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Phytochemical Screening and Toxicity Assessment of *Imperata cylindrica* (L.) P. Beauv. (Poaceae) Raw Extracts with Brine Shrimp (*Artemia salina*) Lethality Assay

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Abstract

Background: Herbal medicinal preparations are used as dietary supplements for disease prevention and as alternative/complementary medicine. The growing interest in herbal medicine raises the question of its safety and efficacy. Numerous recorded cases of intoxication and toxicological studies reveal that medicinal plants can be toxic, which hinders their safe use. Plant intoxications related to a variety of factors include plant organs and many extraction solvents. Little toxicological data on medicinal plants is available. The need to investigate is important for safe use. *Imperata cylindrica*, a poaceae, is one of the medicinal plants for which few toxicological data are available. **Materials & Methods**: To expand toxicological data, water, 70% ethanol, and 30% acetone extracts of the leaves and roots, obtained by maceration and reflux methods, were used for phytochemicals molecules qualitative detection and toxicity test by the brine shrimp lethality assay. **Results**: The qualitative analysis of the different extracts revealed the presence of alkaloids, polyphenolic compounds, saponins, and polyterpenoids. The toxicity endpoint was lethal concentration 50 (LC_{50}). The leaves' extracts LC_{50} was between 489.78 µg/mL and 1066.6 µg/mL. As for the root extracts, the LC_{50} was between 341.98 µg/mL and 1530 µg/mL. **Discussion**: The different compounds' presence justifies the use of *Imperata cylindrica* as a medicinal plant. According to Clarkson classification the root extracts are moderately toxic (LC_{50}: 168.47 µg/mL), and leaf extracts are weakly toxic (LC_{50}: 527.25 µg/mL). The extrapolation

made in relation to the Gosselin, Smith, and Hodge scale, allows us to characterize the *Imperata cylindrica* root and leaf extracts as non-toxic to humans by oral route. **Conclusion**: This result can be a base for more precise toxicological studies.

Keywords

Imperata cylindrica, Poaceae, Phytochemical Analyses, Cytotoxicity, *Artemia salina*

1. Introduction

Herbal medicinal preparations, grouped under the term natural medicines, have been part of the medicinal repertoire of many people since ancient times. They are used as dietary supplements for disease prevention and as alternative/ complementary medicine. The growing interest in herbal medicine raises the question of its safety and efficacy.

The phytochemicals present in plants produce a physiological action on the human body that results in health benefits. Investigate the efficacity of medicinal plants includes the investigation of the suitable extraction method and corresponding extraction parameters which is very important in the plant-based drug discovery process. Extraction technique, solvent, and plant part are some parameters to explore. It is important to keep in mind that the choice of these parameters should be made according to some criteria as Convenient, inexpensive, efficient, and highly applicable extraction technology on a small-middle scale [1] [2].

The safety of herbal medicine is a growing concern because the "natural" character of these drugs somewhat biases the appreciation of their toxicity by the populations. Their safety, judged by the population, is put in motion by the numerous recorded cases of intoxications related to their use. In Malaysia for example, in 2013, 11,473 cases of drug intoxication were recorded of which 0.2% were caused by herbal remedies [3]. The seeds/nuts of *Ginkgo biloba*, one of the oldest tree species in the world alive today, are consumed by Asians as common food and used in Asian medicine for coughs, asthma, bedwetting, pyogenic skin infections, and intestinal tract worm infections. Its leaves are also used to treat memory loss and cognitive impairment, arrhythmias and ischemic heart disease, cancer, diabetes, and thrombosis. Investigations and documented cases of intoxication show that ginkgo can cause mild, transient, and reversible adverse effects (convulsions, vomiting, and loss of consciousness, gastrointestinal symptoms, headaches, nausea, vomiting) but also potentially serious effects (spontaneous bleeding) [4]. Potential side effects are also to be noted with another widely used plant: Ginseng. It is one of the most widely used herbs in traditional Chinese medicine. It is used to maintain, restore or increase health, vitali-

