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Phytochemical Screening by High-Performance Thin-Layer Chromatography, Antioxidant Activities and Acute Toxicity of Trunk Barks Extracts of *Lannea velutina* A. Rich

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Abstract

Lannea velutina is a traditional herbal remedy used to treat various diseases, including hypertension (HBP). Herbal medicines are affordable, and some have minimal side effects; they are rich in bioactive components that encourage prevention and treatment. This work uses appropriate experimental paradigms to investigate the phytochemical composition, antioxidant activity, and acute oral toxicity of *L. velutina* trunk bark extracted in water, methanol, ethyl acetate, dichloromethane, and hexane. According to a high-performance thin-layer chromatography profile, this shrub's bark contains sterols, saponosides, flavonoids, and tannins. Compared to ethyl acetate and aqueous extracts, the methanol extract had the highest total phenolic (607.06 ± 0.6 mg GAE/g DW), flavonoid (20.97 ± 0.23 mg QE/g DW), and condensed tannins (194.50 ± 0.75 CE/g DW) content. The methanol extract displayed the highest antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capability assay (IC_{50} = 8.59 g/mL; AAI = 4.66) compared to the other extracts. It exhibited antiradical action comparable to that of Trolox (IC_{50} = 15.16 g/mL), ascorbic acid (IC_{50} = 11.94 g/mL), and catechin (IC_{50} = 11.64 g/mL). The connection between flavonoid concentration and hydro-

philic antioxidant activity was robust (r = 0.997). Mice were used to evaluate the acute oral toxicity of ethyl acetate, methanol, and aqueous extracts using guidelines 425 of the Organization for Economic Cooperation and Development. All tested extracts have an estimated LD_{50} greater than 2000 mg/kg body weight. The truncal bark of *L. velutina* could be an alternative source for HTA management.

Keywords

Lannea velutina, Phytochemicals, Proanthocyanidins, Antioxidants, LD_{50}

1. Introduction

In many countries, plant therapy has been used traditionally for thousands of years. As the population ages, chronic diseases such as diabetes, hypertension, cancer, and metabolic disorders are rising [1]. Moreover, disease treatment processes focus more on patients' quality of life than symptom relief alone [2]. Today, cardiovascular disease (CVD) accounts for approximately one-third of all deaths worldwide [3], causing over 17.7 million deaths annually. Hypertension-related illnesses are associated with 9.4 million annual deaths [4]. This disease affects around 17.6% of the population in Burkina Faso [5]. Hypertension is a cardiovascular risk that increases the chance of cerebrovascular, coronary, cardiac, and renal accidents [6]. According to a WHO report from 2021, 27 percent of the population has hypertension, with a high prevalence in urban areas [7]. This pathology's predicted hospital mortality rate is 20.80% [8]. This rate remained above the average for African hospitals. Even though its exact causes are unknown, some authors [9] attribute it to oxidative stress.

Consequently, using anti-stress substances known as antioxidants effectively reduces hypertension [9]. Current synthetic antioxidants used in modern medicine are not highly soluble in physiological fluids, and they have several adverse effects and can be hazardous [10]. Modern medical treatments for hypertension are sometimes prohibitively expensive and not accessible to the African masses, whereas herbal medicines are affordable and accessible to low-income populations [11]. Herbal medicines are more productive than other forms of medicine in curing certain diseases. In addition, they tend to offer long-lasting benefits in terms of overall well-being [11].

Hence, the global market for herbal medicine, such as herbal pharmaceuticals and dietary supplements, is expanding. Even though the long-term use of herbal medication is considered safe and beneficial, national health authorities and the general public are concerned about their safety due to a lack of scientific evidence [12]. Several early medical studies indicate that *L. velutina* has been used to treat fever, abscesses, swollen wounds, and hypertension [13] [14]. Previous research has shown that the leaves, trunk, and root bark of *L. velutina* are antibacterial, larvicidal, radical-scavenging, and 15-lipoxygenase-inhibiting [14]

[15]. Although *L. velutina* trunk barks have a long history of widespread use and have demonstrated therapeutic benefits [14] [15], their phytochemistry and safety are poorly understood. To develop new pharmaceuticals and ensure the security of herbal drugs generated from this plant, phytochemical screening and safety testing of *L. velutina* trunk bark extracts are necessary.

This study aims to update the scientific data on the phytochemical profile of the trunk barks, their hydrophilic antioxidant capabilities, and their acute oral toxicity to provide information regarding their non-clinical safety.

2. Materials and Methods

2.1. Plant Material

Trunk barks of *L. velutina* constituted the plant material. The harvesting of the bark of this plant took place in July 2021 near Komkaga, 30 kilometers east of Ouagadougou in the central region. A botanist from the "*Centre National de Semences Forestières du Burkina Faso* (*CNSF*)" identified a plant specimen and deposited it under the reference number N˚ CNSF-1426. The plant material was air-dried at room temperature, and the obtained dry sample was ground into a powder using an electric grinder.

