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Study of the toxicity and phytochemical activity of the aqueous extract of the recipe based on leaves of *Brillantaisia patula* T. Anderson (Acanthaceae) and *Desmodium velutinum* Willd (Fabaceae)

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Abstract

The Republic of Congo has an abundant flora rich in plant species. This study was conducted to evaluate the toxicity and phytochemical activity of the aqueous extract of the recipe (AER) based on *Brillantaisia patula* and *Desmodium velutinum* leaves. For the acute toxicity study, mice were fasted for 4 hours, then divided into groups of 3 mice and treated by gavage with the aqueous extract of the recipe (AER) at a dose of 5000 mg/kg. The behavior of the animals and mortality were observed for 48 hours after administration of the products. In the subacute toxicity study, rats were force-fed daily for 28 days with the aqueous extract of the recipe at a dose of 500 mg/kg/day. Before and throughout the study, weight gain and food consumption were recorded. We also analyzed the hematological and biochemical parameters of the rats at the end of the study. The phytochemical activity of the aqueous extract of the recipe was determined by tube reactions and thin-layer chromatography. The results show that the aqueous extract of the recipe is slightly toxic, with an LD_{50} greater than 5000 mg/kg. Hematological and biochemical analyses show a significant increase ($p < 0.05$) in blood platelet counts and mean corpuscular volume on the one hand, and an increase in ALT and AST levels on the other. Phytochemical activity revealed the presence of alkaloids, tannins, reducing sugars, oses and holosides, flavonoid derivatives, and saponosides. In conclusion, the aqueous extract of the recipe is slightly toxic and very rich in secondary metabolites.

Keywords: Toxicity; Phytochemical; *Brillantaisia patula* - *Desmodium velutinum*; Republic of Congo

1. Introduction

The use of medicinal plants is constantly growing in most countries around the world. This use is mainly based on the idea that plants are a natural means of treatment that is completely risk-free. However, a medicinal plant can be both beneficial and toxic. The difference lies mainly in the dose administered. According to estimates by the World Health Organization [1], more than 80% of the African population still uses medicinal plants for their health needs. The toxicity of contemporary pharmaceutical products, the high cost of medicines in pharmacies, and the scarcity or inaccessibility of health centers, especially in rural areas, limit the effective management of public health issues.

The Republic of Congo is no exception when it comes to the use of medicinal plants for the treatment of various diseases. However, cases of toxicity due to the use of these recognized medicinal plants continue to increase. Previous studies conducted at the Biochemistry and Pharmacology Laboratory have highlighted the pharmacological activity of the

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aqueous extract of the recipe based on *Brillantaisia patula* and *Desmodium velutinum* leaves, which has hypotensive and antihypertensive effects in rats [2,3]. With a view to developing a herbal medicine based on this recipe, it is essential to know its chemical composition, safety, and potential effects on the body. When herbal treatments are administered appropriately, the risks of side effects are very limited. It is in this context that the present study was conducted, the main objective of which is to investigate the toxicity and phytochemical activity of the aqueous extract of the recipe based on *Brillantaisia patula* and *Desmodium velutinum* leaves.

2. Methodology

2.1. Plant material

The leaves of *B. patula* and *D. velutinum* were collected in Makana, a small town located approximately 56 km south of Brazzaville. A sample of each leaf was identified at the National Institute for Research in Exact and Natural Sciences (IRSEN) in the national herbarium under numbers 1384 and 636, respectively. The leaves were dried at room temperature (28 ± 1 °C), away from sunlight, for two weeks. After drying, they were ground using a grinder. The powder obtained was used to prepare the aqueous extract for the recipe.

2.2. Animal material

Two animal species were used:

- Female Swiss mice (Mus musculus), albino strain, weighing between 20 and 30 g and aged 14 ± 2 weeks
- Male and female albino rats of the Wistar strain, aged 5 to 6 months, weighing between 150 and 200 g.

