Evaluation of the antioxidant power and the content of secondary metabolites of the crude compounds of *Talinumtriangulare* (Talinaceae), a plant with antiplasmodial activity

Abstract

Talinumtriangulare is a food plant recognized for its nutritional properties and therapeutic virtues. This plant is used in particular in Daloa (Central-West Ivory Coast) by naturotherapists, for the treatment of malaria. This study aimed to evaluate the antioxidant activity of extracts and fractions of *Talinumtriangulare* leaves and to determine their content of secondary metabolites. Two methods were used for this evaluation: reduction of the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) and reduction of the $ABTS^{+}$ cation radical (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid).

The results show that the IC_{50} could not be determined for the 70% aqueous and hydro-ethanolic extracts with the DPPH test, while it was respectively 0,9 and 2,3 mg/mL for the fractions dichloromethane and aqueous. Regarding the $ABTS^{+}$ radical-cation reduction test, the dichloromethane fraction showed a higher percentage of inhibition (41,59%) compared to the aqueous fraction (40,59%), while the aqueous fraction was a little more important than the hydro-ethanolic extract at 70 % (38,29%).Among the four compounds tested, only the aqueous extract presented a relatively low percentage of inhibition (25,51%).

Spectrophotometric analysis revealed the presence of various phenolic compounds, such as flavonoids, polyphenols and total tannins, at varying concentrations. These results could support the traditional use of *Talinumtriangulare* in the treatment of malaria.

Key words: *Talinumtriangulare*, antioxidant activity, phenolic compounds.

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INTRODUCTION

Over the past twenty years, the incidence of parasitic diseases has increased. Malaria is among these parasitic diseases. Despite being entirely preventable and treatable, it continues to have devastating consequences on the health and livelihoods of populations across the world (Tagne, 2021). This state of affairs has led authors to affirm that this disease is “one of the rare public health scourges that has survived the centuries without ever losing its activity” (Gentilini and Dufflo, 1993; Bagayoko, 2008). Unfortunately, its management faces resistance from most antimalarial drugs (WHO, 2023). It is therefore crucial to search for new

active molecules and medicinal plants appear to be an important source to explore. Furthermore, natural therapies based on medicinal plants are of great benefit. They are better biodegradable and are suspected of having resistance-reversing properties. In addition, the best treatments recommended by the WHO for the therapeutic management of malaria are based on derivatives of molecules from medicinal plants (Salery, 2007; Tano, 2016).

According to the World Health Organization (WHO), more than 80% of the African population uses traditional medicine for their primary health care due to their proximity and accessibility (Nkasa et *al.*, 2020). Ivory Coast is one of the third world countries where medicinal plants are commonly used to solve health problems, especially in rural areas. This is how a significant number of works relating to the biological, pharmacological and phytochemical properties of plants from traditional Ivorian medicine have been carried out. The data provided by these studies made it possible on the one hand to explain the therapeutic action and on the other hand to confirm the use of these different plants in traditional medicine (Mariam et *al*., 2024). *Talinumtriangulare*, a cosmopolitan weed of the Talinum genus, of the Talinaceae family (formerly Portulacaceae), fits into this theme. This plant has great therapeutic values in the traditional medicine system, but is not yet fully exploited (Swarna et *al*., 2013). Preliminary work carried out on the aqueous and hydro-ethanolic extracts (70%) of its leaves made it possible to demonstrate the antimalarial properties of the leaves of this plant on clinical isolates of *Plasmodiumfalciparum* (Okou et *al.*, 2019). According to the work of Tano (2016), when a person has an attack of malaria, plasmodia can lead to oxidative stress through the massive lysis of red blood cells. Hemmer et *al.* (2005) also demonstrated that neutrophils activated by parasites can trigger the destruction of endothelial cells. This can be prevented by *exvivo* intake antioxidants and proteolytic enzyme inhibitors. In sum, antioxidants can attenuate endothelial cell damage due to parasite-induced leukocyte activation, supporting the importance of antioxidant activity in the context of malaria treatment (Pincemail et *al*. 2002). The present study is part of continuing investigations into the antimalarial activity of Talinum triangulare. More specifically, the aim was to evaluate the antioxidant activity of extracts and fractions of *Talinumtriangulare* leaves and to determine their contents of phenolic compounds, particularly total polyphenols, total flavonoids and total tannins.

