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GC/MS Profiling and Antibacterial Activity of Steam-Distilled *Solanecio mannii* Essential Oil and Hydrosol Against Multidrug-Resistant Food Pathogens

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Abstract:

Solanecio mannii is a fast-growing woody shrub of up to 10 m in height of the Asteraceae family. This study aimed to identify the chemical constituents of steam-distilled oil and hydrosol of *Solanecio mannii* leaves and determine their activity against *Escherichia coli* and *Staphylococcus aureus* bacteria. Steam distillation extraction followed by GC/MS analysis was employed to characterize the chemical composition, while the antimicrobial effectiveness was assed using disc diffusion method at 10 µL sample volumes. GC/MS analysis revealed 52 compounds in the essential oil predominantly monoterpenes (48%) and sesquiterpenes (38.5%), and 28 compounds in the hydrosol, consisting mainly of fatty acid methyl esters (28.57%) and other heteroatomic compounds (67.86%). The essential oils demonstrated significant activity against both *E. coli* (12 mm) and *S. aureus* (10 mm) for the oil and 10 mm and 8 mm for the hydrosol respectively. The findings emphasize the potential of *S. mannii* essential oil and hydrosol as clean label agents for food industry applications, particularly in active packaging systems.

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1. INTRODUCTION

Foodborne illnesses pose a major public health concern, resulting particularly from consumption of food contaminated with pathogenic microorganisms. Clean-label technology has emerged as a promising approach, utilizing natural antimicrobial and biological preservatives to improve both the shelf life and safety of food items [1]. This is with the goal to meet the consumers need for clean and safe products free from synthetic ingredients. Natural products including plant essential oil, plant extracts and bacteriocins possess great potential as clean label ingredients in application as antimicrobial agents [2]. Utilizing these natural compounds may enable the food industry to match the consumer demands for safe food while upholding safety and quality requirements in the food industry [3].

Antimicrobial resistance (AMR) is another rapidly growing challenge that significantly challenges the rate at which new antimicrobial drugs are developed [4] Gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* have been named by the World Health Organization in the medium to high priority(21-30%) pathogens on the antimicrobial resistance list [5]. Illnesses caused by *S. aureus* and *E. coli* remain to be among the main foodborne diseases even in the contemporary society [6]. This new disaster necessitates intensive research and technological innovations to ensure food safety consequently improving human health.

Essential oil (EOs) obtained from various plant parts such as leaves, flowers, fruits, and roots, have been traditionally used for their flavoring and medicinal properties [7]. They have been reported to be used in medicine, food industry, and drug discovery due to their minimal toxic effects, affordability, and availability [8]. Essential oil have been explored extensively as potential sources of natural antimicrobial agents due to their antifungal, antiparasitic, antibacterial, and antiviral properties [9].

Plant essential oils typically contain terpenes, terpenoids, and other aromatic and aliphatic compounds [10]. The major components usually determine the biological properties of the essential oil. For instance, the high content of thymol in thyme essential oil has been shown to be responsible for its strong antimicrobial activity [11]. However, Saad *et al*., [12] highlighted that when comparing biological effects of essential oil of plants with the same common name and similar major phytoconstituents, results may differ significantly due to the variations in minor constituents of the oil that contribute to the overall effect. The constituents may be differ based on the climatic and environmental differences where the plants have been sourced.

Antibacterial activity of essential oils have been studied and reported to be through various mechanisms [13], primarily targeting the cell membrane of microorganisms generally as bactericidal- kill the bacteria or bacteriostatic-inhibit the bacterial growth [14]. The hydrophobic nature of essential oils and their constituents allows them to partition into the lipids of bacterial cell membranes, leading to increased permeability and subsequent leakage of vital cell contents [15]. This disruption of the cell membrane structure can result in the loss of ions, reduction of membrane potential, collapse of the proton pump, and depletion of the Adenosine Triphosphate (ATP) pool.

Furthermore, essential oils can interfere with various cellular processes. For instance, components of essential oils, such as thymol and carvacrol, have been shown to inhibit ATPase activity and release of intracellular ATP [16]. Other mechanisms include the inhibition of enzymes involved in energy regulation and synthesis of structural components, as well as the denaturation and coagulation of cytoplasmic proteins [15].

Hydrosols (also called hydrolates or aromatic waters) are byproducts of the steam distillation or hydrodistillation of essential plant oils and consist of the distillation water in which some small amounts of the oils are suspended [17] and are often regarded as waste [18]. Despite limited knowledge of their phytoconstituents, they have been employed in industries such as food, agriculture and cosmetics [19]. Hydrosols are mixtures consisting of varying quantities of volatiles, secondary metabolites, essential oils which contribute typically less than 1g per liter of the hydrosols, and water-soluble compounds [20]. Their constituents are responsible for the organoleptic characteristics of the plant material like odor, taste and color [17]. Hydrosols of various plants have been reported to exhibit antimicrobial effect against a range of food pathogens [17,18,21].

