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Phytochemical Investigation on the Roots of *Solanum Incanum*, Hadiya Zone, Ethiopia

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Solanum incanum (Solanaceae) is bushy herb up to 3 m tall, native to Northern and north-eastern Africa including Ethiopia. It is a well known medicinal plant. Throughout tropical Africa a sore throat, angina, stomach-ache, colic, headache, painful menstruation, liver pain and pain caused by onchocerciasis, pleurisy, pneumonia and rheumatism are treated with *Solanum incanum*. This research project is aimed at isolating, Phytochemical screening and characterizing the chemical constituents of *Solanum incanum*, Hadiya Zone, Ethiopia. The root of *Solanum incanum* (650 g) was macerated and extracted with 1.5 L 80% methanol at room temperature for 48 hours with occasional shaking. This process was repeated twice at room temperature, filtered and concentrated using Rota vapor to give yellowish extracts and partitioned was done with n-hexane, ethylacetate and butanol. Then the resulting extract was filtered using filter paper (What man No 1.5, what man Ltd., England). The methanol extract was evaporated to dryness in vacuum using Rota-vapour at 40 °C to yield 17 g of crude extract. Phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, glycosides, phytosterols, saponins, triterpens, tannins and steroids as a major class of compounds. Phytochemical investigation on the methanol extract of the roots of this plant resulted in the isolation of one steroid derivative SIE2. The structure of compound SIE2 was determined by means of spectroscopic methods (IR, ¹H, ¹³C and 2D NMR, UV and DEPT).

Keyword: *Solanum incanum*, Root, Phytochemical screening, and characterization.

1. Introduction

Solanum incanum is one of about 1,500 *Solanum* species in the world. Widely distributed in the Horn of Africa it is shows characteristic thorny leaves, yellow fruits and blue flowers with yellow pistils. *Solanum incanum* propagated by seeds, which usually don't germinate quickly; one month, is needed to reach a germination rate of 50%. It is common as a weed, around houses, in overgrazed grass land and in road sides. It also found at forest edges and in bush land and grassland, from sea-level up to 2500 m altitude^[1]. Throughout tropical Africa asore throat, angina, stomach- pain, colic, headache, painful menstruation and liver pain are treated with *Solanum incanum*. For these purposes, leaf, root

and fruit decoctions are drank, roots are chewed and sap swallowed, leaf sap is used for washing painful areas, and ash of burnt plants is mixed with fat and applied externally^[2]. Leaves are added to soup to improve the flavor.

The large variation in toxicity makes it dangerous to transfer specific uses from one region to another. The fruit and the seed are used in Africa and Asia to curdle milk and to make cheese. Also, the plant is employed in East and Southern Africa for the treatment of skin diseases, general infections, abdominal pains, fever, stomachache and indigestion. In addition the fruit of *Solanum incanum* is used for the treatment of dandruff, skin diseases, sores and wounds in Tanzania^[3]. Another widespread use of *Solanum incanum* is

in the treatment of venereal diseases. In Senegal, Kenya, Uganda and Zimbabwe different plant parts are used to treat snake bites: a decoction of the roots is drunk, roots are chewed and sap is swallowed, and young chewed leaves or pulped fresh roots are applied to the bite wound. In Niger, Sudan, Rwanda and Namibia the fruits are used as an ingredient of arrow poison and in Mozambique of fish poison [4]. In Ethiopia, the fruit juice is used by peasant farmers to control ticks [5]. The boiled fruits are used as soap and in tanning leather. In southern part of Ethiopia, like haddiya peoples use the root of the plant to get relief from stomach problem, the root is chewed and sap swallowed. Fruit sap is mixed with butter and applied to cattle to control ticks.

In general, *Solanum incanum* is one of the most important medicinal plants in Ethiopia. Never the less, up to now there is no research report on extraction and characterization of active constituents of Ethiopian species. Thus this work is believed to fill the gap.

2. Materials and Methods

2.1 Collection and preparation of the plant Material

Root of *Solanum Incanum* was collected from Hemole Keble in Hadiya Zone about 257 Km far from Addis Ababa close to Homecho high school; located in Southern Nation and Nationalities People Regional Government. The plant was authenticated in comparison with the herbarium specimen of faculty of science, university of Addis Ababa. A voucher specimen has been deposited in the department of Biology, Faculty of Natural science, university of Addis Ababa (Herbarium No 001). The collected plant material was dried in an open air protected from direct exposure to sun light. The dried plant material was ground in mortar and pestle to a fine powder and made ready for extraction.

