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Extraction and GC-MS Analysis of the Essential Oil from the Peel of *Solanum incanum* and its Antibacterial Activity Studies

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Phytochemicals were extracted from the peel of *Solanum incanum* (*S. incanum*) with methanol (70% v/v). The phytochemical screening of the methanolic extract showed the presence of glycosides, steroids, terpenoids, flavonoids, tannins, alkaloids, saponins and phenolic compounds. The successive partitioning of the methanolic extract was carried out with three solvents hexane, diethyl ether and ethyl acetate. The diethyl ether fraction yielded two immiscible fractions. The oil was separated as essential oil fraction and analyzed by GC-MS (gas chromatography-mass spectrometry). The GC-MS analysis of the essential oil indicated 17 compounds including 2,3-butanediol (76.76%), diethyl phthalate (8.32%), benzyl benzoate (3.02%), 2,6-dimethyl-6-nitro-2-hepten-4-one (2.56%) and 1,2-dimethoxy-4-(1-propenyl)benzene (1.88%). Among them, the mass spectral patterns of three compounds were analyzed, discussed and confirmed with NIST database. The antibacterial study of essential oil was conducted using four pathogens (*E. coli*, *K. pneumonia*, *S. aureus* and *S. pyogenes*) at four different concentrations (250, 500, 750 and 1000 µg/mL). The result indicates that the essential oil extracted from the peel of *Solanum incanum* exhibited remarkable antibacterial activity against *E. coli* and *K. pneumonia* in 1000 µg/mL, respectively compared with the positive control gentamicin (10 µg/mL). The result of this study revealed the presence of various organic components and the antibacterial activity of this plant essential oil may be as a result of the major compounds.

Keywords: *Solanum incanum*, Phytochemical, Essential oil, GC-MS, Antibacterial activity.

INTRODUCTION

Medicinal plants are important because of their phytochemicals, which are useful for curing of many human diseases. The treatment with medicinal plants were well known from the time immemorial [1-7]. Essential oils, a complex mixture of terpenoids are known for their aroma and usefulness in food and pharmaceutical applications [6]. The application of herbal products in health care industries is on the raise and predicted to increase further in the years to come. This is because of the reason that they don't have harmful effect for human's body compared to synthetic chemicals [7,8]. The potential applications of essential oils within the fields of agriculture, medicine, pharmacy and biotechnology are because of its ability to function as antimicrobial, analgesic and antioxidative agents [9,10].

Solanum L. (*Solanaceae*) encompasses many versatile blooming plants and many species of this family is medicinally important [11]. It has thorny leaves, yellow fruits, and blue flowers with yellow pistils [12]. *Solanum incanum* L. is a traditionally important plant in Ethiopia. The phytochemicals constituents of the fruits of *Solanum incanum* include terpenoids, flavonoids, bioflavonoid, xanthenes, saponins, cyanates, oxalate, anthraquinones, and steroid glycosides in the form of glycoalkaloids including solanine and solasonine [13,14].

The plant is useful in the management of skin diseases, general infections, abdominal pains, fever, stomachache, and indigestion. In Tanzania, the fruit of *Solanum incanum* is used for the treatment of dandruff, skin diseases, sores and wounds. Further, *Solanum incanum* is used in the treatment of venereal diseases and snakebites. In Ethiopia, the mixture of the powder from the fruit sap and butter are applied on the cattle to control ticks. Haddiya people of Ethiopia use the fruit of *S. incanum* to treat the stomach problem [15].

Even though the phytochemical evaluation of different parts of *Solanum incanum* has been done but there is little report on the identification of organic constituents. Hence, this study was conducted to identify the chemical constituents of the essential oil obtained from *Solanum incanum* and to assess the antibacterial activities.

EXPERIMENTAL

Sample preparation: A healthy ripe fruit of *Solanum incanum* (5 kg) was collected from Wollega University, Nekemte, Ethiopia in the month of May, 2018, identified and authenticated by Dr. Tena Regasa (Botanist), Department of Biology. The fruit was cleaned with tap water and incisions were made on the ripe fruit with the sterilized scalpel blade and the internal viscous fluid was squeezed out of the fruit. The fruit peel was cut into pieces and dried at room temperature till a constant weight was observed. The dried plant material was ground in to a fine powder using a laboratory mill. The weight of the powder was determined to be 1.5 kg and stored for further study.

