European Journal of Medicinal Plants

31(20): 57-64, 2020; Article no.EJMP.65540
ISSN: 2231-0894, NLM ID: 101583475

Isolation of Steroidal Compounds from Plant *Tribulus terristris*

Sagar A. Nalawade[1,2], Manisha S. Badhe[2], Manohar G. Chaskar[3] and Shirish S. Pingale[4*]

[1]Department of Chemistry, B. G. College Sangavi, Pune, India.
[2]Department of Chemistry, Dattatray Govindrao Walse Patil College Pargaon Tarf Awsari, Pune, India.
[3]Department of Chemistry, Ramkrushna More ACS College, Akurdi, Pune, India.
[4]Department of Chemistry, Arts, Commerce and Science College, Narayangaon, Pune, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MGC and SSP designed and supervised the work. Author MSB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i2030359
Editor(s):
(1) Dr. Prem K. Ramasamy, Brandeis University, USA.
(2) Prof. Marcello Iriti, Milan State University, Italy.
Reviewers:
(1) Frederick William Akuffo Owusu, Kwame Nkrumah University of Science and Technology, Ghana.
(2) Anselmo Enrique Ferrer Hernandez, Havana University, Cuba.
Complete Peer review History: http://www.sdiarticle4.com/review-history/65540

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 31 December 2020

ABSTRACT

In the present study three steroidal compounds, Stigmasterol, Cholesterol and Stigmasterol glucoside were isolated from the acetone extract of plant *Tribulus terrestris* L. with the help of column chromatography and TLC techniques. The isolated steroidal compounds were characterized using both proton and carbon-13 NMR.

Keywords: Zygophyllaceae; Tribulus terrestris L.; steroidal compounds.

1. INTRODUCTION

Nature is a great source of medicinal plants and herbal drugs which are potential resources of therapeutic products used in various treatments and preventions of various infections and diseases [1]. A plant *Tribulus terrestris* L. of the family *zygophyllaceae* is an autochthonous plant

**Corresponding author: E-mail: drshirishpingale@gmail.com;*

which has been mentioned in Ayurveda with several chemical properties [2,3]. The usage of plant extracts and plant derived chemicals for disease management become therapeutic modality [4]. The plant *Tribulus terrestris* L. is used in household medicine as a tonic, Aphrodisiac, Palliative, Astringent, Gastric, anti-infective medicines [2,3]. The ash of the plant is good for external application in rheumatic arthritis [5].

In India it is commonly known as *gokharu* which means the spines of fruit that injure the grazing cattles. *Tribulus terrestris* L. is used in folk medicine as health tonic [6]. The extract of *Tribulus terrestris* L. is commonly used in medicine to control blood pressure and cholesterol [7]. The extract decreases the blood cholesterol level in humans, rats and mice. Plant can be found all over the world, especially in moisture less climate viz. China, India, southern USA, Spain, Bulgaria, Bangladesh, Pakistan etc. [3,8].

