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Semi-Quantitatif Phytochemical Profile and Antiradical Potential of Aqueous and 70 % Ethanol Extracts of *Zanthoxylum Zanthoxyloides* (Lam) Zepern & Timler, Leaves Used In Traditional Medicine in the North of Côte D'ivoire

Monon Koné[1]*, Fabrice Armel Zoro[1], Gboko Abiba Ouattara[1], Daouda Touré[1], Ahmont Landry Claude Kablan[2], Abdoulaye Toure[1], Karamoko Ouattara[3], Adama Coulibaly[3]

[1]*Department of Biochemistry-Genetics, Training and Research Unit of Biological Sciences, Peleforo Gon Coulibaly University of Korhogo, Korhogo, Côte d'Ivoire.*

[2]*Department of Mathematics-Physics-Chemistry, Training and Research Unit of Biological Sciences, Peleforo Gon Coulibaly University of Korhogo, Korhogo, Côte d'Ivoire.*

[3]*Department of Biology and Health, Training and Research Unit of Bioscience, University of Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire*

Corresponding Author:** ***Monon Koné*, Department of Biochemistry-Genetics, Training and Research Unit of Biological Sciences, Peleforo Gon Coulibaly University of Korhogo, Korhogo, Côte d'Ivoire.*

Abstract

Medicinal plants have a wide range of therapeutic properties and are therefore used in the treatment of a variety of illnesses. This is the case of Zanthoxylum zanthoxyloides, a plant regularly prescribed in the north of Côte d'Ivoire for a variety of reasons. Our aim is to assess the phytochemical composition and antioxidant activity of the leaves of this plant. Phytochemical compounds were detected using thin layer chromatography (TLC) tests on silica gel 60 F254 chromatoplates on an aluminium support (Merck). Antioxidant activity was assessed by TLC and spectrophotometry. Phytochemical screening revealed the presence of several chemical compounds of interest (flavonoids, tannins, coumarins, terpenes and sterols) and an absence of phenolic acid and alkaloids in the leaf extracts. As for antioxidant activity, the IC50 value of the 70% ethanol extract (IC50 =56.48 µg/mL) is close to that of vitamin C (IC50 =39.81 µg/mL). The 70% ethanol extract of Z. zanthoxyloides is a good antioxidant. This work justifies the use of Z. zanthoxyloides leaves in traditional environments. They could be used to treat pathologies linked to oxidative stress.

Keywords: *Zanthoxylum. zanthoxyloides, antiradical activity, traditional medicine, phytochemical screening.*

1. INTRODUCTION

Herbal medicines have grown in popularity in recent decades, with many people turning to them to maintain their health and prevent and treat various illnesses, particularly chronic diseases. In Europe, the use of traditional medicine varies from 42% in Belgium to 90% in the UK, and from 42% in the USA among adults to 70% in Canada **(**Sarman et Uzuntarla 2022**;** Esmaïl, 2017**)**. In Africa, the use of traditional medicine varies from 60% in Uganda and the United Republic of Tanzania, 70% in Ghana and Rwanda, to 80% in Benin and 90% in Burundi and Ethiopia (Ouoba *et al*., 2022). For millions of people, herbal medicines are the only reliable source of healthcare. This is due to the safety of traditional medicines its easy access and affordability, in contrast to the high cost of health worker services and pharmaceutical drugs. The elimination of natural molecules by the body is easy, and the risk of carcinogenicity or complicated diseases is low. Many therapeutic virtues are attributed to plants, which are generally linked to their richness in phytomolecules known as secondary metabolites (Jamshidi-Kia *et al*, 2017). These secondary metabolites, which contain several classes of compounds, are generally of polyphenolic origin and are the most abundant in plant material (Kouamé *et al.,* 2021). These polyphenolic compounds are endowed with significant antimicrobial, antiplasmodic, antihelmetic, antihistaminic and antioxidant activities (Kouadio *et al*., 2021). As such, they have the potential to treat many emerging chronic pathologies linked to oxidative stress, such as cancer, diabetes, asthma,

premature ageing, endocrine diseases, cardiovascular and neurodegenerative diseases, as well as numerous inflammatory diseases (Cheurfa et Allem, 2016). Being aware of the virtues attributed to medicinal plants, the people in northern Côte d'Ivoire use Zanthozylum zanthoxyloides to treat many illnesses, particularly serious chronic diseases. This plant belongs to the Rutaceae family. Its ethno-medical use remains insufficiently documented. This study, based on the evaluation of the antioxidant activity and the detection of the phytochemical profile of Z. zanthoxyloides extracts, could provide justification for the choice of this plant in the treatment of certain pathologies.

