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(RESEARCH ARTICLE)

Effect of individual factors on the extraction of phenolic compounds from turmeric rhizomes (*Curcuma longa*) and moringa leaves (*Moringa oleifera*)

Bosson Jean-Aimé ASSANVO [1], William Kwithony DISSEKA [1,*], Yves DJINA [1], Martin Luthère King N'GBO [2], Koué Stéphanie Vianney DAYORO [1] and Meuwiah Betty FAULET-AHONZO [1]

[1] *Laboratory of Biocatalysis and Bioprocessing, Training and Research Unit Food Science and Technology, Université NANGUI ABROGOUA, Abidjan, Côte d'Ivoire.*
[2] *Training and Research Unit Agriculture, Fisheries Resources and Agro-Industries, Université de San-Pédro, San-Pédro, Côte d'Ivoire.*

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Abstract

Phenolic compounds are of great interest for their high antioxidant power and their numerous health benefits. These biomolecules are present in large quantities in many food and medicinal plants, such as the rhizomes of *Curcuma longa* and the leaves of *Moringa oleifera*. However, extraction methods for these bioactive compounds underestimate the plant's potential. The aim of this study was therefore to evaluate the influence of seven factors involved in the process of extracting phenolic compounds from *Curcuma longa* rhizomes and *Moringa oleifera* leaves. The seven main factors, i.e. drying time, mesh size, ethanol concentration, solvent/sample ratio, stirring speed, temperature and extraction time, influence the extraction of total polyphenols from *Curcuma longa* rhizomes and *Moringa oleifera* leaves. However, the optimum conditions for sample drying time are 48 to 72 h for Curcuma longa rhizomes and 60 to 72 h for *Moringa oleifera* leaves. Those for mesh size and sample/solvent ratio are 250 to 500 µm and 1/20 to 1/10 respectively. The best solvent concentration was between 60 and 80% for *Curcuma longa* rhizomes and between 40 and 60% for *Moringa oleifera* leaves. In addition, the best stirring speed is between 200 and 300 rpm and the best extraction time between 15 and 30 min. In addition, the best extraction temperatures are between 40 and 50°C for *Curcuma longa* rhizomes and between 50 and 60°C for *Moringa oleifera* leaves. These factors should therefore be taken into account for maximum extraction of total polyphenols from *Curcuma longa* rhizomes and *Moringa oleifera* leaves.

Keywords: Total polyphenols; Bioactive compounds*; Curcuma longa*; *Moringa oleifera*; Extraction conditions.

1. Introduction

Food and medicinal plants are playing an increasingly important role in people's daily lives. According to data from the World Health Organization (WHO), more than 80% of the world's population turns to these natural resources to satisfy their nutritional and health needs, not only for their affordable cost and availability but also for their recognised efficacy [1]. Furthermore, in this age of advances in organic chemistry, it is clear that more than 25% of medicines prescribed in industrialised countries derive their active ingredients directly or indirectly from plants [2]. These therapeutic effects are due to the bioactive compounds present in plants, such as polyphenols, flavonoids and tannins [1,2,3]. These are natural antioxidants that are attracting increasing interest for their potential use in the prevention and treatment of inflammatory and metabolic diseases such as diabetes, hypertension and obesity. [4]. In addition, extracts of secondary plant metabolites have been particularly studied because of their use in pharmaceuticals, cosmetics and food for their beneficial effects on health [2,4].

* Corresponding author: William Kwithony DISSEKA.

Curcuma longa and *Moringa oleifera* are two plants used in food and traditional medicine in Côte d'Ivoire. [5,6,7]. They are distinguished by their hypoglycaemic properties due to their ability to influence the metabolism and assimilation of glucose by the body [7,8,9]. The rhizomes of *Curcuma longa* contains curcumin, a component known for its anti-inflammatory, antioxidant and anti-diabetic properties [10,11]. On the other hand, *Moringa oleifera* leaves extracts contain a diversity of secondary metabolites, including glucosinolates, isothiocyanates, flavonoids, terpenoids and phenolic acids responsible for its hypoglycaemic and antihyperglycaemic properties [9,12].

