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Extraction of Curcumin from Raw Turmeric (Curcuma longa.) – A Comparative Study, Using Soxhlet, Chemical, Chromatographic, and Spectroscopic Methods and Determining its Bioavailability

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***Abstract*: Turmeric (Curcuma longa) is a broadly grown perennial herb, and its organic residences are attributed to the content material of curcuminoids, which abundantly exist in Turmeric. Curcumin, is a crystalline polyphenol, which is extracted from turmeric, and contains various phytochemicals such as desmethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). In this study, specific types of samples of Curcuma longa species (Turmeric) have considered for the extraction of Curcumin, which has highly potential and established medical benefits. According to a human tissue-based angiogenesis, curcumin was found to be anti-angiogenic. This experiment is carried out to compare and investigate the best extraction strategy and the ideal sample of Turmeric which gives out immoderate and optimum yield. Soxhlet extraction technique is carried out to evaluate the results of those obtained from curcumin extraction with dissolving method with assistance of liquid chromatography. One of the influential solvents includes methanol for dilution and extraction procedure. HPLC is completed for the calculation of bioavailability of curcumin, UV- VIS spectroscopy is used for the determination of absorbance. While there are an extensive range of biological experiments about this study, the pharmacological and chemical aspects are comparatively scarce. With this comparative study, the average curcumin consumption for enhancement of everyday diet can be obtained.**

***Keywords*: Turmeric, Curcumin, Soxhlet method, Extraction, Chromatography, Spectroscopy, Bioavailability, Consumption.**

1. Introduction

Turmeric is an enduring and extensively used herb in all the tropical and subtropical regions and therefore India is an enormous producer, consumer, and exporter of curcuma longa across the world. It has huge scientifically proven benefits and medicinal properties such as antioxidant, anti-inflammatory, anti-viral and bacterial, antifungal cancer chemo preventive actions, etc. It is an important dietary source and is also used as traditional medicine and acts as natural antiseptic. It is a major food colouring agent mainly due to the yellow pigment present in curcuminoids. It is a natural disease biding medicinal plant which can be consciously added to everyday diet. Since turmeric contains highly beneficial curcuminoids, which is a natural substitute for anti-inflammatory drugs, cause dependencies and side effects. Turmeric's dietary additives performs an essential position in lowering blood strain and keeping balanced blood sugar levels. One of the most extensive benefits includes cholesterol regulation and it also helps in protective lining of the blood vessels and blood clotting. With the exception of all the physical health benefits, it also helps in maintaining mental stability. As proven, depression is an anti-inflammatory disease, and turmeric is an effective and valuable alternative. Turmeric has pure essential oils, contained with capable components which helps in boosting the mood and improve focus and promotes best sleep. In addition to this, its pure property of repelling viruses adds on to the cleansing benefits, both internally and externally to the body. It slows the histamine release in the body since it has immune boosting antibacterial quality. Brain health is vital and important as we age. This also has the benefit of supporting health of brain functioning. It delays neurodegenerative conditions in brain and plays extensive role in the improvement of cognitive functions and memory improvement. One of the major advantageousness of its component is the ability to restore skin cells. Clumping of the skin cells is prevented by turmeric and it also clogs the pores of skin which is essentially healthy to the skin. Various pores and skin irritations are irradiated because of its presence and it allows in removing the growth of acne causing bacteria in the skin and therefore providing a vibrancy and natural glow in the skin. Since it has many beneficial welfares such as safe ingestion, immune boosting ability, calming the mood and mental state, emotional balance, skin blemish remedy, internal health benefits, and other protective qualities, it is a common-sense step to include turmeric in our diet.