Several chemical constituents were found in different parts of whole plant of *Tribulus Terrestris* L. Studies on the plant indicates that ascorbic acid, calcium carbonate, fat, fibre, iron, oxalates, phosphorous potassium, protein, tribuloside were isolated from the leaf of the plant [2]. β- sistosterol, campesterol, gitogenin, kaempferol-3- β -D-(6"P-coumaroyl)-glycoside, kaempferol-3-rutinoside, reogitogenin, guercetin, stigmasterol were found in flower of plant [2]. From the fruits of plant, aspartic acid, glutamic acid, linoleic acid, nernecogenin-3-0-beta-D-glycopy ranoside aleic acid, palmitic acid, stearic acid were isolated [9]. Shoot of plant consists of duacosterol, desoxydiosgenin, terrestrosides, diosgenin, hecogenin, diosgin, protodisocin, rutin, tribulson were isolated from shoot of plant [10]. Fat, harmin, proteins etc were found in seed of the plant [11,12]. The structure of 26-*O*-β-D-glucopyranosyl-(25*S*)-5α-furostane-20(22)-en-12-one-3*β*, 26-diol-3-*O*-*α*-L-rhamnopyranosy l-(1→2)-[*β*-D-glucopyranosy l-(1→4)]-*β*-D-galactopyranoside was isolated from root of *Tribulus terrestris* L. as a furostanol glycosides [13-19]. *Tribulus terrestris* L. contains about 20 chemical constituents which are isolated from methanolic extract of the whole plant [3]. *Tribulus terrestris L.* extract was subjected to various phytochemical tests and found varies compounds in it such as saponins, amino acid, proteins, glycosides, cardiac glycosides, alkaloids and flavonoids, carbohydrates [20]. The phytochemical screening of seed of *Tribulus terrestris* L. reported to shows the different bioactive compounds like sterols, oils, alkaloids, saponins, phenols tannins and resins [3]. The 16.63% percentage of total protein content was found in arial part of *Tribulus terrestris* L. The amino acid such as phenylalanine, threonine, valine, leucine, lysine, aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine and arginine etc. were also isolated from *Tribulus terristris* [21]. The 6.076% percentage of flavonoid content was reported to found in methanolic extract of the plant. HPLC analysis shows presence of flavonoids such naringin, rutin, hyperoside, quercetrin, naringenin, quercetin, hesperetin, kampferol and apigenin from the methanolic extract [22]. The results of HPLC analysis of phenolic compounds revealed that fourteen identified phenolic compounds which are protocatechuic acid, pyrogallol, gallic acid, chlorogenic acid, p-hydroxybenzoic acid, catechin, catechol, caffeic acid, vanillic acid, salicylic acid, ellagic acid, ferulic acid, coumaric acid and cinnamic acid [9,23-26]. The present study was aimed to isolate various medicinally important ingredients present in the *Tribulus terrestris L.* plant.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plant material was collected in the form of whole plant by S.A.N. from the lake area of Nagapur, Pune, India in November 2018. The plant's flowering is generally observed during January to September, while fruiting takes place throughout the year. It was identified by the depositing it as a voucher specimen at the Botanical Survey of India, Western Circle, Pune (No. SPPG1N).

2.2 Extraction Methodology and Compound Isolation

The material was shade dried and pulverized. Acetone extract of the plant *Tribulus terrestris L.* was prepared in 10 L round bottom flask as described earlier [27]. The whole dried plant was grinded (480 g) and was extracted by maceration with acetone (1 L × 3 for 14 h) at room temperature. At reduced pressure the acetone-soluble fraction was filtered and concentrated which resulted in a green colored acetone extract ATT (42.00 g, 8.4% based on the dry weight of the plant).

2.3 Characterization

2.3.1 Chromatographic methods

Column chromatography (CC) was performed using silica gel 100–200 mesh size and re-chromatography using silica gel 230–400 mesh size (Thomas Baker, Ltd., Mumbai, India) and preparative thin-layer chromatography (TLC) was carried out using glass TLC plates supplied by Merck Ltd. (Whitehouse Station, NJ, USA). Spectra Max Plus 384 plate reader (Molecular Devices, Inc., Sunnyvale, CA, USA) was used. Rifampicin, isoniazid, paclitaxel and MTT were purchased from Sigma-Aldrich, St. Louise, MO, USA. Britelite plus reagent was purchased from Perkin Elmer, Waltham, MA, USA. All the compounds were purified in distilled solvents.

2.3.2 NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance III Ultra Shield NMR instrument (^{1}H: 400 MHz and ^{13}C: 100 MHz) at 25 °C. ESI-MS spectra were recorded with Waters Acquity LC–MS instrument and HR-ESI-MS spectra recorded using Autoconcept Mass Spectrometer (Mass Spectrometry Instruments, West Yorkshire, UK).

3. RESULTS AND DISCUSSION

On column chromatography purification 21 g of ATT was separated using acetone in pet ether. Fractions which were showing similar patterns in TLC were combined to get ten broad fractions (ATT-1 to ATT-10). Fraction ATT-4 (0.50 g) was separated using CC and eluted with acetone in pet ether and eight fractions (ATT-4-I to ATT-4-VIII) were collected. Fraction ATT-4-II was purified using preparative TLC with 2% acetone in pet ether as the mobile phase offered compound 1 and crystallization of which was obtained in 29 mg. Recrystallization of fraction ATT-4-VII was offered white crystalline compound 2 in 18 mg. Fraction ATT-5 (1.5 g) was CC purified using 2% to 20% gradient acetone-petroleum ether and collected five fractions (ATT-5-I–ATT-5-V). Washing of the fraction ATT-5-III using diethyl ether offered white solid compound 3 (16 mg). Based on the PMR, CMR and HR-ESI-MS, the compounds were identified as Stigmasterol 1, Cholesterol 2, and Stigmasterol glucoside 3 as shown in the Fig. 1.

