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Parkia biglobosa: An assessment of phytochemical compounds and antioxidant activities

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***Abstract**: Parkia biglobosa is a plant widely used in Senegal as source of food. Seed as well as the pulp of the fruit are widely consumed by people. The objective of our work is to determine the total polyphenols content (TPC) and the total flavonoids content (TPC) of aqueous and organic solvents extracts of the defatted seed and the pulp of the fruit of Parkia biglobosa. Aqueous extracts are richer in polyphenols with TPC value of 3.00 ± 0.11 mg GAE/gfor pulp and 2.11 ± 0.09 mg GAE/g for defatted seed. Methanol extracts are richer in flavonoids with TFC value of 2.81 ± 0.44 mg QE/g for pulp and 4.22 ± 0.22 mg QE/g for defatted seed. Subsequently, the antioxidant and of the extracts were studied using DPPH˙ and CUPRAC methods. The antioxidant of aqueous extracts are better than those of the organic solvent extracts. The antioxidant capacities values of aqueous extracts (5.94 ± 0.13 mg TrE/g(DPPH) and 14.92 ± 0.06 mg TrE/g (CUPRAC)) for pulp and for defatted seed (3.44 ± 0.09 mg TrE/g(DPPH) and 5.74 ± 0.05 mg TrE/g (CUPRAC)) are correlated to the TPC values of aqueous extracts. The high TPC and the good scavenging activities of the Parkia biglobosa extracts on free radical justify the use of this plant as food source.*

***Keywords**: Parkia biglobosa, polyphenols, flavonoids, DPPH˙, CUPRAC*

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I. Introduction

Reactive Oxygen Species (ROS) are a plague for us, they are produced during metabolic and respiration process in the living. ROS are the cause of irreversible damage on macromolecules[1]. These damages on macromolecules caused by ROS are the main causes of the imbalance between oxidants (free radicals) and antioxidants[2]. A high rate of free radicals in the organism is the reason of several disorders such as diabetes, cancer, cirrhosis, emphysema, cardiovascular diseases, etc. [2–6].

High ROS levels need to be controlled by antioxidants. For this purpose, antioxidants have to be supplement *via* alimentation. Those antioxidants must be able to render inactive the free radicals without generating harmful species.The polyphenols family have the abilities to neutralize the free radicals [7–10]. This family is subdivided into several categories such as phenolic acids, flavonoids, coumarins, etc. Those phytochemical compound are widely distributed in the plant kingdom. They are secondary metabolites from plant metabolism [11–14].

Parkia biglobosa also known as African locust bean is a tree belonging to the Fabaceaefamily, sub-family of theMimosoideaeandLeguminosae[15,16]. They are wild endemic tree of the Sahelian zones. *Parkia biglobosa* are large trees up to 20 meters which are found in the Savannah [17]. The wood is used in the making of house equipment such as mortars seats.[18,19]. Although not cultivated, these plants are well protected by people living on their zones because they are a good source of food. Indeed, the pods enclose a sweet yellow flesh surrounding the seeds.When fermented, the seeds are use as condiments, they are known as "Iru" in Yoruba, "Nététu" in Senegal, "Soumbala" in Burkina Faso[15]. The fermented seeds of *Parkia Biglobosa*are a good sources of vitamins such as vitamin A and B[15].

In Africa where medicine is more traditional, *Parkia biglobosa* is used in the treatment of various disease such as malaria, leprosy, pneumonia using his bark macerate[20,21].

Although the pulp and seeds of *Parkia Biglobosa* are widely consumed, its seeds are considered as a good source of vegetal oil. However, aqueous extracts from these two parts of the plant are associated with various health benefits [22,23]. This study aims to optimize the various parameters that can influence the extraction by focusing on the polyphenol yield, scavenging activity and the antioxidant capacity of the pulp and seed aqueous extracts.

