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EXTRACTION AND CHARACTERIZATION OF CURCUMIN FROM TURMERIC RHIZOMES GROWN IN MÉRIDA, VENEZUELA

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ABSTRACT

The extraction of naturally occurring compounds is one of the fastest-growing industries because of its benefits against its synthetic analogs. Environmental protection must require the use of natural products instead of chemicals to minimize pollution. Thus, this investigation studies the use of some natural product, as curcumin, as naturally occurring acid-base indicators. Curcumin can be used as acid-base indicators since it was found that it possesses pH-dependent solubility. Curcumin, the major active component of turmeric, *Curcuma longa* (Zingiberaceae), is used as a spice in curry and as a coloring agent in yellow mustards, cosmetics, pharmaceuticals, and hair dyes. In this research, the main compound colored rhizome of turmeric (*Curcuma longa*) cultivated in Mérida, Venezuela, is extracted: Curcumin ($C_{21}H_{20}O_6$) (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, in a yield of 3.42% after 8 hours of extraction using soxhlet extractor system with organic solvents (hexane and ethanol). The thin-layer chromatography and column performed separation and purification using a mobile phase, a mixture of chloroform-hexane 3:2. The dye was characterized by spectroscopic analysis of visible ultraviolet (UV-Vis) and infrared (IR), in addition to his studio in steering sensitivity as an acid-base indicator. This dye is useful as an acid-base indicator in strong acid-strong base volumes and did not require large amounts of it as it has high sensitivity. The results indicate that curcumin as an acid-base indicator allows the development of new standards in different chemistry fields that require this type of analysis.

Keywords: *Curcuma longa, curcumin, acid-base indicator, soxhlet extraction, chromatography*.

1. INTRODUCTION:

Of the existing acid-base indicators used in laboratories, the so-called synthetic indicators stand out. These are products whose synthesis and manufacture have a high cost, and their implementation in jobs that involve monitoring the acidity or basicity of a system is simpler and faster (Soltan and Sirry, 2002; Sabnis, 2007; Singh *et al*., 2011). However, synthetic indicators are not available to all interested persons in conducting studies and experiments. On the other hand, are natural indicators whose home collection is quite economical and uses fairly simple extraction methods (Esatbeyoglu *et al*., 2012).

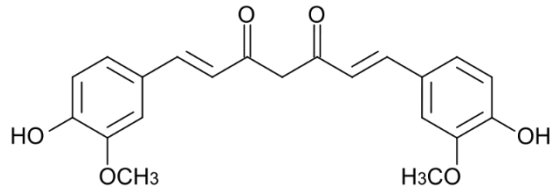
Curcumin, (1E, 6E) -1,7-bis (4-hydroxy-3-methoxy phenyl) -1,6-heptadiene-3,5-dione ($C_{21}H_{20}O_6$), is a natural dye from turmeric (*Curcuma longa*), a herbaceous plant of the family *Zingiberaceae* native to southwestern India. *Curcuma longa* is used as a spice in curry and as coloring agent in yellow mustards, cosmetics, pharmaceuticals, and hair dyes (Ammon and Wahl, 1991; Toda *et al*., 1985; Surh, 2003). It has attracted interest because of its antioxidant, anti-inflammatory, and potential anti-cancer activities (Aggarwal *et al*., 2003; Anand *et al*., 2007; Chattopadhyay *et al*., 2004; Mishra and Palanivelu, 2008; Sharma *et al*., 2005). Besides, curcumin has also been found to bind to β-amyloid proteins in models of Alzheimer's disease (Yang *et al*., 2005).

This dye has an intense yellow color and is extracted from turmeric roots and stems (Wickenberg *et al*., 2010). There are at least two forms of curcumin, both tautomers: the keto form and the enol form. The keto form is in liquid form, while the enol form is solid (Wickenberg *et al*., 2010). Both tautomeric forms of curcumin are shown in Figure 1. This molecule can lose both hydrogens from either of the two -OH groups and the hydrogen located between the two ketones in a basic medium. Loss of any of these hydrogens occurs at pH 8-9 (Heredia, 2006).

