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ISOLATION OF FLAVONOID DERIVATIVE FROM *CURCUMA LONGA*

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ABSTRACT

The present study was carried to isolate flavonoid present in *Curcuma longa* rhizome extracts using column and thin layer chromatography separation techniques. And elucidated the structure by using, UV, IR, NMR & Mass. The spectral data proved it to be 2-(3,4, 5-trihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl oxy]-4*H*-chromen-4-one.

KEY WORDS: flavonoid, UV, IR, NMR & Mass.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicinal systems that have been in existence for thousands of years, and they continue to provide humanity with new remedies [1] The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [2]. In order to promote the use of medicinal plants, it is important to thoroughly investigate their composition and activity and thus validate their use [3]. In the last few years, spectroscopic methods have become firmly established as a key technological platform for secondary metabolite profiling in both plant and non plant species [4-5] Therefore, the presentresearch was conducted to investigate and characterize the bioactive compound of Curcuma longa using UV-VIS, FTIR and Mass spectrum.

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to tropical South Asia but is now widely cultivated in the tropical and subtropical regions of the world. The deep orange-yellow powder known as turmeric is prepared from boiled and dried rhizomes of the plant [6]. The rhizome has

antiseptic, aromatic, alterative, antipyretic, germicidal, carminative, stimulant, tonic and vermifuge properties. The drug cures diseases due to morbid *vata*, *pitha* and *kapha*. It is used in diabetes, eye diseases, ulcers, oedema, anaemia, anorexia, leprosy and scrofula [7].

MATERIAL AND METHOD

Collection of Plant Material

The rhizomes of *Curcuma* were collected on 1st November from *Sanjivani Ayurvedic Nursery Bhopal.* All the plant materials were further identified in the Department of Botany, SNGGPG College Bhopal, (India).

Preparation of Extract

The rhizomes were cut into pieces and air dried at room temperature. The dried rhizomes were coarsely powdered and successfully extracted with methanol using Soxhlet extractor at a temperature of 55-60 0C for a period of 7-8 hrs. The solvents was distilled off at lower temperature under reduced pressure and concentrated to dryness (crude extract). The dried extract was weighed and then stored in a freezer. The crude extract was used for the experiments.

Isolation of compound

The isolation of the methanol extract was subjected to coloum chromatography of ***size*** 100 cms x 3 cms chromatography with silica gel (60-120 mesh) as the stationary phase. The charged column was then eluted with mobile phase Ethyl acetate: glacial acetic acid: formic acid: water [100:11:11:25, v/v/v/v]. The fractions were collected and the solvent recovered by simple distillation. All the concentrated fractions were subjected to TLC for the identification of the desired bands.

TLC was performed on the 20 × 20 cm plates precoated with silica gel (Sigma Aldrich Co., India). TLC analysis of methanolic extracts was performed using Ethyl acetate: glacial acetic acid: formic acid: water [100:11:11:25, v/v/v/v] developing solvent systems.

Identification of similar fraction

The different fractions of column chromatographic elution were monitored by TLC (Ethyl acetate: glacial acetic acid:formic acid: water [100:11:11:25, v/v/v/v]; using UV chamber and derivatization with specific reagent (TLC for each collected fraction was developed and individually observed under UV-256 and UV 366 nm. But each TLC is derivatized with one

specific reagent for identification of single isolated compound with comparison of reference compounds). The fractions which show similar fingerprinting profile on TLC were collected and mixed and subjected to refractionation, to isolate single pure compound.

Characterization of Isolated compound

Isolated compound was characterized by UV- absorption spectra, IR, NMR and Mass Spectra studies. Fraction 103-114 shows single spot at same R_f value rutin and derivatized with 1% $AlCl_3$ reagent so it may be flavanoid compound. Supporting evidence for the structure of the isolated compound is provided by the UV and NMR (125 MHz, DMSO). Spectral data that were recorded on a Bruker AMX 400 NMR spectrometer. Chemical shifts were referenced to the respective residual solvent peaks and the values were recorded in δ.

