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Comparative Studies of the Antioxidant Activities of Different Extracts Obtained From Curcumina Powder at Different Particle Size Classes and From Cumin Powder (95%)

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ABSTRACT

Curcumin is the main active ingredient and the source of most of curcumin's therapeutic power. This molecule has multiple medicinal properties, therefore an essential ingredient in the design of products intended for both consumption and therapy. Curcumin is the subject of many scientific studies and most have attributed anti-carcinogenic and antioxidant qualities to it. It turns out that there is indeed a correlation between antioxidant activity, the presence of phenolic compounds, and pharmacological properties. The object of this study is therefore determined among eight extracts prepared from different fractions of turmeric powder (having different grain sizes), the one having the antioxidant properties (%IP or Ic50), the highest cumin composition and the most interesting total phenolic compounds. The Cs2 is therefore the one with the highest % IP.

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KEYWORDS:
curcumina; polyphenols; antioxidant propreties; cumin; curcumin; HPLC-MS

I. INTRODUCTION

Since ancient times, many civilizations have used medicinal plants to heal themselves, and at least 70 percent of existing medicines are derived from plants. Therefore, several studies are conducted on plants intrinsically regarding their healing effect on the human body and animals. Plants were considered indisputable curative means -natural remedies for humans and animals. Researchers are looking for their biological bio-properties (antioxidants, antimicrobials, bioavailability, possible interactions, adverse effects, their pharmacological uses or their efficacy and safety of use both in animals or humans. The latter fundamental property of polyphenolic compounds in plants which have pro-health activities (antimutagenic, anticarcinogenic, and anti-aging) (Muanda et al., 2023). Polyphenols have the ability to scavenge reactive oxygen (ROS), preserving the genomic stability of cells through elimination of carcinogens and interference with DNA adduct formation. Antioxidants can protect biological systems against potentially harmful effects. Then, when the production balance between ROS and defense systems is disrupted, oxidative stress sets in, promoting many diseas such as cancer, atherosclerosis and other cardiovascular disorders, diabetes, accelerated aging….

Turmeric is the native of tropical South Asia, as a dried rhizome of an herbaceous plant, tumeric is closely related to ginger. The spice sis also sometimes called “Indian Saffron” thanks to its yello color. The turmeric is a spice that comes from the root *curcumalonga L*, a member of the giger family (Zingaberaceae). Its bright yellow pigment is used as a food coloring agent. It has been used for centuries as a spice and a food preservative and for its various medicinal proprieties. That plant is one of the many plants with multiple health benefits that nature provides to humans. Besides its use as a condiment or pigment, turmeric is a source of curcumin, a molecule that is used for its multiple medicinal uses in India (Curcuma longa) for centuries. Curcumin has anti-inflammatory and anti-carcinogenic. These different properties have attracted the interest of scientists for the prevention and treatment of various diseases. Curcumin is the main natural polyphenol found in rhizome of Curcuma longa (turmeric), having antioxidant properties, anti-inflammatory,

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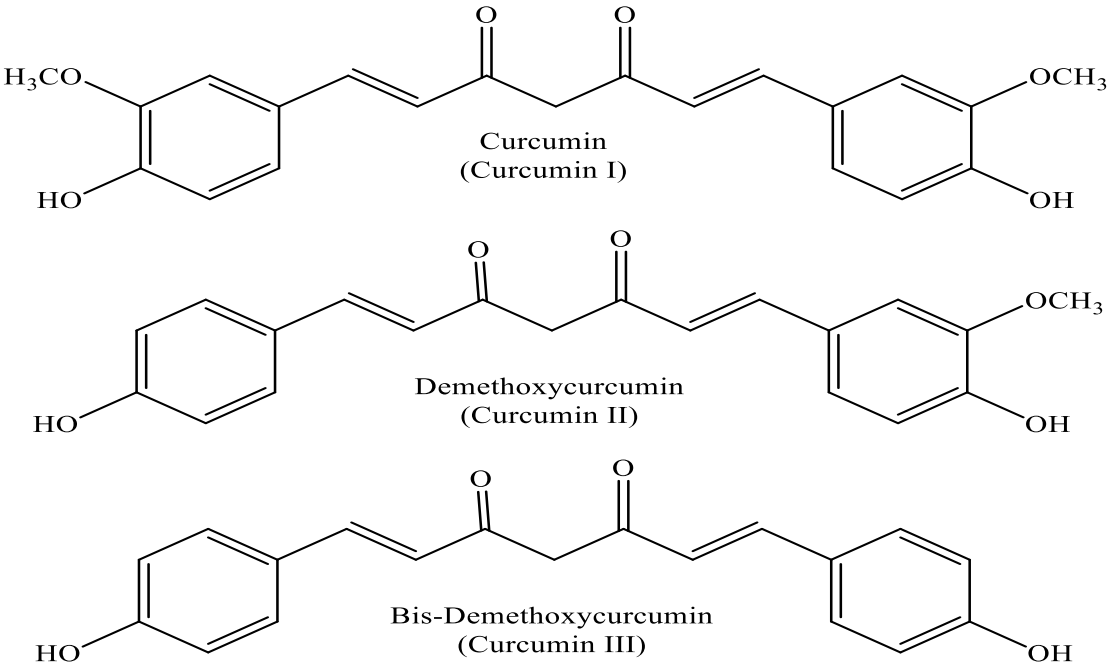
anti-mutagenic, antimicrobial, anti-parasitic and anti-cancer properties. Curcumine is a liposoluble compound and can be easily dissolved into organic solvent such as methanol, ethanol, and acetone. However, poor water solubility often limits its biomedical uses aqueous systems. Curcumin's basic coloring substance in Curcuma longa and two related compounds, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), are all known as curcuminoid (Tønnesen, H.H., Karlsen, J.,1985; Chih-Hung H., Ann-Lii C., 2007; Aria L.A.D. L., Gunawan I., 2014; Kavirayani I., 2019).



