# Structural Characterization of Stigmasterol and β-Sitosterol from the Roots of *Premna herbacea* Roxb.

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## ABSTRACT

One Myanmar Medicinal Plant, *Premna herbacea* Roxb. (Local name-Galon-ohnauk) was selected for this research work. The white needle like crystal compound could be isolated from the roots sample using modern separation techniques such as thin layer and column chromatography. The structure assignments of the isolated compounds were performed by applying nuclear magnetic resonance (NMR) spectroscopic methods such as $^{1}$H NMR, $^{13}$C NMR, HSQC, COSY and HMBC. The structures of the isolated compounds could be assigned as Stigmasterol and β-Sitosterol by careful interpretation of NMR spectral data along with the comparison of the measured NMR spectral data with those in the literature.

**Keywords :** *Premna integrifolia* Linn., NMR spectroscopy, β- sitosterol and stigmasterol



## 1 INTRODUCTION

Different parts of the plants have been used as source of important therapeutic aids since ancient time and numerous drugs are derived from various plants for centuries. [1, 2] Medicinal plants provide the primary health care service to the rural people because they are the potential source of secondary metabolites. [3] More than 80%v of the world's population relies on traditional medicine for their primary healthcare needs according to World Health Organization (WHO). The uses of medicinal plants are effective, safe due to low side effects, low costs, and ease of availability. [1, 4, 5] From the survey (1993) of World Health Organization (WHO), the medicinal practitioners treat about 85% of patients in Myanmar, 90% in Bangladesh and 80% in India. [4]

The genus Premna was transferred into the family Lamiaceae from the family Verbenaceae. This genus currently contains 200 species which are distributed throughout tropical and subtropical areas in Asia, Africa, Australia and the Pacific Islands. The word 'Premna' is derived from the Greek 'premnon', meaning tree stump, which refers to the short and twisted trunks of *Premna serratifolia* L., the first collected species of this genus. [6, 7] Various species of Premna were used as various traditional medicines. These plants were used to treat malaria, stomach disorder, headache, cough, malaria and tuberculosis. *Premna serratifolia* is notably used to treat neuralgia and headache, stomachic, fevers, colds and cough, and also to improve liver- and cardiac- related problems in tropical Asia and East Africa. The leaves, root of the inner bark were used to relieve stomach ache discomfort/ pain, for diuretic or to treat diarrhea by the local people in Myanmar, Thailand, Malay Peninsula and Indonesia. [6] Many different flavonoid compounds were isolated and identified from Premna species. But very litte work has been reported in literature regarding the phytochemical studies of *Premna herbacea* Roxb. [7, 8]

The roots of *Premna herbacea* Roxb. were very useful in the Ayurvedic system of medicine for the treatment of several ailments such as rheumatism, snakebite, scorpion-sting, but it is not an antidote to either snake-venom or scorpion-venom. The different parts of the plant are used as laxative, stomachic, alexipharmic, anemia, diabetes chyluria, inflammation, swelling, bronchitis, dyspepsia, piles, fever, tumors, cold, neuralgia and many other diseases. [9] The alcoholic extract of the roots of *Premna herbacea* was investigated for its antipyretic, antinociceptive and anti-inflammatory potential in mice by N. Narayanan. [10] V. K. Verma reported that Scutellarein (flavone) compound was isolated from the ethyl acetate extract of the plant *Premna herbacea* Roxb. which based on spectral studies. [9] In this research, the roots of *Premna herbacea* Rixb. were used for the determination phytochemical constituents and their structure assignments using spectral analysis.

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## 2 MATERIALS AND METHODS

### 2.1 General Experimental Procedure

Commercial grade solvents were distilled before using them. Precoated siligica gel (Merk. Co. Inc, Kiesel gel 60 $F_{256}$) plate was applied for thin layer chromatography. Iodine vapor and UV- lamp (Lambda – 40, Perkin – Elmer Co, England) were used as visualizing agents in column chromatography. $^{1}$H-and $^{13}$C NMR spectroscopy was carried out on Bruker AVANCE 600, Germany at 600MHz and 150 MHz respectively. Chemical shifts values are given in $\delta$-value (ppm) with tetramethylsilane (TMS) as internal standard.

### 2.2 Plant Materials

The roots of *Premna herbacea* Roxb. were collected from Pyinmanar Township, Mandalay Region, Myanmar. The plant was screened and identified by authorized botanist from Botany Department, Mandalay University, Myanmar. The collected sample was washed properly with water to remove any contaminants.

