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# PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF *Solanum incanum* (L.) FRUITS

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## ABSTRACT

*India has a long history and strong base for the traditional herbal medicinal system. Herbal plants play an important role in preventing and treating of human diseases. People have been using plants as a traditional medicine for thousands of years ago. Plants have been associated with the development of human civilization around the whole world. However, plants are considered as rich sources of phytochemical ingredients which enable to have medicinal value. Medicinal plants are a potential source for the development of new herbal drugs. Usage of many plants are ignored because of lack of knowledge about its medicinal values. One such plant is Solanum incanum, belonging to the solanaceae family, which is considered as toxic due to the bitter taste of its fruits. Hence, present study attempts to bring out the medicinal values of the S.incanum fruits through phyto-chemical screening.*

**KEYWORDS**: *herbal medicinal, Solanum incanum, analgestic properties, herbal drugs*

## INTRODUCTION

*Solanum incanum* (L) is one of the important traditional medicinal plant which belongs to Solanaceae family and is one of about 1,500 Solanum species in the world. It is also known as the bitter garden egg. It is either a perennial bushy herb or shrub up to 1.8m of height with spines on the stem, leaves, stalks and calyces and with velvet hairs on the leaves (Sambo H.S., *et al.*,2000). *Solanum incanum* is propagated by seeds, which usually don't germinate and the germination rate is only 50%. It is common as a weed, around houses, in overgrazed grass land and in road sides. The fruit is botanically classified as a berry and contains numerous small, soft seeds which are edible but are bitter because they contain an insignificant amount of nicotinic alkaloids (Aliyu H.M., 2006). The bitter taste of *Solanum incanum* which reduces palatability may be connected with the presence of cynogenic glycosides. *Solanum incanum* is used for sore-throat, angina, head ache, warts and benign tumours. The herb is used by several East African communities as a remedy for toothache, stomach-ache, skin diseases, fever, chest pains, painful menstruation, lever pain, snake bite, colic and ear ache (Kokwaro,1993). This plant is popular due to its analgestic properties. Reported ethno-pharmocological activities include anti-microbial, anti-tumour, hepatoprotective, hypoglycemic, antinociceptive, analetic and antipyretic effects. The fruits are green in color and turn into yellow when they ripe. The present work is an attempt to identify the Phyto-constituents of *S.incanum* fruits.

**Fig. 1.1** ***Solanum incanum***

## METHODOLOGY

## PLANT COLLECTION

The raw and ripe fruit of *Solanum incanum(L)* were collected from the rural areas of Sathiyamangalam district, Tamil Nadu. The plant was identified by Botanical survey of India, Southern Regional Centre TNAU Campus, Coimbatore.

The raw and ripe fruits were sliced into small pieces and were dried under shade for a period of 20 days to achieve complete dryness and then pulverized to obtain coarse and fine powders. The dried fruit powders were stored for further use.

## EXTRACTION

The extraction was done using soxhlet apparatus (Harwood and Moody, 1989). 50gms of dried powder collected from fruits of both raw and ripe were extracted separately with various solvents according to the increasing order of polarity such as petroleum ether, chloroform, ethyl acetate, methanol and water. 250ml of each solvent were used for extraction with soxhlet apparatus following 1:5 ratio for sample to solvent. The maceration was carried out for one day at room temperature for each solvent and successively repeated for each solvent with the sequence as mentioned above. The extracts from each solvent were collected separately and dried under the hot air oven at 60$^{o}$C. These dried extracts were weighed to calculate the total percentage yield for individual solvent.

## Qualitative Phytochemical Investigations

Identification of phytochemicals in the fruit extracts of Solanum incanum were found by using standard tests (Sofowora, 1993).

## Alkaloids

Presence of alkaloids was detected by Hager's test. To 50mg of solvent free extracts, 5ml of dilute Hydrochloric acid was added, shaken well and filtered. To 1ml of the filtrate, 4-5 drops of saturated picric acid solution (Hager's reagent) was added. The appearance of yellow colour precipitate indicates the presence of alkaloids.

## Glycosides

Glycosides in the samples were detected using Borntrager's test. To 10mg of the solvent extract, 4ml of concentrated Hydrochloric acid (conc. HCl) was added and kept for 2 hours on a water bath and filtered. To 2ml of the filtrate, 3ml of ethyl acetate was added and shaken well. Ethyl acetate forms a separate layer to which 2ml of 10% ammonia solution was added. The formation of pink colour indicates the presence of glycosides.

