Phytochemical characterization of the ethanolic extract, antioxidant activity, phenolic content and toxicity of the essential oil of *Curcuma longa* L.

Caracterización fitoquímica del extracto etanólico, actividad antioxidante, contenido fenólico y toxicidad del aceite esencial de *Curcuma longa* L.

Caracterização fitoquímica do extrato etanólico, atividade antioxidante, conteúdo fenólico e toxicidade do óleo essencial de *Curcuma longa* L.

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Abstract

Curcuma longa L. tubers rhizomes are commonly used as a spice, dye, starch source, and in ancient medicine. Due to its functional properties, extracts and essential oil of ethanolic extract *C. longa* have been used as an antifungal, antimicrobial, antioxidant and anti-inflammatory agent, so it can be an alternative for its potential use in the food industry. Therefore, in this work, the phytochemical characteristics of the extracts, the phenolic content, the antioxidant activity and the toxicity of the essential oil of *C. longa* were evaluated. The phytochemical characterization of the ethanolic extract was carried out through a phytochemical screening (alkaloids, flavonoids, phenols, saponins, tannins, quinones, resins and reducing sugars) and the extraction of the essential oil by means of hydrodistillation. The determination of the total phenolic content (TPC) was carried out using the Folin-Ciocalteu method; the antioxidant activity with the ABTS and DPPH methods and the oil toxicity by the resazurin reduction method using *Escherichia coli* as a biosensor. The results obtained in the phytochemical screening indicate the presence of tannins, alkaloids, flavonoids, saponins, quinones and reducing sugars. The total phenolic content (TPC) was 50.99 mg GAE.g^{-1}, the inhibition coefficient (IC_{50}) of the ABTS radical was 426.943 µg.mL^{-1} and the DPPH radical was 2274.024 µg.mL^{-1}. The mean lethal concentration (LC_{50}) of turmeric essential oil for *E. coli* was 2585.69 mg.L^{-1}. It is concluded that turmeric essential oil has a high phenolic content, high antioxidant activity and low toxicity for *E. coli*, which is why it is recommended for use in the food industry.

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Resumen

Los tubérculos rizomas de *Curcuma longa* L. se utilizan como especia, tinte, fuente de almidón y en la medicina ancestral. Debido a sus propiedades funcionales, los extractos y el aceite esencial de *C. longa* se emplea como agente antifúngico, antimicrobiano, antioxidante del extracto etanolico y antiinflamatorio, por lo que puede ser una alternativa para su uso en la industria de alimentos. En este trabajo se evaluaron las características fitoquímicas de los extractos, el contenido fenólico, la actividad antioxidante y la toxicidad del aceite esencial de *C. longa*. La caracterización fitoquímica del extracto etanólico se realizó a través de un tamizaje fitoquímico (alcaloides, flavonoides, fenoles, saponinas, taninos, quinonas, resinas y azúcares reductores) y la extracción del aceite esencial mediante hidrodestilación. La determinación del contenido fenólico total (TPC) se realizó empleando el método Folin-Ciocalteu; la actividad antioxidante con los métodos ABTS y DPPH y la toxicidad del aceite por el método de reducción de resazurina utilizando *Escherichia coli como* biosensor. Los resultados obtenidos en el tamizaje fitoquímico indican la presencia de taninos, alcaloides, flavonoides, saponinas, quinonas y azúcares reductores. El contenido fenólico total (TPC) fue de 50,99 mg GAE.g^{-1}, el coeficiente de inhibición (IC_{50}) del radical ABTS fue de 426,943 µg.mL^{-1} y del radical DPPH fue de 2274,024 µg.mL^{-1}. La concentración letal media (CL_{50}) del aceite esencial de cúrcuma para *E. coli* fue de 2585,69 mg.L^{-1}. El aceite esencial de cúrcuma presenta un alto contenido fenólico, una alta actividad antioxidante y una baja toxicidad para *E. coli*, por que se recomienda su uso en la industria alimenticia.

Palabras clave: rizomas, metabolitos secundarios, fenoles, antioxidantes, resazurina.

