The GC–MS analysis of the diethylether and ethylacetate fraction of the peel of *Solanum incanum* and the study of their antibacterial activity

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Abstract
The diethyl ether and ethyl acetate fractions of the peel of the fruit of *Solanum incanum* (*S. incanum*) were analyzed using gas chromatography-mass spectrometry (GC–MS). 105 compounds were identified in the diethyl ether fraction and 75 compounds were identified in ethyl acetate fraction. Among them, 5 compounds were analyzed by fragmentation pattern, discussed, and compared with NIST database. The antibacterial screening was also conducted for both diethyl ether and ethyl acetate fractions of the fruit peel of *S. incanum* using four pathogens, two Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pyogenes* (*S. pyogenes*) and two Gram negative bacteria *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), at various concentrations (250, 500, 750 and 1000 μg/ml). The diethyl ether and ethyl acetate fractions of the peel of *S. incanum* exhibited activity against *E. coli* and *K. pneumoniae* at 1000 μg/ml concentration.

Keywords *S. incanum* · Peel · Phytochemical · GC–MS · And antibacterial activity

Introduction
Natural Products are of considerable significance to the health of human beings and animals. The medicinal importance of plants lies in the secondary metabolites, which produce a definite physiological action on the human or animal body. Investigation of the phytochemical constituents and relating them to pharmacological activities furnish a plethora of information. In this line, our group has also reported the phytochemicals and their uses (Tesfaye and Ravichandran 2018; Tamrat and Ravichandran 2018; Ravichandran and Sulochana 2016a, 2016b). Many of the endemic medicinal plants are traditionally used as spices and food plants (Sakha et al. 2018).

*Solanum* L. (*Solanaceae*) is a large and diverse genus of flowering plants. The species of *Solanum* are known for their medicinal importance (Sheeba 2010). *S. incanum* has various traditional applications in many Ethiopian communities. The species is endemic and well distributed in Ethiopia (Abebe et al. 2014). *S. incanum* is commonly seen around local neighborhoods, green-lands, and roadsides (Yrjonen 2004).

The fruit of *S. incanum* is the source of many medicinally important secondary metabolites (Asaolu 2003). Further, solanine and solasonine have already been isolated (Alghamdi 2013). The leaves of *S. incanum* L. were found to contain minerals such as K (Auta and Ali 2011) and Ca (Abdalla 2015).

In recent times, GC–MS analysis is progressively engaged in the analysis of medicinal plants (Asha et al 2017). The GC–MS analysis of the leaf extract (Sundar and Pillai 2015) and the fruits extract of *S. incanum* from India has been reported (Buvaneshwari et al. 2017). We have earlier reported the GC–MS analysis of the essential oil derived from the peel of the fruit from *S. incanum* (Yetayih and Ravichandran 2020) but there is no report on the GC–MS analysis of the diethyl ether and ethyl acetate fraction of the peel of the fruit of the *S. incanum*. Hence, we report the...
GC–MS analysis of the diethyl ether extract and the ethyl acetate fraction of the peel of the *S. incanum*.

**Experimental**

**The survey, collection, and preparation of plant material**

The Fruits of *S. incanum* (5 kg) were collected on May 22, 2018, identified by Dr. Tena Regasa (botanist), Department of Biology. The collected ripe fruits were cleaned and washed with water to remove all the impurities, and ripe fruits were cut with the sterilized blade and the internal content was removed. The peel of the fruit was cut into small pieces and dried till constant weight is achieved. The dried peel was powdered using a laboratory mill and sieved and stored in air-tight containers till it is used.

**Preparation of the extract**

A sample of 1.5 kg *S. incanum* peel powder was soaked in 7-L of 70% methanol solution (Hassim et al. 2014; Khalil et al. 2017). The solution was kept for 7 days with random shaking and the extract was filtered by cotton plugged filtration followed by Whatman No 1 filter paper. The same procedure was repeated twice and the extracts were combined and evaporated to dryness using Rota-evaporator at 60 °C. The obtained methanol extract was used for further portioned into different fractions based on polarity.

**Liquid–liquid extraction**

The methanol extract was partitioned into various solvents based on their polarity. The methanol extract was transferred to a separatory funnel and successively partitioned with various solvents including hexane, diethyl ether, and ethyl acetate. The extracts in each solvent were done successively till the added solvent is colorless. The different fractions obtained were dried over anhydrous sodium sulfate (Khalil et al. 2017). Each of the fractions was combined, and concentrated by a rota-evaporator. Among them, diethyl ether and ethyl acetate fractions were considered for further work.

