International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 3 Issue 6 ǁ June 2014 ǁ PP.41-49

Comparative Evaluation of Phytoconstituents, Antibacterial Activities and Proximate Contents of Fresh, Oven Dried Uncooked and Cooked Samples of *Buchholzia coriacea* Seed and Their Effects on Hepatocellular Integrity

M. I. Nwachukwu[1], M. K. C. Duru[2], B. A. Amadi[3] and I. O.Nwachukwu[1]
[1](Department of Microbiology, Faculty of Biological Science/ Imo State University, Nigeria) [2](Department of Chemical Sciences (Biochemistry unit), College of Basic and Applied Sciences/ Rhema, University, Nigeria) [3](Department of Biochemistry, Faculty of Science/ University of Port Harcourt, Nigeria)

ABSTRACT: *The phytoconstituents, antibacterial activities and proximate contents of fresh, oven dried uncooked and cooked seed samples of Buchholzia coriacea and their effects on hepatocellular integrity following consumption was comparatively evaluated. One hundred and four male Wistar albino rats out of which eight rats were used as control and the rest divided equally among three major groups with four subgroups housing eight rats each in each subgroup, were used for hepatocellular integrity study. The phytoconstituents, antibacterial activities, and proximate contents were evaluated using standard methods. Phytoconstituents found in the investigated seed samples were flavonoids, saponins, oxalates, tannins, phytates, cyanogenic glycosides and alkaloids. Results obtained for antibacterial studies revealed that fresh Buchholzia coriacea seed sample inhibited the activities of the test organisms best than oven dried uncooked and cooked samples. The proximate contents showed that the shelf-life of the studied seed samples followed the order oven dried uncooked seed sample>fresh seed sample > cooked sample. Hepatocellular integrity results showed that the ASL, ALT and ALP of rats placed on compounded feed of fresh B. coriacea seed and oven dried uncooked seed were significantly affected ($p<0.05$) against those of the control. From the observations of the present study, the antibacterial power of the studies seed especially when fresh or in an uncooked form cannot be doubted but the rate at which any of these forms compromise hepatocellular integrity is also acknowledged. Hence those that consume the seed in these forms should take note. This study has shown the phytoconstituents, antibacterial activities, and proximate contents of fresh, oven dried uncooked and cooked seed samples of Buchholzia coriacea and their effects on hepatocellular integrity following consumption.*

KEYWORDS: *Antibacterial activities, Buchholzia coriacea, hepatocellular integrity, phytoconstituents, proximate content.*

I. INTRODUCTION

Traditional medicine which involves the use of plant and plant materials to cure diseases is as old as mankind on earth [1-3]. The practice has been given different names such as alternative medicine, complimentary medicine, botanical medicine or herbalism [1-4]. Research studies have shown that medicinal plants possess bioactive compounds that exhibit physiological activity against bacteria and other microorganisms [5-7]. Osuagwu *et al.* [8] noted that there is a general assumption that the active dietary constituents contributing to the protective effects of medicinal plants are phytochemicals, vitamins and minerals. Different authors have noted that these constituents confer the power to remedy disease conditions such as diarrhoea, dysentery, diabetes, asthma, malaria, rheumatism, measles, ulcer, etc on medicinal plants [1, 9-12]. Burkill [11] and Akubugwo *et al.* [13] noted that trees and shrubs with medicinal and nutritional potentials exist in Nigeria. Studies have also revealed that many of the trees and shrubs possess this power to remedy disease conditions as conferred on them by the active dietary constituents known as phytonutrients and other plant chemicals known as phytochemicals[14-15]. Hence most of the indigenous plants are therefore used as medicinal plants [15-17]. *Buchholzia coriace* known as musk tree is among such indigenous plants that possess the power to remedy disease conditions and are used as medicinal plants. *Buchholzia coriacea* is a perennial plant which grows as a tree. It belongs to the family *Capparaceae* [17]. The plant was named after R. W. Buchholz who collected the plant in Cameroon in the late 19th century [18]. The potency of the seed product of *Buchholzia coriacea* against diseases earned it the popular name "wonderful kola". The seed is also called memory nut because of its ability to enhance the memory. Record has it that different diseases are remedied with the seed of *Buchholzia coriacea* [17]. Among such diseases are cough, chest pain, waist pain, irregular

menstruation, internal piles, malaria, quick ejaculation, headache, hypertension, dysentery, premature ageing, etc. [17, 19-20].

The seed also acts as blood cleanser, facilitates learning ability and strengthens the nervous system [19]. In Africa, the seed of *Buchholzia coriacea* is specially used against migraine headache [19]. In Nigeria, *Buchholzia coriacea* is known as "uworo", "owi", and "uke' among Yoruba, Edo and Igbo tribes respectively [11].Among the people of Central African, the fruit is known as "esson bossi" [21]. The seed of *Buchholzia coriacea* is normally covered in a purple aril. It is eaten raw or cooked. The extract of the seed is also taken when it is allowed to ferment in water, dry gin or any other drinkable alcohol aside beer. Aside the medicinal efficacy of *Buchholzia coriacea* seed, it is a known fact that the seed when fresh has a sharp pungent taste with hot spicy flavour [22-24]. The hot spicy flavour could be the cause of its painful sensation when placed on the skin (especially on parts of the skin with soft tissues such as the eyelids, etc).Due to this effect, and coupled with the rate at which the fresh seed is being consumed especially in Nigeria and other West African countries, there is need to comparatively evaluate the antibacterial activities, phytoconstituents, and proximate contents of fresh, oven dried uncooked and cooked seeds of *Buchholzia coriacea* and their effects on organs. This will help inform the people on the best form of *Buchholzia coriacea* seed to use or eat. Since existing studies [22, 23-30] on *Buchholzia coriacea* plant did not look into these areas of study on the seed, the present study comparatively evaluated the antibacterial activities, phytoconstituents, and proximate contents of fresh, oven dried uncooked and cooked samples of *Buchholzia coriacea* seed and their effects on hepatocellular integrity following consumption.

