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Total Phenolic Content and Antioxidant, antimicrobial Activity from Some Yemani Plants

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Abstract:

The chemical compounds in P. inuloides essential oil include 47.34% of 2-Cyclohexen-1-one, 2-methyl-5-(1-methyl with Hexadecanoic acid (CAS) (12.82%) and Ethane, 1, 2-diethoxy- (9.613%). The major components identified in O. forskolei essential oil included Bicyclo [3.1.1] hept-2-ene, 2 (23.364 %),Bicyclo[3.1.1] hept-2-ene, 2 (9.00 %), Naphthalene, 1,2,3,4,4a,5,6, (19.32 %). P. inuloides showed a higher total phenol content than O. forskolei (55.4 ± 0.1 vs. 35.3 ± 0.2 mg GAE/g extract), lower antioxidant activity (43.97 ± 0.18 % vs. 23.07 ± 0.06 % scavenging activity; IC_{50}, 31.18 ± 0.01 vs. 80.01± 0.03) and β-carotene bleaching (39.9 ± 0.7 % vs. 25. 3 ± 0.3 % inhibition). P. inuloides essential oil inhibited all tested microorganisms except Salmonella typhimurium, Shigella dysenteriae and E. coli.

Conclusion*: The root of P. inuloides and O. forskolei essential oil possesses significant antioxidant and antimicrobial activities are lower.*

Key words: Essential oil; Phenolic content; Antioxidant; Antimicrobial activity, Pulicaria inuloides, Ocimum forskolei, aureus, Streptococcus pneumonia**.**

Introduction

Genus Pulicaria belonging to the tribe Inuleae of the Asteraceae family consists of ca. 100 species distributed in Europe, North Africa and Asia and five species of this genus reported from Yemen [1]. Essential oils are volatile, natural, complex compounds characterized by a strong odour and formed by aromatic plants as secondary metabolites. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals and insecticides. Pulicaria genus is an annual herb producing small bright yellow flowers [2]. The oil of *P. arabica* was characterized by the presence of a high percentage of sesquiterpene hydrocarbons and alcohols, whilst that of *P. undulate* was rich in phenolic compounds and monoterpene hydrocarbons. The oil of *P. undulata was* shown to have insecticidal properties [3]. The oil of another Saudi Arabian Pulicaria species has also been studied [4] and the major components were P-caryophyllene and its oxide. *Pulicaria jaubertii* indigenous to Yemen, locally known as Anssif is traditionally used in the Yemeni folk medicine to reduce the symptoms of flu and common cold [5], treat back-pain, intestinal disorders [2], treat inflammation and also as an insect repellent [6]. The flower of *P. jaubertii* was also used as spice to make various delicious foods. Various biological activities have been reported for some species of Pulicaria, such

as cytotoxic activity of *P. crispa* and *P. orientalis* [7], antibacterial activity of P. *undulata* and *P. dysenterica* [8], antispasmodic activity of *P.* [9] and antihistaminic effect of *P. dysenterica* [10]. No previous phytochemical work has been done on the essential oil of *P.inuloides* up to now. Ocimum is a member of Lamiaceae family with a distinction of the most studied genus among all the aromatic plants. This genus has been identified with up to 160 species [11]. Ocimum plants are also called basil with many widespread medicinal uses. The genus Ocimum pubescent, grows about one meter high, and have an obtusely quadrangular, stem. The leaves, which have grayish-green on the bottom and dotted with dark oil cells, are opposite The leaves of basil are used in folk medicine as a tonic and vermifuge [12]. The oil of the plant has been found to be beneficial for the alleviation of mental fatigue, colds spasm, rhinitis, and as a first aid treatment for wasp stings and snake bites [13]. Moreover the medicinal and aromatic properties of basil are associated with the presence of an essential oil that accumulates in the largest amount in its leaves and flowers. The fresh and dried basil herb is used as an aromatic spice and a source of essential oil, and its main components are also used as plant drugs, since it has antimicrobial, antimutagenic and fungistatic activity [14]. Ocimum plants are also called basil with many widespread medicinal uses. Based on the essential oil composition, there has been many chemotypes reported from basil species, which fall either under terpenoid or phenylpropanoid class. Moreover, monoterpenoids dominate basil essential oils invarious proportions. [15]. 1,8-cineole, linalool , linalool , terpinen-4-ol, citral, anisole and methyl-(E)-cinnamate [16]. Similarly, *O. kilimandscharicum* has been reported with high camphor proportion from various locations [17]. *Ocimum frskoleian* aromatic perennial woody shrub up to 2 m tall. In Rwanda the plant is used in traditional medicine to cure eye infections [18]

