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Phytochemical Study and Evaluation of the Antiradical Activity of Extracts of Oleaginous Seeds of *Panda oleosa* and *Isolona hexaloba* from Gabon

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Abstract

Our study focused on phytochemical tests and evaluation of the anti-free radical activity of seed extracts of two oleaginous plants from Gabon used in traditional medicine or as condiments: *Panda oleosa* and *Isolona hexaloba*. The extraction was carried out by maceration with solvents of increasing polarity: cyclohexane, trichloroethylene, acetone, ethanol and finally distilled water. The total yields of the extracts are about 69.50% for *Panda oleosa* and 34.28% for *Isolona hexaloba*. The phytochemical tests carried out on the extracts of the seeds of Panda and Isolona highlight in both seeds the presence of alkaloids, polyphenols, triterpenes, carotenoids, reducing compounds, flavonoids, total sugars, coumarins, anthraquinones, free quinones, free anthracene derivatives, and terpenoids. Isolona seeds also contain leucoanthocyanins, sterols, cardiac glycosides and saponins. Phytochemical tests revealed the absence of tannins and mucilage in both seeds. The free radical scavenging activity was measured by scavenging the free radical cation of 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS-+) with gallic acid as the reference antioxidant. The results of the free radical scavenging activity of the aqueous and ethanolic extracts of both seeds showed that the aqueous extracts were more active than the ethanolic extracts. The IC50s of the aqueous and ethanolic extracts of Panda seeds are 40 and 60 $\mu g \cdot mL^{-1}$ respectively, and those of the aqueous and ethanolic extracts of Isolona are 37.5

and 95 $\mu g \cdot mL^{-1}$ respectively. Gallic acid, the reference antioxidant (IC50 = 0.37 $\mu g \cdot mL^{-1}$) is about 10 times more active than the aqueous extracts of both seeds, 16 times more active than the ethanolic extract of Panda and 25 times more active than the ethanolic extract of Isolena.

Keywords

Panda oleosa, *Isolona hexaloba*, Oleaginous Seeds, Extracts, Phytochemical Screening, Antiradical Activity, ABTS-+, Gallic Acid

1. Introduction

Fats have a wide range of applications and are a major scientific and economic issue. Indeed, depending on their variety and dietary preferences, oilseeds can be consumed as a main dish, condiment, or fortifier. They thus contribute to the diversity and balance of the population's diet or they can be used as non-food sources. They can be used in the form of medicines or dyeing and tanning products: and in cosmetics [1]. *Panda oleosa* Pierre of the pandaceae family is a very common tree in Gabon. These fruits envelop a thick and very hard core, characterized by a bumpy aspect containing oleaginous seeds in the shape of crescent. Its fruiting is abundant in February. It is a medicinal and food plant. The bark contains tannin. The seeds contain an edible oil [2], produced and sold locally by the populations. In Gabon, the crushed seeds are added to sauces such as *Irvingia gabonensis* fruit kernels [3]. The seed oil is applied to ulcers, the crushed and roasted seeds to bronchial diseases. Screening tests on the bark of *Panda oleosa* have shown an inhibitory activity of HIV [4] [5].

Isolona hexaloba of the annonaceae family is a plant species found in Angola, Cameroon, Congo, Gabon, Equatorial Guinea, Nigeria, CAR, DRC. It is present in evergreen and semi-deciduous forest, in primary as well as secondary forest. It is often found along rivers. Its fruits are green to black with a wrinkled-bossed surface [6]. In the Democratic Republic of Congo, the bark is used in traditional medicine as a purgative and decoctions of bark are administered to treat abdominal pain, constipation, and wounds [7]. Bisbenzylisoquinoline alkaloids, curine and cyclene, have been isolated from the root bark. Both compounds showed significant trypanocidal activity in mice infected with strains of *Trpanosoma cruzi*, the protozoaire causing Chagas disease. A sesquiterpene derivative, cazolobine has also been isolated from the roots [8]. Consumable vegetable oils are essential to our nutritional balance and play a role in our health. If the knowledge surrounding oil plants is relatively well developed elsewhere, it would seem that oil seeds are very little documented in Gabon or even in the Central African sub-region.

The objective of this work is to study the phytochemical and antiradical activity of the oil seeds of two oil plants from Gabon that have not been extensively studied: *Panda oleosa* Pierre and *Isolona hexaloba* Pierre ex Engl. and Diels.

