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(RESEARCH ARTICLE)

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Evolution of antioxidant potential during fruit maturation *Pancovia laurentii* (De Wild.) Gilg ex De (Sapindaceae)

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Abstract

Pancovia laurentii (De Wild.) Gilg ex De (Sapindaceae) is a plant species of the spontaneous flora of Central Africa, which grows easily in the Republic of Congo. It produces very succulent edible fruits. The evaluation of phenolic compounds and antioxidant activity in the pulp and seed of the fruit during its maturation was done by spectrophotometer. The results reveal the increase of total polyphenols, total flavonoids and anthocyanins in the pulp and seed of the fruit during maturation. Flavonoids accumulate more in the seed than in the pulp. At taste maturity, the seed with 400.85 mg EQ/DM is richer in flavonoids than the pulp which has 322.26 mg EQ/DM. Polyphenols and anthocyanins accumulate more in the pulp than in the seed with respectively 432.8 mg EAG/mg DM for polyphenols and 157.50 mg/mL for anthocyanins. The pulp and seed presented a DPPH radical scavenging activity. The antioxidant activity increases during maturation. It is greater than 50%, particularly in the pulp with 0.25 mg/mL and in the seed with 0.37 µg/mL at taste maturity. These results show the existence of a good linear correlation between the content of total polyphenols, total flavonoids, anthocyanins and the antioxidant power of the pulp and seed of the fruit during maturation. With these contents, the fruit can be considered as a potential source of natural antioxidants.

Keywords: Antioxidant; Phenolic compounds; Maturation; *Pancovia laurentii*

1. Introduction

In tropical countries, particularly in the Republic of Congo, fruits abound in the wild, and sometimes throughout the year. They are a source of inexpensive food for populations. However, their nutritional and medicinal virtues remain unknown [1]. Fruits play an important role in feeding at least half of the world's population [2]. The role of fruits in the proper functioning of the body is no longer in doubt. They are major constituents of human nutrition and contribute largely to our intake of vitamins, antioxidants and trace elements. The quality of the fruit can be reflected in nutritional values and/or antioxidant properties. It has been known for many years that fruits and vegetables are beneficial for human health and are useful in the prevention of many diseases. Fruits are therefore natural sources of vitamins, proteins, and antioxidants essential for human health [3]. This is the case of the fruits of *Pancovia laurentii* (De Wild.) Gilg ex De, produced by a fruit tree of the Sapindaceae family. It is native to tropical and subtropical Africa. In Congo, its fruit is known by the vernacular name "akôyi" in the Batéké plateaus, where our study was conducted. In addition, its fleshy fruit is highly appreciated by consumers with a green epicarp at the young fruit stage and becomes yellow-orange at taste maturity [3]. Many studies have been carried out on the bark and leaves, addressing studies relating to medicinal aspects [3], but until now the study on phenolic compounds and antioxidant activity during the maturation of the fruit of *Pancovia laurentii* (De Wild.) Gilg ex De seems to be unknown. This study aims to evaluate the contents of phenolic

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compounds and the antioxidant activity of the pulp and seed of this fruit during maturation, with a view to its valorization.

2. Material and Methods

2.1. Plant material

Pancovia laurentii (De Wild.) Gilg ex De is a species of the Congolese spontaneous flora of the Sapindaceae family, which produces edible fruits that are green when juvenile and orange-yellow when ripe. These fruits are grouped in clusters (Figure 1). The fruit is a drupe that includes a relatively thin epicarp, a fleshy, thick and juicy mesocarp inside which is a seed.

Figure 1 Fruit clusters of *Pancovia laurentii* (De Wild.) Gilg ex De

For the study, the fruits were harvested at each of the main stages of their maturation, on a well-identified tree, in the Nature Reserve of Gorillas of Lesio Louna (RNGLL) in the department of Pool. They were then authenticated by a taxonomist from the Department of Biology, Section of Biology and Physiology of Plants, Faculty of Science and Technology, Marien Ngouabi University, Brazzaville, Congo. The harvested fruits were divided into 4 batches based on visual criteria for establish the different stages of maturity [4]. This is the pigmentation of the epicarp of the fruit (figure 1). Thus, these 4 stages of maturity are :

