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Original Article

Potential of some autochthonous wild plants of Burundi for vegetable oil and valuable compounds production

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(With 6 figures)

Abstract

Twelve species of indigenous plants have been studied in order to valorize some natural resources of Burundi (Eastern Africa) to investigate possibilities of vegetable oil production. Physicochemical properties and oil contents were determined from seeds harvested through five ecogeographic zones. From oilcake extracts, total sugars contents, proteins (TPrC), polyphenolic (TPhC), and flavonoids were quantified using spectrophotometry. Furthermore, antioxidant activity of oilcake extracts was assessed by 2, 2-diphenyl-b-picrylhydrazyl (DPPH) radical-scavenging and ferric reducing antioxidant power (FRAP) assays. All oil contents obtained were found to be quite similar to those of common oleaginous seeds. The two highest were found in *Parinari curatellifolia* (61.44 ± 4.81% Dry Matter) and *Myrianthus arboreus* (48.26 ± 5.96% DM). More than half of the species have shown TPrC ranging from 10 to 24% dry matter of oilcake (DM). *Brachystegia longifolia* was revealed exceptionally stronger antioxidant potential: effectiveness antiradical of 163.06 ± 26.29 mL/µg.min (DPPH assay) and reducing power of 2618.21 ± 161.22 GAE/100 g DM (FRAP assay). TPhC were positively correlated ($p < 0.05$) to the antioxidant activity. This pioneering work on these wild species highlight the potential for producing vegetable oil and valuable biomolecule sources likely for food, cosmetics, pharmacy and industry.

Keywords: autochthonous species, oleaginous plants, oil content, polyphenols, antioxidant activity, Burundi.

Potencial de algumas plantas silvestres autóctones do Burundi para a produção de óleos vegetais e compostos valiosos

Resumo

Doze espécies de plantas indígenas foram estudadas para valorizar alguns recursos naturais do Burundi (África Oriental), para investigar as possibilidades de produção de óleo vegetal. As propriedades físico-químicas e o conteúdo de óleo foram determinados com base em sementes colhidas em cinco zonas ecogeográficas. A partir de extratos de bagaço de óleo, os teores de açúcares totais, proteínas (TPrC), polifenólicos (TPhC) e flavonoides foram quantificados por espectrofotometria. Além disso, a atividade antioxidante dos extratos de bagaços foi avaliada por ensaios de 2,2-difenil-b-picrilhidrazil (DPPH) e antioxidante redutor de ferro (FRAP). Todos os conteúdos de óleo obtidos foram encontrados para ser bastante semelhantes aos das sementes oleaginosas comuns. Os dois maiores foram encontrados em *Parinari curatellifolia* (61,44 ± 4,81% de matéria seca [MS]) e *Myrianthus arboreus* (48,26 ± 5,96% de MS). Mais da metade das espécies mostrou TPrC variando de 10% a 24% de MS de tortas. *Brachystegia longifolia* revelou um potencial antioxidante excepcionalmente mais forte: eficácia antirradical de 163,06 ± 26,29 mL/µg.min (DPPH *assay*) e poder redutor de 2.618,21 ± 161,22 GAE/100 g de MS (ensaio FRAP). TPhC correlacionaram-se positivamente ($p < 0,05$) com a atividade antioxidante. Este trabalho pioneiro sobre essas espécies selvagens destaca o potencial para a produção de óleo vegetal e fontes valiosas de biomoléculas para alimentos, cosméticos, farmácia e indústria.

Palavras-chave: espécies autóctones, plantas oleaginosas, teor de óleo, polifenóis, atividade antioxidante, Burundi.

1. Introduction

The natural terrestrial ecosystems of Burundi provide great biological resource. The vascular flora already inventoried was estimated at 3125 species with 70 endemic (MEEATU, 2013). Forest ecosystems constitute a source of plant material for domestication (Abbo and Gopher, 2017) increase food production and valuable substances

isolation. The edible oil production from oleaginous plants cultivated in Burundi does not reach the national demand. The main oleaginous plant is oil palm (*Elaeis guineensis*). Although, it was introduced in Burundi before 1910 (Ngiye, 2017), its annual oil production (20 000 tons) is still too low to reach the national demand which is estimated at 100 000 tons/year (MINAGRIE, 2008). Unfortunately, intensification of oil palm cultivation leads to deforestation with all the harmful consequences that follow. It has been reported that the conversion of forest ecosystems to oil palm crops has severely impacted biodiversity in South Asia (Gaveau et al., 2009; Koh and Wilcove, 2008). Meanwhile, wild plants can provide valuable vegetable oils (Madzimure et al., 2011; Chan et al., 2013). *Balanites aegyptiaca* oils show interesting properties for food, pharmaceutical and industrial domain (Abasse et al., 2011), while that of Jatropha (*Jatropha curcas*) can be used for biodiesel production (Achten et al., 2008). The diversification of oleaginous plants would not only remedy the problem of the edible oil deficit, but would also provide various raw materials in different application domains (Merrien et al., 2012). Many other studies have shown that oilcakes still contain several valuable substances including fibers, proteins and polyphenols (Hussein, 2009; Merrien et al., 2012; Salgado et al., 2012; Chan et al., 2013). Nowadays, it is well known that phenolic compounds are powerful antioxidants and are therefore involved in different biological activities and in the prevention of many diseases (Aljadi and Kamaruddin, 2004; Amari et al., 2014; Brighenti et al., 2005; Shahidi and Ambigaipalan, 2015).