However, the application of essential oils in the food industry faces limitations due to the rapid degradation from environmental factors such as oxidizing species, light, temperature variations and changes in bioactive composition [22]. The challenge has led to innovative

food packaging methods incorporating various active agents, including essential oils, nanoparticles and natural acids among others [23]. Packaging is crucial in food processing since it greatly impacts a number of characteristics of food, including acceptability, nutritional value, and food safety, the packaging material. Active food packaging (including antimicrobial, pH indicators, antioxidants, and ethylene scavenging) innovations reduces the direct contact of the food with the active ingredients consequently preserving the desired quality attributes such as the flavor of the food [24].

This study focuses on the essential oil of *Solanecio mannii*, a fast-growing woody shrub or a small tree, up to 10 m in height of the Asteraceae family [25]. Bello *et al*., [26] reported that 17 species of the genus Solanecio or senecio exist in the tropic regions of Africa, Madagascar Island and Yemen. Some communities in Western Uganda use this species as a remedy for indigestion, fever and malaria [27]. The plant is also used as live fences and in marking boundaries in some African communities [28]. The dichloromethane extract of *Solanecio mannii* leaves were reported to show antiplasmodial effect with low cell toxicity confirming its use as remedy for malaria in Rwanda [29]. Leaf extracts of *S. mannii* have shown excellent antimicrobial characteristics mostly related to its phenolic compounds [30]. However, the constituents and antimicrobial effects of *Solanecio mannii* steam distilled essential oil and their by-products (hydrosols) have not been assessed.

The aim of the present study was to identify, for the first time, the chemical composition of steam distillation products of *Solanecio mannii* leaves and investigate their antimicrobial effects against two multidrug resistant food pathogenic bacteria strains, *Staphylococcus aureus* and *Escherichia coli,* to evaluate their potential future application as clean label ingredients in the food industry.

2. MATERIALS AND METHODS

2.1. Reagents

Absolute HPLC grade ethanol (99.8%, Sigma-Aldrich, Germany), nutrient agar (HiMedia Laboratories GmbH, GERMANY), Ciprofloxacin hydrochloride monohydrate (Sigma-Aldrich, Germany), DMSO (99.5% (GC), Sigma-Aldrich, Germany) and nutrient agar plates were used.

2.2.Plant Material

Fresh leaves of *Solanecio mannii* were harvested in a clean nylon bag from Ndeffo, Nakuru County, Kenya (0°28'57.0"S 35°58'54.8"E) in December 2024. Oil and hydrosol extraction and analysis were conducted at the food fortification and analytical chemistry laboratories, Jomo Kenyatta University of Agriculture and Technology, Kenya.

2.3. Extraction

Leaves were subjected to steam distillation in batches using round-bottomed flasks placed on electric heating mantles connected to Clevenger apparatus with Liebig condensers in place for 4hrs at 80℃ as described by [31]. The hydrosol and the oil were collected separately into clean amber vials. The procedure was repeated until 1kg of the harvested fresh leaves sample was extracted. Samples were stored in a refrigerator at 4℃ for further analysis.

2.4. GC/MS Profiling of the Extracts

GC/MS is a hyphenated method merging compounds separation by gas chromatography and their identification by mass to charge ratio assisted by the mass spectrometer and is appreciated in research involving organic mixtures such as natural products [32]. Two GC/MS models were used to analyze the chemical composition of the *S. mannii* oil and the hydrosol respectively as described in detail in the following sections.

2.4.1. Essential Oil Analysis

A Shimadzu QP 2010 SE GC/MS (Shimadzu Corporation, Kyoto, Japan) coupled to an auto sampler was used for the analysis of the oil with the method by Mwangi *et al*. (2024). Helium (99.7%) was used as the carrier gas at a linear velocity of 1 mL/min. A BPX5 non polar column 30M long with 0.25mm internal diameter, and 0.25um thickness was used for separation. The GC oven temperature was increased from 50℃ to 200℃ at 5 ℃/min for 10 minutes and ramped to 250℃ at 10 ℃/min for 5 minutes. The Electron Ionization (EI) ion source was set at 200 ℃. The total run time was to set to 40 minutes. Mass analysis was done in scan mode with scanning range of 50-650 m/z. Samples were injected in split mode with a split ratio of 25:1. The mass-to-charge (m/z) ratios were compared to the NIST 14 library of compounds for identification of individual eluted compounds and generation of the chromatogram.