2. Materials And Methods

2.1. Material

2.1.1. Plant Material and Pretreatment

The biological material consisted of Zanthoxylum zanthoxyloides leaves harvested in February 2024 in Korhogo in the Poro region (northern Côte d'Ivoire). The leaves were washed and left to dry in the shade at room temperature in a ventilated area for 15 days. The dried leaves were ground using an electric grinder, and the powder obtained was stored in jars in a dry place protected from light and humidity until use.

2.1.2. Reagents and Chemicals

The main reagents and developers listed below were used in this study. Dragendorff's reagent was used for alkaloid revelation. Iron III chloride (FeCl3) 2% was used to reveal polyphenols, tannins and phenolic acids. For flavonoids, 1% aluminum chloride (AlCl3) was used. Coumarins were revealed with 5% potassium hydroxide (KOH) and sterols and terpenes with vanillin sulfur. DPPH was used to assess the antioxidant activity of phenolic compounds. All thin-layer chromatography (TLC) tests were carried out on 60 F254 silica gel chromatoplates on aluminum support (Merck).

2.2.1. Aqueous Decoction

A 100 g test portion of plant powder was introduced into a flask containing 1000 mL of distilled water. The mixture was kept boiling for 20 minutes. After cooling and filtration, the different filtrates were combined and placed in an oven at 50°C for 3 days (Bohui *et al*., 2018).

2.2.2. Ethanolic Maceration

A 100 g mass of plant powder was introduced into a blender containing 1000 mL of a 70% (v/v) hydroethanolic solution. The whole was kept under agitation for 3 times 5 min. After filtration twice on absorbent cotton and once on Whatmann n°3 filter paper, the filtrate was placed in an oven at 50°C for 24 hours (Zirihi *et al.,* 2003).

2.2.3. Preparation of Selective Fractions

The total extracts obtained were used to prepare the fractions. A mass of 2 g of each extract was dissolved in 30 mL of distilled water. A volume of 20 mL of each prepared solution was exhausted by successive fractionations with 10 mL hexane (C6H14), 10 mL dichloromethane (CH2Cl2) and 10 mL ethyl acetate (AcOEt) (Koné *et al*., 2024 (a); Ouattara *et al.,* 2016).

2.2.4. Phytochemical Screening of Phenolic Compounds on TLC Plates

Flavonoids, coumarins, tannins, alkaloids and phenolic acids were detected on aluminum-supported silica gel 60 F254 chromatoplates (Merck). Fractions were screened according to the methods described in Lagnika, (2005). Using capillary tubes, 2 µL of each fraction was deposited as a dot 0.5 cm from both edges of the chromatoplate. The TLC plates are then placed in the migration tank containing a solvent mixture composed as follows:

- -Dichloromethane (CH2Cl2)/ethyl acetate (AcOEt)/hexane (C6H14) (2:2:1; V/V/V); for dichloromethane extracts;
- -CH2Cl2/AcOEt/C6H14 (3 :4 :2) (V/V/V) and CH2Cl2/AcOEt/ CH3COOH (1:3, 5:1) (V/V/V) for acetate-ethyl extracts.
- -C6H14 /AcOEt (5 :0.375) (V/V) for hexanic extracts

After development, chromatograms were visualized using developers (AlCl3, FeCl3, vanillin sulfur, Dragendorff and KOH) for flavonoids, tannins, terpenoids, alkaloids and coumarins respectively. Plates were then observed under UV 366 nm (Lagnika, 2005). Characteristic colorations, appearing as spots, were recorded and the frontal ratios (Rf) were calculated for each spot using the relationship:

$$Rf = \frac{\text{Distance covered by the component}}{\text{Distance covered by eluent}}$$

2.2.5. Detection of Antioxidant Power by TLC

The detection of antioxidant power by TLC was carried out according to the method described by Takao *et al.* (1994). In this technique, a volume of 10 µL of each extract solution is deposited on a chromatoplate (silica gel 60 F254, on aluminum support (Merck)). The chromatoplate is placed in a chromatography tank saturated with migration solvent. After development, the chromatogram is dried and revealed with an ethanolic solution of DPPH. After 30 min at room temperature, extract constituents with potential free radical scavenging activity are revealed as pale-yellow spots on a violet background.