Solvent extraction of *Solanum incanum* peel: *Solanum incanum* (1.5 kg) peel was soaked in 7 L methanol solvent (70% v/v) at room temperature for 10 days with occasional stirring [16,17]. The extract was screened, filtered by Whatmann No. 1 filter paper. The extract was concentrated by rotary evaporator under reduced pressure to get the methanolic extract.

Phytochemical screening tests of methanolic extract: Freshly prepared methanolic extract of *Solanum incanum* peel was subjected to phytochemical analysis by standard methods [7].

Liquid-liquid extraction: Liquid-liquid extraction was done by organic solvents hexane (1 L), diethyl ether (1.25 L) and ethyl acetate (1.5), respectively as indicated in the literature with slight modifications [18,19]. The different extracts obtained were dehydrated over an anhydrous sodium sulphate for 48 h filtered and concentrated by rotary evaporator under reduced pressure. The diethyl ether fraction yielded two immiscible phases. The two phases were separated using a separatory funnel (the essential oil and diethyl ether fraction). The essential oil (50 mL) was then collected into the sample bottle and stored till further work at 4 ºC.

GC-MS analysis: The GC-MS analysis of the essential oil was carried out 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/s] and an Agilent ChemStation data system by the method reported in the literature [20,21]. The HP-5ms fused silica capillary column made of polymethylsiloxane stationary phase (5% phenyl) with a film thickness of 0.25 µm, length of 30 m and an internal diameter of 0.25 mm was used. Helium was used as carrier gas with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. The inlet temperature and interface temperature were maintained at 200 ºC and 280 ºC, respectively.

The oven temperature of the GC was programed as follows: The initial temperature was maintained at 40 ºC and augmented at 3 ºC/min to 200 ºC and with a hold for 10 min. Further, the temperature was augmented 2 ºC/min to 220 ºC. Sample solution (1 µL of 1% v/v) was injected using a splitless injection technique. The components were identified based on their retention time (RT) determined with reference to a homologous series of normal alkanes and their fragmentation patterns were compared with those reported in the literature and NIST library [20, G1036A, revision D.01.00)/Chem Station data system (G1701CA, version C.00.01.08)].

Assessment of antibacterial activity: Antibacterial activity of essential oil, diethyl ether and ethyl acetate fractions were determined by using the disc diffusion assay with some modifications [22]. The antibacterial activity was done using the American Type Culture Collection (ATCC), and the samples were tested against Gram-positive, *S. aureus* (ATCC 31488) and *S. pyogenes* (ATCC 27853) and *E. coli* (ATCC 25922) and *K. pneumonia* (ATCC 27853) Gram-negative bacteria. Each test bacterium was inoculated on Mueller-Hinton broth, homogenized and swamping on a sterilized Petri-dish to yield a uniform depth. The antibacterial studies were conducted using four different concentrations (1000, 750, 500 and 250 µg/mL), prepared in 10% DMSO and loaded into 6 mm filter paper disc impregnated with 50 µL. Gentamicin (10 µg/disc) disc localized at each petri dish was the positive control, whereas DMSO (10%) was the negative control. The petri dishes were then incubated at 37 ºC for 24 h. The zones of inhibition were then spotted and calculated. The zones of inhibitions were calculated by determining the diameters of zones in millimeters (mm) and expressed as antibacterial activity.

RESULTS AND DISCUSSION

Phytochemical analysis of methanolic extract: The presence of various phytochemicals were analyzed qualitatively using various standard methods. The methanolic extract of S. incanum peel showed the presence of various important phytocompounds like alkaloids, flavonoids, steroids, tannins, terpenoids, glycosids and phenolic compounds. However, the extract depicted the absence of anthraquinones (Table-1), this is in accordance with an earlier investigation, which showed the presence of those phytochemicals with the absence of anthraquinones in the crude extract of *S. incanum* [23,24].

Physical characteristics of essential oil: The essential oil (from diethyl ether fraction) was less dense and insoluble in water but soluble in 70% methanol and dimethyl sulfoxide (DMSO) at a level of 1:1 (v/v). It had a watery viscosity and exhibited a pale yellow color when observed against a white background. This essential oil was liquid at room temperature and maintained as liquid even when stored at -20 ºC.

