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Comparative study of phenolic compound contents and antioxidant potential of *Moringa oleifera* Lam. leaf extracts from the four climatic zones of Mali

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Abstract

Moringa oleifera (MO) due to its medicinal and nutritional virtues has been acclimated in all the climatic zones of Mali. The aim of this work was to evaluate the variation of antioxidant activity of MO leaves harvested in four climatic zones of Mali. Thus, the contents of some important antioxidants compounds (such as total polyphenols and flavonoids) were determined by spectrophotometric method. The evaluation of the antioxidant activity of extracts was done by DPPH method. Some differences have been found between the levels of total polyphenols and flavonoids. The sample from Segou (Sudanian zone) had the highest levels of total polyphenols and flavonoids, while the Sikasso (Sudano-Guinean zone) one had the lowest. In regards to the antioxidant activity is concerned, the Segou sample has been the most active with IC_{50} = 27.79 ± 0.09 µg/mL; that activity is higher than the standard, ascorbic acid (with IC_{50} = 36.72 ± 0.11 µg/mL). Samples from Timbuktu (Saharan zone), Mopti (Sahelian zone) and Sikasso showed statistically similar antioxidant activities. This study showed that MO leaves are rich in metabolites and possess a high antioxidant power; that could help to prevent the malnutrition and certain disorders related to oxidative stress.

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Introduction

Moringa oleifera (MO) is originally a native plant from India, but has become naturalized in tropical and subtropical areas throughout the world. It grows in both wet and warm climates and survives in infertile soils even under drought conditions (Nouman *et al.*, 2014; Leone *et al.*, 2016). The relative easiness with which MO spreads sexually and asexually and its low demand for soil nutrients and water, so able to support poor and dry soils, make it easy to produce and manage (Zaku *et al.*, 2015; Alegbeleye, 2018). All parts of MO (roots, bark, leaves, flowers, sap, fruits and seeds) have shown nutritional, prophylactic and therapeutic virtues (Metwally *et al.*, 2017). The high levels of proteins, vitamins including vitamin A precursors such as beta-carotene, minerals and phenolic compounds in the leaves of MO make the plant in traditional medicine and feeding (Leone *et al.*, 2015; Vongsak *et al.*, 2013). The leaves are eaten crude or as powder form by many African and Asian (Popoola and Obembe, 2013). These chemical and nutritional characteristics make them good candidates to be combined with local foods to improve the diet of people living in developing countries, thereby reducing the risk of malnutrition (Leone *et al.*, 2018). Even, many scientific studies have reported several properties of the plant, such as antimicrobial, anti-inflammatory, thyroid hormone regulating, anti-obesity, anti-ulcer, anti-diabetic, anti-cancer and antioxidant properties (Sy-Ndiaye *et al.*, 2016; Anthanont *et al.*, 2016; Metwally *et al.*, 2017).

The organism of mammal, through oxidative stress phenomena, daily produces free radicals, which are highly reactive unstable compounds with a single electron (Evenamede *et al.*, 2017). The main cause of most pathologies is free radicals which, in order to stabilize themselves, attack some biological molecules by creating others free radicals and so trigger chain reactions having for consequences the damage of many cellular components. (Lobo *et al.*, 2010; Evenamede *et al.*, 2017). Plants are natural sources of antioxidant substances which are intended to protect cells against these oxidative stress (Sarr *et al.*, 2015).

The objective of this study was to compare phenolic compound contents and antioxidant potential of *Moringa oleifera* Lam. leaf extracts from four climatic zones of Mali.

Materials and methods

Plant material

The leaves of Moringa oleifera were used as plant material. They were harvested from September to October, 2018 in the different climatic zones of Mali (Figure 1); Sikasso (Sudano-Guinean zone), Segou (Sudanian zone), Mopti (Sahelian zone) and Timbuktu (Saharan zone). The leaf samples were carefully washed, dried at room temperature (30-35°C) before being powdered and stored away from light and moisture.