2.2.6. Spectrophotometric Determination of Antioxidant Activity

The antioxidant potential of extracts was assessed according to the method of Blois (1958). DPPH was solubilized in absolute EtOH to obtain a solution with a concentration of 20 µg/mL. Different concentrations (2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 mg/mL) of the extract are prepared in absolute EtOH. 2.5 mL extract and 1 mL ethanolic DPPH solution are added to dry tubes. After shaking, the tubes are placed in the dark for 30 min. The absorbance of the mixture is measured at 517 nm against a blank consisting of 2.5 mL pure EtOH and 1 mL DPPH solution. The positive reference control is ascorbic acid (vitamin C). DPPH inhibition percentages are calculated according to the following formula:

%I = (Abscontrol - Absextract) / Abscontrol x 100%

%I: Inhibition percentage; Abscontrol: Absorbance of the control; Absextract : Absorbance of the extract

The concentrations required to trap 50% (IC50) of DPPH are determined on graphs representing DPPH inhibition percentage as a function of extract or vitamin C concentrations.

2.3. Statistical Analysis

Graph pad Prism software 8.0.1 version was used to construct the graphs and determine IC50 values. Statistical analyses of the results were carried out using Statistica software 7.1 version Fisher's LSD test was used to determine significant differences between several means. For all statistical analyses, differences were considered significant at the 5% level.

3. Results And Discussion

3.1. Phytochemical Profile of Phenolic Compounds on TLC Plates

The results in Tables I, II, III, IV and V show the colorations and front ratios (Rf) of the spots corresponding to the groups of secondary metabolites present in the fractions studied, in the visible and/or UV light. Flavonoids appear yellow in both visible and UV light. However, with the same developer, a green spot in the visible at Rf=0.95 and 2 blue spots in the UV at Rf=0.73 and 0.88 were observed in the ethyl acetate fraction of ZZD. Similar results with different Rf were obtained with the ethyl acetate fraction ZZE (Table I and Figure 1a). These results show that the ethyl acetate fractions are rich in various types of flavonoids. They also contain very few tannins (a spot at rf: 0.95), as shown by the results reported in Table II and Figure (1b). Alkaloids were absent in the dichloromethane fraction, which contained very little coumarin (one spot, rf: 0.94, in both visible and UV), as shown in Tables III and IV. Sterols and terpenes were detected in the hexane fraction. Orange and violet spots indicate the presence of terpenes, while the blue color indicates sterols. The ZZD fraction showed 3 orange spots indicating the presence of terpenes, while the ZZE fraction showed the presence of two compounds, a sterol at rf: 0.74 and a terpene at rf: 00 (Table V and figure 1c).

a b c

Figure 1. *Visible detection of ethyl acetate and hexanic fractions*

a) -flavonoids; b) -tanins; c) -sterols and terpenes

Table 1. *Flavonoid detection in CH2Cl2/AcOEt/C6H14 (3 :4 :2) (V/V/V) developer from acetate-ethyl (AcOEt) fractions*

Extraits	R_f (visible color, UV color) : *Possible compound*
ZZ D (AcOEt)	0,95 (Vert, -) : flavonoïde ; 0,88 (-, Bleu) : flavonoïde ; 0,84 (Jaune, -) 0,8 (Jaune, Jaune) : flavonoïde ; 0,73 (-, Bleu) : flavonoïde ; 0,64 (Jaune, Jaune) : flavonoïde ; 0,54 (Jaune, Jaune) : flavonoïde ; 0,44 (-, Jaune) : flavonoïde ; 0,31 (-, Jaune) : flavonoïde ; 0,23 (-, Jaune) : flavonoïde 0,13 (-, Jaune) : flavonoïde ; 0,00 (-, Jaune) : flavonoïde
ZZ E (AcOEt)	0,96 (Vert, -) : flavonoïde ; 0,88 (-, Bleu) : flavonoïde ; 0,8 (Vert, Bleu) : flavonoïde ; 0,70 (Jaune, Jaune) : flavonoïde ; 0,6 (Jaune, Jaune) : flavonoïde ; 0,50 (Jaune, Jaune) : flavonoïde ; 0,44 (-, Jaune) : flavonoïde ; 0,33 (-, Jaune) : flavonoïde ; 0,19 (-, Jaune) : flavonoïde ; 0,09 (-, Jaune) : flavonoïde ; 0,00 (-, Jaune) : flavonoïde

ZZD : Décocté aqueux de *Z. zanthoxyloides*; ZZ E : Macéré éthanolique de *Z. zanthoxyloides*, (-) : nothing

