Department, College of Medical Sciences University of Calabar, Calabar, Nigeria, and used for this study. The rats were divided into three groups with six rats each, as follows:

Group I.Mc (Male control group receiving distilled water as placebo),

Group II.MvitC (Male test group receiving vitamin C),

Group III.MPg (Male test group receiving aqueous extract of *P. guajava* leaves).

The rats in all the groups were acclimatized in the experimental animal house for one week before the commencement of the experimental administrations. The animals, housed in stainless steel cages under standard conditions (ambient temperature, $28.0 \pm 2.0^\circ \text{C}$ and humidity, 46%, with a 12 hr light/dark cycle), were fed with the normal rat pellets. All the rats in both test and control groups were allowed free access to food and water *ad libitum*, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily.

The animals in test groups II and III received 200/mg/kg body weight oral daily doses of vitamin C and aqueous extract of *P. guajava* leaves, respectively, using orogastric tubes for 30 days

while animals in the control group I were administered, by oral gavage, with 2ml of distilled water (placebo). All the animal experiments were carried out in accordance with the guidelines of the Institution's Animal Ethical Committee.

Collection and analysis of blood:

All the animals were anaesthetized with chloroform vapour, twenty-four hours after last day of extract and vitamin C administration, and dissected for blood collection. Blood samples were collected by cardiac puncture into a set of plain sample bottles, and allowed to clot. The clotted blood samples were spun in a bench top centrifuge (MSE, England) to obtain sera. The serum samples were separated into another set of plain sample tubes. The separated serum samples were stored in the refrigerator until required for the assay of lipid profile. All assays were done within 24 hours of the sample collection. Reagents kits obtained from the Randox Chemical Company, UK were used for lipid profile assays. The "high performance" enzymatic colorimetric, CHOD-PAP method described by Richmond (1973), was used to estimate total serum cholesterol. The principle is based on the proportionate formation of hydrogen peroxide (following oxidation of free cholesterol), which is quantified when treated with chromogen, 4-aminoantipyrine. A coloured compound whose intensity is in proportion to the evolved peroxide, and thus total cholesterol concentration in the sample, is formed and assayed spectrophotometrically, using HAICH, DR3000, Germany model of spectrophotometer.

Serum triglycerides were estimated by the modified enzymatic colorimetric test, according to the glycerol phosphate oxidase (GPO) method (Trinder, 1969). It is based on the action of L-d-glycerol phosphate oxidase on glycerol-3-phosphate and glycerol, obtained from the lipase action on triglycerides in serum. Hydrogen peroxide, a by-product of the GPO reaction is estimated as already described for total cholesterol.

HDL-cholesterol estimation employed the method of Richmond (1973). The principle entails the separation of HDL-cholesterol from chylomicrons, VLDL-C and LDL-C with a suitable precipitant, and then estimation of cholesterol by the method described for total serum cholesterol.

VLDL and LDL cholesterol were obtained by calculations using the empirical relationships of Friedwald et al. (1972).

Arterogenic index of plasma was obtained by calculations using the empirical relationships described by Dobiáková (2004).

Statistical Analysis:

The results obtained from this study were analyzed by one-way analysis of variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups. A significant change was considered acceptable at $P < 0.05$.

RESULTS

The results obtained from this study are presented in Table 1 and Figure 1. As indicated in these results, treatment of rats model with aqueous extract of *P. guajava* leaves and vitamin C, respectively, caused a significant increase ($P \leq 0.05$) in the serum HDL-C level, and a significant decrease ($P \leq 0.05$) in serum total cholesterol, triglyceride, LDL-C, VLDL-C levels, TG/HDL-C and AIP within and among the animals in test groups, compared with the levels obtained for rats in the control group (Table 1). However, the percentage increase in serum HDL-C, and decrease in serum TC, TG, (LDL-C), VLDL-C, TG/HDL-C and AIP associated with administration of *P. guajava* extract was observed to be higher than that recorded for vitamin C (Figure 1).

The results of this study indicated that the antiatherogenic properties of aqueous extract of *P. guajava* leaves are higher than that of vitamin C in male rat model.