2.2 Preparation of crude extract

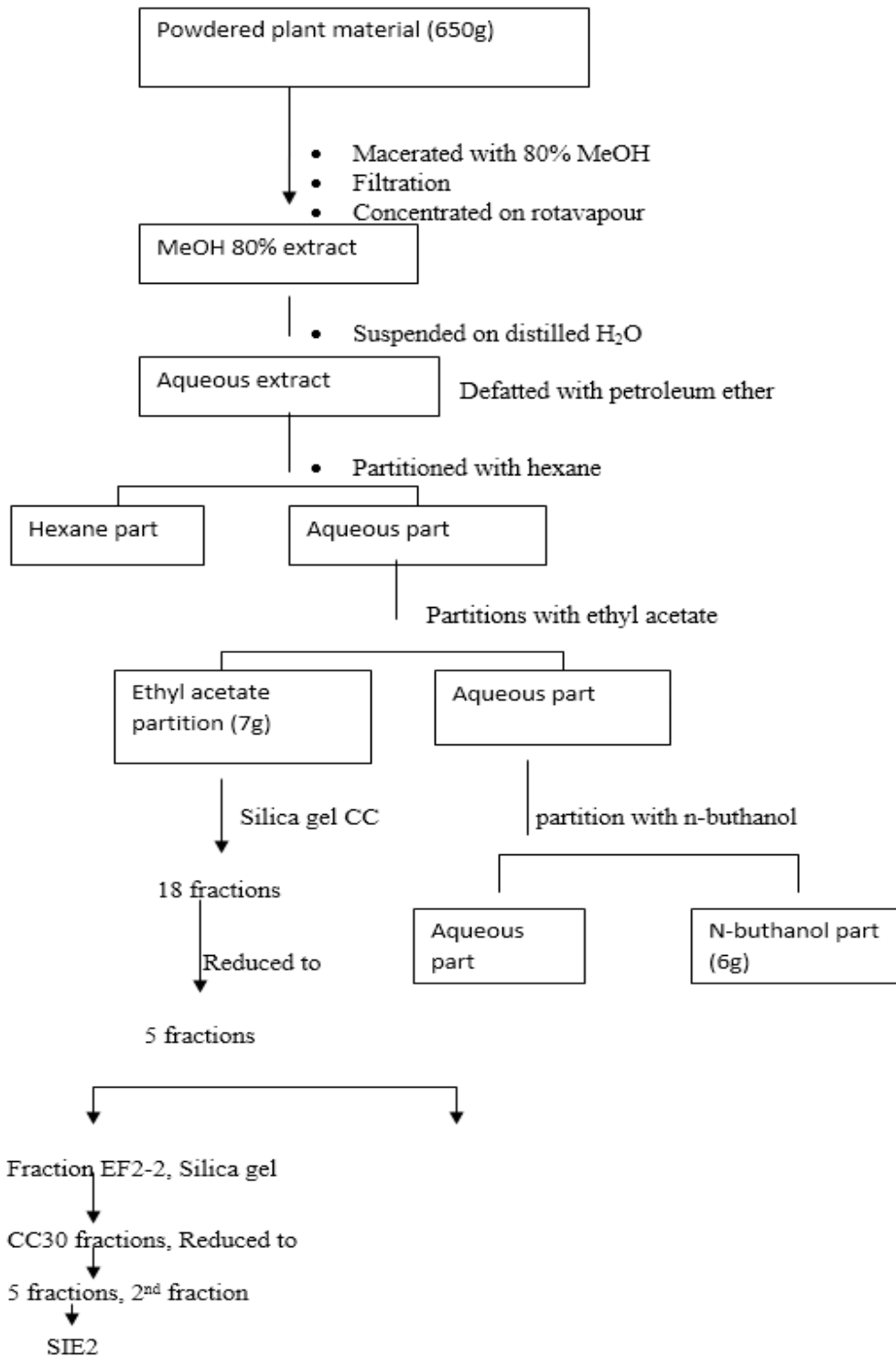
Powdered root of *Solanum incanum* (650 g) was macerated in 1.5 L 80% methanol at room temperature for 48 hours with occasional shaking. This process was repeated twice. Then the

resulting extract was filtered using filter paper (What man No 1.5, what man Ltd., England). The methanol extract was evaporated to dryness in vacuum using Rota-vapour at 40 °C to yield 17 g of crude extract. The obtained crude extract was dissolved in distilled water and defatted with petroleum ether to remove fats, then successively partitioned with hexane, ethyl acetate and butanol in this order by using separator funnel (100 ml) as shown below in (Scheme 6). Then the TLC chromatograms for both ethyl acetate and butanol fractions were developed in chloroform/ethyl acetate/ethanol (40: 40:20) and the plate was visualized by UV light (254 and 365 nm). Then the profile was checked for both ethyl acetate and butanol.

