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Original Article

High-Performance Thin-Layer Chromatography Phytochemical Profiling, Antioxidant Activities, and Acute Toxicity of Leaves Extracts of *Lannea velutina* A. Rich

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ABSTRACT

Leaves of *Lannea velutina* are used to treat several human pathologies, such as high blood pressure (HBP) and oxidative stress. Rich in bioactive components that promote prevention and therapy, herbal medicines are affordable and have no adverse effects. This work aims to evaluate the phytochemical profile, antioxidant activity, and acute toxicity of water, methanol, ethyl acetate, dichloromethane, and hexane leaf extracts of *L. velutina* by using suitable experimental paradigms. This shrub's leaves include sterols, saponosides, flavonoids, and tannins, as revealed by a high-performance thin-layer chromatography (HPTLC) profile. The methanol extract exhibited a significant highest total phenolic (533.9 ± 5.05 mg GAE/g DW), flavonoid (59.4 ± 0.6 mg QE/g DW), and condensed tannins (86.9 ± 0.05 CE/g DW) content compared with ethyl acetate and aqueous extracts. By using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capability experiment, the methanol extract demonstrated the highest antioxidant activity (AAI = 2.60) among the other extracts. There was a strong association between flavonoid concentration and hydrophilic antioxidant activity (r = 0.96). The acute oral toxicity test of ethyl acetate, methanol, and aqueous extracts on mice was evaluated by using Economic Cooperation and Development (OECD) guidelines 423. All investigated extracts exhibit a lethal dose (LD_{50}) estimated higher than 2000 mg/kg body weight. This study constitutes a solid scientific basis that could justify the traditional uses of the leaves of *L. velutina* as a natural source of phenolic compounds for high blood pressure management.

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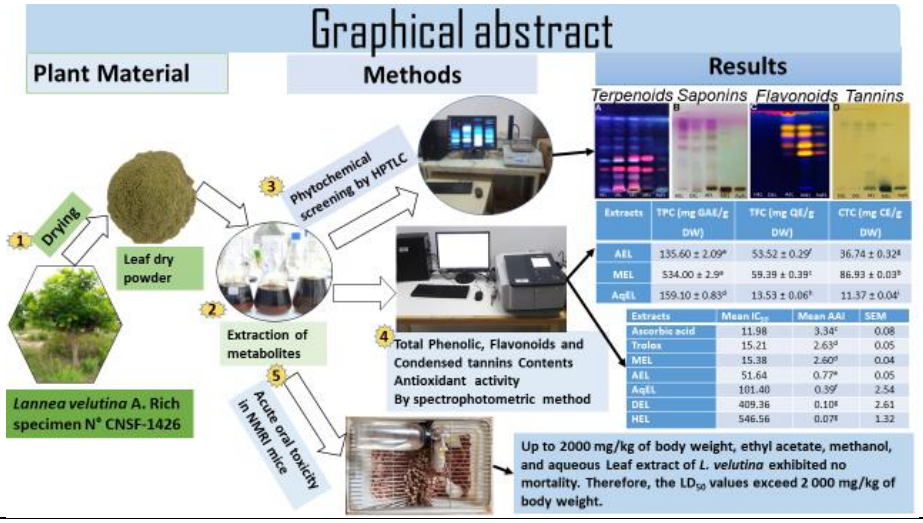
GRAPHICAL ABSTRACT



Introduction

Herbal medicine has been utilized traditionally in numerous nations for millennia. With the rising global population aging in recent years, the number of people with chronic diseases such as diabetes, hypertension, cancer, and metabolic diseases has increased [1]. In addition, disease treatment procedures have placed a greater emphasis on the quality of life of the patients than on symptom relief alone [2]. Nowadays, cardiovascular disease (CVD) causes over 18 million fatalities per year worldwide, accounting for nearly one-third of all deaths [3]. 9.4 million fatalities per year are linked to hypertension-related disorders [4]. This illness affects about 17.6% of the population in Burkina Faso [5]. Hypertension is a cardiovascular risk characterized by increased cerebrovascular, coronary, cardiac, and renal incidents [6]. A study by WHO (2021) found that 27% of the population has hypertension, with a significant prevalence in metropolitan regions [7]. The expected hospital mortality owing to this pathology is 20.80% [8]. This rate remained higher than the average for hospitals in Africa. Even though its specific causes are still unknown, some authors link it to the oxidative stress [9]. Therefore, the usage of anti-stress compounds known as antioxidants would be an efficient method for controlling hypertension. In physiological fluids, the synthetic antioxidants currently employed in modern medicine are not highly soluble [10]. They have numerous adverse consequences and can be dangerous [11]. Modern medicine's hypertension treatments are costly and frequently inaccessible to the African populace, hence the interest in traditional medicine.