The anti-radical activity was measured by scavenging the free radical cation of 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS-+) according to the method developed by Re *et al.* [9] and optimized by N'negue *et al.* [10] with gallic acid as the reference antioxidant.

2. Materials and Methods

2.1. Plant Material

The plant material, namely the seeds of *Panda oleosa* of the pendaceae family and the seeds of *Isolona hexalobade* of the annonaceaes family, were collected in the Sibang garden (Libreville-Gabon) and then transported to the laboratory of the Institute of Pharmacopoeia and Traditional Medicine (Iphametra) where they were sun-dried for several days.

2.2. Extraction Method by Maceration

We chose the method of successive extraction by maceration of the seed powders extracted from the fruits of *Panda oleosa* and *Isolona hexaloba* obtained with the help of a Retsch type grinder, with Iphametra, using the solvents of increasing polarities: cyclohexane, trichloroethylene, acetone, ethanol, and distilled water.

80 g of seed powders were placed in 400 mL of solvent in a 500 mL glass Erlenmeyer flask, closed with a rubber stopper covered with aluminum foil. The mixtures were then placed under stirring at room temperature on a PIERRON type stirrer for 24 h. After 24 h, the mixtures were separated by vacuum filtration with a Whatman n˚4 type filter in a Büchner type funnel. For aqueous filtrates, water was evaporated by freeze-drying. The extractions by organic solvents, are evaporated in a flask pre-weighed with a rotary evaporator, in a bath of 40˚C, then placed in the oven at 40˚C until a constant mass is measured. The extracts are stored in the refrigerator in the closed vials and covered with aluminum foil for the next tests. The percentage of extractables to the initial mass of seed powders used is determined using the following equation:

$$R(\%) = \text{Mext}/\text{Mech} \times 100$$

where:

R: yield of extracts in %;

Mext: mass of the extract after evaporation or freeze-drying in grams;

Mech: anhydrous mass of the sample of seed powders in grams.

2.3. Phytochemical Screening

The reagents used to perform the phytochemical screening of the extracts were prepared and used according to the protocols described by Houghton and Raman [11], Akinjogunla *et al.* [12] and by Badiaga [13]. All the different tests were performed in triplicate. For alkaloids, 10 mL of extract was introduced into a test tube and then a few drops of Dragendorff's reagent solution were added. The appearance of a precipitate of red-orange coloration indicated the presence of alkaloids [14]. For polyphenols, 2 mL of extract was introduced in a test tube,

then a few drops of ethanolic solution of 2% ferric chloride were added. The appearance of a blue-blackish coloration indicates the presence of polyphenols [14]. Sterols and terpenes were detected by introducing 2 mL of the extract into a test tube and then a few drops of concentrated sulfuric acid. The appearance of a purple coloration indicates the presence of triterpenes and a green coloration the presence of sterols [15]. The presence of tannins was demonstrated by adding to 1 mL of extract, 1 mL of distilled water and 1 to 2 drops of $FeCl_3$ solution (iron perchloride or iron (III) chloride) diluted to 1%. The appearance of a dark green color indicates the presence of tannins. For the reducing compounds, 2 mL of extract was introduced into a test tube, followed by 2 mL of Fehling's liquor. The whole was then heated in a boiling water bath for 8 minutes. The appearance of a brick red precipitate indicates the presence of reducing compounds [16]. For flavonoids, 1 mL of extract was introduced into a test tube, then 1 mL of hydrochloric acid, 1 mL of isoamyl alcohol and then some magnesium chips were added. The appearance of a pink-orange coloration indicates the presence of flavonoids [17]. Saponosides were identified by introducing 10 mL of each extract into a test tube which was vigorously shaken with a vortex for 15 seconds. The tube was allowed to stand for 15 minutes. The appearance of a persistent foam indicates the presence of saponosides [14]. For cardiac glycosides, 2 mL of chloroform was added to 1 mL of each extract. The appearance of a reddish-brown coloration after the addition of a few drops of concentrated H_2SO_4 (sulfuric acid), indicates the presence of cardiac glycosides [18]. The presence of free quinones is revealed by adding to 1 mL of each extract a few drops of 1% NaOH (sodium hydroxide). The appearance of a yellow-red or purple color indicates the presence of free quinones [19]. For anthraquinones, to 2 mL of each extract is added 1 mL of 10% NH_4OH. After shaking, the appearance of a purple color indicates a positive test [19]. Leuco anthocyanins are identified by adding to 5 mL of each extract, 5 mL of hydrochloric alcohol and then a few drops of isoamyl alcohol. The mixture is heated for two minutes in a boiling water bath. The appearance of a red coloration indicates the presence of leucoanthocyanins [20]. For carotenoids, to each 2 mL of the extract, 0.5 mL of concentrated sulfuric acid (H_2SO_4) is added. The appearance of a blue coloration that turns red indicates the presence of Carotenoids [21]. To identify the mucilages, 1 mL of extract is introduced into a test tube, then 5 mL of absolute alcohol is added. Obtaining a flaky precipitate after shaking indicates the presence of mucilage [22]. After shaking, the two phases appear and a brown coloration indicates their presence. To highlight the total sugars, to 1 g of each extract, 3 drops of Molish's reagent are added, followed by 1 mL of concentrated sulfuric acid (H_2SO_4). The appearance of a purple-colored interphase indicates their presence [21]. For coumarins, 1 mL of ammonia diluted to 25% is added in 2 mL of extract. The whole is heated in a water bath for 5 minutes and then a UV reading is taken at 365 nm. The appearance of intense fluorescence in the tube (yellow, blue, blue-green, orange, violet, pink) indicates the presence of coumarins [22]. Free anthracene derivatives are detected by adding 1 mL of 25% diluted ammonia to 1 mL of extract in a test tube. After shaking, the appearance