This preliminary study was carried out on *Entada abyssinica* Steudel ex A. Rich, *Annona senegalensis Pers*, *Brachystegia longifolia* Benth, *Caesalpinia decapetala* (Roth) Alston, *Dodonaea viscosa* Jacq, *Ipomoea involucrata* P. Beauv, *Myrianthus arboreus* Beauv., *Maesopsis eminii* Engl, *Parinari curatellifolia* Planch. Ex Benth, *Sterculia tragacantha* Lindl, *Tephrosia vogelii* Hook.f., *Uvaria angolensis* Welw. ex Oliv distributed in 9 families. These species are not yet cultivated but in a few rare cases, some feet of trees were deliberately left on the farm after clearing. They are all wild plants and grow naturally in the forest ecosystems. Their geographical distribution extends through all the savannas of eastern part of Burundi (Buragane, Kumoso and Buyogoma), and through all the vegetation formations that occupy the Congo-Nile ridge (Mumirwa, Bututsi and Mugamba). Locally, these plants are used as firewood, edible fruit, timber and traditional medicines (Supplementary Table 1). However, so far, no report on seed oils and bioactivity of oilcake from these seed species have been presented. The aim of this study was to valorize some wild autochthonous plants by determining their oil content, analyzing the qualitative parameters of the oils and by assessment of the antioxidant properties of oilcake extracts. This study highlights the potential production of oil and natural antioxidants of the country.

2. Material and Methods

2.1. Plant material

The fruits of *E. abyssinica*, *A. senegalensis*, *B. longifolia*, *C. decapetala*, *D. viscosa*, *I. involucrata*, *M. arboreus*, *M. eminii*, *P. curatellifolia*, *S. tragacantha*, *T. vogelii*, *U. angolensis* were sampled from May to December according to their period ripening. They were collected in five ecoclimatic zones, namely: the plain (775-1000 m of altitude), the foothills (1000-1500 m of altitude), the high mountains (1500-2600 m), the central trays (1400-2000 m of altitude) and the depressions (1200-1500 m of altitude) (Table 1). From the shapefile of the natural regions of Burundi, the calculated geometry automatically with arcMap 10.4.1 estimated that distribution occupy a surface of 15033 km^2, more than half of the national territory. Species were harvested in three different sites according to the ecological conditions of each plant. The identification of the plant species was performed at the herbarium of the University of Burundi (BJA) and

Table 1. Seeds sampling.

Plant species	Vernacular name	Sampling period	P	FH	HM	CT	D
Entada abyssinica	umusange	July to October		1		1	1
Annona senegalensis	Umukanda, Umutobe	October to November		1		1	1
Brachystegia longifolia	Ingongo	October to November	1	2			
Caesalpinia decapetala	Uruzira,Umubambangwe	July to October			1	1	1
Dodonaea viscosa	umusasa	July to October			1	1	1
Ipomoea involucrata	Umurandaranda	July to October		1	1		1
Myrianthus arboreus	Umwufe	October to November	2	1			
Maesopsis eminii	Umuhumura, Indunga	July to September		2			1
Parinari curatellifolia	Umunazi	August to October		1		1	1
Sterculia tragacantha	Umutakataka	July to September		2			1
Tephrosia vogelii	Ntibuhunwa	June to August		1		1	1
Uvaria angolensis	Umubungo	July to October		2		1	

P = Plain; FH = foothills of Mumirwa; HM = high mountains; CT = central trays; D = depressions; 1 = the species is sampled on the same site of an ecoclimatic zone; 2 = a species is sampled at two (communes) on the same ecoclimatic zone.

the herbarium of the Burundian Office for the Protection of the Environment. Nine plants were sampled on each specie and three samples were collected per site. The ripe fruits, identifiable by their respective color, were harvested manually. After, the fruits were dried at room temperature in Microbiology Laboratory at University of Burundi. After drying, the seeds were hulled manually and then brought to Cadi Ayyad University (Morocco) in the Food Science Laboratory for further analysis.

2.2. Oil content

Seeds were crushed using a Moulinex blinder (France) and then the extraction was performed with hexane as solvent in Soxhlet apparatus under reflux for 8h. The solvent was evaporated under reduced pressure. Oil content was estimated gravimetrically by weighing the oil after complete removal of the solvent.

2.2.1. Acidity and peroxide value determination

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 O_2)/kg of oil.

2.2.2. Unsaponifiable matter preparation

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. Preparation of ethanol extracts from 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 obtained ethanol extracts were mixed and evaporated to dryness under reduced pressure (vacuum). The dry extract was recovered in 1 mL of 80% ethanol. Finally, yields were measured before storing the extracts at –21 °C for further analysis.

2.4. Determination of Total Sugar (TSC), Total Protein (TPrC), Total Polyphenols (TPhC), Total Flavonoids (TFC) content

TSC was determined using (Dubois et al., 1956) 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 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-Ciocalteau 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).

TFC was determined according to the aluminum chloride colorimetric method (Amezouar et al., 2013). The results were expressed as mg catechin (mg CE /1OO g DMOC).

