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Full Length Research Paper

Bromatological analysis, phytochemical and antioxidant potential of carnauba (*Copernicia prunifera* (Mill.) H.E. Moore) fruit

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Chemical composition as well as phytochemicals and antioxidant activity of underutilized plants, such as carnauba palm (*Copernicia prunifera*), provide an informed decision on how to maximize their benefits. This study aimed to evaluate the chemical composition and antioxidant activity of the fruit of carnauba. The total phenolic content (gallic acid equivalents, GAE) of the ethanol extract of the whole fruit, pulp and kernel were 44.6000, 0.0447 and 0.6930 mg GAE/g extract, respectively. The respective total tannin content was 1590.932, 2977.724 and 147.650 ppm in the whole fruit, pulp and kernel. The fruit of carnauba showed 9.84 mg anthocyanins/50 g of fresh pulp. Ethanol extract of whole fruit had a potent antioxidant activity. Thus, the fruit of carnauba has add-value in food industry.

Key words: Secondary compounds, carnauba ethanol extract.

INTRODUCTION

Carnauba palm or Brazilian tree of life [*Copernicia prunifera* (Miller) H. E. Moore, Arecaceae family] is a palm tree found in the northeastern Brazil and Cerrado biomes, located in some Brazilian regions (D'alva, 2004). The extractive exploitation of the resources offered by This palm was first described around 1648 by Maregravius and Piso, and subsequently ratified by R. Müller, in 1768, upon reporting the first experiments on carnauba wax for candles. Carnauba wax is extracted from the leaves, and used extensively in various

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Figure 1. The unripe fruits of carnauba (*C. prunifera*) used, before full maturity when the external coloring of the bunches were predominantly green.

industrial applications, such as a protective and polishing agent, glazing for paper, coating and encapsulation of pharmaceuticals, and food additives. It is exported to more than forty countries, although the United States, Japan and Germany are the major destinations (Oliveira and Gomes, 2006).

The fruit of carnauba contains protein (5.46% on pulp; 6.89% on kernel), fat (6.25% on pulp; 13.65% on kernel), carbohydrates (64.32 % on pulp; 63.29% on kernel), cellulose (5.81% on pulp; 4.07% on kernel), vitamins (96,20 mg.100g^{-1} on fruit) and other constituents, such as phenols (33,93 mg.100 g^{-1} for flavonoids; 2,42 mg.100 g^{-1} for anthocyanin) (Braga, 1976; Nogueira, 2009). Moreover, this fruit has been evaluated for its antioxidant (DPPH radical scavenging activities with inhibitory concentration of 50% from 15.3±0.4 and 41.9±0.8 µg.ml^{-1} on mesocarp and epicarp, respectively; 3549±184 g.g DPPH^{-1} on fresh matter, and 4877±24.3 g.g DPPH^{-1} on dry matter of whole fruit) (Silva et al., 2005; Rufino et al., 2010, 2011), and antibacterial activities against Gram-positive bacteria (*Staphylococcus aureus*) (Ayres et al., 2008).

The fruit of carnauba can be used for different things, including obtaining milk from the pulp, as well as producing flour from the extracted and dried pulp or the green fruit, for use in biscuit manufacturing (Pereira and Carneiro, 2006; Ferreira, 2009). The mature fruit can be used, for example, to manufacture jelly for human consumption (Nogueira et al., 2009). Furthermore, the ground and roasted kernel may be used as coffee substitute (Pio Correa, 1931) or yield oil, which can also be used in food (Lorenzi, 1996). Recently, use as animal feed (Silva et al., 2015a). Besides that, carnauba leaves are noted for their elevated content of wax which has many applications in the pharmaceutical industry, food industry, cosmetics, and lubricants (Barman et al., 2011).

In this context, in addition to standard chemical analysis to determine the protein, fiber, lipids and mineral salts, the phytochemical approach, which involves the study of the properties of secondary metabolites (phenols, flavonoids, anthocyanins, tannins, among others) present in the food has gained prominence. Namely, the antioxidant (Schenkel et al., 2007), antimicrobial (Kooti et al., 2016), antifungal, antiparasitic, (Camacho et al., 2004) anti-inflammatory (Raso et al., 2001), hypocholesterolemic, immunostimulant (Cozzolino, 2009) and insecticidal properties (Menezes, 2005) demonstrate the various biological effects posed by these bioactive secondary metabolites. .

The aim of this study was to determine the chemical composition, phytochemical profile and antioxidant activity of the extracts obtained from the fruit of the carnauba palm and its separate parts, namely: the pulp and kernel.

MATERIALS AND METHODS

Plant materials

The unripe fruits of *C. prunifera* (Mill.) H. E. moore were collected in the Aracati, Ceará state (northeast of Brazil) (Figure 1). The fruit was then transported to the experimental farm where a sample was immediately frozen at -20°C until use. A separate sample was

Table 1. Evaluation of color tests for anthocyanins, anthocyanins, and flavonoids.

Constituents	Color		
	Acid (pH 3)	Alkaline (pH 8.5)	Alkaline (pH 11)
Anthocyanins and anthocyanidins	Red	Lilac	Blue-crimson
Flavones, flavonols and xanthones	-	-	Yellow
Flavonoids	-	-	Orange-red

Source: Matos (2009).

dehydrated using an outdoor dryer that was revolved several times a day to allow homogeneous dehydration to occur. The dried sample was ground and stored at room temperature until analysis. Analyses were performed on the whole fruit of the dried sample, and the pulp and kernel of the frozen fruit.

Chemical analysis of the fruit

The dry matter (DM), crude protein, ether extract and mineral matter were analyzed according to the procedures described by Silva and Queiroz (2002). The neutral detergent fiber, acid detergent fiber, lignin and cellulose contents were analyzed according to Van Soest et al. (1991).

Phytochemical detection

The whole fruit, kernel and pulp extracts were obtained by respective pulverization. An initial hexane extraction was performed followed by an ethanol extraction, and then the sample left in contact with the solvent for 7 days. This process was repeated three times. After, the extracts were filtered (filter paper, Qualy® - diameter 9 cm) and concentrated by rotary evaporation (model:801, FISATOM, Sao Paulo, Brazil) under reduced pressure, thus obtaining the hexane and ethanol extracts. Detection of phytochemicals were performed according to the method by Matos (2009). The hexane and ethanol extracts were qualitatively analyzed for phenols and tannins, anthocyanins, proanthocyanidins, flavonoids, saponins, catechins, triterpenes and steroids (Libermann–Burchard test), and leucoanthocyanidins.

Detection for phenols and tannins

Three drops of an alcoholic solution of 1% ferric chloride III ($FeCl_3$) was added to 10 mg of extract. Then, the mixture was stirred well for visual assessment of variations in color and precipitate formation, indicating the presence or absence of phenols and tannins (Matos, 2009).

Detection of anthocyanins, anthocyanidins and flavonoids

Approximately, 0.2 mg of extract was added to a test tube in triplicate. The first tube was acidified using 10% HCl until pH 3 was attained, the remaining extracts were basified using 10% (w/v) aqueous sodium hydroxide (NaOH) solution until pH 8.5 and 11, were reached, respectively. The color development in the tubes was evaluated according to Table 1.

Detection of saponins

In a test tube, 50 mg extract was added to 5 to 10 mL of water. Then, the tube was shaken vigorously for 2 to 3 min, and the presence or absence of foam was observed.

Steroids and triterpenoids detection

Based on the Liebermann-Burchard test, 0.2 mg of extract was placed in a vial and then 1 to 2 mL of chloroform added for dissolution. After, the solution was filtered using a small funnel closed with cotton into a second test tube. Then, 1 ml of acetic anhydride was added, and the solution stirred gently before adding three drops of concentrated sulfuric acid (H_2SO_4; 18 M) and stirred again. The development of color was then observed (Matos, 2009).

Leucoanthocyanidins, catechins and flavones detection

Approximately, 0.2 mg of extract was added to two test tubes, respectively. HCl was added to one tube, until pH 1 to 3 was attained. The remaining tube was basified using NaOH until pH 11 was attained. Then, the tubes were heated at 60 to 70°C in a water bath for 3 min, observing changes in color, according to Table 2 (Matos, 2009).

Flavonols, flavanones, flavonols and xanthones detection

Approximately, 10 mg of magnesium ribbon and 0.5 mL of concentrated HCl was added to a test tube with 10 mg of extract. After completion of the reaction, indicated by the end of the effervescence, a color change indicative of the presence of the compounds was observed (Matos, 2009). .

Preparation of extracts

The unripe fruits (whole fruit, pulp and kernel) were initially immersed in hexane for 7 days. After, the mixture was filtered (filter paper - diameter 9 cm) and the organic liquid phase was concentrated on a rotary evaporator under reduced pressure, resulting in the hexane extract. Then, the plant material was placed at room temperature to evaporate any residual hexane and the plant material then immersed in ethanol (70% ethyl alcohol) for 7 days (this procedure was repeated twice with the same plant material to ensure maximum removal of the constituents). After, the liquid phase was concentrated on a rotary evaporator under reduced pressure, resulting (non-volatile portion) in the ethanolic extracts (Freire et al., 2015).

Table 2. Evaluation of colors for leucoanthocyanidins, catechins and flavones.

Constituents	Natural color	
	Acid	Alkaline
Leucoanthocyanidins	Red	-
Catechins (catechin tannins)	Yellow-gray	-
Flavanones	-	Red-orange

Source: Matos (2009).

Determination of total phenols

According to the Folin-Ciocalteu method (Obanda and Owuor, 1997), each extract was dissolved in methanol to a final concentration of 5 mg/ml. Then, 100 µl of the final solution was vortexed with 500 µl of Folin-Ciocalteu reagent and 6 ml of distilled water for 1 min. Then, 2 mL of 15% sodium carbonate (Na_2CO_3) was added and the mixture vortexed for 30 s. Then, 10 mL of distilled water was added, and after 2 h at room temperature, the absorbance was measured in a spectrophotometer (T-2000, TEKNA, Brazil) at 750 nm. Quantitation was done in triplicate and the results expressed as gallic acid equivalents (GAE) per gram of crude extract.

Determination of anthocyanins

According to Teixeira et al. (2008), organic solvent (ethanol/water) in the ratio 70:30 v/v was added to approximately 50 g sample, followed by sufficient HCl to adjust the pH to 2. The material was allowed to stand for 24 h at low temperature (27°C) in the dark light. After, the material was filtered (filter paper, Qualy® - diameter 9 cm) and transferred to a 100 ml volumetric flask, and then centrifuged at 2000 rpm for 10 min. The extract was purified using a mixture of ethyl ether and hexane in the ratio 1:1 to remove chlorophyll (three successive extractions). Finally, an aliquot was transferred to a 10 ml flask and brought to volume using a mixture of ethanol/HCl in the ratio 85:15 v/v. The absorbance was measured at 535 nm (T-2000, TEKNA, Brazil) using ethanol/HCl (85:15 v/v) as the blank. The anthocyanin content was calculated according to equation 1.

Equation 1: Equation for obtaining anthocyanin content of the vegetable extracts of carnauba.

$$AntT\ (mg/50\ g\ sample) = \frac{OD\ x\ VE1\ x\ VE2\ x\ 1000}{Valq\ x\ W\ x\ 982}$$

where,
OD is optical density of the diluted extract, *VE*1 is the total volume of the concentrated extract, *VE*2 is the total volume of the diluted extract, *Valq* is the aliquot volume used to dilute the concentrated extract, *W* is the sample weight, and 982 is the average extinction coefficient of anthocyanins by the single pH method.

Determination of tannins

100 mg aliquot of the plant material was extracted with 1 ml of acetone: water solution (70:30 v/v) in water bath at 30°C, and vortexed every 5 min. Then, the contents were centrifuged at 4000 rpm for 5 min, and the supernatant was collected. The extractions were repeatedly performed until achieving a maximum extraction. The extracts were then evaporated in a water bath at 60°C until near completely dried, at which time 2.5 ml of methanol was added. Then, 0.1 ml of this mixture was added to a test tube containing 0.9 ml of methanol. After, 5 ml of vanillin reagent was added (2.5 mL of a solution containing 1 g of vanillin in 100 mL of methanol, followed by 2.5 mL of a solution containing 8 ml of concentrated HCl in 100 mL of methanol). The tubes were heated in a water bath at 60°C for 20 min. The sample control contained 4% HCl solution in methanol. The absorbance was then measured at 500 nm (T-2000, TEKNA, Brazil) and the tannin content obtained from the calibration curve, previously performed using catechin (Costa et al., 2003).

