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Indigenous Knowledge and Phytochemical Screening of Antihypertensive Plants in Benin with Evaluation of Larval Toxicity

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ABSTRACT

Background and Objective: Due to the high cost of healthcare, several African countries are increasingly turning to medicinal plants as alternative therapies. In Africa, species such as *Persea americana*, *Heliotropium indicum*, *Parkia biglobosa*, *Mangifera indica*, and *Newbouldia laevis* are recognized for their antihypertensive properties; however, their phytochemical composition and toxicity profiles remain poorly documented. This study aimed to analyze the phytochemical composition and larval toxicity of five medicinal plants used for hypertension treatment in Benin. **Materials and Methods:** Plant samples were collected from the Baka experimental site (University of Parakou, Benin), dried, powdered, and extracted using aqueous maceration and decoction methods. Phytochemical screening was performed using standard qualitative tests, and larval cytotoxicity was evaluated using the Artemia salina assay. The LC_{50} values were determined by logarithmic regression analysis of dose-response data. **Results:** Phytochemical screening revealed the presence of several bioactive compounds, including tannins, flavonoids, anthocyanins, saponosides, and reducing compounds. The LC_{50} analysis indicated no significant toxicity at the tested concentrations, with values ranging from 0.42 to 1.18 mg/mL for Heliotropium indicum and Mangifera indica, respectively. Moreover, no toxic compounds, such as cardiotonic heterosides or cyanogenic derivatives, were detected. **Conclusion:** These findings suggest that the studied plant extracts have potential therapeutic value for hypertension management with a favorable safety profile. However, further quantitative phytochemical analysis and preclinical and clinical studies are required to confirm their efficacy and safety.

KEYWORDS

Arterial hypertension, medicinal plants, phytochemistry, larval toxicity, Benin

INTRODUCTION

Arterial Hypertension (AHT) is a widespread condition today, although it has long been perceived as a rare or even absent pathology in Africa. The prevalence of AHT in adults aged 25 and over in the global population was estimated at more than 27% in 2022[1,2]. Estimates of hypertensive people could

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reach 1.56 billion people in 2025[1,3], an increase to be largely observed in low- and middle-income countries[2]. A very common and disabling disease, AHT impacts both men and women because it represents one of the leading causes of mortality linked to cardiovascular diseases in the world[4,5]. Cardiovascular diseases are chronic disorders of the cardiac system involving complex interactions with other body systems[6]. They can be associated with the respiratory, endocrine, renal and nervous systems[4]. Specific risk factors for cardiovascular diseases may include age, gender, obesity, diabetes, excessive alcohol and tobacco use, heredity, blood pressure, cholesterol, stress and depression[1]. Without a shadow of a doubt, there are a variety of well-known synthetic drugs for the treatment of AHT. However, this medication becomes distressing due to the expensive treatment in the long term[7]. Besides the economic implications and side effects of synthetic drugs[5,8], the preference for herbal therapy may be justified by the low compliance of patients[9] who have difficulty taking the recommended doses of modern drugs daily due to side effects.

The use of medicinal plants is a common and traditional practice in the world and especially in Africa[10]. This fact is more verifiable in developing countries in West Africa where more than 80% of the population depends on traditional medicine[11]. In the Republic of Benin, traditional healers and plant traders provide health coverage for the population at both rural and urban levels. A generalized ethnobotanical survey[9] on medicinal plants that can prevent and treat HBP identified 5 *(Persea americana*, *Heliotropium indicum*, *Parkia biglobosa*, *Mangifera indica* and *Newbouldia laevis*) main plants according to the preference of local communities. Mainly administered orally, the leaves, fruits, seeds, roots, barks, etc., of these plants are known to contain phytochemical compounds[1,12] (flavonoids, tannins, alkaloids, reducing compounds) and possess antihypertensive[9], cardioprotective[13], anti-inflammatory[12], antioxidant, etc activities. However, few studies have addressed the increased risk of toxicity due to poor administration in low or high doses for the treatment of AHT on the 5 species of choice according to the preference of the indigenous Beninese populations.