1- Material

1-1 Plant material

The plant material consists of leaves of *Talinumtriangulare*, belonging to the Talinaceae family and well known under the name wild spinach.

1-2 Technical equipment

Most of the technical equipment is made up of:

- Retsch type mechanical crusher to make dried plant material into powder;
- Moulinex type mixer for maceration;
- an electronic scale for the various weighings;
- a Drying Oven DHG-9013A type oven for drying extracts;
- an IKA® MS 3 digital vortex for homogenization;
- a water bath for regulating the temperatures of the environments;
- a Mindray MR-96A type spectrophotometer for reading the different optical densities.

1-3 Reagents and solvents

The main part of the reagents consists of: 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), DPPH (2,2'-diphenyl-1-picrylhydrazyl), potassium persulfate, Vitamin C, Folin - Ciocalteu, sodium carbonate, gallic acid, sodium nitrite (NaNO2), aluminum chloride (AlCl3), sodium hydroxide (NaOH 1N), distilled water, ethanol, methanol, dimethyl sulfoxide (DMSO), hexane, dichloromethane, acetate ethyl, butanol.

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2- Methods

2-1 Harvesting, drying and spraying *Talinumtriangulare* leaves

The leaves of *Talinumtriangulare* were harvested between July and August 2021 at the start of the morning (6:30 a.m.) in Koffikro, a camp located 12 km from the Daloa Department and in the Tazibouo district within the UEESO-CI city near COPRO college of Daloa Department (Haut-Sassandra Region, Ivory Coast). After harvest, they were freed of all other elements, dried in the shade and in the open air for ten weeks then crushed until a fine powder was obtained using a mechanical grinder. The resulting powder was stored at room temperature in glass jars to avoid mold.

2-2 Preparation of extracts and fractions of *Talinumtriangulare* leaves

The extraction of the active ingredients was generally done by cold maceration in a given solvent using a blender for a specific time.

2-2-1 Preparation of the aqueous extract

The fine powder initially obtained was extracted using the method of Zirihi et *al*. (2003). To do this, a quantity of one hundred grams of the vegetable powder was cold macerated in one liter of distilled water using a blender. After three minutes of homogenization, the homogenate obtained was collected in a square of clean white tissue and then pressed by hand. The collected solution was filtered twice through hydrophilic cotton and then once through Whatman filter paper (3 mm). The filtrate obtained was dried in an oven at a temperature of 60 °C for (five) 5 days to obtain the total aqueous extract (Aq).

2-2-2 Preparation of the hydro-ethanolic extract

It followed the same principle as the previous one, with the only difference that instead of water, an ethanol-water mixture (70/30: v/v) was used. It made it possible to obtain the hydro-ethanolic extract (HOH) after drying for 72 hours in an oven at 60°C.

These two extracts (Aq, HOH) were stored separately in airtight jars at laboratory temperature.

2-2-3 Fractionation of the hydro-ethanolic extract

Previous work by Okou et *al.* (2019) revealed that the hydro-ethanolic extract (HOH) had more antiplasmodial action than the aqueous extract (Aq). It is on the basis of these results that the hydro-ethanolic extract (HOH) underwent partition chromatography, using solvents of increasing polarity such as hexane, dichloromethane, ethyl acetate and butanol. It is a partition method which consists of liquid-liquid partitioning with solvents that are immiscible with each other. It was inspired by that of Bolou et *al*. (2011). For its production, a quantity of one hundred grams of the hydro-ethanolic extract (HOH) was dissolved in 500 mL of distilled water then transferred to a 1000 mL separating funnel. Then, to the aqueous solution obtained, 250 mL of hexane was added then the whole was stirred vigorously for five minutes then allowed to settle for a time in order to have a hexanic phase and a residual aqueous phase. After which, the tap of the separating funnel was opened to let the residual aqueous phase pass into a beaker and then the supernatant (hexane phase) was also collected in another beaker. After this first operation, the second consisted of transferring the new residual aqueous phase once again into the separating funnel and then adding 250 mL of hexane again. Then, the whole was vigorously homogenized for five minutes and then left to settle for a while in order to have a new residual hexanic and aqueous phase. After this step, the tap was reopened as before to recover the new residual aqueous and hexanic phases separately in various beakers. The third and final operation was identical to the second in order to obtain