II. Experimental

II.1 Plant material

*Parkia biglobosa*fruits was purchased in a local market (14°45'33.0 N; 17°23'37.3 W) at Dakar (Senegal).The pods were sliced so we can collect the pulps and the seeds. Before extracting the oil from the seeds, they have been ground. Oil were extracted with hexane under agitation during several hours. The defatted seeds have been dried before utilization.

II.2 Extraction process

The extraction method was carried out according to the method described by Gaye et *al.*[1]**.** Total Phenolic Content, scavenging activity by DPPH and Total antioxidant by CUPRAC were used as response value. Firstly, we determined the optimum temperature by weighing 0.5g of powder (pulp and defatted seeds). At this mass we added 50 mL of distillate water at variable temperatures (50, 60, 70, 80, 90, 100°C) for 20 minutes. Secondly, the optimal duration was established by setting the optimal temperature and varying the duration between 5 to 30 minutes. Thirdly, the optimum ratio was determined while using the first two parameters, the mass ranged from 0.5 to 5 g.

For further study, the optimum values of the above three parameters were used. All the extraction was done by using a water bath with well controlled temperature. All extracts were filtered through Whatman filter paper N°1. All extracts were stored at −20°C until use.

II.3 Determination of total phenolic content

The total phenolic content assay was conducted according toMohdaly et *al.*[24]. At 200 µL of the sample were added 150 µL of Folin-Ciocalteu reagent, 600 µL of Na_2CO_3 20% and 2.32 mL of distillate water. After a 30 minutes incubation in the darkness at room temperature, the absorbance was read at 760 nm by using a Perkin-Elmer Lambda 365 UV/Visible Spectrophotometer. Gallic acid was used as a standard. All the results were expressed as mg GAE/g ± Standard deviations.

II.4 Determination of the flavonoids content

Ordoñez et *al.* [25] assay was used in this process. 2.5 mL of each sample were mixed with 2.5 mL of a 2% ethanolic solution of $AlCl_3$. The absorbance was read at 425 nm after an hour of incubation in the darkness. Quercetin was used as a standard. All the results were expressed as mg QE/g ± Standard deviations.

II.5 Determination of Proanthocyanidins

Proanthocyanidins content was determined according to the procedure developed byLi et *al.*[26]. At a 0.5 mL of the sample were added 2.5 mL of vanillin 4% and 1.5 mL of concentrated HCl. This mixture was incubated at room temperature for 15 minutes and the absorbance was read at 500 nm. Catechin was used as a standard and the results were expressed as mg CE/g ± Standard deviations.

II.6 Antioxidant activities

II.6.1 $DPPH^{\cdot}$ free radical scavenging

Scavenging activity by $DPPH^{\cdot}$ were determined according to Akhtar et *al.*[27]. Firstly a 0.1014 mM working solution of $DPPH^{\cdot}$ was prepared in methanol. At 200 µL of each sample were added 3.8 mL of the working solution. Trolox was used as a standard and the absorbance was determined at 517 nm after a 0.5 hour incubation in the darkness.

The kinetic were also determined by reading the absorbance every 5 minutes for a total of 180 minutes. The Inhibition was determined by using the following equation:

$$\% \textbf{ Inhibition} = \frac{\textbf{Abs}_{\textbf{control}} - \textbf{Abs}_{\textbf{sample}}}{\textbf{Abs}_{\textbf{control}}} \times \textbf{100}$$

II.6.2 CUPRAC method

The assay conducted byApak et *al.*[28] were used to determine the total antioxidant capacity. At 1.1 mL of sample were added 1 mL of a 10^{-2} M copper chloride solution, 1 mL of ammonium acetate buffer (pH = 7) and 1 mL of a 7.5 mM ethanolic solution of neocuproine. The mix were incubated at room temperature for 1 h before reading the absorbance at 450 nm. Trolox was used as a standard and results were expressed as mg/g ± Standard deviations.

