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Phytochemical Constituents and Antimicrobial Activities of Libyan *Pituranthos Chloranthus* on Different Species of Bacteria

Yahya Saber E Mansour [1]*, Nusieba A Mohammed Ibrahim [2], Yaroub S Sergiwa [3], Krishna Chaitanya Varma Sagi [4]

[(1, 2)] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Omar Al-Mukhtar University, Al-bayda, Libya

[(3)] Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University, Al-bayda, Libya

[(4)] Qpathlabs, Visakhapantnam, Andhra Pradesh, India

Abstract: *The principal objectives of this current study were to identify the phytochemical constituents and the antimicrobial activities of Libyan Pituranthos chloranthus. The essential oils (EOs) that were obtained from the fresh and dry aerial parts of Libyan P. chloranthus were isolated and analyzed by the Gas Chromatography/Mass Spectrometry (GC/MS) and the hydro-distillation technique. The most constituents of the EOs obtained from the fresh herb of P. chloranthus were found to be α-pinene, sabinene, cis-ocimene, and myrcene whereas the major components of the EOs derived from the dry herb of P. chloranthus were α-phellandrene, Δ-3-carene, and β-phellandrene. The EOs of the fresh and dry herbs were also contained approximate percentages 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 a significant restrictive impact of the EOs obtained from the fresh plant herb against most of the tested microorganisms.*

Keywords: *Pituranthos chloranthus*, essential oils (EOs), northeast of Libya

INTRODUCTION

Plants naturally produce essential oils to shield themselves from infectious microorganisms. These essential oils are utilized in folk medicine for thousands of years as antimicrobial agents **(1)**. Essential oils were often observed because of their natural and environmentally friendly cleaning solutions. They are also used as a substitute for chemicals to make clean and unfold a nice scent within the air **(2)**. They are additionally used to manage human diseases of microbial origin and to cure some diseases such as coronary artery disease (CAD) and cancer **(2)(3)**. The insecticidal properties of essential oils are extensively studied against numerous insect species **(4)**. Moreover, the utilization of antioxidants having natural origin has become a lot of in style as a way to extend the shelf-life of food product, to improve the stability of fats and oils, and to slow down the aging. *P. chloranthus* is a North African endemic plant, domestically named Al-Guezzah (القزاح). The stems of *P. chloranthus* are traditionally used as straw for farmers to dry figs and grapes (5)(6). This plant encompasses a double advantage: i) First, it is used for its aroma and distinctive taste that adhere to the dry fruits, and, ii) Second, it has an insecticidal effect. In some African countries, a tuft of *P. chloranthus* was historically suspended on the surface of the water to clean the underground cisterns of the rainwater storage used for the drink. Furthermore, *Pituranthos* species are used in traditional medicine for the treatment of respiratory illnesses, rheumatism, postnatal care, spasms, pains, fevers, diabetes, lice, hepatitis, digestive difficulties, urinary infections, and scorpions' stings **(6)(7)**. The main aim of this present study was to focus on this nonexploited endemic plant as a new material within the production of essential oils that were worthily evaluated due to their antimicrobial activities and possible exploitation as a natural disinfectant and food preservative.

MATERIALS AND METHODS

1. Plant Sample Collection

Two samples of about 1kg of the aerial parts of *P. chloranthus* were collected on April 2019 in two different random localities (9km north and 10km east) of Al-bayda, Libya. 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. The fresh plant material (500g of each sample) was subjected to the hydro-distillation during approxing time nearly about 5h in a Clevenger-type apparatus. The distilled essential oils of each sample were separately collected. They were dried over anhydrous sodium sulfate (Na_2SO_4) and stored in tightly closed dark vials at 3˚C till use.

2. Gas Chromatography/Mass Spectrometry (GC/MS)

GC analysis was carried out using an Agilent 6890N Network GC System. GC fitted with flame ionization detector and an electronic integrator, using a HP-5 fused silica capillary column (30m×0.32mm) **(8).** 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. The identification of the components was based on, i) the comparison of their GC Refractive Indexes (GC RIs) on a polar column (HP-5) with those of literature data **(6)-(9)**, ii) the 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 **(9) (10)**, and, iii) the identities of some other components were further confirmed by co-injection of pure standards offered in the laboratory beneath the same GC/MS conditions as above.

3. Antimicrobial Assay

The antimicrobial activity of the special essential oils was evaluated by the paper-disc agar diffusion method **(11)** against the *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Escherichia coli* (ATCC 25922) and *Klebsielle 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 agar medium. The inocula were prepared by diluting overnight (24h at 37˚C) cultures in Muller-Hinton broth medium to approximately 106 CFU/ml. Absorbent discs which were Whatman N˚3 discs, 6mm in diameter were impregnated with 10µL of oil and then placed on the surface of inoculated plates. Positive control discs of gentamicin (10µg/disc) were included in each assay. Diameters of growth inhibition zones were measured after incubation at 37˚C for 24h.

RESULTS

As shown in **Table 1**, the main constituents of the EOs there were obtained from the fresh herb of *P. chloranthus* were found to be *α*-pinene (40.7%), sabinene (14.5%), cis-ocimene (6.8%), and myrcene (6.7%). In the dry herb, the percentage of *α*-pinene was less than in the fresh herb and the *cis*-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 chemical compositions of the *P. chloranthus* essential oils obtained from the fresh or dry herbs; however, the main constituents were found to be α-pinene, sabinene, cis-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 content has been modified after drying the biomass was 3-n-butyl phthalide from 2% to 2.8%. The influence of drying the above ground biomass on the chemical composition has been previously reported by quite a few authors **(12) (13)**. A differential response of the aromatic species is accredited generally to the loss of some compounds during the storage of the biomass after deteriorating oil glands and/or due to some physiological processes that continue even after harvesting.

