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**Original Research Article**

# Antimicrobial and Antioxidant Activities of the Essential Oils of Some Aromatic Medicinal Plants (*Pulicaria inuloides*-Asteraceae and *Ocimum forskolei*-Lamiaceae)

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## Abstract

***Purpose:*** *To determine the antimicrobial and antioxidant activities of Pulicaria inuloides and Ocimum forskolei essential oils.*
***Methods:*** *Steam distillation of the aerial parts of P. inuloides and O. forskolei was performed using a Clevenger apparatus. Essential oils were analyzed by gas chromatography–mass spectrometry. Total phenolic content and antioxidant activities were determined by 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) and β-carotene bleaching assays. Disc diffusion and microtiter broth microdilution assays were employed to determine antimicrobial activity.*
***Results:*** *The chemical compounds in P. inuloides essential oil include 2-cyclohexen-1-one, 2-methyl-5-(1-methyl) (55.1 %) and benzene, methyl- (20.6 %). The major components identified in O. forskolei essential oil included bicyclo [3.1.1] hept-2-ene,2, (22.4 %) and naphthalene 1,2,3,4,4a,5,6, (19.3 %). P. inuloides showed a higher total phenol content than O. forskolei (144 ± 5.32 vs. 54.6 ± 30 mg GAE/g extract), higher antioxidant activity (92.92 ± 0.10 % vs. 26.76 ± 0.11 % scavenging activity; IC50, 4.5 ± 0.05 vs. 73.03 ± 0.05) and β-carotene bleaching (90.77 ± 0.21 % vs. 41.03 ± 6.35 % inhibition). P. inuloides essential oil inhibited all tested microorganisms except Salmonella typhimurium and Shigella dysenteriae with a minimum inhibitory concentration (MIC) of 3.0 µg/mL against Escherichia coli. O. forskolei essential oil inhibited only Candida albicans.*
***Conclusion:*** *P. inuloides essential oil possesses significant antioxidant and antimicrobial activities.*

***Keywords:*** *Essential oil; Phenolic content; Antioxidant; Antimicrobial activity, Pulicaria inuloides, Ocimum forskolei, Salmonella typhimurium, Shigella dysenteriae*

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## INTRODUCTION

The genus *Pulicaria*, which belongs to the family Asteraceae (tribe Inuleae), consists of more than 77 species found throughout the world. Members of this genus contain various bioactive compounds such as monoterpenes, flavonoids, acetylenes, isocomene, and sesquiterpene lactones [1]. The leaves of *Pulicaria inuloides* are used to flavor foods and to make an herbal tea [2]. Biological actions reported for *Pulicaria* species include the antibacterial and antispasmodic activities of *P. undulate, P. odora*, and *P. dysenterica* [3]. In addition, members of this genus have been traditionally used to repel insects, to reduce influenza and common cold symptoms, and to treat back pain, intestinal disorders, and inflammation [4].

The genus *Ocimum*, which belongs to the Lamiaceae family, is the most well-studied genus of all the aromatic herbs and contains up to 160 species [5]. *Ocimum*, also called basil, grows to a height of about one meter and has an obtusely quadrangular stem and opposite leaves, which are used in folk medicine as a tonic and vermifuge [6]. The plant oils have been used to alleviate mental fatigue, colds, spasms, rhinitis, wasp stings, and snakebites [6]. The medicinal and aromatic properties of basil are derived from the essential oil, which accumulates primarily in the leaves and flowers. The fresh and dried herb is used as an aromatic spice, and its bioactive components harbor antimicrobial, antimutagenic, and fungistatic activities [7].

Because the chemical composition of the essential oils of *P. inuloides* and *O. forskolei* have not yet been reported, we used gas chromatography-mass spectrometry (GC–MS) to determine their total phenolic content, as well as their antioxidant, antibacterial, and antifungal activities.