Essential oil of *S. incanum* peel: The GC-MS analysis of the essential oil revealed the presence of 17 components (Table-2). The components were identified by representing 100% of the total oil composition. The major constituents of the essential oil were 2,3-butanediol (76.76%), diethyl phthalate (8.32%), benzyl benzoate (3.02%), 2,6-dimethyl-6-nitro-2-hepten-4-one (2.56%) and 1,2-dimethoxy-4-(1-propenyl)-benzene (1.88%).

The empirical formula of this fraction was derived to be $C_{14}H_{12}O_2$ using nitrogen rule and rule of thirteen. The absence

TABLE-1
PHYTOCHEMICAL CONSTITUENTS OF *S. incanum* PEEL

Phytochemicals	Colour formation	Methanolic extract
Alkaloids	Formation of yellow or brown precipitate confirmed the presence of alkaloid	Presence
Tannins	The green precipitate indicated the presence of tannins	Presence
Flavonoids	Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids	Presence
Terpenoids		Presence
Steroids	The upper layer turns red and sulfuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids	Presence
Saponins	Formation of foam indicates the presence of saponins	Presence
Glycosids	A reddish brown colour indicated the presence of the steroidal ring, *i.e.*, the glycone portion of the glycoside	Presence
Phenolic compounds	Formation of blue or green indicates the presence of phenolic compounds	Presence
Anthraquinones	–	Absence

TABLE-2
CONSTITUENTS OF THE ESSENTIAL OIL OF *S. incanum* PEEL

Peak#	Retention time (min)	Area	Area (%)	Name of compounds
1	3.535	9370	0.45	Formamide
2	4.054	862526	41.00	2,3-Butanediol
3	4.251	752194	35.76	2,3-Butanediol
4	32.361	24071	1.14	Methyl anthranilate
5	39.222	39485	1.88	1,2-Dimethoxy-4-(1-propenyl)-benzene
6	40.034	6178	0.29	5-Isothiazolemethanol
7	40.034	4607	0.22	1,1,1-Trifluoro-methanamine
8	42.929	175046	8.32	Diethyl phthalate
9	45.163	53760	2.56	2,6-Dimethyl-6-nitro-2-hepten-4-one
10	48.807	63468	3.02	Benzyl benzoate
11	52.523	28619	1.36	Phthalic acid, 4-bromophenyl heptyl ester
12	55.210	10737	0.51	Trifluoroacetic acid, 2-tetrahydrofurylmethyl ester
13	55.539	29078	1.38	Ethyl N-formyldithiocarbamate
14	57.667	8217	0.39	3-Fluoro-2-propynenitrile
15	60.228	19078	0.91	Trifluoro-acetic acid, 2,2-dimethylpropyl ester
16	63.413	10148	0.48	Isoxazole
17	64.965	6982	0.33	Ethylisopropylmethyl-borane

of M+2 peak indicated the absence of halogen, silicon and sulfur (Fig. 1). The base peak at 105 indicated the presence of benzyl ester. Further, the fragments benzyl cation (91) and phenyl cation (77), cyclopentadienylcation (65) and cyclobutadienyl cation (51) confirmed the presence of benzyl benzoate (**Scheme-I**).

The empirical formula for diethyl phthalate was derived to be $C_{12}H_{14}O_4$ using the nitrogen rule and rule of thirteen (Fig.

