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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS FROM SELECTED SPECIES OF THE GENUS *CUCUMIS* IN ETHIOPIA

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ABSTRACT. Chemical composition of the essential oils obtained by hydrodistillation from various parts of *Cucumis ficifolius*, *Cucumis dipsaceus* and *Cucumis prophetarum* were determined by gas chromatography-mass spectrometry (GC/MS) analysis. Compounds 3,7,11,15-tetramethyl-1-hexadecanol, neophytadiene, and isophytol from leaves, phytol and octacosane from the stems, hentriacontane from the fruits, and octacosane, abietadiene from roots were identified the major constituents of *Cucumis ficifolius*. Hydrocarbons including Octacosane, tricosane, tetracosane, hentriacontane and hexacosane were the principal components of various parts of *Cucumis. dispaceus*. While leaves, stems and roots of *Cucumis prophetarum* contain mainly hentriacontane, neophytadiene and octacosane, respectively. The oils were evaluated for their antibacterial activity using disc diffusion method against four bacterial pathogens including *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*. All tested essential oils were sensitive against the bacterial strains. The essential oil from the leaves of *Cucumis dispaceus* exhibited the highest inhibition zone against *E. coli* (11.87±2.42 mm) while the other displayed modest activity compared with the positive control, ceftriaxone. Therefore, the antibacterial activities displayed by the essential oils along with the literature reported for their constituents suggest the use of these plants as an antibacterial agent. Furthermore, the results presented herein also support the traditional use of these plants against bacteria.

KEY WORDS: *Cucumis ficifolius*, *Cucumis dipsaceus*, *Cucumis prophetarum*, *Antibacteria*

INTRODUCTION

Essential oils are complex mixtures of volatile substances present at low concentrations in the flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots of plant materials. Essential oils, named as essence, fragrant oil, volatile oil, etheric oil, petroleum or aromatic oil, are obtained from natural raw material either by distillation or mechanical pressing [1-3]. The essence or aromas of plants are due to volatile or essential oils, many of which have been valued since antiquity for their characteristic odors. The essential oils have characteristic fragrances and tastes of mixtures of known and unknown compounds [4]. They have wide applications in food as spices, perfumery industry as fragrances and not the least of which is in pharmaceutical industry as medicines [5].

Essential oils have been used for the treatment or betterment of various diseases including, respiratory tract infections, colds, inhalation therapy (to treat acute and chronic bronchitis), acute sinusitis, abdominal pain, abscess, acne, fever, flu, headaches, gingivitis, bronchitis, bruises, burns, influenza, insect bites, insomnia, shock, sinusitis, sore throat, constipation, coughs, cuts, diarrhea, wounds and toothache [6]. Hence essential oils have attracted strong interest among researchers due to their antimicrobial activities for the need of substituting synthetic antibiotics. Some of the traditional uses of essential oils are supported by scientific investigation including antimicrobial [4, 7, 8], antibacterial [9], antiviral [10], antimycotic [11], ant-toxigenic [12], antiparasitic [13], and insecticidal [14] properties. Therefore, the trend of using essential oils as

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natural antimicrobial agents is gradually becoming an attractive approach in the field of drug discovery [15]

The genus *Cucumis* (Cucurbitaceae) comprises of about 52 species [16], indigenous to tropical Africa, also Asia, Australia and some islands in the Pacific. Results obtained in the survey conducted by different researchers indicated that plants of the genus *Cucumis* are traditionally been used to treat various life threatening diseases wounds, burns, cancer, cholera, diabetes, ear disorders, eye disorders, fever, stomachache, gastro-intestinal, goiter, heart disorders, hepatic disorders, infertility, inflammation, malaria, menstrual disorders, paralysis, pox, respiratory tract disorders, skin disorders, sexual disorders, sexually transmitted diseases, sun stroke, tetanus, tuberculosis, typhoid, and vomiting [17]. The major principal secondary metabolites reported from some species of the genus *Cucumis* are the triterpenes like compounds named as cucurbitacins [17, 18]. Furthermore, few plants of the genus *Cucumis* including *Cucumis melo* was reported to have essential oils [19]. Therefore, the immense biological activity of some species of plants in this genus might be accounted to the presence of cucurbitacins and essential oils.

Cucumis ficifolius, *Cucumis dipsaceus* and *Cucumis prophetarum* are some of the plant species in the genus *Cucumis* and traditionally used in Ethiopian folk medicine for the treatments of a wide array of diseases. In recent years, various natural products including plant essential oils have been studied for their antibacterial activities against various arrays of pathogenic bacteria. Despite all these efforts, infectious diseases caused by microorganisms continue to pose a threatening challenge to public health all over the world. The problem is exacerbated by increasing rate of resistance of disease caused by microorganisms to conventional antibiotics [20, 21]. One of the major reasons attributed to the antibiotic resistance crisis is due to the lack of new drug development by the pharmaceutical industry [22]. This, therefore, calls for continuous search for affordable, safe and effective drugs from natural products. Therefore, the absence of literature report on the biological activities of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* prompts us to explore the essential oil composition and antibacterial activities of these plants. Hence, in the present study we report for the first time the GC-MS analysis of the essential oils from roots, fruits, stems and leaves of *C. ficifolius*, *C. dispaceus* and *C. prophetarum*. Also incorporated herein is the antibacterial activity of the essential oils obtained from *C. ficifolius*, *C. dispaceus* and *C. prophetarum.*

EXPERIMENTAL

General

The chemicals used in this work include water, chloroform, and anhydrous sodium sulfate. Milling machine and Whatman no 1 filter paper were used for powdering sample and filtration, respectively. Separatory funnel as used to separate the organic component from the aqueous layer while rotary evaporator (Heidolph Laborata 4000) was used for solvent evaporation.

