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Fluorescence Characteristics of 2-benzo [1, 3] dioxol-5-yl-2, 3-dihydro-furo [2, 3-h] chromone-4-one Isolated from *Pongamia glabra*

Shubashini K Sripathi* and Lalitha. P.
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Coimbatore - 641043; India.

Abstract : The aim of the present investigation is to determine the fluorescence characteristics of 2-benzo[1,3] dioxol-5-yl-2,3-dihydro-furo[2,3-h]chromone-4-one. The compound was isolated by column chromatography of the benzene extract of the galls of *Pongamia glabra.* The structures was characterized by preliminary colour tests and UV and Fluorescence spectra as well as PMR and IR spectra. The emission peaks were located at 426 nm and the compound may be considered to show good emission fluorescence, a property useful in identification and also quantification of organic compounds.

Key words : Flavonoids, Emission fluorescence, *Pongamia glabra.*

Introduction

Fluorescence spectroscopy is a very sensitive way to determine properties about substances. It boasts phenomenal sensitivity for the analytical chemist or the life scientist working at nanomolar concentrations (Allais, 2004). The dynamics of the folding of proteins can be studied. Membrane structure and function may be studied with fluorescence probes. Drug interactions with cell receptors can be investigated. Minute traces of fluorescent materials can be detected and identified in mixtures. The electronic structure and dynamics of an excited state of a molecule may be elucidated. Hence, the interest has been generated in fluorescence studies. Flavonoids, constitute one of the most characteristic classes of compounds in higher plants and consequently selected for study. Flavones bearing a furan ring annelated at C_7-C_8 or C_6-C_7 of ring A belong to the category of furanoflavones (Koysomboon *et al., 2006*) and have diverse biological activities including antitumour, anti inflammatory, quinine reduction, cytotoxic activities (Yadav *et al.*, 2004; Ghanim *et al.*, 1980; Gupta *et al.*,1980) and can be used for insecticide synergistic and even in cosmetics and sunscreens. Some of these compounds mentioned in literature have promise for further development and optimization of their activities to obtain candidates for the drug discovery process (Maurya *et al*, 2005).

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Materials and Methods

The present work deals with the fluorescence characteristics of 2-benzo[1,3] dioxol-5-yl-2,3-dihydro-furo[2,3-h]chromone-4-one designated as TM1.The leafs galls of the plant *Pongamia glabra* were collected and the identity of the plant was confirmed at the herbarium of botanical survey of India, Coimbatore.

Solvents and reagents

Solvents used for the study are petroleum ether, benzene, chloroform, acetone, methanol, ethanol, THF (Tetrahydrofuran), acetonitrile, dichloromethane, ethyl acetate.

Phytochemical examination of galls of *Pongamia glabra*

The galls present in the leaves of *Pongamia glabra* were cut air dried and coarsely powdered. The powdered galls (2.5 kg) were then successively extracted with pet. ether (4x2.5L) benzene (4x2.5L). The benzene extract obtained was clarified by filtration and concentrated under reduced pressure to give a dark green semi-solid residue (60g).

Isolation of compound TM1

The residue from the benzene extract (about 20g) was made into slurry with silica gel and subjected to chromatographic separation over a column of silica gel built in petroleum ether: benzene (80:20) mixture. The column was eluted with (i) petroleum ether: benzene mixtures with increasing amount of benzene (ii) benzene (iii) benzene: ethyl acetate mixtures with increasing amounts of ethyl acetate. Eluates of 200ml were

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Ferric chloride solution	-	Salkowski reagent	-
Schinoda test	+	Dilute ammonia solution	+
Labat test	+		

collected and concentrated by distillation. The residues were checked for homogeneity by TLC on silica gel plates. Similar fractions were combined.

Spectral measurements

UV spectrum of TM1 was recorded in methanol in Shimadzu UV-VIS 160 spectro photometer in the range 200-1100 nm. Fluorescence spectrum was also recorded by using Varian Cary Eclipse Fluorescence Spectrophotometer.

Results and Discussion

Column chromatographic analysis

Column chromatographic analysis of the benzene extract of the galls of *Pongamia glabra* led to the isolation of compound TM1.