and in Kenya it is used as a grain protectant against insect pests [19]. Members of the genus find a number of uses in African traditional medicine [18]. Tanzanians, especially those living along the Indian Ocean coastal regions, use the plants to repel mosquitoes and as flavouring agents. Plants of the genus Ocimum are also reported for many biological activities, such as mosquito repellent and antimicrobial activity [20]; insecticidal activity against crop pest insects [21], antipyretic [22] and antioxidant activity [23]. There is no previous study on the chemical compositions of the essential oils of *O. forskolei*. Therefore, this study reported for the first time the chemical compositions of the *Ocimum forskolei* by gas chromatography mass spectrometry.

The present study was to compares the results of Gas Chromatographic-Mass analyses of *Pulicaria inuloides* root and *O. forskolei* root essential oils and determine their total phenolic content, , as well as their antioxidant, antibacterial, and antifungal Activities.

Fig. 1. *Pulicaria inuloides.*

Fig. 2 ***Ocimum forskolei***

Materials and methods

Plant collection and identification: The root plant of *P. inuloides* and *O. forskolei* was collected in March 2014 from Bany Mater, province of Sana'a at flowering stage (Figs. 1and 2). The samples were air-dried and taxonomically identified by Prof. Abdellah Amine (College of Agriculture, Sana'a University, Yemen). A voucher specimen of the plant material was deposited at the Dept. of Biology (Sana'a University) of the College of Agriculture.

Essential oils isolation

The root plant of *P. inuloides* and *O. forskolei* (200 g) was separately subjected to hydrodistillation for 6 h using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [24]. The obtained oils were dried over anhydrous sodium sulphate and stored in air-tight, amber coloured glass vials at 4 ^{O}C.

GC-MS analysis

The components of the essential oils were identified by GC-MS analysis (Kumar et al., 2010) [25].Gas chromatography-mass spectrometry (Varian 1200L) was incorporated with a relatively no polar capillary column (DB-5, 30 m length, 0.25 mm film thickness, 0.25 internal dia). The injection port and interface were held at 220 and 260 ^{O}C respectively. The temperature was programmed from 50-220 ^{O}C at the 15 ^{O}C per min and a hold at 220 ^{O}C for 25 min with helium as the carrier gas. Mass spectra with electronic impact, ionisation potential of 70 eV, ion source temperature of 200 ^{O}C and mass range of 35-500 Da was carried out. The identification of individual compounds was based on comparison of their relative retention times with those of authentic samples on HP-5MS capillary column and by matching of their mass spectra of peaks with those obtained from authentic samples and/ or the Wiley NIST7 library spectra and published data [26].

Determination of total phenolic content

It was evaluated using a modified colorimetric method described previously by Singleton and Rossi (1965) [27] . In this study, 0.50 mL of the extract was mixed with 3.0 mL of distilled water and 0.25mL of Folin-Ciocalteau reagent. Immediately, 0.75 ml of saturated sodium carbonated and 0.95 ml of distilled water was added. Then, the mixture was incubated for 30 min at 37 ^{o}C, and the absorbance was read at 765 nm using an UV-Vis spectrophotometer (Unicam Heλio α, Cambridge, UK). The measurement was compared to a standard curve prepared with a garlic acid solution (Sigma Chemical). The total phenolic content was expressed as milligrams of garlic acid equivalents per gram of fresh weight (mg GAE g-1 FW).

Determination of DPPH Radical Scavenging Activity

Determination of the free radical scavenging activity of the different extracts was carried out using a modified quantitative DPPH (1,1-diphenyl-2-picrylhydrazyl; Sigma -Aldrich, St. Louis, MO, USA) assay [28]. One ml of 0.2 mM DPPH in methanol was added to 1 ml of the test solution, or standard, plus 1 ml of methanol for dilution and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 517 nm. Inhibition of free radical in percent (I%) was calculated according to the following equation 1: DPPH (%) = {(An – Am)/Am}100 ….. . (1) where An is absorbance of the control (without essential oil), and Am is absorbance of the sample. Measurements were carried out in triplicates.

