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Comparative Hypolipidaemic Effect of Aqueous Extract of *Psidium guajava* Leaves and Ascorbic Acid in Male Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author FEU designed the study, wrote the protocol and the first draft of the manuscript, managed the literature searches, read and approved the final manuscript. Authors GOI and PEE managed the laboratory analyses of the study and performed the statistical analysis. Author IEO identified the plant specimen. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To assess the hypolipidaemic properties of aqueous extract of *Psidium guajava* leaves (AEPGL) and ascorbic acid (vitC) in male albino *Wistar* rats.
Study Design: Three groups of rats were respectively administered distilled water, 200mg/kg of AEPGL and vitC orally, for thirty days, to determine the hypolipidaemic properties of AEPGL and vitC. The hypolipidaemic properties were determined from the serum lipid profile of the rats.
Place and Duration of Study: This work was carried out in Biochemistry Department, University of Calabar, Calabar, Nigeria between January and February, 2012.
Methodology: Eighteen albino Wistar rats, divided in three groups of six rats each, were used in this study. The rats in group I (control) were administered, by oral gavage, with 2ml of distilled water. While the rats in groups II and III received 200/mg/kg body weight oral daily doses of aqueous extract of AEPGL and vitC, respectively, using orogastric tubes for 30 days. The hypolipidaemic properties of AEPGL and vitC were determined from their

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effect on serum lipid profile of the treated rats, using serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), TG/HDL-C and artherogenic index of plasma (AIP). Standard reagent kits were used to determine serum TC, TG and HDL-C, while LDL-C, VLDL-C, TG/HDL-C and AIP were calculated.
Result: The results showed that serum HDL-C level obtained for rats treated with AEPGL (7.10 ± 0.11mg/dl) and vitC (6.93 ± 0.11mg/dl) were significantly ($p<0.05$) increased, compared respectively to that of the control rats (3.73 ± 0.18mg/dl). While the levels of TC, TG, LDL-C, VLDL-C, TG/HDL-C and AIP obtained rats treated with AEPGL (4.63 ± 0.21, 3.45 ± 0.14, -3.15 ± 0.28, 0.68 ± 0.02mg/dl, 0.48 ± 0.02 and -0.32 ± 0.02, respectively) and vitC (4.68 ± 0.18, 3.65 ± 0.15, -2.98 ± 0.24, 0.73 ± 0.03 mg/dl, 0.53 ± 0.03 and -0.28 ± 0.03, respectively) were significantly ($p<0.05$) decreased, compared respectively to the control rats (7.22 ± 0.06, 6.87 ± 0.10, 2.12 ± 0.21, 1.37 ± 0.02mg/dl, 1.88 ± 0.08 and 0.27 ± 0.02, respectively). 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 AEPGL was observed to be insignificantly ($p>0.05$) higher than that recorded for vitamin C.
Conclusion: In conclusion, the results of this study indicate that AEPGL and vitC possess hypolipidaemic, hence antiatherogenic, properties in male rats, thereby supporting the local use of *P. guajava* leaves in the management of hypertensive conditions.

Keywords: Psidium guajava; ascorbic acid; hypolipidaemia; antiatherogenicity.

1. INTRODUCTION

Guava (*Psidium guajava*) is a common shade tree or shrub planted in most door-yard gardens in the tropics. Its' leaves are known to contain vitamin C and other phytochemicals with antioxidant properties [1-4]. However, quercetin has been reported to be the main active constituent in the plant and that the spasmolytic and antidiarrheal effects of *P. guajava* leaves decoctions utilized in the treatment of various gastrointestinal disorders are associated with its quercetin-derived flavonoid glycosides [5]. Literature report shows that guava intake has a beneficial effect on serum total and high-density lipoprotein cholesterol levels and on systemic blood pressure [6]. This beneficial effect may be related to the antioxidant properties reported for *P. Guajava* fruits by Jimenez-Escrig et al. [7].

The long history of guava's use in folk/traditional medicine has motivated the modern-day medicinal plant researchers to study more on the ehnobotanical properties of the extracts from different guava plant parts. Our earlier studies showed that aqueous extracts of *P. guajava* leaves have a beneficial effects on the haematological parameters, liver and reproductive tissue functions in rats [8,9]. The antioxidants content of the guava extracts, including polyphenols, ascorbic acid and carotenoids, have been reported to cause a significant decrease in the serum lipid profile [4,7,10]. Also, oral administration of vitamin C have been reported to cause a significant decrease in the serum total cholesterol (TC), very low-density lipoprotein (VLDL) and a non significant increase in high density lipoprotein (HDL) in albino Wistar rats [11,12].