However, in traditional Ivorian medicine, the extraction of these bioactive compounds is often carried out without taking into account the various factors involved in the process. These include the drying time of the plant matrix, the particle size of the powder, the solvent concentration, the ratio of material/solvent, the speed, the temperature and the extraction time [3,13,14]. These factors, which come into play during the various conventional extraction processes (maceration, infusion and decoction) and which are not taken into account, affect the food or therapeutic quality of the extracts [3,15]. Thus, the general aim of this work is to assess the influence of seven factors involved in the process of extracting phenolic compounds from *Curcuma longa* rhizomes and *Moringa oleifera leaves*.

2. Materials and methods

2.1. Plant materials

The plant material used in this study consisted of turmeric (*Curcuma longa*) rhizomes (Figure 1A) and moringa (*Moringa oleifera*) leaves (Figure 1B). These materials were harvested from well-kept village plots in Dabou (Côte d'Ivoire) and Hermankono-Garo (Côte d'Ivoire) respectively. Once harvested, the plant materials were sent to the Laboratory of Biocatalysis and Bioprocessing of the University NANGUI ABROGOUA (Abidjan, Côte d'Ivoire) for analysis.

Figure 1 Different plant materials used

(A) = *Curcuma longa* rhizomes; (B) = *Moringa oleifera* leaves

2.2. Methods

2.2.1. Preparation of samples

Production of *Curcuma longa* rhizomes powders

The *Curcuma longa* rhizomes were first sorted and cleaned of any foreign matter (insects, sand, etc.). They were then weighed and washed thoroughly with tap water and distilled water. Once drained, they were sliced and divided into several batches. The lamellae obtained were dried at room temperature at various times, then ground using a MOULINEX-type blender (Normandy, France) and sieved to different mesh sizes.

. Production of *Moringa oleifera* leaves powders

The *Moringa oleifera* leaves were sorted and cleaned of all foreign matter (insects, sand, etc.), defoliated, weighed and then washed thoroughly with tap water and distilled water. The leaves were drained, then weighed in batches and left to dry at room temperature for various lengths of time. The dry leaves obtained were ground using a MOULINEX type blender (Normandy, France) and then sieved to different mesh sizes.

2.2.2. Variation in the main factors

Seven (7) main factors were taken into account in this study. These were drying time (24, 48 and 72 h for *Curcuma longa* rhizomes and, 36, 48 and 60 h for *Moringa oleifera* leaves), mesh size (unsieved (NT), 1000, 750, 500 and 250 µm), ethanol concentration (0, 20, 40, 60, 80 and 100%), material/solvent ratio (1/10, 1/20, 1/30, 1/40 and 1/50), stirring speed (0, 100, 200, 300, 400 and 500 rpm), temperature (room temperature (TA), 40, 50, 60, 70 and 80°C) and extraction time (15, 30, 60, 90, 120 min). These factors were considered to determine the best extraction conditions for phenolic compounds.

In practice, the factor to be studied was varied and the other factors were fixed.

2.2.3. Determination of total polyphenol content

Total polyphenol extraction method

Total polyphenol were extracted by maceration in the extraction solvent. A quantity of sieved, dried *Curcuma longa* rhizomes or *Moringa oleifera* leaves powders was introduced into a 250 mL flat-bottomed flask containing 50 mL of solvent with different ethanol ratios and concentrations. The whole mixture was heated on a RET control-visc digital hot plate, IKA LABORTECHNIK (Germany) at different extraction speeds, temperatures and times. All extractions were performed in triplicate. The resulting mixture was filtered through Wattman paper (110 mm diameter) and the extract obtained was used to determine the total polyphenol.

Determination of total polyphenols

The total polyphenol content was determined using the method described by Singleton et al. [16]. One (1) mL of each extract was placed in a test tube and one (1) mL of Folin-Ciocalteu reagent (diluted 1:10, V/V) was added. The tube was left to stand on the bench for three (3) min and then one (1) mL of a 20% (w/v) sodium carbonate solution was added. The contents of the tube were made up to 10 mL with distilled water and the whole set was placed in the dark (protected from light) for 30 min. The optical density was read at 725 nm using a spectrophotometer (UV/VIS Spectophometer, Europe) against a blank. The quantity of polyphenols was determined using a standard curve established from a solution of gallic acid at an initial concentration of 1 mg/mL.

2.2.4. Statistical tests

The data collected was entered into an Excel spreadsheet. Statistical processing was then carried out using STATISTICA software (version 7.1). Statistically significant differences were identified using a one-factor analysis of variance (ANOVA) followed by Duncan's test. Statistical significance was defined at the 5% level.