Plants collection, authentication and preparation

Leaves, fruits, seeds, roots, stems of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum* were collected from Dera town (latitude 08º 20 N and longitude 39º 19 E), Arsi Zone, Oromia region, Ethiopia, in August 2020. The plant materials were authenticated by a botanist Mr. Melaku Wendafirash and voucher specimen numbers CF001, CD002 and CP003 for *C. ficifolius*, *C. dispaceus* and *C. prophetarum* respectively were deposited at the National Herbarium of Addis Ababa University. The collected plant materials were washed with distilled water to remove any dirt, and dried under shade at room temperature in the Chemistry Laboratory of Adama Science and Technology University. The dried plant parts were ground into fine powder by using a dry blender and stored in polyethylene bag at room temperature prior to extraction.

Extraction of essential oils

The air-dried ground leaves part of *C. ficifolius* (50 g) were hydrodistilled using Clevenger type apparatus for 3 h. The distillate was extracted with chloroform and separated by separatory funnel. It was dried over anhydrous sodium sulphate, filtered and concentrated. The distillate was preserved in a sealed sample tube and stored under refrigeration at 4 ºC until analysis. This was repeated for the leaves, fruits, roots, stems of *C. ficifolius*, *C. dipsaceus* and *C. prophetarum.*

GC-MS analysis of essential oils

The analysis of the essential oils of *C. ficifolius*, *C. prophetarum* and *C. dispaseus* was done using Gas Chromatography-Mass Spectrometry Agilent 7890B series GC equipped with mass selective detector [(MSD), Agilent 5977 series operated in the EI mode (electron energy = 70 eV), Scan range = 40-500 amu and scan rate = 10 scans/s] and an Agilent Chem Station Data System. The GC column was an Agilent-5 fused silica capillary with a 5% phenyl-polymethyl siloxane stationary phase, 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 constant flow rate of 1 mL/min. Inlet (injector) temperature was 200 °C; ion-source (interface) temperature was 280 °C. The GC oven temperature program was used as follows: 50 °C initial temperature, hold for 2 min with an increase of 2 °C/min, to 150 °C, increased 5 °C/min, to 220 °C, increased 10 °C/min, to 250 °C and isothermal at 280 °C. Total GC running time was 70 min. A 1% w/v solution of the sample in *n*-hexane was prepared and an injection volume of 1 μL was employed using a split ratio of 1:20.

Retention indices (RI)

Retention indices (RI) using *n*-alkanes were used as the basis. The percent of each compound was based on the peak area divided by the total area of component peaks. The temperature range was from 50-250 °C, with a temperature program rate as follows: 50 °C initial temperature, hold for 2 min with an increase of 2 °C/min, to 150 °C, increased 5 °C/min, to 220 °C, increased 10 °C/min, to 250 °C and isothermal at 280 °C starting at three minutes and finishing at 70 min. The pressure applied in this experiment was 48.7 kpa with a total flow of 50 mL/min and 1 mL/min of column flow. The injection, ion source and the interface temperatures were 200 °C, and 280 °C, respectively. Retention indices (RI) were calculated using the following formula:

$$\textbf{Retention indices (RI)} = 100\left[\frac{(tR - tRz)}{(tR(z+1) - tRz)} + z\right]$$

where RI: retention index value of unknown compound (peak), z: the number of carbons in the alkane preceding unknown compound, tR: the retention time of unknown compound, tRz: the retention time of the preceding alkane and tR(z+1): the retention time of the following alkane.

Identification of components

The components of essential oil were identified on the basis of comparison of their retention indices and mass spectra with published data [23] and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST3.0) libraries provided with computer controlling the GC-MS system, in Adama Science and Technology University, Ethiopia. The spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained. Furthermore, the retention index of each compound was compared with the same compound reported in the literature.

Antibacterial activity

The antibacterial activity of samples was investigated using agar disc diffusion method against four bacterial strains (two Gram negative: *Escherichia coli* and *Pseudomonas aeruginosa* and two Gram positive: *Staphylococcus aurous* and *Streptococcus pyogenes*) obtained from Adama Regional Laboratory. Appropriate colonies of required bacterial strains were selected and standardized with 0.5 McFarland standards turbidity by using UV-Vis spectrophotometer at 625 nm wavelength with absorbance of 0.111 and evaluated *in vitro* using the agar disc diffusion method. Centrifuged pellets of bacteria from a 24 h old culture containing 1.5 x 10^8 CFU/mL was spread using cotton swab on the surface of the nutrient agar (NaCl 0.5%, tryptone1%, yeast extract 0.5%, agar 1%, 1000 mL of distilled water, pH 7.0) which was autoclaved at 121 °C for 20 min then cooled down to 45 °C, 25 mL poured into each Petri dishes and allowed to settle. The essential oil samples were prepared at different concentration of 200, 100 and 50 µg/mL in dimethyl sulfoxide (DMSO). After solidification of media on Petri dishes, the samples (each 8 µL) were loaded onto each paper disk and then placed on to the plate. DMSO has loaded as the negative control. All plates were observed for a zone of inhibition at 35 °C for 18 h. The activity was determined by measuring the diameter of the inhibition zone (in mm). Each inhibition zone was measured by using antibiotic zone reader digital instrument (OK Tested, Optics Technology, Delhi-110034). The experiment was conducted in triplicates and results were reported as Mean ± Standard Deviation (M±SD). Ceftriaxone and DMSO were used as positive and negative controls, respectively.

RESULTS AND DISCUSSION

Yield of essential oil

The yields of the essential oils from *C. prophetarum*, *C. ficifolius* and *C. dispaseus* were calculated in relation to dry weight plant materials. The highest yields were obtained from *C. prophetarum* stem (0.25% w/w) and lowest yields were obtained from *C. ficifolius* root (0.02% w/w). The results obtained presented as there is no much difference in the yields of the essential oils among different parts of *C. prophetarum*, *C. ficifolius* and *C. dispaseus*. However, the essential oil yield of *C. prophetarum* was 0.25% w/w. This is in fact superior than the essential oil obtained from *C. ficifolius* and *C. dispaseus* (Table 1). The oils exhibited a pale yellow colour with a pleasant odor. The essential oils were less dense and insoluble in water. But it was miscible in DMSO, *n*-hexane and chloroform.

Table 1. Essential oil yields (% w/w) of different parts of some species in the genus *Cucumis* (Cucurbitaceae).

