Comparative study of the physicochemical, nutritional and phytochemical properties of the extract of *Sclerocarya birrea* (anacardiaceae) produced in three different localities in Chad

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ARTICLE INFO
Received May 5th, 2023
Received in revised form June 30th, 2023
Accepted June 30th, 2023

Keywords:
Sclerocarya birrea,
Extracts,
physicochemical properties,
Nutritional properties,
Phytochemical properties,
Chad.

ABSTRACT
*Sclerocarya birrea*, a tree belonging to the Anacardiaceae family, is widely distributed across various African countries and is utilized for culinary and medicinal purposes. However, there have been limited studies on the fruits of this plant as produced in Chad. Therefore, the aim of this research is to investigate the diverse chemical compositions and nutritional properties of *Sclerocarya birrea* fruits grown in Chad. Specifically, three primary producing regions have been identified: Sarh (Middle Chari), Koumra (Mandoul), and Bongor (Mayo-Kebbi East). The collected fruits from these regions were subsequently processed into powder and subjected to characterization. Several parameters were analyzed, including secondary macronutrients, micronutrients, and metabolics, which were measured using conventional and standardized methods. The physico-chemical analyses revealed that the fruits from Bongor (Mayo-Kebbi East) exhibited comparatively higher water content (9.72 ± 0.15 g/100g), ash content (7.97 ± 1.35 g/100g DM), and pH level (4.55 ± 0.006) than the fruits from the other two sites. This variation could be attributed to the specific soil type and climate conditions in Bongor. Furthermore, the nutritional analyses of macronutrients in the three different samples demonstrated significant differences at Bongor, with higher carbohydrate (67.22 ± 0.64 g/100g DM), lipid (8.45 ± 0.09 g/100g DM), and protein (6.71 ± 0.003 g/100g DM) contents compared to Sarh and Koumra samples. Bongor also showed a higher density of micronutrients, including nitrogen, calcium, and magnesium, with respective contents of (1.07 ± 0.0005%), (5.7 ± 0.46%), and (243.43 ± 1.05%). Moreover, the phytochemical properties exhibited significant variability, with Bongor samples showing notably higher levels of total phenols (2033.15 mg EAG/100g DM), flavonoids (151.66 mg EQU/100g DM), and tannins (1534.97 mg EAG/100g DM) compared to the other sites. Overall, the results indicate substantial differences in nutrient levels among the fruits collected from the three locations. These findings can be instrumental in guiding the selection of the optimal production site for *Sclerocarya birrea* fruits to enhance their value.
1. Introduction:
*Sclerocarya birrea*, commonly known as the Marula tree, is a shrubby plant belonging to the Anacardiaceae family. It has a long history of traditional use in many countries, particularly in sub-Saharan Africa, where it holds significant cultural and economic importance. This plant is revered for its proven therapeutic properties [1], and its various parts, especially the fruits, hold immense nutritional value. In several communities, *Sclerocarya birrea* is highly regarded for its fruit, which is a rich source of vitamin C and other essential nutrients. The fruits can be consumed fresh, in juice form, or fermented to produce alcoholic beverages. Additionally, they are processed into jelly and jam, extending their shelf life and making them more accessible for consumption throughout the year. Due to its versatility and nutritional benefits, *Sclerocarya birrea* has become an integral part of the local cuisine and dietary habits, contributing significantly to the nutrition of the West African rural population [2]. Among the parts of the plant consumed, both the fruits and leaves are considered crucial dietary supplements, particularly in the Sahelian region, where food scarcity and nutrient deficiencies are common challenges [3]. The incorporation of *Sclerocarya birrea* into daily meals helps improve the nutritional quality of diets, especially for rural communities with limited access to diverse food sources. The leaves and fruits of this plant also play a role in animal husbandry, as they are utilized as supplementary feed for livestock [4]. To fully harness the benefits of *Sclerocarya birrea*, understanding its nutritional composition is essential. The plant contains macronutrients such as carbohydrates, lipids, and proteins, which provide food energy measured in kilocalories (kcal). These macronutrients are essential for meeting the body's energy needs and supporting various physiological functions. Additionally, *Sclerocarya birrea* offers a wealth of micronutrients, including vitamins and minerals, which are crucial for overall health and well-being. These micronutrients play vital roles in metabolic processes, immune function, and disease prevention. For populations facing nutrient deficiencies, the consumption of plant parts rich in vitamins and minerals, like *Sclerocarya birrea*, becomes even more critical in addressing these nutritional gaps. Notably, the Sahelian region, like many other areas in sub-Saharan Africa, faces nutritional challenges, particularly protein and vitamin deficiencies [5]. Therefore, promoting and studying the nutritional potential of plants like *Sclerocarya birrea* could hold the key to combating malnutrition and its associated health issues [6]. Considering the diverse applications and nutritional significance of *Sclerocarya birrea*, researchers have undertaken studies to determine its chemical composition and nutritional properties. One such study aims to compare the physico-chemical, nutritional, and phytochemical parameters of different fruit extracts from *Sclerocarya birrea* collected at three distinct sites in Chad where the plant naturally thrives.

