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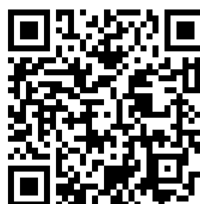

Article

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VALORIZATION OF MEDICINAL PLANTS FROM KORHOGO (CÔTE D'IVOIRE): PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIOXIDANT ACTIVITY OF LEAVES AND STEM BARK OF SABA SENEGALENSIS (A.DC.) PICHON (APOCYNACEAE)

Abstract*: Saba senegalensis is a plant commonly used in traditional medicine in Korhogo (Côte d'Ivoire) for treating diabetes and renal failure. This study is based on phytochemical screening and evaluation of the antioxidant activity of aqueous, hydroethanolic, and ethanolic extracts of S. senegalensis leaves and stem bark. Phytochemical screening of secondary metabolites using thin-layer chromatography (TLC) revealed the presence of flavonoids,*

Clarivate Analytics indexed

tannins, phenolic acids, saponins, sterols, and terpenes in the plant's leaf and stem extracts. Coumarins were found only in the leaves, while alkaloids were absent from both organs studied. Antioxidant activity was highlighted in both organs by qualitative and quantitative methods with respect to the DPPH free radical. Quantitative spectrophotometric analysis showed that both leaves and stems possess antioxidant activity. The antioxidant activity of leaves was better than that of stems. Thus, the plant's leaves and stems could be used as antioxidants capable of preventing and/or treating diseases linked to oxidative stress.

***Key words**: Saba senegalensis, phytochemical screening, antioxidant activity, Korhogo, Côte d'Ivoire*

***Language**: English*

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Introduction

Humans are confronted with several diseases that constitute public health problems and whose treatments are proving increasingly complicated **[1].** These public health problems include oxidative stress-related diseases such as cancer, cataracts, diabetes, Alzheimer's disease, rheumatism, cardiovascular disease, and accelerated skin aging **[2, 3].** However, synthetic antioxidants are available for some treatments, but some are now responsible for undesirable effects on the body **[4].** Moreover, access to conventional medicine remains problematic, on the one hand, because of the inadequacy of specialized health structures and qualified personnel, and the difficulty of transporting patients from rural areas to health centers in urban areas, and on the other because of the very high cost of certain treatments and the resistance of certain pathogens. Faced with this alarming situation, other treatment options are imperative. It is in this context that this study focuses on *Saba senegalensis.* Today, these plants are the subject of massive chemical and biological studies for their possible use as an alternative for protection against oxidation **[5, 6].** This work aims to justify or refute the use of *Saba senegalensis* leaves and stems as antioxidants capable of preventing or treating diseases linked to oxidative stress.

I. MATERIAL AND METHODS

I.1. Material

I.1.1. Plant material

The plant material consisted of *Saba senegalensis* leaves and stem bark. The various organs were collected in October 2022 in Korhogo (9° 27' 28" North, 5° 37' 46" West). The various plant species were authenticated by botanists at Peleforo GON COULIBLY University. The various plant organs were dried for ten (10) days in a room at room temperature, away from the sun. Finally, these dried organs were pounded in a mortar and sieved to obtain fine powders, which were used to prepare the different extracts to be tested.

I.1.2. Laboratory materials and equipment

Laboratory equipment includes the usual glassware, an electronic balance (DENVER INSTRUMENT SI-234), a water bath (Neo-Tech SA), a hot plate (Rommelsbcher), a drying oven (Memmert), and a spectrophotometer (JENWAY 7315).

I.1.3. Reagents and chemical products

The analytical-grade chemicals used were purchased from Polychimie (Côte d'Ivoire). For Thin Layer Chromatography (TLC) tests, we used silica gel 60 F254 chromatoplates on aluminium support. The developers and reagents used were 2% $FeCl_3$, 5% KOH, 1% $AlCl_3$, sulfuric vanillin, Dragendorff reagent, and DPPH.

I.2. Methods

I.2.1. Extractions

I.2.1.1. Aqueous extracts

A mass of 7 g of powder from each organ was decocted in 70 mL of distilled water for 30 minutes at a temperature of 100 °C. After filtration, the different filtrates were placed in an oven at 50 °C for three (3) days. The various dry aqueous extracts of *S. senegalensis* obtained were used to assess antioxidant activity by spectrophotometry.

I.2.1.2. Ethanolic extracts

A mass of 7 g of powder from each organ (leaves and stem bark) of *S. senegalensis* was macerated in 70 mL of ethanol for 24 hours. After filtration, the different filtrates were placed in an oven at 50 °C for two (2) days to provide the ethanolic crude extracts. These extracts were then used to assess antioxidant activity by spectrophotometry.

