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Research Article

# CRITICAL ANALYSIS ON QUANTIFICATION, SEQUESTRATION AND SPECTRAL CHARACTERIZATION OF β-SITOSTEROL IN HEXANE EXTRACT OF MIMOSA PUDICA

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**ABSTRACT**

*Mimosa pudica* is a plant species under the family fabaceae which have been used as a folk medicine in curing assorted ailments. The endeavor of this study was to quantify, isolate and characterize the therapeutically important bioactive constituent β-sitosterol present in n-hexane extract of whole plant of *Mimosa pudica*. The HPTLC scrutiny was done to quantify the amount of β-sitosterol in whole plant extract, which yields 2.901 mg/g. Further, isolation was carried out by preparative TLC with reference standard. The compound was scrapped in accordance with the Rf value of standard. The physical and chemical properties of isolated compound were compared with standard β-sitosterol for identification. The UV spectroscopy, Fourier-transform infrared spectroscopy, $^{1}$H NMR and $^{13}$C NMR analysis was carried out for characterization of isolated compound. The interpretation data obtained through spectral analysis shows the nature of carbon and hydrogen atoms which confirms that the isolated compound was β-sitosterol.

**Keywords**: *Mimosa pudica*, β-sitosterol, HPTLC, UV, FT-IR, $^{1}$H NMR, $^{13}$C NMR.

## INTRODUCTION

The medicinal plant comprises of bioactive constituents that progress the physiological balance of human beings and the facts of these healing properties have been passed down through generations. [1]The plant is rich in abundant therapeutic chemical constituents like alkaloids, flavanoids, tannins, β-sitosterol, stigmasterol, leucoanthocyanidin, linoleic acid, oleic acid, etc.

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The β-sitosterol is a phytosterol or plant sterol in *Mimosa pudica* majorly used for lowering cholesterol. It also act has an antioxidant, anticancer, antiulcer, anti-diabetic, anti-inflammatory, antipyretic and for wound healing The phytochemical assessment is quite necessary to generate assurance about the separation, purification and identification of adulterated and substituted medicinal plants. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents, therefore phytochemical validation is crucial for standardization of traditionally used medicinal plants. The existent inquisition deals with quantification, separation, isolation and characterization of hexane extract of *Mimosa pudica*.

## MATERIALS AND METHODS

### Collection and authentication

The whole plants of *Mimosa pudica* were collected from Cheranmadevi, Tirunelveli district and were authenticated by Dr S. Mutheeswaran M.Sc., M.Phil., Ph.D, Scientist, Centre for Biodiversity and Biotechnology, Xavier Research Foundation, St Xavier's College, Palayamkottai, Tamil Nadu. The collected plants were air dried, powdered, sieved, weighed and stored in air tight container.

### Preparation of extract

Extraction of medicinal plants is a process of separating active plant materials using an appropriate solvent and standard extraction procedure.[2] Phytosterols are usually extracted by soxhlet extraction (Hot continuous extraction) and maceration (single continuous extraction). The most widely used solvents for the extraction of Phytosterols are n-hexane, petroleum ether, ethanol, and methylene chloride. Phytosterols are lipophilic in nature and are freely soluble in non-polar solvents [3]. Reminiscing survey of disputes, maceration method and n-hexane solvent is taken as a choice for better yield. The whole plant powder were dissolved in n-hexane and subjected to maceration for 72 hrs. The macerate was filtered and concentrated to yield extract. The concentrated extract was collected, weighed and stored in an air tight container.

### Chemicals and reagent

Standard compound β–sitosterol was purchased from Sigma-Aldrich. Other chemicals such as n-hexane, diethyl ether, methanol, anisaldehyde sulphuric acid were procured from Merck India LTD. Analytical TLC plates used in the studies were Silica gel 60 F254 TLC plate were procured from Merck, Darmstadt, Germany.

### Quantification of β-sitosterol by HPTLC

### STD Preparation

1 mg of β-Sitosterol was dissolved in 1ml of methanol and further diluted with methanol to obtain 0.2 µl, 0.4 µl, 0.6 µl, 0.8 µl and 1.0 µl.

### Sample preparation

100mg of hexane extract of *Mimosa pudica* was dissolved in 1 ml of methanol and further diluted with methanol to obtain 3 µl, 3 µl, 6 µl, 6 µl.

### Procedure

High performance thin layer chromatography was performed on an aluminum sheet precoated with silica gel 60 F254 HPTLC plates. The STD and Samples were applied on the plates as bands of 7mm width with the help of a Camag Linomat IV sample applicator at the distance of 15mm from the edge of the plates. The plates were developed to a distance of 80 mm in a Camag twin trough chamber previously equilibrated with mobile phase for 20 min. Toluene: diethyl ether (5: 5 v/v) was used as a solvent system. After development, the plate was air dried at room temperature and derivatized with freshly prepared Anisaldehyde reagent in a derivatization chamber for 20 secs and dried at room temperature. After drying, plate was heated in oven at 105°C for 10min before densitometric scanning. Densitometric evaluation of the plates was performed at ʎ = 520nm with a Camag Scanner II in conjunction with winCATS 1.4.3 software for quantification.