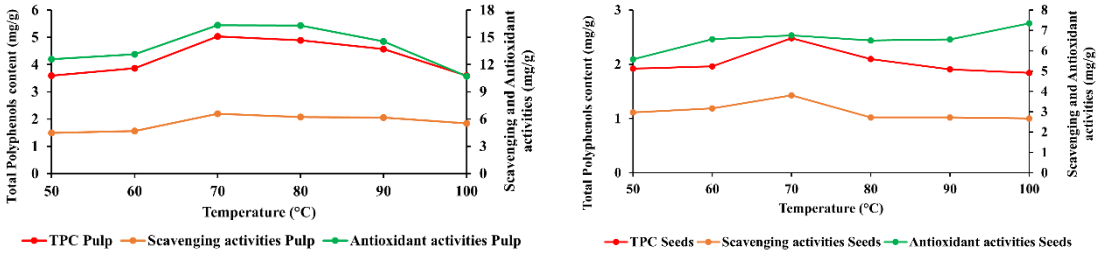
III. Results and Discussion

III.1 Optimization of the extraction

Figure 1 shows that the extraction yields of polyphenols, scavenging activity and antioxidant capacity of the pulp and defatted seed extracts are temperature dependence. In fact, in Figure 1A, the TPC increases from 50 to

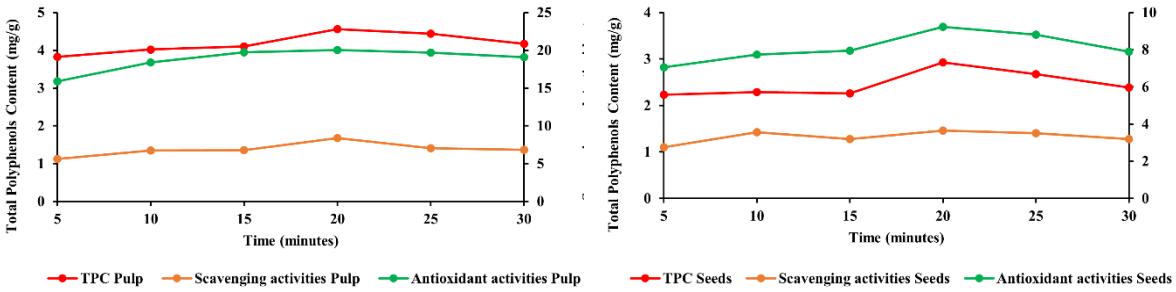
70°C and decreases from 70 to 100°C. This observation may be explained by the thermal decomposition of the compounds as observed by Vuong et *al.* [29] for *Carica papaya*. The scavenging activity and antioxidant capacity curves are similar to the TPC curve. For these three parameters the temperature of 70°C is optimum to have the best results. Figure 1B which shows the evolution of the three parameters for the defatted seed aqueous extract,present a maximum of TPC and scavenging activity at 70°C. For the antioxidant capacity the values increase from 50 to 70°Cbefore decreasing very slowly between 70 to 90°C. The value raised slightly when the temperature grown to 100°C. The optimal temperature of the extraction of the pulp and the defatted seed was fixed at 70°C. Similarly, the optimum time for aqueous extraction of pulp and defatted seed was determined focusing on TPC,scavenging activity and antioxidant capacity. As shown in Figure 2A and 2B, the whole parameters increase from 5 minutes to 20 minutes before decreasing beyond 20 minutes. For the study of the ratio (vegetal material/water) the temperature and the time of extraction were fixed respectively at 70°C and 20 minutes. In Figure 3A and 3B, it was observed that the optimum extraction ratio for TPCin pulp as well as in defatted seed is 1%. The TPC is very slightly higher for a ratio of 1% than for a ratio of 2%. It decreases very quickly when the ratio increases from 2 to 10%. The scavenging activity and antioxidant capacity of pulp and seed are ratio dependence and increase rapidly from 1% with maximum values at 2%. Beyond 2% ratio vegetal material/water the values decrease drastically.

