EVALUATION OF THE ANTIOXIDANT ACTIVITY OF PHENOL EXTRACTS OF TWO MEDICINAL PLANTS *Curcuma longa* L. and *Nigella sativa* L.

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Abstract. As part of the discovery of new antioxidants of natural origin, we have devoted this study to the evaluation of the antioxidant activity of phenolic extracts. of *Curcuma longa* L. and *Nigella sativa* L. a medicinal plant widely used in the traditional pharmacopoeia the polyphenol compounds were obtained by maceration in ethanol with a yield of 10% of the *Nigella sativa* extract and 21% of the *Curcuma longa* extract. The total content of phenol compounds of the ethanol extracts was determined by the method of folin -ciocalteu, The highest content is found in the plant *Curcuma longa*, it is of the order of 251.46mg / mL (7.54mg GAE / g), and for the plant *Nigella sativa*, it is of the order of 167.46mg / mL (5.02mg GAE / g) .The antioxidant activity was evaluated using the free radical reduction method DPPH extract concentration needed to reduce 50% of the radical DPPH (EC50). The ethanol extract of *Curcuma longa* L. is estimated at 0.00596 mg / mL showing a very important activity which is practically similar to that of the ethanol extract of *Nigella sativa* L. which reduces stability to DPPH with an EC50 of 0.050 mg / mL.

From these results it is proved that ascorbic acid remains the most effective antioxidant with an EC50 of 0.0002 mg / mL relative to the ethanol extract of the plants studied.

Keywords: *Curcuma longa* L.; *Nigella sativa* L.; antioxidant activity; Folin-Ciocalteu; phenol compounds.

INTRODUCTION

The use of synthetic antioxidant molecules is currently being questioned because of potential toxicological risks. Now, new plant sources of natural antioxidants are being sought. Indeed, polyphenols are natural compounds widely used in the plant kingdom that have a growing importance thanks to their beneficial effects on health.

Their role as natural antioxidants is attracting more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases. They are also used as additives in the food industry, pharmaceuticals and cosmetics.

Scientific research has been developed for the extraction, identification and quantification of these compounds from different sources such as agricultural and horticultural crops or medicinal plants [1, 30].

Nigella sativa L. and *Curcuma longa* L. are two of the most widely used medicinal plants worldwide. The extracts of the seeds of *Nigella sativa* L. et the rhizomes of *Curcuma longa* L. are widely used in traditional medicine, for centuries against many pathologies such as hypertension, diabetes, fever, inflammation ... etc. Several studies have been made on the chemical composition of these seeds and rhizomes in correlation with their pharmacological properties (anti-inflammatory, antioxidant, antidiabetic, anticancer, antimicrobial), but very little research is conducted on polyphenols; active principles probably responsible for these pharmacological activities [2, 28].

This work is part of the research and recovery of bioactive substances such as natural substances (polyphenols) endowed with antioxidant activity that are of interest in the field of bio pharmacology. The main objective of this work is to evaluate in vitro the antioxidant activity of the ethanol extract of *Nigella sativa* L. and *Curcuma longa* L. according to the DPPH free radical scavenging method.

MATERIALS AND METHODS

Plant material

The plant material used is the seeds of *Nigella sativa* L. and the rhizomes of *Curcuma longa* L. they were crushed, and subsequently preserved until the moment of use.

Extraction of polyphenol compounds

Preparation of the ethanolic extract

The ethanol extract was prepared from 10 g of ground seed of *Nigella sativa* L. which was macerated in 100 mL of ethanol (50 %) at room temperature and protected from light for 24 hours. With maximum agitation, then the mixture is filtered on a filter paper. The operation is repeated a second time on the grounds. The filtrates obtained are added dilapidated and evaporated to dryness with the aid of an oven at a temperature of 45°C dry extract is stored in the refrigerator. And the same step by the rhizomes of *Curcuma longa* L. [3, 25, 29].

Performance determination

The yield refers to the mass of the extract determined after evaporation of the solvent; it is expressed as a percentage relative to the initial mass of the plant subjected to the extraction [4, 26].

$$R = (PE / PA) \times 100$$

R: yield of the extract in percentage.
PE: weight of the extract in gram.
PA: weight of the plant in gram.

Determination of total polyphenols

To 0.3 mL of extract solution (100 g / mL) are added 1.5 mL of folin-ciocalteu reagent (10 % v / v) and 0.2 mL sodium carbonate ($NaCO_3$) (7.5 % w / v), is well stirred, the test tubes are incubated at room

temperature for 30 min in the dark; then the absorbance is measured at 765 nm.

He white containing the solvent instead of the substance tested.