ty, and longevity. *Panax ginseng* has several relatively serious side effects ranging from insomnia, diarrhea, vaginal bleeding, and mastalgia to severe headaches, schizophrenia, and Stevens-Johnson syndrome [5] [6]. Poisonings related to herbal preparations are the result of active components, extraction solvents, metallic contaminants, confusion between two species, interaction with so-called modern drugs and abuse, the origin of the plant, the part of the plant, the type of extract, and the many extraction solvents used today. The species of ginseng for example are often a source of confusion. The lack of clear distinction between Siberian or Russian ginseng (*Eleutherococcus senticosus*) and Asian (Chinese or Korean) ginseng (*Panax ginseng*) generates confusion, as the former does not contain ginsenosides but rather eleutherosides, members of the digitoxin family [7]. Still, with the example of ginseng, the daily doses of ginseng vary according to the type. The intake of *Panax ginseng* extract (G115) is 100 to 400 mg, while the intake of ginseng extract with 20% ethanol is 1 to 2 g. The dosage of Korean red ginseng powder is 0.9 to 6 g, and the intake of Korean red ginseng extract is between 200 mg and a maximum of 60 g per day. The intake of fermented red ginseng powder is 1.5 to 2.7 g and the intake of cultured ginseng extract is 2 g [5]. The toxicological behavior of some plants can also be paradoxical: for example, the consumption of extracts of senna (*Cassia angustifolia*) and neem (*Azadidirachta indica*), two plants commonly used as laxatives and antimalarials, respectively, or green tea (*Camellia sinensis*), whose consumption is touted for its many virtues, have been shown to be toxic to the liver [8] [9] [10]. Licorice root used safely to treat duodenal and gastric ulcers can cause serious side effects such as hypokalemia, high blood pressure, heart failure, and death if overused [11]. One factor sustaining the safety issue of herbal medicine is the herbal component. Due to the multitude of active components in herbal medicine, the therapeutic dose in herbal medicine can be close to toxic levels. An example is *nux vomica* which contains strychnine. Five to ten milligrams of *nux vomica* causes an adverse reaction in adults, while 30 mg can result in death [12]. In short, the convergence of uses of a plant to treat an ailment by successive generations, although an indication of the validity of its use, is not sufficient to attest to the efficacy and safety of a traditional remedy [13]. Pharmacological and toxicological testing of medicinal plants to support and improve the use of herbal remedies is therefore very important. The identification of toxicological parameters such as acute toxicity, lethal dose, and IC_{50} is necessary for remedies already used by the population [12].

To assess the toxicity of natural substances *in vitro* and *in vivo* toxicity of substances have been standardized. However, these tests are long, expensive, and require a large number of animals. In recent years, the world's research and accreditation bodies promote low-cost, time-saving assays, and/or not requiring or reducing animal sacrifice assays. Therefore, *in vitro* toxicity assessment assays, such as cell viability or proliferation tests, hemolysis tests, and *in vivo* toxicity assays on algae (*Chlamydomonas* sp.), zebrafish (*Danio rerio*) and especially ar-

temia (*Artemia* spp.) are in full expansion. What is more, these tests have the advantage of being effective with plant-based preparations of complex compositions and requiring very few specific skills [14] [15] [16] [17].

BSLA (brine shrimp lethality assay) is used for the preliminary study of the natural products toxicity chemicals compounds, heavy metals, metal ions, cyanobacteria, algae, dental materials, nanoparticles and to screen marine natural products [18]. The molecular, cellular, and physiological levels of *Artemia* spp. are drastically altered when in contact with even slight levels of contamination, it's so an excellent predictive preclinical assay to estimate general toxicity, short-term acute (STA) and long-term chronic (LTC) toxicities, and lethal dose [19].

One's medicinal plant to explore toxicological properties is *Imperata cylindrica*. *Imperata cylindrica*, a poaceae (Monocotyledon, Cyperales, Poaceae, Panicoides, Andropogones) is growing in tropical, subtropical, or warm temperate regions. In Côte d'Ivoire, *Imperata cylindrica* is present throughout the territory [20]. It is used as a decoction in anemia, sinusitis, fever, and gonorrhea cases [21] [22] [23]. In Asian pharmacopoeia, it's associated or not with other plants, in decoction or maceration. *Imperata cylindrica* is renowned for its antihypertensive, neuroprotective, antibacterial, anthelmintic, astringent, anti-inflammatory, digestive, diuretic, emollient, febrifuge, hemostatic properties, and in particular its antidiarrheal property [24] [25] [26]. Unfortunately, bibliographic search did not provide toxicological data related to the *Imperata cylindrica* use. *Imperata cylindrica* could be toxic and its use dangerous for humans despite its use in traditional medicine.

