



Research Article

Pharmacognostic Valorization of *Strychnos afzelii* Gilg. Stems Used as Traditional Chewing Sticks in Western Cote d'Ivoire: Phytochemical and Elemental Profile

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Abstract

Traditional chewing sticks remain widely used for oral hygiene in many African communities. In western Cote d'Ivoire, stems of *Strychnos afzelii* Gilg. are sold in local markets and used as traditional chewing sticks. However, information on their phytochemical composition and elemental profile remains limited. This study aimed to contribute to the pharmacognostic characterization of *S. afzelii* stems. Plant material was collected at Glaogouin, approximately 5 km from Man, Cote d'Ivoire, and botanically authenticated at the National Floristic Center of Abidjan (voucher specimen UCJ011524). Aqueous and 70% hydroethanolic extracts were prepared by 24-h maceration. Phytochemical screening was performed using qualitative color and precipitation reactions and thin-layer chromatography (TLC). The elemental composition of the powdered stems was determined by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS). Extraction yields were 14.0% and 16.9% for the aqueous and hydroethanolic extracts, respectively. Both extracts contained polyphenols, flavonoids, catechin tannins, coumarins, aromatic amino acids and proteins. The elemental profile was dominated by calcium (27444.32 mg/kg), followed by potassium (2856.55 mg/kg), sulfur (1992.71 mg/kg), silicon (1868.81 mg/kg), aluminum (1188.71 mg/kg), and magnesium (1020.95 mg/kg). Cadmium was detected at 18.40 mg/kg, whereas lead was detected at 1.30 mg/kg. These findings provide preliminary pharmacognostic data on *S. afzelii* stems and contribute to the scientific documentation of a traditionally used chewing-stick plant in western Cote d'Ivoire.

Keywords

Strychnos Afzelii, Chewing Stick, Phytochemical Screening, Elemental Profile, SEM-EDS, Oral Hygiene

1. Introduction

Traditional plant-based oral hygiene practices constitute an important component of ethnobotanical knowledge in many African communities. Chewing sticks are traditionally used for cleaning the teeth and oral cavity and may provide both mechanical cleaning and exposure to plant-derived chemical constituents [1-3]. Their continued use has stimulated increasing

interest in the phytochemical and biological characterization of the plants used as chewing sticks. The oral-health benefits associated with chewing sticks may depend on several factors, including the plant species, plant organ, preparation method, chemical composition, and frequency of use [1-3]. Previous investigations of chewing-stick plants have reported the presence

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of phenolic compounds, flavonoids, tannins, alkaloids, terpenoids and other secondary metabolites that may contribute to antimicrobial or antioxidant properties [2-5]. The genus *Strychnos* L. is of particular pharmacognostic interest because of its richness in specialized metabolites, especially indole alkaloids. Several *Strychnos* species have been investigated for their chemical diversity and biological activities [6-8]. The genus includes species known for pharmacologically active alkaloids and therefore represents an important group for natural-product research. *Strychnos afzelii* Gilg. is a tropical African species whose traditional uses and chemical composition remain comparatively poorly documented. Earlier investigations demonstrated the presence of alkaloids in the stem bark of *S. afzelii* and reported antimicrobial effects associated with some of these constituents [9]. In particular, bisnor-dihydrotoxiferine, bisnor-C-alkaloid H and caracurine V were identified among the alkaloids associated with antimicrobial activity [9]. Ethnobotanical information also indicates the use of *S. afzelii* stems as chewing sticks in Cote d'Ivoire and other parts of West Africa [10]. However, available information concerning the global phytochemical profile of the stems and their elemental composition remains scarce. Extraction procedures are critical in pharmacognostic investigations because solvent polarity strongly influences the classes and quantities of compounds recovered from plant material [11, 12]. Maceration is a simple and widely applicable extraction technique, while hydroethanolic mixtures can provide a broader extraction range for compounds with different polarities [11, 12]. Therefore, the present study aimed to characterize the stems of *S. afzelii* used as traditional chewing sticks in western Cote d'Ivoire through: (i) determination of aqueous and hydroethanolic extraction yields; (ii) qualitative phytochemical screening; (iii) thin-layer chromatographic characterization; and (iv) determination of the elemental profile using SEM-EDS.

