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Effect of Cooking on Proximate, Phytochemical Constituents and Hematological Parameters of *Tetracarpidium conophorum* in Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the effect of cooking on proximate, phytochemical constituents and their changes in hematological parameters.
Study Design: Determination of proximate and quantitative phytochemical constituents of the cooked and raw *T. conophorum* (CTC and RTC respectively) nut and the effect of the nut on the hematological indices on male albino rats fed with the cooked and raw diet formulations of the nut for 30 days period.
Methodology: Rats were divided into six groups of five rats each. Each feed and walnut was weighed and mixed in the ratio of 1:1 before administration. Group A: Normal animal feed, Group B: Mixture of animal feed and cooked nut (ratio of 1:1).
Group C: Mixture of animal feed and the raw nut (ratio of 1:1), Group D: 100% of the cooked nut, Group E: 100% of the raw nut while Group F: Mixture of raw nut and cooked (ratio of 1:1).
Result: The result showed that crude protein, carbohydrate and crude fibre contents of RTC were significantly higher ($P<0.05$) than the CTC. While the percentage moisture, fat and ash content of the CTC were significantly higher than the RTC. The quantitative phytochemical analysis revealed that there was no significant difference ($P>0.05$)

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between the alkaloid and flavonoid contents of RTC and CTC. Tannin, saponin, glycosides, hydrogen cyanide and steroid contents of RTC were significantly higher (P<0.05) than the CTC while terpenoid content of the CTC was significantly higher (P<0.05) than of the the RTC. The hemoglobin values showed no significant difference between the test groups and control group. The neutrophil values of group E and F were significantly higher (P<0.05) when compared with the control group. Rats in group E had a significant decrease (P<0.05) in lymphocyte value as against the control. Total WBC levels in group B, C and F recorded a significant decrease (P<0.05) when compared with the control.

Conclusion: The study suggests that processing cooking affects some nutrient constituents and some hematological parameters.

Keywords: Tetracarpidium conophorum; proximate composition; phytochemical constituent; haematological parameter.

1. INTRODUCTION

The importance of food in our life cannot be over emphasized. Indigenous food crops and edible seeds and plant products which are widely grown but neglected and rarely consumed by people in urban areas are much more highly nutritious than most exotic foods [1,2]. T. conophorum *T. conophorum* (Mull. Arg.) Hutch. & Dalz (Euphorbiaceae *Euphorbiaceae*), known as conophor (English), ukpa (Igbo-Eastern Nigeria), awusa or asala (Yoruba-Western Nigeria), is a perennial climbing shrub of 3–6m long. In Nigeria, it is found in Uyo, Etinam, Enugu, Lagos and Ibadan. There is a wide distribution of biologically-active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition [3]. The knowledge that these compounds elicit both toxic and advantageous biological responses has given rise to several investigations in recent times as to their possible physiological implications in various biological systems [3]. Some of these chemicals are known as ‘‘secondary metabolites’’ and they have been shown to be highly biologically active [4]. Most of these secondary metabolites elicit very harmful biological responses, while some are widely applied in nutrition and as pharmacologically-active agents [5,6]. Oxalate, phytate and tannins are anti-nutrients, which could be toxic when consumed in an unprocessed food [7]. Chelation property may afford protection against oxidative damage and iron-overload [8]. Enujiugha and Ayodele [9] reported some significant concentrations of oxalates, phytates and tannins in raw conophor nut, while [10] reported that cooking brought about decreases in tannin and phytate contents. The roasted *T. conophorum* has no adverse effect on blood glucose, urea and creatinine; it also has adequate Fe and other nutrients needed to maintain homeostasis of haemoglobin [11]. For a food to be considered safe for human and animal health, its effect on these parameters need to be investigated to understand the nutritional potentials and safety of such foods with a view to determining their acceptability. This study determined the effect of cooking on proximate and phytochemical constituents of Nigerian walnut, as well as its effect on the haematological parameters on male albino rats. This study determined the phytochemical and proximate compositions of raw and cooked *Tetracarpidium conophorum* (walnut) and to ascertain if it could have beneficial effect on hematological parameters on male albino rats as our model for the study.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh *Tetracarpidium conophorum* nut was obtained from its tree at Olido village, Igbo-Eze North L. G. A. of Enugu State and was identified by Ozioko, Alfred of Biodiversity Centre, Nsukka. The method described by [12] was adopted for preparation of the meal. Cooked and raw seeds were ground separately into fine powder with the aid of electric blender.