- Fractions first eluted with pet ether: benzene (80:20) and (30:70) gave a yellow residue. It was a mixture of six compounds.
- Fractions eluted with pet ether: benzene (30:70) and (10: 90) gave a pale white residue. It was a mixture of 3 compounds.
- Fractions eluted with benzene (100%) gave a crystalline white substance designated asTM1 and it was found to be homogenous on TLC..

Compound TM1 was characterized by preliminary tests and UV, IR and PMR spectral data.

Preliminary tests on TM1

Colour test

Preliminary colour test were performed by standard

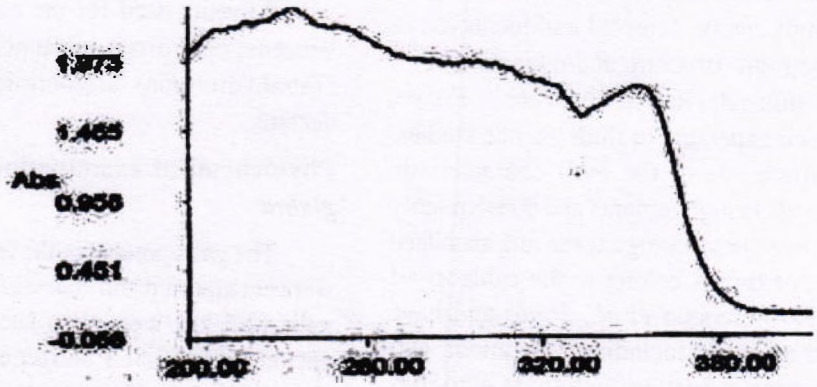


Fig. 1: The UV spectrum of TM1

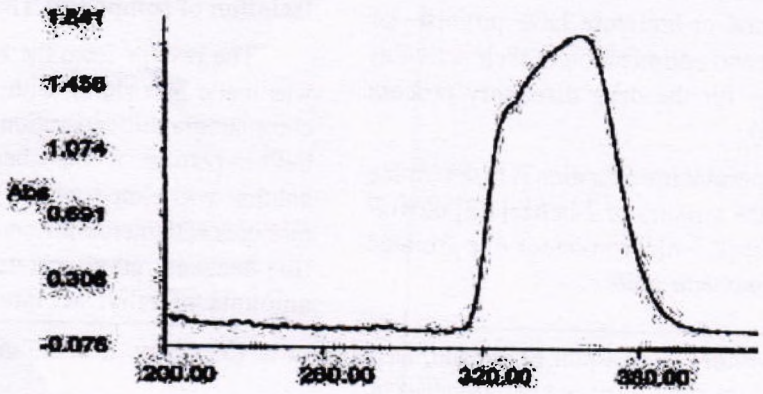


Fig. 2: The effect of mild base NaOMe on the UV absorption of TM1

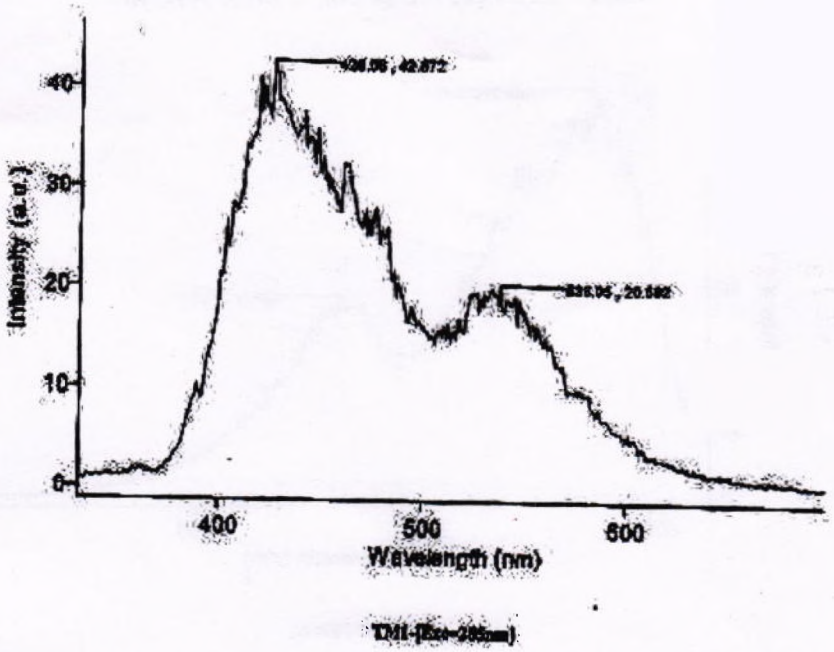


Fig. 3: Fluorescence spectrum of TM1 at excitation wavelengths 285 nm

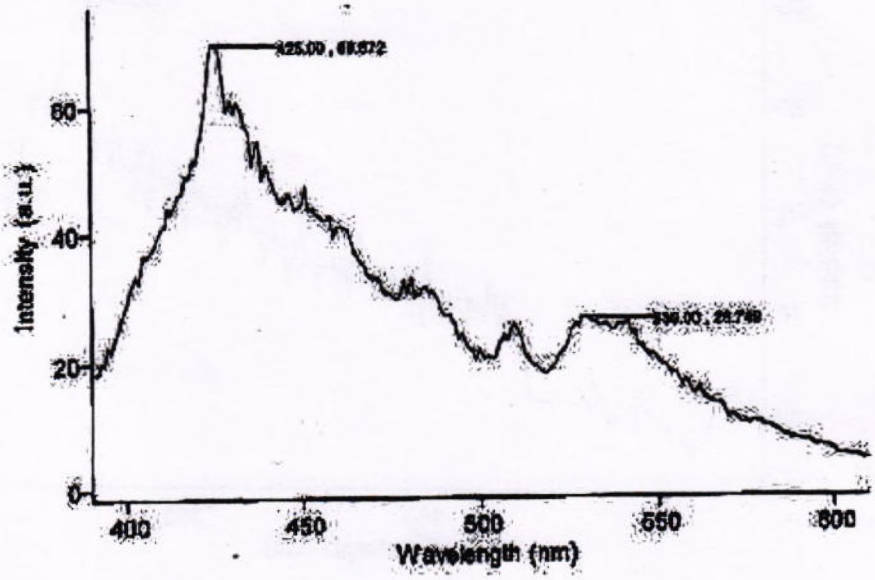


Fig. 4: Fluorescence spectrum of TM1 at excitation wavelengths 373 nm

procedures (Harborne, 1998) for the isolated compound TM1. The results of the colour tests performed with TM1 are given in following table 1.

Physical measurements of TM1

Melting point: TM1 - 222° C

Rf value:TLC of TM1 showed an R_f of 0.64 in benzene: ethyl acetate (9:1) solvent system.

Solubility :TM1 showed good solubility in chloroform and acetone and was partially soluble in ethanol. ethyl acetate benzene.

UV spectrum of TM1

The UV spectrum of TM1 is represented by Fig (1). The effect of mild base NaOMe on the UV absorption of TM1 is represented by Fig (2).

Fluorescence spectrum

Fluorescence spectrum was obtained for the compound TM1 at excitation wavelengths of 285, 373, 360, 426 nm represented by Fig (3, 4, 5, 6).

IR spectrum of TM1 (Fig.7)

The IR data obtained is (in cm^{-1}): 1246, 2912, 2852, 1134, 2797, 1112, 1740, 1065, 1629, 1030, 1581, 904, 1495, 850, 1443, 804, 1399, 746, 1374, 1338, 670.

