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EXTRACTION, ISOLATION AND CHARACTERIZATION OF MANNITOL FROM AERIAL PART OF *STRIGA HERMONTHICA* DEL

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ABSTRACT: *Striga hermonthica (Del.) Benth is a common hemi-parasitic plant growing in a variety of food crops like maize, millet, rice and sorghum roots. It has many medicinal uses include abortifacient, dermatosis, leprosy, ulcer, pneumonia, jaundice and antibacterial activities. The plant material was extracted and concentrated under reduced pressure. Bioassay guided isolation was done by chromatographic methods. Compound coded "003" was isolated as white amorphous substance from combined hexane and ethyl acetate extracts. Spectral analysis was carried out to characterize the isolated compound. The proton Nuclear Magnetic Resonance (^{1}H-NMR) spectrum of isolated compound 003 displayed seven chemical shifts; δ H 4.31ppm, δ H 3.38 ppm, δ H 3.61 ppm, δ H 4.39 ppm, δ H 3.46 ppm, δ H 4.12 ppm and δ H 3.55 ppm. This also showed that there are seven different types of protons in the compound. Interpretation of the spectrum also revealed that signal of alcoholic protons of OH were observed downfield compared with the signal of protons directly attached to carbons. The Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectrum gave three peak signals at δ C 64.3, 71.9 and 70.3 ppm corresponding to C_1/C_6, C_2/C_5 and C_3/C_4 respectively. Based on the comparison of the spectra of the isolated compound with reported spectral data in the literature, the chemical structure of the isolated compound 003 was deduced and identified as Mannitol. The chemical structure was also confirmed by ^{1}H-^{1}H Correlation Spectroscopy (COSY), Hetero-nuclear single quantum correlation (HSQC) and Hetero-nuclear Multiple Bond Correlation (HMBC) spectra.*

KEYWORDS: extraction, isolation, characterization, mannitol, *striga hermonthica*.

INTRODUCTION

Stiga hermonthica (Del.) Benth (family: *Scrophulariaceae*) is a common hemi-parasitic plant growing in a variety of food crops such as maize (*Zea mays* L.), millet (*Pennisetum glaucum* L. Leeke), rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* L. Moench) roots (Tarr, 1962; Hutchinson and Dalziel, 1963; Carson, 1988; Presset *et al*., 2001). It is widely distributed in West and East Africa (Mohamed *et al*., 2001; Musselman *et al*., 1991). *S. hermonthica* has been used

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extensively in folkloric medicine in many parts of Africa (Choudhury *et al*., 1998; Atawodi *et al*., 2003).

Striga hermonthica is an annual plant that grows about 20-60cm tall with bright-green stems. The stems are hairy, hard and quadrangle shape. The leaves are nearly opposite narrowly lanceolate about 1-3cm long with successive leaf pairs perpendicular to one another. It has small and attractive bright purple or pink flowers. The corolla is two-lipped, tube recurved and shortly hairy. The calyx is up to 6mm long, ribbed, unequally lobed. The roots are succulent, round without root hairs and attached to the root system of the hosts (Keindrebeogo *et al*., 2005).

S. hermonthica has been used extensively in folkloric medicine in many parts of Africa (Choudhury *et al*., 1998; Atawodi *et al*., 2003). It has a wide range of medicinal uses which include abortificient effect, dermatosis, leprosy, ulcer, pneumonia and jaundice remedy, trypanocidal effects, antibacterial and anti-plasmoidal activities (Choudhury *et al*., 1998; Hussain and Deeni, 1991; Okpako and Ajaiyeoba, 2004).

MATERIAL AND METHODS

Sample collection and identification

The plant sample was collected from a farm along Azare-Misau Road close to Federal Government College (FGC) Azare, in Bauchi State, Nigeria using standard method. The freshly collected aerial parts of the plant were conveyed in clean polythene bag to Biology Department, Abubakar Tafawa Balewa University (ATBU), Bauchi for identification and voucher specimen was deposited in the Departmental Herbarium (Abdalfatah *et al*., 2013; Zailani *et al*., 2010; Mann *et al*, 2008; Kubmarawa *et al*., 2007).

Drying and Pulverising

The aerial parts of the plants were dried under shade inside a room. The well dried samples were pulverized to fine powder using a wooden mortar and pestle. The powder of each sample was weighed using analytical balance and kept at room temperature until use (Ibrahim *et al*., 2012).

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Extraction

One thousand seven hundred and five grams (1.75Kg) of the pulverized plant material was macerated successively in hexane, ethyl-acetate and methanol for 72 hours and filtered under pressure using Buckner funnel. The extract was concentrated under reduced pressure using a rotary evaporator and later allowed to air dry. The dried extracts were Hexane extract (BSHH), Ethyl acetate extract (BSHE) and Methanol extract (BSHM). The BSHH and BSHE extracts were combined for column chromatography due to the low yield and coded BSH.

