https://doi.org/10.48047/AFJBS.6.13.2024.7579-7587

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Antipromastigote, antiradical activity and phytochemical screening of four plants of traditional Ivorian medicine

Pekoula Yves Patrick[1], Kipré Gueyraud Rolland[1*], Agré Don Josette[1], Asare Kwame Kumi[2], Offoumou M'Baï Rostand[1], N'Guessan Jean David[1], Djaman Allico Joseph[1,3]

[1]Laboratory of Biology and Health, UFR Biosciences, Felix Houphouet-Boigny University, Abidjan, Côte d'Ivoire

[2]Deparment of Biomedical Sciences, College of Allied Health Sciences and Allied Sciences,

University of Cape Coast, Ghana

[3]Pasteur Institute of Côte d'Ivoire, Abidjan

*** Corresponding author** : Kipré Gueyraud Rolland, 28 BP 693 Abidjan 28, +225 0505057136, kip_rolland@yahoo.fr

Volume 6, Issue 13, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.13.2024.7579-7587

ABSTRACT

Human leishmaniasis is a tropical parasitic disease that remains a serious public health problem worldwide. It can also be fatal if left untreated, especially when it progresses to the visceral form. To date, there is still no vaccine available to prevent leishmaniasis. The current treatments available to combat leishmaniasis are inadequate, toxic, expensive, require repeated injectable applications, and are not effective against all species. Extracts from *Ricinodendron heudelotii*, *Enantia polycarpa*, *Kigelia africana*, and *Adenia cissampeloides* have been evaluated for their ability to inhibit the growth of *Leishmania donovani* promastigotes. The results show that *E. polycarpa* has the lowest IC_{50} at 4.79±1.02 µg/mL, indicating strong antipromastigote activity. In contrast, *R. heudelotii* and *K. africana* exhibit respective IC_{50} values of 95.10±1.57 µg/mL and 79.44±2.12 µg/mL, suggesting lower efficacy. *A. cissampeloides* did not show significant activity, with an IC_{50} greater than 100 µg/mL. The activity of *E. polycarpa* is likely due to the presence of polyphenols and alkaloids it contains.

Keywords : Antiradical activity, Leishmaniasis, *Leishmania donovani*, Promastigotes, Phytochemical screening.

INTRODUCTION

Leishmaniasis is an anthropozoonosis caused by a parasite of the genus *Leishmania*, transmitted by the bite of a female sandfly.

It is endemic in certain countries and can take on severe forms (visceral involvement) beyond just cutaneous manifestations. It affects 1.5 million people worldwide each year, particularly the most disadvantaged populations in developing countries, with children being especially vulnerable[1].

Epidemiological data suggest that 12 million people are currently infected with the parasite, and 350 million people are at risk in over 98 countries[2]. Each year, there are between 200,000 and 400,000 new cases of visceral leishmaniasis and up to 1.2 million new cases of cutaneous leishmaniasis.

To date, there is still no vaccine available to prevent leishmaniasis; however, several treatments exist. Among them, the first line of defense is pentavalent antimony and its derivatives. This treatment has been used for many years to treat all forms of leishmaniasis. However, growing resistance issues are associated with it[3].

Pentamidine is the second-choice treatment. Its exact mode of action is not yet known, but it has been reported that it accumulates in the parasite's mitochondria, inhibiting mitochondrial topoisomerase[4,5]. Due to its high toxicity, this medication is only used in cases of antimony resistance. Amphotericin B works by forming pores in the plasma membrane, thereby creating an ionic imbalance. This drug is most commonly used for treating leishmaniasis co-infected with HIV-1[6].

Its side effects include cardiovascular collapse, general malaise, fever, chills, and renal failure. Miltefosine is the first treatment administered orally[7]. It acts on the parasite's membrane, causing its apoptosis. This drug has a leishmanicidal effect on both visceral and cutaneous strains. Unfortunately, miltefosine has teratogenic effects, preventing its use in pregnant women. Leishmaniasis is considered an "orphan disease," so few pharmaceutical companies consider researching new molecules due to the low incidence of this disease in the Global North.

Given this situation, it is urgent to find new effective, low-toxicity, and affordable molecules. Plants, rich in bioactive molecules, can be exploited for use as antileishmanial agents.

MATERIAL AND METHODS

Plant Material

The plant material consists of the barks of *Ricinodendron heudelotii*, *Adenia cissampeloides*, *Enatia polycarpa*, and *Kigelia africana*. The plant parts were collected in the Agboville region and identified at the National Center of Floristics at the Felix Houphouët Boigny University in Cocody. The samples were rinsed with tap water and dried at room temperature in the laboratory, away from light. After drying, the plant parts were ground into a fine powder.

