



E-ISSN: 2278-4136

P-ISSN: 2349-8234

[www.phytojournal.com](http://www.phytojournal.com)

JPP 2022; 11(2): 309-314

Received: 10-12-2021

Accepted: 16-01-2022

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## Phytochemical screening, acute toxicity and anti-anemic activity of aqueous and hydroethanolic extracts of *Adenia lobata* leaves

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**Abstract**

The active ingredients contained in the aqueous and hydroethanolic extracts of *Adenia lobata* was extracted used to conduct a toxicity study and evaluation of the anti-anemic activity of these extracts in anemic- induced rats. The dry powder of *Adenia lobata* was extracted with water (100g/L) for the aqueous extract and in a 70% hydroethanolic solution (ethanol/water 70:30) for the preparation of the hydroethanolic extract. The secondary metabolites contained in the different extracts were identified by thin layer chromatography. Acute oral toxicity was assessed according to the method of the Organization for Economic Cooperation and Development 423. Anemia was induced in rats by intraperitoneal administration of 40 mg/kg/d of phenylhydrazine for two days (J0 and J1) and these anemic rats were treated with extracts of *Adenia lobata* and vitamin B12. *Adenia lobata* extracts are rich in secondary metabolites such as flavonoids, alkaloids, polyterpenes but poor in quinone. The plant is non-toxic to the body because its LD<sub>50</sub> is greater than 5000 mg/Kg per body weight. The aqueous and hydroethanolic extracts of *Adenia lobata* normalize the hematological parameters of anemic rats. *Adenia lobata* could be a solution for cures of anemia encountered in children and pregnant women.

**Keywords:** *Adenia lobata*, anemia, acute toxicity, secondary metabolites

**Introduction**

Anemia is a condition in which the number of red blood cells, or their ability to carry oxygen, is insufficient. According to the World Health Organization (WHO), in children aged 6 months to 5 years, the hemoglobin level below 11 g/dL is a sign of anemia <sup>[1]</sup>. Anemia affects more than 47% of children under 5 worldwide <sup>[1]</sup>. This rate is approximately 40% in South America, 17% in Europe and reaches 64.6% in the African continent, which represents more than 90 million children <sup>[2]</sup>. The situation in Côte d'Ivoire is alarming, in this sub-Saharan African country, anemia is a public health problem because it is found in 40% of children and pregnant women <sup>[3]</sup>. The difficulties related to the diagnosis and treatment of anemia, namely their high costs, put them out of reach of low-income populations, mainly those in sub-Saharan Africa and in particular those in Côte d'Ivoire. As a result, they resort to medicinal plants to treat themselves <sup>[2,3]</sup>.

In Côte d'Ivoire, 761 traditional medicinal plants have been identified <sup>[4,5]</sup>. Among these many substances of diverse natural origin, some are used for the treatment of many pathologies by traditional medicine <sup>[6]</sup>. However, there are still many plants for which the mode of action, biological targets and side effects are not yet studied. The importance of directing research towards the discovery of new avenues is a source of research for new herbal medicines. It is with this in mind that our work focused on *Adenia lobata*, a species of Ivorian flora which is used in the traditional medicine to treat anemia in children and pregnant women. With the aim of contributing to the cure for anemia, we are going to extract the active ingredients contained in the aqueous extract and the hydroethanolic extract of *Adenia lobata* and carry out toxicity studies and evaluation of the anti-anemic activity of these extracts in anemic-induced rats.

**Material and Methods****Material****Plant Material**

The plant material consisted of powder from the aerial part (leaves) of *Adenia lobata* (*Passifloraceae*). The plant was collected in Bregbo village located in the municipal of Bingerville which is in the southern part of Côte d'Ivoire. This plant was identified by a specialist from the National Floristic center of Côte d'Ivoire, where a sample was kept.

## Animal material

Albino rats (*Rattus Norvegicus*) of the Wistar strain consisting of both sexes were used as animal material in the various *in vivo* studies. They were kept in favorable rearing conditions and fed with a standard complete diet in the form of pellets and received tap water *ad libitum*. These animals were acclimatized to a temperature varying between 19° and 21° C. for 5 weeks.