2.2. Animals

The Naval Medical Research Institute (NMRI) femalemice were obtained from the International Research and Development Centre on Livestock in Subhumid Zones, Bobo-Dioulasso, at 1.5 to 3 months of age (Burkina Faso). The mice were confined in plastic cages in a room with 65 percent regulated humidity, a temperature range of 20˚C to 23˚C, a 12:12 h light-dark cycle, and unrestricted access to rat food and water [16]. It was carried out by the procedure approved by Belemnaba [17].

2.3. Chemicals and Standards

All the solvents of analytical quality were obtained from Sigma-Aldrich (Taufkirchen, Germany). MOLSHEIM, France's Millipore apparatus, was used to clean water. Sigma Chemical Co. supplied ferric chloride, DPPH (2, 2-diphenyl-1-picrylhydrazyl), hydrochloric acid, and Folin-Ciocalteu reagent (St. Louis, MO). These standards were purchased from Sigma-Aldrich (St. Louis): gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), catechin, quercetin, and ascorbic acid. All buffer salts and other chemical goods possess an analytical grade.

2.4. Extraction

Two hundred milliliters of n-hexane were macerated at 4 degrees Celsius for twenty-four hours with 20 grams of trunk bark powder at a low temperature. The experiment was conducted repeatedly until the substance lost its color. After filtration with filter paper, the hexane extracts (HEB) were collected and con-

centrated using a rotary evaporator (BUCHI) at t ≤ 40˚C. The remainder of the trunk bark powder was successively extracted with dichloromethane, ethyl acetate, and methanol under the same conditions as before. The dichloromethane (DEB), ethyl acetate (AEB), and methanol (MEB) trunk bark extracts of *L. velutina* were dehydrated and stored in a fridge for future use.

The aqueous extract was produced by macerating 100 grams of trunk bark powder with 1000 milliliters of water at 4 degrees Celsius for 24 hours. The experiment was conducted repeatedly until the substance lost its color. Individual filtrates were collected, frozen, and dehydrated using a freeze-dryer.

A minimal proportion of methanol dissolved the dried extracts for compound screening, determination of phenolic component concentrations, and spectrophotometer evaluation of the hydrophilic and lipophilic antioxidant activity.

2.5. Screening Using High-Performance Thin-Layer Chromatography (HPTLC)

2.5.1. Chromatography

Phytochemical screening of *L. velutina* trunk bark extracts was carried out using the high-performance thin-layer chromatography (HPTLC) method [18], with minor modifications. For this work, 200 mm × 100 mm silica gel 60 F_{254} HPTLC plates were used (Merck, Darmstadt, Germany). On the HPTLC plates, sample solutions were applied using a Linomat 5 applicator (CAMAG, Muttenz, Switzerland) and a 100-microliter syringe. Briefly, volumes of 5 microlitres of samples were used as 8 mm strips. 3.4 millimeters separate each dot. The space between the first patch and the plate's left edge is 20 mm, and the distance between the last patch and the plate's right edge is also 20 mm. A constant rate of 100 nL/s was used for application. A mobile phase (10 mL) was employed for linear ascending development in a filter paper-lined CAMAG twin-trough glass chamber saturated with mobile phase vapor for 30 minutes. The development distance was seventy millimeters. The plates were dried with a hairdryer following development. The mobile phase in the chamber's twin troughs consisted of:

- n-hexane-ethyl acetate 20:4, v/v, for sterols and triterpenes;
- Ethyl acetate-petroleum ether 2:1, v/v, for saponosides.
- Ethyl acetate-formic acid-acetic acid-water, 100:11:11:26, v/v/v/v, for flavonoids;
- Ethyl acetate-methanol-water-chloroform 18:2.4:2.1:6, v/v/v/v, for tannins;

2.5.2. Derivatization and Documentation

Derivatization was done using the spraying equipment and the following substances:

- Sterols and triterpenes: Liebermann Burchard reagent was produced by combining acetic anhydride (5 mL), concentrated sulphuric acid (5 mL), and cold 95 percent ethanol (50 mL) in the order specified [18]. The plates were sprayed with the reagent three minutes after being dried with a hair dryer. The plates were heated for 3 to 5 minutes on the plate heater at 110˚C. The

examination was then conducted under UV light at 366 nm [19].

- Saponosides: anisaldehyde sulphuric acid reagent was prepared by mixing 0.5 mL of anisaldehyde with 10 mL of glacial acetic acid, then adding 85 mL of methanol and 5 mL of sulphuric acid. The plate was sprayed with the reagent and heatedfor approximately ten minutes at 100˚C. The reagent's stability period is exceptionally brief. Under white light, saponosides became visible. [19].
- Flavonoids: Developed plates were sprayed with a mixture containing Natural Products reagent (1 percent 2-aminoethyl diphenylborinate in methanol) and Macrogol reagent (5 percent polyethylene glycol 400 in ethanol). Five minutes were spent heating the plate to 110˚C before drying it in the fume hood. At UV 366 nm, flavonoids were found.
- Tannins: The plate was heated to 100 degrees Celsius for two minutes before spraying with ethanol containing 2% trichloride of iron III reagents. After derivatization, the plaque was dried for 5 minutes in a fume hood. Under white light, tannins become visible [19].