**Gas Chromatography–Mass Spectrometry (GC–MS) analyses**

Gas chromatography-mass spectrometry (GC–MS) is an important analytical technique to analyze the chemical composition of the plant extract. The GC–MS analysis was done using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45–400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system by the method reported in the literature (Yetayih and Ravichandran 2020; Essien et al. 2016). The GC column was an HP-5 ms fused silica capillary with a (5% phenyl) polymethyl siloxane stationary phase, the film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 ml/min. The inlet temperature was 200 °C and the interface temperature was 280 °C. The GC oven temperature program was used as follows: 40 °C initial temperature, hold for 10 min; increased at 3 °C/min to 200 °C; increased 2 °C/min to 220 °C. A 1% w/v solution of the sample was prepared and 1 μL was injected using a splitless injection technique. The compounds were identified based on their retention time (RT), and by the comparison of their fragmentation patterns with those of the reported values and NIST Library (G1036A, revision D.01.00)/Chem Station data system (G1701CA, version C.00.01.08) (Yetayih and Ravichandran 2020; Okhale et al. 2018).

**Antibacterial activity**

Four strains of pathogenic bacteria were used in this study including Gram-positive, *S. aureus* (ATCC 31,488), and *S. pyogenes* (ATCC 27,853), and Gram-negative bacteria, *E. coli* (ATCC 25,922), and *K. pneumonia* (ATCC 27,853). The disk diffusion method was employed for the evaluation of antibacterial activity (Dar et al. 2017). The bacterial strains were inoculated on Mueller Hinton broth, homogenized on a sterilized Petri dish to yield a uniform depth. The different fractions of the plant extracts were dissolved in 10% of dimethyl sulfoxide (DMSO). 250, 500, 750, and 1000 μg/ml (extract soaked by each disc) concentration of the fractions were tested for antibacterial activity respectively. The zone of inhibition was measured. The experiments were done in triplicate. DMSO (10%) and Gentamicin (10 μg/disc) were taken as negative and positive control respectively. Statistical analysis was performed using MS Excel. The graphs were also plotted with MS Excel. The structures are drawn with ChemDraw Ultra 8.0.

**Results and discussion**

**The diethyl ether fraction**

The GC–MS analysis of diethyl ether fraction was confirmed with the presence of 105 components (Table 1). The components were identified by representing 100% of the total sample composition.

The principal compounds identified in the diethyl ether fraction were 8-nonenenoic acid (19.59%),...
The GC–MS analysis of the diethylether and ethylacetate fraction of the peel of *Solanum incanum* …

