International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2024; 12(1): 13-19

Received: 14-11-2023
Accepted: 21-12-2023

Kouadio Kouassi Blaise
Laboratoire de Sciences et Technologies de l'Environnement, UFR Environnement, Université Jean LOROUGNON GUEDE, Daloa, BP 150 Daloa, Côte d'Ivoire

Douhore Gnaoré Yoh Toussaint
Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix HOUPHOUËT-BOIGNY, 22 BP 582 Abidjan 22, Côte d'Ivoire

Ouattara Logopho Hyacinthe
UPR de Chimie Organique, Département de Mathématiques-Physique-Chimie, UFR Sciences Biologiques, Université Peleforo GON COULIBALY, Korhogo, BP 1328 Korhogo, Côte d'Ivoire

Kablan Ahmont Landry Claude
UPR de Chimie Organique, Département de Mathématiques-Physique-Chimie, UFR Sciences Biologiques, Université Peleforo GON COULIBALY, Korhogo, BP 1328 Korhogo, Côte d'Ivoire

Dongui Bini Kouamé
Laboratoire de Sciences et Technologies de l'Environnement, UFR Environnement, Université Jean LOROUGNON GUEDE, Daloa, BP 150 Daloa, Côte d'Ivoire

Corresponding Author:
Ouattara Logopho Hyacinthe
UPR de Chimie Organique, Département de Mathématiques-Physique-Chimie, UFR Sciences Biologiques, Université Peleforo GON COULIBALY, Korhogo, BP 1328 Korhogo, Côte d'Ivoire

Evaluation of the antioxidant activity and study of the acute toxicity of extracts from the leaves of *Turraea heterophylla* Sm. (Meliaceae) from Côte d'Ivoire

Kouadio Kouassi Blaise, Douhore Gnaoré Yoh Toussaint, Ouattara Logopho Hyacinthe, Kablan Ahmont Landry Claude and Dongui Bini Kouamé

DOI: https://doi.org/10.22271/chemi.2024.v12.i1a.12378

Abstract
This research work is based on the evaluation of the antioxidant activity and the study of the toxicity of various *Turraea heterophylla* leaf extracts. The antioxidant activity, carried out by spectrophotometry using the DPPH and ABTS methods, shows that the leaves of *T. heterophylla* have a significant capacity to reduce free radicals compared with those of the reference molecules (ascorbic acid and Trolox). The oral toxicity study using OECD method 423 justifies that *T. heterophylla* leaves can be considered as almost non-toxic with an LD_{50} greater than 5000 mg/kg BW. Taken together, the results obtained show that *T. heterophylla* leaves can be used to treat diseases associated with oxidative stress, while presenting no major danger to the body.

Keywords: *Turraea heterophylla*, antioxidant, toxicity, Ivory Coast

1. Introduction
Oxygen is an essential molecule for all living things. It is involved in oxidative stress, characterised by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralise them [1]. Reactive oxygen species include free radicals such as superoxide ion, hydroxyl ion and hydrogen peroxide, which are normally produced in cells during metabolism. These are highly reactive, toxic molecules responsible for a great deal of damage to the body's cellular components [2]. As this damage is at the root of various pathologies such as cancer, diabetes, cardiovascular disease, rheumatism and accelerated ageing [1, 3], studies on antioxidants aimed at compensating for a deficit in the natural anti-free radical protection system are proving necessary [4, 5, 6]. In recent years, there has been considerable interest in studying the natural antioxidants contained in medicinal plants in relation to their therapeutic properties. Plant resources rich in polyphenolic compounds, such as those found in the leaves of *Turraea heterophylla* [7], can be used to prevent a wide range of diseases. Several scientific studies carried out on *T. heterophylla* leaves have reported antimicrobial [8], antimalarial [9], antidiarrhoeal [10] and aphrodisiac [11] properties. However, although *T. heterophylla* grows abundantly in Côte d'Ivoire [12], very few studies have focused on the phytochemical properties of its leaves. The aim of this study was therefore to evaluate the antioxidant activity using DPPH and ABTS methods, and to study the toxicity of *T. heterophylla* leaf extracts.

2. Materials and Methods
2.1. Materials
2.1.1. Plant Materials
The plant material consisted of *Turraea heterophylla* leaves. The organ was collected in the locality of Gadouan (Daloa) in central-western Côte d'Ivoire according to GPS coordinates (6° 38' 64.0"N; 6° 09' 19.3"W). Identification was carried out at the CNF (Centre National de Floristique) at the Université Félix HOUPHOUËT-BOIGNY (Abidjan), using the specimen

kept under number 31235. The leaves were then dried in a room at room temperature (25 °C) and out of the sun for 3 weeks. Finally, the dry matter obtained was pulverised using an electric grinder (RETSCHSK, 100) to give a fine powder to facilitate extraction of the active ingredients.