- Stage 1. Physiological maturity (mph) : the fruits are green in color ;
- Stage 2. Veraison (ver) : the fruits are light green ;
- Stage 3. Prematurity of taste (pmg) : the color of the fruit epicarp becomes light yellow ;
- Stage 4. Taste maturity (mag) : the fruit becomes dark yellow in color

3. Methods

3.1. Extraction of phenolic compounds

At each stage of maturity, the fruit was separated into two parts : the pulp and the seed. Each part was dried away from light at room temperature in the laboratory. Then, 25 g of the dried pulp or seed was ground in a porcelain mortar and reduced to powder, homogeneous. This powder was macerated in 250 mL of distilled water for 24 hours under magnetic stirring, then filtered through cotton wool and Wattman paper. The filtrate obtained was evaporated at 55 °C using an oven. The concentrated powdered macerate was stored in dry tinted bottles for use in the determination of phenolic compounds and the evaluation of antioxidant activity.

3.2. Determination of phenolic compounds

Determination of total polyphenols, total flavonoids and anthocyanins present in the epicarp, mesocarp and seed at different stages of maturity was carried out using of a spectrophotometer.

3.3. Total polyphenols

The dosage of total polyphenols in the extracts was carried out using the Folin-Ciocalteu reagent. [5]. Indeed, 25 µL of extract was mixed with 125 µL of Folin-Ciocalteu, 2 mL of distilled water and 375 µL of 10% sodium carbonate (Na_2CO_3). The mixture is incubated for 40 minutes at room temperature in the dark. The contents of Total polyphenols were expressed as mg EAG/g extract. The expression made using a calibration curve. Thus, 5 mg of gallic acid was diluted in 50 mL of distilled water. Gallic acid was prepared at concentrations of 20, 40, 60, 80 and 100 µg/ml. These solutions were incubated for 40 minutes at room temperature in the dark. The absorbances test and standard solutions were determined relative to the reagent blank at a wavelength of 725 nm with an ultraviolet-visible spectrophotometer.

3.4. Total flavonoids

The estimation of total flavonoid content was adapted from the method described by [5]. It is based on the ability of these compounds to form complexes chromogenic with aluminum chloride ($AlCl_3$). First, a calibration curve was made using the reference standard which is quercetin. Quercetin solutions at 20, 40, 60, 80 and 100 µg/ml were prepared. The reaction medium composed of 250 µL of The aqueous extract is mixed with 75 µL of a NaNO2 solution (5%). After an incubation At room temperature, 150 µL of a 10% aluminum trichloride solution is added ($AlCl_{3.6}H_2O$) freshly prepared. After 5 minutes, 500 µL of sodium hydroxide (NaOH, 1M) then 2.5 mL of distilled water. The absorbance of the test solutions and the standard solutions was was determined relative to the reagent blank at 510 nm wavelength with a UV/Visible spectrophotometer. Flavonoid content was expressed as mg QE/g of extract.

3.5. Anthocyanins

To measure anthocyanins, 1g of concentrated extract from each part of the fruit at different stages of maturity was dissolved in 10 mL according to the distilled water medium. Then, 1 mL of this mixture, 1 mL of a solution prepared from 95° ethanol acidified to 0.1% of the acid pure hydrochloric acid and 20 mL of a solution prepared from 35% HCl diluted to 2% in distilled water were added in turn to a test tube. To take the reading at spectrophotometer, two tanks are previously prepared [5].

- Tank A : 5 mL of prepared solution and 2 mL depending on the extraction solvent (water, ethanol and water/ethanol)
- Tank B : 5 mL of prepared solution and 2 mL of sodium bisulfite solution The resulting mixture was incubated for 20 minutes and the spectrophotometer reading was performed at a wavelength of 520 nm against an ethanol blank. For reliability of the results, three repetitions were performed.

The variation of anthocyanins is expressed approximately as follows : DOA – DOB.

However, the concentration of anthocyanins in mg/mL was determined by the formula following :

$$C\ (mg/mL) = (DOA - DOB) \times 875$$

DOA = Optical density of tank A

DOB = Optical density of cell B

3.6. Evaluation of antioxidant activity

Antioxidant activity was assessed by the 1,1-Diphenyl free radical scavenging method, 2-pyrrolidone (DPPH). DPPH, a stable, purple-colored free radical, is transformed into a stable yellow compound when reacted with an antioxidant. The radical scavenging test DPPH was estimated by the IC_{50} value. A lower IC_{50} value indicates activity higher antioxidant.