Botanical name	Parts used	Yield (% w/w
C. ficifolius	Leaves	0.16
	Fruits	0.12
	Stems	0.12
	Roots	0.02
C. prophetarum	Leaves	0.25
	Stems	0.25
	Roots	0.21
C. dispaseus	Leaves	0.15
	Fruits	0.20
	Stems	0.12

Chemical composition of essential oil

The essential oils compositions of different parts of *C. ficifolius*, *C. prophetarum* and *C. dispaseus* were analyzed using GC-MS. The results revealed the presence of 50, 21 and 19 components in various parts of *C. ficifolius, C. dispaseus* and *C. prophetarum*, respectively.

Composition of the essential oil of Cucumis ficifolius

The chemical composition of the essential oils of the leaves, stems, fruits and roots *C. ficifolius* were analyzed by GC-MS, which allowed identification of 50 compounds (Table 2). The constituents were identified based on their retention indices and compared with the retention indices reported in the literature for the same compound. Excluding linoleic acid, all the essential constituents are new to the genus *Cucumis.*

Table 2. Compounds identified from the essential oil of various parts of *C. ficifolius.*

No.	Chemical composition	RT	RI	RI of same compound in lit.	% Peak area			
					Leaves	Stems	Fruits	Roots
1	Morpholine[2]	3.509	96	-	0.006	0.24	0.01	0.36
2	2-Heptanol[2]	4.26	899	899.4	-	0.10	-	0.17
3	Hexanoic acid[2]	7.211	997	996.4	0.009	0.21	-	0.49
4	delta-3-Carene[2]	8.684	1029	1011.3	0.05	1.23	-	1.34
5	Pinene hydrate, trans-[2]	13.287	1121	1121.2	0.06	0.52	-	1.26
6	Citronellal[2]	15.314	1154	1153.7	0.03	0.16	-	0.74
7	Linalool oxide (pyranoid), trans-[2]	16.319	1171	1171.0	0.39	-	-	-
8	Phenol,2-(1-phenylethyl)[2]	17.965	1199	-	-	0.29	-	0.21
9	Isobornyl acetate	23.625	1286	1285.9	0.62	0.81	-	3.12
10	Indole[2]	24.439	1298	1298.4	-		-	0.81
11	Carvacrol[2]	24.445	1299	1300.4	0.04	0.88	-	-
12	Undecanal[2]	24.912	1306	1306.5	0.016	0.21	-	-
13	Piperitenone[2]	27.361	1343	1340.7	0.19	-	-	-
14	Cyperene[2]	30.971	1398	1397.8	-	0.19	-	-
15	Thujopsene, cis-[2]	33.287	1435	1432.0	0.058	-	-	2.35
16	Benzyl (S,E)-2-methyldec-3-enoate[2]	36.781	1490	-	0.1	-	-	-
17	1-epi-acetoxy-2-(1-methylethenyl)-5-methyl-cyclohexane[2]	41.632	1570	-	0.11	-	-	-
18	Humulene epoxide II[2]	43.862	1607	1604.7	0.18	-	-	3.28
19	Bicyclo [4.2.1] nonan-1-ol[2]	44.266	1614	-	3.93	-	0.03	2.30
20	γ-Eudesmol, 10-epi[2]	44.595	1620	1618.7	0.59	-	-	-
21	2-(2-Furyl)-1-nitrocyclohexane[2]	47.904	1677	-	0.1	-	-	-
22	Hexadecanal[2]	55.620	1823	1816.5	0.12	-	-	-
23	Methyl hexadecanoate[2]	59.744	1939	1942.5	0.11	-	-	13.2
24	Isophytol[2]	59.934	1945	1946.5	8.07	-	-	6.85
25	3-(Benzyloxycarbonyl)-1-(p-toluenesulfonyl)-5,6-dihydro-2(1H)-2-pyridone[2]	60.258	1956	-	-	-	-	0.38
26	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester[2]	60.552	1967	-	3.23	-	0.02	9.83

27	Tetracosane[2]	61.494	1999		0.05	-	-	-
28	Manool, 13-epi-[2]	61.777	2010	2010.2	1.62	-	-	-
29	Pentadecanoic acid, 14-methyl-, methyl ester[2]	62.158	2026		1.34	-	-	0.47
30	(S)-Methyl 1-(2,5-dimethoxyphenyl)-1H-benzo[f]chromene-3-carboxylate[2]	62.291	2031	-	-	2.23	-	-
31	Neophytadiene	62.672	2047	-	0.41	-	-	-
32	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-[2]	63.209	2069	2080	4.48	54.90	-	-
33	Abietadiene[2]	63.711	2090	2080.5	0.26	-	-	14.15
34	Methyl oleate[2]	63.810	2094	2096.1	0.31	-	-	-
35	2-hexyldecan-1-ol[2]	63.948	2100		0.23	-	-	-
36	Linoleic acid [1]	64.572	2129	2128.9	0.5	4.56	-	-
37	1-Dotriacontanol[2]	66.085	2199		-	-	-	0.76
38	(S)-Methyl 1-(2,5-dimethoxyphenyl)-1H-benzo[f]chromene-3-carboxylate[2]	66.316	2212	-	0.71	-	-	-
39	Carbonic acid, eicosyl vinyl ester[2]	66.767	2236	-	6.18	-	-	-
40	1-Hexadecanol, 3,7,11,15-tetramethyl-[2]	67.414	2270	2111	57.08	1.35	-	-
41	2-hexyl-1-Decanol[2]	68.003	2302	-	0.48	2.95	-	-
42	1-Decanol, 2-hexyl-Pentacosane[2]	68.436	2331	-	1.08	-		-
43	Neophytadiene[2]	68.621	2345	-	12.42	-	-	-
44	9-Eicosyne[2]	68.794	2355	-	-	0.86	-	-
45	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-[2]	68.823	2357	-	0.5	-	-	-
46	7-Hexadecenal [2]	68.990	2369	-	1.51	-	-	-
47	Benzyl methyl ether[2]	69.025	2371	-	-	5.64		-
48	(E)-14-methylhexadec-8-enal[2]	69.158	2380	-	0.93	-	-	-
49	Octacosane[2]	69.273	2388	-	0.90	13.55	-	37.8
50	Hentriacontane[2]	69.510	2399	-	0.38	9.09	99.76	-

[1]Compounds reported from the genus; [2]New to the genus.