The study hypothesis was, therefore, "*Imperata cylindrica* would be toxic and the lethality levels would be related to the extract type".

Thus, the work consisted in evaluating *Imperata cylindrica* various raw extracts toxicity by brine shrimp lethality assay as a predictive acute toxicity data, a guide for toxicity assay, and a guide for the safe use of *Imperata cylindrica* extract by population.

2. Materials and Methods

2.1. Biological Material

Vegetal material consisted of *Imperata cylindrica* leaves and roots. They were harvested in Bouaké in central Côte d'Ivoire. The identification was done by Mr Tano Firmin Agronomist and Dr. Amadou Touré researcher/weed scientist at AfricaRice, Bouaké. The plant material was then washed and dried in the shade for 7 days. It was finally pulverized and then stored in jars away from humidity.

For the animal material, the brine shrimp eggs (*Artemia salina*), one crustacean species, were used. They can be purchased at any pet store where they are sold for use as fish food. Hobby brand eggs were acquired from the company Aqua store (Auvergne-Rhône-Alpes, France). The hatching system from JBL was used.

2.2. Preparation of Crude Extracts

The macerates were obtained after 24 hours of 5 g shredded leaves and roots maceration in 100 mL in different solvents made of distilled water, 70% ethanol, and 30% acetone. Thus, there were 3 macerates for each organ. The decoctions were obtained from 500 g of shredded leaves and roots extracted 3 times under reflux for a 2 successive period, 1 h 30 and 1 h respectively in 2 L, 2 L and 1 L solvent. For each organ, the 3 filtrates were assembled, and the collection constituted the water, ethanolic and acetonic decoction extracts [24] [27] [28] [29]. The various filtrates were freeze-dried and then stored at −4˚C for the analyses.

The choice of solvent based on traditional using for water and exploration study for alcohol 70% and acetone 30%.

2.3. Determination of the Extract's Phytochemical Composition

The phytochemical study based on the coloring and precipitation tests was carried out on *Imperata cylindrica* aqueous, ethanolic and acetonic extracts. The compounds targeted were alkaloids, polyphenolic compounds, terpene compounds, saponins, quinones, cardiac glycosides [30] [31].

2.3.1. Detection of Alkaloids

6 mL of plant extract are evaporated. The residue is taken up in 6 mL of alcohol at 60˚ (alcoholic degree) and the alcoholic solution thus obtained is distributed in two test tubes. In the first tube are added 2 drops of Dragendorff reagent (aqueous solution of potassium iodo-bismuth). The appearance of a precipitate or an orange coloration indicates the presence of alkaloids. In the second tube, 2 drops of Bouchardat's reagent (aqueous solution of iodo-iodide) are added. The appearance of a reddish-brown coloration indicates the presence of alkaloids.

2.3.2. Detection of Polyphenols

To 2 mL of plant extract, one drop of alcoholic solution of 2% ferric chloride is added. The appearance of a more or less dark blue-blackish or green coloration indicates the presence of phenolic compounds.

2.3.3. Identification of Tannins

1) Catechic tannins: 5 mL of extract are evaporated. To the dry residue, 15 mL of Stiasny reagent is added (10 mL of 40% formalin with 5 mL of concentrated HCl). The mixture is kept in a water bath at 80˚C for 30 min. Cool under a stream of water. The observation of large flaky precipitates characterizes the catechic tannins.

2) Gallic tannins: The solution containing catechic tannins is filtered and the filtrate collected is then saturated with sodium acetate. 3 drops of 2% ferric chloride are added to the mixture. The appearance of an intense blue-black coloration indicates the presence of gallic tannins.

