Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(3): 55-61
Received: 26-03-2022
Accepted: 10-04-2022

Marthe Makwena Mpongo
Department of Biology, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo

Didier Lusimbamo Dianzuangani
Department of Biology, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo

Ruth Malobo Katunda
Department of Biology, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo

Florent Biduaya Mukeba
1. Department of Geostrategy, Geography, Environment and Land Management, Center of Research of Human Sciences, Kinshasa, Democratic Republic of the Congo
2. National Pedagogical University, Faculty of Sciences, Department of Biology, Kinshasa, Democratic Republic of Congo

Odette Ngandu Kabena
Department of Biology, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo

Félicien Luyeye Lukoki
Department of Biology, Faculty of Sciences, University of Kinshasa, Kinshasa, Democratic Republic of Congo

Corresponding Author:
Florent Biduaya Mukeba
1. Department of Geostrategy, Geography, Environment and Land Management, Center of Research of Human Sciences, Kinshasa, Democratic Republic of the Congo
2. National Pedagogical University, Faculty of Sciences, Department of Biology, Kinshasa, Democratic Republic of Congo

In vitro evaluation of the cytotoxicity of *Dissotis rotundifolia* (Sm.) Triana (Melastomataceae) and *Emilia sagittata* (Vahl) DC. (Asteraceae) plants used in intimate hygiene among women in Mbandaka, Democratic Republic of the Congo

Marthe Makwena Mpongo, Didier Lusimbamo Dianzuangani, Ruth Malobo Katunda, Florent Biduaya Mukeba, Odette Ngandu Kabena and Félicien Luyeye Lukoki

DOI: https://doi.org/10.22271/phyto.2022.v11.i3a.14415

Abstract
Plants used by Pygmy women in Mbandaka, Equateur Province, Democratic Republic of Congo include *Dissotis rotundifolia* (Sm.) Triana (Melastomataceae) and *Emilia sagittata* (Vahl) DC. (Asteraceae) known as the scarlet gland flower, and are geographically native to parts of Africa, particularly West and East Africa, whose leaves are used for birth spacing, sexual appetite and vaginal shrinkage respectively. The use of these plants as nutraceuticals for several diseases for decades, without any scientific data on their safety is at risk, because although they are natural, they are not completely free of toxic effects or other undesirable effects, hence the evaluation of the toxic effects of these phytomedicines on red blood cells to ensure its safety. For this purpose, the hydro-methanolic extracts of the leaves were prepared by maceration and human red blood cells were used. The results obtained showed a significant hemolytic effect of these extracts, depending on the concentration and the incubation time (15 and 60 minutes). However, the concentrations of 25 and 100 mg/mL of *D. rotundifolia* induced 34.2 and 51.2% of red blood cell lysis while those of *E. sagittata* induced only 20.8 and 38.7% of hemolysis. This led to the conclusion that the hydro-methanol extracts of *D. rotundifolia* are more hemolytic than those of *E. sagittata*. In view of this finding, the application of *D. rotundifolia* to the mucosa would cause more damage than that of *E. sagittata*. However, no significant difference was observed ($p>0.05$), after Student's test.

Keywords: *Emilia sagittata*, *Dissotis rotundifolia*, hemolytic, toxicity, *in vitro*

1. Introduction
Medicinal plants have played a major role in the development of modern orthodox medicine and continue to be widely used in their original form [1]. Published data indicate that approximately 80% of the world's population relies on plant medicine for health care delivery and approximately 70-95% of developing countries rely on plant medicine for primary health care [2, 3]. Implicitly, the practice of plant medicine is gradually becoming the norm worldwide. This may be due in part to the recognition of the value of traditional medical systems and the identification of medicinal plants from indigenous pharmacopeias that have been shown to have a significant pharmacotherapeutic effect, either in their natural state or as a source of new pharmaceuticals.
The use of medicinal plants for various health problems around the world would be supported by choice, but also because of the growing poverty of populations who do not have access to modern medicines because of their very expensive costs [4]. Among the plant preparations that treat various ailments, some are specific to women. The latter are invited to use certain recipes for genital cleansing [4, 5].
The Democratic Republic of Congo (DRC), by its cultural diversity, the richness, and diversity of its flora and fauna, constitutes a real reservoir of biodiversity as indicated by the results of recent work, which is why it has been allowed to occupy a privileged place among the countries of the Congo Basin as traditional medicinal know-how based on plants and animals [6].
In the DR Congolese context, approximately 80% of the population depends on plants for their health care [6, 7]. This is an indication that plant medicine continues to play a key role in maintaining health care.

In the Democratic Republic of Congo, plants have been used as foods and treatments for several diseases for decades with little or no scientific evidence of their safety. The practice has relied heavily on clinical experience. Phytomedicines are widely used and perceived as low risk compared to synthetic drugs [8], as they are natural, although not completely free of toxic or other adverse effects [9]. The popularity of herbal medicines, coupled with the paucity of evidence on their safety, has raised serious concerns about their therapeutic value. Evaluation of the toxic effects of plant drugs in different models is essential to ensure safety. The increasing use of herbal preparations as cosmetics and drugs requires that their toxic profile be evaluated.