Bleaching of β-carotene assay (BBC)

Antioxidant activities of the essential oils were carried out in accordance with [29]. The ß-carotene (0.1 mg) was added to a boiling flask together with linoleic acid(20 mg) and Tween 40 dissolved in chloroform. After evaporating the chloroform under vacuum at 50 °C using a rotary evaporator, 50 mL oxygenated distilled water was added, and the mixture emulsified for 1 min. Thereafter, 5 mg of each essential oil was added separately to 4.8 mL of the emulsion. Absorbance at 470 nm was measured using a spectrophotometer before (t = 0 h) and after a 2-h incubation at 50 °C (t = 2 h).

The antioxidant activity was calculated using equation 2:

Inhibition%=(AA(2h) – AC(2h)/(AC(0h) – AC(2h)}100……..…(2}

is absorbance of the sample at t=2 h, AC(2h) is absorbance of the control at t = 2

h, and AC(0h) is absorbance of the control at t =0h, and AC(2h) is absorbance of the control at t=2h.

Assessment of antimicrobial activity

Microorganisms

Staphylococcus aureus 6538, *Streptococcus pneumoniae* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Shigella dysenteriae* 51302, *Salmonella typhimurium* 50013 and *Candida albicans* ATCC 10231 was purchased from the China General Microbiological Culture Collection Center (Beijing, China).

Disc diffusion assay

The antimicrobial activity of the essential oils was determined by using the disc diffusion assay [36]. The Tryptic soy agar was inoculated with with the microorganism (10^4 colony- forming units/mL). A 6-mm paper filter disc impregnated with 20 µL essential oil diluted in dimethyl sulfoxide was placed on the agar , and the oil was allowed to diffuse into the medium for 30 min at room temperature. The plates were then incubated at 37 °C for 24 h (bacteria) or at ***32***°C for 72 h (yeast). The zone of inhibition was recorded as the mean ± standard deviation (SD of triplicate experiments). Ampicillin (10 µg) and gentamicin (10 µg) were used as reference antibiotics for bacteria, and nystatin (100 µg) was used as the reference antifungal agent for *C. albicans.*

Statistical analysis

Experiments were conducted at least in triplicate. Groups were compared by analysis of variance, and differences between mean values were evaluated by Fisher LSD test; $p < 0.05$ was considered significant. Statistical analyses were carried out using SPSS version 19.0 (SPSS, Chicago, IL, USA).

Results and Discussion

The yield of volatile oil of *P. inuloides* and *O. forskolei* obtained by Hydrodistillation of the finely powdered root plants. About 60 components were identified in the oil of *P. inuloides* , which represented about 100% of the oil and 54 components were identified in oil of *O. Forskolei* , representing 100% of all components of the oil. The qualitative and quantitative essential oil compositions are presented in Table 1,2 and Fig 1,2 respectively.

The results show that the main compounds in *P. inuloides* oil were 47.34% of 2-Cyclohexen-1-one, 2-methyl-5-(1-methyl with Hexadecanoic acid (CAS) (12.82%), Ethane,1,2-diethoxy- (9.613%). The *O. forskolei* oil was rich in Bicyclo [3.1.1] hept-2-ene, 2, (23.364%), Bicyclo [3.1.1] hept-2-ene, 2 (9.00 %) and Naphthalene, 1,2,3,4,4a,5,6 (19.32%). Our results of some components of essential oil of *P. inuloides* showed minor differences when compared with literature [30]. This difference might be due to growth conditions, genetic factors, geographical variations and analytical procedures. In addition, according to previous phytochemical studies, this plant is a considerable source of eudesmanolide, sesquiterpene lactones of the guaianolide and xanthanolides family Asteraceae [1]. To the best of our knowledge, there is no any report on the chemical composition of O. *forskolei* essential oils in the literature. However, there are few reports on the chemical composition of the oils from the other plants belonging to the genus of *O. forskolei*. Previously studied on the composition of *O. basilicum* oil shows that there are some qualitative and quantitative differences which , can be attributed to growth conditions, genetic factors, geographical variations and analytical procedures [31].