2.3.4. Detection of Steroids

To 0.5 ml of extract are added 2 ml of anhydrous acetic acid and 2 ml of sulfuric

acid H_2SO_4. The coloration then changes from purple to blue or green, showing the presence of steroids.

2.3.5. Detection of Flavonoids

2 mL of plant extract are evaporated. After cooling, the residue is taken up by 5 mL of hydrochloric alcohol diluted 2 times in a test tube. Two to three magnesium chips are added (heat release). The addition of 3 drops of isoamyl alcohol intensifies a pink-orange or purplish coloration, indicating the presence of flavonoids.

2.3.6. Detection of Saponosides

10 mL of plant extract are put in a test tube. After shaking for a few minutes, the height of foam is measured. A foam height higher than 1 cm indicates the presence of saponosides. The saponosides can also be highlighted by the persistence of the foam.

2.3.7. Detection of Quinones

We use the reagent of Borntraeger (ammonia diluted 2 times) which allows the detection of quinone substances. 2 mL of plant extract is evaporated to dryness in a capsule. The residue is added to 5 mL of HCl diluted 5 times. The solution is placed in a boiling water bath for half an hour in a test tube. It is then cooled under a stream of cold water and the hydrolysate is extracted with 20 mL of chloroform into a test tube. The chloroform phase is collected in a test tube and 0.5 mL of 2-fold diluted ammonia is added. The appearance of a coloration going from red to purple indicates the presence of quinones.

2.3.8. Detection of Polyterpenes and Sterols

The Liebermann reagent is used for this detection. 5 mL of plant extract are dried under rotary evaporator. The residue is dissolved in 1 mL of acetic anhydride and collected in a test tube. Along the tube, 0.5 mL of concentrated sulfuric acid is dripped. The appearance at interphase of a purple or violet ring, turning blue and then green, indicates the presence of polyterpenes and sterols.

2.3.9. Detection of Cardiac Glycosides (Keller-Killani Test)

5 ml of extract are treated with 2 ml of glacial acetic acid added with a drop of iron chloride solution. The addition of 1 ml of concentrated sulfuric acid reveals a brown ring at the interface indicating the presence of a deoxygenated sugar characteristic of cardenolides.

2.4. *Artemia salina* Larvae Lethality Assay

2.4.1. Brine Shrimp Hatching

Brine shrimp cyst was hatched in a hatching system. Twenty-five (25) mg to 1 g of artemia cyst were incubated at 28˚C in 1 L artificial seawater (23 g NaCl, 11 g $MgCl_2{\cdot}6H_2O$, 4 g Na_2SO_4, 1.3 g $CaCl_2{\cdot}2H_2O$, 0.7 g KCl in 1 L distilled water.) under continuous light and aeration. After 24 h incubation, 15 mL 0.06% yeast solution was added to the hatching media. After additional 24 hours incubation,

the larvae which reached the stage of instar II and III were collected for the lethality assay.

2.4.2. Brine Shrimp Lethality Assay (BLSA)

The different extract at the range concentrations from 10 to 1.25 mg/mL was prepared by the half dilution method. In a hemolysis tubes batch, each containing 0.5 mL of defined concentration's extract, 10 artemia larvae were introduced and then the mixture volume was adjusted to 5 mL with artificial seawater (0.5 mL extract + 4.5 mL sea water + 10 artemia larvae). The final concentration of the extracts in the test tubes was then 1; 0.5; 0.25 and 0.125 mg/mL. The test was done in triplicate. The test tubes were then incubated at 20˚C ± 0.5˚C. Lethality effect of extract was determined after 24 hours. As artemia larvae very mobile, the larva was considered dead after 10 s of immobility. Potassium dichromate was used as a positive control with a final concentration of 0.1 mg/mL; 0.05 mg/mL; 0.025 mg/mL and 0.0125 mg/mL in the test tubes. Artificial seawater was used as negative control [32] [33]. Percentage mortality was calculated according to Equation (1).

$$\text{Mortality}(\%) = (\text{Diedlarvae's})/(\text{Totallarvae's number}) * 100 \quad (1)$$

The mortality percentage is then corrected for the percentage of natural deaths using Abbott's formula Equation (2) [34].

$$P = Pi - C/1 - C \quad (2)$$

P being the corrected mortality rate, Pi the observed mortality rate and C the natural mortality rate.