2.4.2. Hydrosol Analysis

An Agilent 5977B GC/MSD (Agilent Technologies Inc. California, US) with helium carrier gas was used to identify compounds in the hydrosol following the method described by Quimby, [34]. Hydrolates were first filtered using 0.22 µm microfilters then ultrasonicated. A10 µL sample was reconstituted in 5% HPLC grade ethanol. A 30 m by 0.25 mm ID, 0.25 µL Agilent J&W HP-5ms Ultra Inert (UI) column was used with oven temperature programmed from 50 to 250 °C at 5 °C/min. Helium (99.97%) was used as the carrier gas at the flow rate of 1 µL/min. The Electron Ionization (EI) ion source was set at 200 ℃ with a total run time of 40 minutes. Samples were injected in split mode with the split ratio of 10:1. Mass analysis was done in scan mode with scanning range of 40-400 a.m.u. The mass-to-charge (m/z) ratios were compared to the NIST 14 library of compounds and the total ion chromatogram generated.

2.5. Antibacterial Activity of *S. mannii* oil and Hydrosol

Antibacterial activities of the oil and hydrosols were evaluated using disc diffusion method as described by Din *et al*. [35]. Nutrient agar was prepared according to the manufacturer's instruction (28g in 1000 ml of distilled water) and sterilized by autoclaving for 15 minutes at 121℃. Freshly prepared nutrient agar plates were uniformly inoculated with bacterial inoculants *S. aureus* ATCC 11622 and *E. coli* ATCC 25922, using sterile cotton swabs. Sterile filter paper discs were laid onto the bacteria-inoculated nutrient agar plates by help of flame-sterilized forceps. Using micropipettes, 10 µL of each sample were loaded onto respective discs with each containing. Equal volumes of ciprofloxacin hydrochloride and 5% DMSO were used as standard drug and negative control respectively and the plates incubated for 24 hours. After the incubation period, the zones of inhibition were measured and recorded in millimeter and results reported as Mean ± SD.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of *S. mannii* Essential Oil and Hydrosol

The essential oil yield of the *Solanecio mannii* was 3% of wet leaves. Poudel *et al*., [36] reported that plant essential yield can be influenced by several factors such as the method, time and temperature of extraction, the time of harvest, and geographic variations of the location where the plant material is sourced. This yield was lower than the typical 10% oil and 90% hydrosol distillation ratio reported by Fatt *et al*., [37] which may be attributed to the high water content of *S. mannii* leaves, evidenced by their rapid deterioration (less than 5 days) when attempting to shade drying at 24℃ room temperature.

The chemical composition of the essential oil of S. mannii were analyzed using GC/MS. The results depicted that *S. mannii* oil contains fifty-two (52) compounds consisting mainly of terpenes and terpenes derivatives contributing 71% of the identified compounds. The total ion chromatograms (TIC) of the oil and hydrosol are shown in Figure **1** and **2**.

The major classes of terpenes in the oil were sesquiterpenes (15%) followed by monoterpenes (13.5%). The most abundant compounds identified in the oil, each contributing more than 5% were; 6-(1,2-Dimethyl-1-propenyl)-4,5-diazaspiro[2.4]hept-4-ene (6) (44.49%), (1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene (31) (10.33%), β-Ocimene

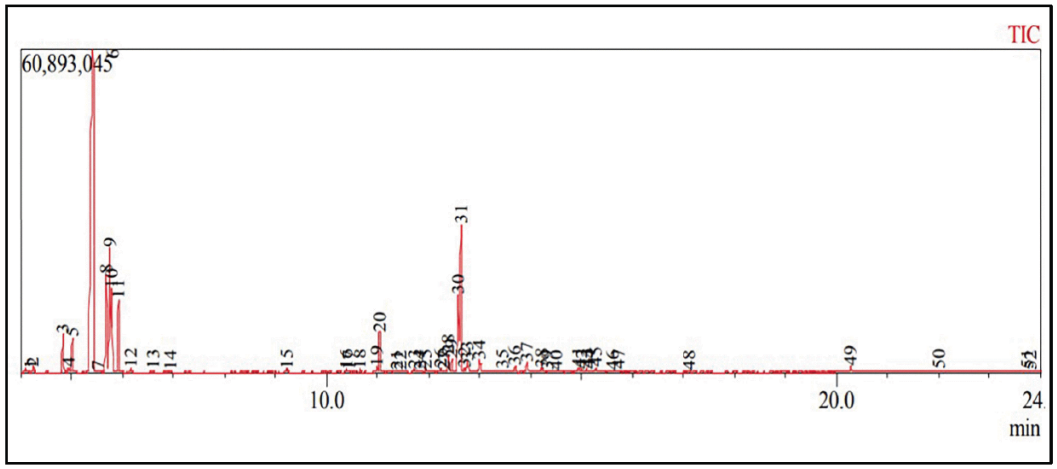

Figure 1: Total ion chromatogram (TIC) of *S. mannii* oils.