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging activity assay

Evaluation of the antioxidant activity of seeds extracts was performed using two approaches. Both the concentration of extract necessary to reduce 50% (IC_{50}) of DPPH$^{\bullet}$ after a duration of 60 minutes and the kinetics of reduction were determined. The DPPH free radical scavenging test was measured using the method described by (Lopes-Lutz et al., 2008) with some modifications. The principle of the method is based on the reduction of this radical which is accompanied by the turn of the violet color (DPPH•) to yellow color measurable at 515 nm. This reduction capacity is determined by a decrease in the absorbance induced by an antioxidant (Popovici et al., 2009). The concentration required to inhibit 50% of the DPPH (antiradical activity) was expressed as IC_{50} (mg/mL). So, a lower IC_{50} value corresponds to a higher antioxidant activity (Patro et al., 2005). The kinetics of reaction and the parameters of calculation were determined using the method described by Sánchez-Moreno et al. (1998).

2.5.1.1. Determination of the equilibrium time TIC_{50}

The parameter TIC_{50} is defined as the time needed to reach the equilibrium with an antioxidant concentration equal to IC_{50}. This parameter is determined graphically.

2.5.1.2. Determination of the Effectiveness Antiradical (EA)

The two parameters IC_{50} and TIC_{50} can be combined to obtain the anti-free radical efficiency parameter: $EA = TIC_{50}/IC_{50}$.

2.5.2. Ferric Reducing Antioxidant Power (FRAP) assay

FRAP assay was measured according to method described by (Mouhoubi-Tafinine et al., 2016). The reducing agents in the extracts induce a reduction of ferric ions (Fe+3) to ferrous ions (Fe+2). This reduction is induced by a resulting intensity of the blue-green color. An increase in absorbance indicates that reducing power increases (Balasundram et al., 2006). Absorbance was measured using a spectrophotometer at a wavelength of 700 nm. The results were expressed as gallic acid equivalents in mg per 100 g of product (mg GAE/100 g).

2.6. Statistical analysis

Data analysis was performed using IBM SPSS statistic 20. Results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Correlation between various parameters was also investigated. Significance was determined at $p < 0.05$ level and the results were expressed as mean values ± standard error (SE). All tests were performed in triplicate.

3. Results and Discussion

3.1. Characterization of the lipid fraction

3.1.1. Oil content and chemical profile

In this study, oil content of species studied varied significantly ($p < 0.05$) from 8.15 ± 0.32 DM to 61.44% ± 4.81 DM (Figure 1). These results are similar to those reported in other seed oil commonly used as soybean (Song et al., 2017; Stahl et al., 1980), and canola seed (Thobani and Diosady, 1997). Similar contents were also recorded in unconventional oils such as *Salvadora persica* Linn. (Hussein, 2009) and *cucumis melo* var. agrestis (Mariod and Matthäus, 2007). Highest contents were observed in the *P. curatellifolia* (61.44% ± 4.81 DM), *M. arboreus* (48.26% ± 5.96 DM), and *M. eminii* (46.58 ± 6.82% DM). The lowest oil content was observed in *B. longifolia* (8.15% ± 0.32 DM). Although some oil contents seem very low, the potential interest in these oil can rise from their cosmetic properties and/or other properties. The corn oil content (5%) (Portugal et al., 2017) is low than these all of our samples, however, it is widely used in pharmaceutical and cosmetic industries (Barrera-Arellano et al., 2019). The cactus with an oil content not exceeding 8.74% (El Hachimi et al., 2015; Ouhammou et al., 2017) is emerging due to its important antioxidant activity, α-glucosidase inhibitory activity, cytotoxicity against human certain cancer cell lines, antimicrobial action, antifungal activity (Santiago et al., 2017).

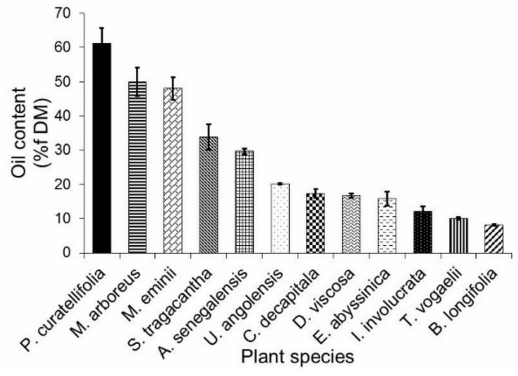

Figure 1. Oil content of the studied plants.

Our results suggested that oil can be produced in all ecogeographical zones of Burundi (Table 1 and Figure 1). In the Imbo plain, only oil palm is produced, another species was found with an oil content of over 50%. In the foothills of the Mumirwa escarpment, ten species were found with oil contents exceeding 16%, among them six have more than 30%. In the depressions, six species were found having an oil content exceeding 16%, three of which exceeded 30%. In the central trays, five species show an oil content that exceeds 16%, two of them have more than 30%. In the mountains, three species have oil contents exceeding 16%, among them one species exceeds 30%.

