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Phytochemical study of the extracts of oilseeds of *Staudtia kamerunensis* var. *gabonensis* (Warb.) from Gabon and evaluation of their antiradical activity

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The report focused on phytochemical tests and evaluation of the antiradical activity of seed extracts of *Staudtia kamerunensis* var. *gabonensis*. The successive extraction of seed powders from *S. kamerunensis* var. *gabonensis* was carried out by maceration at room temperature with solvents of increasing polarities: Cyclohexane, trichlorethylene, acetone, ethanol and distilled water. The antiradical activity was measured by trapping the radical cation of 2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] (ABTS•+) with gallic acid as reference antioxidant. The total extraction yields were the order of 44.6%. Phytochemical tests demonstrated the presence of secondary metabolites of alkaloids, tannins, polyphenols types, reducing compounds, free anthracene derivatives, anthraquinones, total sugars, coumarins, free quinones, sterols and triterpenes, carotenoids, flavonoids, mucilages and traces of cardiac glycosides and saponins. The results of the anti-free radical activity showed that the polar extracts were much anti-free of the free radicals than the non-polar extracts. The ethanolic extract was the most active with an IC_{50} of 20 µgmL^{-1}, followed by the aqueous and acetone extracts with IC_{50} of 25 µgmL^{-1}. The cyclohexane and trichlorethylene extracts have lower antiradical activities with IC_{50} of 400 µgmL^{-1}. Gallic acid, the reference antioxidant, showed an IC_{50} of 0.37 µgmL^{-1}.

Key words: *Staudtia kamerunensis* var. *gabonensis*, oilseeds, extracts, phytochemical screening, antiradical activity, ABTS, gallic acid.

INTRODUCTION

Staudtia kamerunensis var. *gabonensis* (Warb.) is a large tree that extends to Cameroon, Gabon and the Democratic Republic of Congo (Marbeley, 1987). This tree is used in traditional medicine in Central Africa. In the Democratic Republic of Congo, for example, seed pulp mixed with palm oil repels sandflies (Vivien and

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Faure, 1995). In Gabon, a decoction of the bark and seeds of *Staudtia gabonensis* Warb is used in gargles for the treatment of the teeth and in lotions on the head. The seeds yield a fat of yellow color with an aromatic flavor and odor which can be used as an anointing oil, ointment or medicine for scabies. The fleshy, scarlet-red aril of the ovoid or subglobose, tangerine-sized fruit is edible (Raponda- Walker and Sillans, 1961).

The species *S. gabonensis* Warb. has been the subject of some chemical investigations. The essential oils of *S. gabonensis* Warb were obtained from seeds and bark by hydrodisillation. Analyses by GC and GC/MS indicate a sesquiterpenes, germacrene D (35%) and β-caryophyllene (12%) in the seed oil while trans-α-bergamotene (39.5%) and β-caryophyllene (13%) are the major components of the bark oil. These volatile extracts did not reveal important antioxidant and antiradical activities (Agnaniet et al., 2004).

The stem bark and seeds of *Staudtia kamerunensis* Warb is being investigated for the first time. The seed hexane extract is composed mainly of glycerol tetradecanoate (trimyristin, glycerol trimyristate) and six lignans: otaobain, hydroxyotobain, otobaphenol, licarinA, licarin B and (-) dihydroxyguaiaretic acid were isolated. The ethyl acetate extract of the stem bark gave a seventh lignan, otobuiene (Yankep, 1999). A few years earlier, a diterpene acid (staudtienic acid) was isolated and identified in the specie *S. kamerunensis* (Noumbissie, 1992).

Secondary metabolites extracted from oilseeds of *S. kamerunensis* var. *gabonensis* could have a wide range of applications. Fatty acids play an essential role in several processes such as platelet aggregation, inflammation, immunity, etc. (Leclerf, 2011). Today, antioxidants from natural products such as plants, fruits and seeds are widely sought and used in several fields such as dietetics, agri-food, cosmetology and the medical field. It is known that polyphenolic compounds have interesting properties for the human body, both nutritionally and medically (Charrouf and Guillaume, 2007a).

In humans, oxidative damage to DNA, lipids and proteins is associated with certain chronic diseases such as cardiovascular diseases, certain cancers, diabetes, inflammatory diseases, Alzheimer's disease and other neurodegenerative diseases, as well as the aging process (Choi et al., 2018; Klaunig, 2018; Luc et al., 2019; Tönnies and Trushina, 2017; Kozakiewicz et al., 2019). An antioxidant or molecules isolated from the oilseed extracts of *S. kamerunensis* var. *gabonensis* could, for example, help prevent the appearance of these pathologies.

The results of our investigations relate to the polar and apolar extracts of the oilseeds of *S. kamerunensis* var. *gabonensis* in order to carry out phytochemical tests and evaluate their antiradical activity for their potential use in biotechnological fields such as agri-food, cosmetics and pharmacology.

