International Journal of Research and Review
Vol. 9; Issue: 9; September 2022
Website: www.ijrrjournal.com
Research Paper *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Phytochemical Analysis, Antioxidant Activity and Larval Toxicity of Ethanolic and Ethyl Acetate Extracts from Rhizomes of *Curcuma Longa* L.

ADOVELANDE Jacques[1], CACHON Fresnel Boris[2,3], ASSOGBA Mahoudo Fidèle[1], AHOTON Djidénou Paul[1], GBENOU Joachim Djimon[1,4]

[1]Laboratory of Pharmacognosy and Essential Oils, Faculty of Sciences and Techniques, Faculty of Health Sciences, University of Abomey-Calavi, 01 BP 918, Cotonou, Benin
[2]Laboratory of Biochemistry, Molecular Biology and Environment, Faculty of Sciences and Techniques, University of Abomey-Calavi, 04 BP 0320, Cotonou, Benin
[3]Laboratory of Experimental and Clinical Biology, National School of Applied Biosciences and Biotechnologies, National University of Sciences, Technologies, Engineering and Mathematics, Abomey, Benin
[4]Laboratory of Research of perfume, aromatic, food and medicinal plants, FAST-ENS, Natitingou, National University of Sciences, Technologies, Engineering and Mathematics, Abomey, Benin.

Corresponding Author: CACHON Fresnel Boris

DOI: https://doi.org/10.52403/ijrr.20220902

ABSTRACT

Turmeric (*Curcuma longa L.*), a plant native to Asia, was the subject of the present study whose objective was to evaluate the antioxidant properties of the rhizome powder of this plant cultivated at Bopa, a commune of Benin. The methodology used consists in evaluating the toxicity of the extracts by the shrimp larvae, to make the phytochemical analysis of the *Curcuma longa L.* powder in order to obtain information on its biochemical and/or chemical value. It also consists in the determination of polyphenols, flavonoids, tannins and beta-carotene of ethanolic and ethyl acetate extracts, and in the evaluation of the antioxidant activity of these extracts by the FRAP and DPPH methods. Phytochemical screening of the powder revealed the presence of alkaloids, flavonoids, anthocyanins, tannins, steroids and terpenes, reducing compounds, mucilages and anthracene derivatives. The yield of ethanolic and ethyl acetate extracts gave 31.94 % and 14.66 % respectively. The larval toxicity test performed on these two extracts revealed that they will not be toxic. The ethyl acetate extract had a high content of polyphenol (1304.596 mg EqAG) while for the flavonoids (1259.938 mg EqQ/g E), tannins (259.859 mg EqP/g E) and beta-carotene (0.139 mg/100 g) assay, the ethanolic extract had the highest contents. Note also that the ethyl acetate extract showed the best antioxidant activity (IC_{50} = 0.007 mg/mL) compared to the ethanolic extract (IC_{50} = 0.009 mg/mL) after the control (ascorbic acid 0.0074 µg/mL).

Keywords: antioxidant activity, phytochemical analysis, rhizome, larval toxicity

INTRODUCTION

For centuries in tropical and subtropical regions, several genera of the Zingiberaceae family have been used in cooking as spices or food coloring, in cosmetics, in traditional medicine. Among the genera, Turmeric is the most studied.(1) This genus includes about 110 species from tropical Asian and northern Australian regions.(2)

The species *Curcuma longa L.* is a perennial plant produced primarily in India and Southeast Asia.(3) The rhizome is the most used part of the plant. The rhizome is ground into a powder and used as a spice and food coloring. The powder has a peppery and bitter flavor. Its orange-yellow color is attributed to curcuminoids. Among

these, curcumin is the most abundant and most studied molecule. *Curcuma longa L*. is also used as a dye in the textile industry. Moreover, this plant is very well known in traditional Indian and Chinese medicine. Indeed, it is used to treat asthma, allergies, liver diseases, jaundice, anorexia, rheumatism, cold, sinusitis and infectious diseases.(4) Recently, *Curcuma longa L*. has been the subject of much scientific research. The results of this work have confirmed the data of traditional Indian and Chinese medicine. Moreover, the effectiveness of curcumin in the prevention and treatment of many diseases such as colon cancer, diabetes, Alzheimer's disease is now recognized.(5) Nowadays, the importance of turmeric is considerable throughout the world to the point where the plant is cultivated in most tropical and subtropical countries and is the subject of an important international trade.(6) Thus, Turmeric is found in Benin where it is increasingly cultivated for culinary and medicinal purposes. However, very little scientific work has been devoted to this Beninese production. It is in this context that the present study was conducted.

MATERIAL AND METHODS

Plant material

The plant material used is the rhizome of *Curcuma longa L*. harvested in September 2021, in the Valley farm in Lobogo, Commune of Bopa, Department of Mono. Our samples were cut and dried out of the sun. They were then ground into powder for extraction and analysis.

Preparation of the ethanolic extract

To 100 g of *Curcuma longa L*. rhizome powder is added one liter of alcohol at 96 °C. The mixture is left to macerate for 24 hours and then filtered on Whatman filter paper. The ethanolic extract obtained is evaporated under vacuum at 45 °C using a rotavapor and then dried in an oven at 50 °C for 24 hours. The product obtained is scraped into colored vials and stored at 4 °C in the refrigerator.

Preparation of ethyl acetate extract

To 50 g of *Curcuma longa* L. powder is added one liter of ethyl acetate. The mixture is left to macerate for 24 hours and then filtered through Whatman filter paper. The ethyl acetate extract obtained is evaporated under vacuum at 45 °C using a rotavapor and then dried in an oven at 50 °C for 24 hours. The residue obtained is preserved in colored vials and stored at 4 °C in the refrigerator.