As a result, the global market for herbal medicine, including herbal pharmaceuticals and dietary supplements is rising [12]. Even though long-term use of herbal medicines is thought to make them safe and effective, national health authorities and the general public are worried about their safety because there is not enough scientific proof. Several early medical investigations indicate that *L. velutina* has been used to treat fever, abscesses, swelling wounds, and the excessive blood pressure [13, 14]. Previous research has demonstrated that the leaves, trunk, and root bark of *L. velutina* possess antibacterial, larvicidal, radical-scavenging, and 15-lipoxygenase-inhibiting properties [15]. Although the leaves of *L. velutina* have a long history of popular use and proven therapeutic advantages [16], their phytochemistry and safety are not thoroughly understood. Therefore, phytochemical screening and safety testing of *L. velutina* leaf extracts are required for the development of new pharmaceutical pharmaceuticals and to ensure the safety of herbal medicines derived from this plant part.

The purpose of the present study is to update the scientific data on the phytochemical profile of the leaves, their hydrophilic antioxidant activities, and their acute oral toxicity to provide information on their non-clinical safety.

Materials and Methods

Plant material

The plant material was composed of *L. velutina* leaves. The leaves of this plant were harvested in July 2021 near Komkaga, 30 kilometers from Ouagadougou in the central area. A plant specimen was identified by a botanist from the *Centre National de Semences Forestières du Burkina Faso (CNSF)* and deposited with the reference number N° CNSF-1426. The plant matter was air-dried at ambient temperature. By using an electric grinder, a fine powder was produced from the obtained dry sample.

Animals

The Naval Medical Research Institute (NMRI) mice were acquired from the International Research and Development Centre on Livestock in Sub-Humid Zones, Bobo-Dioulasso, at the age of 1.5 to 2 months old (Burkina Faso). The animals were housed in plastic cages in a room with controlled humidity (65%), at temperature range of 20 to 23 °C, a 12:12 h light-dark cycle, and free access to rat food and water. It was conducted according to the protocol certified by Belemnaba [17].

Chemicals and standards

All the analytical-grade solvents were acquired from Sigma-Aldrich (Taufkirchen, Germany). A Millipore instrument (MOLSHEIM France) was used to purify water. Ferric chloride, DPPH (2, 2-diphenyl-1-picrylhydrazyl), hydrochloric acid, and Folin-Ciocalteu reagent were obtained from Sigma Chemical Co. (St. Louis, MO). The following standards were acquired 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 products were of analytical quality.

Extraction

Two hundred milliliters of n-hexane were subjected to a low-temperature maceration of 25 grams of leaf powder at 4 °C for 24 hours. The experiment was performed numerous times until the material lost its color. The hexane extracts (HEL) were collected after filtration with filter paper and concentrated at t < 40 degrees using a rotary evaporator (Bauchi). Under the same conditions as before, the residue of the leaf powder was extracted progressively with dichloromethane, ethyl acetate, and methanol. The dichloromethane (DEL), ethyl acetate (AEL), and methanol (MEL) extracts of *L. velutina* leaves were dehydrated and kept in a cold location for future use. The aqueous extract was obtained by macerating 100 grams of leaf powder at 4°C for 24 hours with 1000 milliliters of water. The experiment was performed numerous times until the material lost its color. By using a freeze drier, the individual filtrates were gathered, frozen, and dried. The dried extracts were dissolved in a minimal volume of methanol for compound screening, measurement of phenolic compound concentrations, and spectrophotometer evaluation of the hydrophilic and lipophilic antioxidant activity.

Screening by using high-performance thin-layer chromatography (HPTLC)

Chromatography

Phytochemical screening of *L. velutina* leaf extracts was conducted by using the high-performance thin-layer chromatography (HPTLC) technique [17], with slight modifications. 200 mm × 100 mm silica gel 60 F_{254} HPTLC plates were utilized for this study (Merck, Darmstadt, Germany). By using a Linomat 5 applicator (CAMAG, Muttenz, Switzerland) with a 100-microliter syringe, sample solutions were applied to the HPTLC plates. Briefly, quantities of 5 µL of samples were applied as 8 mm strips. The space between each dot is 3,4 mm. Both the distance between the first patch and the left edge of the plate and the distance between the last place and the right edge of the plate are 20 mm. A continuous rate of 100 nL/s was employed for application. In a filter paper-lined CAMAG twin-trough glass chamber previously saturated with mobile phase vapor for 30 minutes, 10 mL mobile phase was used for linear ascending development. The distance for development was 70 mm. By using a hairdryer, the plates were dried after

development. The mobile phase in the chamber's twin troughs was:

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, Sterols and triterpenes: n-hexane-ethyl acetate 20:4, v/v, for sterols and triterpenes, and Ethyl acetate-petroleum ether 2:1, v/v, for sapiens.

Derivatization and documentation

By spraying with the spraying apparatus and the following substances:

Flavonoids: Developed plates were sprayed with a mixture of Natural Products reagent (1 percent 2-Aminoethyl diphenylborinate in methanol) and Macrogol reagent (5 percent polyethylene glycol 400 in ethanol). The plate was heated to 110 °C for five minutes before being dried in the fume hood. Flavonoids were identified at UV 366.