Determination of condensed tannins (CT)

Powdered sample (200 mg) was placed in a beaker, and 10 mL of 70% acetone was added. The mixture was then transferred to a test tube, which was capped and then vortexed for 20 min, before centrifugation at 3000 rpm for 10 min. The supernatant was collected and placed in an ice bath. A capped test tube containing 0.5 ml extract, 3 ml of butanol-HCl solution (95:5 v/v) and 0.1 ml of 4% ferric reagent (2.1 g of NH4Fe (SO4)2.12 H_2O + 17 mL concentrated HCl + 33 ml methanol), was then placed in a water bath at 100°C for 60 min. A control sample without extract (no heat treatment was required) was prepared simultaneously. After the tubes had been cooled, the absorbance at 550 nm was measured (T-2000, TEKNA, Brazil). The level of CT was expressed as leucocyanidin equivalents using the following formula:

Reading x 78.26 x dilution factor/DM% (Porter et al., 1986).

Antioxidant potential assessment

The antioxidant potential was evaluated using the DPPH free radical assay, as described by Almeida et al. (2006) with some modifications. Briefly, the extracts were dissolved in methanol and then further diluted with methanol to yield the following concentrations: 10, 20, 40, 70, 100, 200, 600 and 700 ppm. A 1 mL aliquot of each concentration was combined with 1 ml methanolic solution of DPPH 60 µmol L-1. The samples were left to rest for 30 min in the dark at room temperature. Then, the absorbance was measured at 520 nm (T-2000, TEKNA, Brazil) against a blank sample containing methanol. The process was performed in triplicate. The inhibition of the DPPH radical was calculated from the ratio of absorbance of each extract concentration against a reference solution containing 1 mL methanol and 1 mL of DPPH. The percentage inhibition of each extract was then obtained from Equation 2.

Table 3. Chemical analysis of the whole carnaúba fruit, its kernel and pulp.

Chemical composition (%)	Whole fruit	Pulp	Kernel
Dry matter	87.60	96.65	34.81
Crude protein	6.30	5.21	4.50
Ethereal extract	6.36	0.65	4.95
Mineral matter	5.32	6.44	2.07
Neutral detergent fiber	61.03	67.29	50.11
Acid detergent fiber	42.33	37.35	47.69
Lignin	16.08	14.81	11.64
Cellulose	25.21	22.91	35.32

Equation 2: Equation for obtaining the percent inhibition of the DPPH radical of the vegetable extracts of carnauba.

$$\%In = \frac{(Abs_{DPPH} - Abs_{sample})x\ 100\%}{Abs_{DPPH}}$$

Where,

% In is the free radical inhibition percentage of the extract, Abssample is the absorbance of the extract and AbsDPPH is the absorbance obtained from the sample DPPH/methanol.

The respective concentration of the extract (mg/ml) required to inhibit 50% of the free radical scavenging ability was calculated by linear regression, using Origin 7.0 software, of plots, where the x-axis represented the various concentrations of the extracts, while the y-axis represented the average percentage of free radical scavenging ability obtained from the triplicates.

RESULTS AND DISCUSSION

Chemical analyses of foods provide insight into the nutritional composition of such foods, facilitating their maximal efficacy. In the present study, the chemical composition of whole carnauba fruit, and its pulp and kernel were independently assessed, as shown in Table 3.

Silva et al. (2015b) evaluated several fruit species belonging to the Arecaceae family, and observed that for most species, the total protein contents of the pulp (1.50 to 20.60 g 100 g^{-1}) and kernel (2.60 to 11.70 g 100 g^{-1}) resented a wide variation, but was typically higher in the kernel relative to the pulp. One exception was Syagrus *cearensis*, which had the highest content in the pulp as seen in kernel (20.60 g 100 g^{-1} versus 4.30 g 100 g^{-1}) (Silva et al., 2015b). Braga (1960) reported that values of protein relatively close to those obtained in the present study for the pulp content (5.46%) and higher values for the kernel (6.89%), compared to those found in present study (6.30, 5.21 and 4.50% in the dried whole fruit, the pulp and fresh kernel, respectively).

Braga (1960) also assessed the fat content of the fruit pieces, and found 13.65 and 6.25% in the almond and pulp, respectively. The present work presented comparatively lower values of 0.65 and 4.95% in the kernel and pulp, respectively. The ash content of the pulp was 2.07%, similar to that reported in the literature (2.95%) for the pulp, however, the ash content of the kernel was significantly different, at 6.44% in the present study compared to 1.55% reported Braga (1960).

In this instance, the drying process used in this study may have influenced the outcome, as the fruit drying process reduces the fat content and sodium content as described by Alasalvar and Shahidi (2013). Furthermore, it is known that fruit maturation stage influences various physico-chemical characteristics and therefore, nutritional changes in the content of sugars, protein, vitamin C, minerals, as well as pH, among other features (Brito and Narain, 2002; Nogueira et al., 2002; Tlili et al., 2014).

The phytochemical analysis of *C. prunifera* is reported in Table 4. Positive results were obtained for phenols and tannins, and anthocyanins, anthocyanidins and flavonoids at pH 11 (except for the hexane extract of the whole fruit), saponins (exclusive to the ethanol extract of the kernel), free steroids (except for the ethanol extracts of the pulp and almond), leucoanthocyanidins, catechins and flavones (solely in the ethanol extract of the whole fruit), and flavonols, flavanones,flavonoids and xanthones (only in the ethanol extract of the kernel).

Phenols are responsible for the flavor, odor and color of various plants (Vizzoto et al., 2010). They constitute a major chemical group of plants and can be divided into three subclasses, namely phenolic acids, stilbenoids and flavonoids, these being the most studied. Several studies have demonstrated the beneficial effects of diets rich in flavonoids, including anti-inflammatory, anti-cancer, cardiovascular disease prevention, oxygen free radical reduction, anti-allergic and antiviral (Middleton, 1998; Egert and Rimbach, 2011; Mccullough et al., 2012). Rufino et al. (2010) observed that the presence of

Table 4. Qualitative phytochemical analysis of the whole fruit, kernel and pulp hexane and ethanol extracts.

Compounds	Whole fruit		Pulp		Kernel	
	HE	EE	HE	EE	HE	EE
Phenols and tannins	(+)	(+)	(+) for flaboblenics tannins	(+) for flaboblenics tannins	(+) for flaboblenics tannins, condensed or catechin	(+) for flaboblenics tannins, condensed or catechin
Anthocyanins, anthocyanidins and flavonoids	(-)	(-) for pH 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11	(-) for pH 3 and 8.5 (+) for pH 11
Saponins	(-)	(-)	(-)	(-)	(-)	(+)
Steroids and triterpenoids	(+) for free steroids	(+) for free steroids	(+) for free steroids	(-)	(+) for free steroids	(-)
Leucoanthocyanidins, catechins and flavones	(-)	(+)	(-)	(-)	(-)	(-)
Flavonols, flavanones, flavanonols and xanthones	(-)	(-)	(-)	(-)	(-)	(+)

EE = Ethanol extract; HE = hexane extract; (+) = Positive; (-) = Negative.

flavonoids in the pulp of the fruit of carnauba. As this class of compounds (flavonoids) have many beneficial health properties (Sucupira et al., 2012; Moo-Huchin et al., 2015; Cao et al., 2016), their presence in the fruits of the carnauba is a positive factor that contributes to increase their potential use in animal nutrition.

Flavonoids represent an attractive example of plant bioactives with promising uses, including in animal feed (Vasta and Luciano, 2011). As shown in Table 5, the total phenolic content in the ethanol extracts obtained in the present study, was 44.60 mg GAE/g in the whole fruit extract, 0.04 mg GAE/g extract in the pulp and 0.69 mg GAE/g extract in the kernel. In the hexane extract, there was 33.2000 mg GAE/g in the whole fruit extract, 0.8326 mg GAE/g in the kernel extract and none detected in the pulp. These data shows that the phenolic compounds have more solubility in ethanol, justified by the interactions of the hydrogen bond type of the hydroxyl of the alcohol with the hydroxyl groups characteristic of the phenol, presenting, therefore, a higher concentration of phenols in the extract in ethanol Table 5.

The total polyphenol content of carnauba evaluated by Rufino et al. (2010), observed that the aqueous extract of the DM was 830±28.3 mg GAE/100 g. Coimbra and Jorge (2012) evaluated, among other parameters, the total phenolic compounds in three different Brazilian fruits, including the macaúba (*Acrocomia aculeata*), which belongs to the same family as *C. prunifera* (Mill.) H.E. Moore. In the analysis of macaúba, Coimbra and Jorge (2012) found 4.38±0.08 mg GAE/g in the kernel, and 2.21±0.02 mg GAE/g in the pulp. Tannins are polymers with valuable functions in plants, contributing to the taste, odor and color, and responsible for the astringency of

Table 5. Total phenols and tannins in the whole fruit, pulp and kernel extracts, and the anthocyanin content of the pulp and kernel extracts of carnaúba.

Evaluation	Whole fruit	Pulp	Kernel
Phenols (mg GAE/g extract)			
Ethanol extract	44.6000	0.0447	0.6930
Hexane extract	33.2000	Not detected	0.8260
Tannins			
Total	1590.9262 ppm	2977.7234 ppm	147.6531 ppm
Condensed	15.4950 mg eq leucocyanidin/100 g DM	-	-
Anthocyanins (mg/50 g sample)	-	9.84	5.63

Table 6. Antioxidant activity of whole fruit of carnaúba.

Extracts	Antioxidant activity
Ethanol extract	15.41 ppm
Hexane extract	1845.80 ppm

many products. For these reasons, tannins are economically important flavors and colors in foods and beverages (Simões et al., 2007). Plants rich in tannins are used in traditional medicine to treat various diseases because both the hydrolysable and condensed tannins have the ability to complex iron, vanadium, manganese, copper and aluminum, and they display antioxidant and free radical scavenging abilities (Simões et al., 2007). The total tannin content was 1590.93, 2977.72 and 147.65 ppm in the whole fruit, pulp and kernel, respectively. The tannin content in plants can vary according to the climatic and geographic conditions, and they may present a varied chemical composition that is often poorly understood (Battestin et al., 2004). The presence of tannins in the plant kingdom is well known, and this class of compounds has been related to several therapeutic properties, knowing that foods that have the same composition have functional properties in organisms (Sirdaarta et al., 2015).

The fruit of carnauba showed 9.84 mg anthocyanins/50 g of fresh pulp. Evaluating several tropical fruits, including carnauba, Rufino et al. (2010, 2011) found a relatively higher value in the fruit pulp of carnauba, reporting 4.10±0.10 mg anthocyanins/100 g DM. However, the immature green fruit may be one reason for the difference in values between this study and that by Rufino et al. (2010, 2011). The fruit of carnauba was also characterized for their antioxidant capacity. Antioxidants are substances which retard or inhibit oxidative degradation reactions by various mechanisms, such as inhibiting free radicals and complexing metal ions.

In this study, the antioxidant activities of the ethanol and hexane extracts of the whole fruits were 15.4 and 1845.8 ppm, respectively, as shown in Table 6. These data show that the extract in ethanol has antioxidant ability expressively higher than that of the extract in hexane. This is possibly related to the fact that phenols compounds known for their good antioxidant ability (Boulekbache-Makhlouf et al., 2013; Bicudo et al., 2014), are present in a higher proportion in the ethanol extract, justifying the result. The antioxidant capacity of various tropical fruits was studied by Rufino et al. (2010), who reported the pulp of the fruit of carnauba presented a high antioxidant activity of 3549±184 g/g DPPH, for the fresh matter and 4877±24.30 g/g DPPH, based on DM.

The antioxidant activity seems to be directly related to the phenolic content, as noted by Vasco et al. (2008), who evaluated some main fruits of Ecuador and observed that the fruits with relatively higher antioxidant activity were those with high or medium phenol contents.

Conclusion

The chemical and phytochemical analysis of the carnauba fruit showed a significant potential in terms of energy content and a protein content of more than 5% linked to the presence of secondary metabolites such as phenols that have recognized beneficial properties as antioxidants. The fruit of the carnauba is carrying an alternative with potential use in animal feeding, especially in the region where its production is consolidated (northeastern Brazil), which often suffers from prolonged droughts that put the animal feed at risk. Therefore, the strategic use of the fruit of the Carnauba as a food source paved way for expanding the possibilities of using this tree in a sustainable and optimized way.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Alasalvar C, Shahidi F (2013). Composition, phytochemicals, and beneficial health effects of dried fruits: An overview. Dried fruits: Phytochemicals and Health Effects. pp. 1-9.