3. Results

3.1. Effect of drying time on the total polyphenols content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves extracts

The total polyphenol content of *Curcuma longa* rhizomes extracts and that of *Moringa oleifera* leaves extracts increased significantly during drying ($p<0.05$). The highest total polyphenols content in *Curcuma longa* rhizomes extracts (1.09 ± 0.01 mgGAE/mL) was obtained at 72 hour drying (Figure 2A). On the other hand, *Moringa oleifera* leaves extracts showed the highest content after 60 hour drying (Figure 2B).

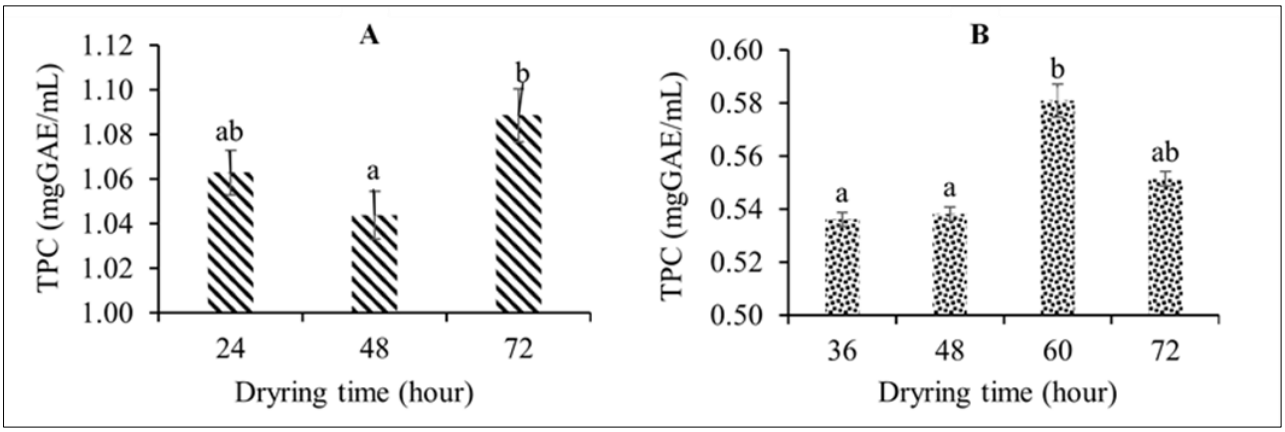

Figure 2 Total polyphenol content (TPC) of extracts of *Curcuma longa* rhizomes (A) and *Moringa oleifera* leaves (B) at different drying times

Histograms with a different letter are significantly different (P<0.05).

3.2. Effect of sieve mesh size on the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves extracts

The total polyphenol content of extracts of *Curcuma longa* rhizomes and *Moringa oleifera* leaves decreased significantly at the 5% threshold with increasing sieve mesh size. In the extracts of *Curcuma longa* rhizomes powder. the total polyphenol content fell from 1.20 ± 0.02 mgGAE/mL to 1.11 ± 0.02 mgGAE/mL for the 250 µm and 750 µm powders respectively (Figure 3A). For *Moringa oleifera* leaves powder, the highest total polyphenol content (0.62 ± 0.00 mgGAE/mL) was obtained with 250 µm mesh and the lowest (0.57 ± 0.00 mgGAE/mL) with 750 µm mesh (Figure 3B).