Scientific research spanning over more than four decades has confirmed the diverse pharmacological effects of cucumin and established its ability to act as a chemopreventive agent as well as a potential therapeutic agent against diseases (Mario P.M., et al., 2016; Kavirayani I., 2019; Camila F. et al., 2020). This observation prompted us to examine turmeric extracts as a curcumin delivery system and to investigate the possibility that turmeric extract itself is a candidate agent for pharmacological evaluation.

The objectives of this study are to evaluate the antioxidant activities, to determine the total polyphenol compounds of these different curcumin powders. Establish a correlation between the antioxidant activity and the presence of total polyphenols, then compare the different bioactivities obtained with respect to the particle size of each sample and compare the results obtained with those obtained by the analysis of the standard sample (recognized for its beneficial effects on health).

2. MATERIALS AND METHODS

2.1. Apparatus

-*1. LC-ESI/MS analyses* were performed on a LC-MS 2020 system (Shimadzu, Tokyo, Japan) coupled with an electrospray ionization source (ESI). Separation was performed on a Gemini 3 µm C18 reversed phase column (Phenomenex, Torrance, CA, USA) of 150 mm length and 4.6 mm i.d.

-*2. UV–visible analyses* were carried out with an UV–visible spectrophotometer Analytikjena Specord 205 (Konrad-Zuse-Strasse 1 Jena, DE 07745).

2.2. Plants materials

Figure 1. Rhizome of Curcuma longa (turmeric)

Eight samples of curcumin powders were provided to us, all of these powders having different particle sizes, represented in Table 1, below (S1 to S8).

Table 1: Curcumin samples analyzed according to particle size

Curcumin sample	
Sample number	*Grain size (nm)*
CS0 (Standard)	*Nd*
CS1	*>160*
CS2	*>50-200*
CS3	*>200-2000*
CS4	*<300*
CS5	*300-500*
CS6	*500-1000*
CS7	*>1000*
CS8	*<50*

CS: curcumin sample, Np: not provided, *S1 to S8 (Sample 1 to sample 8)*

2.3. Chemicals

Chemicals Folin-Ciocalteu phenol reagent, gallic acid, sodium chloride, ethyl acetate, acetic acid, Folin-Ciocalteu's phenol reagent, aluminum chloride catechin, gallic acid, p-coumaric acid, coumarin, rutin, chlorogenic acid, vitamin acid, delphinidin, orietin, ellagic acid, l-cyanidin, ellagic acid, l-cyanidin were purchased from Across Organics. Sodium carbonate, sodium nitrite, chlorhydric acid, ethyl acetate, soduim sulfate anhydrous, ammonium phosphate, ferric ammonium sulfate, acetoninitrile, DPPH (2,2-diphenyl-1picrylhydrazyl) and curcumin (99%) were obtained from Sigma and Roth (France). The chemicals used were all of analytical grade.