Almeida JMD, Santos RJ, Genovese MI, Lajolo FM (2006). Evaluation of antioxidant activity using ß-carotene / linoleic acid system and DPPH radical drying method. Ciência e Tecnologia de Alimentos, 26(2):446-452.

Ayres MCC, Brandão MS, Vieira-Júnior GM, Menor JCAS, Silva HB, Soares MJS, Chaves MH (2008). Antibacterial activity of useful plants and chemical constituents of *Copernicia prunifera* root. Braz. J. Pharmacogn.18(1):90-97.

Barman K, Asrey R, Pal RK (2011). Putrescine and carnauba wax pretreatments alleviate chilling injury, enhance shelf life and preserve pomegranate fruit quality during cold storage. Sci. Hortic. 130: 795–800.

Battestin V, Matsuda LK, Macedo GA (2004). Sources and applications of tannins and tannases in foods. Alimentos e Nutrição, 15: 63-72. Acesso em: 07/2017. Disponível em: http://serv-bib.fcfar.unesp.br/seer/index.php/alimentos/article/viewFile/58/75

Bicudo MOP, Ribani RH, Beta T (2014). Anthocyanins, phenolic acids and antioxidant properties of Juçara fruits (*Euterpe edulis* M.) along the on-tree ripening process. Plant Foods Hum Nutr. 69(2):142-7.

Boulekbache-Makhlouf L, Slimani S, Madani K (2013). Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. Ind.Crops. Prod. 41:85-89.

Braga R (1960). Northeastern Plants: Especially from Ceará. 4 ed. Natal: Universitária UFRN, Brasil.

Braga R (1976). Plants of the Northeast: Especially from Ceará. Special edition for the Oswaldo Lamartine de Faria Virtual Collection. Fortaleza. Editor: Escola Superior de Agricultura de Mossoró, Brasil.

Brito ES, Narain N (2002). Physical and chemical characteristics of sapota fruit at different stages of maturation. Pesquisa agropecuária brasileira, 37(4):567-572.

Camacho MR, Phillipson JD, Croft SL, Yardley V, Solis PN (2004). *In-vitro* antiprotozoal and cytotoxic activities of some alkaloids, quinones, flavonoids, and coumarins. Plant Med. 70:70-72.

Cao W, Hu SS, Ye L.H, Cao J, Pang XQ, Xu JJ (2016). Trace matrix solid phase dispersion using a molecular sieve as the sorbent for the determination of flavonoids in fruit peels by ultra-performance liquid chromatography. Food Chem. 190: 474-480.

Coimbra MC, Jorge, N (2012). Fatty acids and bioactive compounds of the pulps and kernels of Brazilian palm species, guariroba (*Syagrus oleraces*), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*). J .Sci. Food Agric. 92:679-684.

Costa TSA, Lima A, Lima MV (2003). Determination of tannin in cashew peduncle: vanillin method versus butanol acid method. *Quim. Nova,* 26:763-765.

Cozzolino SMF (2009). Nutrient Bioavailability. 3. Ed. São Paulo: Manole, Brasil. www.manole.com.br. ISBN:978-85-204-2828-3

D'alva OA (2004). The extractivism of Carnaúba in Ceará. Dissertation (MSc.) - Universidade Federal do Ceará, Núcleo de Pós-Graduação, Programa Regional de Pós-Graduação em Desenvolvimento e Meio Ambiente, Fortaleza-CE, 2004. Acesso: 24/09/2017 em: http://www.repositorio.ufc.br/handle/riufc/16166

Egert S, Rimbach G (2011). Which sources of flavonoids: Complex diets or dietary supplements? Advances in Nutrition 2:8-14.

Ferreira CS (2009). Behavior of carnauba populations at different stages of development in the city of União-Piauí. [dissertation]. Universidade Federal do Piauí, Brasil. Acesso em: 07/2017.

Freire LCC, Araujo EIM, Valdevino FISP, Silva FFM, Alves LA, bertini, LM (2015). Contribution to the phytochemical study and chemical tests of the extracts of *Citrus limonium* (lemon) and *Capsicum frutescens* l. (chilli pepper). Holos 01:21-29.

Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Samani MA (2016). The role of medicinal plants in the treatment of diabetes: A systematic review. Electronic Physician 8 (1):1832-1842.

Lorenzi H, Souza HM, Costa JTM, Siqueira LSC, Von Brehr N (1996). Palm tree from Brazil – Native and Exotic. Ed. Plantarum, Nova Odessa, Brasil.

Matos FJA (2009). Introduction to experimental phytochemistry. 3rd ed. Fortaleza: Edições UFC. http://www.editora.ufc.br/catalogo/21-ciencias-puras/346-introducao-a-fitoquimica-experimental-3-edicao (ISBN): 85-7282-026-4.

McCullough ML, Peterson JJ, Patel R, Jacques PF, Shah R, Dwyer JT (2012). Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. Am. J. Clin. Nutr. 95:454-464.

Menezes ELA (2005). Botanic insecticides: their active principles, mode of action and agricultural use. Seropédica: Embrapa Agrobiologia: Documentos 205, Rio de Janeiro, Brasil. Acesso: 07/2017. Disponível em: https://www.agencia.cnptia.embrapa.br/recursos/doc205ID-E5DFp9Pf68.pdf

Middleton EJ (1998). Effect of plant flavonoids on immune and inflammatory cell function. Advanc. Exp. Med. Biol. 439:175-182.

Moo-Huchin VM, Moo-Huchin MI, Estrada-León RJ, Cuevas-Glory L, Estrada-Mota IA, Ortiz-Vázquez E, Betancur-Ancona D, Sauri-Duch E (2015). Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. Food Chem. 166:17-22.

Nogueira DH (2009). Quality and potential use of fruits of genotypes of carnaubeira (*Copernicia prunifera*) from the state of Ceará. [PhD. Thesis]. Universidade Federal da Paraíba, Brasil. Acesso em: 07/2017. Disponível em: https://www.alice.cnptia.embrapa.br/alice/handle/doc/697462

Nogueira DH, Lima JR, Alves RE (2009). Production of Fruit Jelly Carnaubeira. Embrapa, Fortaleza. Comunicado Técnico, 143.4p. Acesso em: 07/2017. Disponível em: https://www.embrapa.br/agroindustria-tropical/busca-de-publicacoes/-/publicacao/580684/producao-de-geleia-a-base-de-frutos-da-carnaubeira

Nogueira RJMC, Moraes JAPV, Burity HA, Silva Junior JF (2002). Effect of maturation stage of fruits on the physical-chemical characteristics of acerola. Pesquisa Agropecuária Brasileira, 37(4):463-470. Acesso em 07/2017. Disponível em: http://www.scielo.br/pdf/pab/v37n4/9078

Obanda M, Owuor PO, Taylor SJ (1997). Flavonol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. Journal of the Science of Food and Agriculture, 74(2):209-215. DOI: 10.1002/(SICI)1097-0010(199706)74:2<209::AID-JSFA789>3.0.CO;2-4

Oliveira AMS, Gomes JMA (2006). Requirements and market advantages of carnauba wax. In: Gomes. J. M. A.; Santos, K. B; Silva, M. S. (Org.). Cadeia produtiva da cera de carnauba: diagnóstico e cenários. Teresina: EDUFPI, Brasil.

Pereira DR, Carneiro JGM (2006). Use of green fruits of carnauba in the elaboration of cookies. Teresina, Universidade Federal do Piauí, Comunicado Técnico n. 9. 2p.

Pio Correa MP (1931). Dictionary of Useful Plants of Brazil. Editora: Ministerio da Agricultura. 1ª Edição, Rio de Janeiro, Brasil. http://www.bchicomendes.com/bcm/bcm.htm[ISBN:][BCM: 094.033.02]

Porter LJ, Hrstich LN, Chan BG (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, 25: 223-230.

Raso GM, Meli R, Di Carlo G, Pacilio M, Di Carlo R (2001). Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. Life Sci. 68:921-931.

Rufino MSM, Alves RE, Brito ES, Pérez-Jiménez J, Saura-Calixto F, Mancini-Filho J (2010). Bioactive compounds and antioxidant capacities of 18 nontraditional tropical fruits from Brazil. Food Chem. 121(4):996-1002.

Rufino MSM, Alves RE, Fernandes FAN, Brito ES (2011). Free radical scavenging behavior of ten exotic tropical fruits extracts. Food Res. Int. 44(7):2072-2075.

Petrovick PR, Pharmacognosy: From Plant to Medication. Edição, Porto Alegre: Editora da UFRGS, 1104p.

Schenkel EP, Gosmann G, Athayde ML (2007). Saponins. In: Simões, CMO, Schenkel EP, Gosman G, Mello JCP, Mentz LA, Petrovick PR, Pharmacognosy: From Plant to Medication. Edição, Porto Alegre: Editora da UFRGS, 1104p.

Silva AM, Oliveira CHA, Fernandes CCL, Silva LM, Nunes-Pinheiro DC, Fernandes AAO, Lima IMT, Silva CMG, Rondina D (2015a). Post-partum reproductive activity and estrus synchronization responsiveness in anglonubian x sprd fed with dried carnauba wax palm fruit (*Copernicia prunifera*) long term. Semina: Ciências Agrárias, 36(4):2619-2632.

Silva CG, Herdeiro RS, Mathias CJ, Panek AD, Silveira CS, Rodrigues VP, Renno MN, Falcao DQ, Cerqueira DM, Minto ABM, Nogueira FLP, Quaresma CH, Silva JFM, Menezes FS, Eleutherio ECA (2005). Evaluation of antioxidant activity of Brazilian plants. Pharmacol. Res. 52:229-233.

Silva DJ, Queiroz AC (2002). Food analysis: Chemical and biological methods (3rd ed.) Viçosa: UFV, Brasil.

Silva RB, Silva-Júnior EV, Rodrigues LC, Andrade LHC, Silva SI, Harand W, Oliveira AFM (2015b). A comparative study of nutritional composition and potential use of some underutilized tropical fruits of *Arecaceae*. Annals of the Brazilian Academy of Sciences, 87(3):1701-1709.

Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR (2007). Pharmacognosy: from plant to medicine. Porto Alegre: UFRGS, Brasil.

Sirdaarta J, Matthews B, Cock IE (2015). Kakadu plum fruit extracts inhibit growth of the bacterial triggers of rheumatoid arthritis: Identification of stilbene and tannin component. J. Functional Foods 17:610-620.

Sucupira NR, Silva AB, Pereira G, Costa JN (2012). Methods for determining the antioxidant activity of fruits. Científica Ciências Biológicas e da Saúde, 14(4):263-269.

Teixeira LN, Stringheta PC, Oliveira FA (2008). Comparison of methods for quantification of anthocyanins. Revista Ceres 55(4):297-304. Acesso em 07/2017. Disponível em: http://www.ceres.ufv.br/ojs/index.php/ceres/article/view/3320

Tlili N, Tir M, Benlajnef H, Khemiri S, Mejri H, Rejeb S, Khaldi A (2014). Variation in protein and oil content and fatty acid composition of *Rhus tripartitum* fruits collected at different maturity stages in different locations. Ind. Crops Prod. 59:197-201.

Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci.74:3583-3597.

Vasco C, Ruales J, Kamal-Eldin A (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chem. 111:816-823.

Vasta V, Luciano G (2011). The effects of dietary consumption of plants secondary compounds on small ruminants' products quality. Small Ruminant Research 101:150-159.

Vizzoto M, Krolow AC, Weber GEB (2010). Secondary metabolites found in plants and their importance. Pelotas: Embrapa Clima Temperado: Documentos, 316, Brasil. Acesso: 08/2017. Disponível em: https://www.infoteca.cnptia.embrapa.br/handle/doc/886074.

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Full Length Research Paper

Effects of post-harvest handling on hydrogen cyanide content of cassava products obtained from Ilorin-West urban markets, Nigeria

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Cyanide contents of locally processed cassava based products (CBP) in Ilorin-West urban markets, Nigeria was estimated and compared to established safety levels. Hence, 29 locally processed cassava products samples were randomly collected from retail outlets and tested for hydrogen cyanide content, pH, titratable acidity (TTA), water absorption capacity (WAC) and least gelation capacity (LGC) following standard procedures. Results from the survey showed that hydrogen cyanide contents of the CBP ranged from 3.36 to 37.73 mg/kg. Titratable acidity ranged between 0.22 and 1.79 × 10^{-3} (% w/w); the pH of the samples was between 4.55 and 6.75; WAC ranged from 1.46 to 5.82 g/ml, while the LGC was from 8.07 to 38.08%. Twenty five out of the 29 CBP samples collected had hydrogen cyanide content above the maximum safe level (10 mg/kg), the TTA was low, while the pH was high. These problems might be due to poor processing methods adopted by the processors. Continuous exposure of consumers to sub-lethal dose of hydrogen cyanide may lead to serious health hazards.

