Pharmaceutical and Biosciences Journal
ISSN: 2582-0540
Available at www.ukjp.com

Phytochemical Constituents and Antimicrobial Activities of the Essential Oils of Libyan *Pituranthos chloranthus* on Different Species of Bacteria

Yahya Saber E Mansour¹*, Nusieba A Mohammed Ibrahim¹, Yaroub S Seriwa², Krishna Chaitanya Varma Sagi³

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-bayda, Libya
²Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University, Al-bayda, Libya
³Qpathlabs, Visakhapantnam, Andhra Pradesh, India

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Abstract

Essential oils (EOs) obtained from fresh and dry aerial parts of Libyan *Pituranthos chloranthus* were analyzed and isolated by Gas Chromatography/Mass Spectrometry (GC/MS) and hydro-distillation technique. The main constituents of the EOs obtained from fresh herb of *P. chloranthus* were found to be α-pinene, sabinene, cis-β-ocimene, and myrcene whereas the major components of the EOs derived from dry herb of *P. chloranthus* were α-phellandrene, Δ-3-carene, and β-phellandrene. Minor changes in the phytochemical compositions of the *P. chloranthus* EOs obtained from fresh or dry herbs; however, the major constituents were found to be α-pinene, sabinene, cis-β-ocimene, myrcene, α-phellandrene, Δ-3-carene, and β-phellandrene with approximate amounts of p-cymene, limonene, trans-β-ocimene, γ-terpinene, and cis-verbenol. The paper-disc agar diffusion method was used to evaluate the antimicrobial activities of *P. chloranthus*, and the results showed that the EOs extracted from fresh herb had a significant inhibitory effect against most of the tested bacteria.

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1 Introduction

Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs¹. They are commonly rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans, and lignins¹. These compounds have multiple biological effects including antimicrobial and antioxidant activities. Plants naturally produce EOs to shield themselves from infectious microorganisms. These EOs are utilized in folk medicines for thousands of years as antimicrobial agents¹. Essential oils were frequently referred to as the natural and environmentally friendly cleaning solutions. They are used as a substitute to chemicals to disinfect and spread a pleasant scent in the air². They are additionally used to manage human diseases of microbial origin and to cure some diseases such as coronary artery disease (CAD) and cancer²³. The insecticidal properties of EOs are extensively studied against numerous insect species⁴. Moreover, the utilization of antioxidants having natural origin has become more popular as a means to increase shelf-life of food product, to improve the stability of fats and oils, and to slow down the aging. *P. chloranthus* is an endemic and aromatic plant, locally named “Guezzah” which grows naturally in North Africa and is widespread in northeast of Libya. Yanghui et al., 2009, demonstrated that *P. chloranthus* EOs collected at Sfax (Tunisia) were mainly composed of terpinen-4-ol, 8-hydroxy-p-cymene, myrtenol, p-menth-2-en-1-ol, and α-terpineol and exhibited antioxidant, antifungal, and insecticidal activities¹. Stems of *P. chloranthus* are traditionally used as straw for farmers to dry figs and grapes⁵. This plant encompasses a double advantage: i) it is used for its aroma and distinctive taste that adhere to the dry fruits, and, ii) it has an insecticidal effect. In some African countries such as Tunisia, a tuft of *P. chloranthus* was traditionally suspended on the surface of the water to clean the underground cisterns of the rainwater storage used for the
human drink. Furthermore, *Pituranthos* species are used in
traditional medicines for the treatment of respiratory illnesses
(especially asthma), rheumatism, postnatal care, spasms, pains,
fevers, diabetes, lice, hepatitis, digestive disorders, urinary tract
infections, and scorpions’ stings. The main aim of this present
study was to focus on this non exploited endemic plant as a new
material within the production of EOs that were worthwhile evaluated
because of their antimicrobial activities and possible exploitation
as natural disinfectants and food preservatives.

2 Materials and Methods

2.1 Plant sample collection

The samples of about 1 kg of the same species of *P. chloranthus*
were collected on April 2019 from northeast of Libya (Al-bayda
and Al-mekhely). The botanical identification of *P. chloranthus*
was carried out by the staff members of the Faculty of Pharmacy,
Omar Al-Mukhtar University, Al-bayda, Libya.