2.5. Statistical Analysis

The data obtained from the tests were processed using XLSTAT 2014. The analysis of different average (n = 3) was done with the Student-Newman Keuls test. The population (LC_{50}) lethal concentration at 50% is determined using the Probit regression analysis system [35].

3. Results

3.1. Phytochemical Screening

The phytochemical screening results of *Imperata cylindrica* rhizomes and leaves' extracts, summarized in **Table 1**, indicates the presence of alkaloids, polyphenolic compounds, saponins and polyterpenoid compounds.

3.2. Extracts Effect on Brine Shrimp Larvae

The results of the artemia larvae mortality rate towards the different *Imperata cylindrica* extracts are reported in **Table 2**. The variables given the highest mortality rate are roots, organic solvents (ethanol, acetone) and decoction. From the study of the mortality rate according to the concentration, it appears that it increases with this one. The concentration which gives the highest percentage of

Table 1. Phytochemical screening of *Imperata cylindrica* leaves and roots extracts.

Plant Metabolites	Roots			Leaves		
	Alcohol 70%	Water	Acetone30%	Alcohol 70%	Water	Acetone 30%
Alkaloids	+	+	+	−	+	−
Polyphenols	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Polyterpenoids	−	−	−	+	+	+
Gal Tannins	+	+	+	+	+	+
Cat Tannins	−	−	−	−	−	−
Saponins	−	+	+	−	+	+
Quinones	−	−	−	−	−	−

+: Presence; −: Absence; Gal: Gallic; Cat: Catechin.

Table 2. *Imperata cylindra* raw extracs's mortality rate.

Variables	Value
Roots	39.31 ± 0.46
Leaves	17.22 ± 0.46
Decoction	30.83 ± 0.46
Maceration	25.69 ± 0.46
Acetone	34.58 ± 0.56
Ethanol	32.71 ± 0.56
Water	17.50 ± 0.56
1000 µg/mL	79.72 ± 0.65
500 µg/mL	31.67 ± 0.65
250 µg/mL	1.67 ± 0.65
125 µg/mL	0.00 ± 0.65

mortality (79.72%) is observed at 1000 µg/mL and the lowest rate (0%) at 125 µg/mL.

The lethal concentration 50 (LC_{50}) of the *Imperata cylindrica* extracts and potassium dichromate are given in Table 3 and Table 4.

The aqueous extracts of the leaves proving not to be lethal, the LC_{50} of the ethanolic and acetonic extracts, was between 489.78 µg/mL and 1066.6 µg/mL. The smallest LC_{50} was obtained by the ethanolic macerate. As for the root extracts, the LC_{50} ranged from 341.98 µg/mL to 1530 µg/mL, the smallest value being obtained by the aqueous decocted.

The LC_{50}s of the extracts were all higher than the LC_{50} of potassium dichromate (63.83 µg/mL).

Table 3. Brine shrimp larvae mortality rate in probit at different concentrations, and LC_{50} value of *Imperata cylindrica* extracts.

Extracts	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	LC_{50} µg/mL
ICFEOm	1.03	1.03	1.03	1.03	NL
ICFEEm	1.03	1.03	4.05	8.95	489.78
ICFEAm	1.03	1.03	1.03	6.48	1066.6
ICFEOd	1.03	1.03	1.03	1.03	NL
ICFEEd	1.03	1.03	3.7	6.48	707.95
ICFEAd	1.03	1.03	1.03	8.95	630.96
ICREOm	1.03	1.03	1.03	5.52	1530
ICREEm	1.03	3.12	4.67	8.95	411.62
ICREAm	1.03	1.03	6.88	8.95	399.76
ICREOd	1.03	4.05	6.48	8.95	341.98
ICREEd	1.03	1.03	5.33	8.95	442.89

ICR: *Imperata cylindrica* root; ICF: *Imperata cylindrica* leaves; EO: aqueous extract; EE: alco holic extract 70%; EA: acetone extract 30%; indices m and d: maceration and decoction; NL: Non-Lethal.

