ABSTRACT
Curcumin due to its various medicinal, biological, pharmacological activities is high on demand and has high market potential, high cost. Curcumin naturally occurs from rhizomes of Curcuma longa L. Zingiberaceae (Turmeric). Extraction is the first crucial step in preparation of curcumin formulations. This review article focuses on extraction of curcumin from turmeric using different techniques like, Soxhlet Extraction, Solvent Extraction, Maceration, Microwave Assisted Extraction, Sonication Extraction, Slurry Extraction and Refinement of Curcumin; further Isolation and purification and Identification of curcumin is also done.

KEYWORDS: Curcumin, Soxhlet extraction, Isolation, Identification.

INTRODUCTION
Turmeric (Curcuma Longa Linn) is an important medicinal plant which found throughout India. It is a member of Zingiberaceae family. Turmeric has been valued worldwide as a functional food because of its health promoting properties.[7] Turmeric is of special importance to humans with the discovery that its rhizomes preserves their freshness and imparts a characteristic flavors.[8] Curcuma species has a characteristic dark yellow color, and it has been found to be a rich source of phenolic compounds, viz. Curcuminoids.[9] Curcuminoids contain three different diarylheptanoids Curcumin (diferuloylmethane), Demethoxycurcumin (phdroxycinnamoylferuloylmethane), and Bisdemethoxycurcumin (di- phdroxycinnamoylmethane). Curcumin, which is the major constituent of curcuminoids, is reported to be a natural antioxidant with inhibition effects for cytotoxicity and cancer.[10]
addition, Curcuma species possess several other advantages such as the anti-inflammatory and anti-bacterial activities \[^{[11]}\] anti-human immunodeficiency virus \[^{[7]}\].

Turmeric was described as C. longa by Linnaeus and its taxonomic position is as follows:
Class: Liliopsida
Subclass: Commelinids
Order: Zingiberales
Family: Zingiberaceae
Genus: Curcuma
Species: Curcuma longa \[^{[13]}\]

**Chemical Composition of Turmeric**

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-pheollarene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%)\[^{5}\]. Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%)\[^{6}\]. Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated. Curcumin was first isolated in 1815 and its chemical structure was determined by Roughley and Whiting in 1973. It has a melting point at 176–177°C; forms a reddish-brown salt with alkali and is soluble in Ethanol, Alkali, Ketone, Acetic acid and Chloroform. \[^{[13]}\]
Biological and Medical Properties of Curcumin

Turmeric powder has healing effect on both aseptic and septic wounds in Rats and Rabbits.\textsuperscript{16} It also shows adjuvant chemoprotection in experimental forestomach and oral cancer models of Swiss mice and Syrian golden hamsters.\textsuperscript{17} Curcumin also increases mucin secretion in rabbits.\textsuperscript{18} The ethanolic extract of Curcumin, contains sodium curcuminate, [feruloyl-(4-hydroxycinnamoyl) - methane] (FHM) and [bis-(4-hydroxycinnamoyl)-methane] (BHM) and their derivatives, shows high antiinflammatory activity against carrageenin-induced rat paw oedema.\textsuperscript{19, 20} Curcumin is also effective in formalin induced arthritis.\textsuperscript{19} Curcumin prevents intestinal gas formation\textsuperscript{21} and carbon tetrachloride and D-galactosamine induced glutamate oxaloacetate transaminase and glutamate pyruvate transaminase levels.\textsuperscript{22, 23} It also increases bile secretion in anaesthetized dogs\textsuperscript{24} and rats\textsuperscript{25}, and elevates the activity of various enzymes including pancreatic lipase, amylase, trypsin and chymotrypsin.\textsuperscript{26}
The volatile oil of *C. longa* shows anti-inflammatory, antibacterial and antifungal activities. The petroleum ether extract of *Curcuma longa* shows antiinflammatory activity. Petroleum ether and aqueous extracts have 100% antifertility effects in rats. 50% ethanolic extract of *C. Longa* shows hypolipemic action in rats. Ethanolic extract also possesses antitumour activity. Alcoholic extract and sodium curcuminate can also offer antibacterial activity. The crude ether and chloroform extracts of *C. longa* stem also have antifungal effects. A *C. Longa* fraction containing ar-turmerone has potent antivenom activity.