Key words: Cassava, cyanide, Ilorin, market, survey.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae and it is a perennial woody shrub, producing enlarged tuberous roots (Oghenechavwuko et al., 2013). Cassava being the second important Africa's staple food after maize in terms of calories consumed, is a reliable and inexpensive source of food for more than 700 million people in the world, with Nigeria being the largest producing country (FAO, 2003; Eleazu et al., 2011).

However, the presence of cyanogenic glucosides constitute a major limitation to the utilization of cassava as human food (Asegbeloyin and Onyimonyi, 2007; CAC/RCP, 2013). The leaves and roots of cassava plant contain the cyanogenic glucosides called linamarin and

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small amount of lotaustralin (Orjiekwe et al., 2013). Linamarin is readily hydrolysed to glucose and acetone cyanohydrin in the presence of enzyme linamarase, which is released when the cells of cassava roots are ruptured (CAC/RCP, 2013; Omotioma and Mbah, 2013; Orjiekwe et al., 2013). The acetone cyanohydrin decomposes rapidly in neutral or alkaline conditions to liberate hydrogen cyanide and acetone (Orjiekwe et al., 2013). The levels of the cyanogens vary considerably between tissues depending on many factors such as cultivar, climatic conditions, age of cassava at harvesting and postharvest practices (Ojo et al., 2013).

Hydrogen cyanide is a volatile compound which evaporates rapidly in the air at temperature over 28°C, dissolves rapidly in water, may be toxic to humans and animals, and the severity of the toxicity depend on the quantity consumed (CAC/RCP, 2013). According to Orjiekwe et al. (2013), there are several health disorders which have been associated with regular intake of sub-lethal quantities of cyanogens, some of which have resulted into outright poisoning and death due to cyanide intake from consumption of poorly processed cassava products. The authors further stated that health risks caused by ingestion of these chemicals (cyanogens) include the exacerbation of goiter, cretinism and cardiovascular diseases such as encephalopathy and neuropathy, while severe cyanide poisoning can lead to heart, brain and optic nerve degeneration. The Nigerian Industrial Standards (NIS) and the Codex Alimentarius Commission (CAC) standards for maximum safe level of hydrogen cyanide in most cassava products meant for human consumption is 10 mg/kg (Sanni et al., 2005; Ubwa et al., 2015).

Monitoring of the cyanogenic potentials of cassava is therefore of utmost importance due to the following reasons: determination of safety of different cassava products, evaluation of the efficiency of different existing methods of cassava processing on the removal of cyanogenic glucosides and determination of level of cyanogenic potentials of newly released cassava varieties in breeding programmes (Tivana et al., 2014). Literature evidences have shown that hydrogen cyanide contents of cassava products from eastern and western parts of Nigeria were above the acceptable limit and there were reported cases of occasional deaths resulting from consumption of cassava meal (Adindu et al., 2003; Odoemelam, 2005; Adindu and Aprioku, 2006; Komolafe and Arawande, 2011). Hence, the need to survey and compare the other parts of the country.

Available data have also shown that under appropriate postharvest handlings especially fermentation and/or retting, more than 60% of cyanogen compounds were being detoxified (Onwuka and Ogbogu, 2007; Oghenechavwuko et al., 2013). Cassava products are being processed largely in the rural areas where some of the processors are known to employ short practices that are less effective in removing cyanogen compounds (Adindu et al., 2003). Therefore, the need to monitor these compounds in cassava products cannot be over emphasized. This study is an attempt to evaluate some selected quality parameters of cassava based food products obtained from urban markets within Ilorin-West Local Government Area of Kwara State, Nigeria, with a view to determine whether they are within the acceptable safe level of hydrogen cyanide concentrations.

MATERIALS AND METHODS

Reagents and test samples

All used reagents were of analytical grade and most were products of SIGMA-ALDRICH, Germany and BDH, England. All cassava products displayed in each markets were sampled, these include white gari, yellow gari, ‘lebu’ (fine gari), ‘gari Ijebu’, ‘lafun’ I (edible cassava flour, whole root tubers), ‘lafun’ II (edible cassava flour, pellatized root tubers), tapioca, ‘fufu’ and ‘abacha’.

Sampling area

Five urban markets (designated as A, B, C, D and E) within Ilorin-West Local Government Area of Kwara State, Nigeria were used for sample collection because apart from the facts that most consumers in the area collect their food stuffs from these urban markets, they also serve as major sources of goods for other smaller markets in the city. At least 5 to 10 samples of each product were randomly selected from each market. After collection, each product was thoroughly mixed together to give a representative sample from each market. Finally, a total of 29 samples were obtained for further analyses.

METHODOLOGY

Analysis of cyanide contents

Cyanide content determination was done using the alkaline picrate method (Onwuka, 2005). The method was partially modified and used as follows; 5 g finely ground sample was dissolved in 50 ml distilled water in a corked conical flasks and left to stay overnight for proper extraction of cyanide. The extract was filtered through Whatman number 1 filter paper. To 1 ml of the extract was added 4 ml alkaline picrate solution and incubated in the water bath at 37°C for 15 min for colour development. The absorbance was read with UV-Vis Spectrophotometer (SEARCHTECH: UV1902PC) at 490 nm against the reagent blank containing 1 ml of distilled water and 4 ml of alkaline picrate solution prepared at the same time. Cyanide concentrations of the food samples were extrapolated from the standard curve.

Preparation of the standard cyanide curve

Pure potassium cyanide (KCN) was used as the standard in this determination. From 5 to 50 ppm of the standard was prepared and to each concentration was added 25 ml of 1 N HCl in 500 ml conical flasks. Exactly 1 ml of each solution and 1 ml of distilled water as blank were taken into separate corked boiling tubes followed by addition of 4 ml alkaline picrate solution. The mixture was incubated in water bath at 37°C for 15 min to develop the colour and the absorbance of the standards with the blank was read at 490 nm. The results obtained were plotted into a graph as standard curve (Figure 1).

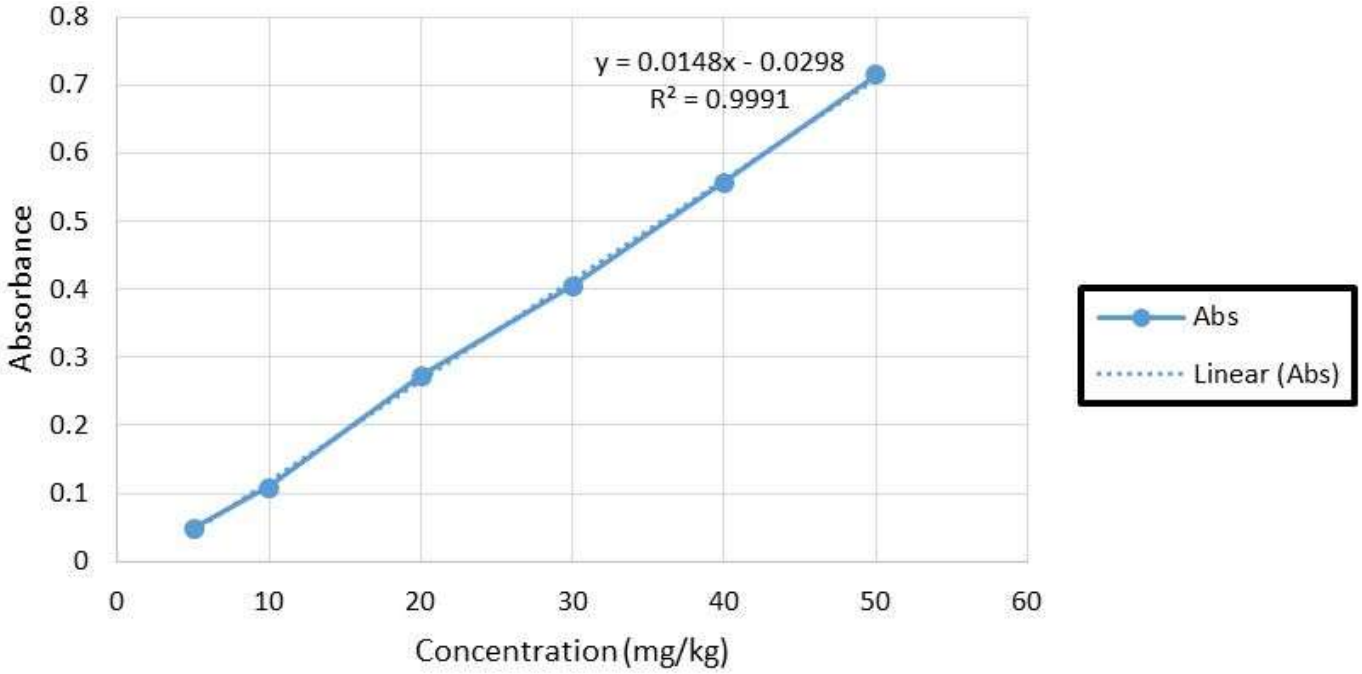

Figure 1. Standard calibration curve of potassium cyanide (KCN). The blue thick line represents absorbance (Abs) while the dotted blue line represents linear plot. The equation of the graph is given by y=0.0148x – 0.0298, where R^2 represents line of regression.

Physicochemical analysis

Total titratable acidity (TTA) and pH of cassava based food samples were determined following the method of Onwuka and Ogbogu (2007). Three grams each of the cassava based food samples was weighed into a conical flask and 30 ml of distilled water was added. The suspension was allowed to stand for 30 min after which it was titrated with a standard base (0.1 N NaOH) using 3 drops of phenolphthalein as indicator. Total titratable acidity was estimated according to the formula:

$$\%TTA\ (w/w) = [N \times V \times Eqwt/W \times 1000] \times 100$$

where N is the normality of NaOH ($MEqmL^{-1}$), V is the volume of 0.1 N NaOH used, Eqwt is the equivalent weight of predominant acid (mg mEq^{-1}) which is lactic acid, W is the weight of sample, and 1000 is the factor relating mg to gram (mg g^{-1}).

The pH was determined by dispersing 1 g of the cassava based food samples in small quantities of distilled water and then making it up to 10 ml. The dispersion was allowed to stand for 30 min after which the electrode of the pH meter (SEARCHTECH: PHS-3C) was inserted and shaken vigorously and allowed to stand till a stable reading was obtained, the value was recorded as the pH.

Determination of functional properties

Water absorption capacity (WAC) and least gelation capacity (LGC) of cassava based samples were determined by following the methods described by Onwuka (2005). One gram of each sample was weighed into a conical graduated centrifuge tube and mixed thoroughly with 10 ml of distilled water for 30 s. The setup was allowed to stand for 30 min at room temperature and then centrifuged at 4800 × g for 30 min using a centrifuge (CENTURION SCIENTIFIC: K2202). The volume of the free water was read directly from the graduated centrifuge tube and expressed as grams of water absorbed per gram of sample. Varying amounts from 2 to 40% (w/v) of sample were prepared in 5 ml of distilled water in glass test tubes. The sample test tubes were then heated in a boiling water bath for 1 h followed by rapid cooling under running tap water. The test tubes were further cooled at 4°C and the gelation capacity was taken as the least gelation concentration determined as the concentration when the sample from the inverted test tube did not fall or slip down.

Data analysis

Results were expressed as mean ± standard deviation (SD) of replicate determinations. All data generated were subjected to statistical analysis by one-way analysis of variance (ANOVA) using the SPSS statistical software package version 20.0.0 (IBM SPSS Statistics, IBM Corporation 2011). The means were separated using New Duncan Multiple Range Tests (DMRT) as described by Duncan (1955). Significance was accepted at 5% probability level (p=0.05).