Phytochemical screening

It is an analysis based on staining or precipitation reactions using relatively specific reagents for each class of active ingredients described by Bruneton and repeated by Assogba et al.(7,8)

Determination of total polyphenols

The determination of total polyphenols was carried out according to the method of Singleton et al and adopted by Assogba et al with some slight modifications.(8,9) This determination was carried out using the Folin-Ciocalteu reagent. Gallic acid is used as a reference compound to make the calibration curve.

Determination of flavonoids

The total flavonoid content of the plant extracts is estimated by the aluminum trichloride (AlCl3) method. Quercetin is used as a reference compound to make the calibration curve.(8,10)

Determination of tannins

The method used to determine the content of condensed tannins is the vanillin sulfur method of Broadhurst et al and repeated by Assogba et al.(8,11) Pyrogallol is used as a reference compound to make the calibration curve.

Antioxidant activity FRAP method (Ferric Reducing Antioxidant Power)

The FRAP (Ferric Reducing antioxidant power) method is based on the ability of extracts to reduce ferric ion to ferrous ion. The total antioxidant capacity of each plant

extract was determined by the method of Hinneburg et al.[12] Thus 1 mL of an aqueous solution of each extract (20 mg/mL diluted to 100 to obtain 0.2 mg/mL), of ascorbic acid, was mixed with 2.5 mL of phosphate buffer (0.2 M ; pH 6.6) and 2.5 mL of the aqueous solution (1 %) of potassium hexacyanoferrate $K_3Fe(CN)_6$. After 30 min of incubation at 50 °C, 2.5 mL of trichloroacetic acid (10 %) was added. The mixture was then centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant was then mixed with the same volume of water and 0.5 mL of a freshly prepared aqueous $FeCl_3$ solution (0.1 %) was added. The absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-200 mg/L). The reducing power was expressed as mol ascorbic acid equivalent per gram of extract (mol EqAA /g E) considering 1 mM equals FRAP of 1 mL of the extract according to the following formula:

C = c x D / M x Ci
c: represents the average concentration of your extract by projecting its optical density on the calibration curve.
D: if you did not dilute your extract before reading, your dilution is 1.
M: molecular weight of ascorbic acid (176.1 g/mol).
Ci: concentration of the extract used for the study.

Antioxidant capacity DPPH (2,2-diphenyl-1-picrylhydrazyl) method

The capacity of the extracts and fractions to trap the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined using the procedure described by Lamien-Meda et al and repeated by Assogba et al.[8,13] The antioxidant capacity of the extracts was determined using an ascorbic acid calibration curve (0-10 mg/mL). Each test is performed in triplicates. Antioxidant capacity is expressed as mmol ascorbic acid equivalent per gram of extract (mmol EqAA /g E) using the formula below:
$C = (C_{read} \times D) \times 100 / (M \times Ci)$
C: concentration of free radical scavenging compounds in mmol EqAA /g E or dry fraction.
C_{read}: concentration of the sample read.
D: dilution factor of the sample.
Ci: concentration of the extract stock solution in mg/mL.
M: molecular weight of ascorbic acid (176.1 g/mol).

Determination of the IC_{50} of extracts

For this test, samples were prepared by dissolving in ethanol.[8] For each extract, a stock solution is prepared in distilled water at 0.1 µg/mL. This solution is then diluted in geometric series of reason 2 to have different concentrations. In dry and sterile test tubes, 1 mL of the test extract solution is introduced, 2 mL of DPPH solution (0.04 mg/mL) is added. After vortexing, the tubes are placed in the dark at laboratory temperature for 30 min. The absorbance is measured at 517 nm with a spectrophotometer (Biomate UV/VIS). For each dilution, a blank is prepared, consisting of 1 mL of distilled water and 2 mL of DPPH. The positive control is represented by ascorbic acid (100 µg/mL) and is treated under the same conditions as the test sample. The antioxidant activity AA % is given by the following formula:

$AA\ \% = 100 - \{[(Abs_{test} - Abs_{blanc}) \times 100] / Abs_{control}\}$
Or $Inhibition\ \% = ((Abs_{control} - Abs_{test}) / Abs_{control}) \times 100$
AA: Antioxidant activity.
Abs: Absorbance at the wavelength of 517 nm.

RESULTS AND DISCUSSION

Yields of the extractions

The yields of the different extractions were obtained by the formula below and the results recorded in Table 1.
Y = Mass of the extract x 100 / Mass of dry matter
Y: yield in percentage
Mass in gram

Table 1: Aspects, colors and yields of extracts of *Curcuma Longa L*.

Extracts	Aspects	Colors	Yields (%)
Ethanolic	Sticky paste	Dark yellow	31.94
Ethyl acetate	Sticky paste	Bright red	14.66

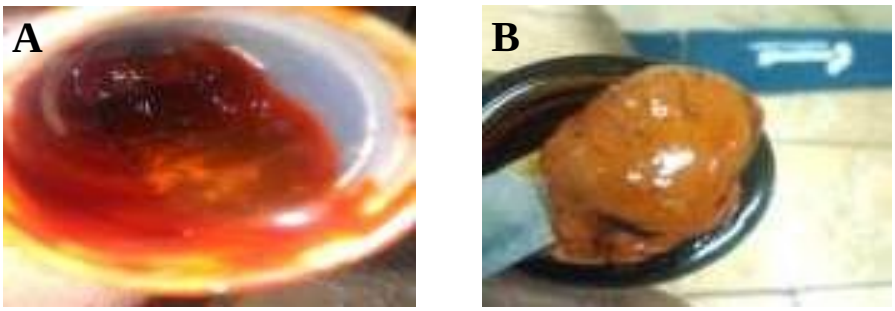

Figure 1: Ethyl acetate extract (A) and ethanolic extract (B)

