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Characterization of the growth promoting substances from *Lantana camara* L. influencing growth in rice and other crops

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Abstract
Two different groups of natural products with growth promoting activity were extracted from the pink and white flowered variety of *Lantana camara* L. One was a gibberellin-like compound with a molecular weight of 468 and m. p. of 227.5 ºC while the other was a phytosterol with molecular weight of 430 and melting point 98.8ºC. The two fractions in combination, acted synergistically and positively influenced the growth and yield of rice.

Keywords: growth - promoting activity, *Lantana camara*, gibberellin-like, phytosterol

Introduction
Increased agricultural productivity is largely dependent on high levels of chemical inputs in the form of fertilizers and pesticides. This trend is not only expensive but also habitat damaging. Currently there is a move towards more sustainable agricultural systems which emphasizes the use of bio-fertilizers and bio-pesticides. Growth promoting natural products of *Lantana camara* L. fruit extract had been observed to significantly affect growth and yield of important crop plant by Roy and Mukherjee (1982). Keeping this in view, a study was originally undertaken to exploit this activity, for improvement in growth and yield of other useful crops, if present from other parts (leaves and young stems) of this weed, rich in several other natural products *viz*. alkaloids, terpenoids etc. It was found, in an earlier study that the crude extract from young leaves of *Lantana camara* (with orange-brown flowers) considerably increased growth and yield of rice under field conditions. The growth promoting compound on analysis was found to be a gibberellin-like compound with molecular weight of 410 (Sukul and Chaudhuri 2001) [7]. Biswas and Chaudhury (2004) in a subsequent study observed a similar effect of the crude extract of leaves of *L. Camara* with pink and white flowers also, which was found to significantly increase rice growth and yield. An attempt was made to characterize the compound (s) from the pink and white flowered plant leaves which is being presented in this communication.

Materials and Methods
Young leaves (1 kg) of *L.camara* (pink-white flower) were sun-dried, powdered, and extracted in a Soxhlet apparatus for 48 hours. The defatted leaf powder was soaked in 95% ethanol for 7 days and the homogenate filtered and charcoalized. The charcoal (F1) and the residual (F2) portions were separated and each further extracted with several solvents viz. petroleum ether (PE), chloroform (CH), ethyl acetated (EA), methanol (ME), acetone (AC) and water(AQ). Each fraction was subjected to rice seeding bio-assy. The acetone (F1AC) and chloroform (F2CH) fractions which showed significant growth promoting activity, were further tested for the nature of the compound (S) persent. These two fractions were separated by TLC. The separated spots with different *Rf* values of each fraction was scraped, dissolved in its respective solvent and centrifuged at 5000 rpm for 20 minutes. Each of the supernatant was gain bio-assayed (rice grain germination). The spots, showing significant bio-activity were selected and characterized. UV absorbance (Shimadzu UV, 160A), IR (Perkin Elmer), Mass (Jeol D 300) and NMR (Bruker DRX-300) analysis was done. The IR spectrum of spots from the acetone fraction (F1AC) were compared with GA3 (Sigma) while those of the chloroform fraction (F2CH) with β-sitosterol. These compounds were further crystallized and their melting points determined.

Results and Discussion
Rice seedling bioassay (Table1) showed that fraction F1AC and only F2CH increased seedling height significantly while maximum increase in height was observed in seedlings where the two fractions were sprayed in combination. The TLC plates of F1AC showed characteristic green-blue fluorescence of gibberellins (*Rf 0.63)* (Table 2). The UV spectrum had a maximum absorbance at 260.5nm suggesting the presence of an organic molecule (Fig1).The principal peaks of the IR spectrum of this compound coincided with that of GA3 indicating that the compound may be a type gibberellin. The mass spectrum showed molecular ion peaks at m/z 233, 241, 248, 257, 285, 299, 309, 323, 339, 353, 365, 407, 423, 435, and 468 (Fig3). Hence from this data the indicated molecular weight of the compound is around 468. The NMR spectrum (Fig.4) showed chemical shifts of methyl (-CH3), methylene (-CH2),-CH, benzylic, alcohol and ester protons. The spectrum also showed the presence of a number of olefinic signals and an indication of an aromatic structure. The compound yielded crystals with petroleum ether and acetone and had a melting point of 227.5 ºC.