2. Materials and Methods

2.1. Plant Material and Botanical Identification

The plant material consisted of stems of *Strychnos afzelii* Gilg. (Figure 1). Samples were collected at Glaogouin, a village located approximately 5 km from Man, in western Cote d'Ivoire. Botanical identification was performed at the National Floristic Center (CNF), Abidjan, Cote d'Ivoire. A voucher specimen was deposited in the herbarium under accession number UCJ011524. Preliminary observations conducted in local markets in Man indicated that stems of *S. afzelii* are commercially available and commonly used as traditional chewing sticks for oral hygiene. This field observation motivated the selection of the species for the present phytochemical and elemental investigation.



Figure 1. Stems of *Strychnos afzelii* Gilg. used as traditional chewing sticks in western Cote d'Ivoire.

2.2. Collection, Drying and Preparation of Plant Material

The stems of *S. afzelii* were collected in the study area and cut into small fragments. The plant material was carefully washed with water to remove superficial impurities and then dried for seven days under continuous air conditioning at approximately 18 °C. After drying, the material was powdered using an electric grinder at the Central Laboratory of the University of Man. The resulting powder was stored in appropriate conditions and used for phytochemical and elemental analyses as well as for extract preparation.

2.3. Preparation of Plant Extracts

2.3.1. Aqueous Extraction

One hundred grams (100 g) of powdered *S. afzelii* stems were macerated in 1 L of distilled water for 24 h under magnetic stirring. Maceration was selected because it is a simple extraction procedure that allows plant constituents to diffuse into a suitable solvent without requiring prolonged heating [11, 12]. After extraction, the macerate was pressed through sterile cloth and subsequently filtered through cotton to obtain a clear filtrate. The filtrate was dried slowly in an oven at 50 °C for 24 h. The resulting dry extract was collected in an airtight container and stored at 4 °C until further analysis.

2.3.2. Hydroethanolic Extraction

One hundred grams (100 g) of powdered stems were macerated in 1 L of 70% ethanol for 24 h under magnetic stirring. Hydroethanolic mixtures are frequently used in plant extrac-

tion because their intermediate polarity can facilitate the recovery of compounds exhibiting different physicochemical properties [11, 12]. The macerate was subsequently pressed through sterile cloth and filtered through cotton. The filtrate was dried slowly in an oven at 40 °C for 24 h. The dry extract was collected in an airtight container and stored at 4 °C until further analyses.

$$\text{Extraction yield (\%)} = \frac{\text{Mass of dry extract (g)}}{\text{Mass of plant powder used (g)}} \times 100 \quad (1)$$

2.5. Qualitative Phytochemical Screening

2.5.1. Tube Characterization Tests

Qualitative phytochemical screening was performed on the aqueous and 70% hydroethanolic extracts of *S. afzelii* stems.

The main classes of secondary metabolites were investigated using color and precipitation reactions according to previously described phytochemical screening procedures [13, 14]. The investigation included polyphenols, flavonoids, catechin tannins, gallotannins, alkaloids, saponins, coumarins, anthocyanins, sterols and polyterpenes, aromatic amino acids, reducing sugars, proteins, gums and mucilages. Results were expressed qualitatively using "+" for detection and "-" for non-detection under the experimental conditions.

2.5.2. Thin-layer Chromatography

Thin-layer chromatography (TLC) was used to complement the qualitative phytochemical screening, following a modified procedure previously described for the chromatographic characterization of plant metabolites [14, 15]. A solution of the dry extract was prepared at 10 mg/mL by dissolving 10 mg of extract in 1 mL of methanol. A volume of 20 µL, corresponding to 200 µg of extract, was applied using capillary tubes onto aluminum-backed silica gel 60 F254 plates. The plates were developed in a saturated chromatographic chamber containing chloroform/ethyl acetate/acetic acid/water (2.5:7:0.2:1, v/v/v/v), with two drops of ammonia solution added to the mobile phase. After development, the plates were dried and examined under visible light and ultraviolet radiation at 254 and 365 nm.