Tannins: The plate was heated at 100 °C for two minutes, and then sprayed with ethanol containing 2 percent trichloride of iron III reagent. After derivatization, the plate was dried in a fume hood for 5 minutes. Under the white light, tannins were uncovered [18].

Sterols and triterpenes: Liebermann Burchard reagent was made by combining acetic anhydride (5 mL), concentrated sulphuric acid (5 mL), and chilled 95% ethanol (50 mL) in the aforementioned order [19]. Next, the plates were sprayed with the reagent after being dried with cold hair for three minutes. Then, the plates were heated at 110 °C for 3 to 5 minutes on the plate heater. Following that, the evaluation was conducted under 366 nm UV light [18].

Saponosides: Anisaldehyde sulphuric acid reagent was prepared by combining 0.5 mL anisaldehyde with 10 mL glacial acetic acid, followed by the addition of 85 mL of methanol and 5 mL of sulphuric acid. The plate was sprayed with the reagent and heated at 100 °C for five to ten minutes. The stability period of the reagent is extremely short. Under the white light, saponosides were uncovered.

Total phenolic, flavonoid, and condensed tannins contents

The total phenolic content (TPC) of leaf extracts was measured by using the Folin-Ciocalteu colorimetric technique [20]. Briefly, 1 mL of plant extract or gallic acid solution was mixed with 1 mL of Folin-Ciocalteu reagent diluted tenfold with distilled water. After 8 minutes of room temperature incubation, 2 mL of a sodium carbonate solution containing 7.5% saturate was added to the mixture. The combinations were placed at 37 °C for 30 minutes in the dark. At 760 nm, the absorbance of the resulting blue color was measured with a SHIMADZU UV-Vis spectrophotometer. The phenolic content of plant extracts was determined by using the equation for the calibration curve ($y = 19{,}532x + 0{,}0236$, $R^2 = 0{,}9993$ (1)). The outcomes are expressed in milligrams of gallic acid equivalents (GAE) per gram of dry weight. All measurements were conducted in triplicate (n = 3). The determination of total flavonoid content (TFC) in leaf extracts was conducted by using the $AlCl_3$ colorimetric method, as previously reported [18]. As the standard, quercetin was utilized, and the concentration was reported as the absorbance on the quercetin calibration curve ($y = 20{,}022x + 0{,}0087$, $R^2 = 0{,}9992$ (2)). The total flavonoid concentration was reported as mg equivalents of quercetin per gram of dry weight. All measurements were conducted in triplicate (n = 3). Minor modifications are made to the procedure described by Wendkouni *et al.* (2021) to determine the concentration of condensed tannins in the extracts [18]. 0.5 mL of each properly diluted sample or the standard is combined with 3 mL of vanillin solution (4% w/v in methanol) and 1.5 mL of concentrated HCl. After agitation, the mixture was incubated at 20°C for 20 minutes. By using a SHIMADZU UV-Vis spectrophotometer, the absorbance of the resultant red color was measured at 500 nm. The catechin (0-1 mg/mL) calibration curve is utilized to derive the condensed tannins concentration ($y = 2{,}7512x + 0{,}0095$, $R^2 = 0{,}9996$ (3)). The outcomes are reported in mg of catechin equivalent per gram of dry weight (mg CE/g). All measurements were conducted in triplicate (n = 3).

Hydrophilic and lipophilic antioxidant activities determined by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

By using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging technique [19], the antioxidant activity of samples and the standards were determined. In a dose-response curve, the procedure is predicated on the ability of plant extracts to scavenge more DPPH radical (DPPH•) as Trolox. DPPH absorbs visible light at max = 517 nm and vanishes upon reduction by an antioxidant agent [19, 21]. Briefly, varying the amounts of 1 mL aliquots of methanol solutions of samples or standards were added to 4 mL of a DPPH• methanol solution. A 0.10 mM DPPH• solution was utilized, which was generated by dissolving 4 milligrams of DPPH• in 100 mL of methanol. 1 milliliter of methanol was added to 4 milliliters of DPPH• to create the blank sample. Tests were conducted in triplicate. After 15 minutes of incubation at room temperature in the dark, the absorbance at 517 nm was measured by using a spectrophotometer (SHIMADZU). The calculation for radical scavenging activity was as follow: I % = [(Abs $_{blank}$-Abs $_{sample}$)/Abs $_{blank}$] * 100 (4). The IC_{50} (concentration producing 50% inhibition) was calculated graphically by using a linear calibration curve by graphing the extract concentrations against the corresponding scavenging action. The antioxidant activity index (AAI) was computed as follows:

$$AAI = \frac{\text{DPPH final concentration (g/mL)}}{IC_{50}(\mu g/mL)} \quad (1)$$

Consequently, the AAI was computed based on the mass of DPPH• and the mass of the tested chemical in the reaction, resulting in a constant for each compound regardless of the concentration of DPPH• and the used sample. In this study, the antioxidant activity of plant extracts is deemed to be poor when AAI is less than 0.5, moderate when AAI is between 0.5 and 1.0, high when AAI is between 1.0 and 2.0, and very strong when AAI is greater than 2.0 [21]. The experiments were performed in triplicate, and all samples, standard solutions, and DPPH• solutions were generated on a daily basis.