3.7. DPPH (1,1-Diphenyl, 2-pycro-hydrazyil) test

The protocol used was described by [4]**.** In a test tube, it was added 1250 µL of a methanolic solution of DPPH (0.04 mg/mL) to 50 µL of each extract at 2, 4, 6 and 8 mg/mL or 50 µL of the solvent (blank). After shaking, incubation was carried out at room temperature and in the dark for 30 min.

The absorbance was measured at λ = 517 nm. Each solution was repeated three times. The Percentage of DPPH free radical inhibition was calculated using the following formula

$$I\% = [(Witness - Aech)\ /Witness] \times 100$$

I% : percentage of inhibition of the DPPH free radical.
Witness : Absorbance of white.
Aech : Absorbance of the sample.

The CI50 values were then determined graphically by linear regression. CI50 or 50% inhibitory concentration, expresses the concentration of the sample that produces 50% anti-radical effect (50% DPPH trap). The IC_{50} values and data from all experimentally obtained results are expressed (mL/mL).

3.8. Data analysis methods

SPSS (Statistical Package for Social Sciences) software, version 22.0 was used for to strip the collected data. The average concentrations were first compared according to the 1-way ANOVA test. Then, when differences were detected, the comparisons were performed using the Student-Newman test. The significance level was set at $p < 0.05$.

4. Results

4.1. Total polyphenol contents

The results recorded in Figure 2 shows the richness of the fruit of *Pancovia laurentii* in total polyphenols at all stages of maturity. An increase in the content of total polyphenols in the pulp and seed of the fruit during its maturation. Polyphenols accumulate preferentially in the pulp. Indeed, at physiological maturity, the contents in total polyphenols are 99.01 mg EAG /g DM in the pulp and 127.9 mg EAG /g DM in the seed (values to be reviewed). At veraison, the total polyphenol contents increase progressively in the 2 compartments of the fruit with 297.95 mg EAG /g DM in the pulp and 182.50 mg EAG /g DM in the seed (values to be reviewed). At taste prematurity, the contents in total polyphenols are in the pulp are statistically equal, with 316.28 mg EAG /g MS in the pulp and 330.53 mg EAG /g MS in the seed. At taste maturity, they are 432.80 mg EAG /g DM in the pulp and 392.04 mg EAG /g DM in the seed.

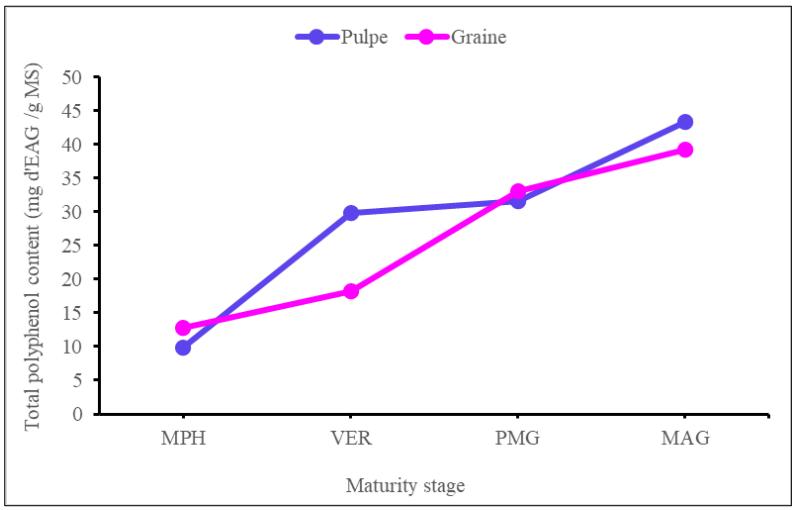

Figure 2 Total polyphenol contents in the epicarp, mesocarp and seed of the fruit of *P. laurentii* during maturation