The TLC plates with F2CH showed characteristic orange fluorescence of a phytosterol (*Rf* 0.21) (Table 2). The UV spectrum showed maximum absorbance at 291.5 nm (Fig.5). The IR spectrum showed the major peaks coinciding with the standard β- sitosterol (Fig.6) suggesting the presence of a phytosterol. The mass spectrum of the compound showed molecular ion peaks at m/z 213, 215, 229, 241, 255, 271, 283, 299, 311, 339, 353 and 430 (Fig.7) suggesting the molecular weight of the compound to be 430. The NMR

spectrum (Fig.8) showed the chemical shifts of methyl, methylene, -CH, ester, benzylic and alcohol protons as well as olefinic signals and an aromatic structure. The compound yielded crystals with chloroform and methanol and had a melting point 98.8ºC.

Grunwald (1975) [3] suggested that plant sterols might act as hormones or more likely be precursons to steroids which act as hormones. Steroid effects may be brought about indirectly through effects on auxins and gibberellin levels. Both steroids and gibberellins are derived from mevalonic acid (Fieser and Fieser 1949) [2]. Thus, the occurrence of both a gibberellins-like compound and a phytosterol in the leaves of *L.camara* is not unusual. In addition, phytosterols like brassinosteroids and tricontanol have been reported to have growth- promoting activity in plants (Mitchell *et al*.1970, Ries and Wert 1982) [5]. Hence, in this instance, the phytosterol compound has a growth promoting activity independent of the gibberellins like compound as evidenced by the bioassay tests.

The range of molecular weights of the 136 gibberellin known till date is between 400 – 470 in methyl ester form. Thus the molecular weight of the gibberellin obtained in the present study is within the above range. Among the phytosterols, the closest to the one present in *L.camara* is tricontanol which has a mol. wt of 438.83 and m.p of 86-87ºC, though they may be different compounds.

Table 1: Rice seedling bioassay test of some promising sub-fractions

Treatment	Rice cv.TN(1) seedling height (cm)		
	Conc. Of sub-fraction (ppm)		
	25	50	100
FII_{CH}	6.5	7.2	9.0
FI_{EA}	6.2	6.6	6.7
FI_{AC}	8.3	9.7	12
FI_{ME}	6.0	6.2	6.7
FII_{CH}+$F1_{AC}$	9.5	11.3	15
Control	6.0	6.0	6.0

Mean of 30 observations.

Table 2: Detection of the nature of compounds in FI_{AC} AND $F2_{CH}$

Fraction	Nature of compound	Fluorescence	Rf value	Solvent system
$F1_{AC}$	Gibberellin	Green -blue	0.63	Benzene:n-butanol:acetic acid (70:25:5)
$F2_{CH}$	Phytosterol	Orange	0.21	Benzene:ethyl acetate(9.1)