Fractionation of the crude extracts and isolation of active compound

The isolation of the active compounds was done by chromatographic methods. BSH extract (31.50g) was absorbed on 30g of activated silica-gel (230-400mesh) and allowed to dry and chromatographed on a glass-column of silica gel i.e. packed with 90.0g of the same activated silica-gel. The elution solvents were mixtures of n-hexane, ethyl-acetate and methanol. 200-400ml of eluent was prepared for each solvent mixture. Eluates were collected in volumes of 200ml and a total of eighteen fractions were collected. The fractions were labeled BSH 1, BSH 2, BSH 3, BSH 4, BSH 5, BSH 6, BSH 7, BSH 8, BSH 9, BSH 10, BSH 11, BSH 12, BSH 13, BSH 14, BSH 15, BSH 16, BSH 17 and BSH 18. The eluates were combined based on TLC profiling performed on pre-coated silica gel k5 glass plates. The mobile phase for the TLC consists of a mixture of hexane and ethyl-acetate in the ratio ranging from 4:1 to 1:1. The combined BSH 6 and BSH 7 were fractionated and the sub-fractions 18-22 yielded a compound that was washed and re-crystallized in methanol and the compound isolated was labeled HES 003. Further characterization using spectral analysis was carried out on the isolated compounds. In this paper we are reporting the isolation and characterization of compound coded 003 and identified as mannitol.

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RESULTS

Spectral data of isolated compound 3: Compound 003 was isolated as white amorphous substance and melting point 163-169°C. The results of the spectral data consisting of ^{1}H-NMR, ^{13}C-NMR (APT), COSY, HSQC and HMBC were presented in table 1.

Table 1: Spectral data summary of isolated compound 003

Carbon atom	^{1}H-NMR	^{13}C-NMR	^{1}H-^{1}H COSY	HSQC	HMBC	
					Proton nuclei	Carbon nuclei
C1/C6	4.31(t, OH) 3.38(q, 2H) 3.61(m, 1H)	64.3	H2, H5	H1/6	H-1/6,H-OH	C1, C2 C3,C4 C5,C6
C2/C5	4.39(d,OH) 3.46(m, 1H)	71.9	H1, H3	H2/5	H-2/5,H-OH	C2, C3, C4, C5
C3/C4	4.12(d, OH) 3.55(t, 1H)	70.3	H2, H5	H3/4	H-3/4 H-OH	C1, C2, C5, C6 C3, C4

DISCUSSION

The proton ^{1}H-NMR spectrum of compound 3 displayed seven chemical shifts each one corresponds to a signal of chemically equivalent protons. The spectrum also showed that there are seven different types of protons in the compound. The signal of alcoholic proton of OH group located on C-1/C-6 was observed downfield at δ H 4.31ppm and the signal on the ^{1}H-NMR spectrum of mannitol. The proton of the OH group on C-1/C-6 has adjacent protons (2H) of C-1/C-6 which coupled with the proton of OH group thereby splitting the signal peak into three peaks (triplet) according (n+1) rule of spin splitting in the ^{1}H-NMR spectrum. The oxygen atom of the OH group being electronegative atom therefore it causes deshielding effect on the proton hence