I.2.1.3. Hydroethanolics extracts

A mass of 7 g of each organ powder was macerated in 70 mL of binary ethanol/water mixture (80 mL/20 mL) for 24 hours. After filtration, the macerates were placed in an oven at 50 °C for two hours to remove the ethanol. The extract obtained for each organ is kept for 24 hours in the refrigerator at 4°C to precipitate lipophilic compounds. After decantation and filtration, a quantity of these extracts was completely dried in an oven at 50 °C for two (2) days, and these crude hydroethanolic extracts were used to assess antioxidant activity by spectrophotometry. The other quantity was used to prepare the selective extracts.

I.2.1.4. Selective Extracts

A volume of 15 mL of each filtrate from the hydro-ethanol mixture of *S. senegalensis* leaves and stem bark was exhausted by successive fractionations with (3 × 10 mL) hexane (C_6H_{14}), dichloromethane (CH_2Cl_2) and ethyl acetate (AcOEt). The various selective organic fractions were then concentrated in an oven at 50 °C. These concentrates were then used for phytochemical screening by TLC and evaluation of antioxidant activity on TLC plates.

I.2.2. Phytochemical screening on TLC plates

Secondary metabolites (sterols-polyterpenes, alkaloids, coumarins, flavonoids, tannins, and phenolic acids) were identified using TLC plate tests. TLC screening of selective extracts was carried out using the methods described by Mamyrbékova-Békro et *al.* **[7].**

Using capillaries, 2 µL of each selective extract is deposited as a dot 0.5 cm from both edges of the chromatographic plate. The TLC plates are then placed in the migration tank containing the migration solvents (developer).

After development, chromatograms were visualized with visible and UV 365 nm developers. Colorations appearing as spots are recorded, and front ratios (Rf) are calculated.

I.2.3. Estimation of antioxidant power

I.2.3.1. DPPH screening of selective extracts by TLC

The antioxidant power evaluation by TLC used is that developed by the method described by Takao et *al.* **[8].**

A 10 µL volume of each plant extract solution is deposited on a chromatoplate (silica gel 60 F254, on aluminium support (Merck)), which is then placed in a chromatography tank saturated with migration solvent. After development, chromatograms are dried and then developed with an ethanolic solution of DPPH (0.2 mg/mL). After 30 minutes of optimal time, extract constituents with potential free radical scavenging activity are revealed as pale-yellow spots on a violet background. The frontal ratios (Rf) associated with the yellow spots are calculated.

I.2.3.2. Assessment of antioxidant activity of aqueous, ethanolic, and hydroethanolic extracts and vitamin C by DPPH spectrophotometry

The antioxidant potential of the extracts was assessed using the Blois method **[9].**

DPPH is solubilized in absolute ethanol to obtain a solution with a 0.3 mg/mL concentration. Each extract has different concentration ranges (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, and 0.0625 mg/mL) prepared in absolute ethanol. 2.5 mL plant extract and 1 mL DPPH ethanolic solution are added to dry, sterile tubes. After shaking, the tubes are placed in a dark place for 30 minutes. The absorbance of the mixture is then measured at 517 nm against a blank consisting of 2.5 mL pure absolute ethanol and 1 mL DPPH solution. The positive reference control, ascorbic acid (vitamin C), was treated under the same conditions as the plant extracts. DPPH inhibition percentages are calculated according to the formula:

$$I(\%) = (A_b - A_e) / A_b \times 100$$

I: inhibition percentage
A_b: absorbance of blank
A_e: absorbance of sample

The concentrations required to trap 50% (IC50) of DPPH are determined from the graphs showing the percentage inhibition of DPPH as a function of extracts or vitamin C concentrations.

I.2.4. Statistical analysis

Analyses of the measurements obtained during the various manipulations were done using EXCEL 2021 software (version 16.0). It was used to plot the various diagrams used to determine the IC_{50} parameter for each extract.

II. RESULTS AND DISCUSSION

II.1. Results

II.1.1. Yields

The various extractions were carried out by decoction with water and maceration with ethanol and a water/ethanol mixture on *Saba senegalensis* leaf and stem bark powders. Yields based on the dry weight of the various plant powders were calculated using the following formula:

Extraction yield = (extracted mass/sample mass) *100, and the results obtained were recorded in Table 1. Yield values ranged from 35.10 ±3.94% to 49.00 ±2.76%.