2.1.2. Chemical products

The solvents methanol, ethyl acetate, dichloromethane, hexane and distilled water were used for extraction and fractionation. DPPH, ABTS and controls (ascorbic acid, trolox) were used to assess antioxidant activity.

2.1.3. Technical and laboratory equipment

The technical equipment consisted of standard laboratory glassware (test tubes, measuring cylinders, Erlenmeyer flasks, separating funnel, etc.), an electric grinder (RETSCHSK, 100), a gastric probe, an evaporator (BUCHI-461) and a Jasco V-530 UV-Visible spectrophotometer.

2.1.3. Animal material

The animals used come from the animal house of the Biology Department of the Ecole Normale Supérieure (ENS) in Abidjan. They are white rats of Wistar strain, 8 to 9 weeks old, weighing between 108 and 112 g. The female animals were chosen for their sensitivity. The female animals were chosen for their sensitivity. The rats were acclimatised to the experimental conditions for 15 days prior to testing. During treatment, the animals were deprived of food but not water. Each animal was identified by a number. The products were administered orally (gavage) using a gastric tube.

2.2. Methods

2.2.1. Extractions

2.2.1.1. Methanol extraction of *Turraea heterophylla* leaf powder

200 g of *T. heterophylla* leaf powder was macerated in 2000 mL of methanol for 24 hours. After filtration on Wattman paper, the filtrate was recovered and the Marc, after drying, was weighed to calculate the yield. This operation was repeated twice and the different filtrates were combined, concentrated and then evaporated under reduced pressure using a rotary evaporator at a temperature of 60 °C. The methanol extract obtained was oven-dried at 50 °C and then stored in an airtight glass jar in the refrigerator at 4 °C. The methanol extract was used to prepare selective extracts by fractionation, to assess antioxidant activity and acute toxicity.

2.2.1.2. Fractionation of the methanolic extract of *Turraea heterophylla* leaves

The crude methanolic extract was fractionated successively using solvents of increasing polarity (hexane, dichloromethane, ethyl acetate) according to the method reported by Attioua *et al.* [13]. Part of the crude methanolic extract (E_{MeOH} =7.2 g) was dissolved completely in 100 mL of distilled water and the resulting solution was transferred to a separatory funnel. The solution was successively stripped with hexane (3x 100 mL), dichloromethane (3x 100 mL) and ethyl acetate (3x 100 mL). The various organic fractions and the aqueous residual phase were oven-dried at 60 °C, then weighed to calculate the yields. This operation was repeated twice. The hexane (F_{HEX}), dichloromethane (F_{DCM}), ethyl acetate (F_{AE}) and aqueous residue (FAq) fractions obtained were used to assess antioxidant activity by spectrophotometry.

2.2.2. Assessment of antioxidant activity: Antioxidant activity was assessed by DPPH (2, 2-diphenyl-l-picrylhydrazyl) and ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) reduction tests.

2.2.2.1. Evaluation of the antioxidant activity of different *T. heterophylla* extracts against DPPH

The antiradical activity of the extracts studied was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test using the method of Dieng *et al.* [14] with a few modifications. DPPH was solubilised in a binary mixture of methanol and water (70/30) (v/v) to obtain a solution with a concentration of 0.3 mg/mL. Next, 1 mL of the DPPH solution was added to 2.5 mL of extract and the mixture was incubated in the dark for 30 min after stirring. The absorbance of the mixture was then measured at 517 nm against a blank consisting of 2.5 mL of the binary mixture methanol/water (70/30) (v/v) and 1 mL of DPPH solution. Plant extracts and the reference molecule (ascorbic acid) were prepared at different concentrations (0.5; 1; 1.5; 2; 3.5; 4; 4.5; 5 mg/mL). For all concentrations of each extract, three absorbance measurements were carried out.
DPPH inhibition percentages were calculated according to the formula.

$$I(\%) \text{ of DPPH} = (A_0 - A_e) / A_0 \times 100$$

- **I:** Percentage of DPPH inhibition.
- **A_0:** Absorbance of the blank.
- **A_e:** Absorbance of the sample or extract.

The concentrations required to trap 50% (IC_{50}) of the DPPH are determined on the graphs representing the percentages of DPPH inhibition as a function of the concentrations of the extracts or vitamin C.

