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The significance of testa in the lipid profile of wistar rats fed oil extracts of *Irvingia gabonensis* and *Irvingia wombolu*

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Abstract
Lipid profiles of experimental wistar rats fed with oil extracts from kernels of Irvingia gabonensis, (Ig) and Irvingia wombolu (Iw) were studied using standard methods. In addition, effect of testa was also investigated on the profiles of the rats. On day zero, before administration of oil extract, rats were sacrificed and this served as the control group. After 7days of oil administration to the rats, the diet containing these oil showed nutritional improvement compared with control. The observation was more significant ($p<0.05$) after 14 days when increase in HDL cholesterol and reduction in TG and LDL cholesterol levels were pronounced in animals fed with Iw, especially. The Irvingia wombolu (Iw) extracts irrespective of treatment, had stable LDL, increase in TC by 24.6%, a remarkable rise in HDL by 115.8% and a decrease in TG by 6.4%. When effect of testa was examined between the periods of administration, Iwt gave increased values of 178.2% TC, 354.5% HDL and 743% decrease in LDL representing ratio 1:2:5 respectively. The study, therefore, revealed that oil extract of Irvingia kernels contain high levels of phenolic substances capable of inhibiting lipid autoxidation.

Keywords: Testa, Irvingia, cholesterol, HDL, LDL

1. Introduction

Lipid profile is a panel of blood test carried out which serves as an initial medical screening tool in detecting abnormalities in lipids such as triglycerides and cholesterol. It usually includes the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and the calculated low- density lipoprotein (LDL) cholesterol. HDLs are the lipoprotein group in the blood plasma with the highest density and smallest size. They are produced by various mechanisms mainly by biosynthesis in the liver and intestine. HDL grows by taking up phospholipids and cholesterol from the walls of blood vessels and thereby changes from disc-like structure to a spherical shape. HDL contributes decisively to reducing the blood cholesterol from peripheral tissues and providing it to the liver directly or via VLDL. In the liver, cholesterol is converted into bile acids or excreted directly into the bile. HDL is therefore, regarded as “good cholesterol” in contrast to LDL which is known as the “bad” cholesterol.

Lipid peroxidation is the oxidative degradation of polyunsaturated lipids. It is a process where free radicals ‘steal’ electrons from the lipids in cell membranes resulting in cell damage. It most often affects polyunsaturated fatty acids because they contain multiple double bonds in between them (Fernandes, 1997) [6]. It can occur both in vivo and in vitro; and involves free radical chain reactions. Rodriguez *et al* (2006) [13] reported that the reaction of oxygen with unsaturated lipids results in a whole range of compounds with hydro peroxide as the initial product. The process may be non enzymic or not enzymically catalyzed. Where the process is non-enzymic, it is referred to as a lipid peroxidation reaction but where the process involves a characterized enzyme that gives a defined set of products, it is usually referred to as a lipoxygenase type of reaction.

Lipid peroxidation is heavily associated with the production of free radicals. Free radicals are either molecules or molecular fragments with unpaired electron and the unpaired electron gives them certain characteristic properties like high chemical reactivity. In lipid peroxidation therefore, a reactive free radical ($R^{\cdot}$) reacts with a polyunsaturated fatty acid to initiate a complex series of reactions that result in a variety of degradation products. Most lipids are barely soluble in water, while some have amphipathic properties, that is, they are both water-soluble and fat-soluble. Lipoproteins are proteins that bind lipids such as fats, cholesterol and triglycerides, to enable them to be transported through the lymphatic and circulatory systems (Marshall, 1992) [8].

1.1 Wild Mango and its description (*Irvingia spp*)

Irvingia is a genus of African and Southeast Asian trees in the family *Irvingiaceae*, sometimes known by the common names wild mango, African mango, or bush mango. There are two varieties of wild mango, one is “sweet” (*I. gabonensis*) and the other has a “bitter” skin (*I. wombolu*). The germination rate of these two species is 80%. *I. gabonensis* can be found in the humid forest zone of Cameroon, Nigeria and other West African countries while *l. wombolu* is more localized in the south west of some of these countries. The fruit of the *l. gabonensis* weighs about 200g, while the *l. wombolu* weighs about 85g when harvested (www.cifor.cgiar.org).