MATERIALS AND METHODS

Purchase of chemicals

ABTS (2,2'-Azinobis [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). All these products were required for analysis. Concentrated H_2SO_4 (sulfuric acid) (95-97%), cyclohexane, trichlorethylene, acetone, absolute ethanol, chloroform, isoamyl alcohol, iron perchloride, magnesium shavings, chloroform, hydrochloric acid, acetic anhydre, Fehling's liquor, Dragendorf's reagent were also purchased from Sigma-Aldrich, Carlo Erba or Prolabo and used without further purification. Distilled water was obtained from Chimie Gabon. Molish's reagent, solutions of 1% iron perchloride, 2% ferric chloride, 1% sodium hydroxide, 10 and 25% ammoniac in water and hydrochloric alcohol were prepared in the laboratory from reagents and grade chemicals using procedures in the literature.

Plant material

The seeds of *S. kamerunensis* var. *gabonensis* (Myristicaceae) were collected in January 2021 from Sibang Herbarium of Pharmacopoeia and Traditional Medicine Institute (IPHAMETRA), Libreville, Gabon and then sent to the Laboratory of IPHAMETRA where they were dried in the sun for several days.

Extraction method by maceration

Preparation of samples of *S. kamerunensis* var. *gabonensis* for the trials began with the fragmentation and crushing of oilseeds. The seeds were first broken with a hammer to facilitate grinding. The seed fragments are then introduced in small quantities, in turns, into a Retsch-type grinder with IPHAMETRA. This approach is necessary to obtain a particle size corresponding to the requirements of ASTM standard No. 1105 (1996) in terms of particle size to quantify the extract rate. The powder collected was sieved to a diameter of less than 2 mm and kept in an oven at 40°C until the time of the tests.

In order to extract all the molecules of interest (polar and apolar), a sequential extraction by maceration using solvents of increasing polarity (cyclohexane, trichlorethylene, acetone, ethanol, distilled water) was carried out taking into account its efficiency in extracting and preserving the molecules of interest. This technique is similar to infusion and is done at room temperature. It is generally used for heat-sensitive compounds.

80 g of *S. kamerunensis* var. *gabonensis* were introduced into a 500-mL glass Erlenmeyer flask containing 400 mL of cyclohexane in order to collect lipid and apolar molecules or eliminate compounds such as oils, fats, sterols. The Erlenmeyer flask is then closed with a rubber stopper covered with aluminum foil. The mixtures were subsequently stirred at room temperature on a PIERRON type stirrer for 24 h. Thereafter, the mixtures were separated by vacuum filtration with a Whatman No. 4 type filter in a Büchner type funnel. The retentate or the extraction cake remaining in the upper part of the filter was immediately put back into solution for a second extraction in 400 mL of trichlorethylene in order to still collect the lipid molecules not extracted by cyclohexane. A third, fourth and fifth extraction were carried out respectively in acetone, ethanol and finally water to extract the phenolic compounds and the polar molecules while following the same extraction steps as the

cyclohexane solvent. After extraction, the organic solvent extracts (cyclohexane, trichlorethylene, acetone, ethanol) were evaporated in a pre-weighed flask using a rotary evaporator under vacuum. For aqueous extracts, freeze-drying will dry them completely. These five successive extractions were repeated on four samples (three evaporated samples for the calculations of the extractable levels and the anti-radical tests, one non-evaporated sample or filtrate for the phytochemical tests). The extracts with organic solvents were placed in an oven at 40°C until a constant mass was measured. All the extracts are stored in the refrigerator in closed bottles and covered with aluminum foil for the next tests. The percentage of extractables compared to the initial mass of seed powders of *S. kamerunensis* var. *gabonensis* used is determined using the following Equation (1):

$$R(\%) = \frac{Mext}{Mech} \times 100 \quad (1)$$

Where: R: yield of extracts in %; Mext: mass of the extract after evaporation or freeze-drying in grammes; Mech: anhydrous mass of the seed powder sample in grammes.

Screening phytochimique

The reagent used to carry out the phytochemical screening of the extracts was prepared according to the protocols described by Houghton and Raman (1998), Akinjogunla et al. (2010) and Badiaga (2011). All the different tests were carried out in triplicate.

Test for alkaloids: 2 mL of extract was placed in a test tube and then a few drops of Dragendorff's reagent solution was added. The appearance of a red-orange precipitate indicated the presence of alkaloids as described by N'Guessan (2009).

Test for polyphenols: 2 mL of the extract was placed in a test tube, thereafter a few drops of the 2% ethanolic ferric chloride solution was added. The appearance of a blue-blackish colour indicated the presence of polyphenols according to N'Guessan (2009).

Test for sterols and triterpenes: This was determined by placing 1 mL of the acetic anhydride which is added to 2 mL of each extract. 1 mL of concentrated H_2SO_4 (sulfuric acid) is then poured into tubes (this is the reaction of Libermann-Buchard). The appearance of a purple colour and coloring of the supernatant blue or purple green indicates the presence of sterols or triterpenes respectively as described by Nze Kamsi et al., (2020).

Presence of tannins: This was determined by adding to 1 mL of extract, 1 mL of distilled water and 1 to 2 drops of $FeCl_3$ solution (Iron (III) chloride) diluted to 1%. The appearance of a dark green colour indicated the presence of tannins (Bentab Lasgaa, 2015 cited by Hamid, 2018).