Table1. Chemical composition of the essential oil of root of *P. inuloides.*

Peak	RT (min.)	Area (%)	Compounds	RT index	Percentage
1	1.592	4.57	Ethane, 1,2-diethoxy-	772	9.613
2	3.173	1.77	No Match	-	0.372
3	14.715	6.96	FILIFOLONE	942	0.146
4	15.699	5.73	dihydroedulan II	909	0.12
5	16.508	1.33	LINALOOL L	917	0.28
6	16.602	1.91	Carvomenthone	955	0.402
7	16.79	3.39	Terpineol, cis-.beta.-	888	0.071
8	17.206	7.03	No Match	-	0.148
9	18.644	2.25	2-Cyclohexen-1-one, 2-methyl-5-(1-methyl	926	47.34
10	18.927	2.03	2-Cyclohexen-1-one, 6-methyl-3-(1-methyl	885	0.004
11	19.06	1.31	Cyclohexanol, 2-methyl-5-(1-methylethyl)	938	2.755
12	19.244	4.89	2-Cyclohexen-1-ol, 2-methyl-5-(1-methyle	883	1.029
13	19.293	3.06	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trim	842	0.643
14	19.414	1.05	.delta.-Cadinene	882	0.221
15	19.548	5.43	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-me	892	1.142
16	19.676	8.21	Benzaldehyde, 4-(1-methylethyl)-	883	0.173
17	19.822	1.72	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl	792	0.361
18	19.92	5.32	Carvotanacetol, cis-	905	1.117
19	20.358	2.57	1-(1'-ACETYL)-2-(2-OXOPROPYL)CYCLOPENTAN	743	0.054
20	20.484	8.73	Geranyl propionate	882	0.184
21	20.592	5.32	Thymohydroquinone dimethyl ether	950	1.117
22	20.955	1.60	Thymyl acetate	897	0.337
23	21.185	2.54	TRANS-2-UNDECEN-1-OL	921	0.053
24	21.28	3.31	3,5-Heptadienal, 2-ethylidene-6-methyl-	771	0.07
25	21.424	1.40	.beta.-Ionone	860	0.293
26	21.744	3.89	Dihydro-.alpha.-ionone	797	0.082
27	21.98	5.31	(-)-Caryophyllene oxide	936	1.117
28	22.291	4.83	E-2-Tetradecen-1-ol	947	1.014
29	22.562	1.12	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-c	918	0.235
30	22.74	1.13	9-Chloro-8-oxatetracyclo[7.3.1.0(2,7).0(	770	0.237
31	22.813	9.26	cis-Z-.alpha.-Bisabolene	831	0.195

32	22.916	7.67	epoxide trans-Z-.alpha.-Bisabolene epoxide	826	0.161
33	23.02	3.20	Nerolidol	782	0.067
34	23.141	4.82	zingiberenol	842	0.101
35	23.224	3.33	2-Pentadecanone, 6,10,14-trimethyl-	916	0.7
36	23.557	1.20	APHA SINENSAL	816	0.253
37	23.652	8.39	Phenol, 2-methoxy-4-(2-propenyl)- (CAS)	795	0.176
38	23.742	3.26	Thymyl acetate	893	0.685
39	23.903	2.11	No Match	-	0.443
40	24.035	7.07	2-Cyclohexen-1-one, 2-methyl-5-(1-methyl	889	1.486
41	24.109	6.41	No Match	-	0.135
42	24.314	4.51	.alpha.-Cadinol	911	0.947
43	24.422	7.66	No Match	-	0.161
44	24.512	4.99	No Match	-	0.105
45	24.677	1.87	1-HYDROXYLINALOOL	806	0.392
46	24.803	1.17	Tetracosane (CAS)	961	0.245
47	24.993	1.96	9,17-Octadecadienal, (Z)-	903	0.412
48	25.251	9.35	Allopregnane-7.alpha.,11.alpha.-diol-3,2	735	0.196
49	25.504	2.90	1-Hexadecanol (CAS)	869	0.61
50	25.61	3.93	cis,cis,cis-7,10,13-	913	0.827
51	25.927	2.83	Hexadecatrienal	805	0.06
52	26.115	1.11	trans-.alpha.-Bergamotene	796	0.233
53	26.197	3.11	Sabinene	787	0.065
54	26.313	4.79	NEROLIDOL-EPOXYACETATE	808	0.101
55	26.481	6.77	2-Methyl-Z,Z-3,13-octadecadienol	779	0.142
56	26.593	7.30	Phenol, 3-(1,1-dimethylethyl)-4-methoxy- Tetracosane	964	1.535
57	26.712	4.41	Acetic acid, 3,7,11,15-tetramethyl-hexad	918	0.927
58	26.901	2.18	Ethyl linoleate	818	0.458
59	27.055	2.59	1,2-Benzenedicarboxylic acid, bis(2-meth	927	0.543
60	27.468	3.07	Hexadecen-1-ol, trans-9-	946	0.646
61	27.62	3.34	Methyl (Z)-5,11,14,17-eicosatetraenoate	872	0.702
62	27.782	8.98	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-	962	1.887
63	28.886	2.03	1,2-Benzenedicarboxylic acid, butyl 8-me	794	0.427
64	29.466	7.17	Cyclopropaneoctanoic acid,	773	0.151