2.6. Contents of Total Phenolic, Flavonoid, and Condensed Tannins

The total phenolic content (TPC) of trunk bark extracts was measured using a colorimetric method with slight modifications to the Folin-Ciocalteu method [20]. Briefly, 1 mL of plant extract or solution of gallic acid was mixed with 1 mL of diluted Folin-Ciocalteu reagent. After eight minutes at room-temperature incubation, two milliliters of a saturated sodium carbonate solution (7.5% in water) were added to the mixture. After thirty minutes in the dark and at 37˚C, the absorbance of the resulting blue hue was measured using a SHIMADZU UV-Vis spectrophotometerat 760 nm. Using the calibration curve equation $y = 19.532x + 0.0236$, $R^2 = 0.9999$ (1), the phenolic content of plant extracts was calculated. The data is reported as the equivalent of milligrams of gallic acid (GAE) per gram of dry weight. Each measurement was conducted twice (n = 3). The aluminum trichloride method was used to evaluate trunk bark extracts' total flavonoid content (TFC), using quercetin as a reference [19]. A calibration curve is made from concentrations ranging from 0.001 to 0.5 mg/mL of a quercetin solution. Then, 1 ml of the sample solution was mixed with 1 ml of a 2% methanol solution of aluminum trichloride ($AlCl_3$). After 30 minutes of room-temperature incubation, the absorbance of the supernatant was measured at 415 nm using a spectrophotometer (SHIMADZU UV-1800, Japan). The TFC of the extract was obtained by relating the absorbance read to the standard curve equation $y = 20.022x + 0.0087$, $R^2 = 0.9992$ (2). The flavonoid concentration was represented as mg of equivalent quercetin per gram of dry substance. Each measurement was conducted twice (n = 3). The method described by Wendkouni *et al.* (2021) is slightly modified [19]. 0.5 mL of each appropriately diluted sample or standard to 3 mL of vanillin solution (4 percent w/v in methanol) and 1.5 mL of concentrated HCl. After vortexing, the mixture was incubated at 20˚C

for twenty minutes. A Shimadzu UV-Vis spectrophotometer was utilized to determine the absorbance of the ensuing red hue at 500 nm. The catechin (0 - 1 mg/mL) calibration curve is utilized to compute the condensed tannin concentration ($y = 2.7512x + 0.0095,\ R^2 = 0.9996$ (3)). The outcomes are expressed in milligrams of catechin equivalent per gram of dry weight (mg CE/g). Each measurement was conducted twice (n = 3).

2.7. Activities of Hydrophilic and Lipophilic Antioxidants by the 2.2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

The antioxidant activity of samples and standards was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging method [18] [20] [21]. In a dose-response curve, the technique depends on the capacity of plant extracts to absorb more DPPH radical (DPPH•) than Trolox. DPPH absorbs visible light at a maximum wavelength of 517 nm and disappears when an antioxidant reduces [18] [21]. In brief, 1 mL aliquots of samples or standards in varying amounts are added to 4 mL of a DPPH-methanol solution. The 0.10 mM DPPH• solution was created by dissolving 4 milligrams of DPPH• in 100 milliliters of methanol. For the blank sample, 1 milliliter of methanol was added to 4 milliliters of DPPH•. After 15 minutes of incubation at room temperature in the dark, the spectrophotometric absorbance at 517 nm was measured (SHIMADZU). The calculation for radical scavenging activity was as follows:
$\mathrm{I}\% = \left[\left(\mathrm{Abs}_{\mathrm{DPPH}} - \mathrm{Abs}_{\mathrm{Mixture}}\right)/\mathrm{Abs}_{\mathrm{DPPH}}\right] \times 100$ (4). The IC_{50} (concentration causing 50% inhibition) was determined graphically using a linear calibration curve by plotting the extract concentrations vs. the associated scavenging action. The antioxidant activity index (AAI) was computed in this manner:

$$\mathrm{AAI} = \frac{\text{DPPH final concentration}\left(\mu\mathrm{g/mL}\right)}{\mathrm{IC}_{50}\left(\mu\mathrm{g/mL}\right)} \tag{5}$$

To account for this variation in DPPH• concentration and sample size, we estimated AAI by dividing the quantity of DPPH• by the amount of the tested chemical in the reaction. In this study, the antioxidant activity of plant extracts was considered weak when the AAI is less than 0.5, moderate when the AAI is between 0.5 and 1.0, high when the AAI ranges from 1.0 to 2.0, and very strong when the AAI is more significant than 2.0 [21]. All samples, standard solutions, and DPPH• solutions were produced daily, and all experiments were conducted in triplicate.

2.8. Acute Toxicity Assessment of Ethyl Acetate, Methanol, and Aqueous Extracts

The acute oral toxicity test was conducted following OECD guidelines 425 [22] with a few minor modifications. Three groupings of animals of three mice each were established. Each animal has a unique identifying mark. After 16 hours of fasting, each mouse's weight was recorded, and a batch-specific dose of plant extract was delivered. After 72 hours of observation, each batch's mortality rate

was determined. Samples were administered by oral gavage using an esophageal tube. Solvents were supplied to mice serving as controls (0.2 percent TWEEN 80). At the beginning of the experiment, three batches of three mice were each administered a single dosage of 2000 mg/kg of plant extracts. The extracts were given to the animals at a maximum volume of 0.5 mL. After receiving the excerpts, the animals were observed for two hours before being fed. The subjects were then observed 24, 48, and 72 hours later. Animal's signs of drunkenness were seen. The lethal dose was determined by tallying the number of deceased mice in each batch (LD_{50}). Those mice that survived 72 hours of observation were observed for fourteen days. The test was conducted twice.