Table 4. Brine shrimp larvae mortality rate in probits at different concentrations and LC_{50} value of potassium dichromate.

Extracts	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	LC_{50} µg/mL
$K_2Cr_2O_7$	4.23	4.25	4.48	5.58	63.827
% Mortality expressed in Probit at different concentrations					

4. Discussion

Compared to other plant families, Poaceae are more generally recognized for properties other than medicinal. *Imperata cylindrica*, a Poaceae member is not left out of this observation. It is much more recognized for its devastating properties than medicinal ones [36] [37]. In Côte d'Ivoire, very few articles mention its use in traditional medicine. The different extracts analysis, however, shows that *Imperata cylindrica* contains secondary metabolites which have shown many medicinal properties in various studies. One compound type revealed in the aqueous, ethanolic and acetone crude extracts of *Imperata cylindrica* roots and leaves are alkaloids. These results are consistent with Laltanpuii *et al.* [38], and Sudha *et al.* [39] results. The alkaloids properties are as diverse as their structure is varied. They are endowed with anti-neurodegenerative, anti-inflammatory, antimicrobial, antioxidant, antiparasitic, antiplasmodial, antioxidant, antibacterial, anti-HIV, neuro-stimulant activities [40] [41]. Our results are consistent with some scientific research concluding the presence of alkaloid.

Another compounds group identified during the study is the saponified com-

pounds group. This result is contrary to Bamba outputs [22], but it agrees with Bashige *et al.* [42] and Lalthanpuii *et al.* results [38]. Plant extracts rich in saponins due to their foamy nature are irritating orally but the less irritating ones are generally used as cough suppressants and expectorants [43]. Saponins have hypo-cholesterolemic, anti-inflammatory and anti-diarrheal properties, particularly on diseases water-related diarrhea and rotavirus infection [44] [45] [46]. The *I. cylindrica* use in feverish states and in diarrhea cases in a traditional environment could therefore be explained by the saponins presence.

Finally, the third large secondary metabolites family revealed in this work are those of polyphenolic compounds, as also noted by Iram *et al.* [47] in aerial part of *I. cylindrica*. Numerous studies have shown the preventive and/or therapeutic effects that polyphenols could have on chronic diseases, metabolic diseases, neuro-degenerative diseases, cell proliferation, inflammatory, diarrheal, viral, bacterial conditions and many other so-called non-communicable diseases [48] [49] [50]. The polyphenols effect on these many conditions is due to its antioxidant property and its ability to regulate certain intra- and inter-cellular signaling pathways. In inflammatory diseases, for example, polyphenols can modulate the pro-inflammatory molecules synthesis (cytokines, tumor necrosis factor α, interleukin (IL)-1β, and IL-6.4) [49] [51] [52].

The presence of these different components can explain the different traditional using of *Imperata cylindrica*

All the molecules responsible for the biological and plants medicinal properties can also be responsible for their toxicity [53]. The brine shrimp larvae first use in bioassays was by Michael *et al.* [54] and since then the method has been standardized [16] [55]. Unlike other *in vivo* study methods which are expensive, time-consuming and which require certain qualifications and infrastructures, the Artemia larvae use as a model has the advantage of being inexpensive, easy to acquire, rapid, does not require specific skills or infrastructures. Artemia larvae also have a high sensitivity to many compounds ranging from heavy metals to medicinal substances [32] [56].

This work that aim was also to evaluate the lethality level of *Imperata cylindrica* crude extracts against brine shrimp, showed that the larvae mortality rate was related to different parameters. The mortality level depends on the extraction type solvent. Here organic extracts (ethanol 70% and acetone 30%) had the highest mortality (32.7% et 34.5%) rate than aqueous extract (17.5%). The same report was done by Sharaibi and Afolayan [57] and Alencar *et al.* [58]. The first authors, who worked on Acetonic, Aqueous and Methanolic of Agapanthus praecox Willd leaf extracts found that methanol extract (78.53%) and acetone extract (50.22%), had highest mortality rate against brine shrimp compared to aqueous extract (42.35%). The second authors also found the high mortality rate of *Palythoa caribaeorum* organic extracts (70% EtOH, dichloromethane (DCM), ethyl acetate (EtOAc) than aqueous ones. This high mortality rate of organic extracts can be explained by the fact they have a higher extracting potential. Fur-

