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Isolation of Pyrrolizidine Alkaloid from *Heliotropium indicum* Leaf for Control of insects of Stored Grains

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ABSTRACT

Fresh leaves of *Heliotropium indicum* L collected from bush in Makurdi were allowed to air dry for two weeks and ground into a coarse powder before extraction in n-hexane, ethyl acetate and methanol to obtain their respective crude extracts. Using standard phytochemical analysis, the methanolic extract which caused higher mortality on insect pests of stored grains than others was screened for the presence of secondary metabolites. Alkaloids, flavonoids, steroids, terpenoids, cardiac glycosides, and tannins were detected. Isolation and purification of bioactive compound from the methanolic extract was performed using chromatographic techniques.The fractions collected were monitored on thin layer chromatography (TLC) until a suitable retention factor (R_f) was achieved on a pre-coated TLC plate to achieve a pure isolate coded as IPM-65. Dragendorf reagent was sprayed on the air-dried chromatogram and a reddish-brown colour was observed. The pure isolate was then subjected to spectroscopic procedure using nuclear magnetic resonance (NMR) and liquid chromatography mass spectroscopy (LCMS). IPM-65 was then characterized as intermedine and was matched with the standards in literature. The IPM-65 was tested for insecticidal activity on the weevils of stored grains and the result was positive because of similarity in effectiveness compared to a standard insecticide, cypermethrin 2.5 E.C.

Keywords: Dragendorff, chromatography, isolation, spectroscopy, cypermethrin

INTRODUCTION

Toxins are naturally occurring secondary metabolites that plants use to defend themselves against a variety of threats from bacteria, fungi, insects, and predators. It has been reported that *Heliotropium indicum* L which is one of the most prevalent weeds found in Asia, Africa and other parts of the world contains a variety of phytoconstituents with pharmacological potential as a herbal remedy [1]. The plant is a member of the genus *Heliotropium* from the Boraginaceae family.

Numerous pathological disorders have been reported to be treated with this plant in traditional medicine [2]. This plant is most commonly used locally to treat variety of diseases. Some important phytochemicals reported to be present in the plant include flavonoids, terpenoids, glycosides, alkaloids, tannins, saponins, steroids, and essential oils [3] and that the alkaloid component of this plant is responsible for anti-inflammatory, antiseptic, antimicrobial, febrifuge, and wound healing effect [4]. Hameed et al. [5] had reported that plant extracts can be toxic, insecticidal, antifeedant, increased inhibition, and decreased fertility, among other negative effects on insects. Numerous secondary metabolites that are present in plants affect the physiology and behavior of insects as well as being crucial for plant defense. This study established the isolation of Pyrrolizidine alkaloid (PA) called intermedine from leaf methanolic extract of *Heliotropium indicum* Linn and may be responsible for the high mortality of insect pest of stored grains, suggesting that methanolic extract of H.indicum has some insecticidal properties. Investigation of three Sri Lankan plants' extracts, *Plearostylia opposita* (Wall), Alston (Celastraceae), *Aegle marmelos* Correa (Rutaceae), and *Excoecaria agallocha* (Euphorbiaceae) has reported by Okwute, [6] revealed that the plants are insecticidal, and for the first time three compounds were found to possess the daphnane orthoester skeleton which may be the constituent of ethyl acetate extract of *E. agallocha* and have been found to be insecticidal. Numerous food items and dietary supplements, particularly tea, herbal products, and honey, contain PAs and their N-oxide derivatives. The European Food Safety Authority (EFSA) [7] has identified a group of 17 PAs and their N-oxide derivatives that commonly contaminate food, including intermedine/lycopsamine, intermedine-N-oxide/lycopsamine-N-oxide,senecionine/senecivernine,senecionine-N-oxide/ senecivernine-N-oxide, seneciphylline, seneciphylline-N-oxide, retrorsine, retrorsine-

N-oxide, echimidine, echimidine-N-oxide, lasiocarpine, lasiocarpine-N-oxide, and senkirkine. There is the need to monitor the presence of PAs in food to avoid human mortality.

Despite the many traditional and medicinal uses of *H. indicum* Linn, it has not been used to control insect pests of stored grains, hence the aim of this study. The objectives of the study are to (I) extract bioactive constituents from the leaf extract of *H.indicum,* (ii) isolate and purify the bioactive constituents using chromatographic techniques, (iii) characterize and elucidate the structure of bioactive compounds using NMR, and LCMS, (iv) test isolate efficacy on the insect pests of stored grains at various concentrations.

MATERIALS AND METHOD

Collection of Plant Samples

The leaves of *H. indicum* L. were harvested and rinsed under running tap water to get rid of dust and other debris. They were air dried for two weeks under shade. The dried leaves were then pounded with a mortar and pestle and kept in a polythene bag prior to extraction using successive maceration.

Preparation of Crude Extracts

Extraction by Successive Maceration

The successive maceration method of Sukhdev et al. [8] was employed for the preparation of crude extracts to obtain crude hexane extract, crude ethyl acetate extract, and crude methanolic extract, respectively. The crude extracts were stored in different glass bottles till further use.

Phytochemical Screening

The method of Samatha [9] was used to screen the crude extracts for the presence of secondary metabolites.