Methods

Preparation of hydro-ethanol extracts

The extracts were obtained by macerating 50 g of *Moringa oleifera* powder in 500 mL of 70% ethanol. The mixture was stirred for 48 h and then filtered under vacuum. The resulting filtrates were evaporated dry under reduced pressure using a rotary evaporator.

Phytochemical screening

The extracts were screened for the presence of main chemical groups (alkaloids, tannins, flavonoids, coumarins, saponins, terpenoids and reducing sugars) using the standard methods described by (Pascale *et al.*, 2018; Vivekraj *et al.*, 2017).

Determination of total phenolic compounds

The content of phenolic compounds in the different extracts was estimated by the Folin-Ciocalteu method described by Balkan *et al.*, (2018). Five hundred microliters (500 µL) of Folin-Ciocalteu reagent (diluted 10% distilled water) are added to 100µL of extract and 400 µL of disodium carbonate (Na_2CO_3) at 75 mg/mL are also added to the reaction mixture. After 2-hours incubation in darkness at room temperature, the absorbances are read at 765 nm. A calibration curve is performed in the same operating conditions using a gallic acid.

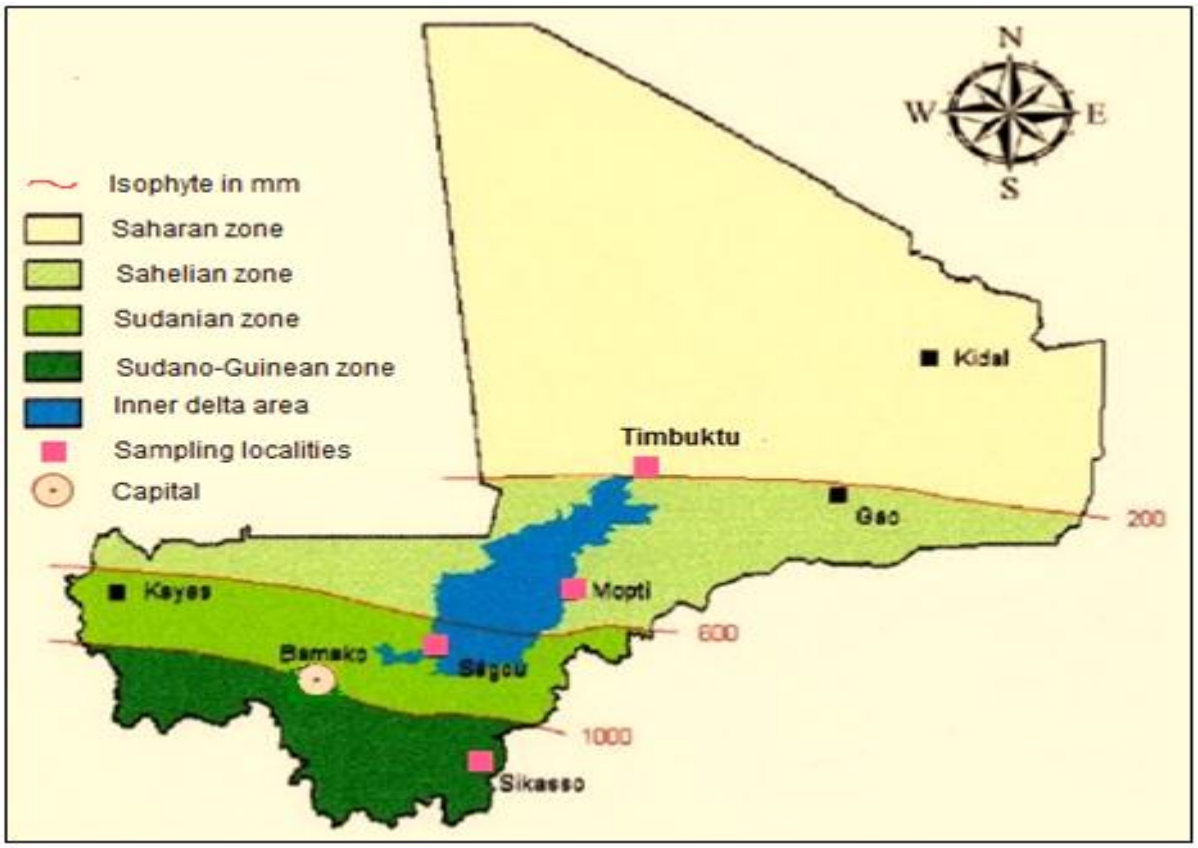

Fig. 1. Map showing the different climatic zones of Mali and the sampling localities.
Source: LaboSEP/IER, 2000 (modified by Hamata).

