The GCMS Analysis of the Diethylether and Ethylacetate Fraction of the Peel of Solanum incanum and the Study of Their Antibacterial Activity

Yesudass Dominic Ravichandran (✉ ydominic64@yahoo.co.in)

Wollega University <https://orcid.org/0000-0002-7372-0389>

Molla Mequanint Yetayih

Wollega University

Original Article

Keywords: Gas Chromatography-mass Spectrometry, Antibacterial Screening, E. coli, K. pneumonia

Posted Date: January 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-163382/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

1. Introduction

Medicinal plants are of great importance to the health of human beings and animals. The medicinal value of plants lies in the phytochemicals that produce a definite physiological action on the human body. Some of these bioactive phytochemicals include alkaloids, tannins, flavonoids and phenolic compounds. Investigation of the phytochemical constituents and relating them to the pharmacological activities furnish a plethora of information. In this line our group has also reported the phytochemicals and their uses [1-4]. Many of the indigenous medicinal plants are used as spices and food plants [5]

Solanum L. (*Solanaceae*) is large and diverse genus of flowering plants. It has several species known for their medicinal importance [6]. *Solanum incanum* L (*S. incanum*). is one of the species with multiple traditional applications in many Ethiopian communities. The species is native to and widely distributed in Ethiopia. It has thorny leaves, yellow fruits, and blue flowers with yellow pistils [7]. *S. incanum* is commonly seen around houses, overgrazed grasslands, wastelands, and roadsides [8].

Fruits of *S. incanum* are found to be the source of several groups of phytochemicals, including terpenoids, flavonoids, xanthenes, and multiple metabolites like tannins, saponins, cyanates, oxalate, and anthraquinones [9]. Further, steroid glycosides in the form of glycoalkaloids such as solanine and solasonine were also reported [10]. Likewise, the leaves of *S. incanum* L. are rich in minerals such as K [11] and Ca [12]

In recent times, GC-MS analysis is increasingly applied for the analysis of medicinal plants [13]. The GC-MS analysis of the leaf extract [14] and the methanolic extract of the fruits of *S. incanum* from India has been reported [15]. We have earlier reported the GC-MS analysis of the essential oil derived from the peel of the fruit from *S. incanum* [16] but there is no report on the GC-MS analysis of the diethylether and ethylacetate 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 ethylacetate fraction of the peel of the *S. incanum*

2. Experimental

2.1. Survey, collection and preparation of plant material

The Fruits of *S. incanum*(5kg) were collected on May 22, 2018, identified by Dr. Tena Regasa(botanist), Department of Biology. The collected ripe fruits were washed with water thoroughly to free from debris, and incisions were made on the ripe fruits with the sterilized scalpel blade and the internal viscous fluid was squeezed out. The fruit peel was cut into pieces and dried at room temperature for 20 days till it acquired a dry constant weight. The dried peel were grounded finely by using a laboratory mill and passed through a sieve and stored for further use.

2.2. Preparation of the Extract

A sample of 1.5k.g from the powdered *S. incanum* peel was dissolved in 7-litre of 70% methanol solution [17 ,18]. The solution was kept for 10 days with random stirring and the extract was filtered by cotton plugged filtration followed by whatmann No 1 filter paper. The extraction was repeated twice for better quantity with the same amount of solvent and filtered again. The 70% methanolic extract was finally evaporated to dryness in vacuum using Rota-vapor at 60°C to yield methanolic extract. The obtained methanolic extract was used for further portioned into different fractions based on polarity.

2.3. Liquid-liquid Extraction

Liquid-liquid extraction was done by organic solvents as indicated in the literature with slight modifications [18]. The methanolic crude extract was transferred to a separatory funnel and successively extracted with organic solvents i.e. hexane, diethyl ether and ethyl acetate, respectively. The extracts in each solvent was done successively till the added solvent is colorless. The different extracts obtained were dehydrated over by an anhydrous sodium sulphate. Different fractions of each extract were combined together, and concentrated by rotary evaporator under reduced pressure to give hexane, diethyl ether, and ethyl acetate fractions. The diethyl ether and ethyl acetate fractions were considered for further work.