## Methods

### Method for extracting aqueous and hydroethanolic extracts from the leaves of *Adenia lobata* (Passifloraceae).

The fresh leaves are dried in the laboratory at 18°C for one week and crushed by a mechanical crusher. The dry powder of *Adenialobata* is extracted with water (100g/L) for the aqueous extract and in a 70% hydroethanolic solution (ethanol/water 70:30) for the preparation of the hydroethanolic extract. The mixtures obtained were homogenized using a magnetic stirrer for 24 hours. The homogenates are filtered through Whatman 3 mm paper. The filtrates collected were concentrated in a rotary evaporator then brought to an oven at 40°C for complete drying for one week [7].

### Characterization of some chemical groups by tube reactions

#### 1. Extraction of Alkaloids

Five (05) grams of the aqueous and hydroethanolic extracts were each weighed into a round bottom flask. Fifty (50) mL of dichloromethane was added to each flask and stirred using a magnetic bar and magnetic stirrer for thirty (30) minutes. The mixtures thus obtained were filtered through Wattman 3 mm paper and each of the residues was dried for twenty (20) minutes under a hood. After drying under the hood, each residue was collected in different flasks to which 10 mL of ammonia and fifty (50) mL of dichloromethane were respectively added into and stirred using a magnetic bar and a magnetic stirrer for thirty (30) minutes. Then, each of the mixtures was filtered on a Wattman 3 mm paper and each filtrate obtained was used to identify the alkaloids in the different extracts.

#### 2. Extraction of polyterpenes

Five (05) grams of aqueous and hydroethanolic extracts were each weighed in a round bottom flask using an electronic scale. Fifty (50) mL of dichloromethane was added to each round bottom flask and stirred using a magnetic bar and a magnetic stirrer for thirty (30) minutes. Each mixture was filtered on Wattman 3 mm paper and each filtrate obtained was used for the identification of *terpenes* in the different extracts.

#### 3. Extraction of flavonoids

Five (05) grams of aqueous and hydroethanolic extracts were each weighed in a round bottom flask using an electronic scale. Ten (10) ml of methanol are added to each of the round bottom flasks and brought to a water bath at 60° C. for five (05) minutes. Each mixture is filtered on Wattman 3 mm paper and each filtrate was used for the identification of the *flavonoid* compounds in the forms of genin and glycosyl in each extract.

#### 4. Extraction of saponins

One (01) gram of aqueous and hydroethanolic extracts of *Adenia lobata* was each weighed in a round bottom flask

using an electronic scale. Thirty (30) mL of sulfuric acid water (0.5M) was added to each flask and heated under reflux at 60°C for one (01) hour on a hot plate. After cooling, each unfiltered mixture was shaken with forty (40) mL of chloroform. The extracted chloroform phases were dried over sodium sulphate, filtered and evaporated to dryness using a hot plate. Each of the residues obtained was dissolved in two (02) mL of a mixture of chloroform-methanol (1:1) and each extract thus obtained was used for the identification of the saponosides in the extracts using a thin layer chromatography (TLC).

### Acute oral toxicity

Acute oral toxicity was assessed according to the method described by the Organization for Economic Co-operation and Development 423 [8]. Healthy, nulliparous and non-pregnant young rats, weighing  $118.9 \pm 0.076$  g and 8 weeks' old were used. They were allowed to fast all-night (12 hours) without water restriction before the experiment. For each extract (aqueous and hydroethanolic), four batches of four animals were constituted and treated as follows:

- Batch 1: control, receiving distilled water;
- Batch 2: treated, receiving 1000 mg/kg Bw of extract;
- Batch 3: treated, receiving 2000 mg/kg BW of extract;
- Batch 4: treated, receiving 5000 mg/kg BW of extract.

Animals were treated as a single dose. After administration of the test products, they were deprived of food for 4 hours. Then they were observed individually for the first 30 minutes, regularly for the first 24 hours, and once daily for 14 days. Observation of the animals was based primarily on external physical appearance, measurable clinical signs, behavior change and mortality.

### Anti-anemic activity

Anemia is induced through intraperitoneal administration of 40 mg/kg/day of phenylhydrazine (PHZ) for two days (J0 and J1) [9]. Seven batches of 5 rats with a body weight between 150 and 230 g, aged 4 to 5 months were made up: The rats of normal control batches 1 and 2 anemic having received distilled water, the rats of batch 3 having received vitamin B12 syrup, the rats of batches 4 and 5 having received respectively 250 mg of aqueous and hydroethanolic extracts per kg of body weight and the rats of batches 6 and 7 having received respectively 250 mg of aqueous and hydroethanolic extracts per kg of body at the time of induction. During the experimental phase, we initially collected blood from the rats by incision of the tail (J0). The hematological parameters were assayed on days J0, J2, J7, J14, J21 and J28 in the seven batches of rats using an automatic blood cell counter and the variations in the mean values of the hematological parameters were calculated by comparing to the mean values on J2.