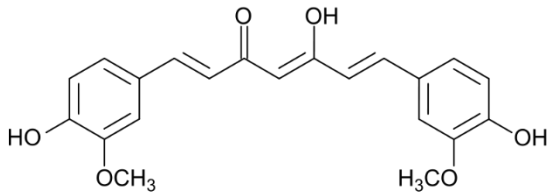
The active ingredient in turmeric is the

polyphenol curcumin (responsible for its yellow color), also known as C.I. 75300, or natural yellow 3 (Breedlove, 1995; Carvalho *et al*., 2002; Priyadarsini, 2014). It is used in food as a yellow dye (E-100i) or a flavoring agent, although it is also marketed for boron detection and as a pH indicator (Wickenberg *et al*., 2010). Among the main physicochemical characteristics that curcumin presents are: an orange-yellowish color, specific gravity: 0.935, the melting point for the keto form: 183 ºC. However, the boiling point for the enol form: decomposes, and density 0.93 g/mL. (Hatcher *et al*., 2008) Curcumin has solubilities in ethanol, chloroform, acetone, glacial acetic acid, alkalis, and ether (Rios *et al*., 2009). On the other hand, some researches have focused on curcumin, as acid-base indicator (Ma *et al*., 2017). This dye is a lipophilic phytochemical that has been found to possess pH-dependent solubility (Wang *et al*., 2019).

This work presents the extraction and purification of curcumin from the rhizomes of *Curcuma Longa* cultivated in Mérida, Venezuela. It is characterized by IR and UV-vis spectroscopic methods. The acid-base indicator character of curcumin is analyzed through the variation of the wavelength (λ) as a function of pH.



keto



enol

Figure 1. *Keto and enol structure of curcumin*

2. MATERIALS AND METHODS

2.1. Obtaining the turmeric rhizomes

The turmeric rhizomes of orange, fleshy, ovate, and piriform color come from the town of Lagunillas, Sucre Municipality of the Mérida state; This population is located at 1070 meters above sea level with an average annual temperature of 23 ºC. A total of 1 Kg of turmeric rhizomes were collected for the study.

2.2. Preparation of the turmeric pulp

The rhizomes were subjected to washing, water and saturated NaCl solution, and chipping process, cutting them into small parts and placing them in a plastic container; the weight of the wet turmeric pulp was 717.0 g (Analytical Balance ± 0.001g, model: AR3130). It was then placed in an oven at 65 ºC (Brand: JP Selecta) for 4 hours, obtaining a final dry weight of 192.0 g. The dried turmeric was ground employing a grain mill (Corona), and 10 g was taken that was then passed through a degreasing process using three 20 mL aliquots of hexane (Fisher Scientific 99.8%) for 8 hours.

2.3. Coloring extraction

The extraction process was carried out for 7 hours using a Soxhlet extraction equipment (Brand: Klax.sa) of 250 mL capacity and 125 mL of ethanol (Reiedel de Haen, 95%) solvent. The orange extract was separated from the solvent in a rotary evaporator (Brand: Heidolph) at 60 °C, for 48 h.

2.4 Separation and purification of curcumin

It was performed by column chromatography, using a vacuum system (brand: Kimax) using Chloroform: Hexane 3:2 as eluent. The column was prepared in a 50 mL capacity burette (brand: Kimax ± 0.1mL) with WN-3 type aluminum oxide: neutral (Sigma-Aldrich). The head of the column was made with 0.5 g of the extract in 2.5 g of alumina. The purity of the separated curcumin was checked by thin-layer chromatography (TLC).

2.5. Colorimetric pH scale

250 mL of sodium hydroxide (Reidel de Haen 99%) 0.105 M were prepared to weigh 1.05 g of the base by dissolving them in distilled water ASTM D1193 (ASTM, 2011). On the other hand, 250 mL of 0.1 M hydrochloric acid (Riedel de Haen 37%) was prepared, dissolving 2.1 mL of it in distilled water ASTM D1193 (ASTM, 2011). The indicator solution was prepared by weighing 7.0 mg of the purified curcumin in 15 mL of 95% ethanol. The proportions of the prepared solutions are shown in Table 6. A pH meter (Model Crison pH Meter Basic 20ºC UPV) was used, and the results are shown in Table 6.

2.6. Characterization and quantification

FTIR spectroscopy: a Perkin-Elmer RX1 spectrometer, model 1605, was used for spectroscopic analysis using KBr pellets. UV-Vis

Absorption Spectroscopy: the solution was prepared in absolute ethanol (Riedel de Haen, reagent grade), scanned from 800 to 200 nm in a Shimazdu UV-mini 1240 spectrophotometer in a 1 cm quartz cell.