DISCUSSION

Various studies indicate that high serum cholesterol levels are strongly related to atherosclerosis and increased risk of various cardiovascular diseases. Cardiovascular diseases, including coronary heart diseases, stroke and hypertension, are known to present some of the major health threats across the globe today (Carr et al., 2000). Elevated plasma lipids, (hyperlipidaemia and other abnormal blood lipid profile), are among the risk factors commonly implicated in most cardiovascular problems. Under these conditions, lipids and other related substances accumulate on arterial wall, forming plaque, which occlude the vascular lumen and obstruct the blood flow to vital organs such as the heart, brain, liver, or kidney. Obstruction of blood supply to the heart, brain, liver or kidney cause coronary heart diseases, stroke or kidney failure, as the case may be. The important lipids whose elevations are implicated in these disease conditions are cholesterol and triacylglycerols.

Lipids are generally transported as lipid-protein complexes called lipoproteins, which are classified based on their density and charges. The high-density lipoprotein cholesterol (HDL-C) transports lipids out of blood cells to the liver, while the low density lipoproteins cholesterol (LDL-C) mobilizes lipids against the cells and blood vessels. Triacylglycerols have been found to be elevated along with total cholesterol elevation. Therefore, elevated low-density cholesterol, triacylglycerols and total cholesterol with reduced HDL-C will enhance the development of atherosclerosis and related cerebrovascular disorders (Carr et al., 2000). However, clinical studies have shown that lowering the levels of serum cholesterol, triacylglycerol, and the related lipid fractions, using diet or drugs, decreases the incidence of coronary heart disease.

In this present study, it is recorded and reported that administration of 200mg/kg of aqueous extract of *P. guajava* leaves and vitamin C expressed a significant decrease in atherogenic risk predisposing indices, (including serum total cholesterol, triglycerides, LDL-C, VLDL-C levels, TG/HDL-C ratio and AIP), to desirable levels, after four weeks of treatment in male rats. Specifically, the results obtained in this study for vitamin C agrees with the literature report that vitamin C produces hypocholesterolaemic effect; lowering the atherogenic predisposing factors (serum total cholesterol and LDL-C), and elevates serum HDL-C level in rats (Eteng et al., 2006). A strong hypolipidaemic and antiatherogenic properties is therefore reported to be associated with aqueous extract of *P. guajava* leaves and vitamin C in male rats. The specific mechanism(s) through which the leaf extract enhances a reduction in the serum lipid profile was not considered in this present study, and hence remain a subject for further studies. However, since the leaf of *P. guajava* is known to be rich in vitamin C and other antioxidants, the reported mechanism of serum lipid reduction role of vitamin C is strongly suggested to be implicated.

One of the possible explanations for the observed decrease in serum lipid profile may be the ability of vitamin C to activate the enzyme, 7 α -hydroxylase, which enhance the conversion of plasma cholesterol to bile acids, thereby reducing the serum cholesterol levels. Particularly, it has been reported that deficiency of vitamin C inhibits 7 α -hydroxylase thereby blocking bile acid synthesis and accumulate cholesterol in the plasma, with subsequent atherosclerosis in scorbutic Guinea pigs (Mayes, 1996). The reduction in serum cholesterol levels reported in this study also agrees with the report of Anderson *et al.* (1999) as well as Bsoul and Terezhalmay (2004) that