Table 1. Yields of various *Saba senegalensis* leaf and stem extracts

	SSF Aq	SSF Et	SSF H-Et	SST Aq	SST Et	SST H-Et
R1	40.57	41.00	49.00	47.14	50.29	49.00
R2	**46.43**	**34.29**	**45.87**	**50.00**	**51.86**	**42.45**
R3	46.14	30.00	43.22	42.29	44.86	47.73
Rmoy	44.38 ±2.54	35.10 ±3.94	46.03 ±1.98	46.48 ±2.79	49.00 ±2.76	46.39 ±2.63

R1: yield 1; R2: yield 2; R3: yield 3; Rmoy: average yield; SS: *Saba senegalensis*; F: leaf; T: stem; Aq: Aqueous; Et: ethanol; H-Et: hydro-ethanol

II.1.2. Phytochemical screening by TLC

Identification of the various secondary metabolites was carried out using the following migration or developing solvents:

- Hexane (C_6H_{14}) / ethyl acetate (AcOEt) (5 : 0,375; V/V) for hexane extracts;
- Dichloromethane (CH_2Cl_2) / ethyl acetate (AcOEt) / hexane (C_6H_{14}) (2 : 2 : 1; V/V/V); CH_2Cl_2/AcOEt/ C_6H_{14} (3:4:2) (V/V/V) and CH_2Cl_2/AcOEt/ CH_3COOH (1 : 3,5: 1) (V/V/V) for dichloromethane extracts ;
- CH_2Cl_2/AcOEt/ C_6H_{14} (3 :4 :2) (V/V/V) and CH_2Cl_2/AcOEt/ CH_3COOH (1 : 3,5 :1) (V/V/V) for acetate-ethyl extracts;
- The same migration solvents were used to assess antioxidant potential by TLC.

The various target metabolites were investigated in hexanolic, dichloromethane, and acetate-ethyl extracts of *Saba senegalensis* leaves and stems. The results obtained are presented in Tables 2 to 6. The various tables provide information on the retention factor (Rf), visible and ultraviolet (UV) observation of the various stains. The reagents: vanillin sulfuric acid, Draggendorff, KOH, $AlCl_3$, and $FeCl_3$ were used to identify seven groups of secondary metabolites: (sterols, terpenes), alkaloids, coumarins, flavonoids, and (tannins, phenolic acids).

Table 2. Visible detection of sterols and terpenes in C_6H_{14}/AcOEt (5: 0.375) (V/V/V) developer from hexane extracts

Extracts	R_f (Color): Possible compounds
SSF H-Et (Hexane)	0.89 (purple): **terpene**; 0.76 (blue): **sterol**; 0.74 (blue): **sterol**; 0.6 (blue): **sterol;** 0.49 (blue): **sterol**; 0.43 (blue): **sterol**; 0.31 (blue): **sterol**; 0.25 (blue): **sterol**; 0.21 (blue): **sterol**; 0.18 (blue): **sterol**; 0.15 (blue): **sterol**; 0.09 (blue): **sterol**; 0.05 (blue): **sterol**; 00 (blue): **sterol**
SST H-Et (Hexane)	**0.83 (purple):** terpene**; 0.7 (blue):** sterol; **0.61 (blue):** sterol**; 0.56 (blue):** sterol: **0.53 (blue):** sterol**; 0.49 (blue):** sterol; **0.38 (blue):** sterol; **0.29 (blue):** sterol**; 0.15 (blue):** sterol**; 0.14 (blue):** sterol**; 0.08 (blue):** sterol**; 00 (rose):** terpene

Table 3. Visible detection of alkaloids in CH_2Cl_2/AcOEt/C_6H_{14} (2: 2: 1) (V/V/V) developer from visible dichloromethane (CH_2Cl_2) extracts

Extracts	R_f (Color): Possible compounds
SSF H-Et (CH_2Cl_2)	**No alkaloid identified**
SST H-Et (CH_2Cl_2)	No alkaloid identified

Table 4. Visible (a) and UV (b) detection of coumarins in CH_2Cl_2/AcOEt/C_6H_{14} (2: 2: 1) (V/V/V) developer from dichloromethane extracts CH_2Cl_2)

Extracts	R_f (Color): Possible compounds
SSF H-Et (CH_2Cl_2)	0.9 (Yellow^a. blue^b): **coumarin;** 0.78 (Yellow^a): **coumarin**; 00 (Yellow^b): **coumarin**
SST H-Et (CH_2Cl_2)	No coumarin identified^{a,b}

Table 5. Visible (a) and UV (b) detection of flavonoids in CH_2Cl_2/AcOEt/C_6H_{14} (3:4:2) (V/V/V) developer from dichloromethane (CH_2Cl_2) and acetate-ethyl (AcOEt) extracts

Extracts	R_f (Color): Possible compounds
SSF H-Et (CH_2Cl_2)	0.98 (green^a): **flavonoid**; 0.8 (Yellow^{a,b}): **flavonoid**; 0.7 (Yellow^{a,b}): **flavonoid**; 0.63 (Yellow^{a,b}): **flavonoid**; 0.58 (Yellow^{a,b}): **flavonoid**; 0.54 (Yellow^a. Blue^b): **flavonoid**; 0.39 (Blue^b):

