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PHYTOCHEMICAL STUDY AND ANTI-RADICAL POWER OF SEEDS OF TETRACARPIDIUM CONOPHORUM, PACHIRA GLABRA AND PENTACLETHRA MACROPHYLLA

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ABSTRACT

Plants are an essential source of molecules of interest capable of responding to a number of conditions including degenerative diseases mainly due to oxidative stress and those caused by micronutrient deficiencies. In Congo and everywhere else, man has always used natural resources to treat his ailments. Plants used in traditional medicine include *T. conophorum*, *P. glabra* and *P. macrophylla*. The methodology used in this work, based on qualitative research of chemical families, allowed us to identify terpenoids in *T. conophorum*. *P. glabra* extracts revealed the presence of flavonoids as well as tannins and polyphenols. Concerning *P. macrophylla*, the aqueous extract showed us the presence of all the chemical families sought except the alkaloids. From the quantitative point of view, the dosage of total polyphenols gave the following results: *P. macrophylla*: 160.73 mg EAG/gDM; *P. glabra*: 8.42 mg EAG/gDM; *T. conophorum*: 5.5 mg EAG/gMS. Regarding total flavonoids, we obtained: *P. macrophylla*: 0.48mgEG/gMS; *T. conophorum*: 0.133 mgEG/gMS; *P. glabra*: 0.183 mg EG/gMS. TLC to demonstrate anti-radical activity gave a positive result for each sample. The assay of anti-radical activity provided the following results with regard to CI 50: *T. conophorum*: 178.09 mg/ml; *P. glabra*: 45.82 mg/mL; *P. macrophylla*: 0.71 mg/mL. The almonds studied have a great phytotherapeutic benefit.

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INTRODUCTION

The Congolese ecosystem, one of the most diverse in Africa, is made up of several animal and plant species which the population uses to meet its health and nutrition needs. Natural products, particularly those of plant origin, have always been an important source of biomolecules of great interest. According to the World Health Organization, 80% of the world's population in developing countries relies primarily on traditional medicinal plants for their primary health care (Organisation mondiale de la santé, 2003). In Congo, the economic situation and increased poverty force many citizens to resort to plants to treat their various ailments. The renewed interest observed by Thomas Silou *et al.* (2004) for unconventional crops is also valid for reasons of traditional self-medication. Degenerative diseases that were once common only in Western countries have become commonplace in developing countries. The production of free radicals being the major cause of these conditions; However, this remains a natural phenomenon. In living organisms, the production of free radicals is a physiological process regulated through various chemical or enzymatic detoxification processes (Adida, 2016).

In fact, the body has its own means of defense allowing it to fight against these free radicals. When this protection system loses its effectiveness or when the number of free radicals increases significantly; oxidative stress occurs (Koechlin, 2006). Synthetic antioxidants such as butyl hydroxy anisole (BHA) and butyl hydroxy toluene (BHT) are certainly very effective, but likely to exhibit side and even toxic effects (Manian et al., 2008). To compensate for the side effects of synthetic products and their toxicities, scientists are faced with the obligation to resort to herbal medicine (Adida, 2016) *T. conophorum*, *P. glabra*, *P. macrophylla* respectively called "Nkah", "Ngouba ya Mputu", "Mpayi" according to the study areas in Congo are unconventional oilseeds belonging respectively to the families *Euphorbiaceae*, *Malvaceae*, *Fabaceae* (Legume Mimosoideae). These plants are present in most savannahs, bushes and forests, mainly in the plateaux region, the Cuvette Ouest, and in the department of Brazzaville where *P. glabra* is quite widespread. Previous studies that have been carried out on the biochemical composition of these almonds and their oils have shown that they are particularly rich from a nutritional point of view (Ossoko Jean Paul Latran, 2021; Enzonga Yoca, 2020 and Ossoko *et al.*, 2020). The results relating to their oils coupled with those of bibliographic

research have suggested a potential that until now has been very little exploited in Congo. Thus, the present work aims to study the phytochemistry of these plants, by carrying out tests, the dosage of total polyphenols and flavonoids, as well as the evaluation of the anti-radical activity of the different extracts of their almonds.