3. RESULTS AND DISCUSSION:

Pure curcumin was obtained by slow evaporation of the solvent, being a rather viscous and oily yellow liquid (Figures 2a and 2b), corresponding to the enol form of the compound of interest. Among the most important characteristic that could be obtained is that, as reported in previous studies, curcumin presented a decomposition reaction at temperatures greater than 80 ºC, as well as a color change in basic medium to intense red. The amount of curcumin obtained is expressed in Table 1.

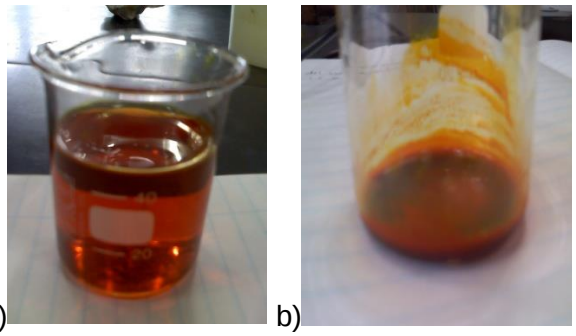


Figure 2. *Photograph of the turmeric extract, (a) dissolution in 95% ethanol. (b) concentrated extract*

Through the analyzes carried out on the substrate of the turmeric rhizomes, the percentage of humidity of these was determined compared to previous studies carried out in Quindio, Colombia, (Rios *et al*., 2009) in which the variation of results based on the difference in altitude, soil characteristics of cultivation and climatological characteristics of each region that consequently alter the chemical compositions in the rhizomes shown in Table 2.

A thin layer chromatography (TLC) was performed of the extract obtained from the turmeric pigments, from the Soxhlet extraction in 95% ethanol, using chloroform as eluent observing three separate spots (Figure 3a); Knowing that the curcumin molecule has a low polarity, it could be assumed that it is the signal with the highest Rf. However, it was not possible to compare it with a standard sample as a reference.

The mixture of pigments contained in the extract was separated by column chromatography (Figure 3b). There was a good separation of the pigments, the yellow pigment corresponding to curcumin coming out first (Figure 3c), showing high purity. This being verified by a thin layer chromatography whose Rf value resulted in 0.85 (Table 3).

The yellow fraction could not be recrystallized but, by slow evaporation of the solvent, a rather viscous and oily yellow liquid was obtained (Figure 3c), corresponding to the enol form of curcumin. Among the most important characteristic that could be obtained is that, as reported in previous studies, curcumin presented a decomposition reaction at temperatures greater than 80ºC, as well as a color change in basic medium to intense red. The amount of curcumin obtained is expressed in Table 4.

3.1. Spectroscopic analysis

The UV-vis spectrum of curcumin in the enol form presented a maximum wavelength λmax in absolute EtOH equal to 426.0 nm with an absorbance of 0.236 (dimensionless) (Figure 4). It was caused by the conjugation of the pi bonds as chromophores in the molecule. This type of transition of medium energy involved for this wavelength is π → π *, observing the yellow color as a complementary color, the wavelength is within the range of 420.0-430.0 nm of the chromatic circle. Likewise, the displacement value of λmax agrees with the data reported for curcumin (Rios *et al*., 2009).

On the other hand, it can be observed that in an aqueous medium, the solution with the curcumin indicator turns bright yellow, occurring transitions of the type n→π* and π→π*. For this spectrum, there is a wavelength λmax equal to 426.0 nm and absorbance of 0.2332, seeing yellow as a complementary color (Figure 5). Something similar happens with the spectrum in an acid medium (Figure 6), with the difference of a slight bathochromic shift in wavelength at 432.0 nm and an increase in absorbance at 0.7028, known as hyperchromic shift because, in the solution in Acidic medium, turbidity is formed which causes the visible radiation that crosses the cell or optical path to be absorbed with greater intensity. In this way, the species that predominate in the aqueous medium is the keto-enolic form, according to the reaction shown in Figure 7.

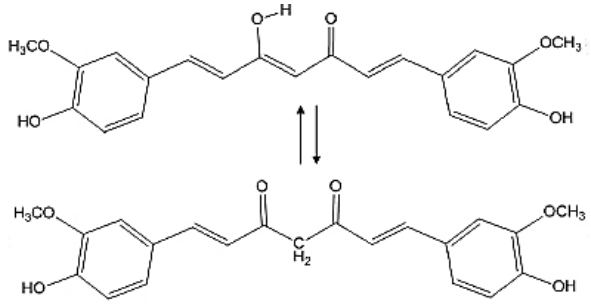


Figure 7. *Keto-enol form of curcumin in the aqueous medium*

Table 5 expresses the wavelength variation λmax concerning pH on the colorimetric scale.