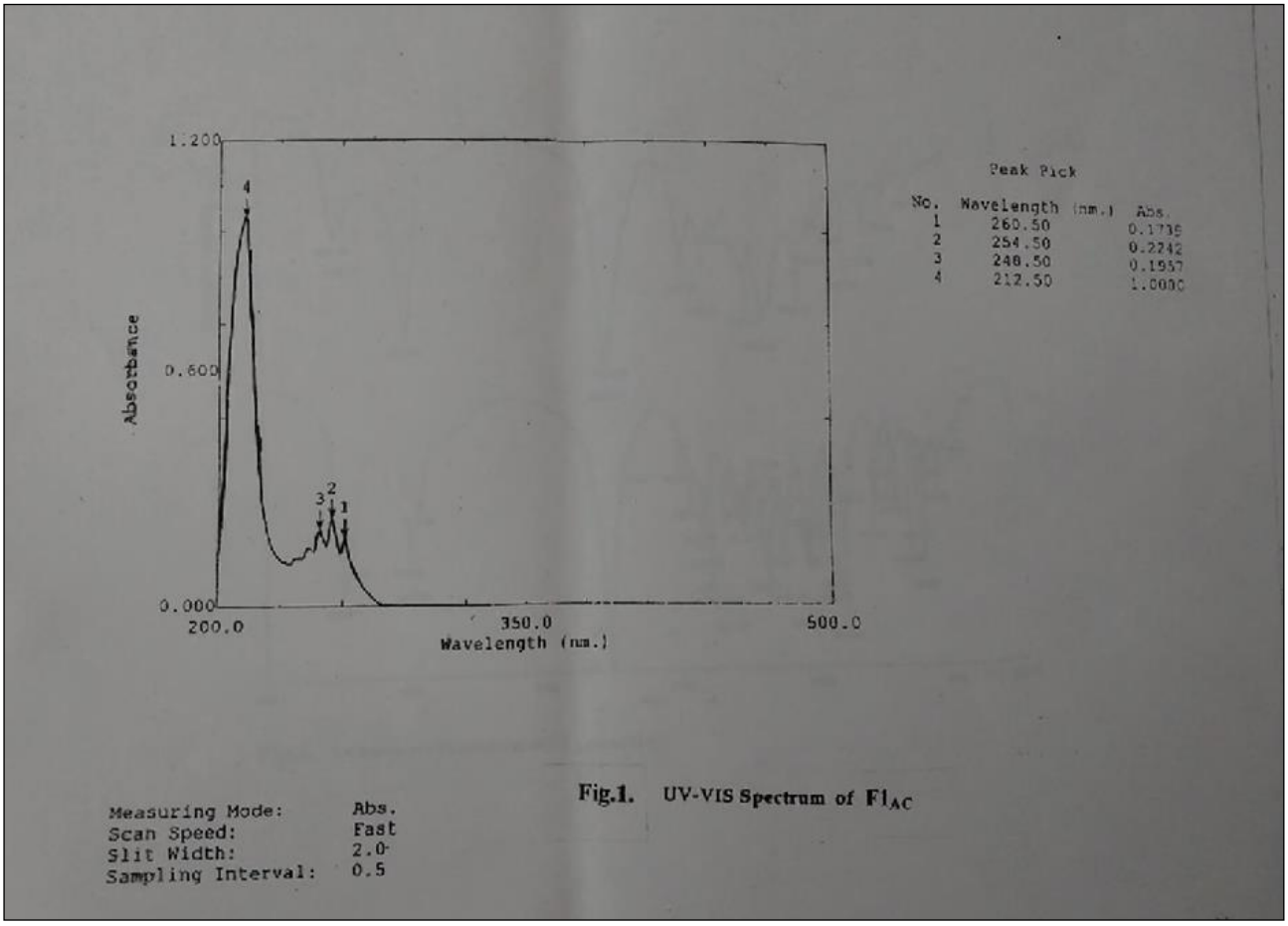

Fig.1. UV-VIS Spectrum of $F1_{AC}$

Fig 1: UV-VIS Spectrum of $F1_{AC}$