the previous results. After these various operations, all of the hexane phases were brought together and subsequently evaporated to constitute the hexane fraction. As for the residual aqueous phase obtained after these various leachings with hexane, it was also subjected to the same previous leaching steps three times, but this time successively with dichloromethane, ethyl acetate and butanol. with the aim of having their respective phase (dichloromethane, ethyl acetate, butanolic and residual aqueous). The different phases obtained were then evaporated separately in order to obtain their respective fraction (dichloromethane (DCM), ethyl acetate, butanolic and aqueous fraction (Faq)).

2-3 Antioxidant activity

2-3-1 Measurement of antioxidant activity by the DPPH test

The DPPH method is a test for the reduction of the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical to 2,2-diphenyl-1-picrylhydrazine (DPPH-H). It makes it possible to evaluate the antioxidant potential of a sample, by its capacity to trap the DPPH• radical used as a chromogen (Brand-Williams et *al.*, 1995; Benzie and Szeto, 1999). This organic radical is stable at room temperature and has a purple color. The antioxidants present in the sample, by transferring an H atom to the DPPH• radical, reduce it to DPPH-H or $DPPH_2$. This reduction causes a change in the color of the solution from purple to pale yellow (Molyneux, 2004). For its production, a quantity of 4 mg of DPPH powder was dissolved in 100 mL of methanol, homogenized then stored away from light to constitute a solution. At the same time, a quantity of 30 mg of each extract to be tested was diluted in 3 mL of sterile distilled water in order to have an extract concentration of 10 mg/mL. Then, a volume of 1,5 mL of methanol was distributed into ten sterile test tubes numbered from 1 to 10 to constitute the experimental series of a given extract. In tube 1 of the experimental series, a volume of 1,5 mL of the extract with a concentration of 10 mg/mL previously prepared was added and then mixed to constitute the stock.It consisted of taking 1,5 mL from tube 1, diluting it in tube 2 then homogenizing it. This operation was repeated up to tube 10. From tube 10, the excess volume was rejected. Thus, the various concentrations varied from 10 to 0,019 mg/mL from tube 1 to tube 10. After which, a volume of 1,5 mL of DPPH was added to every 10 tubes. Alongside this experimental series, a reference series was produced. In fact, it consisted of using vitamin C instead of the plant extract, and always in 10 tubes. The only difference between the experimental and reference series is that the concentrations of the reference product used ranged from 25 to 0,195 µg/mL from tube 1 to tube 10. However, in parallel with the experimental and reference series, a control control was prepared by soliciting only methanol

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and DPPH at equal volume (1,5 mL). All of the tubes were kept away from light for 30 minutes then their absorbance was determined distinctly with a spectrophotometer at 517 nm with methanol as the blank control and vitamin C as a positive control. The percentage of inhibition of the DPPH radical was calculated according to the following equation:solution. After this step, the double dilution technique of geometric ratio ½ in medium was applied :

DPPH inhibition (%) = (A0 – A sample)/A0) x 100

A0: absorbance of the control (drug-free). A sample: absorbance of the sample after 30 min.

2-3-2 Measurement of antioxidant activity by ABTS

The method used was that described by Choong et *al.*, (2007). It is based on the ability of compounds to reduce the $ABTS^+$ cation radical (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). It was produced by the reaction of 8 mM ABTS (87,7 mg in 20 mL distilled water) and 3 mM potassium persulfate (0,0162 g in 20 mL distilled water) in a 1:1 ratio (v/v). The mixture was then incubated in the dark at room temperature for 12 to 16 hours to obtain an $ABTS^+$ solution. It was diluted with methanol to obtain a solution whose absorbance was 0,7 ± 0,02 at 734 nm. Then, a volume of 3,9 mL of this diluted $ABTS^+$ solution was added to 100 µL of each compound to be tested (aqueous and hydro-ethanolic extracts, dichloromethane and aqueous fractions) in different test tubes then shaken vigorously. The initial concentration of these test compounds is 10 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 with a UV-visible spectrophotometer and should be between 20%-80% of the absorbance of the white. The tests were carried out in triplicate and the results were expressed in µmol Trolox equivalent per liter of extract (µmol TE/L). The inhibition rate (%I) of ABTS+ was expressed as follows:

