

Extraction, Isolation, Characterization of Curcumin-I From Curcuminoids

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Abstract

The major goal of this study is to extract, isolate, and characterize curcumin-I, a component of curcuminoids. Turmeric, or *Curcuma longa* Linn., plays a significant role in Ayurvedic medicine. Turmeric, or *Curcuma longa* Linn, is the popular name for this plant. An array of chemicals with biological activity may be more easily extracted with the help of this seasoning. Some of the compounds at play include curcuminoids and turmerone, although this is by no means a full list. When curcumin I, II, and III are combined, a process takes place that creates curcuminoids. Curcumin is a powerful anti-inflammatory, anti-HIV, anti-tumor, anti-viral, anti-cancer, anti-fungal, and anti-parasitic chemical. Some more uses are: It has also been hypothesised that this molecule has anti-parasitic and anti-fungal effects. The fact that it could potentially prevent tumour, viral, and parasite development is just frosting on the cake in terms of its therapeutic potential. In order to collect the data necessary for this study, an extraction of ground turmeric using the Soxhlet technique was performed. Separating curcumin-I from the other synthetic curcuminoids was crucial to achieving this aim. Scientists were able to effectively isolate and purify the molecule using crystallisation and chromatography. The best strategy was crystallisation.

Keywords: *Curcuma longa*, Curcuminoids, TLC, U.V., Mass, NMR

INTRODUCTION

Zingiberaceae family contains the important medicinal plant known as turmeric (*Curcuma Longa* Linn). Turmeric is native to India. Turmeric is regarded as a functional food due to the positive benefits it has on one's health [1]. In addition, turmeric has the ability to provide a distinct flavour to

whatever it is combined with and maintain that flavour for an extended amount of time after the dish has been cooked. Phenolic compounds known as curcuminoids are obtained from the dark yellow spice known as *Curcuma Longa* [2]. The three primary diarylheptanoids that may be discovered in curcuminoids are referred to as curcumin (diferuloylmethane), demethoxycurcumin (phydro xycinnamoylferuloylmethane), and bisdemethox ycurcumin (di-phydroxycinnamoylmethane). It has been shown that the natural antioxidant curcumin lowers cytotoxicity and the risk of developing cancer [3]. In addition to its efficacy against the human immunodeficiency virus, curcumin has properties that make it beneficial as an antimicrobial agent, an anti-inflammatory agent, an antibacterial agent, and astringent [4]. In this study, an attempt is made to isolate and identify the many types of curcuminoids that are found in turmeric.

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MATERIALS AND METHODS

In addition to the standard curcuminoid purchased from Yucca Enterprises Ltd. in Mumbai, India, all other chemicals and solvents used were procured from a local vendor and were of analytical grade.

Extraction

Fifty grammes of dried, ground turmeric powder was added to the thimble-sized Soxhlet extractor. To guarantee that all curcuminoids were extracted, the process took place in methanol (150 ml) for 6 hours. To obtain the supersaturated curcuminoid solution, methanol was recovered using a rotary evaporator. After chilling this solution to 4°C while stirring constantly, the orange-yellow curcuminoids were filtered out [5–7].

TLC Analysis

Preparative thin layer chromatography was used for the separation of extracted curcuminoids. A 20×20 inch glass plate covered with silica gel-G was utilised. At a predetermined height above the base, a dot of the curcuminoid concoction was painted and left to dry (Figure 1). Table 1 displays the optimal solvent system compositions that were found. The development chamber was saturated with dichloromethane: methanol (99.5:0.5) mobile phase for 20 minutes (Figure 2 & Table 2) [8].



Figure 1. TLC of isolated and standard curcuminoids.



Figure 2. Preparatory separation of Curcuminoid mixture.

Table 1. R_f values and absorbance of isolated compounds

S.N..	Spots	Isolated		Standard	
		R_f value	Absorbance	R_f value	Absorbance
01	A	0.48	418.50	0.47	429
02	B	0.22	412.42	0.20	424
03	C	0.10	399.08	0.10	419

Table 2. Composition and optimization of solvent system

S.N.	Solvents systems	Saturation time	Observation
1	Dichloromethane : methanol (60:40)	20 min	Single spot with tailing
2	Dichloromethane : methanol (70:30)	20 min	Single spot with tailing
3	Dichloromethane : methanol (80:20)	20 min	Single spot with tailing
4	Dichloromethane : methanol (90:10)	20 min	Single spot with tailing
5	Dichloromethane : methanol (95:5)	20 min	Two spots with tailing
6	Dichloromethane : methanol (99:1)	20 min	3 spots with tailing
7	Dichloromethane : methanol (99.5:0.5)	20 min	3 distinct spots