### Isolation of β-sitosterol by preparative TLC

1 mg of standard β-Sitosterol was dissolved in 1ml of methanol. 100mg of hexane extract of *Mimosa pudica* was dissolved in 1 ml of methanol. The hexane extract of *Mimosa pudica* were dissolved in the methanol of required solubility and this solution was spotted on TLC plates against reference standard. Then the TLC plates were run by specific solvent system, toluene: diethyl ether (5:5 v/v). The plate was then sprayed with 50% Anisaldehyde sulphuric acid reagent and heated in a hot air oven until the appearance of spot. After identification of specific spot of β-sitosterol it was subjected to preparative TLC. In preparative TLC (20x20 cm), concentrate sample was loaded along with band spotting was done so that highest amount of sample can be placed on the plates which facilitate the maximum separation of the specific compound. The plate was allowed to run in the mobile phase. The plate was dried after complete development. The confirmed spot with those of standard reference compound was noted down. Analogous spot was separated out at 0.56 Rf (violet). The band that shows the 0.56 Rf was marked and the compound was scrapped out and collected separately along with the silica gel along. The separated compound was redissolved in methanol. Subsequently the solution was filtered and the solvent was evaporated to obtain the purified isolated compound.

### Spectral analysis

The isolated compound was dissolved in methanol and subjected to UV to absorb maximum absorption at a wavelength of 292nm. Then FT-IR, $^{1}H$ NMR and $^{13}C$ NMR analysis were performed using the solvent DMSO. The peaks were recorded and the data obtained through spectrometric analysis were compared with the reference standards and literatures.

## RESULTS

### HPTLC quantification of β-sitosterol

The HPTLC visualization plate for STD and sample after the development of spot was plated in figure 1. The fingerprinting 3D display @520 nm were figured in figure 2. The peak of β-Sitosterol was obtained at Rf = 0.56. The typical HPTLC chromatogram of β-sitosterol in

STD and sample at peak display 520nm were given in figure 3 & 4. The overlay spectral display for STD and sample were determined. The typical absorption spectrum obtained from Standard and from in sample and STD were recorded in figure 5 and 6. The linear calibration curve was drawn (figure 7) and the linearity range was found between 200 to 1000 ng for β-sitosterol. The area under the curve for STD (0.2 µl, 0.4 µl, 0.6 µl, 0.8 µl and 1.0 µl) and sample (3µl and 6µl) were found to be 2408.47, 3872.27, 5309.9, 5810.73, 7332.31, 3149.80 and 3091.0 respectively. The Regression Equation (y = a + bc) for β-sitosterol calculated by the calibration curve gives Y = 1410.898 + 5.893*X with a Correlation coefficient r2 =0.9902. The calculated standard deviation was found to be 6.12%. With the above results the quantity of β-sitosterol present in n-hexane extract of *Mimosa pudica* was yields 2.901 mg/g of β-sitosterol .

**Track 1-5** contains β sitosterol standard at varying concentration 0.2, 0.4, 0.6, 0.8, 1µl respectively.
**Track 6-9** contains HEMP contains with concentration 3.0, 3.0, 6.0, 6.0µl respectively.

**Isolation and characterization of β-sitosterol**

The results of the study revealed by physical nature and chemical test confirmed that the compound was steroidal in nature. The physical parameters for the isolated compounds were predicted in **Table 1.** The isolated compound was subjected to chemical test (Liebermann's Burchard's) to identify the phytoconstituents. The test shows green colour which indicates the presence of phytosterols in isolated compound. TLC was performed to identify the phytoconstituents in which the isolated compound exhibits Rf value 0.56 that the correlates with the Rf value of reference standard β-sitosterol.

**Spectral analysis**

**UV spectroscopy**

The UV spectroscopy determines the quality of the compound by absorption value. The spectrum of isolated compound obtained at a wavelength of 292 nm shows a maximum absorption peak at 0.698.The isolated compound shows maximum absorption at 0.698 at a wavelength of 292 nm. The recorded spectrum was given in figure 2.

