

Chemical composition and oral toxicity assessment of *Anisophyllea boehmii* Engl. (Umushindwi) Kernel meal from Burundi, a potential source of animal feed

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Abstract

Anisophyllea boehmii Engler (Umushindwi) is a wild and indigenous species in Burundi with high socio-economic and nutritional interest. Recent studies revealed that *A. boehmii* kernels yield a substantial quantity of oil with physicochemical quality. The present study aims to valorize a meal which is a by-product of *A. boehmii* kernel oil extraction, by evaluating its chemical composition and performing acute toxicity tests. The results show water (8.22-12.68%) and ash (3.27-3.63%) content; and mineral composition comparable to those of most other known meals. The meal under study is particularly rich in protein (21.54-25.29%), potassium (5349-6746 mg kg⁻¹), phosphorus (3582-4202 mg kg⁻¹) and magnesium (949-1356 mg kg⁻¹). The acute oral toxicity test revealed no toxicity of *A. boehmii* kernel meal. The important level of protein and minerals places the *A. boehmii* kernel meal amongst meals which are recognized as good quality resource for animal feed. Furthermore, since the acute toxicity tests did not show any danger of its consumption, the *A. boehmii* kernel meal becomes a potential alternative resource for conventionally used in animal feed. For a better use of this resource, we suggest an analysis of the amino acid composition of the proteins, the different components of the carbohydrates, and subacute and chronic toxicity tests.

Keywords: *Anisophyllea boehmii*; kernel meal; protein; mineral composition; animal feed

Résumé

Anisophyllea boehmii Engler (Umushindwi) est une espèce sauvage et indigène au Burundi qui présente un grand intérêt socio-économique et nutritionnel. Des études récentes ont révélé que les graines d'*A. boehmii* produisent une quantité substantielle d'huiles d'une bonne qualité physico-chimique. La présente étude vise à valoriser son tourteau qui est un sous-produit de l'extraction de l'huile, en évaluant sa composition chimique et en effectuant des tests de toxicité aiguë. Les résultats montrent une teneur en eau de 8,22-12,68% et en cendres de 3,27-3,63% des tourteaux d'*A. boehmii*. Leur composition minérale est comparable à celles de la plupart des autres farines connues. Ces tourteaux sont particulièrement riches en protéines (21,54-25,29 %), en potassium (5349-6746 mg kg⁻¹), en phosphore (3582-4202 mg kg⁻¹) et en magnésium (949-1356 mg kg⁻¹). Le test de toxicité orale aiguë n'a révélé aucune toxicité des tourteaux des graines d'*A. boehmii*. L'importance des protéines et des minéraux place ces tourteaux parmi les ressources de bonne qualité pour l'alimentation animale. Ainsi, les tests de toxicité aiguë n'ayant pas montré de danger de leur consommation, les tourteaux des graines d'*A. boehmii* deviennent une source alternative potentielle de l'alimentation animale conventionnelle. Pour une meilleure valorisation, nous proposons une analyse de la composition en acides aminés des protéines et des différentes composantes des glucides ainsi que des tests de toxicité subaiguë et chronique.

Keywords: *Anisophyllea boehmii*, tourteaux, protéines, composition minérale, alimentation animale.

1. Introduction

Recent studies show that the genus *Anisophyllea* contains a wide range of food and medicinal products. The medicinal side has already been explored in detail for some species of this genus such as *A. boehmii*, *A. dichostyla*, *A. quangensis*, *A. laurina* [Khallouki *et al.*, 2007 & 2009; Kalaba *et al.*, 2009; Balde *et al.*, 2015; Göhre *et al.*, 2016]. This taxon is also known for its food and nutritional importance. This is also the case of *A. boehmii*, *A. quangensis* and *A. laurina* (Kalaba *et al.*, 2009; Göhre *et al.*, 2016; Onivogui *et al.*, 2014). Fruits of *A. boehmii* are consumed and marketed in species distribution area (Nkengurutse *et al.*, 2019; Kalaba *et al.*, 2009). Furthermore, they are the mostly consumed of all wild fruits (Kalaba *et al.*, 2009). They are also processed into juice that can even be fermented (Kalaba *et al.*, 2009; Malaisse, 1985). In addition, in some countries such as Zambia, *A. boehmii* fruits rank first in wild fruit processing (Kalaba *et al.*, 2009). However, only the fruit pulp corresponding to the mesocarp is widely consumed while the consumption of the kernel is sporadic. It is in this prism that we undertook a research project aiming at optimizing the exploitation of this species, is a non-timber forest products. Nkengurutse *et al.* 2016 & 2019 evaluated the chemical composition and toxicity of kernel and the kernel oil of *A. boehmii*. The results revealed significant kernel oil content of up to 29% with an interesting physicochemical quality comparable to that of conventional oilseeds. The optimization of *A. boehmii* kernel exploitation raises the question of valorization of the kernel meal.