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

2. MATERIALS AND METHODS

2.1 Identification and Preparation of Plant Materials

Fresh leaves of *P. guajava* were collected in 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 30ºC for 10hours 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.

2.2 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±2.0ºC 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 [8,9], 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.

2.3 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 [13] 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 chromogem, 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 colorimeric test, according to the glycerol phosphate oxidase (GPO) method [14]. 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 [13]. 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 cholesterols were obtained by calculations using the empirical relationships of Friedwald et al. [15].

Arterogenic index of plasma was obtained by calculations using the empirical relationships described by Dobiáová [16].

2.4 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 $P < 0.05$.

3. RESULTS AND DISCUSSION

The results obtained from this study are presented in Table 1 and Fig. 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 insignificantly ($p>0.05$) higher than that recorded for vitamin C (Fig. 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.

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

Group	TC (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*

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

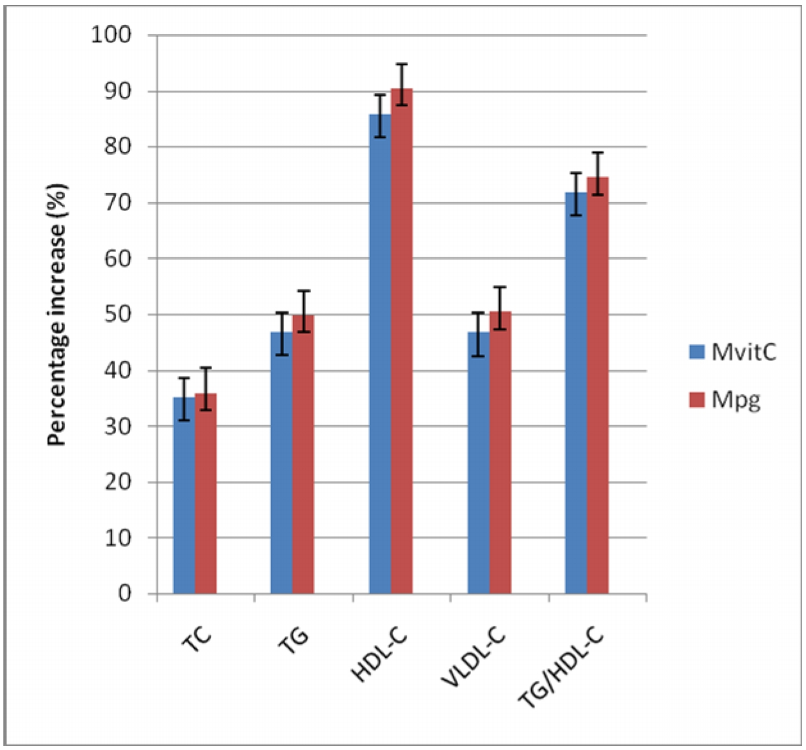

Fig. 1. Comparative percentage changes in serum lipid profile in male rats following oral administration of vitamin C and aqueous extract of *P. guajava* leaves

(MvitC = male rats treated with vitamin C, Mpg = male rats treated with aqueous extract of P. guajava leaves). Mean ± S.E.M = Mean values ± Standard error of means of six determinations.

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 [17]. 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 plague, 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 [17]. 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 [12]. 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 [18]. The reduction in serum cholesterol levels reported in this study also agrees with the report of Anderson *et al.* [19] as well as Bsoul and Terezhalmy [20] that adequate vitamin C intake can reduce the incidence of atherosclerosis, and hence the risk of coronary heart disease.