The details of the chemical shifts of ^{1}H NMR and ^{13}C NMR as observed for the three isolated fractions are given below.

Stigmasterol (1):

^{1}H NMR (400MHz, CHLOROFORM-d) δ= 5.35 (d, J = 5.5 Hz, 1 H), 5.15 (dd, J = 8.2, 15.1 Hz, 1 H), 5.01 (dd, J = 8.7, 15.1 Hz, 1 H), 3.58 - 3.46 (m, 1 H), 2.32 - 2.22 (m, 3 H), 2.05 - 1.95 (m, 3 H), 1.88 - 1.82 (m, 2 H), 1.73 - 1.64 (m, 1 H), 1.57 - 1.46 (m, 8 H), 1.21 - 1.10 (m, 5 H), 1.05 - 0.99 (m, 9 H), 0.98 - 0.87 (m, 3 H), 0.87 - 0.83 (m, 4 H), 0.82 - 0.77 (m, 6 H), 0.70 (s, 3 H)

^{13}C NMR (100 MHz, CHLOROFORM) δ=12.3, 12.6, 19.3, 21.3, 24.5, 25.4, 29.2, 32.1, 36.6, 37.3, 39.7, 40.7, 42.4, 50.3, 51.3, 56.0, 57.1, 71.9, 121.9, 129.1, 138.1, 140.4

Molecular Formula: $C_{29}H_{48}O$

Cholesterol (2):

^{1}H NMR (400MHz, CHLOROFORM-d) δ= 5.39 - 5.29 (m, 1 H), 3.58 - 3.47 (m, 1 H), 2.34 - 2.18 (m, 2 H), 2.06 - 1.91 (m, 3 H), 1.90 - 1.76 (m, 3 H), 1.65 - 1.39 (m, 8 H), 1.39 - 1.22 (m, 4 H), 1.21 - 1.04 (m, 7 H), 1.04 - 0.94 (m, 6 H), 0.91 (d, *J* = 6.4 Hz, 3 H), 0.86 (dd, *J* = 1.8, 6.9 Hz, 6 H), 0.68 (s, 3 H)

^{13}C NMR (100 MHz, CHLOROFORM) δ= 11.6, 18.3, 19.2, 20.5, 22.6, 26.0, 27.8, 31.2, 31.5, 35.7, 36.9, 39.3, 39.5, 42.0, 49.9, 55.9, 56.5, 71.7, 121.5, 140.7

Molecular Formula: $C_{27}H_{46}O$

Stigmasterol glucoside (3):

^{1}H NMR (200MHz, Pyridine) δ= 4.64 - 4.53 (m, 1 H), 4.48 - 4.19 (m, 3 H), 4.16 - 3.82 (m, 3 H), 2.72 (br. s., 1 H), 2.63 - 2.37 (m, 1 H), 2.12 (br. s., 1 H), 2.07 - 1.53 (m, 6 H), 1.53 - 1.20 (m, 9 H), 1.20 - 0.97 (m, 7 H), 0.97 - 0.87 (m, 9 H), 0.85 (br. s., 3 H), 0.67 (s, 3 H)

^{13}C NMR (100 MHz, Pyridine) δ= 12.3, 14.2, 19.0, 19.7, 21.6, 23.4, 24.3, 26.4, 28.6, 29.2, 30.3, 32.3, 34.3, 36.9, 37.6, 39.2, 40.0, 42.4, 46.2, 50.3, 56.6, 62.9, 71.8, 75.3, 78.2, 102.6, 121.8, 140.5.