Table 1. Major active constituents of the essential oils of *Pituranthos choloranthus by* GC/MS.

			Pituranthos chloranthus	
			Dry Herb	**Fresh Herb**
		Refractive Index (RI)	1.537	1.463
		Yield %	0.93	1.98
Major Compounds	***Rt$_{lit}$***	***Rt***	**Density**	0.632	0.845
3-n-butyl phthalide	1720	1722		2.8	2.0
β-eudesmol	1649	1645		1.5	**Trace**
t-cadinol	1640	1638		1.9	1.4
cis-verbenol	1140	1138		0.6	0.6
***γ*-terpinene**	1058	1060		0.4	0.8
trans-β-ocimene	1045	1040		0.8	0.3
***cis*-β-ocimene**	1037	1036		**Trace**	6.8
β-phellandrene	1032	1029		12.5	3.8
limonene	1031	1025		**Trace**	**Trace**
***p*-cymene**	1023	1020		5.3	6.3
Δ-3-carene	1010	1007		4.7	**Trace**
α-phellandrene	1008	995		6.7	3.6

myrcene	986	990	3.5	6.7
β-pinene	980	979	1.7	1.9
sabinene	976	956	11.9	14.5
α-pinene	937	940	29.9	40.7

***Rt*:** Retention time on a HP-5 column**;** ***Rt*$_{lit}$:** Retention time on HP-5 column according to literature data; **Trace** which is less than 1%.

The different essential oils isolated from the *P. chloranthus* harvested at the vegetative, flower growing, fruiting, and flowering stages from two separately different areas of 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 **(2)**. However, these compositions varied with respect to both the geographical area and the season: p-cymene was only detected at the floral budding stage (on February), whereas the vegetative stage (on November) could be distinguished by the presence of α/β-pinene and limonene **(2)**. This differentiation on the compositions between the essential oils suggests that the different chemo-types of *P. chloranthus* exist in Libya. That depends on the period of the collection of the vegetal samples **(6)**. The essential oils of the samples that were collected during November were mainly composed of myrcene, sabinene, limonene, and α-pinene whereas the EOs of the samples that were harvested during April were mainly composed of terpineol, butylidene phthalide, limonene, and sabinene. The major components of the oil prepared by the hydro-distillation technique were β-myrcene, sabinene, and terpinen-4-ol; those predominant in the oil extracted by the simultaneous hydro-distillation solvent (*n*-pentane) extraction.

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

		Pituranthos chloranthus	
Type of Bacteria	**Gentamicin**	**Fresh Herb**	**Dry Herb**
Streptococcus pyogenes	45	35	15
Staphylococcus aureus	35	40	14
Escherichia coli	20	10	0
Klebsielle pneumoniae	15	8	5

The antibacterial activities of *P. chloranthus* with different essential oils collected from the northeast of Libya were evaluated by a paper-disc diffusion technique against some bacteria. As shown in **Table 2** above, the results revealed that the EOs obtained from the fresh herbs of *P. chloranthus* exhibited higher antimicrobial activities than those of dried herbs. Previous studies demonstrated that the essential oils and especially oxygenated monoterpenes such as α-pinene, sabinene, myrcene, and cis-β-ocimene had antimicrobial activities (Loughlin *et al.*, 2008).

DISCUSSION

To the best of our knowledge, the antimicrobial activities and the disinfectant properties of the 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* essential oils contained a high proportion of oxygenated monoterpenes. The antibacterial activities of *P. chloranthus* essential oils were evaluated by a paper-disc diffusion method against some strains of bacteria. As shown in **Table 2**, the results proved that the EOs obtained from the fresh herbs exhibited higher antibacterial activities compared to the dried herbs. Regarding *P. chloranthus* EOs, all tested bacteria were found to be more susceptible against the oil isolated from the fresh herbs than the one extracted from the dried herbs except *Klebsielle pneumoniae* which showed the same weak action with both type of oils. *Escherichia coli* showed a resistant activity against the dried herb oil and a weak activity against the extract of fresh herb oil. The highest activity has been observed for *Streptococcus pyogenes* and *Staphylococcus aureus* with the fresh herb oil (35 and 40mm respectively), while the dried herb oil revealed a weak activity against these two strains (15 and 14mm respectively). These results demonstrated that the essential oils isolated from the fresh *P. chloranthus* exhibited a strong antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus* which was similar as 10µg of gentamicin as a positive control. This critical 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 stains. The minor components such as γ-terpinene and cis-verbenol could also contribute to this activity in the synergism with the major components **(18)-(21)**. Some other components such as trans-β-ocimene, limonene, Δ-3-carene, and β-eudesmol could be also contribute to the antibacterial activity of this essential oil **(14)**.

CONCLUSION

This in vitro experimental study obviously revealed the phytochemical constituents and the efficient antibacterial activities of Libyan *P. chloranthus* essential oils. It also supports the traditional uses and strongly encourages the generously use of this natural product as an antiseptic.

RECOMMENDATIONS

A further study can be conducted to identify the antioxidant activity and the toxicity of Libyan *P. chloranthus.*

CONFLICTS OF INTEREST

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

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