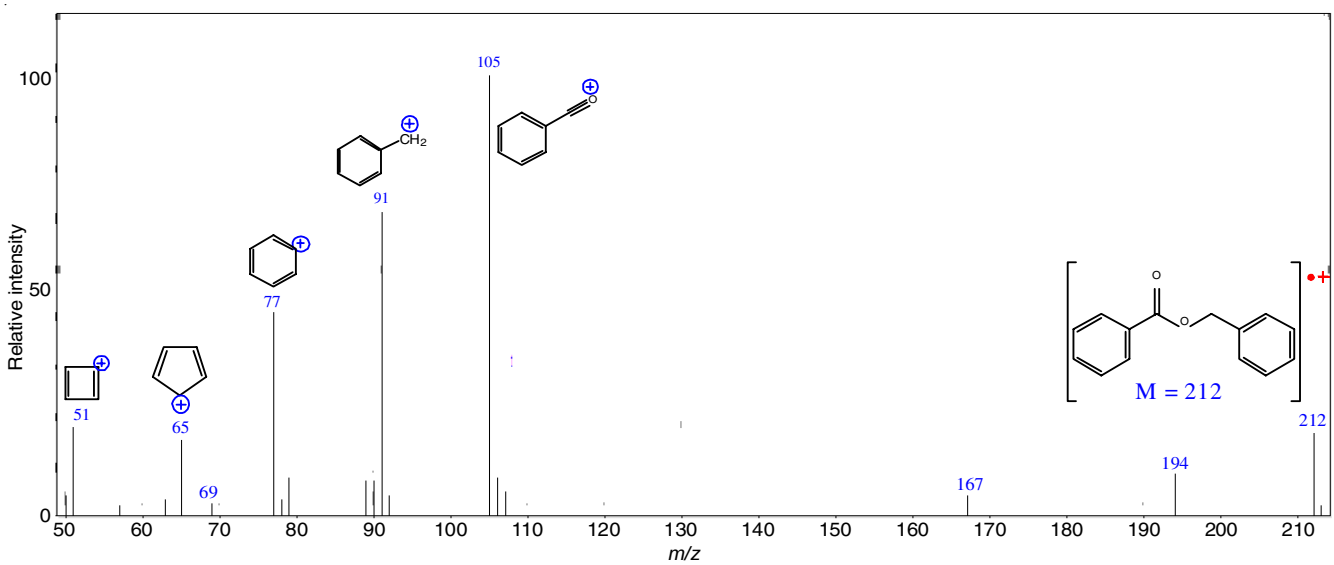
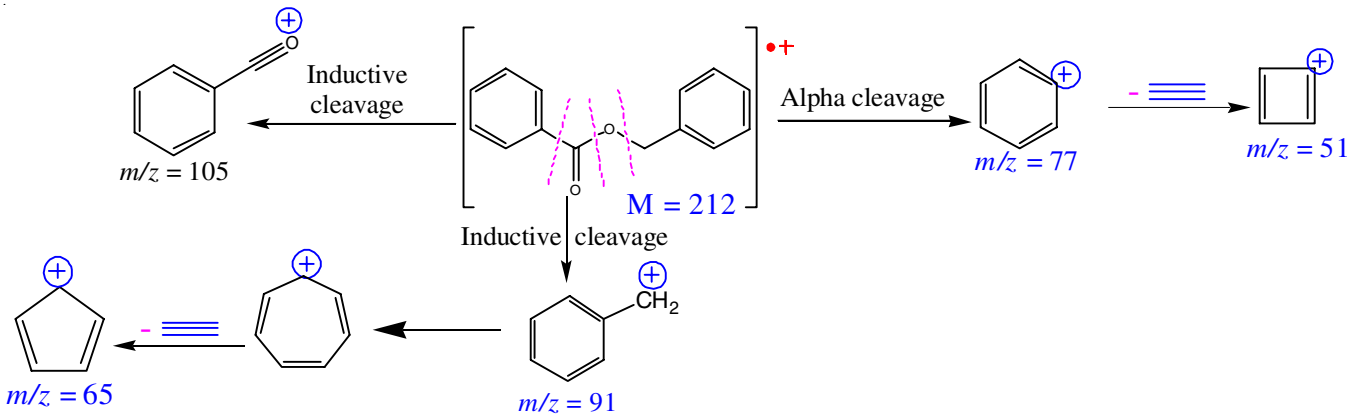


Fig. 1. Mass spectrum and fragmentation of benzyl benzoate



Scheme-I: Mass fragmentation of benzyl benzoate [Ref. 25]

