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Phytochimic screening and cytotoxic, anti-hemolytic and antioxidant activities of leaf extracts of *Tithonia diversifolia* (Hemsl.) a gray

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Abstract
Background: *Tithonia diversifolia* (Hemsl.) A Gray is an invasive plant species belonging to the Asteraceae family. It is native to Mexico, *Tithonia diversifolia* has been introduced to Africa and Asia as an ornamental plant. In Cameroon, this plant is known as the "jealousy flower" and considered as a bad plant. Traditionally, different peoples around the world use this plant for medicinal purposes. For example, it is used as antimalarial, antidiabetic, anti-inflammatory, wound healer, and to certain skin diseases and menstrual pain. Today, there is an increase in the incidence of cancer, metabolic, cardiovascular, neurodegenerative diseases. Oxidative stress is one of the factors that contribute to the occurrence of these diseases.
Objective: This study was to identify secondary metabolites and evaluate the antioxidant and anti-hemolytic activity of leaf extracts of *Tithonia diversifolia*.
Methods: The leaves of *Tithonia diversifolia* were collected in Nkoldongo, an area in Yaoundé, the capital city of Cameroon. The extracts were obtained by maceration in distilled water, ethanol/water mixture (70/30) and 95% ethanol. The DPPH and FRAP methods were used to evaluate the in-vitro antioxidant, anti-hemolytic and cytotoxicity activities of the extracts. The cytotoxic profile of the extracts was determined by evaluating the inhibition percentage of normal cell growth (*Vero* cells). Their anti-hemolytic activity was evaluated by the protection percentage of erythrocytes against hemolysis.
Results: Phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, alkaloids, tannins, saponosides, coumarins and terpenoids in the plant extracts. All the extracts showed good antioxidant activity with the hydroethanolic extract having the highest anti-free radical power with an IC_{50} of 14.882 µg/ml. Hydroethanolic extract had also shown a good ability to protect red blood cells.
Conclusion: Being rich in active metabolites, *Tithonia diversifolia* may offer high therapeutic potentials.

Keywords: *Tithonia diversifolia*, secondary metabolites, antioxidant, antihemolytic, *Vero cells*

Introduction
Plants are photosynthetic organisms capable of producing substances primary metabolites (proteins, carbohydrates and lipids) and secondary metabolites. The secondary metabolites differ depending on the plant species and are involved in the growth or in the defense mechanisms of the plant and its environment [1]. They are probably essential elements of coevolution of plants with living organisms such as parasites, pathogens and predators but also pollinators and disseminators. These different relationships have led to an extreme diversification of secondary compounds [2]. Secondary metabolites constitute a variety of useful substances for the treatment of certain human diseases. This may explains why even today, most people in Africa rely on herbal medicine for treatment. According to the World Health Organsation, more than 80% of African populations use traditional medicine and pharmacopoeia for their primary health care [3, 4]. For decades, plants have been recognised as an important source of biomolecules with several therapeutic properties. However, less than 10% of the 250 000 plant species recorded in the world's flora have been investigated for their therapeutic activities [5]. Therefore, more than 90% of the flora remains unexplored, especially in tropical areas like Cameroon where several plant families such as *Tithonia diversifolia* abound.
Tithonia diversifolia is native to Mexico (Sunflower of Mexico); it is very invasive and commonly called "jealousy flower" in Cameroon. Perennial or annual shrubby plant, it belongs to the family Asteraceae [6]. In Cameroon, it is mostly perceived as a weed because of its high invasiveness, yet it has great therapeutic properties that could be exploited for agricultural, livestock feeding and medicinal purposes. Traditionally, all parts of this plant, particularly the leaves are widely used for the treatment for several disease conditions such as wounds,

malfunctions of the musculoskeletal system, abscesses, dermatological problems but also diabetes, malaria, hepatitis and infectious diseases [3].

Today, there is an increase in the incidence of chronic diseases such as cardiovascular diseases, cancer, and diabetes known to have a negative impact on human health [7]. The accumulation of free radicals in the body remains an important epidemiological factor for oxidative stress-based diseases such as cardiovascular, metabolic, rheumatic, neurodegenerative, and cancerous diseases [8]. Free radicals are wastes from cellular metabolism with the ability to attack cell structures including fatty acids, proteins, and nucleic acids [9]. Red blood cells are among the most sensitive cells to free radicals. In fact, during oxidative stress, free radicals induce early aging of red blood cells thereby promoting their abnormal destruction, which may lead to hemolytic anemia. One of the ways to address these oxidative stress-related diseases lie in more in-depth research on medicinal plants to promote their therapeutic properties including antioxidant activities in the prevention and fight against oxidative stress.

This triggered our interest in evaluating the phytochemical, cytotoxic, anti-hemolytic and antioxidant activities of the leaf extracts of *Tithonia diversifolia*.