2.4. Samples preparations

1. Phenolics compounds extraction for LC-ESI/MS analysis & UV–visible analyses

Several researchers have focused on the development of curcumin extraction methods, among which (Jayaprakasha G.K, et al., 2002; Su, Ke et al., 2023), for this study the extraction of polyphenols from turmeric powder were carried out according to the method of Muanda et al. (2023) with some modifications. 2 g of powder of were macerated during 24 h under stirring at 200 rpm in 10 mL methanol–water (70–30% (v/v)). Then, the methanol–water extract was centrifuged at 6000 rpm for 20 min, the supernatant layer was filtered, brought to 10 mL by adding methanol–water 70–

30% (v/v), and stored at 4 °C until analysis in a sealed dark brown vial.

2. *LC-ESI/MS analyses*

-Analytical conditions

The column oven was fixed at 30°C. The mobile phase used under isocratic conditions, was consisted by a mixture of 10% acetonitrile and 90% of a solution of 0.5% of formic acid in water. The flow rate was fixed at 0.5 ml/min and the injected volume was 20µl.

The ESI source was operated in negative mode. The nebulization gas flow was set at 1.5 L/min, the heat block temperature was fixed at 350 °C, and the desolvation line (DL) temperature at 250 °C. The probe voltage was set at −4500 V.

Compounds were quantified with an external calibration method. Before the analysis, the curcuma extracts were filtered and diluted ten times twice.

Data were expressed in milligrams of corresponding standards per gram of dry matter.

Method sensitivity was assessed by determining the limits of detection (LOD) and the limits of quantification (LOQ), defined as the concentrations leading to signal-to-noise (S/N) values of 3 and 10, respectively.

-Qualitative analyzes

The prepared extracts were analyzed on the LC/MS; the major peak reveals a m/z rapport [M-H] equal to 367. The retention times of the curcumin pure standards was 28.83 mn, were carried out under the same analysis conditions.

-Quantitative analyzes

Calibration method

Quantification of standards was performed using the TIC (Total Ion Chromatogram) in order to improve detection sensitivity in comparison with the full scan mode. The linear calibrations curves of curcumin were established in a range comprised between 1µg/ml to10µg/ml.

3. UV–visible spectrophotometric analyses

-Determination of the Total Phenolic Content

Total phenolic contents (TPC) were measured with the method reported by Muanda et al. (2023). 100 µL of samples were added to test tubes containing 3.16 mL of distilled water followed by addition of 900 µL Folin-Ciocalteu reagent (1 N) and 200 µL sodium carbonate (Na_2CO_3, 20%). Samples were thoroughly mixed and vortexed. After 40 min incubation at room temperature, the absorbance was measured at 725 nm.

For calibration curves, diluted solutions of gallic acid (1, 1.5, 2, 2.5, and 3 mg/mL) were used and total phenolic content was expressed in terms of equivalent amounts of gallic acid per gram of dry matter (GAE/g DM). All experiments were triplicated.

-Antioxidant activity

DPPH tests

The DPPH radical scavenging activity was evaluated according to the method described by (Sharififar et al. (2009); slightly modified. 2.9 mL of 100 µM DPPH solution in methanol was mixed with 1 mL Curcuma extract. The reaction mixture was incubated *in* the dark for 30 min and the optical density was recorded at 517 nm against the blank. For the control, 100 µl of DPPH solution in methanol was mixed with 2.9 mL methanol and optical density of solution was recorded after 30 min. The DPPH radical scavenging activity was expressed in terms of IP% and Ic50.

IP% = (A_0 - At)/$A_{0,IP}$*: inhibition percentage, A_0: absorbance of DPPH solution without sample at zero time, A_t: absorbance of DPPH solution with the sample at 30 minutes. I_C50 (Half percentage inhibition concentration).*

2.5. Statistical analyses

For all experiments, the average value and standard deviation from three replicates were calculated. Statistical analysis (ANOVA) was conducted using SAS statistical software (SAS Institute, NC) with $p < 0.05$ as significance level.

3. RESULTS AND DISCUSSION

1.LC –ESI/MS analyzes

-Identification of major bioactive compounds in Curcuma extract

-Qualitative analyzes

The curcumin was identified and quantified by LC-ESI/MS. The LC-ESI/MS analyses of some polyphenol standards (cumin 95 %) allowed us to determine the retention times (28,83 mn) and the rapport m/z ([M-H] = 367) as the ratios of curcumin compounds (fig.2).

