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Isolation of Stigmasterol from Aerial Plant Part of *Spillanthes Acmella* Murr

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ABSTRACT [ENGLISH/ANGLAIS]

Pulverized aerial plant material (300 g) of *Spillanthes acmella* was exchaustively extracted with methanol and concentrated in vacuo using rotary evaporator at 40oC.the extract was later subjected to solvent partitioning to yield the following extracts petroleum ether, chloroform, Ethyl acetate and methanol. The petroleum ether crude extract was subjected to Vacuum Liquid Chromatographic (VLC) technique to yield various fractions which were combined based on their thin layer chromatography analysis to give SPEC. This was further purified by the Preparatory Thin Layer Chromatography (PTLC) to give SPEC-1.The structure of the isolated compound was established by 1D and 2D NMR spectroscopic analysis and by direct comparison of the data obtained with those reported in literature to be Stigmasterol.

Keywords: Spillanthes,toothache,vacuum liquid chromatography,stigmasterol

RÉSUMÉ [FRANÇAIS/FRENCH]

Pulvérisé matériel végétal aérienne (300 g) de Spillanthes acmella a été extraite avec du méthanol exchaustively et concentrée sous vide en utilisant un évaporateur rotatif àl'extrait a ensuite été soumis 40oC.the à un cloisonnement solvant pour donner l'éther de pétrole extraits ci-dessous, le chloroforme, l'acétate d'éthyle et de méthanol. L'extrait brutd'éther de pétrole a été soumis à un vide chromatographie en phase liquide (VLC)technique pour obtenir différentes fractions qui ont été réunies sur la base de leur analyse chromatographie sur couche mince de donner SPEC. Ceci a été purifiée par lachromatographie sur couche mince préparatoire (PTLC) pour donner SPEC-1.Lestructure du composé isolé a été établi par l'analyse RMN 1D et 2D spectroscopique etpar comparaison directe des données obtenues avec celles rapportées dans la littérature pour être Stigmastérol.

Mots-clés: Spillanthes, les maux de dents, la chromatographie liquide sous vide, le stigmastérol

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INTRODUCTION

Spillanthes acmella Murr is a flowering herb in the family Asteraceae. It is known as toothache plant or pancress as the leaves and the flower heads contain analgesic agent spilanthol that is used to numb toothache. The fresh and cooked leaves are combined with chilies and garlic to add flavour and vitamins to foods [1]. Some chemical constituents have been isolated from the plant such as spilanthol an alkyl amide [2]. This is responsible for the saliva inducing effect of the plant, and some triterpenoids [3]. The leaves along with alum are recommended as emetic [4].Because of this observation and the regular uses of the plant by the natives prompted this research possibly to find out if there could be more metabolites that can be of pharmacological relevance.

MATERIALS AND METHODS

Collection of plant material

The aerial plant part of *Spillanthes acmella* was collected from Okene, in Okene Local Government Area of Kogi State, Nigeria in March 2010. This was identified at the Herbarium of the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria Nigeria. The voucher specimen with number 534 was deposited at the Herbarium. The sample was air-dried, pulverized using wooden pestle and mortar. Finally, this was stored in an air-tight polythene bag and kept away from moisture until when needed for extraction.

Extraction Procedure

The pulverized plant material (300 g) was weighed and extracted exchaustively with redistilled methanol (2 liters) for 72 hours in a soxhlet extractor. Concentration of the extract was done *in vacuo* at 40 °C using rotary evaporator (*Rota vapor*) to give (43.7 g) or (15 %) of the crude extract. The methanol crude extracts was later subjected to solvent partitioning using petroleum-ether, chloroform, ethyl acetate to obtain various crude fractions. The petroleum ether fraction was subjected to vacuum liquid chromatography (VLC) using petroleum ether and ethyl

acetate for the gradient elution to obtain various fractions (SPE1 to SPE 54) in each case 25 ml of eluent was collected.

Purification of the Pure Compound

All the fractions obtained were studied by the process of thin layer chromatographic (TLC) technique using petroleum ether and ethyl acetate in the ratio of 1:9. From the outcome similar fractions were combined to give SPEC. This was further cleaned by preparatory thin layer chromatography (PTLC) using petroleum ether: ethyl acetate (1:9) to obtain SPEC-1 which was a white crystalline substance

RESULTS AND DISCUSSION

The **IR** spectral values for **SPEC-1** are: 3421.78, 2960.78, 1504.47, 1432.61, 1408.32, 1342.20, 1192.77, 1104.70, 1016.92, 952.79, 872.16, 814.51, 724.38, 497.51, 465.85, and 431.90.