of a red coloration indicates their presence [22].

2.4. Antiradical Activity

2.4.1. Material

ABTS (2,2'-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]), gallic acid, potassium persulfate ($K_2S_2O_8$) and hydrated sodium dihydrogen phosphate were purchased from Sigma-Adrilch (Saint-Quentin Fallavier, France). The water used was distilled by the equipment of the "Milli-Q Labo" laboratory (Millipore Japan, Tokyo, Japan). All these products are quality for analysis. The anti-radical activity was determined by UV spectrophotometry: V-200 spectrophotometer (BOECO, Germany). The optical density reading was taken at 734 nm, maximum absorption wavelength of the radical cation ABTS-+.

2.4.2. Material

ABTS (2,2'-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]), gallic acid, potassium persulfate ($K_2S_2O_8$) and hydrated sodium dihydrogen phosphate were purchased from Sigma-Adrilch (Saint-Quentin Fallavier, France). The water used was distilled by the equipment of the "Milli-Q Labo" laboratory (Millipore Japan, Tokyo, Japan). All these products are quality for analysis. The anti-radical activity was determined by UV spectrophotometry: V-200 spectrophotometer (BOECO, Germany). The optical density reading was taken at 734 nm, maximum absorption wavelength of the radical cation ABTS-+.

2.4.3. Preparation of Gallic Acid Solutions, "Reference Antioxidant"

Gallic acid (3,4,5-trihydroxybenzoic acid) is an aromatic organic compound, used as a reference anti-radical compound. Ten working solutions, in decreasing concentrations, ranging from 0.94 to 0.094 µg/mL, were prepared by diluting gallic acid in distilled water.

2.4.4. Preparation of *Panda oleosa* and *Isolona hexaloba* Seed Solutions

Five solutions of increasing concentrations ranging from 0 to 150 µg/mL of the different Panda and Isolona seed extracts are prepared by dissolving the powder in the extraction solvent.

2.4.5. Measurement of the Anti-Radical Activity

The principle of the test for measuring the radical activity by the ABTS method is based on the decrease of the absorbance at 734 nm of the radical cation ABTS-+ (blue-green coloration) in the presence of a potentially anti-free radical compound which reduces the radical cation. The reduction of the radical form of ABTS-+ leads to a decoloration of the solution. The radical ion ABTS.+ is obtained by reacting the ABTS molecule (7 mM) with potassium persulfate (2.45 mM), in distilled water for 16 hours at room temperature and protected from light. The resulting ABTS.+ solution is diluted with sodium phosphate buffer (5 mM, pH = 7.4), to obtain a stock solution with an initial absorbance value at 734 nm between 0.65 and 0.70. The radical cation (ABTS.+) is stable for more than 2

days when stored at room temperature and protected from light. The assays were performed three or two times and the anti-free radical activity is calculated according to the following formula:

$$\text{Anti-free radical activity}(\%) = \left[1-(\text{Ar}-\text{Ab})/(\text{Ai}-\text{Ab})\right]\times 100 .$$

With Ar = remaining activity of ABTS-+; Ai = initial activity of ABTS-+ and Ab = Activity of the blank. In fact, the reduction of the cation radical ABTS-+ is therefore equivalent to determining the anti-free radical activity and in total, the antioxidant properties of the plasma, compared to the antioxidant properties of gallic acid (standard). In fact, the reduction of the ABTS-+ cation radical is therefore equivalent to determining the anti-free radical activity and, in total, the antioxidant properties of Panda and Isolona extracts compared to the antioxidant properties of gallic acid (standard). The free radical scavenging activity was determined by UV spectrophotometry in 1 cm optical path cuvettes (2 mL reaction volume). The incubation time was 6 minutes [10].