Molecular Formula: $C_{35}H_{58}O_6$

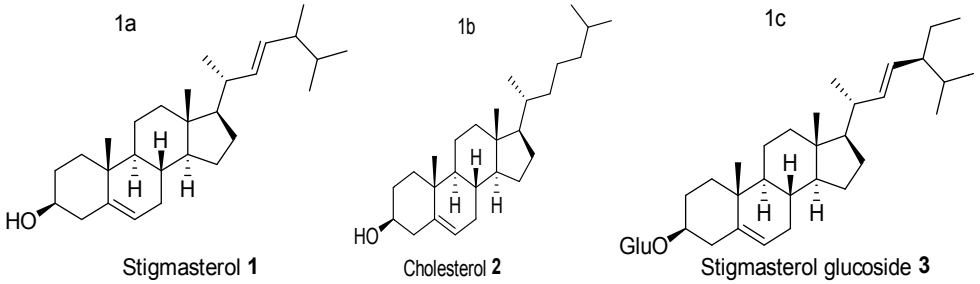
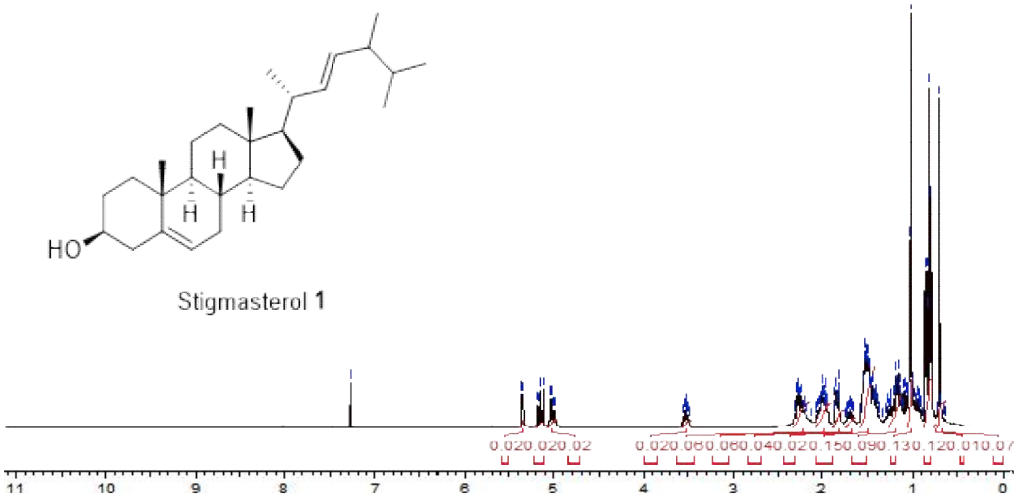


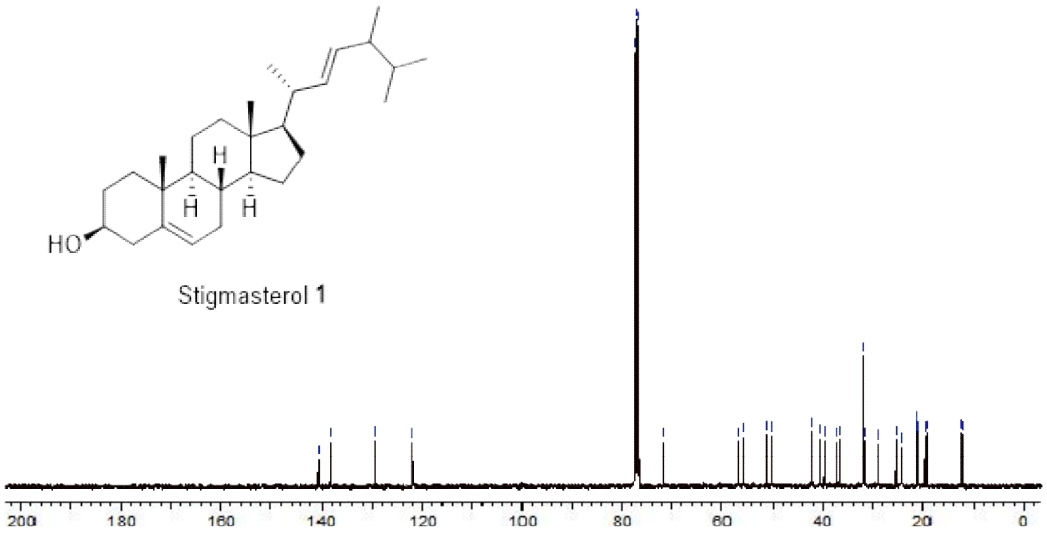
Fig. 1. Structure of the isolated and identified compounds from the plant *Tribulus terrestris* L., 1a. Stigmasterol, 1b. Cholesterol, and 1c. Stigmasterol glucoside

Stigmasterol (1):

