International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 20-06-2021, Accepted: 05-07-2021, Published: 20-07-2021
Volume 6, Issue 4, 2021, Page No. 301-305

Isolation and identification of stigmasterol from *Byttneria herbacea* Roxb. using methanol extract

M Sathish[1], P K Siva[1], T Parvathi[1], R Bhuvaneswari[2], M Arumugam[1*]

[1] PG and Research, Department of Botany, J.J College of Arts and Science (Autonomous), (Affiliated to Bharathidasan University), Pudukkottai, Tamil Nadu, India

[2] Departments of Physics, Government Arts College for Women (Autonomous), (Affiliated to Bharathidasan University), Pudukkottai, Tamil Nadu, India

Abstract
Phytosterols a group of steroidal alcohol, play an important role in the structure and stability of the cell membrane. The present study deals with characterizing the phytosterol from methanolic extract of *Byttneria herbacea* Roxb. The phytochemical was subjected to physical, chemical, and spectral identification using UV- Vis Spectrophotometer, FTIR, ^{1}H-NMR, and ^{13}C-NMR. Based on the spectral data analysis and chemical reactions, the compounds have been recognized as Stigmasterol.

Keywords: phytosterol, stigmasterol, *Byttneria herbacea*, UV- Vis spectrophotometer

Introduction

The contribution of plants to the life of all living things on earth especially to humans is seen as vital. Various parts of the plant are used as food or medicines without side effects in the treatment of various diseases. Herbal remedies have long been used to treat many ailments (Asila *et al.*, 2018) [1]. Plants are being investigated extensively for their pharmacological potential as a source of raw material for drug discovery. Crude extract of medicinal plants was being used to treat various infectious diseases in traditional medicine systems (Singh *et al.*, 2015 and Hemavathy *et al.*, 2019) [36, 13]. According to the World Health Organization, over 80% of the world's people rely on traditional medicine for their primary healthcare needs (Sasidharan, 2011) [33].

Byttneria herbacea Roxb. Belonging to the Malvaceae family is commonly found in peninsular India (Gujarat, Tamil Nadu, Odisha, and Bihar) (CSIR 1988, Saxena *et al.*, 1994, Tarun *et al.*, 2019) [5, 34, 39]. *B. herbacea* has been described on anti-asthmatic activity (Sharma *et al.*, 2018; Bharathi *et al.*, 2016) [35, 2], anti-inflammatory activity (Sarkar *et al.*, 2013) [31], anti-edematogenic activity (Sarkar *et al.*, 2012) [32], and antioxidant activity (Somkuwar *et al.*, 2014) [37].

Stigmasterol, also known as Stigmasterin or Wulzen anti-stiffness factor is an unsaturated plant sterol present in various plants. It is being used to design synthetic and semi-synthetic compounds in the pharmaceutical industry (Navpreet *et al.*, 2011) [25]. It acts as a forerunner in the synthesis of progesterone and acts as an intermediately in the biosynthesis of androgens, estrogens, corticosteroids, or corticoids (Sundararaman *et al.*, 1977) [38] It also plays a vital role in the synthesis of vitamin D3 (Kametani *et al.*, 1987) [17].

It has been previously reported in *Physostigma venenosum* (Wind, 1907) [41], *Emilia sonchifolia* (Gao *et al.*, 1993) [8], *Parkia speciosa* (Jamaluddin *et al.*, 1994) [16], *Eclipta alba* (Zhang *et al.*, 1996) [44], *Eclipta prostrate* (Han *et al.*, 1998) [11], *Gypsophila oldhamiana* (Yang *et al.*, 1999) [42], *Croton sublyratus* (De-Eknamkul *et al.*, 2003) [6], *Aralia cordata* (Peng 2005) [29], *Akebia quinata* (Liu *et al.*, 2005) [21], *Heracleum rapula* (Luu *et al.*, 2006) [22], *Ficus hirta* (Li *et al.*, 2006) [22, 19], *Eucalyptus globules* (Yang *et al.*, 2007) [43] and *Desmodium styracifolium* (Li *et al.*, 2007) [7, 20]. Since the stigmasterol has been isolated from various plant species, the present study has been made in *B. herbacea* because the species is locally available and grows as a weedy plant.