These animals were supplied by the animal facilities of the Faculty of Science and Technology and the National Institute for Health Research. They were raised under standard conditions of approximately 27°C air temperature, 50-60% humidity, and a 12-hour day/night photoperiod. These animals had free access to tap water and were fed pellets.

2.3. Preparation of the aqueous extract of the recipe based on the leaves of *B. patula* and *D. velutinum*

The aqueous extract based on *B. patula* and *D. velutinum* leaves was prepared by 10% maceration. For this purpose, 100 g of leaf powder (50 g of *B. patula* and 50 g of *D. velutinum*) was left to macerate in 1000 mL of distilled water for 48 hours. The resulting macerate was filtered three (3) times using cotton wool; the filtrate obtained was then evaporated to dryness using a rotary evaporator. The powder obtained was used as the aqueous extract in the recipe, which was administered in different doses.

2.4. Acute and subacute toxicity study

2.4.1. Acute toxicity

Acute toxicity was tested in Swiss mice according to the OECD method [4]. For this purpose, the mice were fasted for 4 hours and then divided into two (2) groups of three (3) mice each and treated orally as follows:

- The control group received distilled water at a dose of 1 ml/100 g;
- The test group received the formulation at a dose of 5000 mg/kg.

The general behavior of the animals was observed at ½, 1, 2, 3, and 4 hours after administration of each product (distilled water and the formulation). Mortality was recorded for 48 hours after administration of the products. The mice were kept under observation for 14 days to detect the appearance of any late signs of toxicity. Body weight was recorded every two days.

2.5. Subacute toxicity

The rats were divided into two (2) groups of six (6) animals, with an equal number of males and females. After a 12-hour fast, the products were administered daily by gavage for four (4) weeks to the different groups of animals as follows:

- Group 1 (control) received distilled water at 1 ml/100 g/day;
- Group 2 (test) was treated with the formulation at 500 mg/kg/day.

The rats' behavior was observed daily and their weight measured at the end of each week. At the end of the treatment, the animals were sacrificed by decapitation.

Blood was collected from each animal in an EDTA tube and a dry tube in order to measure hematological and biochemical parameters, respectively. The organs (heart, liver, spleen, kidneys) were then removed and the effects of the products on the color, shape, and weight of these organs were evaluated [5].

2.6. Phytochemical profile

2.6.1. Qualitative analysis

The qualitative analysis was carried out using two approaches:

Detection by tube reactions

Six (6) families were investigated. These were alkaloids, tannins, flavonoids, saponosides, reducing sugars, oses, and holosides. The principle is based on adding a reagent to the extract and then observing the resulting reactions: precipitation, color change, etc. The tests to detect chemical families were carried out in accordance with the methods described by Békro et al. [6].

Detection by Thin Layer Chromatography (TLC)

TLC analysis was performed on the aqueous, hydro-ethanolic, and ethanolic extracts of the recipe. To detect polyphenols, the following eluents and developers were used:

- An ethyl acetate/chloroform/ethanol system in proportions (7/3/1)
- Neu's reagent was then sprayed on, followed by exposure of the plate to a UV lamp at 366 nm, in order to detect polyphenols (phenolic acids and flavonoids);

The Neu reagent used to highlight flavonoids was prepared by mixing 0.5 g of 2-aminoethyldiphenylborate, 5 g of PEG 400, and 100 mL of methanol.

2.7. Statistical analysis

Results are expressed as mean ± standard error of the mean (M±SEM). Comparison of mean measurements between batches was performed using Student's t-test. Differences were considered significant at $p < 0.05$.