**EXTRACTION OF CURCUMIN**

Curcumin is extracted from the dried root of the rhizome Curcuma Longa. For extraction, the raw materials are ground into powder and washed with a suitable solvent that selectively extracts colouring matter. After distillation this process of the solvent yields an oleoresin with colouring matter content in the region of 25-35 percent along with volatile oils and other extractives. The oleoresins are then subjected to further washes using selective solvents that can extract the curcumin pigment from it. This process yields a purified food colour which is known as Curcumin. It contains 90 percent colouring matter and very little volatile oil and other dry matter. The extractability and regulatory criteria depends on the selection of solvent.
Solvent Screening
Different solvents with varying polarity were used for extraction of curcuminoids from turmeric rhizome. After concentrating each extract total yield were determined and percentage composition of individual curcuminoids present in the extract were analysed by HPLC. The identity of each peak was confirmed by determination of retention times and by spiking with standards. Curcumin was found to be the major compound in all of the tested extracts followed by demethoxycurcumin and bisdemethoxycurcumin.

The following is the list of solvents which are considered suitable: [02]

Table 1: Different solvents used for extraction

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropanol</td>
<td>In the curcumin manufacturing process isopropyl alcohol is used as a processing aid for purifying curcumin.</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>With a restriction placed on the use of chlorinated solvents, such as dichloroethane, it is found that ethyl acetate, owing to its polarity, is a reasonable replacement providing acceptable quality of product and commercially viable yields.</td>
</tr>
<tr>
<td>Acetone</td>
<td>This is used as a solvent in the curcumin manufacturing process.</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>This is not currently used in commercial production. However, it is listed in EC Directive 95/45/EC and has potential as a substitute for chlorinated solvents.</td>
</tr>
<tr>
<td>Methanol</td>
<td>This solvent is used occasionally as a processing aid for purification.</td>
</tr>
<tr>
<td>Ethanol</td>
<td>This solvent is used sparingly because curcumin is completely soluble in ethanol.</td>
</tr>
</tbody>
</table>

DIFFERENT METHODS OF EXTRACTION

1. Soxhlet Extraction

Fresh rhizomes were cleaned, washed with deionised water, sliced and dried in the sun for one week and again. Dried at 50˚c, for six hours in a hot air oven. Dried rhizomes were cut in small pieces and powdered using electronic mill.

6 gm of sample were taken into a thimble and placed in a Soxhlet apparatus, were set up with various solvent from non polar solvent to polar solvent. 250 ml of solvent was added and extracted according to their boiling point for seven hours. The solvents used were [1; 4],

- Chloroform (B.P. =61˚c).
- Ethyl acetate (B.P. =77˚c),
- Methanol (B.P. =65˚c) and
- Acetone (B.P. =56.53˚c).

After completion of extraction the dark brown extract was then cooled, it is then concentrated using rotary evaporator to get a crude dried extract which was black orange in colour. After
drying Soxhlet extract were weighted and weight percentage of curcuminoids were calculated those are shown in table (Table 2). Maximum concentration of curcuminoids was obtained in methanol extract in the form of dark black orange colour. [1; 4]

Table 2: weight % of Curcuminoids extracted with different solvents [04]

<table>
<thead>
<tr>
<th>solvent</th>
<th>% of weight extracted</th>
<th>Dry weight from 6gm of Turmeric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>4.6%</td>
<td>0.28gm</td>
</tr>
<tr>
<td>Chloroform</td>
<td>4.3%</td>
<td>0.26gm</td>
</tr>
<tr>
<td>Methanol</td>
<td>5.6%</td>
<td>0.34gm</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>4.5%</td>
<td>0.27gm</td>
</tr>
</tbody>
</table>

Soxhlet Apparatus

One way to extract the compounds is to use a soxhlet apparatus (Fig.4), and it implies that the extraction and filtration of the product is done. The turmeric powder is put in a paper thimble in a glass container. The solvent vapours are then condensed when it reaches the glass container and fills it up. When the volume of the extract reaches a certain level, the filtrate flows back by leverage to the round bottom flask containing the solvent. The extraction stopped when the filtrate becomes colourless. [03]

Fig.3 Soxhlet apparatus [03]