^{1}H NMR

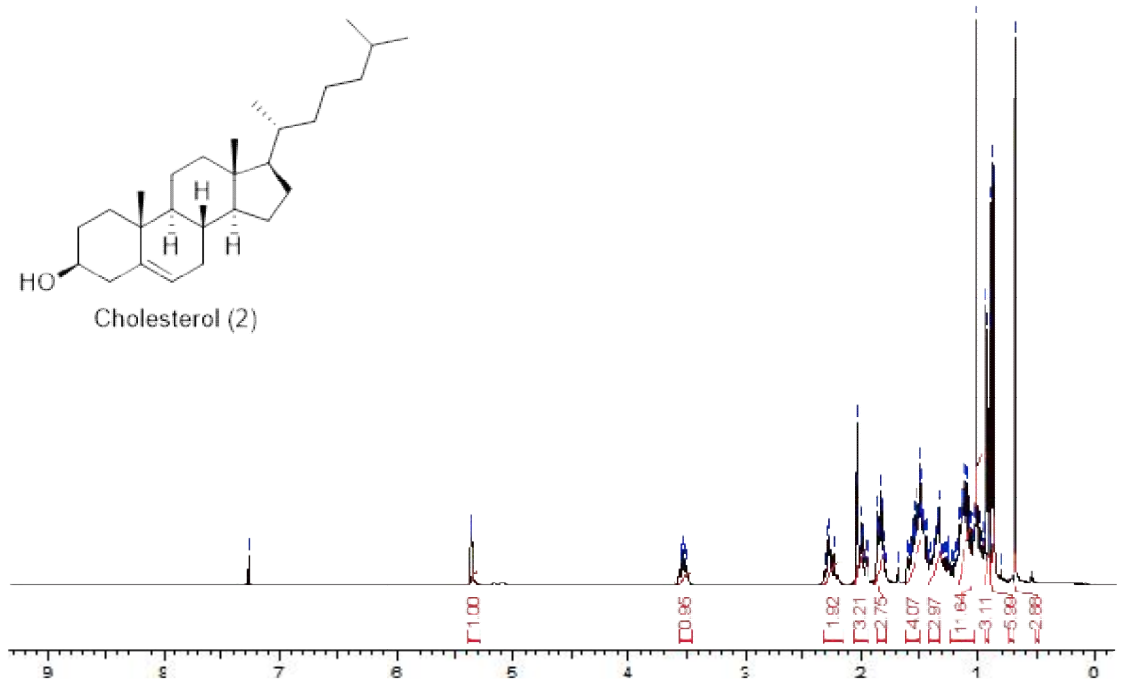


^{13}C NMR

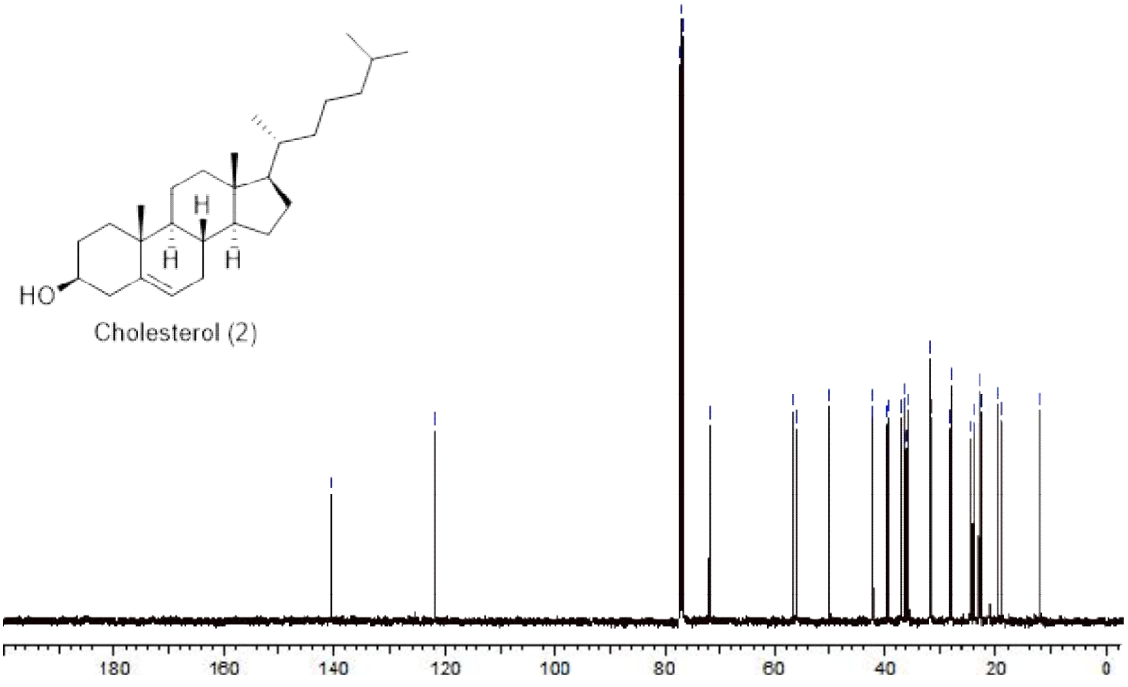


Cholesterol (2):