2. Material and methods:
2.1. Presentation of the study area:
The study focused on three specific regions in Chad: Sarh (Moyen Chari) in Kemkian, Koumra (Mandoul) in Bendoyoh, and Bongor (Mayo-Kebbi Est) in Nahaina. To visually represent the geographical distribution of these areas, the researchers utilized Arc GIS (Arc Geographic Information System) software to create Figure 1, a cartographic representation.

![Figure 1. Geographic coordinates (source: DIVA GIS data and 2019 Administrative Division).](image-url)
2.2. Sample collection:
A preliminary study was carried out, including a field visit to prepare a well-structured survey sheet for systematic sample collection of *Sclerocarya birrea* fruits from three provinces. After collection, the fruits were dried in the shade at an ambient temperature of 37°C for two weeks and then transported to the Physicochemistry Laboratory at the Food Quality Control Center (CECOQDA) in N'Djamena, Chad, for preservation. Subsequently, the fruits were ground into powder using an electronic grinder and stored in a dry oven for future analysis. The plant material mainly comprised of *Sclerocarya birrea* seeds harvested from the mentioned provinces, with the species authentication performed at the Institut de Recherche en l'Élevage pour le Développement (IRED-N'Djamena, Chad).

![Figure 2. Plant material used (fruit and powder of *Sclerocarya birrea*).](image)

2.3. Determination of physicochemical properties:
The physicochemical properties of the samples were determined using various standardized methods. For assessing the moisture content (Humidity Rate - TH), 5g of the sample was weighed and then placed in an oven at 105°C for 1 to 2 hours [7]. The dry matter content was determined using the ISO 1572:1987 method [8]. To measure the ash content, the ISO 1576:1987 method was employed [9]. The hydrometric title was determined using the ISO 20978:2018 standardized method [10]. For pH determination, a pH-meter was used, consisting of an electric housing that displays the pH value and an electrode that measures this value [11].

2.4. Determination of nutritional properties:
The nutritional properties of the samples were assessed, focusing on both macronutrients and micronutrients. For determining the carbohydrate content, the FAO method (2002) was utilized [12]. The fat content was measured using the standardized method ISO 4048:2018 [13], while the protein content was determined using ISO 20483:2013 [14]. Regarding essential micronutrients, the calcium content was evaluated using the standardized method ISO – 90-003:1984 [15], and the magnesium content was measured following the ISO Standard Method – 003:1984 [15].

2.5. Determination of phytochemical properties:
The phytochemical properties of the samples were determined using various established methods. The total phenol content was assessed using the method described by Makkar *et al.*, (1993) [16]. The flavonoid content was measured using the colorimetric method developed by Mitic *et al.*, (2014) [17]. Tannin content was determined following Makkar *et al.* method, (1993) [16]. The saponin content was evaluated using the method of Poornima *et al.*, (2009) [18]. Finally, the terpenoid content was measured using Harborne's method, (1973) [19].

2.6. Statistical analysis:
For the determination of means and standard deviations, the calculations were performed using Microsoft Office Excel 2016. The data were expressed as three standard deviation averages. To assess significant differences between the groups, the Duncan Multi Range Test was utilized with the aid of IBM SPSS Statistics version 26.0 by IBM Corp. in Armonk, NY. The statistical significance level was set at p < 0.05.