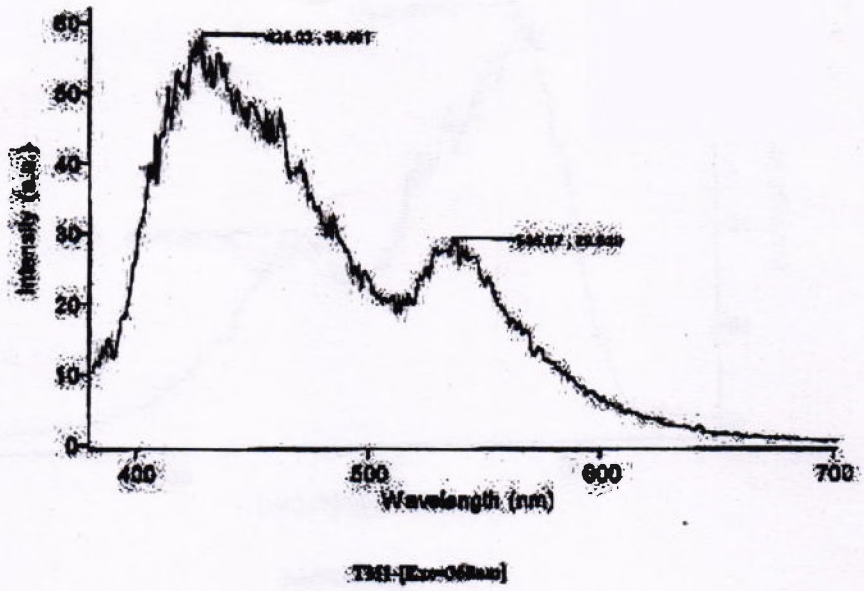


Fig. 5: Fluorescence spectrum of TM1 at excitation wavelengths 360 nm

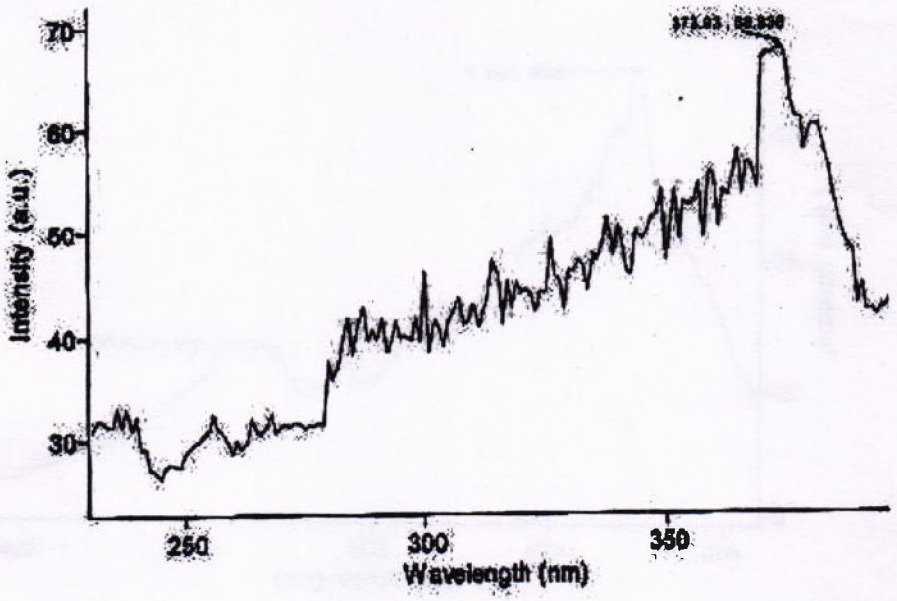


Fig. 6: Fluorescence spectrum of TM1 at excitation wavelengths 426 nm

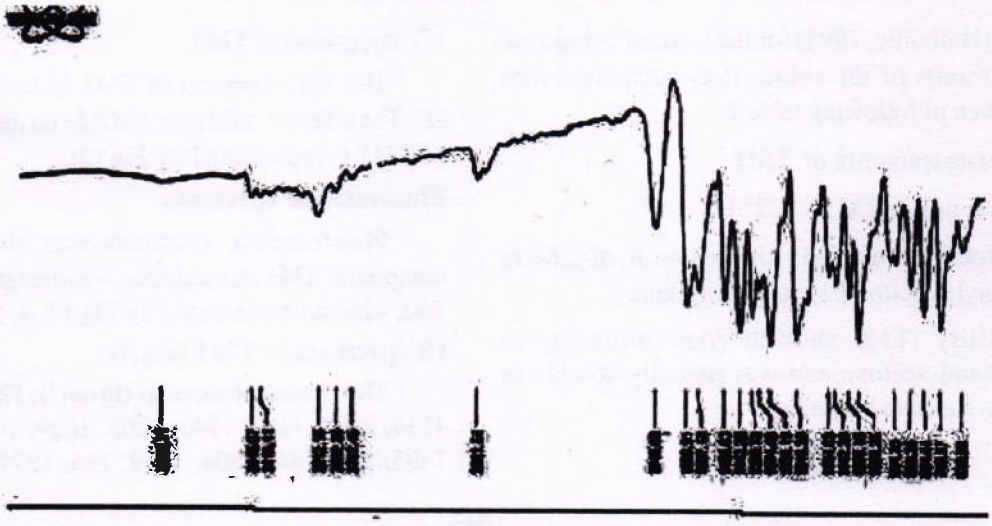
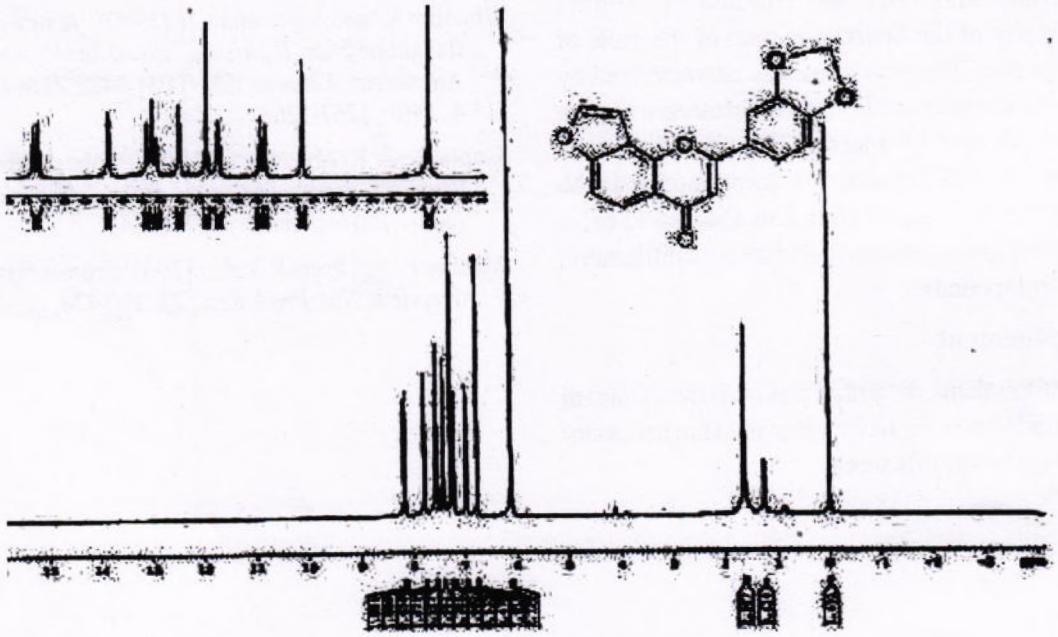


Fig. 7: IR spectrum of TMI

Table 2: PMR spectral data of TM1

Proton position	TM1	Proton position	TM1
2	-	1'	-
3	6.76(s)	2'	-
4	-	3'	7.39-7.41(d)
5	8.14-8.17(d)	4'	-
6	6.95-6.98(d)	5'&6'	7.51-7.57(m)
7	-	1''	-
8	-	4''	7.77-7.78(d)
9	-	5''	7.20-7.21(d)
10	-	O-CH_2-O	6.10(s)

s-singlet: d-doublet: m-multiplet



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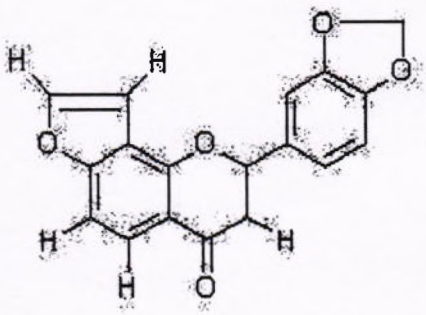
The structure of TM1 given below has been characterized by an analysis of its UV, IR and PMR spectral data:

The UV absorption spectrum of TM1 showed the flavonoidal nature of the compound Fig (1) with wavelength around 348 nm. In presence of base NaOMe, there was not much shift in the absorption showing the absence of hydroxylation Fig (2).The fluorescence excitation spectra pf TM1 showed the maximum at 426 nm (Fig 3,4,5). In contrast to the