## Carbohydrate

Presence of Carbohydrates was detected by Fehling's test. 50mg of the solvent extract was dissolved in 5ml of distilled $H_2O$ and filtered. To the filtrate 1 ml each of Fehling's A and Fehling's B solution were added and boiled.The appearance of red precipitate indicates the presence of reducing sugars.

## Saponins

Saponins in the samples were detected using Foam test. A Small quantity of the extract was diluted with distilled water and made up to 20ml. The suspension is shaken in a graduated cylinder for 15minutes. A 2cm layer of foam stable for 10minutes indicates the presence of saponins.

## Flavonoids

Flavonoids in the sample were tested using Alkaline reagent test. 50mg of the solvent free extract was dissolved in 5ml distilled $H_2O$ and filtered. To the filtrate few drops of Sodium hydroxide solution was added. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl, indicates the presence of flavonoids.

## Tannins

Tannins in the sample were tested using gelatin test. 50mg of the solvent free extract was dissolved in 5ml of distilled $H_2O$ and filtered. To the filtrate 2ml of 1% gelatin solution containing 10% Sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

## Phenols

Presence of phenols in the sample was detected using Lead acetate test. 50mg of the solvent free extract was dissolved in 5ml of distilled $H_2O$ and filtered. To the filtrate 3-4 drops of 5% lead acetate solution was added. Formation of yellow colour indicates the presence of phenols.

## Triterpenes

Triterpenes in the sample were tested using Salkowski's test. 50mg of the solvent extract was dissolved in 5ml of ethyl acetate and filtered. The filtrates were treated with few drops of concentrated Sulphuric acid, shaken well and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

## GC MS Analysis

To identify the Phytochemicals present in *Solanum incanum* (L), GC MS analysis was of the methanolic extract was done. The experiment was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.:2.2, Thermo TSQ QUANTUM XLS. DB 5-MS capillary standard non-polar column, with a dimension of 30Mts, with an ID of 0.25 mm and Film thickness of 0.25μm was used. The Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature (oven temperature) was 40 °C raised to 290 °C at 5 °C/min and injection volume was 1.0 μl. A scan interval of 0.5 seconds with scan range of 40-600 m/z was set. Total GC running time was 35minutes and the results were compared by using Wiley Spectral library search programme.

# RESULTS

## Extraction

The extraction for the raw and ripe fruits of *Solanum incanum (L)* with five different solvents such as Petroleum ether, Chloroform, Ethyl acetate, Methanol and water and the yield is presented in Table 1.

**Table 1 Percentage yield of Raw and ripe fruit extracts of *Solanum incanum* (L) in different solvents**

| S. No | Solvents | Yield in % | |
|---|---|---|---|
| | | RAW | RIPE |
| 1. | Petroleum ether | 2.136 % | 2.554 % |
| 2. | Chloroform | 0.262% | 0.624% |
| 3. | Ethyl Acetate | 1.614% | 9.408% |
| 4. | Methanol | 3.040% | 6.970% |
| 5. | Water | 3.926% | 1.033% |

From table 1, it is evident that raw fruits samples has the highest yield of 3.926% with Water. But, the ripe fruit sample has the highest yield in ethyl acetate which is 9.408%.

## Qualitative analysis

The extracts were treated with reagents for phytochemical screening. The results of phytochemical screening with raw and ripe fruit samples are presented in Tables 2 and 3 respectively.

**Table 2 Phytochemical screening of raw fruits of *Solanum incanum (L)***

| S.No | Phyto chemical | Test | Observation | Solvents | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 |
| 1 | Alkaloids | Hager's test | Formation of yellow colour precipitate | + | + | + | + | + |
| 2 | Glycosides | Borntrager's test | Formation of pink colour | - | - | + | + | + |
| 3 | Carbohydrate | Fehling's test | Formation of blue precipitate | + | + | + | + | + |
| 4 | Saponins | Foam test | Formation of 2cm layer of stable foam | - | - | - | - | + |
| 5 | Flavonoids | Alkaline reagent test | Colourless | - | + | + | + | + |
| 6 | Tannins | Gelatin test | Formation of white precipitate | + | + | + | - | + |
| 7 | Phenols | Lead acetate test | Formation of yellow colour | - | - | - | + | + |
| 8 | Triterpenols | Salkowski's test | Formation of golden yellow colour | + | + | + | + | - |

1=Petroleum ether;2=Chloroform;3=Ethyl acetate;4=Methanol;5=Water

From Tables, 2 and 3, it is obvious that the phytochemical screening for raw and ripe samples yielded similar results except for Phenols and Tannins in ethyl acetate. Alkaloids were present in all the solvent extracts in both the raw and ripe fruits. Similarly were present in all the solvents from both raw and ripe fruits.