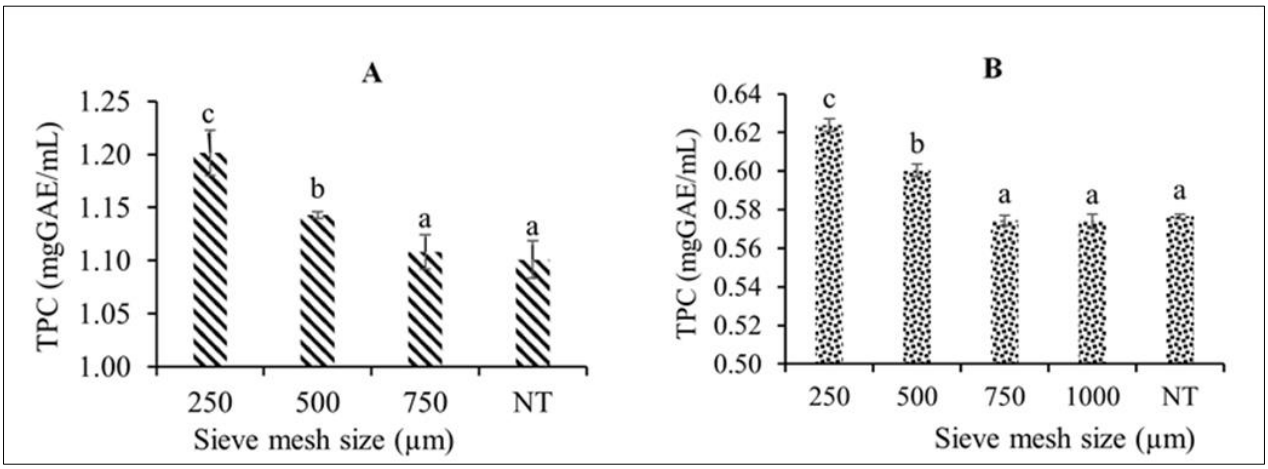

Figure 3 Total polyphenol content (TPC) of extracts of *Curcuma longa* rhizomes (A) and *Moringa oleifera* leaves (B) at different sieve mesh sizes

NT= unsieved ; Histograms with a different letter are significantly different (P<0.05).

3.3. Effect of ethanol concentration on the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves extracts

The ethanol concentration had a significant influence ($p < 0.05$) on the total polyphenol content of extracts of *Curcuma longa* rhizomes and *Moringa oleifera* leaves powders.

The total polyphenol content of extracts of *Curcuma longa* rhizomes powder increased from 0.30 ± 0.04 mgGAE/mL to 1.53 ± 0.04 mgGAE/mL when the ethanol concentration was increased from 0 to 80%. Thereafter, this content falls to reach a value of 1.44 ± 0.01 mgGAE/mL at 100% (Figure 4A).

The total polyphenol content of *Moringa oleifera* leaves powder extracts increased with increasing ethanol concentration, reaching a maximum value of 0.90 ± 0.00 mgGAE/mL at 60% ethanol. Subsequently, a reduction of around 14.45% and 84.45% of the maximum content was observed at ethanol concentrations of 80% and 100% respectively (Figure 4B).

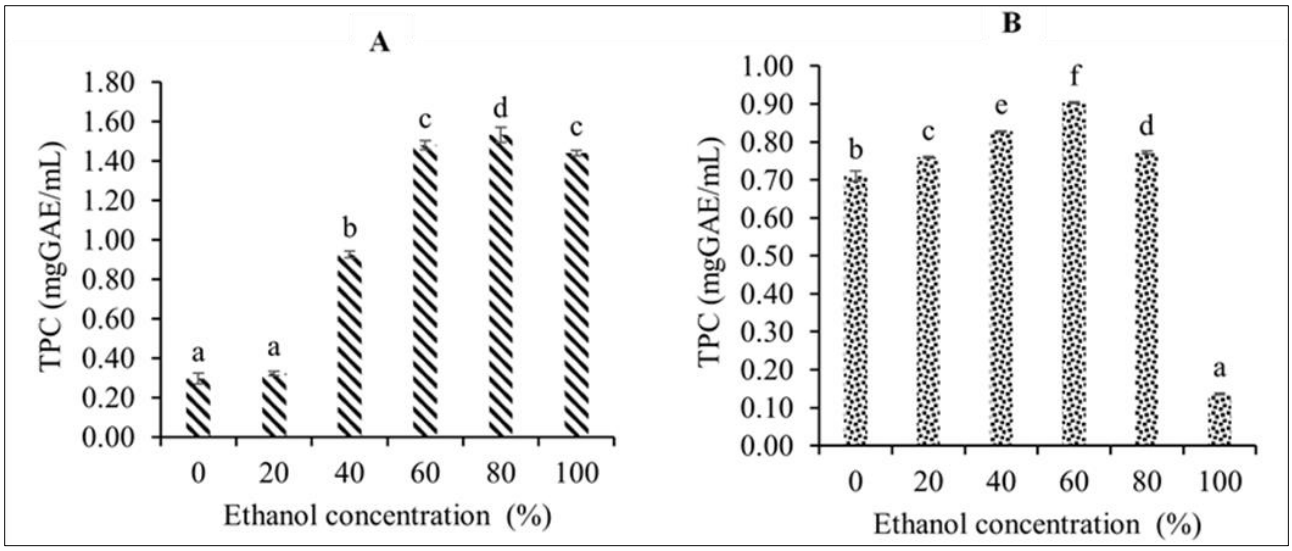

Figure 4 Total polyphenol content (TPC) of extracts of *Curcuma longa* rhizomes (A) and *Moringa oleifera* leaves (B) at different ethanol concentrations

Histograms with a different letter are significantly different (P<0.05).

