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# Isolation of stigmast-5-en-3β-ol (β-sitosterol) from dichloromethane extract of *Sterculia setigera Leaves* (Sterculiaceae)

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**ABSTRACT**

*Ethnomedical survey of plants used for the treatment of tuberculosis in North Eastern Nigeria was undertaken primarily for the purpose of documentation and screening for possible drug leads. The leaves of Sterculia setigera has been implicated in the folkloric treatment of Tuberculosis by the Fulani people of Bauchi state, Nigeria. Preliminary phytochemical screening of the leaves of S. setigera (sterculiaceae) revealed the presence of steroids, terpenoids, tannins, fatty acids and cardiac glycosides, alkaloids and flavonoids were not detected. In this work, the pulverized leaves of S. setigera was defatted in cold maceration with hexane and then exhaustively extracted with dichloromethane. The Dichloromethane soluble extract was subjected to normal Phase column chromatography (NPCC) using gradient elution. Further purification led to isolation of colourless, shiny needle-like crystals which was subjected to physical, chemical and spectroscopic analyses using IR, 'H-NMR, $^{13}$C-NMR and GC-MS. The compound was confirmed to be Stigmast-5-en-3β-ol (β-sitosterol) (1), a known sterol hitherto unreported in S. stigera leaves.*

**Keywords**: *Sterculia setigera*; Ethnomedicine; Tuberculosis, phytochemical, stigmasterol

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## INTRODUCTION

Natural products continue to play a significant role in the drug discovery and development process, and plants are recognized as a reservoir of myriads of active anti-mycobacterial natural products [1, 2]. Overtime, folkloric medicine has had the chance to become refined based on efficacy and safety. It has been hypothesized that plants that were used in the past and are still used today to treat microbial infections are more likely to contain anti-microbial principle(s) than plants that have not been used continuously [3]. Undoubtedly, many higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective and chemotherapeutic agents.

*Sterculia setigera* is a tree, wide spread in the savannah area of Tropical Africa, which is often characterised of stoney hills. It is deciduous, grows up to 12m high and 15m in girth. The bark is pale purplish, smooth with thin scales which peel off expose yellowish patches, exuding gummy sap. The Hausa people of Northern Nigeria refer to it as Kukkuki; Bo'poli in Fulani and Sugubo in Kanuri. The plant has been used in fokloric treatment of Malaria, Jaundice, Measles, Syphilis, Tuberculosis and Leprosy [4,5]. Very little information is available in the literature on phytochemicals and pharmacological properties of the leaves of *S. setigera* compare to its roots, seeds and stem/bark. Anti-tubercular activity of the plant leaves extract against virulent strains of *Mycobacterium tuberculosis*, $H_{37}$Rv (ATCC27294) in Alamar blue assay has been reported [6]. The primary aim of this research is to identify and characterize the bioactive metabolites from the leaves of *Sterculia setigera*. This paper reports on the isolation and characterization of Stigmast-5-en-3β-ol, a known phytosterol but not previously reported in *Sterculia setigera*,

## MATERIALS AND METHODS

Fresh leaves of *Sterculia setigera* were collected in July, 2011 at Narabi village (Hill side), Bauchi state. The plant was taxonomically identified and authenticated by Dr. I. Abdulkareem of the department of Horticulture, Federal College of Forestry, Jos, Plateau state, Nigeria. The plant material were air-dried and pulverized into coarse form.

***Extraction and isolation of the compound***

250 g of the plant material was defatted with hexane for 76 hours by cold maceration. The marc was extensively extracted with dichloromethane for another 76 hours. The extract was concentrated in vacuo using rota vapour and stored in refrigerator (yield 2.5%).

The chloroform extract was chromatographed on Silica gel (0.046-0.63mm; 230-400mesh, Product of Marc KGa A; 64271 Darmstadt, Germany). Gradient elution of Hexane-Ethylacetate (5% stepwise increase) afforded 154 fractions. Thin layer chromatography (TLC) was used to monitor the eluates, similar fractions were pooled together. Further purification of fraction 16-24 (which gave single spot with traces of impurity) on a smaller column (360x15mm) afforded a shiny colourless needle-like crystals coded IBF018 (64 mg). Repeated TLC of the crystals in solvent systems of varying polarity gave single spot confirming homogeneity. The melting point of the crystal was determined on a Perkein Elmer141 polarirmeter in chloroform selection (135-138$^0$C). The structure was comfirmed by NMR spectra ($^1$H, $^{13}$C, DEPT), IR and GL-MS. Spectra (Perkin Elmer Auto System).