Preparation of Extracts

Hydro-ethanolic extracts were prepared by solid-liquid extraction according to the method described by Zirihi et al[8]. One hundred grams (100 g) of plant powder were mixed in one liter (1 L) of 70% ethanol using a Moulinex blender, three times for three minutes. The resulting homogenate was first squeezed through a piece of cloth to remove plant debris, then filtered three times through cotton wool to eliminate debris that may have passed through the cloth mesh. Finally, a last filtration was done using Whatman filter paper. The filtrate obtained was dried in an oven at 45°C for 48 hours. The powder thus obtained is the total hydro-ethanolic extract.

Phytochemical Screening

Phytochemical screening is a method used to highlight the presence of major families of secondary metabolites in a given plant material. In this study, it was performed on medicinal plant extracts using color or precipitation reactions. The techniques used refer to those described in the works of Békro et al[9], N'Guessan et al[10], Koffi et al[11], Kallo et al[12] and Tojola et al[13].

Detection of Polyphenols

To a 2 mL solution of extract (at a concentration of 5 mg/mL), one drop of a 2% alcoholic solution of ferric chloride was added. The appearance of a dark bluish-black or greenish coloration indicates the presence of polyphenols.

Detection of Tannins

- **Catechin Tannins:** To a 5 mL solution of extract (at a concentration of 5 mg/mL), 15 mL of Stiasny's reagent was added. The mixture was heated in a water bath at 80°C for 30 minutes. The observation of large flocculent precipitates indicates the presence of catechin tannins.
- **Gallic Tannins:** A 5 mL sample of plant extract was filtered, saturated with sodium acetate, and then added with 3 drops of 2% FeCl3 (2 g in 100 mL). The appearance of a specific blue-black coloration reveals the presence of gallic tannins.

Detection of Quinones

A sample of 30 mg of plant extract was triturated with 5 mL of diluted hydrochloric acid (1:5). The mixture was heated in a boiling water bath in a test tube for 30 minutes. After cooling, the hydrolysate was extracted with 20 mL of chloroform in a test tube. It was then saturated with 0.5 mL of half-diluted ammonia. The appearance of a red or violet coloration indicates the presence of quinones.

Detection of Alkaloids

The detection of alkaloids was carried out using Dragendorff's and Wagner's reagents. A sample of 30 mg of plant extract was mixed with 6 mL of 60° ethanol. The resulting solution was divided into 2 test tubes.

- In the first tube, two drops of Dragendorff's reagent were added. If an orange precipitate forms, the reaction is positive.
- In the second tube, 2 drops of Wagner's reagent were also added. If flocculation or a precipitate appears, the test is positive.

Detection of Saponins

A 2 mL solution of the extract was mixed with 2 mL of distilled water in a test tube. After shaking for 15 seconds, the formation of a stable foam indicates the presence of saponins in the extract.

Detection of Sterols and Terpenes

To 2.5 mL of an extract solution at a concentration of 0.8 mg/mL, 1 mL of chloroform was added. The mixture was left to rest for 30 seconds. Then, 1.5 mL of sulfuric acid was added to the solution. The appearance of a brown-reddish coloration at the interface is characteristic of the presence of terpenes.

Detection of Flavonoids

Flavonoids were detected in the extracts using the lead acetate test. To 2 mg of each extract, a few drops of lead acetate solution were added. If the solution turns yellow or orange, flavonoids are present.

Detection of Anthocyanins

To 2.5 mL of an extract solution, 1 mL of 20% HCl was added. A pink-red coloration develops. Then, the addition of 5 mL of NH4OH turns the solution blue-violet, indicating the presence of anthocyanins.

Detection of Leuco-Anthocyanins

To 5 mL of an extract solution, 4 mL of hydrochloric alcohol (ethanol/HCl pure 3/1 v/v) was added. After heating in a water bath at 50°C for a few minutes, the appearance of a cherry-red color indicates the presence of leuco-anthocyanins.

Detection of Coumarins

To 2 mL of ethanolic solution, 0.5 mL of 10% NaOH was added. After heating and cooling, 4 mL of distilled water was added. The solution becomes transparent compared to the control (without alkaline solution). The reaction is positive if acidifying the transparent solution with a few drops of concentrated HCl turns it cloudy or if a precipitate forms.