However, in a basic medium, there is a wavelength λmax whose value is 461.0 bathochromic shift towards the lower energy red and absorbance of 0.4070, observing the red color as a complementary color with transitions of the type π→π* (Figure 9), predominating the molecular species shown below in Figure 8. It should be noted that these values agree very well with the values calculated theoretically for the transitions n → π * and π → π * of 483 and 343 nm, respectively (Rios *et al*., 2009).

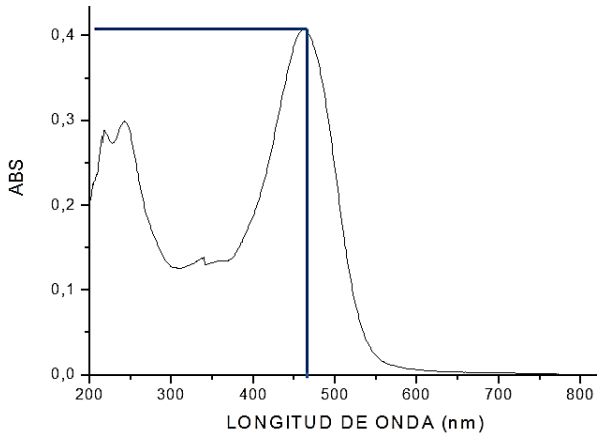


Figure 9. *UV-vis spectrum of curcumin in basic medium*

Concerning the characterization by FTIR spectroscopy of the purified curcumin (Figure 10), the main bands of the functional groups that coincide with the structure of curcumin in enol form are observed, identifying the strong tension signal OH at 3432 cm^{-1}, the voltage signal of the C=O link at 1650 cm^{-1} of medium intensity. This unusual intensity of this band is due to the delocalization of charge motivated by keto-enolic tautomerism that the curcumin molecule presents (Figure 1). Finally, bands of weak intensity are also observed between 2000 and 1900 cm^{-1} corresponding to conjugated C=C double bonds in the molecular skeleton.

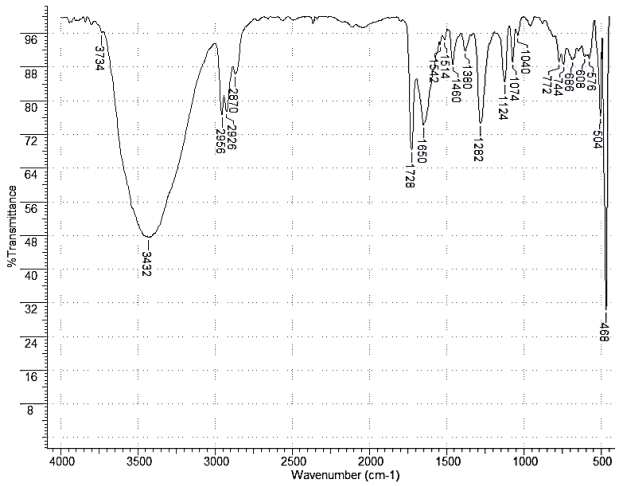


Figure 10. *FTIR spectrum for purified curcumin in liquid (enol form)*

The colorimetric pH scale was carried out to know the pH range of the change, being useful between pH 6-8. Table 6 shows the proportions of the solutions of the colorimetric scale with their pH value.

4. CONCLUSIONS:

It was possible to extract curcumin from the turmeric rhizomes through a simple method with a yield close to that reported in the bibliography and of high purity, verified by spectroscopic and chromatographic methods. Depending on the region where the crop is located, the concentration of curcumin can vary as well as the moisture content. This dye was useful as an acid-base indicator in strong acid-strong base volumes and did not require large amounts of it as it has high sensitivity summarize the data discussed in the Results and Discussion showing the relevance of the work and how different it is from others researches. Also, point out the benefits and improvements that can be observed to develop new science standards that can change something in the related field.

