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

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**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.

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**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]. Otuagwu 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, 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 phytoneutrients and other plant chemicals known as phytochemicals[14-15]. Hence most of the indigenous plants are therefore used as medicinal plants [15-17]. *Buchholzia coriacea* 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 “essen 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 Oriie Amaraku market in Isiala Mbano L.G.A of Imo State, Nigeria. The seeds were identified by Dr. Mbogwu, 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 dirt. The dirt 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 washed 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°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 Ezeifeke 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. pneumoniae,

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 pelleted 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<sub>5</sub>= 5% of fresh *B. coriacea* seed + 95% of normal feed + portable water; Group A<sub>10</sub>= 10% of fresh *B. coriacea* seed + 90 of normal feed + portable water; Group A<sub>15</sub>= 15% of fresh *B. coriacea* seed + 85% of normal feed + portable water; Group A<sub>20</sub>= 20% of fresh *B. coriacea* seed + 80% of normal feed + portable water. Group B<sub>5</sub>= 5% of oven dried uncooked *B. coriacea* seed + 95% of normal feed + portable water; Group B<sub>10</sub>= 10% of oven dried uncooked *B. coriacea* seed + 90 of normal feed + portable water; Group B<sub>15</sub>= 15% of oven dried uncooked *B. coriacea* seed + 85% of normal feed + portable water; Group B<sub>20</sub>= 20% of oven dried uncooked *B. coriacea* seed + 80% of normal feed + portable water. Group C<sub>5</sub>= 5% of cooked *B. coriacea* seed + 95% of normal feed + portable water; Group C<sub>10</sub>= 10% of cooked *B. coriacea* seed + 90% of normal feed + portable water; Group C<sub>15</sub>= 15% of cooked *B. coriacea* seed + 85% of normal feed + portable water; Group C<sub>20</sub>= 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’-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*.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Fresh seed</th>
<th>Oven dried uncooked seed</th>
<th>Cooked seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>++++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>++++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytates</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxalates</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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, antiiallergic, 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 concentration 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*.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Control</th>
<th>Fresh seed</th>
<th>Oven dried uncooked seed</th>
<th>Cooked seed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>+</td>
<td>48.0±0.13</td>
<td>21.0±0.15</td>
<td>7.0±0.01</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>+</td>
<td>53.0±0.10</td>
<td>19.0±0.02</td>
<td>4.0±0.08</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>+</td>
<td>40.0±0.06</td>
<td>23.0±0.01</td>
<td>1.0±0.02</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>+</td>
<td>46.0±0.07</td>
<td>18.0±0.09</td>
<td>5.0±0.01</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>+</td>
<td>59.0±0.10</td>
<td>30.0±0.13</td>
<td>3.0±0.09</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>+</td>
<td>37.0±0.21</td>
<td>17.0±0.08</td>
<td>9.0±0.11</td>
</tr>
</tbody>
</table>

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* 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. pneumoniae* 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 phytoconstituents could be behind the inhibitory effects of the investigated seed samples against test organisms.
The proximate contents of fresh, 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 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.
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*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fresh seed sample</th>
<th>Oven dried uncooked seed sample</th>
<th>Cooked seed sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>A₁</td>
<td>A₁₀</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>22.03 ± 1.18</td>
<td>32.70 ± 0.56</td>
<td>35.70 ± 0.40</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>56.72 ± 0.22</td>
<td>60.04 ± 1.53</td>
<td>63.26 ± 1.02</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>19.84 ± 0.22</td>
<td>26.84 ± 1.62</td>
<td>29.01 ± 0.37</td>
</tr>
<tr>
<td>TB (mg/dl)</td>
<td>0.45 ± 0.02</td>
<td>0.51 ± 0.07</td>
<td>0.60 ± 0.01</td>
</tr>
<tr>
<td>CB (mg/dl)</td>
<td>0.20 ± 0.01</td>
<td>0.24 ± 0.06</td>
<td>0.25 ± 0.03</td>
</tr>
</tbody>
</table>

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 agents 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) increase in ALT, AST and ALP levels of rats in groups A (A1-A3) and B (B1-B20) 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 comparatively 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.

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