Most plant species have therapeutic properties, as they contain active ingredients that act directly on the body [10], and also, any biologically active substance is likely, at high or low doses and for prolonged administration, to produce undesirable or even harmful effects [11]. This is the particular case of plant products rich in saponosides, terpenes, alkaloids, and in general other chemical substances [12]. Ali-Risasi *et al*. [13] observed low grade or worse squamous intraepithelial lesions in women practicing intra-vaginal insertion of plants in Kinshasa.

Among the plants used by pygmy women in Mbandaka, Equateur Province, Democratic Republic of Congo are *Dissotis rotundifolia* (*D. rotundifolia*), is a member of the Melastomataceae family, and *Emilia sagittata* known as a scarlet gland flower, belongs to the Asteraceae family and are geographically native to parts of Africa, especially West and East Africa [14-16] whose leaves are used for birth spacing, sexual appetite seeking and vaginal shrinkage respectively [17]. Evidence for the toxicological evaluation of hydro-methanol extracts of the leaves of *Dissotis rotundifolia* (Sm.) Triana (Melastomataceae) and *Emilia sagittata* (Vahl) DC. (Asteraceae) is necessary to guarantee their safety to consumers. Thus, this study was designed to evaluate the potential toxicity of these extracts on red blood cells.

2. Materials and Methods

2.1 Materials

2.1.1 Plant material

In the present study, the plant material consisted of leaves of *Dissotis rotundifolia* (Sm.) Triana (Melastomataceae) and *Emilia sagittata* (Vahl) DC. (Asteraceae) collected in October 2019, at the Jardin Botanique d'Eala Ville of Mbandaka in the Province of Equateur, Democratic Republic of Congo. They were identified at the Herbarium of the Institut National d'Etudes et de Recherches Agronomiques and confirmed by the Laboratoire de Botanique systématique et Ecologie des plantes, Département de Biologie, Faculté des Sciences de l'Université de Kinshasa (UNIKIN).

2.1.2 Animal model

Human red blood cells were used in this study to evaluate the *in vitro* toxicity of hydro-methanol extracts of *E. sagittata* and *D. rotundifolia* leaves.

2.1.3 Inclusion criteria

To participate in this study, it was necessary to be a female subject, to have consented to donate blood, to be between 18 and 30 years of age, and to present red blood cells of type AA at the end of electrophoresis. Thus, three human subjects were selected.

2.1.4 Exclusion criteria

Female subjects with Hepatitis C infection, malaria infection, HIV infection, or with red blood cells of type AS or SS after electrophoresis were excluded.

2.2 Methods

2.2.1 Preparation of phosphate-buffered saline (sodium) (PBS)

1 tablet of 193 mg PBS buffer was dissolved in 200 mL of distilled water. The pH was readjusted by gradually adding NaOH solution (40%) to obtain pH=7.4.

2.2.2 Preparation of plant extracts

a) Conditioning of the plants

The samples of the leaves of *D. rotundifolia* and *Emilia sagittata* dried at room temperature (± 27 °C) and were protected from light (Biology Department) for two weeks and were ground to obtain a fine powder of 50.0g and 59.4g respectively. The different parts used are described in the figures below (Figure 1).

Then, place, 20 g of each plant powder into Erlenmeyer flasks and cover with 200 mL of 70% methanol (60 mL distilled water and 140 mL methanol). Homogenize the mixtures for about 10 minutes, then let them stand for 24 hours. After filtration (Whatman paper No. 4), the macerates obtained were placed in the Rotavapor to obtain a dry extract to be stored in a 500 mL glass bottle protected from light. The residue of each extract was recovered and weighed.

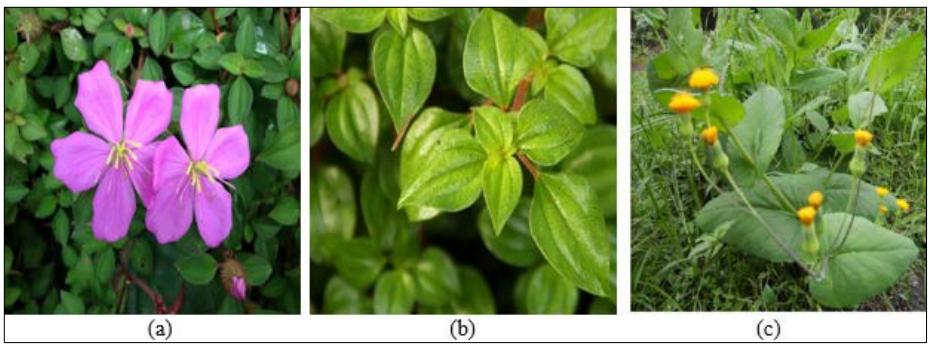


Fig 1: (a) flowering leaves and (b) leaves of *Dissotis rotundifolia* (Sm.) Triana (Melastomataceae), (c) whole flowering plants of *Emilia sagittata* (Vahl) DC. (Asteraceae).

b) Preparation of extracts at different concentrations

Different concentrations of hydro-methanol extracts were prepared in PBS (250 mg/10 mL PBS, 500 mg/10 mL and 500 mg/5 mL).

