

Characterization of the seed of *Myrianthus holstii* Engl. from Burundi: chemical composition of oil and oilcake

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Abstract

In order to valorize the *Myrianthus holstii* species and assess its potential contribution to improving food security in Burundi, a study was conducted on the chemical composition of their seeds. After the extraction of oils with soxhlet, both the oil and oilcake were subsequently analyzed. Then after the analysis of the physicochemical properties of the lipid fraction, fatty acids and phytosterols were identified using chromatography gas. In the oilcakes, the content of total sugars, proteins and polyphenols were also determined. The seed of *M. holstii* was found to be with higher oil content (48.34%). Among nine fatty acids were identified, linoleic acid was exceptionally represented at 85.55%. The phytosterol content obtained was estimated at 3629.37 ± 40.06 mg / kg of oil. The three dominant phytosterols among the nine identified were β -sitosterol, sitostanol, and campesterol (3173.60 ± 36.20 , 311.91 ± 5.38 , and 63.69 ± 0.11 mg / kg of oil respectively). The oilcake analysis indicated that the seed of *M. holstii* contains a great amount of total sugars, proteins and polyphenols. In the light of these results, oil of *M. holstii* seeds could be used in the food and cosmetic industries. In addition, the oilcakes could be used in animal feeding and they can also be used in the food as an antioxidant factor and pharmaceutical domain thanks to their polyphenol content.

Keywords: *Myrianthus holstii*, oils, phytosterols, fatty acids, proteins, Burundi

Résumé

Dans l'objectif de valoriser l'espèce *Myrianthus holstii* et d'évaluer sa potentielle contribution dans l'amélioration de la sécurité alimentaire, une étude a été réalisée au Burundi sur la composition chimique de leurs semences. Après l'extraction des huiles au soxhlet, on a ensuite analysé chacun de l'huile et du tourteau. Après la détermination des propriétés physico-chimiques de la fraction lipidique, les acides gras et les phytostérols ont été identifiés par chromatographie en phase gazeuse. Dans les tourteaux, les teneurs en sucres totaux, protéines et polyphénols ont également été déterminées. La graine de *M. holstii* s'est avérée avoir une teneur en huile très élevée (48.34%). Parmi neuf acides gras identifiés, l'acide linoléique était exceptionnellement représenté à 85,55%. La teneur en phytostérols obtenue a été estimée à $3629,37 \pm 40,06$ mg/kg d'huile. Trois phytostérols dominants parmi les neuf identifiés étaient le β -sitostérol, le sitostanol et le campesterol ($3173,60 \pm 36,20$, $311,91 \pm 5,38$ et $63,69 \pm 0,11$ mg/kg d'huile respectivement). L'analyse des tourteaux a montré que la graine de *M. holstii* contient des quantités importantes de sucres totaux, de protéines et de polyphénols. A la lumière de ces résultats, l'huile de graines de *M. holstii* pourrait être utilisée dans les industries alimentaires et cosmétiques. De plus, les tourteaux pourraient être utilisés dans l'alimentation du bétail et ils peuvent également être utilisés dans le domaine alimentaire comme facteur antioxydant et pharmaceutique grâce à leur teneur en polyphénols.

Keywords: *Myrianthus holstii*, huiles, phytostérols, acides gras, protéines, Burundi