3. Results

3.1. Extraction Yields

The results of the extractives content of Panda and Isolona oilseeds are shown in Table 1. The results of the extractives content of Panda and Isolona oilseeds are shown in Table 1.

According to the results obtained (Table 1) the extractables rates vary from one solvent to another. For the seeds of Panda, the rates of extractables with cyclohexane are the highest (35.97%), followed by the rates of extractables with water (14.16%) and that with trichloroethylene (11.40%). Then followed the rates of extractables in acetone (5.26) and ethanol (2.71%). For Isolona seeds, the rate of extraction with cyclohexane is always the highest (16.08%), followed by that with water (4.37%) and that with trichloroethylene (4.28%). The acetone (2.89%) and ethanol (2.19%) extract rates are the lowest and similar. The overall extract rates of isolona seeds are 34.28% and those of Panda are 69.50%.

Table 1. Rate of seed extracts obtained by maceration.

Extract rate (average of three trials ± standard deviation)		
Solvent	**Extraction rate (%)**	
	Panda	**Isolena**
Cycohexane	35.97 ± 0.05	16.08 ± 0.08
Trichloroethylene	11.40 ± 0.09	4.28 ± 0.11
Acetone	5.26 ± 0.07	2.89 ± 0.25
Ethanol	2.71 ± 0.15	2.19 ± 0.40
Distilled water	14.16 ± 0.07	4.37 ± 0.07
Total	**69.50**	**34.28**

3.2. Phytochemical Tests

In order to get an idea of the composition of each extract, several qualitative tests to identify the main chemical groups present in the different extractives fractions were performed. The results of the phytochemical screenings are reported in Table 2 for Panda seeds and in Table 3 for Isolona seeds.

According to the results in Table 2, all the extracts of *Panda oleosa* seeds contain the alkaloids, and reducing compounds. The acetone extract contains the majority of the tested chemical compounds except leucoanthocyanins, sterols, saponisides, mucilages and cardiac glycosides. The aqueous extract contains only alkaloids and reducing compounds; while the ethanolic extract contains in addition to these two compounds polyphenols, total sugars and coumarin. We find a high amount of triterpenes in the cyclohexanic extract and a low amount of coumarins in the trichloroethylene extract (Table 2). According to the results in Table 3, all extracts of *Isolona hexaloba* contain the alkaloids, reducing compounds, carotenoids, total sugars and coumarins. Leucoanthocyanins and terpenoids are absent only in the aqueous extract. Sterols are present only in the acetone

Table 2. Results of phytochemical tests performed on *Panda oleosa* extracts.

Compounds	Solvents				
	Cyclohexane	Trichloroethylene	Acetone	Ethanol	Water
Alkaloids	+++	+++	++	++	++
Polyphenols	–	–	+++	++	–
Leucoanthocyanins	–	–	–	–	–
Sterols	–	–		–	–
Triterpenes	+++		+++		
Carotenoids	–	–	++	–	–
Tannins	–	–			
Reducing compounds	++	++	+++	+++	+++
Flavonoids	–	–	+	–	–
Total sugars	–	–	+++	++	–
Saponins	–	–	–	–	–
Coumarins	–	+	+++	+++	+
Anthraquinones	–	–	++	–	–
Free quinones	–	–	++	–	–
Mucilages	–	–	–	–	–
Free anthracene derivatives	–	–	++	–	–
Cardiac glycosides	–	–	–	–	–
Terpenoids	–	–	++	–	–

Coloration: +++: very intense, ++: moderately intense, +: not very intense, –: absent.

Table 3. Results of phytochemical tests performed on *Isolona hexaloba* extracts.