Materials and Methods

Plant Collection

Byttneria herbacea was collected from the Narthamalai hills, Pudukkottai District, Tamil Nadu, India. The plant material was identified by the Department of Botany, St. Joseph College (Autonomous), Tiruchirappalli, Tamil Nadu, India and the herbarium voucher specimen had been deposited at the PG and Research Department of Botany, J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India. The fresh plant was collected and dried at room temperature. The dried material was fine powdered by an electric blender and stored in an airtight container.

Extraction

The powdered plant material was extracted with methanol (60-80° C) in the Soxhlet apparatus. The elute was dried and refluxed with water for 8 hrs. The methanol extract was filtered and concentrated using a rotary vacuum evaporator and the dried extract was stored in an airtight container.

Chromatographic Analysis

The methanol extract of *B. herbacea* was subjected to Thin Layer Chromatography (TLC) using silica gel as stationary phase and chloroform: methanol (1:1) as mobile phase. The chromatograms were developed in Iodine chamber. Column chromatography of *B. herbacea* methanol extract (10g) was conducted by wet packing method using silica gel (Mesh 100-200).

The column was run using chloroform, methanol successive by gradient elution technique, and obtained fractions were further studied. A white crystal was formed in any one of the eluates. The crystal was named compound X.

Test for Steroid

The compound X has been tested against Liebermann Buchard and Salkowski reagent for the conformation of the steroidal nucleus (Kandati *et al*., 2012; Victor and Chidi, 2009) [18, 40].

Spectroscopic Analyses

Spectrophotometric analysis was conducted for the *B. herbacea* methanol extract using a UV-Vis Spectrophotometer. The plant elute was centrifuged for 15 minutes at 3000 rpm and sieved through Whatman No. 1 filter paper. The diluted (1:10) sample was examined under visible and UV lights in wavelengths ranging from 200-1100nm. The characteristic peak values were observed.

FT-IR (Model Shimadzu 8700) has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown sample (Eberhardt *et al.*, 2007 and Hazra *et al*, 2007) [7, 12]. FT-IR analysis of *B. herbacea* was performed using Perkin an Elmer Spectrophotometer system to denote the characteristic peaks and their functional groups.

The NMR experiment was carried out in BRUKER-AMX400 MHz instrument with 5mg of the purified compound in $DMSOd_6$. Tetra Methyl Silane was used as the internal standard and chemical shifts were expressed in ppm.

Result and Discussion

The methanolic extract was yielded 30.62g/kg from aerial parts of *B. herbacea.* The extract was further subjected to column chromatography and verified using TLC in hexane-ethyl acetate 8:2 (v/v) and chloroform-methanol 4:4 (v/v) solvent system. Rf value of the chromatogram was 0.68. The Rf value also corresponds with the previous report on *Acacia nolotica* (Padmasri and Sarada, 2011) [27]. The eluted compounds underwent thin layer chromatography with a slight modification of n-hexane instead of hexane. The isolated compound is confirmed as a steroid by Salkowski and Liebermann-Burchard reaction. Finally, the yield of the isolated compound (figure 5) was 74mg. The isolated compound was powdery with a melding point of 144° - 146 ° C, and the absorbance of UV- λ_{max}: 255nm (figure. 1 and Table. 1). The molecular formula of the isolated compound also been described by GC-MS data as $C_{29}H_{30}O$ [m/z 413.3(m+). FT-IR analysis was performed to identify the functional groups and to identify the formation of hydrogen bonds (Xue *et al*., 2004; Bao *et al*., 2006) [30, 4]. Based on the peak values in the region of IR radiation, the functional groups of the compound were separated. The result of FT-IR analysis confirmed the presence of functional groups at the peak value 3430.14 (N-H stretching primary amine), 2922.07 (C-H stretching alkane), 2852.67 (C-H stretching alkane), 2086.39 (N=C=S stretching isothiocyanate), 1642.63 (C=C stretching alkene), 1469.84 (C-H bending alkane methylene group), 1378.52 (S=O stretching sulfonyl chloride), 1249.75 (C-O stretching alkyl aryl ether), 1215.65 (C-O stretching vinyl ether), 1113.16C-O stretching secondary alcohol 1070.96 (S=O stretching sulfoxide), 752.82 (C=C bending alkene) (Figure 2 and Table 1). These absorption frequencies resembled the prevoiuos reports of Stigmasterol by Muthukrishnan *et al*., (2015) and Olawumi (2019) [24, 26].