1. Introduction

Healthy diet contributes to productivity, economic development as it reduce poverty by improving physical work capacity, cognitive development, school performance and health; therefore, by reducing diseases and mortality. Unfortunately, in sub-Saharan Africa there is a high prevalence of malnutrition (Magadi, 2015; Grudziak et al., 2017). This region accounts for 64% of global mortality in the 0-4 age group and 78% of the mortality in the 5-14 age group (Albertyn et al., 2006; Forjuoh, 2006; Grudziak et al., 2017; Nthumba, 2016). However, such situation differs from country to country. In Burundi, calorie intake is the lowest in the East African region with an average of 1,834 calories per person per day (DSIA and ISTEEBU, 2018). However, Burundi contains an immense rich indigenous flora that could be used for production increasing and food diversification. *Myrianthus holstii* Engl. (vernacular name: amwufe) is one of the 3,125 species of higher plants that Burundi records (MEEATU, 2013). It is a shrub that belongs to the Cecropiaceae family. It is found in submontane and in the forest mountains of central and southern Africa (Kissa & Sheil, 2012). The fleshy and edible mesocarp surrounding a seed protected by a hard endocarp, was among the main foods at the time when Burundians lived by hunting and gathering. The shrub is also used as firewood but does not exhibit timber properties. The young leaves and fruits are very appreciated by frugivores such as primates (Kissa & Sheil, 2012). In Burundi, *M. holstii* Engl. is widely distributed on the high mountains of the Congo Nile ridge. It grows naturally in forests and forest galleries that have not yet been disturbed, including Kibira National Park, Vyanda Nature Reserve and Bururi Nature Reserve. They can also be found on farms where they were deliberately left, after forest clearing, to produce stakes for twining beans.

There are not enough researchers have worked on *M. holstii* Engl. However, *Myrianthus arboreus* species, which shares the genus with *M. holstii* Engl, and having perfect morphological resemblances, this has been more examined on several parameters. As *M. arboreus* is concerned, its leaves chemical composition has been attentively analyzed (Otitoju et al., 2016) which are also edible, seeds (Niyukuri et al., 2019, 2020) and bark (Kasangana et al., 2015). Very few biochemical studies were realized on *M. holstii* and reported on its nutritional potential (Omuja et al., 2019) and fatty acid composition (Minzangi et al., 2011). Extracts from its parts of the vegetative apparatus exhibited a lot of biological activity (Agyare, 2014). The present work was carried out with the overall objective of characterizing the chemical composition of the seed of *M. holstii* Engl from Burundi. The specific objectives were to identify fatty acids and phytosterols in oil and to determine the total sugar, protein and polyphenols contents in oilcake. Extraction of the oil from the seeds made it possible to characterize the lipid fraction and the chemistry composition of the oilcakes. This study highlighted the possibilities of using the seeds of *M. holstii* Engl. in the fields of food, cosmetics and livestock breeding.

2. Material and Methods

2.1. Plant material

The fruits of *M. holstii* were sampled in the period from August to October 2020, which corresponds to the ripening

period. All the seeds were collected in the high mountains in Burundi (1500-2600 m) possessing the optimal ecological conditions of this plant. The sampling sites were located in Kibira National Park, Vyanda Nature Reserve and Bururi Nature Reserve. Manual harvests were carried out on 9 feet of each site. The identification of the plant species was performed at the herbarium of the University of Burundi (BJA) and the herbarium of the Burundian Office for the Protection of the Environment. The fruits are inserted on a receptacle in the order of 22 to 33. The seeds were separated from the pericarps and dried at room temperature under the shelter of the sun in the Microbiological Analysis and Food Science Laboratories.

2.2. Oil extraction

The oil extraction was performed from the powder of the seeds well dried with hexane as solvent in Soxhlet apparatus under reflux for 8 hours. The extraction led to two materials, oils and oilcakes, on which analyzes were made.

2.3. Analysis of the lipid fraction

2.3.1. Physicochemical properties

Three parameters namely, acidity, peroxide index and the unsaponifiable fraction were determined. The acidity was determined according to the method of ISO 660:2009 (ISO, 2009) norms and expressed in g of oleic acid per 100 g of oil (g OA /100 g). The peroxide value was analyzed according to the methodology described by ISO 3960:2007 (ISO, 2007) and it was expressed in milliequivalents of active oxygen (meq O₂)/kg of oil. The extraction of the unsaponifiable material was carried out as described by ISO 18609:2000 (ISO, 2000) and it was expressed in g of extract/100 g of oil.

2.3.2. Fatty acid analysis

Fatty acid methyl esters (FAMES) were prepared in two ways according to the oil acidity. Oils with an acidity < 3,3% were methylated following alkaline conditions as described by Bannon et al. (1982), while oils with a free fatty acid content ≥ 3,3% were performed as described by Jham et al. (1982). A Shimadzu GC-2010 Plus equipped with a flame ionization detector and capillary column (30 m × 0.25 mm; film thickness 0.25 μm) was used. Parameters were column temperature, 180°C; injector temperature, 225°C; detector temperature, 250°C; injection volume, 1 μl; carrier gas, nitrogen; and flow rate, 1 ml/min. Total running time was 20 min. Identification of individual fatty acids was performed by comparing their retention times with a certified fatty acid methyl ester mix and quantified as percentage of total fatty acids.