4. CONCLUSION

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Begum S, Hassan SI, Siddiqui BS. Two new triterpenoids from the fresh leaves of *Psidium guajava*. Planta Med. 2002a;68:1149-1152. PMID: 12494352
2. Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur MN, Gilani AH. Triterpenoids from the leaves of *Psidium guajava*. Phytochemistry 2002b;61:399-403. PMID: 12377233
3. Suntornsuk L, Gritsanapun W, Nilkamhank S, Paochom A. Quantitation of vitamin C content in herbal juice using direct titration. J Pharm Biomed Anal. 2002;28:849-855. PMID: 12039626.
4. Qian H, Nihorimbere V. Antioxidant power of phytochemicals from *Psidium guajava* leaf. J Zhejiang Univ Sci. 2004;5:676-683. doi: 10.1631/jzus.2004.0676; PMID: 15101101.
5. Lozoya X, Reyes-Morales H, Chavez-Soto M, Martinez-Garcia Mdel C, Soto-Gonzalez Y, Doubova SV. Intestinal anti-spasmodic effect of a phytodrug of *Psidium guajava* folia in the treatment of acute diarrheic disease. J Ethnopharmacol. 2002;83:19-24. PMID: 12413703.
6. Singh RB, Ghosh S, Niaz AM, Gupta S, Bishnoi L, Sharma JP et al. Effects of guava intake on serum total and high-density lipoprotein cholesterol levels and on systemic blood pressure. Am. J. Cardiol. 1992;70(15):1287-91. PMID: 1332463.
7. Jimenez-Escrig A, Rincon M, Pulido R, Saura-Calixto F. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *J Agric Food Chem*. 2001;49:5489-493. PMID: 11714349.
8. Uboh FE, Okon IE, Ekong MB. Effect of aqueous extract of *P. guajava* leaves on liver enzymes, histological integrity and hematological indices in rats. Gastroenterology Research. 2010a;3(1):32-38. doi:10.4021/gr2010.02.174w.
9. Uboh FE, Edet EE, Eteng MU, Eyong EU. Comparative effect of aqueous extract of *P. guajava* leaves and ascorbic acid on serum sex hormones levels in male and female rats. Journal of Applied Sciences Research. 2010b;6(4):275–279. http://www.aensiweb.com/jasr/jasr/2010/275-279.
10. Yamashiro S, Noguchi K, Matsuzaki T, Miyagi K, Nakasone J, Sakanashi M et al. Cardioprotective effects of extracts from *Psidium guajava* L and *Limonium wrightii*, *Okinawan* medicinal plants, against ischemia reperfusion injury in perfused rat hearts. Pharmacology. 2003;67:128-135. doi: 10.1159/000067799.
11. Hillstrom RJ, Yacapin-Ammons AK, Lynch SM. Vitamin C inhibits lipid peroxidation in human HDL. J. Nutr 2003;133(10):3047–3051. PMID: 14519782.
12. Eteng MU, Ibekwe HA, Amatey TE, Bassey BJ, Uboh FE, Owu DU. Effect of Vitamin C on serum lipids and electrolyte profile of albino wistar rats. Nig J Physiol Sc 2006; 21(1-2): 15–19. Available online/abstracted at http://www.biolineinternational.org.br/njps; www.ajol.info/journals.njps; www.cas.org, 2006.
13. Richmond W. Cholesterol enzymatic colorimetric test chop-PAP method of estimation of total cholesterol in serum. Clin. Chem. 1973;191:1350-1356.
14. Trinder P. Triglycerides estimation by GPO-PAP method. Ann. Clin. Chem. 1969;6:24–27.

15. Friedewald WT, Levy RT, Fredickson DS. Estimation of the concentration of LDLcholesterol without use of plasma ultracentrifuge Clin. Chem. 1972;18:499–520.
16. Dobiáová M. Atherogenic Index of Plasma [Log(Triglycerides/HDL-Cholesterol)]: Theoretical and Practical Implications. Clin Chem 2004;50:1113–1115. doi: 10.1373/clinchem.2004.033175.
17. Carr AC, Zhu B, Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and α-tocopherol (vitamin E). Circulation Research. 2000;87:349–354. doi: 10.1161/01.RES.87.5.349.
18. Mayes PA. Cholesterol Synthesis, Transport and Excretion, in; Murrary, R.K. et al. Harper's Biochemistry, 24th Ed, Prince- Hall Int.Inc., California. U.A. 1996;271-280.
19. Anderson JW, Gowri MS, Turner J. Antioxidant supplementation effects low density lipoprotein oxidation for individuals with type 2 diabetes mellitus. J. Am. Coll. Nutr. 1999;18:457–461.
20. Bsoul SA, Terezhalmy GT. Vitamin C in health and disease. J. Contemp. Dent. Pract. 2004;15:1–13.

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