## EXPERIMENTAL

### Plant collection and identification

The aerial parts of *P. inuloides* (flowers) and *O. forskolei* (leaves) were collected in August 2013 during the flowering stage in the Sana'a and Dammar areas of Yemen. The plant specimens were air-dried and taxonomically identified by Prof. Abdellah Amine (College of Agriculture, Sana'a University). A voucher specimen (SANB N. 196, DAMO N. 179) of the plant material was deposited at the Department of Biology in the College of Agriculture at Sana'a University.

### Extraction of essential oil

The fresh aerial parts of *P. inuloides* and *O. forskolei* (3 kg) were ground in a blender, and essential oils were obtained by hydrodistillation at 100 °C using a Clevenger-type apparatus for 4 h with 3 L distilled water. The extracted oils were dried over sodium sulfate ($Na_2SO_4$) and stored at 4 °C until use.

### Analysis of essential oil

The essential oil components were identified by GC–MS [8] using a Varian 1200L system (electronic impact mode, ionization potential 70 eV, ion source temperature 200 °C, mass range 35–500 Da) fitted with a relatively nonpolar capillary column (DB-5, 30 m × 0.25 mm, 0.25 µm film thickness). The injection port and interface were held at 220 °C and 260 °C, respectively. The column temperature was increased from 50 °C to 220 °C (15 °C/min) and then the temperature was maintained at 220 °C for 25 min. Helium was the carrier gas. Most of the compounds were identified using two different analytical methods: (a) KI, Kováts Indexes in reference to n-alkanes (C8- C32) [9]; and (b) mass spectra (authentic chemicals and Wiley spectral library collection). Identification was considered tentative when it was based on mass spectral data only.

### Determination of total phenolic content

Total phenolic content was estimated as milligrams of gallic acid equivalent (mg GAE) per gram plant extract, as previously described [10]. In brief, a 100 µL aliquot of dissolved extract was transferred to a volumetric flask containing 46 mL distilled $H^2O$, and 1 mL Folin–Ciocalteu reagent was added. After 3 min, 3 mL $Na_2CO_3$ (2 %) was added. The sample was incubated at 25 °C for 2 h, after which absorbance was measured at 760 nm. Gallic acid (0.2–1 mg/mL; Sigma) was used as the standard for the calibration curve, and total phenolic content was expressed as mg GAE/g extract ($Y = 0.0792x + 0.00302$; $R^2 = 0.9739$) [10].

### Determination of radical scavenging activity

The antioxidant activities of the essential oils of *P. inuloides* and *O. forskolei* were determined by the 2, 2-diphenyl-1,1-picrylhydrazyl (DPPH) free radical scavenging assay, as previously described [11]. Each concentration of essential oil was mixed individually into 1 mL methanol solution containing 0.1 mM DPPH radicals. The reaction mixture was shaken thoroughly, incubated in the dark for 30 min at room temperature, and measured at an absorbance of 517 nm (2100, Unico, Shanghai, China). The free radical scavenging activity was calculated as follows using equation 1:

$$DPPH\ (\%) = \{(An - Am)/Am\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where An is absorbance of the control (without essential oil), and Am is absorbance of the sample.

### β-carotene bleaching assay (BCB)

Antioxidant activities of the essential oils were determined as previously described [11], with some modifications. The β-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). All measurements were performed in duplicate, and antioxidant activity, assessed as percent inhibition of β-carotene bleaching, was calculated using equation 2:

$$\% \text{ inhibition} = \{AA(2h) – AC(2h)/(AC(0h) – AC(2h)\}100 \ldots (2)$$

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

### 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 were purchased from the China General Microbiological Culture Collection Center (Beijing, China).

#### *Disc diffusion assay*

Antimicrobial activity of the essential oils was determined by using the disc diffusion assay [12] with some modifications. Tryptic soy agar was inoculated 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*.