From the results reported in Table 1, the yield of the ethanolic extract is at least twice as high as that of the ethyl acetate extract. It is therefore deduced that the best yield is obtained with the ethanolic extract. The work done by Ameur et al resulted in 11 % yield of ethanolic extract of *Curcuma longa L.*, which is lower than our study.[14] This difference may be related to the genetic properties of the plants as well as the geographical origin, the conditions and duration of storage of the harvest and also the conditions under which the extractions were performed.[15] The remarkable colors of these two extracts (Figure 1) would be due to the strong coloring character of turmeric and their pasty aspects could possibly depend on the concentration of vegetable oil in the rhizomes of *Curcuma longa L*.

Phytochemical screening

The results of the phytochemical analysis of turmeric are presented in the table 2.

The phytochemical screening on the rhizome powder of *Curcuma longa L*. allowed us to obtain information on the biochemical and/or chemical value through identified chemical tests. The phytochemical analyses reveal the presence of alkaloids, flavonoids, anthocyanins, quinone derivatives, tannins, steroids and terpenes, reducing compounds, mucilages and anthracene derivatives, but we note the absence of leuco-anthocyanins, saponosides, cyanogenic derivatives, c-heterosides and coumarins. The turmeric powder contained the alkaloids, which is also the case with Boukri on the crude extract of *Curcuma longa L*. making from the spices contained in the Ras-el-hanout mixture.[16] But these results show a contradiction compared to those of Leela et Anchana on a range of different spices.[17] These compounds have microbicidal properties used to treat certain cancers.[18] In addition, they have remarkable physiological action on the central nervous system or sympathetic and parasympathetic autonomic nervous system of which they act in small amounts. Other pharmacological effects also attributed to alkaloids such as analgesic (cocaine), anti-cholinergic (atropine), anti-malaria (quinine), anti-hypertensive (reserpine), antitussive (codeine), central stimulant (caffeine), cardiac depressant and narcotic diuretic (morphine), anti-tumor and sympathomimetic (ephedrine). [19] Our work reveals the existence of gallic and/or catechic tannins. A study by Shamsuddeen revealed that the alcoholic extract of Zingiber officinale does not contain tannins.[20] But the same author confirmed the presence of tannins in the aqueous extract of ginger. Tannins (especially gallic) are considered good remedies in the treatment of respiratory diseases and against coughs. Internally, tannins have an antidiarrheal activity. Its antiseptic, antibacterial and antifungal properties are clearly demonstrated in the treatment of infectious diarrhea and dermatitis. Tannins have a strong antioxidant activity, they are very good scavengers of free radicals and

inhibit the formation of superoxide radical.[19] The phytochemical screening of the rhizome of *Curcuma longa L*. powder reveals the presence of flavonoids. Our results corroborate the results of Tacouri et al which testify to the richness of flavonoids in spices.[21]

Table 2: Results of phytochemical analysis of *Curcuma longa L*.

Chemical compounds	Results
Alkaloids	+
Flavonoids	+
Anthocyanins	+
Leuco-anthocyanins	-
Quinone derivatives	+
Catechic tannins	+
Gallic tannins	+
Triterpenoids	+
Steroids	+
Cardenolides	+
Saponosides	-
Cyanogenic derivatives	-
Reducing compounds	+
Mucilages	+
Free anthracene	+
o-heterosides	+
c-heterosides	-
Coumarins	-
(+) present ; (-) absent	

These compounds are known mainly for their "veinoactive" role, i.e., reducing the permeability of blood capillaries and strengthening their resistance.[22] Some anti-allergic, hepatoprotective and antispasmodic properties are also attributed to these compounds. Flavonoids have antioxidant and anti-inflammatory activities and play a positive role in the treatment of cardiovascular and neurodegenerative diseases.[7] In some cases, they are known for their antiviral and antitumor activities. They have been shown to be antimicrobial substances active in vitro against a wide spectrum of microorganisms. This activity would be due to their ability to complex with soluble extracellular proteins and the bacterial wall.[23] Anthocyanins which represent one of the subclasses of flavonoids revealed their presence in the turmeric powder used here and their absence in the research work of Boukri on Turmeric.[16] These powerful antioxidants cleanse the body of free radicals and maintain good blood circulation.[7] This absence may be due to the fact that these substances are fragile and rapidly degradable. Terpenoids rare in many other genera are present in *Curcuma longa L*. as also demonstrated by the results of Boukri.[16] Quinone derivatives, reducing compounds, mucilages, steroids, cardenolides, o-heterosides, free and combined anthracenics are also present in *Curcuma longa L*.

Total polyphenol contents

The determination of total polyphenols was carried out and the results obtained are expressed as mg gallic acid equivalent per gram of extract (mg EqAG/g E), using the equation of the linear regression of the calibration curve plotted for gallic acid.