Test for reducing compounds: 2 mL of extract was placed in a test tube, followed by 2 mL of Fehling's liquor. The whole content was then poured into a boiling water bath for 8 min. The appearance of a brick red precipitate indicated the presence of reducing compounds as reported by Bentab and Lasgaa (2015) cited by Hamid (2018).

Test for flavonoids: 1 mL of extract was introduced into a test tube; thereafter 1 mL of hydrochloric acid was added, followed by 1 mL of isoamyl alcohol and then a few shavings of magnesium. The appearance of a pinkish-orange colour indicated the presence of flavonoids as reported by Harborne and Grayer (1988).

Test for saponosides: These were identified by placing 10 mL of each extract in a test tube which was shaken vigorously with a vortex for 15 s. The tube was left to stand for 15 min; thereafter the appearance of persistent foam indicated the presence of saponins as reported by N'Guessan (2009).

Test for cardiac glycosides: 2 mL of chloroform was added to 1 mL of each extract. The appearance of a reddish-brown colour after adding a few drops of concentrate H_2SO_4 (sulfuric acid) indicated the presence of cardiac glycosides as reported by Yam et al. (2009).

Presence of free quinones: This was determined by adding a few drops of 1% NaOH (sodium hydroxide) to 1 mL of each extract. The appearance of a colour turning yellow red or purple indicated the presence of free quinones as reported by Oloyede (2005).

Test for anthraquinones: To 2 mL of each extract was added 1 mL of 10% NH_4OH (ammoniac). After shaking, the appearance of a purple colour indicated a positive test according to Oloyede (2005).

Test for leucoanthocyanins: These were identified by adding to 5 mL of each extract, 5 mL of hydrochloric alcohol and then a few drops of isoamyl alcohol. The mixture was heated for two minutes in a boiling water bath. The appearance of a red coloration indicated the presence of leucoanthocyanins as reported by Fournet (1979).

Test for carotenoids: To each 2 mL of the extract, 0.5 mL of concentrated H_2SO_4 (sulfuric acid) is added. The appearance of a blue colour that turned red indicated the presence of carotenoids according to Ntsame Nze (2020).

Identification of mucilages: 1 mL of extract was introduced into a test tube followed by 5 mL of absolute alcohol. Obtaining a fluffy precipitate after shaking indicated the presence of mucilage as described by Awor et Samseny (2003) cited by Hamid (2018).

Determination of total sugars: To 1 g of each extract, 3 drops of Molish's reagent (for a volume of 100 mL, mix 0.25 g of 1-Naphthol + 50 mL of ethanol and 50 mL of 20% sulfuric acid) were added, followed by 1 mL of concentrated H_2SO_4 (sulfuric acid). The appearance of a purple interphase indicated their presence as reported by Feuya et al., (2015**).**

Test for coumarins: 1 mL of NH_4OH (ammoniac) diluted to 25% was added in 2 mL of extract. The whole content was heated in a water bath for 5 min and then a UV reading was taken at 365 nm. The appearance of an intense fluorescence in the tube showing either yellow, blue, blue-green, orange, purplish pink indicated the presence of coumarins as described by Awor et Samseny (2003) cited by Hamid (2018).

Determination of free anthracene derivatives: This was obtained by adding 1 mL of NH_4OH (ammoniac) diluted to 25% to a 1 mL of extract in a test tube. After stirring, the appearance of a more or less red colour indicated their presence as described by Awor et Samseny (2003) cited by Hamid (2018).

Anti-radical activity

The anti-free radical activity was determined by UV spectrophotometry using a V-200 spectrophotometer (BOECO, Germany). The optical density was read at 734 nm recording the maximum absorption wavelength of the radical cation ABTS • +.

Table 1. Result of the levels of extracts of *Staudtia kamerunensis* var. *gabonensis.*

Extract rate (average of three tests ± standard deviation)	
Solvent	**Extraction rate (%)**
	S. kamerunensis* var. *gabonensis
Cyclohexane	22.9±1.4
Trichloroethylene	5.9±0.6
Acetone	4.6±1.6
Ethanol	4.8±0.8
Distilled water	6.4±1.2
Total	44.6

Source: Authors

Preparation of "reference antioxidant" gallic acid solutions

Gallic acid (3,4,5-trihydroxybenzoic acid) is an aromatic organic compound, used as a reference anti-free 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.

Preparation of seed solutions of *S. kamerunensis* var. *gabonensis*

Ten solutions of increasing concentrations ranging from 2.5 to 200 µg/mL of different *S. kamerunensis* var. *gabonensis* extracts were prepared by dissolving the powder in the extraction solvent.

Measurement of anti-radical activity

The principle of the test for measuring the anti-radical activity by the ABTS method was based on the decrease in the absorbance at 734 nm of the radical cation ABTS • + (blue-green colouration) in the presence of a potentially anti-radical compound which reduced the cation radical. The reduction in the radical form of ABTS • + led to a discolouration of the solution. The radical ion ABTS • + was obtained by reacting the ABTS molecule (7 mM) with potassium persulfate (2.45 mM) in distilled water for 16 h at room temperature and protected from light.

