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Phytochemical Analysis of Leaf, Stem Bark, and Root Extracts of *Cassia abbreviata* Grown in Zambia

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Abstract

Background: Plants have for a long time been used as medicines to treat ailments. *Cassia abbreviata* (*C. abbreviata*) is one of the plants that has been widely used in the management of a range of ailments, including malaria, diarrhea, and abscesses. **Aim:** This study was conducted to investigate the phytochemical composition of crude extracts of the roots, leaves, and stem bark of *C. abbreviata*. **Materials and Methods**: This was a laboratory-based study in which the fresh leaves, stem bark, and roots of *C. abbreviata* were collected, thoroughly cleaned, chopped into small pieces, and kept at room temperature until fully dry. The dried plant parts were powdered separately and stored separately in airtight containers for the phytochemical investigation. Ethanol (96% v/v) or distilled water was used for the extraction of components of the coarse powder. The mixtures were separately filtered, and the filtrates were reduced to a concentrated semisolid mass by drying in a water bath at 40˚C. Thereafter, phytochemical analysis for flavonoids, phenolics, tannins, saponins, alkaloids, terpenoids, glycosides, carbohydrates, and proteins was done. **Results**: The phytochemical analysis of the ethanolic extracts revealed the presence of high concentrations of tannins, reducing sugars, and sterols in the stem bark and roots. Flavonoids, phenolics, and proteins were present in high concentrations in the stem bark, while anthraquinone, glycosides, and alkaloids were present in the leaves and roots, respectively. **Conclusion:** *C. abbreviata* plant contains a wide range of phytochemical constituents. Depending on the solvent used for extraction, various compositions of phytochemicals are obtained in each part of the tree. However, extraction with ethanol showed a better concentration than with water for most of the phytochemicals.

Keywords

Cassia abbreviata, Flavonoids, Phytochemicals, Stem Bark, Sterols, Tannins

1. Introduction

The use of plants has increased over the past number of years to become one of the most widespread sources of complementary treatments [1]. Studies have shown that people living in Sub-Saharan Africa are almost completely dependent on folk medical practices as a source of their primary health care needs [2] [3]. This may probably be attributed to such challenges as limited availability and affordability, and ease of accessibility of pharmaceutical drugs.

Numerous plants have been used as medicines for thousands of years, playing a crucial role in the discovery of new as well as improved versions of already existing drugs [4]. Different parts of plants from different parts of the world have been used as essential components in the management of various sorts of ailments in many indigenous communities, and Africa is no exception to this [5].

Cassia abbreviata (*C. abbreviata*) is one of the plants with vast medicinal values [6]. It is a small, umbrella-shaped deciduous shrub or small tropical tree in the genus *Cassia* that belongs to the family *Caesalpiniaceae* (*Leguminosae*) [6]. The shrub grows between 2 and 15 m in height, with a medium-round canopy. It has a dark grey to brown stem bark that is very rough on older trees, as well as young branchlets that are glabrous, pubescent, or puberulous [7].

In Zambia, *C. abbreviata* is referred to as Umunsokansoka by the Bemba-speaking people, Matholisi or Mulesa by the Chewa-speaking people, and as Mululwe by the Ila-speaking people [8]. The word "Mululwe" means "bitter", which is characteristic of the taste of the herb. A variety of ethno-therapeutic properties and pharmacological actions have been attributed to *C. abbreviata* [9].

The most widely used parts of the plant are the leaves, stem bark, and roots [10]. The different plant parts of *C. abbreviata* have been used in the management of many ailments like malaria, stomachache, diarrhea, and skin lesions in the Zambian traditional medicine system [11]. The roots are dried, dissolved in water and then taken orally to treat malaria, and venereal diseases, as well as alleviate stomachache and symptoms associated with pre-menstrual syndrome. The stem bark is also crushed, soaked in water and taken orally to treat diarrhoea, and applied to help treat abscesses and many other types of skin lesions.