Isolation and Purification Procedures

Methanol extract was subjected to isolation and purification using chromatographic techniques. Briefly, about 9.2 g of the crude extract was allowed to adsorb onto silica gel to make a slurry. The resultant slurry was allowed to dry and was loaded onto the already packed columns and was allowed for gradient elution using 5% gradual increase amounts of ethyl acetate in hexane, ethyl acetate and finally methanol in

ethyl acetate until 10% methanol in ethyl acetate. Fractions were collected using 25 mL beaker and were allowed to evaporate, and monitored by TLC. Similar fractions agreeing to similar R_f values were combined, washed with acetone, and subjected to TLC with n-hexane:methanol (7:3, v/v) which gave a distinct R_f spot of 0.42. The resulting chromatogram, after air-drying for 30 min was viewed under UV lamp of short and long wavelengths of 254 nm and 366 nm, respectively. Compounds identity were detected by spraying with Dragendorff reagents according to the methods of Resman*et al*.[10].The observation of an orange red colour indicated the presence of alkaloids, and the resulting whitish crystal which was washed with acetone was coded as IPM-65 (65 mg) and was subjected to spectroscopic analysis.

Spectroscopic Analysis

Nuclear Magnetic Resonance (NMR) Analysis of Pure Isolate

The pure isolate was subjected to ^{1}H and ^{13}Carbon- NMR as well as 2D NMR including COSY, HSQC, and HMBC. The spectra of ^{1}H, ^{13}C, and 2D NMR were verified with the aid of acetone as solvent on a Bruker Avance III HD spectrophotometer. Briefly, approximately 7.0 mg of IPM-65 was dissolved in 0.5 mL deuterium chloroform ($CDCl_3$) and was shaken well before being filtered into a 5 mm cleaned NMR tube which surface was cleaned with acetone. Using a Top-spin 3.2 software, a ^{1}H NMR spectrum was obtained on a Bruker Avance III HD spectrometer (400 MHZ Bruker, Ultrashield 2). The spectrometer was outfitted with a 5 mm BBO-z Prodigy cryoprobe with Bruker Automatic 120 holder sample changer. Following insertion into the magnet, the temperature was given five (5) minutes to stabilize. Subsequently, the sample was adjusted, matched, and shimmied. The ^{1}H pulse duration was measured on the sample and was generally approximated to 10 μs. A 5-second relaxation delay was used to the Carr-Purcell-Meiboom-Gill (CPMG) spin echo sequence, which was utilized to attenuate the wide signals of larger molecules and minimize macromolecular signals) [11]. To avoid broad signals, free induction decays were multiplied by an exponential window function (LB = 0.3 Hertz) before the Fourier transform. The spectra were allowed for a baseline and phase corrections manually. Tetra Methyl Silane (TMS) signal at 0 ppm was used as the internal reference standard for all spectra. The signal levels of the remaining molecules were measured with a 2D COSY, HSQC, and HMBC pulse sequence.

Liquid Chromatography Mass Spectrometer (LCMS)

The exact mass of the compound was investigated using a dual source LCMS, an Agilent 6130 with 1200 series LC. Briefly, about 1.0 mg of IPM-65 was dissolved in methanol preparatory for the LCMS analysis. LC-MS was acquired using the following parameters: Flow rate was 1.0 mL/min, injection volume was 10.0 mL. Run time was 10 min and solvent composition was acetoniltrile:water in ratio 1:1. For the mass spectrometer (MS), the ionization mode with combined electron-spray ionization and atmospheric pressure chemical ionization source (ESI/APCI) in both positive and negative polarity and mass range was from 100–1000 mass units. The column was Agilent Infinity Lab Poroshell 120, 4.6x100mm, 2.7 micron C18, while the detector was a single wavelength UV and Quadrupole Mass Spectrometer.

RESULTS AND DISCUSSION

Phytochemical Analysis

Table 1 shows the result of phytochemical analysis. of leaf methanolic extract of *H.indicum*

Table 1: Results of phytochemical screening of *H.inducm* leaf methanolic extract

Phytochemicals	Observation
	Methanolic extract
Tannin	+
Saponin	+
Alkaloids	++
Cardiac glycosides	+
Terpenoids	+
Flavonoids	++
Steroids	+
Phenols	+

- = absent + = present in trace amount, ++ = present

Spectroscopic analyses

Nuclear Magnetic Resonance: Proton (^{1}H NMR ($CDCl_3$, 400 MHz)

Figure 1 showed the proton NMR spectrum of IPM 65, while Table 2 showed the protocol for proton NMR signal of IPM-65

