SOME BIOCHEMICAL STUDIES ON THE LEAVES AND FRUITS OF *PERSEA AMERICANA*

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ABSTRACT
Vitamin, amino acid and haematological studies on leaves and fruits of *Perseaamericana* was carried out. Results obtained for vitamins, showed higher concentrations of niacin,ascorbic acid and tocopherol in the studied fruitsthan leaves. Aside serine and tyrosine, other amino acids investigated in both samples were appreciably high.Rats placed on leaves and fruits of the studied sample showed insignificant ($p>0.05$) effect in levels of RBC,Hb, and WBC when compared to those of the control. Neutrophils, lymphocytes, MCH, and MCHC were significantly affected ($p<0.05$) in test rats against those of the control rats. The study has shown that consumption of leaves or fruit of *P.americana* may induce a hypochromic or normocytic condition in the body.

Keywords: *Vitamins, amino acid, haematological status, Perseaamericana.*

1. INTRODUCTION

Medicinal plants are plants used in herbalmedicine to treat diseases. They are as old as mankind on earth. Excellent research works and reviews [1, 5, 6, 7, 13, 16, 21, 22, 24, 28, 34, 36, 39, 41] have documented a list of medicinal plants used in African continent and other part of the world for the treatment of a variety of disorders. Disorders such as asthma, diabetes, malaria, elephantiasis, cold, etc.,have been treated with medicinal plants for as long as history can remember [24, 27, 25, 30]

[27]reported that many of these medicinal plants possess bioactive compounds that exhibit physiological activity against different illness. [24, 16,25,36] noted that these plant bioactive compounds are in chemical forms, and are called phytochemicals. Studies have shown that some of these phytochemicals aside their medicinal values, can as well act as body nutrients.

Perseaamericana (*Lauraceae*), the plant that produces alligator pear as fruit, is among such plants whose constituents act as medicine and body nutrients. It is a tall evergreen tree that can grow up to 65 feet in height [36]. The fruit is edible when ripped. In herbal medicine, infusion, concoction and extracts made from any part of the plant are effective against hypertension, cancer, menstrual problems, inflammation, wounds, etc., [12,15,22,31,37,43].

As part of our continued research and to add knowledge to the existing limited literature on *P.americana*, the present study investigated the phytonutrients composition and haematological study on leaves and fruits of *P.americana* using rat model.

2. MATERIALS AND METHODS

Sample collection and preparation: The fruits and leaves of *Perseaamericana* used in this study were collected from Imo State University school farm in Owerri, Imo State and were identified by Dr. F. N. Mbagwu of Plant Science and Biotechnology Department, Imo State University,Owerri, Imo State, Nigeria. The identified leaves got separated and only fresh ones were obtained. The fruits at their onset of ripening were cut open to obtain the edible portion. The required plant materials were air dried for one week. The dried samples were ground to fine powder and stored in airtight bottles for analysis.

Experimental animals and design: A total of thirty-six (36) male Wistar albino rats weighing between 100- 120g were purchased from the animal colony of Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria and kept in standard cages for 4 days to enable them adapt to their new environment.Pelletized commercial rat feed (Pfizer livestock co.ltd, Aba, Nigeria) and sachet water (Marlin purified water,Owerri, Imo State, Nigeria) were given to the rats *ad libitum* within this period. After adaption period, the rats were allocated to 8 groups of 4 rats each and their weights were equalized as nearly as possible. Aside the control group, compounded feeds were given to the rats after allocation. The feeds for the different groups were compounded as follows:

Control group = Normal feed + sachet water ,Group I_a = 5% leaf sample + 95% pelletized feed + sachet water,Group I_b = 10% leaf sample + 90 % pelletized feed + sachet water,Group I_c = 15% leaf sample + 85% pelletized feed + sachet water andGroup I_d = 20% leaf sample + 80% pelletized feed + sachet water, whileGroup II_a = 5% fruit

sample + 95% pelletized feed + sachet water;Group II_b= 10% fruit sample + 90 % pelletized feed + sachet water;Group II_c = 15% fruit sample + 85% pelletized feed + sachet water andGroup II_d = 20% fruit sample + 80% pelletized feed + sachet water.

Blood sample collection: At the end of the feed and water administration periods (45days), rats from the various groups were weighed and sacrificed after being put to sleep in a closed container with chloroform. Blood was collected by direct cardiac puncture into heparin treated tubes. The tubes were properly labelled and used for haematological analyses.