RESULTS AND DISCUSSION

Cyanide contents of cassava based food samples sold in urban markets in Ilorin-West, Nigeria

The standard calibration curve of absorbance against the concentration of KCN is as shown in Figure 1. Hydrogen cyanide contents of cassava based food samples sold in Ilorin-West urban markets (Figure 2) varied from as low as 3.36 mg/kg (tapioca, market C) to as high as 37.73

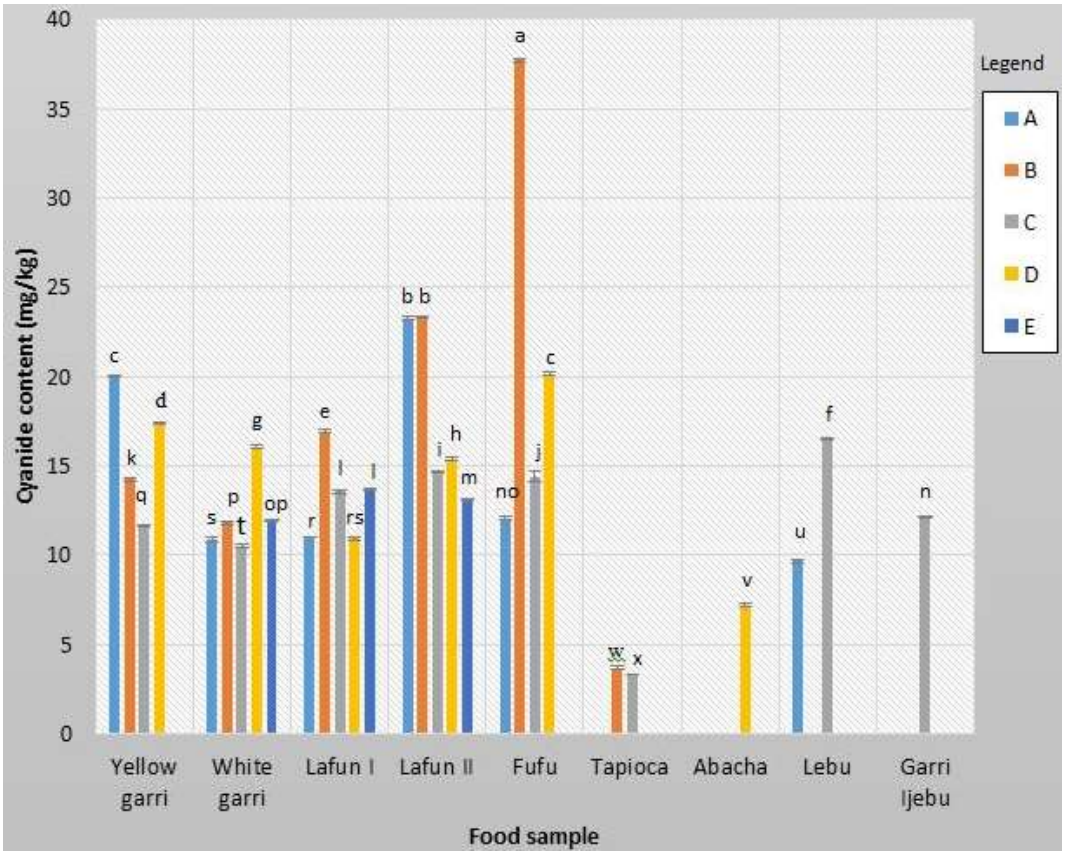

Figure 2. Cyanide contents (mg/kg) of cassava based products sold in Ilorin-West markets. Each bar represents the mean of triplicate determinations while the error bars represent the standard deviations; bars with different alphabets differ significantly (p=0.05). Light blue bars represents market A, Orange bars represents market B, grey bars represents market C, yellow bars represents market D while deep blue bars represents market E.

mg/kg ('fufu', market B). Data collected from each market differ significantly (p=0.05) in their cyanide contents except in few cases; especially in white 'gari' where there was no significant (p=0.05) difference between market B and market E, lafun I where there was no significant (p=0.05) difference between market C and market E; also in 'lafun' II where there was no significant difference (p=0.05) between market A and market B. Only one market each displayed 'abacha' sample (market D) and 'gari Ijebu' (market C) during the survey exercise.

The data obtained in this present study showed that fufu had the highest significant (p=0.05) hydrogen cyanide contents. This might be attributed to poor processing of the product. Similar results were also obtained in previous studies. For instance, Orjiekwe et al. (2013) observed that out of three commodities (gari, fufu and tapioca) sampled in Okada area of Edo State Nigeria, fufu had the highest significant (p=0.05) value of cyanogenic glycosides.

The NIS and CAC standards for maximum safe level of hydrogen cyanide in most cassava products meant for human consumption is 10 mg/kg (Sanni et al., 2005; Ubwa et al., 2015). Only four out of the 29 samples collected had hydrogen cyanide contents below the maximum safe level of hydrogen cyanide concentration in foods meant for human consumption. This again might be due to poor processing and/or handling by the processors. Similar results have been published in respect of other locations in Nigeria. For instance, field evaluation conducted in Nigeria on three cassava products (garri, fufu and tapioca) revealed that total cyanogen content exceeded the safe level (10 mg/kg) recommended for cassava products (Adindu et al., 2003). The cyanide levels of cassava products reported for eastern and western parts of Nigeria were also above the acceptable limit and there have been reported cases of occasional deaths resulting from consumption of cassava meal (Odoemelam, 2005; Adindu and Aprioku, 2006; Komolafe and Arawande, 2011). Epidemiological studies have shown that exposure to small doses given over a long period of time can result in increased blood cyanide levels with the following symptoms; dizziness, headache, nausea and vomiting, rapid breathing, restlessness, weakness and even severe cases of paralysis, nerve

Table 1. Physicochemical and functional properties of cassava based food products sold in Ilorin-West Urban markets.

Sample	Market	Titratable acidity ($\times 10^{-3}$ % w/w)	pH	Water absorption capacity (g/ml)	Least gelation capacity (%)
Yellow 'gari'	A	$1.67^{ab} \pm 0.10$	$5.35^{i} \pm 0.01$	$4.15^{cde} \pm 0.15$	$10.12^{kl} \pm 0.02$
	B	$1.68^{ab} \pm 0.14$	$5.14^{m} \pm 0.01$	$4.81^{cd} \pm 0.19$	$8.07^{m} \pm 0.12$
	C	$0.82^{im} \pm 0.07$	$6.64^{b} \pm 0.01$	$5.29^{b} \pm 0.10$	$12.14^{i} \pm 0.02$
	D	$1.46^{cde} \pm 0.07$	$5.47^{f} \pm 0.01$	$4.66^{def} \pm 0.05$	$10.08^{l} \pm 0.02$
White 'garri'	A	$1.31^{defg} \pm 0.05$	$5.07^{o} \pm 0.01$	$3.88^{h} \pm 0.06$	$12.12^{ij} \pm 0.02$
	B	$1.12^{ghij} \pm 0.06$	$5.44^{g} \pm 0.01$	$4.54^{cf} \pm 0.06$	$14.06^{h} \pm 0.03$
	C	$1.07^{hijk} \pm 0.07$	$5.39^{h} \pm 0.01$	$5.35^{b} \pm 0.05$	$12.04^{j} \pm 0.02$
	D	$0.99^{ijkl} \pm 0.07$	$5.07^{o} \pm 0.01$	$4.08^{h} \pm 0.10$	$12.15^{i} \pm 0.01$
	E	$1.46^{cde} \pm 0.18$	$5.06^{o} \pm 0.01$	$4.31^{g} \pm 0.23$	$14.04^{h} \pm 0.05$
'Lebu'	A	$1.79^{a} \pm 0.16$	$5.73^{d} \pm 0.01$	$4.73^{cde} \pm 0.09$	$14.07^{h} \pm 0.03$
	C	$1.51^{bcd} \pm 0.11$	$5.42^{g} \pm 0.02$	$4.73^{cde} \pm 0.06$	$14.04^{h} \pm 0.05$
'Garri Ijebu'	C	$1.38^{cdef} \pm 0.06$	$5.32^{j} \pm 0.01$	$3.94^{h} \pm 0.25$	$12.12^{ij} \pm 0.02$
'Lafun' I	A	$1.14^{ghi} \pm 0.03$	$5.14^{m} \pm 0.01$	$1.85^{jkl} \pm 0.07$	$34.07^{c} \pm 0.03$
	B	$1.30^{efg} \pm 0.06$	$4.97^{r} \pm 0.01$	$1.77^{klm} \pm 0.07$	$34.14^{c} \pm 0.05$
	C	$1.23^{fgh} \pm 0.02$	$5.22^{e} \pm 0.01$	$2.06^{j} \pm 0.06$	$38.08^{a} \pm 0.02$
	D	$0.94^{jkl} \pm 0.07$	$5.55^{e} \pm 0.01$	$1.62^{mn} \pm 0.12$	$36.08^{b} \pm 0.02$
	E	$1.37^{cdef} \pm 0.09$	$5.27^{k} \pm 0.01$	$1.78^{klm} \pm 0.10$	$36.14^{b} \pm 0.13$
'Lafun' II	A	$0.88^{kl} \pm 0.02$	$5.10^{n} \pm 0.01$	$1.92^{jkl} \pm 0.21$	$10.04^{l} \pm 0.02$
	B	$1.30^{efg} \pm 0.06$	$5.22^{l} \pm 0.01$	$1.72^{lm} \pm 0.09$	$16.07^{f} \pm 0.03$
	C	$0.81^{lmn} \pm 0.06$	$5.02^{p} \pm 0.02$	$1.96^{jk} \pm 0.05$	$16.02^{f} \pm 0.02$
	D	$0.81^{lmn} \pm 0.03$	$4.98^{r} \pm 0.01$	$1.46^{n} \pm 0.05$	$32.08^{d} \pm 0.10$
	E	$1.25^{fgh} \pm 0.17$	$5.00^{q} \pm 0.01$	$1.90^{jkl} \pm 0.11$	$32.03^{d} \pm 0.03$
'Fufu'	A	$0.43^{o} \pm 0.09$	$4.83^{s} \pm 0.01$	$3.39^{i} \pm 0.09$	$16.04^{f} \pm 0.02$
	B	$0.62^{n} \pm 0.15$	$4.97^{r} \pm 0.01$	$3.39^{i} \pm 0.11$	$16.04^{f} \pm 0.05$
	C	$0.67^{mn} \pm 0.07$	$5.23^{l} \pm 0.01$	$3.28^{i} \pm 0.11$	$18.07^{e} \pm 0.03$
	D	$0.39^{op} \pm 0.04$	$6.75^{a} \pm 0.01$	$3.35^{i} \pm 0.12$	$16.06^{f} \pm 0.03$
Tapioca	B	$0.68^{mn} \pm 0.04$	$4.55^{u} \pm 0.01$	$5.82^{a} \pm 0.15$	$10.09^{kl} \pm 0.01$
	C	$0.22^{p} \pm 0.05$	$4.75^{t} \pm 0.01$	$4.93^{c} \pm 0.16$	$10.17^{k} \pm 0.03$
'Abacha'	D	$1.51^{bc} \pm 0.35$	$5.95^{c} \pm 0.02$	$4.47^{fg} \pm 0.09$	$14.15^{g} \pm 0.03$

Results show mean ± SD of triplicate readings. Values with the same superscripts along the same column show no significant difference (p=0.05).

lesions, hyperthyroidism and miscarriage (Orjiekwe et al., 2013).

Physicochemical properties of cassava based food samples sold in urban markets in Ilorin-West, Nigeria

Physicochemical properties of cassava based food products sold in Ilorin-West urban markets were presented in Table 1. Total titratable acidity (TTA) of cassava based food samples from five different urban markets within Ilorin-West Local Government was between 0.22 and 1.79 × 10^{-3} (% w/w). There was no significant (p=0.05) difference between the TTA of yellow gari from markets A and B. For white gari, no significant (p=0.05) difference in the TTA of samples from market A, B and E, as well as those samples from markets B, C and D. Similarly, no significant (p=0.05) difference in the TTA of lafun I from markets A, B and C, as well as between those from markets B and E. For lafun II samples, no

significant (p=0.05) difference in the TTA of samples from markets A, C and D, while those from markets B and E also showed no significant (p=0.05) differences in their TTA values. Fufu samples from markets A and D with those from markets B and C had no significant (p=0.05) difference in their TTA values.

The pH values (Table 1) of cassava based food samples from urban markets in Ilorin-West Local Government ranged from 4.55 to 6.75. Fufu sample from market D was significantly (p=0.05) higher in the pH value than all other food samples, while Tapioca from the same market D had the least pH reading and the value was significantly (p=0.05) lower.

The NIS limit for total acidity of gari and edible cassava flour was set at 1.0% w/w maximum, while that of pH was set at 5 to 7 units (Sanni et al., 2005). Research works have shown that TTA increases as fermentation time increases (Onwuka and Ogbogu, 2007; Oghenechavwuko et al., 2013). In addition, 48 h fermentation caused increased acidity of processed fufu samples (Onwuka and Ogbogu, 2007). Low TTA values in the present study might be an indication of poor processing which means that they were partially or inadequately fermented.