^{1}H NMR

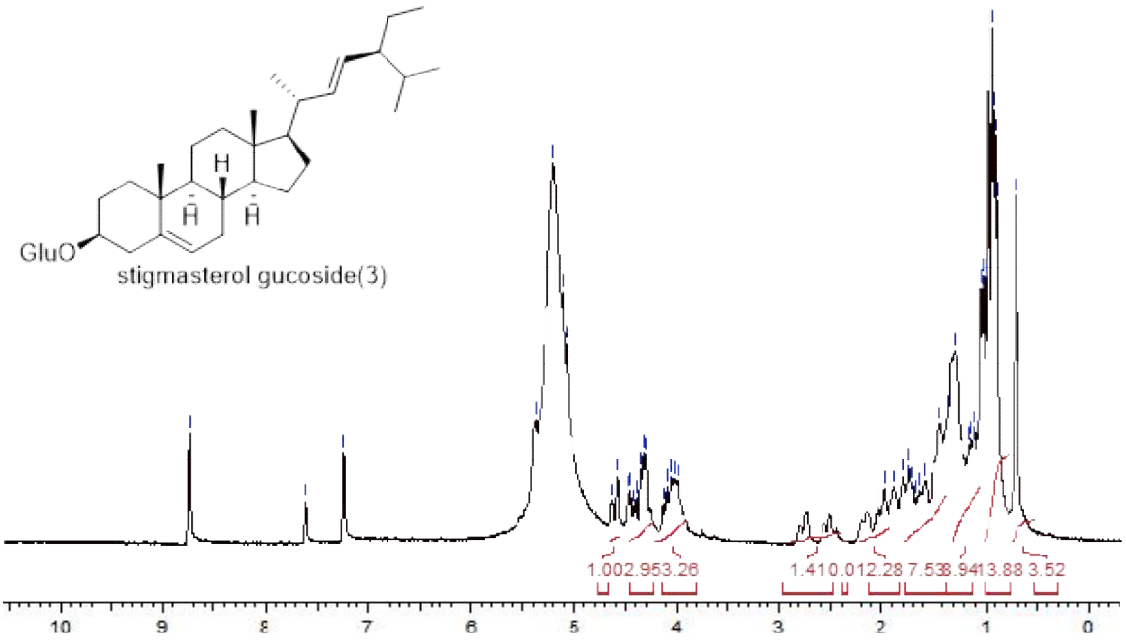


^{13}C NMR

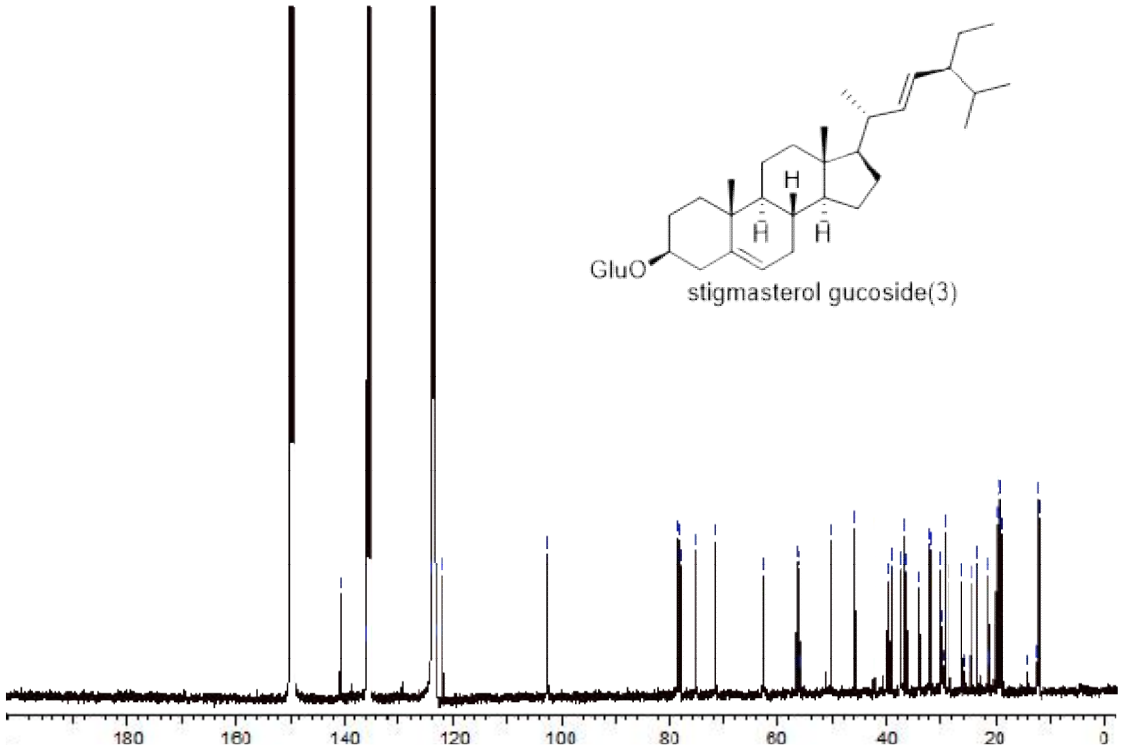


Stigmasterol glucoside (3):

^{1}H NMR



^{13}C NMR



Similar compounds were reported in the literature from the various parts of the plant *Tribulus terrestris* L [2,3,28]. The potential uses of the extracted compounds were reported in the literature as follows. Stigmasterol is reported to be found in the plant which is reported to be a part of medicine to control the blood pressure as it has potential to reduce the risk of cardiovascular diseases [29]. While the Stigmasterol glucoside is reported to be medicinally important as it shows anthelmintic activity, antituberculosis activity, antioxidant and anti-inflammatory functionality [28].

4. CONCLUSION

In this present study column chromatography technique was carried out on acetone extract of plant *Tribulus terrestris L.* Fractions of acetone extract of plant were separated by using TLC technique and medicinally important three steroidal compounds viz. Stigmasterol, Cholesterol and Stigmasterol glucoside have been isolated which are verified by ^{1}H NMR and ^{13}C NMR spectra.

CONSENT AND ETHICAL APPROVAL

It's not applicable.

ACKNOWLEDGEMENT

SAN would like to thank Dattatray Govindrao Walse Patil College, Pargaon Tarf Awsari, Pune for their constant support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shawky E, Nada AA, Ibrahim RS. Potential role of medicinal plants and their constituents in the mitigation of SARS-CoV-2: Identifying related therapeutic targets using network pharmacology and molecular docking analyses. RSC Advances. 2020;10(47):27961-27983.
2. Ukani MD, Nanavati DD, Mehta NK. A review on the ayurvedic herb *Tribulus terrestris* L. Ancient Science of life. 1997;17(2):144-150.
3. Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S. Phytopharmacological overview of Tribulus terrestris. Pharmacognosy Reviews. 2014;8(15):45-51.