Acute toxicity assessment of ethyl acetate, methanol, and aqueous extracts

The acute oral toxicity test was performed according to the Organization for Economic Co-operation and Development (OECD) guideline 423 [22] with slight modifications. The animals were divided into three (3) batches of three (3) mice. Each animal is identified by a different mark. After 16 hours of fasting, the weight of each mouse was recorded, and a batch-specific amount of plant extract was administered. After 72 hours of observation, the mortality rate of each batch was established. The extracts were delivered by using an esophageal tube for oral gavage. Control mice were administered solvent (0.2% tween 80). For the evaluation of acute toxicity of the extracts, three batches of three mice were given a single dose limit of 2,000 mg/kg of plant extracts once at the start of the experiment. The extracts were given to the animals in a volume no greater than 0.5 mL. After administration of the extracts, the animals were watched for 2 hours, and then they were fed. Thereafter, they were observed after 24, 48, and 72 hours. Symptoms of intoxication of the animal were noted. Mice that died in each batch were counted for the determination of the lethal dose (LD_{50}). Mice that did not die during 72 hours were observed for a fortnight. The test was carried out twice.

Statistical analysis

Graph Pad Prism version 6.0 was utilized to handle and analyze the data. The data were presented as Mean ± Standard Error of the Mean (SEM). By using One-Way ANOVA (Analysis of Variance) and the Bonferroni test, the researchers compared the means of usage values. If the p-value is less than 0.05, the differences are statistically significant.

Results and Discussion

High-performance thin-layer chromatography (HPTLC) screening

On HPTLC (glass) and silica gel F_{54} (Merck) plates, various extracts of *Lannea velutina* leaves were screened for their phytochemical groups. The results are displayed in Figure 1. Under UV/366

nm and white light, various hues of the patches (orange, yellow, blue, green, pink, and violet) on a chromatogram may correspond to various classes of secondary metabolites. To determine what the UV/366 nm tests revealed about the chemicals, the Natural Products reagent (for flavonoids), the trichloride of iron reagent (for tannins), the Liebermann Burchard reagent (for triterpenes and sterols), and Anisaldehyde sulphuric acid reagent (for saponosides) were utilized.

Sterols, triterpenes, and saponosides detection

Sterols typically fluoresce at UV/366 nm as blue, yellow, and green, whereas triterpenes fluoresce at blue, yellow, green, and violet [19]. After being treated chemically with Liebermann-Bürchard reagent, the HPTLC plates were heated to 110 °C. Triterpenes and steroids are disclosed in the ultraviolet light by the Liebermann-Burchard reagent as blue, green, pink, brown, and yellow hues (Figure 1**a**). In visible light, the anisaldehyde-sulphuric acid reagent revealed sterols and triterpenes as blue and reddish-purple colors (Figure 1**b**). In visible light, the same reagent exhibited yellow and green colors for saponosides [23]. At UV/366 nm, the Liebermann-Bürchard reagent reveals sterols as yellow and yellow-green. This reagent classifies triterpenes as oleanane and ursane types if the patches have a red fluorescence and as lupine types if the patches have a yellow-orange fluorescence [24]. This information from the bibliography enabled us to attribute the chromatograms that we acquired (Figures 1**a** and 1**b**). The Liebermann-Bürchard reagent revealed that each extract included oleanane and ursane triterpenes (Rf patches = 0.05, 0.29, and 0.39), lupine triterpenes (Rf patches = 0.19 and 0.27), and sterols (Rf patches = 0.02, 0.38, 0.48, 0.58, 0.79, and 0.95). The anisaldehyde-sulfuric acid reagent (Figure 1**b**) revealed that only hexane, dichloromethane, and ethyl acetate extract have triterpene and sterols (Rf patches = 0.81 and 0.88). Figure 1**b** demonstrates that dichloromethane and ethyl acetate extracts (Rf patches = 0.10, 0.53 and 0.69) and methanol extracts (Rf patches = 0.63 and 0.74) contain saponosides (yellow and green).

