



**Phytochemical composition and antiradical activity of *Sakersia africana* Hook. f. medicinal plant from Gabon**

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**Abstract**

The valorization of the medicinal plants of our country and determination of their impact on health due to their abundance of substances with various pharmacological effects are our principal objective. This study was evaluated the phytochemical screening and radical 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of different extracts of *Sakersia africana* Hook. f.. The results revealed that *Sakersia africana* Hook. f. is rich in phenols compounds, sterols, triterpenes, alkaloids and reducing compound. The values in total phenols and proanthocyanidines are ranging respectively from  $391.58 \pm 0.04$  to  $777 \pm 0.03$  mg/100 g of drugs and  $113.5 \pm 3.17$  to  $653.5 \pm 36.83$  mg/100 g of drugs. Results also show that different extracts tested present antiradical activity with values of  $IC_{50}$  ranging from  $164.21 \pm 0.014$  to  $195.54 \pm 0.012$  % and abundance in bioactive compounds. This study could justify the use of *Sakersia africana* of some chronic diseases.

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## Introduction

The plants were used for prehistory by Human for nutritional and therapeutic requirement. Medicinal plants are the major source of drugs through their rich secondary metabolites (Fouché *et al.*, 2000; Nostro *et al.*, 2000). Thus, medicinal plants are the main means to fight against diseases by including populations of underdeveloped countries. This practice was abandoned in favor of modern medicine. We are witnessing a return to use of medicinal plants over the past decade. World Health Organization (WHO), in 2007 estimated that about 80 % of the population of developing countries can be cured from plants (Obame, 2009). In 2001, the studies have revealed that approximately 25 % of prescriptions in the world for drugs herbal and 60 to 70 % of antibacterial and anticancer drugs are substances of natural origin (Rate, 2001). Hence the useful achieve the phytochemical studies of medicinal plants for discovery of news bioactive compounds. As such the WHO is currently supporting clinical validation of some traditional medicines. It is a shrub of the Melastomataceae family that grows on moist soils and in secondary forest (Walker and Sillans 1961). The leaves and twigs in a hairy very steep, and the protruding ribs of a green-red, turn yellow or red before falling. Fruit globular form berries are very small and contain many tiny seeds. The decoction of the leaves has healing properties crushed. It is used as healing by gabonese populations in southern and the treatment of several diseases as dysentery, diarrhea, stomach ulcers and stomach aches (Walker and Sillans, 1961). The objective of this study was to evaluate the phytochemical characteristics and radical scavenging activity of different extracts of *Sakersia africana* Hook. f.

## Material and methods

### Plant material

The branches of *S. africana* were collected in september 2012 in Mbomo village, at 28 km of Oyem city (Northern Province of Gabon). The plant was

taxonomically authenticated at the Laboratory of Plant Biology and Ecology of University of Science and Technology of Masuku (Franceville, Gabon), where a voucher specimen was deposited.

### Preparation of plant extract

The branches of *Sakersia africana* was air-dried at room temperature for a total period six weeks and pulverized to powder using a clean electric blender (Model Phillips 190). A 25 g sample of the pulverized branches of *Sakersia africana* was soaked in 500 mL of solvent (ethanol, ethanol-water (50/50 v/v) and water) and allowed to stand for 72 h with intermittent stirring. This was filtered through a whatman No. 1 filter paper and the filtrate obtained was evaporated to a dry mass using a rotary rotavaporator at 40 °C. The residues recovered were dried in an oven at a temperature of 65 °C. The extract obtained is stored in vials protected from light until the completion of various tests. The yields of the extracts (%) were calculated (Boulenouar *et al.*, 2009; Salem, 2009).

### Phytochemical screening

Phytochemical tests the dry extracts (ethanolic, ethanolic-water and aqueous) bioactive compounds was realized using standard procedures. Two extractions methods (maceration and decoction) were used (Bruneton, 1999; Bidié *et al.*, 2008). The extracts were tested qualitatively for the presence of chemical constituents such as sterols, terpenoids, tannins, polyphenols, flavonoids, quinones and saponins (Paris and Moyses, 1969; Bouquet and Debray 1971). The alkaloids were tested by the method of Dragendorff and Meyer (Bruneton, 1999).