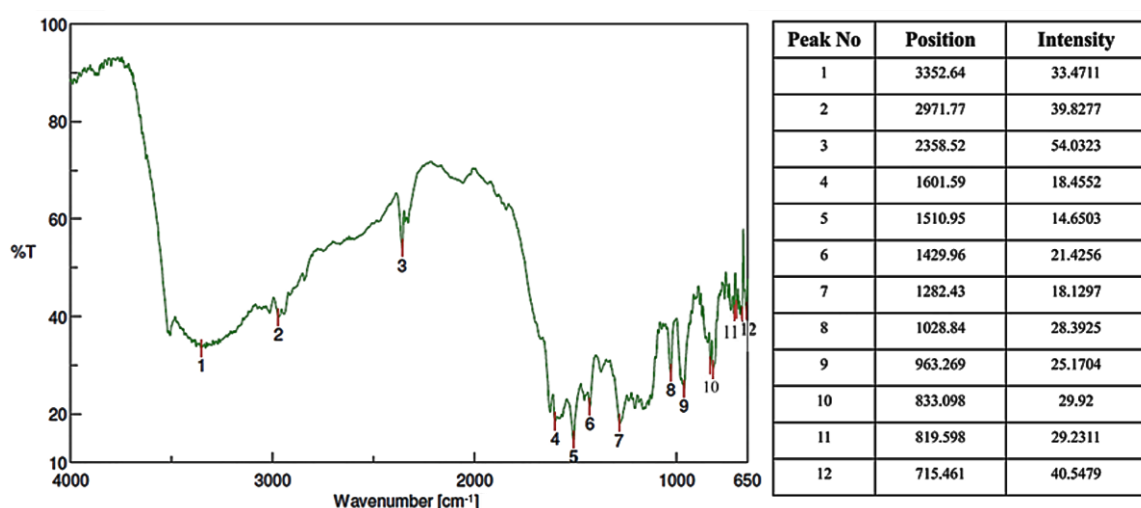


Figure 3. I.R. Spectra of isolated Curcumin.

I.R. Spectroscopy

The moisture was removed from the separated component A and the Potassium Bromide (KBr) by placing them in an oven at 105 ± 5 °C for 1.5 hours. A Hydraulic Pellet Machine was used to compress the sample with KBr into a solid disc or pellet at a 1:300 ratio. Table 3 and Figure 3 show the results of an FTIR analysis of pellets, in which the results were compared to previously published spectra [9].

Table 3. The frequencies of functional groups of isolated curcumin

S.N.	Isolated Curcumin	
	Frequency cm^{-1}	Functional group
1	3352.64	O-H stretch (ali)
2	2971.77	=C-H stretch(ali.)
3	2358.52	=C-H stretch (aro.)
4	1601.59	C=C stretch (aro.)
5	1510.95	C=C (aro.)
6	1429.96	C=C (aro.)
7	1282.43	C=O
8	1028.84	C-O stretch
9	963.269	C-H bend

S.N.	Isolated Curcumin	
	Frequency cm^{-1}	Functional group
10	833.098	C-H bend
11	819.598	C-H bend
12	715.461	C-H bend