Specific spraying reagents were used for the detection of different phytochemical groups: 2% ferric chloride (FeCl₃) for phenolic compounds and tannins, 5% ethanolic aluminum chloride (AlCl₃) for flavonoids, 5% methanolic potassium hydroxide (KOH) for Anthrone/anthraquinone derivatives, and 25% ammonia (NH₃) for coumarins [14, 15].

The retention factor (Rf) was calculated according to equation (2):

2.4. Determination of Extraction Yield

The extraction yield was calculated as the ratio between the mass of dry extract obtained after solvent removal and the initial mass of plant powder used for extraction [11, 12].

The extraction yield was calculated using equation (1):

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}} \quad (2)$$

2.6. Elemental Analysis

The elemental composition of *S. afzelii* stem powder was determined after mineralization of the plant material. Powdered samples were incinerated at 550 °C for 24 h in a Naberthern MORE THAN HEAT furnace at the National Center for Agronomic Research (CNRA), Bingerville, Cote d'Ivoire. The resulting ash was analyzed at the Analysis and Research Center (CAR) of PETROCI, Abidjan, Cote d'Ivoire. Elemental analysis was performed using a Zeiss Supra 40 VP FEG scanning electron microscope coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) and equipped with an Oxford Instruments X-ray detector and an INCA Dry Cool microanalysis platform. Acceleration voltages ranging from 0.1 to 30 keV were used according to analytical requirements. Different magnifications, ranging from 12× to 1.000.000×, were applied depending on sample characteristics. The reported analytical resolution was 2 nm. The concentrations were initially reported by the analytical system mainly as oxide forms. To facilitate interpretation of the elemental composition of the plant material, oxide concentrations were converted into elemental concentrations using the corresponding elemental mass fraction. The elemental concentrations were expressed as percentage (%) and milligrams per kilogram of dry matter (mg/kg). The elemental analysis was performed in triplicate and mean values were reported.

2.7. Data Processing

Extraction yields and qualitative phytochemical results were organized and processed using Microsoft Excel. Elemental analytical data were transcribed from the laboratory analytical report and converted from measured oxide forms into elemental concentrations. The conversion from percentage to mg/kg was performed according to equation (3):

$$\text{Elemental concentration (mg/kg)} = \text{elemental concentration (\%)} \times 10.000 \quad (3)$$

Only the corrected elemental concentrations obtained after conversion were used for interpretation of the elemental profile.

3. Results

3.1. Extraction Yield

The extraction yields obtained from *S. afzelii* stems are presented in Table 1.

Thus, the hydroethanolic extraction yield was 2.9 percentage points higher than that obtained with water.

Table 1. Extraction yields of aqueous and 70% hydroethanolic extracts from *Strychnos afzelii* stems.

Extracts of <i>S. afzelii</i>	Plant powder (g)	Dry extract (g)	Yield (%)
Aqueous	100	14.0	14.0
Hydroethanolic 70%	100	16.9	16.9

3.2. Phytochemical Screening

The qualitative phytochemical screening results are presented in Table 2.

Both aqueous and hydroethanolic extracts showed positive

reactions for polyphenols, flavonoids, catechin tannins, coumarins, aromatic amino acids and proteins. Gallotannins, alkaloids, saponins, anthocyanins, sterols and polyterpenes, and reducing sugars were not detected in either extract. Gums and mucilages were detected only in the aqueous extract.

Table 2. Qualitative phytochemical screening of aqueous and 70% hydroethanolic extracts of *Strychnos afzelii* stems.

Phytochemical group	Aqueous extract	Hydroethanolic extract
Polyphenols	+	+
Flavonoids	+	+
Catechin tannins	+	+
Gallotannins	–	–
Alkaloids	–	–
Saponins	–	–
Coumarins	+	+
Anthocyanins	–	–
Sterols and polyterpenes	–	–
Aromatic amino acids	+	+
Reducing sugars	–	–
Proteins	+	+
Gums and mucilages	+	–

+ = detected; – = not detected under the experimental conditions.