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2. Methods and Materials

A. Collection and sterilization of samples

Samples were collected from the plant species Curcuma longa. Fresh and dry samples were collected from different regions of Chennai. They were taken in a clean sterile bag and processed within few hours after sampling. The isolation work was done freshly to avoid contamination. The Curcumin longa samples collected in the sterile bags were rinsed in the running water in order to remove the dust and debris. The root and leaves part were cut carefully and separated from the wet sample. Highly aseptic condition was required for the isolation of the sample. Therefore, all the process was done in the laminar air flow chamber to prevent any contamination. Clean and aseptic glass wares and mechanical things, such as scalpels, blades, forceps, etc were used. The separated sample were then rinsed in sterile water for three times. Then they were blotted on the sterile blotting paper aseptically. Once the wet sample was free of moisture they were packed to sterile bags.

B. Extraction of the compound

Fresh aseptic raw sample were measured and 10g was taken for the preparation of sample. Once the grinding of sample was finished the grinded powder have been transferred withinside the beaker with 10ml methanol solution and dissolved well. After the sample was mixed with the methanol solution, they were sonicated for 10 minutes in ultra sonicator for adequate mixing. This sample extraction was stored in a beaker and was labelled as sample 1.

The preparation of sample 2 was done using Soxhlet extraction method. The dry sample of turmeric were first measured and 10.44g of sample weight was taken for sample. The turmeric was then kept in mortar and crushed partially using pestle. As for the Soxhlet extraction method, the solvent used was methanol. Methanol was used as a solvent for extraction because Curcumin is soluble in methanol solution. The solvent was added to the round bottom flask which was then attached to the Soxhlet extractor and condenser on a heating mantle. The solvent was filled about 75% of round bottom flask. Glass beads were added to the round bottom flask along with the solvent to stabilize the heat. The crushed turmeric sample was transferred in a filter paper and was loaded into the thimble placed inside the Soxhlet extractor. The Soxhlet extraction process heats the solvent to temperature below 60°C. The solvent heated through heating mantle gets evaporated and moves to the condenser through the apparatus. Then it dripped into the reservoir containing the thimble. Once the extent of solvent reached the siphon, it poured again into the flask and this becomes the first cycle. The Soxhlet extraction was done for 4hours till one cycle. Then the process was stopped and let to cool down. Then after some time the round bottom flask was removed from the Soxhlet apparatus setup. The solvent extraction was then transferred to a beaker. The solvent was cooled for some more time. Then the solvent extraction was concentrated using heating mantle leaving a small yield of extracted Curcumin. The method needs to be completed under a fume hood in case of escape. The extracted sample was labelled as sample 2. The concentrated Curcumin was added with 10ml of diluent or solvent mixture before running in chromatography.

The preparation of sample 3 was also done using Soxhlet extraction method. The raw turmeric was first measured and 10.19g of sample was taken for sample. Similar procedure is continued and Soxhlet extraction is done for the extraction of sample 3. It is followed by concentration. The extracted sample was labelled as sample 3. The concentrated Curcumin was added with 10ml of diluent or solvent mixture before running in chromatography.

C. Estimation of active content of curcumin

High performance liquid chromatography method was used for the estimation of active content of Curcumin present in the sample. The buffer solution was prepared by dissolving 3g of Citric acid in 300ml of water in a beaker. pH of the solution was adjusted using ammonia solution. For proper dissolving the buffer solution was sonicated in ultrasonicator for 5 minutes. The mobile phase solution was prepared by adding the prepared buffer solution with tetrahydrofuran. 100ml of mobile phase solution was prepared in proportion to 65ml buffer solution and 35ml of tetrahydrofuran. After dissolving the mixture of buffer solution and tetrahydrofuran it was transferred into mobile phase container. Methanol was used as solvent mixture and the mixture was dissolved completely. The solvent mixture is also known as the diluent and it was also used as the blank solution

Table 1
Chromatographic condition for curcumin in HPLC

1	Liquid chromatography system	Liquid chromatography equipped with injection, a variable UV wavelength detector and a suitable data collection system.
2	Column	C_{18}, 250 X 4mm, 5$\mu\ell$ or equivalent
3	Detection	UV 282nm
4	Flow rate	1.0 ml/min
5	Injection volume	20$\mu\ell$
6	Run time	10 minutes