II. MATERIALS AND METHODS

2.1 Sample collection and preparation

Buchholzia coriacea seeds used in the present study were obtained from Orie Amaraku market in Isiala Mbano L.G.A of Imo State, Nigeria. The seeds were identified by Dr. Mbagwu, F. N in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The identified seeds were washed with distilled water to free them from dirts. The dirts free seeds were also cleaned by double disinfection methods. The disinfected and washed seeds were immersed in 80% ethanol for half an hour before they were removed and washed with sodium hypochlorite in aqueous form to reduced surface contamination. The seeds were finally wished with distilled water to free them from sodium hypochlorite. After the final washing of the seeds, a portion was taken and boiled using pressure cooker until confirmed done, another portion was kept under sterile condition to preserve the freshness. A portion was also oven dried at 50^{o}C for 72 hours. The ground powder of the prepared samples was obtained with the help of Thomas-Wiley milling machine and use for preparation of extracts for microbial study, and compounding feed for rat study.

2.2 Extract preparation

The method described by Ezeifeka *et al* [31] and Azuzu [32] was used for preparation of the extract used in the present study.

2.3 Test organisms

The bacterial species used in this study were obtained from Microbiology unit of Imo State University, Owerri, Nigeria. The bacteria were cultured and maintained using the method of Cruickshank *et al.* [33]. The isolates were identified using biochemical methods as described by Holt *et al.*[34]. Bacterial species used were *B. subtilis, S. epidermidis, S. aureus, E. coli, E. faecalis* and *K. pneunoniae,*

2.4 Antimicrobial test

The methods described by Ezeifeke *et al.*[31] were used for antimicrobial study.

2.5 Phytoconstituent analysis

Phytoconstituent screening was carried out using the methods of Harbone [35]; and Odebiyi and Sofowora [36] on the studied *Buchholzia coriacea* seed samples in the present study. The samples were screened for flavonoids, saponins, oxalates, tannins, phytates, cyanogenic glycosides and alkaloids.

2.6 Proximate content analysis

The ground samples were used for proximate content analysis in the present study. Moisture, ash, fat, and protein contents of the studied samples were determined following the standard methods of AOAC [37]. CHO and energy contents of the samples were obtained by difference method and Buradbury`s equation respectively.

2.7 Experimental animals

One hundred and four male albino rats of Wistar strain weighing between 60-70 grams were purchased from the animal colony of University of Port Harcourt Rivers State, Nigeria and housed in the animal house of Imo State University, Owerri, Nigeria under required condition. The animals were allowed free access to pelletized commercial rat feed (Pfizer Livestock Co., Ltd, Aba, Nigeria) and water *ad libitum*. After acclimatization of four weeks, the animals were allocated to three major groups A, B, and C. Each of the major groups had subgroups. Each of the subgroups housed eight rats. Group A rats were placed on compounded feed of fresh *B.coriacea* seed, group B rats were placed on compounded feed of oven dried uncooked *B. coriacea* seed, and group C rats were placed on compounded feed of cooked *B. coriacea* seed. Eight rats were used as control (control group). The rat weights were equalized as nearly as possible. The feed and water administration lasted for twenty-eight days. Treatments of the rats were as follows; Control group= Normal feed+ portable water. Group A_5= 5% of fresh *B. coriacea* seed + 95% of normal feed + portable water; Group A_{10} = 10% of fresh *B. coriacea* seed + 90 of normal feed + portable water; Group A_{15}= 15% of fresh *B. coriacea* seed + 85% of normal feed + portable water; Group A_{20}= 20% of fresh *B. coriacea* seed + 80% of normal feed + portable water. Group B_5= 5% of oven dried uncooked *B. coriacea* seed + 95% of normal feed + portable water; Group B_{10} = 10% of oven dried uncooked *B. coriacea* seed + 90 of normal feed + portable water; Group B_{15}= 15% of oven dried uncooked *B. coriacea* seed + 85% of normal feed + portable water; Group B_{20}= 20% of oven dried uncooked *B. coriacea* seed + 80% of normal feed + portable water. Group C_5= 5% of cooked *B. coriacea* seed + 95% of normal feed + portable water; Group C_{10} = 10% of cooked *B. coriacea* seed + 90 of normal feed + portable water; Group C_{15}= 15% of cooked *B. coriacea* seed + 85% of normal feed + portable water; Group C_{20}= 20% of cooked *B. coriacea* seed + 80% of normal feed + portable water. The treatment of experimental animals was in accordance to the National Institute of Health [38] guidelines for the care and use of laboratory animals.

2.8 Hepatocellular integrity studies

Alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were determined using Reitman and Frankel [39] method. Alkaline phosphatase (ALP) was estimated by phenolphthalein monophosphate method as described by Babson *et al.*[40]. Doumas *et al.*[41] method was used for assay of bilirubin.

2.9 Statistical analysis

Students't-distribution was used in this study and value for each subgroup was directly compared to control at 5% significant level. Results were means and standard errors of mean (SEM).

III. RESULTS AND DISCUSSION

Table 1: Phytoconstituents of fresh, oven dried uncooked and cooked seed samples of *Buchholzia coriacea*.

Phytoconstituents	Fresh seed	Oven dried uncooked seed	Cooked seed
Flavonoids	++++	+++	++
Saponins	+++	++	++
Tannins	++++	++	+
Alkaloids	++++	++	+
Cyanogenic glycosides	++	+	+
Phytates	+++	+	+
Oxalates	++	+	+

Key:++++ = very high concentration;+++ = high concentration;++ = moderate concentration; + = low concentration.