MATERIAL AND METHODS

Plant material: The plant material for our study consists of almonds extracted from the seeds of *T. conophorum*, *P. glabra* and *P. macrophylla*, collected respectively in Lékana, located in the Plateaux Department, in Manianga in the Brazzaville department, and in the forests of Ontogo in the Cuvette Department in the Republic of Congo.

Figure 1. ***Tetracarpidium conophorum*** **(a: Fruits; b: dried almonds; c: crushed almonds)**

Figure 2. ***Pachira glabra*** **(a: Pod; b: dried almonds; c: crushed almonds)**

METHODS

Preparation of the different extracts: To detect the presence of total polyphenols and flavonoids, three extracts were prepared: the aqueous extract (AE), the hydroethanolic extract (HE) and the ethanolic extract (EE). The extracts were prepared by mixing respectively 40 g of plant material and 80 ml of solvent (water, ethanol). The different solvents were water, ethanol and the water-ethanol mixture in the proportions 50 (v/v).

Figure 3. ***Pentaclethra macrophylla*** **(a: Seeds; b: dried almonds; c: crushed almonds)**

The mixtures were then macerated by stirring for 72 hours, then filtered on filter paper. The filtrates obtained were concentrated to dryness at 50°C under reduced pressure, using an N-1 rotary evaporator (Eyela, Tokyo Rikakikal co, ltd. Japan). Everything was then stored at room temperature pending analysis.

Highlighting some chemical families by tube reactions:In order to highlight the presence or absence of chemical families, we carried out specific phytochemical tests based on coloring, turbidity or precipitation reactions. Several families were searched. These are alkaloids, terpenoids, reducing compounds, total polyphenols and flavonoids.These analyzes were carried out in accordance with the protocols described in the literature (Békro et al., 2007) and summarized in Table 1 below:

Table 1. Characterization of chemical groups (MIAKAYIZILA BAONDA Snelle, 2011)

Family Chemical	Extract at test	Reagents	Reagent composition
Alkaloids	Acidified aqueous	Dragendorf	Bismuth nitrate + acetic acid
		Mayer	Potassium Iodide (KI) + Chloride mercury (HgCl2)
Saponosides	Aqueous	Foam index	
Tanins	Aqueous	Stiany	Hydrochloric acid + formalin
Quinones	Aqueous	Borntrager	Ammonia 10 %
Flavonoids	Ethanolic	Shinoda	Ethanol 95 % + HCl N/2
	Hydro Ethanolic	Ethanol/ hydrochloric acid (50%, V/V) + Mg + iso amyl alcohol	
Terpenes et sterols	Chloroformic	Lieberman	Sulfuric anhydride + acid concentrated sulfuric
Anthocyanin	Ethanolic	Hydrochloric acid 20%	
Heteroside cyanogenetic	Toluene – water	Picrosodized paper	
Heteroside cardiotonic	Chloroformic	Acetic anhydride + concentrated sulfuric acid	
Anthraquinone	Chloroformic	KOH 10 %	
Compounds reducers	Aqueous	Fehling Liqueur	
Mucilages	Aqueous	Ethanol	
Dare and holosides	Aqueous	Sulfuric acid + thymol saturated alcohol	

Demonstration of polyphenolic compounds by Thin Layer Chromatography: Polyphenolic compounds were determined by TLC on a 60 F254 silica gel plate on a 20 cm x 20 cm aluminum foil support from Merck. The eluent system used was ethyl acetate/formic

acid/water in the ratio 9/0.5/0.5. The chromatogram was revealed using Neu's solution (0.5 g of 2-amino diphenyl borinate + 0.5 g of PEG 400 + 100 mL of ethanol). The plates were observed under UV at 366 nm before and, in some cases, after revelation with Neu.