2.4. Gas Chromatography–Mass Spectrometry (GC-MS) analyses

To determine the chemical composition of the most active plant extracts, gas chromatography–mass spectrometry (GC-MS) was used. One μL of solvent containing the plant extract was injected into the GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70eV), 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 [19]. The GC column was an HP-5ms fused silica capillary with a (5% phenyl) polymethylsiloxane stationary phase, film thickness of 0.25 μm , a length of 30m, and an internal diameter of 0.25mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0mL/min. 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. Identification of components was achieved based on their retention time (RT), and by comparison of their mass spectral fragmentation patterns with those reported values in the literature [20] and stored in the MS library [NIST database (G1036A, revision D.01.00)/Chem Station data system (G1701CA, version C.00.01.08)].

2.5. Antibacterial Activity

Four pathogenic bacterial strains were used in this study for assessing the antibacterial activity of *S. incanum* peel extracts, including Gram-positive, *S. aureus* (ATCC 31488) and *S. pyogenes* (ATCC 27853), and Gram-negative bacteria, *E. coli* (ATCC 25922) and *K. pneumonia* (ATCC 27853). The antibacterial activity of plant extracts was carried out using the disk diffusion method [21]. Each test bacterium was inoculated on Mueller Hinton broth, homogenized and swamping on a sterilized Petri dish to yield a uniform depth. The diethyl ether fraction and ethyl acetate fraction were dissolved in 10% of dimethyl sulfoxide (DMSO). The concentration and volume of the extracts used for the analysis of antibacterial activity were, 250, 500, 750 and 1000 $\mu\text{g/mL}$ (extract soaked by each disc) respectively. The antibacterial activity was assessed by measuring the zone of inhibition surrounding the disks and each experiment was carried out in triplicate. In the present study, DMSO (10%) and Gentamicin (10 $\mu\text{g/disc}$) were placed at each

petri dish and served as negative and positive control in a separate petri dish. The petri dishes were then incubated at 37°C for 24 hours in an incubator. The plates were then observed for zones of inhibition. The antibacterial potential was evaluated by measuring the diameters of zones of inhibition in millimeters (mm).

3. Results and Discussion

3.1. The Diethyl Ether Fraction

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

Table 1. Constituents of diethyl ether fraction of the peel of *S. incanum*

Peak#	RT	Area	Area%	Name compounds
1	4.142	6464154	1.27	2,3-Butanediol
2	4.321	11966968	2.35	[R-(R*,R*)]-2,3-Butanediol
3	14.198	254929	0.05	2-pentyl-Furan
4	16.899	4962483	0.97	Benzyl alcohol
5	17.747	92527	0.02	1-ethyl-1-methyl-Cyclopentane
6	18.042	167251	0.03	2-Methylbutanoic anhydride
7	18.326	350838	0.07	Butan-2-one, 4-[pyrrolidin-2-one-5-yl]-
8	19.074	212311	0.04	7-Octen-2-ol, 2-methyl-6-methylene-
9	20.017	107019	0.02	Mequinol
10	20.532	134141	0.03	5-Methyl-1-heptanol
11	20.830	533001	0.10	3,7-dimethyl-1,6-Octadien-3-ol
12	21.045	420417	0.08	5-Nonen-2-one
13	21.316	596171	0.12	Phenylethyl Alcohol
14	22.587	172497	0.03	7-Bromo-1-heptanol, trimethylsilyl ether

15	24.204	263407	0.05	Acetic acid, phenylmethyl ester
16	24.569	138830	0.03	2-ethyl-Phenol
17	24.898	217662	0.04	Benzeneacetic acid, methyl ester
18	25.398	290521	0.06	L-.alpha-Terpineol
19	25.725	1192480	0.23	Decanal
20	26.608	7076080	1.39	Catechol
21	26.959	2346275	0.46	Benzoic acid
22	27.269	8743987	1.72	2,3-dihydro- Benzofuran
23	29.964	6106067	1.20	Benzeneacetic acid
24	30.282	423731	0.08	Indole
25	30.541	363009	0.07	Nonanoic acid
26	30.974	993622	0.20	2-t-Butyl-5-propyl-[1,3]dioxolan-4-one
27	31.298	1647954	0.32	2-Methoxy-4-vinylphenol
28	31.508	158721	0.03	Isomyrcenyl acetate
29	31.871	1000593	0.20	2-Decen-1-ol
30	32.308	289326	0.06	2-t-Butyl-5-propyl-[1,3]dioxolan-4-one
31	32.855	2765290	0.54	2,5-Dimethylcyclohexanol
32	33.082	3208997	0.63	(S)-2,7-Octadien-4-ol, 2-methyl-6-methylene
33	33.448	1512440	0.30	dihydro-5-pentyl-2(3H)-Furanone
34	33.612	7675007	1.51	dihydro-5-pentyl-2(3H)-Furanone
35	34.084	2795101	0.55	1-methoxy-2,2-dimethyl-3-(3,3-dimethyl-1-propynyl)- Cyclopropane
36	34.202	5229121	1.03	3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol
37	34.390	9749572	1.91	trans-5,6-Epoxydecane
38	34.994	2369512	0.47	2-Isopropylidene-5-methylhex-4-enal
39	35.659	1069366	0.21	4-Ethylthiane
40	36.075	270006	0.05	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin
41	36.308	514672	0.10	4-Dimethylsilyloxypentadecane