			2-[[2-[(2-eth		
65	30.099	5.80	No Match	-	0.122
66	31.597	7.01	(12Z)-ABIENOL	785	0.147
67	32.848	6.10	Hexadecanoic acid (CAS)	947	12.82
				Total =99.993	

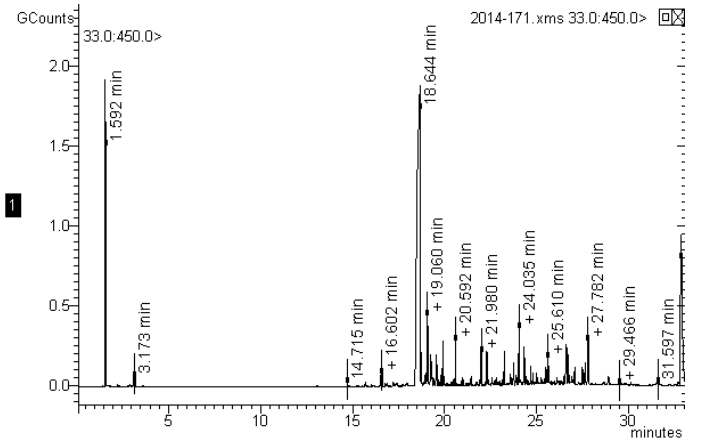

Fig. 1.Typical chromatogram of *P. inuloides* essential oil components.

Table 2. Chemical composition of *Ocimum forskolei* roots essential oil

Peak	RT (min.)	Area (%)	Compounds	RT index	Percentage
1	2.338	4.18	2-Propanone (CAS)	988	0.30
2	14.006	3.29	Bicyclo[2.2.1] heptan-2-one,	958	0.21
3	15.201	5.42	alpha.-Cubebene	939	0.425
4	15.819	1.88	Copaene	952	1.48
5	16.145	9.24	Bicyclo[2.2.1] heptan-2-one,	936	0.07
6	16.236	3.26	beta. BOURBONENE	960	0.252
7	16.545	9.87	1H-Cyclopenta[1,3]cyclopropa	940	0.73
8	16.599	5.00	Naphthalene, 1,2,3,4,4a,5,6,	860	0.392
9	17.136	7.95	alpha.-Ylangene	900	0.623
10	17.532	2.83	Bicyclo[3.1.1] hept-2-ene, 2,	933	23.364
11	17.554	1.12	Bicyclo[3.1.1] hept-2-ene, 2,	933	9.00
12	17.694	4.442	trans-Caryophyllene	959	3.238
13	17.774	3.03	gamma.-Gurjunene	927	0.22
14	18.09	1.07	beta.-Cedrene	876	0.844
15	18.374	2.20	Estragole	881	1.733
16	18.498	3.74	alpha.-Humulene	863	2.955
17	18.633	5.82	Cedrene	886	1.00