% I = [(A0 – Compound Abs) / A0] x 100

A0 = diluted ABTS absorbance.Compound Abs = diluted ABTS absorbance + sample

2-4 Spectrophotometric determination of certain secondary metabolites

2-4-1 Determination of total flavonoids

The method of Marinova et *al*. (2005) was used for the determination of total flavonoids. It consisted of putting a volume of 0,75 mL of sodium nitrite ($NaNO_2$) at 5% (m/v) into a 25 mL flask, adding 2,5 mL of extract then homogenizing it. To this mixture, 0,75 mL of aluminum chloride (AlCl3) at 10% (m/v) was added, then incubated for 6 minutes in the dark. After the incubation, a volume of 5 mL of sodium hydroxide (1N NaOH) was added and then the volume was made up to 25 mL with distilled water. The mixture was shaken vigorously before being measured with a UV-visible spectrophotometer. The reading was taken at 510 nm. The tests were carried out in triplicate. The flavonoid content was expressed in grams per liter of quercetin equivalent extract.

2-4-2 Determination of total polyphenols

The determination of total polyphenols was carried out according to the method described by Wood et *al*. (2002). To do this, to a volume of 30 µL of extract or fraction, 2,5 mL of Folin-Ciocalteu reagent diluted 1/10 was added. The mixture obtained was kept for 2 minutes in the dark at room temperature (27 ± 3 °C) then 2 mL of sodium carbonate solution at 75 g/L was added and then homogenized. The solution obtained was then incubated at 50°C for 15 minutes. The absorbance reading was carried out with a UV-visible spectrophotometer at a wavelength of 760 nm against a blank consisting of distilled water. Gallic acid was used as a reference standard for the quantification of total polyphenol contents expressed in grams per liter of gallic acid equivalent extract (g.L-1, Eq AG). The tests were carried out in triplicate for each sample.

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2-4-3 Dosage of total tannins

The condensed tannin content was determined according to the method described by Julkunen-Titto (1985). To carry it out, a volume of 50 mL of each fraction or the hydro-alcoholic extract was added to 1500 µL of the 4% solution of vanillin in methanol then stirred vigorously. To this mixture, 750 µL of concentrated hydrochloric acid was added and the whole mixture was then left to stand at room temperature for 20 minutes. The absorbance was measured at a wavelength of 550 nm against a blank consisting of the 4% solution of vanillin in methanol. The tests were carried out in triplicate for each sample. A stock solution of tanic acid was used as a reference standard for the establishment of the calibration curve and for the quantification of the contents of condensed tannins expressed in milligram equivalent of tanic acid per gram of dry matter (mg EAT /g of dry matter). The tests were carried out in triplicate for each sample.

2-5 Statistical analyzes

The curves and histograms are drawn using Microsoft Excel 2013 and GraphPad Prism 5 software. The IC_{50} values (50% inhibitory concentration) were calculated by the linear regression method from the curve [% inhibition = f (concentrations)].

RESULTS

1- Antioxidant activity by the DPPH test

The antioxidant activity of extracts ((aqueous (Aq) and hydro-ethanolic (HOH)) and fractions (dichloromethane (DCM) and aqueous (Faq)) of *Talinumtriangulare* leaves and that of ascorbic acid (vitamin C) was represented by graphs showing the variation in the percentage of free radical inhibitory power as a function of the concentration of each compound (figure 1 and 2). The DCM and Faq fractions of *Talinumtriangulare* leaves gave respective IC_{50s} of 0,9. and 2,3 mg/mL As for the aqueous (Aq) and hydro-ethanolic (HOH) extracts, their IC_{50} could not be defined from the linear regression equations of the graphs. Regarding vitamin C (reference molecule), it provided an IC_{50} of 3,056 µg/mL. It is also noted that the reduction of free radicals at the level of the two fractions (DCM and Faq) and the reference molecule (vitamin C) is a function of the concentration of the substance used.