thermore, this study showed that decocted extracts had high mortality rate. High temperatures cause a decrease in viscosity and surface tension, which allows better penetration of the solvent into the sample matrix. Also, it increases the molecular movement by accelerating the mass transfer into solvent of intracellular bioactive compounds from the plant matrix [59]. These events can elevate extraction yield and compound extracted which could influence toxicity rate [28]. Moreover, the role of solvent polarity in the quantity and quality of crude extracts, secondary metabolites, and biological activities has been previously reported [60] [61] [62]. This can explain why most of the same family compounds are present in the different extract but the toxicity rate is different. Moreover, the mortality rate average was concentration dependent with the maximum at 1000 µg/mL and the minimum at 250 µg/mL. This tendency is corroborated by Aliyu *et al.* [63] which concluded at the end of their study on ten poaceae toxicity that the mortality growth with concentration and the maximum and the minimum mortality rate take place at respectively 1000 µg/ml and 10 µg/mL.

In addition to the above parameters, our study showed that plant material is an important parameter in *Imperata cylindrica* toxicity assessment. Indeed, root extracts (39.31%) showed a significantly higher mortality rate ($p < 0.0001$) than leaves (17.22%) by about 2.3 times. This finding is shared by Wakawa & Fasihunddi [64], who during their toxicity study of *Abrus precatorus*, observed that root extracts had about 1.3 to 4.2 times higher toxicity than leaf extracts. And this result of our study could explain the preferential use of roots [65] in traditional medicine.

The toxicity evaluation parameter of the extract in this study is the lethal concentration 50 (LC_{50}) which is the concentration that causes 50% brine shrimp larvae mortality. The lower the LC_{50}, the greater the toxicity of the analyzed element is. The plant extracts toxicity is generally interpreted based on Meyer's or Clarkson's toxicity indices. According to Meyer, toxic bioactive extracts and non-toxic extracts are extracts with an LC_{50} respectively lower and higher than 1000 µg/mL [18] [55]. Referring therefore to Meyer classification, herein study showed that apart from the aqueous leaves' extracts, the aqueous'roots (1530 µg/mL) and leaves acetone macerates (1066 µg/mL), all the others were toxic and biologically active, because having an LC_{50} less than 1000 µg/ml (**Table 3**). These results disagree with Wong *et al.* [66] work, which showed that *Imperata cylindrica* roots ethanolic (90%) extract with an LC_{50} of 5490.2 µg/ml could be considered non-toxic. Iram *et al.* [47] showed that *Imperata cylindrica* aerial part ethanolic and acetone extracts could be toxic with LC_{50} of 11.00 µg/mL and 7.93 µg/mL, respectively. So, these results are conformed to herein study. In fact, aqueous decoction, roots ethanolic and acetone extracts LC_{50} are less than 1000 µg/mL (**Table 3**). The polarity of the extraction solvent and therefore the quantity and quality of the extracted compounds can explain the difference between the toxicity results.

Besides Meyer classification, Clarkson classification is used in toxicity assess-

ment. It allows being a little more precise in the toxicity degree assessment. According to Clarkson, substances with an LC_{50} > 1000 µg/mL are considered non-toxic, weakly toxic those with 500 µg/mL < LC_{50} < 1000 µg/mL, moderately toxic those with 100 µg/mL < LC_{50} < 500 µg/ml and extremely toxic those with an LC_{50} < 100 µg/mL [67]. Thus, according to the present study (**Table 3**), and as Sunandar Ihsan *et al.* [68] and Aliyu *et al.* [63] also observed, the root extracts are moderately toxic (LC_{50}: 168.47 µg/mL), and leaf extracts are weakly toxic (LC_{50}: 527.25 µg/mL).