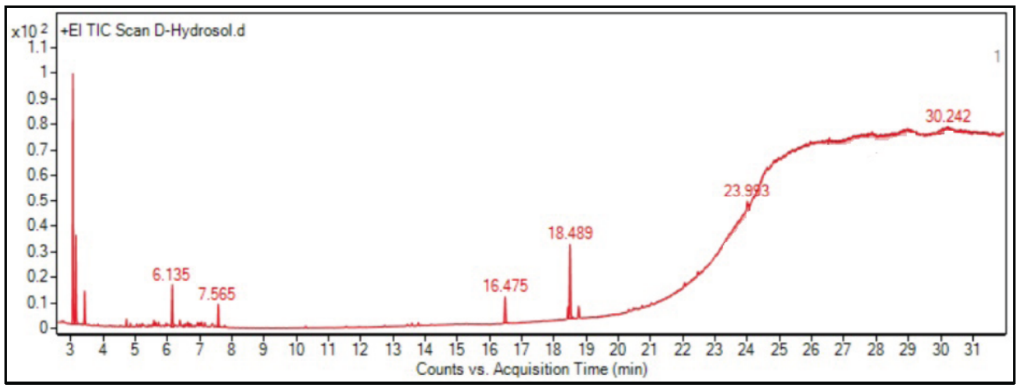

Figure 2: Total ion chromatogram (TIC) of *S. mannii* hydrosol.

Table 1: Chemical Composition of *S. mannii* Oil

Peak #	Compound name	R.Time	Area%	A/H
1	β-ocimene	4.098	0.21	2.63
2	Pinene	4.248	0.36	2.05
3	4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane	4.837	2.61	1.95
4	β-Pinene	4.949	0.29	1.97
5	β-Myrcene	5.018	2.43	2.02
6	6-(1,2-Dimethyl-1-propenyl)-4,5-diazaspiro[2.4]hept-4-ene	5.424	44.49	3.91
7	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	5.523	0.06	1.49
8	3,7,7-trimethyl-1,3,5-Cycloheptatriene	5.681	5.87	1.72
9	β-Ocimene	5.756	7.77	1.78
10	4-methylene-1-(1-methylethyl)-Bicyclo[3.1.0]hexane	5.789	4.32	1.48
11	β-Ocimene	5.922	3.94	1.55
12	γ-Terpinene	6.167	0.18	1.44
13	3-methyl-6-(1-methylethylidene)-Cyclohexene	6.604	0.09	1.53
14	(E)-4,8-Dimethylnona-1,3,7-triene	6.945	0.06	1.41
15	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	9.229	0.16	1.66
16	1-methyl-4-(1-methylethyl)-, trans-2-Cyclohexen-1-ol	10.390	0.13	1.73
17	3-Isopropyl-4-methyl-1-pentyn-3-ol	10.437	0.07	1.58
18	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	10.665	0.13	1.54
19	octahydro-1-7a-dimethyl-5-(1-methylethyl)-[1S-2S]-1,2,4-Metheno-1H-indene	10.997	0.31	1.75
20	α-Copaene	11.053	2.33	1.68
21	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	11.382	0.07	1.79
22	2,6,6-trimethyl-Bicyclo(3.1.1)heptane-2,3-diol	11.455	0.09	1.67
23	Caryophyllene	11.724	0.13	1.66
24	3-(hydroxymethyl)-6-(1-methylethyl)-2-Cyclohexen-1-one	11.839	0.09	3.44
25	(E)-β-Famesene	11.951	0.17	1.91
26	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	12.232	0.19	1.73
27	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	12.298	0.10	1.97
28	1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,3-diene	12.386	0.96	1.64
29	1-(1,5-dimethyl-4-hexenyl)-4-methyl- Benzene	12.450	0.77	1.72