| Peak# | RT     | Area     | Area% | Name compounds                        |
|-------|--------|----------|-------|---------------------------------------|
| 1     | 4.142  | 6,464,154| 1.27  | 2,3-Butanediol                        |
| 2     | 4.321  | 11,966,968| 2.35  | [R-(R*,R*)]-2,3-Butanediol            |
| 3     | 14.198 | 254,929  | 0.05  | 2-Pentyl-Furan                        |
| 4     | 16.899 | 4,962,483| 0.97  | Benzyl alcohol                        |
| 5     | 17.747 | 92,527   | 0.02  | 1-ethyl-1-methyl-Cyclopentane         |
| 6     | 18.042 | 167,251  | 0.03  | 2-Methylbutanoic anhydride            |
| 7     | 18.326 | 350,838  | 0.07  | Butan-2-one, 4-[pyrrolidin-2-one-5-yl]- |
| 8     | 19.074 | 212,311  | 0.04  | 7-Octen-2-ol, 2-methyl-6-methylene-   |
| 9     | 20.017 | 107,019  | 0.02  | Mequinol                              |
| 10    | 20.532 | 134,141  | 0.03  | 5-Methyl-1-heptanol                   |
| 11    | 20.830 | 533,001  | 0.10  | 3,7-dimethyl-1,6-Octadien-3-ol        |
| 12    | 21.045 | 420,417  | 0.08  | 5-Nonen-2-one                         |
| 13    | 21.316 | 596,171  | 0.12  | Phenylethyl Alcohol                   |
| 14    | 22.587 | 172,497  | 0.03  | 7-Bromo-1-heptanol, trimethylsilyl ether |
| 15    | 24.204 | 263,407  | 0.05  | Acetic acid, phenylmethyl ester       |
| 16    | 24.898 | 217,662  | 0.04  | Benzeneacetic acid, methyl ester      |
| 17    | 25.398 | 290,521  | 0.06  | L-.alpha-Terpineol                   |
| 18    | 25.725 | 1,192,480| 0.23  | Decanal                               |
| 19    | 26.608 | 7,076,080| 1.39  | Catechol                             |
| 20    | 26.959 | 2,346,275| 0.46  | Benzoic acid                          |
| 21    | 27.269 | 8,743,987| 1.72  | 2,3-dihydro-Benzofuran               |
| 22    | 29.964 | 6,106,067| 1.20  | Benzeneacetic acid                    |
| 23    | 30.282 | 423,731  | 0.08  | Indole                                |
| 24    | 30.541 | 363,009  | 0.07  | Nonanoic acid                         |
| 25    | 31.298 | 1,647,954| 0.32  | 2-Methoxy-4-vinylphenol              |
| 26    | 31.508 | 158,721  | 0.03  | Isomyrcenyl acetate                  |
| 27    | 31.871 | 1,000,593| 0.20  | 2-Decen-1-ol                          |
| 28    | 32.308 | 289,326  | 0.06  | 2-t-Butyl-5-propyl-[1,3]dioxolan-4-one |
| 29    | 32.855 | 2,765,290| 0.54  | 2,5-Dimethylcyclohexanol              |
| 30    | 33.082 | 3,208,997| 0.63  | (S)-2,7-Octadien-4-ol, 2-methyl-6-methylene |
| 31    | 33.448 | 1,512,440| 0.30  | dihydro-5-pentyl-2(3H)-Furanone       |
| 32    | 33.612 | 7,675,007| 1.51  | dihydro-5-pentyl-2(3H)-Furanone       |
| 33    | 34.084 | 2,795,101| 0.55  | 1-methoxy-2,2-dimethyl-3-(3,3-dimethyl-1-propynyl)- Cyclopropane |
| 34    | 34.202 | 5,229,121| 1.03  | 3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol |
| 35    | 34.390 | 9,749,572| 1.91  | trans-5,6-Epoxydecane                 |
| 36    | 34.994 | 2,369,512| 0.47  | 2-Isopropylidene-5-methylhex-4-enal   |
| 37    | 35.659 | 1,069,366| 0.21  | 4-Ethylthiane                         |
| 38    | 36.075 | 270,006  | 0.05  | 2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin |
| 39    | 36.308 | 514,672  | 0.10  | 4-Dimethylsilyloxypentadecane         |
| 40    | 36.742 | 2,637,720| 0.52  | 15-(2,2-dimethylcyclopropyl)-2-methyl-4-methylenepentene |
| 41    | 37.383 | 14,571,016| 2.86  | o-Toluic acid, 6-ethyl-3-octyl ester  |
| 42    | 37.809 | 2,594,865| 0.51  | 2,6-dimethyl-7-Octene-2,6-diol        |
| 43    | 38.242 | 1,334,860| 0.26  | 2-Dodecen-1-yl(-)succinic anhydride   |
| 44    | 38.654 | 2,164,695| 0.42  | 5-Hepten-3-yn-2-ol, 6-methyl-5-(1-methylethyl)-  |
| 45    | 39.288 | 2,497,131| 0.49  | dl-Mevalonic acid lactone            |
| 46    | 39.505 | 9,958,474| 1.95  | Nonanoic acid, 9-hydroxy-, methyl ester |
| 47    | 39.700 | 2,451,540| 0.48  | 5-(2-tetrahydrofurfuryl)-Heptan-2-ol  |
| 48    | 39.805 | 11,623,593| 2.28  | Butylated Hydroxytoluene              |
| Peak# | RT   | Area  | Area% | Name compounds                                      |
|------|------|-------|-------|----------------------------------------------------|
| 51   | 40.108 | 930,279 | 0.18  | 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-(R)-2(4H)-Benzo|furanone                      |
| 52   | 40.603 | 3,614,576 | 0.71  | Nerolidolobutyrate                                 |
| 53   | 41.402 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 54   | 42.356 | 11,945,857 | 2.34  | Fumaric acid, ethyl 2-methylallyl ester             |
| 55   | 43.721 | 76,729,146 | 15.06 | 8-Nonenoic acid                                    |
| 56   | 43.975 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 57   | 44.332 | 20,089,247 | 3.94  | 3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol           |
| 58   | 44.457 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 59   | 44.615 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 60   | 45.721 | 11,945,857 | 2.34  | Fumaric acid, ethyl 2-methylallyl ester             |
| 61   | 45.975 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 62   | 46.356 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 63   | 46.457 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 64   | 46.615 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 65   | 46.721 | 11,945,857 | 2.34  | Fumaric acid, ethyl 2-methylallyl ester             |
| 66   | 46.975 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 67   | 47.125 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 68   | 47.275 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 69   | 47.425 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 70   | 47.575 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 71   | 47.725 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 72   | 47.875 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 73   | 48.025 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 74   | 48.175 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 75   | 48.325 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 76   | 48.475 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 77   | 48.625 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 78   | 48.775 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 79   | 48.925 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 80   | 49.075 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 81   | 49.225 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 82   | 49.375 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 83   | 49.525 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 84   | 49.675 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 85   | 49.825 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 86   | 50.075 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 87   | 50.225 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 88   | 50.375 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 89   | 50.525 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 90   | 50.675 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 91   | 50.825 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 92   | 51.075 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 93   | 51.225 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 94   | 51.375 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 95   | 51.525 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 96   | 51.675 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
| 97   | 51.825 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
| 98   | 52.075 | 9,336,554 | 1.83  | Nonanedioic acid, dimethyl ester                    |
| 99   | 52.225 | 6,284,027 | 1.23  | Benzoic acid, 4-hydroxy-3-methoxy                  |
|100   | 52.375 | 4,044,024 | 0.79  | Nonanedioic acid, monomethyl ester                  |
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3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (3.94%), 2,3-butanediol (3.62%), acetoxycetic acid, tridec-2-ynyl ester (3.46%), o-toluic acid, 6-ethyl-3-octyl ester (2.86%), 3-butyllindolizidine (2.64%), \([R-(R^*,R^*)]-2,3\)-butanediol (2.35%), Fumaric acid, ethyl-2-methylallyl ester (2.34%), and butylated hydroxytoluene (2.28%) along with minor constituents.