18	18.739	1.67	trans-Caryophyllene	886	1.37
19	18.788	1.38	1,6,10-Dodecatriene, 7,11-di	906	1.087
20	19.189	2.45	Naphthalene, 1,2,3,4,4a,5,6,	911	19.32
21	19.28	7.86	Azulene, 1,2,3,5,6,7,8,8a-oc	934	6.21
22	19.341	3.83	Naphthalene, 1,2,3,4,4a,5,6,	939	3.01
23	19.367	5.04	alpha.-selinene	936	3.98
24	19.483	3.70	1H-Cyclopenta[1,3]cyclopropa	889	0.028
25	19.701	7.37	Naphthalene, 1,2,3,4,4a,5,6,	894	5.927
26	19.889	1.38	alpha.-Cubebene	892	0.107
27	19.989	1.56	Naphthalene, 1,2,4a,5,6,8a-h	910	0.12
28	20.414	1.42	Naphthalene, 1,2,3,4-tetrahy	951	0.113
29	20.984	2.55	alpha.-Patchoulene	814	0.202
30	21.553	1.56	1-Hydroxy-1,7-dimethyl-4-iso	827	0.12
31	22.105	1.26	(E)- ß-Caryophyllene	935	0.2
32	22.222	7.21	Benzene, 1,2-dimethoxy-4-(2-	936	0.56
33	22.287	6.17	trans-Z-.alpha.-Bisabolene e	775	0.041
34	22.392	1.40	Hexadecanal (CAS)	948	0.11
35	22.795	3.14	Cubenol	839	0.246
36	22.871	3.63	Cubenol	893	0.028
37	22.943	8.81	Cyclohexanemethanol, 4-ethen	918	0.695
38	23.153	6.36	(-)-Caryophyllene oxide	814	0.71
39	23.355	8.49	2-Pentadecanone, 6,10,14-tri	835	0.67
40	23.428	3.19	Isoaromadendrene epoxide	835	0.02
41	23.869	2.55	tau.-Cadinol	929	2.017
42	24.001	3.65	tau.-Muurolol	880	0.285
43	24.232	1.54	alpha.-Bisabolol	914	0.12
44	24.434	4.44	alpha.-Cadinol	888	0.33
45	24.781	1.25	Isophytol	952	0.099
46	25.322	5.63	CYCLOPENTANEACETIC ACID, 3-O	933	0.044
47	25.551	9.34	(+)-.alpha.-Cyperone	893	0.07
48	25.606	2.57	5,9,13-Pentadecatrien-2-one,	809	0.2
49	25.868	8.54	Cyclopentanecarboxylic acid,	842	0.061
50	26.689	9.51	Octacosane	912	0.071
51	27.069	1.27	GERANYL LINALOOL ISOMER	901	0.1
52	27.166	1.17	Palmitaldehyde, diallylacet	798	0.093
53	28.098	8.48	Phytol	953	4.700
54	29.042	1.27	Pentacosane	956	0.1
					Total =100%

Rt: Retention time time
RI: Retention Index

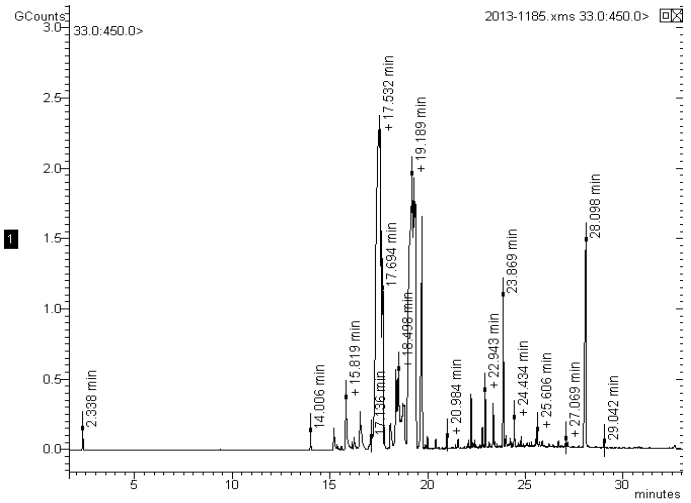

Fig 2. Typical chromatogram of *Ocimum forskolei* essential oil components.

Total phenolic content and antioxidant activity

It is well known that there is a strong relationship between total phenolic content and antioxidant activity, as phenols possess strong scavenging ability for free radi -cals due to their hydroxyl groups. Therefore, the pheno -lic content of plants may directly contribute to their an -tioxidant action [32].

The oil extracts were assessed for their capacity to scavenge DDPH free radical and bleaching of ß-carotene with gallic acid as a positive control.