3.4. Effect of the material/solvent ratio on the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves extracts

The total polyphenol content of *Curcuma longa* rhizomes powder decreased significantly at the 5% threshold (from 1.33 ± 0.01 to 0.80 ± 0.0 mgGAE/mL) as the M/S ratio decreased (Figure 5A). Thus, the highest total polyphenol content (1.33 ± 0.01 mgGAE/mL) is found at the 1/10 ratio and the lowest content (0.80 ± 0.01 mgGAE/mL) at the 1/50 ratio.

Similarly, for *Moringa oleifera* leaf powder extracts, the total polyphenol content decreased significantly ($p<0.05$) with the M/S ratio (Figure 5B). The 1.29 ± 0.01 mgGAE/mL content was highest for the 1/10 ratio and lowest (0.43 ± 0.00 mgGAE/mL) for the 1/50 ratio.

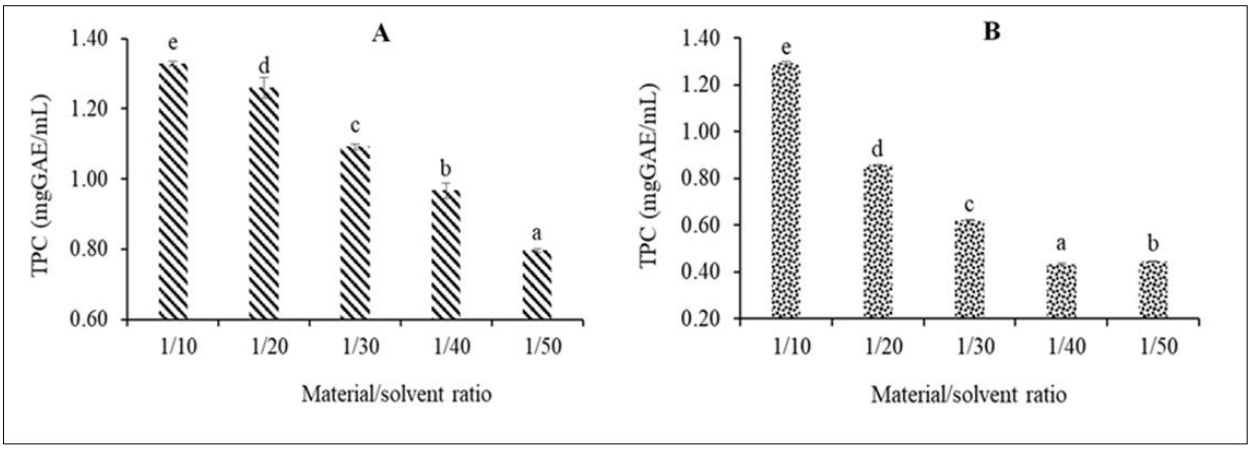

Figure 5 Total polyphenol content (TPC) of extracts of *Curcuma longa* rhizomes (A) and *Moringa oleifera* leaves (B) at different material/solvent ratios

Histograms with a different letter are significantly different (P<0.05).

3.5. Effect of stirring speed on the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves extracts

An increase in the total polyphenol content of *Curcuma longa* rhizomes extracts was observed at stirring speeds between 0 rpm (1.25 ± 0.00 mgGAE/mL) and 300 rpm (1.29 ± 0.02 mgGAE/mL) (Figure 6A). Above this value (300 rpm), there was a decrease in this content. The total polyphenol content of *Moringa oleifera* leaf powder extracts varied significantly ($p < 0.05$) with stirring speed (Figure 6B). This content increased when the extract was subjected to a stirring speed ranging from 0 rpm (1.16 ± 0.00 mgGAE/mL) to 200 rpm (1.35 ± 0.00 mgGAE/mL). The content then decreased to 1.08 ± 0.00 mgGAE/mL at a stirring speed of 500 rpm (Figure 6B).