Materials and Methods

Plant material

The leaves of *Tithonia diversifolia* were harvested in the city of Yaoundé more precisely in the locality of Nkolndongo during the month of December 2018. The plant was identified with the Cameroon National Herbarium. The leaves were dried for two weeks under the shade and ground into a fine powder.

Extract preparation

The dry powdered leaves of *T. diversifolia* (500 g) was macerated with 2 L of each of the solvents (distilled water, hydroethanol mixture, 95 % ethanol) at room temperature for 72 h with solvent replacement every 24 hours. The macerates were filtered successively using the No. 4 coffee filter paper. The aqueous filtrate was oven dried at 45 °C while the ethanol and ethanolic filtrates were first allowed to evaporate under reduced pressure (Rotavapor) before oven drying. Finally, the dry extracts were weighed to determine the extraction yield on the basis of 100 g of dry matter.

Phytochemical Screening

The chemical screening was carried out to verify the presence of phenols, flavonoids, tannins, alkaloids, saponosides, steroids, terpenoids and coumarins. Phytochemical screening was performed according to the method of Bruneton (1999) [10].

Evaluation of antioxidant activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging method DPPH (2,2-diphenyl-1-picrylhydrazyl) was one of the first free radicals used to study the structure-antioxidant relationship of phenolic compounds [10, 12].

The reduction of the radical DPPH • (2,2-diphenyl-1-picrylhydrazyl) was evaluated according to the modified protocol described by Bassene (2012) [13].

The extracts were diluted to obtain the following final concentrations of 250 extracts; 125; 62.5; 31.25; 15.625; 7.8125; 3.90625; 1.953125 and 0.9765625 µg/ml. In a 96-well microplate, 100 µl of ethanol were introduced into all the wells except that of line 1. Thereafter, 200 µl of the different extracts were introduced into the wells of line 1 and then 100 µl of each extract of the line 1 were removed to make second-order dilutions of concentrations ranging from (250 to 0.97µg / mL) from line 2 to 10. Line 11 was the negative control (without extract). Each dilution (25µl) was added to a new microplate to which 75µl of the 0.01% DPPH solution were added. Optical densities were read at 517 nm after 30 minutes of incubation in the dark at room temperature. The negative control consisted of DPPH without extract and the positive control (ascorbic acid) considered as extract. The tests were performed in duplicates. The results were expressed as inhibition percentage according to the following formula:

$$PP\ \% = \left[\frac{(Ac - Ae)}{Ac}\right] * 100$$

Where PP = trapping percentage
Ab = Absorbance of the negative control
Ae = Absorbance of the sample
The results were expressed in IC_{50}; EC_{50} and PA values were deduced where:
IC_{50} (inhibitory concentration 50) = Concentration to trap 50% of free radicals
The IC_{50} is obtained from the graph representing the trapping percentage (% PP) as a function of the concentration (µg /ml) of the extract.
EC_{50} (Effective concentration 50) = this is the concentration of extract necessary to trap half a mole of DPPH and is calculated by the following formula:

$$CE50 = \frac{CI50}{[DPPH]}$$

The anti-free radical power (PA) is the opposite of the effective concentration; the higher it is, the better the anti-radical power of the extract is and is given by the following formula:

$$PA = \frac{1}{CE50}$$

Feric Reducing Antioxidant Power (FRAP) method

The Fe^{3+} reduction test was performed according to the modified protocol described by Path Canada (1994) [14].

In a 96-well microplate, 100 µL of solvent (ethanol) were introduced into all the wells except those of the first line 1. Then, 200 µL of the extracts were introduced into the wells of line 1; a serial dilution was carried out until line 10. Then, 25µl of each dilution were introduced into a new microplate with 25µl of Fe^{3+} solution at a concentration 1.2 mg/ml; the mixture was incubated for 15 min. After incubation, 50 µl of 0.2% ortho Phenantroline solution were added and the plate was re-incubated for 20 minutes again at room temperature. At the end of this incubation, the optical densities of the wells were read at 505 nm. The negative control representing 0% reduction was made up of the solvent, Fe^{3+} and orthophenantroline while the positive control corresponding to 100% reduction was made up of the hydroxylamine acting as an extract. The results were expressed as reducing power (OD) as a function of the concentration of extract.

Antihemolytic activity

Evaluation of cytotoxic activity

The cytotoxicity activity of the plant extracts was carried out on the Vero cell line according to the colorimetric method

with MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide) as described by Mosmann [15].

Cell culture

Vero cells were maintained in culture in 75 cm2 T-Flask containing DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) and 1% streptomycin-penicillin antibiotic, standard condition with 5% CO2 at 37 °C. The medium was renewed every 3 days after cultivation.

The cells were detached by introducing 5 ml of 0.25% trypsin-EDTA into the T-Flask after triple rinsing with PBS (Phosphate Buffer Saline). After 5 minutes of incubation at 37 °C, the detached cells were centrifuged at1800 rpm for 3 minutes and the resulting pellets were suspended in 1 ml of medium. The suspension (20 µl) was added to 20 µl of trypan blue solution in order to evaluate cell viability and the calibrated cell load via the Neubauer hematimeter.