The phytoconstituents of the studied seed samples of *Buchholzia coriacea* are present in Table 1. The Table shows the presence of flavonoids, saponins, tannins, alkaloids, cyanogenic glycosides and phytates in the studied seed samples. The concentrations of all the phytoconstituents investigated in this study were highest in fresh seed sample of *Buchholzia coriacea*. The roles of these constituents to well-being of plants and health of animals cannot be over emphasised. Flavonoids have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombic and vasodilatory activity [42]. The foaming and bitter tastes of saponins have been confirmed by different authors [15, 42-44]. Although saponins are mostly non-toxic in nature but they are known to produce cytotoxic and growth inhibition against a variety of cell hence making then to have anti-inflammatory and anticancer properties [45]. Tannins are known for astringency [43].

The sharp pungent taste with hot spicy flavour of *Buchholzia coriacea* seed could be traceable to its tannin content.

The observed flavonoid, saponin, tannin, alkaloid, and glycoside contents of this study agree with the earlier works of Ibrahim and Fagbonun [25] and Chinedu *et al.*[46]; oxalates and phytates agree with the earlier work of Amaechi [47]; while the presence of tannins is inconsistent with earlier work on the seed by Adediwura *et al.*[30]. Generally, the phytoconstituents found in the investigated samples of *Buchholzia coriacea* in the present study are known to affect the growth of one or more microbial forms. The concentrations of the observed phytoconstituents followed the order fresh seed > oven dried uncooked seed > cooked seed. Processing of the oven dried uncooked and cooked samples could be the cause of the difference in concentrations observed in the present study.

Table 2: Antibacterial activities of fresh, oven dried uncooked and cooked seed samples of *Buchholzia coriacea*.

	Zone of inhibition (mm)			
Test organism	Control	Fresh seed	Oven dried uncooked seed	Cooked seed
B. subtilis	+	48.0±0.13	21.0±0.15	7.0±0.01
S. epidermidis	+	53.0±0.10	19.0±0.02	4.0±0.08
S. aureus	+	40.0 ±0.06	23.0±0.01	1.0±0.02
E. coli	+	46.0±0.07	18.0±0.09	5.0±0.01
E. faecalis	+	59.0±0.10	30.0±0.13	3.0±0.09
K. pneunoniae	+	37.0±0.21	17.0±0.08	9.0±0.11

Results are means and standard errors of mean.

Evidences have shown that *B. subtilis* exists as a commensal in gut of human [48]. *S. epidermidis* is part of human skin flora, and consequently part of human flora [48-50]. Due to contamination, *S. epidermidis* is probably the most common species found in laboratory tests [51].It is not usually pathogenic but patients with compromised immune systems are often at risk for developing an infection [49-50]. *S. aureus* is frequently found in the human respiratory tract and on the skin [49]. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteraemia, and sepsis[49-50]. *E. coli* is a facultative anaerobic rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms [52-53]. *E. faecalis* is a commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals but can cause serious diseases [53-54]. *K. pneunoniae* can cause destructive changes to human lungs if aspirated [54]. The result of antibacterial activities as observed in the present study showed inhibitory zones produced by fresh seed as the highest (39.0-59.0 mm), followed by the inhibitory zones produced on the test organisms by oven dried uncooked seed (17.0-30 mm) while cooked seed produced the least inhibitory zones (1.0-9.0 mm) (Table 2). Ezekiel and Onyeozir [24]; Ibrahim and Fagbonun [26] had earlier noted the antibacterial activities of *B.coriacea* seed. The antibacterial activities of the *B.coriacea* seed as observed in this study followed the order fresh seed >oven dried uncooked seed> cooked seed. The observed inhibitory zones in this study followed the order of phytoconstituent concentrations as observed in Table 1 above. Hence phytoconstitutents could be behind the inhibitory effects of the investigated seed samples against test organisms.

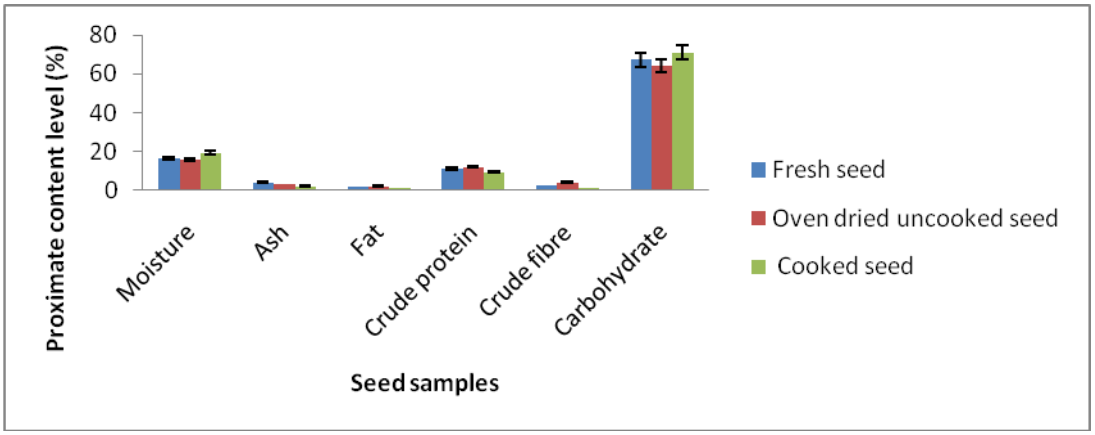

Figure 1: Proximate contents of fresh, oven dried uncooked and cooked seed samples of *Buchholzia coriacea* (%).