2.3.3. Sterols analysis

The extraction of the unsaponifiable material was carried out as described by ISO 18,609:2000 (ISO, 2000). An internal standard, 5α-cholestane, was used for sterols quantification. The sterol fraction from the unsaponifiable matter was purified by thin-layer chromatography (TLC) on 20 × 20 cm silica gel, 0.25 mm thickness of layer using hexane/ diethyl ether (1/1:v/v). The extracted sterol fraction from the TLC has been subjected to silylation. Thus, trimethylsilyl (TMS) ether derivatives of sterols were prepared as described by Savage et al. (1997) for further analyses in a Shimadzu GC-2010 Plus equipped with a flame ionization detector and column SUPELCO SAC-5 (30 m × 0.25 mm × 0.25 μm). A sample of 1.0 μl was injected in a split mode.

The column was held at isocratic temperature of 280°C during 35 min. The detector temperature was set at 300°C. Hydrogen was used as a carrier gas at a flow rate of 0.35 ml/min. Sterols were identified by comparing their retention times relative to 5 α -cholestane, and results were expressed as mg/kg of oil.

2.4. The oilcakes chemical composition analysis

2.4.1. Organic compounds

The first step was the extraction of polar compounds from the oilcake. Ten grams of oilcake were mixed with ethanol (80%) and homogenized for 30 minutes on a magnetic stirrer. The separation was done by centrifugation at 4000 rpm for 20 minutes. The ethanol extract was recovered and the pellet was re-extracted for two times. The three kinds of ethanol extracted were mixed and evaporated to dryness under reduced pressure (vacuum). Thus, the oilcake extracts were ready for determination of Total Sugar (TSC), Total Protein (TPRC), Total Polyphenols (TPHC), Total Flavonoids (TFC).

TSC was determined using (Dubois et al., 1951) method. The results were obtained by reference to a glucose standard range made under the same conditions as the samples and expressed as g glucose equivalent / 100 g dry matter of oilcakes (% of DMOC). The TPRC was estimated colorimetrically using the method of (Bannon et al., 1982) Bradford (1976). The results were expressed as mg Bovine Serum Albumin equivalent per 100 g dry matter of oilcakes (% of DMOC). The TPHC of the samples was determined using Folin-Ciocalteu colorimetric method as described by (Slinkard and Singleton, 1977). The results were expressed as mg gallic acid equivalent per 100 g dry matter of oilcakes (mg GAE/100 g DMOC).

2.4.2. Statistical analysis

Data analysis was performed using IBM SPSS statistic 20. Results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test. Correlation between various parameters was also computed. Significance was determined at $p < .05$ level, and the results were expressed as mean values \pm standard error (SE).

3. Results and discussion

3.1. Characterization of *M.holstii* oil

3.1.1. Oil content

The study of the lipid fraction (Table 1) revealed that the oil content from the seeds of *M. holstii* was overall 48.34% \pm 5.65. As there are not enough researches that have been realized on *M. Holstii* the Burundi forest, some parameters studied in this present investigation were compared also with those of *Myrianthus arboreus* sufficiently studied. Thus, this oil content was found to be comparable to that of the seeds of *M. arboreus* (48.26% \pm 5.96) performed by (Niyukuri et al., 2019). However, significant differences ($p < 0.05$) in oil content were observed in between sites; order being Bururi Nature Reserve (BNR) > Vyanda Nature Reserve (VNR) > Kibira National Park (KNP). Among the parameters that could justify this difference, we can cite the microclimate. The accumulation of lipids depends on sugars (Fan et al., 2014) which in turn depend on light intensity. The oil content in the seeds of *M. holstii* was found to be higher than that of some conventionally exploited oils. Sunflower is reported to

be 37% (Pérez-Vich et al., 1998) while the rapeseed is 50% (Ara et al., 2015). In light of the data for this parameter of oil content, the high altitude region that covers the Congo Nile ridge can produce *M. Holstii* oil.