**Benzyl alcohol (MW108)**

The empirical formula \(C_7H_{10}O\) was derived using the nitrogen rule and rule of thirteen. The absence of the \(M + 2\) peak indicated the absence of halogen, silicon, and sulfur. The base peak at 79 indicated the presence of phenyl groups. The other fragments benzyl cation(91), phenyl cation(77), cyclopentadienyl cation(65), and cyclobutadienyl cation(51) confirmed the presence of Benzyl alcohol (Fig. 1 and Scheme 1).

**2, 3-dihydro-Benzofuran (MW120)**

The empirical formula of \(C_8H_8O\) was derived using the nitrogen rule and rule of thirteen. The absence of the \(M + 2\) peak indicated the absence of halogen, silicon, and sulfur. The base peak at 120 indicated the presence of very stable benzyl ether. The presence of 2, 3-dihydro-Benzofuranyl cation(119), benzyl cation(91), cyclopentadienyl cation(65), and cyclobutadienyl cation(51) was confirmed with the presence of 2, 3-dihydro-Benzofuran (Fig. 2 and Scheme 2).

**Butylated hydroxytoluene (MW 220)**

According to the nitrogen rule and rule of thirteen, the derived empirical formula was \(C_{15}H_{24}O\). The absence of the \(M + 2\) peak indicated the absence of halogen, silicon, and sulfur. The base peak at 205 indicated the presence of butylated hydroxytoluene. The presence of 2-tert-butyl-1-methoxy-4-methylphenyl cation(177), 1-tert-butylbenzyl cation(145), 2-methylpropyl cation(57) confirmed the presence of Butylated Hydroxytoluene (Fig. 3 and Scheme 3).

**Ethyl acetate fraction of *S. incanum* Peel**

The GC–MS analysis of ethyl acetate fraction was confirmed with the presence of 75 components (Table 2). The

### Table 1 (continued)

| Peak# | RT    | Area    | Area% | Name compounds                                      |
|-------|-------|---------|-------|-----------------------------------------------------|
| 101   | 66.946| 2,105,734| 0.41  | Nerolidolisobutyrate                                 |
| 102   | 67.537| 1,109,923| 0.22  | 9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol |
| 103   | 67.985| 1,108,356| 0.22  | 1-Heptatriacetanol                                   |
| 104   | 68.808| 2,400,883| 0.47  | Hexanedioic acid, bis(2-ethylhexyl) ester            |
| 105   | 70.275| 692,075  | 0.14  | 3,5-dimethyl- Cyclohexanol                           |
|       |       | 2,103,564 | 100.00|                                                     |
components were identified by representing 100% of the total sample composition.

The ethyl acetate fraction revealed 75 constituents (Table 2), the major constituents were \([R-(R^*,R^*)]-2,3\)-butanediol (51.02%), 2,3-butanediol (22.65%) followed by 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2\(H\)-chromene (3.17%) and trans-linalool oxide (furanoid) (1.64%) along with major constituents, and minor constituents were also reported.

According to the nitrogen rule and rule of thirteen, the derived empirical formula can be \(C_{12}H_{20}O\). The base peak at 124 indicated the presence of 5, 6-dihydro-2-methyl-2\(H\)-pyryl-2-ethyl radical cation. The presence of 1,1-dimethylcyclohexyl-2, 3-dimethylcynation(135), 3-methylene-penta-1,4-dienyl cation(79), 4-methylpent-1-enyl cation (69), 5, 5, 8a-trimethyl-3, 5, 6, 7, 8,8a-hexahydro-2\(H\)-chromene(MW 180)

\[\text{Scheme 1 Mass fragmentation of Benzyl alcohol}\]

\[\text{Scheme 2 Mass fragmentation of 2, 3-dihydro-Benzofuran}\]

\[\text{Fig. 2 Mass spectrum and fragmentation of 2, 3-dihydro-Benzofuran}\]
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Fig. 3 Mass spectrum and fragmentation of Butylated Hydroxytoluene

Scheme 3 Mass fragmentation of Butylated Hydroxytoluene

2-methylprop-1-enyl cation (55) confirmed the presence of 5, 5, 8a-trimethyl-3, 5, 6, 7, 8,8a-hexahydro-2H-chromene (Fig. 4 and Scheme 4).

1-docosene(MW 125)

The empirical formula of 1-docosene was derived as C_{22}H_{44} to using the nitrogen rule and rule of thirteen. The base peak at m/z 57 indicated the presence of straight alkane. Further, the presence of butenyl cation(55), pentenyl cation(69), hexenyl cation(83), heptenyl cation(98), octenyl cation(111) and nonenyl cation(125) confirmed the presence of 1-docosene (Fig. 5 and Scheme 5, Table 3).

Antibacterial assay

The diethyl ether and ethyl acetate fractions of the peel of S. incanum exhibited antibacterial activity against E. coli, K. pneumonia, S. pyogenes, and S. aureus. The results were shown in Table 4. The results indicated that the antibacterial activity was specific in action against the growth of bacterial species.