The final concentrations of hydro-methanol extracts obtained were 25 mg/mL, 50 mg/mL and 100 mg/mL respectively.

2.2.3 Collection and Conditioning of Human Blood

a) Collection

15 mL of blood was collected intravenously for each subject and placed in a dry hemolysis tube.

b) Preparation of the erythrocyte suspension

15 mL of collected and retained blood was centrifuged at 4000 rpm for 5 minutes, after removal of the supernatant, the pellet was washed twice with 10 mM PBS, pH 7.4, and then the pellet, thus obtained, was re-suspended by PBS with the same volume of plasma removed [18].

2.2.4 Evaluation of the toxicity of hydro-methanolic extracts of *D. rotundifolia* (Sm.) Triana and *Emilia sagittata* (Vahl) DC leaves.

a) Principle

The red blood cell (Erythrocyte or Hematopoietic) was chosen as a model, because of its ease of isolation, its relative simplicity, moreover, the erythrocyte membrane is a valuable tool for the study of transmembrane ionic transport, and the presence of this transport system similar to those in some tissues.

The erythrocyte is a blood cell responsible for gas transfer, its biconcave disc shape allows it to offer a maximum exchange surface.

Lysis of red blood cells can be achieved simply by placing red blood cells in a hypotonic solution (osmotic hemolysis). The erythrocyte changes from its biconcave disc shape to an elongated spherical shape characterized by the passage of K+ ions to the extracellular medium and Na+ ions into the intracellular medium, thus the cell membrane is broken, resulting in the release of the contents into the extracellular medium.

b) Evaluation

The test of the cytotoxic effect of hydro-methanolic extracts of the leaves of *Dissotis rotundifolia* (Sm.) Triana (Melastomataceae) and *Emilia sagittata* (Vahl) DC. (Asteraceae) was performed according to the method described by Elalaoui [18].

Put in hemolysis tubes 594 μL of the erythrocyte suspension prepared with 6 μL of the hydro-methanol extract of the leaves at different initial concentrations (25 mg/mL, 50 mg/mL, and 100 mg/mL) to obtain final concentrations (0.05 mg/mL, 0.1 mg/mL and 0.2 mg/mL), then incubate the tubes at 37°C for 1 hour, then take 100 μL at the 15th and 60th minute of incubation, add 300 μL of PBS and mix the tubes gently to avoid mechanical hemolysis, re-suspend in an ice-cold wash of 2 mM MgCl2 (500 μL) and finally centrifuge the tubes at 4000 rpm for 5min, the pellet is thus eliminated.

A negative control tube was prepared under the same experimental conditions. It is composed of 100 μL of erythrocyte suspension and 500 μL of PBS buffer (without extract) and a positive control tube, named total hemolysis tube (HT), containing 100 µl of erythrocyte suspension and 500 μL of distilled water, has been prepared (without extract)

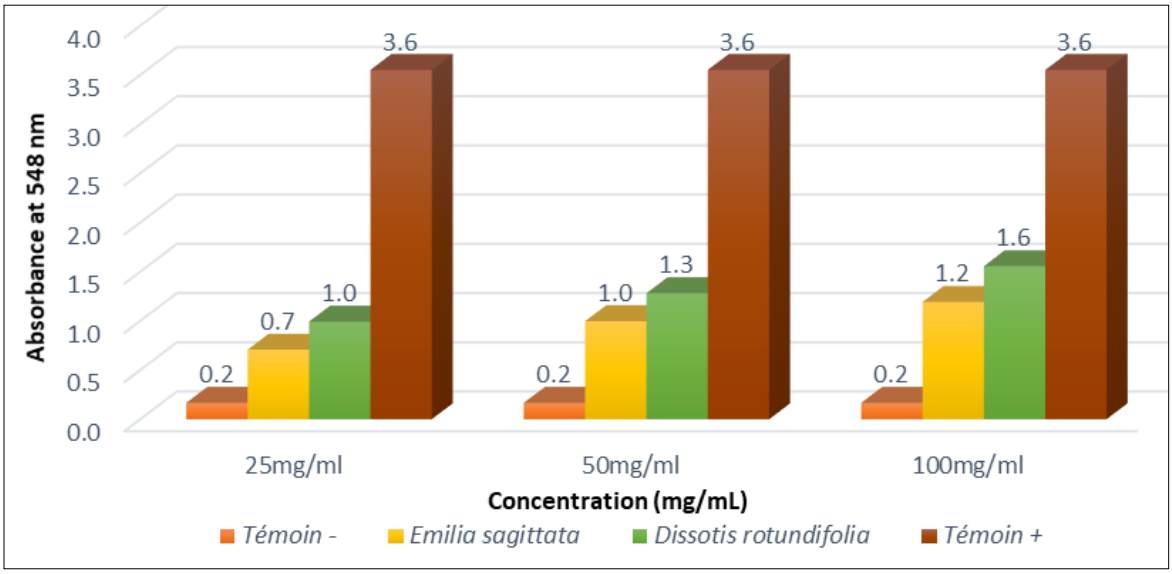
The absorbance of each tube was read at 548 nm (characteristic wavelength of hemoglobin) using a double beam UV-Vis spectrophotometer (Thermoscientific, type Genesys 10S UV-Vis) against a blank containing PBS. The data observed at the 15th minute was considered for comparison of each sample concentration of each plant.

