



Extraction and Configuration of an Isolate from *Chrysanthellum Indicum*

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ABSTRACT

Bioactivity guided column chromatography of the ethylacetate fraction of the plant *chrysanthellum indicum* led to the isolation of a white chrysaline compound which melted at a temperature range of (257-259°C) with decomposition ¹H nmr, ¹³C nmr, DEPT and IR spectroscopic techniques were use to analyzed and propose a steroidal structure for the compound.

Keywords: Bioactivity, column chromatography, isolation, spectroscopic techniques.

INTRODUCTION

Chrysanthellum indicum also known as “Dunkufe” among the Hausas (Kano) is a faintly aromatic branching annual herb which belongs to the family of composite (Dalziel and Hutchinson 1976). The leaves are depurative while the flowers are apereint and bitter. The Hausas (Kano) use the plant in the treatment of fever and it is used among the Chinese to treat eye ailments . In conjunction with black pepper, it is used in the treatment of gonorrhoea (Carvalho, 2005)., the flowers are also used in the treatment of scrofula, deep rooted boils, inflammation of the throat eyes and cervix, eczema and itchiness of the skin. An essential oil obtained from the plant contains chryssanthenone, which is active on the brain centre affected by parkinson’s disease (van and Heuvelink 2006).

Chrysanthellum is economically important as a natural source of insecticide. The active components, called pyrethrins (Lee and Lim 2011) which ocqcur in the achenes, are extracted and sold in the form of an oleoresin. Pyrethrins attack the nervous systems of all insects, and inhibit female mosquitoes from biting. In sublethal doses they have insect repellent effect. They are harmful to fish, but far less toxic to mammals and birds than many synthetic insecticides. Pyrethroids such as permethrin are synthetic insecticides based on natural pyrethrum. The other compounds detected in the plant include: Apigenin, acacetin-7-O-beta-D-glucopy ranoside and apignein-7-O-beta-D-glucopy ranoside (Lu *et al.*,2009).

The aim of this work is to isolate a single pure compound whose chemotherapeutic index equals or even exceeds those of the presently used drugs.

MATERIALS AND METHODS

Materials

Solvents were used without further purification except ethanol, which was redistilled before use. Column chromatography was performed on silica gel (Merck 60 – 120 mesh), filter agent celite (R) 521 was mixed with sample before loading on the column, thin layer chromatography (TLC) was performed on 20x5cm glass plates coated with 0.5mm silica gel G (merck, tlc grade), with gypsum binder and florescent indicator. Bands were viewed under uv lamps (254nm and 365nm) or by exposure to iodine. The silica gel plates were prepared by coating the glass plates with slurry of silica gel in distilled water (1g: 3ml) and activated in an oven set at about 110°C for at least 24 hours before use.

Plant material:

The whole plant of *Chrysanthellum indicum* was collected from the fields in Federal College of Education, Kano and authenticated by the herbarium of Bayero University Kano. The plant was air dried and crushed into fine powder before use.

Extraction and Isolation:

Dried and ground powder (1.5kg) of *Chrysanthellum indicum* was percolated with 95% ethanol (6litres) for two weeks. The extract was decanted filtered and concentrated using rotary evaporator (R110) at 40°C and the concentrated crude extract was labeled as CI₀₁. Maceration of the crude ethanol extract was done using n-hexane, chloroform and ethylacetate, respectively. These give the n-hexane (CI₀₂), chloroform (CI₀₃) and ethylacetate (CI₀₄), fraction.

The column chromatography of the ethylacetate fraction was carried out using a standard method (Fatope *et al.*, 2000). A Slurry of CI_{04} was prepared by mixing silica gel (5g) with fraction CI_{04} (5 g) after which silica gel (60 – 120 mesh) 150g) was loaded on a column. The slurry was then loaded on top of the adsorbent. The column was eluted with a gradient of solvent mixtures petroleum ether: chloroform; chloroform: ethylacetate after the gradient elution the column was washed with 100% methanol (Table1). Eluents collected in fractions of 250ml each were evaporated to dryness on Rata vapor (R110) and transferred into weighed beakers. Identical eluents were combined based on their TLC patterns to give a total of six fractions. These fractions were subjected to brine shrimp lethality test (BST). Pooled Fraction (CI_{04} . 34 to CI_{04} – 37) labelled as CI_{04} 34-35 showed the highest activity against BST and was selected for further purification.

Purification of fraction CI_{04} (34-35)

The fraction (1.2g) was mixed with ethylacetate (5ml) and adsorbent (1.2g) to form the slurry. It was loaded on a finger column (diameter 1.4cm) packed with silica gel (32g) to a length of 45cm. The column was eluted using a gradient solvent system of petroleum ether: ethylacetate (Table 2).