RESULT AND DISCUSSION

The rhizome of *Curcuma longa* have been found to contain 2-(3,4, 5-trihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl oxy]-4*H*-chromen-4-one a rutin derivative.The qualitative UV spectrum profile of *Curcuma longa* methanolic extract was selected at wavelength from 200 to 400 nm due to sharpness of the peaks and proper baseline. The UV profile of methanol extract of *Curcuma longa* chosen wavelength of 200 to 400 nm and the profile showed two major peaks at 264nm and 350 nm with the absorption 3.382 and 0.684 respectively (fig:1), to reveal a flavonoid skeleton.

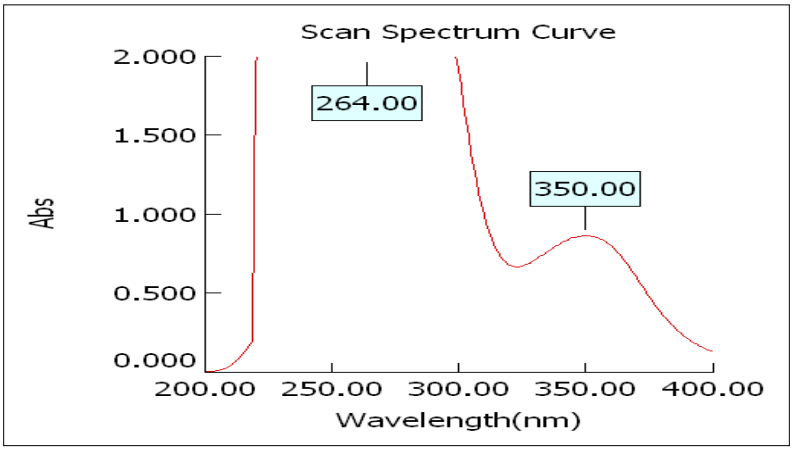

Figure 1: UV spectrum of methanolic extract of *Curcuma longa*

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 1. The FTIR spectrum profile was illustrated in

the Fig. 2 The IR spectra showed the broad peak [8-9] at 3284.77 was observed due to –OH stretching, peaks at 2918.88, 2851.42 revealed aliphatic stretching in a compound. A sharp peak at 1739.96 confirmed the presence of C=O stretching in a compound. Peak at 1496.99 and 1428.13 revealed presence of double bond (characteristic ring stretching) and a coupled peak at 1071 and 1012 was due to C-O stretching.

Table 1: FTIR peak values and functional groups of *Curcuma longa*

S. No.	Frequency (cm^{-1})	Inference
1.	3284.77	OH str.
2.	2918.88, 2851.42	CH str.
3.	1739.96	C=O str.
4.	1071.00	C-O-C str.
5.	722.03	Out of plan bending of aromatic H