Compounds	Solvents				
	Cyclohexane	Trichloroethylene	Acetone	Ethanol	Water
Alkaloids	+++	+++	+++	++	++
Polyphenols	–	–	+++	++	–
Leucoanthocyanins	+++	+++	+++	++	–
Sterols	–	–	+++	–	–
Triterpenes	+++	++		–	–
Carotenoids	+++	++	+++	++	+
Tannins	–	–		–	–
Reducing compounds	+++	+++	+++	+++	+++
Flavonoids	–	–	++	++	–
Total sugars	+++	+++	+++	+++	++
Saponins	–	–	–	–	+++
Coumarins	+++	+++	+++	+++	++
Anthraquinones	–	–	–	–	–
Free quinones	–	–	++	–	–
Mucilages	–	–	–	–	–
Free anthracene derivatives	–	–	+++	–	–
Cardiac glycosides	+	–	+++	–	–
Terpenoids	+++	++	+++	++	–

Coloration: +++: very intense, ++: moderately intense, +: not very intense, –: absent.

extract. Triterpenes are present in the cyclohexane and trichloroethylene extracts. Flavonoids are found in the acetone and ethanol extracts, and saponosides in the aqueous extracts. Free quinones and free anthracene derivatives are found in the acetone extracts. Mucilages and tannins are absent in all extracts of *Isolona hexaloba*.

3.3. Anti-Radical Activity of Gallic Acid According to the Concentration

The percentage of free radical scavenging activity increases linearly with the concentration of the reference antioxidant: gallic acid (Figure 1). The IC50 value (concentration necessary to reduce the free radical scavenging activity by 50%) of gallic acid deduced from the curve is 0.37 µg/mL (2 µM).