In recent decades, meals have elicited the interest of researchers and investors. Indeed, the rise in cereal price has led farmers to reconsider the animal feeding. The underlying principle is to maintain a balanced diet while minimizing the cost, especially by valorizing products so far considered as waste such as meals. This increases the diversity and availability of food, thereby contributing to the realization of sustainable development goals, namely by reducing hunger, improving nutrition and achieving food security (ONU, 2018). Sabikhi & Kumar (2012) suggest that these initiatives should be further focused on in developing countries, where food security is still a challenge. The purpose of the present study is twofold. On the one hand, it seeks to determine the chemical composition of the kernel meal of *A. boehmii* in terms of proteins, carbohydrates and mineral matter (ash and mineral elements). On the other hand it intends to carry out toxicity tests in order to eventually turn this resource into animal food use. No-previous study has investigated the chemical composition and toxicity test of *A. boehmii* kernel meal.

2. Material and methods

2.1. Plant material

Plant material consisted of six samples of *Anisophyllea*

boehmii fruits (Fig. 2.A) collected from two sites: Ruvubu National Park (Kigamba district, samples K1, K2 and K3) and Gisagara Protected Landscape in East of Burundi (samples G1, G2 and G3), both located in eastern Burundi. The details on the location and the site characterization are published by Nkengurutse *et al.* (2019). The meal studied here are by-products derived from *A. boehmii* kernel oil extraction whose results have also been reported by Nkengurutse *et al.* (2019). The meal (Fig. 1.E) was stored at 4 °C in glass bottles until analysis.

2.2. Evaluation of dry matter and moisture content

The moisture and dry matter of the meal was determined by drying at 100°C for 24 hours according to method 950.46 of the Association of Official Analytical Chemists (AOAC, 2005).

2.3. Determination of total protein

Total proteins were evaluated by Kjeldahl method. The total protein content was expressed by multiplying the amount of nitrogen obtained by the conventional factor 6.25, according to the method of the Association of Official Analytical Chemists (AOAC, 2005). This method is based on the mineralization of *A. boehmii* kernel meal using sulfuric acid, alkalization of the ammonium sulphate produced, distillation of the ammonia released and its recovery in excess by boric acid and its titration with sulfuric acid solution.

2.4. Determination of total sugars

Fifty mg of *A. boehmii* kernel meal were weighed into a 12 ml airtight tubes and 6 ml of H₂SO₄ (1 M) were added. The tubes were hermetically sealed, mixed vigorously with vortex and placed in an oven at 100°C for 3 h. After cooling, the tube solutions were neutralized with NaOH 10 N solution to pH 7. The solution was then filtered through a filter paper placed on a Buchner and vacuum flask and rinsed several times. The filtered solutions were transferred to a 25 ml volumetric flask and adjusted to constitute the solution for total sugars titration. In order to determine the total reducing sugar, 300 µl of the filtered solutions were successively mixed with 1000 µl of DNS (3,5-Dinitrosalicylic acid) and 700 µl of distilled water in a glass tube. Then the whole was homogenized and placed in a boiling water bath (95°C) for 5 min. After cooling, 5 ml of distilled water was added and the absorbance was measured using a UV-visible spectrometer (RAYLEIGH UV1800) at 540 nm. The results were expressed in % (g of sugars per 100 g of meal) with reference to a standard curve established from a range of glucose concentrations ranging from 50 to 500 µg.ml⁻¹.

2.5. Determination of ash content and mineral composition

Ash content was quantified by incineration at 550°C for 24 hours in accordance with the official method of the Association of Official Analytical Chemists (AOAC, 2005). The contents of

potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) were determined after mineralization by atomic absorption spectrometry (Perkin Elmer Atomic Absorption, modèle Analyst 400, USA). To do this, 1 g of meal was weighed into a teflon container with 6 ml of nitric acid 65% and 1 ml of hydrogen peroxide 35%. The mineralization was carried out in a high performance microwave digestion unit (MLS 1200 meg) using the following program: 250 W: 2 min; 0 W: 2 min; 250 W: 6 min; 400 W: 5 min; 600 W: 5 min; ventilation: 10 min. The total meal phosphorus was evaluated after mineralization of the samples by the molybdenum blue method (Scheel, 1936) at 700 nm using a Shimadzu UV-1205 type spectrophotometer (Shimadzu, Kyoto, Japan).