The proximate contents of fresh seed, oven dried uncooked seed and cooked seed of *Buchholzia coriacea* are present in Figure 1. The cooked seed sample was the highest in moisture content as against fresh seed and oven dried uncooked seed samples in this study. Since moisture is related to growth of microorganisms and shelf-life [54], it there implies that the shelf-life of the investigated seed samples will follow the order oven dried seed > fresh seed> cooked seed. The observed values of moisture in the present study are comparable to the value reported earlier on the seed by Ezekiel and Onyeoziri [24] (Figure 2) but higher than the value of Amaechi [46] on the seed (Figure 2). Different methods of preparation of the samples for analysis could be behind the observed difference in moisture contents. Ash in food material relates to minerals [56-57]. The ash contents of the studied samples are comparable though the order fresh seed > oven dried uncooked seed > cooked seed was followed in the study. The observed ash contents of the present study are comparable to values of Ezekiel and Onyeoziri [24] and Amaechi [47] on the seed (Figure 2). It is therefore important to ascertain the mineral contents of the investigated samples since they are required for tissue functioning and necessary for human nutrition. Fats retain flavour in foods [58]. They therefore play important role in food palatability [58]. According to Mongeau and Bassard [59], fibre in food is inversely related to moisture content. The fat, protein, fibre contents; and energy values of *B.coriacea* seed samples in the present study are comparable. The observed values of fibre in in the present study are comparable to values reported on the seed sample by Ezekiel and Onyeoziri [24] and Amaechi [47] (Figure 2). Carbohydrate content followed the order cooked > fresh seed > oven dried uncooked seed. Processing methods may have influenced the availability of the observed carbohydrate. Observed energy values are lower than values reported on the seed by Ezekiel and Onyeoziri[24] and Amaechi [47] (Figure 3). Different methods of processing or analysis for contents could be behind the observed difference.

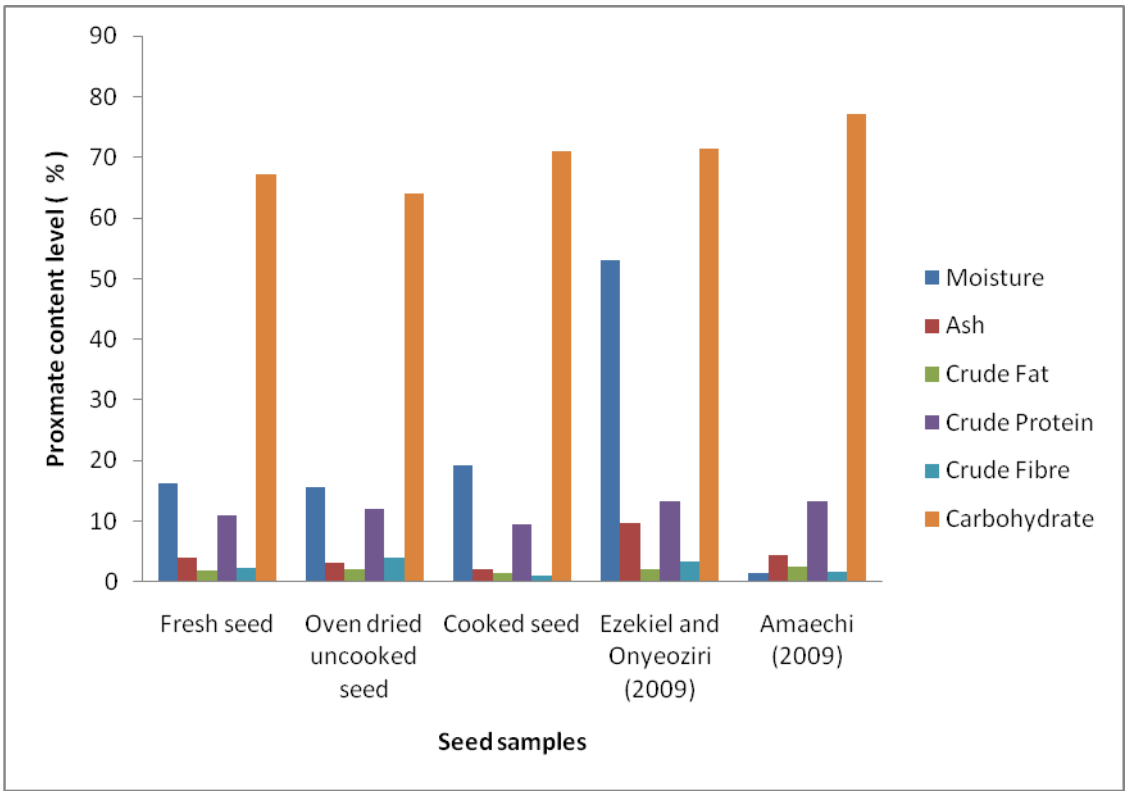

Figure 2: The proximate contents of fresh, oven dried uncooked and cooked seed samples of *B.coriacea* compared to those of Ezekiel and Onyeoziri [24] and Amaechi [46].