The results of the 1-way ANOVA test revealed that total polyphenol contents vary in the pulp and seed during fruit ripening (p-Value = 0.02). At the stage of physiological maturity, averages of total polyphenol contents of pulp and seed are statistically different according to the 1-way ANOVA test (p-value = 0.00). According to the test Student-Newman, total polyphenol contents accumulate more in the seed than in the pulp with respectively 127.90 mg EAG /g DM and 99.01 mg EAG /g DM. A About the comparison of the averages of total polyphenol contents in the pulp and the seed of the fruit at veraison, the Student-Newman test was applied. The results reveal that the averages of the total polyphenol contents of these two compartments of the fruit are very highly and significantly different (p-value = 0.000). The pulp has contents of higher total polyphenols with 297.95 mg EAG/g DM and the seed with 182.50 mg of EAG /g DM. Regarding the comparison of the averages of the total polyphenol contents in these compartments of the fruit at pre-taste maturity, the Student-Newman test was applied. The results show that the averages of the total polyphenol contents of these 2 compartments of the fruit are not significantly different (p-value = 0.61). The analysis Comparison of total polyphenol contents at taste maturity reveals a high content of total polyphenols in the pulp (Table I). We note the existence of 2 homogeneous groups (a and b) of averages of the total polyphenol contents in the 2 compartments of

the fruit according to Student -Newman test. This is group a, represented by the total polyphenol content in the pulp, characterized by a high content with 432.80 mg EAG /g DM and group b, constituted by the total polyphenol content in the seed, marked by a very low content in total polyphenols with 392.04 mg EAG/g DM (Table I).

Table 1 Classification of total polyphenol contents (mg EAG/g DM) in the 2 fruit compartments during ripening according to the Student-Newman test

Compartment	Maturity stage			
	MPH	VER	PMG	MAG
Pulp	9,901 [a]	29,795 [b]	31,628 [a]	43,280 [b]
Seed	12,790 [b]	18,250 [a]	33,053 [a]	39,204 [a]

Legend: MPH*: Physiological maturity; **VER** * : Veraison; **PMG :** Taste prematurity; **MAG*** : Taste maturity

The same letters are not significantly different at the 5% level according to the Student t test Newman

4.2. Total flavonoid contents

Figure 3 shows us variations in total flavonoid contents in the pulp and seed of the fruit of *Pancovia laurentii* during maturation. The evaluation of the contents of total flavonoids revealed a significant increase ($p < 0.005$) in the contents of total flavonoids in both compartments of the fruit during ripening. These contents are more important in the pulp at the stage of taste maturity with 400.85 mg EQ /g MS compared to the seed which has 322.26 mg EQ/g MS. These same trends are observed in the pulp of the fruit at veraison (287.00 mg EQ/g MS), at taste prematurity (396.47 mg EQ /g MS) and at taste maturity (400.85 mg EQ /g MS). The lowest contents are recorded in the seed at veraison (249.45 mg EQ/g MS), at taste prematurity (314.89 mg EQ/g MS) and at taste maturity (322.26 mg EQ/g MS) in the pulp. The lowest content is recorded in the seed of the fruit at physiological maturity with 25.77 mg of EQ /g MS (figure 3).

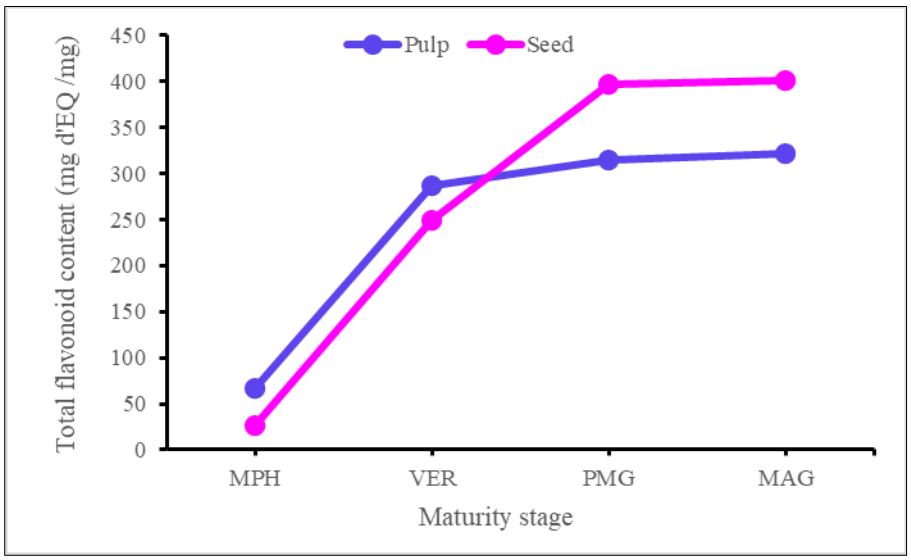

Figure 3 Total flavonoid contents in the pulp and seed of the fruit of *P. laurentii* during maturation