3. Results and discussion:
Most plants are recognized in Africa like Chad as both medicinal and food plants. This use gives the population an effective factor in the preventive and curative fight against several diseases, protecting it and providing the nutrients it needs. Physico-chemical characterization of *Sclerocarya birrea* fruit samples from three provinces. The results of the different water, dry matter, ash, hydrometrical titre and pH levels are shown in Table 1. The study found that the water content was significantly different (p<0.05) between the three samples. Compared to Sarh (9.40%), the Koumra (9.81%) and Bongor (9.72%) samples have different water content concentration (p<0.05). In addition, the dry matter (DM) content of the samples also showed a significant difference (p<0.05) between the three samples. MS values of these samples from the three cities are almost identically similar: Sarh (3.62%), Bongor (3.61%) and Koumra with 3.60% respectively. Similarly,
the percentage of hydrometric titre is higher in the samples of Sarh (286.96%) and Bongor 248.46% compared to that of Koumra 183.28%. It should be noted that in the pH results there is a significant difference (p<0.05) between the three samples. The pH values of Koumra (4.55) and Bongor (4.55) are significantly different (p 0.05) between the three cities, Sarh, Koumra and Bongor with an average of 7.40%, 7.61% and 7.97%, respectively. The physicochemical parameter is important for this study because it allows to have an idea about the lifespan, and hardness of our fruits. The different samples of the three cities have high water contents in Sclerocarya birrea and appreciated from the other two cities with a threshold of variable significance. This can be explained by the permeability of the soil to the received water making its acidity less dense and also its ash content. Our results are close to those obtained in Chad with a water content of 9.81±0.02% in the fruits of Adansonia digitata L. in the Laï site [20]. In addition, the hydrogen potential value of our sample is similar to that obtained by Lepeengue et al. (2020) [21] in Gabon on the study of some physicochemical and biochemical characteristics of Wavé-fortex a natural dietary supplement giving a pH (4.02). However, the ash results are not consistent with those found by Prisacaru et al. (2017) [22] when estimating heavy metal levels in green leafy vegetables.

Table 1. Characterization of physicochemical properties.

<table>
<thead>
<tr>
<th>Province</th>
<th>Water content (g/100g DM)</th>
<th>Matter dry (g/100g DM)</th>
<th>Content ash (g/100g DM)</th>
<th>Title hydrometric (°f)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarh</td>
<td>9.40±0.14a</td>
<td>3.62±0.006c</td>
<td>7.40±0.6c</td>
<td>286.96±0.52c</td>
<td>4.51±0.01a</td>
</tr>
<tr>
<td>Koumra</td>
<td>9.81±0.04b</td>
<td>3.61±0.001a</td>
<td>7.61±0.60a</td>
<td>183.28±0.36c</td>
<td>4.55±0.006b</td>
</tr>
<tr>
<td>Bongor</td>
<td>9.72±0.15b</td>
<td>3.62±0.0004b</td>
<td>7.97±1.35a</td>
<td>248.46±0.58b</td>
<td>4.55±0.006b</td>
</tr>
</tbody>
</table>

The different letters indicate statistically significant differences between the groups (p < 0.05).

It emerges from this analysis that the carbohydrate content presents a significant difference (p < 0.05) between the three extracts from the three cities. As a result, the grades at Koumra (70.73%) and Sarh 69.77% are different compared to that at Bongor (67.22%). In addition to the study conducted, the statistical analyzes of the fat (lipid) content showed a significant difference (p<0.05) between the three samples represented. Thus, there is a slightly high concentration increase at Bongor 8.45% compared to the two samples from Koumra (6.68%) and Sarh (6.76%) with a slight drop in concentration. In addition, the protein levels in the three provinces fall within the scope of this study that there is a significant difference (p<0.05) between the three samples represented. There was a slight increase in almost similar values between Bongor 6.71% and Sarh samples (6.46%) compared to a slight decrease in Koumra (5.17%). Nutritional properties are important by their energetic, constructing and building values that the body needs for its proper functioning, maintaining homeostasis for a normal physiology. They are mainly composed of carbohydrates, lipids and proteins. According to our results, Bongor’s samples had the most important nutritional values overall than the other two provinces. These results are almost similar to those obtained in Ivory Coast by Maxime et al. (2022) [23] on the characterization of some leafy vegetables most consumed in the city of Daloa of which they reported that the carbohydrate content of leafy vegetables is between 71.16±0.25% and 90.85±0.12%. But, on the other hand, our results are different from those obtained by Lepeengue et al., 2016 [21] in Gabon giving a carbohydrate content of Wavé-fortex a natural food supplement 45.69 ± 3.75% the lipid values obtained by Maxime et al. (2022) [23] in Ivory Coast with low concentrations (0.29±0.02% to 0.69±0.04%). Similar results have also been observed in many previous studies Ponka et al. (2016) [24].