Data analysis

For data processing, some statistical variables were calculated such as arithmetic mean, variance, and standard deviation. The Student's t-test was used to compare the results of two plants.

3. Results and Discussion

Figure 2 below, constructed with absorbance averages, shows the cytotoxic effect of *D. rotundifolia* and *E. sagittata* plant leaf extracts on red blood cells.

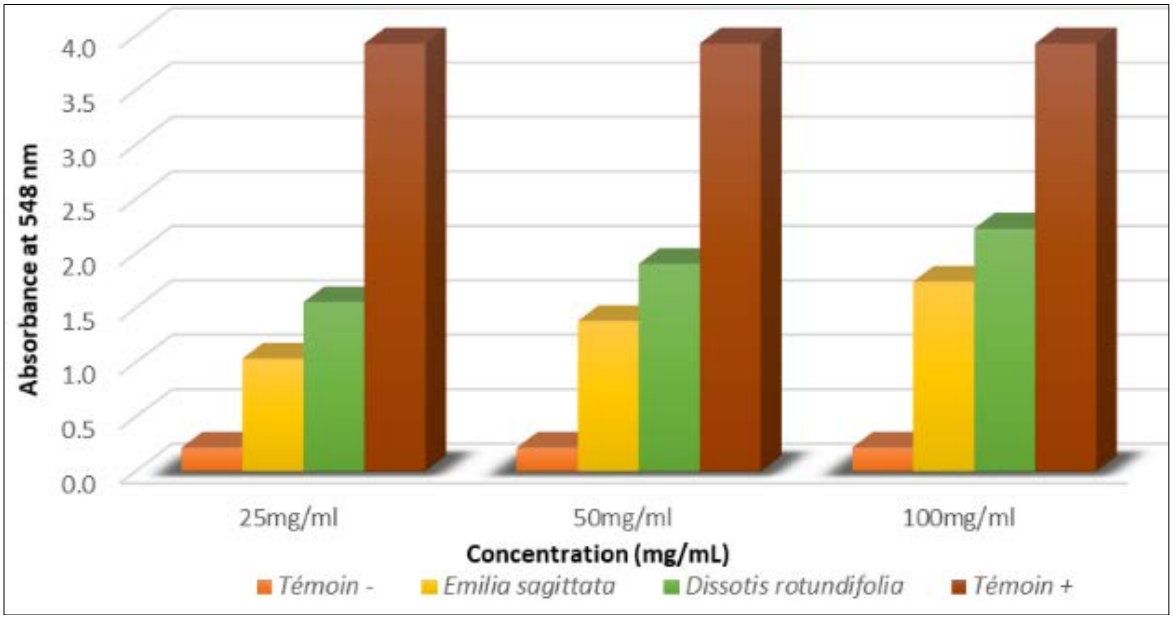


Negative control: Phosphate-buffered saline (PBS)
Positive control: Distilled water

Fig 2: Evolution of absorbance after 15 minutes of incubation of red blood cells in the presence of extracts from the leaves of Negative control (-), *Emilia sagittata* (Vahl) DC., *D. rotundifolia* (Sm.) Triana and Positif control (+)

Figure 2 shows that the absorbance varies from 1.0 to 1.6 for *D. rotundifolia* and from 0.7 to 1.2 for *E. sagittata* after 15 minutes of incubation of red blood cells, in the presence of the different concentrations of these extracts. At all concentrations, the absorbance values induced by the presence of *D. rotundifolia* are higher than those induced by *E.*