2. Solvent extraction

500 g of dried turmeric is first grinded in a mixer grinder and then subjected to separation through a vibrating sieving machine. Particles are separated as 250μ size, 44 mesh sizes, and 30 mesh sizes and above 30 mesh sizes. The powders are accumulated and then sealed separately with labeling for further analysis. The solvent extraction turmeric powder was carried using ethanol and water. From each particle size 2 g of sample is taken and mixed with 30 ml of ethanol and 30 ml of water respectively, separately and then filtered. The
concentration of each of the filtrate is kept same and then the absorbance is measured using spectrophotometer at 425nm. \[35\]

Curcumin content (g/100g) is measured using this formula:

\[
0.0025 \times \text{Absorbance at 425 nm} \times \text{volume made up} \times \text{Dilution factor} \times 100
\]

\[
0.42 \times \text{weight of sample} \times 1000
\]

Since 0.42 absorbance at 425 nm =0.0025 g of curcumin.

Table 3: showing the values of curcumin extracted \[35\]

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Curcumin Content g/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent ---Ethanol</td>
</tr>
<tr>
<td>250 μ</td>
<td>0.01238</td>
</tr>
<tr>
<td>44 mesh</td>
<td>0.01337</td>
</tr>
<tr>
<td>30 mesh</td>
<td>0.01239</td>
</tr>
<tr>
<td>Above 30 mesh</td>
<td>0.01139</td>
</tr>
</tbody>
</table>

The above procedure is repeated, with the exception of using timed interval of 2hour, 3hour and 4hour respectively for solvent extraction of turmeric using equivalent amounts of ethanol and water, that is 30 ml of each, separately filtering and keeping the concentration of the filtrate same. The filtrate is subsequently then taken for spectrophotometric analysis at 425nm and calculations are done accordingly with the above specified formula. \[35\]

Table 4: Showing the different percentages of curcumin extracted at different time intervals. \[35\]

<table>
<thead>
<tr>
<th>Time (Hour)</th>
<th>Curcumin Content g/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent ---Ethanol</td>
</tr>
<tr>
<td>2</td>
<td>0.0168</td>
</tr>
<tr>
<td>3</td>
<td>0.01679</td>
</tr>
<tr>
<td>4</td>
<td>0.01652</td>
</tr>
</tbody>
</table>

By using the mixture of solvent of ethanol and water by keeping the size of particle, amount and time constant at 1.5 hour different ratio of solvents are utilized to find the effective extraction parameter. Two different sets are made with water and ethanol separately of 30 ml each respectively and three sets of mixed solvents are used with 80%, 70% and 50% ethanol in ratio with water, keeping the total volume at 30 ml of each set constant respectively. The amount of curcumin extracted is again calculated using the gravimetric method mentioned earlier for the two different set of water and ethanol while another approach is taken into account for the mixed solvents. The 10 ml of filtrate from the mixed solvents are taken, same
as that for the pure solvents, and are left to evaporate the ethanol in water bath and subsequently transferring the petriplates into hot air oven at 130°C for 1.5 hours and then repeating the previously mentioned method again.\textsuperscript{[35]}

**Table 5: Showing the percentage of curcumin extracted using mixture of solvents in ratio.\textsuperscript{[35]}**

<table>
<thead>
<tr>
<th>Volume of Water (ml)</th>
<th>Volume of Ethanol (ml)</th>
<th>Total (ml)</th>
<th>Percentage of Curcumin extracted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>---</td>
<td>30</td>
<td>9.890</td>
</tr>
<tr>
<td>---</td>
<td>30</td>
<td>30</td>
<td>28.656</td>
</tr>
</tbody>
</table>

By using petroleum ether and keeping constant for 1 hour, with 250 micron particle size and at 2 g sample weight, solvent extraction of turmeric is carried out using a different solvent of pet ether with successive volume of 50 ml, 60ml, 70ml and 80 ml respectively. Filtration is carried out and the filtrates are collected in a petriplate each of 10 ml quantity and the solvent is evaporated at atmospheric pressure in water bath. After drying the residual weight is noted down and subtracted from the initial empty weight of the petriplate, and the amount of curcumin being extracted is calculated.\textsuperscript{[35]}