Table 2. Characterization of nutritional properties (macronutrients).

<table>
<thead>
<tr>
<th>Province</th>
<th>Carbohydrate (g/100g DM)</th>
<th>Lipid (g/100g DM)</th>
<th>Protein (g/100g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarh</td>
<td>69.96 ±0.18c</td>
<td>6.76 ±0.18c</td>
<td>6.46 ±0.08c</td>
</tr>
<tr>
<td>Koumra</td>
<td>70.72±0.24b</td>
<td>6.68±0.15a</td>
<td>5.17±0.08c</td>
</tr>
<tr>
<td>Bongor</td>
<td>67.14±0.64a</td>
<td>8.45±0.09b</td>
<td>6.71±0.003c</td>
</tr>
</tbody>
</table>

The different letters indicate statistically significant differences between the groups (p < 0.05).

Table 3. Characterization of nutritional properties (micronutrients).

<table>
<thead>
<tr>
<th>Province</th>
<th>Nitrogen (%)</th>
<th>Calcium (mg /L)</th>
<th>Magnesium (mg /Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarh</td>
<td>1.03 ±0.08b</td>
<td>9 ±0.6b</td>
<td>277.96 ±0.088c</td>
</tr>
<tr>
<td>Koumra</td>
<td>0.83±0.04a</td>
<td>8±0.56b</td>
<td>175.48±0.4a</td>
</tr>
<tr>
<td>Bongor</td>
<td>1.07±0.0005c</td>
<td>5.7±0.46a</td>
<td>243.43±0.05b</td>
</tr>
</tbody>
</table>

The different letters indicate statistically significant differences between the groups (p < 0.05).
It appears from this study that the nitrogen content has a concentration similar to Bongor (1.07%) and Sarh (1.03%) compared to Koumra (0.83%) with a somewhat low concentration decrease. Similarly, this difference in significance (p<0.05) is found in the calcium levels of the three samples are shown above with a low Bongor concentration (5.70%) compared to Koumra and Sarh samples with high concentrations of 8.00% and 9.00% respectively. Of the above, the variable homogeneity test yielded averages of different levels of magnesium in representative form with high values at Sarh (277.96%) and Bongor (243.43%) and a decrease compared to Koumra (175.48%). In addition, micronutrients are food substances that have no energy role in the body but which are involved in the functioning of all metabolisms. The micronutrients analyzed are: nitrogen, calcium and magnesium. Our values obtained are different from those found by Glew et al. (2004) [25] with high rates. These results are in agreement with those observed by N’guessan et al. (2019) [26], who said that the variation in macronutrient content differs from one region to another due to environmental and climatic conditions.

Table 4. Characterization of Phytochemical Properties.

<table>
<thead>
<tr>
<th>Province</th>
<th>Total phenols (mg EAG/g)</th>
<th>Flavonoid (mg EQU/g)</th>
<th>Tannins (mg EAG/g)</th>
<th>Saponins (mg/g)</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarh</td>
<td>1964.29±31.86ab</td>
<td>166.26±12.55a</td>
<td>1313.52±26.55a</td>
<td>88.90±5.54a</td>
<td>+++</td>
</tr>
<tr>
<td>Koumra</td>
<td>1915.63±28.38a</td>
<td>164.33±5.58a</td>
<td>1523.23±26.52b</td>
<td>89.95±1.35a</td>
<td>+</td>
</tr>
<tr>
<td>Bongor</td>
<td>2033.15±53.81b</td>
<td>151.66±3.78a</td>
<td>1534.97±41.34b</td>
<td>82.44±4.69a</td>
<td>+</td>
</tr>
</tbody>
</table>

+++ : Very abundant ; ++: Presence; +: Low presence
mg EAG/g: milligram gallic acid equivalent per gram; mg EQU/g (milligram quercetin equivalent per gram);
mg/g: milligram per gram.
The different letters indicate statistically significant differences between the groups (p < 0.05).

