



Phytochemical Screening and Determination of Polyphenols in the Hydro-Ethanollic Extract of Trunk Bark and Its Fractions of *Stereospermum kunthianum* Cham (Bignoniaceae)

Abdou Sarr^{1*}, Serigne Ibra Mbacké Dieng¹, Kady Diatta-Badji¹,
Amadou Ibrahima Mbaye¹, William Diatta¹, Awa Ka¹,
Alioune Dior Fall¹ and Emmanuel Bassène¹

¹Laboratory of Pharmacognosy and Botany, FMPO, UCAD, Dakar, B.P: 5005 Dakar Fann, Sénégal.

Authors' contributions

This work was carried out in collaboration among all authors. Author AS designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author SIMD managed the analyses of the study. Author KDB managed the analyses of the study. Authors AIM, WD, ADF and AK performed the statistical analysis. Author EB managed the literature searches and approved the final corrections. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2021/v7i1130142

Editor(s):

(1) Dr. Langa Tembo, University of Zambia, Zambia.

Reviewers:

(1) Ressiliane Ribeiro Prata Alonso, Centro Universitário Araguaia (UNIARAGUAIA), Brazil.

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(3) Carlos Eduardo Silveira da Silva, Federal Rural University of Rio de Janeiro, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63347>

Original Research Article

Received 10 October 2020
Accepted 13 December 2020
Published 15 January 2021

ABSTRACT

Background : *Stereospermum kunthianum* Cham., is a medicinal plant from the Bignoniaceae family, used in Ferlo (Senegal) against stomach aches, as a healing agent and aphrodisiac.

Aims/Objective : The aim of this study was to contribute to the valorisation of the plant by carrying out a phytochemical screening followed by an assay of the polyphenols of the hydro-ethanollic extract of the bark and its fractions.

Methods : Phytochemical screening was carried out by coloring and/or precipitation reactions. The total polyphenol and tannin contents were evaluated by the Folin-Denis method and the flavonoid content by a method using aluminium chloride (AlCl₃) and sodium nitrite (NaNO₂).

Results: The search for chemical compounds revealed the presence of various secondary

*Corresponding author: E-mail: as2mar@yahoo.fr;

metabolites such as gallic and catechic tannins, flavonoids, saponosides, alkaloids, sterols and polyterpenes. The total polyphenol contents of the samples ranged from 0.81 ± 0.02 to 13.52 ± 0.06 mg EAT / g. Those of tannins and flavonoids were between 0.03 ± 0.01 and 4.56 ± 0.02 mg EAT / g and between 1.13 ± 0.04 to 31.88 ± 0.19 mg ER / g respectively.

Conclusion : These different metabolites would be responsible for the activities noticed for this plant.

Keywords: *Stereospermum kunthianum*; trunk bark; polyphenols; tannins; flavonoids.

1. INTRODUCTION

Humans have always used plants for food and medicine. However, the chemical composition of many plants is still unclear. Today, the search for bioactive molecules of plant origin is unavoidable in most research laboratories.

Among these molecules are polyphenols, which are widespread in the plant kingdom. These compounds are products of the secondary metabolism of plants [1].

The fundamental structural element which characterizes polyphenols is the presence of at least one benzene ring to which is directly linked at least one free hydroxyl (OH) group or involved in another function: ether, ester or heteroside [2, 3].

Several studies have shown that many biological activities of plant extracts are often linked to their polyphenol content. These are antioxidant [4], anti-inflammatory [5] anti-tumour [6] and healing [7] activities. They also have a stimulating action on immune system cells. This plant is also used in the treatment of wounds, diarrhoea, snake and scorpion bites, fever and diabetes [8-11]. Biological investigations have shown that *S. kunthianum* extracts possess antibacterial [12, 13], anti-inflammatory [14], anticonvulsant [15], antihelmintic [16], antidiarrhoeal [17] and analgesic [18] activities. Various morphological parts of *Stereospermum kunthianum* are used in traditional medicine to treat an array of human ailments. The pods are chewed with salt to treat coughs and are used in treatment of ulcers, leprosy, skin eruptions and venereal diseases, while the stem bark decoction or infusion is used to cure bronchitis, pneumonia, coughs, rheumatic arthritis and dysentery. The twigs are chewed to clean teeth and to treat toothache. The roots and leaves have been found useful in treating venereal diseases, respiratory ailments and gastritis [19]. The efficacy of the water extract of *Stereospermum kunthianum* in human complement system fixation in-vitro has been reported [20]. The antiplasmodial activity of

naphthoquinones and one anthraquinone from the lipophilic extract of the root bark of *Stereospermum kunthianum* has also been reported [21]. In Senegal, the use of plants for treatment is a common practice in both rural and urban areas. Traditional medicine, particularly herbal medicine, is the primary care remedy for almost the majority of the population. Senegal possesses an important flora with many species including, *Stereospermum kunthianum* constituting a richness provided by its value.