Both varieties do not produce during the same season. *I. gabonensis* produce fruit from April to June, while *I. wombolu* does so from November to March. The *gabonensis* variety has an edible yellowish pulp when ripe, with a turpentine flavour while the *wombolu* variety has a bitter inedible and acrid pulp (Ekpo *et al*, 2007) [5].

The aim of this research is to study the lipid profiles of albino rats fed with *Irvingia spp* kernel oils. And specifically, to investigate the significance of testa in the lipid profiling of the studied rats.

2.0 Materials and Methods

Sample collection and handling

Mature fruits of *Irvingia gabonensis* and *Irvingia wombolu* were plucked directly from trees at Kogi State University Anyigba, Nigeria. The fleshy pulp was peeled using a stainless steel knife to release the seeds which were then sun-dried for a week. The shells were then cracked manually to obtain the kernels which were creamy and bi-lobed. The kernels were then oven-dried at 105ºC to storable moisture content and finally ground to powder in readiness for experimentation.

The percentage moisture content of the kernel samples after drying in the oven to a constant weight was then calculated as:

$$\frac{\text{Loss in weight}}{\text{Weight of sample}} \times \frac{100}{1}$$

Pelletized Broiler Starter feed manufactured by Vital Feeds; a division of Grand Cereals & Oil Mills Limited. Jos, Nigeria was used as normal feed. Diagnostic kits used were manufactured by Agape Diagnostics, Switzerland Gmbh. Other solvents and reagents used in this study were of analytical grade and available commercially.

2.1 Oil Extraction and Determination of percentage oil extracted

Each sample type was subjected to solvent extraction using Soxhlet method (1879). *Irvingia* species kernel powder (100g) were placed in a cellulose paper (thimble), placed in the extraction chamber and extracted using ethanol as solvent. The extraction was carried out for eight (8) hours and the resulting extract was evaporated under reduced pressure by the aid of a rotary evaporator, (HB4 Basic, IKA-WERKE rotary evaporator) to remove the solvent. The percentage oil extracted was calculated using the following formula:

$$\%\text{ residue extract} = \frac{\text{Wt of sample before extraction} - \text{Wt of sample after extraction}}{\text{Wt of sample}} \times \frac{100}{}$$

2.2 Effect of the oil extracts on rat lipid profile

The lipid profile was determined according to procedure described by Dzeufit *et al* (2009), Matsinkou *et al* (2012) [10] and Ngondi *et al* (2005) [11] with some modifications. Briefly, forty-eight (48) healthy, age-matched wistar rats weighing 110.12±20g were used. All aspects of animal care were complied with the ethical guidelines and technical requirements approved by the institution's Animal Ethnics Committee. The rats were divided into five diet groups of eight animals each namely, 1, 2, 3, 4 and control. The animals were housed in individual wooden cages covered with net. They were left to acclimatize for one week within which they were fed normal feed.

To feed, animals were provided with their normal feed and water. Except for the control group, one (1) ml of oil extract was additionally administered orally with the aid of syringe, shortly after being warmed. The groups were:

1 given *Irvingia wombolu* oil extract with testa (*Iwt*),
2 given *Irvingia wombolu* oil extract without testa (Iwt_o),
3 given *Irvingia gabonensis* oil extract with testa (*Igt*),
4 given *Irvingia gabonensis* oil extract without testa (Igt_o) and 5 given only normal feed and water as control.

At the expiration of one week (day zero), rats from the control group were sacrificed to serve as control. After 7 days of oil administration, 4 rats from each group were sacrificed, and blood samples were withdrawn by cardiac puncture. Similar treatment was given to the remaining half of each group after fourteen (14) days. Blood serum was separated with the aid of a centrifuge (Uniscope Laboratory centrifuge Model SM800B, UK), and used for analysis. Standard diagnostic kits for the determination of total cholesterol, HDL cholesterol, Triglyceride and LDL cholesterol available commercially from Agape Diagnostic (Switzerland GmbH) were used to analyze the serum from the rats (Dzeufiet *et al*., 2009). All analyses were performed in triplicate.