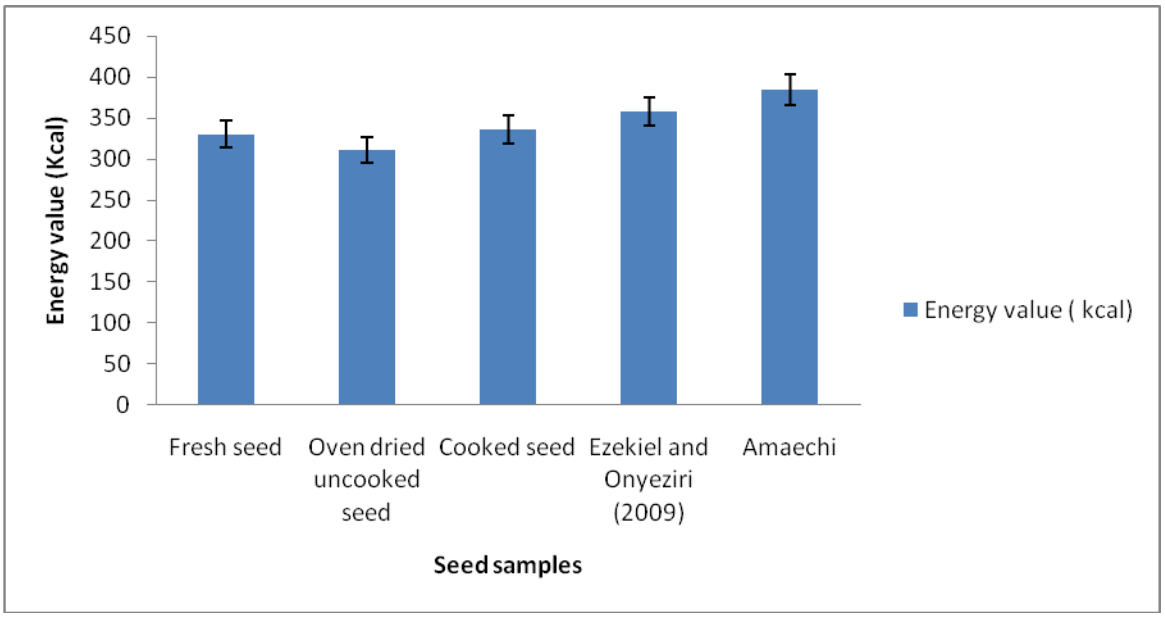

Figure 3: Energy values of fresh, oven dried cooked and uncooked seed samples of *B.coriacea* compared to those of Ezekiel and Onyeziri [24] and Amaechi [46].

Although the efficacy of medicinal plants against diseases has been confirmed in traditional medicine practice but in recent times there are quite a number of accumulating reports about organ injuries and other effects after intake of formulations made from some medicinal plants [60]. The liver is an organ that performs vital functions for the healthy survival of the body. Aside bile secretion into the intestine, the liver also detoxifies, stores, and synthesizes important biomolecules among other functions. These functions of the liver are made possible by hepatocytes [61]. The hepatocytes also house enzymes among which are aspartate aminotransferase (AST) and alanine aminotransferase (ALT). They are among the enzymes used to determine hepatocellular integrity though ALT is more specific indicator of liver inflammation than AST [62]. Different authors have noted that transaminases (AST and ALT) found in the liver leak in the general circulation when hepatocellular integrity is compromised [60-63].

Table 3: Hepatocellular integrity result of fresh, oven dried uncooked and cooked seed samples of *B.coriacea.*

		Fresh seed sample				Oven dried uncooked seed sample				Cooked seed sample			
Parameters	Control	A_5	A_{10}	A_{15}	A_{20}	B_5	B_{10}	B_{15}	B_{20}	C_5	C_{10}	C_{15}	C_{20}
AST (U/L)	22.03 ± 1.18	32.70 ± 0.56	35.70 ± 0.40	40.11 ± 0.12	43.93 ± 2.05	27.01 ± 3.28	29.72 ± 1.10	31.85 ± 0.16	33.50 ± 1.29	23.17 ± 0.14	24.03 ± 1.32	24.78 ± 3.02	24.17 ± 2.19
ALT(U/L)	56.72 ± 0.22	60.04 ± 1.53	63.26 ± 1.02	67.30 ± 0.12	69.30 ± 1.59	58.12 ± 2.86	59.08 ± 1.20	61.80 ± 0.37	62.48 ± 2.06	56.90 ± 1.32	57.12 ± 0.20	58.12 ± 1.80	58.07 ± 1.04
ALP (U/L)	19.84 ± 0.22	26.84 ± 1.62	29.01 ± 0.37	34.93 ± 3.02	37.87 ± 2.04	21.05 ± 1.72	24.84 ± 2.84	27.40 ± 1.01	27.98 ± 1.31	19.75 ± 1.40	20.36 ± 0.84	21.16 ± 2.21	22.42 ± 1.56
TB (mg/dl)	0.45 ± 0.02	0.51 ± 0.07	0.60 ± 0.01	0.61 ± 0.09	0.68 ± 0.01	0.48 ± 0.02	0.55 ± 0.03	0.54 ± 0.01	0.57 ± 0.06	0.46 ± 0.01	0.46 ± 0.03	0.47 ± 0.05	0.47 ± 0.05
CB (mg/dl)	0.20 ± 0.01	0.24 ± 0.06	0.25 ± 0.03	0.25 ± 0.01	0.26 ± 0.05	0.22 ± 0.05	0.23 ± 0.03	0.23 ± 0.01	0.25 ± 0.02	0.21 ± 0.07	0.21 ± 0.04	0.21 ± 0.02	0.22 ± 0.01

Results are means and standard errors of mean.

Key: AST=Aspartate aminotransferase; ALT= Alanine aminotransferase ; ALP=Alkaline Phosphatase; TB= Total Bilirubin; CB= Conjugated Bilirubin.

Alkaline phosphatase (ALP) is the enzyme most frequently used to detect obstruction in the biliary system. Elevation of alkaline phosphatase could be indication of disorders such as gallstone disease, alcohol abuse, drug-induced hepatitis, biliary cirrhosis (PBC) or biliary tumors since the enzyme is found in both the liver and bile as well in other organs as the case with AST [64-65]. For this reason, alkaline phosphatase is usually measured with gamma-glutamyl transpeptidase (GGT) or 5'-nucleotidase (5'-NT) to be able to ascertain its origin [62]. Microbial attack and toxic substances are among the gents that can enhance increased state of these enzymes in the liver [66].Phytoconstituents are very useful in the system but become toxic at high levels. The observed significant ($p<0.05$) increased in ALT, AST and ALP levels of rats in groups A (A_5-A_{20}) and B (B_5-B_{20}) against those of the control (Table 3) could be due to high levels of the observed phytoconstituents in the investigated samples (Table 1). Bilirubin is formed primarily from the breakdown of heme found in red blood cells [64]. It is relatively non-specific but useful since it reflects the liver's ability to take up, process, and secrete bilirubin into the bile [61-62]. Bilirubin levels (both total and conjugated bilirubin) were insignificantly ($p>0.05$) affected in all the groups against those of the control in this study.