2.2 Experimental Animals

A total of 30 male albino rats weighing 120-150g were obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. They were acclimatized for one week under standard environmental conditions and maintained on a regular feed and water *ad libitum.*

2.3 Animal Grouping and Feed Formulation

Rats were divided into six groups of five rats each. Each feed and walnut was weighed and mixed in the ratio of 1:1 before administration. The rats were fed *ad libitium* for 30 days. The animals were handled according to the guidelines of the Ethical Committee on the use and care of experimental animals of the Department of Biochemistry, University of Nigeria, Nsukka. Rats in the following groups were fed as follows:

Group A: Normal animal feed, Group B: Mixture of animal feed and cooked nut (ratio of 1:1). Group C: Mixture of animal feed and the raw nut (ratio of 1:1), Group D: 100% of the cooked nut, Group E: 100% of the raw nut while Group F: Mixture of raw nut and cooked (ratio of 1:1).

2.4 Proximate Analysis

Proximate analysis of raw and cooked *T. conophorum* nut was carried out using the standard procedures of AOAC by micro-Kjeldhal method and crude protein content calculated as N × 6.25.

The readings were three times and the average taken as mean

2.5 Phytochemical Constituents Analysis

Quantitative phytochemical analysis of *Tetracarpidium conophorum* was carried out for the following bioactive compounds: Alkaloids and flavonoids by the method of [13]; saponins by the method of [14]; tannins by the method of [15] and steroids by the method of [16]; Cyanide by method of [17].

2.5.1 Determination of alkaloids

5g of the samples were weighed into 250ml beaker and 200ml of 20% acetic acid was added and covered to stand for 4hr.This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was

allowed to settle and the precipitate was collected by filtration and weighed (Harborne, [13]; Obadoni and Ochuko, [14]).

2.5.2 Determination of flavonoids

10g of the plant samples were extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The extract was filtered through whatman filter paper no. 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Harborne [13]; Obadoni and Ochuko [14]).

2.5.3 Determination of saponins

20 g of each sample was dispersed in 200ml of 20% ethanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200ml of 20 % ethanol. The combined extracts were reduced to 40ml over water bath at about 90ºC. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content were calculated in percentage (Obadoni and Ochuko [14]).

2.5.4 Determination of tannins

The tannin content of the Conophor nut of the plants was determined using the Folin Dennis spectrophotometric method as described by Pearson (1976). The powered sample (2.0g) was mixed with 50ml of distilled water and shaken for 30 minutes in the shaker. The mixture was filtered and the filtrate used for the experiment. A volume of 5.0ml of the filtrate was measured into 50ml volume flask and diluted with 3 ml of distilled water. Similarly, 5ml of standard tannic acid solution and 5 ml of distilled water was added separately. One millitre (1ml) of Folin- Dennis reagent was added to each of the flask which was followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was made up to a mark and incubated for 90 minutes at room temperature. The absorbance of the developed colour was measured at 760 nm with the reagent blank at zero. The process was repeated three times to get an average.

2.5.5 Determination of Steroid content

This was determined by the method described by Okeke and Elekwa [17]. A measured weight of each sample was dispersed in 100ml freshly distilled water and homogenized in a laboratory blender. The homogenate were filtered and the filtrate was eluted with normal ammonium hydroxide solution (pH 9). Two milliliters (2ml) of the eluate were put in test tube and mixed with 2ml of chloroform. A known volume of 3ml of ice-cold acetic anhydride was added to the mixture in the flask and 2 drops of conc. H_2SO_4 were cautiously added to cool. Standard sterol solution was prepared and treated as described above. The absorbance of standard and prepared sample was measured in a spectrophotometer at 420nm.