Antiradical Activity

The antiradical activity of the hydro-ethanolic extracts was evaluated using the DPPH test according to the method described by Molyneux[14] with slight modifications. In the presence of free radical scavengers, the violet-colored DPPH radical is reduced to 2,2-diphenyl-1-picrylhydrazine, which is yellow.

A DPPH ethanolic solution was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol. Then, 800 µL of each extract solution or vitamin C (reference antioxidant) at different concentrations (800, 400, 200, 100, 50, 25, 12.5 µg/mL) was added to 3200 µL of the DPPH solution. The control tube (negative control) received 800 µL of 70% ethanol and 3200 µL of the DPPH solution. After 30 minutes of incubation in the dark at room temperature, the absorbance (Abs) was measured at 517 nm. The experiment was repeated three times, and the inhibition percentage (IP) was calculated using the following equation:

$$\mathbf{IP = [(Abs\ contrôle - Abs\ test) / Abs\ contrôle] \times 100}$$

The IC50, which is the concentration of plant extract or vitamin C responsible for 50% inhibition of the DPPH radical, was determined from the curve of inhibition percentage as a function of extract or vitamin C concentration.

Antipromastigote Activity

Antileishmanial tests were performed on promastigotes of *Leishmania donovani* (responsible for visceral leishmaniasis). These cells were obtained from the University of Cape Coast (Ghana).

One hundred microliters (100 µL) of promastigote cells (500,000 cells/mL) were distributed into 96-well culture plates using M199 medium supplemented with 10% fetal bovine serum (Gibco, USA). The cells were then exposed to different concentrations of plant extracts (0-200 µg/mL). The plates were incubated at 25 ± 1°C for 48 hours. Cell viability was assessed using a standard WST-8 colorimetric assay (Abutaha, 2015), and readings were taken at 490 nm using a spectrophotometer. The percentage of cell viability was calculated using the following formula:

$$\mathbf{\%Cell\ Viability = (Mean\ absorbance\ in\ control\ well / Mean\ absorbance\ in\ test\ well) \times 100}$$

The IC_{50} values were used to assess the antileishmanial potency of the extracts.

Data Analysis

The results of the antiradical and antipromastigote tests, obtained from three independent trials, were processed using Excel and GraphPad Prism version 9 for mean comparisons. The mean value is presented with the standard

error of the mean (Mean ± Standard Deviation). Statistical analysis was performed using an analysis of variance (ANOVA). The significance level was set at 5% ($p < 0.05$). When a significant difference was observed, Tukey's and Dunnett's multiple comparison tests were conducted.

Phytochemical Screening

The results of the phytochemical screening conducted on the extracts of the four medicinal plants are presented in Table 1. All four extracts contain coumarins and alkaloids. Saponins and anthocyanins are absent in all four extracts. The extract of *R. heudelotii* is the only one that contains tannins and quinones, while *E. polycarpa* is the only one that contains flavonoids.

Table 1: Results of Phytochemical Screening

Families of secondary metabolites												
Plant name	Plp	Ta		Qu	Sa	Co	Ant	L.Ant	St	Fl	Al	
		C	G								D	W
R. heudelotii	++	++	++	+	-	++	-	++	+	-	+	++
A. cissampeloides	-	-	-	-	-	+	-	-	-	-	+	+
E. polycarpa	+	-	-	-	-	+	-	++	++	++	+	+
K. africana	+	-	-	-	-	+	-	-	+	-	+	+

***Plp**: Polyphenols, **Ta**: Tannins, **C**: Catechic Tannins, **G**: Gallic Tannins, **Qu**: Quinones, **Sa**: Saponins, **Co**: Coumarins, **Ant**: Anthocyanins, **L. ant**: Leuco-Anthocyanins, **St**: Sterols, **Fl**: Flavonoids, **Al**: Alkaloids, **D**: Dragendorff (Reagent for Alkaloids Detection), **W**: Wagner, (Reagent for Alkaloids Detection),*
*+: Presence, ++: Strong Presence, +++: Very Strong Presence, **-**: Absence*

Antiradical Activity

The antiradical activity of our extracts was evaluated using the DPPH test, and the percentage of trapped DPPH free radicals was expressed as a function of the extract or vitamin C concentration. Figure 1 shows the inhibition percentage of our hydro-ethanolic extracts and vitamin C as a function of concentration. The IC50 was graphically determined from the inhibition percentage curve in relation to concentration. It serves as an indicator of the antiradical capacity of the extract or reference molecule. A lower IC_{50} value indicates a higher antiradical activity of the extract[15].