Haematologictest: Blood percentage (Hb) and RBC levels were determined using Sahi`s and [2] methods respectively.Westergreen`smethodwas used for erythrocyte sedimentation rate(ESR), Counting chamber and slide methods were used for white blood cell total count (WBC_{Total}) and differential counts respectively.Haematocrit method was used for packed cell volume (PCV) whereas, mean corpuscular volume (MCV),mean corpuscularhaemoglobin (MCH) and mean corpuscularhaemoglobin concentration (MCHC), were determined as described by [3].

Vitamin screening: The vitamins which include retinol (Vitamin A), thiamine (Vitamin B1), riboflavin (Vitamin B2), Niacin (Vitamin B3), ascorbic acid (Vitamin C) and Tocopherol(Vitamin E) were carried out using the methods described by [29].

Amino acid analysis: The method described by [39] was usedfor amino acids analysis.
Statistical analysis: Values presented for haematological study and amino acids were mean and standard deviations of triplicate determinations. Significance was established using least significance difference (LSD) at $p<0.05$as described by [20].

3. RESULTS AND DISCUSSION

Table 1: Vitamin screening inleaves and fruits of*P americana*.

Vitamins	Leaves	Fruits
Retinol (Vitamin A)	++	++
Riboflavin (Vitamin B1)	+	+
Thiamine (VitaminB2)	+	+
Niacin(VitaminB3)	+	++
Ascorbic acid(Vitamin C)	+	+++
Tocopherol (Vitamin E)	+	++

Key
+ =Slightlypresent; ++ = Moderately present; +++ = Highly present

Table 1 shows the vitamin screening in the leaves and fruits of *P.americana*. Retinol was found to be moderately present while riboflavin, thiamine, niacine, ascorbic acid and tocopherol were slightly present in the leaves but in the fruits, riboflavin and thiamine were slightly present; retinol, niacine and tocopherol moderately present while ascorbic acid was highly present.These vitamins become important when their nutritional and health benefits are considered in the body system. The benefits of these vitamins, for example retinol helps to regulate cell development, promotes bones and teeth development, and boost immune system. Retinol is particularly needed for good vision and healthy skin [17]. Thiamine, niacin and riboflavin, the vitamin B complex vitamins are required for converting blood sugar to energy, keeping the nervous system healthy, etc ,[9]. Ascorbic acid is required for healthy skin and muscles, and plays important role in the production of collagen, a structural material (tissue), that holds bones together and in place at the joints. Tocopherol is important in slowing down ageing process, absorption of iron and its role in fertility.As a powerful antioxidant, tocopherol protects the cells from being damaged by free radicals [22, 40]. The fruit of *P.americana* may be in position to offer more of the above mentioned benefits on consumption due to higher concentrations of the screened vitamins in it.

Table 2: Amino acid composition of the leaves and fruits of *P americana*(mg/g Nitrogen).

Amino acids	Leaves	Fruits
Arginine	12.31±2.84	11.81±0.21
Alanine	11.92±4.12	9.21±1.87
Asparagine	15.40±0.16	12.90±3.57
Cystine	2.87±0.80	3.54± 0.69
Methionine	13.59±4.43	8.89±0.47
Isoleucine	11.94±2.11	12.69±4.98
Lysine	9.86±1.89	8.42±0.47
Leucine	10.57±0.09	13.76±1.19
Glycine	9.12±1.87	4.43±0.14
Serine	2.81±0.10	1.84±0.74
Threonine	14.01±2.57	3.53±0.28
Phenylalanine	16.86±2.39	17.23±2.31
Tyrosine	2.41±0.03	1.90±0.01
Valine	11.59±3.53	12.83±3.42
Glutamine	8.31±5.07	13.78±3.94
Histidine	10.96±0.14	8.18±1.64

Values are means ± standard deviations of triplicate determinations.

Amino acids in the investigated samples revealed the presence of essential amino acids such as methionine lysine,leucine, isoleucine, threonine, phenylalanine, valine, and histidine including non-essential amino acids such as arginine, alanine, asparagine, glycine, serine, and tyrosine. Aside serine and tyrosine, the values of other amino acids observed in the samples investigated are appreciably high. This could be an indication that these samples may offer these amino acids to the body on consumption. Apart from structural functions, amino acids are the chief precursors for the manufacture of many important substances in the body of living organisms [45].In the body, amino acids and vitamins interact in interesting and important ways. Riboflavin, niacin, thiamine, pyridoxine, etc., are among the vitamins that interact with amino acids to form transaminases and other important enzymes that facilitate metabolic processes in body [23]. Amino acids apart from their known precursor functions, could also serve as valuable sources of energy especially in the absence of carbohydrate and fats in the body [45].

