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CHEMICAL STUDY AND EVALUATION OF THE INFLUENCE OF TWO PHYSICAL PARAMETERS ON POLYPHENOLS EXTRACTION FROM *CARAPA PROCERA* LEAVES.

Gbohaïda Virginie[1], Agbangnan D. C. Pascal[1]*, Nonviho Guévara[1], Gnansounou Martial[2], Bothon F. T. Diane[2], Bogninou G. S. Reine[2], Avlessi Félicien[2], Sohounhloué C. K. Dominique[1]

[1]URIM/LERCA/EPAC/UAC, 01 BP 2009 Cotonou, Republic of Benin.
[2]UREV/LERCA/EPAC/UAC, 01 BP 2009 Cotonou, Republic of Benin.

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***Corresponding Author**
Dr. Agbangnan D. C. Pascal
URIM/LERCA/EPAC/UA,
01 BP 2009 Cotonou,
Republic of Benin.

ABSTRACT

Background: Various specific biochemical properties of polyphenols are known and their study increasingly attracts the attention of scientists and researchers all over the world. But their extraction from the plant matrix is the major obstacle to their availability. **Objective:** Present work was carried out to study the extraction kinetic of bioactive polyphenols. **Material and Methods:** We evaluated the influence of two physical parameters, (particle size and temperature) on the bioactive polyphenols extraction kinetic from *Carapa procera* leaves. This plant is more known in Benin's traditional pharmacopoeia for the treatment of common affections. **Results:** The phytochemical screening revealed the presence of several secondary metabolites such as saponins, coumarins, reducing sugars, combined anthraquinones and polyphenols (anthocyanins, catechic tannins and flavonoids). *Carapa procera* leaves contain very few leucoanthocyanins, proteins and alkaloids, while the mucilages and Gallic tannins aren't detected. The ethanolic extraction on *Carapa procera* leaves gave a yield of 17.8% and a phenolic compounds content of 62.28 $mg.g^{-1}$withanaverage antiradical power (IC_{50} = 0.16mg/mL) close to one of quercetin (IC_{50} = 0.1mg/mL). The granulometry's influence evaluation revealed that the extraction solvent diffuses more easily inside the small particles for extracting polyphenols molecules. Considering the high cost implicated by the temperature application and the disintegration risk of the compounds extracted, 50°C might be according to our results, the optimal temperature for better extraction yield of polyphenols from *Carapa procera* leaves.

Conclusion: Physical parameters influence revealed that the extraction solvent diffuses more easily inside the small particles at 50°C to extract polyphenol molecules.

KEYWORDS: phytochemical screening, polyphenols, extraction, granulometry, temperature.

INTRODUCTION

Fromthe Meliaceae family, *Carapa procera* is a large tree reaching 30 to 35 m high and 1m in diameter. Belonging to the family of Meliaceae its young foliage is red. The paripinnate leaves covering 8-16 leaflets end with an aborted leaf bud. The accumulation of spine ground is characteristic of the species. The stem bark tends to flake in rectangular plates. Fruit ripening requires one year and is in open capsule containing large pyramidal seeds dispersed by rodents. Its wood has a pleasant smell typical of Meliaceae.[1] Originally from the west coast of tropical Africa, this plant is found in South America in Brazil, on the Guyana Shield, in West Africa and Central Africa from Senegal to Angola.[2] It is usually used in African villages in the development of local treatments such as malaria, skin diseases,.[3] Despite the many virtues recognized to this plant in traditional medicine, very little data have been reported in the literature regarding its phenolic composition and anti-radical activity.

The extraction of its secondary metabolites has never been subject of scientific investigation. In addition, thousands of scientific studies have examined the bioactive compounds from medicinal plants and found that polyphenols are their most important secondary metabolites.[4] Indeed, they are known for their antioxidant, anti-inflammatory, antifungal, antiviral and anticancer properties.[5] The antioxidants in our diet are, for the most, polyphenols. Over two hundred studies were conducted on the effect of plant consumption on health. Most of them showed a decrease of the risk factor for many diseases (heart, lung, colon, stomach, kidney, prostate and breast cancer.). Polyphenols having antioxidant activity are increasingly studied. Indeed, oxidation is a widely spread as well in food (lipid oxidation) and physiological (oxidative stress) phenomenon. Due to their antioxidant properties, polyphenols have the ability to scavenge free radicals, which are generated continuously by the body or formed in response to attacks from our environment (smoking, pollutants, infections.) and are, for the most, the base of the reduction of our perishable foodstuffs life.