The proton NMR (Figure 3 and Table 1) showed the proton of H-3 appeared as a multiplet at 3.51ppm and this reveals the existence of signal for olefinic proton at 4.98, 5.14, and 5.31. The angular methyl protons showed at 0.71, 0.80, and 1.02 corresponds to C19, C20, and C21 protons respectively (Habib *et al*., 2007) [9, 10].

^{13}C-NMR supporting evidence for the structure of the Stigmasterol compound was provided by the analysis of ^{13}C-NMR data and a complete assignment is given below (Figure 4 and Table 1). Due to hydroxylation at 3-position, C-3 carbons absorb at δ 72.12ppm. The ^{13}C has recognizable signals at 141.15ppm and 121.85ppm which are assigned C5 and C6 double bonds respectively. The value at 21.62, 138.72, and 129.60ppm corresponds to angular carbon C19, C20 and C21. Spectra show twenty-nine carbon signals including six methyls, nine methylene, eleven methane, and three quaternary carbon (Jamal *et al*., 2019) [14, 15].

Conclusion

The phytochemical assessment of the methanol fraction of the aerial parts of *B. herbacea* belonging to the family Malvaceae was efficiently carried out. From these chemical and spectral pieces of evidence, compound X was confirmed as Stigmasterol (Figure 5). Now, it is the turn of the pharmacologists/scientists to research the plant to explore the content by individual bioactivity of the stigmasterol. So, the current study will upgrade scientific networks to accomplish more work on this significant therapeutic plant in not so distant future.

Acknowledgment

The authors are sincere thanks to the Management and Principal of J.J. College of Arts and Science (Autonomous), Pudukkottai, Tamil Nadu for their continuous encroachments and support. The authors are thanks to Dr. S. Soosairaj, Assistant Professor, Department of Botany, St.Joseph College, Tiruchirappalli for identification and authentication of the plant sample.