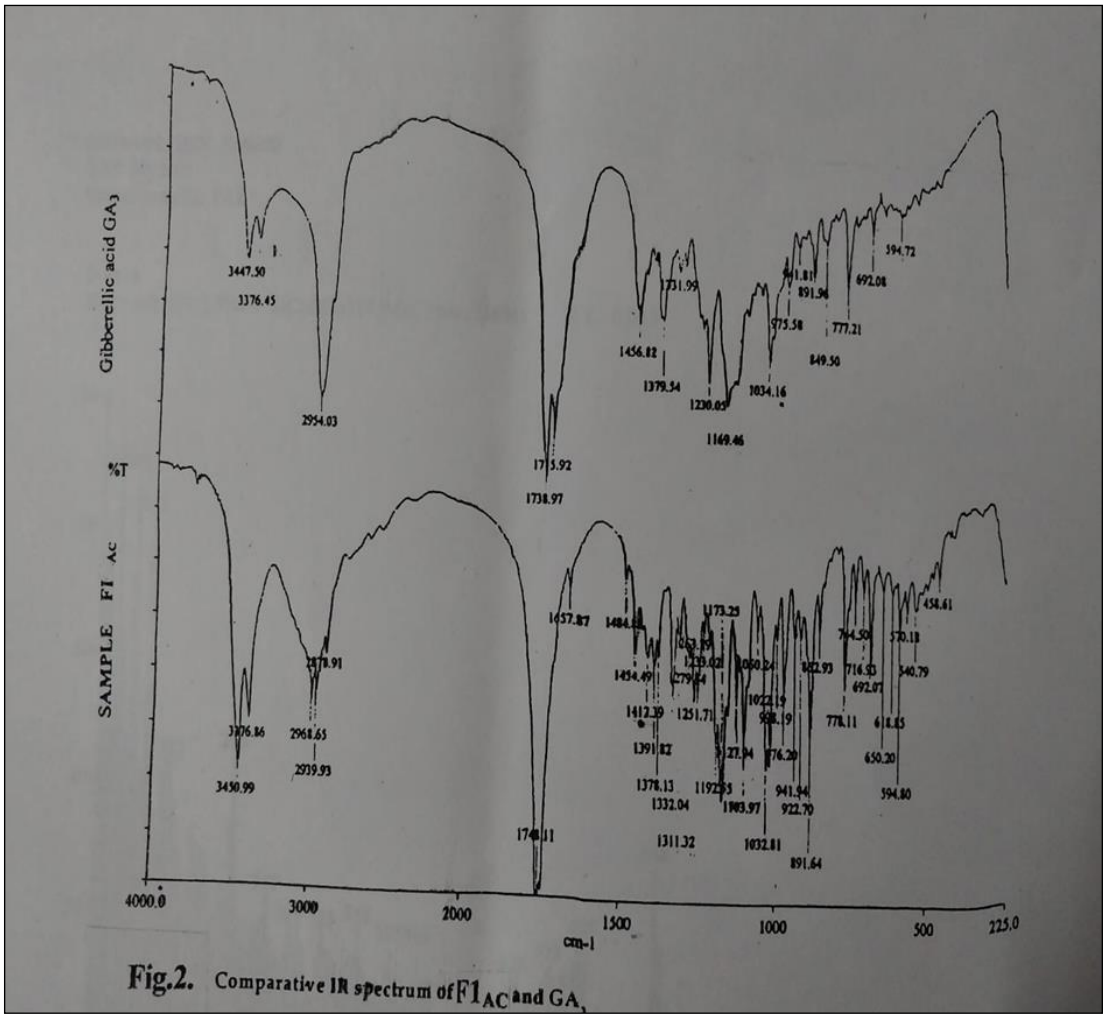

Fig 2: Comparative IR Spectrum of $F1_{AC}$ and GA_3

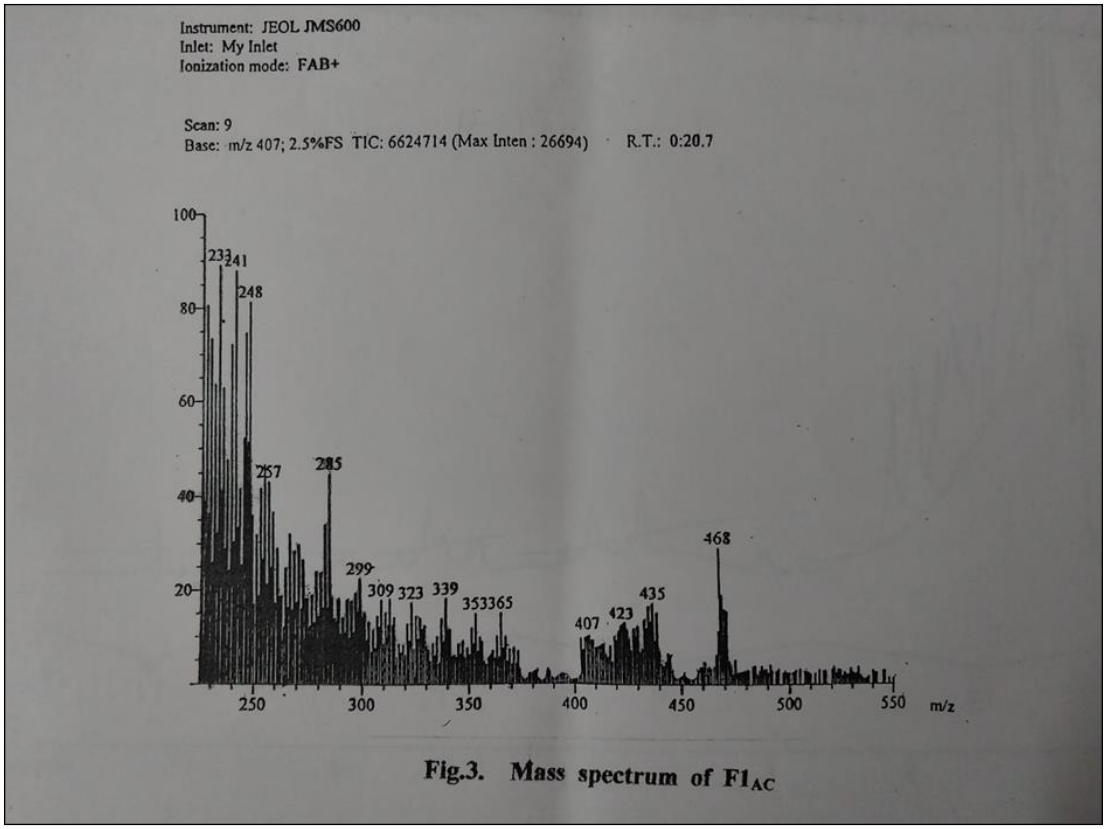