Total Cholesterol (mg/dl) was calculated using the formula:

$$\text{Abs of sample X 200 Abs of standard}$$

(200mg/dL is the standard concentration)

HDL-Cholesterol concentration was calculated using the following formula:

$$\text{HDL-Cholesterol (mg/dL)} = \frac{\text{Abs of sample X N X 2}}{\text{Abs of Standard}}$$

(N is the standard concentration of 50mg/dl and 2 is the dilution factor)

Triglycerides concentration is calculated as follows,

$$\text{Triglycerides conc (mg/dL)} = \frac{\text{Abs of sample X 200}}{\text{Abs of standard}}$$

(200mg/dl is the standard concentration)

$$\text{LDL-Cholesterol (mg/dL)} = \text{Total Chol} - \frac{(\text{HDL-Chol} + \text{Triglycerides})}{5}$$

2.3 Statistical analysis

The statistical analysis was carried out using Graphpad instat 3 statistical solution software. Results are presented as mean value ± standard deviation of three replicate determinations. Statistic analysis among treatments were determined at the significance level of $P < 0.05$.

3.0 Results

Table 1: Some physical properties of oil extract of *Irvingia* varieties (*wombolu and gabonensis*).

Kernel types	% Oil extracted (w/w)	Specific gravity (SG)	Refractive Index (RI)	Melting point (ºC)
Iwt	27.30±0.80	0.67±0.01	1.4425±0.41	38 - 40
Iwt_o	14.40±1.20	0.65±0.02	1.3940±0.03	37 – 40
Igt	34.02± 1.40	0.92±0.01	1.3606±0.31	35 - 38
Igt_o	32.00±0.90	0.91±0.01	1.3443±0.00	36 – 38

Values are mean±SD of triple determinations at ambient temperature of 29ºC.

Iwt = Irvingia wombolu kernel with testa; Iwt_o *= Irvingia wombolu kernel without testa; Igt = Irvingia gabonensis*

kernel with testa; Igt_o = Irvingia gabonensis kernel without testa

Table 2: Lipid Profile of wistar rats before being subjected to oil extracts. (Day zero)

Oil extracts	Total Cholesterol (TC)	HDL-cholesterol	Triglycerides (TG)	LDL-cholesterol
Iwt	150.5±0.01	18.8±0.06	105±1.10	110.7±1.12
Iwt_o	153.1±2.60	18.2±0.01	108±1.20	113.3±0.01
Igt	154.0±3.00	20.0±0.03	110±0.00	112.0±0.30
Igt_o	152.0±2.20	20.0±0.01	107±0.10	110.6±0.00
control	157.4±2.30	19.1±0.10	111±0.13	116.1±1.65

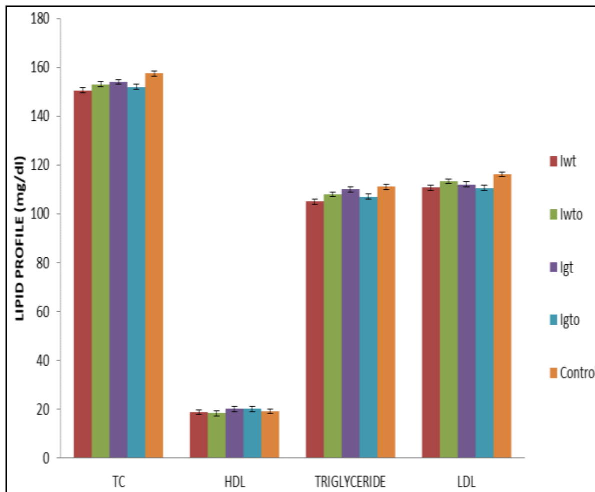

Fig 1: Lipid profile of wistar rats before administration of oil extracts (day zero).

Table 3: Lipid Profile of wistar rats subjected to the extracts for 7-days (mg/dL).