Gallic acid (0-300 µg / mL) is used as a standard to establish the calibration curve from which the concentration of the total polyphenols in the extracts is calculated (Figure 1). The result is expressed in mg equivalents of Gallic acid per gram of extract (mg GAE / g extract) [5, 6].

Evaluation of the antioxidant activity of the extracts

Trapping of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) DPPH (2,2-diphenyl-1-picrylhydrasyl) is generally the most used substrate for rapid and direct assessment of antioxidant activity due to its free radical form stability and simplicity of analysis. It absorbs in the visible at the wavelength of 515-520 nm [7]. The DPPH method has several advantages because it is independent, simple and fast. The test consists in putting the DPPH radical (of violet color) in the presence of so-called "antioxidant" molecules in order to measure their capacity to reduce the radical DPPH [8, 19].

150 µL of the sample (different concentration) is added to 1.35 ml of a solution of DPPH prepared in methanol. The reaction mixture was kept in the dark for 30 minutes at 37 °C he absorbance of the reaction medium was measured at 517 nm against the methanol as white and the DPPH reagent + methanol as a negative control. All the tests carried out in duplicate. A blank containing each of the various extracts without DPPH was prepared to remove the absorbance of the extracts [5, 13].

Expression of results

Calculation of inhibition percentages(PI%) we thus calculate the percentages of inhibition by the following formula:

$$PI\% = [(AC-AE) / AC] * 100$$

AC: Absorbance of the negative control

AE: Absorbance of the radical after 30 min of contact with the antioxidant in the dark.

Calculation of IC_{50}

IC50 or 50 % inhibitory concentration (also called EC50 for Efficient Concentration 50), is the concentration of the tested sample needed to reduce 50 % of DPPH radical. The IC50 are calculated graphically by the linear regression of the plotted graphs, percentages of inhibition as a function of different concentrations of the fractions tested [9-12].

Extraction of phenolic compound

Yield of the ethanolic extract

The yield is expressed as a percentage of the mass of extract relative to the mass of the dry plant.

Table 1. Represents the yield obtained from the ethanolic extract

Plant	Mass of the dry plant (g)±SD	Extract mass (g)±SD	Productivity %
Nigella sativa	10g ± 0.0002	1g ± 0.0025	10%
Curcuma longa	10g ± 0.0031	2.1g ± 0.0012	21%

Dosage of total polyphenols

The total polyphenols were determined by the method of Folin-Ciocalteu. In the presence of polyphenols, the Folin-Ciocalteu complex changes its color from yellow to blue; gallic acid was used as standard, absorbance was read in a wavelength of 765nm. The results obtained are shown in a calibration curve.

The levels obtained are expressed in mg, gallic acid equivalent per gram of dry vegetable matter (mg GAE / g), using the equation of the linear regression of the traced gallic acid calibration curve.

A single factor analysis of variance (p = 0.05) was used in this study and is processed by. MINITAB 2000 statistics software, the test of meaning (post hoc) is done by Newman Keuls.

RESULTS

For both plants studied *Curcuma longa* L. and *Nigella sativa* L. we noticed a variability of the total polyphenol contents; the highest content is found in the plant *Curcuma longa* L., it is of the order of 251.46 mg / mL (7.54 mg GAE / g), and for the plant *Nigella sativa* L., it is of the order of 167.46 mg /mL (5.02 mg GAE/g). The levels of total polyphenols in the ethanol extracts of *Nigella sativa* L. are high as the levels found by [30] (163.33 µg / mg). However, the values noted in the present study are lower than the levels recorded by [10, 24] (191.06 µg / mg) in the chloroform extract of *Nigelle sativa* L.

The total polyphenol values in the ethanol extracts of *Curcuma longa* L. are high as the levels found by [11, 22] recorded values of (4.14 mg GAE / g).

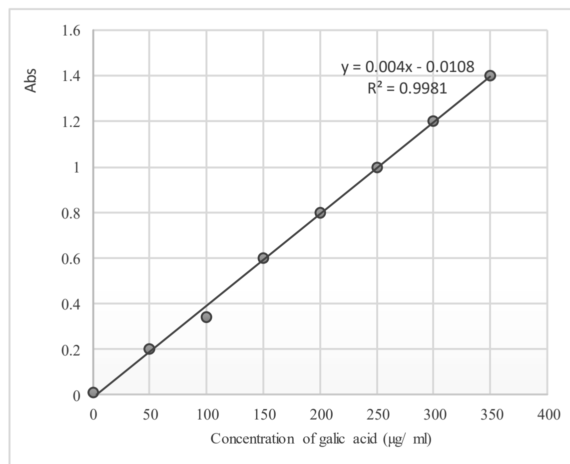

Figure 1: Calibration curve of gallic acid for the determination of total polyphenols

Table 2: Determination of Total Polyphenols in the seeds of *Nigella sativa* L. and *Curcuma longa* L.