2.2.2.2. Evaluation of the antioxidant activity of different *T. heterophylla* extracts against ABTS

This method is based on the ability of the compounds to reduce the ABTS+ (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) radical-cation. The test was carried out according to the technique used by Dieng *et al.* [14]. The $ABTS^+$ radical-cation was produced by reaction of 8 mM ABTS (87.7 mg in 20 mL distilled water) and 3 mM potassium persulphate (0.0162 g in 20 mL distilled water) in a 1:1 (v/v) ratio. The mixture was then incubated in the dark at room temperature for 4 hours. This mixture was progressively diluted with methanol (MeOH) until an absorbance of 0.7±0.02 was obtained at 734 nm. A 3.9 mL sample of this diluted $ABTS^+$ solution was added to 100 µL of extract or reference (Trolox) at various concentrations (3.5; 5; 6.25; 10; 11.25; 13.75; 15 mg/mL). After shaking, the mixture was incubated for 6 minutes in the dark (T=30±2 °C). The residual absorbance of the $ABTS^+$ radical was then measured at 734 nm using a UV-visible spectrophotometer and should be between 20%-80% of the absorbance of the blank. Three trials were carried out for each concentration of product tested. ABTS inhibition percentages were calculated using the formula.

$$I(\%) \text{ of ABTS} = (A_0 - A_e) / A_0 \times 100$$

- **I:** Percentage inhibition of ABTS.
- **A_0:** Absorbance of diluted ABTS (blank).
- **A_e:** Absorbance of diluted ABTS + sample or extract.

Calibration lines for the percentage inhibition of ABTS as a function of the concentrations of the different samples and the reference were constructed to determine the IC_{50} (mg/mL) of the plant extracts and Trolox (reference).

2.2.3. Assessment of acute toxicity

The acute toxicity of the methanolic crude extract of *Turraea heterophylla* leaves was assessed according to OECD protocol 423 [15].

Several batches of three rats were set up for the tests. For the initial dose, one of four was chosen: 5, 50, 300 and 2000 mg/kg body weight (BW). The dose chosen was generally the one at which mortality could be expected to occur in some of the treated rats. In this study, the initial dose chosen was 5 mg/kg BW. Female rats from each test batch were each gavaged with 1mL of crude methanolic extract recovered in distilled water at the doses previously indicated. After gavage, the rats were observed regularly (every 30 min). When the treated batch showed no dead animals and no signs of toxicity (aggressiveness, mobility, vigilance, stool condition, vomiting, mortality, etc.), the next batch was treated in turn. A control batch of spleens (blank) was also set up and each spleen was force-fed with 1 mL of distilled water. At the end of the experiment, the treated rats were observed continuously on the first day, and regularly for the next 14 days.

2.2.4. Statistical analysis of experimental results: EXCEL 2019 (Version 16.0) was used to process the experimental measurements obtained and plot the various graphs.

3. Results and discussion

3.1. Results

3.1.1. Yields of the different extractions

The yield results for all the different extractions are given in Table 1. The yield from methanol maceration of *Turraea heterophylla* leaves was 6.75±0.28. As for fractionation extractions, yields varied from 13.19±0.14 for the hexane fraction to 37.51±0.32 for the aqueous residual fraction.

Table 1: Yields of methanolic extract of *T. heterophylla* leaves and its fractions

Samples	Extracted mass (g)	Yield (%)
E_{MeOH}	13.5±0.93	6.75±0.28
Fraction Hex	0.95±0.22	13.19±0.14
Fraction DCM	1.18±0.25	18.88±0.21
Fraction AE	1.42±0.17	28.01±0.15
Fraction Aq.	3.43±0.30	37.51±0.32

MeOH: Methanol, **Hex:** Hexane, **DCM:** Dichloromethane, **AE:** Ethyl acetate, **Aq:** Aqueous residual

3.1.2. Antioxidant activity

3.1.2.1. Antioxidant activity against DPPH

3.1.2.1.1. Percentage inhibition of DPPH by plant extracts and ascorbic acid

Table 2 shows the inhibition percentages of the different extracts and ascorbic acid with respect to the DPPH free radical. These percentages increase progressively with concentration. They vary from 20.12% to 62.57% for the different plant extracts studied, compared with values ranging from 39.00% to 97.55% for the reference substance (ascorbic acid).