All extracts demonstrated antiradical power except for the extract of *Adenia cissampeloides*, which exhibited an IC_{50} greater than 250 µg/mL. The results of this test allowed us to classify our extracts into three categories based on IC_{50} values (Table 2). The first category includes plants with very high activity, with IC_{50} values below 20 µg/mL. This group includes *Enantia polycarpa* (10.47 ± 6.47 µg/mL). The second category consists of plants with high activity, with IC_{50} values between 20 and 50 µg/mL. This group includes *Ricinodendron heudelotii* and *Kigelia africana* with respective IC_{50} values of 47.711 ± 11.68 µg/mL and 40.94 ± 9.60 µg/mL.

The last category includes plants with low antiradical power ($IC_{50} \geq 250$ µg/mL), such as *Adenia cissampeloides*.

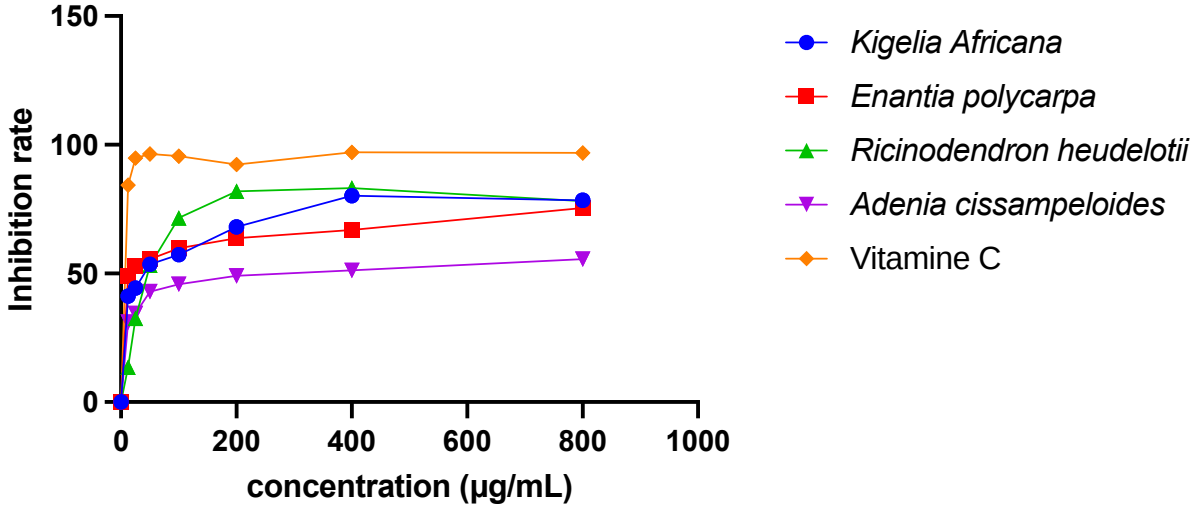

Figure 1 : Inhibition rate of promastigotes as a function of concentrations

Plant name	**IC_{50} (µg/mL)**	**p-value**
R. heudelotii	47,711 ± 11,684	ns
A. cissampeloides	268,81 ± 0,91	*
E. polycarpa	10,47 ± 6,47	ns
K. Africana	40,94 ± 9,60	ns
Vitamine C	8,22 ± 1,25	ns

ns**: Non-significant difference compared to vitamin C **: Significant difference compared to vitamin C (p-value < 0.05)*

Table 2 : IC_{50} of extracts and Vitamin C

Antipromastigote Activity

The tests of antipromastigote activity for the four extracts showed that all of them are effective against the promastigote forms of *L. donovani* (Figure 2). As the concentration of the product increases, the survival percentage of promastigotes decreases, indicating a dose-dependent effect. The IC_{50} values of the different extracts are as follows : 4.79 ± 1.02 µg/mL for *Ricinodendron heudelotii*, 95.10 ± 1.57 µg/mL for *Enantia polycarpa*, and 79.44 ± 2.12 µg/mL for *Kigelia africana*. As for *Adenia cissampeloides*, its IC_{50} was greater than 100 µg/mL. The reference molecule, Nifuroxazide, had an IC50 of 1.70 ± 0.52 µg/mL (Table 3).