3.4. Anti-Radical Activity of Panda Oleosa Extracts

1) Evaluation of the antiradical activity of the aqueous extract

According to the results obtained (Figure 2), the anti-free radical activity of

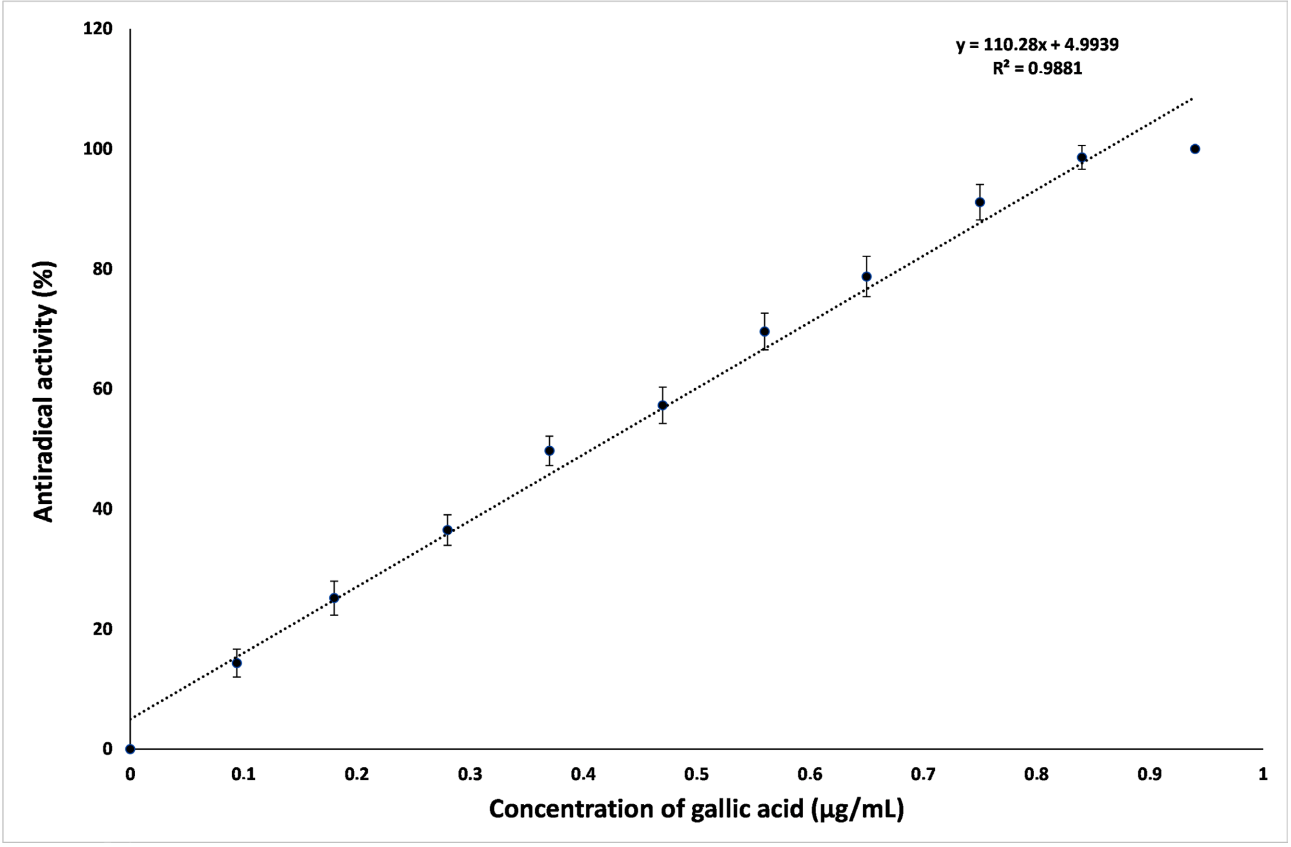

Figure 1. Anti-radical activity as a function of the concentration of gallic acid after 6 minutes of incubation. The proportion ABTS•+ transformed into ABTS+ in the presence of gallic acid is calculated from the change in absorbance at 734 nm measured by spectrophotometry. The equation on the right is: $y = 110.28x + 4.9$ ($R^2 = 0.98$); n = 3.

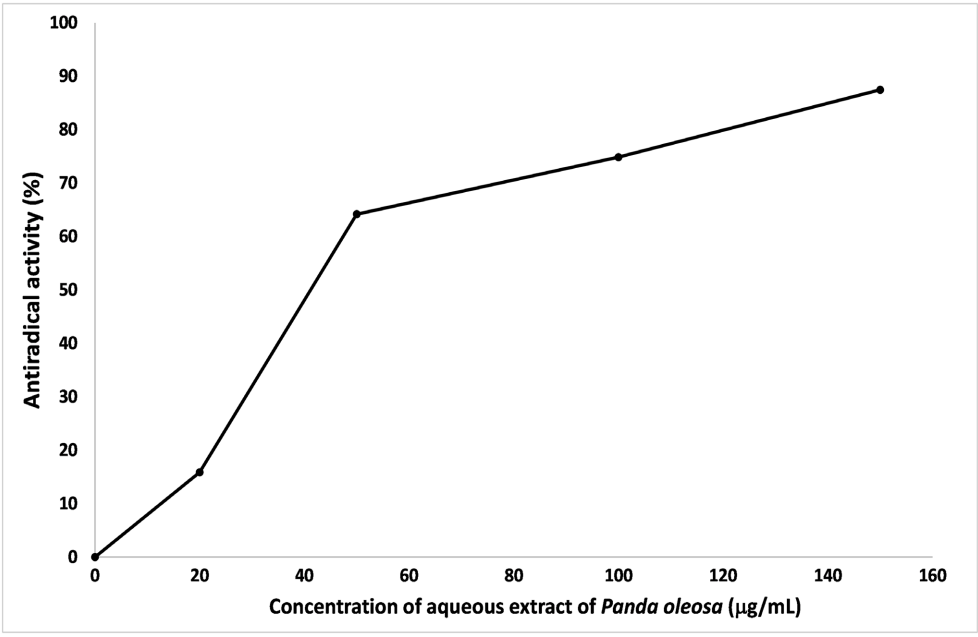

Figure 2. Anti-radical activity according to the concentration of aqueous extract of *Panda oleosa* after 6 min of incubation. The proportion ABTS•+ transformed into ABTS+ is calculated from the change in absorbance at 734 nm measured by spectrophotometry: n = 3.

Figure 3. Anti-radical activity according to the concentration of ethanol extract of *Isolona hexaloba* after 6 min of incubation. The proportion ABTS•+ transformed into ABTS+ is calculated from the change in absorbance at 734 nm measured by spectrophotometry: n = 3.

the aqueous extract increases from 15.88% ± 0.14% for a concentration of 20 µg·mL^{-1} to 87.46% ± 0.08% for concentrations of 100 to 200 µg·mL^{-1}. The IC50 deduced from our results is 40 µg·mL^{-1}.

2) Evaluation of the antiradical activity of the ethanolic extract

The results (**Figure 3**) show an increasing anti-radical activity with the concentration of ethanolic extract of *Panda oleosa*. The free radical scavenging activity is 40.26% ± 0.19% for a concentration of 20 µg·mL^{-1} and 82.78% for a concentration of 150 µg·mL^{-1} ethanolic extract. The IC50 of the ethanolic extract is 60 µg·mL^{-1}. It is 1.5 times higher than that of the aqueous extract. The ethanolic extract would thus present a lower activity than the aqueous extract.

