INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 17, No. 1, p. 46-56, 2020

RESEARCH PAPER OPEN ACCESS

Ethnobotanical, phytochemical and nutritional characterization of *Bixa Orellana* Linn. seeds of Benin Ecology

Ezéchiel Akakpo[1], Marius Eric Badoussi[1,2], Claude Kouassi Gnacadja[1], Hermance Houngbo[1], Alphonse Dossou[1], Fernand Gbaguidi[3], Paulin Azokpota[1*]

[1]*Laboratoire de Sciences des Aliments (LSA) Faculté des Sciences Agronomiques, Université d'Abomey-Calavi; 03 BP 2819 Jéricho, Cotonou, Bénin*

[2]*Ecole Nationale Supérieure des Biosciences et Biotechnologies Appliquées (ENSBBA), Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), BP: 14 Dassa-Zoumé, Bénin*

[3]*Laboratire National de Pharmacognosie et des Huiles essentielles, Centre Béninois de la Recherche Scientifique et Technique, Porto-Novo, BP 03-1665, Benin*

Key words: Biocolorant, food industries, socio-cultural group, pharmacological properties, Bénin.

http://dx.doi.org/10.12692/ijb/17.1.46-56 Article published on July 17, 2020

Abstract

Bixa orellana seeds, providers of biocolorant, are found in some localities of Benin. However, its local names, its uses such as the phytochemical and nutritional characteristics are not documented. This paper focuses on characterizing the ethnobotanical, phytochemical, and nutritional plan of *Bixa orellana* seeds of Benin ecology. An ethnobotanical investigation was first carried out close to 148 seeds users in two agro-ecological zones. The seeds collected at the investigation time were characterized through their phytochemical screening and evaluation of their nutritional value by reference methods. Various designations are associated to Annatto according to the socio-cultural groups of Benin such as "timati winiwini", "timati kouinon" or "Kpokpo" (Fon and Mahi, in Zou and the Collines); "Kpararou", "Dodokpara", "Kparara" or "Timati borou" (Bariba and Dendi, in the North); "Disonri", "Dipersonri" or "Monsonri" (Ditamari, in the North). These designations refer to its use in sauces as a substitute for tomatoes due to its red color (64.78%). Ripe fruit (10.81%) and seeds powder (8.10%) is used to treat anemia. Phytochemical screening of seeds revealed gallic tannins, flavonoids, anthocyanins, leucoanthocyanins, saponins, triterpenoids, steroids, mucilage, reducing compounds, and C-heterosides. Nutritionally, the seeds contain protein (12.83%), fats (4.64%), Potassium (14.59 mg/g), Sodium (6.93 mg/g), Phosphorus (4.79 mg/g), Calcium (2.89 mg/g), Magnesium (1.04 mg/g), Manganese (0.19 mg/g), Copper (0.19 mg/g), and Iron (0.01 mg/g). In addition to being an excellent source of dyes usable for various purposes, Annatto is a potentially nutritious product. It is therefore of paramount importance in the improvement of the quality of traditional food.