But extraction is the most important step in the production and characterization of active ingredients from plant material. It is influenced by the extraction method selected according

to phytochemical compounds investigated. Several factors such as pH, temperature, amount of material to the volume of solvent, time intervals, particle size, number and steps of individual extraction, play an important role in this process.[6] Phenolic compounds are heat sensitive, so it is urgent to find suitable methods for making available these active principles. Thus, the main objective of this study is to evaluate the influence of the particle size and temperature on the kinetic of polyphenols extraction from *Carapa procera* leaves. But first, it is important to identify secondary metabolites present in the plant and assess its phenolic content as well as antioxidant properties of its polyphenols.

MATERIAL AND METHODS

Plant material

The plant material used in the present study is constituted of *Carapa procera*'s leaves collected at "Sakété" in the department of Plateau in Benin. After drying at the laboratory temperature (20°C±5°C) for stabilization of their mass and to avoid risk of polyphenols oxidation, the samples were reduced in powder to facilitate solvent penetration.

Methods

We used three different mesh sieves (300 µm, 600 µm and 1.18 mm) for particle size influence and a thermostat bath of refluxing equipped with thermocouple for temperature control during the extraction.

Large families of chemical compounds were detected according to the methods reported by Houghton.[7] For determining the yield and the phenolic compounds content, the solid-liquid extraction was performed by soaking in ethanol (95°) with a ratio of 5%. 50 g of dried plant material were mixed with 500 mL of solvent. The mixture is maintained under magnetic stirring for 24 hours at room temperature.[8] The solution obtained was filtered through Whatman N°1 paper (0.16 mm in diameter) under reduced pressure. The filtrate was then recovered and the operation was repeated 3 times (72 hours total extraction) with 250 mL of solvent from the second day. The total volume of the filtrate was concentrated under vacuum at 60°C on a rotavapor. The dry extract was then collected, weighed, labeled and stored at 4°C until use. The extraction yield was calculated using the following formula:

$$\mathbf{Y = [(M_{ext})/ M_{hd}] \times 100}.$$

Y: yield (%); Mext: extract mass; M_{hd}: herbal drug mass.

To quantify the phenolic compounds, the ethanol extract was assayed by colorimetric UV-Visible spectrophotometry; total polyphenols were measured by the Folin-Ciocalteu,[9] the total flavonoids by aluminium trichloride,[10] the condensed tannins by sulfuric vanillin[11] and anthocyanins by sodium sulphite.[12] The antiradical activity was determined by DPPH (2,2-diphenyl-1-pycrilhydrazil) method reported by Agbangnan *et al.*[13] The percentage of free radical scavenging DPPH was calculated using the formula:

$IP = [(A_{bl}-A_s)/A_{bl}] \times 100$.

IP:Inhibition percentage; A_{bl}: absorbance of the blank; A_s: absorbance of the sample.

For extraction kinetic study, the influence of two very critical physical parameters (size and temperature) was evaluated. Colorimetric method based on the use of UV-Visible spectrophotometer was used to monitor the polyphenols extraction kinetic from the plant material. 2g of the crushed (particle size 300 µm, 600µm or 1.18 mm) are macerated in 200 mL of distilled water. For the influence of the temperature, 5 different temperatures, 25°C to 125°C with a step of 25°C, were used. The polyphenols' diffusion in distilled water was observed through the color change of the medium over time, according to the particle size and temperature. Samples were then taken every 10 min until 1hour and polyphenols were quantified on colorimeter after filtration and adequate dilution (1/5). Distilled water was the reference solvent used as blank.[6]