In the rest of the work, the extractions will be carried out with the optimized parameters: temperature at 70°C, extraction time of 20 minutes and vegetal material/water ratio of 2%to quantify total phenolic content, totalflavonoid content, and proanthocyanidins content.



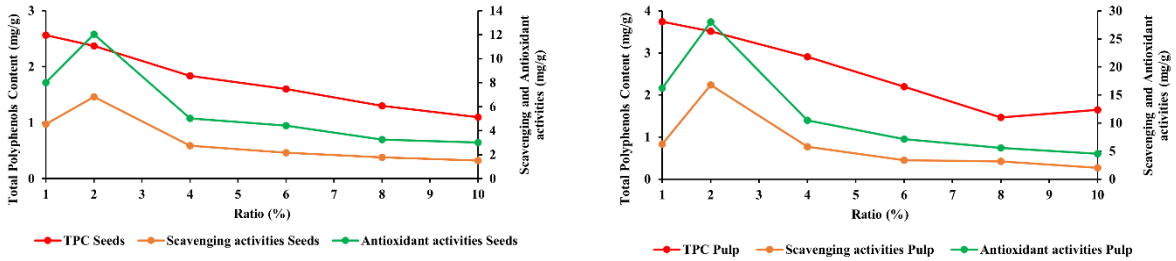
(**A**) (**B**)

Figure 1. Optimization of the temperature of the aqueous extraction: (**A**) Pulp; (**B**) Defatted seed.



(**A**) (**B**)

Figure 2. Optimization of the time of the aqueous extraction: (**A**) Pulp; (**B**) Defatted seed.



(**A**) (**B**)

Figure 3. Optimization of the ratio of the aqueous extraction: (**A**) Pulp; (**B**) Defatted seed.

III.2 Total phenolic and total flavonoid content from the pulp and the defatted seed of Parkia biglobosa

Table 1. Total phenolic and total flavonoid contents of the pulp and defatted seed.

Pulp					
	Water	MeOH	EtOH	Acetone	Ethyl Acetate
Polyphenols (mg GAE/g)	3.00 ± 0.11	1.84 ± 0.04	1.27 ± 0.07	0.32 ± 0.08	0.13 ± 0.09
Flavonoids (mg QE/g)	1.06 ± 0.14	2.81 ± 0.04	1.65 ± 0.07	2.52 ± 0.07	0.33 ± 0.07
Proanthocyanidins (mg CE/g)	n/a	n/a	n/a	n/a	n/a
Defatted seeds					
	Water	MeOH	EtOH	Acetone	Ethyl Acetate
Polyphenols (mg GAE/g)	2.11 ± 0.09	0.71 ± 0.02	0.61 ± 0.07	0.47 ± 0.01	0.38 ± 0.08
Flavonoids (mg QE /g)	2.03 ± 0.14	4.22 ± 0.22	7.22 ± 0.11	n/a	1.13 ± 0.06
Proanthocyanidins (mg CE/g)	n/a	n/a	n/a	n/a	n/a