**Table 3 Phytochemical screening of ripe fruits of *Solanum incanum (L)***

| S.No | Phyto chemical | Test | Observation | Solvents | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 |
| 1 | Alkaloids | Hager's test | Formation of yellow colour precipitate | + | + | + | + | + |
| 2 | Glycosides | Borntrager's test | Formation of pink colour | - | - | + | + | + |
| 3 | Carbohydrate | Fehling's test | Formation of blue precipitate | + | + | + | + | + |
| 4 | Saponins | Foam test | Formation of 2cm layer of stable foam | - | - | - | - | + |
| 5 | Flavonoids | Alkaline reagent test | Colourless | - | + | + | + | + |
| 6 | Tannins | Gelatin test | Formation of white precipitate | + | + | - | - | - |
| 7 | Phenols | Lead acetate test | Formation of yellow colour | - | - | + | + | + |
| 8 | Triterpenols | Salkowski's test | Formation of golden yellow colour | + | + | + | + | - |

1=Petroleum ether;2=Chloroform;3=Ethyl acetate;4=Methanol;5=Water

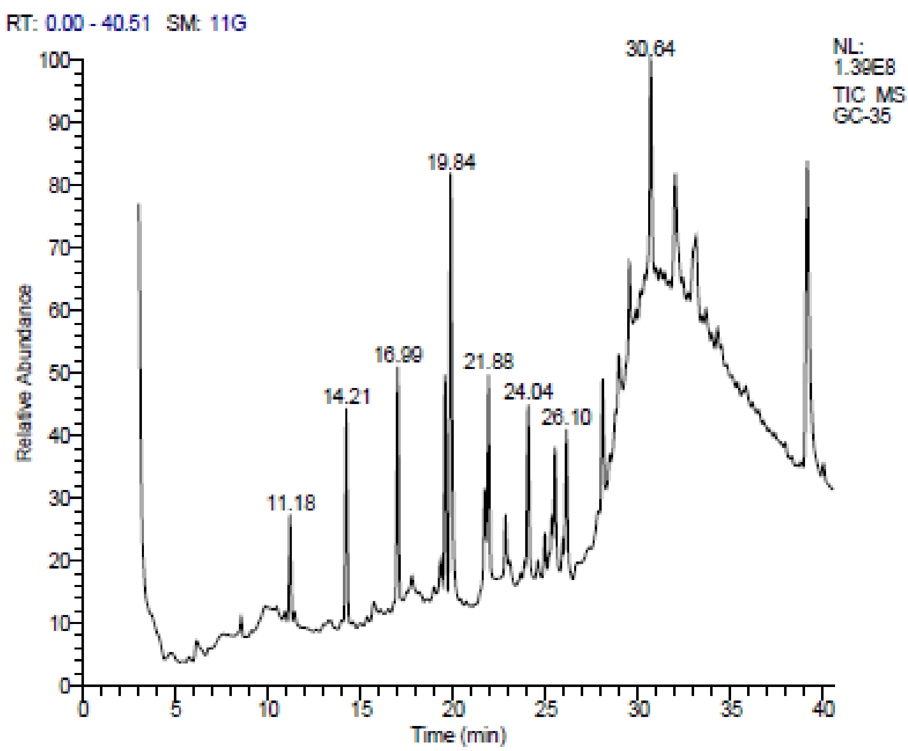
But, Glycosides were present only in the Ethylacetate, Methanol and water in both raw and ripe fruits. Saponins test showed positive results only in the water extracts of raw and ripe fruits.