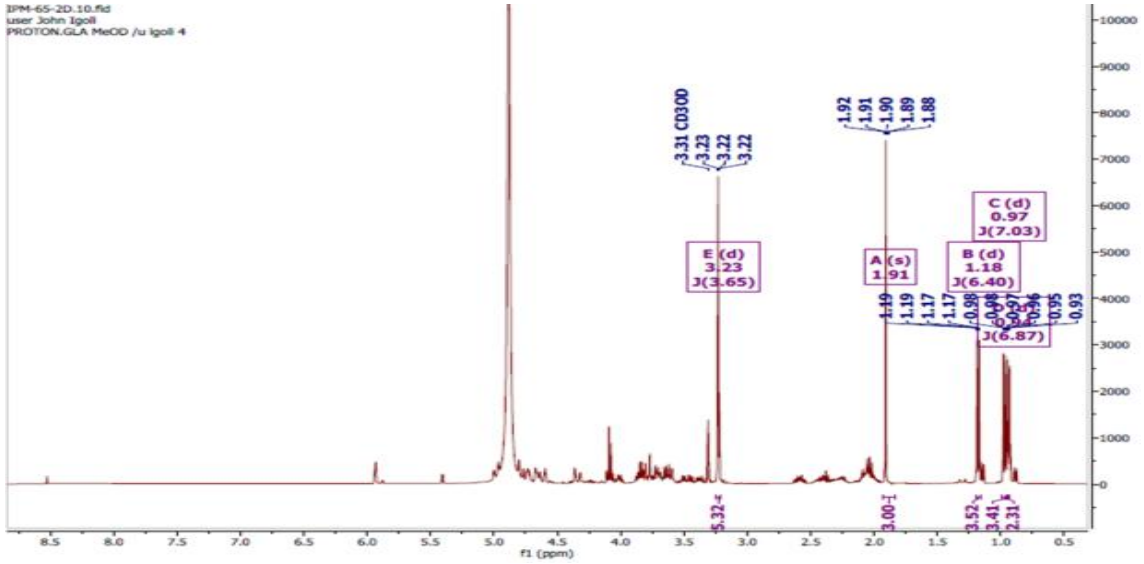

Figure 1:[1]H-NMR Spectrum of IPM-65

Table 2: Protocol for proton NMR spectrum of the pure isolate, IPM-65.

Node	Multi-plicity	δ shift (ppm)	Standard Range	Proton type	Proposed Structure (ppm rel. to TMS)
CH_3	d	0.97	0.1-1.3	R-CH_3	Methyl, 1 beta –CC,1 beta -C
CH_3	d	0.97	0.1-1.3	R-CH_3	Methyl, 1 beta –CC,1 beta -C
CH_3	d	1.18	0.1-1.3	R-CH_3	Methyl, 1 beta –CC,1 beta -
CH	s	1.91	1.6-2.6	-CH	Necid acid
CH	-	4.10	3.5-4.8	R-C=O-OCH	From necic acid
CH	-	2.10	0.1-1.3	-C-H	Pyrrolidine,1 unknown substituent(s),1-C from N-CHx
CH_2	-	2.55	1.0-5.0	R-N-H2	Pyrrolidine,1 unknown substituent(s),1 -C from N-CHx
CH_2	-	3.23;3.60	3.2-3.8	RO-C-R’	Pyrrolidine,1 gamma - N-CHx
CH_2	d	3.80	3.2-3.8	RO-C-R’	Methylene,1 gamma-C-C,1alpha -N(C)C
CH_2	-	4.40	3.5-4.8	R-C=O-O-C-H	Methylene,1alpha-C=C,1alpha-OC=O-C
CH	-	4.65		R-C=C-H	Pyrrolidine: Methine
OH	-	4.90	1.0-5.0	R-OH	Alcohol,1 -CCO
OH	-	5.45	4.0-7.0	R-OH	Alcohol,1 -CCO
OH	-	5.90		R-OH	Alcohol -2-beta-C
H	-	3.45	4.5-6.5	R-C=C-H	1-ethylene,1 -C-O cis,1 -C-N trans, 1 -C-N gem

^{13}C-NMR

Figure 2 showed the ^{13}C-NMR of pure isolate, IPM-65; while Table 3 expressed the protocol for ^{13}carbon NMR of IPM-65.

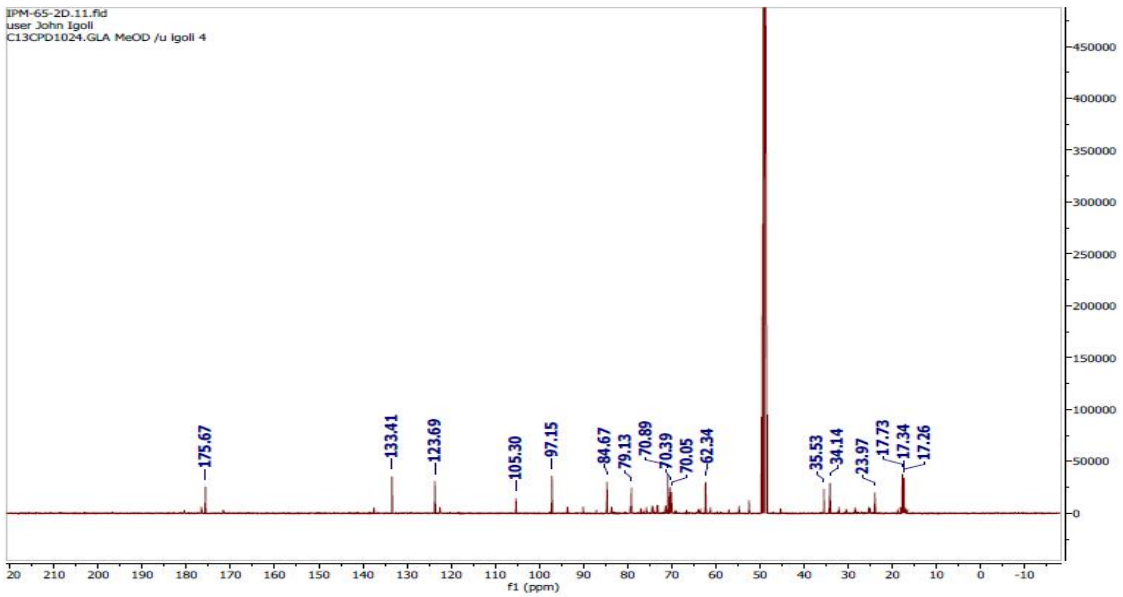

Figure 2: ^{13}C-NMR spectrum of IPM-65

Table 3: ^{13}CNMR of IPM-65.