The principal components of the essential oils of the leaves of *C. ficifolius* were found to be 3,7,11,15-tetramethyl-1-hexadecanol (57.08%) **(40)**, neophytadiene (12.42%) **(43)**, isophytol (8.07%) **(24)**, eicosyl vinyl ester carbonic acid (6.18%) **(39)**, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(4.48%) **(45)**, bicyclo [4.2.1] nonan-1-ol (3.93%) **(19)**. Most of the chemical constituents identified from the leaves are mainly hydrocarbons (27.5%), alcohols (25%), carboxylic acids (15%), aldehyde (12.5 %), esters (12.5%) and ketone (7.5%).

As displayed in Table 2, the second principal component of the essential oil of the leaves of *C. ficifolius* is neophytadiene. Various scientific reports revealed that this compound had antipyretic, anti-inflammatory, anti-microbial and antioxidant activity [24]. Therefore, the presence of this compound in the leaves of this plant is one positive attributes of *C. ficifolius*. The stem of *C. ficifolius* showed the presence of 20 components with the major constituents found to be 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-2-hexadecen-1-ol (54.90%) **(45)**, octacosane (13.55%) **(49)**,

hentriacontane (9.09%) **(50)**, Benzyl methyl ether (5.64%), **(47)**, linoleic acid (4.56%) **(36)**, and 2-hexyl-1-decanol (2.95%) **(35)**. The principal components of the stem of *C. ficifolius* are mainly categorized under alcohols and hydrocarbons. 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-2-hexadecen-1-ol is a diterpene reported to have immense biological activity including anti-microbial, antioxidant, anti-tumor, anti-cancer, anti-arthritic, immunostimulatory, anti-diabetic, chemopreventive, pesticidal and diuretic agent and has sunscreen properties [25]. Linoleic acid was reported to be used as an emollient and thickening agent in cosmetics, antioxidant and an anti-inflammatory agent in the treatment of burns, cold sores and other minor wounds [26].

The GC-MS analysis of the fruits of *C. ficifolius* revealed the presence of four components with the major constituents found to be the hydrocarbon hentriacontane (99.76%) **(50)**. The roots of *C. ficifolius* showed the presence of 20 components with the major constituents found to be octacosane (37.8%) **(49)**, abietadiene (14.15%) **(33)**, methyl hexadecanoate (13.2%) (**23)**, bis(2-methylpropyl) ester-1,2-benzenedicarboxylic acid (9.83%) **(26)**, isophytol (6.85%) **(79)**, humulene epoxide II (3.28%) **(18)**, isobornyl acetate (3.12%) **(9)**, and thujopsene, cis-(2.35%) **(15)**. The major components in the roots of *C. ficifolius* are mainly hydrocarbons, alcohols and esters. The number of components detected in the leaves of the essential oil of *C. ficifolius* was superior to those detected from the stems, roots and fruits (Table 2). The least number of components was identified from the essential oil of the fruit of *C. ficifolius*. Literature report showed that the seeds of *Cucumis melo* had 40 components with the highest yield identified as 9,12-octadecadienoic acid, methyl ester (15.27%) [27]. This compound was also identified from the leaves and stems of *C. ficifolius* but the composition is inferior compared with the one reported from *C. melo.*

Composition of the essential oil of C. dispaceus

The chemical composition of the essential oils of the leaves, stems and fruits of *C. dispaceus* were analyzed by GC-MS which led to identification of 21 components (Table 3). Excluding linoleic acid, the remaining compounds presented in Table 3 are new to the genus *Cucumis*.

Table 3. Compounds identified from the essential oils of different parts of *C. dispaceus*.

No.	Chemical composition	RT	RI	RI of same compound in lit.	Percent peak area		
					Leaves	Stems	Fruits
51	Verbenol, cis-[2]	14.73	1145	-	-	-	0.1
7	Linalool oxide (pyranoid), trans-[2]	16.301	1171	1171.0	0.19	-	-
9	Isobornyl acetate[2]	23.63	1286	1285.9	0.11	0.1	1.66
11	Carvacrol[2]	24.44	1299	1300.4	0.009	0.02	0.1
12	Undecanal[2]	24.918	1306	1306.5	0.005	0.03	0.1
52	δ-Elemene[2]	27.026	1337	1337.0	0.1	-	-
15	Thujopsene, cis-[2]	33.287	1435	1432.0	0.02	0.04	
53	Geranyl butanoate[2]	41.037	1560	1562.5	-	-	0.11
18	Humulene epoxide II[2]	43.86	1607	1604.7	0.03	0.16	-
19	Bicyclo [4.2.1] nonan-1-ol[2]	44.27	1614	-	0.29	-	-
23	Methyl hexadecanoate[2]	59.744	1939	-	0.12	0.02	-
24	Isophytol[2]	59.934	1945	1946.5	1.15	-	-
26	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester[2]	60.55	1967	-	0.36	-	-
54	Tricosane[2]	62.23	2029	-	33.40	-	-
33	Abietadiene[2]	63.711	2090	2080.5	0.20	-	-
36	Linoleic acid[1]	64.555	2129	2128.9	-	0.15	-

55	Ethyl linoleate[2]	66.899	2242	2158.8	0.17	-	0.02
56	Tetracosane[2]	67.37	2268	2400	32.49	-	-
57	Hexacosane[2]	67.604	2280	-	0.1	98.87	-
49	Octacosane[2]	69.27	2388	-	0.22	-	-
50	Hentriacontane[2]	69.91	2399	-	30.61	0.25	96.65

[1]Compounds reported from the genus; [2]New to the genus.

