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Phytochemical Analysis and Antioxidant Activity of Aqueous and Ethanolic Extracts from *Bombax costatum* Pellegr. and Vuillet Used in the Treatment of Hypertension in Niger

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ABSTRACT

Bombax costatum Pellegr. & Vuillet belonging to the Bombacaceae family, is a medicinal plant commonly used in Niger by traditional practitioners to treat cardiovascular diseases. The aim of the present study is to justify scientifically the use of *B. costatum* leaves in the management of hypertension in Niger. Aqueous and ethanolic extracts were prepared by decoction. Phytochemical screening was performed by tube reactions. Total phenolics were determined by UV-Vis spectrophotometry and the antioxidant capacity was assessed by molybdate ion reduction. Phytochemical screening of the decocts revealed the presence of flavones, saponosides, coumarins, polyphenols, catechic tannins, gallic tannins, sterols and triterpenes, alkaloids and combined anthrocenosides-C. Quantitative analysis showed that the ethanolic decoctate of the leaves had the highest levels of total polyphenols (178.51±7.2 mg EAG/g ES) and total tannins (20.86±0.98 g/L), compared with the amounts found in the aqueous extract (84.33±9.79 mg EAG/g ES and 1.84±0.57 g/L) respectively. Total flavonoid contents ranged from 62.55±6.48 mg EQ/g ES to 65.88±7.96 mg EQ/g ES for aqueous and ethanolic leaf extracts respectively. The antioxidant activity values obtained ranged from 0.07±0.01 to 0.09±0.005 mg EAA/g ES. These results provide a scientific basis that could justify the traditional use of this plant in the treatment of diseases involving oxidative stress such as hypertension in Niger.

Keywords: *Bombax costatum*, polyphenols, antioxidant, Hypertension, Niger.

I. INTRODUCTION

African populations, particularly those of Niger, are facing the emergence of metabolic diseases such as cardiovascular disease, diabetes and asthma. Their management is a public health concern. Arterial hypertension (AH) is a chronic metabolic disease with a high mortality rate, and a major public health problem worldwide. It's present in both developed and developing countries. The number of hypertensives doubled between 1990 and 2019, from 650 million to 1.3 billion, and is responsible for around 17 million of deaths per year in the world. AH is responsible for at least 45% of deaths from cardiovascular disease (WHO, 2023).

The prevalence of the disease in sub-Saharan Africa is high among adults 18 old and over, ranging from 16% to 40%. In some studies, the prevalence exceeds 60% in people 65 old and over (Houehanou *et al.*, 2018).

The national survey on risk factors of non-communicable diseases (STEPS Niger, 2021) reports that the prevalence of high blood pressure (HBP) at the threshold of 140/90 mmHg was 27.4%. It is insignificant by gender, but increases significantly with age up to 59 old. Various risk factors contribute to the acceleration of hypertension in sub-Saharan Africa. These factors are linked to genetics marked by a metabolic anomaly of salt regulation, the growing development of diabetes, obesity, smoking, sedentary lifestyle, increasing urbanization, eating habits, psychosocial stress, poverty, beliefs and taboos all contribute to the scale of the phenomenon (WHO, 2021).

Like endothelial dysfunction, overproduction of reactive oxygen species (ROS) can induce hypertension (Frey *et al.*, 2009). In physiological conditions, ROS are present within the cell and regulated by the balance between their production and elimination rates: this is known as maintaining the pro-oxidant/antioxidant balance, sometimes referred to as redox status. However, in certain pathophysiological conditions, this homeostasis can be disrupted when ROS are formed in excessive quantities, as an inevitable by-

product of many biochemical processes. This disturbance in redox status is referred to as oxidative stress, and results in tissue and cell damage (Halliwell, 1994).