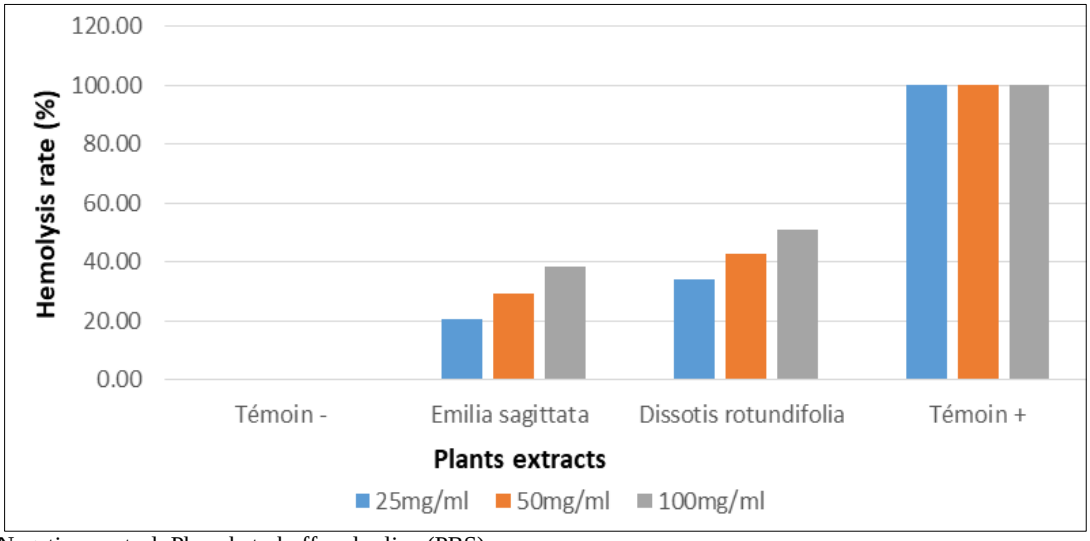
sagittata. The absorbance observed in the presence of distilled water (positive control) is 18 times higher than that obtained with the negative control (PBS buffer).
Comparing the absorbance read with the positive control and that of the extracts, it can be seen that the total hemolysis obtained with distilled water is 5, 3.6, and 3 times higher respectively for 25, 50, and 100 mg/mL of *E. sagittata*. In front of *D. rotundifolia*, the absorbance values are respectively 3.6; 2.7, and 2.2 times less than the absorbance value of distilled water.



Negative control: Phosphate-buffered saline (PBS)
Positive control: Distilled water

Fig 3: Evolution of absorbance after 60 minutes of incubation of red blood cells in the presence of hydro-methanolic extracts of Negative control (-), *Emilia sagittata* (Vahl) DC., *Dissotis rotundifolia* (Sm.) Triana and Positif control

Figure 3 shows that the absorbance increased as a function of concentration, at the 60th minute of incubation of the red blood cells against the plant extracts at different concentrations. Its values increase respectively from 1.0 and 1.7 for *E. sagittata* and from 1.6 to 2.2 for *D. rotundifolia*; for the concentration of 25 and 100 mg/mL.
The figure also shows the same evolution of absorbance against the negative and positive controls. The extracts of *E. sagittata* cause an absorbance of 3.9; 2.7 and 2.3 times less than that induced by distilled water. The latter causes absorbance values 2.4, 2, and 1.8 times higher than that of *D. rotundifolia* extracts.
The release of intracellular components of erythrocytes, especially hemoglobin, is progressive and the cloudiness in the tubes increases with time. The low concentration (25 mg/mL) of *E. sagittata* and *D. rotundifolia* extracts induced 3.9 and 5 times less hemolysis than the positive control, respectively. These results show the existence of a very intense hemolytic effect, depending on the concentration and time.
The fact that the absorbance increases with the increasing concentration of the extract are observed by many researchers, having used different biological models. The values taken in 60 minutes of incubation are close to values found by [18, 19] who worked respectively with the seeds of *Nigella sativa* L. (Ranunculaceae) and the aerial parts of *Fredolia aretioides* (Amaranthaceae) and *Echium vulgare* (Boraginaceae). These observations deviate, on the contrary, from the absorbance values obtained in 60 minutes by [20], which did not exceed 0.5; working with the aerial part of *Ammoïdes verticillata* (Apiaceae).
Since the absorbance values induced by the presence of *D. rotundifolia* are higher than those induced by *E. sagittata*, for the incubation times of 15 and 60 minutes. Thus, from the comparison of absorbance variances using the Student's t-test, it was found that the absorbance values obtained comparatively between *E. sagittata* and *D. rotundifolia* at the different concentrations did not show a significant difference.
However, it was noted that after 15 and 60 minutes, the 50 mg/mL concentration of *E. sagittata* leaf extracts showed an insignificant difference compared to the 25 mg/mL concentration ($p<0.05$). However, no significant difference is observed when considering the rest of the comparisons ($p>0.05$).
Figure 3 below shows the rates of hemolysis of red blood cells in the presence of hydro-methanol extracts of *D. rotundifolia* and *E sagittata* leaves compared to controls.



Negative control: Phosphate-buffered saline (PBS)
Positive control: Distilled water

Fig 4: Rate of hemolysis of red blood cells at different concentrations of hydro-methanol extracts of Negative control (-), *Emilia sagittata* (Vahl) DC., *Dissotis rotundifolia* (Sm.) Triana and Positif control (+)

As presented in Figure 4, the hydro-methanolic extracts of *D. rotundifolia* showed levels of hemolytic activity ranging from 34.2% at a concentration 25 mg/ml to 51.2% at a concentration 100 mg/ml at 60 minutes of incubation. In contrast, the hydro-methanol extracts of *E. sagittata* showed hemolytic activity levels ranging from 20.8% at the 25 mg/ml concentration to 38.7% at the 100 mg/mL concentration at 60 minutes of incubation.