42	36.742	2637720	0.52	15-(2,2-dimethylcyclopropyl)-2-methyl-4-methylene-Pentene
43	37.383	14571016	2.86	o-Toluic acid, 6-ethyl-3-octyl ester
44	37.809	2594865	0.51	2,6-dimethyl-7-Octene-2,6-diol
45	38.242	1334860	0.26	2-Dodecen-1-yl(-)succinic anhydride
46	38.654	2164695	0.42	5-Hepten-3-yn-2-ol, 6-methyl-5-(1-methylethyl)-
47	39.288	2497131	0.49	dl-Mevalonic acid lactone
48	39.505	9958474	1.95	Nonanoic acid, 9-hydroxy-, methyl ester
49	39.700	2451540	0.48	5-(2-tetrahydrofurfuryl)-Heptan-2-ol
50	39.805	11623593	2.28	Butylated Hydroxytoluene
51	40.108	930279	0.18	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-(R)- 2(4H)-Benzofuranone
52	40.603	3614576	0.71	Nerolidolisobutyrate
53	41.402	9336554	1.83	Nonanedioic acid, dimethyl ester
54	42.356	11945857	2.34	Fumaric acid, ethyl 2-methylallyl ester
55	43.721	76729146	15.06	8-Nonenoic acid
56	43.975	6284027	1.23	Benzoic acid, 4-hydroxy-3-methoxy
57	44.332	20089247	3.94	3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol
58	44.457	4044024	0.79	Nonanedioic acid, monomethyl ester
59	44.615	10543547	2.07	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol
60	45.608	4084381	0.80	2,6-dimethyl-3,7-Octadiene-2,6-diol
61	45.753	3452490	0.68	3,5,5-trimethyl-4-(3-oxobutyl)- 2-Cyclohexen-1-one
62	46.153	4566263	0.90	Ethyl homovanillate
63	46.983	23082760	4.53	8-Nonenoic acid
64	47.067	4535308	0.89	4-(3-hydroxybutyl)-3,5,5-trimethyl-2-Cyclohexen-1-one
65	47.866	2382404	0.47	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol

66	48.355	2278911	0.45	Acetoxyacetic acid, tridec-2-ynyl ester
67	48.846	5431308	1.07	Benzyl Benzoate
68	49.068	4081760	0.80	Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl este
69	49.201	1318247	0.26	1,8-epoxy-2,6,6,9-tetramethyl Tricyclo[6.3.0.0(5,7)]undecane
70	49.360	2583465	0.51	2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin
71	50.074	15335267	3.01	Acetoxyacetic acid, tridec-2-ynyl ester
72	50.522	13441969	2.64	3-Butylindolizidine
73	50.639	2930674	0.58	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-.alpha.,.alpha.,4a,8-tetramethyl
74	50.920	1603293	0.31	Isophytol, acetate
75	51.051	3377676	0.66	4,5-dimethyl-2-pentadecyl-1,3-Dioxolane
76	51.927	5260540	1.03	2,5,5,8a-Tetramethyl-4-methylene-4a,5,6,7,8,8a-hexahydro-4H-chromene
77	52.369	6570511	1.29	5-methyl-2-(1-methylethyl)- 2-Cyclohexen-1-one
78	52.610	2487193	0.49	Cyclopropanepropionic acid, 2-[(2-decylcyclopropyl)methyl]-, methyl ester
79	53.080	5307267	1.04	Nonanoic acid, 4-benzyloxyphenyl ester
80	53.202	2771060	0.54	Erucic acid
81	53.899	4496459	0.88	2,6-Dimethyltridecanenitrile
82	54.606	3300316	0.65	Hexadecanoic acid, methyl ester
83	55.208	5049225	0.99	2(1H)-Naphthalenone, 3,4,4a,5,6,7,8,8a.alpha.-octahydro-5.alpha.-hydroxy-4a
84	55.947	7860390	1.54	n-Hexadecanoic acid
85	56.703	7245242	1.42	6-Benzyloxy-2,6-dimethyl-octa-2,7-dien-1-ol
86	57.675	2862371	0.56	[[[(3,7-dimethyl-2,6-octadienyl)oxy]methyl]-, (E)-Benzene
87	58.075	2582193	0.51	sesquicineole
88	58.786	9061356	1.78	2-Dodecen-1-yl(-)succinic anhydride