The ABTS • + solution obtained was diluted with sodium phosphate buffer (5 mM, pH = 7.4), in order to obtain a stock solution having an initial absorbance value at 734 nm between 0.65 and 0.70. The radical cation (ABTS • +) was stable for more than 2 days when stored at room temperature and protected from light. All the assays were carried out three times and the anti-free radical activity was calculated according to the following Equation (2):

$$\text{Anti-free radical activity (\%)} = [1 - (Ar-Ab) / (Ai-Ab)] \times 100 \quad (2)$$

With Ar = remaining activity of ABTS • +, Ai = initial activity of ABTS • + and Ab = White activity.

In fact, the reduction of the ABTS • + radical cation therefore amounted to determining the anti-free radical activity and in total, the antioxidant properties of *S. kamerunensis* var. *gabonensis* extracts compared to the antioxidant properties of gallic acid (standard). The anti-free radical activity was determined by UV spectrophotometry in cuvettes with an optical path of 1 cm (reaction volume of 2 mL). The incubation time was 6 min as reported by N'Negue et al. (2020).

RESULTS AND DISCUSSION

Extraction yields

Results of the extractable contents of the oilseeds of *S. kamerunensis* var. *gabonensis* are presented in Table 1. These results indicate that the levels of extractables vary from one solvent to another. Cyclohexane extraction rates are the highest with 22.9%. The lowest yields of extracts are obtained with the solvents distilled water with 6.4%, trichlorethylene with 5.9%, ethanol with 4.8% and finally acetone with 4.6%. The overall extract levels of oilseeds of *S. kamerunensis* var. *gabonensis* are 44.6%.

Cyclohexane, the first solvent used during the successive extraction and the least polar, mainly extracts fat-soluble substances (oils, fats, terpenes) from the oilseeds of *S. kamerunensis* var. *gabonensis*. Cyclohexane thus contains significant fractions of lipids, hence its high yield with 22.9%. Trichlorethylene, another apolar solvent, showed a very low yield with 5.9% indicating that the seed powder of *S. kamerunensis* var. *gabonensis* was practically de-oiled or delipidated by the first solvent, cyclohexane. Polar solvents (acetone, ethanol, water) subsequently solubilise polar compounds such as polyphenols and polar molecules. The successive extraction thus combines the apolar and polar solvents. It makes it possible to partition the extractables into different fractions facilitating subsequent analyses. The sum of the extracts with each solvent gives an idea of the overall extract content of the seeds of *S. kamerunensis* var. *gabonensis* with 44.6%.

Silou (2014) studied one hundred and thirty samples of oils and fats extracted from 77 species from the Congo Basin constituting 35 botanical families and divided them into three classes of equal amplitude between 15 and 75% fat content. Plants with low fat content have a rate between 15 and 35%; those with a medium fat content have a rate of between 35-55% and finally plants with a high fat content have a rate of between 55-75%. In view of these percentages and the levels of extracts obtained with the two apolar solvents (Cyclohexane and trichloroethylene), *S. kamerunensis* var. *gabonensis* is not a high fat plant.

Phytochemical tests

Results of the phytochemical screening are classified according to the criteria for observing the reactions:

Intense: +++
Moderately intense: ++
Not very strong: +
Absent: -

Table 2. Results of the phytochemical tests the extract of *Staudtia kamerunensis* var. *gabonensis.*

Compounds	Solvents				
	Cyclohexane	Trichloroethylene	Acetone	Ethanol	Distilled water
Alkaloids	+++	+++	+++	+++	+++
Polyphenols	-	-	+++	+++	+++
Leucoanthocyanins	-	-	-	-	-
Carotenoids	+	+	++	++	-
Tannins	-	-	-	+++	+++
Reducing compounds	+++	++	+++	+++	+
Flavonoids	+++	++	+++	++	+++
Saponines	-	-	-	-	+
Anthraquinones	-	+	+	++	++
Cardiacglycosides	+	+	-	+	+
Free quinones	+	++	++	+++	+++
Mucilages	+++	-	-	-	-
Total sugars	+++	+	++	+++	+++
Sterols/triterpenes	+++	-	-	-	-
Coumarins	+	-	-	++	+
Free anthracene	-	-	-	+++	-

Source: Authors

The results of the phytochemical tests are presented in Table 2. The alkaloids were present in all the extracts (cyclohexane, trichlorethylene, acetone, ethanol and water). Polyphenols were abundant in polar extracts (acetone, ethanol and water) and absent in less polar extracts (cyclohexane, trichlorethylene).

The tannins were present in the ethanolic and aqueous extracts and absent in the other extracts (cyclohexane, trichloroethylene, acetone). Carotenoids were in low quantity in the cyclohexane and trichlorethylene extracts, moderately present in the acetone and ethanol extract, and absent in the aqueous extract. The flavonoids were present in the cyclohexane, acetone and aqueous extracts; they are moderately present in the trichloroethylene and ethanol extracts. Saponins were in very low quantity in the aqueous extract and absent in the rest of the extracts.

Anthraquinones were moderately present in the ethanolic and aqueous extracts, very low in the trichloroethylene and acetone extracts, and absent in the cyclohexane extract. Cardiac glycosides were weakly present in the cyclohexane, trichlorethylene, ethanol and water extracts, and absent in the ethanolic extract. Free quinones were present in the ethanolic and aqueous extracts, moderate in the acetone and trichlorethylene extracts, and very low in the cyclohexane extract. Mucilages were present in the cyclohexane extract and absent in the rest of the extracts.