4. Discussion

In this study, we first calculated the extraction yields of the different solvents from *Panda oleosa* and *Isolona hexaloba* seeds. The results obtained indicate that cyclohexane, the first solvent used during the successive extraction and the least polar, extracts mainly liposoluble substances (oils, fats, terpenes) from the seeds of the two oleaginous plants (**Table 1**). The cyclohexane thus contains the important fat fractions. The yields of cyclohexane are the highest; 35.97% for Panda seeds and 16.08% for Isolena seeds. These results reflect that the seed powders were virtually de-oiled by cyclohexane. Polar solvents subsequently solubilize polar compounds such as polyphenols. The successive extraction thus combines apolar and polar solvents. It allows partitioning the extractables in different fractions facilitating the later analyses, and the sum of the extracts with

each solvent gives an idea of the global content in extracts of the seeds. The overall extract content is 69.50% for Panda seeds and 34.28% for Isolena seeds (**Table 1**). Silou [23] studied one hundred and thirty samples of oils and fats extracted from 77 species of the Congo Basin belonging to 35 botanical families and divided them into three classes of equal magnitude between 15% and 75% fat content. The plants with low fat content have a rate between 15% and 35%; those with medium fat content have a rate between 35% - 55% and finally the plants with high fat content have a rate between 55% - 75%. According to these percentages, Panda is a high-fat plant and Isolena a low-fat plant.

The phytochemical tests (**Table 2**) carried out on the seeds showed the presence of alkaloids, polyphenols, reducing compounds, free anthracene derivatives, terpenoids, anthraquinones, total sugars, coumarins, free quinones, leucoanthocyanins, sterols and triterpenes, carotenoids, flavonoids and cardiac glycosides. We note in Panda seeds, the absence of leucoanthocyanins, sterols, tannins, saponins, cardiac glycosides and mucilages. While in Isolena seeds, we note only the absence of tannins and mucilages. These bioactive compounds have multiple therapeutic properties and support the use of *Panda* and *Isolena* seeds in traditional medicine in the treatment of several pathologies. Indeed, *Panda* is used in the Democratic Republic of Congo as an antidiabetic and *Isolona* as a purgative and for the treatment of abdominal pain, constipation and wounds. Several authors have demonstrated that the consumption of foods rich in polyphenols reduces the development of numerous pathologies, such as cancer, vascular diseases, hypertension, atherosclerosis [24] [25] [26]. The seeds of *Panda* and *Isolena* contain a high quantity of alkaloids. These compounds are sought after for their physiological effects and pharmacological activities that are exerted in various fields. Alkaloids also play the role of antibiotics [13]. Cardiac glycosides highlighted in phytochemical tests of *Isolena* seeds could be major drugs for heart failure. They exert their activity on the heart at several levels: contraction forces, frequency, conductivity. These effects are reflected in the electrocardiographic changes [27]. Sterols and triterpenic alcohols present in both types of seeds, have anti-inflammatory, antidiabetic, anticancer, antidiarrheal, antiviral and anti-HIV activities [28]. Carotenoids are well-known phytomicronutrients. Carotenoids in *Panda* and *Isolena* seeds may have pro-vitamin A activity. Vitamin A is involved in maintaining good vision and preventing diseases that could affect the eyes. Carotenoids are also known to be antioxidants. They have a potential protective effect on the prevention and progression of cancers, cardiovascular diseases and cataracts [29]. The carotenoids contained in *Panda* and *Isolena* seeds can also be used in the food industry as additives as food coloring or in cosmetic products [29] [30].

In a second step, we evaluated the anti-free radical activity of aqueous and ethanolic extracts of *Panda* and *Isolena* seeds, by scavenging the free radical ion ABTS-+ according to the method of Re *et al.* [9] optimized by N'negue *et al.* [10] with gallic acid as reference antioxidant. The results of the antioxidant activity of gallic acid (a synthetic molecule with high antioxidant activity) validate the chosen method. Moreover, the IC50 value of gallic acid deduced from our results is

more or less equivalent to that obtained by Sadat *et al.* [31] *i.e.* 0.47 µg/mL and N'negue *et al.* [10] [32] *i.e.* 0.47 and 0.37 µg/mL. These authors worked under the same conditions.