3.3. TLC Characterization

TLC analysis provided additional evidence for several groups of secondary metabolites in the extracts.

In the hydroethanolic extract, tannins were detected following spraying with 2% FeCl₃, with dark spots observed under visible light. Anthrone/anthraquinone derivatives were detected following treatment with 5% KOH, producing yellow-

orange spots. Flavonoid-related spots were observed following treatment with 5% AlCl_3 . Coumarins were revealed by green fluorescence following exposure to 25% ammonia.

In the aqueous extract, migration of tannins, anthrone/anthraquinone derivatives and flavonoids was not observed under the experimental TLC conditions, whereas coumarin-related spots were detected.

Table 3. Compounds detected by TLC in aqueous and 70% hydroethanolic extracts of *Strychnos afzelii* stems.

Reagents Extracts	Tannins FeCl_3 2%	Anthrone/anthraquinone derivatives KOH 5%	Flavonoids AlCl_3 5%	Coumarins NH_3 25%
Hydroethanolic extract	Rf: 0.50; 0.75 (dark spots)	Rf: 0.3025; 0.3025 (yellow-orange spots)	Rf: 0.9375; 0.9375 (yellow-orange fluorescence)	Rf: 0.9375; 0.60; 0.25 (green fluorescence)
Aqueous extract	Rf: 0	Rf: 0	Rf: 0	Rf = 0.375; 0.750 (green fluorescence)

Rf= Retention factor.

3.4. Elemental Profile of *Strychnos Afzelii* Stems

SEM-EDS analysis revealed a diverse elemental composition in the stems of *S. afzelii*. After conversion of the measured oxide forms into elemental concentrations, calcium was the predominant element, reaching 2.74443% (27444.32 mg/kg). It was followed by potassium (0.28566%; 2856.55 mg/kg), sulfur (0.19927%; 1992.71 mg/kg), silicon (0.18688%; 1868.81 mg/kg), aluminum (0.11887%; 1188.71 mg/kg), and magnesium (0.10210%; 1020.95 mg/kg). Sodium, phosphorus, manganese, iron, barium, lanthanum, chlorine, strontium, and cesium were detected at intermediate concentrations, whereas the remaining elements occurred at lower or trace levels. Cadmium and lead were detected at 18.40 and 1.30 mg/kg, respectively, while several elements, including arsenic, selenium, mercury, thallium, and bismuth, were below the detection limits (Figure 2).

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These results indicate a mineral profile characterized by a marked predominance of calcium, accompanied by substantial amounts of potassium, sulfur, silicon, aluminum, and magnesium, together with a broad diversity of minor and trace elements.

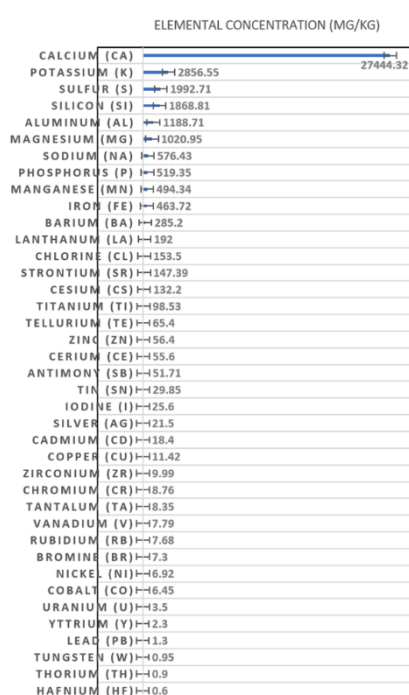


Figure 2. Corrected elemental profile of *Strychnos afzelii* stems determined by SEM-EDS after conversion of measured forms into elemental concentrations.