Statistical analyses revealed a significant compartment effect at the 5% threshold according to the Student Newman tests (Table II). In the pulp and seed during fruit ripening, variance analyses highlighted the existence of two homogeneous groups of total flavonoid contents (a and b). According to the Student-Newman test, the contents of total flavonoids accumulate more in the pulp than in the seed at physiological maturity with respectively 25.77 mg EQ/g MS and 66.4 mg EQ/g MS. About the comparison of the averages of total flavonoid contents in the pulp and seed of the fruit at veraison, the Student-Newman test was applied. The results reveal that the means total flavonoid contents of these fruit compartments are very high and significantly different (p-value = 0.000). The pulp has flavonoid contents larger totals with 287.00 mg EQ/g MS and the seed with 249.45 mg EQ/g MS. Regarding the comparison of the averages of total flavonoid contents in these compartments of the fruit with taste prematurity, the Student-Newman test was applied results show that the averages of the total flavonoid contents of these 2 compartments of the fruit are

significantly different (p-value = 0.01). The comparative analysis of the contents of total flavonoids at taste maturity reveals a high content of total flavonoids in the pulp (Table II). We note the existence of 2 homogeneous groups (a and b) of means of the total polyphenol contents in the 2 compartments of the fruit according to the Student-Newman test (Table II).

Table 2 Classification of total flavonoid contents (mg EAG/g DM) in the 2 fruit compartments during ripening according to the Student-Newman test

Fruit compartment	Maturity stage			
	MPH	VER	PMG	MAG
Pulp	0,664 [a]	28,700 [b]	39,647 [b]	40,085 [b]
Seed	2,577 [b]	24,945 [a]	31,489 [a]	32,226 [a]

Legend: MPH*: Physiological maturity; **VER** * : Veraison; **PMG :** Taste prematurity; **MAG*** : Taste maturity

The same letters are not significantly different at the 5% level according to the Student t test Newman

4.3. Anthocyanin content

Figure 4 shows the results of increasing anthocyanin contents in the pulp and the seed during the ripening of the fruit of *Pancovia laurentii* dosed from the extract aqueous. This figure shows the presence of anthocyanins in all parts of the fruit at Student each stage of maturity. At physiological maturity, the anthocyanin contents are relatively low in the pulp with 14.333 mg/ml and the seed with 25.958 mg/ml compared at other stages of maturity (figure 4). It is at taste maturity that these contents are the pulp with 25.958 mg/mL and 14.333 mg/mL respectively. About the comparison of higher in the pulp with 157.500 mg/mL and the seed with 79.187 mg/mL (Figure 3).