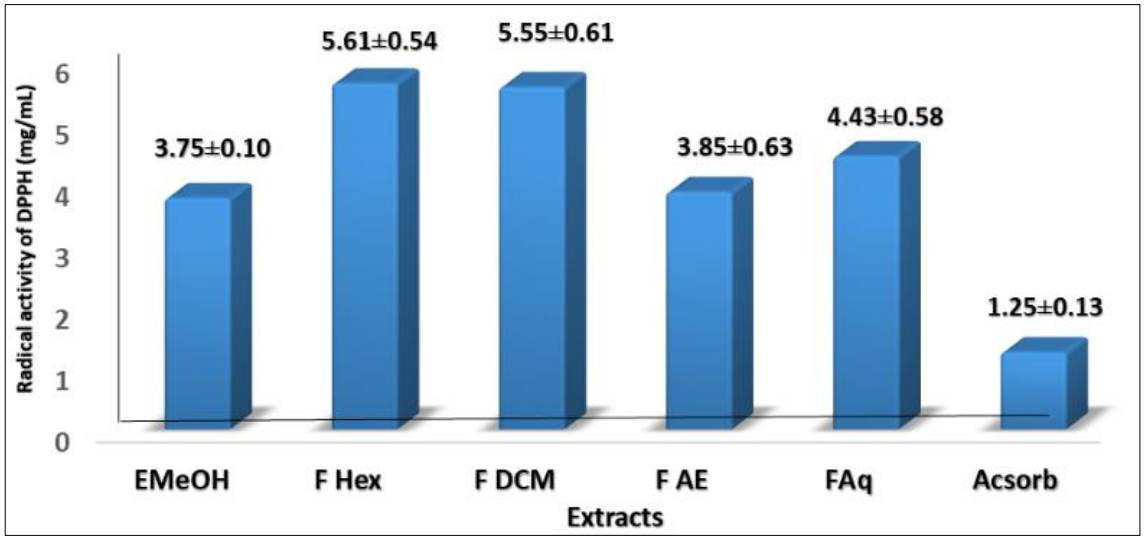
Table 2: DPPH absorbance values for methanolic extract, fractions and ascorbic acid

C (mg/mL)	0.5	1	1.5	2	2.5	3.5	4	4.5	5
I (%) (E_{MeOH})	20.62	20.67	28.14	30.57	30.21	49.15	50.74	62.10	62.57
I (%) (F_{Hex})	21.58	23.61	32.77	30.05	35.75	36.72	36.59	37.39	55.78
I (%) (F_{DCM})	20.12	21.70	22.75	33.25	32.16	33.75	33.58	46.33	50.59
I (%) (F_{AE})	31.86	32.10	32.90	36.30	40.05	46.77	54.78	55.31	55.72
I (%) (F_{Aq})	30.50	30.69	34.29	31.50	36.35	41.31	48.17	50.82	56.67
I (%) (A. a.)	39.00	46.10	54.15	60.50	68.70	82.62	86.15	96.31	97.55

C: Concentration, **I (%):** Percentage of DPPH inhibition, **E_{MeOH}:** Methanolic extract, **F_{Hex}:** Hexanic fraction, **F_{DCM}:** dichloromethane fraction, **F_{AE}:** Ethyl acetate fraction, **F_{Aq}:** Residual aqueous fraction, **A. a:** Ascorbic acid

3.1.2.1.2. Determination of IC_{50} for plant extracts and ascorbic acid using the DPPH method

To better assess the antioxidant power of the extracts studied compared to the reference molecule (ascorbic acid), IC_{50} values (mg/mL) were determined. The IC_{50} represents the concentration of a substance that inhibits 50% of the free radicals represented by the DPPH solution *in-vitro*. These values are determined graphically using calibration lines for the percentages of inhibition as a function of the different concentrations of the chemical substances tested. The various IC_{50} values for the organic extracts, ranging from 3.75±0.10 to 5.61±0.54, compared with 1.25±0.13 for ascorbic acid, are all shown in Figure 1.



E_{MeOH}: Methanolic extract, **F_{Hex}:** Hexane fraction, **F_{DCM}:** Dichloromethane fraction, **F_{AE}:** Ethyl acetate fraction, **F_{Aq}:** Residual aqueous fraction, **Acsorb:** Ascorbic acid

Fig 1: Diagram of the different IC_{50} values for plant extracts and ascorbic acid in relation to the DPPH radical

3.1.2.2. Antioxidant activity towards ABTS

3.1.2.2.1. Percentage inhibition of ABTS by plant extracts and Trolox

The results of the antioxidant activity by the ABTS method verify the antioxidant activity by a method other than the DPPH method. Table 3 shows the percentages of inhibition of the various organic extracts and Trolox by ABTS. Generally speaking, these percentages increase progressively with concentration. They varied from 20.81% to 67.75% for the different plant extracts studied, compared with values ranging from 19.29% to 74.41% for the reference substance (Trolox).