3.1.2. Physicochemical properties

The triglyceride content for good oil quality should be greater than or equal to 95%. However, enzymes from the seeds (Beisson et al., 2001) and the extraction processes hydrolyze fatty acids from triglycerides. Acidity is one of the parameters, which results in the number of fatty acids hydrolyzed from the triglyceride. The acidity of *M. holstii* oil was found to be 0.69 \pm 0.46 g OA / 100 g (Table 1). Seed oils from different sites recorded significant differences ($p > 0.05$) of acidity, thus the order is like VNR > KNP > BNR. Nevertheless, is much lower than 1.8 mg AO / 100 determined in *M. arboreus* oils (Niyukuri et al., 2019). Furthermore, it is slightly higher than that fixed by ISO 660:2009 (E) norms (ISO, 2009) on extra virgin olive oil (\leq 0.343 g / 100 g) and comparable to those fixed on sunflower oil (\leq 0.60 g / 100 g) and on coconut oil (\leq 0.830 g / 100 g).

A large number of polyunsaturated fatty acids improve the quality of oils due to its multiple benefic on human body. Nevertheless, the major problem remains their susceptibility to oxidation. Although the latter depends on storage techniques and time, analysis of *M.holstii* oil, just after extraction, revealed very low peroxide value. While CODEX STAN 33-1981 norms (FAO, 2001a) fixed peroxide value less than or equal to 20 meq O₂ / kg on virgin olive oils, that of *M. holstii* oil was qualified to be 5.04 \pm 1.10 meq O₂ / kg (Table 1). However, significant ($p > 0.05$) variations between sites (VNR, BNR, and KNP) were observed. They were respectively 6.19 \pm 0.87 > 5.53 \pm 0.64 > 3.39 \pm 0.29.

The unsaponifiable material is also a parameter, which indicates the quality. It is a portion, which contains several non-lipidic compounds, the most predominant of which are phytosterols, triterpenes, tocopherols and pigments (Boskou & Morton, 1976). Małecka (1994) reported that the content can vary from 0.5 to 2.5% of oil, with some exceptions, reaching 5 to 6%. In this study, unsaponifiable material content of *M.holstii* was estimated to be 1.51 \pm 0.87g of extract/100 g of oil (Table 1). The results for the three forests showed significant differences at $p > 0.05$: VNR > BNR > KNP.

The results of the physicochemical parameters studied are equivalent or are even better than those of conventional edible oils. Thus, our findings indicated that, if other parameters are ignored, the oils of *M.holstii* could be used as edible oils.

3.1.3. Chemical profile

Fatty acids

The results on fatty acids are depicted in Table 1. In 11 compounds found, 9 fatty acids (C14 : 0, myristic acid ; C16 : 0, palmitic; C16 : 1n-7, palmitoleic; C18 : 0, stearic; C18 : 1n-9, oleic; C18 : 2n-6, linoleic; C20 : 0, arachidic; C18 : 3n-3, linolenic; C24 : 1, selacholeic) were identified.

Therefore, the fatty acid profile of *M. holstii* is almost similar to that of *M. arboreus*. In *M. holstii*, the saturated fatty acids content were 2.9%, monounsaturated fatty acids content were 8.1% and polyunsaturated fatty acids content were 86.13 % while in *M.*

arboreus they were found to be 2.5, 6.9, and 89.77% respectively (Niyukuri et al., 2020).

Table 1: Oil contents, physicochemical properties and chemical composition (KNP, Kibira National Park; VNR, Vyanda Nature Reserve; BNR, Bururi Nature Reserve and X, average).

