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In vitro antioxidant activity of trunk bark hydro-ethanolic extract of *Stereospermum kunthianum* Cham. and its fractions

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Abstract
The aim of this study was to contribute to the valorisation of the plant by determining the antioxidant activity of *Stereospermum kunthianum* trunk bark hydroethanolic extract and its fractions obtained with solvents of increasing polarity. Antioxidant activity was screened by bioautography and assessed by DPPH, ABTS and FRAP assays. All samples shown an antioxidant activity by the bioautography test. The ethyl acetate fraction had shown better activity in DPPH and ABTS assays with CI_{50} of 4.8 ± 0.24 µg/ml and 78.6 ± 0.38 µg/ml respectively. In the FRAP method, the ethyl acetate fraction shown also better activity with a percentage reduction close to that of the ascorbic acid (94.44 ± 0.25% and 96.01 ± 0.04% respectively, at the highest concentration (10.68 µg/ml). Trunk bark hydroethanolic extract of *Stereospermum kunthianum* possess an antioxidant activity which increase significatively after fractionation with ethyl acetate and butanol.

Keywords: *Stereospermum kunthianum*, trunk bark, antioxidant activity, IC_{50}

1. Introduction
Oxidative stress is an imbalance between free radicals and antioxidants in your body [1]. More free radicals in the body can start doing damage to fatty tissue, DNA, and proteins and lead to a vast number of diseases over time. Therefore, the search for antioxidant extracts or molecules is more than necessary. Medicinal plants are a rich repository of various molecules that have many biologically effects [2]. *Stereospermum kunthianum* Cham *also* known as *Bignonia lanata* R. Br. Exn Frese or *Dolichandrone smithii* Baker is a species of plant belonging to the Bignoniaceae family and native to tropical Africa. In Senegalese traditional medicine, this plant is used against syphilis, bronchitis, gastritis and erectile dysfunction. This plant is also used in the treatment of wounds [3]. Biological investigations of the stem bark have shown that *S. kunthianum* extracts possess anti-inflammatory and analgesic [4, 5], antibacterial [6] and antidiarrhoeal [7] activities. Based on this data we set ourselves to research and evaluate by different methods, the antioxydant activity of hydroethanolic trunk bark extract of this plant and its fractions.

2. Materials and Methods
2.1 Plant material
Trunk barks of *Stereospermum kunthianum* belonging to the *Bignoniaceae* family were used for this study. These barks were harvested in Widou, in the municipality of Téssékéré (Louga region, Senegal) in March 2019. The plant has been identified by Pr Diatta William at the Pharmacognosy and Botany Laboratory of the Faculty of Medicine, Pharmacy and Odontology (FMPO) of Cheikh Anta Diop University in Dakar and a voucher specimen (n° 1410) was deposited in the herbarium of the same institute (FMPO) for future reference. The bark (1 kg) was dried out of the sun during fifty days at room temperature, then pulverized using a Brabender-type electric grinder.

2.2 Preparation of plant extracts
2.2.1 Extraction
The plant extracts were prepared at the Pharmacognosy and Botany Laboratory of the Faculty of Medicine, Pharmacy and Odontology (FMPO) of Cheikh Anta Diop University in Dakar. The extraction was done by decoction under reflux of 50 g of trunk bark powder in 500 ml of

an ethanol/water mixture (80-20; v/v) for 30 minutes. Pumice stone was added to stabilise the boiling. After filtration, the solution was concentrated using a rotary evaporator at 70 °C. The result was a pasty extract which was dried in a desiccator. The dry extract thus obtained constitutes the hydro-ethanolic extract (EHE).