2.3 Solvent extraction of *Solanum incanum*

The ground *Solanum incanum* root was extracted with 80% MeOH at room temperature, filtered and concentrated using Rota vapor to give yellowish extracts and partitioned with n-hexane, ethylacetate and butanol. TLC of the EtOAC soluble part using Hexane/EtOAC/EtOH (1:1:1) solvent system revealed presence of four spots when visualized by UV light (254 and 365 nm). While TLC of n-butanol soluble part using CHCl₃/EtOAC/MeOH (30:50:20) revealed presence of five spots when visualized by UV light (254 and 365 nm). The spot with the highest R_f value was labeled as SIE1 and SIB1 the lowest as SIE4 and SIB5 respectively. The EtOAC soluble part was subjected to column chromatography and fractions were monitored by TLC. Those fractions that had similar retention factor were combined and purified by precipitation.

In this work it was possible to isolate from the EtOAC soluble part of methanol extract two compounds exhibiting R_f value of 0.36 SIE2 and R_f value 0.75 SIE3 by using chloroform:ethanol:butanol (4.0:1.1:4.9). The characterization of this compound was based on IR, UV, ¹H, ¹³C and 2D NMR spectra. Details about the extraction and isolation of this compound are given in the Experimental Section.



Scheme:1 - Isolation of the components of SIE

In this work it was possible to isolate from the EtOAC soluble part of methanol extract two compounds exhibiting R_f value of 0.36 SIE2 and R_f value 0.75 SIE3 by using chloroform:ethanol:buthanol (4.0:1.1:4.9). The characterization of this compound was based on IR, UV, ¹H, ¹³C and 2D NMR spectra. Details about the extraction and isolation of this compound are given in the Experimental Section.

2.4 Fractionation of ethyl acetate soluble parts with column chromatography:

After the solvent

removed by Rota vapor to yield 7 g of pale yellow solid from the EtOAC soluble part and 6 g of a yellow powder from the BtOH soluble part as show in (Scheme 1). The EtOAC part (7 g) was subjected to column chromatography on silica gel (230-400 mesh, 80 g) using a gradient of EtOAC in chloroform, a total of 18 fractions were obtained. Fractions that showed the same R_f value and the same characteristic color on TLC was combined (Table 1).

Table 1: Ethyl acetate ex tracts fractions.

Solvent system	Ratio	Fractions	Volume (ml)	Fractions Combined	code
Chloroform-ethyl acetate	9 :1	1-4	60 ml	1-4	EF-1
Chloroform-ethyl acetate	8 :2	5-7	60 ml	5-7	FE-2
Chloroform-ethyl acetate	1 :1	8-13	60 ml	8-13	FE-3
ethyl acetate	1	14-16	60 ml	14-16	FE-4
ethyl acetate	1	17-18	60 ml	17-18	EF-5

EF = Ethyl acetate fraction

Fraction 5-7 (EF-2) was applied on a small column chromatography silica gel (30 g) and

further fractionated in to 30 fractions of 10 ml each.

Table 2: Fractions of EF-2

Solvent system	Ratio	Fractions	Volume (ml)	Fractions Combined	Code	Dried weight (mg)
Chloroform-ethyl acetate	1:1	1-5	10 ml	1-5	EF2-1	10.60 mg
Chloroform-ethyl acetate	2:8	6-10	10 ml	6-10	EF2-2	40 mg
Chloroform-ethyl acetate	9:1	11-18	10 ml	11-18	EF2-3	26.61 mg
ethyl acetate	1	19-23	10 ml	19-23	EF2-4	15.6 mg
ethyl acetate-methanol	9:1	24-30	10 ml	24-30	EF2-5	59.45 mg

Based on TLC analysis, fractions that showed the same characteristics of spots were combined. Fraction 6-10 (40 mg) was have five compounds that have the same R_f value in different solvent system and have similar color of spot when the TLC was visualized by UV light (254 and 365 nm).

2.5 Coding system

In the coding system S stands for the genus name *Solanum*, I stand for species name *Incanum*, E stands for ethyl acetate extract and SIE indicate the position of the compound starting from the highest R_f value to the lowest. Thus, SIE2, SIE3 SIE4 stands for the second, third and fourth compounds respectively.

2.6 Phytochemical screening

The extracts obtained were subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by [6], [7].

A) Test for alkaloids

Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

B) Test for flavonoids

One ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow color was produced in the plant extract, which become colorless on addition of a few drops of dilute acid indicates the presence of flavonoids.

C) Test for glycosides

The extract was hydrolyzed with HCl for few hours on a water bath. To the hydrolysate, 1 ml of pyridine was added and a few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides.