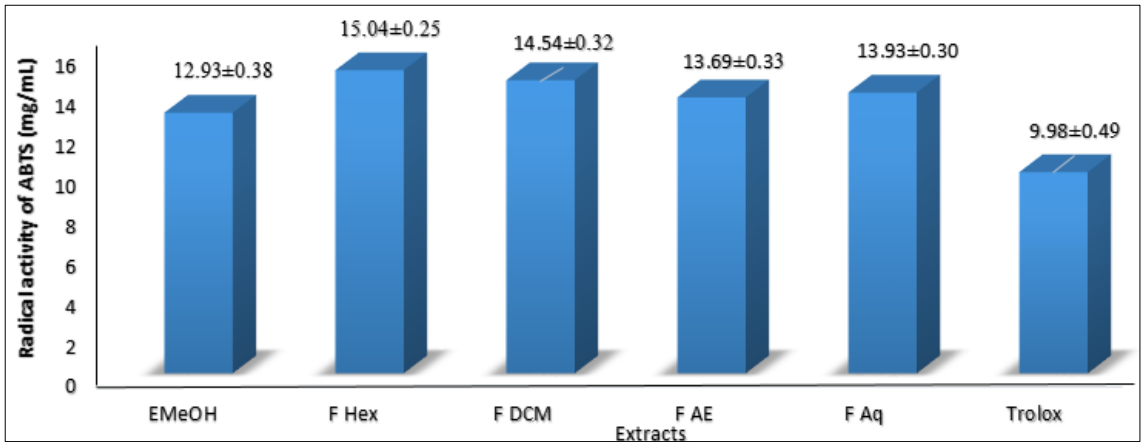
Table 3: Absorbance values for the ABTS test for plant extracts and Trolox

C (mg/mL)	3.75	5	6.25	10	11.25	11.5	13.75	15
I' (%) (E_{MeOH})	21.31	21.58	21.66	35.03	33.24	45.13	55.10	67.75
I' (%) (F_{Hex})	31.61	32.92	34.90	34.03	28.58	24.53	56.81	58.87
I' (%) (F_{DCM})	21.05	25.50	29.15	23.74	36.54	29.58	50.50	65.05
I' (%) (F_{AE})	21.58	27.30	25.50	37.80	40.15	45.15	46.67	57.09
I' (%) (F_{Aq})	20.81	31.50	34.07	36.05	35.15	45.12	47.57	55.87
I' (%) (Trol.)	19.29	27.69	33.46	51.05	55.43	60.25	68.62	74.41

C: Concentration, **I' (%):** Percentage of ABTS inhibition, **E_{MeOH}:** Methanolic extract, **F_{Hex}:** Hexanic fraction, **F_{DCM}:** dichloromethane fraction, **F_{AE}:** Ethyl acetate fraction, **F_{Aq}:** Residual aqueous fraction, **Trol:** Trolox

3.1.2.2.2. Determination of IC_{50} for plant extracts and Trolox using the ABTS method

The IC_{50} values for plant extracts and Trolox were also determined graphically using calibration lines for percent inhibition as a function of the concentrations of each product analysed. The various IC_{50} values for organic extracts ranged from 12.93±0.38 to 15.04±0.25, compared with 9.98±0.49 for Trolox. The different IC_{50} values are shown in Figure 2.



E_{MeOH}: Methanolic extract, **F_{Hex}:** Hexane fraction, **F_{DCM}:** Dichloromethane fraction, **F_{AE}:** Ethyl acetate fraction, **F_{Aq}:** Residual aqueous fraction, **Acsorb:** Ascorbic acid

Fig 2: Diagram of different IC_{50} values for plant extracts and Trolox in relation to ABTS

3.1.3. Acute toxicity of *Turraea heterophylla* methanolic extract: The toxicity of the methanolic extract of the leaves of *T. heterophylla* was determined by administering a single dose of this extract and then observing the effects on the

behaviour and life of the treated animal. After oral administration of the extract to rats at various doses, a number of clinical signs were observed (skin and fur, eyes, mucous membranes, diarrhoea, salivation, lethargy, heart rate, aggressiveness, somnolence, feeding, mobility, mortality) and are listed in Table 4.