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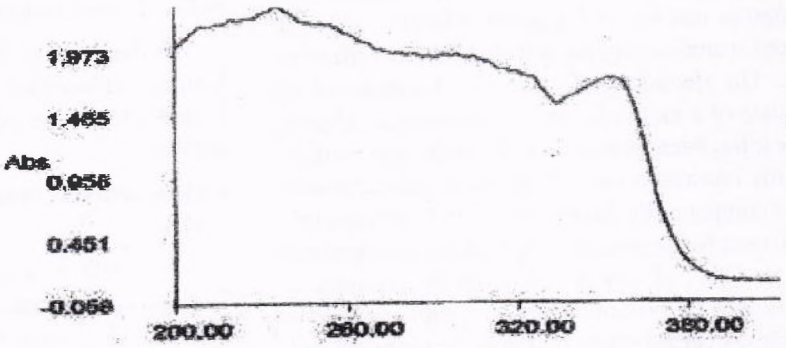


Fig. 1: The UV spectrum of TM1

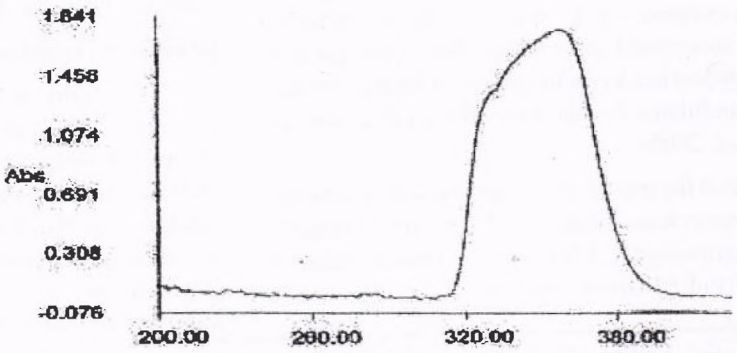


Fig. 2: The effect of mild base NaOMe on the UV absorption of TM1

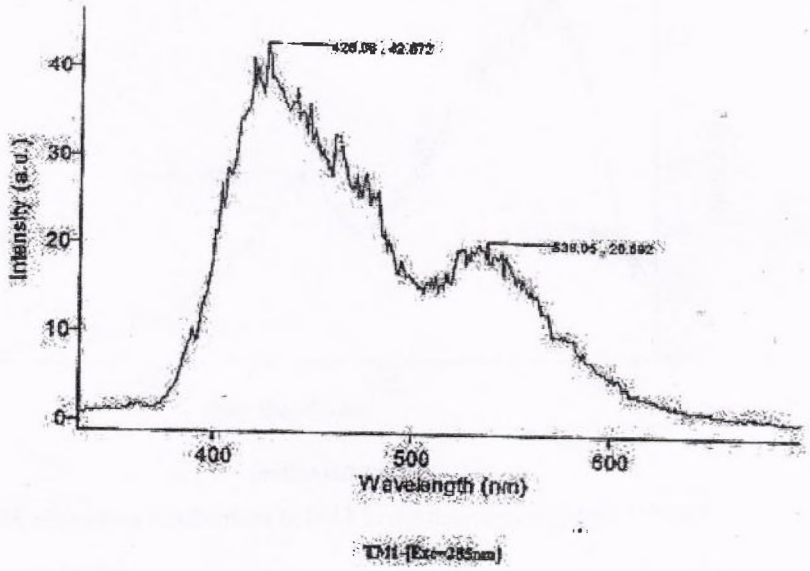
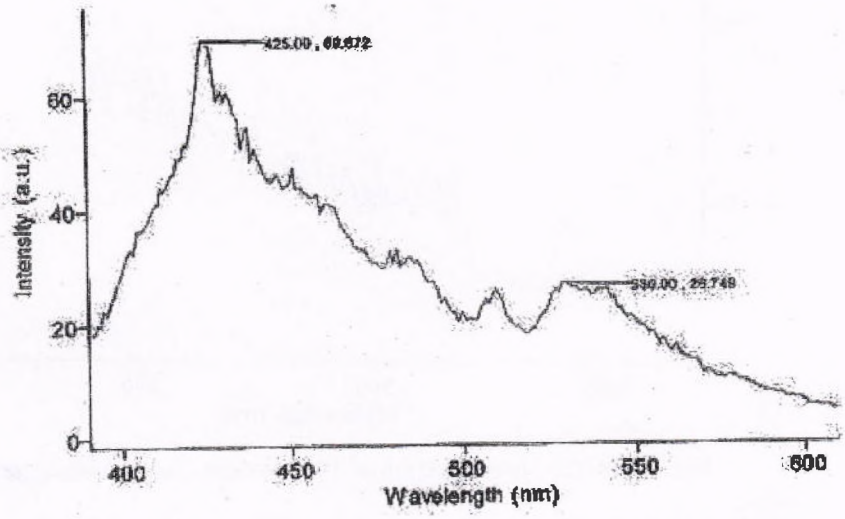