Determination of total polyphenols: The total phenol content of the different extracts of our plants was determined using the Folin-Ciocalteu method. The dry extracts previously obtained were resolubilized in a mixture of equal proportions of hydroethanolic solvent 50 (v/v). The masses and quantities of solvents (water and ethanol) were weighed so as to obtain stock solutions of 40 g/l for each plant extract.From the mother solutions, we prepared the daughter solutions of concentration: 20g/l, 10g/l, 5g/l, 2.5 g/l, 1.125g/l. To determine the polyphenol content of the extracts studied, we took 0.1 mL of each extract (hydroethanolic) to which 0.9 mL of distilled water was added followed by 0.9 mL of the Folin-Ciocalteu reagent. Immediately 0.2 mL of the sodium carbonate solution was added (20% Na_2CO_3). Said mixture was incubated at an ambient temperature of 25°C for approximately 40 minutes away from light.The absorbance was measured with a spectrophotometer at 725nm against a methanol solution used as a blank. The results obtained were expressed in mg gallic acid equivalent per gram of dry matter (EGA/gMs).

Dosage of the Anti-Radical Activity of the different extracts

Inhibition percentage: The evaluation of the anti-radical activity was carried out using 5 mL of the solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH at 10 mg in 250 mL of ethanol) and 100 µL of each extract diluted to a concentration of 0.625 to 20 or even 40 mg/mL, all mixed in EDTA type glass tubes. After 40 minutes of incubation in the dark, the anti-radical activity was measured in a spectrophotometer at 517 nm. The percentage of inhibition was calculated by the following relationship

$$\%\, I = \frac{D.O_{white} - D.O_{El}}{D.O_{white}} x100$$

With DO $_{white}$: 1.113; DO $_{El\ (Tc)}$: 1.023; DO $_{El\ (Pg)}$: 0.870; DO $_{El\ (Pm)}$: 0.788

Inhibitory concentration 50% (IC50): Taking into account the percentage of inhibition, the IC50 was determined by deduction from the variation curves of said percentage. The value of the anti-radical activity, such that y = 50%, corresponds to the inhibitory concentration of IC50 of the extract studied (Mensor *et al.,* 2001; Sanchez-Moreno et al., 1998 and Nouioua, 2012).

Table 2. Results of chemical screening of almond extracts

Plant species	Extract used	Alkaloids	Flavonoids	Reducing compounds	Tannins	Terpenes/ Sterols
T. conophorum	Aqueous	-	-	-	-	
	Hexanic					+
P. glabra	Ethanolic	-	+	-	+	
P. macrophylla	Aqueous	-	+	+	+	
	Hexanic					+

Legend: += present; - = absent;

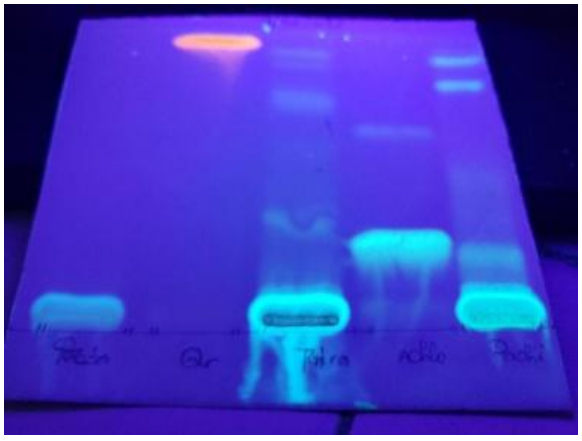

Figure 4. TLC of water/ethanol extracts (50% V/V) of *T. conophorum* (*Tc*), *P. glabra* (*Pg*), *P. macrophylla* (*Pm*); Eluent: Ethyl acetate / formic acid / water (9/0.5/0.5; v/v/v)

Assay of total flavonoids: From the previously prepared daughter solutions, the total flavonoid content of the different extracts was obtained using aluminum trichloride (AlCl3) and sodium nitrate ($NaNO_2$ at 5%) (Mansouri, 2000). In a 100 mL flask, 250 µL of each hydroethanolic extract were successively introduced. 1 mL of distilled water, 7.5 µL of sodium nitrate solution (5% $NaNO_2$); the mixture was allowed to sit for 5 minutes. Then, 75 µl of aluminum trichloride (10% $AlCl_3$) was added. After 6 minutes, 500 µL of sodium hydroxide NaOH with a concentration of 1N and 2.5 mL of distilled water were added successively to the mixture. Absorbance was measured with a UV spectrophotometer at 413 nm and the results were expressed as mg of catechin equivalent per gram of dry matter (ECa/gMs).