The results are expressed in equivalent milligrams of gallic acid per gram of extract (mg EAG/g).

Determination of flavonoids

Total flavonoid levels were estimated using the method described by Fofié *et al.*, (2017). 500 µL of each extract were added to 1500 µL of 95% methanol, 100 µL of 10% (w/v) $AlCl_3$, 100 µL of 1 M sodium acetate and 2.8 mL of distilled water. The mixture was stirred and incubated in darkness at room temperature for 30 min. The blank is made by replacing the extract with 95% methanol and the absorbance is measured at 415 nm using a UV spectrophotometer. The results are expressed in equivalent mg of quercetin /gram of dry matter (mg EQ/g).

Antioxidant activity in vitro

The evaluation of this activity was done by the method using diphenylpicryl-hydrayl (DPPH), according to the protocol described by (Sy *et al.*, 2018; Fofié *et al.*, 2017). Briefly, 50 µL of each ethanolic solution, at different concentrations (12.5; 26; 40; 60; 80 µg/ml), were added to 1.95 mL of the ethanolic solution of DPPH (0.024g/L). At the same time, a negative control was prepared by mixing 50 µL of ethanol with 1.95 mL of the DPPH ethanolic solution. The absorbance reading was done using spectrophotometer against a blank at 515nm after 30min incubation in darkness at room temperature.

Control using ascorbic acid as standard antioxidant is included in the experiment. The antioxidant activity related to the scavenging effect of the DPPH radical is expressed as percentage inhibition (PI) calculated using the absorbances obtained according to the following formula:

$[PI] = (A_0-A_1)/A_0 \times 100$

A0 = DPPH absorbance; A1= sample absorbance. The IC_{50} (concentrations that inhibit 50% of the DPPH radical) were deduced from the linear regression line obtained from the graph of the percentage inhibition of DPPH (Fofié *et al.*, 2017).

Data analysis

The data were collected on Excel® version 2013 and analyzed with Minitab 18.1 software. The one ANOVA test using the Fisher's test was chosen to compare the means of three trials in each case to the 5% probability threshold. The averages are considered significantly different at p-value < 0.05 probability level.

Results and discussion

Phytochemical screening

The results of phytochemical screening of ethanol extracts from the leaves of *Moringa oleifera* are summarized in the Table 1.

Table 1. Phytochemical screening of leaves of Moringa oleifera Lam. found in Mali.

	Localities			
Chemical groups	Mopti	Segou	Sikasso	Timbuktu
Alkaloids	-	-	-	-
Tannins	++	+++	+	++
Flavonoids	++	+++	+	++
Coumarins	++	+++	+	++
Saponins	+	+		+
Terpenoids	+	+	+	+
Reducing sugars	++	+++	+	++

The analysis presented in Table 1 shows the presence of tannins, alkaloids, saponins, flavonoids, coumarins, reducing sugars and terpenoids in all samples of *Moringa oleifera*. These results are similar to those of Millogo-Koné *et al*., (2012) carried out at Gourcy, northwestern Burkina Faso and to those of Kasolo *et al* (2010). However, Kasolo *et al* (2010) noted the absence of coumarins in their sample.

Table 2. Total polyphenol and flavonoid contents of leaves of *Moringa oleifera* Lam. found in Mali.