3. Results

Study of the acute and subacute toxicities of the aqueous extract of the recipe

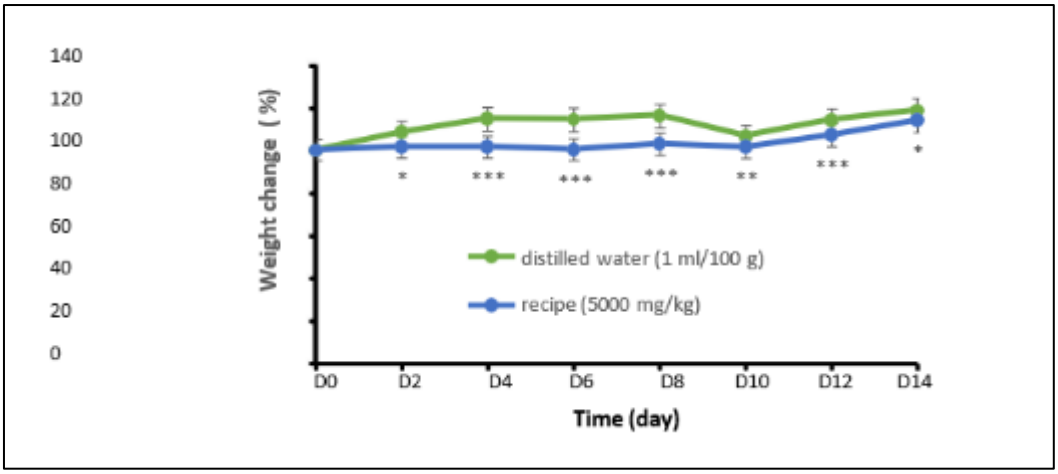
3.1. Acute toxicity

3.1.1. Effect of the aqueous extract of the recipe on the general behavior and mortality of mice

The results obtained show that oral administration of the aqueous extract of the recipe does not alter the general behavior of the treated mice compared to the control mice. No mortality was observed in mice treated with the recipe at 5000 mg/kg b.w. (body weight). The LD50 of the AER is therefore greater than 5000 mg/kg bw.

3.1.2. Effect of the aqueous extract of the recipe on the weight of mice

Figure 1 shows that mice treated with AER did not show any significant weight gain from day 1 to day 10, unlike the control mice. On day 14, weight gain was observed in the treated mice (114.09 ± 8.54%), but this remained lower than that of the control mice (118.73 ± 5.61%).



Each point is a mean ± SEM, n = 3; *p < 0.05, **p < 0.01, ***p < 0.001 significant differences compared to controls (distilled water)