^{1}H NMR: 5.3358, 5.3229, 5.1632, 5.1418, 5.1254, 5.1040, 5.0258, 5.0042, 4.9879, 4.9663, 3.5084, 3.4983, 2.2888, 2.2837, 2.2683, 2.2650, 2.2563, 2.2509, 2.2432, 2.2376, 2.2153, 2.2101, 2.0285, 2.0104, 2.0064, 1.9956, 1.9874, 1.9769, 1.9647, 1.9558, 1.9463, 1.9327, 1.8544, 1.8462, 1.8374, 1.8224, 1.8132, 1.8022, 1.7907, 1.7098, 1.6954, 1.6865, 1.6763, 1.6620, 1.6535, 1.6390, 1.5713, 1.5223, 1.5118, 1.5079, 1.4974, 1.4868, 1.4779, 1.4740, 1.4607, 1.4462, 1.4375, 1.4274, 1.4192, 1.4095, 1.3997, 1.3942, 1.3856, 1.3755, 1.3663, 1.3569, 1.3479, 1.3257, 1.2942, 1.2826, 1.2630, 1.2571, 1.2483, 1.2438, 1.2315, 1.2159, 1.2053, 1.1976, 1.1817, 1.1632, 1.1516, 1.1393, 1.1147, 1.0901, 1.0637, 1.0545, 1.0344, 1.0299, 1.0094, 0.9908, 0.9615, 0.9476, 0.9353, 0.9293, 0.9204, 0.9092, 0.8986, 0.8931, 0.8821, 0.8438, 0.8337, 0.8245, 0.8178, 0.8059, 0.8029, 0.7846, 0.7671, 0.7440, 0.6782, 0.6599, 0.5306 and 0.5160.

^{13}C NMR: 140.7669, 138.3258, 129.2863, 121.7248, 77.2189, 71.8260, 56.8779, 55.9664, 51.2485, 50.1705, 42.3209, 42.2267, 40.4976, 39.6918, 37.2667, 36.5242, 31.9098, 31.6811, 28.9270, 26.0852, 25.4145, 24.3726, 21.2215, 21.0904, 19.4076, 18.9861, 12.2565 and 12.0547.

SPEC-1 was obtained as a white solid crystal. The IR (Figure 1) signal absorption band observed at 3421.78 cm^{-1} is characteristic of O-H stretching. Absorption at 2960.78 cm^{-1} is typical of cyclic olefinic –HC=CH- stretching while the absorption at frequencies such as 1504.47 cm^{-1} is as a result of C=C absorption. However, this signal is weak .the absorption at 1432.6 cm^{-1} was assignable to cyclic $(CH_2)_n$ bending absorption frequency. While the one at 1342.20 cm^{-1} is attributable to OH deforming absorption. The absorption at 1016.92 cm^{-1} is typical of cycloalkane moieties. These absorption frequencies were in very close agreement with the ones observed for Stigmasterol [5].

The ^{1}HNMR spectrum (Figure 2) of SPEC-1 completely corresponds to the data for Stigmasterol [6].It showed peaks for two tertiary methyl groups (angular methyl proton) at 0.8438 and 0.8337 and further had two multiplet at 5.3358 (1H) and 5.1632 (2H) for three olefinic protons and another multiplet at 3.4983 for carbinylic protons. The signal at 1.0094 corresponds to C-18 and C-19 protons respectively of Stigmasterol.

The ^{13}C NMR (Figure 3) shows some recognizable signals at 140.7669 and 121.7248 ppm which is assignable to the double bond at C-5 and C-6 [7].The δ value observed at 71.8260 ppm is due to C-3 β-hydroxyl group [8]. Again the signals observed at 21.2215 and 12.0547 ppm correspond to angular carbon atoms at C-19 and C-18 respectively (Table 1). It had been reported that the lower value for C-18 is attributable to γ-gauche interaction that increases the screening of C-18 thereby causing lower chemical shift.

Figure 1: This figure shows infra-red spectra for SPEC-1

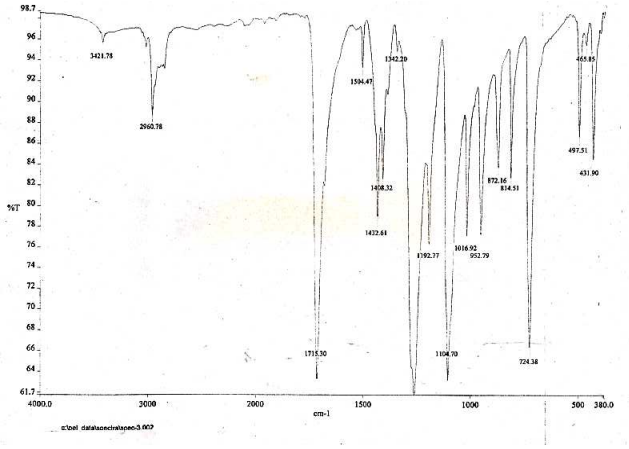
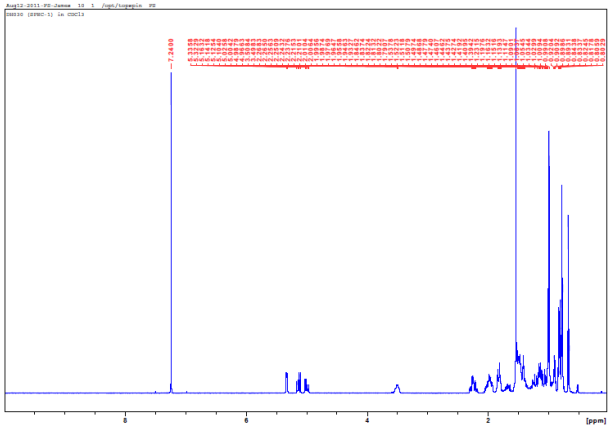