IV. CONCLUSION

The present study has shown that phytoconstituents, antimicrobial activities and ability to affect hepatocellular integrity by the studied seed samples were in the following order; fresh seed > oven dried uncooked seed > cooked seed. The effects of fresh and oven dried uncooked seed samples on the hepatocellular integrity could be due to high levels of phytoconstituents. The hot spicy and painful sensation of fresh seed sample may have aided its effect on hepatocellular integrity than oven dried uncooked seed and cooked seed samples. The essence of this study is for those that consume the studied seed due to its perceived medicinal values to take note.The present study has comparative evaluated the phytoconstituents, antimicrobial activities, and proximate contents of fresh, oven dried uncooked and cooked samples of *Buchholzia coriacea* seed and their effects on hepatocellular integrity.

REFERENCES

[1]]J. Okpuzor, O. Adebesin, H. Ogbunugafor, and I. Amadi, The potential of medicinal plants in sickle cell disease control; A review. International Journal of Biomedical and Health Sciences 4(2), 2008, 47-54. W.C. Evans, Trease and Evans. Pharmacognosy, 15th edition (Edinburgh, 2002)585.

[2] A. Sofowora, A medicinal plants and traditional medicine in Africa. (Ibadan, Spectrum Books, 1993) 289. M.M. Iwu, Traditional Igbo medicine Institute of African studies publication, University of Nigeria, (Nsukka, 1983)112-144

[3] C. U. Okeke, and I. Elekwa, Phytochemical study of the extract of Gongronema latifolium Benth (Asclepiadaceae). J. Health and Visual Sci. 5, 2003, 47-55.

[4] D.E. Okwu and O. Ekeke, Phytochemical screening and mineral composition of chewing sticks in south Eastern, Nigeria. Global J Pure and Applied Sci. 9, 2003, 235-238.

[5] C. Nnimh, The encyclopedia of medicinal plants (Dorling Kindersley London, 1996).G. G. E. Osuagwu, I.C. Okwulehie. and J. O. Emenike, Phytochemical and mineral content of leaves of four Nigeria Pterocarpus(JACQ) species. International Journal of Molecular Medicine and Advance Science 3(1), 2007, 6-11.

[6] T. Bartram, Encyclopedia of Herbal medicinal (Macmillian London,1998). F. Stray, The National guide to medicinal herbs and plants (Tiger Books International London, 1998) 12-16.

[7] H. M. Burkill, The useful plants of West Africa (Royal garden,Kew, 1985)319.

[8] L.L. Zaika, Spices and Herbs; their antimicrobial activity and its determination. J. Food Safety 9, 1988, 97-118.
E.I.Akubugwo , A.N.Obasi, G. C. Chinyere and A. E. Ugbogu, Mineral and phytochemical contents in leaves of Amarantus hybridus L and Solanum nigrum L. Subjected to different processing methods. African Journal of Biochemistry Research 2(2), 2008, 040—044.

[9] A.O. Adebanjo, C.O. Adewumi and E.E. Essien, Anti-infective agents of higher plants.5th International Symposium in Medicinal Plants, University of Ife (OAU), 1983, 152-158.

[10] D. E. Okwu, Phytochemicals, vitamins and mineral contents of two Nigeria medicinal plants. J.Mol.Med. Adv. Sci. 1, 2004, 378-381.

[11] E. A. Sofowora, Medicinal Plants and Traditional medicine in Africa (John Wiley and Sons Ltd, 2008) 1-10.
B. Oliver, Nigeria`s useful plants. Nig. Field 24, 1959, 13-34.

[12] J. W. R. Keay, Tress ofNigeria(Clarendon press, Oxford, 1989)42-44.
J. Oseto, Medicinal uses of wonderful kola. Ezinne article.com. http://ezinearticles.com?/Medicinal-uses-of-wonderful-kola. (Retrieved 7/April/ 2010)

[13] L. S. Gill, Ethno medical uses of plants in Nigeria (University of Benin Press Benin, Nigeria, 1992).
F. L. S. Quattroachi-Umbelto, C.R.C. World Dictionary of plant names-common names scientific names, eponyms, synonyms, and entomology(C.R.C. Press. 2007) 367-368.

[14] A. A. Fred-Jaiyesimi, A. Adepoju, and O. Egbebunmi, Anthelminthic activities of Chloroform and methanol extract of Buchholzia coriacea Engle seed. Parasitology Research., 2011 Doi10.1007/s00436-011-2224y.
R. A. Adisa, M. J. Choudhary , and O.O. Olorunsogo, Hypoglycemic activity of Buccholzia coriacea (Capparaceae) seeds in streptozotacin-induced diabetic rats and mice. Exp Toxicol., 2010,

[15] Ptol 2010 doi:10.1016/j.etp.2010.05.002 O. O Ezikiel, and N. F. Onyeoziri, Preliminary studies on the antimicrobial properties of Buccholzia coriacea (wonderful kola). African Journal of Biotechnology 8 (3), 2009, 472-474

[16] T. A. Ibrahim and E. D. Fagbonun, Phytochemical and mineral quality of dried seeds of Buchholzia coriacea. Journal of Applied hytotechnology in Environment Sanitation 2(4), 2013a, 121-126.

[17] T. A. Ibrahim and E. D. Fagbonun, Antibacterial and Antifungal Activity of Ethanolic and Methanolic Extract of Dried Seeds of Buchhloziacoriacea.Greener Journal of Agricultural Sciences 3 (6), 2013b, 458-463.

[18] N. E. Nweze, L.B. Fakae and I. U. Asuzu, Anti-trypanosomal activity of ethanolic extract of Buchholzia coriacea seeds. WOCMAP IV Book of Abstracts, (Cape Town, South Africa, 2008) 103.
N. Nwaeze and I. Asuzu, Antihelminthic effect of Buchholzia coriacea seeds. Niger Vet J., 27 (2), 2006, 60-65. E. O. Ajayeoba, P. A. Onocha, S.O. Nwozo, and N.O. Sama, Antimicrobial and cytotoxicity evaluation of Buchhlozia coriacea stem bark. Fitoterapia 74(7-8), 2003, 706-709.