This article focuses on highlighting the phytochemical potential and toxicity of these 5 plants of choice in the prevention and treatment of AHT in Benin through phytochemical screening and measurement of their larval toxicity.

MATERIALS AND METHODS

Study area and duration: For the preparation of aqueous extracts, samples of plant parts (Table 1) were collected from the Baka experimental site (9°18′47.1′′N, 2°42′07.7′′E) of the Faculty of Agronomy, University of Parakou, Republic of Benin, then dried at room temperature (33-35°C) in a dry, well-ventilated, dark place for one month. Each dried plant part was then transformed into a fine powder using an electric grinder, then preserved in a dry and hermetically sealed container to prevent contamination. This research was conducted between the period of October, 2024 and December, 2025.

Phytochemical characterization of medicinal plant species

Plant selection: The plant species included in this study were selected based on the results of an ethnobotanical survey[9] of local communities in the Republic of Benin. The relative frequencies of citation made it possible to retain *Persea americana* Mill., *Heliotropium indicum* L., *Parkia biglobosa* (Jacq.) R.Br. ex G.Don, *Mangifera indica* L. and *Newbouldia laevis* (P.Beauv.) Seem. ex Bureau. The plants collected for this study were authenticated on the basis of regional botanical flora and by comparison with reference specimens available at the Benin National Herbarium.

Phytochemical screening of extracts: Qualitative phytochemical screening of aqueous extracts from different species (Table 1) was carried out[14], based on coloring and/or precipitation reactions specific to the main groups of chemical compounds. Phytochemical screening and larval toxicity testing *(Artemia salina* larvae) were carried out in the Organic Chemistry and Pharmaceutical Chemistry Laboratory of the Faculty of Health Sciences in Cotonou, Benin.

Table 1 : List of medicinal plants, their families, and plant parts used in the study

N°	Name of plant extract	Family	Organ used
1	*Persea americana* Mill.	Lauraceae	Leaves
2	*Heliotropium indicum* L.	Boraginaceae	Whole plant
3	*Parkia biglobosa* (Jacq.) R.Br. ex G.Don	Fabaceae	Bark
4	*Mangifera indica* L.	Anacardiaceae	Leaves
5	*Newbouldia laevis* (P.Beauv.) Seem. ex Bureau	Bignoniaceae	Leaves

Table 2 : Correspondance between LC_{50} et toxicity

LC_{50}	Toxicity
$LC_{50} \geq 100$ µg/mL ou 0,1mg/mL	-
100 µg/mL$> LC_{50} \geq$ 50 µg/mL	+
50 µg/mL$> LC_{50} \geq$ 10 µg/mL	++
$LC_{50} <$ 10 µg/mL	+++

-: Non toxic, +: Weak, ++: Moderate abd +++: Strong

Preparation of crude extracts: For the preparation of aqueous extracts, 50 g of powder are dissolved in 500 mL of distilled water. The mixture is left to stir continuously for 72 hrs and the macerate obtained is filtered three times successively on hydrophilic cotton. Then, the filtrate obtained was placed in an oven at 40°C for 24 hrs to obtain the crude aqueous extract.

The decoction is made in the same proportions 1m/10v i.e. 50 g of powder in 500 mL of distilled water and the mixture is brought to a moderate boil on a hot plate for 30 min. Then, the decoction is filtered, evaporated and dried at 40°C.

The residues obtained are weighed to calculate the yield according to the following relationship:

$$\text{Yield (\%)} = \frac{\text{Mass of dry extract obtained (g)}}{\text{Initial mass of powder (g)}} \times 100$$

Evaluation of larval cytotoxicity of extracts from different plant species: The toxicity assessment of organ extracts was carried out using the shrimp larvae survival test (*Artemia salina*) in seawater in the presence of extract solutions. This is a bioassay method used to assess preliminary toxicity in the context of the search for active natural substances. This test is very useful for knowing the degree of toxicity of plant extracts and is widely used in the literature[15]. For this work a colony of 16 larvae was used in each solution.