### 2.3 Extraction, Isolation and Purification of Organic Compound

The small pieces of the roots of *Premna herbacea* Roxb (500 g) were extracted with ethanol (2.5 L) and concentrated using rotatory evaporator. The dried crude sample was extracted with ethyl acetate and then concentrated to give the ethyl acetate extract. The ethyl acetate extract was subjected to column chromatography on silica gel (Merck 70- 230 Mesh). The column was eluted gradient wise with n- hexane and ethyl acetate from non- polar to polar. The fractions were analyzed by thin layer chromatography (TLC) and similar fractions were combined to obtain (10) combined fractions which include fraction (VI). 24.1 mg of the combined fraction (VI) was subjected to repeated column chromatography over silica gel eluted with the same solvent system to afford white needle shape crystals (15 mg). The structure of the isolated compound (1) was assigned by NMR analysis such as $^{1}$H-NMR, $^{13}$C-NMR, COSY, HSQC and HMBC spectroscopic measurements.

## 3 RESULTS AND DISCUSSION

### 3.1 Structure Identification of Isolated Compound

The compound (1) was isolated from the ethyl acetate soluble fraction of the ethanol extract from the roots of *P. herbacea* by a combination of thin layer and column chromatography. The $^{1}$H NMR spectrum of compound varied between 0.65 ppm to 5.35 ppm. This spectrum showed the presence of high intensity peaks indicating the presence of methyl groups between 0.65 ppm and 1.05 ppm. The proton corresponding to the H-3 of a sterol moiety was appeared as a multiplet at δ 3.52 ppm. The proton signals at δ 5.05 ppm, 5.15 ppm and 5.35 ppm correspond to two double bonds with one quaternary carbon and three methine protons indicating the double bond between C-5 and C-6, and C-22 and C-23. $^{13}$NMR spectrum of the compound (1) has given signals at $\delta_C$ 140.743, 121.691 and 121. 681ppm for C5=C6 double bond of stigmasterol and β-sitosterol respectively. This spectrum also reflected the signals at 71.775 for C-3 β-hydroxyl group. The singlet and doublet signals of the methyl groups were shown in HSQC at 11.80, 11.97, 12.01, 12.20, 18.60, 18.90, 19.10, 19.41, 21.0 and 21.50 ppm respectively.



According to the literature β-sitosterol and Stigmasterol are always in a mixture form in which may have maximum portion of stigmasterol. It is very difficult to obtain Stigmasterol and β- sitosterol in pure state. The only difference between the two compounds is the presence of C22=C23 double bond in Stigmasterol and C22-C23 single bond in β-sitosterol. Furthermore, literatures have shown that sitosterol is difficult to be obtained in pure state. [11-15] Stigmasterol and beta-sitosterol have the same $R_f$ value despite the use of several solvent systems. Therefore, compound (1) is a mixture of β-sitosterol and Stigmasterol. Complete assignment of all protons and carbons was confirmed by $^{1}$H-$^{1}$H COSY. All one- and two-dimensional NMR spectroscopic spectral data indicated that Compound (1) is the mixture of Stigmasterol and β-Sitosterol. From the comparison of the experimental $^{13}$C NMR spectral data of the compound (1) with literature [15], the structure assignment of the two sterol compounds could be confirmed. The $^{1}$H and $^{13}$C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations and were given in the tables (1 and 2).

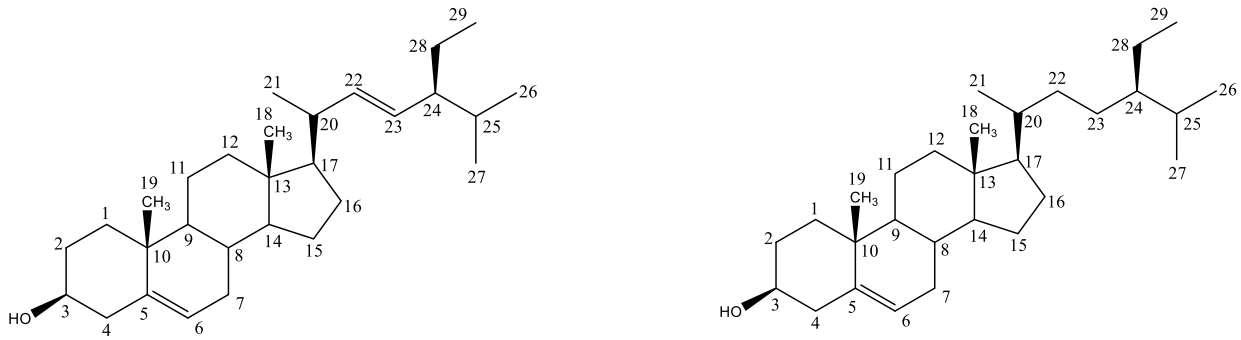

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Fig.1 Complete structure of stigmasterol compound