Responsible for most, if not all, of the uses of medicinal plants, are chemical compounds referred to as phytochemicals. Phytochemicals are a large group of plant-derived compounds that are hypothesised to be responsible for much of the disease protection conferred by diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine. Based on their chemical structure, phytochemicals can be grouped into such groups as tannins, flavonoids, glycosides, saponins, alkaloids, triterpenoids, and sterols [12]. Therefore, the medicinal properties of *C. abbreviata* are due to its phytochemical composition [13] [14].

Although different parts of *C. abbreviata* are extensively used in the Zambian system of traditional medicine, there has not been much research done to investigate and document the phytochemical components of the leaf, stem bark, or root of the plant grown in Zambia. In view of this, and the therapeutic importance of *C. abbreviata,* this research was aimed at investigating the phytochemical constituents of *C. abbreviata* grown and used in Zambia.

2. Materials and Methods

2.1. Collection of Plant Materials

This was a laboratory-based study in which the fresh leaves, stem bark, and roots of *C. abbreviata* were collected in the month of November 2014 from Liteta district, Zambia. A voucher herbarium specimen was prepared, and botanical identification and authentications were done at the University of Zambia (UNZA), School of Natural Sciences, Department of Biological Sciences.

2.2. Preparation of the Plant Extracts

The leaves, stem bark and roots of *C. abbreviata* were thoroughly cleaned, chopped into small pieces, and kept under the shade at room temperature for 14 days until fully dry. The dried plant parts were then powdered separately using a mortar and pestle and stored separately in airtight containers for the phytochemical investigation. 100 g of each coarse powder was extracted with 500 mL of either 96% ethanol (EtOH) or distilled water by intermittent shaking for 72 hours. The six mixtures were then separately filtered using Whatman® number one filter paper to obtain the filtrate. The filtrates were then reduced to a concentrated semisolid mass by drying in the water bath at 40˚C and packed into separate airtight containers.

2.3. Phytochemical Analysis

Test for Carbohydrate

A small quantity of each extract was first dissolved separately in 5 ml distilled water and filtered. The filtrates were then subjected to the test below.

Benedict's test: Extract filtrates were treated with equal volumes of Benedict's reagent (Sodium citrate, sodium bicarbonate and copper sulfate solution) in test tubes separately. The mixtures were then boiled for 5 - 10 minutes in a water bath. Solution appearance of a brick-red precipitate was used as an indication of the presence of reducing sugar(s) in each filtrate. The intensity of the colour obtained was used to indicate whether the filtrate contained a high, medium, or low concentration of the reducing sugar(s). The results of this phytochemical screening were recorded in **Table 3** and **Table 4**.

Test for Glycosides

Borntrager's test: The three separate extracts were boiled with 1 ml of dilute

Sulphuric acid in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with equal volumes of dichloromethane. The lower levels of dichloromethane were separated and shaken with half its volume with dilute ammonia. A rose-pink to red color was used as an indication of the presence of Anthraquinone glycosides. The intensity of the rose colour was used as a measure to indicate whether the filtrates contained a high, medium, or low concentration of Anthraquinone glycoside(s). The results of this phytochemical screening were recorded in Table 3 and Table 4.

Tests for Amino Acids and Proteins

Biuret's test: The three separate extracts were treated with 1 ml 10% Sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet colour would indicate the presence of proteins. To indicate whether the extracts contained a high, medium, or low concentration, the colour intensity of the resultant solution was used. The results of this phytochemical screening were recorded in Table 3 and Table 4.

Tests for Terpenoids, Alkaloids, Saponins, Tannins, Phenols and Flavonoids

Tests for terpenoids, alkaloids, saponins, tannins, phenols and flavonoids were all done according to protocols adapted from Trease and Evans [15].

3. Results

As shown in Table 1, the percentage extraction value of *C. abbreviata* extracts after aqueous extractions of the leaves, stem bark and root were 5.15%, 7.51% and 6.90% respectively.

As shown in Table 2, the percentage extraction value of *C. abbreviata* extracts after ethanol extractions of the leaves, stem bark and root were 6.65%, 10.92% and 10.18% respectively.