### Statistical analysis

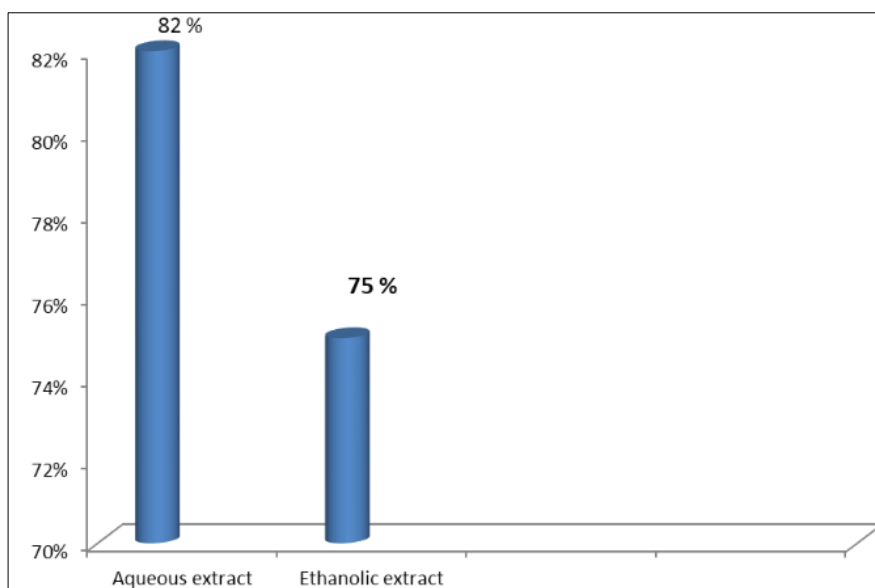
With the exception of the phytochemical screening results, the other results were expressed as a mean plus or minus standard deviation (SD) of the mean (mean  $\pm$  SD). Data representation was performed using Graph Pad Prism 5.0 software (Microsoft USA). The analysis of the results was carried out using the analysis of variances (ANOVA ONE WAY). The differences between the means were determined according to Dunnett's test,  $p < 0.01$ : (very significant difference) and  $p < 0.05$ : (significant difference).

**Result****Extraction of aqueous and hydroethanolic extracts from the leaves of *Adenialobata*(*Passifloraceae*):** The different

yields after aqueous and hydroethanolic extraction of *Adenia lobata* leaf powder gave the following results:

Yield of aqueous extract = 82%

Yield of hydroethanolic extract = 75%



**Fig 1:** Yield of aqueous and hydroethanolic extracts of *Adenia lobata* leaves

**Characterization of secondary metabolites**

The phytochemical screening made it possible to reveal chemical groups contained in the aqueous and hydroethanolic extracts of the leaves of *Adenia lobata* (*Passifloraceae*). The chemical groups are: saponins, flavonoids, polyphenols and catechic tannins. Alkaloids, sterols and polyterpenes (Table I). The aqueous extract contains more of saponins, flavonoids,

alkaloids and catechic tannins and in lower quantity polyphenols and sterols. Quinones are absent in the aqueous extract. The hydroethanolic extract contains the same chemical groups but in smaller quantities; saponins, flavonoids, alkaloids and catechic tannins. Quinones are also absent in the hydroethanolic extract.

**Table 1:** Phytochemical composition of aqueous and hydroethanolic extracts of *Adenia lobata* leaves

Secondary Metabolites Group	<i>Adenia lobata</i>	
	Aqueous extract	Hydroethanolic extract
Polyphenols	++	+
Flavonoïdes	++	+
Saponines	+++	++
Quinones	-	-
Sterols and Terpenes	+	+
Alcaloïdes	++	+
Tanins catechic	++	+

(+) reflects the presence of the chemical group highlighted in the extract;

(++) Reflects a stronger presence of the chemical group highlighted in the extract;

(-) indicates that the highlighted chemical group is absent in the extract

**Acute oral toxicity**

The results of the acute oral toxicity of the aqueous and hydroethanolic extracts indicated that the single administration of the doses of 1000, 3000 and 5000 mg/Kg of bw to the animals did not reveal any clinical sign of

intoxication and did not result in no death in rats during 24 hours of observation and even 14 days after treatment. All the animals survived at the end of the 2 weeks of observation (Tables 2 and 3).