The results of the quantitative determination of polyphenol and flavonoids in pulp and defatted seed of Parkia *Biglobosa* are summarized in Table 1. Different solvents basedon polarity were usedbecause the extraction of polyphenol and flavonoid are significantly affected by the dielectric constant of the solvent (ε_{water} = 80,$\varepsilon_{methanol}$ = 33,$\varepsilon_{ethanol}$ = 24.55, $\varepsilon_{acetone}$ = 21 and $\varepsilon_{ethyl\ acetate}$=6.02).The extractions were done at room temperature with organic solvent using the optimized ratio and the optimized time. For water extract it is noted that the pulp is much richer in polyphenol than the defatted seed. The TPC are respectively 3.00 ± 0.11mg GAE/g and 2.11 ± 0.09mg GAE/g. These values are comparable to those reported in the literature [30]. Both pulp and defatted seed extract are richer than the steam bark of *Parkia Biglobosa* studied in the literature [31]. On the other hand, the defatted seed is richer in flavonoid than the pulp with respective values of 2.03 ± 0.14mg QE /g and 1.06 ± 0.14mg QE /g. When methanol is used the TPC decreases for pulp (1.84 ± 0.04mg GAE/g) and defatted seed (0.71 ± 0.02 mg GAE/g) while the flavonoids contentsincrease considerably with values of 2.81 ± 0.04mg QE /g and 4.22 ± 0.22mg QE /g. The higher yield of TPC obtained in water can be explained by the high polarity of water and the high dipole moments of polyphenols. In contrast, the low dipole moments of flavonoids reduce their solubility in solvent with high polarity. The methanol solvent, which is less polar than water, better extract flavonoids as shown by the results in Table 1.The same tendency is observed when using solvents with lower dipole moments. Indeed, when passing from ethanol to acetone and then to ethyl acetate, there is a decrease in polyphenol extraction yields for pulp and defatted seedas shown in the table 1.For pulp, the flavonoids are best extracted by methanol with a value 2.81 ± 0.04mg QE/g. Ethanol is the best solvent for extracting flavonoids in defatted seed with 7.22 ± 0.11mg QE/g. The flavonoid content of the defatted seed is in accordance with the value reported by Donatien et *al*. [32]. Ethyl acetate, which is the less polar solvent, gives the lowest extraction yields for polyphenols and flavonoids in both pulp and defatted seed (Table 1). For all solvents used in this study, pulp is richer in polyphenols than defatted seed extract, while flavonoids are more present in defatted seed than in pulp extract.

III.3 Antioxidant Capacity

The antioxidant capacities of the pulp and the defatted seeds extracts of *Parkia Biglobosa* were analyzed using the free radical scavenging capacityof $DPPH^{\cdot}$(Figure 4) andCUPRAC (Figure 5).

The health benefits of polyphenols are widely studied in the scientific literature. Indeed, it is reported that polyphenols can contribute to the prevention of many diseases such as cancer, cardiovascular risks, diabetes and can also contribute to the better functioning of the immune system[33,34]. The benefits of polyphenols are linked to their antioxidant properties. They can scavenge free radicals and prevent the damage that free radicals can cause to cells. To determine the ability of the polyphenols, present in the pulp and defatted seed extracts to scavenge free radicals and the total antioxidant activity, a correlation analysis was performed. Aqueous and organic solvent extracts from the pulp and defatted seeds are examined.

While water is the best solvent for the extraction of polyphenols, the yields of flavonoids and saponins are much lower than for methanolic, ethanolic and acetone extracts. The $DPPH^{\cdot}$ free radical activity correlates well with the presence of polyphenols present in the extracts. As shown in Tables 1 and 2 the aqueous extracts which contain more polyphenols exhibit greater scavenging radical activity suggesting that these compounds are responsible for the radical scavenging activity. Indeed, it appears that the scavenging radical activity $DPPH^{\cdot}$ is correlated with the presence of polyphenol in water (*i.e*., in pulp : 5.94 ± 0.13 mg TrE/g) and organic solvents. The activity in methanol (*i.e.*, in pulp : 3.05 ± 0.01 mg TrE/g) is greater than that of ethanol (*i.e.*, in pulp : 2.80 ± 0.04 mg TrE/g) which is followed by that of the acetone extract (*i.e*., in pulp : 0.78 ± 0.07 mg TrE/g). The activity of the ethyl acetal extractwhich contains the lowest polyphenols contentsin pulp is0.31 ± 0.01 mg TrE/g). The same tendency is observed for the defatted seeds extract. The decreasing order of the $DPPH^{\cdot}$

scavenging radical activity of the extracts is observed : water > methanol > ethanol > acetone > ethyl acetate as show in Figure 4 and Table 2.

Table 2. The antioxidant capacity (DPPH$^{\cdot}$ and CUPRAC) for pulp and defatted seed of *Parkia Biglobosa*.