Figure 2: HPLC-MS /Chromatograms of samples extracts

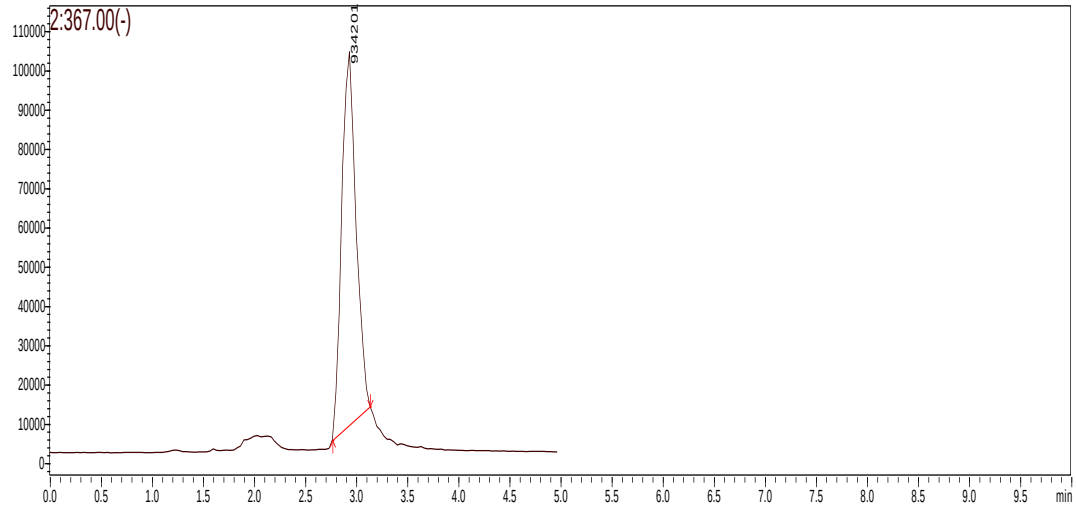


Fig.2.1: C_S1: sample extract (>160 nm)

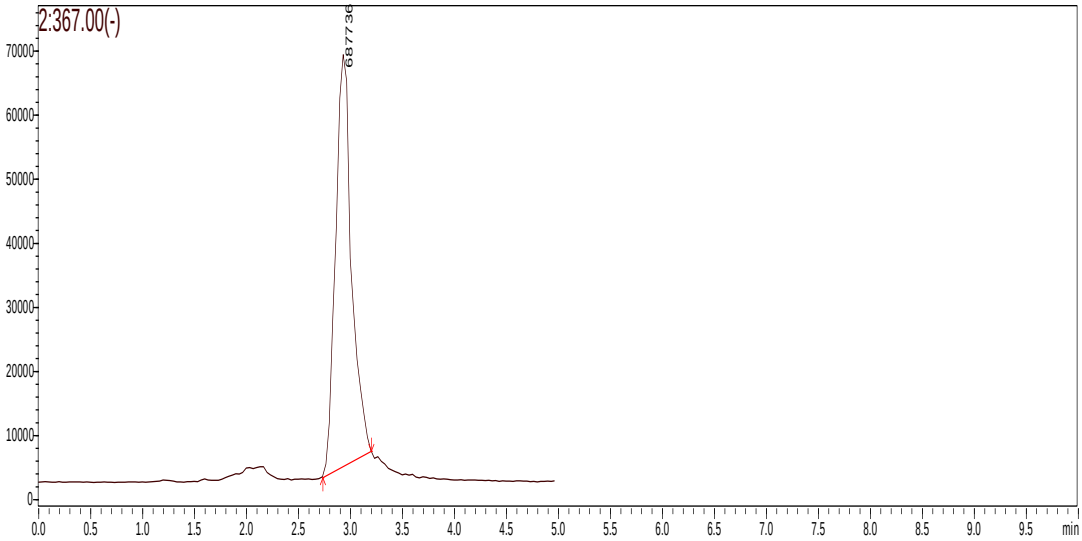


Fig.2.2: C_S2: sample extract > (50-200 nm)