Evaluation of cytotoxicity at 1000 µg/mL

In a 96-well microplate, 100 µl of cell suspension loaded with 5×10^4 cells / wells were introduced into all the wells. At the end of a 24h incubation period, the medium contained in the wells was removed and 90 µl of a new medium were introduced, then 10 µl of the extracts at a concentration of 1000 µg /ml were also added. The plates were incubated for 48 h under a humidified atmosphere at 37 °C and 5% CO2. The positive control consisted of 90 µl of cell solution plus 10 µl of DMSO (10%) while the negative control consisted of 100 µl of the cell solution. At the end of 48 hours of incubation, 20 µl of MTT solution (Sigma) were introduced into each well and the whole mixture was homogenised and incubated for 4 hours at 37 °C. At the end of this incubation period, the content of the wells was carefully drained, replaced with 100 µl of DMSO in order to dissolve the formazan deposit. The latter was quantified by reading the optical density at 570 nm using the TECAN plate reader (Infinite M200).

Determination of the cytotoxic concentration 50 (CC_{50})

The cytotoxic extracts at 1000 µg/mL were diluted to obtain the following final concentrations of extracts: 500; 250; 125; 62.5; 31.25; 15.625µg/ml.

In a 96-well microplate, 100 µl of culture medium were introduced into all the wells except for line 1. Then, 180 µl of culture medium plus 20 µl of extracts were introduced into the wells of line 1; then, 100 µl of the content of line 1 were removed to make second-order dilutions of concentrations ranging from (500 to 15.625 µg/ml) from line 2 to 7; the content of the tip was latter discarded. The experiment was done in triplicates.

From the optical densities, the percentages of viable cells were calculated using the formula below:

$$\text{Inhibition}\% = \frac{\text{DO positive control} - \text{DO test}}{\text{DO positive control}} * 100$$

Cytotoxic concentrations (CC_{50}) were determined using the Statgraphics software. Following the American National Cancer Institute (NCI) criteria, an extract is considered cytotoxic if CC_{50} on the host cells is less than 30 µg/ml [16].

Evaluation of anti-hemolytic activity

Isolation of red blood cells from albino rats

The antihemolytic activity of the extracts was evaluated using hydrogen peroxide (H_2O_2) to induce hemolysis following the protocol described by Singh and Rawal (2012) [17].

Before the rats were sacrificed, they were first exposed to ether for a few seconds for stunning purpose. The rats were then sacrificed and blood was collected from the ventricle with a syringe. The collected blood was distributed into sterile heparinised tubes and centrifuged at 1500 rpm for 5 min. After centrifugation, the supernatant was discarded and the erythrocytes were washed three times with 0.9% NaCl solution. A suspension of red blood cells was made from the erythrocytes in order to adjust the hematocrit to 10%. The suspension was kept at the refrigeration temperature (4 °C).

Principle

Before starting the experiment, serial of dilutions of the extracts were performed. It was a second order dilution to achieve the following concentrations: 1000; 500; 250; 125; 62.5 µg / ml. Thus, at 0.5 ml of each of extract and at different concentrations, 0.25 ml of the erythrocyte suspension (10% hematocrit) was added and incubated for 5 min at room temperature. After incubation, 0.25ml of 0.3% H_2O_2 solution was added and then incubated again for 4 hours at 37 °C. After incubation, the mixture was centrifuged at 2500 rpm for 10 min and then 100 µl of the supernatant were transferred to a microplate and the absorbance corresponding to the release of hemoglobin was measured at 540 nm using the plate reader (TECAN Infinite M200 Flat Reader). The negative control consisted of 0.9% sodium chloride and erythrocyte while the positive control consisted of 0.9% sodium chloride, erythrocyte and hydrogen peroxide. The results were expressed as follows:

$$\text{Hemolysis } \% = \left(\frac{\text{AE}}{\text{AC}}\right) * 100$$

Where: AE is the absorbance of the sample

AC is the absorbance of the positive control (complete hemolysis)

Protection % = 100-Hemolysis %

Results

Extraction efficiency

The extraction yields of the plant extracts are described in Figure 1 below. The extraction yields varied based on the solvents used during extraction. The highest extraction yield (6.74%) was recorded by the aqueous extract followed by the hydroethanolic extract (6.058%); the ethanol extract had the least extraction yield (4.196%).