2.6. Acute oral toxicity tests

The toxicity of *A. boehmii* kernel meal was evaluated on Albino mice by oral gavage. The meal extract was prepared by maceration. Fifty grams (50 g) of meal were dissolved in 500 ml of water. After stirring for one hour, the mixture was filtered through a sieve with a mesh size of 200 μm . The filtrate was dried in an oven at 40°C until complete water evaporation. The acute oral toxicity of *A. boehmii* kernel meal was performed on mice by gavage according to the Organization for Economic Cooperation and Development guidelines 420 (OECD-420, 2011) and following the same protocol as (Nkengurutse *et al.*, 2019). 24 mice (20-30 g) fasting 12 to 14 hours were divided into 4 groups ($n = 6$; ♂ / ♀ = 1) including one control and three treated groups. The treated groups were force-fed with meal extract by receiving respectively 3, 7 and 10 g per kg of body weight. The control group was given 10 g of distilled water. It should be noted that the meal extract required a relatively high quantity of water (5 g in 10 ml) to constitute a gavage solution. In accordance with the principle of OECD-420 of the maximum administrable quantities (not exceeding 1 ml per 100 g of the body weight), the doses 7 and 10 g of meal extract per kg of body weight were administered in two times at six-hour intervals. After administration of studied-meal extract, the mice were observed for 14 days for possible behavioral and motor autonomy alterations or death. At the end of the experiment, the mice were sacrificed by cervical dislocation and vital organs were observed for eventual anomalies. The mice were provided by the Laboratory of Genetics, Physiology and Ethnopharmacology (Faculty of Science-Oujda, Morocco).

2.7. Statistical analysis

The results were expressed as the mean values \pm standard deviation (SD) for each measurement carried out in triplicate. The statistical analysis consisted of One-Way ANOVA and Duncan's Post-hoc test to determine the significant differences between sample means. Differences between the two sites (Gisagara and Ruvubu) were assessed by t-Student test. The significant difference threshold was set

at 5%. Statistical analyses were carried out using software for Windows: IBM Statistical Package for the Social Sciences (IBM SPSS. 20).

3. Results and discussion

3.1. Moisture and dry matter content

The food is much preserved from microbiological deterioration at low water content (Chapeland-Leclerc *et al.* 2005). Furthermore, the market's value of a product is often exaggerated if the water content is high (Hinton, 2007). Therefore, it is crucial to know the water content of a food and possibly define the standards. The water content of *A. boehmii* kernel meal varies between 8.22 and 12.68% (Table 1). These results show that there are significant differences ($p < 0.05$) in water content between the trees studied. A variation in humidity implies the variation of the dry matter content. In fact, the results of the dry matter content of *A. boehmii* kernel meal range between 87.62 and 91.78%. These values are comparable to those reported in other meals. Hinton (2007) reported that the average dry matter content of most grains and seeds is 90%. Sunflower, cotton, rapeseed, soya and palm kernel meals contain 89 to 94% of dry matter (Grieshop *et al.*, 2003; Sundu, Kumar and Dingle, 2006; Adeola and Kong, 2014). However, dry matter contents between 97 and 98% have been reported in safflower and sesame meals (Roy *et al.*, 2014; Mansouri *et al.*, 2018). We believe that for a better conservation of *A. boehmii* meal, drying conditions should be reconsidered.

3.2. Protein content

Protein content is one of the key criteria to take into consideration when selecting meals (Woyengo, Beltranena and Zijlstra, 2014). This explains the expansion of oleaginous varieties with high protein content despite their relatively lower oil content. This is the case for soybeans, with crude protein content often exceeding 50% of the meal's dry matter (Smartt and Nwokolo, 1996; Slominski *et al.*, 2012; Woyengo, Beltranena and Zijlstra, 2014).

The protein content of *A. boehmii* kernel meals ranges between 21.54% and 25.29% (Table 1). These values are comparable to those reported in other meals commonly used in animal feed. This is notably the case of palm kernel meal with protein content between 14% and 21% (Sundu *et al.*, 2006). The palm kernel meal is very common in livestock feed in Burundi. It is also used in poultry and pig feed (Smartt and Nwokolo, 1996; Sundu *et al.*, 2006). Other meals with comparable crude protein content include sesame, sunflower and safflower meals (Lomascolo *et al.*, 2012; Roy *et al.*, 2014). However, the meal's protein composition is based on content and availability of amino acids, mainly essential amino acids (Sundu *et al.*, 2006). For example, compared to soybean meal, the safflower meal is less attractive for monogastric animals not only because of its lysine, methionine and isoleucine deficiency, but also for their poor availability (Heuzé *et al.*, 2012). It is therefore relevant to complete this study by an analysis of the amino acid

composition.

Table 1. Proximal meal composition of *Anisophyllea boehmii boehmii* kernels harvested in eastern Burundi (expl).

	G1	G2	G3	K1	K2	K3	Gisagara	Kigamba
Moisture	10.74±0.10 ^c	12.38±0.09 ^d	9.32±0.03 ^b	9.70±0.03 ^b	8.22±0.08 ^a	12.68±0.37 ^d	10.81±1.33 ^A	10.20±1.98 ^A
Dry matter	89.26±0.10 ^b	87.62±0.10 ^a	90.67±0.03 ^c	90.29±0.03 ^c	91.78±0.08 ^d	87.31±0.37 ^a	89.18±1.32 ^A	89.79±1.98 ^A
Ash	3.40±0.01 ^b	3.27±0.03 ^a	3.44±0.04 ^{bc}	3.55±0.05 ^c	3.63±0.05 ^d	3.62±0.05 ^d	3.37±0.08 ^A	3.60±0.61 ^B
Crude protein	24.88±0.27 ^c	25.01±0.56 ^c	22.72±0.75 ^b	21.54±0.28 ^a	25.29±0.10 ^c	24.35±0.20 ^c	24.20±1.21 ^A	23.73±1.70 ^A
Carbohydrate	71.71±0.27 ^a	71.70±0.59 ^a	73.84±0.71 ^b	74.91±0.25 ^b	72.01±0.20 ^a	72.02±0.21 ^a	72.42±1.16 ^A	72.66±1.73 ^A

G1, G2, G3, K1, K2, K3 correspond to meal of six trees of *A. boehmii* kernel from the two sampling sites: G, Gisagara; K, Kigamba. Significant differences ($p < 0.05$) among trees are shown by different lowercase letters (a–d) and significant differences ($p < 0.05$) between sites are indicated by different capital letters (A and B).