Table 2
System suitability test

S.No.	Solution	No. of Injection	System suitability parameter	Acceptance criteria
1.	Specificity Blank	1	Baseline should be stable. Disregard any peak appearing in the chromatogram less than 0.05% of the size of the peak of interest in all subsequent chrromatograms.	Should be complies
2	Standard solution	1	The tailing factor of Curcumin	NMT 2.0
			Theoretical Plates	NLT 2000
			Tolerance for RT of peaks due to Curcumin	NMT± 2.0 minutes

for chromatography. The standard solution of Curcumin was prepared by adding 2.55mg of accurately weighed quantity of standard curcumin in form of powder in solvent mixture. The solution was made up to 25ml. The standard mixture was diluted completely to obtain a solution having a known concentration of about 0.1mg per ml. The prepared standard solution was then added in vial to run in chromatography for active content estimation. The chromatographic condition used to run the samples used in this study is given in the table 1.

The chromatographic system was equilibrated until a stable baseline was observed.

The three samples were made to run spontaneously in order sample 1,2,3 respectively. The chromatograms were recorded as stated.

The percentage of the assay was calculated using the formula mentioned below.

$$\% \text{ Assay} = \frac{U_r}{S_r} \times \frac{S_{wt}}{U_{wt}} \times \text{Potency of the standard}$$

Where,

U_r = Area of peak due to Curcumin obtained with the sample solution.

S_r = Mean peak area of Curcumin peak obtained with standard.

S_{wt} = Weight of working standard (standard solution) in mg/ml.

U_{wt} = Weight of test sample in mg/ml.

D. Spectroscopic analysis of curcumin samples

The three samples i.e., the dissolved extract, Soxhlet extracted (dry) sample and the Soxhlet extracted (wet) sample were analysed using UV-VIS spectroscopic method. The cuvette was first properly cleaned with ethanol to avoid contamination and errors in the data. First, the blank was filled in two cuvettes and then placed in the spectroscopy. They were made to run and the data was recorded. Then one of the cuvettes with blank was removed and was filled with the standard solution in 0.1 concentration. Once the peak of standard solution was observed, the cuvette was then emptied and then filled with the sample. The sample was filled in 0.1 concentration by diluting the samples in solvent mixture. All the three samples were made to run one after another. The peak formed was observed and the maximum absorbance was found out. The wavelength was 282 nm. By comparing the peak level of maximum absorbance of Curcumin standard solution, the maximum absorbance in sample with nearly similar to standard solution was concluded as the curcumin peak.

3. Results and Discussion

The results obtained are as discussed below.

A. Active content estimation by HPLC

During the process of chromatography, the peak was observed and the chromatogram was recorded for each of the samples. The active content of Curcumin in the sample was estimated by comparing the peak observed in the standard Curcumin sample along with the retention time.