Table 1. Percentage extraction yield of aqueous *C. abbreviata* extracts.

Plant Part	Weight of Powder (g)	Weight of Extract (g)	Percentage Yield (%)
Leaves	100.12	5.16	5.15
Stem bark	100.15	7.52	7.51
Root	100.10	6.91	6.90

Table 2. Percentage extraction yield of ethanol *C. abbreviata* extracts.

Plant Part	Weight of powder (g)	Weight of extract (g)	Percentage Yield (%)
Leaves	100.09	6.66	6.65
Stem bark	100.07	10.93	10.92
Root	100.14	10.19	10.18

The results of the chemical screening of the two (2) extracts from the leaves, stem bark, and roots are presented in Table 3 and Table 4. According to these results, tannins, reducing sugars, and sterols were in high concentrations in ethanolic extracts of stem bark and roots. Flavonoids, phenolics and proteins were in high concentrations of ethanolic extracts from stem bark, while anthraquinone and alkaloids were in high concentrations of ethanolic extracts from leaves and roots, respectively. Phytochemical analysis of *C. abbreviata* aqueous

Table 3. Phytochemical analysis of *C. abbreviata* ethanolic extracts.

	PLANT PART		
	LEAF	STEM BARK	ROOT
Tannins	–	+++	+++
Reducing sugars	++	+++	+++
Flavonoids	+	+++	++
Anthraquinone glycosides	+++	++	++
Alkaloids	+	++	+++
Terpenes	+	++	++
Sterols	++	+++	+++
Saponins	++	+	+
Phenolics	+	+++	++
Proteins	++	+++	–

KEY; +++ High concentration, ++ Moderate concentration, + Low concentration, (–) Absent.

Table 4. Phytochemical analysis of *C. abbreviata* aqueous e.xtracts.

	PLANT PART		
	LEAF	STEM BARK	ROOT
Tannins	+	+++	++
Reducing sugars	+++	++	+
Flavonoids	+++	++	++
Anthraquinone glycosides	–	+	+
Alkaloids	+	+	+
Terpenes	–	–	–
Sterols	–	–	–
Saponins	+++	++	++
Phenolics	++	++	+++
Proteins	+	+	+

KEY; +++ High concentration, ++ Moderate concentration, + Low concentration, (–) Absent.

extracts showed that reducing sugars, flavonoids, and saponins were in high concentrations in the leaves, while tannins and phenolic compounds were in high concentrations in the stem bark and roots, respectively. Terpenes and sterols were absent in all aqueous extracts of leaves, stem bark, and roots.

4. Discussion

This study investigated the phytochemical content of *C. abbreviata* grown and used among the Zambian population. Medicinal plants contain some chemical constituents that provide significant physiological action on the human body, and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids [16] [17]. Phytochemical analysis showed that *C. abbreviata* has a vast phytochemical constituent list. However, a better concentration was achieved with ethanol than with water for most of the phytochemicals. This observation may be attributed to the differences in polarity between ethanol and water. Another factor that could have influenced the results is that phenols are degraded by phenol oxidase in water extracts but not in ethanol, so phenols may be present in higher concentrations in the ethanol extracts [12].

Lim *et al.* 2012 stated that alkaloids have antimicrobial, antidiarrhoeal, and anthelminthic activity and have been extensively used as centrally acting stimulants, anaesthetics, and analgesics [18] [19]. Our study revealed that the ethanolic extracts of *C. abbreviata* contained more alkaloids than the aqueous extracts. Ethanolic root extracts contained the highest concentration of alkaloids, followed by the stem bark, while the rest of the extracts contained a lower concentration of alkaloids. This may be because alkaloids are more soluble in alcohol and sparingly soluble in water. Similarly, a study conducted in Burkina Faso by Traore *et al.* (2017) found that alkaloids were absent in the aqueous extracts of both the root bark and leaves [20]. A study in Kenya also reported the absence of alkaloids in the aqueous root bark [21]. However, a study conducted by Hikaambo *et al.* (2022) on the stem bark did not detect the presence of alkaloids in ethanolic extracts [22].