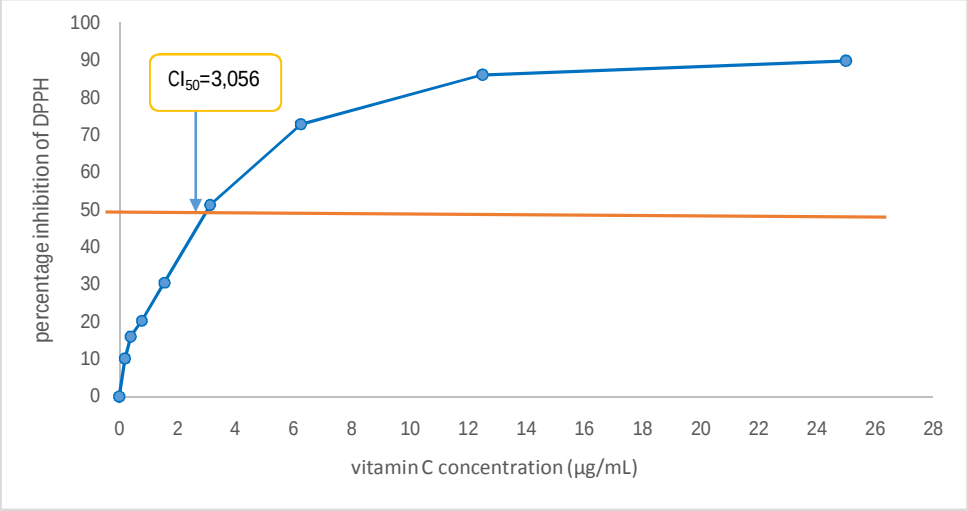

Figure 1: Percentage of DPPH inhibition as a function of different concentrations of ascorbic acid (reference molecule)

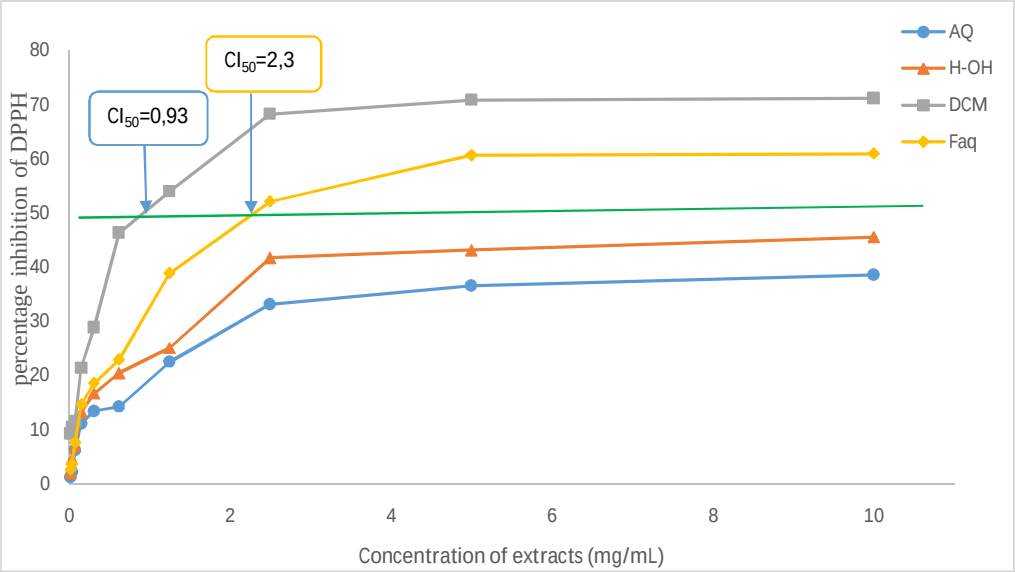

Figure 2: Percentage of DPPH inhibition as a function of concentrations of various extracts and fractions of *Talinumtriangulare* leaves