The leaves of *C. dispaceus* revealed the presence of 18 components with the major constituents found to be tricosane (33.40%) **(54)**, tetracosane (32.49%) **(56)**, hentriacontane (30.61%) **(50)** isophytol (1.15%) **(24),** and Bis(2-methylpropyl) ester-1,2 Benzenedicarboxylic acid (0.36%) **(26)**. The compounds identified from the leaves of *C. dispaceus* are mainly hydrocarbons. The fruits of *C. dispaceus* showed the presence of seven components with the major constituents found to be the hydrocarbon hentriacontane (96.65%) **(50)** and the ester isobornyl acetate (1.66%) **(9)**. The stem of *C. dispaceus* showed the presence of nine components with the major constituents found to be the hydrocarbon hexacosane (98.87%) **(57)**. The constituents of the essential oils of the fruits of *C. dispaceus* were found to be inferior to the number of components detected from the leaves and stems while the components in the leaves were superior to those identified from the stems and fruits.

Composition of the essential oil of C. prophtarum

The essential oil constituents of *C. prophetarum* were analyzed with GC-MS which has led to the identification of nineteen (19) components (Table 4).

Table 4. Compounds identified from the essential oil of various parts of *C. prophtarum.*

No.	Chemical composition	RT	RI	RI of same compound in lit.	Percent peak area		
					Leaves	Stems	Roots
1	Morpholine[2]	3.51	96	-	0.01	0.01	0.62
58	1-(4-methylcyclohex-3-enyl) ethanol[2]	10.49	1067	-	-	0.13	-
59	2-Phenylethyl acetate[2]	21.811	1258	1258.8	-	0.02	0.53
9	Isobornyl acetate[2]	23.625	1286	1285.9	-	1.1	-
15	Thujopsene, cis-[2]	33.287	1435	1432.0	-	-	1.57
18	Humulene epoxide II[2]	43.861	1607	1604.7	-	-	9.90
19	Bicyclo [4.2.1] nonan-1-ol[2]	44.271	1614	-	0.1	0.12	-
23	Methyl hexadecanoate[2]	59.732	1939	-	-	-	4.87
24	Isophytol[2]	59.934	1945	1946.5	-	0.38	-
25	3-(Benzyloxycarbonyl)-1-(*p*-toluenesulfonyl)-5,6-dihydro-2(1H)-2-pyridone[2]	60.258	1956	-	-	0.1	-
26	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester[2]	60.558	1967	-	0.02	0.67	2.73
60	Hexadecanoic acid, methyl ester[1]	62.158	2025	-	-	0.05	0.75
33	Abietadiene[2]	63.711	2090	2080.5	-	0.03	8.22
50	Hentriacontane[2]	65.586	2176	-	99.75	-	1.18
37	1-Dotriacontanol[2]	66.045	2199	-	-	0.01	-
55	Ethyl linoleate[2]	66.879	2242	2158.8	-	-	17.79
43	Neophytadiene[2]	68.638	2345	-	-	96.31	-
61	(E)-14-methylhexadec-8-enal [2]	69.158	2380	-	0.01	0.1	-
49.	Octacosane[2]	69.27	2388	-	-	-	51.79

[1]Compounds reported from the genus; [2]New to the genus.

The GC-MS analysis of the leaves of *C. prophetarum* revealed the presence of five components with the major constituents found to be the hydrocarbon hentriacontane (99.75%) **(50)**. Hentriacontane, has reported to have various pharmacological activities including anti-inflammatory, antitumor and antimicrobial activities [28]. The presence of this compound is one positive attributes of this plant against various deleterious diseases. The stem of *C. prophetarum* showed the presence of 13 components with the major constituents found to be neophytadiene (96.31%) **(43)**. This compound was also identified as a major constituent in the leaves of *C. ficifolus*. The second component detected from the stem of *C. prophetarum* was isobornyl acetate (1.1%) **(9)**. Isobornyl acetate's aroma makes it a common ingredient of perfumes used in air fresheners, cleaners, and personal care products. It is reported to have anti-inflammatory, analgesic, antibiotic, and sedative properties [29].

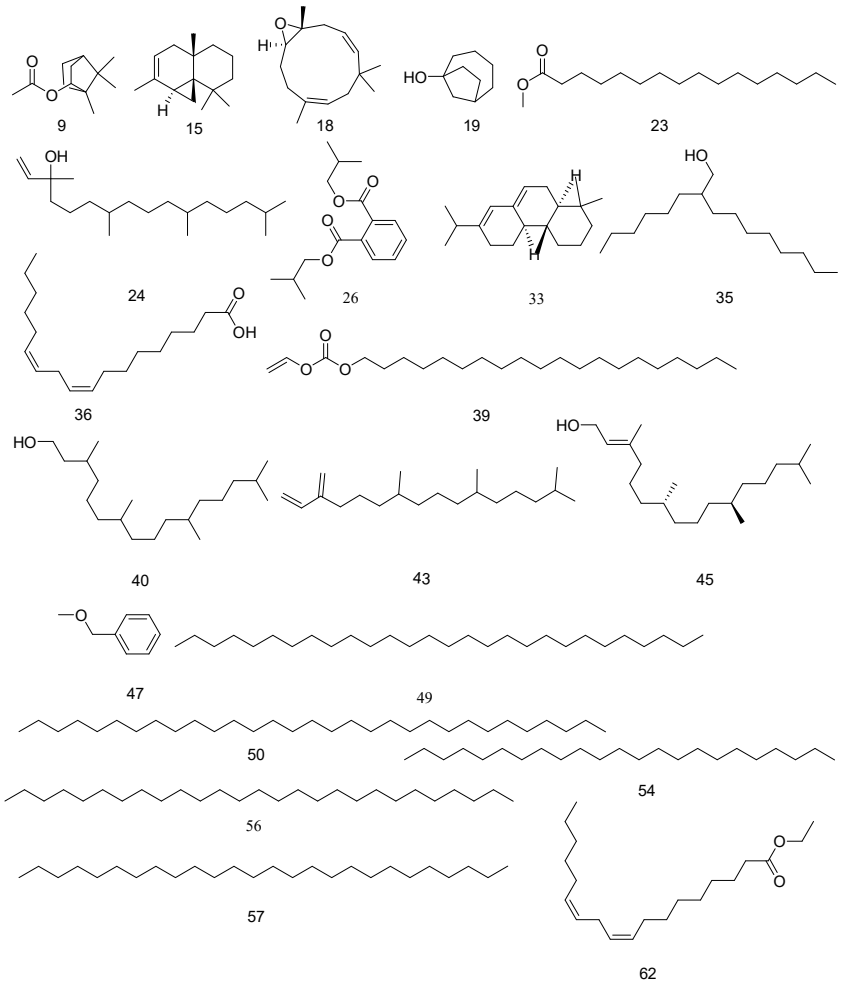

Figure 1. Structures of the major constituents of the essential oils of C. *ficifolius, C. dispaceus* and *C. prophtarum.*