Table 4: Clinical signs observed in rats

Observations	Control batch		Experimental batch	
	6 hours	12 hours	6 hours	12 hours
Skin and fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membranes	Normal	Normal	Normal	Normal
Diarrhoea	Absence	Absence	Absence	Absence
Salivation	Absence	Absence	Absence	Absence
Lethargy	Absence	Absence	Absence	Absence
Heart rate	Normal	Normal	Normal	Normal
Aggression	Absence	Absence	Absence	Absence
Drowsiness	No	No	No	No
Feeding	Yes	Yes	Yes	Yes
Mobility	Yes	Yes	Yes	Yes
Mortality	No	No	No	No

The data in Table 4 show that the methanolic extract of the leaves of *T. heterophylla* administered at different doses to female rats did not cause any deaths over 24 hours of observation. To ensure that the extract had no harmful effect, the observation period was extended to 14 days and the animals were weighed regularly. The body weights of rats with daily increases are shown in Table 5.

Table 5: Monitoring animal body weights over a 14-day period

	Lots	Day 1	Day 3	Day 5	Day 9	Day 12	Day 14
Weight M (T)	1	110±2.3	111±2.7	113±2.7	115±3	117 ±3	119±3
	Δ		+1	+2	+2	+2	+2
Weight M (5 mg/kg)	2	110±2.7	112±2.7	115±3.6	118±2.7	121±2.7	124±2.7
	Δ		+2	+3	+3	+3	+3
Weight (50 mg/kg)	3	110±3	112±2.7	115±2.7	119±3.3	122±2.7	126±2.7
	Δ		+2	+3	+3	+3	+4
Weight M (300 mg/kg)	4	112±2.7	115±4	118±3	122±3	125±3	127±2.7
	Δ		+3	+3	+3	+3	+2
Weight M (2000 mg/kg)	5	112± 3	114±2.7	117±2.7	121±3	125±2.3	129±3
	Δ		+2	+4	+4	+4	+4

T: Indicator, **M:** Medium, **Δ:** Increase in body weight

3.2. Discussion

This research was carried out to assess antioxidant activity using spectrophotometry based on free radical reduction methods (DPPH and ABTS) and to study the toxicity of organic extracts from *Turraea heterophylla* leaves.

The leaves of the plant were extracted by maceration with methanol and part of the crude extract obtained was recovered in distilled water and then successively exhausted with solvents of increasing polarity: hexane, dichloromethane and ethyl acetate. The methanolic extract of *T. heterophylla* leaves gave a yield of 6.75±0.28%. This yield indicates that these leaves contain methanol-extractable secondary metabolites, some of which are phenolic compounds. Methanol is a solvent commonly used to extract polyphenols [16]. With regard to the yields of the selective fractions, that of ethyl acetate (28.01±0.15%) was higher than that of dichloromethane (18.88±0.21%), which in turn was higher than that of hexane (13.19±0.14%). This difference in selective yields could be linked to the polarity of the solvents used for fractionation. Ethyl acetate (1.78) is more polar than dichloromethane (1.14), which is more polar than hexane (0.1). The vast majority of compounds remained in the residual aqueous phase during fractionation, with a proportion of 37.51±0.32%. In addition, ethyl acetate and water, which have the highest percentages of compounds in the organic fractions, are hydrophilic solvents and therefore more likely to extract water-soluble compounds [17].

Evaluation of the antioxidant activity carried out on methanolic, hexanolic, dichloromethane, ethyl acetate and aqueous residual extracts gave significant inhibition percentages regardless of the analytical method used (DPPH and ABTS). The percentages of these extracts obtained by the DPPH method varied between 20.12% and 62.57%, compared with 39.00% and 97.55% for ascorbic acid (reference). As for the ABTS method, they ranged from 20.81% to 67.75% for plant extracts from the plant studied, compared with 19.29% to 74.41% for Trolox (reference).

For a better estimate of antioxidant activity, it was wise to calculate IC_{50}. The inhibition percentage of a plant extract only reflects its capacity to trap free radicals. On the other hand, the IC_{50} represents the concentration of an extract that causes a 50% loss in DPPH activity [18]. The lower the IC_{50} of an extract, the greater its antioxidant activity. The IC_{50} of the extracts using the DPPH method ranged from 3.75±0.10 to 5.61±0.54 mg/mL, compared with 1.25±0.13 mg/mL for ascorbic acid. Using the ABTS method, the IC_{50} of the extracts ranged from 12.93±0.38 to 15.04±0.38 mg/mL, compared with 9.98±0.49 mg/mL for Trolox. However, these two methods give the same order of magnitude for the antioxidant power of the plant extracts tested. It was noted for both methods that the IC_{50} (E_{MeOH}) < IC_{50} (F_{AE}) < IC_{50} (F_{Aq}) <