**FT-IR**

The literature of analysis reveals a broad peak at 3549.99 cm−1 for the OH group, 2935.73 cm−1 for the CH2 group, 2867.38 cm−1 for CH group, 1637.63 cm−1 for the C=C group, and 1063.34 cm−1 for the C–O group. [4] The molecular weight determination indicates $C_{29}H_{50}O$ as its molecular formula. Similar results were observed for isolated compound in which IR peaks were obtained for OH at 3294.78, CH3 at 2864.74, CH2 at 2935.13, unconjugated olefinic (C=C) at 1701.87, bending OH group at 1460.81, isopropyl group at 1375.96 and C–OH of secondary alcohol at 1329.68. The obtained peaks also matches with the literature [5] tabulated in **table 2.**

**NMR spectroscopy**

The $^{1}H$ NMR and $^{13}C$NMR analysis was performed for the identification of phytosterols in an isolated compound. The spectra were recorded by using DMSO as a solvent. The values of chemical shifts to specify the carbon and hydrogen atoms have been discussed according to the **[5] [6]** and summarized in **Table 3**. The $^{1}H$ NMR spectrum (figure 9**)** showed the presence of fifty hydrogen which comprises of 6 (CH3), 11(CH2), 9(CH) and 1(OH) groups). The $^{13}C$ NMR spectrum analysis shows 29 carbons with 6(CH3), 10(CH2), 10(CH) and 3(C) according to their chemical shift obtained through spectrum recorded in figure 10. The comparison of $^{1}H$ NMR and $^{13}C$NMR with reference to literature was illustrated in table 3.

**Table 1: Physical parameters of the isolated compound**

| S. No | Properties | Isolated compound | STD β-sitosterol |
|---|---|---|---|
| **1.** | Nature | Crystalline | Crystalline |
| **2.** | Colour | White | White |
| **3.** | Melting point | 141°C | 143.5 °C |
| **4.** | Rf value | 0.56 | 0.56 |
| **5.** | Solubility | Methanol > Acetone | Methanol > Acetone |
| **6.** | Density | 0.9 g/cm3 | 1 g/cm3 |

**Table 2: FT-IR bands for isolated compound.**

| IR bands ($cm^{-1}$) | Literature (Azeez et al., 20001) | Interpretations |
|---|---|---|
| 3294.78 | 3399.5 – 3328.28 | Stretching vibration of OH |
| 2864.74 | 3863.24 – 2990 | Stretching vibration of CH alkane |
| 2935.13 | 3110 | Stretching vibration of CH alkene |

| | | |
|---|---|---|
| 1701.87 | 1640 | Stretching vibration of C=C |
| 1460.81 | 1580 | Bending vibration of OH |
| 1375.96 | 1460.8 – 1374.30 | Bending vibration of isopropyl |
| 1329.68 | 1312.72 | Bending vibration of C-O of 2° alcohol |

**Table 3: $^{1}$H NMR and $^{13}$C NMR chemical shifts for isolated compound with literature**

| S.NO | Experimental | | Literature (Azeez et al., 2001) | | Literature (Mesfin Medihin ododo et al., 2016) | | Interpretation |
|---|---|---|---|---|---|---|---|
| Carbon atoms | $^{13}$CNMR | $^{1}$HNMR | $^{13}$CNMR | $^{1}$HNMR | $^{13}$CNMR | $^{1}$HNMR | Nature of carbon and hydrogen atoms |
| C-1 | 37.2 | | 37.39 | | 37.28 | | $CH_2$ |
| C-2 | 31.7 | | 31.76 | | 31.69 | | $CH_2$ |
| C-3 | 71.6 | 3.54 | 71.98 | 3.55 | 71.82 | 3.53 | CH |
| C-4 | 41.8 | | 42.39 | | 42.33 | | $CH_2$ |
| C-5 | 140.8 | 5.20 | 140.88 | 5.37 | 140.70 | 5.36 | C |
| C-6 | 121.8 | | 121.80 | | 121.72 | | CH |
| C-7 | 32.0 | | 32.06 | | 31.69 | | $CH_2$ |
| C-8 | 31.8 | | 29.30 | | 31.93 | | CH |
| C-9 | 50.8 | | 50.28 | | 50.17 | | CH |
| C-10 | 37.7 | | 36.65 | | 36.52 | | C |
| C-11 | 21.1 | | 21.22 | | 21.10 | | $CH_2$ |
| C-12 | 39.8 | | 39.92 | | 39.80 | | $CH_2$ |
| C-13 | 42.7 | | 42.39 | | 42.33 | | C |
| C-14 | 56.5 | | 56.91 | | 56.79 | | CH |
| C-15 | 25.9 | | 23.21 | | 24.37 | | $CH_2$ |
| C-16 | 30.4 | | 28.39 | | 28.25 | | CH |
| C-17 | 56.2 | | 56.21 | | 56.09 | | CH |
| C-18 | 12.0 | 0.80 | 12.12 | 0.70 | 11.86 | 0.63 | $CH_3$ |
| C-19 | 19.3 | 1.15 | 19.18 | 1.03 | 19.40 | 1.01 | $CH_3$ |
| C-20 | 36.1 | | 36.29 | | 36.52 | | CH |
| C-21 | 19.3 | 0.92 | 18.92 | 0.95 | 18.79 | 0.93 | $CH_3$ |
| C-22 | 33.9 | | 34.09 | | 33.98 | | $CH_2$ |
| C-23 | 26.4 | | 24.44 | | 26.14 | | $CH_2$ |
| C-24 | 46.1 | | 45.98 | | 45.88 | | CH |
| C-25 | 27.2 | | 26.23 | | 28.91 | | CH |
| C-26 | 21.0 | 0.86 | 19.96 | 0.87 | 19.80 | 0.84 | $CH_3$ |
| C-27 | 19.4 | 0.82 | 19.18 | 0.85 | 18.79 | 0.83 | $CH_3$ |
| C-28 | 21.0 | 0.80 | 22.83 | | 23.10 | | $CH_2$ |
| C-29 | 12.2 | | 9.69 | 0.84 | 11.99 | 0.81 | $CH_3$ |