Preparation of test solutions: The aqueous extracts of the plants were prepared at an initial concentration of 50 mg/mL. We then proceeded to ten[10] successive dilutions to half (1/2) of the stock solution with seawater. The concentrations expressed in mg/mL of the diluted solutions contained in test tubes numbered from 1 to 10 were, respectively 50/2; 50/4; 50/8; 50/16; 50/32; 50/64; 50/128; 50/256; 50/512 and 50/1024. The tests were repeated to ensure the fidelity of the results.
Determination de la LC_{50}.

The LC_{50} lethal half-concentration values for the various extracts tested were determined using the expression[15]:

$$LC_{50} = e^{\left[(8-\beta)/\alpha\right]}$$

With β the y-intercept and α the directrix of the equation (y = alnx+β) of the logarithmic regression curve plotted on the sensitivity graph. These values express the concentrations necessary for the mortality of 50% of the larval population introduced into the extract solutions tested.

Data analysis: Phytochemical screening data were entered into an Excel spreadsheet and analyzed in tabular and graphical form. The dose-response data obtained from the toxicity test were log-transformed to determine the lethal concentration 50 (LC_{50}) by linear regression[16]. To assess the level of toxicity from the LC_{50} valeurs, we used the correspondence scale (Table 2) established by16 and widely used in the literature[15,17].

RESULTS AND DISCUSSION

Phytochemical composition of 5 plant species for therapeutic use against Arterial hypertension: Phytochemical compounds identified in the plant species used to treat arterial hypertension are summarised in Table 3. Regardless of the species, cardiotonic heterosides and cyanogenic derivatives were absent from the extracts. These compounds are generally known to be acutely toxic to heart failure patients.

***Persea americana* Mill. (Lauraceae):** Avocado (*Persea americana*) leaf powder contains tannins, catechic tannins, gallic tannins, flavonoids, mucilages, reducing compounds, free anthracenics, coumarins, O-heterosides, and some traces of quinone derivatives.

***Heliotropium indicum* L. (Boraginaceae):** Phytochemical screening carried out on the powder of the whole plant of *Heliotropium indicum* highlighted the following bioactive compounds: Gallic tannins, saponosides, triterpenoids, reducing compounds and O-heterosides.

***Parkia biglobosa* (Jacq.) R.Br. ex G.Don (Fabaceae):** Phytochemical screening carried out on the powder of the bark of the trunk of *Parkia biglobosa* highlighted the following compounds : alkaloid, catechic tannins, gallic tannins, anthocyanins, leuco-anthocyanins, saponosides, reducing compounds and free anthracenics.

***Mangifera indica* L. (Anacardiaceae):** *Mangifera indica* leaf powder contains: alkaloids, gallic tannins, flavonoids, anthocyanins, leuco-anthocyanins, mucilages, saponosides, reducing compounds and coumarins.

Table 3 : Phytochemical screening of plant extracts showing detected metabolites and their relative presence.

Metabolite samples	Detection tests/Reagents	1	2	3	4	5
Alkaloids	Mayer's reagent	-	-	+	++	+
Tannins	1% ferric chloride	+	+	+	+	-
Catechin tannins	Stiasny's reagent	+	-	+	-	-
Gallic tannins	1% ferric chloride, saturated with sodium acetate	++	++	++	+	-
Flavonoids	Shinoda test (hydrochloric alcohol + Mg powder)	++	-	-	++	++
Anthocyanins	5% hydrochloric acid and 50% ammonia	-	-	+	++	++
Leucoanthocyanins	Shinoda reaction	-	-	++	+	-
Mucilages	Absolute alcohol test	+	-	-	+	+
Cyanogenic derivatives	Picric acid / HCN release	-	-	-	-	-
Saponosides	Foam index test	+	++	++	++	+
Triterpenoids	Liebermann–Burchard reagent	-	+	-	-	-
Steroids	Kedde reagent	-	-	-	-	-
Reducing compounds	Fehling's solution test	+	+	+	+	-
Cardiotonic heterosides	Kedde reagent	-	-	-	-	-
Coumarins	Ether and 25% ammonia	+	-	-	++	-
Free anthracenics	Chloroform and ammonia test	++	-	+	-	-
O-heterosides	Hydrochloric acid+chloroform+ammonia	++	+	-	-	-
C-heterosides	FeCl +chloroform+ammonia	-	-	-	-	+
Quinone derivatives	Born–Träger reagent	+	-	-	-	-
Proportion of presence (%)		57.89	31.58	47.37	52.63	31.58