Table 3: Haematological study of *P. americana*leaves and fruits using rats

Groups / Parameters	Control	I_a	I_b	I_c	I_d	II_a	II_b	II_c	II_d
RBCx10^{12}/l	5.39±2.1	4.78±1.2	4.15±0.9	4.34±1.0	5.01±0.5	5.93±1.1	6.01±1.2	6.12±1.7	5.46±1.4
Hb (g/dl)	11.75±0.3	10.94±1.3	10.42±0.6	10.12±1.2	11.03±0.5	12.03±0.0	12.14±0.1	12.18±0.3	12.32±1.7
PCV(%)	37.64±0.3	37.80±2.6	36.75±1.3	36.56±1.1	38.79±0.8	37.41±5.9	38.58±1.5	38.72±2.6	39.47±0.9
WBCx10^{9}/l	17.03±4.2	18.14±2.6	18.94±2.3	18.94±0.2	18.46±1.6	16.82±5.3	16.56±3.1	16.83±2.4	16.92±1.7
N(%)	38.50±6.2	20.25±5.1	21.61±3.9	20.84±7.2	21.24±9.0	24.82±7.0	23.11±6.1	22.48±9.2	25.61±1.0
L(%)	60.25±2.8	69.50±4.9	68.15±1.8	67.22±2.1	69.10±3.2	64.60±6.3	63.81±5.4	64.02±2.0	63.01±1.9
MCV(fl)	79.80±1.4	80.09±3.2	87.89±5.6	84.30±7.4	87.21±5.5	63.46±9.8	64.91±4.2	63.67±4.0	71.23±1.1
MCH(pg)	21.80±1.0	22.60±1.9	22.90±1.5	23.43±1.4	22.14±2.0	18.36±1.3	17.20±2.4	17.22±1.8	17.54±4.5
MCHC(g/dl)	31.22±6.0	28.94±3.9	28.32±4.6	27.68±6.1	28.4±3.2	32.16±1.4	31.39±1,9	31.46±2.2	31.21±1.8
ESR(mm/hr)	8.19±0.1	8.07±2.5	7.69±1.0	7.69±0.3	7.93±0.6	8.01±0.7	8.10±2.6	8.12±0.4	8.19±1.7

Values are means ± standard deviations of triplicate determinations. L= Lymphocytes

N= Neutrophils

[46] reported that decrease in RBC could involve the polymerization of haemoglobin S.Table 3 shows that RBC, Hb, and PCV levels in all the test groups were insignificantly affected ($p>0.05$) against the control group. This may imply that neither the leaves nor the fruits of *Perseaamericana* could induce erythropoiesis (Red blood cell production) in test rats. White blood cells are considered as soldiers of the body due to their defensive mechanism [11]. The observed WBC levels in test rats were insignificantly affected ($p>0.05$) when compared to those of thecontrol group. Lymphocytes, the source of serum immunoglobulin cellular immune response which attack foreign substances in the body, were significantly ($p<0.05$) increased in test rats against the control rats. This may be

an indication that the leaves or fruits under study could boost immunity [46]. The ESR levels in test groups were insignificantly affected ($p>0.05$) when compared to ESR levels of the control group. This could be due to little or no increase in theblood parameters investigated in test rats hence the rate at which whole blood sediments was not altered. MCV, MCH and MCHC unlike other blood parameters are used for future possible disease condition in the body [11,2, 3,44]. MCHC levels reduced significantly ($p<0.05$) in rats placed on leaves whereas MCH levels were significantly ($p<0.05$) reduced in rats placed on fruit of *P.americana.* [19] noted that normocytic and hypochromic conditions occur when there are reduction in MCH or MCHC respectively. Hence, consumption of leaves of *P.americana*may induce hypochromic condition while that of thestudied fruit may lead to normocyticcondition in the body.

4. CONCLUSION

The present study has revealed the vitamins and amino acids present in the leaves and fruits of *P.americana*and the possible haematologic status of these leaves and fruits on consumption in the body system. The implication of this study could be that consumers of leaves, (or extract, infusion, and concoction made from the leaves) and the fruits of *P. americana* may be exposed to these effect.

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