The data on the larvae mortality rate according to different extracts concentrations define the harmlessness at maximum and minimum concentrations in order to prevent possible acute intoxication. These data could also serve as a basis for future *in vivo* toxicity testing Ohikhena *et al.* [69]. Besides, many previous works have shown a good correlation between toxicity on brine shrimp larvae and many *in vivo* and *in vitro* cytotoxicity tests with 80 to 90% correlation coefficients order [18] [70] [71]. Thus, Parra *et al.* [72] showed a good correlation (r = 0.85) in acute oral toxicity in mice between LC_{50} and the LD_{50} (LD stands for "Lethal Dose". LD_{50}, used to measure acute toxicity, is the amount of a material, given all at once, which causes the death of 50% of a group of test animals). According to them, when the lethality test gives an LC_{50} < 10 µg/mL, the extract LD_{50} is between 100 and 1000 mg/kg; LC_{50} < 20 µg/mL the LD_{50} is between 1000 and 2500 mg/kg, and when the LC_{50} > 25 µg/mL the LD_{50} is between 2500 and 8000 mg/kg. Thus, according to the correlation defined by Parra *et al.* [72], the crude extracts LD_{50} in the present study are between 2500 and 8000 mg/kg. These results are consistent with Nayim *et al.* [73] and Chunlaratthanaphorn *et al.* [74] observations. Of course, they determined a LD_{50} > 5000 mg/kg in rats for *Imperata cylindrica* methanolic and aqueous extracts. Thus, herein results could make it possible to classify *Imperata cylindrica* crude total extracts as slightly to Almost non-toxic in mice on the Hodge and Sterner scale (**Table 5**) [75]. Also, for the LD_{50} range (2500 mg/kg - 8000 mg/kg) of our crude extracts. So, 70 kg person

Table 5. Hodge and sterner scale.

		Route administration		
		LD_{50} oral	LC_{50} inhalation	LD_{50} dermal
Toxicity Index	**Terms**	**(Single dose to rats) mg/kg**	**(4 h rats' exposure) ppm**	**(Single dermal application rabbit skin) mg/kg**
1	**Very toxic**	1 or less	10 or less	5 or less
2	**Highly toxic**	1 to 50	10 to 100	5 to 43
3	**Moderately toxic**	50 to 500	100 to 1000	44 to 340
4	**Slightly toxic**	500 to 5000	1000 to 10,000	350 to 2810
5	**Almost nontoxic**	5000 to 15,000	10,000 to 100,000	2820 to 22,590
6	**Relatively harmless**	15,000 or more	100,000	22,600 or more

Table 6. Gosselin, Smith and Hodge scale.

Probably lethal oral dose (human)			
Toxicity Index	**Terms**	**Dose (mg/kg)**	**For 70 Kg weight person**
6	Super toxic	Less than 5	1 grain (a pinch less than 7 drops)
5	Extremely toxic	5 to 50	4 mL
4	Very toxic	50 to 500	30 mL
3	Moderately toxic	500 to 5000	30 to 600 mL
2	Slightly toxic	5000 to 15,000	600 to 1200 mL
1	Almost not toxic	More than 15,000	More than 1200 mL

should receive a dose in the range (2500 mg/kg × 70 - 8000 mg/kg × 70) or (175 g - 560 g) of extract in one go orally to run the same risks. On the Gosselin, Smith and Hodge scale, our crude extracts could be classified as almost non-toxic to humans by the oral route [75] (**Table 6**).

5. Conclusion

The chemical composition and toxicity of brine shrimp larvae of *Imperata cylindrica* roots and leaves crude aqueous, ethanolic, and acetone extracts have been characterized. The results showed the presence of alkaloids, polyphenolics, saponins, and polyterpenoids. All the extracts have an LC_{50} between 341.98 and 1530 µg/mL on brine shrimp larvae. These results correspond to a LD_{50} between 2500 mg/kg and 8000 mg/kg in mice. According to Clarkson classification, root extracts are moderately toxic (LC_{50}: 168.47 µg/mL), and leaf extracts are weakly toxic (LC_{50}: 527.25 µg/mL) on brine shrimp. These extracts are almost non-toxic to humans orally. These results justify *Imperata cylindrica* safe use and especially the roots in a traditional environment. Another toxicological assay must be done to confirm and expand data on sub-acute toxicity and the effect on different organs.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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