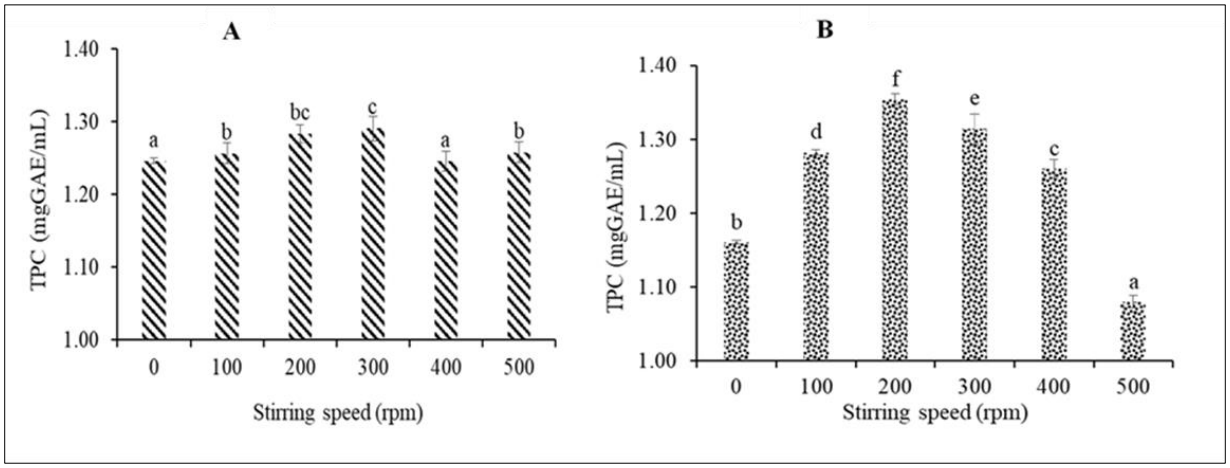

Figure 6 Total polyphenol content (TPC) of *Curcuma longa* rhizomes (A) and *Moringa oleifera* leaves (B) extracts at different stirring speeds

Histograms with a different letter are significantly different (P<0.05).

3.6. Effect of extraction temperature on the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves extracts

The total polyphenol content of *Curcuma longa* rhizomes extracts decreased significantly at the 5% threshold in proportion to the increase in extraction temperature. These values ranged from 1.48 ± 0.00 mgGAE/mL to 1.44 ± 0.01 mgGAE/mL (Figure 7A). In *Moringa oleifera* leaf powder extracts, the total polyphenol content increased to reach a maximum value of 1.31 ± 0.00 mgGAE/mL at a temperature of 60°C, then decreased above 60°C (Figure 7B).

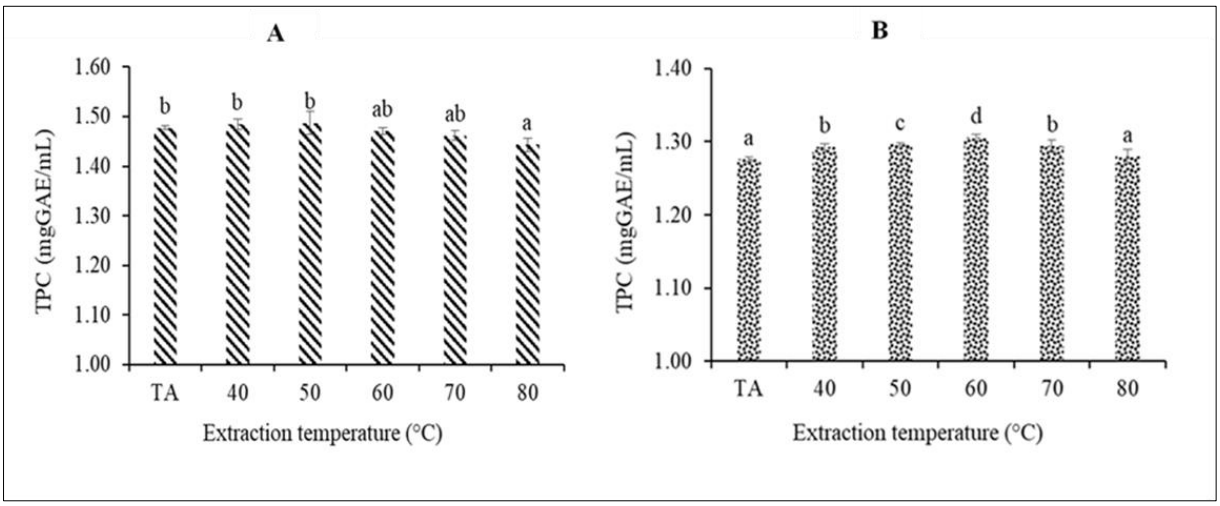

Figure 7 Total polyphenol content (TPC) of *Curcuma longa* rhizomes (A) and *Moringa oleifera* leaves (B) extracts at different extraction temperatures