A. boehmii meal's crude protein content varies significantly ($p < 0.05$) among different trees although there is no significant difference between the two sites (Ruvubu and Gisagara). This suggests that the difference could be largely related to the genetic factor rather than pedoclimatic conditions. Other studies have shown that crossbreeding can improve protein content (Slominski et al., 2012). This entails research perspectives on genetic variability evaluation and selection of interesting phenotypes when the domestication of *A. boehmii* starts.

3.3. Carbohydrate content

The carbohydrate content of *A. boehmii* is presented in Table 1. It varies between 71.70% and 74.91%. These values show significant differences ($p < 0.05$) between different trees. Balanced human or animal nutrition contains carbohydrates up to 50% of the calorie intake. However, most research is not concerned with carbohydrates as such. It rather focuses on various components, including simple sugars and dietary fiber. The composition of the meal, especially its fiber content determines their use. Rich meals in fiber are preferred for ruminant feed while other meals (poor in fiber) are better for feeding monogastric animals (Van Soest et al., 1991). Thus, the results of the present work are only preliminary and are not sufficient enough to assign specific uses for the various meals based solely on the composition of carbohydrates.

3.4. Ash content

Ashes are residues of mineral components resulting from the incineration of organic matters. The ash content of *A. boehmii* kernel meal varies between 3.27 and 3.63% (Table 1). These results are comparable to those reported in other meals including safflower and palm kernel (Abdollahi et al.,

2015; Mansouri et al., 2018). However, other meal sources could contain much more ash. For instance sesame, sunflower and rapeseed meals could bear an ash content varying between 6 and 10% (Lin and Humbert, 1974; Lomascolo et al., 2012; Slominski et al., 2012; Roy et al., 2014).

The ash content of *A. boehmii* kernel meal varies significantly ($p < 0.05$) among trees within and between sites of our sampling area. Mansouri et al. (2018) suggest that the differences in ash content for safflower meals could be explained by the varietal effect. We believe that the significant differences in our case would find explanation in genetic and pedoclimatic differences.

3.5. Mineral content

Mineral elements are involved in the constitution and proper functioning of organisms. Meals rich in some mineral elements are particularly well indicated in fish nutrition (Roy et al., 2014). In this work, we determined the mineral composition of some important elements in the meal of *A. boehmii* kernels. The respective contents of the studied mineral elements show that potassium is the main element, followed by phosphorus, magnesium and calcium (Table 2).

Potassium is involved in muscle contractions and blood pressure (Ajayi et al., 2007). Its content in the meals of *A. boehmii* kernels ranged from 5349.66 to 6746.33 mg kg⁻¹ (Table 2). This composition is close to the one reported in the palm kernel meal (6400 mg kg⁻¹) (Abdollahi et al., 2015). Higher contents were reported in the rubber seed meal (9300 mg kg⁻¹) (Smartt and Nwokolo, 1996).

Potassium supplementation reduces the effects of Lysine-Arginine imbalances. Potassium requirements would increase with protein intake as the excretion of uric acid is potassium-dependent. Values show significant differences ($p < 0.05$) among trees and between the sites.

Table 2: Mineral composition (mg/kg of dry matter) of *Anisophyllea boehmii* kernels harvested in eastern Burundi.

Min.*	G1	G2	G3	K1	K2	K3	Gisagara	Kigamba
P	3582.7±30.51 ^a	3789.7±14.5 ^{bc}	3723.7±2.5 ^b	4202.0±19.0 ^d	3751.3±40.5 ^{bc}	3818.3±40.5 ^c	3698.7±93.12 ^A	3923.9±213.2 ^B
Ca	784.7±3.8 ^c	872.7±10.06 ^d	645.3±10.5 ^b	461.0±57.0 ^a	465.0±24.0 ^a	456.0±2.4 ^a	767.6±99.6 ^A	460.78±31.5 ^B
K	6746.3±11.7 ^c	5986.3±41.3 ^{ab}	6389.7±46.5 ^{bc}	5539.6±97.5 ^a	5609.7±299.5 ^a	5349.7±578.5 ^a	6374.1±330.8 ^A	5499.7±349.3 ^B
Mg	1157.7±15.5 ^c	1356.7±19.5 ^d	1308.3±4.5 ^d	968.7±13.5 ^{ab}	1019.7±33.5 ^b	949.3±38.6 ^a	1274.2±90.8 ^A	979.4±1.1 ^B
Fe	36.0±1.3 ^c	24.98±1.36 ^{ab}	22.2±3.3 ^a	32.5±1.15 ^c	27.1±0.7 ^b	27.2±0.30 ^b	27.7±6.6 ^A	28.9±2.7 ^A