2- Results of antioxidant activity by ABTS$^+$

Figure 3 shows the proton trapping capacity of the cationic radical ABTS$^+$ of the various products used (plant extracts and fractions). These results generally reveal that the products requested inhibited the absorbance of the ABTS$^+$ radical. However, the percentage of inhibition is greater for the DCM fraction (41,59%) than that of Faq (40,59%). While that of Faq is more remarkable than that of the HOH extract (38,29%). Of the two extracts, only the Aq extract has the lowest percentage of inhibition (25,51%).

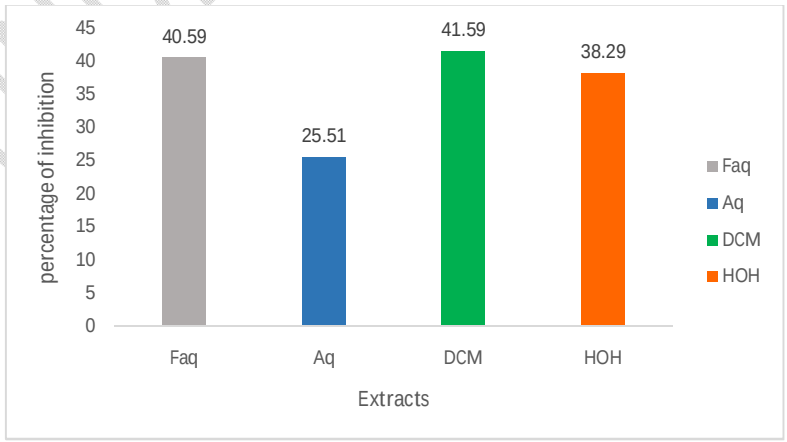

Figure 3: Percentage of inhibition of the absorbance of the $ABTS^{+}$ radical various extracts and fractions of *Talinumtriangulare* leaves

3- Total flavonoid contents

Figure 4 is the result of the total flavonoid contents of the extracts (aqueous and hydro-ethanolic) and fractions (DCM and Faq) of the leaves of *Talinumtriangulare* (Talinaceae). It generally reveals that the aqueous extract (Aq) contains more total flavonoids (10,16667 ± 0,289 mg EQ/g of extract) than the DCM fraction and the HOH extract with approximately equal respective values of 2,16667 ± 0,577 and 1,8333 ± 0,577 mg EQ/g of extract. As for the Faq fraction, it is characterized by a low presence of total flavonoids (0,5 ± 0.00 mg EQ/g).

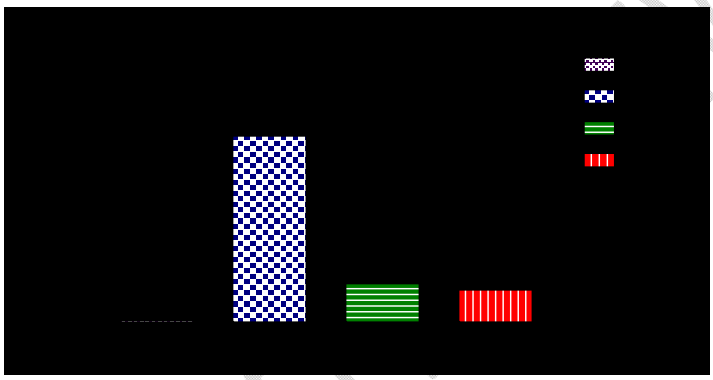

Figure 4: Total flavonoid contents of the Aq and HOH extracts, and of the DCM and Faq fractions of the leaves of *Talinumtriangulare* (Talinaceae).

4- Total polyphenol contents

The results of the polyphenol contents are presented in Figure 5. It should generally be noted that this content is greater in the HOH extract (4,8333 ± 0,289 mg EAG/g) than in the Faq fraction (4,5 ± 0,289 mg EAG/g), 0,500 mg EAG/g). After these, follow the Aq extract (4,333 ± 0,289 mg EAG/g) and the DCM fraction (3,8333 ± 0,289 mg EAG/g).