++: Very positive reaction, +: Positive reaction and -: Negative reaction

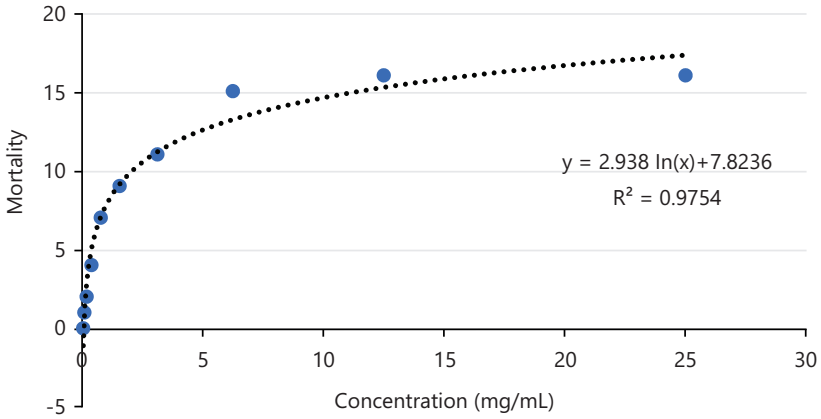

Fig. 1: Sensitivity curve for *Artemia salina* larvae to *Persea americana* extract

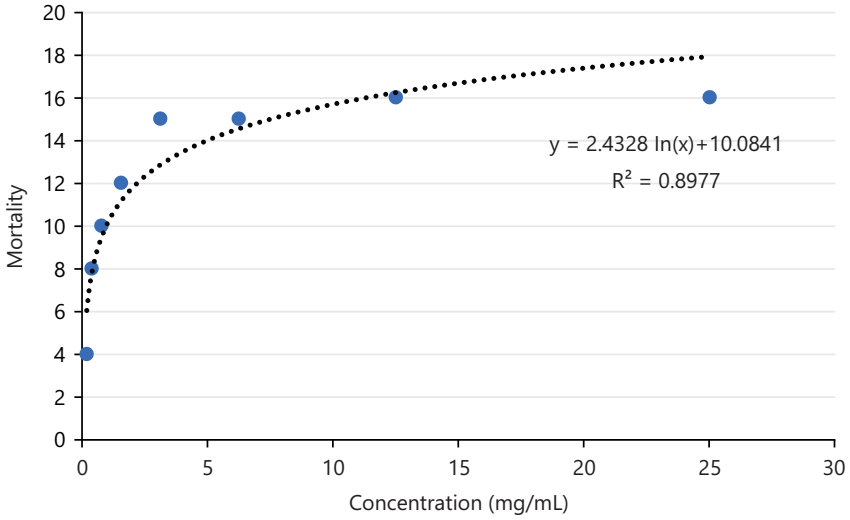

Fig. 2: Sensitivity curve for *Artemia salina* larvae to *Heliotropium indicum* extract

Table 4: Percentage yield of plant extracts

Extracts	*Persea americana*	*Heliotropium indicum*	*Parkia biglobosa*	*Mangifera indica*	*Newbouldia laevis*
Yield (%)	6.08	8.72	5.43	12.48	10.22

***Newbouldia laevis* (P.Beauv.) Seem. ex Bureau (Bignoniaceae):** *Newbouldia laevis* leaf powder contains: alkaloids, flavonoids, anthocyanins, mucilages, saponosides and C-heterosides.

Extraction yield: The mass percentage yield is calculated as the average of the two tests with the standard deviation. The values found are listed in Table 4.

The yield varies depending on the type of extract and the species studied. The highest yield is obtained with *Mangifera indica* (12.48%) against 5.43% minimum value for the plant *Parkia biglobosa*. Water being the main solvent used to carry out the extractions, we can say that all the species studied contain chemical principles which have an affinity with water, and therefore partly contain polar molecules.