	KNP	VNR	BNR	X
Oil content (%)	42.23±5.20^b	45.98±8.01^b	56.82±5.14^a	48.34±5.65
Acidity (g OA /100 g)	0.54±0.03 ^b	1.38±0.15 ^a	0.14±0.01 ^b	0.69±0.46
Peroxide value (meq O ₂ /kg of oil)	3.39±0.29 ^b	6.19±0.87 ^a	5.53±0.64 ^a	5.04±1.10
Unsaponifiable material (g of extract/100 g of oil)	2.82±0.07 ^a	1.02±0.14 ^a	0.70±0.07 ^b	1.51±0.87
Fatty acid (%)				
Ind1	0.06±0.00	0.07±0.00	0.07±0.00	0.07±0.00
C14 : 0	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00
Ind2	0.06±0.00	0.06±0.00	0.06±0.00	0.06±0.00
C16 : 0	2.70±0.29	2.98±0.84	2.85±0.24	2.84±0.10
C16 : 1	0.21±0.07	0.16±0.07	0.19±0.07	0.19±0.02
C18 : 0	2.61±0.64	2.27±0.54	2.24±0.14	2.37±0.16
C18 : 1	7.57±0.54	7.16±0.09	8.50±0.34	7.74±0.50
C18 : 2	85.92±0.92	84.67±5.05	86.07±7.71	85.55±0.59
C20 : 0	0.08±0.00	0.07±0.00	0.07±0.00	0.07±0.00
C18 : 3	0.21±0.00	0.19±0.04	0.23±0.08	0.21±0.01
C24 : 1	0.22±0.05	0.17±0.07	0.20±0.01	0.20±0.02
SFA	2.84±0.02	3.12±0.74	2.99±0.40	2.98±0.10
MUFA	8.00±0.87	7.49±0.34	8.89±0.47	8.12±0.51
PUFA	86.13±8.01	84.86±5.55	86.29±8.10	85.76±0.60
PUFA/SFA ratio	30.35±2.57	27.20±2.90	28.87±6.02	28.81±1.07
Phytosterol contnt (mg/kg of oil)	3321,02±245,62	3602,36±132,62	3596,29±66,97	3629,37±40,06
Cholesterol	1.52±0.94	1.16±2.33	1.04±1.04	1.24±0.18
Campesterol	63.52±3.81	63.75±8.41	63.81±5.20	63.69±0.11
Stigmasterol	0.33±0.07	0.36±0.05	0.36±0.01	0.35±0.01
Δ-7-campesterol	8.25±0.56	9.99±0.01	8.55±0.47	8.93±0.71
Clerosterol	30.52±9.45	33.86±6.40	35.26±8.08	33.22±1.79
β-sitosterol	3227.90±72.32	3148.43±100.35	3144.48±58.14	3173.60±36.20
Sitostanol	319.97±9.07	309.74±10.01	306.01±4.85	311.91±5.38
Δ-5-24-stigmastadienol	19.19±0.78	17.07±2.44	17.71±5.21	17.99±0.80
Δ-7-avenasterol	18.26±1.24	18.00±2.37	19.07±2.5	18.44±0.42

M. holstii oil was characterized by high contents especially in linoleic acid (85.55%±0.59) while Minzangi et al (2011) found 80.2% of see from Kahuzi-Biega National Park and surroundings. This content gives it all the properties of being a linoleic oil. These contents are slightly lower than those found in *M. arboreus* (89.54%) (Niyukuri et al., 2020). This indicated that the oil of *M. holstii* could be used in several domains. Linoleic sunflower oil (65 % of linoleic), is used as biofuel and paint while soybean oil which contains 55% is used as biofuel, paints, inks, lubricants, mold release agent,

anti-dust agent and phytosanitary (Merrien et al., 2012). However, this oil cannot be used directly in human nutrition. An adult person requires lipids which can provide 15.1%; 12.4% and 4.0% of total calories intake of saturated, monounsaturated and polyunsaturated fatty acids, respectively (Razanamahefa et al., 2005). Furthermore, the required calorie intake for linoleic acids is 4% of the total energy requirements. In light of these results, the 38% caloric intake provided by total lipids would be provided by linoleic fatty acids alone. This indicated that *M. holstii* oil could be used to improve other oils low in saturated fatty acids.

We would cite the palm oil which is very rich in saturated fatty acids. The palm oil is the only one type of oil produced in Burundi and it is consumed by over 90% of the population. Regarding the ecogeographic influences, no significant difference ($p > 0.05$) was observed in the contents of the respective fatty acids from the different sites. This could not be explained by the fact that these seeds were collected in the same natural region of high mountains characterized but the microenvironment failed to change the chemical composition.