The eluents were collected in fractions of 25mls and allowed to evaporated to dryness in already weighed test tubes. The fractions were analyzed on TLC and similar ones were combined

to give a total of six fractions four of which were found to be active on brine shrimp larvae. A white crystalline solid was filtered out of the fraction CI_{04} 13-16 and this was labeled as A4.

Brine Shrimp Lethality Test (BST):

Pooled fractions from the column were evaluated for lethality on brine shrimp larvae (Meyer *et al.*, 1982). Solution of instant sea salt was made by dissolving the salt (2.86 g) in distilled water (75ml), *Artenia salina* (heach) eggs (50mg) were added in a hatching chamber. The hatching chamber was kept under an inflorescent bulb for 48 hrs for the eggs to hatch into shrimp larvae. Each test extract fraction (20 mg) was separately dissolved in methanol (2 ml) from which 500 μ l, 50 μ l, and 5 μ l of each solution was transferred into vials corresponding to 1000, 100 and 10 μ g/ml respectively. Each dosage was tested in triplicate. The vials (9 per test fraction) and one control containing 500 μ l of solvent (methanol) were allowed to evaporate to dryness in about 48 hrs at room temperature. The instant ocean sea salt solution (4.5 ml) was added to each vial and 10 larvae of *Artenia salina* (taking 48 – 72 hrs) after initiation of hatching were added to each vial, the final volume of the solution in each vial was adjusted to 5ml with the salt solution, immediately after adding shrimps 24 hrs later, the number of surviving shrimps at each dosage was counted and recorded, LC_{50} values were determined with 95% confidence interval before analyzing the data on a kintech AT-compatible computer loaded with a “Finney program”.

Table 1: Column Chromatography of the ethylacetate Fraction (CI_{04})

Fraction No.	Pooled into	Fraction code	BST LC_{50}
0-12	12	CI_{04} , 0-12	> 1000
13-27	27	CI_{04} , 13-27	389.0
28-33	33	CI_{04} , 28-33	366.04
34-35	35	CI_{04} , 34-35	>1000
36-50	50	CI_{04} , 36-50	>1000
51-72	72	CI_{04} , 51-72	422.27

Table 2: Column Chromatography of CI_{04} (34-35)

Fraction No.	Pooled into	Fraction code	BST LC_{50}
0-4	4	CI_{04-35} , 0-4	> 1000
5-8	8	CI_{04-35} , 5-8	389.0
9-12	12	CI_{04-35} , 9-12	366.04
13-16	13	CI_{04-35} , 13-16	>1000
17-20	17	CI_{04-35} , 17-20	>1000
21-24	24	CI_{04-35} , 21-24	422.27
Crystal A4			416.80

RESULT AND DISCUSSION

^1H nmr, ^{13}C nmr and DEPT of the compound was used in combination with prominent absorption frequencies in the infrared to propose the structure of the compound.

In ^1H nmr the cluster of methyl and methylene protons (Fig 1) suggests the steroidal nature of the compound while this cluster makes it complicated to pick out the various CH_3 and CH_2 protons. However the chemical shift δ 7.85 indicates the presence of an acetylenic proton, that at δ 5.25 points to a hydroxyl proton and those at δ 3.58 and 4.8 are due to the residual methyl and hydroxyl protons respectively resulting from incompletely deuterated solvents (CH_3OH) used as the NMR solvents. Methyne protons are also observed at δ 2.8 and δ 3.18.

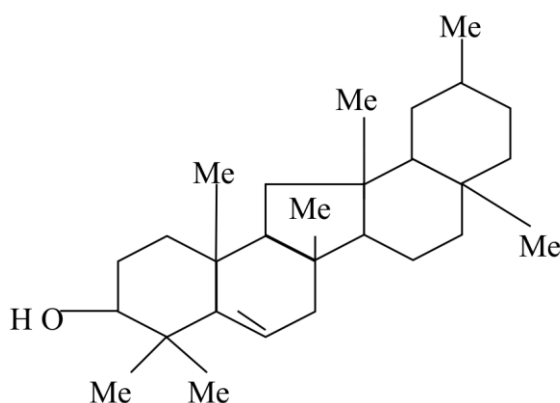
The carbon -13 nmr decoupled spectra (fig.2) is also used to arrive at the structure of compound A4 by the display of the right number of signal at proper values for the carbon resonance as expected of the compound. The ^{13}C spectra revealed a total of 28 signals without those due to solvent (CD_3OD and CD_3Cl) used. The signals at 143.999 and 122.469 falls within the alkenes region, the signal at 78.577 shows the presence of $>\text{CH-O}$ - linkage while the signal between 5 and 52 ppm corresponds to the presence of both primary,

secondary, tertiary and quaternary carbon. The signals at around 80 and those between 47 and 49 are attributed to solvents CDCl_3 and CH_3OD used.