D) Test for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid; 3 ml of acetic anhydride was added followed by few drops of Conc. H₂SO₄. Appearance of bluish green color showed the presence of phytosterol.

E) Test for saponins:

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

F) Test for steroids

One ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

G) Test for tannins

Five ml of the extract and a few drops of 1% lead acetate were added. A yellow precipitate was formed, indicates the presence of tannins.

H) Test for triterpenoids (Salkowski test)

10 mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H₂SO₄. Formation of reddish violet color indicates the presence of triterpenoids.

3. Results and Discussion

3.1 Characterization of SIE2

Compound SIE2 was isolated as a pale yellow solid. Its TLC plate gave pale blue color under UV light 365 nm, which is characteristic of steroids.

In the IR (KBr) spectrum (Appendix 1), the absorption band at 2938 cm⁻¹ and 2913 cm⁻¹ showed the presence of C-H asymmetric stretch for CH₃ and CH₂ stretch, respectively. The absorption band at 1600 cm⁻¹ and a weak band at 1635 cm⁻¹ showed the presence of conjugated ketone C=O stretches which is attached to aromatic ring with oxygen in Para position and conjugated C=C stretches, respectively. The presence of absorption bands between 1300-1000 cm⁻¹ illustrated C-O stretches of the ether functional groups. A band at 2852-2879 C-H stretch for CH₂-O (oxymethylene group). Therefore the IR spectrum depicts the presence of Carbonyl group, an aromatic group, methylene and a methyl group attached to a quaternary carbon in the compound isolated.

The UV spectrum (in CHCl₃) of compound SIE2 (Appendix 8) showed maximum absorption bands at λ_{max} 280 nm, which may support that the

compound has C=C double bond and C=O absorbance may tell us the possibility of (carbonyl functionality). This region of conjugation.

Table 3: The phytochemical screening of the *Solanum incanum* was tabulated below.

S.No.	Phytochemical	Reagents	Chloroform Extract [+ means Present; - means Absent]
1.	Alkaloids	Wagner Test	+
		Hager Test	+
2.	Polyphenols	Salkowski Test	+
3.	Flavonoids	Alkaline reagent test	+
		Mg turning test	+
4.	Tannin	2 ml Ferric chloride 2% and 5 ml gelatin solution 1%	-
5.	Glycosides (Oligosaccharides)	2 ml glacial acetic acid and a drop of FeCl ₃	+
6.	Phytosterols/withanoids	CHCl ₃ /conc. H ₂ SO ₄ Liebermann-Bruchard's	+
7.	Saponins	Honey comb froth formation	+
8.	Triterpens	Anisaldehyde	+
9.	Steroids	10 ml CHCl ₃ and 10 ml conc. H ₂ SO ₄	+

The presence of an aromatic group in compound SIE2 is also supported by its ¹H NMR spectrum (Appendix 2, Table 1). The ¹H NMR spectrum revealed the presence of substituted benzene ring it showed typical two ortho-coupled doublets with J=8.65 Hz at δ 7.6 and 7.4, each integrating for two protons, which were assigned to the H-22 and H-26, H-23 and H-25, respectively. More specifically, the ¹H NMR of compound SIE2 shows the presence of two methine protons in the aromatic region at δ 7.6 (1H, d) and δ 7.4 (1H, d) indicating Para-substituted benzene ring. The chemical shift at δ 4.25 corresponding to a proton attached to a carbon bearing an electronegative atom such as oxygen. Peaks at δ 2.4 (2H, m, H-28) correspond to methylene proton. Peaks at δ 1.4-0.9 were assigned for methyl protons. A proton resonance appear at δ 2.3-1 were assigned for methine and methylene groups.

The ¹³C NMR spectrum (Appendix 3, Table 2), indicated that SIE2 has 27 carbon spectrum. The multiplicity assignments were determined by DEPT-135 experiments, which showed 3-methyl, 12-methylene, 7-methine and 5-quaternary carbon atoms. The ¹³C NMR spectrum of SIE2 in CDCl₃ analyzed with DEPT 135 shows the presence of two quaternary carbons in the aliphatic region at δ 31.53 and at δ 29.63, one quaternary carbon in the aromatic region at δ 132.53 and two quaternary oxygenated carbon at δ 179.29 and 169.38. In addition, two methine carbons in the aromatic region at δ 130.69 and δ 128.76. It can be seen that C-21 is deshielded by the electronegative oxygen atom directly connected to it. Moreover, C-21 is influenced by the resonance effect.