Functional properties of cassava based food samples sold in urban markets in Ilorin-West Local Government Area

The results of WAC of cassava based food samples sold in Ilorin-West Local Government urban markets was as presented in Table 1. The WAC of cassava based food samples ranged from 1.46 g/ml (Lafun II, market D) to 5.82 g/ml (Tapioca, market B). The WAC of tapioca (market B) was significantly (p=0.05) higher than that of all the other food samples collected. There was no significant (p=0.05) difference found in the WAC of yellow 'gari' from markets A, B, and D. For white gari samples, no significant (p=0.05) difference was found in the WAC of samples from markets A and D. The lafun I samples from markets A, B, and E had no significant (p=0.05) difference in their WAC values. Similarly, lafun II from markets A, B, and D had no significant (p=0.05) difference in their WAC readings. Also, there was no significant (p=0.05) difference in WAC of lebu samples from markets A and C and fufu samples from markets A, B, C, and D.

The results of least gelation capacity (LGC) of cassava based food samples sold in Ilorin-West Local Government urban markets was as displayed in Table 1. The LGC of these food samples ranged from 8.07% (yellow gari, market B) to 38.08% (lafun I, market C). There was no significant (p=0.05) difference in the LGC of yellow gari samples from markets A and D. For white gari, no significant (p=0.05) difference was found in the LGC of samples from markets A, C and D, then B and E. For lafun I, there was no significant (p=0.05) difference between the LGC of samples from markets A and B, also between markets D and E. Similarly, no significant (p=0.05) difference was found in the LGC of lafun II samples from markets B and C, also between markets D and E. The two lebu gari samples (markets A and C), two tapioca samples (markets B and C) and three fufu samples (markets A, B and D) had significantly (p=0.05) the same LGC values. It was generally observed in the present study that values from different markets in many of the parameters analyzed were significantly (p=0.05) the same, this might simply suggests that some sellers from different markets under the survey probably got their goods from a common source.

Conclusion

It has been evidently shown that two days' (48 h) fermentation is enough to detoxify up to 60% of hydrogen cyanide in most species of cassava. The present study revealed that hydrogen cyanide concentrations of cassava food products from these urban markets in Ilorin-West Local Government, Nigeria were as high as 4 times (in some cases) the maximum safe level in food meant for human consumption. In addition, low TTA observed in the majority of the samples under this study is an indication of poor processing. Since cassava based products are major staple in most Nigeria societies and many of them are ready-to-eat (RTE) with minimal or no further processes, continuous exposure of unsuspecting consumers to sub-lethal dose of hydrogen cyanide may pose serious health hazards. Therefore, introduction of new breeds of cassava with minimal cyanogenic glycosides and guidelines for processors in sourcing and handling their raw materials will be of tremendous help to reduce the level of cyanide in cassava products. Also, as part of CAC/RCP 73 recommendations, food safety authorities and public health monitoring bodies may consider the introduction of scientific kits such as picrate kits to monitor cyanide concentrations in cassava products at the point of sale or use and the urinary thiocyanate concentration in the population.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Abbreviations: CBP, Cassava based products; **TTA,** titratable acidity; **WAC,** water absorption capacity; **LGC,** least gelation capacity.

REFERENCES

Adindu MN, Aprioku ABI (2006). Cyanogenic content of gari from some processing centres in Rivers State, Nigeria. Nig. Food J. 24(1):135-138.

Adindu MN, Olayemi FF, Nze-Dike OU (2003). Cyanogenic potential of some cassava products in Port-Harcourt markets in Nigeria. J. Food Compost. Anal. 16:21-24.

Asegbeloyin JN, Onyimonyi AE (2007). Effect of different processing methods on the residual cyanide of 'Gari'. Pak. J. Nut. 6:163-166.

CAC/RCP 73 (2013). Codex Alimentarius Commission: Code of Practice for the reduction of Hydrocyanic Acid (HCN) in cassava and cassava products. [Internet Document]: Available at: http:// www.fao.org/input/download/standards/13605/CXP_073e.pdf.

Duncan PB (1955). New Multiple Range and Multiple F-tests. Biometrics 11:1-42.

Eleazu CO, Oti E, Amajor JU, Ikpeama AI, Awa E, Eleazu KC (2011a). Nutritional evaluation and Microbial load of cassava (*Manihot esculenta*) Sieviates and peels fermented in brine. Int. J. Adv. Sci. Tech. Res. 1:1-23.

Food and Agricultural Organization (FAO) (2003). Cassava Market Assessment. [Internet Document]. Available at: http://www.fao.org/docrep/019/i3473e/i3473e.pdf

Komolafe EA, Arawande AO (2011). Quality characteristics of gari produced in some selected cassava processing centres in Owo, Ondo State, Nigeria. J. Res. Nat. Dev. 8:1.

Odoemelam SA (2005). Studies on residual hydrocyanic acid (HCN) in gari flour made from cassava (*Manihot* spp.). Pak. J. Nutr. 4(6):376-378.

Oghenechavwuko UE, Saka GO, Adekunbi TK, Akanbi CT (2013). Effect of Processing on the Physico-Chemical Properties and Yield of *Gari* from Dried Chips. J. Food Proc. Technol. 4:255.

Ojo RT, Obi NP, Akintayo CO, Adebayo-Gege GI (2013). Evaluation of Cyanogen contents of cassava and cassava based food products in Karu, Nasarawa State, North-Central Nigeria. IOSR J. Environ Sci. Toxicol. Food Technol. 6(1):47-50.

Omotioma M, Mbah GO (2013). Kinetics of natural detoxification of hydrogen cyanide contained in retted cassava roots. Int. J. Exp. Res. 1(2).

Onwuka GI, Ogbogu NJ (2007). Effect of fermentation on the quality and physicochemical properties of cassava based fufu products made from two cassava varieties NR8212 and Nwangbisi. J. Food Technol. 5(3):261-264.

Onwuka GI (2005). Food Analysis and Instrumentation. Theory and Practice. Naphtali Prints, Lagos. Nigeria. pp. 122-128.

Orjiekwe CL, Solola A, Iyen E, Imade S (2013). Determination of Cyanogenic glycosides in Cassava products sold in Okada, Edo State, Nigeria. Afr. J. Food Sci. 7(12):468-472.

Sanni L, Maziya-Dixon B, Akanya J, Okoro CI, Alaya Y, Egwuonwu CV, Okechukwu RU, Ezedinma C, Akoroda M, Lemchi L, Ogbe F, Okoro E, Tarawali G, Mkumbira J, Patino M, Ssemakula G, Dixon A (2005). Standards for cassava products and guidelines for export, IITA Ibadan, Nigeria. pp. 34-41.

Tivana LD, Francisco JDC, Zelder F, Bergenstahl B, Dejmek P (2014). Straightforward rapid spectrophotometric quantification of total cyanogenic glycosides in fresh and processed cassava products. Food Chem. 158:20-27.

Ubwa ST, Otache MA, Igbum GO, Shambe T (2015). Determination of cyanide content in three sweet cassava cultivars in three Local Government Areas of Benue State, Nigeria. Food Nutr. Sci. 6:1078-1085.

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Full Length Research Paper

Acute toxicity evaluation of mixture of neem *(Azadirachta indica)* and moringa (*Moringa oleifera)* seed oils in rats

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The toxicity of the mixture of neem and moringa seed oils in the ratio of 1:3 was evaluated based on some biomarkers of liver and kidney functions of Wistar Albino rats. Thirty male Wistar albino rats were randomly divided into six groups of five rats each. Group A served as control. Groups B, C, D, E and F received doses of 100, 1000, 1600, 2900 and 5000 mg/kg body weight of ratio 1:3 neem-moringa seed oil, respectively. The albino rats were observed for any changes for seven days; during this period, they were allowed free access to food and water *ad-libitum.* The rats were weighed and made to fast overnight. The serum obtained was used to determine the serum level of alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST). Similarly, liver and kidney tissues were removed and homogenized separately in a normal saline in ratio of 1:10 w/v. The homogenate of liver was centrifuged and the supernatant was used to determine total protein and billirubine while that of kidney was used for determining creatinine and urea. The results of all the biochemical parameters tested did not show any significant difference (P>0.05) from the control up to the dose of 5000 mg/kg body weight and did not produce any visible toxic effect. The dosage of 1:3 mixtures of neem-moringa seed oils appeared to be safe for humans.

Key words: Wistar albino rats, acute toxicity, neem-moringa seed oil, safety, biomarker.

INTRODUCTION

Insecticides are classified according to their mammalian toxicity, chemical origin or composition, mode of entry, formulation and their usage in stored grains (Van-Valkenburg, 2000; Ware, 1995). Based on mammalian toxicity, Van-Valkenburg (2000) reported that toxicological studies are conducted to determine the threshold limit

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of a chemical which an animal or a human being is capable of building without significant biological effects. The usual beginning in any toxicological evaluation is the assessment of the acute toxicity that is the effects of a single dosage of the chemical. Botanical insecticides have eco-toxicological advantages compared to traditional synthetic insecticides because they can have favourable eco-toxicological properties (low human toxicity), rapid degradation and reduced environmental impact which make them suitable insecticides for organic agriculture (Zanuncio et al., 2016).

According to a report by the United Nations Environment Program (UNEP) and the World Health Organization (WHO), pesticides are responsible for poisoning around 3 million people and causing about 200 thousand deaths each year world-wide. Such cases of poisoning are reported more in developing countries (95%) than in developed countries (World Health Organization, 1990; Yadav et al., 2015).

Neem oil is a natural occurring pesticide found in the seed of neem tree. It is yellow to brown in colour, has a bitter taste, and a garlic/sulphur smell. Azadirachtin is the most active component and is used for repelling and killing pests. It works as an effective non-toxic insect control agent in agriculture. Neem is considered harmless to humans, animals, birds, beneficial insects and earthworms and has been approved by the United States Environmental Protection Agency (USEPA) for use on food crops and bed bugs. USEPA also reported that based on the data available, it has been determined that there were no unreasonable adverse effects to the American population and the environment when labelled instructions are followed and good agricultural practices are employed. Laboratory studies indicate that the active ingredient is not toxic, following the oral inhalation or dermal exposure (http://draxe.com>neemoil).

Neem oil contains more than a dozen azadirachtin analogs but the major contributor to the insecticidal activity is azadirachtin. The remaining triterpenoids including nimbin, salannin, and their derivatives contribute little to the efficacy (Isman, 2006). Interestingly, neem oil is non-toxic to mammals, birds and fishes and exhibit fewer chances of resistance due to its multiple mode of actions on insect (Chaudhary et al., 2017).

Moringa oil is a nice cooking oil. It is used for deep frying sautéing. Its smoke point is about 200°C which is good for deep frying purpose. Moringa seed oil is preferred as salad oil in many places. Moringa oil is generally safe to use and it can also be consumed internally. Moringa oil is made up of monounsaturated fatty acid (MUFA) and saturated fat. It is exceptionally high in oleic acid (Omega-A). About 70% of the oil is oleic acid. This may lead to blood pressure lowering effect which is similar to what is seen while using olive oil (http://oilhealthbenefits.com/moringa-oil-ben-oil/).

According to O'Brien (2005), the general technique for acute toxicity evaluation is the determination of the LD_{50} (the dosage necessary to produce death or reproducible effect in 50% of the animal population tested). The compound is administered on a weight/weight basis (milligram or gram of compound per kg of body weight of test animals) in a suitable solvent and suspension system. This is evaluated by acute tests, orally (AO) or dermally (AD), chronic oral tests (CO), vapour toxicity tests (VA), chronic vapour tests (VC) and inhalation test (IT) (O'Brien, 2005).

According to Davis and Freed (2000), the insecticides used in stored product treatment is supposed to be of low mammalian toxicity in a formulation that is likely to be effective against the species involved, persistent for the required period of time under given storage conditions and will not alter the flavour, colour and odour of the stored commodity. It was reported on the Nigerian Television Authority (NTA) Channel-7 (2006) that a particular insecticide used in the preservation of cowpea led to the death of some individuals in Lagos, Nigeria which scared people away from buying and consuming cowpea at that period. Also in August 2011 in Gombi Local Government Area of Adamawa state Nigeria, it was reported on the NTA and Adamawa Television (ATV) Yola (local television stations) that a whole family was wiped out after consuming cowpea due to the chemical used in storing the produce. Some people blamed these unfortunate incidences on the fact that the chemical was not allowed to expire before the cowpea was cooked and consumed but the crux of the matter is that the people died of some certain insecticides.