Oil extracts	Total Cholesterol (TC)	HDL-cholesterol	Triglycerides (TG)	LDL-cholesterol
Iwt	165.2$\pm$ 1.90^a	34.1$\pm$ 2.95^a	171.4$\pm$ 1.80^a	97.4$\pm$ 3.38^b
Iwt_o	160.0$\pm$ 1.32^a	33.0$\pm$ 2.18^a	157.0$\pm$ 2.60^a	96.0$\pm$ 3.50^b
Igt	247.0$\pm$ 3.61^c	44.0$\pm$ 2.29^b	186.0$\pm$ 2.29^b	166.0$\pm$ 2.00^d
Igt_o	187.2$\pm$ 5.22^b	31.7$\pm$ 1.11^a	204.1$\pm$ 3.69^b	115.1$\pm$ 4.22^c
Control	229.0$\pm$ 3.00^d	64.0$\pm$ 3.12^c	311.4$\pm$ 2.01^c	103.0$\pm$ 3.04^c
SEM	14.7	6.9	23.0	13.3

Values are mean $\pm$ SD of triplicate determinations.

Values with the same superscripts within a given column are not significantly different (p>0.05) (Dunnett multiple comparisons test).

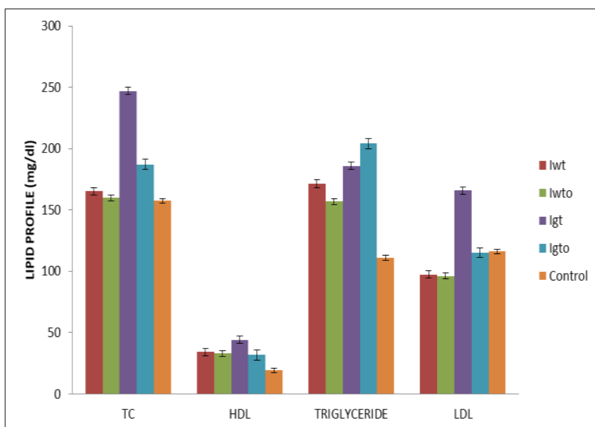

Fig 2: Lipid profile of wistar rats after seven days administration of oil extracts.

Table 4: Lipid Profile of wistar rats subjected to the extracts for 14-days (mg/dL).

Oil extracts	Total cholesterol (TC)	HDL-cholesterol	Triglycerides (TG)	LDL – cholesterol
Iwt	195.0 $\pm$ 2.65^c	75.0 $\pm$ 3.00^b	162.0 $\pm$3.61^{cd}	88.0 $\pm$ 2.65^c
Iwt_o	210.0 $\pm$ 2.00^c	70.0 $\pm$ 3.04^a	145.0 $\pm$ 2.00^c	111.0 $\pm$ 1.32^d
Igt	147.0 $\pm$ 3.61^b	72.0 $\pm$ 3.46^b	135.0 $\pm$ 2.65^c	48.0 $\pm$ 4.00^a
Igt_o	140.0 $\pm$ 4.58^b	67.0 $\pm$ 1.73^a	31.0 $\pm$ 3.91^a	67.0 $\pm$ 1.73^b
Control	95.0 $\pm$ 4.36^a	65.0 $\pm$ 1.70^a	71.0 $\pm$ 1.00^b	61.0 $\pm$ 2.78^b
SEM	17.0	2.9	21.6	9.9

Values are mean $\pm$SD of triplicate determinations. SEM= Standard Error of Mean.

Values with the same superscripts within a given column are not significantly different (p>0.05) (Dunnett multiple comparisons test).

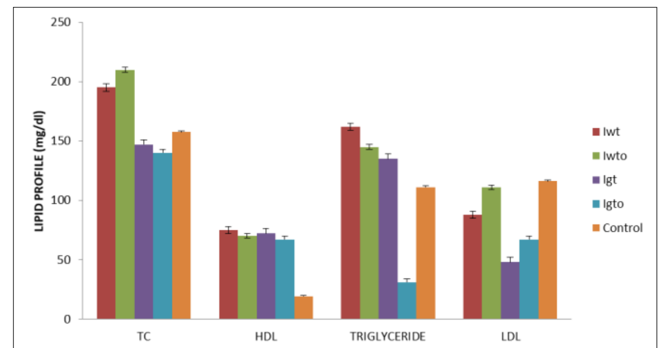

Fig 3: Lipid profile of wistar rats after fourteen days administration of oil extracts.