2.2.2 Fractionation
The fractionation of the hydro-ethanolic extract (EHE) was carried out using a 1000 ml separating funnel, with solvents of increasing polarity. Thus, a quantity of 2.5 g of dry extract was dissolved in 300 ml of distilled water and then introduced into a separating funnel. This solution is subsequently exhausted three times with 300 ml of dichloromethane. The dichloromethane phases thus obtained are combined and evaporated to dryness to give the dichloromethane fraction (DF). The aqueous phase was subsequently treated in the same manner as previously with ethyl acetate and then with butanol to give the ethyl acetate fraction (EAF), the butanol fraction (BF) and the aqueous fraction (AF) respectively.

2.3 Antioxidant activity
2.3.1 Bioautography of antioxidant activity
The Bioautography of antioxidant activity was done using the described method [8]. The constituents of an extract are separated by thin-layer chromatography using as migration solvent the mixture: hexane / ethyl acetate / methanol (4/16/3.5; v/v). The extracts in solution (10 mg /ml) are deposited on a silica plate in an amount of 10 µl per extract. After migration and drying of the plate, the visualization is done with a 2% DPPH solution. Extracts with antioxidant activity have one or more spots colored in yellow.

2.3.2 DPPH. Radical scavenging assay
The determination of the DPPH free radical scavenging activity of samples was done using the described method [9]. An ethanol solution of DPPH. Was prepared by dissolving 4 mg of DPPH. In 100 ml of ethanol, followed by a cool incubation between 4-8° for at least 16 hours. An aliquot of each sample (0.8 ml) at appropriate concentration was added to 3.2 ml of ethanol solution of DPPH. The extract and its fractions of *S. kunthianum* trunk bark and ascorbic acid were tested at different concentrations. The absorbance of each sample was measured at 517 nm after 30 min. Each experiment was done in triplicate
The antioxidant activity related to the DPPH. Free radical scavenging effect was expressed as IC_{50} (concentration of sample required to scavenge 50% of free radicals).

2.3.3. ABTS assay
Reduction of free radical ABTS.+ (2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) was investigated using the described method [10]. Two stock solutions of 7.4 mM ABTS.+ and 2.6 mM potassium persulfate were prepared and mixed in equal volumes before allowing them to react for 12 h at room temperature in darkness. This mixture was diluted by adding ethanol, in order to obtain an absorbance 0.7 at 734 nm. Samples (2 ml) were mixed with 2 ml of $ABTS^{\cdot+}$ solution and the mixture was left at room temperature for 2 h in darkness. The absorbance of each sample was measured at 734 nm after 30 min. Each experiment was done in triplicate.

2.3.4 FRAP assay
The ferric reducing power was determined according to the described method [11]. An aliquot of 0.20 ml of each sample at appropriate concentration was mixed with 0.5 ml of phosphate buffered saline (0.2 M; pH 6.6) and 0.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50 °C for 30 min and 0.5 ml of 10% trichloroacetic acid was added. After centrifugation for 10 minutes at 3000 rpm, the supernatant (0.5 ml) was mixed with distilled water (0.5 ml) and 0.1% ferric chloride (0.1 ml). The experiments were done in triplicate. Absorbance was measured at 700 nm. Ascorbic acid was used as positive control. Absorbance increasing relatively to that of concentration represented the reducing capacity of tested sample.

2.4.5 Statistical analyses
Data were expressed as mean ± SD. Statistical analysis were done by Stat view 4.5 software using the Fischer test. The difference was considered as significant when $p<0.05$ compared to the negative control.

3. Results
3.1. Extraction and fractionation yields
After extraction of 50 g of *S. kunthianum* trunk bark powder, a dry extract weighing 10.135 g was obtained, giving a yield of 20.27%. The weights and yields of the fractions from the dry hydro-ethanolic extract are given in Table 1.

3.2. Antioxidant activity
3.2.1. Bioautography of antioxidant activity
The figure 1 illustrate the antiradical activity of the EHE and its fractions. All spots colored in yellow mean the corresponding extract or fraction had an antioxidant activity. AF had shown less yellow color intensity suggesting less antiradical activity.