The chosen study was *Stereospermum kunthianum* Cham, a plant from the Senegalese flora with various uses in traditional medicine: syphilis, bronchitis, gastritis, erectile dysfunction etc. [22]. The aim of this study is to determine the secondary metabolites present in the trunk bark of *Stereospermum kunthianum* Cham and to subsequently quantify the total polyphenols, tannins and total flavonoids.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consisted of the bark of the trunk of *Stereospermum kunthianum* belonging to the Bignoniaceae family. These barks were harvested in Widou, in the municipality of Tèssékéré (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 ambient temperature, then pulverized using a Brabender-type electric grinder.

2.2 Methodology

2.2.1 Preparation of plant extracts

The plant extracts was 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 (EAF), butanol (BF) and aqueous fractions (AF) respectively.

2.2.3 Phytochemical screening

The main phytochemical families were searched for using staining and/or precipitation reactions, referring to the tests described by Bassène [23], Bekro et al. [24] and Longanga et al.[25]. The aim was to highlight the presence of flavonoids, tannins, anthracene heterosides, cardiotoxic heterosides, alkaloids, sterols and triterpenes and saponosides in the hydro-ethanolic extract of the trunk bark of *Stereospermum kunthianum* Cham. and its fractions.

2.2.4 Dosage of total polyphenols

The method used is that described by Joslyn [26], slightly modified. In a haemolysis tube, 2500 µl of sample at 25 mg/l (extract or fractions) and 500 µl of Folin Denis reagent were mixed. After 3 min incubation, 500 µl of 25% sodium carbonate was added before centrifuging the tube at 4000 rpm for 4 min. The absorbance was measured at 760 nm against a blank (methanol) using a BioSystem BTS/340 UV-visible spectrophotometer. The dosage was done in triplicate (n = 3).

A calibration range carried out with tannic acid at different concentrations (0; 2.5; 5; 7.5; 10; 12.5; 15; 17.5; 20 mg/l) was treated in the same way as the samples. The results were expressed in mg tannic acid equivalent per gram of dry extract (mg EAT/g) according to the formula:

$$T = C.V/M$$

T: Total polyphenol content expressed in milligram tannic acid equivalent per gram of dry extract (mg EAT/g ES),

C: concentration of polyphenols established from the equation of the calibration line in mg/l,

V: volume of extract in litre

M: mass of the extract studied in g.

2.2.5 Dosage of tannins

To 30 ml of the previously prepared 25 mg/l samples, 3 grams of casein were added. The whole was then magnetically stirred for 3 hours to fix the tannins. After filtration, the determination of residual polyphenols was made from the filtrate, under the same conditions as those described for the determination of total polyphenols. The tannin concentration represents the difference between total polyphenols and residual polyphenols [27]. The determination was made in triplicate (n = 3).

2.2.6 Dosage of flavonoids

The method described by Zhishen et al. [28] was used with some modifications. For this, 400 µl of sample (or standard or distilled water for the control) was placed in a glass haemolysis tube with 120 µl of 5% NaNO₂. After 5 minutes of incubation, 120 µl of 10% AlCl₃ was added and mixed in the vortex. Then 800 µl of 1 M NaOH was added 6 minutes later. After homogenisation, the absorbance was read immediately at 510 nm against the control. The test was repeated 3 times for each sample (n = 3).

A calibration range performed with rutin at different concentrations (0; 10; 20; 30; 40; 50; 60; 70 mg/l) was treated in the same way as the samples. The results were expressed in milligram rutin equivalent per gram of dry extract (mg RE/g).

2.2.7 Statistical analysis

The statistical analysis were done by Statview 4.5 software using the Fischer test. The difference was considered

significant when $p < 0.05$ compared to the negative control.

3. RESULTS

3.1 Extraction and Fractionation

After extraction of 50 g of *S. kunthianum* 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 extract are given in Table 1.