The essential oils of the roots of *C. prophetarum* also showed the presence of 11 components with the major constituents found to be octacosane (51.79%) **(49)**, ethyl linoleate (17.79%) **(62)**, humulene epoxide II (9.90%) **(18)**, abietadiene (8.22%) **(33)**, methyl hexadecanoate (4.87%) **(23)** and Bis(2-methylpropyl) ester-1,2-Benzenedicarboxylic acid (2.73%) **(26)**, thujopsene, cis- (1.57%) **(15)** and hentriacontane (1.18%) **(50)**. The structures of some of the major constituents are depicted in Figure 1.

This study further found out that the essential oil compositions of the various parts of *C. ficifolius, C. dispaceus* and *C. prophetarum* were significantly different. The difference in the composition of these plants arises as a result of sister species used in the study. Same factors were reported by Lis-Balchin while analyzing the chemical composition of the essential oils from different *Lavandula* species [30]. This study further demonstrated that the leaves, fruits, roots and stems of the same species of the plants of the genus *Cucumis* studied herein displayed different percentage of chemical compositions. The result is in good agreement with the study reported by Bouyahya, *et al*., on leaves and fruits of *Pistacia lentiscus* which revealed differences among intra species [31]

Antibacterial activity of essential oils

In this study, the bacterial activity of essential oil of the leaves, stems, fruits and roots of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* were evaluated using agar disc diffusion method against four bacterial strains (two Gram negative: *Escherichia coli* and *Pseudomonas aeruginosa* and two Gram positive: *Staphylococcus aureus* and *Streptococcus pyogenes*) in Adama regional laboratory. Table 5 show the antibacterial activity of the essential oils of the leaves, stem, fruit and root of the three plants against four pathogenic bacteria species. The essential oil of these three plant species showed varying degree of inhibitory activity against *E. coli*, *P. aeruginosa*, *S. aureus and S. pyogenes* at different concentrations.

Among the essential oil of the three plants, *C. dispaceus* fruit exhibited the highest inhibition zone against *E. coli* (11.87 ± 2.42 mm) at concentration of 100 µg/mL and 50 µg/mL (11.27 ± 1.67). Whereas essential oil from root *C. prophetarum* was the second with inhibition zone of 11.75 ± 0.07mm against *E. coli* at 100 µg/mL and 11.0 ±1.13 at concentration 200 µg/mL. The least inhibition zone was observed with the fruit of *C. dispaceus* and root *C. prophetarum* against *S. aureus* and *S. pyogenes* with inhibition diameter of 6.0 ± 0.06. It was found out that the activity of the essential oil in most cases inhibit the bacterial pathogens in a dose dependent manner. The essential oil of fruit of *C. dispaceus* was ineffective against *S. aureus* and *P. aeruginosa*. *E. coli* was the most susceptible test organism for all essential oil from all plants. *P. aeruginosa* and *S. pyogenes* was the second susceptible test organism for the stems and leaves of *C. dispaceus* and leaves of *C. prophetarum. S. aureus* was relatively the least susceptible test organisms displaying least inhibition zones by the essential oil constituents of C. *ficifolius* stem, *C. dispaceus* stem and leaves, and *C. prophetarum* stems and roots. The *in vitro* antibacterial activity evaluation of the essential oil of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* were compared with *C. melo*. Results showed that the essential oil of *C. melo* showed comparable antibacterial activity with the essential oil of *C. ficifolius, C. dispaceus and C. prophetarum* against three strains of Gram-positive and Gram-negative bacteria [27].

The activity displayed herein by the essential oils of the three species of *Cucumis* is superior in Gram negative bacteria (*E. coli*) than Gram positive bacteria. This is against findings reported by Bouyahya *et al*. where Gram-negative bacteria were turned out to be resistant to the essential oils of *Centaurium erythraea* [31]. However, similarities to our finding were also reported by the same author on the essential oils of *O. compactum* which found out significant antibacterial activity against *E. coli*, Gram negative bacteria [15]. Since, the essential oils of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* had several kinds of chemical constituents; it seems very difficult to attribute the activity to one component alone. Some of the chemical constituents responsible

for the antibacterial activity of the essential oil of *C. erythraea* and *O. compactum* were detected in the essential oils of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* [32]. Hence, the action of essential oils against the tested bacteria might be via membrane permeability, enzymes inhibitory and morphological perturbation of the bacteria. Furthermore, the mechanism of action of the essential oil against *E. coli* may be due to the irreversible damage caused by the essential oils on the cell wall and membrane, leading to the leakage of proteins and genetic materials (DNA and RNA). This agrees very well with the finding reported by Bouyahya *et al.* [15].

Table 5. The effect of essential oil from the leaves, stem, fruit and root of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* against the bacterial test organism.