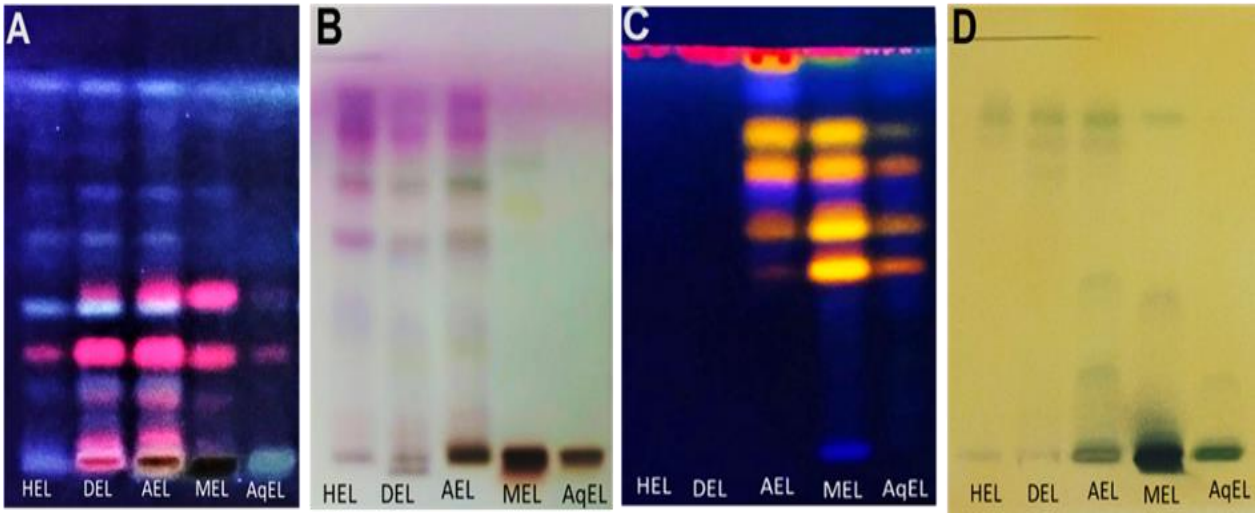
Flavonoids detection

The method of high-performance thin-layer chromatography was employed to obtain the chromatographic profile of flavonoids in leaf extracts (HEL, DEL, AEL, MEL, and AqEL), as displayed in Figure 1**c**. Under 366 nm, the derivatized plate revealed blue, green, yellow, yellow-orange, greenish-yellow, and fluorescent dots in all of the plant samples. The chromatogram demonstrated that the number of flavonoids in the methanol extracts of the leaves differentiated between various strains [19]. It is known that flavonoids interact with various reagents (natural products and aluminum chloride) to generate complexes with brilliant colors that glow brightly under UV/366 nm or visible light. Yellow (Rf patches = 0.46, 0.54, 0.68, and 0.75) and greenish-yellow (Rf patches = 0.96) colored patches on the plate revealed the flavonols presence (Figure 1**c**). The presence of flavones, ethylated flavone, isoflavones, flavanones, and chalcones led to the discovery of blue regions (Rf patches = 0.40, 0.61, and 0.87). The presence of flavanols and aurones was indicated by the presence of green patches. Characterization of flavonoids in crude extracts revealed that these phenolic components are prevalent in leaf extracts, especially methanol extracts (MEL). In conclusion, Neu reagent, which rendered the flavonoids in question as yellow and brown patches, was able to demonstrate the flavonoids presence in the investigated leaves. Under the impact of UV/366 nm, these hues look more vivid and diverse [25].

Tannins detection

Tannins are luminous, like many other secondary metabolic products. Condensed tannins acquire a brown-green hue [26]. Hydrolysable tannins account for the blue-black patches [27]. Several specific revelators are utilized to confirm that this fluorescence is due to tannins (for example, the solid blue salt B and $FeCl_3$). Tannins generate well-colored visible-range complexes with $FeCl_3$ [27]. $FeCl_3$ demonstrated the existence of hydrolysable tannins in ethyl acetate, methanol, and aqueous extracts (Rf patches = 0.02 and 0.05) (Figure 1**d**). This reagent also demonstrated the existence of condensed tannins in ethyl acetate and methanol

leaf extracts of *L. velutina* (Rf patches = 0.18; 0.24; 0.36; 0.40; 0.72 and 0.79) (Figure 1**d**).



HEL: N-hexane extract of L. velutina leaves
DEL: DCM extract of L. velutina leaves
AEL: AcOEt extract of L. velutina leaves
MEL: MeOH extract of L. velutina leaves
AqEL: Aqueous extract of L. velutina leaves

Figure 1: Chromatogram for the detection of triterpenes and sterols (a and b), saponosides (b), flavonoids (c), and tannins (d)

By using HPTLC, secondary metabolites such as sterols, triterpenes, flavonoids, and tannins were detected in leaf extracts of *L. velutina*. The secondary metabolites are substances with biological activity. The therapeutic activities of *L. velutina* leaves could be explained by their presence in the extracts. Due to their potential pharmacological effects and commercial viability, the therapeutic qualities of plants have been explored globally in light of recent scientific advancements [28]. Numerous aromatic and therapeutic plants include chemical components with antioxidant activity (flavonoids, tannins, and triterpenes) [29, 30]. It has been observed that phenolic substances suppress amylase and glucosidase linked with lipid peroxidation, type 2 diabetes, and regulation of blood pressure [31].