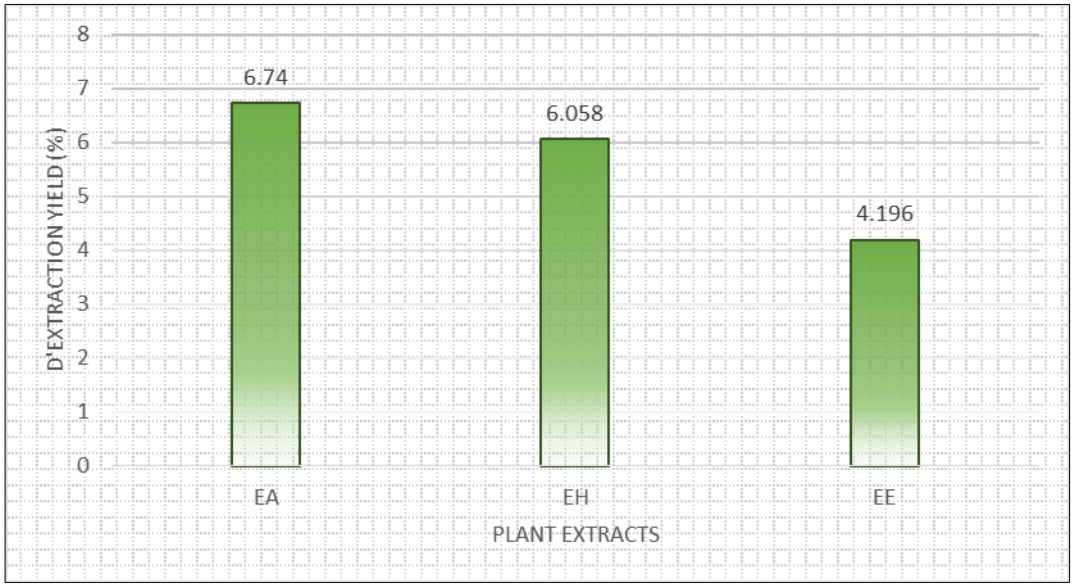

Fig 1: Extraction yield of the leaves of *Tithonia diversifolia*
EA= Aqueous extract; EH = Hydroethanolic extract; EE= Ethanol extract

Phytochemical screening

Table 2 below illustrates the different classes of secondary metabolites present in the plant extracts that may be responsible for the antioxidant activity. Results indicate that steroids were absent in all the three extracts whereas coumarins were present only in the aqueous extract. Moreover, saponosids and terpenoids were absent only in the ethanol extract and aqueous extract, respectively.

Table 1: Phytochemical screening of leaf extracts of *Tithonia diversifolia*

Secondary metabolites	Leaf extracts of *Tithonia diversifolia*		
	Aqueous extract (EAF)	Hydro-ethanolic extract (EHF)	Ethanol extract (EEF)
Polyphenols	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Coumarins	+	-	-
Alkaloids	+	+	+
Saponosids	+	+	-
Terpenoids	-	+	+
Steroides	-	-	-

+: Present □: Absent

The antioxidant activity of plant extracts

Free radical sequestration by DPPH

Figure 2 below describes the anti-radical activity of the plant extracts. Results show that at a concentration equal to 250 µg/ml, the hydro-ethanolic extract registered the highest anti-radical activity (65.34%) followed by the aqueous extract (60.21%) and lastly by the ethanol extract (59.02%).

On the other hand, Figure 3 describes the IC_{50} values (Inhibitory concentration 50) of the plant extracts. Results indicate that the aqueous and ethanol extracts had similar IC_{50} values (30.77 and 30.60 µg/ml, respectively) whereas the hydro-ethanolic extract recorded the least IC_{50} value (14.88 µg/ml).

Table 2 described other parameters of antiradical activity such as CE_{50} and PA. The results in Table 2 show that the hydroethanolic extract had the highest anti-radical power (67.2×10^{-5}) followed by the ethanol extract (41.9×10^{-5}) and the aqueous extract registered the least anti-radical power (32.5×10^{-5}). The anti-radical power values of extracts were inversely proportion to the their CE_{50} values (Table 2).

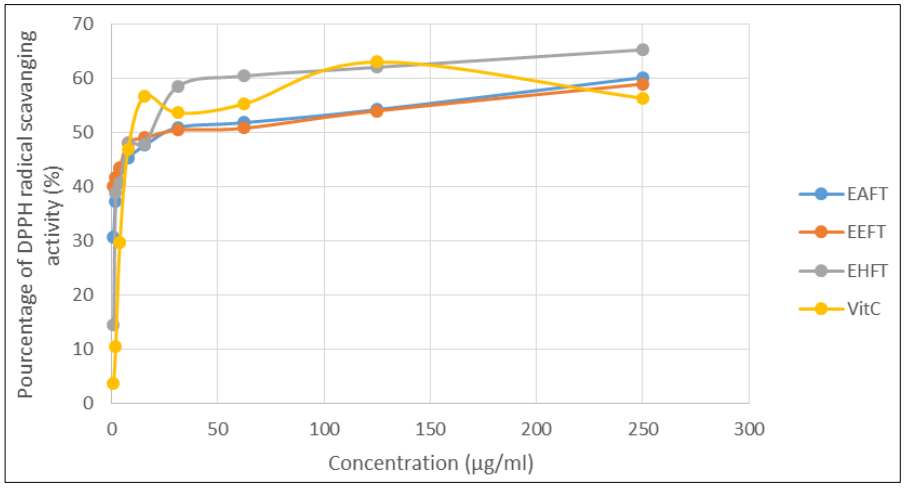

Fig 2: Antioxidant activity by capturing the free radical DPPH
EAFT = Aqueous leaf extract; EEFT = Ethanol leaf extract; EHFT = Hydro-ethanolic leaf extract; Vit C = Vitamin C (Ascorbic acid).