Resumo

Os tubérculos rizomas de *Curcuma longa* L. são comumente usados como tempero, corante, fonte de amido e na medicina antiga. Pelas suas propriedades funcionais, os extratos e óleos essenciais de *C. longa* têm sido utilizados como agente antifúngico, antimicrobiano, antioxidante e antiinflamatório, podendo ser uma alternativa para potencial uso na indústria alimentícia. Portanto, neste trabalho, foram avaliadas as características fitoquímicas dos extratos, o teor fenólico, a atividade antioxidante e a toxicidade do óleo essencial de *C. longa*. A caracterização fitoquímica do extrato etanólico foi realizada por meio de uma triagem fitoquímica (alcalóides, flavonóides, fenóis, saponinas, taninos, quinonas, resinas e açúcares redutores) e da extração do óleo essencial por meio de hidrodestilação. A determinação do conteúdo fenólico total (CTP) foi realizada pelo método de Folin-Ciocalteu; a atividade antioxidante pelos métodos ABTS e DPPH e a toxicidade do óleo pelo método de redução da resazurina usando *Escherichia coli* como biossensor. Os resultados obtidos na triagem fitoquímica indicam a presença de taninos, alcalóides, flavonóides, saponinas, quinonas e açúcares redutores. O conteúdo fenólico total (TPC) foi de 50,99 mg GAE.g^{-1}, o coeficiente de inibição (IC_{50}) do radical ABTS foi de 426,943 µg.mL^{-1} e do radical DPPH foi de 2274,024 µg.mL^{-1}. A concentração letal média (CL_{50}) do óleo essencial de cúrcuma para *E. coli* foi de 2585,69 mg.L^{-1}. Conclui-se que o óleo essencial de cúrcuma possui alto teor fenólico, alta atividade antioxidante e baixa toxicidade para *Escherichia coli*, por isso é recomendado para uso na indústria alimentícia.

Palavras-chave: rizomas, metabólitos secundários, fenóis, antioxidantes, resazurina.

Introduction

Turmeric (*Curcuma longa* L.) is a native plant of Southeast Asia and belongs to the Zingiberaceae family (Du *et al*., 2006). It is a perennial plant that grows between 90 to 150 centimeters in height and is cultivated in countries with tropical climates (Du *et al*., 2006). It is generally used as a spice, dye, source of starch (Ferreira *et al*., 2013) and ancestral medicine for the cure of various diseases. Among its many therapeutic properties, its anti-inflammatory, hepatoprotective, antimicrobial, antioxidant, antitumor and antiviral activity stands out (Parveen *et al*., 2013). In addition, its therapeutic potential for the treatment of neurodegenerative, cardiovascular, pulmonary, metabolic and autoimmune diseases is highlighted (Aggarwal & Harikumar, 2009).

Essential oils EOs (EA) are made up of a complex mixture of volatile substances, and can be found in the leaves, stems, bark, roots, fruits and seeds of plants (Sánchez-González *et al*., 2011). They are considered as secondary metabolites and for the most part they are responsible for giving it the characteristic aromas and flavors (Sánchez-González *et al*., 2011).

The increase in demand for products that contain ingredients or additives from natural sources and given that the Food and Drug Administration (FDA) classifies essential oils as "generally recognized as safe" (GRAS) products, due to the fact that they have a low toxicity and do not present adverse effects on health, has generated great interest for the use of these compounds for use as an additive, preservative, antimicrobial agent, antifungal agent, among others.

Turmeric essential oil has a wide variety of uses reported as: nutritional additive in fish farming (Negrete and Secaira, 2016), additive for edible coatings (Campo *et al*., 2017), antifungal agent (Damalas, 2011; Naveen *et al*. , 2016; Abdelgaleil *et al*., 2019), antimicrobial agent (Parveen *et al*., 2013; Teles *et al*., 2019), antioxidant and anti-inflammatory agent (Vijayasteltar *et al*., 2011). These uses may be determined by the functional properties of this oil, and it is important to note that these depend on factors such as the type of soil and other climatic variables of the growing area of the plant species.

Therefore, the objective of this study was to evaluate the phytochemical characteristics of the extracts, the phenolic content, the antioxidant activity and the toxicity of the essential oils obtained from the rhizomes of *Curcuma longa* L. cultivated in the province of Manabí for their potential use in the food industry.