Fig. 3: Fluorescence spectrum of TM1 at excitation wavelengths 285 nm



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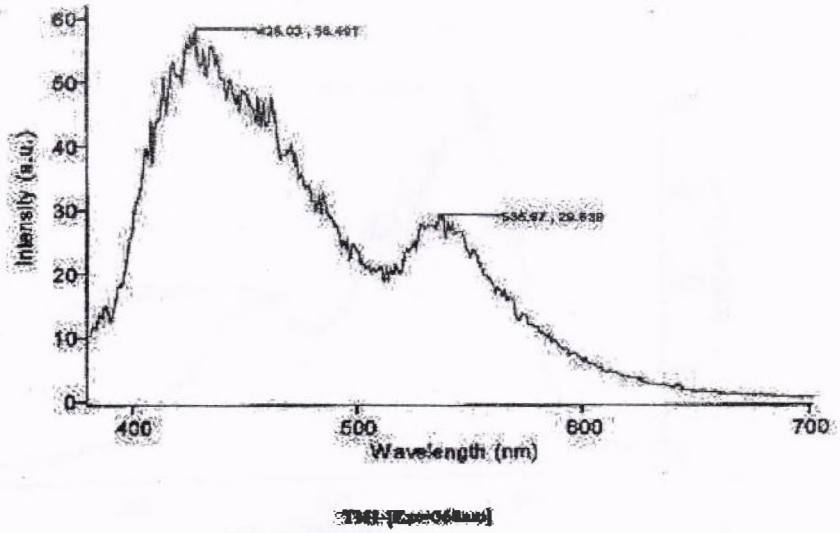