The antibacterial activity of diethyl ether fraction and ethyl acetate fraction was analyzed by in vitro disk diffusion method using paper discs (Indhumathi and Mohandass 2014; Elisha et al. 2017). Figures 6 7 and 8 summarize the results. The diethyl ether fraction exhibited higher activity against E. coli at 1000 μg/ml whereas the ethyl acetate fraction showed higher activity against K. pneumonia at 1000 μg/ml. Further, the extracts were found to show greater activity against E. coli, K. pneumonia.

Conclusion

In the present study 105 and 75 chemical constituents have been identified from diethyl ether and ethyl acetate extracts, respectively by GC–MS analysis. The GC–MS analysis of these extracts revealed various constituents; 2, 3-butanediol (76.76%), [R-(R*,R*)]-2,3-butanediol (51.02%), 2,3-butanediol (51.02%), 2,3-butanediol (22.65%) diethyl Phthalate (8.32%), 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene (3.17%), benzyl benzoate (3.02%), 2, 6-dimethyl-6-nitro-2-hepten-4-one (2.56%), 1, 2-dimethoxy-4-(1-propenyl)-benzene (1.88%), and trans-linalool oxide (furanoid) (1.64%) and were considered as major compounds on the basis of percentage peak area. The mass spectral pattern of 5 compounds was analyzed and confirmed with NIST database. The antibacterial activity of the extracts may be a result of the major compounds.
Table 2  Constituents of ethyl acetate fraction of the peel of *S. incanum*

| Peak# | R.Time | Area     | Area% | Name of compounds                                                      |
|-------|--------|----------|-------|-----------------------------------------------------------------------|
| 1     | 4.533  | 27,132,087 | 51.02 | [R-(R*,R*)]-2,3-Butanediol                                             |
| 2     | 4.686  | 12,044,321 | 22.65 | 2,3-Butanediol                                                        |
| 3     | 5.560  | 283,025    | 0.53  | 4-hydroxy-4-methyl-2-Pentanone                                         |
| 4     | 8.642  | 71,867     | 0.14  | 4-hydroxy-Butanoic acid                                                |
| 5     | 10.308 | 484,574    | 0.91  | 2,3-dimethoxy-1,4-Dioxane                                              |
| 6     | 10.790 | 704,676    | 1.33  | 12-Crown-4                                                            |
| 7     | 16.641 | 31,559     | 0.06  | 3-(2-Methoxyethoxymethoxy)-2-methylpentan-1-ol                         |
| 8     | 16.879 | 94,782     | 0.18  | 1-(bicyclo[3.2.1]oct-2-en-4-yl)-4-phenyl-1,2,4-Triazolidine-3,5-dione |
| 9     | 20.218 | 53,521     | 0.10  | Thymine                                                               |
| 10    | 22.620 | 370,827    | 0.70  | Formic acid, 1-methylpropyl ester                                      |
| 11    | 22.909 | 535,671    | 1.01  | 1-Butaneboronic acid                                                  |
| 12    | 23.267 | 22,508     | 0.04  | 3-Methyl-2-butoenoic acid, cyclobutyl ester                            |
| 13    | 23.454 | 114,612    | 0.22  | 1-phenyl-2-Propen-1-one                                               |
| 14    | 23.775 | 29,808     | 0.06  | Dehydromevalonic lactone                                               |
| 15    | 25.650 | 44,968     | 0.08  | Di-n-hexyl-diselenide                                                  |
| 16    | 25.873 | 118,206    | 0.22  | 2,3-dihydro-Thiophene                                                  |
| 17    | 26.767 | 51,548     | 0.10  | 2,3-dihydro-Thiophene                                                  |
| 18    | 27.183 | 85,660     | 0.16  | 2,3-dihydro- Benzofuran                                                |
| 19    | 28.577 | 64,558     | 0.12  | 2-isocyanato-2-methyl- Propane                                         |
| 20    | 30.261 | 38,520     | 0.07  | Indole                                                                |
| 21    | 30.483 | 80,397     | 0.15  | 2-Oxopentanedioic acid                                                |
| 22    | 31.257 | 50,200     | 0.09  | 2-Methoxy-4-vinylphenol                                               |
| 23    | 32.352 | 39,405     | 0.07  | Methyl anthranilate                                                   |
| 24    | 32.608 | 27,865     | 0.05  | 3-ethyl-3-methyl-Pentane                                              |
| 25    | 33.471 | 126,691    | 0.24  | (2-methylpropyl)- Oxirane                                             |
| 26    | 33.947 | 69,361     | 0.13  | Methyl 2-oxo-1-pyrrolidine acetate                                     |
| 27    | 34.857 | 872,593    | 1.64  | Trans-Linalool oxide (furanoid)                                       |
| 28    | 37.121 | 68,054     | 0.13  | 1-(bromomethyl)-3-nitro-Benzene                                       |
| 29    | 38.353 | 47,612     | 0.09  | 1,3-Dioxane, 2,4-dimethyl-                                             |
| 30    | 38.832 | 29,411     | 0.06  | 5-(pentyloxy)-(E)- 2-Pentiene                                          |
| 31    | 39.218 | 228,778    | 0.43  | Methyleugenol                                                         |
| 32    | 39.862 | 115,752    | 0.22  | Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters                |
| 33    | 40.027 | 38,043     | 0.07  | 2-Phenoxyethyl isobutyrate                                            |
| 34    | 40.200 | 97,274     | 0.18  | Cyclobutanecarboxylic acid, 2-methoxyethyl ester                      |
| 35    | 40.664 | 122,641    | 0.23  | Cyclobutanecarboxylic acid, 2-methoxyethyl ester                      |
| 36    | 41.346 | 52,283     | 0.10  | 4-pentyl-Phenol                                                       |
| 37    | 41.583 | 13,536     | 0.03  | 4-Fluorobenzoic acid, tridec-2-ynyl ester                             |
| 38    | 41.770 | 47,806     | 0.09  | 4'-Ethoxy-2'-hydroxyoctadecanophenone                                  |
| 39    | 41.922 | 61,347     | 0.12  | 3-Hexen-1-ol benzoate                                                |
| 40    | 42.039 | 43,992     | 0.08  | 2-bromo-Hexane                                                        |
| 41    | 42.500 | 38,691     | 0.07  | 3,3-Diethoxy-1-propyne                                               |
| 42    | 42.944 | 440,841    | 0.83  | Didodecyl phthalate                                                  |
| 43    | 44.073 | 238,753    | 0.45  | 2-methyl-Bicyclo[3.3.1]nanonan-2-ol                                   |
| 44    | 44.162 | 353,788    | 0.67  | 9-(3,3-Dimethylxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol             |
| 45    | 44.401 | 550,694    | 1.04  | 9-(3,3-Dimethylxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol             |
| 46    | 45.169 | 276,838    | 0.52  | Cyclopentaneacetie acid, 3-oxo-2-pentyl-, methyl ester                |
| 47    | 45.417 | 51,473     | 0.10  | 1-O-acetyl-4-O-p-toluenesulfonyl-2-Deoxythreitol                      |
| 48    | 45.628 | 62,642     | 0.12  | 2,6-dimethyl-1,7-Octadiene-3,6-diol                                   |
| 49    | 48.339 | 65,432     | 0.12  | 2-ethyl-2,4,5-trimethyl-1,3-Dioxolane                                 |
| 50    | 48.810 | 437,395    | 0.82  | Benzyl Benzoate                                                      |
The GC–MS analysis of the diethylether and ethylacetate fraction of the peel of Solanum incanum…