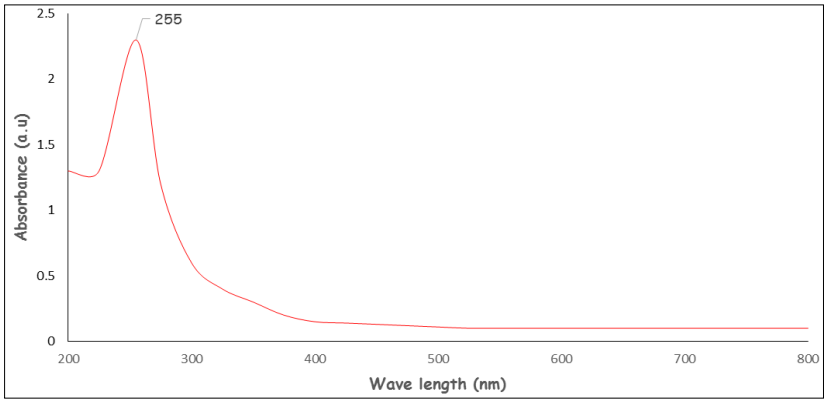

Fig 1: UV–visible spectrophotometer

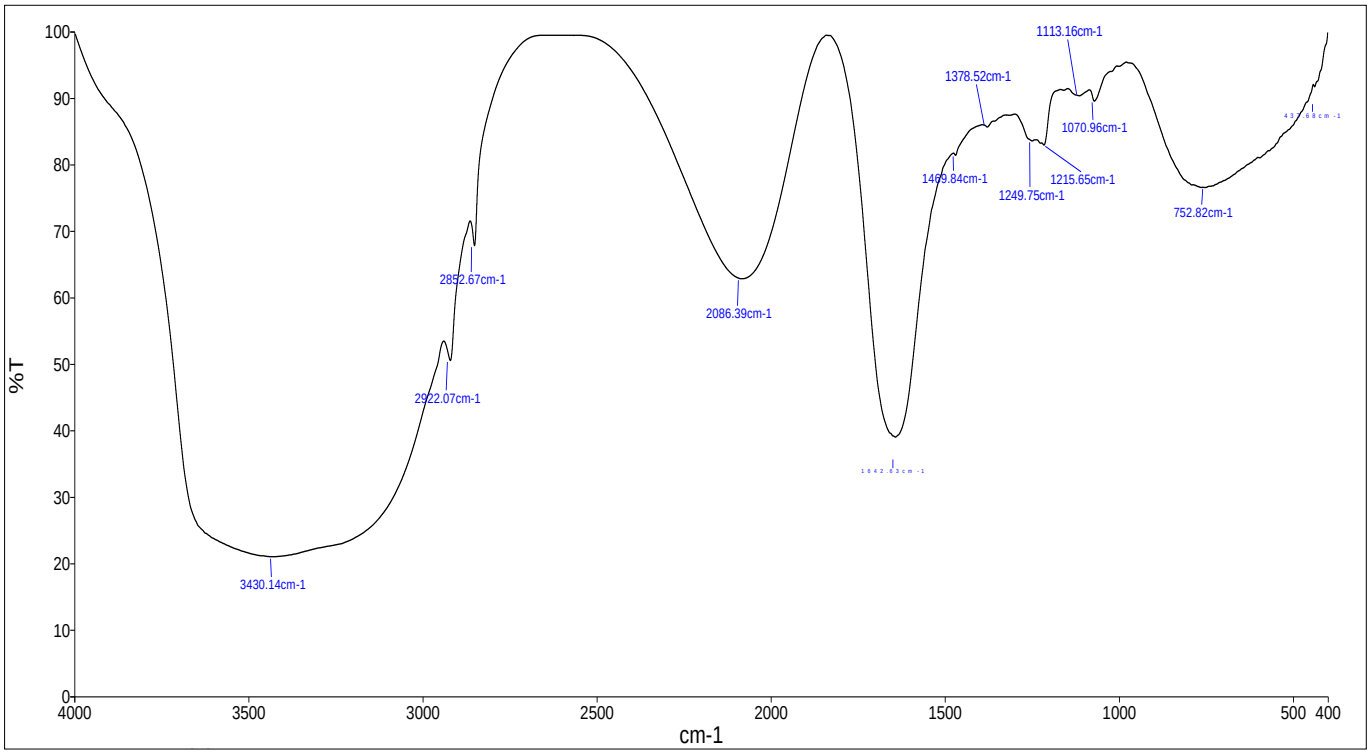

Fig 2: Fourier transform infrared spectrum (FTIR) Analysis

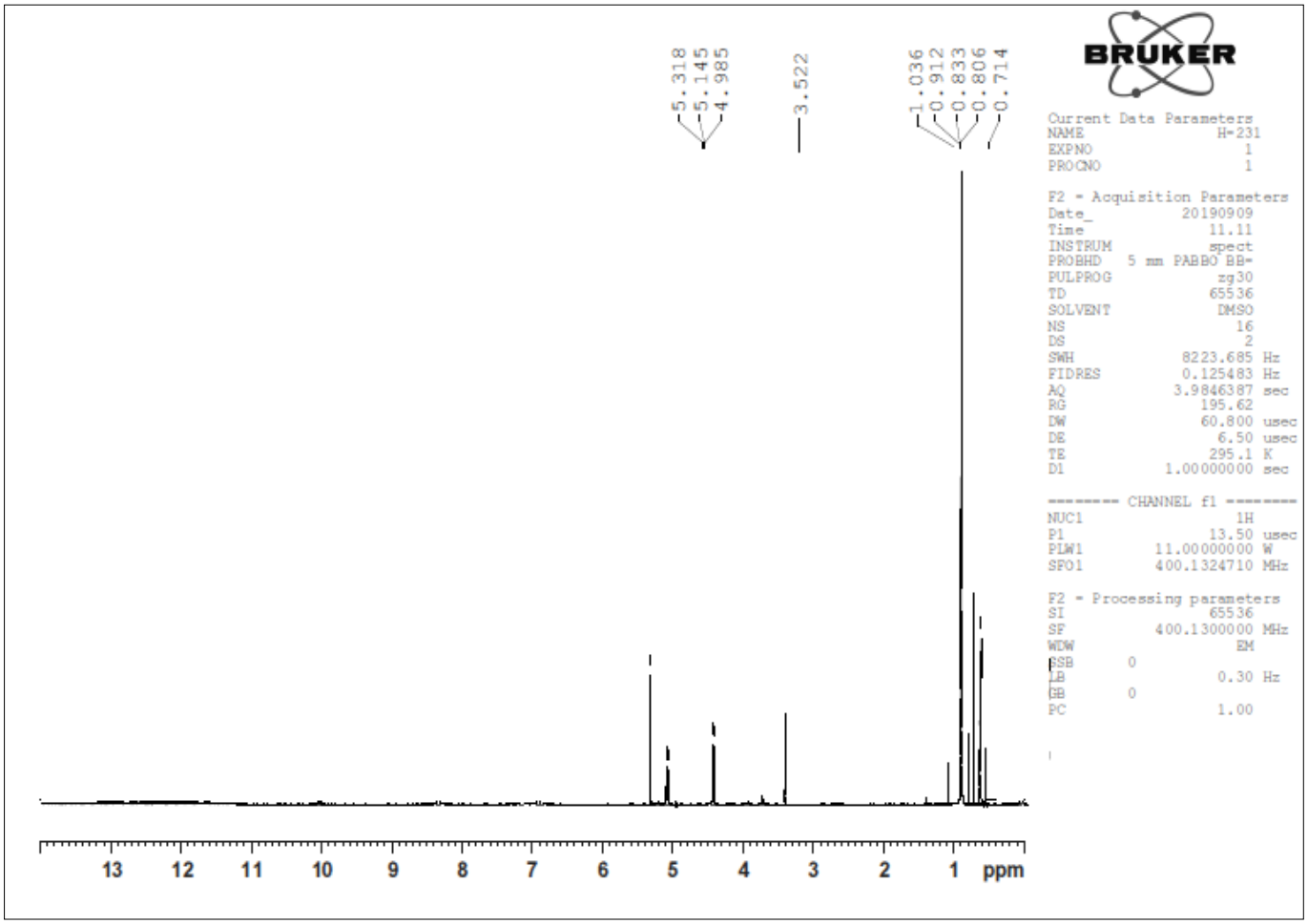

Fig 3: ^{1}H-NMR Spectrum

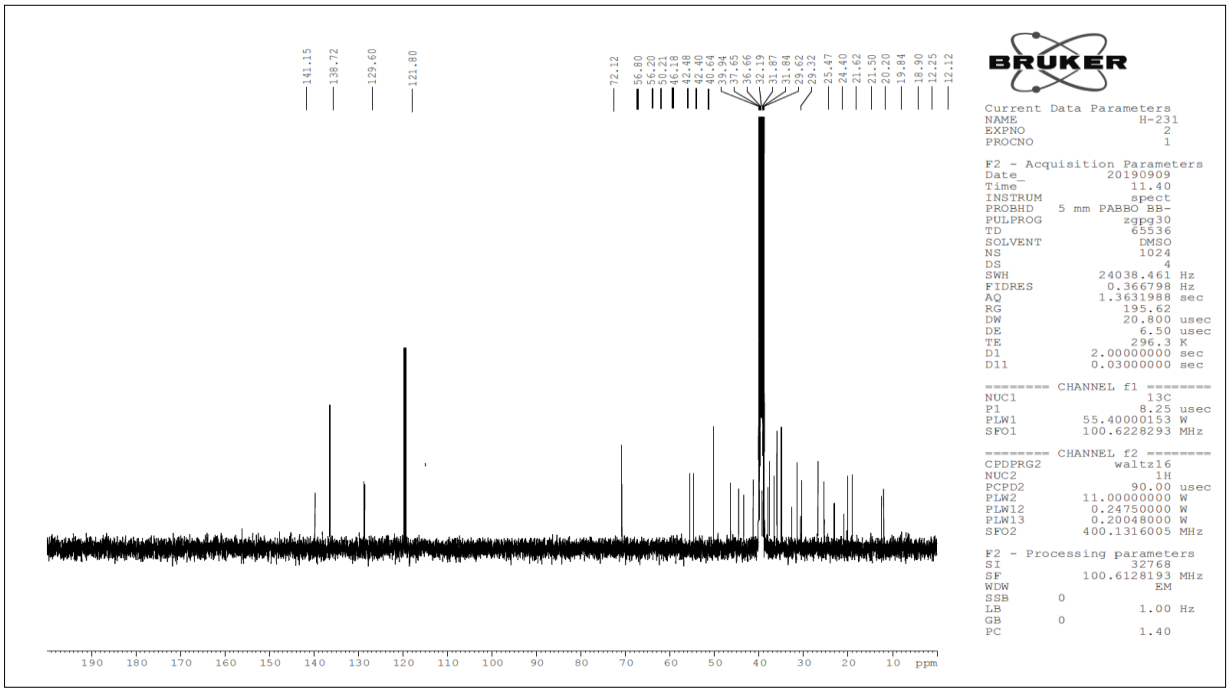

Fig 4: ^{13}C-NMR Spectrum

Table 1: Spectroscopic Data of Isolated Compound from Methanol Fraction of *B. herbacea*

Spectroscopic techniques	Data
UV λ max	255nm
IR (ranges in cm-1)	3430.14 (N-H stretching primary amine), 2922.07 (C-H stretching alkane), 2852.67 (C-H stretching alkane), 2086.39 (N=C=S stretching isothiocyanate), 1642.63 (C=C stretching alkene), 1469.84 (C-H bending alkane methylene group), 1378.52 (S=O stretching sulfonyl chloride), 1249.75 (C-O stretching alkyl aryl ether), 1215.65 (C-O stretching vinyl ether), 1113.16(C-Ostretching secondary alcohol), 1070.96 (S=O stretching sulfoxide), 752.82 (C=C bending alkene) functional groups