Table 2. *Detection of tannins and phenolic acids in CH2Cl2/AcOEt/ CH3COOH (1:3,5:1) (V/V/V) developer with ethyl acetate extract*

Extraits	Rf (visible color) : Possible compound
ZZ D (AcOEt)	0,95 (gris) : tanin ; 0,90 (gris) : tanin
ZZ E (AcOEt)	0,95 (gris) : tanin ; 0,90 (gris) : tanin
ZZ D (AcOEt)	Aucun acide phénolique identifié
ZZ E (AcOEt)	Aucun acide phénolique identifié

Table 3. *Detection of alkaloids in CH2Cl2/AcOEt/C6H14 (2 :2 :1) (V/V/V) developer with dichloromethane fraction*

Extraits	Rf (visible color) : Possible compound
ZZ D (DCM)	Aucun alcaloïde identifié
ZZ E (DCM)	Aucun alcaloïde identifié

Table 4. *Detection of coumarins in CH2Cl2/AcOEt/C6H14 (2 :2 :1) (V/V/V) developer with dichloromethane fraction*

Extraits	Rf (visible color ; UV color) : Possible compound
ZZ D (DCM)	0,94 (Jaune, Bleu) : coumarine
ZZ E (DCM)	0,94 (Jaune, Bleu) : coumarine

Table 5. *Detection of terpenes and sterols in C6H14/AcOEt (5 :0.375) (V/V) developer with hexane fraction*

Extraits	Rf (visible Color) : Possible compound
ZZ D (Hex)	0,95 (Orange) : terpène ; 0,88 (Orange) : terpène ; 0,81 (Orange) : terpène
ZZ E (Hex)	0,74 (Bleu) : stérol ; 0,00 (Violet) : terpène

3.2. Antioxidant Activity

3.2.1. DPPH Radical Inhibitory Power by TLC

The DPPH radical inhibitory power of the phenolic compounds studied was highlighted through the appearance of a pale-yellow color on a violet background. The calculated head-to-head ratios (Rf) are given in Tables VI, VII, VIII and IX. The ethylacetate fractions of ZZD and ZZE showed the same number of spots (13). In ZZD, 3 spots were identified as flavonoids, at rf: 0.96; 0.84; 0.00; the other 10 spots were compounds not identified (NI) during phytochemical screening. As for ZZE, 7 spots were identified, flavonoids and 6 NI spots (table VI and figure 2a). The analysis of Table VII shows that a DPPH radical-scavenging compound, tannins, was identified at rf: 0.90 in the ZZD fraction, while in the ZZE fraction 2 compounds were identified at rf: 0.95 and 0.90. With regard to the coumarins (Table VIII and Figure 2b), the two dichloromethane fractions (ZZD and ZZE) showed an identified spot, coumarin at rf: 0.94. The hexane fractions ZZD and ZZE also showed DPPH radical scavenging power. One spot identified terpene at rf: 0.81 in ZZD and 2 compounds identified sterol at rf: 0.74 and terpene at rf: 0.00 in ZZE (table IX and figure 2c). This analysis shows that, in addition to the compounds identified in the phytochemical screening, several unidentified compounds have free radical scavenging activity.

Table 6. *DPPH scavenging phytocompounds in CH2Cl2/AcOEt/C6 H14 (3 :4 :2) (V/V/V) developer with ethyl acetate fraction for flavonoids*

Extraits	R_f (Color) : *Possible compound*
ZZ D (AcOEt)	0,96 (Jaune) : flavonoïde ; 0,91 (Jaune) : NI ; 0,84 (Jaune) : flavonoïde ; 0,75 (Jaune) : NI ; 0,71 (Jaune) : NI ; 0,61 (Jaune) : NI ; 0,50 (Jaune) : NI ; 0,43 (Jaune) : NI ; 0,35 (Jaune) : NI ; 0,25 (Jaune) : NI ; 0,20 (Jaune) : NI ; 0,19 (Jaune) : NI ; 0,00 (Jaune) : flavonoïde
ZZ E (AcOEt)	0,95 (Jaune) : flavonoïde ; 0,88 (Jaune) : flavonoïde ; 0,81 (Jaune) : NI ; 0,73 (Jaune) : flavonoïde ; 0,66 (Jaune) : NI ; 0,61 (Jaune) : NI ; 0,53 (Jaune) : flavonoïde ; 0,46 (Jaune) : NI ; 0,36 (Jaune) : NI ; 0,28 (Jaune) : NI ; 0,19 (Jaune) : flavonoïde ; 0,09 (Jaune) : flavonoïde ; 0,06 (Jaune) : NI ; 0,00 (Jaune) : flavonoïde