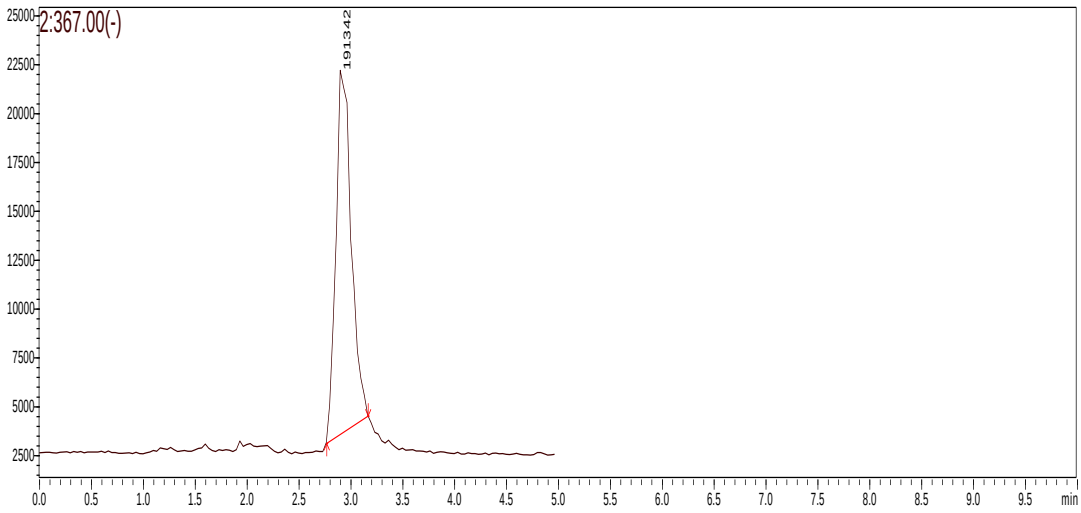


Fig2.3 C_S3:sample extract (>200-2000 nm)

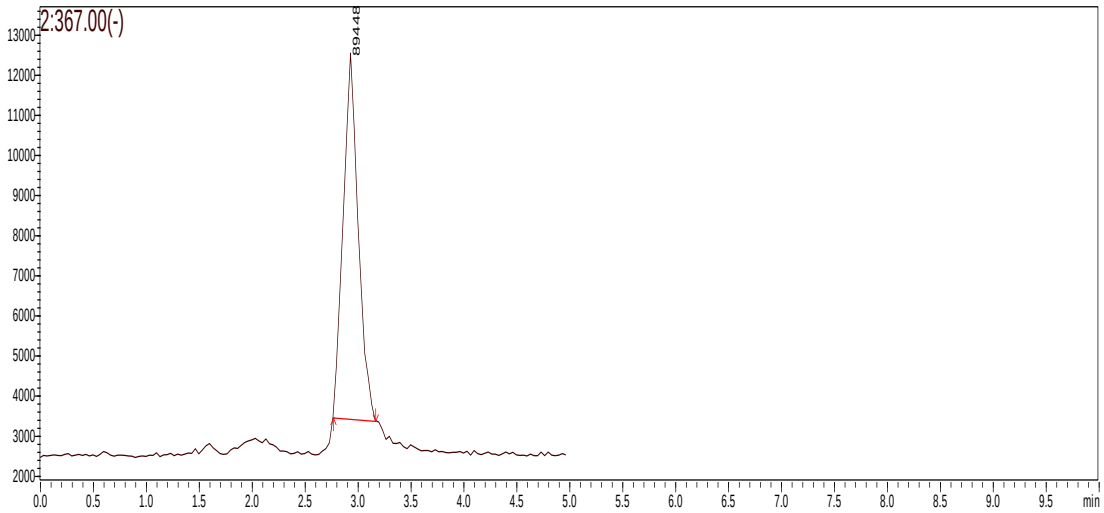


Fig.2.4 CS4:sample extract (<300 nm)