^{1}HNMR (DMSO)	δ0.71, δ0.80, δ0.82, δ0.83, δ0.91, δ1.03 (each 3H, Me × 6), δ3.51 (^{1}H, m, H-3), δ5.31 (1H, t, H-6), δ5.14 (^{1}H, s, H- 22), δ4.98 (^{1}H, s, H-23) ppm.
^{13}CNMR (DMSO)	δ 37.65, δ32.19, δ 72.12, δ 42.48, δ 141.15, δ 121.85, δ 31.84, δ 31.87, δ 50.21, δ 36.66, δ 21.50, δ 39.94, δ 42.40, δ 56.80, δ 24.40, δ 29.32, δ56.20, δ40.64, δ21.62, δ 138.72, δ129.60, δ46.18, δ25.47, δ12.12, δ29.62, δ20.20, δ19.84, δ18.90, δ12.25.

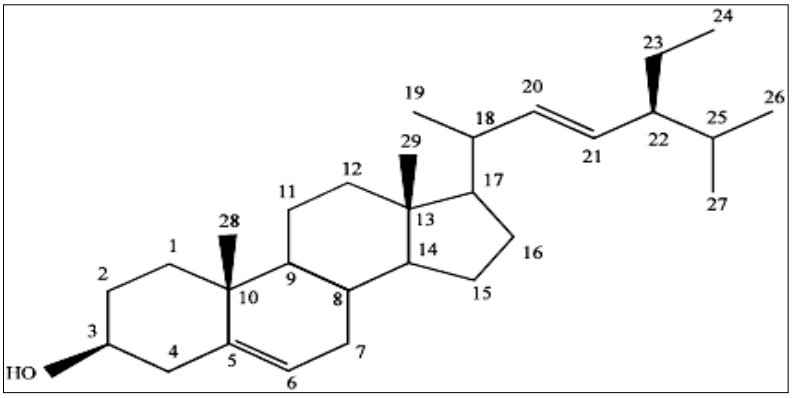

Fig 5: Structure of Stigmasterol

Reference

1. Asila Ahmed Salim Al Habsi, Mohammad Amzad Hossain. Isolation, structure characterization, and prediction of antioxidant activity of two new compounds from the leaves of *Dodonaea viscose* native to the Sultanate of Oman. Egyptian Journal of Basic and Applied Sciences,2018:5:157-164.
2. Bharathi KN, Vidyashree N, Raju HV. Evaluation of the antiasthmatic activity of root extracts of *Byttneria herbacea*. Indo Am J Pharm Sci,2016:5:456-61.
3. Chaturvedula VSP, Prakash I. Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavissimus*. International Current Pharmaceutical Journal,2012:1(9):239-242.
4. Chunyan Bao, Ran Lu, Ming Jin, Pengchong Xue, Changhui Tan, Tinghua Xu *et al*. Helical Stacking Tuned by Alkoxy Side Chains in p-Conjugated Triphenylbenzene Discotic Derivatives. Chem. Eur. J,2006:12:3287-3294.
5. Council of Scientific and Industrial Research. The Wealth of India. New Delhi: Council of Scientific and Industrial Research, 1988, 2.
6. De-Eknamkul W, Potduang B. Biosynthesis of β-sitosterol and stigmasterol in *Croton sublyratus* proceeds