IC_{50} (F_{DCM}) < IC_{50} (F_{Hex}) so the antioxidant powers of the plant extracts from the leaves of *T. heterophylla* are ranked in descending order as follows: E_{MeOH} > F_{AE} > F_{Aq} > F_{DCM} > F_{Hex}. The antioxidant powers of these extracts are negligible compared with those of the two reference molecules used (ascorbic acid and Trolox). The antioxidant activity observed is thought to be due to the synergistic presence of secondary metabolites in *T. heterophylla* leaves. It was noted for both methods that the IC_{50} (E_{MeOH}) < IC_{50} (F_{AE}) < IC_{50} (F_{Aq}) < IC_{50} (F_{DCM}) < IC_{50} (F_{Hex}) so the antioxidant powers of the plant extracts from the leaves of *T. heterophylla* are ranked in descending order as follows: E_{MeOH} > F_{AE} > F_{Aq} > F_{DCM} > F_{Hex}. The antioxidant powers of these extracts are negligible compared with those of the two reference molecules used (ascorbic acid and Trolox). The antioxidant activity observed is thought to be due to the synergistic presence of secondary metabolites in *T. heterophylla* leaves. In fact, the work of Kouadio *et al*. showed the presence of polyphenols, flavonoids, alkaloids, saponins, sterols and polyterpenes in the leaves of this plant [7]. These secondary metabolites are known to have antioxidant properties [19, 20]. The antioxidant power of *T. heterophylla* leaves thus observed would justify the use of the plant's leaves in the traditional treatment of conditions such as skin and eye diseases [10], typhoid fever, diarrhoea, malaria, bronchitis, hypofertility [9] and sexual impotence [11], some of which are conditions linked to oxidative stress [1, 3, 21].

The toxicity study carried out on the methanolic extract of the leaves of *T. heterophylla* did not result in any deaths in the rats, whatever the dose administered, up to 2000 mg/kg BW. Furthermore, no clinical signs were observed and, on the contrary, the body weights of the rats increased until the end of the experiment. Since the dose of 2000 mg/kg BW did not cause any deaths, the extract was classified as almost non-toxic according to the Hodge and Sterner toxicity scale [22], with a lethal dose (LD_{50}) of over 5000 mg/kg BW. *T. heterophylla* leaves are therefore harmless to the human organism, justifying its use in traditional medicine.

4. Conclusion

The aim of this research was to evaluate the antioxidant activity and study the acute toxicity of *Turraea heterophylla* leaves.

The antioxidant activity, carried out against DPPH and ABTS using quantitative methods, shows that the leaves of this plant contain antioxidant activity and could therefore be recommended as antioxidants to prevent or treat damage caused by oxidative stress.

Oral toxicity studies have shown that the leaves of this plant can be considered almost non-toxic with a lethal dose (LD_{50}) of over 5000 mg/kg BW.

Taken together, these results highlight the antioxidant effects of *Turraea heterophylla* leaves, which are not thought to present any major danger to the body. However, it would be interesting to continue the work by assessing the biological activities of these leaves in relation to oxidative diseases and by elucidating certain active molecular structures that they contain.

5. Conflict of interest

The authors state that they have not received any funding for this work.

6. Acknowledgements: The authors would like to thank the heads of the laboratories at the various institutions and the technicians for their help in carrying out this work.