* **Corresponding Author:** Paulin Azokpota ✉ azokpotap@yahoo.fr

Introduction

Global food resources consist of a limited number of species that represent the basis of global nutrition. This globalization of food resources has led to the gradual disappearance of many cultivated species while others remain known only in restricted geographical areas (Djè *et al.,* 2006). Such species occur, especially in tropical regions, where secular beliefs and traditions contribute to the maintenance of several crops designated as minor crops (Djè *et al.,* 2006). This is the case of Annatto (*Bixa orellana* L.) which is a species native to central and south American, but now grown in the tropics (Periyasamy and Kumar, 2016) including Benin (Akoègninou *et al.,* 2006; Fagbohoun, 2014; Akakpo *et al.,* 2019). From a botanical point of view, *Bixa orellana* L. is an evergreen shrub or a small tree, measuring about 3-10 meters in height and belongs to the family of Bixaceae. It is cultivated not only because of its beautiful thorny ornamental flowers and red fruits but also for its socio-economic value (Akakpo *et al.,* 2019). Different parts of the species are used in agro-food as well as for certain treatments, particularly by people in developing countries (Russell *et al.,* 2005; Lans, 2006). Also, the roots and leaves of *Bixa orellana* are used to treat epilepsy, diarrhea, dysentery, fever, jaundice, parasitic diseases, cough and urinary infections, and some gastrointestinal and pulmonary diseases (Giorgi *et al.,* 2013; de Araújo Vilar *et al.,* 2014) in several countries including Brazil, Peru, Philippines, Nigeria, and Côte d'Ivoire (Omonhinmin *et al.,* 2013; de Araújo Vilar *et al.,* 2014). The different medicinal properties of these organs are linked to their phytochemical composition (Akakpo *et al.,* 2019). However, of all the organs of the tree, the red seeds called roucou or rocou (French), annatto (English), and achiote (Spanish) are the most used (Venugopalan *et al.,* 2011). Furthermore, apart from their traditional use as a condiment or seasoning due to their richness in nutrients (Senthil *et al.,* 2007; Akakpo *et al.,* 2019), annatto seeds provide the second natural colorant of economic importance (after caramel) (Venugopalan *et al.,* 2011). Also, the seed extract, rich in tannins, contains a mixture of eight dyes from the carotenoid group. However, the main dyes are bixin and norbixin (Gulrajani *et al.,* 2002; Das *et al.,* 2007). These dyes are used in the textile, cosmetic, and craft industries to dye fibers (basketry), fabrics, and various masks. They are also used in the food industry, particularly in the coloring of ice creams, dairy products, juices and liqueurs, sauce, and cakes (Venugopalan *et al.,* 2011; Fagbohoun, 2014; Akakpo *et al.,* 2019). There are types of *Bixa orellana* with white flowers, but trees with pink flowers are much more common. Although there are many cultivated types, there is no official classification for cultivars (Jansen, 2005). However, despite the presence of the species in Benin, endogenous knowledge related to its uses is not yet documented. Also, the phytochemical and nutritional characteristics of the seeds of the cultivar(s) of *Bixa orellana* found in Benin are not yet known. A directory of information concerning the plant, the cultivar present in Benin localities, the seeds and their virtues, the zones of production and consumption as well as the evaluation of its nutritional compounds is, therefore, necessary for a better valorization of the resource. Thus, this study focuses on characterizing ethnobotanically, nutritionally, and phytochemically the *Bixa orellana* seeds of Beninese ecology.

Materials and methods

This study consisted, firstly, in inventorying endogenous knowledge relating to the use of different parts of the species. Seed samples were collected and characterized through phytochemical screening and evaluation of their nutritional value.

Ethnobotanical characterization of Bixa orellana

The study was conducted among the populations of the townships of NIKKI and N'DALI in agro-ecological zone III and the township of OUESSE in agro-ecological zone V. These different localities were chosen based on the presence of the species and its use by the population made up of several socio-cultural groups. The reasoned choice method as described by Kpètèhoto *et al.* (2017) was used. Thus, the interviewees were selected taking into account their proximity to the identified plants, the presence

of the species in their field, and the frequency of seed harvesting as indicated by some owners of fields/hut gardens with the species. A total of 148 people (102 women and 46 men) were surveyed and distributed as follows: 50 respondents at NIKKI, 44 at N'DALI, and 54 at OUESSE. The information collected concerns the local names of the species, its availability (dry season or rainy season), the different parts of the plant used, the different uses that these parts constitute, and the places of collection of the organs of the plant. The table 1 presents the summary of the survey areas as well as the numbers registered in each locality.

Characterization of Bixa orellana seeds

Sampling plan: Seed samples were taken from three different fields, one field per municipality. At each field, the seeds were extracted from ripe and dried fruit picked from three different trees. The seeds collected from the three trees in the same field were combined in a bottle that was labeled, and this made up a sample. The samples thus collected were kept in the laboratory for the evaluation of the different phytochemicals and physicochemical parameters.