NI: unidentified compound

Table 7. *DPPH scavenging phytocompounds in CH2Cl2/AcOEt/ CH3COOH (1:3,5:1) (V/V/V) developer with ethyl acetate fraction for tannins*

Extraits	R_f (Color) : *Possible compound*
ZZ D (AcOEt)	0,98 (Jaune) : NI ; 0,90 (Jaune) : tanin ; 0,83 (Jaune) : NI
ZZ E (AcOEt)	0,95 (Jaune) : tanin ; 0,90 (Jaune) : tanin ; 0,81 (Jaune) : NI ; 0,73 (Jaune) : NI ; 0,65 (Jaune) : NI

NI: unidentified compound

Table 8. *DPPH-scavenging phytocompounds in CH2Cl2/AcOEt/C6H14 (2 :2 :1) (V/V/V) developer with Dichloromethane extract for coumarins*

Extraits	R_f (Color) : *Possible compound*
ZZ D	0,94 (Jaune) : coumarine ; 0,86 (Jaune) : NI ; 0,79 (Jaune) : NI
ZZ E	0,94 (Jaune) : coumarine ; 0,85 (Jaune) : NI ; 0,79 (Jaune) : NI ; 0,71 (Jaune) : NI ; 0,63 (Jaune) : NI ; 0,23 (Jaune) : NI ; 0,16 (Jaune) : NI ; 0,09 (Jaune) : NI ; 0,00 (Jaune) : NI

NI: unidentified compound

Table 9. *DPPH-scavenging phytocompounds in AcOEt/C6H14 (5:0.375) developer (V/V) with hexane extract for sterols and terpenes*

Extraits	R_f (Color) : *Possible compound*
ZZD (Hex)	0,84 (Jaune) : NI ; 0,81 (Jaune) : Terpène ; 0,69 (Jaune) : NI ; 0,40 (Jaune) : NI ; 0,30 (Jaune) : NI ; 0,21 (Jaune) : NI ; 0,13 (Jaune) : NI ; 0,06 (Jaune) : NI
ZZE (Hex)	0,89 (Jaune) : NI ; 0,74 (Jaune) : Stérol ; 0,59 (Jaune) : NI ; 0,43 (Jaune) : NI ; 0,29 (Jaune) : NI ; 0,23 (Jaune) : NI ; 0,13 (Jaune) : NI ; 0,06(Jaune) : NI ; 0,00 (Jaune) : Terpène

NI: unidentified compound

a **b** **c**

Figure 2. *Screening for antioxidant activity of fractions*

a) -flavonoids; b) -coumarins; c) -sterols and terpenes

3.2.2 Inhibitory Power of the DPPH Radical by Spectrophotometer

The curves in Figure 3 show the percentages inhibition of the DPPH radical by aqueous, 70% ethanol and vitamin C extracts. The results show a dose-dependent effect at different DPPH concentrations. Curve analysis revealed IC50 values ranging from 39.815 ± 0.003 µg/mL (vitamin C) through 56.481 ± 0.003 µg/mL (ZZ E) to 110.186 ±0.006 µg/mL (ZZ D).