Cells employ numerous antioxidant strategies and consume a great deal of energy to control their ROS levels. Antioxidants can be preventative, i.e. suppressing ROS formation, or radical scavengers, to suppress initial reactions and/or prevent chain propagation. Antioxidants can also repair ROS damage, as in the case of proteolytic and DNA repair enzymes (Rahman *et al.*, 2006). Once the disease of hypertension has been declared, treatment with modern drugs is extremely costly for both the individual and society. In recent years, there has been a resurgence of interest in herbal treatment, due to the decline in people's purchasing power, the high cost of conventional drugs and the fear of synthetic products (Perroti, 1999).

According to WHO (2002), 80% of the population uses traditional herbal medicine in primary health care. In Niger the uses of several plants as antihypertensives were reported in an ethnobotanical survey carried out by Jazy *et al.*, 2017. Among these plants, *B. costatum* was chosen for the present work. To our knowledge, *B. costatum* Pellegr. & Vuillet of the Bombacaceae family, is one of the medicinal plants of the Niger flora for which little scientific data on its chemical composition and antihypertensive activity are available.

It was therefore in the interest of contributing to the development of Niger's medicinal plants that we initiated this investigation. The overall aim of the present study is to scientifically justify the use of *B. costatum* leaves in the treatment of hypertension in Niger. This involves phytochemical characterization of the plant's leaf extracts, quantification of phenolic compounds (polyphenols, flavonoids and tannins) in aqueous and ethanolic decocts of *B. costatum* leaves, and assessment of the in vitro antioxidant capacity of *B. costatum* leaf extracts.

II. MATERIAL AND METHODS

2.1 Plant material

2.1.1 Plant material

B. costatum leaves were used as plant material. The samples were collected in October 2024 in a field located in Bouka-Gorou commune of Farrey, Department and Region of Dosso/Niger. The plant was identified at the Mounkaila Garba botany laboratory in the Biology Department of the Faculty of Science and Technology at Abdou Moumouni University. Samples of the plant were washed and left to dry in a ventilated room at room temperature. The dried samples were ground using a mill, and the powder obtained was stored at room temperature, in a dry place protected from humidity and light, until use for extraction.

2. 2 Methods

2.2.1. Extractions and yields

50 g of powdered *B. costatum* leaves were added to 500 mL of distilled water or ethanol. The mixture was heated under reflux for 1 hour. After cooling, the mixture was filtered into a beaker using a funnel and filter paper. A sand bath at around 45°C was used to evaporate the solvents. The dry extracts obtained were then stored at 18°C, away from direct sunlight and humidity. The yields were expressed as a percentage and calculated according to the following formula:

$$R = \frac{M_1}{M_2} \times 100$$

M_1 : Weight of extract obtained ;

M_2 : Weight of plant material before extraction;

R: yield.

2.2.2. Phytochemical screening

For phytochemical screening, the method described by Bruneton, 1999 ; Evans *et al.*, 2002 with some modifications was used. The aim of this technique is to determine the chemical groups present in the leaves. These are: tannins, flavonoids and polyphenols (Brunneton., 2016; Jian-Wei *et al.*, 2009). Polyphenols and tannins were identified by the $FeCl_3$ test and the Stiasny reagent;

flavonoids by the cyanidine reaction; saponosides by the foam test; triterpenes and steroids by the Liebermann-Burchard test and finally alkaloids by the Mayer and dragendorff tests.

2.3. Spectrophotometric assay

2.3.1. Quantification of total polyphenols

The total phenolic contents of the crude extracts of *B. costatum* were determined by the methods describe by Singleton *et al.*, 1999, using the Folin–Ciocalteu reagent. This quantification was done at the concentration of 1 mg/mL. To 0.5 mL of the extract, 2.5 mL of the reagent had been diluted 10 times, and the test tubes were then allowed to sit at room temperature for 5 minutes. Following the in-cubation time, 2 mL of a sodium carbonate solution (75 mg/mL) was added, and the mixture was then agitated. The etalon for the calibration curve was made up using gallic at diferent concentrations of 0, 20, 40, 60, 80, and 100 mg/L. The absorbance was obtained by a spectrophotometer at 760 nm against the blank. The total polyphenol content is determined using the linear regression equation yP = 0.009x + 0.0584 with a correlation coefficient R^2 = 0.9897. Values are expressed in milligram equivalents of gallic acid per gram dry extract (EAG/g ES).