RESULTS AND DISCUSSION

Phytochemical screening

Various secondary metabolites were identified in the plant by a series of reactions of precipitation and colouring more or less specific to each class of plant active ingredients. The results of phytochemical screening of *Carapa procera* leaves reported in table1 revealed the presence of saponins, anthocyanins, catechic tannins, flavonoids, coumarins, reducing sugars and combined anthraquinones. This organ of the plant contains very few leucoanthocyanins, proteins and alkaloids while the mucilage and Gallic tannins are not detected. Our results are similar to those of Adjè who identified in the leaves of *Carapa procera* the presence of chemical compounds such as anthocyanins, flavonols and phenolic acids.[14] Also, the investigations of Ononga *et al.* revealed in *Carapa procera* plant the presence of flavonoids, saponins and alkaloids. Tannins, quinones, steroids and terpenoids aren't detected.[15] According to the literature, environmental factors influence the production of secondary

metabolites in plants. The differences observed could be related to the fact that samples were collected in different regions and at different times of the year.[16]

Table 1: Metabolites identified in *Carapa procera* leaves

Class of substances		Results
Tannins	Total tannins	+
	Cathechic tannins	+
	Gallic tannins	-
Flavonoids	Anthocyanins	+
	Free flavonoids	+
	Leucoanthocyanins	±
Mucilage		-
Alkaloids		±
Sterols and terpenes		±
Proteins		±
Reducing sugars		+
Free quinones		-
Combined anthraquinones	O-heterosids	-
	Reduced genine O-heterosids	+
	C-heterosids	+
Coumarins		+
Saponins	Foam index	105

+ : Present ; ± : Trace; - : absent.

Phenolic content

The aqueous extract of *Carapa procera* leaves has shown an extraction yield of 17.8%. The results of quantitative analyses of phenolic compounds content in the extract of *Carapa procera* leaves are reported in Fig 1. It appears that the *Carapa procera* leaves extract is very rich in total polyphenols (62.28mg Gallic Acid equivalent/g of dry matter) and has an average content of condensed tannins (24.49 mg Catechin equivalent/g dry matter) and flavonoids (37.61 mg Catechin equivalent/g dry matter). It should be noted that this organ of the plant contains very few anthocyanins (1.92 mg Cyanidin equivalent/g dry matter). The investigations of Adje on *Carapa procera* leaves indicated yields of 5 to 10% powders of polyphenolic extracts (5.1-27.2 mg GAeq.g^{-1}) obtained by atomization.[14] The differences between our results and those of Bothon may be related to methods of extraction and quantification, which are two factors that may affect the phenolic content of plants.[17]

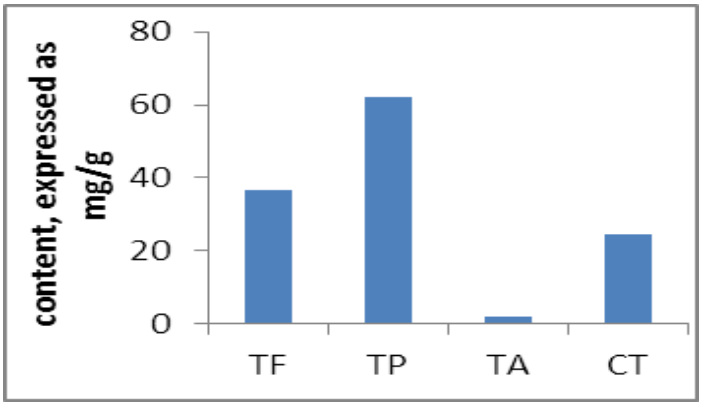

Fig 1: Phenolic composition of *Carapa procera* leaves extract *TF: total flavonoids; TP: total polyphenols; TA: total anthocyanins; CT: condensed tannins*

Antiradical activity

DPPH is the best, easiest and widely used method for testing preliminary free radical scavenging activity of a compound or a plant extract.[16] The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The radical scavenging activity of *Carapa procera* leaves' ethanolic extract was determined by DPPH method using quercetin as reference antioxidant. *Carapa procera*'s leaves ethanolic extract had an average antiradical power (IC_{50} = 0.16 mg/mL) close to one of quercetin (IC_{50} = 0.1 mg/mL). Adjè in his investigations, has also notified the antioxidant capacity determined by DPPH method of *Carapa procera* leaves.[14] We note a correlation between antiradical activity of our extracts and their phenolic content. This observation corroborates those already made earlier by Medoatinsa *and al.*[18] Several studies support the antiradical activity by the presence of total polyphenols.[19] The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[16]

Kinetic study

The absorption curves of aqueous extracts of *Carapa procera* leaves (Fig 2) revealed two peaks in the Ultra-Violet rang (240 nm and 279 nm) corresponding to the absorption of polyphenols in general.[20] Moreover, the perfect overlapping of curves recorded as a function

of time shows that the extraction of phenolic compounds remain the same regardless of the extraction time.