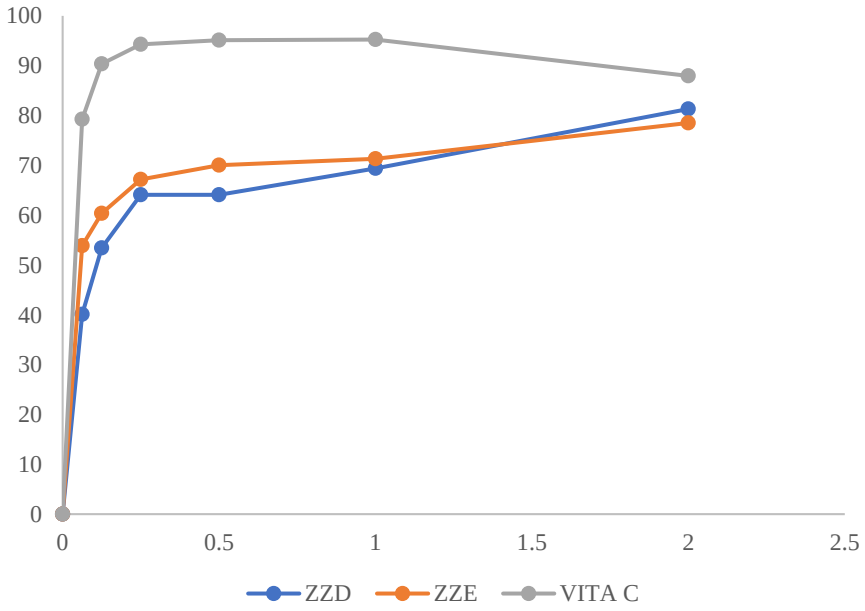

Figure 3. *DPPH radical inhibition curves for extracts and vitamin C*

4. DISCUSSION

The aim of this study was to determine the phytochemical profile and antiradical activity of 70% ethanol and aqueous extracts of Zanthoxylum zanthoxyloides leaves. Zanthoxylum zanthoxyloides is used alone or in combination in the treatment of a number of pathologies, including urinary tract infections, dental caries, sickle cell anemia and inflammatory diseases (pain, fever). Determining its phytochemical profile may be necessary to provide scientific evidence for its use in traditional environments. The two polar solvents used in this study (water, ethanol) were chosen to facilitate continuous extraction of the active ingredients in a solid-liquid system, and to come as close as possible to domestic realities (Poirot, 2007; Koné et al., 2024(b)). Microplates made from selective extracts obtained after fractionation of aqueous (ZZD) and 70% ethanol (ZZE) extracts showed several spots of varying color depending on the reagents and compounds highlighted. Flavonoids were co-present with tannins, coumarins, sterols and terpenes in both extracts (Koné *et al*., 2024 (b); Cissé *et al*.,2024). Tannins, coumarins, sterols and terpenes were poorly represented, and an absence of phenolic acids and alkaloids was observed in the same extracts studied (Cissé *et al*.,2024). These results show that flavonoids were the predominant phenolic compounds in the 70% ethanol and aqueous extracts of Z. zanthoxyloides. The scientific literature reports similar results (Adou *et al*., 2029; Koné *et al*., 2024 (b)). A comparison of our results with those of other researchers showed that, in terms of Z. zanthoxyloides leaves, plants from Côte d'Ivoire and Mali have more or less the same phytochemical composition, but differ from those from the DRC. The difference in phytochemical composition could be linked to several factors, notably geographical origin and ecological conditions (altitude, degree of soil fertilization, wild or cultivated nature of the plant) (Togola *et al*., 2023; Olushola-siedoks *et al*., 2020). Phenolic compounds such as flavonoids, tannins, coumarins, sterols and terpenes are endowed with biological and pharmacological activities (Ouedraogo *et al.,* 2021; Juang et Liang, 2020). The physiological and pharmacological properties recognized in the leaves of this plant could be linked to the high presence of bioactive compounds. These compounds may have analgesic Koné et al., 2024 (b) and non-antibacterial properties (Del Prado-Audelo *et al*., 2021). These secondary metabolites highlighted in the phytochemical screening of selective extracts could be the basis for screening the antiradical activity of each fraction. In the ethylacetate fractions, flavonoids were more active than tannins. In the chloroformic fractions, DPPH scavengers were coumarins, while in the hexanic fractions, sterols and terpenes were more active. Most of the phytocompounds found to be free-radical scavengers were flavonoids (Maguirgué *et al*., 2022; N'Guessan *et al*., 2011). The results of the anti-free radical screening of Z. zanthoxyloides leaf extracts were reinforced by the determination of IC50 values (see figure 3). The IC50 of the aqueous extract was 2 times higher than that of the 70% ethanol extract, which is close to that of vitamin C. The 70% ethanol extract therefore has the best anti-free radical activity, and could be more active than the aqueous extract in the treatment of pathologies linked to oxidative stress.

5. CONCLUSION

Leaf extracts from Z. zanthoxyloides harvested in the northern Côte d'Ivoire town of Korhogo are potential sources of flavonoids, tannins, coumarins, sterols and terpenes. However, these leaves do not contain alkaloids or phenolic acids. All extracts show anti-free radical activity. The best activity was obtained with the ethylacetate fraction of the leaves. The 70% ethanol extract was found to have the best anti-free radical activity. This result was confirmed by the IC50 value of this extract, which is close to that of the reference molecule (vitamin C). Z. zanthoxyloid leaves are potentially anti-free radical and therefore good antioxidants. They can be recommended in the treatment of pathologies linked to oxidative stress.

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