2.3.2. Quantification of total tannins

The assay is based on the property of proanthocyanidins to transform, by cleavage of the interflavane bond in an acid medium and at 100° C. To determine total tannins, 3 mL hydrochloric acid (12N or 37%) was added to 2 mL (1 mg/mL) of extract in a hydrolysis tube. The tube was then sealed with a Teflon-lined stopper and placed in a water bath at 100°C for 30 min. At the same time, a control tube containing the same solution is left at room temperature. After cooling the hydrolyzed tube, the optical density was read at 550 nm Bate-Smith *et al.*, 1965. Total tannin content was calculated using the following formula:

$$\mathbf{F_t = 19{,}33(\mathit{Doh} - \mathit{Dot})}$$

F_t : Total tannin content in g/L;

Doh : Optical density of hydrolyzed tube;

Dot : Optical density of control tube.

2.3.3. Quantification of total flavonoids

The total content of flavonoids was determined by the method described by Arvouet-Grand *et al.*, (1994). The flavonoid content of extracts from the leaves of this plant was assessed using the $AlCl_3$ aluminum trichloride colorimetric method. Flavonoid content was determined from the linear regression equation of the following calibration curve: yf = 0.0034x + 0.02 with R2 = 0.9047. Values are expressed in milligram equivalents of quercetin per gram dry extract (EQ /g ES).

2.3.4. Determination of antioxidant power

The total antioxidant capacity (TAC) of plant extracts was assess using the phosphomolybdenum method according to the procedure described by Prieto *et al.*, 1999. Ascorbic acid was used as standard. The antioxidant capacity of each extract was calculated from the calibration curve expressed in milligrams of ascorbic acid equivalent per gram of dry matter (mg EAA/g ES).

3. Statistical analysis

Graph Pad Prism 8.4.3 was used for statistical analysis. T-test was performed for comparison between groups. Results are expressed as mean ± standard deviation.

III. RESULTS

3.1 Extraction yield from *B. costatum* leaves

Table 1 shows the yield results for the aqueous and ethanolic extractions of *B. costatum* leaves. The ethanolic leaf extract showed a higher yield (18.80%) than the aqueous leaf extract (12.50%).

Table 1: Aqueous and ethanolic extraction yields of *B. costatum* leaves

***B. costatum* leaf extracts**	**Yields (%)**
Aqueous leaf decoction	12,50 % b
Ethanolic leaf decoction	18,80 % a

Values are expressed as mean ± standard deviation, each performed in triplicate at 3 separate concentrations. Different letters (a > b) show significant differences (p value < 0.05) between groups.

3.2 Phytochemical screening

Table 2 reports the results of phytochemical screening of *B. costatum* leaf extracts. This result revealed the presence of several chemical groups in the leaves with high intensity: flavones, saponosides, coumarins, polyphenols, catechic tannins, gallic tannins, sterols and triterpenes. Combined-C alkaloids and anthrocenosides are only slightly present.

Table 2: Phytochemical screening of *B. costatum* leaf extracts

Chemical groups	**Leaves**
Flavones	+++
Saponosides	+++
Coumarins (UV 366)	+++
Polyphnols	+++
Gallic tannins	+++
Catechin tannins	+++
Sterols and triterpenes	+++
Anthracenosides C. (c-heterosides)	+
Alkaloids	+

Abundant (+++) ; Present (++) ; Trace (+) ; not detected (-)

3.3 Spectrophotometric assay results

3.3.1 Total polyphenols and antioxidant activity

Table 3 shows the results for total polyphenols, total tannins, total flavonoids and total antioxidant capacity of *B. costatum* leaf extracts. The ethanolic leaf decoctate shows the highest amounts of total polyphenols (178.51 ± 7.2 mg EAG/g ES) and total tannins (20.86 ± 0.98 g/L) compared with the amounts observed respectively in the aqueous leaf decoctate (84.33 ± 9.79 mg EAG/g ES and 1.84 ± 0.57 g/L). As for the quantity of total flavonoids, a non-significant difference was observed for these decocts at p value > 0.05).