Determination of phytochemical characteristics: The phytochemical compounds were determined through a phytochemical screening. This is a qualitative analysis based on coloring and/or precipitation reactions carried out on dry seeds. These were at first reduced into powder in a mortar using a pestle and then by standard reactions described by Houghton and Raman (1998) and Adjatin *et al,* (2013). The main secondary metabolites were sought using the methods grouped in Table 2.

Evaluation of the nutritional value of Bixa orellana seeds

The sample was analyzed for moisture, crude protein, crude fat, and ash content. Crude protein was determined by using the Kjeldahl method (Nair *et al.*, 2013). The moisture and crude fat were determined according to the procedure of the Association of Official Analytical Chemists (AOAC, 1990). The percentage was calculated based on the dry weight. Ash was determined after incineration in a muffle furnace following Bangash *et al.* (2011). Mineral composition of the samples was determined according to methods recommended by the Association of Official Analytical Chemists (AOAC, 1990) and Badau *et al.* (2013). The samples were incinerated in the oven at a temperature of 550°C for 3 hours. The samples of *Bixa orellana* seeds were each digested using a mixture of concentrated nitric (HNO_3), perchloric ($HClO_4$), and sulphuric (H_2SO_4) acids in the ratio 9:2:1 (v/v) respectively (Nair *et al.*, 2013). Copper (Cu), iron (Fe), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and Manganese (Mn) were determined by Atomic Absorption Spectrophotometer (AAS) (PerkinElmer AAnalyst 700, England). Phosphorus contents of the samples were determined using a Flame photometer as specified in Alinnor and Oze (2011). The concentration of each element in the sample was calculated from the dry matter. The analysis was performed with triplicates for the needs of statistical analysis.

Statistical data analysis methods

The data obtained from the investigation and the physicochemical analysis were entered and processed with the Microsoft Excel spreadsheet. The different local designations as well as the uses of the various organs of the species have been grouped in the form of a table and figure. The different physicochemical parameters evaluated as a function of the sample collection area were compared using an analysis of variance. This was followed by a Fisher LDS test at the 5% threshold with STATISTICA 7.1 software.

Results and discussion

Ethnobotanical characteristics of Bixa orellana

In the areas surveyed, there is only one type of *Bixa orellana*. This is the type with pink flowers that produces fruit during the rainy season (April-May) and the dry season (November-December). However, only the production of fruit during the dry season is often harvested because the seeds of this season do not rot under the effect of rainwater. Local people traditionally classify name, and group the species of

plants they use withthe introduction, ecology, morphology, and technological traits (Dansi *et al.,* 2008). Although the local names for *Bixa orellana* vary from one socio-cultural group to another (Table 3), the majority of the names given to the species relate to its technological traits.

This means that the names "timati winiwini" or "timati kouinon" given by Fon and Mahi or "Timati borou" by Bariba and Dendi or "Timati atchidjitô" by Adja brings out the use of seeds as tomato for various food preparations (64.18% of respondents) (Fig. 1). Similarly, "tomate mougou" in Côte d'Ivoire and "tomati enshee" in Burkina-Faso which relate to the use of seeds as tomato had been reported (Akakpo *et al.,* 2019). On the other hand, the seeds are used in food preparations, either crushed alone and directly added to the sauce, or crushed in a mixture with cornflour to use as tomato powder. Also, the name Kpokpo given by Fon to the leaf sheath of Sorghum bicolor to bring out its coloring power is given to the seeds of *Bixa orellana*. *Bixa orellana* seeds, like leaf sheaths, could be used to color traditional foods such as local cheese wagashi. The use of *Bixa orellana* seeds has been reported in dairy technology for the production of butter, ice cream, margarine, and snacks. It is also used in cosmetics (Guiliano *et al.,* 2003; Akakpo *et al.,* 2019).

Table 1. Distribution of respondents by sex and locality.