3.1.2. Physicochemical properties

Usually, vegetable oils contain more than 95% triglycerides. Nevertheless, enzymatic hydrolysis intrinsic lipases contained in the seeds (Beisson et al., 2001) and some chemical reactions during oil extraction (Khattab and Zeitoun, 2013; Seneviratne and Dissanayake, 2015) can alter the composition. The quantification of the free fatty acids thus formed gives a good idea of the quality of the oil. Our results, Figure 2A show that the highest acidity observed, *U. angolensis* (7.81 g/100 g), was slightly higher than that fixed for a technologically accepted palm oil (≤ 5 g /100 g) by (≤ 5 g /100 g) by CODEX STAN 210-1999 norms (FAO, 2017). Low acidity levels were found in oils from *P. curatellifolia* 0.19 ± 0.05 g OA/100 g, *E. abyssinica* 0.55 ± 0.01 g OA/100 g, and *T. vogaelii* 0.73 ± 0.03 g OA/100 g. These values are comparable to those fixed by ISO 660:2009 (E) norms (ISO, 2009) on extra virgin olive oil (≤ 0.343 g /100 g); on sunflower oil (≤ 0.60 g /100 g) and on coconut oil (≤ 0.830 g/100 g).

The Peroxide Value (PV) indicates the degree of primary oxidation of the oil. Internal factors such as the fatty acid composition, enzymes (Choe and Min, 2009; Doblado-Maldonado et al., 2012) and external factors like temperature (Sathivel et al., 2008) and oxygen

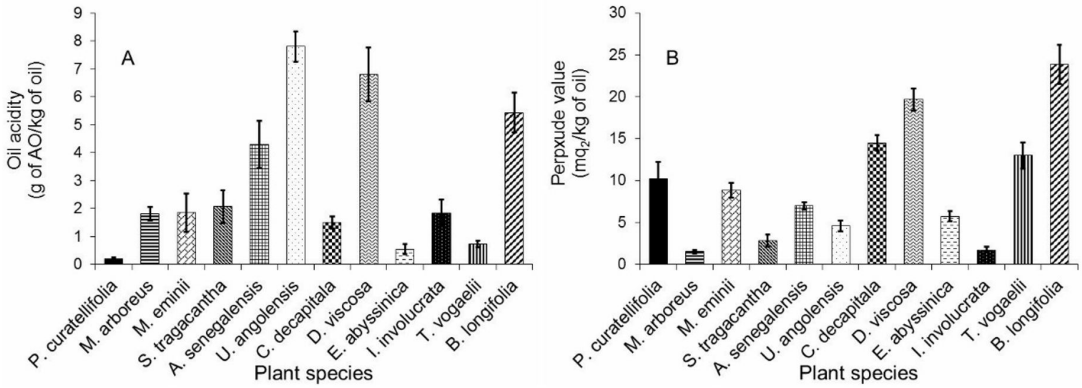

Figure 2. Acidity (A) and peroxide value (B) of the studied oils. q_2.

partial pressure (Tan et al., 2002) were the main cause of oil oxidation. Deterioration of oil quality will lead to sensorial (rancidity) and lossing nutritional values (loss of polyunsaturated fatty acids and vitamin E) (Cuvelier and Maillard, 2012). In fact, the results of the oxidation state on our samples estimated by PVs are mentioned in Figure 2B. Except one sample, obtained PVs range from 1.51 to 19.66 g meq O_2/ kg and under the limit fixed by CODEX STAN 33-1981 norms (FAO, 2001a) on virgin olive oils (≤ 20 meq O_2 / kg). However, they were similar to those reported on cold pressed oil of sunflower (4.19 meq O_2/kg), peanut (8.39 meq O_2/kg), rapeseed (9.46 meq O_2/kg), and olive (6.39 meq O_2/kg) (Bozdogan et al., 2018). Oil from *B. longifolia* was found to be more oxidized (24.23 ± 2.31 g meq O_2/ kg) but this parameter is greatly dependent on the quality of raw material and the processing parameter.

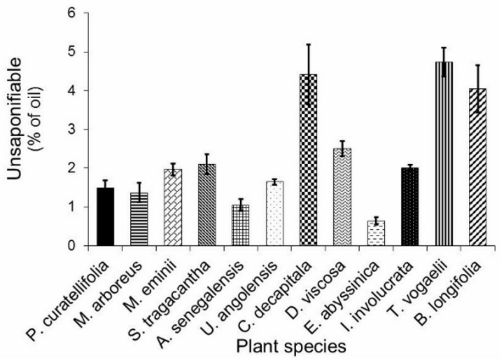

Figure 3. Unsaponifiable content of the studied oils.

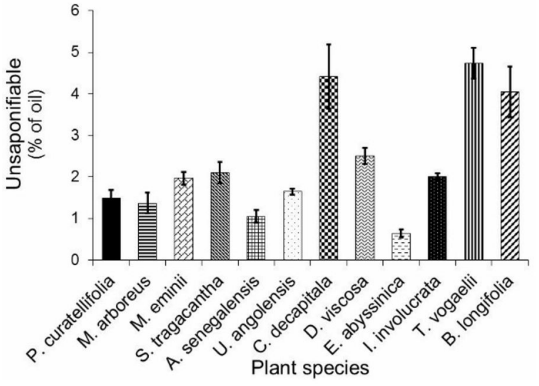

Figure 4. Ethanolic extracts from oilcakes.