Node	δ shift (ppm)	Standard Range(ppm)	Carbon Type	Proposed Structure (ppm rel. to TMS)
CH_3	17.34	0-35	R-CH_3	Aliphatic: 1-alpha -C, 1 beta –C, 1 beta – O, 1 gamma – C(=O)-O, 1 gamma – C, 1 gamma – O, 2-delta – C.
CH_3	17.34	0-35	R-CH_3	Aliphatic: 1-alpha -C, 2 beta –C 1 gamma –C(=O)-O, 1 gamma – C, 1 gamma – O, 1-delta – C, 1 delta - O.
CH_3	17.26	0-35	R-CH_3	Aliphatic: 1-alpha -C, 2 beta –C, 1 gamma –C(=O)-O, 1 gamma – C, 1 gamma – O, 1-delta – C, 1 delta - O.
CH_2	23.97	15-55	R_2-CH_2	Pyrrolidine: 2 – alpha –C from aliphatic, 1 beta –C from Aliphatic, 1-beta – N from aliphatic, 1 gamma –C=C from aliphatic, 1 gamma –C from aliphatic, 1 delta –C from aliphatic
CH	34.14	30-40	R-C	Pyrrolidine: 2 –alpha –C from aliphatic, 1 beta –C=C from aliphatic, 1 beta – C from aliphatic, 3 gamma –C from aliphatic, 1 delta –O-C=O from aliphatic
CH	35.53	30-40	R_1,R_2,-C	Aliphatic: 3 alpha –C, 1 beta –C(=O)-O, 1 beta –C, 1 beta –O, 1 gamma –C, 1 gamma –O, 1 delta –C,
CH_2	62.34	10-65	-C-X X=N	Pyrrolidine: 1 alpha –C-C,1 beta –C, 3 gamma –C,1 gamma -N
CH_2	70.05	50-90	-C-C	Pyrrolidine: 1 alpha –C-C,1 beta –C, 3 gamma –C,1 gamma -N
CH_2	70.39	50-90	-C-O	Aliphatic: 1 alpha –C=C, 1 gamma –N, 1 alpha –O-C=O
CH	70.89	50-90	-C-X	Aliphatic: 1 alpha –C, 1 beta –C(C=O)-O.
CH	79.13	50-90	-C-C	Pyrrolidine: 1 –alpha –C-C from aliphatic,1 delta – N
C	84.67	80-150	R_1R_2C=	Aliphatic: 1 –alpha –C(=O)-O, 3 beta -C, 1 beta –O, 1 gamma –C, 1 delta –C=C
CH	123.69	110-170	Aromatic ring	1-ethylene: 1 –C-C-C-C, 2 –C-O, 1 –C-N
C	133.41	110-170	Aromatic ring	1-ethylene: 1 –C-C-C-C, 1 –C-O, 3 –C-N
C	175.67	165-175	R_1-C=O-OR_2	1-caroxyl: 1 –C(CC) CC,1 –C from O-carboxyl

Based on the analysis of the proton and carbon spectra data and comparison with data in literature, a new structure of compound (IPM-65) was proposed using the pyrrolizidine skeleton shown in Figure 3.

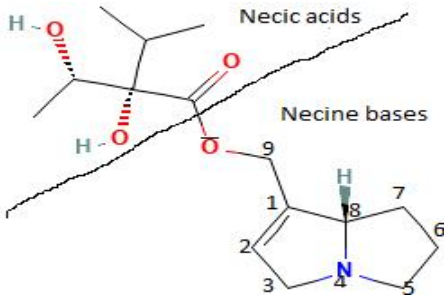

Figure 3: Proposed structure of the compound

This predicted compound was further elucidated on 2D NMR using COSY, HSQC and HMBC.

2D COSY NMR

Figure 4 showed the 2D COSY NMR of pure isolate (IPM-65); while Table 4 showed the two-dimensional spectrum from a 2D COSY experiment indicating the chemical shift, and the coupling hydrogen. It revealed the frequencies for a single isotope, most commonly hydrogen (^{1}H) along both x and y axes.