Materials and methods

Plant material

The plant material used in the present study (turmeric rhizomes) was collected in the Ricaurte locality of the Chone canton, Manabí province, Ecuador (North Latitude 0°34'24", West Longitude 80°2'39"). The microorganism used for the toxicity test was *Escherichia coli*, identified and isolated in the microbiology laboratory of the Universidad Técnica de Manabí. The tests described below were performed in triplicate.

Phytochemical screening

Before phytochemical screening, an extraction of the turmeric rhizomes was carried out by the Soxhlet method using ethanol (96 %) as solvent. To do this, 30 g of previously scratched vegetable matter (wet mass) were weighed, placed in a cartridge and extraction

was carried out with 250 mL of ethanol. The process was concluded after three recirculations in the equipment. The phytochemical tests were carried out on the ethanolic extract obtained, according to the methodology described by Ramos and Solorzano (2016) with some modifications.

Ferric chloride assay (phenolic compounds and/or tannins): Two (2) mL of ethanolic extract were used in a test tube, to which 100 µL 5% ferric chloride (Sigma Aldrich) solution were added in physiological saline solution. The test is considered positive (+++) when the solution is colored: wine-red, deep green or blue.

Wagner's test (alkaloids): Two (2) mL of the extract were added to a test tube to later acidify the medium with three (3) drops of concentrated hydrochloric acid (Fisher Scientific). Then six (6) drops of Wagner's reagent were added, to proceed to observe the color changes. It is considered positive in the following ranges: presence of opalescence (+), presence of defined turbidity (++) and presence of precipitate (+++).

Shinoda test (flavonoids): Two (2) mL of extract were added to a test tube and it was diluted with two (2) mL of concentrated hydrochloric acid, then a piece of metallic magnesium tape was added to allow it to react by five (5) minutes. After the established time, two (2) mL of amyl alcohol were added, mixing the phases, and then allowed to stand until separated. The test is considered positive when the amyl alcohol is colored yellow, orange, brown or red.

Foam test (saponins): One (1) mL of extract was diluted with five (5) mL of distilled water and stirred vigorously for 10 minutes. This test is considered positive if foam appears on the surface of the solution with a height of more than two (2) mm and it remains for more than two (2) minutes.

Benedict's test (reducing sugars): 0.5 mL of the extract was placed in a test tube and eight (8) mL of Benedict's reagent were added; shaken gently and placed in a 70 °C water bath for 15 minutes. The test is considered positive when there is a change from the blue hue of the reagent to an orange (+), brick (++) and brown (+++) color.

Bornträger test (quinones): To one (1) mL of ethanolic extract contained in a test tube, two (2) mL of 20 % sodium hydroxide (Merk) were added, subsequently, it was stirred and then allowed to stand until phase separation. The test is considered positive when the upper phase is colored pink (++) or red (+++).

Resin test: One (1) mL of the extract and five (5) mL of distilled water were added, the appearance of a precipitate indicates the presence of resins.

Preparation of essential oil

The turmeric essential oil was obtained by hydrodistillation using a Clevenger hydroditillation equipment (Brand Glassco). For the extractions of the turmeric EO, 60 g (wet mass) of striped rhizomes and 400 mL of distilled water were used. The extraction time was three (3) hours.

Determination of phenolic content of essential oil

The determination of the phenolic content was carried out from the Folin-Ciocalteu test according to the methodology described by Rover & Brown (2013) with modifications. The starting point was a stock solution of essential oil with a concentration of 2000 mg.L^{-1} in methanol ACS (Merk). Two-hundred (200) µL of sample were taken from the stock solution, to then add 1.5 mL of distilled water and 100 µL of the Folin-Ciocalteu reagent (Sigma Aldrich) and let stay for five (5) minutes. After 200 µL of 20 % m/v sodium carbonate (Merk) was added, the resulting solution was stirred and allowed to stand at room temperature in the dark for one hour. Absorption was measured with a wavelength of 730 nm in a UV-vis spectrophotometer (Model Genesys 180, ThermoFisher brand), for quantification a gallic acid calibration curve was prepared (5, 25, 50, 75, 100, 125, 150 mg.L^{-1}). The results were expressed as mg equivalent of gallic acid.g^{-1} of essential oil.