Peak #	Compound name	R.Time	Area%	A/H
30	β-copaene	12.582	5.47	2.02
31	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl) bicyclo[3.1.0]hex-2-ene	12.640	10.33	1.99
32	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4)	12.710	0.25	1.89
33	(1R,4aS,8aR)-1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	12.762	0.78	2.24
34	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	12.997	1.12	2.83
35	Nerolidyl acetate	13.449	0.15	2.67
36	1,5-dimethyl-8-(1-methylethylidene)-(E,E)-1,5-Cyclodecadiene	13.693	0.36	1.94
37	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	13.929	0.63	2.00
38	Z- α-trans-Bergamotol	14.221	0.23	2.11
39	(1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]de	14.335	0.08	4.26
40	(1S,3aS,4S,5S,7aR,8R)-5-Isopropyl-1,7a-dimethyloctahydro-1H-1,4-methanoic	14.502	0.08	2.07
41	τ-Muurolol	14.936	0.44	3.07
42	Aristol-1(10)-en-9-yl isovalerate	15.006	0.17	2.16
43	α-Cadinol	15.112	0.24	2.15
44	decahydro-α-4a-trimethyl-8-methylene-2-Naphthalenemethanol	15.174	0.12	2.05
45	Silphiperfola-4,7(14)-diene	15.291	0.33	2.14
46	β-Muurola-4,10(14)-dien-ol	15.617	0.07	1.76
47	3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-[1R]- Cyclohexanol	15.740	0.13	2.04
48	α-Caryophylla-4(12),8(13)-dien-5-ol	17.116	0.06	1.88
49	Isophytol, acetate	20.278	0.35	1.88
50	Tricosane	22.008	0.09	1.60
51	Eicosane	23.747	0.08	1.73
52	n-Tetracosanol-1	23.822	0.06	1.87
	Name		100	

(9) (7.77%), 3,7,7-trimethyl-1,3,5-Cycloheptatriene (8) (5.87%) and β-copaene (30) (5.47%) as shown in Table **1**.

About 70% of the 52 compounds in the *S. mannii* essential oil were terpenes including monoterpenes and sesquiterpenes Table **1**. Tuei *et al*. [38] reported that GC/MS analysis of methanolic extracts of *S. mannii* contained mainly of flavonoids and essential oil. However, the chemical composition of steam-distilled *S. mannii* oils differed from that of hydrodistilled oils of *S. mannii* reported by [39] who reported eight components in the oil. The current results indicate superiority of steam distillation over hydrodistillation extraction method for *S. mannii* essential oils. Steam distillation is advantageous in oils extraction due to minimal degradation of the organic compounds [21]. Some common essential oil components reported in many plants essential oils are own in Figure **3**.

The GC/MS analysis of the hydrosol identified 28 compounds as outlined in Table **2**. Major compounds identified in the hydrosol include ethylbenzene (1) (34.00%), (Z)-15-octadecenoic acid methyl ester (16.90%), o-xylene (2) (13.72%), 4,5-dimethyl-nonane (11) (6.87%), and hexadecanoic acid methyl ester (21) (6.00%). The hydrosol consisted of 27.5% fatty acids methyl esters (FAMES). The presence of FAMES in the hydrosols may result from natural esterification reactions during the high temperature steam distillation process [21]. Esters have also been reported in hydrosols of other plants [21].

The hydrosol contained mainly of FAMEs with α-phellandrene as the only identified terpene (Table **2)**. The results agreed with those reported for hydrodistilled hydrosol of arecanut and coconut [40]. Fourier Transform Infrared (FTIR) analysis of Algerian lemongrass hydrodistilled hydrolates revealed that they mainly contained aromatic compounds, alkanes, aldehydes, and alkenes functional groups [41]. The major constituents of the hydrosol of *Solanecio mannii* are shown in Figure **4**.

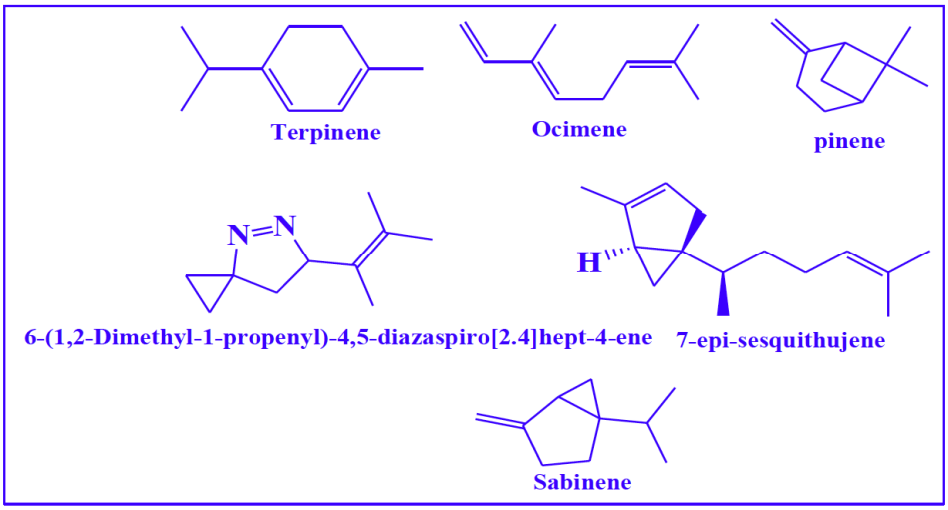

Figure 3: Some components identified in *Solanecio mannii* oil.