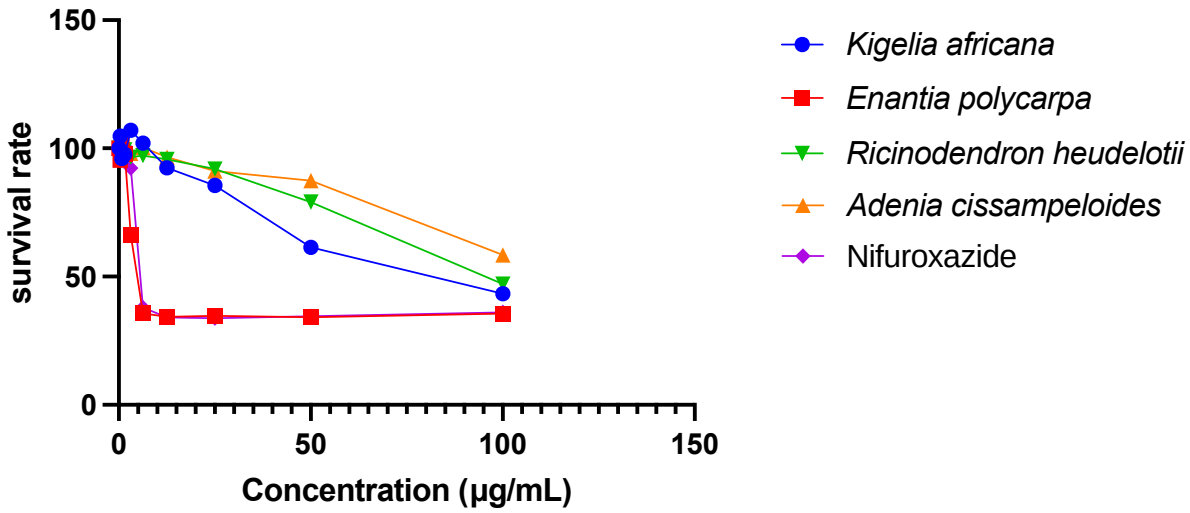

Figure 2 : Survival rate of promastigotes as a function of concentrations

Nom de la plante	CI50 (µg/mL)
Ricinodendron heudelotti	95,10 ± 1,57
Adenia cissampeloides	>100
Enantia polycarpa	4,79 ± 1,02
Kigelia africana	79,44 ±2,12
Nifuroxazide	1,70 ± 0,52

Table 3 : IC_{50} of extracts and Nifuroxazide on *L. donovani* promastigotes

DISCUSSION

The antioxidant activity of the extracts was tested against the DPPH radical, a simple, rapid, and widely used method for estimating the ability of substances to act as free radical scavengers or hydrogen donors, thereby assessing their antioxidant activity[16]. This study showed that among the four plants studied, three exhibited notable antiradical activity, with *Enantia polycarpa* demonstrating particularly strong activity.

The antiradical activity of these plants is likely due to the presence of polyphenols. The phytochemical screening revealed the presence of polyphenols in the three extracts with good antiradical capacity. Several studies have confirmed that phenols discolor DPPH due to their hydrogen-donating ability[17]. Additionally, Li et al.[18] highlighted that polyphenols and flavonoids play a crucial role in the antioxidant properties of plants.

The results of this study also show that the extract of *Enantia polycarpa* exhibits strong antipromastigote activity against *Leishmania donovani*, with an IC_{50} of 4.79 ± 1.02 µg/mL, a value close to that of nifuroxazide, the reference molecule (IC_{50} = 1.70 ± 0.52 µg/mL), indicating an interesting antiparasitic potential for this plant. *Kigelia africana* also demonstrated activity, albeit weaker, with an IC_{50} of 79.44 ± 2.12 µg/mL.

In comparison, *Ricinodendron heudelotii* showed moderate activity with an IC_{50} of 95.10 ± 1.57 µg/mL, suggesting low efficacy. *Adenia cissampeloides* did not show significant activity, with an IC_{50} greater than 100 µg/mL, indicating that it may not be a viable candidate for applications against *L. donovani*.

These results align with the traditional uses of these plants against malaria and other parasitic diseases in West and Central Africa. *E. polycarpa* is notably used in Ivorian traditional medicine to treat malaria[19]. The strong antipromastigote activity observed for *Enantia polycarpa* could indeed be attributed to the presence of secondary metabolites such as flavonoids and alkaloids, as suggested by several studies. An in vivo study showed that the ethanolic extract of the stem bark of *E. polycarpa* possesses promising antimalarial activity against *Plasmodium berghei* in mice, with a chemo-suppression of 75.8% at a dose of 600 mg/kg[20].