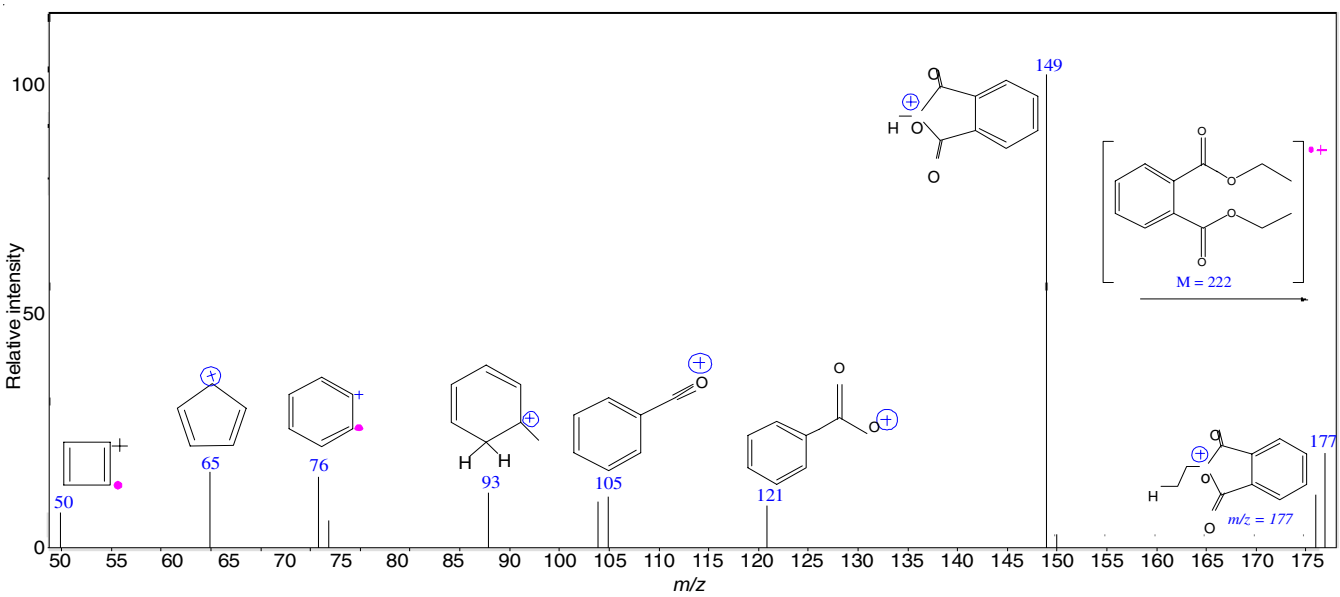
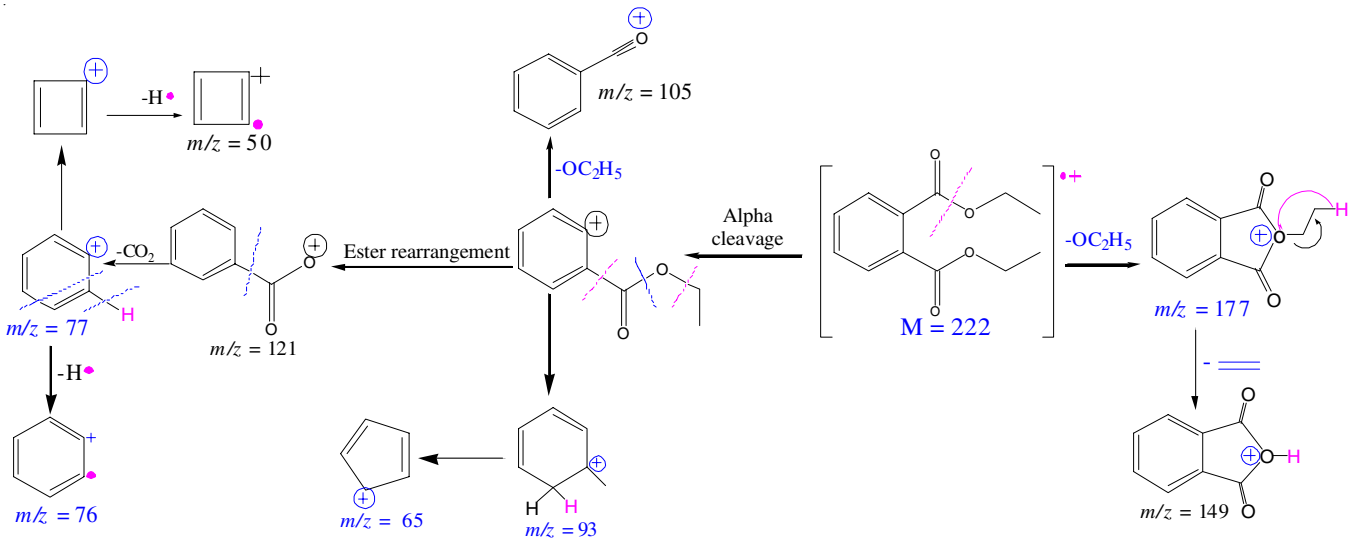