89	59.207	1424986	0.28	3,7,11,15-tetramethyl-, acetate, (E,E,E)- 2,6,10,14-Hexadecatetraen-1-ol
90	59.742	4040882	0.79	9,12-Octadecadienoic acid, methyl ester
91	59.944	7303869	1.43	11-Octadecenoic acid, methyl ester
92	61.001	9342907	1.83	(Z,Z)-9,12-Octadecadienoic acid
93	61.889	8608746	1.69	Butyl 6,9,12-hexadecatrienoate
94	62.643	3703501	0.73	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol
95	63.300	3126546	0.61	decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.1H-Cycloprop[e]azulen-4-ol
96	64.142	4011265	0.79	2-Dodecen-1-yl(-)succinic anhydride
97	64.470	1424960	0.28	Nerolidolisobutyrate
98	64.675	3800804	0.75	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate
99	65.088	1494317	0.29	2-ethyl-6-methyl- Pyridine
100	65.341	6273408	1.23	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
101	66.946	2105734	0.41	Nerolidolisobutyrate
102	67.537	1109923	0.22	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol
103	67.985	1108356	0.22	1-Heptatriacotanol
104	68.808	2400883	0.47	Hexanedioic acid, bis(2-ethylhexyl) ester
105	70.275	692075	0.14	3,5-dimethyl- Cyclohexanol
		2103564	100.00	

The principal compounds identified in the diethyl ether fraction were 8-nonenoic acid (19.59%), 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (3.94%), 2,3-butanediol (3.62%), acetoxyacetic acid, tridec-2-ynyl ester (3.46%), o-toluic acid, 6-ethyl-3-octyl ester (2.86%), 3-butylinolizidine (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)

Figure 1. Mass spectrum and fragmentation of Benzyl alcohol

The empirical formula C_7H_8O was derived using the nitrogen rule and rule of thirteen. The absence of $M+2$ 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.

Scheme 1. Mass fragmentation of Benzyl alcohol

2, 3-dihydro-Benzofuran(MW120)

Figure 2. Mass spectrum and fragmentation of 2, 3-dihydro-Benzofuran

The empirical formula of C_8H_8O was derived using the nitrogen rule and rule of thirteen. The absence of $M+2$ 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) confirmed with the presence of 2, 3-dihydro-Benzofuran.

Scheme 2. Mass fragmentation of 2, 3-dihydro-Benzofuran

Butylated Hydroxytoluene(MW 220)

Figure 3. Mass spectrum and fragmentation of Butylated Hydroxytoluene

According to the nitrogen rule and rule of thirteen the derived empirical formula was $C_{15}H_{24}O$. The absence of $M+2$ 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.

Scheme 3. Mass fragmentation of Butylated Hydroxytoluene

3.2. Ethyl Acetate Fraction of *S. incanum* Peel

The GC-MS analysis of ethyl acetate fraction confirmed with the presence of 75 components (Table 2). The components were identified by representing 100% of the total sample composition.