TA = Room temperature (25 ± 2°C) ; Histograms with a different letter are significantly different (P<0.05).

3.7. Effect of extraction time on the total polyphenol content of Curcuma longa rhizomes and Moringa oleifera leaves extracts

The total polyphenol content of *Curcuma longa* rhizomes extract and *Moringa oleifera* leaves powder varied significantly at the 5% threshold (Figure 8). The total polyphenol content of extracts of *Curcuma longa* rhizomes powder increased from 1.38 ± 0.01 mg/mL to 1.47 ± 0.00 mg/mL at 15 and 30 min of extraction, respectively. This value then fell to a minimum of 1.31 ± 0.00 mg/mL after 120 min of extraction (Figure 8A). The total polyphenol content of *Moringa oleifera* leaves powder extracts increased from 1.34 ± 0.01 to 1.38 ± 0.00 mg/mL from 15 to 30 min respectively (Figure 8B). After 30 min of extraction, a decrease in total polyphenol content was observed until the 120th min of extraction.

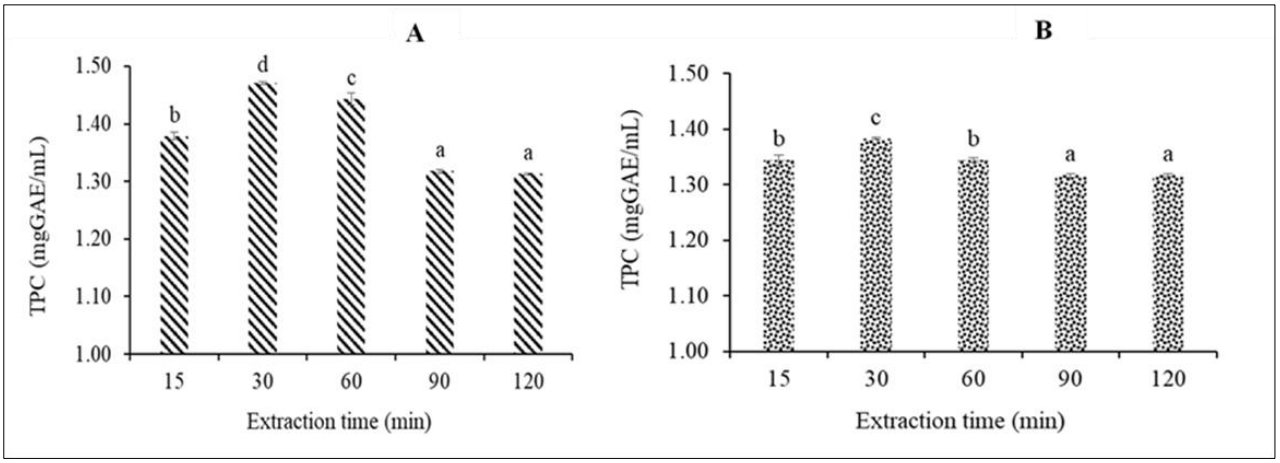

Figure 8 Total polyphenol content (TPC) of *Curcuma longa* rhizomes (A) and *Moringa oleifera* leaves (B) extracts at different extraction times

Histograms with a different letter are significantly different (P<0.05).

4. Discussion

Phenolic compounds are of great interest in the management of metabolic diseases. Indeed, they are good scavengers of free radicals and exhibit strong antioxidant activity [17]. Furthermore, according to Fahmy et al. [12] and Leone et al. [9], these compounds reduce plasma glucose levels and improve glucose tolerance through their hypoglycaemic effects. However, the extraction of these phenolic compounds remains problematic as it differs from one plant to another and is strongly influenced by several factors [3,18].