3.2.2 DPPH assay
The trunk bark hydro-ethanolic extract of *S. kunthianum* had an IC_{50} of 9.88 ± 0.16 µg/ml. Among the fractions, the EAF had shown the lowest IC_{50} value (4.28 ± 0.24 µg/ml). The AF showed less antiradical activity with a highest IC_{50} (57.92 ± 1.14 µg/ml). The ascorbic acid solution used as reference, had an IC_{50} value of 0.84 ± 0.03 µg/ml (Figure 2).

3.2.3 ABTS assay
According to the Figure 2, all tested samples had shown significative ability to scavenge the free radical ABTS ($p<0.05$ *versus* negative control). The IC_{50} value of ascorbic acid (9.76 ± 0.06 µg/ml) was lowest than those of the EHE (142.6 ± 0.8 µg/ml) and EAF (78.6 ± 0.38 µg/ml) ($p<0.05$).

3.2.4. FRAP assay
As shown in figure 3, at low concentrations (0.73; 1.47; 2.94 µg/ml) the trunk bark hydro-éthanolic extract and its fraction showed similar ability to reduce ferric iron. Since the concentration of 5.87 µg/ml, the EAF, the BF and AA had exhibited the best ferric reducing abilities than the other samples ($p<0.05$).

Table 1: Fractionation yields of hydro-ethanolic extract (EHE)

Fractions	Quantities (g)	Yield (%)
DF	0.200	1.98
EAF	1.495	14.75
BF	1.403	13.85
AF	2.980	29.41

DF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanolic Fraction; AF: Aqueous Fraction

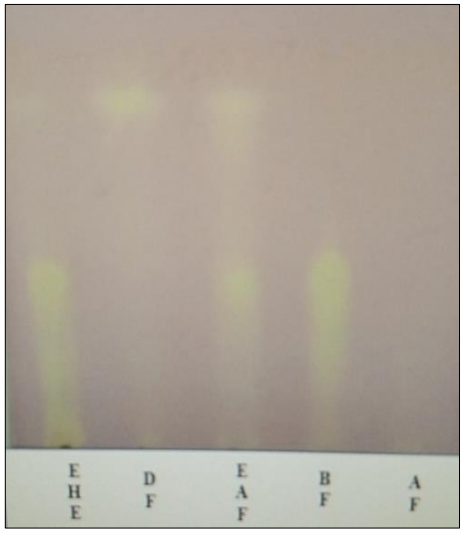

Fig 1: Antioxidant activity Bioautography of the HEE and its fractions

EHE: Hydro-Ethanolic Extract; DF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanolic Fraction; AF: Aqueous Fraction

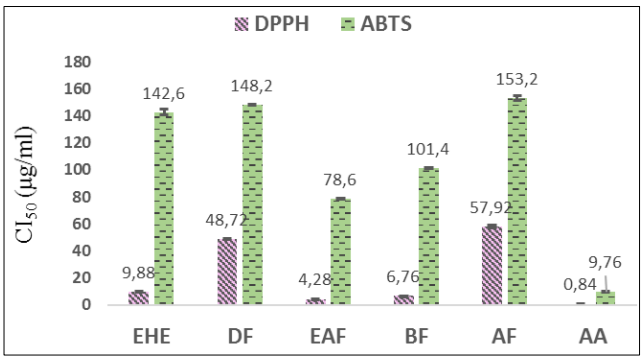

Fig 2: IC_{50} of EHE and its fractions on DPPH and ABTS tests.

EHE: Hydro-Ethanolic Extract; DF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanolic Fraction; AF: Aqueous Fraction; Ascorbic Acid