### Table 2 (continued)

| Peak# | R.Time | Area   | Area% | Name of compounds                                      |
|-------|--------|--------|-------|--------------------------------------------------------|
| 51    | 49.465 | 663,822| 1.25  | Endo-1,5,6,7-Tetramethylbicyclo[3.2.0]hept-6-en-3-ol    |
| 52    | 49.879 | 1,685,648| 3.17  | 5,5,8a-Trimethyl-3,5,6,7,8a-hexahydro-2H-chromene      |
| 53    | 50.154 | 576,294| 1.08  | 1-Nonadecene                                           |
| 54    | 50.283 | 143,226| 0.27  | (-)-Nortrachelogenin                                   |
| 55    | 50.965 | 77,154 | 0.15  | 3,7,11-trimethyl-, (Z,E)- 1,3,6,10-Dodecatriene        |
| 56    | 51.468 | 182,249| 0.34  | 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- 2-Butenal |
| 57    | 51.597 | 205,823| 0.39  | 3-Cyclopropylpropionic acid, 2-isopropoxyphenyl ester  |
| 58    | 52.148 | 138,020| 0.26  | 1-(p-Toluidino)-1-deoxy-.beta.-d-idopyranose           |
| 59    | 52.267 | 75,943 | 0.14  | Monobenzone                                            |
| 60    | 52.526 | 63,889 | 0.12  | Phthalic acid, cyclobutyl tridecyl ester               |
| 61    | 53.127 | 26,495 | 0.05  | 5-ethyl-2,4-dimethyl-4-Hepten-3-one                    |
| 62    | 53.585 | 420,244| 0.79  | 2-propyl-2-Heptenal                                    |
| 63    | 54.575 | 122,605| 0.23  | Tridecanoic acid, methyl ester                         |
| 64    | 55.206 | 93,530 | 0.18  | 1-bromo-2-methyl- Butane                               |
| 65    | 55.544 | 92,156 | 0.17  | Phthalic acid, cyclobutyl heptyl ester                 |
| 66    | 56.685 | 468,296| 0.88  | 1-Nonadecene                                           |
| 67    | 56.933 | 37,156 | 0.07  | Tert-Butyl cyclopropylmethyl sulfoxide                 |
| 68    | 57.660 | 51,367 | 0.10  | 2,6-dimethyl-1,5-Heptadiene                            |
| 69    | 58.037 | 57,807 | 0.11  | 2-Oxa-3-methylbicyclo(3.3.0)octane                    |
| 70    | 59.880 | 129,863| 0.24  | 8,11,14-Docosatrienoic acid, methyl ester             |
| 71    | 60.229 | 145,782| 0.27  | Phytol                                                |
| 72    | 62.663 | 269,407| 0.51  | 1-Docosene                                             |
| 73    | 63.415 | 145,519| 0.27  | Phytol, acetate                                        |
| 74    | 64.971 | 97,166 | 0.18  | (E)- 5-Eicosene                                        |
| 75    | 68.602 | 80,146 | 0.15  | 11-Tricosene                                           |
|       |        | 53,176,493| 100.00|                                                        |