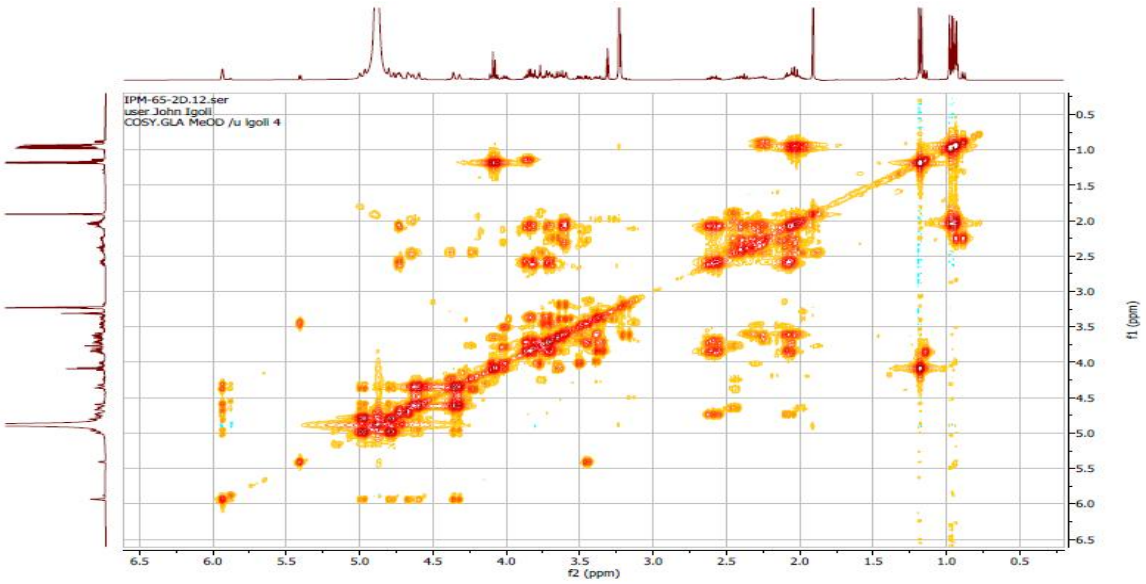

Figure 4: 2D COSY NMR of IPM-65 from Leaf Methanol Extract of *H.indicum L*

Table 4: Proton-proton 2D COSY NMR

COSY ^1H_x ^1H_y		Predicted Structure
0.97	0.97	Methyl, 1 beta –CC,1 beta -C
0.97	2.10	Methyl, 1 beta –CC,1 beta -C
2.10	0.97	Methyl, 1 beta –CC,1 beta -C
2.10	2.10	
2.10	2.55	Pyrrolidine,1 unknown substituent(s),1 -C from N-CHx
2.10	3.52	Pyrrolidine,1 unknown substituent(s),1 -C from N-CHx
2.10	3.58	-
2.10	4.10	-
2.25	0.97	-
1.18	1.18	Methyl
1.18	4.10	Methyl coupling methine on necic acid
3.23	3.23, 3.60	Pyrrolidine,1 gamma - N-CHx
3.80	2.10, 2.55	Methylene,1 gamma -C-C,1 alpha -N(C)C
4.10	1.18, 4.10	R-C=O-OC From necic acid
4.65	1.91, 2.55,4.40, 4.65,4.90,5.90	-
4.90	4.90	-
5.45	3.45, 5.45	Alcohol,1 -CCO
5.90	4.45, 4.65, 4.90, 5.90	Alcohol,1 -CCO

HSQC

Figure 5 showed the 2D HSQC NMR of pure isolate (IPM-65); while Table 5 showed a direct ^1H- ^{13}C correlation in the HSQC spectrum.

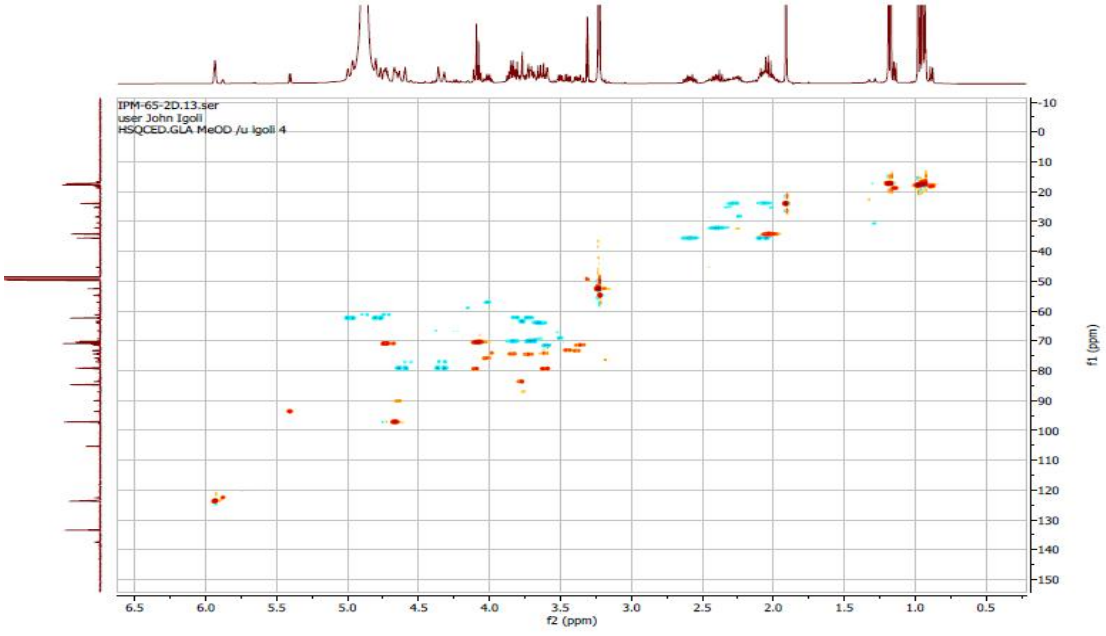

Figure 5: HSQC of IPM-65 compound

Table 5: Proton-Carbon HSQC NMR of pure Isolate IPM-65

δH_{xppm}	$\delta C_{y\ ppm}$
0.97	17.34
1.18	17.26, 17.34, 17.73
1.91	23.97
2.10	23.97, 34.14, 35.53
2.55	35.53
3.23	62.34
3.45	70.05, 70.39,70.89
3.52	79.13, 89.90
3.80	74.00
4.10	70.05,79.13
4.40	79.13
4.65	79.13, 97.15
4.75	62.34, 70.89
4.90	62.34
5.45	94.00
5.90	123.69

HMBC-NMR

Figure 6 showed the 2D HMBC of pure isolate (IPM-65); while Table 6 showed a direct ^{13}C-^{1}H correlation in the HMBC spectrum.