Table 2: Chemical Constituents of *S. mannii* Leaf Hydrosol

Peak #	Compound	RT	AH	%Area	m/z
1	Ethylbenzene	3.052	476542	34.00	91
2	o-Xylene	3.142	192307	13.72	91
3	2-Propenoic acid, butyl ester	3.394	8009	0.57	55
4	Ethylbenzene	3.415	70455	5.03	91
5	2,2-Dimethyl-propyl-2,2-dimethyl-propanesulfinyl sulfone	4.715	13111	0.94	57
6	α-Phellandrene	4.838	6916	0.49	93
7	3,3,5-trimethyl- Heptane	5.033	4386	0.31	71
8	N-cyclopropylcarbonyl-L-Proline methyl ester	5.571	9718	0.69	71
9	5H-Tetrazol-5-amine	5.62	7028	0.50	57
10	4-ethyl-2,2,6,6-tetramethyl-Heptane	5.715	8198	0.58	57
11	4,5-dimethyl- Nonane	6.135	96288	6.87	57
12	(Z,Z)-5,10-Pentadecadien-1-ol	6.372	6382	0.46	81
13	1-Bromo-3-butene-2-ol	6.385	4758	0.34	57
14	Sulfurous acid, 2-ethylhexyl hexyl ester	6.528	4292	0.31	71
15	2-hexyl- Furan	6.612	4766	0.34	81
16	Orcinyl di-tiglate	6.674	5147	0.37	83
17	Pentanoic acid, 1,1-dimethylpropyl ester	6.991	6827	0.49	71
18	5H-Tetrazol-5-amine	7.051	7616	0.54	57
19	4-ethyl-2,2,6,6-tetramethyl- Heptane	7.148	7006	0.50	57
20	1,1'-oxybis-Heptane	7.567	54106	3.86	57
21	Hexadecanoic acid, methyl ester;	16.47	84144	6.00	74
22	2-methyl- Hexanedinitrile	18.42	4144	0.30	55
23	2-isobutyl-Norbornane	18.43	16424	1.17	67
24	(Z)-15-Octadecenoic acid methyl ester	18.49	236768	16.90	55
25	Methyl 8-methyl-nonanoate	18.76	29384	2.10	74
26	Methyl 2-O-methyl-β-D-xylopyranoside	22.46	5201	0.37	74
27	4-ethyl-2,2,6,6-tetramethyl- Heptane	24	18948	1.35	57
28	2-Propenoic acid, 2-methoxyethyl ester	30.13	12533	0.89	55

Fatty acid methyl esters (FAMEs)=8(28.57%) Terpenes=1(3.57% Others= 20 (67.86%).

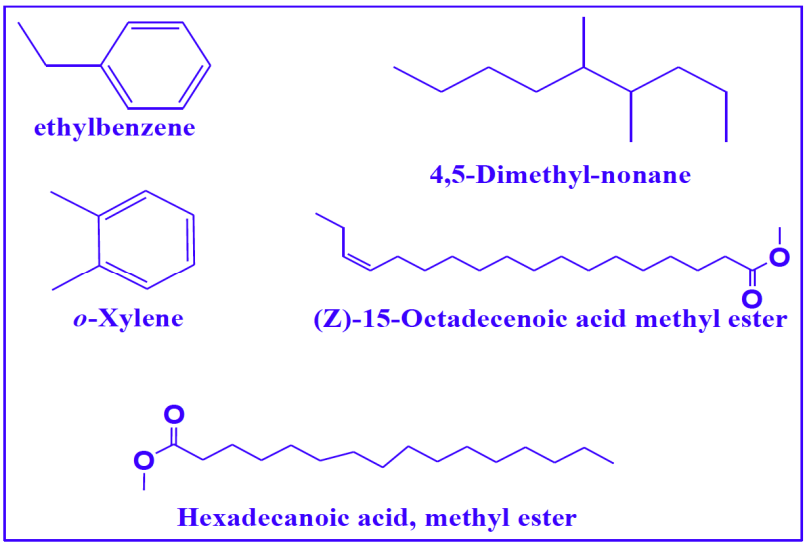

Figure 4: Some compounds in the *S. mannii* hydrosol.