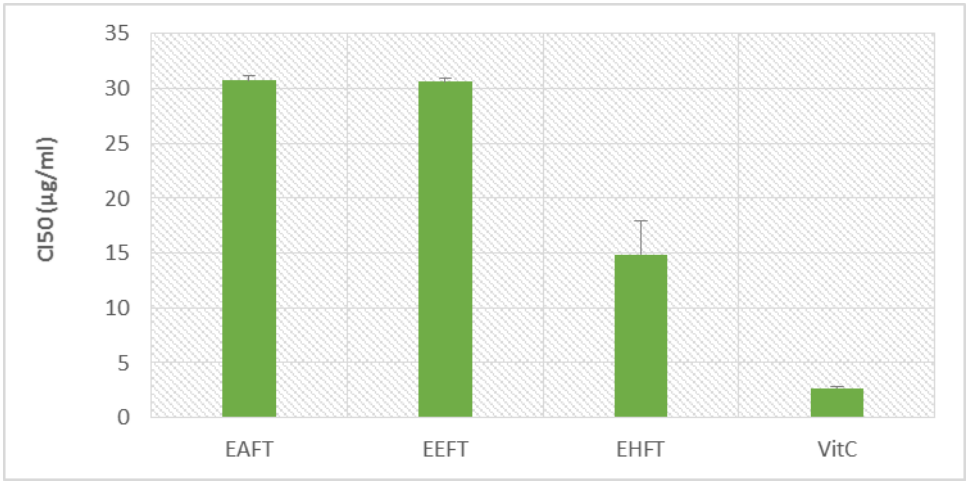

Fig 3: The IC_{50} values (Inhibitory concentration 50) of the extracts and vitamin C
EAFT = Aqueous leaf extract; EEFT = Ethanolic leaf extract; EHFT: hydro-ethanolic leaf extract; Vit C = Vitamin C (ascorbic acid).

Table 2: Parameters for antiradical activity of the various extracts and vitamin C

Extract	IC_{50} (µg/ml)	CE_{50} x 10^3 (µg/ml)	PA x 10^{-5}
EAFT	30.770 ± 0.370^c	3.077 ± 0.037^c	32.5 ± 0.389^c
EEFT	30.600 ± 0.310^c	3.060 ± 0,031^c	41.9 ± 26.591^c
EHFT	14.882 ± 3.048^b	1.488 ± 0,304^b	67.2 ± 14.466^b
Vit C	2.710 ± 0.080^a	0.271 ± 0,008^a	368.9 ± 11.088^a

EAFT = Aqueous leaf extract; EEFT = Ethanolic leaf extract; EHFT: hydroethanolic leaf extract; VitC: Vitamin C (ascorbic acid)

FAP (Reduction activity of Fe^{3+}) method

The Fe^{3+} Reduction activity of plant extracts was described in Figure 4 below. At concentration equal to 500, the aqueous extract exhibited the highest Fe^{3+} Reduction activity (OD = 0.99) followed by the hydro-ethanolic extract (OD = 0.84). The least Fe^{3+} Reduction activity was recorded by the ethanol extract (OD = 0.78). Results in Figure 4 shows that the absorbance of the Fe^{2+}-orthophenantroline complex increased with the concentration of the plant extract.

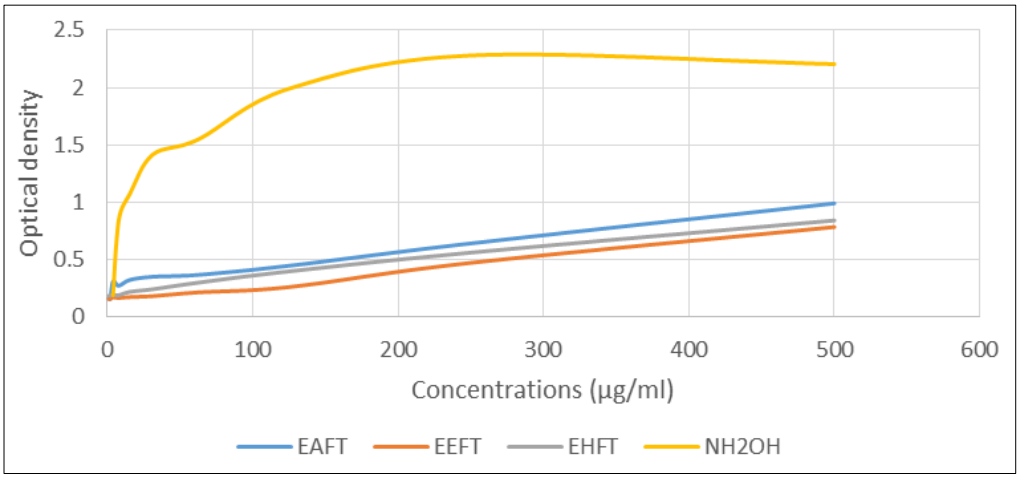

Fig 4: Absorbance of the Fe^{2+} -orthophenantroline complex as a function of the concentration of plant extracts
EAFT = Aqueous leaf extract; EEFT =Ethanolic leaf extract; EHFT= hydroethanolic leaf extract; Vit C =Vitamin C (ascorbic acid)