4. Ansari J, Inamdar N. The promise of traditional medicines. International Journal of Pharmacology. 2010;6.
5. Chandrasekar R, Chandrasekar S. Natural herbal treatment for rheumatoid arthritis - a review. Int J Pharm Sci Res. 2017;8(2):368-384.
6. Duke JA. Handbook of Medicinal Herbs. Second Editio ed. CRC Press; 2002.
7. Khare CP, Indian medicinal plants, an illustrated dictionary. Springer-Verlag New York; 2007.
8. The wealth of India: A dictionary of Indian raw materials and industrial products (Industrial Products—Part I). The Indian Medical Gazette. 1949;84(10):476-477.
9. Wu TS, Shi LS, Kuo SC. Alkaloids and other constituents from Tribulus terrestris. Phytochemistry. 1999;50(8):1411-1415.
10. Jin JM, Zhang YJ, Yang CR. Spirostanol and furostanol glycosides from the fresh tubers of polianthes tuberosa. Journal of Natural Products. 2004;67(1)5-9.
11. Nadkarni KM. Indian materia medica. Popular Prakashan, Bombay. 1976;1.
12. Xu Y, Liu Y, Xu T, Xie S, Si Y, Liu Y, Zhou H, Liu T, Xu D. A new furostanol glycoside from tribulus terrestris. Molecules (Basel, Switzerland). 2010;15(2):613-618.
13. Bedir E, Khan IA. New steroidal glycosides from the fruits of tribulus terrestris. Journal of Natural Products. 2000;63(12):1699-1701.
14. Conrad J, Dinchev D, Klaiber I, Mika S, Kostova I, Kraus W. A novel furostanol saponin from tribulus terrestris of Bulgarian origin. Fitoterapia. 200475(2):117-122.
15. Hammoda HM, Ghazy NM, Harraz FM, Radwan MM, ElSohly MA, Abdallah II. Chemical constituents from tribulus terrestris and screening of their antioxidant activity. Phytochemistry. 2013;92:153-159.
16. Kang LP, Wu KL, Yu HS, Pang X, Liu J, Han LF, Zhang J, Zhao Y, Xiong CQ, Song XB, Liu C, Cong YW, Ma BP. Steroidal saponins from tribulus terrestris. Phytochemistry. 2014;107:182-189.
17. Perrone A, Plaza A, Bloise E, Nigro P, Hamed AI, Belisario MA, Pizza C, Piacente S. Cytotoxic furostanol saponins