Fig.2 Complete structure of β-sitosterol compound

Table (1) $^{1}$HNMR, $^{13}$CNMR, HSQC, COSY and HMBC Spectral Data of Isolated Stigmasterol Compound (at 600 and 150 MHz, resp. in $CDCl_3$; 298 K; $\delta$ in ppm, $J$ in Hz)

| No. | Type of Carbon | Experimental | | | | Literature [15] |
|---|---|---|---|---|---|---|
| | | $\delta_C$ ($CDCl_3$) | $\delta_H$ ($CDCl_3$) | COSY | HMBC | $\delta_C$ ($CDCl_3$) |
| 1 | $CH_2$ | 37.24 | 1.08 (o), 1.85 (o) | H-2a, 2b | H-2a, 19 | 37.6 |
| 2 | $CH_2$ | 31.64 | 1.52 (o), 1.84 (o) | H-1a, 1b,3 | H-1a, 1b, 4a, 4b | 32.1 |
| 3 | CH | 71.78 | 3.52 (m) | H-2b, 4a,4b | H-1a, 1b, 4a, 4b | 72.1 |
| 4 | $CH_2$ | 42.20 | 2.24 (m), 2.28 (m) | H-3 | H-6 | 42.4 |
| 5 | C | 140.74 | | | H-4, 6, 7a,7b,8,19 | 141.1 |
| 6 | CH | 121.68 | 5.35 (brd) | H-7a,7b | H-4, 7a,7b, | 121.8 |
| 7 | $CH_2$ | 31.90 | 1.50 (o), 1.96 (o) | H-6 | H-6 | 31.8 |
| 8 | CH | 31.88 | 1.49 (o) | H-9, 14 | H-6 | 31.8 |
| 9 | CH | 50.12 | 0.93 (o) | H-8, 11a | H-8, 11a, 11b,12a, 12b, 19, | 50.2 |
| 10 | C | 36.49 | | | H- 6, 11a, 11b, 19 | 36.6 |
| 11 | $CH_2$ | 21.02 | 1.45 (o), 1.50 (o) | H-9,12a, 12b | | 21.5 |
| 12 | $CH_2$ | 39.67 | 1.17 (o), 1.99 (o) | H-11a,11b | H-11a, 11b, 18 | 39.9 |
| 13 | C | 42.31 | | | H- 8, 11a, 11b, 12a, 12b, 14, 18 | 42.4 |
| 14 | CH | 56.75 | 0.98 (o) | H-8, 15b | H-8, 9, 15a, 15b,18,21 | 56.8 |
| 15 | $CH_2$ | 24.35 | 1.15 (o), 1.57 (o) | H-14, 16b | H-14 | 24.4 |
| 16 | $CH_2$ | 28.90 | 1.24 (o), 1.73 (o) | H-15a, 15b, 17 | H-15a, 15b | 29.3 |
| 17 | CH | 56.04 | 1.15 (o) | H-16a, 16b | H-21, 22 | 56.2 |
| 18 | $CH_3$ | 11.80 | 0.65 (s) | | H-12a, 14, 17 | 12.0 |
| 19 | $CH_3$ | 19.10 | 1.05 (s) | | H-1a, 9 | 18.9 |
| 20 | CH | 40.48 | 2.04 (o) | H-21, 22 | H-21, 22, 23 | 40.6 |
| 21 | $CH_3$ | 21.51 | 1.02 (d) | H-20 | H-17, 22 | 21.7 |
| 22 | CH | 138.30 | 5.15 (brd) | H-20, 23 | H-17, 21,23 | 138.7 |
| 23 | CH | 129.26 | 5.05 (brd) | H-22, 24 | H-22 | 129.6 |
| 24 | CH | 51.22 | 1.53 (o) | H-25, 28b | H-22, 23 | 46.1 |
| 25 | CH | 36.13 | 1.37(o) | H-24, 26,27 | H-24, 26, 27 | 29.6 |
| 26 | $CH_3$ | 19.80 | 0.83(d) | H-25 | H-24, 25, 27 | 20.2 |
| 27 | $CH_3$ | 18.91 | 0.80 (d) | H-25 | H-24, 25, 26 | 19.8 |
| 28 | $CH_2$ | 25.39 | 1.16 (o), 1.43 (o) | H-24, 29 | H- 23, 24, 25, 29 | 25.4 |
| 29 | $CH_3$ | 11.97 | 0.65 (t) | H-28 | H-24, 28 | 12.1 |