Table 2. Constituents of ethyl acetate fraction of the peel of *S. incanum*

Peak#	R.Time	Area	Area%	Name of compounds
1	4.533	27132087	51.02	[R-(R*,R*)]-2,3-Butanediol
2	4.686	12044321	22.65	2,3-Butanediol

3	5.560	283025	0.53	4-hydroxy-4-methyl-2-Pentanone
4	8.642	71867	0.14	4-hydroxy-Butanoic acid
5	10.308	484574	0.91	2,3-dimethoxy-1,4-Dioxane
6	10.790	704676	1.33	12-Crown-4
7	16.641	31559	0.06	3-(2-Methoxyethoxymethoxy)-2-methylpentan-1-ol
8	16.879	94782	0.18	1-(bicyclo[3.2.1]oct-2-en-4-yl)-4-phenyl-1,2,4-Triazolidine-3,5-dione
9	20.218	53521	0.10	Thymine
10	22.620	370827	0.70	Formic acid, 1-methylpropyl ester
11	22.909	535671	1.01	1-Butaneboronic acid
12	23.267	22508	0.04	3-Methyl-2-butenic acid, cyclobutyl ester
13	23.454	114612	0.22	1-phenyl-2-Propen-1-one
14	23.775	29808	0.06	Dehydromevalonic lactone
15	25.650	44968	0.08	Di-n-hexyl-diselenide
16	25.873	118206	0.22	2,3-dihydro-Thiophene
17	26.767	51548	0.10	2,3-dihydro-Thiophene
18	27.183	85660	0.16	2,3-dihydro- Benzofuran
19	28.577	64558	0.12	2-isocyanato-2-methyl- Propane
20	30.261	38520	0.07	Indole
21	30.483	80397	0.15	2-Oxopentanedioic acid
22	31.257	50200	0.09	2-Methoxy-4-vinylphenol
23	32.352	39405	0.07	Methyl anthranilate
24	32.608	27865	0.05	3-ethyl-3-methyl-Pentane
25	33.471	126691	0.24	(2-methylpropyl)- Oxirane
26	33.947	69361	0.13	Methyl 2-oxo-1-pyrrolidine acetate
27	34.857	872593	1.64	trans-Linalool oxide (furanoid)
28	37.121	68054	0.13	1-(bromomethyl)-3-nitro-Benzene
29	38.535	47612	0.09	1,3-Dioxane, 2,4-dimethyl-
30	38.832	29411	0.06	5-(pentyloxy)-(E)- 2-Pentene

31	39.218	228778	0.43	Methyleugenol
32	39.862	115752	0.22	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters
33	40.027	38043	0.07	2-Phenoxyethyl isobutyrate
34	40.200	97274	0.18	Cyclobutanecarboxylic acid, 2-methoxyethyl ester
35	40.664	122641	0.23	Cyclobutanecarboxylic acid, 2-methoxyethyl ester
36	41.346	52283	0.10	4-pentyl-Phenol
37	41.583	13536	0.03	4-Fluorobenzoic acid, tridec-2-ynyl ester
38	41.770	47806	0.09	4'-Ethoxy-2'-hydroxyoctadecanophenone
39	41.922	61347	0.12	3-Hexen-1-ol benzoate
40	42.039	43992	0.08	2-bromo-Hexane
41	42.500	38691	0.07	3,3-Diethoxy-1-propyne
42	42.944	440841	0.83	Didodecyl phthalate
43	44.073	238753	0.45	2-methyl-Bicyclo[3.3.1]nonan-2-ol
44	44.162	353788	0.67	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol
45	44.401	550694	1.04	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol
46	45.169	276838	0.52	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester
47	45.417	51473	0.10	1-O-acetyl-4-O-p-toluenesulfonyl-2-Deoxythreitol
48	45.628	62642	0.12	2,6-dimethyl-1,7-Octadiene-3,6-diol
49	48.339	65432	0.12	2-ethyl-2,4,5-trimethyl-1,3-Dioxolane
50	48.810	437395	0.82	Benzyl Benzoate
51	49.465	663822	1.25	endo-1,5,6,7-Tetramethylbicyclo[3.2.0]hept-6-en-3-ol
52	49.879	1685648	3.17	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene
53	50.154	576294	1.08	1-Nonadecene
54	50.283	143226	0.27	(-)-Nortrachelogenin