[19] A. Fred-Jaiyesimi, O. Ogbole, O. Anthony, and O. Egebunmi, Larvicidal effect of pet, ether, chloroform fractions and methanol extract of Buccholzia coriacea Engle seed. International Journal of Pharmaceutical Sciences and Research 2(7), 2011, 1736-1738.
G. O. Ezeifeka, M. U. Orji, T.I. Mbata, and A. O. Patrick, Antimicrobial activities of Cajanus cajan, Garcinia kola and Xylopia aethiopica on pathogenic microorganisms. Biotechnology 3(1), 2004, 41-43.

[20] I. U. Azuzu, Pharmacological evaluation of folkore of sphenostylia slenocarpa.J.Ehnophamacol 16, 1986, 236-267.

[21] R. Cruickshank, J. P. Daguid, B.P. Marnino and R.H. Swain, Medical Microbiology (12th edition) (Churchhill, Living stone, London, 1980) 180-188.

[22] J. G. Holt, N. R. Krieg, P.H.A. Sneath, J.T. Stanley and S.T. Williams, Bergey`s manual of systematic Bacteriology, 4th edition., (William and Wilkins Company, Batimore Maryland, 1994).

[23] J. B. Harbone, Introduction of ecological biochemistry (3rd edition) (Academic press, 1973).

[24] A. Odebiyi, and A. E. Sofowara, Phytochemical screening of Nigerian medicinal plants. Part III. Lloydia 41, 1978, 234-246.

[25] A.O.A.C , Official methods of analysis (13th ed). Association of Official Analytical Chemists.(Washington D. C, 1990).

[26] National Institute of Health , Guide for the Care and Use of Laboratory Animals.(U.S. Department of Health Education and Welfare, NIH Publication, 1985) 85-123.

[27] S. Reitman and S. Frankel, Colorimetric GOT and GPT determination. Am. J. Clin. Pathol. 28, 1957, 56-63. L. A. Babson, S. L. Greeley, C. M. Coleman, and G. D. Philips, Serum alkaline phosphatase determination. Clin. Chem. 12, 1966, 482-490.

[28] B. T. Doumas, B. W. Peny, E. A. Seasse, and J. V. Straumfjord, Standardization in bilirubin assay: evaluation of selected methods and stability of bilirubin solutions. Clin. Chem.19, 1973, 984-993.

[29] L.Alan, and N. D. Miller, Antioxidant flavonoids; structure, function and clinical usage. Alt. Med.Rev.1, 1996, 103-111. D. Okwu, and C. Josiah, (2006): Evaluation of chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology 5(4), 2006, 357-581.

[30] O. A. Sodipo, J. A. Akiniyl, and J. U. Ogunbamosu, Studies on certain charcteristics of extracts of bark of Pensnystalia macrucean(K. schemp) pierre Exbelie. Global J. Pure Appl. Sci.6, 2000, 83-87.

[31] A. A. Akindahunsi, and S. O. Salawu, Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. Afr.J.Biotec. 4, 2005, 497-500.

[32] F. A. Chinedu, I. Chibeze, E. Emma, and E. Chukwuenweiwe, The phytochemical, antispasmodic and antidiarrheal properties of the methanol extract of the leaves of Buchholzia coriacea family Capparaceae. International Journal of Pharmaceutical Research 4 (3), 2012, 52-55.

[33] N. C. Amaechi, Nutritive and anti-nutritive evaluation of wonderful kola (Buccholzia coriacea) seeds. Pakistan Journal of Nutrition 8 (8), 2009, 1120-1122.

[34] A. H. Huynh, K. Reena, M. K. T. Nguyen, C. Alessia, T. Sisareuth, U. Maria, B. Alain, G. Antonio, B. Ian, and M. C. Simon, Bacillus subtilis isolated from the human gastrointestinal tract.Research in Microbiology160 (2), 2009, 134–143.[49] M. W. Joanne, M. S. Linda, and J. W. Chritopher,Prescott`s microbiology, eight edition. (McGraw-Hill International. New York, 2011).

[35] M. Otto, Staphylococcus epidermidis — the 'accidental pathogen", Nature Reviews Microbiology7 (8), 2009, 555–567. S. Y. Queck, and M. Ott, Staphylococcus epidermidis and other Coagulase-Negative Staphylococci". Staphylococcus: Molecular Genetics. (Caister Academic Press.2008).

[36] J. Kluytmans, A. Van Belkum and H. Verbrugh, Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev.10 (3), 1997, 505–20.
A. Ogston, On Abscesses. Classics in Infectious Diseases. Rev Infect Dis6 (1), 1984, 122–28.

[37] K. J. Ryan, and C. G. Ray, Sherris Medical Microbiology (4th ed.) (McGraw Hill, 2004) 294–295.
J. C. Okaka, and A. N. C. Okaka, Foods: composition spoilage, shelf-life extension (OcJANCO Academic Publishers, Enugu26J. O. Olusanya,Essential of food and nutrition. 1st edition (Apex Book Limited, Lagos, Nigeria, 2008). G. I. Onwuka, Food analysis and instrumentation (Theory and practice), Ist edn. (Napthal prints, Surulere, Lagos, Nigeria.,2005) 64-68.

[38] B. S. Anita, E. J. Akpan, P. A. Okon, and I. U. Umoren, Nutritive and anti-nutritive evaluation of sweet potatoes (Ipomea batatas) leaves. Pak.J.Nutr.5, 2006, 166-168.

[39] R. Mongeau and R. Bassard, A comparison of three methods for analysing dietary fibre in 38 foods. J. food Composition and Analysis 2, 1989, 189-199.

[40] M. K. C. Duru, E. N. Agomuo, and B. A. Amadi, Biochemical studies on "udu" an antimalarial concoction used in Umunchi village, Isiala Mbano L.G.A of Imo State, Nigeria. Continental J. Pharmacology and Toxicology Research 5 (2), 2012, 28 – 34.
H. Lodish, A. Berk, S. L. Zipursky, P. Matsudaira, D. Baltimore, and J. E. Darnell, Molecular Cell Biology (Fifth Edition). (W. H. Freeman and Company. New York, 2000) 10-200.
E. U. Friday, Clinical uses of enzymes, In: Conceptual Enzymology, (Innolex Printers and Publishers, Calabar, 2004) 190-212
U. Wurochekke, A. E. Anthony, and W. Obidah, Biochemical effects on Liver and kidney of rats administered aqueous stem bark extract of Xemenia americana. African Journal of Biotechnology 7 (16), 2008, 2777-2780.