The DEPT-135 spectrum also confirms the presence of methylene carbon at δ 67.94 is most

probably an oxygenated carbon as it is relatively found down field of the other methylene groups. HSQC also shows the correlation of oxygenated methylene group at 4.25 with carbon at δ 67.94. The spectra show three methyl carbons at δ 10.97, 14.08 and 14.15. The presence of ketone functional group C-27 which is conjugated with aromatic ring was shown by the peaks at δ 179.5

and numerous overlapping signals in the region 28.92 - 29.72 ppm was observed. The observations indicated that the compound contained a several aliphatic groups. The longest peak with two carbons at δ 29.48 is for two carbon atoms that overlapped.

From all the above data, the following structure suggested for compound SIE2

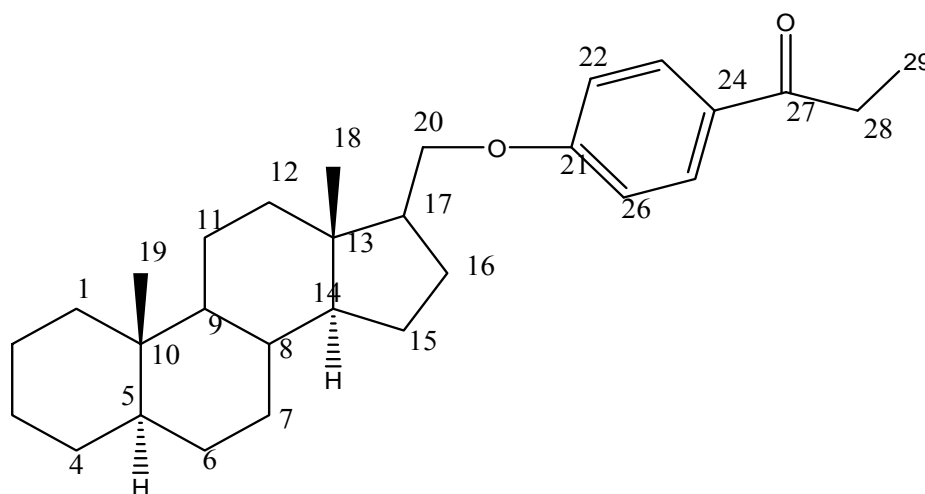


Fig 2: The proposed structure of compound SIE.

The 2D NMR spectra of the compound SIE2 further supported the proposed structure. From HSQC spectrum (Appendix-5), the protons at δ 7.6 (1H, d) and 7.4 (1H, d) correlated with the carbon peaks at δ 130.69 and 128.76, respectively. Also the proton peak at δ 4.25 (2H, d,) correlated with the carbon peak at δ 68.17 which confirms that there is methyleneoxy group. The COSY spectrum (Appendix-6) also supported the predicted structure of compound SIE2. In the ^1H - ^1H Correlation Spectroscopy (COSY) spectrum of compound SIE2, a strong correlation is observed between the two methine protons (δ 7.6, d) of C-22,26 & (δ 7.4, d) of C-

23,25 (δ 130.69 and 128.76) respectively. A correlation is also observed between H- 20 (δ 4.25, d) and H-17 (δ 1.75, m), between H-28 (δ 2.4, q) and H-29 (δ 1.4, t). Table 4 shows the COSY correlation of SIE2, which shows the nearby hydrogen. From HMBC (appendix 7), again there is correlation between H-22 and C-21, H-20 and C-21, indicating a quaternary carbon C-21 has to be connected with oxygen bearing methylene group. To confirm the suggested structure is correct or not, the HMBC data given in Table 5 was found to be most useful.

These above correlations can be shown in the following figure.

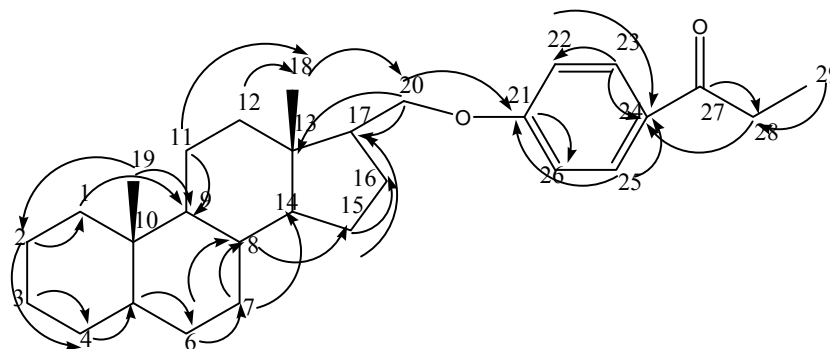
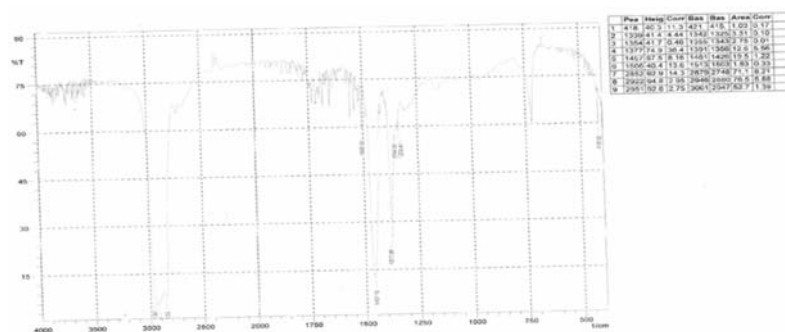