Extracts	Total polyphenol content
Nigella sativa	167.46 mg/mL ± 5.02 mg GAE/g
Curcuma longa	251.46 mg/mL ± 7.54 mg GAE/g

Antioxidant activity

Test of DPPH free radical trapping

The antioxidant activity of the ethanol extracts of *Nigella sativa* L. and *Curcuma longa* L. and of the standard antioxidant (ascorbic acid) of the radical DPPH was evaluated using a spectrophotometer by following the reduction of this radical which is accompanied by its passage from the violet color (DPPH) to the yellow color (DPPH-H) measurable at 517 nm (Figure 2 and 3).

This reduction capacity is determined by a decrease in the absorbance induced by anti-free radical substance [12, 25].

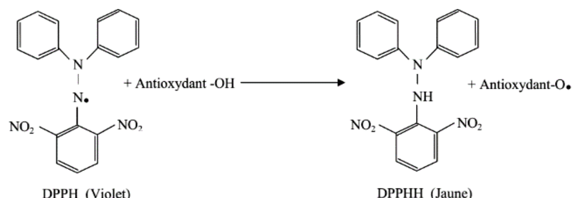

Figure 2: Reaction of an antioxidant with the radical DPPH

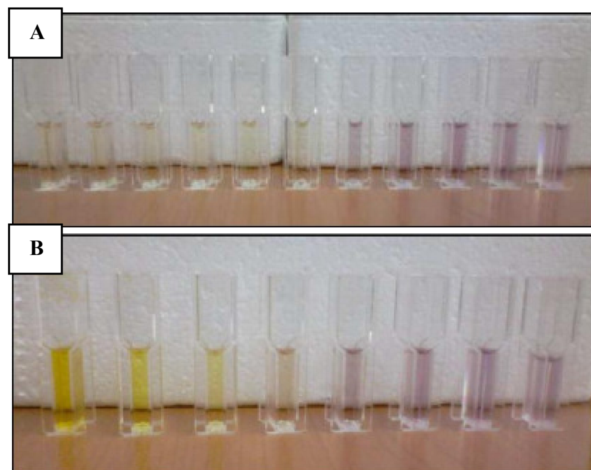

Figure 3: Discoloration of the DPPH solution of purple in yellow as a function of the concentration of the extract *Nigella sativa* L. (**A**) and *Curcuma longa* L. (**B**)

Measurements of the inhibition of DPPH absorbance caused by the presence of the ethanol extract of *Curccuma longa* L. and ethanol extract of *Nigella sativa* L. as well as ascorbic acid control made it possible to determine the (PI%) percentage inhibition (PI) of each dilution of these extracts. It is calculated by applying the formula quoted previously (material and methods).

After calculating the PI of the different dilutions, we obtain a concentration range. The values obtained make it possible to draw curves from which we can determine the value of the EC50.

Determination of value EC50

Concentrations that trap 50 % free radicals or effector concentrations (EC50) are calculated and shown in Figure 7.

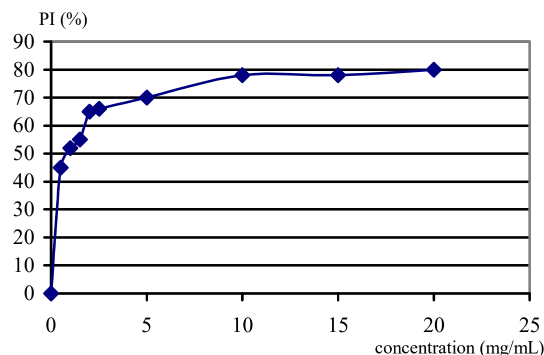

Figure 4. Percent inhibition of the free radical DPPH as a function of the different concentrations of the ethanol extract of *Nigella sativa* L.

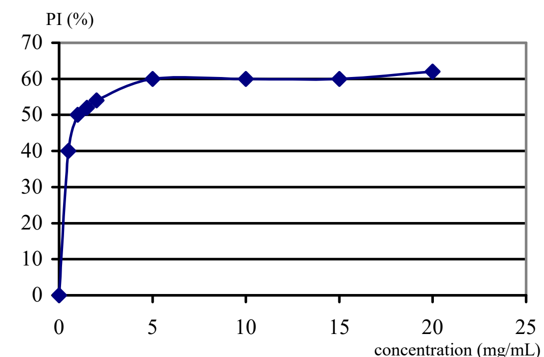

Figure 5. Percentages of inhibition of the free radical DPPH as a function of the different concentrations of the ethanol extract of *Curcuma longa* L.