Table 3: Polyphenol content, tannin content, flavonoid content and antioxidant power of aqueous and ethanolic *B. costatum* leaf decocts.

Content and Antioxidant activity of extracts	**Aqueous leaf decoction**	**Ethanolic leaf decoction**
Polyphenol content (mg GAE/g ES)	84,33 ± 9,79 b	178,51 ± 7,2 a
Tannin content (g/L)	1,84 ± 0,57 b	20,86 ± 0,98 a
Flavonoid content (mg EQ/g ES)	62,55 ± 6,48 a	65,88±7,96 a
Antioxidant power (mg EAA/g ES)	0,07 ± 0,01 b	0,09 ± 0,01 a

Values are expressed as mean ± standard deviation, each performed in triplicate. Different letters (a > b) show significant differences (p value < 0.05) between groups. Total polyphenol content is expressed as milligram equivalent of gallic acid per gram of dry extract (mg EAG/g ES). Total tannin content is expressed in grams of dry extract per liter (g/L). Flavonoid content is expressed as milligram quercetin equivalent per gram dry extract (mg EQ/g ES). Total antioxidant capacity is expressed as milligram ascorbic acid equivalent per gram dry extract (mg EAA/g ES).

IV. DISCUSSION

Aqueous and ethanolic crude extracts of *B. costatum* leaves were obtained by decoction. According to the results shown in Table 1, a significant difference in yield ($p < 0.05$) was observed between the plant extracts. The best yield was obtained with the ethanolic extract of *B. costatum* leaves. The difference in yield could be explained by the high content of polar compounds in the ethanolic decoctate.

Analysis of the phytochemical screening results in this study revealed the presence of secondary metabolites such as flavones, saponosides, polyphenols, gall tannins, catechic tannins, sterols and triterpenes, alkaloids and anthracenoside-Cs. Previous work has confirmed these results. The work of Tilaoui *et al.*, 2021, which focused on phytochemical screening of *B. costatum*, reveals the presence of alkaloids, terpenes, sterols, flavonoids, tannins and saponins in the various extracts with varying intensities. Mohammed *et al.*, 2018 reported the presence of alkaloids, flavonoids, glycosides, saponosides, triterpenes and tannins. Several factors could explain the differences in chemical compounds observed. Indeed, environmental conditions, soil type, climate and age of the plants when the organs were harvested, as well as extraction solvents and genetic factors, could influence the presence of secondary plant metabolites (Naczk and Shahidi, 2006 ; Mrabti *et al.*, 2018).

Tannins and flavonoids have inhibitory activities on angiotensin-converting enzyme. Angiotensin inhibition induces cardiac slowing and vasodilation, correcting hypertension and bringing blood pressure back to normal (Bruneton., 1999 and Jian-Wei *et al.*, 2009).

The results of the polyphenol assay show that there is a significant difference between the contents of the different extracts, which ranged from 136.10±3.58 mg EAG/g ES to 228.51±5.52 mg EAG/g ES respectively for the aqueous and ethanolic extracts of the bark of the plant, and from 84.39 ± 9.69 mg EAG/g ES to 178.51± 7.20 mg EAG/g ES respectively for the aqueous and ethanolic extracts of the leaves of the same plant. The ethanolic extract of *B. costatum* leaves contained higher polyphenol levels (178.51± 7.20 mg EAG/g ES) than the aqueous extract (84.39 ± 9.69 mg EAG/g ES). This variation may be linked to the difference of polarity of the solvents used. The solubility of polyphenols depends mainly on hydroxyl groups, molecule size and hydrocarbon chain length (Franco *et al.*, 2008). These polyphenols are capable of inhibiting the activity of certain enzymes, by forming complexes with proteins thanks mainly to their functional groups (Arts *et al.*, 2001). Polyphenols act as reducing agents and hydrogen donors, trapping free radicals (Valko *et al.*, 2001).