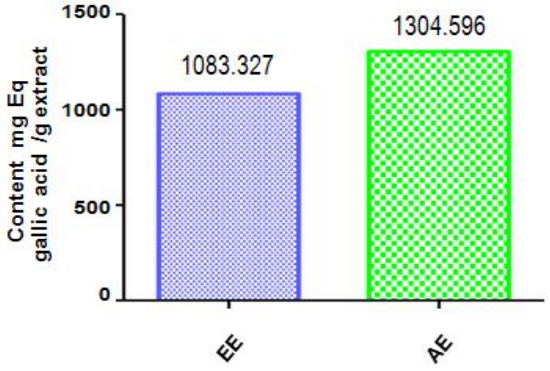

Figure 2: Histogram representing the polyphenol content of EE and AE extracts of *Curcuma longa L*.
Legend: EE=Ethanolic extract, AE=Ethyl acetate extract

Comparison of the polyphenol values of ethanolic and ethyl acetate extracts highlights the polyphenol richness of these extracts (Figure 2). The highest content is obtained with the ethyl acetate extract (1304.596 mg EqAG/g E). These results are largely superior to those obtained by the study performed on *Curcuma longa L*. leaves and stems by Brahimi et al which gives 10.64 µg EqAG /mg and 11.71 µg EqAG /mg for ethanolic and ethyl acetate extracts, respectively.[24] But the study of Marfak on the methanolic extract of *Curcuma longa L*. records a value of 4.14 mg Eq AG/g ES and that of Seggani et al a value of 18.125 mg Eq AG/g ms.[25,26] These differences in concentration may be caused by the low specificity of the Folin-Ciocalteu reagent. The reagent is extremely sensitive to the reduction of all hydroxyl groups not

only those of phenolic compounds, but also of some sugars and proteins, etc.[27,28] The extraction solvent carries away non-phenolic substances such as sugars, proteins and dyes that may interfere during any phenolic evaluation.[29] The assay by this reagent therefore gives a crude evaluation of all phenolic compounds in an extract. It is not specific to polyphenols, but many compounds can react with the reagent, giving an apparently high phenolic content, which is the case here for *Curcuma longa L*. rhizomes.[30] The distribution of secondary metabolites may change during plant development. This may be related to harsh climatic conditions (high temperature, solar exposure, drought, salinity), which stimulate the biosynthesis of secondary metabolites such as polyphenols.[31] The phenolic content of a plant depends on a number of intrinsic (genetic) and extrinsic (climatic conditions, cultural practices, maturity at harvest and storage conditions) factors.[31,32] The main reason for choosing this class of polyphenols is that flavonoids are the most important polyphenolic class, with more than 5000 compounds already described.[28]

Flavonoid content

The determination of total flavonoids was performed. The results obtained are expressed as mg quercetin equivalent (Q) per gram of extract (mg EqQ/g E), using the linear regression equation of the calibration curve plotted for quercetin.

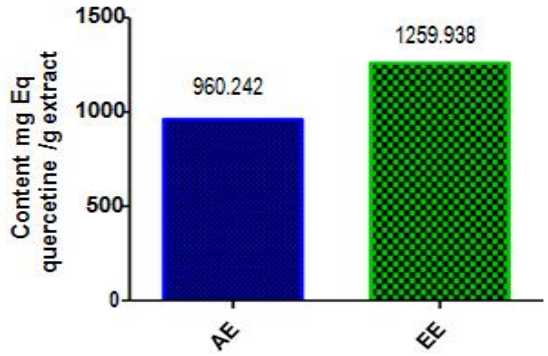

Figure 3: Histogram representing the flavonoid content of EE and AE extracts of *Curcuma longa L*.
Legend: EE=Ethanolic extract, AE=Ethyl acetate extract

The comparison of the flavonoid values of the ethanolic and ethyl acetate extracts shows the richness in flavonoids of these extracts (Figure 3). Of the two extracts the highest content is obtained with the ethanolic extract (1259.938 mg EqQ/g E). But Boukri in his study on the crude extract of *Curcuma longa L*. obtains 6.52 mg EQ EC/g ms and flavonoid contents of the leaves and stems of *Curcuma longa L*. give 16.31 µg EQ EC /mg for the ethanolic extract and 7.98 µg EQ EC /mg for that of ethyl acetate according to Brahimi et al.[16,24] *Curcuma longa L*. from Malaysia was reported to contain 0.094 mg /g flavonoid content of a dry sample, while Sumazian et al reported 4.05 mg /g flavonoid content of a dry sample of *Curcuma domestica*.[33,34] Similarly, Tilak et al reported that the flavonoid content of turmeric in India ranged from 3.58 to 7.86 mg /g.[35] Our values are significantly higher than those of all these researchers. Flavonoids are widely studied in turmeric for their broad advantage of pharmacological activities.[36] Flavonoids are antioxidants that can prevent or delay oxidation of substrates even when present at low concentrations.

Tannin content

The tannin assay was performed and the results obtained are expressed as mg pyrogallol equivalent (P) per gram of extract (mg EqP/g E), using the equation of the linear regression of the calibration curve plotted for pyrogallol.