Figure 1 Effects of the aqueous extract of the recipe on mouse weight

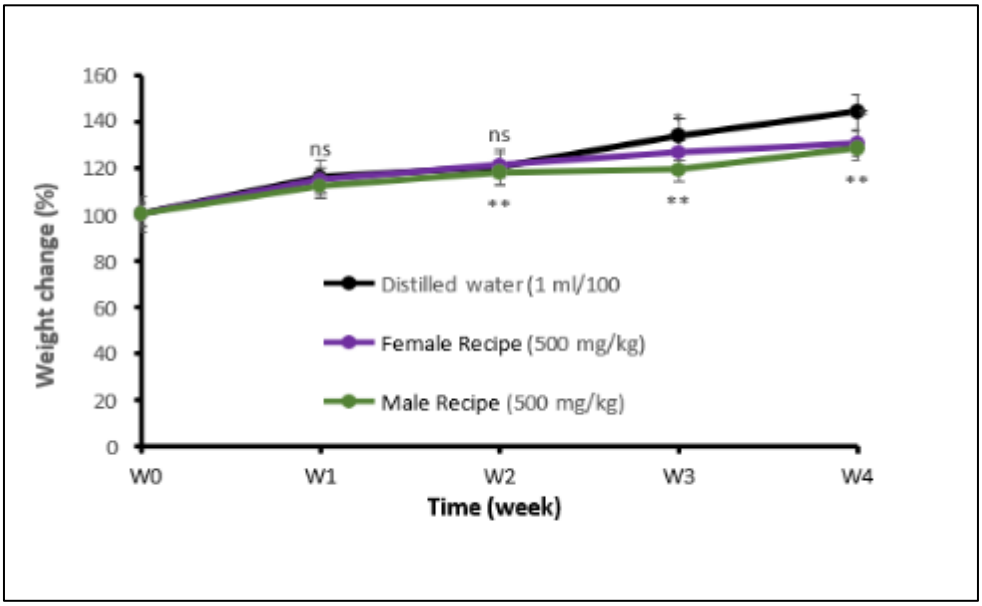
3.2. Subacute toxicity

3.2.1. Effect of the aqueous extract of the recipe on body weight

Figure 2 shows that male and female rats treated with AER (500 mg/kg) gain weight like control rats until the second week.

However, between the third and fourth weeks of treatment, body weight gain in treated rats was significantly lower ($p < 0.01$) than that observed in control rats.

The weight of male and female rats increased in the fourth week to 128.13 ± 5.12 and 130.25 ± 7.94, respectively, in rats treated with AER, compared with 144.16 ± 1.99 in control rats.



Each point is a mean ± SEM, (n = 3); ns = no significant difference compared to controls (distilled water). *p < 0.05; **p < 0.01

Figure 2 Effects of the aqueous extract of the recipe on weight change in rats

3.2.2. Effect of the aqueous extract of the recipe on the weight of internal organs

Table 1 shows that administration of the aqueous extract of the recipe at 500 mg/kg did not cause any significant variation in the weight of internal organs in either male or female rats compared to control rats. Furthermore, no

significant difference was observed between the relative organ weights of female and male rats. Visual inspection of the organs revealed no abnormalities.

3.2.3. Effect of the aqueous extract of the recipe on hematological and biochemical parameters

Effects on hematological parameters

Table 2 shows that administration of the aqueous extract of the recipe at 500 mg/kg did not cause any significant change in blood platelet count in male rats, whereas it caused a significant increase in female rats.

This number increased to 324.33 ± 28.04 10^3/mm^3 ($p < 0.05$) compared to 235.50 ± 16.50 10^3/mm^3 in control rats, representing an increase of 37.72%. In addition, this extract caused an increase in mean corpuscular volume in male and female rats to 60 ± 1.52 ($p < 0.05$) and 58.33 ± 1.45 µm3 ($p < 0.05$), respectively, compared to 34.65 ± 8.35 µm3 in control rats. The respective percentages of increase are 73.16% and 68.34%. However, this extract has no significant effect on the other hematological parameters studied.

Effects on biochemical parameters

Table 3 shows the effects of the aqueous extract of the recipe at 500 mg/kg on biochemical parameters. This table shows that this extract has a significant effect in female rats only on blood glucose, which increased from 0.86 ± 0.05 to 1.09 ± 0.76 g/l ($p < 0.05$), representing an increase of 26.74%. In male rats, this extract caused significant increases in AST (59.77 ± 11.15; $p < 0.01$), ALT (50.12 ± 21.94; $p < 0.01$), and blood glucose levels (1.30 ± 0.29; $p < 0.05$) compared to 6.13 ± 1.23; 4.34 ± 0.24 and 0.86 ± 0.05 in control rats, respectively. In addition, in male rats, this extract caused a significant decrease in triglyceride levels (2.50 ± 0.95 g/l; $p < 0.05$) and total cholesterol (0.54 ± 0.10 g/l; $p < 0.05$) levels compared to control rats, which were 4.69 ± 0.10 and 0.66 ± 0.02 g/l, respectively (Table 3).