### Phenols and proanthocyanidins content extracts

The Folin-Ciocalteu method was used to measure total amount of total phenols content (Singleton *et al.*, 1999). Aliquots of 0.25 mL of leaf extracts (1 mg/mL) were mixed with 1.25 mL Folin–Ciocalteu reagent (0.2 N diluted in Methanol). A reagent blank using methanol instead of sample was prepared. After 5 min

incubation at room temperature, 1 mL sodium carbonate solution (7.5 %) was added. Samples were incubated at room temperature for 1 h and the absorbance was measured at 765 nm versus the

prepared blank. All tests were carried out in triplicate and total phenols content was expressed as mg of gallic acid equivalents (GAE) per 100 g of drug.

**Table 1.** Composition phytochemical extracts of *Sakersia africana* Hook.f.

Chemical compounds	Aqueous	Ethanollic-water	Ethanollic
Saponins	+++	+	-
Gallic tannins	++	+++	+++
Catechic tannins	+++	+++	+++
Anthraquinones	-	-	-
Total phenols	+++	+++	+++
Total flavonoids	-	+++	+++
Flavanonols	-	+++	-
Flavones	-	-	+
Flavanones	-	-	-
Coumarins	+++	+++	++
Digitoxin	+++	+++	++
Digitoxigenin	-	-	-
Gitoxin	-	-	-
Gitoxigenin	-	-	-
Alkaloids	+++	+++	+++
Sterols and triterpenes	+++	+++	+++
Reducters compounds	+++	+++	+++

+++ = High, ++ = Moderate; + = Low; -: negative test.

**Table 2.** Comparison of total phenolic compound, proanthocyanidins and antiradical activity of extracts de *Sakersia africana* Hook.f.

Extracts	Yields (%)	Total phenols (mg GAE/100 g of drug)	PAs (mg APE/100 g of drug)	Quota of PAs in Total phenols (%)	DPPH: IC <sub>50</sub> (µg/ml)
Aqueous	4.04	507.42 ± 0.17	113.5 ± 3.17	22.37 ± 3.17	195.54 ± 0.012
Ethanollic-water	4.36	391.58 ± 0.04	341.83 ± 36.5	87.30 ± 36.5	164.21 ± 0.014
Ethanollic	5.56	777 ± 0.03	653.5 ± 36.83	84.11 ± 36.83	177.02 ± 0.013

Proanthocyanidins (PAs) were quantified with the hydrolysis test of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavans-3-ols constituting the polymers (Prigent, 2005). The heating step destroys the anthocyanidins pigments generated by flavan-4-ols and eliminates part of the chlorophyll

pigments. The routine assay is performed by mixing 0.16 mL (1 mg/mL) of the extract with 2.33 mL of 30 % HCl-butanol solution (v/v). The mixture was put in tightly closed tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, the absorbance was read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as

mentioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

#### *Antiradical activity*

DPPH spectrophotometric (quantitative) assay was performed with some modifications (Brand-Williams *et al.*, 1995). Method was widely used to test the ability of antioxidant bioactive compounds to activity as free radical scavengers or hydrogen donors. This test is based on the capacity of stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen (H) donors, including phenols. It is used for the quantification of antioxidants in the complex of biological systems (Miliauskas *et al.*, 2004). Each sample of extract was prepared at different concentrations (100, 200, 300 and 400 µg/mL). The reaction mixture contained 1 mL of DPPH prepared at a concentration 20 mg/L in methanol, and 1 ml of test samples. After a 30 min reaction, the absorbance was read at 517 nm and converted into percentage of antiradical activity (AA %), using the following the formula (Abdoul-Latif *et al.*, 2010).

$$\%DPPH \text{ radical scavenging} = ((Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}) \times 100$$

Where,

Abs<sub>control</sub> is the absorbance of the blank;

Abs<sub>sample</sub> is the absorbance of the sample.

Control contained 1 mL of DPPH solution and 3 mL of methanol. The measurements of DPPH radical scavenging activity were carried out for three sample replications, and values are an average of three replicates. IC<sub>50</sub> is defined as the concentration of the test sample leading to a 50 % inhibition of the DPPH free radicals. IC<sub>50</sub> value was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against concentration of the test compounds (mg /mL) obtained from three replicate assays.