* Minerals; G1, G2, G3, K1, K2, K3 correspond to meals of six trees of *A. boehmii* kernel from the two sampling sites: G, Gisagara; K, Kigamba. Significant differences ($p < 0.05$) among trees are shown by different lowercase letters (a–d) and significant differences ($p < 0.05$) between sites are indicated by different capital letters (A and B)

The average content of this element is exceptionally high ($6374.11 \text{ mg kg}^{-1}$) in the Gisagara site compared to Kigamba ($5499.67 \text{ mg kg}^{-1}$). Soil type, more than genetic variability, may have played a big role in the potassium amount of *A. boehmii* kernel meal. Soil analyses would be interesting for further research.

Phosphorus is a functional element whose needs increase during pregnancy, lactation and growth. It is also a constitutive element of teeth and bones. Husbandry of monogastric animals requires phosphorus supplementation. However, the notion of phosphorus "availability" is still an ongoing discussion in scholarly circles, particularly because of the phytic phosphorus fraction. Roy et al. (2014) suggest that the process of meal fermentation reduces phytates and thus increases the availability of phosphorus in animal feed. As Table 2 shows, the phosphorus content of *A. boehmii* kernel meal is between 3582.66 and $4202.00 \text{ mg kg}^{-1}$. These results are close to those reported in palm kernel and rubber meals (Smartt and Nwokolo, 1996; Abdollahi et al., 2015). However, these values are lower than those reported in sesame seeds, rapeseed, cotton and sunflower seeds (Lomascolo et al., 2012; Adeola and Kong, 2014; Roy et al., 2014). The phosphorus content of our meals showed significant differences ($p < 0.05$) among trees in the same site and between the sites. Unlike the previous case, the phosphorus content is higher in the Kigamba site compared to Gisagara. We believe that these differences in values would have the same explanations as potassium related to the nature of the soil.

Magnesium was also detected at considerable concentrations in the analyzed samples. Its content varies between 949.33 and $1356.66 \text{ mg kg}^{-1}$ (Table 2). These results show significant differences ($p < 0.05$) among trees at the same site and between the sites. The values of our results are lower than the values reported for other meals, namely palm kernels, soybean, rubber and safflower meals (Smartt and Nwokolo, 1996; Abdollahi, Hosking and Ravindran, 2015; Mansouri et al., 2018). Needs are estimated at 400 mg kg^{-1} for pigs, but ruminants have relatively higher needs (Ferrando et al., 1989). The magnesium content of our

samples is an added value to the quality of *A. boehmii* kernel meal compared to other sources containing more of this mineral. In fact, magnesium is involved in bone formation and plays a key role in many enzymatic reactions. Magnesium deficiencies are rare and supplementation is almost always inappropriate. On the other hand, problems arise when this element is excessive in food. It causes diarrhea and reduces the use of calcium and phosphorus (Ferrando et al., 1989).

With regard to calcium, its content varies significantly ($p < 0.05$) between 456.00 and $872.66 \text{ mg kg}^{-1}$, with a remarkable variability between sites (767.5 and $460.78 \text{ mg kg}^{-1}$ respectively for Gisagara and Kigamba). This element is constitutive and an indicator of bone health. It reduces fractures and bone loss (Recker et al., 1996). However, an excess of calcium causes deficiency in other mineral elements including zinc in the growing rabbit or a phosphorus deficiency in the suckling rabbit (Ferrando et al., 1989). The results of this work are comparable to those reported for sesame seeds, rubber and rapeseed meals (Smartt and Nwokolo, 1996; Lomascolo et al., 2012; Roy et al., 2014). They are still lower than those reported in palm kernel or safflower and sunflower meals that exceed 1700 mg kg^{-1} and sometimes 3000 mg kg^{-1} (Lomascolo et al., 2012; Abdollah et al., 2015; Mansouri et al., 2018).

Concerning iron, the results reveal the presence of low quantities. The iron content of *A. boehmii* kernel meals ranged between 22.23 and 36.01 mg kg^{-1} with significant differences ($p < 0.05$) among trees within the sites (Table 2). These values are among the lowest values known in cereal grains, which often range between 30 and 60 mg kg^{-1} (Underwood, 1999). In addition, they are lower than those reported in other sources, particularly in palm kernel or sesame seeds, rubber and rapeseed meals (Smartt and Nwokolo, 1996; Roy et al., 2014; Abdollahi et al., 2015). The meals of *A. boehmii* kernels seem unsuitable to be a source of iron, especially for rabbits whose needs can reach 100 mg kg^{-1} of food (Ferrando et al., 1989). *A. boehmii* kernel meal should be supplemented with other more iron-rich sources to be balanced. This element is a constituent of hemoglobin and myoglobin. Iron deficiency causes anemia. However, excess in iron would interfere with copper and zinc uptake and could be toxic (Ferrando et al., 1989).