Localities	Total phenolic compounds (mg EAG/g)	Total flavonoids (mg EQ/g)
Segou	32.7 ± 1.41^{a}	8.23 ± 0.21^{a}
Timbuktu	27.13 ± 2.3^{b}	7.33 ± 0.25^{b}
Mopti	22.23 ± 0.5^{c}	5.8 ± 0.31^{c}
Sikasso	17.47 ± 1.69^{d}	5.37 ± 0.21^{d}
p-value	0.000017	0.00000036

*Averages not sharing any letters are significantly different.

The presence of these chemical groups in the leaves of *Moringa oleifera*, would justify their use especially in the prevention of cardiovascular diseases and other diseases (such as hepatitis and ulcers as wall as) and the management of many infections suggested by Millogo-Koné *et al*., (2012). Indeed, secondary metabolites such as tannins have astringent, anti-inflammatory, antidiarrheal, antioxidant, antiviral, antibacterial, antifungal and anti-tumor properties. (Veluri *et al*., 2006; Kang *et al*., 2008; Kumar *et al*., 2013). They also inhibit HIV replication and are used as diuretics. (Mohan and Krishna, 2019). Flavonoids are known to have cardio-protective, antioxidant and anti-diabetic properties (Martinez-Fernandez *et al*., 2015; Zhang *et al*., 2014; Yeon *et al*., 2015). Glycoside compounds are used in the treatment of congestive cardiac insufficiency and cardiac arrhythmia (Mohan and Krishna, 2019).

Regarding the terpenoids, they have been shown to be useful in the prevention and treatment of cancer and also possess antimicrobial, antifungal, antiparasitic, antiviral, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulating properties (Rabi *et al.*, 2009; Shah *et al.*, 2009).

Fig. 2. Fresh leaves of Moringa oleifera lam.

Determination of total polyphenols and flavonoids

The levels of total polyphenols and flavonoids are presented in Table 2. The results showed that the levels of total polyphenols and flavonoids have been varied according to the sources of MO. Other studies have shown the variation in the rate of these metabolites in different areas (Zakawa *et al.*, 2020; Sreelatha and Padma, 2009; Ndhlala *et al.*, 2014).

Thus, we obtained the highest levels of total polyphenols and flavonoids with the *Moringa oleifer*a from Segou (Sudanian zone), followed by those from Timbuktu (Saharan zone) and Mopti (Sahelian zone). The sample from Sikasso (Sudano-Guinean zone) had the lowest levels of total polyphenols and flavonoids.

According to Bajalan *et al.*, (2016), the variation in the levels of these compounds could possibly be explained by both genetic variation and the geographical origins of the plants. This phenomenon would also be linked to adverse environmental conditions. (Bale *et al.*, 2015; Ndhlala *et al.*, 2014).

In vitro Antioxidant activity

A comparative study in order to determine the variation in the antioxidant activity of *Moringa oleifera* leaf extracts in different climatic zones of Mali was carried out. The results (figure 3) show that there is a significant difference between all samples (p-value = 0.000002 < 0.05).

The Segou sample had the highest antioxidant activity with IC_{50} of 27.79 ± 0.09 µg/mL, followed by the Timbuktu and Mopti ones with respectively IC_{50} of 35.25 ± 1.44 µg/mL and 36.44 ± 1.12 µg/mL.

The leaves of *Moringa oleifera* from these three localities showed higher antioxidant activities than these of ascorbic acid used as a standard control with IC_{50} value of 36.72 ± 0.11 µg/mL. The sample from Sikasso was the most active with IC_{50} of 38.19 ± 1.43 µg/mL.

However, statistical tests shown that there is no significant difference between the samples of Mopti and Timbuktu on the one hand (p-value = 0.191) and Mopti and Sikasso on the other hand (p-value = 0.067). Also the antioxidant activity of the Sikasso sample is similar to that of the standard (p-value = 0.114).

These data revealed the capacity of *Moringa oleifera* leaf extracts to eliminate free radicals. That would justify its use in the prevention of diseases related to the oxidative stress.