## **Results and discussion**

### *Phytochemical screening*

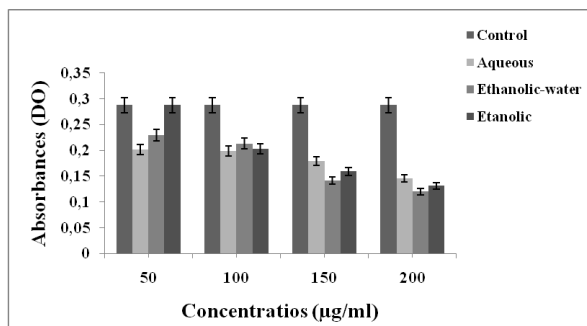
The results show that all extracts are rich in sterols, triterpenes, tannins (catechic and gallic), polyphenols, alkaloids, and reducing compounds. Coumarins and cardiac glycosides (digitoxins) are abundant in aqueous and ethanolic-water extracts. Only the aqueous extract contains saponins and no anthraquinones free flavanones, digitoxigenin, and gigitoxin gigitoxigenine. Also, some extracts (ethanolic and ethanolic-water) are rich in flavonoids total (Table 1).

Phytochemical analysis is very useful in the evaluation of some active biological components of *Sakersia africana*. The qualitative and quantitative analyses were carried out in both dry extract show that *Sakersia africana* is rich in phenolic compounds (polyphenols, tannins, flavonoids) in terpenic and nitrogen compounds. This wealth of pharmacological compounds conferred to said plant several pharmacological properties. The abundance of tannins explains the use of *Sakersia africana* ethnotherapy for the treatment of pathology identified during this investigation. These are diseases such as hypertension, dysentery, diarrhea, stomach ulcers and stomach aches. Indeed, it has been shown that the higher the plant is rich in tannic compounds will be more effective for the treatment of diseases of hypertension, dysentery, diarrhea, hemorrhoids, stomach ulcer, stomach ache and some various venereal diseases (Kerharo and Adjanohoun 1989; Pousset, 1989). In addition, the healing properties of plants are correlated with the abundance of tannins. This is the case for example *Lannea acida*, rich in tannins, is used in traditional medicine for wound healing (Sereme *et al.*, 2008). This could also justify the healing properties attributed to *Sakersia africana* (Walker and Sillans 1961).

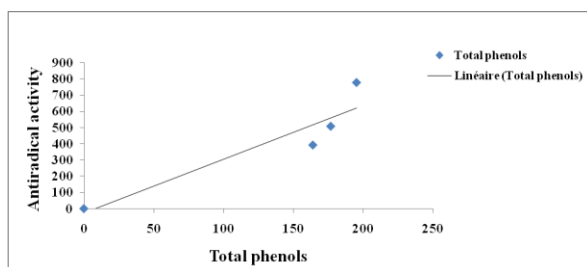
### *Total polyphenols, proanthocyanidins contents*

The yields of different extracts of *Sakersia africana* were respectively 4.04 % (aqueous), 4.36 % (ethanolic-water) and 5.56 % (ethanolic) (table 2). The concentrations of total phenols in the different extracts of the plant of study are more significant in

comparison with those of the cereals (0,481 à 0,896 mg/g of matter) appreciably equal with those of other plants such as Broccolis (11.7 mg /g of matter), the gallic ones (9.9 mg/g of matter) and the fruits (23.1 mg/g of matter for the blackberry) (Vinson *et al.*, 1995; Wang and Lin, 2000).



**Fig. 1.** Antiradical activity extracts of *Sakersia africana* Hook.f.



**Fig. 2.** Correlation between antioxidant activity and total phenols, ( $R^2 = 0.8605$ ).

Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the right and side of the proportioning of total phenolic content by the method of Folin-Ciocalteu gave  $Y = 0.0012 X - 0.0004$  with  $R^2 = 0.9902$  (Abdoul-Latif *et al.*, 2012).

The HCl/butanol assay used here for the determination of proanthocyanidins is more specific than many other tests such as the vanillin assay (Makkar, 2000; Santos-Buelga and Scalbert, 2000). The interferences, which might result from flavan-4-ols conversion into proanthocyanidins or from chlorophylls, may have been minimized during the heating step (Prigent, 2005; Santos-Buelga and Scalbert, 2000).