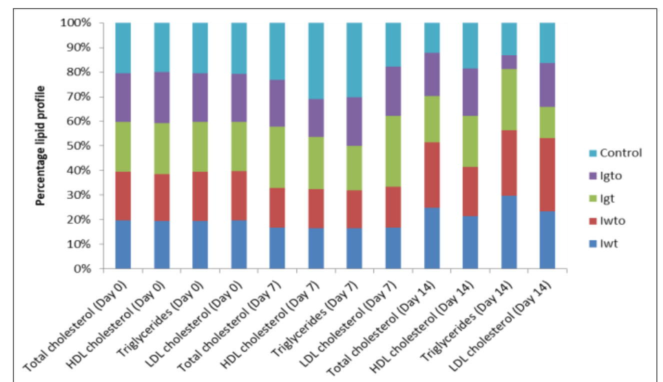

Fig 4: Percentage contribution to the lipid profile of the oil extracts as at day 0, 7 and 14 at a glance.

4.0 Discussion

The average moisture content of the freshly plucked kernels were gotten to be 26% and 6.4% for *Irvingia gabonensis (Ig)* and *Irvingia wombolu (Iw)* respectively. The value for *Ig* was above the critical equilibrium moisture content (EMC) of 14.1% as reported by Arogba (2001) [3] for *Irvingia gabonensis* kernel and could subject these kernels to rapid deterioration from mould as well as early sprouting. On the contrary, the *Irvingia wombolu* kernels which were plucked few months earlier due to seasonal variations appeared drier. However, all kernel types were oven-dried to approximately common dry matter content before experimentation.

From these results, it could be noticed that *Irvingia* oils were in the range of 14 – 34%. This indicated that the *Irvingia gabonensis* variety particularly, was a richer oil seed. Matos *et al.* (2009) [9] also reported that *Irvingia* seed

kernels are richer in lipids than other conventional oil seeds such as cotton seeds, soybean, rapeseed and palm fruit. It was further reported that *Irvingia gabonensis* oil content varies between 34.3 and 62.7%. The value of 34.02% in this study is in the lower range. Differences in literature values could be due to environmental as well as geographical factors.

Table 4 and figure 2 shows the lipid profile of the extracts after 7-days of oral administration of the oil. It was observed that among oil types, Ig gave the highest total cholesterol (TC), triglyceride (TG) and LDL cholesterol but significantly ($p<0.05$) lower than those of the control. Literature reports (NCEP, 2010) gave average borderline high levels of TC, HDL, LDL cholesterols and TG at 200 - 239, 40 - 50, 130 -159 and 150 - 199 mg/dl respectively, in favor of *Iw* oil extracts.

After 14 days of administration as shown in Table 5 and *figure 3*, the TC for *Mi* remained stable though with a rise in HDL cholesterol of 21.4% due to decrease of 12% in TG (triglycerides) and a further decrease in LDL by 27%. This result showed that after 14 days of administration, the oils improved the health of the animals studied. The *Irvingia wombolu* (*Iw*) extracts irrespective of treatment, had stable LDL, increase in TC by 24.6%, a remarkable rise in HDL by 115.8% and a decrease in TG by 6.4%. When effect of testa was examined between the periods of administration, *Iwt* gave increased values of 178.2% TC, 354.5% HDL and 743% decrease in LDL representing ratio 1:2:5 respectively. The observation re-emphasized the positive contribution of testa of *Irvingia* seed in nutrition. This is in agreement with our earlier work where the effect of testa was favourable in increasing HDL cholesterol and remarkably lowering LDL cholesterol (Omale *et al*., 2014) [12].

The reduction pattern in TG concentrations observed in the rats fed with all oil extracts indicated increased rate of lipolysis in favor of HDL-cholesterol production and concomitant lowering of serum TG and LDL-cholesterol concentration. Literature citations also mentioned studies that are in agreement with this study (Ali *et al*., 2004; Dzeufiet *et al*., 2009; Nangue *et al*., 2011 and Matsinkou *et al*., 2012) [4, 10]. According to the American Heart Association (AHA), a triglyceride level of 100mg/dl (1.3mml/l) or lower is considered “optimal” and will improve our heart health but adoption of a responsible lifestyle is the key. This is because triglycerides usually respond well to dietary and lifestyle changes (MayoClinic.com).