2.5.6 Determination of cyanide content

The cyanide contents of the cooked and raw samples were determined by spectrophotometric method of Bradbury et al. [18]. A quantity (0.1g) of each sample was weighed into a flat bottom bottle with a screw cap lid. 0.5ml of 0.1M phosphate buffer at pH 6 was added with a pipette. A yellow picrate paper attached to a plastic strip placed immediately in the flat bottom bottle contained the buffer. The picrate buffer was not allowed to touch the liquid in the bottle. The bottles were immediately closed with screw cap lids. A blank for each of the sample was also prepared as above into another screw capped bottle. Linamarin standard stock solutions were also prepared using 10mg linamarin in 10ml of 0.1M phosphate buffer at pH 6. This was diluted to give concentrations of 25ppm to 100 ppm (i.e. 25, 50, and 75,100).This was used to standardize and calibrate the spectrophotometer. Linamarin paper of 50 ppm concentration each was treated as samples above and put in a separate screw capped plastic bottles containing phosphate buffer and linamarase enzyme bottles were closed immediately. All the bottles containing samples, blank and linamarin standard paper were allowed to stand for 16-24hr at room temperature; after which, the bottles were opened, plastic backing sheets of picrate paper were removed and placed in a test tube. Distilled water (5ml) was pipetted into each of the test tubes containing picrate paper and was allowed to stand for 5 minutes with occasional gentle stirring. The absorbance of all the solution in the test tubes including linamarin standard solution were measured against blank on spectronic 20 spectrophotometer at a wavelength of 510nm.

The total cyanide content was calculated using the formular.

Total cyanide content = 396 x Absorbance (ppm) or mg/kg.

$$\text{Total cyanide content (\%)} = \frac{\text{ppm cyanide}}{10{,}000}$$

The readings were three times and the average taken as mean.

2.6 Hematological Analysis

Full blood counts including PCV, Hb, RBC, WBC (packed cell volume, haemoglobin, red blood cell, white blood cell respectively) platelet count, differential WBC (lymphocytes, neutrophils and mixed), and red cell indices (MCHC= mean cell haemoglobin concentration, MCH= mean cell haemoglobin, MCV= mean cell volume.), were determined using the Sysmsex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan, in Centre for Disease and Control Laboratory, University of Nigeria Teaching Hospital (UNTH) Enugu.

2.7 Data Analysis

The results were analyzed using a statistical software package – SPSS Version 18. Data were expressed as mean ± standard error of the mean (mean ± SEM). Student's t-test was employed for comparison between two sets of data. Where the variables to be compared are three or more, one-way analysis of variance (ANOVA) was used, Duncan test was used for post-hoc. $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Proximate composition

The results of the proximate analysis of the cooked and raw *T. conophorum* showed that the moisture contents of CTC were significantly higher (P<0.05) than those of RTC. Similarly, the values for the crude fat and ash content of the CTC were significantly higher (p<0.05) than those of the raw (RTC) nut while the carbohydrate as well as the crude fibre contents of RTC were significantly higher (p<0.05) than those of the CTC nuts.

Crude protein content of the cooked *Tetracarpidium conophorum* (CTC) was significantly higher (p<0.05) than that of the raw *Tetracarpidium conophorum* (RTC).

3.1.2 Phytochemical constituents

Phytochemical constituents of *T. conophorum* indicates that tannin, soluble carbohydrate, saponin, glycosides, steroid and hydrogen cyanide values of RTC were significantly higher (P<0.05) than that of the CTC. However, terpenoid content of the cooked *Tetracarpidium conophorum* (CTC) was significantly higher (P<0.05) than the content of the RTC. Both alkaloid and flavonoid showed no significant difference (P>0.05) between RTC and CTC values.

3.1.3 Hematological Parameters

The mean haemoglobin concentrations of rats in group F (50% cooked + 50% raw) and E (100% raw) were slightly elevated when compared with the group A (control group). However mean Hb concentration (Fig. 1) of group B, C and D showed no change when compared with that of the control (group A). The mean neutrophil values (Fig. 2) of group E (100% raw diet) and group F (50% cooked + 50% raw) were significantly elevated (P<0.05) when compared with the group A (control) while Group E (100% raw) diet had a significant decrease (P<0.05) in lymphocyte count (Fig. 3) when compared with the control. The Total WBC count (Fig. 4) seem to have been lowered by raw walnut meals in groups B (50% feed + 50% cooked), C (50% feed +50% raw), E (100% raw walnut) and F (50% cooked + 50% raw) while the cooked walnut seem not to have significant effect on the total WBC count.