Communes	Villages/Districts	Sex		Number of respondents
		Men	Women	
Ouessè	Odougba	9	21	30
	Dokoundoho	8	16	24
Nikki	Danri	9	18	27
	Gourou	6	17	23
N'Dali	Travo	8	11	19
	Ouénou	6	19	25
Total	-	46	102	148

The seeds of *Bixa orellana* are variously used within prospecting zones. Fig. 1 shows some uses of the various organs of the species of Beninese ecology.According to Fig. 1 which illustrates the proportions of the use of *Bixa orellana* organs in the study areas, it appears that the organs with great use in these zones are the dry seeds in food followed by the leaves and roots, ripe fruits, and dry seeds for medicinal uses.

Table 2. Metabolites sought and research methods.

Test N°	Secondary metabolites sought	Methods used
1	Alkaloids	Mayer's test
2	Quinone derivatives	Borntrager's test
3	Catechic tannins	Stiasny test
4	Gallic tannins	Ferric chloride test after saturation with sodium acetate
5	Flavonoids	Shinoda test and magnesium powder
6	Cyanogenic derivatives	Picric acid test
7	Triterpenoids	Acetic acid test + mixture of acetic anhydride-sulfuric acid
8	Steroids	Kedde reaction
9	Saponins	test index foam
10	Cardiac glycosides	Raymond Marthoud reaction
11	Anthocyanins	Test with hydrochloric acid and ammonia diluted to half
12	Leucoanthocyanes	Shinoda test
13	Mucilages	Test of absolute alcohol
14	Reducing compounds	Test with Fehling's solution
15	Coumarin	Test with ether and ammonia
16	Free anthracene derivatives	Test with chloroform and ammonia
17	Combined anthracene derivatives	Test with chloroform and ammonia

Apart from their use as an ingredient for various food preparations, dry seeds are sometimes powdered and incorporated into the porridge to relieve people suffering from anemia, particularly children (8.10%). Non-food uses of seeds have also been identified. The seeds are also used not only in convents for the adornment of the followers of certain endogenous religions during different ceremonies but also for occult purposes. Also, the use of dye from seeds to color traditional masks has been reported (Fagbohoun, 2014). Other parts of the tree, especially the decoction leaves and roots are used to treat stomach aches, diarrhea, and urinary infections (14.86% of respondents). Similar uses of these organs have been reported in the Brazilian Pharmacopoeia (Venugopalan *et al.,* 2011; Akakpo *et al.,* 2019). Undried ripe fruit is used as a decoction in children to relieve anemia problems (10.81% of respondents).

Table 3. Some local appellations of *Bixa orellana* according to socio-cultural groups.

Socio-cultural groups	Local names	Meaning
Mahi/Fon	Timati winiwini,	Small tomato
	Timati kouinon	Seeds tomato
	Kpokpo	-
Bariba/Dendi	Pkararou, Dodokpara, Kparara	-
	Timati borou	Powdered Tomato
Ditamari	Risonri, Disonri, Dipersonri ou Monsonri	-
Ayizô	Sokplè	-
Kotafon	Ebiahou dékou	Which is redder than palm oil
Adja	Yovogbo, Yovogbo atchidjitô	Tomato from tree

Phytochemical Profile of Bixa Orellana Seeds

Phytochemical screening revealed the presence of gallic tannins, flavonoids, anthocyanins and leucoanthocyanins, saponins, triterpenoids, steroids, mucilage, reducing compounds, and C-heterosides (Table 4). According to Table 4, it appears that *Bixa orellana* seeds samples have the same large groups of chemical compounds.

Table 4. Secondary metabolites examined and detected in the *Bixa orellana* seeds powder.