Determination of the antioxidant activity of essential oil

DPPH method

The determination of the *in vitro* antioxidant activity of turmeric essential oil was carried out from the DPPH (2,2-diphenyl-1-picrylhydrazyl) test, according to the methodology described by Mimica-Dukić *et al*. (2003) with modifications. A 0.1 mM solution of DPPH (Sigma Aldrich) in methanol was prepared and allowed to incubate for 24 hours in the dark. Starting from a stock solution of essential oil with a concentration of 5000 mg.L^{-1} in methanol, different dilutions were made (1000, 1600, 2000, 3000, 4000, 5000 mg.L^{-1}). For the measurement of antioxidant activity, one mL of DPPH solution was added to one mL of the different samples prepared and it was left to stand for one hour at room temperature in the dark. Subsequently, the absorbance was measured in a UV-vis spectrophotometer (model Genesys 180, brand ThermoFisher) at a wavelength of 517 nm using a Trolox calibration curve (Sigma Aldrich) (2.5; 5; 10; 15, 30 and 40 µM). The results were expressed as mg equivalent of Trolox.g^{-1} of essential oil, and the IC50 (mean inhibitory concentration), was obtained graphically with the values of the percentage of inhibition determined by Equation 1 and the concentrations of the prepared solutions.

$$\% Inh = \frac{Abs.control - Abs.muestra}{Abs.control} * 100 \qquad \text{Equation 1}$$

ABTS method

The determination of the *in vitro* antioxidant activity of turmeric essential oil, using the ABTS test (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)), was carried out according to the methodology established by Proestos *et al*. (2013), with modifications. For the preparation of the ABTS solution, a 7 mM solution of ABTS (Sigma Aldrich) in water was mixed with a 2.45 mM potassium persulfate solution (ACROS Organics) in water and left to incubate for 12 hours in the dark at room temperature. Starting from a stock solution of essential oil with a concentration of 2000 mg.L^{-1} in methanol, different dilutions were made (100, 200, 400, 600, 800, 1000 mg.L^{-1}). To measure the antioxidant activity, one mL of ABTS solution was added to one mL of the different samples prepared and it was left to rest for one hour at room temperature in the dark, to then measure the absorbance in a UV-vis spectrophotometer (Model Genesys 180, brand ThermoFisher) at a wavelength of 734 nm and with the aid of a Trolox calibration curve (1.25, 2.5, 5, 10, 15 and 20 µM). The results were expressed as mg equivalent of Trolox.g^{-1} of essential oil, and the IC50 was obtained graphically with the solutions prepared and the percentage of inhibition determined with equation 1.

Determination of essential oil toxicity

The resazurin reduction method for insoluble chemicals developed by Liu (1986) with modifications was used to determine the toxicity of turmeric essential oil. For the bioassay, a bacterial culture was prepared in which a colony of the selected microorganism (*E. coli*) was transferred into 100 mL of nutrient broth (TM MEDIA), covered and placed in an incubator for 18 hours at 37 °C. After the time had elapsed, 15 mL of the broth was transferred under aseptic

conditions into test tubes to be centrifuged at 4000 rpm for five (5) minutes. The supernatant from the tubes was discarded and the precipitate was resuspended with physiological saline to centrifuge again, repeating the process until the supernatant was clear. Next, the absorbance of the suspension of microorganisms was measured at 600 nm and serial dilutions were carried out at which absorbances were measured and in parallel they were seeded on Standard agar (Sigma-Aldrich) in order to build a graph of UFC. mL^{-1} vs absorbance, with this the dilution factor necessary to obtain a suspension with a concentration of 106 UFC.mL^{-1} was calculated.