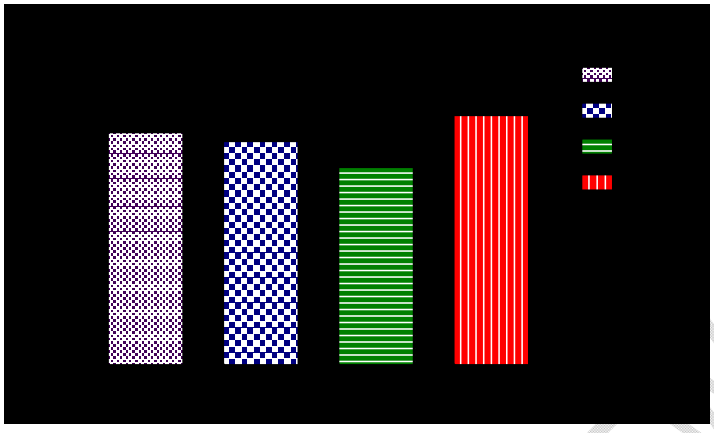

Figure 5: Total polyphenol contents of the Aq and HOH extracts, and of the DCM and Faq fractions of the leaves of *Talinumtriangulare* (Talinaceae).

5- Total tannin contents

The results of the total tannin dosage test are given in Figure 6. They show overall that the total tannin content is relatively low in all of the *Talinumtriangulare* compounds studied. However, the tannin level is a little more abundant in the HOH extract (3,3228333 ± 0,0254 µg EAT/g of dry extract) than in the Aq extract (2,970667 ± 0,00318 µg EAT/ g of dry extract). After these, the DCM fraction has more total tannins than the Faq fraction with respective contents of 2,5096667 ± 0,00029 and 1,835333 ± 0,01384 µg EAT/g of dry extract.

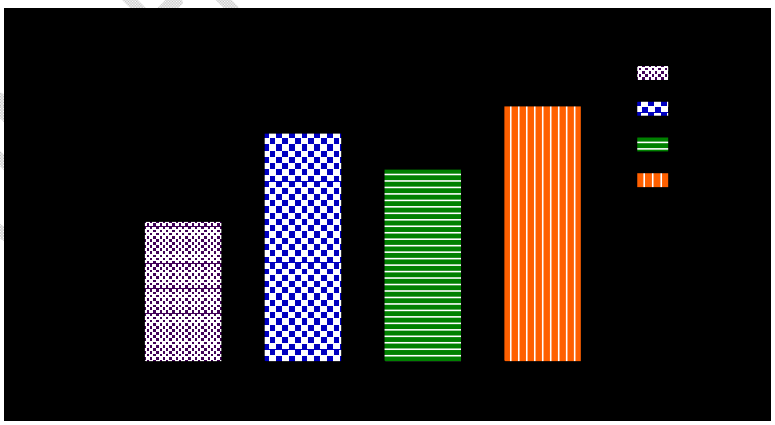

Figure 6: Total tannin contents of the Aq and HOH extracts, and of the DCM and Faq fractions of the leaves of *Talinumtriangulare* (Talinaceae).