Chemical compounds	E1(OUESSE)	E2 (NIKKI)	E3 (N'DALI)
Alkaloids	-	-	-
Polyphenol compounds			
• Cathetic tannins	-	-	-
• Gallic tannins	+	+	+
• Flavonoids	+	+	+
• Anthocyanins	+	+	+
• Leucoanthocyanins	+	+	+
Quinone derivatives	-	-	-
Saponins	+	+	+
Triterpenoids	+	+	+
Steroïds	+	+	+
Cyanogenic derivatives	-	-	-
Mucilages	+	+	+
Coumarins	-	-	-
Reducing compound	+	+	+
Anthracene derivatives	+	+	+
• Free anthracene derivatives	-	-	-
• Combined anthracene derivatives O-heterosids	-	-	-
• Combined anthracene derivatives C-heterosides	+	+	+
Cardiac glycosides	-	-	-

(+): Presence; (-): Absence.

A study carried out by Fleisher *et al.* (2003) had revealed, in the *Bixa orellana* seeds, the presence of flavonoids, saponins, and reducing compounds. It also revealed the absence of alkaloids, coumarins, free anthracenes, and quinone derivatives as shown by the present study. On the other hand, the study carried out by Prathima *et al.* (2016) identified in *Bixa orellana* seeds the presence of alkaloids and Cardiac glycosides. These differences observed could be related to the origin of the different seeds used.

It also recognized flavonoids, antiviral, antitumor, anti-inflammatory drugs, anti-allergic, and anti-cancer activities (Morel, 2011). Tannins enable the reduction of the biodisponibility of iron and other micronutrients and present an antibacterial activity from weak to moderate. Besides, the bacteria as an anticryptococcus activity (yeasts), antiviral, anti-inflammatory, anti-hypertensive, antimutagenic, immuno-stimulative, antitumor, and anti-diarrheal are relatively high (Feldman *et al.,* 1999).

Table 5. Content of organic compounds in *Bixa orellana* seeds.

	Seeds' origins		
Parameters	E1(OUESSE)	E2 (NIKKI)	E3 (N'DALI)
Moisture (%)	2.7± 0.1 (a)	2.7± 0.1(a)	2.8±0.1(a)
Crude protein (%)	12.5±0.2(a)	13.0±0.1(b)	13.0±0.2(b)
Crude lipid (%)	4.3±0.1(a)	4.9±0.1(b)	4.6±0,1(b)

The seeds also contain steroids and anthocyanins. These compounds could, therefore, be responsible for the antimicrobial activity of *Bixa orellana* seeds against *Staphylococcus aureus*, *Baccilus Cereus,* and *Escherichia coli* (Abhishek *et al.,* 2010). Thus, the presence of gallic tannins, flavonoids, anthocyanins and leucoanthocyans, saponosides, triterpenoids, steroids, mucilage, reducing compounds, and C-heterosides could explain the use of seeds in the traditional pharmacopoeia for various medicinal treatments (Akakpo *et al.,* 2019) as a purgative and against oral tumors (Fleischer *et al.,* 2003). The seeds have anti-leishmanial, anticonvulsant, antidiabetic, and cardio-protective properties (Viuda-Martos *et al.,* 2012; Akakpo *et al.,* 2019). Furthermore, the absence of toxic compounds such as quinone derivatives, cardiac glycoside, and cyanogenic derivatives in the three samples studied could explain to some extent the consumption of these seeds without risk of food poisoning. The studies of toxicity indicated that *Bixa orellana* did not produce toxic effects in rats or mice if it is administered orally (26 mg/day for rats, and a drop of 10% in soya oil/day for mice). Its consumption is thus without risk for the human food (de Paula *et al.,* 2009). Therefore, consumption is safe for human food.

Table 6. Ash content (g / 100g DM), and individual minerals of the three seed samples.