#### *Determination of minimal inhibitory concentration (MIC)*

The MIC of the essential oil was determined using the microtiter broth microdilution assay described by Amsterdam [13]. The essential oils were diluted to 50 µL/mL and subjected to a serial dilution in a microtiter plate containing tryptic soy broth (for bacteria) or Sabouraud dextrose broth (for yeast). The bacterial and yeast strains were suspended in the liquid culture medium at a final concentration of $10^4$ colony-forming units/mL. After incubation at 37 °C for 24 h (bacteria) or at 32 °C for 72 h (yeast), optical density was measured at 520 nm using a spectrophotometer. MIC was defined as the lowest concentration of the essential oil at which the microorganisms did not exhibit visible growth.

### 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

### Chemical composition of the essential oils

The compositions of the essential oils obtained by hydrodistillation of the flowers and leaves of *P. inuloides* and *O. forskolei* were determined by GC–MS. Ten components accounted for 87.468 % of the *P. inuloides* essential oil, with 2-cyclohexen-1-one, 2-methyl-5-(1-methyl (55.1 %), benzene, methyl- (20.6 %), and Z-Citral (2.915 %) being the major constituents (Table1). Ten components were identified in the essential oil of *O. Forskolei*, with Bicyclo [3.1.1] hept-2-ene, 2, (22.4 %), naphthalene, 1,2,3,4,4a,5,6, (19.3 %), and phytol (6.7 %) being the major constituents (Table 2).

### Total phenolic content and antioxidant activity

The total phenolic content of *P. inuloides* essential oil (144 ± 5.32 mg GAE/100 g extract) was considerably higher than that of *O. forskolei* essential oil (54.6 ± 3.0 mg GAE/100 g extract) (Table 3). In addition, the antioxidant activity of *P. inuloides* essential oil (90.77 ± 0.21 % inhibition) was stronger than that of *O. forskolei* essential oil (41.03 ± 6.35 % inhibition), as assessed by the BCB assay. The half maximal inhibitory concentration ($IC_{50}$) was graphically obtained to determine concentration needed to inhibit 50 % of free DPPH radicals. The $IC_{50}$ values of *P. inuloides* and *O. forskolei* were 4.95 ± 0.05 and 73.54 ± 0.05 µg/mL, respectively.

**Table 1:** Main components of the essential oil of *Pulicaria inuloides*

| RT (min) | Area (%) | Compound | Content (%) |
|---|---|---|---|
| 5.785 | 3.68 | Benzene, methyl- (CAS) | 20.6 |
| 18.966 | 9.83 | 2-Cyclohexen-1-one, 2-methyl-5-(1-methyl | 55.1 |
| 19.081 | 1.95 | Zingiberene | 1.094 |
| 19.182 | 5.20 | Z-Citral | 2.915 |
| 19.355 | 2.09 | alpha-Farnesene | 1.169 |
| 19.684 | 3.09 | Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)- | 1.734 |
| 19.961 | 2.74 | 7-Oxabicyclo[4.1.0]heptane, 1-methyl- (C | 1.538 |
| 20.609 | 3.15 | Geranyl propionate | 1.511 |
| 22.097 | 1.76 | (-)-Caryophyllene oxide | 0.985 |
| 26.679 | 1.47 | Pentacosane | 0.822 |
| | | | ***Total*** = 87.468 |

*RT = retention time*

**Table 2:** Main components of the essential oil of *Ocimum forskolei*

| RT (min) | Area (%) | Compound | Content (%) |
|---|---|---|---|
| 17.532 | 2.83 | Bicyclo[3.1.1] hept-2-ene, 2, | 22.369 |
| 17.554 | 1.12 | Bicyclo[3.1.1] hept-2-ene, 2, | 8.877 |
| 17.694 | 4.442 | trans-Caryophyllene | 3.496 |
| 18.498 | 3.74 | alpha-Humulene | 2.956 |
| 19.189 | 2.45 | Naphthalene, 1,2,3,4,4a,5,6, | 19.327 |
| 19.28 | 7.86 | Azulene, 1,2,3,5,6,7,8,8a-oc | 6.212 |
| 19.341 | 3.83 | Naphthalene, 1,2,3,4,4a,5,6, | 3.03 |
| 19.367 | 5.04 | alpha-Selinene | 3.983 |
| 19.701 | 7.37 | Naphthalene, 1,2,3,4,4a,5,6, | 5.827 |
| 28.098 | 8.48 | Phytol | 6.704 |
| | | | ***Total*** = 82.781 |