**Figure 1: HPTLC visualization of STD and sample**

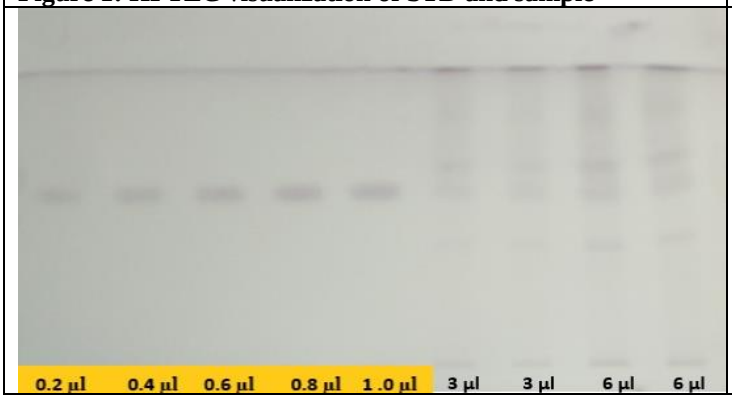



**Figure 2: Fingerprinting 3D display @520 nm**

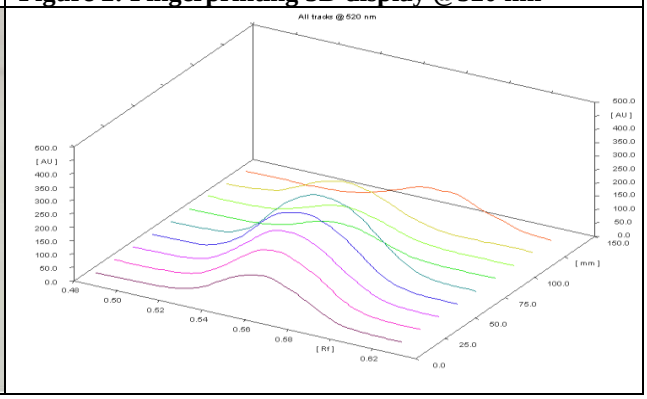


**Figure 3: Typical HPTLC chromatogram of B-sitosterol in STD**

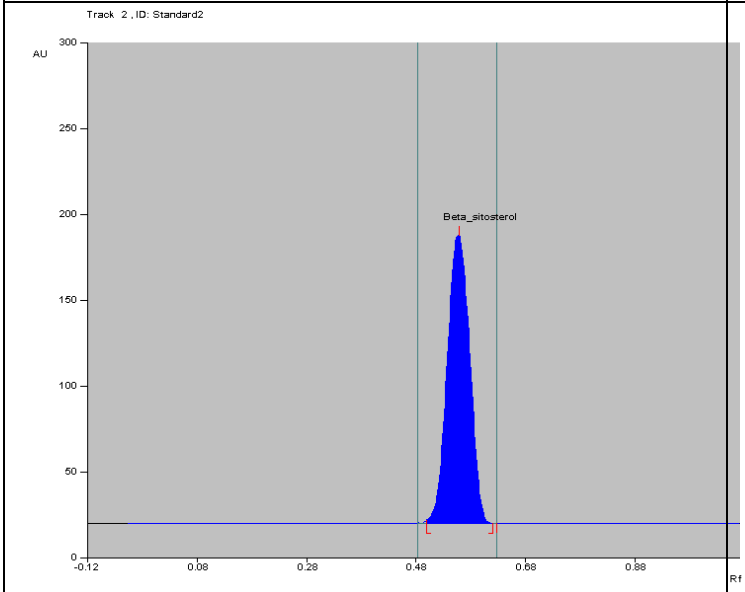



**Figure 4: Typical HPTLC chromatogram of β-sitosterol in STD from Sample**

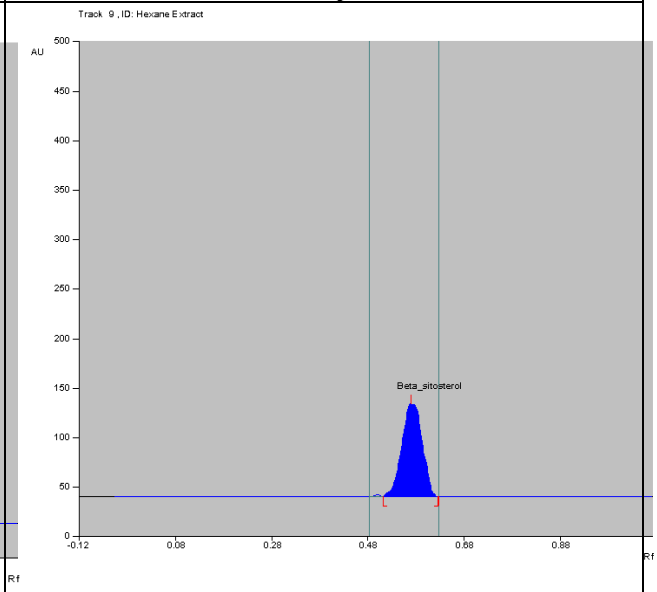



**Figure 5: Typical absorption spectrum obtained from Standard**

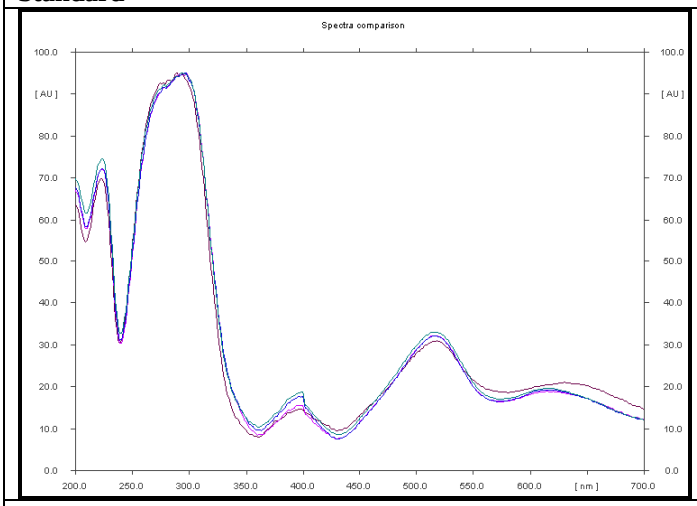



**Figure 6: Typical absorption spectrum obtained from in STD and sample**

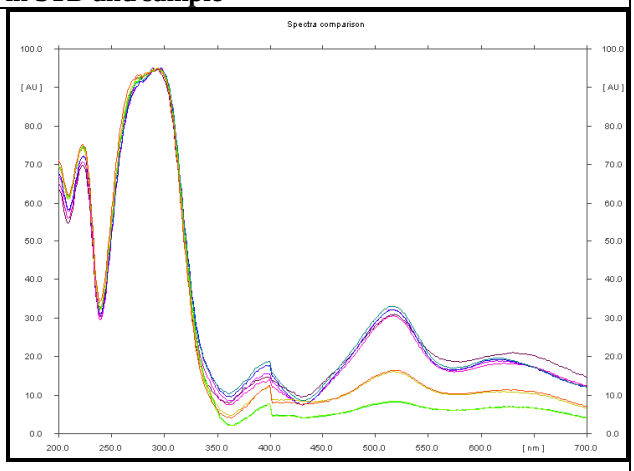



**Figure 7: Linear calibration curve**

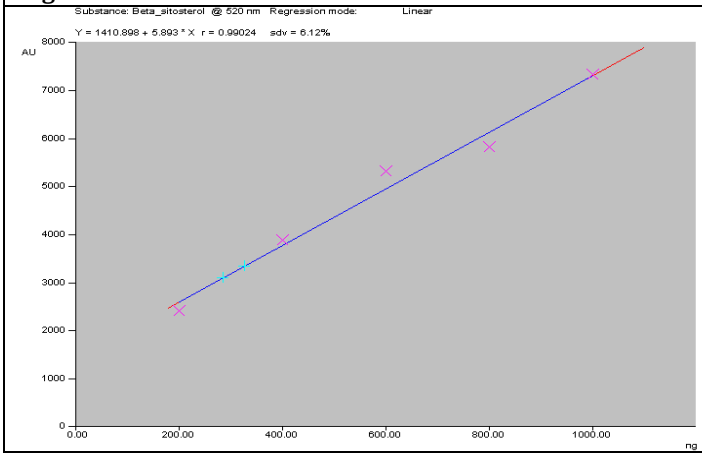