Fig 3: Mass spectrum of $F1_{AC}$

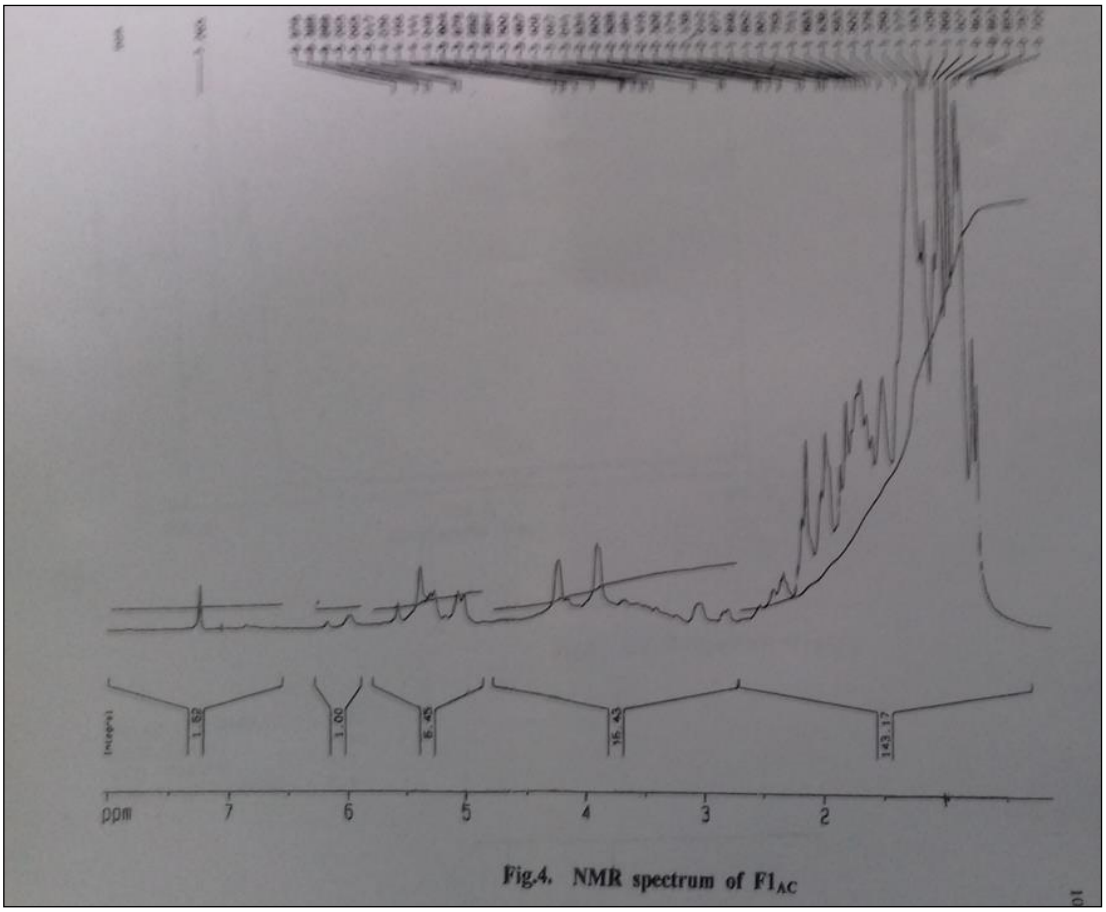

Fig 4: NMR spectrum of F1 $_{AC}$