*RT = retention time*

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

| Botanical name | Total phenolic content (mg GAE/g DW) | Inhibition of DPPH (%) | $IC_{50}$ (µg/ml) | β-carotene bleaching (% inhibition) |
|---|---|---|---|---|
| *P. inuloides* | 144±5.32[a] | 92.92±0.10[a] | 4.95±0.05[a] | 90.77±0.21[a] |
| *O. forskolei* | 54.6±3.0[b] | 26.76±0.11[b] | 73.54±0.05[b] | 41.03±6.35[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

The *in vitro* bacteriostatic activity of *P. inuloides* essential oil was greater than that of *O. forskolei* essential oil, as assessed by the disc diffusion assay (Table 4) and by the microtiter broth microdilution assay (Table 5).

## DISCUSSION

To the best of our knowledge, the chemical compositions of *P. inuloides* and *O. forskolei* essential oils have not previously been reported. However, essential oils of plants belonging to the same genera (*P. dysenterica* and *O. basilicum*) show qualitative and quantitative differences, which can be attributed to different growth conditions, genetic factors, geographical variations, and analytical procedures [3].

Medicinal plants have been extensively studied for their antioxidant and free radical scavenging activity in the last few decades. Among these natural antioxidants, phenolic compounds have been shown to quench oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical [11]. The total phenolic content of the essential oil of *O. forskolei* (54.6 ± 30 mg GAE/g) determined in this study is in agreement with that previously reported for *O. basilicum* (55 ± 122 mg GAE/g) [13]. Phenolic compounds may contribute directly to antioxidant activity.

Compared with *O. forskolei* essential oils, *P. inuloides* essential oils strongly inhibited β-carotene bleaching and had a higher total phenolic content in our study, suggesting greater

**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] (zone of inhibition, mm) |
|---|---|---|---|
| ***Gram-positive bacteria*** | | | **Ampicillin** |
| *Staphylococcus aureus* | 16.6 ±1.16 | ND | 25±0.9 |
| *Streptococcus pneumoniae* | 18.2±2.24 | ND | 25.2±2.18 |
| *Bacillus subtilis* | 18.0±0.4 | ND | 20.0±1.1 |
| ***Gram-negative bacteria*** | | | **Gentamicin** |
| *Shigella dysenteriae* | ND | ND | 17.7±1.22 |
| *Salmonella typhimurium* | ND | ND | 26±1.1 |
| *Escherichia coli* | 14.7±1.37 | ND | 25±0.9) |
| ***Yeast*** | | | **Nystatin** |
| *Candida albicans* | 26±2.5 | 10.1±1.85 | 26.4±1.20 |

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

**Table 5:** Minimal inhibitory concentration (MIC) of the essential oils of *Pulicaria inuloides and Ocimum forskolei* against bacteria and yeast

| Microorganism | MIC (µl/mL) | |
|---|---|---|
| | ***Pulicaria inuloides*** | ***Ocimum forskolei*** |
| *Staphylococcus aureus* | 6.25 | ND |
| *Streptococcus pneumoniae* | 3.7 | ND |
| *Bacillus subtilis* | 3.0 | ND |
| *Shigella dysenteriae* | ND | ND |
| *Salmonella typhimurium* | ND | ND |
| *Escherichia coli* | 3.0 | ND |
| *Candida albicans* | 3.12 | 35.3 |

*ND = Not detectable, i.e., essential oil has no antimicrobial activity against this microorganism*

antioxidant capacity [11]. In addition, *P. inuloides* essential oils exhibited higher radical scavenging activities than *O. forskolei* ($IC_{50}$, 4.95 ± 0.05 µg/mL vs. 73.54 ± 0.05 µg/mL). These results are consistent with a previous report on the radical scavenging activities of *Pulicaria* species [14]; however, there are few studies reporting β-carotene assay results for essential oils [14]. Differences in values may be due to differences in geographical areas, genus, reproductive stage, climate, season of harvest, and extraction methods [15].