**Table 6: Showing the percentage of curcumin extracted using pet ether as a solvent\textsuperscript{[35]}**

<table>
<thead>
<tr>
<th>Volume of Pet ether used (ml)</th>
<th>Percentage of Curcumin Extracted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.1</td>
</tr>
<tr>
<td>60</td>
<td>0.63</td>
</tr>
<tr>
<td>70</td>
<td>1.56</td>
</tr>
<tr>
<td>80</td>
<td>1.79</td>
</tr>
</tbody>
</table>

3. Maceration

The dried powder (3 g) is extracted with 70% ethanol (30ml) on a shaker with 210 rpm at room temperature for 2 days. The extract is filtered through Whatmann no. 1 filter paper. Other portions of the solvent were added to the solids and the extraction was repeated until the extractant is colorless. The extracts are combined and filtered. The filtrates are concentrated under reduced pressure at 50 °C using a rotary evaporator. The crude extract was then heated on a boiling water bath until constant weight was obtained and stored for injection to HPLC. The experiment is repeated three times. The total curcuminoids yield was 12.39%.
4. Microwave assisted extraction (MAE)
For MAE accurately weighed 2 g of the homogenous 40, 20, 10 mesh drug powder is used. The samples are mixed thoroughly with a suitable modifier (methanol) in accordance with the experimental method. A saturation time of 10 min was allowed for the powdered drug to absorb methanol. The powdered drug was then placed into the extraction vessel, and 40 ml of the extracting solvent (acetone) was added. MAE was carried for different time of irradiation with the microwave extractor operating at different power levels. The sample was then treated under microwave irradiation in an intermittent way, i.e. irradiation–cooling–irradiation. The irradiation time was kept for 1 min and 1 min was taken to cool the sample solution between two irradiations. The samples were then centrifuged at 4000 rpm (3520×g) for 10 min. The supernatant was filtered, concentrated under vacuum, dissolved in methanol for quantified by HPTLC. The extraction efficiency (%) for MAE is defined as follows

Relative extraction efficiency (%) =
Percentage extraction of curcumin (w/w) obtained from MAE×100
Percentage extraction of curcumin (w/w) obtained from 24 h of exhaustive Soxhlet extraction

5. Sonication extraction
An ultrasonic cleaning bath and a double jacket cell were used for extraction procedure. The cell was kept at constant temperature (25°C) by circulating water from a controllable thermostat bath through the jacket, to avoid rising temperature caused by ultrasonic exposure. The following samples were filtered, washed with 15 ml solvent, evaporated to dryness using a rotary evaporator and diluted for injection to HPLC. In order to obtained total
curcuminoids, after certain dilution, the optical density of the samples at 420 nm was measured by using a Shimadzu Multi spec-1501 photo diode array spectrophotometer.

6. Slurry extraction
20 g of turmeric powder was mixed in 200 ml of acetone and stirred for 48 hours. The solution was filtered on a silica filter and the obtained product was concentrated. The obtained concentrated yield was in the range of 2.0 g – 2.2 g in different attempts with varying stirring times. The optimal time of stirring was found to be 48 hrs. The extract was then transferred to a column for separation and further purification.

7. Refinement of Curcumin
Curcumin composes only 4% of the turmeric rhizome, therefore necessitating the need for highly precise refinement processes.[05]

**Filtration:** Filtration is the first step in the refinement of curcumin. In this process, the turmeric powder is converted into a mixture called the Oleoresin. Ethylene dichloride (EDC or C₂H₄Cl₂) was used as a solvent to dissolve impurities. Ethylene dichloride was added to the turmeric powder in the ratio of 3 ml/gm of turmeric powder. Ethylene dichloride (300 ml) was added to 100 g of turmeric powder in steady and equal increments and mixed. The resulting mixture was heated to 60°C, 1 hour. Then, the mixture was cooled, the leftover solvent was decanted, and the extract was filtered. The same process was repeated twice, the Ethylene dichloride extract was collected, and the leftover solvent was decanted. This process removes impurities from the turmeric. The mixture is the oleoresin. 100 g of turmeric powder yielded 25.2 g of Oleoresin. This process is used to filter out the remaining impurities.