Cytotoxic activity of extracts: Based on the number of dead larvae recorded at the end of the test, we drew curves showing the variation in larval sensitivity as a function of extract concentration. These curves are shown in the Fig. 1-5 below.

We note for all the graphs obtained that the correlation coefficient R^2 is greater than 0.8. There is therefore a good correlation between the concentrations applied and the responses obtained.

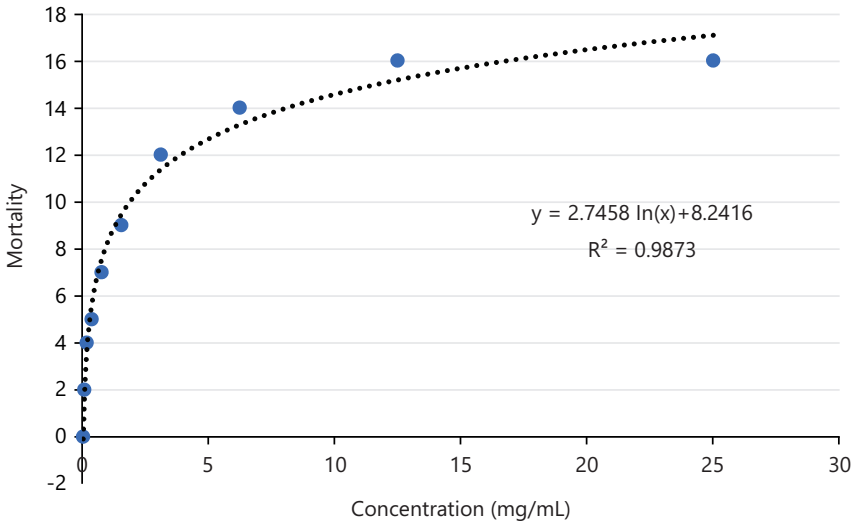

Fig. 3: Sensitivity curve for *Artemia salina* larvae to *Parkia biglobosa* extract

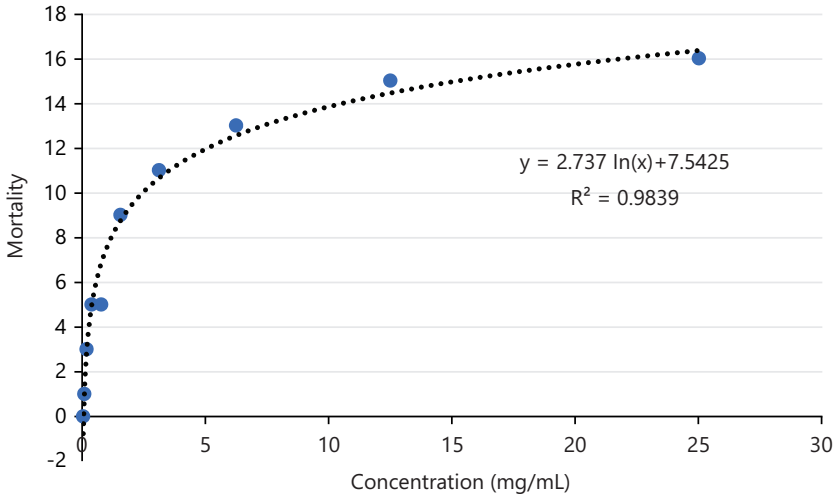

Fig. 4: Sensitivity curve for *Artemia salina* larvae to *Mangifera indica* extract