Determination of polyphenols in the hydro-methanolic extract of *B. costatum* leaves by Luís Catarino *et al.*, 2019 yielded a value of 36.0 ± 0.5 mg EAG/g. The study conducted by Gandji *et al.*, 2019 gave a value of 21.31 ± 1.36 mg EAG/g in the determination of polyphenols in the aqueous extract of *B. costatum* leaves. All these values are lower than those obtained in our study. This difference may be due to extraction conditions on the one hand, and harvesting periods on the other.

The results of the tannin assay show that there is a highly significant difference between the contents of the different extracts. Studies conducted by Adebowale *et al.*, 2015 on the determination of total tannin content reveal a content of 0.07 ± 0.00 mg/100g. This value obtained is lower than our values obtained on ethanolic leaf extracts.

The results show that there is a highly significant difference between the contents of the different extracts. The ethanolic extract of *B. costatum* leaves contains higher levels of flavonoids than the aqueous extract. This variation may be linked to the different polarity of the solvents used.

Reactive oxygen species (ROS) are normally present in cells and are regulated by a balance between their rates of production and elimination. ROS are formed in excessive quantities, as inevitable by-products of certain biochemical processes, and when antioxidant defenses are deficient (Halliwell, 1994). Oxidative stress is implicated in a variety of metabolic pathologies, including inflammatory diseases, cardiovascular disease and hypertension. In the peripheral nervous system, ROS interact with the mechanisms involved in the development of hypertension. Some of the drugs used to treat this condition use this mechanism of action, as in the case of allopurinol (Morgan *et al.*, 2018), with antioxidants such as vitamin C (Del Rio *et al.*, 2010) or Super Oxide Dismutase mimetics (Peng and Prabhakar 2004). Products of plant origin with antioxidant properties are of great importance in the treatment of these pathologies. The antioxidant activity of the various extracts was assessed using the (CAT) *in vitro* method. Antioxidant potency was 0.07±0.01 and 0.09±0.01 mg EAA/g ES for aqueous and ethanolic decoctates respectively. Statistical analysis showed a significant difference ($p < 0.05$) in antioxidant power. The ethanolic decoctate with the highest total polyphenol content also showed the best antioxidant power. The same extract showed the highest content of flavonoids and total tannins. Some authors have found a correlation between antioxidant activity and total polyphenol content (Bakasso *et al.*, 2013; Compaoré *et al.*, 2016; Mahamane *et al.*, 2020). The differences in the activities (CAT) of the extracts could be attributed to the contents of the important phenolic compounds in these extracts. The antioxidant capacity of the different extracts could also be attributed to the presence of phytochemical compounds such as phenolics and flavonoids. This class of metabolites is also involved in antioxidant activities, thanks to their hydroxyl groups. This plant has potential that can be exploited.

V. CONCLUSION

This study enabled us to identify the phytochemical groups present in *B. costatum* leaf extracts. These investigations revealed the presence in the leaves of alkaloids, flavones, saponosides, polyphenols, gallic tannins, catechic tannins, sterols and triterpenes. This plant contains high levels of phenolic compounds and antioxidant activity, which would justify its use in the treatment of cardiovascular disease and hypertension. Nevertheless, it would be important to continue work on this plant by fractionating its extracts in order to isolate and identify all the bioactive constituents responsible for its antihypertensive and antioxidant effects. Studies to assess the antihypertensive activity in vivo will confirm the use of this plant and provide a scientific basis for the formulation of a phytomedicine.

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