55	50.965	77154	0.15	3,7,11-trimethyl-, (Z,E)- 1,3,6,10-Dodecatetraene
56	51.468	182249	0.34	2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)- 2-Butenal
57	51.897	205823	0.39	3-Cyclopentylpropionic acid, 2-isopropoxyphenyl ester
58	52.148	138020	0.26	1-(p-Toluidino)-1-deoxy-.beta.-d-idopyranose
59	52.267	75943	0.14	Monobenzene
60	52.526	63889	0.12	Phthalic acid, cyclobutyl tridecyl ester
61	53.127	26495	0.05	5-ethyl-2,4-dimethyl-4-Hepten-3-one
62	53.585	420244	0.79	2-propyl-2-Heptenal
63	54.575	122605	0.23	Tridecanoic acid, methyl ester
64	55.206	93530	0.18	1-bromo-2-methyl- Butane
65	55.544	92156	0.17	Phthalic acid, cyclobutyl heptyl ester
66	56.685	468296	0.88	1-Nonadecene
67	56.933	37156	0.07	tert-Butyl cyclopropylmethyl sulfoxide
68	57.660	51367	0.10	2,6-dimethyl-1,5-Heptadiene
69	58.037	57807	0.11	2-Oxa-3-methylbicyclo(3.3.0)octane
70	59.880	129863	0.24	8,11,14-Docosatrienoic acid, methyl ester
71	60.229	145782	0.27	Phytol
72	62.663	269407	0.51	1-Docosene
73	63.415	145519	0.27	Phytol, acetate
74	64.971	97166	0.18	(E)- 5-Eicosene
75	68.602	80146	0.15	11-Tricosene
		53176493	100.00	

The ethyl acetate fraction revealed 75 constituents (Table 6), 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.

5, 5, 8a-trimethyl-3, 5, 6, 7, 8,8a-hexahydro-2H-chromene(MW 180)

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

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-2H-pyryl-2-ethyl radical cation. The presence of 1,1-dimethylcyclohexyl-2, 3-dimethylenyl cation(135), 3-methylene-penta-1,4-dienyl cation(79), 4-methylpent-1-enyl cation (69), 2-methylprop-1-enyl cation (55) confirmed the presence of 5, 5, 8a-trimethyl-3, 5, 6, 7, 8,8a-hexahydro-2H-chromene.

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

1-docosene(MW 125)

Figure 5. Mass spectrum and fragmentation of 1-docosene

The empirical formula of 1-docosene was derived as $C_{22}H_{44}$ using the nitrogen rule and rule of thirteen. The base peak at 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.

Scheme 5. Mass fragmentation of 1-docosene

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

Fraction	Major Compound	Structure	Mol. formula	Mol.wt	Reported biological activities
	8-Nonenoic acid		C ₉ H ₁₆ O ₂	156.225	Streptomyces & antimicrobial
	3, 7, 11-trimethyl-1, 6, 10-Dodecatrien-3-ol		C ₁₅ H ₂₆ O	222.3663	Antibacterial and Anti-inflammatory
	Acetoxyacetic acid, tridec-2-ynyl ester		C ₁₇ H ₂₈ O ₄	296.4018	No activity report
Diethyl ether	o-Toluic acid, 6-ethyl-3-octyl ester		C ₁₈ H ₂₈ O ₂	276	No activity report
	3-Butylindolizidine		C ₁₂ H ₂₃ 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

3.3. Antibacterial Assay

In this investigation the diethyl ether, and ethyl acetate fractions of the peel of *S. incanum* exhibit antibacterial activity against *E. coli*, *K. pneumonia*, *S. pyogenes*, and *S. aureus*. The antibacterial potential of each fraction was assessed in terms of zone of inhibition of bacterial growth. The triplicate results of the effects of this plant material on bacterial species were shown in Table 4. The results clearly showed that the plant material was specific in action against the growth of bacterial species.