CONCLUSION

This study highlights the antiparasitic potential of certain plants used in African traditional medicine, particularly *E. polycarpa*. These results encourage further investigations into this species for the development of new natural antileishmanial treatments. However, additional *in vivo* and toxicity studies are necessary before considering therapeutic application.

REERENCES

1. WHO. 2020. https://www.who.int/fr/news-room/fact-sheets/detail/leishmaniasis
2. Alvar J, Ivan DV, Caryn B, Merce H, Philippe D, Jorge C, Jean J, Margriet DB, WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*, 2012 : 7(5) : e 35671.
3. Desjeux P. Therapeutic options for visceral leishmaniasis. *Médecine et Maladies Infectieuses*, 2005 : 35 (2) : S74-6.
4. Filipi C, Malherbe L, Julia V, Glaichenhaus N. L'immunité contre leishmanies. *Médecine/Sciences*, 2001 : 17 : 1120-1128.
5. Singh N, Mishra BB, Bajpai S, Singh RK, Tiwari VK. Natural product based leads to fight against leishmaniasis. *Bioorganic & Medicinal Chemistry*, 2014 : 22(1) : 18-45.
6. Monge-Maillo B and Lopez-Velez R. Therapeutic options for visceral leishmaniasis. *Drugs,* 2013 : 73(17) :1863-1888.
7. Sundar S and Chakravarty J. Leishmaniasis : an update of current pharmacotherapy. *Expert Opinion Pharmacotherapy*, 2013 : 14(1): 53-63.
8. Zirihi GN, Kra AM. and Guédé-Guina F. Evaluation de l'activité antifongique de *Microglosa pyrifolia* (Lamarck) O. Kunze (Asteraceae) « « PYMI » » sur la croissance *in vitro* de *Candida albicans*. *Revue de Médecine et pharmacologie Africaine*, 2003 : 17 : 11-18.

9. Bekro YA, Mamyrbekova J, Boua BB, Bi FT and Ehile EE. Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae). *Sciences & Nature*, 2007 : 4(2) : 217-225.
10. N'Guessan K, Kadja B, Zirihi G, Traoré D and Aké-Assi L. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). *Sciences & Nature*, 2009 : 6(1) : 1-15.
11. Koffi A, Bla K, Yapi H, Bidie A, Djaman A. Phytochemical Screening of Some Medicinal Plants in Côte D'ivoire and Evaluation of their Extraction Efficiency. *International Journal of Pharmacognosy and Phytochemical Research*, 2015 : 7(3): 563-569.
12. Kallo MS, Adamou R, Sawadogo J, Mahamane AA, Maarouhi ML and Ikhiri K. Enquête ethnobotanique et criblage phytochimique de quelques plantes tinctoriales du Niger en vue d'une valorisation en énergie solaire. *International Journal of Biological and Chemical Sciences*, 2018 : 12(2) : 867-883.
13. Tojola OB, Lajide L, Owolabi BJ, Olaleye MT and Okoh SO. Phytochemical screening & antibacterial activity of ethyl acetate & methanol extracts of Annona muricata aerial part. *Journal of Medicinal Plants Studies*, 2019 : 7(6) : 01-05.
14. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin. *Journal of Sciences and Technology*, 2004 : 26(2) : 211-219.
15. Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M, Abdelly C. Phenolic composition of *Cynara cardunculus L.* organs, and their biological activities. *Comptes Rendus Biologies*, 2008 :331 (5): 372-379.
16. Milardović S, Ivekovic D, Grabarić BS. A novel amperometric method for antioxidant activity determination using DPPH free radical. *Bioelectrochemistry*, 2006 : 68(2):175-80
17. Bougandoura N, Bendimerad N. Évaluation de l'activité antioxydante des extraits aqueux et méthanolique de *Satureja calamintha* ssp. Nepeta (L.) Briq. *Nature & Technologie, Sciences Agronomiques et Biologiques*, 2013 : 9 : 14-19.
18. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, 2007 : 102: 771-776.
19. Koudio YJC, Kpangui KB, Yao NO, Tiébré MS, Ouattara D et N'Guessan KE. Disponibilité des plantes utilitaires dans la zone de conservation de biodiversité du barrage hydroélectrique de Soubré, Sud-Ouest, Côte d'Ivoire. *Afrique Science*, 2020 : 16(6) (2020) 65 – 74.
20. Anosa GN, Udegbunam RI, Okoro JO, Okoroafor ON. *In vivo* antimalarial activities of *Enantia polycarpa* stem bark against *Plasmodium berghei berghei* in mice. *Journal Ethnopharmacology*, 2014, 153(2) : 531-534.