Dilutions of essential oil and 10 % v/v DMSO (100, 250, 500, 750, 1000, 2000, 5000 mg.L^{-1}) were prepared. For the analysis of the samples, in a test tube, 2.75 mL of nutrient broth, 250 µL of the oil dilution, one (1) mL of suspension of 106 CFU.mL^{-1} and 400 µL of resazurin (Biotum) were added. For the reagent control, 3.75 mL of nutrient broth, 250 µL of DMSO solution (Merck) 10 % v/v and 400 µL of resazurin were added. 2.75 mL of nutrient broth, 250 µL of 10 % DMSO solution, v/v, one (1) mL of suspension of 106 CFU. mL^{-1} and 400 µL of resazurin. The tubes were homogenized and incubated at 37 °C for five (5) hours. After the established time, the absorbances were read at a wavelength of 600 nm. The determination of the mean lethal concentration (LD_{50}) was determined according to the change from resazurin (blue) to resofurin (pink) expressed as a percentage of inhibition of bacterial growth. The calculation of the LD_{50} value was carried out through equation 2, obtained by adjusting the data obtained in a dose-effect model carried out with the Origen Lab2019 software.

$$\%\ de\ inhibition = \frac{(Abs_{ctrl\ react.} - Abs_{ctrl\ m.o.}) - (Abs_{ctrl\ react.} - Abs_{sample})}{(Abs_{ctrl\ react.} - Abs_{ctrl\ m.o.})} * 100\%$$

Equation 2

Statistic analysis

The data obtained in the bacterial growth inhibition assays were analyzed using an analysis of variance (ANOVA) and the significance was calculated with a confidence level of 95 % with the Origen Lab2019 software.

Results and discussion

Qualitative phytochemical characterization of *Curcuma longa* L.

Phytochemical characterization of the ethanolic extract of *Curcuma longa* are presented in table 1.

Table1. Phytochemical compounds present in the ethanolic extract of rhizomes of *Curcuma longa* L.

Phytochemical compound	Presence
Tannins	+++
Alkaloids	++
Flavonoids	+++
Saponins	+++
Phenols	+++
Reducing sugars	+
Quinones	++
Resins	-

+++ = abundant ++ = moderate + = low - = absent.

Phytochemical screening of the ethanol extract of *C. longa* indicates abundant presence of tannins, flavonoids, saponins and phenols. Moderate presence of alkaloids and quinones and low for reducing sugars (table 1). Freire and Vistel (2015), reported similar results in the determination of secondary metabolites in turmeric rhizome powder, identifying tannins, flavonoids, saponins, quinones, reducing sugars, carotenes, coumarins and alkaloids. On the other hand, Ema and Torres (2018) performed the phytochemical screening of an ethanolic extract of *C. longa*, obtaining better observable results for triterpenes, polysaccharides and phenolic compounds. In this sense, Sánchez-González *et al*. (2011), indicate that the difference in the presence of secondary metabolites may vary according to genotype, environmental conditions, geographic location, solvent used, extraction techniques, among other factors.

Phenolic content of *Curcuma longa* essential oil

The total phenolic content (TPC) of the essential oil of *C. longa* was 50.99 mg GAE.g^{-1}. Lower concentrations were reported by Chen *et al*. (2007) and Choi *et al*. (2020), who obtained values of 21.4 mg GAE.g^{-1} for methanolic extracts and 8.81 mg GAE.g^{-1} for ethanolic extracts, respectively.

The essential oil of *C. longa* has a higher phenolic content compared to methanolic and ethanolic extracts. In this regard, Bouarab-Chibane *et al*. (2019), state that a high phenolic content improves antimicrobial activity, because this type of compound favors interaction with the cell membrane of microorganisms, thus inducing irreversible damage to the cytoplasm and could even lead to intracellular enzyme inhibition. Therefore, essential oils with a high phenolic content, such as the essential oil of *Curcuma longa* L., can be an alternative for use in the agri-food industry, for example, to avoid post-harvest food losses, which are around 55 % in Latin America and the Caribbean (Food and Agriculture Organization of the United Nations (FAO, 2019), the main factors of food spoilage being fungi and pathogenic bacteria (Aguilar-Veloz *et al*. , 2020).

Antioxidant activity of *Curcuma longa* essential oil

ABTS radical inhibition activity

Figure 1 shows the inhibition percentages of the ABTS radical by action of the essential oil of *C. longa* in a concentration range of 50-500 mg.L^{-1}, reaching an inhibition of the radical between 7.918 % and 56.305 %, respectively.