**Fig. 4** Mass spectrum and fragmentation of 5, 5, 8a-trimethyl-3, 5, 6, 7, 8a-hexahydro-2H-chromene
**Scheme 4** Mass fragmentation of 5, 5, 8a-trimethyl-3, 5, 6, 7, 8,8a-hexahydro-2H-chromene

![Scheme 4](image)

**Fig. 5** Mass spectrum and fragmentation of 1-docosene

![Mass spectrum and fragmentation of 1-docosene](image)

**Scheme 5** Mass fragmentation of 1-docosene

![Scheme 5](image)
The GC–MS analysis of the diethylether and ethylacetate fraction of the peel of *Solanum incanum*...

Table 3  The major active principles identified from *S. incanum*(peel)

| Fraction     | Major Compound                               | Structure | Mol. formula | Mol. wt  | Reported biological activities                  |
|--------------|----------------------------------------------|-----------|--------------|----------|--------------------------------------------------|
| Diethyl ether| 8-Nonenoic acid                               | C_9H_{16}O_2 | 156.225      | Streptomyces & antimicrobial                      |
|              | 3, 7, 11-trimethyl-1, 6, 10-Dodecatrien-3-ol | C_{11}H_{20}O | 222.3663     | Antibacterial and Anti-inflammatory               |
|              | Acetoxyacetic acid, tridec-2-yynyl ester     | C_{15}H_{24}O_4 | 296.4018   | No activity report                               |
|              | o-Toluic acid, 6-ethyl-3-octyl ester         | C_{16}H_{26}O_2 | 276         | No activity report                               |
|              | 3-Butylindolizidine                           | C_{12}H_{22}N | 181.323      | Anti-inflammatory and antioxidant                 |
|              | Fumaric acid, ethyl 2-methylallyl ester      | C_{10}H_{14}O_4 | 198.218     | Antibacterial                                    |
|              | Butylated Hydroxytoluene                     | C_{15}H_{24}O | 220.356      | Insecticidal, insect antifeedant, anti-tumor, anti-inflammatory, antioxidant, antibacterial, and fungicidal properties |
| Ethyl acetate| [R-(R*, R*)]-2, 3-Butanediol                  | C_{4}H_{10}O_2 | 90.121       | Pesticides                                       |
|              | 5, 5, 8a-Trimethyl-3, 5, 6, 7, 8,8a-hexahydro-2H-chromene | C_{12}H_{20}O | 180.291      | Antimicrobial and treatment of respiratory tract disorders |
|              | Trans-Linalool oxide (furanoid)              | C_{10}H_{18}O_2 | 170.2487    | Antimicrobial                                    |

Source Dr. Duke’s phytochemical and ethnobotanical databases

Table 4  Antibacterial activities of *S. incanum* peel at different concentration

| Fractions (µg/ml) | *E. coli* (mm) | *K. pneumonia* (mm) | *S. aureus* (mm) | *S. pyogenes* (mm) |
|-------------------|----------------|---------------------|------------------|-------------------|
| DDE: 250          | –              | –                   | –                | –                 |
| 500               | –              | –                   | –                | –                 |
| 750               | 11.5 ± 0.5     | 10.5 ± 0.5          | 9.5 ± 0.4        | 9.3 ± 0.58        |
| 1000              | 21.5 ± 0.5     | 18.3 ± 0.14         | 17.3 ± 0.14      | 16.3 ± 0.14       |
| Gentamicin: 10    | 18.5 ± 0.5     | 18.7 ± 0.3          | 17.5 ± 0.4       | 14.5 ± 0.5        |
| EAE: 250          | –              | –                   | –                | –                 |
| 500               | 7.5 ± 0.5      | 7.3 ± 0.14          | 6.3 ± 0.14       | –                 |
| 750               | 7.6 ± 0.6      | 12.7 ± 0.3          | 7.3 ± 0.58       | 6.7 ± 0.3         |
| 1000              | 16.3 ± 0.58    | 20.7 ± 0.3          | 15.7 ± 0.3       | 13.5 ± 0.5        |
| Gentamicin: 10    | 17.3 ± 0.14    | 20.5 ± 0.4          | 16.5 ± 0.5       | 13.3 ± 0.14       |

10% DMSO had not an effect on the bacterial growth
Fig. 6  Zones of inhibition in millimeter at 1000 µg/ml of each fraction of *S. incanum* peel

Fig. 7  Zones of inhibition in millimeter at 750 µg/ml of each fraction of *S. incanum* Peel

Fig. 8  Zones of inhibition in millimeter at 500 µg/ml of EAC of *S. incanum* peel
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Code availability Chemdraw was used for drawing the Chemical structures.