and a megastigmane glucoside from tribulus parvispinus. Journal of Natural Products. 2005;68(10):1549-1553.
18. Wu G, Jiang S, Jiang F, Zhu D, Wu H, Jiang S. Steroidal glycosides from tribulus terrestris. Phytochemistry. 1996;42(6):1677-1681.
19. Yuan WH, Wang NL, Yi YH, Yao XS. Two furostanol saponins from the fruits of tribulus terrestris. Chinese Journal of Natural Medicines. 2008;6(3):172-175.
20. Tomova M, Panova D. Steroid sapogenins. Isolation of diosgenin from tribulus terrestris L. Farmatsiya. 1965;15:211-214.
21. Ammar NM, El-Hawary SSED, Mohamed DA, Afifi MS, Ghanem DM, Awad G. Phytochemical and biological studies of tribulus terrestris L. Growing in Egypt. International Journal of Pharmacology. 2018;14:248-259.
22. Mattila P, Astola J, Kumpulainen J. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. Journal of Agricultural and Food Chemistry. 2000;48(12):5834-5841.
23. Panova D, Tomova M. Screening of tribulus terrestris L. for phenolic compounds. Farmtsiya. 1970;20:29-31.
24. Ganzera M, Bedir E, Khan IA. Determination of steroidal saponins in tribulus terrestris by reversed-phase high-performance liquid chromatography and evaporative light scattering detection. Journal of Pharmaceutical Sciences. 2001;90(11):1752-1758.
25. Lee ST, Mitchell RB, Wang Z, Heiss C, Gardner DR, Azadi P. Isolation, Characterization and Quantification of Steroidal Saponins in Switchgrass (Panicum virgatum L.). Journal of Agricultural and Food Chemistry. 2009;57(6):2599-2604.
26. Zheng W, Wang F, Zhao Y, Sun X, Kang L, Fan Z, Qiao L, Yan R, Liu S, Ma B. Rapid characterization of constituents in tribulus terrestris from different habitats by UHPLC/Q-TOF MS. Journal of the American Society for Mass Spectrometry. 2017;28(11):2302-2318.
27. Said MS, Chinchansure AA, Nawale L, Durge A, Wadhwani A, Kulkarni SS, Sarkar D, Joshi SP. A new butenolide cinnamate and other biological active chemical constituents from Polygonum glabrum. Natural Product Research. 2015;29(22):2080-2086.
28. Deepak M, Dipankar G, Prashanth D, Asha MK, Amit A, Venkataraman BV. Tribulosin and β-sitosterol-D-glucoside, the anthelmintic principles of tribulus terrestris. Phytomedicine. 2002;9(8):753-756.
29. Ștefănescu R, Tero-Vescan A, Negroiu A, Aurică E, Vari CE. A comprehensive review of the phytochemical, pharmacological and toxicological properties of *Tribulus terrestris* L. Biomolecules. 2020;10(5):752.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/65540