3.2 Phytochemical Screening

Phytochemical characterisation tests, carried out on the hydro-ethanolic extract of the plant's bark and its fractions, gave the results mentioned in Table 2.

3.3 Dosage of Total Polyphenols, Tannins

The total polyphenol contents as well as the residual polyphenol contents after precipitation of the tannins from the samples were calculated using the equation $y = 0.0463x$ ($R^2 = 0.99$) from

the calibration line obtained with tannic acid shown in Fig.1.

The results showed that the hydro-ethanol extract had a total polyphenol content of 6.4 ± 0.01 mg EAT/g dry extract and a low tannin content of 1.24 ± 0.1 mg EAT/g. Of the fractions, ethyl acetate had the highest total polyphenol and tannin content with 13.52 ± 0.06 and 4.56 ± 0.02 mg EAT/g, respectively, as shown in Fig. 2.

3.4 Dosage of Total Flavonoids

The flavonoid contents of the hydro-ethanolic extract of bark and its fractions were obtained from the equation $y = 0.0062x$ ($R^2 = 0.99$) of the rutin calibration line shown in Fig. 3.

The flavonoid content of the hydro-ethanolic extract of the bark was 14.07 ± 0.52 mg RE/g. Of all the fractions, ethyl acetate had the highest content with 31.88 ± 0.19 mg RE/g. The butanol fraction had a content of 29.97 ± 0.29 mg RE/g while the dichloromethane and aqueous fractions contained 1.13 ± 0.04 and 2.07 ± 0.04 mg RE/g respectively. Fig. 4 shows the flavonoid contents of the samples.

Table 1. Fractionation yields of hydro-ethanol extract (EHE)

Fractions	Quantities (g)	Yield (%)
DF	0.200	1.98
EAF	1.495	14.75
BF	1.403	13.85
AF	2.98	29.41

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

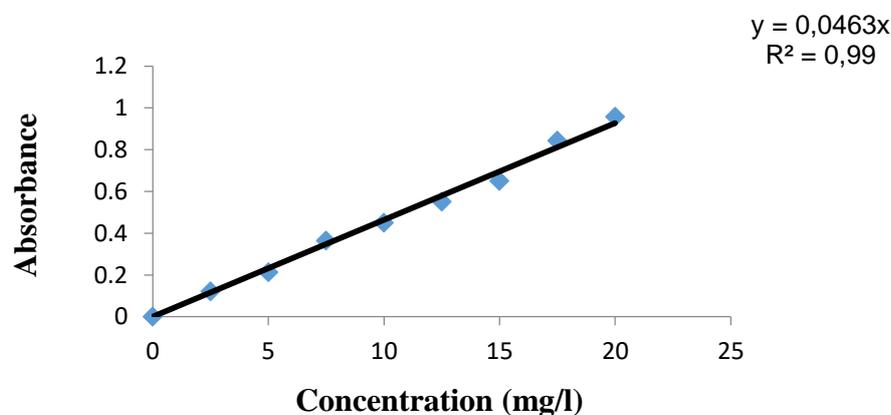


Fig. 1. Calibration line obtained with tannic acid

Table 2. Chemical screening of the hydro-ethanolic extract of the bark of *Stereospermum kunthianum* and its fractions

	EHE	DF	EAF	BF	AF
Condensed tannins	+	+	+	-	-
Hydrolyzable tannins	+	-	+	+	-
Anthracenic heterosides	-	-	-	-	-
Flavonoids	+	+	+	+	+
Alkaloids	+	-	-	-	+
Cardiotonic heterosides	-	-	-	-	-
Saponins	+	-	+	+	+
Sterols and triterpenes	+	+	-	-	-

(+): Presence; (-): Absence; EHE: Hydro-Ethanolic Extract; DF: Dichloromethane Fraction; EAF: Ethyl Acetate Fraction; BF: Butanolic Fraction; AF: Aqueous Fraction