Fig. 5: Fluorescence spectrum of TM1 at excitation wavelengths 360 nm

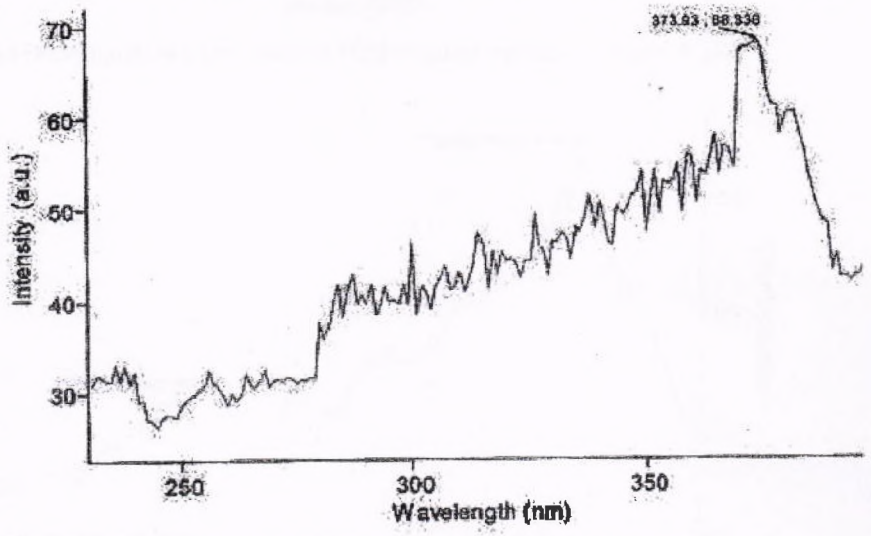
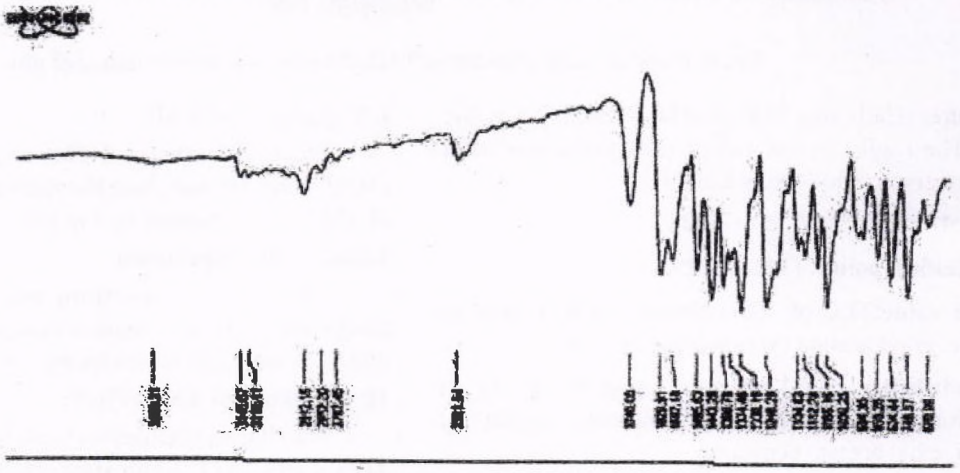


Fig. 6: Fluorescence spectrum of TM1 at excitation wavelengths 426 nm



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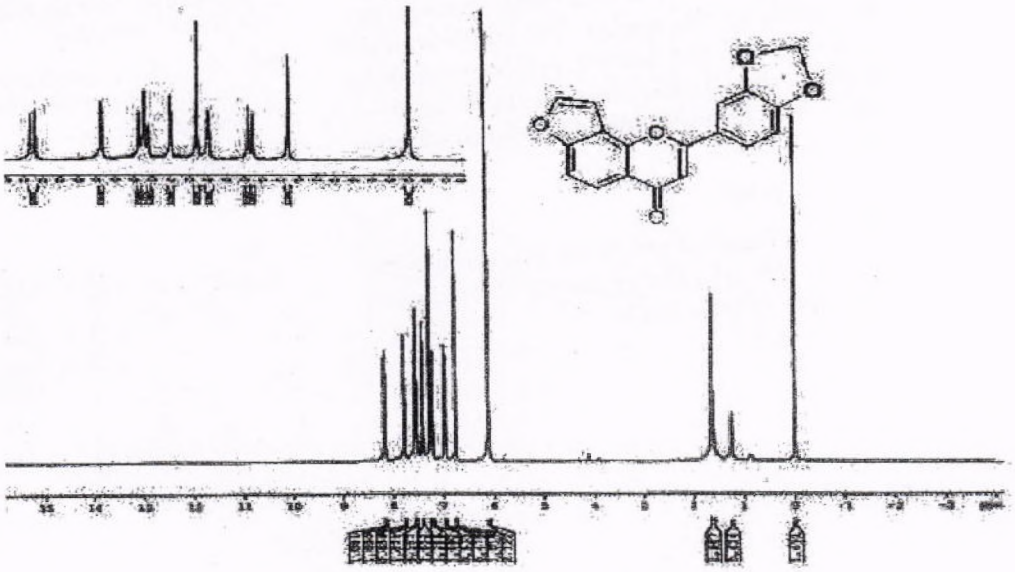

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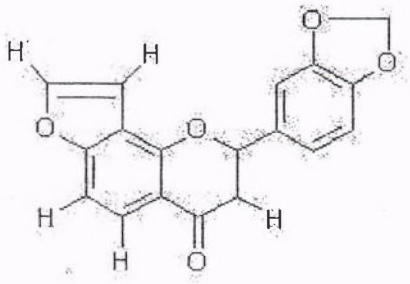

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