Table 1 Effect of the aqueous extract of the recipe on internal organs in rats after 28 days of oral treatment

	Treatments		
Organs (g/100g body weight)	**D.W (1mL/100g)**	**Rec. (500 mg/kg) Female**	**Rec. (500 mg/kg) Male**
Heart	1.03 ± 0.05	0.96 ± 0.07 ns	1.09 ± 0.05 ns
Liver	7.86 ± 0.55	7.93 ± 0.72 ns	8.07 ± 0.23 ns
Spleen	0.37 ± 0.05	0.51 ± 0.03 ns	0.48 ± 0.02 ns
Left kidney	0.90 ± 0.04	0.90 ± 0.07 ns	0.84 ± 0.04 ns
Right kidney	0.91 ± 0.03	0.88 ± 0.06 ns	0.81 ± 0.03 ns

Values are means ± standard errors, with each batch comprising 3 animals (n=3). D.W. = distilled water; Rec = recipe; ns = no significant difference compared to the control.

Table 2 Effect of the aqueous extract of the recipe on the hematological parameters of rats treatments

	Treatments		
Hematological parameters	**D.W (1mL/100g)**	**Rec. (500mg/kg) Female**	**Rec. (500mg/kg) Male**
WBC 10^3/mm^3	3.50 ± 0.10	4.26 ± 1.43 ns	4.93 ± 1.35 ns
RBC (10^6/mm^3)	4.99 ± 0.41	3.74 ± 0.61 *	5.18 ± 1.23 ns
HGB (g/dl)	9.95 ± 1.05	7.03 ± 1.08 ns	9.10 ± 2.11 ns
HCT B (%)	31.4 ± 2.60	22.63 ± 4.37 ns	30.43 ± 7.44 ns
PLT (10^3/mm^3)	235.50 ± 16.50	324.33 ± 28.04 *	227.00 ± 29.80 ns
MCV B (µm^3)	34.65 ± 8.35	60 ± 1.52 *	58.33 ± 1.45 *

MCH B (Pg)	19.95 ± 0.45	18.83 ± 0.76 ns	17.56 ± 0.29 ns
MCHC (g/dl)	31.75 ± 0.75	31.40 ± 1.62 ns	30.13 ± 0.49 ns
RDW (%)	13.40 ± 0.10	13.40 ± 0.32 ns	13.40 ± 0.57 ns
MPV B (μm^3)	7.20 ± 0.20	6.53 ± 0.26 ns	6.06 ± 0.43 ns

Each value is an average ± SEM (n = 3); *p < 0.05 significant difference compared to the control (distilled water); DW: distilled water; Rec: recipe; ns: no significant difference compared to the control (distilled water): WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; PLT: Platelet; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red blood cells distribution width; MPV: Mean platelet volume.

Table 3 Effect of the aqueous extract of the recipe on the biochemical parameters of rats Treatments

Treatments			
Biochemical parameters	**D.W (1mL/100g)**	**Rec. (500mg/kg) Female**	**Rec. (500mg/kg) Male**
ALT (IU/L)	6.13 ± 1.23	8.2 ± 2.35 ns	59.77 ± 11.15 **
AST (IU/L)	4.34 ± 0.24	7.45 ± 2.32 ns	50.12 ± 21.94**
TG (g/L)	4.69 ± 0.10	3.39 ± 1.09 ns	2.50 ± 0.95 *
Cho.T. (g/L)	0.66 ± 0.02	0.67 ± 0.07ns	0.54 ± 0.10*
Crea (mg/dL)	0.56 ± 0.07	0.63 ± 0.08 ns	0.61 ± 0.10 ns
GLY (g/L)	0.86 ± 0.05	1.09 ± 0.76 *	1.30 ± 0.29*

Each value is a mean ± SEM (n = 3); *p < 0.05, **p < 0.01; significant difference compared to controls (distilled water); ns: non-significant difference compared to controls (distilled water). DW: Distilled water; Rec: Recipe; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TG: Triglycerides; TC: Total cholesterol; Crea: Creatinine; GLY: Blood glucose

3.3. Phytochemical profile

3.3.1. Identification of major chemical families

Table 4 presents the results of phytochemical screening of the aqueous extract of the recipe based on the leaves of *B. patula* and *D. velutinum*. This aqueous extract of the recipe revealed the presence of several chemical families. There is a high presence of alkaloids, tannins, reducing sugars, oses, and holosides of flavonoid derivatives with anthocyanidol nuclei and free types. There is also a moderate presence of saponosides in the aqueous extract of the recipe.