3.2 Discussions

Proximate composition of food crop is a major index of the nutritional potential of crops. The proximate composition of raw *T. conophorum* nut (RTC) (Table 1) differed from those reported by previous studies other workers. For example, RTC nut was assayed to contained 40.10% moisture, 1.77% crude fibre, 4.75% fat, 17.68% protein and 33.29% carbohydrate – were much lower than those reported by [10] to have 6.34% fibre, 48.90% oil 29.09% protein and 12.58% carbohydrate. Food and Agriculture Organisation [18] reported that the raw seed contained 3.70% fibre, 56.0% fat (ether extract), 22.7% crude protein, and 9.10% carbohydrate. These differences may be attributed to environmental and agronomic factors as has been suggested by other researchers [19,20]. The FAO report was an average of varying findings from different researchers in different countries while the present study was carried out in Nsukka locality, Enugu state, Nigeria. The raw *T. conophorum*

(RTC) protein was significantly higher (P<0.05) than that of the cooked *T. conophorum* (CTC).

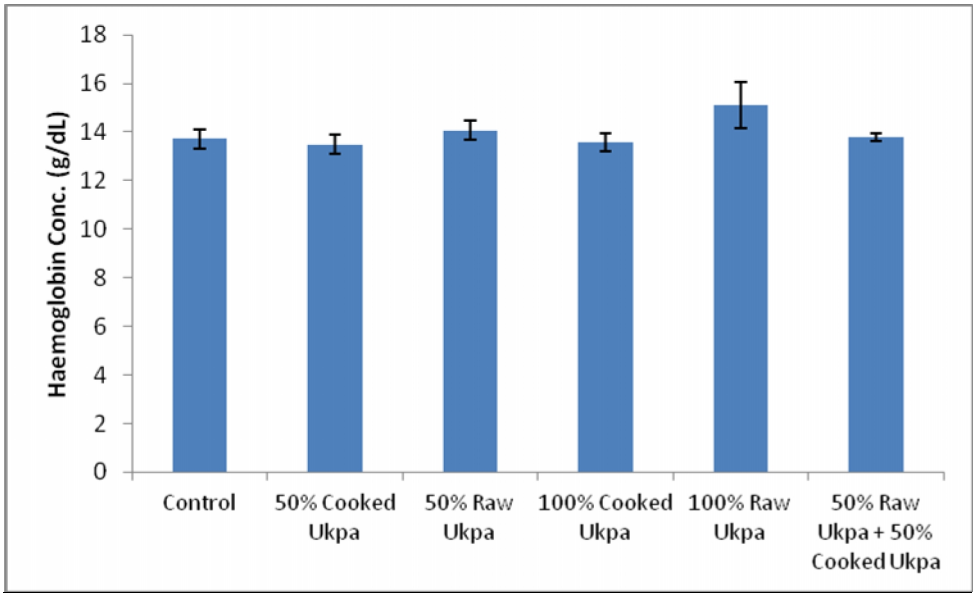

Fig. 1. Effect of cooked and raw *T. conophorum* on Haemoglobin concentration of different groups