For most edible oils, Unsaponifiable material (USM) represent 0.5 to 2.5% of oil, with some exceptions, reaching 5 to 6% (Malecka, 1994). It contains hydrocarbons, terpene alcohols, sterols, tocopherols and other phenolic compounds (Boskou and Morton, 1976). Depending on its chemical constituents, it can contribute significantly to oxidative stability of oil (Chanioti and Tzia, 2017; Pazhouhanmehr et al., 2016). Our results on USM contents are presented in Figure 3. Thereby, USM contents found in seed oils from *E. abyssinica* (0.63% ± 0.10), *A. senegalensis (*1.04% ± 0.15) *M. eminii* (1.3%7 ± 0.19), and *P. curatellifolia* (1.49% ± 0.25) were in agreement with the values fixed by CODEX STAN 210-1999 norms (FAO, 2001b) on virgin olive oil (<1.5%), oil palm (<12%), and soybean oil (<1.5%). Furthermore, similar contents was reported on olive-pomace oils (2.52-4.38%) (Chanioti and Tzia, 2017), and on rapeseed oil (0.26%) (Schröder and Vetter, 2012).

3.2. Characterization of oilcakes

3.2.1. Extract yield, Total Tugars (TSC), Total Proteins (TPrC), Total Polyphenols (TPhC) and Total Flavonoids (TFC) contents

TSC, TPrC, TPhC, TFC analyses were performed on extracts obtained with ethanol 80%. Extract yields on oilcakes are depicted on Figure 4. It was found that the ethanolic extraction yields was exceeded 10% in all species. The highest extract content was observed in *C. decapitala* (24% dry matter of oilcake) while the lowest was for *S. tragacantha* (10.24%). Most of the studied species has shown no significant difference ($p > 0.05$) between them.

The TSC from oilcakes determined using Dubois method and expressed as mg glucose equivalent are shown in Figure 5A. Based on Tukey's statistical test, the studied species are divided into two groups low sugar contents (< 10%) and a groups of high sugar contents (> 10%). Thus, Oilcakes of *B. longifolia* were found to have the highest TSC (22.23%). It was followed by *C. decapetala*

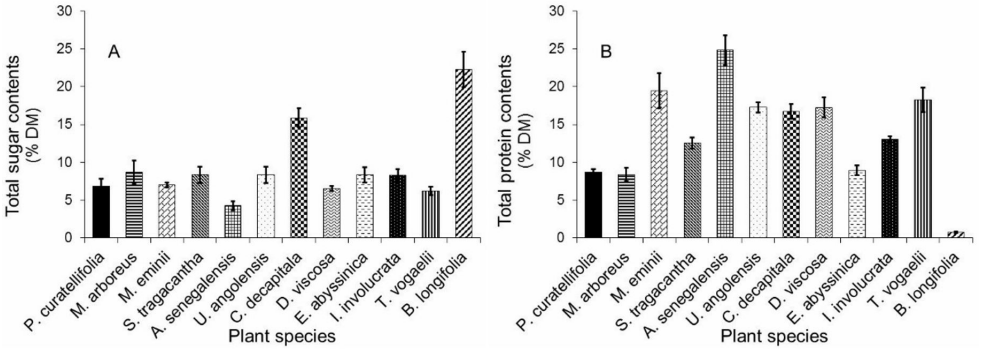

Figure 5. Total sugars content (A) and total proteins content (B).

(15.87%), the difference was found significant ($p < 0.05$). A serie of 9 species showed TSC significantly different from the previous one ($p < 0.05$), but not between them. Lowest TSCs were found in oilcakes of *A. senegalensis* (4.25%) and *T. vogaelii* (6.21%). This results revealed that species with high oil content were low in total sugar cotents (Table 1 and Figure 5A). Furthermore, a negative correlation ($p < 0.05$) was observed between this two parameters (Table 2). This trend of the results is in accordance with the scientific literature. Lipids and sugars are both primary metabolites (Coutinho et al., 2018; Jia-xi et al., 2019) while sugars are also involved in lipid biosynthesis (Sun et al., 2018).

The results of the TPrC evaluation from oilcakes in different species studied are reported in Figure 5B. Using the Tukey test, six groups were found. The group of high TPrC (> 20%) and that of low TPrC (< 1%), each one, was constituted by single specie. All other species are regularly distributed between 7 and 20% TPrC. Thus, 4 significantly different ($p < 0.05$) groups were found. Each group consisted of 5 species of TPrC not significantly different ($p > 0.05$). However, overlaps were observed when one specie could belong to two groups. The highest TPrC were found in oilcake extracts of *A. senegalensis* (24.82% ± 3.18), *M. eminii* (19.45% ± 3.06), *and T. vogelii* (18.27% ± 1.48). *B. longifolia* oilcakes contained almost no protein. Its TPrC (0.75%) was the lowest of the other species with a strong significant difference ($p < 0.05$) compared to that of the penultimate (*M. arboreus*) which had 8.38%. For most species, TPrCs allow them to be used as feed in livestock. Our results were found to be lower than those of soybean oilcake (48,26) (Février et al., 1952) and sunflower oilcake (31,7%) (Salgado et al., 2012) but comparable to those of cottonseed oilcake (16%) (Zotte et al., 2013), Palm kernel cake (22.9%) (Ribeiro et al., 2018) rapeseed oilcake (24.76%) (Nájera et al., 2017). The confrontation between the data of Figure 1 and 5B made it possible to reveal many proteaginous seeds. The species that can supply both oil and protein at relatively high levels were *A. senegalensis, M. emnii, U. angolensis, C. decapitala, and D. viscosa.*