Fig. 2. Mass spectrum and fragmentation of diethyl phthalate

2). The base peak at 149 indicated the presence of phenyltetrahydrofuranyl cation (149) and the fragments ethylformate benzalate cation (177), benzyl ester cation (121), benzalate cation (105) and cyclopentadienyl cation (65), phenyl radical cation (76), cyclobutadienyl radical cation (50) confirmed the presence of diethyl phthalate (**Scheme-II**).



Scheme-II: Mass fragmentation of diethyl phthalate [Ref. 25]

The empirical formula of 1,2-dimethoxy-4-(1-propenyl)-benzene was derived be to be $C_{11}H_{14}O_2$ from the nitrogen rule and rule of thirteen. The absence of M+2 peak indicated the absence of halogen, silicon and sulfur (Fig. 3). The base peak at 178 indicated the presence of very stable 1,2-dimethoxy-4-(1-propenyl)benzene molecular ion, the fragments styrenyl-cation (103), benzyl cation (91), 1,2-dimethoxybenzyl-4-vinyl cation (163), anisolyl cation (107) confirmed the presence of 1,2-dimethoxy-4-(1-propenyl)benzene [24] (**Scheme-III**).

Antibacterial assay: In this investigation, an essential oil of the peel of *S. incanum* exhibit antibacterial activity against *E. coli*, *K. pneumonia*, *S. pyogenes* and *S. aureus*. The antibacterial potential activity of the essential oil at different concentrations 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-3. The results clearly showed that the plant material was specific in action against the growth of bacterial species.

The antibacterial activity of the plant was assayed by *in vitro* disk diffusion method using paper discs. The maximum antibacterial activity was shown against *E. coli*, *K. pneumonia* followed by *S. aureus*, respectively, and a minimum activity was shown against *S. pyogenes*. The maximum antibacterial activity against Gram-negative, *E. coli* and *K. pneumonia* indicate that this can be useful for the treatment of stomach pain and diarrhea andthe minimum antibacterial activities against Gram-positive, *S. aureus* and *S. pyogenes*, suggest that it may not be that beneficial for the wound healing and skin infections [25].

The distinction between Gram-negative and Gram-positive bacteria could be due to the difference in their cell wall structure.