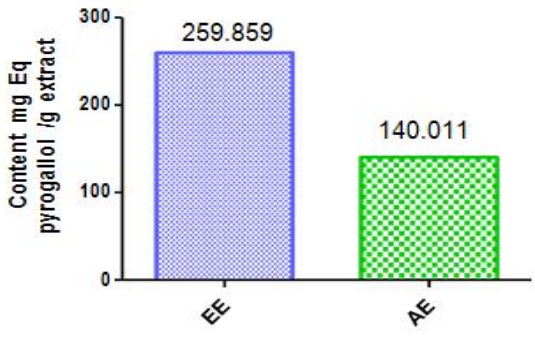

Figure 4: Histogram representing the tannin content of EE and AE extracts of *Curcuma longa L*.
Legend: EE=Ethanolic extract, AE=Ethyl acetate extract

The comparison of the tannin values of ethanolic and ethyl acetate extracts shows the richness in tannins of these extracts (Figure 4). The content of the ethanolic extract (259.859 mg EqP/g E) prevails over that of ethyl acetate (140.011 mg EqP/g E). The tannin contents obtained for our ethanolic and ethyl acetate extracts are higher than the work of Tanvir et al whose varieties Mura de Khulua, Chora de Khulua, Mura de Chittagong and Chora de Chittagong yielded 7.74, 8.93, 11.78, 7.15 g tannic acid equivalent (T) per 100 g extract (g TEQ /100 g extract), respectively.[37] The turmeric varieties studied differed significantly, reflecting the effects of geographical variation between the Chittagong, Khulna and Benin divisions. Tannins are substances of plant origin that have the property of transforming fresh skin into a rot-proof material, leather.[7]

Antioxidant activity by the FRAP method

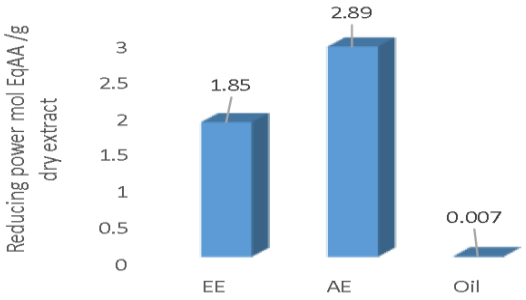

Figure 5: Reducing power of extracts in mol EqAA /g dry extract

Legend: EE=Ethanolic extract, AE=Ethyl acetate extract

The evaluation of the antioxidant activity by the iron reduction method allows us to better understand the antioxidant power of *Curcuma longa L.* extracts. Ascorbic acid is used as a control and the extracts are at a concentration of 0.2 mg/ml (Figure 5).

Antioxidant activity by DPPH method

The DPPH free radical allowed the estimation of the antioxidant activity of the prepared extracts. The main results of the antioxidant activity of *Curcuma Longa L.* are summarized below (Figure 6).

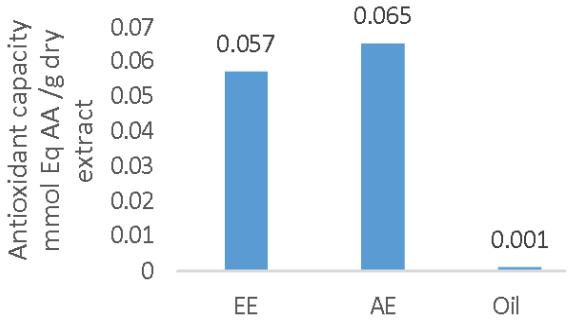

Figure 6: Antioxidant capacity of extracts for the determination of antioxidant activity of *Curcuma Longa L.*

Legend: EE=Ethanolic extract, AE=Ethyl acetate extract

Determination of the IC_{50}

The curves below were used to determine the IC_{50} of each extract (Figure 7).

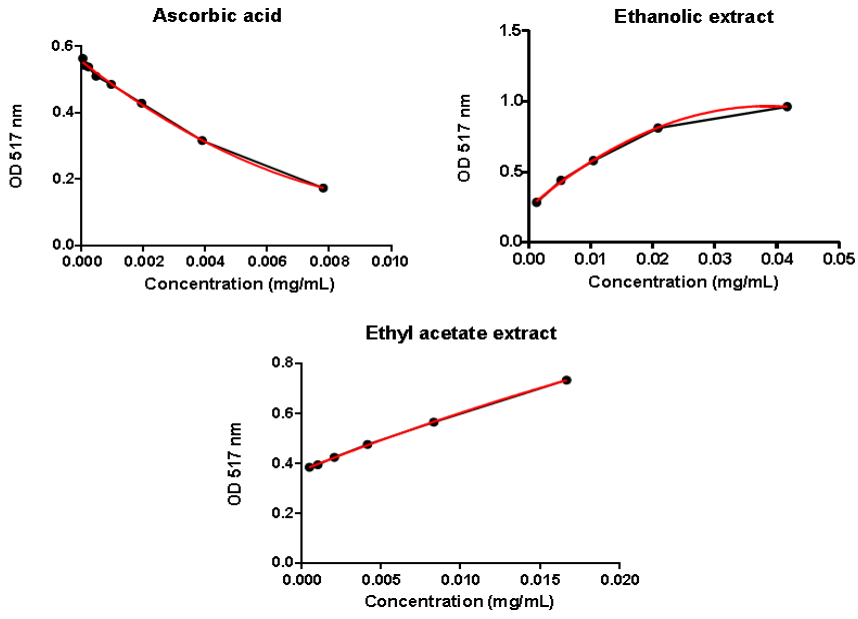

Figure 7: Antiradical activity of different extracts of *Curcuma Longa L.*

The values obtained allowed us to draw curves with these different gaits. From these curves we can determine the IC_{50} value of the extracts and the oil. The smaller the IC_{50} value, the higher the antioxidant activity of the extract. Another parameter calculated from the effective concentration is the antiradical activity. The more these values do not tend and move away from zero, the more the antioxidant power increases. The values of the inhibitory concentrations and the antiradical activity are reported in the table 3.

Table 3: The values of IC_{50} and the antiradical power (ARP)

Extracts	IC50	ARP	R^2
Ascorbic acid	0.0074 (µg/mL)	135.13	
Ethanolic	0.009 (mg/mL)	111.111	0.9992
Ethyl acetate	0.007 (mg/mL)	142.857	0.9997

Compared to ascorbic acid (IC_{50} = 0.0074 µg/mL), ethyl acetate extract (IC_{50} = 0.007 mg/mL) has a lower antioxidant activity but better than ethanolic extract (IC_{50} = 0.009 mg/mL). The ethyl acetate extract is therefore the one, which has an antiradical power that is farther from zero (142.857).