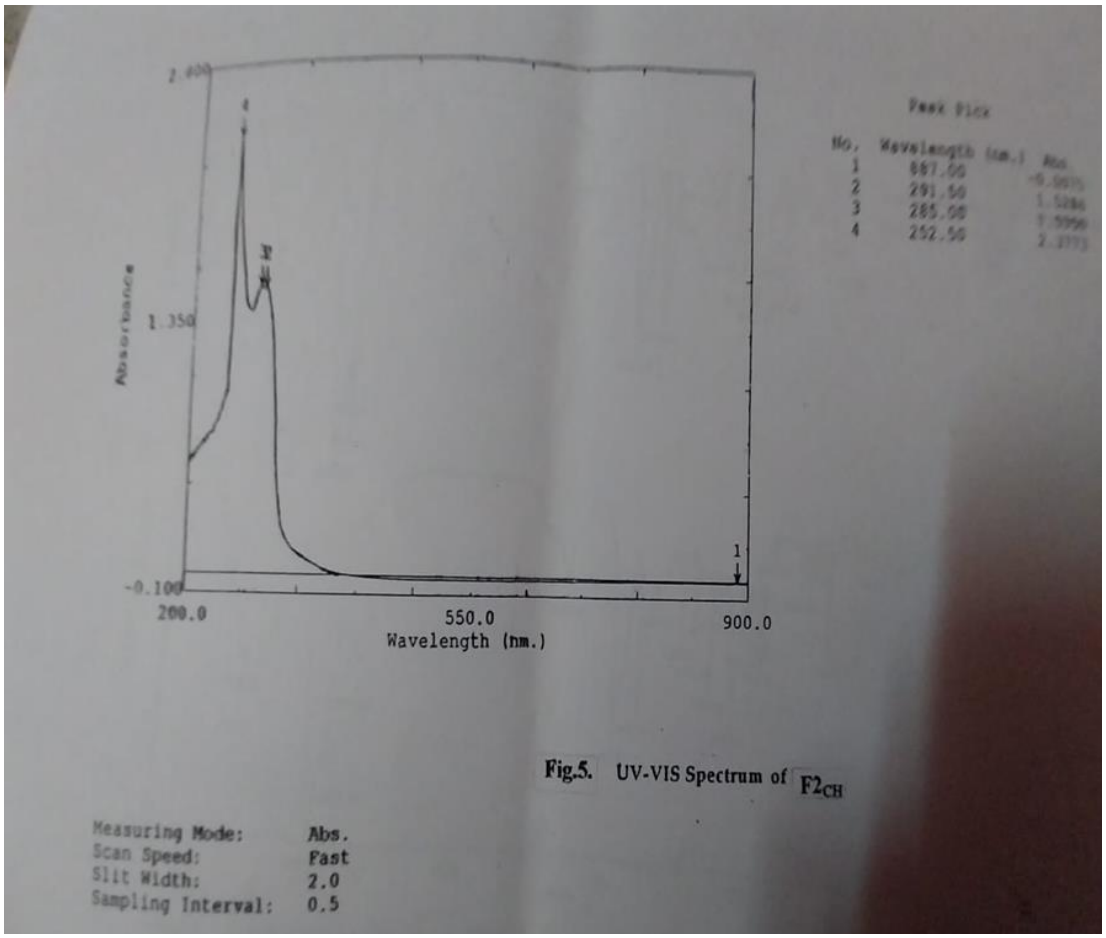

Fig 5: UV-VIS Spectrum of F2$_{CH}$

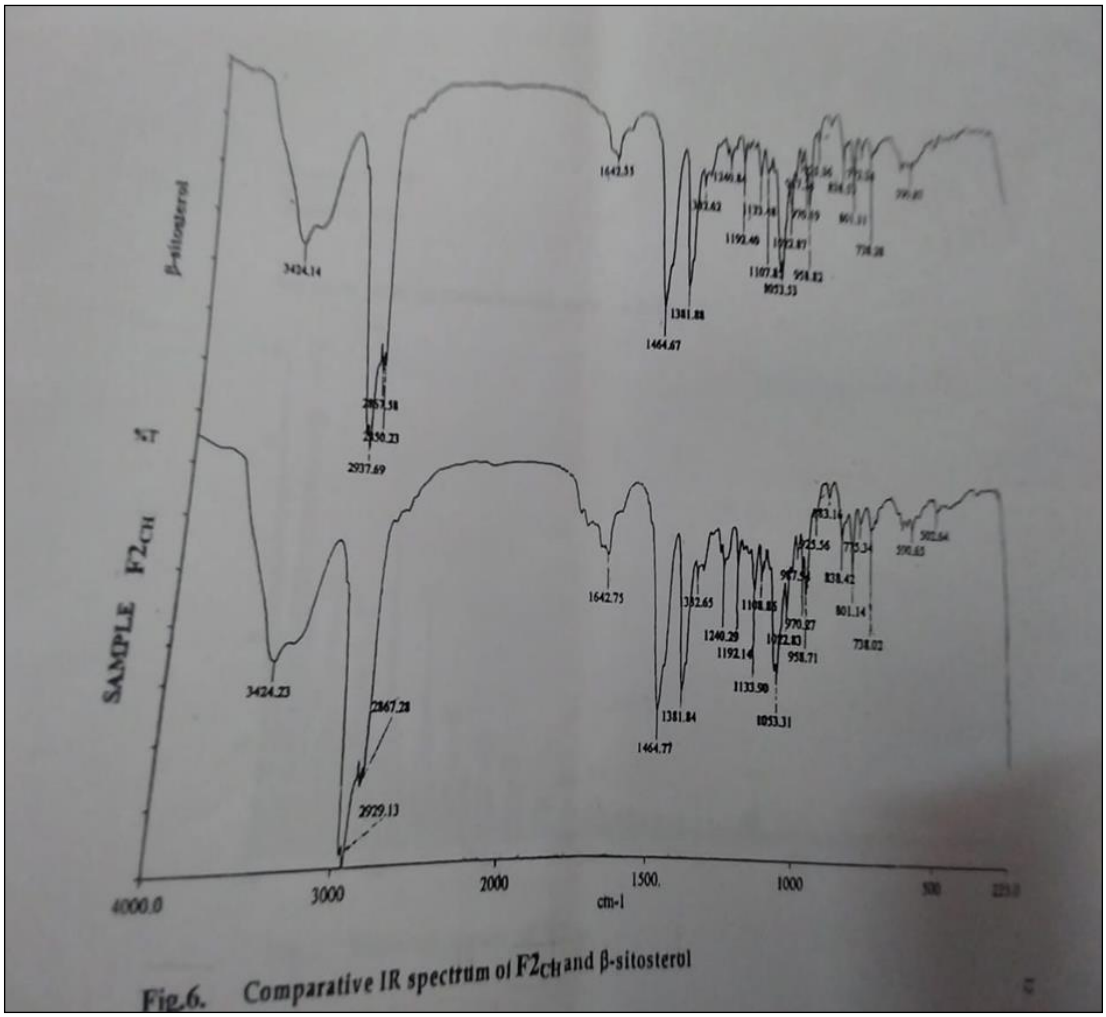