2.2 Essential oil extraction

The collected samples were divided into two groups, one used
as fresh herb and the other air-dried during 20 days in shade at
ambient temperature for the EO extraction with a modified
Clevenger-type apparatus for 5 h. The EOs were collected, dried
by anhydrous sodium sulfate (Na$_2$SO$_4$), and stored at 4°C in
tightly closed dark vials until analysis.

2.3 GC and GC/MS analysis

GC analysis was carried out using an Agilent 6890N Network GC
system fitted with flame ionization detector (FID) and an
electronic integrator, using a HP-5 fused silica capillary column
(30m×0.25mm i.d., film thickness 0.25mm) was
The oven temperature was programmed from
50°C-280°C at 7°C/min; injector temperature: 220°C; detector
temperature: 240°C; carrier gas: nitrogen (1.0ml/min); sample
manually injected: 0.2ml. EOs constituents were also analyzed by
GC/MS using the Agilent 6890N GC system combined with Agilent 5975B Inert MSD detector (quadrupole)
with electron impact ionization (70 eV). A HP-5-MS fused silica
capillary column (30m×0.25 mm i.d., film thickness 0.25mm) was
used. The column temperature was programmed to rise from
50°C-280°C at 7°C/min. The carrier gas was helium adjusted to a
linear velocity of 34 cm/s. Scan time and
mass range were 2.2 s and 50-550 m/z, respectively. Samples (0.1 ml) were injected
with a split ratio of 1:100. Identification of the components was
based on, i) the comparison of their GC Retractive Indexes (GC
RI) on a polar column (HP-5) with those of literature data
by comparison of their recorded mass spectra with those of
a computer library (Wiley 275 library and NIST98 Database/Chem
Station Data System) provided by the instrument software and
MS literature data, and, ii) identities of some other
components were further confirmed by co-injection of pure
standards available in the laboratory under the same GC/MS
conditions as above.

2.4 Antimicrobial assay

The antimicrobial activities of the special EOs were evaluated by
the paper-disc agar diffusion method against
*Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes*
(ATCC 19615), *Escherichia coli* (ATCC 25922), and *Klebsiella pneumoniae* (ATCC 13883). These clinical strains were obtained from the Department of Microbiology, Faculty of Science, Omar
Al-Mukhtar University, Al-bayda, Libya. The microorganisms
were maintained on Muller-Hinton (MH) agar medium. Inocula
were prepared by diluting overnight (24 h at 37 °C) cultures in
Muller-Hinton Broth (MHB) medium to approximately 106
CFU/ml. Absorbent discs (Whatman N˚3 discs, 6 mm in diameter)
were impregnated with 10 μl of oil and then placed on the
surface of inoculated plates. Positive control discs of gentamicin
(10 μg/disc) were included in each assay. Diameters of growth
inhibition zones were measured after incubation at 37 °C for 24
h.

3 Results and Discussion

As shown in table 1, the main constituents of the EOs obtained
from fresh herb of *P. chloranthus* were found to be α-pinene
(40.7%), sabinene (14.5%), cis-β-ocimene (6.8%), and myrcene
(6.7%). In dry herb, the percentage of α-pinene was less than in
fresh herb, and the cis-β-ocimene was there as a trace, but the
major constituents were α-phellandrene (6.7%), Δ-3-carene
(4.7%), and β-phellandrene (12.5%). There were slight changes
in the phytochemical compositions of *P. chloranthus* EOs
obtained from fresh or dry herbs; however, the main constituents
were found to be α-pinene, sabinene, cis-β-ocimene, myrcene,
α-phellandrene, Δ-3-carene, and β-phellandrene with approximate amounts of p-cymene, limonene, trans-β-ocimene,
γ-terpinene, and cis-verbenol. The only compound that its
composition has been modified after drying the herb was 3-n-
buty1 phthalide from 2.0% to 2.8%. The influence of drying the
above herb on the phytochemical compositions has been previously reported by several authors. A differential response of the aromatic species is accredited generally to the
loss of some compounds during the storage of the herb after
deteriorating oil glands and/or due to some physiological
processes that continue even after harvesting.