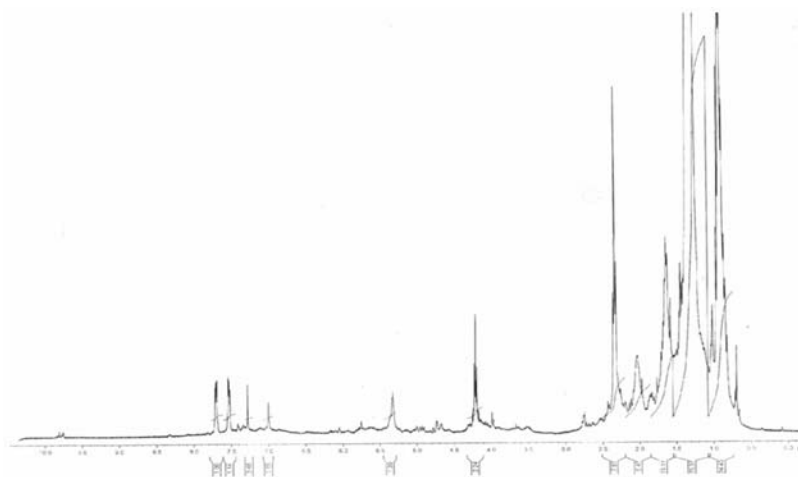
Fig 2: HMBC Correlations of compound SIE2

From this information and the whole data of HMBC,HSQC, ^1H - ^1H COSY,DEPT, ^{13}C NMR, ^1H NMR and UV, the suggested structure is most

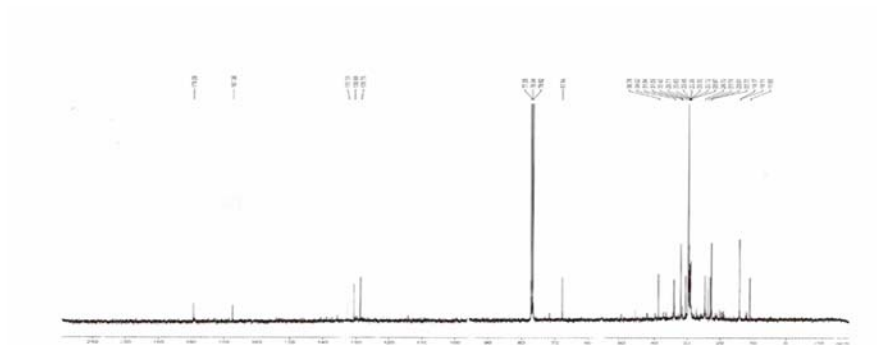
probably the correct structure of compound SIE2 and to our knowledge compound has not been reported from *Solanum Incanum*.



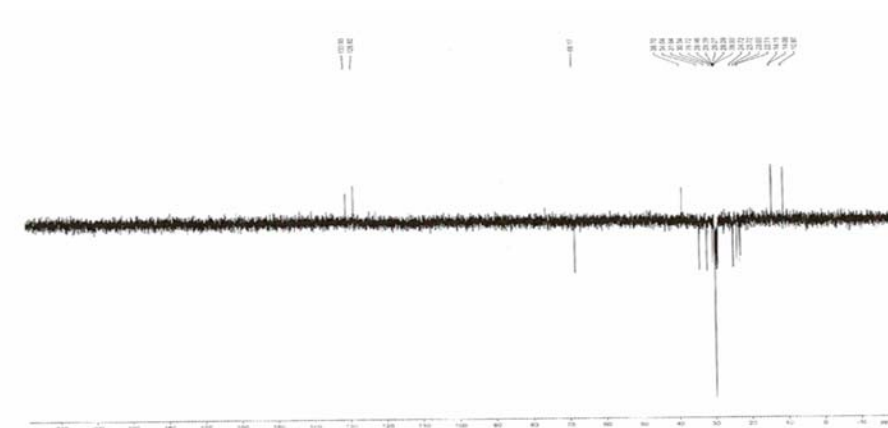
Appendix 1: IR Spectrum of Compound SIE2