Total phenolic, flavonoid, and condensed tannin contents

Table 1 displays the total phenolic (TPC) and flavonoid (TFC) concentrations of *L. velutina* leaf extract determined in ethyl acetate (AEL), methanol (MEL), and water (AqEL). Phenolic concentrations varied from 135.6 ± 2.1 mg gallic acid equivalent/g dry weight for the ethyl acetate extract (AEL) to 534.0 ± 2.9 mg gallic acid equivalent/g dry weight for the methanol extract (MEL). The total phenolic content of the leaves of *L. velutina* was highest in the methanol extract, followed by the aqueous and ethyl acetate extracts. The total phenolic content of the three extracts increased as follows: ethyl acetate extract, aqueous extract, and methanol extract. The significant discrepancies between the results were attributable to the varying polarity of the employed solvents, and also to the affinity between the secondary metabolites and the extraction solvent [32]. Known phenolics with antioxidant properties include phenolic acids, flavonoids, and proanthocyanidins. The total phenolic content determined by the Folin-Ciocalteu technique does not provide an accurate depiction of the quality or quantity of phenolic components. The total flavonoid content (TFC) trended from 13.53 ± 0.06 mg quercetin equivalent/g dry weight for the aqueous extract to 59.39 ± 0.39 mg quercetin equivalents/g dry weight for the methanol extract. The *L. velutina* leaf methanol extract has the greatest total

flavonoids. As presented in Table 1, the methanol extract has the highest total flavonoid content, followed by ethyl acetate extract and aqueous extract. In addition to flavonoids and phenolic acids, proanthocyanidins (condensed tannins) play a crucial role in the prevention of human diseases and the maintenance of good health in the fight against cardiovascular accidents [33]. The condensed tannin or proanthocyanidins content (CTC) was determined by spectrophotometer in leaf extracts of *L. velutina*. The CTC values were between 11.37 and 86.93 mg catechin equivalents/g of dry weight for the aqueous and methanol extracts, respectively (Table 1). According to Table 1, the methanol crude extract of *L. velutina* leaves contained the highest concentration of proanthocyanidins, followed by the ethyl acetate and aqueous extracts, respectively. There was a linear connection (r = 0.92) between the concentrations of total proanthocyanidins and total phenolic.

Table 1: Contents of total phenolics, total flavonoids, and condensed tannins (CTC)

Extracts	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	CTC (mg CE/g DW)
AEL	135.60 ± 2.09^{e}	53.52 ± 0.29^{f}	36.74 ± 0.32^{g}
MEL	534.00 ± 2.9^{a}	59.39 ± 0.39^{c}	86.93 ± 0.03^{b}
AqEL	159.10 ± 0.83^{d}	13.53 ± 0.06^{h}	11.37 ± 0.04^{i}

Different letters correspond to significant difference (P < 0.05)

Hydrophilic and lipophilic antioxidant activity evaluated by using the DPPH assay

The results for extracts and standards are presented in Table 2. The IC_{50} determination for each drug should be performed in the linear region. On each day of analysis, a calibration curve was produced for each tested substance, and a linear region deemed satisfactory was noted (Table 2). According to a prior study, at 25 °C in the light presence, the DPPH absorbance in acetone and methanol fell by 35% and 20%, respectively [34]. Nonetheless, no substantial alteration was detected for 150 minutes in the dark [34]. The AAI was calculated by using Equation (5), where the final concentration of DPPH in the 0.10 mM solution was 40 µg/mL. The AAI score for gallic acid was the highest, followed by catechin and ascorbic acid compared with one another and Trolox (Trolox). The antioxidant effects of plant samples are linked to their bioactive components, phenolics being the most important [35]. Due to the higher intricacy of flavonoid molecules, the structure-activity correlations (SARs) of flavonoids are frequently more intricate than those of phenolic acids. The hydroxylation level, the positioning of the hydroxyl groups, and a double bond associated with a hydroxyl group enhanced the radical scavenging ability of flavonoids [36]. Table 2 demonstrates the hydrophilic and lipophilic antioxidant activity of five investigated *L. velutina* leaf extracts. As listed in Table 2, the hydrophilic antioxidant activities of three types of leaf extracts (AEL, MEL, and AqEL) significantly contributed in all instances. In contrast, the lipophilic antioxidant capabilities (HEL and DEL) were significantly less important. The results indicate that the level of antioxidant activity varies by extract type. The significant linear relationships exist between hydrophilic antioxidant activity and total phenolic (r = 0.74), flavonoid (r = 0.96), and proanthocyanidins (r = 0.94) concentrations. The DPPH test revealed that among the examined leaf extracts, the methanol extract exhibited an extremely strong antioxidant activity. For the same sample, the IC_{50} value and the DPPH index (I %) fluctuated based on the final DPPH concentration, whereas the AAI value remained constant [21]. With an AAI value compared with that of Trolox, the methanol extract exhibited a significant antioxidant activity. This extract also included substantial concentrations of phenolics, flavonoids, and proanthocyanidins (Table 1), the three most important hydrophilic antioxidants found in nature [37]. The lack of phenolics in the hexane and dichloromethane leaf extracts may account for their inability to inhibit DPPH. Several epidemiological studies have demonstrated that

antioxidant consumption can have a substantial impact on health [37]. Natural compounds derived from plants have been utilized to prevent and treat no communicable diseases with low adverse effects and toxicity [38]. Therefore, an acute toxicity study is conducted on NMRI mice by using extracts with antioxidant activity (MEL, AEL, and AqEL). The individual with hypertension is frequently stressed. In response to stress, the body releases a surge of chemicals, including adrenaline and cortisol into the bloodstream. They quicken the heartbeat and constrict the blood vessels, hence increasing blood pressure.