In case of flavonoids, Petroleum ether extracts of both raw and ripe did not show positive results, but all other solvents exhibited presence of Flavonoids. Tannins were present in Petroleum ether and chloroform extracts of raw fruit but absent in the other three solvent extracts. But, Ethyl acetate extract of ripe fruits along with Petroleum ether and chloroform extracts showed presence of tannins. Phenols were present in Methanol and water extracts of raw fruit but absent in the other three solvent extracts. But, Ethyl acetate extract of ripe fruits along with methanol and water extracts showed presence of phenols. Triterpenols showed positive results with all the solvents except water in both raw and ripe fruits.

## GC-MS Analysis

Since the preliminary phytochemical screening in both raw and ripe fruits yielded similar results and the methanolic extracts contains all active phytochemical ingredients, methanolic extracts of the ripe fruits of *Solanum incanum (L)* was subjected to GC-MS analysis. The GC-MS analysis revealed the presence of 22 compounds (Table 4) from the methanol leaf extract of *S.incanum* (Figure 2). The major constituents were Siloxanes namely Tetradecamethylcycloheptasiloxane (RT=8.52), Hexadecamethyl cyclooctasiloxane (RT=11.18), Cyclononasiloxane, octadecamethyl (RT=14.21), and Cyclodecasiloxane, eicosamethyl (RT=16.99).

**Fig. 2 GC-MS Chromatogram of methanol ripe fruit extract of *S. incanum***



The other constituents were Benzene chloroethyl (RT=6.12), Methyl N-(n-benzyloxy carbonyl-beta-l-aspartyl)-beta-d-glucosaminide (RT=7.53), 1,3-diazacyclooctane-2-thione (RT=9.83), Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19s) (RT=10.45), 2,2,3,3,4,4 hexadeutero octadecanal (RT=13.23), 4-azido-2-methylbenzoic acid (RT=17.77), Diethyl 2,6-dimethyl-4-(3-pyridazinyl)-1,4-dihydropyridine-3,5-dicarboxylate (RT=15.70), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (RT=22.82), Dibutyl phthalate (RT=24.94), Octadecanoic acid, methyl ester (25.49), 2-benzyloxy-4,5-methylenedioxybenzyl alcohol (28.93), 2,6-dimethoxybenzoic acid benzyl ester (RT=30.64), Quercetin 7,3',4'-trimethoxy (RT=31.20), 1H-purin-6-amine, [(2-fluoro phenyl ) methyl](RT=31.99), Cholesterol (33.11), 2-acetyl-5a,6-dihydroxy-5a-methylthio-6-phenoxyazeto[1,2-d]thieno[2,3-b][1,4]thiazin-7(5h)-one (RT=34.27) and 13-docosenamide, (z) (RT=39.12).

Quercetin 7,3',4'-trimethoxy is a flavonoid, 1H-purin-6-amine, [(2-fluoro phenyl ) methyl], 2-benzyloxy-4,5-methylenedioxybenzyl alcohol, 1,3-diazacyclooctane-2-thione, Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, Diethyl 2,6-dimethyl-4-(3-pyridazinyl)-1,4-dihydropyridine-3,5-dicarboxylate and 2-acetyl-5a,6-dihydroxy-5a-methylthio-6-phenoxyazeto[1,2-d]thieno[2,3-b][1,4]thiazin-7(5h)-one are alkaloids.

## CONCLUSION

The fruit sample contains rich amounts of alkaloids and flavonoids confirming their medicinal importance. Among the alkaloids, 2-acetyl-5a,6-dihydroxy-5a-methylthio-6-phenoxyazeto[1,2-d]thieno[2,3-b][1,4]thiazin-7(5h)-one is found to be novel as it was not available in any of the databases. The work can be extended to analyze the medicinal properties of the Phyto-constituents. Another interesting phyto-constituent, siloxanes are sources of oil with insecticidal properties. Thus, *S. incanum* is a rich source of medicinal as well as economic value.