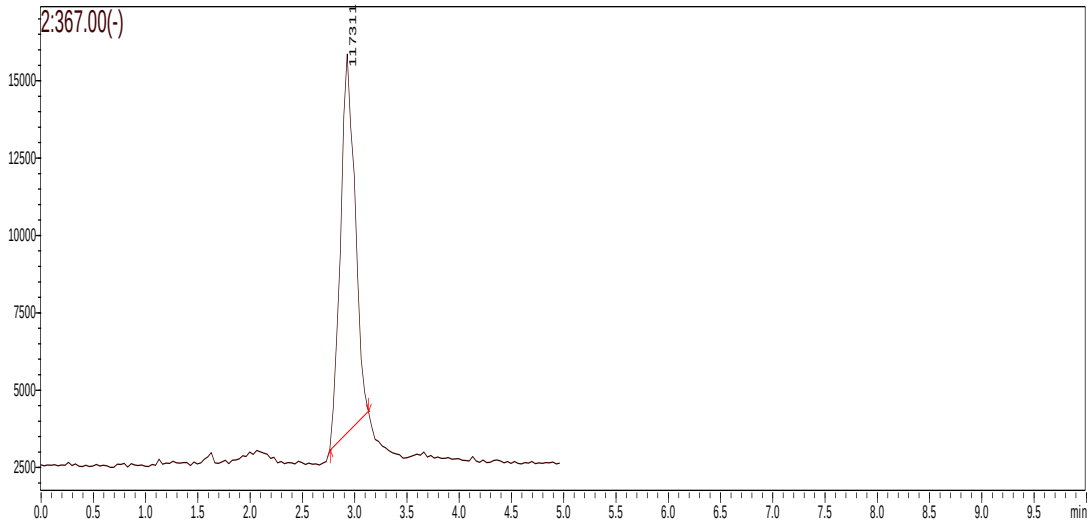


Fig.2.5.C_S5sample extract: (300-500 nm)

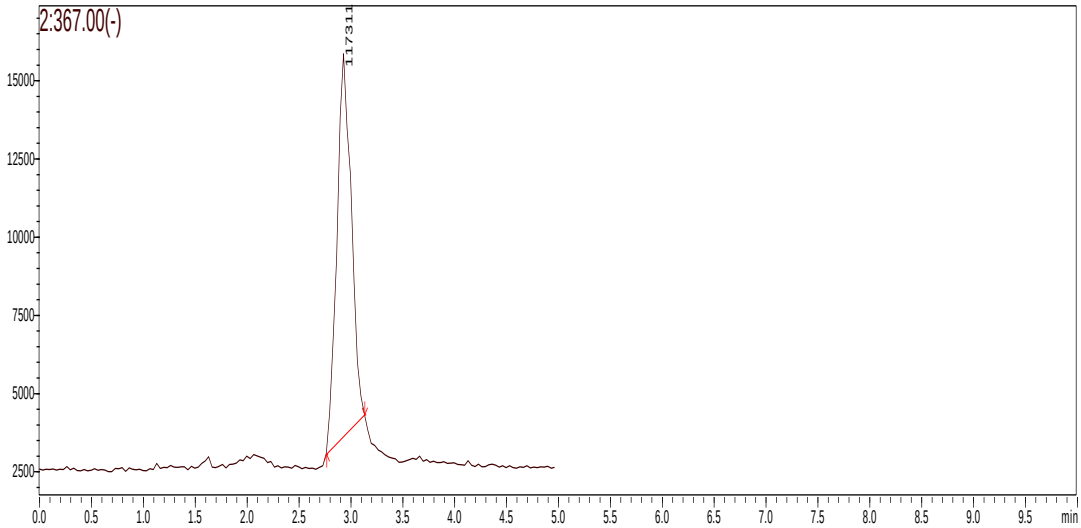


Fig.2.6. C_S6:sample extract (500-1000 nm)