Evaluation of Anti-Radical Activity by Thin Layer Chromatography: Qualitative research aimed at revealing substances with antioxidant activity was carried out using the "bioautography" method by thin layer chromatography where the antiradical activity was revealed by DPPH, according to the method of Takao *et al.* (Takao *et al.,* 2010).

RESULTS AND DISCUSSION

Chemical families observed by reactions in tubes: The results of the tube reactions carried out to determine the major chemical families present in *T. conophorum*, *P. glabra*, *P.macrophylla* are reported in Table II below. Phytochemical tests carried out on the hydroethanolic and hexanic extracts of the three plants revealed disparate results. We note the presence of a single chemical family in the hexanic extract of *T. conophorum*: terpenoids. The aqueous extract revealed no alkaloids, reducing compounds or polyphenolic compounds. Furthermore, the ethanolic extracts of *P. glabra* revealed the presence of flavonoids as well as tannins; polyphenols and alkaloids being absent. Concerning *P. macrophylla*, the aqueous extract allowed us to note the presence of all the chemical families sought except the alkaloid family, terpenes were observed thanks to the hexanic extract. These results allowed us to distinguish the richness in certain secondary metabolites of the almonds of the selected plants. It should be noted that the chlorophyll parts of the plants are generally those which contain the most metabolites, the presence of these few

families suggests that these plants as a whole are richer in the aforementioned compounds.

Thin-layer chromatography

Research of Polyphenols: Figure 4 below shows the result of the qualitative research of polyphenols by thin layer chromatography. The chromatographic profile of the different hydro-ethanolic extracts in the presence of two (02) reference compounds (Quercetin and Chlorogenic Acid) obtained after exposure of the plate to the UV lamp (Figure 4) shows a succession of tasks materializing the presence of polyphenolic compounds. The orange-yellow and blue fluorescence spots at Rf (0.92 and 0.25) observed in the figure below correspond to the chosen reference compounds: quercetol and chlorogenic acid. *P. glabra* and *P. macrophylla* present a significant number of spots. The different spots corresponding to Rf: 0.1375; 0.237; 0.7875; 0.8625 for *P. glabra* and: 0.15; 0.3625; 0.7375; 0.8625; 0.9 for *P. macrophylla* show the diversity of polyphenolic compounds that the two plants include. Concerning *T. conophorum* we do not observe the formation of any spots. However, this result can be justified by the constitution of the eluent used, we can imply the presence of polyphenolic compounds which have not migrated and therefore have a zero frontal ratio.

Polyphenol and total flavonoid content: The content of polyphenols and flavonoids in the hydro-ethanolic extracts of *T. conophorum*, *P. glabra* and *P. macrophylla* is represented in the graph below.

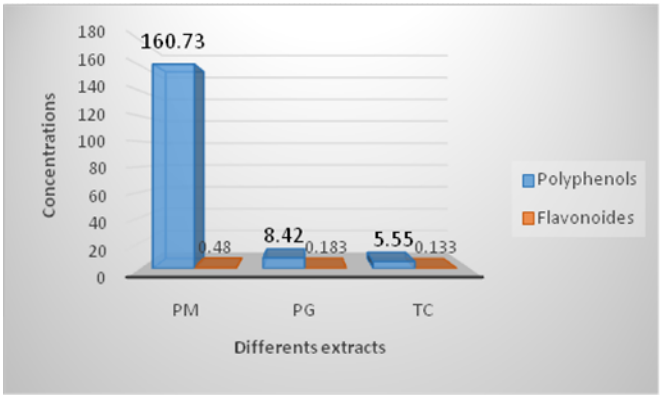

Figure 5. Determination of total polyphenols and flavonoids in hydro-ethanolic extracts

The results obtained during these analyzes showed varied contents of total polyphenols. The highest content is that of *P. macrophylla* 160.73 mg EAG/gMS followed by *P. glabra* 8.42 mgEAG/gMS and *T. conophorum* 5.5 mg EAG/gMS (Figure 5). While 2.92 mgEAG/gDM separates *P. glabra* from *T. conophorum*, the polyphenol content of *P. macrophylla* is almost three times higher than those of the other two plants. Concerning the flavonoid content in the different plants (Figure 5), we note that the highest level remains that of *P. macrophylla*: 0.48 mg/gDM. While the rest of the extracts contain 0.133 mgEG/gDM for *T. conophorum* and 0.183 mgEG/gDM for *P. glabra*.