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

Fractions($\mu\text{g/mL}$)	<i>E. coli</i> (mm)	<i>K. pneumonia</i> (mm)	<i>S. aureus</i> (mm)	<i>S. pyogenes</i> (mm)
DDE: 250	-	-	-	-
500	-	-	-	-
750	11.5 $\pm$ 0.5	10.5 $\pm$ 0.5	9.5 $\pm$ 0.4	9.3 $\pm$ 0.58
1000	21.5 $\pm$ 0.5	18.3 $\pm$ 0.14	17.3 $\pm$ 0.14	16.3 $\pm$ 0.14
Gentamicin: 10	18.5 $\pm$ 0.5	18.7 $\pm$ 0.3	17.5 $\pm$ 0.4	14.5 $\pm$ 0.5
EAE:250	-	-	-	-
500	7.5 $\pm$ 0.5	7.3 $\pm$ 0.14	6.3 $\pm$ 0.14	-
750	7.6 $\pm$ 0.6	12.7 $\pm$ 0.3	7.3 $\pm$ 0.58	6.7 $\pm$ 0.3
1000	16.3 $\pm$ 0.58	20.7 $\pm$ 0.3	15.7 $\pm$ 0.3	13.5 $\pm$ 0.5
Gentamicin: 10	17.3 $\pm$ 0.14	20.5 $\pm$ 0.4	16.5 $\pm$ 0.5	13.3 $\pm$ 0.14

10%DMSO had not an effect on the bacterial growth

Figure 6. Zones of inhibition in millimeter at 1000 $\mu\text{g/mL}$ of each fraction of *S. incanum* peel

Figure 7. Zones of inhibition in millimeter at 750 $\mu\text{g/mL}$ of each fraction of *S. incanum* Peel

Figure 8. Zones of inhibition in millimeter at 500 $\mu\text{g/mL}$ of EAC of *S. incanum* peel

The antibacterial activity of this plant was assayed by in vitro disk diffusion method using paper discs. Figure 6-8 summarize the bacterial growth inhibition of the sample of the plant material. The maximum antibacterial activity was shown by ethyl acetate extract than diethyl ether extract. The ethyl acetate extract showed greater activity against *E. coli*, *K. pneumonia* followed by *S. aureus*, respectively, and a minimum activity was shown against *S. pyogenes*. The investigated plant material showed maximum antibacterial activity against Gram-negative, *E. coli*, and *K. pneumonia* which are the main cause for many human diseases [22].

The cell wall of Gram-positive bacteria comprises of 70-100 layers of peptidoglycans. Peptidoglycan consists of two polysaccharides, N-acetylglucosamine and N-acetyl-muramic acid and cross-linked and cross-bridged by peptide side chains. This is just a simple explanation, but

other mechanisms may also play a role. The resistance exhibited by Gram-negative bacteria against antibiotics like penicillin may be attributed to the secretion of the lactamase enzyme in the periplasmic space between thin layer of the outer membrane and the cytoplasmic membrane [23].

4. 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 (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 as a result of the major compounds.

5. Acknowledgements

The authors thank the management of Wollega University and Ministry of Science and Higher Education, Ethiopia for providing required facilities to carry out this research work.

6. Conflict of interest:

The authors declare that there is no conflict of interests.

7. References

1. Tesfaye T, Ravichandran YD (2018), Traditional Uses, Pharmacological Action and Phytochemical analysis of *Carrissa carandas* Linn. Natural Product Chemistry and Research 6:334

2. Tamrat T, Ravichandran YD(2018), A Review on Anticancer Activity of Some Plant-Derived Compounds and their Mode of Action. *Natural Product Chemistry and Research* 6:330.
3. Ravichandran YD, Sulochana N, (2006)¹H and ¹³C assignment of Passiflorine from *Passiflora edulis*. *Asian Journal of Chemistry* 18 (4): 3092
4. Ravichandran YD, Sulochana N, (2006) Isolation and Characterization of 8-Methyluteolin 5-O-6''-(2-Methyl Propanoyl) Glucoside and Patuletin 3-O-Glucuronide from *Tithonia tagetiflora*. *Asian Journal of Chemistry* 18(4):3173
5. Dhakal D, Hora R, Shrestha S, Acharya S, Sakha H, Thapaliya S, & Prajapati K, (2018). Antimicrobial Activity of Ethanolic Extract of Medicinal Plants against Human Pathogenic Bacteria. *Tribhuvan University Journal of Microbiology*, 5(1): 1-6.
6. Sheeba E (2010) Antibacterial activity of *Solanum surattense* burm. F. Kathmandu University Journal of Science, Engineering and Technology 6(1):1–4, 2010.
7. Abebe H, Gebre T, Haile A(2014) Phytochemical investigation of the roots of *Solanum incanum*, Hadiaya Zone, Ethiopia. *Journal of Medicinal Plants Studies* 2(2): 83–93.
8. Yrjönen T(2004) Extraction and planar chromatographic separation techniques in the analysis of natural products, University of Helsinki, Helsinki, Finland, Doctoral dissertation.
9. Asaolu MF(2003) Chemical composition and phytochemical screening of the seeds of *Garcinia kola*. *Pakistan Journal of Science and Industrial Research* 46(3): 145–147.
10. Alghamdi SSK(2013) Topical depilatory and method of removing hair, Patent No. 8,551,187.