3.6. Toxicity tests

Toxicity tests are often conducted in medicine as well as for molecules and extracts of medicinal plants. Feed and food testing is relatively rare because in most cases there is a long tradition of consumption and use and therefore they are empirically known to be non-toxic. However, in cases of new feed and food products, toxicity tests are necessary. This is also the case with the use of *A. boehmii*. The consumption of its kernel is not systematic in Burundi (Nkengurutse *et al.*, 2016, 2019). Recent studies have shown that *A. boehmii* kernel oil is not toxic (Nkengurutse *et al.*, 2019). We opted to realize the same tests regarding the meals. Three doses were used, the highest of which corresponds to the maximum dose allowed in the acute toxicity tests (10 g kg^{-1}). After 14 days of monitoring the mice, no case of mortality was recorded in either case. Additionally, no apparent sign of toxicity was observed on vital organs. These results represent

an interesting step towards safe consumption of seeds and derived products. However, the absence of acute toxicity risk does not rule out subacute and chronic toxicity. It is thus necessary to carry out the other two types of tests. Indeed, the use of meal may be limited by certain compounds such as phytic acid, tannins, glucosinolates, matairesinol- β -glucoside and 2-hydroxyarctiin- β -glucoside (Heuzé *et al.*, 2012; Roy *et al.*, 2014). On the other hand, it has been reported that some meals, such as those from rubber seeds, contain compounds that cause infertility in pigs (Smartt and Nwokolo, 1996). It is possible that *A. boehmii* kernel meal may contain harmful compounds, although no acute toxicity has been identified. Whatever, we believe that the non-consumption of *A. boehmii* kernels would be related to their bitter aftertaste rather than the existence of possible toxicity. However, subacute and chronic tests are still necessary. The search for taste improvement processes and proposal of the seed-based menus and its by-products are interesting.

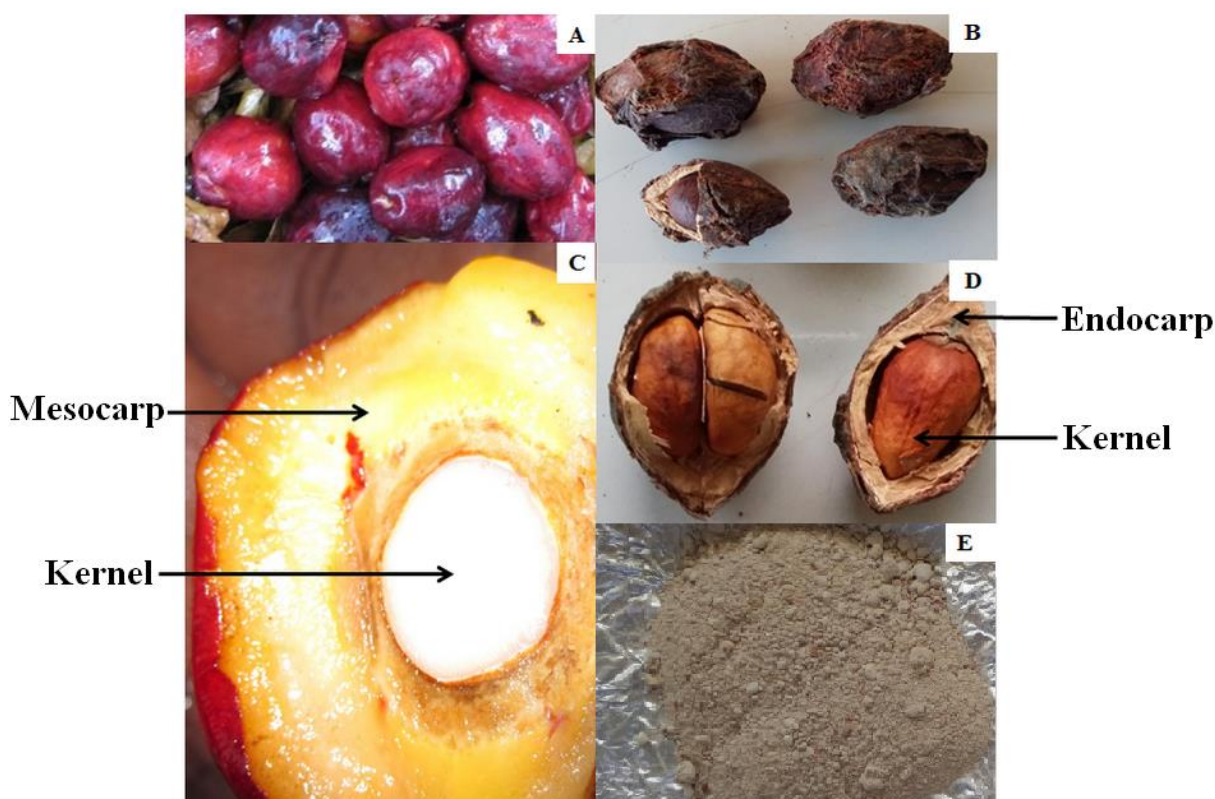


Figure 1. *Anisophyllea boehmii* fresh (A) and dried (B) fruits; kernels (C & D) and kernel meal (E).