Phytosterol

Through this study, 9 compounds (cholesterol, campesterol, stigmasterol, Δ -7-campesterol, clerosterol, β -sitostanol, sitostanol, Δ -5-24-stigmastadienol, Δ -7-avenasterol) were identified (Table 1) while it can be found globally over 250 phytosterols from different plants (Yoshida & Niki, 2003). The total phytosterol contents of *M. holstii* oil have been found to be higher (3629.37 mg / kg) than those from some conventional oils such as extra-virgin olive oil which has 1865.14 mg / kg of oil (Rocco & Fanali, 2009) and sunflower oil which contains 2861 mg / kg of oil (Hassanien, 2012). However, it was found to be far lower than the phytosterol

content from rapeseed (13,260 mg / kg oil), corn (15,320 mg / kg oil) and evening primrose (21,940 mg / kg oil) oils (Phillips et al., 2002). About the phytosterol composition, the first three highest contents were β -sitosterol (3173.60 mg / kg of oil), followed by the sitostanol (311.91 mg / kg of oil) and lastly the campesterol (63.69 mg / kg of oil). In most other oils, sitostanol is not among the three most abundant, rather it is stigmasterol which occupies this position. Nevertheless, the lowest recorded content was found to be stigmasterol in *M. holstii* oil while Niyukuri et al. (2020) could not report nothing in *M. arboreus* oils. Cholesterol, which is known to have negative health effects if it is consumed in large amounts, as it has a high content (1.24 ± 0.18 mg / kg of oil). This chemical content is slightly lower than those found in extra virgin olive oil, (2.03 mg / kg of oil) (Rocco & Fanali, 2009) and rapeseed, extra virgin (2.6 ± 0.33 mg / kg of oil) (Phillips et al., 2002). However, other phytosterols have several benefic effects on the human body. It has been reported that β -sitosterol has anti-inflammatory and antipyretic activities (Backhouse et al., 1994), exhibited anthelmintic and antimutagenic activities (Villaseñor et al., 2002), and inhibits tumor cell growth (Awad et al., 2007). Sitostanol reduce serum cholesterol by inhibiting its absorption (Miettinen et al., 1995).

Table 2: Extraction yield and chemical composition of the oilcake from *M. holstii* seeds ((KNP, Kibira National Park; VNR, Vyanda Nature Reserve; BNR, Bururi Nature Reserve and X, average).

	KNP	VNR	BNR	X
Extraction yield (%)	10.1 \pm 1.3 ^b	13.5 \pm 1.8 ^{ab}	14.5 \pm 2.1 ^a	12.7 \pm 1.7
Total sugar(g G/100g)	6.27 \pm 0.51 ^b	4.25 \pm 0.97 ^c	8.41 \pm 0.23 ^a	6.71 \pm 0.57
Total protein (g BSA/100g)	10.95 \pm 0.10 ^b	13.41 \pm 5.90 ^a	6.58 \pm 0.11 ^b	10.31 \pm 4.27
Total polyphenols (mg d'Ac Asc/100g)	518.23 \pm 63.50 ^a	214.79 \pm 81.00 ^b	314.70 \pm 44.62 ^b	349.24 \pm 139.50

3.2. Characterization of *M. holstii* oilcakes

3.2.2. Extraction yield

After the oil extraction, this study look into how to value the oilcake. The Table 2 shows the extraction of the polar compounds using 80% ethanol. The results revealed that the three sites had a slight significant difference ($p < 0.05$) among the extraction yields: BNR > VNR > KNP. This may be due to the degree of grinding of the flour used for the solid liquid extraction. Flour consisting of finer particles gives a high extraction yield compared to those of coarse particles. It is from these extracts that the total sugar, total protein, and total polyphenols contents were analyzed.