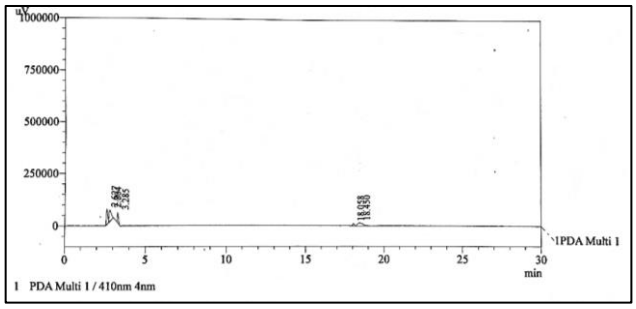

Fig. 1. Chromatogram of the blank solution

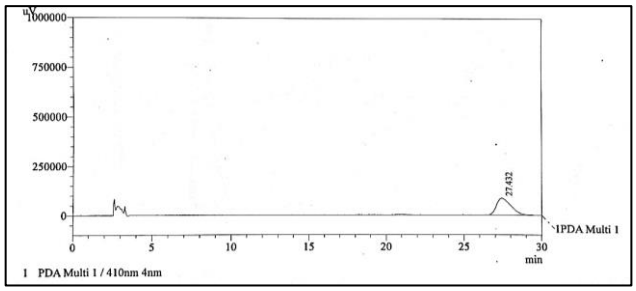

Fig. 2. Chromatogram of the standard curcumin solution

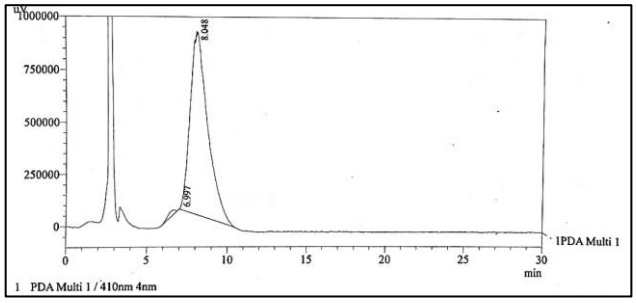

Fig. 3. Chromatogram of Sample 1

Table 4
Readings of the standard curcumin solution

	Retention time	Area	Height	Area %	Height %
Curcumin	27.432	5574165	86740	100.00	100.00
		5574165	86740	100.00	100.00

Table 3
Readings of the blank solution

Name	Retention time	Area	Height	Area %	Height %
RT 2.627	2.627	462060	72934	27.603	36.057
RT 2.804	2.804	520609	54576	31.101	26.616
RT 3.285	3.285	244687	49207	14.618	23.998
RT 18.058	18.058	89596	11802	5.352	5.756
RT 18.450	18.450	356975	15526	21.326	7.572
		1673927	205046	100.00	100.00

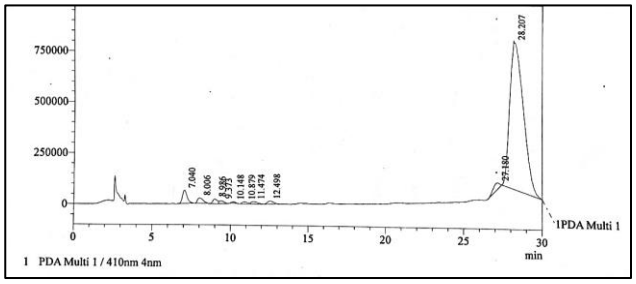

Fig. 4. Chromatogram of Sample 2

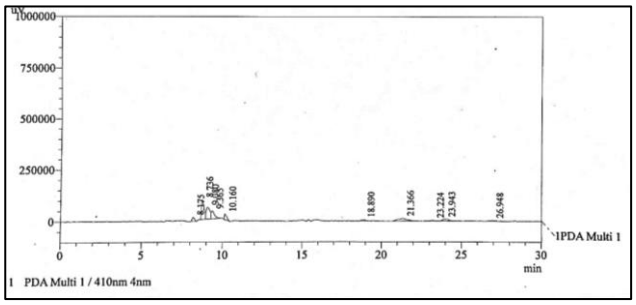

Fig. 5. Chromatogram of Sample 3

B. *Spectroscopic analysis of curcumin*

The maximum absorbance of the samples were checked using the UV VIS spectroscopy. The range used was 200nm-800nm. The peaks of all the sample solutions were observed and recorded. Referring to the peak formed in standard solution, other peaks of samples were compared and the maximum absorbance was concluded.