(s = singlet, d = doublet, t= triplet, m= multiplet, o= overlap)

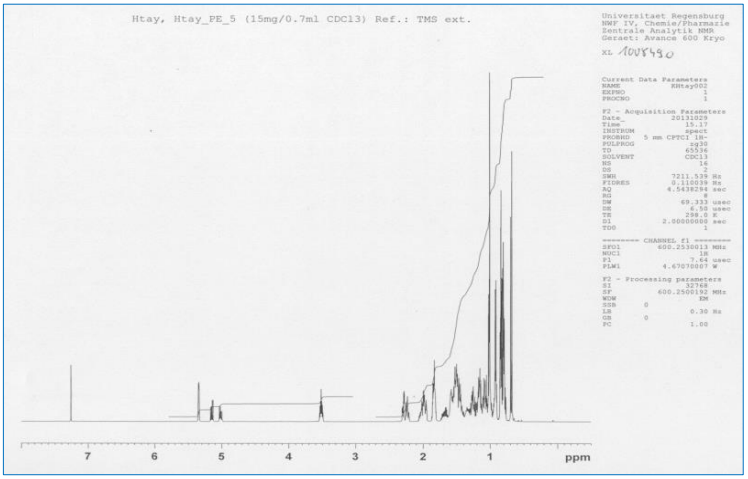

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Fig. 3 $^{1}$H NMR spectrum of isolated compound (1)

Table (2) $^{1}$HNMR, $^{13}$CNMR, HSQC, COSY and HMBC Spectral Data of Isolated β-Sitosterol Compound (at 600 and 150 MHz, resp. in $CDCl_3$; 298 K; $\delta$ in ppm, $J$ in Hz)

| No. | Type of Carbon | Experimental | | | | Literature [15] |
|---|---|---|---|---|---|---|
| | | $\delta_C$ ($CDCl_3$) | $\delta_H$ ($CDCl_3$) | COSY | HMBC | $\delta_C$ ($CDCl_3$) |
| 1 | $CH_2$ | 37.243 | 1.08 (o), 1.85(o) | H-2a.2b | H-2a, 19 | 37.5 |
| 2 | $CH_2$ | 31.864 | 1.52 (o), 1.84 (o) | H-1a,1b, 3 | H-1a | 31.9 |
| 3 | CH | 71.775 | 3.52 (m) | H-2b, 4a, 4b | H-1a, 1b, 4a, 4b | 72.0 |
| 4 | $CH_2$ | 42.285 | 2.23 (m), 2.28 (m) | H-3 | H- 6 | 42.5 |
| 5 | C | 140.743 | | | H-4, 7a, 7b, 19 | 140.9 |
| 6 | CH | 121.691 | 5.35 (brd) | H-7a, 7b | H-4,7a, 7b, 8 | 121.9 |
| 7 | $CH_2$ | 31.897 | 1.95, 1.98(o) | H-6, 8 | | 32.1 |
| 8 | CH | 32.401 | 1.47(o) | H-7a, 7b, 9, 14 | H-6, 7a, 7b, 9 | 32.1 |
| 9 | CH | 50.143 | 0.93(o) | H-8 | H- 7a, 7b, 8, 19 | 50.3 |
| 10 | C | 36.498 | | | H-1a, 1b, 8, 9, 19 | 36.7 |
| 11 | $CH_2$ | 21.05 | 1.46 (o), 1.49(o) | H-12a, 12b | H-9, 12a, 12b | 21.3 |
| 12 | $CH_2$ | 39.761 | 1.16 (o), 1.19(o) | H-11a, 11b | H-9, 11a, 11b, 18 | 39.9 |
| 13 | C | 42.305 | | | H- 8, 11a, 11b, 12a, 12b, 14, 18 | 42.6 |
| 14 | CH | 56.851 | 1.06(o) | H-15a, 15b | H-8, 9, 12a, 12b | 56.9 |
| 15 | $CH_2$ | 25.500 | 1.16 (o), 1.43(o) | H-14, 16b | H-14 | 26.3 |
| 16 | $CH_2$ | 28.233 | 1.26 (o), 1.85(o) | H-15b, 17 | H- 17 | 28.5 |
| 17 | CH | 56.096 | 1.11(o) | H-16a, 16b | H-16a, 16b | 56.9 |
| 18 | $CH_3$ | 12.20 | 0.68 (s) | | H-12a, 12b,14, 17 | 12.0 |
| 19 | $CH_3$ | 19.41 | 1.01 (s) | | H- 9, 1a, 1b | 19.0 |
| 20 | CH | 35.865 | 1.38 (o) | H-21 | H-21, 22a | 36.3 |
| 21 | $CH_3$ | 18.60 | 0.92 (d) | H-20 | H-22a | 19.2 |
| 22 | $CH_2$ | 33.930 | 1.02, 1.34 (o) | H-23a, 23b | H-21, | 34.2 |
| 23 | $CH_2$ | 26.062 | 1.16, 1.18 (o) | H-22a | H-22b, 24, 25 | 26.3 |
| 24 | CH | 45.818 | 0.92 (o) | H-25 | H-25,26,27 | 46.1 |
| 25 | CH | 29.137 | 1.67 (o) | H-24, 26, 27 | H-24,26,27 | 29.4 |
| 26 | $CH_3$ | 21.00 | 0.86 (d) | H-25 | H-24, 27 | 20.1 |
| 27 | $CH_3$ | 18.60 | 0.90 (d) | H-25 | H-24, 25, 26 | 19.6 |
| 28 | $CH_2$ | 22.90 | 1.23, 1.28 (o) | H-29 | H-23a, 23b, 24, 25, 29 | 23.3 |
| 29 | $CH_3$ | 12.01 | 0.86 (t) | H-28a, 28b | H-28a, 28b, | 12.2 |