Fig 6: Comparative IR spectrum of $F2_{CH}$ and β- sitosterol

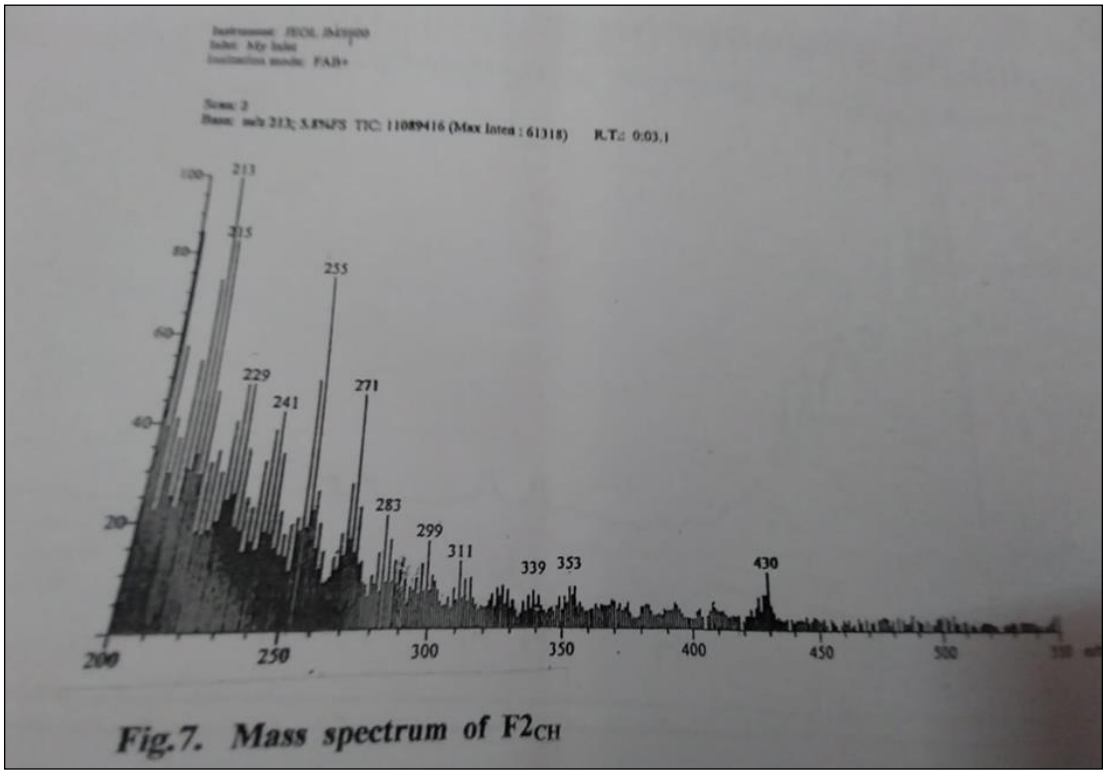

Fig 7: Mass spectrum of $F2_{CH}$

