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ISOLATION & CHARACTERIZATION OF CURCUMIN FROM ALCOHOLIC EXTRACT OF *CURCUMA LONGA*.

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ABSTRACT

The objective of this work is to isolate Curcumin from ethanolic extract of *Curcuma longa* and to determine the physico-chemical characteristics of Curcumin. The isolated Curcumin is characterized by using Fourier Transform Infrared spectroscopy (F.T.I.R.) and Ultra-violet spectroscopy (U.V.)

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Key Words

Curcuma longa, Curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), Thin Layer Chromatography (TLC), F.T.I.R, U.V. spectrophotometer.

INTRODUCTION

Curcuma longa (Family- Zingiberaceae) is a widely cultivated spice in India. It has been used in traditional medicine as a household remedy for various diseases. It also plays a very important role in various fields like fragrance, food, pharmaceutical and aromatherapy (Jae *et al*, 2006). *Curcuma longa* is rich in curcuminoids, they vary in chemical structure, physico-chemical characteristics as well as the functional properties. Curcumin and two related compounds, i.e., DMC and BDMC are altogether known as Curcuminoid (V.S. Govindarjan, 1980). Its active ingredient is Curcumin. Curcumin is main colouring substance of *Curcuma longa* and it has a distinctly earthy, slightly bitter, slightly hot peppery flavour and a mustardy smell. Recently, many studies on health benefits associated with Curcumin have been reported.

ETHNOPHARMACOLOGICAL CHARACTERS

In Medieval Europe, *Curcuma longa* became known as an alternative to the far more expensive saffron spice. It is also used for Wound healing (Gujral *et al*, 1953). *Curcuma longa* is currently being investigated in Alzheimer's disease (BBC news, 2011), Cancer (Report, 2005). As an example of preliminary laboratory research, *Curcuma longa* ameliorated the severity of Pancreatitis-associated lung injury in Mice (Sew S W *et al*, 2011).

Curcumin (diferuloylmethane) , the main yellow bio-active component of *Curcuma longa* has been shown to have a wide spectrum of biological actions. These include its Antioxidant activity (Sharma, O.P., 1976), Anticarcinogenic effect (Chan *et al*, 1998) etc... Ethanolic extract also possess Antitumour activity (Kuttan *et al*, 1985), Anti-inflammatory (Yegnanarayan *et al*, 1976).

MATERIALS & METHODS

COLLECTION OF PLANT MATERIAL

Curcuma longa (Zingiberaceae) was collected from the agricultural fields around Vidisha, M.P., India. Identification of plant was carried out by Dr. Jagrati Tripathi HOD, Department of Botany, Unique College, Bhopal, M.P. It was cleaned, thoroughly washed with deionised water and was kept for drying in shade at

room temperature for 20 days. The thoroughly air dried material was grinded to about 40 – 60 mesh size.

SOLVENT EXTRACTION

400 gm of powder of *Curcuma longa* is taken in a reflux condenser and Ethanol solvent was used for extraction. Now powder is reflux with 250 ml of Ethanol for two and a half hour. Cool the extract and filter quantitatively into a 100 ml volumetric flask and wash thoroughly and dilute to mark with alcohol (I.S. No. 2446-1980).

PHYTOCHEMICAL SCREENING

Phytochemical screening were performed to assess the qualitative chemical composition of different crude extracts using commonly employed precipitation and coloration reactions. Moisture, Ash-content and Acid insoluble ash was determined by standard method (A.O.A.C.2000). The presence of Calcium, Magnesium, Iron and Phosphate was determined by method described in Indian Pharmacopoeia (2007). Protein, crude fibre was determined by standard method (A.O.A.C. 1995).

ISOLATION AND PURIFICATION

Isolation of Curcumin from *Curcuma longa* was done by **Thin Layer Chromatography** (TLC). Different solvents were used for the separation and purification of Curcumin from *Curcuma longa*. Then various tests were performed for the identification of Curcumin and to determine the net content of Curcumin in the sample by F.T.I.R. and U.V. respectively.

Identification of Curcumin content by F.T.I.R.

The 80 ml of the extracted solution was evaporated until dryness to get solid crystals. The standard, sample and Potassium Bromide (KBr) are kept in an oven at 105+5 ° C for one and a half hour to get them moisture free. Now Standard and KBr are taken in the ratio of 1: 300 to make a solid disc or pellet with the help of Hydraulic Pellet Machine. Same procedure was done with the sample. Now identification of sample with respect to sample is done on F.T.I.R.

Determination of Curcumin content by U.V. (Ultra-violet) Spectrophotometer

Pipette out 20 ml of the filtered extract into a 250 ml volumetric flask and dilute to volume with alcohol. Measure the absorbance of the standard solution and

the extract at 425 nm in 1 cm cell against an alcohol blank.

CALCULATION

$$\% \text{ of Curcumin} = \frac{\text{Sample reading} \times 100 \times \text{Strength of Standard}}{\text{Standard reading} \times \text{Strength of Sample}}$$

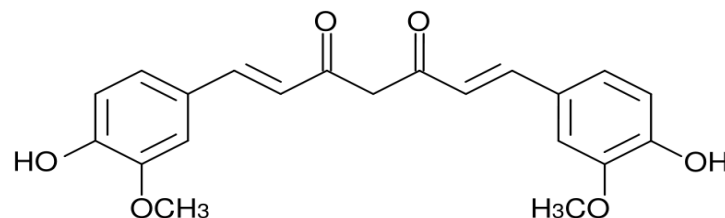
RESULTS & DISCUSSION

Characterization of isolated Curcumin by U.V. and F.T.I.R

The characterization of the isolated Curcumin was done on F.T.I.R and U.V. Spectrophotometer. I.R. spectra was obtained on F.T.I.R. The value of sample was found to be in between $1600 - 1800 \text{ cm}^{-1}$ (for C=O stretching), $1400 - 1800 \text{ cm}^{-1}$ (for O-H Bending). The results were found to be come under the range of standard graph. The U.V.

Curcumin was subjected to physico-chemical analysis. The obtained results are depicted in table-1.

absorption maxima of isolated compound was recorded using ethanol as a solvent. A U.V. spectrum of the isolated compound shows peak of almost same intensity at 424.8 nm. The Curcumin content of the sample was found to be 101.37%.



STRUCTURE OF CURCUMIN

The structure of Curcumin was identified by J. Milobedzka *et al* in 1910.

TABLE-1. Showing physico-chemical properties of Curcumin.

Sr.No.	Tests	Results	PFA Standards
1	Color	Yellow	–
2	Odour	Mustardy smell	–
3	Taste	Bitter	–
4	Melting Point	182.5 °C	183 °C
5	Moisture	7.84%	13.0%
6	Ash Content	7.24%	9.0%
7	Acid insoluble ash	0.98%	1.5%

BIO-CHEMICAL EVALUATION OF CURCUMIN

TABLE-2. Showing bio-chemical evaluation of Curcumin.

S.NO.	Tests	Results
1	Protein	Present
2	Tannin	Present
3	Calcium	Present
4	Magnesium	Present
5	Iron	Present
6	Phosphate	Present
7	Carbohydrate	Present
8	Crude fibers	Present

The results came out were matched with the standard and the results came were found to be within the standard range. To determine the presence of various elements, qualitative tests were performed. Identification of Curcumin was done on F.T.I.R. and determination of the net Curcumin content was done on U.V. Thus based on the results of the tests carried out and from spectral studies, the observed data was found to match well with that of standard data for Curcumin. Thus, we can say that the isolated compound was may be Curcumin.

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