These values suggest that *D. rotundifolia* is more hemolytic than *E. sagittata*; yet no significant difference was observed ($p>0.05$) with Student's t-test. However, it is found that the cytotoxic effect of these extracts is concentration-dependent, as it is related to the increase of the concentrations of the studied extract. Indeed, the concentration of 25 mg/mL of *D. rotundifolia* leads to 34.2% of lysis of red blood cells while that of *E. sagittata* allows only 20.8% of hemolysis. At high concentrations (100 mg/mL), the levels of hemolytic activity reached 51.2% and 38.7% respectively for *D. rotundifolia* and *E. sagitta Because of* w of this finding, the application of *D. rotundifolia* to the mucosa would cause more damage than *E. sagittata*.

In agreement with [21, 22], the results obtained suggest that at high concentrations, *D. rotundifolia* shows toxicity in certain organs. On the other hand, other studies report that *D. rotundifolia* extracts do not have significant effects on red blood cells [23]; they improve testicular histology, enzymatic antioxidant levels and semen parameters [22].

The results of this study, however, indicated that after 60 minutes, the concentration of *E. sagittata* leaf extracts is significantly higher at 50 mg/mL compared to 25 mg/mL ($p<0.05$). However, no significant difference is observed when considering the comparisons made between 25mg/mL and 100mg/mg as well as 50mg/mL and 100mg/mL ($p>0.05$).

These results are in agreement with those [24, 25] regarding the use of blood cells in the determination of the cytotoxic power of plant extracts since they showed high hemolytic effects against human erythrocytes at a high concentration of plant extracts.

The fact that at the same concentration, the rate of hemolysis is different in the presence of the extracts would indicate that *D. rotundifolia* and *E. sagittata* contain phytochemicals that act differently on red blood cells.

Previous phytochemical analyses performed on *D. rotundifolia* leaves revealed the presence of flavonoids, phenols, polyphenols, tannins, anthocyanins, saponins, anthraquinones, cyanogenic glycosides, alkaloids, and cardiac glycosides [21, 26-28]. The presence of high amounts of retinol, tocopherol, cholecalciferol, thiamine, and low amounts of B vitamins (Pyridoxine, niacin, riboflavin, Cobalamin), Vitamin K_1 (Phylloquinone) [29] and Vitamin C (ascorbic acid) in fresh and dry leaves of *D. rontuifolia* [30].

Studies have also revealed the presence of saponins, tannins, alkaloids, flavonoids (flavones), steroids, reducing sugars, phenols [31, 32], oxalate [33], triterpenoids, anthraquinones, coumarins, proanthocyanins, digitoxin (cardiac glycoside) [34] in extracts of *Emilia sagittata* leaves (aqueous, ethanol-water, and ethanol extract).

According to [35], the methanolic extract of *Emilia sagittata* leaves did not induce any observable toxic effects after 24 hours. However, at 1000 mg/kg, the extract significantly shortened the estrous cycle, prolonged the estrous phase, increased uterine weight and altered the histology of uterine and ovarian tissues, and female reproductive hormones, indicating possible antifertility effects in rats. *Emilia sagittata* leaf extracts were found to exhibit anovulatory and estrogenic activities which would support the traditional use of the plant in Nigeria.

Several analyses have revealed that alkaloids, at high doses, are highly toxic to the body although they are beneficial for the treatment of multiple diseases or dysfunctions of the human body [36].

A cytotoxicity study carried out in rats by [37], revealed that the extract of *Emilia sagittata* leaves has an effective potentiality to protect the stomach, muand cous membranes against indomethacin and ethanol induced ulcerations.

Conclusions

This study consisted of the determination of the cytotoxic power of the extracts of leaves of *D. rotundifolia* and *E. sagittata*. A double beam UV- Visible spectrophotometer was used to read the absorbance of the extract solutions at 548nm. This study revealed that the extracts of *D. rotundifolia* and *E. sagittata* leaves exhibit cytotoxic power on red blood cells. The results obtained indicate that at the concentration of 100 mg/ml, the hemolysis rate is 51.2% for *D. rotundifolia* and

38.7% for *E. sagittata*. Although *D. rotundifolia* is more cytotoxic than *E. sagittata*, the statistical analysis shows that this difference is not significant. It was also noted that the rate of red blood cell lysis induced by these extracts increased with increasing concentration and time.

The present study, having been registered with the aim of defining the consequences of the inappropriate use of medicinal plants in intimate hygiene, *D. rotundifolia* and *E. sagittata* could be counted among those with unfortunate consequences. Women who use these plants in vaginal insertion must use them with great care, better to avoid using them regularly and in very concentrated solutions.

The blood cells as a model of choice in the determination of the *in vitro* cytotoxic power of the plant extracts were judicious since it allowed us to obtain good results with ease in the obtaining and the manipulation. The plant being the seat of an intense metabolic activity, the cytotoxicity of the leaves of *D. rotundifolia* and *E. sagittata* would be due to the richness of these plants in the different secondary metabolites that they constitute.

It is necessary to deepen researches by varying the different solvents of extraction and the types of organs of plants used by the women in vaginal insertion; to evaluate the mutagenic effects of these plants.