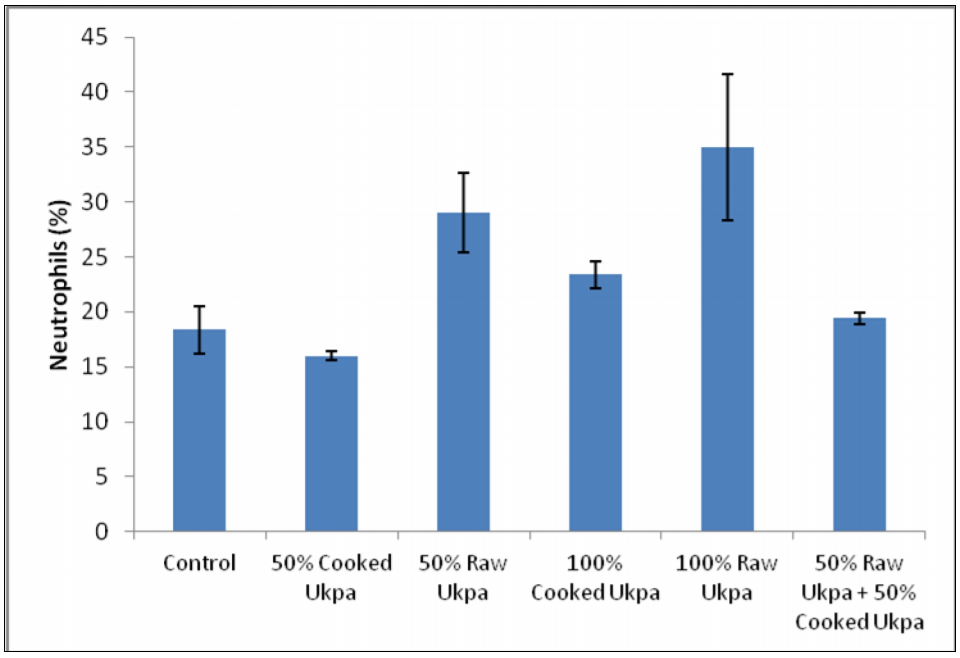

Fig. 2. Effect of cooked and raw *T. conophorum* on neutrophil count of different groups

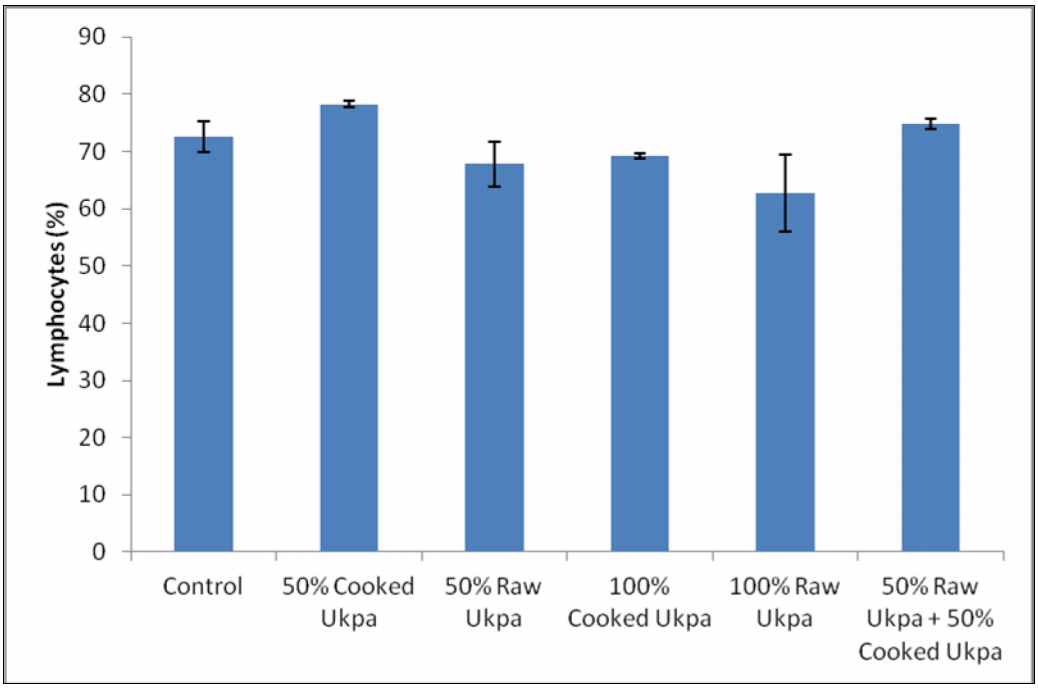

Fig. 3. Effect of cooked and raw *T. conophorum on* lymphocyte counts of different groups