Figure 2: This figure shows ^{1}H NMR for SPEC-1



Likewise the loss of H in C-6 results in decrease in screening of C-19 leading to an increase in ^{13}C chemical shift to higher frequency [9].This explanation is also accepted for 21.2215 and 12.0547 ppm (for C-19 andC-18 respectively). From the spectra above coupled with the dept-135 (Figure 4) and with the comparison made so far it was concluded that sample SPEC-1 is β- Stigmasterol (Figure 5).

Figure 3: This figure shows ^{13}C NMR for SPEC-1

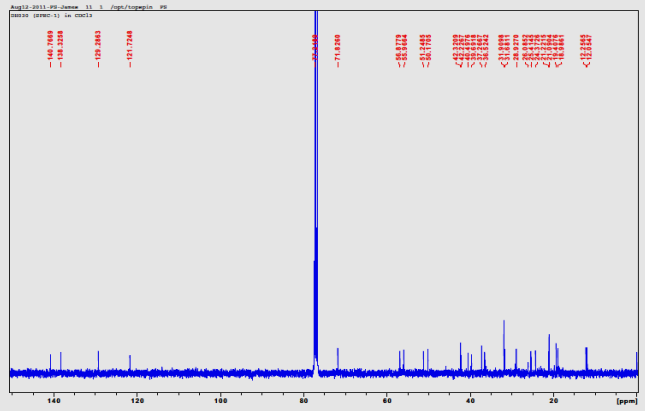


Figure 4: This figure shows dept – 135 for SPEC-1

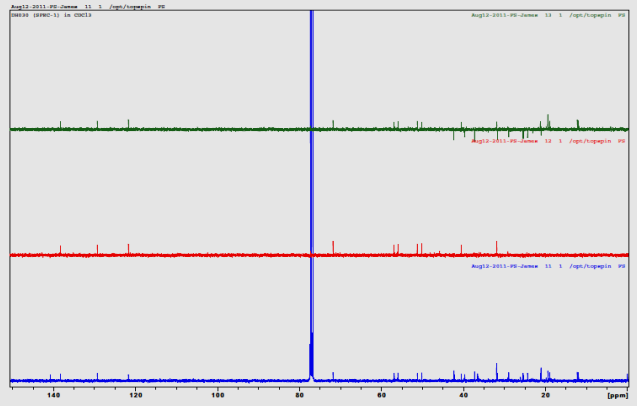


Figure 5: This figure shows β-Stigmasterol

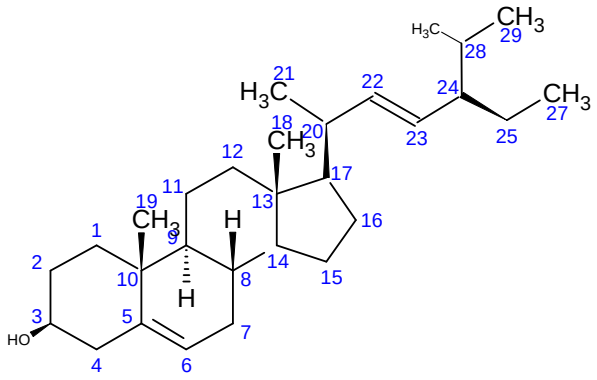


Table 1: This table shows ^{13}NMR data of Stigmasterol

S/No	β-Stigmasterol[6] Literature Value ^{13}C	β-Stigmasterol Experimental Value ^{13}C
1	37.3	37.2667
2	31.6	31.6811
3	71.8	71.8260
4	42.3	42.3209
5	140.8	140.7669
6	121.7	121.7248
7	31.9	31.9098
8	31.9	31.9098
9	51.2	51.2485
10	36.5	36.5242
11	21.1	21.0904
12	39.7	39.6918
13	42.3	42.3209
14	56.9	56.8779
15	24.4	24.3726
16	28.4	28.9270
17	56.1	55.9664
18	11	12.0547
19	21.2	21.2215
20	40.5	40.4976
21	21.2	21.2215
22	138.3	138.3258
23	129.3	129.2863
24	51.2	50.1705
25	31.9	31.6811
26	21.2	21.2215
27	19	19.4076
28	25.4	25.4145
29	12.1	12.2565

Source: Pateh et al [6]

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CONFLICT OF INTEREST

No conflict of interests was declared by authors