DISCUSSION

The evaluation of the antioxidant activity of the 70% aqueous and hydro-ethanolic extracts, as well as the residual dichloromethane and aqueous fractions of *Talinumtriangulare*, reveals respective IC_{50s} of 0,9 and 2,3 mg/mL for the fractions. The aqueous and hydro-ethanolic extracts did not allow the IC_{50} to be determined using the DPPH test. In comparison, vitamin C, used as a reference molecule, showed an IC_{50} of 3,056 µg/mL. These results suggest through the inhibition activity curves that the reduction of free radicals is a function of the concentration of the substances tested, confirming the dose-dependent nature of the antioxidant activity. Compared to the work of Kouakou (2022), who reported IC_{50s} of 0,398 and 0,235 mg/mL for methanolic extracts of Shea hulls and cakes, *Talinumtriangulare* fractions show lower anti-radical activity. Vitamin C is approximately 294 times (0,9 mg/3,056 µg) more effective than the fractiondichloromethane and approximately 752 times (2,3 mg/3,056 µg) more effective than the residual aqueous fraction, highlighting the superior effectiveness of vitamin C as a pure antioxidant. In contrast, the dichloromethane fraction is approximately twice as effective as the residual aqueous fraction (2,3/0,9). This result is supported by the work of Athamena et *al.* (2010), who showed that the percentage of inhibition of free radicals varies depending on the solvent used for the extraction and the concentrations of the anti-radical compounds. The results of Tidiane et *al.* (2021) report a lower IC_{50} of vitamin C in Vitellaria paradoxa (shea) extract, but our IC_{50} for vitamin C (3,056 µg/mL) is approximately three times higher than theirs, which may reflect differences in sample quality or experimental conditions.According to Barkat and Laib (2011), a lower IC_{50} is associated with better antioxidant activity. However, despite the higher IC_{50} of *Talinumtriangulare* fractions compared to vitamin C, their antioxidant activity remains significant.

The results of the $ABTS^{+}$ radical absorbance inhibition test revealed a higher percentage for the dichloromethane fraction (41,59%) compared to the residual aqueous fraction (40,59%), the hydro-ethanolic extract (38,29 %), and the aqueous extract (25,51%). They thus corroborate those obtained with the DDPH test on the antioxidant capacity of the different compounds contained in the plant products tested and show comparable performance, but highlight the hydro-ethanolic extract and the aqueous extract as having antioxidant capacities notable, although lower than the dichloromethane fraction.This study also highlighted the presence of phenolic compounds such as flavonoids, polyphenols and total tannins at different

proportions in the plant products used. In fact, they are present in descending order when switching from one plant product to another. Thus, the content of total flavonoids is 10,16667 ± 0,289 mg EQ/g for the aqueous extract, 2,16667 ± 0,577 mg EQ/g for the dichloromethane fraction, 1,8333 ± 0,577 mg EQ/g for the hydroethanolic extract and 0,5 ± 0,00 mg EQ/g of extract for the residual aqueous fraction. Regarding the content of total polyphenols, it is 4,8333 ± 0,289 mg EAG/g for the hydro-ethanolic extract, 4,5 ± 0,500 mg EAG/g for the residual aqueous fraction, 4,333 ± 0,289 mg EAG/g for the aqueous extract and 3,8333 ± 0,289 mg EAG/g for the dichloromethane fraction. As for the total tannin content, it is 3,3228333 ± 0,0254 µg EAT/g for the hydroethanolic extract, 2,970667 ± 0,00318 µg EAT/g for the aqueous extract, and 2,5096667 ± 0,00029 µg EAT/g and 1,835333 ± 0,01384 µg EAT/g of dry extract respectively for the dichloromethane fraction and the residual aqueous fraction.These results confirm the observations of N'guessan et *al.* (2007) and Bidié et *al.* (2011), who found a correlation between the level of total phenols and antiradical activity. Furthermore, the work of Chen and Ho (1995) demonstrated that the functional groups of phenolic compounds, such as flavonoids and polyphenols, are capable of donating electrons or protons to neutralize free radicals. Thus, the antioxidant activity of the requested plant products previously demonstrated by the DDPH test and the $ABTS^{+}$ radical-cation reduction test could be due to the presence of these phenolic compounds (polyphenols, flavonoids and total tannins) in the leaves of *Talinumtriangulare*. The results of this study are different from those of Soro (2023) who revealed the absence of phenolic compounds such as polyphenols and total flavonoids in Rhynchospora corymbosa extract.

CONCLUSION

The study demonstrated that *Talinumtriangulare* leaf extracts and fractions have variable antioxidant activity, with notable effectiveness of the dichloromethane fraction. Although vitamin C remains the most potent antioxidant among the compounds tested, the plant's fractions offer significant antioxidant potential, justifying their traditional use in the treatment of malaria. The results suggest that phenolic compounds present in *Talinumtriangulare* compounds contribute to this antioxidant activity. These results support the interest of this plant in traditional pharmacopoeia and encourage further research to isolate and characterize the active compounds responsible for its therapeutic properties.

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