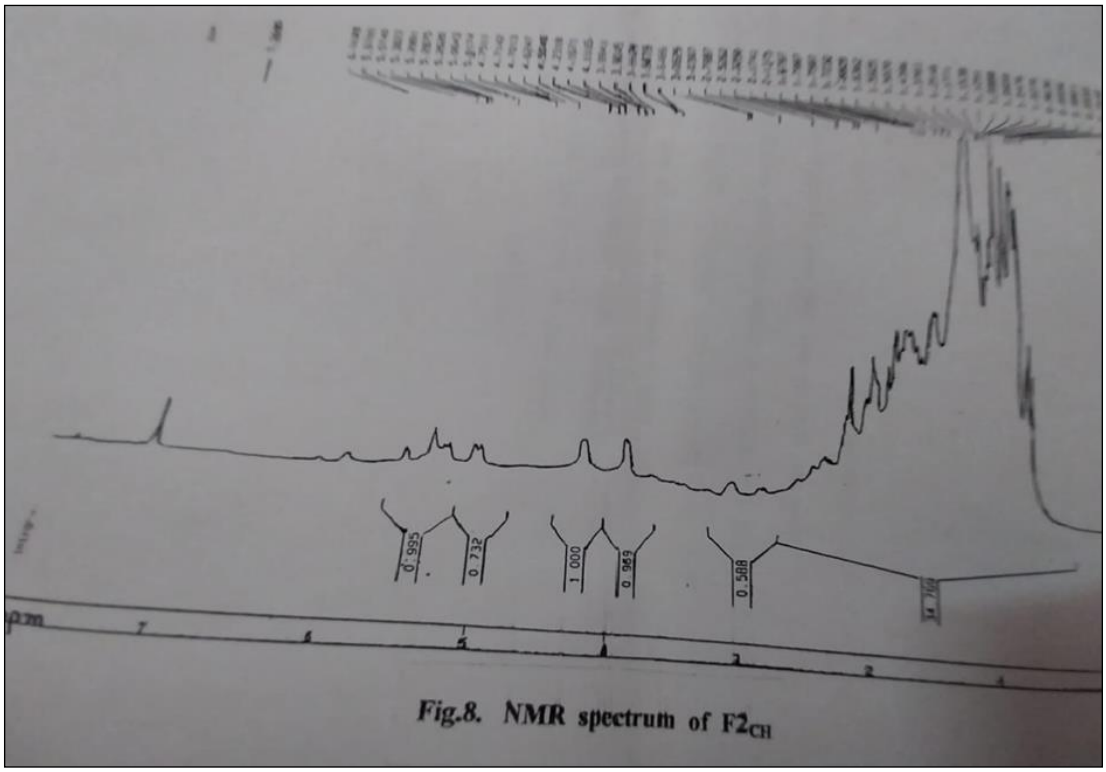

Fig 8: NMR spectrum of $F2_{CH}$

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