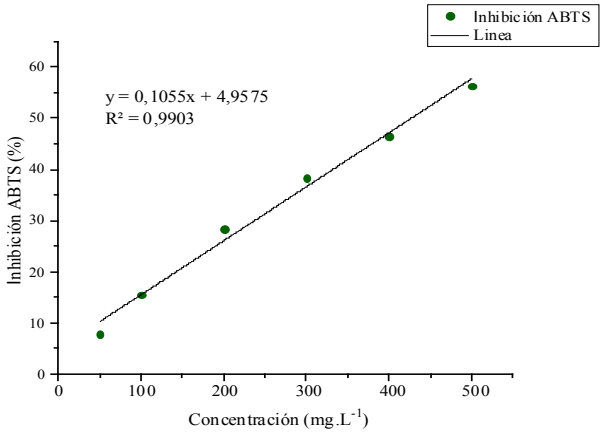

Figure 1: Inhibition of the radical ABTS by action of turmeric essential oil.

The Trolox equivalent value (TEAC) of the essential oil of turmeric is 30.43 µmol.g^{-1} sample and the concentration of essential oil of *C. longa* necessary for the inhibition of the ABTS radical by 50 % (IC50) was 426.943 µg .mL^{-1}. This value is similar to those reported by Avanço *et al*. (2016) and Li & Li (2009), which were 540 µg.mL^{-1} and 699.57 µg.mL^{-1}, respectively. On the other hand, Priya *et al*. (2012) and Kuttan *et al*. (2011), obtained higher IC50 values of 1541 µg.mL^{-1} and 1000 µg.mL^{-1}; respectively. For samples of fresh, dried and cured turmeric, Kutti & Lingamallu (2012), reported IC50 of 3300 µg.mL^{-1}, 1900 µg.mL^{-1} and 2100 µg.mL^{-1}, respectively, showing that there are differences in the results. due to the treatment carried out on the sample before extraction. Therefore, the evaluated essential oil has a greater capacity to reduce the ABTS radical than those compared, since at lower IC_{50} values, greater antioxidant capacity. Therefore, the essential oil of *C. longa* grown in Manabí has a high potential as an antioxidant agent, being able to be used in foods with a significant content of lipids, which due to oxidation generate an unpleasant taste and odor; also in the beverage industry, for functional beverages, cereals, snacks, as a fish preservative and also as an active component in packaging (Hashemi *et al*., 2017; Bhavaniramya *et al*., 2019).

DPPH radical inhibition activity

Figure 2 shows the percentages of inhibition of the DPPH radical by action of the essential oil of *C. longa* in a concentration range of 500-2500 mg.L^{-1}, reaching an inhibition of the radical between 34.305 % and 51.630 % in the concentration highest.

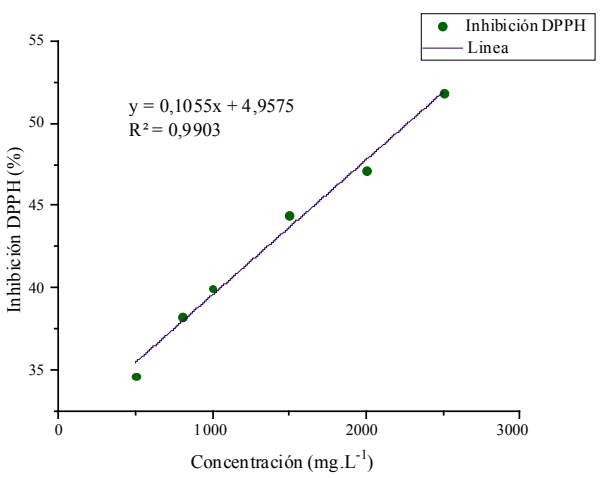

Figure 2: Inhibition of the radical DPPH by action of turmeric essential oil.