According to the lead acetate test for flavonoids, the aqueous leaf extract and the ethanolic extract of the stem bark contained a high concentration of flavonoids, while the aqueous extracts of the root and stem bark contained a medium concentration of flavonoids. The ethanolic leaf extract, on the other hand, was found to contain the lowest concentration of flavonoids. Similarly, studies conducted by Hikaambo *et al.*, (2022) and Njagi *et al.*, (2016) also reported the presence of alkaloids in aqueous extracts of stem bark and root bark, respectively [21] [22]. Flavonoids can therefore be responsible for the anti-diarrhoeal properties that are seen with the stem bark and the root of *C. abbreviata*. The anti-diarrhoeal properties of flavonoids are reported to be elicited via inhibition of the release of autocoids and prostaglandins, thereby inhibiting motility and secretion [23]. Flavonoids exert their antimicrobial activity via complexing with cell walls as well as binding to adhesins [24]. Flavonoids have also been reported to be the ones responsible for the antiplasmodial activity of *C. abbreviata* [25].

Tannins were found to be in very high concentrations in the root and the stem bark of both the aqueous and ethanolic extracts of *C. abbreviata*. This is because tannins are soluble in both water and ethanol. The concentrations in the leaf extracts, on the other hand, were very low and absent in the ethanol and water extracts, respectively. In a study conducted in South Africa [26], the presence of tannins in the methanolic stem bark extract of *C. abbreviata* was also revealed. Tannins are reported to exert antimicrobial effects by complexing with proteins through both covalent and non-covalent interactions [27]. Therefore, tannins may be responsible for the antidiarrhoeal properties seen with the use of *C. abbreviata* root and stem bark in managing diseases such as dysentery, gonorrhoea and syphilis.

Sterols are reported to have antidiarrhoeal activity elicited by enhancing intestinal absorption of sodium and water [28]. In this study, ethanolic extracts of the leaves, roots, and stem bark contained high concentrations of sterols. However, none of the aqueous extracts contained sterols according to Salkowski's test. This indicates that sterols may not be responsible for the antidiarrhoeal activity seen with *C. abbreviata* in the traditional system of medicine.

A test for saponins showed that the aqueous extracts of *C. abbreviata* contained more saponins than the ethanolic extracts, with the leaves having the highest concentration. This is similar to the study conducted in Zambia, which revealed the presence of saponins in the aqueous extract of stem bark [22]. However, saponins were not detected in the ethanolic extract [22]. The presence of saponins is also linked to the antibacterial effects of *C. abbreviate* [29].

Phenols are natural colour pigments responsible for the colour of fruits and plants. They are hypothesised to have antimicrobial, antidiarrhoeal, and anthelminthic activities [14] [29] [30]. In the current study, the ferric chloride test for phenols showed that the aqueous root and ethanolic stem bark extracts of *C. abbreviata* contained the highest concentrations of phenols. Phenols may also be responsible for the antimicrobial activity of the stem bark and root extracts of *C. abbreviata*. Similarly, a study in South Africa revealed the presence of phenols in a *C. abbreviata* methanol extract [26]. Kumar and Goel have reported that phenols are responsible for antioxidants and other medicinal properties [31].

Polypeptides are known to have antiviral properties via blockade of viral fusion or adsorption and the formation of disulfide bridges [32]. The highest concentrations of proteins were observed in the ethanolic stem bark extracts, however, low concentrations were seen in all the aqueous extracts.

5. Conclusion

This study revealed that *Cassia abbreviata* grown in Zambia possesses phytochemicals that may be responsible for its medicinal properties. Both ethanolic and aqueous extracts indicated the presence of reducing sugars, flavonoids, saponins, phenols, and alkaloids in the leaves, bark, and roots of *C. abbreviata*.

There is a need to continue to explore plants that contain medicinal properties and understand the constituents responsible for their use as herbal or traditional medicines.

6. Recommendation

There is a need for more research into bioassay-guided isolation and purification of extracts to determine the exact phytoconstituents responsible for their various pharmacological activities.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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