11. Auta R, Ali, I(2011) Nutritional and chemical value of *Solanum incanum* (L) (bitter garden egg). International Journal of Tropical Medicine and Public Health 1(1): 96–107.
12. Abdalla IMF(2015) Leaves value of *Solanum incanum* (L) at Khartoum, North Sudan. International Journal of Engineering Science and Innovative Technology 4(1): 25–28.
13. Asha RK, Priyanga S, Hemmalakshmi S, Devaki K, (2017). GC-MS Analysis of the Ethanolic Extract of the whole Plant *Drosera indica* L. International Journal of Pharmacognosy and Phytochemical Research 9(5): 685-688.
14. Sundar S, Pillai YJK (2015) Phytochemical screening and gas chromatograph-mass spectrometer profiling in the leaves of *Solanum incanum* L. Asian Journal of Pharmaceutical and Clinical Research 8(3):179-188
15. Buvaneshwari PJ, Jenifer SA, Ramya D, Sharmila C, Dhamayanthi C, Selvarajan S(2017) Preliminary Phytochemical Screening and GC-MS Analysis of *Solanum incanum* L. Fruits. EPRA International Journal of Research and Development 2(9)
16. 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 journal of Chemistry 32(8): 2001-2006.
17. Hassim N, Markom M, Anuar N, Baharum SN (2014). Solvent Selection in Extraction of Oil and Bioactive Compounds from *Polygonum minus*. Journal of Applied Sciences 14:1440-1444.
18. Khalil AA, Rahman U, Khan RM, Sahar A, Mehmood T, Khana M (2017). Essential Oil Eugenol: Sources, Extraction Techniques and Nutraceutical Perspectives. Royal society of chemistry Advances 7(52): 32669–32681.

19. Essien EE, Walker MT, Newby MJ, Ogunwande AI, Setzer NW, Ekunday O (2016). The Floral Essential Oil Composition and Biological Activity of *Solanum Macranthum* Dunal. American Journal of Essential Oils and Natural Products, 4(1) 36-39
20. Okhale SE, Ugbabe GE, Oladosu PO, Ib-rahim JA, Egharevba HO, Kunle OF, Elisha EP, Chibuike AJ, Ettah UO (2018). Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* L (Rubiaceae) collected from North Central Nigeria International. Journal of Bioassays, 7(4): 5630-5637.
21. Dar BK, Bhat HA, Amin S, Zargar AM, Masood A, Amin S, Malik HA, Ganie AS, (2017). Evaluation of Antibacterial, Antifungal and Phytochemical Screening of *Solanum Nigrum*. Biochemistry and Analytical Biochemistry 6(1):309-320.
22. Indhumathi T, Mohandass, S. (2014). Efficacy of Ethanolic Extract of *Solanum incanum* Fruit Extract for its Antimicrobial Activity. International Journal of Current Microbiology and Applied Science 3(6): 939-949.
23. Elisha IL, Botha, FS, McGaw LJ, Eloff JN (2017). The Antibacterial Activity of Extracts of Nine Plant Species with Good Activity against *Escherichia Coli* against Five Other Bacteria and Cytotoxicity of Extracts. BioMed Central Complement Alternative Medicine, 17(1), 133.

Figures

Figure 1

Mass spectrum and fragmentation of Benzyl alcohol

Figure 2

Mass spectrum and fragmentation of 2,3-dihydro-Benzofuran

Figure 3

Mass spectrum and fragmentation of Butylated Hydroxytoluene

Figure 4

Mass spectrum and fragmentation of 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2Hchromene

Figure 5

Mass spectrum and fragmentation of 1-docosene

Figure 6

Zones of inhibition in millimeter at 1000 µg/mL of each fraction of *S. incanum* peel

Figure 7

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

Figure 8

Zones of inhibition in millimeter at 500 $\mu\text{g/mL}$ of EAC of *S. incanum* peel