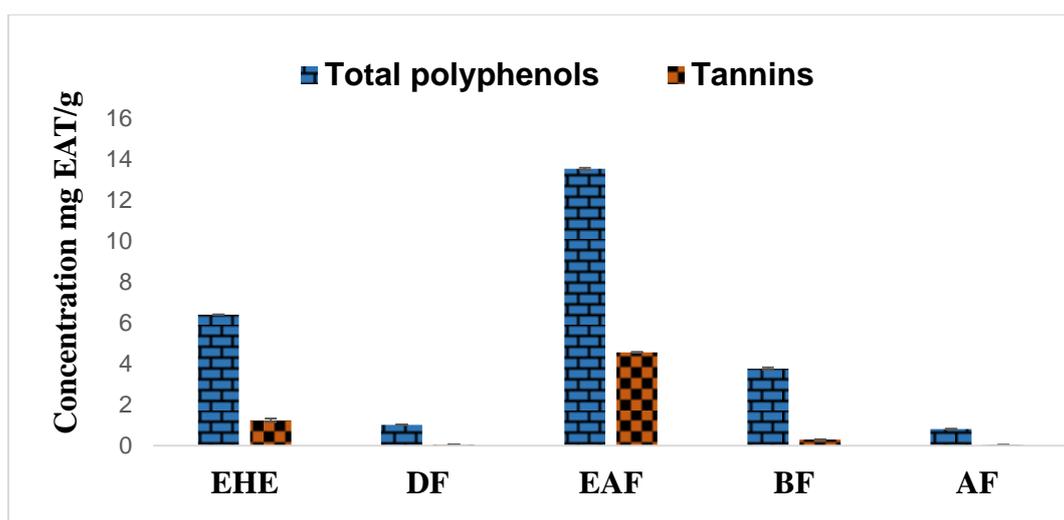


Fig. 2. Concentration of total polyphenols and tannins in the hydro-ethanolic extract of the bark and its fractions of *Stereospermum kunthianum*.