**Oleoresin to Curcumin:** From the filtration process 25.2 g of oleoresin was obtained. Using the same 3 ml of solvent per 1g compound ratio, 75 ml of Isopropyl alcohol (IPA) was measured in a graduated cylinder. An experimental set-up as shown in Figure 2 was assembled to enable the refinement process. [05]
A piece of 200 mesh filter paper was weighed and then put into a funnel and placed over a Buchner flask with vacuum attachment. The 25.2 g was transferred to the filter paper on funnel. A small quantity of isopropyl alcohol was added to the funnel. This process was repeated, adding the alcohol in small amounts. The filter cloth and the oleoresin on top were taken out of the funnel and weighed to be 6.4 g. The resin was then broken up and using the following dimensional analysis, the approximate volume of isopropyl alcohol was calculated:

\[
= 6.4 \text{ g oleoresin} \times 3 \text{ ml Isopropyl alcohol} \\
1 \text{ g oleoresin} \\
= 19.2 \text{ ml of IPA}
\]

The process was repeated with 19.2 ml of alcohol. The filter paper or the cloth was placed in the flask, and again in equal amounts the alcohol was poured in. Finally the filter cloth and its contents (pure curcumin) were dried in a hot air oven at 60°C for 1 hour. After an hour, the filter cloth and the contents was weighed (dry Curcumin weight). The mass of the filter cloth was subtracted from the total mass, to get a mass of curcumin equal.\[05\]

It was found that 100 g of turmeric powder yielded 5% of pure Curcumin. Typically, raw turmeric contains 3-10% curcumin, the oleoresin contains 25-30% curcumin, and the curcumin sample that is refined contains 90-98% pure curcumin.\[05\]
ISOLATION, PURIFICATION AND IDENTIFICATION

- **Purity Testing Techniques**
  In this study, two commonly used test techniques Spectrophotometry and Chromatography were employed. One of the major goals of this analysis following the research/experiment was to compare these two commonly used techniques specifically for Curcumin characterization.

- **Physico-chemical Tests**
  1. *Description* Reddish brown thick paste with characteristics odour & characteristics taste.
  2. *Physical Evaluation* In physical evaluation, ash values including, total ash, acid insoluble ash and water soluble ash, as well as extractive values including, alcohol soluble extractive value and water soluble extractive were determined. The ash values represent the presence of inorganic salts.
  3. *Determination of Total Ash Value* Two gram of *C. longa* powder was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon. The resultant ash was then cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.
  4. *Acid Insoluble Ash Value* The total ash obtained from 2g of *C. longa* powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was then washed with hot water, ignited and then weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.
  5. *Water Soluble Ash Value* The total ash obtained from 2g of *C. longa* powder was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.
  6. *Separation of Curcumin by Thin layer chromatography* For thin layer chromatographic studies of curcumin, precoated Silca gel F254 aluminum plates (20 X 20cm) were used. The Curcumin was separated using n-hexane: ethyl acetate (7:3). The colour and Rf values were recorded using spraying the plates with 1% alcoholic KOH solution.
  7. *Determination of Total Phenolic contents* The Folin-Ciocalteu reagent assay was used to determine the total phenolic contents. The extract 1ml (10mg/ml) was mixed with 0.5ml Folin-Ciocalteu reagent previously diluted with 7ml deionized water. The solution was
allowed to stand for 3 min. at 250 °C before adding 0.2 ml of saturated sodium carbonate solution. The mixture was allowed to stand for another 120 min and absorbance was measured at 425 nm. Gallic acid was used as standard for the calibration curve. The total phenolic contents of the extract were calculated in terms of Gallic acid equivalent [GAE]

\[ C = c \times \frac{V}{m} \]

Where,
- \( C \) = total phenolic compound in mg/gm of the extract
- \( c \) = concentration of Gallic acid (established via calibration curve)
- \( V \) = volume of the extract in ml
- \( m \) = wt. of extract in gm

**SUMMARY AND CONCLUSION**

Medicinal plants are important for discovery and identification of new therapeutic compounds. Extraction method plays an important role in separation and characterization of different phytochemicals from herbs, and screening plant extracts for novel leads. This review article focuses on the extraction of curcumin from turmeric powder with the help of different solvents and with different techniques.

**REFERENCES**

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