The FRAP and DPPH tests allowed to highlight the antioxidant activity of the studied extracts.

The concordance found between the FRAP and DPPH tests is that both tests retain the ethyl acetate extract as a powerful antioxidant.

From these curves we can determine the percentages of inhibition obtained according to the concentrations used as well as the IC_{50} value of each extract. Another parameter calculated from the effective concentration, is the anti-radical activity. The more these values do not tend and move away from zero, the more the antioxidant power increases.

The anti-radical activity of our *Curcuma longa L*. shows that the ethyl acetate extract has the highest antioxidant activity (IC_{50} = 0.007 mg/mL) compared to the ethanolic extract (IC_{50} = 0.009 mg/mL) and the oil (IC_{50} =3.767 mg/mL) compared to the IC_{50} of the control (ascorbic acid 0.0074 µg/mL) The smaller the IC_{50} value, the higher the antioxidant activity of the extract and consequently the higher the free radical scavenging power.

Our results are contrary to the previous report of Tanvir et al which says that according to IC_{50} values ethanolic extracts show stronger scavenging activity.[37]

On the other hand we can also say that our ethanolic, ethyl acetate extracts of *Curcuma longa L*. are all found to be antioxidant as Lee et al says that any extract with antioxidant activity less than or equal to 10 mg/mL is an extract with antioxidant activity.[38]

The results obtained indicate that the free radical scavenging activity can be attributed to the high levels of total polyphenols for the ethyl acetate extract.[39]

The crude extract of turmeric present in the spice mixture "Ras el hanout" studied by Atti on the Wilaya region of Ouargla (Algeria) gives an antioxidant activity with an IC_{50} of 0.55 mg/mL against 0.06 mg/mL for ascorbic acid.[40]

Nesrine in his study on rhizomes marketed in herbalists of the wilaya of M'sila showed an antiradical activity of *Curcuma longa L*. with an IC_{50} of about 4.39 mg/mL against an IC_{50} of 2.51 mg/mL for the positive control.[41]

The antioxidant activity of curcumin is mediated by antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Curcumin is an acceptor in the Michael reaction, allowing it to react with glutathione and thioredoxin. The reaction of curcumin with these compounds reduces intracellular glutathione in cells.[42]

Also, curcumin acts as a free radical scavenger. It protects hemoglobin from oxidation. In vitro, curcumin can significantly inhibit the generation of reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide H_2O_2, as well as the generation of nitrite radicals by activating macrophages. The latter play an important role in inflammation. Curcumin can also decrease the production of ROS in vivo. Its derivatives,

demethoxycurcumin and bis-demethoxycurcumin also exert antioxidant effects.

Since ROS are involved in the development of various pathological conditions, curcumin has the potential to control these diseases through its antioxidant activity.(43)

Larval toxicity

The study of the toxicity on the shrimp larvae of the extracts allowed to determine the lethal concentrations LC_{50} from the regression curves which express the percentage of the dead larvae according to the concentration of the extracts (Figure 8a, 8b).

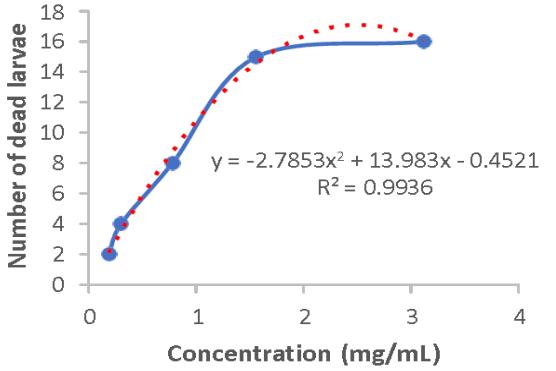

Figure 8a: Curve of larval mortality as a function of the concentration of the ethanolic extract of *Curcuma longa L.*

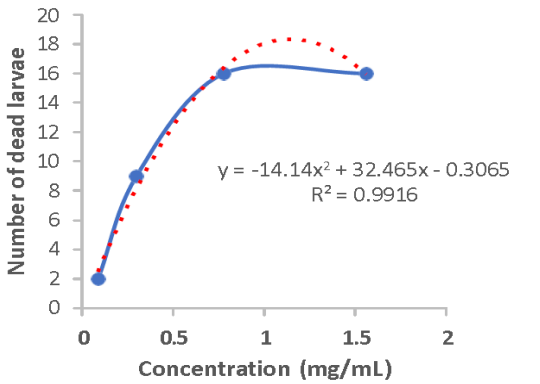

Figure 8b: Curve of larval mortality as a function of the concentration of ethyl acetate extract of *Curcuma longa L.*

Table 4: LC_{50} values of extracts

Extracts	LC_{50} (µg/mL)
Ethanolic	293.3
Ethyl acetate	702.8

Toxicity analysis of our extracts gives us values above 100 µg/mL for lethal concentrations (Table 4). Our extracts will then not be toxic on shrimp larvae.

In view of the results obtained, we note that the ethanolic and ethyl acetate extracts of *Curcuma longa L.* are non-toxic. Indeed, in a toxicological evaluation of plant extracts by the toxicity test, an LC_{50} value <1000 µg/mL is considered bioactive.(44) The "brine shrimp" test is a preliminary screening to determine not only the degree of toxicity of a product, but also the presence of potential anticancer compounds. Therefore, ethanolic and ethyl acetate extracts of *Curcuma longa L.* could not be cytotoxic against cancer cells.