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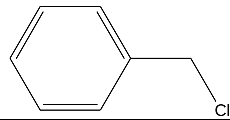
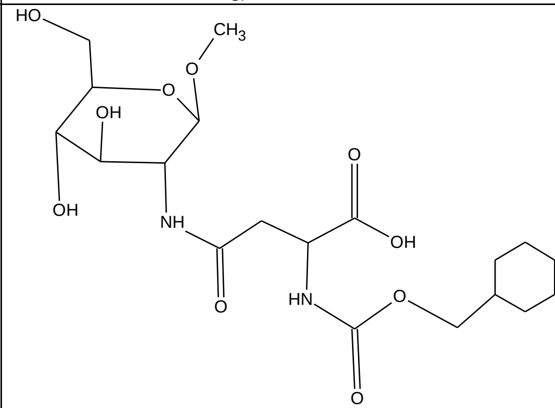
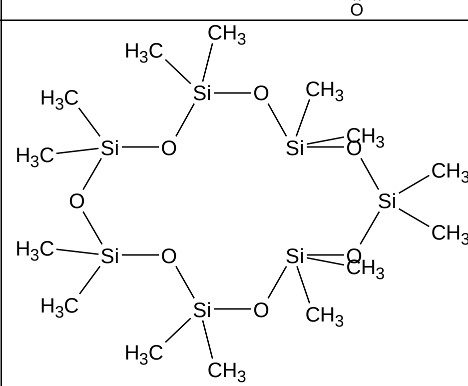
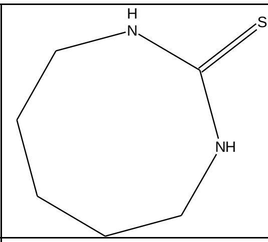
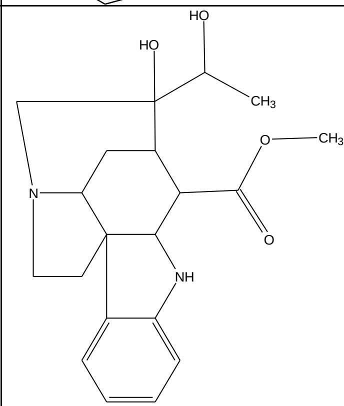
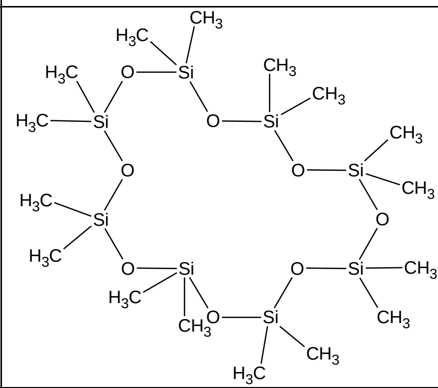
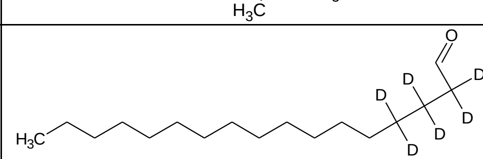
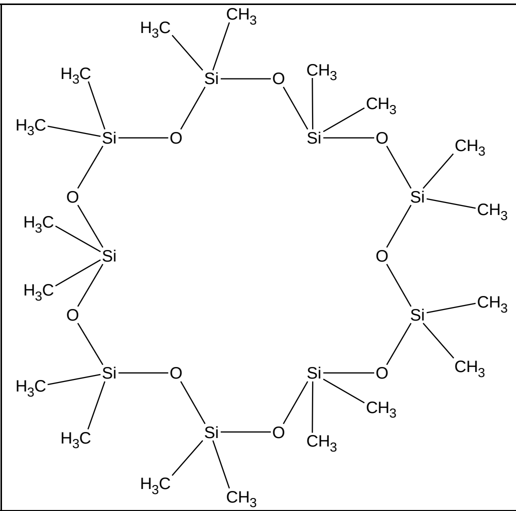
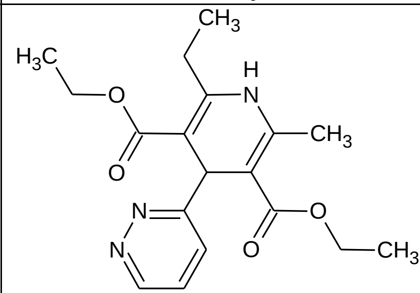
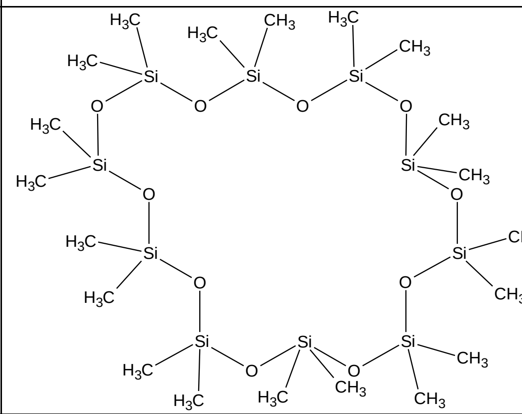
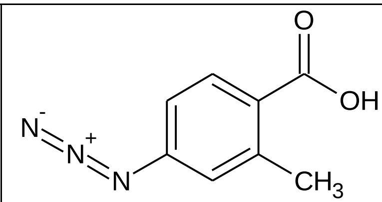
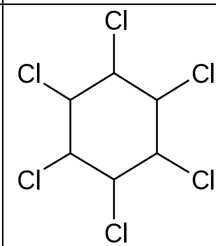
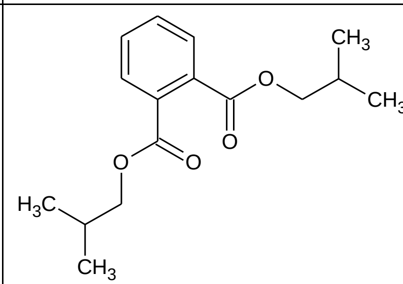
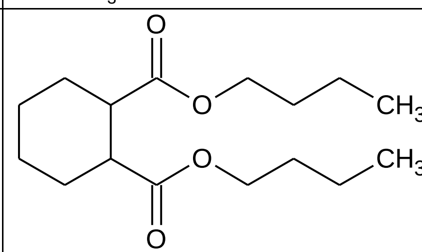
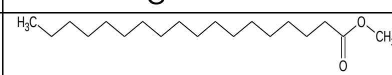
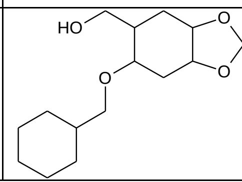
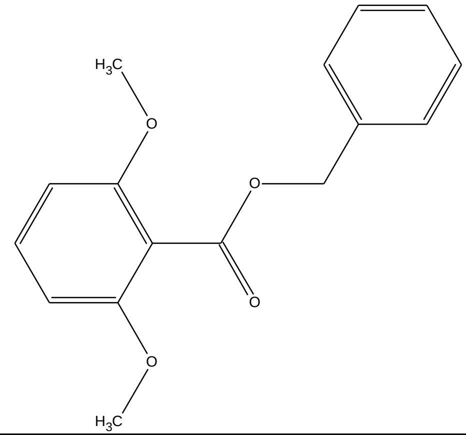
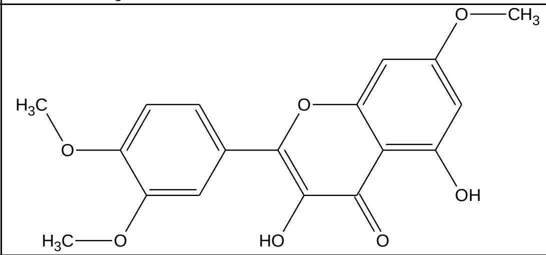
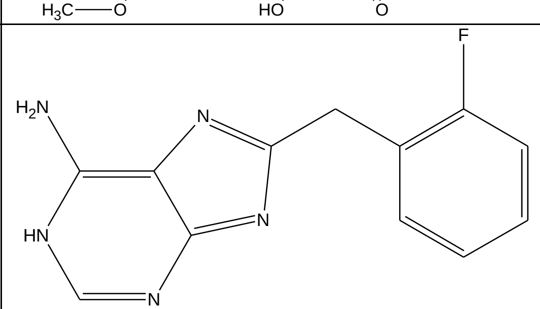
**Table 4 Phytocomponents Identified in the methanol ripe fruit Extract of *S.incanum***