(s = singlet, d = doublet, t= triplet, m= multiplet, o= overlap)

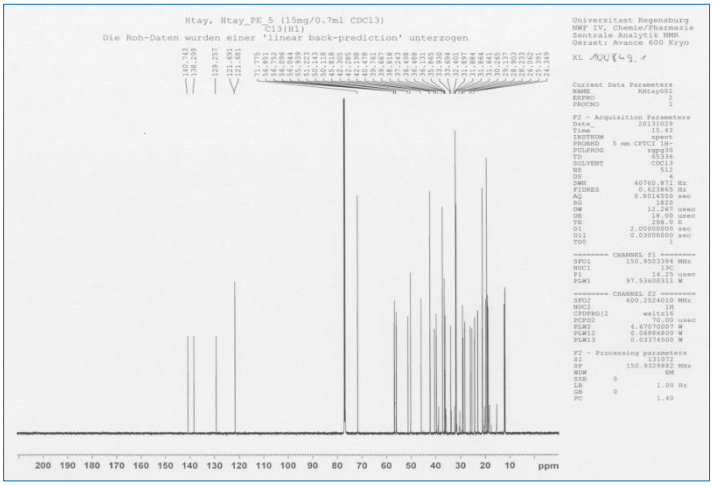

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Fig. 4 $^{13}C$ NMR spectrum of isolated compound (1)