Levels of proanthocyanidins were expressed in terms of apple proanthocyanidins equivalent (APE). The

equation of the right-hand side of the proportioning of the proanthocyanidins by the HCl- Butanol method gave  $Y = 0.0006 X + 0.0024$  with  $R^2 = 0.9869$  (Abdoul-Latif *et al.*, 2012) Among extracts, proanthocyanidins contents ranged between 113.5 and 653.5 mg APE/100 g of drug (Table 2).

The total contents of phenols ranged between 391.58 and 777 mg GAE/100 g of drug (table 2). However, 87.30 and 84.11 % of total phenols in ethanolic-water extracts and ethanolic respectively are proanthocyanidins. Only the aqueous extract has a low percentage of proanthocyanidins (22.37 %).

#### *Correlation between antioxidant activity and total phenols*

Several comprehensive works have been done on the effects of phenolic compounds on total antioxidants (Li *et al.*, 2008; Kim *et al.*, 2009; Kouamé *et al.*, 2009 and Abdoul-Latif *et al.*, 2010), and correlations between phenolic compounds and total antioxidants (Chanwitheesuk *et al.*, 2005; Li *et al.*, 2008). This same trend was also obtained in our study. There was a good linear correlation ( $R^2 = 0.8605$ ,  $p < 0.05$ ) between the total phenolic content and the scavenging of DPPH radical in each extract (Figure 1). These results are similar to those obtained by Njintang *and collaborators* in their study on antiradical activity and polyphenol content of ethanolic extracts of *Propolis* deed. These authors showed that the antiradical activity was correlated with the polyphenol content in different extracts (Njintang *et al.* 2012).

#### *Antiradical activity*

Antioxidant activity using DPPH radical-scavenging assay expressed as  $IC_{50}$  value is showed in table 2 lower  $IC_{50}$  indicating the higher antioxidant activity of extract. The results of DPPH antiradical activity were differed significantly between different extracts. This difference is explained by the fact that an antiradical activity of phenolic compounds depends on their molecular structure, on the availability of phenolic

hydrogens and on the possibility for stabilization of the resulting phenoxy radicals formed by hydrogen donation (Catherine *et al.*, 1996; Ramarathnam *et al.*, 1997; Sundaram *et al.*, 2011). These results of antiradical activity of *Sakersia africana* show that extracts exhibit antiradical activity from a concentration of 50 µg/mL (aqueous and ethanolic-water) (figure 1 and table 2). The radical scavenging activity of ethanolic extract starts at concentrations that are higher than 50 µg/mL. The lowest concentration IC<sub>50</sub> that inhibits half of the DPPH radical was obtained with ethanolic-water extract (164.21 mg/mL) followed by ethanol extract (177.02 mg/mL). The aqueous extract show the highest inhibitory concentration (IC<sub>50</sub> = 195.54 mg/mL) (table 2). These results also show that *Sakersia africana* have a significant antiradical activity. However, ethanolic-water and ethanol extract were the higher antioxidant activity with an IC<sub>50</sub> respectively 164.21µg/mL and 177.02 mg/mL. Aqueous extract show lower activity (IC<sub>50</sub> = 195.54 mg/mL). In terms of concentrations of phenolic compounds in different extracts, it appears that ethanolic-water extract is less rich in total polyphenols (391.58 mg EGA/g of drug) and more active (IC<sub>50</sub> = 164.21 mg/mL). It is observed probably result from the fact that the determination by the Folin-Ciocalteu reagent is not specific to polyphenols. Other compounds may interact with said reagent and provide a high level of polyphenols (Catherine *et al.*, 1996; Ramarathnam *et al.*, 1997). Thus ethanolic and aqueous extracts are apparently rich while the actual content of total phenols is low.

### Conclusion

The present study was aimed to evaluate the phytochemical and antiradical allow us to infer that the use of *Sakersia africana* traditherapy by people against various diseases would depend on its relative wealth in saponins, phenolic compounds (tannins, Coumarins and flavonoids) and nitrogen (alkaloids) endowed of pharmacological properties. This abundance of active plant gives remarkable properties,

which could justify its multiple therapeutic indications for which it is used traditherapy. These preliminary results could provide a scientific basis for the research of new therapeutic molecules. Further pharmacological investigations allow us to determine precisely the different biological activities and to evaluate the acute and subacute *Sakersia africana*.

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