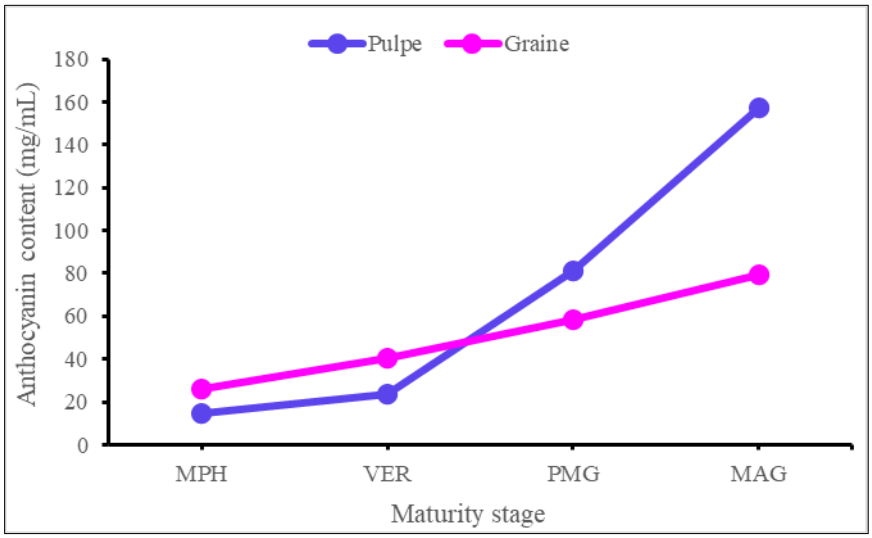

Figure 4 Anthocyanin contents in the pulp and seed of the fruit of *P. laurentii* during maturation

The results of the 1-way ANOVA test revealed that anthocyanin contents varied in pulp and seed during maturation (p-Value = 0.0001). At the maturity stage physiological of the fruit, the averages of the anthocyanin contents of the pulp and the seed are statistically different according to the 1-way ANOVA test (p-value = 0.00). According to the ANOVA test Student-Newman, anthocyanin contents accumulate more in the seed than in the pulp with 25.958 mg/mL and 14.333 mg/mL respectively. About the comparison of average anthocyanin contents in the pulp and seed of the fruit at veraison, the test Student-Newman was applied. The results reveal that the averages of the contents of anthocyanins of these fruit compartments are very highly and significantly different (p-value = 0.000). The seed has higher anthocyanin contents with 40.250 mg/mL and the pulp with 23.625 mg/mL. Regarding the comparison of the average contents in anthocyanins in these compartments of the fruit at pre-taste maturity, the Student Newman was applied. The results show that the averages of the anthocyanin contents of these 2 compartments of the fruit are significantly different (p-value = 0.01). The pulp has the highest contents compared to the seed. The comparative analysis of the contents in anthocyanins at taste maturity reveals a high content of soluble proteins in the pulp (Table III). We note the existence

of 2 homogeneous groups (a and b) of means of the anthocyanin contents in the 2 compartments of the fruit according to the Student-Newman test. It this is group a, represented by the anthocyanin content in the pulp, characterized by a high content with 157,500 mg/mL and group b, consisting of the anthocyanin content in the seed, marked by a very low anthocyanin content with 79.187 mg/mL (Table III).

Table 3 Classification of Anthocyanin contents (mg EAG/g DM) in the 2 fruit compartments during ripening according to the Student-Newman test

Fruit compartment	**Maturity stage**			
	MPH	**VER**	**MPH**	**MAG**
Pulp	14,333 [a]	23,625 [b]	81,375 [c]	157,500 [d]
Seed	25,958 [a]	40,250 [b]	58,625 [c]	79,187 [d]

Legend : MPH*: Physiological maturity; **VER** * : Veraison; **PMG :** Taste prematurity; **MAG*** : Taste maturity

The same letters are not significantly different at the 5% level according to the Student t test Newman

4.4. Anti-radical potential

At taste maturity, a strong antioxidant activity is noted in the pulp and the seed. In both parts of the fruit, the 50% inhibition concentration (IC_{50}) is respectively 0.250 mg/mL and 0.379 mg/mL (Figure 8). In the pulp, the antioxidant activity increases at during maturation. Indeed, the IC_{50} assayed were respectively 0.659 mg/mL (at physiological maturity), 0.555 mg/mL (at veraison) ; 0.393 mg/mL (at prematurity taste) and 0.250 mg/mL (at taste maturity). These IC_{50} s were stronger than those recorded in the seed at physiological maturity (0.728 mg/mL), at veraison (0.653 mg/mL), to taste prematurity (0.455 mg/mL) and to taste maturity (0.379 mg/mL) (Figure 5).