	flavonoid; 0.35 (Yellow[a]): **flavonoid**; 0.24 (Blue[b]): **flavonoid**; 0.13 (Yellow[a]): **flavonoid**; 00 (Yellow[b]): **flavonoid.**
SST H-Et (CH_2Cl_2)	**0.9 (Blue[b]):** flavonoid**; 0.79 (Blue[b]):** flavonoid**; 0.68 (Blue[b]):** flavonoid**; 0.5 (Yellow[a]. Blue[b]):** flavonoid**; 0.4 (Blue[b]):** flavonoid**; 0.28 (Yellow[a]. Blue[b]):** flavonoid**; 0.20 (Blue[b]):** flavonoid**; 0.15 (Yellow[a]):** flavonoid**; 00 (Yellow[b]):** flavonoid.
SSF H-Et (AcOEt)	0.86 (Blue[b]): **flavonoid**; 0.81 (Yellow[a]): **flavonoid**; 0.75 (Yellow[b]): **flavonoid**; 0.71 (Yellow[a]): **flavonoid**; 0.65 (Yellow[b]): **flavonoid**; 0.6 (Yellow[a,b]): **flavonoid**; 0.46 (Yellow[b]): **flavonoid**; 0.38 (Yellow[a]): **flavonoid**; 0.36 (Yellow[a]): **flavonoid**; 0.25 (Yellow[a,b]): **flavonoid**; 0.23 (Yellow[b]): **flavonoid**; 0.13 (Yellow[a,b]): **flavonoid**; 00 (Yellow[b]): **flavonoid.**
SST H-Et (AcOEt)	**0.95 (Blue[b]) :** flavonoid**; 0.74 (Blue[b]):** flavonoid**; 0.45 (Yellow[a]. Blue[b]):** flavonoid**; 0.25 (Yellow[a]):** flavonoid**; 0.18 (Blue[b]):** flavonoid**; 0.08 (Yellow[a]):** flavonoid.

Table 6. Visible detection of tannins and phenolic acids in CH_2Cl_2 / AcOEt / CH_3COOH (1: 3,5:1) (V/V/V) developer from dichloromethane (CH_2Cl_2) and acetate-ethyl (AcOEt) extracts.

Extracts	R_f (Color)**: Possible compounds**
SSF H-Et (CH_2Cl_2)	0.96 (green): **phenolic acid**; 0.65 (green): **phenolic acid**; 0.56 (grey): **tannin**; 0.49 (grey): **tannin.**
SST H-Et (CH_2Cl_2)	**0.9 (green):** phenolic acid**; 0.68 (grey):** tannin**; 0.49 (grey):** tannin.
SSF H-Et (AcOEt)	0.68 (green): **phenolic acid**; 0.6 (grey): **tannin**; 0.55 (grey): **tannin**; 0.46 (grey): **tannin**; 0.36 (grey): **tannin**; 0.26 (grey): **tannin**; 0.19 (grey): **tannin**; 0.09 (grey): **tannin.**
SST H-Et (AcOEt)	**0.9 (green):** phenolic acid**; 0.69 (grey):** tannin**; 0.42 (grey):** tannin**; 0.31 (grey):** tannin**; 0.2 (grey):** tannin**; 0.11 (grey):** tannin**; 00 (grey):** tannin.

The summary results of phytochemical screening on TLC plates of the various secondary metabolites of *Saba senegalensis* leaf and stem extracts are given in Table 7. Both organs were found to contain sterols, terpenes, flavonoids, tannins, and phenolic acids. However, only the leaves contain coumarins, while alkaloids are absent in both plant organs.

Table 7. Summary table of phytochemical screening of secondary metabolites

	Sterols	**Terpenes**	**Alkaloids**	**Coumarins**	**Flavonoids**	**Phenolics acids**	**Tannins**
SSF	+	+	-	+	+	+	+
SST	+	+	-	-	+	+	+

Presence (+) ; absence (-)

II.1.3. Evaluation of antioxidant activity

II.1.3.1. Evaluation of antioxidant activity by TLC

Analysis of the hexanolic, dichloromethane, and acetate-ethyl extracts of the two organs studied revealed the appearance of pale-yellow spots on a violet background, and the values of their frontal ratios (Rf) have been recorded in tables 8, 9, 10, and 11. It can thus be concluded that all these extracts contain significant antioxidant activity in view of the high number of pale-yellow spots observed.

Table 8. DPPH radical scavenging phytocompounds in C_6H_{14}/AcOEt (5 : 0.375) (V/V/V) developer from hexane extracts

Extracts	R_f (Color)**: Possible compounds**
SSF H-Et (Hexane)	0.89 (yellow): **terpene**; 0.25 (yellow): **sterol**; 0.09 (yellow): **sterol**; 0.00 (yellow): **sterol.**
SST H-Et (Hexane)	**0.99 (yellow): NI; 0.9 (yellow): NI; 0.83 (yellow):** terpene**; 0.38 (yellow):** sterol**; 0.29 (yellow):** sterol**; 0.08 (yellow):** sterol**; 0.00 (yellow):** terpene.