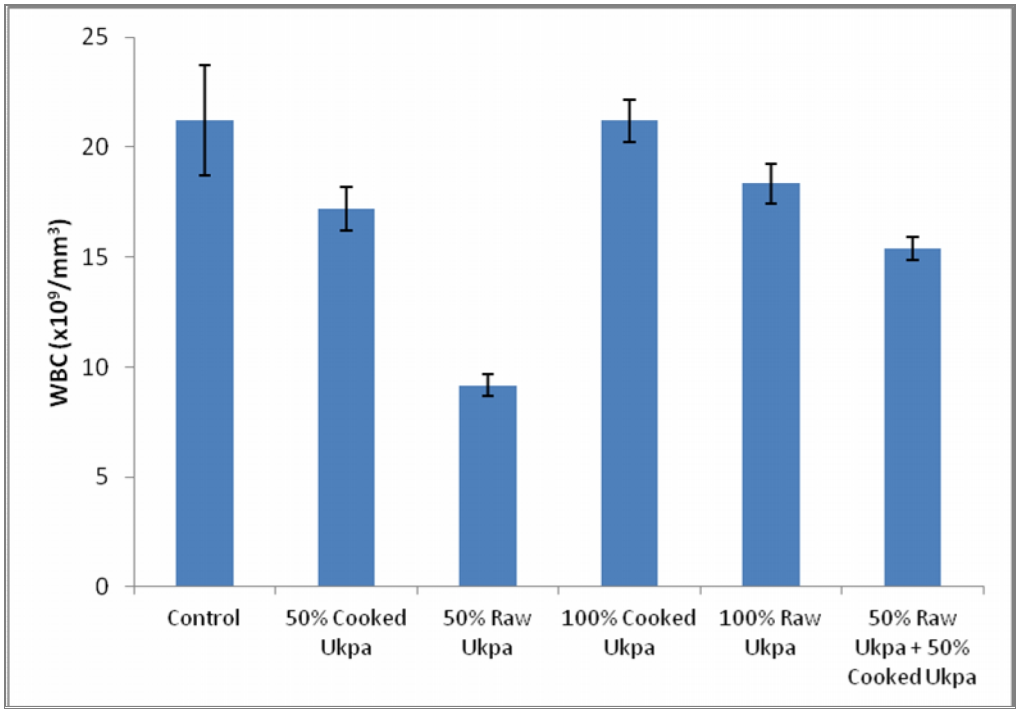

Fig. 4. Effect of cooked and raw *T. conophorum on* total WBC count of all the groups

The reduced level of protein in CTC sample could be due to leaching loss and solubility of nitrogen as supported by [21] in his research on cowpea. The fibre content of the RTC was significantly higher (P<0.05) than that of the CTC which could be attributed to loss of solid particles during boiling [22]. The crude fat content of CTC (4.9%) as recorded in this study, was lower than that reported by [18] (56.0%) and [10] (48.9%), but significantly higher (P<0.05) than that of the RTC (4.7%).There was also significant increase (P<0.05) in carbohydrate and ash content of CTC when compared with that of the RTC. This increase may be attributed not only to heat treatment which hydrolyses some organic bonds to release more free nutrients such as fats, ash and sugars but also to geographical location, varietal difference and growth conditions [19,21]. The percentage moisture content of CTC (46.5%) was significantly higher than that of the RTC (40.11%) which would be due to water intake as a result of hydrothermal application. These findings were consistent with the report of [23] that this increase in the boiled sample was due to the absorption of water by simple diffusion.

The results of the phytochemical composition as shown in Table 2 revealed that the plant, *T. conophorum* is rich in as alkaloids, flavonoids, tannins, saponins, glycosides,Terpenoid, and steroids. The presence of these secondary metabolites could have contributed to its medicinal value as well as physiological activity [24]. For instance, flavonoids have been shown to have antibacterial, anti – inflammatory, anti allergic, antiviral antineoplastic activities [25]. Many of these alleged effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chellators [26]. Steroidal compounds are of importance in pharmacy because of their relationship with compounds used as sex hormones [27]. Saponins have been reported to show tumor inhibiting activity on experimental animals (*Rattus novergicus*) [28]. Saponins may also enhance nutrient absorption and aid in animal digestion [29]. Alkaloid has pharmacological effects and is used as medication and recreational drugs [30]. Our results showed that there was no significant difference (P>0.05) between alkaloid and flavonoid contents of the CTC and RTC while Tannin, saponin, glycosides and steroid contents were significantly higher (p<0.05) in RTC when compared with that of the CTC. It was observed therefore that cooking decreased the phytochemical contents especially the anti nutritional factors in CTC when compared with those of the RTC walnuts. Perhaps because some secondary metabolite leached into the processing water in the course of cooking and at higher temperature [22]. This is in consonance with the observations that soluble secondary metabolite get lost by dissolving into cooking water [31,32]. The concentration of hydrogen cyanide of RTC (0.039mg/100g) was significantly higher than the value of the CTC (0.032mg/100g) in which could be attributed to leaching of cyanide into the cooking water [31] or the evaporation of cyanide during cooking.