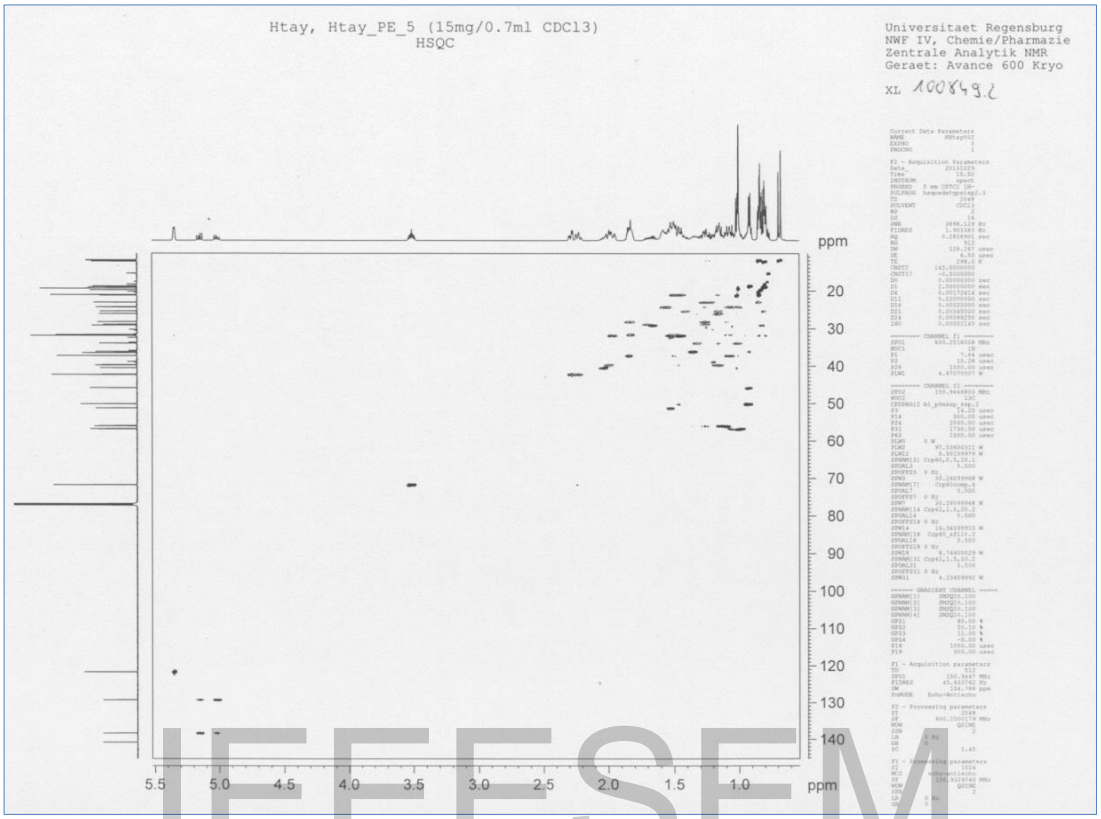




Fig. 5 HSQC spectrum of isolated compound (1)

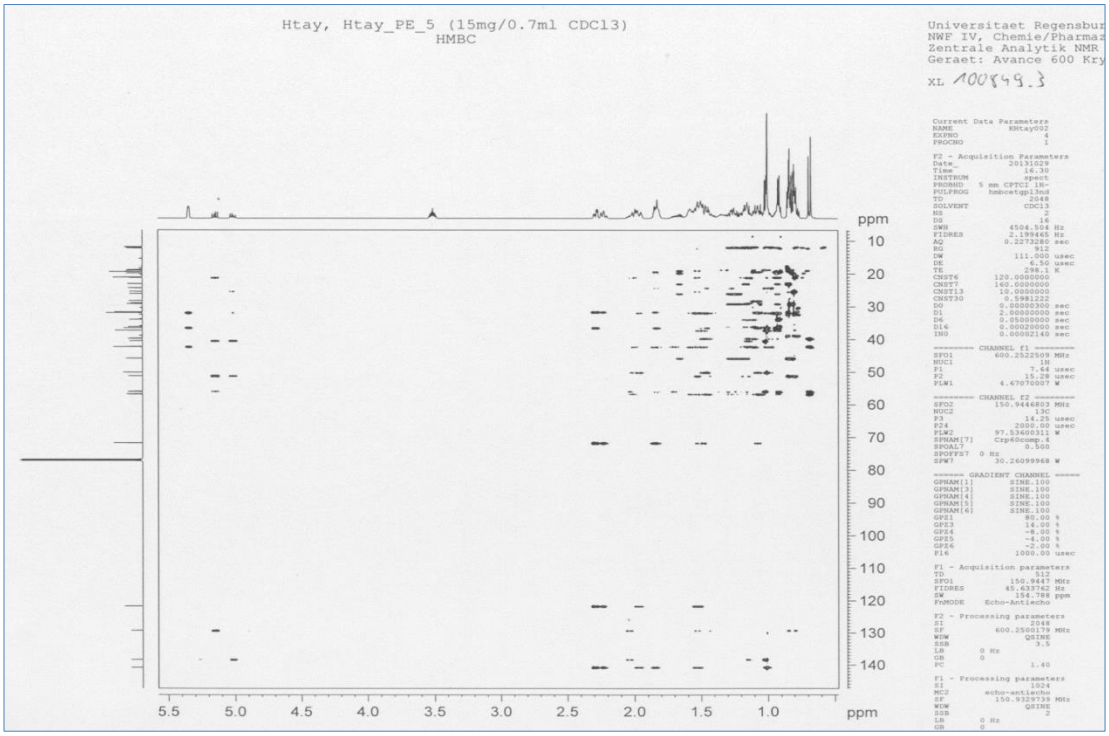

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Fig. 6 HMBC spectrum of isolated compound (1)