Similar to the essential oils described in this report, the essential oils of *Thymus* species contain the aromatic monoterpenes caryophyllene oxide and thymol, and their biological activities are often attributed to these compounds [2]. In this study, *P. inuloides* essential oils demonstrated higher antibacterial activities against all bacteria tested except *Salmonella typhimurium* and *Shigella dysenteriae*. Furthermore, the Gram-positive bacteria *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Bacillus subtilis* were more sensitive to this essential oil than the Gram-negative bacteria *Escherichia coli*. Similarly, a previous study reported that the essential oils of *Pulicaria astephanocarpa* showed high antimicrobial activity against *Candida albicans* and all tested bacteria except *Pseudomonas aeruginosa* [13]. Our results suggest that *P. inuloides* essential oils may be useful in the treatment of infectious diseases caused by *S. aureus, B. subtilis, S. pneumoniae, E. coli*, and *C. albicans*.

The essential oils of *O. forskolei* demonstrated no antibacterial activity against all tested bacteria (Table 4), which is consistent with a previous report [16]. 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 [17].

In the present study, we found that *P. inuloides* essential oils demonstrated activity against *C. albicans*. This activity may be related to its high level of oxygenated monoterpenes, especially its major constituent, 2-cyclohexen-1-one, 2-methyl-5-(1-methyl) (55.1 %). In addition, the presence of two phenolic isomers of thymyl acetate (0.143 and 0.354 % of the total content) may synergistically contribute to the antifungal activity of *Pulicaria* essential oils. Phenolic compounds in essential oils (e.g., carvacrol, thymol, and eugenol) are thought to be primarily responsible for their biological properties [18]. The antimicrobial activity of these phenolic compounds can be attributed to the presence of

an aromatic nucleus and a phenolic OH group, which can form hydrogen bonds with disulfide groups at the active sites of target fungal enzymes, resulting in their deactivation [18].

MIC values indicate that *P. inuloides* essential oils have a greater inhibitory action against Gram-positive bacteria than Gram-negative bacteria. A previous study showed that the antimicrobial effects of spices and herbs against *C. albicans*, *E. coli*, and other Gram-negative bacteria were due to their complex chemical composition, which included compounds such as thymol, carvacrol, methyl eugenol, linalool, α-pinene, 1, 8-cineole, and camphor [19]. This discrepancy in antibacterial potential may be caused by variations in chemical composition, which may be influenced by the distillation and extraction techniques as well as geographical origin.

In our study, *O. forskolei* essential oils were completely inactive against all tested microorganisms other than *C. albicans*. This result may be due to evaporation of the oil during the boiling process. The antifungal property of the oil was likely due to the eugenol [18]. Nanasombat and Lohasupthawee [19] described the antibacterial action of the essential oils derived from another Ocimum species, *O. basilicum*, against clinically determined multidrug-resistant isolates of *Staphylococcus*, *Enterococcus*, and *Pseudomonas*, reporting MIC values of 0.0030 % to 0.0007 % (v/v). In another study, *O. gratissimum* essential oils demonstrated significant antimicrobial action, whereas essential oils of *O. basilicum* were found to have less activity, and *O. sanctum* oils were completely inactive against microorganisms [19]. Our findings were consistent with the results of these studies analyzing *Ocimum* species. After reviewing the literature, we found no studies evaluating the antimicrobial activity of essential oils and/or plant extracts of *P. inuloides* or *O. forskolei*. Therefore, we believe our study is the first to report on this topic, thus making a significant contribution to the scientific field.

## CONCLUSION

The essential oils of *P. inuloides* exerted strong antimicrobial actions against gram-positive bacteria and *C. albicans*, whereas *O. forskolei* essential oils inhibited the growth of *C. albicans* only. The strong antioxidant activity of *P. inuloides* essential oil suggests its potential as a food additive and candidate for clinical development as a new broad-spectrum bioactive compound.

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