**Table 2:** Clinical signs observed during the first 24 hours after injection of the aqueous and hydroethanolic extracts of the leaves of *Adenia lobata*.

Parameters	Aqueous and hydroethanolic extract of the leaves of <i>Adenia lobata</i>			
	Control	1000 mg/Kg	3000 mg/Kg	5000 mg/Kg
Number of animals	4	4	4	4
Mobility	+	+	+	+
Aggressiveness	-	-	-	-
Appetite	+	+	+	+
pain sensitivity	+	+	+	+

+: Presence

-: Absence

**Table 3:** Rats mortality according to the dose of aqueous and hydroethanolic extracts of *Adenia lobata* leaves received and time.

		Aqueous and hydroethanolic extract of <i>Adenia lobata</i> leaves					
		Aqueous extract			Hydroethanolic extract		
	Treatment	1000 mg/Kg de pc	3000 mg/Kg de pc	5000 mg/Kg de pc	1000 mg/Kg de pc	3000 mg/Kg de pc	5000 mg/Kg de pc
	Number of animals	4	4	4	4	4	4v
	Time after Treatment						
15 min		0	0	0	0	0	0
30		0	0	0	0	0	0
1h		0	0	0	0	0	0
8h		0	0	0	0	0	0
16h		0	0	0	0	0	0
24h		0	0	0	0	0	0
14 J		0	0	0	0	0	0
Mortality percentage		0	0	0	0	0	0
DL <sub>50</sub> (mg/Kg of bw)		>1000	> 3000	> 5000	> 1000	> 3000	> 5000

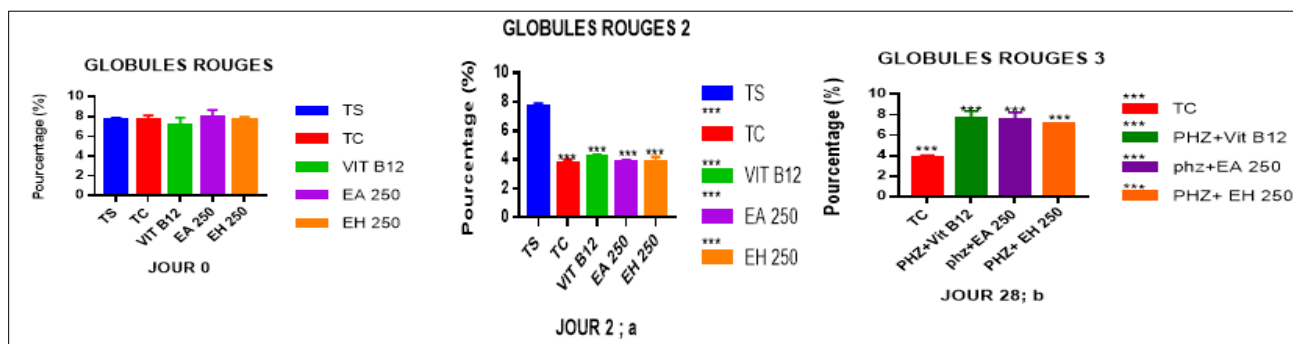
## Evaluation of anti-anaemic activity

### Effects of aqueous and hydroethanolic extracts of *Adenialobata* leaves on hematological parameters

#### 1. Red blood cells

After injection of phenylhydrazine into six rat groups except the positive control group, there was a reduction in red blood cells (47.95%) on day J2. An increase in the number of red blood cells was observed after treatment on the following

days. The results showed that the rats treated with aqueous and hydroethanolic extracts of *Adenia lobata* and vitamin B12 recovered almost completely by the 4th week (79.66%, 78.33% and 79% recovery respectively compared to the positive control (Figure 2). results also showed that the aqueous extract at a dose of 250 mg/Kg/bw allows better recovery compared to that of the hydroethanolic extract



**Fig 2:** Effect of aqueous and hydroethanolic extracts of *adenia lobata* leaves on the number of red blood cells during and after induction of anemia by phenylhydrazine in rats.