			Pulp		
	Water	MeOH	EtOH	Acetone	Ethyl Acetate
DPPH (mg TrE/g)	5.94 ± 0.13	3.05 ± 0.01	2.80 ± 0.04	0.78 ± 0.07	0.31 ± 0.01
CUPRAC (mg TrE/g)	14.92 ± 0.06	6.28 ± 0.06	4.31 ± 0.15	0.84 ± 0.05	0.065 ± 0.003
			Defatted seeds		
	Water	MeOH	EtOH	Acetone	Ethyl Acetate
DPPH (mg TrE/g)	3.44 ± 0.09	1.23 ± 0.07	0.51 ± 0.01	0.25 ± 0.02	0.21 ± 0.03
CUPRAC (mg TrE/g)	5.74 ± 0.05	1.88 ± 0.07	0.45 ± 0.13	0.21 ± 0.08	0.087 ± 0.007

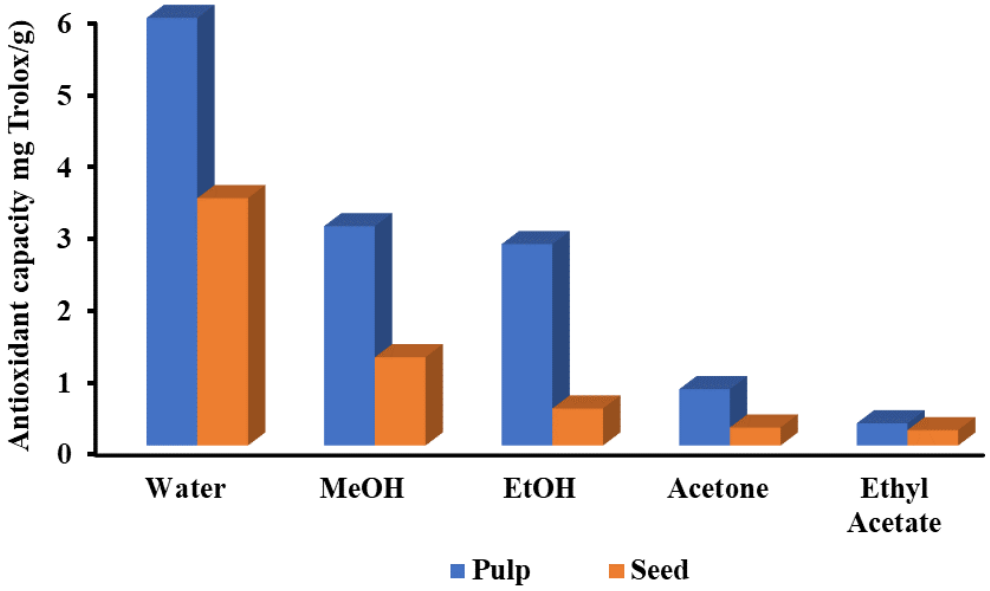


Figure 4. Antioxidant activity of pulp and defatted seed byDPPH$^{\cdot}$.