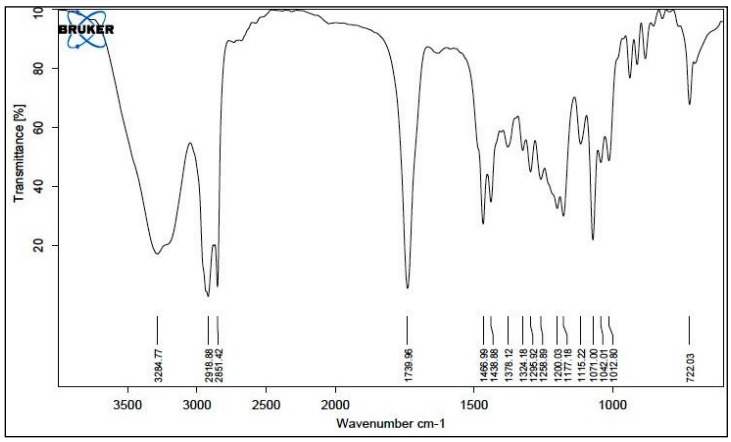

Table 2: Mass spectrum base peak value

S. No.	m/z	Inference
1.	625.2	Base peak

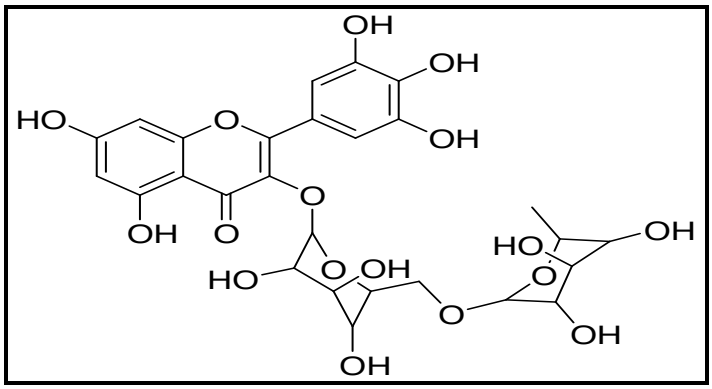

Figure2: FTIR spectrum of *Curcuma longa*

The NMR spectra (Fig:3) showed that presence of multiplets at 2.17-2.55 was due to straight chain CH_2 and 3H of CH_3 at cyclic ether ring. Singlet at 3.63 was due to solvent and multiplets between 6.41-7.39 showed splitting due to aromatic ring.

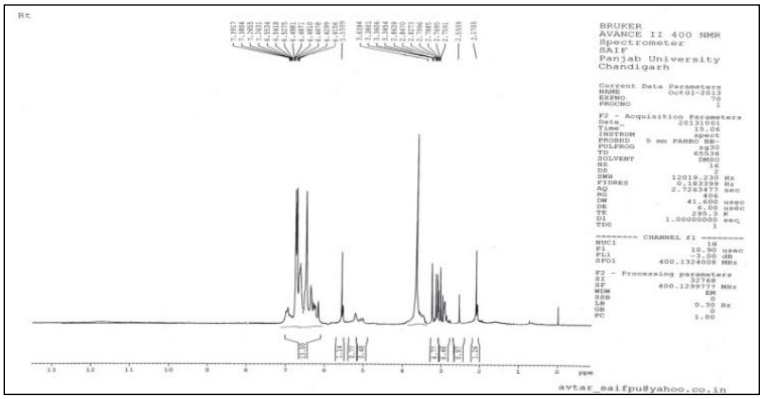

Figure 3: NMR spectrum of Curcuma *longa extract*

The Mass spectra (Fig:4) showed the base peak value at 625.2 m/z.

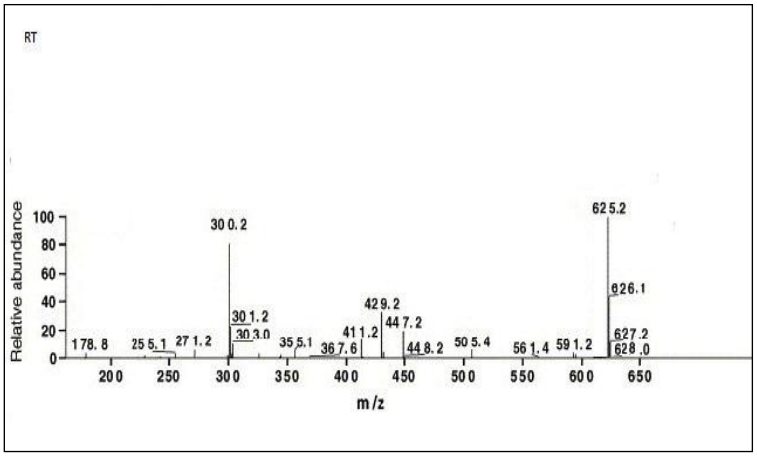

Figure 4: Mass spectrum of Curcuma *longa extract*

Figure:5 2-(3,4, 5-trihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl oxy]-4*H*-chromen-4-one.

CONCULSION

All the spectral data of isolated compound shows the isolate is rutin derivative, 2-(3,4, 5-trihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl oxy]-4*H*-chromen-4-one.

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