In a study evaluating the variation in antioxidant activity of thirteen cultivars of Moringa oleifera from different parts of the world, Ndhlala *et al.*, (2014) obtained IC_{50} ranging from 14.57 to 32.56 µg/mL. Sy *et al.*, (2018) recorded an IC_{50} of 87.86 µg/mL with *Moringa oleifera* leaves from the city of Dakar in Senegal. Several studies have established a correlation between the content of phenolic compounds and the antioxidant activity of plant extracts (Fofié *et al.*, 2017; Togola *et al.*, 2019; Bagewadi *et al.*, 2019; Konaré *et al.*, 2020). However, the antioxidant activity of *Moringa oleifera*. Can't

always be associated only with levels of phenolic compounds (Ndhlala *et al.*, 2014). That is confirmed by the results obtained with the samples of Sikasso and Mopti which have presented statistically different levels of phenolic compounds (p-value = 0.007 < 0.05) but identical antioxidant activities (p-value = 0.067 > 0.05).

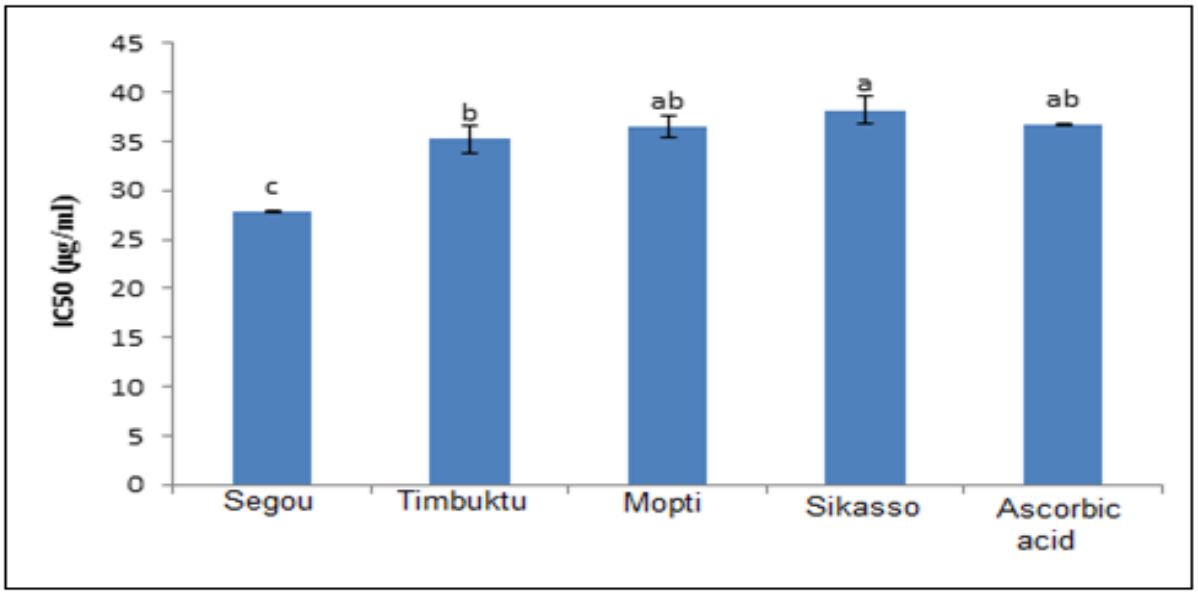

Fig. 3. IC_{50} variations of samples and standard (Ascorbic acid).
*Averages not sharing any letters are significantly different.

Conclusion

A comparative study of the phytochemistry and antioxidant potential of Moringa oleifera Lam leaves harvested in four climatic zones of Mali was carried out. The sample from Segou (Sudanian zone) showed the highest content of total phenolic compounds and flavonoids and the highest antioxidant activity.

The ones from other climatic zones of Mali showed the similar antioxidant activity which is consistent to that of vitamin C. *Moringa oleifera* leaves of from Mali are therefore excellent source of bioactive compounds and their antioxidant. These compounds would therefore contribute to protect adequately the population against the diseases due to the metabolic disorders and the malnutrition.

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