The DEPT was used to identify multiplicity (quaternary), CH , CH_2 , or CH_3 peaks in a ^{13}C spectrum. It helps to distinguish between the CH , CH_2 and CH_3 carbons but quaternary carbons are not observed in DEPT. A combination of the ^{13}C and DEPT nmr spectra showed that the compound contains six quaternary carbons, six CH methines, nine methylene and 7 methyl carbons thus giving an attached formular of $\text{C}_{28}\text{H}_{45}$.

The IR spectra (Fig 3) of sample A4 indicated prominent absorption frequencies at 3445.67cm^{-1} being characteristic of hydroxyl functional group. The absorption peaks observed at 2924.19cm^{-1} and 2853.90cm^{-1} are characteristic of C-H stretching of aliphatic groups e.g CH_3 , CH_2 , CH . It is obvious that there is no carbonyl stretching vibration. The aliphatic C-H bending vibration are observed at $(1458.02$ and $1376.44)\text{cm}^{-1}$. C-O stretching vibration is observed at 1196.90cm^{-1} while C=C stretching vibration is also observed at 1635.59cm^{-1} .

The information obtained from the spectra analysis ^1H nmr, ^{13}C nmr and Dept and infrared spectra lead to the proposed structure of the isolated compound (Steroid) shown below.