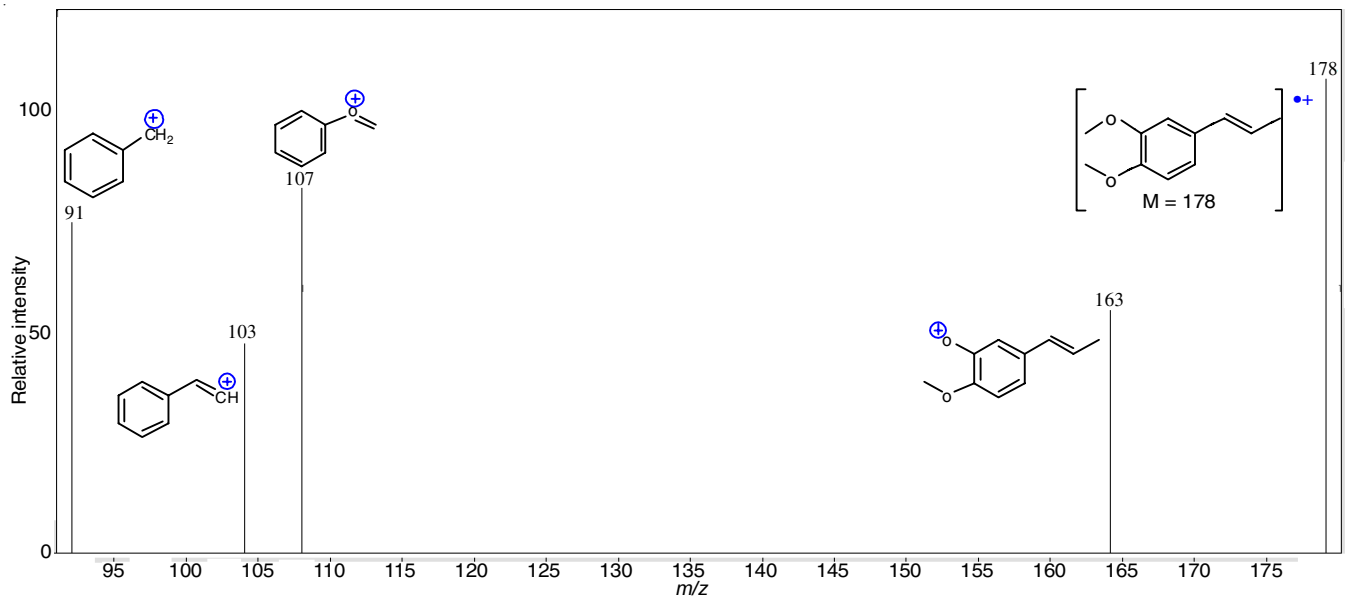
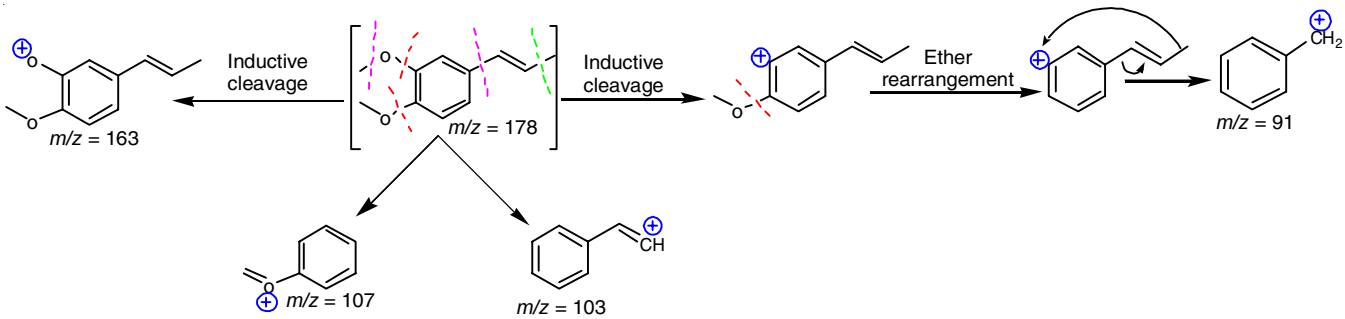


Fig. 3. Mass spectrum and fragmentation of 1,2-dimethoxy-4-(1-propenyl)benzene



Scheme-III: Mass fragmentation of 1,2-dimethoxy-4-(1-propenyl)benzene [Ref. 25]

TABLE-3
ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OIL OF *S. incanum* PEEL AT DIFFERENT CONCENTRATIONS

Fractions (µg/mL)		*E. coli* (mm)	*K. pneumonia* (mm)	*S. aureus* (mm)	*S. pyogenes* (mm)
Oil:	250	–	–	–	–
	500	7.7 ± 0.3	7.3 ± 0.14	6.5 ± 0.4	6.3 ± 0.14
	750	13.7 ± 0.3	7.5 ± 0.5	7.3 ± 0.14	7.5 ± 0.5
	1000	22.3 ± 0.14	18.3 ± 0.14	17.3 ± 0.14	16.5 ± 0.5
Gentamicin: 10		13.3 ± 0.58	16.7 ± 0.3	14.5 ± 0.5	14.3 ± 0.58

10% DMSO had not an effect on the bacterial growth

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 [26].

Conclusion

In the present study, the phytochemical screening of the methanolic extract showed the presence of glycosides, steroids, terpenoids, flavonoids, tannins, alkaloids, saponins and phenolic compounds with the absence of anthraquinones. The methanolic extract was partitioned with hexane, diethyl ether, and ethyl acetate fractions, but diethyl ether yielded essential oil and other diethyl ether fractions. The essential oil was subjected to GC-MS analysis, and the GC-MS analysis of the essential oil revealed the presence of 17 compounds. The mass spectral pattern of the three compounds was analyzed, and confirmed with NIST database. The antibacterial activity of this plant essential oil may be as a result of the major compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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