Références

1. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. Toxicol Appl Pharmacol. 2010;243:198-216.
2. Karunamoorthi K, Jegajeevanram K, Vijayalakshmi J, Mengistie E. Traditional medicinal plants: A source of phytotherapeutic modality in resource-constrained health care settings. Evid Based Complement Altern Med. 2013;18(1):67-74.
3. Ladoh-Yemeda CF, Vandi D, Dibong SD, Mpondo Mpondo E, Wansi JD, Betti JL, *et al*. Étude ethnobotanique des plantes médicinales commercialisées dans les marchés de la ville de Douala, Cameroun, p. 9453, Journal of Applied Biosciences. 2016;99:9450-9468. ISSN 1997–5902.
4. Moroh JL, Bahi C, Dje K, Loukou YG, Guede-Guina F. Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de *Morinda morindoides* (Baker) milne-redheat (*Rubiaceae*) sur la croissance *in-vitro* des souches d'Escherichia coli. Bulletin de la Société Royale des Sciences de Liège. 2008;77:44-61.
5. Gafos M, Mzimela M, Sukazi S, Pool R, Montgomery C, Elford J. Intravaginal insertion in KwaZulu-Natal: sexual practices and preferences in the context of microbicide gel use. Culture, Health and Sexuality. 2010;12(8):929-942.
6. Ngbolua KN, Moke EL, Baya JL, Djoza R, Ashande CM, Mpiana PT. A mini-review on 14. The pharmacognosy and phytochemistry of tropical medicinal plant: *Annona senegalensis* Pers. (Annonaceae). Tropical Plant Research. 2017;4(1):168-175.
7. Kabena NO, Lukoki LF, Mpiana PT, Ngombe KN, Fatiany RP, Robijaona B. Phytochemical Screening of some 16. medicinal plants traditionally used by African Women in Kinshasa city (DR Congo) for their intimate hygiene and Evaluation of the pH of derived recipes. J of Modern Drug Discovery and Drug Delivery Research. 2014;2348-3776. V1I3. DOI: 10.15297/JMDDR.V1I3.04.
8. Adinortey MB, Sarfo JK, Adukpo GE, Dzotsi E, Kusi S, Ahmed MA, *et al*. Acute and sub-acute oral toxicity assessment of hydro-alcoholic root extract of Paullinia pinnata on haemato- logical and biochemical parameters. Biol Med. 2012;4(3):121-5.
9. Gilbert N. Regulations: herbal medicine rule book. Nature. 2011;480(7378):S98-9.
10. Hadjer Rabhi et Bachiri Khawla. Etude de l'activité antioxydante *in vitro* extraites à partir des plantes: *Punica granatum, Olea europaea, Ficus carica*. Master en Sciences de la Nature et de la Vie, Université des Frères Mentouri Constantine, 2016, 23.
11. Ouedraogo Y, Nacouima Guisson IP, Guede Guina FJ. Evaluation *in vivo* et *in vitro* de la toxicité des extraits aqueux d'écorces de tige et de racines de *Mitragyna inermis* (Rubiaceae), 2001.
12. Elalaoui Moulay Driss. Contribution à l'étude phytochimique et l'évaluation de l'effet hémolytique d'extrait brut hydroalcoolique des graines de *Nigella sativa* L., Université Abou Bekr Belkaid —Tlemcen-Faculté des sciences de la nature et de la vie et des sciences de la terre et de l'univers Département de Biologie. 2014;21:27-28
13. Ali-Risasi C, Verdonck K, Padalko E, Vanden Broeck D, Praet M. Prevalence and risk factors for cancer of the uterine cervix among women living in Kinshasa, the Democratic Republic of the Congo: a cross-sectional study. Infect Agent Cancer. 2015;(10):20. DOI: 10.1186/s13027-015-0015-z
14. Abere TA, Onwukaeme DN, Eboka CJ. Pharmacognostic evaluation of the leaves of Dissotis rotundifolia Triana (Mela- stomataceae). Afr J Biotechnol. 2009;8(1):113-5.
15. Addo-Fordjour P, Obeng S, Anning AK, Addo MG. Floristic composition, structure and natural regeneration in a moist semi- deciduous forest following anthropogenic disturbances and plant invasion. Int J Biodivers Conserv. 2009;1(2):21-37.
16. Teke GN, Kulate JR, Ngouateu OB, Gatsing D. Antidarrhoeal and antimicrobial activities of Emilia coccinea (Sims) G. Don extracts. J of Ethnopharm. 2007;112(2):278-283.
17. Kabena NO, Katunda MR, Bikandu KB, Botefa IC, Ngombe KN, Pius Mpiana T, *et al*. Ethnobotanical study of plants used by Pygmies for reproductive health in Mbandaka and surrounding areas / Equateur Province, DR Congo. 2018;36(1):21-24.
18. Elalaoui Rachida. Contribution à la recherche de l'effet hémolytique à partir d'extraits *Berberis vulgaris* L., Mémoire de fin d'études en vue de l'obtention du diplôme de Master en Biologie, Option Biochimie appliquée, Université Abou Bekr Belkaïd –Tlemcen, 2015, 5-11.
19. Kherbouche Hanane. Evaluation de l'activité cytotoxique des extraits de plantes: *Fredolia aretioides* et d'*Echium vulgare* vis-à-vis des globules rouges humain, Mémoire de l'Université Abou Bekr BelkaÏd –Tlemcen, 2014, 17.
20. Haoulia Amina. Tests phytochimiques, dosage et recherche d'effet hémolytique des polyphénols totaux extraits de la partie aérienne d'Ammoïdes verticillata, 2015, 4.
21. Abere Tavs A, Pius E Okoto, Freddy O Agoreyo. Antidiarrhoea and toxicological evaluation of the leaf extract of *Dissotis rotundifolia* triana (Melastomataceae), BMC Complementary and Alternative Medicine. 2010;10:71, 2.