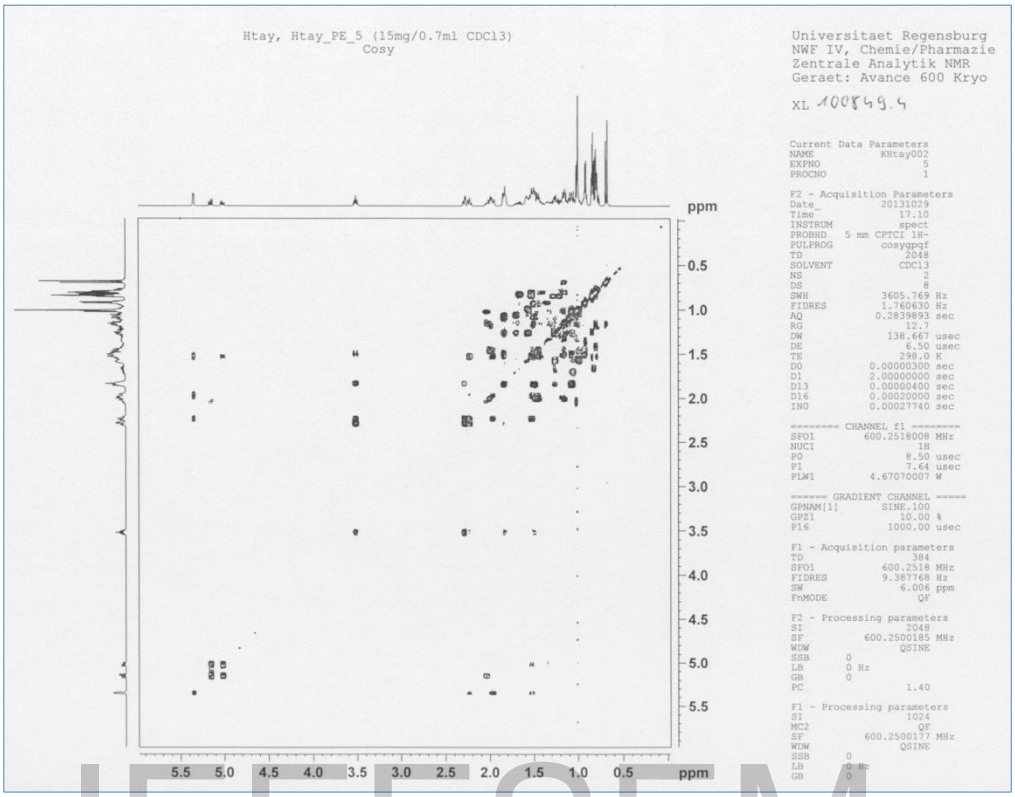




Fig. 7 COSY spectrum of isolated compound (1)

## 4 CONCLUSION

The two steroid compounds (Stigmasterol and β- sitosterol) could be isolated from the roots of *Premna herbacea* Roxb. using chromatographic separation techniques such as thin layer and column chromatography. The structural assignments of these steroid compounds could be done by some modern spectroscopic techniques such as COSY, HSQC and HMBC. There are only five $sp^2$ carbon signals at $\delta_C$ 140.743, 138.299, 129.257, 121.691 and 121. 681 ppm indicating the presence of C5=C6 double bonds in both stigmasterol and β- sitosterol compounds and C22= and C23 double bond in stigmasterol compound. There are a lot of methyl signals in $^{13}C$ NMR spectrum reflecting the existence of six methyl groups in both compounds. Careful interpretation of 1D NMR such as $^{1}H$ and $^{13}C$ NMR and 2D NMR such as HSQC, COSY and HMBC spectra indicated the existence of stigmasterol and β-sitosterol together in the compound (1). Their structures could be confirmed by comparing the experimental carbon data with those in literature.

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## REFERENCES

[1] P. Garg and S. Sardana, "Pharmacological and Therapeutic Effects of Ocimum sanctum", European Journal of Pharmaceutical and Medical Research., vol. 3, No.8, pp.637-640, 2016.