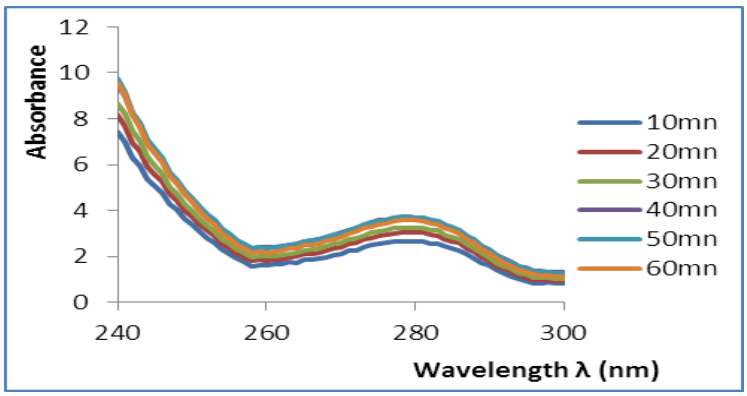

Fig 2: UV spectra of aqueous extracts from *Carapa procera* leaves

The graph TPI (Total Polyphenols Index) versus time (Fig 3) reflecting changes in the amount of polyphenols extracted versus time shows an increasing trend with a steep slope during the first 20 minutes. In addition, average growth was observed between 20 and 40 minutes. After 40 minutes, the extraction rate does not evolve practically.

In general, at first stage, the TPI increased fast, followed by a slow increment and then remained practically constant till the end of the process. This asymptotic behavior was found previously by other authors.[21, 22, 23]

Most of the phenolic compounds were therefore transferred from the vegetable matrix to the solvent during the first 20 minutes and 40 minutes would be sufficient for an almost complete extraction of the polyphenols from the leaves of *Carapa procera*. However, the extraction time can be extended up to 60 minutes without extracted compounds degradation risk.

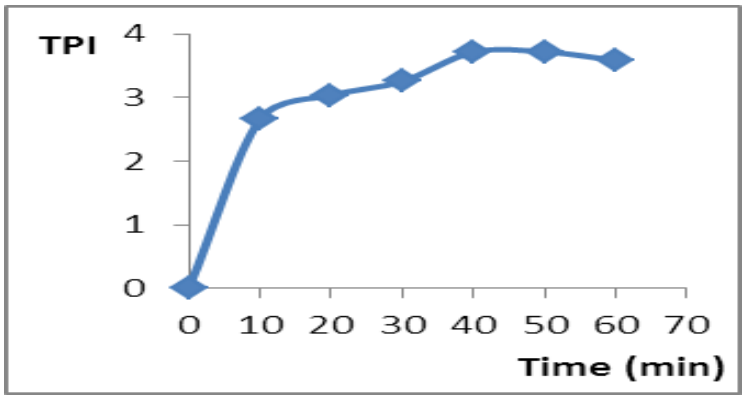

Fig 3: Polyphenol extraction kinetic of *Carapa procera* leaves

Influence of particle size

The results of the influence of particle size on the polyphenols extraction kinetic from *Carapa procera* leaves are shown in the graph of Fig 4. There was a high extraction rate of the particle size of 300 microns. While the other two sizes, we didn't observe a significant difference in the evolution of the extraction rate over time. This had be explained by the fact that the solvent diffuses more easily inside the small particles to extract polyphenols molecules. This confirms our previous results according to which, with the fine particles, chemical compounds are more easily transferred from the plant material to the extraction solvent.[8] Penchev, in his work on bioactive products also notified the significant influence of particle size on the extraction rate in the sense that, the best result is obtained with small particles, this due to their larger specific surface.[6] The finer particles thus have a greater solid-solvent contact surface.