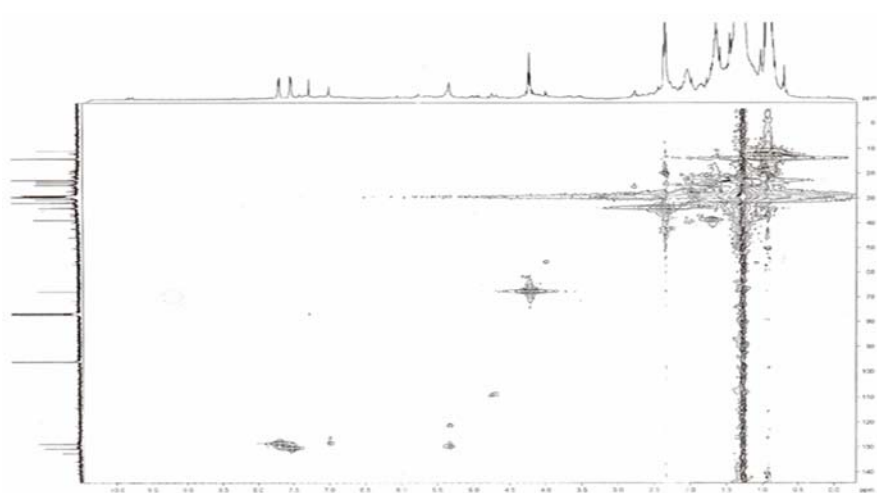
Appendix 2: ^1H NMR Spectrum of Compound SIE2



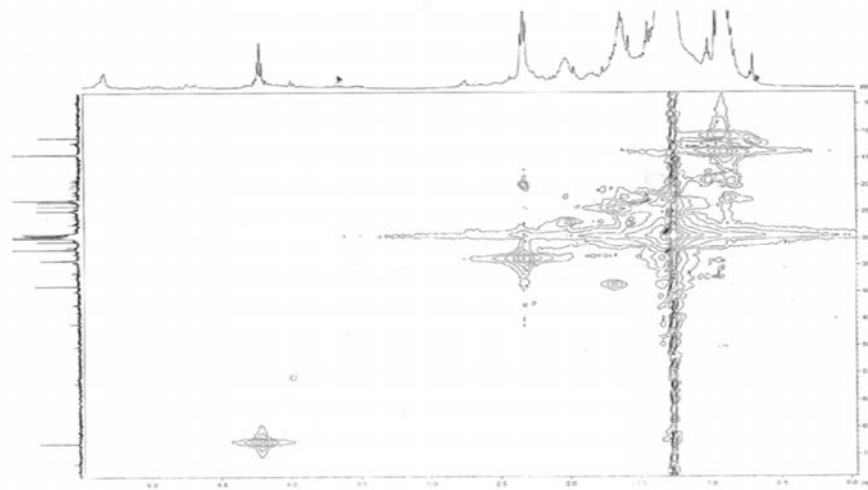
Appendix 3: ¹³C NMR Spectrum of Compound SIE2



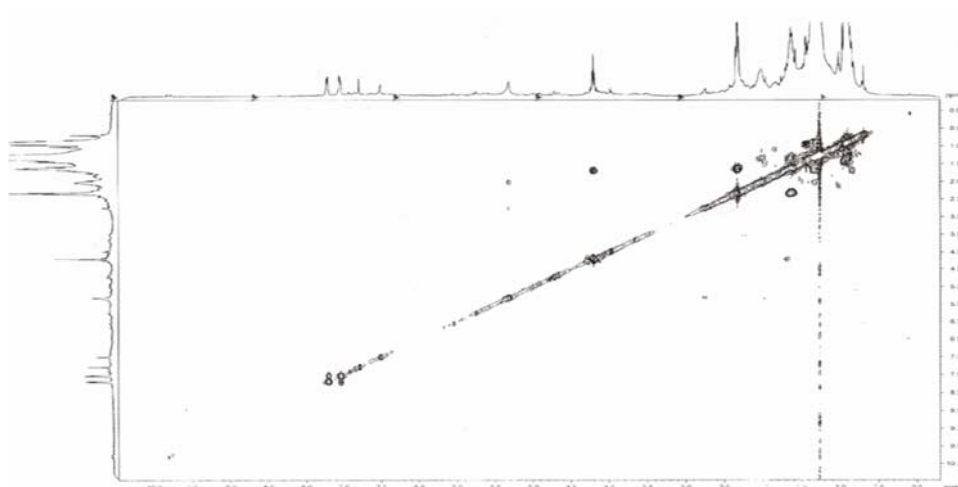
Appendix 4: DEPT Spectrum of Compound SIE2