	Seeds' origins				
Composition	E1 (OUESSE)	E2 (NIKKI)	E3 (N'DALI)	Mean	CV (%)
Ash (g/100g)	6,2±0,07(a)	5,1±0,1(b)	5,2±0,1(b)	5,5	11,06
individuals Mineral (mg/g)					
Potassium (K)	18,17	13,02	12,59	14,59	0,21
Sodium (Na)	9,66	5,64	5,49	6,93	0,34
Phosphorus (P)	6,15	4,11	4,09	4,78	0,25
Calcium (Ca)	3,92	2,43	2,33	2,89	0,31
Magnesium (Mg)	1,39	0,94	0,79	1,04	0,29
Manganese (Mn)	0,24	0,17	0,17	0,19	0,21
Copper (Cu)	0,20	0,18	0,18	0,19	0,48
Iron (Fe)	0,02	0,01	0,01	0,01	0,01

DM = Dry Matter.

Also, the research results of Paumgartten *et al.* (2002) showed that the extract of *Bixa orellana* is neither maternally toxic nor embryotoxic. Shilpi *et al.* (2006) reported that *Bixa orellana* does not have any mortal effect 24 hours after the administration of its extract, even to the highest amount (4.000 mg/kg) for mice. According to Rajib *et al.* (2009), the extracts of *Bixa orellana* have a very high hepatoprotective activity.

Nutritional characteristics of Bixa orellana seeds

Table 5 shows the water, crude protein, and total lipid content of the *Bixa orellana* seed samples. The water content of the *Bixa orellana* seed samples is 2.7% and does not reveal any significant difference between the different samples (Table 5). This value is lower than those reported by Valério *et al.* (2015) and Dike *et al.* (2016) which are 6.74% and 4.89%, respectively. The low water content recorded for these seeds could allow their conservation for a relatively long period without altering their nutritional qualities. Thus, the high humidity generates a greater activity of water-soluble enzymes and coenzymes required for metabolic activities (Badau *et al.,* 2013).

The protein content of *Bixa orellana* seeds varies from 12.5 ± 0.2 to 13.0 ± 0.2% with a significant difference between the samples from the Municipality of Ouèssè (agro-ecological zone V) and the Communes of N'dali and Nikki (agro-ecological zone III). This same observation was noted at the level of lipid content which varies from 4.3 ± 0.1 to 4.9 ± 0.1% (Table 5). These recorded differences could be linked to the effects of agro-ecological zones.

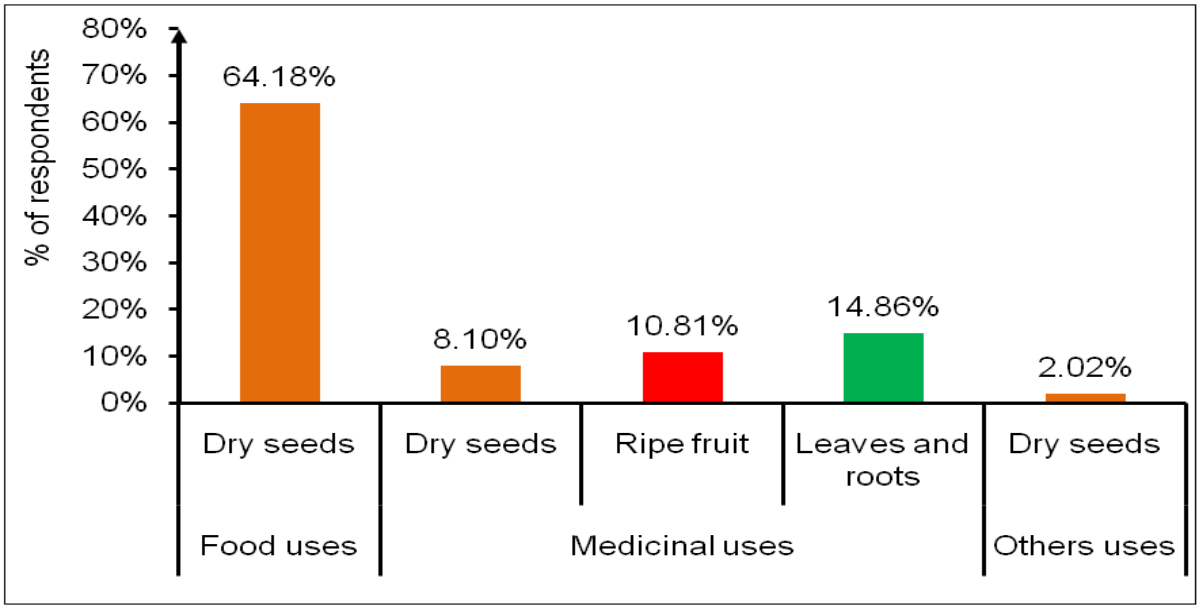

Fig. 1. Different uses of *Bixa orellana* organs identified in the study areas.