Thus, a high absorbance reflects the presence of high level of Fe^{2+} -orthophénantroline complex thereby indicating a strong reducing activity of extracts.
However, the positive control (hydroxylamine) had a Fe^{3+} reducing capacity greater (OD = 1.99 at a concentration equal to 125 µg/ml) than that of the extracts.

Anti-hemolytic activity

The evaluation of cytotoxicity

The cytotoxic profile of plant extracts on the Vero cells was presented in Figure 5 below. Results reveal that at a concentration of 1000 µ/ml, all the plant extracts were toxic except the aqueous extract. At this very concentration, the cell inhibition capacity of ethanol extract was higher than that of hydroethanol extract. Moreover, the results of the serial dilutions of plant extract capable of lysing 50% of cell are also described in Figure 5.

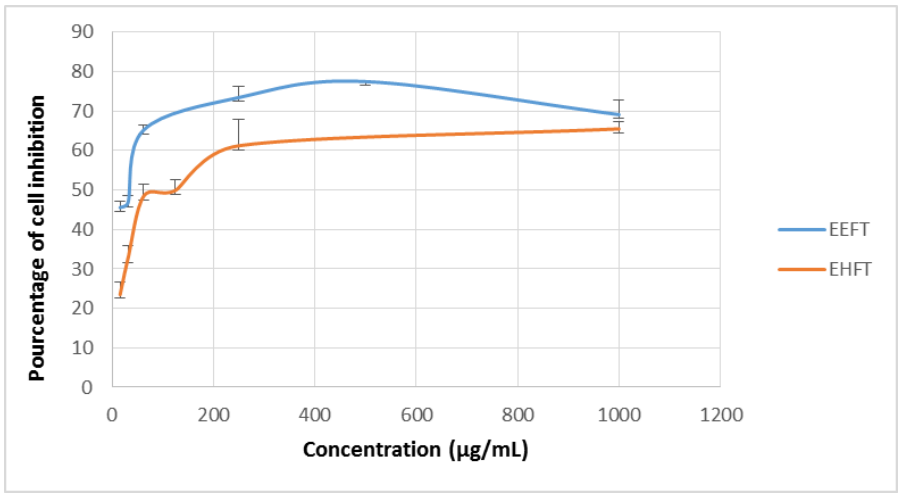

Fig 5: Percentage inhibition of *Vero* cells as a function of the concentration of cytotoxic extracts.
EEFT =Ethanol leaf extract; EHFT = Hydro-ethanolic leaf extract

The curve profile in Figure 4 above shows that the percentage of cells inhibition increased as the concentration of extract increased.

Results in Table 3 below describe the cytotoxic concentration 50 of the plant extracts on *Vero* cells. The CC_{50} values on *Vero* cells registered by the aqueous and hydroethanol extracts were above 30 µg/ml whereas those recorded by the ethanol extract was below 30 µg/ml indicating the cytotoxicity of the latter.

Table 3: The cytotoxic concentration 50 (CC_{50}) of extracts

Plant part	Extract	CC_{50} (µg/ml)
Leaves (FT)	Aqueous extract (EA)	> 1000
	Ethanol extract (EE)	17.675 ± 1.4
	Hydro-ethanolic extract (EH)	112 ± 0.7

Anti-hemolytic activity of plant extracts

The protection ability of the extracts on the red blood cell membrane are presented in Figure 6 & 7. Results in Figure 6 indicate that the aqueous leaf extract of *Tithonia diversifolia* at different concentrations, had greater hemolysis percentages than the cell protection percentages.

The cell protection percentage of hydro-ethanolic extract decreased as its concentration decreased. Hemolysis occurred highest at the lowest concentration (62.5 µg/ml) for the hydro-ethanolic extract.