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

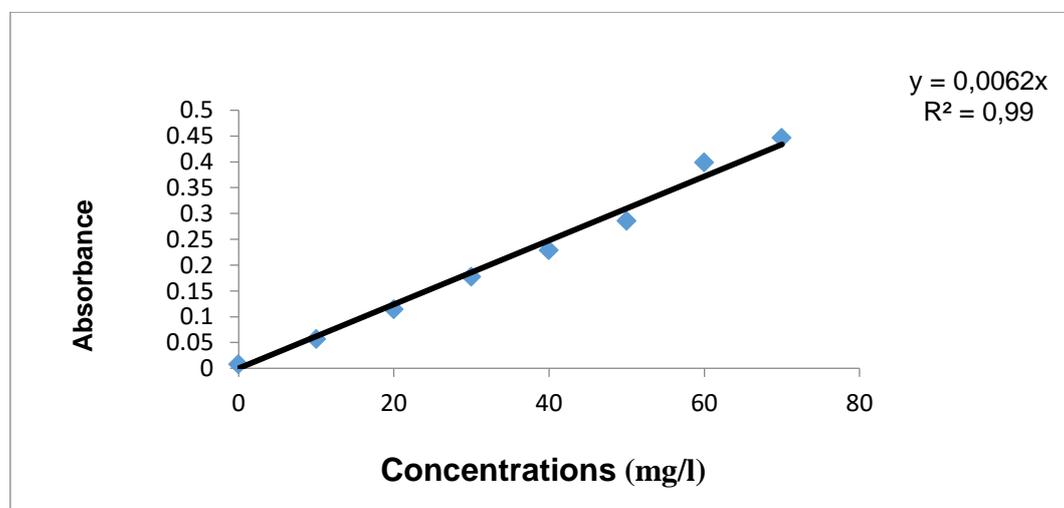


Fig. 3. Calibration line obtained with rutin

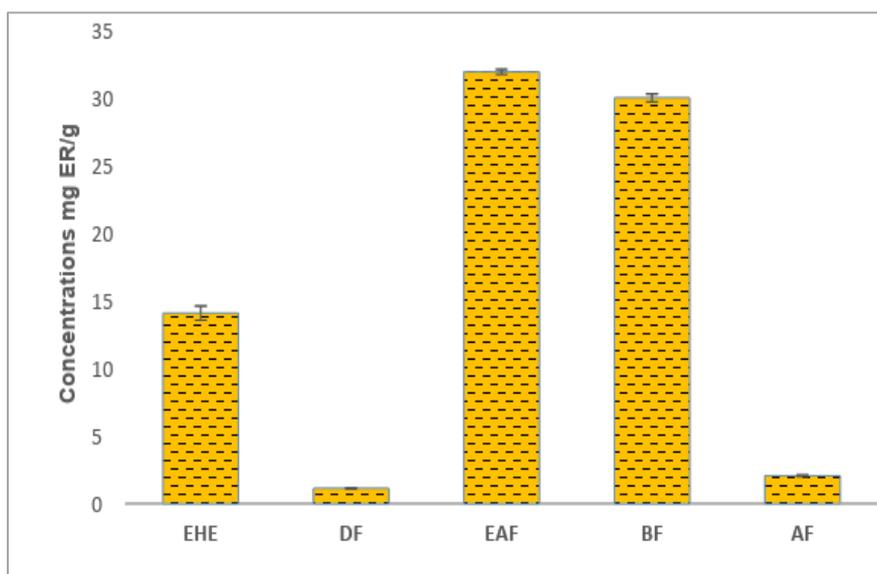


Fig. 4. Total flavonoid concentrations of the hydro-ethanolic extract of the bark and its fractions of *S. kunthianum*

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

4. DISCUSSION

The extraction solvent used was the ethanol/water mixture (80 ; 20 v/v). Ethanol and water are polar solvents that can extract polar compounds such as polyphenols. Ethanol can also be used to extract non-heterosidic compounds such as alkaloids but also certain lipids [29]. This implies that most of the active ingredients of the *Stereospermum kunthianum* bark powder would be well extracted by this mixture.

Phytochemical studies carried out on the samples revealed the presence of several chemical families. The polar samples (EHE, EAF, BF and AF) contained mainly tannins, flavonoids and saponosides. Sterols and triterpenes were found in the apolar fraction of dichloromethane. Alkaloids were found in EHE and FA. These results are in agreement with those of Ching et al. [30] who revealed the presence of alkaloids, tannins, saponins in an aqueous extract of stem bark of *Stereospermum kunthianum*.

The assay of total polyphenols revealed their differential distribution between the hydro-ethanolic extract of the bark and its fractions. In fact, EAF is by far the richest in total polyphenols and tannins with respective concentrations of 13.52 ± 0.06 mg EAT/g and 4.56 ± 0.02 mg EAT/g dry extract. It is followed by EHE (6.4 mg

EAT/g ES for total polyphenols and 1.24 mg EAT/g ES for tannins). AF is the lowest in total polyphenols (0.81 ± 0.02 mg EAT/g ES) and tannins (0.03 ± 0.01 mg EAT/g ES). This could be explained by the fact that most of the polyphenols were extracted by ethyl acetate and butanol.

For total flavonoids, the ethyl acetate fraction was also richer (31.88 ± 0.19 mg RAE/g), followed very closely by the butanolic fraction (29.97 ± 0.29 mg RAE/g). The normal analysis of variance (ANOVA) followed by the Fischer test, however, shows a significant difference ($p < 0.05$) between the concentrations of these two fractions. This may explain the fact that these two solvents are recommended in the extraction of flavonoids [31]. These fairly close concentrations could also be explained by the presence of compounds with intermediate solubility between ethyl acetate and butanol. In view of these results, the polyphenols were found to be concentrated in the EAF and BF where they are purified compared to the EHE. The dichloromethane fraction contained less flavonoids (1.13 ± 0.04 mg EAR/g ES) which would be apolar flavonic aglycones and therefore soluble in dichloromethane. The phytochemical screening indicates the presence of various bioactive constituents in the powdered *Stereospermum kunthianum* stem bark. Bioactive constituents in plants suggest possible drug

basis [32]. Antiinflammatory, analgesic and anti diarrhoeal activities were detected by Ching on the barks of stems of this plant. Activities could be due to secondary metabolites [33,34, 35]. The use of *Stereospermum kunthianum* bark in traditional medicine would probably be linked to the presence of the different groups of chemical compounds we have identified.

5. CONCLUSION

The chemical screening revealed the presence of tannins, flavonoids, saponins and alkaloids. The results of the assay showed that the ethyl acetate fractions and the hydro-ethanolic extract contained more polyphenols and tannins. On the other hand, for the flavonoids, the ethyl acetate and butanolic fractions were the richest.

Stereospermum kunthianum Cham. would be a significant source of polyphenols and more particularly of flavonoids. Lastly, follow-up studies could lead to ways to isolate and identify the flavonoids and polyphenols compounds by spectroscopy methods and the antioxidative molecules by bioactivity-guided methods, as well as permitting determination of acute and sub-acute toxicity of the bark trunk.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

We thank all the Laboratory peoples for their precious helps.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI: 10.4103/0250-474X.51943.

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