5. ACKNOWLEDGMENTS

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6. REFERENCES:

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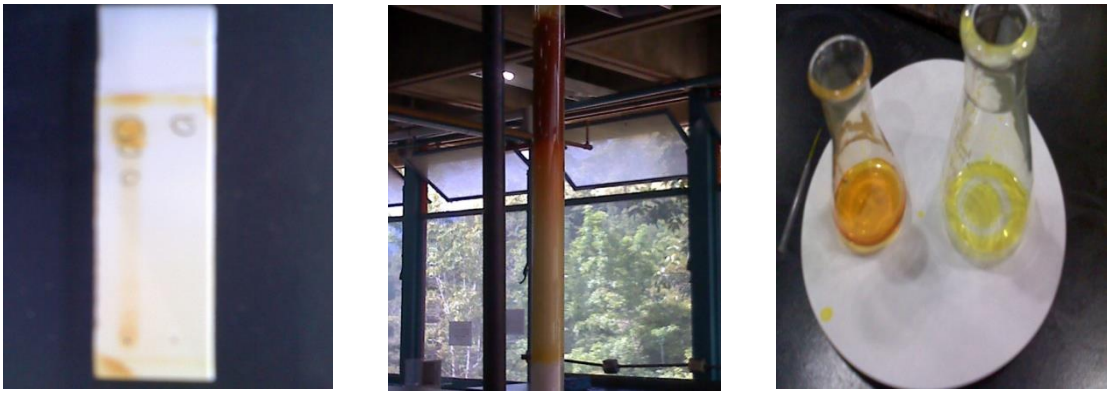

(a) (b) (c)

Figure 3. *Photographs of separation and chromatographic purification. (a) Thin-layer chromatography of pure curcumin (application 2). (b) Separation of pigments in column (c) Pigments obtained*

