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Extraction and Antibacterial Studies of Curcumin

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Curcuminoids are the polyphenolic pigments found in the turmeric. The major curcuminoids are curcumin, demethoxy curcumin, and bis demethoxy curcumin. These substances comprises 3 to 6% of *curcuma longa L.* curcumin makes up 70 to75% of the curcuminoids, demethoxy curcumin 15 to20% and bisdemethoxy curcumin about 3%. The present study is planned to study the purity and antibacterial study of curcumin. Curcumin from dried, powdered rhizome are extracted with the polar solvents by cold and hot extraction. Some of the polar solvents used in which curcumin is soluble are IPA, Ethyl acetate, Acetone. A comparative study was done with three different solvents for extraction and the yield of crude curcumin was analysed by spectrophotometric method and curcuminoids separation was carried out by high performance liquid chromatography (HPLC) a separation process. The same was used for the antibacterial study and *Bacillus stearothermophilus* inhibition was studied by Kirby-Bauer disk diffusion method. Standard aseptic microbiological methods are followed throughout this antibacterial susceptibility studies. The purity obtained through HPLC was found to be 96.22% and percentages of different kinds of curcuminoids are cucumin 69.9%, demethoxy curcumin 22.36%, bisdemethoxy curcumin 3.9%. The obtained purity 93.09% is very close to the normal high purity value of 95%. *Bacillus stearothermophillus* inhibition was studied by kirby- bauer disk diffusion method.we can conclude that curcumin has the high purity and also possesses significant antibacterial activity.

Key words: Curcumin; Extraction; Spectrophotometry; HPLC; *Bacillus stearothermophilus*; Kirby Bauer Method.

Curcuma longa L. belongs to the family Zingiberaceae, commonly known as turmeric. It is cultivated primarily in Bengal, China, Taiwan, Sri Lanka, Java, Peru, Australia and the West Indies[1]. Turmeric is the rhizome or underground stem of a ginger-like plant. The whole turmeric is a tuberous rhizome, with a rough, segmented skin. The rhizome is yellowish-brown with a dull orange interior that looks bright yellow when powdered. Rhizome measures 2.5-7 cm (length), 2.5 cm (diameter) with small tuber branching off[2]. India has a rich history of using plants for medicinal purposes.

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Turmeric (c*urcuma longa* L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases[3]. 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*- hellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%)[4]. Curcumin (diferuloyl-methane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%)[5] Curcumin was first isolated [5] in 1815 and its chemical structure was determined by Roughley and Whiting[6] in 1973. Turmeric is a well known indigenous herbal medicine[7]. Turmeric powder, curcumin and its

derivatives and many other extractives from the rhizome were found to be bioactive[8]. Its major constituents, curcumin, various curcuminoids, curcuma oil-particularly all-ar-turmerone exhibit a wide range of biological activities e.g. antibacterial[2], anti-inflammatory hypolipidemic, hepatoprotective[9]. Hence, the present study was planned to study the antibacterial activity of curcumin against strains of (Gram positive) *Bacillus steareothermophilus* bacteria.

MATERIAL AND METHODS

Plant material

In the present study the rhizome powder of *Curcuma longa* L. plant was used.

Solvents

The polar solvents used for the extraction and purification of curcumin were Isopropyl alcohol, Ethyl acetate, Acetone.

Organism used

Bacillus stearothermophilus was used for the present study to test the antibacterial activity

Cold and hot extraction setting

Curcumin was extracted from various extraction methods by using different solvents. Cold extraction (50g of turmeric powder/ 350ml solvent) was done by percolation method and hot extractions (10g / 150ml solvent) were done by soxhlet method[10] and both were compared for curcumin yield. The cold extraction by acetone gave high yield (98.43%) and high purity of curcumin(93.09%), so further bulk extractions were carried out by cold extraction. The percentage of curcumin present in the oleoresin was determined by spectrophotometric analysis. The physical and chemical yield of curcumin in both the extraction method was calculated by the following formulas

$$\text{Percentage of Curcumin content} = \frac{\text{absorbance}}{E_1\%} \times \frac{\text{dilution}}{\text{weight}} \times \text{further dilutions}$$

$$\text{Physical yield as crude extract} = \frac{\text{Weight of the crude extract}}{\text{weight of the turmeric powder taken}} \times 100$$

$$\text{Chemical yield as curcumin} = \frac{\text{Weight of the crude extract}}{\text{weight of the turmeric powder taken}} \times 100$$

Spectrophtometric analysis

Curcumin was quantitatively extracted by refluxing the material in acetone and was estimated spectrophotometrically at 425nm[11].

Purification

Curcumin was purified by using 3 different solvents such as Ethanol, Acetone and Isopropyl alcohol.purification was carried out by taking few grams of oleoresin and solvent in 1:4 ratios[12]. It was kept for overnight for the crystal formation. Among the three different solvents used; IPA was found to be the best solvent to attain better purification and better purity of curcumin crystals.

HPLC

The curcuminoids such as curcumin, demethoxy curcumin and bisdemethoxy curcumin, were separated using HPLC to find out the percentage of different curcuminoids by HPLC[13].