5.0 Conclusion

The main lipids in the blood are triglyceride, an important energy substrate, and cholesterol, a component of the membranes of cells and their organelles. Cholesterol and triglycerides are insoluble in water and are transported in the blood by lipoproteins (Marshall, 1992) [8]. This study has shown that the oil extracts of *Irvingia wombolu* and *Irvingia gabonensis* proved to contain effective and potent serum cholesterol and TG lowering component. These beneficial effects support the work of Dzeufiet *et al*, (2009) [4] and Arogba (2001) [3] that these kernels contributed about 11% by weight to the intact kernels and contains equivalent level of reducing substances, polyphenolic compounds inclusive (Omale. 2014) [12].

6. Acknowledgement

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7. References

1. Abdalla AEM, Saeid MD, Eman HEA, Rehman ME. Egyptian mango by-product: Compositional quality of mango seed kernel. Food Chemistry. 2007; 103:1134-1140.
2. Arogba SS. Physical, chemical and functional properties of Nigerian mango (Mnagifera indica) kernel and its processed flour. Journal Science and Food Agriculture. 1997; 73:321-328.
3. Arogba SS. The effect of testa on the physico-chemical properties, browning index and functional properties of wild mango (Irvingia gabonensis var. gabonensis) kernel. Inter-World Journal Science and Technology. 2001; 1(1):159-167.
4. Dzeufiet DPD, Ngeutse DF, Dimo T, Tedong L, Ngueguim TF, Tchamadeu MC, Nkouambou NC, Sokeng DS, Kamtchouing P. Hypoglycemic and hypolipidemic effects of Irvingia gabonensis (Irvingiaceae) in diabetic rats. Pharmacologyonline. 2009; 2: 957-962.
5. Ekpo IW, Amor ID, Morah FNI. Seed oils and nutritive studies on the seeds of gabonensis and wombolu varieties of Irvingia gabonensis. The Nigerian Academic Forum, 2007, 13(1).
6. Fernandes G. Lipid Peroxidation During n-3 fatty acid Vitamin E Supplementation in Humans. LIPIDS. 1997; 32:535-541.
7. Maisuthisakul P. Antiradical scavenging activity and polyphenolic compound extracted from Thai mango seed kernels. Asian Journal of Food and Agro-Industry. 2008; 1(2):87-96.
8. Marshall WJ. Clinical Chemistry, London: Grower Medical Publishing. 1992, 222-237. 2nd edition.
9. Matos L, Nzikou JM, Matouba VN, Pandou-Yembe VN. Studies of Irvingia gabonensis seed kernels: Oil Technological Applications. Pakistan Journal of Nutrition. 2009; 8(2):151-157.
10. Matsinkou RS, Ngondi JL, Kuate D, Mbofung C, Oben JE. Antioxidant and anti-hyperglycemic potential of pulp extracts of Irvingia wombolu fruits. Biology and Medicine. 2012; 4(1):10-19.
11. Ngondi J, Oben J, Minka S. The effect of Irvingia gabonensis seeds on body weight and blood lipid of obese subject in Cameroon. Lipids in Health and Disease, 2005, 4:12.
12. Omale J. Antioxidant activity and lipid profile of wistar rats fed with Mangifera indica, Irvingia wombolu and Irvingia gabonensis kernel oil extracts. M. Sc Thesis, Kogi State University, Anyigba Nigeria, 2014.
13. Rodriguez J, Di Pierro D, Gioia M. Effects of a natural extract from mangifera indica L. and its active compound, mangiferin, on energy state and lipid peroxidation of red blood cells. Biochem Biophy Acta. 2006; 1760(9):1333-1342.
14. www.cifor,cgiar.org. (Ecoregional Center, Yaounde, Cameroon, 2008. Retrieved 8/7/2012.