2.9. Statistical Analysis

GraphPad Prism version 6.0 was utilized to manage and analyze the data. The data was shown as the Mean ± Standard Error of Mean (SEM). Researchers compared utilization value averages using One-Way ANOVA (Analysis of Variance) and the Bonferroni test. The differences are statistically significant if the "p-value is less than 0.05."

3. Results and Discussion

3.1. High-Performance Thin-Layer Chromatography (HPTLC) Screening

On HPTLC (glass) and silica gel F_{254} (Merck) plates, several phytochemical groups of *L. velutina* trunk bark extracts were tested. Figure 1 illustrates the outcomes. While exposed to UV/366 nm and standard white light, the different colors of the spots on a chromatogram (orange, yellow, blue, green, pink, and violet) may correspond to various classes of secondary metabolites. The Natural Products reagent (for flavonoids), the trichloride of iron reagent (for tannins), the Liebermann Burchard reagent (for triterpenes and sterols), and the anisaldehyde sulphuric acid reagent (for saponosides) were used to clarify the nature of the compounds revealed at UV/366 nm.

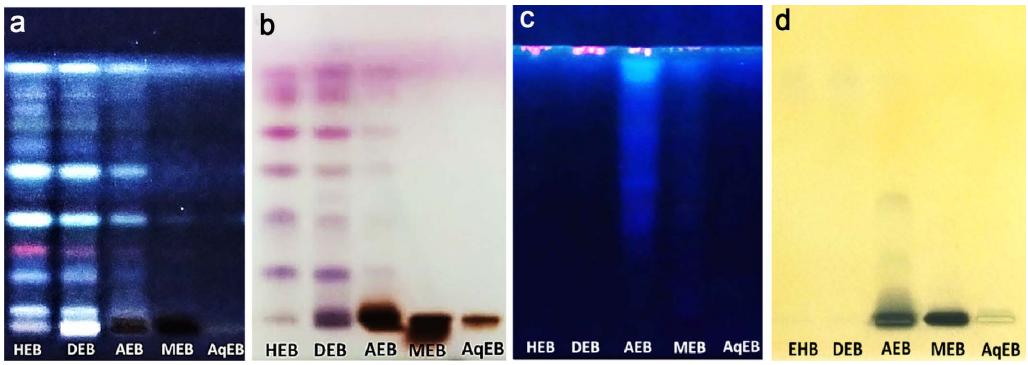

Figure 1. Chromatogram for detection of sterols and triterpenes (a), Saponosides, sterols, and triterpenes (b), flavonoids (c), and tannins (d). HEB: n-hexane extract of *L. velutina*trunk barks; DEB: DCM extract of *L. velutina*trunk barks; AEB: AcOEt extract of *L. velutina*trunk barks; MEB: MeOH extract of *L. velutina*trunk barks; AqEB: aqueous extract of *L. velutina*trunk barks.

3.1.1. Sterols and Saponosides Detection

After the spots' deposition, the plates were chemically treated with Liebermann-Bürchard reagent and heated to 110 degrees Celsius. The Liebermann-Burchard reagent reveals triterpenes and steroids in ultraviolet light as blue, green, pink, brown, and yellow (**Figure 1(a)**). The anisaldehyde-sulphuric acid reagent displayed sterols and triterpenes as blue and reddish-purple hues under visible light (**Figure 1(b)**). The same reagent showed yellow and green shades for saponosides in visible light [23]. The Liebermann-Bürchard reagent exposes sterols as yellow and yellow-green at UV/366 nm. This reagent defines triterpenes as oleanane and ursane types if the fluorescence is red and lupine types if the fluorescence is yellow-orange [24]. This information from the bibliography allowed us to attribute the acquired chromatograms (**Figure 1(a)** and **Figure 1(b)**). The Liebermann-Bürchard reagent revealed that only the hexane, dichloromethane, and ethyl acetate extracts exhibited oleanane and ursane triterpenes (Rf spot = 0.27) as well as lupine triterpenes (Rf spots = 0.01, 0.05, 0.38, 0.58, and 0.95) and sterols (Rf spots = 0.19, 0.40, 0.67, 0.79, and 0.86). The blue stains (Rf spots = 0.40, 0.58, and 0.95) observed in the methanol, and aqueous extracts could indicate the presence of sterols in these extracts. With the anisaldehyde reagent (**Figure 1(b)**), only the hexane, dichloromethane, and ethyl acetate extracts exhibited triterpenes and sterols as purple spots (Rf = 0.18, 0.37, 0.53, 0.67, 0.81, and 0.87).