Coumarins were weakly present in the cyclohexane extracts, aqueous, medium in the ethanolic extract and absent in the trichlorethylene and acetone extracts. Free anthracene derivatives were abundant in the ethanolic extract and absent in the rest of the extracts. Leucoanthocyanins were absent in all extracts.

Phytochemical tests carried out on the seeds of *S. kamerunensis* var. *gabonensis* revealed the presence of alkaloids, tannins, polyphenols, reducing compounds, free anthracene derivatives, anthraquinones, total sugars, coumarins, free quinones, sterols and triterpenes, carotenoids, flavonoids, mucilages and traces of cardiac glycosides and saponins. These compounds have multiple therapeutic properties in the literature. Indeed, several authors have demonstrated that the consumption of foods rich in polyphenols reduces the development of much pathology, such as cancer, vascular diseases, hypertension, astrosclerosis (Hertog et al., 1993; Middleton et al., 2000; Leong and Shui, 2002).

The seeds of *S. kamerunensis* var. *gabonensis* contain a high proportion of alkaloids in the five extracts, but these compounds could involve interesting biological activities. Alkaloids have several pharmaceutical applications in humans, as these applications have been clinically proven (Mc Calley, 2002; Silvestrini et al., 2002). Alkaloids are researched for their physiological effects and their pharmacological activities which are exerted in various fields. They also act as antibiotics (Badiaga, 2012). Sterols and triterpenes are demonstrated in extracts of *S. kamerunensis* var. *gabonensis* or these compounds are recognized to have anti-inflammatory, antidiabetic, anticancer, antidiarrheal, antiviral and anti-HIV activities (Raman et al., 2012). We note the absence of leucoanthocyanins in our extracts, where the only flavoinoides present are free flavonoids. It is recognized that flavonoids have antioxidant and anti- inflammatory

activities and play a positive role in the treatment of cardiovascular and neurodegenerative diseases (Wang et al., 2002). In some cases, they are known for their antiviral, antimicrobial and antitumor activity (Narayana et al., 2001; Seyoum et al., 2006).

A strong presence of tannins is marked in the ethanolic and aqueous extracts as these compounds exert an antidiarrheal, antiseptic, antibacterial and antifungal activity, as well as dermatitis (Aron and Kennedy, 2008). Tannins also have strong antioxidant activity. They are very good free radical scavengers. Phytochemical screening has detected the presence of coumarins which are known for their antioedematous properties in patients with advanced cancer: they are immunostimulants and would develop cytotoxic activity (Diallo, 2000). Traditionally, coumarin is used to facilitate urinary and digestive elimination functions. Mucilages are present in the cyclohexane extract of the seeds of *S. kamerunensis* var. *gabonensis*. Mucilages have confirmed functional and physical properties in the cosmetic fields (Chen et al., 2006). The commercial potential of mucilage in the pharmaceutical fields is based on its emollient and softening properties (Bemiller et al., 1993).

The carotenoids of the seeds of *S. kamerunensis* var. *gabonensis* could have potential for a pro-vitamin A activity. Vitamin A indeed participates in the maintenance of good vision and in the prevention of 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 (Jaswir et al., 2011). The carotenoids contained in these seeds could also be used as additives in the food industry as food coloring or in cosmetic products (Swiss Confederation, 2017; Jaswir et al., 2011).

Anthraquinone, free quinones and its natural derivatives are evidenced in the seeds of *S. kamerunensis* var. *gabonensis*. These compounds have proven therapeutic power to treat all functional intestinal disorders such as functional colopathy, laxophobia or constipation (Müller-Lissner, 1993). Some quinones have antioxidant, bronchial antispasmodic properties (Bruneton, 1997). Anthracene derivatives are responsible for laxative activity (Bruneton, 1987). Anthracene glycosides described as antidepressants are said to have antimicrobial properties (Cronquista, 1981).

In summary, the phytochemical screening of *S. kamerunensis* var. *gabonensis* reveals potential pharmacological, cosmetic and food-processing activities by the presence of numerous chemical families. By correlating the properties of the identified classes of secondary metabolites mentioned above, oilseeds of *S. kamerunensi* var. *gabonensis* constitute a target of choice and research work in the fields of biotechnology.

It is worth noting that phytochemical tests on seeds or barks of the species *S. kamerunensis var. gabonensis* have not been included in the literature to corroborate our results.

Anti-radical activity

Anti-free radical activity of gallic acid depending on the concentration

The anti-radical activity of the standard antioxidant "gallic acid" increased linearly with its concentration (Figure 1). The value of the IC_{50} which is the concentration necessary for the reduction of 50% of the anti-radical activity of gallic acid deduced from the curve is 0.37 µg/mL (2 µM). The antioxidant activity of gallic acid which is a strongly antioxidant synthetic molecule, validates the method chosen in the present work.

Anti-free radical activity of *Staudtia kamerunensis* var. *gabonensis* extracts

Evaluation of the anti-radical activity of the aqueous extract

According to the results obtained (Figure 2A), the anti-radical activity of the aqueous extract increased by 7.34 92 ± 0.254% for a concentration of 2.5 μgmL^{-1} to approximately 90% for concentrations of 100 to 200 μgmL^{-1}. The IC_{50} is 25 μgmL^{-1}.