Chrysenol

Me = CH_3

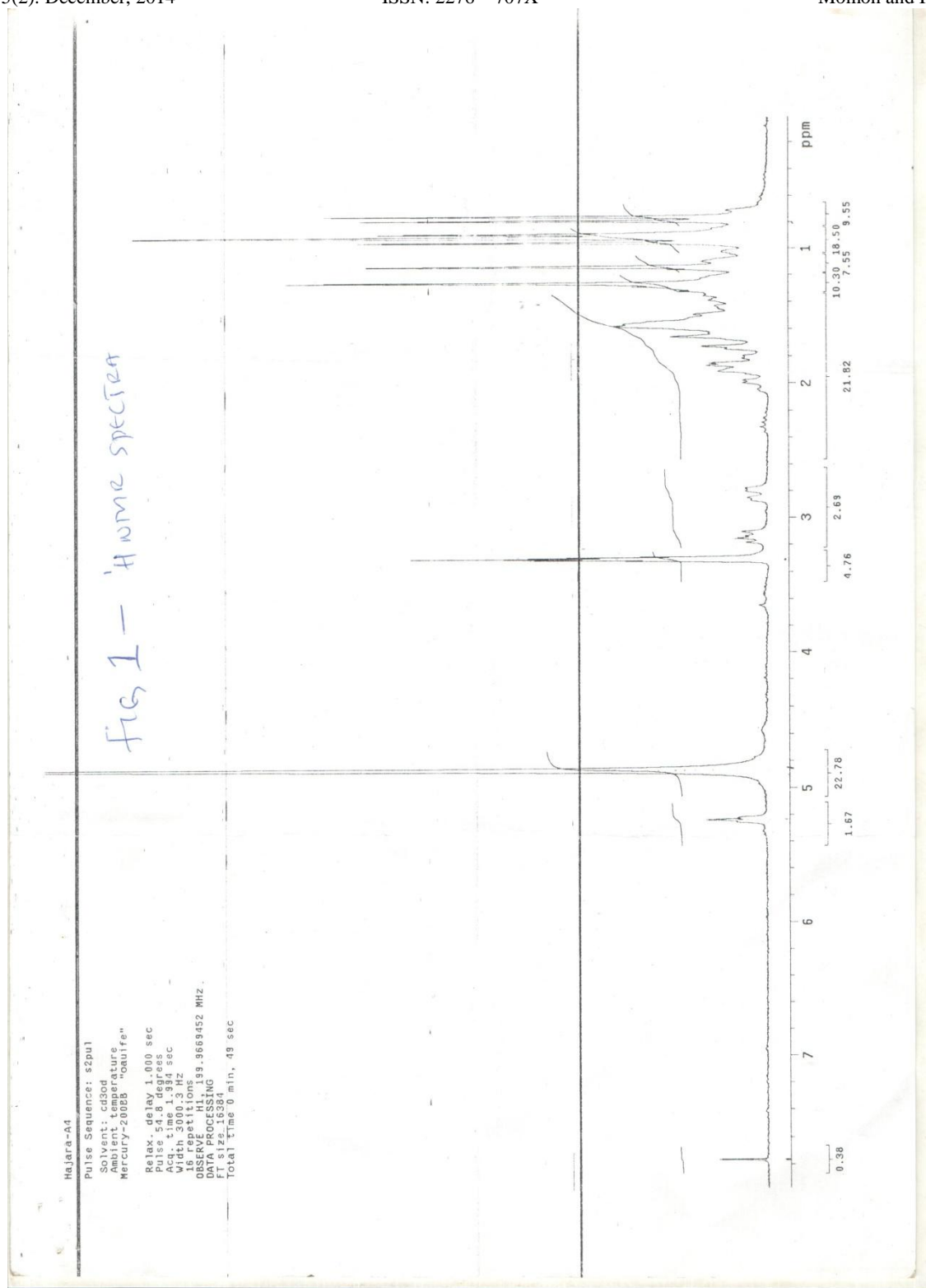
CONCLUSION

Bioactivity guided chromatography of the ethylacetate fraction of the plant led to isolation of a steroidal structure which have been shown to have activity in the brine shrimp test, thus justifying the ethnomedical claims of curing many diseases.

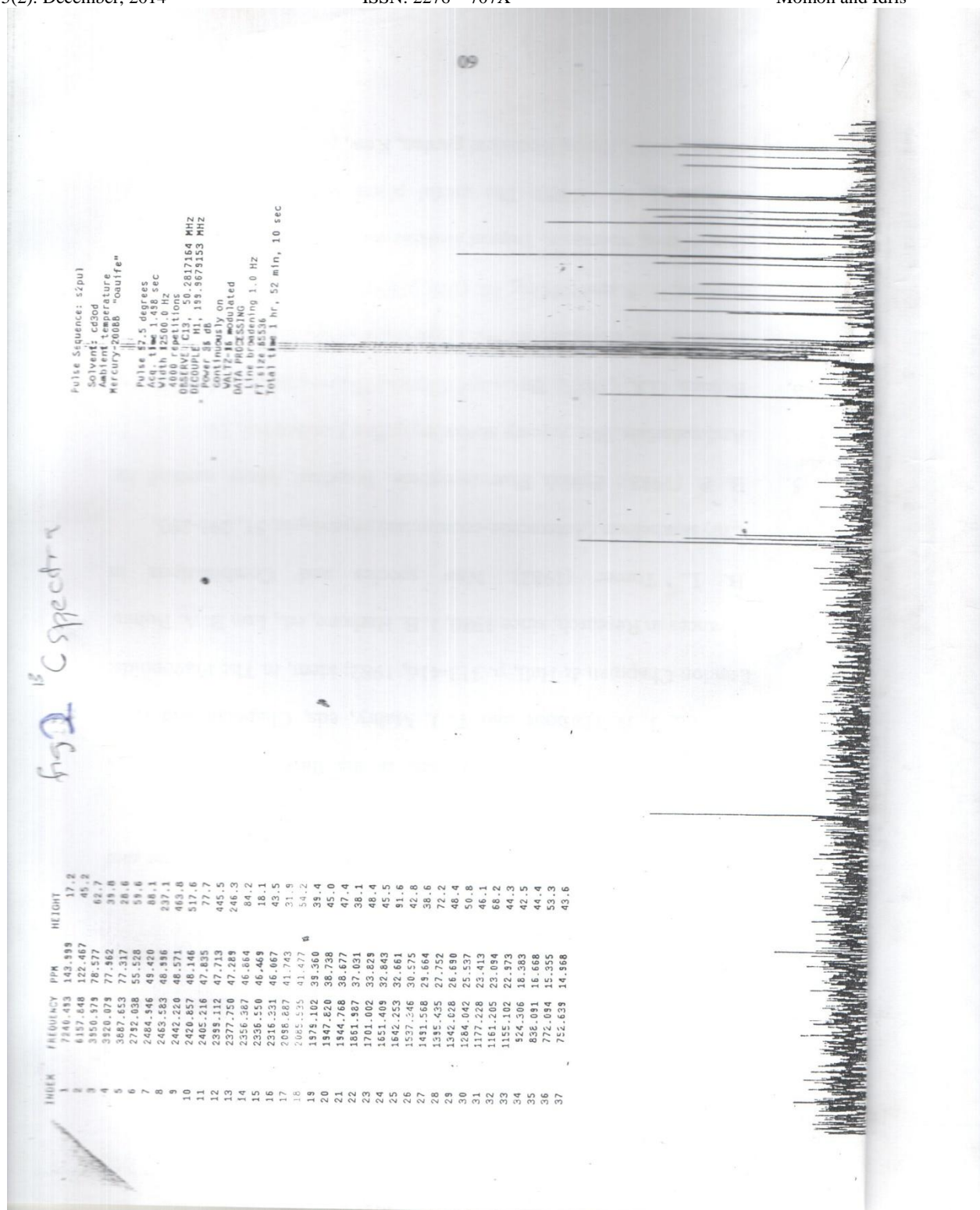
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Appendix 1



Appendix 2

Fig 3 Depi Spectra