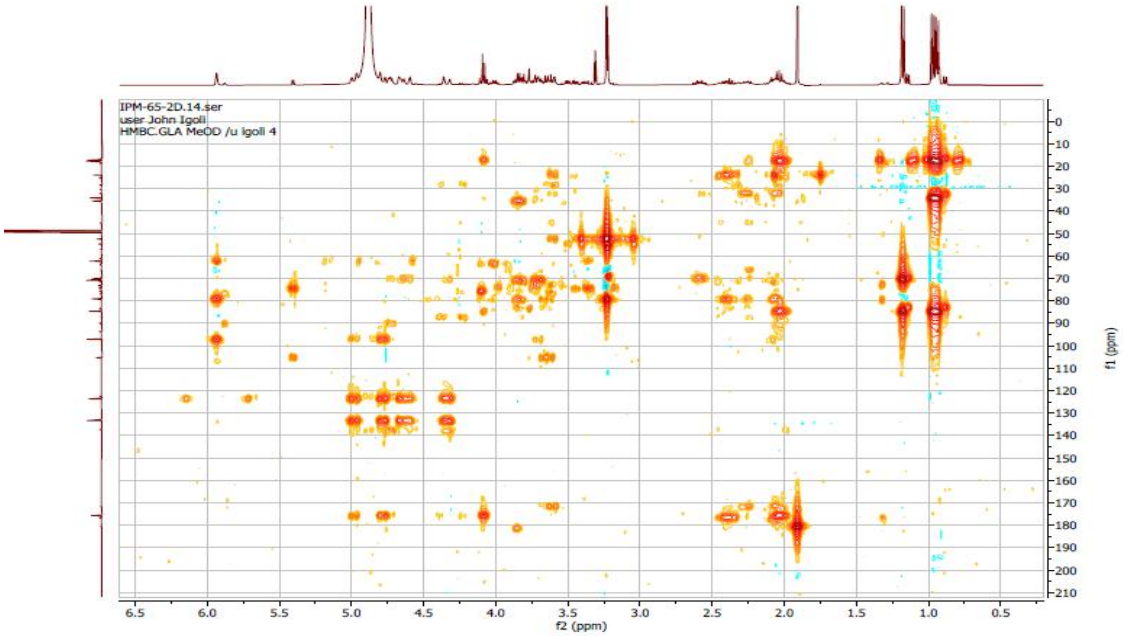

Figure 6: HMBC of Isolated Compound IPM-65

Table 6: Carbon-proton HMBC of Isolated Compound IPM-65

$\delta C_{y\ ppm}$	$\delta H_{x\ ppm}$
17.26	0.97
17.34, 17.73	0.97
17.26, 23.97	1.18
23.97	1.91
17.26, 84.67,175.67	2.10
23.97,79.13,175.67	2.55
70.05	2.60
62.34, 70.39,79.13	3.23
62.34	3.45
23.97, 175.67	3.60
35.53, 70.89, 79.13,175.67	3.80
62.34,175.67	4.10
62.34, 70.05,123.69,134.00	4.65
97.15, 123.69,133.41, 175.67	4.90
123.69	5.75

Assignment of Proton and Carbon

Based on the available data on Tables 2-6, proton and carbon can be assigned on the proposed structure of IPM-65 (Figure 7)

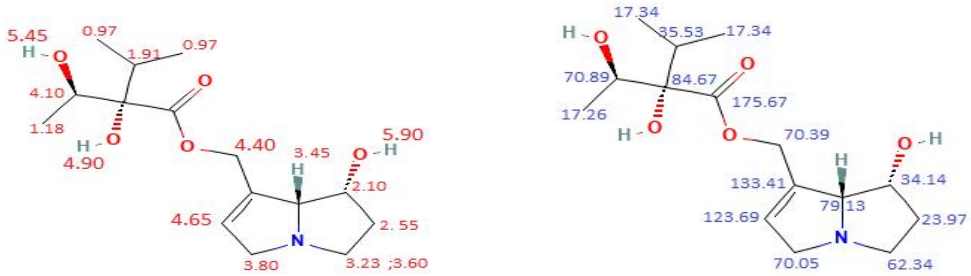

Figure 7: Assignment of protons and carbons on Compound IPM-65.

LC-MS

Figures 8 showed the LCMS chromatogram and spectrum result of compound IPM-65 run on High Resolution Electron Ionization Mass Spectrometry (HREIMS).