For 20 plant extracts tested using in vivo (mouse) and in vitro methods, the results showed a good correlation ($r = 0.85$; $p < 0.05$), suggesting that the Artemia test is a relatively useful alternative toxicity model.(45) The crucial point in discussing the relevance of the LC_{50} values obtained through the shrimp larva test is whether the mortality data can be related to a more specific activity. A general correlation of this test with special types of bioactivity does not seem to be accepted. Nevertheless, in many cases it has been shown to be possible.(46) McLaughlin et al in a study of the utility of this assay as an antitumor prescreening of plant extracts, was able to determine a positive correlation between Artemia larval mortality and cytotoxicity against KB cells.(47) This led to the discovery of acetogenins from Annonaceae as a new class of natural pesticides and active antitumor agents.(47) Similarly, Solis et al found this test predictive of toxicity in KB cells except for compounds requiring metabolic activation, as Artemia larvae lack the necessary cytochrome P450 enzymes.(48) Richardson et al consider this test suitable for predicting the toxicity of trichothecenes (mycotoxins) in roasted chickens.(49)

CONCLUSION

Medicinal plants are the source of the majority of natural nutrients and antioxidants, but they remain under-exploited in the medical field. The development of new treatment by these plants must be on the agenda because

Curcuma longa L. cultivated in Benin at Bopa proved to be non-toxic and would be rich in nutrients and polyphenolic compounds.

From the results, it could be said that the consumption of *Curcuma longa L*. could fight against nutritional deficiencies and could have a good antioxidant activity in the capacity of free radical scavenging; which would contribute to a good functioning of the body.

Acknowledgement: None
Conflict of Interest: None
Source of Funding: None

REFERENCES

1. Jourdan JP. Curcuma et curcumine : de l'histoire aux intérêts thérapeutiques. Université de Caen Normandie; 2015.
2. Gui L, Jiang S, Xie D, et al. Analysis of complete chloroplast genomes of Curcuma and the contribution to phylogeny and adaptive evolution. Gene. 2020;732:144355.
3. Martin RC, Aiyer HS, Malik D, et al. Effect on Pro-inflammatory and Antioxidant Genes and Bioavailable Distribution of Whole Turmeric vs Curcumin: Similar Root but Different Effects. Food Chem Toxicol. 2012;50(2):227-31.
4. Verma S, Singh DC, Singh R, et al. A review--Curcuma Longa (Haridra): Emerging as magical herb from traditions to the pharmaceutical industries. Ayushdhara. 2016; 3:607-12.
5. Qadir MI, Naqvi STQ, Muhammad SA. Curcumin: a Polyphenol with Molecular Targets for Cancer Control. Asian Pac J Cancer Prev. 2016;17(6):2735-9.
6. Verma S, Singh S, Sharma S, et al. Assessment of genetic diversity in indigenous turmeric (Curcuma longa) germplasm from India using molecular markers. Physiol Mol Biol Plants. 2015;21(2):233-42.
7. Bruneton JF. Pharmacognosie Phytochimie Plantes médicinales. 5° Éd. Lavoisier; 2016. 1504 p.
8. Assogba M, Ganfon H, Lokossou J, et al. Comparative Study of Phenolic Compounds Leaflets and Ribs of Elaeis guineensis Jacq (Arecaceae) Extracts and Evaluation of their Antioxidant Activity. Chemistry Research Journal. 2021;6(5):31-9.
9. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic. 1965;16(3):144-58.
10. Kim DO, Chun OK, Kim YJ, et al. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem. 2003;51(22):6509-15.
11. Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture. 1978;29(9):788-94.
12. Hinneburg I, Damien Dorman HJ, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry. 2006;97(1):122-9.
13. Lamien-Meda A, Lamien CE, Compaoré MMY, et al. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules. 2008; 13(3):581-94.
14. Ameur I, Dechoucha S. Evaluation de l'activité antioxydante in vitro des plantes Lavandula stoechas et Curcuma longa. 2018. Available from http://dspace.univ-msila.dz:8080//xmlui/handle/123456789/15516
15. Lee SE, Hwang HJ, Ha JS, et al. Screening of medicinal plant extracts for antioxidant activity. Life Sci. 2003;73(2):167-79.
16. Boukri N. Contribution à l'étude phytochimique des extraits bruts des épices contenus dans le mélange Ras-el-hanout. Université Kasdi Merbah; 2014.
17. Leela K, Anchana DC. Isolation, Purification and Application of Secondary Metabolites from Lichen Parmelia Perlata. Biosciences Biotechnology Research Asia. 2017;14(4):1413-28.
18. Omulokoli E, Khan B, Chhabra SC. Antiplasmodial activity of four Kenyan medicinal plants. Journal of Ethnopharmacology. 1997;56(2):133-7.
19. Badiaga M. Etude ethnobotanique, phytochimique et activités biologiques de Nauclea latifolia Smith, une plante médicinale africaine récoltée au Mali. Université Blaise Pascal - Clermont-Ferrand II; 2011
20. Shamsuddeen U. Microbiological quality of spice used in the production of Kilishi, a traditionally dried and grilled meat product.