[2] C. R. Singh, R. Nelson, P. M. Krishnan and B. Pargavi, "Identification of Volatile Constituents from Premna serratiofolia L. through GC- MS", International Journal of Pharm Tech Research, vol.3, No.2, pp. 1050- 1058, 2011.

[3] P. Bharali, P. Dutta, M.C. Kalita, A.K. Das, H. Tag and A.M. Baruah, "Evaluation of Antioxidant and Proximate Compositions of the Leaf Extract of Gnetum gnemon L", International Research Journal of Pharmacy, vol. 9, No. 10, pp. 101- 105, 2018.

IEEE-SEM, Volume 7, Issue 8, August-2019
ISSN 2320-9151

[4] P. Prakash and N. Gupta. "Therapeutic Uses of *Ocimum sanctum* Linn (Tulsi) with a Note on Eugenol and Its Pharmacological Actions: A Short Review", Indian J Physiol Pharmacol, vol 49, No. 2, pp. 125- 131, 2005.

[5] R. K. Joshi, W.N. Setzer and J. K. daSilva, "Phytoconstituents, Traditional Medicinal Uses and Bioactivities of Tulsi (*Ocimum sanctum* Linn.): A Review", American Journal of Essential Oils and Natural Products., vol. 5, No. 1, pp.18–21, 2017.

[6] R. Dianita and I. Jantan, "Ethno medicinal Uses, Phytochemistry and Pharmacological Aspects of the Genus Premna: A Review", Pharmaceutical Biology, vol. 55, No. 1, pp. 1715- 1739, 2017.

[7] Z. Hu, Y. Xue, G. Yao, Z. Luo, Y. Wang and Y. Zhang, "Chemical Constituents from the Leaves of *Premna microphylla* Turcz", Journal of Chinese Pharmaceutical Sciences, vol. 22, No. 5, pp. 431- 434, 2013.

[8] G. Chen, C. Dai, T. Wang, C. Jiang, C. Han and X. Song, "A New Flavonol from the Stem Bark of *Premna fulva*", ARKIVOC, (ii), pp. 179- 185, 2010.

[9] V. K. Verma, N. U. Siddiqui and M. Aslam, "Isolation of Scutellarein from *Pygmaeopremna herbacea* Roxb.", Journal of Applied Pharmaceutical Science, vol. 2, No. 6, pp. 241- 242, 2012.

[10] N. Narayanan, P. Thirugnanasambantham, S. Viswanathan, M. Kannappa Reddy, V. Vijayasekaran and E. Sukumar, "Antipyretic, Antinociceptive and Anti-inflammatory Activity of *Premna herbacea* Roots", Fitoterapia, vol. 71, No. 2, pp. 147- 153, 2000.

[11] I.S. Okoro, T. A. Tor-Anyiin, J. O. Igoli, X. S. Noundou and R. W. M. Krause, "Isolation and Characterization of Stigmasterol and β- Sitosterol from *Anthocleista djalonensis* A. Chev.", Asian Journal of Chemical Sciences, vol. 3, No. 4, pp. 1- 5, 2017.

[12] L. L. Pierre and M. N. Moses, "Isolation and Characterization of Stigmasterol and β- Sitosterol from *Odontonema strictum* (Acanthaceae)", Journal of Innovations in Pharmaceuticals and Biological Sciences, vol. 2, No. 1, pp. 88- 95, 2015.

[13] S. Khanam and R. Sultana, "Isolation of β- Sitosterol & Stigmasterol as Active Immunomodulatory Constituents from Fruits of *Solanum xanthocarpum* (Solanaceae)", International Journal of Pharmaceutical Sciences and Research, vol.3, issue-4, pp. 1057-1060, 2012.

[14] A. Kamboj and A. Kumarsaluja, "Isolation of Stigmasterol and β- Sitosterol from the Petroleum Ether Extract of Aerial Parts of *Ageratum conyzoides* (Asteraceae)", International Journal of Pharmacy and Pharmaceutical Sciences, vol. 3, No. 1, pp. 94- 96, 2011.

[15] V.S.P. Chaturvedula and I. Prakash, "Isolation of Stigmasterol and β- Sitosterol from Dichloromethane Extract of *Rubus suavissimus*", International Current Pharmaceutical Journal, vol.1, No. 9, pp. 239- 242, 2012.