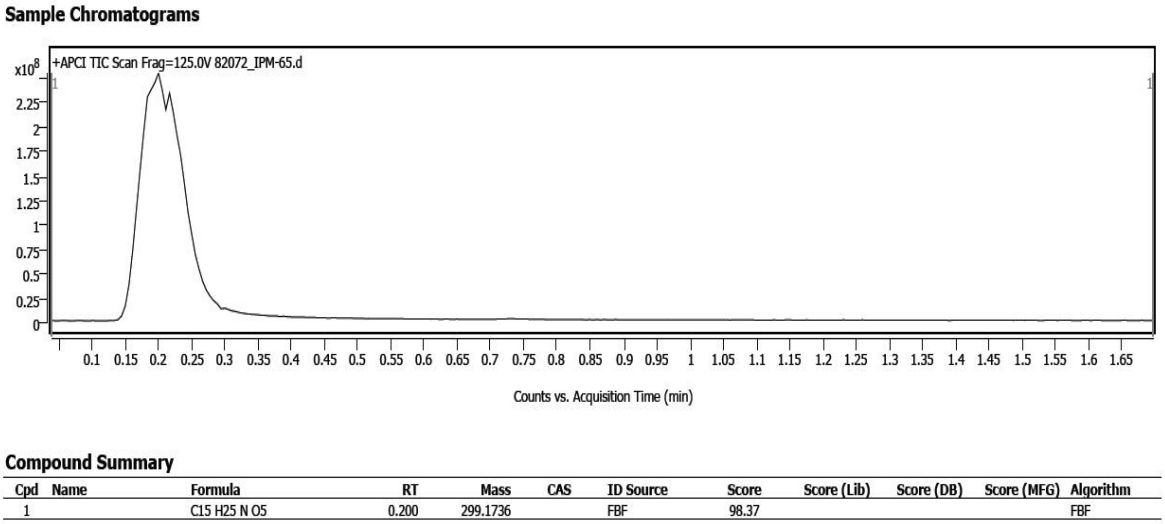

Figure 8: Chromatogram of IPM-65 showing Intermedine

In line with the proposed compound structure in Figure 5 and according to the analysis of all proton and carbon spectra data; and comparison with reported data in literature, the proposed structure of (IPM-65) having pyrrolizidine skeleton were found to be intermedine based on its exact mass as revealed by LCMS.

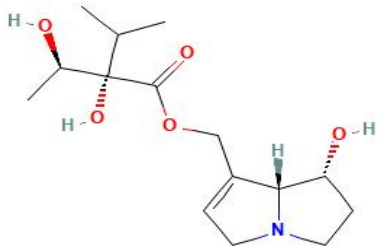

Figure 9: Intermedine

Insecticidal Activity Test of IPM-65 on Stored Grains' Insects

Insect Mortality

The isolated pure compound, IPM-65 was tested for its potency to cause mortality on weevils of rice, maize, sorghum and cowpea at two different concentrations of 5.0 mg/mL and 10.0 mg/mL, respectively. The positive and negative controls were cypermethrin 2.5 E.C, and untreated substrates, respectively, (Table 7).

Table 7: % mortality of stored grains' insects Exposed to IPM-65

IPM-65	Conc	substrate	Exposure time (Mean ±SEM)		
	g/Ml	Rice	Sorghum	Maize	Cowpea
@24 h	5.0	3.33±1.67[a]	1.67±1.67[a]	6.67±1.67[a]	3.33±1.67[a]
	10.0	6.67±1.67[a]	8.33±1.67[a]	10.00±0.00[a]	6.67±1.67[a]
@48 h	5.0	8.33±1.67[a]	15.00±2.89[b]	46.67±3.33c	51.67±6.00[c]
	10.0	11.67±1.67[a]	15.00±0.00[a]	55.00±5.77b	58.33±9.27[b]
@72 h	5.0	35.00±2.89[a]	38.33±4.40[a]	75.00±2.89[b]	70.00±5.77[b]
	10.0	46.67±1.67[a]	48.33±1.67[a]	81.67±1.67[b]	81.67±1.67[b]
@96 h	5.0	66.67±3.33[a]	75.00±2.89[a]	86.67±1.67[a]	88.33±1.67[a]
	10.0	81.67±1.67[a]	85.00±0.00[a]	96.67±3.33[a]	98.33±1.67[a]
Control	0.00	0.00±0.00[a]	0.00±0.00[a]	1.67±1.67[a]	1.67±1.67[a]
Cypermethrin	0.5 mL	68.33±4.41[a]	76.67±6.67[a]	96.67±3.33[a]	100.00±0.00[a]

Values are expressed as Mean ±SEM

Phytochemical Analysis

As revealed by the phytochemical analysis of *Heliotropium indicum* leaf extract in Table 1, the plant contains a wide range of important phytochemicals, including alkaloids, tannins, terpenoids, glycosides, steroids, and flavonoids, which is consistent with the findings of Ogidi et al. [3]. The presence of the alkaloid component is responsible for the plant's anti-inflammatory, antiseptic, antimicrobial, febrifuge, wound healing effect.

Insecticidal Activity of IPM-65

The result showed that the pure isolate (IPM-65) had a high degree of insecticidal activity on the insect pests of stored grains. From Table 8, the highest mortality observed on rice weevil (*Sitophilus oryzae*) was 66.67%, and 75% on sorghum weevil (*Sitophilus granarius*), while maize and cowpea weevils (*Sitophilus zeamais*, and *Callosobruchus maculatus*) experienced mortality of 96.67% and 98.33%, respectively at 10 mg/mL 96 h post exposure. The least mortality caused by the pure isolate was 1.67% on sorghum weevil (*Sitophilus granarius*).The ability of IPM-65 to cause mortality of *S.oryzae, S.zeamais, S.granarius and C.maculatus* may be attributed to its toxic effects on the weevils which agrees with the report of Lajide et al. [12] on the effectiveness of some plant powders in controlling *Sitophilus zeamais* by using insect adult mortality. The pure isolate caused high mortality to *Sitophilus zeamais* and *C. maculatus* which suggested that IPM-65 is lethal to the insect pests. Cypermethrin has been very effective in controlling adult *S. zeamais* and *C. maculatus* which agrees with Asawalam et al. [13] who reported 100% mortality to *Sitophilus zeamais* when treated with Cypermethrin stored maize.