Polyphenols are secondary metabolites found in all plants. Nevertheless, their contents vary widely depending on the species, and the environmental factors (Connor et al., 2002; Ksouri et al., 2008). As shown in Figure 6A, the results obtained on TPhCs were characterized by a high variability. The three highest TPhCs were found in extracts of *B. longifolia, T. Voegeli*, and *M. emnii* (1029.77 ± 152.78 > 745.10 ± 84.41 > 486.72 ± 12.0 mg GAE/100 g, respectively). The differences in TPhCs were found significant of $p < 0.05$. Next highest contents were in group of six species whose TPhCs ranged, without significant difference ($p > 0.05$), from 200 to 350 mg GAE/100 g. Lowest recorded TPhC was found in *P. curatellifolia* oilcake (84.81 ± 5.72 mg GAE/100 g). Phenolic contents are reported to widely vary among the plant species even in the same specie (Hatamnia et al., 2014), and depending on several factors physiological and environmental (Rabeta and Nur Faraniza, 2013). Our results were similar to those found on defatted kenaf seed meal (339.93 mg GAE/100 g defatted material) (Chan et al., 2013) and on

Table 2. Antioxidant activity of seed oilcake extracts with FRAP assay.

Plant species	mg GAE /100 g
P. curatellifolia	16.13 ± 1.44[b]
M. arboreus	115.31 ± 4.06[b]
M. eminii	24.47 ± 27.99[b]
S. tragacantha	16.81 ± 4.57[b]
A.senegalensis	110.31 ± 6.90[b]
U. angolensis	55.50 ± 4.51[b]
C.decapitala	87.06 ± 6.22[b]
D. viscosa	37.08 ± 5.84[b]
E. abyssinica	78.33 ± 6.98[b]
I. involucrata	15.75 ± 2.91[b]
T. vogaelii	43.29 ± 10.91[b]
B. longifolia	2618.21 ± 161.22[a]

Results are mean of three replicates with standard errors (Means ± S.E, n = 3). In each column, values with different letters mean that are significantly different ($p < 0.05$).

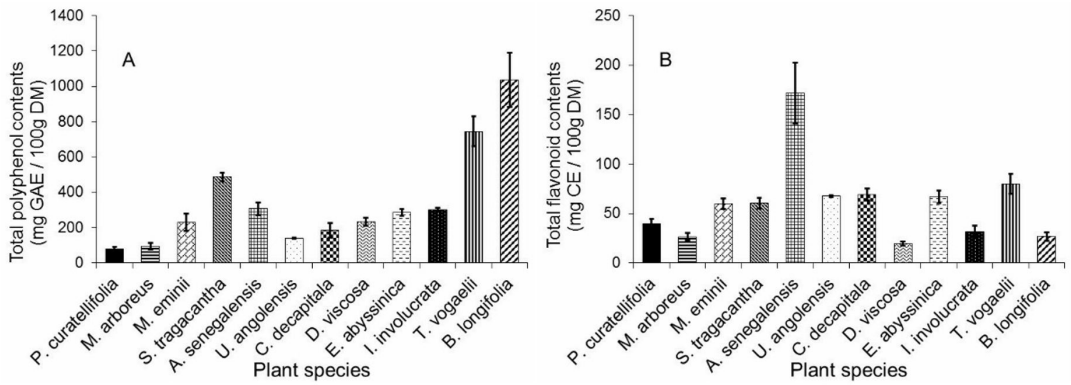

Figure 6. Total phenols content (A) and total flavonoids content (B) of oilcakes.

Cynometra cauliflora fruit (847.31 +26.82 mg GAE/100 g dried sample) (Rabeta and Nur Faraniza, 2013). Many of them are suggested to be a source of polyphenols. Flavonoids are the most abundant polyphenolic compounds found in plants (Ghasemzadeh and Ghasemzadeh, 2011; Jin et al., 2012). Due to their high bioactivity, they play an important role in human health and disease prevention (Kulkarni et al., 2016). TFC analysis from the twelve studied oilcake species, Figure 6B, revealed that all TFCs ranged from 20 to 80 mg CE/100 g) excepting the TFC obtained from *A. senegalensis* (171.80 ± 30.76 mg CE/100 g) which significantly exceeded the range ($p < 0.05$) . Nevertheless, considering the TFC / TPhC ratio (Figure 6B, A), only *B. longifolia and D. viscosa* were found to have proportions of 2% and respectively 9%. For all other species, proportions were greater than 10%, among them, highest were 57%, 49% and 41% for *A. senegalensis*, *U. angolensis and P. curatelifolia* respectively.