The different EOs isolated from *P. chloranthus* harvested at the
vegetative, flower growing, fruiting, and flowering stages from
northeast of Libya (Al-bayda and Al-mekhely). They were mainly
composed of α-pinene, sabinene, α-phellandrene, myrcene, β-
phellandrene, p-cymene, and Δ-3-carene. However, these
components varied with respect to both the geographical area
and the season: p-cymene was only detected at the floral
budding stage (February), whereas the vegetative stage (November) could be distinguished by the presence of αβ-
pinene and limonene. This differentiation on the compositions
between the EOs suggests that different chemo-types of *P.
chloranthus* exist in Libya. That depends on the period of the

Pharm & Biosci J. 2019: 7(4): 28
The antibacterial activities of *P. chloranthus* EOs were evaluated by a paper-disc diffusion technique against some strains of bacteria. As shown in table 2, the results revealed that the EOs obtained from fresh herb of *P. chloranthus* exhibited higher antibacterial activities than those of dried herb. Loughlin *et al.*, 2008, demonstrated that the EOs especially oxygenated monoterpenes such as α-pinene, sabinene, myrcene, and cis-β-ocimene had antimicrobial activities.

To the extent of our knowledge, the antimicrobial activities, and the disinfectant properties of Libyan *P. chloranthus* essential oils have never been reported. Therefore, this study was the first report on the biological characteristics of this herb in the arid zones. Table 1 indicated that the Libyan *P. chloranthus* EOs contained a high proportion of oxygenated monoterpenes. Regarding *P. chloranthus* EOs, all tested bacteria were found to be more sensitive against the oil isolated from fresh herb than the one extracted from dried herb except *Klebsiella pneumoniae* which showed the same weak action with both type of EOs (from fresh and dried herbs). Some strains such *Escherichia coli* which were resistant against dried herb oil, showed a weak activity against fresh herb oil. The highest activity has been observed for *Streptococcus pyogenes* and *Staphylococcus aureus* with fresh herb oil (35±0.4 and 40±0.9 mm respectively), while dried herb oil revealed a weak activity against these two strains (15±0.8 and 14±0.3 mm respectively). These results demonstrated that the EOs isolated from fresh *P. chloranthus* exhibited a strong antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus*, which was similar as 10 μg of gentamicin.
as a positive control. This significant activity could be attributed to the high amount of α-pinene, sabinene, myrcene, and cis-β-ocimene known to have exhibited potent activity against these strains. Minor components such as γ-terpinene and cis-verbenol could also contribute to this activity in the synergism with major components. Some other components such as trans-β-ocimene, limonene, Δ3-carene, and β-eudesmol could also be attributed to the antibacterial activity of this EO.

Table 2: Antimicrobial activities of the EOs of Pituranthos chloranthus and the standard antibiotic (gentamicin) against four different species of bacteria (inhibition zone diameters: mm)

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>Gentamicin</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh herb</td>
<td>Dry herb</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>45±0.6</td>
<td>35±0.4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>35±0.2</td>
<td>40±0.9</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>20±0.7</td>
<td>10±0.7</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>15±0.5</td>
<td>8±0.2</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (Triplicate reading)

4 Conclusion

This in vitro experimental study showed that the EOs isolated from fresh aerial parts of Libyan P. chloranthus had a potent antibacterial activity against both Streptococcus pyogenes and Staphylococcus aureus. Our results suggest that these EOs can be utilized as natural food preservatives, as well as possible sources of antimicrobial ingredients for the food and pharmaceutical industries.

5 Recommendations

Further studies should be undertaken on the EOs of dried herb, which could exhibit other biological activities due to their phytochemical composition which is different from fresh herb. Additional research can be also conducted to identify the antioxidant activity and the cytotoxicity of Libyan P. chloranthus.

6 Acknowledgment

We would like to express our gratitude to the entire staff at the Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University, Al-bayda, Libya, who have been so helpful and cooperative in giving their support at all times to help us achieve our goal.

7 Conflicts of Interest

We hereby declare that there are no conflicts of interest regarding the publication of this research article.

8 Author’s contributions

This work was carried out in collaboration between all authors. Author YSEM designed the study, wrote the protocol, collected the plant samples, and extracted the EOs. Author NAMI drafted the manuscript, managed the literature searches, and performed the statistical analysis. Author YSS performed the antimicrobial screening techniques. Author KCVS analyzed the phytochemical constituents via GC/MS. All authors read and approved the final manuscript.

9 References

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