3.5. Elements Below the Detection Limits

Gallium, germanium, selenium, arsenic, niobium, molybdenum, mercury, thallium and bismuth were below the detection limits of the analytical method (Table 4).

Table 4. Elements below the detection limits of the SEM-EDS method.

Element	Measured form	Elemental concentration (%)	Elemental concentration (mg/kg)
Gallium (Ga)	Ga	<0.00005	<0.50
Germanium (Ge)	Ge	<0.00005	<0.50
Selenium (Se)	Se	<0.00005	<0.50
Arsenic (As)	As ₂ O ₃	<0.000053	<0.53
Niobium (Nb)	Nb ₂ O ₅	<0.000098	<0.98
Molybdenum (Mo)	Mo	<0.00010	<1.00
Mercury (Hg)	Hg	<0.00010	<1.00
Thallium (Tl)	Tl	<0.00010	<1.00
Bismuth (Bi)	Bi	<0.00010	<1.00

Values preceded by "<" indicate concentrations below the analytical detection limit.

4. Discussion

The extraction yields obtained from *S. afzelii* stems were 14.0% for the aqueous extract and 16.9% for the 70% hydroethanolic extract. The higher yield obtained with the hydroethanolic solvent may be explained by the ability of water-ethanol mixtures to solubilize compounds with different polarities [11, 12]. Solvent polarity is a major determinant of extraction efficiency and strongly influences the chemical composition of plant extracts [11, 12]. The phytochemical screening demonstrated the presence of polyphenols, flavonoids, catechin tannins, coumarins, aromatic amino acids and proteins in both extracts. These groups of compounds are frequently reported in medicinal and traditionally used plants and may contribute to several biological properties, including antioxidant and antimicrobial activities [3, 13]. Chewing-stick plants constitute an important but still comparatively under-investigated source of phytochemicals, and further characterization of their chemical composition and biological properties has been recommended [16]. The detection of tannins, flavonoids and coumarins by TLC further supports the qualitative screening results. However, TLC provides mainly qualitative or semi-quantitative information. Consequently, the detected groups should not be interpreted as evidence of the presence of specific individual compounds without complementary chromatographic or spectrometric identification. The absence of detectable alkaloids in both extracts deserves particular consideration. The genus *Strychnos* is well known for

its indole alkaloids [6-8]. More specifically, previous investigations of *S. afzelii* demonstrated the presence of several alkaloids in stem bark extracts and reported antimicrobial activity associated with some of these compounds [9]. The absence of a positive alkaloid reaction in the present investigation may therefore be related to differences in the plant organ examined, extraction solvent, extraction conditions, concentration of the compounds or sensitivity of the qualitative test. Consequently, the negative alkaloid result should be interpreted as non-detection under the experimental conditions used, rather than as evidence that *S. afzelii* stems are completely devoid of alkaloids. The elemental analysis revealed a strongly calcium-dominated profile. Calcium reached 27444.32 mg/kg, representing the highest elemental concentration detected. Potassium (2856.55 mg/kg), sulfur (1992.71 mg/kg), silicon (1868.81 mg/kg), aluminum (1188.71 mg/kg) and magnesium (1020.95 mg/kg) were the next most abundant elements. The relatively high concentrations of calcium and potassium indicate that these elements constitute major components of the elemental profile of the stems. Nevertheless, elemental detection alone does not demonstrate nutritional, therapeutic or pharmacological efficacy. The biological significance of these elements depends on their chemical forms, bioavailability, exposure levels and interactions with other constituents.