4. Conclusion and perspectives

Anisophyllea boehmii is a wild and indigenous species in Burundi with a high socio-economic and nutritional interest. Recent studies showed that *A. boehmii* kernel is an oil-rich source. The analysis of the chemical composition of *A. boehmii* kernel meals aimed to valorize this by-product particularly for animal feed. The results show that water and ash content as well as mineral composition are comparable to

those of most other known meals. *A. boehmii* kernel meals have an interesting composition for a good mineral intake. However, the iron content seems too low to ensure the necessary inputs for animal feed. *A. boehmii* kernel meals would need to be supplemented by an iron-rich source. The protein content, up to 25%, places the *A. boehmii* kernel meals among protein-rich sources for animal feed. Acute-toxicity tests seem to entail safe consumption of *A. boehmii* kernel meal. *A. boehmii* kernel meals could be a potential complementary resource for conventional

meal used in animal feed. For a better assignment of this resource, it is necessary to complete the present research by analyzing the amino acid composition of the proteins as well as the different components of the carbohydrates and subacute and chronic toxicity tests.

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References

- [1] Abdollahi, M. R., Hosking, B. and Ravindran, V., 2015. Nutrient analysis, metabolisable energy and ileal amino acid digestibility of palm kernel meal for broilers. *Animal Feed Science and Technology*, 206, pp. 119–125.
- [2] Adeola, O. and Kong, C., 2014. Energy value of distillers dried grains with solubles and oilseed meals for pigs. *Journal of Animal Science*, 92, pp. 164–170.
- [3] Ajayi, I., Oderinde, R., Ogunkoya, B., Egunyomi, A. and Taiwo, V., 2007. Chemical analysis and preliminary toxicological evaluation of *Garcinia mangostana* seeds and seed oil’, *Food Chemistry*, 101, pp. 999–1004.
- [4] AOAC (2005) Official Methods of Analysis of the Association of Official Analytical Chemists, 18th ed. Maryland.
- [5] Balde, M., Traore, M., Diane, S., Diallo, M., Tounkara, T., Camara, A., Baldé, E., Bah, F., Ouedraogo, U. and Drame, H., 2015. Ethnobotanical survey of medicinal plants traditionally used in Low and Middle - Guinea for the treatment of skin diseases. *Journal of plant sciences*, 3, pp. 32–39.
- [6] Chapeland-Leclerc, F., Papon, N. and Noël, T., 2005. Moisissures et risques alimentaires (mycotoxines). *Revue Francophone des Laboratoires*, 373, pp. 61–66.
- [7] Chen, X. I. N., He, H. A. I. and Zhang, L., 2015. A monograph of the Anisophylleaceae (Cucurbitales) with description of 18 new species of Anisophyllea. *Phytotaxa*. Auckland: Magnolia Press.
- [8] Ferrando, R., Blum, J., Bourdon, D., Cabrera-Saadoun, M., Fevrier, C., Henry, Y., Larbier, M., Laury, V., Lebas, F. and Leclercq, B., 1989. L’alimentation des animaux monogastriques: porc, lapin, volaille. *Nutrition*. Paris
- [9] Göhre, A., Toto-Nienguesse, Á.B., Futuro, M., Neinhuis, C. and Lautenschläger, T., 2016. Plants from disturbed savannah vegetation and their usage by Bakongo tribes in Uíge, Northern Angola. *Journal of Ethnobiology and Ethnomedicine*, 12, pp. 1–28.
- [10] Grieshop, C.M., Kadzere, C.T., Clapper, G.M., Flickinger, E.A., Bauer, L.L., Frazier, R.L. and Fahey, G.C., 2003. Chemical and Nutritional Characteristics of United States Soybeans and Soybean Meals. *Journal of Agricultural and Food Chemistry*, 51, pp. 7684–7691.
- [11] Heuze, V., Tran, G., Chapoutot, P., Renaudeau, D., Bastianelli, D. and Lebas, F., 2012. Safflower (*Carthamus tinctorius*) seeds and oil meal. *Feedipedia*.
- [12] Hinton, D. G., 2007. Supplementary feeding of sheep and beef cattle. *FAO*. Washington, D.C: Landlinks Press.
- [13] Kalaba, F., Chirwa, P., Prozesky, H. and Ham, C., 2009. The role of indigenous fruit trees in rural livelihoods: the case of communities in the Mwekera area, copperbelt province, Zambia. *Acta Hort.* (ISHS), 806, pp. 129–136.
- [14] Kalaba, F. K., Chirwa, P. W. and Prozesky, H., 2009. The contribution of indigenous fruit trees in sustaining rural livelihoods and conservation of natural resources. *Journal of Horticulture and Forestry*, 1, pp. 1–6.
- [15] Khallouki, F., Bartsch, H. and Owen, R. W., 2007. Isolation, purification and identification of ellagic acid derivatives, catechins, and procyanidins from the root bark of *Anisophyllea dichostyla* R. Br. *Food and Chemical Toxicology*, 45, pp. 472–485.
- [16] Khallouki, F., Hull, W. E. and Owen, R. W., 2009. Characterization of a rare triterpenoid and minor phenolic compounds in the root bark of *Anisophyllea dichostyla* R. Br. *Food and Chemical Toxicology*, 47, pp. 2007–2012.
- [17] Lin, M. J. Y. and Humbert, E., 1974. Functional properties of sunflower meal products. *Journal of Food Science*, 39, pp. 368–370.
- [18] Lomascolo, A., Uzan-Boukhris, E., Sigoillot, J. C. and Fine, F., 2012. Rapeseed and sunflower meal: A review on biotechnology status and challenges. *Applied Microbiology and Biotechnology*, 95, pp. 1105–1114.
- [19] Malaisse, F. and Parent, G., 1985. Edible wild vegetable products in the Zambezi woodland area: A nutritional and ecological approach. *Ecology of Food and Nutrition*, 18, pp. 43–82.
- [20] Mansouri, F., Ben Moumen, A., Richard, G., Fauconnier, M.-L., Sindic, M., Elamrani, A. and Serghini Caid, H., 2018. Proximate composition, amino acid profile, carbohydrate and mineral content of seed meals from four safflower (*Carthamus tinctorius* L.) varieties grown in north-