The research conducted by Ilesanmi and Gungula (2010) established that mixture of neem and moringa seed oils in ratio 1:3 at a concentration of 0.5 ml/200 g cowpea recorded over 90% success when used for cowpea storage. Also, the result of cookability and palatability test conducted by Ilesanmi and Gungula (2011) at the end of 180 days of storage of cowpea using 0.5 ml/200 g cowpea revealed that there is no significant difference between the control (untreated) cowpea and the mixture of neem-moringa seed oils 1:2 and 1:3 treated cowpea, suggesting that it was well accepted. But it is not clear yet whether the oil has any toxic effect on mammals, this is therefore the focus of this research. Thus, the objective is to assess the acute toxicity of mixture of neem and moringa seed oils in 1:3 using rat models.

MATERIALS AND METHODS

Plant materials

The neem seeds used for the oil extraction were handpicked in Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria while the *Moringa oleifera* seeds were partly purchased

Table 1. Effect of mixture of neem and moringa seed oils (1:3) administration on the body weights of Wistar strain albino rats.

Treatment (mg/kgbwt)	Body weight (in g)		
	Initial weight	Final weight	% Difference in body weight
Control	64.60±2.17^{d}	80.90±2.07^{c}	25.17±2.04^{a}
100	67.00±1.90^{d}	81.00±2.40^{c}	20.82±3.72^{ab}
1000	72.40±2.30^{c}	83.10±3.00^{c}	14.83±2.27^{c}
1600	85.40±3.07^{b}	99.40±2.20^{b}	16.35±2.75^{bc}
2900	88.60±1.55^{b}	103.40±4.20^{b}	16.75±0.46^{bc}
5000	111.80±4.50^{a}	131.90±4.00^{a}	14.73±2.30^{c}

Results are presented as means ± SEM of five replicates.

from Girei market in Girei Local Government Area of Adamawa State and partly from Kaltungo in Kaltungo Local Government of Gombe State, Nigeria.

Experimental animal

The Wistar strain albino rats for the toxicity experiment were purchased from the Nigerian Institute for Trypanosomiasis Research, Vom, Jos, Nigeria and the cages to house these Wistar strain albino rats were obtained from the Department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria. All the rats were allowed free access to commercially formulated rat feed and water *ad-libitum*. The rats were then allowed to acclimatize for one week.

Extraction of neem seed oil

The processing of neem seed for oil extraction involved cleaning, de-hulling and oil extraction. The cleaning process was dry-cleaning. The seeds were de-hulled with a mortar and pestle and then winnowed. The de-hulled seeds were milled using hammer mill and 1000 g of the resultant powder was used for extraction. The oil was extracted manually using hands to knead the paste with occasional addition of cold water until the oil exuded out. The extracted oil was sieved to remove impurities.

Extraction of moringa seed oil

The dried *M. oleifera* seeds were de-hulled using mortar and pestle, the kernels were then milled using hammer mill and 1000 g of the powder obtained was used for the extraction. The extraction was done manually by kneading the moringa paste with hand with occasional mixing of the paste with water. The extracted oil was then sieved to remove impurities.

Experimental design

The experimental design here was completely randomized design (CRD). Thirty male Wistar strain albino rats aged 6 to 8 weeks weighing 80 ± 20 g were randomly divided into six groups of five rats each. Group A served as the normal control. Groups B, C, D, E, and F received doses of 100, 1000, 1600, 2900, and 5000 mg/kg body weight of ratio 1:3 neem-moringa seed oil, respectively as described by Dietrich (1983). Group A, which is the control were administered water. All dosages were administered orally (intragastically). All the experimental rats were allowed free access to food (commercially formulated rat feed) and water *ad-libitum* after the oil was administered. Their cages were cleaned daily and food and water changed daily. The experimental Wistar strain albino rats were allowed to stay for a period of seven days and were observed frequently from the day of treatment. The nature and time of adverse effect was noted. Observations were carried out for seven days and the experiment terminated. All rats were then weighed.

The Wistar strain albino rats were allowed to fast overnight then blood samples were collected by heart puncture under diethyl ether anesthesia. The blood was left for 15 min and was centrifuged at 3000 rpm for 15 min. The serum obtained was used to determine the serum levels of alanine aminotransferase (ALT), aspartate amino transferase (AST) as described by Reitman and Frankel (1957) and alkaline phosphatase (ALP) as described by Rec. Gscc (DGKC) (1972) using commercial kits (Ramdox Co. Atrium UK). Creatinine was determined according to the method described by Henry (1974), bilirubin was determined according to the method described by Malloy and Evelyn (1937), the serum was also used to determine the serum level of urea and total protein as described by Weatherburn (1967) and Reinhold (1953), respectively using commercial kits (Ramdox Co. Atrium UK). Similarly, liver and kidney tissues were removed and homogenized separately in normal saline in the ratio of 1:10 w/v. The homogenate of liver was centrifuged and the supernatant was used to determine total protein (Reinhold, 1953) and billirubin (Malloy and Everlyn, 1937), while that of the kidney was used for determining creatinine (Henry, 1974) and urea (Weatherburn, 1967). All these were carried out using commercial kits (Ramdox Co. Atrium UK).

Statistical analysis

Analysis of variance was used to ascertain the significant differences between means. Least significant differences (LSD) test was used to compare means that were significantly different at $p<0.05$ using generalized linear model procedure of the SAS/STAT® software Release 9.2.

RESULTS AND DISCUSSION

After the mixture of neem-moringa seed oils (1:3) was administered to the Wistar strain albino rats, it was observed that in the first 2 h of administration, the experimental rats were weak and sleepy. Thereafter, they became very active throughout the experimental period. The effect of mixture of neem and moringa (1:3) seed oils on the body weight of Wistar strain albino rats is presented on Table 1. The percentage average final body

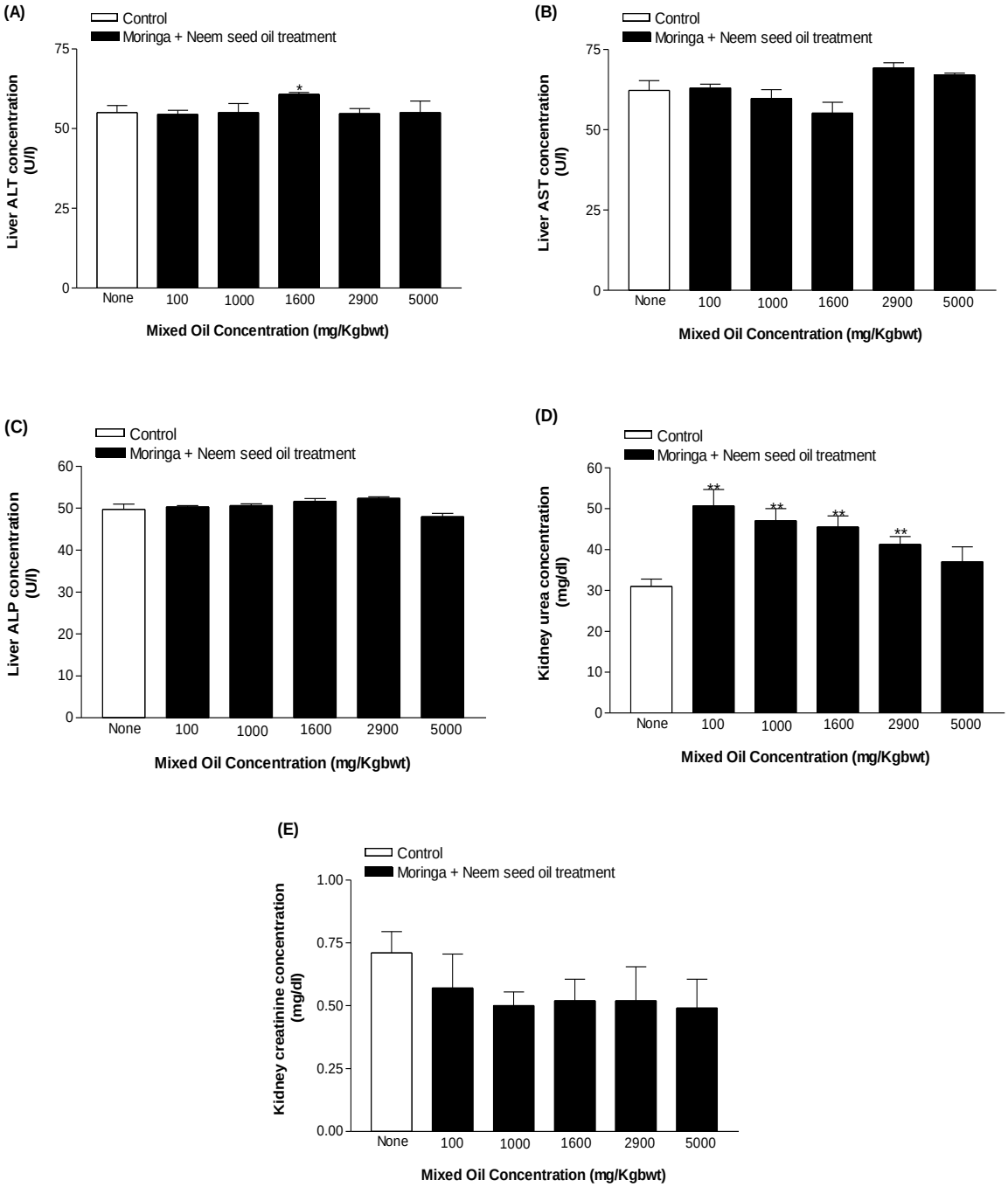

Figure 1. Level of some biochemical parameters in liver and kidney of Wistar albino rats administered with ratio 1:3 neem - moringa seed oils. Values are mean ± SEM with n = 5. *p<0.05 compared to control.

weight gain of the treated albino rats ranged between 14.73 to 25.17%. The highest percentage average body weight gain (25.17%) was recorded for the saline-treated group (control) while the group treated with 5000 mg/kg body weight recorded the lowest percentage of average body weight gain (14.83%). There was a drop in percentage body weight gain in all the groups treated with the mixture of the oils when compared with the control group.

The biochemical parameters in liver and kidney of Wistar strain albino rats treated with the mixture of neem and moringa (1:3) seed oil is presented in Figure 1A to E.

There were no significant differences ($p > 0.05$) in organ ALT, AST and ALP activities of the treated Wistar strain albino rats compared to saline-treated (control) rat at different treatment doses. In the same vein, the creatinine level of the Wistar strain albino rats treated with different doses of ratio 1:3 neem and moringa seed oils are significantly ($p<0.05$) the same. The urea concentration of the group treated with 100, 1000, 1600 and 2900 mg/kg body weight of the oil mixture had no significant difference ($p > 0.05$) from the control. The group treated with 5000 mg/kg body weight of the oil mixture was not significantly different ($p > 0.05$) from the saline-treated group (control). There was also no death observed even at 5000 mg/kg body weight of the experimental rats.

Figure 2A to G presents the results of acute toxicity of mixture of neem and moringa seed oils on the serum biochemical parameters of Wistar strain albino rats. There were no significant differences ($p > 0.05$) in the serum ALT, AST and ALP activities of treated rats and control rats, but there was an insignificant reduction in the serum level of ALT, AST, and ALP, no significant ($p > 0.05$) difference was observed in the level of total protein, bilirubin, urea and creatinine of ratio 1:3 neem-moringa oil-treated albino rats compared to saline-treated Wistar strain albino rats. There was a slight increase in the enzyme activities in serum ALT of groups administered with 100, 2900 and 5000 mg/kg body weight of ratio 1:3 neem-moringa oil but did not differ significantly from that of the control rats.

DISCUSSION

The observation made in the first 2 h of administration of the ratio 1:3 mixture of neem and moringa seed oil to the experimental albino rats is in agreement with the findings in organic facts (http://www.organicfacts.net>oil) that moringa oil aids sleep when taken internally because of its soothing properties. It is known to relax the body and promote a sense of calmness. The finding opined that moringa oil can be used for aroma therapy treatment or topically applied to the chest or temples for sedative effect. It appeared that the percentage weight gain is age dependent, as the rats that are six weeks old (64 to 67 g) appeared to have higher percentage weight gain (20 to 25%) while rats that are 7 to 8 weeks old (72 to 111 g) has lower percentage weight gain (14 to 16%). This low percentage weight gain may be due to their age, as younger mammals tend to grow more rapidly than the older ones. The observation is in accordance with those of Whiteny and Rolfes (1993) who observed that infant rapid growth and metabolism demands an ample supply of all needed nutrients. They also observed that because infants are small, they need smaller total amount of the nutrients than adults do, but when comparison are based on body weights, infants need over twice as much nutrients. With this, they have greater percentage weight gain. It is therefore not clear if the drop in percentage body weight gain is due to the oil administered or the age and weight.