The mode of action of IPM-65 (Intermedine) is not known, but may have acted on the insects by disrupting the normal respiratory activities of the weevils; resulting in asphyxiation and subsequent death [14].

Spectroscopic analysis

The characterization of IPM-65 as intermedine [1*R*,7a*R*)-1-hydroxy-2,3,5,7a-tetrahydro-1-*H*-pyrrolo[1,2-a]pyrrol-7-yl]methyl(2*S*,3*R*)-2,3-dihydroxy-2-isopropyl butanoate] was achieved by NMR and LCMS analyses. The proton spectrum (Figure1) indicated a compound with necic acid moiety, and a necine base containing pyrrolizidine skeleton [15]. It showed signals for 25 protons and 15 carbons. There are three terminal methyl group (δ_H0.97), (δ_H0.97), and (δ_H1.18) ppm (Figure 3, Table 2), Two doublets at δ_H3.80 and δ_H4.40 ppm for C-3 and C-9 , a proton signal at δ_H4.65 ppm for C-2 suggesting IPM-65 as a pyrrolizidine-type alkaloid. A doublet at δ_H3.23 ppm linking 1 alpha -C-C from methylene, 1 beta to a proton, and 1 gamma -C from N-CHx. Also a doublet at δ_H 2.55 ppm on C-6 and a proton at δ_H5.90 revealed atom corresponding to a signal for olefin (Table 2). The pyrrolizidine methylene protons were the most shielded and were observed at δ_H2.55 ppm and 3.23 ppm. The pyrrolizidine and necic acid methine protons were not identical and observed at δ_H 4.65 and 2.10 ppm for pyrrolizidine, and δ_H4.10 and 1.91, respectively. The rest of the proton signals were envelop of OH protons from parts of the pyrrolizidine skeleton. The ^{13}C-DEPT spectrum (Figure 2) showed 15 carbons which are one carbonyl at δc 175.7, two signals for olefinic double bonded carbons at δc 134.4 and 123.7 ppm, necic acid group; two identical terminal methyl carbons at δc 17.34 ppm (methyl), and a single terminal methyl carbon at δc 17.26 ppm (Figure 2 and Table 3). Two methine carbons were observed on the necic acid group at δc 35.5, 70.89 ppm, and another one on the necine base observed at δc 34.14 ppm. Other prominent carbons on the pyrrolizidine compounds were methylene carbons observed at δc 23.97, 62.34,70.05, and 70.39 ppm, while the rest were quaternary carbons observed at δc 79.13, 84.67,133.41, and 175.67 ppm, respectively. Following 2D NMR experiments (Figures 4-6), the structure of the compound was deduced as and identified to be (1*R*,7a*R*)-1-hydroxy-2,3,5,7a-tetrahydro-1-*H*-pyrrolo[1,2-a] pyrrol-7-yl] methyl (2*S*,3*R*)-2,3-dihydroxy-2-isopropylbutanoate) (Figures 3,7 and 9) and was confirmed

by the exact mass of 299.1736 g/mol in its LC-MS chromatogram (Figure 8). The full chemical shift assignments are given in Figure 7.

However, the major mechanism of action of pyrrolizidine alkaloids against insects is not known, but may exert insecticidal activity by acting on membrane phospholipids and phosphate groups in insects leading to damaging the structures of the insect's cellular membrane, and eventually resulting in cell death. Also worthy of note is that the action of PAs on insects may be connected to inhibition of acetylcholine enzyme known as acetylcholinesterase (AChE). This enzyme is a serine hydrolase that terminates the action of the neurotransmitter, acetylcholine, by hydrolyzing it into choline and acetic acid as reported by Silman *et al*. [16]. The PAs may bind to AChE and interfere with the breakdown of acetylcholine, leading to the deposition of acetylcholine in the nerve synapses and causing disrupted neurotransmission [17]. It is important to note that Cholinesterase inhibitors increase parasympathetic nervous system (cholinergic) activity indirectly by inhibiting AChE, thereby preventing the breakdown of acetylcholine. The AChE inhibitors are used in the treatment of Alzheimer's disease [18], the treatment and diagnosis of myasthenia gravis [19] and the treatment of diabetic urinary bladder [20].

Lycospamine, a diastereomer of intermedine which is the major PA alkaloid present in pure compound IPM-65 isolated from *Heliotropium indicum*, had been reported to show significant anti-proliferative effects in A549 lung cancer cells in a dose-reliant manner [21]. The antiproliferative effects of lycopsamine were associated with its autophagy inducing, apoptosis inducing, and inhibiting IL-2 expression. Overall, lycospamine is a potential anti-lung cancer agent and can be a lead molecule in lung cancer treatment.

RECOMMENDATION AND CONCLUSION

The isolation and characterization of pyrrolizidine alkaloids as intermedine was successfully carried out from the leaf methanolic extract of *Heliotropium indicum.* The chemical identification of this compound were validated using NMR and LCMS as pyrrollizidine alkaloid (PA).This is the first time of reporting the isolation of Intermedine from the methanolic leaf extract of *Heliotropium indicum L.* Pyrrolozidine alkaloids from the Boraginaceae family have been reported to be toxic,

suggesting that intermedine compound isolated from the leaf of *Heliotfopium indicum* is a potential biopesticide and may find use in the control of insect pests of stored grains at moderately recommended dosage**.**

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