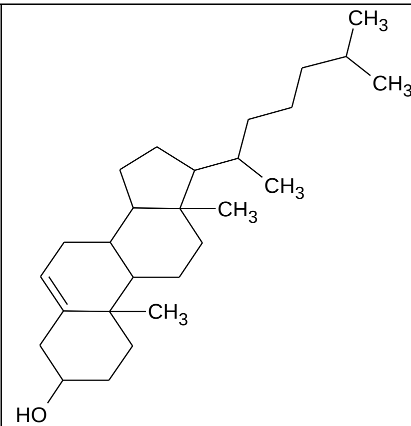
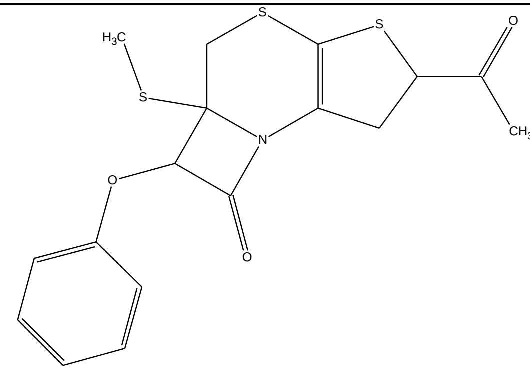
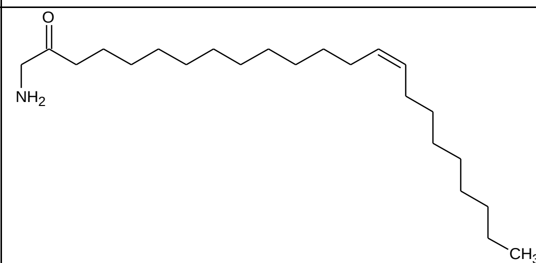
| S.No | Compound name | Molecular Formula | Structure | RT | Area % |
|---|---|---|---|---|---|
| 1. | Benzene, (chloromethyl) | C7H7Cl |  | 6.12 | 0.94 |
| 2. | Methyl N-(n-benzyloxy carbonyl -beta-l-aspartyl)-beta-d-glucos aminide | C19H26N2O10 |  | 7.53 | 0.78 |
| 3 | Tetradecamethyl cycloheptasiloxane | C14H42O7SI7 |  | 8.52 | 0.53 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 1,3-diazacyclooctane-2-thione | C6H12N2S |   | 9.83 | 2.12 |
| 5 | Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19s) | C20H26N2O4 |   | 10.45 | 0.54 |
| 6 | Hexa decamethyl cyclooctasiloxane | C16H48O8SI8 |   | 11.18 | 2.62 |
| 7 | 2,2,3,3,4,4 hexadeutero octadecanal | C18H30D6O |   | 13.23 | 0.84 |