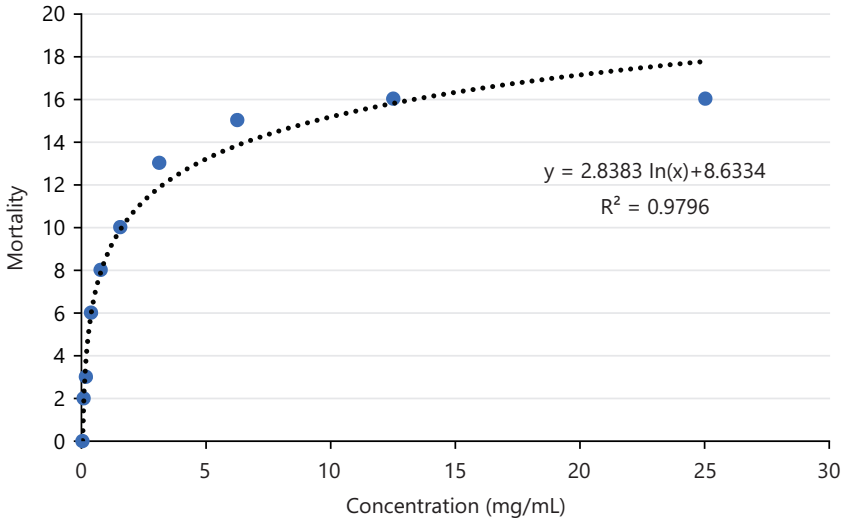

Fig. 5: Sensitivity curve for *Artemia salina* larvae to *Newbouldia laevis* extract

Furthermore, In addition, the larvae were sensitive to all the extracts tested. The number of dead larvae increases with the concentration, therefore the sensitivity of the larvae to the extracts follows a dose-response relationship. Different values found are summarized in Table 5.

Table 5 : LC_{50} values of the tested plant extracts

Extracts	α	β	LC_{50} (mg/mL)
Persea americana	2.938	7.8236	1.061879917
Heliotropium indicum	2.4328	10.081	0.425116103
Parkia biglobosa	2.7458	8.2416	0.915771016
Mangifera indica	2.737	7.5425	1.181936054
Newbouldia laevis	2.8338	8.6334	0.799701995

α and β represent the intercept and slope of the logarithmic regression equation ($y = \alpha \ln x + \beta$) used for LC_{50} determination. LC_{50} (mg/mL) indicates the lethal concentration required to cause 50% mortality of Artemia salina larvaeWe

Analysis of the LC_{50} values of the extracts tested, in relation to Table 5 of correspondence, allows us to say that the extracts tested do not present any toxicity in the range of concentrations analyzed, because LC_{50} values obtained are between 0.42 and 1.18 mg/mL respectively for the *Heliotropium indicum* and *Mangifera indica* extracts, which values are higher than the set limit (0.1 mg/mL).

The convergence between the absence of cytotoxicity and the screening results suggests that the safety of these extracts stems from the absence of hazardous chemical classes, such as cardiotonic glycosides and cyanogenic precursors, which are usually associated with undesirable biological responses[15].

The abundance of bioactive compounds in plant extracts is an important indicator for the potential for biological activity against arterial hypertension (AHT). Secondary metabolites responsible for antihypertensive activity have been highlighted by several studies[18,19]. The present study highlighted the different phytochemicals and evaluated the toxicity of plant extracts *Persea americana*, *Heliotropium indicum*, *Parkia biglobosa*, *Mangifera indica* and *Newbouldia laevis* in the prevention and treatment of AHT.

Avocado tree or *Persea americana* is an edible fruit tree used in traditional medicine and cosmetics[20]. Leaves of this species contains various phytochemical compounds namely: gallic tannins, flavonoids, mucilages, saponosides, reducing compounds, coumarins, free anthracenic and O-heterosides. *Heliotropium indicum* is a herbaceous plant containing mainly gallic tannins, saponosides, triterpenoids, reducing compounds, O-heterosides. Considered as a weed by several people, *Heliotropium indicum* is an erect or spreading annual herbaceous plant found in most plant formations[12]. The mango tree is a multi-purpose tree[21]. It is a fruit tree whose leaf powder contains: alkaloids, gallic tannins, flavonoids, anthocyanins, leucoanthocyanins, mucilages, saponosides, reducing compounds and coumarins. *Parkia biglobosa* is known for the multiple uses (medicine, fodder, fiber, food and drink, timber) of these organs by indigenous African populations Phytochemical screening of trunk bark powder highlighted the following compounds: Alkaloids, catechic tannins, gallic tannins, anthocyanins, leucoanthocyanins, saponosides, reducing compounds and free anthracenic compounds. *Newbouldia laevis* is a tree with therapeutic use in traditional medicine[22]. The powdered leaves of this species contains: alkaloids, flavonoids, anthocyanins, mucilages, saponosides, C-heterosides.