Several trace elements were also detected. Of particular interest were zinc (56.40 mg/kg) and copper (11.42 mg/kg), which are essential elements involved in numerous biological processes. However, their presence in the plant material should not automatically be interpreted as a nutritional advantage because the actual amount released during chewing

and subsequently absorbed by the human organism was not determined. The detection of cadmium at 18.40 mg/kg deserves particular attention. Lead was detected at 1.30 mg/kg. Because plants can accumulate potentially toxic elements from their growing environment, the occurrence of heavy metals in plant materials intended for human use warrants appropriate monitoring and risk assessment [17]. Because the stems are reportedly used repeatedly as chewing sticks, the presence of potentially toxic elements warrants further investigation. Nevertheless, the SEM-EDS results should be interpreted cautiously because this technique is primarily suitable for elemental screening and semi-quantitative characterization. Confirmation by a validated quantitative technique such as ICP-MS, ICP-OES or AAS would be desirable before drawing conclusions concerning toxicological safety [18]. The detection limits obtained for arsenic, mercury, selenium and other elements were low, with arsenic below 0.53 mg/kg, selenium below 0.50 mg/kg, mercury below 1.00 mg/kg and bismuth below 1.00 mg/kg. These results provide useful preliminary information on the elemental composition of the material but should also be confirmed by a quantitative analytical method [18]. The combined phytochemical and elemental characterization therefore provides a broader pharmacognostic profile of *S. afzelii* stems. The results are particularly relevant because the plant material is traditionally commercialized and used as a chewing stick in western Cote d'Ivoire.

However, the present study has several limitations. The phytochemical investigation was qualitative, and no quantitative determination of total phenolics, flavonoids or tannins was performed. Likewise, the individual chemical constituents responsible for the observed TLC profiles were not identified. Further investigations using HPLC-DAD, LC-MS/MS, GC-MS or NMR would therefore be useful for chemical characterization.

Similarly, the elemental data obtained by SEM-EDS should

be considered preliminary. Confirmatory quantitative analysis would be necessary to establish reliable concentrations and assess possible exposure during repeated use as a chewing stick. Recent advances in plant elemental analysis highlight the importance of appropriate sample preparation and validated quantitative analytical procedures for reliable determination of major and trace elements [18].

5. Conclusion

The present study provides preliminary pharmacognostic data on the stems of *Strychnos afzelii* Gilg. used traditionally as chewing sticks in western Cote d'Ivoire. The extraction yields were 14.0% for the aqueous extract and 16.9% for the 70% hydroethanolic extract. Qualitative phytochemical screening demonstrated the presence of polyphenols, flavonoids, catechin tannins, coumarins, aromatic amino acids and proteins in both extracts. TLC further supported the presence of tannins, flavonoids and coumarin-related compounds. The elemental profile was dominated by calcium (27444.32 mg/kg), followed by potassium, sulfur, silicon, aluminum and magnesium. Several trace elements were also detected, including zinc, copper and cadmium. The detection of cadmium and lead highlights the need for further quantitative and toxicological investigations before any conclusion can be drawn regarding the long-term safety of repeated use. Overall, these findings contribute to the scientific documentation and pharmacognostic valorization of *S. afzelii* as a traditional chewing-stick plant in Cote d'Ivoire. Future studies should focus on the quantitative determination and identification of its bioactive constituents, evaluation of antimicrobial and antioxidant activities, and confirmation of its elemental composition using more sensitive quantitative analytical techniques.

Abbreviations

AAS	Atomic Absorption Spectroscopy
AlCl ₃	Aluminum Chloride
CAR	Analysis and Research Center
CNF	National Floristic Center
CNRA	National Center for Agronomic Research
EDS	Energy-Dispersive X-ray Spectroscopy
FeCl ₃	Ferric Chloride
FEG	Field Emission Gun
GC-MS	Gas Chromatography–Mass Spectrometry
HPLC-DAD	High-Performance Liquid Chromatography with Diode-Array Detection
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
KOH	Potassium Hydroxide
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
NH ₃	Ammonia
NMR	Nuclear Magnetic Resonance
PETROCI	National Oil Operations Company of Cote d'Ivoire

Rf	Retention Factor
SEM-EDS	Scanning Electron Microscopy Coupled With Energy-Dispersive X-ray Spectroscopy
TLC	Thin-Layer Chromatography

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Author Contributions

Monyn-Kouamé Ebalah Delphine: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Kouamé N'guessan Brigitte: Investigation, Project administration, Resources, Validation, Visualization, Writing – review & editing

Conflicts of Interest

The authors declare no conflict of interest.

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