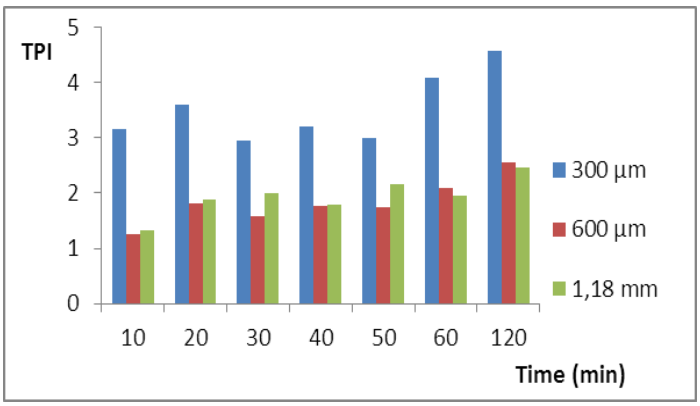

Fig 4: Influence of granulometry on the polyphenols extraction kinetic from *Carapa procera* leaves

Effect of temperature

The influence of temperature on the polyphenols extraction kinetic from *Carapa procera* leaves was carried out on the powder of particle size 300 microns to give the best extraction efficiency in consideration of particle size influence. The results obtained are shown in the graph of Fig 5. The shift range of the temperature used (25°C to 125°C) of the envelope values starting from room temperature (25°C) and up to above the boiling point of the solvent (100°C). The analysis of this graph showed that extraction rate of polyphenols increases with temperature up to 100°C and fall beyond. 100°C would be the maximum temperature tolerable by the extracted molecules would deteriorate beyond. Moreover, we noted that the polyphenols quantity extracted from the leaves of *Carapa procera* in 10 min at 50°C (IPT =

5.72) was higher than that obtained after 120 min at 25°C (IPT = 4.57). But beyond 50°C, the increase of the extraction yield in function of time is low indeed null.

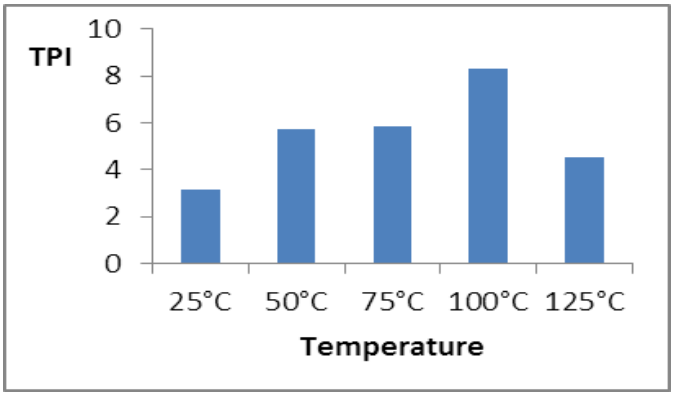

Fig 5: Influence of temperature on the kinetic of extraction of polyphenols from *Carapa procera* leaves

Considering the cost it would take to increase the temperature and the risk of degradation of the extracted compounds, 50°C would be according to our results, the optimal temperature for proper extraction of phenolic compounds from the leaves of *Carapa procera*. These results are consistent with those of Penchev *et al.* showed that the extraction rate increases with temperature and 50°C is the optimal temperature, since maintaining this temperature is more economic in terms of energy consumption.[24] The work of Penchev has also shown that 60°C is a reasonable value for temperature, preserving, on the one hand the active components of the plant with a thermal destruction and, secondly, ensuring intensive kinetic regime.[6] Agbangnan *et al.* have shown that increasing the temperature affects positively the extraction of polyphenols without necessarily offset the cost that would result from the application of this temperature increase.[20]

CONCLUSION

This study revealed the importance of controlling the extraction conditions such as temperature and particle size to obtain an extract with the highest polyphenol content with best antioxidant activity. The extraction solvent diffuses more easily within small particles to extract polyphenol molecules. 50°C is the optimum temperature for better extraction yield of polyphenols from the leaves of *Carapa procera*.

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