The increase in total polyphenol content in extracts of *Curcuma longa* rhizomes and *Moringa oleifera* leaves during drying could be explained by the evaporation of water from the plant matrix, which would have led to a concentration of these compounds in the samples [19]. These results corroborate those of Pham et al. [14], when extracting phenolic compounds from *Codonopsis javanica* roots.

The mesh size of *Curcuma longa* rhizomes and *Moringa oleifera* leaves powders is also a factor influencing the phenolic compound extraction process. The small mesh size of the samples resulted in high extraction of total polyphenols from the extracts of *Curcuma longa* rhizomes and *Moringa oleifera* leaves powders. Indeed, the fine particles of the samples would increase the contact surface of the samples with the solvent and consequently the diffusion of the compounds within the extraction solvent [14,20].

The concentration of the ethanol solvent has a huge effect on extraction efficiency. In fact, the increase in the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves due to increasingly high concentrations of ethanol up to 60% is thought to be the cause of the increase in ethanol polarity with the addition of water [21]. According to Mohammedi and Atik [22], ethanol extraction power is optimal at concentrations close to 70%. However, the decrease in the total polyphenol content of extracts above 60% ethanol would be due to the decrease in its polarity with a low proportion of water. These results corroborate those of Chan et al. [17] and Bouterfas et al. [23], who worked on *Centellas asiatica* and *Marrubium vulgare* respectively. Furthermore, the high polyphenol content of C. longa extracts at 80% ethanol can be explained by the solubility of the major phenolic compound (curcumin) in organic solvents such as ethanol [24].

The material/solvent ratio also affects compound dissolution and therefore extraction efficiency. Indeed, the decrease in extraction yield of total polyphenols as a function of the ratio could be explained by saturation and the decrease in the quantity of samples in the extraction solvent. Similar results have already been obtained by Le et al. [25] and Al-Hatim et al [21] for the extraction of anthocyanins from *Carissa carandas* fruits and total polyphenols from tea leaves respectively.

The increase in the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves extracts with agitation speed is thought to be due to an increase in the exchange surface between the plant matrix and the extraction solvent [14]. However, the drop in total polyphenol content from 200 rpm onwards is explained by the high agitation, which causes a loss of solvent and consequently a decrease in extraction efficiency [26].

Furthermore, according to Maillard and Breset [27] and Antony and Farid [28], the increase in total polyphenol content of *Moringa oleifera* leaves extracts at extraction temperatures below 60°C could be explained by hydrolysis of the bonds between lignin and phenolic acids or by degradation of the lignin at elevated temperatures. Furthermore, these results corroborate those of Teh and Birch [29] who observed an increase in the total polyphenol content of hemp and canola seed cakes at temperatures between 50 and 60°C. However, the decrease in total polyphenol content at temperatures above 60°C in *Moringa oleifera* leaves extracts and during the study in *Curcuma longa* rhizomes extracts could be explained by the thermosensitivity of certain flavonoid derivatives such as quercetin and catechin [30,31,32]. These results corroborate those obtained by Pham et al. [14] who obtained similar results in their work on *Codonopsis javanica* root extract.

The decrease in total polyphenol content in extracts of *Curcuma longa* rhizomes and *Moringa oleifera* leaves at extraction times of less than 30 min can be explained by the dissolution of these compounds in the extraction medium [33]. However, the drop in total polyphenol content at extraction times greater than 30 min could be explained by oxidation of the polyphenols due to their prolonged exposure to temperature and oxygen [34].

5. Conclusion

The extraction of phenolic compounds is a crucial step for the valorisation of these active ingredients because, it strongly depends on the extraction method. This study determined the influence of extraction conditions on the total polyphenol content of *Curcuma longa* rhizomes and *Moringa oleifera* leaves. The study showed that the longer the drying time for the various plant organs, the higher their total polyphenol content. In addition, increasing the mesh size and the E/S ratio reduced this content. On the other hand, ethanol concentrations of 80% and 60% gave the best extractions of total polyphenols for *Curcuma longa* rhizomes and *Moringa oleifera* leaves respectively. The best stirring speeds and times for extracting total polyphenols from *Curcuma longa* rhizomes and *Moringa oleifera* leaves were between 200 and 300 rpm and between 15 and 30 min, respectively. In addition, the optimum extraction temperatures for total polyphenols are 40 to 50°C for *Curcuma longa* rhizomes and 50 to 60°C for *Moringa oleifera* leaves.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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