3.2.2. Chemical composition of oilcakes: total sugar (TSC), total protein (TPrC) and total polyphenols (TPhC) contents

The results of the chemical composition of the *M. holstii* oilcakes are depicted in Table 2. Thus, the analysis of on TSC were found with contents that reached 6.71g G / 100g of Dray Oilcake. These contents are slightly lower compared to those found in the *M. arboreus* (Niyukuri et al., 2020). Significant differences ($p < 0.05$) were noticed between the sites; order being BNR > KNP > VNR. However, we cannot conclude that it was related to the environment effect. It could be due to the handling and especially the grinding. This content (6.71g G /

100g), which is calculated in relation to the oilcake, would become even lower when it is expressed in relation to the dry seed. Since the oil content is very high, the low sugar content is acceptable because oils are synthesized from sugars (Sun et al., 2018).

Regarding the TPrC, the results of the analysis revealed that the contents of 10.31 \pm 4.27 g BSA/100g from *M. holstii* oilcake is economically exploitable. The three sites for research were found to be highly significant ($p < 0.05$) containing total protein contents. The highest contents (13.41 \pm 5.90 g BSA/100g) recorded were reported from the oilcakes of seed from VNR. The lowest contents (6.58 \pm 0.11 g BSA/100g) were reported from the oilcakes extracted from seeds collected in BNR. This supposed that the three sites have undergone eco-geographic influences differently. Protein synthesis depends on photosynthesis and the availability of nitrogen in the soil (Deroche, 1983). Although TPrC was low, it is not much lower compared to the cotton seed oilcake (16%) (Dalle Zotte et al., 2013). Considering that the parameter of toxicity is verified, these results indicated that the oilcake from *M. holstii* seed could be used as source of protein. The valorization of *M. holstii* seed as oilcake could be of great contribution in Burundi, a country where over 90% of the total population is living by agriculture and livestock. Furthermore, throughout the country, only one type of oilcake provided by palm nuts can be found.

Polyphenols are known as powerful antioxidant compounds and are reported to be able to prevent oxidative damage, reduce inflammation (Zhang & Tsao, 2016), and cancer chemo-preventive agents (Stoner & Mukhtar, 1995). Due to its important properties with benefic effects on health, many studies have already been performed, but there is a need to conduct more investigations on this aspect. In the present study, the analysis done on *M.holstii* oilcake had revealed the TPhC which reached 349.24 ± 139.50 mg d'Ac Asc/kg. While TSC and TPrC were characterized by little environmental influence, TPhC recorded strong variations from site to site. The difference was highly significant at $p < 0.05$ among the study sites: KNP (518.23 ± 63.5 mg d'Ac Asc/100g) > BNR (314.70 ± 44.62 mg d'Ac Asc/100g) > VNR (214.79 ± 81.00 mg d'Ac Asc/100g). This shows that the KNP plants were under a lot of stress compared to those at other sites. Polyphenols which are secondary metabolites are synthesized to adapt the plant to the conditions of environmental stress (Zhang & Tsao, 2016). In the light of these results on the TPhCs, the seeds of *M.holstii* could be valued as sources of polyphenols and can also be used in different fields such as in breeding to feed animals, food industries, pharmaceuticals among others.

4. Conclusion

The results of this study show that *M.holstii* contains very high oil content (48.34%). This oil was characterized by a high content of polyunsaturated fatty acids (85.76%). Linoleic acid was very exceptionally high with contents reaching to 85.55%. *M.holstii* oil could be used to improve oils with low content in omega 3 or 6 fatty acids. It could be mixed with palm oil which is very low in unsaturated fatty acids and very high in saturated fatty acids. The oil has also been found with high levels of phytosterols (3629.37 ± 40.06 mg/kg of oil): the most important were β -sitosterol, bitostanol, and campesterol with 3173.60 ± 36.20 , 311.91 ± 5.38 , and 63.69 ± 0.11 mg/kg of oil content respectively. Thus, *M.holstii* oil could serve as a raw material for the food and cosmetics industry. The total sugar, protein and polyphenols content of *M. holstii* oilcake are interesting in different domain such as livestock feeding, food industries as antioxidant factor, and pharmaceuticals. The present study show that *M.holstii* can really contribute to food security but more studies are recommended to analyze other nutrients content (such as minerals, vitamins), the toxicity of oils and oilcake, the profitability and productivity of *M. holstii*.

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