These results corroborate with those obtained in thin layer chromatography and in the search for chemical families. In addition, we generally note that all the extracts are comparatively rich in polyphenols while their flavonoid content is low. This difference in content between the different compounds can be explained by the fact that total polyphenols include several groups of compounds including the flavonoids themselves. According to the literature, there is a particular affinity between alcohol and polyphenolic compounds. This affinity is supported by several studies (Abdille, 2005). This is because of the ability of alcohol to inhibit the action of polyphenol oxidase which causes the oxidation of polyphenols in plant tissues (Abdille *et al*., 2005). This information would justify the results obtained thanks to the hydro-methanolic extracts used.

Anti-Radical Activity of the different extracts

Thin layer chromatography

Anti-radical activity: The qualitative analysis of the antiradical activity of the hydroethanolic extracts of *T. conophorum*, *P. glabra*, as well as *P. macrophylla* was carried out by thin layer chromatography and revealed with DPPH. The results relating to this analysis can be observed on the chromatogram below (Figure 6).

Figure 6. TLC of water/ethanol extracts (50% V/V) of *T. conophorum* (*Tc*), *P. glabra* (*Pg*), *P. macrophylla* (*Pm*);

Eluent: Ethyl acetate / formic acid / water (9/0.5/0.5; v/v/v)

The anti-radical activity marked by the change of the DPPH reagent from purple to yellow, we notice on all the samples the formation of different spots of yellow color characteristic of positive antioxidant activity. The spots located at different heights demonstrate the diversity of families or chemical compounds exerting antioxidant activity in the almonds of the plants analyzed. *T. conophorum* seems to present only one chemical family also found in the two other samples (*P. glabra* and *P. macrophylla*) at Rf: 0.869. *P. glabra* presents two spots, both near the front line (Rf: 0.728 and Rf: 0.869), these strongly apolar compounds would probably be polyphenols or terpenoids. The greatest quantity of spots corresponds to the chromatogram of *P. macrophylla*. Located at variable heights (Rf: 0.217; 0.510; 0.597; 0.869), the almonds of *P. macrophylla* present a diversity of compounds with corresponding anti-radical activity.

Assay of antiradical activity

Percentage of DPPH radical inhibition: The results of the anti-radical activity of the different extracts on DPPH are presented in the series of Tables below. Tables III to V show, at the chosen concentrations, different optical densities characterized by the relative inhibition percentages. From 0.625 mg/ml to 40 mg/ml, it can be noted that the anti-radical activity values increase depending on the concentration of the extracts. At a concentration of 20 mg/ml, *P. glabra* presents an inhibition percentage of 21.83% higher than that of 19.38% obtained from *T. conophorum*. The free radical inhibitory activity is even stronger with *P. macrophylla* which has a concentration of 10 mg/ml at an inhibition percentage of 88.36%. This result is all the more remarkable because of the concentration used, for a concentration twice as low as that of *P. glabra* and *T. conophorum* the inhibitory activity of *P. macrophylla* is nevertheless twice as high. As polyphenolic compounds are known for their strong antioxidant potential, the results obtained above are consistent with those relating to the determination of polyphenols.

Table 3. Optical densities depending on the different concentrations of *P. macrophylla* extracts

Concentrations	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.625mg/ml
Optical Density (D.O)	0.117	0.133	0.163	0.315	0.564
Inhibition Percentage (%I)	88.36%	86.77%	83.79%	68.68%	43.93%

Tableau 4. Optical densities depending on the different concentrations of *P. glabra* extracts

Concentrations	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml
Optical Density (D.O)	0.346	0.870	0.890	1.175	1.228
Inhibition Pourcentage (%*I*)	68.91%	21.83%	20.03%	-	-

Tableau 5. Optical densities depending on the different concentrations of *T. conophorum* extracts

Concentrations	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml
Optical Density (D.O)	0.988	1.023	-	-	-
Inhibition Pourcentage (%***I***)	11.23%	19.38%	-	-	-