The reported results of total phenols yielded high concentrations in Bongor (2033%) and Sarh (1964%) compared to a slight decrease in this concentration in Koumra (1915%). In addition, the Sarh tannin concentration (1313%) differs from the corresponding higher Bongor (1534%) and Koumra (1523%) concentrations. In addition, the qualitative values of the terpenenoid concentration are very abundant at Sarh (+++) compared to those with a low presence at Koumra (+) and Bongor (+) respectively. However, it emerges from this statistical analysis that there is no significant difference (p>0.05) in flavonoids and saponins between the three extracts of the three cities mentioned. These values of flavonoids, although not significant, have little different and decreasing contents in Sarh (166%), Koumra (164%) and Bongor (151%) respectively. Similarly, the saponin levels. However, it appears from this statistical analysis that there is no significant difference (p> 0.05) in flavonoids and saponins between the three extracts from the three cities mentioned. These values of flavonoids, although not significant, have little different and decreasing contents in Sarh (166%), Koumra (164%) and Bongor (151%) respectively. Similarly, the saponin contents have distinctly variable concentrations in Koumra (89.95%), Sarh (88.90%) and Bongor (82.44%). Phytochemical studies are crucial parameters for evaluating interactions between samples. From the above, total phenols, flavonoids, tannins, saponins, terpenoids were the subject of our parameters. Saponins and terpenoids had low concentrations in Bongor compared to the other two provinces (Sarh and Koumra), but on the contrary with high levels of total phenols and tannins. Our results are different from those obtained in Chad by Maguirgue et al. (2022) [27] on the yield of the crude extract of Commelina benghalensis in water, with contents of total phenols (74.00%), flavonoids (51.00 %), tannins (16.00%) respectively. This justifies that total phenols, flavonoids, and tannins possess antioxidant activities, enabling them to combat free radicals effectively. As a result, they contribute to slowing down cell damage, exhibit anti-inflammatory properties, and act as immunostimulants [28-29].

5. Conclusion:
At the conclusion of this comparative study, focused on the physicochemical, nutritional, and phytochemical properties of Sclerocarya birrea extracts produced in three different localities in Chad, significant findings were obtained. The analysis of various parameters revealed that the powdered fruit of Sclerocarya birrea displayed low levels of water, dry matter, and ash content, but exhibited a higher water hardness (hydrometrical title). Despite its low-fat content, the extract yielded a high energy value. Moreover, the powder was rich in essential mineral elements such as calcium and magnesium, while nitrogen content was relatively low.

Additionally, the study highlighted the presence of vital phytonutrients in the Sclerocarya birrea extract, including total phenols, flavonoids, tannins, saponins, and terpenoids, all of which displayed abundant content. These phytonutrients play a crucial role in promoting health and preventing oxidative stress. Based on these favorable results, it is recommended to incorporate Sclerocarya birrea fruits regularly into the diet as a beneficial food ingredient for the Chadian population. Furthermore, the usage of these fruits could serve as an essential measure in the fight against malnutrition and the prevention of oxidative stress-related conditions. Overall, the study provides valuable insights that can guide the selection of the most
suitable production sites for Sclerocarya birrea fruits, thus optimizing their nutritional value and potential benefits for the well-being of the population in Chad.

Acknowledgment:

We would like to thank the respondents and the Graduate School of Science, Technology and Environment (ED-STE) of the University of N’Djamena for the authorization of research as well as the local administrative authorities of the various cities used to collect the data. At the Laboratory of Physicochemistry of the Center for Food Quality Control (CECOQDA) and the laboratory of the National Institute of Agro-Industrial Sciences (INSAI) of N’Gaoundéré for the analysis of samples of this study.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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