**Figure 8: UV spectrum of isolated compound**

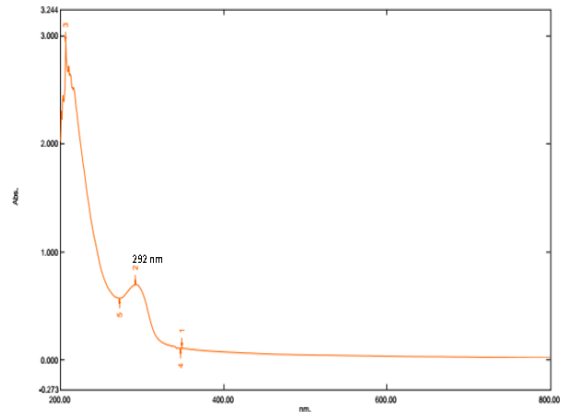
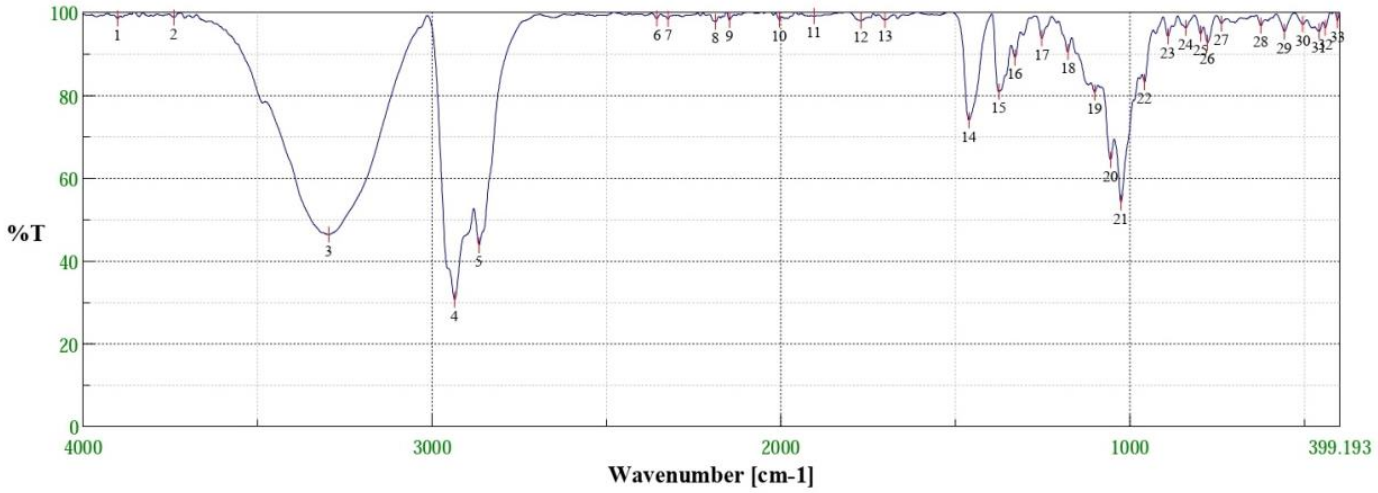


**Figure 9: FT-IR spectrum of isolated compound**




| | | | |
|---|---|---|---|
| Accumulation | 0 | Resolution | 0 cm-1 |
| Zero Filling | ON | Apodization | Cosine |
| Gain | 0 | | |
| Date/Time | 23-003-22 11:06AM | Update | 28-003-22 5:01PM |
| Operator | | | |
| File Name | HEMP.txt | | |
| Sample Name | | | |
| Comment | | | |

| No. | cm-1 | %T | No. | cm-1 | %T | No. | cm-1 | %T | No. | cm-1 | %T | No. | cm-1 | %T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3900.32 | 98.6506 | 2 | 3739.3 | 98.9136 | 3 | 3294.78 | 46.4611 | 4 | 2935.13 | 30.7567 | 5 | 2864.74 | 43.8954 |
| 6 | 2355.62 | 98.582 | 7 | 2323.8 | 98.5278 | 8 | 2186.88 | 97.7982 | 9 | 2147.35 | 98.2872 | 10 | 2004.64 | 98.4219 |
| 11 | 1905.32 | 99.1593 | 12 | 1770.33 | 98.1073 | 13 | 1701.87 | 98.3309 | 14 | 1460.81 | 74.0845 | 15 | 1375.96 | 80.9749 |
| 16 | 1329.68 | 89.2287 | 17 | 1252.54 | 93.8091 | 18 | 1178.29 | 90.5789 | 19 | 1101.15 | 80.7933 | 20 | 1055.84 | 64.4574 |
| 21 | 1025.94 | 54.2507 | 22 | 958.448 | 83.2861 | 23 | 891.916 | 94.3107 | 24 | 839.847 | 96.3742 | 25 | 797.421 | 94.962 |
| 26 | 778.136 | 92.8676 | 27 | 737.638 | 97.4207 | 28 | 624.823 | 96.8982 | 29 | 557.327 | 95.4747 | 30 | 505.258 | 97.2277 |
| 31 | 458.011 | 95.5233 | 32 | 440.655 | 96.4065 | 33 | 405.942 | 98.1222 | | | | | | |