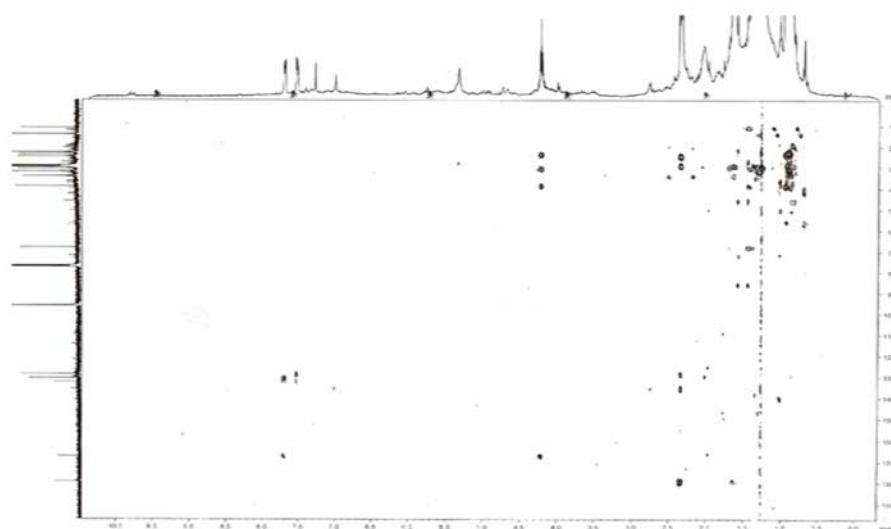
Appendix 5 (I & II): HSQC spectrum of Compound SIE2 (I).



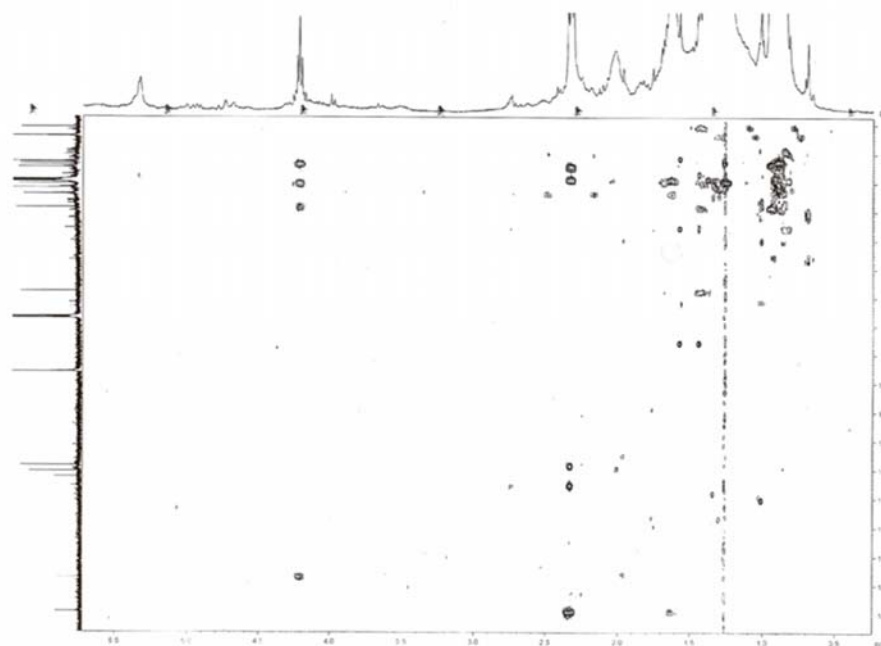
II



Appendix 6: ^1H - ^1H COSY spectrum of Compound SIE2



Appendix 7(I & II): HMBC spectrum of compound SIE2. (I)



(II)

4. Conclusion

Solanum incanum is a medicinal plant widely used around the world as antifungal, anti-ulcerogenic, laxative, anti-microbial, hemorrhoids and snake bites. The phytochemical work embodied in this thesis is based on the isolation and structure elucidation of bioactive compounds purified from the crude extract of *Solanum incanum*.

This investigation was conducted because of the importance of compounds in the genus *Solanum*, especially the steroids which are of great interest for their cytotoxic properties. Since many steroids, steroidal alkaloids and steroidal saponins are also antimicrobial agents, it is possible that they also exert their action by altering the microbial composition. One compound (SIE2) were isolated and characterized from the root extract of *Solanum incanum*. Moreover the structural elucidation of this compound were done by using spectroscopic methods NMR, IR and UV-vis. To the best of our knowledge compound SIE2 is reported for the first time.

5. References

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