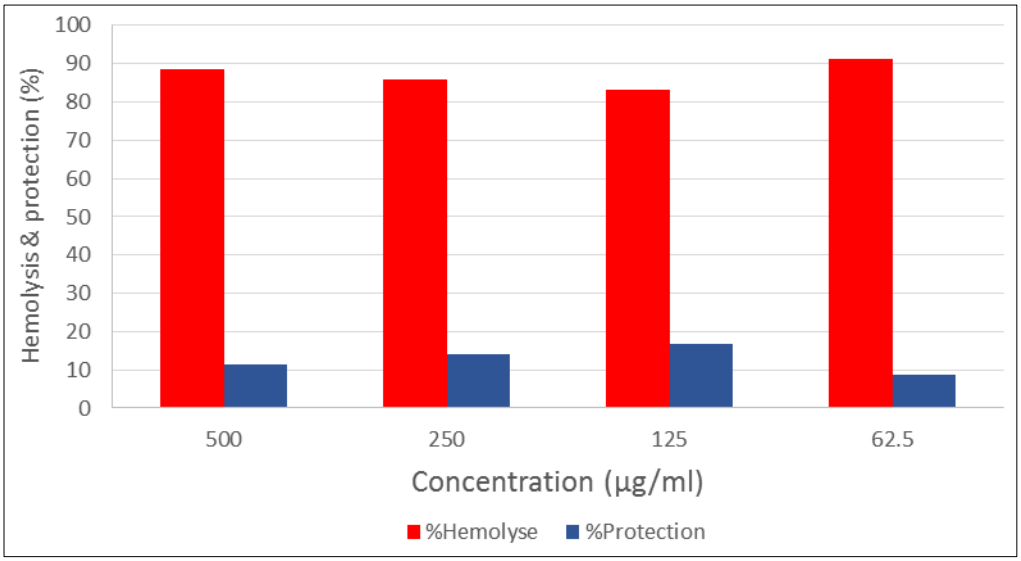

Fig 6: Protection and hemolysis percentages of the aqueous leaf extract

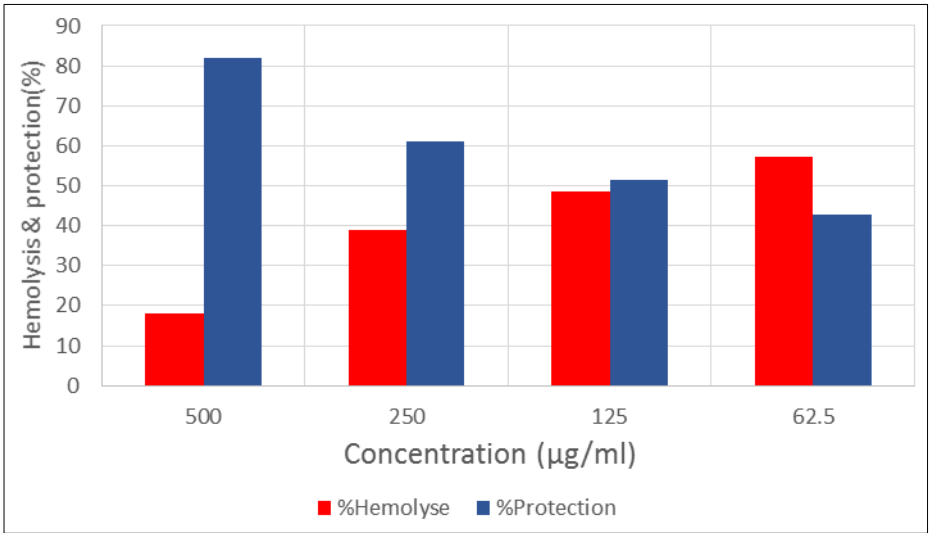

Fig 7: Protection and hemolysis percentages of the hydro-ethanolic extract

Discussion

The highest extraction yield (6.74%) recorded by the aqueous extract underlines the fact that distilled water is the solvent that enables maximum extraction. This could be explained by the fact that most of the constituents of the plant might be polar and therefore more soluble into a polar solvent such as water.

Our results are superior to those obtained by Olanyika and others who reported extraction yields of 1.4% and 1.2% for the ethanol and aqueous extracts, respectively [18]. This may be due to differences in the maceration time and quantity of the plant material used for extraction.

Our study revealed almost similar phytochemical composition for the aqueous and ethanol extracts except that coumarins were only present in the aqueous extract; while terpenoids were absent in the aqueous extract, saponosids were absent in the ethanol extract. In contrast, Olanyinka and his teammates reported that both aqueous and ethanol extracts had all the classes of secondary metabolites except coumarins and steroids [18]. The absence of steroids was also noticed in all the extracts used in the present study.

Results indicate that the aqueous and ethanol extracts had similar IC_{50} values (30.77 and 30.60 µg/ml, respectively) whereas the hydro-ethanolic extract recorded the least IC_{50} value (14.88 µg/ml). The anti-radical power values of extracts were inversely proportional to the values of their CE_{50} (Table 2), that is the smaller the IC50, the greater the antiradical power.

The results in Table 2 show that the hydro-ethanolic extract had the highest anti-radical power (67.2×10^{-5}) followed by the ethanol extract (41.9×10^{-5}) and the aqueous extract registered the least anti-radical power (32.5×10^{-5}).