3.1.2. Flavonoids and Tannins Detection

As illustrated in **Figure 1(c)**, high-performance thin-layer chromatography was used to produce the chromatographic profile of flavonoids in trunk bark extracts (HEB, DEB, AEB, MEB, and AqEB). Under 366 nm, the derivatized plate displayed blue, green, yellow, yellow-orange, greenish-yellow, and fluorescent dots in every plant sample. The chromatogram revealed that the number of flavonoids in the methanol extracts of the trunk barks varied among the different stains [18]. Flavonoids interact with several reagents (natural products, aluminum chloride) to create complexes with beautiful colors that emit a spectacular glow under UV (366 nm) or visible light. Blue regions (Rf spots = 0.40, 0.48, 0.81, 0.88, and 0.91) were discovered due to the presence of flavones, ethylated flavones, isoflavones, and flavanones (**Figure 1(c)**). The characterization of flavonoids in crude extracts found that these phenolic substances are abundant in trunk bark extracts, particularly in ethyl acetate (AEB) and methanol (MEB) extracts. The Neu reagent, which was rendered as blue and yellow spots, conclusively confirmed the existence of flavonoids in the analyzed trunk barks.

Similar to numerous other secondary metabolic products, tannins are luminescent. Hydrolyzable tannins and condensed tannins produce blue-black and brown-green luminescence, respectively. Several unique illuminators are used to confirm that these fluorescences are caused by tannins (for example, solid blue salt B and $FeCl_3$) [18]. Tannins with $FeCl_3$ form visible-range compounds with a rich coloration. Hydrolyzable tannins were detected in ethyl acetate, methanol,

and aqueous extracts (Rf spots = 0.02 and 0.05) using $FeCl_3$ (**Figure 1(d)**). This reagent also established the presence of condensed tannins in ethyl acetate and methanol trunk bark extracts of *L. velutina* (Rf spots = 0.19, 0.39, and 0.79). (**Figure 1(d)**).

In *L. velutina* trunk bark extracts, secondary metabolites such as sterols, triterpenes, flavonoids, and tannins were identified using HPTLC. Secondary metabolites are biologically active chemical substances, and their existence in the bark of the *L. velutina* trunk could explain their medicinal properties. Due to their potential pharmacological effects and commercial viability, the medicinal properties of plants have been investigated worldwide [25] in light of contemporary scientific breakthroughs. Numerous aromatic and medicinal plants include antioxidant-active chemical compounds (flavonoids, tannins, sterols, triterpenes) [20] [26]. Observations have shown that phenolic compounds inhibit amylase and glucosidase associated with lipid peroxidation, type 2 diabetes, and blood pressure regulation [27].

3.2. Total Phenolic, Flavonoid, and Condensed Tannin Contents

The concentrations of total phenolic (TPC), flavonoid (TFC), and condensed tannin (CTC) in *L. velutina* trunk bark extract evaluated in ethyl acetate (AEB), methanol (MEB), and water (AqEB) are in **Table 1**. Phenolic concentrations ranged from 195.36 ± 0.95 mg gallic acid equivalent/g dry weight for the aqueous extract (AqEB) to 607.06 ± 0.6 mg gallic acid equivalent/g dry weight for the methanol extract (MEB). The methanol extract of *L. velutina* trunk barks had the highest total phenolic content, followed by the ethyl acetate and aqueous extracts. The entire phenolic content of the three extracts increased in the following order: aqueous extract, ethyl acetate extract, and methanol extract. Among the known phenolic compounds with antioxidant characteristics are phenolic acids, flavonoids, and proanthocyanidins. The total phenolic content calculated by the Folin-Ciocalteu method does not accurately reflect the quality or quantity of phenolic components. The total flavonoid content (TFC) increased from 3.64 ± 0.02 mg of quercetin equivalent/g dry weight in the aqueous extract to 20.97 ± 0.23 mg in the methanol extract. The *L. velutina* trunk bark methanol extract contains the highest concentration of flavonoids. According to **Table 1**, the methanol extract has the highest total flavonoid content, followed by ethyl acetate and aqueous extracts. In addition to flavonoids and phenolic acids, proanthocyanidins (condensed tannins) are essential in preventing human

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), and condensed tannins content (CTC).

Extracts	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	CTC (mg CE/g DW)
MEB	607.06 ± 0.6	20.97 ± 0.23	194.50 ± 0.75
AEB	425.85 ± 12.5	16.17 ± 0.92	141.01 ± 0.61
AqEB	195.36 ± 0.95	3.64 ± 0.02	77.72 ± 0.19

disease and maintaining good health in the fight against cardiovascular accidents [28]. The condensed tannins or proanthocyanidins contents (CTC) of *L. velutina* trunk bark extracts were measured spectrophotometrically. CTC values ranged between 77.72 ± 0.19 and 194.50 ± 0.75 mg catechin equivalents/g of dry weight for the aqueous and methanol extracts (Table 1). According to Table 1, the *L. velutina* trunk bark methanol extract contained the highest concentration of proanthocyanidins, followed by the ethyl acetate and aqueous extracts, respectively. The relationship between total proanthocyanidins and phenolic concentrations was linear (r = 0.99, Table 3).

Significant differences in the results were associated with the varying polarity of the appropriate solvent. The polarity of phenolic compounds varies from polar to nonpolar [29]. Optimal extraction of these compounds is generally achieved in polar solvents at low temperatures, which have better solvation efficiency due to interactions (hydrogen bonds) between the opposing sites of the antioxidant compounds and the solvent than in nonpolar solvents [29]. Therefore, aqueous solutions and aqueous alcohol mixtures are frequently used to recover polyphenols. Due to its ability to extract and maintain the chemical structure stability of phenolic compounds from the trunk bark of *L. velutina*, methanol has yielded many antioxidant compounds.