Table 1. Proximate composition of raw and cooked *Tetracarpidium conophorum*

Proximate parameters	Raw walnut Mean ± SD	Cooked walnut Mean ± SD
1. Moisture	40.11±0.01	46.52±0.10
2. Ash	2.33±1.09	2.38±0.30
3. Fibre	1.77±0.40	1.65±0.06
4. Fats	4.76±0.01	4.94±0.00
5. Proteins	17.69±0.10	14.01±2.01
6. Carbohydrates	33.32±0.03	30.49±0.01

Table 2. Phytochemical constituents of raw and cooked *Tetracarpidium conophorum*

Phytochemical constituents	Raw walnut Mean ± SD	Cooked walnut Mean ± SD
1. Tannins	0.82±0.60	0.78±1.01
2. Soluble Carbohydrates	0.34±0.08	0.14±0.50
3. Saponins	0.94±0.10	0.93±0.03
4. Glycosides	1.52±0.01	1.51±0.10
5. Terpenoids	0.26±0.32	0.27±0.31
6. Reducing Sugars	0.00±0.60	0.00±0.10
7. Steroids	1.07±0.00	0.85±0.26
8. Flavonoids	1.12±2.01	1.12±0.30
9. Alkaloids	1.64±0.52	1.64±0.09
10. Cyanide	0.39±0.00	0.32±0.00

Hematological parameters provided vital information regarding the status of bone marrow activity and possible immune response. Significant changes in the hematological indices do not follow any particular trend that could be attributed to the inclusion levels of diets [32].This makes variations in leukocyte subpopulations are more difficult to interpret. The significant increase in the blood components especially neutrophil ($p<0.05$) of groups fed with 50% CTC + 50% RTC and 100% RTC (Fig. 2) could suggest increased maturation of neutrophils as a result of antigenic challenge from the RTC nut. Neutrophils are phagocytic type of WBC that attack and destroy invading bacteria, viruses and other injurious agents. There was no significant different ($P>0.05$) in hemoglobin (Hb) values (Fig. 1), implying that the components of the nut are not hemolytic to the red blood cells. The significant decrease in lymphocyte count ($P<0.05$) in rats fed with 100% RTC (Fig. 3), could suggest decreased proliferation of the lymphocytic cells or the mobilisation of lymphocyte from bone marrow into the extravascular tissues as hypothesized by [33]. Significant decrease ($P<0.05$) in total white blood cell (TWBC) in rats fed with 50% feed + 50% CTC, 50% feed + 50% RTC and 50% CTC + 50% RTC was observed. It is possible that the decrease was caused by the white blood cells attempt to fight the damage caused by the constituents of the raw nut. Some antinutritional factors and cyanide may have interacted synergistically especially in the RTC containing diets to suppress the homeopoitic activity of WBC and lymphocyte hence the decrease. The total WBC of any animal is a function of the immunity and resistance to diseases [34]. Basophils, monocytes and Eosinophils were produced in insignificant proportion and were therefore not included in the results.

4. CONCLUSION

In conclusion, the result shows that cooking affected the values of the proximate and phytochemical constituents, especially the antinutritional factors. The group that fed on RTC containing diets had high neutrophils; we conclude that RTC could probably be a good source of innate immunity.

However there is a need for further studies to be carried out to elucidate the influence of agronomic factors on the biochemical responses of different species of walnut. Studies are also needed to determine neutrophil phagocytic activity.

CONSENT

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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