Evaluation of the anti-radical activity of the ethanolic extract

The results (Figure 2B) show an increasing anti-free radical activity with the concentration of ethanolic extract of *S. kamerunensis* var. *gabonensis*. The anti-radical activity is 6.99 ± 0.99% for a concentration of 2.5 μgmL^{-1} and 91% for concentrations of 75 to 200 μgmL^{-1} of ethanolic extract. The IC_{50} of the ethanolic extract is 20 μgmL^{-1}. It is 1.25 times lower than that of the aqueous extract. The ethanolic extract would therefore present a slightly higher activity than that of the aqueous extract.

Evaluation of the anti-radical activity of the acetone extract

The results presented in Figure 2C show an increasing anti-free radical activity ranging from 4.088±0.73% for a concentration of 2.5 μgmL^{-1} in acetone extract of *S. kamerunensis* var. *gabonensis,* at 88% for concentrations of 100 to 200 μgmL^{-1}. The IC_{50} is equal to 25 μgmL^{-1} and is equivalent to that of the aqueous extract.

Evaluation of the anti-radical activity of the trichlorethylene extract

According to the results (Figure 2D), the anti-radical activity increased weakly with the concentration of

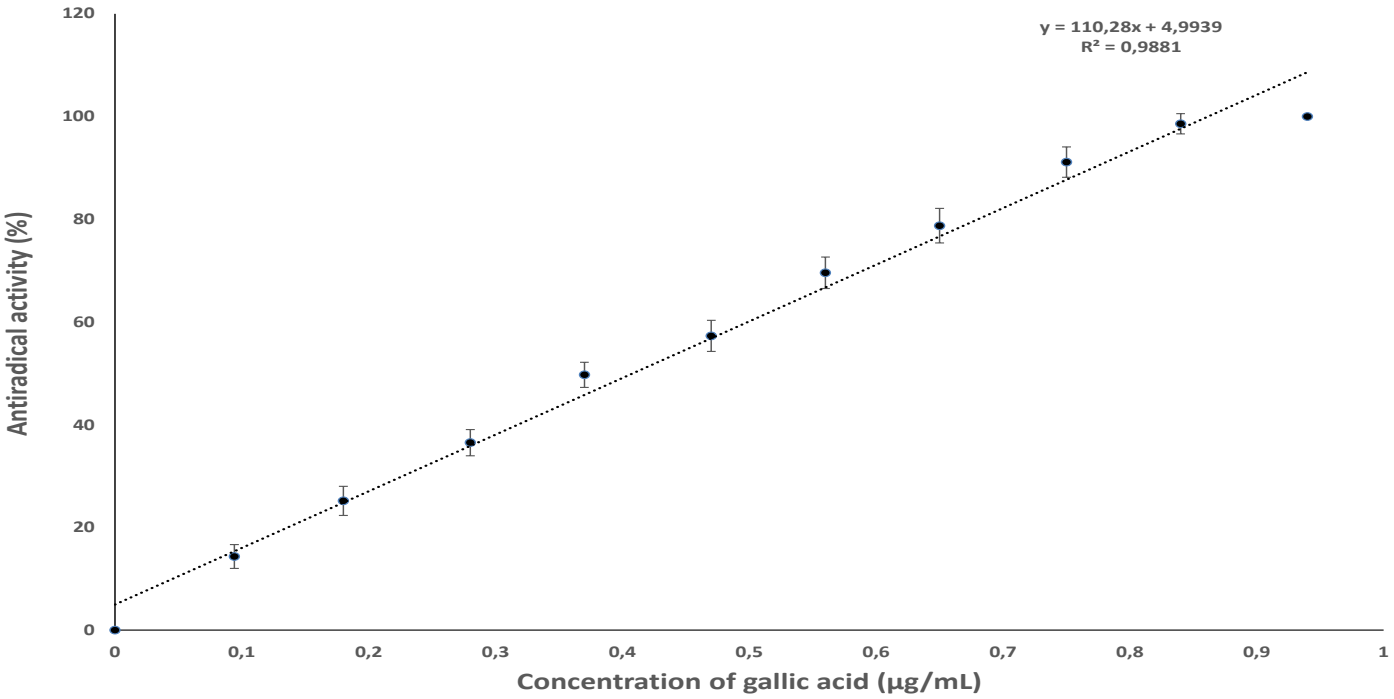

Figure 1. Anti-radical activity as a function of the concentration of gallic acid after 6 min of incubation. The proportion ABTS•$^{+}$ transformed into $ABTS^{+}$ in the presence of gallic acid is calculated from the change in absorbance at 734 nm measured by spectrophotometry [The equation on the right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

trichloroethylene extract of *S. kamerunensis* var. *gabonensis*. It is 1.47 ± 0.14% for a concentration of 2.5 μgmL^{-1}; 8.78 ± 1.05% for a concentration of 25 μgmL^{-1} and only 11.597 ± 2.67% for a concentration of 50 μgmL^{-1}. The IC_{50} of the trichlorethylene extract is greater than 200 μgmL^{-1}. Indeed, for a concentration of 200 μgmL^{-1}, the antiradical activity is only 24.95 ± 2.06%, and is a percentage of activity corresponding to the $IC_{50}/2$. By extrapolation, we can deduce that the IC_{50} of the cyclohexane extract would be around 400 μgmL^{-1}.