via a mixed origin of isoprene units. Phytochemistry,2003:62(3):389-398.
7. Eberhardt TL, Li X, Shupe TF, Hse CY. Chinese tallow tree (*Sapium Sebiferum*) utilization: Characterization of extractives and cell-wall chemistry. Wood Fiber Sci,2007:39:319-324.
8. Gao JJ, Cheng DL, Liu XP. Chemical constituents of *Emilia sonchifolia* L. DC. Zhongguo Zhong Yao Za Zhi,1993:18(2):102-3,127.
9. Habib MR, Nikkon F, Rahman M, Haque ME, Karim MR. Isolation of stigmasterol and beta-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn). Pakistan Journal of Biological Sciences,2007:10(22):4174-4176.
10. Habib MR, Nikkon F, Rahman ME, Karim MR. Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of *Calotropis gigantean* (Linn). Pak. J. Biol. Sci,2007:10:4174-4176.
11. Han Y, Xia C, Cheng X, Xiang R, Liu H, Yan Q. Preliminary studies on chemical constituents and pharmacological action of *Eclipta prostrata* L. Zhongguo Zhong Yao Za Zhi,1998:23(11):680-2,703.
12. Hazra KM, Roy RN, Sen SK, Laska S. Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengi* Linn. Afr. J. Biotechnol,2007:6(12):1446-1449.
13. Hemavathy A, Shanthi P, Sowndharya C, Thiripura Sundari S, Priyadharshni K. Extraction and Isolation of Bioactive Compounds from a Therapeutic Medicinal Plant - *Wrightia tinctoria* (Roxb.) R. Br. International Journal of Pharmacognosy and Phytochemical Research,2019:11(3):199-204.
14. Jamal AK, Yaacoh WA, Laily B. Din. Triterpenes from the root bark of *Phyllanthus Columnaris*. Aus Journal of Basic and Applied Sciences,2009:3(2):1428-1431.
15. Jamal AK, Yaacob WA, Din LB. A Chemical Study on *Phyllanthus columnaris*, European Journal of Scientific Research,2009:28:76-81.
16. Jamaluddin F, Mohamed S, Lajis M. Hypoglycaemic effect of *Parkia speciosa* seeds due to the synergistic action of βsitosterol and stigmasterol. Food Chemistry,1994:49(4):339-345.
17. Kametani T, Furuyama H. Synthesis of vitamin D3 and related compounds. Med Res Rev,1987:7(2):147-171.
18. Kandati V, Govardhan P, Reddy CS, Nath AR, Reddy RR. *In-vitro* and *in-vivo* anti-inflammatory activity of *Andrographis serpylli-folia* (Rottl. Ex Vahl.) Wt. Int. Curr. Pharm. J,2012:1:199-204
19. Li C, Bu PB, Yue DK, Sun YF. Chemical constituents from roots of *Ficus hirta*. Zhongguo Zhong Yao Za Zhi,2006:31(2):131-3.
20. Li XL, Wang H, Liu G, Zhang XQ, Ye WC, Zhao SX. Study on chemical constituents from *Desmodium styracifolium*. Zhong Yao Cai,2007:30(7):802-5.
21. Liu G, Zheng J, Yu Z, Zhang J and Lin R. Study on sterols and triterpenes from the stems of Akebia quinata. Zhong Yao Cai,2005:28(12):1060-2.
22. Luu YY, Li L, Zhang C, Xiao YQ. Studies on chemical constituents in roots of *Heracleum rapula*. Zhongguo Zhong Yao Za Zhi,2006:31(8):667-8.
23. Mohamed Hajji, Rodham Arrayal, Mien Lassoed, Ones Masmoudia, Mohamed Damak, Moncef Nasri. GC/MS and LC/MS analysis and antioxidant and antimicrobial activities of various solvent extract from *Mirabilis jalapa* tubers. Process Biochemistry,2010:45:1486-1493.
24. Muthukrishnan, Prakash P, Jeyaprabha B, Shankar K. Stigmasterol extracted from *Ficus hispida* leaves as a green inhibitor for the mild steel corrosion in 1M HCl solution. Arabian Journal of Chemistry Arabian Journal of Chemistry,2015:12(8):3345-3356.