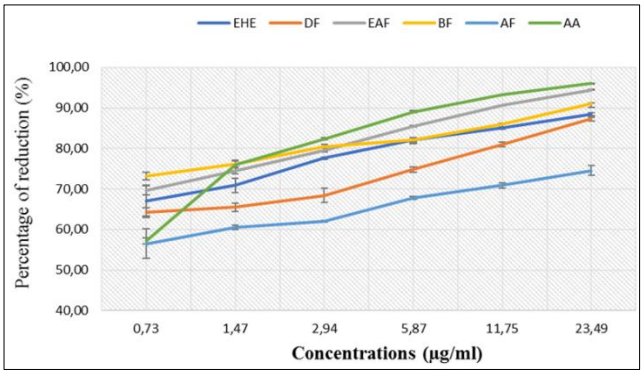

Fig 3: Percentage reduction of different samples by FRAP assay

EHE: Hydro-Ethanolic Extract; DF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanolic Fraction; AF: Aqueous Fraction; Ascorbic Acid

4. Discussion

Antioxidants are substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress [12]. The antioxidant potential of *S. kunthianum* trunk bark hydro-ethanolic extract and its fractions was investigated by different methods. For extraction, the ethanol/water mixture (80; 20 v/v) was used by its ability to extract polar compounds such as polyphenols and non-heterosidic compounds such as alkaloids but also some lipids [13].

This study was about to detect first, the antioxydant activity of samples by a bioautography research and on another hand to measure the ability of extract and its fractions to scavenge free radicals like DPPH. And ABTS. +. In FRAP test it's about to measure their capacity to reduce metals (Fe^{3+} to Fe^{2+}) [14].

The bioautography research showed that all samples possessed an antioxydant activity by the presence of one or more spots yellow colored for all deposits after revelation with a 2% DPPH solution. The three tests used to measure the antioxidant potential of extract and its fractions confirmed the results of the bioautography research. So for DPPH method, all samples reduced the DPPH. Radical and the best antioxydant activity is noticed for the EAF with an IC_{50} of 4.28 ± 0.24 µg/ml. It was followed by the butanolic fraction (6.76 ± 0.12 µg/ml) and the EHE (9,88 ± 0,16 µg/ml) with a significant difference ($p < 0.05$) between the concentrations of these three samples. The other fractions as the DF and the AF presented higher IC_{50} (148.2 ± 0.36 µg/ml and 153.2 ± 1.63 µg/ml) respectively. So these two fractions was less effective because a higher IC_{50} means smaller antioxidant activity [15]. Ascorbic acid (IC_{50}: 0.84 ± 0.03 µg/ml) was seen to be more active than the plant extract and its fractions. Regarding the ABTS method, this same trend was observed but with higher IC_{50} for ABTS method. This is explained by the fact that DPPH an ABTS tests have similar action mechanisms. Indeed, it has been found that antioxidant molecules reduce and decolorize DPPH. And $ABTS^{\cdot+}$ due to their hydrogen-donating capacity [16]. In the FRAP assay, the reducing power is due to their electron-donating capacity that could convert free radicals to more stable molecules [17]. At all tested concentrations, all samples showed good ability to reduce ferric (Fe^{3+}) ion to ferrous ion (Fe^{2+}). The higher reducing powers is obtained with the EAF, the BF and the EHE.

Previous reports suggested that the antioxydant properties of plant materials is linked to their phenolic content such as flavonoids and tannins which have potent antioxidant activities [18]. So the good antioxidant activity of our samples could be correlated to their polyphenol contents and especially to flavonoids. Indeed, according to Sarr and *al*. [19]. who worked with the same extract and its fractions, the EAF, the BF and the EHE which presented best antioxydant activity respectively in this study, was also richer in flavonoids (31.88 ± 0.19 mg RAE/g; 29.97 ± 0.29 mg RAE/g and 14.07 ± 0.52 mg RAE/g, respectively).

In view of these results, *S. kunthianum* trunk bark have real potential to fight oxidative stress. Therefore, many diseases with an oxidative mechanism could be managed through the development of improved traditional drugs based on *S. kunthianum* trunk bark.

5. Conclusion

The hydro-ethanolic trunk bark extract of *S. kunthianum* and its fractions had shown high antioxidant activity. Samples like the ethyl acetate fraction and the butanolic fraction was more effective than the extract itself. So further studies may be done on isolation of active compounds of these active fractions.

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