The photosynthetic reactions responsible for the production of organic compounds in plant organs are subject to sunshine, which is not the same in the different survey areas. Therefore, it can differentiate the content of organic compounds such as proteins and lipids in plant organs (Le Gall *et al.,* 2015). Also, a variation in the biochemical composition of different parts (leaves, pulp, and seeds) of baobab of Beninese ecology has already been observed depending on the area of origin of the samples (Assogbadjo *et al.,* 2012). Furthermore, the difference observed could presage the probable existence of two cultivars. The protein contents obtained are higher than those obtained by Valério *et al,* (2015), which is 11.5%. This is, however, similar to the value of 12.55% reported by Dike *et al,* (2016). The lipid contents are found in the range of reported values which varied from 2.23 to 7.20% (Valério *et al.,* 2015; Dike *et al.,* 2016; Akakpo *et al.,* 2019). The seeds of *Bixa orellana*, therefore, contain significant amounts of protein and lipid which are organic compounds that are beneficial for the body of consumers.

The ash content of Ouessè sample is higher than that of Nikki and N'dali samples (Table 6). This difference could be linked to the types of soil in the areas where the samples come from. Ouessè is in an agro-ecological zone different from that of Nikki and N'dali. According to INSAE (2016), tropical ferruginous soils are dominant in the township of N'Dali where they are deep, not concreted, and subjected to leaching. Ferralitic soils are found in the township of Nikki while the township of Ouessè is characterized by tropical ferruginous soils on a crystalline base and colluvial soils. The differences observed could, therefore, be linked in particular to the mineral content of these soils.

However, the values obtained are in the range of values reported in the literature which varies from 5.05% to 6.32% (Senthil *et al.,* 2007; Valério *et al.,* 2015; Dike *et al.,* 2016). The ash content is a reflection of the mineral content in food products. Therefore, *Bixa orellana* seeds constitute a significant source of mineral elements which are important components of diets because of their physiological and metabolic function in the body (Adjatin *et al.,* 2013). The average calcium content of *Bixa orellana* seed samples is 2.89 mg. Foods colored with the extract of these seeds can be considered as a good source of calcium because the dye brings its calcium supplement to the food it colored. Magnesium was determined and evaluated at 1.04 mg/g for the seed samples analyzed. This value is found in the range of the values reported by Akakpo *et al.* (2019).

The manganese content of *Bixa orellana* seeds was evaluated at 0.19 mg, and this value is lower than 0.25 mg obtained by Senthil (2007). Copper is necessary for the body for the production of enzymes and the biological transport of electrons (Alinnor and Oze, 2011). The copper content, of the dry weight of the seeds, was 0.19 mg. The iron content of *Bixa orellana* seeds found in this study (0.01 mg/g) is lower than the value (0.03 mg/g) reported by Akakpo *et al.* (2019). These seeds can be considered as a significant source of mineral elements for various food formulations.

Conclusion

The local designations of *Bixa orellana* in the regions explored vary from one socio-cultural group to another and sometimes within the same socio-cultural group. These seeds used mainly in the food sector are potential sources of proteins and mineral salts. They also contain certain active phytochemical conferring on the seeds and certain therapeutic properties which are usable in the treatment of certain human pathologies. These chemical compounds in *Bixa orellana* seeds vary from one agro-ecological zone to another. Because of these different results, it, therefore, becomes necessary to promote this species as a sector through the promotion of its culture and the development of its value chain.

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