Table 4 Results of chemical screening of the aqueous extract of the recipe based on the leaves of *B. patula* and *D. velutinum*

Chemical families	**Alkaloids**	**Tannins**	**Flavonoids**		**Reducing sugars**	**Sugars and holosides**	**Saponosides**
			Anthocyanins	**Free**			
Aqueous extract	+	+	+	+	+	+	+

+ = Presence

3.3.2. Detection by Thin Layer Chromatography (TLC)

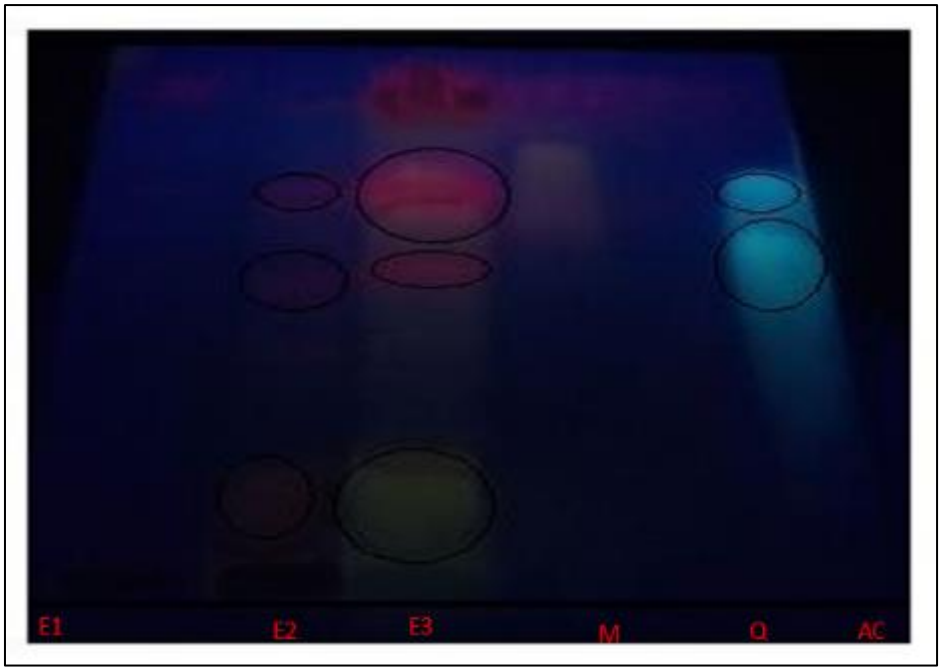
Search for polyphenolic compounds

The chromatographic profiles of the aqueous, hydro-ethanolic, and ethanolic extracts (Figure 3) show a succession of spots indicating the presence of compounds with polyphenolic structures.

Thus, after spraying the plate with Neu and viewing it under a UV-366 nm lamp, the following can be observed:

- Spots of strong yellow, pinkish orange, pink, and pale pink fluorescence with frontal retentions of 0.18, 0.22, 0.58, and 0.72;
- Orange (E2) and orange-yellow (E3) fluorescence spots with frontal retentions of 0.12 and 0.93;

- Orange fluorescence spots and orange stains for the hydro-ethanolic extract with frontal retentions (0.14 and 0.77) and pale yellow and orange-yellow fluorescence with frontal retentions (0.15 and 0.80) for the ethanolic extract;
- Blue fluorescence spots for the hydro-ethanolic and ethanolic extracts with frontal retentions (0.48 and 0.53).