3.3. Activities of Hydrophilic and Lipophilic Antioxidantsby the DPPH Assay

Table 2 presents the outcomes of antioxidant properties of extracts and standards. The IC_{50} must be determined in the linear region for each extract. A calibration curve was generated for each chemical tested on each analysis day, and an excellent linear part was identified (Table 2). According to a previous study, the DPPH absorbance in acetone and methanol decreased by 35% and 20% at 25 degrees Celsius [30]. Nonetheless, there was no discernible change after 150 minutes in the dark [30]. The AAI was computed using Equation (5), with the final

Table 2. Values for the antioxidant activity index (AAI) with the final concentration of DPPH•.

Extracts	I			II			III			IC_{50}	AAI	SD
	R^2	$^{A}IC_{50}$	AAI	R^2	IC_{50}	AAI	R^2	IC_{50}	AAI			
Gallic acid	0.9955	4.65	8.61	0.9967	4.63	8.64	0.9968	4.63	8.65	4.63	8.63	0.01
Catechin	0.9922	11.64	3.44	0.9936	11.60	3.45	0.9944	11.61	3.44	11,60	3.44	0.02
Ascorbic acid	0.9981	11.94	3.35	0.9987	11.93	3.35	0.9989	12.07	3.31	11.98	3.34	0.08
Trolox	0.9987	15.16	2.64	0.9992	15.21	2.63	0.9994	15.25	2.62	15.21	2.63	0.05
MEB	0.9948	8.58	4.65	0.9949	8.58	4.65	0.9949	8.60	4.65	8.59	4.66	0.01
AEB	0.9982	27.50	1.45	0.9982	27.12	1.48	0.9983	27.12	1.48	27.25	1.47	0.22
AqEB	0.9945	98.67	0.41	0.9966	98.55	0.41	0.9967	98.00	0.41	98.41	0.41	0.36
DEB	0.9922	1880.64	0.02	0.9939	1887.99	0.02	0.9945	1869.56	0.02	1879.40	0.02	9.27
HEB	-	-	-	-	-	-	-	-	-	-	-	-

DPPH concentration in the 0.10 mM solution being 40 µg/mL. Gallic acid had the highest AAI score among the employed standards, followed by catechin and ascorbic acid, which had comparable results, and Trolox.

The antioxidant properties of plant samples are correlated with their bioactive constituents, phenolics being the most significant [31]. Due to the increased complexity of flavonoid molecules, flavonoids' structure-activity correlations (SARs) are often more complex than those of phenolic acids. The degree of hydroxylation, the position of the hydroxyl groups, and the presence of a double bond with a hydroxyl group boosted the radical scavenging activity of flavonoids [32]. The hydrophilic and lipophilic antioxidant activities of the five examined *L. velutina* trunk bark extracts are displayed in **Table 2**. As demonstrated in **Table 2**, the hydrophilic antioxidant activities of the three types of trunk bark extracts (AEB, MEB, and AqEB) contributed considerably in every case. In contrast, the lipophilic antioxidant capacities (HEB and DEB) were far less significant.

The results reveal that the level of antioxidant activity differs depending on the type of extract. Hydrophilic antioxidant activity has significant linear associations with total phenolic (r = 0.97), flavonoid (r = 0.99) and proanthocyanidins (r = 0.96) contents (**Table 3**). The DPPH assay demonstrated that the methanol extract had the most potent antioxidant activity among the trunk bark extracts studied. The IC_{50} value and the DPPH index (I %) varied based on the final DPPH concentration for the same sample. However, the AAI value stayed constant [21]. Except for gallic acid (AAI = 8.6), the methanol extract (AAI = 4.66) exhibited much greater antioxidant activity than the standards. This extract also included significant amounts of phenolics, flavonoids, and proanthocyanidins (**Table 1**), the three most important natural hydrophilic antioxidants [33]. The inability of hexane trunk bark extracts to inhibit DPPH may be due to the absence of phenolics. Several epidemiological studies have shown that consuming antioxidants can significantly impact health [33]. Plant-derived substances with low adverse effects and toxicity have been used to prevent and treat no infectious diseases. Therefore, an acute toxicity test is performed on NMRI mice using antioxidant-rich extracts (MEB, AEB, and AqEB). The hypertensive individual is frequently stressed [9]. In response to stress, the body produces a surge of hormones into the bloodstream, including adrenaline and cortisol [34]. They increase blood pressure by quickening the heartbeat and constricting the blood vessels.

Table 3. Correlation coefficient (r) between phenolic, flavonoid, condensed tannins, content, and hydrophilic antioxidant activities.