25. Navpreet K, Jasmine C, Akash J, Lalit K. Stigmasterol: A Comprehensive Review. IJPSR,2011:2(9):2259-2265.
26. Olawumi O, Koma O. Stigmasterol and stigmasterol glycoside: isolated compounds from *Tetrapleura tetraptera* extracts. Journal of Biochemistry International,2019:6(1):21-48.
27. Padmasri Govindarajan, Sarada DVL. Isolation and characterization of stigmasterol and β-sitosterol from *Acacia nilotica* (L.) Delile ssp Indica (benth.) Brenan. Journal of Pharmacy Research,2011:4(10):3601-3602.
28. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, anti per oxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. *Fitoterapia*,2009:80:123-126.
29. Peng T, Dong X, Deng Y, Tu Y, Li X. Research on chemical ingredients of the root from cultivar *Aralia cordata* Thunb. Zhong Yao Cai,2005:28(11):996-8.
30. Pengchong Xue, Ran Lu, Ying Huang, Ming Jin, Changhui Tan, Chunyan Bao *et al*. Novel Pearl-Necklace Porous CdS Nanofiber Templated by Organogel. Langmuir,2004:20(15):6470-6475.
31. Sarkar L, Bera R, Sen T, Karmakar S. Comparative study of the fractions of a relatively unexplored plant *Byttneria herbacea* on histaminergic inflammation. Int. J. Pharm. Pharm. Sci,2013:5(3):862-6.
32. Sarkar L, Bhuvaneswari N, Samanta SK, Islam MN, Sen T, Fukui H. A report on anti-oedemogenic activity of *Byttneria herbacea* roots possible involvement of histamine receptor (Type I). J Ethnopharmacol,2012:140:443-6.
33. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plant extracts. Afr J Tradit Complement Altern Med,2011:8(1):1-10.
34. Saxena HO, Brahmam M. The Flora of Orissa. Bhubaneswar: Orissa Forest Development Corporation Ltd, 1994, 1.
35. Sharma T, Acharya R. Review on Ethnomedicinal Claims, Pharmacological Activity, and Phytochemical Constituents of Samarakhadyam (*Byttneria herbacea* Roxb.). J Drug Res Ayurvedic Sci,2018:3:173-80.
36. Singh R, Kakkar A, Mishra VM. Phytochemical analysis of *Wrightia tinctoria* barks extract in water using GC-MS. International Journal of Pharmacy and Pharmaceutical Sciences,2015:7(3):470-473.
37. Somkuwar SR, Dongre UJ, Chaudhary RR, Chaturvedi A. *In vitro* screening of an antioxidant potential of *Byttneria herbacea* Roxb. Int J Curr Microbiol Appl Sci,2014:3:622-9.
38. Sundararaman P, Djerassi C. A convenient synthesis of progesterone from stigmasterol. J Org Chem,1997:42(22):3633-3634.
39. Tarun Sharma, Rabinarayan Acharya, Harisha CR, Shukla VJ. International Journal of Green Pharmacy,2019:13(4):332-337.
40. Victor O, Chidi O. Phytochemical constituents of some selected medicinal plants. Afri. J. Pure Appl. Chem,2009:3:228-23.
41. Wind from A and Hauth A: Over Stigmasterin, a new Phytoserin from Calabar beans. In: Reports of the German chemical society,1907:39:4378-4384.
42. Yang S, Zhong Y, Luo H, Ding X, Zuo C. Studies on chemical constituents of the roots of *Gypsophila oldhamiana* Miq. Zhongguo Zhong Yao Za Zhi,1999:24(11):680-1,703.
43. Yang XW, Guo QM. Studies on chemical constituents in fruits of *Eucalyptus globules*. Zhongguo Zhong Yao Za Zhi,2007:32(6):496-500.
44. Zhang M, Chen Y. Chemical constituents of *Eclipta alba* (L.) Hassk. Zhongguo Zhong Yao Za Zhi,1996:21(8):480-1,510.