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the signal of the proton appeared down field. The signal of protons ($1H_c$) of C-1/C-6 was observed at δ H 3.38 ppm on the ^{1}H-NMR spectrum, they coupled with adjacent 1H of OH group and 1H of C-2/C-5 and 1Ht lead to spin splitting of the signal into five (5) peaks (quintet). The signal of protons ($1H_t$) of C-1/C-6 was observed at δ H 3.61 ppm and labeled by a letter 'D' on the H-NMR spectrum the protons coupled with adjacent 1H of OH group, 1H of C-2/C-5 and alcoholic proton of C-2/C-5 which lead to spin splitting of the signal into eight (8) peaks (ddd) and the signal was clearly observed on the spectrum. The signal of the alcoholic proton of the OH group located on C-2/C-5 in the mannitol structure was observed down field at δ H 4.39 ppm. The signal displayed a doublet peaks due to spin coupling with a single proton (1H) of C-2/C-5. Since oxygen atom causes deshielding effect and C-2 has only one proton bonded to it. The deshielding effect is more therefore the proton of the OH group of C-2/C-5 appeared a little more down field than the other alcoholic protons of the OH groups in mannitol molecule. The protons of C-1 /C-6 were in the same chemical environment due to their chemically equivalent protons therefore they displayed a similar signal with same chemical shift. Therefore, the protons of C-1 /C-6 gave single signal which was observed as a singlet signal. The signal of single proton (1H) of C-2/C-5 was observed at δ H 3.46 ppm on the ^{1}H-NMR spectrum of Mannitol. The adjacent protons 2H of C-1/C-6 coupled with the proton of C-2/C-5, 1H of C-3/C-4 and alcoholic proton of C-2/C-5 hence the signal of the proton appeared as doublet of triplet of doublet (dtd). The protons of C-2 /C-5 were in the same chemical environment due to their chemically equivalent protons therefore they displayed a similar signal with same chemical shift. Therefore, the protons of C-2 /C-5 gave single signal which was observed as a singlet signal. The signal was assigned to the proton of the OH group located on C-3/C-4. The signal was observed down field at δ H 4.12 ppm. The coupling splitting of the signal afforded two peaks i.e doublet. This was due to single proton on C-3/C-4 adjacent to the alcoholic proton of C-3/C-4. Since the oxygen atom of the OH group causes deshielding effect the signal appears downfield. The single proton of C-3/C-4 was observed at δ H 3.55 ppm, a little upfield from the alcoholic proton of C-3/C-4. The spin coupling with 1H of adjacent C-2/C-5 and1H of OH of C3/C4 caused the splitting of the signal into three peaks. The signal appearing upfield could be explain based on the fact that protons attached to carbon atoms

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usually appear upfield than the protons of the OH group. The proton of C-4 has same chemical shift as the proton of C-3 because they in the same chemical environment. Therefore, the protons of C-3/C-4 gave a single peak. The result summary of the ^{1}H-NMR spectrum was presented in table 1 above.

The APT (^{13}C-NMR) spectrum of compound 003 displayed three (3) signals which showed a single peak for each chemically non-equivalent carbon atom unlike ^{1}H-NMR which showed multiplets for each proton position leading to more complex spectrum than ^{13}C-NMR. The ^{13}C-NMR clearly gave three peak signals at δ C 64.3, 71.9 and 70.3 ppm corresponding to C_1/C_6, C_2/C_5 and C_3/C_4 respectively. This result agreed with the work of Rodrigues *et al.* (2010), who also reported three peaks in ^{13}C-NMR (CDCl3, 125MHz) of mannitol at δ C 63.371, 69.434 and 70.998 ppm for C1/C6, C2/C5 and C3/C4 respectively. The slight differences in the chemical shifts values of carbon atoms could be due to the differences in the solvents used in the study. The ^{13}C-NMR spectra showed a single peak for each chemically non-equivalent carbon atom unlike ^{1}H-NMR which displayed multiplets for each proton position leading to more complex spectrum than ^{13}C-NMR. Therefore, the signals of ^{13}C-NMR are better resolved than in ^{1}H-NMR spectra thus, ^{13}C-NMR spectrum separate signals for each chemically non-equivalent carbon. The chemical shift reference for ^{13}C-NMR spectrum is the carbon atoms in TMS whose chemical shift was assigned to be 0.0ppm like in ^{1}H-NMR spectra however the range of ^{13}C-NMR chemical shifts were δ C 8-220 ppm which are much large than for ^{1}H-NMR range (δ H 0-10 ppm). The result summary of the APT (^{13}C-NMR) spectrum was presented in table 1 above.

The ^{1}H-^{1}H Correlation Spectroscopy (^{1}H-^{1}H COSY) of compound 003 shows correlation between H-2 and H-5 while the proton H-3 correlates with proton H-1. The spectrum displayed both the diagonal and off diagonal peaks but the off diagonal peaks are the important signals which indicate the positions of coupling between protons on the x-axis and the protons of the y-axis on the COSY spectrum. when interpret a COSY spectrum, one starts from any cross peak and draw two perpendicular lines (parallel to each 1H-NMR spectrum axis) leading back to the diagonal, the peaks intersected on the diagonal by these lines are coupled to each other. There is a mirror