*ali = Aliphatic != Aromatic

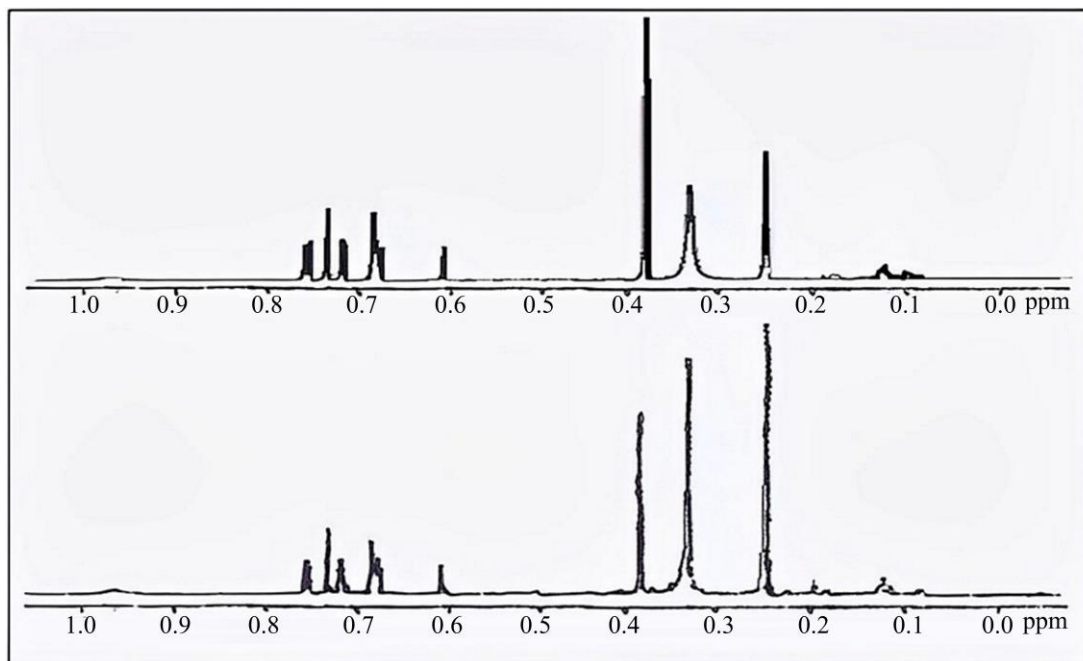


Figure 4. NMR spectra of standard (A) and isolated (B) sample (Curcumin)

Table 4. Assignment of peaks of NMR spectra of standard and isolated sample

S.N.	Standard		Isolated	
	Assignment	PPM	Assignment	PPM
1	m,2H	10.04-9.45	m,2H	9.66
2	d,2H	7.74-7.51	d,2H	7.56
3	m,2H	7.41-7.31	m,2H	7.32
4	d,2H	7.30-7.08	d,2H	7.16
5	d,2H	6.91-6.81	d,2H	6.83
6	d,2H	6.80-6.71	d,2H	6.78
7	m,1H	6.18-6.00	m,1H	6.05
8	m,6H	4.02-3.75	m,6H	3.83

NMR Spectroscopy

NMR spectroscopy was performed on compound A, and the results were compared to the published spectrum (see Figure 4 and Table 4).

Mass Spectrometry

Table 5 shows the settings of the mass spectrometer used to calculate the mass spectrum of the isolated sample, which was then compared with the mass spectrum published by [10], Which showed a mass peak at 368 m/z (53.7% R.I.). Curcumin (M-, 53.81%) is found with a mass of 368.8 (relative intensity) on the mass spectrometer.

Mass spectrum m/z (rel intensity): Curcumin 368.8 (M-, 53.81%)

Table 5. Mass spectroscopy parameters

S.N.	Parameter	Specification
1.	Make	Waters (Switzerland)
2.	Model no.	Quadrupole
3.	Ionization source	Electron spray ionization

RESULT AND DISCUSSION:

Curcumin, a kind of polyphenol, is responsible for turmeric's medical and culinary benefits. Turmeric is also used in traditional Indian medicine. The rhizome of the turmeric plant is what gives the spice its characteristic bright yellow colour, and for centuries, it has been used in the traditional medical practises of both China and India. It has been suggested that curcumin may decrease inflammation in both acute and chronic forms [11], which is one of its many purported benefits. Researchers were able to determine the generation of curcuminoids by drying and weighing the various components (Figure 2) [12]. The absorbance of the spots that were scraped and collected from the plate was measured using a UV spectrophotometer, and the findings are presented in Table 2. To put it simply, curcumin inhibits the progression of cancer in a wide variety of places. Curcumin acts as both a suppressive agent, which inhibits the growth of malignant cells, and a blocking agent, which prevents the activation of carcinogens [13]. This allows curcumin to play a role in both the promotion and development of carcinogenesis. Curcumin activity has been proven to have radioprotective benefits in healthy cells and radiosensitizing effects in cancer cells. This phenomenon is noteworthy for the reasons that both of these effects have been shown. Curcumin is hypothesised to have pleiotropic effects due to the complex chemical composition it has as well as its capacity to activate a number of different signalling pathways. It is necessary for chemical producers to get curcumin I in its pure form from turmeric powder in order for them to characterise and synthesise it in sufficient quantities to meet the high demand. Curcumin I is effective in the treatment of a number of ailments. As a result, we focused on acquiring it in its most unadulterated form at the most reasonable price possible.

We used preliminary thin layer chromatographic procedures in addition to column chromatographic methods in order to separate the curcuminoids. We found that the latter procedure was both a lot easier to do and more effective than the former one when it came to separating curcumin I from the other curcuminoids. This conclusion is consistent with the results made by Susanna and Zetterstrom.