Acute toxicity assessment of ethyl acetate, methanol, and aqueous extracts

Influence on the general health of mice

Two and four hours were spent observing the test animals, respectively. Observations were recorded consistently over fourteen days. Within the first 30 minutes, the groups administered the extracts displayed a reduction in feeding, drinking, and activity. Activity and feeding resumed to normal after two and four hours, respectively. Both the hair color and the stool consistency were typical. There was no aberrant eye, ear, mouth, or nasal secretions, and no signs of poisoning or death. After 14 days, hair color and activity were normal in the control group. Feeding and defecation were regular, and there were no irregular discharges from the eyes, ears, mouth, or nose. All other metrics, including eyes, coat and skin, drooling, and sleep, were healthy throughout the trial [39]. The specific observations are exhibited in Table 3.

Water ingestion, food Intake, and body weight

The results revealed that the extracts did not significantly affect the mice's water and food intake. During the research, it grew marginally. Throughout the trial, food consumption increased somewhat. All treated groups consumed almost the same amount of water (Figure 2**a**) and food (Figure 2**b**) as the control group. Figure 2**c** depicts the body weights of all animals, both untreated and treated. After treatment with the various extracts of *L. velutina* leaves, there was no significant difference in the body weight of the animals compared with the control group ($P > 0.05$).

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

Extracts	First analysis			Second analysis			Third analysis			Mean IC_{50}	Mean AAI	SEM
	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[a]	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[b]	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[c]	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[d]	0.05
MEL	0.9939	15.42	2.59	0.9940	15.34	2.61	0.9972	15.39	2.60	15.38	2.60[d]	0.04
AEL	0.9915	51.68	0.77	0.9917	51.67	0.77	00.9964	51.59	0.78	51.64	0.77[e]	0.05
AqEL	0.9978	103.29	0.39	0.9986	102.39	0.39	0.9993	98.52	0.41	101.40	0.39[f]	2.54
DEL	0.9895	411.34	0.10	0.9925	410.34	0.10	0.9927	406.41	0.10	409.36	0.10[g]	2.61
HEL	0.9856	546.98	0.07	0.9872	547.62	0.07	0.9874	545.08	0.07	546.56	0.07[g]	1.32

R^2: Linear correlation coefficient

SEM: Standard error mean. Different letters correspond to significant difference ($P < 0.05$). [A] µg/mL

Table 3: The influence of extracts on mice's behavior in studies of acute toxicity

Parameters	Observations of the control groups and the leaves extract-treated groups																	
	2 hours			24 hours			48 hours			72 hours			7 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

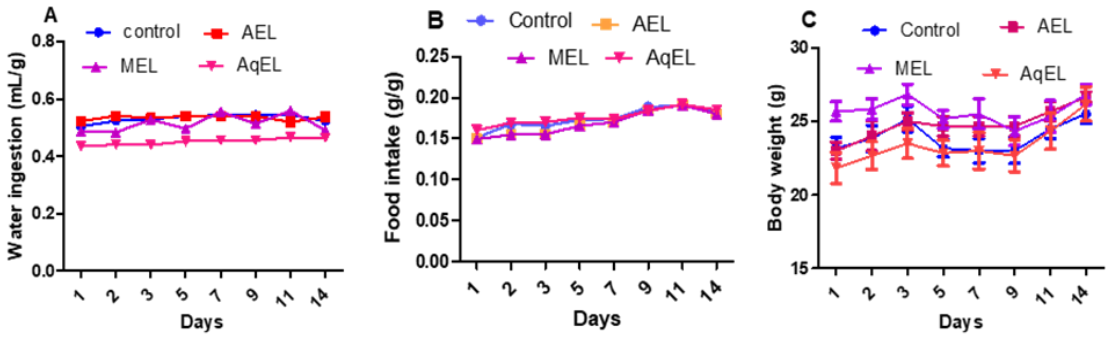


Figure 2: The effect of the extracts on the mice's water ingestion (a), food intake (b), and body weight (c)**.** The control group got 0.2% tween 80, whereas the other groups (AEL, MEL, and AqEL) received a suspension containing 2000 mg/kg of the extracts; values are shown as Mean ± SEM, n = 6

Mouse autopsy and organ index

The organs (heart, liver, spleen, lung, kidney, thymus, stomach, intestines, etc.) exhibited no visible pathological alterations, which were noticed with the naked eye following dissection of the mice and examination of the organs (Figure 3**a**). As illustrated in Figure 3B, the organ index was computed by weighing the major organs (liver, heart, lungs, spleen, and kidney). The organ index of mice in the AEL, MEL and AqEL groups did not differ substantially from the control group (Figure 3**b**; P > 0.05) when compared with the control group.