The antioxidant activity data are presented as percent of free radical. Inhibition and bleaching of ß-carotene in **Table 3.** The essential oil (by water) extracts of the roots of *P. inuloides* and *O. forskolei* exhibited pronounced lower antioxidant activity were 43.97± 0. 18 and 32.07±***0.06*** DPPH and 39.9 ± 0.7, 25.3 ± 0.3 % BBC respectively at a concentration of 1000 µg/ml, different to gallic acid. For comparison of DPPH and BCB antioxidant activity methods *P. inuloides* showed significantly higher ($P < 0.05$) antioxidant activity than *O.*

forskolei in the DPPH and higher ability to prevent the bleaching of ß-carotene.

The IC_{50} values of *P. inuloides* and *O. forskolei* were 33.3 ± 0.01 and 21.8 ± 0.05 µg/mL, respectively. Polyphenolic compounds are also believed to have chemopreventive and suppressive activities against cancer cells by inhibition of metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle [33]. Nevertheless, a compound with strong antioxidant potential can also contribute to DNA protection and prevent apoptosis [34]. Further studies are therefore required to detect potential anticancer activities of the extracts reported here.

Table 3. Essential oil antioxidant activity and total phenolic content of *Pulicaria inuloides* and *Ocimum forskolei.*

Botanical name	Total phenolic content (mgGAE/g DW)	Inhibition of DPPH (%)	IC_{50} (µg/ml)	ß-carotene bleaching (% inhibition)
P. inuloides	55.4±0.1 [a]	43.97± 0. 18[a]	31.18 ± 0.17[a]	39.9 ± 0.7[a]
O. forskolei	35.3±0.2[b]	23.07±***0.06*** [b]	80.01 ± 0.03[b]	25.3 ± 0.3[b]

Different letters indicate significant differences between the two essential oils ($p < 0.05$). DW - dry weight. Results are expressed as mean ± SD.

Antimicrobial activity

In this study, *P. inuloides* essential oils demonstrated lower antibacterial activities against all bacteria tested except *Salmonella typhimurium, Shigella dysenteriae* and *Escherichia coli* . Furthermore, the Gram - positive bacteria *Staphylococcus aureus, Streptococcus pneumoniae,* and *Bacillus subtilis* were more sensitive to this essential oil than the Gram - negative bacteria. The essential oils of *O. forskolei* demonstrated no antibacterial activity against all tested bacteria (Table 4). which is consistent with a previous report [35]. That study reported that extracts

derived from another Ocimum species, *O. gratissimum*, showed no activity against 11 tested bacterial strains, including *S. aureus* (four strains), *E. coli* (two strains), *Pseudomonas aeruginosa* (one strain), Proteus spp. (three strains), and Shigella (one strain). Similarly, the essential oils of *O. basilicum* were ineffective against gram-positive and gram-negative bacteria tested in another study [36].

Table 4: Antimicrobial activity of *Pulicaria inuloides* and *Ocimum forskolei* essential oils

Test microorganism	*Pulicaria inuloides* (zone of inhibition, mm)	*Ocimum forskolei* (zone of inhibition, mm)	Standard antibiotic [b] (zon of inhibition, mm)
Gram-positive bacteria			Ampicillin
Staphylococcus aureus	9.7 ± 0.59	ND	20.1±0.5
Streptococcus pneumoniae	9.3 ±0.58	ND	19.0±0.1
Bacillus subtilis	10.2 ± 0.76	ND	21.2±0.3
Gram-negative bacteria			Gentamicin
Shigella dysenteriae	ND	ND	15.3±0.3
Salmonella typhimurium	ND	ND	15.1±0.7
Escherichia coli	ND	ND	23.0±0.5
Yeast			Nystatin
Candida albicans	10.7±0.57	ND	22.1±0.2

[b]Standard antibiotics used as positive control; ND = Not detectable, essential oil has no antimicrobial activity against this microorganism

Conclusion

The essential oils of *P. inuloides* roots exerted lower antimicrobial actions against gram-positive bacteria and C. *albicans*, whereas *O. forskolei* essential oils were ineffective against gram-positive and gram-negative bacteria tested in

this study. The antioxidant activity of P. *inuloides* and *O. forskolei* essential oil roots were lower in this study.

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