3.2.2. Antioxidant activities

3.2.2.1. DPPH radical scavenging assay

DPPH assay was used to evaluate the antioxidant capacity of oilcake extracts in comparison to ascorbic acid. The IC_{50} were determined directly as a concentration of the ethanolic extract and then assuming that mostly such antioxidant activity may be due to the phenolic compounds it is the calculated as concentration of polyphenols in the extract. In addition to the determination of IC_{50}, an optimization of the method was performed by determining the kinetic of DPPH reduction by our extracts. For IC_{50} results obtained with concentrations of extracts, the Tukey analysis on differences between estimated averages allowed us to distinguish between 6 groups significantly different ($p < 0.05$) (Table 3). Thus, antioxidant activity had decreased from the first group to the sixtieth. However, overlaps have been observed between groups sharing one or more species. The first group, consisting of antioxidant activity from 3 species extracts, had the highest antioxidant potential. They were *B. longifolia* (0.13 ± 0.00 mg of extract/mL) > *M. eminii* (5.583 ± 1.67 mg of extract/mL) > *E. abyssinica* (5.89 ± 0.6600 mg of extract/mL) and no significant differences ($p > 0.05$) were observed between them. These three species showed antioxidant capacity comparable to that of ascorbic acid. The next group that showed high antioxidant activity was composed of species sharing the letter « d ». They were no significantly different at $p > 0.05$; order being *M. eminii* > *E. abyssinica* > *T. vogaelii* > *M. arboreus*. Oilcake extracts from (*P. curatellifolia* and *S. tragacantha)* were unable to reduce 50% of DPPH. With maximum concentration, *P. curatellifolia* and *S. tragacantha* had reduced 31.45 ± 3.33 and 46.09 ± 3.54% of DPPH, respectively. However, compared to ascorbic acid, the antioxidant activity of all extracts were generally promising. This was revealed very well when expressing IC_{50} as a concentration of polyphenols in the extract.

The application of Tukey test on IC_{50} calculated related to the polyphenol concentrations divided the results into 5 groups significantly different at $p < 0.05$ (Table 3). The first powerful group for antioxidant activity was constituted by *B. longifolia* (0.005 ± 0.00 mg GAE/mL) extract slightly higher ($p < 0.05$) than that of ascorbic acid. The first group was followed by that of 5 species whose averages did not differ significantly ($p > 0.05$) from that of ascorbic acid and order is as: *M. eminii* > *U. angolensis* > *E. abyssinica* > *I. involucrata* > *M. arboreus*. This suggested that the purification of the extracts will lead to a promising bioactive molecules that could at least be effective as the standard.

Table 3. Antioxidant activity of seed oilcake extracts with DPPH assay: TIC_{50}, IC_{50} expressed as mg GAE/mL was calculated relative to the total polyphenol, IC_{50} expressed as mg/mL was determined in crude extract, EA calculated relative to the concentration of crude extract.

Plant species	IC_{50} (TPhC)	IC_{50} (extracts)	TIC_{50}	EA
	mg GAE/mL	mg/mL	min	mL/µg.mm
P. curatellifolia	-	-	-	-
M. arboreus	0.32 ± 0.02^{cd}	10.99 ± 0.75^{cde}	35.55 ± 0.13b	2.41 ± 0.84^{c}
M. eminii	0.24 ± 0.14^{cd}	5.583 ± 1.67^{ef}	27.77 ± 0.22^{c}	5.10 ± 0.54^{c}
S. tragacantha	-	-	-	-
A.senegalensis	0.58 ± 0.14^{ab}	18.45 ± 4.35^{b}	26.11 ± 0.12^{c}	1.53 ± 0.46^{c}
U. angolensis	0.25 ± 0.02^{cd}	17.27 ± 1.29^{bc}	20.83 ± 0.07^{d}	1.30 ± 0.27^{c}
C.decapitala	0.43 ± 0.10^{bc}	65.56 ± 8.38^{a}	30.55 ± 0.15^{c}	0.46 ± 0.01^{c}
D. viscosa	0.37 ± 0.13^{bc}	19.47 ± 0.76^{b}	51.66 ± 0.33^{a}	2.65 ± 0.12^{c}
E. abyssinica	0.27 ± 0.17^{cd}	5.89 ± 0.66^{ef}	13.89 ± 0.05^{e}	1.97 ± 0.45^{c}
I. involucrata	0.29 ± 0.03^{cd}	13.82 ± 0.99^{bcd}	34.99 ± 0.16^{b}	2.54 ± 0.06^{c}
T. vogaelii	0.67 ± 0.18^{a}	7.99 ± 0.80^{de}	51.66 ± 0.33^{a}	6.54 ± 0.74^{c}
B. longifolia	0.005 ± 0.00^{e}	0.13 ± 0.00^{f}	15.55 ± 0.08^{e}	163.06 ± 26.29^{a}
Ascorbic acid	0.09 ± 0.00^{de}	0.09 ± 0.00^{f}	5.00 ± 0.02^{f}	52.63 ± 1.58^{b}

Results are mean of three replicates with standard errors (Means ± S.E, n = 3). In each column, values with different letters mean that are significantly different ($p < 0.05$).

Table 4. Pearson's correlation between, Oil content ethanolic extracts (Eth Extr), total sugar contents (TSC), total polyphenol contents (TPhC), Total flavonoid contents (TFC), total protein contents (TPrC), IC_{50} of ethanolic extracts (IC_{50} Extract), IC_{50} calculated based on total polyphenols (IC_{50} TPhP), Ferric reducing antioxidant power (FRAP) assay, and effectiveness antiradical (EA).