***Phytochemical method***

The presence of steroid was confirmed using standard phytochemical methods (Salkowski and Liebermann Burchard reaction) as described in literature [7].

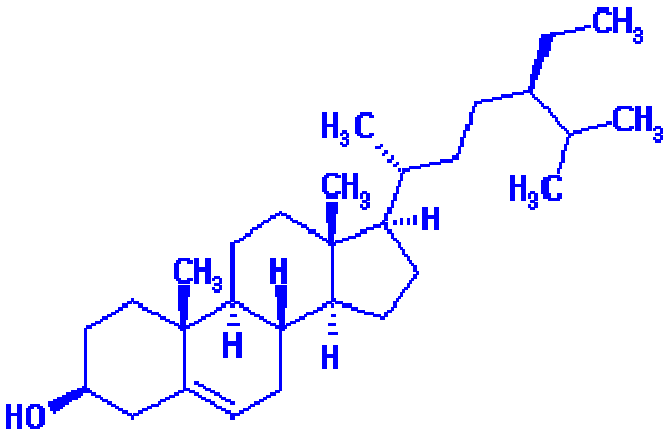


**Fig. (1) Stigmast-5-en-3β-ol**

## RESULTS AND DISCUSSION

IBF018 was isolated as a white crystalline compound, m.p. 135-139$^o$c. The IR spectra showed absorption bands at 3372.4cm$^{-1}$; characteristic of O-H stretching. 2940.5 and 2865.6cm$^{-1}$ absorptions are attributed to aliphatic $CH_2$ and CH stretching respectively. The absorption frequency at 1037cm$^{-1}$ signifies cycloalkane, while 1063.34cm$^{-1}$ indicates C-O stretch. Other absorption frequencies include 1641.6cm$^{-1}$ as a result of carbon-carbon double bond stretching, however, this bond is weak. These absorption frequencies are agreeble to those reported of stigmast-5-en-3β-ol [8].

The mass spectra of IBF18 suggested a molecular formula of $C_{29}H_{50}O$ as deduced from the mass spectrum (observe m/z 414.73[M+H]).

IBF018 showed positive Salkowski's and Liebermann-Burchard reaction confirming its steroidal nature. The $^1$H and $^{13}$C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlation and were shown in Table 1.

The $^1$H NMR Spectra of IBF18 shows that the compound has six methyl, eleven methylene and three quaternary carbons with a hydroxyl group. The carbons of alkenes conjugated are at 140.7ppm (C5) and 121.7ppm (C6) which was confirmed from the $^{13}$C NMR. The structure was simulated to obtain chemical shifts for both proton and carbon.

IBF018 was confirmed stigmast-5-en-3β-ol (β –sitosterol) on the basis of comparison of experimental and simulated data. The data is in accord with previously reported data for stigmast-5-en-3β-ol [8-10].

Steroids are essential secondary metabolites and they have been isolated in a number of plants. They are involved in the synthesis of many important hormones like progesterone, androgens, estrogens, corticoids, and in the synthesis of vitamin $D_3$. The pharmacological activities of steroids as anti-osteoarthritic, anti-hypercholesterolemic, cytotoxic, anti-tumour, hypoglycaemic, anti-mutagenic, anti-oxidant and anti-inflammatory have been reported in literature [8,11,12]. Steroids isolated from plants have been implicated in a number of reviews of reported anti-mycobacterial natural products as the anti-tubercular agents, with significant minimum inhibitory concentrations [13-15].