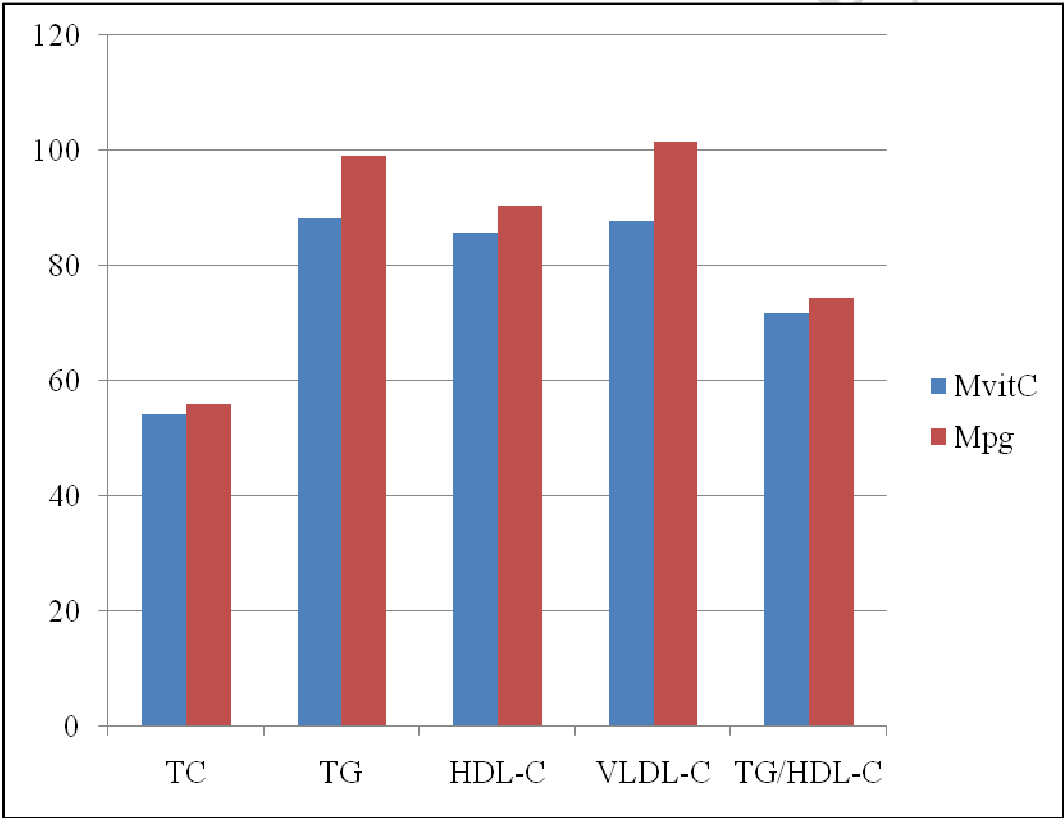
adequate vitamin C intake can reduce the incidence of atherosclerosis, and hence the risk of coronary heart disease.

On account of the ability of vitamin C and aqueous extract of *P. guajava* leaves to lower the AIP and other atherogenic predisposing factors (serum total cholesterol, LDL cholesterol and triacylglycerol), and elevate HDL cholesterol, it may be concluded that moderate to high intake of aqueous extract of *P. guajava* leaves can produce a hypocholesterolaemic effect. The results of this study therefore indicated that *P. guajava* and vitamin C possess hypolipidaemic, hence antiatherogenic properties, which may be useful in the management of atherosclerosis, and the attendant coronary heart disease (CHD) and hypertension. These properties may also be the underlying mechanism associated with the local use of *P. guajava* in the management of hypertensive conditions.

201 **Table 1.** Serum lipid profile of male rats treated with vitamin C and aqueous extract of *P.*
 202 *guajava* leaves.

Group	T-C (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	TG/HDL	AIP
Mc	7.22±0.06	6.87±0.10	3.73±0.18	2.12±0.21	1.37±0.02	1.88±0.08	0.27±0.02
MvitC	4.68±0.18	3.65±0.15	6.93±0.11	-2.98±0.24	0.73±0.03	0.53±0.03	-0.28±0.03
Mpg	4.63±0.21	3.45±0.14	7.10±0.11	-3.15±0.28	0.68±0.02	0.48±0.02	-0.32±0.02

203 Values are presented as mean ± SEM, n = 6, P ≤ 0.05 compared to the control. Mc = male rats in
 204 the control group, MvitC = male rats treated with vitamin C, Mpg = male rats treated with
 205 aqueous extract of *P. guajava* leaves.



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 210 **Figure 1.** Comparative percentage changes in serum lipid profile in male rats following oral
 211 administration of vitamin C and aqueous extract of *P. guajava* leaves. (MvitC = male rats
 212 treated with vitamin C, Mpg = male rats treated with aqueous extract of *P. guajava* leaves).

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