NI: unidentified compound

Table 9. DPPH radical scavenging phytocompounds in CH_2Cl_2/AcOEt/ C_6H_{14} (2 : 2 : 1) (V/V/V) developer from dichloromethane extracts (CH_2Cl_2)

Extracts	R_f (Color)**: Possible compounds**
SSF H-Et (CH_2Cl_2)	0.98 (yellow): NI; 0.9 (yellow): **coumarin**; 0.85 (yellow): NI; 0.78 (yellow): **coumarin**; 0.69 (yellow): NI; 0.61 (yellow): NI; 0.54 (yellow): NI; 0.48 (yellow): NI; 0.4 (yellow): NI; 0.35 (yellow): NI; 0.28 (yellow): NI; 0.2 (yellow): NI; 0.16 (yellow): NI; 0.1 (yellow): NI; 00 (yellow): **coumarin.**
SST H-Et (CH_2Cl_2)	**0.98 (yellow): NI; 0.93 (yellow): NI; 0.85 (yellow): NI; 0.79 (yellow): NI; 0.71 (yellow): NI; 0.71 (yellow); 0.66 (yellow): NI; 0.59 (yellow): NI; 0.55 (yellow): NI; 0.55 (yellow): NI; 0.48 (yellow): NI; 0.4 (yellow): NI; 0.35 (yellow): NI; 0.28 (yellow): NI; 0.21 (yellow): NI; 0.15 (yellow): NI; 0.1 (yellow): NI; 00 (yellow): NI.**

NI: unidentified compound

Table 10. DPPH radical scavenging phytocompounds in CH_2Cl_2/ AcOEt/ C_6H_{14} (3 :4 :2) (V/V/V) developer from dichloromethane (CH_2Cl_2) and acetate-ethyl (AcOEt) extracts

Extracts	R_f (Color)**: Possible compounds**
SSF H-Et (CH_2Cl_2)	0.98 (yellow): **flavonoid**; 0.8 (yellow): **flavonoid**; 0.7 (yellow): **flavonoid**; 0.69 (yellow): NI; 0.63 (yellow): **flavonoid**; 0.54 (yellow): **flavonoid**; 0.39 (yellow): **flavonoid**; 0.24 (yellow): **flavonoid**; 0.13 (yellow): **flavonoid**; 0.1 (yellow): NI; 0.06 (yellow): NI; 00 (yellow): **flavonoid.**
SST H-Et (CH_2Cl_2)	**0.94 (yellow): NI; 0.9 (yellow):** flavonoid**; 0.79 (yellow):** flavonoid**; 0.68 (yellow):** flavonoid**; 0.64 (yellow): NI; 0.5 (yellow):** flavonoid**; 0.45 (yellow): NI; 0.4 (yellow):** flavonoid**; 0.28 (yellow):** flavonoid**; 0.2 (yellow):** flavonoid**; 0.15 (yellow):** flavonoid**; 0.11 (yellow): NI; 0.08 (yellow): NI; 00 (yellow):** flavonoid.
SSF H-Et (AcOEt)	0.94 (yellow): NI; 0.86 (yellow): **flavonoid**; 0.81 (yellow): **flavonoid**; 0.75 (yellow): **flavonoid**; 0.65 (yellow): **flavonoid**; 0.6 (yellow): **flavonoid**; 0.49 (yellow): NI; 0.46 (yellow): **flavonoid**; 0.36 (yellow): **flavonoid**; 0.25 (yellow): **flavonoid**; 0.23 (yellow): **flavonoid**; 0.13 (yellow): **flavonoid**; 0.06 (yellow): NI; 00 (yellow): **flavonoid.**
SST H-Et (AcOEt)	**0.95 (yellow):** flavonoid**; 0.85 (yellow): NI; 0.78 (yellow): NI; 0.74 (yellow):** flavonoid**; 0.61 (yellow): NI; 0.54 (yellow): NI; 0.45 (yellow):** flavonoid**; 0.36 (yellow): NI; 0.25 (yellow):** flavonoid**; 0.18 (yellow):** flavonoid**; 0.13 (yellow): NI; 0.08 (yellow):** flavonoid**; 00 (yellow): NI.**

NI: unidentified compound

Table 11. DPPH radical scavenging phytocompounds in CH_2Cl_2/ AcOEt/CH_3COOH (1 : 3,5 :1) (V/V/V) developer from dichloromethane (CH_2Cl_2) and acetate-ethyl (AcOEt) extracts

Extracts	R_f (Color)**: Possible compounds**
SSF H-Et (CH_2Cl_2)	0.96 (yellow): **phenolic acid**; 0.84 (yellow): NI; 0.78 (yellow): NI; 0.71 (yellow): NI; 0.65 (yellow): **phenolic acid**; 0.54 (yellow): NI; 0.58 (yellow): NI; 0.56 (yellow): **tannin**; 0.49 (yellow): **tannin**; 0.4 (yellow): NI; 0.35 (yellow): NI; 0.28 (yellow): NI; 0.23 (yellow): NI; 0.15 (yellow): NI; 0.08 (yellow): NI; 00 (yellow): NI.