**Table 1. $^{1}H$ and $^{13}C$ NMR Chemical Shrift values for stigmast-5-en-3β-ol (1) recorded in CDCl3**

| Position | Group | $^{1}H$ | $^{13}C$ |
|---|---|---|---|
| 1 | CH2 | 1.48 | 37.2 |
| 2 | CH2 | 1.56 | 32.9 |
| 3 | CH | 3.40 | 71.8 |
| 4 | CH2 | 2.27 | 42.3 |
| 5 | C | - | 140.7 |
| 6 | CH | 5.37 | 121.7 |
| 7 | CH2 | 2.03 | 29.7 |
| 8 | CH | 1.50 | 31.6 |
| 9 | CH | 1.49 | 50.1 |
| 10 | C | - | 36.5 |
| 11 | CH2 | 1.45 | 21.0 |
| 12 | CH2 | 1.49 | 39.7 |
| 13 | C | - | 42.3 |
| 14 | CH | 1.5 | 56.7 |
| 15 | CH2 | 1.60 | 24.3 |
| 16 | CH2 | 1.80 | 29.1 |
| 17 | CH | 1.47 | 56.0 |
| 18 | CH3 | 0.70 | 39.7 |
| 19 | CH3 | 1.02 | 19.4 |
| 20 | CH | 1.62 | 33.9 |
| 21 | CH3 | 0.92 | 26.0 |
| 22 | CH2 | 0.86 | 45.8 |
| 23 | CH3 | 1.05 | 23.0 |
| 24 | CH | 1.50 | 11.9 |
| 25 | CH | 1.70 | 29.7 |
| 26 | CH3 | 0.82 | 19.8 |
| 27 | CH3 | 0.85 | 19.0 |
| 28 | CH2 | 1.04 | 18.7 |
| 29 | CH3 | 0.87 | 11.8 |
| 30 | **OH($C_3$ position)** | 2.00 | - |

*Assignments made on the basis of COSY, HMQC and HMBC correlations; chemical shift values are in 0 (ppm) coupling constants are in HZ.*

## CONCLUSION

Secondary metabolites are responsible for the various bioactivity observed by the indigenous people in medicinal plants. The occurrence of biologically active steroid in *Sterculia setigera* leaves provides credence for its use in several tradition remedies.

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## REFERENCES

[1] DJ Newman; GM Cragg; KM Snader. *Journal of Natural Products*, **2003**; 66: 1022-1037
[2] GF Pauli; RJ Case; T Inui; Y Wang; S Cho; NH Fischer; SG Franzblau. *Life Sciences*, **2005**; 78:485-494.
[3] IT Babalola IT. *Yobe Journal of Environmental Sciences*, **2012**; Vol.4 (1), pg.37-42.
[4] JM Dalziel. The Useful Plant of West Tropical Africa. Crown Agents for Overseas Government and Administration, **1937** pg. 109.
[5] JO Igoli; OG Ogaji; TA Tor-anyiin; NP Igoli. *African Journal of Complementary and Alternative Medicine*, **2005**; 2: 44

[6] IT Babalola; EA Adelakun; Y Wang; FO Shode. *Journal of pharmacognosy and phytochemistry*, **2012**; Vol.1 issue 3, pg 19-26.
[7] JB Harbone. Phytochemical methods, A guide to modern techniques of plant analysis. Chapman and Hall. London, **1998**; 3rd edition, pg 302.
[8] P Arjun; S Jha; PN Murthy; A Manik; A Sharone. *International Journal of pharm. Sci. and Res, 2010*; Vol. (2), 95-100.
[9] MIC Azizudin. *Turkish Journal of Chemistry*, **2008**; 32: 201-204.
[10] MR Habib; F Nikken; M Rahman; ME Haque, MR Karim. *Pakistan Journal of Biological Sciences, 2007*; 10(22): 4174-4176.
[11] N Kaur; J Chaudary; A Jain; And L Kishore. *Int. Journal of pharmaceutical Sciences and Research*, **2011**; Vol. 2 (9): 2259-2265.
[12] VS Chaturvedula; I Prakash. *International Current Pharmaceutical Journal*; **2012**; 1(9): 239-242
[13] BR Copp. *Nat. Prod. Rep*., **2003**, 20 535-557.
[14] AL Ounade; MPF Elvin-Lewis; WH Lewis. *Phytochemistry*, **2004**, (65): 1017-1017.
[15] LN Rogoza; NF Salakhutdinov and GA Tolstikov. *Opportunity, challenge and scope of Natural Products in Medicinal Chemistry*, **2011**, 103-120