[41] M. N. Chatterjea, and R. Shinde, Uses of enzymes, in; Textbook of Medical Biochemistry, 5th edn. (Jaypee Brothers, New Delhi, 2000a) 118.
[42] . C. P. Prince, and K. G. Albert, Biochemical assessment of liver function, in: Liver and Biliary diseases-pathophysiology, diagnosis and management (R. Wright, K.G. Albert, S. Karran and G. Millivard-Sadler, eds), (Saunders Co. Ltd., London., 1976) 381-416.[66]
[43] K. C. D. Majesty, A. A. Eze, C.O. Prince, A. Benjamin, and R. O. Chima-Ezika, Consequencies of long-term consumption of water from Nworie River (Owerri, Nigeria) on haematological,
[44] hepatic and renal function using rats model. Biokemistri 24(1), 2012, 52-57.
[45] F. A. Chinedu, I. Chibeze, E. Emma, and E. Chukwuenweiwe, The phytochemical, antispasmodic and antidiarrheal properties of the methanol extract of the leaves of *Buchholzia coriacea* family Capparaceae. *International Journal of Pharmaceutical Research 4 (3),* 2012, 52-55.
[46] N. C. Amaechi, Nutritive and anti-nutritive evaluation of wonderful kola (*Buccholzia coriacea*) seeds. *Pakistan Journal of Nutrition 8 (8),* 2009, 1120-1122.
[47] A. H. Huynh, K. Reena, M. K. T. Nguyen, C. Alessia, T. Sisareuth, U. Maria, B. Alain, G. Antonio, B. Ian, and M. C. Simon, *Bacillus subtilis* isolated from the human gastrointestinal tract. *Research in Microbiology 160 (2),* 2009, 134–143.
[48] M. W. Joanne, M. S. Linda, and J. W. Chritopher, *Prescott`s microbiology*, eight edition. (McGraw-Hill International. New York, 2011).
[49] M. Otto, *Staphylococcus epidermidis* — the 'accidental pathogen", *Nature Reviews Microbiology 7 (8),* 2009, 555–567.
[50] S. Y. Queck, and M. Ott, Staphylococcus epidermidis and other Coagulase-Negative Staphylococci". *Staphylococcus: Molecular Genetics*. (Caister Academic Press. 2008).
[51] J. Kluytmans, A. Van Belkum and H. Verbrugh, Nasal carriage of *Staphylococcus aureus:* epidemiology, underlying mechanisms, and associated risks. *Clin. Microbiol. Rev. 10 (3),* 1997, 505–20.
[52] A. Ogston, On Abscesses. Classics in Infectious Diseases. *Rev Infect Dis 6 (1),* 1984, 122–28.
[53] K. J. Ryan, and C. G. Ray, *Sherris Medical Microbiology* (4th ed.) (McGraw Hill, 2004) 294–295.
[54] J. C. Okaka, and A. N. C. Okaka, *Foods: composition spoilage, shelf-life extension* (OcJANCO Academic Publishers, Enugu, 2005) 26-33.
[55] J. O. Olusanya, *Essential of food and nutrition.* 1st edition (Apex Book Limited, Lagos, Nigeria, 2008).
[56] G. I. Onwuka, *Food analysis and instrumentation (Theory and practice),* Ist edn. (Napthal prints, Surulere, Lagos, Nigeria., 2005) 64-68.
[57] B. S. Anita, E. J. Akpan, P. A. Okon, and I. U. Umoren, Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomea batatas*) leaves. *Pak.J.Nutr.5,* 2006, 166-168.
[58] R. Mongeau and R. Bassard, A comparison of three methods for analysing dietary fibre in 38 foods. *J. food Composition and Analysis 2,* 1989, 189-199.
[59] M. K. C. Duru, E. N. Agomuo, and B. A. Amadi, Biochemical studies on "udu" an antimalarial concoction used in Umunchi village, Isiala Mbano L.G.A of Imo State, Nigeria. *Continental J. Pharmacology and Toxicology Research 5 (2),* 2012, 28 – 34.
[60] H. Lodish, A. Berk, S. L. Zipursky, P. Matsudaira, D. Baltimore, and J. E. Darnell, *Molecular Cell Biology* (Fifth Edition). (W. H. Freeman and Company. New York, 2000) 10-200.
[61] E. U. Friday, *Clinical uses of enzymes, In: Conceptual Enzymology*, (Innolex Printers and Publishers, Calabar, 2004) 190-212.
[62] A. U. Wurochekke, A. E. Anthony, and W. Obidah, Biochemical effects on Liver and kidney of rats administered aqueous stem bark extract of *Xemenia americana*. *African Journal of Biotechnology 7 (16),* 2008, 2777-2780.
[63] M. N. Chatterjea, and R. Shinde, *Uses of enzymes, in; Textbook of Medical Biochemistry*, 5th edn. (Jaypee Brothers, New Delhi, 2000a) 118.
[64] C. P. Prince, and K. G. Albert, Biochemical assessment of liver function, in: Liver and Biliary diseases-pathophysiology, diagnosis and management (R. Wright, K.G. Albert, S. Karran and G. Millivard-Sadler, eds), (Saunders Co. Ltd., London., 1976) 381-416.
[65] K. C. D. Majesty, A. A. Eze, C.O. Prince, A. Benjamin, and R. O. Chima-Ezika, Consequencies of long-term consumption of water from Nworie River (Owerri, Nigeria) on haematological, hepatic and renal function using rats model. *Biokemistri 24(1),* 2012, 52-57.