TS; positive control: NaCl (sodium chloride)

TC: negative control (Phenylhydrazine hydrochloride = PHZ)

EA: Aqueous extract of *adenialobata*

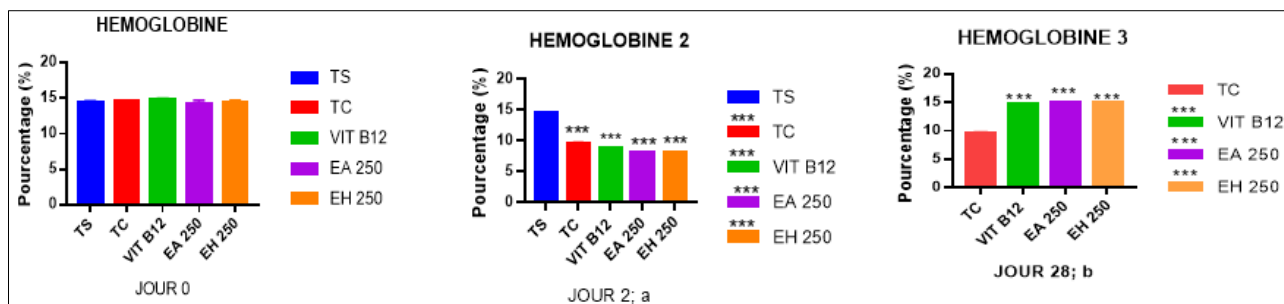
EH: Hydroethanolic extract of *adenialobata*

Vit B12: vitamin B12

#### 2. Hemoglobin

The hemoglobin concentration was strongly restored ( $p < 0.01$ ) in the batches treated with vitamin B12, the aqueous extract 250mg/kg, and the hydroethanolic extract 250mg/kg

of body weight to reach respectively 84,47%, 82,51% and 81,97% compared to the group of positive control rats (Figure 3).



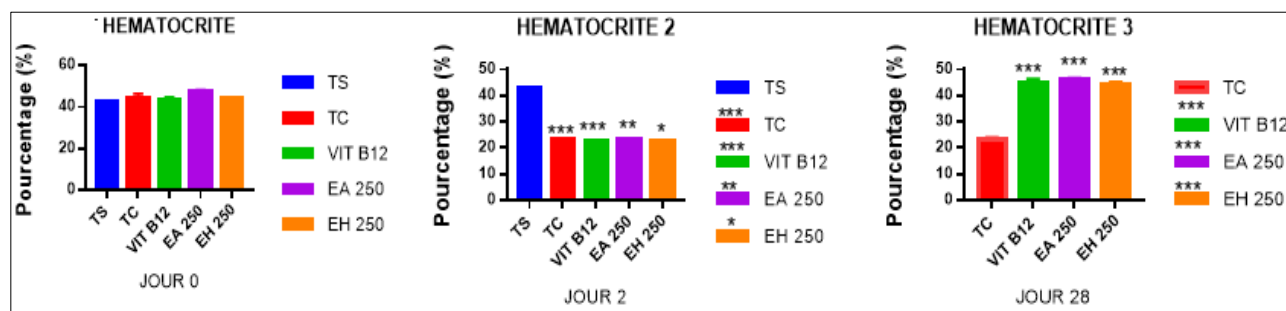
**Fig 3:** Effect of aqueous and hydroethanolic extracts of the leaves and stems of *adenia lobata* on the number of hemoglobin during and after induction of anemia by phenylhydrazine in rats

TS; positive control: NaCl (sodium chloride)  
 TC: negative control (Phenylhydrazine hydrochloride = PHZ)  
 EA: Aqueous extract of *adenialobata*  
 EH: Hydroethanolic extract of *adenialobata*  
 Vit B12: vitamin B12

### 3. Hematocrit

The administration of phenylhydrazine also reduced the hematocrit on day 1. This reduction is 47.65%, 50.21%, and 48.18% respectively in the batches treated with vitamin B12, the aqueous extract 250mg / kg and the hydroethanolic extract

250 mg/kg. After treatment, the hematocrit increased with percentages of 99.34%; 96.42% and 93.95% respectively (vitamin B12, aqueous extract and hydroethanolic extract) compared to the control group (Figure 4).



**Fig 4:** Effect of aqueous and hydro-methanolic extracts of *adenia lobata* leaves on the hematocrit number during and after induction of anemia by phenylhydrazine in rats.