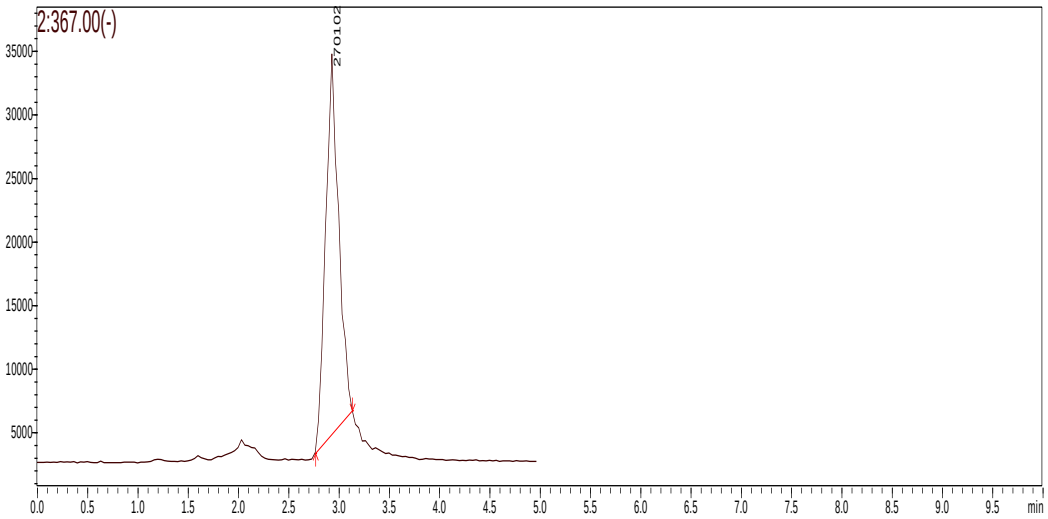


Fig.2.7.Cs7:sample extract >1000 nm

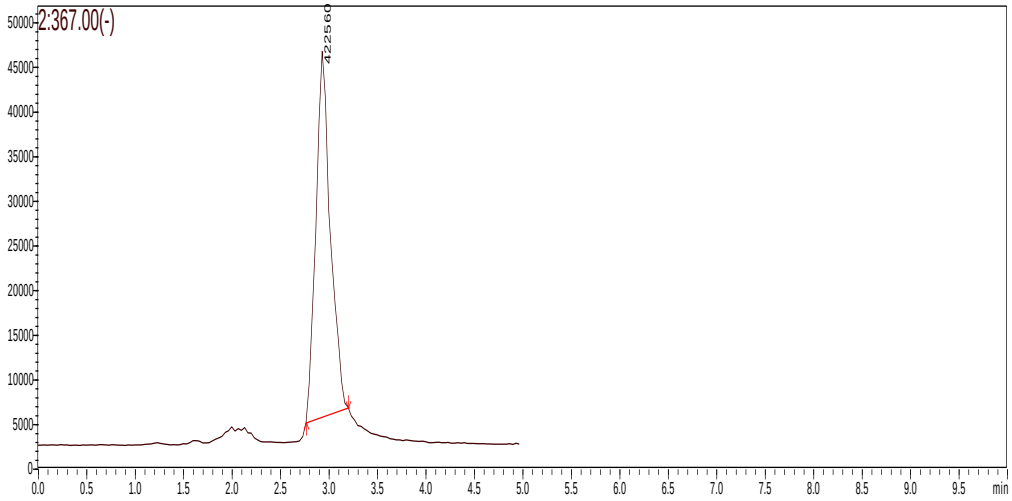


Fig.2.8.sample extract <50 nm

-Quantitative analyzes

For quantitative analyses, we determined the calibration curve from the standard (curcumin sample standard, CS0). The calibration line (y = 4675.8 x - 0.1096) gave a value of R^2 = 0.999. From these calibration curves, the CS0 contain 224 ± 0.15 µg/ml. The concentrations of the curcumin in the powder extracts were calculated from the calibration curve using the mean response values of three sequential injections. Thus, the curcumin extracts contained are represented in the table 2.

Table 2: Concentrations of the curcumin contained in the Curcumina extracts

Curcumin samples		DPPH	Cc	TPC (mg/ml)
		%IP	mg/gDM	
Cs8	<5 nm	90.17	10.05	77,4
Cs1	>160	89.93	21.70	59,3
Cs2	>50-200	92.36	16.30	55,6
Cs4	< 300	90.3	3.60	45,7
Cs5	300-500	89.42	3.20	45,8
Cs6	500-100	89.88	3.80	48,2
Cs7	>1000	86.47	7.20	65,7
Cs3	>200-2000	87.82	5.40	45,6
Cs0	Standard	56	2.50	37,6

Cs: Curcumin sample, IP: inhibition percentage, TPC: total phenolics compound, cc: concentration, mg/ml (milli gramme/ml); mg/gDM (milli gramme/gramme Dry mater)

The results of these analyses show that, curcumin in the curcuma extracts sampled were higher than in the standard sample (C_S0). The table 2 show that, the C_S1, C_S2, C_S8, with 21,7; 16,3 and 10,5 mg/g DM respectively have the highest values. But the standard (C_S0), with the C_S5 have the lowest with respectively 2,5 mg/g DM (Table2).

2. Bioactivity correlation

Starting from the particle size of the sample, (Cs2) with a particle size (>50-200) is the one with the highest %IP (92.16%), and has a concentration of curcumin and has a total composition of phenolic compounds (TPC) relatively high, i.e., 16.30 mg/g DM and 55.6 mg/ml. These results are comparable to those of Ali Zaiter et al., 2016; Mahta Mousavi et al., 2019. who worked on Salix alba and Prunus powders, these confirm that the particle size does have an impact on the powder antioxidant activity. By comparing the elution spectra of these different samples (Rt), we find that the retention time of curcumin is almost identical (Fig.2.1-2.8).

The values of R^2, (0.96) and (0.82) show that there is indeed a correlation between the presence of phenolic compounds and the concentration of curcumin in the sample (% IP/TPC) and (% IP/Cc) fig.3.1-3.2.