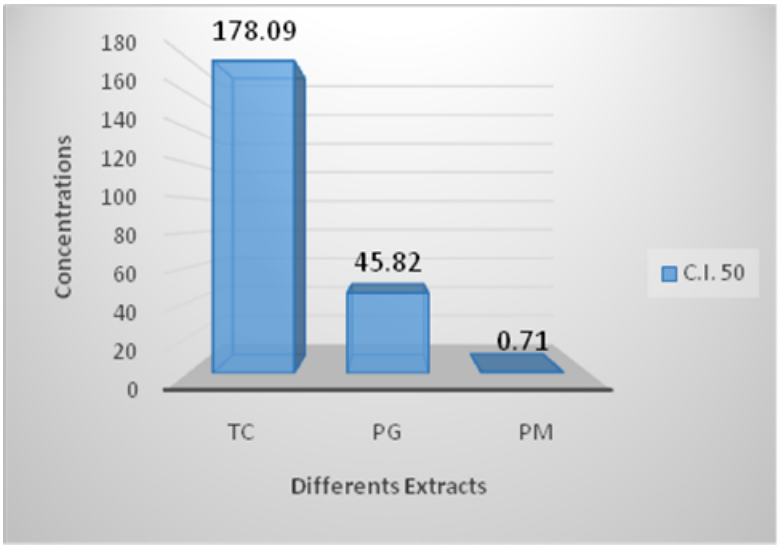

Figure 7. Anti-radical activity of hydroethanolic extracts of *T. conophorum*, *P. glabra* and *P. macrophylla*

Inhibitory Concentrations 50: The inhibitory concentrations 50 of the plants evaluated using the inhibition percentages are presented in Figure 7. The IC50 (50% inhibitory concentration) is defined as the substrate concentration that causes 50% loss of DPPH activity. The evaluation of the IC 50 of the different extracts (Figure 7) gave 178.09 mg/ml for *T. conophorum*, 45.82 mg/ml for *P. glabra* and 0.71 mg/mL for *P. macrophylla*. It should be remembered that the lower the IC50 value, the higher the antioxidant activity of the extracts [18]. As a result, the low value of the inhibitory concentrations at 50% (IC50) of the *P. glabra* and *P. macrophylla* extracts show that they have an antioxidant power significantly greater than that of the *T. conophorum* extract. *P. macrophylla* turns out to be the plant with the highest activity. This could be justified by the high concentration of phenolic compounds known to be compounds with powerful reducing power (Bruneton, 1993 and Huang et al., 2009).

CONCLUSION

The aim of this work is to search for the presence of chemical families, to measure total polyphenols and flavonoids, to evaluate the anti-radical activity of our plants; the objective of this study was achieved. The chemical family search of the different extracts revealed the presence of tannins, terpenoids, reducing compounds and flavonoids. Thin layer chromatography for polyphenols was positive for *P. glabra* and *P. macrophylla*. The determination of total polyphenols and flavonoids gave more or less similar values for *T. conophorum* and *P. glabra*. The polyphenol content of *P. macrophylla*, on the other hand, was found to be three times higher than that of the other two plants (160.73 mg EAG/gDM) while its flavonoid level was substantially identical to those of the others.

The qualitative evaluation of the antioxidant activity of the hydroethanolic extracts of *T. conophorum, P. glabra* and *P. macrophylla* by TLC was positive for all the extracts. This result, justified by the chemical profile determined by tube reaction and by TLC for the search for polyphenols, was confirmed by the dosage of the anti-radical activity materialized by the IC50 values below: 178.09 mg/ml for *T. conophorum*, 45.82 mg/mL for *P. glabra* and 0.71 mg/mL for *P. macrophylla*. All of these results allow us to confirm the possibility of the curative potential of each of these plants. These almonds, already consumed by the Congolese population, nevertheless require additional analyzes to encourage or prohibit their consumption. For example, this will involve assessing their toxicities. This work leaves room for several perspectives: The results obtained particularly for *P. macrophylla* and *T. conophorum* lead us to anticipate a fractionation and purification of the active molecules considered to highlight the anti-radical power and other biological activities of the identified families.

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