- eastern Morocco. Oilseeds and fats, Crops and Lipids, 25, pp. 1–9.
- [21] Nkengurutse, J., Houmy, N., Mansouri, F., Ben Moumen, A., Caid, H.S. and Khalid, A., 2016. Preliminary Chemical Characterization of Amashindwi (*Anisophyllea boehmii* Engl.) Kernels and Kernel oil. Journal of Materials and Environmental Science, 7(6), pp. 1996–2005.
- [22] Nkengurutse, J., Mansouri, F., Bekkouch, O., Moumen, A.B., Masharabu, T., Gahungu, G., Serghini, H.C. and Khalid, A., 2019. Chemical composition and oral toxicity assessment of *Anisophyllea boehmii* kernel oil: potential source of new edible oil with high tocopherol content. Food Chemistry, 278, pp. 795–804.
- [23] OECD-420, 2011. Guideline for testing of chemicals: acute oral toxicity – fixed dose procedure. p. 14.
- [24] Onivogui, G., Zhang, H., Mlyuka, E., Diaby, M. and Song, Y., 2014. Chemical composition, nutritional properties and antioxidant activity of monkey apple (*Anisophyllea laurina* R. Br. ex Sabine). Journal of Food and Nutrition Research, 2, pp. 281–287.
- [25] ONU, 2016. Rapport sur les objectifs de développement durable. New York.
- [26] Recker, R.R., Hinders, S., Davies, K.M., Heaney, R.P., Stegman, M.R., Lappe, J.M. and Kimmel, D.B., 1996. Correcting calcium nutritional deficiency prevents spine fractures in elderly women. Journal of bone and mineral research, 11, pp. 1961–1966.
- [27] Roy, T., Banerjee, G., Dan, S.K., Ghosh, P. and Ray, A.K., 2014. Improvement of nutritive value of sesame oilseed meal in formulated diets for rohu, *Labeo rohita* (Hamilton), fingerlings after fermentation with two phytase-producing bacterial strains isolated from fish gut. Aquaculture International, 22, pp. 633–652.
- [28] Sabikhi, L. and Kumar, M. H. S., 2012. Chapter four – Fatty acid profile of unconventional oilseeds In J. Henry (ed.) Advances in Food and Nutrition Research. Cambridge: Academic, pp. 141–184.
- [29] Scheel, K., 1936. Colorimetric determination of phosphoric acid in fertilizers with the Pulfrich photometer. Z Anal Chem., 105, pp. 256–269.
- [30] Slominski, B.A., Jia, W., Rogiewicz, A., Nyachoti, C.M. and Hickling, D., 2012. Low-fiber canola. Part 1. Chemical and nutritive composition of the meal. Journal of Agricultural and Food Chemistry, 60, pp. 12225–12230.
- [31] Smartt, J. and Nwokolo, E., 1996. Food and feed from legumes and oilseeds. FAO.
- [32] Van Soest, P. J., Robertson, J. B. and Lewis, B. A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of dairy science, 74, pp. 3583–3597.
- [33] Sundu, B., Kumar, A. and Dingle, J., 2006. Palm kernel meal in broiler diets: Effect on chicken performance and health. World's Poultry Science Journal, 62, pp. 316–325.
- [34] Underwood, E. J., 1999. The mineral nutrition of livestock. Oxon: Cabi.
- [35] Woyengo, T. A., Beltranena, E. and Zijlstra, R. T., 2014. Controlling feed cost by including alternative ingredients into pig diets: A review. Journal of Animal Science, 92, pp. 1293–1305.