The changes in weight of the Wistar strain albino rats during the period of observation also suggest that the mixture of the oils might have caused an interference in the absorption of nutrients making them unavailable or the intake of the oil might have made them to feel satisfied for some moment thereby reducing their feed intake as it is generally noted that oil or oily foods intake makes one to be satisfied even when less quantity of meal is consumed. This may be due to the fact that 1 g of fat gives 9 kcalories of energy while a gram of protein or carbohydrate gives 4 kcalories of energy (Whitney and Rolfes, 1993). Besides, the percentage weight gain as observed from the study was acceptable over that period of one week. According to Graziela et al. (2011), moringa oil is a monounsaturated oil which is liquid at room temperature. Monounsaturated oil may help lower cholesterol level when used in place of saturated fat and nutritionally its consumption is associated with healthier serum lipid and low cholesterol content of the blood (A Calorie Counter, 2016, www.acaloriencounter.com>diet>mono).

Acute toxicity test gives clues on the range of doses that could be toxic to the animals; it could also be used to estimate the therapeutic index (LD_{50}/ED_{50}) of drugs and xenobiotics (Maikai et al., 2008). The range of ALT (54.5 to 60.8 U/l), AST (55.3 to 69.2 U/l), ALP (48.01 to 52.39 U/l), urea (31.0 to 50.6 m/dl) and creatinine (0.485 to 0.708 mg/dl) activities in the Wistar strain albino rats' organs were observed in the study. The result was all within the range of normal laboratory values reported by Johnson-Delaney (1996): AST (45.7 to 80 Ul), ALP (56.8 to 128 u/l), creatinine (0.2 to 0.8 mg/dl) but for ALT and urea that are not within this range they still showed no significant variation from the control. Davies and Freed (2000) classified insecticides according to their toxicity based on the LD_{50} and said that an insecticide is relatively not toxic if there is absence of lethal death at 5000 mg/kg body weight. $AOLD_{50}$ = 5000 mg/kg body weight, this ratio 1:3 mixture of neem and moringa seed oil can be classified as relatively non-toxic to mammals.

The concentration ranges for ALT (14.60 to 22.60 u/l), AST (20.20 to 41.0 U/l), ALP (48.18 to 51.26 u/l), total protein (3.72 to 5.03 u/l), creatinine (0.30 to 1.09 mg/dl) from this study are all in the range reported by Johnson-Delaney (1996) as normal laboratory values for Wistar strain albino rats biochemical reference range. The results showed no significant difference ($p > 0.05$) between the serum level of ALT, AST and ALP of treated Wistar strain albino rats and the normal. The serum urea level range of (19.20 to 35.60 mg/dl) and that of Billirubin (0.94 to 1.58 mg/dl) are slightly above those reported by Johnson Delaney (1996) but however not significantly different from group treated with normal saline (control).

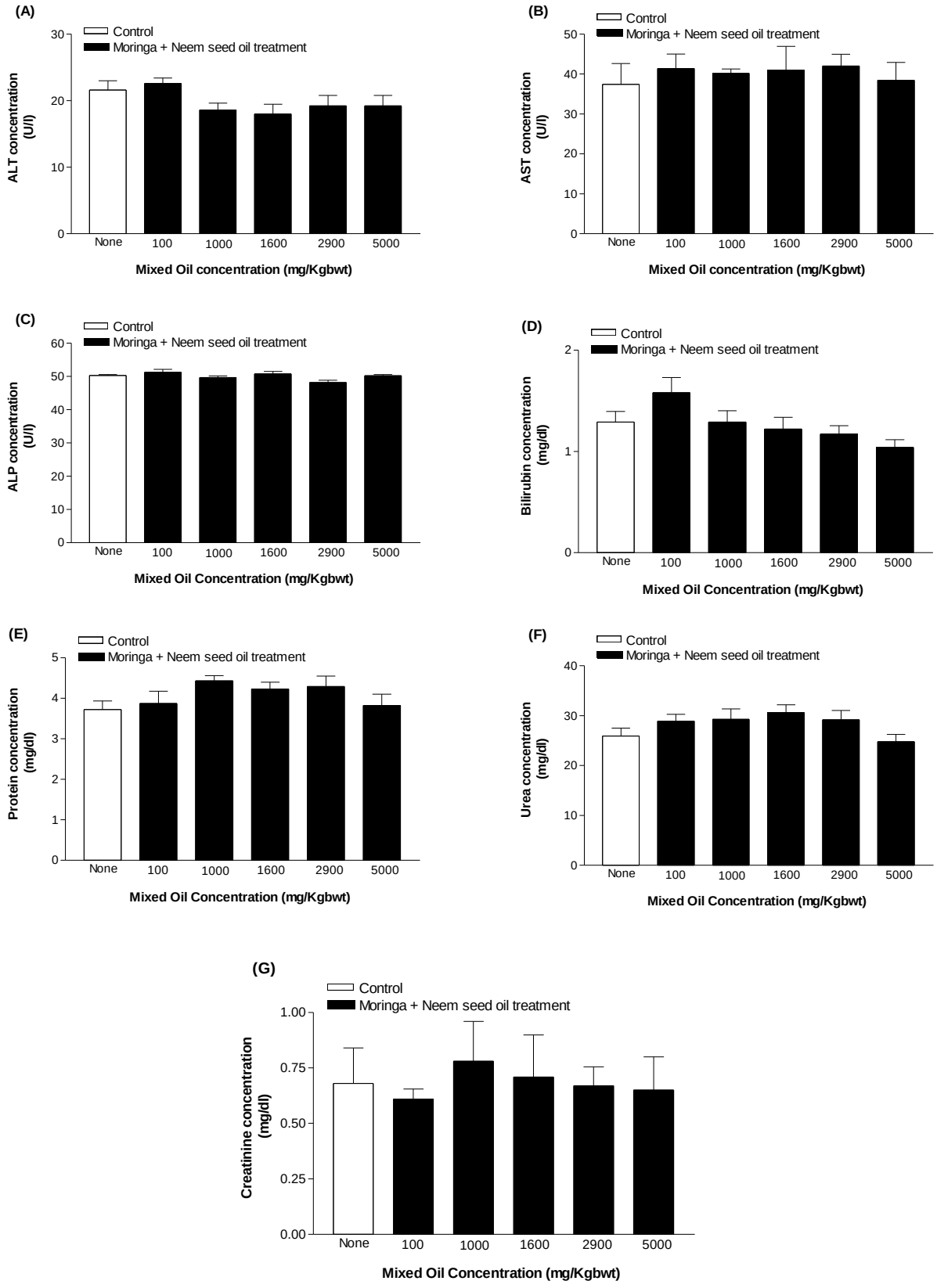

Figure 2. Level of some biochemical parameters in serum of Wistar albino rats administered with ratio 1:3 neem - moringa seed oils. Values are mean ± SEM with n = 5.

The slight reduction in the level of serum AST, ALT and ALP as the dosage increased as shown in the study is in agreement with the finding of Olatosin et al. (2013); that moringa oil helps to lower enzymes markers of liver damage (ALT, AST and ALP) in the serum even at a single dose.

Renal or hepatic injuries as well as major alteration in serum proteins were not induced by the tested oils. This is in line with the result of Graziela et al. (2011) who reported on biological evaluation of crude and degummed oil from *M. oleifera* seeds.

Conclusion

From the observation taken after the administration of the mixture of neem and moringa seed oils to the Wistar albino rats, it was concluded that the mixture of the oils has sedative effects. It is suggested that further investigation be carried out to ascertain if the nature of these oils in ratio 1:3 can be used to treat insomnia. The percentage gain in body weight of experimental rats may not be due to the single dose of the oil administered but the age range of the experimental rats. Since the serum and organ biochemical analysis showed no significant differences in any of the parameter tested even at 5000 mg/kg body weight dosage, then the mixture of these oils may not be toxic. Finally, there was no death, no renal or hepatic injury recorded even at 5000 mg/kg body weight dosage, it is therefore concluded that the mixture of neem and moringa seed oils in ratio 1:3 may not have any adverse effect on humans, and it is suggested that further investigation be carried out to ascertain if a dosage above 5000 mg/kg body weight will be disastrous to albino rats. It is also suggested that the mixture of these oils be used to treat cowpea grains meant for storage against infestation because of its non-toxic characteristics and its insecticidal activities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

A Calorie Counter (2016). What is monounsaturated fat and polyunsaturated fat? The Healthy Good Fats. Retrieved on 19/5/2017 from www.acaloriencounter.com>diet>mono.

Davies FB, Freed VH (2000). An Agro medical approach to pesticide management: Some health and environmental considerations. Consortium for International Crop Protection. 327p.

Dietrich L (1983). A new approach to practical acute toxicity testing. Arch Toxicol. 54:275-287.

Graziela de FAI, Tânia MSM II, Cláudia DG II, Kátia MN II; Rinaldo C dos S III; Marcelo ES II (2011). Biological evaluation of crude and degummed oil from *Moringa oleifera* Seeds. Brazilian Archives of Biology and Technology. Int. J. 54(5):1003-1006.

Henry RJ (1974). Clinical chemistry principles and techniques**.** Second Edition. Harper & Row. 525p.

Ilesanmi JO, Gungula DT (2010). Preservation of cowpea *[Vigna unguiculata* (L.)] walp grains against cowpea Bruchids *(Callosobruchus maculatus)* using Neem and Moringa Seed Oils. Int. J. Agron. 2010:1687-8159.

Ilesanmi JO, Gungula DT (2011). Effects of neem and moringa seed oil on cookability and palatability of cowpea grains stored for six months. Afr. J. Food Sci. 5(9):522-528

Isman MB (2006). Botanical insecticides deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51:45-66. 10.1146/annurev.ent.51.110104.151146

Johnson-Delaney C (1996). Exotic Animal Companion Medicine Handbook for Veterinarians, (2 Vol. Set). Lake Worth, FL: Zoological Education Network.

Maikai VA, Kobo PI and Adaudi AO (2008). Acute toxicity studies of aqueous stem back extract of *Ximenia americana.* Afr. J. Biotechnol*.* 7(10):1600-1603.

Malloy H, Everlyn KA (1937). The determination of bilirubin with the photoelectric colorimeter. J. Bio. Chem. 119:481-490.

Nigerian Television Authority (NTA) Channel 7, Lagos, Nigeria (2006).

Nigerian Television Authority (NTA) Yola, Nigeria (2011).

O'Brien RD (2005). Insecticides – action and metabolism**.** Academic Press. P 333.

Olatosin TM, Akinduko DS, Uche CZ (2013). Evaluation of the hepatoprotective efficacy of *Moringa oleifera* seed oil on CCl_4 – induced liver damage in Wistar Albino rats. Int. J. Eng. Sci. 11(2):13-18.

Organic facts: moringa oil benefit, uses and side effect. http://www.organicfacts.net>oil retrieved 2016/2017

Rec. Gscc (DGKC) (1972). Optimized standard colorimetric methods. J. Clin. Chem. Clin. Biochem.10:182.

Reinhold JG (1953). Determination of total protein and albumin in: Standard methods of clinical chemistry. Reiner M (edited). Academic Press, New York and London. P 88.

Reitman S, Frankel S (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamate pyruvate transaminases. Am. J. Clin. Path. 28:56-63.

Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ, Sehgal R, Kanwar JR (2017). Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. Frontiers in plant science published online 2017 May 8.doi:10.3389/fpls 2017.00610

Van-Valkenburg W (2000). Pesticides formulations. Marcel Dekker, New York.

Ware GW (1995). Pesticides: An auto tutorial approach. W.H. Freeman and company, San Francisco.

Weatherburn MW (1967). Phenol hypochlorite reaction for determination of ammonia. Anal. Chem. 39:971- 974.

Whitney EN, Rolfes SR (1993). Understanding nutrition. West Publishing Company Minneapolis New York. pp. 28-37.

World Health Organization (1990). Public health impact of pesticides used in agriculture Geneva: World Health Organization.

Yadav IC, Devi NL, Syed JH, Cheng Z, Li J, Zhang G (2015). Current stature of persistent organic pesticides residues in air, water and soil and their possible effect on neighbouring countries: a comprehensive review of India. Sci. Total Environ. 511:123-137. 10.1016/j.scitoteni.2014

Zanuncio JC, Serrao JE, Mourao SA (2016). Toxic effects of the neem oil (*Azadirachta indica*) formulation on the stink bug predators, *Podisus nigrispinus* (Heteroptera: pentatomidae). Sci Rep. 6(6):30261. doi: 10.1038/srep30261.

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