Evaluation of the anti-radical activity of the cyclohexane extract

The percentage of antiradical activity increased slightly with the concentration of cyclohexane extract of *S. kamerunensis* var. *gabonensis* (Figure 2E). Indeed, it is 3.1 ± 0.309% for a concentration of 2.5 μgmL^{-1}; 8.63 ± 0.08% for a concentration of 25 μgmL^{-1}; 10.98 ± 1.54% for a concentration of 50 μgmL^{-1} and only 26.25 ± 1.54% ($IC_{50}/2$) for a concentration of 200 μgmL^{-1}. The IC_{50} of the cyclohexane extract would be around 400 μgmL^{-1}.

The anti-radical activity of the various extracts of the seeds of *S. kamerunensis* var. *gabonensis* was evaluated by trapping the ABTS•+ radical ion according to the method of Re et al. (1999) optimized by N'Negue et al. (2020) with gallic acid as reference antioxidant. The results of the antioxidant activity of gallic acid (strongly antioxidant synthetic molecule) validate the chosen method. Moreover, the IC_{50} value of gallic acid deduced from our results is more or less equivalent to that obtained by Sadat et al. (2011), which is 0.47 µg/mL and N'Negue et al. (2020, 2021) which is 0.47 and 0.37 µg/mL. These authors worked under the same conditions. The results of the evaluation of the anti-radical activity of the extracts of the seeds of *S. kamerunensis* var. *gabonensis* showed a variation of scavenging activity with the mode of extraction. Indeed, according to our results, the ethanolic extract which presents a strong antiradical activity, is the most active with an IC_{50} of 20 μgmL^{-1}. The activity of the ethanolic extract is slightly higher than that of the aqueous and acetonic extracts, which presented quite similar activities with an IC_{50} of 25 μgmL^{-1}.

However, according to the results obtained, the extracts of the apolar solvents "cyclohexane and trichlorethylene" have lower activities than those of the polar solvents "water, ethanol and acetone". The IC_{50} of apolar solvents was greater than 200 μgmL^{-1}. We can explain these results by the presence of a large quantity of polyphenols in the polar extracts and their absence in the apolar extracts. Indeed, according to the literature, polyphenolic compounds are able to trap free radicals, which explain their strong anti-radical power (Biradar 2016; Nga et al., 2017; Becker et al., 2019).

The results of the phytochemical study of the seed extracts of *S. kamerunensis* var. *gabonensis* also showed