SST H-Et (CH_2Cl_2)	**0.9 (yellow):** phenolic acid**; 0.84 (yellow): NI; 0.76 (yellow): NI; 0.68 (yellow):** tannin**; 0.63 (yellow): NI; 0.55 (yellow): NI; 0.49 (yellow):** tannin**; 0.39 (yellow): NI; 0.33 (yellow): NI; 0.25 (yellow): NI; 0.16 (yellow): NI; 0.11 (yellow): NI; 0.08 (yellow): NI; 00 (yellow): NI.**
SSF H-Et (AcOEt)	0.96 (yellow): NI; 0.85 (yellow): NI; 0.78 (yellow): NI; 0.73 (yellow): NI; 0.68 (yellow): **phenolic acid**; 0.6 (yellow): **tannin**; 0.55 (yellow): **tannin**; 0.51 (yellow): NI; 0.46 (yellow): **tannin**; 0.36 (yellow): **tannin**; 0.31 (yellow): NI; 0.26 (yellow): **tannin**; 0.19 (yellow): **tannin**; 0.09 (yellow): **tannin**; 00 (yellow): NI
SST H-Et (AcOEt)	**0.94 (yellow): NI; 0.9 (yellow):** phenolic acid**; 0.79 (yellow): NI; 0.7 (yellow): NI; 0.69 (yellow):** tannin**; 0.55 (yellow): NI; 0.45 (yellow): NI; 0.42 (yellow):** tannin**; 0.31 (yellow):** tannin**; 0.2 (yellow):** tannin**; 0.11 (yellow):** tannin**; 0.05 (yellow): NI; 00 (yellow):** tannin.

NI: unidentified compound

II.1.3.2. Assessment of antioxidant activity by spectrophotometry

II.1.3.2.1. Inhibition percentages for vitamin C, aqueous, ethanolic, and hydroethanolic crude extracts

Percentage inhibition is the ability of an extract to scavenge free radicals. The different percentages of DPPH inhibition by aqueous, ethanolic, and hydroethanolic extracts and vitamin C are shown in Figures 1 and 2. Inhibition percentages for plant extracts range from 05.839 ± 03.499% to 79.774 ± 0.579%, and for vitamin C from 63.316 ± 0.405% to 84.201 ± 0.116%, at concentration ranges from C6=0.062 5mg/mL to C1=2 mg/mL.

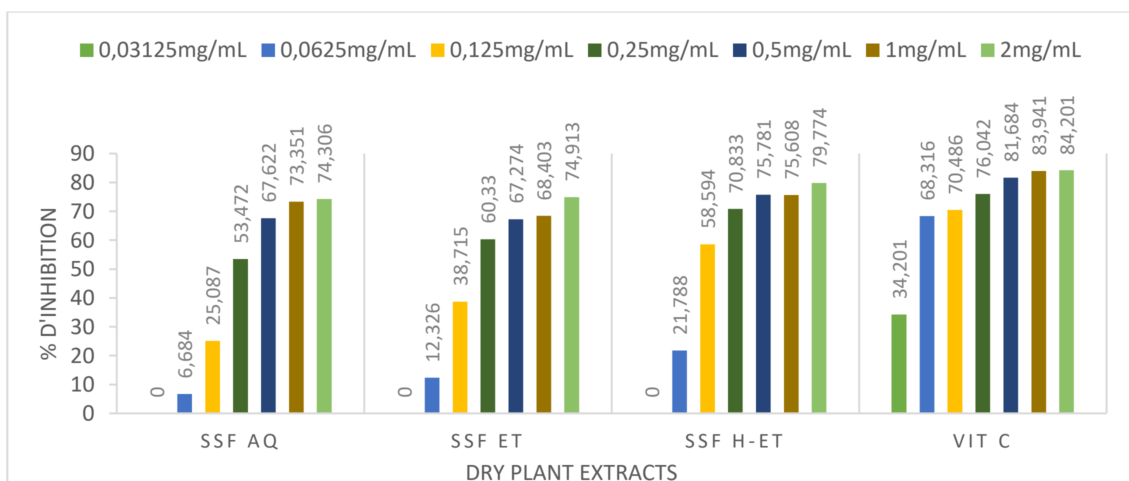

Figure 1. DPPH inhibition by aqueous, ethanolic and hydroethanolic extracts of *Saba senegalensis* leaves