4. CONCLUSION

Several studies have shown pharmacological properties related to the use of turmeric roots, for this study it was a question of studying the different fractions of turmeric with different grain sizes and to study its pharmacological effects (related to the antioxidant properties of the presence of polyphenols). The emphasis was placed on the determination of the percentages of inhibitions and the indices of inhibitions (IC_{50}) of the different fractions, these percentages see IC_{50} was compared to the fraction of pure curcumin. It therefore turned out that the fraction containing the highest percentage of pure curmin is not the one which has the most interesting antioxidant activity (%IP). This study shows that, the Cs2 extract which particles sizes > 50-200, is the most interest because of his (%IP: 92.6); cumin concentration (16.30 mg/g DM) and 55.6 of (TPC (mg/ml)).

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Figure 3

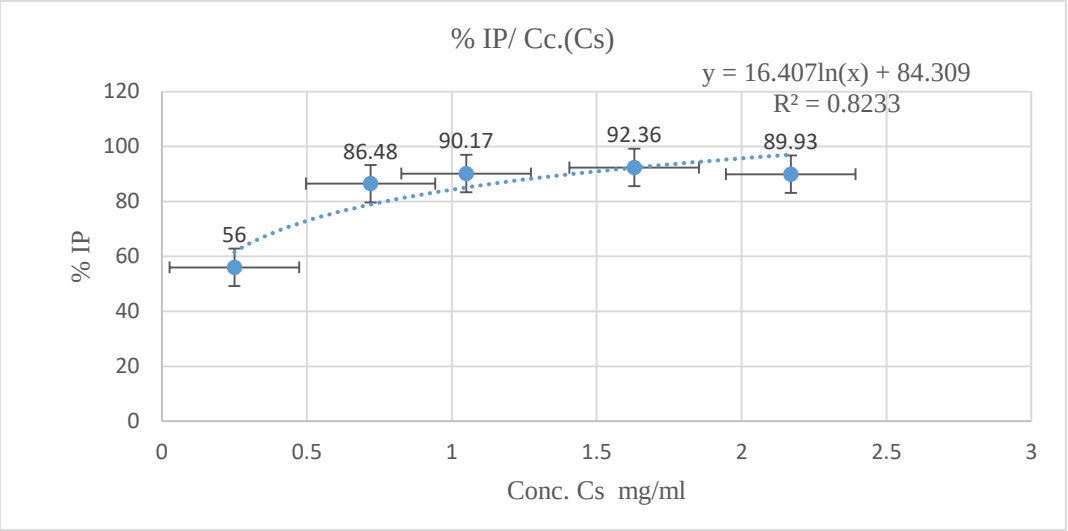

Fig.3.1. Correlation between % IP/ the concentration of sample

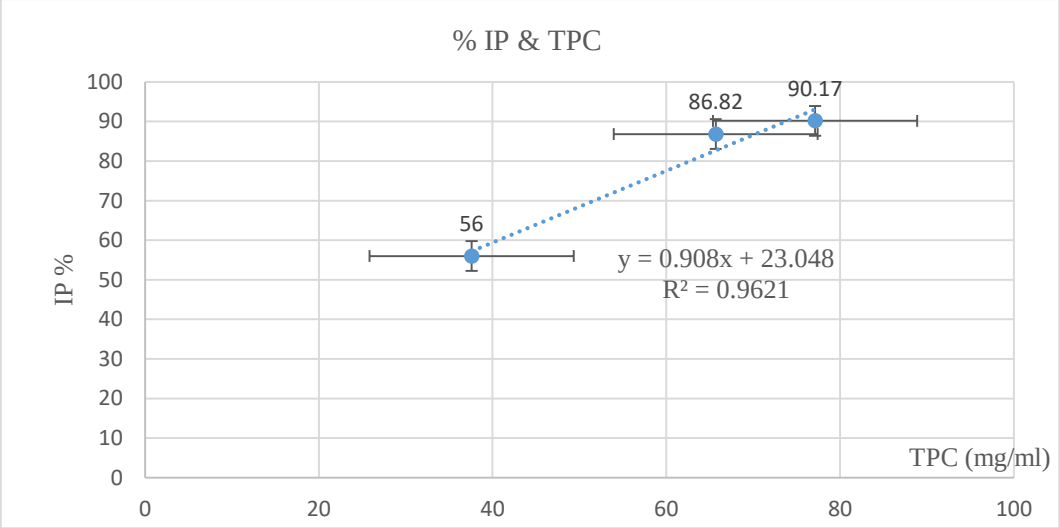

Fig.3.2. Correlation between % IP/ and TPC