Declarations

Ethical statement This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

Conflict of interest Yesudass Dominic Ravichandran has no conflict of interest. Mequanint Yetayih has no conflict of interest.

References

Abebe H, Gebre T, Haile A (2014) Phytochemical investigation of the roots of Solanum incanum, Hadiaya Zone, Ethiopia. J Med Plants Stud 2(2):83–93

Alghamdi SSK(2013) Topical depilatory and method of removing hair. Patent No. 8,551,187

Asaolu MF (2003) Chemical composition and phytochemical screening of the seeds of Garcinia kola. Pak J Sci Ind Res 46(3):145–147

Auta R, Ali I (2011) Nutritional and chemical value of Solanum incanum (L) (bitter garden egg). Int J Tropical Med Pub Health 1(1):96–107

Abdalla IMF (2015) Leaves value of Solanum incanum (L) at Khartoum, North Sudan. Int J Eng Sci Innov Technol 4(1):25–28

Asaolu MF, Olayinka AO, Adesanya OA (2014) Phytochemical composition and anti-bacterial activity of the leaf extracts of Solanum incanum var. minus. J Toxicol 2014:1–6. https://doi.org/10.1155/2014/311368

Ashokkumar B, Suresh Babu K (2014) Phytochemical screening and antioxidant activity of Solanum incanum L. fruit extract for its antimicrobial activity. Int J Phytomed. 2014. 1440. 1444

Essien EE, Walker MT, Newby MJ, Oguneawde AI, Setzer NW, Ekunday O (2016) The floral essential oil composition and biological activity of Solanum Macranthum Dunal. Am J Essent Oil Nat Prod 4(1):36–39

Hassim N, Markom M, Anuar N, Baharum SN (2014) Solvent selection in extraction of oil and bioactive compounds from Polygonum minus. J Appl Sci 14:1440–1444. https://doi.org/10.3923/jas.2014.1440.1444

Indhumathi T, Mohandass S (2014) Efficacy of ethanolic extract of Solanum incanum fruit extract for its antimicrobial activity. Int J Curr Microbiol Appl Sci 3(6):939–949

Khalil AA, Rahman U, Khan RM, Sahar A, Mehmood T, Khana M (2017) Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives. RSC Adv 7(52):32669–32681. https://doi.org/10.1039/C7RA04803C

Okhale SE, Ugbabe GE, Oladosu PO, Ibahram JA, Eghevrebva HO, Kunle OF, Elisha EP, Chibuike AJ, Etta UO (2018) Chemical constituents and antimicrobial activity of the leaf essential oil of IXora coccinea L (Rubiaceae) collected from North Central Nigeria. Int J Bioassays 7(4):5630–5637

Ravichandran YD, Sulochana N (2006a) 1H and 13C assignment of Parsilinone from Passiflora edulis. Asian J Chem 18(4):3092–3096

Ravichandran YD, Sulochana N (2006b) Isolation and characterization of 8-methyluteolin 5-O-6¢¢-(2-methyl propanoyl) glucoside and patuletin 3-O-glucuronide from Tithonia taggefillia. Asian J Chem 18(4):3173–3175

Sheeba E (2010) Antibacterial activity of Solanum surattense burm. F. KUISET 61(1):1–4. https://doi.org/10.3126/kuset.v61i1.3278

Sundar S, Pillai YJK (2015) Phytochemical screening and gas chromatograph-mass spectrometer profiling in the leaves of Solanum incanum. Asian J Pharm Clin Res 8(3):179–188

Sakha H, Hora R, Shrestha S, Acharya S, Dhakal D, Thapaliya S, Sundar S, Pillai YJK (2015) Phytochemical screening and gas chromatograph-mass spectrometer profiling in the leaves of Solanum incanum. Asian J Pharm Clin Res 8(3):179–188

Tesfaye T, Ravichandran YD (2018) Traditional uses, pharmacological action and phytochemical action of Carrissa carandas Linn. Nat Prod Chem Res 6:334. https://doi.org/10.4172/2329-6836.1000334

Tamrat T, Ravichandran YD (2018) A review on anticancer activity of some plant-derived compounds and their mode of action. Nat Prod Chem Res 6:330. https://doi.org/10.4172/2329-6836.1000330

Yetayih MM, Ravichandran YD (2020) Extraction and GC-MS analysis of the essential oil from the peel of Solanum incanum and its antibacterial activity studies. Asian J Chem 32(8):2001–2006. https://doi.org/10.14233/ajchem.2020.22770

Yrjönen T (2004) Extraction and planar chromatographic separation techniques in the analysis of natural products, University of Helsinki, Helsinki, Finland, Doctoral dissertation

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