	DPPH	TPC	TFC
TPC	0.97	-	-
TFC	0.997	0.98	-
CTC	0.96	0.9998	0.98

3.4. Acute Toxicity Assessment of Ethyl Acetate, Methanol, and Aqueous Extracts

3.4.1. Influence on the General Health of Mice

The test animals were observed for two and four hours, respectively. Over fourteen days, consistent observations were made. Within the first 30 minutes, the extract-treated groups demonstrated decreased feeding, drinking, and activity. Regular physical activity and eating are resumed after two to four hours. The hair color and stool consistency were both averages. Eye, ear, mouth, and nasal secretions were normal, and there was no evidence of poisoning or death. In the control group, hair color and activity were normal after 14 days. There were no abnormal discharges from the eyes, ears, mouth, or nose. Feeding and excrement were routine. All other parameters were healthy, including watches, coats, skin, drooling, and sleep throughout the trial [22]. **Table 4** outlines the specific observations.

3.4.2. Food and Water Intake and Body Weight

The results showed that the extracts had no significant effect on the mice's water and food consumption. Throughout the study, it grew marginally, and food consumption climbed slightly throughout the experiment. All treated groups ingested about the same amount of water and food as the control group (**Figure 2(a)** and **Figure 2(b)**, respectively).

Table 4. The influence of extracts on mice's behavior in acute toxicity studies.

Parameters	Observations of the control groups and the trunk barks extract-treated groups											
	2 hours		24 h		48 h		72 h		Seven days		14 days	
	T	Ext.	T	Ext.	T	Ext.	T	Ext.	T	Ext.	T	Ext.
Skin	N	N	N	N	N	N	N	N	N	N	N	N
Hair	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Breathing	N	N	N	N	N	N	N	N	N	N	N	N
Heart Pool	N	N	N	N	N	N	N	N	N	N	N	N
Behavior	N	N	N	N	N	N	N	N	N	N	N	N
Convulsion	A	A	A	A	A	A	A	A	A	A	A	A
Trembling	A	A	A	A	A	A	A	A	A	A	A	A
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhea	A	A	A	A	A	A	A	A	A	A	A	A
Lethargy	A	A	A	A	A	A	A	A	A	A	A	A
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Coma	A	A	A	A	A	A	A	A	A	A	A	A

T: The control group got 0.2% tween 80; Ext.: The extract groups received 2000 mg/kg of extract suspension; A: Absent; N: Normal.

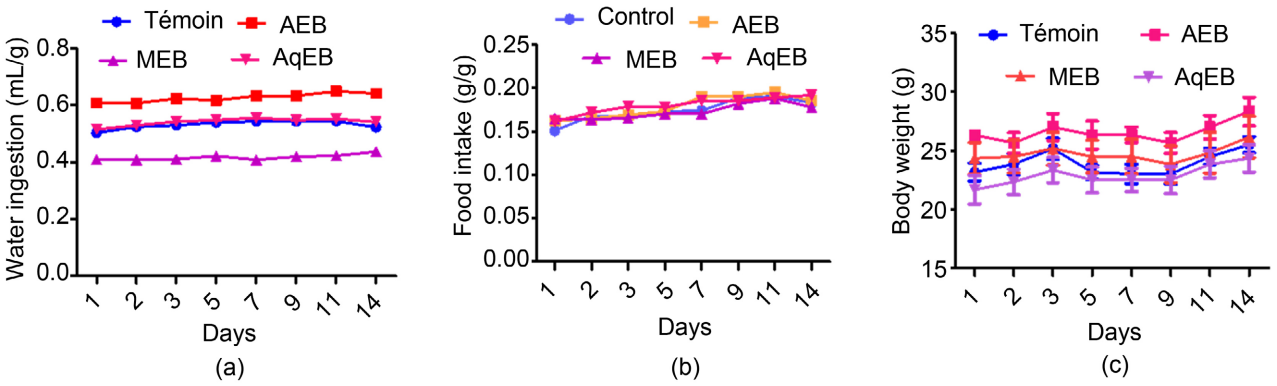

Figure 2. The effect of the extracts on the mice's water (a), food intake (b), and body weight (c). The control group got 0.2% tween 80, whereas the other groups (AEB, MEB, and AqEB) received a suspension containing 2000 mg/kg of the extracts; values are shown as Mean ± SEM, n = 6.

Figure 2(c) displays the body weights of both untreated and treated mice. After treatment with various extracts of the trunk bark of *L. velutina*, there was no significant difference in body weight between the treated and control groups ($P > 0.05$).

3.4.3. Mouse Autopsy and Organ Index

The organs (heart, liver, spleen, lung, kidney, thymus, stomach, and intestines) displayed no abnormal changes in the naked eye following dissection and examination of the organs from mice (Figure 3(a)). The organ index was estimated by weighing the major organs, as shown in Figure 3(b). (liver, heart, lungs, spleen, and kidney). Compared to the control group, the organ index of mice in the AEB, MEB, and AqEB groups did not change significantly (Figure 3(b); $P > 0.05$).

3.4.4. Lethal Dose 50 (LD_{50})

L. velutina trunk bark ethyl acetate, methanol, and aqueous extracts revealed no mortality up to 2000 mg/kg of body weight (Table 5). The LD_{50} values, therefore, exceed 2000 mg/kg of body weight. Considering the OECD toxicity guidelines 425 [22], oral administration of macerates of *L. velutina* trunk bark extracts is safe for NMRI mice. The low toxicity of this species could justify its use for medicinal purposes.