Table 3: Antimicrobial Activity of *S. mannii* Essential Oil and Hydrosol

Sample	*E. coli* (mm)	*S. aureus* (mm)
Essential Oil	12.0 ± 0.5	10.0 ± 0.3
Hydrosol	10.0 ± 0.4	8.0 ± 0.2
Ciprofloxacin (positive control)	24.0 ± 0.8	22.0 ± 0.7
DMSO (negative control)	0	0

3.2. Antimicrobial Activity of *S. mannii* oil and Hydrosol

The essential oil and hydrosol from steam distillation of *S. mannii* leaves were screened for antibacterial activity using agar disc diffusion method. Ciprofloxacin hydrochloride (positive control) showed the highest inhibition for both bacterial strains. Both the oil and the hydrosol demonstrated antimicrobial activity against *S. aureus* and *E. coli* at 10 µL volumes, though to different extents shown by the inhibition zones diameters (Table **3**).

The hydrosol showed moderate antibacterial activity compared to the oil and ciprofloxacin. Ciprofloxacin (positive control) exhibited the highest inhibition against both *E. coli* and *S. aureus*. DMSO (negative control) had no notable zone of inhibition against either tested strain as depicted in Figure **5**. Antibacterial activity of S. mannii oil and hydrosol was greater against gram-negative *E. coli* ATCC 25922 compared to gram-positive *S. aureus* ATCC 11622. Generally, gram-positive bacteria have been identified demonstrate higher antimicrobial resistance compare to gram-negative bacteria [42].

In comparing the antibacterial activities of some plants used in Cameroon as medicine, Mbosso *et al*., [43] reported that cyclohexane extracts of *Solanecio mannii* were the most active against pathogenic bacteria among the examined plants. This could be attributed to the terpene compounds present in the leaves of *S. mannii* as shown in Table **1**. Terpene molecules identified in various plant volatile oil have been proved to show significant effects against varieties of food pathogens [44].

The oil's superior antimicrobial activity compared to the hydrosol can also be attributed to oxygenated terpenes like aristol-1(10)-en-9-yl isovalerate, τ-Muurolol, α-Cadinol, α-caryophylla-4(12),8(13)-dien-5-ol, Nerolidyl acetate and isophytol acetate. Oxygenated terpenes have been proven to show strong antibacterial activity against bacteria, especially gram-negative bacteria compared to hydrocarbon terpenes [45]. Nerolidol is approved by the US Food and Drug Administration (FDA) as a safe natural ingredient and has also been shown to exhibit antimicrobial effects against various bacterial strains including against *Staphylococcus aureus* and *Klebsiella pneumoniae* [46–48]. The

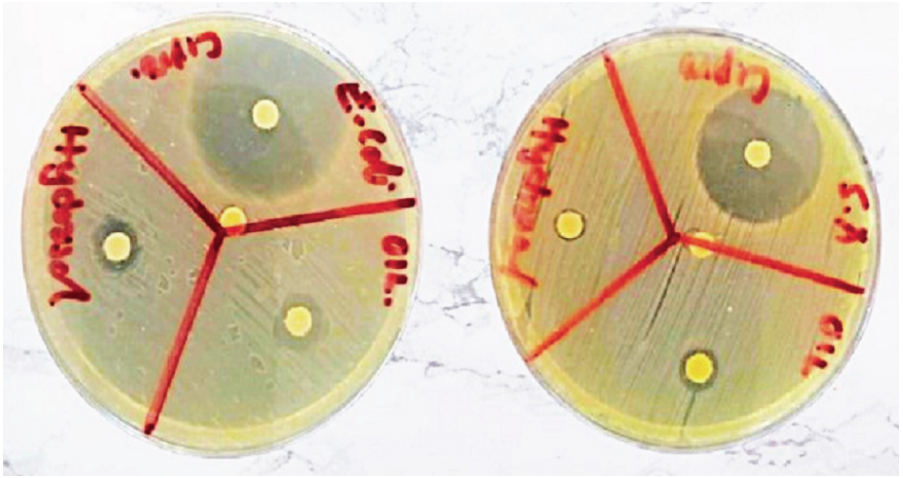

Figure 5: Zones of inhibition exhibited by *S. mannii* essential oil and hydrosol against *E. coli* and *S. aureus* respectively.

essential oil of *Solanecio gigas* were reported to be the most effective against E. coli *ATCC 25922* and *S. aureus ATCC 31488* bacteria [49].

The dominant compound in the oil, 6-(1,2-Dimethyl-1-propenyl)-4,5-diazaspiro[2.4]hept-4-ene (6) (44%), belongs to the spiro cyclopropanes group of compounds which exhibits desirable characteristics including antibacterial, antifungal and antiviral effects in many native plants [50]. Peysan *et al,* 2017 notes that cyclopropyl group containing spiro moieties exhibit interesting range of pharmacological characteristics including antibacterial inhibition in many plant materials [50].