	Oil content	Eth Extr	TSC	TPhC	TFC	TPrC	FRAP	IC_{50} Extract	IC_{50} TPhP	EA
Oil content	1	-.342*	-.355*	-.529**	-.027	-.006	-.295	-.258	-.268	-.330*
Eth Extr		1	.409*	.229	-.216	-.268	.131	.477**	.235	.184
TSC			1	.536**	-.311	-.568**	.782**	.210	-.325	.781**
TPhC				1	-.042	-.371*	.712**	-.304	-.037	.717**
TFC					1	.523**	-.198	.184	.448**	-.239
TPrC						1	-.533**	.341*	.599**	-.577**
FRAP							1	-.196	-.305	.871**
IC_{50} (Extrait)								1	.403*	-.243
IC_{50}(TPhP)									1	-.332*
EA										1

*The correlation is significant at the 0.05 level (bilateral); **The correlation is significant at the 0.01 level (bilateral).

As mentioned in Table 3, steady states differ greatly from one species to another. With Tukey statistic, obtained group number reached to six with different species composition and different level of significance at $p < 0.05$. There was no overlap from one group to another. A complementarity between data from IC_{50} and TIC_{50} was observed. To reach steady state, species that had high antioxidant activity considering IC_{50} realized a shorter time relatively to those with low antioxidant activity. After the EA calculation by IC_{50} and TIC_{50} parameters, two groups (*B. longifolia* > all other species) significant difference ($p < 0.05$) were obtained. This suggested that there was synergy of these two parameters for an estimate close to reality. Thus, with the exception of *B. longifolia* (163.06 ± 26.29 mL/µg.mm), all species submitted to the Tukey test were found to have no significant difference ($p > 0.05$) for antioxidant activity. Their values varied from 0.46 to 6.54 mL/µg.mm.

3.2.2.2. Ferric ions reducing activity

Assessment of antioxidant potential in the extracts from different oilcakes was also carried out using the FRAP method. The results are shown in Table 2. Among the 6 groups forme, extracts of *B. longifolia* (2618.21 ± 161.22mg GAE/100 g) were shown to be the much more powerful reducers of the ferric ions to ferrous ion. It was 22 times more reductive than that of the next specie. A similar value was reported in propolis extract (Mouhoubi-Tafinine et al., 2016). The following species (*M. arboreus*, *A. senegalensis*, *C. decapitala* and *E. abyssinica*) were, respectively, 115.31 ± 4.06 > 110.31 ± 6.90 > 87.06 ± 6.22 > 78.33 ± 6.98 mg GAE/100 g with no significant difference between them. As in the DPPH method, *P. curatellifolia* (16.813 mg GAE/100 g) and *S. tragacantha* (16.133 mg GAE/100 g) showed a very low reducing power of the ferric ions to ferrous ion. However, the last low content was that of *I. involucrate* 15.75 ± 2.91 mg GAE/100 g.

The highest antioxidant power obtained in these species may be related to their high polyphenol contents (Figure 6A) and flavonoid (Figure 6B). Significant correlation ($p < 0.05$) was observed between polyphenols content and antioxidant activity, on the one hand, and significant correlation ($p < 0.05$) between flavonoid contents and antioxidant activity, on the other hand (Table 4). Several studies reported positive correlation between TPhC and antioxidant activity (Hodzic et al., 2009; Zheng and Wang, 2001). Generally, free radical scavenging and antioxidant activity of phenolic (e.g. flavonoids, phenolic acids) mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules, and is also affected by other factors, such as glycosylation of aglycones, other H-donating groups (-NH, -SH) (Cai et al., 2004). *B. longifolia* had a high TPhC and a powerful antioxidant activity nevertheless its TFC is low. In some species, the presence of large TPhC does not necessarily lead to the large TFC (Mouhoubi-Tafinine et al., 2016). The important antioxidant activity of *B. longifolia* is explained by the presence of other polyphenols than flavonoids. In addition to flavonoids, antioxidant also depends of phenolic acids (Nakatani, 2000; Rice-Evans et al., 1996; Zheng and Wang, 2001) and tannins, coumarins, lignans, quinones, stilbenes and curcuminoids (Cai et al., 2004).

4. Conclusion

It is for the first time that some potential oleaginous seeds from Burundi were studied. Our study highlighted the potential of some natural resources to be used as source of edible oil and valuable antioxidant compounds. Important oil contents were found in most plants studied through the five ecogeographic zones of the country. Six highest oil contents were ranged from 20 to 62%. Oilcake obtained after oil extraction was found to contain significant protein contents that could be used in livestock feed. While synthetic antioxidant components are reported to be toxic

(Houbairi et al., 2015; Rabeta and Nur Faraniza, 2013), our results suggested that most of species studied can be resource of natural bioactive molecules. Important TPhC were observed in 8 species and were ranged from 229.061 to 1035.55mg GAE/100 g DM. Exceptional antioxidant activity was found in *B. longifolia* with 163.06 ± 26.29 mL/µg.min in DPPH assay and with 2618.21 ± 161.22 GAE/100 g DM in FRAP assay. This makes it understandable that they contain bioactive molecules which, once isolated, could be useful in the agri-food, pharmaceutical and cosmetic fields. It may also be one of the sustainable ways to protect the environment since the exploited part of the plant is a seed and give rise to the domestication

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Supplementary Material

Supplementary material accompanies this paper.
Supplementary Table 1: Local uses of *E. abyssinica, A. senegalensis, B. longifolia, C. decapetala, D. viscosa, I. involucrata, M. arboreus, M. eminii, P. curatellifolia, S. tragacantha, T. vogelii, U. angolensis* plant in Burundi
This material is available as part of the online article from http://www.scielo.br/bjb.

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