4. Conclusion

This study reveals that *L. velutina* trunk bark extracts contain recognized antioxidant micro-constituents. High-performance thin-layer chromatography profiles detected sterols, terpenes, tannins, and flavonoids. The quantitative analysis demonstrates that methanol extracts have the highest levels of total phenolic compounds, total flavonoids, and condensed tannins. According to the DPPH method, trunk bark extracts' antioxidant properties vary according to the solvent used for extraction. The trunk bark methanol extract of *L. velutina* has the highest antioxidant activity with an IC_{50} of 8.59 µg/mL. A significant correlation

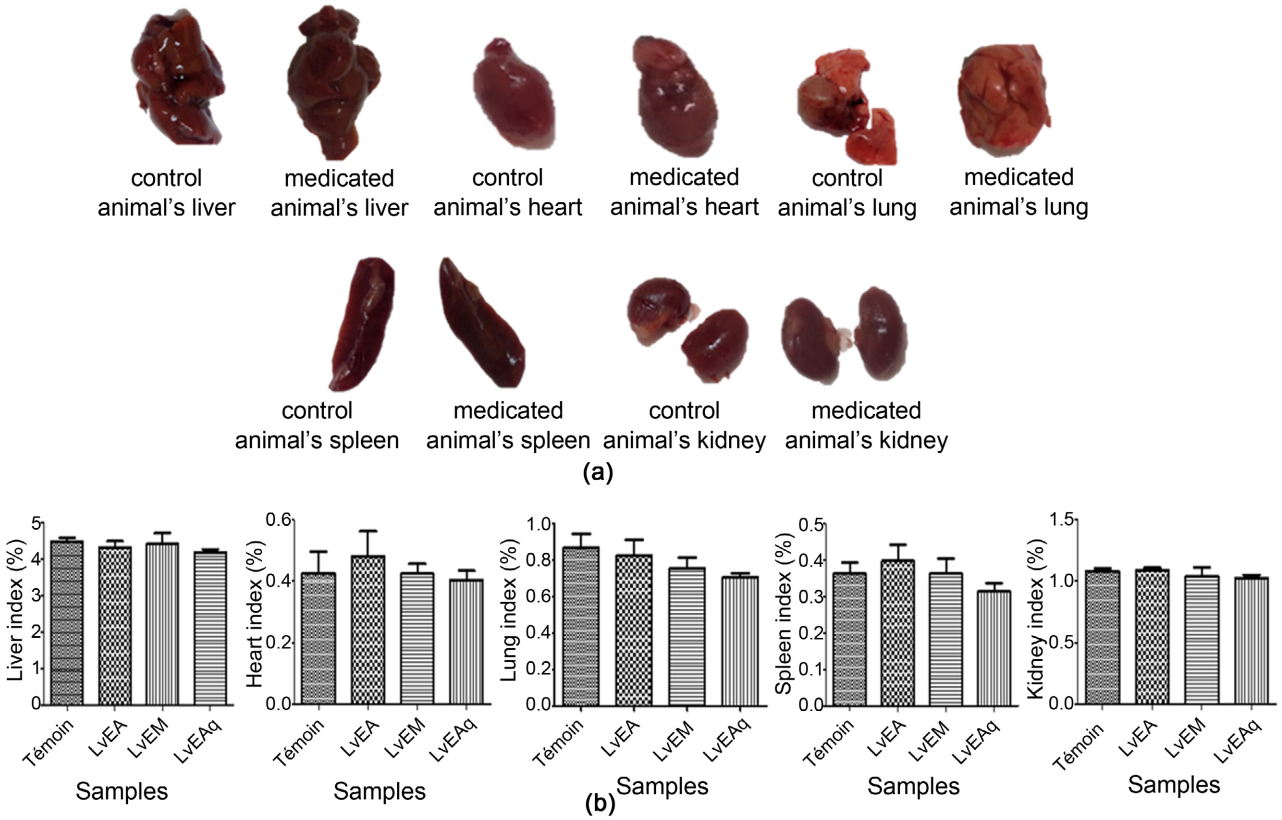

Figure 3. Images of organs as seen by the visible light after the dissection of mice (a) and the effect of extracts on mouse organ indexes (b). The control group received 0.2% tween 80, while AEB, MEB, and AqEB groups received 2000 mg/kg of extracts.

Table 5. Acute toxicity of the extracts.

Extracts	Doses (mg/kg)	Number of mice	Average weight (g)	number of deaths	% of mortality
AEB	2000	06	24.1 ± 2.0	00	00
MEB	2000	06	22.2 ± 3.1	00	00
AqEB	2000	06	22.3 ± 2.5	00	00

exists between flavonoid content and antioxidant activity. The total flavonoids are responsible for over 99 percent of the radical-scavenging effect. NMRI mice given 2000 mg/kg of ethyl acetate, methanol, and water extracts showed no weight loss, mortality, or gross lesions 14 days after treatment. Each sample's estimated LD_{50} is more significant than 2000 mg/kg in mice. Regarding acute oral toxicity, up to 2000 mg/kg of these extracts are safe for NMRI mice. Testing the antihypertensive effect of these extracts on mice will allow us to distinguish the majority of the less toxic and bioactive compounds.

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Conflicts of Interest

The authors have announced no potential conflicts of interest.

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