**Figure 10: $^{1}$H NMR spectrum of isolated compound**

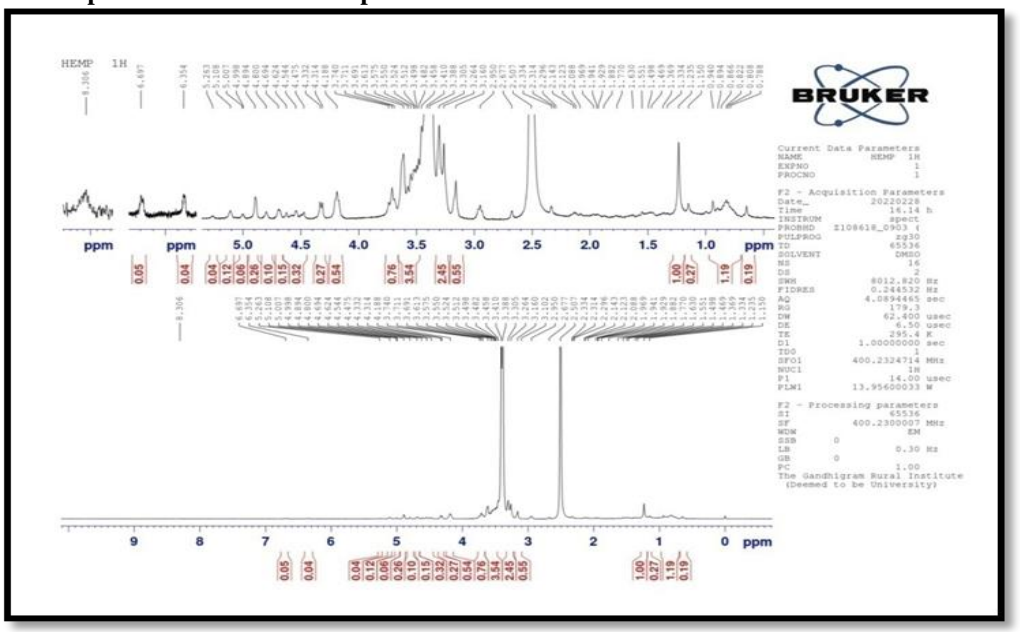
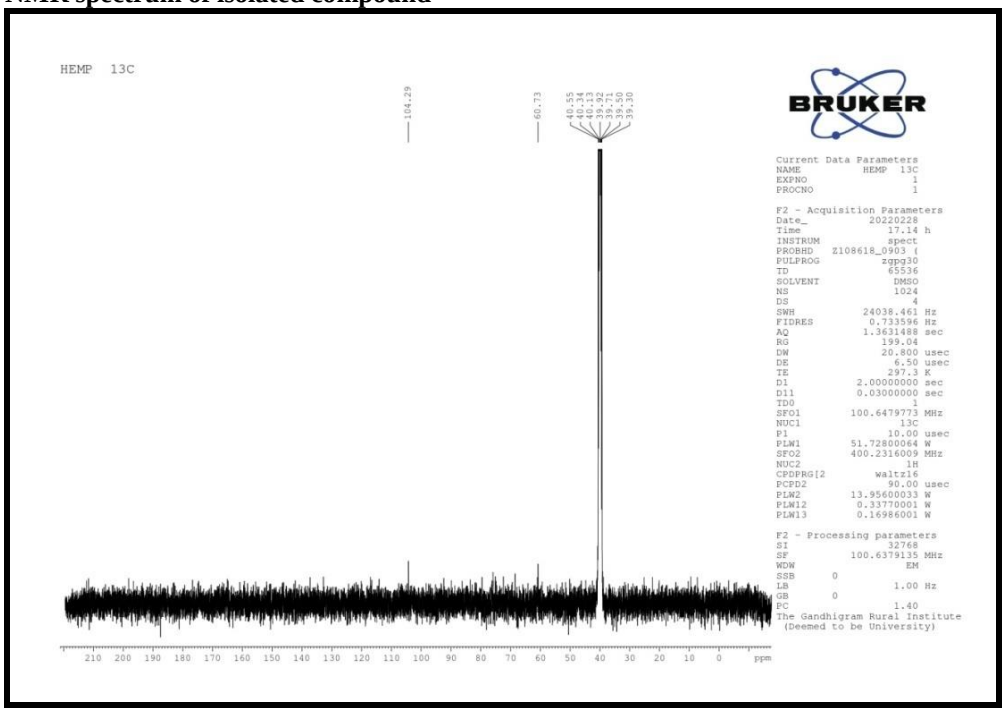