22. Makanjuola VO, Tams GE, Ippiniwa DA. The effect of methanolic extract of *Dissotis Rotundifolia* on cadmium induced testicular damage in whistar rats. IOSR, Journal of Pharmacy. 2014;4:56-65.
23. Ansah Charles, Michael Buenor Adinortey, Jerry Asiedu-Larbi, Benjamin Aboagye, Du-Bois Asante, Alexander Kwadwo Nyarko. *In vivo* assessment of the toxic potential of *Dissotis rotundifolia* whole plant extract in Sprague–Dawley rats; Asian Pac J Trop Biomed. 2016;6(7):574-579.
24. Elalaoui Rachida. Contribution à la recherche de l'effet hémolytique à partir d'extraits *Berberis vulgaris* L., Mémoire de fin d'études en vue de l'obtention du diplôme de Master en Biologie, Option Biochimie appliquée, Université Abou Bekr Belkaïd –Tlemcen, 2015, 5-11.
25. Bensalah Fouzia. Contribution à l'étude phytochimiques et l'effet hémolytique de l'extrait brut hydroalcoolique de la partie aérienne de *Marrubium vulgare* L., 2014, 6-10.
26. Balogun OS. Phytochemical and antimicrobial investigation of *Dissotis rotundifolia* (sm) Triana, Natural Product, an Indian journal. Volume 9 Issue 2, NPAIJ. 2013, 9(2):77-80. ISSN: 0974-7508.
27. Aja PM, Alum EU, Ezeani NN, Ibiam UA, Egwu C. Comparative Phytochemical Evaluation of *Dissotis rotundifolia* Root and Leaf, Ebonyi State University, Abakaliki, Nigeria, 2015, 422.
28. Kweku Darkwah Williams, Micheal Adinortey and Alexander Weremfo. Phytochemical Constituents and Aromatic Content of Methanol and Aqueous Extracts of Dissotis rotundifolia Whole Plant; South Asian Research Journal of Natural Products. 2018;1(1):1-5. Article no. SARJNP.39334
29. Offor CE. Determination of vitamin composition of *Dissotis rotundifolia* leaves. International Journal of Current Microbiology and Applied Sciences. 2015;4(1):210-213.
30. Okeri HA, Alonge PO. Determination of the ascorbic acid content of two medicinal plants in Nigeria. Pakistan journal of pharmaceutical sciences. 2006;19(1):44-48.
31. Nwachukwu Viola A, Stanley Udedi C, Ezeonu FC, Bartholomew Brai IC, Chika Scholastica Ezeanyanaso, Gloria Nwakaego Elemo. Bioactive Agents, Nutraceuticals Potentials, Phytochemistry, and Food Value of *Emilia coccinea* Leaf: A Review, Journal of Complementary and Alternative Medical Research. 2017;4(1):1-15. Article no. JOCAMR.29435, ISSN: 2456-6276
32. Nwachukwu Viola A, Udedi SC, Ezeonu FC, Orji FA, Ezeanyanaso CS, Bartholomew Brai IC, *et al*. An Analysis of Food Value and Some Selected Secondary Metabolites of *Emilia coccinea* (Asteraceae) Leaf. Journal of Complementary and Alternative Medical Research. 2017;2(2):1-11. Article no.JOCAMR.29763
33. Unegbu CC, Ajah O, Amaralam EC, Anyanwu OO. Evaluation of phytochemical contents of Emilia coccinea leaves. Journal of Medicinal Botany. 2017;1:47-50.
34. Ondo Joseph Privat, Louis-Clément Obame, Gontran Nsi Akoue, Jean-Pierre Medzo Engono, Soulemane Parkouda, Edouard Nsi Emvo et Jacques Lebibi, 2013. Studies on phytochemical screening, total phenolic content and antiradical activity of three extracts of Emilia sagittata DC. (Asteraceae). International Journal of Biosciences. 2013;3(4):50-57.
35. Elvis-Offiah *et al*., 2016.
36. Mohammedi Zohra. Etude Phytochimique et Activités Biologiques de quelques Plantes médicinales de la Région Nord et Sud-Ouest de l'Algérie, Thèse de Doctorat en Biologie, Université Abou Bekr Belkaid, 2013, 24pp.
37. Eweka KI, Oviawe O, Aiworo CN. Analysis of the Effects of Ethanol Extract of Emilia Coccinea on Induced Gastric Ulcer in Rats, 2017.