Sample	Plant parts	Conc. µg/mL	Inhibition zone in mm (Mean ± Standard Deviation)			
			E. coli (ATCC-25922)	*P. aeruginosa* (*ATCC-27853*)	*S. aureus* (*ATCC-25923*)	*S. pyogenes* (*ATCC-19615*)
C. ficifolius	Leaves	200	7.87 ± 0.31	6.6±0.20	6.3 ± 0.15	7.13±0.21
		100	7.73 ± 0.31	6.27±0.23	6.13 ± 0.12	6.60±0.26
		50	7.1 ± 0.95	6.27±0.23	6.13 ± 0.12	6.50±0.36
	Stem	200	6.9 ± 0.85	7.6 ± 1.4	7.2 ± 0.3	6.83 ± 0.67
		100	7.2 ± 1.38	7.6 ± 0.56	6.37 ± 0.15	7.80 ± 0.96
		50	6.63± 0.57	6.33 ± 0.31	6.1 ± 0.1	6.47 ± 0.32
	Fruit	200	7.0 ± 0.92	6.43 ± 0.35	7.07 ± 0.57	6.80 ± 0.44
		100	6.13± 0.12	6.0 ± 0.0	6.5 ± 0.62	6.30 ± 0.2
		50	6.07± 0.12	6.13 ± 0.12	6.27 ± 0.46	6.17 ± 0.15
	Root	200	7 ± 0.92	6.43 ± 0.35	7.07 ± 0.57	6.80 ± 0.44
		100	6.13± 0.12	6.00 ± 0.0	6.5 ± 0.62	6.30 ± 0.20
		50	6.07± 0.12	6.13 ± 0.12	6.27 ± 0.46	6.17 ± 0.15
C. dispaceus	leaves	200	10.0 ± 1.3	9 ± 4.1	6.43 ± 0.75	8.27 ± 2.56
		100	11.87± 2.42	7.5 ± 2.18	6.17 ± 0.21	7.2 ± 1.99
		50	11.27± 1.67	7.5 ± 1.95	6.27 ± 0.38	7.0 ± 1.56
	Stems	200	7.53 ± 0.58	7.87 ± 0.40	7.4 ± 1.15	8.50 ± 0.56
		100	6.93 ± 0.12	7.53 ± 1.31	7.2 ± 0.8	7.6 ± 0.72
		50	6.93 ± 0.38	6.83 ± 0.29	8.33 ± 1.80	8.13 ± 1.10
	Fruit	200	8.63± 2.55	6.00 ± 0.00	6.0 ± 0	6.03 ± 0.06
		100	10.53 ± 0.35	6.00 ± 0.00	6.0 ± 0	6.07 ± 0.12
		50	11.03 ± 0.70	6.0 0± 0.00	6.0 ± 0	6.03 ± 0.06
C. prophetarum	Leaves	400	9.2 ± 3.29	9.40 ± 3.63	7.43 ± 1.0	6.20 ± 0.10
		200	8.5 ± 3.90	8.20± 3.12	6.5 ± 0.44	6.10 ± 0.10
		100	8.23 ± 3.53	7.77 ± 2.72	6.23 ± 0.32	6.00 ± 0.00
	Stem	200	7.03 ± 0.50	6.67 ± 0.35	7.7 ± 0.4	7.40 ± 0.96
		100	7.13 ± 0.15	6.77 ± 0.38	7.07 ± 0.38	7.40 ± 0.72
		50	7.17 ± 0.57	6.33 ± 0.41	6.03 ± 0.06	6.13 ± 0.23
	Root	200	11.0 ± 1.13	7.15 ± 0.49	7.35 ± 0.21	7.55 ± 0.21
		100	11.75± 0.07	6.7 ± 0.57	6.20 ± 0.28	6.05 ± 0.07
		50	10.15± 0.92	6.40 ± 0.56	6.8 ± 0.56	6.00 ± 0.00
Ceftriaxone		200	16.97± 1.56	16.67 ± 1.00	16.9 ± 0.65	17.5 ± 1.29
		100	14.33± 0.65	15.43 ± 1.55	13.8 ± 2.15	14.7 ± 3.27
		50	12.53± 0.71	14.13 ± 3.31	13.23 ± 0.29	12.87 ± 0.36

Results are mean ± standard deviation of triplicates; Ceftriaxone was used as positive control.

CONCLUSION

The major constituents of the essential oils of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* were found to be 3,7,11,15-tetramethyl-1-hexadecanol, neophytadiene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-2-hexadecen-1-ol, hentriacontane, octacosane, hexacosane abietadiene,

tricosane, tetracosane and ethyl linoleate. This study revealed that the essential oil of *C. ficifolius, C. dispaceus* and *C. prophetarum* were active against the tested microbials. However, further studies should be conducted to offer comprehensive investigations on the isolation of molecules responsible for the antibacterial activity and the detailed mechanism of action. Therefore, the antibacterial activity displayed by the essential oils of various parts of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* along with the literature reported for their constituents suggest the use of the essential oils of these plants as antibacterial agent. Therefore, the activity displayed by the various parts of *C. ficifolius*, *C. dispaceus* and *C. prophetarum* corroborate the traditional use of these plants against bacteria.

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REFERENCES

1. Schnaubelt, K. *Medical Aromatherapy Healing with Essential Oils*, Frog Books: Berkeley, California, USA; **1999**; 126–131.
2. ISO. International Standards Organization, *http://www.iso. org/iso/home.htm* (accessed on 12 December 2014).
3. Başer, K.H.C.; Demirci, F. Chemistry of essential oils *in Fragrance and Flavours: Chemistry, Bioprocessing and Sustainability*, 1st ed.; Berger, R.G. (Ed.), Springer: Leipzig, Germany; **2007**; pp. 768–769.
4. Aslan, S.; Firat, M.; Konuklugil, B. Essential oil of *Cyclotrichium longiflorum* Leblebici. *J. Chem. Natural Compd.* **2007**, 43, 724–725.
5. Maffei, M.E.; Gertsch, J.; Appendino, G. Plant volatiles: Production, function and pharmacology. *Nat. Prod. Rep.* **2011**, 28, 1359–1380.
6. Silva, J.; Abebe, W.; Sousa. S.; Duarte, V.; Machado. M.; Matos, F. Analgesic and anti-inflammatory effects of essential oils of eucalyptus. *J. Ethnopharmacol.* **2003**, 89, 277–283.
7. Carson, C.F.; Cookson, B.D.; Farrelly, H.D.; Riley, T.V. Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia*. *J. Antimicrob. Chemother.* **1995**, 35, 421–424
8. Silva, T.D.K. *A Manual on the Essential Oil Industry*, United Nations Industrial Development Organization: Vienna; **1996**; pp. 265–259.
9. Mourey, A.; Canillac, N. Anti-listeria monocytogenes activity of essential oils components of conifers. *Food Control* **2002**, 13, 289–292.
10. Bishop, C.D. Antiviral activity of the essential oil of *Melaleuca alternifolia* (Maiden and Betche) Cheel (tea tree) against tobacco mosaic virus. *J. Essent. Oil Res.* **1995**, 7, 641–644.
11. Mari, M.; Bertolini, P.; Pratella, G.C. Non-conventional methods for the control of post-harvest pear diseases. *J. Appl. Microbiol.* **2003**, 94, 761–766.
12. Juglal, S.; Govinden, R.; Odhav B. Spice oils for the control of co-occurring mycotoxin-producing fungi. *J. Food Prot.* **2002**, 65, 683–687.
13. Pandey, R.; Kalra, A.; Tandon, S.; Mehrotra, N.; Singh, H.N.; Kumar, S. Essential oil compounds as potent source of nematicidal compounds. *J. Phytopathol.* **2000**, 148, 501–502.
14. Karpouhtsis, I.; Pardali, E.; Feggou, E.; Kokkini, S.; Scouras, Z.G. Mavragani-Tsipidou, P. Insecticidal and genotoxic activities of oregano essential oils. *J. Agric. Food Chem.* **1998**, 46, 1111–1115.
15. Bouyahya, A.; Abrini, J.; Dakka, N.; Bakri, Y. Essential oils of *Origanum compactum* increase membrane permeability, disturb cell membrane integrity, and suppress quorum sensing phenotype in bacteria. *J. Pharm. Anal.* **2019**, 9, 301–311.
16. Abebe, W. An overview of Ethiopian traditional medicinal plants used for cancer treatment. *Eur. J. Med. Plants* **2016**, 14, 113–116.