Sesquiterpenes such as silphiperfola-4,7(14)-diene, 7-epi-sesquithujene, β-caryophyllene, α-Curcumene, γ-Curcumene and Germacrene D were also identified in the oil. Sesquiterpenes have an advantage in their use in food industry due to their nontoxic nature [51]. Minimum inhibitory concentration of β-caryophyllene sesquiterpene against gram positive *Bacillus cereus* was reported to be 2.5% (v/v) [52]. Umerenewaza *et al* [30] reported novel pyrrolizidine alkaloid, silphiperfolanol angelate ester compounds and several known secondary metabolites from dichloromethane extracts of *S. mannii*. These compounds showed weak antibacterial activities against *E. coli* and *Bacillus subtilis* and anticancer activity against MFC-7 breast cancer cells. Curcuminoids (such as α-Curcumene and γ-Curcumene) present in abundance in turmeric have been directly linked to the plant's excellent antibacterial capabilities [53].

Germacrene D sesquiterpene has been reported to display mild inhibitory effects compared to monoterpenes [54]. The compound 7-epi-Sesquithujene (10.33%), a bicyclic sesquiterpene isolated from phoebe oil, an essential oil of the Brazilian walnut tree [55]. In vitro assessment of antibacterial effects of acetone extracts of bisabol myrrh containing 8.8% 7-epi-sesquithujene revealed significant activity against a range of microbes with the best activity against *Candida tropicalis* [56]. Sesquithujene has also been reported in mature unripe bananas [57], and ginger water extracts [58,59] with proven antimicrobial effectiveness [60].

The moderate antimicrobial activity of *S. mannii* hydrosol (8-10 mm inhibition zones) (Figure **5**) can be attributed to its distinct chemical composition, which significant terpene compounds present in the hydrosol. The antimicrobial capacities of plant hydrolates generally depend on the chemical constituents that differ from plant to plant and part to part of the individual plant examined and on the pathogen of interest [17]. Oregano hydrosols have been reported to reduce *E. coli* and *S. aureus* on fresh-cut parsley [61].

Some chemicals identified in the *S. mannii* hydrosol could reveal the reason for their antimicrobial activity. For instance, the analysis showed 0.49 % of α-phellandrene terpene. In vitro and in vivo studies on α-Phellandrene have proved the compound's multi-pharmacological effects antibacterial and antioxidant capabilities [62]. The antimicrobial activity of present in the hydrosol can also be attributed to the abundance of FAMEs. Fatty acids methyl esters extracts have been reported to show antibacterial and antifungal activities [63-65].

Another compound of known antimicrobial effect identified in the hydrosol is the 5H-Tetrazol-5-amine (0.54%). Schiff bases of 5-amino tetrazole have been

reported to portray remarkable antibacterial and antifungal activity against a wide range of pathogens. These compounds exhibited superior antimicrobial capacities against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* compared to oxazepine products [66]. Mekheimer *et al*., [67] synthesized novel 5-amino tetrazole compounds which portrayed greater antimicrobial suppression against gram negative *Escherichia coli* and *Pseudomonas aeruginosa* compared to the standard drug ciprofloxacin [67].

This study, however, has several limitations that should be acknowledged. The absence of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) testing limits our understanding of the precise antimicrobial potency. Additionally, the study focused on only two bacterial strains, broader antimicrobial spectrum testing would provide more comprehensive insights.

4. CONCLUSION AND FUTURE PROSPECTIVES

This study highlighted the chemical composition and antimicrobial activity of steam distilled *S. mannii* leaf oil and hydrolates using GC/MS and antimicrobial testing. GC/MS revealed sesquiterpenes and monoterpenes as the major components of the oil while the hydrosol contain mainly of FAMEs. Results revealed that both *S. mannii* oil and hydrosol possess antimicrobial effectiveness against both gram-positive and gram-negative bacterial, though lower than that of ciprofloxacin at 10 µL volumes. The antimicrobial effectiveness of the *S. mannii* leaves oil was superior found to that of the hydrosol, attributed to their distinct chemical compositions.

The essential oil of *S. mannii* exhibited promise as a clean label ingredient for application in the food industry; however, further research on formulation stability, cytotoxicity, and packaging integration is needed. Future research should focus on determining the minimum inhibitory concentrations and maximum inhibitory zones of both *S. mannii* oil and hydrosol. This may contribute significantly to the food industry's standard maintenance of food safety and quality.

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DATA AVAILABILITY STATEMENT

All the data used in this work has been supplied in the manuscript and any other supplementary materials attached.

DISCLOSURE STATEMENT

The authors declare no potential conflicts of interest associated with this work.

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