Lethal dose 50 (LD_{50})

Up to 2000 mg/kg of body weight, ethyl acetate, methanol, and aqueous extracts of *L. velutina* leave exhibited no mortality. Therefore, the LD_{50} values exceed 2 000 mg/kg of body weight. Taking into account the OECD toxicity guideline 423 [22], the macerates of *L. velutina* leaf extracts are not harmful to NMRI mice when administered orally. The low toxicity of this species could justify its usage by the population for medical purposes.

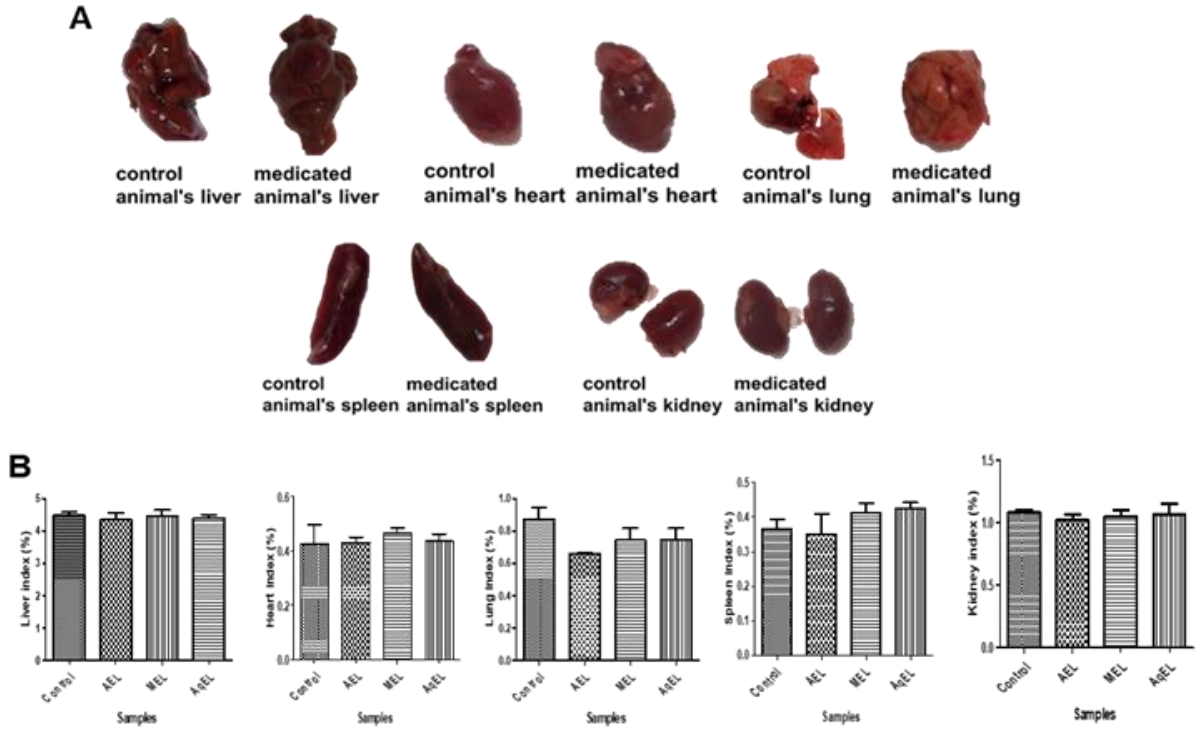


Figure 3: Images of organs as seen by the visible light after the dissection of mice (a) and the effect of extracts on mouse organ indexes (b). The control group received 0.2% tween 80, while AEL, MEL, and AqEL groups received 2000 mg/kg of extracts

Conclusion

This study demonstrates that the leaf extracts of *L. velutina* contain micro constituents known to be antioxidants. Profiles of high-performance thin-layer chromatography showed the presence of sterols, terpenes, tannins, and flavonoids. The ethyl acetate, methanol, and aqueous extracts contain an abundance of phenolic chemicals. The concentration of phenolic compounds is highly dependent on extraction solvents. The quantitative study reveals that the methanol extracts have the highest concentrations of total phenolic compounds, total flavonoids, and condensed tannins. According to the DPPH technique, the antioxidant capabilities of leaf extracts vary depending on the extraction solvent. The *L. velutina* leaf methanol extract has the best IC_{50} with 15.42 g/mL value. The relationship between flavonoid concentration and antioxidant action is substantial. Almost 96% of the free radical scavenging action is attributable to the total flavonoids. 14 days after receiving a single oral dose of 2000 mg/kg of the ethyl acetate, methanol, and water extracts, female NMRI mice exhibited no toxicity-related body weight loss, animal death, or gross lesions. The estimated LD_{50} of each sample in mice is larger than 2000 mg/kg. In terms of acute oral toxicity, these extracts are safe up to 2000 mg/kg in mice NMRI. Testing the diuretic action of these extracts on rats will help us separate the majority of less toxic and bioactive chemicals.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

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