TS; positive control: NaCl (sodium chloride)  
 TC: negative control (Phenylhydrazine hydrochloride = PHZ)  
 EA: Aqueous extract of *adenialobata*  
 EH: Hydroethanolic extract of *adenialobata*  
 Vit B12: vitamin B12

### Discussion

Our study focused at phytochemical screening, acute toxicity and the evaluation of the anti-anemic activity of aqueous and hydroethanolic extracts of the leaves of *Adenia lobata*. This species of the Ivorian flora is used in traditional medicine to treat anemia in children and pregnant women. The phytochemical screening made it possible to reveal chemical groups contained in the aqueous and hydroethanolic extracts of the leaves of *Adenia lobata* (*Passifloraceae*). The chemical groups are: saponins, flavonoids, polyphenols, alkaloids and catechic tannins. These chemical compounds are more in terms of concentration in the aqueous extract. In both extracts, we observed the absence of quinones. The yield of the aqueous extract is 82% against 75% for the hydroethanolic extract. These results are similar to the work of several researchers who showed that the fruits of *H. madagascariensis* also contained these bioactive compounds [10,11]. These molecules are involved in the management of several diseases such as tumors, cancers, diabetes, inflammation, sickle cell disease and oxidative stress [12, 13]. Phenolic compounds possess various biological activities such as antioxidant, anti-inflammatory, antiviral, antimicrobial and antiseptic [14]. This antioxidant activity is due to their ability to scavenge free radicals and chelate metal ions [15]. Our results showed that there is a significant difference between the yields of aqueous and hydroethanolic extracts of *Adenia lobata*. This better yield obtained with the aqueous extract is contrary to the work of certain researchers who have shown that the percentage of extraction is higher with the water-alcohol mixture (hydroethanolic extract [16]. Medicinal plants have been used for centuries to treat different diseases and contain many metabolites [17]. These metabolites have interesting biological activities but they can also be linked to toxic effects. Therefore, the assessment of the toxicity of *Adenia lobata* leaves is very important. Therefore, the present

study was conducted to evaluate its toxicological profile by performing acute oral toxicity in female rats for 14 days. The aqueous and hydroethanolic extracts of *Adenia lobata* at doses of 1000; 3000 and 5000 mg/kg as a single dose oral treatment induced no signs of behavioral toxicity and mortality in the treated groups. The aqueous and hydroethanolic extracts have an  $DL_{50} > 5000$  mg/kg, are classified as a non-toxic substance or category 5 according to the criteria of the OECD in its Globally Harmonized System of Classification (GHS) of chemical substances and mixtures [8]. This suggests that the oral  $LD_{50}$  of *Adenia lobata* is greater than 5000 mg/kg, so our plant is a non-toxic substance. Our results are similar to those of other studies which showed that single oral doses of 1000, 2000 and 5000 mg/kg of aqueous extract of *Piper umbellatum* leaves did not induce mortality or toxic effects in wistar rats [18]. Intraperitoneal administration of 40 mg/kg/bw of phenylhydrazine for two days (J0 and J1) in wistar rats caused a significant drop in hemoglobin levels, number of red blood cells and hematocrit. Our results are in agreement with those of Ryu who observed a decrease in the number of red blood cells and hematocrit respectively of 50% and 55% [19]. Indeed, in our study, at the end of the treatment of anemic rats treated with aqueous and hydroethanolic extracts of *Adenia lobata* and those treated with vitamin B12 saw the levels of red blood cells, hemoglobin and hematocrit increased significantly. These results are similar to those of the work of Saravanan and Manokara, which showed that the treatment of anemic rats with hydroalcoholic extracts of *Mukia maderaspatana* normalizes the hematological parameters of anemic rats [20]. The anti-anemic activity of *Adenia lobata* leaves could be partly attributed to the chemical constituents present in the aqueous and hydroethanolic extracts of *Adenia lobata* leaves. Indeed, saponins and alkaloids have shown anti-anemic properties [21].

## Conclusion

Our study made it possible to determine the composition of the secondary metabolites contained in the aqueous and hydroethanolic extracts of *Adenia lobata*. The study of acute toxicity has shown that this plant is non-toxic to the body and the anti-anemic study has shown that this plant has anti-anemic properties thanks to these chemical constituents such as alkaloids and saponins. The aqueous and hydroethanolic extracts of *Adenia lobata* could therefore help to find a cure for the anemia encountered in children and pregnant women.

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