The Trolox equivalent value (TEAC) of the essential oil of *C. longa* is 10.86 µmol.g^{-1} sample and the inhibition coefficient for the radical DPPH is 2274.024 µg.mL^{-1} compared to that obtained with the ABTS radical, it is observed that approximately five (5) times more essential oil is needed to be able to inhibit the radical by 50 %, so in the evaluated samples the amount of compounds capable of stabilizing the DPPH radical are very low, this is because the ABTS method evaluates both lipophilic and hydrophilic compounds and the DPPH method only evaluates lipophilic compounds, so it can be deduced that the amount of lipophilic compounds in the essential oil of *C. longa* L. are less than the amount of hydrophilic type compounds. This value is similar to that obtained by Li & Li (2009), which was 28421.83 µg.mL^{-1}. On the other hand, the essential oil of *C. longa* extracted from species cultivated in the Ecuadorian Amazon has a lower antioxidant activity than the species cultivated in the province of Manabí - Ecuador, as observed in the study carried out by Pino *et al*. (2018), who obtained an IC_{50} of 14500 µg.mL^{-1} for the one grown in the Ecuadorian Amazon.

***Curcuma longa* L. essential oil toxicity**

The selected bioassay showed sensitivity of *E. coli* to the essential oil of *C. longa*, since effects of the change from resazurin to resofurin were observed (figure 3), translated into a statistically significant inhibition of bacterial growth for the essential oil ($p < 0.001$). The dose-response model (figure 4) presented a regression coefficient of 0.99.

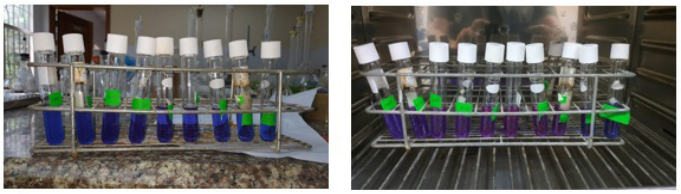

Figure 3. Inhibition of *Escherichia coli* growth measured through change from resazurin (blue) to resofurin (pink) a) t = 0 h, b); t = 5 h.

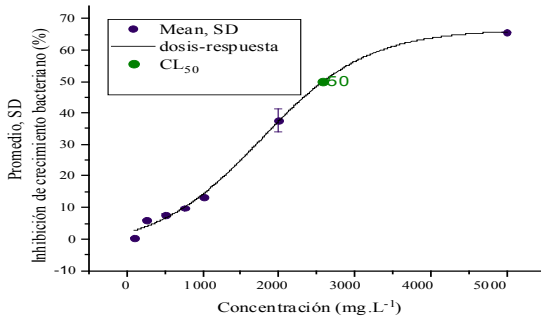

Figure 4. Dose-effect relationship of the concentration of essential oil of *Curcuma longa* L. in the growth of *Escherichia coli*.

Considering that the inhibition of resazurin reduction indicates a deterioration of cellular metabolism (Dayeh *et al*., 2004) and based on the statistical evaluation of the dose-response relationship, it was determined that the mean lethal concentration (LD_{50}) of turmeric essential oil for *E. coli* it is 2585.69 mg.L^{-1}, a value that reflects a relatively low toxicological response for the microorganism evaluated, since high concentrations are required to show an inhibitory response of 50 %. This result coincides with those reported by Khattak *et al*. (2005), for ethanolic extracts of *C. longa*, in which the mean lethal concentration to inhibit the growth of *Artemia salina* was 33 mg.L^{-1}, and *E. coli* in an incubation time of 24 h showed no inhibition in the growth of it. It is necessary to mention that the test described in the present investigation corresponds to a method that assesses toxicity in significantly smaller units of time, than those established in the tests carried out by other authors and although bacteria such as *E. coli* turn out to be

a good biosensor for toxicity (Charrier *et al*., 2011) these types of results are not decisive for the toxicological evaluation of products intended for human consumption. Although it is appropriate to indicate that the description of the results regarding the toxicity of the essential oil of *C. longa* coincide with those established in other investigations where the test model (subject) is different (Funk *et al*., 2010; Vijayasteltar *et al*. , 2013). It is also appropriate to highlight that the acute administration of turmeric essential oil at a dose of up to five (5) g of EA per kilogram of weight in rats did not represent adverse clinical signs or metabolic changes (Vijayasteltar *et al*., 2013).

Conclusions

The essential oil of *C. longa* has a phenolic content similar to other essential oils used in food formulations, it also has a high antioxidant activity against ABTS and DPPH radicals, which makes it a product of potential use for the food industry. Taking into account that the oil has a low toxicity compared to the test carried out, its application could be considered safe.

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