Bayero Journal of Pure and Applied Sciences. 2022;2(2): 66-69.
21. Tacouri DD, Ramful-Baboolall D, Puchooa D. In vitro bioactivity and phytochemical screening of selected spices used in Mauritian foods. Asian Pacific Journal of Tropical Disease. 2013;3(4):253-61.
22. Wichtl M, Anton R. Plantes thérapeutiques. Tradition, pratique officinale, science et thérapeutique. 2° Éd. TEC-DOC et EMI; 2003. 632 p.
23. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4): 564-82.
24. Brahimi I, Terrai R. Evaluation de l'activité antioxydante des deux plantes Rosmarinus officinalis et Curcuma longa. Université des Frères Mentouri Constantine 1; 2018.
25. Marfak A. Radiolyse gamma des flavonoïdes : étude de leur réactivité avec les radicaux issus des alcools. Université de Limoges; 2003.
26. Seggani S, Boukehil H. Corrélation entre le contenu polyphénolique et l'activité antioxydante et antimicrobienne in vitro de Curcuma longa L. Université des Frères Mentouri Constantine; 2017.
27. Vuorela S, Salminen H, Mäkelä M, et al. Effect of Plant Phenolics on Protein and Lipid Oxidation in Cooked Pork Meat Patties. J Agric Food Chem. 2005;53 (22): 8492-7.
28. Gómez-Caravaca AM, Gómez-Romero M, Arráez-Román D, et al. Advances in the analysis of phenolic compounds in products derived from bees. J Pharm Biomed Anal. 2006;41(4):1220-34.
29. Djeridane A, Yousfi M, Nadjemi B, et al. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food Chemistry. 2006;97(4):654-60.
30. Tawaha K, Alali FQ, Gharaibeh M, et al. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry. 2007;104(4):1372-8.
31. Falleh H, Ksouri R, Chaieb K, et al. Phenolic composition of Cynara cardunculus L. organs, and their biological activities. C R Biol. 2008;331(5):372-9.
32. Podsędek A. Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. LWT - Food Science and Technology. 2007;40(1):1-11.
33. Ghasemzadeh A, Azarifar M, Soroodi O, et al. Flavonoid compounds and their antioxidant activity in extract of some tropical plants. JMPR. 2012;6(13):2639-43.
34. Sumazian Y, Syahida A, Hakiman M, et al. Antioxidant activities, flavonoids, ascorbic acid and phenolic content of Malaysian vegetables. JMPR. 2010;4(10):881-90.
35. Tilak JC, Banerjee M, Mohan H, et al. Antioxidant availability of turmeric in relation to its medicinal and culinary uses. Phytother Res. 2004;18(10):798-804.
36. Krup V, Prakash HL, Harini A. Pharmacological Activities of Turmeric (Curcuma longa linn): A Review. Traditional Medicine and Clinical Naturopathy. 2013;2(133):2167-1206.
37. Tanvir EM, Hossen MS, Hossain MF, et al. Antioxidant Properties of Popular Turmeric (Curcuma longa) Varieties from Bangladesh. Journal of Food Quality. 2017; 2017:e8471785.
38. Lee BB, Park SR, Han CS, et al. Antioxidant Activity and Inhibition Activity against α-Amylase and α-Glucosidase of Viola mandshurica Extracts. Journal of The Korean Society of Food Science and Nutrition. 2008; 37(4):405-409.
39. Priyadarsini KI, Maity DK, Naik GH, et al. Role of phenolic O-H and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. Free Radical Biology and Medicine. 2003;35 (5):475-84.
40. Atti I. Evaluation Des Activités Antioxydant Et Antiradicalaire D'un Mélange D'épices Ras El Hanout. Université Kasdi Merbah Ouargla; 2014.
41. Nesrine A. Etude de l'activité antioxydante et antibactérienne des extraits méthanoliques de Curcuma longa L. et Zingiber officinale (Rosc.) commercialisés dans la région de M'sila. Université Mohamed Boudiaf de M'Sila; 2019.
42. Yoo GY, Kim E, Kang H, et al. Mass spectrometric investigation of concentration-dependent effect of curcumin and oxidative stress on intracellular glutathione levels. Anal Bioanal Chem. 2020;412(12):2873-80.
43. Chattopadhyay I, Biswas K, Bandyopadhyay U, et al. Turmeric and curcumin: Biological actions and medicinal applications. Current Science. 2004;87(1): 44-53.

44. Meyer BN, Ferrigni NR, Putnam JE, et al. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982;45(5):31-4.
45. Lagarto Parra A, Silva Yhebra R, Guerra Sardiñas I, et al. Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine. 2001;8 (5): 395-400.
46. Hartl M, Humpf HU. Toxicity assessment of fumonisins using the brine shrimp (Artemia salina) bioassay. Food and Chemical Toxicology. 2000;38(12):1097-102.
47. McLaughlin JL, Rogers LL, Anderson JE. The Use of Biological Assays to Evaluate Botanicals. Drug Information Journal. 1998;32(2):513-24.
48. Solis PN, Wright CW, Anderson MM, et al. A microwell cytotoxicity assay using Artemia salina (brine shrimp). Planta Med. 1993;59(3):250-2.
49. Richardson KE, Hamilton PB. Comparative Toxicity of Scirpentriol and Its Acetylated Derivatives1,2. Poultry Science. 1990;69 (3):397-402.

How to cite this article: ADOVELANDE Jacques, CACHON Fresnel Boris, ASSOGBA Mahoudo Fidèle et.al. Phytochemical analysis, antioxidant activity and larval toxicity of ethanolic and ethyl acetate extracts from rhizomes of Curcuma Longa L. *International Journal of Research and Review*. 2022; 9(9): 13-24.
DOI: *https://doi.org/10.52403/ijrr.20220902*

******