It was calculated by the formula

$$\text{Percentage of curcumin} = \frac{\text{sample area x standard weight x standard purity}}{\text{standard area x sample weight}}$$

Percentage of curcumin

Antibacterial studies

The bacterial culture was continuously maintained in the nutrient agar slants. The culture was periodically sub cultured in the slants and streak plate to keep them alive and free of contaminations. Antibacterial activity[14]of the curcumin compound was determined using pure culture of *Bacillus Steareothermophilus* by agar plate method. Antibacterial activity was done by Kirby-Bauer disk diffusion method. The culture was diluted in 1:10 ratios; in control plate one loop full of culture was used for the growth of *Bacillus stearothermophilus* (Fig. 1). In test plate after organism inoculation discs were placed. After 24 hours incubation, measure the diameter of the zone of inhibition around each disk keeping the lid of the plates, the plate was measured in mm.

RESULTS

Yield and purification of curcumin

The Curcuminoids were extracted from turmeric by two methods namely hot and cold extraction methods. The result obtained shows the higher yield of curcuminoids, when extracted by cold extraction procedure (Table 1). Curcumin content found to be greater (70%) in turmeric when compared to other curcuminoids(25%,5%).

Curcumin obtained (Table 2) was of 93.09% pure in contrast to normal purity form of curcumin (95%). The separation of curcuminoids by HPLC is shown in table (Table 3 & 4). The percentage of curcumin present in the purified curcuminoids was 69.9% which correlates well with the results of previous study.

Antibacterial study of curcumin

The purified curcumin was subjected to antibacterial activity by employing the culture *Bacillus steareothermophilus*. The various zones of inhibition are evident from the plates. Curcumin showed as 25mm maximum zone of inhibition and as 13mm minimum zone of inhibition (Fig. 2).

Fig. 1. Control plate

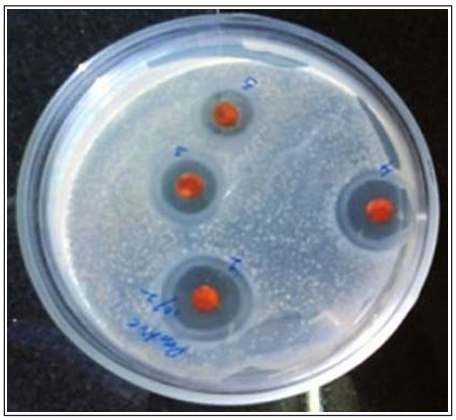

Fig. 2. Antibacterial activity of Curcumin

Table 1. Comparision of Cold and Hot extraction

Solvents	Assay (%)	Physical yield (%)		Chemical yield (%)		
	Cold extraction	Hot extraction	Cold extraction	Hot extraction	Cold extraction	Hot extraction
IPA	26.03	22.65	8.36	8.88	85.33	78.86
Ethyl acetate	24.23	24.48	7.76	7.44	73.82	71.45
Acetone	27.96	25.98	8.97	8.69	98.43	88.54

Table 2. Purified Curcumin Assay

Solvent	Assay (%)	Physical yield (%)	Chemical yield (%)
Isopropyl alcohol	93.09	11.53	38.44

Table 3. Standard curcumin-HPLC result Table (Uncal-CUR1009.0)

	Reten.Time (min)	Area(mV.s)	Height(%)(mV)	Height (%)	Area (%)
1	19.573	4323.339	82.755	76.3	72.6
2	23.460	1395.616	22.402	20.6	23.4
3	27.937	238.159	3.354	3.1	4.0
	Total	5957.113	108.511	100.0	100.0

Table 4. Sample curcumin-HPLC result Table (Uncal-CUR1009.0)

	Reten.Time (min)	Area(mV.s)	Height(%)(mV)	Height (%)	Area (%)
1	19.573	4472.382	82.923	76.1	72.7
2	23.460	1430.198	22.542	20.7	23.2
3	27.937	250.885	3.437	3.2	4.1
	Total	6153.465	108.901	100.0	100.0

DISCUSSIONS

Turmeric pastes and ointments can be made from turmeric. The turmeric, with its antibacterial action, will prevent the bacterial wound infections. The bacteria is a thermopile and are widely distributed in soil, hot springs, ocean sediment, and are a cause of spoilage in food products. It is commonly used as a challenge organism for sterilization validation studies and periodic check of sterilization cycles[15].

Turmeric powder is an essential ingredient in various Indian food preparations for taste and coloring and is an essential ingredient in various herbal preparations. It is also used in preparation of Bakery products, Sources, Confectionary, Compressed vitamin tablets, Snack foods, Ice cream, Desserts, Culinary, Meat products, Fruits preparations pickles and fish. The promising results obtained in this study prove the significance of curcumin. Comparative studies were done in three different solvents (IPA, Ethyl acetate, Acetone) used for cold and hot extractions. From the comparative study acetone gives high chemical yield 98.43% in cold extraction and as well as 88.54% in hot extraction. So the bulk extraction of curcumin was carried out by cold extraction method using acetone as the best solvent for highest yield of curcumin (98.35%).

Isolation of curcumin was done by using IPA. The same was used for the determination of total curcuminoids as well as different kinds of curcumionids such as curcumin, demethoxy curcumin, bisdemethoxy curcumin by HPLC. The purity obtained through HPLC was found to be 96.22% and percentages of different kinds of curcuminoids are cucumin 69.9%, demethoxy curcumin 22.36%, bisdemethoxy curcumin 3.9%. The obtained purity 93.09% is very close to the normal high purity value of 95%. *Bacillus stearothermophillus* inhibition was studied by kirby- bauer disk diffusion method. This study (Antibacterial study) suggests that curcumin possesses significant antibacterial activity at minimal concentration (2g) on gram positive (*Bacillus steareothermophilus*) organism.

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