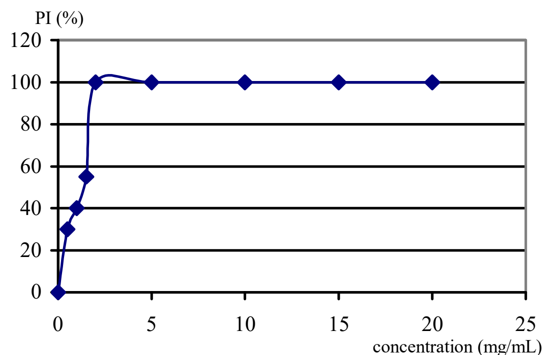

Figure 6. Percentage of inhibition of DPPH as a function of different concentrations of ascorbic acid

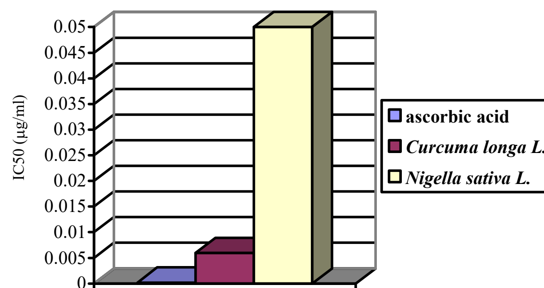

Figure 7. IC50 values for ascorbic acid (AA); *Nigella sativa* (Ns) and *Curcuma longa* (Cl)

DISCUSSION

EC50 is inversely proportional to the antioxidant capacity of a compound, because it expresses the amount of antioxidant required to decrease the free radical concentration by 50%. The lower the EC50 value, the greater the antioxidant activity, of a compound. The EC50 values of the ethanol extract of *Curcuma longa* L. *and Nigella sativa* L. are shown in Figure 4 and 5.

The EC50 values for ascorbic acid, ethanol extract of *Curcuma longa* L. and *Nigella sativa* L. are shown in Figure 6. The ethanol extract of *Curcuma longa* L. makes the free radical stable (2.2 diphenyl-1-picrylhydrazyl) yellow-colored (diphenyl-picrylhydrazine) with an EC50 of 0.00596 mg / mL showing a very important activity which is practically similar to that ethanol extract of *Nigella sativa* L. which reduces stability to DPPH with an EC50 of 0.050 mg / mL. From these results it is proved that ascorbic acid remains the most effective antioxidant with an EC50 of 0.0002 mg / mL relative to the ethanol extract of the plants studied.

In another study carried out by Hebi [13] and Imran [14], *Curcuma longa* L. rhizomes of the Morocco region have a strong anti-radical activity determined by the DPPH test, whose ethanol extract has an activity. Antioxidant EC50 = 24 µg / mL (0.024 mg / mL), in the same study the ethanol extract of *Curcuma zedoaria* (other species of curcuma) showed an antioxidant activity with an EC50 = 40 µg / mL (0.04 mg / mL). So the antioxidant activity obtained for turmeric extracts are low by comparison to our results.

Moreover, another study by [15, 16] the methanol extract of *Nigella sativa* L. showed a low free radical antioxidant activity with an EC50 = 0.64 mg / mL compared to our result EC50 = 0.050 mg / mL. As well as [17-20], this showed that EC50 of the aqueous extract of seeds of *Nigella sativa* L. is 447.67 µg / mL (0.447 mg / mL)

In herbal medicine, medicinal plants or plant extracts are used in the treatment of many infectious diseases and are also used in pharmaceutical preparations. These plants are widely used to cure many diseases.

In our work, we have in practice carried out the study of antioxidant activity of the extracts of *Nigella sativa* and *Curcuma longa*, and we will make a comparison between the two extracts of the medicinal plants tested to determine the most effective extract concerning the ability to reduce free radicals.

The results obtained show that the ethanol extracts studied have important antioxidant activities *in vitro*. They show a very significant inhibition of the DPPH radical and an excellent reducing power.

In addition, for both plants studied *Curcuma longa* L. and *Nigella sativa* L. we noticed a variability of the total polyphenol contents; the highest content is found in the plant *Curcuma longa* L.

Furthermore, according to the results found, the ethanol extract of *Curcuma longa* L. showing a very important antioxidant activity which is practically similar to that of the ethanol extract of *Nigella sativa* L. [18-22]. Have a moderate antioxidant capacity, which is greater than that of ascorbic acid, it has been shown that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins reduce and discolor the DPPH in because of their ability to give up hydrogen. The polyphenols contained in the extracts of *Nigella sativa* L. and *Curcuma longa* L. are probably responsible for the antioxidant activity of these extracts.

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