17. Dwivedi, N.K.; Dhariwal, O.P.; Gopala Krishnan, S.; Bhandari, D.C. Distribution and extent of diversity in *Cucumis* species in the Aravalli ranges of India. *Genet. Resour. Crop Evol*. **2010**, 57, 443–452.
18. Rajasree, R.S.; Sibi, P.I.; Francis, F.; William, H. Phytochemicals of Cucurbitaceae family a review. *Int. J. Pharmacogn. Phytochem.* **2016**, 8, 113–123.
19. Azhari, S.; Elmuez, A.; Yanshun, X.; Qixing, J.; Wenshui, X. Chemical composition and antibacterial activity of the essential oil isolated from seinat (*Cucumis Melo* Var. Tibish) seeds. *Int. J. Eng. Res. Technol.* **2014**, 2, 2347–4289.
20. Patini, A.; Mangino, A.; Martellacci, L.; Quaranta, G.; Masucci, L.; Gallenzi, P. The effect of different antibiotic regimens on bacterial resistance: A systematic review. *Antibiotics* **2020**, 9, 22–37.
21. Bouyahya, A.; Dakka, N.; Et-Touys, A.; Abrini, J.; Bakri, Y. Medicinal plant products targeting quorum sensing for combating bacterial infections, *Asian Pac. J. Trop. Med.,* **2017**, 10(8), 729–743.
22. Ventola, C.L. The Antibiotic Resistance Crisis, Part 1: Causes and Threats, *P and T*, **2015**, 40 (4), 277–283.
23. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. *Retention Indices for Frequently Reported Compounds of Plant Essential Oils*, National Institute of Standards and Technology: Gaithersburg, Maryland, USA; **2011**, 40(4), 5–16.
24. Carretero, M.E.; López-Pérez, J.L.; Abad, M.J.; Bermejo, P.; Tillet, S.; Noguera, P.B. Preliminary study of the anti-inflammatory activity of hexane extract and fractions from *Bursera simaruba* (Linneo) Sarg. (Burseraceae) leaves. *J. Ethnopharmacol*. **2008**, 116, 11–15.
25. Jayashree, E.; Zhang, C.; Kumar, P.S.; Nair, M.G. Antioxidant and antiinflammatory compounds in nutmeg (*Myristica fragrans*) pericarp as determined by invitro assays. *Nat. Prod. Commun.* **2015**, 10, 1399–1402.
26. Koroma, L.; Yormah, T.B.R; Kamara, L.M.; Robert, G.M.T. Extraction and characterization of linoleic acid from the leaves of the traditional medicinal plant *Caloncoba Echinata* in Sierra Leone. *Am. Sci. Res. J. Eng. Technol. Sci.* **2018**, 45, 185–206.
27. Siddeeg, A.; Alsir, E.; Wenshui-Xia, Y.Q. Chemical composition and antibacterial activity of the essential oil isolated from seinat (*Cucumis melo* var. tibish) seeds. *Int. J. Eng. Res. Technol.* **2010**, 2, 121–122.
28. Khajuria, V.; Gupta, S.; Kumar, N.S.A. |Anti-inflammatory potential of hentriacontane in lps stimulated raw 264.7 cells and mice model. *Biomed. Pharmacother*. **2017**, 92, 175–186.
29. Martino, L.D.; Feo, V.D.; Fratianni, F.; Nazzaro, F. Chemistry, antioxidant, antibacterial and antifungal activities of volatile oils and their components. *Nat. Prod. Commun.* **2009**, 4, 1741–1750.
30. Lis-Balchin, M. Chemical composition of essential oils from different species, hybrids and cultivars of *Lavandula in Lavender*, CRC Press: Online publication; **2002**; pp. 1–12.
31. Bouyahya, A.; Assemiana, I.C.C.; Mouzounta, H.; Bouraisa, I.; Et-Touysa, A.; Fellah, H.; Benjouad, A.; Dakka, N.; Bakri, Y. Could volatile compounds from leaves and fruits of *Pistacia lentiscus* constitute a novel source of anticancer, antioxidant, antiparasitic and antibacterial drugs? *Ind. Crops Prod.* **2019**, 128, 62–69.
32. Bouyahyaa, A.; Belmehdi, O.; Jemli, M.E.; Marmouzi, I.; Bourais, I.; Abrini, J.; Faouzic, Dakka, A.N.; Bakri, Y. Chemical variability of *Centaurium erythraea* essential oils at three developmental stages and investigation of their in vitro antioxidant, antidiabetic, dermatoprotective and antibacterial activities. *Ind. Crops Prod.* **2019**, 132, 111–117.