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reflection of these cross peaks. Only cross peaks on one side of the diagonal need to be interpreted (Manna, 2017). ^{1}H-^{1}H correlation spectroscopy (1H- COSY) displays the coupled protons nuclei only, therefore using 1H-COSY one can determine whether there is a coupling between a pair of protons or not. The ^{1}H-COSYspectrum of compound 003 consists of two frequency axes (F1 and F2) where the ^{1}H-NMR spectra are displayed. The COSY spectrum is a symmetrical spectrum that has the ^{1}H-NMR spectrum of a compound as both of the chemical shift axes. The diagonal signals showed chemical shifts in ^{1}H-NMR and do not provide any additional information. The off diagonal peaks (also called crossed peaks) are symmetrically appeared about the diagonal. They are the important signals which indicate the positions of coupling between a proton on the F1 axis and a proton on the F2 axis. Generally, the diagonal running from the upper right to the lower left corner has a series of signals (Breitmaier, 1993). To interpret a COSY spectrum, one starts from any cross peak and draw two perpendicular lines (parallel to each 1H-NMR spectrum axis) leading back to the diagonal, the peaks intersected on the diagonal by these lines are coupled to each other. Normally, there is a mirror reflection of these cross peaks and only cross peaks on one side of the diagonal need to be interpreted for accurate interpretation (Manna, 2017; Rodrigues *et al.,* 2010). The result summary of the ^{1}H-COSYspectrum was presented in table 1 above

The result of the heteronuclear single quantum correlation (HSQC) of the isolated compound 003 was interpreted and presented in table 1 and the HSQC spectrum showed the correlation of protons that are directly attached to the carbon atoms in the compound. From the spectrum, it was observed that proton at chemical shift (δ H 3.46 ppm) correlated with carbon atom at the chemical shift of δ C 72.2 ppm on the HSQC spectrum of compound 003. The drawn line from the carbon resonance intersected only one cross point. The drawn parallel line from the ^{13}C-NMR peak (δ C 72.2 ppm) intersected one cross peak then from this cross peak a line was drawn perpendicular to the x-axis that intersected ^{1}H-NMR signal bearing 1H proton. Similarly, proton at chemical shift (δ H 3.55 ppm) correlated with carbon atom at the chemical shift of δ C 70.7 ppm on the HSQC spectrum of the isolated compound 003. When line was drawn from the carbon resonance, it intersected one cross point. The drawn parallel line from the ^{13}C-NMR peak (at δ C 70.7 ppm) intersects one cross peak from which a line was drawn perpendicular to the x-axis that intersected ^{1}H-NMR signal that

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have 1H proton. It was observed that the two protons that resonate at chemical shifts (δ H 3.61and 3.38 ppm) correlated with carbon atom at the chemical shift of δ C 64.6 ppm on the HSQC spectrum of the isolated compound 3. The line drawn from the carbon resonance intersected two cross points. The drawn parallel line from the ^{13}C-NMR peak intersects two cross peaks then the lines were drawn perpendicular to the x-axis that intersected ^{1}H-NMR signal. The Heteronuclear Multiple Bond Correlation (HMBC) spectrum of Compound 3 showed the correlations between chemical shifts of hydrogen nuclei and carbon nuclei that was separated by two or more bonds. The HMBC spectrum showed that H nuclei of C-1/C-6 correlated with C-2/C-5 and C-1/C-6. The H of the alcoholic proton of C1/C6 also correlated with C2/5 and then with C1/C6 as shown on the HMBC spectrum in Figure 4. The H of C2/C5 correlated with carbon nuclei of C1/C6 and also coupled with C3/C4 as shown. The H of the alcoholic proton attached to C2/C5 was coupled to C1/C6 as well as C3/C4 while the H of alcoholic proton of C3/C4 only correlated with C3/C4. But the H of C3/C4 correlated to C2/C5 and C1/C6 carbons nuclei. Similar to HSQC, the HMBC has also no diagonal peaks. Therefore, the peaks always appeared at the crossing points where the carbons nuclei on the y-axis coupled with the protons (H) on x-axis through intervening bonds. The coupling correlations among the peaks were interpreted with drawn parallel lines to x-axis (^{1}H-NMR) from the peaks of carbon resonance and drawn lines parallel to the y-axis (^{13}C-NMR) from each proton resonance peaks. In HMBC spectrum, all the peaks appeared at the crossing points. Each crossing point corresponded to a particular carbon which correlated with the particular hydrogen nuclei. The result summary of the HSQC and HMBC spectra were presented in table 1 above.

CONCLUSION

Based on the comparison of the spectra of the isolated compound 003 with reported spectra in the literature (Manna, 2017; Rodrigues *et al.,* 2010) the chemical structure of the isolated compound 003 was deduced as Mannitol which was confirmed by ^{13}C-NMR spectral analysis and 2D spectroscopy (COSY, HSQC and HMBC spectra). Therefore, interpretation of the spectral results

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and comparison with the literature the chemical structure below was assigned to the isolated compound 003.

Compound 003

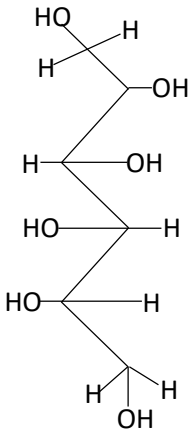

Fig. 1: Proposed chemical structure of isolated Mannitol

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