| | | | | | |
|---|---|---|---|---|---|
| 8 | Cyclononasiloxane, octadecamethyl | C18H54O9Si9 |  | 14.21 | 5.44 |
| 9 | Diethyl 2,6-dimethyl- 4-(3-pyri dazinyl)-1,4-dihydropyridine-3,5- dicarboxylate | C17H21N3O4 |  | 15.70 | 0.88 |
| 10 | Cyclodecasiloxane, eicosamethyl | C20H60O10Si 10 |  | 16.99 | 5.26 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | 4-azido-2-methylbenzoic acid | C8H7N3O2 |  | 17.77 | 1.79 |
| 12 | Benzene, hexachloro- | C6Cl6 |  | 19.84 | 11.58 |
| 13 | 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester | C16H22O4 |  | 22.82 | 2.07 |
| 14 | Dibutyl phthalate | C16H22O4 |  | 24.94 | 0.76 |
| 15 | Octadecanoic acid, methyl ester | C19H38O2 |  | 25.49 | 4.13 |
| 16 | 2-benzyloxy-4,5-methylene dioxybenzyl alcohol | C15H14O4 |  | 28.93 | 2.36 |

| | | | | | |
|---|---|---|---|---|---|
| 17 | 2,6-dimethoxybenzoic acid benzyl ester | C16H16O4 |  | 30.64 | 8.93 |
| 18 | Quercetin 7,3',4'-trimethoxy | C18H16O7 |  | 31.20 | 0.51 |
| 19 | 1H-purin-6-amine, [(2-fluoro phenyl ) methyl] | C12H10FN5 |  | 31.99 | 4.19 |

| | | | | | |
|---|---|---|---|---|---|
| 20 | Cholesterol | C27H46O |  | 33.11 | 3.79 |
| 21 | 2-acetyl-5a,6-dihydroxy-5a-methylthio-6-phenoxyazeto[1,2-d]thieno[2,3-b][1,4]thiazin-7(5h)-one | C17H15NO3S3 |  | 34.27 | 1.02 |
| 22 | 13-docosenamide, (z)- | C22H43NO |  | 39.12 | 10.76 |