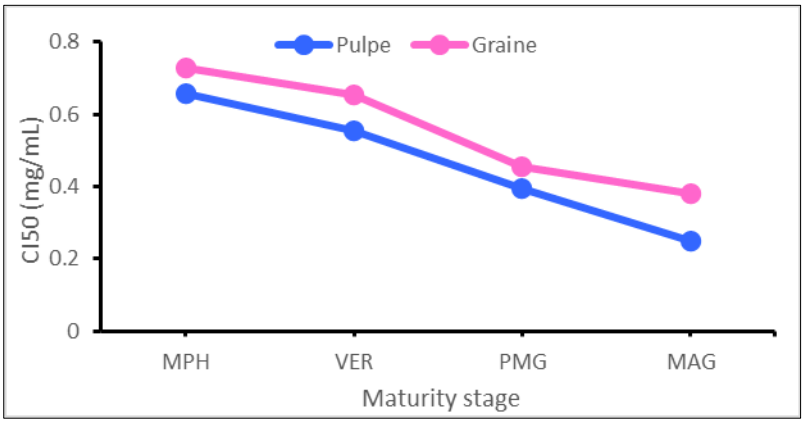

Figure 5 Variation of **IC_{50}** in the pulp and seed of *P. laurentii* fruit during maturation

5. Discussion

During maturation, the contents of phenolic compounds accumulated in the fruit of *Pancovia laurentii* and evaluated the antioxidant activity in the pulp and seed. For the total polyphenol, total flavonoid and anthocyanin contents in pulp and seed, results showed the richness of this fruit in these compounds. These total polyphenol contents, total flavonoids and anthocyanins increased significantly in pulp and seed of the fruit during its maturation. This increase would be due to the acquisition by the fruits of their potential due to the high synthesis activities. These results corroborate those obtained by [4] on the antioxidant activity of phenolic compounds accumulated during the maturation of the fruit of *Dacryodes edulis* ((G. Don) HJ Lam.). The results of the variation of phenolic compound contents during maturation reported in other fruits are not similar to those obtained in this study. [6] showed that during fruit ripening continuing at a stage ripe to overripe, a decrease in phenolic compounds is observed. This author suggests that the decrease is due to the activity of polyphenol oxidase. In addition, the implementation of the purple coloration of the epicarp of safou during maturation could be explained by the presence of flavonoids [7]. In addition, the high levels of these compounds phenolics were obtained at the stage of the fruit at taste maturity. Furthermore, the low contents were noted in the fruit at physiological maturity. The increased contents at taste maturity could be explained by the fact that the fruits acquire high potentials in due to intense metabolic activities, especially syntheses. These are completed at the taste maturity,

the peak point where most of the metabolites accumulate which give to the fruit its juiciness and organoleptic qualities. Similar results were obtained by [8]. Moreover, of all these compounds, it is the total flavonoids and anthocyanins that accumulate more in the pulp. These results are in agreement with those of [5]. These authors proved that total flavonoids accumulate more in the pulp of the fruit of *Grewia coriaceae.* This difference could be explained by numerous structural and biochemical events that took place between the pulp and the seed at during fruit ripening. Anthocyanins are natural pigments responsible for red and orange colors of many fruits and vegetables [9]. [8] demonstrated that low anthocyanin contents at maturity physiological are due to the influence of chlorophyll (a) which is very abundant especially in the pulp green fruits at physiological maturity. For antioxidant activity, the results showed that the pulp was more active than the seed. This activity increases during maturation of the fruit. The antioxidant activity of *P. laurentii* was shown by [5]. The high antioxidant activity in the pulp and the seed of the *P. laurentii* fruit during maturation would be due to their high content of total flavonoids, total polyphenols and anthocyanins [9 ;10 ;11 ; 12]. Fractions of the fruit of *Pancovia laurentii* have a good antioxidant activity.

6. Conclusion

In the fruit of *Pancovia laurentii,* the contents of total polyphenols, flavonoids and anthocyanins are higher in the pulp and than in the seed at taste maturity. There are a good linear correlation between phenolic compound contents and power antioxidant of different parts of the fruit during ripening. The fruit of *P. laurentii* is considered a source of natural antioxidants. The DPPH method has made it possible to highlight the antioxidant activity of the pulp and seed of the fruit of *P. laurentii.* Our work show that this fruit can now be used both for food and therapeutic.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author contributions

Attibayeba designed the research project and edited the manuscript. Waltta Itoua Ingoba and Etou Ossibi Grace Jokael executed this project and wrote the manuscript. Mbama Okandzé, Mvoumbi Tété Chanelle, Waltta Itoua Ingoba, Mbama Okandzé and Mbon Nguékou Chrichina manipulated in the laboratory. Mpika Joseph and Etou Ossibi Grace Jokael analyzed the obtained results.

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