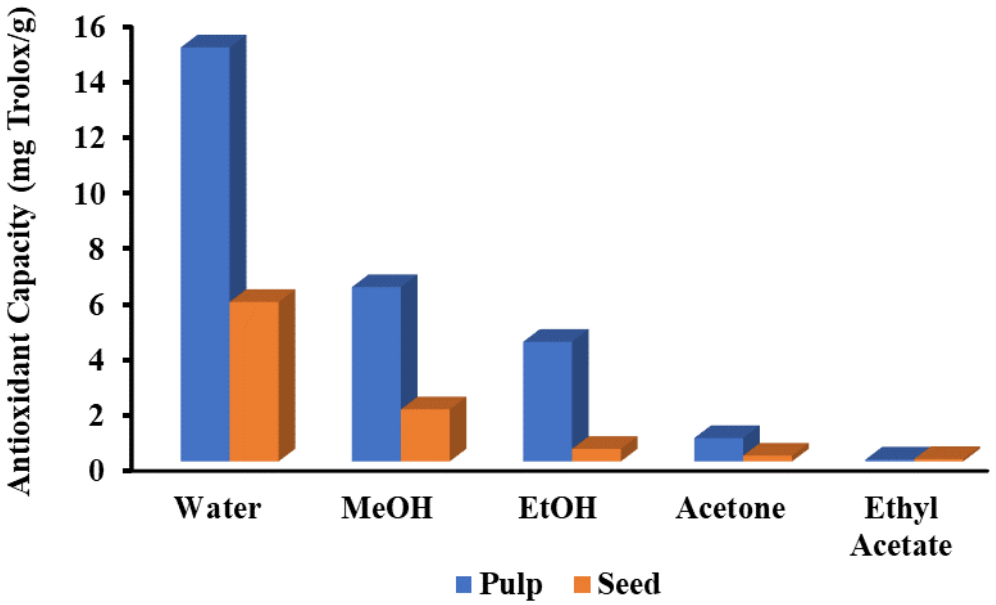


Figure 5. Total Antioxidant capacity of *Parkia biglobosa* pulp and defatted seeds by CUPRAC method.

The CUPRAC method of total antioxidant capacity (TAC) assay uses the chelate cation bis(2,9-dimethyl-1,10-phenanthroline: (neocuproine)Cu(II) as a chromogenic oxidant, which is reduced in the presence of antioxidants in (neocuproine)Cu(I) which absorbs visible light at a maximum wavelength of 450 nm. Color development in the CUPRAC method is based on the following reaction:

$$Cu(Nc)_2^{2+} + \text{reductant} \rightleftharpoons Cu(Nc)_2^{+} + \text{oxidized product} + H^{+}$$

Neocuproine $Cu(Nc)_2^{2+}$has shown the ability to oxidize all flavonoids and phenolic compounds and can be used to determine total antioxidant capacity (TAC)[28].

The total content of polyphenols and flavonoids in methanolic extracts is greater than that found in the aqueous extracts for both the pulp and the defatted seeds. Despite the higher overall content of polyphenols and flavonoids in the methanolic extract, the scavenging radical activity is largely higher for the aqueous extracts which have a higher content of polyphenols than the organic extracts.The CUPRAC radical scavenging of the aqueous extracts (14.92 ± 0.06 mg TrE/g (pulp) and 5.74 ± 0.05 mg TrE/g (defatted seed)) were significantly higher than those of methanol extracts (6.28 ± 0.06 mg TrE/g (pulp) and 1.88 ± 0.07 mg TrE/g (defatted seed)). The same tendency is observed with organic solvents. The decreasing of the total polyphenol content of the extracts is correlated with the decreasing of the scavenging radical activity.These results show that the polyphenolsmaterials are primarily responsible of the scavenging radical activity.

The scavenging activities for CUPRAC method are higher than those found when $DPPH^{\cdot}$ is used for aqueous extracts (5.94 ± 0.13 mgTrE/g (pulp) and 3.44 ± 0.09 mgTrE/g (defatted seed)) and methanol extracts (3.05 ± 0.01 mgTrE/g (pulp) and 1.23 ± 0.07 mgTrE/g (defatted seed)). When considering the organic extracts for pulp and defatted seeds, the following decreasing order of the scavenging radical capacity was found:methanol > ethanol > acetone > ethyl acetate.

These results are in accordance with those reported in the literature. Indeed, as reported previously, CUPRAC is more efficient than $DPPH^{\cdot}$ to oxidize polyphenols and flavonoids[28].

III.4 DPPH. Kinetic

The kinetic reactions are followed. Since the methanolic solution of $DPPH^{\cdot}$ is stable for up to 180 min at room temperature (23°C), the study was carried out over this period. Figure 6 gives the evolution of the scavenging radical activity of the extracts.