The results of the evaluation of the anti-free radical activity of the aqueous and ethanolic extracts of *Isolona* seeds also showed a variation of anti-free radical activity with the extraction solvent. According to the results obtained (**Figure 2** and **Figure 3**), the aqueous extract with an IC50 of 37.5 µg·mL^{-1} is more active than the ethanolic extract with an IC50 of 95 µg·mL^{-1}. The IC50 of the ethanolic extract is thus 2.5 times higher than that of the aqueous extract. As with the *Panda* seeds, the results obtained with the *Isolena* seeds seem surprising. Indeed, the aqueous extracts of *Isolona* seeds showed after the phytochemical study an absence of polyphenols and a low level of carotenoids which are both antiradical compounds [33] [34] [35]. While the ethanolic extract presented average amounts of carotenoids and polyphenol. This can be explained by the fact that during the successive extraction by maceration, the level of antioxidant compounds (polyphenol and carotenoids) present in the ethanol may be lower than the level of saponosides (antiradical compounds) strongly present in the aqueous extract. On the other hand, the aqueous extract may contain an unidentified polar anti-radical compound.

A comparison of the antiradical activity of the two oil plants shows that the aqueous extracts of the two seeds have quite similar antiradical activities, with however a slight advantage for the aqueous extract of *Isolona*. (IC50 = 37.5 µg·mL^{-1} against 40 µg·mL^{-1} for the aqueous extract of *Panda*). This can be explained by the presence in the *Isolena* aqueous extract of carotenoids and saponins, absent in the *Panda* aqueous extract. The comparison of the antiradical activities of the ethanolic extracts of the two oleaginous plants shows a more important activity for the *Panda* extract. Indeed, the IC50 of the ethanolic extract of *Panda* is equal to 60 µg·mL^{-1}, and that of the *Isolena* extract 95 µg·mL^{-1}. This result could be explained by the presence of a type of polyphenolic compounds much more active in the ethanolic extract of *Panda* seed.

The comparison of the free radical scavenging activity of *Panda* and *Isolena* oil seeds with that of gallic acid (IC50 = 0.37 µg·mL^{-1}), a pure chemical compound "reference antioxidant", shows that gallic acid is about 10 times more active than the aqueous extracts of the two seeds, 16 times more active than the ethanolic extract of Panda and 25 times more active than the ethanolic extract of *Isolena*. Indeed, the active principle responsible for the antiradical activity representing only about 10% of the total compounds of the extract, IC50 of 37.5; 40 µg·mL^{-1}; 60 µg·mL^{-1} or 95 µg·mL^{-1} of the total extract, would be equivalent to IC50 of 3.7 µg·mL^{-1}; 4 µg·mL^{-1}; 6 µg·mL^{-1} or 9.5 µg·mL^{-1} of the active principle. The aqueous extracts of the seeds would thus be more active than the ethanolic extracts.

5. Conclusion

The work showed that the extraction rates of *Panda oleosa* and *Isolona hexaloba*

seeds varied from one solvent to another. Extraction yields were highest with cyclohexane, water and trichloroethylene for Panda seeds and with cyclohexane for *Isolena* seeds. Panda is a highly oleaginous plant as its global extract rate was 69.5%, and *Isolena* a low oleaginous plant with a global extract rate of 34.28%. The phytochemical tests carried out showed that these oleaginous seeds contain major chemical groups such as alkaloids, polyphenols, sterols, triterpenes, carotenoids, tannins, reducing compounds, total sugars, saponins, coumarins and terpenoids, leucoanthocyanins, flavonoids, free anthracene derivatives, and cardiac glycosides. These bioactive compounds having multiple therapeutic properties, these seeds could thus be used in traditional medicine, in the treatment of several pathologies. Aqueous and ethanolic extracts of *Panda* and *Isolena* seeds have antiradical activity. Aqueous extracts have a higher antiradical activity than ethanolic extracts. Gallic acid, the "reference antioxidant", is about 10 times more active than the aqueous extracts of both seeds, 16 times more active than the ethanolic extract of Panda and 25 times more active than the ethanolic extract of *Isolena*. A number of studies have revealed the important role that antioxidants play in our body. The extracts with strong anti-free radical activity of Panda and Isolena, due to their antioxidant properties, would therefore have preventive potential in the fight against pathologies associated with oxidative stress (cardiovascular diseases, aging, diabetes, cancer, inflammation, neuronal or genetic diseases). Moreover, these extracts could be used as natural antioxidants fighting against oxidation in food, pharmaceutical and cosmetic industries.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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