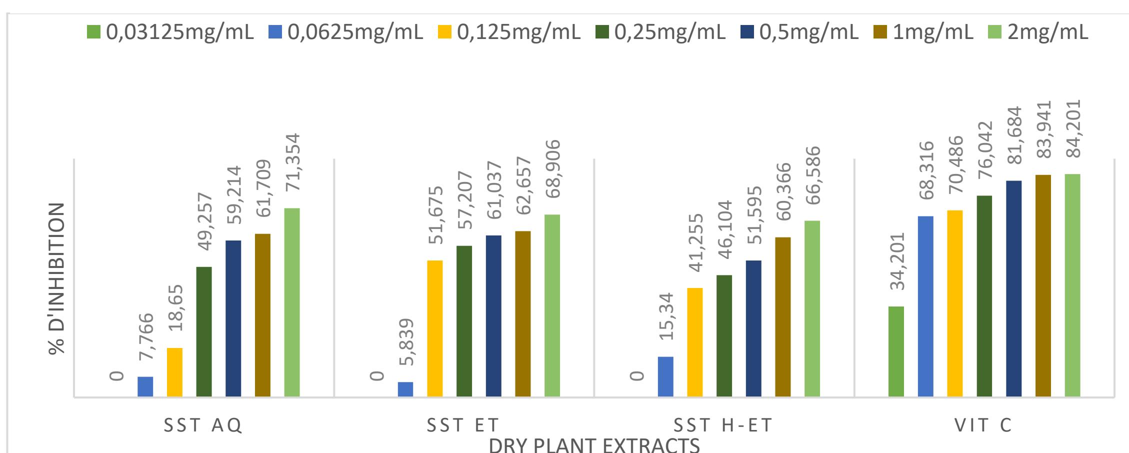

Figure 2. DPPH inhibition by aqueous, ethanolic and hydroethanolic extracts of *Saba senegalensis* stems

II.1.3.2.2. Determination of IC50s for vitamin C and aqueous, ethanolic, and hydroethanolic crude extracts

The 50% inhibitory concentrations were used to calculate the concentrations of the samples studied required to reduce 50% of DPPH radicals. The IC_{50} of an extract is the concentration that results in a 50% loss of DPPH activity **[10].** The lower the IC_{50}, the greater the antioxidant activity of the extract.

IC_{50} values for aqueous and organic extracts range from 0.11041 mg/mL to 0.42738 mg/mL, while that for vitamin C is 0.04575 mg/mL. These mean values are shown in Table 12.

Table 12. Summary table of IC_{50} values (mg/mL) for various extracts

Extracts	Right equations	IC_{50} (mg/mL)
Vit C	Y= 1091 X + 0.086	**0.04575**
SSF Aq	Y= 227.08 X – 3.298	**0.23471**
SSF Et	Y= 172.92 X + 17.1	**0.19026**
SSF H-Et	Y= 588.9 X – 15.018	**0.11041**
SST Aq	Y= 39.828 X + 39.3	**0.26865**
SST Et	Y= 733.38 X – 39.997	**0.12271**
SST H-Et	Y= 21.964 X + 40.613	**0.42738**

II.2. Discussion

Leaf yields ranged from 35.10 ±3.94 for the ethanolic extract to 46.03 ±1.98 for the hydroethanolic extract, while stem bark yields varied from 46.39 ±2.63 for the hydroethanolic extract to 49.00 ±2.76 for the ethanolic extract. The aqueous extracts of leaves and stems each achieved the second-highest yields for the different extractions of their respective organs. Yield values varied from one organ to another, depending on the solvent used, the extraction method and the extraction conditions. All extraction yields for stems are higher than those for leaves. However, these values are relatively high in comparison with the research work of several other authors **[11, 12].** This may justify the routine use of these extraction methods in many research projects. In fact, extraction by maceration with alcohols (ethanol) is said to be more effective in extracting many groups of phytocompounds **[13].** Moreover, the presence of water in extractions allows the permeability of plant tissues, and favors the phenomenon of mass diffusion in the extraction stage **[14, 15, 16].** Therefore, the use of water, ethanol or a mixture of the two is partly responsible for these relatively high yields.

Phytochemical screening by TLC was carried out on *Saba senegalensis* leaves and stems. Sterols, terpenes, flavonoids, tannins and phenolic acids were identified in both organs studied, coumarins only in leaves, and alkaloids were absent in both organs. These results are in harmony with those of Serigne et *al.* **[17]** and Traoré **[18]** on *S. senegalensis* leaves. Indeed, the work of Serigne et *al.* showed the presence of non-hydrolyzable tannins, and flavonoids and the absence of alkaloids in the ethanolic and aqueous extracts of the plant's leaves **[17].** As for Traoré's work, he highlighted the presence of coumarins, flavonoids, tannins, sterols and triterpenes in *S. senegalensis* leaf extracts, and also noted the absence of alkaloids **[18].** Phytochemical TLC screening of *S. senegalensis* leaves and stems revealed several secondary metabolites with numerous pharmacological properties. These include antibacterial **[19, 20]**, antiparasitic **[21]**, analgesic **[22]**, anti-inflammatory **[23, 24]**, antimicrobial **[21]**, antiviral **[20]**, hemostatic **[25]**, and antioxidant **[21]** properties. This work could justify the use of *S. senegalensis* leaves and stems in the traditional treatment of numerous pathologies. Indeed, the leaves are used to treat headaches, rectal prolapse, otitis, anorexia, food poisoning, dysentery, and urinary schistosomiasis, while also having hemostatic properties **[26].** Stems are used to treat infectious diseases (lung diseases, boils, diarrhea), parasites (urinary schistosomiasis), inflammatory diseases, and headaches **[27].**