Peak threshold: 0.0100
Range: 800.00nm to 200.00 nm

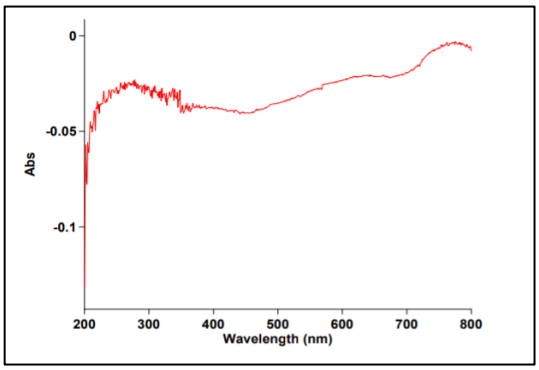

Fig. 6. Spectrum of blank solution

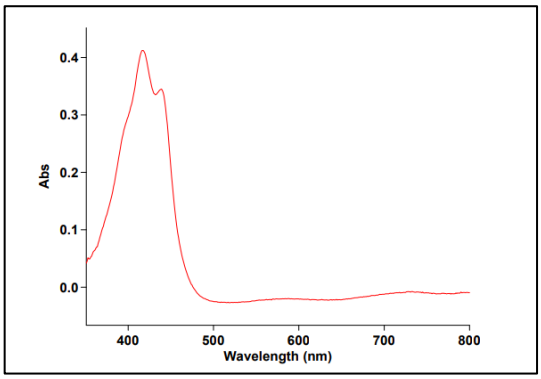

Fig. 7. Spectrum of standard solution

Table 5
Readings of the Sample 1

Name	Retention time	Area	Height	Area %	Height %
RT 3.052	6.997	889454	423	1.314	0.048
RT 3.466	8.048	666788586	877613	98.686	99.952
		67678040	878036	100.000	100.000

Table 6
Readings of the Sample 2

Name	Retention time	Area	Height	Area %	Height %
RT 7.040	7.040	1343602	64927	2.753	6.956
RT 8.006	8.006	672883	26888	1.379	2.881
RT 8.986	8.986	433934	22130	0.889	2.371
RT 9.373	9.373	305427	14105	0.626	1.511
RT 10.148	10.148	145533	8126	0.298	0.871
RT 10.879	10.879	234979	9979	0.481	1.069
RT 11.474	11.474	304020	12375	0.623	1.326
RT 12.498	12.498	424363	15245	0.869	1.633
RT 27.180	27.180	1021347	30238	2.093	3.239
Curcumin	28.207	43921979	729407	89.989	78.143
		48808066	933420	100.000	100.000

Table 7
Readings of the Sample 3

Name	Retention time	Area	Height	Area %	Height %
RT 8.175	8.175	193653	18809	4.735	6.884
RT 8.736	8.736	1061368	95381	25.950	35.908
RT 9.080	9.080	921984	57125	22.542	20.907
RT 9.365	9.365	549444	37510	13.434	13.728
RT 10.160	10.160	277266	27810	6.779	10.178
RT 18.890	18.890	101321	6384	2.477	2.337
RT 21.366	21.366	520833	13141	12.734	4.809
RT 23.224	23.224	64349	3678	1.573	1.346
RT 23.943	23.943	239350	8581	5.852	3.141
Curcumin	26.948	160437	4815	3.923	1.762
		4090005	273234	100.000	100.000

Table 8
Peak table of blank solution

Wavelength (nm)	Absorbance
278.00	-0.023
215.00	-0.039
202.00	-0.057

Table 9
Peak table of standard solution

Wavelength (nm)	Absorbance
417.00	0.413
343.00	0.059
299.00	0.057
253.00	0.089
207.00	0.261
205.00	0.261