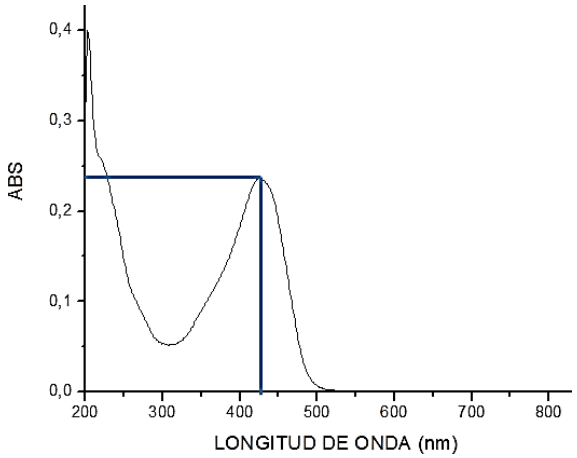

Figure 4. *UV-Vis spectrum of curcumin in absolute ethanol*

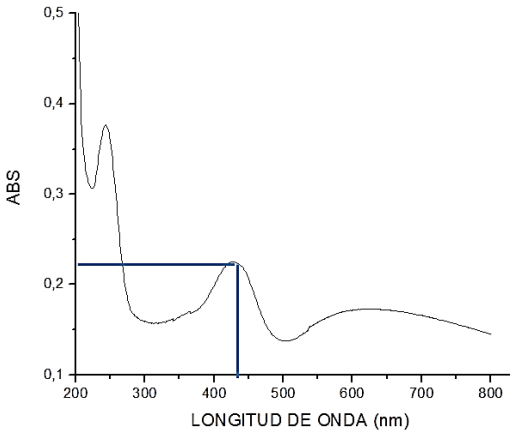

Figure 5*. UV-vis spectrum of curcumin in the aqueous medium*

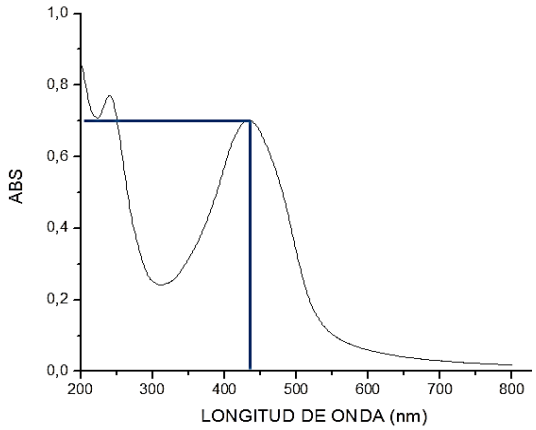

Figure 6. UV-vis spectrum of curcumin in acid medium

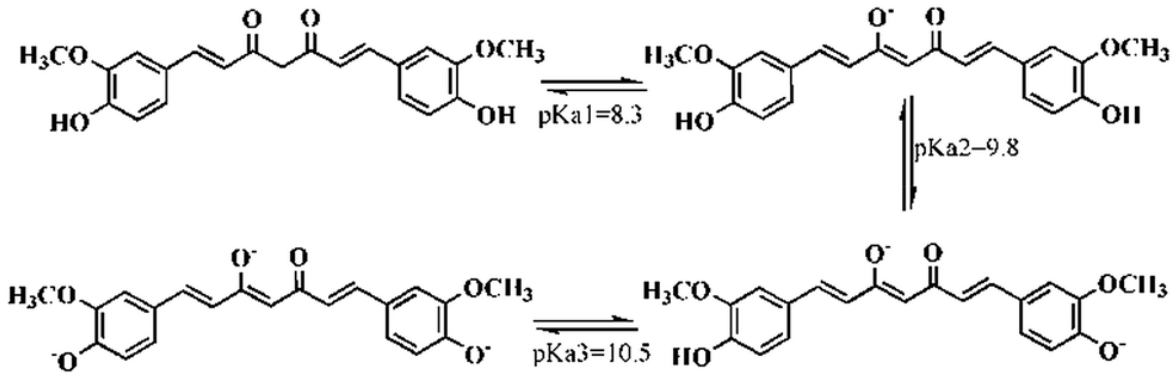

Figure 8. *Mechanism of deprotonation of curcumin in basic medium*

Table 1. *The average amount of extract and curcumin obtained during the extraction processes with Soxhlet (ethanol).*

m (flour subjected to extraction) (g)	m (extract) (g)	extraction yield (%)	m (curcumin) (g)	curcumin yield (%)
9.8 ± 1.6	0.9± 0.1	8.9 ± 0.7	0.030 ± 0.006	3.4±0.3

Table 2. *Determination of relative humidity and percentage of the fatty extract of the turmeric rhizomes studied and compared with a crop from another region*

Region	Altitude (masl)	Annual average temperature (ºC)	Initial substrate mass (g)	Dry substrate mass (g)	% of relative humidity (g)	% of fatty extract
Mérida-Venezuela	1070	21-23	716.975	192.035	72.9 ± 1.3	6.8 ± 0.8
Quindio-Colombia	900-4750	18-21	NR	NR	84.7 ± 3.0	0.05 ± 0.02

Table 3. *Thin-layer chromatography performed on the extract of turmeric pigments*

Aplication	signal	signal height (cm)	solvent front (cm)	Rf
1	A	2.3	4.0	0.57
	B	3.0		0.75
	C	3.4		0.85

Table 4. *Amount of extract and curcumin obtained during the extraction processes with Soxhlet (ethanol)*

Experiment	m_{flour} subjected to extraction (g)	$m_{extract}$ (g)	extraction Yield (%)*	$m_{Curcumin}$ (g)	curcumin Yield (%)**
Exp. 1	10.0	1.1	10.7	0.039	3.7
Exp. 2	10.0	0.8	8.4	0.026	3.1
Exp. 3	9.3	0.7	7.6	0.024	3.5
Average	9.8 ± 1,6	0.9± 0.1	8.9 ± 0.7	0.030 ± 0,006	3.4±0.3

* The extraction yield was determined with the mass of the oily extract of the pigment mixture concerning the initial mass of the dry turmeric. ** The yield was calculated considering the mass of the oil obtained concerning the concentrated extract of the pigment mixture.

Table 5. *Variation of wavelength and absorbance with the change of pH*

Medium	wavelength λ_{max}(nm)	Absorbance	Color	Medium
	631.0	0.1732	Yellow	
aqueous	426.0	0.2253	Yellow	aqueous
	243.0	0.3765	Yellow	
acid	432.0	0.7028	Yellow	acid
	240.0	0.7708	Yellow	
	461.0	0.4070	Red	
basic	243.0	0.2996	Red	basic
	219.0	0.2883	Red	
	631.0	0.1732	Yellow	
aqueous	426.0	0.2253	Yellow	aqueous
	243.0	0.3765	Yellow	

***Table 6**. Variation of pH as a function of the variation of the acid and base proportions of the colorimetric scale*

Solution	**$V_{NaOH\ 0.1M}$ (mL)**	**$V_{HCl\ 0.1M}$ (mL)**	**number of indicator drops**	**pH**	**Solution color**
1	0	10	10	1.07	Yellow
2	1	9	10	1.78	Yellow
3	2	8	10	2.01	Yellow
4	3	7	10	2.66	Yellow
5	4	6	10	3.17	Yellow
6	5	5	10	6.52	Orange
7	6	4	10	10.66	Red
8	7	3	10	11.40	Red
9	8	2	10	11.54	Red
10	9	1	10	11.88	Red
11	10	0	10	12.59	Red