**Figure 11: $^{13}$C NMR spectrum of isolated compound**




## DISCUSSION

From the physical, chemical methods, the isolated compound with white powder of melting point 141-143°C and spectroscopic measurements of UV, $^{1}$HNMR, $^{13}$CNMR and FT-IR with reference to literature the compound was identified as β-sitosterol. The above mentioned spectral data also coincides with the literature [7][8]. The HPTLC quantification is necessary to determine the amount of therapeutically effectual constituents present in the extract. The peak obtained in HPTLC of β-sitosterol shows Rf = 0.56. Regression analysis of calibration data showed that the linearity of standard β-sitosterol was observed over a concentration range of 200-1000ng with regression coefficient of 0.0992. The calculated amount of β-sitosterol was found to be 2.902 mg/g in n-hexane extract of *Mimosa pudica*, which yields higher quantity of phytoconstituents when compared to ethanolic extract (0.071mg) according to [9]. This research specifies that maximum amount of β-sitosterol can be extracted by the solvent n-hexane. The spectroscopic analysis is crucial for identification, molecular weight determination, functional group confirmation and interpretation of various atoms. The obtained data through UV, $^{1}$HNMR, $^{13}$CNMR and FT-IR reveals the nature of carbon and hydrogen atom, functional group present in the identified compound β-sitosterol.

## CONCLUSION

The isolated β-sitosterol have shown significant role in producing major health benefits. The aforementioned literature of *Mimosa pudica* shows that β-sitosterol acts in boosting the immune system, preventing different types of cancer, lowering cholesterol, curing gallstones, hair loss, bronchitis, migraine, headache, enlarged prostate (benign prostatic hyperplasia or BPH), irregular periods, breast enlargements and hormonal regulations. The EFSA, USFDA, joint FAO/WHO published a safety report on phytosterols as food supplements without any specific emphasis on individual compound β-sitosterol. In spite the individual compound β-sitosterol has many benefits than combined phytosterols. In considering various literatures, we conclude β-sitosterol may have future scope to act as therapeutic medicines to generalize the individual effect than combined effect of different phytosterols.

## REFERENCE

1. Owolabi, O.O, James, D.B, Sani, I. *et al*. Phytochemical analysis, antioxidant and anti-inflammatory potential of Feretia apodanthera root bark extracts. *BMC Complement Altern Med* 18(12), 2018.
2. Abubakar AR, & Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J Pharm Bioallied Sci*: 12(1), 2020, 1-10.

3. Uddin MS, Sahena Ferdosh, Md, Jahurul Haque Akanda, Kashif Ghafoor, Rukshana A.H, Md. Eaqub Ali, B. Y. Kamaruzzaman, Fauzi M. B., Hadijah S., Sharifudin Shaarani & Md. Zaidul Islam Sarker. *et al* Techniques for the extraction of phytosterols and their benefits in human health: a review, *Separation Science and Technology*, 53(14), 2018, 2206-2223,
4. Sayeed, Muhammad, Karim, Selim, Sharmin, Tasnuva, Morshed, Mohammed. *et al* Critical Analysis on Characterization, Systemic Effect, and Therapeutic Potential of Beta-Sitosterol: A Plant-Derived Orphan *Phytosterol*. *Medicines*. 3, 2016, 29.
5. Rand A Azeez, Ibrahim S Abaas, Enas J Kadhim. Isolation And Characterization Of B-Sitosterol From Elaeagnus Angustifolia Cultivated In Iraq. *Asian J Pharm Clin Res, Vol* 11 (11), 2018, 442-446
6. Arjun, P; Jha, S; Murthy, PN; Manik, AS .Isolation and Characterization of Stigmast-5-en3β-ol (β - sitosterol) from the Leaves of Hygrophila spinosa T. Anders. *In. J. Pharm. Sci.Res*. 1(2), 2010, 95-100
7. Aliba, M.O, Ndukwe, I.G, Ibrahim, H. *et al* . Isolation and characterization of B-sitosterol from methanol extracts of the stem bark of large- leaved rock fig (Ficus abutilifolia Miq). *Journal of Applied Sciences and Environmental Management*. 22, 2018, 1639.
8. P.A.Champanerkar, V.V.Vaidya, M.B.Kekare. *et al* Chromatographic techniques for quantification of sterol fromMimosa pudica Linn. whole plant powder. *ACAIJ*, 9(2), 2010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