These results are different from those of Mayara and others who obtained higher IC_{50} values (2273 µg/ml and 630 µg/ml) for the leaf aqueous and ethanol extracts of *Tithonia diversifolia* [19]. These differences could be related to the extraction procedures, the concentration of ethanol used for extraction and the geographical variation of plant materials. However, like in the present study, they also concluded that the ethanol extract had the highest antioxidant power. Generally, the antioxidant activities of our extracts might be attributed mostly to phenolic compounds. Polyphenolic compounds are known to exhibit a high antioxidant potential due to their hydroxyl groups and protect effectively against certain free radical-related diseases [20]. Natural phenolic compounds can directly capture free radicals and break down oxidative reactions; they can also chelate pro-oxidants such as transition metal ions that stimulate the formation of free radicals [21] and play an important role as catalysts for the oxidation processes, leading to the formation of hydroxyl radicals and to hydroperoxide decomposition reactions by the Fenton reaction [22].

The absorbance of the Fe^{2+}-orthophenantroline complex increased with the concentration of the plant extract and the aqueous extract exhibited the highest Fe^{3+}Reduction activity (OD = 0.99) followed by the hydro-ethanolic extract (OD = 0.84). A high absorbance reflects the presence of high level of Fe^{2+}-orthophenantroline complex thereby indicating a strong reducing activity of extracts. Similarly, Barboza and colleagues showed that the leaf extract of *T. diversifolia* exhibited with sodium chloride a relatively higher Fe^{3+} iron-reducing activity [23].

While ethanol extract was toxic (CC_{50} of 17.675 µg/ml) and hydroethanolic extract less toxic (CC_{50} of 112 µg/ml), the aqueous extract was non-toxic (CC_{50} > 1000 µg/ml). This is based on the criterion defined by the American National Cancer Institute (NCI) [16]. Equally, Omokhua and others when evaluating the in-vitro cytotoxicity of leaf extracts of *T. diversifolia*, concluded that only the aqueous extract was not toxic on VERO cells [24]. The cytotoxicity test is crucial to guide in the therapeutic use of medicinal plants vis-à-vis the consumers. This explains why only the less or non-toxic extracts are always chosen for anti-hemolytic activity.

Of the two extracts selected for testing cell protection, only the hydro-ethanolic extract showed good anti-hemolytic activity in comparison with the aqueous extract. The inability of the aqueous extract to protect red blood cells may be due to its rich saponosides content. Saponosides have been known to be toxic to red blood cells. Saponins have a hemolytic effect, resulting from their interaction with the sterols of the erythrocyte membrane. The interaction induces an increased membrane permeability to sodium and the water, causing cell burst due to a high osmotic pressure. This justifies why saponins are never administered intravenously [25].

On the other hand, the high red blood cells protection capacity of hydro-ethanolic extract against hydrogen peroxide correlated positively with its antioxidant power. A strong antioxidant activity protects the membrane of red blood cells against lesions and delays time of hemolysis. The anti-hemolytic effect of phenolic compounds may also explain the

good red blood cell protection power of hydro-ethanolic extract. Phenolic compounds are known to prevent methemoglobin and hydroxyl radical formation by trapping hydrogen peroxide thereby protecting red blood cells against oxidative damage and the production of hemolysis [26]. The mechanism of action of the hydro-ethanolic extract does not only consist in inhibiting free radicals, but also favours the stability of the erythrocyte membrane. Many authors have also demonstrated that flavonoids can be incorporated into the erythrocyte membrane and improve its stability [21].

In sum, a good part of the antioxidant activity of hydroethanolic extract of *T. diversifolia* could be attributed to polyphenols and flavonoids. Our results have shown that this plant has a good protective and stabilising effect on the cell membrane, and may possess anti-atherogenic properties [21].

Conclusion and perspectives

The antioxidant and antihemolytic activities of leaf extracts suggest that *Tithonia diversifolia* is a plant rich in active metabolites with extraction yields of 6.74%; 6.06% and 4.2% for the aqueous, hydroethanolic and ethanolic extracts respectively. Phytochemical profile revealed the presence of total phenols, flavonoids, tannins, saponosides, terpenoids, alkaloids and coumarins in the plant extracts. The rich polyphenol and flavonoids content of the plant extract may explain the antioxidant power of *T. diversifolia*.

All the plant extracts exhibited a strong antiradical power and ferric iron reducing power. Based on their antihemolytic activity, only the hydroethanolic extract had a good ability to protect the membrane integrity of erythrocytes with protection percentages greater than 80% at a concentration of 500 µg/ml. The presence of phenolic compounds such as flavonoids, polyphenols and tannins in the extracts of *T. diversifolia* provides this plant with pharmacological properties thereby supporting its traditional use for the relief of various diseases.

Finally, given the results obtained in the present study, as future perspective, we intend to evaluate some biological activities such as the Fe^{2+} iron chelating activity, isolation of active compounds and their synergistic actions, in-vivo studies for better understanding of molecular and cellular mechanisms of the biological activities of the plant extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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