For both pulp and defatted seed extracts, a rapid change in the reaction rate is observed during the first ten minutes (Figure 6). The aqueous pulp extract reaches 29 % of inhibition while the methanolic and ethanolic pulp extracts achieve 16.8 % and 16.1 % of inhibition of the radical activity of $DPPH^{\cdot}$, respectively.The scavenging radical activity of the aqueous extract continues to increase and reaches 50 % of inhibition after 50 min while the methanolic and ethanolic extracts of the pulp reach 19 % and 18 % of inhibition from 15 min and increase very slightly to reach a maximum of 22 % (methanol) and 19 % of inhibition (ethanol) after 180 min.The scavenging radical activity of the aqueous extract of the pulp continues to increase and reaches 71 % of inhibition after 180 min.

For the defatted seeds, the scavenging radical activity of the aqueous and methanolic extracts evolve in the same way over the first ten minutes with a percentage of inhibitionof *ca.* 8%. It evolves slowly and reaches its maximum after 120 min with 21.5% of inhibition (water) and 25.1% of inhibition of methanol.

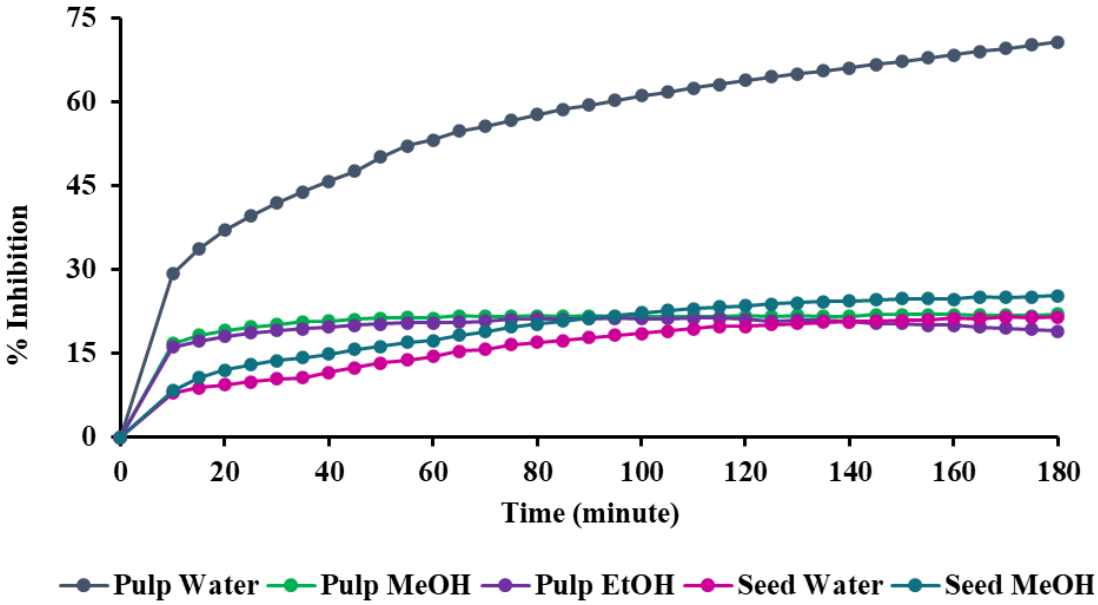


Figure 6: Percentage of inhibition of DPPH˙during the reaction with extracts.

IV. Conclusion

Polyphenols and flavonoids are present in both fruit and defatted seed of *Parkia biglobosa*. However, there is a low presence of proanthocyanidin in both studied parts of the plant. The assay results revealed water is the more suitable solvent to extract the polyphenols while methanol and ethanol are suitable for the extraction of flavonoids. The quantification of polyphenolic compounds and flavonoids made it possible to deduce that the recurrent use of the different parts of this plant would be linked to their relative richness in secondary metabolites based on polyphenols and flavonoids. The evaluation of the antioxidant power with DPPH˙ and CUPRAC revealed that the fruit aqueous extract possess an interesting antioxidant activity which would justify the use of this plant as food source.

Conflicts of Interest

The authors declared no conflicts of interest regarding the publication of this article.

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