7. References

1. Kodjo SE, Kafui K, Oudjaniyobi S, Pakoupati B, Amegnona A, Messanvi G, *et al*. Etude comparative des activités antioxydantes d'extraits éthanoliques de feuilles, d'écorces et de racines de *Cassia sieberiana*. Int. J Biol. Chem. Sci. 2017;11(6):2924-2935.
2. Codoner-Franch P, Valls-Belles V, Arilla Codoner A, Alonso-Iglesias E. Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress. Transl Res. 2011;158(6):369-384.
3. Thayyil AH, Amuthu K, Ibrahim M. *In vivo* antioxidant and lipid peroxidation effect of various extracts from aerial parts of *Chomelia asiatica* (Linn) in rats fed with a high-fat diet. Afr J Pharmacol. 2016;10(38):810-816.
4. Koné M, Toure A, Ouattara K, Coulibaly A. Phytochemical Composition, Antioxidant, and Antibacterial Activities of Root of *Uvaria chamae* P. Beauv. (Annonaceae) Used in Treatment of Dysentery in North of Côte d'Ivoire. Int. J Pharmacogn Phytochem Res. 2015;7(6):1047-1053.
5. Gnaoré YTD, Koffi BA, Yaya S, Faustin AK, Landry CAK, Marc V, *et al*. Nor-cucurbitacins from the leaves of *Mareya micrantha* (Benth.) Müll. Arg. (Euphorbiaceae). Fitoterapia. 2020;143:104538.
6. Kouamé KT, Siaka S, Kassi AB, Soro Y. Determination des teneurs en polyphénols totaux, flavonoïdes totaux et tanins de jeunes feuilles non encore ouvertes de *Piliostigma thonningii* (Caesalpiniaceae). Int. J Biol. Chem. Sci. 2021;15(1):97-105.
7. Kouadio KB, Kablan ALC, Ahoua ARC, Konan DJ, Oussou KR, Attioua KB, *et al*. Criblage phytochimique, dosages des polyphénols totaux et flavonoïdes totaux, et évaluation de l'activité antibactérienne des feuilles de *Turraea heterophylla* Smith (Meliaceae). Int. J Innov. Appl. Stud. 2021;33(2):405-413.
8. Akrofi R. Extraction, Isolation et caractérisation de certains limonoïdes et composants de l'écorce de racine de *Turraea heterophylla* Smith (Meliaceae). Thèse de Doctorat, Département de Chimie de l'école des Sciences physiques, Université de Cape Coast (Ghana); c2011. p. 90.
9. N'Guessan K, Tra Bi FH, Koné MW. Etude ethno pharmacologique de plantes antipaludiques utilisées en médecine traditionnelle chez les Abbey et Krobou d'Agboville (Côte d'Ivoire). J Ethnopharmacol. 2009;44:42-50.
10. N'Guessan K, Kadja B, Zirihi G, Traoré D, Aké AL. Screening phytochimique de quelques plantes médicinales ivoiriennes. J Nat Sci. 2009;6:1-15.
11. Irvine FR. Woody plants of Ghana, with special reference to their uses. London, Oxford University Press; c1961. p. 868.
12. Kerahro J, Adam JG. La Pharmacopée Sénégalaise et Traditionnelle: Plantes Médicinales et Toxiques. Ed. Vigot et Frères: Paris; c1974. p. 1011.
13. Attioua KB, Ramiarantsoa H, Boti JB, Vincent AA, Félix ZT, Léon AD, *et al*. Isolation and identification of alkaloids from Croton lobatus. Institute of Pharmaceutical Education and Research. 2012;13(2):1-4.
14. Dieng SIM, Fall AD, Diatta BK, Sarr A, Sene M, Mbaye A, *et al*. Evaluation de l'activité antioxydante des extraits

hydro-éthanoliques des feuilles et écorces de *Piliostigma thonningii* Schumach. Int. J Biol. Chem. Sci. 2017;11(2):768-776.
15. OECD. Ligne directrice de l'OCDE pour les essais de produits chimiques: Toxicité orale aiguë - Méthode par classe de toxicité aiguë, N° 423, Lignes directrices de l'OCDE pour les essais de produits chimiques (section 4: effets sur la santé); c2002. p. 14.
16. Trabelsi N, Megdiche W, Ksouri R, Falleh H, Oueslati S, Soumaya B, *et al*. Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. LWT - Food Sci. Technol. 2010;43(4):632-639.
17. Silverstein RM, Webster FX, Kiemle D. Spectrometric identification of organic compounds, seventh Edition, John Wiley and Sons; c2005. p. 512.
18. Falleh H, Ksouri R, Abdelly C. Activité antioxydante et contenu en polyphénols dans les différents organes de l'artichaut sauvage *Cynara cardunculus*. Revue des Région Arides; c2009. p. 341-344.
19. Sivapriya M, Srinivas L. Isolation and purification of a novel antioxidant protein from the water extract of Sundakai (*Solanum torvum*) seeds. Food Chem. 2007;104:510-517.
20. Kolak U, Kabouche A, Öztürk M, Kabouche Z, Topçu G, Ulubelen A, *et al*. Antioxidant diterpenoids from the roots of *Salvia barrelieri*. Phytochem. Anal. 2009;20:320-327.
21. Droupy S. Épidémiologie et physiopathologie de la dysfonction érectile. EMC (Elsevier SAS, Paris), Urologie; c2005. p. 18-720-A-10.
22. Hodge AC, Sterner JH. In études de toxicité: quelques données fondamentales (A.K DONE). Tempo Medical Afrique. 1980;7:18.