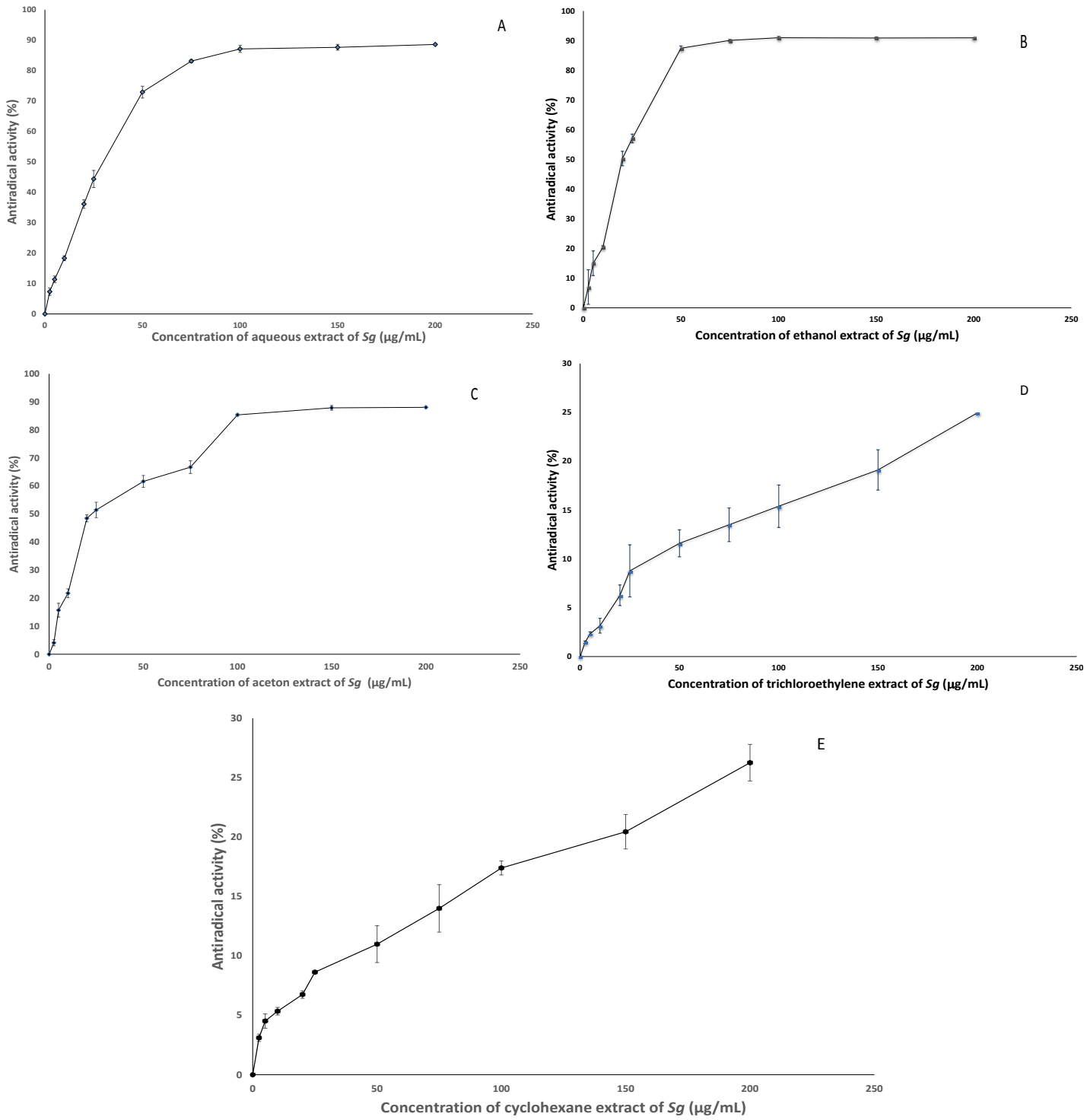

Figure 2. Anti-radical activity according to the concentration of Aqueous extract (A), Ethanol (B), Acetone (C), Trichloroethylene (D), Cyclohexane (E) of *S. kamerunensis* var. *gabonensis* after 6 min of incubation. The proportion $ABTS^{\bullet+}$ transformed into $ABTS^{+}$ is calculated from the change in absorbance at 734 nm measured by spectrophotometry: $n = 3$.
Source: Authors

the presence of tannins in the ethanolic and aqueous extracts and that of carotenoids in the acetone and ethanolic extracts. However, the carotenoids were not found in the aqueous extract but in the acetone and ethanol extract. These phytochemical results explain the slightly higher activity of the ethanolic extract compared

to the aqueous and acetone extract. Indeed, in the ethanolic extract, there is an abundant presence of polyphenols, tannins and an average presence of coumarins, flavonoids and then carotenoids which are recognized in the literature as high-level antioxidants because of their ability to trap free radicals such as singlet oxygen, superoxide free radicals and hydroxyl radicals (Treml and Smejkal, 2016; Kawamura et al., 2011; Jaswir et al., 2011; Anderson et al., 1996).

The very low quantity of carotenoids, coumarins, the total absence of polyphenols and tannins in the oils or fats of apolar extracts (extracts with cyclohexane and trichloroethylene) would explain their less significant antiradical activity.

This result corroborates that of Agnaniet (2004) who evaluated the antioxidant and antiradical activities of the essential oils of *S. kamerunensis* var. *gabonensis* by comparison with a commercial sample of di-t-buylhydroxytoluene widely used as a preservative. Biological screening for antioxidant action did not reveal significant activity, but a slight positive concentration effect was observed. The antioxidant activity of the seeds of *S. kamerunensis* var. *gabonensis* for IC_{50} was 37% for a concentration of 666 μgmL^{-1}. The DPPH test showed a weak activity confirmed by the measurement of the antiradical activity which proved to be very weak compared to BHT. Agnaniet (2004) results indicate that the essential oils of *S. kamerunensis* var. *gabonensis* were much less active than the commercial sample. These essential oils are therefore of no interest in the fields of biotechnology.

However, according to our results, polar extracts are more active than apolar extracts. It seems that the more the extraction solvent is polar, the more it solubilizes the antiradical compounds. The activity of polar extracts is comparable to that of “reference antioxidant” gallic acid. Indeed, in the active ingredient responsible for the antiradical activity representing only about 10% of the total compounds of the extract, IC_{50} of 20 or 25 μgmL^{-1} of the total extract would be equivalent to an IC_{50} of 2 or 2.5 μgmL^{-1} of the active ingredient, which is only 5.4 or 6.75 times greater than that of the reference antioxidant, a pure chemical compound “gallic acid with IC_{50} = 0.37 μgmL^{-1}”. Polar extracts would therefore have potential antioxidants.

Conclusion

The results showed that the total extract levels of oilseeds of *S. kamerunensis* var. *gabonensis* were 44.6%. The phytochemical tests carried out showed that the seeds of *S. kamerunensis* var. *gabonensis* are rich in major chemical groups such as alkaloids, tannins, polyphenols, flavonoids, reducing compounds, free anthracene derivatives, anthraquinones, total sugars, coumarins, free quinones, sterols, triterpenes, carotenoids and mucilages.

Strong anti-radical activity is observed with the ethanolic extract followed by the aqueous extract and with acetone. On the other hand, the cyclohexane and trichloroethylene extracts rich in fat or oil did not show significant antiradical activity. Extracts with high antiradical activity of *S. kamerunensis* var. *gabonensis*, because of 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 the food, pharmaceutical and cosmetic industries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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