After the TLC plate had been built, it was placed in the incubator to begin the process of extracting the curcuminoids. While the curcuminoids travelled 4.9 centimetres, 3.9 centimetres, and 3.2 centimetres, respectively, the solvent run reached a maximum of 6.6 centimetres. Spectroscopy techniques such as NMR, Mass, IR, UV, and IR were used to the investigation of the separated chemicals. The TLC analysis of the curcuminoids using dichloromethane and methanol as the solvent solution gave an R_f value for curcumin I of 0.48, which was higher than the R_f value of the referenced standard, which was 0.47. The mixture of dichloromethane and methanol in the proportion of 99:1 is the most efficient solvent solution for isolating curcuminoids from one another. Despite this, we were successful in optimising this system such that it could function with dichloromethane and methanol at a ratio of 99.5 to 0.5 with a saturation period of 20 minutes.

The most important ones are the O-H stretch, which was measured at 3410.06 and compared to the standard of 3417; the C-H stretch, which was measured at 2854.45 and compared to the standard of 2840; the C=O stretch, which was measured at 1714.60 and compared to the standard of 1720; and the C-C stretch, which was measured at 1466.16 and compared to the standard of 1473 [12, 13]. This reveals that the isolated sample had curcumin I-specific peaks that were comparable to the standard reference.

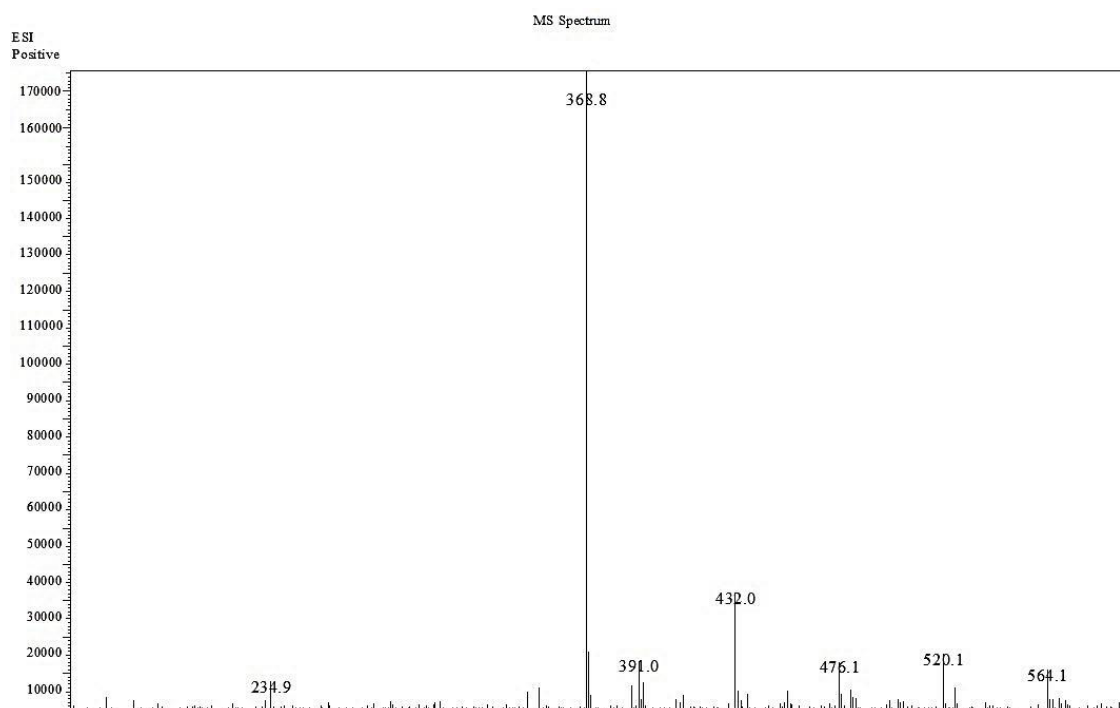


Figure 5. Mass spectra of isolated curcumin.

Spectral range of the NMR for purified curcumin I disclosed my results to the general public on the norm sample. The molecular ion peak of the reference standard is 368, however mass measurement of the pure curcumin found that it had a peak of 368 (Table 5 & Figure 5) [14].

CONCLUSION

The research elucidated a straightforward method for isolating pure curcumin I, which can then be put into an appropriate dose form. Further effort can be done to stabilise C-I, as it has been established through this study that it is photo instable. Since this substance is a dye, the solution containing it had to be filtered using centrifugation rather than filter paper.

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