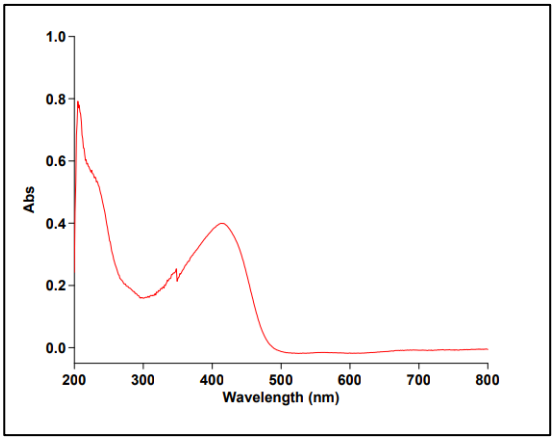

Fig. 8. Spectrum of Sample 1

Table 10
Peak table of Sample 1

Wavelength (nm)	Absorbance
413.00	0.400
348.00	0.254
205.00	0.792

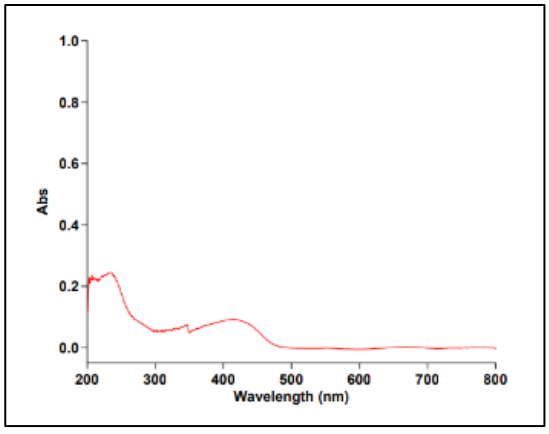

Fig. 9. Spectrum of Sample 2

Table 11
Peak table of Sample 2

Wavelength (nm)	Absorbance
415.00	0.093
347.00	0.076
234.00	0.245
207.00	0.235
203.00	0.228

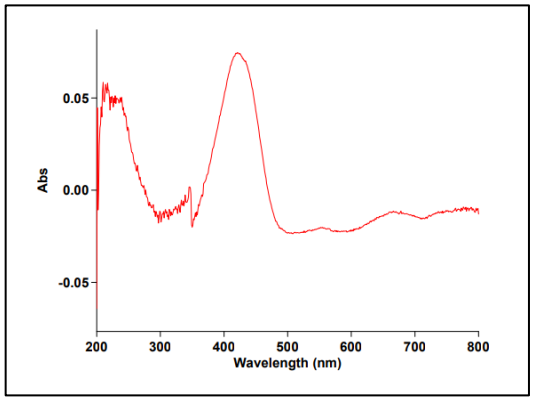

Fig. 10. Spectrum of Sample 3

Table 12
Peak table of Sample 3

Wavelength (nm)	Absorbance
421.00	0.075
346.00	0.002
217.00	0.058
210.00	0.058
201.00	0.045

Calculations:

The collected data obtained from chromatography was then used for calculation purpose in order to find out the percentage recovery of Curcumin that was extracted from the sample of Turmeric.

Formula:

$$\% \text{ Assay} = \frac{U_r}{S_r} \times \frac{S_{wt}}{U_{wt}} \times \text{Potency of the standard}$$

- Recovery percentage of Curcumin in sample 1 = 0 %
- Recovery percentage of Curcumin in sample 2 = 0.08 %
- Recovery percentage of Curcumin in sample 3 = 0.003%

4. Conclusion

As stated in the hypothesis, three different samples were collected and observed. After undergoing various processes such as extraction, HPLC and UV spectroscopy, it was found that out of three samples, sample 2 i.e., dry sample of turmeric has high content and recovery rate of curcumin when compare to other two samples. The curcumin which was extracted from the dry sample (Sample 2) was extracted using the Soxhlet extraction method, which is comparatively a better method and a better alternative than the chemical method according to the experimental data obtained.

As per the experimental studies, the intake of Curcumin in the form of Turmeric is 250mg per day at a maximum amount. Therefore, by following the required calculations it is concluded that we approximately take 30g - 32.46g of turmeric for obtaining 250mg Curcumin content. We require maximum amount of 500mg – 2000mg of turmeric content for daily intake diet and essentials. In case of separate intake of Curcumin

extract we can obtain 250mg from 33g of Turmeric. The required dosage amount can be adjusted according to the diseases and needs such as Osteoarthritis (400mg- 500mg), Cholesterol (700mg- 750mg), etc.

The experiments confirmed that, curcumin can be recovered from Dry turmeric using Soxhlet method, which is more convenient than other chemical methods, and the data obtained supports the hypothesis that comparatively, dry turmeric has more recovery rate of curcumin than powdered turmeric (sample 1) and raw/wet turmeric (sample 3).

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