Antioxidant activity was assessed by TLC and spectrophotometry. The TLC profile identified several compounds responsible for the antioxidant activity of the two organs studied. By comparing the chromatographic profiles of the phytochemical screening with those of the antioxidant activity screening, the correspondence between the active zones and the phytocompounds responsible for this activity was established. In hexane extracts, some yellow spots correspond to the antioxidant activity of sterols and terpenes (Rf: 0.89; 0.25; 0.09; 0.0 for

leaves / Rf: 0.83; 0.38; 0.29; 0.08; 0.0 for stems). Similarly, the antioxidant power of the dichloromethane and acetate-ethyl extracts from the two plant organs is deduced from flavonoids, phenolic acids, tannins, and coumarins only in the leaves. However, several other compounds with antioxidant activity could not be identified (NI) in the various selective extracts of the two *Saba senegalensis* organs studied.

As for the evaluation of antioxidant activity by spectrophotometry, the aim was to determine the inhibition percentages of the extracts with respect to DPPH and to determine their inhibitory IC_{50} concentrations. The study showed that the various extracts exhibited antioxidant potential, irrespective of their concentration. This oxidizing activity observed for these aqueous and organic extracts is partly due to the synergistic action of all the secondary metabolites in *Saba senegalensis* leaves and stems. Indeed, a study by Kang et *al.* **[28]** attributed the extracts' anti-free radical activity to the richness of phenolic compounds detected within them. This study suggested that the polar molecules present in plant extracts contribute to increased anti-free radical activity **[28].** In addition, other studies on certain plant extracts have shown a high, positive correlation between total phenolic compounds (flavonoids, phenolic acids, tannins, and coumarins) and anti-free radical activity **[29, 30].**

These inhibition percentages represent the extracts' capacity to trap free radicals and have been used to calculate the IC_{50}, an even more precise constant for interpreting results. This concentration represents the capacity of an extract to cause a 50% loss of DPPH activity **[10].** The lower the IC_{50}, the greater the antioxidant activity of the extract.

By comparison: IC_{50} (Vit C) < IC_{50} (SSF H-Et) < IC_{50} (SSF Et) < IC_{50} (SSF Aq), so the antioxidant power of the hydroethanolic leaf extract is greater than that of the ethanolic extract, which in turn is greater than that of the aqueous extract. Similarly, we can see that IC_{50} (Vit C) < IC_{50} (SST Et) < IC_{50} (SST Aq) < IC_{50} (SST H-Et), so for stems, the antioxidant power of the ethanolic extract is greater than that of the aqueous extract, which in turn is greater than that of the hydroethanolic extract. Generally speaking, the antioxidant power of leaves is greater than that of stems.

At the end of this quantitative analysis of antioxidant capacity, the crude aqueous, ethanolic, and hydroethanolic extracts of *Saba senegalensis* leaves and stems revealed significant DPPH neutralizing power. These results are in perfect harmony with those of Traoré, who had previously confirmed this activity by TLC but especially by spectrophotometry **[18].** Therefore, these two *Saba senegalensis* organs are antioxidants that could be recommended to prevent or curb the damage caused by oxidative stress, namely cancer, accelerated aging, high blood pressure, Alzheimer's, Parkinson's, and diabetes **[31, 32].** These results seem to justify the *de facto* use of these two plant organs in traditional medicine in Korhogo (Côte d'Ivoire).

CONCLUSION

This work aimed to confirm or invalidate the use of *Saba senegalensis* leaves and stem bark as a good antioxidant capable of traditionally treating various pathologies.

The results of phytochemical screening of the various extracts using TLC highlighted the presence of flavonoids, tannins, phenolic acids, saponins, sterols, and terpenes in the extracts of *S. senegalensis* leaves and stem bark. Coumarins were found only in the leaves. Alkaloids, on the other hand, were absent in both organs studied.

Antioxidant activity towards DPPH was assessed using qualitative (TLC) and quantitative (spectrophotometry) methods on both organs' aqueous, ethanolic, and hydroethanolic extracts. The results showed that these organs have a good antioxidant profile, partly due to secondary metabolites detected in them. Quantitative analysis of antioxidant capacity showed that, in general, the antioxidant capacity of leaves was higher than that of stems. However, both organs can be considered antioxidants that could prevent or treat diseases linked to oxidative stress.

In the future, this work on *S. senegalensis* leaves and stems should continue to prove their safety for the human organism and produce an antioxidant phytomedicine within the reach of all social classes.

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