A: TLC of the 3 extracts from the recipe Eluent: ethyl acetate/chloroform/ethanol (7/3/1)

Figure 3 Thin-layer chromatographic profiles of extracts and reference compounds. Developer: Neu; Observation: UV-366 nm. E1: aqueous extract, E2: hydroethanolic extract, E3: ethanolic extract, Q: quercetin, M: myricetin, AC: caffeic acid

4. Discussion

4.1. Acute and subacute toxicity

The acute toxicity study in mice showed that after 14 days at a dose of 5000 mg/kg, AER caused no mortality or noticeable signs of toxicity. These results allow this extract to be classified in category 5 (unclassified) of the GHS (Globally Harmonized System) with a LD50 greater than 5000 mg/kg, corresponding to substances with relatively low acute toxicity according to OECD 423 [4], guidelines on the acute toxicity of chemicals.

Regarding the subacute toxicity study, a slight weight loss was observed in rats treated with the aqueous extract of the recipe from the second week onwards compared to the control rats. No significant difference in organ weight was observed between the control group and the groups treated (females and males) with the aqueous extract of the recipe. This insignificant difference could be explained by the low toxicity of the extract. The results of hematological analyses show a significant increase ($p < 0.05$) in blood platelet levels in female rats compared to control rats. This significant increase ($p < 0.05$) is also observed in the mean corpuscular volume in female and male rats compared to control rats. This suggests that the aqueous extract of the recipe may have a stimulating effect on hematological parameters. Transaminase levels (ALT and AST) were significantly ($P < 0.01$) elevated in male rats treated with the aqueous extract of the recipe at a dose of 500 mg/kg. Transaminase levels in the blood increase when there is cell damage, mainly in the liver, heart, kidneys, or muscles, and they decrease during pregnancy or vitamin B6 deficiency [7]. Prolonged consumption of this recipe could therefore cause damage to these organs.

Our results also showed a significant decrease ($P < 0.05$) in triglyceride and total cholesterol levels. Identical results on cholesterol levels have already been obtained by Mosaddegh et al. [8] and Sharmila et al. [9] respectively with aqueous extracts of Papiliurus spina-christin and Trichosanthes dioica Roxb in rats. Lowering total cholesterol levels has been

widely proven to be effective in reducing the incidence of cardiovascular disease [10]. Thus, this recipe, which has a cholesterol-lowering effect, could have a protective effect on the cardiovascular system.

4.2. Phytochemical profile

The pharmacological properties of plants depend mainly on their chemical composition. The recipe contains high levels of alkaloids, tannins, reducing sugars, oses and holosides, anthocyanidol-based flavonoid derivatives and free flavonoids, as well as moderate levels of saponosides. Several authors have demonstrated the presence of certain chemical families in the extracts of each plant in this recipe, with therapeutic effects [11,12,13]. These different chemical families are generally used in several pharmaceutical and cosmetic preparations [14]. This explains the results of the toxicity study. The high levels of total polyphenols and flavonoids obtained in this study could be justified by the very clear evidence observed by thin-layer chromatography (TLC) and the presence of these metabolites reported by several authors [15, 16]. It should be noted that flavonoids refer to a wide range of compounds belonging to the polyphenol family. Therefore, the low toxicity observed for AER could be attributed to the antioxidant action of flavonoids.

5. Conclusion

This study shows that AER based on the leaves of *B. patula* and *D. velutinum* is low in toxicity, with an LD50 > 5000 mg/kg. The phytochemical study of the aqueous, hydroethanolic, and ethanolic extracts of this recipe indicates that these extracts are very rich in secondary metabolites. Qualitatively, thin-layer chromatography analyses on silica gel showed, under UV light, a wide variety of polyphenolic compounds, particularly prominent in the ethanolic extract.

Compliance with ethical standard

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All experimental procedures involving animals were conducted in accordance with institutional guidelines and approved by the relevant ethics committee.

Statement of informed consent

Not applicable. This study did not involve human participants.

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