The majority of molecules identified were present in the identified species. Chemical compounds found in most plant extracts are gallic tannins, flavonoids, alkaloids, saponosides, reducing compounds and anthocyanins. Flavonoids are known for their ability to dilate blood vessels and inhibit adrenergic receptors[23], proteins that can regulate cardiovascular functions. Tannins in general are polyphenolic compounds that prevent and stop bleeding and fight infections[24]. The presence of tannins in medicinal plants has proved their effectiveness[25] antioxidant[20], anti-inflammatory and antihyperglycemic. The antioxidant activity of a plant has the ability to prevent the deposition of fat in arteries. The antioxidant activity of our plant extracts could be explained by the abundance of polyphenolic substances such as tannins, flavonoids, anthocyanins having the capacity to trap free radicals that play a role in the condition of AHT, especially atheroma leading to atherosclerosis[26]. Flavonoids have demonstrated their ability to prevent cardiovascular diseases thanks to the antioxidant activity of plant extracts. Tannins present in plant

xtracts have properties that promote circulation in small blood vessels, strengthening venous tone, increasing capillary resistance and stabilizing collagen. Tannins are also known for their analgesic and anti-inflammatory effects and their ability to reduce and maintain systolic and diastolic blood pressure. The presence of saponins in each extract demonstrates plants' capacity for antimicrobial and anti-inflammatory defense system[27].

Given the observed relationship between cytotoxic effects on shrimp larvae and on KB cells (human nasopharyngeal carcinoma) as well as A-549 lung carcinoma and HT-29 colon cancer cells, it may be inferred pending additional studies that the tested extracts do not exhibit significant cytotoxic activity[28]. Cases of cytotoxicity associated with phytotherapeutic substances used since ancient times and widely documented among populations are rarely reported in the clinical literature. The safety of the extracts from each plant is confirmed by the results of phytochemical screening, revealing the absence of cardiotonic heterosides, cyanogenic derivatives and quinonic derivatives, generally recognized as toxic compounds[23,29]. The correlation coefficient R^2 following each plant extract was greater than 0.8, indicating a good relationship between the applied concentrations and the observed responses. This shows that the sensitivity of the larvae to the tested extracts increases with the number of dead larvae as the LC_{50} concentration increases. The different values of half-lethal concentrations LC_{50}, represent the concentrations necessary to ensure the survival of 50% of the larval population exposed to each plant extract tested. The plant extract *Heliotropium indicum* showed a low LC_{50}, which was less toxic than the extract of *Mangifera indica* leaves, which recorded the highest LC_{50}value, proving the usefulness of respecting the dose for the consumer.

Although initial pharmacological studies provide encouraging evidence, confirmation through strictly controlled clinical protocols remains essential. However, the implementation of such research is hampered by significant financial constraints and difficulties specific to translational research, which currently limits the practical application of these findings in medical practice. In this context, it is essential to continue and deepen investigations in order to better understand the therapeutic and preventive potential of plant-based resources. A more in-depth analysis of these five plants would contribute to improving the management of high blood pressure through scientifically validated phytotherapeutic approaches.

CONCLUSION

Phytochemical screening of *Persea americana*, *Heliotropium indicum*, *Parkia biglobosa*, *Mangifera indica*, and *Newbouldia laevis* revealed the presence of diverse bioactive compounds, including tannins, flavonoids, anthocyanins, and saponosides. Larval toxicity assessment using *Artemia salina* showed LC_{50} values ranging from 0.42 mg/mL (*Heliotropium indicum*) to 1.18 mg/mL (*Mangifera indica*), indicating low toxicity. No cardiotoxic heterosides or cyanogenic compounds were detected. These results support the potential therapeutic use of these plants in traditional hypertension management and suggest a favorable safety profile, while further quantitative analyses and preclinical studies are needed to confirm their efficacy.

SIGNIFICANCE STATEMENT

This study provides scientific evidence supporting the traditional use of these five medicinal plants in hypertension management in Benin. By confirming the presence of bioactive compounds and LC_{50} values within safe ranges, it highlights their potential as safe, natural alternatives or complements to conventional antihypertensive therapies and lays the groundwork for future pharmacological studies.

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